The Response of Anaerobic Prokaryotic Processes and Communities in Severn Estuary Sediments to Environmental Change

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"And heaved and heaved, still unrestingly heaved the black sea, as if its vast tides were a conscience; and the great mundane soul were in anguish and remorse for the long sin and suffering it had bred."
Herman Melville, Moby Dick

"I cannot think of the deep sea without shuddering at the nameless things that may at this very moment be crawling and floundering on its slimy bed"
H. P. Lovecraft, Dagon

"There is no point trying to hide from your bacteria… This is their planet, and we are only on it because they allow us to be"
Bill Bryson, A Short History Of Nearly Everything

Summary

The Severn Estuary in the south-western UK is one of the most tidally dynamic environments on the planet. However, despite this the sediments of the estuary remain relatively understudied with regards to their biogeochemical potential. The aim of this project was to investigate how the constantly changing sedimentary environment in the estuary, in which millions of tonnes of sediment are eroded and deposited over the tidal cycle, affects the prokaryotes within the sediments and the processes they control, and also to determine what effect environmental changes in the estuarine system might have on these processes.

The study showed that the sediments of the Severn Estuary have high rates of sediment oxygen demand (SOD) indicating a high degree of organic matter (OM) degradation. However, the sediments have low rates of the anaerobic processes that are expected to dominate in shallow marine systems (e.g. sulphate reduction and methanogenesis), suggesting that most OM degradation must be linked to processes further up the redox cascade. The sediments also showed a lack of microbial guild depth zonation, with methanogenesis occurring above or alongside sulphate reduction. Both of these unusual factors can be linked to the regular re-suspension of the estuary's sediments by tidal action, resulting in large-scale oxidation and mixing of the sediment column and the suppression of anaerobic processes while potentially stimulating aerobic and dysaerobic activity. This same mixing would also distribute guilds of organisms throughout the sediment, creating isolated pioneer populations with a general lack of competition. Re-suspension is also likely responsible for the high cell counts that persist to significant depth around the estuary, as the mixing of sediment and entrainment of OM would produce high and homogeneous cell profiles upon deposition, which in turn can be linked to the high SOD of the estuary's sediments.

Despite this dominance of aerobic and dysaerobic processes throughout most of the estuary some isolated sites do show increased rates of anaerobic processes, particularly at locations that have undergone significant environmental change. These include fluidised mud pools in the deeper areas of the estuary, salt marsh peat deposits at St Brides Wentlooge (especially within the "activated interface") and Cardiff Bay, an anthropogenic lake and former mudflat environment which shows significant methanogenic potential.

Overall this study has shown that the dynamic conditions in the Severn Estuary promote the activity of aerobic and dysaerobic prokaryotic groups over the anaerobic groups traditionally thought to dominate in shallow marine sediments. However, this promotion is not uniform across the estuary, instead varying with topography/bathymetry and the degree of sediment disturbance.

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ANME – anaerobic methanotroph

Anammox – anaerobic ammonium oxidation

AODC – acridine orange direct counts

AOM – anaerobic oxidation of methane

AVS – acid-volatile sulphide

BCE – before common era

BP – before present

CRS – chromium (II) reducible sulphide

DMA – dimethylamine

DMEA – *N*, *N*-dimethylethanolamine

DMF – *N*, *N*-dimethylformamide

DMPD – *N*, *N*-dimethyl-*p*-phenylenediamine

DNRA – dissimilatory nitrate reduction to ammonium

DO – dissolved oxygen

DOC – dissolved organic carbon

DOM – dissolved organic matter

DSR – dissimilatory sulphate reduction

$\Delta G_o'$ – Gibbs free energy

E_a – activation energy

E_o' – redox potential under standard conditions

E_H – redox potential

ETM – estuarine turbidity maximum

GWP – global warming potential

HMW – high molecular weight

K_m – half saturation constant (Michaelis constant)

LMW – low molecular weight

lws – litre of wet sediment

MMA – monomethylamine/ methylamine

MMEA – *N*-monomethylethanol amine

MPB – microphytobenthos/ microphytobenthic

MPR – methane production rate

MQ H_2O – Milli-Q water

NADH – nicotinamide adenine dinucleotide (reduced)

NAO – North Atlantic oscillation

OC – organic carbon

OFN – oxygen free nitrogen

OM – organic matter

PCA – principal component analysis

PSS – practical salinity scale

Q_{10} – temperature coefficient

RCF – relative centrifugal force

SMTZ – sulphate-methane transition zone

SOD – sediment oxygen demand

SPM – suspended particulate matter

SRB – sulphate reducing bacteria

SRR – sulphate reduction rate

SSSI – site of special scientific interest

SST – sea surface temperature

TMA – trimethylamine

TMEA – *N*, *N*, *N*-trimethylethanol amine/choline

T_{opt} – optimum growth temperature

TRIS – total reducible inorganic sulphur

VFA – volatile fatty acid

w/v – weight:volume

Chapter 1. An Introduction to Estuarine Sediment Biogeochemistry and the Severn Estuary

This chapter will introduce the main biogeochemical processes that occur in estuarine sedimentary systems as well as the external factors that can influence them. It will also provide an introduction the study area itself, namely the Severn Estuary in the southwestern UK.

1.1 The Redox Cascade

Estuaries are one of the most biogeochemically active and diverse environments on the planet (Bianchi, 2007). This is mainly due to the large amounts of compounds (organic matter, nitrate, sulphate etc.) that enter estuaries both from the land, via rivers, and from the marine environment. In terms of organic matter (OM) mineralisation most of this activity occurs within the sediments of the estuary where it is carried out by guilds of chemoorganoheterotrophic prokaryotes (with lithotrophic groups oxidising the reduced inorganic waste products). These organisms usually occur in specific biogeochemical depth zones within the sediment column, the positions of which are dictated by a number of factors including: the efficiency of the organism's respiration reaction; the concentration and type of organic matter; the availability of electron donor/acceptor compounds, and particularly the presence/absence of oxygen. This (usually vertical) sequence of depth-zonated microbial communities is referred to as the redox cascade (Froelich *et al.* 1979, Canfield and Thamdrup, 2009) (Fig. 1.1).

Within these zonated sediments the organisms with the most efficient respiration pathway are usually present in the uppermost zone, at or near the surface, with respiration pathways gradually becoming less efficient moving downward through the sediment. This is due to competition for common organic substrates, which occurs because, despite different guilds of microorganisms having different levels of respiration efficiency and different electron-acceptor substrates, most still utilise similar organic carbon substrates (such as acetate, lactate etc.) to serve as electron donors (as well as hydrogen) and carbon sources. However, the guilds with the more efficient respiration pathways (such as the aerobes or denitrifiers) will usually outcompete those with less efficient pathways (such as the

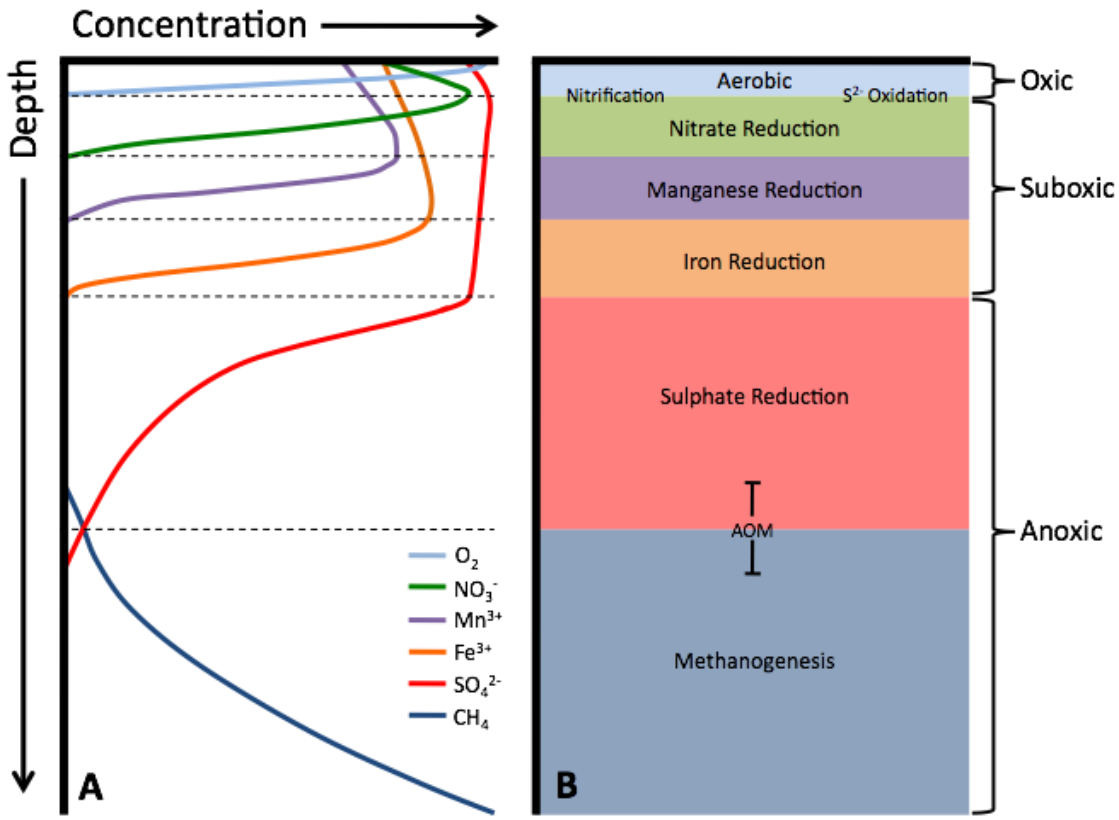


Fig. 1.1 – Diagram of the standard arrangement of geochemical zones present in shallow aquatic sediments, generated by variations in redox potential and metabolic efficiency (the "redox cascade"). The diagram shows: A) changes in the concentrations of major compounds with depth (concentrations not to scale) and; B) the main microbial processes controlling the production and consumption of these compounds and the O_2 availability in each zone. The relative size of these metabolic zones will vary depending on location, linked to the availability of electron acceptor compounds and organic carbon.

sulphate reducers and methanogens) and as such will occur higher in the sediment column where concentrations of bioavailable carbon substrates are higher. The less efficient prokaryotes are, therefore, relegated to the lower levels of the sediment where the higher-efficiency organisms are not dominant. This decrease in higher-efficiency organisms with depth is due to the electron acceptors used in these reactions (such as O_2, NO_3^-, Fe^{3+} and SO_4^{2-}) being a limited resource, usually derived from the surface. Therefore, when removed, these compounds restrict the activity of the prokaryotes that consume them. As a result of this, descending through the sediment, the levels of these compounds will decrease as they are consumed (shown in Fig. 1.1), until the concentrations become too low for the prokaryotes to take up, limiting the activity of the guild utilising that particular electron acceptor (Ribes *et al.* 2004). This enables a new guild, utilising a lower-energy-yielding electron acceptor to take over. This process then repeats itself down through the sediment.

As well as this competition, some guilds of prokaryotes are relegated to the deeper portions of the sediment column because they are only capable of metabolising certain low molecular weight (LMW) groups of organic carbon compounds. These compounds are usually produced as waste products by other groups of prokaryotes (e.g. fermenters), and therefore, these LMW-utilising guilds can only occur alongside fermenters, which are predominantly confined below the aerobic layer of the sediment (see below).

In addition, certain guilds are also limited in their positioning in the sediment due to the presence of oxygen and redox potential of the sediment. Within the upper layers of the sediment where oxygen is present, the microbial populations are dominated by aerobic (oxygen consuming) organisms (obligate and facultative) and the sediment is referred to as the oxic zone. Below this surface layer oxygen does not penetrate as it is consumed by aerobes as fast as it can diffuse into the sediment, however, oxidised compounds are still present. As such the zone below is referred to as the oxidised/suboxic/dysoxic/dysaerobic zone, where redox potentials (E_H) are greater than 0-100 mV and can be as high as +400 mV (Jørgensen, 2006). Prokaryotes in this zone can be: microaerophilic (preferring small amounts of oxygen); facultative anaerobes (able to respire both with and without O_2); or obligate anaerobes (only respire in the absence of O_2) utilising electron acceptors such as nitrate or metal oxides. Finally, beneath the oxidised/suboxic zone is the anoxic zone. In these sediments (where the E_H is <0-100 mV and as low as -200 mV) most, if not all, of the chemical species are reduced and it is here that organisms which cannot tolerate oxygen (obligate anaerobes) dominate.

Since the redox cascade described above leads to the production of large amounts of reduced compounds (NH_4^+, Fe^{2+}, S^{2-} etc.), an environment that contained only reducing bacteria would soon develop high concentrations of reduced species. This would be detrimental to the reducing guilds, as not only would they quickly run out of electron acceptors, but high concentrations of these reduced waste products could also inhibit further metabolism by making their respiratory reactions thermodynamically inefficient. In addition certain reduced compounds (e.g. H_2S) can also be toxic at high concentrations (OFlaherty *et al.* 1998). However, this circumstance does not occur in nature due to the presence of guilds of (predominantly) chemolithoautotrophic prokaryotes that live slightly above the

reducers and obtain energy by oxidising the upwardly diffusing reduced compounds produced by them back to oxidised species, which then diffuse downward again.

The sections below will detail the various metabolic processes carried out by microorganisms (both eukaryotic and prokaryotic) that are commonly present in estuarine sediments, beginning with those that occur at the top of the sediment column. Most sections will deal with both the reduction and oxidation reactions that occur in each zone of the redox cascade detailed above.

1.1.1 Aerobic Respiration

Both prokaryotic and eukaryotic microorganisms in the top layers of the sediment carry out aerobic respiration. This reaction involves the oxidation of organic carbon compounds (e.g. acetate) to carbon dioxide (CO_2), with oxygen (O_2) acting as an electron acceptor and itself being reduced to water (H_2O) (Eq. 1.1):

$$CH_3COO^- + H^+ + 2O_2 \Rightarrow 2CO_2 + 2H_2O \quad (1.1)$$

This reaction is very efficient as O_2 has a high redox potential (+820 mV) compared with NADH, the cell's internal electron carrier (E_o'= -320 mV) (Jørgensen, 2006), and as such the Gibbs free energy (ΔG_o') produced by the reaction is high (-871 kJ mol^{-1} of acetate using Eq. 1.1) (Jankowski *et al.* 2008). Since aerobic respiration is so efficient in comparison to other metabolic pathways it is usually the dominant respiration mechanism in environments where O_2 is plentiful (such as in the water column of unstratified estuaries). This high energy yield also enables a large range of enzymes to function, which increases the range of carbon substrates that aerobes can utilise (Freeman e*t al.* 2001). However, within most shallow water sediments O_2 is not able to permeate deep into the subsurface (usually only a few millimetres to centimetres) due to its rapid consumption by aerobes, and as such acts as a self limiting factor on the distribution of these organisms, restricting them to this oxic, surface zone (Sass *et al.* 2003). Despite the efficiency of aerobic respiration, aerobic organisms are rarely able to degrade all of the organic material that is deposited on the sediment surface and as such a proportion of this material becomes buried in the anoxic zone of the sediment beneath, where it can be utilised by other organisms with less efficient metabolic pathways (Jørgensen, 1982).

1.1.2 Nitrogen Cycling - Denitrification, DNRA and Nitrification/Ammonium Oxidation

Nitrate reduction occurs within the suboxic zone where O_2 levels are negligible and involves the oxidation of organic carbon compounds coupled to the reduction of nitrate (NO_3^-) to either nitrogen gas (N_2) in a process called denitrification; or to ammonium (NH_4^+), known as dissimilatory nitrate reduction to ammonium (DNRA) – in contrast, assimilatory nitrate reduction involves uptake of nitrogen for synthesizing amino acids, proteins etc. Bacteria capable of carrying out these reactions include: *Pseudomonas* spp., *Paracoccus denitrificans*, and *Thauera* spp. (denitrification); and *Escherichia coli*, *Desulfovibrio desulfuricans* and *Wolinella succinogenes* (DNRA) (Kraft *et al.* 2011). The equations for these reactions are shown below:

Denitrification:

$$5CH_3COO^- + 8NO_3^- + 13H^+ \Rightarrow 4N_2 + 10CO_2 + 14H_2O \quad (1.2)$$

DNRA:

$$CH_3COO^- + NO_3^- + 3H^+ \Rightarrow 2CO_2 + NH_4^+ + H_2O \quad (1.3)$$

These respiration pathways have redox potentials of +751 mV (Eq. 1.2, $NO_3^- \Rightarrow N_2$) and +363 mV (Eq. 1.3, $NO_3^- \Rightarrow NH_4^+$) and as such have sizeable energy yields: -771 kJ mol^{-1} and -489 kJ mol^{-1} for denitrification and DNRA respectively (Jankowski *et al.* 2008). This range in energy yields (which also change with different carbon substrates) means that the nitrate reduction zone can sometimes overlap with the manganese reduction zone depending on which Mn-containing compounds are present in the sediment (Froelich *et al.* 1979). As well as oxidising organic matter, nitrate reduction can also be coupled to the oxidation of inorganic compounds such as hydrogen, ferrous iron (Straub *et al.* 1996), sulphur (Burgin *et al.* 2012), or H_2S and iron sulphide (Krishnakumar and Manilal, 1999; Vaclavkova *et al.* 2014)

The oxidation process associated with nitrate reduction is nitrification (or ammonium oxidation). The bacteria that carry out this process convert ammonium to nitrite (NO_2^-) and nitrate with O_2 as the electron acceptor. This reaction is a two-step process with one group, the nitrosofiers (including the genera *Nitrosomonas* and *Nitrosospira*), converting ammonium to nitrite (Eq. 1.4) (Konhauser, 2007):

$$NH_4^+ + 1.5O_2 \Rightarrow NO_2^- + H_2O + 2H^+ \quad (1.4)$$

While the other, the nitrifiers (including the genera *Nitrobacter* and *Nitrococcus*), oxidise nitrite to nitrate (Eq. 1.5):

$$NO_2^- + 0.5O_2 \Rightarrow NO_3^- \quad (1.5)$$

The energy yields from these reactions are -92 kJ/2 e^- and -74 kJ/2 e^- respectively, well below half the energy yielded by denitrification (-226 kJ/2 e^-). However, a group of bacteria has been identified that are able to oxidise ammonia directly to nitrogen gas without the requirement for O_2. These anaerobic ammonia oxidising (anammox) bacteria utilise ammonia and nitrite (Strous *et al.* 1998) in the reaction below (Eq. 1.6) (Konhauser, 2007):

$$NH_4^+ + NO_2^- \Rightarrow N_2 + 2H_2O \quad (1.6)$$

This results in a ΔG_0' of -358 kJ/reaction. The organisms that carry out this reaction belong to the order *Planctomycetales* (Strous *et al.* 1999) and are present in a wide variety of environments, ranging from sewage farms and stratified basins to estuaries and mid-ocean ridges (Byrne *et al.* 2008; Nicholls and Trimmer 2009; Bale *et al.* 2014).

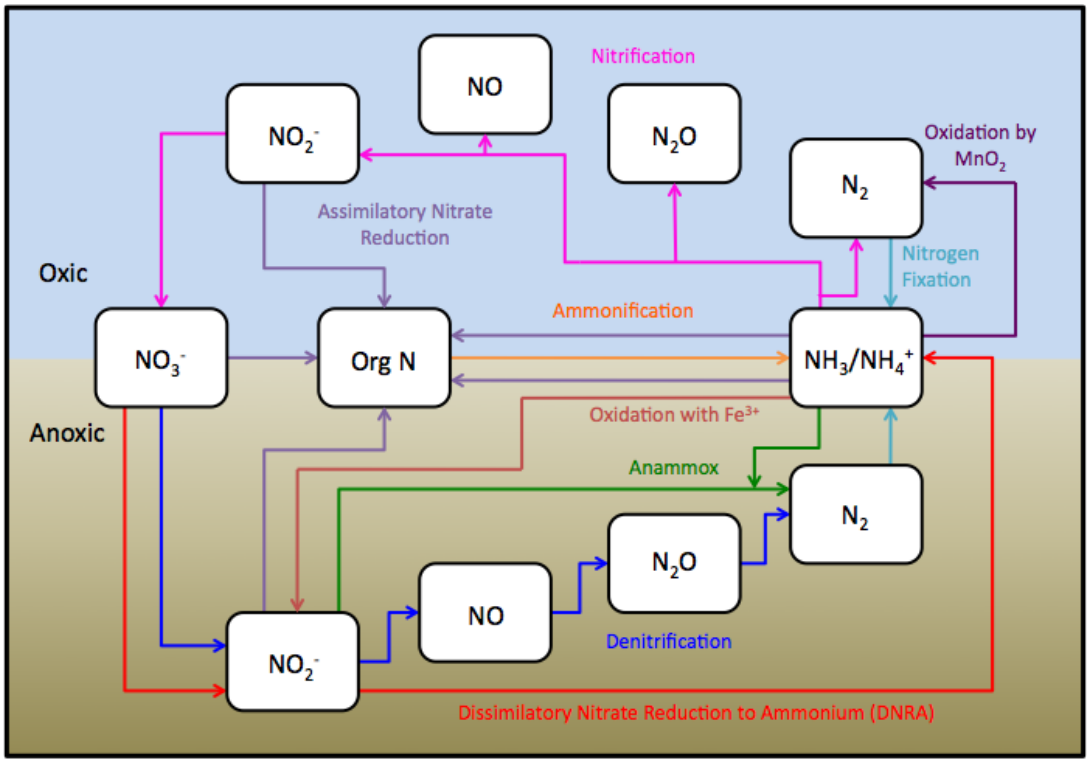


Fig.1.2 – Diagram showing the chemical reactions involved in the sedimentary nitrogen cycle. Processes in the upper section are aerobic/microaerophillic and occur primarily at or near the sediment surface or within the water column. Processes in the lower section of the diagram are anaerobic and usually occur within the dysoxic/anoxic portion of the sediment.

The various processes described in this section, along with the ammonification of organic matter, and nitrogen fixation, in which atmospheric nitrogen is converted to ammonia (and then into biomass), in the oceans mainly by phototropic cyanobacteria such as *Trichodesmium* and *Synechococcus* (Karl *et al.* 1997), combine to form the marine nitrogen cycle (Fig 1.2), an extremely important cycle in estuarine and coastal systems due to the role of nitrogen in influencing primary production as a limiting nutrient (Herbert, 1999; Hulth *et al.* 2005).

1.1.3 Dissimilatory Manganese Reduction and Oxidation

Dissimilatory manganese (Mn) reduction often occurs in the suboxic zone and involves the oxidation of organic carbon compounds (Eq. 1.7) or H_2 (Eq. 1.8) with manganese oxides (generalised formula MnO_2) acting as electron acceptors and being reduced to Mn^{2+} ions in the process (Vandieken *et al.* 2014) - although several groups of Mn reducers can also grow as facultative aerobes, using O_2 as an electron acceptor when available. This process is carried out by a number of bacterial genera including *Bacillus*, *Geobacter*, *Pseudomonas* and *Shewanella* (Thamdrup, 2000; Bräuer *et al.* 2011). The equations for these reactions are shown below (Konhauser, 2007):

$$CH_3COO^- + 4MnO_2 + 3H_2O \Rightarrow 4Mn^{2+} + 2HCO_3^- + 7OH^- \quad (1.7)$$

$$H_2 + MnO_2 \Rightarrow Mn^{2+} + 2OH^- \quad (1.8)$$

This respiration pathway is also quite efficient as MnO_2 again has a high redox potential (+390 mV) resulting in a relatively large energy yield from the reaction (-558 kJ mol^{-1} acetate and -166 kJ mol^{-1} H_2 respectively). However this yield varies depending on the Mn-containing mineral that is being reduced (Froelich, 1979), for example the $\Delta G_o'$ obtained from the reduction of birnessite ($(Na_{0.3}Ca_{0.1}K_{0.1})(Mn^{4+},Mn^{3+})_2O_4 \cdot 1.5\ H_2O$) is higher than that obtained from the reduction of pyrolusite (MnO_2). Due to this variation in energy yields, the position of the Mn reduction zone within the sediment column is variable and can therefore occur either at the same level as the nitrate reduction zone or just below it, dependent on both the Mn-containing substrate present and the type of nitrate reduction that is occurring. In addition, if concentrations of Mn oxides in the

sediment are high then Mn reduction can become the dominant OM degradation pathway (Canfield *et al.* 1993a, b).

The opposite reaction to Mn reduction is Mn oxidation. Under abiotic conditions this process occurs relatively slowly due to the high activation energy needed to convert Mn^{2+} to Mn^{3+} or MnO_2 (although this can depend on the pH of the environment in question). Therefore, it is thought that most of the MnO_2 produced in marine sediments is present as a result of bacterial metabolism. The organisms that carry out this reaction belong to the genera *Pseudomonas* and *Bacillus* (and also potentially *Shewanella* and *Rhodobacter*) and it is believed that they utilise a 2-step reaction:

$$2Mn^{2+} + 0.5O_2 + 2H^+ \Rightarrow 2Mn^{3+} + H_2O \quad (1.9)$$

$$2Mn^{3+} + 0.5O_2 + 3H_2O \Rightarrow 2MnO_2 + 6H^+ \quad (1.10)$$

This first converts Mn^{2+} to Mn^{3+} (Eq. 1.9) and then Mn^{3+} to MnO_2 to gain energy (Eq. 1.10) (Konhauser 2007). Oxidation of upwardly diffusing Mn^{2+} can then in turn lead to elevated MnO_2 concentrations at the oxic:suboxic interface in sediments (Aller, 1990; Canfield *et al.* 1993b)

1.1.4 Dissimilatory Iron Reduction and Oxidation

Below the Mn reduction zone the next process to occur is usually iron reduction. The bacteria that carry out this process include the genera *Geobacter*, *Shewanella*, *Geospirillum* and *Geovibrio*. This respiration pathway involves the oxidation of organic carbon compounds (Eq.1.11) or H_2 (Eq. 1.12) coupled to the reduction of compounds containing ferric iron (Fe^{3+}) to produce ferrous iron ions (Fe^{2+}) (Konhauser, 2007):

$$CH_3COO^- + 8Fe(OH)_3 \Rightarrow 8Fe^{2+} + 2HCO_3^- + 15OH^- + 5H_2O \quad (1.11)$$

$$0.5H_2 + Fe(OH)_3 \Rightarrow Fe^{2+} + 2OH^- + H_2O \quad (1.12)$$

This reaction has a redox potential of only +150 mV and as such the energy yield from the reaction is quite low (-337 kJ mol^{-1} acetate and -110 kJ/$2e^-$ respectively). However, even more so than with manganese, the yield varies depending on the Fe-containing mineral being reduced, with haematite (Fe_2O_3) reduction having a higher ΔG_o' than the reduction of goethite (FeOOH) (Froelich *et al.* 1979). Certain

acidophilic organisms (e.g. *Acidthiobacillus* spp. and *Sulfobacillus* spp. see below) are also capable of coupling iron reduction to the oxidation of other inorganic compounds, such as elemental sulphur (Pronk *et al.* 1992) and tetrathionate (Bridge and Johnson, 1998; Johnson, 1998).

Unlike Manganese oxidation, under abiotic conditions Fe^{2+} oxidises rapidly with O_2 to form Fe^{3+} and subsequently to Fe hydroxides and oxyhydroxides. As such any organism that couples its metabolism to the oxidation of Fe^{2+} has to compete with O_2 for substrate. Prokaryotes get around this problem in two ways; the first is by living under acidic conditions where the reaction rate between O_2 and Fe^{2+} is slower (or ceases completely). Organisms that carry out this mode of life are known as acidophiles (e.g. *Acidthiobacillus ferrooxidans* and *Ferroplasma acidiphilum*) and are often found in environments such as acid-mine-drainage lakes and volcanic sulphur springs (Oren, 2010) which can reach pH values as low as 1-2 (Golyshina *et al.* 2000). These organisms utilise the reaction below to generate ATP (Eq. 1.13):

$$2Fe^{2+} + 0.5O_2 + 2H^+ \Rightarrow 2Fe^{3+} + H_2O \quad (1.13)$$

However, at pH 2 this reaction generates only 33 kJ mol^{-1} of Fe^{2+} (Konhauser, 2007), and therefore acidophilic organisms must oxidise large amounts of Fe^{2+} to be able to survive. The other group of Fe oxidising bacteria live under neutral pH conditions, but at redox conditions and O_2 concentrations lower than normal surface water. These organisms are called neutrophiles and are often found in iron springs and stratified water bodies. Under neutral conditions the energy generated by Fe^{2+} oxidations is substantially larger than under acidic conditions (due to differences in the electrode potential of the Fe^{3+}/Fe^{2+} couple). Therefore neutrophilic Fe oxidisers can generate much more energy from a similar amount of substrate than their acidophilic counterparts (Konhauser, 2007).

1.1.5 Dissimilatory Sulphate Reduction and Sulphide Oxidation

Beneath the iron reducers the next redox zone in the sediment is the sulphate reduction zone. The prokaryotic microorganisms that inhabit this zone gain energy by oxidising organic substrates or H_2 to CO_2 or H_2O respectively (Sørensen *et al.* 1981), while reducing sulphate ions (SO_4^{2-}) to hydrosulphide ions (HS^{2-}) via

dissimilatory sulphate reduction, and are therefore known as sulphate-reducing bacteria (SRB). These bacteria are predominantly members of the δ-Proteobacteria and spore-forming Firmicutes and include the genera *Desulfovibrio*, *Desulfobulbus, Desulfotomaculum, Desulfobacter*, *Desulfosarcina*, *Desulfococcus* and *Desulfonema*. They are able utilise a wide variety of electron donors, such as H_2 (either derived from larger molecules like lactate and pyruvate, or directly from the environment), and reduce sulphate (Eq. 1.14):

$$4H_2 + SO_4^{2-} + H^+ \Rightarrow HS^- + 4H_2O \quad (1.14)$$

This produces a ΔG_o' of -152 kJ mol^{-1}. Other organisms can also utilise acetate as an electron donor, completely oxidising it to CO_2 (Eq. 1.15):

$$CH_3COO^- + SO_4^{2-} + 2H^+ \Rightarrow 2CO_2 + HS^- + 2H_2O \quad (1.15)$$

This in turn produces a ΔG_o' of -41 kJ mol^{-1} (Jørgensen, 2006). These groups can often also completely oxidise other compounds, including: lactate, longer chain fatty acids, alkanes and benzoic acid (Thauer *et al.* 2007). In addition some groups (e.g. *Desulfovibrio vulgaris*) only incompletely oxidise compounds such as lactate, producing acetate in the process (Eq. 1.16):

$$2CH_3CH(OH)COO^- + SO_4^{2-} + H^+ \Rightarrow 2CH_3COO^- + 2CO_2 + HS^- + 2H_2O \quad (1.16)$$

This reaction yields a ΔG_o' of -196.4 kJ mol^{-1} (Thauer *et al.* 2007). However, despite these low energy yields, SRB respiration is the dominant anaerobic OM mineralisation process in many shallow marine sediments, at least equalling (and possibly exceeding) the amount of aerobic organic carbon mineralisation (Jørgensen, 1982; Canfield *et al.* 1993a).

As well as sulphate some groups of prokaryotes (including some SRB) are also able to couple their metabolism to the reduction of other sulphur containing compounds such as elemental sulphur (S^0) and sulphite (SO_3^{2-}) and the oxidation of either acetate or H_2:

S^0 Reduction:

$$CH_3COO^- + 4S^0 + 4H_2O \Rightarrow 4H_2S + 2HCO_3^- + H^+ \quad (1.17)$$

$$H_2 + S^0 \Rightarrow H_2S \quad (1.18)$$

SO_3^{2-} Reduction:

$$3CH_3COO^- + 4SO_3^{2-} + 5H^+ \Rightarrow 4H_2S + 6HCO_3^- \quad (1.19)$$

These reactions produce ΔG_o' values of -7 kJ mol^{-1} of acetate (Eq. 1.17), -28 kJ mol^{-1} of H_2 (Eq. 1.18) and -126 kJ mol^{-1} of acetate (Eq. 1.19) respectively. This means that sulphur reduction with acetate is theoretically impossible, as it does not produce the minimum energy necessary to be used as a metabolic substrate (≈-20 kJ mol^{-1}, the energy required to move one proton across the cell membrane) (Schink, 1997). However, sulphur reducers have been found that can grow in this fashion (Pfennig and Biebl, 1976) and these organisms appear to grow best in the presence of bacteria which utilise the metabolic waste products of the sulphur reducers (e.g. *Chlorobium*), which makes the above equation more energetically favourable (Biebl and Pfennig, 1978; Jackson and McInerney, 2002).

The reverse reaction to sulphate reduction is sulphur oxidation. This process is carried out by 3 main of groups of bacteria that obtain their energy by oxidising reduced sulphur species (such as S^{2-} or S^0) to sulphate. The first of these groups are colourless sulphur bacteria. These organisms grow at or near the sediment surface in areas where O_2 concentrations are low and concentrations of upward-diffusing S^{2-} are high. They can grow either as individual cells (*Thiobacillus*, *Macromonas*) or, in areas with high productivity, as dense mats covering large areas of sediment (*Beggiatoa*, *Thioploca*). These organisms oxidise S^{2-} to SO_4^{2-} to obtain energy (Fenchel and Bernard, 1995), although some will only partially oxidise the S^{2-} to S^0, which is then stored intracellularly and can be further oxidised to SO_4^{2-} to obtain energy if S^{2-} levels run low:

Sulphide oxidation to sulphate:

$$H_2S + 2O_2 \Rightarrow SO_4^{2-} + 2H^+ \quad (1.20)$$

Sulphur oxidation:

$$S^0 + 1.5O_2 + H_2O \Rightarrow SO_4^{2-} + 2H^+ \quad (1.21)$$

These produce ΔG_o' values of -199 kJ/2 e^- (Eq. 1.20) and -196 kJ/2 e^- (Eq. 1.21) respectively (Konhauser, 2007). In more dysoxic/anoxic environments where O_2 is not available, these organisms are also capable of using compounds such as NO_3^- or Fe^{3+} as electron acceptors (Marzocchi *et al*. 2014; Vaclavkova *et al.* 2014). As well as

SO_4^{2-}, compounds such as thiosulphate ($S_2O_3^{2-}$) and SO_3^{2-} can also be produced via sulphide oxidation as by-products or metabolic intermediates (Thamdrup *et al.* 1994).

The second group of sulphide/sulphur oxidisers are acidophiles that thrive in acid-mine-drainage lakes associated with coal or metal mining (Oren, 2010) and include the genera *Acidthiobacillus* and *Sulfolobus*. Some members of these two chemolithoautotrophic groups are also capable of oxidising $S_2O_3^{2-}$ or SO_3^{2-} to SO_4^{2-} as well as S^{2-} using the equations below:

Thiosulphate oxidation:

$$0.5S_2O_3^{2-} + O_2 + 0.5H_2O \Rightarrow SO_4^{2-} + H^+ \quad (1.22)$$

Sulphite oxidation:

$$SO_3^{2-} + 0.5O_2 \Rightarrow SO_4^{2-} \quad (1.23)$$

These yield -205 kJ/2 e^- (Eq. 1.22) and -258 kJ/2 e^- (Eq. 1.23) respectively. Some groups are also able to oxidise thiosulphate to tetrathionate ($S_4O_6^{2-}$), often using nitrogen compounds as electron acceptors (Sorokin *et al.* 1999), while others can oxidise $S_4O_6^{2-}$ itself (Kupka *et al.* 2009)

The third group of sulphide oxidisers are different from the others in that they are anoxygenic photoautotropic bacteria. These green and purple sulphur bacteria, which include the genera *Pelodictyon* and *Chlorobium* are a strictly anaerobic group that are often found at the base of microbial mats and in the hypolimnion of stratified lakes. These organisms utilise H_2S in a similar manner to the way oxygenic photoautotrophs use water (Brune 1989), oxidising it to SO_4^{2-} (Eq. 1.24):

$$3H_2S + 6CO_2 + 6H_2O \Rightarrow C_6H_{12}O_6 + 3SO_4^{2-} + 6H^+ \quad (1.24)$$

In the process organic matter (in this case glucose) is synthesised. Like the colourless sulphur bacteria detailed above, the phototrophic sulphur bacteria are also capable of converting S^{2-} to S^0, storing it in either within the cell in vacuoles (e.g. *Chromatium*) or on the outside of the cell membrane (e.g. *Ectothiorhodospira*) and then utilising it as an emergency energy source if S^{2-} levels become dangerously low (Eq. 1.25):

$$12S^0 + 18CO_2 + 30H_2O \Rightarrow 3C_6H_{12}O_6 + 12SO_4^{2-} + 24H^+ \quad (1.25)$$

Also, like the colourless sulphur bacteria, many are able to utilise $S_2O_3^{2-}$ instead of S^2, oxidising it to SO_4^{2-} (Eq. 1.26):

$$3S_2O_3^{2-} + 6CO_2 + 9H_2O \Rightarrow C_6H_{12}O_6 + 6SO_4^{2-} + 6H^+ \quad (1.26)$$

As well as reducing and oxidising, some groups of bacteria (such as *Desufovibrio sulfodismutans*) are also capable of coupling their metabolism to the disproportionation (simultaneous oxidation and reduction) of sulphur compounds (Bak and Cypionka, 1987; Bak and Pfennig, 1987; Finster *et al*. 1998) including SO_3^{2-}, $S_2O_3^{2-}$ and S^0:

Sulphite disproportionation:

$$4SO_3^{2-} + 2H^+ \Rightarrow 3SO_4^{2-} + H_2S \quad (1.27)$$

Thiosulphate disproportionation:

$$S_2O_3^{2-} + H_2O \Rightarrow SO_4^{2-} + H_2S \quad (1.28)$$

Sulphur Disproportionation:

$$4S^0 + 4H_2O \Rightarrow SO_4^{2-} + 3H_2S + 2H^+ \quad (1.29)$$

These yield ΔG_o' values of -236 kJ/reaction (Eq. 1.27), - 22 kJ/reaction (Eq. 1.28) and +41 kJ/reaction (Eq. 1.29) respectively (Konhauser, 2007). As sulphur disproportionation is energetically unfavourable (it has a positive ΔG_o' value) it can only take place in environments where H_2S is rapidly removed from the system, either by oxidation by other microorganisms or precipitation with an Fe or Mn bearing mineral (Thamdrup *et al.* 1993). As is the case with nitrate, the processes detailed above do not act in isolation and as such are all linked together in the marine sedimentary sulphur cycle (Fig 1.3).

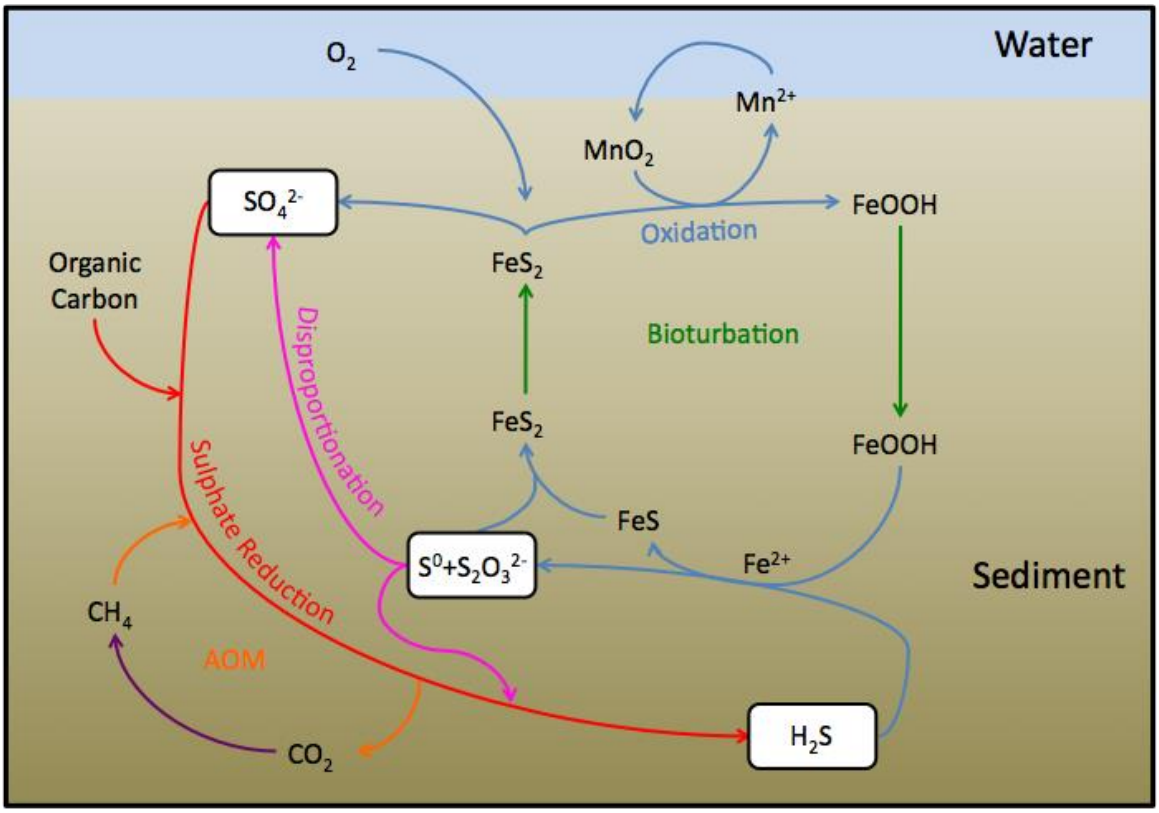


Fig. 1.3 – Diagram of the processes and major compounds involved in the sedimentary sulphur cycle (modified from Jørgensen and Kasten, 2006). Also shown are interactions with the iron and manganese cycles and the effects of bioturbation (discussed further in Chapter 2).

1.1.6 Methanogenesis, Fermentation and AOM

The deepest diagenetic redox zone in estuarine sediments is called the methanogenic or methanic zone, as it is within this zone that biogenic methane is produced due to the activity of *Archaea* called methanogens. These organisms belong to the Euryarchaeota and include the genera *Methanobacterium*, *Methanococcus* and *Methanosarcina*. The metabolic reactions carried out by these *Archaea* (known as methanogenesis) involve either the disproportionation of organic carbon compounds (such as acetate) to produce methane (CH_4) and CO_2 (known as acetoclastic methanogenesis – Eq. 1.30) or the reduction of HCO_3^-/CO_2 to CH_4 coupled to the oxidation of H_2 to water (Eq. 1.31). The equation for these reactions are shown below:

Acetoclastic Methanogenesis

$$CH_3COO^- + H^+ \Rightarrow CH_4 + CO_2 \quad (1.30)$$

H_2/CO_2 Methanogenesis

$$4H_2 + HCO_3^- + H^+ \Rightarrow CH_4 + 3H_2O \quad (1.31)$$

The ΔG_o' values for these reactions are -28 kJ mol^{-1} (Eq. 1.30) and -136 kJ mol^{-1} (Eq. 1.31) respectively (Jørgensen, 2006). Due to competition from other groups, methanogenesis is usually confined to the lower depths of the sediment column and is often the terminal organic carbon oxidation process in the redox cascade. However, in some environments certain groups of methanogens can occur much nearer the sediment surface as they consume so called non-competitive substrates. These are organic carbon substrates that fulfil a similar role to acetate in the respiration reaction. However, these compounds are not as widely utilised by other groups of prokaryotes and as such, the methanogens do not have to compete so much with the other groups for them. This allows these methanogens to live at any depth in the anoxic zone of the sediment column. These non-competitive substrates are usually compounds that contain methyl groups (CH_3-R) such as methanol and trimethylamine (Oremland *et al.* 1982). The reactions for the reduction of methanol, with (Eq. 1.32) and without H_2 (Eq. 1.33), are shown below:

$$CH_3OH + H_2 \Rightarrow CH_4 + H_2O \quad (1.32)$$

$$4CH_3OH \Rightarrow 3CH_4 + CO_2 + 2H_2O \quad (1.33)$$

The ΔG_o' values for these reactions are -113 kJ (Eq. 1.32) and -319 kJ (Eq. 1.33) respectively (Madigan and Martinko, 2006).

It is important to note that while methanogens are able to utilise short chain organic compounds such as acetate, these compounds are rarely directly deposited in the sediment, and if they are, they are usually consumed by other groups closer to the surface (Sørensen *et al.* 1981; Parkes *et al.* 1989). What's more, those that are currently known are unable to directly metabolise fatty acids with more than two carbons (propionate, butyrate etc.), or aromatic compounds. Therefore the methanogens living at depth rely on the activity of other microbial metabolic guilds to break down more complex compounds into acetate, H_2 etc. These organisms are generally known as fermenters and they are capable of breaking down an extremely wide variety of substrates by using organic material as both an electron donor and an electron acceptor. However, while fermenters in general are able to use a wide variety of substrates each species is only able to utilise a select few. Therefore,

fermenters often work in sequence, with one group degrading a larger compound to a smaller one which is subsequently utilised as a substrate by another group (Fig.

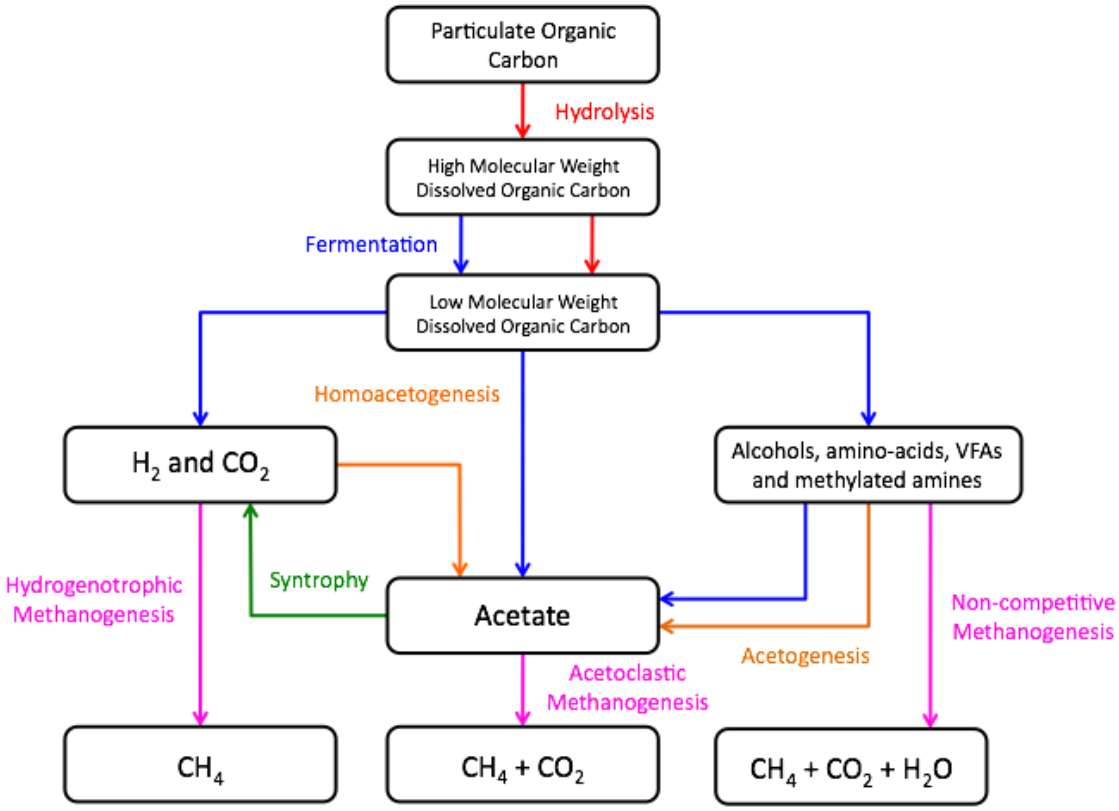


Fig. 1.4 – Diagram showing the mineralization pathway of organic carbon from large polymeric molecules to CO_2 and CH_4 via fermentative and methanogenic pathways. Modified from Weston and Joye (2005) and Madigan and Martinko (2006).

1.4), with the final step being the conversion to acetate, formate or methyl-containing compounds which are then utilised by methanogens (Glombitza *et al.* 2014). In addition to fermentation, some of the heterotrophic processes described above are capable of supplying substrates to the methanogens. For example incomplete oxidising SRB such as *Desulfovibrio* spp. are capable of producing acetate due to the incomplete oxidation of lactate (Thauer *et al.* 2007). Also a group of organisms called the homoacetogens are capable of producing acetate from H_2 and CO_2 (Diekert and Wohlfarth, 1994).

Another important process occurring in marine sediments is the anaerobic oxidation of methane (AOM) (Iversen and Jørgensen, 1985). This process usually occurs at the depth in the sediment column where methanogens take over from SRB as the dominant prokaryotic group, in an area known as the sulphate-methane

transition zone (SMTZ). At this depth biogenic methane (produced by methanogens below the SMTZ) is converted to HCO_3^- in a reaction coupled to the reduction of sulphate by SRB. The net formula for this reaction is shown below in Eq. 1.34 (Jørgensen and Kasten, 2006):

$$CH_4 + SO_4^{2-} \Rightarrow HCO_3^- + HS^- + H_2O \quad (1.34)$$

However, as yet no single organism has been found that is capable of carrying out the above reaction. Environmental and mixed culture data suggest that AOM may be carried out by a consortium of two groups of organisms (Boetius *et al.* 2000): one, an archaeon (referred to as an anaerobic methanotroph, or ANME) oxidises methane; while a second, an SRB, metabolises the end products of the *Archaea* along with sulphate and in doing so allows the first reaction to remain energetically favourable. Several reaction pathways have been put forward for such a process (Valentine, 2002; Caldwell *et al.* 2008), the first of which (called reverse methanogenesis) involves the oxidation of methane to produce CO_2 and hydrogen (Eq. 1.35), which is subsequently used to as a electron donor by SRB (Eq. 1.36):

ANME:

$$CH_4 + 2H_2O \Rightarrow CO_2 + 4H_2 \quad (1.35)$$

SRBs:

$$SO_4^{2-} + 4H_2 + H^+ \Rightarrow HS^- + 4H_2O \quad (1.36)$$

However, Nauhaus *et al.* (2005) found that adding H_2 to the SRB in AOM communities did not uncouple the process, suggesting that H_2 may not be the electron transfer molecule involved. Also, while this reaction pathway could work, it does not account for the fact that SRB involved in AOM have low $\delta^{13}C$ values (characteristic of biogenic methane) which would indicate some form of carbon transfer between the ANME and SRB groups working in the consortia (Jørgensen and Kasten, 2006). An alternative pathway (acetogenesis) was, therefore, suggested in which methane was converted to either acetic acid and hydrogen (Eq. 1.37) or acetate (Eq. 1.40) (which would carry the light isotopic signal), and then utilised by SRB – Eqs. 1.38, 1.39 and 1.41 (Valentine and Reeburgh, 2000):

Acetic acid and Hydrogen:

ANME:

$$2CH_4 + 2H_2O \Rightarrow CH_3COOH + 4H_2 \quad (1.37)$$

SRB:

$$4H_2 + SO_4^{2-} + H^+ \Rightarrow HS^- + 4H_2O \quad (1.38)$$

$$CH_3COOH + SO_4^{2-} \Rightarrow 2HCO_3^- + HS^- + H^+ \quad (1.39)$$

Acetate:

ANME:

$$CH_4 + HCO_3^- \Rightarrow CH_3COO^- + H_2O \quad (1.40)$$

SRBs:

$$SO_4^{2-} + CH_3COO^- \Rightarrow HS^- + 2HCO_3^- \quad (1.41)$$

A third mode of substrate transfer (methylogenesis) was also proposed by Moran *et al.* (2008) in which methyl sulphides act as the energy transfer substrate within the consortia (Eqs. 1.42 and 1.43):

ANME:

$$CH_4 + \tfrac{1}{3}HCO_3^- + \tfrac{5}{3}H^+ \tfrac{4}{3}HS^- \Rightarrow \tfrac{4}{3}H_3CSH + H_2O \quad (1.42)$$

SRB:

$$\tfrac{4}{3}H_3CSH + SO_4^{2-} \Rightarrow \tfrac{4}{3}HCO_3^- + \tfrac{7}{3}HS^- + \tfrac{5}{3}H^+ \quad (1.43)$$

The authors proposed this reaction after conducting experiments in which AOM consortia were grown under high partial pressures of hydrogen (0.43 mM), since the reverse methanogenesis pathway is not energetically favourable under high hydrogen concentrations. They also concluded that because only a select number of SRB groups can utilise methyl sulphides (*Desulfococcus*, *Desulfosarcina* and relatives of *Desulfobulbus*) this might explain why the diversity of SRB in AOM consortia is low. The net energy yield of AOM reactions is between -22 to -35 kJ mol^{-1} of methane, which is near the threshold (≈-20 kJ mol^{-1}) required by the cell to generate ATP (Schink, 1997). However, because the SRB scavenge the ANME's waste products the reactions are able to remain energetically favourable albeit near the minimum value required for microbial respiration (Caldwell *et al.* 2008)

As well as having an unusual respiration pathway, the identities of the organisms involved in AOM have also proved difficult to discern. This is mainly due to their syntrophic nature, which makes it all but impossible to study them in pure culture. Genetic analyses of AOM consortia though, have revealed that the ANME involved come from a broad range of groups within the *Euryarchaeota* (Jørgensen and Kasten, 2006) but can be grouped into three clades: ANME-1 which consists of organisms distantly related to the *Methanosarcinales* and *Methanomicrobiales*; ANME-2 which occurs within the *Methanosarcinales* and ANME-3 which are closely related to the *Methanococcoides* (Caldwell *et al.* 2008). Genetic studies have also revealed that the SRBs involved generally belong to the *Desulfosarcina-Desulfococcus* group within the δ-Proteobacteria, although some related to the genus *Desulfobulbus* have also been found (Lösekann *et al.* 2007).

Although sulphate coupled AOM appears to be the main method of methane oxidation in marine sediment (mainly due to sulphate being the first electron acceptor that methane encounters as it diffuses up through the sediment column) it is not the only AOM pathway that occurs. For example, AOM coupled to both nitrate (Raghoebarsing *et al.* 2006) and nitrite (Luesken *et al.* 2011) reduction has been shown to occur in freshwater environments where sulphate concentrations are generally low, whereas nitrate concentrations can become quite high, especially in eutrophic environments. Nitrate reduction is also more energetically favourable than sulphate reduction, so in freshwater environments (particularly those where high levels of methane are produced, e.g. rice paddies) nitrate-coupled AOM may play a major role in the global methane cycle (Deutzmann and Schink, 2011; Norei and Thamdrup, 2014), and could also have links to anammox (Shi *et al.* 2013; Chen *et al.* 2014). In addition, AOM coupled to manganese and iron reduction has also been found to occur, both in saline (Beal *et al.* 2009; Riedinger *et al.* 2014) and freshwater (Sivan *et al.* 2011) environments, suggesting that AOM is a much more diverse process than was previously thought.

As can be seen in the sections above, the microbial processes that occur in sediments are often interdependent on one another, as well as on other processes occurring within the overlying water column (e.g. photosynthesis), to provide them with substrates and/or to remove their waste products. This means that each process

cannot operate sustainably in isolation and is instead part of a larger and more complex interlinked microbial food web (Fig. 1.5), which is capable of operating on depth scales of between a few millimetres to over a kilometre depending on the environment in question (Ciobanu *et al.* 2014). As such when examining sediment

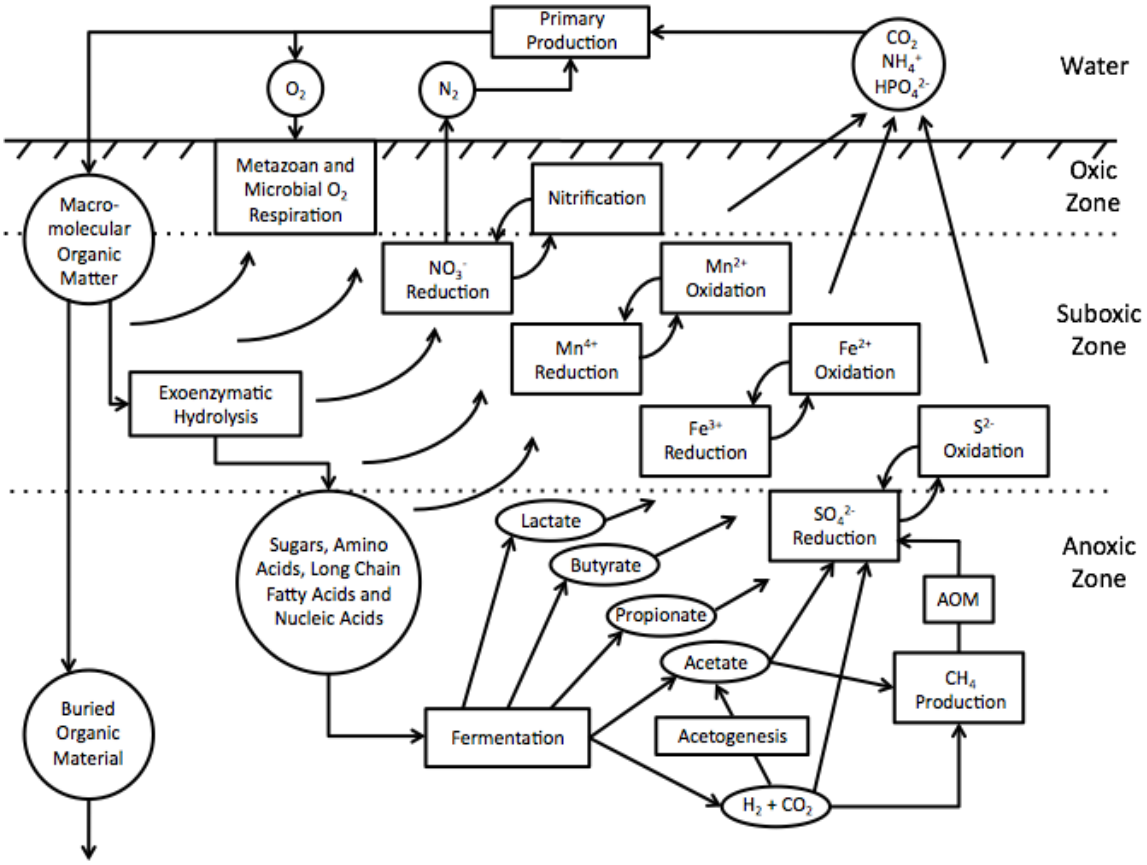


Fig. 1.5 – Diagram showing the processes and compounds described in section 1.1 and how they connect to each other to form a microbial food web. Modified from Jørgensen (2006).

microbial communities it is always important to consider how changes in environmental parameters might influence the community as a whole rather than focussing strictly on one single process.

1.2 Environmental Factors influencing Prokaryotic Growth

1.2.1 Salinity

Salinity is a measure of the dissolved salt content in a body of water and can be expressed in parts per thousand (‰), with freshwater having a salinity of <0.5‰ and saline water having a salinity of 30-50‰ (seawater has an average salinity of 35‰). However, it is usually measured using the Practical Salinity Scale (PSS),

which measures the conductivity of seawater, compared to a standardised potassium chloride (KCl) solution (Dauphinee 1980, Hill *et al.* 1989). On this scale seawater has an average value of 30-35 (euhaline) and brackish waters have a salinity of 0.5-29 (oligohaline-polyhaline).

Since estuaries are locations where freshwater and seawater mix, variations in salinity play an important role in the estuarine environment. The salinity of the water body can influence, and in turn be influenced by, the degree of mixing in an estuary, which can furthermore influence the pattern of sedimentation within the estuary (Dyer, 1997). Salinity also has an impact on the prokaryotes living within estuarine sediments due to the fact that saline waters contain different levels of nutrients (e.g. SO_4^{2-}) than freshwater. For example Purdy *et al.* (2003) utilised 16S rRNA probing of sediment slurries from the Colne Estuary, UK and found that prokaryotic populations varied along the estuarine salinity gradient. At a brackish/freshwater site (East Hill Bridge) at the estuary's head, acetate consumption was dominated by methanogens (*Methanosarcinales*) however, in the more saline sediments at the estuary's mouth (Colne Point) acetate respiration was dominated by SRB, particularly *Desulfobacter* spp. The authors also found that after a tidal intrusion *Desulfobacter* also became dominant at East Hill Bridge, indicating that higher salinities inhibited the growth of methanogens by raising the levels of sulphate in the sediment, and thereby promoting the growth of SRB groups, which outcompete them for common organic substrates. O'Sullivan *et al.* (2013) also found changes in microbial diversity over the salinity gradient in the same estuary, but in addition noted changes in the rates of both sulphate reduction and methanogenesis between sampling sites with differing salinities, indicating that salinity can affect not only which microbial processes are dominant in an environment but also how rapidly these processes metabolise substrates. Finally Weston *et al.* (2006a, 2006b), and Edmonds *et al.* (2009) examined the effects of saline intrusion on tidal creek sediments and found that although sulphate reduction increased and methanogenesis decreased with rising salinities, the overall species composition of the sediments remained relatively constant, suggesting that the effect of salinity on the proportion and rates of differing biogeochemical processes can be somewhat variable.

As well as affecting which groups of prokaryotes dominate a particular environment, salinity can also influence which genera within a group occur in that environment, for example Lozupone and Knight (2007) suggested that salinity is the main factor controlling the diversity of microbial communities. In a study carried out on the Tama River in Japan, Purdy *et al.* (1997) found that different groups of SRBs occurred at the saline and freshwater ends of the estuary. At the mouth of the estuary (Haneda Airport) sulphate reduction was dominated by the acetate-utilising *Desulfobacter* spp., whereas at the upper estuary site (Gasu-Bashi) the propionate-utilising genus *Desulfobulbus* was the dominant SRB. Experiments utilising molybdate to inhibit sulphate reduction also indicated that the marine SRB were much more physiologically reliant on sulphate reduction than the freshwater species, possibly since the freshwater species may also have been capable of utilising nitrate as an alternative electron acceptor. This agrees with Purdy *et al.* (2003) who found that *Desulfobulbus* is capable of carrying out non-sulphate-dependant H_2 consumption in freshwater sediments when sulphate levels are low by utilising alternative electron acceptors (e.g. nitrate). Leloup *et al.* (2006) also found similar results in a study of the sediments of the Seine Estuary, where under brackish conditions, sulphate reduction was controlled by members of the gram-negative genus *Desulfobacterales* whereas under freshwater conditions, the gram-positive genus *Desulfotomaculum* dominated. Salinity can also affect which archaeal genera are present in an estuary. For example, Purdy *et al.* (2003) found that populations of methanogens in the Colne Estuary varied with salinity, with the phylotypes found at Colne Point being related to *Methanoculleus* and *Methanococcoides*, while those at East Hill Bridge were related to *Methanosarcina* and *Methanocorpusculum*. The authors also found that some methanogenic groups (e.g. *Methanogenium*) appear to be able to cope with a wide range of salinity conditions, while others, such as *Methanosaeta*, require more specific conditions in order to thrive.

Finally salinity can also play a role in controlling which substrates a particular group of organisms utilise. For example, it has long been considered that in environments with higher salinities total methanogenesis is usually dominated by the H_2/CO_2 pathway, while in freshwater environments acetoclastic methanogenesis is dominant (Whiticar *et al.* 1986). This is because in higher salinity environments

sulphate reduction tends to dominate over methanogenesis, which results in the SRB outcompeting the acetoclastic methanogens for their common substrate (i.e. acetate) and also producing a sizeable bicarbonate pool for H_2/CO_2-utilising methanogens to consume (Whiticar, 1999). Such variation in dominance has been observed in the natural environment (Banning *et al.* 2005; Hershey *et al.* 2014), however, as a rule it does not appear to be universally applicable, as will be seen in later chapters.

1.2.2 Temperature

Changes in temperature can have a major effect on prokaryotic populations with regard to the amounts of substrate/waste products they consume/produce, and the number and type of prokaryotic organisms in a given environment. This is because temperature affects the rate at which chemical reactions occur (a doubling of reaction rate occurs with a temperature change of around 10°C), as at higher temperatures more particles have the activation energy (E_a) needed to initiate a chemical reaction (Timberlake, 2003). In biological systems this is even more the case, as the cumulative effect of temperature on the many different reactions occurring in the cell means that temperature coefficient (Q_{10}) values for biological systems tend to be in the range of ≈2-3, although they can exceed this in productive environments (Kätterer *et al.* 1998; Shiah *et al.* 2000). This means that at higher temperatures turnover of substrates by prokaryotic organisms is generally higher as their metabolic reactions occur at an increased speed. This also means that they produce more waste products and that their population size can increase as they are able to divide more often. However, temperature can also have a deleterious effect on the growth of microorganisms, as the enzymes that they use to catalyse the chemical reactions both inside and outside of their cells are sensitive to temperature. At high temperatures the atoms that make up the enzyme proteins gain more energy and their atomic vibration increases. This destabilises the bonds holding the enzyme's complex structure together causing it to change shape or even break apart. This loss of shape is known as denaturation and results in a loss of function for the enzyme as, without its specific shape and active site, it is no longer able to bind to substrate molecules and catalyse chemical reactions (Madigan and Martinko, 2006). Denaturation of enzymes can also occur at very low temperatures

(cryodenaturation) as the hydrophobic interactions between protein strands become weaker at lower temperatures, which can again cause enzymes to lose their shape (Privalov, 1990). Changes in temperature can also have a deleterious effect on the cell membranes of prokaryotes, both via denaturation of the membrane proteins that control the uptake and removal of compounds (Welker, 1976), and by effecting the fluidity and stability of the membrane lipids themselves (Rose, 1989).

This sensitivity to extremes of heat and cold means that groups of microorganisms have optimum growth conditions under which the temperature is high enough that their respiratory reactions can occur at a fast rate but not so high as to denature the enzymes within their cells rendering them non-functional. As there are a wide variety of temperature zones across the Earth inhabited by microorganisms, ranging from brine channels and permafrost at -10-17°C (Bakermans *et al.* 2003) to deep sea hydrothermal vents at 122°C (Takai *et al.* 2008), different groups of microorganisms have adapted to have different optimum growth temperatures (T_{opt}) in order to live in these areas. As such a change in temperature in a given location can result in a change in the makeup of a prokaryotic population from one containing organisms adapted to the old conditions to one containing new organisms adapted to the current conditions.

For example Robador *et al.* (2009) carried out a study on the effects of temperature change on the activity of SRB in arctic and temperate marine sediments. Their results showed that under *in situ* conditions (1.6 °C) the arctic community had a maximum sulphate reduction rate (SRR) of 22.5 nmol cm^{-3} d^{-1} while the temperate community (*in situ* temperature 10 °C) had a maximum SRR of 32 nmol cm^{-3} day^{-1}. The authors also carried out thermal gradient incubations of the two communities and found that the T_{opt} of the arctic community was 22 °C with a maximum SRR of 546 ± 136 nmol cm^{-3} day^{-1} while the T_{opt} of the temperate community was 33 °C with a maximum SRR of 1333 ± 293 nmol cm^{-3} day^{-1}. They also repeated the thermal gradient incubations after storing sediment from the two sites at 0 °C, 10 °C and 20 °C for 2, 6, 12 and 24 months to determine the effects of long term temperature change on the communities. After 24 months at 20 °C the T_{opt} of the temperate community had changed relatively little while the T_{opt} of the arctic community had increased by 8 °C indicating that the community had changed from one made up of psychrophilic organisms to one made up of more mesophillic

strains. This shows that not only are bacterial communities able to operate over a wide temperature spectrum, but also that their T_{opt} can be much greater than the *in situ* temperatures in their native environment. In addition it shows that long term increases in temperature can cause shifts in microbial diversity without changing the overall process occurring in the sediments.

Avery Jr *et al.* (2003) carried out a similar experiment looking at the effects of temperature on rates of methanogenesis. The authors collected material from two sites: White Oak River, a tidal freshwater estuary in North Carolina (January temperature = 8.5 °C, July temperature = 27 °C); and Buck Hollow Bog, a peat bog in southern Michigan (January temperature = 0 °C, July temperature = 20 °C). They found that the White Oak community had an annual methane production rate of 55.3 mM yr^{-1}, an order of magnitude higher than the Buck Hollow community (5.5 mM yr^{-1}). However, the authors do suggest that some of this discrepancy may be due to competition for acetate from non-methanogenic organisms at Buck Hollow Bog for a large portion of the year. Even so, the methanogenesis rates of the two communities at *in situ* temperatures did vary greatly throughout the year, with the White Oak community having a minimum methane production rate (MPR) of 1.18 μM hr^{-1} in December (9.5 °C) compared to a maximum MPR of 11.57 μM hr^{-1} in June at a temperature of 21.5 °C. A similar situation was also observed in the Buck Hollow community which had a minimum MPR of 0.14-0.18 μM hr^{-1} in the winter months (*in situ* temperature of 0 °C) and a maximum MPR of only 2.79 μM hr^{-1} in June at 20 °C indicating that temperature does play a role in controlling rates of methanogenesis at these sites.

As has been shown in the preceding sections both salinity and temperature can play a major role in controlling which groups of organisms, and therefore which metabolic processes, dominate in an area, as well as controlling the rates at which these processes occur. As such when investigating the biogeochemistry of temperate estuarine sediments, where these conditions can vary dramatically on both a spatial and temporal basis, it is always important to consider the role of both salinity and temperature in affecting the prokaryotes and the biogeochemical cycles they control.

1.3. An Introduction to the Severn Estuary

This section will provide an introduction to the Severn Estuary. It will provide an overview of the general geography of the region as well as an in depth review of the biology, sedimentology and hydrology of the region with regard to how it might effect the sediment biogeochemistry. In addition, the main aims and objectives of this study will be discussed along with the reasoning behind the selection of sites for study.

1.3.1 Geographical Setting

The Severn Estuary (*Môr Hafren* in Welsh) is located in the southwest of the UK between Monmouthshire, Newport, Cardiff and the Vale of Glamorgan in southeast Wales; and Gloucestershire and Somerset in southwest England (Fig. 1.6). The estuary is the second largest estuarine system in the UK (after the Wash in East Anglia) and is roughly 42 km in length, from Aust in Gloucestershire to the mouth of the estuary at Lavernock Point, near Cardiff in South Wales where it empties into the Bristol Channel.

The estuary is fed mainly by the River Severn, the longest river in the UK, which rises in the Cambrian Mountains of Mid-Wales and meanders through the Welsh Borders and West Midlands before flowing out into the Bristol Channel. The estuary is also fed by a number of smaller secondary rivers that flow through south Wales and southwest England, including the Taff, Usk, Wye and Avon (Fig. 1.7). The land immediately surrounding the estuary is generally low lying and made up of former saltmarsh and alluvial wetlands, much of which has been reclaimed for agriculture by human action over the past two thousand years. These former wetlands, such as the Wentlooge and Caldicot Levels on the northern side of the estuary, retain a wide variety of animal and plant species while the tidal flats they border are considered to be important habitats for wading bird species such as redshank (*Tringa totanus*) and dunlin (*Calidris alpina*) (Burton *et al.* 2010). The waters of the estuary, despite their high turbidity, are home to a wide variety of fish species, including several rare and migratory species such as sea and river lampreys (*Petromyzon marinus* and *Lampetra fluviatilis*), sea trout (*Salmo trutta* morpha *trutta*) and the twaite shad (*Alosa fallax*) (Henderson *et al.* 2011).

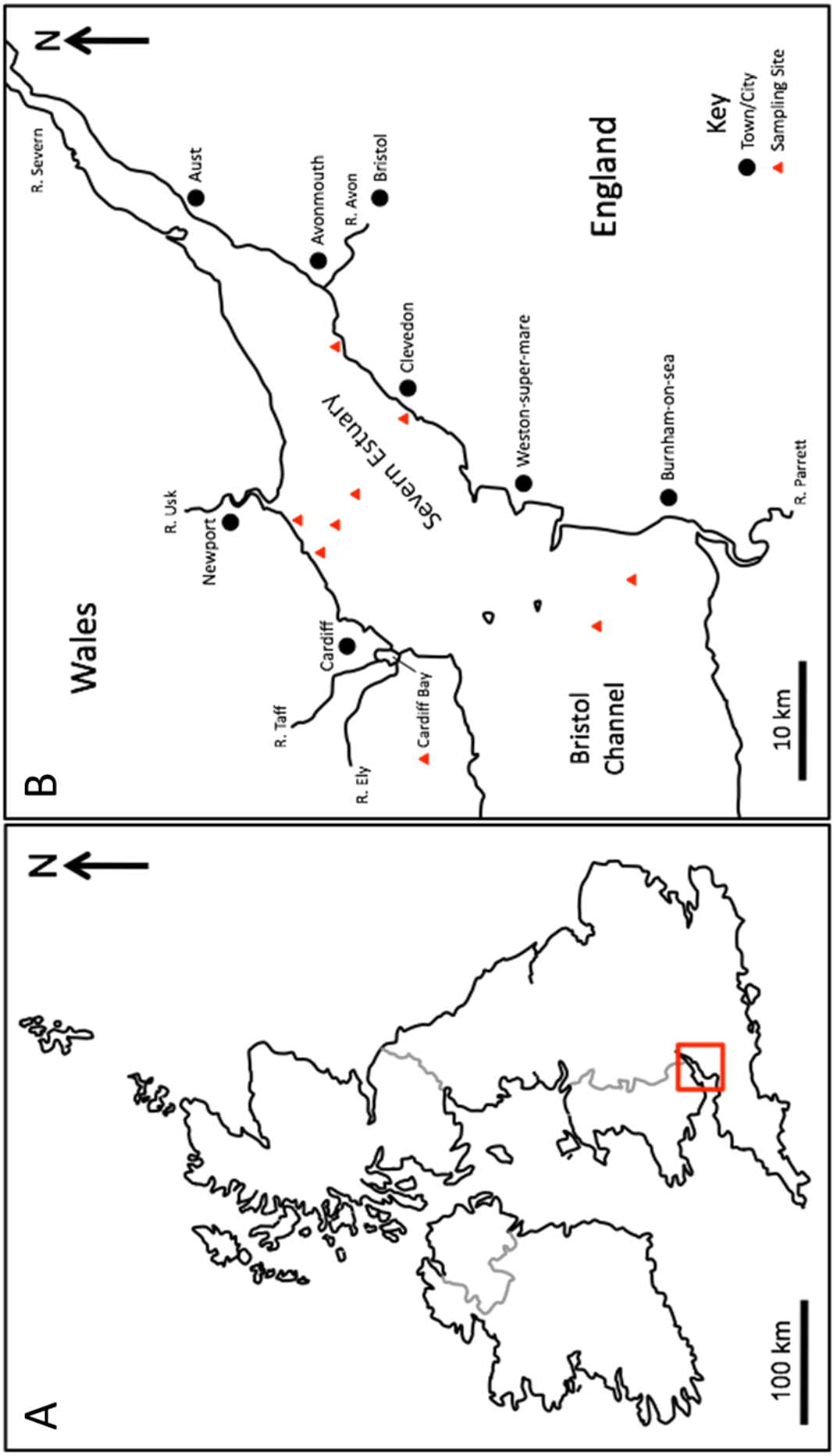


Fig. 1.6 – A) Line map of the United Kingdom showing the location of the Severn Estuary and inner-Bristol Channel highlighted in red. B) Magnified view of the Severn Estuary and inner-Bristol Channel showing the location of major rivers and coastal conurbations. The sampling sites used in this study are also highlighted.

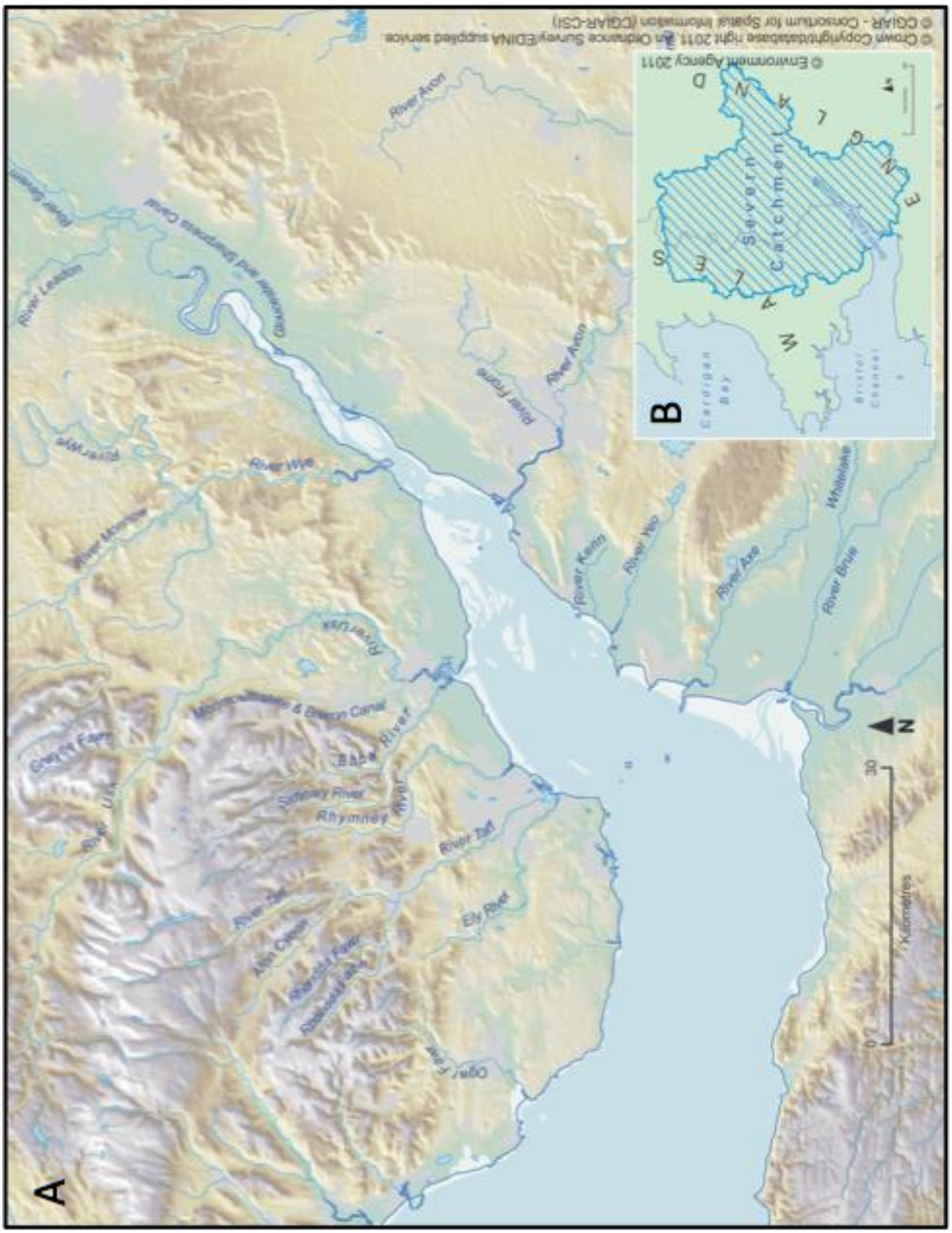


Fig. 1.7 – A) Topographical map of the landscape surrounding the Severn Estuary and Bristol Channel showing the major rivers that flow into the estuary. Offshore areas highlighted in white indicate mudflats and sandbanks exposed at low tide. B) Map showing the catchment area of the rivers flowing into the Severn Estuary. (Severn Estuary Partnership, 2011).

The region surrounding the estuary has also been a major industrial centre for the last 200 years, with the South Wales valleys in particular being a major locus of coal mining and steel production from the 1830's up until the 1980's. This industry, coupled with coal mining in the Forest of Dean and intensive agriculture in the southwest of England has meant that by the latter part of the 20th century the estuary had become polluted with both organic and inorganic contaminants. However, in recent years the cessation of large-scale industry in South Wales coupled with new guidelines on the levels of pollutants that can be disposed of in rivers have resulted in a "clean up" of the estuarine environment (Owens 1984; Jonas and Millward 2010; Severn Estuary Partnership, 2011). This in turn, has led to much of the land immediately surrounding the Estuary, particularly the mudflats of the upper estuary, being designated as sites of special scientific interest (SSSIs) (Fig. 1.8).

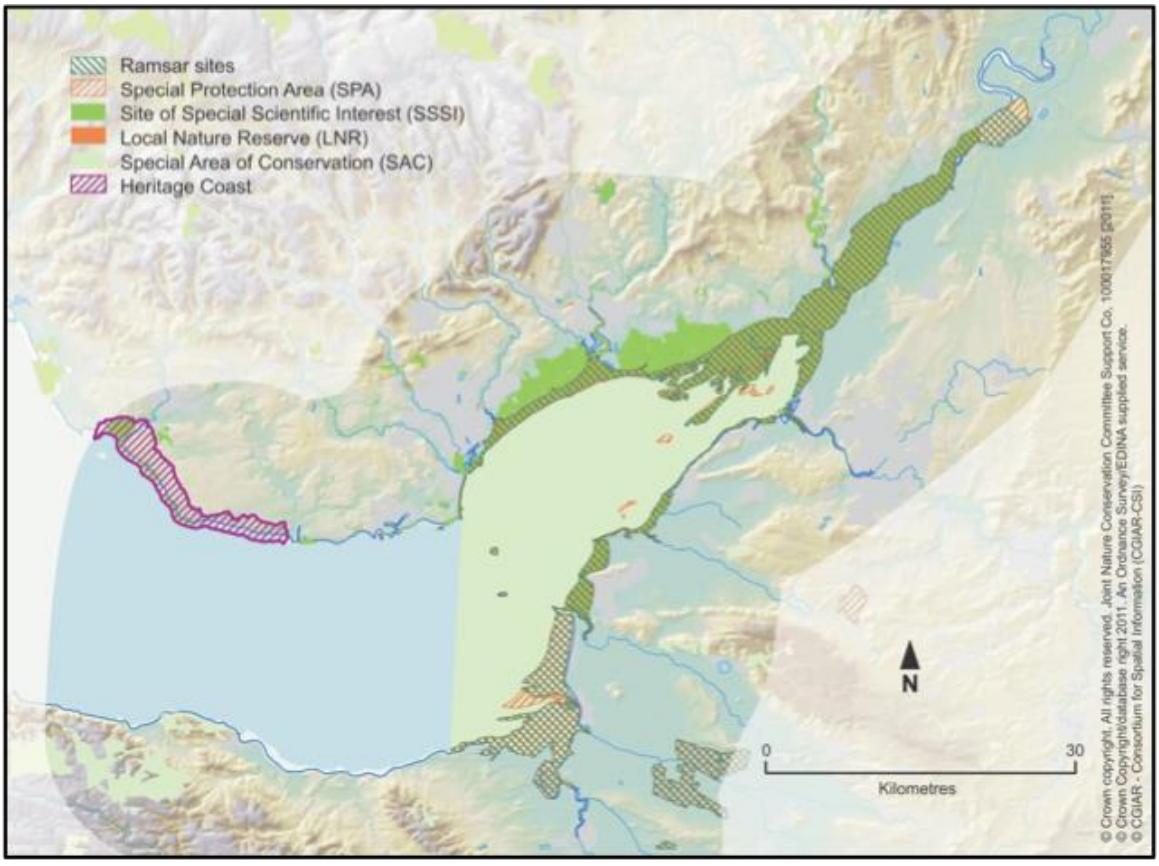


Fig. 1.8 – Map showing the special protective statuses assigned to the Severn Estuary and its surrounding environs (Severn Estuary Partnership, 2011).

1.3.2 Geological History and Formation

The history of the geographical feature now called the Severn Estuary begins during the Devensian epoch (110-12 Kya), when the area that is now the estuary was a large river valley formed by seasonal meltwater rivers flowing from the Weichselian ice sheet that covered much of the UK and northern Europe (Allen, 1987a). These fast flowing rivers, rich in eroded sediment, carved a deep canyon (the inner estuary) through the underlying Triassic mudstones and Carboniferous limestones, before fanning out and depositing their sediment onto a wide strand-plain, spotted with lakes and marshes (the current Bristol Channel). However, as the ice sheets started to recede due to increasing global temperatures during the Flandrian stage of the Holocene (12 Kya – present), global sea levels began to rise. This eustatic sea level rise combined with isostatic rebound led to a relative sea level rise of around 30 metres (Shennan and Horton, 2002), which gradually inundated the Severn Valley, in turn leading to the formation of the estuary as it is seen today (Ings *et al.* 2011).

Due to its mode of formation the Severn Estuary is referred to as a "drowned river valley" type estuary (Bianchi, 2007). These estuary types are commonly found in temperate regions of the World where sediment discharge from a river mouth is unable to keep up with regional sea level rise during highstand periods. They usually maintain the cross-sectional profile of the original river valley and are often no more than 30 metres deep at their deepest point (the maximum depth of the Severn Estuary is ≈27 m below chart datum (Fig. 1.9).

1.3.3 Tidal Range

One of the most notable features of the Severn Estuary is its extreme tidal range. At the head of the estuary near Avonmouth the highest astronomical tide can reach 14.7 m (Kirby, 2010), which classifies it as a hypertidal regime (Dyer, 1997) or more specifically a Hypertidal-C to Hypertidal-E regime, according to the classification system of Archer (2013) (Fig. 1.10). In fact, the Severn Estuary has the third highest tidal range in the World after the Minas Basin in the Bay of Fundy, New Brunswick/Nova Scotia (16.3 m recorded, 17 m estimated) (O'Reilly *et al.* 2005); and the Leaf Basin in Ungava Bay, Nunavik (*ungava kangiqluk*/ᐅᖓᔪᖅ ᑲᖏᖅᓗᑦ in Inuktitut, 16.2 m recorded, 16.8 m estimated) (Arbic *et al.* 2007). This remarkable

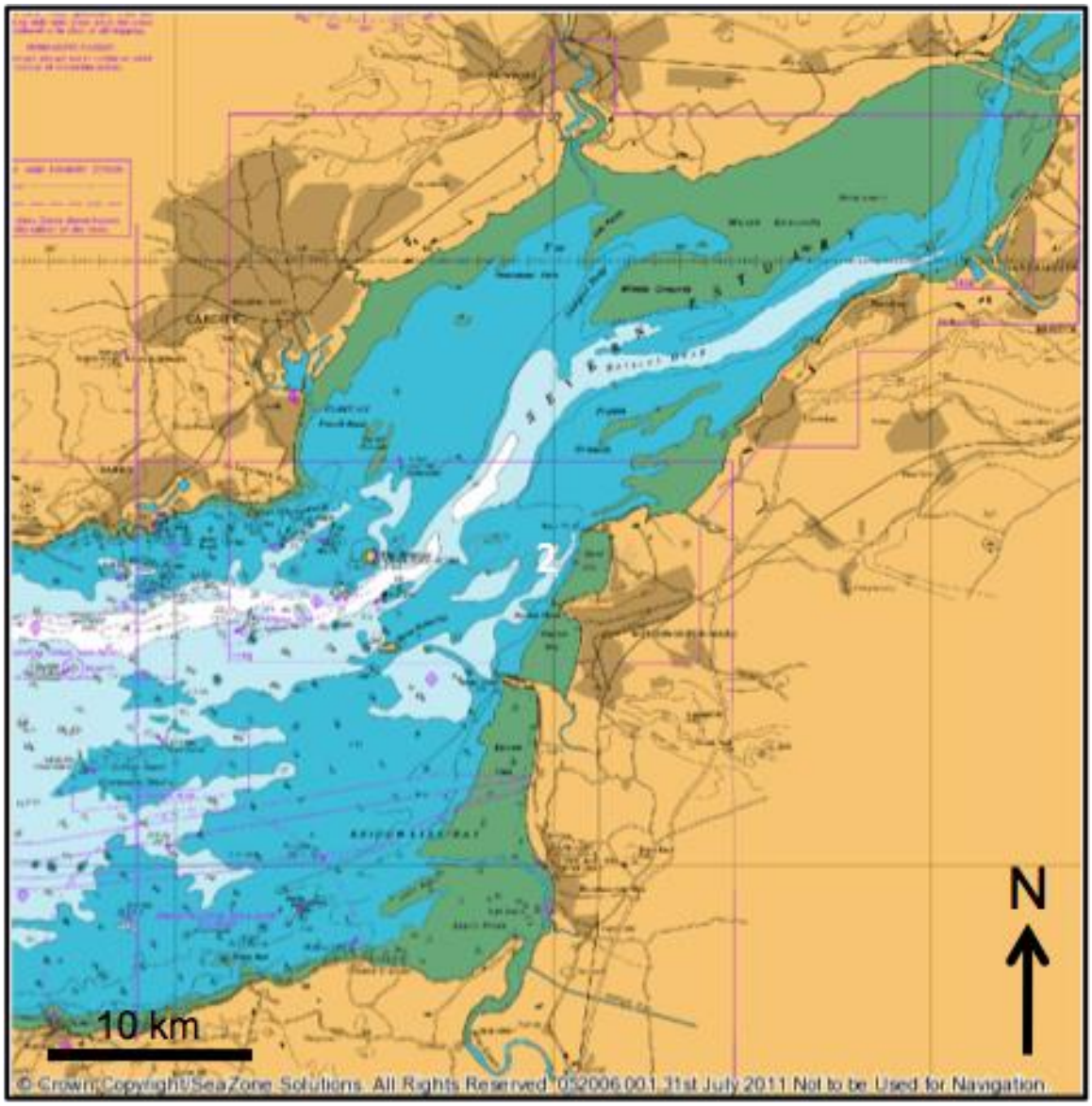


Fig. 1.9 – Chart showing the bathymetry of the Severn Estuary and inner-Bristol Channel. Green shading indicates areas exposed at the lowest astronomical tide (chart datum), while blue shading signifies subtidal areas - lighter shading signifies greater water depth, all depths marked are relative to chart datum. Note that the palaeochannel of the River Severn can be clearly seen running through the south-central portion of the upper-estuary forming what is now the Bristol Deep. (© Copyright: Sea Zone Solutions)

change in water levels is brought about by a combination of two main factors: the shape of the estuary and tidal resonance.

Due to its previous existence as a river valley, the Severn Estuary has a characteristic conical shape with a very wide mouth, which quickly narrows toward the head of the estuary. This rapid narrowing means that the estuary can be classified as a hypersynchronous-type (Dyer, 1997). Due to their shape most hypersynchronous estuaries have relatively large tidal ranges because as the tidal wave moves up the estuary with the rising tide, the large tidal prism of the estuary is

compressed into a laterally confined space at the head. This leads to the water level being forced vertically upwards at the head of the estuary, which results in very high tidal ranges (Hashemi *et al.* 2008).

The other factor controlling the high tides of the Severn Estuary is a phenomenon called tidal resonance (Fong and Heaps, 1978). This occurs when a tidal wave front moves across a continental shelf with a depth and width that is a specific fraction of the tidal wavelength (a shelf around 100-200 m deep with a width equivalent to a quarter of a tidal wavelength, >300 km, produces the most pronounced affect). As the tidal wave travels across the shelf the wave will reflect off the sea floor and these secondary reflected waves can interact with the original wave, producing a resonating frequency and leading to the creation of a much larger amplitude of tidal wave (Brown *et al.* 2006). As the Severn Estuary sits on the western coast of the UK, the distance between the mouth of the Bristol Channel and the continental slope is around 500 km, which is more than enough distance to develop a degree of shelf resonance.

Another form of tidal resonance can also occur within a bay or estuary, when a tidal wave is reflected back on itself by the head of the estuary and meets another primary tidal wave coming the other way. This collision of waves, if timed correctly (related to the length of the estuary and the duration of the tidal period), can again lead to an interaction between primary and secondary tidal waves and a subsequent increase in wave amplitude. In the Severn Estuary the time taken for a tidal wave to travel up the estuary, get reflected and travel back, is close to the tidal period thus leading to a strong tidal resonance and higher than normal tides (Liang *et al.* 2014).

Due to its exceptional tidal range, the Severn Estuary has often been suggested as a location for a variety of tidal power generation projects. The size and scope of these proposed projects has varied with time and available funding, however, they would all have the potential to generate a large amount of relatively carbon neutral energy, up to 16 TWh yr^{-1} (Xia *et al.* 2012). The most widely discussed of these potential projects is the construction of a tidal barrage, similar to (though on a larger scale than) those at La Rance in Brittany (Kirby and Retière, 2009) or Annapolis Royal in Nova Scotia. Several sites have been suggested for such a barrage (Xia *et al.* 2010; Ahmadian *et al.* 2014a), including an 8.1 km wide barrage 1 km downriver from the 2nd Severn Bridge (referred to as the Shoots Barrage); a

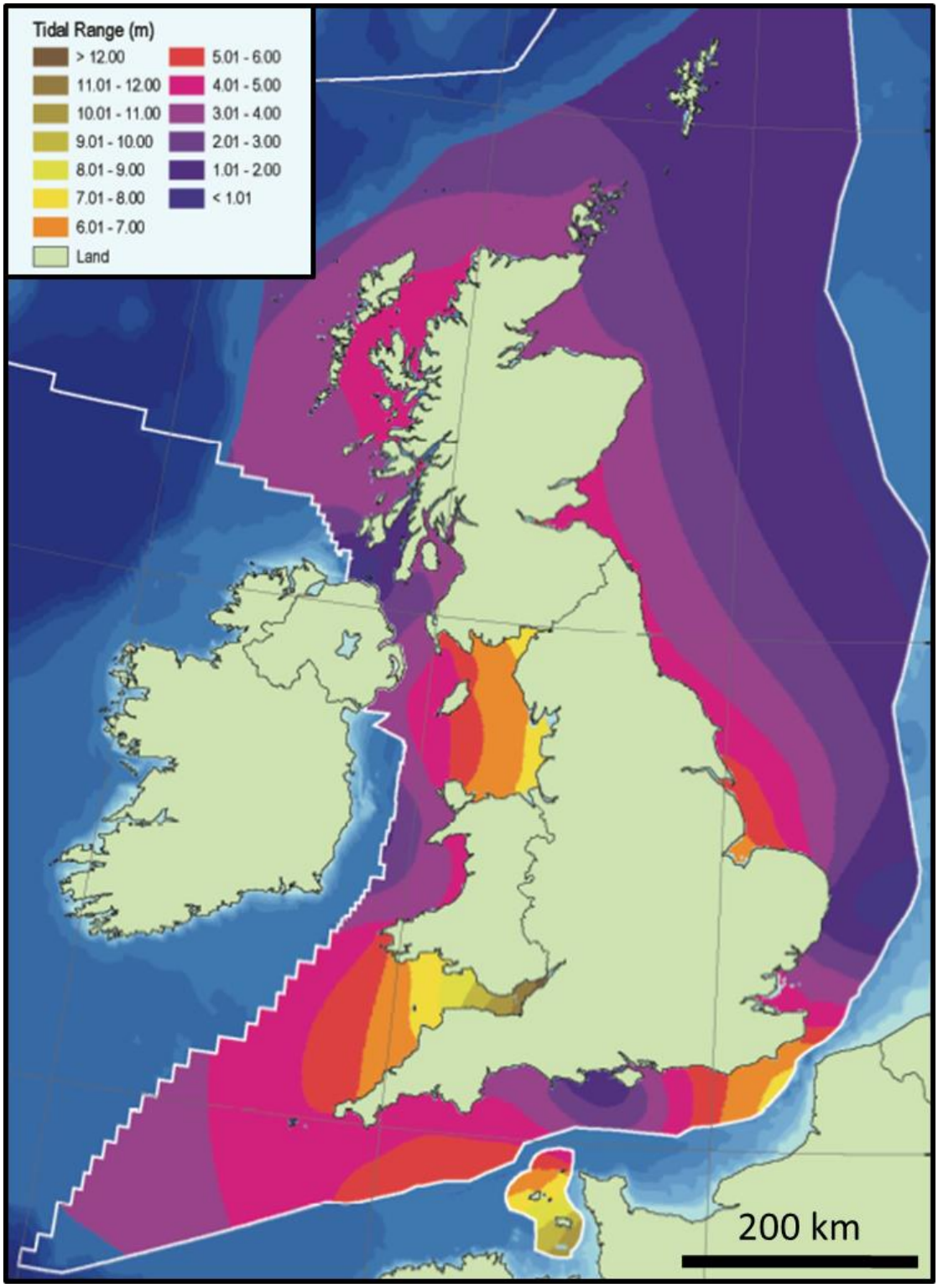


Fig. 1.10 – Map showing the variation in tidal ranges around the UK (ABPmer, 2008). Note how tidal ranges increase moving into the Severn Estuary as it becomes progressively narrower towards its head. The maximum range in the Severn Estuary is ≈14.7 metres at Avonmouth (Kirby, 2010).

smaller (≈1.5 km wide) barrage upstream of the Severn Bridges (the Beachley Barrage); or a much larger 16.1 km barrage extending from Lavernock Point in South Wales, to Brean Down in Somerset (referred to as the "Cardiff-Weston Barrage" and/or "Severn Barrage"). Another barrage-type project would involve the construction of a large 85 km^2 tidal lagoon (known as the Fleming Lagoon) on the northern side of the estuary centred on an area of mudflats known as the Welsh Grounds. The walls impounding this lagoon would be fitted with turbines and would therefore function in a similar fashion to the other barrages described above. Similar lagoons have also been suggested at other locations in the vicinity of the estuary, including: the Peterstone Flats, the Langford Grounds, and Bridgewater Bay in Somerset (Fig. 1.11), as well as further west in Swansea Bay.

However, all three of these projects would be extremely expensive to construct (estimates for the Cardiff-Weston Barrage range up to £34 billion) (Hutchinson, 2010), hence smaller scale projects have been suggested that might provide similar outputs at a fraction of the cost. One of the most popular of these is the deployment of an array of freestanding tidal stream turbines (Ahmadian *et al.* 2012). These structures, which resemble submarine windmills, would be sunk to the sea floor of the estuary and function in a similar fashion to the turbines in a barrage, although without the need for a retaining wall. The turbines could then be emplaced either in specific locations in the estuary where current speeds are at their highest (often due to the funnelling effect of the submarine topography) or strung out in a line across the width of the estuary to form a "tidal fence" (Pelc and Fujita, 2002).

1.3.4 Biogeochemistry of the Severn Estuary

Despite the fact that the Severn Estuary is one of the most dynamic estuarine environments in the world, relatively little is known about the biogeoghemistry and geomicrobiology of the estuary and its surrounding environs. At present only two published articles exist on the prokaryotic populations within the sediments of the estuary, both of which are detailed below.

Wellsbury *et al*. (1996) were the first to examine the biogeochemical potential of the sediments of the Severn Estuary. These authors examined and compared the prokaryotic activity in sediments at two sites with varying environmental conditions, with a previously studied sheltered estuarine location at

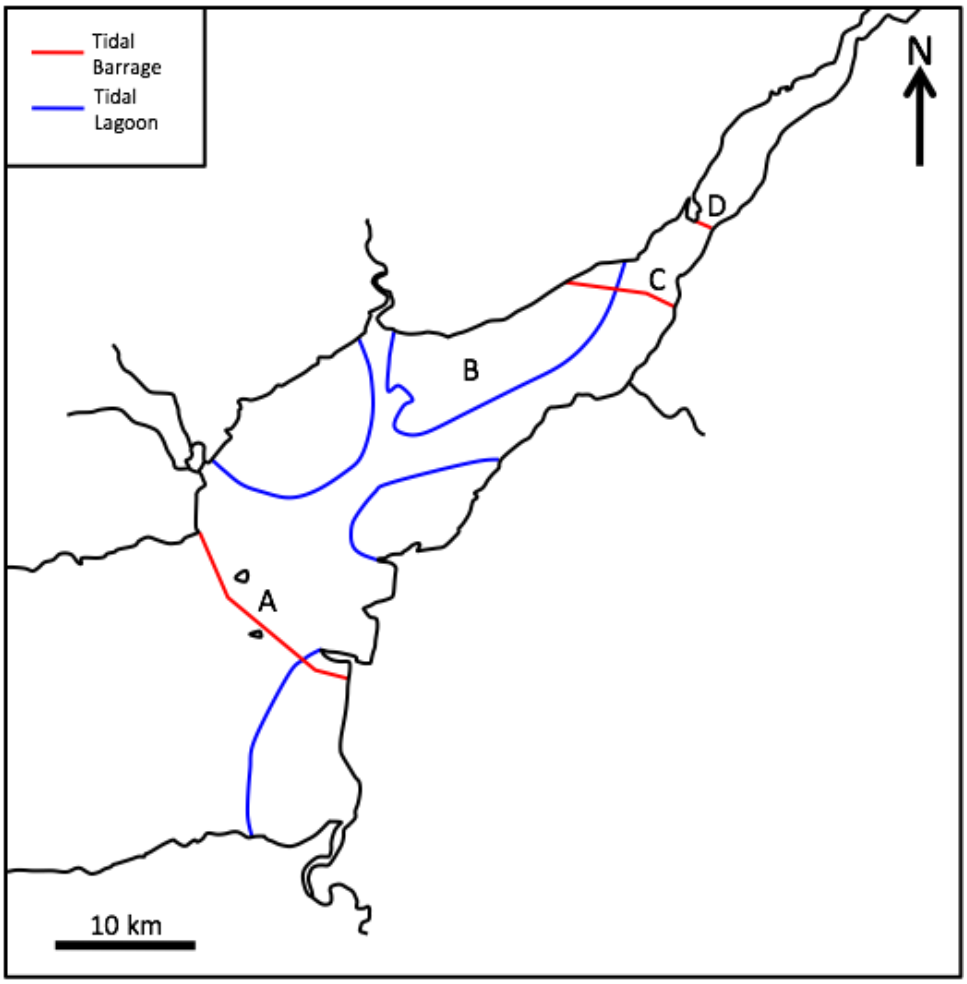


Fig. 1.11 – Map showing proposed locations for tidal barrage (red) and lagoon (blue) projects in and around the Severn Estuary. Including: A) the "Cardiff-Weston"/Severn barrage; B) the Flemming lagoon; C) the Shoots barrage and D) the Beachley barrage. Modified from Knight and Hill (2007).

Kingoodie Bay 16 km from the mouth of the River Tay in Scotland. The two Severn Estuary sites were: a tidally dynamic estuarine location at Aust Warth; and a freshwater site, Ashleworth Quay, on the River Severn 68 km upstream from Aust Warth. At each site rates of sedimentary oxygen uptake, sulphate reduction and methanogenesis were measured alongside cell growth (via thymidine incorporation) and total cell counts. The results of this study showed that anaerobic processes were the dominant method of carbon mineralization at each of the sites, with sulphate reduction being dominant at Aust Warth and Kingoodie Bay (61.4% and 75.6% respectively) whilst methanogenesis was dominant at Ashleworth Quay (55.7%). The results also showed an unusual depth profile at the Aust Warth site with bacterial numbers being relatively uniform in the top 8 cm compared to the other two sites. This top 8 cm also had a higher than normal porosity suggesting a

recent depositional event (not unusual in the tidally dynamic Severn, see below) which indicated that the Severn Estuary's extreme tide and current patterns may play a major role in the distribution and activity of the microorganisms in its sediments.

Webster *et al*. (2010) carried out a study of the metabolic diversity of prokaryotes in the Severn Estuary by examining sediment slurries from tidal flat sediments at Woodhill Bay, Portishead on the southern side of the estuary. The slurries were treated according to their depth position in the sediment and the assumed dominant biogeochemical activity: Slurry A representing the aerobic zone, Slurry B the dysaerobic zone, Slurry C the anaerobic sulphate reduction zone and Slurries D and E representing the anaerobic methanogenic zone. The sediments and slurries were then studied using pore water and gas analysis to determine the biogeochemical activity of the sediment, plus ^{13}C-SIP and PCR amplified 16s rRNA analysis (^{12}C and ^{13}C DNA) to determine the identity of the active prokaryotic populations (Radajewski *et al.* 2000). The results of the sediment pore water analysis showed that sulphate concentrations decreased downwards into the core from 29 mM at the surface to around 10 mM 60 cm below the surface, while methane concentrations increased with depth, from zero in the top 10 cm, to 16-25 μmol l^{-1} of sediment below 50 cm depth. However, geochemical analysis of the slurries showed no sign of the expected sulphate reduction in C, or methanogenesis in D or E. Also, analysis of bacterial 16s rRNA in the slurries did not identify any known active sulphate-reducing bacteria, although some SRB were detected using more selective *dsr*A gene analysis. Similarly, no active methanogenic archaea were detected in Slurries D and E using the 16s rRNA gene. The authors therefore, suggested that though SRBs and methanogens were probably present in the slurries, they were present at low numbers and included as yet uncultured groups, which also may have a broad range of metabolic pathways making their activity hard to detect. It was also suggested that the reduced compounds produced by the SRBs and methanogens may be being rapidly re-oxidised by other groups of organisms (eg. sulphide oxidising bacteria), and therefore these compounds were not readily detected.

As well as these investigations of sedimentary prokaryotic groups and biogeochemistry, a few studies have also been conducted to determine the diversity

of epibenthic and planktonic microorganisms within the Severn Estuary. It is important to examine these groups as they play a vital role in the survival of the endobenthic sedimentary prokaryotes in two ways: 1) their dead biomass and excreted dissolved organic carbon (DOC) provide both electron donors and a carbon source for the heteretrophic endobenthic groups such as the SRB; 2) many phytobenthic groups of organisms that grow on the sediment surface secrete extracellular polysaccharides, forming biofilms, which help stabilise the sediment modifying the endobenthic groups' habitat. A review by Underwood (2010) details the distribution and diversity of the microphytobenthic (MPB) and phytoplanktonic organisms in the Severn Estuary. The review indicates that the overall MPB biomass in the estuary is reduced compared to other estuaries in the UK, with the majority of the biomass occurring on the mudflats, and to a lesser extent, the saltmarshes, in the upper estuary. This reduced biomass is likely due to the estuary's strong tidal currents, which erode, deposit and re-mobilise sediments on a daily basis, resulting in most areas of sediment not being stable enough for MPB colonies to develop. In addition, this low-density level of MPB organisms, and therefore lack of sediment binding biofilms, results in the mudflats in the upper estuary having an increased susceptibility to erosion (Kirby and Kirby, 2008). As well as being depleted in MPB biomass the Severn Estuary also has a relatively low level of phytoplankton due to the high turbidity of the waters, which prevents light from penetrating down into the water column, thereby limiting photosynthesis (Joint, 1984; Kadiri *et al.* 2014a).

1.3.5 Benthic Macrofauna of the Severn Estuary

Benthic macrofauna are thought to play a major role in determining the diversity and positioning of the various prokaryotic guilds within the sediment column. Studies carried out on anaerobic benthic prokaryotes on tidal salt marshes have shown that bioturbation can have a dramatic effect on the rates of respiration of these organisms (Kostka *et al.* 2002), with rates being both increased and decreased in areas that have been bioturbated depending on the mode of bioturbation and the prokaryotic guild concerned (Banta *et al.* 1999). It has been suggested that the mixing of the sediment by burrowing organisms allows compounds such as acetate and sulphate, that are necessary for microbial respiration, to permeate deeper into the sediment to the depth that anaerobic prokaryotes inhabit. It is also likely that

the rapid turnover of sediment causes the reoxidation of metabolic products such as sulphide making respiration more energetically favourable and providing more sulphate for the prokaryotes to consume (Kristensen, 2000). However, Nielsen *et al*. (2003) suggest that bioturbation may also decrease the amount of sulphate reduction occurring in the sediment by increasing the amount of Fe^{3+} in the areas in close proximity to burrows, which would promote the growth of Fe^{3+}-reducing species over the SRB. Finally, Koretsky *et al*. (2005) suggest that bioturbation in areas with high levels of organic matter can result in different guilds of bacteria (Fe/Mn reducers and SRB) coexisting in the same area and not being confined to their respective biogeochemical depth zones. It is therefore necessary to know the diversity of macrobenthic organisms in the Severn Estuary to determine what effect their activities might have on the prokaryotes they share the sediment with.

Warwick and Somerfield (2010) carried out a review of various studies into the benthic macrofaunal diversity of the Severn Estuary and found that the overall diversity of organisms was very low. This is due to a number of reasons, including high water turbidity and the lack of a clear sediment/water interface, which inhibits the growth of filter-feeding organisms such as bivalves and polychaetes. Deposit feeding organisms are slightly more diverse than filter-feeders, although high rates of sediment erosion and basal scouring caused by the strong currents and tides prevent larger burrowing organisms from living in an area for a prolonged period of time. This suggests that while macrobenthic bioturbation may be a factor in microbial distributions and activity in some localised and more stable areas, it is unlikely to play a significant role in the majority of the estuary. These findings agree with the results of an earlier study by Radford (1994) who also found the Severn Estuary to be impoverished in benthic organisms, especially filter-feeders, except in a few exceptional localities such as sheltered bays. The exception was the honeycomb worm *Sabellaria alveolata*, a variety of reef-building polychaete that is found in abundance in the outer estuary particularly in an area between the Cardiff and Langford Grounds. This worm is able to survive even in the highly dynamic Severn Estuary due its construction of reefs from coarse-grained sediment, thereby self-stabilising its environment and protecting it from the harsh currents of the estuary (Mettam *et al.* 1994). *S. alveolata* also require a constant supply of

suspended coarse sediment in order to construct their reefs and therefore ironically the conditions that prevent most macrobenthos from colonising the estuary (i.e. strong currents and high suspended sediment concentrations) make it the perfect environment for *S. alveolata*.

However, while the numbers of larger benthic organisms may be severely reduced in the Severn Estuary, a study by Cullen (1973) shows that smaller meiobenthic organisms could play a role in sediment mixing. In this study, samples of sediment and macrobenthic organisms from the Severn Estuary were collected and kept in tanks until the larger organisms bioturbated the sediment. These larger organisms were then removed and the tanks were left undisturbed, however, over time the tracks made by the macrofauna were gradually disrupted. Observation and fine sieving found that small meiofaunal organisms such as ostracods, nematodes and copepods (some of which were smaller than the sediment grains) were responsible for the destruction of the original tracks by their own bioturbating activities. The activity of these organisms was shown to be enough to mix and aerate the sediment to 1.5cm depth within a few hours of emplacement. This suggests that while macrobenthic organisms may be impoverished and therefore play a minor role in sediment mixing in the Severn Estuary, these much more abundant meiofauna may play a significant mixing role, at least in the upper layers of the sediment.

A review by Kirby (2010) also details the effects that a tidal barrage might have on the benthic macrofauna of the estuary. It suggests that the biodiversity of benthic organisms behind the barrage would increase in a similar fashion to the increase observed at the La Rance, Annapolis Royal and Kislogubskija barrages. In particular, numbers of filter-feeding organisms would dramatically increase. This could lead to a dramatic increase in large-scale sediment bioturbation, which would likely have consequences for the benthic prokaryotic populations living in the sediments of the tidal lagoon behind the barrage.

1.3.6 Sediments of the Severn Estuary

Due to the extreme conditions that occur in the Severn Estuary, the dynamics of sediment transport and deposition within the estuary have been extensively studied. Since the prokaryotic communities that this study is concerned with reside

within these sediments it is important to gain a broad understanding of the processes at play within the estuary and its surrounding environs.

Allen (1991) carried out a study of the composition of the sediments of the estuary to determine their origins. To do this the author collected 22 sediment samples from tidal areas (mainly mud flats) around the edge of the estuary and another 22 from the floodplains of rivers that drain into the estuary. The dried sediment samples were examined using an X-ray diffractometer to determine the types and proportions of clay minerals they contained. The results of this analysis showed that the riverine and estuarine sediments had assemblages of clay minerals which strongly suggested that most of the fine sediment in the Severn Estuary is derived from material carried into the estuary by rivers. However, while most of the fine estuarine sediment is fluvial in origin the coarser, sandy component of the sediment appears to have a marine origin and may have originated in the Celtic Sea before being transported eastward after the last glaciation.

Kirby (2010) and Manning *et al*. (2010) carried out complimentary reviews of the distribution of and behaviour of sediments in the Severn Estuary and what effects a tidal barrage might have upon them. The reviews indicate that much of the base of the outer estuary and inner channel is scoured clean of sediment by strong tidal currents exposing large areas of bedrock. Those areas of the main channel not scoured clean are covered by coarse sands and gravels with the finer muddier sediments concentrated in less tidally dynamic areas such as Bridgwater Bay, deep water areas such as the Newport and Bristol Deeps and the Cardiff Roads, and on tidal mudflats/subtidal mud patches on the edge of the Gwent, Caldicot and Somerset Levels, all of which act as sediment sinks (Kirby, 1994) (Fig. 1.12). The mudflats (which can be 3 km wide in places) also exhibit low, concave cross-sectional profiles indicating that they are being rapidly eroded possibly due to a rise in sea levels (estimated at 2.4 mm yr^{-1}) (Phillips and Crisp, 2010), which could lead to their destruction in the near future (Allen and Duffy, 1998a). These intertidal sediments also have a very low biogenic carbonate content due to the low diversity of shelled benthic organisms living in the estuary's sediments. The high degree of erosion by tides and currents also means that the sediments of the estuary are

Fig. 1.12 – Diagram taken from Kirby (2010) showing the distribution, and variation in type, of intertidal and subtidal sediment cover in the Severn Estuary and Bristol Channel. Not that large areas of seafloor, particularly in deeper water, are scoured clean of sediment by strong tidal currents.

exceptionally mobile, with an estimated $1.3x10^7$ tonnes of sediment being in suspension over the spring tide (70% of which is deposited during the neap period) (Collins, 1987; Manning *et al.* 2010). This high degree of suspended sediment also leads to the formation of lutoclines, distinct vertical zones of varying turbidity in the water column for which the Severn Estuary is a type locality. The extreme mobility of the sediment also means that the estuary has two estuarine turbidity maxima (ETMs). These are areas of the estuary where the concentration of suspended particulate matter (SPM) is at its highest, and are in Bridgwater Bay and upstream of Sharpness in the estuary's upper reaches.

Manning *et al*. (2010) also detail the flocculation behaviour of sediments in the estuary and show that fine and coarse sediments often accumulate together to form mud/sand mixtures. This could be important from a microbiological standpoint, as Al-Raei *et al*. (2009) found that mud/sand mixtures were the most productive type of sediment for the growth of SRB in the Wadden Sea. Perkins *et al*. (2004) and Manning *et al.* (2010) also suggest that the formation of mud/sand flocs is aided by biological activity, such as that of diatoms, which secrete extracellular polysaccharides that might help bind sediment together in the water column. This is another way in which the action of aerobic microbes might aid the development of anaerobic communities, similar to the findings of Underwood (2010).

The reviews also describe another important sedimentary phenomenon that occurs within the estuary, namely the formation of fluid mud pools in the deeper parts of the estuary. These are non-Newtonian gel-like accumulations of fine sediment mixed with water, with SPM concentrations of 10-400 g l^{-1} (Aller *et al.* 2010; Green and Coco, 2014) that form in areas such as the Newport and Bristol Deeps after spring tide events erode fine sediment from the base and edges of the estuary (Fig. 1.13). This fine sediment then forms cohesive bodies (colloquially referred to as "slugs"), which migrate to the deeper areas of the estuary where they remain until the next spring tide. Due to the very fine nature of the sediment it can stay in suspension for a prolonged period of time, forming pools up to 5m thick at the bottom of the deeper channels which are distinct enough from the overlying water that they can be detected using an echo sounder (Kirby, 2010). These pools are also anoxic for most of their "life-cycle" except for when they are re-mixed by the next spring tide, and therefore could provide a potential habitat for anaerobic

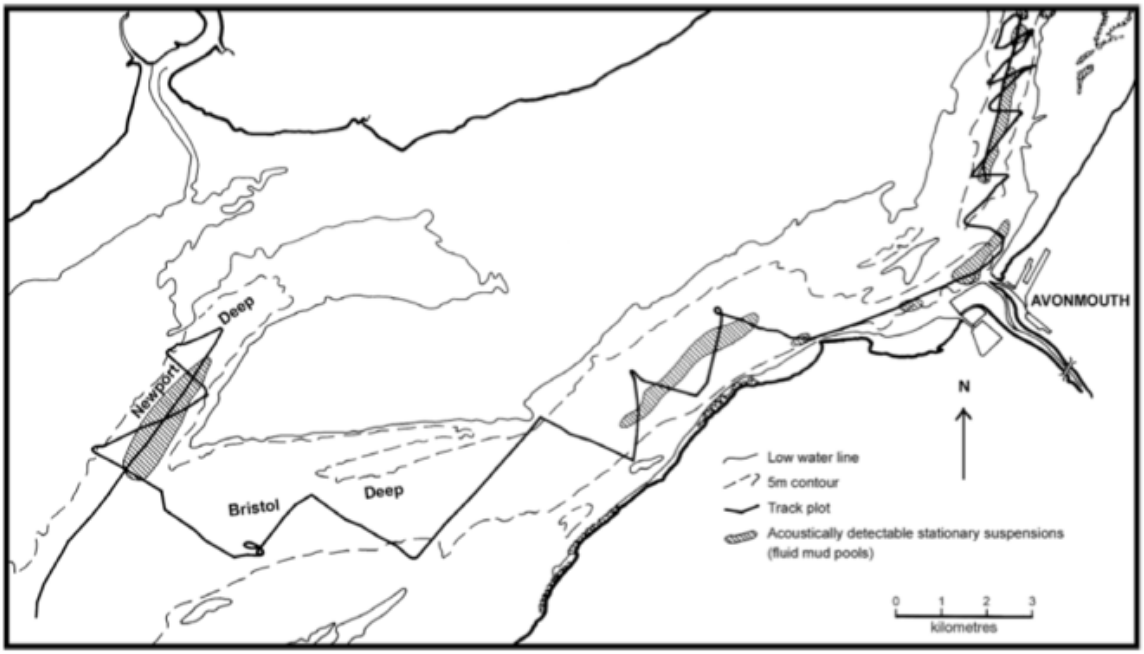


Fig. 1.13 – Diagram taken from Kirby (2010) showing the position of fluid mud pools detected via echo-sounder over a neap tide. Note that the pools are confined to the deeper areas of the estuary (see Fig. 2.4), particularly the Newport and Bristol Deeps.

prokaryotes (Madrid *et al.*, 2006, Aller *et al.*, 2010), possibly by recycling reduced metabolic compounds and making them available once more for use by prokaryotes, in a similar fashion to activated sludge bioreactors (Aller, 1998). A study by McAnally *et al*. (2007) also describes how the fluid mud pools in the Severn Estuary act as distinct units and do not mix with the surrounding water, and that they can be moved up and down the deeper parts of the estuary by tidal currents and the influence of gravity.

Kirby (2010) again describes what potential affects a tidal barrage might have on the sediment regime of the estuary. For example construction of a tidal barrage would severely reduce suspended sediment resulting in the loss of the fluid mud "slugs" and lutoclines. Large scale cycling of sediment between aerobic and anaerobic states by currents would also cease and while this might be beneficial for anaerobic prokaryotes it might also decrease the natural self cleansing properties of the sediments leading to a build up of toxic materials (such as heavy metals) in the sediment.

One of the most hotly debated areas within the study of the sediment regime of the Severn Estuary relates to how sediment is transported into, out of, and around the

estuary. For example, Culver (1980) described the patterns of sediment transport within the estuary and suggested that the estuary may have a differential two-way transport regime. The author proposed that the estuary's coarser sediments are transported westward along the southern side of the estuary as bed-load by deep-water currents, while the presence of stenohaline benthic foraminifera tests in the fine sediments of the estuary would indicate that this sediment is derived from deeper waters in the west and that it has therefore been transported eastward into the estuary. These findings disagree with those of Allen (1991) who suggested that the coarse sediment in the estuary had travelled eastward from the outer channel while the finer sediment had travelled westward and was derived from fluvial sediments.

Later, Harris and Collins (1985) also carried out a study into sediment transport paths in the Severn Estuary. Their results indicated that there was a much more complex current, and therefore sediment transport, regime than was previously thought and agreed with Culver (1980), in that sediment is transported into the estuary from the Celtic Sea. However, the authors disagreed with Culver (1980) over the path this sediment takes, suggesting that sediment is transported eastward along the northern and southern edges of the estuary and westward through the centre of the estuary, whereas Culver (1980) suggested that sediment was transported out of the estuary along its southern margin.

This marginal-eastward/central-westward transport model put forward by Harris and Collins (1985) was disputed by Stride and Belderson (1990), who suggested that most of the sand transport in the Severn Estuary is in a westward direction away from the Bristol Channel Bed Load Parting Zone (which extends from Barry to Bridgewater Bay). They also suggested that sand transport is dominated by the ebb, rather than the flow tide, though they conceded that some small-scale marginal-eastward transport may occur in localised areas due to interactions between currents and local topography such as in the area north of Scarweather Sands.

However, this unidirectional flow regime is rejected in a later publication by Harris and Collins (1991), who maintain their advocacy of a model of marginal flood-based (eastward), and axial ebb-based (westward) flow. The authors argued that in order to sustain the unidirectional model proposed by Stride and Belderson

(1990) $1.9x10^{10}$ tonnes of sand would have to have been removed from the Bristol Channel/Severn Estuary over the past 3000 years. This would amount to an increase in the mean depth of the channel from ≈9 m to ≈20 m, however, there is no evidence for this having occurred in the sediment record. They also argued that flood-dominated eastward transport occurs in thin bands (≈1 km wide) at the margins of the Bristol Channel, which, while not detectable using the numerical methods promoted by Stride and Belderson (1990), can be observed using current meters.

Observation of the Severn Estuary, either in person or via satellite/aerial images, shows from the colour of the waters that the estuary has a high level of suspended sediment carried within it. Kirby and Parker (1982) carried out a study into lateral discontinuities in this suspended sediment load. While it was already known that the estuary was vertically stratified with regard to suspended sediment (i.e. lutoclines) it was not known how suspended particulate matter (SPM) concentrations varied horizontally across the estuary. In order to investigate this, the authors carried out a series of traverses across the estuary in a survey ship at various states of the tide towing optical turbidity meters, a pressure/depth sensor and a conductivity meter. The results of this study showed that there is a large and distinct suspended sediment front present in the Severn Estuary, which extends along an axis between The Shoots and the Holm Islands before angling southwest into Bridgewater Bay. This front can be over 5 km wide in the southwest but can narrow to only 2 m at its northernmost extent in The Shoots. The SPM concentrations of the front vary with the spring/neap tidal cycle as well as with the diurnal tidal cycle, with the highest SPM concentrations during the ebb of a spring tide. Within the front itself, SPM also varies with depth, as would be expected in the sediment stratified Severn, with concentrations of 1 g l^{-1} at the surface in Bridgwater Bay on a spring tide which increase to >4 g l^{-1} at the seabed at the same location. The causal mechanism for the front is unknown, although the authors suggested that it may be linked to interactions between the estuary's waters and the local topography coupled with the Coriolis Effect, which induces the formation of secondary circulation cells within the estuary leading to a convergence of sediment laden water along the main axis of the estuary. The fact that the front is slightly off centre of the main axis, with SPM concentrations being higher on the English side of the estuary,

may be due to the fact that Bridgwater Bay on the Somerset coast is one of the main sources of fine sediment in the estuary.

As well as documenting the subtidal sediments it is also important to understand the processes occurring on the mudflats and saltmarshes that border the estuary as these areas can form important habitats for sedimentary prokaryotes, with an area of 225-258 km^2, depending on source (Kirby, 2010; Zhou *et al.* 2014a). The mudflats are also one of the main sources/sinks for the estuary's suspended sediment load as well as acting as a source of organic matter and nutrients for prokaryotic activity in subtidal sediments. Allen and Duffy (1998b) studied the variation in the types of sediment deposited on mudflats and salt marshes in the estuary during the different parts of the year between 1991-1993. They did this by setting up monitoring stations on both sides of the upper estuary, which collected samples of the sediment that was deposited during each diurnal tidal cycle over the course of eight spring/neap tidal cycles. The results of the study show that the types of sediment deposited in the estuary varied both spatially and temporally, with the silts that were deposited on the mudflats being slightly coarser grained than those deposited on the marshes and with both mudflat and marsh sediments getting gradually more clay-rich towards the mouth of the estuary. The type of sediment also varied with the time of year, with sediments that were deposited on the vernal equinox being more sandy than those deposited on the autumnal equinox. Since the tidal regimes at both these times are virtually identical the authors suggest that increased storminess of the winter weather, possibly coupled with changes in temperature, viscosity and differences in the amount of biological activity over the course of the year, may be the cause for the observed variation in sedimentation between the two equinoxes.

In another earlier study, Allen (1987b) investigated the effects of sediment reworking in the Severn Estuary. The author found that the sediments of the estuary were particularly susceptible to reworking due to three factors: 1) the strongly erosive tidal currents that are present in the estuary; 2) the presence of fine sand laminations between layers of finer muds and silts which encourage splitting; and 3) the drying out of exposed mudflats in the summer months (with subsequent development of mud cracks). The results of the study also indicated that the annual

turnover of reworked sediment ranges between $7.2x10^4$ t yr^{-1} and $7.2x10^5$ t yr^{-1}. These figures indicate that sediment reworking amounts to between 7-70% of the annual fluvial supply of fine sediment to the Severn Estuary, and is probably responsible for around 0.7-7% of the estuary's suspended sediment load.

Allen (1987c) and French (1998) both investigated the levels of coal dust in the muddy intertidal sediments of the Severn Estuary. Since the catchment area of the Severn Estuary contains many of the historical coal producing areas in the Southern UK that have been exploited over the past 300 years (including the South Wales coalfield and the Forest of Dean), and since in the past rivers and estuaries were considered a good place to dispose of unwanted coal material, it was hypothesised that the quantities of coal dust in the estuary might be high and pose a risk of pollution. In order to determine the coal content of the sediment, the author collected sediment cores from a number of intertidal sites in upper and lower estuary and analysed their coal content. The results showed that coal dust was concentrated in the upper 50 cm of the sediment cores with concentrations in some locations peaking as high as 6-8% of the total sediment content. This would indicate that overall the intertidal mudflat sediments of the estuary may hold as much as 10^5-10^6 tonnes of coal material which could potentially act as a potential future pollutant in the estuarine system, due to the extensive sediment reworking and re-mobilisation potential of the waters of the estuary. However, in the context of this study it is important to consider the potential effects the coal might have on benthic prokaryotes. For example, the crystalline FeS_2 in the coal could be oxidised on exposure to seawater or groundwater, providing a source of sulphate, which might aid the growth of the SRB. Or alternatively the coal itself (and it's breakdown products) could be utilised as a carbon substrate source by SRB and methanogens (Tang *et al.* 2012). Another possibility is that zones within the sediment pile with high concentrations of coal dust could affect the flow of pore-waters due to the hydrophobic nature of the coal material.

1.3.7 Hydrology and Water Chemistry of the Severn Estuary

The waters of the Severn Estuary are likely to play a major role in affecting the growth and development of prokaryotic communities. Factors such as currents, dissolved electron acceptors, nutrients and the presence of heavy metals etc. might

influence where, and how well, benthic prokaryotes develop in the estuary. Therefore it is important to understand some of the hydrological processes within the estuary.

Uncles (2010) carried out a review of the physical properties of the waters of the Severn Estuary. The review indicates that while currents and tides in the estuary are strong compared to many other estuaries, the waters of the estuary are flushed comparatively slowly (ca. 200 days), although cyclonic density currents in the Celtic Sea also enhance sediment transport from the Bristol Channel into the Celtic Sea. The average sea-surface temperature (SST) of the estuary varies with proximity to the open sea. For example, in the Celtic Sea temperatures range from 8-10 °C in February to 16-17 °C in August, whereas in the Bristol Channel winter temperatures can drop to 8 °C at the mouth of the channel and <6 °C in the upper Severn Estuary, while in summer temperatures in the inner channel can reach >20 °C (Norris, 2001). The average salinity of the Celtic Sea is 35, which decreases upon entering the Bristol Channel and Severn Estuary due to the increasing influence of fluvial input. In total 26 river systems discharge into the estuary, 19 on the north side and 7 on the south. Salinity also varies with fluvial input, and therefore weather, with salinities reaching as low as 20 in winter in the upper estuary due to increased rainfall. The tidal currents generated by the estuary's high tidal range can be very fast, with current speeds >1 m s^{-1} at the mouth of the channel and exceeding 2.5 m s^{-1} in the upper channel and estuary (Fig. 1.14). These tidal currents also ensure that the waters of the estuary remain unstratified with regard to salinity and temperature (Vijith and Shetye, 2012). Annual mean wave heights in the channel decrease moving from the channel mouth (where wave heights are around 2 m) eastward into the estuary (ABPmer, 2008). However the relatively steep gradient of the seafloor results in large waves reaching close to shore and during periods of high wind speeds, storm surges of >1.5 m can occur in the Bristol Channel. Uncles (2010) also collected data on temperature, salinity, SPM and currents at a number of sites across the Severn Estuary and the greater Bristol Channel. The results of this study showed that in the channel, near-bed density-driven currents were directed eastward into the channel while middle or upper water column currents were directed westward out of the channel. It is suggested that these eastward up-estuary currents are generated by the prevailing winds. The results also indicated that there

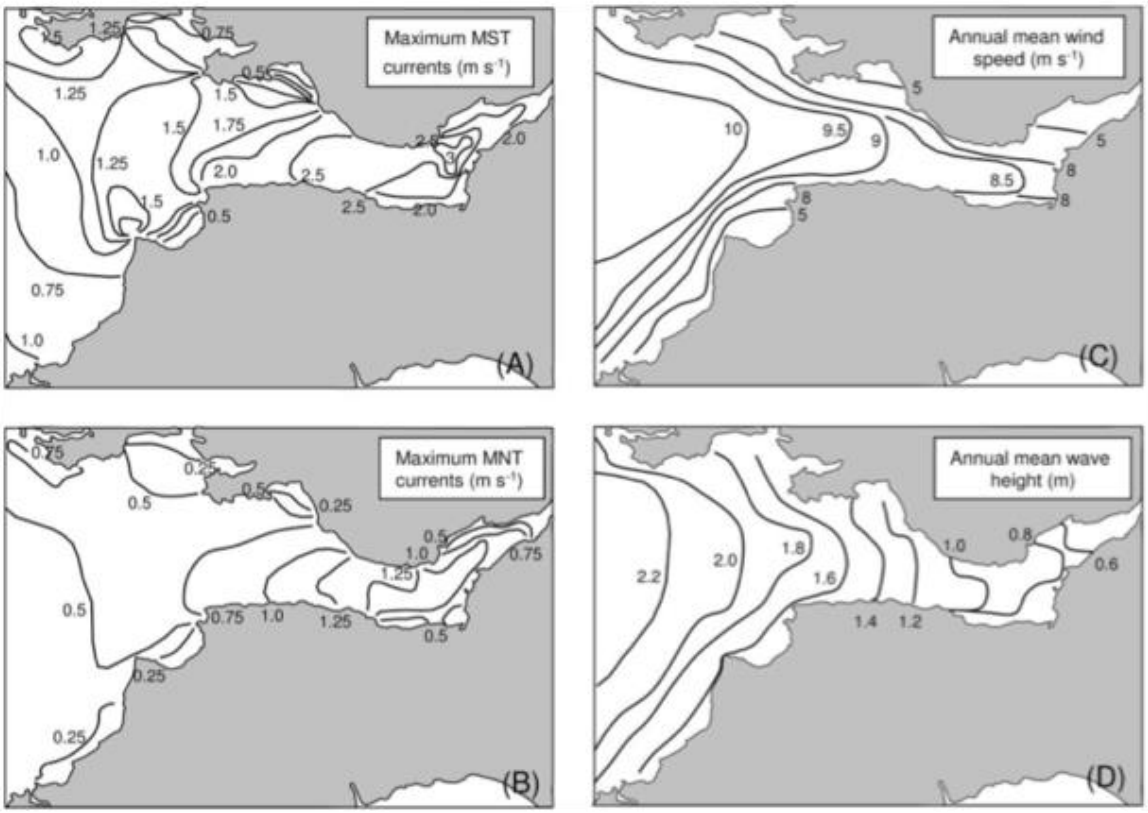


Fig. 1.14 – Diagram taken from Uncles (2010) showing simulated parameters for water currents and wind speeds in the Severn Estuary and Bristol Channel. A) Maximum mean spring tide (MST) current speed. B) Maximum mean neap tide (MNT) current speeds. C) Annual mean wind speed at 80 metres above sea level. D) Annual mean significant wave height.

is a potential for strong up-estuary transport of fine sediment on spring tides, which coupled with gravitational circulation, would lead to a large amount of fine sediment storage and subsequently high SPM in the estuary's upper reaches.

Jonas and Millward (2010) studied the concentrations of dissolved compounds in Severn Estuary waters. They measured concentrations of SPM, dissolved and suspended metals, dissolved nutrients (nitrate, nitrite, ammonia and reactive phosphorous and silicate), dissolved oxygen, chlorophyll and salinity. These parameters were measured in three main areas: the inner estuary, the outer estuary and the inner channel using monitoring stations at river discharge points on both sides of the estuary as well as on a series of ship-based transects along the estuary's axis. The results of the study showed the long term mean annual flow of the River Severn (termed the base flow) was 111 m^3 s^{-1} while the total long-term mean fluvial input into the Severn Estuary and Bristol Channel (2000-2003) was 375.7 m^3 s^{-1}. The mean location of the saline/freshwater interface was around 8 km down-

estuary from the tidal limit near Elmore Back. The mean dissolved oxygen (DO) saturation in the parts of the estuary >80 km downriver from the tidal limit was >95% while in the areas <80 km from the limit, saturations were lower by around 10%, probably linked to an increase in SPM. The DO data gave no indication of wide spread oxygen depletion either at the surface or at depth in the estuary however, the results indicate that benthic anoxia could occur in the sediments in the upper estuary in the tidally quiescent period following a spring tide. The salinity gradient in the estuary was steep <50 km from the tidal limit at which point the gradient became more gentle until ≈121 km where salinity became constant at 30.3. The mean chlorophyll content of the water increased towards the tidal limit, however, overall the concentrations of chlorophyll in the estuary were low, probably due to the estuary's high turbidity limiting light penetration and hindering photosynthesis. The mean dissolved nitrate concentrations were around two orders of magnitude greater than the levels of dissolved nitrite and ammonia, and all three, along with dissolved silicate and phosphate, reached maximum concentrations 8 km down-estuary of the tidal limit. This concentration of dissolved nitrogen species in a turbid, low salinity region has also been observed in macro-tidal estuaries (Morris *et al.* 1985; Uncles *et al.* 1998). Finally the results of the investigation into suspended metals showed that the Severn Estuary has concentrations similar to the world averages for riverine and estuarine particulate material.

1.3.8 Research Objectives and Site Selection

As the previous sections have shown, the Severn Estuary is an extremely dynamic environment with regard to both sedimentary and hydrological conditions, which can have an extremely negative effect on both sedimentary macrofauna and planktonic and benthic photosynthetic organisms. Therefore, based on the following hypothesis:

> If the dynamic sedimentary conditions present in the Severn Estuary dramatically affect both the macrobenthos and photosynthetic prokaryotes living within the estuary, these dynamics must also have a significant effect on the prokaryotes living within the sediments.

the main research objectives of this project were:

1. To determine and quantify, using a variety of biogeochemical analyses, what effect these dynamic conditions might have on the metabolism of the prokaryotic organisms living within the sediments, and in turn the geochemical processes they control.
2. To assess how environmental changes, both past and present might have influenced these organisms and also to put forward predictions about how future changes in the estuarine system might subsequently affect them.

In order to do this sediment cores were collected from a variety of sites around the estuary from both subtidal and intertidal settings (shown in Fig. 1.6B) and subjected to biogeochemical analyses (see Chapter 2). These sites were selected based on a variety of parameters including:

- Water depth – to assess whether this played a role in how affected the sites were by erosion driven by currents or surface waves.
- Proximity to the shoreline – to assess how affected sites were by terrestrially linked processes.
- Degree of exposure – for tidal flat sites, to see how sites were affected by erosion and re-suspension.
- Position within the estuary – to see if conditions changed on the northern and southern sides of the estuary.
- Proximity to sediment sources/sinks – to see how conditions varied within known important areas of large scale sediment accumulation and removal, such as Bridgwater Bay.
- Any knowledge of previous environmental change occurring in an area.

This last point led to the in-depth study of two more unusual sites within the greater Severn Estuary area, St Brides Wentlooge and Cardiff Bay. St Brides Wentlooge is a tidal flat environment in the northeast of the estuary that contains buried deposits of Holocene salt marsh peat formed due to sea level rise over the past several thousand years. As such it presented an interesting environment of study with relevance to both past and future climatic change. Cardiff Bay on the other hand represents a more recent site of anthropogenic environmental change. Prior to 1999

the site was a tidal flat environment open to the rest of the Severn Estuary, however, following the construction of a barrage across the mouth of the bay it was transformed into a 200 ha freshwater lake (Crompton 2002; Platt, 2002; Williams, 2008). This transformation of the environment obviously had a significant effect on the sedimentary geochemistry and as such Cardiff Bay became an important site to investigate how such extreme changes over a relatively rapid period of time could have affected the metabolism of the organisms living within the sediments and how they have adapted to their new habitat.

In total 11 sites were sampled across the Severn Estuary and Cardiff Bay, with several others investigated and abandoned due to unfavourable conditions (e.g. strong tidal currents, lack of seafloor sediment, high sand content of sediment). The majority of these cores were collected from sediments described as muds or sandy muds (see Fig. 1.12) based on previous knowledge of sediment distribution in the estuary (Kirby, 2010). Higher resolution maps of these sites showing seafloor topography (for subtidal sites) or the surrounding landscape (for intertidal sites) can be found at the start of their respective results chapters (Chapters 3-8) alongside detailed descriptions of the conditions at each site and the lithological characteristics of the sediments studied.

Chapter 2. - Methodology

2.1 Sediment Coring

The subtidal sediment cores used in this study were collected using a multi-corer (Duncan and Associates, Cumbria, UK), operated from Cardiff University's research vessel "Guiding Light" (Fig. 2.1). The multi-corer was lowered into the water from the stern deck of the research vessel using a hydraulic winch and was then allowed to gently descend until it landed on the seabed. Once the legs of the outer frame had settled on the sediment surface the inner section of the corer containing the core tubes would automatically disengage and slowly sink into the sediment. The speed at which the central section descended into the sediment could be controlled by a valve connected to the central piston. This meant that in very soft sediment the central section could be lowered more slowly, minimising disturbance of the upper layers of sediment. After a prescribed period of time, usually two minutes but varying depending on sediment type (shorter time spans for unconsolidated lacustrine/re-suspended sediments, longer for more compacted, clay-rich sediments) the corer was winched back up to the surface. As the inner section rose up out of the sediment and returned to its original position relative to the outer frame, four plastic bungs would be released, sealing the top of the core tubes. Simultaneously four spring-loaded arms attached to the top of the inner section would be released and would swing downwards, capping the bottom of the core tubes and preventing any sediment from escaping. Once the corer had been winched back to the surface it would be lowered onto the rear deck and the core tubes could be detached from the corer and capped with rubber bungs ready for transport to the laboratory. New core tubes were then attached to the corer ready for the next descent. The corer was capable of recovering four intact sediment cores of up to ≈40-45 cm in length and 6.5 cm in diameter on each descent. Once these cores had been returned to the laboratory they were stored either in a cold room at a constant temperature of 4 °C or in a specially constructed water bath (Fig 2.2) at *in situ* temperature until they were ready to be sectioned or used for O_2 uptake measurements (usually within 24-48 hours).

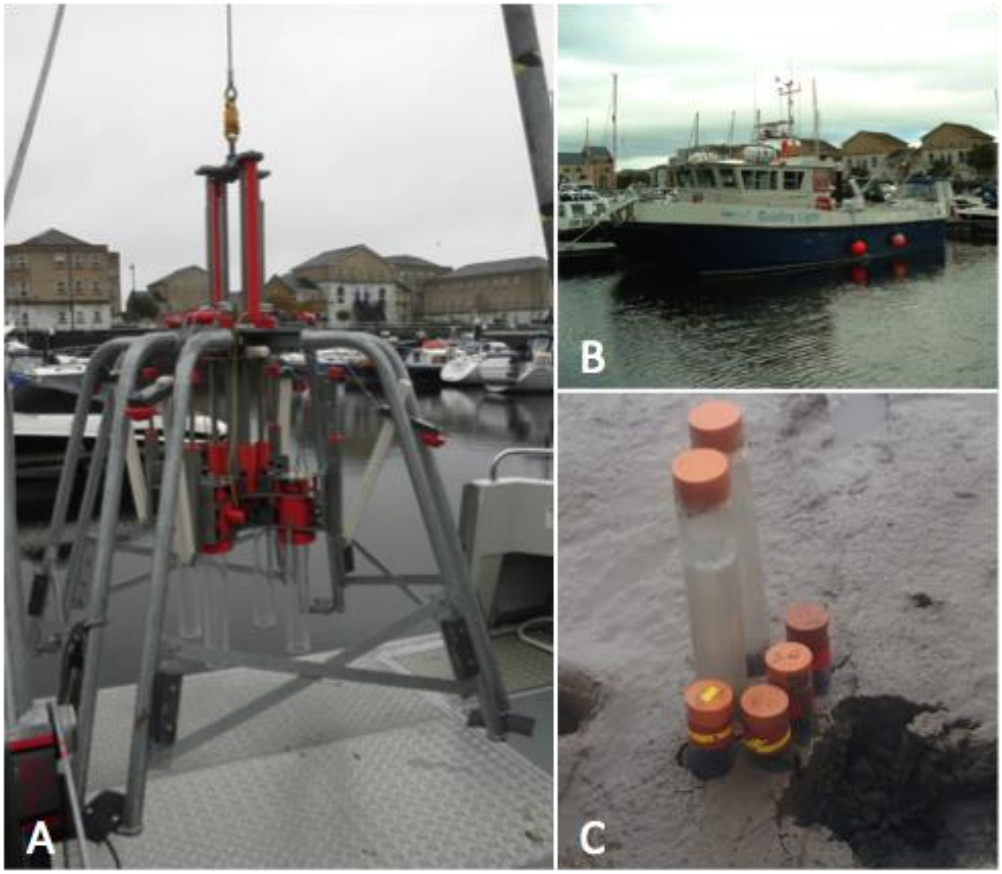


Fig. 2.1 – A) Photograph of the multi-corer used for subtidal sampling. Corer is shown in it's "armed" position with sealing-arms raised and held in place by bungee cords. B) Photograph of the Research Vessel Guiding Light used for subtidal sampling, moored at Penarth Marina, Cardiff Bay (© Copyright Wigmore Wright Marine Services). C) Photograph showing the collection of sediment cores from intertidal mudflat sites (samples in question are core StB2 and duplicates).

Due to the extreme tidal conditions present in the Severn Estuary, subtidal coring was restricted to days around the neap tides and as such only three to four days every month were considered viable for coring. In addition coring could only take place during slack water to minimise the effects of tidal currents, limiting operations to 2-3 hours per day. Attempts were initially made to core outside these windows, however, buffeting by currents resulted in the corer being dragged along the seafloor and subsequently no sediment was recovered. In addition, several prospective sampling sites had to be abandoned due to the fact that the corer appeared unable to core through sediments with a high sand content, such as those found in the near-shore regions of Bridgwater Bay or in the vicinity of the English Grounds, either due to the

compacted nature of the sediment or a lack of sediment cohesiveness and/or improper sealing of the core tubes during recovery.

Intertidal coring was carried out using the same core tubes used for the multi-corer or (if the sediment proved very dense) longer 1 m tubes with sharpened ends (diameter = 5 cm). These tubes were gently pushed into the sediment while twisting by hand so as not to disturb the surface layers of sediment and then subsequently hammered in if the sediment became denser beneath. The tubes were then dug out by hand, capped with rubber bungs and immediately returned to the laboratory for storage in the 4 °C room. Cores were again sectioned within 24-48 hours depending on measurements taken. During both subtidal and intertidal sampling trips, additional duplicate cores were taken at each site to allow for O_2 uptake and radiotracer measurements to be conducted alongside the geochemical analyses. Under ideal conditions, four cores were taken from each site: one for geochemistry, one for O_2 uptake, and two for radiotracer work.

3.2 Oxygen Uptake Measurements

In order to measure total oxygen uptake/sediment oxygen demand (SOD) as a proxy for total organic carbon degradation within the sediments (Parkes and Buckingham, 1986), one of the duplicate cores collected from each site was placed in a water tank at *in situ* temperature and salinity and left uncapped overnight to equilibrate (Fig. 2.2). The following day the core tubes were sealed with mechanical stirring units, in order to prevent influx of O_2 while also allowing a for a well-mixed water column similar to conditions in the Severn Estuary (Boynton *et al.* 1981). Water samples were then taken from the headspace of the core tubes along a time series (0, 2, 4, 6 and 8 hours) to determine any change in oxygen concentration over time. Samples were taken by attaching a syringe to the top of the stirring unit (via a 3-way stopcock) and pushing the stirrer further into the core tube, forcing water into the syringe in the process. This allowed samples to be collected without creating a vacuum in the headspace and prevented any O_2 from entering during sampling. All incubations were carried out in the dark to avoid O_2 production by photosynthetic organisms and care was taken when fitting the stirrers so as not to disturb the sediment surface micro-layer. Oxygen

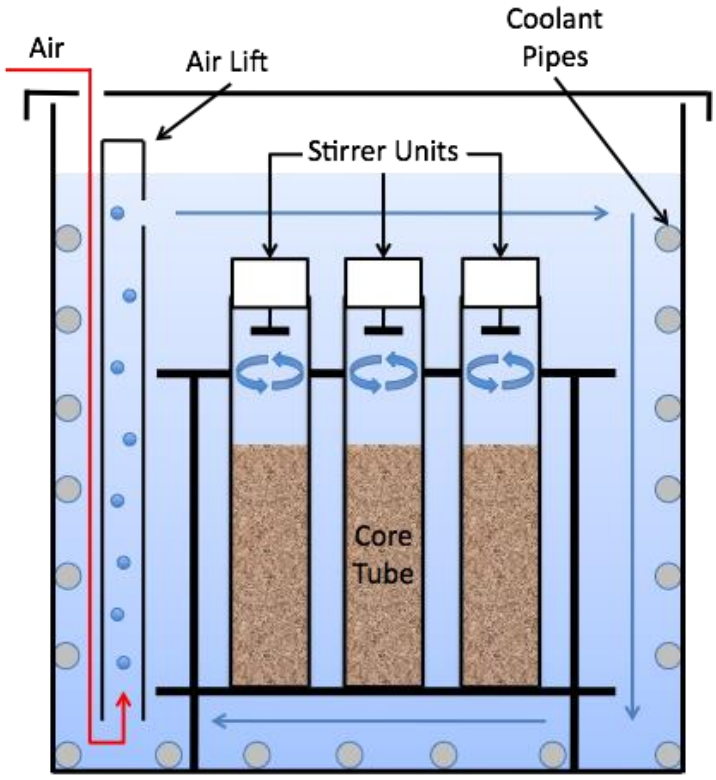


Fig. 2.2 – Cross section diagram of the core bath used for the incubation of whole cores for O_2 uptake measurements. The core bath itself was constructed from a reinforced, opaque plastic cold water tank and had a capacity of c. 200 litres. The water was oxygenated and mixed using an airlift connected to an external aquarium air pump fitted with an air stone. During operation the tank was stored in a temperature controlled room and cooling was achieved using a heat exchanger constructed from sections of marine grade (SAE 316) stainless steel pipe separated by reinforced plastic tubing and connected to an external refrigeration unit running ethylene glycol coolant (50:50 ethylene glycol:water).

concentrations were measured by a modified Winkler titration using potentiometric detection of endpoint, via a method modified from Strickland and Parsons (1960) and Oudot *et al.* (1988).

The water samples were collected using a 10 ml glass syringe (Weber Scientific, New Jersey) fitted with a 3-way stopcock (Heinz Herenz, Hamburg) to prevent contact with atmospheric oxygen. The samples were then immediately injected with 0.1 ml of 0.215 M manganese sulphate ($MnSO_4$) and 0.1 ml of alkaline iodide solution (6 M NaI in 10 M NaOH) to trap the dissolved oxygen as manganese hydroxide/oxyhydroxide - $Mn(OH)_3/MnO(OH)_2$. This precipitate was then allowed to settle for 15 minutes before the addition of 0.2 ml of concentrated (86%) phosphoric acid (H_3PO_4) to dissolve the precipitate back to $MnSO_4$ and iodide (I^-) ions, and subsequently into iodine (I_2). The acidified solution was then titrated with an excess of 1.25 mM sodium thiosulphate (NaS_2O_3) and then back titrated with 1.25 mM potassium iodate (KIO_3) to determine the dissolved oxygen concentration. All titrations were carried out using a Mettler Toledo DL50 autotitrator fitted with two 10 ml burettes and a Mettler Toledo DM140-SC platinum ring combination redox electrode. The results were then analysed using LabX

graphical analysis software. In order to accurately determine the dissolved oxygen concentration the thiosulphate and iodate solutions were standardised at the beginning of each day by running each titrant against a known volume of the other to determine it's exact normality and to create a correction factor (Eqs. 2.1-2.4):

$$\text{Normality of thiosulphate (forward titration)} = \frac{(0.00125 \times 10)}{V_f} \quad (2.1)$$

Where V_f is the volume of thiosulphate added at the endpoint of the forward titration (VEQ)

$$\text{Normality of thiosulphate (backward titration), } N_b = \frac{(0.00125 \times V_b)}{5} \quad (2.2)$$

Where V_b is the volume of iodate added at the endpoint of the backward titration (VEQ)

Normality correction, N_c = normality of K iodate/normality of thiosulphate

$$= \frac{0.00125}{N_b} \quad (2.3)$$

The dissolved oxygen concentration was then determined using the following formula (Eq. 4.4):

$$[O_2] = \frac{\{[V_x - (V_i \times N_c)] \times k_1\}}{[k_2 \times (0.0125 \div N_b)]} \quad (2.4)$$

Where: $[O_2]$ is the dissolved oxygen concentration (in mg l^{-1})

- V_x is the volume of excess thiosulphate added, derived from V_f
- V_i is the volume of iodate added at endpoint
- N_c is the normality correction
- k_1 is a correction factor (usually 102.04) to compensate for the weight of the water sample, density etc.
- k_2 is a correction factor (usually 9.8) to compensate for the volume of the sampled displaced by the Winkler reagents
- N_b is the normality of thiosulphate as determined by backwards titration.

At the same time that the cores were capped with the stirring units a second, time zero (T0), sample was collected in a 30 ml glass biological oxygen demand (BOD) bottle with a ground-glass stopper and incubated alongside the sealed core tubes. The

concentration of oxygen in this sample was measured at the end of the time course and the difference between it and the T0 values from the cores used to determine the oxygen uptake by heterotrophic pelagic/planktonic organisms in the water column. This value was then used to correct the results obtained from the incubated cores. The volume of the core headspace was also measured at the beginning and end of the experiment in order to calculate the number of moles of O_2 present.

Core incubations were selected as the preferred method of oxygen uptake measurement over other methods, such as benthic landers or flux chambers (Schulz, 2006; Spooner and Maher, 2009; Abhilash *et al.* 2012; Reimers *et al.* 2012), as these structures were not suitable for use in the highly physically dynamic Severn Estuary.

2.3 Sectioning of Sediment Cores

Subtidal sediment cores were sectioned in the laboratory using a hydraulic core-cutter assembly (Duncan and Associates, Cumbria, UK). The bung in the bottom of the core tube was removed and replaced with a plastic piston. The core was then quickly screwed into the base of the cutter, the top bung removed and the sectioning blade assembly was screwed to the top of the core. Water was then pumped through the base of the cutter and the piston in the bottom of the core tube slowly rose up pushing the sediment ahead of it. As the sediment rose up through sectioning assembly it was cut into 2 cm thick sections, which were subsequently sampled using cut-off 5 ml syringes (BD Plastipak, New Jersey).

The sediment in the intertidal cores was usually denser than that found in the subtidal cores resulting in more friction during extrusion. In several cases the friction was too great for the core-cutter to cope with and the o-ring seal around the base of the core tube would give way before the plunger began to move. In these cases (and when the longer core tubes were used) the cores were extruded and sectioned using a hand-held plunger.

2.4 Pore Water Analysis

In order to analyse the chemical content of the pore waters within the sediment cores a minimum of 6 cm^3 of sediment (3 x 2 cm^3 plugs) was collected from each section of the

core using a cut off 5 ml syringe. This sediment was then injected into 15 ml centrifuge tubes and gassed with oxygen free nitrogen (OFN) using a gas jet syringe. The tubes were then capped and immediately centrifuged (Hettich Rotanta 460 R – Hettich Rotanta, Massachusetts) at 1270 x G (RCF) at 4 °C for 15 minutes to separate the pore waters from the sediment. The resulting supernatant pore water was then pipetted out of the centrifuge tubes and syringe filtered (0.22 μm, Millex® - Millipore,) into 1.5 ml glass vials (Chromacol, Thermo Fisher Scientific) before being stored at -20 °C prior to analysis. In the intertidal sediment cores containing peat (cores ST5, StB1 and StB2) the lower layers of sediment were so dry that this method did not yield sufficient quantities of pore water necessary for geochemical analysis (≈0.5 ml). With these cores ≈30 cm^3 of the peat sediment was placed into 50 ml falcon tubes (VWR, Pennsylvania), centrifuged at 1270 x G (RCF) and the pore waters extracted as above. If this still did not produce the necessary volume of pore water, the peat was packed into a pore water press (KC Denmark Research Equipment, Silkeborg) and compressed for ≈30 minutes to extract the required 0.5 ml.

Analysis of the anionic content of the pore waters was carried out as described by Watkins *et al.* (2012). One millilitre of the filtered pore water from each section was pipetted into a 1.5 ml Chromacol glass vial with a rubber septum cap. For saline/brackish cores the samples were diluted 1:10 with ultra-pure Milli-Q water (MQ H_2O, >18.2 MΩ; Milli-Q system, Millipore), usually 100 μl of sample in 900 μl of MQ H_2O although less was used if pore water was limited, whereas freshwater samples were run undiluted. Blank vials containing 1 ml of MQ H_2O were interspersed after every five samples to reduce cross contamination. The samples were then run on a Dionex ICS-2000 ion chromatograph fitted with an AS50 Autosampler running KOH eluent (flow rate 0.9ml min^{-1}). The system had two Ionopac AS15 columns in series and separation was carried out using an anion self-regenerating suppressor unit (4-mm ASRS-Ultra II) along with a DS6 heated conductivity cell (Dionex Ltd). The gradient program was set at 6 mM KOH (38 min), 16 mM KOH min^{-1} to 70 mM (17 min), and 64 mM KOH min^{-1} to 6mM (12 min) (Webster *et al.* 2009). The resulting values were compared against previously made standards of a known composition using Chromeleon graphical

analysis software. This system allowed for the detection of chloride, nitrite, sulphate, phosphate, nitrate, bromide and thiosulphate as well as volatile fatty acids (VFAs) such as acetate, formate and propionate and other organic acids such as lactate and glycolate.

For the analysis of the pore water cations, 1 ml of sample was pipetted into glass 1.5 ml Chromacol vials with a rubber septum cap. As with the anion samples, the pore waters were diluted depending on their environment of origin with saline/brackish samples being diluted 1:10 (100 μl of sample in 900 μl of MQ H_2O) while freshwater samples were run undiluted. Initially the samples were then run on a Dionex DX-120 ion chromatograph fitted with an AS40 autosampler, an IonPac CS16 column, a CSRS 300 4-mm suppressor and a conductivity detector with methanosulfonic acid eluent (32 mM) at a flow rate of 0.75 ml min^{-1} (Parkes *et al.* 2012). Later samples (cores StB1, ST6, ST7, ST8 and StB2) were analysed on a second Dionex ICS-2000 ion chromatograph fitted with an AS50 autosampler, DS6 heated conductivity cell, IonoPac CS16 column, CSRS 300 4-mm suppressor and methanosulfonic acid eluent (flow rate 0.9 ml min^{-1}). Again as above, the samples were interspersed with MQ H_2O-filled blank vials to reduce contamination and compared against standards of a known composition using Chromeleon graphical analysis software. This setup allowed for the detection of the major cationic groups present in aqueous environments; such as sodium, calcium, potassium, magnesium and ammonium as well as methylated amines such as methylamine, dimethylamine (DMA) and trimethylamine (TMA) and amino group-containing alcohols such as *N*-monomethylethanolamine (MMEA), *N*,*N*-dimethylethanolamine (DMEA) and choline.

2.5 Sediment Gas Content

To analyse the gas content of the sediment cores 4 cm^3 of sediment (2 x 2 cm^3 plugs) was taken from each core section using a cut-off 5 ml syringe. The sediment was then injected into 22.2 ml headspace vials (Chromacol, Thermo Fisher Scientific) containing 5 ml of 10 mM KCl and three glass balls. The vials were then plugged with rubber stoppers and sealed with crimped aluminium caps before being shaken to form a slurry

and then stored upside down for ≈1-2 days to allow the gas content of the sediment slurry and headspace to equilibrate.

Immediately before the analysis took place the vials were shaken for an hour at 300 oscillations min^{-1} using an auto-shaker (Stuart Scientific Flask Shaker SF1 – Keison, Chelmsford) to re-suspend the sediment, and thereby free any trapped gas. This also removed any sediment that might have collected around the neck of the vials. The gas within the vials was then extracted, using a 2 ml syringe (BD Plastipak, New Jersey) with a 0.5 mm diameter needle (BD Microlance, New Jersey) connected via a three-way stopcock to prevent gas escape. Gas samples were analysed using a modified Perkin Elmer/Arnel Clarus 500 Natural Gas Analyser (Watkins *et al.* 2012) fitted with a thermal conductivity detector and flame ionization detector (packed column; oven temperature of 110 °C; detector temperature of 250 °C with helium as a carrier gas). Before each sample was injected the system was flushed with pure nitrogen to reduce the chances of contamination. Blanks containing air (one from an empty headspace vial containing only KCl and another from the surrounding atmosphere) were run at the beginning and end of the cycle to help detect any potential contamination. Finally the data obtained was analysed using TotalChrom analysis software and compared with industrially made standards of a known composition (Scott Speciality Gases, Pennsylvania). This system allowed for the detection of most gases commonly found in sediments that are indicators of microbial activity, including methane, carbon dioxide and hydrogen. Carbon monoxide was analysed using a Peak Performer 1 gas analyser (Peak Laboratories, Mountain View, USA) with a reducing compound photometer and a 1/8 column. Detector and column temperatures were 265 °C and 100 °C respectively with OFN used as a carrier gas. Results were then analysed using Chrom Viewer graphical analysis software.

2.6 Total Cell Counts

To determine the size of the prokaryotic populations within the sediment at different depths acridine orange direct counting (AODC) was used (Hobbie *et al.* 1977; Cragg and Parkes, 1994; Kepner and Pratt, 1994). During sectioning, 1 cm^3 plugs of sediment were collected from each depth interval of the core being studied using autoclave sterilised,

cut-off 5 ml syringes. The plugs were then injected into 10 ml serum vials filled with 9 ml of filter sterilised (0.2 μm Vacucap 90 - Pall Corporation, New York) formaldehyde solution (2% v/v formaldehyde in MQ H_2O, with an NaCl concentration appropriate to the core), which were mixed and then stored at room temperature ready for counting. The vials had previously been sterilised in a furnace (Carbolite, Derbyshire) at 450 °C for 4 hours to remove any organic material, including dead bacterial cells, that might contaminate the samples.

In order to count the prokaryotic cells the vials were vortex mixed for 15 seconds to re-suspend the sediment within, 10 μl of sediment was then pipetted into a test tube (20 ml – Sterilin, Newport) containing 10 ml of formaldehyde solution (same as described above, filter sterilised using a 0.1μm syringe filter - Whatman Anotop, GE Healthcare Life Sciences) and 50 μl of acridine orange dye (1 g l^{-1}). The tube was then shaken and left for 3 minutes to allow time for the fluorochrome to penetrate the prokaryotic cells and bond to their nucleic acids. The contents of the tube were then vacuum filtered through a 0.22 μm black polycarbonate filter (GE Water and Process Technologies) and the filter was then washed with 10 ml of formaldehyde solution to remove any excess dye.

The filter was mounted on a microscope slide under a cover slip using paraffin oil and examined under UV light at 1000x magnification using an epifluorescence microscope (Axioskop – Zeiss, Oberkochen) fitted with an acridine orange filter (450-490 nM). The cells observed on the slide were divided into four categories: attached to sediment particles, unattached to sediment particles, dividing and divided and counted separately. The number of cells classified as attached was doubled to take into account those that would have been obscured by sediment particles (see Eq. 4.5). Dividing cells (those that showed a distinct invagination) were counted as one cell while divided cells (morphologically identical cells that were adjacent to each other) were doubled as well. Cells were counted using a 10x10 grid attached on the eyepiece of the microscope and counting continued until at least 200 cells and 20 grids had been counted to reduce potential errors caused by the uneven distribution of cells. For samples from the upper sediment layers the size of the grid was reduced (usually to 5x5) in order to

compensate for the increased number of cells (this was then factored into in the final calculation of cell numbers). This procedure was then repeated for each depth interval in order to increase the accuracy of the results obtained and to determine the margin of error. The number of cells on each filter was obtained using Eq. 2.5

$$FC = \left[\left[\left(2xC_{on}\right)+C_{off}+C_{dg}+\left(2xC_{dd}\right)+\left[\left[\frac{C_{on}}{\left(2xC_{on}\right)+C_{off}}\right]+\left[C_{dg}+\left(2xC_{dd}\right)\right]\right]\right]\div Vc\right] x \frac{326851300}{Ac}$$

(2.5)

Where: FC is the number of cells on the filter

C_{on} is the number of cells counted on particles

C_{off} is the number of cells counted off particles

C_{dg} is the number of dividing cells counted

C_{dd} is the number of divided cells counted

Vc is the number of fields of view counted

326851300 is the total number of possible views

Ac is the area counted (determined from the size of the grid)

The total count was then obtained using Eq. 2.6:

$$TC = Log_{10}\left[\frac{FCx\left[\frac{1000}{Vol_C}\right]}{Sed_{dil}}\right]$$

(2.6)

Where: TC is the total number of cells cm^{-3} (expressed as Log_{10})

FC is the filter count (see above)

Vol_C is the volume counted (usually 10µl)

Sed_{dil} is the dilution of sediment in formaldehyde solution (usually 1:10 and therefore expressed as 0.1)

2.7 Rates of Anaerobic Processes

2.7.1 Active Sulphate Reduction Rate Measurements

Rates of *in situ* bacterial sulphate reduction (SRR) were determined using a modification of the cold chromium distillation method detailed by Kallmeyer *et al.* (2004). Sub-cores were collected by slowly pushing 20 mm diameter Plexiglas coring tubes into one of the larger cores from the sampling sites. These tubes had been drilled with a fine diameter bit at 1 cm intervals along the length of the tubes to allow for the injection of the radiotracer, and had then been sealed using non-toxic, aquarium grade silicone sealant (Selsil, Istanbul). As the sub-core tubes were pushed into the sediment, a hand-held vacuum pump was used to maintain pressure inside the sub-core tube to prevent compression of the sediment surface. Up to four sub-cores could be taken per large core, one being a control core. Once the sub-cores had been pushed all the way into the large core they were extruded using the hydraulic core cutter assembly or manual pistons as detailed above (section 2.3). Once collected the sub-cores were capped with rubber bungs and stored in the dark at *in situ* temperature overnight to allow them to re-equilibrate.

The following day the sub-cores were then injected every 2 cm with 2 μl of ^{35}S labelled sulphate (approx 0.16-0.17 MBq/injection, American Radiolabeled Chemicals Inc.) using a glass 10 μl syringe (Hamilton, Nevada). The sub-cores were then incubated for 6 hours before being sectioned into 2 cm slices (volume = 6.28 cm^3/sample) The sections were stored in vials containing 7 ml of 20% w/v zinc acetate solution and vortex mixed in order to cease further activity. Vials were then stored in the dark at room temperature after sectioning. Recent experiments by Røy *et al.* (2014) have found that storage of sediment samples at room temperature can cause a loss of ^{35}S-labelled total reducible inorganic sulphur (TRIS) resulting in an underestimation of the true SRR. However, in experiments conducted here radiolabeled samples were distilled within ≈28 days of injection, which should mean that any underestimation of the true SRR was kept to a minimum and therefore results should be comparable with the freezing storage method recommended by Røy *et al.* (2014). Initially replicate sub-cores were incubated for a variety of time periods (6, 18 and 24 hours) to determine the best

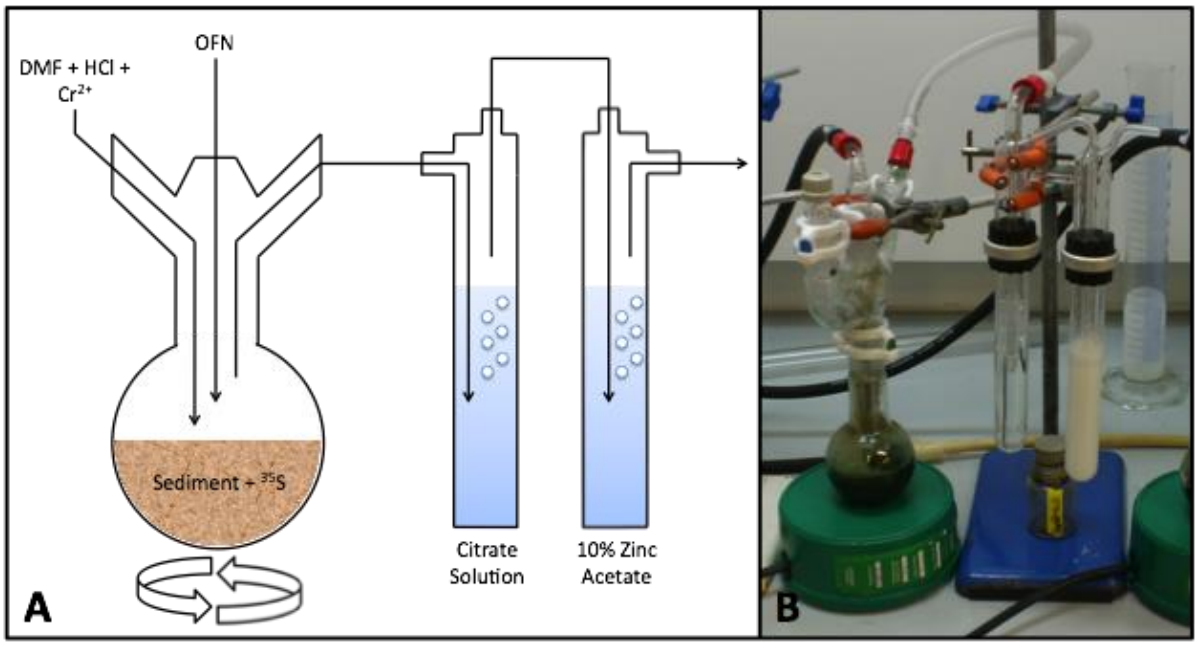


Fig. 2.3 – A) Schematic diagram for the rig used for the cold chromium reduction of ^{35}S-labelled sedimentary sulphur (modified from Kallmeyer *et al.* 2004). B) Photograph of the rig in action (analysis of core ST5) note the opaque white colour of the zinc acetate (right-hand test tube) indicating high levels of reduced sulphur.

incubation time. Six hours was eventually chosen as the standard incubation time due to the fact that in active sediments (particularly those from freshwater sites) over half the $^{35}SO_4^{2-}$ pool had been reduced within six hours making longer incubation times unnecessary and unadvisable. Injection of sub-cores with radiotracers was chosen as a method over the addition of tracers to slurried sediment samples in order to reduce the potential dilution and handling errors inherent to slurrying and short-term incubations (Jørgensen, 1978; Burdige, 1989; Meier *et al.* 2000).

During analysis the sediment samples were shaken to re-suspend the sediment and then poured into 50 ml centrifuge tubes (Corning, Thermo Fisher Scientific) and spun at 2140 x G (RCF) in a centrifuge (Hettich Rotanta 460 R – Hettich Rotanta, Massachusetts) at 10°C for 15 minutes. The supernatant was then removed to reduce the amount of non-reduced radioactive sulphate in the sample and the remaining sediment was re-suspended in 5% w/v zinc acetate solution. The sample was then poured into a 100 ml round-bottomed flask along with a magnetic stirrer and then attached to a separate three-way neck. This flask was in turn connected via PVC tubing (7 mm diameter – Portex, Smiths Medical, Minnesota) to a double trap – pvc tubing was

used rather than silicone to prevent oxygen diffusing into the reaction apparatus. The double trap was made up of two boiling tubes: the first containing 25 ml of 100 mM citrate solution (19.3 g citric acid, 4 g NaOH in 1 L of MQ H_2O at pH 4) to act as an aerosol trap; and the second containing 20 ml of 10% w/v zinc acetate solution and a drop of silicone antifoam solution (Thermo Fisher Scientific) to act as a trap for the radiolabeled sulphide. Before starting the distillation the flask was flushed with OFN at approx 150 ml min^{-1} for ten minutes to remove any oxygen in the system. The OFN flow was then decreased to approx 60 ml min^{-1}, and 20 ml of *N, N*-dimethylformamide (DMF) was injected into the flask via a rubber septum in order to break down any S-S bonds in the sample and allow for the detection of radiolabeled elemental sulphur (Hsieh and Yang, 1989). 8 ml of 6 N HCl was then injected in order to liberate the acid volatile sulphide (AVS) component of the sample: iron monosulphide (FeS) and dissolved sulphide (S^{2-}_{aq}). Finally 25 ml of acidified 1 M Cr^{2+} solution (1 M $CrCl_2$ in 0.5 M HCl) was added to liberate the chromium (II) reducible sulphur (CRS) component: iron pyrite (FeS_2). The apparatus was then left for two hours to allow the liberation of the radiolabelled sulphide to occur (Fig. 2.3). The zinc acetate trap was then removed and a 2 ml subsample was taken and mixed with 10 ml of ScintiSafe 3 scintillation fluid (Thermo Fisher Scientific) in a 20 ml plastic scintillation vial (LabLogic, Sheffield). These samples were then placed in a Packard Tri-Carb 2900TR scintillation counter and counted for 3 x 20 minutes per sample with a counting window of 4-167 keV. Two "blank" vials were also run, the first "background blank" contained 10 ml of ScintiSafe 3 and was run to estimate the background radioactivity as well as any radioactivity in the scintillation fluid itself. The second "sample blank" was made by injecting 2 μl of radiolabelled sulphate into a vial containing sediment that had already been mixed with 20% ZnAc to stop sulphate reduction. This blank was then distilled as described above and thus gave the total number of counts produced in the sample that were not related to bacterial sulphate reduction plus any counts associated with carry-over of radioactive sulphur species still within the rig from previous experiments. The size of the sulphate cold pool used for calculating rates for these experiments was derived from the *in situ* pore water sulphate concentration as determined via ion

chromatography. The sulphate reduction rate was then calculated using Eq. 2.7 (Kallmeyer *et al.* 2004):

$$SRR = \left[SO_4^{2-}\right] x P_{SED} x \frac{a_{TRIS}}{a_{TOT}} x \frac{1}{t} x 1.06 x 1000$$

(2.7)

Where: SRR is the sulphate reduction rate (nmol cm^{-3} d^{-1})

$[SO_4^{2-}]$ is the porewater sulphate concentration (mmol l^{-1})

P_{SED} is the porosity of the sediment (ml of pore water/cm^{-3} of sediment) obtained by standard methods (see section 2.8)

a_{TRIS} is the total radioactivity of TRIS (dpm)

a_{TOT} is the total radioactivity of the injected substrate (dpm)

t is the incubation time in days

1.06 is the isotopic correction factor (Jørgensen and Fenchel, 1974)

1000 is the correction from mmol l^{-1} to nmol cm^{-3}

In order to produce the Cr^{2+} solution needed for this experiment a method modified from Fossing and Jørgensen (1989) was used (Fig. 2.4). A two litre modified duran aspirator bottle was filled with 1 kg of mossy zinc (Sigma-Aldrich) which was then washed with 1 M HCl. One litre of Cr^{3+} solution (1 M $CrCl_3$ in 0.5 M HCl) was then added to the bottle, which was then sealed with a rubber septum and the headspace flushed with OFN. The septum was pierced with a 1.1 mm syringe needle fitted with a 3-way stopcock and a length of 3mm diameter PVC tubing to allow both the OFN and the H_2 gas produced by the reaction to escape. The other end of the tubing was submerged in silicon oil to prevent atmospheric O_2 from entering the reaction vessel, and between the two was fitted a 165 ml serum bottle filled with OFN to prevent any oil from refluxing back into the reaction vessel. The apparatus was then left for between 12-24 hours to allow the reaction to take place and for the dark green Cr^{3+} solution to be reduced to bright blue Cr^{2+} solution (Fig. 2.5). Once the solution had turned blue, 165 ml serum bottles filled with OFN and sealed with "mushroom" bungs were attached to a port at the base of the aspirator bottle via a length of 7 mm diameter neoprene tubing (Masterflex, Oldham), a 1 ml Luer Lok syringe (BD, Massachusetts), two 3-way

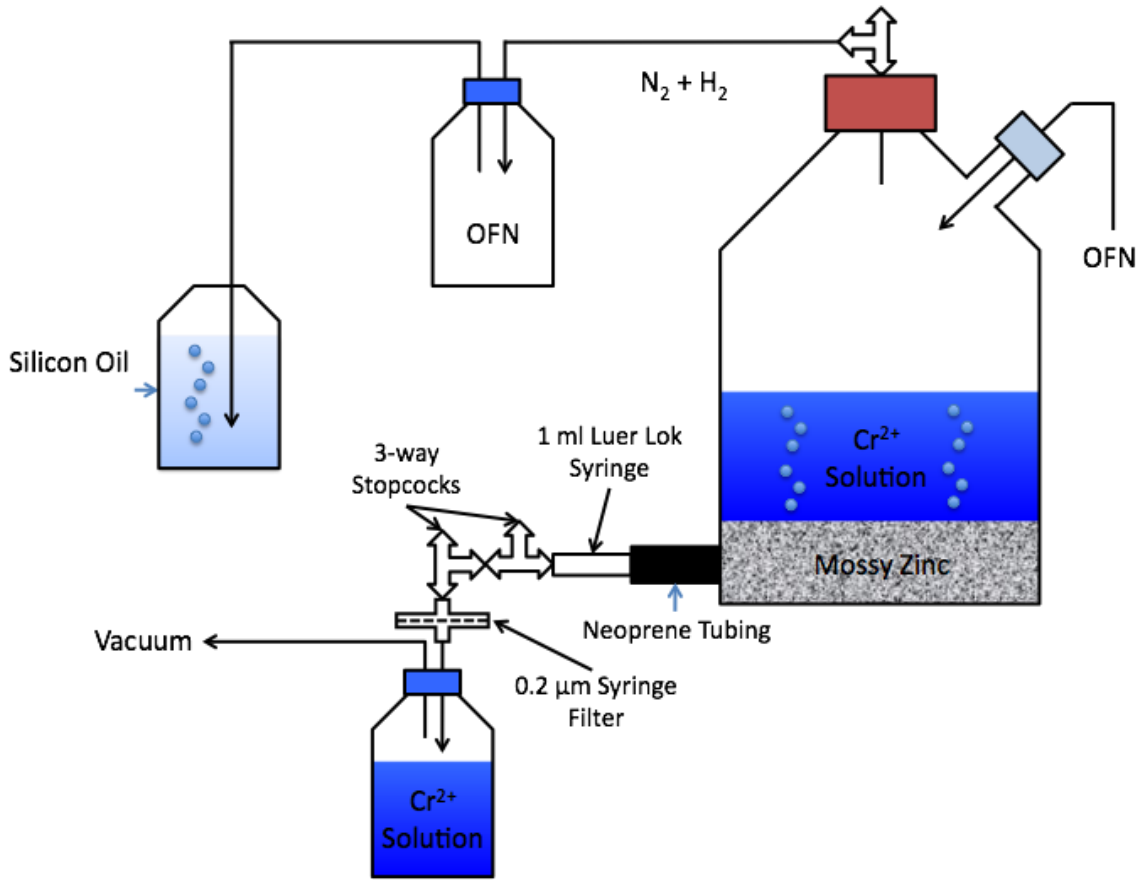


Fig. 2.4 – Schematic diagram of the procedure used to produce acidified Cr^{2+} solution for use in cold chromium reduction of sedimentary sulphur based on Fossing and Jorgensen (1989).

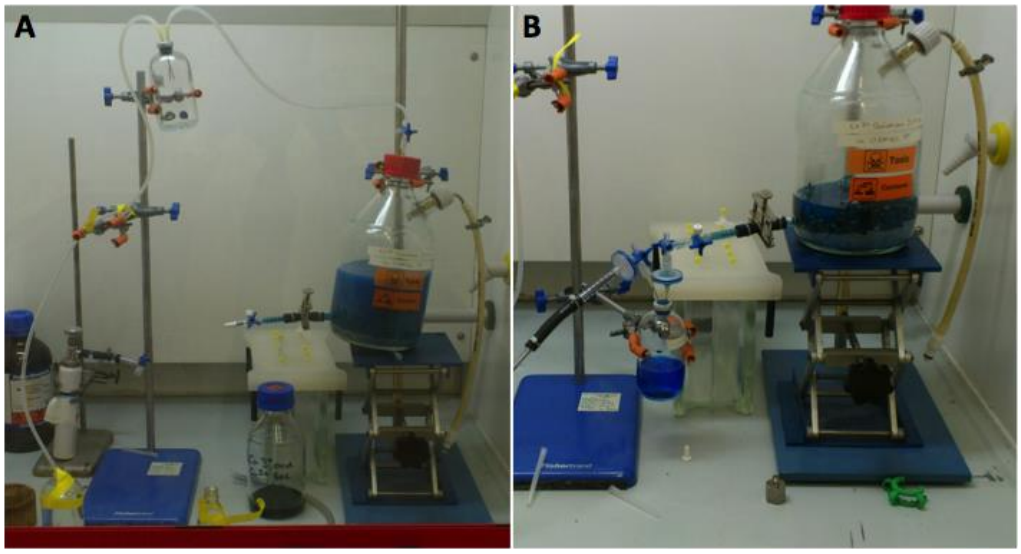


Fig. 2.5 – A) Photograph of the set-up used for the large-scale production of Cr^{2+} solution showing the reaction vessel and gas release system made up of N_2 and silicon oil filled bottles (centre-top and bottom left respectively) – photograph taken approximately 5 hrs after initiation of reaction. B) Photograph taken after the reaction was complete (c. 20 hrs, note the vivid blue colour of the solution) showing the dispensing of the Cr^{2+} into evacuated bottles for long-term storage.

stopcocks and a 1.1 mm diameter syringe needle. The 3-way stopcock at the top of the reaction vessel was closed and 100 ml of the Cr^{2+} solution was then drawn into each of the vials using a handheld vacuum pump attached to a syringe needle. After it was initially found that bottles filled in this fashion often contained a small quantity of a particulate Zn compound (either elemental Zn powder or $ZnCl_2$) that was produced within the reaction vessel, a 0.2 μm syringe filter (Sartorius, Goettingen) was fitted between the 3-way stopcocks and the syringe needle. The OFN filled serum bottles were also evacuated using the hand pump before attaching them to the syringe needle; this increased the flow rate of the Cr^{2+} solution into the bottles and prevented the filters from becoming clogged too quickly. Once all of the Cr^{2+} solution was removed from the reaction vessel the mossy zinc was washed with MQ H_2O to remove the particulate Zn compound that remained in the vessel. The bottle was then flushed with compressed air to dry out the zinc and subsequently filled with OFN to prevent the zinc from reacting with moisture in the surrounding atmosphere. When more Cr^{2+} solution was required the zinc was again washed with 1 M HCl and the procedure was repeated. This method allowed for the production of large volumes of Cr^{2+} solution that could be stored for months in serum bottles in a reduced state. It also has an advantage over the alternative method of Cr^{3+} to Cr^{2+} reduction using a Jones reductor column (Mendham *et al.* 2000), in that it does not require the use of highly toxic mercuric nitrate or mercuric chloride solution to produce a zinc-mercury amalgam (Canfield *et al.* 1986; Fossing and Jørgensen, 1989).

To determine the total percentage of sediment sulphide retrieved by this process, sediment samples taken from sub-cores injected with 100 mM Na_2S solution (final concentrations 2 mM, 5 mM and 10 mM) were distilled along with an un-amended sediment sample and a sample containing 5 mM of Na_2S in 20% w/v zinc acetate solution. These "cold" sub-cores were injected with Na_2S and then incubated at room temperature for 2 days to allow the injected sulphide to equilibrate with the sulphide already present in the sediment. After distillation the total sulphide concentration was determined using a method modified from Cline (1969). 1 ml of each distilled sample

(in 10% w/v zinc acetate) was pipetted into test tubes to which was added 1 ml of mixed diamine reagent – 2 g of *N, N*-dimethyl-*p*-phenylenediamine sulphate (DMPD) and 3 g of ferric chloride hexahydrate ($FeCl_3.6H_2O$) dissolved in 50 ml of 50% v/v HCl. The volume in each tube was then made up to 10 ml with MQ H_2O, the tubes were stoppered with rubber bungs, shaken and left for 1 hour for the colour to develop. After an hour, 0.4 ml of each sample was pipetted into tubes containing 10 ml of MQ H_2O, which were stoppered with rubber bungs, shaken and left for 20 minutes. 1 ml from each tube was then pipetted into disposable plastic semi-micro cuvettes (Fisherbrand, Thermo Fisher Scientific) and analysed on a Cary 50 Probe UV-Visible Spectrophotometer (Varian, California) at a wavelength of 670 nm. The absorbance results obtained were then compared with those from previously made standards containing a known concentration of total sulphide (0 mM, 0.05 mM, 0.1 mM, 0.5 mM, 1 mM, 5 mM and 10 mM) prepared in the same manner as the samples, using Cary WinUV software, in order to determine the sulphide concentration. The results of this test indicated that the recovery of sulphide from the sediment samples had an average error of ± 9.3%.

2.7.2 Active Methanogenesis Rate Measurements

Rates of *in situ* methanogenesis were determined using ^{14}C radiolabeled substrates injected into sub-cores sampled in the same fashion as those used for the SRR measurements described above. The cores were injected with a variety of ^{14}C labelled compounds utilised by methanogenic *Archaea* including: acetate [1,2-^{14}C], bicarbonate, DMA (bi-labelled ^{14}C) and choline [methyl-^{14}C] using a 10 μl glass syringe (Hamilton) – all isotopes were from American Radiolabeled Chemicals Inc. The cores were injected with 2 μl of substrate every 2 cm (acetate = 0.0251 MBq, bicarbonate = 0.00107 MBq, DMA = 0.0076 MBq and choline = 0.0087 MBq) and were then incubated at *in situ* temperature for varying time periods depending on the substrate used (6 hours for acetate and DMA, 18 hours for bicarbonate and choline). After incubation the sub-cores were sectioned every 2 cm and the radiolabeled sediment (vol = 6.28 cm^3/sample) was placed in vials containing a magnetic stirrer bar and 7 ml of 2 M NaOH and vortex mixed

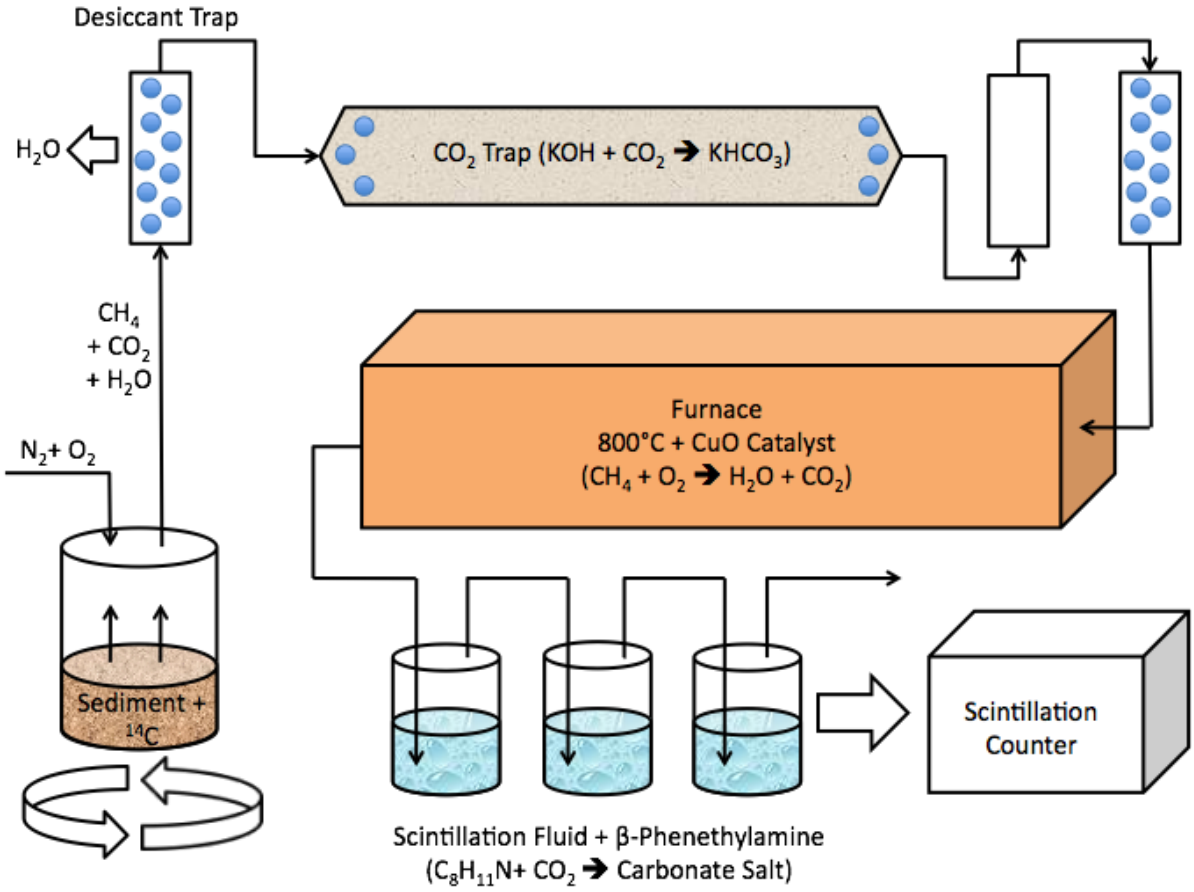


Fig. 2.6 – Schematic diagram of the procedure used for the extraction of ^{14}C-radiolabelled methane from sediment samples with reactions occurring at each stage shown in brackets (desiccant traps are filled with blue silica gel beads).

to prevent further activity. The vials were sealed with rubber bungs and then stored upside down ready for analysis. A control sample was also created in which a 2 cm slice of sediment from a separate sub-core was mixed with NaOH before being injected with isotope in order to act as a sediment blank.

Radiolabeled methane was measured following a method modified from Parkes *et al.* (2010) utilising a methane capture rig (Fig. 2.6) The vials were first vortex mixed and then placed on magnetic stirrers to ensure continuous movement of the sediment and gaseous equilibrium between sediment and headspace. An N_2/O_2 gas mix was then flushed through the headspace at ≈35 ml min^{-1} for 20 minutes to flush out the headspace gases (a mixture of N_2, O_2, CH_4, CO_2 and H_2O). The gas was then passed through multiple traps containing dehydrated silica-gel beads to remove the H_2O component and then through a CO_2 trap (Supelco, Sigma Aldrich) containing KOH that

converted the CO_2 to $KHCO_3$. The remaining gas then passed through a furnace (Carbolite, Derbyshire) at 800 °C, containing a CuO catalyst, that oxidised the $^{14}CH_4$ to $^{14}CO_2$ (Cragg *et al.* 1990). The CO_2 was then bubbled through three sequential 20 ml plastic scintillation vials (LabLogic, Sheffield) containing a mixture of scintillation fluid (ScintiSafe 3, Thermo Fisher Scientific) and β-phenethylamine (140 ml of phenethylamine in 1860 ml of ScintiSafe 3) in order to fix the ^{14}C via the formation of a carbonate salt. The vials were then detached from the rig and placed in a Packard Tri-Carb 2900TR scintillation counter. Each scintillation vial (3 per depth interval) was counted for 3 x 20 minutes with a counting window of 4-156 keV and the average DPM results from the three vials were added together to produce a final count. Methanogenesis rates were then calculated using Eq. 2.8:

$$MR = \left[\frac{DPM_{out} x ID}{IJ_{vol} x DPM_{in}}\right] x \frac{t}{24} x C_{pool} \quad (2.8)$$

Where: MR is methanogenesis rate (in pmol cm^{-3} d^{-1})

DPM_{out} is the total DPM from the three scintillation vials (minus blanks)

ID is the isotope discrimination factor (1.06 for substrates with a methyl group, 1.12 for HCO_3^-) (Parkes *et al.* 2010).

IJ_{vol} is the volume of radioisotope injected (in μl)

DPM_{in} is the DPM injected μl^{-1} (determined from pre-made standards)

t is the incubation time (in hours)

C_{pool} is the *in situ* concentration of the cold pool (in pmol cm^{-3} of sediment, modified from the pore water concentration using sediment porosity when necessary).

DPM_{in} values for acetate were halved to take into account the ^{14}C-labelled carboxyl carbon that cannot be used by methanogens. On the other hand for DMA, DPM_{out} values were doubled to account for the 2 molecules of CH_4 produced per DMA and in the case of choline the DPM_{out} was tripled to account for the three CH_4/choline produced in this reaction. The cold pool sizes used for calculating the rate measurements were derived

from the *in situ* concentrations as determined via ion chromatography (acetate, DMA and choline) or gas chromatography (free CO_2). In the case of DMA and choline, where the cold pool was near or below the detection limits of the ion chromatograph, the concentration of the hot pool was also added to the cold pool (as determined from the material data sheets supplied with the isotope). As well as measuring the production rates based on substrate pool size, the turnover of substrate to methane was also calculated in order to provide the relative potential methanogenic capability of the sediment independent of the cold pool size.

2.8 Sediment Porosity

Sediment porosity is the percentage volume of sediment composed of pore space, which is filled by pore water in saturated sediment. A measure of porosity was required for the radiotracer analyses as well as as a potential proxy metric for re-suspension, and was derived from the difference in weight between wet and dry sediment of a known volume (Breitzke 2006). During sectioning of the cores for geochemical analysis 4 cm^3 of sediment was collected from each depth interval using cut-off 5 ml syringes. The sediment was then injected into pre-weighed glass vials and then weighed again, with the new weight subtracted from the old to determine the weight of wet sediment. The vials were then placed in an oven at 90 °C for several days to allow the sediment pore water to evaporate without dehydrating any minerals within the sediment. The vials were then weighed again to determine the weight of the dry sediment and the dry weight subtracted from the wet weight to determine the weight of pore water in the original sample. Since the density of water is approximately 1 g cm^{-3} this meant that the volume of pore water in the original sample could also be determined. This value was then divided by the total volume of sediment sampled (4 cm^3) to determine the percentage volume of the original sample that was filled by pore water (i.e. the porosity). For example, if the 4cm^3 plug weighed 4 g when wet, and 1 g after drying it had lost 3 g of water. Since the weight:volume ratio for water is approx 1:1 this means it had lost 3 cm^3 of water, which is 75% of it's original volume, yielding a porosity value of 75%.

Chapter 3. – The Severn Estuary Transect: Cores ST1, ST2 and ST3

3.1 Site Locations and Lithology

All three cores described in this chapter were collected using the multi-corer from the northern half of Severn Estuary, along a transect running NW-SE across the width of the Estuary (shown in Fig. 3.1). The cores were collected during a neap tidal period in August 2011 when the water temperature was 18 °C and the salinity was 28.7‰.

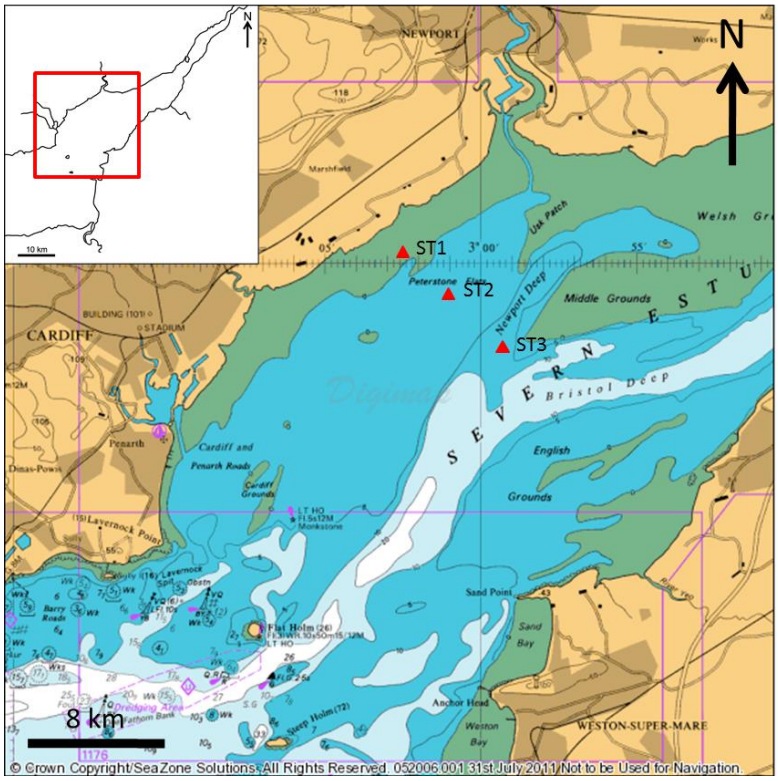


Fig. 3.1 - Bathymetric chart showing the positions of the sampling sites for cores ST1, ST2 and ST3 in the upper estuary (red triangles), relative to submarine topography. Zones shaded white represent the areas with greatest water depth while those in blue represent progressively shallower water. Green represents the extent of intertidal areas at lowest astronomical tide. (bathymetric chart ©Crown Copyright SeaZone Solutions)

Core ST1 (Fig. 3.2A) was collected from an intertidal/shallow water area approximately 0.75 nautical miles (1.28 km) from the northern shore of the estuary (51° 30’ 20.1” N, 03° 02’ 12.9” W), from a water depth of 8.3 m. This core was 18 cm long and made up of fine sand/mud that was brown in colour at the surface, changing to grey and then black at around 9 cm depth. At 15 cm depth there was a small (c. 1 cm diameter) circular patch of sandy material with a brown colouration, suggesting an input of more oxygenated water at this point. This patch could possibly represent the remains of an old burrow as there appeared to be some evidence of a hole in the centre (though it had no obvious connection to the surface)

however, this cannot be confirmed as no evidence of any burrowing organisms was found in the core and the Severn Estuary is known to be impoverished with regard to burrowing benthic macrofauna (Warwick and Somerfield, 2010).

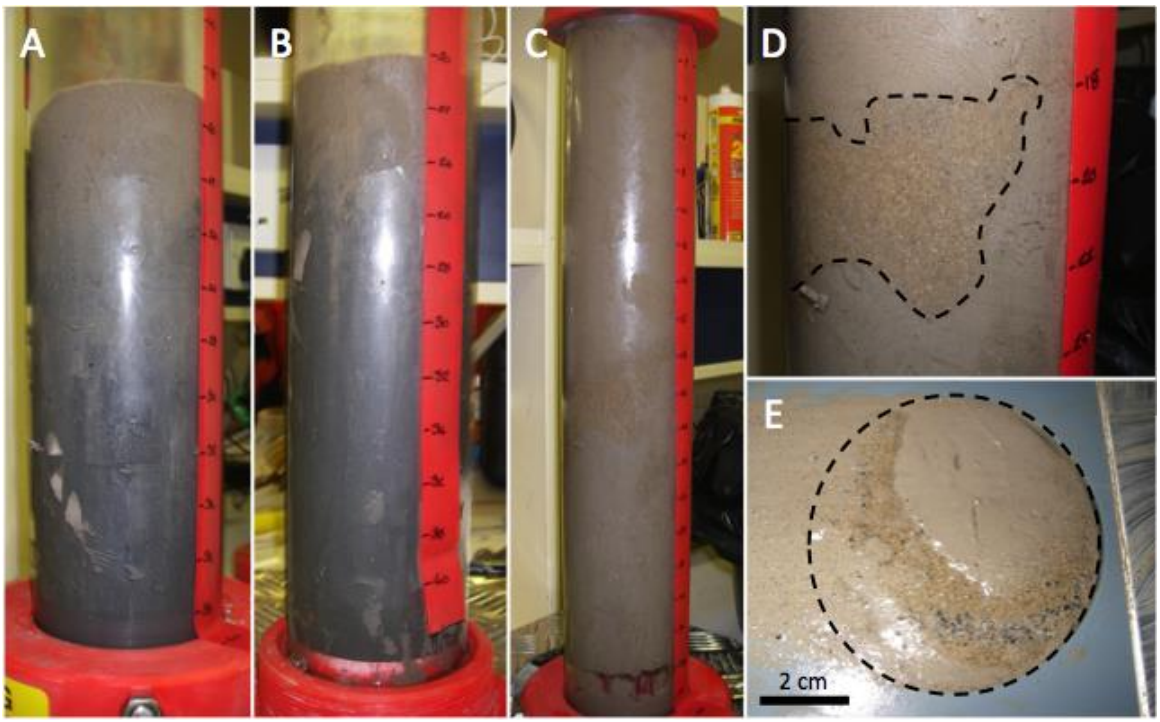


Fig. 3.2 – Photographs of cores A) ST1, B) ST2 and C) ST3 showing changes in sediment colour with depth (tape marked at 2 cm intervals for scale). D) Magnified view of one of the sand bodies observed in core ST3 showing its irregular shape (dashed line shows boundary of sand body). E) View of a section through a sand body taken during sub-sampling, again showing its heterogeneous composition and irregular shape (dashed line shows the circumference of the core tube).

Core ST2 (Fig. 3.2B) was collected from a site on the Peterstone Flats, around 1.5 nautical miles (2.8 km) from the northern shore (51° 29’ 35.6” N, 03° 01’ 06.9” W), from a water depth of 9.7 m. This core was 20 cm in length and like core ST1 was also made up of fine sand/mud, though in this case the colour transition from brown to grey/black occurred few millimetres beneath the surface.

Core ST3 (Fig. 3.2C) was collected from a site at the mouth of the Newport Deep, a blind-ended deep water channel around 3.3 nautical miles (6 km) from the northern shore (51° 28’ 23.4” N, 02° 59’ 14.7” W), from a water depth of 15 m. This core was 34 cm long however, when this core was recovered sediment was oozing from the top of the core tube and as such the top of this core may not represent the true sediment-water interface. The core was mainly made up of fine sand/muddy sediment similar to cores ST1 and ST2, however, the sediment was much less

consolidated and there was not a noticeable increase in cohesion with depth. Also, this core contained discontinuous bodies of coarse sand at intervals down the core (Figs. 3.2D and 3.2E). These sand bodies were irregular in shape, unsorted and did not form distinct horizons suggesting that they did not represent the conventional planar or lens-shaped sand layers that would be expected to form from the gradual settling out of a homogeneous mud-sand mixed sediment or due to scouring/channelised current flow (Boggs Jr., 2006).

3.2 The Severn Estuary Transect - Results

3.2.1 Biogeochemistry of Core ST1

In core ST1, the concentration of organic acids such as acetate, lactate and formate (shown in Fig. 3.3) decreased overall with with depth, from local highs of 27.3 μM (acetate), 2.4 μM (lactate) and 23.4 μM (formate) at the surface to 4.1 μM, 1.6 μM and 7.2 μM respectively at 17 cm depth. This decrease with depth was not uniform however, as at 5 cm depth there was a peak in acetate and formate concentrations to 22.5 μM and 97.3 μM, while lactate showed smaller peaks in concentration at 9 cm and 17 cm (to 1.9 μM and 1.6 μM respectively). This pattern would suggest overall heterotrophic consumption of these compounds with depth with possible local increases due to changes in degradative processes (e.g. at 5 cm for acetate and formate).

Chloride and sodium concentrations at the surface were lower than the seawater-norm (550 mM) as would be expected in an estuary. Despite being conservative ions, concentrations showed a small decrease with depth (16% and 29% respectively), dropping from 449 mM and 482 mM respectively at the surface, down to 379 mM and 340 mM at depth. This decrease was not linear, with the drop in concentrations over the first 3 cm being steeper than that below. This could reflect the disturbance of the upper few centimetres of the core by waters with a higher salinity – possibly related to erosion/re-suspension by more marine-derived waters flowing up the estuary on a rising tide. Alternatively it could be caused by the forcing of more saline waters into the upper layers of the sediment via wave pumping or fluid shear (Santos *et al.* 2012). The overall decrease in salinity down core is more unusual but will be discussed further later (section 3.3.4).

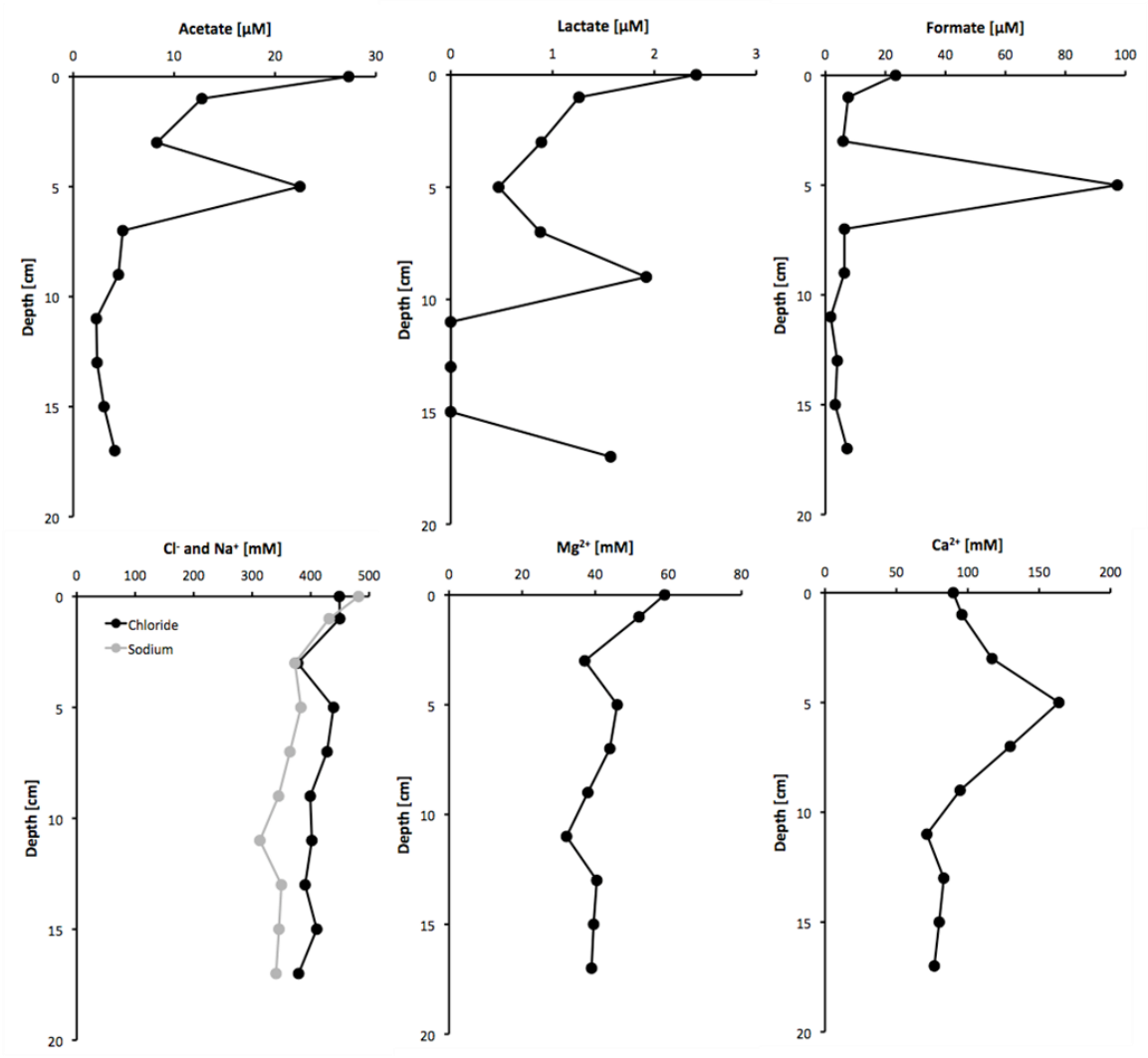


Fig. 3.3 – Biogeochemical profiles obtained from core ST1 showing changes in the dissolved concentrations of: organic acids, chloride, sodium, magnesium and calcium with depth.

Magnesium decreased down-core from 58.9 mM at the surface down to 38.9 mM at the base. However, as with chloride and sodium the decrease was greatest over the top 3 cm possibly also indicating an influx of more saline waters. Calcium on the other hand, whilst decreasing overall down-core (from 89.8 mM at the surface to 76.5 mM at the base), exhibited a broad peak in concentration around 3-7 cm depth where values rose to 164 mM. This increase corresponds with the increase in acetate and formate concentrations at 5 cm with the two possibly being related, this again will be discussed further later (section 3.3.4).

Nitrate was present only in low concentrations (10-30 µM) near the top of the core and decreased rapidly with depth, being removed completely by 9 cm (shown in Fig.

3.4), indicating that denitrification and/or dissimilatory nitrate reduction to ammonium (DNRA) - was occurring in these sediments. Ammonium on the other hand increased consistently from zero at the surface up to 1.7 mM at the core's base producing an almost sigmoidal profile indicating that ammonification/deamination of organic matter was occurring at depth. The concave-upward nature of the profile in the top 7 cm of the core also mirrors the nitrate profile suggesting that this near-surface decrease in ammonia is likely linked to nitrification in the dysaerobic zone near the sediment surface.

Both the sulphate and SO_4^{2-}:Cl^- ratio profiles for this core exhibit an initial slight increase with depth (possibly indicating some sulphide oxidation) followed from 3 cm by a decrease down-core, with sulphate values dropping from 23.4 mM down to 13.9 mM at the base of the core, indicative of dissimilatory sulphate reduction. This decrease in sulphate values begins to occur at around 5 cm, which coincides with the increase in organic acids described above and is also the depth at which methane starts to increase (see below). It also corresponds with the depth that nitrate becomes depleted which suggests that it is around this depth that the sediment becomes anoxic (though not necessarily reducing) as this would stimulate the growth of SRB, fermenters and methanogens. The sulphate gradient, however, is not constant but instead varies with depth, with the drop in values between 5-11 cm being steeper than between 11-17 cm. This may indicate that sulphate reduction at the base of the core is being partially inhibited - the cause of this suppression is unknown but may be related to the decrease in salinities with depth (discussed in section 3.3.4). No free phosphate was detected in this core possibly indicating a limited supply of iron phosphate minerals or low degree of iron sulphide (FeS) formation.

The methane concentrations from core ST1 increased with depth from 1.09 μmol l^{-1} of wet sediment (lws^{-1}) at 1cm depth up to 6.4 μmol lws^{-1} at the base showing that methanogenesis was active within these sediments. In the top 5 cm of the core the increase in methane concentrations was low, which coupled with the decrease in ammonium and the apparent lack of sulphate reduction in this layer may indicate that the sediment was partially oxidised. Between 5-11 cm methane concentrations

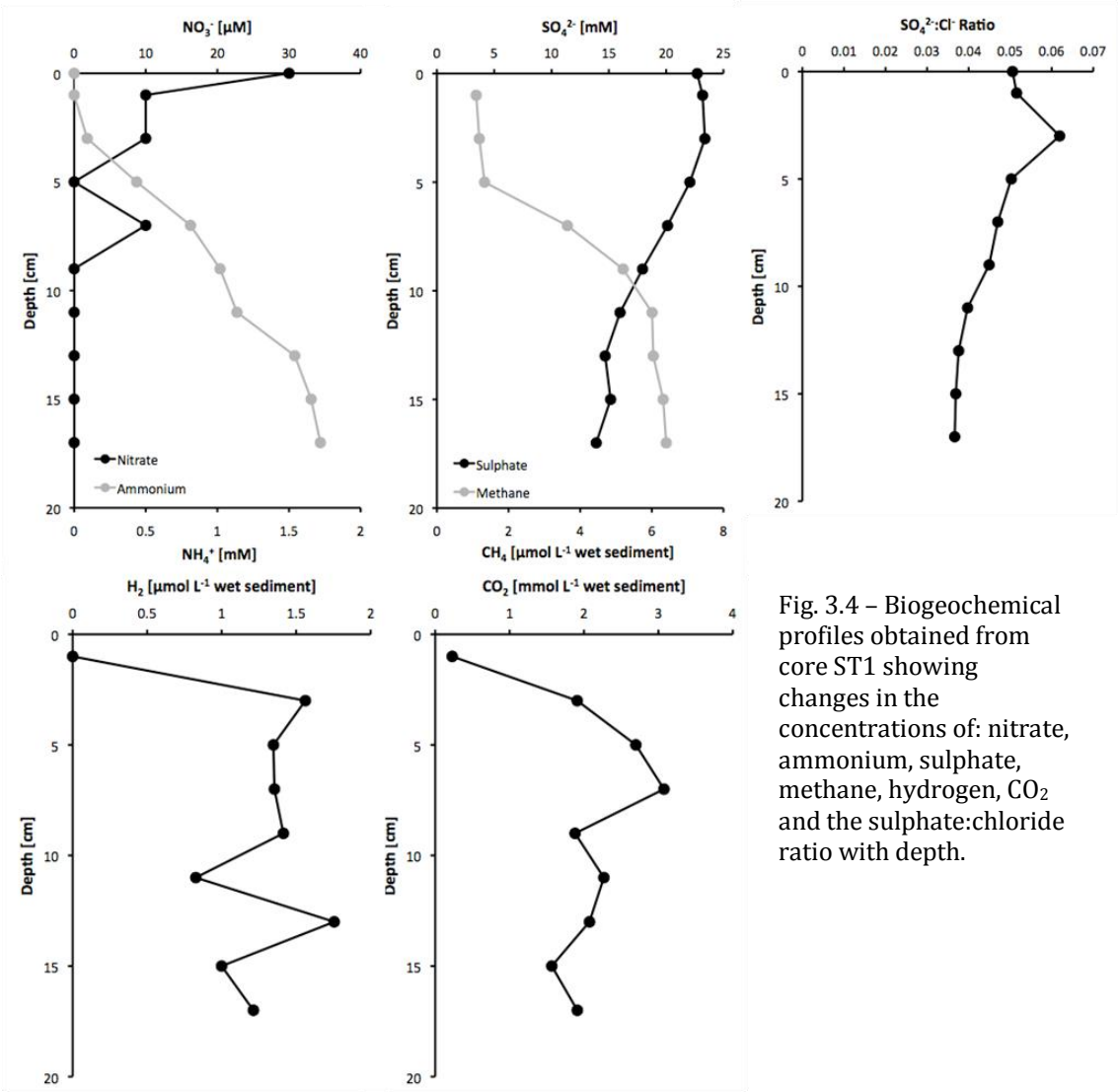


Fig. 3.4 – Biogeochemical profiles obtained from core ST1 showing changes in the concentrations of: nitrate, ammonium, sulphate, methane, hydrogen, CO_2 and the sulphate:chloride ratio with depth.

rose rapidly, possibly linked to the peaks in some of the organic acid concentrations and increases in hydrogen concentrations at this depth. However, below 11 cm the profile became much more vertical indicating a potential decrease in methanogenesis at this depth. This change in profile shape occurs at the same depth as the change in sulphate profile described above and may again indicate a degree of inhibition, possibly linked to changes in salinity (see section 3.3.4).

As mentioned above hydrogen concentrations increased from below detection limits at 1 cm to 1.5 µmol lws^{-1} at 3 cm and remained relatively constant down to the base of the core (with the exception of a local minimum at 11 cm depth). This increase in H_2 roughly corresponds to the increase in organic acids described above and as such may be related to changes in degradative processes. The absence of H_2 above 3 cm could be explained by the fact that this top layer of sediment had been recently re-

suspended or subject to hydraulic pumping (as indicated by the chloride and sodium values) and was therefore still relatively oxic, which would have inhibited of anaerobic fermentation.

Carbon dioxide concentrations showed an initial increase with depth, from 0.2 mmol lws^{-1} at 1 cm to 3 mmol lws^{-1} at 7 cm, likely linked to the metabolism of heterotrophic and fermentative prokaryotic groups. However, there appears to be no direct link between the NH_4^+ and CO_2 profiles, suggesting that processes other than organic matter degradation are also controlling the CO_2 concentrations. Below 7 cm CO_2 levels began to decrease again, down to 1.9 mmol lws^{-1} at the base of the core, possibly due to H_2 consumption or removal via carbonate precipitation (as Ca^{2+} values also decrease at depth). Since this decrease in CO_2 also corresponds with the increase in methane concentrations it may indicate that lithotrophic H_2/CO_2 utilising methanogens were active at this depth.

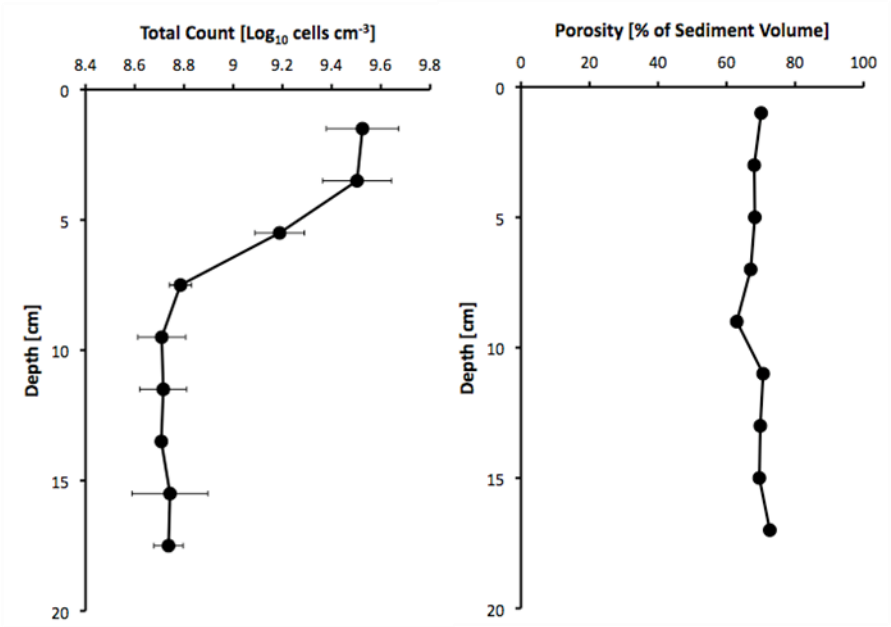


Fig. 3.5 – Total cell count (AODC) and sediment porosity profiles obtained from core ST1 showing variations with depth. Error bars represent the 95% confidence limit derived from repeated cell counts.

The total cell counts for this core (shown in Fig. 3.5) were fairly consistent (around $1x10^{9.5}$ cells cm^{-3}) over the top 3.5 cm of the core. However, below this depth cell numbers dropped sharply, down to $\approx 1x10^{8.7}$ at 7.5 cm, a level at which they stayed at until the base of the core. This rapid decrease in numbers at around 5 cm matches the geochemical results obtained from this core which potentially indicates higher numbers of dysaerobic nitrate and ammonium-utilising organisms at the top of the core and lower numbers of anaerobic SRB and methanogenic communities nearer

the base. This dramatic change in numbers cell numbers has been observed elsewhere in Severn Estuary sediments by Wellsbury *et al.* (1996) who suggested a different explanation that will be discussed later in section 3.5.7. The porosity of the sediment was relatively consistent down-core, ranging from between 68-72% of the total volume of the sediment.

4.2.2 Biogeochemistry of Core ST2

In core ST2 the concentrations of acetate, lactate and formate remained low throughout the top 11 cm of the core (shown in Fig. 3.6). Below this depth, concentrations increased dramatically, reaching maximum values of 8.48 μM (acetate), 4.82 μM (lactate) and 91.8 μM (formate) at the base of the core. This sharp increase in concentrations down-core (particularly in the case of formate) suggests that a change in degradative processes is occurring at depth in this core below 11 cm – either an increase in fermentation or a decrease in organic acid consumption.

Chloride and sodium concentrations showed a slight linear decrease with depth from 453 mM to 412 mM (chloride) and 407 mM to 342 mM (sodium) likely for similar reasons to core ST1 (see section 3.3.4). Unlike ST1 however, no sharp decrease in salinity occurred over the top 3 cm. This may be because core ST2 was sampled from a deeper water locality where the tidal currents/wave action may have been less vigorous which would have meant that the erosion and/or hydraulic pumping that may have induced the sharp gradients seen in ST1 might not have occurred (at least to the same degree).

Magnesium showed a linear decrease down-core, though at an overall lower rate than in core ST1, from 45.5 mM at the surface to 39.4 mM at the base and with no change in gradient. Calcium again showed an initial increase with depth, from 67.2 mM at the surface up to 118 mM at 5 cm depth, before gradually decreasing to 77.4 mM at the base of the core.

Nitrate was depleted much more quickly in this core compared to ST1, being only present at the surface (40 μM) and 1 cm depth (10 μM) before becoming undetectable (shown in Fig. 3.7), this possibly indicates a more rapid rate of denitrification/DNRA in this core compared to core ST1. Ammonium again rose

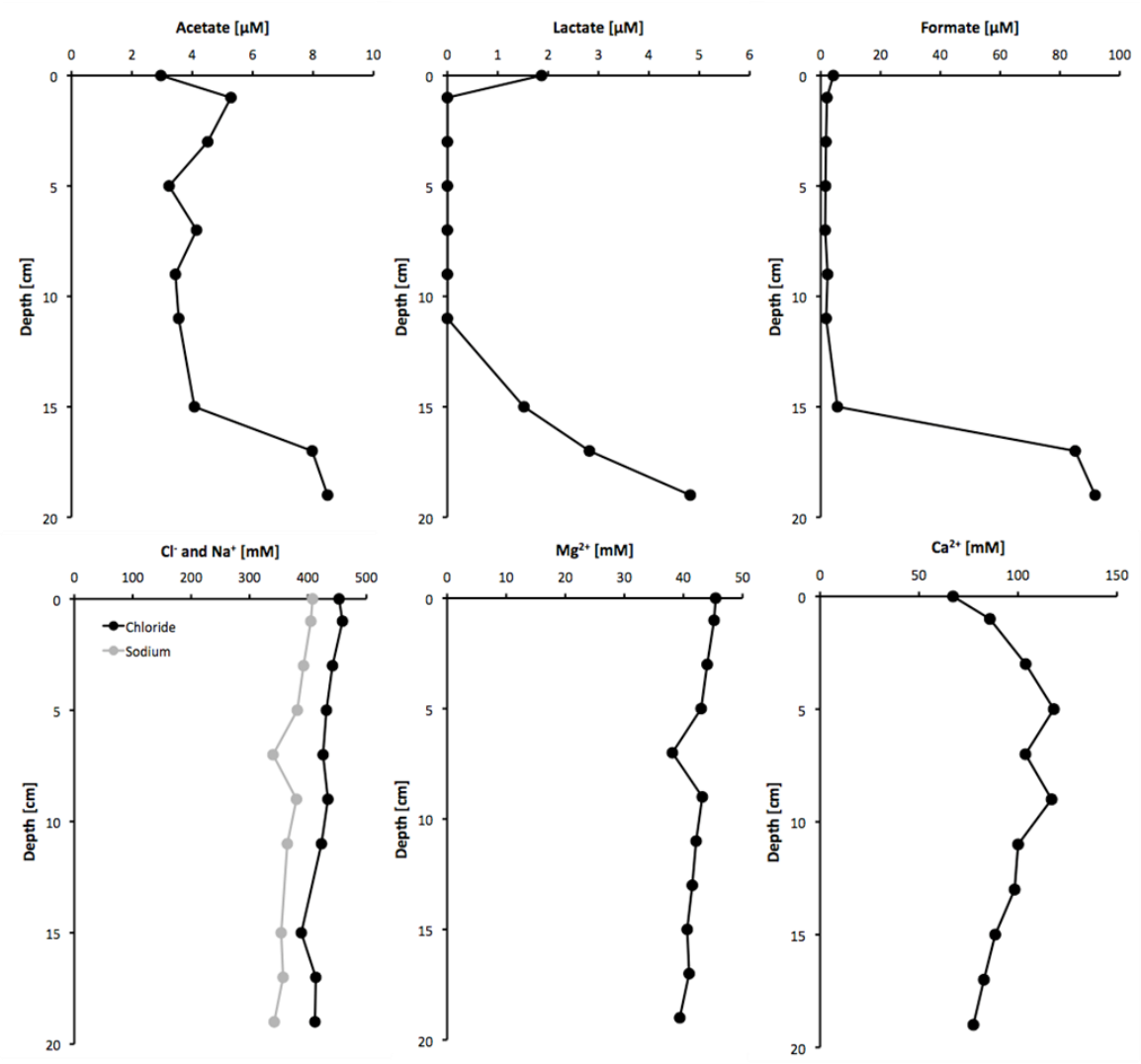


Fig. 3.6 – Biogeochemical profiles obtained from core ST2 showing the changes in pore water concentrations of: organic acids, chloride, sodium, magnesium and calcium with depth

dramatically, from zero at 1 cm up to 2.19 mM at the base of the core. This time however, the ammonium profile was much more linear, representing a more constant rate of production via ammonification of organic matter. Unlike in core ST1 the profiles from core ST2 show no broad zone where ammonium and nitrate coexist making the activity of nitrifiers hard to recognise.

Both the sulphate concentrations and SO_4^{2-}:Cl^- ratio in core ST2 produced a more linear profile than in core ST1 with values decreasing from 23.4 mM at 1 cm to 10 mM at the base of the core indicating active sulphate reduction throughout most of the core. Unlike in core ST1 no obvious subsurface sulphate peak was present in this core, possibly because the upper layers of the sediment were not as oxidised as in

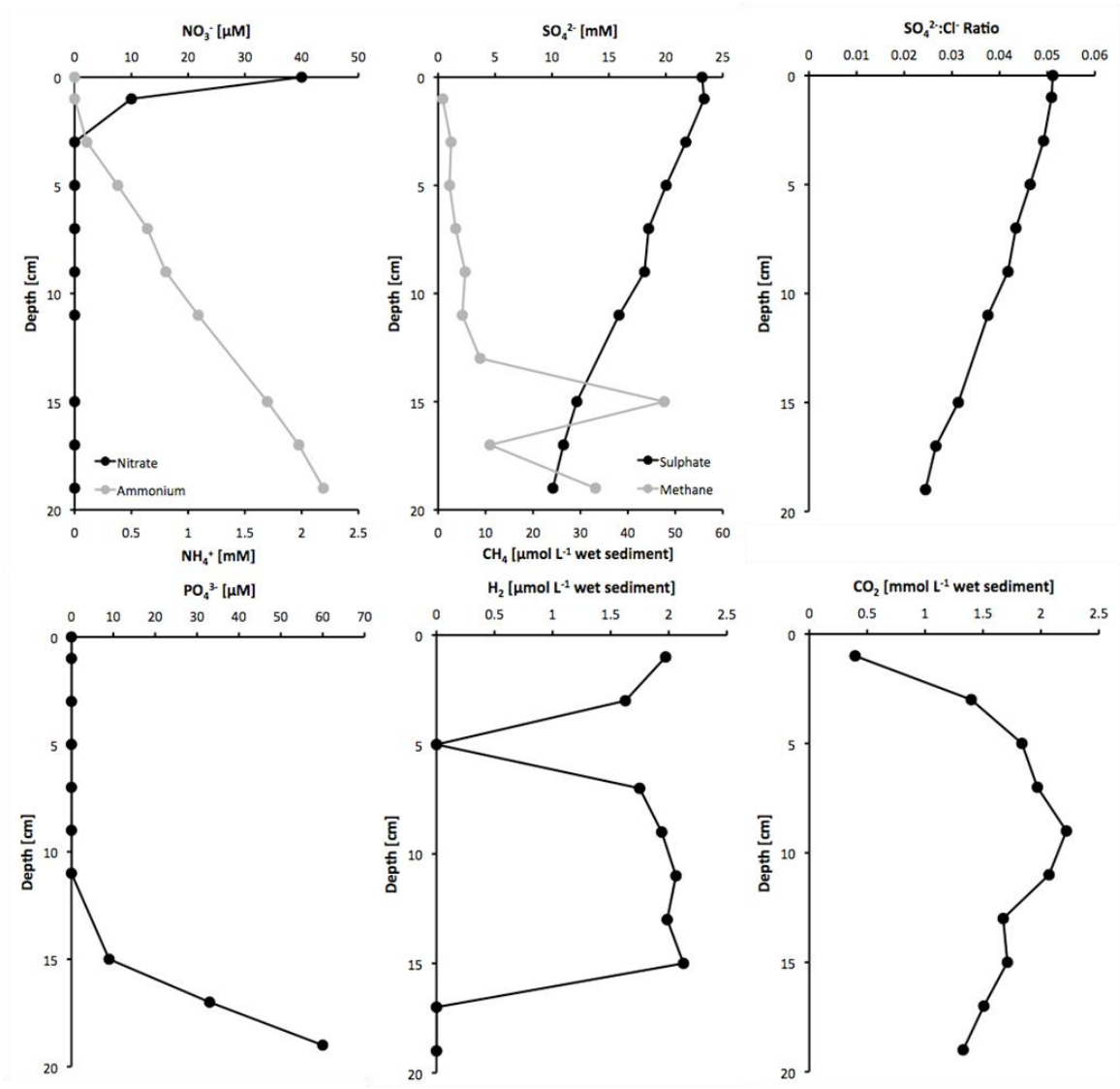


Fig. 3.7 – Biogeochemical profiles obtained from core ST2 showing the changes in the concentration of: nitrate, ammonium, sulphate, methane; phosphate, hydrogen, CO_2 and the sulphate to chloride ratio with depth.

ST1 (the colour change to black occurred within a few millimetres of the surface in core ST2 compared to 9 cm depth in ST1), which would have prevented large-scale sulphide oxidation from occurring. However, there was a slight increase in concentrations between the surface and 1 cm indicating that some oxidation may have been taking place in the upper few millimetres of the sediment. In addition phosphate was detected at 15 cm (10 µM) in core ST2, and rose to 60 µM at the base of the core. The presence of free phosphate here could be linked to FeS production due to sulphate reduction (Rozan *et al.* 2002), as when the sulphide produced by the SRB reacts with iron phosphate minerals to form FeS the phosphate molecules bound within the mineral structure can be released into the porewaters. However, if

sulphide production were the only source for phosphate in this core it would be expected to be detectable much closer to the surface as sulphate reduction was occurring throughout this core, as was the black colouration of the sediment indicative of FeS formation. As such it may be the case that this increase in phosphate is related to changes in lithology with depth (e.g. changes in iron mineral composition) or may be linked with other processes that appear to be more confined to the lower reaches of the core - e.g. increases in fermentation products (Welch *et al.* 2002).

Interestingly methane concentrations also increased dramatically in this zone and showed a much greater range than in ST1. Over the top 13 cm of the core levels rose gradually from 0.95 μmol lws^{-1} at 1cm depth to 8.8 μmol lws^{-1} at 13 cm, before rising sharply to a maximum of 33.2 μmol lws^{-1} at 15cm, below which the values began to fluctuate but remained elevated. This increase towards the base of the core again shows that methanogenesis is occurring in these sediments particularly at the base of the core where it may be associated with the increase in organic acid concentrations described above.

Hydrogen values stayed broadly between 1.5-2 μmol lws^{-1} over the top 15 cm of the core before dropping below detection limits beneath 15 cm. This decrease at depth coincides with increases in the concentrations of the organic acids, phosphate and methane suggesting a possible link between these processes (e.g. consumption of H_2 by methanogens at depth).

As in core ST1, CO_2 concentrations showed an initial increase with depth, from 0.4 mmol lws^{-1} at 1 cm to 2.2 mmol lws^{-1} at 9 cm, followed by a subsequent decrease towards the base of the core (1.3 mmol lws^{-1} at 19 cm). This initial increase is again probably attributable to the heterotrophic oxidation of organic carbon, although the profile shape does not match that of ammonium (the other compound largely controlled by organic matter degradation) suggesting that, in a similar situation to core ST1, other processes (possibly relating to changes in mineral geochemistry and pH) may also be controlling CO_2 concentrations. Below 9 cm CO_2 begins to decrease again, this could again be linked to H_2/CO_2 methanogenesis (as methane also increases at depth, although to much lower concentrations than the

CO_2 decrease), but could also be due to an increase in carbonate formation as Ca^{2+} values begin to decrease at this depth as well.

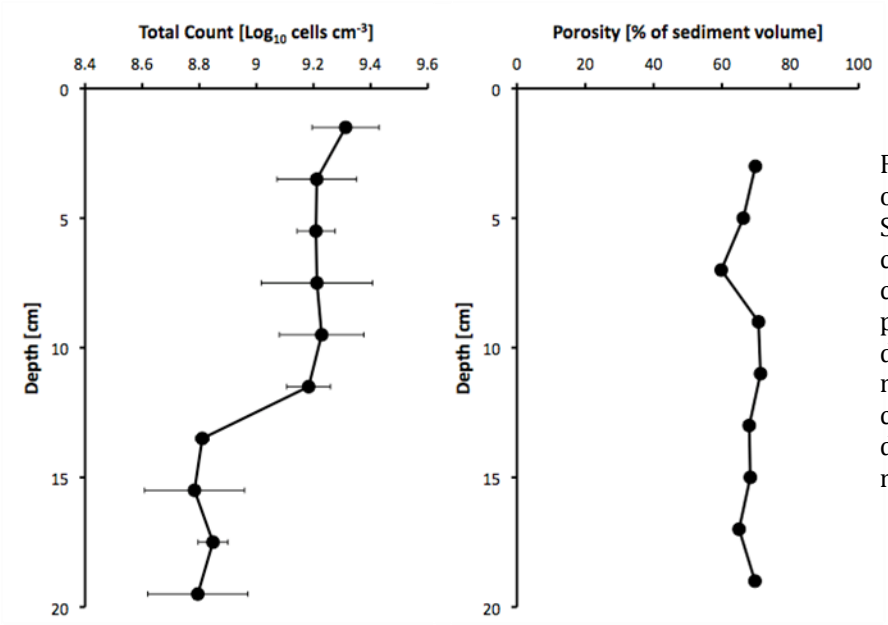


Fig. 3.8 – Profiles obtained from core ST2 showing the changes in total cell count (AODC) and porosity values with depth. Error bars represent the 95% confidence limit derived from repeated cell counts.

The total cell counts for core ST2 (Fig. 3.8), exhibited a similar pattern to core ST1 with marked decreases in cell numbers with depth, however these decreases occurred at very different depths in the two cores. In core ST2 the drop in cell numbers occurred deeper in the sediment with values in the upper 11 cm staying around $1x10^{9.2}$-$10^{9.3}$ cells cm^{-3}, below which numbers dropped sharply down to around $1x10^{8.8}$, and remained so to the base of the core. The reason for this drop in cell numbers is not as clear cut as in core ST1 as the change does not coincide with the switch from dysaerobic-anaerobic processes. However, it could relate to the increase in organic acids and methane at depth, as in core ST1, reflecting a change to a smaller community dominated by fermentative and methanogenic processes. The porosity values of core ST2 were similar to ST1 and also relatively consistent, ranging from 66-71% for most of the core.

4.2.3 Biogeochemistry of Core ST3

The geochemical profiles for core ST3 are considerably different from either core ST1 or ST2. Concentrations of acetate and lactate (Fig. 3.9) showed no apparent increase or decrease with depth but instead fluctuated throughout the length of the core around average concentrations of 6.96 µM (acetate) and 2.51 µM (lactate).

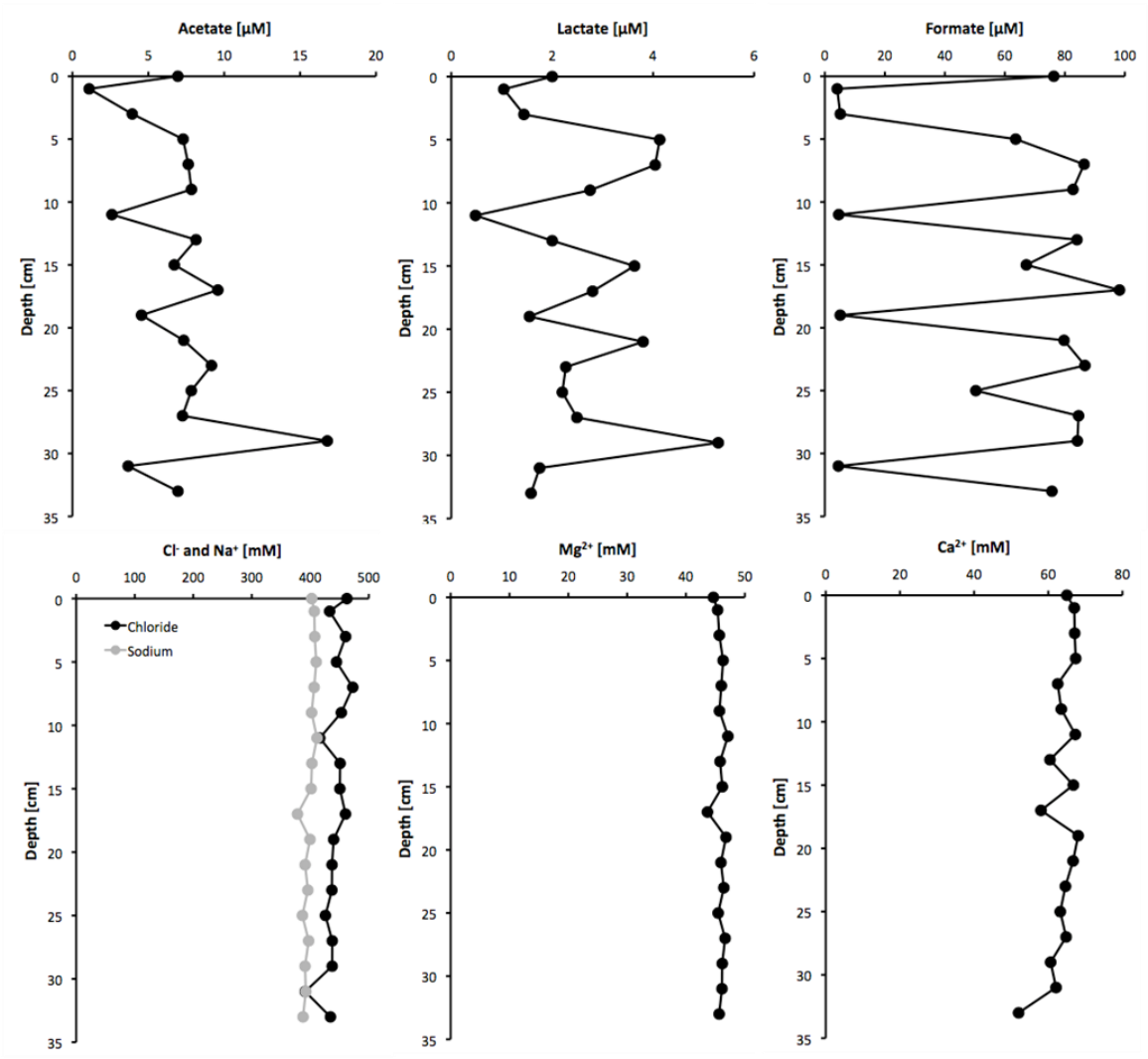


Fig. 3.9 – Biogeochemical profiles obtained from core ST3 showing changes in the pore water concentrations of: organic acids, chloride, sodium, magnesium and calcium with depth.

Formate exhibited a similar pattern, with no clear increase with depth but with a much greater degree of fluctuation (between 4-80 µM). These unusual profiles are much more erratic than those seen previously and would suggest that the anaerobic prokaryotes driving the production and consumption of these compounds in these sediments are unable to form stable, depth-zonated communities – the potential reasons for this, and the overall unusual nature of core ST3 will be discussed later.

Chloride and sodium again decreased down-core, but to a much lesser extent than in the previous two cores, with chloride decreasing from 463 mM at the surface to 435 mM at the base and sodium from 402 mM at the surface down to 388 mM. The magnesium and (particularly) calcium values for core ST3 were more consistent than the other two cores, with values for magnesium staying around 46 mM for the

duration of the core. Calcium remained around 64 mM over the top 20 cm of the core but showed a slight decrease below this (also reflected in the Ca^{2+}:Na^{+} ratio) that may indicate a change in mineral geochemistry with depth.

No nitrate was detected in this core, however, ammonium was still present, rising from zero at in the top 5 cm to 1.66 mM at core-bottom (Fig. 3.10). This increase in ammonium shows that ammonification was also occurring at this site The increase in ammonium with depth, however, is not linear and shows a greater degree of production below 15 cm.

Both sulphate and the SO_4^{2-}:Cl^{-} ratio, again showed a decrease with depth however, the decrease was much lower (23.3 mM at the surface down to 19.2 mM at the base) and more linear than in previous cores suggesting that although sulphate reduction was occurring it was at a lower rate than in the other two cores. In addition no subsurface sulphate peak was detected nor was phosphate detected (though this was not unexpected as the brown colour of the sediment indicated that little FeS was present). As with ammonium the sulphate profile also shows a slight change in gradient around 15 cm suggesting a slight increase in sulphate removal below this depth. The SO_4^{2-}:Cl^{-} ratio shows a similar small change in slope around this depth and may indicate that sulphate reduction was in fact only occurring below 15-20 cm.

Methane concentrations increased down-core but again to much lower concentrations than previously, rising from 0.59 μmol lws^{-1} at 1cm depth up to only 3.43 μmol lws^{-1} at the base of the core (33 cm depth). Again, as with ammonium and sulphate, methane showed a change in gradient at 15 cm indicating an increase in production below this depth. This change in gradients, may suggest that the sediment above 15 cm was very disturbed and as such provided an unstable habitat for anaerobic groups, a subject further considered in section 3.3. Interestingly however, this change in methane gradient also exhibits a concave-upward profile, one of the indicators of anaerobic methane oxidation (AOM), which also coincides with the change in the sulphate gradient. However, since the changes in sulphate and methane concentrations are on different orders of magnitude it would be impossible to confirm AOM activity without further analyses. Below 25 cm the

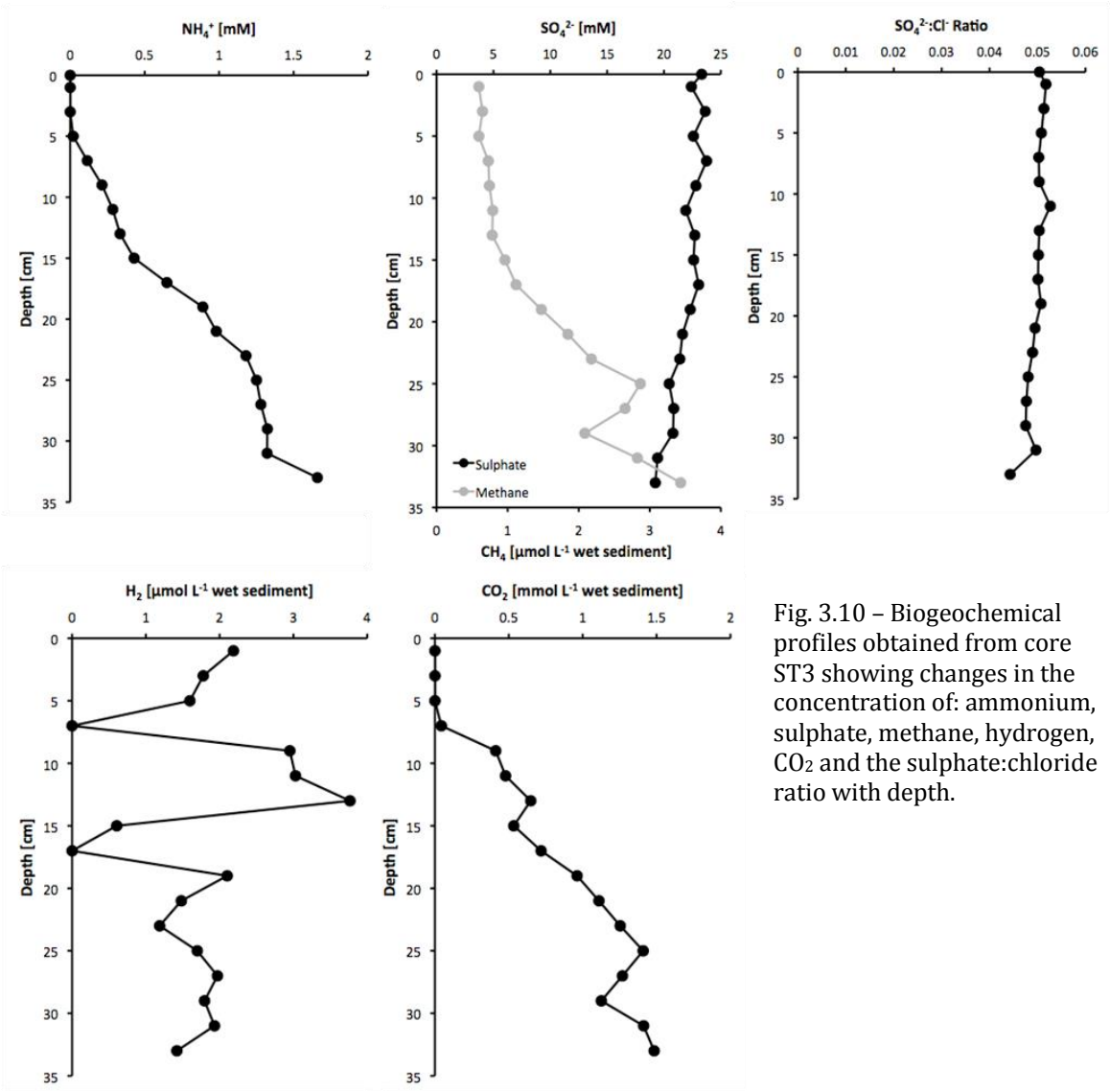


Fig. 3.10 – Biogeochemical profiles obtained from core ST3 showing changes in the concentration of: ammonium, sulphate, methane, hydrogen, CO_2 and the sulphate:chloride ratio with depth.

methane profile changes shape again, showing an initial decrease followed by a further increase in concentrations towards the bottom of the core in a similar pattern to the CO_2 profile described below.

Hydrogen concentrations, like the organic acids, are quite erratic except for the steady decrease over the top 5 cm of the core from 2.2-1.6 µmol lws^{-1}. Below this concentrations varied erratically between 0-3.8 µmol lws^{-1} down to 20 cm before the profile became more stable around 2 µmol lws^{-1} down to the base of the core. Interestingly, the H_2 concentrations below 25 cm are very similar to those in the near-surface sediments, this coupled with the overall highly variable nature of the profile may again indicate that the sediments in this core originated in an unstable environment.

The CO_2 profile for core ST3 is again different from the two previous cores and shows an overall increase with depth, from zero at 1 cm to 1.5 mmol lws^{-1} at the base. This increase is not linear however, as little to no CO_2 was detected in the top 7 cm of the core possibly indicating only low levels of heterotrophic activity, while below this values increased steadily down to 25 cm. This increase correlates with the ammonium profile indicating a clear stimulation in organic matter degradation with depth. Below 15 cm this increase becomes more pronounced, matching the sulphate and methane profiles and indicating that degradation was enhanced below this depth, possibly again due to enhanced sediment stability. Interestingly, unlike the other two cores, values continue to increase with depth to the base of the core, while Ca^{2+} values also exhibit a different pattern (with no subsurface peak). This may indicate that the process controlling the levels of these compounds in cores ST1 and ST2 (e.g. the dissolution and formation of carbonate at depth) is not occurring in this core, or at least not to the same degree.

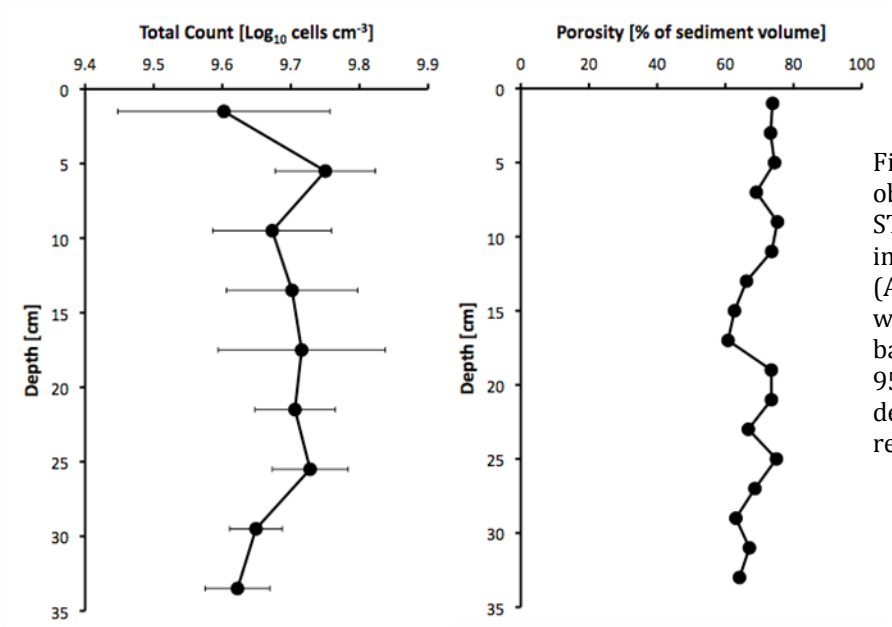


Fig. 3.11 – Profiles obtained from core ST3 showing changes in the total cell count (AODC) and porosity with depth. Error bars represent the 95% confidence limit derived from repeated cell counts.

The total cell counts for core ST3 (Fig. 3.11), were very different from the other two cores with no marked subsurface decrease in cell numbers. Unusually cell numbers showed an initial increase with depth, from $1x10^{9.6}$ cells cm^{-3} at 1.5 cm to $1x10^{9.75}$ at 3.5 cm, below which numbers stayed around $1x10^{9.7}$ down to 25 cm. Below 25 cm counts showed a slight decrease down to $1x10^{9.6}$, coinciding with the changes in methane (decrease then increase) and sulphate (increase then slight decrease)

profiles and potentially suggesting lower numbers of SRB and methanogenic communities at the base of the core.

The porosity of core ST3 varied mainly between 64-74%, but did show a slight decreasing trend with depth indicating that the sediment at the top of the core was potentially less cohesive than at the base (values in the top 11 cm were also higher than those from cores ST1 and ST2). This could indicate that the sediment had only recently settled out of suspension a possibility further considered in the discussion (section 3.3).

3.3 The Severn Estuary Transect - Discussion

3.3.1 Sedimentology

Despite originating in intertidal and subtidal settings respectively, the sedimentary structure of cores ST1 and ST2 was broadly similar with both cores being made up of similar fine sandy/muddy sediment throughout the length of the cores. The change in colour from brown to black associated with a shift from broadly oxidised to reducing conditions (and the concomitant production of black FeS) however, occurred at different depths in the cores (9 cm and a few millimetres respectively). This variation in depth may reflect differences in the amount of re-suspension and/or hydraulic pumping that the sediments in each core underwent with the upper layers of core ST1 being more recently deposited/extensively pumped than ST2, which would make sense as ST1 was originated in shallower waters where wave and current action are likely to be more intense. Core ST3 on the other hand exhibited a very different sedimentary structure, containing discontinuous bodies of coarse sand throughout its length, which suggests that the material in the core settled out of suspension very quickly. The poorly-sorted nature of this core, coupled with the fact that during collection it was evident that sediment had flowed out of the top of the core tube (and was therefore at least partially mobile) leads to the suggestion that this core may represent a section through a body of fluidised mud (referred to colloquially as a "slug"). These fluid mud pools (which are described in detail in the Chapter 1) can be several metres thick and partially anoxic, and are known to be present in the Severn Estuary, particularly in the deeper water channels such as the Newport Deep and Bristol Deep, during specific periods of the tidal cycle (Kirby, 2010, Manning *et al.* 2010).

3.3.2 Organic Acids – Acetate, Lactate and Formate

With regard to the organic acids, all three cores show different profile patterns. In core ST1 all three acids show an overall decrease in concentration down-core, likely indicative of them being used efficiently as electron donor substrates/organic carbon sources by coupled fermentative and terminal oxidising prokaryotes. However, at 5 cm depth acetate and formate show a profound increase in concentration (particularly formate). This increase in concentrations occurs around the depth where nitrate reduction and nitrification appear to cease (as indicated by the nitrate and ammonium profiles) and sulphate reduction and methanogenesis begin in earnest. This suggests that this depth represents an important geochemical horizon between the dysoxic and anoxic zones of the sediment column. This change from dysoxic to anoxic conditions (and the concomitant transition in prokaryotic guilds that goes with it) may cause an uncoupling of the normal pattern of production and consumption, with the production of organic acids temporarily outpacing demand as the number of nitrate reducing organisms decreases with depth while the SRB and methanogenic communities still remain small. With increasing depth however, the SRB and methanogen activities increase (as seen from the geochemical profiles) and the normal production/consumption coupling may resume. Comparible results were observed by Jørgensen and Parkes (2010) in sediment cores taken from a Danish fjord, and also by Parkes *et al.* (2012) from the Colne Estuary, where in both cases acetate concentrations increased at the depth in the sediment where sulphate reduction transitioned to methanogenesis.

Unlike acetate and formate, lactate shows two subsurface peaks (at 9 cm and 17 cm) however, values again begin to increase below 5 cm indicating that anoxia is required for lactate production to occur. In addition the lactate peak at 9 cm corresponds with the depth at which the colour of the sediment changed from brown to black indicating that the processes producing lactate may require a lower environmental redox state that those relating to the other organic acids. Finally it may also be the case that the supply of acetate and formate is being supplemented by other sources than just surface-derived organic matter, such as decaying meiofauna, a possibility that will be expanded on in section 3.3.4.

In core ST2 all three acids (but particularly formate) increase with depth, especially below 15cm depth. This profile shape is what would be expected as the result of fermentative bacteria breaking down low molecular weight dissolved organic carbon (LMW DOC) compounds into organic acids at depth. In addition since the increase in acetate is occurring deep in the sediment column (and in close proximity to active methanogenesis) it is also possible that homoactogenesis may be occurring – especially as H_2 also decreases markedly beneath this depth. However, since organic acid concentrations are governed not just by their rate of production, but also by their consumption by heterotrophic organisms it is also important to note how their profiles relate to others observed in this study. For example the increase in organic acids coincides with the increase in methane concentrations and decrease in AODC counts below 10 cm. This suggests a change in community structure around this depth, which could again lead to uncoupling of the production and consumption rates similar to that seen in core ST1. However, without sampling deeper into the sediment (to see of organic acid values decrease again as the populations stabilise) this cannot be confirmed. Finally the increase in organic acids with depth also coincides with the increase in phosphate levels, indicating that elevated organic acid production may be dissolving phosphate-bearing minerals (e.g. apatite) and releasing free phosphate into the pore waters (Welch *et al.* 2002; Uroz *et al.* 2009).

Unlike the other two cores, core ST3 showed fluctuating levels of organic acids throughout its length (particularly in the case of formate). The lack of any overall increase or decrease with depth would indicate that no definitive fermentative (or coupled organic acid consumption) zone is present in the sediment, and that instead the organic acids may be being produced in isolated reduced microniches within an oxidised sediment column (Jørgensen, 1977). This would fit with the suggestion that this core originated in a fluidised mud pool as the turbulent mixing of the muds along with frequent oxidation of the sediment could prevent stable communities from forming, leading to a constant uncoupling of producing and consuming processes and a lack of prokaryotic guild zonation. This would be a similar situation to that which causes temporary increases in organic acid concentrations during the initial creation of sediment slurries (Parkes *et al.* 1989). In addition, it may be the case that in the location that ST3 came from, organic acid production was stimulated

by the input of fresh organic material to the sediment at depth, allowing for the maintenance of a fluctuating but relatively constant profile. This topping up would be possible if the sediment did indeed come from an actively mobile fluid mud pool where the constant movement of the sediment would allow compounds from the water column to permeate to all parts of the mud pool creating the overall homogeneity seen in the profiles. It should also be noted that the average and depth integrated (over the top 17 cm of the cores) concentrations for lactate and formate were significantly higher in core ST3 than in the other two cores (acetate was highest in core ST1, mainly due to the peak in concentrations at 5 cm). It has been previously documented that fluidised muds, due to their continuously cycling redox states, are extremely active with regard to the mineralisation of organic matter with much of the organic matter entrained within them being completely oxidised to CO_2 (Aller and Blair, 2006; Abril *et al.* 2010). However, these results indicate that it may also be the case that these sediments are able to produce higher-than-normal quantities of small organic carbon substrates as an intermediary to complete oxidation, assuming that the compounds in question originated within the sediments and were not derived externally from mixing.

3.3.3 Nitrate and Ammonium

Nitrate is present in both the overlying water and the near surface sediment of cores ST1 and ST2 at levels higher those reported by Webster *et al.* (2010) for Severn Estuary tidal mudflat sediments at Portishead. It also appears at considerable depth (7cm) in core ST1, which is unusual as nitrate is usually depleted by denitrification/dissimilatory nitrate reduction to ammonium within a few centimetres of the surface in muddy sediments (Canfield *et al.* 1993a; Hulth *et al.* 2005). The reason for the deep penetration of nitrate into the sediments of ST1 may again relate to re-suspension as recent deposition of oxidised sediment at this site might allow nitrate to be present at greater depths than it might reach purely by diffusion from surface waters. Alternatively it may be the case that increased wave or current strength at this shallower water site might force seawater, containing nitrate, into the upper layers of the sediment (Santos *et al.* 2012). In addition such re-suspension/hydraulic pumping might result in the upper layers of the sediment remaining relatively oxic, which might limit dysaerobic processes such as

denitrification and DNRA to some degree. With regard to nitrification neither core ST1 or ST2 exhibit the "classic" subsurface nitrate peak caused by nitrification directly beneath the sediment surface (e.g. Hensen *et al.* 2006). However, core ST1 shows changes in the gradient of its ammonium profile that may be indicative of ammonium oxidation via nitrification, as the profile exhibits a concave-upward shape in the upper 7 cm of the core (Henriksen *et al.* 1981; Schulz, 2006; Carini and Joye, 2008) - this nitrification could also explain the presence of nitrate in the top 7 cm of ST1. Since nitrification is an aerobic process, if it was occurring in the top 7 cm of core ST1 this would also mean that oxygen would have to penetrate (at least to some degree) to this depth. Normally in coastal sediments O_2 does not penetrate more than a few millimetres into un-bioturbated sediment (Jørgensen and Revsbech, 1985). However, in this case it could be that the re-suspension and/or hydraulic pumping that may have affected these sediments significantly increased the O_2 penetration depth allowing nitrification to occur at depth. Alternatively it may be the case that the ammonium in question is being oxidised by compounds other than oxygen such as via the reduction of iron and manganese oxides (Anschutz *et al.* 2000; Clement *et al.* 2005), although the prevalence and importance of this pathway in natural systems is debated (Crowe *et al.* 2012).

Unlike the first two cores nitrate was not detected in core ST3. This may be due to the fact that fluid muds are biogeochemically active and dominated by redox processes such as iron, manganese and nitrate reduction (Abril *et al.* 2000, Abril *et al.* 2010) and as such nitrate would likely be quickly depleted by denitrifying and dissimilatory nitrate-reducing bacteria, particularly as the sediments comprising core ST3 were collected during a neap tide and so may not have been fully replenished with electron acceptors for up to 7 days.

Ammonium is present in all three cores and with profiles that all form a similar pattern, rising from zero at the surface and increasing with depth (reaching a maximum of 2.2 mM at the base of core ST2). This increase with depth is likely caused by the action of dissimilatory nitrate-reducing bacteria converting nitrate to ammonium in the near surface and also by the ammonification/deamination of nitrogen-containing organic compounds by heterotrophic bacteria throughout the

cores. Though core ST3 contains ammonium, it again differs from the other two cores in that the level of ammonium produced at similar depths is lower. For example (as seen in Fig. 3.12), at 17 cm depth, the ammonium concentration in ST1 is 1.7 mM and 1.97mM in ST2 while in ST3 it is only 0.65 mM. This again may be due to core ST3 originating in a fluid mud pool as it is thought that the conditions prevalent in fluid muds favour denitrification over DNRA as the main mode of nitrate reduction (Abril *et al.* 2000), thus any nitrate present in these sediments (albeit ephemerally) would quickly be converted to N_2 rather than ammonium. On the other hand, an alternative hypothesis might be that the fluid mud that core ST3 came from was completely depleted in nitrate and therefore its ammonium profile was generated entirely by ammonification of organic matter, without the additional ammonium supplied by dissimilatory nitrate reduction in the other two cores, which would have increased the concentration of ammonium found within them. The ammonium profile in core ST3 also differs from the other two cores in that the gradient is not constant with depth but instead appears to show an increase in production below 15 cm. The reason for this is not clear but may indicate that the top half of the sediment core was too disturbed for optimal anaerobic organic matter degradation (and hence ammonification) to occur - possibly again due to this sediment's origin in a fluid mud pool. This might be another reason for the lower levels of ammonium observed in this core, and is also consistent with the lowered levels of SO_4^{2-} removal and methane production.

3.3.4 Chloride and the Alkali Metals

The chloride and sodium profiles for all three cores from this transect show a decrease in concentration with depth. Since chloride and sodium ions are conservative and not actively utilised by microbial processes, this depletion must be the result of physical processes. The decrease in salinity may be the result of an influx of freshwater from deeper in the sediment caused by submarine groundwater discharge (SGD) (Burnett *et al.* 2003). This phenomenon, caused by meteoric water flowing into the sediments from a submerged coastal aquifer, can have a major impact on the biogeochemistry of the surrounding sediments by providing an injection of nutrients such as nitrate and phosphate into the deeper layers of the sediment pile (Slomp and Van Cappellen, 2004) However, without the use of tracer

elements (Moore, 1999), it would be impossible to say for certain whether this decrease in salinity was due to freshwater input or other unknown factors. It should also be noted that this decrease in salinity with depth became less pronounced with increasing distance from the coast (shown in Fig. 3.12). For example at 17 cm depth chloride had decreased by 16% in core ST1, 9% in ST2 and only 1% in ST3, which may reflect the decreasing influence of a coastal submarine aquifer. In addition an influx of SGD could also be the cause of the apparent partial inhibition of sulphate reduction and methanogenesis below 11 cm in core ST1 (whilst NH_4^+ production continued), if the groundwater in question contained other anaerobic electron acceptors and therefore a higher redox potential than was suitable for these processes (Porubsky *et al.* 2014).

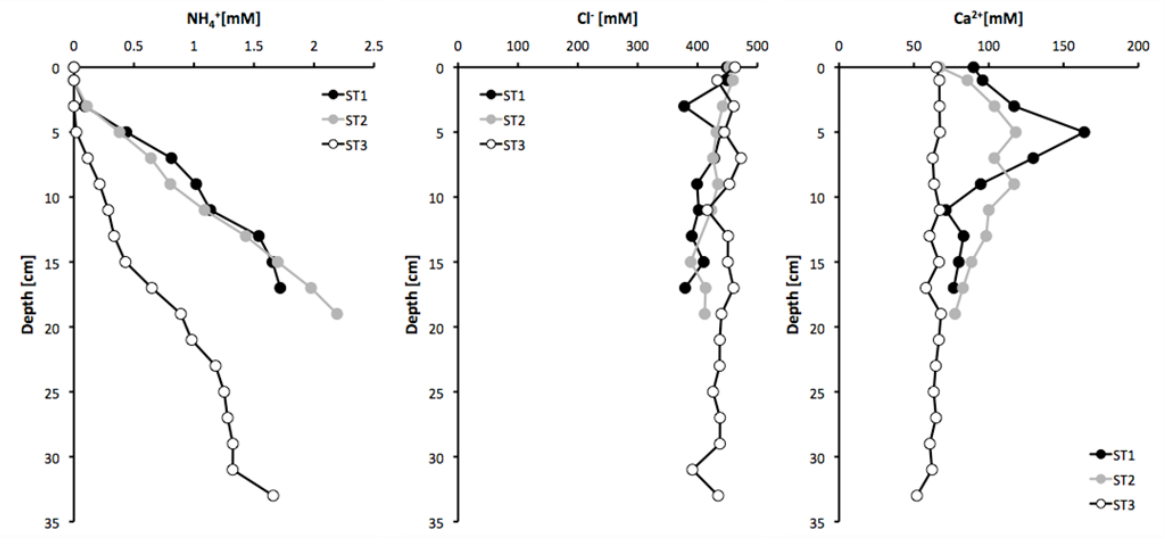


Fig. 3.12 – Comparison of the biogeochemical profiles of pore water ammonium, chloride and calcium concentrations obtained from cores ST1, ST2 and ST3 showing the changes in values with depth.

Magnesium shows a similar pattern in cores ST1 and ST2, with its profiles exhibiting a slight decrease in concentration with depth, while in core ST3 its concentration remains stable all the way through the core. This again may reflect dilution at depth by less saline SGD at the more coastal sites.

Calcium on the other hand shows a more unusual pattern in cores ST1 and ST2, with both cores displaying an initial increase and subsequent decrease with depth, with peak in concentrations around 5-10 cm depth (shown in Fig 3.12). In core ST1 this peak is much sharper (occurring at 5 cm) while in ST2 the trend is more spread out with the peak occurring between 5-9 cm. One possible explanation for this calcium spike could be the presence of high levels of calcium carbonate at

this depth, possibly originating from the shells of calcareous organisms. If this is the case it is unlikely that these organisms were macrofauna as no evidence of large benthic organisms (shell fragments etc.) was found in either of the cores and the Severn Estuary is considered to be underpopulated with regard to large benthic organisms (Warwick and Somerfield, 2010). It is possible however, that the calcium could have been released by the dissolution of the shells of benthic (and possibly planktonic) meio/microfauna such as ostracods and foraminifera, which are known to exist in the estuary's sediments (Cullen, 1973). Another piece of evidence that might suggest that the calcium peaks (at least in core ST1) are the result of the breakdown of calcareous fauna is the increase in acetate and formate concentrations observed at 5 cm depth in ST1, which could have been released by the decomposition of the organic constituents of these organisms by heterotrophic bacteria.

Alternatively it may be the case that changes in the microbial populations within the sediment (possibly connected to the switch in redox conditions from dysoxic to anoxic mentioned above that occurs at this depth in ST1) could also be effecting the porewater chemistry and causing calcium ions to dissolve out into solution, possibly by altering the pH of the pore waters. Sediment pore waters are generally thought to be well-buffered with respect to pH, with values maintained between ≈6.9-8.3 (Ben-Yaakov, 1973). However, there is evidence that even small shifts in pH due to microbial activity can still have an effect on the dissolution of carbonates possibly leading to a release of Ca^{2+} ions. For example Meister (2013) suggests that sulphate reduction can initiate a pH decrease significant enough to induce carbonate dissolution and the geochemical profiles for both core ST1 and ST2 indicate that sulphate reduction is occurring at the same depth as the carbonate peak. However, other studies have linked sulphate reduction with increases in carbonate precipitation rather than dissolution (Visscher *et al.* 2000; Wright and Wacey, 2005), while Gallagher *et al.* (2012, 2014) suggest that both increases and decreases in pH can be caused by SRB activity depending on which electron donor substrates they utilise. Another process that has been shown to be capable of lowering sediment pH is bacterial sulphide oxidation (Jørgensen and Revsbech 1983; Reaves, 1986). However, while this process could be the cause of the Ca^{2+} peak in core ST1, as the peak in this core occurs within a few centimetres of the

small subsurface peak in sulphate values (indicating sulphide oxidation), in ST2 the Ca^{2+} peak occurs within the darker reduced sediments where no oxygen is likely to be present, making sulphide oxidation unlikely. The decrease in Ca^{2+} concentrations below 5-10 cm can also be linked to sulphate reduction via the formation of FeS, which would remove free S^{2-} from the sediment, raising the pH (Ben-Yaakov, 1973), and allowing carbonate to precipitate (Alongi *et al.* 1996).

There may also be a link between calcium concentrations and CO_2 production as both profiles have broadly similar shapes in cores ST1 and ST2 and both peak at around the same depth. It could therefore be the case that the increased CO_2 in the porewaters (derived from the aerobic and anaerobic oxidation of organic matter) causes a decrease in pH, via carbonic acid formation, that again could lead to dissolution of $CaCO_3$ in the sediment (Boudreau, 1987; Walter and Burton 1990; Alongi *et al.* 1996). Moving downward through the cores the CO_2 concentrations begin to decrease again (possibly due to autotrophic consumption), which might raise the pH once more, allowing $CaCO_3$ to precipitate and thus lowering the Ca^{2+} concentration. Finally, the organic acids could also have played a role in the increase in Ca^{2+}, as production of such metabolic acids via organic matter degradation has been shown to play an important role in carbonate dissolution at some localities (Martin and Sayles, 1996). However, while the organic acids could have contributed to carbonate dissolution in core ST1 (where the subsurface peaks in acetate and formate at 5 cm coincide with the Ca^{2+} peak) there is much less evidence of a connection in core ST2, where the organic acids instead peak at depth.

In core ST3 on the other hand, calcium has a much more linear profile and appears more linked to overall salinity. Since sulphate reduction is reduced in this core (see below) this may mean that it does not affect the pH of the sediment as much as in ST1 and ST2. Also in this case there does not appear to be a link to CO_2 levels, as concentrations of the gas increased throughout the core without any dramatic change in the Ca^{2+} profile. This may be because the CO_2 levels in core ST3 were not high enough to affect the carbonate solubility, as CO_2 concentrations were significantly lower than in either core ST1 or ST2.

3.3.5 Sulphate

Both cores ST1 and ST2 show clear decreases in sulphate concentration with depth due to bacterial sulphate reduction (Fig. 3.13). With relation to previous studies carried out on the Severn Estuary the concentrations of sulphate in these cores are lower than those in Portishead mudflats (≈30 mM) (Webster *et al.* 2010) but higher than those found by Wellsbury *et al.* (1996) at the mudflats at Aust Warth (≈18 mM). It is however, worth noting that Aust Warth is considerably further towards the head of the estuary than the sites used for this study and, therefore, would have been more influenced by freshwater, resulting in inherently lower sulphate concentrations even before sulphate reduction had occurred (Sass *et al.* 2003). The depth at which sulphate reduction appears to start to occur also varies between these two cores. In core ST2 sulphate begins to decrease below 1 cm whereas in ST1 a decrease is not noticeable until below 3-5 cm. In core ST1 there is also a slight increase in sulphate values in the top 3 cm of the core potentially indicating that sulphide oxidation is occurring. As with the nitrate profiles described above this would again indicate that the top layers of core ST1 are more oxidised than ST2 further suggesting that this intertidal site is more subject to sediment re-suspension and/or hydraulic pumping than the subtidal ST2 site. The gradient of the sulphate profile in ST1 also becomes less steep with depth than in ST2, possibly due to the influence of SGD described above. This decrease in sulphate depletion also correlates with a slight change in the NH_4^+ profile indicating that the apparent drop in sulphate reduction below ≈11-13 cm could also be causing a decrease in overall organic matter degradation (and hence NH_4^+ production).

Core ST3 also showed clear evidence of active sulphate reduction but at a much lower level than in cores ST1 and ST2, and only significantly at depth (below ≈17 cm). For example, as can be seen in Fig. 3.13, in core ST1 total sulphate decreased by 40% from the top of the core to the bottom, and at 17 cm depth (the base of core ST1), the sulphate content of core ST2 had decreased by 53% while in core ST3 sulphate had fallen by only 1%. In fact over the whole of core ST3 sulphate levels only decreased by 17%, despite ST3 being around twice the length of the other cores. This lower level of sulphate reduction is another indicator that core ST3 may have come from a fluid mud pool, as fluid muds that have been studied in other parts

of the world (Abril *et al.* 2010) have been found to be dominated by more energetically efficient chemical reactions further up the redox cascade such as nitrate, iron and manganese reduction (Canfield and Thamdrup, 2009) due to their continuously changing redox state. There is however, some evidence that sulphate reduction does still occur within fluid muds, as in a study by Madrid *et al.* (2001), sequence analysis of 16s rDNA from mobile fluid muds retrieved from the coast of French Guiana indicated the presence of potentially sulphate-reducing bacteria. In a further study (Madrid *et al.* 2010), dissimilatory sulphate reductase (*dsr*) genes were successfully amplified from mobile fluid mud beds from French Guiana and Papua New Guinea indicating the presence of SRB. The same study also found, through the use of radiotracers, that active sulphate reduction was occurring within the muds, albeit at low levels and without a net increase in sulphide (which may indicate why there was no colour change in core ST3 despite the decrease in sulphate).

3.3.6 Methane

Methane concentrations increased down-core in all three of the sediment cores studied, indicating that active methanogenesis was occurring (shown in Fig. 3.13). This increase took place despite the presence of significant concentrations of sulphate, which is unusual as when sulphate is not limited SRB are usually thought to outcompete methanogens for common substrates (due to their more efficient metabolisms), making significant co-occurrence unusual without the presence of non-competitive substrates. This co-occurrence may again be due to the overall disturbed nature of the sediments of the Severn Estuary and the high degree of re-suspension that they are subjected to - a subject that will be discussed further in Chapter 9. The increase in methane was greatest in core ST2 where maximum concentrations reached 47 μmol lws^{-1} at 15 cm depth, a level much higher than that present in a previously studied Severn Estuary inter-tidal mudflat, where concentrations only reached around 25 μmol lws^{-1} at 50 cm depth (Webster *et al.* 2010). In core ST1 the maximum methane concentration at 15cm depth was lower, at 6.3 μmol lws^{-1}, a concentration more in line with the inter-tidal mudflat results. In both ST1 and ST2 methanogenesis coincided with changes in the concentrations of organic acids, hydrogen and CO_2 suggesting that both acetoclastic and H_2/CO_2

methanogenesis may have been occurring in these cores. Again as with sulphate reduction, methanogenesis in ST2 began to occur at a shallower depth than in ST1. In ST2 methane began to increase steadily from 1 cm depth while in ST1 a marked increase did not occur until below 5 cm depth. Again this may indicate that the top layers of core ST1 were more disturbed by physical processes than in core ST2. The reason for the marked difference in values between these two cores at depth on the other hand might be due to partial inhibition of methanogenesis beneath 11 cm in core ST1, possibly caused by more redox-positive SGD.

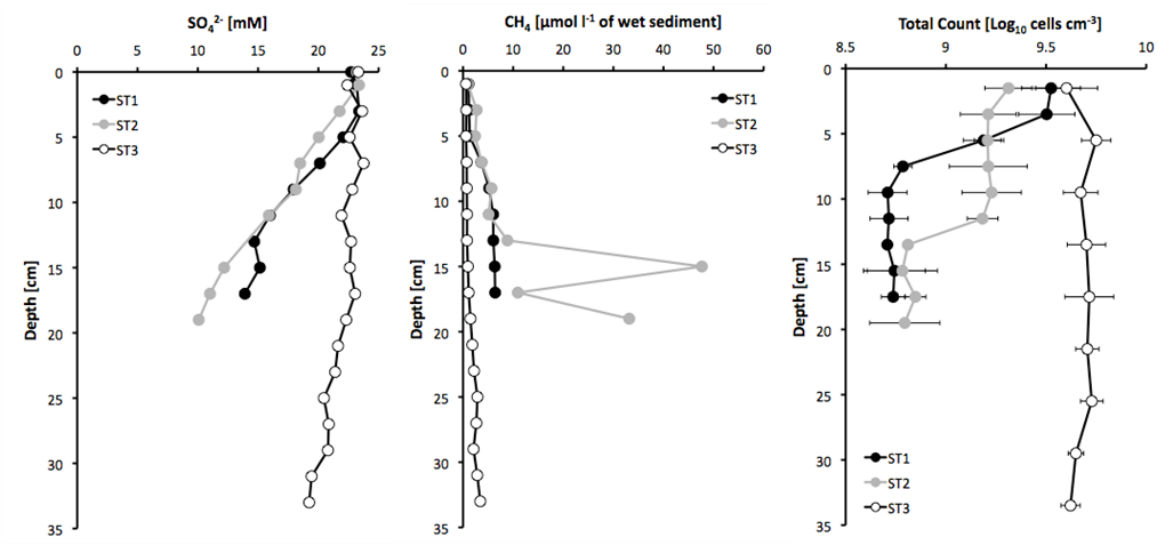


Fig. 3.13 – Comparison of the biogeochemical profiles of sulphate, methane, and the total cell counts (AODC) obtained from cores ST1, ST2 and ST3, showing changes in values with depth. Error bars represent the 95% confidence limit derived from repeated cell counts.

The methane levels in core ST3 were much lower than the other cores, with a maximum concentration of only 3.4 µmol lws^{-1} at the base of the core and ≈1 µmol lws^{-1} at 15cm depth. This low level of methane production again indicates that core ST3 is likely derived from a fluidised mud, as the constant cycling of electron acceptor compounds within the mud would inhibit methanogenesis by allowing more efficient organisms to outcompete the methanogens for common substrates (e.g. acetate and H_2) at every level. However, methanogenesis can still occur in fluidised muds as observed by Abril *et al.* (2010), possibly due to the action of methanogens utilising non-competitive substrates such as methylated amines or methanol. It may also be the case (as with ammonium production) that the disturbed nature of the sediments within the fluidised mud made it difficult for stable methanogenic communities to develop. Instead methanogenesis may have

been limited to isolated microniches (possibly linked to organic acid production), with diffusion of CH_4 gas creating the smooth profile seen in the core.

Core ST3 is also notable for possessing a concave-upward methane profile, one of the indicators of anaerobic methane oxidation (Iversen and Jørgensen, 1985; Valentine and Reeburgh, 2000; Knittel and Boetius, 2009). Abril *et al.* (2010) suggested that AOM could occur in fluidised muds, possibly via the action of nitrate reducing bacteria similar to the freshwater consortia described by Raghoebarsing *et al.* (2006). However, the lack of nitrate detected in this core coupled with the difference in magnitude between the methane and ammonium profiles (in addition to the fact that the ammonium profile is linear) means that a direct comparison between compounds is very difficult. Also the methane concentrations in ST3 are low compared the minimum methane concentrations for active AOM observed for other marine sites (Dale *et al*. 2008; Knittel and Boetius, 2009; Meulepas *et al.* 2009). Therefore without further work (e.g. using ^{14}C-CH_4 radiotracers and/or $\delta^{13}C$-CH_4 values) it would be impossible to confirm that AOM is occurring within these sediments.

One important point that should be raised here is in regard to why, when they are supposed to be homogenously mixed, do fluid muds possess decreasing or increasing profiles with depth (e.g. ammonium, sulphate and methane). This is likely because the fluid mud pools found in the Severn Estuary are thought to potentially be re-suspended and re-mixed on a two-week basis by the actions of the spring tides. Since the cores used in this study were collected on a neap tide it is possible that the mud pool that core ST3 originated from may have been gradually settling out of suspension in a relatively quiescent environment for up to seven days before it was sampled, thus allowing the profiles to form.

3.3.7 Total Cell Counts

In all three cores the total counts of prokaryotic cells (as determined by AODC) were high compared to the global average for marine sediments (Parkes *et al.* 2014). In cores ST1 and ST2 the counts showed a relatively similar pattern with depth (shown in Fig. 3.13). In both cores the number of cells near the surface was high (around

$1x10^{9.5}$ cells cm^{-3} in ST1 and $1x10^{9.3}$ in ST2), then at a distinct depth in the sediment (7.5 cm in ST1 and 13.5 cm in ST2) numbers dropped sharply to around $1x10^{8.8}$ and then stayed at this lower level down to the base of the core. Higher numbers of cells near the surface are often observed in tidal flat and shallow marine sediments due to the large amounts of bioavailable organic matter in near-surface sediments which allow large prokaryotic populations to develop (Beck *et al.* 2009; Webster *et al.* 2011). With increasing depth within the core the amount of these compounds decreases as they are gradually metabolised, and as such the size of the prokaryotic population decreases with depth (Cragg *et al.* 1995; Parkes *et al.* 2000). However, in most shallow sedimentary environments this decrease usually begins to occur within the top 1-2 cm and it is unusual to find such high numbers of cells down to ≈14 cm depth. Similar results, however, have previously been found for Severn Estuary mudflat sediments (Wellsbury *et al.* 1996), with cell numbers remaining close to surface values down to around 7-8 cm depth. The authors attributed this unusual profile to the fact that the sediment in question had recently been re-deposited by the strong tidal currents of the estuary which would have resulted in a uniform distribution of bacteria throughout the top of the core. Since both cores ST1 and ST2 come from an area of the estuary where tidal currents can be stronger than at Aust Warth (Uncles, 2010) it is likely that a similar re-suspension event could have caused the pattern seen in cores ST1 and ST2.

The problem with this hypothesis is that the depth of the change from high to lower counts is much deeper in core ST2 than in ST1, which would imply that re-suspension had occurred to a greater depth in ST2. This would seem to contradict the geochemical evidence (nitrate/ammonium, sulphate and methane profiles), which indicate that ST1 was the more disturbed site. In addition since core ST1 was collected from a shallower water depth, it stands to reason that it should be more affected by erosive processes than the deeper water ST2 site. As such it may be that the sudden shifts in cell counts seen in these two cores do not reflect re-suspension but rather are driven by other changes in the biogeochemistry of the sediments – for example the switch from dysaerobic to anoxic conditions at 5 cm depth in core ST1 and the transition to a methanogenic community below 11 cm in core ST2. Alternatively it could be the case that the decrease represents a change in makeup of the organic carbon pool, with the high cell numbers occurring in sediments with

higher amounts of labile OC while the lower cell counts come from sediments where much of this labile OC had been degraded, leaving behind only more refractory material (Cragg *et al.* 1995). If this was case then it would suggest that the sediments at site ST1 were more biogeochemically active with regard to OC mineralisation, as the cell counts would indicate that the labile OC was depleted closer to the surface than at site ST2 (provided OC deposition was uniform across the sites). This could again be due to disturbance of the sediment (by re-suspension, or hydraulic pumping), which might supply the sediments with limiting compounds allowing larger populations of more metabolically-active redox-positive organisms (aerobes etc.) to develop (this will be discussed further in Chapter 9).

The total count of cell numbers in core ST3 was very different from the other two cores, with numbers averaging much higher ($1x10^{9.6}$-$1x10^{9.7}$ cells cm^{-3}) than in either core ST1 or ST2 and remaining relatively uniform with depth, with only a slight decrease near the base of the core. This unusual profile is probably again linked to core ST3 originating in a fluid mud pool. The uniformity of cell numbers with depth can be explained by the fact that the fluid mud had regularly been (or at the very least had been recently) re-suspended and mixed leading to the production of a homogeneous profile. The overall higher cell counts can be explained by the fact that the re-suspension of the fluid mud pool could have resulted in a re-oxidation of reduced former electron acceptor species thus allowing them to be utilised again by the incumbent prokaryotes. In addition it would allow the microbial communities to continue using more efficient electron acceptors (such as nitrate, iron etc.), which would have produced a higher overall growth yield. In this way the mud pool could act in a similar fashion to a microbial bioreactor (Aller, 1998) making it an ideal habitat for certain guilds of prokaryotes and accounting for the higher than average cell numbers found in the core.

3.4 The Severn Estuary Transect - Conclusions

- This study represents the first time that full biogeochemical profiles and prokaryotic cell counts have been obtained from the sub-tidal sediments of the Severn Estuary, and also to the author's knowledge the first time this data has been obtained from a hypertidal estuary.

- Despite representing a transect across only half of the Severn Estuary, cores ST1, ST2 and ST3 have markedly different geochemistry.
- The geochemistry of ST1 reflects an environment subjected to processes that lead to the oxidation of the upper 0-5 cm of sediment (either sediment re-suspension or hydraulic pumping of seawater), resulting in the suppression of anaerobic processes in these upper layers and the probable re-oxidation of reduced chemical species (e.g. NH_4^+, S^{2-}). However, deeper in the sediment anaerobic processes are well developed.
- Core ST2 represents a more stable environment, likely subject to les re-oxidation and with anaerobic processes closer to the sediment surface (this may also be enhanced by the lack of bioturbation).
- Both the sedimentology and geochemistry of core ST3 indicate that it was likely derived from a fluidised mud bed with suppressed levels of anaerobic processes (i.e. ammonification, sulphate reduction and methanogenesis). However, there is a higher total population of prokaryotes than at the other sites, with high counts continuing throughout the sediment core, which may be related to the previous fluidised and mixed state of the sediment.
- The sites at which cores ST1 and ST2 were sampled may be affected by a degree of submarine groundwater discharge (SGD), which could be resulting in the partial inhibition of some of the more redox-negative metabolic processes at depth
- Despite the presence of different competing metabolic processes within the sediment column these processes do not seem to be mutually exclusive (i.e. sulphate reduction and methanogenesis are co-occurring in all cores). This may indicate that the normal redox cascade structure based on competition for common substrates may not be occurring in these sediments.

Chapter 4. – Severn Estuary Tidal Flats, Part 1: Cores ST4 and ST5

4.1 Site Locations and Lithology

Both cores ST4 and ST5 were collected from tidal mudflats on opposing sides of the Severn Estuary during early October 2012. Core ST4 was collected from Woodhill Bay near the town of Portishead, Somerset on the southern side of the estuary (51° 29' 30.94" N, 02° 46' 28.91" W; OS grid reference ST 46345 77379), while core ST5 was collected from tidal flats southeast of the village of St Brides Wentlooge, Newport on the northern bank of the estuary, offshore of the Wentlooge Levels (51° 31' 41.2" N, 03° 00' 32.5" W; OS grid reference ST 30099 81508). These sites were selected to determine what affect the different conditions, both hydrological and sedimentological, occurring on opposite sides of the estuary might have on the biogeochemistry of the tidal flat sediments.

With regard to topography, the tidal flats at Portishead occur in an embayment backed by high ground and are sheltered by headlands to the northeast and southwest. The flats near St Brides on the other hand form part of a much larger expanse of mudflats extending along much of the Welsh coast of the Severn Estuary, from Cardiff in the west to Chepstow in the east. Unlike Portishead these mudflats are backed by the low-lying Wentlooge and Caldicot Levels and are therefore exposed to the full force of the erosive processes occurring within the estuary. As such the sedimentary regime at this location is likely to be much more dynamic than at Portishead and is known to be both a site of net erosion (O'Brien *et al.* 2000; Severn Estuary Partnership, 2001) or deposition (Kirby, 1994) depending on the prevailing conditions (season, tide, weather etc.). In addition to differing topography, the mudflats are exposed to different hydrological conditions, as within the Severn Estuary the rising and falling tides follow an asymmetrical pattern, with the rising tide flowing strongest up the northern side of the estuary while the falling tide flows out along the southern side (Dr Chris Wooldridge pers. comm.). This, in addition to the fact that the main channel of the estuary sits near the south bank, means that the tidal flats on the estuary's southern flank are more likely to be influenced by riverine conditions than the north bank, which in turn is more likely to be shaped by marine processes.

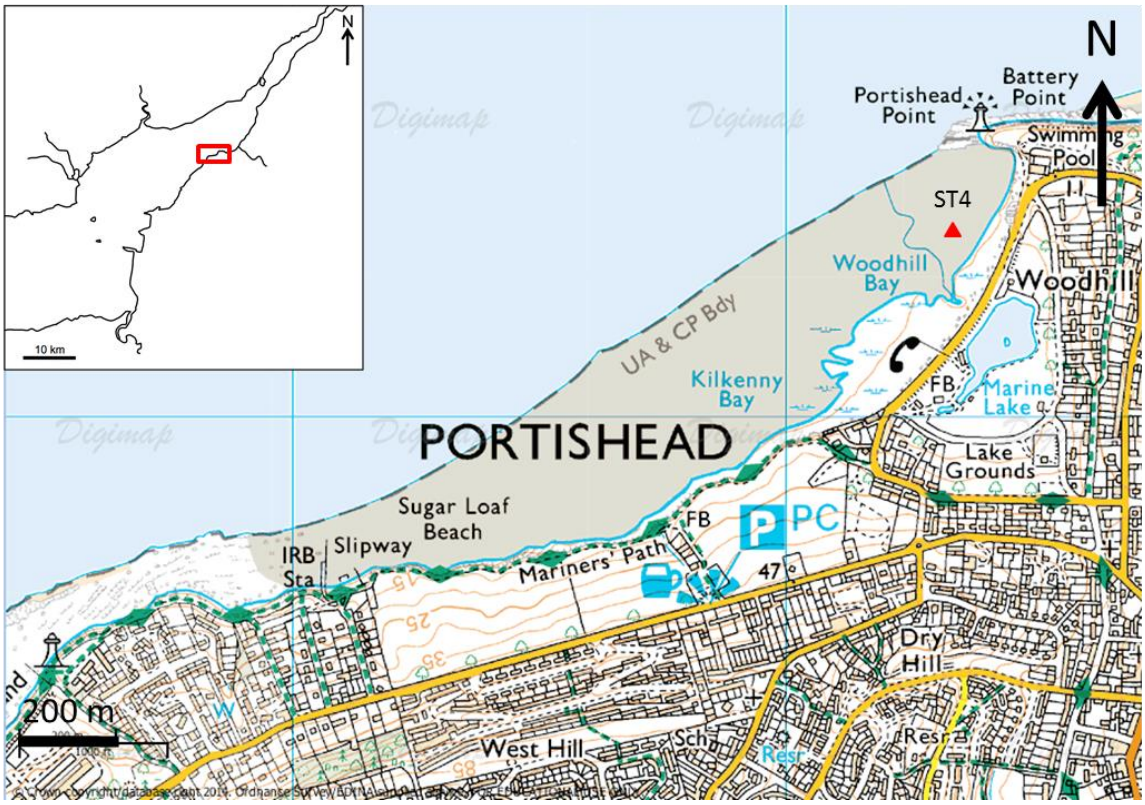


Fig. 4.1 – Ordnance Survey map showing the location of the sampling site for core ST4 in Woodhill Bay, Portishead. Sampling site denoted by red triangle. Brown shading indicates intertidal mudflats exposed at low tide. © Crown Copyright/database right 2014 Ordnance Survey/EDINA.

The mudflats at Portishead - which have been previously studied at one site by Webster *et al.* (2010) – extend for ≈1.5 km across Kilkenny Bay and Woodhill Bay from Battery Point in the northeast to Black Nore Point in the southwest (Fig. 4.1) At the lowest astronomical tide they extend ≈200-300 metres from the shoreline and are backed by a short expanse of vegetated saltmarsh representing the Northwick Surface (Allen and Duffy, 1998a), which is itself backed by man-made sea defences. Topographically the sediment surface is rather uniform with only a few tidal channels cutting across it (shown in Fig. 4.2). Average core lengths for this site were 30-35 cm with the top 13 cm being light brown in colour while below this the sediment changed rapidly to dark grey/black, denoting a build up of iron sulphide (FeS) minerals indicative of sulphate reduction. The lithology of the sediment remained consistent throughout the core, being a fine-grained mud/silt however, particularly in the darker zone, the sediment was very dense and capable of maintaining its shape even when extruded from the core tube. The density was so

high in fact that the hydraulic core cutter used to sample the sub-tidal cores collected in this study was not strong enough to push the sediment out of the core tubes so sectioning was carried out using a hand-held plunger instead. This also meant that the sediment did not readily break down when immersed in the gas/AODC/radiotracer vials and therefore had to be shaken/vortexed vigorously before storage. With regard to resident macrofauna the upper brown layers of the core showed evidence of bioturbation by polychaete worms though no activity was observed below the brown-black (oxidised/reduced) interface.

Fig. 4.2 – A) Mudflats at Kilkenny/Woodhill Bay, Portishead showing drainage channels, salt marsh and cliffs behind (© Copyright Dr Duncan Pepper). B) Mudflats south of St Brides Wentlooge showing ridge and runnel bedforms perpendicular to the shoreline and rip-rap sea defences landward of the mudflats.

As previously mentioned the mudflats at St Brides are more exposed than at Portishead however, they are also much more extensive, reaching a maximum extent of ≈1100 metres from the shoreline on the lowest astronomical tide (shown in Fig. 4.3). The flats are backed by artificial sea defences (riprap) behind which sit the Wentlooge Levels, a large expanse of agricultural marshland, reclaimed from the sea during the Roman to medieval period (Allen and Haslett, 2007). The surface topography of the mudflats here differs greatly to those at Portishead, with the surface being covered in large ridge and runnel bedforms similar to those described by Carling *et al.* (2009) that run perpendicular to the shoreline. Streams of (presumably non-saline) water originating beneath the riprap, also flow through these runnels. Another noticeable feature at this location is that the soft sediment cover is much thinner than at Portishead, reaching a maximum depth of ≈20 cm, whereas at Portishead the sediment depth is greater than 1 m (Webster *et al.* 2010).

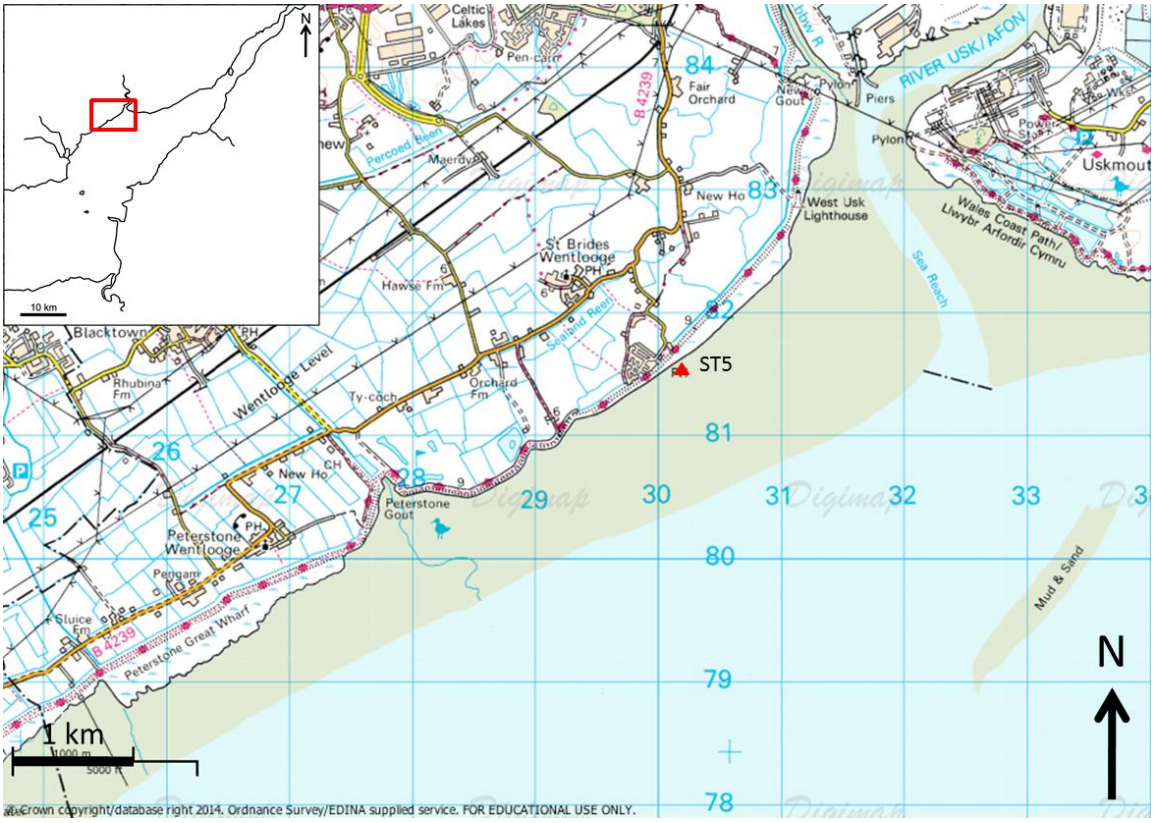


Fig. 4.3 – Ordnance Survey map showing the location of the sampling site for core ST5, near St Brides Wentlooge, Newport. Sampling site denoted by red triangle. Brown shading indicates intertidal mudflats exposed at low tide. © Crown Copyright/database right 2014 Ordnance Survey/EDINA

Below the soft sediment cover lays the compacted greenish clay of the Wentlooge Formation (Allen and Rae, 1987), a Holocene estuarine facies deposited between the end of the last glaciation and the period when the Wentlooge levels were embanked 800-1800 years ago. Also part of this formation are layers of compacted, fibric peat, made up of a mixture of salt marsh (*Phragmites*) and fen carr vegetation that formed during low-stand periods over the last 3000-7000 years when the area was above sea level (Allen and Haslett, 2007). The resilience of these peats to erosion leads to the formation of distinct "ledges" and "cliffs" at many locations along the northern shore of the estuary (Allen and Haslett, 2002), at least one of which can be seen exposed at the St Brides site (shown in Fig. 7.1B) As expected considering the differences in environment described above, the lithological characteristics of core ST5 are different to ST4. The top 10 cm of core ST5 were made up of brown mud/silt similar to core ST4 (albeit less compacted and sticky) however, below this, instead of turning black, the mud ceases and is replaced by the salt marsh peat described

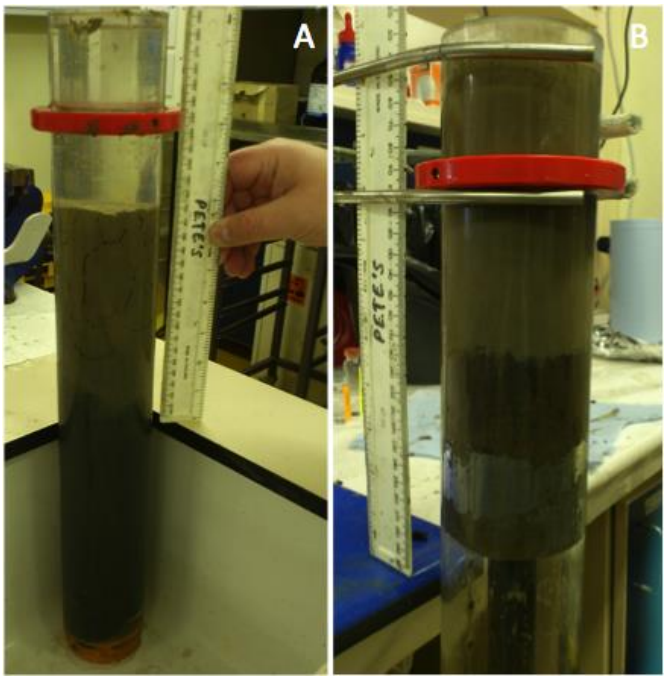


Fig. 4.4 – A) Photograph of core ST4 showing colour change at depth and bioturbation of the upper oxidised sediment layers (30 cm ruler for scale). B) Photograph of core ST5 showing bioturbation of upper sediment layers and abrupt change in lithology to Holocene peat at depth. 30 cm ruler again for scale.

above (Fig. 4.4). This peat then persists for 4 cm down to the base of the core. As a result, core ST5 was therefore considerably shorter (total length = 14 cm) than core ST4 (total length = 32 cm) due to the fact that the compacted nature of the peat prevented the core tubes from being pushed any further into the sediment by hand. Further down the foreshore where the peat bed forms a ledge it can be see that it had a thickness greater than 20 cm, below which was the greenish, Wentlooge Formation clay seen exposed elsewhere. The compacted nature of the peat again required the use of a hand held piston for sectioning and for the vials containing samples from the bottom of the core to be shaken/vortexed before storage. With regard to macrofauna, bioturbation was evident in the top 9 cm of the core and possibly to a greater degree than at Portishead (although with smaller burrows), with evidence of polycheate (likely *Nereis diversicolor*), bivalve and potentially gastropod burrowing occurring (O'Brien *et al.* 2000). However, no evidence of burrowing was found in the peat, and due to its compact nature (and the sulfidic smell it gave off when sectioned) it is unlikely any macrofauna would have been present.

4.2 Severn Estuary Tidal Flats, Part 1 - Results

4.2.1 Biogeochemistry of Core ST4

Throughout core ST4 pore water acetate concentrations exhibited a degree of variability (Fig. 4.5), but showed a general trend of increasing concentration with depth, from 9.5 μM at 1cm depth to 23.3 μM at the base of the core, suggesting an increase in fermentation activity with depth. In addition, two localised zones of elevated concentrations were evident, one between 7-11 cm and the second below 25 cm. The upper of these two zones (7-11 cm) occurs just above the change in sediment colour (13 cm) indicating a switch from oxidised to reduced conditions. It also coincides with a decrease in sulphate reduction rates and a subsequent increase in methane levels suggesting a change in the terminal oxidising community. This switch-over (and the subsequent decrease in terminal-oxidising organisms) could have resulted in an uncoupling of the production and consumption pathways of acetate, with fermentation occurring at a faster rate than either sulphate reduction or methanogenesis, thus accounting for the acetate peak (Jørgensen and Parkes, 2010; Parkes *et al.* 2012). The second increase in concentrations (below 25 cm) occurs deep in the reduced zone of the sediment column and may indicate a secondary source of acetate as well as fermentation, such as homoacetogenesis.

Both lactate and formate concentrations showed no clear trend with depth and were quite variable, although both appear to show a slight initial decrease from 20 μM at 1 cm to 5.1 μM at 5 cm (lactate) and 76.2 μM at 1 cm to 37.2 μM at 5 cm (formate) – possibly indicative of heterotrophic consumption. Below this, concentrations vary between 3-25 μM (lactate) and 31-87 μM (formate) down to 29 cm, below which values increase again to 33 μM (lactate) 133 μM (formate) potentially indicating a lower zone of fermentation at a similar depth to the 2nd zone of elevated acetate concentrations. Propionate concentrations displayed a different pattern to the other organic acids, with a dramatic initial decrease from 25.2 μM at 1 cm to 0.36 μM at 9 cm – again likely linked to heterotrophic consumption. Concentrations remained low down to 29 cm, below which concentrations rose slightly, reaching 5 μM at the base of the core, again indicating a possible zone of fermentation.

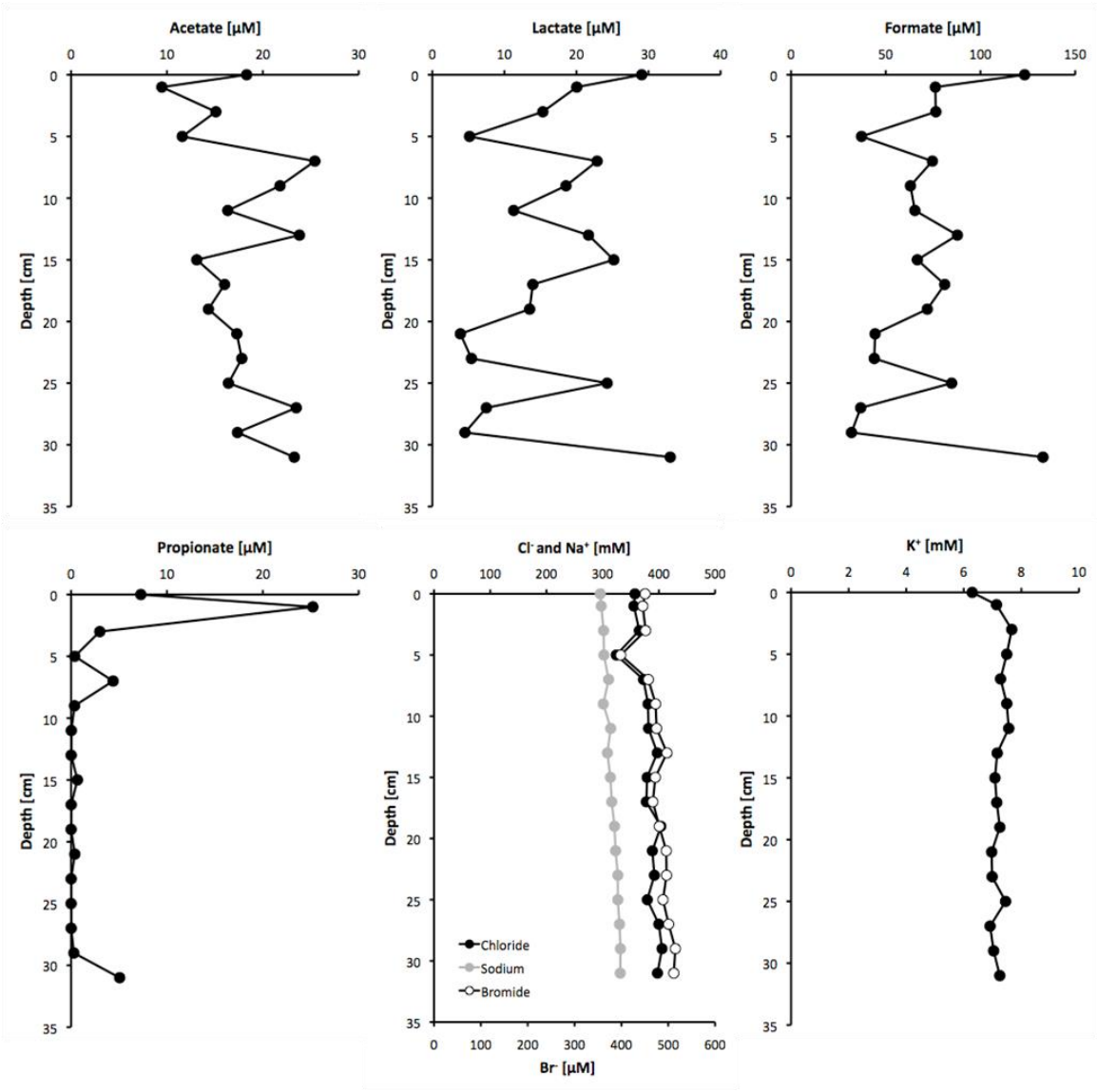


Fig. 4.5 - Biogeochemical profiles obtained from core ST4 showing changes in the concentration of pore water: organic acids, chloride, sodium, bromide and potassium with depth.

Chloride, sodium and bromide concentrations all showed a slight increase with depth from 355 mM, 297 mM and 445 μM respectively at 1 cm; to 397 mM, 331 mM and 511 μM respectively at the base of the core. This increasing salinity with depth is likely caused by the flow of freshwater over the top of the tidal flats gradually diffusing down into the sediment. This increase in salinity with depth may have been more pronounced than normal during the time that the core was taken, due to it coinciding with period (several weeks in duration) of intense rainfall that left portions of the land behind the tidal flats flooded and may have resulted in a greater than average degree of surface water runoff. Potassium concentrations increased over the top 3 cm possibly also due to low-salinity surface water infiltration.

However, the calcium profile (Fig. 4.6), increased downward over the whole length of the core, and at a greater rate than sodium, suggesting a secondary source of calcium at depth - possibly derived from the dissolution of $CaCO_3$ (sediment from this site fizzed intensely when HCl was added to it during cold chromium reduction).

Nitrate concentrations in the surface waters of core ST4 were high (60 μM), but rapidly decreased to 11.1 μM at 1cm and then to 2.3 μM by 3 cm depth, indicating that denitrification/dissimilatory nitrate reduction to ammonium was occurring. Below this concentrations mainly stayed between 0.5-7.9 μM down to the base of the core – no shallow subsurface nitrate peak usually indicative of nitrification was detected. At 25 cm concentrations did increase to 16.5 μM before dropping back down again rapidly. Since this depth is well below the expected zone of oxygen penetration it is unlikely that this peak is caused by nitrification and it therefore may be an indicator that anaerobic ammonium oxidation (anammox) is occurring in these sediments as nitrate is a minor by-product of the anammox reaction (Egli *et al.* 2001), however, without more data it is impossible to confirm if this is the case. Alternatively, since no nitrite was detected in the core it may be linked to a flow of more redox-positive groundwater as there is a small decrease in the chloride profile at this depth indicating possible freshwater input.

Ammonium increased with depth throughout the core from 0.4 μM at 1cm to 1.4 mM at the base. In the top 7-9 cm of the core the ammonium profile possessed a concave-upward shape indicating that nitrification was likely occurring in these sediments despite the lack of a subsurface nitrate peak (though nitrate was still present at this depth). Beneath 9 cm concentrations increased in a more linear fashion, indicating production via the ammonification of organic matter. No obvious decrease in ammonium concentrations was detected at 23 cm that could be linked with the increase in nitrate. However, since the levels of ammonium at this depth (995 μM) were many times higher than the nitrate levels it is possible that any depletion of ammonium caused by anammox could be masked by the general increasing trend caused by ammonification.

Phosphate concentrations stayed between 0-3 μM through the top 13cm of the core. Below this, levels began to rise, particularly below 19 cm where values rose sharply to 21.8 μM at 23 cm and from then on varied from 2-16 μM down to the base

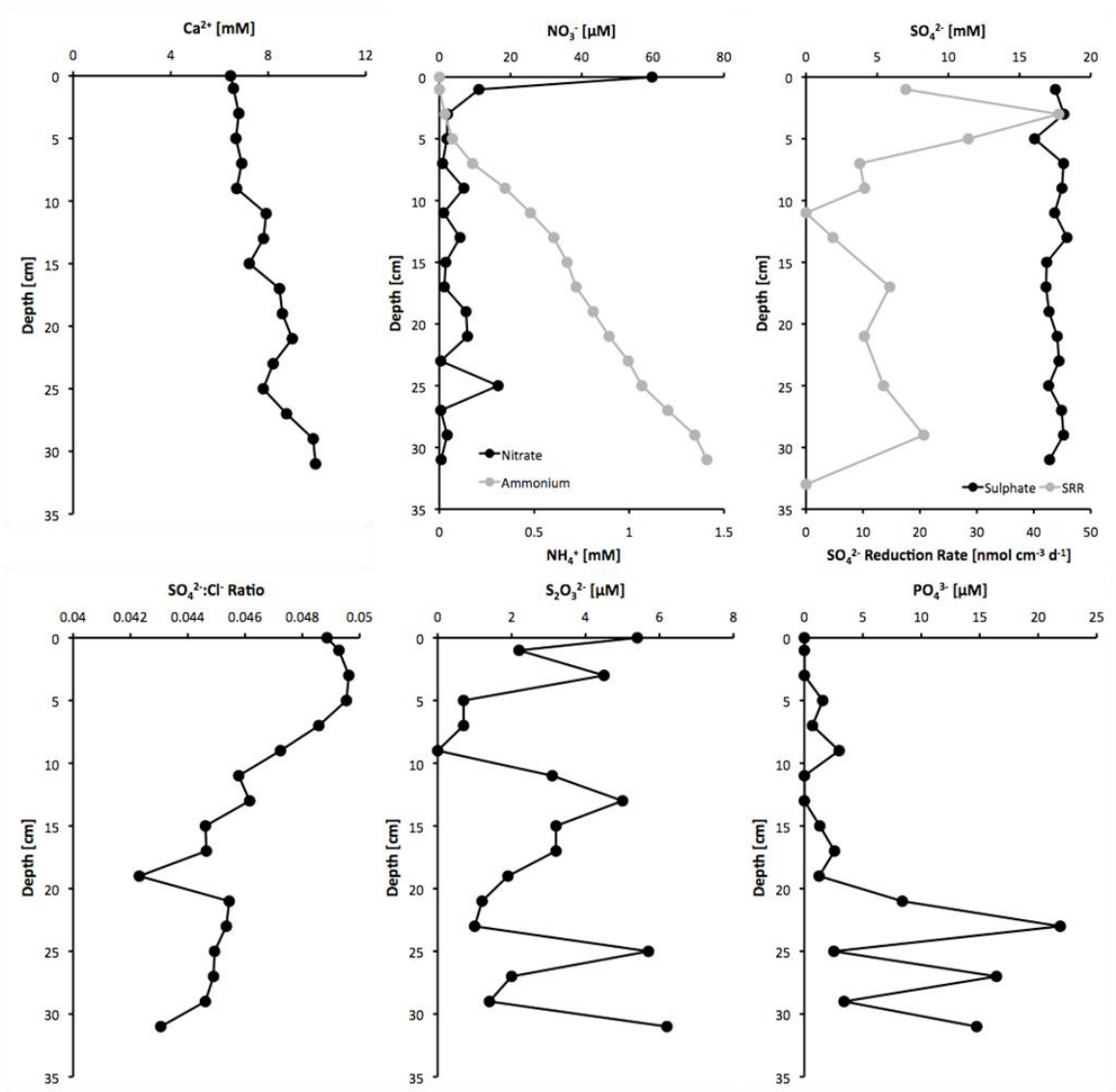


Fig. 4.6 - Biogeochemical profiles obtained from core ST4 showing changes in the concentration of pore water: calcium, nitrate, ammonium, sulphate, thiosulphate and phosphate with depth. Also shown are profiles for the sulphate:chloride ratio and *in situ* rates of bacterial sulphate reduction (SRR).

of the core. This increase coincides with the change in colouration of the sediment and as such is likely caused by the reaction of free sulphide with iron minerals contained within the sediment to form FeS, which in turn would release phosphate, that was previously complexed with the oxidised iron compounds, into the pore waters (Rozan *et al.* 2002).

Concentrations of sulphate showed little sign of decrease down-core with values of 17.5 mM at 1 cm and 17.1 mM at 33 cm. The SO_4^{2-}:Cl^- ratio however, did change with depth, indicating that the relatively static sulphate profile may have been caused by

the decrease in salinity at the top of the core rather than by a lack of sulphate reduction. In addition the SO_4^{2-}:Cl^- ratio shows a subsurface peak at 3-5cm indicative of sulphide oxidation which could also have contributed to straightening out the sulphate profile. This peak coincides with the concave-upward section of the ammonium profile, which would indicate that oxygen (at least in small concentrations) is able to penetrate up to 5-7 cm into the sediment, extending the dysoxic/anoxic boundary to this depth. Below 5 cm the SO_4^{2-}:Cl^- ratio decreases substantially indicating that sulphate reduction is active below this depth. There is also a notable change in gradient with depth, with a steeper gradient indicating elevated sulphate reduction rates in between 5-11 cm and a more vertical gradient beneath this extending to the base of the core. Sulphate reduction rate measurements utilising $^{35}SO_4^{2-}$ radiotracers also indicate that active sulphate reduction was occurring. Rates were high in the top 9 cm of the core (as predicted by the SO_4^{2-}:Cl^- profile) with the highest values (44.4 nmol cm^{-3} d^{-1} at 3 cm depth) occurring above 7 cm depth. Values then decreased down to zero at 11 cm and then rose again below 13 cm (to a second peak of 20.7 nmol cm^{-3} d^{-1} at 29 cm) matching the second, less steep, SO_4^{2-}:Cl^- gradient. This kind of bi-peaked sulphate reduction profile is often observed in sediments and may represent two SRB communities utilising different pools of organic carbon at different depths, with an upper community (above 10 cm) rapidly utilising labile OC compounds and a lower community utilising the remaining more refractory pool at a slower rate (Westrich and Berner, 1984). Interestingly the highest rates of sulphate reduction occurred in the top 5 cm of the sediment, an environment that would appear to still contain (at least some) oxygen. Despite the fact that they are anaerobes, SRB can still exist in such conditions within isolated anaerobic microniches (such as within macrofaunal faecal pellets) (Jørgensen, 1977) and in this case would appear to be more metabolically active in such an environment compared to the completely anoxic sediments below. Therefore results such as these indicate the importance of using radiotracer studies to study sulphate reduction as by merely examining the *in situ* geochemical profiles the activity (which show no decrease in the top 5 cm) the activity of these organisms might have been overlooked.

Thiosulphate concentrations show an initial increase from 2.2 μM at 1cm to 4.5 μM at 3 cm before dropping to zero at 9cm depth. This initial increase matches

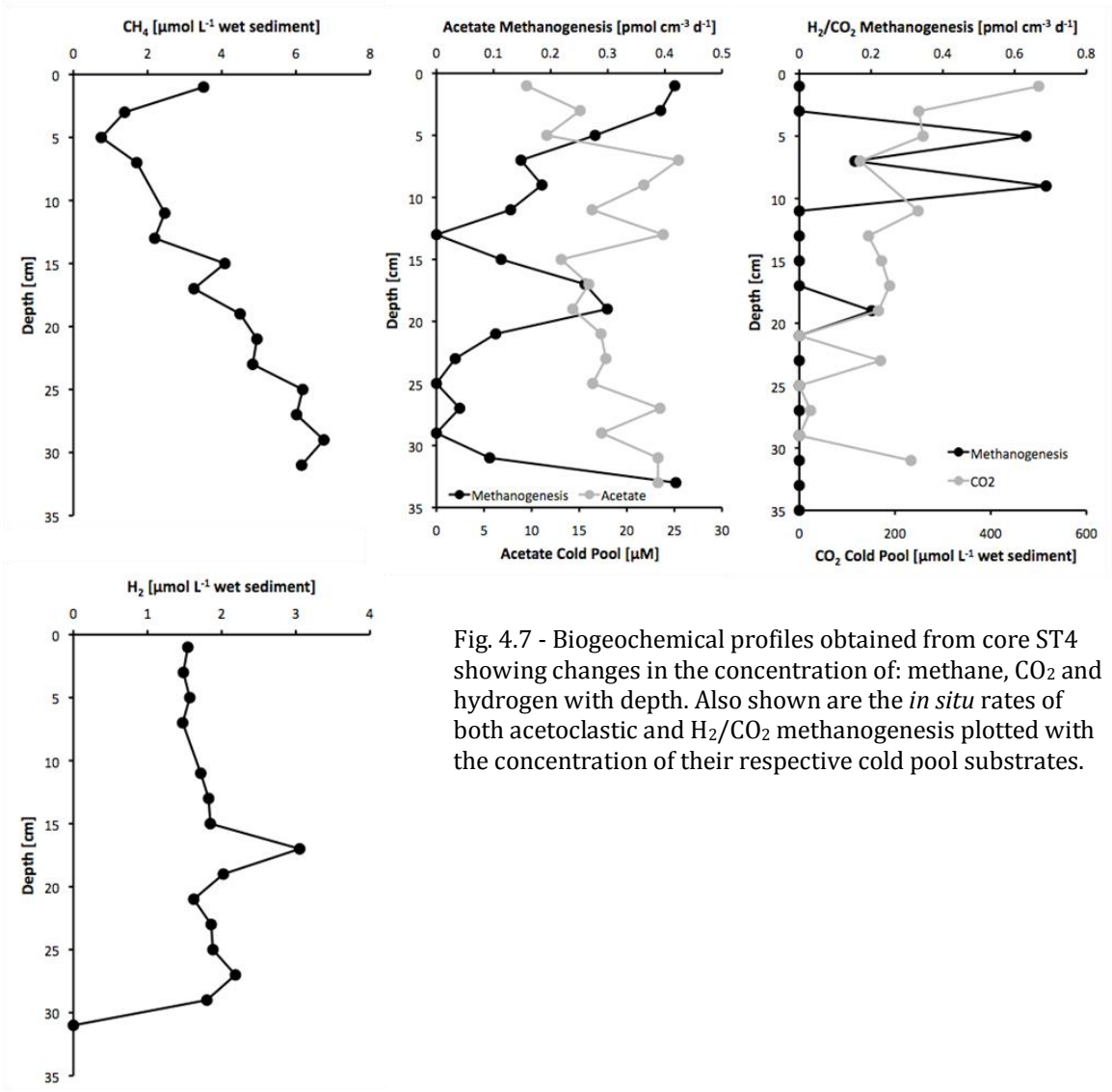


Fig. 4.7 - Biogeochemical profiles obtained from core ST4 showing changes in the concentration of: methane, CO_2 and hydrogen with depth. Also shown are the *in situ* rates of both acetoclastic and H_2/CO_2 methanogenesis plotted with the concentration of their respective cold pool substrates.

the SO_4^{2-}:Cl^- profile is likely due to the chemical oxidation of sulphide diffusing from below while the subsequent decrease matches both the SO_4^{2-}:Cl^- and SRR profiles and is therefore most likely due to disproportionation or reduction by SRB (Thamdrup *et al.* 1994). Concentrations then increase again to 5 µM at 13cm and subsequently decrease again steadily to 1 µM over the next 10 cm before rising again to 5.7 µM at 25 cm and continuing to vary between 1.4-6.2 µM, down to the base of the core. This increase at 25 cm coincides with the increase in nitrate levels described above again indicating a potential flow of groundwater at this depth. Alternatively the presence of nitrate at this depth might be acting as an electron acceptor allowing for the anaerobic oxidation of sulphide (Fossing *et al.* 1995), which in turn could lead to increasing thiosulphate concentrations.

Methane concentrations (Fig. 4.7) showed an initial decrease from 3.5 μmol l^{-1} of wet sediment at 1 cm to 0.75 μmol lws^{-1} at 5 cm. Levels then increased gradually to 6.15 μmol lws^{-1} at the base of the core. This increase in methane with depth below 5 cm coincides with the switch from dysoxic to anoxic conditions described above and indicates that methanogenesis is occurring in these sediments. To determine which methanogenic metabolisms were occurring in these sediments rates were measured using radiolabelled ^{14}C-acetate and ^{14}C-HCO_3^-. Overall methanogenesis rates were low compared to sulphate reduction – on the picomolar rather than nanomolar scale. The acetoclastic methanogenesis rate profile shows an initial decrease with depth, from 0.4 pmol cm^{-3} d^{-1} at 1cm down to zero at 13cm. Rates then increase again to 0.3 pmol cm^{-3} day^{-1} at 19 cm before decreasing to zero at 25 cm and then rising again to a peak of 0.42 pmol cm^{-3} d^{-1} at the base of the core. The reason for this variation with depth is unknown as the results do not match the porewater acetate profile, and in fact some of the lowest rate values occur between 5-13 cm when acetate is at it's highest concentrations. H_2/CO_2 methanogenesis rates showed a peak in activity between 5-9 cm (maximum value of 0.69 pmol cm^{-3} d^{-1} at 9 cm) though little activity occurring elsewhere. This peak occurs just below the peak in SRR suggesting possible competition between the two groups for common substrates (eg. H_2). The higher rate of lithotrophic methanogenesisis compared to organotrophic methanogenesis is to be expected since H_2/CO_2 methanogenesis is thought to dominate over acetoclastic methanogenesis in marine environments (Whiticar *et al.* 1986) though the rates are still quite low overall.

Hydrogen concentrations were relatively uniform in the top 27 cm of the core with values showing a slight increase with depth from 1.5 μmol lws^{-1} at 1 cm to 2.2 μmol lws^{-1} at 27 cm. The only departure from this trend was around 17 cm where values rose to 3 μmol lws^{-1}, though the reason for this peak remains unknown as it does not appear to match any of the other profiles. Below 27 cm H_2 concentrations decreased again to below detection limits at 31 cm. This decrease coincides with the peak in acetate below 25 cm and maybe evidence that this peak is indeed the result of homoacetogenic activity.

CO_2 concentrations show a decrease with depth from 500 μmol lws^{-1} at 1 cm to below detection limits at 23 cm. Below this concentrations become more variable

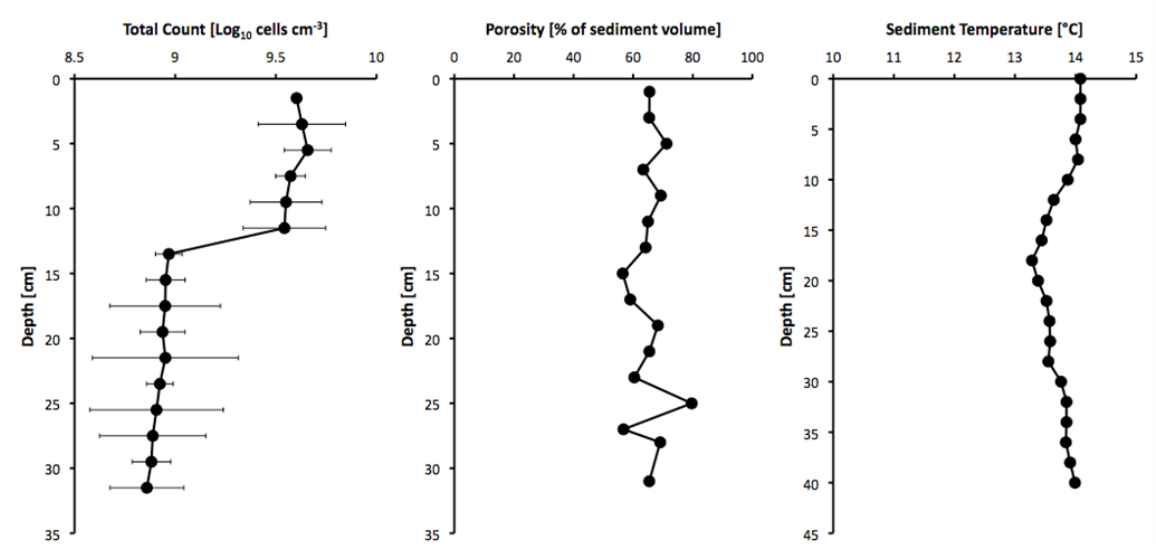


Fig. 4.8 - Profiles obtained from core ST4 showing changes in: total cell counts (AODC), sediment porosity and temperature with depth. Error bars represent the 95% confidence limits obtained from repeated counts.

with no overall trend with depth. The reason for this initially decreasing profile may be linked to consumption by autotrophic processes, as it broadly matches the trends in the H_2/CO_2 methanogenesis and acetate profiles, potentially suggesting consumption by lithotrophic methanogens and homoacetogens.

Porosity values for this core (Fig. 4.8) were relatively constant with depth (65.5% at 1cm and 65.4% at 33 cm) indicating that the sediment had probably not been re-suspended recently. There was an increase in pore water content at 25 cm (79%), which may indicate some flow-through of pore water at this depth. Total cell counts show a distinct change in population size between 11.5-13.5 cm, with values at and above 11.5 cm averaging $1x10^{9.5}$ cells cm^{-3} and values at and below 13.5 cm averaging $1x10^{8.9}$ cells cm^{-3}. This abrupt change in cell numbers is odd considering there seems to be little evidence for re-suspension at this site. However it may be due to increased numbers of more redox-positive organisms (aerobes, nitrate, iron and manganese reducers) in the oxidised sediment above 11.5 cm compared to lower numbers of more redox-negative organisms (SRB, methanogens and homoacetogens) in the darker reduced sediments below 13.5 cm. Alternatively since the change in cell numbers occurs at around the same depth that sulphate reduction and methanogenesis rates decrease, it may reflect a significant reduction in the size

of their communities, in some cases possibly due to a decrease in the availability of more labile OC compounds with depth.

4.2.2 Biogeochemistry of Core ST5

Acetate concentrations in core ST5 (Fig. 4.9), showed an initial decrease with depth from 21.5 μM at 1 cm to a minimum of 8.8 μM at 5cm, indicating consumption by heterotrophic bacteria. Levels then increased drastically into the peat layer, with concentrations reaching 445 μM at the base of the core suggesting that a high degree of acetogenesis (either via fermentative or homoacetogenic pathways) was taking place. Concentrations of lactate varied between 20-28 μM in the top 11 cm of the core however, below this in the peat layer levels rose to a peak of 52 μM at 13 cm again indicating that fermentation is occurring within the peat. Formate decreased with depth from 81 μM at 1 cm down to 56 μM at 11 cm before rising to 209 μM at 13 cm again, indicating heterotrophic consumption followed by fermentation. This increase in fermentation is likely driven by the presence of the peat layer, as the intact plant material contained within it is likely to be a rich source of the fatty acids, sugars and amino acids (and their precursors) utilized by fermentative bacteria. Propionate levels exhibited a different pattern to the other VFAs, initially increasing from 2.9-3.3 μM between 1-3 cm. Below 3 cm concentrations dropped rapidly to 0.012 μM at 5 cm and stayed around these levels until 11 cm at which point they rose to a maximum of 3.7 μM at the base of the core.

Chloride, sodium and bromide all showed a slight decrease with depth within the mud and upper centimetres of the peat, from 378 mM (chloride), 319 mM (sodium) and 474 μM (bromide) at 1 cm to 355 mM (chloride) 300 mM (sodium) and 443 μM (bromide) at 9 cm. Below this, at 13 cm, levels rose again slightly to 361 mM (chloride), 303 mM (sodium) and 446 μM (bromide) indicating a potential flow of less saline groundwater at depth within the core, possibly along the top surface of the peat. Potassium exhibited a similar trend, with an overall decrease in values down to 9 cm followed by an increase into the peat layer, again indicating potential groundwater flow along the mud/peat interface. Calcium on the other hand showed

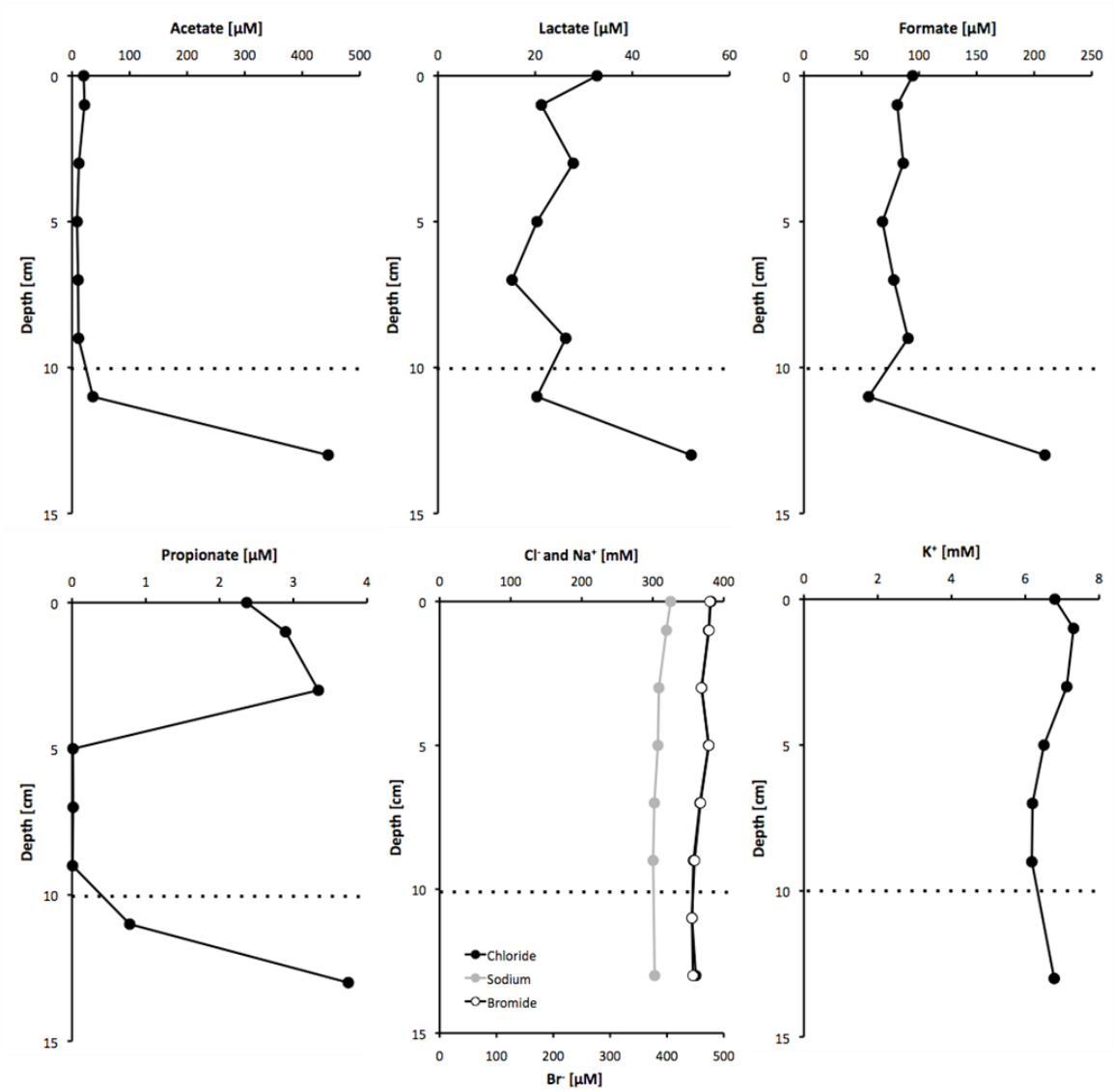


Fig. 4.9 -Biogeochemical profiles obtained from core ST5 showing changes in the concentrations of pore water: organic acids, chloride, sodium, bromide and potassium with depth. The black dashed line indicates the mud:peat boundary.

a decrease in values down to a minimum at 3 cm followed by a gradual increase with depth. (Fig. 4.10).

Nitrate concentrations peaked at 37 µM in the overlying waters before decreasing rapidly to 6.9 µM at 1cm and subsequently to a minimum of 0.3 µM at 5cm, indicating that denitrification/dissimilatory nitrate reduction to ammonium was occurring. Concentrations then increased to 5.9 µM at 9 cm and then rose again dramatically to 24.4 µM at 13 cm. As in Core ST4, this could indicate the possibility of anammox metabolism within the peat or may instead be caused by the

heterotrophic degradation of the peat releasing nitrogen-containing compounds into the pore waters (see Chapter 7).

Ammonium initially increased with depth from 15 μM at 1 cm to a maximum of 485 μM at 9 cm before decreasing again to 357 μM at 13cm in the peat. The concave upward shape of the ammonium profile between 0-7 cm again indicates that nitrification was likely occurring at this depth (despite the lack of a clear nitrate peak). If this is the case then it would mean that the dysoxic zone in these sediments extended to between 5-7 cm depth. The decrease in ammonium in the peat layer, which occurs at the same depth as the nitrate increase, again hints to the possibility of anammox at this depth, as the amount of nitrate produced during anammox is roughly 15-19% of the amount of nitrite oxidised - and therefore ammonium as it is a 1:1 stochiometric ratio - (Strous *et al.* 1998; Egli *et al.* 2001). In this case the increase in nitrate at 13 cm (22 μM) is approximately 17% of the decrease in ammonium observed (128 μM). Anammox is thought to be relatively common in estuarine environments (Nicholls and Trimmer, 2009; Rooks *et al*. 2012) however, it is important to note that for this relationship to work it is likely that nitrite would have to be present in these sediments at similar levels to ammonium, however no such nitrite levels were observed and as such the results remain inconclusive.

Phosphate did not appear consistently throughout core ST5 with concentrations only detected in the surface waters (1.4 μM), at 3 cm (1 μM) and 13 cm (1.5 μM). The reason for the presence of phosphate at 3 cm is unknown as no sulphate reduction was detected at this depth and the brown colouration of the sediment indicated that there was probably no sulphide present. The presence of phosphate at 13 cm is more easy to explain however as the peat, as described above, gave off a strong sulphidic smell during sectioning. As such it is likely that there was abundant sulphide in the peat to complex with any iron minerals present allowing for the release of phosphate.

Unusually for environmental samples methylated-amine compounds were detected at the base of core ST5. Both dimethylamine (DMA) and trimethylamine (TMA) were detected at concentrations of 65.4 μM and 1.9 μM respectively within the peat at 13 cm. Choline was also detected at 3 cm (11.3 μM) and again at 13 cm (2.2 μM). Due to the depth at which they occur, the source of the DMA and TMA in this core is likely

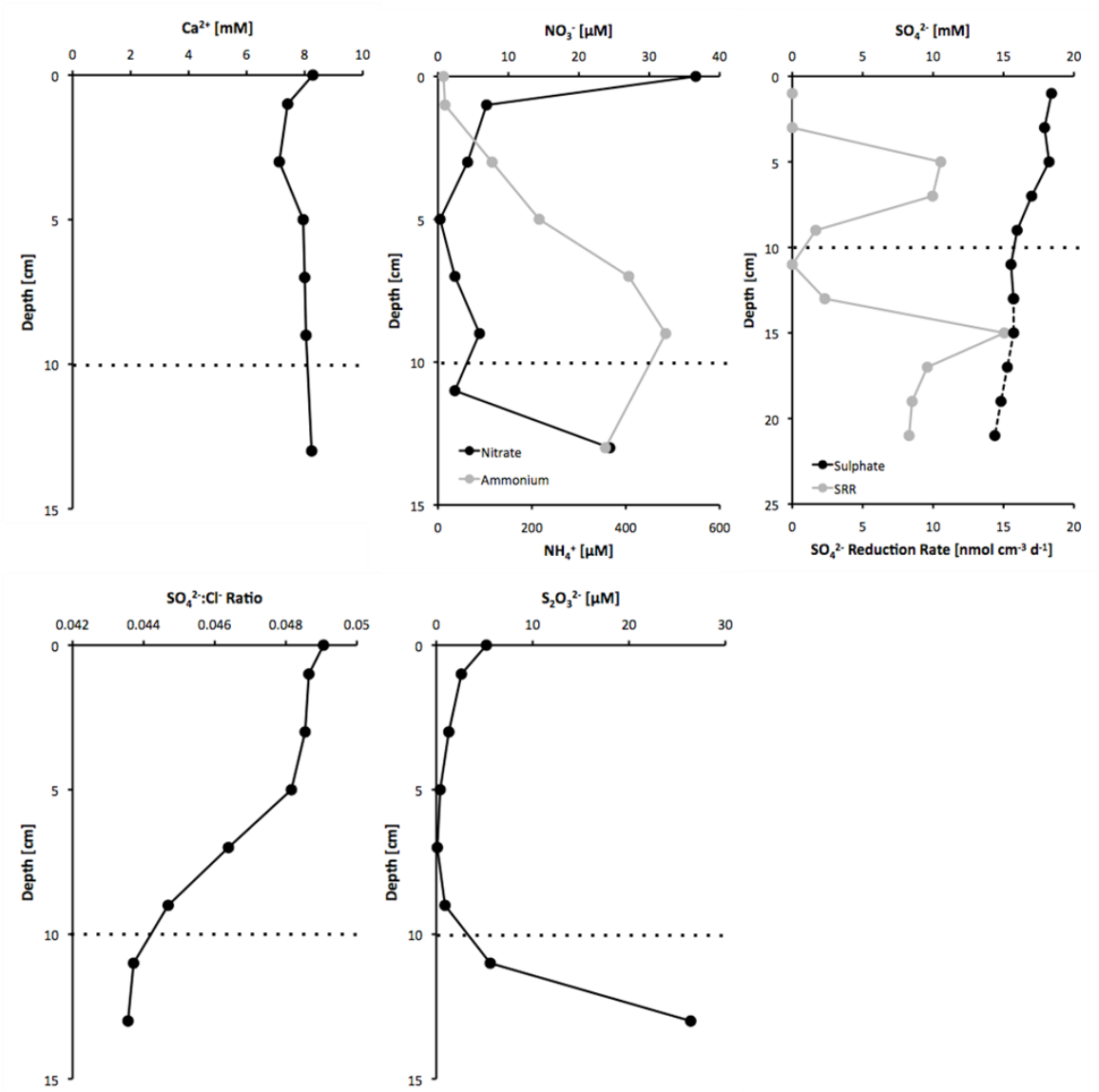


Fig. 4.10 - Biogeochemical profiles obtained from core ST5 showing changes in porewater concentrations of: calcium, nitrate, ammonium, sulphate and thiosulphate with depth. Also shown are profiles for the sulphate:cloride ratio and rates of *in situ* bacterial sulphate reduction (SRR). Note that the profile for SRR is longer than the others as the core duplicates used for radioisotope work were longer than that which was used for the porewater analyses. As such sulphate values below 13 cm (represented by the dashed line on the profile) were derived from data obtained from core StB1, taken from the same site ≈1 month after core ST5 was sampled. The horizontal black dashed line indicates the mud:peat boundary.

to be related to the peat layer in some way – for example it is possible that they are released during degradation of the vegetation contained within the peat (Wang and Lee, 1994). In the case of the case of choline on the other hand, since it was detected in the overlying sediments as well it is likely to be derived from other, more contemporary sources of organic matter – such as from the breakdown of bacterial cell membranes (Watkins *et al.* 2012). The potential sources of these compounds

and the role that they play in the biogeochemistry of the sediments at this site will be further expanded upon in Chapter 7.

Sulphate concentrations decreased with depth, from 18.4 mM at 1cm down to 15.7 mM at 13 cm. In addition the SO_4^{2-}:Cl^- ratio also decreases with depth indicating that the decrease in sulphate is not solely linked to a drop in salinity and therefore that sulphate reduction is occurring within the sediments. Both the sulphate and SO_4^{2-}:Cl^- profiles show a distinct change in gradient between 5-11 cm, indicating that sulphate reduction is stimulated above the mud/peat boundary and then decreases again in the upper 3 cm if the peat. This is confirmed by the $^{35}SO_4^{2-}$ sulphate reduction rate measurements which show an initial increase from zero at 1 cm to 10.5 nmol cm^{-3} d^{-1} at 5 cm before decreasing down to zero again at 11 cm. Moving further down into the peat, rates rise to a peak of 15 nmol cm^{-3} d^{-1} at 15 cm before gradually decreasing to 8.3 nmol cm^{-3} d^{-1} at 21 cm (measurements of SRRs extend to a greater depth than other measurements as the duplicate core used for radiotracer analysis was longer than the other cores taken – c. 22cm). The increase in rates (and change in SO_4^{2-} and SO_4^{2-}:Cl^- profile shape) between 5-10 cm correlates broadly with the ammonium profile, again suggesting that full anaerobic conditions do not occur in these sediments until 5-7 cm depth. The decrease and subsequent increase in rates within the peat would seem to indicate that the peat layer (at least near it's top surface) can both suppress and stimulate sulphate reduction. This phenomenon will be discussed again in detail later in Chapter 7 but may be linked to the higher levels of acetate and other organic acids found in the peat, which would make ideal e^- donor substrates for the SRB, coupled with potentially higher levels of humic compounds and sulphide that might inhibit their growth. Additionally as with core ST4 the two peaks could represent two separate communities of SRB, one in the overlying muds consuming fresh, labile OC and another below consuming older, more refractory peat-derived compounds.

Levels of thiosulphate showed an initial decrease with depth, from 2.6 μM at 1cm depth to 0.1 μM at 7 cm, indicating reduction or disproportionation by SRB (levels were lowest where SRRs were at their highest in the muddy sediments) – no near-surface peak, like the one seen in core ST4 was detected likely due to the lack of sulphide in the oxidised overlying muds. Concentrations then increased into the

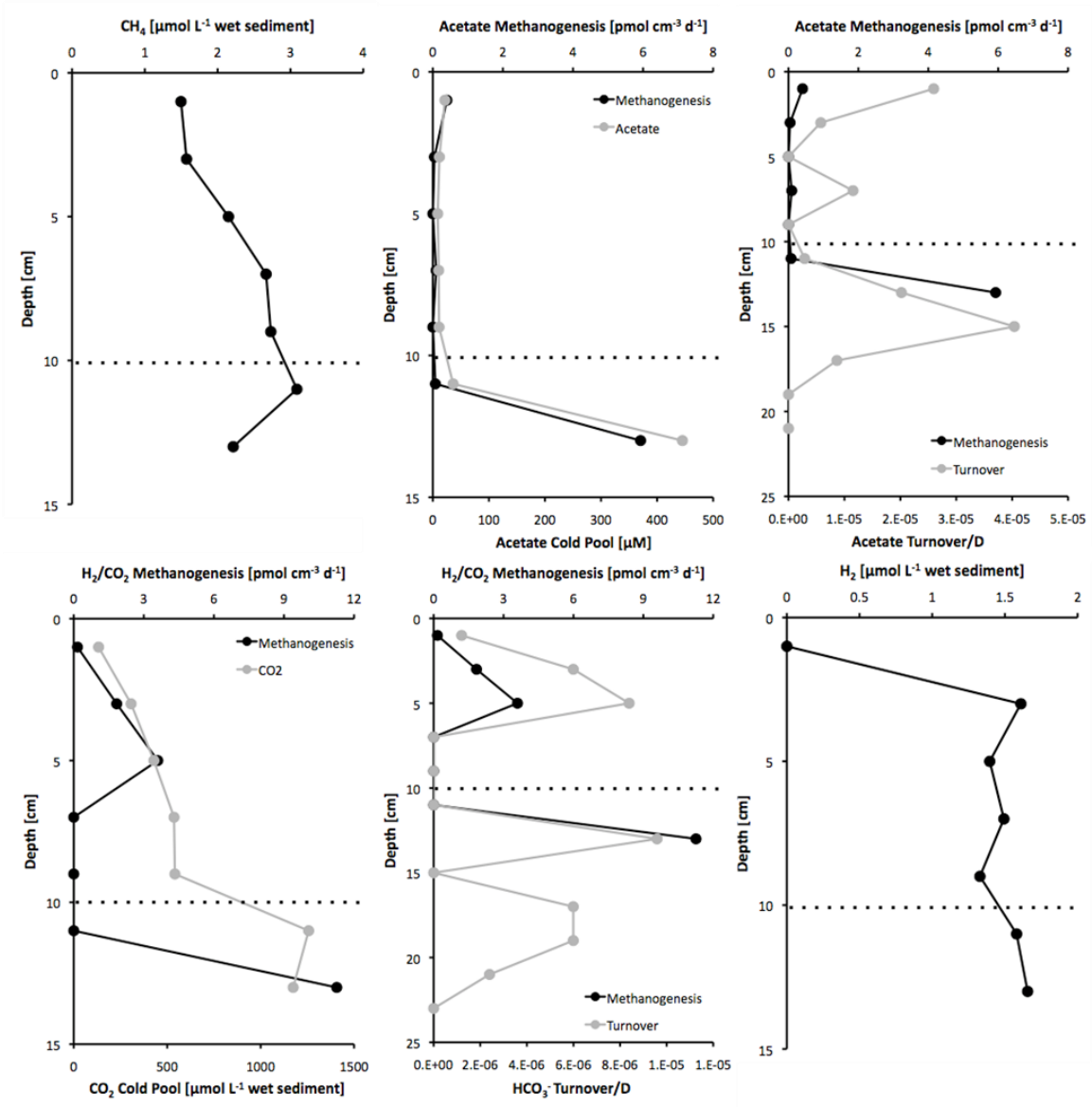


Fig. 4.11 - Geochemical profiles obtained from core ST5 showing changes in the concentration of: methane, hydrogen and CO_2 with depth. Also shown are rates of *in situ* acetoclastic and H_2/CO_2 methanogenesis plotted with the concentrations of their respective cold pool substrates as well as the turnover rate of their radiolabeled substrates to methane. The black dashed line indicates the mud:peat boundary.

peat layer, reaching a maximum of 26.4 µM at 13 cm. The cause of this increase is unknown but may relate to sulphide oxidation and will be discussed further in Chapter 7.

Methane concentrations were low overall in core ST5 (Fig, 4.11), but did show an increase from 1.5 µmol l^{-1} of wet sediment at 1 cm to a maximum of 3.1 µmol lws^{-1} at 11 cm before decreasing back down to 2.2 µmol lws^{-1} at the base of the core (in a similar profile to ammonium). Despite these low *in situ* methane concentrations radiotracer experiments showed that active methanogenesis was occurring within

these sediments. As in core ST4 rates were low compared to sulphate reduction. Rates of acetoclastic methanogenesis decreased from 0.4 pmol cm^{-3} d^{-1} at 1cm to zero at 5cm. Below this, rates stayed between zero and 0.09 pmol cm^{-3} d^{-1} until 13cm at which point rates increased to 6 pmol cm^{-3} d^{-1} indicating that the peat layer at the base of the core may be stimulating methanogenesis (again likely due to the elevated concentrations of acetate within it). As with the SRRs, acetate turnover measurements for core ST5 extend to a greater depth than the geochemistry and indicate that methanogenesis continued to increase down to 15 cm. Below this depth turnover decreased, down to zero at 19 cm indicating that acetoclastic methanogenesis was not occurring within the main body of the peat bed (at least not on the timescales used in this experiment). This decrease below 19 cm also means that the depth integrated methanogenesis data shown below in section 4.3 may not be too great an underestimation of the true values. H_2/CO_2 methanogenesis was also present within core ST5, both within the peat and the overlying muds. Rates showed an initial increase with depth, reaching a peak of 3.6 pmol cm^{-3} d^{-1} at 5 cm below which values decreased down to zero at 7 cm. Within the peat rates rose to a maximum of 11.3 pmol cm^{-3} d^{-1} at 13 cm, again suggesting that the presence of the peat might be enhancing H_2/CO_2 methanogenesis in the core, this time due to increased hydrogen and CO_2 concentrations (see below).

Hydrogen first appeared at 3 cm in core ST5 at a concentration of 1.6 μmol lws^{-1}. Below this depth concentrations began to decrease slightly, likely due to the metabolism of hydrogenotrophic SRB and H_2/CO_2 methanogens, reaching a minimum of 1.3 μmol lws^{-1} at 9 cm. Going down into the peat values increased again, reaching a maximum value of 1.7 μmol lws^{-1} at 13 cm. This increase could be caused by the apparent inhibition of sulphate reduction in the upper 1-3 cm of the peat or could be due to increased levels of hydrogen formation relating to the elevated amounts of fermentative processes that appear to be occurring in the peat. CO_2 concentrations show a gradual increase within the mud layer, from 133 μmol lws^{-1} at 1 cm to 541 μmol lws^{-1} at 9 cm, likely due to production via heterotrophic respiration. Moving into the peat layer values increase dramatically to a maximum of 1.3 mmol lws^{-1} at 11 cm probably due to the increased levels of fermentation and organic matter degradation at this depth (also seen in the ammonium profile).

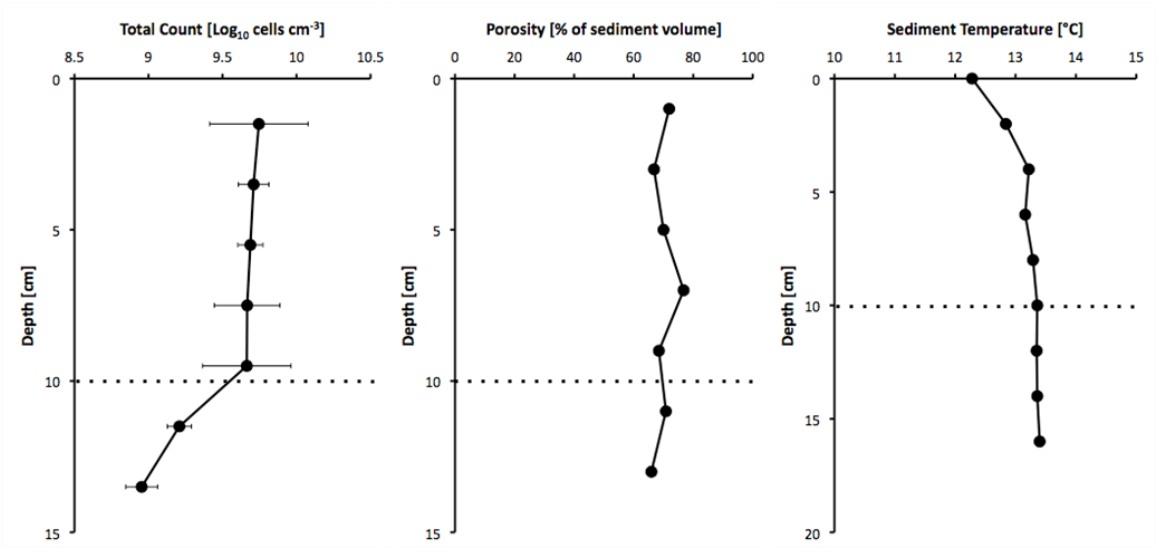


Fig. 4.12 - Profiles obtained from core ST5 showing the changes in: total cell counts (AODC), sediment porosity and temperature with depth. Error bars represent the 95% confidence limits obtained from repeated counts.

Porosity values for this core decreased gradually with depth (Fig. 4.12), from 71.9% at 1 cm to 66% at the base of the core, except at 7 cm depth where porosity increased to a maximum of 76.8%. Total cell counts showed a similar profile to those in core ST4 with numbers in the top 9.5 cm averaging at $1x10^{9.6}$ cells cm^{-3} below which numbers dropped to $1x10^{8.9}$ cells cm^{-3} at the base of the core. However, unlike at Portishead, here the explanation for the sudden drop in numbers has a more obvious explanation and is likely linked to the shift from recently deposited sediment to the ≈3400 year old peat.

4.3 Severn Estuary Tidal Flats, Part 1 - Discussion

As has been detailed above both the lithology and geochemistry of these two sites differ considerably. Since the peat layer present in core ST5 does not occur at Portishead a direct comparison based purely on location cannot be made (it is unknown whether any differences seen in the cores would be driven by the location of the sites or their by their differing lithologies). Nevertheless since the peat beds are a major component of the lithology on the Welsh side of the estuary it is still interesting to compare the sites to determine how different the biogeochemistry of the sediments is on the northern and southern shores.

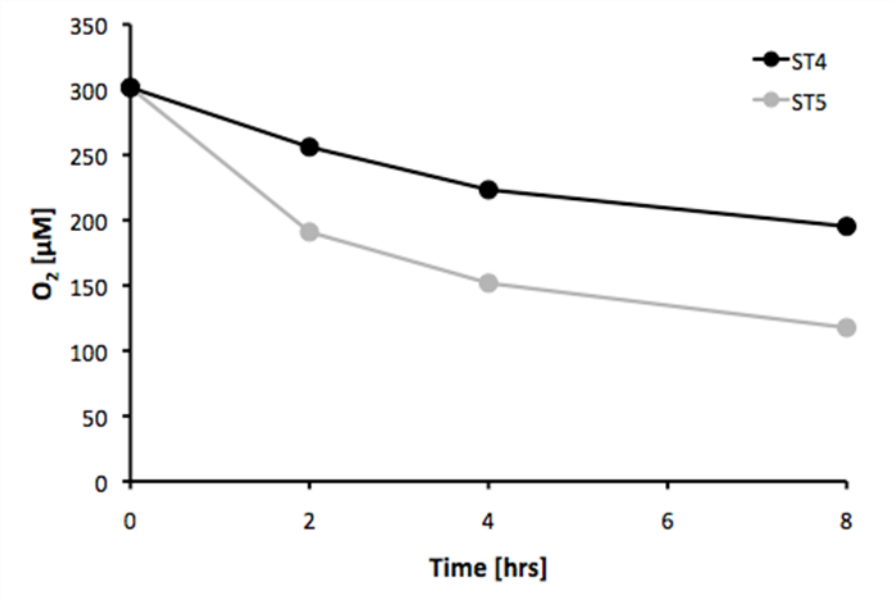


Fig. 4.13 - Line graph showing the consumption of oxygen by copies of cores ST4 and ST5 incubated in darkness at *in situ* temperature for 8 hours. Data obtained from the decrease in oxygen concentrations in the overlying waters of the cores corrected for loss by planktonic heterotrophic activity.

Total oxygen consumption by an incubated duplicate of core ST4 was quite high with concentrations of dissolved oxygen (DO) in the overlying water of the core decreasing from 302 µM to 198 µM after 8 hours (Fig. 4.13), this equates to a sediment oxygen demand (SOD) of 52 mmol m^{-2} d^{-1}. Oxygen consumption by the incubated ST5 replicate on the other hand was higher than in core ST4 with DO concentrations in the overlying water decreasing from 302 µM to 121 µM after the 8 hour incubation – equating to an O_2 uptake rate of or 81 mmol m^{-2} d^{-1}, 1.6x higher than in ST4. This increased rate of O_2 consumption at St Brides could potentially be attributed to the more exposed aspect of the tidal flats at this site which might lead to greater erosion and re-deposition of fresh sediment (the higher porosity values for the surficial muds at this site may also indicate more recent deposition). This increased turnover of the sediment coupled with the higher levels of bioturbation at this site compared to Portishead could lead to more of the sediment column becoming oxic/dysoxic. This would allow an increased number of mircoorganisms dependant on O_2 (aerobes, nitrifiers etc.) to exist within the sediments at St Brides, as well as increasing the amount of chemical oxidation of reduced compounds (S^{2-} NH_4^+ etc.) occurring in the sediments, leading to a greater demand for O_2 (the higher numbers of macrofauna carrying out the bioturbation would also increase the O_2

demand relative to Portishead). Alternatively the increased erosion and re-deposition of the sediments might lead to increased levels of labile OC burial, with fresh organic matter being mixed deeper into the sediment column than would usually occur via conventional sedimentation. This in turn could lead to an increased rate of microbial metabolism (though not necessarily across the board – see below) resulting in the higher O_2 uptake values.

With regard to sulphate reduction, the values obtained from Portishead produce a depth integrated total sulphate reduction rate of 4.7 mmol m^{-2} d^{-1}, compared to 1.31 mmol m^{-2} d^{-1} from St Brides. This difference in values can be attributed to two factors, the first of which is the differing lengths of the cores. Since depth integrated values, as their name suggests, take depth into account during calculation the longer a core is, the larger the potential total sulphate reduction rate can be. As such if two cores with similar sulphate reduction rates but different lengths are compared, the shorter core will produce the lower total result. To avoid this problem, the average of the individual depth zone values of sulphate reduction (expressed as mol cm^{-3} d^{-1}) over the same depth can be compared between cores. Doing this results in an average sulphate reduction rate of 15.5 nmol cm^{-3} d^{-1} for the top 21 cm of ST4, 2.6x higher than the rate of 6 nmol cm^{-3} d^{-1} obtained from ST5. If only the muddy sediments at each site are compared the difference becomes even starker, with an average rate of 22 nmol cm^{-3} d^{-1} in the top 9 cm in core ST4, a value 5x higher than the 4.4 nmol cm^{-3} d^{-1} from ST5. This shows that even with core depth and lithology taken into account average sulphate reduction at Portishead is 2-5 fold higher that at St Brides. This difference can again be linked to the more exposed aspect of the St Brides site and its increased levels of sediment re-suspension. As has already been mentioned, the higher degree of re-suspension (and bioturbation) at St Brides would have increased the amount of O_2 and other oxidised compounds (NO_3^-, Fe^{3+} etc.) in the sediments. This in turn would have raised the redox state of the sediment, which would have made it more difficult for the anaerobic SRB to grow (due to their metabolism requiring a more negative environmental redox potential) resulting in lower *in situ* sulphate reduction rates. This suppression of lower-redox potential anaerobic processes could potentially also cancel out any increase in rates driven by an increase in labile OC burial like that described above. This therefore

could explain why a possible increase in sediment OC, that should theoretically stimulate heterotrophic microbial activity across all redox states, appears to be increasing O_2 uptake over sulphate reduction.

Depth integrated rate measurements for acetoclastic methanogenesis also differed between sites with total rates of 58 nmol m^{-2} d^{-1} for Portishead, 2.3x lower than the 131 nmol m^{-2} d^{-1} obtained for St Brides. However, since rates were increasing at the base of core ST4, it is likely that the zone of methanogenesis extends beneath the base of the core and as such this rate is likely to be an underestimation of the true depth integrated methanogenesis rate at this site. H_2/CO_2 methanogenesis follows a similar pattern with depth-integrated rates at St Brides (503 nmol m^{-2} d^{-1}) substantially higher (≈15x) than at Portishead (33.5 nmol m^{-2} d^{-1}). However, this higher total rate of methanogenesis at St Brides is largely due to the sharp increase in rates within the top of the peat layer. When the methanogenesis rates in the top 9 cm at both sites are compared it can be seen that the average rate of acetoclastic methanogenesis at Portishead is 171 fmol cm^{-3} d^{-1}, 1.7x higher than the 102 fmol cm^{-3} d^{-1} from St Brides. This would indicate that the muds at Portishead are a better habitat for acetoclastic methanogens than those at St Brides, likely due to the more settled sediment regime at that site. However, when it comes to H_2/CO_2 methanogenesis, average rates in the top 9cm at St Brides (1119 fmol cm^{-3} d^{-1}) remain significantly higher (≈4x) than those at Portishead (295 fmol cm^{-3} d^{-1}), possibly suggesting that re-suspension has a greater influence on acetoclastic methanogenesis than on H_2/CO_2 methanogenesis.

As can be seen from the data the two sites described above show markedly different rates of biogeochemical processes with St Brides exhibiting (for the most part) increased rates of more redox-positive reactions while Portishead shows increased rates of more redox-negative reactions. With regard to how the sites' positioning on opposite sides of the estuary might affect the biogeochemistry, it would appear that topographical setting (sheltered bay vs. exposed mudflat) is the main differentiating factor as this controls variables such as wind and wave strength which in turn govern the amount and frequency of sediment re-suspension Since sheltered bays like Portishead are a feature restricted to the upper reaches of the estuary it is also

important to look at the more exposed mudflats that occur further to the west on the estuary's southern side in order to determine if re-suspension of sediment is the only factor governing respiration rates or whether the other factors described above (marine vs. riverine input etc.) also play a part. This will be done in Chapter 5.

Another important component of these cores that requires further study is the peat layer found at the base of core ST5. As described above this layer appears to be a significant source of methylated amines, compounds not usually found in abundance *in situ* due their rapid turnover by methanogens (Parkes *et al.* 2012), and as such further investigation of this peat is required in order to asses the source and degree of methylated amine metabolism occurring. In addition the peat also contains abundant levels of organic acids and as such appears to be an area of very active fermentation. Since organic acids can act as important substrates for both SRB and methanogens it is again important to investigate the prevalence of fermentation within these sediments in order to determine whether the rates of sulphate reduction and methanogenesis are in turn enhanced within the peat layer. This further investigation will be detailed in Chapter 7.

5.4 Severn Estuary Tidal Flats, Part 1 - Conclusions

- Despite existing on different sides of the same estuary, the mudflats at Portishead and St Brides Wentlooge exhibit noticeably different trends in the biogeochemistry of their sediments.
- Portishead (core ST4) appears to be a more stable environment with higher rates of anaerobic processes with low redox potentials such as sulphate reduction and acetoclastic methanogenesis.
- The sedimentary environment at St Brides (core ST5) appears to be more dynamic with higher rates of aerobic/dysaerobic processes with higher redox potentials as evinced by higher oxygen uptake.

The sediments at St Brides are underlain by a bed of ≈3400 year old estuarine peat which seems to be a significant source of compounds both utilised, and produced by prokaryotes (e.g. nitrate, thiosulphate, organic acids and methylated amines), that appear to be stimulating microbial metabolism.

Chapter 5. - Severn Estuary Tidal Flats, Part 2: Core ST6

5.1 Site Location and Lithology

Core ST6 was recovered in April 2013 from tidal flats southwest of the town of Clevedon, Somerset (Fig. 5.1). The sampling site was around 20 metres from the mean high water line, south of Gullhouse Point and north of Kingston Pill (OS grid reference ST 384628 693859; 51° 25' 12.5"N, 02° 53' 10.7"W). The tidal flats in this area are more extensive than those at Portishead to the northeast (≈540 m from high-low water line), mainly as they are not confined by the palaeochannel of the River Severn. The flats form part of a much larger area of mudflats and sandbanks known as the Langford Grounds, an area of shifting submarine topography infamous for shipwrecks, and are backed by a short expanse of salt marsh, behind which extend the low-lying marshlands of the North Somerset Levels (shown in Fig. 5.2).

The main reason for selecting this site was as a comparison with cores collected from other tidal flat localities around the estuary, namely core ST4 taken from Portishead and core ST5 from St Brides Wentlooge. The reason for comparing core ST6 with ST4 was the difference in topography between the two sites – an open, exposed flat vs. a sheltered bay. Since surrounding topography can have an effect on many of the physical processes occurring on a tidal flat (wave speed, current strength etc.) that can in turn affect the biogeochemistry of the sediments (Santos *et al.* 2012), it is important to consider these variables when looking at the Severn Estuary as a whole - especially as the Severn Estuary possesses a wide range of tidal flat environments including salt-marshes, sheltered bays, and large expanses of both coastal-fringing and open water mudflats. The comparison with ST5 on the other hand was designed to compare two mudflats with similar topographic settings but on different sides of the estuary in order to discern if the hydrology of the estuary played a part in influencing the sediment biogeochemistry.

With regards to lithology, the composition of core ST6 was fairly uniform with depth, being made up entirely of fine mud. The colour and cohesion of the mud did change with depth, from light brown and very fluid at the surface to dark grey/black and compacted at the base, indicating the presence of FeS - the colour change from brown to black occurred at around 15-17 cm. The upper brown layer

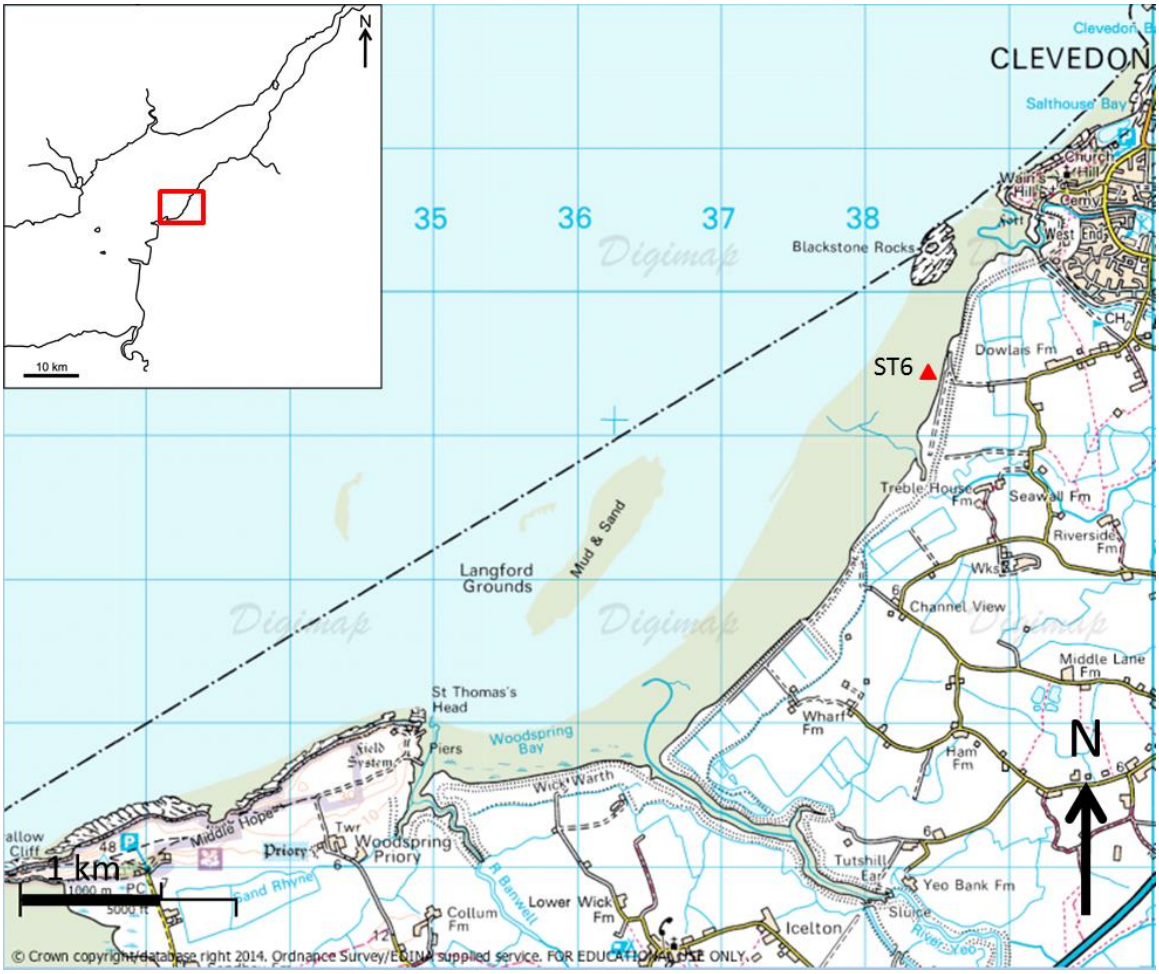


Fig. 5.1 – Ordnance Survey map showing the location of the sampling site for core ST7 (red triangle) near Kingston Pill, southwest of Clevedon, Somerset. Brown shading denotes intertidal mudflats exposed at low water. © Crown Copyright/database right 2014 Ordnance Survey/EDINA.

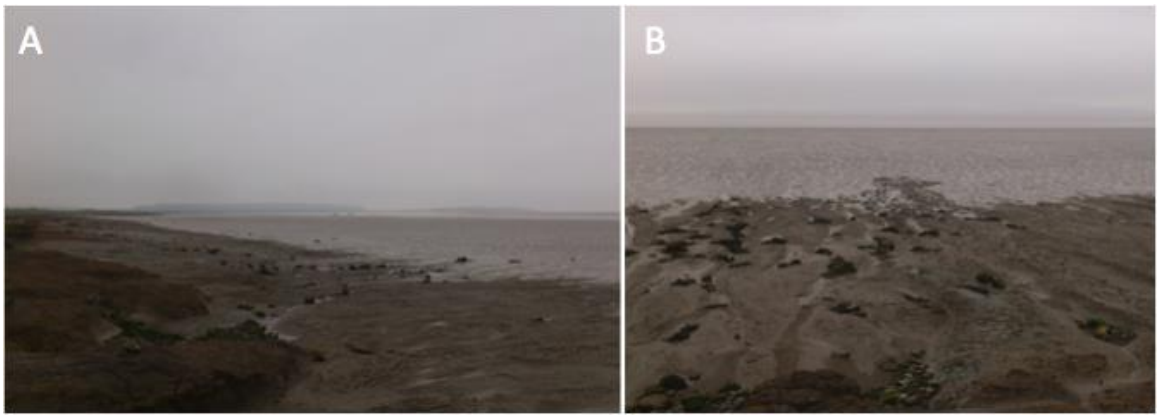


Fig. 5.2 - A) View of the mudflats southwest of Clevedon looking south from ST6 sampling site - showing eroded, former salt marsh deposits landward of mudflats. B) View of ST6 sampling site relative to the high water mark (base of photograph), showing drainage channels and transition from soft, freshly deposited sediment (top half of photograph) to older, more compacted material (bottom half of photograph).

was also heavily bioturbated (more so than ST4) and contained abundant polychaetes as well as small shallow-infaunal bivalve species.

5.2 Severn Estuary Tidal Flats, Part 2 - Results

5.2.1 Biogeochemistry of ST6

Acetate concentrations in core ST6 showed an initial decrease with depth, from 8.47 μM at 1cm depth down to 4.47 μM at 13 cm, (Fig. 5.3) likely connected to consumption by heterotrophic organisms. Below this, concentrations increased dramatically to 54.8 μM at 15 cm before dropping down to 8.7 μM at 17 cm. Levels stayed low down to 25 cm at which point they again rose sharply to a peak of 81 μM at 27 cm before dropping again to 6.15 μM at 33 cm and finally rising again to 53.5 μM at the base of the core (35 cm). This bi-peaked profile might indicate the presence of two distinct zones of acetogenesis, one at 15 cm and another below 25 cm, possibly linked to two different metabolic pathways (fermentation and homoacetogenesis respectively), while the gap between is again likely linked to consumption via heterotrophic processes – possibly sulphate reduction (see below). The fact that the upper of these two zones (at 15 cm) occurs just above the depth in the sediment where the colour change from brown to black occurs (signifying the change from oxidizing to reducing conditions) suggests that the two communities may also have different redox potentials, with the upper community being less sensitive to more positive redox conditions than the community at the base of the core. Alternatively the peaks in acetate may be linked to uncoupling of production and consumption processes, as the peaks also coincide with apparent changes in the terminal oxidizing communities (Jørgensen and Parkes, 2010). The upper peak (at 15 cm) coincides with the switch from nitrate reduction to sulphate reduction, while the lower peak (below 25 cm) coincides with a dramatic decrease in sulphate reduction rates (see below).

Lactate remained relatively constant at around 2.8 μM over the top 7 cm of the core however, below this, concentrations dropped dramatically down to zero at 15 cm before rising again to 1.6 μM at 17 cm and subsequently varying between 0-2.7 μM down to 35 cm. This profile would indicate an initial period of production followed by rapid consumption (again probably linked to heterotrophic processes)

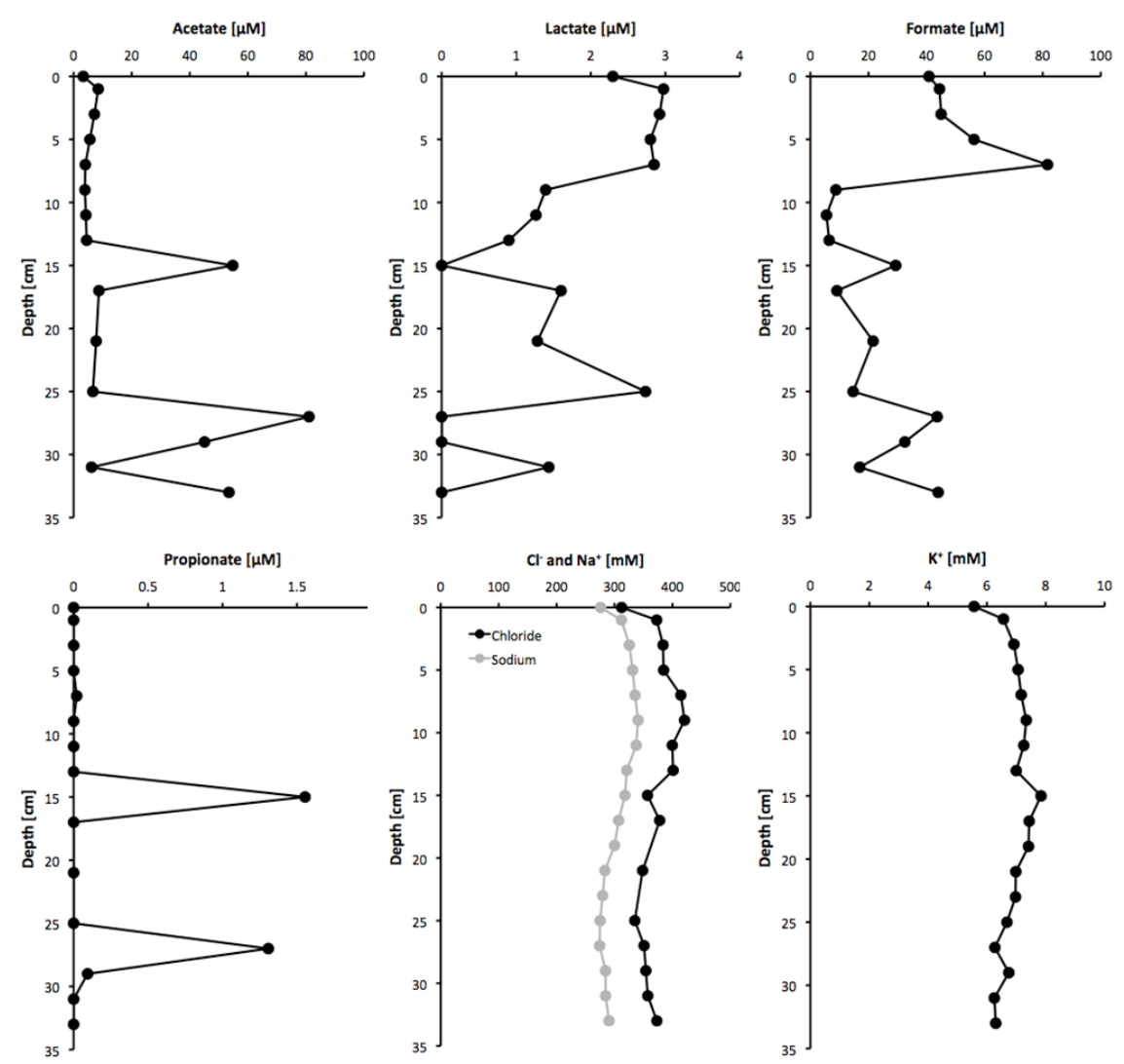


Fig 5.3 – Biogeochemical profiles obtained from core ST6 showing the changes in concentration of pore water: organic acids, chloride, sodium, and potassium with depth.

in the upper oxidized sediments and subsequent production via fermentation in the reduced sediments below.

Formate concentrations showed an initial increase with depth, from 40.8 µM at 1cm to a maximum of 81.7 µM at 7 cm, before decreasing rapidly to a low of 5.52 µM at 11 cm. Below this, levels gradually increased again with depth (though remained very variable) reaching 44 µM by the base of the core. Propionate was not detected consistently throughout the core, interestingly however, when it was detected at levels above 1 µM it was at 15 and 27 cm, the same depths at which the peak concentrations of acetate occurred suggesting a possible link between the processes producing both compounds (such as uncoupling of production and consumption).

Chloride concentrations showed an initial increase with depth from 373 mM at 1 cm to a maximum of 421 mM at 9cm. Levels then dropped down to a minimum of 335 mM at 25 cm before rising again to 373 mM at the core's base. Sodium followed a similar pattern although with rather less variance, rising from 312 mM at 1 cm to a maximum of 340 mM at 9cm. Levels then decreased to a minimum of 274 mM at 27 cm before rising to 290 mM at 33 cm. The initial increase in salinity with depth is likely caused by the diffusion of freshwater into the sediment from the small streams and tidal channels that ran across the sediment surface (possibly aided by bioturbation). The secondary decrease in salinity around 27 cm depth is possibly related to groundwater flow from inland and may also be responsible for injecting other compounds such as nitrate (see below) into the deeper layers of the sediment. Potassium values exhibit a different pattern, with an increase in concentrations from the surface down to ≈10 cm followed by a subsequent decrease in concentrations from ≈15 cm down to the base of the core. This change in gradient seems to correspond to the colour change in the sediment from brown to black and may therefore indicate that the change in redox state of the sediment at this depth is affecting the potassium levels in the pore waters. Calcium on the other hand shows a much more linear profile, indicating a gradual increase in concentrations with depth, though the reasons for this remain unknown (Fig. 5.4).

Nitrate concentrations show an initial decrease from 2.1 μM to below detection limits in the top 3 cm before dramatically increasing to 17.9 μM at 5cm. Levels then decrease gradually down to 0.9 μM at 17 cm before rising dramatically again in the lower levels of the core, reaching 13.1 μM at 25 cm and then gradually decreasing again toward the base of the core. This is very unusual, as nitrate is usually depleted close to the surface due to the combined effects of denitrification and dissimilatory nitrate reduction to ammonium (DNRA). The subsurface nitrate peak seen at 5 cm is likely caused by the nitrification of ammonium diffusing up from deeper in the sediment. Nitrification requires oxygen and therefore usually only occurs in the very topmost layers of the sediment column (0-2 cm) where oxygen can diffuse into the sediment. However, as detailed above the upper 5-10 cm of the core were loosely consolidated and heavily bioturbated by polychaetes and bivalves which would have

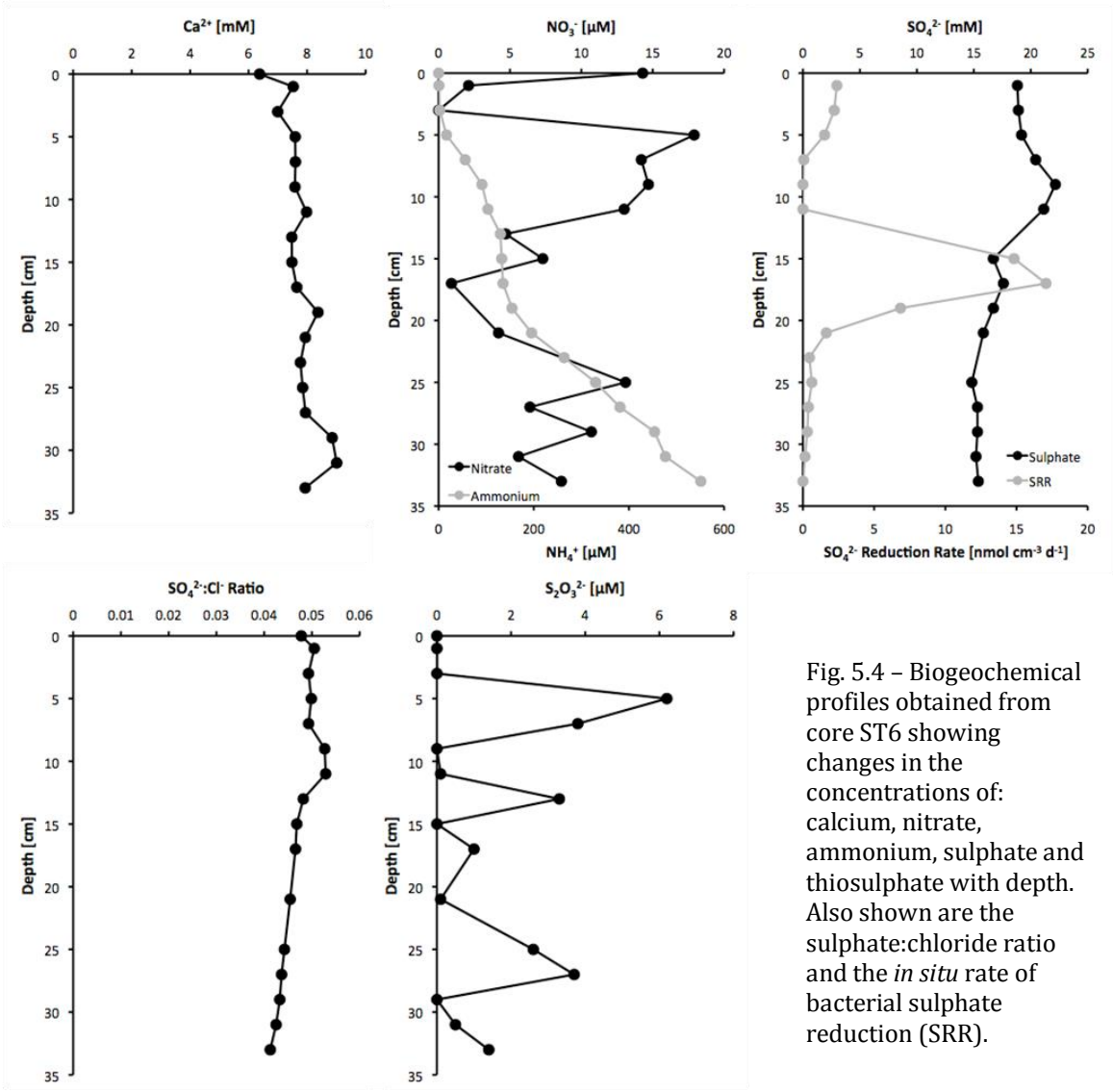


Fig. 5.4 – Biogeochemical profiles obtained from core ST6 showing changes in the concentrations of: calcium, nitrate, ammonium, sulphate and thiosulphate with depth. Also shown are the sulphate:chloride ratio and the *in situ* rate of bacterial sulphate reduction (SRR).

likely allowed O_2-rich seawater to be mixed deeper into the sediment, both by tidal and current activity and also possibly via bioirrigation, thus facilitating nitrification. The subsequent decrease in nitrate levels below 5 cm is due to the action heterotrophs carrying out DNRA and/or via denitrification. The secondary peak in nitrate concentrations at 25 cm is unusual as there is one main biological process capable of producing nitrate at this depth, namely anaerobic ammonium oxidation, otherwise known as anammox (Strous *et al.* 1998; Egli *et al.* 2001). Anammox is considered to be quite an important process within estuarine and shelf sediments and can play a major role in nitrogen cycling (Crowe *et al.* 2012; Teixeira *et al.* 2012; Bale *et al.* 2014; Teixeira *et al.* 2014) - though it's relative contribution is debated in other marine environments (Koop-Jakobsen and Giblin 2009; Trimmer *et al.* 2013). In this case however, neither a drop in ammonium concentrations (of ≈100 µM) or

the presence of nitrite (two signatures/requirements of anammox) are indicated at this depth (Meyer *et al.* 2005), suggesting that this process may not be responsible for the increase in nitrate. The nitrate peak at 25 cm does however, coincide with the drop in chloride concentrations mentioned above. As such it may be possible that groundwater flow from further inland is supplying nitrate to the deeper sediment layers (Capone and Slater, 1990), which is also (judging by the decrease in nitrate below 25 cm) stimulating a second zone of DNRA/denitrification near the base of the core (Papaspyrou *et al.* 2014).

Ammonium concentrations in the top 9 cm of the core exhibit a concave-upward profile which would corroborate the suggestion that nitrification is active in the upper layers of these sediments. Below 9 cm levels of ammonium increase gradually, reaching 135 μM by 17 cm. Below this depth however, concentrations increase again much more rapidly reaching 551 μM at the base of the core. This change in the ammonium gradient with depth roughly coincides with the change in sediment colour and is possibly caused by a switch from DNRA in the upper more oxidized layers (≈9-17 cm) to a more enhanced breakdown of organic matter containing amino-groups by heterotrophic bacteria (deamination/ammonification) in the reduced sediments nearer the base of the core (≈19-33 cm).

Sulphate concentrations down-core exhibited a similar trend to chloride, with an initial increase from 18.8 mM at 1 cm to a maximum of 22.2 mM at 9 cm, before decreasing again down to 15.4 mM at the base of the core. The SO_4^{2-}:Cl^- ratio also showed this initial increase and subsequent decrease which suggests that it was not solely linked to variations in salinity and that other biogeochemical processes were involved. It is likely that the initial increase in the top ≈7-11 cm is linked to the oxidation of upwardly diffusing sulphide to sulphate, as the sulphate peak exists at a similar depth to the concave-upward ammonium profile suggesting that that oxygen (at least in small amounts) is present at this depth. Below 11 cm the sulphate profile shows a pronounced decrease with depth down to 25 cm followed by slight increase down to the bottom of the core (at a similar depth to the second decrease in nitrate). However, since chloride values vary considerably with depth in this core (as described above), and considering that sulphate concentrations are closely tied to salinity, in this case it is important to examine the SO_4^{2-}:Cl^- ratio before drawing any

conclusions. In this case the SO_4^{2-}:Cl^- ratio shows a gradual decrease down to the base of the core indicating that sulphate reduction is occurring in these sediments.

This is backed up by the SRR radiotracer measurements which indicate two zones of active sulphate reduction, one at 1-5 cm where rates are around 2 nmol cm^{-3} d^{-1} and another between 15-25 cm where rates peak at 17 nmol cm^{-3} d^{-1}. The upper zone of sulphate reduction is within what could be considered the oxidized oxic/dysoxic zone of the sediment and as such it is likely that any reduction occurring here is probably happening within anoxic microniches (Jørgensen, 1977) possibly located inside the faecal pellets of the polychaetes living in the sediment or other particles of organic matter. The second larger zone of sulphate reduction is beneath the dysoxic zone boundary (at ≈11 cm) and well within the anoxic zone of the sediment. It corresponds well with the decrease in sulphate and SO_4^{2-}:Cl^- values seen below 11 cm, as well as with the change in colour of the sediment from brown to black indicating FeS formation (though strangely no free phosphate was detected in this core). It also correlates well with the decrease in acetate concentrations between 17-25 cm described above, suggesting that sulphate reduction may be one of the main acetate consuming processes occurring in the sediment. The one puzzling feature of the SRR profile is that little sulphate reduction appears to be occurring below 25 cm despite there being ample sulphate and acetate below this depth (Tarpgaard *et al.* 2011). One possibility is that sulphate reduction is being inhibited by the nitrate present at these depths (Xu *et al.* 2014). This inhibition can either be directly caused by nitrate (Laverman *et al.* 2012), or the incomplete reduction of this nitrate by DNRA bacteria can lead to the production of nitrite which can also have an inhibitory effect on SRB (Nemati *et al.* 2001) - however, as stated above, no nitrite was observed at this depth which would present a problem for this particular hypothesis. Alternatively it may be the case that the SRB are utilizing the nitrate as an electron acceptor instead of sulphate (Dalsgaard and Bak, 1994), which would lead to a drop in SRR. However, as sulphate concentrations at this depth are not in the μM range this switch in metabolism is unlikely. Another potential reason for this apparent inhibition relates to the influx of fresh groundwater that probably carried the nitrate to these sediments. It is possible, since they still contain nitrate, that these waters are still partially oxic and therefore have a relatively high redox potential which would inhibit sulphate reduction and

also methanogenesis (see below), as both require lower environmental redox potentials in order to be energetically viable

Thiosulphate values showed a peak of 6.2 μM at 5 cm, again possibly due to sulphide oxidation, before decreasing down to below detection limits at 21 cm. This decrease broadly corresponds to the second peak in SRR and therefore may be due to the disproportionation of thiosulphate by SRB. Below 21 cm thiosulphate values showed a second peak of 3.7 μM at 27 cm, which like nitrate, may be related to a flow of more oxidized groundwater at depth. This groundwater could supply thiosulphate directly to the sediments at depth, or be causing the production of thiosulphate *in situ*, either via direct chemical oxidation of sulphide with more oxidized compounds such as nitrate (Elsgaard and Jørgensen 1992), or bacterial oxidation of sulphide using nitrate as an electron acceptor (Fossing *et al.* 1995).

Methane concentrations in core ST6 were low overall with concentrations peaking at 4 μmol l^{-1} of wet sediment at 3 cm before decreasing down to a minimum of 1 μmol lws^{-1} at 13 cm (Fig. 5.5). Levels then increased again slightly to 2.44 μmol lws^{-1} at 15 cm and stayed at this level down to the base of the core. Radiotracer measurements showed that active rates of methanogenesis were also low in this core, to such a degree that no H_2/CO_2 based methanogenesis was detected in the core at any depth. Acetoclastic methanogenesis was detected however, with rates decreasing from 0.186 pmol cm^{-3} d^{-1} at 1cm down to zero at 13 cm. At 15 cm rates increased dramatically to 0.755 pmol cm^{-3} d^{-1} before decreasing again down to zero by 21 cm, and remaining at zero down to the core base. This decrease in rates (and turnover of acetate) from the near-surface down to 13 cm matches the *in situ* methane profile, as does the increase in rates at 15 cm (with the exception of lower methane concentrations at 1cm which may be due to gas escaping from the unconsolidated sediment). The increase in acetoclastic methanogenesis at 15 cm also correlates with the increase in acetate concentrations at this depth described above (and the change from oxidized to reducing conditions). It is not known why acetoclastic methanogenesis appears to not be occurring at the base of the core where there are higher concentrations of acetate (up to 81 μM) than above and relatively little sulphate reduction to inhibit methanogenesis through competition for substrates. However, it may be the case that, like sulphate reduction,

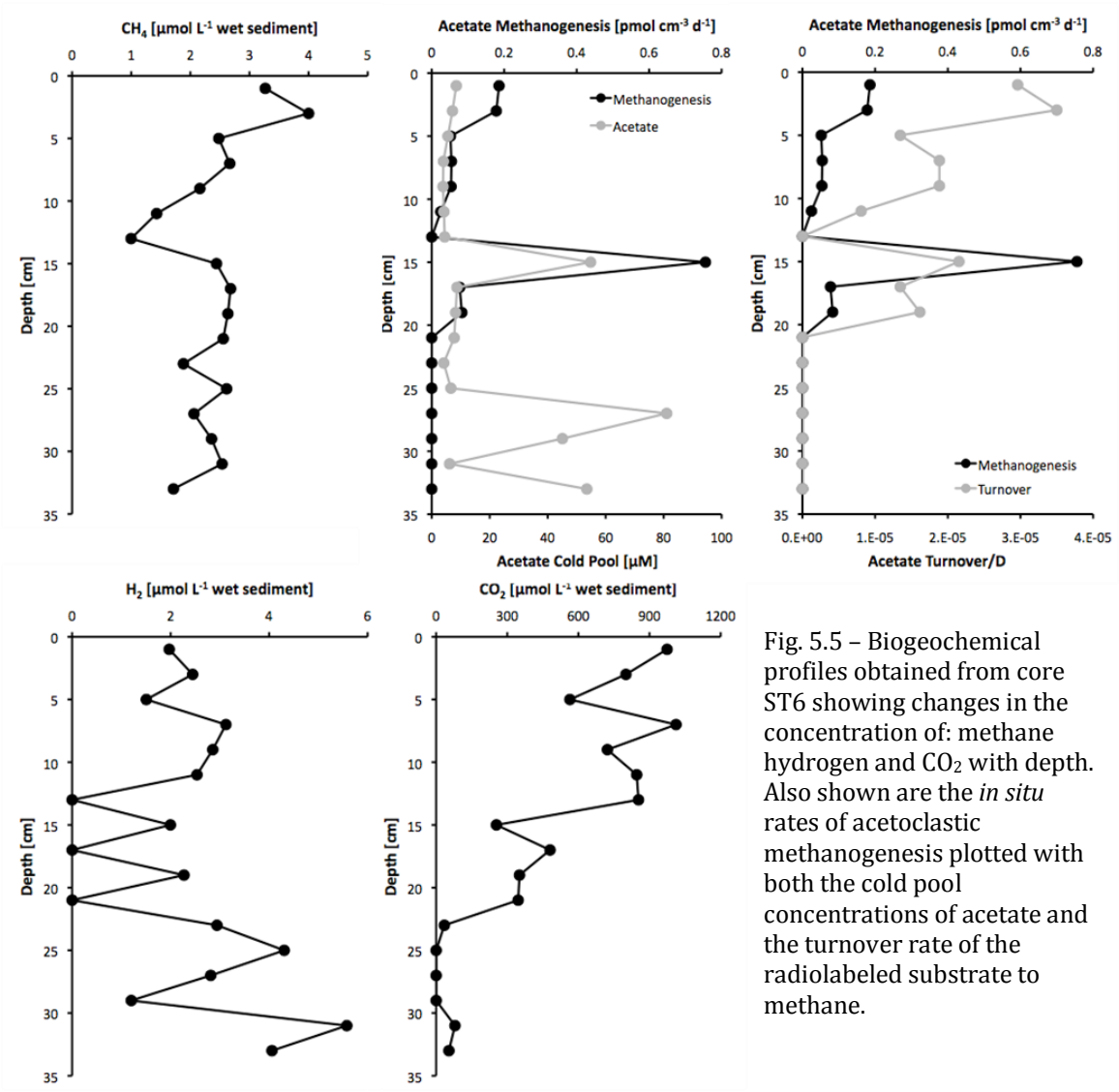


Fig. 5.5 – Biogeochemical profiles obtained from core ST6 showing changes in the concentration of: methane hydrogen and CO_2 with depth. Also shown are the *in situ* rates of acetoclastic methanogenesis plotted with both the cold pool concentrations of acetate and the turnover rate of the radiolabeled substrate to methane.

methanogenesis is being inhibited at this depth, either by the by the presence of nitrate (Klüber and Conrad, 1998) or due to the inflow of oxidised groundwater and the subsequent change in environmental redox potential. The lack of H_2/CO_2 methanogenesis in these sediments is unusual as this process is thought to dominate over acetoclastic methanogenesis in marine sediments, due mainly to competition for acetate by the more metabolically efficient SRB and the subsequent production of CO_2 which can be utilized as a substrate by H_2/CO_2 methanogens (Whiticar, 1999). In these sediments however, acetate concentrations may be high enough that both SRB and methanogens can be active together. Alternatively it may be that the SRB in these sediments are mainly utilizing hydrogen rather than acetate and as such are out-competing the H_2/CO_2 methanogens for their common substrate (Abram and Nedwell 1978a, Abram and Nedwell 1978b).

Hydrogen concentrations varied between 1.5-3.1 μmol lws^{-1} over the top 11 cm of the core before dropping below detection limits at 13 cm. Below this levels rose sharply (though remained very variable) to a maximum of 5.6 μmol lws^{-1} at 31 cm. This dramatic increase corresponds to the colour (and therefore redox) change in the sediment as well as the increases in organic acids and ammonium suggesting a potential link between these processes and hydrogen production (e.g. the generation of hydrogen as a waste product of LMW DOC fermentation).

CO_2 values also varied over the top 13 cm of the sediment (between 0.6-1 mmol lws^{-1}) before decreasing down to 254 μmol lws^{-1} at 15 cm. Below this concentrations rose again to 480 μmol at 17 cm before decreasing again, down to 53 μmol lws^{-1} at the base of the core. This decrease with depth is unusual as the ammonium profile continues to increase with depth suggesting that organic matter degradation (the major source of CO_2 in the sediment) is continuing down to the base of the core. Therefore it is possible that the decrease in concentrations below 13 cm could indicate consumption via an autotrophic process however, as detailed above H_2/CO_2 methanogenesis did not appear to be occurring in these sediments. The decreases on CO_2 however, do correspond to the peaks in the acetate profile which may indicate that homoacetogenesis is occurring in these sediments.

Total cell counts in core ST6 show an initial increase with depth from $1x10^{9.6}$ cells cm^{-3} at 1.5 cm to $1x10^{9.8}$ at 9.5 cm (Fig. 5.6). This lower number of cells at the surface seems counterintuitive considering that this is where the highest levels of OC are likely to be, however, it may indicate that these upper sediment layers are more disturbed (possibly by re-suspension) than those below making them a more challenging habitat for prokaryotes. Below 9.5 cm cell numbers decrease in a more normal fashion back down to $1x10^{9.6}$ at 17.5 cm before dropping sharply down to $1x10^{9}$ at 19.5 cm and then subsequently decreasing more gradually down to $1x10^{8.6}$ at the base of the core. This sharp drop in cell numbers neatly corresponds with the colour change in the sediment from brown to black noted above, and likely represents increased numbers of organisms carrying out the processes at the top of the redox cascade (aerobes, denitrifiers, Fe reducers etc.) living in the oxidized surface sediments, compared to those at the base of the cascade (SRB, ammonifiers,

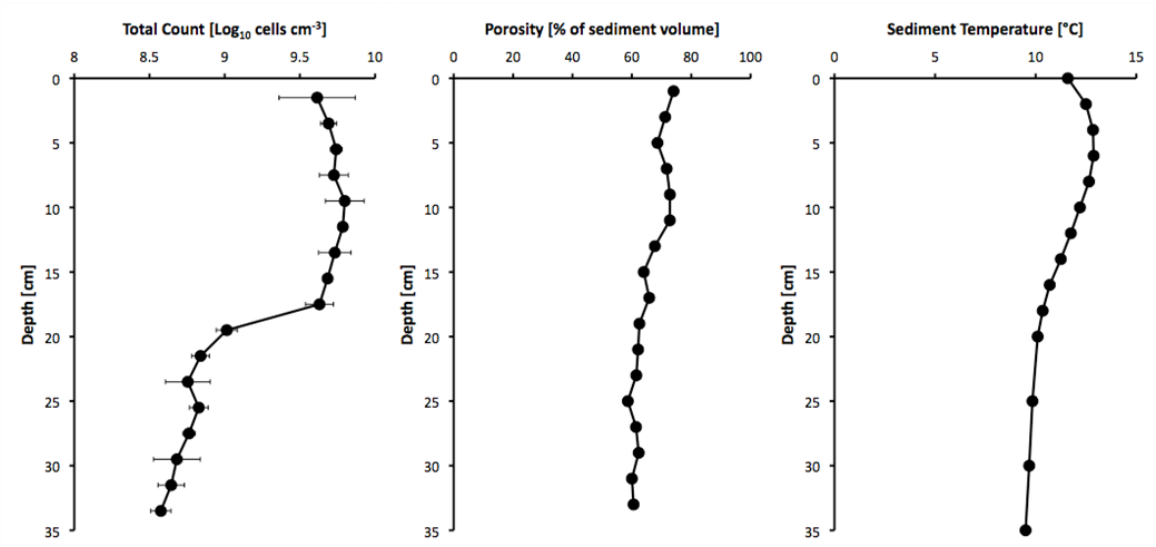


Fig. 5.6 – Profiles obtained from core ST6 showing the changes in: total cell count (AODC), sediment porosity and temperature with depth. Error bars represent the 95% confidence limits obtained from repeated counts.

methanogens etc.) living in the reduced sediments at depth. These lower numbers at the base of the core may also correlate with the potential inhibition of sulphate reduction and methanogenesis below ≈21 cm observed above. Porosity values for core ST6 decrease with depth from 74% at 1 cm down to 60.5% at the base of the core. There also appears to be a slight break in slope around 13-15 cm that may indicate that the upper oxidized layers of the sediment have been re-suspended more recently than the more reduced sediments beneath.

5.3 Severn Estuary Tidal Flats, Part 2 - Discussion

5.3.1 Comparison with Core ST4

When compared with Portishead further to the northeast, the mudflats at Clevedon present a drastically different topographical setting. At Portishead the mudflats are primarily constrained within the confines of Woodhill Bay and protected at either end by rocky promontories while at Clevedon the mudflats, while more extensive (≈500 m from mean high-water line to low-water line) are much more exposed to the full force of the Severn Estuary's tidal currents. Since these currents are known to re-suspend large amounts of sediment (Kirby 2010, Manning *et al.* 2010) it seems reasonable that this difference in geography between the two sites may have an effect on the biogeochemical processes occurring within.

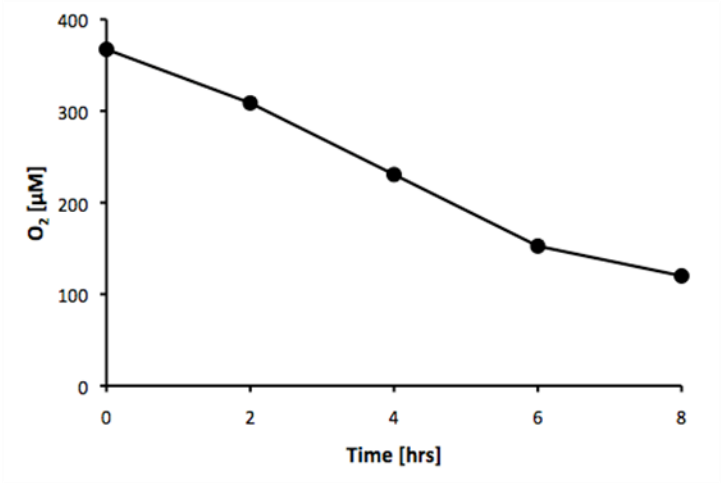


Fig. 5.7 – Line graph showing the consumption of oxygen by a copy of core ST6 incubated in darkness at *in situ* temperature for 8 hours. Data obtained from the decrease in oxygen concentrations in the overlying waters of the core corrected for loss by planktonic heterotrophic activity.

Total O_2 uptake at Clevedon (shown in Fig. 5.7) occurred at a rate of 99 mmol m^{-2} d^{-1}, which is nearly double that measured at Portishead (52 mmol m^{-2} d^{-1}). This is despite the fact that sediment temperatures at Clevedon (10-12 °C) were lower than at Portishead (13-14 °C). This higher rate of oxygen consumption may be related to the more open aspect of the Clevedon site as this would likely have led to more erosion and re-deposition of sediment than at Portishead. This in turn would have lead to a more oxidized sediment column containing higher numbers of aerobic and dysaerobic prokaryotic guilds that may have used up O_2 faster than the more stable and anaerobic Portishead sediments. The higher degree of macrofaunal bioturbation at Clevedon may also have played a role in increasing O_2 uptake, both by direct consumption of O_2 by macrofauna and by exposing reduced compounds in the sediment to oxygenated water via bioirrigation (Banta *et al.* 1999; Webb and Eyre 2004). This re-suspension of the sediment could also have mixed more "fresh" organic carbon compounds deeper into the sediment column, enhancing metabolic rates even further. Evidence for different rates of re-suspension can also be seen in the AODC profiles for both sites which show that the dramatic switch from high cell numbers ($\approx 1x10^{9.5}$ cells cm^{-3}) to lower cell numbers ($\approx 1x10^{8.8}$ cells cm^{-3}) occurs higher in the sediment column in core ST4 than core ST6 suggesting that ST4 was more stable and dominated more by lower-redox potential groups than than ST6 (the colour change from brown to black also occurred higher in core ST4 than in ST6, 13 cm as opposed to 17 cm). This variation in cell numbers with depth is also reflected in the average cell numbers in each core, with core ST6 having an average total count of $1x10^{9.3}$ cells cm^{-3} compared to $1x10^{9.2}$ in core ST4, which also suggests that re-suspension is having a positive effect on prokaryotic growth. Porosity

measurements for the two cores also indicate that at least the top half of ST6 had been re-suspended more recently than ST4 with values averaging 70% in the top 15 cm of core ST6 and 60% in ST4.

In terms of sulphate reduction per unit area, rates are reasonably low for core ST6 with depth-integrated rates of 1.1 mmol m^{-2} d^{-1}, compared to 4.7 mmol m^{-2} d^{-1} at Portishead. Taking into consideration the fact that neither site appears to be substrate limited with regard to OC electron donors or sulphate (Ingvorsen *et al.* 1984a, Taarpgard *et al.* 2011), this difference is likely due to the rather constrained nature of the sulphate reduction zone in the sediments of ST6. In core ST4 sulphate reduction is occurring at relatively high rates (10-40 nmol cm^{-3} d^{-1}) over almost the entire depth of the core whereas in core ST6 the highest SRR only reaches 15 nmol cm^{-3} d^{-1}, and even then only for a brief depth interval between 11-21 cm. This constraining of sulphate reduction in ST6 can again be linked to the fact that the sediments at Clevedon are more exposed, as SRB require anaerobic conditions with a negative redox potential to function which means that the more stable sediments at Portishead would provide a better habitat than those at Clevedon that are likely subject to more re-suspension and hence exposed to oxic, redox-positive conditions on a more regular basis. The constraint on SRR in core ST6 is also present at the bottom of the core where it is likely due to the flow-through of more redox-positive groundwaters containing nitrate. A similar nitrate-enhanced groundwater flow was also potentially seen in core ST4 however, it appeared to have little to no affect on the SRR values. The reason for this discrepancy between sites is not known but may indicate differences in the populations of SRB at the two sites with one being more redox-sensitive than the other.

With regard to methane production per unit area, core ST6 again shows low values, with a depth integrated acetoclastic methanogenesis rate of 29.2 nmol m^{-2} d^{-1} compared to 58.3 nmol m^{-2} d^{-1} at Portishead. This difference is again likely linked to the sediments at Clevedon being more disturbed as, like SRB, methanogens require a stable, anoxic, low redox potential environment in which to thrive. In the case of H_2/CO_2 methanogenesis, activity was detected at Portishead (33.5 nmol m^{-2} d^{-1}) but none was found at Clevedon again suggesting that the higher degree of re-suspension at this sight may be having a negative effect on the methanogenic

community – as might the presence of nitrate throughout the sediment column as well as potentially more redox-positive groundwater flow.

From the evidence provided above it can be seen that the differing topographies of the two sites, and in turn the different processes acting upon them, would appear to play a dramatic role in influencing the biogeochemistry of the sediments. The results would indicate that while sediment oxygen demand and overall cell populations are increased on exposed mudflats (possibly indicating a relative increase in aerobic and dysaerobic guilds), strictly anaerobic processes decrease in activity when compared to more sheltered sites. As such carbon mineralization on these more exposed mudflats may be less dominated by SRB and methanogens than would normally be expected for coastal and shallow marine sediments (Jørgensen 1982, Canfield 1989). Also since exposed mudflats are the dominant topographical type on the southern side of the estuary, and make up almost all of the mudflats on the northern shore, these findings could indicate that sulphate reduction and methanogenesis play much less of a role in the Severn Estuary system than might otherwise be thought.

5.3.2 Comparison with Core ST5

The mudflats near Clevedon from which core ST6 was sampled share many topographical similarities with the site near St Brides Wentlooge on the opposite side of the estuary from which core ST5 was taken. At both sites the mudflats are extensive (extending >0.5 km from the shore) and backed by low-lying marshlands. In addition they are not protected by headlands or other rocky promontories, and as such are exposed to the full force of the Severn Estuary's tidal power – though the Clevedon site is protected somewhat by the presence offshore of the Langford Grounds. In addition, despite being taken at different times of the year, *in situ* sediment temperatures at the two sites (as well as at the Portishead site mentioned above) were similar (≈10-14°C). This means that any differences in processes between the Clevedon and St Brides sites should be relatively independent of topographical influence or temperature and therefore can be attributed to differences in hydrology or possibly sedimentology - no peat was found in core ST6 though it is found inland on the Somerset Levels and exposed on the coast further to the southwest (Allen 1990b, Allen and Haslett 2002).

Total O_2 uptake measurements for core ST6 were higher than those for ST5 (99 vs. 81 mmol m^{-2} d^{-1}) but were more similar to each other than to the sediments from Portishead. Average cell numbers in the surficial sediment were also similar ($1x10^{9.71}$ in core ST6 compared to $1x10^{9.69}$ in ST4, over the top 10 cm) as were porosities. Depth integrated sulphate reduction rates on the other hand were lower at Clevedon than St Brides (1.1 vs. 1.3 mmol m^{-2} d^{-1}) despite core ST5 being considerably shorter (core ST5's duplicate was ≈21 cm long compared to ST6 which was 33 cm in length). Average sulphate reduction rates were also lower with a value of 3 nmol cm^{-3} d^{-1} for core ST6 compared to 6 nmol cm^{-3} d^{-1} in ST5. In addition if only the values from the upper 9 cm of the core are taken (to remove the values from the peat bed in ST5) the average value for ST5 drops to 4.4 nmol cm^{-3} d^{-1}, though the average for ST6 also drops to 1.2 nmol cm^{-3} d^{-1}. Finally acetoclastic methanogenesis rates were again lower in ST6 than ST5 (29.2 vs. 131 mol m^{-2} d^{-1}) as were rates of H_2/CO_2 methanogenesis (0 vs. 503 nmol m^{-2} d^{-1}). However, if the top 9 cm of both cores are again compared the average acetoclastic methanogenesis values are very similar, 102 fmol cm^{-3} d^{-1} for core ST4 and 105 fmol cm^{-3} d^{-1} for core ST6.

These results would seem to indicate two things; firstly they confirm that mudflat topography is very likely the main reason for the differences observed between Portishead and Clevedon as the two exposed-flat sites are more similar to each other than either is to Portishead. This also confirms that the increased oxic/decreased anoxic pattern of microbial metabolism first described in Chapter 4 is prevalent over exposed mudflats on both sides of the estuary – probably due to higher rates of sediment re-suspension on these flats. The second point is that compared to St Brides, Clevedon is even more enriched in oxic processes and depauperate in anoxic processes, suggesting that re-suspension is increased here relative to St Brides. This potential higher rate of re-suspension may be due to the fact that the mudflats at St Brides are wider than at Clevedon (≈1100 m as opposed to ≈540 m) as the more extensive a mudflat is the more it may be able to dissipate wave and current energy (Rulkötter 2009; Mariotti and Fagherazzi, 2010; Adam *et al.* 2011), decreasing the relative erosive power in that area (St Brides also possesses a wide subtidal mud apron in front of it that Clevedon does not which may further buffer wave and current activity). In addition, sediment maps of the estuary show that the seabed

directly offshore of the Clevedon site is clear of sediment (Kirby, 2010). Since bare patches of seafloor such as this are thought to be caused by strong bottom currents scouring the seafloor clean of sediment, it is possible that the currents in this area might be stronger than those on the northern side of the estuary further adding to the increased erosion/re-suspension regime at the Clevedon site.

5.4 Severn Estuary Tidal Flats, Part 2 - Conclusions

- The sediments from the tidal flats south of Clevedon (core ST6) share aspects with both cores ST4 (origin on the southern side of the estuary, uniform composition with depth) and ST5 (mudflats with an open topography, disturbed upper layers of sediment.
- Core ST6 also shares geochemical similarities with cores ST4 (variable salinity, subsurface nitrate peak) and ST5 (isolated peaks of sulphate reduction and methanogenesis), however, it also exhibits differences - such as the apparent inhibition of anaerobic processes at depth possibly caused by the presence of nitrate at depth and/or a flow of more redox-positive groundwater.
- Compared to Portishead (ST4) the sediments at Clevedon (ST6) show lower rates of lower redox potential processes (sulphate reduction and methanogenesis) but higher rates of O_2 uptake suggesting higher levels of more redox positive processes. The step-change in total cell numbers also occurs lower in the sediment column in core ST6 suggesting a greater degree of re-suspension.
- This makes the mudflats at Clevedon similar to those at St Brides (ST5), though the contrast is even greater, suggesting that Clevedon may be subject to even more sediment re-suspension than St Brides.
- The low overall rates for sulphate reduction and methanogenesis on the tidal flats detailed so far would indicate that these processes may not play as dominant a role in carbon mineralization in the Severn Estuary as would normally be expected for a shallow marine environment (Jørgensen, 1982).

Chapter 6. - Bridgwater Bay: Cores ST7 and ST8

6.1 Site Locations and Lithology

Cores ST7 and ST8 were collected in June 2013 from Bridgwater Bay off the coast of Somerset on the southern side of the Severn Estuary. Although excluded from some definitions of the Severn Estuary system, Bridgwater Bay is thought to play a major role in the sedimentary regime of the estuary. This is due to the fact that the mudflats and shallow subtidal areas within the bay act as one of the major sources and sinks of sediment in the greater Severn Estuary area, and as such, it also contains one of it's two turbidity maxima (Kirby, 2010; Manning *et al.* 2010). Within the bay itself net erosion, deposition and steady-state conditions occur in localised areas (Kirby, 1994; Kirby and Kirby 2008), with a general trend towards erosion in shallower areas and steady-state conditions or accretion of sediment in deeper regions. In order to assess the biogeochemical processes occurring within Bridgwater Bay sampling sites were selected across different water depths, this also allowed for the study of the potential affects that the different erosional/ depositional regimes within the bay might have on the sediment microorganisms.

Core ST7 was collected from a shallow shelf 4.8 km offshore between Weston-super-mare and Burnham-on-sea (51° 16' 47.2"N, 03° 04' 59.6"W) from a water depth of ≈10 m (3-4 m below chart datum) (Fig. 6.1). The core was 34 cm long and made up of poorly-consolidated mud and fine sand with no obvious bedding or bioturbation. The top 12 cm of the core was brown in colour but darkened to black beneath indicating reducing conditions and the presence of FeS (Fig. 6.2).

Core ST8 was collected from a deeper area 8.3 km offshore (51° 17' 51.9"N, 03° 07' 48.7"W) from a water depth of ≈14 m (7-8 m below chart datum). The core was 36 cm in length and compositionally similar to core ST7 but also contained patches of coarser sand. It was also much less cohesive than ST7 and multiple samplings were required at this site to retrieve cores with an intact surface as the sediment had a tendency to flow out of the top of the core tubes, indicating that the main body of the corer was itself sinking into the sediment. Again the core showed no clear bedding structures, nor evidence of bioturbation, and was a uniform brown colour throughout, indicating oxidised conditions and little (if any) FeS formation.

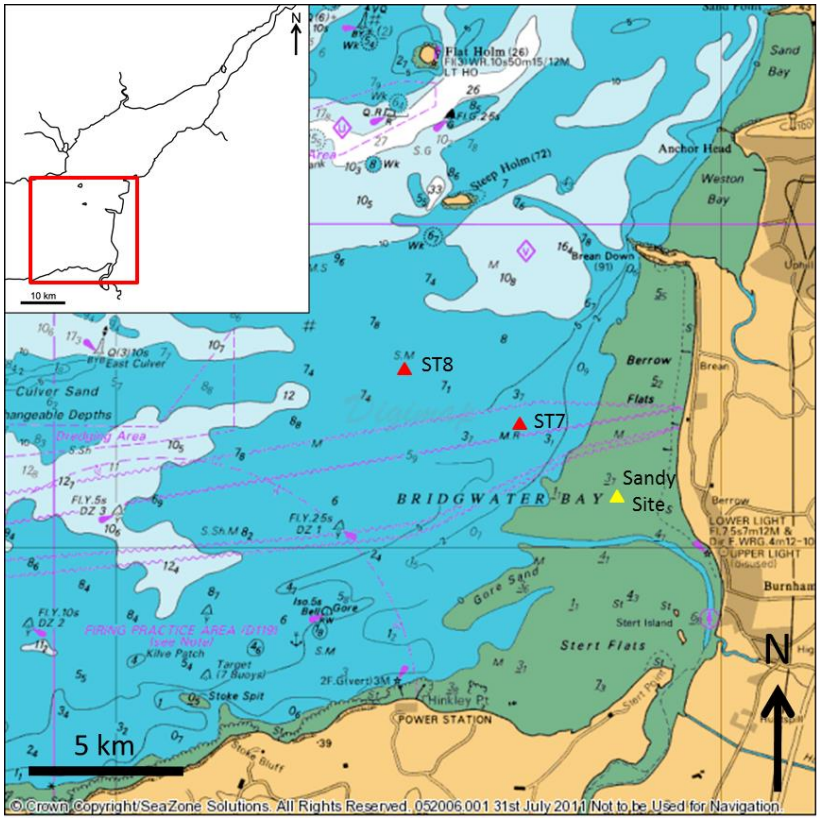


Fig. 6.1 – Bathymetric chart showing the location of the sampling sites in Bridgewater Bay for cores ST7 and ST8 (red), and the prospective third site (yellow) where coring was abandoned due to the high sand content of the sediment, with respect to submarine topography. Areas in white represent the deepest water while those in blue represent shallower water, green represents the extent of intertidal areas at lowest astronomical tide (bathymetric chart ©Crown Copyright SeaZone Solutions).

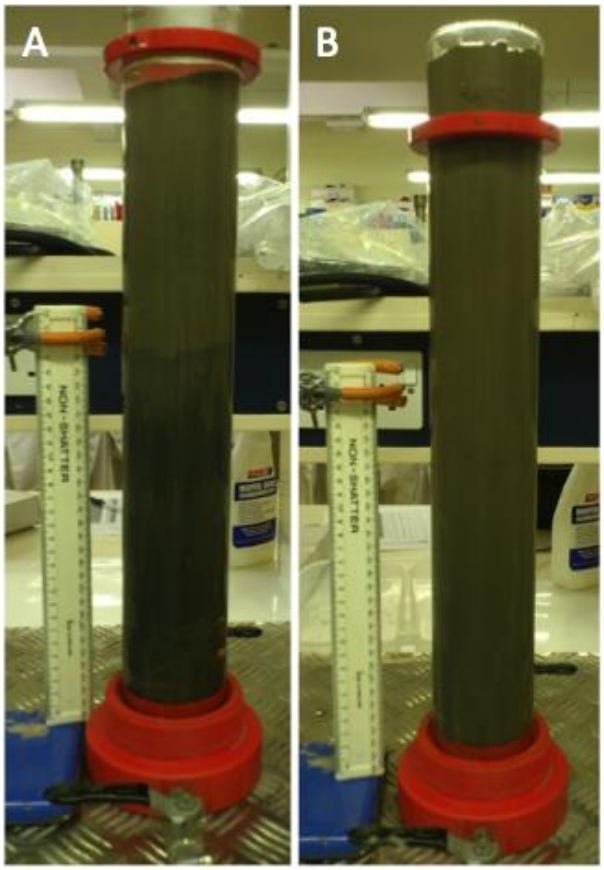


Fig. 6.2 – Photographs of cores A) ST7 and B) ST8 showing the differences in the change in sediment colour with depth. The change in colour from brown to black denoting a change in redox state is clearly visible in core ST7 but no such delineation appears in core ST8. 30 cm ruler is shown for scale

In addition it was initially planned to collect a third core from a site on the tidal flats around 2 km offshore of Berrow (51° 15' 50.2"N, 03° 02' 47.2"W), from a water depth of ≈3.5 m (seafloor 3 m above chart datum) however, multiple deployments of the multicorer at this site yielded no intact cores. Sampling with a grab bucket determined that the sediment was a coarse sand/mud mixture which may either have been too hard for the blunt-ended core tubes to cut through or lacked enough cohesion to remain in the core tubes long enough to get the corer back on board the sampling vessel.

6.2 Bridgwater Bay - Results

6.2.1 Biogeochemistry of Core ST7

Acetate concentrations remained low throughout core ST7 (Fig. 6.3), varying erratically between 0-10 μM throughout the length of the core, however, acetate was only present in the core at levels greater than 1 μM at 11 cm and below, the same depth that the sediment began to darken in colour. This demonstrates that the acetate present at depth is not derived from the overlying waters but is instead produced by fermentation in the anaerobic zone of the core. The formate profile is in agreement with this proposition as levels only rose above 7 μM below 11 cm – reaching a maximum of 66 μM at 15 cm. Lactate concentrations were low overall and showed a more gradual increase with depth, until ≈14 cm, but then exhibited a distinct peak at 15 cm, before concentrations decreased sharply and began to rise again with depth, reaching 4.5 μM at the base of the core. Propionate also had low overall concentrations (<2 μM) with only a slight increase with depth, but with a sharply elevated peak in concentration at 9 cm (5.6 μM), possibly linked to more microaerophillic fermenting groups as it was just above the redox change at 12 cm depth. Alternatively it (and the peaks in the other organic acid profiles) might represent an uneven distribution of organic matter throughout the core with isolated colonies of fermenting organisms occurring close to regions with higher OM content.

Chloride and sodium values were relatively constant down core, varying between 343-376 mM (chloride) and 341-370 mM (sodium) throughout the core. Potassium exhibited a similar pattern, remaining around between 7-8 mM for the length of the

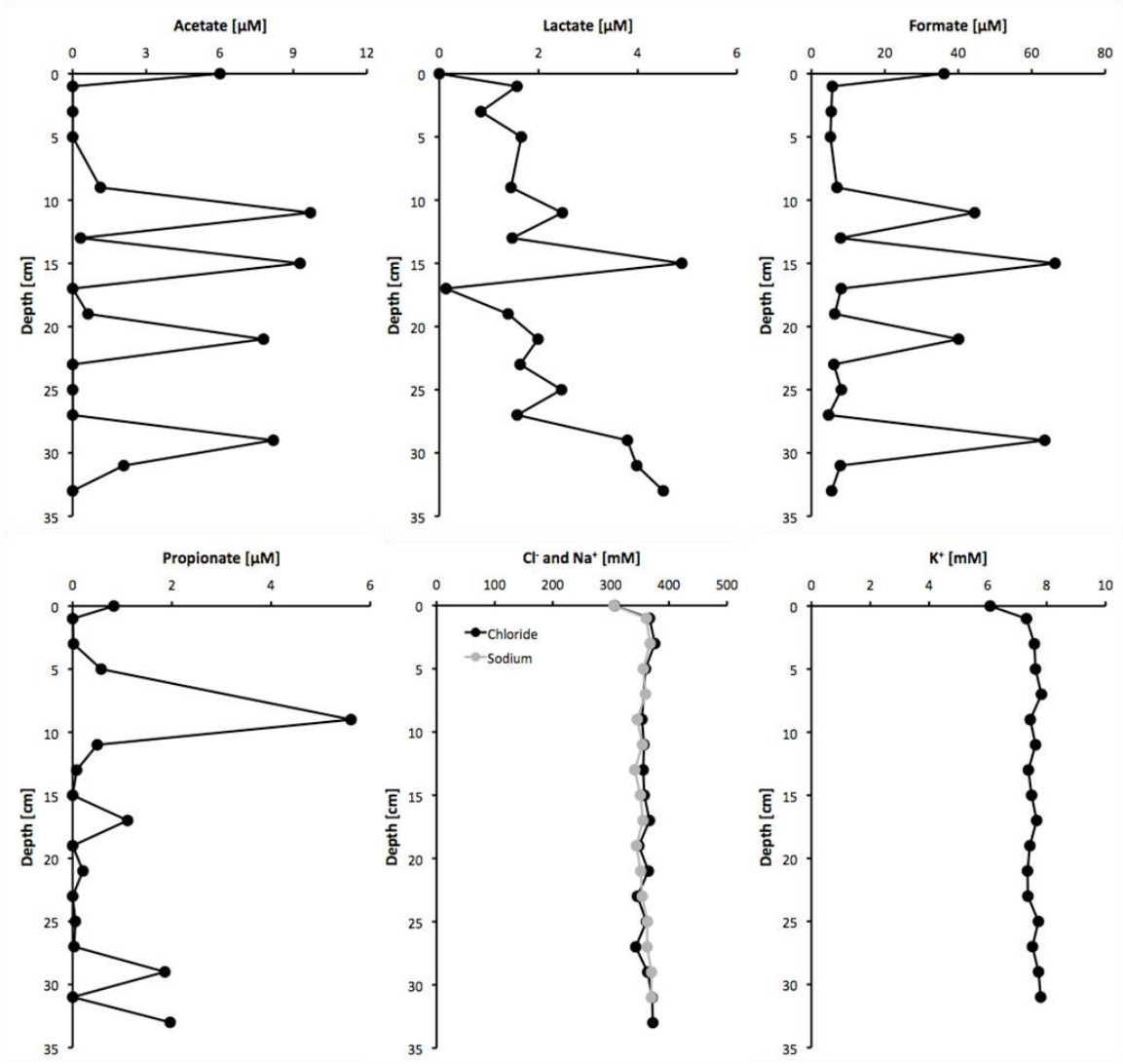


Fig 6.3 - Biogeochemical profiles obtained from core ST7 showing changes in the pore water concentration of: organic acids, chloride, sodium and potassium with depth.

core. Calcium on the other hand showed a different pattern (Fig. 6.4), increasing with depth from 6.5 mM at 1 cm to a maximum of 12.8 mM at 23 cm and then decreasing to 10.4 mM at the base of the core. The reason for this increase (and peak) at depth is unknown but may relate to the dissolution of carbonate, possibly derived from the shells of meio/microfauna, particularly around 23 cm, and linked to increasing CO_2 concentrations at depth (e.g. between 17-23 cm).

Nitrate concentrations decreased from 25 µM in the overlying waters down to zero by 9 cm indicating that denitrification and/or DNRA was occurring in the upper regions of the core. A small peak in nitrate values was observed at 3 cm depth possibly indicating that nitrification was occurring however, no concave-upward

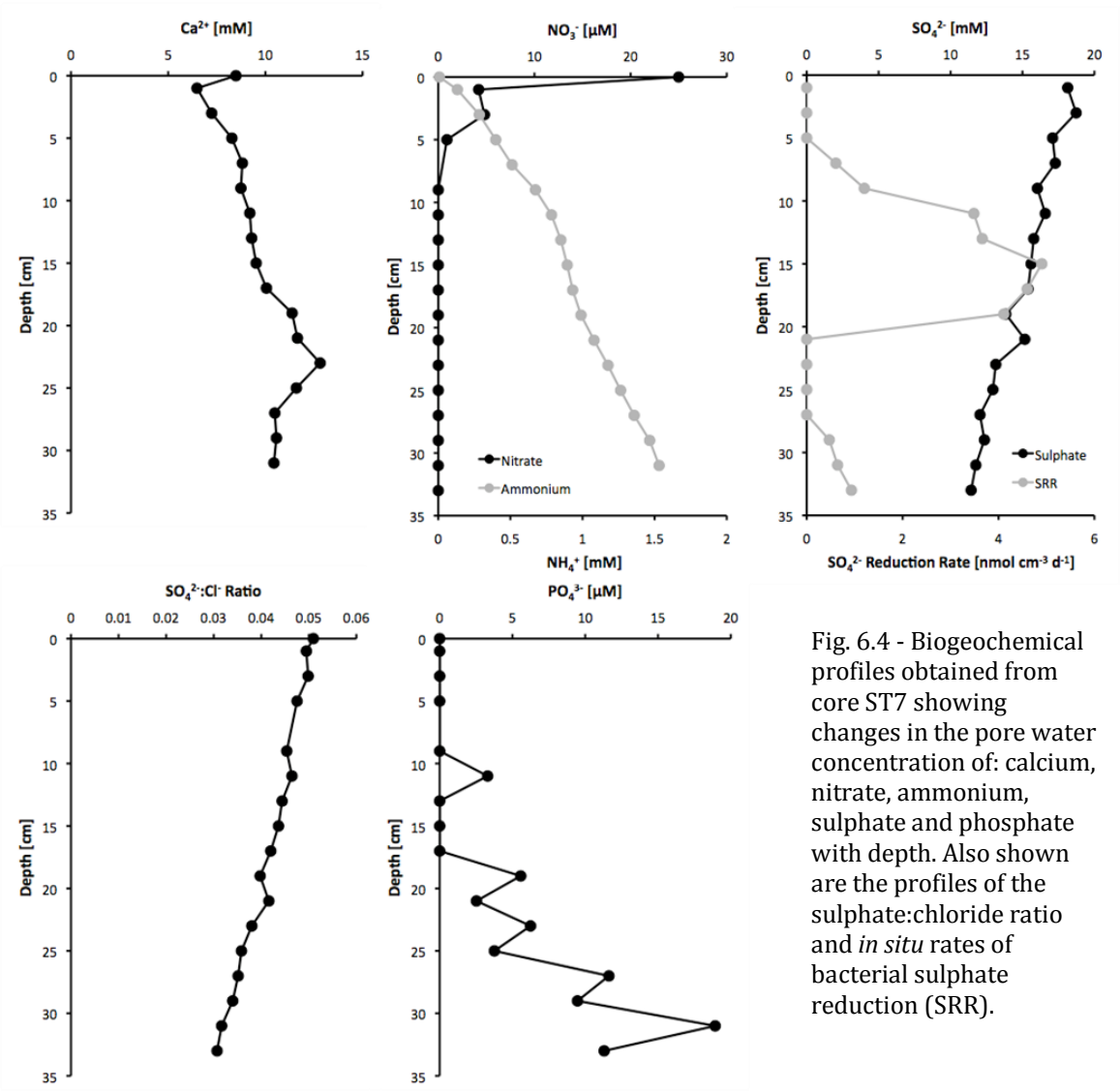


Fig. 6.4 - Biogeochemical profiles obtained from core ST7 showing changes in the pore water concentration of: calcium, nitrate, ammonium, sulphate and phosphate with depth. Also shown are the profiles of the sulphate:chloride ratio and *in situ* rates of bacterial sulphate reduction (SRR).

trend was noticeable in the ammonium profile at this depth that might correlate with this peak. Ammonium concentrations increased with depth, from 130 µM at 1 cm to 1500 µM at the base of the core, demonstrating that OM deamination/ ammonification was occurring in these sediments. The ammonium profile was not constant with depth however, with the steepest gradient occurring within the oxidised upper 11 cm of the core. Below this the gradient became more vertical down to 20 cm depth before steepening again down to the base of the core. This profile shape might indicate that ammonium production decreased somewhat between 11-20 cm, though the reason for such a decrease is unclear.

Sulphate decreased from 18 mM at 1 cm down to 11 mM at 33 cm indicating that sulphate reduction was occurring in these sediments. Active SRR measurements

show that sulphate reduction began to occur at 7 cm but was highest in a zone between 11-19 cm – with a maximum rate of 4.9 nmol cm^{-3} d^{-1} at 15 cm. At 21 cm rates dropped down again to zero before showing a secondary increase below 27 cm. This zone of elevated SRR below 11 cm neatly matches the depth at which the sediment in the core begins to darken (12 cm), confirming that the colour change is indeed likely due to FeS production by SRB. The phosphate profile from this core also indicates FeS production in the lower half of this core as phosphate was first detected at ≈12 cm and shows a dramatic increase in concentrations below 16 cm. Since free phosphate is produced in sediments by liberating it from iron oxy-hydroxides when they react with FeS (Rozan *et al.* 2002), an increase in phosphate can be used as a proxy to indicate elevated FeS levels in the sediment at these depths.

Methane concentrations were low in core ST7 but increased with depth (Fig. 6.5), reaching a maximum of 3.8 μmol l^{-1} of wet sediment at 31 cm. Between 3-27 cm this increase was relatively linear, but superimposed on this, between 17-25 cm the values appear to produce a slight concave-up profile. Since concave-up profiles are usually indicators of consumption (often via microbial processes)(Schulz, 2006), and since this change in gradient is occurring below the oxidised-reduced transition at 12 cm, it is possible that this profile shape could indicate that anaerobic oxidation of methane (AOM) is occurring at this depth. The upper part of this concave upward profile also coincides with the maximum SRR described above and considering that SRB are one of the main groups of organisms known to be involved in AOM consortia this may provide further evidence that AOM is occurring at this depth. However, the above suggestion needs to be considered with caution as little is known about the environmental controls on AOM and therefore further analyses, possibly utilising $^{14}CH_4$ radiotracers, should be carried out before the presence of AOM in these sediments is confirmed. Below ≈25 cm methane values become more variable possibly due to both production and consumption co-occurring at the same depth – which might in turn relate to the slight increase in sulphate reduction occurring near the base of the core.

Radiotracer experiments showed that active methanogenesis was occurring in these sediments, albeit at low levels, which is consistent with the increasing

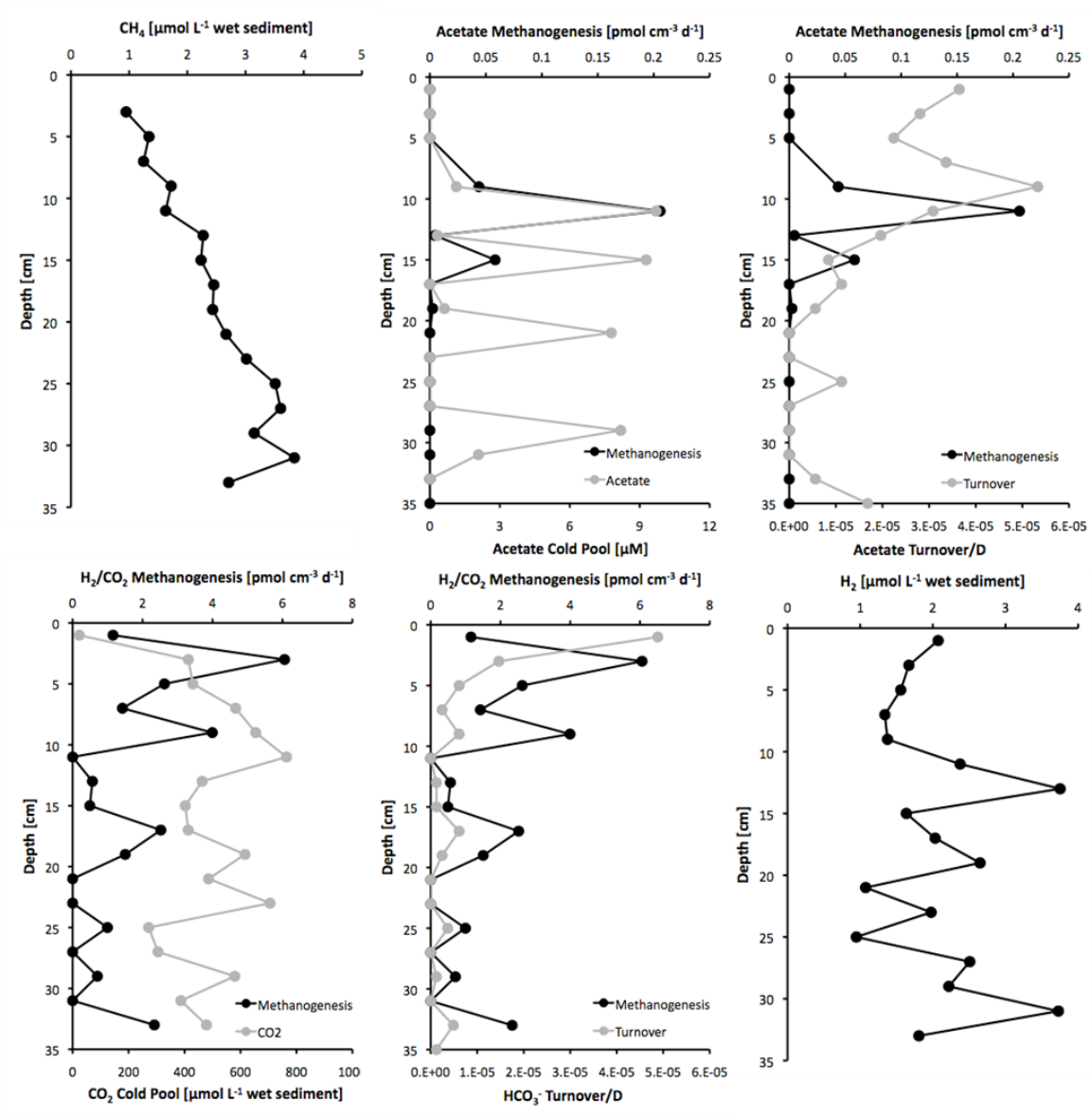


Fig. 6.5 – Biogeochemical profiles obtained from core ST7 showing changes in the concentration of: methane, CO_2 and hydrogen with depth. Also shown are profiles of the *in situ* rates of both acetoclastic and H_2/CO_2 methanogenesis plotted with the cold pool concentrations of their respective substrates and the rate of turnover of their radiolabeled substrates to methane.

methane profile. Both the turnover of added ^{14}C-acetate to methane and rates of acetoclastic methanogenesis peak between 9-11 cm (maximum rate of 0.2 pmol cm^{-3} d^{-1}). The combined peaks coincide with the acetate peak described above and also indicate that methanogenesis is occurring in oxidised sediments - in fact turnover of the label to methane decreases drastically moving downward into the more reducing sediments below 12 cm depth. H_2/CO_2 methanogenesis also occurred in the cores with rates up to 30x higher than those of acetoclastic methanogenesis - peaking to 6.06 pmol cm^{-3} d^{-1} at 3 cm before decreasing gradually towards the base

of the core. As with the acetoclastic methanogenesis results above, rates appear to be highest in the oxidising sediments above 12 cm with turnover of $^{14}C\text{-}HCO_3^-$ to methane in particular highest near the surface. Again this would indicate that the methanogens here are clearly capable of being active in oxidised (possibly overall oxic) sediments, probably within reduced microniches (Jørgensen, 1977). No methylated amine-related compounds (MMA, DMEA, choline etc.) that could be utilised as non-competitive substrates by methanogens were detected in the core.

Hydrogen concentrations were low in the top 9 cm of sediment but showed a slight decrease with depth, from 2.1 μmol lws^{-1} at 1 cm down to 1.4 μmol lws^{-1} at 9 cm. Below the oxidised-reduced boundary concentrations increased, probably linked to increased fermenter activity, reaching a peak of 3.8 μmol lws^{-1} at 13 cm. Deeper again, values decreased erratically down to a minimum of at 25 cm, before rising again near the bottom of the core. CO_2 concentrations increased over the top 11 cm of the core to a maximum of 764 μmol lws^{-1} at 11 cm, most likely driven by heterotrophic respiration in the upper sediment layers, before dropping down again to 403 μmol lws^{-1} at 15 cm. Below this values remained variable, but did show a notable increase between 15-23 cm which could be attributable to increased heterotrophic activity at this depth (as SRRs also reached their maximum around this depth). This increase in CO_2 could also be responsible for the increased calcium values described above as increased carbonic acid formation could have caused a dissolution of carbonate in the sediment leading to a release of Ca^{2+} ions.

Total cell counts for core ST7 also show a change around 11 cm (Fig. 6.6), with counts in the upper 10 cm of the core showing a decrease from $1x10^{9.6}$ cells cm^{-3} down to $1x10^{9.5}$ at 9.5 cm. Below this numbers drop sharply down to $1x10^{9.2}$ at 11.5 cm followed by a more steady decrease to $1x10^{8.8}$ at the base of the core. This sharp drop in numbers occurs at around the same depth that the sediment changes colour and could be the result of the upper layers of sediment being re-suspended and re-supplied with substrates, which could lead to an increase in cell numbers. If such re-suspension had occurred then the cell count profile in the re-suspended sediment should be relatively vertical, due to homogeneous mixing of the sediment (as seen in some of the other cores in this study). However, in this case the cell counts above

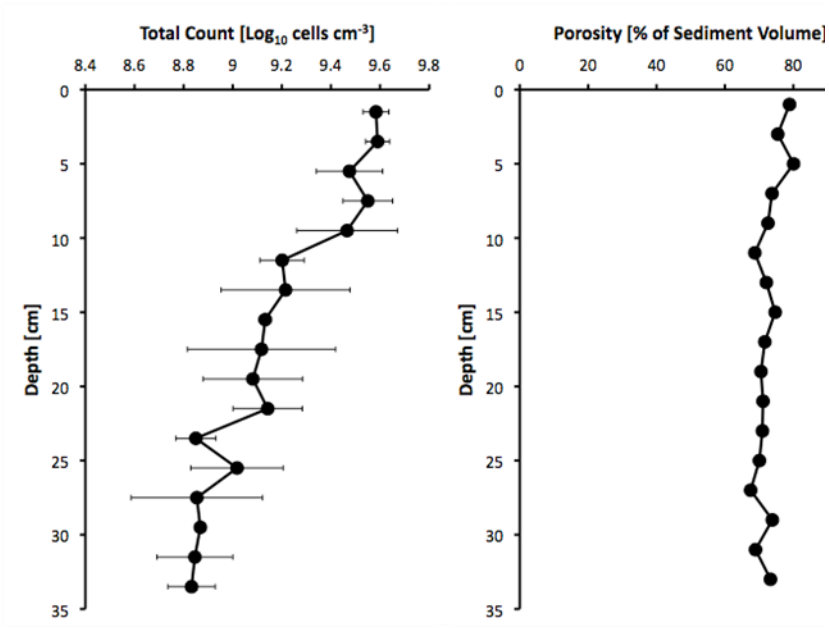


Fig. 6.6 – Profiles obtained from core ST7 showing the changes in total cell counts (AODC) and sediment porosity with depth. Error bars represent the 95% confidence limits obtained from repeated counts.

11.5 cm still decrease with depth, which suggests that the sediment in question may not have been re-suspended for some time (as do the geochemical gradients). Further discussion of the role of sediment re-suspension in shaping cell count profiles will be presented in Chapter 9. Porosity values for this core decreased with depth, as would be expected, from 79% at 1 cm to 73 at 33 cm. There was however a change in the porosity gradient around 11 cm with the decrease below this depth becoming much more gradual. This may indicate that the upper, oxidised layers of the core had been more recently deposited than the rest of the core and had therefore not fully settled when the core was taken.

6.2.2 Biogeochemistry of Core ST8

Acetate concentrations were relatively low in core ST8 (Fig. 6.7), with levels generally remaining between 6-10 μM throughout the length of the core. Concentrations remained stable at around 7 μM over the top 10 cm of the core but became more variable below this. Values showed a slight increase with depth (reaching a maximum of 11.3 μM at 29 cm), again indicating the possibility of fermenter communities being active in these sediments (particularly below 10 cm depth). However, this increase was erratic particularly below 21cm possibly due to increases in sulphate reduction around this depth (see below). Lactate on the other hand showed an initial increase with depth to a maximum of 7 μM at 5 cm,

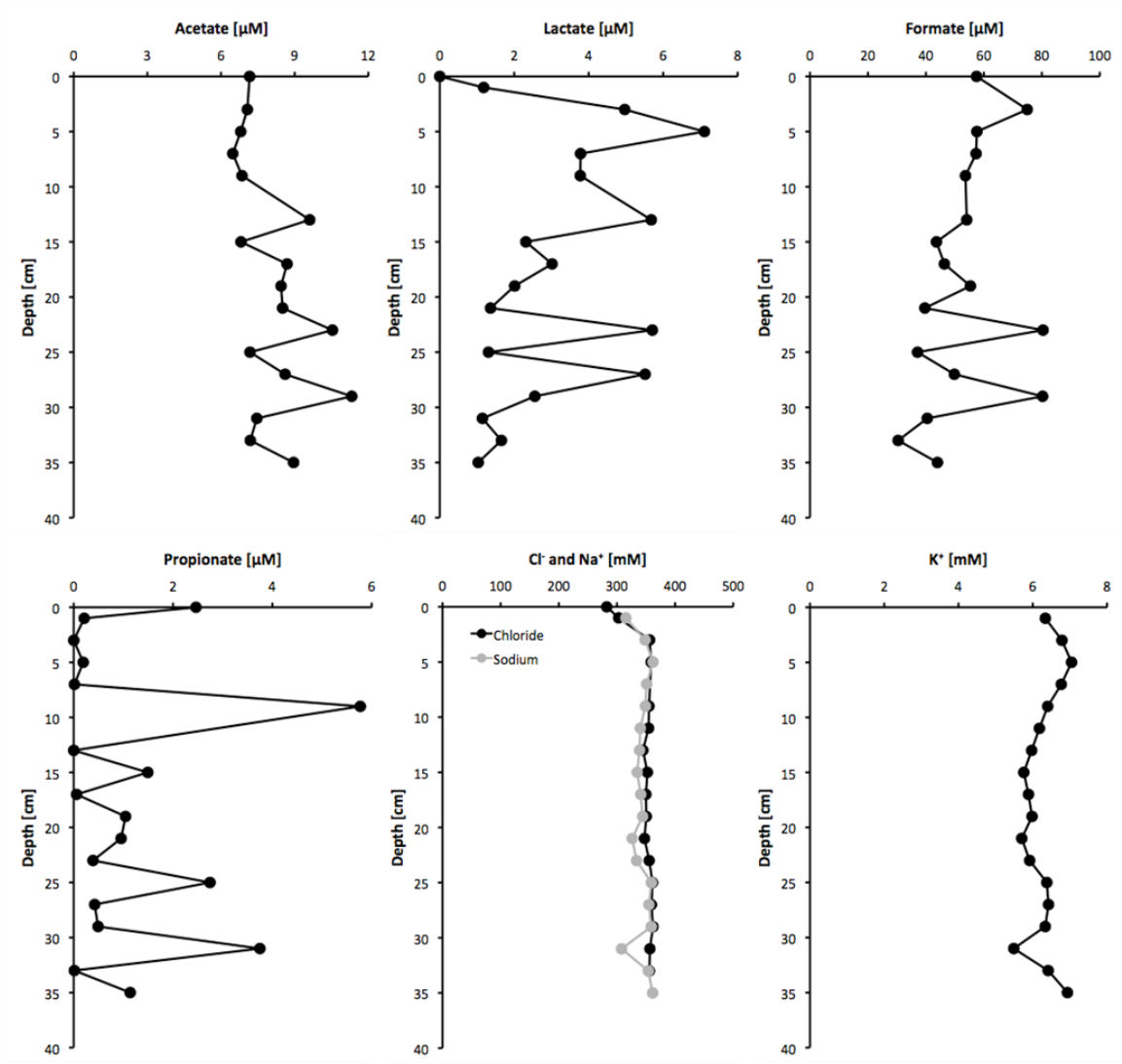


Fig 6.7 – Biogeochemical profiles obtained from core ST8 showing changes in the pore water concentration of: organic acids, chloride, sodium, and potassium with depth.

indicating that fermentation was active near the top of the core (likely within anaerobic microniches considering the oxidised state of the sediment). Below this concentrations decreased down to 1.4 µM at 21 before peaking again to between 23-27 cm and then decreasing towards the base of the core. This profile would indicate a zone of heterotrophic consumption below 5 cm followed by a second zone of fermentative production at depth, which coincides with peaks in the acetate profile. Formate values were higher than the other organic acids and show a more consistent and linear decrease within the upper layers of the core, from 75 µM at 3 cm down to 40 µM at 21 cm, again likely due to heterotrophic consumption. Between 21-31 cm levels increase again, indicating a zone of fermentation similar to that seen in the acetate and lactate profiles, before dropping again towards the base

of the core. Propionate values are more variable than the other organic acids but again indicate a zone of production between 21-31 cm. Unlike the other acids however, propionate also shows a substantial peak (5.8 μM) at 9cm, though the reason for this peak is unclear as it does not seem to relate to the other profiles.

Taken together these results would indicate that organic acid production is very variable throughout this core with different compounds being produced at different depths. However, the profiles do show presence of a common zone of organic acid production between ≈20-30 cm indicating an enhanced zone of fermentation (though the degree that this zone affects the different organic acids varies from compound to compound). Production within this zone appears to be somewhat erratic, with distinct peaks and troughs apparent in the profiles, however this may be due to simultaneous production and consumption of organic acids linked to sulphate reduction which is also occurring at this depth (see below).

Chloride and sodium concentrations in core ST8 are similar to those in core ST7 and are relatively constant down-core, staying around 340-350 mM. The only exception to this is in the top 2 cm where values drop closer to 300 mM, which may indicate that the top of the core was being disturbed by waters with a lower salinity than those in which the majority of the core was deposited, though this would be unusual considering that the core was collected during the slack water period following a rising tide. Potassium concentrations show a similar increase over the top 5 cm of the core, but then exhibit a decrease down to 15 cm followed by an overall increase towards the base of the core. This initial increase with depth could again be linked with disturbance/diffusion of less-saline overlying water however the variations below 5 cm are harder to explain as the K^+:Na^+ ratio values (data not shown) indicate that the changes are not wholly linked to changes in overall salinity. Since potassium it not an element usually associated with microbial activity it is unlikely that these changes are driven by biological processes and therefore they may instead indicate some change in sediment geochemistry/lithology with depth. Calcium values on the other hand show an overall increase with depth (Fig. 6.8), independent of changes in salinity (as indicated by the Ca^{2+}:Na^+ ratio, data not shown), this would indicate a potential source of Ca^{2+} at depth, possibly relating to the dissolution of carbonate. However, the profile also shows distinct peaks along its

length, despite matching peaks not appearing in the CO_2 profile. This might potentially indicate isolated zones of increased carbonate dissolution related to the presence of micro/meiofanal shell material (since no macrofaunal material was found).

Nitrate concentrations again exhibited a rapid decrease in the upper layers of the sediment, from 22 μM in the surface waters down to zero by 7 cm, indicating that denitrification/DNRA was occurring in the top of the core. A subsurface peak was present at 3 cm indicating that nitrification might have been occurring however, as in core ST7, no concomitant concave-upward pattern in the ammonium profile was noticeable. On the contrary ammonium exhibited a concave-downward profile over the whole depth of the core indicating that ammonification of organic matter was occurring throughout the core with concentrations reaching 1.7 mM by 35 cm depth.

The sulphate and SO_4^{2-}:Cl^- ratio profiles for core ST8 exhibit similar patterns, with sulphate showing a decrease in values from 18-17 mM over the top 9 cm of the core (the small increase in sulpate between 1-3 cm is likely linked to the changes in salinity at this depth described above). Below 9 cm, concentrations stay around 17 mM down to 19 cm before decreasing again down to 14 mM at the base of the core. This indicates that sulphate reduction is occurring in this core and may in fact be occurring in two distinct zones, an upper zone between the surface and 9 cm, and a lower zone beneath 19 cm. In between these two zones, where sulphate concentrations are stable it may be the case that sulphate reduction is not occurring (unlikely as the NH_4^+ and CO_2 profiles continue to increase with depth), or that reoxidation of sulphide is keeping pace with sulphide reduction, producing a "steady-state" profile. The change in gradient at 19 cm also coincides with the increase in organic acid concentrations which suggests that a boundary between the dysoxic and anoxic zones of the sediment may be somewhere around this depth. The static nature of the sulphate and SO_4^{2-}:Cl^- profile between 9-19 cm would also add support to this, as if this stasis was caused by sulphide oxidation (either by chemical or biological means) oxygen (or other oxidised compounds) would have to be present in the sediments to some degree.

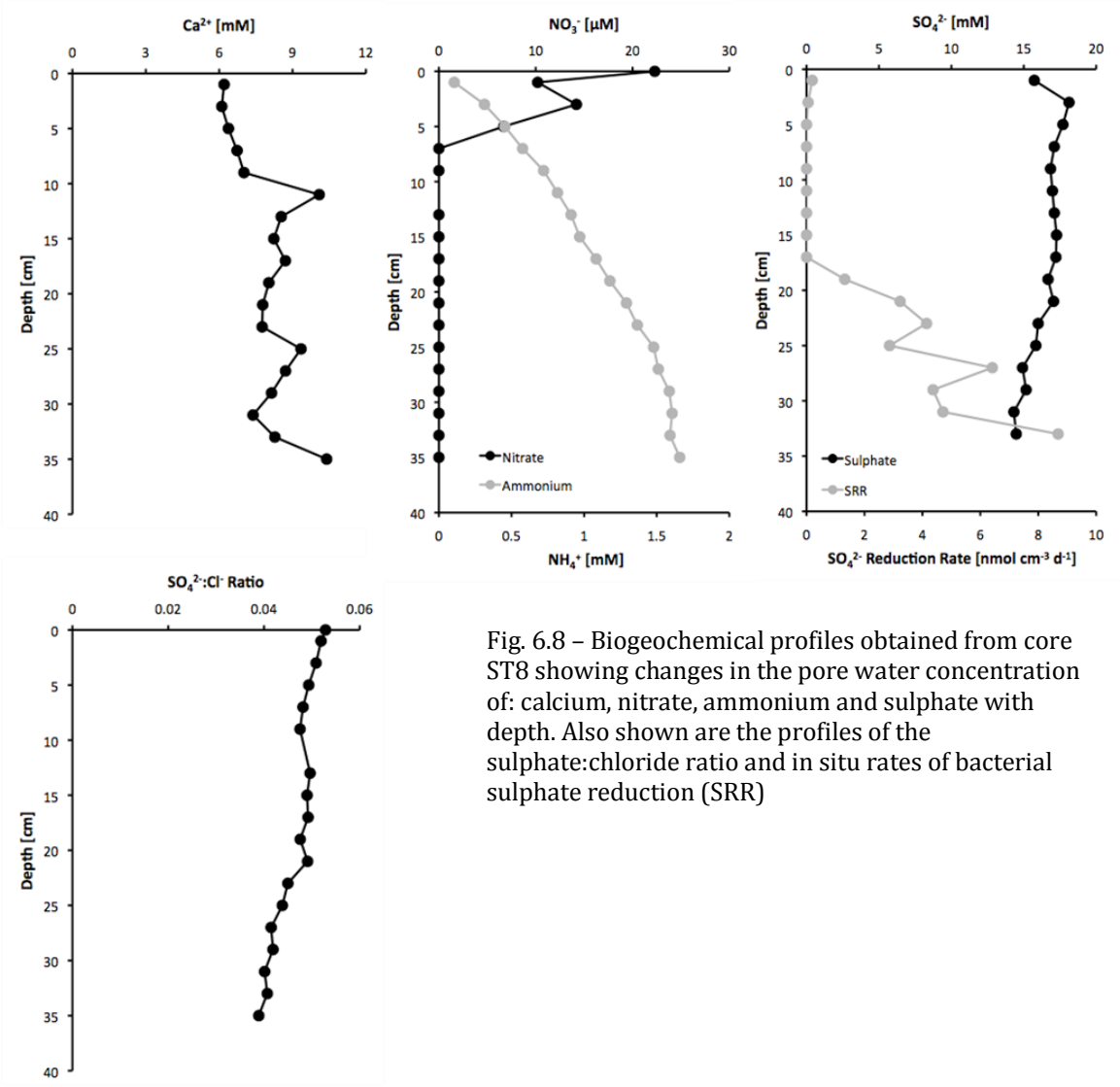


Fig. 6.8 – Biogeochemical profiles obtained from core ST8 showing changes in the pore water concentration of: calcium, nitrate, ammonium and sulphate with depth. Also shown are the profiles of the sulphate:chloride ratio and in situ rates of bacterial sulphate reduction (SRR)

The SRR measurements show that there are indeed two zones of sulphate reduction within core ST8. In the top 4 cm of the core trace levels of sulphate reduction were detected though these did not rise above 0.19 nmol cm^{-3} d^{-1}. Below this rates dropped to zero until 19 cm at which point they began to rise again, reaching a maximum of 8.7 nmol cm^{-3} d^{-1} at the base of the core. The occurrence of sulphate reduction in the top few cm of the core suggests that the organisms here were living within anaerobic microniches (Jørgensen, 1977) as, judging by the presence of a subsurface nitrate peak, the sediment would have almost certainly contained some oxygen. The apparent cessation of activity between 4-17 cm matches the more vertical section of the sulphate profile and could indicate that sulphate reduction was not occurring or that sulphide oxidation was co-occurring at roughly the same rate and converting the radio-labelled $^{35}S^{2-}$ back into $^{35}SO_4^{2-}$ which

would not have been detected by the analytical procedures used (Kallmeyer *et al.* 2004). The increase in rates below 17 cm coincides with both the change in sulphate gradient and the erratic peaks in organic acid concentrations. This provides more evidence of a dysoxic-anoxic transition around 15-20 cm but also indicates that the SRB at depth may be utilising the organic acids produced at this depth as electron donors and/or as a carbon source.

Strangely despite the presence of active SRB in the core at depth, based on sediment colour no significant levels of FeS seem to be forming in the lower levels of the sediment column. Similarly the lack of free phosphate in the pore waters also indicates that there is little to no free FeS formation occurring. In some environments sulphate reduction can occur with a limit on the usual concomitant creation of FeS. For example, if the sediments in question are depleted in iron (Berner 1984, Sternbeck and Sohlenius, 1997), or are rich in humics that can trap the sulphide as organosulphur compounds (Casagrande *et al.* 1977, Brown 1986, Ferdelman *et al.* 1991). However, in this situation these scenarios are very unlikely, especially considering the proximity of a site (ST7) where FeS formation is occurring in what appears to be very similar sediment. A more likely explanation, given the turbulent nature of the Severn Estuary, is that the sediment at this site had been recently re-suspended and the FeS produced within oxidised by oxygenated seawater. This might also explain why sulphate reduction was only occurring significantly in the bottom half of the core as the layers above may not be stable enough for SRB to grow outside of microniches, or may still contain large quantities of substrates for organisms further up the redox cascade that could out-compete the SRB (possibly as a result of the re-oxidation of previously reduced compounds with oxic seawater upon mixing – e.g. the re-oxidation of FeS to produce iron oxides). Further discussion of this differential re-suspension of core sediment will be presented in section 6.3.

Methane concentrations in core ST8 were low but increased with depth from 0.8 μmol l^{-1} of wet sediment at 1 cm to 2.5 μmol lws^{-1} at the base of the core (Fig. 6.9). This increase with depth was not linear however, with the profile exhibiting a break in slope between 11-19 cm in a similar fashion to sulphate. Below 19 cm the profile steepened again and remained so to the base of the core. The reason for this

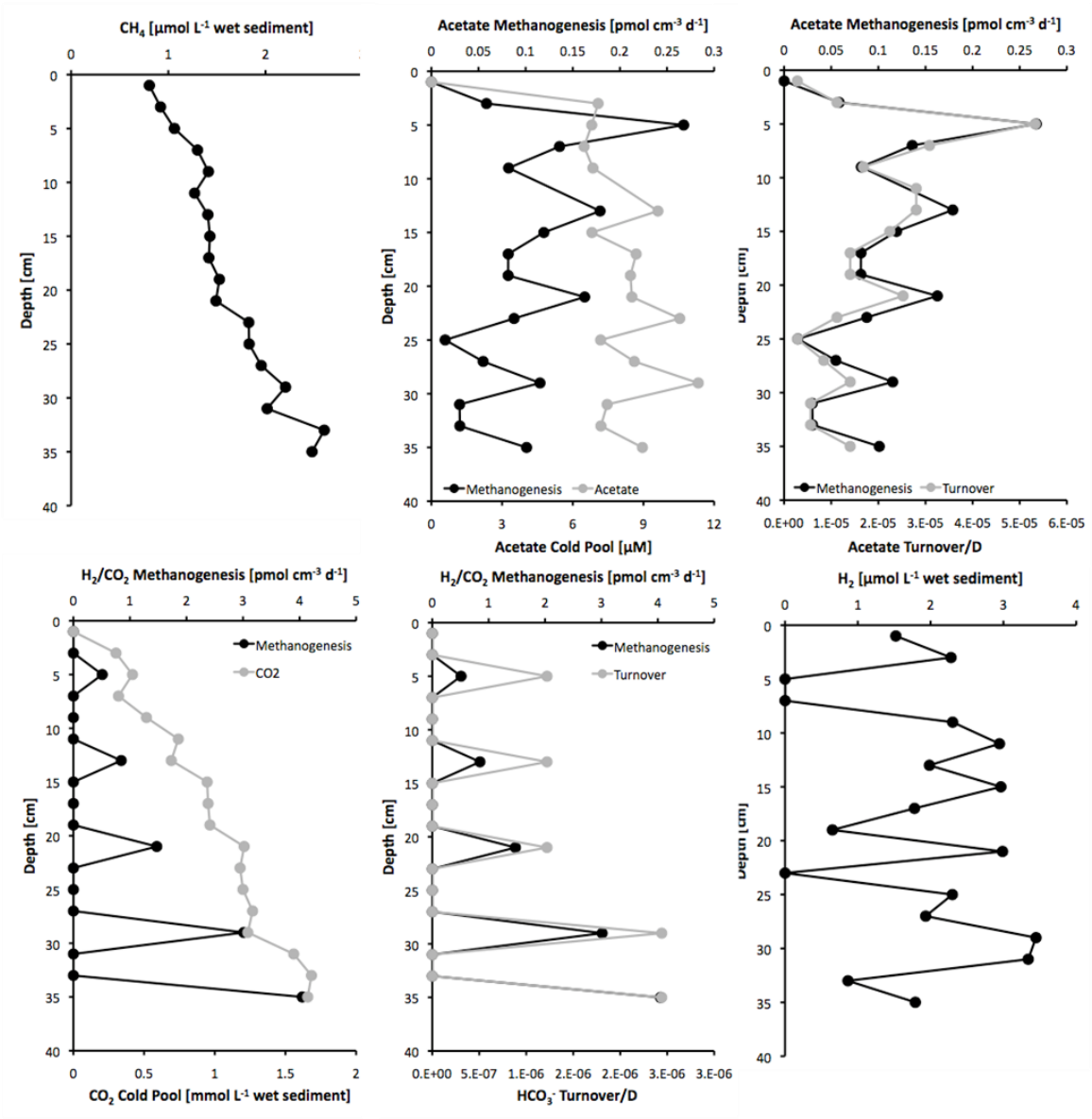


Fig. 6.9 – Biogeochemical profiles obtained from core ST8 showing changes in the concentration of: methane, CO_2 and hydrogen with depth. Also shown are profiles of the in situ rates of both acetoclastic and H_2/CO_2 methanogenesis plotted with the cold pool concentrations of their respective substrates and the rate of turnover of their radiolabeled substrates to methane.

apparent decrease in production between 11-19 cm is unknown, as the profile does not show the concave-upward shape characteristic if AOM and for the most part the break in slope occurs at a depth where little to no sulphate reduction is occurring. However, this does not rule out AOM completely as methane oxidation coupled to iron reduction and manganese reduction has been documented before (Beal *et al.* 2009; Segarra *et al.* 2013), although as with core ST7 the presence of AOM in these sediments cannot be confirmed without more direct evidence (e.g. $^{14}CH_4$ radiotracers). The increase in methane below 19 cm on the other hand, again

coincides with the changes in the other profiles detailed above, thus providing further evidence for a dysoxic-anoxic transition at around this depth.

Activity measurements showed that acetoclastic methanogenesis was occurring in these sediments but again at low levels – the maximum rate was 0.27 pmol cm^{-3} d^{-1} at 5 cm depth. Somewhat incongruously both acetoclastic methanogenesis rates and turnover of ^{14}C-acetate to methane showed a decreasing trend with depth despite the fact that both methane and acetate concentrations were increasing slightly. In addition there was no major change in the depth profile shape around 17-19 cm that might indicate the impact of a change in the oxygen content or oxidation state of the sediment. H_2/CO_2 methanogenesis was also present in the core and had an increasing (though erratic) trend with depth reaching a peak rate of 4.05 pmol cm^{-3} d^{-1} at 35 cm. Unlike acetoclastic methanogenesis, both H_2/CO_2 methanogenesis rates and turnover of ^{14}C-HCO_3^- to methane did increase below 19 cm, again indicating a potential change from dysoxic to anoxic conditions. This increase in methanogenesis with depth may help to account for the increase in methane with depth, although the overall rate of methanogenesis in this core is still low. As with core ST7 no methylated amines or other non-competitive substrates were detected in the core.

Hydrogen concentrations within core ST8 showed a sharp initial decrease in the top 5 cm of the core (possibly linked to nitrate reduction). Below this values were erratic with no obvious trend with depth, varying between 0-3.4 μmol lws^{-1} throughout the core. CO_2 concentrations on the other hand showed a distinct increase with depth, reaching a maximum value of 1.7 mmol lws^{-1} at the base of the core, most likely driven by increasing amounts of organic matter degradation with depth (similar to the NH_4^+ profile).

Total cell counts in core ST8 were higher than in ST7 with counts comparable at the surface ($1x10^{9.5}$ cells cm^{-3}) but remaining relatively constant with depth, reaching $1x10^{9.4}$ cells cm^{-3} at the base of the core (Fig. 6.10). No sharp drop in cell numbers with depth, like that seen at 10 cm in core ST7, was noted in ST8 although there was a slight drop in numbers (down to $1x10^{9.3}$) at 19 cm that might again correspond to

a change from dysoxic to anoxic conditions. Porosity values showed no overall trend with depth with values varying between 65-80% throughout the length of the core.

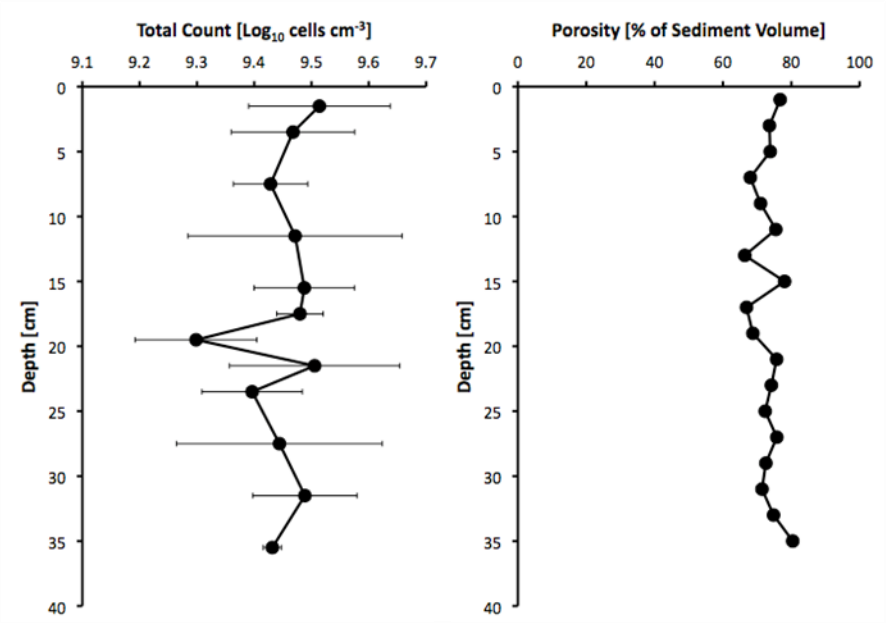


Fig. 6.10 – Profiles obtained from core ST8 showing the changes in total cell counts (AODC) and sediment porosity with depth. Error bars represent the 95% confidence limits obtained from repeated counts.

6.3 Bridgwater Bay - Discussion

Since Bridgwater Bay is one of the largest sources and sinks of transient sediment in the Severn Estuary (along with the tidal flats on it's northern shore) (Kirby, 2010), it probably plays a major role in the estuary's biogeochemical system. As such it is important to understand what effect the erosive power of the Severn Estuary's tides might have on the sediments, the incumbent microorganisms and the biogeochemical processes they control. By examining two sites: one shallower site deemed to be primarily "erosive"; and another deeper water site deemed to be "stable", we can compare the biogeochemical profiles and rates to see what affect the tides and currents are having on the processes occurring within the sediment.

A problem occurs however, when examining the two cores side by side as there are a number of indicators that show that the "erosive" nature of core ST7 and the "stable" nature of core ST8 may not be as clear cut as previously thought. For example when examining the nitrate profiles of ST7 and ST8 (shown in Fig. 6.11), it can be seen that nitrate is depleted more quickly in ST7 than ST8 indicating that the

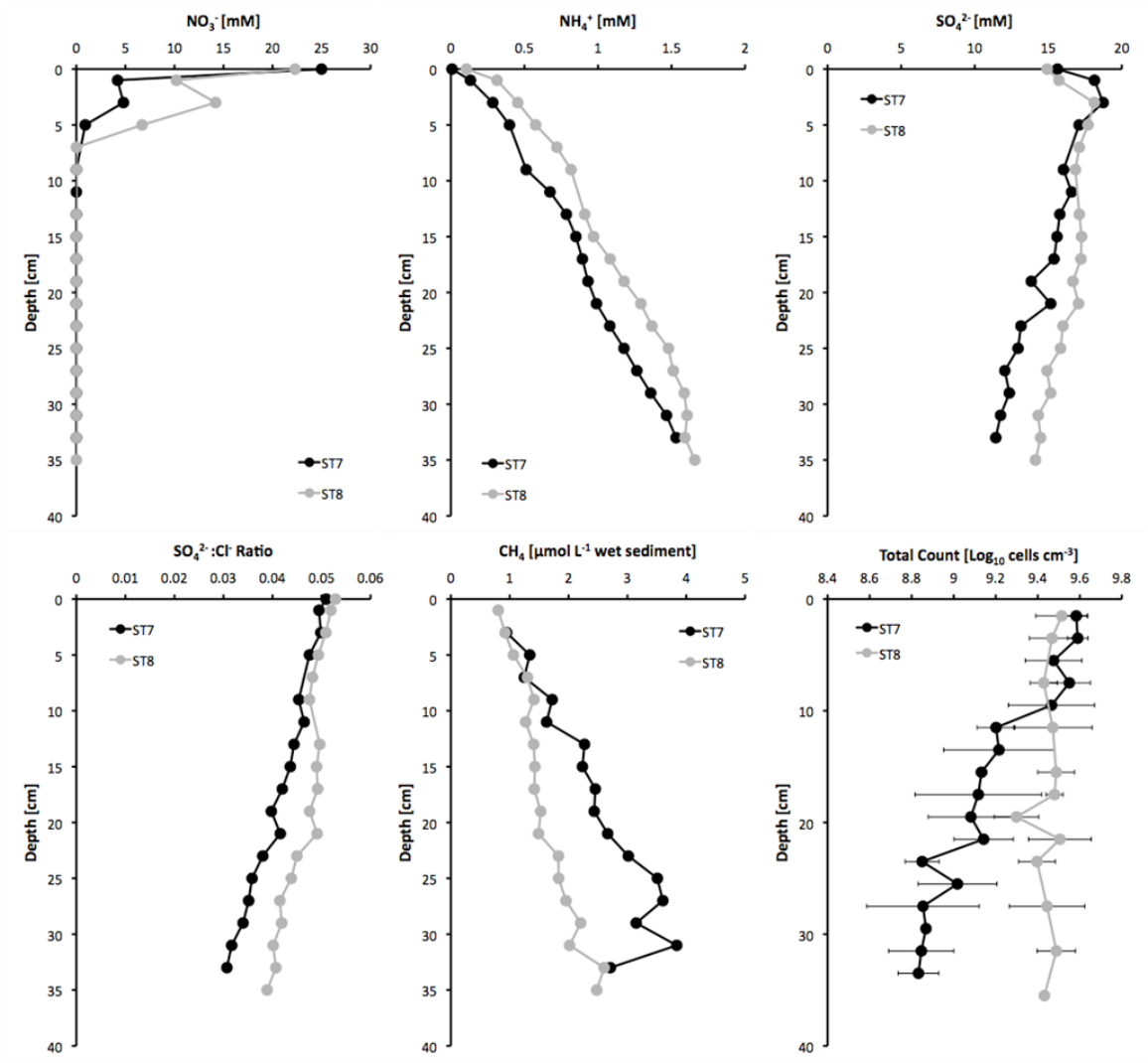


Fig. 6.11 – Comparison of the profiles of: nitrate, ammonium, sulphate, the sulphate:chloride ratio, methane and total cell counts obtained from cores ST7 and ST8 illustrating the differences in the *in situ* biogeochemistry likely linked to the variation in depositional environment between the two cores.

bacterial community carrying out denitrification/DNRA may be more stable in ST7. Since these organisms need hypoxic conditions in which to respire it seems that the sediments in core ST7 may have been stable and in place longer than those in ST8 in order for these conditions to occur.

Further evidence is provided by comparing the sulphate and SO_4^{2-}:Cl^- profiles of the two cores. Within the top 11 cm of both cores values are similar, however, below 12 cm, values in core ST7 start to decrease more sharply than in ST8. This divergence coincides with the change in sediment colour (and therefore change in redox state) in core ST7 and indicates that the while conditions in ST7 become reducing below 12 cm (thus promoting sulphate reduction) sediments in ST8 may

have retained oxidised conditions down to the base of the core, with dysaerobic, surface-like conditions prevailing down to ≈20 cm. This is corroborated by the SRR measurements, as in core ST7 sulphate reduction starts to occur in earnest below ≈5 cm however, in ST8 it does not occur above trace levels until 19 cm depth. Again this would indicate that stable anoxic conditions were present in core ST7 for longer than in ST8 as this would allow the redox zones to migrate up through the sediment column. As mentioned above, the lack of any dark colouration (or free phosphate) in the sediment in ST8 would indicate an absence of FeS, which again points to the fact that that the sediments in ST8 had been exposed to oxidising conditions more recently than in ST7.

Methane profiles for the two cores exhibit a similar depth trend, with values in the top 7 cm of the cores being roughly comparable (probably due to the presence of nitrate which would limit methanogenesis). Below this depth however, values in core ST7 increase more rapidly while in ST8 the gradient remains relatively similar down to the base of the core (with the exception of the slight change in slope below 19 cm). This again indicates that ST8 may have been exposed to oxidising conditions more recently or that the sediments that make up ST8 had been re-suspended more recently and thus had much of the methane stored within them oxidised or released into the water column.

Finally, when comparing the total cell count profiles for the two cores it can clearly be seen that the decrease in numbers with depth expected in surficial sediment cores is much more pronounced in core ST7 than in ST8. In fact in ST8, with variances caused by counting error taken into account, the difference in numbers between the top and bottom of the core is negligible. As previously described for core ST3 (see Chapter 3) this relatively uniform AODC profile may be an indication of recent sediment suspension, mixing and re-deposition as the numbers of cells in a mobile, continuously mixed, water-sediment mixture would be uniform with depth and would likely remain so for a time after deposition, until the limited availability of surface-derived substrates led to a reduction of the numbers of organisms at depth.

Taken together the evidence described above indicates that rather than being “erosive” in nature, the sediments sampled in core ST7 were in all likelihood much

more stable than those in ST8, which may in fact have only been very recently deposited after being exposed to an oxic environment - slurries of well mixed Severn Estuary sediment incubated anaerobically tend to turn black after a period of 3-5 days (S. Thomas, unpublished results). These findings would initially seem to disagree with those of Kirby (1994) however, there are two potential reasons why this might not be the case. The first is that in the time since Kirby's 1994 study was carried out some of the sediment stability zones may have shifted position. However, since these zones are mainly determined by seafloor topography/water depth and since this has not changed drastically over the last 20 years this seems unlikely (though small-scale changes may have occurred). A second possibility is that the proxies used by Kirby for sediment accretion might not be applicable for this study. The main proxy utilised was the abundance of ^{137}Cs and other anthropogenic radionuclides in the sediment column – the deeper the nuclides were present in the sediment, the higher the inferred rate of sedimentation (Pennington *et al.* 1973). While this proxy works well for long term depositional trends in undisturbed sediment (Albrecht *et al.* 1998), it does not help determine how long surficial sediment has been in place – e.g. over a weekly-monthly basis - as any changes in sedimentation on these timescales would not be reflected in the radionuclide levels, especially if the same sediment was being continuously eroded and re-deposited. As such the sediment map produced by Kirby (1994) would possibly have very little bearing on any trends observed in this study. This however, would still not explain why a site with greater water depth would contain more recently deposited sediment, as the general rule that deeper water leads to more stable deposition should still apply. The answer to this may lie in the composition of sediment within core ST8 itself. As stated above ST8 was much less cohesive during sectioning than ST7 and also contained sand bodies. In addition during coring at the ST8 site several cores had to be discarded as sediment had oozed out of the top of the core tubes, this meant that the corer had sank further than normal into the sediment indicating that it was much more fluid than normal. Therefore, it is possible that actually core ST8 once again represents sediment derived from a fluidised mud bed, similar to core ST3 taken from the Newport Deep (with which it shares other physical and geochemical characteristics). This would fit in with the findings of Kirby (1994) who describes the presence of fluidised muds within the

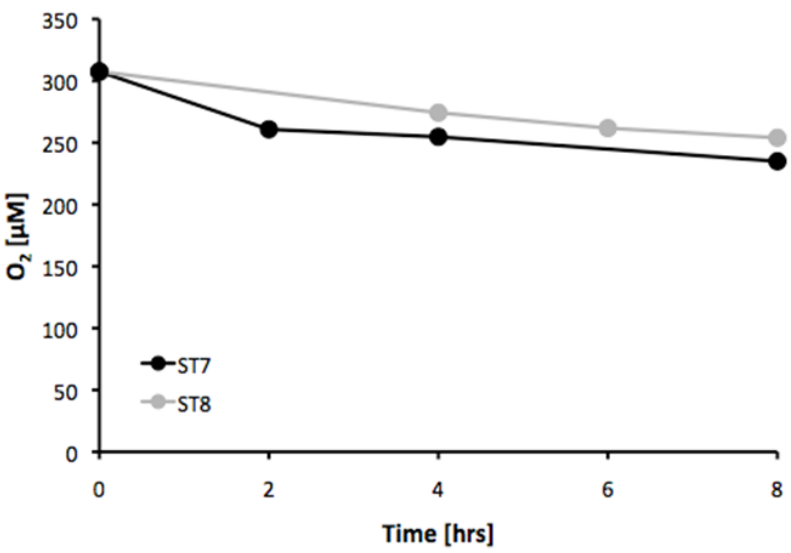


Fig. 6.12 – Line graph showing the consumption of oxygen by copies of cores ST7 and ST8 incubated in darkness at *in situ* temperature for 8 hours. Data obtained from the decrease in oxygen concentrations in the overlying waters of the cores corrected for loss by planktonic heterotrophic activity.

more stable areas of Bridgewater Bay during neap tides, particularly in the area around the ST8 site where near-bed SPM concentrations can reach 10,000 mg l^{-1} (Kirby, 2010). If this were the case then it would also agree with the findings of Madrid *et al.* (2006) who found that bacteria in fluidised muds were capable of carrying out sulphate reduction without the muds in question becoming sulphidic – a similar situation to that observed in core ST8 (with its lack of dark colouration).

When comparing the rates of processes from the two cores it can be seen that total O_2 uptake was higher in core ST7 than ST8 (44 mmol m^{-2} d^{-1} vs 37 mmol m^{-2} d^{-1}) (Fig. 6.12). This is unusual as in the other cores described so far in this study, those with the higher degree of disturbance tended to have a higher O_2 demand. There are several reasons why this might be the case, the first being that due to the mobile nature of the sediment in core ST8 the aerobic zone of the fluidised mud might have been lost when sampling. This is unlikely however, as care was taken when sampling to only retain cores with an intact surface layer. A second possibility is that the upper zones of the fluid mud, where O_2 penetrated in high enough concentrations to make aerobic respiration feasible, might have been too disrupted by mixing etc. for stable communities to develop. Alternatively, though no macrofaunal bioturbation was observed in either core it is possible that the more stable sediments in core ST7 made a better habitat for aerobic meiofauna (ostracods, benthic foraminifera etc.), the metabolism of which might have enhanced the O_2 demand of the sediments. Finally it is possible that the low O_2 uptake of the sediments in ST8 is an artifact of

the sampling procedure, since the O_2 uptake measurements were carried out on stabilised sediment in core tubes, rather than in the fluidised mud bed itself. Though fluidised muds are known to be very active at mineralising carbon (Aller and Blair 2006), this is only because they are being re-supplied with substrates on a regular basis via sediment mixing. It may therefore be the case that after the sediment in core ST8 settled out in it's core tube a potentially very active aerobic community quickly used up the (now limited) OC substrate supply within the aerobic zone and it's growth became suppressed, producing lower O_2 uptake rates by the time the measurements were taken. Meanwhile other microbial groups with typically slower metabolic rates (such as the SRB and methanogens), which occupy a larger portion of the sediment column, might not use up their OC substrate supply so quickly and as such could continue to respire at their more normal rates for longer.

Despite being more recently deposited than core ST7 (or possibly not truly deposited at all) the sediments at the ST8 site still had a higher depth-integrated total SRR value of 720 μmol m^{-2} d^{-1}, compared to 492 μmol m^{-2} d^{-1} at site ST7 (especially considering the SRR profile of ST8 was still increasing at the base of the core to a much greater degree than in ST7, which has significantly lower rates below 20 cm depth). Total acetoclastic methanogenesis values, while low, were also higher at ST8, reaching 34.6 nmol m^{-2} d^{-1} compared to 6.7 nmol m^{-2} d^{-1} at ST7. This would indicate that the re-suspension of sediments at this site as fluidised mud does not appear to have a detrimental effect on the metabolic rates of the heterotrophic anaerobic prokaryotes within. On the contrary it seems to enhance the rates of the processes – by as much as 6 fold in the case of acetoclastic methanogensis. This metabolic enhancement is likely driven by the repeated mixing of the fluidised muds over the semi-lunar cycle which provides a ready supply of both terrigenous and authigenic organic matter and oxidised electron-acceptor substrates at all depths in the sediment, as well as re-oxidising already depleted substrates back into usable forms (Aller 1998, Abril *et al.* 1999, Abril *et al.* 2010). For example SRR measurements from core ST8 are much higher than from ST7 but the sulphate gradient in ST8 is shallower, this could indicate that sulphate is periodically re-supplied to the lower levels by mixing, allowing high SRRs to occur without the usual concomitant decrease in the sulphate profile. This form of mixing provides ideal growth conditions for anaerobic prokaryotes, essentially "short circuiting" the

normal redox cascade of depth dependant substrate utilization, provided that the organisms in question are able to tolerate short periods of oxic/hypoxic conditions during the extensive mixing of the sediment that occurs over the spring tidal period. This also means that the normal stratification of anaerobic groups will not necessarily occur in these sediments. For example, in core ST7 sulphate reduction and acetoclastic methanogenesis occurred at similar depths, whereas in core ST8 acetoclastic methanogenesis predominantly occurred above sulphate reduction despite being less energetically favorable. However this disturbance of the redox cascade is unlikely to be universal and some vertical control of processes may still be occurring (e.g. nitrate and presumably O_2 consumption), though without further *in situ* rate measurements this cannot be confirmed.

On the other hand, despite the apparent enhancement of both SRR and acetoclastic methanogenesis rates, H_2/CO_2 methanogenesis is lower at site ST8 than ST7 with depth integrated rates in the fluidised mud of ST8 (197 nmol m^{-2} d^{-1}) substantially lower than in the mud patch sediments of ST7 (487 nmol m^{-2} d^{-1}). This may be because H_2/CO_2 methanogenesis relies on compounds that are predominantly found as gases and may quickly diffuse out of the sediments during fluidisation, decreasing the substrate pool in the sediments. However, this is unlikely as the pool sizes of these gases measured in the cores were similar in the case of hydrogen and higher in core ST8 in the case of CO_2. Alternatively it may be the case that H_2/CO_2 methanogens are less tolerant of oxygen than the SRB or acetoclastic methanogens and as such their growth may be more hindered by the regular exposure of the muds to oxic conditions during fluidization (Kato *et al.* 1993; Horne and Lessner, 2013) In addition the re-supply of the sediment with OC and electron donors during mixing may not enhance the growth of the chemolithoautotrophic H_2/CO_2 methanogens as much as other heterotrophic groups as rather than relying on surface derived compounds they rely on compounds produced *in situ* in the final stages of the OC degradation pathway (Fig. 1.4), which may not be occurring to their full extent in these sediments (either due to disturbance or O_2 stress). If this is true then it may be the case that the enhancement of microbial growth in fluidised muds described by Aller *et al.* (1998) and others is not universal and is in fact dependant on the metabolism of the organisms concerned (ie. heterotrophs vs. lithotrophs).

As a final point, these findings also highlight the importance of using radiotracers (or other similar means) to directly study rates of microbial respiration in sediments, rather than just examining the geochemical profiles they produce. For example if the sulphate or SO_4^{2-}:Cl^- profiles of cores ST7 and ST8 were compared it might be thought that sulphate reduction was less active in core ST8 than in ST7 since the decrease in values from the top to the bottom of the core was less. However, when we compare the active SRR measurements it can be seen that the reverse is true. This means that in dynamic sedimentary environments, such as the Severn Estuary, where directly measured geochemical profiles have been the only means used to assess processes such as sulphate reduction, the degree to which these processes might be occurring could have been significantly underestimated. This would especially be the case if the sediment in question was frequently re-suspended, or subjected to other such process in which sulphate (or other growth-limiting compounds) might be delivered to the sediment at depth (e.g. via groundwater flow); or if the sediment was subject to oxidised conditions that might promote processes (such as sulphide oxidation), that would decrease the apparent depletion of these compounds shown in the geochemical profiles.

6.4 Bridgwater Bay - Conclusions

- Despite being sampled from locations in relatively close proximity within the same bay cores ST7 and ST8 exhibit some notable lithological and geochemical differences.
- Core ST7, sampled from an area deemed to be erosive, shows signs of having relatively stable and reducing geochemical conditions beneath ≈11 cm.
- Core ST8, sampled from a supposedly stable/accreting area, shows evidence of dysaerobic conditions being present down to ≈20 cm depth and oxidised conditions possibly all the way to the base of the core.
- Multiple lines of evidence suggest that core ST8 may be derived from a fluidised mud pool while ST7 originated in a more normal, subtidal mud-patch environment, subject to occasional surficial resuspension.
- Total O_2 uptake was lower in core ST8, possibly due to the unstable nature of the sediment, lack of meiofauna or problems during analysis (i.e. settling of the sediment in the core tubes).

- Rates of sulphate reduction and acetoclastic methanogenesis were higher in core ST8 suggesting that it's regularly fluidised state may enhance the growth of anaerobic heterotrophs – possibly by incorporating more bioavailable OM into the sediment or due to the re-supply and/or reoxidation of electron-donor substrates.
- H_2/CO_2 methanogenesis was lower in core ST8 suggesting that the enhancement of anaerobic processes in these sediments may not extend to hydrogen-utilizers.
- The fact that core ST8 contains clear geochemical profiles that change with depth, despite likely being recently fluidised, suggests that the sediments of Bridgwater Bay are very biogeochemically active - despite the turbulent conditions to which they are exposed.

Chapter 7. - St Brides Wentlooge: Cores StB1 and StB2

7.1 Core Descriptions and Lithology

Due to the unusual sedimentological and biogeochemical characteristics of core ST5 (i.e. the presence of a peat layer and elevated methylated amine concentrations) a series of longer cores were taken from this site to further study the Holocene peats and clays of the Wentlooge Formation. Two samplings were conducted at the same site that core ST5 was taken from (see Fig. 4.3), using longer (c. 60cm) core tubes with sharpened bases suitable for cutting through the fibrous peat layers and heavily compacted clays beneath. Sampling took place in November 2012 (core StB1) and July 2013 (core StB2), which also allowed any seasonal changes in the biogeochemical processes occurring at this site to be documented.

Lithologically cores StB1 and StB2 were superficially similar to core ST5, with a layer of soft, modern estuarine mud overlying the peat surface (shown in Fig. 7.1). The depth of this mud varied between cores however, with core StB1 having an overlying mud layer around 8 cm thick while the mud layer in StB2 was over double this thickness at 17-18 cm. The colour and texture of the mud also varied between cores with StB1 having an overlying layer composed entirely of loosely consolidated mud of a uniform light brown colour, while in StB2 the mud was more cohesive and showed a distinct darkening with depth (at ≈12 cm) indicative of the presence of iron sulphide. This variation in mud thickness and cohesiveness initially appears to be due to the fact that StB1 was taken over the spring tide portion of the tidal cycle in winter whereas StB2 was taken during a neap tide in the summer. Since in the Severn Estuary spring tides are periods of net erosion compared to neap tides, which are periods of deposition (Manning *et al.* 2010), it seems reasonable that a mud layer deposited on a spring tide would be thinner and less cohesive than a similar layer deposited on a neap tide. However, O'Brien *et al.* (2000) showed that while mudflat height on the Wentlooge Flats is higher in summer than winter, paradoxically the flats are also higher during the spring tide period than the neap. As such the main driving force for the varying sediment thickness at this site is likely to be seasonal rather than tidal while cohesiveness may be linked to both seasonal and tidal effects. The variation in season and tide would also have been a driving factor on the colour differences between the mud layers, as the mud layer in core StB2

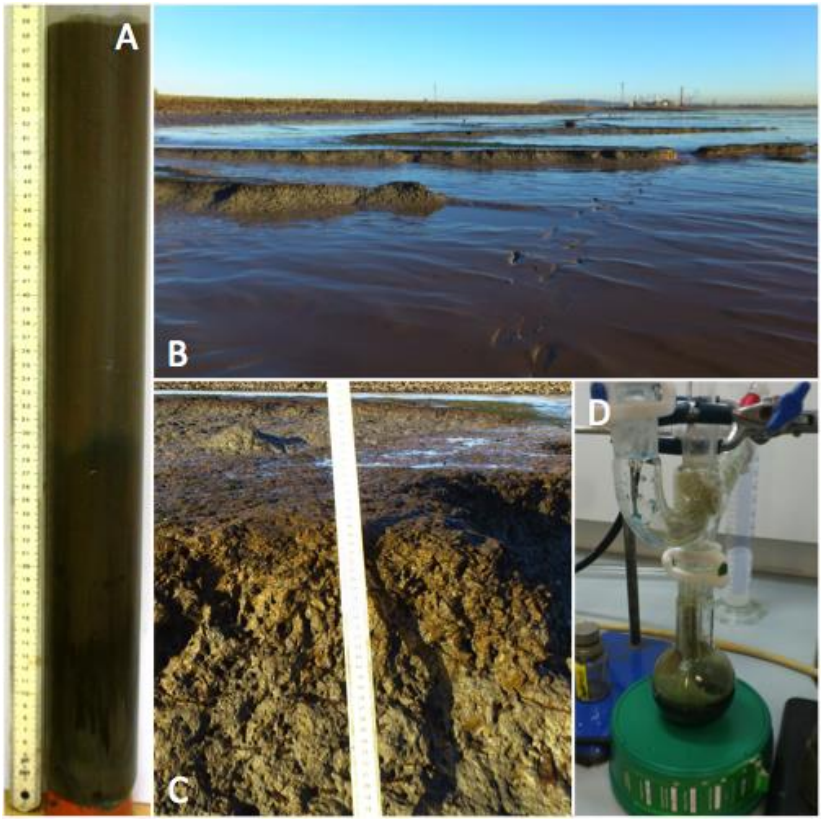


Fig. 7.1 – A) Photograph of core StB2 showing changes in lithology with depth, from light brown surficial muds down into dark brown-black peat and subsequently to grey clay at the base of the core. B) View of the peat bed at the St Brides Wentlooge site. The peat bed is resistant to erosion, forming distinct ledges around the shoreline, which are covered by a veneer of poorly consolidated modern sediment (seen in foreground). C) Close-up view of the peat bed ledge (50 cm ruler for scale) showing it grading into the grey Wentlooge Clay beneath. The peat bed here is approx. 20 cm thick and fragments of intact plant material can be seen, both within the peat and the clay below. D) Photograph of a cold chromium reduction vessel (Kallmeyer *et al.* 2004) during a reaction with the St Brides peat. As can be seen in the photograph large amounts of green foam were produced after addition of the $CrCl_2$ solution indicating potentially high levels of CRS.

would likely have been in place longer which would have allowed time for it to become fully anoxic and for sulphate reduction to occur (hence the darkening colour), which may not have happened in the more disturbed StB1. In addition when core StB2 was collected during the summer months sediment temperatures ranged from 18-21 °C whereas core StB1 was taken in late autumn when temperatures were as low as 6 °C. As such it is likely that the higher summer temperatures would have increased prokaryotic metabolic rates, including those of the sulphate reducers in the overlying muds, which combined with the lack of disturbance of the sediments during the summer period, would have led to more reduced conditions and a higher production of FeS in the mud layer of core StB2 compared to StB1.

Beneath the mud layer in both cores was a bed of densely compacted salt-marsh peat ≈25-35 cm thick. The peat itself was dry and crumbly in texture and gave off a strong sulphidic smell when exposed during sectioning. Compositionally the peat also changed with depth, with the upper portions of the bed being greenish-brown in colour and made up of the semi-intact remains of reeds and rushes, while the base was much darker brown/black and appeared to be composed of well decomposed woody material. This darker peat made up the bottom 7-8 cm of the bed in both cores with the transition occurring at 32 cm in StB1 and 36 cm in StB2. A change in peat composition such as this agrees with the account of Allen (2001) and likely indicates a change of depositional environment over time. The peat at the base of the core was likely formed in a raised bog/fen carr environment (Walker *et al.* 1998) dominated by alder (*Alnus*) and willow (*Salix*) while at the top of the bed the peat probably developed in a more marine-dominated, organogenic salt-marsh environment populated by reeds and sedges (e.g. *Phragmites*), examples of which occurred across the southern UK during the Flandrian (Allen 1990a). This change of depositional environment would have been driven by coastal flooding, caused by relative sea level rise over the course of the Holocene (Allen 2003, Allen and Haslett, 2007). It is thought that the peat layer described at this site (based on thickness, location and ledge-forming nature) matches with the upper portion of the "4th peat" described by Allen and Haslett (2007) from their nearby study site at Redwick - if this is the case it gives the peat a radiocarbon age of ≈3400 years BP or 1500-1900 years BCE.

Near the base of the cores (at 40 cm and 42 cm in StB1 and StB2 respectively) the peat layer rapidly grades into a pale grey bed of dense, compacted clay. This clay intercalates with other peat beds over much of the Wentlooge Formation. (Allen 1987d), and was deposited on mudflats and minerogenic saltmarshes during periods within the Holocene when sea level rise was too rapid to allow the formation of peat deposits (Allen 1995).

7.2 St Brides Wentlooge - Results

7.2.1 Biogeochemistry of Core StB1

Organic acid concentrations within core StB1 were high overall and varied markedly with depth (Fig. 7.2). Acetate, formate and propionate showed similar profile shapes

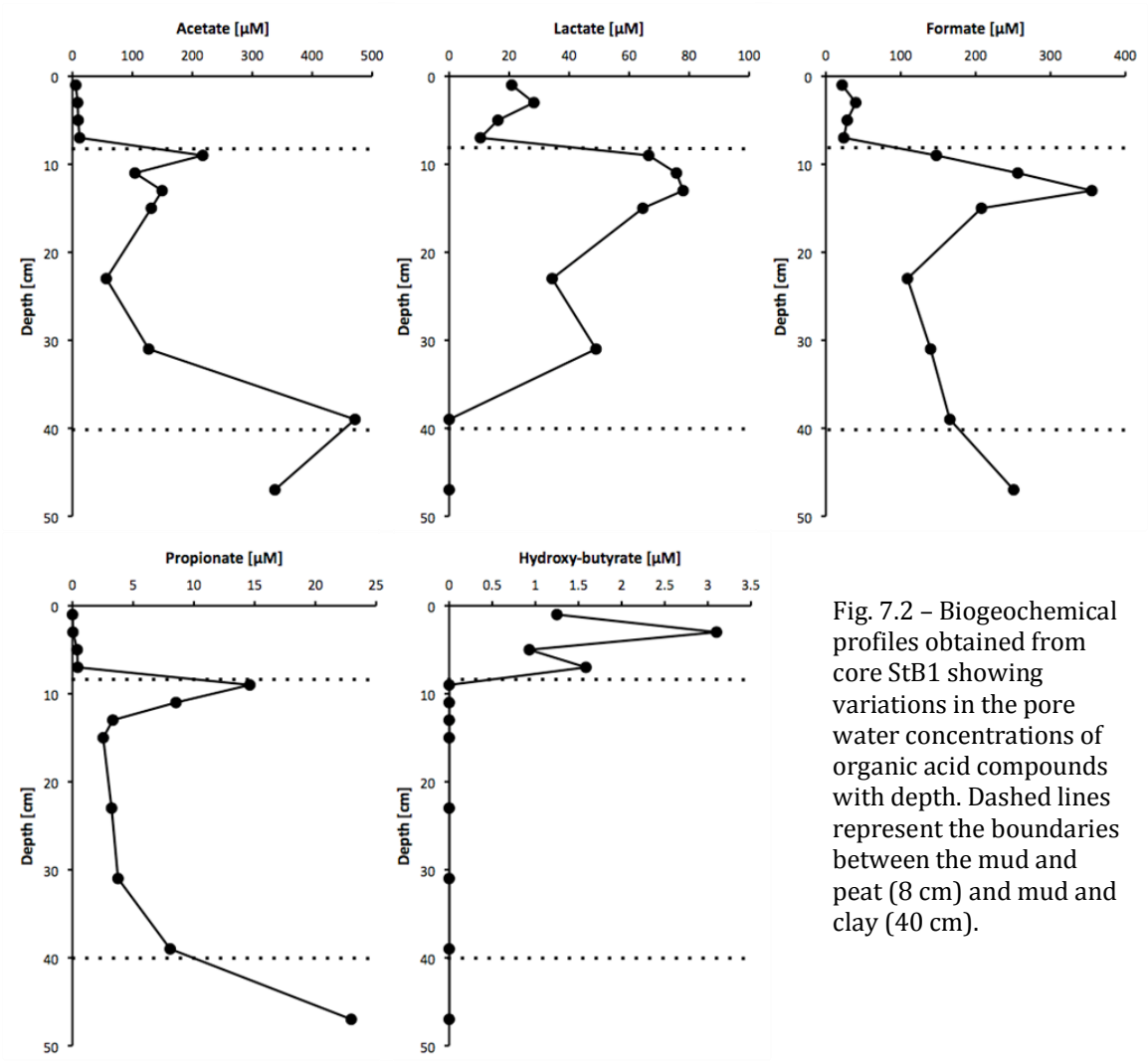


Fig. 7.2 – Biogeochemical profiles obtained from core StB1 showing variations in the pore water concentrations of organic acid compounds with depth. Dashed lines represent the boundaries between the mud and peat (8 cm) and mud and clay (40 cm).

with concentrations remaining low in the overlying muds before increasing dramatically in the top 5 cm of the peat – in the case of acetate there is a 206 µM increase over 2 cm. Below 15 cm, concentrations gradually decrease again down through the peat layer before increasing again towards the peat-clay boundary, in the case of acetate reaching a maximum concentration of 471 µM at 39 cm. Lactate concentrations initially exhibit a similar trend to the three acids detailed above, with concentrations rising from 10 µM at 7 cm to a maximum of 78 µM at 13 cm. Below this however, concentrations fell and continued to do so down to the base of the core where no lactate was detected. Finally hydroxy-butyrate had a different pattern to the other organic acids, peaking within the overlying muds (at concentrations of 3.1 µM) and then decreasing with depth before dropping below detection limits upon entering the peat layer.

The sudden spike in organic acid concentrations at the top of the peat layer (c. 8-15 cm) would indicate that bacterial fermentation (the main pathway for the production of these compounds) is very active at this depth (Kane *et al.* 2013). Since organic acid production is low in the overlying sediment it is likely that the compounds being fermented are derived from the peat itself rather than from "fresh" organic material present in the recently deposited muds. Being rich in organic macromolecules, the peat would make an excellent substrate for fermenting organisms, however, if this is the case it is puzzling that concentrations of organic acids decrease downwards into the peat layer. A possible explanation for this is that the peat, though rich in fermentation substrates, is lacking in other limiting nutrients that the fermenters require to survive. In the upper 5 cm of the peat these compounds could be provided via diffusion from seawater or recently deposited sediment, to which the top of the peat bed is regularly exposed. Alternatively, since the top surface of the peat is in contact with oxidised sediments and (during re-suspension events) seawater it is possible that it regularly comes into contact with oxygen. Such contact (and the oxidase reactions that follow), could lead to enhanced break down of the recalcitrant macromolecular compounds (e.g. lignin) within the upper surface of the peat bed (Yavitt *et al.* 1997; Killops and Killops, 2005), which could in turn result in an increased degree of fermentative degradation – leading to the higher organic acid concentrations. If this is the case then fermenter activity in the peat could be limited to the this upper "activated interface" layer, with the sediments beneath being a microbial "dead zone" with very little *in situ* activity and only the diffusion of compounds from the interface above controlling the geochemistry. Towards the bottom of the peat, and into the clay layer beneath, concentrations of acetate, formate and propionate increased again (and in the case of acetate and propionate reached their maximum) indicating that whatever was inhibiting fermentation in the peat was no longer a factor. From examining the chloride profile for this core (Fig. 7.3) it can be seen that there is a decrease in salinity at the base of the core which may indicate a flow of terrestrially derived groundwater – possibly along the bedding plane between peat and clay. If this is the case then this could be providing the limiting nutrients needed to restart fermentation, possibly leading to the creation of a second "activated interface" at the

base of the peat. Or on the other hand the groundwater could be supplying the organic acids to the sediment directly from a source elsewhere.

An unusual component of the biogeochemistry of core StB1 was the presence of glycolate and oxalate in the cores (Fig. 7.3), compounds not usually present in abundance within sediments. Both of these compounds followed a similar trend with depth; concentrations were low in the overlying muds but then dramatically increased at the top of the peat layer - up to 2.2 mM and 48 μM for glycolate and oxalate respectively. Levels then decreased slightly down-core until 47 cm where both increased markedly again, reaching a maximum of 3.9 mM for glycolate and 523 μM for oxalate. The occurrence of glycolate in sediment cores is unusual as it is usually associated with phytoplankton blooms, which excrete the compound during photosynthesis (Watt, 1966). As such it is usually degraded within the water column or at the sediment surface by aerobic heterotrophs (Steinberg and Bada 1984, Edenborn and Litchfield 1985, Edenborn and Litchfield 1987). If, for whatever reason, glycolate is not consumed within the aerobic zone it can be degraded anaerobically by fermenters (Friedrich *et al.* 1991) or by SRB (Friedrich and Schink, 1995). Therefore where glycolate is found in sediments it is not found in abundance and concentrations tend to decrease with depth as it is consumed (Peltzer and Bada 1981, Llobet-Brossa *et al.* 2002). Since the levels of glycolate in core StB1 were several orders of magnitude higher than those reported above and increase rather than decrease with depth it is unlikely that the compound is derived from a phytoplanktonic source. A more likely explanation is that the glycolate could be produced *in situ* from the breakdown of the vegetative material preserved in the peat, as studies have shown that low molecular weight carboxylic acids, including glycolate, can be extracted from low-rank coals via chemical oxidation (Miura *et al.* 1996) and that they are a breakdown product of humic/fulvic acid degradation by bacterial metabolism – particularly in estuarine environments (Rocker *et al.* 2012). The increase in oxalate in the peat could also be linked to this process, as oxalate is an oxidation product of glycolate and therefore could potentially be produced by the metabolism of glycolate by anaerobes. Alternatively it could be derived directly from the peat itself as several species of coastal plants, such as the sea beet (*Beta vulgaris* subsp. *maritima*), contain high levels of oxalate in their tissues which could be

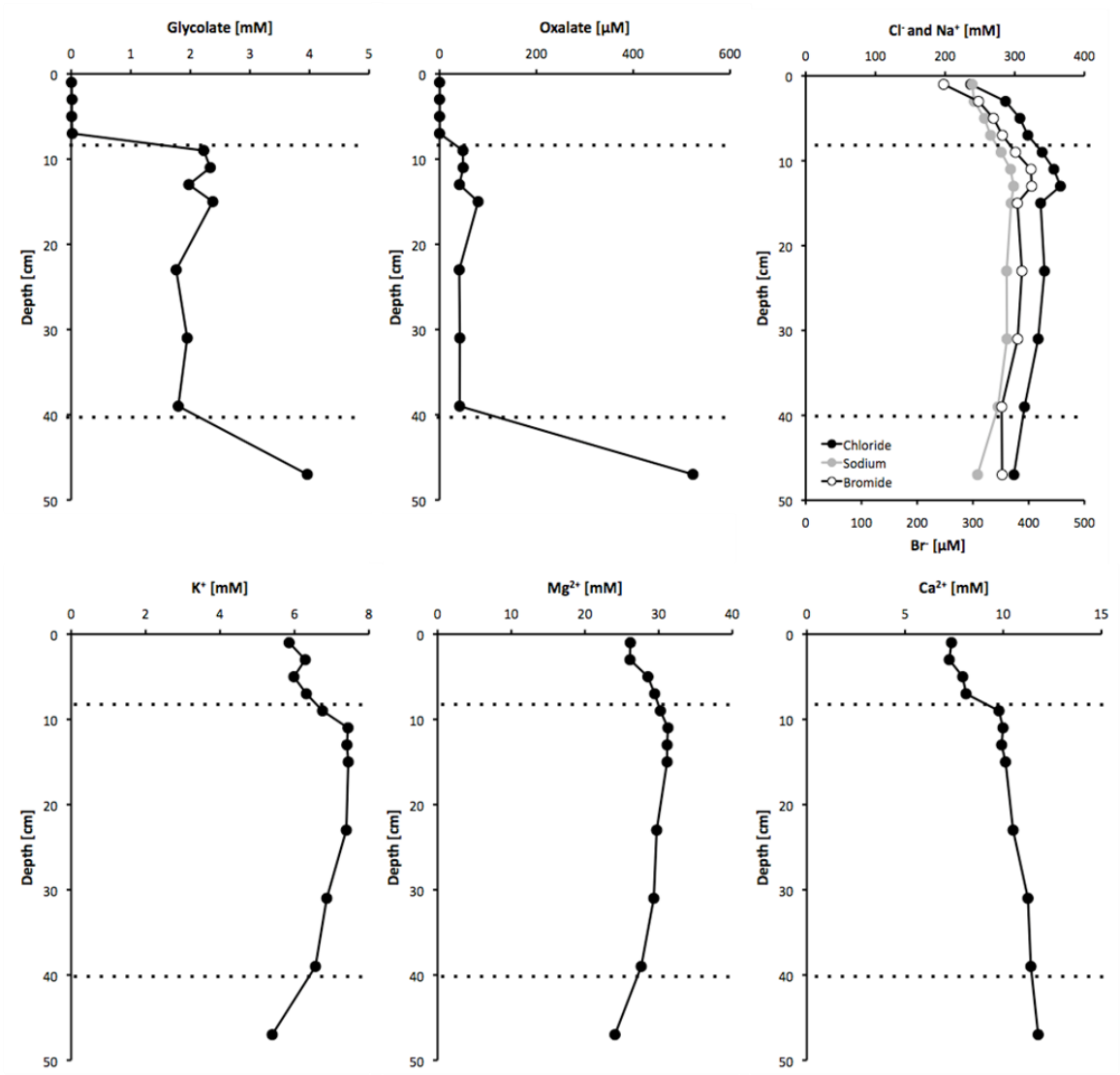


Fig. 7.3 – Biogeochemical profiles obtained from core StB1 showing the variations in the pore water concentrations of: glycolate, oxalate, chloride, sodium, bromide, potassium, magnesium and calcium with depth. Dashed lines represent the boundaries between the mud and peat (8 cm) and mud and clay (40 cm).

released into the pore waters upon decomposition (Franceschi, 1984). In addition oxalate has been shown to be present in extracts derived from buried lignite deposits, suggesting that it can also occur in relation to older organic material (Fry *et al.* 2009).

The lack of glycolate and oxalate in the overlying muds (and the lack of an obvious diffusion gradient between the two units) suggests that both compounds are being rapidly removed near the surface. This removal could be linked to recent re-suspension of the muddy sediments (which would destroy any diffusion gradient present) or it may be due to the microbial degradation of glycolate and oxalate in

these sediments by heterotrophic bacteria (see section 8.4). The decrease in both glycolate and oxalate values below 15 cm would suggest that these compounds are also being consumed within the peat bed itself (though possibly at a similar rate to which they are produced at considering the relatively stable profiles). The increase in both glycolate and oxalate at 47 cm is harder to explain however, as below 40 cm there is no peat present. A possible explanation is that the groundwater flow along the peat-clay boundary described above had previously flowed through the peat bed at some point. This could have the effect of leaching the glycolate out of the peat upstream of the sample site and enriching the groundwaters with the compound, which might then percolate into the clay bed downstream.

Chloride, sodium and bromide concentrations all show an increase over the top 13 cm of the core likely driven by the diffusion of fresher surface waters into the sediments (large numbers of streams flow across the tidal flats at this site, originating from the base of the rip-rap sea defences). Concentrations then remained fairly stable within down to 31 cm, below which then began to decrease again possibly indicating the flow of fresher groundwater at depth described above. Potassium values followed a similar pattern with an initial increase down to 11 cm followed by a clear decrease below 23 cm. This decrease was greater than that showed by sodium (as evinced by the K^+:Na^+ ratio, data not shown) suggesting that the potential groundwater flow was proportionally more depleted in potassium than sodium. Similar results are indicated by the magnesium values, which again show an increase down to 11 cm followed by a decrease down to the base of the core. As with potassium the Mg^{2+}:Na^+ ratio (data not shown) indicates that this decrease with depth is greater than that of sodium suggesting that the groundwater is also comparatively more depleted in Mg^{2+} ions. Calcium on the other hand shows a different profile shape, exhibiting a continuous increase with depth (albeit with a break in slope at the mud-peat boundary, possibly indicating precipitation). This suggests that either the groundwater flow was enriched in calcium relative to the other alkali metal ions or that dissolution of carbonate (possibly driven by a decrease in pH in the peat – see below) is occurring at depth.

Nitrate levels show an initial decrease with depth within the overlying muds, from 36 μM at 1 cm to 2 μM at 7cm (Fig. 7.4). At the start of the peat (at 8 cm) however, concentrations increase dramatically to 425 μM at 9cm, before decreasing again to 250 μM at 23 cm and then and remaining between ≈200-300 μM down to the base of the peat. Within the clay layer beneath the peat concentrations increase again, reaching to a peak of over 600 μM at 47 cm. Ammonium values also show in increase with depth, from 64 μM at 1 cm to 614 μM at the base of the core. Unlike many of the other cores in this study this increase with depth is not linear, but instead forms a concave-down profile, possibly indicating a drop in ammonification rates near the base of the peat. The profile also exhibits a small peak at 15 cm suggesting that ammonium-producing processes (such as the breakdown of amino acids and proteins) may also be stimulated in the activated interface layer.

The initial decrease of nitrate in the overlying sediment described above is consistent with the occurrence of denitrification/dissimilatory nitrate reduction to ammonium (DNRA), processes which are common in coastal sediments and one of the initial zones of the redox cascade (Froelich *et al.* 1979). The increase in nitrate below 9cm is therefore puzzling, as since nitrate is such a desirable electron acceptor for bacteria it is usually entirely consumed within the top few centimetres of the sediment. Its occurrence at depth is sometimes linked with anaerobic ammonia oxidation (anammox), although the ammonia profile for core StB1 gives no obvious indication of consumption of ammonia with depth. Also the amount of nitrate produced by anammox is very small (c. 15% of the ammonium/nitrite metabolised in the reaction), so any small variations that are present in the ammonium profile could not account for the very high levels of nitrate measured in the core (Egli *et al.* 2001). This, combined with the fact that no nitrite (the other substrate necessary for annamox) was detected in the core, rules out anammox as the origin of the nitrate in the peat. The most likely explanation therefore is that the peat itself is the source of the nitrate as it is rich in nitrogen-containing compounds (proteins etc.) that could generate nitrate upon being broken down by bacteria. However, a problem occurs with this hypothesis as breakdown of the peat would first produce ammonium, which would need to be oxidised to form nitrate, and as stated above the mechanism by which such oxidation could proceed is unknown, as O_2 and other oxidised compounds are unlikely to be found in the sulphidic peat. As

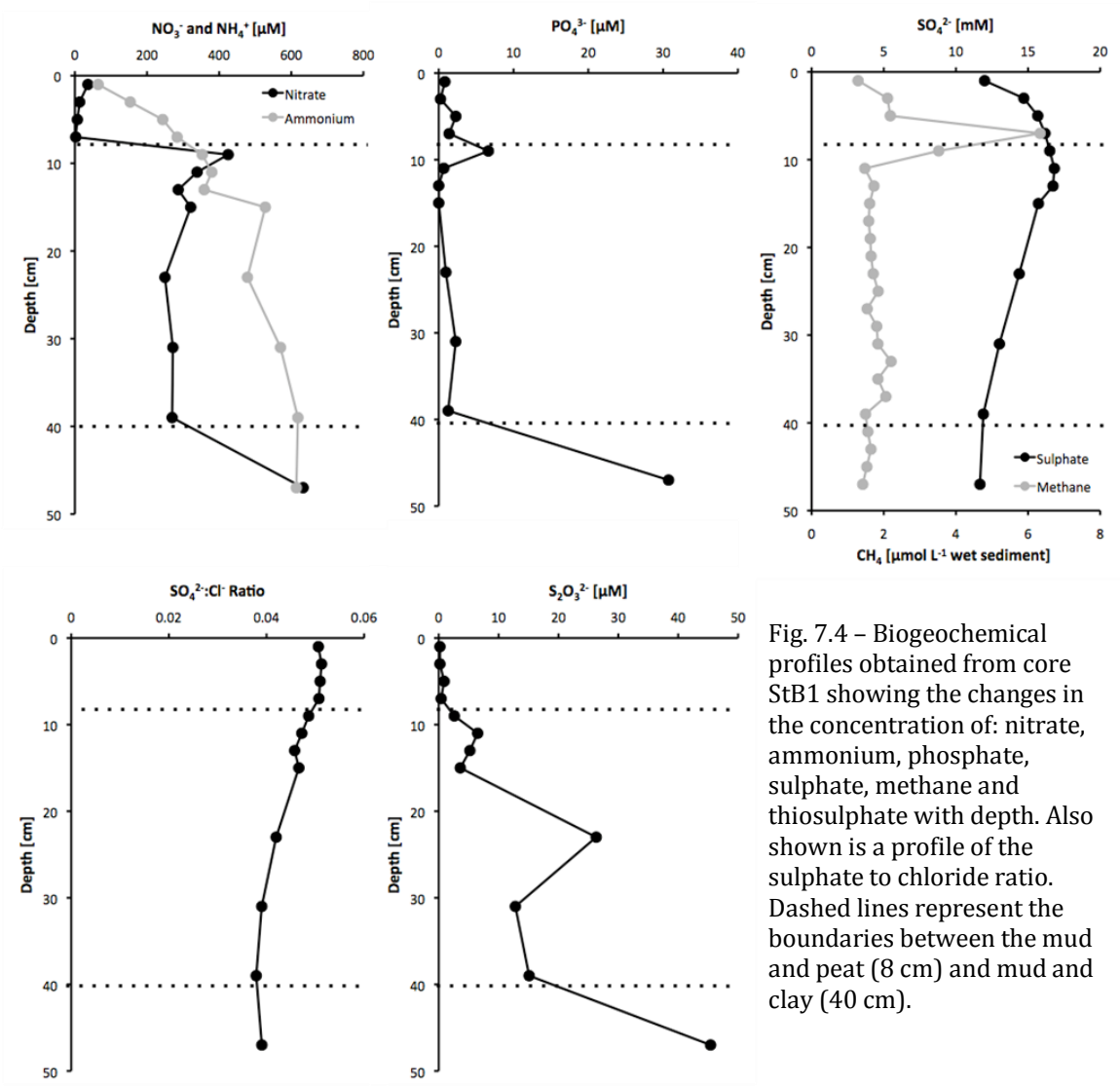


Fig. 7.4 – Biogeochemical profiles obtained from core StB1 showing the changes in the concentration of: nitrate, ammonium, phosphate, sulphate, methane and thiosulphate with depth. Also shown is a profile of the sulphate to chloride ratio. Dashed lines represent the boundaries between the mud and peat (8 cm) and mud and clay (40 cm).

with the organic acids, the highest concentrations of nitrate within the peat are found in the activated interface between 9-15 cm suggesting that the process(es) generating nitrate are enhanced in this layer. The very highest concentrations are found where the peat comes into contact with the oxidised sediments above which may indicate that nitrification is the origin of some of this nitrate. Finally it should be noted that as with the organic acids, nitrate concentrations increased again at 47 cm in the clay layer, which again may indicate a flow of groundwater rich in nitrate (Capone and Slater, 1990). Since land use on the Wentlooge Levels (the area landward of the St Brides site) is dominated by agriculture it is possible that groundwater in the area could be contaminated with higher than average levels of compounds found in chemical fertilizers or animal waste (phosphate for example is also elevated at 47 cm), especially as the water table in this area is often very close to the surface and the area is criss-crossed by drainage channels due to it's low lying topography.

The sulphate profile for core StB1 shows an unusual increase and then decrease with depth uncharacteristic of the sulphate reduction-associated decrease common in tidal flat sediments. However, since the chloride profile from this core, as mentioned above, is very variable and as salinity is a major factor influencing sulphate concentrations it is important to examine the SO_4^{2-}:Cl^- ratio to determine the true effect of bacterial respiration on sulphate concentrations. As can be seen from the SO_4^{2-}:Cl^- plot, the ratio over the top 8 cm is relatively constant indicating that little to no sulphate reduction was occurring - which agrees with the discussion of sediment colour detailed above (though some sulphide oxidation may be occurring). Within the peat layer the ratio begins to decrease indicating that a decrease in sulphate concentrations – almost certainly associated with bacterial sulphate reduction - is occurring. This decrease is not linear however, as there is a change in the slope of the SO_4^{2-}:Cl^- profile at around 15 cm, with the profile below this depth becoming more vertical. This suggests that sulphate reduction may be more active in the upper 6-8 cm of the peat, possibly due to the increased concentrations of electron-donor substrates present at this depth, and therefore again linked to the presence of an "activated interface" (phosphate concentrations also increased at this depth possibly indicating an increased rate of FeS production). Below 15 cm the profile gradually becomes more vertical, suggesting a decrease in sulphate reduction. Since sulphate concentrations were between 10-15 mM at this depth, this change in rate cannot be due to sulphate limitation and may instead indicate a possible inhibition of sulphate reduction by another source (this will be discussed further in section 7.2.2). Near the base of the core a further change in slope occurs within the clay layer at 47 cm, where the ratio begins to increase, once again in line with the organic acids and nitrate. This could again be linked to groundwater movement with the with the waters in question being either enriched in sulphate or possibly enriched in compounds that could lead to the oxidation of the sulphide produced by SRB back into sulphate – such as nitrate (Krishnakumar and Manilal, 1999; Vaclavkova *et al.* 2014).

As well as sulphate, thiosulphate was also detected within core StB1 – an unusual occurrence considering thiosulphate's desirability as a substrate in both reduction and disproportionation reactions by SRB (Bak and Pfennig 1987). Concentrations of thiosulphate were initially low in the muddy overlying sediments

(<1 μM) but began to rise upon entering the peat, reaching 26 μM at 23 cm. Since thiosulphate is an intermediary in the oxidation of sulphide to sulphate this increase in thiosulphate may be linked to biological oxidation of sulphide in the peat by anaerobic lithotrophic bacteria (Jørgensen 1990a, Jørgensen and Bak 1991, Thamdrup *et al.* 1994) or possibly to chemical oxidation of sulphide by nitrate (Elsgaard and Jørgensen 1992). Salt marsh peats similar to those found at St Brides are known to contain high levels of sulphide in the form of pyrite (Dellwig *et al.* 2001), and cold chromium reduction of peat from this site produced large amounts of sulphide, which combined with the pungent smell of the core indicates that there is likely to be a plentiful supply of sulphide that could be oxidised to thiosulphate. This large-scale increase in thiosulphate concentrations only became apparent below 15 cm, the depth at which the SO_4^{2-}:Cl^- profile suggests sulphate reduction decreased. This could suggest that thiosulphate can only occur in the peat in high quantities at depths where sulphate reduction is (at least partially) suppressed, as this would prevent the SRB metabolising it. Alternatively, the apparent decrease in sulphate reduction at this depth could instead be due to the presence of thiosulphate, as the oxidation and/or disproportionation of this compound could "top up" the sulphate pool (Jørgensen 1990a; Holmkvist *et al.* 2011). Below the peat, in the clay layer thiosulphate concentration levels rose again, reaching a maximum of 45 μM at 47 cm. This increase could be caused by further oxidation of sulphide (and matches the increase in SO_4^{2-}:Cl^- described above) or, in a similar fashion to nitrate and phosphate potentially caused by an influx of nutrient-rich groundwater as thiosulphate is again a common ingredient in agricultural fertilizers. (e.g. Grant *et al.* 2004).

Methane concentrations remained, for the most part, low and stable at around 1.5 μmol l^{-1} of wet sediment throughout core StB1. However, just above the mud-peat interface (around 7 cm) concentrations peaked to 6.3 μmol lws^{-1}, before decreasing again to ≈1.5 μmol lws^{-1} by 11 cm. This peak in methane concentrations likely indicates that methanogenesis is being stimulated in this zone in a similar fashion to the "activated interface" layer observed in the organic acid profiles. Since acetate is one of the main substrates for methanogenesis, it may well be that the acetate

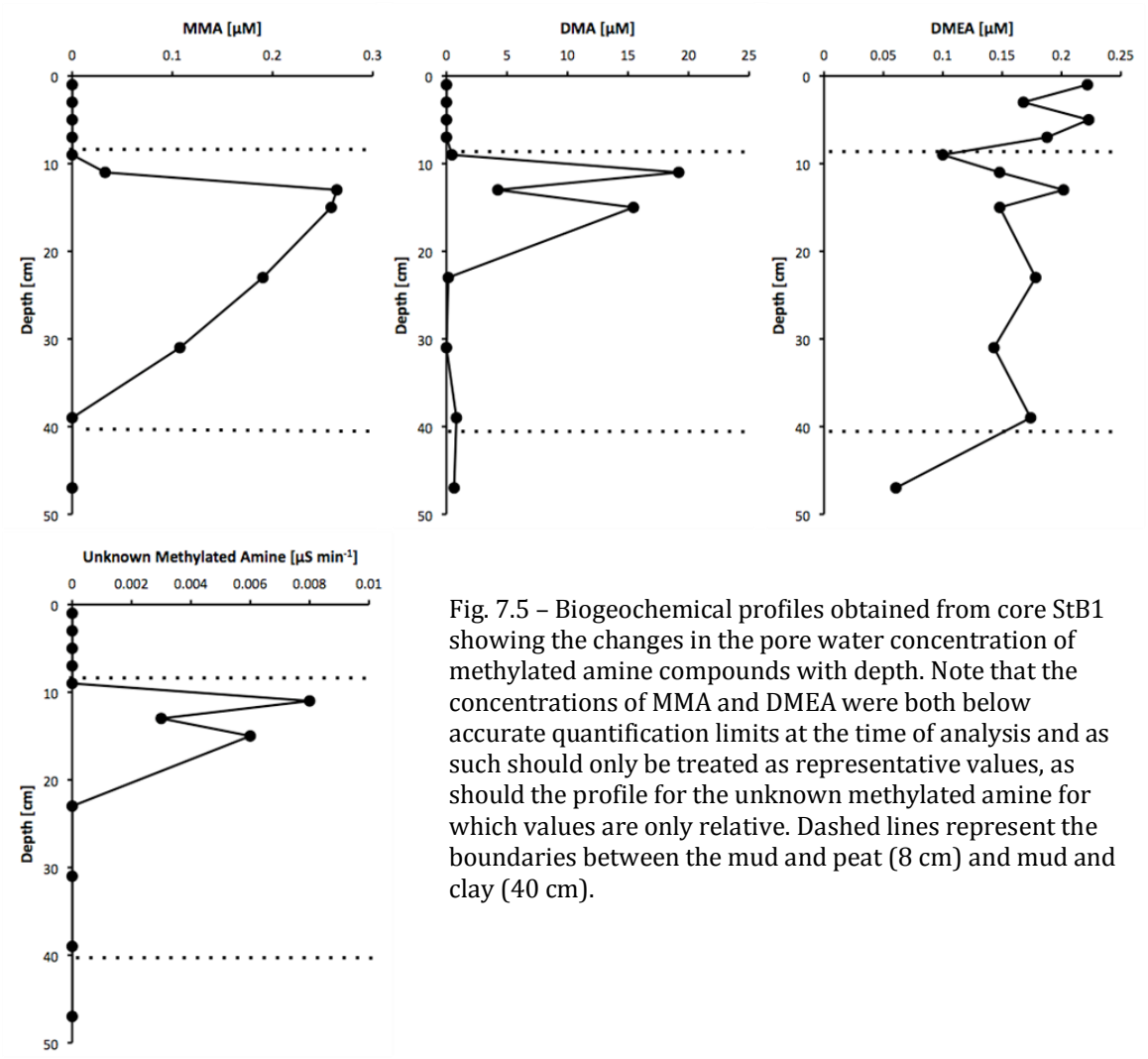


Fig. 7.5 – Biogeochemical profiles obtained from core StB1 showing the changes in the pore water concentration of methylated amine compounds with depth. Note that the concentrations of MMA and DMEA were both below accurate quantification limits at the time of analysis and as such should only be treated as representative values, as should the profile for the unknown methylated amine for which values are only relative. Dashed lines represent the boundaries between the mud and peat (8 cm) and mud and clay (40 cm).

produced in the activated interface is diffusing into the overlying sediments, fuelling methanogenesis and leading to the enhanced methane levels at 7 cm. Another possible reason for enhanced methanogenesis close to the activated interface is the presence of methylated amines in the peat. These compounds act as non-competitive substrates for methanogens (Hippe *et al.* 1979, Oremland 1982) but are not commonly detected in marine sediments due to their low *in situ* concentrations and fast turnover time (Parkes *et al.* 2012). However, as can be seen in the profiles (Fig. 7.5), monomethylamine (MMA) dimethylamine (DMA), dimethylethanolamine (DMEA) and an unidentified methylated amine (UMA, possibly trimethylamine) are all detectable in these sediments (although some are below accurate quantification limits). DMA in particular reaches a peak of 19 µM at 11cm, several times higher than concentrations reported by other studies (Wang and Lee 1990, Fitzsimons 1997, Fitzsimons *et al.* 2001), including samples taken from salt marshes – environments known for their high methylated amine levels (Fitzsimmons *et al.*

2005). As with the organic acids, production of DMA, TMA, and to a lesser extent MMA, is predominantly between 9-15 cm, within the activated interface at the top of the peat. The production of these aliphatic methylated amines is likely once again linked to the degradation of the vegetation preserved in the peat, as decaying salt marsh plants (e.g. *Spartina alterniflora*), similar to those found in the peat, are known to be a significant source of methylated amines in coastal sediments (Wang and Lee 1994; Yuan *et al.* 2014). Unlike the other amines DMEA does not show a peak of production in the activated interface and is also detected in the overlying muds. This compound is a common head group of phospholipids and is also known to be a substrate for methanogenesis as well as a degradation product produced during the consumption of choline by methanogens (Watkins *et al.* 2012). Since it is present in both the mud and peat it is unlikely that DMEA is produced exclusively by peat degradation and it is more likely that it is generated via lysis of modern bacterial cells or via the metabolism of choline, another common component of cell membrane lipids, which would be present in both the peat and the mud (choline itself was not detected, though this may be because choline is consumed by methanogens more rapidly than DMEA).

As well as methane other gases related to microbial metabolism, including hydrogen, carbon dioxide and carbon monoxide were also measured in core StB1 (Fig. 7.6). Hydrogen was first detected in the core at 5 cm depth and the concentrations remained between 0.1-0.7 μmol lws^{-1} for most of the core, except at 15 cm where concentrations rose to 1.4 μmol lws^{-1}. This rise in H_2 corresponds with the maximum concentrations of the methylated amines described above, as well as with increasing organic acid and ammonium concentrations and hence could be linked to amino-acid degradation.

Carbon dioxide levels show an initial peak in the overlying muds, likely caused by heterotrophic respiration of organic carbon. Concentrations then decrease into the peat layer before increasing again dramatically, to a peak of 2.1 mmol lws^{-1} at 37 cm. This peak at depth coincides with the change in peat composition to a darker, more woody texture and as such it may be the case that degradation of this woody peat is driving the increase in CO_2 levels (and also possibly acetate concentrations which peak at 39 cm). Within the clay layer CO_2

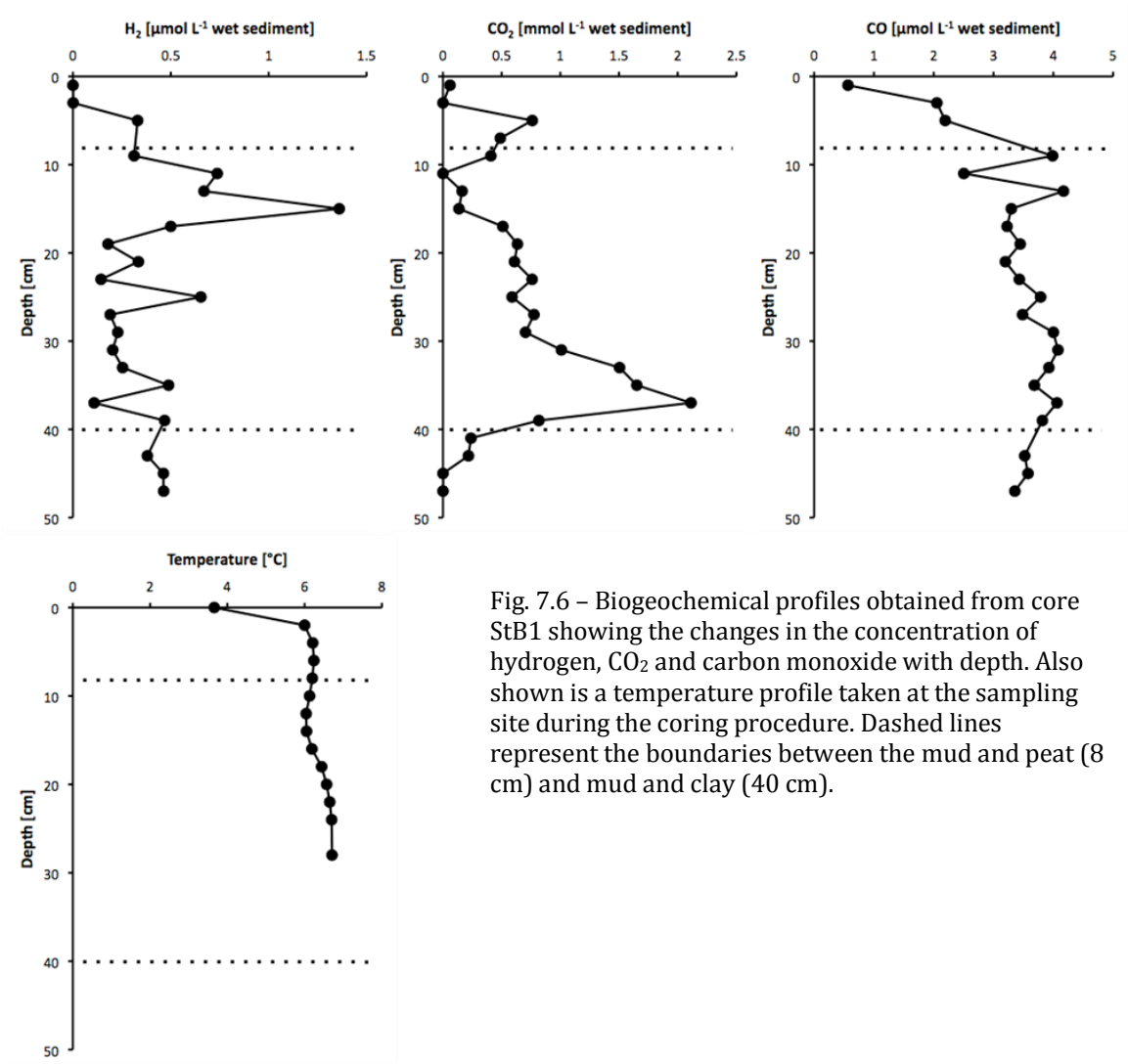


Fig. 7.6 – Biogeochemical profiles obtained from core StB1 showing the changes in the concentration of hydrogen, CO_2 and carbon monoxide with depth. Also shown is a temperature profile taken at the sampling site during the coring procedure. Dashed lines represent the boundaries between the mud and peat (8 cm) and mud and clay (40 cm).

levels drop significantly, reaching zero by 45 cm. This decrease may indicate that autotrophic processes are occurring at these depths or that the groundwater that may be flowing along the base of the peat bed may be low in CO_2.

Finally, carbon monoxide concentrations exhibit a distinct increase in the peat layer reaching concentrations between 3-4 µmol lws^{-1}, with concentrations being slightly lower in both the overlying muds and the clay beneath. The pathways leading to the production of this gas in aquatic sediments are poorly understood (Conrad 1996), but in anaerobic fermenting chambers CO is thought to be produced as an intermediary or by-product during autotrophic methanogenesis (Eikmanns *et al.* 1985), acetoclastic methanogenesis or homoacetogenesis (Hickey and Switzenbaum 1990). It is not commonly measured in environmental samples, but in anaerobic environments CO can be used as a substrate by homoactogens, methanogens and SRB (Conrad 1996, King 2007). In core StB1 concentrations are

high compared to other marine sites (Bird *et al.* 2001), however, the profile of CO does not show any trends relating to either the acetate, methane, or sulphate profiles so it may be that none of the groups present in these sediments utilise CO as a substrate (although it should be considered that the concentrations of CO are so low compared to acetate and sulphate that any variations that correlate between them might not be easily seen).

8.2.2 Biogeochemistry of Core StB2

Organic acid concentrations in core StB2 (Fig. 7.7) were substantially lower than in StB1. Acetate concentrations varied between 13-20 μM over the top 17 cm of the core (the soft overlying mud) but then decreased downward into the peat to a minimum of 10 μM at 35 cm before rising again to 18 μM at the base of the peat and then decreasing further in the clay layer beneath. Lactate concentrations were overall higher in the overlying mud (1.3-4.8 μM) than in the peat (1.1-3.7 μM) and continued to decrease further within the clay, reaching a minimum of 0.5 μM at the base of the core (47 cm). Formate also showed a general decrease from 73 μM at 1 cm down to zero at 35 cm. However, below this, as with acetate, it increased markedly to a maximum of 95 μM at 37 cm before decreasing again downward into the clay (though it did show a peak near the peat-clay boundary). Propionate levels were close to undetectable throughout most of the core except at 7 cm and 31 cm, where concentrations rose to 9 and 14 μM respectively. However, unlike the other organic acids hydroxy-butyrate concentrations were actually higher in StB2 than in StB1, reaching a maximum of 9.9 μM at 9 cm. Values also followed a similar pattern to those in StB1, with maximum values occurring in the overlying muds and lower concentrations in the peat. Unlike in StB1 however, values showed a secondary increase with depth, rising to 4.1 μM at 39 cm – the same depth as the increases in acetate and formate described above.

There are two obvious points of difference between the organic acid profiles in cores StB1 and StB2. The first is that the "activated interface" layer present in the top ≈5 cm of the peat in core StB1, notable for it's elevated organic acid concentrations, does not appear to be present in core StB2. In fact for most of the compounds concentrations are actually higher in the overlying muds than in top of

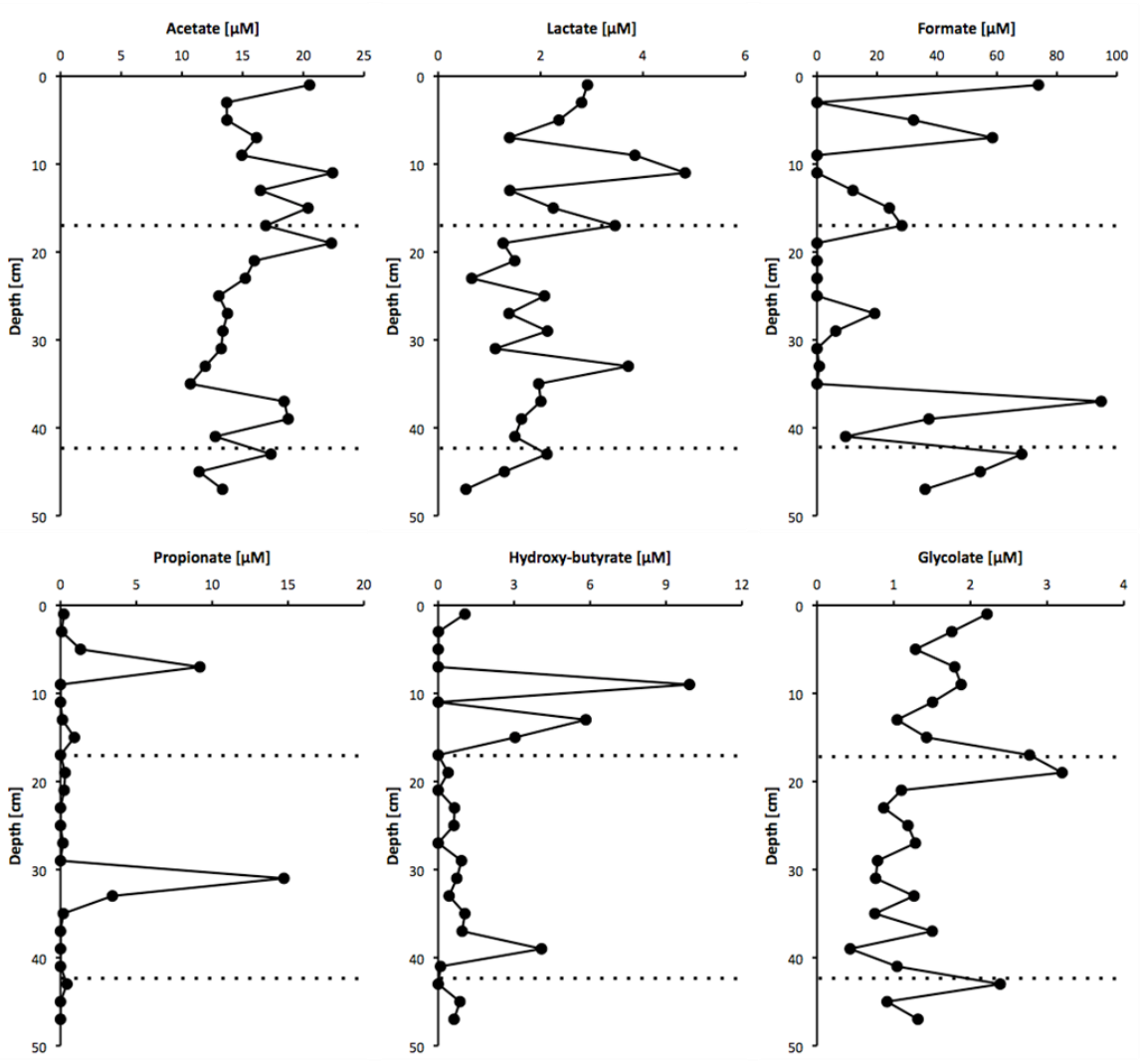


Fig. 7.7 – Biogeochemical profiles obtained from core StB2 showing the changes in the pore water concentration of organic acid compounds with depth. Dashed lines represent the boundaries between the mud and peat (17 cm) and mud and clay (42 cm).

the peat (e.g. the average formate concentration is 25 µM in the mud and 14 µM in the peat). Despite this variance however, the second lower zone of organic acid production (possibly related to fermentation of woody peat at the base of the bed) appears to be still present – at least for acetate and formate – though to a much smaller extent than in StB1. The second difference between the cores relates to the total amounts of organic acids present in core StB2 compared to StB1. For example, in StB1 acetate concentrations reached a maximum of ≈500 µM, whereas in StB2 acetate concentrations never reach above 25 µM. A similar situation occurs for lactate, formate and to a lesser degree propionate. This would suggest that the fermentative processes that were occurring when StB1 was sampled were much more limited when core StB2 was taken, or that the compounds produced by these

processes were being consumed at a faster rate in StB2 than in StB1. The potential reasons for these changes are discussed in section 8.3.

Glycolate concentrations in core StB2 were markedly different to those observed before, with concentrations ≈1000 fold lower than in StB1. Over the majority of the core concentrations were between 0.4-2.4 μM, except around the top of the peat layer where values peaked at 3.2 μM indicating that production was still elevated in the activated interface. These low concentrations reveal two interesting details about the role of glycolate in this system. The first is that glycolate is not a refractory "relict compound" that gradually accumulates over time via general decay, as the considerable difference in concentrations between the two time points indicates that it must be produced and consumed in large amounts over the course of the year. The second is that processes that control the production and consumption of glycolate may be closely controlled by temperature, with glycolate production possibly able to function more effectively at lower temperatures than consumption, thus allowing glycolate to accumulate at high levels during the winter months. It then appears that in summer, with rising temperatures, that the consuming process/processes are able to consume almost all of the glycolate produced over the winter while the producing process/processes are likely operating at a similar rate to consumption to produce the consistent low glycolate concentrations present in StB2. Oxalate on the other hand was not detected in core StB2 suggesting that like glycolate, it too might accumulate in winter and be degraded quickly in summer. However, unlike glycolate the oxalate producing process is either not active in the summer or oxalate turnover is so fast that *in situ* oxalate concentrations are kept below detection limits.

Chloride, sodium and bromide (Fig. 7.8) also showed significantly different depth profiles in core StB2 compared to StB1. Values showed an initial decrease with depth within the overlying muds, suggesting downward-diffusion of higher salinity waters. This is not unexpected as core StB2 was collected on a falling tide however, it does suggest that the majority of the mud present at the time the core was sampled was deposited by waters with a markedly different salinity to those that had previously covered it. This adds further weight to the suggestion that the muds

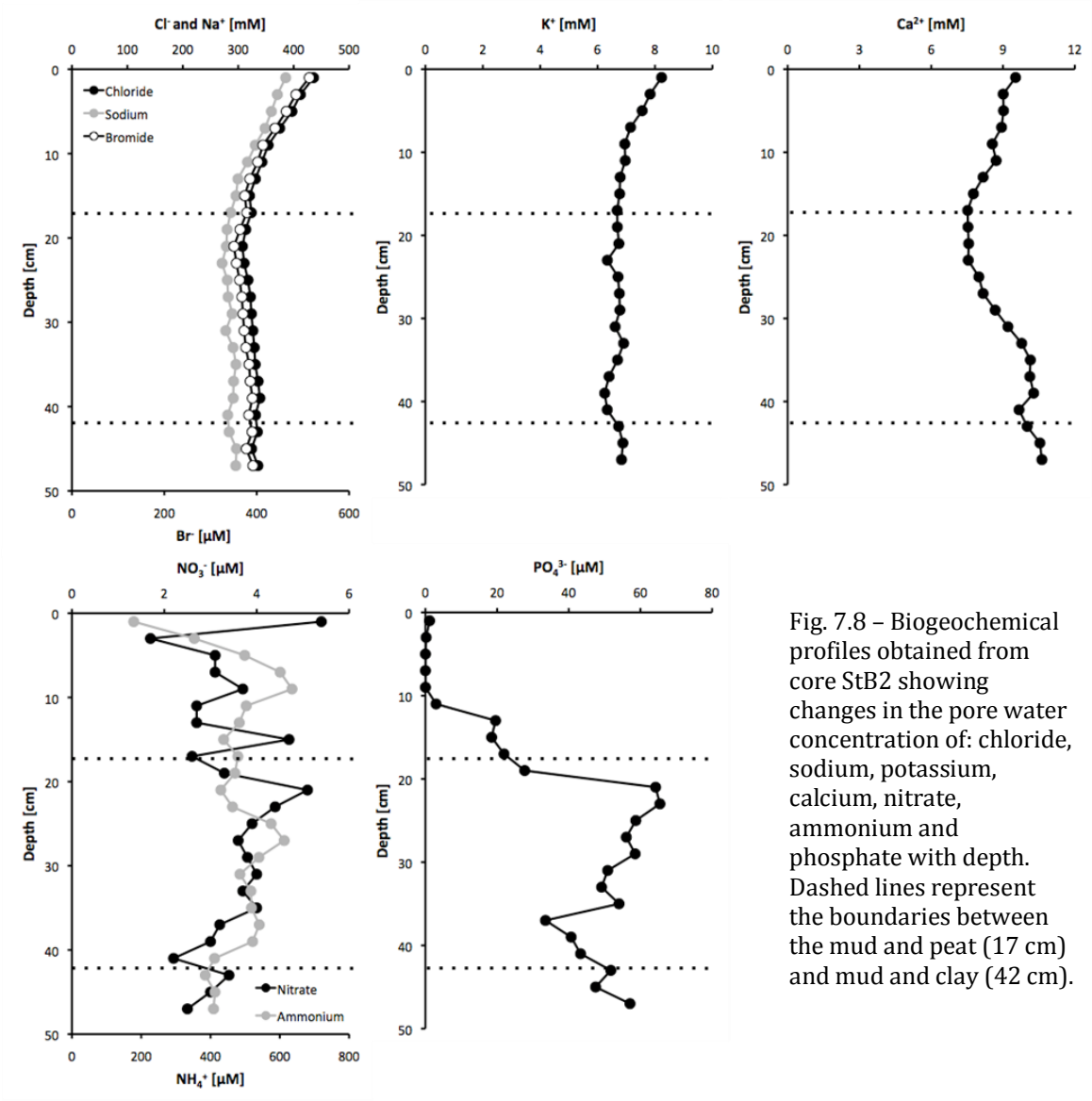


Fig. 7.8 – Biogeochemical profiles obtained from core StB2 showing changes in the pore water concentration of: chloride, sodium, potassium, calcium, nitrate, ammonium and phosphate with depth. Dashed lines represent the boundaries between the mud and peat (17 cm) and mud and clay (42 cm).

sampled in core StB2 had been emplaced for longer than those taken in StB1. Within the peat layer chloride concentrations were similar to those seen in core StB1 (≈330 mM), however, unlike in StB1 concentrations remained relatively constant, suggesting that there was little to no input of lower-salinity groundwater at depth (though sodium values did decrease slightly at the peat-clay boundary suggesting the possibility of a small amount of groundwater movement). Since the timeframe during which core StB2 was sampled (July 2013) was period of exceptionally low rainfall with elevated temperatures (Met Office, 2013 [Online] Accessed: 03/04/14), groundwater flow would have been reduced compared to the period when StB1 was sampled. This could also help to explain why the secondary increase in organic acid concentrations around the peat:clay boundary is much less notable in StB2 compared to StB1 as the supply of limiting nutrients (or organic acids

themselves) could have been severely reduced by the lack of groundwater flow. Potassium values again followed the changes in salinity, although like sodium there also appeared to be a small decrease around 40 cm suggesting a small degree of groundwater flow. Unlike in core StB1, calcium also showed a similar pattern of decrease over the top 17 cm of the core, then stabilised down to 23 cm. Below this concentrations began to increase, reaching a maximum at the base of the core. Once again this might suggest a dissolution of carbonate at depth, especially as the pH of this core has a notable decrease with depth (possibly relating to increasing levels of humic acids or sulphide in the peat) (Fig. 8.12).

Similar to core StB1, nitrate is present even at depth in core StB2 though concentrations are much lower than before (≈100x). Nitrate values showed an initial, sharp decrease between 1-3 cm, very probably linked to denitrification/ DNRA. Below this, levels vary considerably over the next ≈15 cm of the core before increasing upon entering the peat layer and then subsequently decreasing down to the base of the core. Again this is very different to core StB1 where concentrations were much higher overall (up to 420 μM in the peat and 630 μM in the clay) and also showed an increase, rather than a decrease with depth. This would again suggest that the process responsible for producing nitrate in the peat is not as efficient in the summer as in the winter, or that the nitrate is being consumed at a faster rate in the summer. There is also no dramatic increase in nitrate within the clay layer, which again might indicate that whatever process was supplying nitrate and other compounds to the deeper sediment layers (e.g. nutrient rich groundwater flow) is no longer occurring.

The ammonium increase in core StB2 was initially much steeper than in StB1, with concentrations reaching 635 μM within the top 10 cm (in StB1 ammonium had not quite reached this concentration by the base of the core). Concentrations then decreased in the lower layers of the mud before increasing again in the upper layers of the peat and then decreasing consistently down to the base of the core. The initial increase with depth is concomitant with DNRA however, the decrease below this is more puzzling as it cannot be due to nitrification (as nitrate concentrations do not consistently increase and it is within the darker reduced layer of the sediment). Also again there is no obvious sign of anammox occurring as there is no nitrite present

and no ≈15% relative increase in nitrate. Both nitrate and ammonia have slight increases in concentration within the top 5 cm of the peat layer. Although not as dramatic as the increases in concentration in StB1, this may indicate that the activated interface of the peat is still present, and though potentially not as active was seen before, is still able to stimulate organic matter (e.g. amino acid) degradation.

The sulphate profile for core StB2 has an initial sharp decrease within the mud layer (Fig. 7.9), from 23 mM at 1cm down to 13 mM at 15 cm depth. Below this, concentrations continue to decrease, but at a much more gradual rate down to 12 mM at the base of the core. However, since chloride also showed a significant decrease over the top 15 cm (>100 mM) it is again important to consider the SO_4^{2-}:Cl^- ratio profile as sulphate concentrations are closely linked to salinity. The SO_4^{2-}:Cl^- profile still has a significant decrease over the top 15 cm indicating that sulphate reduction is actually occurring in the overlying mud (which matches the black colour of the mud observed during sectioning). Below this, in the upper layers of the peat, the ratio increases again suggesting a decrease in sulphate reduction, then below 23 cm the ratio continues to decrease downward through the peat, although to a lesser degree indicating that sulphate reduction is occurring here as well - though as in StB1, at a slower rate. Within the clay layer on the other hand, the SO_4^{2-}:Cl^- profile becomes more vertical suggesting a potential decrease in sulphate reduction within this layer.

Sulphate reduction rates measured with ^{35}S radiotracers at *in situ* temperature (18 °C, Fig. 8.12) correlate with the geochemical profiles. They show a broad peak of sulphate reduction occurring within the top 17 cm, with a maximum rate of 44 nmol cm^{-3} d^{-1} at 11 cm which also coincides with the colour change in the sediment. Within the peat layer values remain low but constant at between 1-4 nmol cm^{-3} d^{-1} except in the top five centimetres where rates reach 12 nmol cm^{-3} d^{-1}, suggesting that the activated layer is also stimulating sulphate reduction, though not to the same degree as the overlying sediments (which is unusual considering the increase in the SO_4^{2-}:Cl^- profile at this depth). Interestingly the highest SRR does not perfectly match up with the steepest portions of the sulphate and SO_4^{2-}:Cl^- profiles. This suggests that these profiles are also controlled by diffusion, with the high SRRs

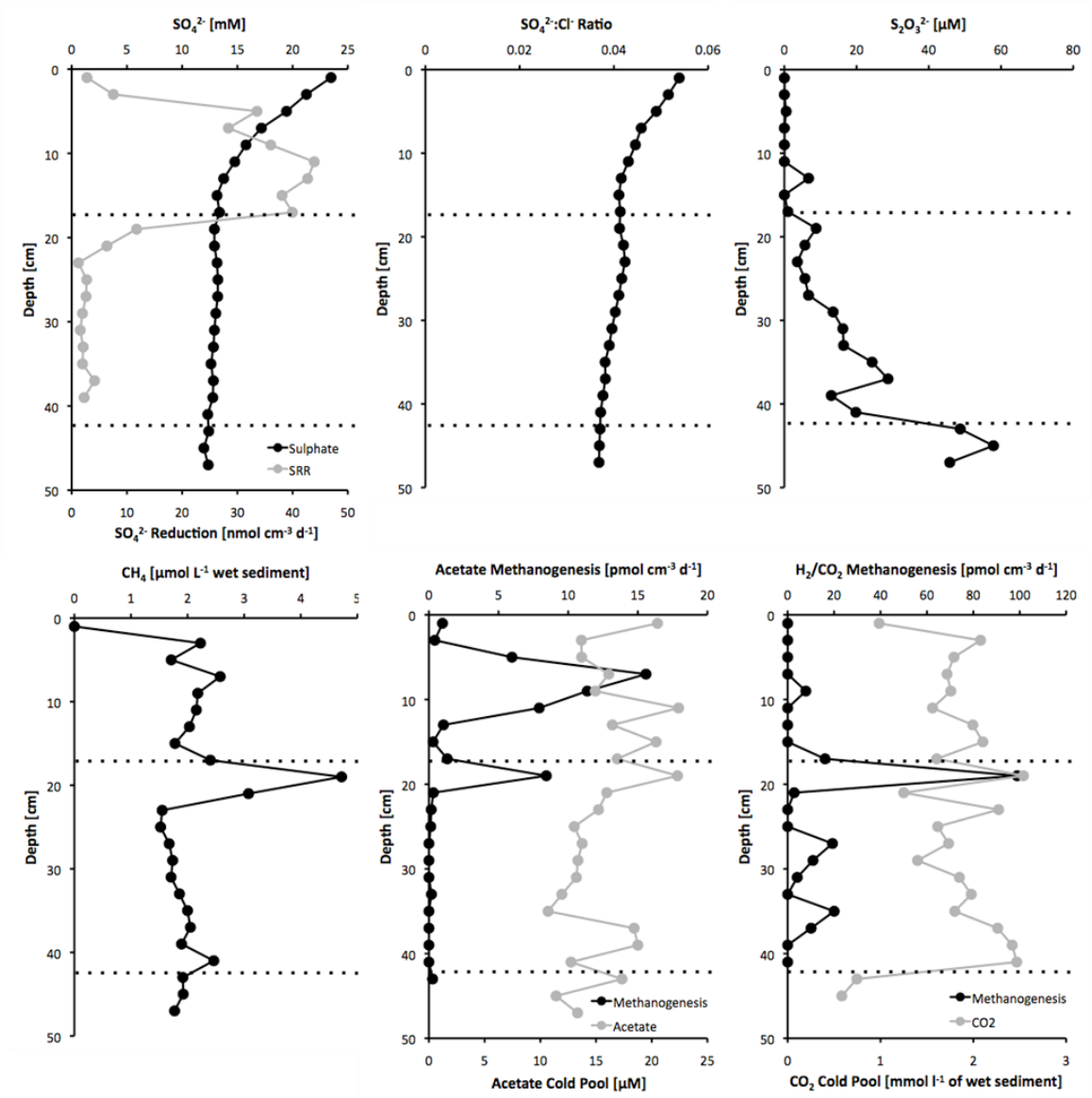


Fig. 7.9 – Biogeochemical profiles showing the changes in the concentration of sulphate, thiosulphate, methane and the sulphate:chloride ratio with depth. Also shown are profiles of the *in situ* rates of bacterial sulphate reduction (SRR), acetoclastic methanogenesis and H_2/CO_2 methanogenesis (plotted with the concentrations of their respective cold pool substrates, acetate and CO_2). Dashed lines represent the boundaries between the mud and peat (17 cm) and mud and clay (42 cm).

around 11 cm "pulling down" the sulphate values above them by removing sulphate in their immediate vicinity. This therefore provides another example of why radiotracers are necessary when studying sediment microbial processes, as the geochemical profiles themselves do not provide the whole picture regarding the distribution of SRB activity. The cause of the higher SRRs in the overlying muds is likely due to an abundance of bioavailable OM (e.g. acetate) in these sediments. However, the situation within the peat layer is harder to explain as the sulphate

profile shows that concentrations are still far in excess of the minimum threshold value needed for sulphate reduction and yet barely any is occurring (Parkes *et al.* 1993, Tarpgaard *et al.* 2011). Two possibilities exist for why sulphate reduction could be reduced in the peat, the first is that activity is restricted by the lack of availability of electron donor substrates in the peat, as the profiles described above show that substrates such as acetate and lactate are lower in the peat compared to the overlying mud. However, this decrease in the organic acid concentrations (≈50%) is minimal when compared to the decrease in SRR (≈10x) and as such is unlikely to be the cause of the decrease in rates. The second possibility is that compounds present in the peat are inhibiting sulphate reduction in some way. The identity of this proposed inhibitory compound is not definitively known, but it is possible that high concentrations of sulphide or humic acids in the peat could be affecting the SRB. At the pH range seen in the peat (≈6.9, Fig. 8.12) SRB in pure cultures can be inhibited by total free sulphide concentrations of between 7-18 mM (O'Flaherty *et al.* 1998). However, although the peat layer probably contains high levels of sulphide, the reactions that occurred during cold chromium reduction indicate that most of this sulphide is likely within the chromium reducible sulphur (CRS) fraction composed of elemental sulphur and pyrite (Fossing and Jørgensen, 1989). Therefore since sulphate reduction is only inhibited by free sulphide, which is part of the acid volatile sulphide (AVS) fraction of total sulphides, it is unlikely that the high levels of CRS in this core could have affected SRRs to the degree seen in the profiles. An alternative possibility is that sulphate reduction is being inhibited by the peat itself, or more specifically the HMW organic compounds contained within it. For example, Minderlein and Blodau (2010) found that growth of SRB was inhibited when they were exposed to peat derived DOM rich in humic substances, although the authors were unsure as to what the exact mechanism for this inhibition might be. Cooling *et al.* (1996) however, found that anthraquinones (a common analogue for humic acids) are capable of inhibiting sulphate reduction by uncoupling ATP synthesis from electron transfer across the cell membrane. In addition some condensed tannins, another group of polyphenolic compounds, have also been shown to suppress the growth and activity of SRB (Whitehead *et al.* 2013) particularly those of the genus *Desulfobulbus*, an important SRB group present in estuarine sediments (Oakley *et al.* 2010). Finally Lovley *et al.* (1996) found that

some microorganisms are capable of using humic compounds as electron acceptors. This could mean that some prokaryotic groups (e.g. SRB) could use humics in preference to their normal electron acceptor (sulphate), which would lead to an apparent inhibition of the original process (sulphate reduction). Since both humic acids and tannins are commonly derived from decaying vegetation and are known to occur in peat deposits it is not unreasonable to consider that these compounds may be inhibiting SRB activity (either by directly inhibiting growth or due to the SRB using them as alternative electron acceptors in preference to sulphate) and thus have a role to play in the suppression of SRRs within the peat bed at this site.

Thiosulphate concentrations in core StB2 were low in the overlying mud but began to increase with depth below 17 cm, particularly within the peat. The mechanism for this increase may again be chemical or biological sulphide oxidation, possibly involving nitrate since it decreases with depth at around this point (though as noted above, in this core levels of nitrate were significantly lower than in StB1). Alternatively it could also relate to the apparent inhibition of SRR (and therefore presumably thiosulphate disproportionation) within the peat which occurs at a similar depth, although the curved shaped of the thiosulphate profile would seem to indicate that production of this compound is occurring. As in core StB1, levels continued to increase into the clay layer, again possibly linked to groundwater flow. However, due to the lack of a nitrate increase and chloride decrease at this depth, any flow of groundwater would likely be of a considerably lower magnitude than that seen in core StB1.

Phosphate concentrations in core StB2 were overall much higher than in StB1, although depth trends were similar. In the top 10 cm of the overlying muds phosphate concentrations were very low, then increased markedly at the top of the peat layer reaching a maximum of 65 μM at 23 cm (a similar, though much smaller, peak occurred in StB1). Below this, concentrations began to decrease with depth through the peat before rising again at the base of the core near the boundary with the clay layer. The initial increase in phosphate below ≈10 cm coincides with the maximum SRR as well as the colour change in the mud from brown to black, and therefore it is likely caused by an increase in FeS in the sediments, as the reduction of phosphate-containing Fe(III) oxides by sulphide to form FeS and pyrite can cause a release of soluble phosphate to pore waters (Rozan *et al.* 2002). The secondary

increase in phosphate in the peat would suggest that the peat is a source of phosphate and the elevated concentrations in the activated interface (≈21-23 cm) suggests that microbial activity is facilitating the production of this phosphate. Since many organic macromolecules (proteins, phospholipids etc.) contain phosphate, the breakdown of the peat, which contains large amounts of such macromolecules, by microorganisms, would release considerable amounts of phosphate into the pore waters. Another possibility is that the high levels of sulphide in the peat could again be liberating phosphate during FeS formation. Deeper in the peat phosphate concentrations decrease again down to a local minimum at 37 cm, possibly caused by the drop in sulphate reduction (and hence free sulphide) within the peat, before increasing again into the clay. Since the top surface of the peat layer at St Brides is likely stripped of mud and exposed to estuarine waters during portions of the tidal cycle and as the Severn Estuary is deficient in microphytobenthos (Underwood, 2010) that might consume released phosphate it is possible that peats at this site and others similar to it may be an important source of phosphate to the estuarine system. Also as phosphate is a limiting nutrient for algal growth in freshwater and some estuarine environments (Bianchi 2007, and references therein) the peats may have an important role in controlling eutrophic algal blooms if in future turbidity and salinity in the estuary were to become reduced (such as by the construction of a tidal barrage).

Methane concentrations in core StB2 in were similar to StB1 with concentrations remaining around 2 μmol lws^{-1} for most of the core (≈5 μmol lws^{-1} in StB1), except around 19 cm where concentrations peaked at 4.7 μmol lws^{-1}, indicating that the activated interface was still a zone of enhanced methanogenesis in summer. Active methanogenesis rate measurements with ^{14}C radiotracers showed that acetoclastic methanogenesis was highest in the oxidised zone of overlying muds, concomitant with the highest acetate concentrations, reaching a peak of 15.6 pmol cm^{-3} d^{-1} at 7cm. Below this rates decreased into the darker reducing muds before increasing again in the peat to a second peak at 19 cm, corresponding to the methane peak and indicating that acetoclastic methanogenesis was stimulated within the activated interface. Below 20 cm rates remained negligible, not above 0.32 pmol cm^{-3} d^{-1} throughout the rest of the peat. In contrast, H_2/CO_2 methanogenesis was low in the

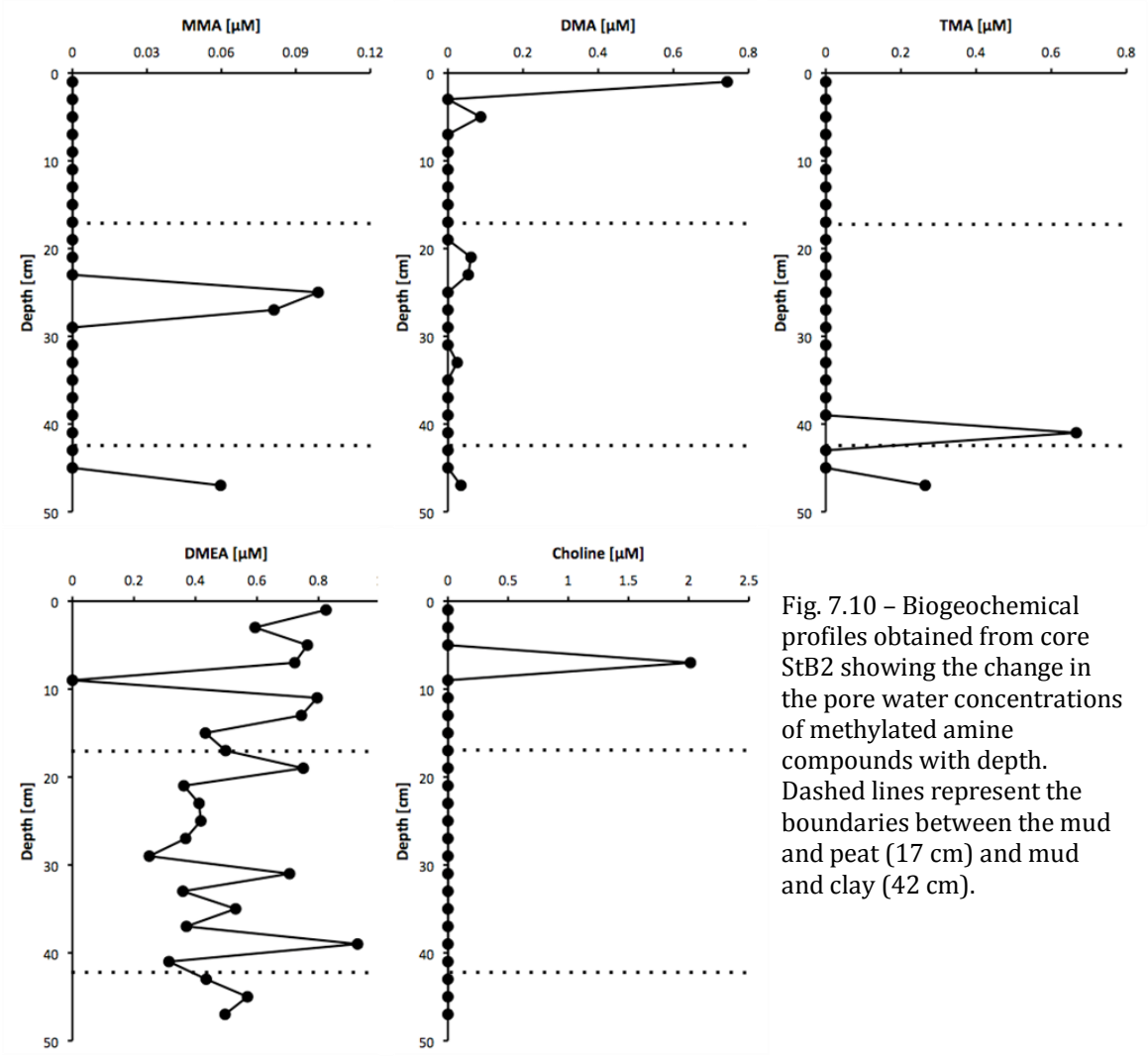


Fig. 7.10 – Biogeochemical profiles obtained from core StB2 showing the change in the pore water concentrations of methylated amine compounds with depth. Dashed lines represent the boundaries between the mud and peat (17 cm) and mud and clay (42 cm).

overlying mud but showed a maximum rate of 99 pmol cm^{-3} d^{-1} at 19 cm again matching the methane peak and indicating that activity was stimulated in the activated interface. Rates then decreased again below 20 cm in the body of the peat bed, with no obvious trend with depth. This lack of methanogenesis below the activated layer may again be related to the presence of plant-derived compounds, as tannins have been shown to have a negative impact on methanogenesis as well as sulphate reduction (Whitehead *et al.* 2013; Kumar *et al.* 2014). Also Cervantes *et al.* (2000) and Minderlein and Blodau (2010) showed that both humic DOM and anthraquinones (acting as humic analogues) can have inhibitory effects on methanogenesis, either due to direct toxicity or competition for common substrates with quinone-reducing bacteria. Also as with the SRR, the use of humics as alternative electron acceptors by methanogens might be contributing to the apparent inhibition of methanogenesis (Lovley *et al.* 1996).

Methylated amines were again detected in core StB2 (Fig. 7.10) with MMA, DMA TMA, DMEA and choline all being present (though choline was only detected at 7cm at ≈2 μM). Both MMA and DMA once again had elevated concentrations within the activated interface while MMA and TMA also show peaks at the base of the peat and in the clay. Concentrations of these aliphatic amines are lower than in core StB1 (>20x so in the case of DMA) suggesting that, as with glycolate, the ratio of production:consumption may vary through the year. As in StB1 the DMEA depth profile is different to the other methylated groups with no obvious depth trend (though there is a significant peak in the darker peat close to the clay boundary). Also DMEA concentrations are on average 2-4x higher in StB2 than StB1, which may be linked to the fact that DMEA is potentially derived from recent organic matter (cell membrane lipids etc.) rather than from the degradation of the peat, as is likely the case with the other amines.

In order to determine whether the elevated amine concentrations in the peat layer were acting as non-competitive substrates and stimulating methanogenesis, active rate measurements were conducted using ^{14}C-labelled DMA and choline (Fig. 7.11). DMA consumption by methanogens was rapid when compared to acetate and HCO_3^-, and was utilised for methanogenesis within both the overlying mud and the top 12 cm of the peat. Turnover to methane was most rapid within the activated layer producing a potential peak methanogenesis rate of 1750 pmol cm^{-3} d^{-1} at 19 cm (based on the size of the ^{14}C-hot and cold pools), while below the activated layer (31 cm) no activity was detected, indicating that DMA-methanogenesis could indeed be in-part responsible for the methane peak in the activated interface. This high potential turnover of DMA to methane also supports the idea that the activated interface is able to produce high levels of DMA, at least at certain times of the year. Choline turnover to methane was much slower than DMA and rates were highest in the oxidised, overlying muds, reaching a peak at 7 cm. This was also the only depth where choline was detected in the pore waters, producing a potential peak methanogenesis rate of 0.95 pmol cm^{-3} d^{-1} (based on combined ^{14}C-hot and cold pool size). Below 7 cm, rates decreased (like acetate) through the reduced muds, before peaking again at 17 cm, just at the mud:peat boundary. Rates then decreased exponentially through the activated layer itself and remained low throughout the

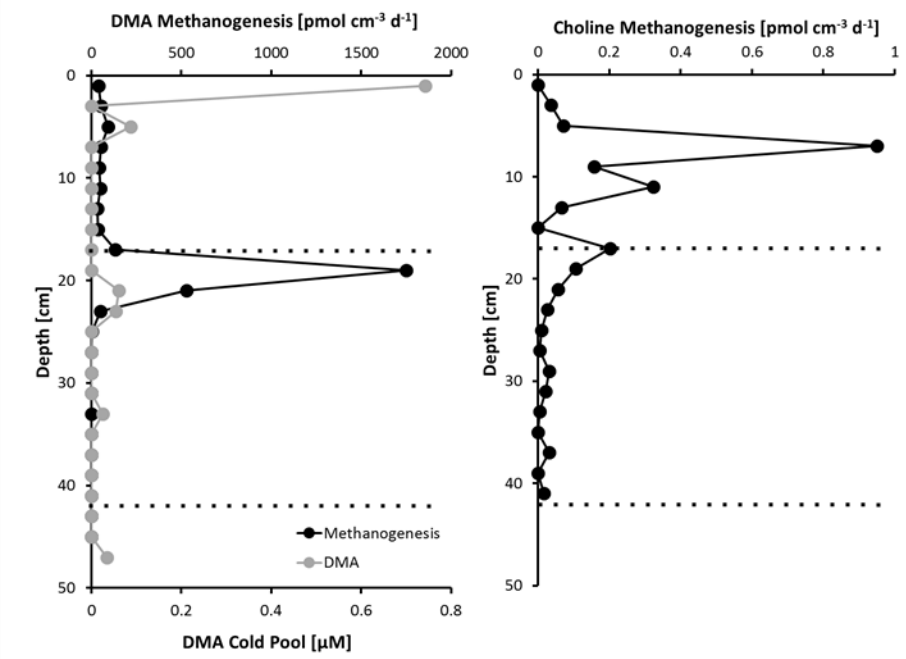


Fig. 7.11 – Biogeochemical profiles obtained from core StB1 showing the changes in the *in situ* rates of DMA and choline methanogenesis with depth (plotted alongside the cold pool concentrations in the case of DMA). Dashed lines represent the boundaries between the mud and peat (17 cm) and mud and clay (42 cm).

peat with only trace levels of activity. This prevalence of choline-methanogenesis in the overlying muds as opposed to the peat suggests that the choline these organisms are utilising *in situ* is likely (as with DMEA) to be derived from "fresh" organic matter deposited from the estuary waters rather than from the breakdown of the peat. However, the fact that turnover is also stimulated at the mud-peat interface (possibly by diffusion of compounds up from the peat) and that rates decrease through the activated layer rather than abruptly terminating suggests that some peat-derived choline could be used as a substrate for methanogens (though at concentrations below detection limits). On the other hand it could be the case that the choline that stimulates this methanogenesis is in fact still "fresh" but is derived instead from the recent lysing of prokaryotic cells that are growing in elevated numbers within the activated interface layer.

Hydrogen concentrations in core StB2 (Fig. 7.12) were more variable than in StB1 but again peaked within the activated interface at 23 cm indicative of fermentative activity. CO_2 increased just below the mud surface and then remained around this concentration until the clay layer, where concentrations decreased to <1 mmol lws^{-1}.

Local maximum concentrations occurred at the mud:peat boundary and the peat:clay boundary which correspond to some peaks in the organic acid profiles – possibly indicating increased fermentation.

Cell counts were conducted on core StB2 to assess if the changes in sediment lithology seen in the cores, or the age of the sediments, affected the size of the prokaryotic population. Cell numbers in the surficial muds decreased gradually with depth, from $1x10^{9.6}$ cells cm^{-3} at 1.5 cm to $1x10^{9.4}$ at 15.5 cm. Unusually, unlike the other tidal flat sites studied herein there is no sharp drop in numbers where the sediment darkens at 12 cm. Moving down into the peat, numbers do drop sharply to $1x10^{9.1}$ at 17.5 cm and continue to decrease down to 23.5 cm. Values then increase again with depth through the peat bed, reaching a subsurface maxima of $1x10^{9.2}$ at 35.5 cm, before decreasing again sharply upon entering the darker woody peats at the base of the unit. Numbers then gradually increase through the woody peat before showing a final decrease upon entering the Wentlooge Clay beneath, down to $1x10^{8.4}$ at the base of the core.

Overall these cell numbers are similar to those obtained at the other tidal flat sites in the estuary described in this study (see Chapter 10) and as such it would appear that, despite radiotracer results, the peat does not have an inhibitory affect on overall bacterial growth. This may mean that the apparent inhibition described above is indeed due to humic compounds acting as alternative electron acceptors, as such a situation would not suppress overall growth. However, the variation in numbers seen within the peat is unusual, for example cell numbers seem to decrease in the upper 5 cm of the peat layer despite the fact that this is where (according to the radiotracers) much of the metabolic activity is taking place. The reason for this, as well as the apparent increase in numbers moving down through the peat layer, may relate to the structure of the peat itself, as in the upper layers the peat contains more intact plant material. This means that the sediment/fibre grain size is comparatively large making pipetting during AODC difficult, and therefore counts in the upper layers of the peat may be an underestimation of the true cell numbers present at those depths. The AODC profile also appears to show a relationship to the SO_4^{2-}:Cl^- profile, with higher cell numbers in areas where sulphate appears to be being removed (i.e. the top 15 cm and between 23-35 cm) and lower numbers

where the SO_4^{2-}:Cl^- profile is increasing or static (i.e. between 15-23 cm and below 41 cm). The reason for this relationship is unknown as SRB are likely to represent only a small percentage of the total prokaryotic population. However, they do consume the waste products of other groups further up the redox cascade and as such their population size may be driven by trends in the overall population of prokaryotes in the sediment.

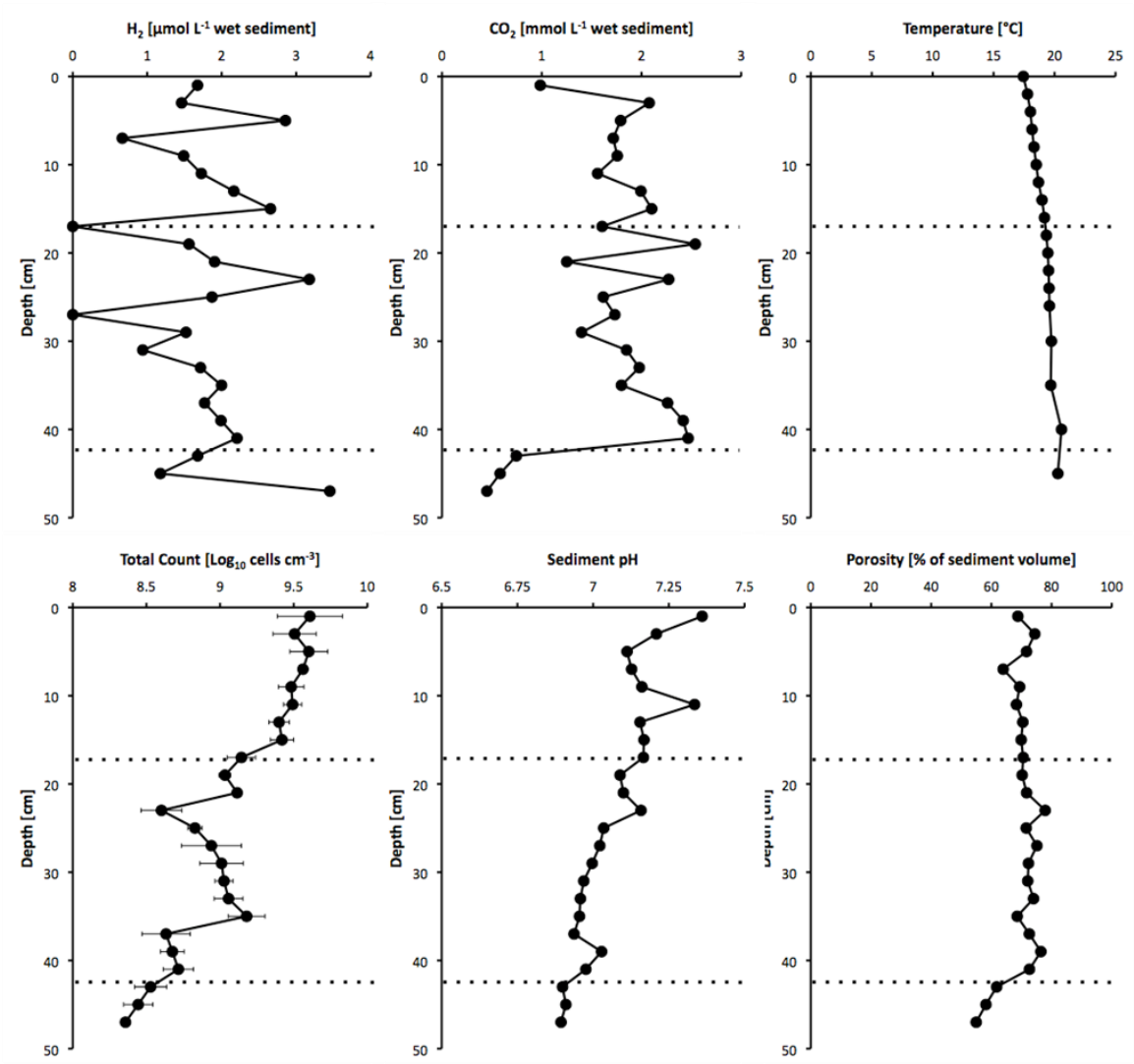


Fig. 7.12 – Biogeochemical profiles obtained from core StB2 showing changes in the concentration of hydrogen and CO_2 with depth. Also shown are profiles of: sediment temperature, total cell count (AODC) sediment pH and sediment porosity. Error bars depicted on the cell count profile represent the 95% confidence limits obtained from repeated counts. Sediment pH was measured during sectioning of the core using an InLab ®423 pH electrode connected to a SevenMulti base station (Mettler Toledo). Dashed lines represent the boundaries between the mud and peat (17 cm) and mud and clay (42 cm).

7.3 St Brides Wentlooge - Discussion

Salt marsh peats similar to those at St Brides Wentlooge are found exposed along much of the north shore of the Severn Estuary as well as a few locations on the southern shore (Allen 1990b). In addition buried intercalated peats form a part of the Holocene succession deposited over much of the greater Severn Estuary area and as such can be considered a major component of the estuary's coastal sedimentary environment (Allen and Haslett, 2002). Similar peat deposits are present elsewhere in the UK (Waller *et al.* 2006), Europe (Baeteman *et al.* 1999, Dellwig *et al.* 2001, Dellwig *et al.* 2002, Kolditz *et al.* 2009) and further afield (Nikitina *et al.* 2000, Hiroshi *et al.* 2001) and can be exposed at the surface or deeply buried under metres of sediment. During formation at the surface, salt marsh sediments are known to be highly biogeochemically active with a wide range of prokaryotic guilds present in the sediment, in large part due to the significant amounts of organic substrate and respiratory compounds provided by the marsh vegetation and seawater respectively (Killops and Killops, 2005). However, little is known about what biogeochemical processes are occurring in buried salt marsh sediments that have been preserved for thousands of years. The results obtained from the examination of the St Brides cores show that the peat beds provide considerable amounts of substrates for fermentative bacteria, which upon utilisation provide a variety of secondary bacterial substrates (organic acids, methylated amines etc.) and in some cases in large quantities (hundreds-thousands of μM in the case of glycolate). This in turn stimulates growth of terminal oxidising prokaryotic guilds such as the SRB and methanogens.

An interesting factor the geochemistry of this site is the presence of nitrate in the sediments even at considerable depth. Since this compound is an electron acceptor with a high energy yield it is usually consumed rapidly by prokaryotes and as such is often only detected close to the surface (see previous chapters). However, in both core StB1 and StB2 nitrate was detectable all the way to the bottom of the cores and in the case of StB1 at very high levels (≈200-600 μM). The reason for the presence of nitrate in these cores is unknown as both cores show evidence of nitrate consumption (either by denitrification or DNRA) in the overlying muds. However, within the peat layer (and the clay in StB1) concentrations increase again, very

dramatically so in the case of StB1. As mentioned in section 8.2.1, production of nitrate in anoxic sediments is usually caused by anammox (Egli *et al.* 2001) however, there does not appear to be any evidence of this process occurring in either of these cores. Another possibility is groundwater flow, which might account for the increased concentrations in the clay layer in core StB1 (with groundwater flowing along the peat:clay boundary) and for the overall lower levels in core StB2 (since it was collected during a period of low rainfall). However, this explanation would not account for the presence of nitrate throughout the peat bed and particularly in the activated interface where concentrations are higher than deeper in the peat. This increase in the activated interface might suggest that the origin of the nitrate is linked to biological processes with the activated interface enhancing the rate of this process as it appears to do with the production of other compounds (organic acids, methylated-amines etc.). The identity of this process is still unknown though, as breakdown of the peat would form ammonium which would still need to be oxidised to form nitrate, this could be occurring (e.g. via nitrification) in the oxidising conditions at the mud:peat interface but not at depth in the anoxic peat.

Another interesting feature of the nitrate profile is that the nitrate produced in the peat does not seem to be rapidly consumed. This could again be due to inhibition by humic compounds, possibly due to them acting as alternative electron acceptors (Lovley *et al.* 1996). However, experiments by Fork and Heffernan (2014) have shown that humics do not appear to inhibit denitrification, while the results of Cervantes *et al.* (2008) indicate that in the presence of multiple electron acceptors AQDS (anthraquinone-1,6 disulfonate), a humic acid analogue, is degraded after complete denitrification has occurred, suggesting that nitrate is used preferentially over humic compounds as a substrate. On the other hand, a study by Dodla *et al.* (2008) showed that phenolic compounds can have a negative effect on denitrification. Since these compounds occur in vegetation and soils, and are one of the precursors of humic compounds (Hättenschwiler and Vitousek, 2000) it is possible that phenols could be the cause of the apparent low degree of nitrate removal within the peat bed. Unusually, the presence of nitrate also does not seem to be having a dramatic effect on the activity of SRB or methanogens. Normally nitrate is thought to inhibit the activity of these groups (Klüber and Conrad, 1998; Laverman *et al.* 2012; Xu *et al.* 2014). However, in this case it may be that due to the

high level of organic matter in the peat, nitrate-reducing organisms are not outcompeting the SRB and methanogens for common OC substrates, and therefore the normal rules concerning inhibition may not apply to the same degree.

Sulphate reduction at this site is concentrated in the overlying muddy sediments, fuelled either by freshly deposited organic material and/or by upward diffusion of substrates such as acetate and glycolate from the peat layer. Depth integrated rates of sulphate reduction at this site reach 6.23 mmol m^{-2} d^{-1}, 6x higher than those obtained from Kingston Pill on the southern side of the Estuary (a site with no peat in the immediate vicinity), despite only a 4-6 °C temperature difference between the two sites. This would suggest that the presence of the peat is providing a boost to sulphate reduction in the surficial sediments not found at other sites, although within the peat itself sulphate reduction is drastically reduced compared to the overlying sediment. Since sulphate and OC substrate concentrations are well above threshold values this decrease is likely caused by inhibition by compounds found in abundance in the peat (i.e. free S^{2-} or humics) or by the SRB utilising humic compounds as electron acceptors in preference to sulphate (Lovley *et al*. 1996). As such, overall while the peat itself may not provide an ideal habitat for SRB, its presence does appear to benefit those living in the sediments in close proximity to it.

Methanogenesis on the other hand, was shown to be stimulated in the upper layers of the peat, with a variety of substrates being utilised (acetate, HCO_3^-, DMA and choline). In addition acetate, DMA and especially choline were also metabolised in the overlying muds suggesting that upward diffusion of compounds from the peat may well be stimulating methanogenesis in addition to "fresher" substrates deposited from the waters of the estuary. When compared to other sites, depth integrated rates for acetoclastic methanogenesis again have higher values (1.09 μmol m^{-2} d^{-1}), with rates at this site ≈37x higher than those at Kingston Pill (core ST6). H_2/CO_2 methanogenesis was also elevated compared with other sites with depth integrated values (3.8 μmol m^{-2} d^{-1}) >7x higher than any other site in the estuary. This again suggests that the presence of peat at this site enhances microbial processes compared to similar sites without peats. Within the peat bed itself rates of methanogenesis (as well as substrate and methane concentrations) are highest

within the top 5-10 cm of the peat, the depth referred to here as the "activated interface". The reason for the presence of this layer may be due to the fact that some or all of the muddy sediments at St Brides can be eroded and re-deposited over the course of the tidal cycle, allowing the top surface of the peat to come into contact with fresh sediment/seawater on a regular basis. This could mean that the top surface of the peat bed is regularly supplied with fresh substrates, electron acceptors and other growth-essential compounds from the seawater while at the same time remaining relatively anoxic (due to it's compacted nature) and rich in some electron donor and organic carbon substrates. This combination of a stable environment coupled with a regular, enhanced supply of substrates and the potential for enhanced breakdown of recalcitrant peat-derived compounds due to temporary contact with oxygen (Pind *et al.* 1994; Freeman *et al.* 2001; Fenner and Freeman, 2011), would create an ideal environment for heterotrophic prokaryotes. If these organisms in turn degraded the peat then their enhanced metabolism would lead to the production of large amounts of waste products capable of stimulating other groups whose substrates are not commonly found in seawater. In addition it may be the case that the activated layer, as well as being rich in substrates, is also an excellent methanogen habitat (relative to the rest of the peat) due to the fact that regular contact with seawater/new sediment may have a leaching effect on the toxic humics and tannins likely present in the peat, thus reducing their concentrations in the upper layers to levels that the methanogens (and SRB) can tolerate. It is also possible that these low levels of humics may in turn further enhance methanogenic activity as Xu *et al.* (2013) found that low levels of anthraquinones can in fact stimulate methanogenesis in paddy field soils in the long term - especially in the presence of iron minerals (Zhou *et al.* 2014c). Since this hypothesis relies on the regular re-suspension of large amounts (10-20 cm) of sediment, it could only occur in environments with strong tidal currents. Therefore if correct, the phenomenon of "activated peats" may be unique to areas with hypertidal regimes such as the Severn Estuary. On the other hand it may be the case that while the occurrence of activated peats at the surface may be limited to hypertidal areas, it is possible that they may also be present in other areas at depth in the sediment column. For example, peats such as those described by Dellwig *et al.* (1999) that occur >10 metres below the surface could come into contact with groundwater flowing horizontally along

bedding planes. If this groundwater was anoxic while also being rich in limiting nutrients then it its possible the upper and/or lower surfaces of the buried peat bed could become "activated" in a similar fashion to those at St Brides Wentlooge. In addition if the peat was particularly porous then the groundwater could flow through the whole thickness of the peat bed "activating" it on a much larger scale. The generation of new substrates by the organisms in the peat could then further stimulate growth in the sediments surrounding the peat, creating a zone of enhanced metabolic activity not directly connected to the normal downward flux of substrates from the surface. Some lines of evidence indicate that such a deep activated layer may in fact be present within the peats at the St Brides site, with concentrations of organic acids being higher close to the peat:clay boundary, suggesting that groundwater flow along the boundary might be stimulating fermentation at this depth. However, since these changes could also be linked to changes in peat lithology or higher concentrations of compounds in the groundwater itself, the presence of a second activated layer cannot be confirmed without further work (e.g. tracking groundwater). Secondary stimulation of growth at depth has been observed elsewhere however, for example by Köpke (2007) and Beck *et al.* (2009) in cores taken from the Wadden Sea, where the presence of buried shell beds (at ≈2 metres depth) allowed sulphate-rich groundwaters to flow through the sediment column forming a second zone of sulphate reduction separated from the surface. In addition the stimulation of methanogenesis due to presence of peat-derived DOC was also observed to in the groundwaters of a Canadian aquifer by Aravena and Wassenaar (1993), while lignite sequences (Fry *et al.* 2009), brine flow and diatom beds (Parkes *et al.* 2005) have also shown the ability to enhance microbial activity at depth. Since this type of buried activated peat would not require a hypertidal regime to form it might be more common than the surficial type present at St Brides and may in some areas provide an important *in situ* source of substrates to the shallow subsurface that has not previously been considered.

As well as drastic differences in biogeochemistry between the peat and overlying muds, differences have also been shown between the two cores taken at different times of the year (November and July). In November (core StB1) the peat layer was abundant with a wide variety of fermentation products and other substrates

whereas in July (core StB2) almost all of these compounds had been severely depleted or had disappeared altogether. In addition to this, when compared to core ST5 (taken at the same site in October 2012,) it can be seen that the rates of sulphate reduction and methanogenesis also differ greatly between the seasons. There are several possible reasons for this change in biogeochemistry with time; the first and most obvious of which is temperature. It is well known that environmental temperatures have a dramatic effect on the rates of microbial metabolic processes (Nedwell and Abram, 1979; Jørgensen and Sørensen, 1985; Yvon-Durocher *et al.* 2014). As such it is likely that the reduced temperatures in October can account for the decreased rates of sulphate reduction and methanogenesis in core ST5 compared to StB2. In addition, it is possible that the change in temperature could account for the variation in organic acid and methylated amine concentrations between cores as well. If the processes that produce these compounds (fermentation etc.) are able to operate at lower temperatures more effectively that the processes that consume them (sulphate reduction, methanogenesis etc.) then it is possible that these compounds could accumulate in the peat over the winter months leading to the high concentrations present in core StB1. Subsequently in summer as temperatures rose, the rates of consuming processes would increase, depleting the stock of compound to produce the profiles seen in StB2. The relative effects of summer temperatures on the production processes is not known but they must be able to, at the very least, keep up with the consumption processes in order for the compounds they produce to be detectable in core StB2 (with the exception of oxalate).

This potential variation in rates of production and consumption of OC compounds with temperature would agree with the findings of Weston and Joye, (2005) who examined variations in labile OC accumulation with temperature in coastal sediment slurries and bioreactors, which the authors linked to a decoupling of hydrolytic/fermentative and terminal-oxidising processes producing and consuming LMW-DOC. Similar results were obtained by Tabuchi *et al.* (2010) who also suggested that changes in temperature could affect acetate accumulation by controlling which groups of SRB (partial vs. complete oxidisers) were active in the sediment.

The second potential cause for the variation between the two cores relates to the amount of sediment re-suspension that occurred prior to the time the cores were collected. As detailed above, core StB1 was collected during the spring tide portion of the tidal cycle (tidal range ≈9 m) while core StB2 was collected during a neap tide (tidal range ≈5.5 m). The hypertidal regime of the Severn Estuary results in large amounts of sediment being eroded and re-deposited on a fortnightly basis (Manning *et al.* 2010), therefore it is possible that the overlying muds present in StB1 were emplaced for less time before coring than those in StB2. In addition, increased wind and wave activity in the Severn Estuary during the winter months is known to re-suspend large amounts of sediment and affect the degree of erosion and deposition on the intertidal mudflats (O'Brien *et al.* 2000, Kirby and Kirby 2008). This difference in deposition duration could explain the very sharp gradients between peat and mud seen in StB1, as if the overlying muds had only been in place for a relatively short space of time diffusion between the two units could have been minimal. In core StB2 on the other hand, where the overlying muds were likely emplaced for longer, the gradient between peat and mud is not so sharply defined as compounds would have had longer to diffuse between the two units and be utilised. The duration of mud emplacement could also account for the decreased levels of compounds (VFAs, glycolate etc.) in the peat bed in core StB2, as the longer the overlying muds were in place the greater chance they had of becoming anoxic. Once this had occurred sulphate reduction and other anaerobic processes can become established which could utilise compounds diffusing up from the peat as electron donor substrates. This could potentially have the effect of "draining" the peat of substrate compounds over the tidal cycle resulting in the low-concentration profiles seen in core StB2. In addition to this, when cut off from fresh substrates/nutrients by tens of centimetres of anoxic mud and without regular contact with oxygen to help break down the peat, the activated interface of the peat may become much less efficient at producing compounds, contributing again to the lower concentrations seen in the summer core.

Finally the third potential cause for the variation between the two cores relates to changes in groundwater flow between the two sampling periods. As described above, core StB1 was collected during a period of prolonged heavy rainfall. This would have generated a substantial hydraulic head landward of the

tidal flats, which could have resulted in an enhanced flow of groundwater through its sediments (Ward and Trimble, 2003). In contrast when core StB2 was collected rainfall over the previous month had been low, this would have resulted in a lower hydrostatic head and therefore probably a lower flow of groundwater through the tidal flats. As such, if the increases in compounds around the peat:clay boundary in core StB1 are derived from groundwater – either directly (e.g. nitrate) or via enhancement of *in situ* processes (e,g. acetate) then is reasonable to suggest that these concentrations would be higher in the winter (StB1) than in the summer (StB2)

Without further study (e.g. sampling repeatedly over a tidal cycle and over a range of seasons) it is impossible to determine which of the factors outlined above is the main contributor to the marked changes in biogeochemistry documented at the St Brides site. In addition it is important to remember that the these processes would not be working in isolation (e.g. since both temperature and deposition are greater in the summer) and therefore could both be contributing to a greater or lesser degree to the changes occurring at this site.

7.4 St Brides Wentlooge - Glycolate Slurry Experiment

The results from core StB1 show that the sediments at the St Brides site are capable of containing high levels of the α-hydroxy acid glycolate, which is not commonly detected in large quantities in marine sediments (Peltzer and Bada, 1981; Llobet-Brossa *et al.* 2002). Since glycolate is a known substrate for both aerobic (Edenborn and Litchfield, 1985) and anaerobic bacteria (Friedrich *et al.* 1991, Friedrich and Schink 1995), and is severely depleted in core StB2 it must be being consumed by some unknown microbial process, and in addition it may be one of the compounds responsible for stimulating microbial metabolism at this site. Therefore in order to directly confirm that glycolate was potentially capable of being utilised by the *in situ* microbial populations, and to see if metabolic pathways differed between the surficial muddy sediment and peat, a time course slurry experiment was conducted.

7.4.1 Methodology

Six 2 litre modified aspirator bottles were filled with 750 ml of sulphide-reduced marine medium (plus a magnetic flea) and autoclaved at 120 °C (Köpke *et al.* 2005).

Three of the bottles were then inoculated with 250 cm^3 of surface sediment from the St Brides site (slurries S1, S2 and S3) while the other three were inoculated with 250 cm^3 of peat from the upper "activated interface" layer of the peat bed (slurries P1, P2 and P3) – all sediment was collected at the same time as core StB2. Sediment was added to the bottles under a continuous upward flow of OFN to minimise oxidation of the medium. The bottles were then sealed with rubber septa, well shaken, and placed on magnetic stirrer plates for ≈2 hours to suspend the sediment. Slurries S1, S2, P1 and P2 were then supplemented with a glycolate solution to a final concentration of 10 mM. Slurries S3 and P3 were not supplemented in order to act as controls. The slurries were then incubated in the dark at *in situ* temperature (≈18 °C) for 2 months with samples taken for gas and pore water analysis every 3-4 days. Before sampling, each slurry was shaken vigorously in order to fully re-suspend and homogenize the sediment and therefore reduce sampling heterogeneity. Gas samples were taken with a 3 ml luer-lock syringe through the septum and analysed for methane via gas chromatography. Pore water samples were taken from a valve at the base of the bottle with a 5 ml syringe. 2 ml of slurry was then injected into an eppendorf tube and spun at 15,800 x G (RCF) in a centrifuge 5415 C (Eppendorf) for 15 minutes at room temperature. The supernatant was then extracted, filtered and frozen at -20 °C ready for analysis via ion chromatography to determine sulphate and glycolate concentrations.

7.4.2 Results and Discussion

Glycolate concentrations decreased over time in all of the spiked slurries (Figs. 7.13 and 7.14), reaching a minimum of 1.4 mM in slurry S2. The decrease in concentrations was greater in the surface slurries than in those containing peat. This indicated that the communities/conditions present in the surficial sediment result in a more active degradation of glycolate than those in the "activated" peat layer. Sulphate in the supplemented slurries also decreased over time and followed a similar pattern to glycolate, suggesting that glycolate was being used as a SRB substrate. As with glycolate, sulphate tended to be more rapidly removed in the surficial slurries than in the peat slurries (with a minimum concentration of 4.2 μM in slurry S2), however, there was variation between replicates. This again suggests a more active SRB community in the surface muds. In the control slurries no

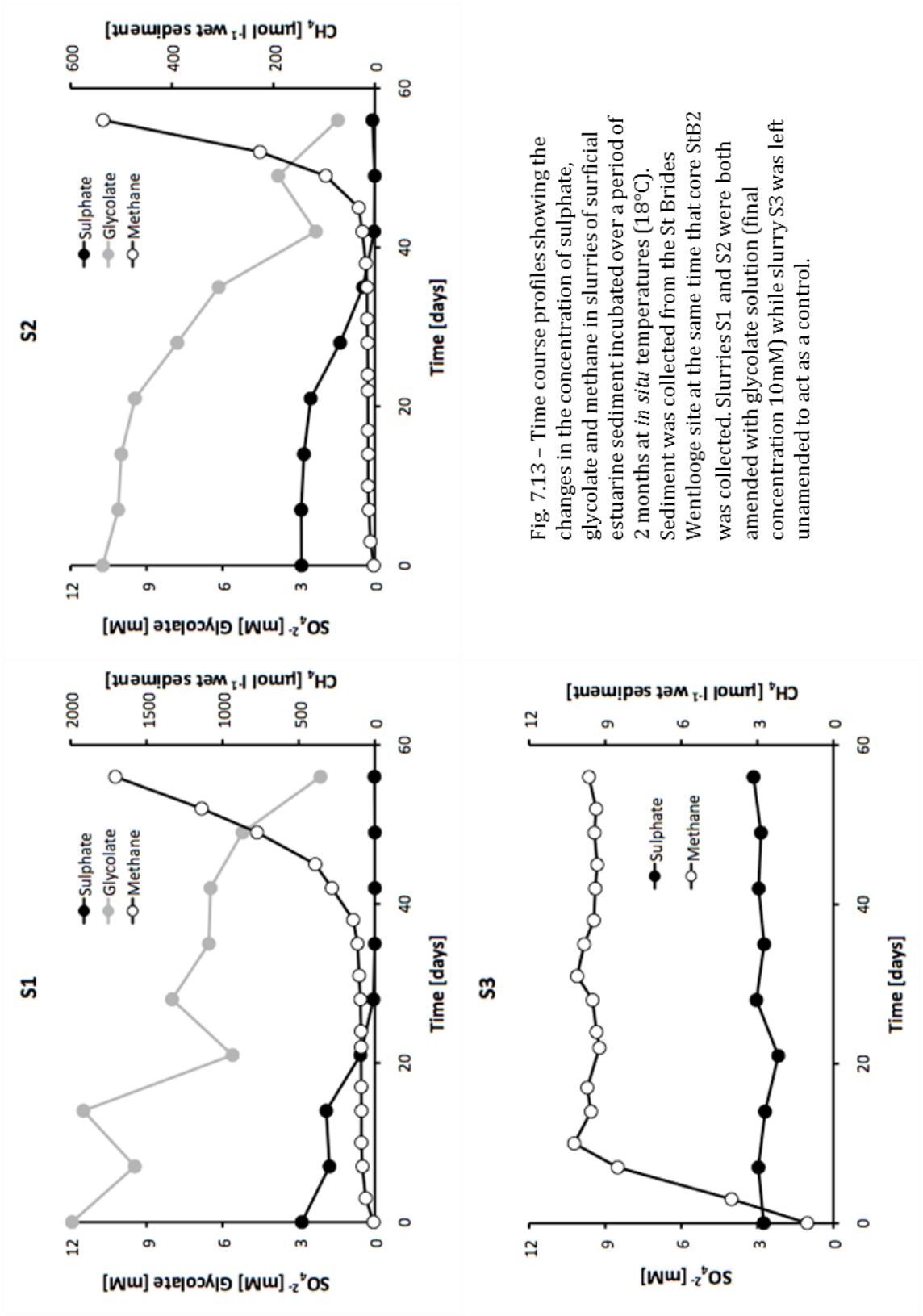


Fig. 7.13 – Time course profiles showing the changes in the concentration of sulphate, glycolate and methane in slurries of surficial estuarine sediment incubated over a period of 2 months at *in situ* temperatures (18°C). Sediment was collected from the St Brides Wentlooge site at the same time that core StB2 was collected. Slurries S1 and S2 were both amended with glycolate solution (final concentration 10mM) while slurry S3 was left unamended to act as a control.

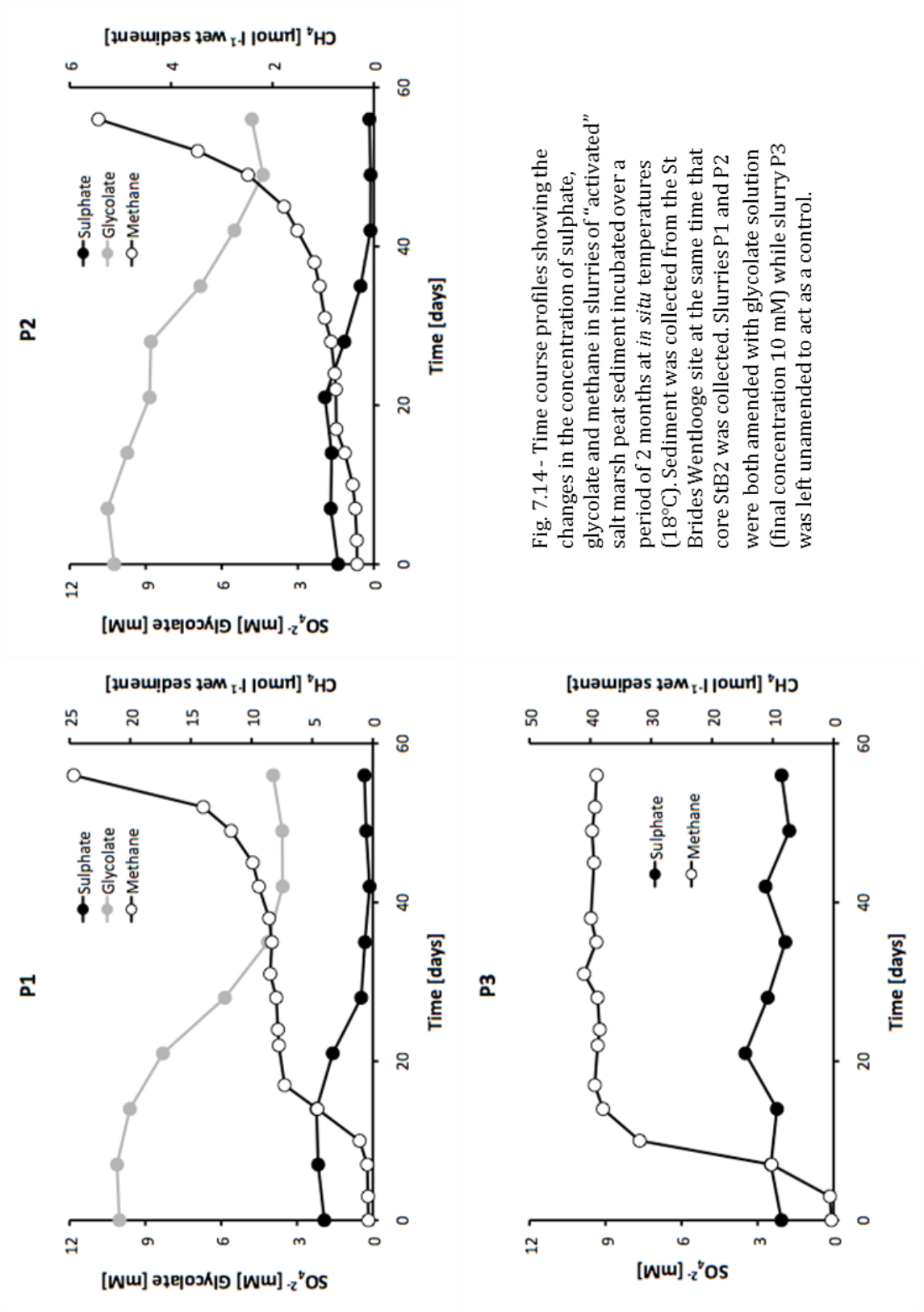


Fig. 7.14 - Time course profiles showing the changes in the concentration of sulphate, glycolate and methane in slurries of "activated" salt marsh peat sediment incubated over a period of 2 months at *in situ* temperatures (18°C). Sediment was collected from the St Brides Wentlooge site at the same time that core StB2 was collected. Slurries P1 and P2 were both amended with glycolate solution (final concentration 10 mM) while slurry P3 was left unamended to act as a control.

significant sulphate reduction occurred, demonstrating that glycolate can substantially stimulate sulphate reduction in these sediments. The fact that no appreciable increase in acetate was detected over time suggests that the SRB in the slurries were oxidising the glycolate completely to CO_2, possibly via a pathway similar to this equation (7.1) (Friedrich and Schink, 1995):

$$4CH_2OHCOO^- + 3SO_4^{2-} + 7H^+ \Rightarrow 8CO_2 + 3HS^- + 8H_2O \quad (7.1)$$

Although this cannot be confirmed without further pure culture or radiotracer experiments.

As well as sulphate reduction, methanogenesis was also occurring in the slurries. In the surface slurries methane concentrations only increased markedly once sulphate was depleted and this corresponded with a second decrease in glycolate concentrations. This would indicate that glycolate is also able to be utilised by methanogenic pathways - possibly via the cleaving of the methanol group from the glycolate by a syntrophic partner or via interspecies hydrogen transfer (Friedrich *et al.* 1991). The fact that methane only increased once sulphate had been depleted also suggests that the SRB and the methanogens (or their precursors) are competing for glycolate (or intermediate compounds) as a common substrate and that the SRB are capable of out-competing the other group(s) in that regard. Methane also increased in a similar fashion in the peat slurries but to a much lesser degree than in the surficial slurries (the maximum methane concentration in the surface slurries was 1700 μmol lws^{-1} compared to 24.7 μmol lws^{-1} in the peat slurries). The increase in methane also corresponded to a decrease in glycolate but not to the same extent as in the surficial sediment (glycolate consumption also seemed to stop at around 3-4 mM in the peat slurries). This suggests that the methanogenic pathway that is capable of metabolising glycolate is more active (by several orders of magnitude) in the surficial sediments than in the peat. Methane concentrations also increased in the control slurries but followed a different pattern to the supplemented slurries, with methane increasing to relatively low levels (10-40 μmol lws^{-1}) quickly over the first ten days and remaining constant at this level for the rest of the experiment. Since this increase was greater in the peat than the surface it is likely that it was caused by the presence of other methanogenic substrates (such as DMA etc.) that could be present in elevated concentrations in the

peat relative to the surficial sediments. Interestingly methane levels in slurry P3 were higher than in either P1 or P2. This might indicate that the addition of glycolate could be inhibiting methanogenesis in the peat, however, the results from the surface slurries (where methane concentrations are much higher in the supplemented slurries than the control) indicate that high glycolate concentrations are unlikely to be inhibitory to all methanogens.

Taken together the evidence from this experiment shows that the microorganisms native to the sediments at the St Brides site are capable of anaerobically degrading the glycolate that is present within the peat bed during the winter months. It appears that glycolate acts as a competitive substrate capable of stimulating both sulphate reduction and methanogenesis (either directly or indirectly). In addition, this data shows that the microbial communities in the surficial sediment are more active in metabolising glycolate than those present within the peat (particularly the methanogens/methanogenic precursors). This enhanced consumption could also help to explain the dramatic differences in glycolate concentrations between the peat and overlying muds in core StB1. When considered in conjunction with the *in situ* geochemical data from cores StB1 and StB2, the data from this experiment adds to the hypothesis that the peat bed at St Brides stimulates growth in the surficial sediments by providing additional electron donor substrates via upward diffusional leaching. In addition it helps explain the varying glycolate values between the two cores as likely being caused by large-scale consumption of glycolate both inside and outside the peat during the summer months

7.5 St Brides Wentlooge - Conclusions

- St Brides Wentlooge represents an unusual sedimentary environment where a ≈3400 year old Holocene salt marsh is exposed to recent tidal flat deposits due to the dynamic nature of the Severn Estuary's tides.
- Geochemical evidence shows that during the winter months the salt marsh peat beds at this site are significant source of anionic and cationic microbial substrates including organic acids (acetate, lactate etc.) and methylated amines. These also include compounds not normally detected at high concentrations in marine sediments including glycolate (>2-4 mM) and DMA (≈20 μM).

- The production of these compounds is particularly high in a ≈10 cm thick "activated interface" layer at the top of the peat, likely due to the regular contact of the surface of the peat bed with seawater or fresh, re-suspended sediment (Fig. 7.15).
- A second activated layer may also be present at depth at the interface between the peat and the underlying Wentlooge Clay – possibly related to nutrient-rich groundwater flow derived from the Wentlooge Levels.
- Despite evidence of denitrification/DNRA in the surficial muds nitrate is consistently present at depth in both cores with levels exceeding 400 μM in core StB1. The reason for the persistence of this compound is unknown however, it does not appear to be inhibiting sulphate reduction or methanogenesis but may be oxidising sulphide leading to the unusually high levels of thiosulphate (≈50-60 μM) present in the cores.
- During the summer months substrate concentrations in the peat bed are dramatically lower – likely due to the higher temperatures preferentially stimulating substrate consumption relative to production; changes in the rates of re-suspension and deposition of the overlying sediments; and changes in groundwater flow.
- Radiotracer measurements confirm that metabolic activity is concentrated in the overlying muds (sulphate reduction, acetoclastic methanogenesis, choline methanogenesis) and in the "activated interface" (H_2/CO_2 methanogenesis, DMA methanogenesis). In some cases (e.g. sulphate reduction) maximum rates do not necessarily match with the steepest portions of the geochemical profile suggesting that the profiles are being "pulled down" by the high rates lower in the sediment via diffusion.
- Little activity appears to be occurring in the main body of the peat bed, possibly as a result of inhibition by peat-derived organic compounds (tannins, quinones etc.) either directly or due to them being used as alternative electron acceptors. However, neither this apparent inhibition, nor the age of the sediment appears to have negatively affected the total cell counts in the sediment.
- Incubated slurries show that compounds likely derived from the peat (e.g. glycolate) are consumed both within the peat and in the overlying sediment and appear to be able to stimulate both sulphate reduction and methanogenesis. Such stimulation may also be occurring *in situ* at the sampling site potentially helping to

account for the elevated rates of anaerobic processes relative to other sites described in this study (discussed further in Chapter 9).

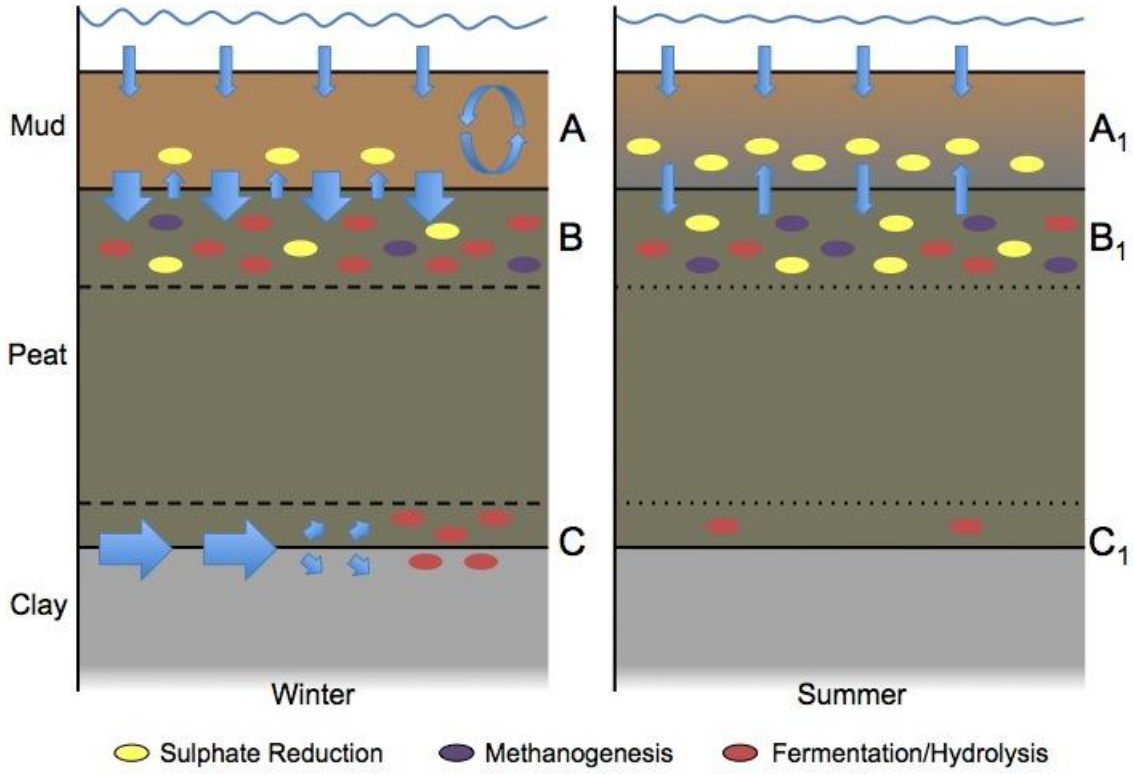


Fig. 7.15 – Diagram summarising the processes thought to be occurring at the St Brides Wentlooge study site throughout the year. During the winter months, increased wave activity leads to intense mixing and re-suspension of the overlying muddy sediments (A) resulting in an overall oxidised state and a high degree of solute and/or oxygen diffusion into the underlying peat (blue arrows). This allows for the formation of the "activated interface" (B), with high fermenter and hydrolytic activity (red) leading to high concentrations of organic acids and methylated amines alongside relatively low levels of sulphate reduction (yellow) and methanogenesis (purple), due to the low *in situ* temperatures. Upward diffusion of these peat-derived compounds may also help to stimulate sulphate reduction in the overlying muds. At the peat:clay boundary nutrient-rich groundwater flow (blue arrows) allows a second activated interface to develop (C). During the summer months, reduced wave activity results in less sediment re-suspension, leading to the overlying muds (A_1) being emplaced for longer. This allows the muds to become reduced and for more sulphate reduction (yellow) to occur. Within the activated interface (B_1) less input of fresh solutes and/or oxygen from the overlying muds (blue arrows) coupled with higher temperatures results in an increase in sulphate reduction and methanogenesis (purple) relative to fermentation/hydrolysis (red), leading to lower organic acid and methylated amine concentrations and higher rates of terminal oxidation processes. At the peat:clay boundary (C_1) lack of groundwater flow means that the 2nd activated interface is largely inactive, though some organic acid production does occur – possibly linked with changes in peat lithology.

Chapter 8. – Cardiff Bay: Cores CB2-CB8

8.1 Site Locations and Lithology

As described in Chapter 1, Cardiff Bay is an artificial freshwater lake located on the northwest shore of the Severn Estuary, created by the impoundment of an area of former tidal mudflats by a man made barrage (Fig. 8.1). It is fed by the Taff and Ely rivers, is ≈2 km wide at its widest point and has an area of approximately 200 ha (Platt, 2002). The majority of the bay is relatively shallow with water depths averaging 2-3 m, although depths can reach ≈8 m in the centre of the bay within the former drainage channel of the River Taff. Both the rivers and the bay itself are deemed eutrophic-hypertrophic based on water chemistry and diatom assemblages (Jüttner *et al.* 2010) and the shallow nature of the bay (coupled with a rapid throughput of river water) ensures that it remains in a polymictic state with regard to stratification.

All cores from Cardiff bay were from three sites along a transect running approximately southwest-northeast across the width of the bay. Cores CB2, CB3 and CB4 were collected in February 2011; cores CB5, CB6 and CB7 in January 2012; and core CB8 was recovered in May of 2012. Cores CB2, CB5 and CB8 were from a site in the southwest of the bay (Site 1: 51° 26’ 56.3”N 03° 10’ 17.8”W, OS grid ref. ST 18680 72872) in a shallow water area between the drainage channels of the Taff and Ely rivers (approximate water depth of 3 m). Cores CB3 and CB6 were from the centre of the bay (Site 2: 51° 27” 10.9”N 03° 10’ 11.2”W, OS grid ref. ST 18814 73320) at a deeper water site within the drainage channel of the River Taff (approximate water depth = 7 m). Finally cores CB4 and CB7 were from a second shallow water site in the northeast of the bay (Site 3: 51 27’ 22.6” 03° 09’ 59.3”W, OS grid ref. ST 19050 73680) between the Taff drainage channel and the remains of the Roath Basin dredging channel (approximate water depth = 3 m).

The cores from Cardiff Bay all had a broadly similar lithology (Figs. 8.2A, B and C). The upper portions of the cores were composed of dark brown-black coloured muddy sediment, containing abundant un-degraded organic material (leaves, twigs, bones etc.) and anthropogenic detritus – characteristics of a lacustrine gyttja (Hansen, 1959; Wetzel, 1975). The surface of this unit was composed of flocculant material with evidence of extensive burrowing activity (Fig.

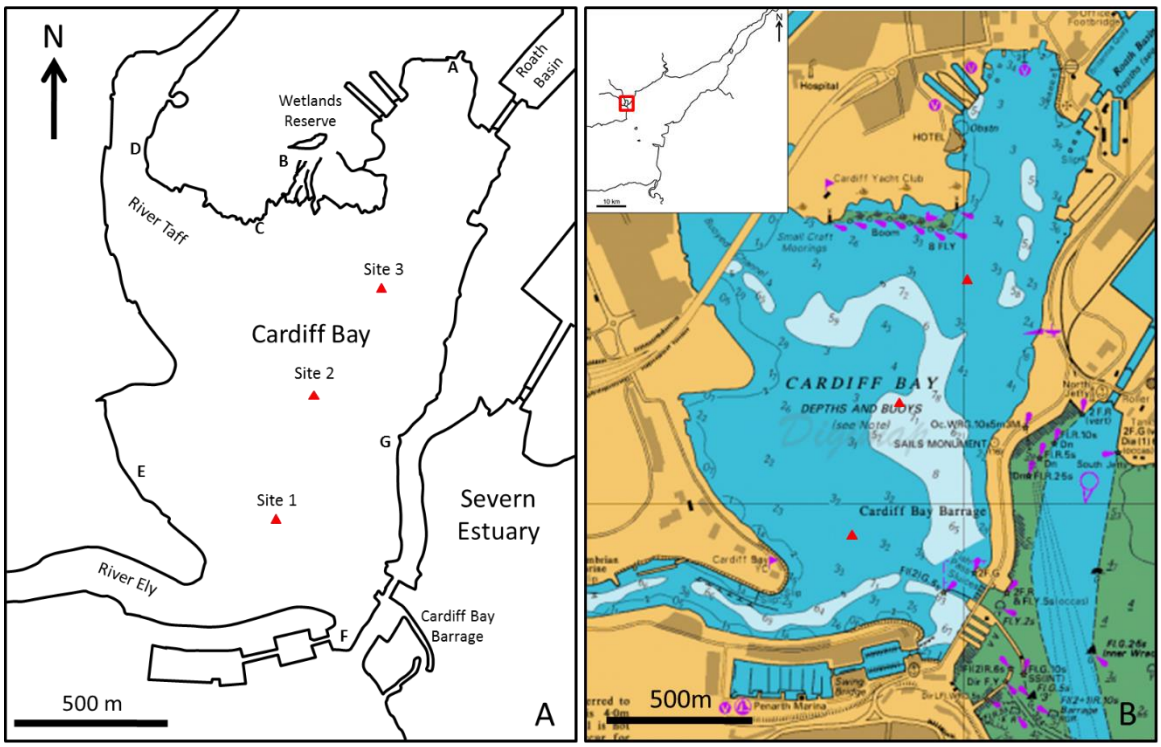


Fig. 8.1 – A) Line map showing the location of sediment sampling sites within Cardiff Bay (red triangles) along with surface water sampling sites (letters A-G). B) Bathymetric chart showing the lakebed topography of Cardiff Bay with sediment sampling sites marked (red triangles) and an inset line map showing the position if Cardiff Bay within the Severn Estuary (red box). Darker Blue indicates shallow water and lighter blue, deeper water. Green indicates intertidal areas outside of the bay. Bathymetric chart © Crown Copyright Seazone Solutions.

8.2D). The sediment surface was also occupied by a variety of invertebrate organisms (Vaughan *et al.* 2008), including chironomid larvae, gastropods, leeches and at Site 3, colonies of zebra mussels (*Driessena polymorpha*). The lower portions of the cores were made up of dense, greyish clay. This clay had no evidence of burrowing but did contain the intact and articulated shells of marine bivalves (*Cerastoderma* sp.). The boundary between these two lithologies was quite distinct, with the gyttja grading into the clay over only 2 cm. This strong demarcation, coupled with the presence of the marine derived shell material, suggests that the clay may have been deposited while Cardiff Bay was still a intertidal mudflat; and that the gyttja was deposited after the conversion to a freshwater lake; with the division between the two marking the point of barrage-closure. The proportion of gyttja to clay also varied between sites, with the interface occurring between 12-22 cm depth at Site 1, 24-25 cm depth at Site 2 and 7-12 cm depth at Site 3. This variance may reflect different rates of sedimentation at the three sites, as Site 2,

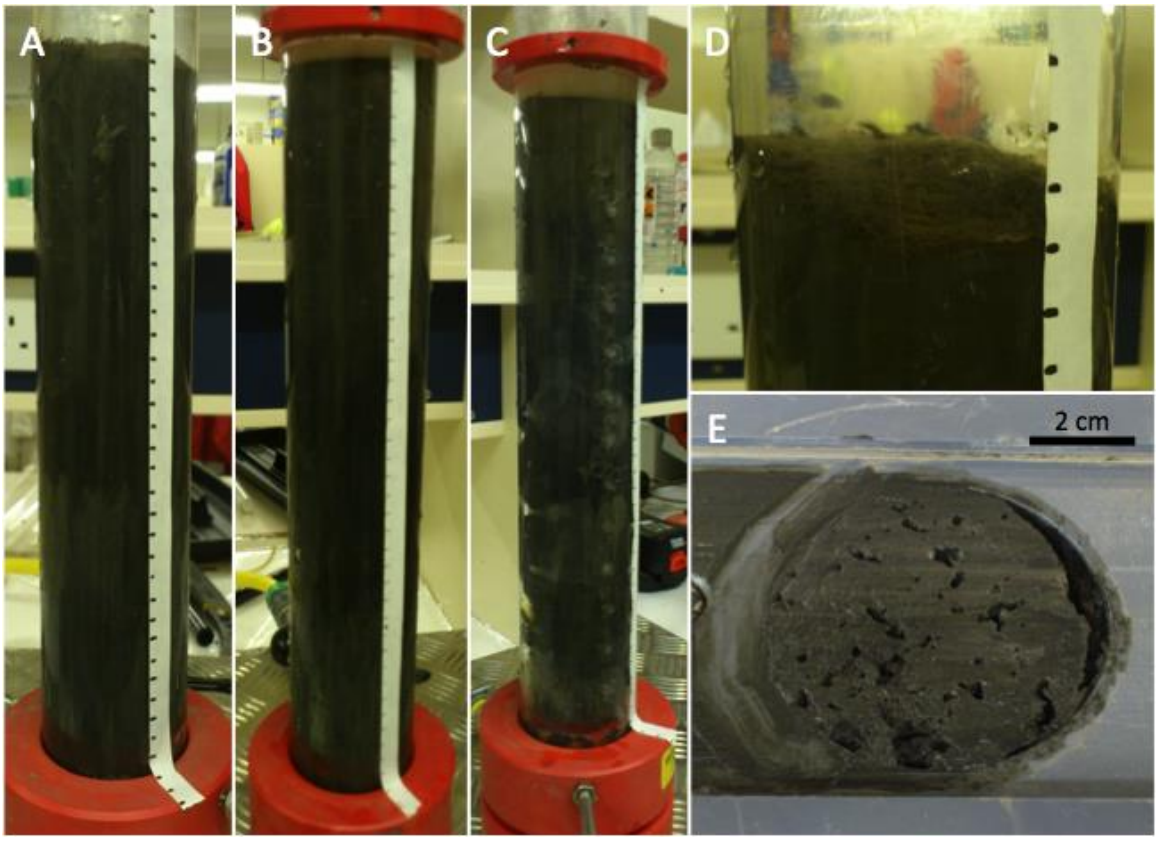


Fig. 8.2 – Photographs of cores A) CB5, B) CB6, and C) CB7 illustrating the change in lithology with depth (tape marked at 1 cm intervals for scale). Voids also be clearly seen against the plastic of the core tube in core CB7. The overlying waters of cores CB6 and CB7 are murkier than in CB5 due to the release of gas bubbles during emplacement within the sectioning rig. D) Magnified view of the surface of core CB5 showing the flocculent surface layers of sediment. E) Section through the base of core CB6 showing voids present in the sediment.

which has the highest proportion of gyttja relative to clay, sits within the Taff drainage channel and would, therefore, likely receive the largest amount of new, fluvially-derived sediment. Site 1 on the other hand is between two river channels and so would likely receive a slightly lower flux, and Site 3 is in a backwater away from any river channels, and hence would receive the least amount of fresh sediment. In addition to the two lithologies described above, core CB4 also contained a thin layer of sand (≈2cm thick) between 21-23 cm. Cores from all three sites also showed evidence of voids within the sediment (Fig. 8.2E). These voids mainly occurred within the grey clay but were also present within the gyttja at depths with particularly high amounts of un-degraded organic material. The amount and size of voids varied between cores with core CB5 having the largest concentration. These voids had no obvious connection to the surface and more than likely represent trapped gas bubbles, as during sectioning of the cores (and also

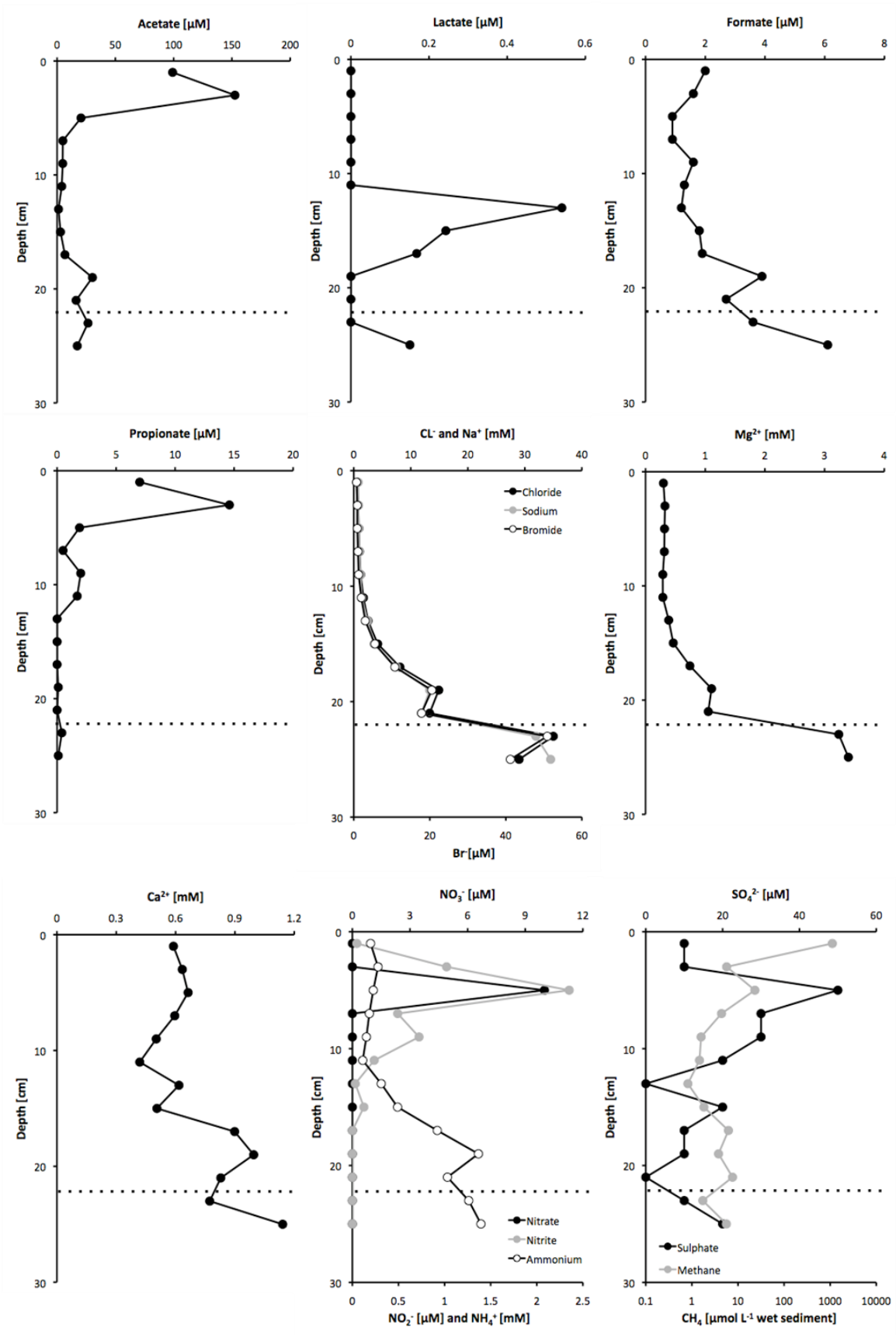
particularly during sub-coring for radiotracer analysis) the cores would often release large bubbles of gas if the subsurface sediment was disturbed.

8.2 Cardiff Bay - Results

8.2.1 Biogeochemistry of Site 1 – Cores CB2, CB5 and CB8

The pattern of the organic acid concentration profiles varied between cores. In core CB2 (Fig. 8.3), acetate decreased from a maximum of 152 μM at 1cm to a low of 1.2 μM at 13 cm before slowly rising again with depth to 17.3 μM at the base of the core. Lactate was not present in the core until 13 cm, where it occurred only at low concentrations (0.5 μM) and then decreased down to zero at 19 cm before rising again slightly to 0.15 μM at the bottom of the core. Formate had a general increase with depth from 1.6 μM at 1 cm to 6.1 μM at the base, particularly below 17 cm (similar to acetate). Propionate also exhibited a similar depth profile to acetate, decreasing from a maximum of 14.6 μM at 1 cm to zero by 11 cm and then rising slightly to 0.1-0.4 μM towards the base of the core. This bi-peaked profile pattern suggests that high levels of some acids (acetate, formate and propionate) are being produced in the upper oxidised layers of the sediment, likely as a byproduct of the decomposition of freshly deposited organic matter, and subsequently consumed by heterotrophic processes deeper in the sediment column at a faster rate than they are produced. The secondary increase within the clay layer at the base of the cores is likely driven either by fermentation of potentially marine-derived organic matter (see below) or possibly by a decrease in the activity of heterotrophic processes due to a depletion of electron acceptors with depth. Acetate and formate also exhibited localised peaks in concentration at ≈19 cm, which coincided with an increase in the amount of intact plant material present in the sediment. This material could have provided additional fermentation substrates and could also account for other changes in the geochemical profiles described below.

In core CB5 (Fig. 8.4), acetate concentrations were ≈10x lower than in CB2 but with a similar depth profile, again exhibiting an initial decrease from a maximum of 16 μM at 1cm down to a minimum of 1.7 μM at 9cm before rising to 11-15 μM in the lower part of the core. Lactate was relatively high in the overlying waters (1.8 μM) but was below detection limits in the sediment until 17 cm depth (0.25 μM),


Acetate [µM]
Lactate [µM]
Formate [µM]
Propionate [µM]
CL⁻ and Na⁺ [mM]
Mg²⁺ [mM]
Ca²⁺ [mM]
NO₃⁻ [µM]
SO₄²⁻ [µM]
Depth [cm]
Chloride
Sodium
Bromide
Br⁻[µM]
Nitrate
Nitrite
Ammonium
NO₂⁻ [µM] and NH₄⁺ [mM]
Sulphate
Methane
CH₄ [µmol L⁻¹ wet sediment]

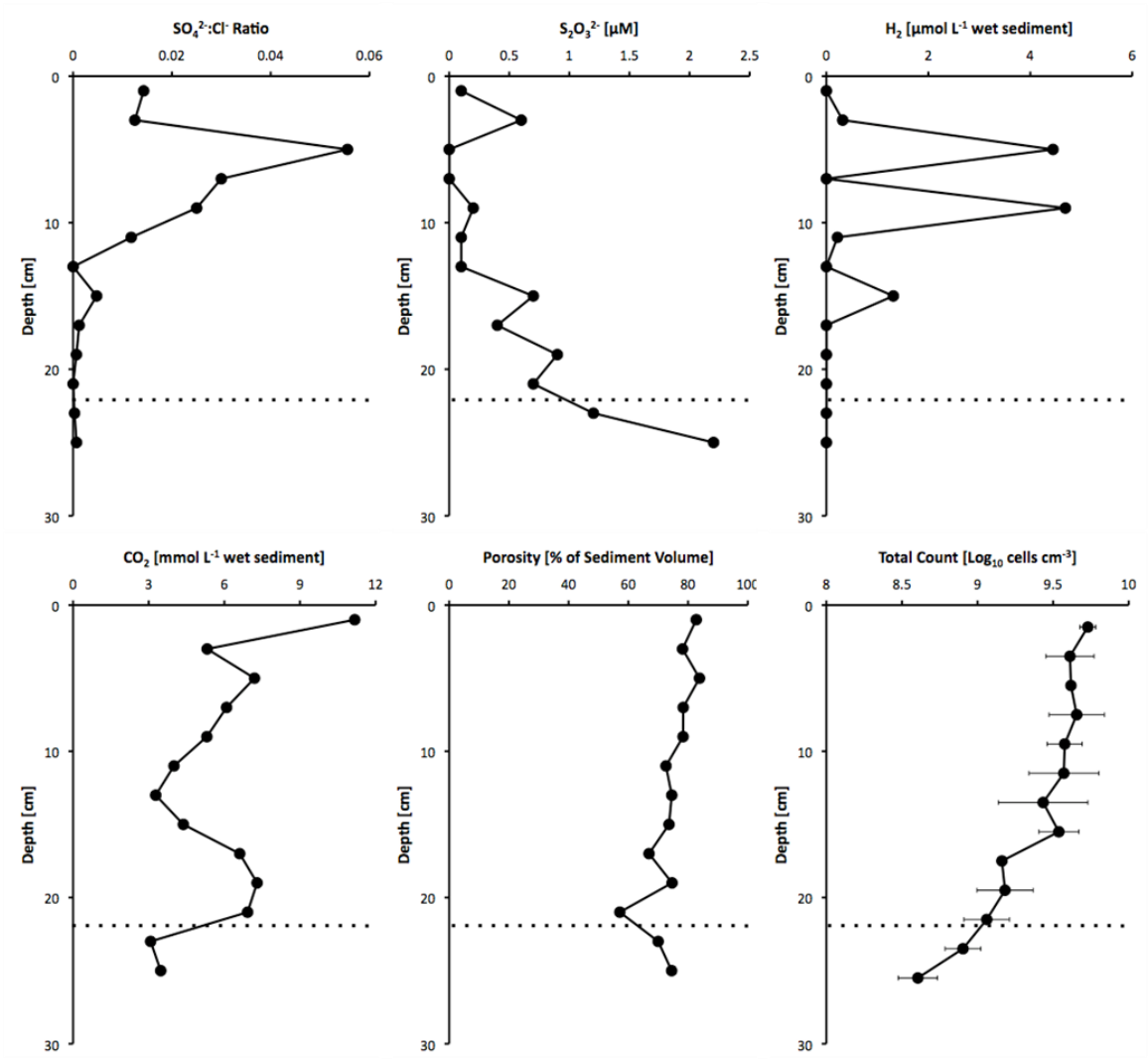


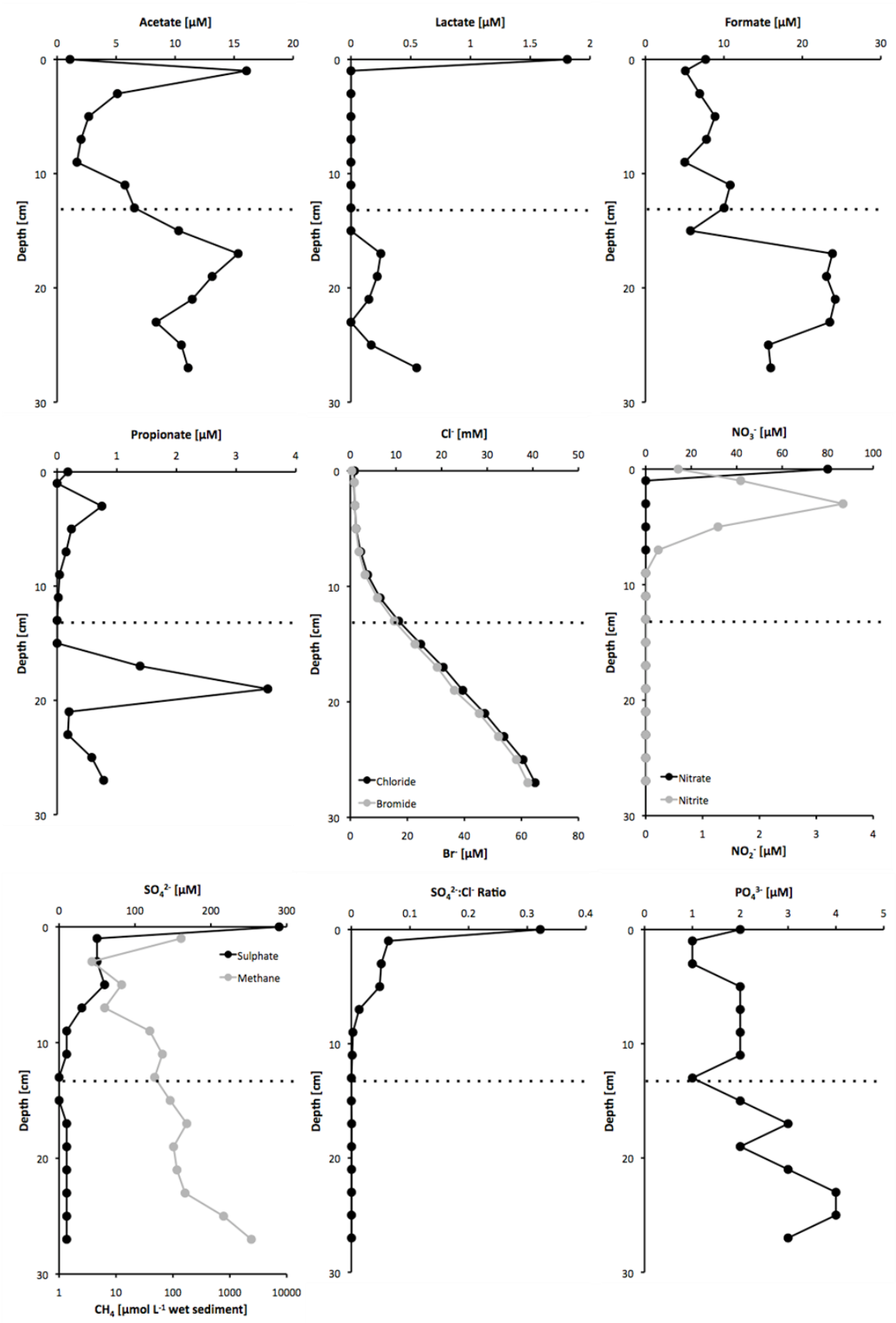
Fig. 8.3 (on this, and previous page) – Biogeochemical profiles obtained from core CB2 showing variations in the pore water concentrations of organic and inorganic solutes with depth. Also shown are sediment gas concentrations, porosity and total cell counts (AODC) - error bars represent the 95% confidence limits obtained from repeated counts. The dashed line represents the boundary between the overlying lacustrine gyttja and the grey clay.

and then rose to 0.55 µM by the bottom of the core. Formate had a generally steady concentration between 5-10 µM in the upper 15 cm of the core but below this the concentrations rose to around 23-24 µM down to 25 cm (similar to core CB2), before decreasing again to ≈15 µM. Propionate had a depth profile broadly similar to formate, with concentrations between 0.75 µM (3cm) and zero down to 15 cm before rising sharply to 3.5 µM at 19 cm and then decreasing to 0.5-0.7 µM towards the core's base. As with core CB2 these profiles again indicate production (at least of acetate) at the surface followed by heterotrophic consumption and subsequently

secondary production at depth within the clay layer via fermentation pathways (and/or a decrease in heterotrophic activity).

In core CB8 (Fig. 8.5), acetate increased with depth from 1.4 μM at 2.5 cm up to 10.5 μM at 17.5 cm and then remained relatively constant to the base of the core. Lactate was present in the overlying waters (≈2 μM) and in the sediment at 2.5 cm in this core (0.3 μM), and increased gradually with depth to 1.1 μM at the base of the core. Formate remained relatively constant with a slight increasing depth trend from 5 μM at 2.5 cm to 11.3 μM at 22.5 cm. However, below this concentrations increased markedly to 40 μM by 27.5 cm. Propionate exhibited an initial decrease with depth from 0.7 μM at 2.5 cm down to zero at 12.5 cm, it then increased rapidly to 2.4 μM by 22.5 cm before decreasing back down to 0.5 μM at the base of the core. Again this general increase with depth would indicate production via fermentation near the base of the core particularly around the transition from gyttja to clay, although compared to cores CB2 and CB5 there seems to be little production of organic acids near the surface and/or greater consumption. This may be due to the warmer temperatures in May causing the rates of heterotrophic processes to increase, allowing them to outstrip the supply of organic acids, and thus preventing a build-up near the surface (Weston and Joye, 2005).

All three cores from this site had similar exponentially increasing chloride depth profiles and concentrations, from 0.6-0.8 mM at 1 cm depth to between 29-40 mM at the base of the cores (CB2 = 25 cm CB5 = 27cm and CB8 = 27.5cm). A similar profile occurred for bromide, which increased from 0.7-1.4 μM at 1cm depth to 41-62 μM at the base of the cores; and sodium (in CB2), which rose from 0.7 mM at 1cm in core CB2 to 34 mM at the base of the core. Magnesium and calcium concentrations also increased down-core in CB2, from 0.3 and 0.6 mM respectively at 1cm depth to 3.4 and 1.1 mM respectively at the base of the core. The rate of increase varied between the compounds with magnesium having a similar exponential increase to chloride, while calcium was much more gradual. The increase in all these compounds is likely driven by the change in lithology and the differing origins of the two sediments and will be discussed later in section 8.3.3.


Acetate [µM]
Lactate [µM]
Formate [µM]
Propionate [µM]
Cl⁻ [mM]
Br⁻ [µM]
Chloride
Bromide
NO3⁻ [µM]
NO2⁻ [µM]
Nitrate
Nitrite
SO4²⁻ [µM]
CH4 [µmol L⁻¹ wet sediment]
Sulphate
Methane
SO4²⁻:Cl⁻ Ratio
PO4³⁻ [µM]
Depth [cm]

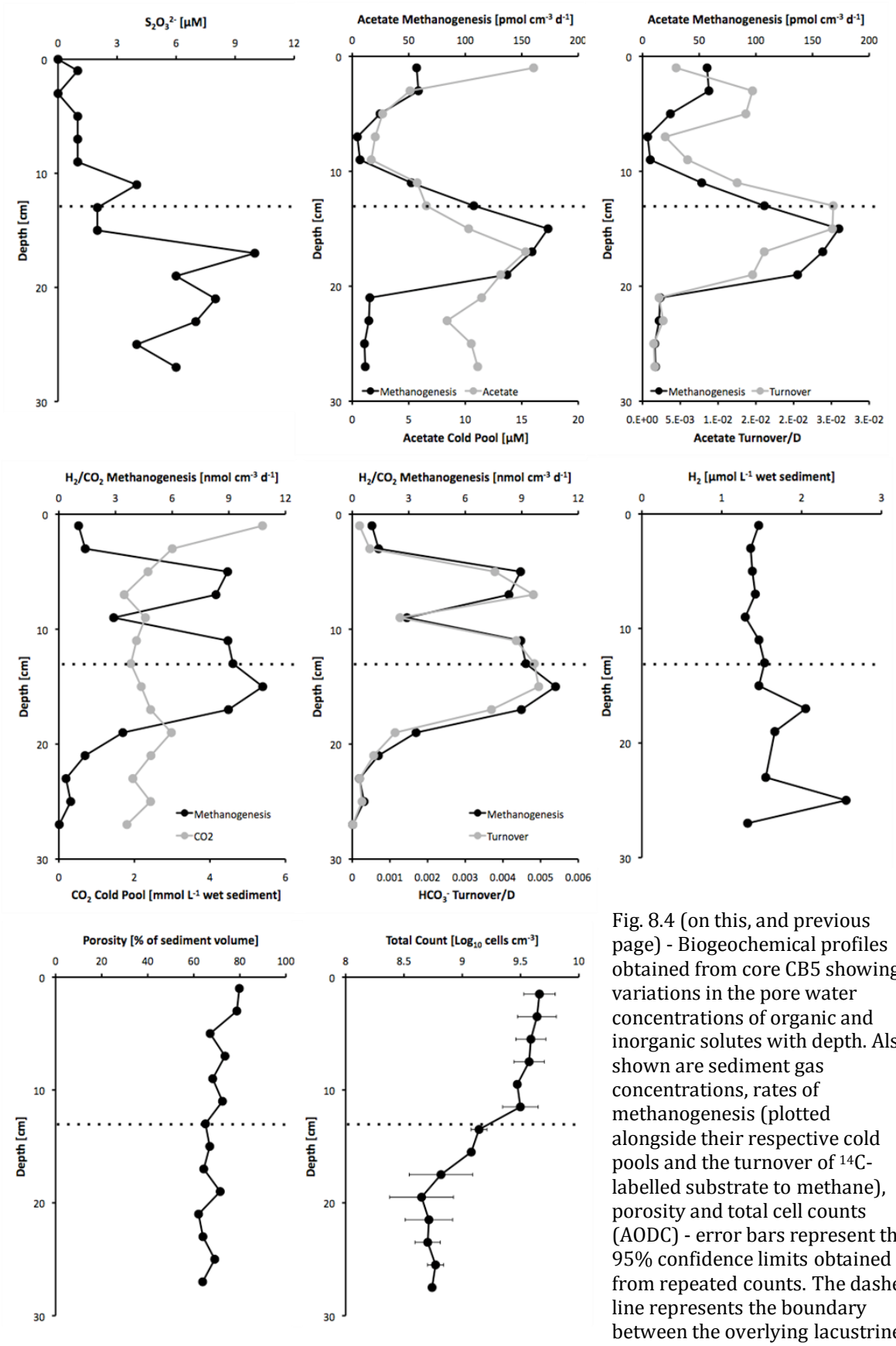


Fig. 8.4 (on this, and previous page) - Biogeochemical profiles obtained from core CB5 showing variations in the pore water concentrations of organic and inorganic solutes with depth. Also shown are sediment gas concentrations, rates of methanogenesis (plotted alongside their respective cold pools and the turnover of ^{14}C-labelled substrate to methane), porosity and total cell counts (AODC) - error bars represent the 95% confidence limits obtained from repeated counts. The dashed line represents the boundary between the overlying lacustrine gyttja and the grey clay.

Nitrate was detected in the sediment only in core CB2 at 5 cm depth (10 μM), although it was detected in the overlying waters of both CB5 (80 μM) and CB8 (60 μM), however, nitrite was detected in all three cores. In core CB2 nitrite showed an initial increase from 0.05 μM at 1 cm to 2.4 μM at 5cm before gradually decreasing to zero by 17 cm depth. A similar depth profile occurred in core CB5, with an initial increase from 1.7 μM to 3.5 μM between 1-3 cm followed by a subsequent decrease in concentration down to zero by 9 cm. Core CB8, however, had a different depth profile with nitrite concentrations in the top 15 cm being around 2-4 μM before increasing to 12.7 μM at 22.5 cm and then decreasing back to zero by 27.5 cm. In core CB2 ammonium decreased from 0.27 mM at 3cm depth to 0.11 mM at 11 cm and then consistently increased with depth up to 1.39 mM at the base of the core. This increase with depth is likely driven by the deamination/ammonification of organic matter, particularly nearer the base of the core. The increase in ammonium with depth was not linear however, instead there was a local peak around 19 cm. This corresponds with the peaks in some the organic acid profiles for this core and suggests that the increased amounts of intact organic matter at this depth might be enhancing ammonification as well as fermentation. Ammonium profiles were not obtained for cores CB5, CB6, CB7 and CB8 as the data sets for these cores were collected specifically to examine rates of active methanogenesis and sulphate reduction respectively.

Both sulphate and the SO_4^{2-}:Cl^- ratio showed the expected pattern of decreasing values with depth in all three cores indicative of dissimilatory sulphate reduction. However, in core CB2 sulphate initially increased from 10 μM at 1cm to 50 μM at 5 cm before decreasing down to 13 cm and then remaining constant, between 0-20 μM, through the rest of the core. In core CB5 sulphate concentrations in the surface waters reached 290 μM but decreased within the sediment to 50 μM at 1cm depth. Values then increased to 60 μM at 5cm, then decreased down to zero by 13 cm, and remained between zero and 10 μM throughout the rest of the core. Sulphate concentrations in core CB8 were similar, decreasing from 260 μM in the overlying water down to 10 μM at 12.5 cm, a level at which it remained constant until 27.5 cm at which point it rose back to 50 μM (possibly due to the more saline conditions at

this depth). The increase in sulphate near the surface in cores CB2 and CB5, occurs above the colour transition from brown to black (at 6 and 5 cm respectively), and therefore, is likely driven by the re-oxidation of sulphide in the upper most oxidised layers of the sediment. This colour change also matches a change in the slope of the sulphate and SO_4^{2-}:Cl^- profiles demonstrating that sulphate reduction was more active beneath this oxidised-reduced boundary. The occurrence of sulphide production in the sediment would be hard to gauge by darkening of the sediment as the sediment was already dark in colour, probably due to it's high organic content. However, the fact that free phosphate was present at near the base of both cores CB5 and CB8 (and that it was increasing with depth) suggests that FeS formation was occurring (Rozan *et al.* 2002) (although no phosphate was detected in core CB2). With regard to the SO_4^{2-}:Cl^- ratio, in core CB2 it shows a distinct change in slope at 13 cm. This corresponds with the lactate peak described above and may indicate that some uncoupling in the production and consumption of organic acids was occurring at this depth probably due to sulphate limitation of sulphate reduction and a subsequent change in the terminal-oxidising communities (Jørgensen and Parkes, 2010; Parkes *et al.* 2012).

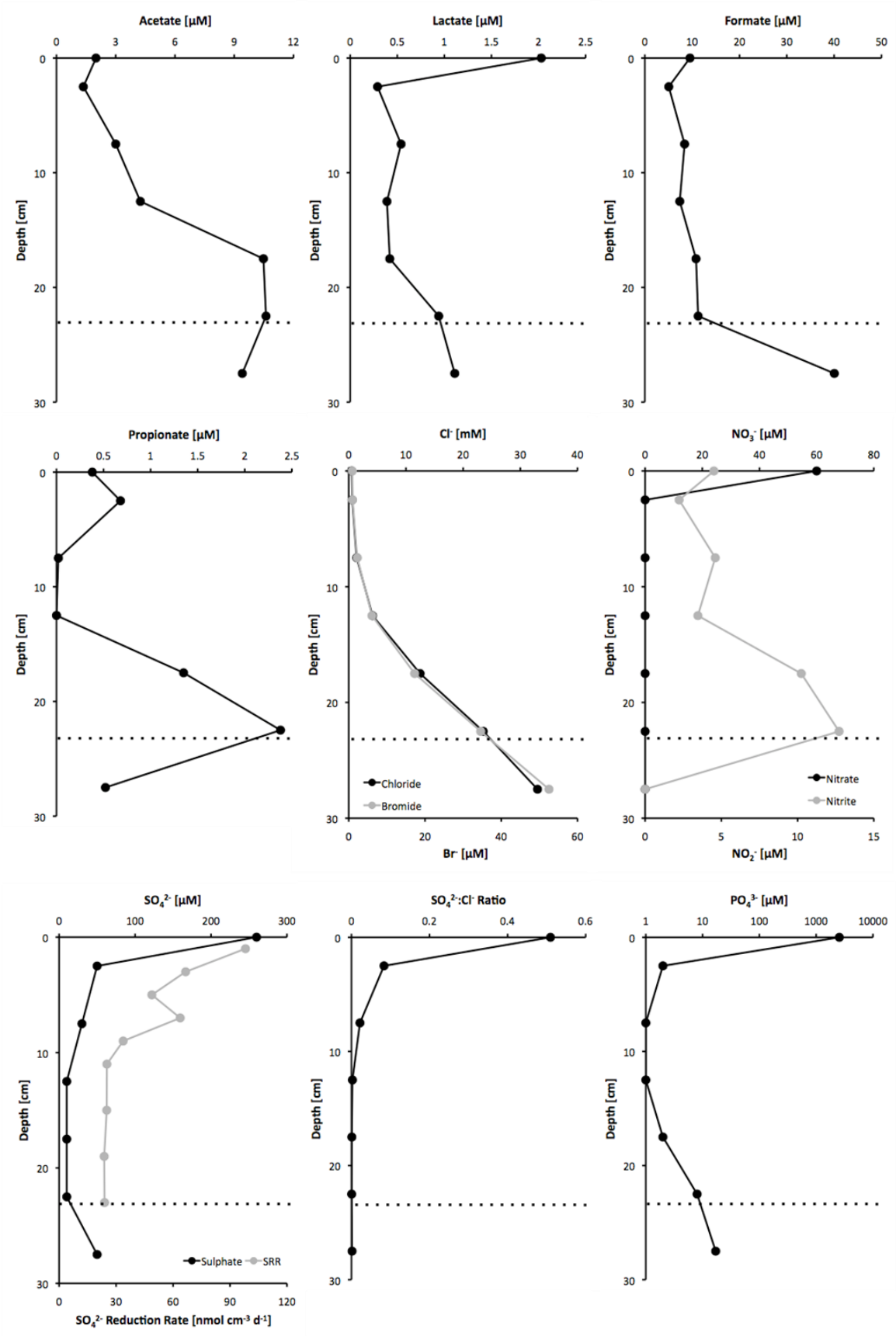
In addition to measuring *in situ* pore water concentrations, active sulphate reduction rate (SRR) measurements were also carried out on core CB8 using $^{35}SO_4^{2-}$ radiotracers. The results showed that despite the low levels of sulphate in Cardiff Bay compared to other sites in this study, the rate of sulphate reduction in the sediments was high, with rates peaking at 98 nmol cm^{-3} d^{-1} in the top 1 cm and decreasing with depth (following the decrease in sulphate) down to 24 nmol cm^{-3} d^{-1} at 23 cm, producing a depth integrated SRR of 9.6 mmol m^{-2} d^{-1}.

Thiosulphate was also present within the cores, showing a general trend of increasing concentrations with depth, particularly below 15 cm. In core CB2 $S_2O_3^{2-}$ increased from 0.1 μM at 1 cm depth to 2.2 μM at the base of the core. A similar increase was found in CB5 where concentrations rose from 1 μM at 1cm to 10 μM at 17cm, before falling back down to 6 μM at the base of the core. Finally in CB8 concentrations increased from 2 μM at 7.5 cm up to 53 μM at the core's base. The reason for these increases with depth is unknown but may relate to the changes in lithology and salinity present in the cores as the largest increases in concentration

seem to occur beneath the gyttja-clay boundary or to the oxidation of sulphide at depth (Aller and Rude, 1987; Jørgensen and Bak, 1991; Marzocchi *et al.* 2014).

Methane was present at very high concentrations (1100 μmol l^{-1} of wet sediment) in the top 1cm of core CB2, although below this concentrations decreased to between 1-6 μmol lws^{-1} throughout the rest of the core. In core CB5 methane concentrations also initially decreased (from 139 μmol lws^{-1} at 1cm to 3.76 μmol lws^{-1} at 3cm), however, when sulphate began to be depleted below 5 cm, methane concentrations began to increase with depth, up to a maximum of 2400 μmol lws^{-1} at the base of the core. In core CB8 the methane profile was similar to the acetate profile, increasing with depth from 12.6 μmol lws^{-1} at 2.5 cm up to 929 μmol lws^{-1} at 22.5 cm before dropping back down to 338 μmol lws^{-1} at the base of he core. However, in replicate cores taken at the same time as CB8, methane concentrations reached as high as 3600-4900 μmol lws^{-1} at 22.5 cm depth, suggesting intense methanogenic activity at depth.

Rates of methanogenesis, as determined by radiotracers in core CB5, varied greatly throughout the core. For acetoclastic methanogenesis, in the top 3 cm of the core the rate was around 57-59 pmol cm^{-3} d^{-1}. Below this rates dropped to a low of 4.5 pmol cm^{-3} d^{-1} at 7cm before rising again to a high of 173 pmol cm^{-3} d^{-1} at 15 cm and then decreasing to 15 pmol cm^{-3} d^{-1} at 21 cm, a level which remained fairly constant down to the base of the core. These results correspond well to the acetate profile described above and produce a depth integrated rate of 16.7 μmol m^{-2} d^{-1}. Despite Cardiff Bay being a freshwater environment, where acetoclastic methanogenesis is usually considered to be dominant (Whiticar *et al.* 1986), chemolithotrophic H_2/CO_2 methanogenesis was also occurring at high levels in core CB5. Rates showed a distinct increase with depth below the brown-black redox transition (5 cm), with maximum rates (10.8 nmol cm^{-3} d^{-1}) occurring between 5-17 cm before decreasing again towards the base of the core. As such, the depth integrated rate of 1.33 mmol m^{-2} d^{-1} was much higher than that for acetoclastic methanogenesis. The reason for the apparent decrease in rates for both methanogenic pathways below 21 cm is not known as the decrease is observed in both the rate and turnover (to methane) profiles indicating that the decrease is not just due to decreasing substrate concentrations. Interestingly the profiles obtained


Acetate [µM]
Lactate [µM]
Formate [µM]
Propionate [µM]
Cl⁻ [mM]
NO3⁻ [µM]
SO4²⁻ [µM]
SO4²⁻:Cl⁻ Ratio
PO4³⁻ [µM]
Depth [cm]
Chloride
Bromide
Br⁻ [µM]
Nitrate
Nitrite
NO2⁻ [µM]
Sulphate
SRR
SO4²⁻ Reduction Rate [nmol cm⁻³ d⁻¹]

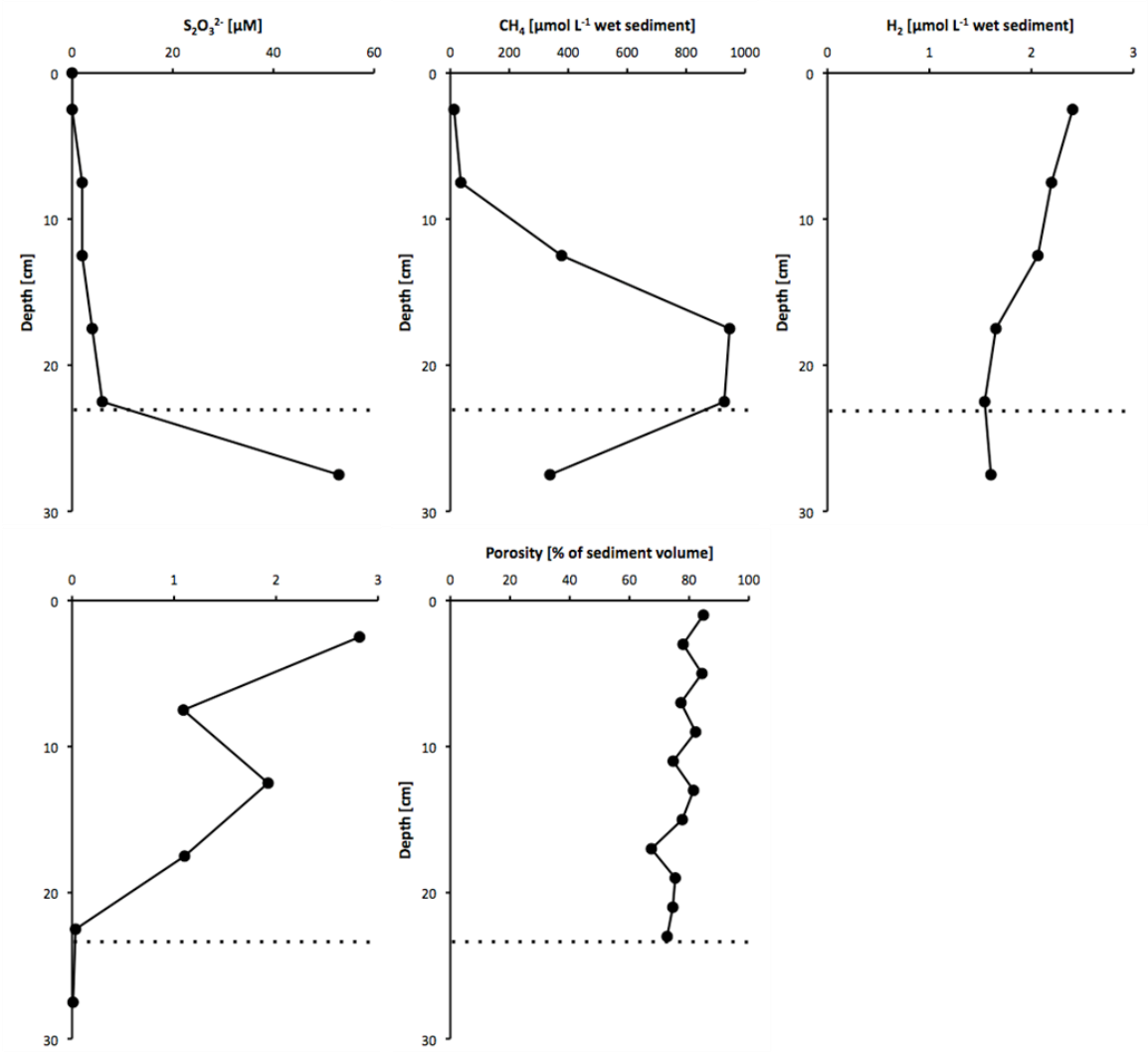


Fig. 8.5 – Biogeochemical profiles obtained from core CB8 showing variations in the pore water concentrations of organic and inorganic solutes with depth. Also shown are sediment gas concentrations, rates of sulphate reduction (SRR), porosity and total cell counts (AODC) - error bars represent the 95% confidence limits obtained from repeated counts. The dashed line represents the boundary between the overlying lacustrine gyttja and the grey clay.

for active methanogenesis also do not seem to correlate with the *in situ* methane profile, with the largest increase in methane values (from ≈100 µmol lws^{-1} to 2400 µmol lws^{-1}) occurring below 21 cm, a depth where methanogenesis rates appear to be low. This would indicate that either the methanogens producing these high methane levels are utilising substrates other than the two measured here (such as methylated amines or methanol) (Lovley and Klug, 1983), or that the sediments at this locality are able to store large amounts of methane without it being degraded or mobilized over time (possibly due to the low sulphate and nitrate concentrations limiting AOM).

Hydrogen concentrations in core CB2 were quite variable, however, they do show an initial increase with depth (from zero at 1cm depth to 4.68 μmol lws^{-1} at 9cm) and then a subsequent decrease back down to zero by 17 cm. This could suggest a zone of production via fermentation centered around the redox boundary at 6cm, followed by consumption by processes such as sulphate reduction in the sediments below. In core CB5 hydrogen concentrations were relatively stable (around 1.5 μmol lws^{-1}) down to 15 cm, below which they were more variable, reaching a maximum concentration of 2.6 μmol lws^{-1} at 25 cm. This increase matches with the changes in the organic acid profiles and again suggests a possible zone of enhanced fermentation below the gyttja-clay interface. Core CB8 on the other hand shows a gradual decrease in hydrogen from 2.4 μmol lws^{-1} at 2.5 cm down to 1.2 μmol lws^{-1} at the base of the core possibly indicating that hydrogenotrophic processes (such as sulphate reduction) were more active at depth than hydrogen-producing processes at the time of year when this core was taken.

CO_2 concentrations in core CB2 decreased initially with depth, from 11 mmol lws^{-1} at 1 cm to 3.3 mmol lws^{-1} at 13 cm. Below this there was a zone of increasing concentrations, reaching a maximum of 7.3 mmol lws^{-1} at 19 cm before decreasing again towards the base of the core. This increase around 19 cm again coincides with the increase in plant material observed at this depth further indicating an enhancement in fermentative metabolism and OC degradation. In core CB5 CO_2 values show a similar pattern, decreasing from 5.4 mmol lws^{-1} at 1 cm 1.7 cm at 7 cm, possibly driven by the increasing rates of H_2/CO_2 methanogenesis around the redox transition at 6 cm. Below 7 cm concentrations remain relatively stable, except around 19 cm where there is a slight peak possibly related to fermentation and the higher levels of organic acids at this depth. Finally in core CB8 CO_2 concentrations decreased from 2.8 mmol lws^{-1} at 2.5 cm down to 1.1 mmol lws^{-1} at 7.5 cm. Concentrations then increased again to a localised peak at 12.5 cm before decreasing again down to zero by 22.5 cm. This drastic decrease would seem to indicate high levels of autotrophic processes occurring at depth – possibly relating to H_2/CO_2 methanogenesis and/or homoacetogenesis considering the other profiles. However, as with methane, the duplicate cores from this site contained higher levels of CO_2 near their base (up to 4.4 mmol lws^{-1}) suggesting that gas concentrations in these

sediments may be quite heterogeneous (possibly due to the presence of gas bubbles – discussed in section 8.3.6), making the overall link between activity and gases within these sediments difficult to determine.

Total counts of cell numbers were high in the near surface and then decreased gradually with depth in core CB2, from $1x10^{9.7}$ cells cm^{-3} at 1cm down to $1x10^{9.5}$ at 15.5 cm, then began to decrease more sharply down to $1x10^{8.6}$ at the base of the core. The initial decrease below 15.5 cm may be due to the presence of the higher levels of vegetative matter at this depth as (in a similar fashion to core StB2) this hindered accurate AODC counting procedures. The even sharper decrease in cell numbers below 21.5 cm depth was then likely caused by the transition to the older, and more OC poor sediments in the clay layer. Core CB5 had a similar depth profile with cell numbers gradually decreasing between $1x10^{9.6}$-$1x10^{9.5}$ cm^{-3} in the top 11.5 cm of the core before dropping sharply to $1x10^{8.7}$ at 19.5 cm, a level that then remained constant down to the base of the core again likely relating to the transition from higher OC gyttja to lower OC clay.

Porosity also decreased down-core in CB2 from 82.7% at 1cm to 74.5% at the base of the core, with a sharp drop down to 57.1% found at 21 cm. Core CB5 also showed a gradual decrease in porosity with depth, from 79.7% at 1cm down to 63.8% at the core's base, as did CB8, from 84.7% at 1cm down to 72.6% at the base of the core. Oxygen uptake measurements were also carried out on cores CB5, CB6 and CB7 with CB5 having the highest rate of the three sites over an 8 hour incubation with a an uptake rate of 61 mmol m^{-2} d^{-1}.

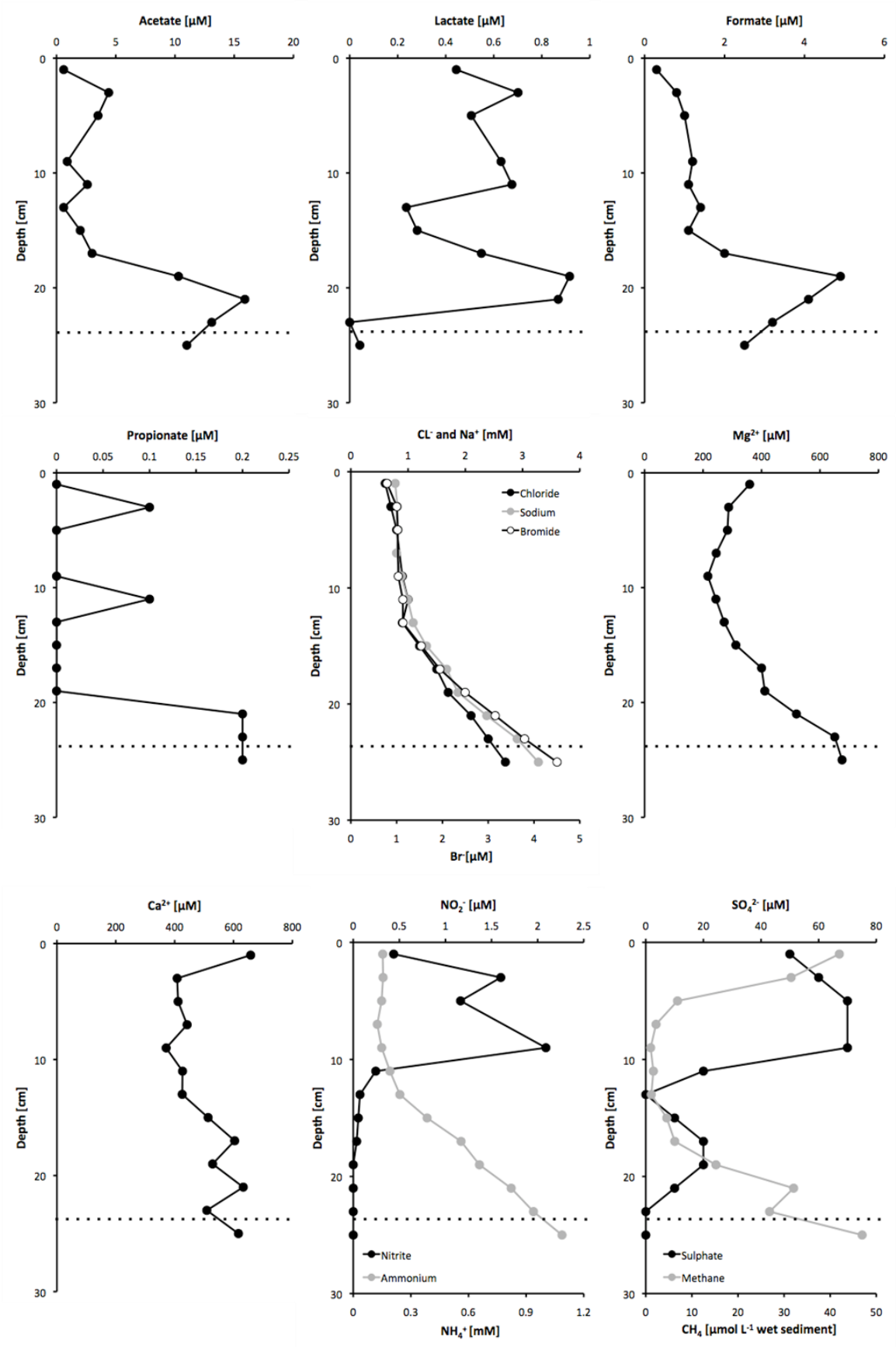
8.2.2 Biogeochemistry of Site 2 – Cores CB3 and CB6

In core CB3 (Fig. 8.6), acetate remained relatively stable in the top 17 cm of the core with values ranging between 0.4-4 μM, below this values increased sharply to a maximum of 15.9 μM at 21 cm before decreasing down to 11 μM at the base of the core. Lactate concentrations were low and generally consistent over the top 11 cm of the core, ranging from 0.4-0.7 μM. Below this, concentrations decreased sharply to 0.2 μM at 13 cm before increasing (with acetate) to a maximum of 0.9 μM at 19 cm, and then subsequently decreased again to 0.04 μM into the clay layer at base of

the core. The formate concentration profile was similar to that of acetate, although concentrations were much lower (≈10x). Formate concentrations gradually increased from 0.3-1 μM in the top 15 cm, then rose rapidly to 4.9 μM at 19 cm before decreasing to 2.5 μM at the core's base. Propionate, despite only being detected in isolated peaks in the top 19 cm of the core, like the other organic acids increased significantly below 20 cm. This increase in compounds with depth suggests the presence of a zone of enhanced fermenter activity close to the gyttja-clay boundary. As in core CB2 this can be linked to an observed increase in relatively intact plant material at this depth that could provide ample OC substrates for the fermenters responsible for producing these compounds.

In core CB6 (Fig. 8.7), the organic acid depth profiles were similar to CB3. Acetate concentrations below ≈10 cm increased with depth and showed two distinct peaks at 13 cm and 21 cm (and a maximum of 19.4 μM) before decreasing again toward the base of the core. Lactate concentrations were relatively high in the overlying waters (3.2 μM) and in the sediment peaked at 13 cm (2 μM), but otherwise remained between 0.05-0.5 μM in the rest of the core. Formate also had a similar depth profile to acetate, with peaks at 13 cm (maximum concentration 18.1 μM) and 23 cm before decreasing to 6.6 μM at the core's base. Finally propionate, despite low concentrations (>0.6 μM) also shows a similar depth profile, though more spikey, with peak values at 15 cm 21 cm and 25 cm before decreasing at the base of the core. This prevalent bi-peaked profile would suggest two distinct zones of enhanced fermentation, one at 13-15 cm and the other at 21-25 cm. The upper of these zones seems to coincide with the switch over from sulphate reduction to methanogenesis (see below), and therefore, again may relate to the uncoupling of the production and consumption of organic acids due to the change in terminal-oxidising communities (and the concomitant decrease in cell numbers). The lower zone on the other hand may be linked to an increase in intact plant material just above the gyttja-clay boundary, which would have provided increased amounts of OC substrates for the fermenters and may have (either directly or indirectly) been affecting other processes that will be discussed below.

Chloride concentrations in core CB3, like cores from Site 1, increased with depth but to a much lesser extent. In the top 15 cm concentrations remained fairly constant


Acetate [µM]
Lactate [µM]
Formate [µM]
Propionate [µM]
CL⁻ and Na⁺ [mM]
Chloride
Sodium
Bromide
Br⁻[µM]
Mg²⁺ [µM]
Ca²⁺ [µM]
NO₂⁻ [µM]
Nitrite
Ammonium
NH₄⁺ [mM]
SO₄²⁻ [µM]
Sulphate
Methane
CH₄ [µmol L⁻¹ wet sediment]
Depth [cm]

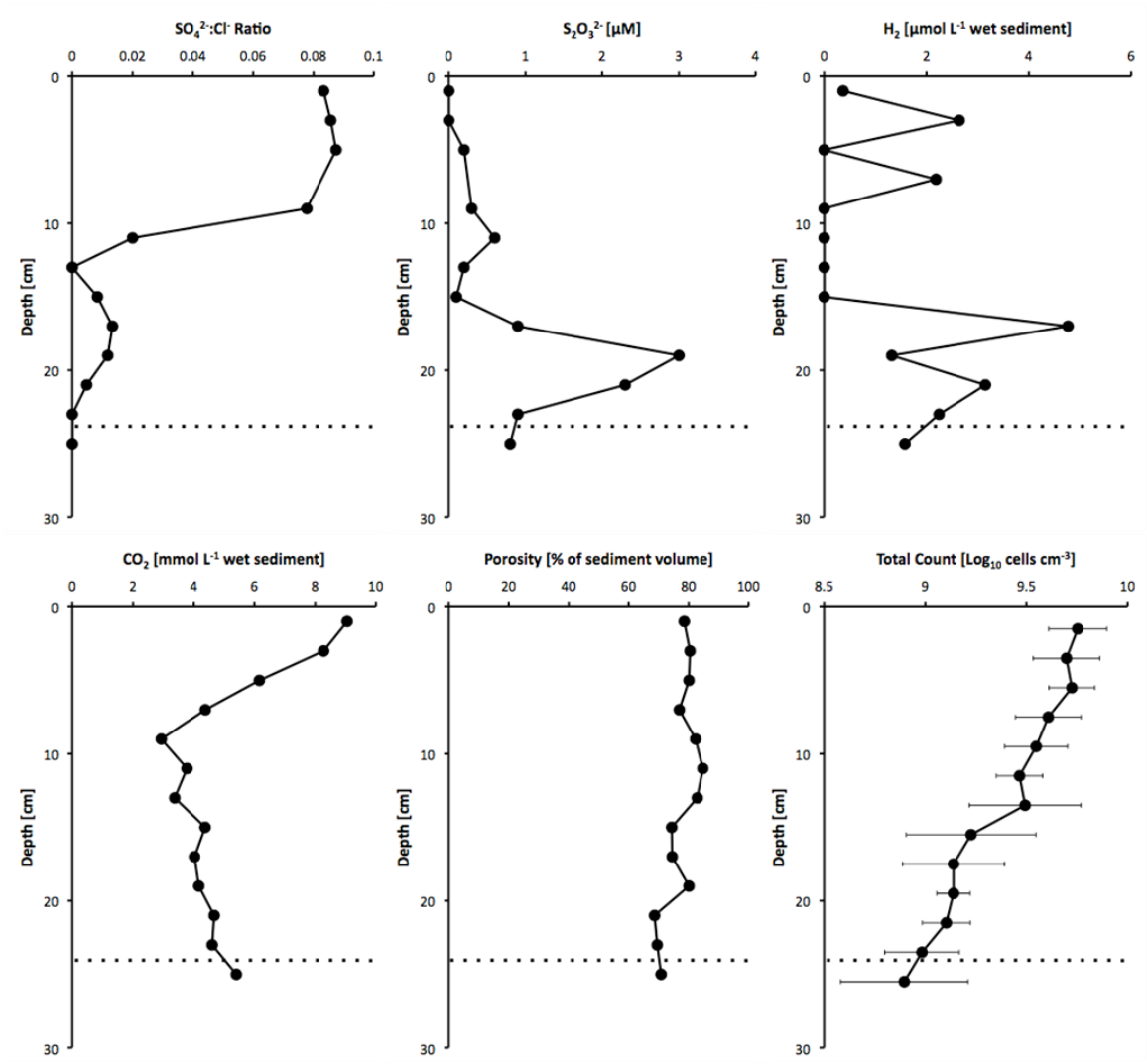


Fig. 8.6 (on this, and previous page) - Biogeochemical profiles obtained from core CB3 showing variations in the pore water concentrations of organic and inorganic solutes with depth. Also shown are sediment gas concentrations, porosity and total cell counts (AODC) - error bars represent the 95% confidence limits obtained from repeated counts. The dashed line represents the boundary between the overlying lacustrine gyttja and the grey clay.

between 0.6-0.9 mM, but below this concentrations increased up to 2.7 mM at the the base of the core (25 cm depth). The core CB6 chloride depth profile was very similar but reached much higher concentrations. In the top 13 cm of the core concentrations slowly rose from 0.7-2.5 mM, while below this the chloride concentration increased markedly to 32.6 mM at the core's base (31 cm depth) – similar to the values obtained from Site 1. Bromide concentrations were also low in CB3, again slowly increasing in the top 15 cm (0.7-1.1 µM) before increasing sharply to 4.5 µM by the base of the core. Similarly, in CB6 bromide rose slowly in the top 13 cm (0.9-4.1 µM), then increased rapidly to 61 µM at 31 cm. Sodium had a similar

depth profile to chloride in CB3, between 0.7-1 mM in the top 15 cm before increasing to 3.2 mM at the core's base. Magnesium and calcium depth profiles in CB3 both exhibited a similar pattern, initially dropping from 300 μM and 600 μM respectively at 1 cm to minima of 210 μM and 370 μM at 9 cm before increasing again to 670 μM and 610 μM respectively at the base of the core.

Nitrate was not detected in core CB3 however, it was present in CB6 in the overlying waters (100 μM) and at very low concentrations (10 μM) in the upper 5 cm of sediment, beneath which, concentrations were below detection (likely due to denitrification/DNRA). Nitrite, however, was again present in both cores. In core CB3 concentrations increased from 0.4 μM at 1cm to a maximum of 2 μM at 9cm before decreasing sharply to zero by 19 cm. In core CB6 nitrite again increased from 1.1 μM at 1 cm to a peak of 4.5 μM at 3 cm before dropping off sharply and was below detection by 13 cm. This peak coincided with the change in sediment colour indicating that the sediments above 3 cm were more oxidising, which might suggest that the nitrite peak may relate to nitrification of ammonium as well as denitrification/DNRA.

The ammonium depth profile in core CB3 was the opposite of the nitrite profile, remaining between 0.12-0.16 mM in the top 11 cm before a linear increase to 1 mM at the base of the core, demonstrating that ammonification was occurring at depth. The concave upward shape of the profile (combined with the peak in nitrite values) around 9-15 cm might also indicate that nitrification was occurring, a possibility expanded upon in section 8.3.4.

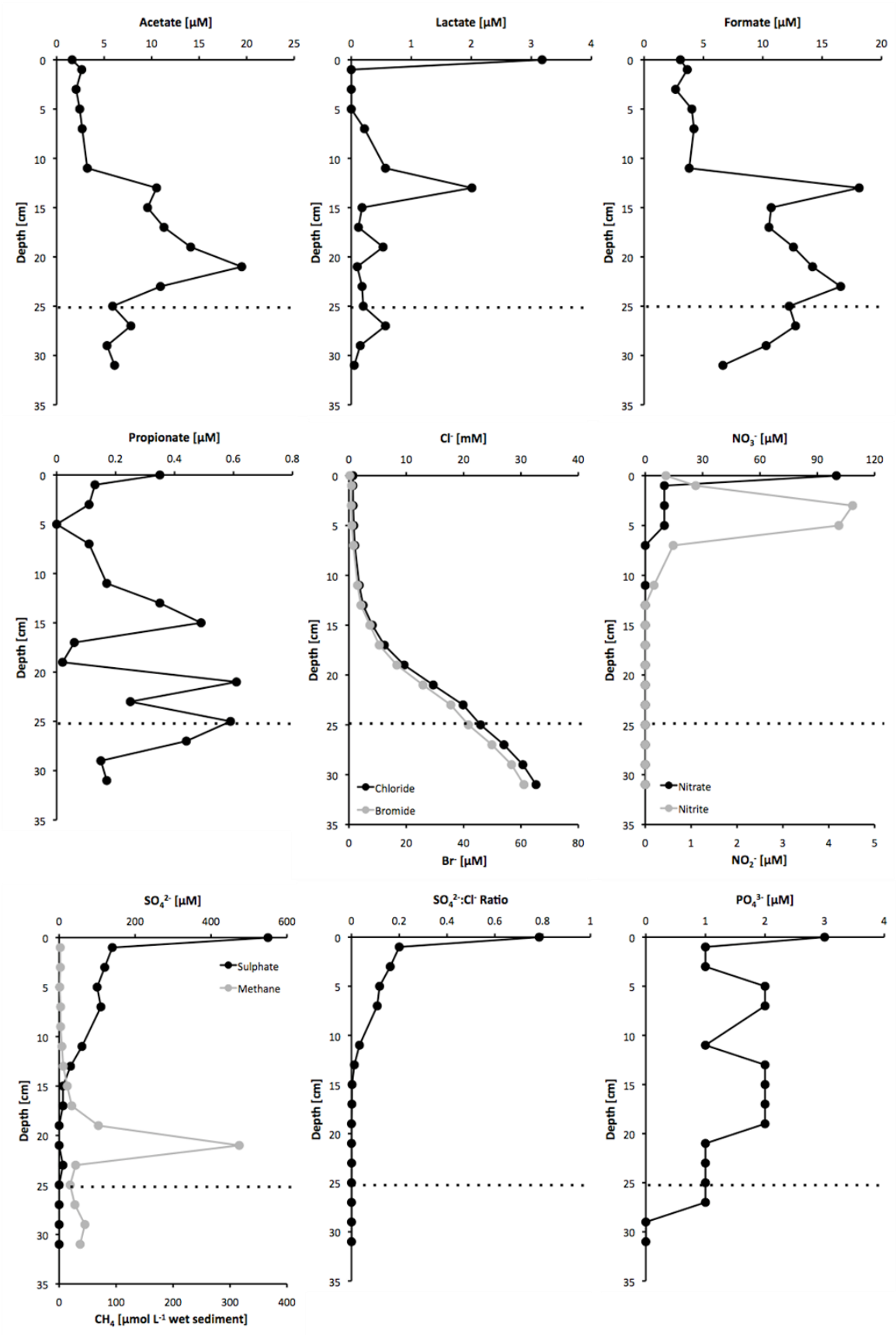
Sulphate concentrations and SO_4^{2-}:Cl^- ratios initially increased with depth in core CB3, from 50 μM at 1 cm to 70 μM at 5cm, indicating sulphide oxidation. This would correlate with the nitrite and ammonium concentration profiles in this core and suggest that dysoxic conditions were present down to at least 9 cm depth. This is an unusual occurrence in eutrophic lake sediments (Sass *et al.* 2003), but possible if the sediment was disturbed by bioturbation or water movement. Below this depth concentrations then decreased sharply to between zero and 20 μM (17cm) to the base of the core, demonstrating that sulphate reduction was occurring at this site – particularly between 9-13 cm. In core CB6 both sulphate and SO_4^{2-}:Cl^- values

decreased sharply, from 550 μM in the overlying waters to 140 μM at 1cm, indicating intense sulphate reduction in the top 1 cm of sediment. Sulphate removal continued with depth with values decreasing to 10 μM at 15 cm and then staying between 0-10 μM down to the base of the core.

The concentration of thiosulphate in core CB3 increased slowly with depth from zero at 1cm to 15 cm, then increased sharply to a maximum of 2.3 μM at 19 cm before decreasing to 0.8 μM at the core bottom. In core CB6 thiosulphate had a similar pattern, increasing from zero at 1cm to a peak of 6 μM at 21 cm before decreasing sharply to 2 μM towards the core's base. In both these cores the thiosulphate profiles appear to closely reflect the profiles of the organic acids (particularly acetate and formate) though the reason for this similarity is unclear.

Similar to Site 1 described above, methane was present in the top 1 cm of core CB3 although at much lower concentrations (41.9 μmol lws^{-1}) than previously described. The concentrations then decreased rapidly to a minimum of 1.05 μmol lws^{-1} at 9cm before exponentially increasing to 46.9 μmol lws^{-1} at the core base, indicating methanogenesis at both the top and bottom of the core. This lower zone of methanogenesis again coincides with the increases in intact plant material and organic acid concentrations suggesting that the increased acetate concentrations derived from the fermentation of the plant material could be enhancing methanogenesis. In addition the breakdown of the plant material could be releasing other substrates (such as methylated amines) that could also be used by the methanogens, although none were detected – possibly due to their rapid turnover (Parkes *et al.* 2012). The methane profile between 11-19 cm also exhibits a concave-upward shape that can be indicative of AOM (Iversen and Jørgensen 1985). This also correlates with decrease in sulphate values at 13 cm indicating a potential enhancement of sulphate reduction linked to methane oxidation.

By contrast, in core CB6 only very low levels of methane (1.95 μmol lws^{-1}) were present at the surface. However, the concentration then increased gradually with depth to 36.8 μmol lws^{-1} at the core bottom. Superimposed on this gradual increase was a local peak of 316 μmol lws^{-1} at ≈21 cm. As in core CB3, this peak coincides with the increase in plant material and some organic acids at depth,


Acetate [µM]
Lactate [µM]
Formate [µM]
Propionate [µM]
Cl⁻ [mM]
NO3⁻ [µM]
SO4²⁻ [µM]
SO4²⁻:Cl⁻ Ratio
PO4³⁻ [µM]
Depth [cm]
Chloride
Bromide
Br⁻ [µM]
Nitrate
Nitrite
NO2⁻ [µM]
Sulphate
Methane
CH4 [µmol L⁻¹ wet sediment]

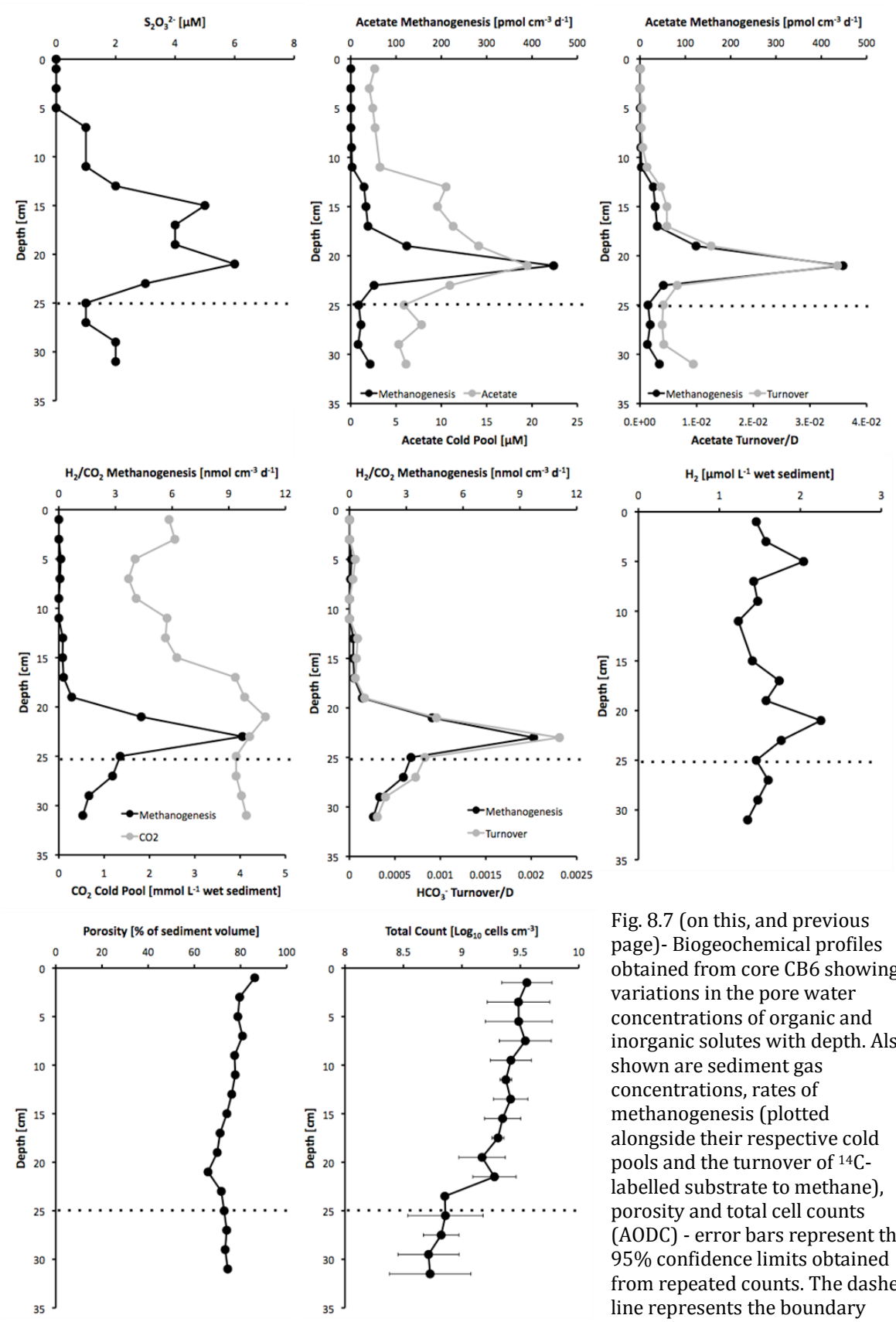


Fig. 8.7 (on this, and previous page)- Biogeochemical profiles obtained from core CB6 showing variations in the pore water concentrations of organic and inorganic solutes with depth. Also shown are sediment gas concentrations, rates of methanogenesis (plotted alongside their respective cold pools and the turnover of ^{14}C-labelled substrate to methane), porosity and total cell counts (AODC) - error bars represent the 95% confidence limits obtained from repeated counts. The dashed line represents the boundary between the overlying lacustrine gyttja and the grey clay.

suggesting an increase in methanogenic substrates and activity in this zone. This was confirmed by the radiotracer results which showed acetoclastic methanogenesis rates were low in the top 11 cm of core CB6, ranging from 0.1-3.1 pmol cm^{-3} d^{-1}, but then rose sharply to a maximum of 448 pmol cm^{-3} d^{-1} at 21 cm before decreasing to 43 pmol cm^{-3} d^{-1} at 31 cm (the depth integrated rate was 16.6 μmol m^{-2} d^{-1}). Acetate turnover (to methane) values matched this profile, demonstrating that there was an increase in activity independent of the increase in acetate concentrations. H_2/CO_2 methanogenesis followed a similar pattern, with rates low in the top 19 cm. Below this rates increased dramatically to a peak of 9.7 nmol cm^{-3} d^{-1} at 23 cm before decreasing again towards the base of the core (the depth integrated rate was 491 μmol m^{-2} d^{-1}, much higher that for acetoclastic methanogenesis). This would indicate that the increase in intact plant material below 20 cm might also be stimulating lithotrophic methanogenesis, as the breakdown of this organic material could be producing elevated levels of H_2 and CO_2 (see below) that could be utilised by the lithotrophic methanogens. Finally, the methane profile in core CB6 exhibits a concave-upward shape between 13-19 cm indicating that methane is being consumed. Since sulphate is also present around this depth and is also being consumed this may again indicate that AOM is occurring at this depth.

In core CB3 hydrogen concentrations in the top 9cm fluctuated between zero and 2.6 μmol lws^{-1} before falling below detection limits between 9-15 cm. Concentrations then increased to a peak of 4.7 μmol lws^{-1} at 17cm before decreasing to 1.5 μmol lws^{-1} at the core base. This decrease in values below 9 cm corresponds to the highest rate of sulphate removal (as shown on the sulphate and SO_4^{2-}:Cl^- profiles) suggesting that increased hydrogenotrophic sulphate reduction may be responsible for the depletion in hydrogen. The increased concentrations between 17-21 cm on the other hand again correspond to the increase in plant material and organic acids, indicating that fermentation may be the process responsible for these higher concentrations at depth. The subsequent decrease below 21 cm also correlates with the increase in methane values suggesting that H_2/CO_2 methanogenesis may be responsible for this increase in CH_4, in addition to the acetoclastic pathway. In contrast, in core CB6 H_2 concentrations were relatively

stable between 1.4-1.7 μmol lws^{-1} throughout the whole core except for isolated peaks at 5 and 21 cm (maximum value of 2.3 μmol lws^{-1} at 21 cm). This lower peak also coincides with the increase in plant material and peaks in organic acid concentrations, suggesting again that fermentation may be a causative factor. In addition it occurs near to the peak in H_2/CO_2 methanogenesis rates, suggesting that heightened H_2 concentrations (derived from the fermentation of un-degraded plant material) may be one of the reasons for the enhancement of methanogenesis at this depth.

CO_2 concentrations in core CB3 decreased initially with depth, from 9 mmol lws^{-1} at 1 cm to a minimum of 3 mmol lws^{-1} at 9 cm. Below this, concentrations began to increase relatively linearly down core, reaching 5.4 mmol lws^{-1} at 25 cm. The clear change in slope at 9 cm occurs at the same depth that nitrite and sulphate values begin to decrease which may represent a change in redox state in the sediment. As such the decrease in CO_2 values above 9 cm might represent a decrease in aerobic and dysaerobic heterotrophic metabolism with depth alongside uptake of CO_2 by chemoautotrophic processes such as sulphide and ammonia oxidation. The increase in concentrations below 9 cm (which matches the ammonium profile, could then be linked to increased levels of anaerobic organic matter degradation in the anoxic sediments. In core CB6 CO_2 concentrations decrease from ≈2.5 mmol lws^{-1} in the upper, 3 cm to a miniumum of 1.5 mmol lws^{-1} at 7 cm. Concentrations then increase again with depth reaching 4.1 at the base of the core. As with the hydrogen concentrations, a peak also occurs at 21 cm (4.6 mmol lws^{-1}), again suggesting that heterotrophic (and possibly fermentative) metabolism is enhanced by the plant material at this depth. This increase in CO_2 could provide another reason why the rates of H_2/CO_2 methanogenesis are enhanced at this depth, and provide another example of how increased levels of organic material can in turn enhance this lithotrophic process.

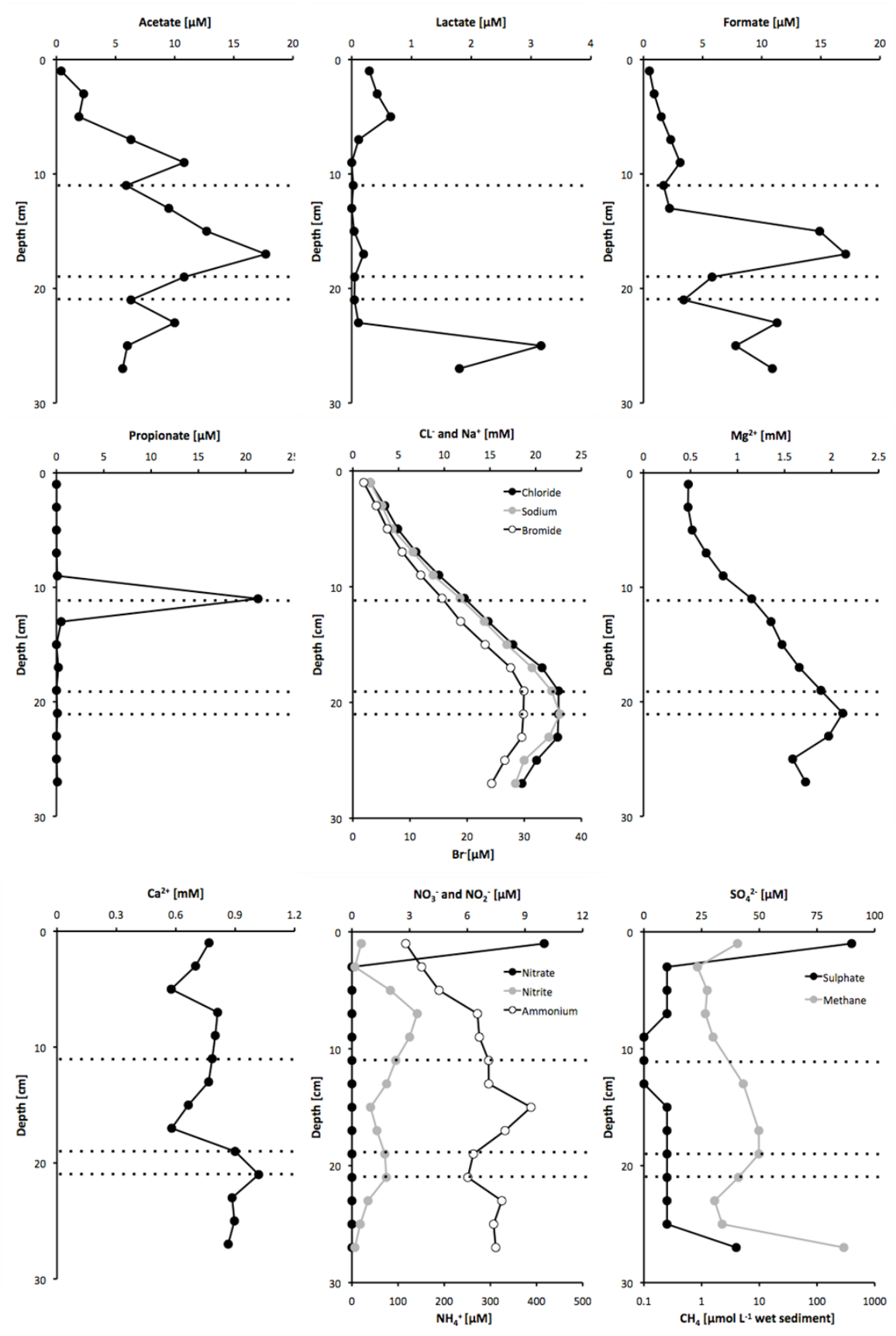
The total cell counts from core CB3 show a decrease in numbers with depth from $1x10^{9.8}$ cells cm^{-3} near the surface down to $1x10^{8.7}$ at the base of the core. In core CB6 a similar decrease (from $1x10^{9.6}$-$1x10^{9.3}$) occurred in the top 21.5 cm, followed by a sharp drop to $1x10^{8.9}$ at 23.5 cm and a subsequent second decrease down to $1x10^{8.7}$ at the core-bottom. In core CB6 the change in slope occurs coincides with the

increase in plant material and may be due to the counting problems described in section 8.2.1, while the continued lower values beneath this depth are likely due to the significantly lower levels of degradable OC in the clay layer below 25 cm.

The porosity of core CB3 was variable but showed a general decrease from 78.5% at 1cm to 70.8% at 25 cm. Core CB6 on the other hand showed a much more linear decrease in porosity with depth, from 86.1% at 1 cm to a minimum of 65.1% at 21 cm. Porosity then increased sharply ≈23 cm (corresponding to the increase in intact OM) and subsequently gradually increased to 74.4% at 31 cm. Oxygen uptake at Site 2 was lower than at Site 1, with an uptake rate of 51 mmol m^{-2} d^{-1}.

8.2.3 Biogeochemistry of Site 3 – Cores CB4 and CB7

The concentration of acetate in core CB4 (Fig. 8.8), initially increased with depth, from 0.4 μM at 1cm, reaching a peak of 17.7 μM at 17 cm, before decreasing to 5.6 μM at the core's base. Lactate exhibited a slight increase with depth, from 0.29 μM at 1cm to 0.65 μM at 5cm, before decreasing to 0.11 μM at 7cm. Concentrations were then consistently low (0-0.19 μM) down to 23 cm, before increasing sharply to 3.2 μM at 25 cm and then decreasing to 1.8 μM at the base of the core. Formate concentrations, like acetate, had an initial increase with depth, rising from 0.5 μM at 1 cm until ≈13 cm. Below this there was a rapid increase to 17.1 μM at 17 cm and then a decrease to 3.4 μM within the sand layer at 21 cm. Formate concentrations then increased again to 10.9 μM at the core bottom. Propionate was largely undetectable in core CB4, with concentrations only ranging between 0-0.2 μM throughout the core. The exception to this was at the gyttja-clay boundary at 11 cm where concentrations peaked to 21.3 μM. These results would suggest that organic acid production via fermentation is greatest within the clay layer at this site with activity mainly concentrated in two zones, one at ≈17 cm and a second at ≈23-25 cm separated by the sand layer. Production in the sand layer appears to be suppressed possibly due to lower levels of OM or more oxidizing conditions caused by groundwater flow. There might also be a third zone of elevated activity around 9-11 cm (corresponding to increases in acetate, formate and propionate), which again


Acetate [µM]
Lactate [µM]
Formate [µM]
Propionate [µM]
CL⁻ and Na⁺ [mM]
Chloride
Sodium
Bromide
Br⁻[µM]
Mg²⁺ [mM]
Ca²⁺ [mM]
NO₃⁻ and NO₂⁻ [µM]
Nitrate
Nitrite
Ammonium
NH₄⁺ [µM]
SO₄²⁻ [µM]
Sulphate
Methane
CH₄ [µmol L⁻¹ wet sediment]
Depth [cm]

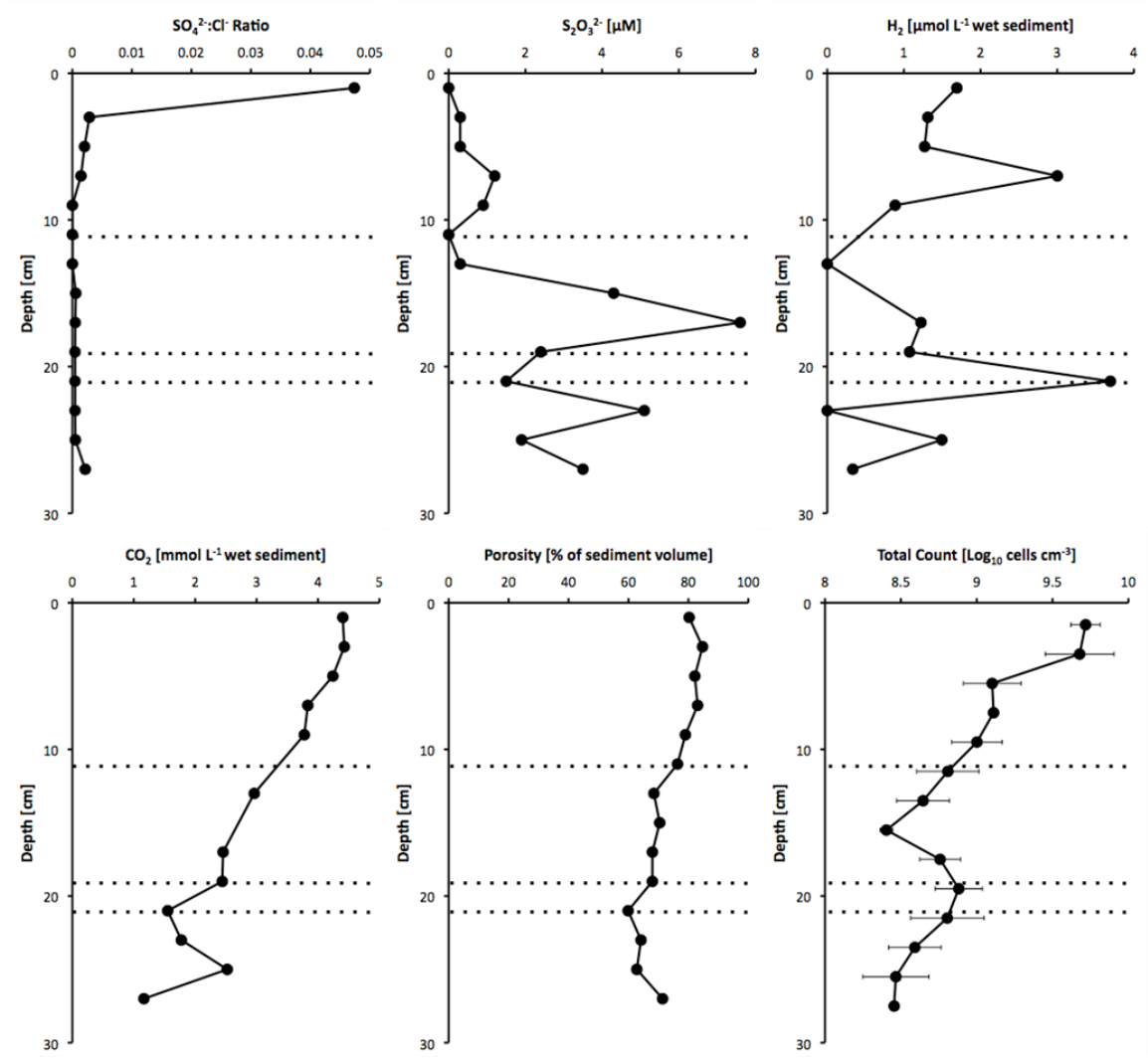


Fig. 8.8 (on this, and previous page) – Biogeochemical profiles obtained from core CB4 showing variations in the pore water concentrations of organic and inorganic solutes with depth. Also shown are sediment gas concentrations, porosity and total cell counts (AODC) - error bars represent the 95% confidence limits obtained from repeated counts. The dashed lines represent the boundary between the overlying lacustrine gyttja and the grey clay (11 cm) and the extent of the sand layer (19-21 cm).

could be due to metabolic uncoupling at this depth where sulphate reduction may become sulphate-limited.

In core CB7 (Fig. 8.9), acetate concentrations were similar to those in CB4 and initially increased from 2.75 µM at 1cm to 11.8 µM at 5cm and then fluctuated around this concentration down to 21 cm, below which concentrations decreased to 5.6 µM at the base of the core. Lactate concentrations were ≈1.2 µM in the overlying waters and in the sediment showed an initial increase (from zero at 1cm to 0.33 µM at 9cm) before decreasing throughout the rest of the core, down to 0.09 µM at 29 cm and then rising sharply to 2.52 µM at the bottom of the core at 31 cm. Formate

increased with depth, from 3.23 μM at 1cm to 28.7 μM at 13 cm before decreasing stepwise to 14.5 μM at the base of the core. Propionate concentrations were more variable, betweem ≈0-1 μM except for a single peak of 1.95 μM at 13 cm, These results, as for the other sites, indicate organic acid generation via fermentation in the clay layer of the sediments, however, in these cores the compounds (with the exception of lactate) appear to be being generated mostly in the middle of the core before being consumed more actively at the base.

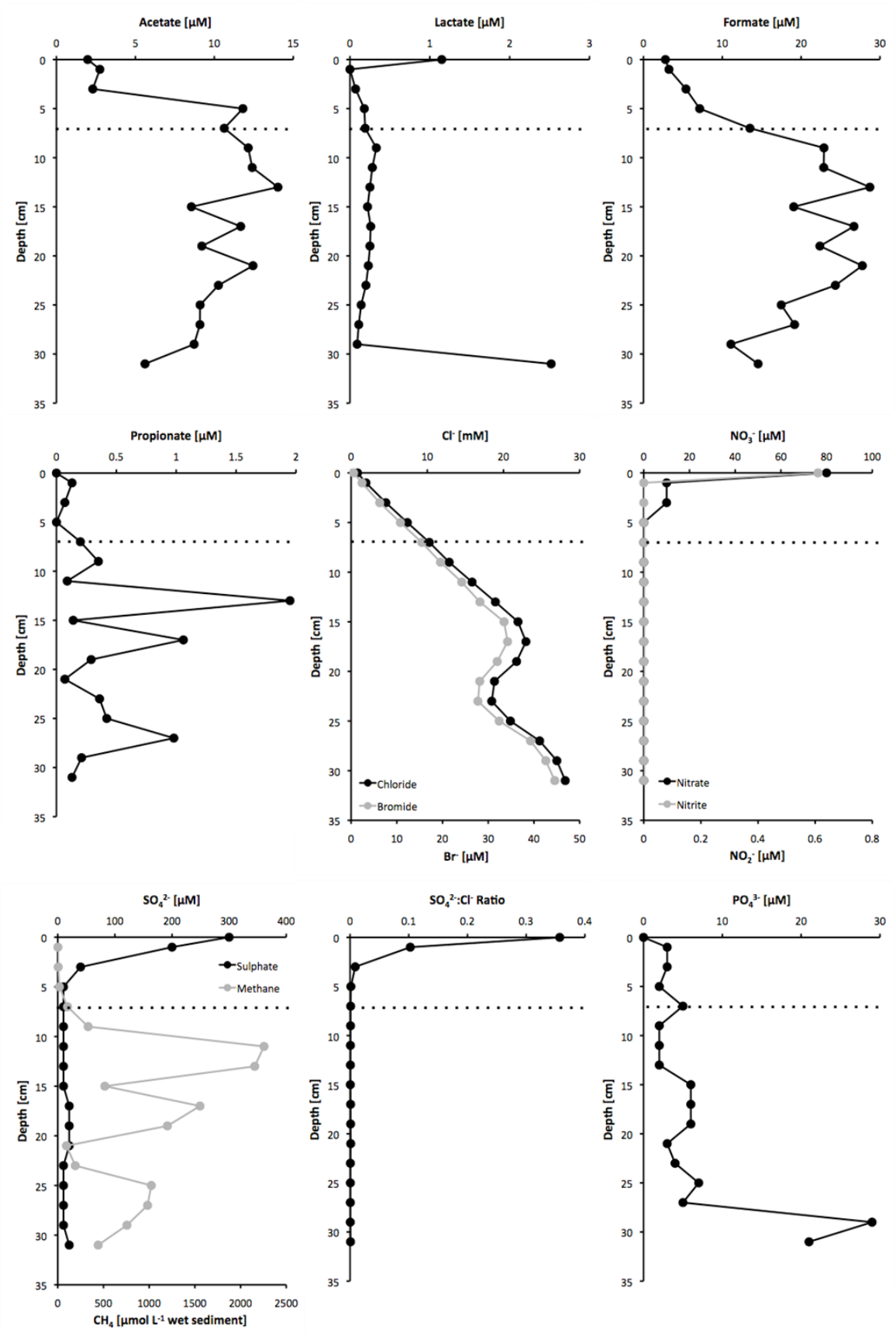
Chloride concentrations in core CB4 increased with depth from 1.9 mM at 1cm to a maximum of 22.5 mM around the sand layer at 19 cm before decreasing to 18.5 mM at the base of the core (27 cm depth). Core CB7 chloride concentrations had a similar depth profile, increasing from 0.84 mM at 1 cm to 22.9 mM at 17 cm before decreasing to 18.4 mM at 23 cm, however, unlike in CB4 no sand layer was present and concentrations then increased again to a maximum of 28.1 mM at the core's base (31 cm depth). This variation in salinity could indicate a subsurface input of terrestrially-derived freshwater into the sediment at around 27 cm in CB4 and 23 cm in CB7. In CB4 Bromide concentrations had a similar depth profile to chloride, initially increasing with depth from 1.9 μM at 1cm to 29.9 μM at 19cm, before decreasing down to 24.2 μM at 27 cm. In CB7 bromide again followed the chloride concentrations throughout the core. The sodium profile in CB4 also almost exactly matched chloride, while the magnesium profile followed a similar pattern as well. In contrast calcium concentrations were more stable down to 13cm (≈0.7-0.8 mM), then decreased at 17 cm (0.57 mM) before increasing sharply into the sand layer at 19-21 cm (≈1 mM) and then becoming more stable towards the bottom of the core.

Nitrate was only detected at 1 cm below the surface in core CB4 (10 μM). However, nitrite followed a similar depth profile to the cores previously described, initially increasing from 0.48 μM at 1cm to a maximum of 3.3 μM at 7 cm before decreasing slowly down-core. The decrease was much more gradual though, with nitrite still detectable at 27 cm (0.15 μM) suggesting that nitrate/nitrite reducing processes might not be as active at this site as at the other two sites. Nitrite also had a second subsurface increase between 15-23 cm, which correlates with the presence of the sand layer (≈19cm). This may indicate that the there is a secondary source of nitrite

at depth, possibly relating to the groundwater flow that may have caused the decrease in salinity described above. The decreased degree of nitrite reduction would seem to be to be supported by the ammonium concentrations, as maximum concentrations are less than a third of those at the other sites. The ammonium depth profile itself is also different as concentrations increased with depth from 116 μM at 1cm up to 387 μM at 15 cm before a zone of decreased concentrations (15-23 cm) and then remaining relatively stable down to the base of the core. This suggests that ammonium production was highest around 15 cm, but then concentrations decreased around the sand layer with a concomitant increase in nitrite concentrations, suggesting that an influx of fresher groundwater at depth facilitated ammonium oxidation. Also ammonium values above 15 cm are comparable with the other two sites. In contrast in core CB7 nitrite was only detected in the overlying water (0.6 μM), although nitrate was present in both the overlying water (80 μM) and at low concentrations (10 μM) in the upper 3 cm but was not detected below that (again likely due to active denitrification/DNRA).

Sulphate concentrations and SO_4^{2-}:Cl^- ratios decreased with depth in core CB4 with concentrations decreasing from 90 μM at 1cm to 10 μM at 3 cm indicative of sulphate reduction. Sulphate concentrations then stayed between 0-10 μM down to 25 cm, below which concentrations increased to 40 μM, possibly linked to the input of groundwater at this depth. Sulphate profiles were similar in core CB7 where sulphate decreased from 300 μM at the surface to 10 μM at 5 cm, again indicating active sulphate reduction, concentrations then stayed consistently between 10-20 μM down to the core's base.

Thiosulphate concentrations in CB4 initially increased from zero at 1cm to 1.2 μM at 7cm. There was then a second rapid increase to 7.6 μM at 17 cm, before concentrations decreased sharply into the sand layer (1.5 μM) and then remained variable down to the bottom of the core. A similar depth profile occurred in core CB7, with thiosulphate concentrations steadily increasing from zero at 1 cm to 18 μM at 13 cm and then slowly decreasing to 5 μM by 31 cm. This subsurface increase in thiosulphate may again indicate that sulphide oxidation is occurring at depth in both cores while the subsequent decrease may relate to disproportionation (which


Acetate [µM]
Lactate [µM]
Formate [µM]
Propionate [µM]
Cl⁻ [mM]
NO3⁻ [µM]
SO4²⁻ [µM]
SO4²⁻:Cl⁻ Ratio
PO4³⁻ [µM]
Depth [cm]
Chloride
Bromide
Br⁻ [µM]
Nitrate
Nitrite
NO2⁻ [µM]
Sulphate
Methane
CH4 [µmol L⁻¹ wet sediment]

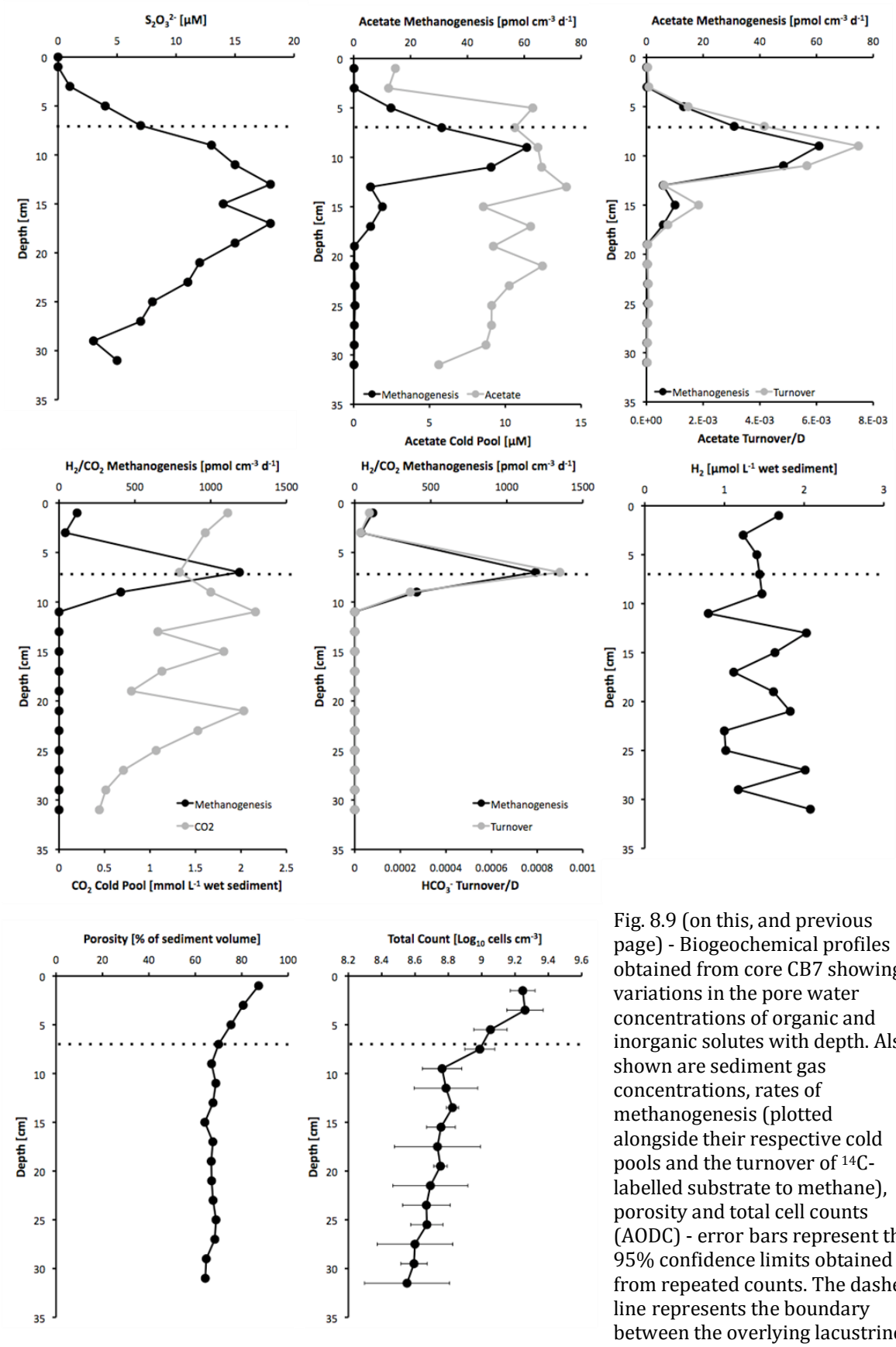


Fig. 8.9 (on this, and previous page) - Biogeochemical profiles obtained from core CB7 showing variations in the pore water concentrations of organic and inorganic solutes with depth. Also shown are sediment gas concentrations, rates of methanogenesis (plotted alongside their respective cold pools and the turnover of ^{14}C-labelled substrate to methane), porosity and total cell counts (AODC) - error bars represent the 95% confidence limits obtained from repeated counts. The dashed line represents the boundary between the overlying lacustrine gyttja and the grey clay.

may account for some of the sulphate increase at depth in CB4) (Holmkvist *et al.* 2011). Once again thiosulphate values in both cores seem to mimic the profiles of the organic acids acetate and formate, though the reason for this similarity remains elusive since thiosulphate-producing processes (such as S^{2-} oxidation) are lithotrophic (Jørgensen and Bak, 1991). Phosphate also showed a significant increase near the base of core CB7 again suggesting the possibility of FeS formation at this depth, although it could also be supplied directly by groundwater.

Methane remained relatively stable between 0.8-9.8 μmol lws^{-1} throughout most of core CB4, except at 27 cm depth where levels swiftly rose to 291 μmol lws^{-1} indicating active methanogenesis at depth. There is also a notable decrease in concentrations between 19-23 cm coinciding with the presence of the sand layer. This could again be due to lower levels of OM within the sand, or could indicate that methane oxidation is occurring – possibly due to a flow of more oxidized groundwater. In core CB7 methane concentrations started to increase markedly with depth upon entering the clay layer and once sulphate had become depleted. Values increased with depth from 1.5 μmol lws^{-1} at 1cm to 2200 μmol lws^{-1} at 11 cm, before decreasing stepwise to 441 μmol lws^{-1} at the base of the core. There was also a notable decrease in values between 20-25 cm, which coincided with the decrease in salinity at this depth, again indicating the possibility of methanogenic inhibition linked to oxidized groundwater flow.

Acetoclastic methanogenesis rates in CB7, however, did not reflect the full methane profile with values rising from 0.1 pmol cm^{-3} d^{-1} at 1cm to a peak of 61 pmol cm^{-3} d^{-1} at 9cm before decreasing to 0.3 pmol cm^{-3} d^{-1} at 19cm and staying at this rate throughout the rest of the core (depth integrated rate = 3.5 μmol m^{-2} d^{-1}). Rates of H_2/CO_2 methanogenesis had a sharp peak around 7cm (1200 pmol cm^{-3} d^{-1}) before decreasing to zero at 11 cm and below (depth integrated rate = 35 μmol m^{-2} d^{-1}). Both of these results indicate that methanogenesis was greatest at or just above the gyttja-clay boundary (roughly corresponding to the maximum methane peak) with little activity occurring below this, despite apparently ample substrate concentrations. The reason for this decrease in rates is puzzling although it may relate to the potential influx of more-oxidised groundwater at depth, which might

inhibit the methanogens (by raising the environmental redox potential), as *in situ* methane values are also low between 20-25 cm where salinity decreases.

H_2 concentrations in core CB4 were low and varied greatly, ranging between 0-3.6 μmol lws^{-1} throughout the core (especially below 10 cm). Unlike in cores previously described, concentrations do not appear to correlate with the organic acids, sulphate or methane, although they do show a distinct increase within the sand layer at 19-21 cm. The H_2 concentrations in CB7, while still quite variable, had a slight increase with depth in the clay layer, from 1.68 μmol lws^{-1} at 1cm to 2.07 μmol lws^{-1} at 31 cm. However again there was no obvious relationship to the other hydrogen producing/consuming processes.

CO_2 concentrations in core CB4 decreased overall with depth from 4.4 mmol lws^{-1} at 1 cm to 1.2 mmol lws^{-1} at the base of the core suggesting a decrease in heterotrophic activity with depth, or more likely, a consumption via autotrophic pathways (e.g. H_2/CO_2 methanogenesis) as NH_4^+ increases over the top 15 cm. There was however a small peak (up to 2.5 mmol lws^{-1}) at 25 cm which could be linked to the apparent second peak in fermentation activity below 21 cm, or the potential oxidation of methane discussed above. In core CB8 CO_2 values also decreased overall with depth (from 1.9 mmol lws^{-1} at 1 cm to 0.44 mmol lws^{-1} at 31 cm), although values were much more variable in the region where the organic acids (except lactate) are at their highest, suggesting a possible link between these compounds. Below ≈20 cm concentrations consistently decreased (again matching acetate and formate) possibly indicating that OM-degrading processes were decreasing at this depth.

The total cell counts from core CB4 show high numbers in the top 4 cm (≈$1x10^{9.7}$ cells cm^{-3}) possibly linked to the higher concentrations of nitrate and sulphate at this depth. Below this, numbers decrease down to $1x10^{8.4}$ at 15.5 cm potentially due to the depletion of these electron acceptor compounds. Numbers then increase again into the sand layer ($1x10^{8.9}$ at 19.5 cm), indicating that even though it appears to be negatively impacting some processes (fermentation, ammonification etc.), the sand layer could be enhancing overall microbial growth, possibly by groundwater re-oxidising ammonium to nitrite (and/or nitrate that might be rapidly consumed).

Below the sand layer cell counts decrease again to $1x10^{8.5}$ at the core base. The cell numbers for core CB7, in contrast, have a depth profile more similar to those of the cores previously described, with cell numbers relatively constant around $1x10^{9.2}$ in the upper brown, oxidised sediments above ≈5 cm. Numbers then decrease sharply down through the darker reduced sediment and at the gyttja-clay boundary, reaching $1x10^{8.8}$ at 9.5 cm, followed by a subsequent slower decline down through the rest of the clay layer to $1x10^{8.6}$ at 31.5 cm.

Porosity in core CB4 declined from 84.7% at 2 cm to a minimum of 59.8% at 21 cm before rising back to 71.3% at the core bottom. In core CB7 the porosity decreased sharply in the top 9 cm from 87.2% to 67%, but below this decreased at a slower rate to 64.3% at the core's base, indicative of the switch from gyttja to the more dense clay. Finally oxygen consumption at Site 3 was similar to Site 2 with an uptake rate of 51 mmol m^{-2} d^{-1}.

8.3 Cardiff Bay - Discussion

8.3.1 Core Lithology

As described above, the lithology of the Cardiff Bay cores is unusual in that they have a distinct bi-layered structure with a lower layer of dense, fine grained greyish clay overlain by dark brown-black sediment, rich in intact plant material, with a flocculent surface. The boundary between these two units was usually quite distinct at Site 1 and Site 3 (but less so at Site 2), suggesting that whatever event caused the change in depositional processes happened quite rapidly – a slower change would have resulted in a gradual gradation of one unit into the other. It seems likely that the bi-layering of the sediment represents sedimentation in the bay before and after construction of the barrage. The grey clay representing sediment deposited on brackish tidal flats while the bay was still open to the Severn Estuary, and the gyttja representing river-borne sediment from the Taff and Ely rivers deposited post-closure. The closure of the barrage was a swift event on sedimentary timescales, and therefore, would be capable of producing the distinct demarcation present in the sedimentary record.

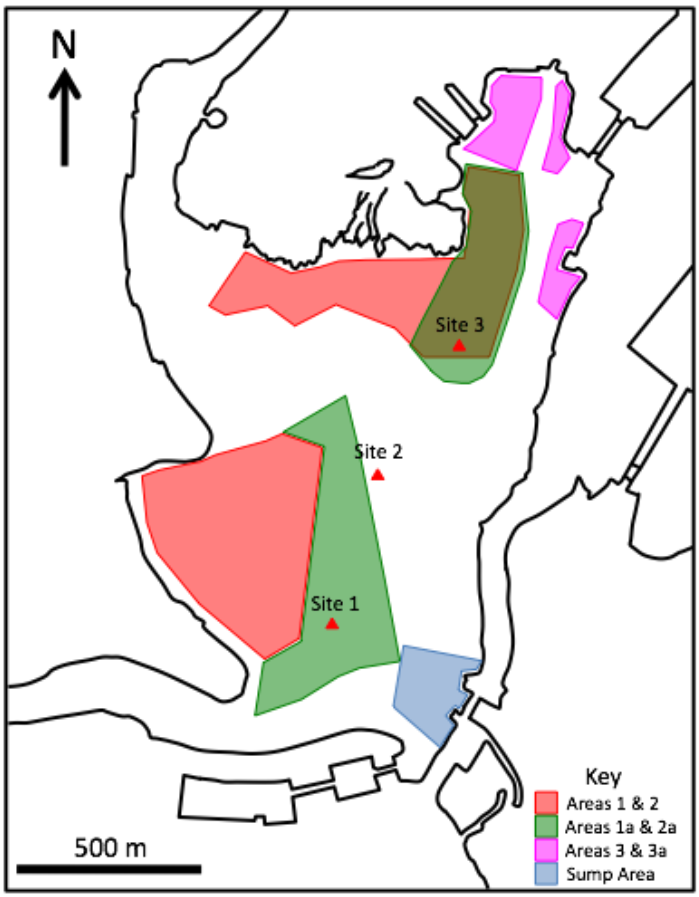


Fig. 8.10 – Line map showing the areas of Cardiff Bay that have dredged during construction of the barrage alongside the locations of the sediment sampling sites described in this study (red triangles). Also shown is the position of the sump used for the collection of saline estuary waters that enter the bay via the barrage lock gates. Modified from: Dredging Areas, Harbour Authority, Cardiff County Council, September 2000.

Further evidence that the grey clay originated in a marine environment comes in the form of infaunal marine bivalve shells (*Cerastoderma* sp.) found within core CB4 at 15 cm depth. Since the valves of the organism were found in an articulated fashion it is likely that the organism died in it's life position and was not transported and re-deposited post-mortem (Brenchley and Harper, 1998). The common cockle (*Cerastoderma edule*, Linnaeus 1758) can tolerate minimum salinities of 15‰ and the lagoon cockle (*Cerastoderma glaucum*, Poiret 1789) is capable of surviving down to 5‰ (Boyden and Russel 1972). Therefore neither of the two species commonly present in estuarine environments in the UK could have survived inside Cardiff Bay at its current salinity (0.058‰). This suggests that the cockle lived in the bay while it was still open to the estuary, and therefore was still brackish, and so the sediment it resided in must have been deposited in such an environment. A question does arise however, with regard to the age of these estuarine clays as soon after the construction of the barrage the bay was extensively dredged. This dredging removed large amounts of the former estuarine sediment from a wide area of the bay including around Sites 1 and 3 (Fig. 8.10). The exact depth of sediment removed is

unknown so it cannot be said with certainty if the clay present in the cores at Sites 1 and 3 represents recent near-surface estuarine deposits, re-suspended dredged material, or sediments that had been emplaced many years in the past. It is also a possibility that the dredging may have removed all of the more modern estuarine sediment from the sampling sites and that the grey clay may in fact represent older facies that underlie the bay, such as the Awre and Northwick Formations or even the Holocene Wentlooge and Rumney Formations (Allen, 1987d; Edwards 1997; Allen, 2001). However, all of these formations were deposited in estuarine settings (representing conditions over the last several thousand years) and as such would likely have a similar geochemistry to the modern estuarine sediments. Therefore, it would be difficult to differentiate them without detailed dating techniques (such as anthropogenic radionuclide dating).

The variation in the thickness of the gyttja relative to the clay between the sites is likely due to their proximity to the channels of the Taff and Ely rivers, and therefore, their proximity to sources of new fluvial sediment. Site 3 was furthest from either of the river channels and as such had the lowest proportion of gyttja:clay with the interface between the two occurring ≈7-12 cm. Site 1, which was between the Taff and Ely channels had an interface ≈12-22 cm indicating that it was receiving more fresh sedimentary material than Site 3, possibly overflowing from one, or both, of the river channels. Finally the interface at Site 2, which was within the Taff river channel itself, was ≈24-25 cm suggesting that it received the most new sediment of the three sites. The interface at Site 2 was also much less distinct than at the other two sites, suggesting that the sediment at Site 2 might have been reworked during the switch between estuarine and lacustrine facies, probably by the erosive action of the waters of the River Taff. If the interface between gyttja and clay does indeed represent the point of barrage closure in 1999 (Platt, 2002), and since the bay has not been dredged extensively since the initial period described above, then the depth of sediment above this point can be used to estimate the average net sedimentation rate at the different sites post-closure. Using this method it can be estimated that the net sedimentation rates at Sites 1, 2 and 3 are 1.83 cm a^{-1}, 2 cm a^{-1} and 1 cm a^{-1} respectively. However, this rate does not necessarily represent the gross rate of sediment deposition, as some sediment (especially at Site 2 with its

enhanced river-driven water movement) is likely to be re-suspended soon after deposition.

8.3.2 Sediment Oxygen Demand and Organic Acids

Oxygen uptake rates for Cardiff Bay (Fig. 8.11) were in the middle of those obtained from sites in the Severn Estuary (see Chapter 9). This is likely due to the Cardiff Bay sediments having high levels of organic matter but relatively low amounts of re-suspension compared to the sediments of the estuary. In addition the cores from Cardiff Bay were taken when *in situ* temperatures were lower (6°C) than when the estuary cores were taken (11-14°C) which may have negatively affected the overall metabolic activity of the sediments. Within Cardiff Bay itself the highest rates came from Site 1 (61 mmol m^{-2} d^{-1}), with Sites 2 and 3 having lower but roughly equal values (≈51 mmol m^{-2} d^{-1}). The reason for this is unknown, as Site 2 (located in the Taff river channel) should have received the highest amount of fresh organic material. However, the majority of this material may have been relatively recalcitrant (e.g. terrestrial plant matter) and as such, hard for prokaryotic organisms to degrade (Killops and Killops, 2005). Site 3, which was located away from the river channels may instead have received less organic material overall, but due to the lack of currents what it did receive may have contained more labile autochthonous carbon (from phytoplankton etc.), which would have been easier to degrade. Since Site 1 was located between two river channels it likely received a mix of both autochthonous labile carbon and high amounts of allochthonous carbon, which combined, could account for it's higher oxygen uptake values.

Another group of compounds likely influenced by carbon deposition are the organic acids. Concentrations of these compounds varied greatly both between sites and between sampling periods. For example concentrations of acetate near the surface at Site 1 changed by a factor of ten over the course of a year (CB2=153 μM, CB5=16 μM) while concentrations above 5 cm at the other two sites never exceeded 5 μM. This variation is most likely linked to changes in the deposition patterns of organic matter across the bay both spatially and temporally. As stated above, the position of each site may influence the amount (and type) of organic matter it receives, both from the rivers and autochthonously from water column. In addition changes in meterological conditions (e.g. precipitation and insolation) over the two

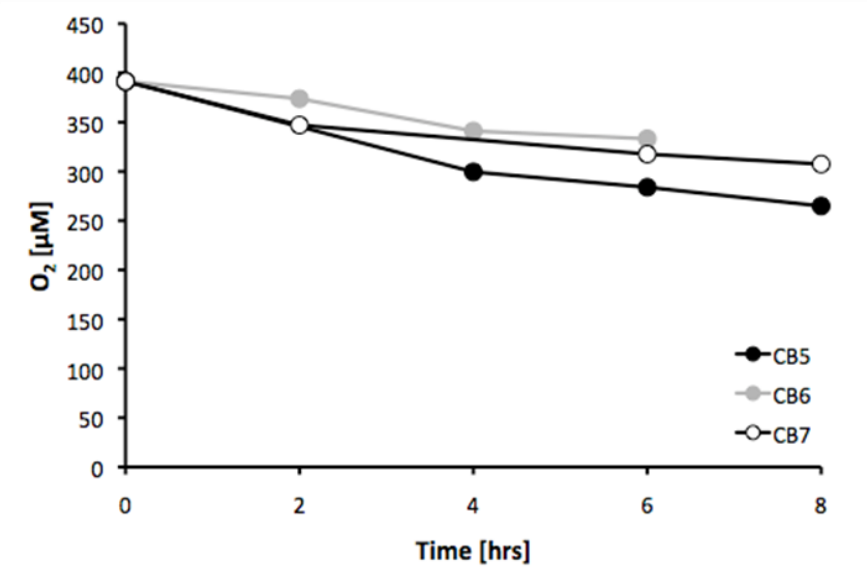


Fig. 8.11 – Line graph showing the consumption of oxygen by duplicates of cores CB5, CB6 and CB7 incubated in darkness at *in situ* temperature for 8 hours. Data obtained from the decrease in oxygen concentrations in the overlying waters of the cores corrected for loss by planktonic heterotrophic activity.

years of the study may have influenced the amount of terrestrial OC transported to the bay via rivers and the amount produced *in situ* via photosynthesis. Therefore, a combination of these two factors, coupled with interconnected production and consumption of these compounds *in situ* by prokaryotes is likely responsible for the variations observed in the profiles described here.

8.3.3 Chloride, Bromide and Sodium

Chloride, bromide and sodium concentrations increased with depth in all Cardiff Bay cores. This is again likely to be linked to the switchover from an estuarine to lacustrine environment, with the estuarine grey clay retaining some of the salinity of the brackish environment in which it was originally deposited. However, in the years since the barrage has been closed the clay has lost much of it's previous salinity, with maximum levels in CB5 of only 2.36‰ at it's base compared to a current salinity of 23.7‰ found in the sediments near Penarth, on the seaward side of the barrage. However, since Cl^-, Br^- and Na^+ concentrations were still increasing with depth at the base of several of the cores it is possible that salinities closer to the estuarine norm could be present deeper in the sediment. The depth within the cores that salinity began to increase also indicates that the grey clay is likely to be estuarine in origin, as in several cores the increase was not constant with depth but instead was most marked within the grey clay. For example in core CB2 chloride concentrations were only 15 mM at 20 cm depth, whereas in CB4, which had a gyttja:clay interface much higher in the core, concentrations reached 15 mM at

around 13 cm and in CB3, which had the highest proportion of gyttja, the maximum chloride concentration was only 2.7 mM. This again suggests that only the grey clay was deposited in an environment with salinities higher than those currently present within the bay.

Similar instances of "relict" salinity have been documented in other environments where there has been a shift from saline to freshwater conditions and vice versa. For example Ross *et al.* (1970) and Knab *et al.* (2009) both detail the existence of a zone of lacustrine sediments buried beneath the seafloor of the Black Sea that was deposited between 10-20 Kya when the Black Sea was a freshwater lake. The Black Sea was eventually flooded by the Mediterranean Sea between 7-12 Kya due to rising sea levels and current sediments have saline pore water profiles. However, the previously lacustrine sediments maintained their freshwater chemistry, which has in turn affected the rates of biogeochemical processes such as sulphate reduction and AOM in the sediments.

The alternative possibility is that the increase in salinity with depth could be related to an influx of modern seawater from outside the barrage. The influx of higher-salinity waters in the bay was deemed undesirable for ecological reasons (Hunter and Gander, 2002), and as such measures have been put in place to prevent such an influx from occurring. The major source of influx would be through the sluices that permit watercraft to enter and exit the bay, therefore, to mitigate any saltwater inflow during sluice operation the sluices are not opened when the level of water outside the bay is higher than inside such as during high spring tides (Crompton, 2002). Any saltwater that does enter through the sluices collects (due to it's higher density) in sumps dug at the opening of the sluice gates and is then flushed via a culvert back into the locks at low tide where it can re-enter the estuary. However, if the increasing salinity profile with depth is due to saline intrusion it is more likely that it would be as a result of subsurface saline water flow underneath or indeed through the barrage itself. This though is unlikely as the lakebed of Cardiff Bay sits higher than the seabed outside the barrage so any saltwater flowing underneath the barrage would have to flow "uphill". In addition for most of the monthly tidal cycle the water level within the bay is higher than outside (the mean water level in the bay post-closure is 4.2 m higher than pre-closure) (Heathcote *et al.* 2003) creating a large hydrostatic head that would prevent any inflow of saltwater

either underneath or through the barrage - although theoretically some tidal pumping could occur on the highest of spring tides (Burnett *et al.* 2003, Santos *et al.* 2012). If such tidal pumping was occurring it may explain why certain chemical species associated with higher salinities but usually depleted by microbial metabolism (e.g. sulphate) could be present at depth in some cores such as CB8 (Beck *et al.* 2009).

The effect that this increase in salinity could have on the microbial communities within the sediment column could be profound (Luzopone and Knight, 2007). As detailed in Chapter 1, salinity can have an effect on which prokaryotic guilds dominate a particular environment either directly; depending on their tolerances to Cl^-/Na^+ concentrations or indirectly; with regard to differing concentrations of electron acceptor substrates present in fresh and saline water (Weston *et al.* 2011). The change in salinity could also effect which genera of organisms within a particular guild are present at particular depths in a similar fashion to the distribution of genera along an estuarine salinity gradient (O'sullivan *et al.* 2013, Campbell and Kirchman 2013), although in this case on a much smaller scale and in a vertical rather than horizontal arrangement.

As well as the general increase in salinity with depth, cores from Site 3 (CB4 and CB7) also showed clear decreases in salinity at depth. In core CB4 this decrease occurs beneath the sand layer (≈25cm) and continues to the base of the core whereas in CB7 it occurs in a distinct zone between 17-25 cm, below which salinity increases again. The reason for this decrease is unknown but may (as in several of the tidal flat cores in this study) relate to terrestrially-derived groundwater flow. Such a flow of potentially more-oxidised groundwater could also account for some of the changes seen in the geochemical profiles at depth including: increases in nitrite/decreases in ammonium in core CB4 possibly linked to ammonium oxidation; and decreases in the methane (and possibly organic acid) profiles in both CB4 and CB7 caused by inhibition due to the higher redox potential of the groundwater.

8.3.4 Nitrate, Nitrite and Ammonium

Nitrate was present only in small amounts (≈10 μM) in the surficial sediments of the cores taken from the three sites in Cardiff Bay despite it being present in the overlying waters of the bay at concentrations >90 μM. This is likely due to high rates

of DNRA/denitrification in the sediment rapidly converting nitrate to ammonium or nitrogen gas. Nitrite was however present in both the surface waters of the Bay (<5 μM) and in the sediment where it's depth profile had a similar subsurface peak to that exhibited by nitrate (Hensen *et al.* 2006) - maximum concentration of 4.5 μM in core CB6 at 3 cm depth. For nitrate this peak is caused by the production of nitrate in the suboxic zone of the sediment by nitrification of upwardly diffusing ammonium and then the subsequent removal of this nitrate by denitrification/DNRA. Therefore, to obtain the same profile shape nitrite must be being produced within the sediment and then utilised by other organisms. Since nitrite is rarely measured in sediments (Canfield and Thamdrup, 2009) this peak is unusual, although accumulation of nitrite at depth has been observed in anaerobic freshwater sediments (particularly with high labile carbon contents) (Kelso *et al.* 1997). Since nitrite is an intermediate compound in both denitrification and DNRA (Konhauser, 2007) this peak could indicate that active nitrate reduction is occurring at this depth (Stief *et al.* 2002), despite the lack of a nitrate profile – and possibly at a very high rate considering the presence of ammonium even at 1 cm depth in some cores. High rates of nitrate removal have also been observed at Haringvliet Lake, a coastal freshwater lake (and former estuary) similar to Cardiff Bay (Laverman *et al.* 2007). Alternatively since nitrite is also an intermediary compound in nitrification it is possible that this process is responsible for the nitrite peak. Though since nitrification only occurs in dysoxic environments this would require an unusually deep O_2 penetration (≈ 5cm in CB2) for eutrophic lake sediments (Sass *et al.* 2003), although this might be possible with bioturbation. The depletion in nitrite below ≈10 cm could be caused by one of two factors, either continued denitrification/DNRA (i.e. the conversion of nitrite to N_2 or ammonium) or anaerobic ammonium oxidation (anammox). Anammox is a possibility, as at Site 1 and Site 2 ammonium increases with depth (likely due to the ammonification of organic nitrogen compounds) but only after nitrite has been severely depleted. Since anammox bacteria utilize nitrite as an electron acceptor to oxidize ammonium it is possible that in the zone where both nitrite and ammonium are depleted anammox is occurring (Meyer *et al.* 2005). Alternatively the decrease in ammonium towards the surface could be caused by nitrification (as the profiles in cores CB2 and CB3 show a distinct concave-upward shape), with the ammonium being converted to nitrite (hence the peak) and then

oxidised further to nitrate however, as detailed above, this reaction requires O_2 to act as an electron donor and this could only occur with an unusually substantial O_2 penetration depth for this environment (up to 10-15 cm).

Unusually, nitrite is also present at considerable depth in core CB4 and appears (along with ammonium) to be influenced by the presence of the sand layer, with nitrite concentrations increasing (and ammonium decreasing) at around this depth (15-23 cm). Such a change could be due to nitrification, which would be unusual at this depth due to the lack of oxygen, but might be possible if the groundwater flow discussed above was partially oxygenated. Alternatively the groundwater could instead contain higher concentrations of nitrite (and lower ammonium) than the *in situ* environment and be affecting the geochemistry directly. Nitrite also occurs at depth in core CB8, although in this case it does not seem to be connected to a decrease in salinity. In addition no ammonium values were taken for core CB8 so further conclusions on the origin of the nitrite in this core are hard to draw. However, this nitrite peak (and/or the nitrite present at depth in core CB4) could relate to the anaerobic oxidation of ammonium coupled to iron reduction (Clément *et al.* 2005), which could also help to account for the decrease in ammonium associated with the nitrite peak in CB4.

8.3.5 Sulphate and Thiosulphate

Sulphate concentrations decreased in all Cardiff Bay cores in the subsurface, indicative of bacterial sulphate reduction. Concentrations in the surface waters of the bay ranged from 390-440 μM with concentrations near the sediment-water interface between 290-550 μM. These concentrations are within the normal range for a eutrophic lake and towards the higher end of the spectrum for mesotrophic and oligotrophic lakes (Holmer and Storkholm, 2001), which is to be expected considering the trophic state of the bay (Jüttner *et al.* 2010). At Sites 1 and 2 sulphate was generally depleted (down to a concentration of 10-20 μM) by between 9-15 cm however, at Site 3 sulphate was removed more quickly, reaching 10 μM at between 3-5 cm. This difference in profiles is unusual considering the fact that sulphate concentrations in the surface waters of the Bay are relatively uniform and that in the top 1 cm of sediment sulphate values at Site 3 were on average higher (145 μM) than Site 1 (30 μM) or Site 2 (95 μM). One possibility might be that this

difference in sulphate depletion depth is due to variability in chloride concentrations between the three sites. At 5 cm depth at Site 3 the mean chloride concentration was 6.14 mM whereas at Sites 1 and 2 mean concentrations at 5 cm depth were 2.14 mM and 0.83 mM respectively. When comparing the sulphate and chloride profiles from the three sites a similar pattern emerges, with sulphate depletion occurring at greater depths at sites with lower salinities (such as Site 2) than at sites with higher salinities (such as Site 3) where depletion occurs at much shallower depths, possibly indicating that sulphate reduction is more active under higher salinity conditions. Alternatively, since Site 3 was further away from the river channels than the other two sites it may have received a greater percentage of labile autochthonous carbon (e.g. derived from phytoplankton) than the other sites, which might have a greater proportion of recalcitrant allochthonous carbon (e.g. derived from terrestrial vegetation via the rivers), thus increasing the immediately bioavailable carbon pool for sulphate reduction.

Sulphate reduction rates obtained from Core CB8 were high with up to ≈77% of the label turned over during the 6 hour incubation. Compared to other sites in this study rates in Cardiff bay were considerably higher, with both cm^{-3} and depth integrated rates ≈1.5-2x higher than the estuarine sites. This is not unexpected as despite the lower concentrations of sulphate in freshwater (≈0.1mM) compared to seawater (≈29 mM) (Jørgensen and Kasten, 2006; Bianchi 2007), freshwater SRB have a high affinity for sulphate (Roden and Tuttle, 1993a). This, coupled with active re-oxidation of sulphide back into sulphate (indicated by the subsurface sulphate peaks in CB2 and CB4) allows freshwater SRB to metabolise rapidly even in the presence of very low sulphate concentrations. Further comparison of the sulphate reduction rates obtained from Cardiff Bay will be presented in Chapter 9.

Thiosulphate is also present in the sediments of Cardiff Bay at concentrations (18 μM in core CB7 and up to 50 μM in core CB8) much higher than those usually found in freshwater sediments - <1 μM (Jørgensen, 1990b) - although concentrations up to 37 μM have been documented in lagoon sediments (Bertolin *et al.* 1997). Since thiosulphate concentrations increase with depth it must be being produced within the sediment column, probably as a result of the oxidation of sulphide by anaerobic bacteria (Jørgensen and Bak, 1991; Thamdrup *et al.* 1994) likely utilising Fe^{3+} as their terminal electron acceptor due to the depletion of nitrate

and nitrite at the depth concerned (Konhauser, 2007). This endogenous production of thiosulphate may also explain the shape of the thiosulphate profiles, as in several of the cores after an initial increase, thiosulphate concentrations decrease again, likely due to the consumption of thiosulphate by SRBs via disproportionation. However, this consumption of thiosulphate occurs at the base of the zone of sulphate reduction which is unusual as thiosulphate is a preferred electron acceptor of SRB (Bertolin *et al.* 1997), therefore, it would be expected that sulphate reduction would only occur one the thiosulphate present in the sediment had been depleted. This unusual situation can however, be explained by the fact that most (if not all) of the thiosulphate in the sediment is generated *in situ*. For example, the SRB near the surface of the sediment (where thiosulphate concentrations are low) utilise sulphate as their electron acceptor and in turn produce sulphide. This sulphide is then converted to thiosulphate by sulphide-oxidising bacteria (producing the thiosulphate peak at the base of the sulphate reduction zone), which is then disproportionated again by SRB. This disproportionation then yields sulphide and sulphate (Konhauser, 2007), which could help to account for the elevated sulphate values observed at the base of some of the cores (e.g. CB3 and CB4) (Holmkvist *et al*. 2011).

8.3.6 Methane

Concentrations of methane increased down-core in the majority of the Cardiff Bay (with the exception of core CB2, likely due to it's short length, though it still contained abundant methane) indicating that active methanogenesis is occurring within the sediments of the bay. The depth at which methane concentrations began to increase varied between cores but was often concomitant with the depletion of sulphate to around 10 μM. This indicates that sulphate concentrations were (at least partially) inhibiting methane production above this depth, likely due to the action of SRB outcompeting methanogens for common electron donor substrates (Lovley *et al.* 1982). Below the zone of sulphate depletion methane concentrations often rose rapidly to very high levels, up to 3.5 mmol lws^{-1} (4.9 mmol l^{-1} of pore water) at 25 cm depth in a duplicate core of CB8. Concentrations also varied with the seasons with lower concentrations in winter (cores CB2 and CB5) compared to spring (core CB8) and summer where concentrations at Site 1 reached 3.1 mmol lws^{-1} (4.6 mmol

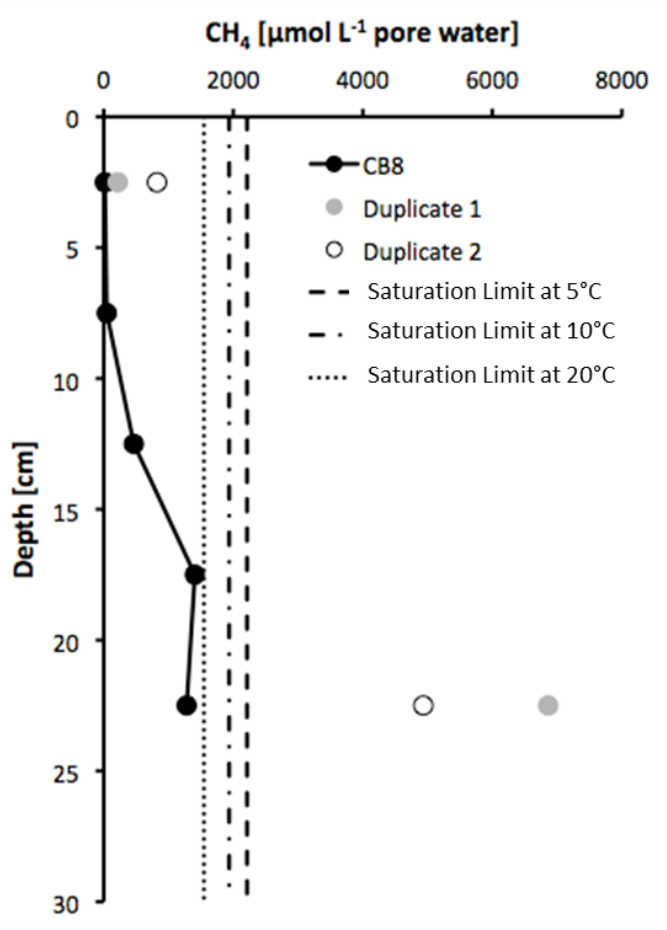


Fig. 8.12 – Graph showing the concentrations of methane in Core CB8 and in duplicate cores taken at the same time. Also plotted are the saturation concentrations of methane in freshwater at surface pressure, at a range of temperatures that Cardiff Bay sediments can be subject to. Saturation values deduced using Bunsen coefficients taken from Wiesenburg and Guinasso Jnr. (1979).

l^{-1} of pore water) at 23 cm (core CB9, data not shown). Indicating that higher temperatures probably enhance methane production in these sediments.

These concentrations are far above the theoretical saturation limit of methane at surface pressures and *in situ* temperatures (Wiesenburg and Guinasso Jr., 1979; Abegg and Anderson, 1997) (Fig. 8.12), meaning that methane gas could have been driven out of solution at these depths. This degassing of the pore waters may explain the voids present in some of the cores, as these voids may in fact be bubbles originally filled with methane that had been forced out of solution. The presence of such methane bubbles would also explain the ebullition of gas observed when the cores were disturbed during sectioning/sub-coring. Large-scale ebullition was also observed during core collection when the multi-corer made contact with the sediment surface. However, it is unknown if ebullition is a natural process in the bay (via pockmarks etc. Bussmann *et al.* 2011) or whether it is a phenomenon only encountered when the sediment surface is disturbed, and whether under natural conditions methane gradually diffuses out of the sediment instead.

Such high levels of methane have been measured in other freshwater environments particularly in artificial lacustrine settings with high rates of OC burial. Sobek *et al.* (2012) found methane concentrations of around 5 mM (at ≈15 cm depth) in the pore waters of sediments from Lake Wohlen, a hydroelectric reservoir in Switzerland. The authors attributed the high methane concentrations to the extremely rapid deposition of OC-rich sediment (5.2-11.5 cm a^{-1}), which led to large amounts of reactive OC reaching the deeper methanogenic zones of the sediment. Such high rates of OC burial are characteristic of artificial lacustrine environments created by river empoundment (Mulholland and Elwood, 1982). As such it would not be unexpected to find high OC burial rates in Cardiff Bay (evinced by the presence of intact plant material deep in the cores – e.g. 20-25 cm in CB6), and hence high levels of methane. DelSontro *et al.* (2010) and Sobek *et al.* (2012) also noted that when such high levels of methane build up in sediments beneath a shallow water column these sediments can become a significant source of atmospheric methane. This is because if the water column is shallower than 10 metres then most of methane bubbles rising from the sediment surface will reach the surface of the water body before they have been fully oxidised, and therefore, the methane will be released into the atmosphere. In addition, a shallow water depth results in low hydrostatic pressure which makes it easier for methane to come out of solution and form bubbles (Xiao *et al.* 2013). Since Cardiff Bay is a very shallow lake (mainly ≈2-3 m water depth), it is feasible that if any methane is being released from the sediments by gas bubble ebullition then most of it will be able to reach the atmosphere - although as with methane production the amount is likely to vary with the seasons (Martinez and Anderson 2013).

As well as peaking at depth, in some Cardiff Bay cores methane occurs in high concentrations in the upper 2 cm of the cores (up to 1.1 mmol lws^{-1} in core CB2). This peak indicates that methane is either being produced *in situ* near to the surface, possibly via the consumption of non-competitive substrates (methanol, methylated amines etc.) (Oremland *et al.* 1982; Lovley and Klug, 1983) since sulphate reduction is still occurring at this depth; or is rising from deeper in the subsurface and becoming trapped at or near the surface. Of the two hypotheses *in situ* methanogenesis is more likely as the sediment between the deep methanogenic zone detailed above and the surface is relatively free of methane, with

concentrations in this middle zone in the range of 1-100 μmol lws^{-1} depending on the concentration of methane beneath. This would imply that anaerobic methane oxidation is occurring in this middle zone, evidence for which can be seen in the concave-upward methane profiles from cores CB3 and CB6 (and possibly ≈19-25 cm in CB4). Such oxidation would limit methane rising to the surface from deeper below, unless such a rise occurred rapidly, for example through bubble formation and/or via small conduits and pockmarks (Bussmann *et al.* 2011; Scandella *et al.* 2011). The presence of large amounts of decomposing vegetation at the top of the cores would also provide methylated compounds for the surface methylotrophic methanogens to utilize (Wang and Lee, 1994), though none were detected possibly due to their quick turnover time (Parkes *et al.* 2012). The reason that this surface methane peak is only present in some of the cores may be linked to the concentrations of sulphate in the sediment pore waters, as even when produced via the breakdown of methylated compounds methane can still be anaerobically degraded in the presence of sulphate by AOM consortia. From the core profiles it can be seen that the cores that exhibit a shallow subsurface methane peak (CB2, CB3, CB4, and CB5) have some of the lowest sulphate concentrations recorded in the top two centimeters (<90 μM). In addition the cores with the lowest surficial sulphate concentrations also have the highest methane values (e.g. core CB2: SO_4^{2-}=10 μM, CH_4=1344 μmol lws^{-1}; core CB4: SO_4^{2-}=90 μM, CH_4=5.2 μmol lws^{-1}). Therefore, it would appear that the near surface methane peak can only occur when sulphate levels are close to depletion as this would limit the amount of AOM occurring (due to the SRB members of the consortia requiring sulphate as an electron acceptor). The lower sulphate levels might also allow the methanogens to utilise competitive substrates as competition from sulphate reduction might be (at least partially) suppressed (Lovley and Klug, 1986). These results would agree with the findings of Conrad *et al.* (2007) who found that methanogens from the upper layers of a sediment core from Lake Stechlin were capable of producing large amounts of methane provided that sulphate was not present.

Measured rates of methanogenesis (acetoclastic and CO_2 reduction) in cores CB6 and CB7, broadly matched the depth profiles of methane concentrations. However, in core CB5 methanogenesis rates were highest within the top 20 cm while methane only began to accumulate dramatically below 20 cm. In all three

cores rates of H_2/CO_2 methanogenesis were greater than acetoclastic methanogenic rates. This is unusual as acetoclastic methanogenesis is thought to dominate in freshwater environments compared with CO_2 reduction/hydrogenotrophic methanogenesis (Whiticar *et al.* 1986), which usually only contributes ≈33% of the total methanogenesis in anaerobic freshwater sediments (Conrad, 1999). The potential reasons for this situation will be discussed in Chapter 9.

The highest rates of methanogenesis occurred around 21-23 cm in core CB6. This likely corresponds to the large amounts of intact plant material present at this depth in CB6, which would have provided an elevated supply of carbon substrates (see acetate profile) and H_2/CO_2 for the methanogens. Strangely although core CB6 has some of the highest rates of acetoclastic methanogenesis (and the second highest rate of H_2/CO_2 methanogenesis) its pore waters have some of the lowest concentrations of *in situ* methane. However this may be due to the fact that unlike cores CB5 and CB7 (which have higher methane concentrations), CB6 is mainly made up of gyttja and contains relatively little clay. This could mean that any methane produced in core CB6 is more easily able to travel upwards through the more porous gyttja (either via diffusion or ebullition), whereas in CB5 and CB7 it is trapped as bubbles in the more impermeable clay. Alternatively it may be that because core CB6 has a higher concentration of pore water sulphate that AOM is more pronounced in CB6, which would lead to a depletion of methane (as evinced by the concave-upward methane profile around 15 cm).

In terms of areal acetoclastic methanogenesis rates Sites 1 and 2 have similar production rates (16.7 and 16.6 μmol m^{-2} d^{-1} respectively), over 4x the rate present at Site 3 (3.5 μmol m^{-2} d^{-1}). The reason for this difference is unknown, as although it could reflect the lower rate of sediment deposition at Site 3 (which would have resulted in a lower rate of OC burial and thus lower rates of substrate production), the pore water acetate concentrations at this site are no lower than those at Sites 1 and 2 suggesting that this is not the case. In terms of areal H_2/CO_2 methanogenesis, rates decrease along the transect, with rates at Site 1 (1327 μmol m^{-2} d^{-1}) 38x higher than at Site 3 (35 μmol m^{-2} d^{-1}). The reason for this may relate to the greater thickness of the gyttja layer at Site 1, as H_2/CO_2 methanogenesis seems to be restricted to this layer in all cores. However, for Site 2, the areal H_2/CO_2 methanogenesis rate (491 μmol m^{-2} d^{-1}) is between the rates at the other two sites,

despite it having the thickest gyttja layer. This may be due to enhanced sediment erosion at site 2 (due to the flow of the river Taff), which might inhibit methanogenesis in the upper portions of the sediment pile by disturbing the sediments.

8.3.7 Total Cell Counts

Total cell counts decreased with depth in all cores, as would be expected due to the decrease in bioavailable carbon with depth (Parkes *et al*. 2000). In most of the cores there was a particularly sharp decrease at the boundary between the gyttja and the clay, followed by a continued decrease through the clay layer. This may be because the (probably estuarine) clay layer is older than the lacustrine gyttja and therefore contains even less bioavailable OC. Unusually the cell counts for Core CB4 show an increase at depth within the clay, centred around the sand layer at ≈20 cm. This increase in cell numbers may reflect a flow of groundwater around this depth, as the input of fresh oxidised substrates, or the oxidation of reduced compounds *in situ* by oxidised groundwaters, may be stimulating microbial growth (e.g. nitrification, methane oxidation), which is reflected in the geochemical profiles (e.g. NO_2^-, NH_4^+ and CH_4). Similar increased cell numbers coinciding with increases in such processes, and linked to fluid flow, have been documented by Parkes *et al.* (2005) and Wilms *et al.* (2007).

8.4 Cardiff Bay - Conclusions

- Despite the conversion of Cardiff Bay from an area of tidal mudflats to an artificial freshwater lake, the sediments are biogeochemically active with high prokaryotic populations that appear to have adapted well to the new conditions.
- Sediments across the width of the bay display a distinct change in lithology with depth, from a dark, organic-rich lacustrine mud (gyttja) to a pale grey, denser clay. The depth at which this transition occurs in the sediments varies relative to the proximity of the Taff and Ely rivers. This change in lithology also dramatically affects the geochemistry of the sediments.
- Sediments from Cardiff Bay possess a vertical salinity gradient, with salinity increasing down-core – particularly within the clay layer. This, coupled with the

presence of the intact remains of marine/estuarine fauna, suggests that the clay was deposited under estuarine conditions before the closure of the barrage.

- Microbial processes appear to be more active in the overlying gyttja than in the clays, particularly at depths with large amounts of un-degraded allocthonous OC. Those processes that are active in the clay are often most active nearest the upper boundary, and decrease with increasing depth. Total cell count numbers are high also show distinct drops in numbers moving downward into the clay layer (with some isolated increases at depth e.g. CB4).
- Despite the low *in situ* levels of sulphate, SRB are active in the lacustrine sediments, with high SRR appropriate for eutrophic lake environments (see Chapter 9). AOM may also be occurring at depth.
- Methane concentrations in the sediments of Cardiff Bay are high, and in some locations exceed saturation. Such high levels are likely due to the anthropogenic nature of Cardiff Bay and the resultant high OC burial rate. This, combined with the shallow water column and the occurrence of ebullition during sampling, indicates the potential for significant methane release from Cardiff Bay to the atmosphere.
- Rates of lithotrophic methanogenesis exceed acetoclastic rates, going against the general rule for freshwater sites – the reasons for this will be expanded upon in Chapter 9.

Chapter 9. – General Discussion

This study has shown that the Severn Estuary contains a number of "classic" prokaryotic sedimentary habitats (mudflats, subtidal mud-patches etc.) as well as several more unusual habitats (fluid muds, Holocene peats and an artificial freshwater lake) that contain significant prokaryotic populations and all of which are biogeochemically active. This section compares and contrasts these various sites and examines how the two main anaerobic processes that occur in marine sediments, namely sulphate reduction and methanogenesis, vary across the greater estuary environment and how they relate to oxygen uptake and carbon mineralization. The unusual conditions in the Severn Estuary will be examined in terms of their effects on the size and distribution of prokaryotic populations, and the Severn Estuary (and some of the more unusual microenvironments found within) will be compared with other sites around the world to see how the estuary fits into the global scheme of estuarine biogeochemical processes. In addition conclusions will be drawn on the current state of the Severn Estuary's microbial communities and their influence on potential changes to the estuarine system that may occur due to future environmental change.

9.1 Biogeochemical Processes in the Severn Estuary

9.1.1 Sulphate reduction in the Severn Estuary

The highest rates of sulphate reduction (both on a cm^3 and m^2 basis) measured in Severn Estuary sediments were from cores ST4 (Portishead) and StB2 (St Brides). These results are not unexpected as these two sites provide good habitats for bacterial sulphate reduction within the estuary albeit for different reasons – a (relatively) sheltered environment in the case of Portishead and a large supply of substrates derived from peat in the case of St Brides. However, since *in situ* temperature is one of the main factors controlling the metabolic rate of prokaryotes it is difficult to directly compare respiration rates at these two sites as they were sampled at different times of the year (October and July respectively). Therefore, in order to be able to discuss the possible reasons for rates differing between sites differences in temperature need to be considered. To do this we can utilise Q_{10} values determined for sulphate reduction to extrapolate the potential rates of

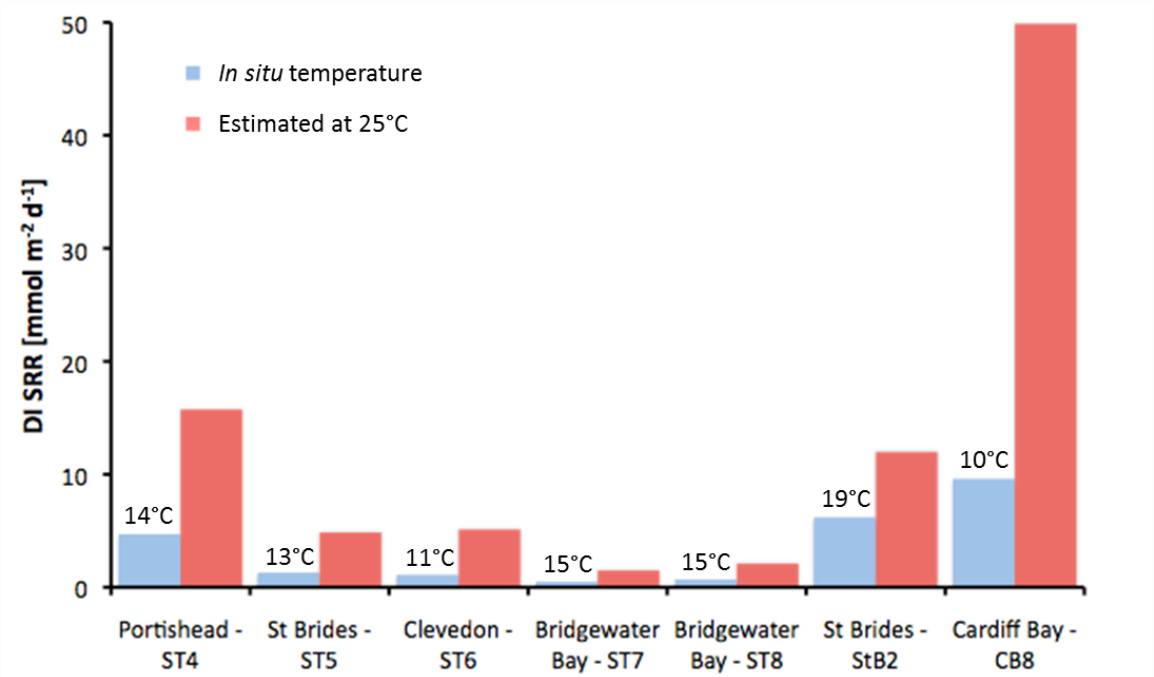


Fig. 9.1 – Graph showing depth integrated sulphate reduction rates (DI SRR) measured in this study at *in situ* temperatures (blue) plotted with the estimated rates at 25°C (red, based on a Q_{10} value of 3). *In situ* temperature values are marked above each column.

sulphate reduction at each site at a constant temperature (e.g. 25 °C). The Q_{10} value chosen for this was 3, as this was the value used by Roden and Tuttle (1993b) as an average value for estuarine and shallow marine environments, although it should be noted that these extrapolated values should always be treated with caution, as Q_{10} values can vary significantly between environments and organisms (Kätterer *et al.* 1998; Shiah *et al.* 2000), and that by their nature as logarithmic functions, small changes in Q_{10} can have drastic effects on rate values. Utilising this method the overall areal rate of sulphate reduction in the main estuary (at 25°C) is highest at Portishead, despite the fact that the core ST4 was significantly shorter than core StB2 (Fig. 9.1). This would suggest that sediment stability is likely to be an important control on sulphate reduction rate (SRR) in the estuary, and that even when an environment is rich in electron-donor substrates, if it is too disturbed (as St Brides is during significant portions of the year) (O'Brien *et al.* 2000) sulphate reduction will be reduced.

The lowest SRRs in the estuary were from Bridgwater Bay, specifically core ST7 (the subtidal mud-patch). Despite the fact that the Bridgwater Bay cores (ST7 and ST8) were incubated at the 2nd highest temperature used in this study (15°C) their SRRs are still significantly lower than those from any other site, both on a

volumetric and areal basis. This again would suggest that sediment re-suspension plays a major role in determining SRR, as of all the sites where SRR was measured in this study these are likely to be the some of the most disturbed, being subtidal and seaward-facing and therefore constantly exposed to the strong tidal currents of the estuary as well as stormy weather conditions originating in the Irish Sea/Atlantic (Kirby, 2010). Interestingly the SRRs from core ST8 were 1.4x higher than those from ST7 despite the fact that, based on sediment colour (and the size of the subsurface nitrate peak), it was more oxidised. This would agree with Aller (1998), and suggests that fluid muds are capable of promoting sulphate reduction relative to similar un-fluidised sediments nearby.

Sulphate reduction rates in the Severn Estuary compare well with some other estuarine habitats at similar latitudes (Table. 9.1) such as the Tamar Estuary in Cornwall (Wellsbury and Parkes, 1995), Himmerfjärden in Sweden (Thang *et al.* 2013) and even others at lower latitudes such as Tomales Bay in California (Chambers *et al.* 2000). They are also not greatly dissimilar to the worldwide average of inner-shelf SRRs (1.07 mmol m^{-2} d^{-1}) documented by Bowles *et al.* (2014). In addition the rates from core ST8 are very similar to those obtained by Madrid *et al.* (2006) from fluid mud beds off the coasts of French Guiana and Papua New Guinea. However, when compared to other estuaries and near-shore environs such as Cape Lookout Bight (Crill and Martens, 1983), the Seine Estuary (Leloup *et al.* 2006) or Bjørnsholm Bay and the Livø Strait (Jørgensen and Parkes, 2010), the rates obtained from the Severn in this study are conspicuously lower. In fact the average SRR from the Severn Estuary sites in this study is 2.42 mmol m^{-2} d^{-1}, 5x lower than the average of other estuarine sites in Table. 9.1 (12.16 mmol m^{-2} d^{-1}). This would indicate that on a global scale the Severn Estuary is towards the lower end of the scale for estuarine SRR measurements, despite the fact that pore water values show that the sediments are not sulphate-limited. This is not totally surprising as the high degree of sediment erosion and re-suspension in the estuary is (as detailed before) likely to lead to a decrease in sulphate reduction over the whole estuary area. However, no other hypertidal estuaries have been studied with regard to sulphate reduction (e.g. the Seine Estuary is macrotidal with a tidal range of ≈6 m), making direct comparison with the extremely dynamic environment of the Severn difficult.

Site Locality	Temperature /Month	Maximum SRR [nmol cm^{-3} d^{-1}]	Depth Integrated SRR [mmol m^{-2} d^{-1}]	Reference
Severn Estuary				
ST4 - Portishead	14°C/Oct	44.4	4.7	This Study
ST5 - St Brides	13°C/Oct	15	1.3	
ST6 - Cleveland	11°C/April	17.1	1.1	
ST7 - Bridgwater Bay	15°C/June	4.9	0.5	
ST8 - Bridgwater Bay	15°C/June	8.7	0.7	
StB2 - St Brides	19°C/July	43.9	6.2	
CB8 - Cardiff Bay	10°C/May	98.1	9.6	This Study
Estuaries and Deltas				
Randers Fjord, Denmark - Winter	3°C		1.9	Sørensen *et al.* 1979
- Summer	18°C		7.8	
Kysing Fjord, Denmark - Winter	3°C		5.8	
- Summer	18°C		15.1	
Norsminde Fjord, Denmark - mean values	2°C/Jan		2	Jørgensen and Sørensen 1985
	10°C/May		6	
	20°C/Aug		13	
	12°C/Oct		10	
Mississippi Delta, USA			35.6	Canfield 1989
Kolding Fjord, Denmark - fish farm	5°C/March	2400		Holmer and Kristensen 1994
Tamar Estuary, England	March	45	3	Wellsbury and Parkes 1995
Kingoodie Bay, Tay Estuary, Scotland	14.5°C/July	260	21.7	Wellsbury *et al.* 1996
Aust, upper Severn Estuary	16.5°C/July	95	9.4	
Great Ouse Estuary, England - mouth	August		37	Trimmer *et al.* 1997
	November		1.3	
Tomales Bay, California	12°C	48		Chambers *et al.* 2000
Seine Estuary, France	17-22°C	25-159		Leloup *et al.* 2006
Scheldt Estuary - Appels (freshwater)	21°C/Feb+Apr	823-1092		Pallud and Van Cappellen 2006
- Waarde (brackish)	21°C/Feb	168-182		
- Rattekai (marine)	21°C/Feb+Mar	720-768		
Bjornsholm Bay, Denmark	13°C	147	13.1	Jørgensen and Parkes 2010
Livo Strait, Denmark	13°C	125	11.9	
Colne Estuary, England - Hythe (low salinity)	7°C/Feb	145		O'Sullivan *et al.* 2013
- Alresford	5°C/Feb	38		
- Brightlingsea	5°C/Feb	20		
Himmerfjarden, Sweden	May-June	40-80		Thang *et al.* 2013
Coastal				
Cape Lookout Bight, North Carolina	May		23	Crill and Martens 1983
	October		106	
Gulf of Papua, Coral Sea	28°C		3.6-6.8	Alongi 1995
Chilean Continantal Shelf	7-12°C/March	170-4670	9.6-74.5	Ferdelman *et al.* 1997
Ise Bay, Japan	24°C/Sept	225		Fukui *et al.* 1997
Tidal Flats				
Wadden Sea (mixed flats) - Winter	5°C		6	Al-Raei *et al.* 2009
- Spring/Autumn	10°C		15	
- Summer	20°C		30	
Fjords				
Loch Linnhe, Scotland	Jan		5.1	Overnell *et al.* 1996
Loch Goil, Scotland	Jan-Nov		2.6	
Loch Fyne, Scotland	Feb-Oct		1.32	
Loch Etive, Scotland	May		1.34	
Malangenfjorden, Norway	7°C		0.99	Sagemann *et al.* 1998
Hornsund, Svalbard	2.6°C		4.15	
Van Mijenfjorden, Svalbard	0.2°C		0.9	
Storfjorden, Svalbard	-1.7°C		1.22	
Bellsund, Svalbard	3.2°C		2.41	
Salt Marsh				
Great Sippewisset Marsh Peat, New England	Summer	6000	420	Howarth and Teal 1979
	Winter		20	
Colne Point, England - saltmarsh pan		15-150	1.8-8.5	Senior *et al.* 1982
- drainage creek		6-180	2.5-12	

Lagoons				
Ningaloo Reef, Western Australia	22°C		1	Alongi *et al.* 1996
Mangrove Bay, Western Australia	22-27°C		6.1-25.3	
Aarhus Bay, Denmark	8°C	38	4.68	Holmkvist *et al.* 2011
	December	95		Webster *et al.* 2011
Ocean				
NE Atlantic		0.1-2.2		Battersby *et al.* 1985
Kattegat, Denmark	5.5°C	19		Jorgensen and Bak 1991
Chilean Coast - 1000-1500 m water depth		0.001-0.012	0.28-1	Canfield *et al.* 2010
Jones Bank, Celtic Sea	10-11°C	7.9-8.2	0.68-1.1	Larsen *et al*. 2013
Rivers				
Ashleworth Quay, River Severn, UK	15.5°C/July	52.5	2.1	Wellsbury *et al.* 1996
Great Ouse Estuary, East Anglia, UK - upriver	August		11	Trimmer *et al.* 1997
	December		46	
Lakes - Eutrophic				
Lake Mendota, Wisconsin	0.5-13°C	50-600		Ingvorsen *et al.* 1981
Wintergreen Lake, Michigan	Summer	96-148		Lovley *et al.* 1982
Lake Kinneret, Israel		5-1600		Hadas and Pinkas 1995
Haringvliet Lake, Netherlands - former estuary			3.59	Canavan *et al.* 2006
Lakes - Mesotrophic				
Lake Washington, Seattle		1.8	0.117	Kuivila *et al.* 1989
Lake Kizaki, Japan	4-6.5°C	0.5-10.1	0.004-0.33	Li *et al.* 1996
Lake Constance, Germany		190	19	Rothfuss *et al.* 1997
Lakes - Oligotrophic				
Lake Michigan		9.1		Namsaraev *et al.* 1994
Little Rock Lake, Wisconsin		8.8	5.2	Urban *et al.* 1994
Lake Baikal, Russia		1.2		Namsaraev *et al.* 1995
Lake Stechlin, Germany	5-7°C	1-13	0.24-2.64	Sass *et al.* 2003
Bog Lakes				
Gerritsfles, Netherlands		180-225	0.27-11.2	Marnette *et al.* 1992
Hypersaline				
Baja California, Mexico - microbial mat	20°C		22-24	Canfield and Marais 1993
	30°C		66-73	
Um Alhool Sabkha, Qatar - microbial mat	15-26°C/Dec	713	15.6	Al-Thani *et al*. 2014
Fluid Muds				
French Guiana	20°C	10-27		Madrid *et al.* 2006
Gulf of Papua	20°C	10		
Cold Seeps				
Green Canyon, Gulf of Mexico	Summer	16-2520		Formolo and Lyons 2013

Table. 9.1 (on this, and previous page) – Table of maximum and depth integrated SRRs from this study and others conducted around the world. *In situ*/incubation temperature and month of sampling are provided for comparison when available.

In the only published study on sulphate reduction rates in the Severn Estuary (Wellsbury *et al.* 1996), the rates from Aust Warth in the upper estuary were considerably higher than those obtained in this study. The reason for this is unknown, although it may be the case that because Aust, like Portishead, is partially protected by rocky shoals and headlands (the English Stones and Goblin Ledge to the south and Aust Cliff to the North) it may be subject to less current-driven re-suspension than the sites in this study, allowing a more stable anoxic environment

to develop. Additionally the Aust site is backed by salt marshes, which provide a potential source of OC substrates (Bianchi, 2007), which might enhance heterotrophic activity.

Compared to the other sites in this study the SRR measurements from Cardiff Bay stand out. Both the maximum and average sulphate reduction/cm^3 are more than double that of the highest Severn Estuary sites even though Cardiff Bay had the lowest *in situ* temperature and sulphate concentrations of all the sites sampled. In addition the depth-integrated rates at this site are also considerably higher than those from the estuary (Table 9.1) despite the fact that the bay cores were shorter than the majority of the estuarine cores. The turnover rate of $^{35}SO_4^{2-}$ was also much greater than at any of the Severn Estuary sites, with up to 77% of the radiolabeled pool metabolised over a 6 hour incubation (at Portishead turnover was <0.09% of the hot pool after 6 hrs). This high rate of sulphate reduction at a freshwater site might seem incongruous considering the low levels of sulphate inside the bay relative to outside, however, there are three factors to consider when comparing the bay to the sediments in the estuary proper. The first is the high level of organic matter in the bay's sediments compared to those outside. Since the upper layers of the sediment column in Cardiff Bay are made up of semi-intact plant material, the sediment is rich in organic carbon compounds - ≈17-18% of sediment weight measured by loss on ignition (Miriam Olivier, unpublished results). This would provide an abundance of electron donor substrates for the heterotrophic SRB, thus promoting their activity. The second factor is that compared to outside the estuary the bay sediments undergo relatively little re-suspension (with the possible exception of Site 2 due to the influence of the River Taff). As such they will retain a more stable anoxic environment promoting the growth of obligate anaerobes like the SRB. Finally, it has been noted that freshwater SRB species tend to have very low half saturation (K_m) values for sulphate, this allows these "high affinity" groups to carry out sulphate reduction even at the low levels of sulphate present in fresh water sediments (Ingvorsen and Jørgensen, 1984b). If these groups are indeed present in the sediments of Cardiff Bay it would help to account for the very active rates of sulphate reduction occurring within the sediments.

Comparing the SRR from Cardiff Bay to other eutrophic lakes, such as Wintergreen Lake in Michigan (Lovley *et al.* 1982) and Lake Mendota in Wisconsin (Ingvorsen *et al.* 1981), Cardiff Bay rates are in the range expected for natural lakes of this type within temperate climate zones (Table. 9.1). In addition, the rates for the bay sediments are of the same order of magnitude to those obtained by Canavan *et al.* (2006) from Haringvliet Lake, a former estuary and now artificial freshwater eutrophic lake in the Netherlands, probably the closest analogue to Cardiff Bay. This suggests that SRRs from Cardiff Bay are not unusual on a worldwide scale and that in terms of sulphate reduction Cardiff Bay is acting as a normal eutrophic lake.

9.1.2 Methanogenesis in the Severn Estuary

Of the estuarine sites examined in this study it is the sediments at St Brides that have the highest rates of methanogenesis. On both a volume and areal basis cores ST5 and StB2 had some of the highest rates of both acetoclastic and H_2/CO_2 methanogenesis, despite being one of the more disturbed sites with regard to sediment re-suspension. This probably relates to the presence of the peat layer at St Brides supplying the overlying sediment with OC and other substrates. Despite being from the same site, core StB2 had higher rates than core ST5, although this is likely due to increased *in situ* temperatures and less re-suspension of the recent overlying sediment during the summer when core StB2 was sampled. Methanogenesis rates were not extrapolated to 25°C in this case due to the extreme variability in methanogenic Q_{10} values (between 1.3-28) (Segers, 1998; van Hulzen *et al*. 1999). In terms of acetoclastic methanogenesis the lowest areal rates came from the subtidal mud patch in Bridgwater Bay (core ST7) and Clevedon (core ST6) suggesting that, as with sulphate reduction, high levels of re-suspension inhibit acetoclastic methanogenesis. Interestingly in core ST8 areal rates of acetoclastic methanogenesis are stimulated relative to core ST7 by ≈5x, again suggesting that fluidised muds may promote methanogenesis, albeit at low levels. With regard to H_2/CO_2 methanogenesis, the lowest depth integrated rates were from Clevedon and Portishead. This, coupled with the fact that one of the highest rates came from core ST7, suggests that sediment re-suspension appears to have less of an influence on the rates of lithotrophic methanogenesis than it does on heterotrophic

methanogenesis and that other factors (possibly salinity or quality of organic matter) may play a more important role.

When compared with rates of methanogenesis at Aust Warth (Wellsbury *et al.* 1996), the rates for the Severn Estuary sites in this study are considerably lower. As with the SRR described above this is possibly due differences in the geochemistry between the two sites relating to re-suspension or OC supply. Globally the results from this study are also rather low (Table. 9.2), compared to results obtained from salt marshes (Senior *et al.* 1982, Parkes *et al.* 2012) and coastal lagoons (Deborde *et al.* 2010), and considerably lower than other coastal sites such as Cape Lookout Bight (Crill and Martens, 1983), Saanich Inlet (Kuivilla *et al.* 1990) and Kiel Harbour (Schmaljohann, 1996). The reason that methanogenesis rates are on the low end of the scale is again likely due to the fact that hypertidal estuaries (and other areas with high rates of sediment re-suspension) have been poorly studied with regard to their biogeochemistry, and as such there are few sites that are directly comparable to the Severn Estuary in terms of environmental conditions.

Interestingly at all of the estuarine sites studied here, methanogenesis was not just confined to the lower sections of the cores and in several cases (e.g. cores ST4 and ST7) both turnover to methane, and production rates were highest near the top of the core even when active sulphate reduction was occurring. This would indicate that there is not a strict zonation of metabolisms as described by Froelich *et al.* (1979) and Canfield and Thamdrup (2009) - with methanogenesis occurring only once sulphate had been depleted – present in these dynamic estuarine sediments. It is possible that this standard "redox cascade" model does not occur in these estuarine sediments to the same extent as in more passive marine systems, due to their regular disturbance by strong tidal currents. This disturbance might prevent the formation of a steady-state biogeochemical cascade, instead creating a constant state of flux in which small "pioneer" communities of SRB, methanogens etc. are distributed throughout the sediment, constantly trying to become established and grow wherever and whenever they can before they are inevitably re-suspended again. Since the SRB and methanogen communities would be kept from growing to too great a size by the regular re-suspension of their habitat, which could lead to

Site Locality	Temperature /Month	Maximum Acetoclastic Methanogenesis [pmol cm^{-3} d^{-1}]	Maximum H_2/CO_2 Methanogenesis [pmol cm^{-3} d^{-1}]	Maximum Total Methanogenesis [nmol cm^{-3} d^{-1}]	Depth Integrated Acetoclastic Methanogenesis [µmol m^{-2} d^{-1}]	Depth Integrated H_2/CO_2 Methanogenesis [µmol m^{-2} d^{-1}]	Depth Integrated Total Methanogenesis [µmol m^{-2} d^{-1}]	Reference
Severn Estuary								
ST4 - Portishead	14°C/Oct	0.42	0.69	0.00091	0.058	0.0335	0.0915	This Study
ST5 - St Brides	13°C/Oct	5.93	11.26	0.01719	0.131	0.503	0.634	
ST6 - Clevedon	11°C/April	0.76	0	0.00076	0.029	0	0.029	
ST7 - Bridgwater Bay	15°C/June	0.21	6.06	0.00606	0.0067	0.487	0.4937	
ST8 - Bridgwater Bay	15°C/June	0.26	4.05	0.00414	0.035	0.197	0.232	
StB2 - St Brides	19°C/July	15.6	98.9	0.10732	1.087	3.798	4.885	
CB5 - Cardiff Bay	6°C/Jan	173	10796	10.969	16.66	1327	1343.66	This Study
CB6 - Cardiff Bay	6°C/Jan	447	9725	9.776	16.58	491	507.58	
CB7 - Cardiff Bay	6°C/Jan	60.9	1190	1.221	3.548	35	38.548	
Estuary								
Kingoodie Bay, Tay Estuary, Scotland	14.5°C/July		≈700			80		Wellsbury *et al.* 1996
Aust Warth, Severn Estuary	16.5°C/July		≈700			130		
Kolding Fjord, Denmark - fish farm	5°C/March			1300				Holmer and Kristensen 1994
Beaulieu Estuary, England	16°C/April	5000	11000					Banning *et al*. 2005
Colne Estuary, England - Hythe (low salinity)	7°C/Feb	1150	180	1.33				O'Sullivan *et al.* 2013
- Alresford	5°C/Feb	2.4	4.8	0.0072				
- Brightlingsea	5°C/Feb	0.3	20	0.0203				
Himmerfjarden, Sweden	4°C/May-June		3250			2630-2980		Thang *et al.* 2013
Coastal Lagoon								
Cape Lookout Bight, North Carolina	February						960	Crill and Martens, 1983
Balandra Bay, Baja California - mangrove fluvisol	25-28°C/March-April			1-23				Giani *et al.* 1996
Arcachon Lagoon, France							1.44-811.2	Deborde *et al.* 2010
Aarhus Bay, Denmark	December	10						Webster *et al.* 2011
Salt Marsh								
Colne Point, England - saltmarsh pan	July		140					Senior *et al.* 1982
- drainage creek	July		90					
Sippewisset Marsh, Massachusetts - microbial mat	May					172		Buckley *et al.* 2008
	Sept					30		
Arne Peninsula, England	20°C	4800	38	4.84				Parkes *et al.* 2012
Fjord								
Saanich Inlet, British Columbia				0.84-6.68				Kuivila *et al.* 1990
Princess Louisa Inlet, British Columbia				1				
Marine								
Kiel Harbour, Baltic Sea	January						981.6	Schmaljohann 1996
	Oct			2.4-14.4			1963	
Mississippi Canyon, Gulf of Mexico - cold seep			110					Bowles *et al.* 2011
Monterey Bay, California			360					
River								
White Oak River, North Carolina - tidal	Oct-Nov						1250-13000	Kelley *et al.* 1995
Ashleworth Quay, River Severn	15.5°C/July		170,000			8750		Wellsbury *et al.* 1996

Site Locality	Temperature /Month	Maximum Acetoclastic Methanogenesis [pmol cm^{-3} d^{-1}]	Maximum H_2/CO_2 Methanogenesis [pmol cm^{-3} d^{-1}]	Maximum Total Methanogenesis [nmol cm^{-3} d^{-1}]	Depth Integrated Acetoclastic Methanogenesis [µmol m^{-2} d^{-1}]	Depth Integrated H_2/CO_2 Methanogenesis [µmol m^{-2} d^{-1}]	Depth Integrated Total Methanogenesis [µmol m^{-2} d^{-1}]	Reference
Eutrophic Lake								
Wintergreen Lake, Michigan - sulphate depleted/profundal				960				Lovley *et al.* 1982
Dotkas, Latvia - 3 m water depth	June			184.73-660.41				Dzyuban 2002
- 1 m water depth	July			29.89-655.95				
- 3 m water depth	July			700.58-946				
Mustyarv, Estonia - 7 m water depth - chthonoeutrophic				69.16-134.76				
Drukshyai, Estonia - 2 m water depth				0.446				
- 5 m water depth				24.09-81.21				
- 30 m water depth				700.58-919.23				
Linoyarv, Estonia - 10 m water depth - hypereutrophic				3145-4730				
Lake Dagow, Germany	4°C/July			7.92				Glissmann *et al.* 2004
	30°C/July			116.16				
Mesotrophic Lake								
Stropu, Latvia - 2 m water depth				<0.446-15.17				Dzyuban 2002
- 6 m water depth				14.27-104.8				
Vishki, Latvia - 2 m water depth				0.446-722.89				
- 12 m water depth				15.61-28.55				
Tivera, Estonia - 5 m water depth				20.08-299.86				
Lake Biwa, Japan	8°C			≈20				Koizumi *et al.* 2003
Oligotrophic Lake								
Dridzas, Latvia - 3 m water depth				<0.446-51.31				Dzyuban 2002
- 30 m water depth				0.892-17.84				
Lake Uttersee, Antarctica - water column	<1°C/Feb	0.017-0.082	0.25-439.6	0.000281-0.439.6				Wand *et al.* 2006
- sediment	<1°C/Feb	5-23.3	258.1-874.2	0.277-0.880				
Lake Stechlin, Germany	30°C/November			39.8-415				Conrad *et al.* 2007
Diamond Lake, Michigan	September			≈30				West *et al.* 2012
Monomictic Lake								
Lake Kinneret, Israel				21.6				Adler *et al.* 2011
Meromictic Lake								
Knaack Lake, Wisconsin	10°C			2.88-18.24				Winfrey and Zeikus 1979
Peat Bog/Fen								
Bakchar Bog, Siberia	4°C	36	21	0.06				Kotsyurbenko *et al.* 2004
	15°C	408	268	0.624				
	25°C	576	346	0.9				
Michigan Hollow - mineratrophic fen	22°C			86				Sun *et al.* 2012
Chicago Bog - ombrotrophic bog	22°C			59				
Mclean Bog (New York State) - ombrotrophic kettle bog	22°C			0.031				

Table. 9.2 (on this, and previous page) - Table of maximum and depth integrated methanogenesis rates from this study and others conducted around the world. *In situ*/incubation temperature and month of sampling are provided for comparison when available.

exposure to toxic oxygen or physical break-up of the community, they would not compete for common substrates to the same degree as the developed communities present in more stable sediments. This would explain why methanogens can be found throughout the sediment column and also why the rates of both methanogenesis and sulphate reduction in these sediments are low. Similar co-occurrence of supposedly competing microbial processes has been described by O'Sullivan *et al.* (2013) from the macrotidal Colne Estuary, where the lack of relationship between prokaryotic community depth profiles and geochemical changes, coupled with the homogeneous nature of the DNA profiles at this location, was suggested to be possibly related to recent disturbance of the sediments. This lack of specialised zonation with depth may also be the reason that Webster *et al.* (2010) were unable to detect either active sulphate reduction or methanogenesis in their Severn Estuary slurries from Portishead (despite the results from this study proving that both processes are occurring *in situ* at this site), as the geochemical depth zones that the authors attempted to sample may not in fact have been sufficiently enriched in the organisms in question relative to the rest of the sediment.

Due to the high concentrations of methylated compounds present at the St Brides site, core StB2 was collected specifically to measure the utilisation of methylated compounds (using ^{14}C-DMA and ^{14}C-Choline). Despite being important substrates for methanogenesis in certain environments (Oremland *et al.* 1982, Summons *et al.* 1998) where they allow methanogens to metabolise even in the presence of active sulphate reduction, the rates of methanogenesis from methylated amine consumption have not been widely measured. When compared with the other radiotracer substrates used in this study, DMA produces the highest depth integrated rate of methanogenesis for the Severn Estuary sites (57.4 μmol m^{-2} d^{-1}), whereas choline produced one of the lowest (42 nmol m^{-2} d^{-1}). It should be noted however, that the DMA and choline rates were calculated using the concentration of the ^{14}C-substrate (the hot pool) added to the concentration of the corresponding compound measured *in situ* (the cold pool) as the *in situ* concentration was often too low to be reliably quantified. This comparatively high rate of DMA metabolism (92% of total methanogenic activity in core StB2) is not unusual as other studies have

found that methylated amine compounds are often preferentially utilised by methanogens in shallow sediments (Parkes *et al.* 2012), due to them acting as non-competitive substrates and can form a major part of the overall methanogenic potential of the sediments (King *et al.* 1983). The methanogenic rate measurements for choline are harder to put in context, as choline has only recently been confirmed as a direct methanogenic substrate (Watkins *et al.* 2012), and hence, this is the first *in situ* rate measurement of choline methanogenesis. When compared to other methanogenic substrate rates from the Severn Estuary however, it appears that choline metabolism plays a relatively minor role in total methanogenesis (0.04% in core StB2). This may be because choline can act as a substrate for other prokaryotic groups such as fermenters (King, 1984) and SRB (Nielsen *et al.* 1999, Alazard *et al.* 2003, Pecheritsyna *et al.* 2012), which could out-compete the methanogens for the (apparently) limited supply of choline in the sediments. If these observations are repeated elsewhere then it may be that direct choline metabolism plays only a minor role in influencing global methanogenesis budgets. However, considering that methanogenesis as a whole appears to be dramatically affected by the unusual sedimentary regime in the Severn Estuary (i.e. re-suspension and peat beds at this site) it may be premature to extrapolate these results more widely when considering the overall global importance of choline as a direct methanogenic substrate - particularly when considering the widespread sources of choline in the marine environment (Summons *et al.* 1998).

As with the SRRs, Cardiff Bay methanogenesis rates contrasted starkly with those from the Severn Estuary (Table. 9.2). When compared with core ST4 from Portishead (the most topographically similar site to the Cardiff Bay pre-impoundment) it would appear that the creation of the barrage has dramatically increased the methanogenic potential of the bay sediments. At Portishead the average rate of acetoclastic methanogenesis was 171 fmol cm^{-3} d^{-1} compared to an average rate of 11-60 pmol cm^{-3} d^{-1} in Cardiff Bay (dependant on site). These rates equate to an areal acetoclastic methane production rate of 58 nmol m^{-2} d^{-1} at Portishead, substantially lower than the 3.5-17 μmol m^{-2} d^{-1} from Cardiff Bay. When H_2/CO_2 methanogenesis rates are compared the difference is even more startling with the average rate at Portishead (93 fmol cm^{-3} d^{-1}) several orders of magnitude

lower than in Cardiff Bay (117-4739 pmol cm^{-3} d^{-1}). The difference is equally large on an areal basis, with the depth-integrated rate at Portishead (33.5 nmol m^{-2} d^{-1}) lower than those obtained from Cardiff Bay (35-1327 μmol m^{-2} d^{-1}) by a factor of ≈1000-40,000. This difference is not surprising as methanogenesis is a much more important pathway for anaerobic organic matter mineralization in freshwater environments than in marine systems, and shows that the conversion of Cardiff Bay from a tidal flat to a eutrophic lake (with low levels of re-suspension and high OC loading) has dramatically altered the biogeochemistry of its sediments.

Of particular interest is the increased rate of H_2/CO_2 methanogenesis compared to acetoclastic methanogenesis in Cardiff Bay's freshwater sediments. This is unusual as acetoclastic methanogenesis is conventionally thought to be the dominant pathway in freshwater systems (Whiticar *et al.* 1986; Whiticar, 1999). However, there are some freshwater environments where CO_2 reduction makes a greater contribution to the methane budget. For example: Lansdown *et al.* (1992) found that in peats from Kings Lake Bog in Washigton State, USA, CO_2 reduction made up >99% of the methanogenesis occurring in the sediment; and Horn *et al.* (2003) found that CO_2 reduction was the major source of methane in sediments from a peat bog in the Fichtelgebirge, a forested mountainous region in northeastern Bavaria. Although, both of these locations were acidic peat bogs, a quite different environment to Cardiff Bay. With regard to lacustrine environments Nozhevnikova *et al.* (2007) found that in sediments taken from Lake Baldegg in Switzerland, up to 98% of methanogenesis could be via the H_2/CO_2 pathway, however, this was only the case in enrichments incubated under thermophilic conditions (50 °C), whereas under psychrophilic (5 °C) and mesophilic (30 °C) conditions acetate was the dominant precursor of 95% and 80% of the methane produced, respectively. This decrease of H_2/CO_2 methanogenesis at low temperatures was also noted by Schulz and Conrad (1996) and Glissmann *et al.* (2004), which conflicts with the results obtained from the Cardiff Bay cores as they were collected in winter when *in situ* temperatures were well within the range for psychrophilic/psychrotolerant growth (<15 °C, Madigan and Martinko 2006). On the other hand, studies by Wand *et al.* (2006) and Mandic-Mulec *et al.* (2012) on cold-water sites (Lake Untersee, Antarctica and Lake Bled, Slovenia) both indicated that CO_2 reduction was the main route for methanogenesis at these locations at *in situ* temperatures (1-6 °C). This

discrepancy in results would appear to indicate that temperature is not the controlling factor determining the dominant methanogenic pathway occurring in lacustrine sediments, such as those of Cardiff Bay. It has also been suggested that the relative proportion of acetoclastic:hydrogenotrophic methanogenesis is controlled by the amount of organic carbon substrates entering the lake environment, with acetoclastic methanogenesis dominating in areas with a high level of OC and hydrogenotrophic methanogenesis dominating in OC poor environments. However, since Lake Untersee is oligotrophic and Lake Bled and Cardiff Bay are eutrophic (Jüttner *et al.* 2010) it is unlikely that the overall quantity of organic matter entering the lake environment is the controlling factor either. Instead it could be possible that the "quality" of the organic matter being deposited in the lake is the key factor governing the type of methanogenesis occurring (specifically the quantity of labile OC vs. refractory OC) as acetoclastic methanogens are usually associated with higher levels of labile OC while hydrogenotrophic groups tend to be linked to environments dominated by more refractory compounds (Hornibrook *et al.* 2000). Since Cardiff Bay is considered a eutrophic system (Jüttner *et al.* 2010) it would be expected that the supply of labile organic matter (from diatoms, water-borne bacteria etc.) would be high. However, since these cores were collected in winter, when temperatures and solar luminosity are low, it is possible that very little diatomaceous/bacterial production was occurring in the overlying waters and entering the sediment, and thus the production of labile OC remained low. In addition, during the summer months algal blooms that are typical of eutrophic lakes do not occur in the bay, due in part to the management of the bay's water quality by the harbor authority and the high flushing rates within the bay, which prevent the build-up of limiting nutrients (Andrews and Gulson, 2002, Jüttner *et al.* 2010). As such though the bay may be technically classified as a eutrophic system it does not appear to be able to produce the high levels of labile OC usually associated with such environments. During the winter months large amounts of decaying vegetation are washed down the Taff and Ely rivers and into the bay by heavy rains, however, being terrestrially derived this material is likely too refractory for acetoclastic methanogens to degrade. As such, at least during the winter months, conditions in Cardiff Bay would likely lead to limited labile OC input to the sediments, favouring lithotrophic H_2/CO_2 methanogenesis over the acetoclastic pathway.

Compared to other sites around the world (Table. 9.2), Cardiff Bay methanogenesis rates are similar to other eutrophic and mesotrophic lakes such as Lake Dagow in Germany (Glissmann *et al.* 2004), Drukshyai in Estonia and Stropu in Latvia (Dzyuban 2002). It is also comparable with tidal rivers such as the White Oak River in North Carolina (Kelley *et al.* 1995). On the other hand the rates are considerably lower than some Eutrophic lakes such as Dotkas in Latvia (Dzyubahn 2002) and Wintergreen Lake in Michigan (Lovley *et al.* 1982), however, this is likely due to the fact that the measurements in this study were carried out during the coldest part of winter, and therefore, probably represent the minimum rates for this site.

9.1.3 Oxygen Uptake and Organic Matter Degradation in the Severn Estuary

Oxygen uptake (and by proxy total OM degradation) was high in all the sites studied. The highest uptake values obtained came from Clevedon (99 mmol m^{-2} d^{-1}) and St Brides (81 mmol m^{-2} d^{-1}), which were also the tidal flat sites considered to be most effected by re-suspension (O'Brien *et al.* 2000) (Table. 9.3). However, the lowest rates of O_2 uptake were from Bridgwater Bay, which is also considered to be regularly subjected to re-suspension events (Kirby 2010). This suggests that the re-suspension of sediment prevalent across the Severn Estuary can both promote and hinder microbial growth. On tidal flats such as Clevedon and St Brides occasional re-suspension events could increase prokaryotic activity by regularly supplying the sediments with fresh OC substrates and electron acceptors. However, in subtidal environments such as Bridgwater Bay the sediments might be subject to more (and potentially harsher) re-suspension resulting in the sediment being more disturbed, and suppressing bacterial activity and populations unable to cope with their unstable environment, meaning that optimum communities cannot develop. Another possible explanation is the variation in sediment composition between the sites, as Trimmer *et al.* (2000) found that sediments from sites in the Thames Estuary with a higher sand content tended to have lower O_2 uptake values than muddy sites. This can in part be due to the OM content of the sediment, which tends to be lower in sandy sediments (Kristensen *et al.* 2000). Since the subtidal sediments of the Severn where O_2 uptake was measured (cores ST7 and ST8) contained a higher percentage of sand than those from the coastal mudflats (cores

Site Locality	Temperature /Month	Sediment O_2 Uptake [mmol m^{-2} d^{-1}]	Reference
Severn Estuary			
ST4 - Portishead	14°C/Oct	52	This Study
ST5 - St Brides	14°C/Oct	81	
ST6 - Clevedon	11°C/April	99	
ST7 - Bridgwater Bay	15°C/June	44	
ST8 - Bridgwater Bay	15°C/June	37	
CB5 - Cardiff Bay	6°C/Jan	61	This Study
CB6 - Cardiff Bay	6°C/Jan	51	
CB7 - Cardiff Bay	6°C/Jan	51	
Estuaries			
Chesapeake Bay, USA	May	47-66	Boynton and Kemp 1985
	August	47-97	
Norsminde Fjord, Denmark - mean values	2°C/Jan	7.5	Jørgensen and Sørensen 1985
	10°C/May	19	
	20°C/Aug	38	
	12°C/Oct	35	
Kingoodie Bay, Tay Estuary, Scotland	14.5°C/July	57.3	Wellsbury *et al.* 1996
Aust, Severn Estuary, England	16.5°C/July	30.6	
San Francisco Bay, California	Feb-May	7-48	Grenz *et al.* 2000
Thames Estuary, UK		36	Trimmer *et al.* 2000
Brunswick Estuary, New South Wales	15-30°C	12-37	Ferguson *et al.* 2003
Simpsons Estuary, New South Wales	14-30°C	29-36	
Sandon Estuary, New South Wales	14-31°C	15-32	
Neuse River Estuary, North Carolina	May-July	14-34	Fear *et al.* 2004
Danshuei River, Taiwan	20°C	20-57	Liu *et al.* 2009
Narragansett Bay, Rhode Island	6°C	15	Fulweiler *et al.* 2010
	24°C	64	
Charlotte Harbor, Florida	March	46.5	Kim *et al.* 2010
	Sept	32.1	
Cochin Backwater, India - dry	April	61.6	Abhilash *et al.* 2012
- wet	August	34.3	
Weeks Bay, Alabama	21°C	17.1	Mortazavi *et al.* 2012
Himmerfjärden Estuary, Sweden	3.1-5.3°C/June	10-30	Bonaglia *et al*. 2014
	3.5-7.6°/Oct	6-17	
Providence River Estuary, Rhode Island	24°C/Sept	40.3	Brin *et al.* 2014
Salt Marshes			
Plum Island Sound, Massachusetts		30-189	Vieillard and Fulweiler 2012
Fjords			
Fanafjorden, Norway	6-10°C	16-30	Wassmann, 1984
Loch Linnhe, Scotland	Jan	14.1	Overnell *et al.* 1996
Loch Goil, Scotland	Jan-Nov	19.4	
Loch Fyne, Scotland	Jan-Oct	14.3	
Loch Etive, Scotland	May	15.6	
Lagoons			
Ningaloo Reef, Western Australia	22°C	2.1	Alongi *et al.* 1996
Mangrove Bay, Western Australia	22-27°C	10.5	
Corunna Lake, New South Wales		11-42	Spooner and Maher 2009

Coastal			
Georgia Bight, USA	11°C/Jan 11°C/Nov	38 67.5	Hopkinson Jr. 1985
Cape Lookout Bight, North Carolina	11°C/Feb 25°C/August	44 126	Chanton *et al.* 1987
Gulf of Papua, Coral Sea	28°C	18-47	Alongi 1995
East Anglia, England		12-60	Trimmer *et al.* 2000
Pine Island Sound, Florida		4.3-101	Kim *et al.* 2010
Ocean			
Northeast Pacific	June-July	2.9-21	Devol and Christensen 1993
Mud Volcano, Gulf of Cadiz	May	4.4-13.2	Sommer *et al.* 2008
Oregon continental shelf	≈7°C/Jun-Aug	1.1-9.8	Reimers *et al.* 2012
Jones Bank, Celtic Sea	10-11°C	5.8-9	Larsen *et al*. 2013
Rivers			
Ashleworth Quay, River Severn, UK	15.5°C/July	31.4	Wellsbury *et al.* 1996
River Thames, England		90	Trimmer *et al.* 2000
Old Woman Creek, Ohio	20-23°C/July	29-53	McCarthy *et al.* 2007
Keelung River, Taiwan	20°C	8-49	Liu 2009
Lakes - Eutrophic			
Lake Ton-Ton. Uraguay	April August	54 28	Sommaruga 1991
Lake Myvatn, Iceland	10-12°C/June 2-6°C/Oct	80-120 30-80	Thorbergsdóttir *et al.* 2004
Lakes - Mesotrophic			
Lake Erie, Ohio	20-23°C/July	13-22	McCarthy *et al.* 2007
Lakes- Oligotrophic			
Lake Stechlin, Germany	4-8°C/October	8.4	Sass *et al*. 2003
Marshes			
Busatello River, Italy	4°C 17°C	12.7 56.9	Longhi *et al.* 2013

Table. 9.3 (on this, and previous page) – Table of depth integrated sediment oxygen uptake rates from this study and others conducted around the world. *In situ*/incubation temperature and month of sampling are provided for comparison when available.

ST4, ST5 and ST6) it is possible that this could also account for the differences in O_2 uptake between sites. Finally the tidal flat sites in this study were notable for the presence of benthic macrofauna, which were not apparent in the subtidal cores. Since these organisms are by default aerobes they could also contribute to the sediment oxygen demand (SOD) and thus increase the O_2 uptake at the tidal flat sites. However, this would not explain why Portishead, a site with ample evidence of bioturbation by polychaetes would have an SOD substantially lower than the other tidal flat sites. This would suggest that re-suspension, possibly coupled with sediment composition is the driving force governing the rate of O_2 uptake.

Site Locality	% Contribution of Process to Organic Carbon Degradation		
	Sulphate Reduction	Methanogenesis	Other
Portishead - ST4	18	0.0004	81.9
St Brides - ST5	3.2	0.002	96.7
Clevedon - ST6	2.2	0.00006	97.8
Bridgwater Bay - ST7	2.3	0.002	97.7
Bridgwater Bay - ST8	3.8	0.001	96.2
Kingoodie Bay	75.7	0.28	24
Aust Warth	61.4	0.85	37.7
Ashleworth Quay	13.4	55.7	30.9

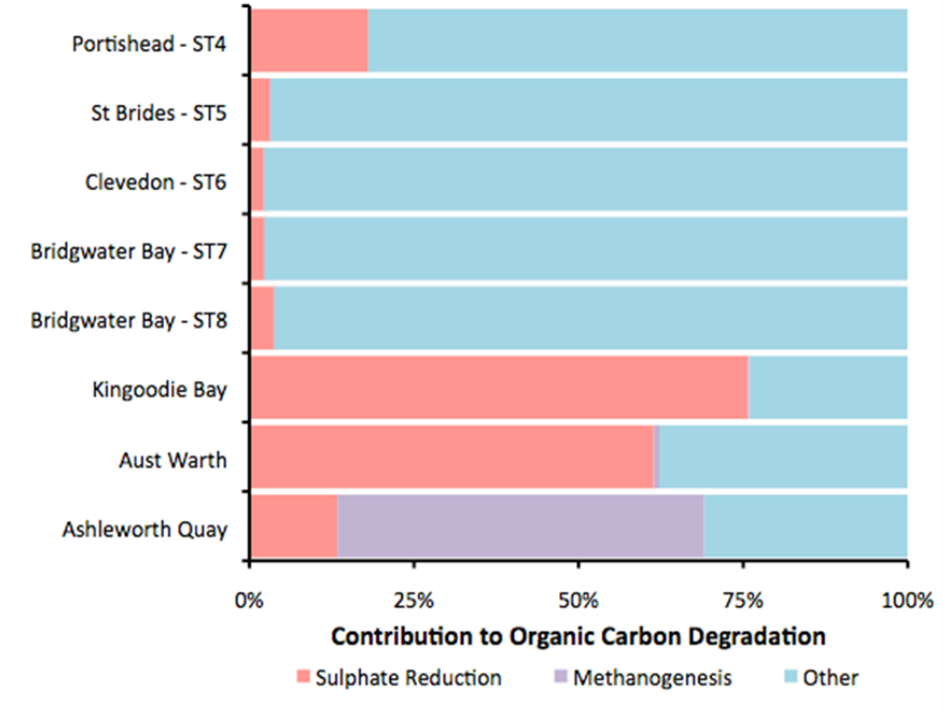


Table 9.4 and Fig. 9.2 – Table and graph showing the percentage contribution of sulphate reduction, methanogenesis and other unmeasured processes (e.g. aerobic respiration, nitrate reduction etc.) to organic carbon degradation in the Severn Estuary (as determined from sediment oxygen uptake). Results from this study (cores ST4-ST8) are shown alongside those obtained by Wellsbury *et al.* (1996) from the authors' previous study of the Tay (Kingoodie Bay) and Severn Estuaries/Rivers (Aust Warth and Ashleworth Quay).

Compared to total oxygen uptake (Table 9.4 and Fig. 9.2) sulphate reduction (via sulphide oxidation) makes up a relatively small percentage of the total oxygen demand of the sediments (≈2-4%) in most of the Severn Estuary sites investigated here. This would indicate that even though the sediments of the estuary are biogeochemically active, sulphate reduction itself does not play a major role in carbon mineralisation at these sites, the only exception being at Portishead where sulphate reduction makes up 18% of the total SOD. Since the percentage of O_2 uptake/OC degradation due to methanogenesis is also very low (<0.002%) it is probable that the majority (90-99%) of the O_2 demand in the sediments of the

Severn Estuary is accounted for by processes further up the redox cascade such as aerobic respiration, nitrate reduction (including via nitrification), and Fe^{3+} and Mn^{3+} reduction (including via Fe^{2+} and Mn^{2+} oxidation). This dominance of higher redox-state processes is again likely due to the high degree of re-suspension in the estuary which would oxygenate the sediment, favouring aerobic and microaerophillic organisms even in an environment usually dominated by sulphate reduction (Jørgensen, 1982). As a case in point, the site with the highest percentage of O_2 uptake accounted by sulphate reduction was Portishead, the site thought to be the most stable in terms of sediment resuspension (the second highest percentage site is ST8 which will be discussed later).

In Cardiff Bay the O_2 uptake rates show that carbon mineralisation is lower on average than in the Severn Estuary (average 63 mmol m^{-2} d^{-1}) with values between 50-60 mmol m^{-2} d^{-1} (average 54 mmol m^{-2} d^{-1}). This may reflect the fact that, despite the high influx of OM, the sediments inside the bay are not subject to the same amount of re-suspension as those outside and as such aerobic bacterial metabolism is not stimulated to the same degree (though it should be remembered that when the sediment samples from Cardiff Bay were collected the *in situ* temperature was significantly lower than during the estuary sampling which likely effected the rate of O_2 uptake). In addition, anaerobic sulphate reduction rates are probably sulphate limited while significant amounts of methane from methanogenesis may escape into the atmosphere, thereby reducing these processes' contribution to the SOD. With regard to sulphate reduction the relative contribution to carbon degradation is around 30% although this should be taken with a degree of caution as the O_2 uptake and SRR measurements were taken at different times of the year (January and May respectively) and as such may not be comparable. Methanogenesis measurements on the other hand are directly comparable and show that 0.2-4.4% of the OM degradation in the bay is via this pathway, considerably more than in the Severn Estuary. This is to be expected as methanogenesis plays a greater role in freshwater environments than marine environments due to the limited sulphate concentrations in freshwater systems. The greater importance of methanogenesis is also likely linked to the lack of re-suspension and higher amounts OC present in the sediments of the bay.

When compared to a previous study of the Severn Estuary by Wellsbury *et al.* (1996), the O_2 uptake rates from the Severn Estuary sites presented here are up to 3x higher than those at Aust Warth. In addition, the relative percentage of OC degradation accounted for by SRB and methanogens is much higher at Aust than in the sites studied here. This difference is, as with the SRR and methanogenesis results described above, likely linked to Aust being a more sheltered site and experiencing less regular and/or less severe re-suspension events, resulting in both a more stable environment for SRB and methanogens, and less stimulation of the more redox-positive processes. On a global basis the Severn Estuary O_2 uptake rates in this study are high but still fit within the normal range of 0.1-140 mmol m^{-2} d^{-1} described by Santschi *et al.* (1990) (Table. 9.3). These high values may again be because few other estuaries with a similar tidal range, and therefore, a similar degree of sediment re-suspension and subsequent aerobic stimulation, have been studied. The uptake rates for Cardiff Bay are similar to those measured in other shallow, eutrophic lakes under cold temperatures (2-6°C), such as Lake Mývatn in Iceland (Thorbergsdóttir *et al.* 2004).

Taken together the data presented here on SRR, methanogenesis rates and SOD suggests that the unusual tidal state of the Severn Estuary, with it's strong erosive currents and rapid removal and re-deposition of sediment, creates an environment that promotes the development of highly active aerobic and dysaerobic microbial communities but probably at the expense of more redox-negative anaerobic groups such as the SRB and methanogens.

9.1.4 Prokaryotic Cell Numbers in the Severn Estuary

Average total cell numbers in the sediments of the Severn Estuary (Table 9.5) are relatively high suggesting that despite the unstable sediment regime in the estuary significant prokaryotic populations are still able to thrive in these conditions. In addition these populations are metabolically active, particularly (as described above in section 9.1.3) with regard to processes nearer the top of the redox cascade. When plotted together with O_2 uptake, it can be seen that the depth-integrated numbers of prokaryotic cells in the top 13 cm of the estuarine cores and those from Cardiff Bay correlate well with the SOD suggesting a strong link between

Core	Average Total Cell Count [Log_{10} cells cm^{-3}]	Average TC Above Step Down [Log_{10} cells cm^{-3}]	Average TC Below Step Down [Log_{10} cells cm^{-3}]	Depth Integrated Cell Count Whole Core [Log_{10} cells cm^{-2}]	Depth Integrated Cell Count Top 13 cm [log_{10} cells cm^{-2}]	% of DI TC Above Step	% of DI TC Below Step
ST1	8.96	9.41	8.73	10.05	10.01	71	29
ST2	9.06	9.23	8.81	10.10	10.03	80	20
ST3	9.68	n/a	n/a	10.91	10.53	n/a	n/a
ST4	9.17	9.59	8.92	10.50	10.39	74	26
ST5	9.52	n/a	n/a	10.44	10.44	n/a	n/a
ST6	9.27	9.71	8.76	10.71	10.58	91	9
ST7	9.17	9.53	9.01	10.48	10.31	57	43
ST8	9.45	n/a	n/a	10.65	10.24	n/a	n/a
StB2	9.06	n/a	n/a	10.59	10.37	n/a	n/a
Core	**Average Total Cell Count [Log_{10} cells cm^{-3}]**	**Average TC Above Clay [Log_{10} cells cm^{-3}]**	**Average TC In Clay [Log_{10} cells cm^{-3}]**	**Depth Integrated Cell Count Whole Core [Log_{10} cells cm^{-2}]**	**Depth Integrated Cell Count Top 13 cm [log_{10} cells cm^{-2})**	**% of DI TC Above Clay**	**% of DI TC In Clay**
CB5	9.15	9.57	8.82	10.46	10.38	80	20
CB6	9.21	9.35	8.78	10.50	10.32	92	8
CB7	8.81	9.19	8.72	10.07	9.88	40	60

Table. 9.5 – Table showing the average and depth integrated total cell counts (TC) for the Severn Estuary and Cardiff Bay sites in this study. For Severn Estuary sites averages are given for the whole core and for the sections of the core above and below the step-down in cell numbers (when one is present). For Cardiff Bay averages are given for the sections above and below the gyttja:clay boundary. Depth integrated (DI) values are given for the whole core and the top 13 cm, as well as the percentage of the DI value above and below the step-down (Severn Estuary) and the clay boundary (Cardiff Bay).

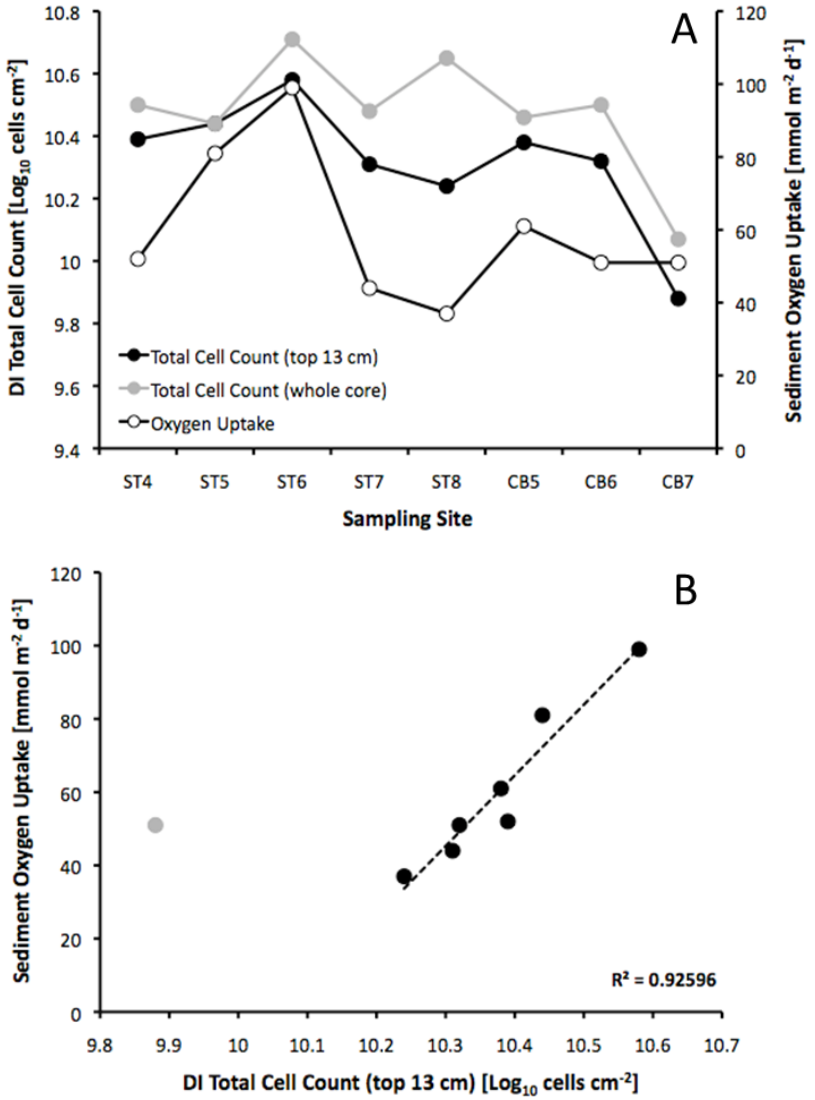


Fig. 9.3 – Graphs showing: A) the relationship between depth integrated (DI) cell counts in both the top 13 cm, the whole core and oxygen uptake for cores taken from the Severn Estuary and Cardiff Bay. B) The correlation between DI cell counts in the top 13 cm and oxygen uptake. The data point marked in grey represents core CB7 which does not fit the pattern of the other cores likely due to the shallow depth of the gyttja clay interface in this core.

increased total cell numbers and an increased demand for O_2 (Fig. 9.3). The exception to this is core CB7, likely due to the fact that the gyttja:clay interface was near the surface in this core. On the other hand, when depth-integrated cell numbers from the full length of the cores are compared with O_2 uptake the correlation is much less obvious, probably reflecting that the processes carried out by prokaryotes deeper in the sediment column have less of an effect on the overall SOD than those nearer the surface. This is consistent with the limited contribution of anaerobic activity to the SOD observed in the cores in this study.

An interesting feature of the AODC depth profiles obtained from the Severn Estuary is the presence of a sharp and distinctive "step-down" in cell numbers with depth at most sites (Fig. 9.4). The depth at which this decrease in numbers occurs varies between cores but it usually occurs over a space of only 2-4 cm, often coinciding with a change in sediment colour from brown to black indicating a change from oxidised to reducing conditions. Although prokaryotic cell numbers generally decrease with depth in the sediment, this decrease is usually in a more constant and regular fashion (Rublee and Dornseif 1978; Parkes *et al*. 2007; O'Sullivan *et al*. 2013), making this sudden drop in cell numbers unusual. When plotted against the global depth trends in cell numbers with sediment depth (Parkes *et al.* 2000; Parkes *et al.* 2014) it can be seen that above the step-down in cell numbers, cell counts remain at relatively constant near-surface values even at significant depth (e.g. ≈18 cm in core ST6). Below the step-down, cell numbers decrease to values within the predicted upper limits and then continue to follow this trend with depth. This would suggest that some process occurring in the Severn Estuary is affecting the upper layers of the sediment and allowing surface-sized populations to occur at considerable depths. In addition, that the depth at which the step-down occurs varies between sites suggests that the degree to which this process is occurring must also vary across the estuary.

As previously discussed in Chapter 3, Wellsbury *et al.* (1996) found similar surficial cell numbers at depth in sediments from Aust Warth in the upper Severn Estuary, with cell numbers of 14-15x10^9 cells cm^{-3} (1x$10^{10.2}$) down to ≈8 cm depth. The authors suggested that this lack of variation in the upper sediment layers was the result of a recent re-suspension event that could have eroded and mixed the top 8 cm of sediment before quickly depositing it again. Such an event would have produced a homogeneous layer of sediment with constant cell counts throughout.

Such re-suspension events could be the cause of the step-down in cell numbers in the cores examined in this study as well, as similar erosive conditions to those present at Aust Warth occur throughout the Severn Estuary and can indeed be more severe in some of the more open-aspect sites in this study (e.g. Clevedon, core ST6). In addition, the fact that the sediment above the step-down was usually brown in colour (e.g. cores ST1, 4, 6 and 7) may suggest that it had been recently re-suspended and oxidised upon mixing with oxygenated seawater. This would also

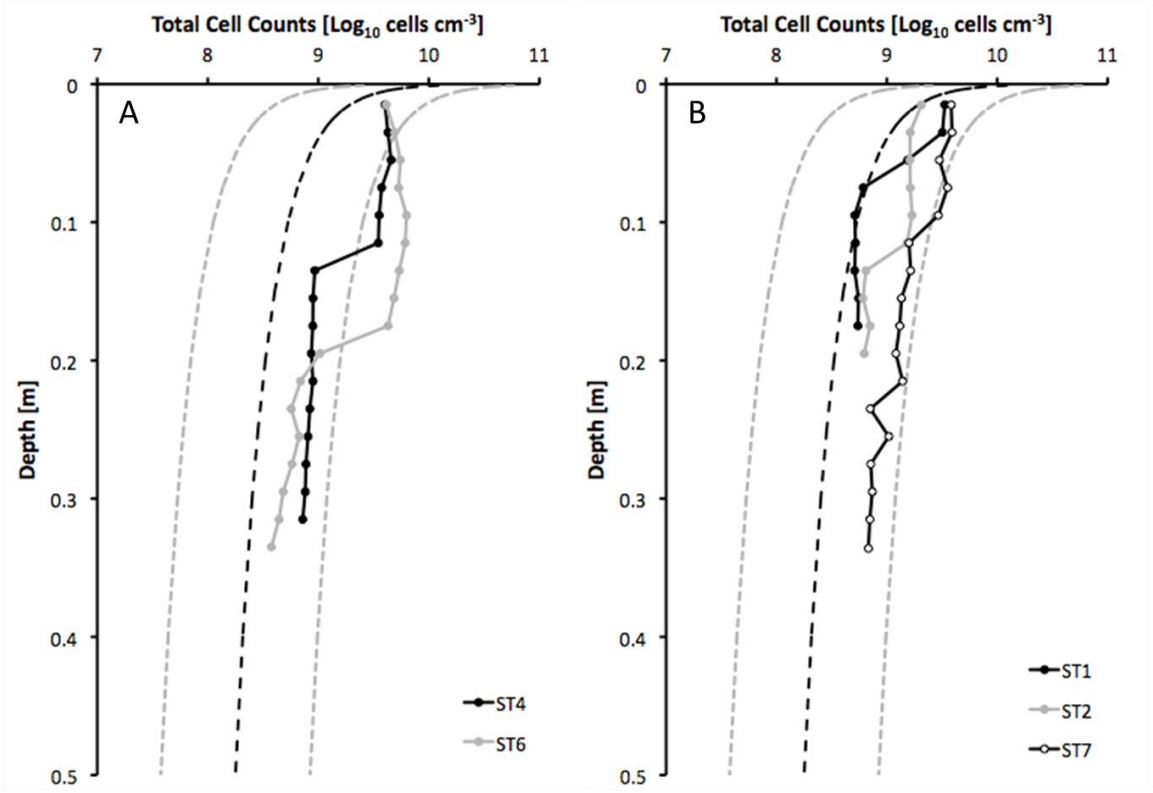


Fig. 9.4 – Depth profiles of total cell counts obtained from A) tidal flat sites and B) subtidal sites in the Severn Estuary plotted against the global predicted decrease in cell numbers with depth (black dashed line) and 95% upper and lower prediction limits (grey dashed line) (Parkes *et al.* 2014). Note the "step-down" in cell numbers evident at depth, most evident in the tidal flat cores. Core ST5 has been omitted due to the change in lithology with depth occurring in this core.

mean that the step-down itself could represent the erosional boundary between the oxidised, recently re-suspended sediment and the reduced, more continuously emplaced sediments below. Finally, in the cores from more exposed sites (e.g. core ST6) the step-down occurs at greater depth than in those from more sheltered sites (e.g. core ST4), which suggests that the process causing the step down may be linked to the extent of erosion at a site. The only core that does not fit with this hypothesis is core ST2 as in this case the step-down occurs at a considerable depth (≈11 cm) despite the fact that the colour change in the sediment (indicating a change in redox state) occurs within a few millimetres of the surface. Also core ST2 was from a deeper water location than core ST1 and as such erosive conditions would be thought to be less harsh. However, the step-down in core ST2 occurs at a greater depth than in ST1, which would mean that a greater degree of re-suspension would have needed to have occurred at this site. This discrepancy could though, be explained if the upper sediment section in core ST2 had been emplaced longer than

at the other sites. If so then it could be the case that the metabolic activity of the prokaryotes could have lead to the sediments becoming reduced before the prokaryotic cell populations had had time to adjust to the more reducing conditions. This delay in the formation of a "normal" cell profile could be prolonged if the re-suspension of the sediment mixed labile OC homogeneously into the sediment prior to deposition. As such, after the sediment had been deposited the deeper layers of sediment would still contain a high OC content, and thus elevated cell numbers, without relying on a gradually decreasing diffusive OC supply from the surface. As for the question of erosion, it is possible that while site ST2 is less likely to be subject to wave-driven re-suspension of sediment it may be that strong tide-driven bottom currents at this location could be capable of eroding and re-depositing the ≈11 cm of sediment needed to generate this cell profile.

Another possibility is that the raised cell counts in the upper layers of sediment are caused by porewater flow supplying the subsurface sediments with organic and inorganic nutrients derived from seawater. Such porewater-driven stimulation was suggested by Gittel *et al.* (2008) as an explanation for the presence of surface-like cell numbers at up to 5 metres depth in tidal flat sediments from the Wadden Sea. The authors also suggested that the high sand content of the sediments aided the flow of porewater to the deeper sediment layers promoting prokaryotic growth. As such it is less likely that such water flow is the main driver for the step-down cell profiles in the Severn Estuary sediments in this study, as the depth of the step-down does not positively correlate with the amount of sand in the sediments. For example in core ST7, which contained more sand than other cores (excluding those from the fluid mud beds), the step down occurred at lesser depth (9.5 cm) than in cores ST4 (11.5 cm) or ST6 (17.5 cm) and was not of the same magnitude as either of the two tidal flat cores. Therefore, while porewater flow may have played a role in maintaining the elevated cell counts above the step-down via wave pumping or advection of nutrient-rich waters (Santos *et al.* 2012), it is more likely that erosion, mixing and re-deposition of sediment were the main causes for this phenomenon at the Severn Estuary sites.

Prokaryotic cell numbers from Cardiff Bay sediments were also high at the surface and were similar to those obtained from Portishead, indicating impoundment of the

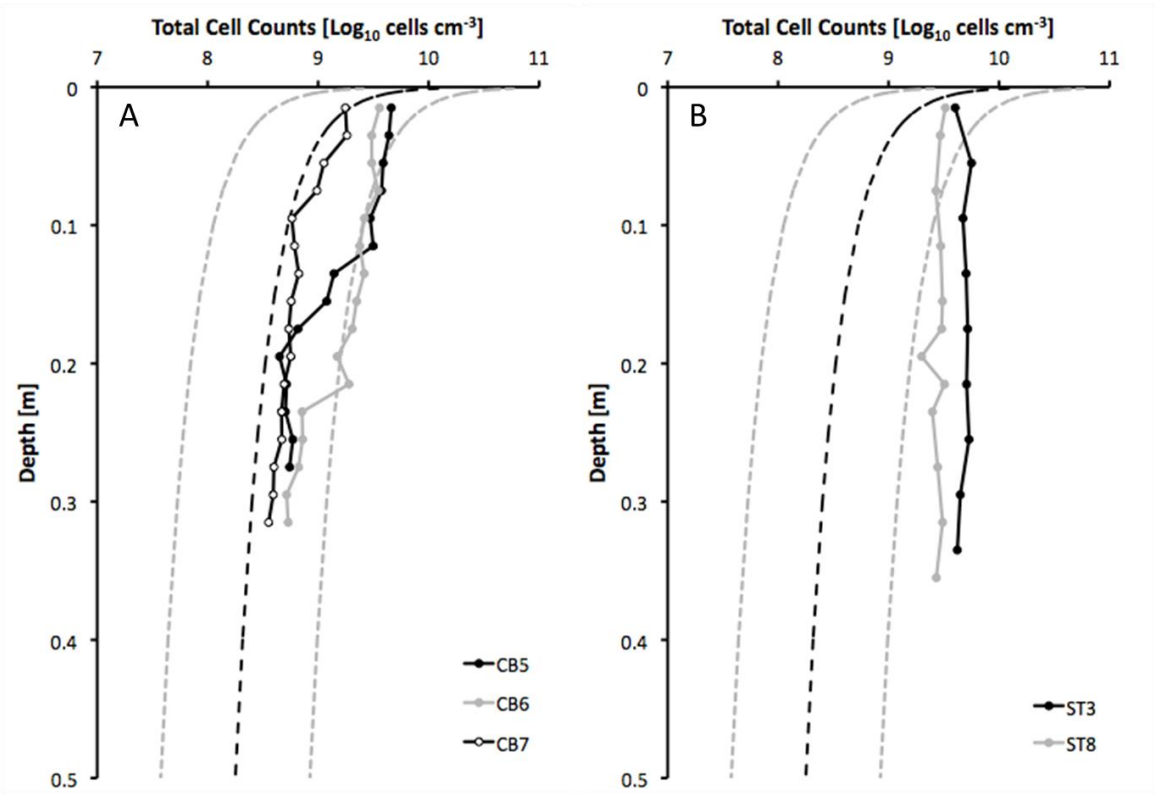


Fig. 9.5 - Depth profiles of total cell counts obtained from A) Cardiff Bay and B) the fluidised mud cores ST3 and ST8, plotted against the global predicted decrease in cell numbers with depth (black dashed line) and 95% upper and lower prediction limits (grey dashed line) (Parkes *et al.* 2014).

bay has not negatively impacted the overall size of its sedimentary prokaryotic population (Fig. 9.5A). There was some variation in whole-core averages across the bay however, which correlated with the relative proportion of clay to gyttja in the cores, suggesting that the clay may be a poorer habitat for prokaryotes than the overlying gyttja. Several of the Cardiff Bay cores also show a distinct step change in cell numbers with depth. However, unlike in the estuary, in this case the cell numbers in the surficial sediment do show a gradual decrease with depth in line with the upper prediction limits of global cell numbers, before dropping relatively sharply (over 2-8 cm) to values more in line with the average predicted trend and then continuing to decrease with depth following this line. Since the sediments in Cardiff Bay are not subject to re-suspension in the same manner as sites in the estuary, there must be an alternative explanation for the step-down in cell numbers. In this case it is likely to be due to the change in lithology from organic-rich, gyttja to more organic-poor clay which is likely causing the decrease in cell numbers, especially as the variation in depth that the step-down occurs at in each core

matches the depths at which the gyttja transitions into the clay. Similar lithology-related changes in cell numbers to these were also observed by Kieft *et al.* (1995) in a borehole in Washington State, USA and by Beck *et al.* (2009) in the German Wadden Sea. This would also further confirm that the clay layer is a less suitable habitat for prokaryotes than the gyttja.

Two cores that did not show a sudden step-down in cell numbers with depth were cores ST3 and ST8 (Fig. 9.5B). In contrast these cores had a relatively constant and vertical AODC depth profile with no decline in cell numbers with depth. This lack of decrease with depth (as described in Chapter 3 and Chapter 6) is likely due to these cores originating in a fluid mud pool where the frequent mixing of the sediment would lead to a homogeneous profile not unlike those seen above the step-down in cell numbers in the other estuarine cores. If this is the case then these would represent one of the first total cell count profiles for a fluidised mud pool (Abril *et al.* 1999). This consistency of cell numbers with depth means that when average and depth-integrated cell numbers are compared cores ST3 and ST8 have some of the highest whole core values. However, when numbers in just the top 13 cm of the cores are compared the difference is much less marked, possibly indicating that conditions in the upper re-suspended and oxidised sections of the other cores are similar to those that persist throughout cores ST3 and ST8.

Unlike the other tidal flat cores the AODC profile for the St Brides core (StB2) shows three separate step-downs in cell numbers with depth (Fig. 9.6). However, these decreases have more in common with those from Cardiff Bay than from the other Severn Estuary sites, as they relate more to changes in sediment lithology rather than re-suspension. The total count values for this core are also more variable than in the others described previously, mainly due to the problems encountered when trying to sample small quantities of peat with a pipette (detailed in Chapter 7). Although despite this, three distinct drops in cell numbers can still be seen – at 15 cm, 21 cm and 35 cm respectively. The first of these drops corresponds to the change in lithology from estuarine mud (where cell numbers are comparable to those in the near-surface of other tidal flat sites) to Holocene saltmarsh peat, and may reflect the decrease in the availability of seawater-derived compounds (such as

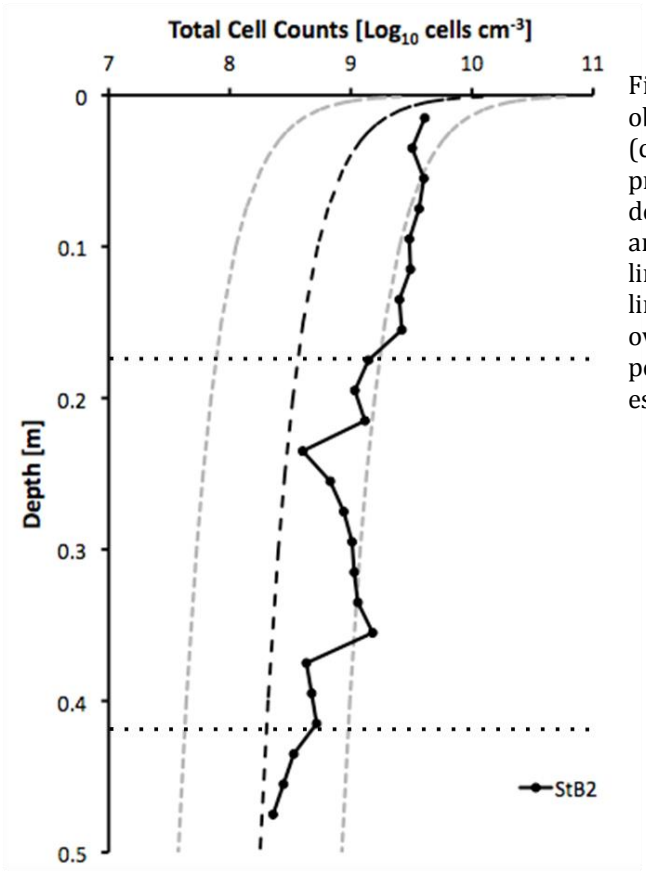


Fig. 9.6 - Depth profile of total cell counts obtained from the St Brides Wentlooge site (core StB2) plotted against the global predicted decrease in cell numbers with depth (black dashed line) and 95% upper and lower prediction limits (grey dashed line) (Parkes *et al.* 2014). Horizontal dotted lines represent changes in lithology from overlying recent estuarine mud to Holocene peat (17 cm) and from peat to Holocene estuarine clay (41 cm).

fresh labile organic carbon) that likely coincides with this transition. The second occurs at near the base of the "activated interface" layer and may correlate with the decrease in active sulphate reduction and methanogenesis rates occurring here. Finally the third drop coincides with a change in the textural lithology in the peat bed, from a predominantly reed-dominated composition to one containing more woody material and then subsequently into the Wentlooge Clay beneath. This again may cause a change in the availability and quality of the organic carbon pool, which could cause a decrease in the overall prokaryotic population of the sediment. When compared to the global predicted trendline, values are still high, reaching just above the upper prediction limit even at 37 cm. This suggests that even in the centre of the peat bed microbial populations are still reasonably high despite the apparent inhibition of several major anaerobic processes, as detailed in Chapter 7, and especially when compared with sediments of a similar age (Parkes *et al.* 2000), probably due to the high organic carbon content of the peat.

9.2 Statistical Analyses

In order to further elucidate any relationships between the sites previously described in this study, statistical multi-parametric relationship analyses were carried out on the data sets obtained from the cores. Hierarchical cluster analysis was performed using Spotfire™ (TIBCO, Boston, MA) using default settings for Euclidean distance analysis. Principal component analysis was performed utilising the XLSTAT add-on for Microsoft Excel (Addinsoft, New York, NY). Hierarchical clustering allows data relationships to be examined without data reduction and is suitable for both large and small data sets and retains all information in the resulting heatmaps and dendrograms allowing further visual analysis of the clusters generated. Principal component analysis (PCA) was used as an independent analysis method to confirm the results obtained by hierarchical clustering, however this data reductionist approach is less suitable for data sets with small number of parameters, and the resulting principal component scatterplots can be less informative of causative relationships.

Both the dendrogram and the heatmap generated by the cluster analysis of the whole data set (Fig. 9.7) clearly show several distinct clusters within the data. At the top of the heatmap there is a distinct cluster (A) made up of samples from Cardiff Bay, below which is a large cluster comprising the data obtained predominantly from the subtidal sites within the Severn Estuary (B). Below this is another smaller cluster containing data from core StB2 alongside cores ST4 and ST5 (C) and finally at the base of the heatmap is a larger cluster of Cardiff Bay data interspersed with data from the St Brides cores (D).

Zooming in on the top of the heatmap (Fig. 9.8) shows that the top Cardiff Bay cluster is made up mainly of data from the cores taken from the bay in February 2011 (CB2, 3 and 4) with some data from CB6 and CB7 also present. This may reflect changes in the *in situ* geochemistry that could have occurred over the course of the year between sampling dates, similar to those discussed in Chapter 8. Within the large Severn Estuary cluster mentioned above (Fig. 9.9), it appears that the sites that are thought to be subject to more re-suspension activity (e.g. ST3, 6, 7 and 8) also cluster together well, again indicating that sediment disturbance is one of the factors contributing to the shape of their geochemical profiles. Also included in this cluster

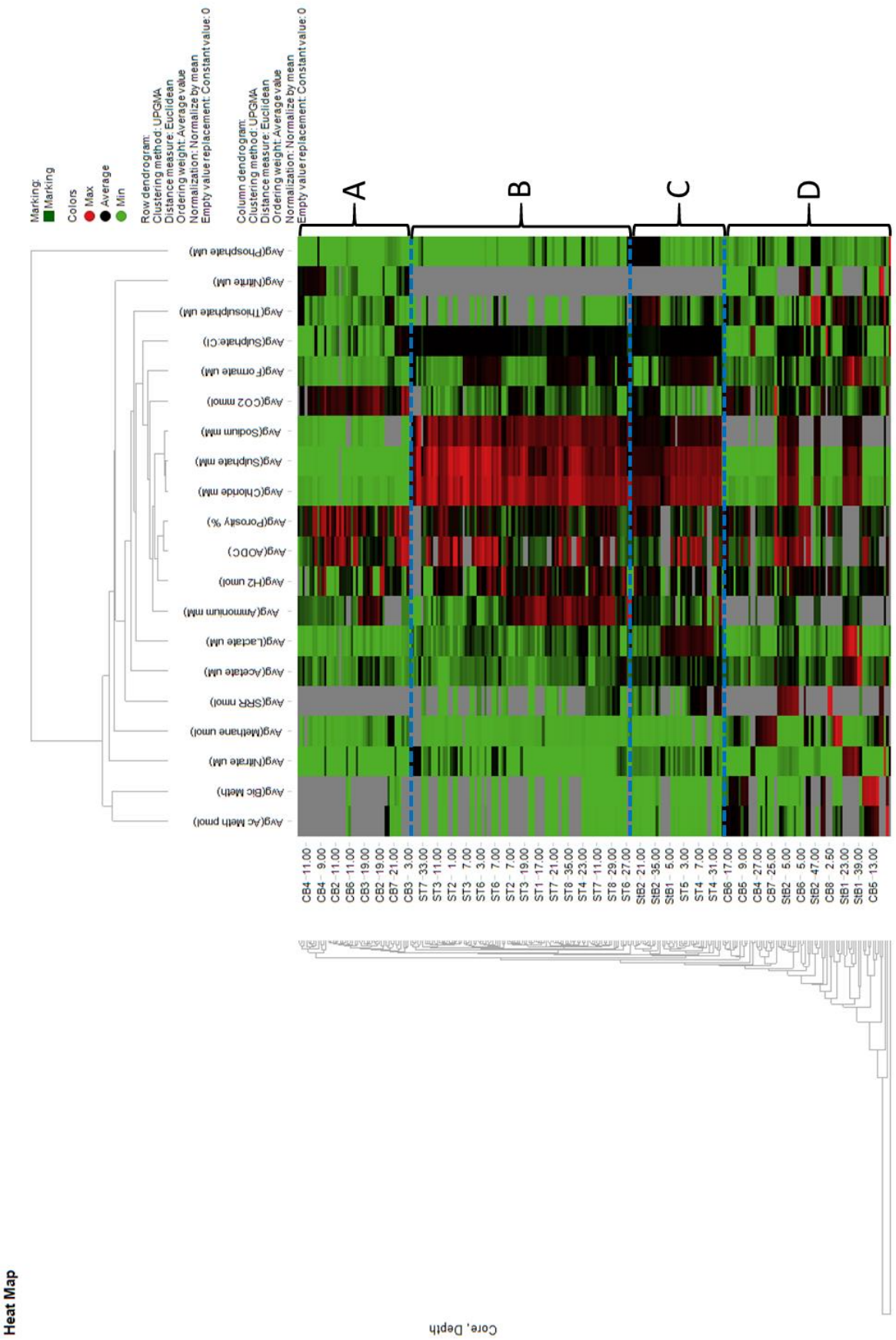


Fig. 9.7 – Dendrogram and heatmap produced from the hierarchical clustering analysis of the data set obtained from this study. Divisions between large-scale clusters are denoted by blue dashed lines.

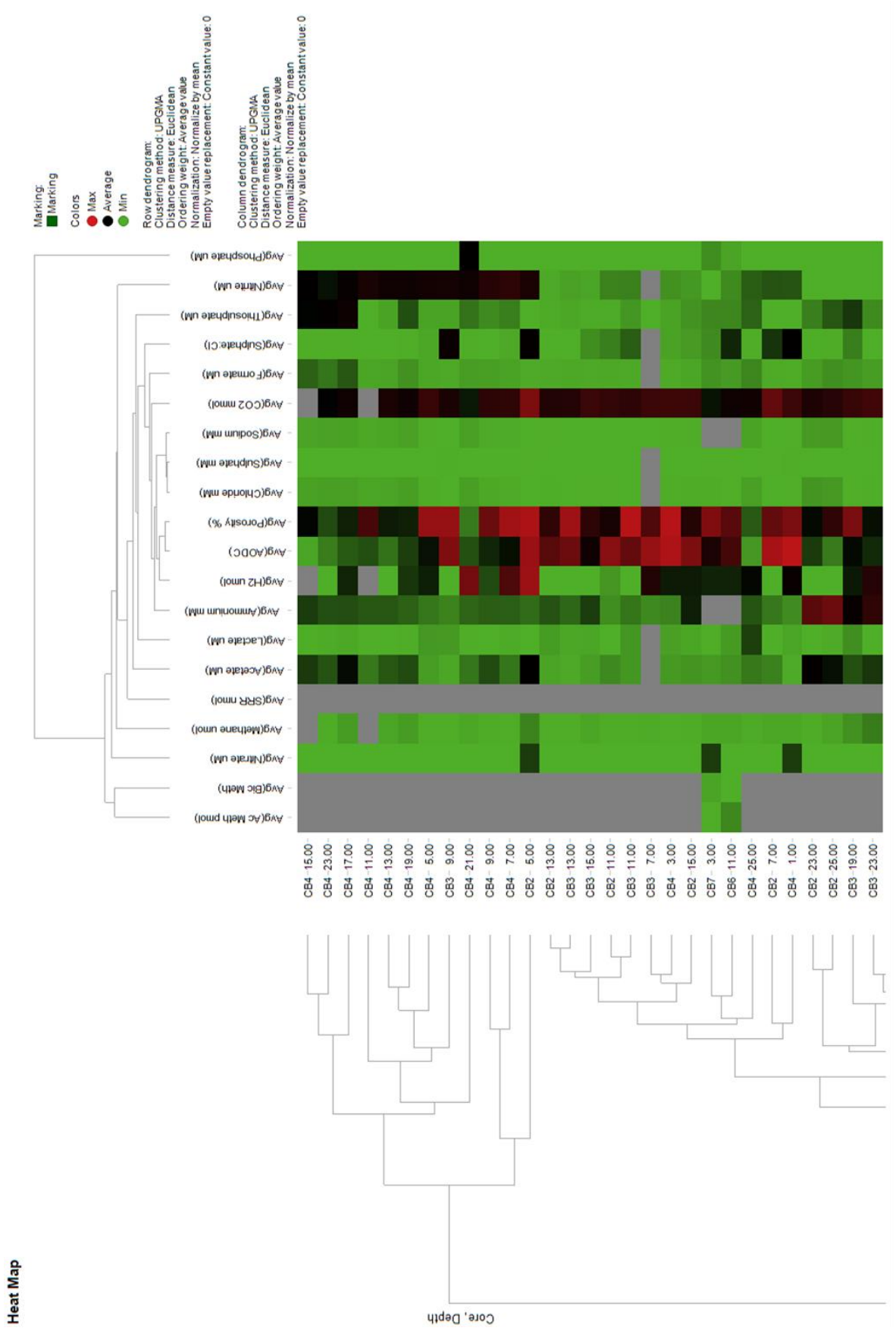


Fig. 9.8 – Magnified view of Fig. 9.7A, showing the clustering of the Cardiff Bay data mostly obtained from February 2011.

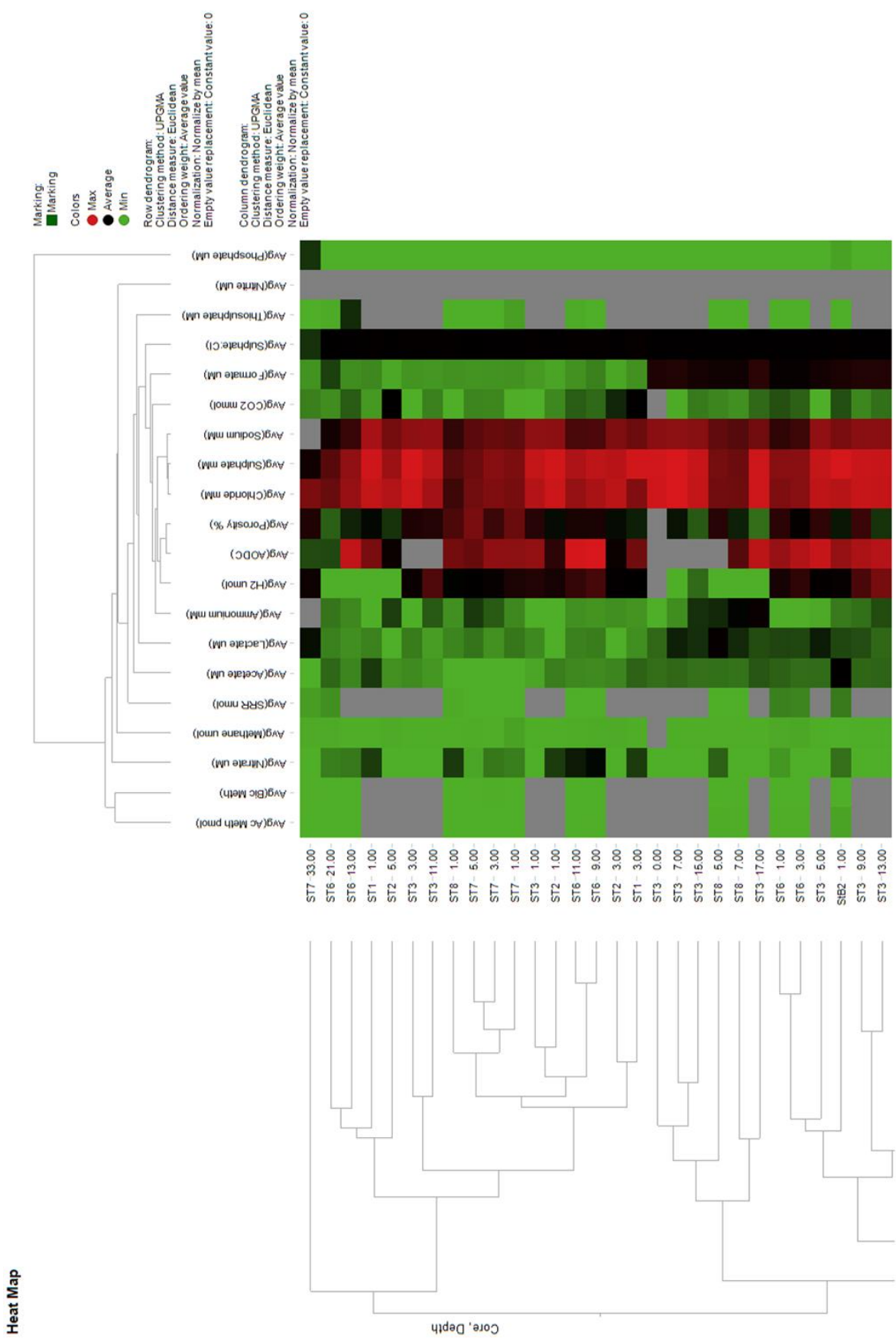


Fig. 9.9 – Magnified view of Fig. 9.7B showing the clustering of the more disturbed subtidal and intertidal sites alongside surficial data from less disturbed sites.

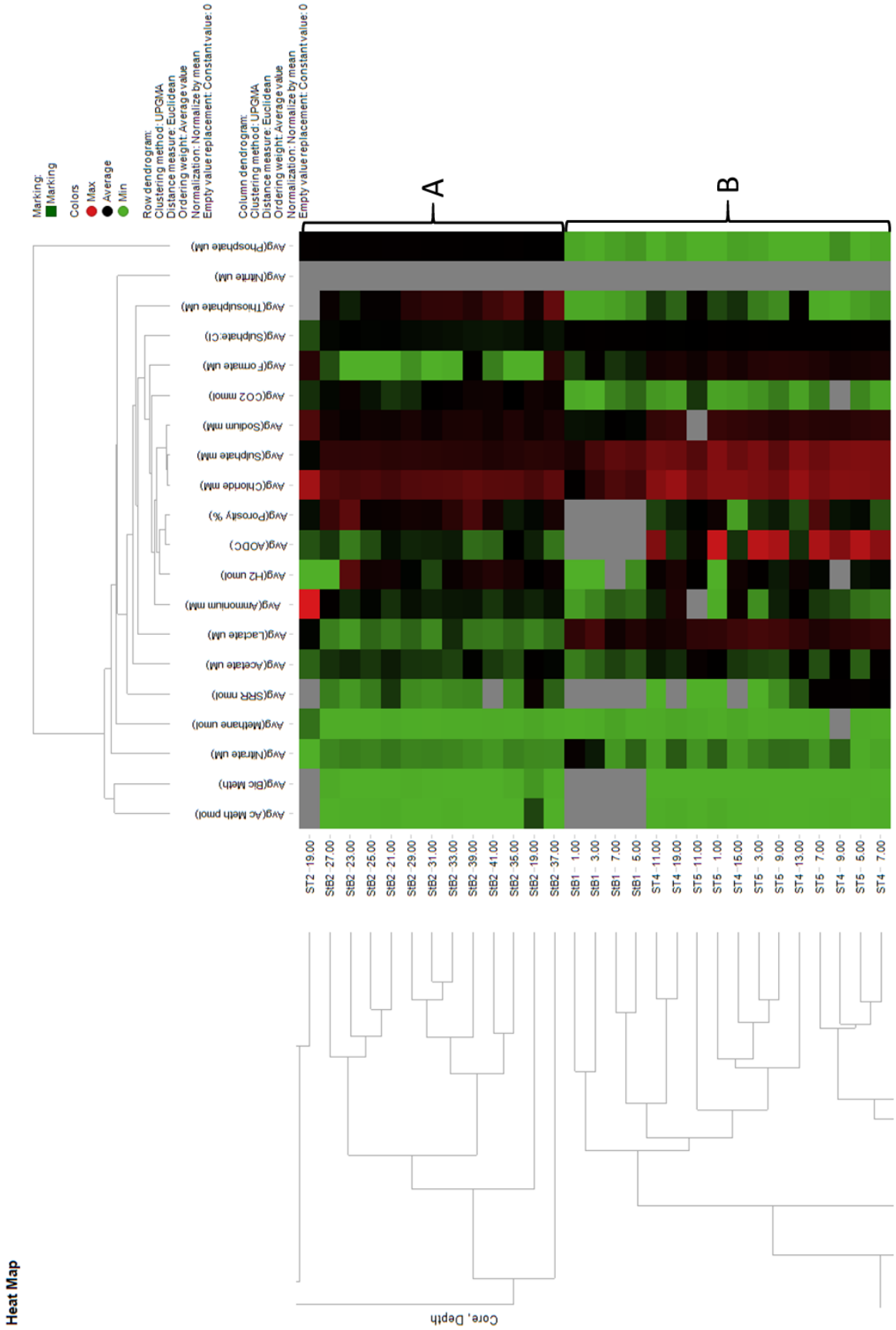


Fig. 9.10 – Magnified view of Fig. 9.7C showing the clustering of data from: A) the peat layer of StB2, and B) core ST4 and the muddy surface sediments of cores ST5 and StB1.

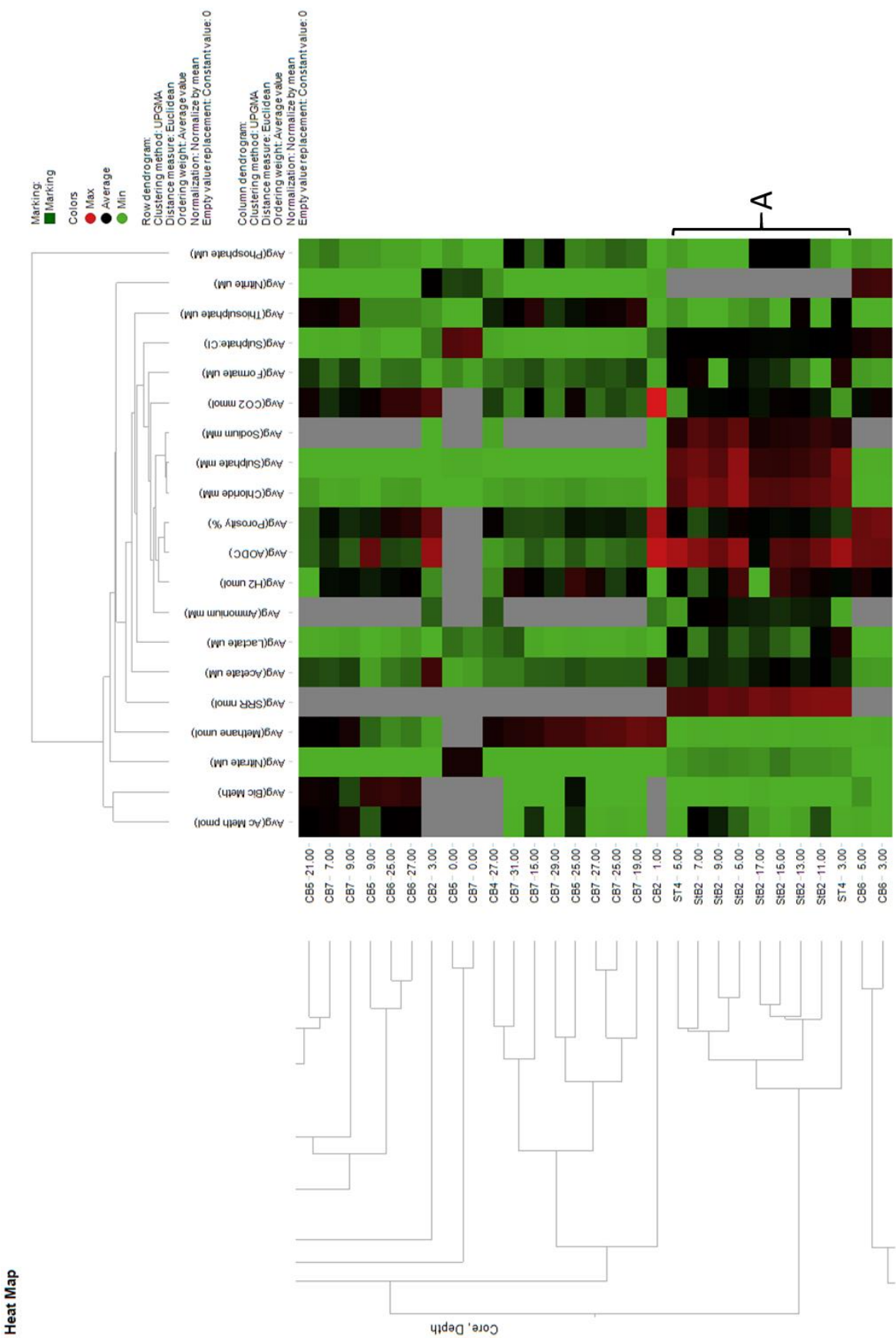


Fig. 9.11 – Magnified view of Fig. 9.7D showing part of the second lower Cardiff Bay cluster mainly containing data from January 2012. Within this cluster is a second (A) containing data from core ST4 and the surficial muddy layers of StB2.

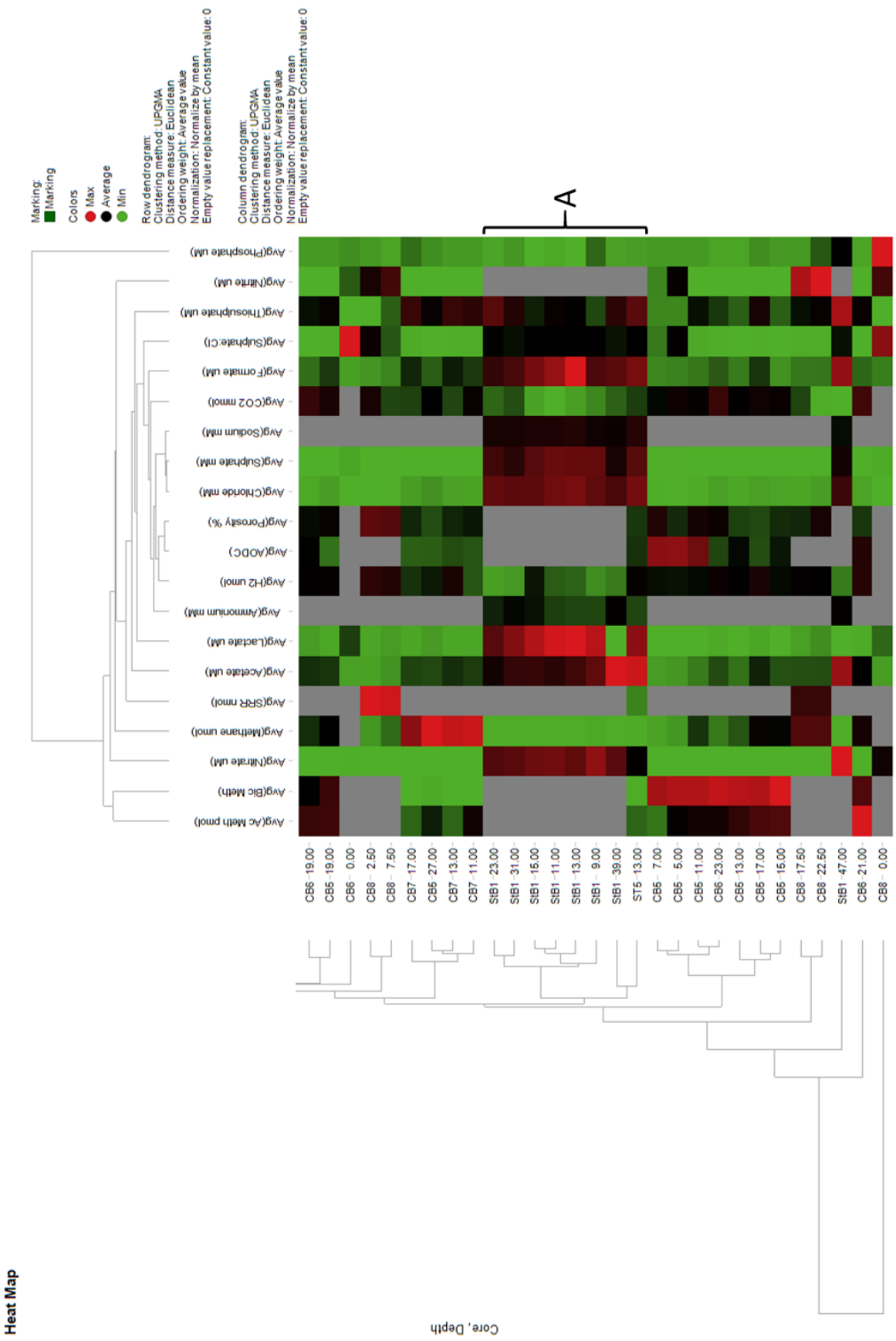


Fig. 9.12 – Second magnified view of Fig. 9.7D showing the base of the lower Cardiff Bay cluster mainly containing data from January 2012. Within this cluster is a second (A) containing data from the peat layer of cores ST5 and StB1.

are data series from the surficial layers of less disturbed cores (ST1, 2 and StB2) indicating that the conditions present near the surface in these cores are similar to those present at significant depth in the more disturbed samples. Data from the peat bed in core StB2 forms a distinct cluster (Fig. 9.10A), which is not surprising considering the radically different geochemistry within this sedimentary facies. On the other hand the surfical data from core StB1 clusters alongside data from cores ST4 and ST5 (Fig. 9.10B) which may indicate seasonal as well as spatial control of geochemistry reflected in the clustering, as these three cores were all taken from tidal flats in the autumn of 2012. A temporal trend in clustering is also observed in the second lower Cardiff Bay cluster (Figs. 9.11 and 9.12), which is mainly made up of data from cores collected in January 2012 (CB5, 6 and 7). Within this second large Cardiff Bay cluster is a smaller cluster containing the data for the surficial, muddy sediments from core StB2, bracketed by data series obtained from core ST4 (Fig. 9.11A). Since Cores ST4 and StB2 were thought to be two of the more stable cores examined in this study (due to surrounding topography and weather conditions respectively) it is possible that this cluster represents an archetype for more stable tidal flat environments in the Severn Estuary, though if this is the case it is puzzling that the rest of the data from core ST4 is not within it, and instead clusters with ST5 and StB1 further up the heatmap (Fig. 9.10B). This may indicate that both temperature and sediment disturbance play a major role in affecting sediment biogeochemistry, with changes in temperature (or possibly season) affecting tidal flat sites more than subtidal sites. Temperature is likely to vary much more dramatically at the intertidal sites as they are not permanently covered by seawater which could buffer atmospheric temperature changes. Finally at the base of the heatmap there is another clear cluster contained within the Cardiff Bay data made up of data from St Brides Wentlooge (Fig. 9.12A). This cluster is made up of data series from 9 cm and below in core StB1 and from 13 cm in ST5 and as such represents data from the peat bed obtained during the autumn of 2012. In addition, since this data clusters a significant distance away from the peat data series from core StB2 (Fig. 9.10A), it again illustrates the dramatic differences in geochemistry within the peat layer over the course of a year as described in Chapter 7.

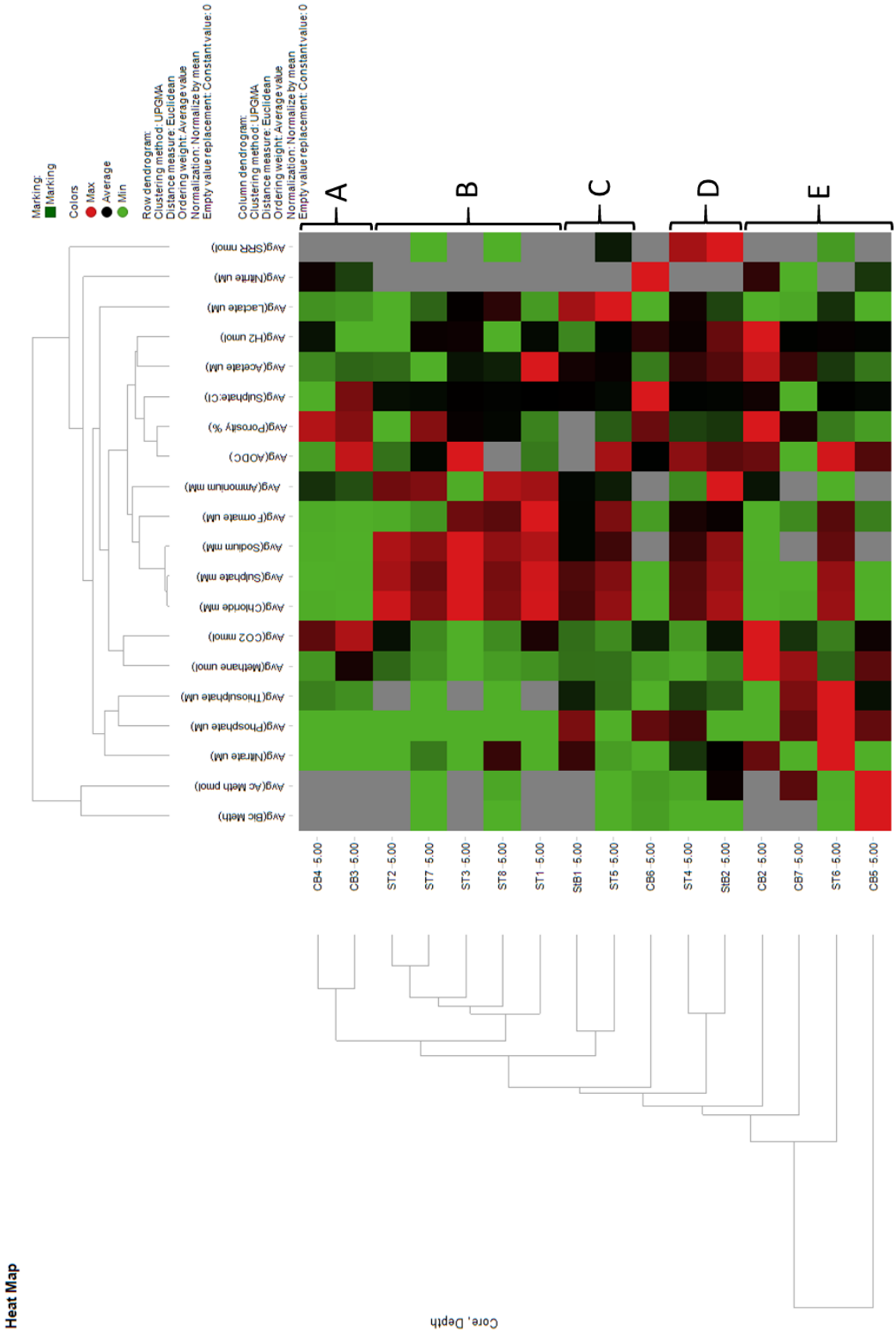


Fig. 9.13 – Hierarchical cluster analysis of the data series obtained from 5 cm depth in each of the cores studied. Clustering is not as clear as previously seen but still contains two Cardiff Bay clusters (A and E), two disturbed Severn Estuary clusters (B and C) and a less disturbed Severn Estuary cluster (D).

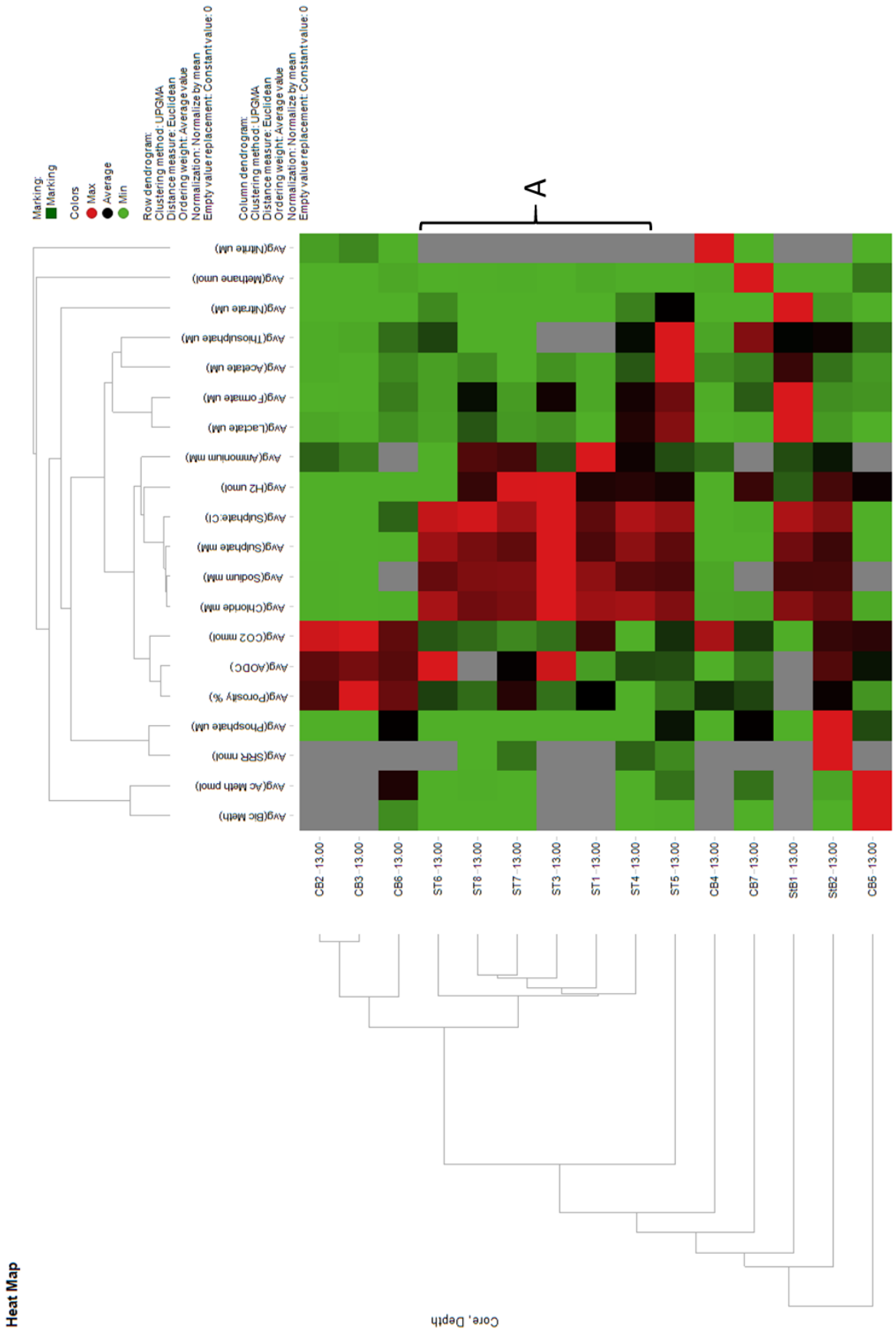


Fig. 9.14 – Hierarchical cluster analysis of the data series obtained from 13 cm depth in each of the cores studied. A distinct Severn Estuary cluster (A) has now formed in the centre, though cores ST5, St B1 and StB2 are no longer part of it, possibly due to the effects of the peat bed.

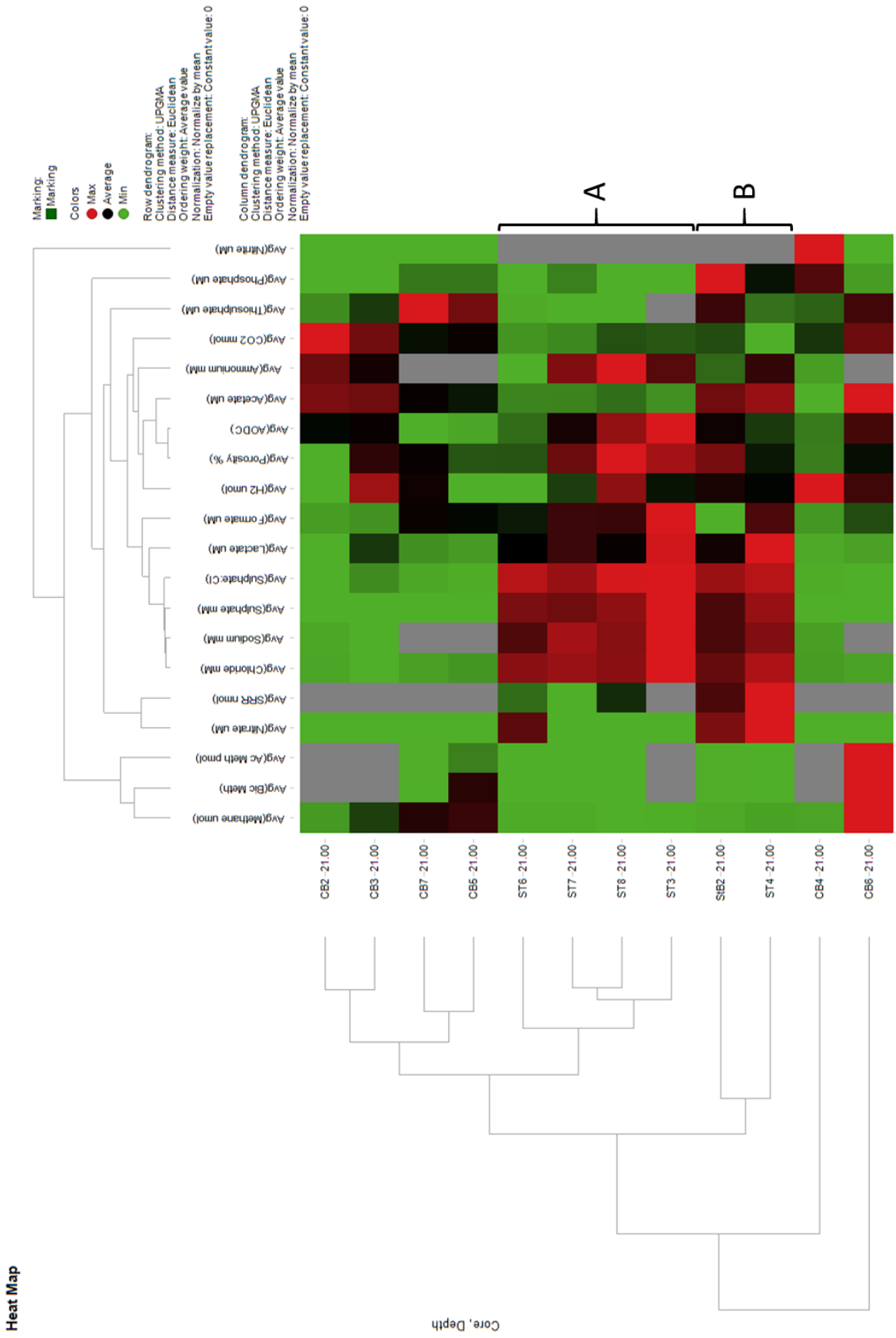


Fig. 9.15 – Hierarchical cluster analysis of the data series obtained from 21 cm depth in each of the cores studied. Two distinct Severn Estuary clusters can now be seen: A) more disturbed, and B) less disturbed.

In order to highlight data trends and similarities between cores and sampling sites further cluster analyses were carried out on data series from three specific depths from each of the cores (5, 13 and 21 cm); these depths were chosen to represent the top, middle and bottom of the cores respectively, where the most data from each of the cores was available. At 5cm depth (Fig. 9.13) clustering is not as obvious as before, though data from Cardiff Bay still clusters at the top and bottom of the heatmap (A and E) and the more disturbed Severn Estuary cores still cluster in the middle (B and C), while the more stable estuarine cores (ST4 and StB2) cluster nearer the base (D) around the 2nd Cardiff Bay cluster (which also includes ST6, possibly due to high surficial nitrate, phosphate and thiosulphate values). Clustering was much more pronounced at 13 cm (Fig. 9.14), with the Severn Estuary sites in particular forming a distinct cluster in the centre of the heatmap (A). Within this cluster cores ST3, 7 and 8 in particular grouped closely together likely due to the close geographical proximity of ST7 and 8 and ST3 and ST8's shared history as fluid muds. At this depth cores ST5, StB1 and StB2 begin to separate from the others due to the presence of the peat bed affecting their geochemistry. Finally a similar pattern can again be observed at 21 cm (Fig. 9.15), where the data series from the Severn Estuary can now be clearly divided into a "more disturbed" cluster (A) containing ST3, 6, 7 and 8 (with ST3, 7 and 8 again clustering more closely) and a "less disturbed" cluster (B) containing ST4 and StB2. This apparent increase in degree of cluster pronouncement with depth is likely due to a combination of sediment disturbance/re-suspension and prokaryotic activity.

Taken together these analyses clearly indicate that near the surface of the cores sediment re-suspension is likely to affect all of the cores relatively equally creating a more homogeneous geochemistry across the sites studied (and hence less distinct clustering). However with increasing sediment depth some sites will be affected less by sediment re-suspension and their data series will begin to diverge from those more exposed sites still affected by it. In addition, with increasing depth the cumulative effect that the metabolism of sedimentary prokaryotes have on the geochemistry of the sediment increases leading to further divergence (and therefore more distinct clusters), particularly since the activity of these organisms will in turn be affected by any re-suspension that the sediment undergoes.

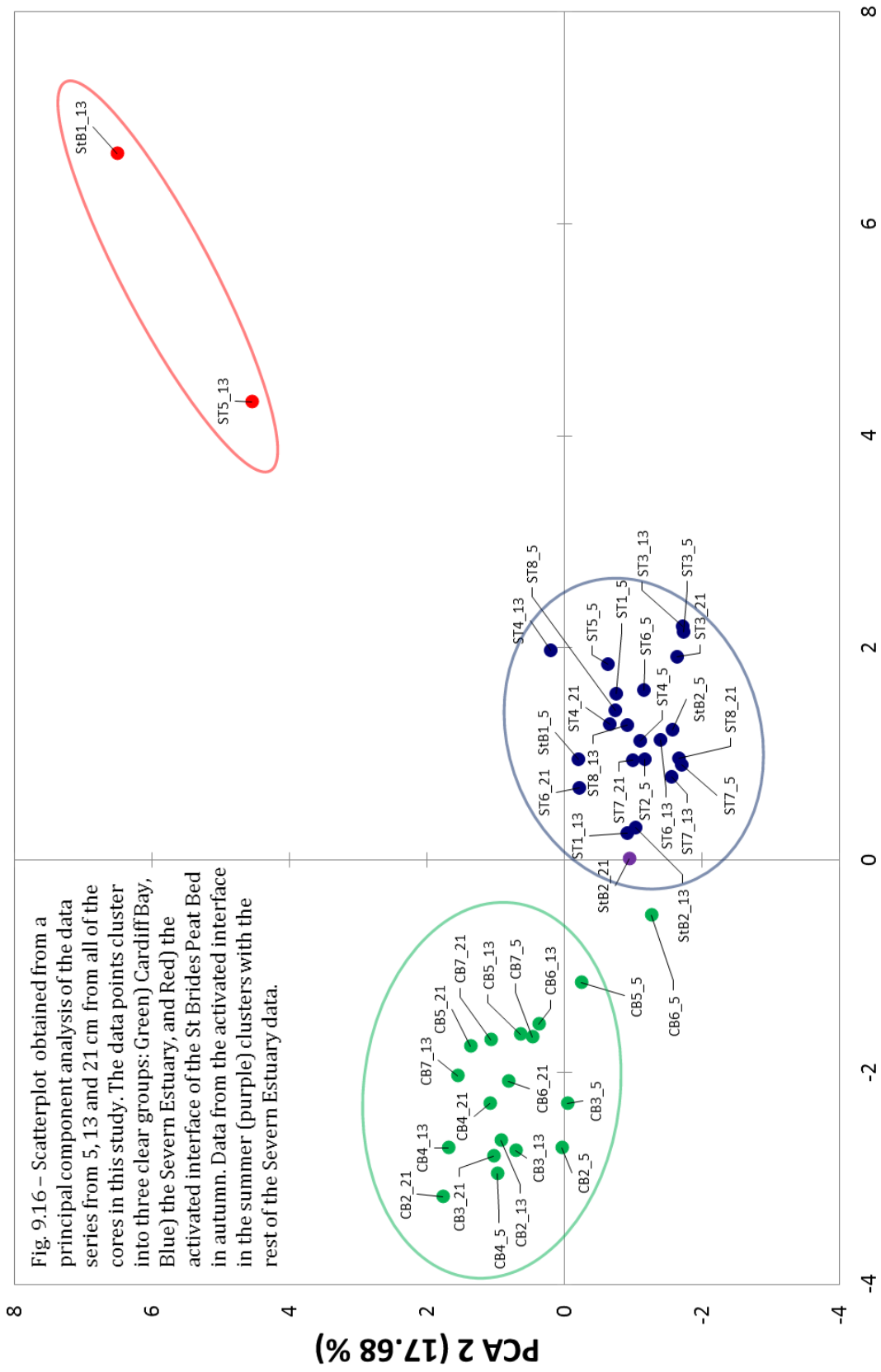


Fig. 9.16 – Scatterplot obtained from a principal component analysis of the data series from 5, 13 and 21 cm from all of the cores in this study. The data points cluster into three clear groups: Green) Cardiff Bay, Blue) the Severn Estuary, and Red) the activated interface of the St Brides Peat Bed in autumn. Data from the activated interface in the summer (purple) clusters with the rest of the Severn Estuary data.

As well as cluster analysis, data series from the three depths described above were also examined using principal component analysis (PCA). The data simplification process inherent in PCA does not allow the same degree of data examination afforded by hierarchical clustering, however the results from PCA broadly confirmed the data segregation described above, grouping the data into three main clusters. These clusters (Fig. 9.16) were predominantly based on core location with data series from Cardiff Bay (green) clustering together (with the exception of CB6_5), separated from data from the Severn Estuary (blue) which is not unexpected considering the substantial differences in geochemistry between the two sites. However, within each of these clusters there appeared to be relatively little sub-clustering of the data points, with the exception of core ST3 which grouped slightly apart, likely due to its history as a fluid mud. Interestingly the third cluster (red) contained only two points however, these represent data series obtained from the activated interface of the St Brides peat bed in autumn. This again highlights how different the geochemistry of the activated interface is from the other intertidal sites around the estuary and also how much the geochemistry of the peat bed can vary depending on the season as the data point corresponding to the activated interface in core StB2 (StB2_21, purple) sits comfortably within the main Severn Estuary cluster.

Finally, in addition to examining the geochemistry of the cores at individual depths the areal rates of processes (SOD, SRR and methanogenesis) from each of the estuarine cores were also subjected to cluster analysis, alongside the average porosity of each core, in order to determine whether sediment re-suspension contributed to the differing rates at each site (PCA was also performed but was not informative on this small data set). Both the dendrogram and heatmap produced by this analysis clearly show four distinct clusters which can be related to sediment re-suspension (Fig. 9.17):

- Cluster A - containing subtidal cores thought to be subject to a high degree of sediment disturbance (cores ST7 and ST8).
- Cluster B - closely linked to the first and containing intertidal cores thought to be subject to high degrees of re-suspension (cores ST5 and ST6).

- Cluster C - containing a core from a tidal flat thought to be a more stable environment with less re-suspension due to its topographical setting (core ST4).
- Cluster D - containing a core from a tidal flat thought to be subject to less re-suspension due to tidal and meteorological conditions coupled with enhanced substrate supply from saltmarsh peat (core StB2).

This clustering pattern indicates that sediment re-suspension does indeed play a role in controlling the metabolic processes occurring within the sediments of the Severn Estuary. In addition when the top dendrogram, indicating analyte correlations, is taken into consideration it is apparent that porosity (and by proxy, re-suspension) is particularly closely linked to rates of SOD and to a lesser extent SRR. This link between re-suspension and rates also appears strong enough to override the geographical proximity of sites (e.g. ST4 and ST6) confirming that it is one of the main drivers (possibly alongside whether a site is sub- or intertidal) of variations in sediment biogeochemistry in the Severn Estuary.

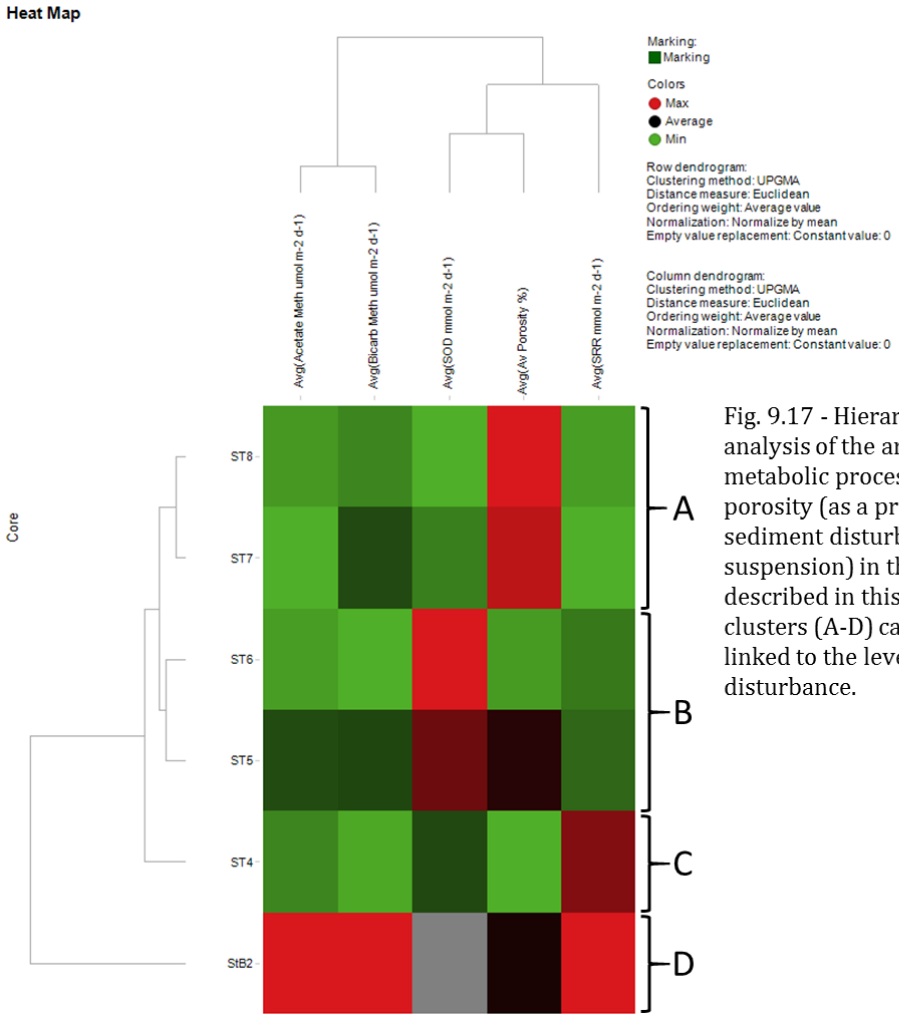


Fig. 9.17 - Hierarchical cluster analysis of the areal rates of metabolic processes and porosity (as a proxy for sediment disturbance/re-suspension) in the cores described in this study. Clear clusters (A-D) can be observed, linked to the level of sediment disturbance.

9.3 Unusual Microbial Habitats of the Severn Estuary and the Effects of SGD

The Severn Estuary contains a wide variety of sedimentary environments, some of which are unusual and relatively poorly studied with regard to their biogeochemical activity. This section summarises two of these unusual sedimentary environments: fluidised mud beds and buried salt marsh peat deposits. It also details the effect that submarine groundwater discharge (SGD) has on sites throughout the estuary.

9.3.1 Fluidised Muds

The first of the unusual sedimentary features of the Severn Estuary is the presence of distinct bodies of fluid mud (sometimes called "slugs") that occur in the deeper regions of the estuary (Kirby 2010, Manning *et al.* 2010). These fluidised muds occur in other regions of the world and tend to be associated with estuaries with energetic tidal regimes and/or high levels of suspended sediment such as the Humber (Uncles *et al.* 2006) Gironde (Abril *et al.* 1999, Tseng *et al.* 2001), Yangtze/Changjiang (Li and Zhang, 1998; Wan *et al.* 2014) and South Alligator River estuaries (Wolanski *et al.* 1988); or with tropical near-shore areas with a high sediment load such as the Amazon Delta (Kineke *et al.* 1996, Aller *et al.* 2010) and the Gulf of Papua (Todorov *et al.* 2000, Madrid *et al.* 2006). Thin layers of fluidised mud can also occur on tidal flats, particularly in estuaries with higher tidal rages, as documented by Christie *et al.* (1999) and Bassoullet *et al.* (2000). During this study two sediment cores were taken (cores ST3 and ST8) that probably originated in fluidised mud beds. Without the use of echo sounder data it would be difficult to confirm for certain that the cores came from a fluid mud, however, the locations at which they were sampled coupled with their unusual lithology (isolated sand bodies and an extreme lack of cohesion) points to this as their likely point of origin.

Biogeochemically these sediments tended to show more vertical geochemical gradients (especially in ST3) and little to no FeS formation, as evidenced by the lack of free phosphate and no darkening of the sediment colour. They also have unusual near-vertical cell count profiles, as previously described (Fig. 9.5B). In addition, in core ST8, despite having a lower SOD, the rates of sulphate reduction and acetoclastic methanogenesis appear to be slightly stimulated relative to those in the mud patch nearby (core ST7). This suggests that *in situ* the sediments are constantly being re-worked resulting in the re-oxidation of reduced compounds like sulphide

(hence the lack of FeS), which would provide ample electron acceptor substrates at all depths. Constant mixing would also allow organic carbon compounds to reach deeper into the sediment, resulting in the higher rates of sulphate reduction and methanogenesis (though the organisms concerned would probably need to occupy reduced microniches) (Jørgensen, 1977). The lower SOD of these sediments is unusual as fluid muds are known to have one of the highest rates of OC mineralization in coastal environments (Aller and Blair 2006). However, in the case of these sediments it may be because the O_2 uptake measurements were carried out when the sediment was within core tubes and had settled. Therefore, the more redox-positive processes were probably no longer being re-supplied with substrates and rapidly became substrate limited, resulting in decreased activity, thus underestimating the true environmental SOD. On the other hand SRB and methanogens, with their typically slower metabolic rates, would still probably have an adequate substrate supply, and hence, activity continued and perhaps became slightly elevated due to increasingly reduced conditions. Despite this, comparing the O_2 uptake rates from ST8 with the sulphate reduction and methanogenesis rates indicates that even with the low overall SOD, more redox-positive processes still dominate these sediments. This is in agreement with the results of Abril *et al.* (2000) and Abril *et al.* (2010), that processes such as aerobic respiration, denitrification and manganese reduction are dominant in fluidised mud beds – although results presented here demonstrate that this situation appears widespread in Severn Estuary sediments as a whole.

9.3.2 Buried Salt Marshes

The second unusual sedimentary feature described in this study are the Holocene buried peat beds sampled at St Brides Wentlooge. Similar peats, formed by the burial of salt marsh sediments due to sea level rise since the last glaciation are present at a number of sites around northern Europe including: Romney Marsh in East Sussex (Waller *et al.* 2006), the Belgian coastal plain (Baeteman *et al.* 1999) and on the coast of the German Bight (Dellwig *et al.* 1999, 2001, 2002), as well as further afield in Delaware Bay (Nikitina *et al.* 2000) and Okayama Prefecture, Japan (Hiroshi *et al.* 2011). They are also present to a varying extent along much of the Severn Estuary coastline, extending as far up river as Gloucester and up to 40 km inland in

the low-lying Somerset Levels (Allen and Haslett, 2002). As such these peat beds can be considered an important facet of the sedimentary regime of the coastal regions of the estuary.

The peats in the Severn Estuary however, differ from those at other locations as they are near to/at the surface whereas other buried salt-marsh deposits (hence the name) tend to be covered by layers of more recent strata, for example, those described by Dellwig *et al.* (1999) are buried beneath >7 m of more recent tidal flat and lagoonal sediments. Having such old sediments (c. 3400 years old, Allen and Haslett, 2007) exposed at the surface is unusual and is a direct result of the strong erosive force of the estuary's currents stripping away the overlying sediments while leaving the more structurally robust peat behind. As detailed in Chapter 7, this has led to the formation of active microbial communities in both the top layers of the peat and the overlying sediments, fuelled by compounds derived from the degradation of the peat itself such as methylated amines and glycolate. To date little research has been conducted on the current biogeochemistry of ancient salt-marsh peats from elsewhere in the world, with most of the focus being on the geochemistry relating to their formation. Although Dellwig *et al.* (2001) found that peat made a good substrate for SRB growth provided it was broken down by fungi beforehand, and Howarth and Teal (1979) found that modern salt marsh peats have some of the highest SRR recorded. As such it is not known whether the microbial stimulation and formation of an "activated interface" layer seen at St Brides is present in all peats or whether this is a consequence of the erosive forces in the Severn. Since the "activated interface" at St Brides was a zone of elevated methanogenesis (particularly from DMA) it may represent a regionally significant source of methane, and as such it would be interesting to investigate other peat-bearing sites around the world to see if the "activated interface" seen at St Brides is repeated elsewhere.

9.3.3 Submarine Groundwater Discharge

Many of the sites examined in this study have shown evidence of being affected to some degree by submarine groundwater discharge (SGD). This phenomenon involves the flow of terrestrial groundwater through marine sediments before it discharges into the sea (Burnett *et al.* 2003), and is driven both by the size of the hydraulic head and by variations in hydraulic pressure caused by changes in the

state of the tide (Santos *et al.* 2012). Such movement of pore water at depth can dramatically affect the geochemistry of sediments (Slomp and Van Cappellen, 2004) as shown at other locations (Jahnke *et al.* 2003; Beck *et al.* 2009; Porubsky *et al.* 2014). The Severn Estuary, with it's high tidal range, would be expected to have SGD to some degree and the results of this study show that several sites and processes appear to be affected by SGD. For example several cores from both the Severn Estuary (ST1, 2, 3, 5, 6 and StB1) and Cardiff Bay (CB6 and 7) show evidence of changes in chloride concentrations at depth possibly due to an influx of fresh SGD. SGD may also be responsible for introducing oxidised compounds such as nitrate, nitrite and thiosulphate into deeper sections of some of the cores (e.g. ST4, 5, and 6, StB1, CB4, 7 and possibly 8). Or it may be that oxidised groundwaters are generating these compounds *in situ* through chemical or biological means (i.e. nitrification, ammonium oxidation and sulphide oxidation). At some sites (e.g. cores ST1 and ST6) the SGD may also be inhibiting anaerobic processes, either directly by raising the redox state of the sediment, or due to the presence of inhibitory compounds such as nitrate. Finally at the St Brides site there is evidence that groundwater flow along the base of the peat bed may be stimulating a second "activated interface" the occurrence of which varies throughout the year depending on the degree of groundwater flow. However, it should be stated here that the biogeochemical data presented in this study likely only gives a small snapshot of the true role that SGD may play in the Severn Estuary system and that further study is needed to fully elucidate this role and to predict how it might change in the future (see section 9.4.2).

9.4 The Role of Sedimentary Prokaryotes in Future Environmental Change

9.4.1 The Effects of Climate Change in the Severn Estuary

Climate change predictions for the Severn Estuary provided by the Severn Estuary Partnership and IMCORE (2011) show that by the year 2080 mean winter land temperatures in the area surrounding the estuary are projected to rise by ≈2.8°C while mean summer temperatures could rise by ≈3.9°C - marine air and sea surface temperatures (SST) are expected to increase in line with these trends. Seasonal weather conditions are expected to become more polarised, with precipitation increasing by an average of 12-23% in winter and decreasing by around 18-24%

during the summer months. Relative sea level rise of around 2.4 mm yr^{-1} (Philips and Crisp, 2010) is also predicted, with levels at Cardiff expected to be 30-40 cm higher in 2080 compared to the 1990 baseline values (Severn Estuary Partnership, 2011) or 48 cm higher by the end of the century (Ahmadian *et al.* 2014a). However, sea levels trends in this area are strongly correlated with the North Atlantic Oscillation (NAO), which can lead to a degree of unpredictability (Philips *et al.* 2013). These increases in sea level are predicted to impact more on the Welsh side of the estuary, where between 1993-2007 higher maximum-extreme and lower minimum-extreme sea levels were recorded (likely due to the direction of the prevailing on-shore winds). Rising sea levels are also predicted to lead to increased wave energy, which coupled with a potential increase in the frequency of storms and severity of storm surges (up to an 0.8mm increase in surge height per year) (Severn Estuary Partnership, 2011), is likely to lead to higher rates of coastal erosion and steepening of beach profiles around the estuary – though again this may vary with changes in the NAO (Kirby and Kirby, 2008).

With regard to temperatures, the overall predicted yearly increase would be unlikely to have a negative effect on the sedimentary microorganisms. The summer of 2013 during which cores ST7, ST8 and StB2 were collected was particularly warm with sediment temperatures between 15-20°C in both subtidal and intertidal environments. A ≈3.9°C increase in summer temperatures would push the *in situ* sediment temperatures close to 25°C, still well within the growth limits of mesophillic and psychrotolerant organisms, the groups that are likely predominant in the sediments studied herein. In addition, if any of the groups currently present in the sediments did gradually decrease in number due to a lack of high-temperature tolerance, then it is likely that they would soon be replaced by other organisms with a similar metabolism but with higher optimum growth temperatures (Parkes *et al.* unpublished results from Portishead sediments), resulting in little if any change in sediment biogeochemistry.

Temperature increase might impact negatively on the sedimentary communities by lowering the oxygen content of the estuary's waters, since a large proportion of the carbon degradation in the estuary occurs via aerobic/dysaerobic pathways. However, considering the constant overturning of the estuary by tides,

which would mix oxygenated surface waters with potentially O_2 depleted bottom waters, coupled with the fact that the mudflats would be exposed to atmospheric O_2 during low tide it is unlikely that increasing temperature would directly lead to any degree of oxygen depletion in the sediments of the estuary.

On the contrary rather than hindering the sedimentary prokaryotes it is likely that the increased temperatures would aid the growth of these organisms, increasing the rates at which they respire. Estimations of SRRs at 25°C using Q_{10} value calculations have already been carried out for section 9.1.1 and show a 5-12 fold increase in rates from those measured at current temperatures. Similar increases would also be expected for the other processes occurring in the sediments, and therefore, since Severn Estuary sediments do not appear to be limited in substrates that might hinder higher activity, if temperature is taken in isolation the future changes in climate would likely stimulate sedimentary prokaryotes. Nevertheless when compared to other sites around the world (Table. 9.1) an increase in temperature to ≈25°C would still not make the Severn Estuary comparative with other estuarine and tidal flat sites in terms of SRR (or likely other processes) and as such would probably not cause any dramatic harm (mass eutrophication and anoxia etc.) to the overall biogeochemistry of the Severn Estuary system to a significant degree. On the other hand, small temperature increases might have a significant effect on the aerobic/dysaerobic processes that are currently dominant in the estuary. Elevated rates of these processes would result in increased oxygen uptake, which would lead to decreased oxygen penetration into the sediment during quiescent tidal periods. This would generate more reduced conditions favouring the anaerobic SRB and methanogens and potentially further increasing their activity.

Compared to temperature the predicted rise in sea levels coupled with increased storm and wave activity is likely to have a more mixed impact on the sedimentary prokaryotic communities in the Severn Estuary. For example the predicted 30-40 cm rise in sea levels will result in more areas of mudflat becoming permanently inundated. This loss of tidal flat area could reach up to 35 km^2 by 2100 (Ahmadian *et al.* 2014a), 14-16 % of the total current intertidal area (225-258 km^2, depending on source) (Kirby, 2010; Zhou *et al.* 2014a). Comparison of mudflat and subtidal data

collected in this study (e.g cores ST6 and ST7) indicates that this inundation could result in decreases in rates of sulphate reduction and total OM degradation across the whole estuary. However, on the other hand in low-lying coastal areas of the estuary without sea defences, the increase in sea level could lead to increased coastal flooding (Hall *et al.* 2006). This in turn could generate new tidal flat and salt-marsh environments providing new habitats for sedimentary prokaryotes and possibly compensating for any loss of habitat caused by the inundation of current mudflats. Increases in wave and storm activity on the other hand are more likely to have a deleterious effect on the anaerobic communities in the sediments as the increases in water movement will likely lead to higher rates of sediment re-suspension further inhibiting the growth of SRB and methanogens. Finally the increased rates of winter rainfall would increase the discharge rate of the rivers flowing into the Severn Estuary, this in turn would increase the amount of terrestrially-derived OC entering the estuary, which might promote the growth of heterotrophic groups in the sediment, provided that the OC is deposited and not quickly flushed from the system into the Irish Sea (Mantoura and Woodward, 1983), as the lower winter temperatures would reduce immediate consumption of the OM. The predicted drop in rainfall during the summer months on the other hand would lead to a decrease in river flow and a concomitant decrease in the amount of terrestrial OC entering the estuary potentially decreasing microbial growth (although this could be counteracted by the effects of temperature on growth rates described above). As such it is difficult to discern any trends in prokaryotic activity relating to rainfall due to there not being any clear net increase or decrease in OC flux.

As well as examining the potential effects of climate change on the Severn Estuary it is also important to discuss how the results of this study could be extrapolated to provide information on the effects of climate change on sedimentary biogeochemistry in a more global context. Since estuaries are by their nature products of sea level rise it stands to reason that rising eustatic sea levels, driven by the melting of the continental ice sheets would lead to the creation of more estuarine environments in the future. Due to the potential rapidity of this sea level rise these estuaries are more likely to be of the drowned river valley-type (similar to

the Severn Estuary) and as such are more likely to have a high degree of synchronicity, and therefore, have higher tidal ranges (Dyer, 1997). In addition, increases in sea level could also convert estuaries with more hyposynchrous shapes (such as bar-built estuaries) into more synchronous forms, increasing their tidal range as well. Finally, rising sea levels could deepen estuaries, increasing their tidal range (Dyer, 1995) even in environments already subject to higher than average tides such as the Bay of Fundy (Greenberg *et al.* 2012) Taken together this could lead to an increase in the number of environments similar to the Severn Estuary, where high tidal current strengths lead to a high degree of sediment re-suspension, resulting in the dominance of aerobic/dysaerobic OM degradation present in the Severn Estuary becoming more widespread.

In addition to this, the increase in global warming-related storm activity described above is not a phenomenon confined to the Severn Estuary but is instead likely to occur on elsewhere as well (Carretero *et al.* 1998, Christensen *et al.* 2007). This may again lead to even more estuaries developing conditions similar to the Severn (at least on a seasonal basis), with storm-driven wave activity resulting in more regular sediment re-suspension. If this scenario proves to be the case then even estuaries without particularly dramatic tidal ranges might develop conditions similar to the Severn Estuary, fundamentally altering the biogeochemistry and macrobenthic ecology of these environments as well. This could result in many estuaries having greater areal CO_2 production (from increased OM degradation), but lower methane production and potentially lower endobenthic macrofaunal diversity (due to increased re-suspension). As such the Severn Estuary, although currently an unusual environment with respect to biogeochemistry, could prove to be an important example of environmental conditions that may become much more prevalent in the future.

9.4.2 The Effects of Tidal Barrages in the Severn Estuary

Aside from climate change the other major future environmental change that may potentially occur in the Severn Estuary is the modification of the estuarine system for power generation via the construction of tidal barrages. Several barrage models have been suggested including large structures impounding the whole estuary, such as the Cardiff-Weston Barrage, or smaller structures enclosing only sections of the

estuary such as the Fleming Lagoon and the Shoots Barrage (Xia *et al.* 2010). The most commonly discussed barrage-type is the large Cardiff-Weston Barrage, and in order to understand the potential ecological impacts of such a barrage on the Severn Estuary, studies have been carried out on impounded estuaries elsewhere in the world to act as analogues, including the La Rance Estuary in Northern France (Kirby and Retière 2009), the Schelde Estuary in the Netherlands (Pethick *et al*. 2009) and Annapolis Royal on the Bay of Fundy (Morris 2013). Using data collected from these sites predictions have been made about the potential environmental and ecological effects on the Severn Estuary (Kirby 2010, Hooper and Austin 2013), however, these studies do not address the potential impacts on the sedimentary biogeochemistry of the estuary. For example, it is thought that the construction of a Cardiff-Weston Barrage would increase sea levels by up to 3 m within the impounded basin whilst also decreasing the tidal range to around 4.5 m (Hooper and Austen, 2013). In addition, the act of closing a barrage to generate the head of water required for power generation would increase the duration of the high tide period. Coupled together these increases in water height would lead to the loss of approximately 81-127 km^2 of the current intertidal area, with this loss increasing to 133 km^2 with sea level rise taken into account (Zhou *et al.* 2014a, Ahmadian *et al.* 2014a), which could seriously impact the microorganisms living in those mudflat sediments. Pethick *et al.* (2009) and Morris (2013) also suggest that the creation of a barrage would lead to increased rates of coastal erosion in the headpond lagoon, removing more of the remaining mudflats and further depriving the microorganisms of habitat – although this may be balanced out by the development of new saltmarshes in the longer term.

In addition, the construction of a barrage would reduce both tidal currents within the lagoon – by as much as 45-50% in the case of the Cardiff-Weston Barrage (Xia *et al.* 2010, Zhou *et al.* 2014a), as well as wave activity (Fairley *et al.* 2014). This would reduce sediment re-suspension making the environment more suitable for SRB and methanogens although also probably decreasing the amount of aerobic respiration in the sediments due to reduced mixing with aerated seawater. Kirby (2010) also suggests that the decrease in re-suspension following the construction of a barrage would stop the formation of fluid mud slugs in the upper estuary, removing this unusual microbial habitat from the ecosystem. These fluidised muds represent a large prokaryotic biomass and hence are likely to be highly

biogeochemically active (the full extent of which is probably underestimated here as previously described). As such the effect on the wider environment of removing these habitats is unknown, although in the Gironde Estuary fluid muds have been shown to play an important role in nitrogen (Abril *et al.* 2000) and trace metal cycling (Robert *et al.* 2004). The decrease in re-suspension would also allow benthic macrofauna such as bivalves and polychaetes to colonise more of the estuary and also allow new species (including more filter-feeders) to colonise the area (Radford 1994, Kirby and Shaw 2005, Warwick and Somerfield, 2010). This could result in more bioirrigation of the sediment, leading to increased rates of aerobic respiration and decreases in sulphate reduction (Banta *et al.* 1999, Nielsen *et al.* 2003) mitigating some of the direct consequences of the decrease in re-suspension. Less suspended sediment in the water column would also result in greater light penetration allowing an increase in phytoplankton growth (Radford 1994, Underwood 2010). These plankton along with their OC exudate compounds would form a new source of labile autochthonous carbon for the sedimentary organisms to consume, which coupled with the increased amounts of allochthonous, terrestrially-derived material that would settle in the lagoon due to lower levels of re-suspension, would further increase the potential rates of anaerobic processes.

As well as reducing currents the barrage could also decrease the flushing time of the upper estuary lagoon behind the barrage (Hooper and Austen, 2013), which in turn may have four primary effects. Firstly, along with the decrease in tidal currents, it could decrease the O_2 content of the bottom waters of the lagoon, which would further decrease the proportion of aerobic respiration occurring in the sediments while promoting sulphate reduction and methanogenesis. Secondly it could also lead to the accumulation of growth-limiting compounds from agricultural run-off, such as nitrate and phosphate, in the lagoon waters. This in turn could lead to eutrophication of the lagoon allowing for the formation of algal blooms (particularly as the water would also be clearer due to the lack of sediment re-suspension) (Kadiri *et al.* 2014a). Kirby (2010) argued against the possibility of eutrophication in the Severn Estuary post-barrage based on previous studies at La Rance although it is unknown how comparable the two estuaries are considering their significant differences - e.g. size, tidal range, shape, SPM concentrations, sediment availability etc. (Morris, 2013). However, assuming that algal blooms could

occur, the consumption of these algae by heterotrophic organisms coupled with the already decreased O_2 content of the lagoon waters could lead to hypoxic/anoxic conditions occurring in the bottom-waters of the estuary further increasing the potential habitat and growth of SRB and methanogen populations. Thirdly, the reduction in flushing would likely lead to a decrease in the salinity of the lagoon waters which in turn could decrease the available sulphate pool stimulating methanogenesis. However, Zhou *et al.* (2012) predict that salinity upstream of the barrage will only decrease by a maximum of 5‰ which is unlikely to dramatically alter the balance of sulphate reduction:methanogenesis as seen in Cardiff Bay. Finally the reduction in tidal range could affect the amount of SGD into the estuary by reducing the amount of tidal pumping occurring and also reducing the size of the hydraulic head that generates groundwater flow (Burnett *et al.* 2003).

Due in-part to the potential negative environmental impacts described above a new barrage design has recently been suggested for the Severn Estuary. This new model of barrage would generate power on both the ebb and flow tide, and as such would not cause the reduction in current speeds or SPM associated with the older barrage design described above (Ahmadian *et al.* 2014b; Zhou *et al.* 2014b). This would also mean that the potential problems with eutrophication in the back-barrage lagoon could be mitigated (Kadiri *et al.* 2014b) and that the sediment biogeochemistry in the lagoon would likely remain in a similar state to that at present, though further study might be necessary to confirm this.

9.4.3 The Role of Severn Estuary Microbiota in Effecting the Environment and Climate

Since anaerobic prokaryotes are responsible for controlling large portions of important biogeochemical cycles (e.g. the carbon, nitrogen and sulphur cycles) it is important not only to examine what effect environmental changes may have on them, but also, how they themselves may effect the environment. In it's current state the Severn Estuary is not a major site of sulphate reduction or methanogenesis, although it's high O_2 uptake rates demonstrate that a large amount of organic matter degradation is taking place in the sediments via aerobic respiration (and other processes high up the redox cascade). These processes produce large volumes of CO_2 most of which likely enters the atmosphere due to the fact there is a general lack of

CO_2-consuming autotrophic processes (photosynthesis or H_2/CO_2 methanogenesis) occurring in the estuary. With rising temperatures and increasing wave energy leading to more re-suspension it is likely that aerobic respiration in these sediments could increase leading to more CO_2 production. In addition, the flooding of low-lying coastal areas by sea level rise could generate more mudflat environments further increasing CO_2 generation.

Alternatively, if a barrage were constructed (on any scale) in the Severn Estuary the resulting decrease in re-suspension within the lagoon would likely decrease the rate of aerobic respiration resulting in less CO_2 release. This is because the anaerobic groups that could take over from the aerobes would still produce CO_2 though probably at a lower rate and the increased light penetration in the water column would allow for photosynthesis to increase and act as a carbon sink. The decrease in re-suspension could also reduce the amount of nitrification occurring in the sediment (due to a lack of O_2 penetration). This, coupled with a possible increase in ammonium production due to increased anaerobic degradation of OM, could result in ammonium escaping the sediment into the water column. Increasing anaerobic metabolism would also mean an increase in sulphate reduction and methanogenesis in the sediments (especially so if coupled with eutrophication and anoxia) causing other problems to arise. Since H_2S (one of the main products of suphate reduction) is toxic to most organisms a large-scale increase in sulphate reduction could have a deleterious effect on the sediment ecosystem by poisoning the resident macrofauna. This would lead to reduction in bioturbation and therefore further increase sediment anoxia. Increased sulphate reduction and sediment anoxia could also affect conditions outside the sediment, as the diffusion of H_2S to into the water column or onto the tidal flats (Al-Raei *et al.* 2009) would have an adverse effect of the resident micro- and macrofauna (e.g. wading birds). In addition this H_2S could also react with the oxygen in the water, further increasing the potential for bottom-water hypoxia/anoxia. This would both deprive benthic organisms of a suitable habitat as well as extending the potential habitat for SRB into the water column creating a positive feedback loop leading to more H_2S production and increasing anoxia. In addition an increase in methanogenesis could also increase water column anoxia (due to chemical methane oxidation), and would potentially have negative effects on the climate as well. Since freshwater wetlands are the

ecosystem with the greatest rate of methane production (Sheppard *et al.* 1982) the conversion of an estuarine environment to one more closely resembling a lake could make the Severn Estuary a much more significant source of atmospheric methane than it is in it's current state. As methane is a potent greenhouse gas with a global warming potential (GWP) 28-86x higher than CO_2 depending on timescale and climate-carbon feedbacks (Myhre *et al.* 2013), this would likely result in a dramatic increase in the global warming footprint of the Severn Estuary system.

In addition to the methane produced in the muddy sediments of the estuary, it is also important to consider methane production within one of the more unique environments in the Severn Estuary – the buried salt marshes. From a climate change perspective St Brides Wentlooge is one of the most important sites examined within this study when considering future changes in both the Severn Estuary and the wider global system. Firstly, the environment of deposition for the peat and clay that make up the bulk of the cores StB1 and StB2 was created by a previous climate change event - i.e. flooding of salt marshes caused by rising sea levels at the end of the last glaciation (Allen, 1990a). As such, St Brides may serve as an example of a sedimentary environment that could become much more common in the future as sea levels continue to rise due to global warming and threaten to flood low lying coastal areas. In addition, since buried salt marsh peats exist around much of the Severn Estuary and extend far inland in low lying areas (Allen and Haslett, 2002), rising sea levels coupled with higher rates of coastal erosion due to increased storm frequency could result in more of the salt marsh peats that are currently buried being exposed. This in turn will likely lead to more of these peats developing an "activated interface" layer with similar biogeochemistry to those at St Brides. As similar peat deposits are found buried elsewhere around the world, eustatic sea level rise could therefore, result in the formation of "activated peat" beds occurring on a more global scale.

Since the St Brides cores had significantly enhanced methanogenesis rates compared to cores taken elsewhere in the estuary where peat is absent, an increase in salt marsh peat exposure could lead to an increase in global methanogenesis rates. This increase in rates could have a dramatic effect on global temperatures – particularly when you consider that methanogenesis rates at St Brides were >8x

higher in summer than in winter which could generate a positive feedback loop if global temperatures rose further. Finally the radiotracer measurements carried in this study show that methylated amine-methanogenesis is actively occurring at this site at high rates. Methylated amines are not commonly detected *in situ* but they have been shown to be act as an important source of methane in marine environments especially where sulphate is not limited (Winfrey and Ward 1983, King *et al.* 1983, Parkes *et al.* 2012). This study has shown that Severn Estuary salt marsh peats with an "activated interface" are both a considerable source of methylated amines and that they are converted to methane at a rapid rate within this layer – the depth integrated DMA-methanogenesis rate at this site was 57.4 $\mu mol\ m^{-2}\ d^{-1}$. As such, an increase in the formation of "activated peats" could lead to the creation of a source of both MAs and methane not previously documented. In addition to this, a recent study has shown that DMA is an important compound for cloud nucleation (Almeida *et al.* 2013). Since clouds increase the Earth's albedo, and hence decrease global warming, the consumption of DMA by organisms that subsequently produce a potent greenhouse could act as a "double whammy", potentially increasing global temperatures even further. Taken together these three factors indicate that the St Brides site can act as a useful proxy for both a sedimentary environment and metabolic process that, though much overlooked up until now, may have a more important role to play in the future with relation to the Earth's climate.

9.5 Future Investigations

9.5.1 Simulating respiration rates in fluidised mud beds

The fluidised mud sampled in core ST8 had higher rates of sulphate reduction and acetoclastic methanogenesis than the nearby more-stable sediments (core ST7). However, in contrast, SOD and H_2/CO_2 methanogenesis rates were found to be lower in ST8 than ST7. As described in section 9.2.1 this is puzzling considering the supposed high rate of OC consumption by fluidised muds (Aller and Blair, 2006), and may be due to the lack of movement of the sediment in core ST8 following sampling. In order to test this hypothesis it could be possible to create an artificial fluidised mud bed using a fluidised bed reactor similar to those described by Parkes and Buckingham (1986) or Abril *et al.* (2010) (Fig. 9.18). Such a reactor would allow the

sediment within it to remain in suspension allowing for a more accurate simulation of the *in situ* conditions in a fluidised mud bed. The reactor could then be sampled periodically to measure changes in the concentrations of the major ions over time and to predict the rates of the prokaryotic processes occurring within. In addition, if the unit was temporarily sealed then the overlying waters of the reactor could be sampled for oxygen uptake analysis in a similar manner to incubated core tubes. Under these conditions it would also be possible to inject radiolabeled substrates ($^{35}SO_4^{2-}$, ^{14}C-acetate etc.) into the reactor in order to directly measure the rates of anaerobic processes in an actively fluidised bed. By modifying the rate of mixing in the reactor it might also be possible to simulate the behaviour of a fluid mud pool at

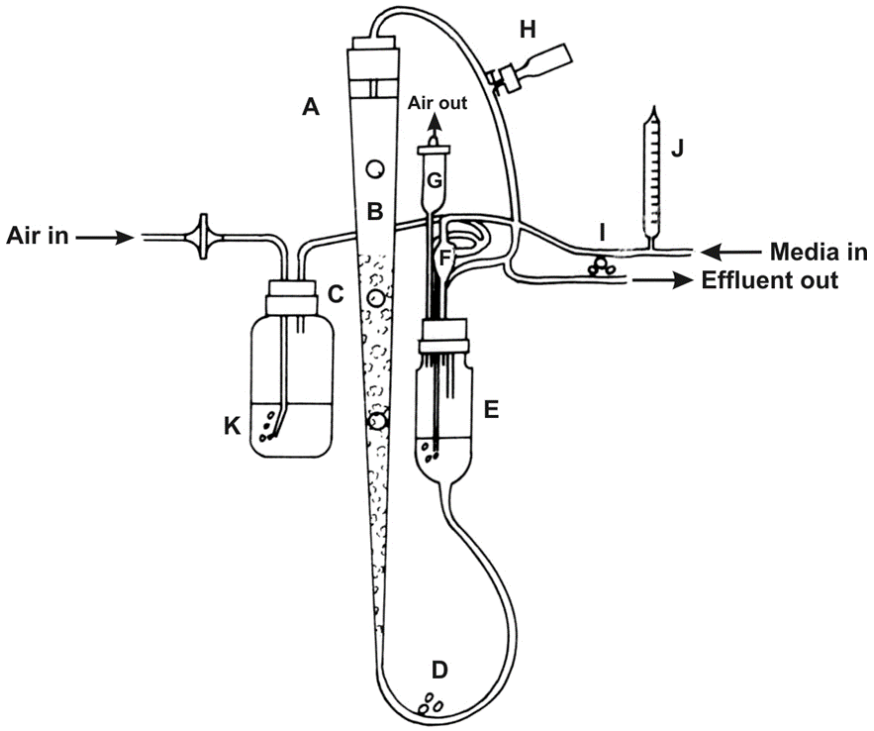


Fig. 9.18 – Diagram of a chemostat fluidised bed reactor used for the incubation of sedimentary prokaryotes modified from Parkes and Buckingham (1986). Sediment is contained within a glass cone (A) with ports allowing sampling at varying depths (C). Peristaltic pumps (I and D) keep a constant flow of media through the base of the cone and out of the top, maintaining a constantly fluidised state with a clear sediment-water interface (B). Media flowing from the top of the cone can be sampled (H) and is then returned to the base of the cone (with a small proportion removed as effluent to allow for the introduction of fresh substrates). The circulating media flows through a mixing chamber (E) in order to maintain homogeneity with regard to new and old substrates. Mixing is achieved by bubbling the media with filtered gas (e.g. air), which has been first bubbled through water to maintain moisture content (K), gas then escapes the mixing chamber via a filtered one-way valve (G). New media is added to the mixing vessel at a constant rate under a flow of gas (F) to prevent backflow of potential contaminants. The flow of media can be measured with the use of a syringe attached to the media-inflow tubing (J).

different stages of the tidal cycle to determine how this effects OM degradation and the different prokaryotic processes involved. Finally by shutting off the circulation/mixing system and letting the sediment in the reactor settle out of suspension it might be possible to examine the post-depositional behaviour of the incumbent microbial communities. For example, by sampling the sediment pore waters at regular intervals, the time taken for normal biogeochemical profiles to form within these sediments could be determined. In addition oxygen uptake measurements could be taken at regular intervals to determine how the deposition timeframe affects the SOD. Repeated sub-sampling and injection with radiotracer compounds, could be used to quantify changes in the rates of prokaryotic processes post-deposition. Finally re-suspension of the reactor after a period of quiescence would allow the re-oxidation of anaerobic end-products to be studied.

9.5.2 Examining the effects of varying temperatures on microbial processes in Severn Estuary Sediments

Changes in temperature can have dramatic effects on the rate at which microbial processes occur (see section 1.2.2). However, in this study it was difficult to attribute any variance in respiration rates between sites directly to changes in *in situ* temperature due to other differences between the cores (site lithology, core length etc.). In order to assess the direct impact of changes in temperature on the sediments of the Severn Estuary, sediment samples collected from sites around the estuary could be injected with radiolabeled substrates and incubated at a range of different environmental temperatures. This would allow any changes in respiration rates attributable to temperature to be directly quantified and could help show the influence of seasonal temperature changes on prokaryotic processes. In addition if cores were also incubated at higher temperatures than those currently measured in the estuary, such as those predicted by UKCP09 (Murphy *et al.* 2009) the Severn Estuary Partnership (2011) or the IPCC (Collins *et al.* 2013; Kirtman *et al.* 2013), then the potential effects of global climate change on the sedimentary biogeochemistry of the estuary could also be quantified.

9.5.3 Studying the inhibition of prokaryotic processes by peat-derived compounds

The results obtained from St Brides (core StB2) indicated that anaerobic processes such as sulphate reduction and methanogenesis may have been inhibited in the middle of the peat bed at this site. In order to test whether this inhibition, as suggested in section 7.2.2, was caused by an abundance of humics and/or tannins in the peat these compounds could be extracted from the peat via chemical oxidation (Miura *et al.* 1996; Fong *et al.* 2007). Active sediment slurries from the overlying sediment at the St Brides site and from the "activated interface" of the peat bed could then be amended with varying concentrations of these peat-derived compounds (or a similar artificial substitute) in order to determine if these compounds had a deleterious effect on the incumbent organisms (either due to toxicity or by acting as competitive electron acceptors) – and if so at what threshold this inhibition occurred at.

9.5.4 Measuring NO_3^-, Fe^{3+} and Mn^{3+} reduction in Severn Estuary sediments

Results obtained from throughout the Severn Estuary showed that organic carbon degradation in the estuary's sediments was dominated by processes other than sulphate reduction and methanogenesis. In order to further elucidate which processes control OC degradation, rate measurements of processes further up the redox cascade could be conducted. For example, rates of nitrate reduction in incubated cores could be measured using ^{15}N isotopes (Dong *et al.* 2011), while iron and manganese reduction rates could be obtained using the methods of Canfield *et al.* (1993b). These results could then be compared with the SOD, SRR and methanogenesis measurements in order to provide a much fuller picture of what is happening in an environment where more redox-positive processes appear to dominate sediment metabolism.

9.5.5 Measuring the effusive methane flux of the sediments of Cardiff Bay

Methane measurements from Cardiff Bay showed very high concentrations of methane present in the sediments of this shallow anthropogenic lake – up to ≈ 5 mmol l^{-1} of wet sediment (≈6.9 mmol l^{-1} of pore water) in core CB8 – potentially leading to the formation of gas bubbles within the sediment. Methane was also detected close to the surface in several cores (e.g. CB2, CB3 and CB5) and large

amounts of gas bubbles were observed being released from the sediment during coring. These factors, combined with the shallow depth of the bay, indicate that methane might be being released from the sediments of the bay and reaching the atmosphere. In order to test this hypothesis, flux chambers (Duchemin *et al.* 1995; Grinham *et al.* 2011; Xiao *et al.* 2013) could be placed on the sediment/water and water/air interfaces of the bay in order to measure how much methane is being released from the sediment, and also, whether this methane is reaching the atmosphere. Since methane can also escape *en masse* from lake sediments via pockmarks, sonar mapping and diver surveys of the lakebed could be carried out to determine the location of any pockmarks (Bussmann *et al.* 2011), which could then be fitted with flux chambers in order to determine the amount of methane released. Such a study would be important, as anthropogenic lakes are known produce large amounts of methane and as such could prove to be significant sources to the atmosphere if more lakes are created in the future (DelSontro *et al.* 2011; Sobek *et al.* 2012), especially if future climate change-related temperature increases are taken into account.

9.5.6 Examining the biogeochemistry of other hypertidal estuaries

Hypertidal estuaries occur all around the world (Archer and Hubbard 2003; Archer 2013) but have been little studied with regard to their biogeochemistry. This study represents the first in depth investigation into how the unusually dynamic conditions in a hypertidal estuary affect the biogeochemistry of its sediments. As such it is important to expand this study in order to determine whether the unusual features observed in the Severn Estuary (low rates of sulphate reduction and methanogenesis, elevated total cell counts etc.) are a common feature of hypertidal systems the world over (e.g. the Bay of Fundy, Nova Scotia; Cook Inlet, Alaska) or for some reason are confined to the Severn Estuary itself. In addition, by examining hypertidal systems in warmer latitudes, such as King Sound in western Australia (Wolanski and Spagnol, 2003), or the Gulf of Khambat/Cambay in western India (Nayak and Shetye, 2003), it may be possible to make predictions about changes that might occur in the Severn Estuary in the future as a result of global climate change.

9.6 Conclusions on the Biogeochemistry of the Severn Estuary

This study has shown that the extremely dynamic sedimentary environment within Severn Estuary has led to the formation of a variety of contrasting prokaryotic habitats ranging from tidal mudflats and subtidal mud patches to fluidised muds and near-surface Holocene salt marsh deposits. Conditions in these environments can change dramatically across the estuary, often resulting in a great deal of variance between the different sites studied, with both geochemical profiles and rate measurements showing significant differences even over geographically short distances. However, as a result of this study it can be seen that there are some factors that are common throughout the Severn Estuary.

For example, high oxygen uptake measurements, coupled with relatively low rates of sulphate reduction and methanogenesis are a signature of almost all of the sites in this study (Fig. 9.19). This indicates that OM mineralisation across the Severn Estuary is high but is mainly the result of prokaryotic processes near the top of the redox cascade such as aerobic respiration, nitrate reduction and iron and manganese reduction. This high degree of microbial activity also allows geochemical profiles to become established within disturbed sediments in relatively short spaces of time, probably within hours to days of the sediment being deposited after an erosive event.

In addition, many of the cores in this study lacked the usual depth succession of microbial processes that is associated with anoxic environments based on metabolic efficiency. At several sites active methanogenesis was detected co-occurring with sulphate reduction and often near the surface in apparently oxidised sediments. This may be due to the regular re-suspension of the estuary's sediments, which prevent the formation of distinct dominant metabolic communities at specific depths, instead creating a sedimentary environment full of small opportunistic communities of different competitive anaerobic guilds occurring at a range of depths (probably within reduced microniches in the case of those near the sediment surface). Such a disruption of the normal depth sequence of prokaryotic guilds may also help to explain the results previously obtained by Webster *et al.* (2010) in their biodiversity study of the Severn Estuary at Portishead, and also why the rates of anaerobic processes measured in this study were low.

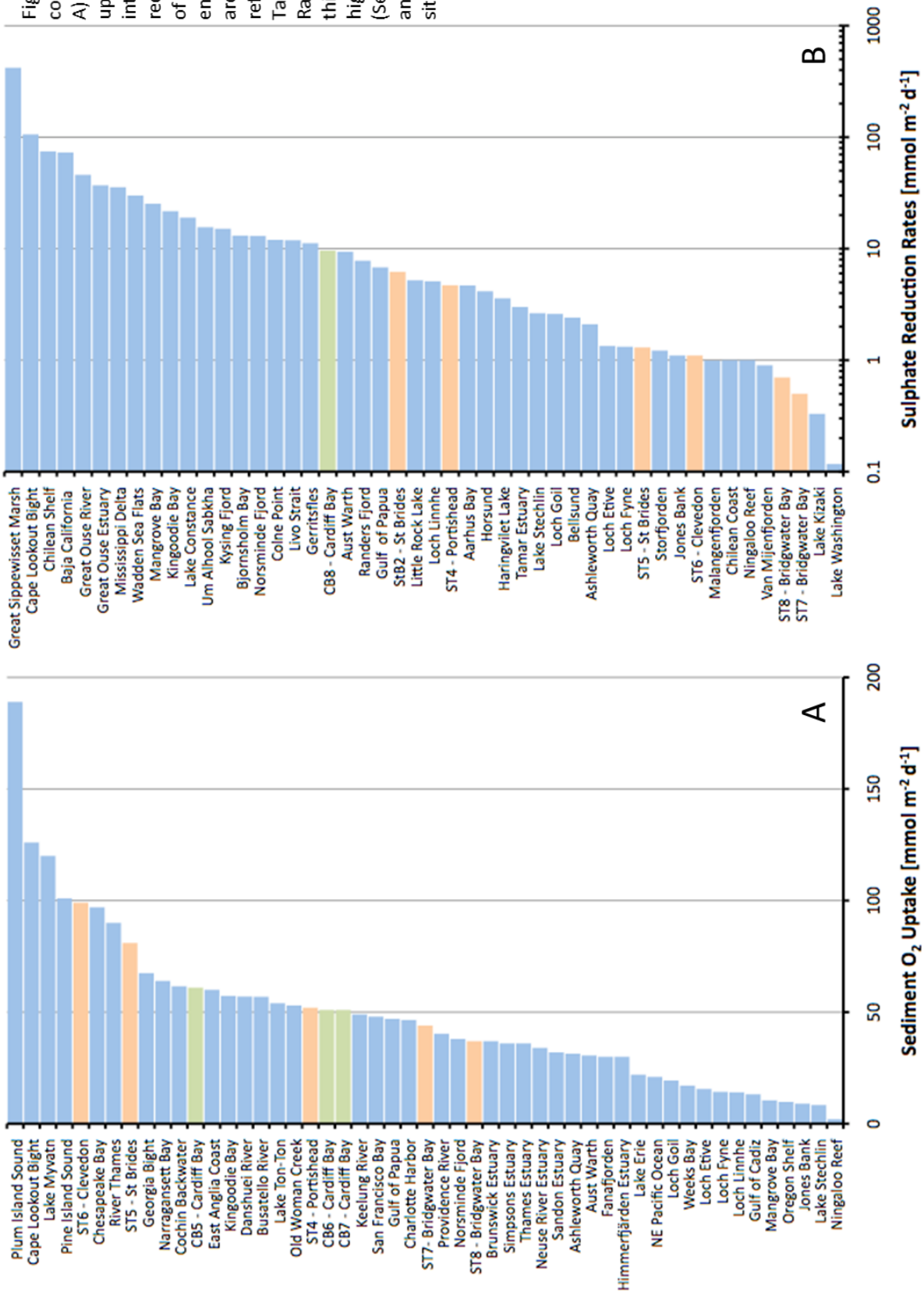


Fig. 9.19 – Graphs comparing areal rates of A) sediment oxygen uptake and B) depth integrated sulphate reduction from a variety of marine and lacustrine environments from around the world (for references consult Tables 9.1 and 9.3). Rates obtained during this study are highlighted in orange (Severn Estuary sites) and green (Cardiff Bay sites).

Statistical analysis of the data obtained in this study also indicates that re-suspension plays a major role in controlling biogeochemical activity, with data from both individual depths within cores and and areal rates of processes from whole cores clustering together in accordance with the degree of disruption the sediment in the cores was thought to have undergone. In addition analysis of the areal rates showed a strong relationship between porosity and SOD, again indicating that re-suspension has a role in controlling microbial metabolisms.

Total cell count profiles from across the estuary were high reinforcing the fact that the sediments are biogeochemically active. Counts showed distinct breaks in slope with depth, often corresponding to a change in sediment colour from brown (oxidised) to black (reduced). This step-down meant that near-surface sized prokaryotic populations remained in the sediment down to significant depths. This preservation of large surficial communities may help to explain the higher than average oxygen uptake measurements obtained from the cores, and is likely the result of regular mixing of the top layers of sediment, leading to the production of elevated and relatively homogeneous near-surface depth profiles. Such mixing is exemplified in the two fluidised mud cores examined in this study where near-constant mixing resulted in the formation of constant and elevated cell counts throughout the whole core. The presence of such elevated cell counts, and the associated step-down in numbers with depth was first described by Wellsbury *et al.* (1996) from a study site at Aust Warth, indicating that some factors of the Severn Estuary's unusual geomicrobiological environment are consistent on both a temporal as well as a spatial scale.

This study has also examined some of the more unusual environments in and around the Severn Estuary that have been created by both natural and anthropogenic means. The buried salt marsh peat deposits at St Brides Wentlooge have shown the potential of being significant sources of methylated amine compounds and provide an active methanogenic habitat compared to the rest of the estuary. The "activation" of these peat deposits is likely driven by the unusually dynamic nature of sedimentation in the Severn Estuary making the peats a unique habitat to study. Fluidised muds from both the Newport Deep and Bridgwater Bay contained elevated cell numbers, and in the case of Bridgewater Bay, heightened anaerobic activity compared to nearby more-stable sediments. The investigation of

Cardiff Bay showed that the conversion from a tidal mudflat to a freshwater lake has fundamentally changed the sediment geochemistry but does not appear to have harmed the size of the prokaryotic populations. In addition, methane and methanogenesis measurements have shown that Cardiff Bay sediments, like those in other anthropogenic lakes, produce high levels of methane, which has potential environmental consequences if this methane is released into the water column and atmosphere. Finally, many of the sites in this study showed evidence of being impacted to some degree by SGD. The full extent of this groundwater flow is unknown but could play a significant role in the subsurface biogeochemistry of sites around the estuary and as such warrants further examination.

In conclusion, this study has shown that the Severn Estuary is an extremely dynamic and unusual environment for sedimentary prokaryotes to inhabit. The system promotes the rapid growth of significant microbial communities, particularly aerobic/dysaerobic groups at the expense of anaerobic populations and processes, and not in the depth succession typically present in more quiescent estuaries. Despite this, isolated environments exist around the estuary where anaerobic processes are enhanced (particularly in locations affected by natural or anthropogenic environmental change), however, these still play a relatively small part in overall organic carbon degradation.

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