SHORT TERM EFFECTS OF HYPOXIA AND ANOXIA ON SEDIMENT BIOGEOCHEMISTRY

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ABSTRACT

Hypoxia and anoxia alter the behaviour, diversity and habitat of the benthic communities and cause accumulation of organic matter and nutrients. Most studies have focused on the effect of anoxia on benthic organisms and little information is available on nutrient sediment-water fluxes and rates of key biogeochemical processes. This study aimed to evaluate the oxygen concentration at which harmful effects start to occur, the rates that the system responds to different levels of oxygen saturation in the overlying water and the recovery times of system processes when re-aeration are restored. Sampling was carried out at Breydon Water, Great Yarmouth in June, 2010 and key biogeochemical processes were monitored in sediment/artificial seawater mesocosms maintained at constant temperature in the laboratory. The experimental work assessed the short term effect of different levels of oxygen by manipulating oxygen into five different treatments; oxic (96% saturation in the overlying water), hypoxia (25% saturation), one day of anoxia (24 hours without oxygen followed by re-aeration of up to 6 days), four days of anoxia (4 days without oxygen and subsequent re-aeration for 3 days) and seven days of anoxia (0% saturation). The effect of these oxygen concentrations on different biogeochemical processes (solute transport, oxygen uptake, nutrient fluxes, porewater chemistry, and enzyme activities) was quantified. Bromide used in solute transport assessments were analysed using inductively coupled plasma mass spectrometry and oxygen was measured using an oxygen minielectrode. Nutrient analysis was carried out using the SKALAR autoanalyser and enzyme activities were measured using microplate based assays. With decreasing oxygen supply, ammonium and phosphate are rapidly released into the water column, and nitrate concentrations decrease, reflecting the reducing conditions. In oxic conditions, rapid transport of water and oxygen into the sediments occurs through irrigation fluxes. Slower transport was observed from the porewater of anoxic treatments, as only molecular diffusion occurs. Vertical profiles of alkaline phosphatase, cellobiohydrolase, β-glucosidase and chitinase activities in the sediment reflect organic matter inputs decreasing with depth. Urease activity was enhanced following anoxic incubations of 7 days, with an inverse relationship with porewater ammonium concentrations and increased total organic carbon input. The biogeochemical resilience of the system is dependent on the duration of anoxic events. The effect of 1 day anoxia is relatively reversible and the system recovers to normal conditions within the 7 days of treatment. However, hypoxia (25% oxygen saturation) or longer duration (4 days) anoxia followed by re-aeration are enough to cause negative effects on the biogeochemical functioning of the system. So, sediment biogeochemistry has some resilience towards short term anoxic events, but more prolonged hypoxia or anoxic events that are continued for only a few days can have major effects on ecosystem function.

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CHAPTER 1 INTRODUCTION

1.1 Estuaries - Their Ecological Importance and Exposure to Environmental Pressures

Estuaries are a semi-enclosed coastal body of water which receive both seawater and freshwater mixing and are subject to daily tidal action (Church, 1975; Hobbie, 2000). They are among the most productive habitats in the world and serve as a receptacle for chemical flows originating from land. They account for high levels of nutrient present in the water column and sediments from seawater and freshwater inflows (Charles, 2004). Recognising their ability to store, bury and recycle chemicals and nutrients from the flow of overlying water to the sediments; estuaries play a vital role in regulating the biogeochemical cycle of nutrients and elements, climate change and organic matter mineralisation (Libes, 2009).

Accelerating agricultural activities, industrial development and population growth near estuaries have caused over enrichment of nutrients and organic matter accumulation (Nedwell et al., 2002; Doney, 2010). These problems are drastically enhanced by human population growth in coastal areas and near estuaries (Meyer-Reil and Koster, 2000). Human activities have resulted in increasing nutrient flows into the water and sediments, dumping or discharging of industrial wastes and municipal sewage sludges and altering the chemical composition of atmosphere through global warming (Libes, 2009). Excessive nutrient inputs, primarily nitrogen and phosphorus further increase the primary production by algae and macrophytes, resulting in eutrophication (Cai et al., 2011; Howarth et al., 2011). Subsequent decay of high plant biomass causes an increase in oxygen consumption which may lead to progressive oxygen depletion within the ecosystem (Meyer-Reil and Koster, 2000; Nedwell et al., 2002). Therefore, the impact of low oxygen conditions (hypoxia) and zero oxygen conditions known as anoxia (Gray et al., 2002; Rabalais et al., 2002; Diaz and Rosenberg, 2008) on sediment biogeochemistry is selected in this study as large number of estuaries is susceptible to this condition.

1.2 Hypoxia and Anoxia

In seawater, anoxia is defined as zero oxygen concentrations (Riedel et al., 2008; Rabalais, 2010) whereas hypoxia is defined as dissolved oxygen concentrations below ~25% of water oxygen saturation or ≤2ml of O_2/l and ~2.8 mg O_2/l (Gray et al., 2002; Wu, 2002; Larson and Sundback, 2008; Vaquer-Sunyer and Duarte, 2008). Hypoxia is influenced by natural phenomenon of the system itself, by human influences or as a result of both natural and anthropogenically-induced processes (Middelburg and Levin, 2009). Naturally occurring hypoxia is associated with water column stratification, which restricts water exchange in natural intrusions or upwelling of shelf systems, in silled basins and fjords such as the Black Sea (Wu, 2002; Diaz and Rosenberg, 2008). Human influences on coastal hypoxia include growing agricultural and industrial development which exerts increase amount of nutrients in water bodies, accumulation of pollutants and modification of the natural status of estuarine ecosystem (Gray et al., 2002; Middelburg and Levin, 2009).

Hypoxia starts with accumulation of organic matter in the system by production, respiration and decomposition processes. The deposition of organic matter enhances microbial growth, which exerts greater demand for oxygen through respiration. If stratification of the water column occurs, dissolved oxygen concentration will be further depleted. Then, the second phase is initiated where hypoxia occurs for a brief period or short-lived, which causes mortality of benthic aerobic organisms (Vaquer-Sunyer and Duarte, 2008). As time goes on, increasing amounts of nutrients and organic matter in the sediments result in the progression of the third phase of hypoxia, seasonal or periodic hypoxia. This phase particularly affects the animal population, with altering behaviour, physiology and mortality of organisms (Steckbauer et al., 2011). If nutrients and organic matter continue to accumulate and hypoxia persists, the dissolved oxygen concentration will further deteriorate until anoxia happens. During anoxia, microbially generated hydrogen sulphide is released (Diaz and Rosenberg, 2008). As a consequence of hypoxia, the number of fishes reduced and food chain are being disrupted (Karlson et al., 2007).

There has recently been increased concern about the oxygen status of the North Sea due to evidence of potentially hypoxic regions and decreasing oxygen levels (Greenwood et al., 2010; Jonasson et al., 2012). The causes of these low oxygen levels are related to isolation of a shallow bottom mixed layer and the re-mineralisation of the organic matter produced during the spring bloom. In the Humber Estuary, UK, large scale oxygen depletion has been recorded historically in particular at Trent Falls, the upper reaches of the estuary and River Ouse (Uncles et al., 1998). The oxygen depletion is attributed to direct discharges of sewage and industrial waste to the estuary. However, in recent years this event has become irregular, reducing in temporal frequency as well as spatial extent (Cutts et al., 2008). Parts of the Mersey estuary may be particularly vulnerable to oxygen depletion, as changes in water quality is highest in semi-enclosed water bodies with long retention times, and where stratification of the water column occurs (Langston et al., 2006).

Based on the duration and dominant time scales of low oxygen, four types of hypoxia can be identified in the ecosystem, which are 1) permanent, 2) seasonal, 3) periodic and 4) episodic hypoxia. The first type, permanent hypoxia occurs in shelf regions, large fjords and inland seas where strong stratification leads to bottom water hypoxia/anoxia in this region (Gilbert et al., 2010). Table 1.1 showed some of the ecosystems experiencing hypoxia distributed all over the world based on the different types of hypoxia.

The second type, seasonal hypoxia is the most commonly occurring hypoxia, responsible for about half of the known dead zones. Seasonal hypoxia usually occurs once in a year, generally during summer after spring blooms. In summer, water stratification is the strongest and water is the warmest which enhance the declining of oxygen concentration in the water bodies (Diaz and Rosenberg, 2008). Seasonal hypoxia may occur in both stratified temperate estuaries and shelf regions as well as throughout the water column of shallow well-mixed estuaries and tidal rivers, subjected to summer heat strengthen stratification (Kemp et al., 2009). This is the most well studied type of hypoxia in the reported literature (Diaz and Rosenberg, 2008; Selman et al., 2008; Kemp et al., 2009; Turner et al., 2012).

Table 1.1 Global distribution of hypoxia in estuaries, coastal and marine ecosystems based on the type of hypoxia (adapted from Diaz and Rosenberg, 2008 and Rabalais et al., 2010).

System	Year	Hypoxia Type	Impacts	References
Pacific Oceans	2002	Permanent	Widespread foraminiferans and meiofaunal nematodes	Helly and Levin, 2004
North Indian Oceans	1998	Permanent	Fish death and plankton blooms	Navqi et al., 2010
Donegal Bay, Ireland	2000	Episodic	Harmful algal blooms	Silke et al., 2005
Tokyo Bay, Japan	1970	Episodic	Decline of Mantis shrimp	Kodama et al., 2006
North Carolina estuaries	2004	Episodic/Diel	Fish abundance decreased, replaced with resilience estuarine and freshwater species	Stevens et al., 2006
Harvey Estuary, Australia	1980	Periodic	Algal bloom	Hearn and Robson, 2001
Westerchelde Estuary, Netherlands	1990	Periodic	Affects nitrification/denitrification	Nobre et al., 2005
Chesapeake Bay, USA	1950-2001	Seasonal	Mortality with annual recolonisation	Hagy et al., 2004
Northern Gulf of Mexico	1970	Seasonal	Blooms of dinoflagellates	Rabalais et al., 2002
Dokai and Ise Bays, Japan	1990	Seasonal	Reduce shellfish populations	Ueda et al., 2000
Mersey Estuary, England	1850	Seasonal	Fish kills	Jones, 2006
Thames Estuary, England	1815	Seasonal	Mortality of fish and benthic macroinvertebrates	Shand, 2005
Loire Estuary, France	1990	Seasonal	Mortality of migratory species and mullet, *Liza ramada*	Abril et al., 2003
Waquoit Bay, USA	1995-2000	Seasonal	Overgrowth of macroalgae	Wenner et al., 2004
California Current Large Marine ecosystems, USA	2000	Seasonal	Absence of all fish from rocky reefs	Chan et al., 2008
Corpus Christi Bay, USA	1980	Seasonal	Reduced benthic organisms	Montagna and Froeschke, 2009
Scheldt Estuary, Belgium	1990	Seasonal	Fluctuations of fish	Maes et al., 2007
Pearl River Estuary, China	2000	Seasonal	High partial pressure of carbon dioxide and high ammonium concentrations	Dai et al., 2006
Caspian Sea, Turkmenistan	1990	Seasonal	Mortality with reduced benthos	Dumont, 1998
St Lawrence Estuary, Canada	1980	Seasonal	Avoidance of fisheries	Benoit et al., 2006

Periodic hypoxia is less severe than seasonal hypoxia, which tends to occur more frequent than seasonal hypoxia, lasting from days to weeks only. This type of hypoxia generally occurs in productive, shallow, slightly deeper (3-8 m) weakly stratified micro tidal coastal systems (Park et al., 2007; Kemp et al., 2009). Relatively, this type of hypoxia happens because of local weather events such as storm and turbulence. It is also influenced by wind and spring neap-tidal cycles, which may increase water stratification for a certain period of time. Daily diel cycles may also caused periodic hypoxia since this processes affects production and respiration, which generally lasts only for a few hours, but has a daily reoccurrence (Kemp et al., 2009).

Episodic hypoxia or diel hypoxia is the least frequent type to occur compared to seasonal and periodic hypoxia, with less than one event per year and it may also happen with years elapsing between events. This type of hypoxia tend to appear and disappear on short (hours to days) time scales in very shallow tidal estuaries (1-5m) (D'Avanzo and Kremer, 1994; Tyler et al., 2009). Normally, this type of hypoxia is triggered by excessive night time respiration of organic matter exceeding oxygen replenishment by air-water exchange during the day by photosynthetic oxygen production (Kemp et al., 2009). When a system has reached this stage, it is an indicator that eutrophication problem within the area is reaching a critical point along with physical factors that stratify the water column, which results in the progression of hypoxia. Episodic hypoxia can be exacerbated by major storm events that deliver large pulses of organic loading. Effect of storm events in estuaries caused a rapid decrease in dissolved oxygen concentrations, which might play a crucial importance in determining the initial response and resilience of the system towards prolonged negative consequences associated with oxygen depletion (Diaz and Rosenberg, 2008).

Large numbers of cases of hypoxia and anoxia have been documented, but some of the systems are already in the state of recovery as a result of measures that have been taken to reduce nutrient concentrations (Rabalais et al., 2010). The complexity of eutrophication processes associated with other environmental pressures such as global warming and ocean acidification (Vaquer-Sunyer and

Duarte, 2008; Howarth et al., 2011), may influence the rate of recovery (Mee, 2001). The intensity, duration and frequency of hypoxia and anoxia are the key factors determining the response on coastal waters and marine ecosystems.

At present, no studies in the literature have looked at the effect of short term anoxia and re-aeration on nutrient fluxes associated with enzyme activities in estuarine systems. Therefore, this study focused on the effect of short term hypoxia and anoxia on sediment biogeochemistry, particularly nitrogen and phosphorus cycle and enzyme activities related to carbon, nitrogen and phosphorus cycle. Additionally, the information about solute transport, oxygen fluxes and total organic carbon and total nitrogen within the system may help in understanding the changes in nutrient fluxes, within the sediment-water interface and enzyme activities in the sediment.

1.3 Solute Transport and Oxygen Fluxes

The study of solute transport has received attention because it shows how water is distributed within the system and provides clues of how harmful substances are spread within the system (Ohrstrom et al., 2004). Transport rates of chemicals are often used in biogeochemical studies of sediments to produce estimates of sediment-water fluxes (Berg et al., 2001). Solute transport in sediment affects the distributions of reactants and metabolites, the location and magnitude of early diagenesis reactions and the microbial processes (Meile and Cappellen, 2003; Dornhoffer et al., 2012). Solute exchange is driven by tides and currents which give way to molecular diffusion through porous medium of the sediment (Meile and Cappellen, 2003). In the uppermost layers of marine sediment, however, biologically induced solute transport (bioirrigation) can exceed transport due to molecular diffusion (Kristensen and Hansen, 1999; Meile et al., 2001). An effective means for describing and quantifying solute exchange through various biological and physical processes between the sediment water interfaces is by using tracers (Bradshaw et al., 2006).

Some ions which are used as tracers in seawater are present as conservative ions and some exist as non-conservative ion. When ions are present in nearly constant proportions in the system, these ions are termed as conservative since their

concentrations are mostly controlled by physical processes associated with water movement, such as transport by currents, mixing via turbulence, evaporation and rainfall. If the ions are not present in constant proportions and are altered by chemical reactions that occur faster than the physical processes responsible for water movement, these ions are termed as non-conservative (Libes, 2009). Bromide is a conservative tracer that does not undergo sorption or chemical and biological transformations (Keefe et al., 2004). So, it can be used as a non-reactive tracer of the mass flow of water through the sediments (Kazemi et al., 1998). Bromide is commonly spiked into the water as a solute transport tracer in sediment (Forster et al., 1995; 1999; Quintana et al., 2011). The use of bromide as a tracer avoids the regulatory constraints and complexities associated with the use of radioactive tracers (Sayles and Martin, 1995). In this study, bromide is used to assess the effect of oxygen depletion and re-aeration on the water movement of fine-grained sediment by measuring solute transport and related fluxes between the sediment and the water.

The effect of changing the oxygen concentration in the water overlying fine-grained sediment was assessed on the flux of oxygen across the sediment-water interface and on the distribution of oxygen within the sediments. This flux provides information on the oxygen penetration depth in fine-grained sediment and how oxygen is consumed. In estuaries, oxygen concentration in the overlying seawater may vary due to water stratification, tidal effects, storm occurrence or hydrodynamic effects (Lewis et al., 2007). Oxygen penetration depth (OPD) was determined from oxygen profiles as the depth below sediment water interface where oxygen permeating from the water column is exhausted (Katsev et al., 2007). The consumption of oxygen by coastal sediments is responsible for extensive hypoxia in estuaries and eutrophicated coastal zones (Zimmerman and Canuel, 2000; Reimers et al., 2012). Oxygen distribution results from a complex interplay between physical processes (diffusion, sedimentation), chemical reactions (oxidation of reduced species) and biogeochemical processes such as photosynthesis or respiration (Rowe et al., 2002; Lansard et al., 2003; Kim and Kim, 2007; Glud, 2008; Glud et al., 2010).

1.4 Organic Matter

Organic matter is derived from bacteria, phytoplankton and zooplankton in the water column, bacteria and diatom from the upper surface of the sediments and through the production by algae (Cowie and Hedges, 1994; Fernandes et al., 2007). Natural inputs of organic matter originate from land-derived nutrients which are washed off into the coastal ecosystems. External sources of organic matter inputs by humans are largely through increase human population and settlement along coastal and estuarine areas (Kristensen, 2000; Meyers et al., 2002). This leads to increase sewage input to the coastal areas (Mudge and Duce, 2005).

Biogeochemical processes in sediments are influenced mainly by conditions in the water column. Easily degradable organic matter within the water column and the composition of bottom waters are the major factor fuelling the sediment biogeochemistry (Soetart et al., 2000). In the water column, eddy diffusion and particle settling are the transport processes. Organic matter produced from primary production within the water column is deposited to heterotrophic consumers inhabiting sediment as means for getting nutrients and energy. These organic matters are then processed by various microbes, protozoans and metazoans (Kristensen, 2000). Processes which occur in the sediments are different from the water column because of different mechanism of transport processes. In sediments, molecular diffusion and bioturbational mixing prevails. Most of biogeochemical processes involved in sediments are linked with decomposition of organic matter. Organic matter is synthesised in the euphotic zone, where primary production takes place since sunlight may penetrate the area and early diagenesis process can occur (Ingalls et al., 2004). Some of the organic matter which are too large to be grazed and sink to the seabed along with faeces and particulate organic matter are degraded in the water column (Hedges et al., 2001).

A minor portion is subjected to sediment burial, contributing to refractory organic matter (Middelburg and Levin, 2009; Burdige, 2011). Secondary production by heterotrophic organisms assimilates the organic matter by respiration or mineralisation. These two processes consume high amount of oxygen, thus

contributing to hypoxic and anoxic conditions within the upper few centimetres of the sediment surface.

Redox reactions are a means of obtaining energy and essential elements for marine and estuarine organisms. This process is controlled by the spontaneous reduction of oxygen and oxidation of organic matter. When oxygen is low, microbes engage in metabolic strategies that involve reduction of oxidised solutes, such as nitrate, Mn (IV), Fe (III), sulphate and organic matter. Therefore, how organic matter is produced and processed based on the most energetically favourable redox reaction is important to understand the different processes occurring in the sediments.

Due to high demand of oxygen in the upper sediments, organic matter degradation in sediments proceeds using the most available oxidant producing the greatest free energy (Figure 1.1). Organic matter degradation follows a certain sequence of reactions, with each successive reaction starting when the previous oxidant is depleted or when chemical and biological conditions favour certain type of organisms to flourish (Bianchi, 2007; Libes, 2009). The rate of decay depends on a few factors, mainly the quality of organic matter, whether labile or refractory, the major composition of organic matter such as protein, cellulose and lignin, at which decomposition stage it is going through and temperature effect (Sullivan et al., 2010). Generally, the formula for chemical composition of organic matter in estuarine sediments, are:

$$(CH_2O)_x(NH_3)_y(H_3PO_4)_z,$$

where x, y and z varies according to the origin and age of the material. The composition for freshly produced marine organic matter is usually close to the Redfield ratio: x = 106, y = 16 and z = 1 (Kristensen, 2000; Schneider et al., 2003).

Oxygen limitations in the upper sediments initiate a cascade of alternative electron acceptor use by anaerobic organisms to continue the decomposition process into anoxic layers. In the oxic (oxygen containing) zone, aerobic respiration, nitrification and sulphide oxidation takes place. Anaerobic decomposition involves different types of bacteria for the processes of manganese oxide reduction, denitrification, iron oxide reduction, fermentation, sulphate reduction and carbon

dioxide reduction (Figure 1.1). Although the strict vertical distribution of electron acceptors shown in Figure 1.1 is an oversimplification of the true spatial distribution; generally this is the pattern of aerobic and anaerobic decomposition process within zones. The decomposition process starts off with hydrolysis and fermentation of complex polymeric organic molecules into water soluble monomers such as amino acids, monosaccharides and fatty acids. During the process of hydrolysis and fermentation, energy and inorganic nutrients are being released (Libes, 2009). The stoichiometries for denitrification and sulphate reduction are as follows:

Denitrification (**Equation 1.1**):

$$\mathbf{CH_3COOH + 1.6\ NO_3^- + 1.6\ H^+ \rightarrow 2CO_2 + 0.7N_2 + 2.8\ H_2O}$$

Sulfate reduction (**Equation 1.2**):

$$\mathbf{CH_3COOH + SO_4^{2-} \rightarrow 2CO_2 + S^{2-} + 2H_2O}$$

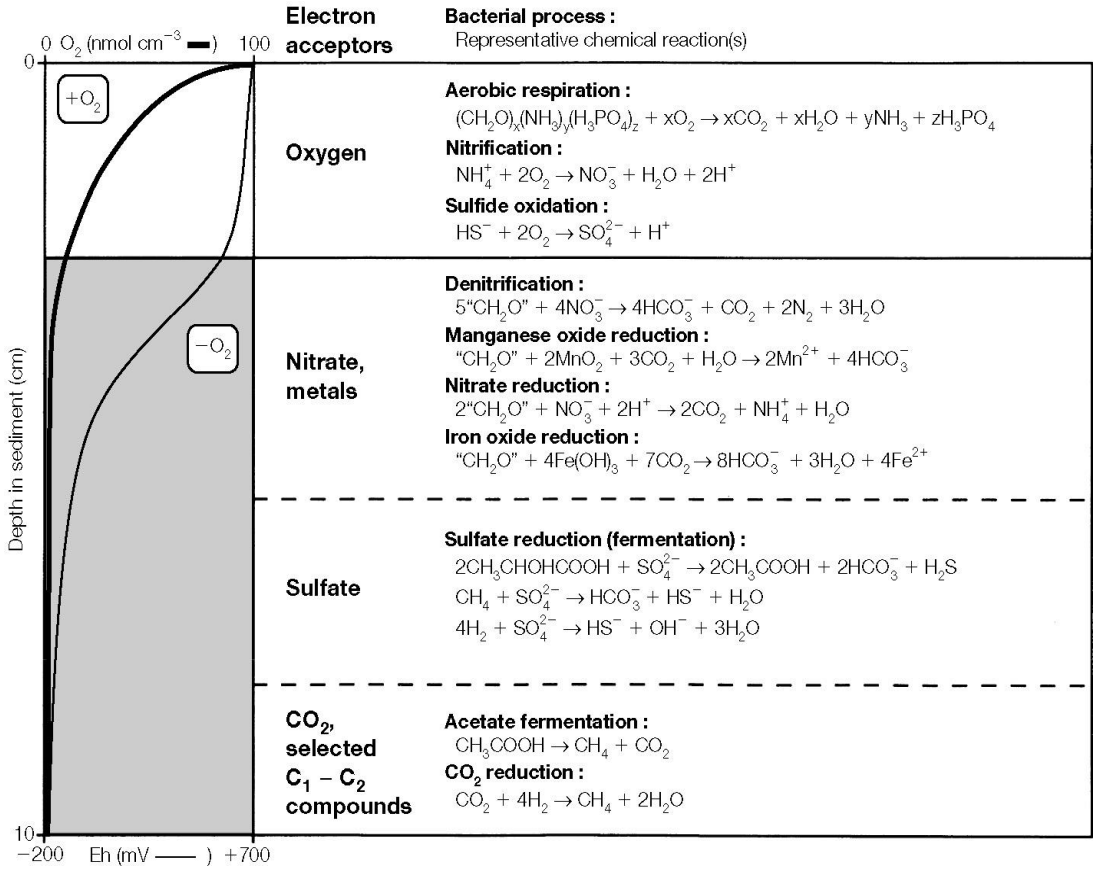


Figure 1.1 Vertical processes of diagenetic reactions in sediments. Source: from Libes, (2009).

Methane is the end product when all oxidants have been consumed during organic matter degradation. During anaerobic respiration, various reduced substances are produced such as ammonium, iron (II), manganese (II), hydrogen sulphide and methane. These reduced substances will eventually be re-oxidised since they contain enough energy from the organic matter and mediated by microorganisms (Kristensen, 2000). Ammonium produced from organic nitrogen mineralisation can be oxidised either aerobically or anaerobically through the process of anammox (Jetten, 2008). Reduced iron and manganese in dissolved form is released by dissimilatory reduction of particulate metal oxides. Sulfide produced by sulphate reduction is also reoxidised due to reactions with iron and other metals (Leloup et al., 2007). Since sulphate is the second most abundant anion in seawater, majority of anaerobic mineralisation occurs through sulphate reduction. During sulphate reduction, sulphide reacts with iron to produce iron sulphide whereas another part reacts with organic matter or is involve in oxidative sulphur cycle (Middelburg and Levin, 2009). The end product from this process depends on bioturbation of reduced particulate sulphur and non-steady state diagenetic processes stimulating contact between reduced and oxidised compounds. Reduced sulphur is re-oxidised by oxygen, nitrate, metal oxides or more oxidised sulphur compounds (Riedinger et al., 2010). Majority of methane is oxidised anaerobically involving bacteria and archaea.

Due to the high demand of oxygen within coastal sediments and estuaries, anaerobic mineralisation usually dominates because of high organic carbon loading (Middelburg and Levin, 2009). The majority of oxygen consumption is attributable to reoxidation processes and less than 25% is directly attributable to aerobic respiration processes.

1.5 Nutrients in Estuaries

Since hypoxia and anoxia are largely driven by excessive nitrogen and phosphorus inputs, the following focuses on the role of estuaries in governing nitrogen and phosphorus cycling and the changes caused by oxygen.

1.5.1 Nitrogen Cycling

Nitrogen is one of the most important elements required by biological systems and in estuaries; its availability is frequently implied as one of the factors limiting productivity (Jjemba, 2004; Ward et al., 2007). Given its major role within these ecosystems, there is a need to establish the forms in which nitrogen is present, the routes by which they are transformed, the microorganisms concerned with these processes and factors associated with its presence (Herbert, 1999). Nitrogen is present in many oxidation states with commonly found species in the form of nitrate ion (NO_3^-), nitrogen dioxide gas (NO_2), nitrite ion (NO_2^-), nitrous oxide gas (N_2O), ammonia gas (NH_3), and ammonium ion (NH_4^+) (Thamdrup and Dalsgaard, 2008; Libes, 2009). Nitrogen cycling is largely controlled by redox transformations between the states which are mediated by organism such as phytoplankton, bacteria and probably archaea. With the exception of dissolved nitrogen gas, the principal forms of nitrogen occurring in marine and estuarine ecosystems are nitrate, nitrite, ammonium and organic nitrogen. Sources of nitrogen include precipitation, agricultural and urban run-off, sewage discharges, sludge dumping and biological nitrogen fixation (Herbert, 1999).

The first step in initiating nitrogen cycle is nitrogen fixation (Jjemba, 2004). Most of nitrogen in estuaries is present as nitrogen gas which is biologically inaccessible except to those few microbes, called nitrogen fixers that can break nitrogen's strong triple bond. Nitrogen fixers can be photoautotrophs, photoheterotrophs, or chemoheterotrophs showing that nitrogen fixation may occur both in light and dark. The following sections discuss various processes involved in nitrogen cycling, starting with nitrogen fixation, followed by ammonium assimilation through solubilisation process, ammonification, ammonium oxidation, nitrate reduction, denitrification and dissimilatory nitrate reduction to ammonium.

1.5.2 Nitrogen Fixation

Nitrogen fixation is an enzyme-mediated reduction of nitrogen gas (N_2) to ammonium, a major source of fixed nitrogen to the biosphere and is particularly

important in nitrogen limited environments (Ward et al., 2007). The stoichiometry of nitrogen fixation requires 8 electrons and 16 molecules of ATP to reduce one molecule of N_2, as shown below:

Equation 1.3:

$$N_2 + 8H^+ + 8e^- + 16MgATP \xrightarrow{\text{nitrogenase}} 2NH_3 + H_2 + 16MgADP + 16P_i$$

Nitrogen fixation is energetically expensive, and only prokaryotes can fix nitrogen such as *Trichodesmium* and the cyanobacteria, *Crocosphaera* (Libes, 2009). The reduced nitrogen or ammonium is then incorporated into organic matter and released again when organic nitrogen compounds are degraded (Thamdrup and Dalsgaard, 2008). The enzyme required to facilitate nitrogen fixation is nitrogenase. Iron is an essential cofactor and is required in large amounts, making iron availability a major factor limiting nitrogen fixation. Nitrogenase is inactivated by oxygen, so, aerobic prokaryotes (*Cyanobacteria, Azotobacter, Rhizobium*) form thick cell walls that prohibit oxygen exchange, creating an anoxic intracellular environment (Libes, 2009). Some free-living cyanobacteria protect their nitrogenase against oxygen exposure by conducting nitrogen fixation only at night when photosynthesis is not occurring. Recent investigation has revealed that the rate of nitrogen fixation is underestimated since no estimates have yet been made in hydrothermal systems, anoxic zones in estuarine waters and anoxic microzones within sinking detrital particulate organic matter (Libes, 2009).

1.5.3 Ammonification and Ammonium Assimilation

In the process of nitrogen fixation, N_2 gas is reduced to ammonium which is assimilated into organic nitrogen through anabolic activities of the autotrophs and their consumers (Thamdrup and Dalsgaard, 2008). During the process of ammonium assimilation, ammonium is transported across the cell membrane, mediated by permeases enzyme. Once ammonium has crossed the cell membrane, it can participate in anabolic reactions. For example, ammonium is incorporated into amino acids by first reacting with α-ketoglutaric acid, followed by transamination reactions (Jjemba, 2004). Other nitrogenous biomolecules are similarly created by these reactions, producing nucleotides, amino sugars, photosynthetic pigments, vitamins

and other metabolites. If ammonium concentrations in seawater are low, nitrate and nitrite will be assimilated through the process of assimilatory nitrogen (nitrate or nitrite) reduction. This process is also mediated by permeases and once inside the cell, these dissolved inorganic nitrogen (DIN) species are transformed into ammonium via redox reactions (Libes, 2009). But, phototrophs prefer to assimilate ammonium compared to nitrate and nitrite as less redox energy is required. Some phototrophs may also assimilate dissolved organic nitrogen (DON), such as urea to meet their nitrogen needs (Bianchi et al., 2004).

The process of solubilisation comes into play when particulate organic nitrogen (PON) is decomposed into DON by fragmentation of biomolecules into compounds small enough to be classified as dissolved (Madsen, 2008). The organic nitrogen can be decomposed by heterotrophic bacteria back into ammonium (Jjemba, 2004). The process of cell lysis, hydrolysis of protein, and metabolism by heterotrophs is a three step known as ammonification (Figure 1.2).

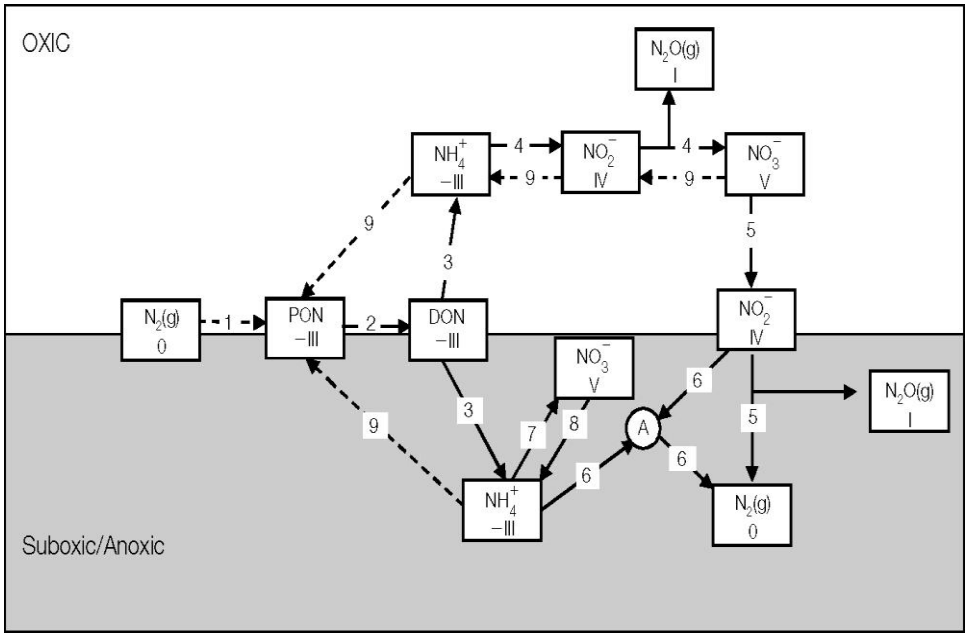


Figure 1.2 Nitrogen cycle through various redox and phase transformations. The boxes contain the nitrogen species and its oxidation number. The arrows represent transformation reactions as follows: 1) nitrogen fixation, 2) solubilisation, 3) ammonification, 4) nitrification, 5) denitrification, 6) anammox, 7) anaerobic nitrification mediated by manganese reduction, 8) DNRA, 9) assimilatory nitrogen reduction. A=anammox microbes (Source: from Libes, 2009).

1.5.4 Ammonium Oxidation

Two processes are involved in ammonium oxidation which are nitrification and anaerobic ammonium oxidation or anammox (Libes, 2009). In oxic conditions, NH_4^+ is thermodynamically unstable, chemoautolithotrophic bacteria of the family *Nitrobacteraceae* mediate the stepwise oxidation of ammonium to nitrite and then to nitrate known as nitrification (Madsen, 2008). The first step is conducted by organisms such as *Nitrosomonas* and *Nitrocystis* (an autotrophic organism) by oxidising ammonium to nitrite in an aerobic environment and the second step by *Nitrobacter*, *Nitrospira*, and *Nitrococcus*, by oxidising nitrite to nitrate (Jjemba, 2004; Madsen, 2008; Thamdrup and Dalsgaard, 2008; Libes, 2009).

Anammox occurs under anaerobic conditions in the water column and sediments when ammonium is oxidised through two pathways (Libes, 2009). First, ammonium is oxidise using nitrite as the electron acceptor and contributes as a dissimilatory nitrogen reduction type, by which DIN is converted to N_2 (Shivaraman and Shivaraman, 2003). This process is an alternative to denitrification in removing fixed nitrogen from the marine and estuaries ecosystem. Second type of anammox involves the oxidation of ammonium using manganese, Mn (IV) as the electron acceptor in anoxic sediments. This causes the manganese to be reduced to Mn^{2+} and the nitrogen to be oxidised to N_2 (Libes, 2009).

1.5.5 Nitrate Reduction

In the presence of oxygen, nitrate is the thermodynamically stable form of nitrogen, whereas in the anoxic part of the sediment, it is a reactant in dissimilatory redox reactions that lead to the formation of N_2 or ammonium. The first step in this reductive pathway is the reduction of nitrate to nitrite, which is catalysed by nitrate reductases, which contain a molybdenum cofactor (Thamdrup and Dalsgaard, 2008). This is often treated as part of the denitrification pathway. A catalyse is required because of the high stability of nitrate ion in the environment. From nitrite, three pathways of reduction follows, which are denitrification and anammox, leading to N_2 and dissimilatory nitrate reduction to ammonium (DNRA).

1.5.6 Denitrification

Denitrification is the reduction of nitrate to N_2 by heterotrophic bacteria. The stoichiometry is:

Equation 1.4:

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 84.8\,HNO_3 \longrightarrow 106\,CO_2 + 148.8\,H_2O + 42.4\,N_2 + 16\,NH_3 + H_3PO_4$$

Denitrification proceeds through a series of steps with nitrate reduced to nitrite (nitrate reduction process) followed by subsequent reduction of nitrite to N_2. Under some conditions, N_2O is also produced. Some of the nitrogen that is denitrified is derived from organic matter undergoing oxidation. Two fates of this process occur when first, organic nitrogen present in the reactant organic matter is oxidised to N_2 and another organic nitrogen in the reactant organic matter is ammonified. The relation between anammox and denitrification occurs when some of the ammonium produced during denitrification is used by anammox bacteria and converted to N_2 (Libes, 2009). Two equations to describe the stoichiometry of denitrification are: Equation 1.4 and equation 1.5 below:

Equation 1.5:

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 94.4\,HNO_3 \rightarrow 106\,CO_2 + 177H_2O + 55.2\,N_2 + H_3PO_4$$

1.5.7 Dissimilatory Nitrate Reduction To Ammonium (DNRA)

Under anaerobic conditions, heterotrophic bacteria will use organic matter as an electron donor to drive the reduction of nitrate to ammonium. This process is known as dissimilatory nitrate reduction to ammonium (DNRA). It is coupled to nitrate reduction or nitrite ammonification (Thamdrup and Dalsgaard, 2008). This process is important as a sink of nitrate in coastal and estuarine sediments. Some sedimentary bacteria perform DRNA by using sulphide as an electron donor, for example in giant sulphur bacteria such as *Thioploca* sp. and *Beggiatoa* sp. (Hulth et al., 2005).

As a result of DIN transformations in response to different oxygen levels, we would expect nitrate to be dominant in the surface layers of the sediment in aerobic conditions, whereas in anoxic conditions, ammonium is expected to prevail. Re-

aeration effect of 1DA (1 day without oxygen followed by re-aeration of 6 days) and 4DA (4 days without oxygen and subsequent re-aeration of 3 days) would cause negative effect to nitrogen cycling, resembling anoxic conditions.

1.5.8 Phosphorus Cycling

Phosphorus (P) availability is crucial in estuaries since it is known as a limiting nutrient, which in turn gives an impact to the primary production and sequestration of atmospheric carbon dioxide in organic matter (Paytan and McLaughlin, 2007). Unlike nitrogen, P cannot be fixed from the atmosphere, and only a minor portion is contributed by dust deposition (Libes, 2009). P occurs naturally in the form of inorganic phosphate minerals and organic phosphate derivatives in rock and soil. In estuaries, organic P only constitutes a small portion of the bulk weight, and is much less abundant than inorganic P (Paytan and McLaughlin, 2007).

The main source of P in estuaries is through river runoff and the sink is through deposition and burial in the sediments (Libes, 2009). P is transported to coastal marine and estuaries in the form of particulate and dissolved phases via riverine flux. Anthropogenic sources of P includes from fertiliser, sewage, soil erosion, livestock and paper pulp manufacturing (Paytan and McLaughlin, 2007).

P burial in the sediments depends on the redox conditions of the sediment. Oxic surface sediments are usually rich in manganese and ferric iron phases which take up large amount of phosphate by adsorption and mineral formation. The opposite is for anoxic sediments, which are depleted of these phases and P is more bound to calcium minerals (Rozan et al., 2002).

In sediments, initial burial of P occurs with organic carbon or P associated with iron oxyhydroxides (Steenbergh et al., 2011). Once in the sediment, labile forms of P are transformed to authigenic forms in a variety of redox states. In oxidising sediments, the majority of the flux of organic phosphorus from the water column is mineralised within the sediments. The released phosphate is partitioned between the porewater and surface adsorption sites. Surface adsorbed phosphate is released into

the porewater to replace the dissolved phosphate that escapes the sediment/water interface and into the water column. If conditions become reducing, phosphate is released to porewaters from the reduction of iron oxides (Belias et al., 2007). Sediments from anoxic conditions desorb or release phosphate from the sediments exceeding the adsorption capacity, whereas in oxic conditions, desorbed phosphate is rapidly readsorbed by iron oxyhydroxides or precipitated as apatite (Paytan and McLaughlin, 2007). In relation to the context of this study, we would postulate that phosphate is immobilised in oxic conditions as a result of Fe (III) binding to phosphate. In contrast, phosphate will be released in periods of anoxic condition due to Fe (III) reduction to Fe (II).

1.6 Enzyme Activities in Sediments as Indicators of Biogeochemical Processes

Enzyme activities come from various sources of biotic, abiotic compounds, cells, mineral complexes and the soil solution phase (Taylor et al., 2002). Estuarine benthic environments are characterised by rapid sediment accumulations (Alongi et al., 2005) and high supplies of organic material (Sobczak et al., 2002). Large quantities of riverine materials, organic loads, and autochthonous organic material produced in the estuary are deposited at the sediment surface. The organic matter remineralisation is initiated by microbial extracellular enzymes (Arnosti et al., 2009; Arnosti, 2011), which have been studied extensively in marine environments (Meyer-Reil, 1986; Mayer, 1989; Boetius et al., 2000; Danovaro et al., 2001; Arnosti and Holmer, 2003). The amount and composition of organic matter are cues for the synthesis of enzymes. Specific enzymes in the sediments are produced for the decomposition of specific compounds of organic matter (Meyer-Reil, 1986; Arnosti and Holmer, 2003). Substrate induction is an important mechanism controlling the activity of enzymes (Witte et al., 2003).

Vertical patterns of enzyme activity are variable and related to the specific characteristics of the benthic environment and the specific enzyme considered. Enzyme activities tend to co-vary with changes in substrate content and temperature (Meyer-Reil, 1986; Mayer, 1989; Boetius et al. 2000; Arnosti and Holmer, 2003;

Ziervogel and Arnosti, 2009). Changes in enzyme activity with depth may depend on the characteristics of the enzyme, the sediment organic matter or both (Poremba, 1995). However, enzyme activity can be quite constant with depth irrespective of substrate content (Poremba, 1995; Arnosti and Holmer, 2003). This study provides information on vertical distributions of five types of extracellular enzyme activities alkaline phosphatase (AP), cellobiohydrolase (CB), β-Glucosidases (BG), chitinase and urease activities in fine estuarine sediments to assess changes to the phosphorus, nitrogen cycle and organic carbon decomposition.

1.6.1 Alkaline Phosphatase (AP)

Phosphatases have a broad range of organic substrate specificities, thus hydrolyse a wide variety of organic phosphomonoesters (Shi et al., 2011). Phosphatase enzymes are attached to cell surfaces or freely dissolved in the water column resulting from cell lyses or excretion (Hoppe, 2003). Upon enzyme hydrolysis, organic phosphomonoesters release inorganic phosphate into the water, thereby increasing inorganic phosphate availability for planktonic as well as benthic organism in shallow marine system (Zhang et al., 2007; Koch et al., 2009). The role of AP is important during periods when there are low inorganic phosphorus concentrations in the water column (Wright and Reddy, 2001). AP contributes as an essential component of microbial phosphorus activity, regenerating inorganic phosphorus through the catalysis of organic phosphorus esters to inorganic phosphate (Wright and Reddy, 2001). The relationship of AP and phosphate is shown below:

Equation 1.6:

$$\text{Organic Phosphorus} \xrightarrow{\text{AP}} \text{Phosphate}$$

AP has been identified and associated with all major groups of eukaryotic algae (Litchman and Nguyen, 2008), cyanobacteria (Koch et al., 2009), heterotrophic bacteria (Cotner et al., 2000) and submerged macrophytes, including seagrass (Martinez-Crego et al., 2006). Phosphatase activity is widely studied in freshwater lakes (Wright and Reddy, 2001; Yiyong et al., 2008; Chen et al., 2011; Jiang et al., 2011), coastal waters and deep-sea sediments (Paytan and McLaughlin, 2007; Caruso, 2010; Steenbergh et al., 2011), lagoon sediments (Sabil et al., 1995),

estuaries (Koch et al., 2009) and forest soil ecosystem (Caldwell, 2005). The interest in phosphatase activity stems from its potential role in evaluating phosphorus and carbon limitation within these ecosystems (Wojewodzic et al., 2011).

Although numerous investigations have been carried out on AP activities in lake sediment (Zhou et al., 2008; Deng et al., 2009) and coastal and deep sea sediment (Coolen and Overmann, 2000; Danovaro et al., 2001), only one study has assessed the effect of overlying water aeration on AP activity in surface sediment of a freshwater lake (Chen et al., 2011). Taylor et al., (2009) have assessed AP in suspended and sinking organic particles within the anoxic Cariaco Basin. The present study attempted to examine what factor influences AP in fine-grained sediment.

1.6.2 Cellobiohydrolase (CB)

Cellobiohydrolase (CB) depolymerises cellulose to oligomers and monomers (Wittmann et al., 2004; Snajdr et al., 2008). CB activity catalyses cellulose into cellobiose according to the following relationship:

Equation 1.7:

$$\text{Cellulose} \xrightarrow{\text{Cellobiohydrolase}} \text{Cellobiose}$$

Cellulose is a homopolymer consisting of glucose units joined by β-1,4-bonds (Leschine, 1995; Sakamoto and Toyohara, 2009). Cellobiose is a disaccharide, consisting of two glucose molecules linked by a β(1→4) bond (Leschine, 1995). CB is produced by a wide range of micro-organisms, including fungi, actinomycetes, and bacteria (Rasmussen et al., 2002). The potential activity of this enzyme could reflect the input of cellulose into the sediments (Sakamoto and Toyohara, 2009; Maki et al., 2009).

The majority of the studies available in the literature focus on measuring CB in soil ecosystems. CB plays an important role in the carbon cycle in the rhizosphere of grasses (Sanaullah et al., 2011), in alpine and forest soils ecosystem (Grandy et al., 2007; Kelley et al., 2011). The effect of N deposition has also been assessed to CB activities in litter decomposition and agricultural soils (Saiya-Cork et al., 2002;

Sinsabaugh et al., 2005; Manning et al., 2008). Only one study has assessed CB activities in lake sediments (Hakulinen et al., 2005). The present study investigates CB activity in estuarine sediments.

1.6.3 β-Glucosidase (BG)

β-Glucosidases (BG) are one group of enzymes that bacteria produce to cleave the β-linkages of combined carbohydrates, belonging to 2 of the 82 families of enzymes grouped as glycosyl hydrolases (Bhatia et al., 2002; Bhaskar and Bhosle, 2008). The activity of BG is the rate at which the enzyme cleaves β-glycosidic bonds (Bhatia et al., 2002) and thus, can be measured using the rate at which glucose is hydrolysed from methylumbelliferyl molecules. BG catalyses cellobiose to form glucose (MacLellan, 2010) based on the relationship shown:

Equation 1.8:

$$\text{Cellobiose} \xrightarrow{\beta\text{-glucosidase}} \text{Glucose}$$

Glucosidases are influenced by temperature and their rates regulated by substrate availability (Talbot et al., 1997). Various researchers have used glucosidase enzyme activity to relate the quality and sources of the organic matter to microbial activity in the near shore, estuarine waters and marine sediments (Koster et al., 1997; Fabiano and Danovaro, 1998; Azua et al., 2003). BG activities are measured because of their role as important energy sources for microorganisms and its involvement in biogeochemical transformation of carbon in decomposition process (Arnosti and Holmer, 2003).

A number of studies have also focused on BG activities because BG is involved in the degradation of cellulose, which is the most abundant organic compound on earth, providing the primary structural components for plants (Hakulinen et al., 2005). It is therefore not surprising that the profound interest in this enzyme has led to extensive studies in grassland soils (Turner et al., 2002), peat-accumulating wetlands (Freeman et al., 1997), lowland rivers (Wilczek et al., 2005) and agricultural soils (Wang and Qin, 2006). The interest in shallow coastal waters, estuaries and deep sea sediments has developed later than enzyme studies in soils,

although the organic matter within these ecosystem also originates from autochthonous sources, algal exudates and detritus as well as allochthonous sources, e.g. decaying, dissolved and particulate material from terrestrial vascular plants (Wilczek et al., 2005).

1.6.4 Chitinase

Chitin is a homopolysaccharide, a linear polymer made of monomers of N-acetyl glucosamine connected by glucoside β-(1-4) bonds (Warren, 1996). It is the supporting substance of the outer skeleton of a number of animals, insects, arachnids and nematodes (Poulicek et al., 1998) and in fungal cell walls (Boetius, 1995). The main producer of chitin is crustacea and phytoplankton is the second most important source (Donderski and Trzebiatowska, 1999). Bacteria are considered to be the primary mediators of chitin degradation in aquatic environments (Gooday, 1990, Poulicek et al., 1998). The first enzymatic step in the chitin-degradation pathway is performed by chitinases, one of the glycosyl hydrolases families (LeCleir and Hollibaugh, 2006).

Chitin hydrolysis is carried out by heterotrophic bacteria assessed with the MUB-labelled N-acetyl glucosaminidase hydrolysis, an analogue for chitinase activity to break down chitin for acquisition of organic nitrogen (Souza et al., 2010). Chitinase is catalysed based on the following relationship (LeCleir and Hollibaugh, 2006; Beier and Bertilsson, 2011):

Equation 1.9:

$$\text{Chitin} \xrightarrow{\text{chitinase}} \text{N-acetylglucosamine + diacetylchitobiose}$$

The mineralisation of chitin is of great ecological significance as it enters both carbon and nitrogen cycles (Gooday, 1990; Kang et al., 2005; Kelley et al., 2011; Sanaullah et al., 2011). Figure 1.3 shows the relationship between chitin and the carbon and nitrogen cycles (Beier and Bertilsson, 2011). Chitin is hydrolysed prior to assimilation by microbial cells producing N-acetylglucosamine (GlcNAc). Chitin deacetylates into glucosamine or chitosan by the removal of acetyl groups from the molecular chain of chitin. Deamination removes the complete amine group and produces glucose. GlcNAc can be transported across the bacterial cell

membranes where they undergo further enzymatic processing to acetate (LeCleir and Hollibaugh, 2006). These compounds may be incorporated into cell material or undergo mineralisation process producing ammonium and carbon dioxide. Ammonium enters the nitrogen cycle by converting into nitrogen through denitrification process. Nitrogen fixation produces ammonium, which can be assimilated into the bacteria. Carbon fixation converts carbon dioxide into organic matter, which can be assimilated by bacteria (Figure 1.3).

Chitinase activity has received far less attention than AP or BG activity although it plays a vital role in degrading chitin, an important polysaccharide to the marine food web (Olander and Vitousek, 2000; Sinsabaugh et al., 2005, Souza, et al. 2010). Most studies have focused on chitinase activity in planktonic, benthic and epiphytic bacteria (Donderski and Tzebiatowska, 1999) bacteria and fungi isolated from shrimp exoskeletons (Swiontek-Brzezinska et al., 2007), marine fish (Goodrich and Morita, 1977) and chitin catabolism in marine bacteria (Keyhani and Roseman, 1999). Chitinase activity has been reported in marine environments (Hoppe et al., 2002), pulp mill-contaminated lake sediment (Wittman et al., 2004), and sediments of the Ythan (Hillman et al., 1989) and Delaware (Kirchman and White, 1999) estuaries.

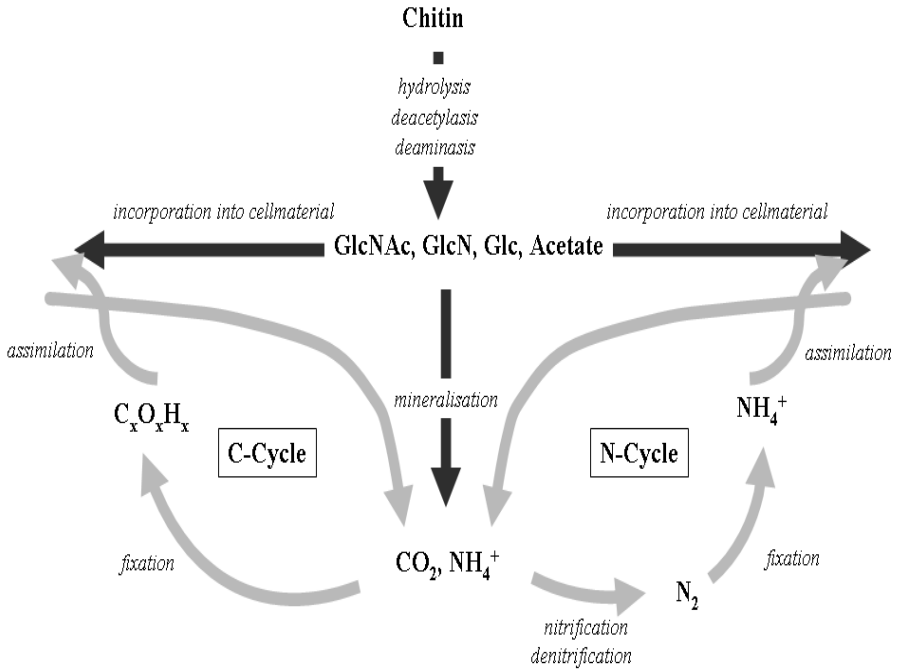


Figure 1.3 Chitin synthesis, assimilation and product in bacteria (adapted from Beier, 2010). Abbreviations: GlcNAc- N-acetylglucoamine, GlcN- glucosamine, Glc- glucose, NH_4^+- ammonium, CO_2-carbon dioxide, N_2-nitrogen, C- carbon, N- nitrogen

1.6.5 Urease

Urea is a nitrogenous waste product being excreted by many aquatic organisms, including zooplankton, molluscs, fish and mammals (Jorgensen, 2006). In addition, bacteria produce urea during degradation of purines and other nitrogen compounds such as arginine and allantoin (Satoh, 1980; Braga and Torres, 2010). Urease is synthesised by many eukaryotes including some invertebrates and numerous microorganisms (Therkildsen and Lomstein, 1994; Solomon et al., 2010). Urease activity has been found in a variety of different bacterial divisions comprising bacteria with aerobic and anaerobic metabolism (Peers et al., 2000; Solomon et al., 2010). Urease catalyses the hydrolysis of urea:

Equation 1.10:

$$CH_4N_2O + H_2O \xrightarrow{\text{urease}} CO_2 + 2NH_4$$

The enzymatic, colorimetric, endpoint method for urea measurement is based on urease-catalysed hydrolysis of urea followed by the quantitation of ammonium by

reaction with salicylate and dichloroisocyanate, which can be measured spectrophotometrically (Alef and Nannipieri, 1995). Ureases can exist in two possible states in the sediments. They may be intracellular, present within the cells of ureolytic microorganisms or alternatively extracellular, released from disrupted plant and microbial cells. Extracellular urease is largely adsorbed on clay and organic colloids which have a high affinity for urease. This adsorption on sediment colloids possibly protects ureases from destruction by sediment microorganisms, while the activity of enzymes remains essentially unimpaired (Alef and Nannipieri, 1995). Interest in this enzyme stems from its role in the decomposition of urea fertiliser in agricultural systems (Gianfreda et al., 2005; Rahmansyah et al., 2006). It is also considered an important agent for nitrogen mineralisation in terrestrial and aquatic systems (Kandeler et al., 1999; Sinsabaugh et al., 2000).

1.7 The Significance and Aims of This Study

Estuarine nutrient biogeochemical cycles are being modified by anthropogenic impacts increasing pollutions and wastes, reducing species abundance and diversity and causing imbalance to the ecosystem functions (Libes, 2009; Jjemba, 2004; Madsen, 2008). Human intervention has also affected the marine phosphorus cycle by doubling the source of phosphorus through sewage discharges and fertiliser runoff (Paytan and McLaughlin, 2007). Oxygen is one of the important factors mediating these biogeochemical cycles, which may also influence organic matter pathways in the estuaries (Bianchi et al., 2004). As a consequence of this, nutrients and organic matter accumulate in the system increasing the productivity of the system. This problem may further complicate the situation by generating eutrophication, causing algal blooms (Meyer-Reil and Koster, 2000; Howarth et al., 2011). Therefore, the link between oxygen and biogeochemistry of estuaries is a vital issue to look at in order to evaluate the oxygen concentration and at which harmful effects start to occur, the rates that the system responds to different levels of oxygen saturation in the overlying water and the recovery times of system processes when re-aeration are restored.

This will be achieved by investigating the effects of different oxygen saturation in the overlying water on the exchange and transformations of nitrogen and the fluxes of phosphate at the sediment-water interface. The linkage between nutrients in the overlying water and the nutrients in the porewater of the sediments were also investigated. Besides nutrients, five enzyme activities were also measured in the sediment (alkaline phosphatase, cellobiohydrolase, β-glucosidase, chitinase and urease) as enzyme activities can give information on the processes that may underlie an overall change in fluxes and are easier to use in small scale experiments (Marx et al., 2001; Saiya-Cork et al., 2002; DeForest, 2009). Additionally, the effect of different oxygen saturation to the pH, total organic carbon, total nitrogen, bromide and oxygen fluxes were also examined as parameters that may influence the nutrient fluxes and enzyme activities in the treatment.

Based on the aims above, the following questions were addressed to fill in the gaps in the related research:

1) Does short term hypoxia and anoxia affect pH in the overlying water and vertical profiles of total organic carbon and total nitrogen in the sediments?
2) Are differences in oxygen saturation in the overlying water reflected by changes in bromide solute transport between sediment-water interfaces and the oxygen consumption in the sediments?
3) How does oxygen affect nutrient cycling, which in turn influences nutrient fluxes?
4) Does the duration of hypoxia and anoxia influence the nitrogen and phosphorus transformations in the estuarine sediment-water system?
5) What factors affect enzyme activities in sediments?

1.8 The Structure of the Thesis

This thesis has been divided into 5 chapters:

Chapter 1 provides the introduction to the current knowledge of hypoxia and anoxia distribution, processes affecting nitrogen and phosphorus cycles in estuarine

sediments and the background of the five enzyme activities selected in this study. The aims of the study and significant research questions were also given. Chapter 2 covers the experimental design, the methodology applied for the field sampling and laboratory experiments and the statistical analyses opted. Chapter 3 focuses on the result of sediment physico-chemical characteristics, solute transports, oxygen uptake, nutrients and porewater concentrations and enzyme activities in this study. Chapter 4 discusses the outcome and interpretation of the pattern observed in this study, and finally, Chapter 5 summarises the findings, stressing the importance of oxygen on modifying nutrient cycles and enzyme activities. The final section details the possible further work to be carried out.

CHAPTER 2 METHODOLOGY

2.1 Introduction

The aim of Chapter 2 is to present the method used in the field sampling and the experimental work of this study. This chapter begins with an explanation of the experimental design, the fieldwork sampling and the experimental setup in the lab. Section 2.6 describes the procedure to determine the physico-chemical characteristics. Section 2.7 and 2.8 explains the method for analysing bromide and oxygen, respectively. This is followed by the determination of nutrients and porewater nutrient concentrations, as well as their fluxes (section 2.9-2.10). Section 2.11 explains the method and calculation for enzyme activities. The final section states the statistical analysis used in this study.

2.2 Experimental Design

The experimental design for the sample collection and processing of the sediment cores were shown in Figure 2.1. Samples were collected in June, 2010 using 8.4 cm diameter and 30 cm high Plexiglas cylindrical cores. The samples were used for analysing the sediment physico-chemical characteristics, nutrient and porewater nutrients, oxygen fluxes, and enzyme analysis. Samples were collected from Breydon Water, Great Yarmouth at the start and on day 7 of laboratory incubations. Bromide fluxes were taken on day 4 and day 7 of the laboratory incubation.

2.3 Field Sampling

Sediment samples were collected during low tide in June 2010 from Breydon Water estuary, Great Yarmouth (Figure 2.2). Plexiglas tubes were pushed 10 cm into the sediment, leaving 20 cm for the overlying water column. Care was taken as not to disturb the cores. The specification used for handling sediment cores was taken from the NICE (Nitrogen Cycling in Estuary) protocol handbook by Dalsgaard et al., (2000). By using undisturbed sediment, the natural diversity of organism as well as heterogeneity could be retained. Samples were returned to the University within approximately 45 minutes where processing of the cores commenced immediately.

Sediments which were not used straightaway were placed in the dark at 4^{o}C as soon as possible and kept under these conditions until analysis.

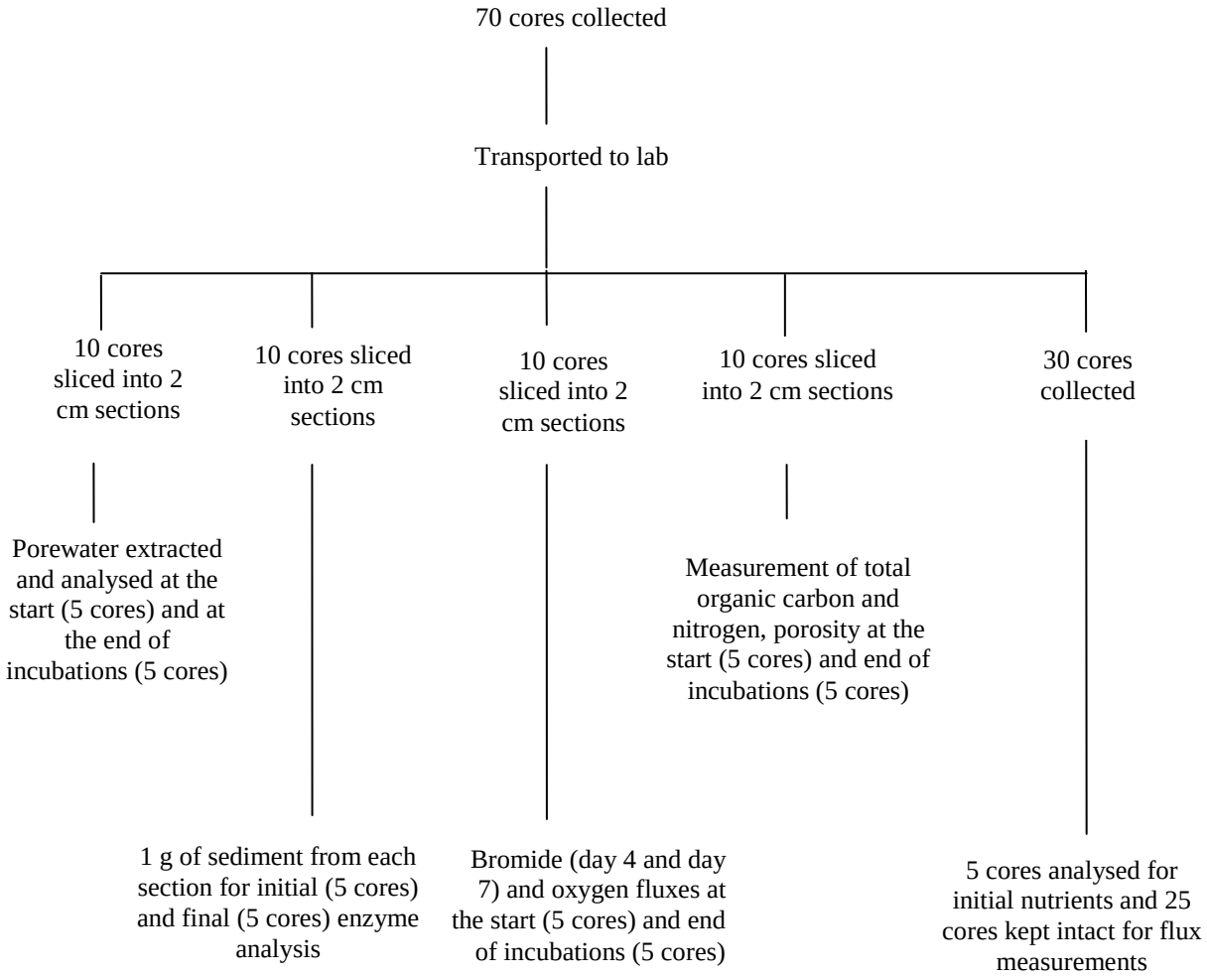


Figure 2.1 Schematic representation of processes carried out on the cores collected from Breydon Water, Great Yarmouth in June 2010.

Initial surveys were carried out before selecting the most appropriate sampling location in Great Yarmouth. Breydon Water, Great Yarmouth was selected as the sampling location because of its accessibility and the short walking distance required transporting samples and equipment back to the lab. It is also an area with a low risk of natural hypoxia. The selection of a location without a history of natural hypoxic or anoxic condition was to ensure that the system was not hypoxic and that the only influence of hypoxia comes from the experimental setup in the study. This procedure follows the recommendation of Larson and Sundback, (2008).

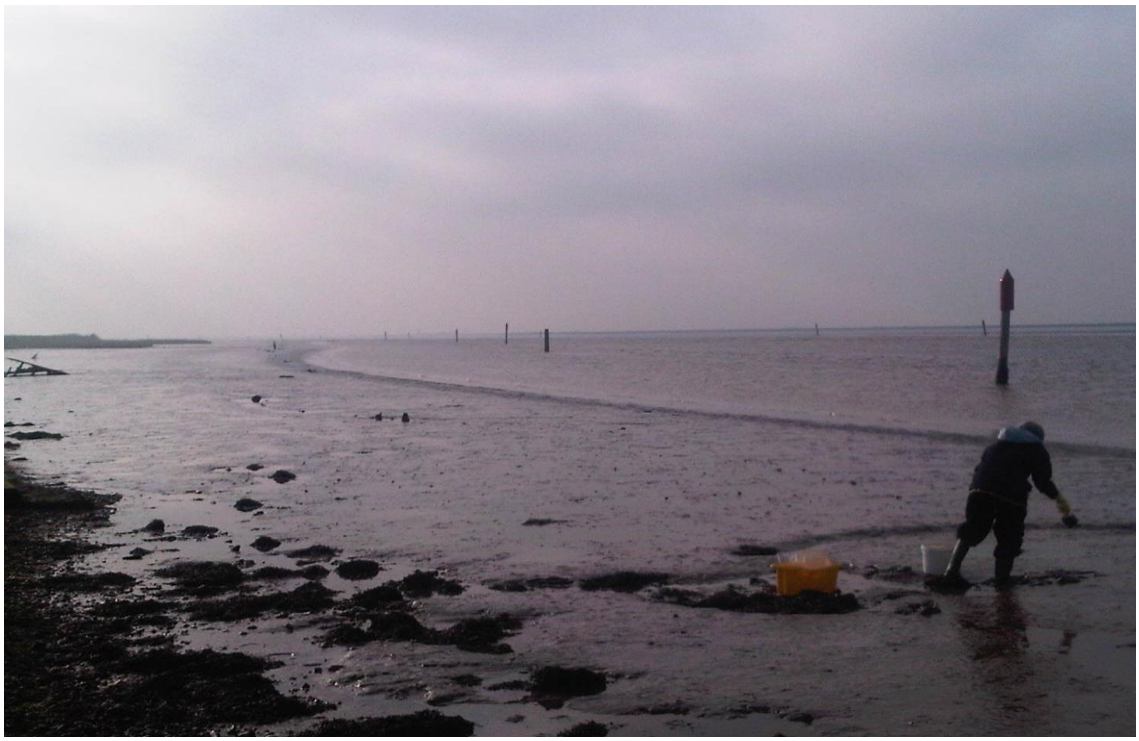


Figure 2.2 Sampling during low tide in June 2010 at Breydon Water, Great Yarmouth

Breydon Water is an inland tidal estuary located in Norfolk, East Anglia, England, lying just to the east of Great Yarmouth (Figure 2.3). It is situated at the mouth of the River Yare and its confluence with the River Bure and Waveney. Rivers Yare and Waveney enter Breydon Water before discharging into the North Sea (Baban, 1997). Breydon Water is relatively shallow, with brackish and well mixed water, and a mean tidal range of 1.5 m. It covers an area of 15.34 km^2, with a mean width of 1-1.2 km and an area of 7 km^2 in high tide whereas during low tide, the water is 10-12 m in width along the entire length of the estuary. The residence time of tidal waters within this estuary including the estuaries of River Yare and Waveney is very short, no more than 1-2 days (Sabri, 1977).

Extensive areas of mud are exposed at low tide and these form the only intertidal flats occurring on the east coast of Norfolk (Baban, 1997). The mudflats are characterised by growths of green algae, *Ulva* sp and two rare species of eelgrass, *Zostera marina* and Z. *noltii*. These plants, together with an abundant invertebrate fauna, particularly the polychaetes, *Nereis diversicolor*, attract large number of ducks

and waders to feed in the estuary, particularly during winter (Norfolk County Council, 2011).

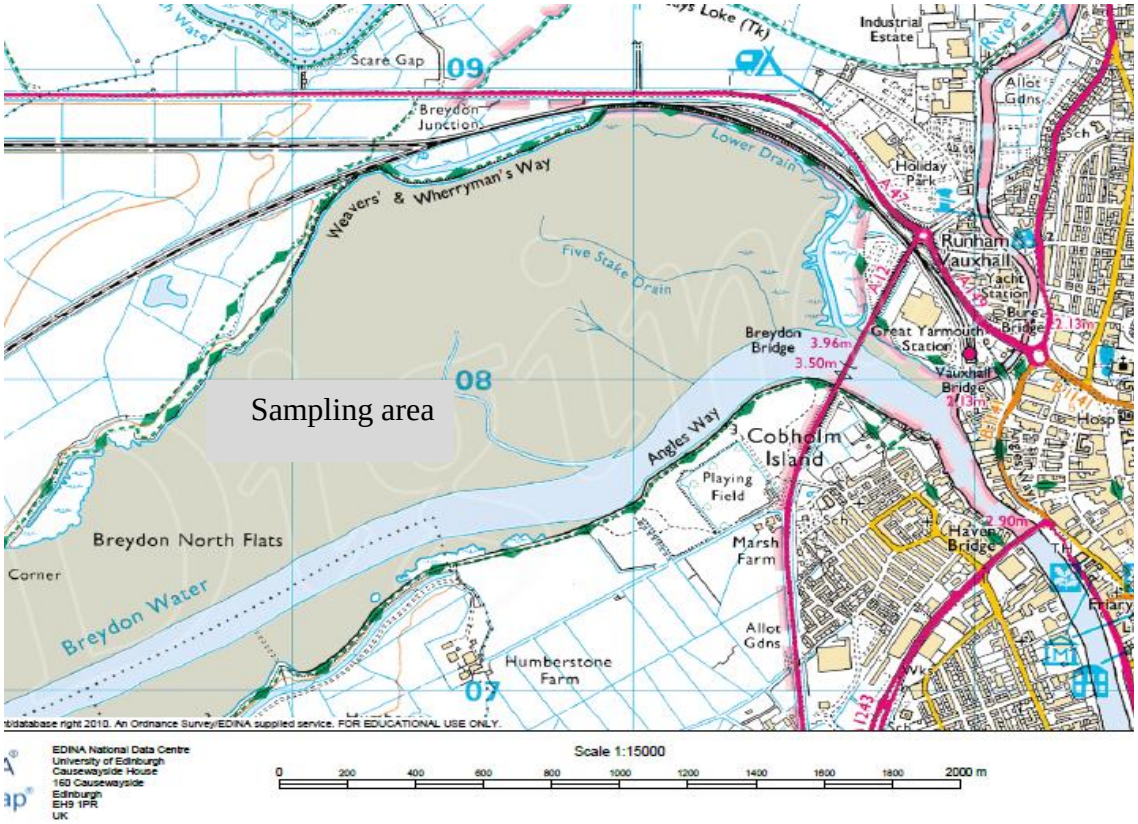


Figure 2.3 Map of sampling site in Breydon Water, Great Yarmouth (source: EDINA Digimap)

2.4 Experimental Setup

Upon arrival to the lab, the cores containing 10 cm depth sediments were covered with 900 ml nutrient-free artificial seawater (Tropic Marin salt [Aquarium System]) dissolved in MQ at a salinity matching that of the sampling location (32 ‰). The use of artificial seawater is to allow full control of the experimental design and reduce variation between replicates. Natural seawater could contain high concentrations of suspended or dissolved material that are not appropriate for experimental systems. Water taken from shallow areas of estuaries may have been subjected to large diurnal changes in light, temperature, nutrients or dissolved oxygen that could affect the experimental results. It is possible that the use of artificial seawater could cause rapid nutrient escape from the *in-situ* sediment into the overlying artificial seawater, with the consequences of disrupting natural processes. The artificial seawater was slowly

added into the cores up to 900 ml to avoid sediment resuspension (Rubao et al., 2002). The depth above the sediment should be 10-20 cm to give a volume of 0.5-1 l of overlying water, following the recommendation by the NICE protocol handbook (Dalsgaard et al., 2000).

The cores were covered with a plastic lid with two holes, one with a rubber stopper for water sampling and the other one for the tubing to allow air or nitrogen bubbling (Figure 2.4). Aeration tubes were placed 10 cm above the sediment surface with the bubbling rates at 50 ml/min for oxic treatments, 100 ml/min gas mixtures for hypoxic treatments and 100 ml/min nitrogen gas flow for anoxic treatments. The flow of both gases was regulated through valves and set using a gas flow regulator. The cores were maintained in the absence of light to quantify benthic respiration (dark fluxes). The cores were kept at a constant temperature of 12 °C in a cold room. This is approximately equivalent to the *in-situ* temperature in the study area. Prior to carrying out the experiments, a preliminary check was conducted to ensure the correct oxygen concentrations were obtained for each treatment. The appropriate gas/gas mixture was allowed to bubble in each sediment core for approximately 1 hour. Dissolved oxygen concentrations were then measured and adjustments to the gas flow in each sediment core were made each additional 0.5 hour. This procedure was repeated until the concentration were within the concentrations desired which generally took 4 to 5 hours. Any potential leaking of nitrogen gas from loose connection of the gas cylinders to the aeration tubes and cores were also checked. Leakage of nitrogen into the cold room was also monitored using an oxygen meter to give the alarm if the oxygen concentration in the cold room has reduced.

The experimental setup consisted of five treatments, n = 25; 1) oxic from continuous aeration of the treatment with air pump for 7 days, 2) hypoxic conditions in the overlying seawater obtained by continuous bubbling with 3% oxygen, 97% nitrogen gas mixtures for 7 days, 3) 1 day anoxic (1DA) by bubbling with oxygen free nitrogen, followed by subsequent re-aeration for another 6 days with air pump, 4) 4 days anoxic (4DA) by bubbling with oxygen free nitrogen, followed by re-aeration with air pump for 3 days and 5) anoxic bubbled with oxygen free nitrogen for 7 days. These five different treatments were selected as to induce oxic

conditions (95% oxygen saturation), hypoxic conditions (25% oxygen saturation), anoxic conditions (0% oxygen saturation), and to determine how fast the system can recover from short term anoxic events (4 day and 1 day anoxic) followed by re-aeration over 3 days and 6 days, respectively. The 7 day experiment was selected in order to capture the consequences of episodic hypoxia, which often lasts from several days up to a week, with a typical duration of 5 days (Sagasti et al., 2003). During the laboratory incubations, the cores were sealed with plastic lids to prevent oxygen dissolution to the overlying seawater from the air in the cold room and to maintain the oxygen concentration needed for the different treatments.

Figure 2.4 Experimental setup for the different aerations conducted in a constant temperature room.

2.5 Laboratory Protocol

Apparatus used in the laboratory was cleaned of visible dirt then washed and soaked in 'phosphate free' detergent (Decon 90) at least overnight. The apparatus was then rinsed three times and on subsequent occasions with milliQ water (MQ). Next, it was placed in 10% HNO_3 overnight to further remove contaminants, re-rinsed three times with MQ and allowed to air-dry. Apparatus used in association with standards and diluted samples were rinsed with milliQ water before use. All dilutions of samples and reagents were made up in milliQ water. Apparatus used in the field was scrubbed with detergent and rinsed with milliQ water.

2.6 Determination of Physico-chemical Parameters

2.6.1 Determination of Particle Size Distribution of Sediments

Particle size analysis was measured with 1 g sediment samples at 1, 3, 5, 7 and 9 cm depth for general characteristics of the field site. Sediments were suspended in 70 ml distilled water and wet sieved through a 1.7 mm Fisherbrand test sieve (Fisher Scientific). The slurry was treated with 1 ml of 10 % Calgon (sodium hexametaphosphate) to avoid flocculation (Bartoli et al., 2009). Then, the samples were sonicated in an ultrasonic bath for 1.5 minutes and stirred with a magnetic stirrer to ensure full mixing. Samples were analysed using a laser diffraction method with a Malvern Mastersizer 2000.

2.6.2 Determination of Overlying Water pH

pH values was measured with a pH meter (HI 02885) which was calibrated using pH 4, 7 and 9 buffers. The pH electrode was dipped into the overlying water daily for 7 days.

2.6.3 Determination of Porosity in Sediments

Sediment samples were sliced into 2 cm thick sections from the surface to 10 cm depth of the sediment at the start and on day 7 of the laboratory incubations. Sediment slicing on day 7 was carried out under gas mixtures for hypoxic treatments and an oxygen free environment in a glove box for anoxic treatments. Sediment samples were placed in a weighing boat, labelled with a marker and the mass of each boat were carefully determined on an analytical balance (Sartorius). A 12-ml plastic syringe with the tip removed was used to collect a 2-ml subsample from each corer. The syringe was used like a miniature corer, pushing the syringe into the mud while simultaneously pulling back on the plunger. The sub-sampled mud was extruded into appropriate weighing boat and the sediment wet weight was recorded. Sediment samples were dried to constant weight for 24 hour at 70 °C (Hietanen and Lukkari, 2007) and the dry weight of the sediments recorded.

Sediment porosity (ϕ) was determined as:

Equation 2.1:

$$\phi = \frac{ww-dw}{vol}$$

Where ww and dw are the wet weight and dry weight of sediment, respectively and vol is the sediment volume.

2.6.4 Determination of Total Organic Carbon and Total Nitrogen in Sediments

Sediment samples of approximately 10 g were dried from each depth of 0-2 cm depth intervals until 10 cm depth at 80 °C for 24 hour in a foil tray. Samples were taken at the start and on day 7 of the laboratory incubations. The dried sediments were crushed into fine particles with a mortar and pestle under gas mixtures for hypoxic treatments and oxygen free environment in a glove box for anoxic treatments. The mortar and pestle were cleaned and air-dried with three successive washes of methanol, acidic-methanol, and dichloromethane prior to adding the respective sediment layers to avoid cross-contamination. Powdered samples were stored at room temperature flushed with gas mixtures for hypoxic treatment and nitrogen in

anoxic treatment before being capped in pre-combusted glass scintillation vials until analysis.

Prior to analysis, the powdered sediments were weighed to about 18-22 mg and then folded into a tin capsule for total nitrogen analysis. Sediment samples for total organic carbon analysis were initially treated similarly except that samples were weighed into double tin capsules, one inside the other, and the inner capsules were not sealed until after treatment to remove the inorganic carbon. Inorganic carbon was removed by treating the weighed samples with 8% sulphurous acid according to the method of Verado et al., (1990). Two tin capsules were used in order to prevent any sample lost due to weakening of the inner capsule by the acid.

Three procedural blanks were prepared from tin capsules treated with acid but without sediments. All reagents and glassware used to analyse samples are combusted at 400 °C for a minimum of 4 hours. Acetanilide standards were also weighed to approximately 2 mg and folded in a tin capsule. Samples were analysed using a CHN EA1108 Elemental Analyser (Hansen and Kristensen, 1998). The analytical technique used for the determination of carbon and nitrogen is based on the quantitative dynamic flash combustion method. This method is the complete and instantaneous oxidation of the sample, where all the organic and inorganic substances are converted into combustion products. The samples were placed inside the autosampler drum where they were purged with a continuous flow of helium and then dropped at preset intervals into a vertical quartz tube (combustion reactor) maintained at 1000 °C. When the samples dropped into the furnace, the helium stream was temporarily enriched with a precise volume of pure oxygen. The sample and its container melt and the tin promote a violent reaction called flash combustion in the temporarily enriched atmosphere of oxygen, when the temperature rises to around 1800 ºC.

This instrument was calibrated at the beginning, end and every fifteen samples during a run using sulphanilamide standards and empty capsules as blanks. Five replicates of each sediment sample were made from 2 cm depth intervals from the five different treatments, n = 125. Percentage organic carbon and nitrogen values

were calculated using Eager 200 software using the K-factor method. This K value is determined by analysing an organic standard of a known elemental composition.

2.7 Bromide Tracer

Movement of bromide tracer into the sediment was assessed from day 4 to day 7 of the laboratory incubation. This procedure is done to determine the effect of oxygen and re-aeration in the overlying water on solute transport between the sediment and water at the end of the incubation. 1 ml of 6.4 mol L^{-1} calcium bromide was added to the water column, corresponding to 16 000 µmol L^{-1} of initial bromide concentration in the overlying seawater. This concentration was selected as the bromide concentration spiked to the cores should be substantially higher than the background value (Martin and Banta, 1992). It is recommend that the concentration should be at least 10 times higher and is in line with the usual concentration of the bromide spike used in solute transport experiments which ranges between 10 000-20 000 µmol L^{-1} (Martin and Banta, 1992; Forster et al., 1995; 1999; Keefe et al., 2004; Quintana et al., 2007). The background porewater concentration in the cores was 700 to 900 µmol L^{-1}.

On day 4 to day 7 of the incubation, 20 ml overlying water samples were taken with a syringe for the determination of bromide concentration. In the case of hypoxic and anoxic treatments, the syringe was flushed for a few seconds with gas mixtures and nitrogen gas before taking water samples for analysis. The water samples were filtered and stored in 30 ml universal bottles at -20 °C until further analysis using inductively coupled plasma mass spectrometer (Thermo X5 series I ICP-MS, Thermo Electron Corporation, USA).

All samples for ICP-MS analysis were sampled through a Cetec 500 auto-sampler. ICP-MS was tuned using the standard mode with a solution containing lithium, indium and uranium at 10 µg L^{-1} each. The multielement tune A from Thermo Electron Corporation containing barium, beryllium, bismuth, cerium, cobalt, indium, lithium and uranium at 10 mg L^{-1} was used to prepare the tuning solution.

The passing criterion for this tune is shown in Table 2.1. The operating conditions applied for bromide concentrations are listed in Table 2.2.

Table 2.1 Passing criteria for standard mode of ICP-MS

Isotope	Counts per second
^{7}Li	>1 000
^{115}In	>10, 000
^{238}U	>10,000
$^{7}Li/^{5}Bkg$	>2 000
$^{115}In/^{101}Bkg$	>10,000
$^{156}Ce\ O/^{140}\ Ce$	<0.02
$^{238}U/^{220}\ Bkg$	>10,000

Table 2.2 ICP-MS operating conditions for bromide measurements

Radio Frequency power	1100W
Argon gas flow rates:	
-plasma	15.0 L/min
-auxiliary	0.8 L/min
-make up	1. 1 L/min
Sample flow rate	0.85 mL/min

Bromide was monitored at mass to charge ratio, *m*/*z* 79 and 81. Linear calibration curves were found for standards in the range of 200 to 1000 µmol L^{-1} prior to sample measurements using the software Plasma Lab. Porewater bromide concentrations were diluted to 1:20 using a Varian SP55 autodilutor.

The experiment was terminated after 3 days of incubation with bromide. Core slicing was carried out in an oxygen free environment using a glove box to assess the movement of bromide throughout the 3 days of incubation in each treatment. Before extruding the sediments, the water above the sediment was removed with a syringe. The rest of the sediment was sectioned at 2 cm intervals by extruding it upwards from the core tube and sectioning it with a thin plastic slicer. A clean slicer was used for each sectioning to avoid contamination between slices (Bradshaw et al., 2006). 10 ml porewater samples were centrifuged from the sediment at 4,500 rpm for 10

minutes and transferred to 15 ml Corning polypropylene centrifuge tubes stored at -20 °C and analysed with the same procedure as seawater.

2.7.1 Total Bromide Incubation, Diffusive and Irrigation Fluxes Measurements

Total bromide incubation fluxes were calculated based on the equation:

Equation 2.2:

$$\text{Flux} = \frac{(C_f - C_i) \times V}{A \times t}$$

Where;

Flux = flux of bromide and nutrients (μmol m^{-2} h^{-1})

C_f = final concentration of bromide and nutrients (μmol L^{-1})

C_i = initial concentration of bromide and nutrients (μmol L^{-1})

A = area of sediment surface in core (m^2)

V = volume of water in core (L)

t = incubation time (h)

The diffusive bromide fluxes (μmol m^{-2} h^{-1}) were calculated from the bromide profile with depth according to Fick's first law of diffusion:

Equation 2.3:

$$J = - \phi D_s (\partial C/\partial x)$$

Where ϕ is porosity

Ds is the whole sediment diffusion coefficient

$\partial C/\partial x$ is the concentration gradient (mol m^{-4})

The sediment diffusion coefficient, Ds, is determined according to:

$$D_s = D_o/\theta^2 \ (m^2\ h^{-1})$$

Where D_o is the effective temperature-corrected diffusion coefficient at 12 °C for bromide, yielding a value of 1.4389×10^{-9} m^2 s^{-1} (Li and Gregory, 1974; Boudreau, 1997). For conversion of seconds to hour, D_s are multiplied by 3600.

$\theta^2 = 1 - \ln(\phi^2)$, ϕ is the porosity which accounts for tortuosity correction according to Boudreau and Meysman, (2006).

$\partial C/\partial x$ is determined from linear regression of the concentration profiles in the sediment (mol m^{-4}).

Irrigation fluxes were calculated from the difference between total bromide incubation fluxes with the diffusive bromide fluxes. Since the sediment was not sieved, we assumed that there was active solute transport through irrigation.

2.8 Determination of Oxygen Penetration Depth in Sediments

Oxygen profiles were measured with a dip-type oxygen minielectrode (Microelectrodes, Inc., USA) at the start of the incubation and just before the end of the incubations. Five oxygen profiles to depths were measured from each core. The minielectrode consists of an Ag reference electrode with a tip diameter of 3 mm and up to 8.6 cm in length. The oxygen minielectrode was connected to an oxygen adapter (O2-ADPT) and a voltage reading device (HI 9025 pH meter). Oxygen vertical profiles of the surface sediments were measured in increments of 0.5 mm depths because dissolved oxygen concentration shows an abrupt change within the upper 1 cm (Kim and Kim, 2007). The 90% response time is 150 seconds.

To measure oxygen profiles within the sediment, the electrode was attached to a rod, to allow penetration into the sediment, which was lowered using a retort stand equipped with a precision screw slide to ensure measurement of 0.5 mm vertical resolution. A hand lens is used to magnify the view of the minielectrode tip and the tip of the probe was carefully lowered to the sediment surface. Measurements were made at 0.5 mm vertical intervals starting from 1 mm above the sediment followed by 0.5 mm intervals down to a depth in the sediment where milivolt (mV) reading reached near zero, indicating a zero oxygen concentration. Before measurement, the minielectrode was calibrated using a two point calibration, one

with 0 % gas bubbled with nitrogen for approximately 30 minutes and the other with 20.9 % oxygen (ambient air). At 20.9 %, -300 ± 7.9 mV reading was obtained and at 0 % oxygen, -0.7 ± 0.4 mV was recorded, n= 5. The mV readings obtained from the calibration process were then converted to percent oxygen of the overlying seawater in the treatment. The percent oxygen saturation was converted to oxygen concentrations using values from the table provided by Ramsing and Gundersen, (2001) (available at http://www.unisense.com/).

2.8.1 Measurements of Diffusive Oxygen Fluxes in Sediments

Diffusive oxygen fluxes or sediment oxygen consumption within the sediment was estimated from the oxygen gradient within the sediment utilising Fick's 1st Law using Equation 2.3. This procedure assumes molecular diffusion-limited rates in the sediment (Berner, 1980). But, the values were multiplied by 86 400 to convert the flux data from hours to day. For this calculation, we used the temperature-dependent oxygen diffusion coefficient according to Ramsing and Gundersen, (2001), yielding the value of D_o=1.5702 x 10^{-9} m^2 s^{-1} at 12 °C and a salinity of 32.0 ‰ (available at http://www.unisense.com/). Porosity was measured in centimetre scale (section 2.6.3), which is different than the sediment oxygen profiles which are measured at milimetre scale (section 2.8), therefore, porosity was estimated from extrapolation (using polynomial curve fit) of the measured porosities (Hulth et al., 1994). $\partial C/\partial x$ is determined from the linear portion of the oxygen gradient in the top layer of the sediment.

2.9 Nutrients Sampling in the Overlying Seawater

To measure nutrient fluxes (nitrate, nitrite, ammonium and phosphate), 20 ml of overlying water were sampled daily for 7 days to capture rapid changes to the system and to assess nutrient changes for benthic respiration (dark fluxes). The water samples were filtered using 0.45 µm cellulose filters (Jiang et al., 2006; Belias et al., 2007). At the end of the incubation, the height of water in the cores and the volume of water over the cores were noted. The 20 ml nutrient samples were transferred to

30 ml universal bottles and kept at -20 °C and were analysed by the standard autoanalyser method using SKALAR autoanalyser.

2.9.1 Determination of Nitrate and Nitrite Using a SKALAR Autoanalyser

The method is based on the cadmium reduction method. The sample was buffered at pH 8.2 and passed through a column containing granulated copper cadmium to reduce the nitrate to nitrite. The nitrite (originally present plus reduced nitrate) was determined by diazotising with sulphanilamide and coupling with N-(1-naphtyl) ethylenediamine dihydrochloride to form a highly coloured azo dye. The pink coloured dye produced was measured at an absorbance of 540 nm.

Working standards, ranging from concentrations of 0 to 20 µmol L^{-1} sodium nitrate (Figure 2.5 a) and 0 to 5 µmol L^{-1} sodium nitrite (Figure 2.5 b) were prepared fresh daily and run before the analysis of samples every day.

2.9.2 Determination of Ammonium Using a SKALAR Autoanalyser

The automated procedure is based on the modified Berthelot reactions. Ammonium was chlorinated to monochloroamine which reacted with phenol. After oxidation and oxidative coupling, a green coloured complex was formed. The reaction was catalysed by nitroprusside; sodium hypochlorite was used for chlorine donation. The absorbance of the coloured complex was measured at 630 nm.

Working standards were prepared from concentrations of 0 to 200 µmol L^{-1} ammonium chloride (Figure 2.5 c). A 10 fold serial dilution was carried out for porewater ammonium concentrations. The working standards were prepared fresh daily from the stock solution before the ammonium analysis was carried out.

2.9.3 Determination of Phosphate Using a SKALAR Autoanalyser

Phosphate was determined by the reaction of ammonium heptamolybdate and potassium antimony (III) oxide tartrate in an acidic medium with diluted solutions of phosphate. This step formed an antimony-phospho-molybdate complex. This complex was reduced to an intensely blue-coloured complex by L (+) ascorbic acid and measured at 880 nm absorbance.Working standards of 0 to 5 µmol L^{-1} (Figure 2.5 d) were prepared fresh daily.

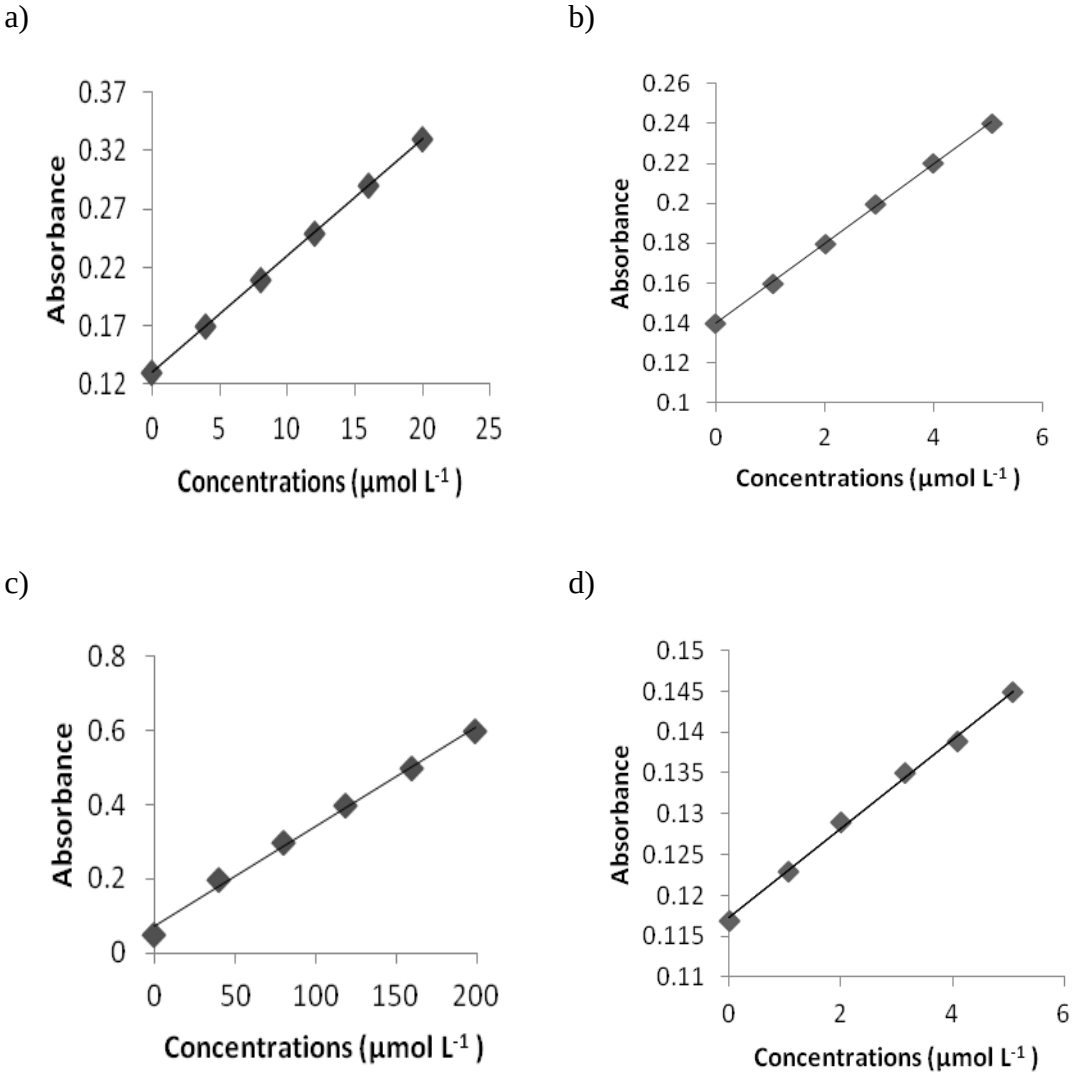


Figure 2.5 Nutrients standard calibration curve for a) nitrate, b) nitrite, c) ammonium and d) phosphate. Standards are presented as mean ± SE, n = 3.

2.9.4 Measured Nutrient Fluxes in the Overlying Water

Fluxes (μmol m^{-2} h^{-1}) were determined from the concentration changes, incubation time and the measured height of the water column in core during incubation. Nutrient fluxes were calculated using equation 2.2. Positive flux values represent a concentration increase in the overlying water, from the flux of the sediment to the overlying water. Negative flux values represent a decrease in the concentration of overlying water, which may be an indication of a flux into the sediment, or loss to other forms.

2.10 Analysis of Porewater Nutrients in the Sediment

All sectioning of cores, centrifugation and handling of unpreserved porewaters was done in an oxygen free environment. This process was carried out in a glove box which is flushed with oxygen free nitrogen (OFN) to minimise oxidation of reduced species in the porewaters (Gillespie, 2005). Samples taken from the cores immediately upon arrival to the lab, 45 minutes represented the start condition. The cores were extruded carefully to minimise compaction at the top and leakage of water from the base. Porewater was obtained by centrifugation for 10 min at 3000 rpm in 50 ml sterile polypropylene centrifuge tubes, which were pre-flushed with OFN before use and sealed in the glove box. Before and after the 7 day incubation period, sediment samples were taken to determine pore water solute concentrations by cutting the cores into 0-2, 2-4, 4-6 and 6-8 cm sections. From each section, roughly around 70 g of sediment were obtained which produced between 5-8 ml of porewater. The porewater was then filtered using a 0.2 μm disposable filter into 15 ml sterile polypropylene tubes.

The supernatant was analysed for nitrate, nitrite, ammonium and phosphate. Once extracted, these samples were frozen and later analysed within a week. They were defrosted for about 2 hours at room temperature before analysis was carried out using the SKALAR autoanalyser. The method used for porewater nutrients analysis is the same using the SKALAR method mentioned under section 2.9.1 to 2.9.3.

2.10.1 Calculated Porewater Diffusive Fluxes in the Sediment

Theoretical diffusive fluxes were calculated from porewater gradients using equation 2.3. The effective diffusion coefficient (Ds) of a nutrient in the sediment was corrected for a temperature of 12 °C from molecular diffusion coefficients, D_o given by Boudreau, (1997). This yields a value of 1.33×10^{-9} m^2 s^{-1} for nitrate, 1.34×10^{-9} m^2 s^{-1} for nitrite, 1.36×10^{-9} m^2 s^{-1} for ammonium and 0.41×10^{-10} m^2 s^{-1} for phosphate. To estimate the gradient close to the sediment-water interface, different calculations were applied depending on the vertical profile of the nutrients considered. For nitrate and nitrite concentrations, the gradient was calculated using the third order polynomial curve fitting. For ammonium and phosphate concentrations, a linear regression over the upper 9 cm depth was considered.

2.11 Measurement of Enzyme Activities in the Sediment

Linearity test and substrate saturation test were carried out to validate the measurement of enzyme activities (section 2.11.1 to 2.11.2). Section 2.12 describes the reagents used for enzyme activities measurement. Section 2.13 explains the two types of assays used in the measurement of enzyme activities.

2.11.1 Linearity Test

The validation process indicated that fluorescence intensity of the fluorochrome analogs, released due to alkaline phosphatase, cellobiohydrolase, β-glucosidase, and chitinase activity in the sediment, increased linearly with time (Figure 2.6 a-d). This linear association shows that fluorescence intensity increases with the amount of fluorochrome in solution and that enzyme activity was not induced or repressed within the 4 h incubation. Absorbance intensity for urease increased linearly with time for up to 20 hours of incubation in Figure 2.6 e.

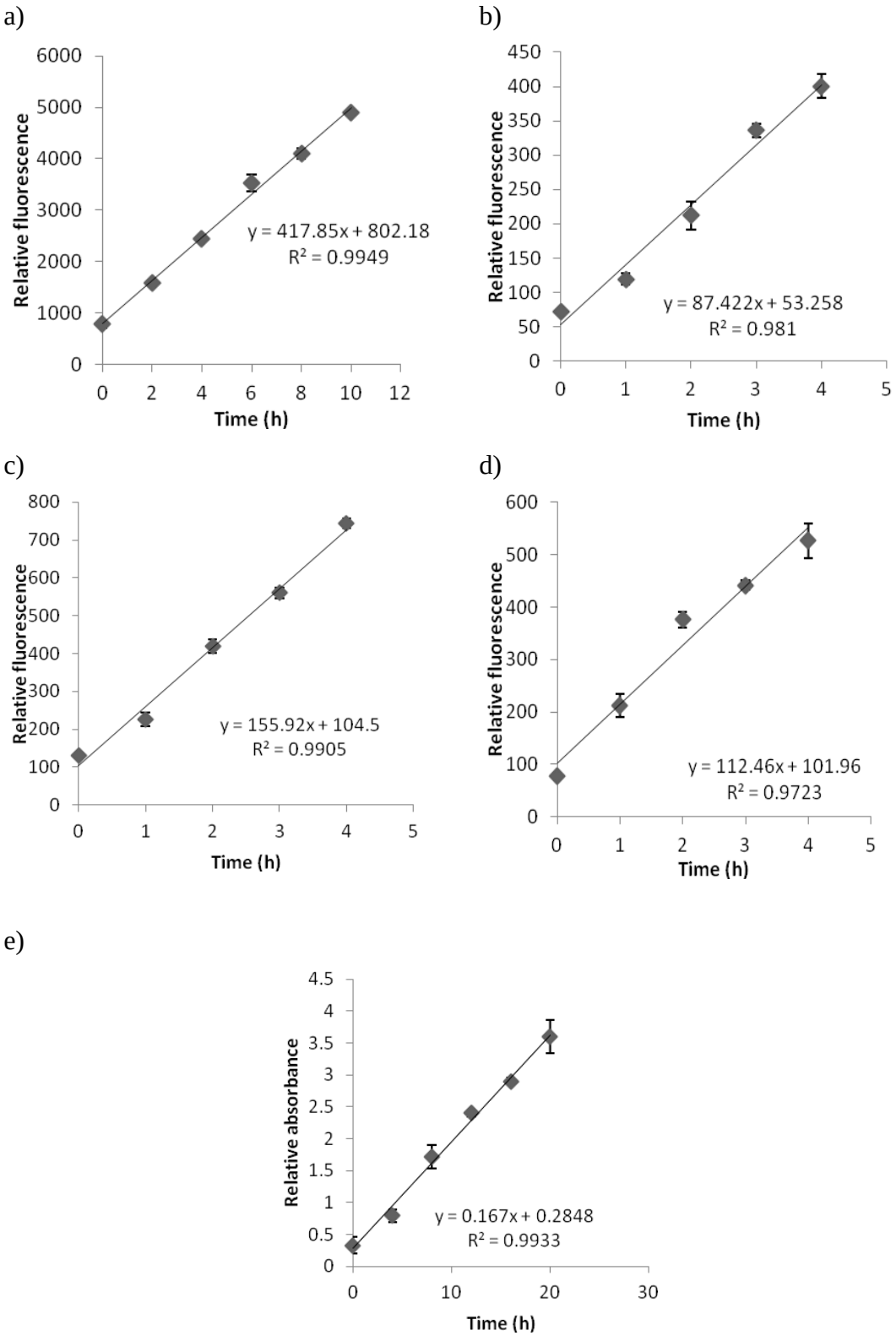
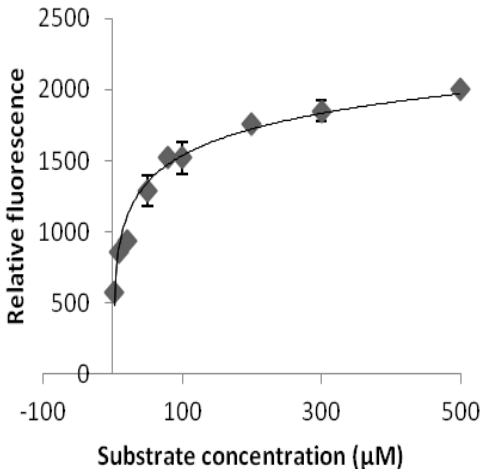


Figure 2.6 Analog fluorescent as a function of incubation time for a) alkaline phosphatase, b) cellobiohydrolase, c) beta-glucosidase and d) chitinase. e) Analog absorbance as a function of incubation time for urease. Values are reported as mean ± SE, n= 5.

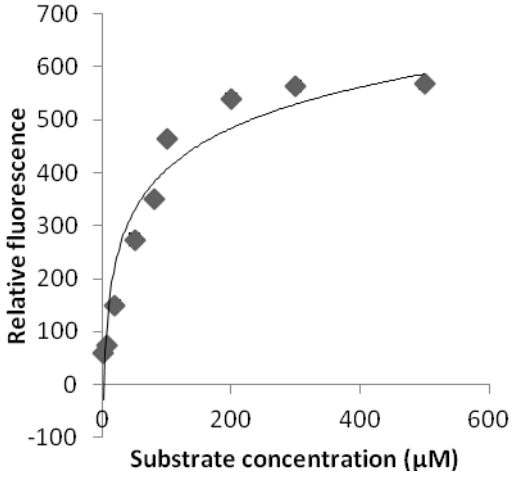
2.11.2 Saturation Test

Substrate saturation curves were carried out for all 5 enzymes measurement to determine which substrate concentration to be used in this study. For all the enzymes measured using fluorometric methods, 200 µM substrate was sufficient to saturate the enzyme. This is shown in Figure 2.7 a-d for alkaline phosphatase, cellobiohydrolase, β-glucosidase and chitinase. This concentration was proposed as the saturated substrates concentration of these 4 enzymes by Sinsabaugh et al., (2000); Saiya-Cork et al., (2002) and DeForest, (2009). Urease was saturated at 20 mM as shown in Figure 2.7 e. Alef and Nannipieri, (1995) have reported substrate saturation of urease between 5-40 mM, depending on the sediment used.

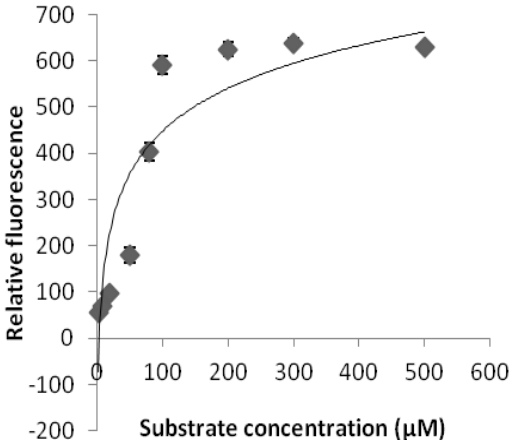
a)



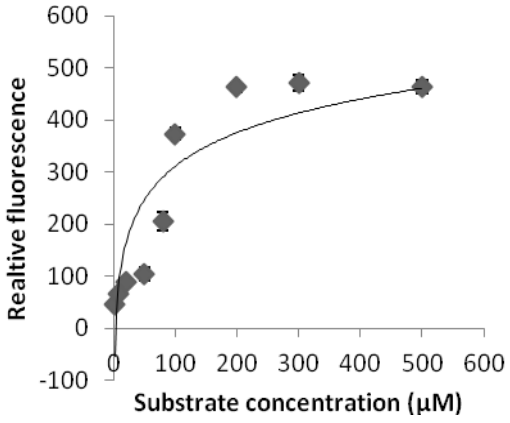
b)



c)



d)



e)

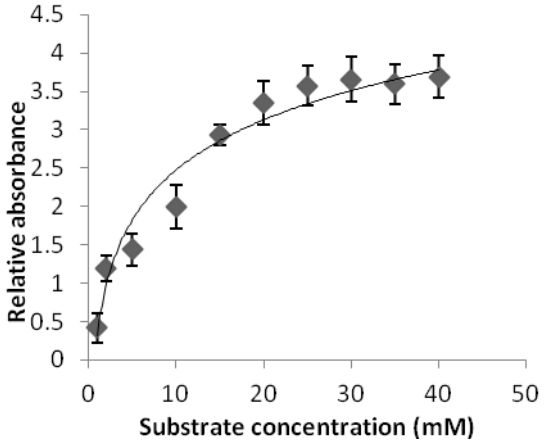


Figure 2.7 Substrate saturation curves on fluorescence intensity of a) alkaline phosphatase, b) cellobiohydrolase, c) beta-glucosidase d) chitinase and absorbance intensity of e) urease. Values are reported as mean ± SE, n= 5.

2.12 Reagents

2.12.1 Substrates

Methylumbelliferyl substrates (MUB) were used for 4 of the enzyme measurement, 4-methylumbelliferyl (MUB)-phosphate, 4-MUB- β-D cellobioside, 4-MUB-β-D-glucopyranoside and 4-MUB-N-acetyl- β-D glucosaminidase. All substrate analogues were purchased from Sigma-Aldrich Co. Ltd in crystalline form. The use of MUB substrates has a close relationship to naturally occurring processes (Hoppe, 1993). All substrates were made up to 10 ml with sterile deionised water and the resulting stock solutions of 10 mM were kept at 4°C. Working solutions for each substrate was diluted to 1000, 100 and 10 µM with buffer in sterile 30 ml universal bottles to establish substrate saturation curves. Solutions of MUB-standards and MUB-linked substrates were prepared fresh during the day of the analysis, to prevent rapid changes of MUB-standard and MUB-linked substrates that can occur within the first 24 hours (DeForest, 2009). The final substrate concentrations in the plates were 2, 5, 8, 20, 50, 80, 100, 200, 300, 500 and 700 µM.

2.12.2 Buffers

All enzyme assays were carried out in buffered conditions in order to reduce the effects of pH changes on the results. The use of buffer also stabilises the fluorescence intensity which is highly dependent on pH (Alef and Nannipieri, 1995) and allow comparisons to be made with other system (German et al., 2011). A 50 mM acetate buffer solution was used by mixing sodium acetate trihydrate (certified ACS grade, crystalline, Sigma-Aldrich) with deionised water for fluorescence assays. Various studies for enzyme measurements have used 50 mM acetate buffer in their study for sediments (Niemi and Vepsäläinen, 2005; Sinsabaugh et al., 2005). The pH was adjusted to 7.8 for all enzyme measurements using either sodium hydroxide (NaOH) or hydrochloric acid (HCl). Generally most of the sediments in this study exhibited pH ranges of 7.5 to 8.0 within the sediment porewaters, therefore average range of pH 7.8 was selected for all enzymes. For alkaline phosphatase (AP) activity measurement in the sediment, usually a near neutral pH was used (Alef and Nannipieri, 1995) and AP was found to react optimally in pH range of 7.6 – 9.6 (Cunha et al., 2010). Niemi and Vepsäläinen, (2005) have observed a pH optimum of 7.5 or higher for alkaline phosphatase activity, chitinase and β-glucosidase activity.

2.12.3 Standards

A 10 mM stock solution of 4-MUB was prepared by dissolving 0.1762 g of 4-MUB in 100 ml of methanol. This stock solution was diluted to concentrations, ranging from 1-10 µM with acetate buffer and prepared fresh on the day of the analysis. These concentrations are good for the sediment samples in this study.

2.13 Measurement of Potential Enzyme Activity

Initial enzyme activities were measured from sediment cores immediately upon arrival to the laboratory. The effect of different oxygen concentrations on enzyme activities was measured at the end of the incubation on day 7. Substrates, buffers, standards and incubation assays in the microplates were purged with gas mixtures for hypoxic treatments and nitrogen gas for anoxic treatments prior to use. Each

sediment core was placed over a core extruder and sectioned with a metal spatula at 2 cm intervals down to 10 cm. Enzyme assays were conducted in five replicates for each depth interval (0-2, 2-4, 4-6, 6-8 cm). For clarity, each depth interval was depicted as 1, 3, 5, 7 and 9 cm on the y-axis of graphs. The enzyme activity assay gives the maximum potential activity of enzymes occurring in sediment rather than the actual enzyme activity (Alef and Nannipieri, 1995). Enzyme activity is determined by measuring the increase in fluorescence signal over a fixed time interval (Boetius et al., 2000).

2.13.1 Fluorometric Assay

1.0 g of sediment suspensions was mixed with 125 ml 50 mM acetate buffer at a pH of 7.8 in a 250 ml beaker using an ultrasonicator water bath for 15 minutes. Sediment slurries are necessary to reduce diffusion barriers and ensure that enzymes have adequate access to substrate (Allison et al., 2007). Ultrasonication provides better homogenisation of the samples and released extracellular enzymes previously immobilised in humic colloids (De Cesare et al., 2000). It also completely break up macro aggregates (>250 µm) but does not disrupt smaller particles (Stemmer et al., 1998). The resulting suspensions were continuously stirred using a magnetic stirrer plate while samples were dispensed into 96-well microplates. The combination of using both ultrasonication and magnetic stirrer is adopted to reduce variability in enzyme assays using microplate technology and to homogenise the sediment slurry thoroughly as to represent the initial material. Before pipetting the assay solution containing the reaction product into new plates, the particles were ensured to be settled during the incubation period. This step is to prevent particles from sample homogenate causing light beam scattering of the microplate reader (Allison et al., 2007).

The dispensing of buffer, sample, standards and substrates followed a strict order and position on the well plate. First, 200 µl of buffer was dispensed in wells that served as the blank, reference and substrate control. Next, 50 µl of buffer was dispensed in columns that served as the sample controls for the sediment. Sample controls and blank was analysed to account for naturally occurring fluorescence of

the buffer or sediment within the well plate. Sediment turbidity exerts a quenching effect on the fluorescence intensity of MUB. Therefore, a quench correction procedure was applied to the sediment samples. Then, 200 µl of sediment samples was dispensed into wells that served as the quench, sample control and sample assay (Saiya-Cork et al., 2002; DeForest, 2009). The steps in the sediment samples, references and substrate in each well are summarised in Table 2.3. Fifty microliters of a 10 µM MUB solution was dispensed into wells that served as reference standard and quench standard. Lastly, 50 µl of a 200 µM MUB-linked substrate was dispensed into plates that served as a substrate control and the sample assays. A substrate concentration of 200 µM was used according to the substrate saturation curves employed (Figure 2.7). The addition of the MUB-linked substrate marked the start of the incubation period and time was noted. The microplates were incubated in the dark at 12 °C for up to 4 h (Saiya-Cork et al., 2002; DeForest, 2009). The selection of the assay temperature is equivalent to the time of sampling and incubations.

The samples to be assayed, buffer, substrates and standard concentrations of 250 µl are pipetted into 96 well, 300 µl black microwell plates (Nunclon Inc.) and fluorescence measured with a microplate reader (Spectramax M2). The fluorescence intensity was measured with 365 nm-excitation and 450 nm emission filters (Saiya-Cork et al., 2002). This analytical equipment allows incubation of the microplate at constant temperature. For the measurements, the microplate reader was programmed to shake the microplate for 5 s in order to homogenise the reaction medium. After finishing the readings, the computer software (Softmax Pro) was used to calculate the relative fluorescence intensity increase in each well. Since the rate of fluorescence increased was measured rather than the absolute amount of fluorescence at the end of the incubation period, a time zero blank was not needed but anyway, only accounted for minimal background fluorescence intensity (Marx et al., 2001). Initial experiments demonstrated that background fluorescence did not change during the incubation time. Calculation of enzyme activity is based on subtraction from control and for standards, with quench and reference standard, based on DeForest, (2009).

Enzyme activities were expressed in units of nmol g^{-1} h^{-1} and calculated from the raw data to final values by the following incubations and formula:

Table 2.3 Incubation of sample assay (SA), sample quench (SQ), reference standard (RS), quench coefficient (QC), substrate control (SuC) and sample control (SaC) of AP, CB, BG, and chitinase activities in wells

SA : Mean fluorescence of 200 µL sample + 50 µL substrate
SQ : Mean fluorescence of 200 µL sample +50 µL standard
RS : Mean fluorescence of 200 µL buffer + 50 µL standard
QC : SQ/RS
SuC : Mean fluorescence of 200 µL buffer + 50 µL standard
SaC : Mean fluorescence of 200 µL sample +50 µL buffer

Formula for enzyme activities calculation:

Equation 2.4:

$$\text{Activity (nmol g}^{-1}\text{ h}^{-1}\text{)} = \frac{\text{NF} \times 125 \text{ ml total sediment slurry}}{\text{EmC} \times 0.2 \text{ ml sediment slurry per well} \times \text{Time (h)} \times \text{sediment in fresh sample (g)}}$$

Where:

Equation 2.5:

$$\text{NF} = \frac{(\text{SA} - \text{SaC})}{(\text{QC})} - \text{SuC}$$

Equation 2.6:

$$\text{EmC (relative fluorescence unit nmol}^{-1}\text{)} = \frac{\text{RS}}{0.5 \text{ nmol per well}}$$

NF = net fluorescence
SA = sample assay
SaC = sample control
SuC = substrate control
QC = quench coefficient
EmC = Emission coefficient
RS = Reference standard

The 125 ml refers to the volume of sediment slurry and 0.5 nmol is the amount of MUB-standard added to a well. Fluorescence values were means from six analytical wells subtracted from the sample control.

Inhibition of the reaction and maximisation of the fluorescence intensity through alkalinisation of the medium was not carried out because at pH 7.8 used in this study, the fluorescence intensity and high sensitivity were detected. The elimination of this procedure further minimise the time consumed in sample

preparation and analysis, offering a better potential for use in routine enzyme measurements.

2.13.2 Colorimetric Assay

Urease activity was measured colorimetrically in clear, flat bottomed polystyrene (Nunclon, Inc.) 96-well, 300 µl microplates. Urease activity was calculated from ammonium concentration using 96 clear microplates after 8 hours of incubation at 12 ºC (Sinsabaugh et al. 2000). The incubation time and temperature selected were based on the linearity test (Figure 2.6) and the temperature during sampling. A sample suspension was created by homogenising 1.0 g sediment in 100 ml of 50 mM, pH 7.8 borate buffer, using an ultrasonicator and a magnetic stirrer. More concentrated suspensions have more activity but they generate a higher absorbance background (Sinsabaugh et al., 2000). pH 7.8 buffer was used to approximate the pH of bulk sediment in this study and to control the intensity of the absorbance. The optimum pH for urease activity is within 7-10 (Alef and Nannipieri, 1995). While stirring vigorously, 200 µl aliquots were withdrawn and dispensed into the microplate wells using a pipette. Three microplate columns (24 wells) were used per sample. Aliquots (10 µl) of substrate solution (400 mM urea in deionised water), were dispensed into the wells of two columns (16 wells) yielding a final urea concentration of 20 mM based on the substrate saturation curve (Figure 2.7).

The third column of wells acted as negative controls; each well received 10 µl of water. Each microplate also contained a substrate control: a column of wells that each contained 200 µl of buffer and 10 µl of substrate solution. Ammonium released was quantified by dissolving 8.5 g of sodium salicylate in 85 ml of water and adding 0.06 g of sodium nitroprusside and diluted to 100 ml. Cyanurate reagent was prepared by dissolving 2.4 g of sodium hydroxide in 90 ml of water, and when it has cool, 0.5 g of sodium dichloroisocyanurate was added and diluted to 100 ml. The reagents were kept in amber bottles and wrapped with aluminium foil. The sample in the microplate (150 µl) was treated with 60 µl of salicylate reagent, followed by 60 µl of cyanurate reagent and 30 µl of water.

Standards, prepared from ammonium chloride, were added to the microplate. Standards ranging from 1 to 10 µM were used for the calibration curve, which are good within the incubation time and analysis of the sediment samples in this study. Wells in standard columns contain 200 µl of standard and 10 µl of water. The wells containing standards constituted the positive controls for the assay. Standards were also prepared using sample suspensions which allows for the potential adsorption of ammonium to particles. In this case, 10 µl aliquots of ammonium chloride used as standard, ranging from 20 to 200 µM, were added to wells containing 200 µl of sample suspension. The absorbance of each well was then read at 610 nm using a spectrophotometric microplate reader. Well to well variation can be minimised by adequate homogenisation, vigorous mixing of samples while dispensing, good pipetting technique (Sinsabaugh et al., 2000) and by running a larger number of replicate samples and controls (i.e. 16). Enzyme activities were expressed in units of nmol g^{-1} h^{-1} and calculated by the following formula and summarised as below in Table 2.4 (Sinsabaugh et al., 2000; Saiya-Cork et al., 2002):

Table 2.4 Incubation of SA, SuC, SaC and extinction coefficient (ExC) for urease activities.

SA : Mean absorbance of 200 µL sample + 10 µL substrate
SuC : Mean absorbance of 200 µL buffer + 10 µL standard
SaC : Mean absorbance of 200 µL sample + 10 µL buffer
ExC : Slope of absorbance versus concentration of NH_4^+ under the conditions of the assay

Formula for enzyme activities calculation:

Equation 2.7:

$$\text{Activity (nmol } g^{-1} h^{-1}) = \frac{FA \times 100 \text{ ml}}{ExC \times (0.2\text{ml}) \times \text{Time (h)} \times \text{Sediment (g)}}$$

Where

Equation 2.8:

$$FA = SA - SuC - SaC$$

FA = final absorbance
SA = sample assay
SuC = substrate control
SaC = sample control
ExC = extinction coefficient (nmol)

Absorbance is the absorbance at 450 nm, 100 ml refers to buffer volume and 0.2 ml is the sample volume in the microplate.

2.14 Statistical Analysis of Results

One way ANOVA was conducted to test the effect of different treatments on total bromide incubation fluxes, porewater oxygen diffusive fluxes and porewater nutrient diffusive fluxes. When there were significant interactions, post hoc Tukey test was carried out (Field, 2009). Differences between treatments and day of pH and nutrient fluxes and differences in porosity, total organic carbon, total nitrogen, CN ratio, porewater nutrient concentrations, porewater bromide concentrations and enzyme activities between treatments and depths were tested using two-way ANOVA. Post hoc Bonferroni test was conducted to identify between which treatments, day and depth, significant differences occurred. Linear regressions and non-linear regressions between enzyme activities with porewater nutrients and enzyme activities and sediment physico-chemical characteristics were also assessed. The relationship between dissolved oxygen with nutrient concentrations and dissolved oxygen with nutrient fluxes in the overlying water were processed using principal component analysis (PCA). In the sediments, porosity, total organic carbon, total nitrogen, porewater ammonium concentrations, alkaline phosphatase, beta-glucosidase, cellobiohydrolase, chitinase and urease activities were also assessed using PCA. Statistical analyses were performed with software package SPSS 19.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6.0 software (GraphPad Prism Software, San Diego, USA).

CHAPTER 3 RESULTS

3.1 Introduction

This chapter presents all the data obtained in this study following the methodology described in Chapter 2. This chapter begins with a description of the physico-chemical characteristics of the sampling area and the effect of different treatments to the physico-chemical characteristics. Section 3.3 describes the effect of different treatments to the bromide concentration in the overlying water and the porewater. Section 3.4 explains the effect of different treatments to the oxygen penetration depth and oxygen diffusive fluxes. Section 3.5 explains the initial nutrient and porewater nutrient concentrations of Breydon Water and the effect of different treatments to the water column nutrients and porewater nutrients concentrations (nitrate, nitrite, ammonium, and phosphate), followed by the nutrient fluxes and porewater diffusive fluxes. The final section explains the enzyme activities in the sediment (alkaline phosphatase, ß-glucosidase, cellobiohydrolase, chitinase and urease). The interrelationships between the different parameters measured in this study were also presented in this chapter.

3.2 Physico-chemical Characteristics of Breydon Water, Great Yarmouth

The overlying water during the sampling carried out in June 2010 had a salinity of 32 ‰, a pH of 7.83 ± 0.06 at a temperature of 12.4 ± 0.02 °C and a dissolved oxygen saturation of 75 %. Table 3.1 summarises the sediment characteristics in Breydon Water, Great Yarmouth. The porosity of the sediment ranged from 0.54 to 0.77, decreasing with depth due to sediment compaction that occurred below 5 cm depth. The sediment was muddy with high total organic carbon (TOC) and total nitrogen (TN) at the surface, which decreased with depth, showing a pattern of organic matter mineralisation.

Table 3.1 Sediment physico-chemical characteristics from samples taken at Breydon Water, Great Yarmouth sampled at 1 to 9 cm depth in June 2010, reported as mean ± SE, n=5.

Depth (cm)	Porosity (g/mL)	TOC (%)	TN (%)	CN ratio	Particle size (%)		
					<8µm	8-64 µm	> 64 µm
1	0.77 ± 0.01	3.86 ± 0.11	0.44 ± 0.02	8.88 ± 0.34	70.3 ± 6.8	15.8 ± 6.1	7.21 ± 2.3
3	0.73 ± 0.02	3.59 ± 0.15	0.45 ± 0.02	8.03 ± 0.57	66.4 ± 7.7	10.3 ± 4.6	5.43 ± 1.8
5	0.70 ± 0.01	3.27 ± 0.09	0.35 ± 0.01	9.27 ± 0.28	65.7 ± 6.0	11.7 ± 5.4	8.90 ± 3.7
7	0.58 ± 0.02	2.83 ± 0.07	0.34 ± 0.01	8.26 ± 0.44	63.4 ± 8.4	9.3 ± 2.6	6.62 ± 0.7
9	0.54 ± 0.03	2.21 ± 0.08	0.29 ± 0.03	8.08 ± 0.97	71.4 ± 5.0	12.1 ± 3.3	6.84 ± 3.4

3.2.1 Effect of Different Treatments on Overlying Water pH during Laboratory Incubations

pH measures the active hydrogen ions concentrations; therefore data are presented in terms of their hydrogen ion concentrations in the overlying water. This data is measured to evaluate the effect of different treatments and days on pH as shown in Figure 3.1. Hydrogen ion concentrations are higher in the oxic treatment; indicating that the pH is lower in the oxic treatment relative to the anoxic treatment. Conversely, low concentrations of hydrogen ions in anoxic treatment; means that the water is more alkaline and has a higher pH than oxic. Statistically significant difference was found between treatments (two-way ANOVA, $p<0.0001$) but were not significant with days (two-way ANOVA, $p>0.05$).

3.2.2 Effect of Different Treatments on Porosity during Laboratory Incubations

Generally, all treatments showed higher porosity at the sediment surface and decreasing pattern with depths (two-way ANOVA, $p< 0.0001$, depth effect, Table 3.2). But, no significant differences were detected among treatments due to no changes in the sediment profiles (two-way ANOVA, $p > 0.05$, treatment effect). Similar with initial treatments (Table 3.1), the influence of depth is likely due to compaction, leaving denser solid particles below 5 cm depth in the sediments.

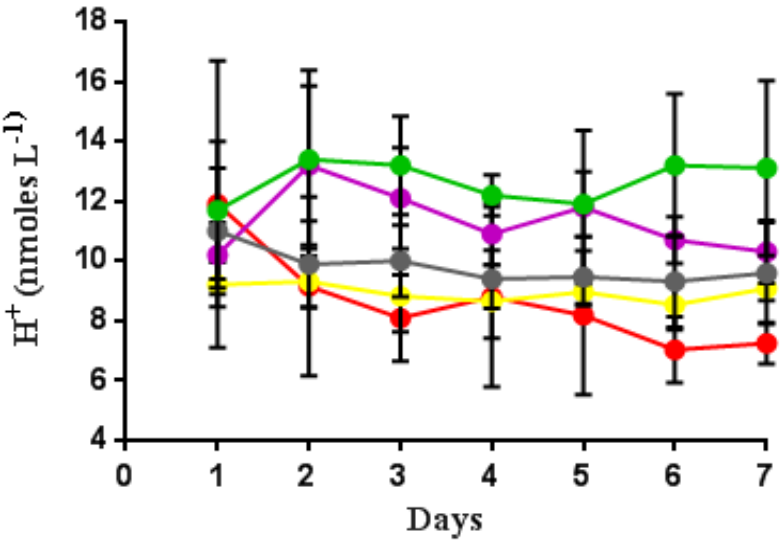


Figure 3.1 Daily hydrogen ion concentrations maintained under different oxygen concentrations in overlying water samples taken during the 7 day laboratory incubations. Samples are reported as mean ± SE, n=5. Red circles - anoxic treatments, yellow circles - four days without oxygen and re-aerated for 3 days (4DA), grey circles - hypoxic conditions, purple circles - 1 day without oxygen and re-aerated for 6 days (1DA), green circles - oxic treatments.

Table 3.2 Porosity (g/mL) measured at 1 to 9 cm depth sediments from five different treatments, oxic, hypoxic, 1DA, 4DA and anoxic taken on day 7 of laboratory incubations. Samples are reported as mean ± SE, n=5.

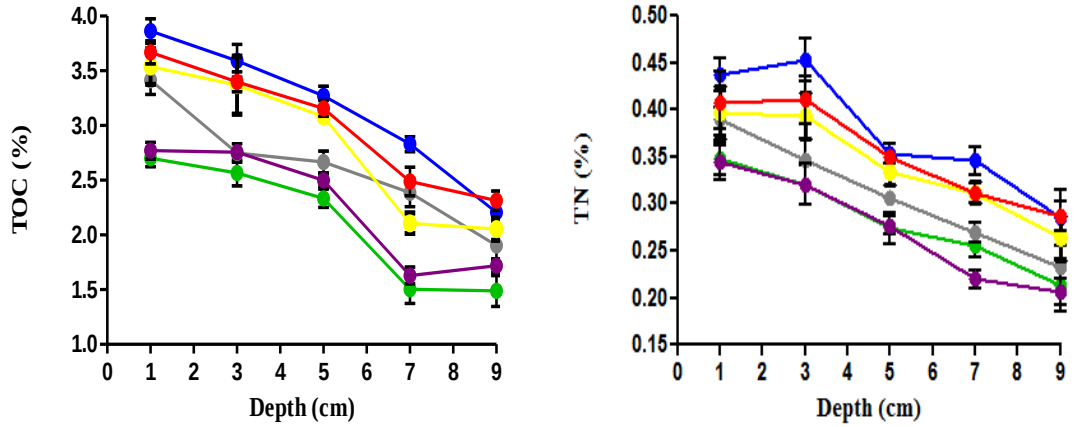
Depth (cm)/ Treatments	1	3	5	7	9
Oxic	0.78 ± 0.11	0.76 ± 0.02	0.72 ± 0.01	0.60 ± 0.03	0.52 ± 0.05
Hypoxic	0.78 ± 0.02	0.72 ± 0.05	0.69 ± 0.05	0.55 ± 0.05	0.50 ± 0.05
1DA	0.80 ± 0.04	0.71 ± 0.06	0.61 ± 0.06	0.53 ± 0.05	0.49 ± 0.05
4DA	0.80 ± 0.06	0.71 ± 0.05	0.61 ± 0.04	0.53 ± 0.03	0.49 ± 0.04
Anoxic	0.81 ± 0.06	0.75 ± 0.04	0.70 ± 0.01	0.60 ± 0.01	0.52 ± 0.01

Abbreviations, 1DA = 1 day anoxic, 4DA = 4 day anoxic

3.2.3 Effect of Different Treatments on Total Organic Carbon (TOC), Total Nitrogen (TN) and CN ratio Taken at the Start and on Day 7 of Laboratory Incubations

During the 7 days of the experiment, all treatments showed rapid reductions in sediment TOC and TN relative to initial conditions (Figure 3.2 a and b). On average, the reduction of TOC in the anoxic treatment is 0.1% and 1% in the oxic treatment relative to initial condition. The loss of TOC and TN affects the mol/mol CN ratio particularly in the oxic and 4DA treatments at 7 cm depths relative to other depths (Figure 3.2 c).

a) b)



c)

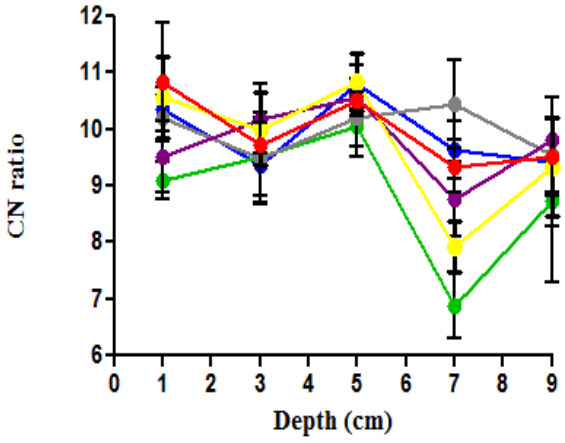


Figure 3.2 Vertical profiles of a) TOC, b) TN and c) CN ratio taken at the start and on day 7 of laboratory incubations. Samples are reported as mean ± SE, n=5. Red circles - anoxic treatments, yellow circles - four days without oxygen and re-aerated for 3 days (4DA), blue circles- initial treatments, grey circles - hypoxic conditions, purple circles - 1 day without oxygen and re-aerated for 6 days (1DA), green circles - oxic treatments.

3.3 Effect of Different Treatments on Bromide Concentration and Total Bromide Incubation Fluxes in the Overlying Water and Porewater Bromide Concentrations and Diffusive Fluxes in the Sediments

At the start of the incubation with Br spiked into the overlying water on day 4, Br concentration in the overlying water was 16 287 ± 10 µmol L^{-1}. After 3 days of incubation until day 7, Br concentrations in the overlying water were 14 900 ± 6 µmol L^{-1} for oxic, 15 200 ± 2 µmol L^{-1} for 1DA, 15 900 ± 2 µmol L^{-1} for hypoxic, 16 400 ± 4 µmol L^{-1} for 4DA and 16 600 ± 2 µmol L^{-1} for anoxic treatments. The difference in Br concentrations in the overlying water after 3 days of incubation reflects differences between treatments in the extent of movement of Br into the sediments. There is also a smaller contribution caused by changes in the volume of overlying water due to evaporation (Quintana et al., 2007).

The background porewater Br concentration at the start of the incubation ranged from 700- 900 µmol L^{-1}. After 3 days of incubations, Br was transported from the overlying water to the sediments in all treatments; concentrations decreasing with increasing depth in the sediment (two-way ANOVA, $p< 0.001$, depth effect, Figure 3.3). The vertical profiles of bromide were significantly different among treatments (two-way ANOVA $p< 0.001$, treatment effect). The significant treatment and depth effect resulted from higher irrigation fluxes into the sediment (Table 3.3). Br penetrated up to 9 cm depth in the oxic, 1DA and hypoxic treatment, however Br concentrations were low from 5 cm depths downwards, relative to those in the oxic treatments. The 4DA treatment showed zero bromide transport beyond 7 cm. The anoxic treatment revealed a steep decrease, reaching the background level at 5 cm depth (Figure 3.3). Reduced bromide exchange in the hypoxic, 4DA and anoxic treatments were driven by the higher diffusive fluxes compared to irrigation fluxes (Table 3.3).

Total bromide incubation fluxes were calculated using equation 2.2 from the difference of Br concentration in the overlying water at the start of the incubation and during the 3 days of incubation. The calculation also includes the volume of water in the cores and the incubation time (Table 3.3). There were statistically

significant difference of total bromide incubation fluxes between treatments (one-way ANOVA, $p<0.001$). Total bromide incubation fluxes from the overlying water in the oxic and 1DA treatments were significantly higher than in the hypoxia, 4DA and anoxic treatments (Tukey, $p<0.001$). This results from higher irrigation fluxes in the oxic and 1DA treatments (Table 3.3). Total incubation fluxes in the hypoxic treatment were significantly lower than in the oxic and 1DA treatment (Tukey, $p< 0.001$), showing lower irrigation fluxes (Table 3.3).

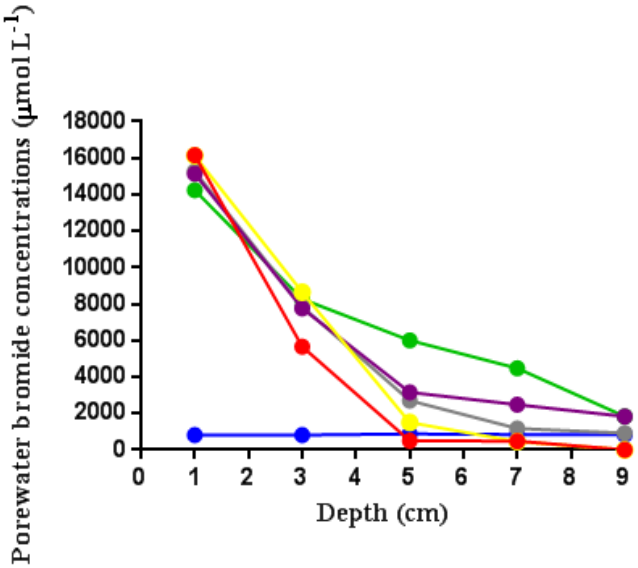


Figure 3.3 Porewater bromide concentrations from five different treatments, oxic, hypoxic, 1DA, 4DA and anoxic taken at the start and after 3 days incubation with bromide spiked into the overlying water. Values are reported as mean ± SE, n=5. No vertical bar indicates standard error is smaller than the symbol size. Red circles - anoxic treatments, yellow circles - four days without oxygen and re-aerated for 3 days (4DA), blue circles - initial treatments, grey circles - hypoxic conditions, purple circles - 1 day without oxygen and re-aerated for 6 days (1DA), green circles - oxic treatments.

Table 3.3 Mean total bromide incubation, diffusive and irrigation fluxes (µmol m^{-2} h^{-1}) after 3 days of incubation. Samples are reported as mean ± SE, n=5.

Treatments	Total incubation fluxes (µmol m^{-2} h^{-1})	Diffusive (µmol m^{-2} h^{-1})	Irrigation (µmol m^{-2} h^{-1})	Ratio Total/Diffusive
Oxic	1475 ± 5	537 ± 25	937	2.7
Hypoxic	1307 ± 28	889 ± 41	417	1.5
1DA	1450 ± 24	809 ± 30	611	1.8
4DA	1335 ± 30	1298 ± 22	90	1
Anoxic	1388 ± 24	1386 ± 14	2	1

Abbreviations, 1DA = 1 day anoxic, 4DA = 4 day anoxic

3.4 Effect of Different Treatments on Oxygen Penetration Depth and Oxygen Diffusive Fluxes Taken at the Start and on Day 7 of Laboratory Incubations

Vertical profiles of oxygen showed decreasing trends with depth as oxygen are consumed within the sediment (Figure 3.4). The oxygen profiles were collected beginning at 1 mm above the sediment-water interface. Oxygen concentrations declined steeply at 0.5 mm above the sediment-water interface in the oxic and 1DA treatments (Figure 3.4). This portion of the profile indicates the development of a slight benthic boundary layer due to the apparent vertical profile of the bottom waters accompanied by a linear decline of oxygen into the sediment.

Initially, oxygen penetrated up to 4 mm into the sediments (Figure 3.4). After being bubbled with air in the oxic treatment, oxygen penetrated up to 5.5 mm depth on day 7 with an oxygen concentration of 265 µmol L^{-1} in the overlying water (Figure 3.4 and Table 3.4). Diffusive oxygen fluxes were significantly different among treatments (one-way ANOVA, $p<0.001$). This difference is influenced by higher concentration gradient in the oxygenated treatments than in the low oxygen treatments. The highest diffusive oxygen fluxes were found in the oxic treatments (Table 3.4), followed by the 1DA treatments (6.87 ± 0.06 mmol m^{-2} d^{-1}). Low oxygen treatments in the hypoxic, 4DA and anoxic treatments showed limited oxygen penetration beyond depths of 2.5 mm and low diffusive fluxes (Figure 3.4 and Table 3.4). This pattern reflects the negative effects of anoxia to the sediment biogeochemistry. The oxygen penetration depth indicates the thickness of the oxic zone and is determined mainly by organic carbon degradation and the supply of oxygen from the bottom water (Cai and Sayles 1996; Kim and Kim 2007).

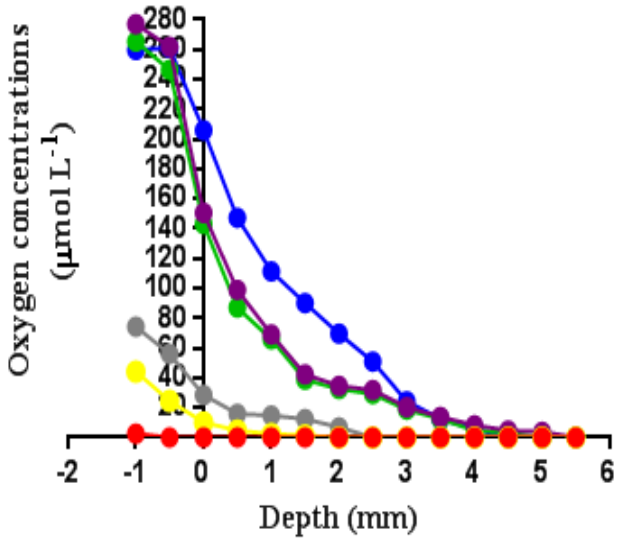


Figure 3.4 Oxygen penetration depths from five different treatments, oxic, hypoxic, 1DA, 4DA and anoxic taken at the start and on day 7 of laboratory incubations. Values are reported as mean ± SE, n=5. No vertical bar indicates standard error is smaller than the symbol size. Red circles - anoxic treatments, yellow circles - four days without oxygen and re-aerated for 3 days (4DA), blue circles - initial treatments, grey circles - hypoxic conditions, purple circles - 1 day without oxygen and re-aerated for 6 days (1DA), green circles - oxic treatments.

Table 3.4 Diffusive oxygen fluxes (mmol m^{-2} d^{-1}) in the sediment calculated on day 7 of laboratory incubations using Fick's first Law of diffusion. Samples are reported as mean ± SE, n=5.

Treatments	DO in the overlying water (% saturation)	DO in the overlying water (µmol L^{-1})	Flux (mmol m^{-2} d^{-1})
Oxic	95	265	7.90 ± 0.04
Hypoxic	25	74	0.95 ± 0.29
1DA	93	276	6.87 ± 0.06
4DA	22	44	0.81 ± 0.05
Anoxic	0	0.6	0.01 ± 0.01

Abbreviations, 1DA = 1 day anoxic, 4DA = 4 day anoxic

3.5 Initial Nutrient and Porewater Nutrient Concentrations of Breydon Water, Great Yarmouth

Table 3.5 shows the nutrient concentration in the overlying water and porewater nutrient concentrations of Breydon Water, Great Yarmouth. The nutrients in the overlying water were dominated by ammonium, followed by nitrate and to a lesser extent by nitrite and phosphate. Porewater nitrate displayed a gradual decrease with depth. There was no change in porewater nitrite and an increase of porewater ammonium with depth. The high concentrations of ammonium in the porewater compared to nitrate and nitrite could be explained by organic matter mineralisation process taking place. Higher nitrate concentrations in the overlying water compared to porewater, illustrated that porewater nitrate were either transformed to other dissolved inorganic nitrogen (DIN) or released to the overlying water. The constant nitrite concentrations with depth, suggests diffusion is taking place. Ammonium profiles displayed drastic reduction of ammonium at the sediment surface relative to that in the overlying water. This might show extensive exchange at the sediment-water interface, influenced by organic matter degradation process. Similar with ammonium, porewater phosphate concentrations increased with depth, possibly as a result of the absence of oxygen in deeper sediments.

Table 3.5 Initial water column nutrients and porewater nutrient concentrations (µmol L^{-1}) from samples taken at Breydon Water, Great Yarmouth. Samples are reported as mean ± SE, n=5.

	Depth (cm)	Nitrate (µmol L^{-1})	Nitrite (µmol L^{-1})	Ammonium (µmol L^{-1})	Phosphate (µmol L^{-1})
Overlying water	-	15.07 ± 1.54	0.35 ± 0.14	44.17 ± 7.89	0.32 ± 0.04
Porewater	1	0.57 ± 0.09	0.48 ± 0.01	2.02 ± 1.64	0.43 ± 0.02
	3	0.27 ± 0.04	0.48 ± 0.01	53.80 ± 4.64	0.57 ± 0.03
	5	0.07 ± 0.02	0.48 ± 0.02	482.57 ± 17.15	0.72 ± 0.03
	7	0.06 ± 0.01	0.48 ± 0.01	786.67 ± 10.22	1.20 ± 0.15
	9	0.05 ± 0.01	0.49 ± 0.01	812.16 ± 3.85	2.41 ± 0.46

3.5.1 Effect of Different Treatments on Nutrients Concentrations and Measured Nutrient Fluxes to/from the Overlying Water During 7 days of Laboratory Incubations

This section begins with the description of different oxygen saturation supplied in the five treatments (Figure 3.5 a). This is followed by the nutrient concentrations measured in the overlying water (Figure 3.5 b-e). The measured nutrient fluxes (Figure 3.6) were calculated from the daily nutrient concentration changes in the overlying water in Figure 3.5. The calculation also includes the incubation time and the volume of overlying water in the core during incubations according to equation 2.2 under section 2.7.1.

The dissolved oxygen saturation in the overlying water of oxic, hypoxic and anoxic treatments was maintained at 95 %, 25 % and 0 % respectively throughout the 7 days experiment (Figure 3.5 a). Recovery processes from anoxia were assessed in two other treatments, with varying length of exposure to anoxia, 1 day and 4 days (1DA and 4DA, respectively). In the 1DA treatments, re-aeration was carried out for 6 days and 3 days in the 4DA treatment. DO % was maintained at 0 % on day 1 and increased to 93 % after re-aeration was supplied in the 1DA treatment. In contrast, DO saturation only reached up to 22 % in the overlying water at the end of the experiment in the 4DA treatments (Figure 3.5 a). That indicates that there was a very substantial oxygen demand after the period of anoxia in the 4DA treatments.

Nitrate concentrations were high initially in all 5 treatments and started to decrease with low oxygen conditions (in the hypoxic, 4DA and anoxic treatments in Figure 3.5 b). A minor shift of increasing nitrate was observed in the 1DA treatment after re-aeration was introduced to the treatments on day 3 and day 4 (Figure 3.5 b).

On the other hand, at the start of the incubation, nitrite concentrations in the water column of all treatments were low and increased in the oxic and 1DA treatments (Figure 3.5 c). Nitrite concentration also increased in the hypoxic treatment after day 3. Only a slight increased of nitrite concentration was observed in

the 4DA treatments after day 5. Nitrite remains low in the anoxic treatment (Figure 3.5 c).

Ammonium concentrations increased at first in all treatment and decreases in the oxic treatment (Figure 3.5 d). In the oxic treatment, decreasing ammonium concentration was measured simultaneous with increasing water column nitrite (Figure 3.5 c and d). The pattern suggests ammonium oxidation to nitrite in the overlying water in the oxic treatment.

In the 1DA treatment, ammonium concentration was lower than in the hypoxic treatment (Figure 3.5 d). This pattern implies the effect of re-aeration in decreasing the ammonium concentrations.

In the hypoxic treatment, ammonium concentration increased and nitrate reduced (Figure 3.5 b and d). The pattern inferred from the hypoxic treatments, reflects nitrate reduction to ammonium.

Substantial increased of ammonium concentration was measured in the 4DA and anoxic treatments (Figure 3.5 d). At the same time, very low nitrate and nitrite concentrations in the 4DA and anoxic treatment suggest a net source of ammonium in these two treatments.

The concentration of phosphate was very low in the oxic treatment, but continues to increase in the hypoxic and anoxic treatment (Figure 3.5 e). The low phosphate concentration in the oxic treatment might reflect phosphate binding with iron oxides. Increasing phosphate concentrations in the hypoxic and anoxic treatment shows desorption from iron oxides, releasing phosphate to the water column. Phosphate concentration remains low in the 1DA treatments and decrease from day 4 onwards. In the 4DA treatments, phosphate concentration increased until day 4 and decreased from day 5 onwards. The pattern in the 1DA and 4DA treatment shows that the effect of re-aeration was probably sufficient to capture the released phosphate back to the sediment surface.

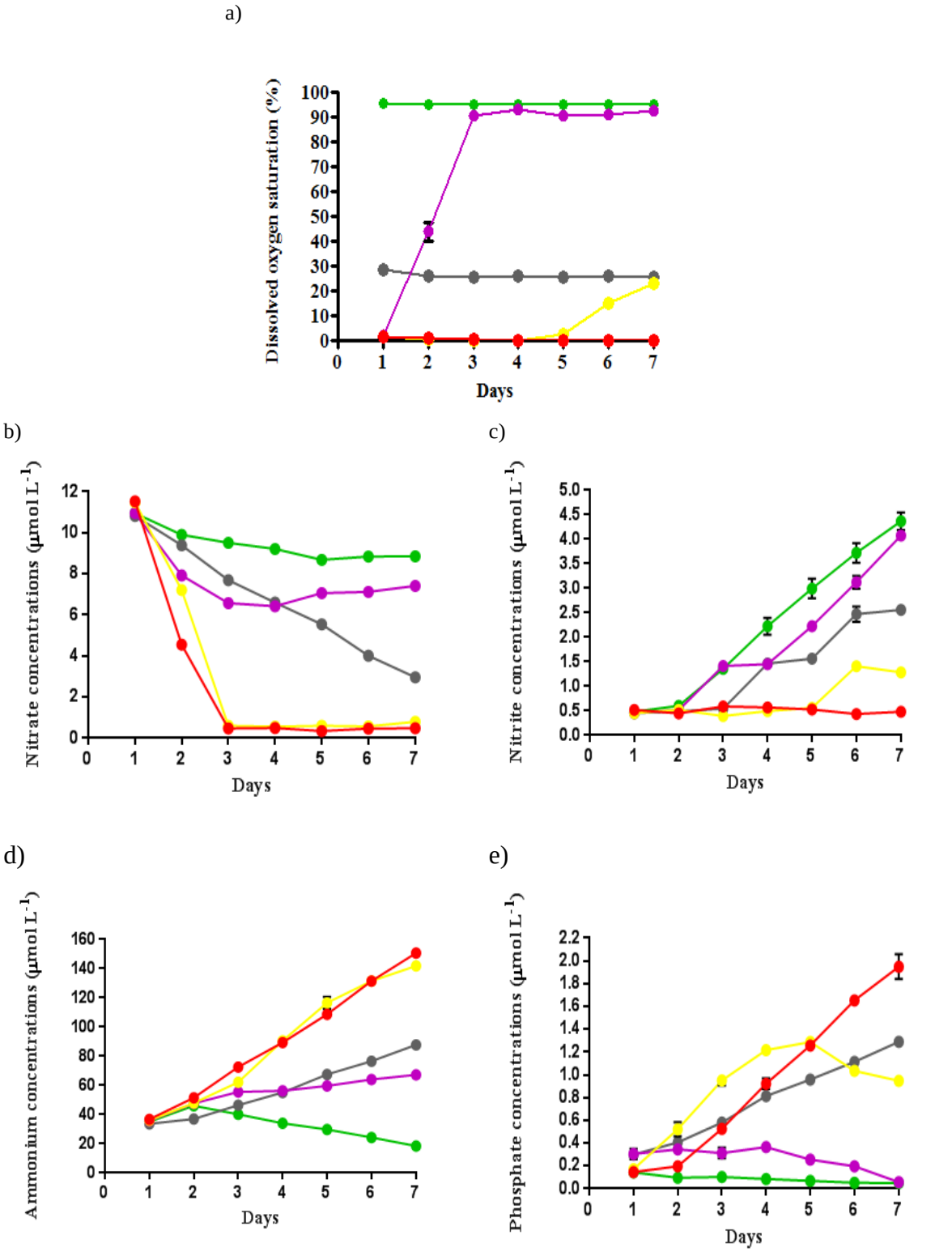


Figure 3.5 The daily evolution of a) oxygen saturation, b) nitrate, c) nitrite, d) ammonium and e) phosphate concentrations exposed to different oxygen treatments during the 7 day laboratory incubations. Values are reported as mean ± SE, n=5. No vertical bar indicates standard error is smaller than the symbol size. Red circles - anoxic treatments, yellow circles - four days without oxygen and re-aerated for 3 days (4DA), grey circles - hypoxic conditions, purple circles - 1 day without oxygen and re-aerated for 6 days (1DA), green circles - oxic treatments.

Daily nutrient fluxes in Figure 3.6 a-d were calculated from the water column nutrient concentration changes in Figure 3.5 b-e. Generally, the magnitude and/or direction of all measured nutrient fluxes in Figure 3.6 a-d differed significantly with day and treatment (two-way ANOVA, $p<0.0001$).

On day 2, nitrate fluxes (Figure 3.6 a) were significantly lower in the 4DA and anoxic treatments compared to the oxic treatments (Bonferroni corrected, $p<0.0001$). On day 7, there was a significant nitrate flux into the overlying water in the 4DA treatments in Figure 3.6 a (Bonferroni corrected, $p<0.01$).

On day 2, nitrite flux was significantly lower in the 1DA treatment compared to the oxic treatment in Figure 3.6 b (Bonferroni corrected, $p< 0.05$). On days 4 and 6, Figure 3.6 b shows that nitrite fluxes were significantly lower in all other treatments than in the oxic treatments (Bonferroni corrected, $p = 0.0001$-0.01).

Figure 3.6 c shows that substantially higher ammonium fluxes out to the overlying water in the 4DA and anoxic treatments from day 3 onwards (Bonferroni corrected, $p<0.01$). This pattern demonstrates the dominance of ammonium, consistent with very low nitrate and nitrite fluxes in low oxygen conditions (Figure 3.6 a-c). High release of ammonium in the overlying water might be due to decreases in the sediment demand for ammonium, i.e for nitrification and assimilation.

But, on day 7, ammonium flux was into the sediments in the 4DA treatment in Figure 3.6 c (Bonferroni corrected, $p<0.0001$). Nitrate flux was simultaneously observed into the overlying water on day 7 in the 4DA treatments (Figure 3.6 a). This shows ammonium conversion to nitrate by the end of the experiment.

On the first day, phosphate flux was to the overlying water in all except the oxic treatments (Figure 3.6 d). Phosphate flux to the overlying water is greatest on day 2 in the 4DA treatments (Figure 3.6 d), potentially as a response to drastic oxygen fluctuations. Phosphate flux into the overlying water continues the following day until day 7 in the anoxic treatment. This flux presumably results from the reduction of iron (III) oxides.

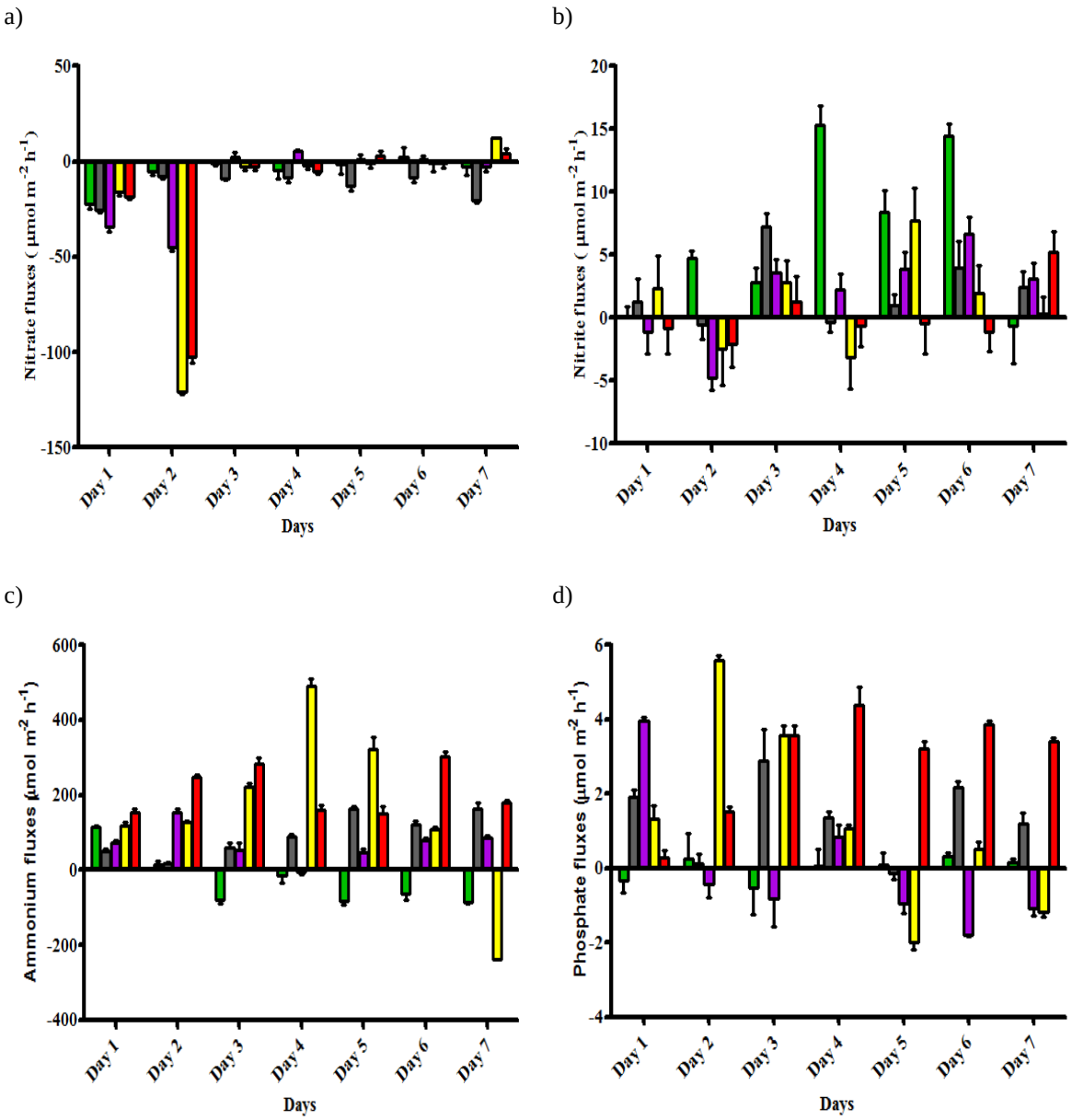


Figure 3.6 Daily a) nitrate, b) nitrite, c) ammonium and d) phosphate fluxes calculated from the water column nutrient concentration changes in Figure 3.6 b-e from five different treatments; oxic, hypoxic, 1DA, 4DA and anoxic. Values are reported as mean ± SE, n=5. Red bar- anoxic treatments, yellow bar- four days without oxygen and re-aerated for 3 days (4DA), grey bar- hypoxic conditions, purple bar- 1 day without oxygen and re-aerated for 6 days (1DA), green bar- oxic treatments.

3.5.2 Interrelationships between Nutrient Concentrations with Dissolved Oxygen; and Nutrient Fluxes with Dissolved Oxygen in the Overlying Water during 7 days of Laboratory Incubations

Principal Component Analysis (PCA) was used to summarise the data on nutrient concentration and dissolved oxygen in the overlying water (Figure 3.7 a - b). Two components accounted for 88% of the overall variability of the data. The first component, which accounted for 66% of the variability of the data, was associated with nitrate, ammonium, phosphate and dissolved oxygen (Table 3.6). Component 1 contrasted sample with increasing nitrate concentration and dissolved oxygen and decreasing ammonium and phosphate concentrations. The water chemistry in each treatment evolves along its own trajectory (Figure 3.7 a).

PCA was also use to show the evolution of nutrient fluxes in response to changing dissolved oxygen in the overlying water during the 7 days laboratory incubations (Figure 3.8 a - b). Component 1 was positively correlated with dissolved oxygen and negatively correlated with ammonium and phosphate fluxes whereas nitrate and nitrite fluxes was positively correlated with both components in Table 3.7 (component 1 44.17%, component 2 18.8%).

Nitrate concentrations are high when dissolved oxygen saturations are high and started decreasing when dissolved oxygen saturations were low (Figure 3.7 a). The largest nitrate uptake into the sediment occurred when the dissolved oxygen was low as a response to oxygen depletion during the experiment (Figure 3.8 a). A scatterplot between nitrate concentration and nitrate fluxes also shows high concentrations and fluxes in the presence of oxygen (Figure 3.9 a). In the hypoxic treatments, nitrate is partly reduced to nitrite with most of the DIN converted to ammonium (Figure 3.9 a, c and e).

Nitrite concentration accounted for the second component (22% of the variability of the data) in Table 3.6. Nitrite concentrations were higher in the presence of oxygen and low in anoxic conditions (Figure 3.7 a). The highest nitrite flux into the overlying water was at high dissolved oxygen saturation (Figure 3.8 a).

This is consistent with the scatterplots of nitrite concentrations and nitrite fluxes in Figure 3.9 b. Nitrite accumulates in the oxic treatment but in the anoxic treatment, this is reduced further to ammonium (Figure 3.9 c and e).

Ammonium is high when the dissolved oxygen is low (Figure 3.7 a). Ammonium fluxes out into the overlying water when oxygen was low (Figure 3.8 a). This indicates ammonium accumulation in the absence of oxygen. Ammonium is inevitably produced from both nitrate and nitrite reduction in the hypoxic, 4DA and anoxic treatments (Figure 3.9 a, c and e).

In the presence of oxygen, phosphate remains relatively low. Hypoxia and anoxia, in turn increased phosphate concentrations in the overlying water (Figure 3.7 a). The greatest phosphate flux into the overlying water occurred when oxygen was low, potentially because of iron (III) reduction to iron (II) (Figure 3.8 a – b). Re-aeration was sufficient to maintain phosphate at low concentrations in the 1DA treatment and led to decreased phosphate concentrations in the 4DA treatment (Figure 3.9 g – h). Overall, principal component analysis revealed a distinct pattern of evolution of nutrients in the water in response to different treatments during the 7 days of incubation.

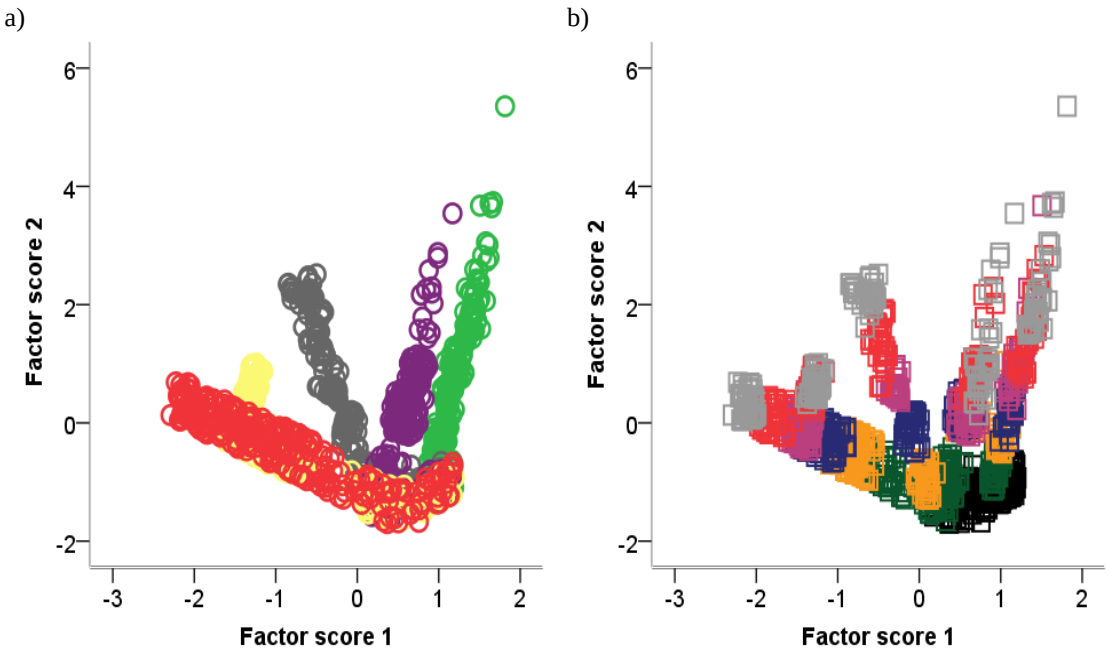


Figure 3.7 Scatterplots of the principal component analysis of dissolved oxygen, nitrate, nitrite, ammonium and phosphate concentrations in the overlying water according to different a) treatments and b) day. Red circles - anoxic treatments, yellow circles - four days without oxygen and re-aerated for 3 days (4DA), grey circles - hypoxic conditions, purple circles - 1 day without oxygen and re-aerated for 6 days (1DA), green circles - oxic treatments. Black square – day 1, green square – day 2, orange square – day 3, blue square – day 4, pink square – day 5, red square-day 6, grey square-day 7.

Table 3.6 Eigenvectors extracted based on values greater than 0.6 and above for component 1 and 2 of Principal Components Analysis

Variables	Component	
	1	2
Nitrate concentration	**.905**	-.241
Nitrite concentration	.368	**.906**
Ammonium concentration	**-.902**	.198
Phosphate concentration	**-.930**	.155
Dissolved Oxygen	**.845**	.245

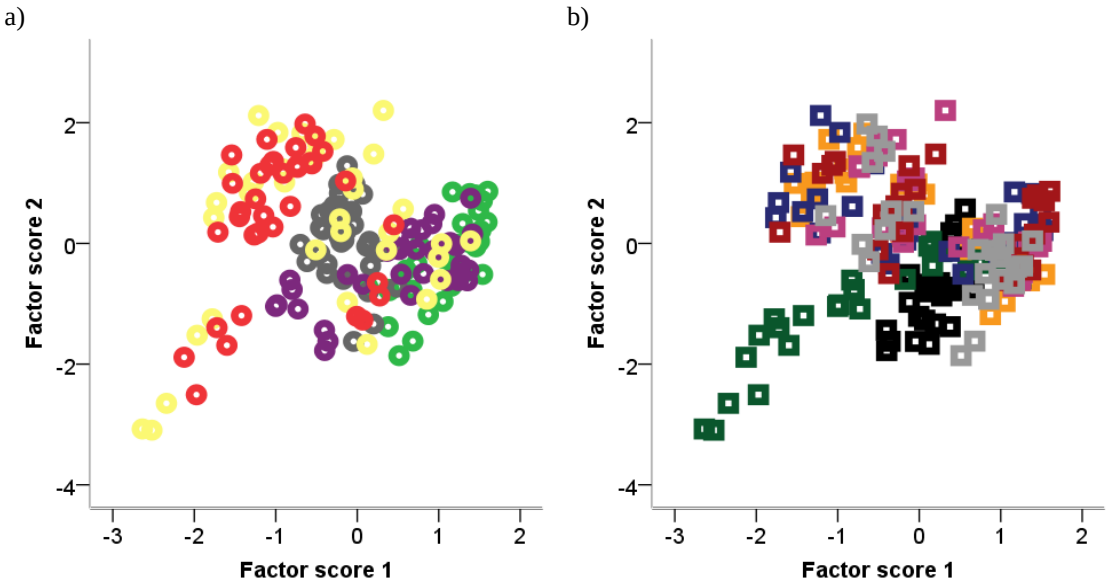
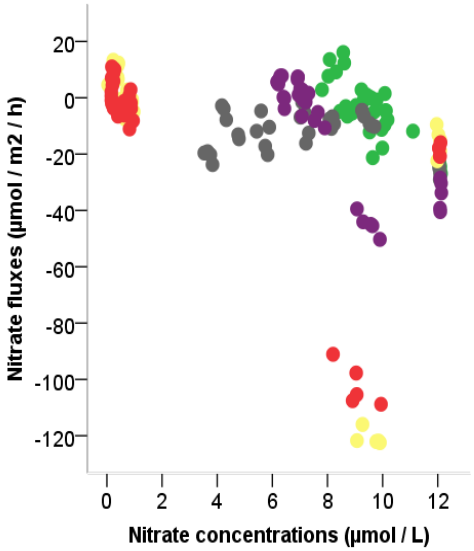


Figure 3.8 Scatterplots of the principal component analysis of dissolved oxygen, nitrate, nitrite, ammonium and phosphate fluxes in the overlying water according to different a) treatments and b) day. Red circles - anoxic treatments, yellow circles - four days without oxygen and re-aerated for 3 days (4DA), grey circles - hypoxic conditions, purple circles - 1 day without oxygen and re-aerated for 6 days (1DA), green circles - oxic treatments. Black square – day 1, green square – day 2, orange square – day 3, blue square – day 4, pink square – day 5, red square-day 6, grey square-day 7.

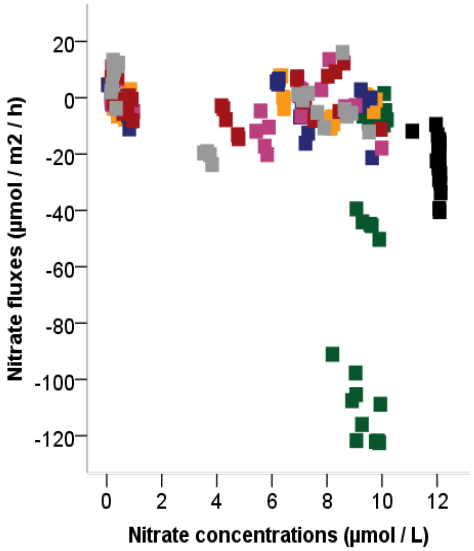
Table 3.7 Factors extracted using Principal Component Analysis by fixing the number of factors to extract and suppressing factor loadings of less than 0.4.

Variables	Component	
	1	2
Dissolved Oxygen	**.794**	
Ammonium fluxes	**-.754**	
Phosphate fluxes	**-.667**	
Nitrate fluxes	.543	.625
Nitrite fluxes	.520	.534

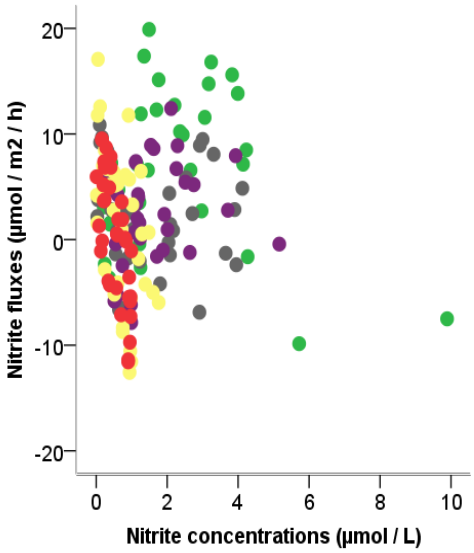
a)


Nitrate fluxes (µmol / m2 / h)
20
0
-20
-40
-60
-80
-100
-120
0
2
4
6
8
10
12
Nitrate concentrations (µmol / L)

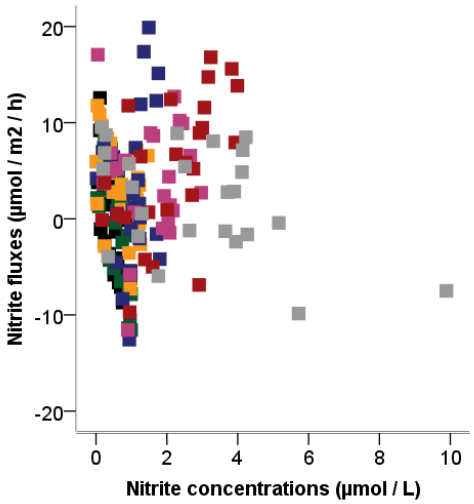
b)


Nitrate fluxes (µmol / m2 / h)
20
0
-20
-40
-60
-80
-100
-120
0
2
4
6
8
10
12
Nitrate concentrations (µmol / L)

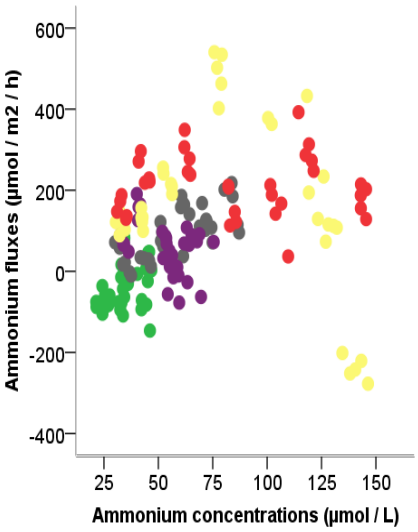
c)


Nitrite fluxes (µmol / m2 / h)
20
10
0
-10
-20
0
2
4
6
8
10
Nitrite concentrations (µmol / L)

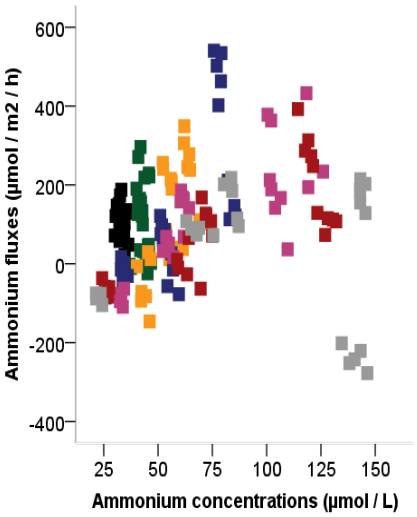
d)


Nitrite fluxes (µmol / m2 / h)
20
10
0
-10
-20
0
2
4
6
8
10
Nitrite concentrations (µmol / L)

e)


Ammonium fluxes (µmol / m2 / h)
600
400
200
0
-200
-400
25
50
75
100
125
150
Ammonium concentrations (µmol / L)

f)


Ammonium fluxes (µmol / m2 / h)
600
400
200
0
-200
-400
25
50
75
100
125
150
Ammonium concentrations (µmol / L)

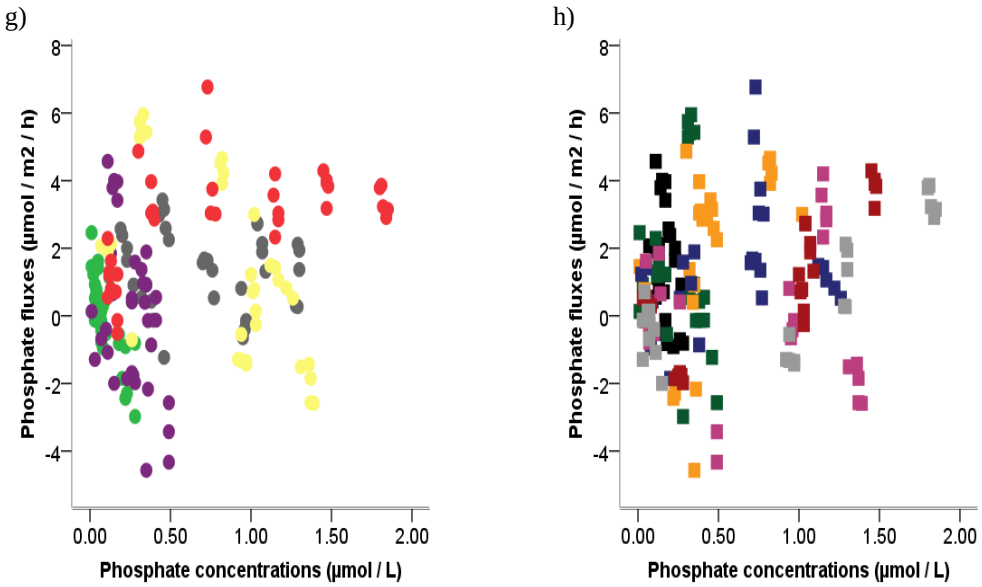


Figure 3.9 Relationships of nutrient concentrations to nutrient fluxes according to treatment (a, c, e and g) and day (b, d, f and h). Samples are reported as mean ± SE, n=5. Red circles - anoxic treatments, yellow circles - four days without oxygen and re-aerated for 3 days (4DA), grey circles - hypoxic conditions, purple circles - 1 day without oxygen and re-aerated for 6 days (1DA), green circles - oxic treatments. Black square – day 1, green square – day 2, orange square – day 3, blue square – day 4, pink square – day 5, red square-day 6, grey square-day 7.

3.5.3 Effect of Different Treatments on Porewater Nutrient Concentrations in the Sediment During 7 days of Laboratory Incubations

In general, the concentrations of porewater nitrate and nitrite were variable with depth, but distinct increasing patterns were observed in porewater ammonium and phosphate concentrations with depth (two-way ANOVA, $p < 0.0001$, depth effect in Figure 3.10 a - d).

A significant difference in the concentration of porewater nitrate was measured between the treatments (two-way ANOVA, $p < 0.0001$). There was a significant interaction between treatments and depth effects on porewater nitrate concentrations (two-way ANOVA, $p < 0.0001$). The significant interaction shows that the relationship with depth varied between treatments. Porewater nitrate concentration peaked at 1 cm depth in the oxic and initial treatments and decreased

with depth (Figure 3.10 a). Porewater nitrate concentrations decreased beyond 3 cm depth in the anoxic treatment. But, there was little change with depth in the hypoxic, 1DA and 4DA treatments. Porewater nitrate concentration is lower in the 4DA and anoxic treatments throughout the sediment depth than in the oxic, 1DA and hypoxic treatment (Figure 3.10 a). This pattern results from conversion of porewater nitrate to ammonium in the anoxic treatment (Figure 3.10 a and c). However, it is unclear whether porewater nitrate in the 4DA treatment is converted to porewater ammonium.

There were significant effects of treatment and depth on porewater nitrite concentrations (two-way ANOVA, $p < 0.001$, Figure 3.10 b). Porewater nitrite concentrations did not show major changes with depth but increasing concentration was observed at 5 cm depth in the 4DA treatments (Figure 3.10 b). Porewater nitrite concentrations decreased at 9 cm depth in the 1DA, hypoxic and 4DA treatments. Porewater nitrite concentrations also decreased beyond 5 cm depth in the anoxic treatments (Figure 3.10 b). The patterns observed in the hypoxic, 1DA, 4DA and anoxic treatments at 9 cm depth; suggest nitrite reduction to ammonium in the porewater of these treatments.

Porewater ammonium concentrations significantly increased from initial values, particularly below 3 cm depth in all treatments. The significant increase shows extensive ammonium accumulation due to organic matter mineralisation.

Porewater phosphate concentrations increased extensively below 5 cm depth in the hypoxic, 4DA and anoxic treatments. Porewater phosphate concentrations remained fairly constant in the oxic and 1DA treatments (Figure 3.10 d). The pattern may be explained by the release of phosphate that is sorbed with iron (III) in low oxygen.

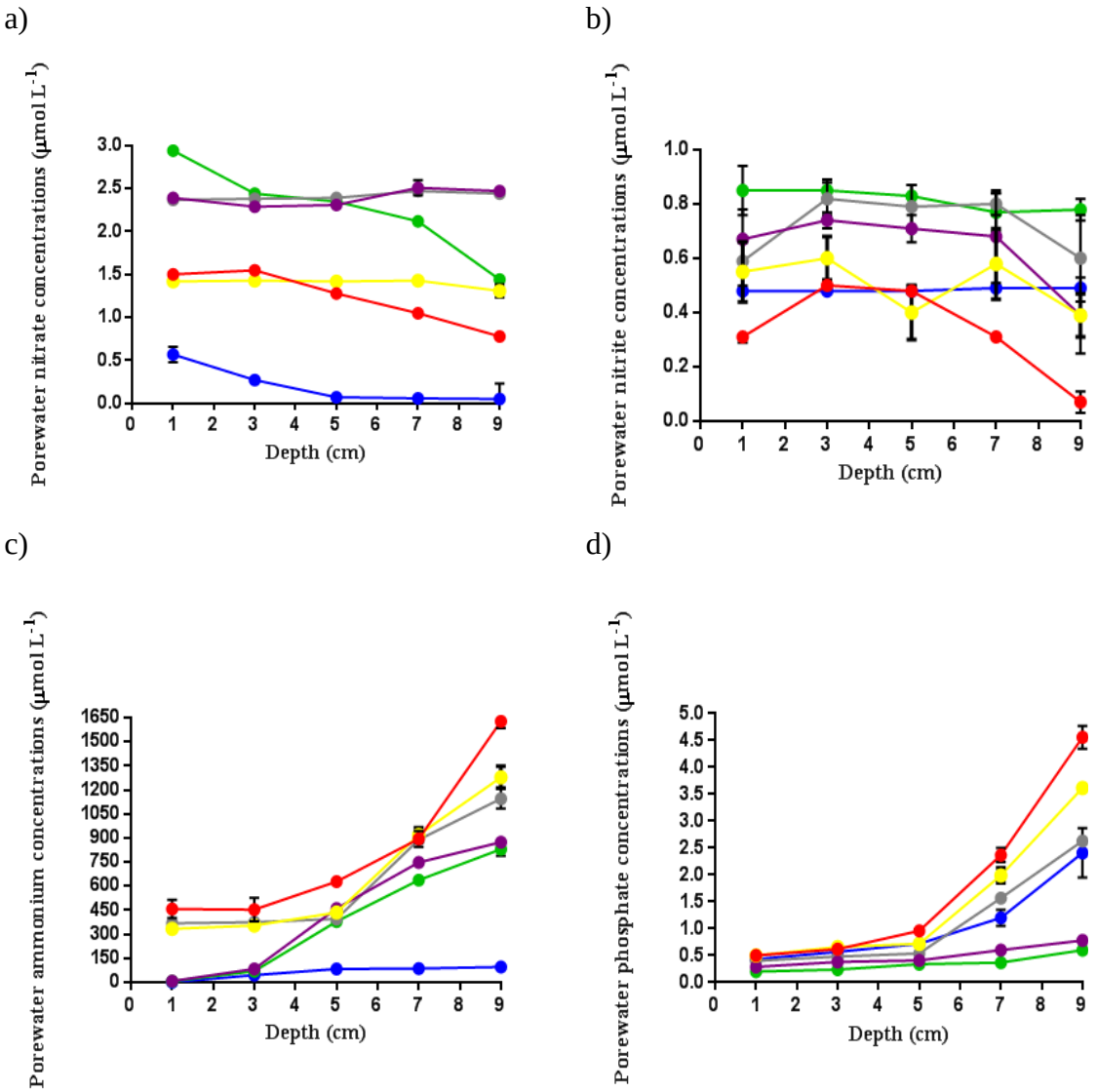


Figure 3.10 Porewater a) nitrate, b) nitrite, c) ammonium and d) phosphate concentrations taken at the start and on day 7 of laboratory incubations from five different treatments, oxic, hypoxic, 1DA, 4DA and anoxic . Values are reported as mean ± SE, n=5. Red circles - anoxic treatments, yellow circles - four days without oxygen and re-aerated for 3 days (4DA), blue circles - initial treatments, grey circles - hypoxic conditions, purple circles - 1 day without oxygen and re-aerated for 6 days (1DA), green circles - oxic treatments.

3.5.4 Effect of Different Treatments on Nutrient Concentrations and Porewater Nutrient Concentrations During 7 days of Laboratory Incubations

The water column nitrate concentrations in the oxic, hypoxic, 1DA, 4DA and anoxic treatment are similar (Figure 3.11 a). But, porewater nitrate concentration is the highest in the oxic treatment followed by the 1DA, hypoxic, 4DA and anoxic treatments (Figure 3.11 a). The highest porewater nitrate concentration in the oxic treatment might imply nitrification whilst low porewater nitrate concentration in the anoxic treatment might suggest conversion into ammonium (Figure 3.11 c).

Low nitrite concentrations occur in both the overlying water and porewater. Porewater nitrite is the highest in the oxic and 1DA treatments relative to those in the anoxic treatment. This might suggest porewater nitrite conversion to porewater ammonium in the 4DA and anoxic treatment (Figure 3.11 b).

Ammonium has the highest concentration in comparison to the other nutrients in both the overlying water and porewater (Figure 3.11 c). Ammonium is highest in the porewater of the 4DA and anoxic treatments due to organic matter mineralisation. Low ammonium concentrations in the oxic and 1DA treatment may imply nitrification.

Low phosphate concentrations in both the overlying water and porewater in the oxic and 1DA treatment is due to the buffering mechanism of iron oxides (Figure 3.11 d). Higher porewater phosphate concentrations in the hypoxic, 4DA and anoxic treatment relative to those in the oxic and 1DA treatment might be due to the reduction mechanism of insoluble $FePO_3^-$ into soluble $Fe_3(PO_4)^2$ (Figure 3.11 d).

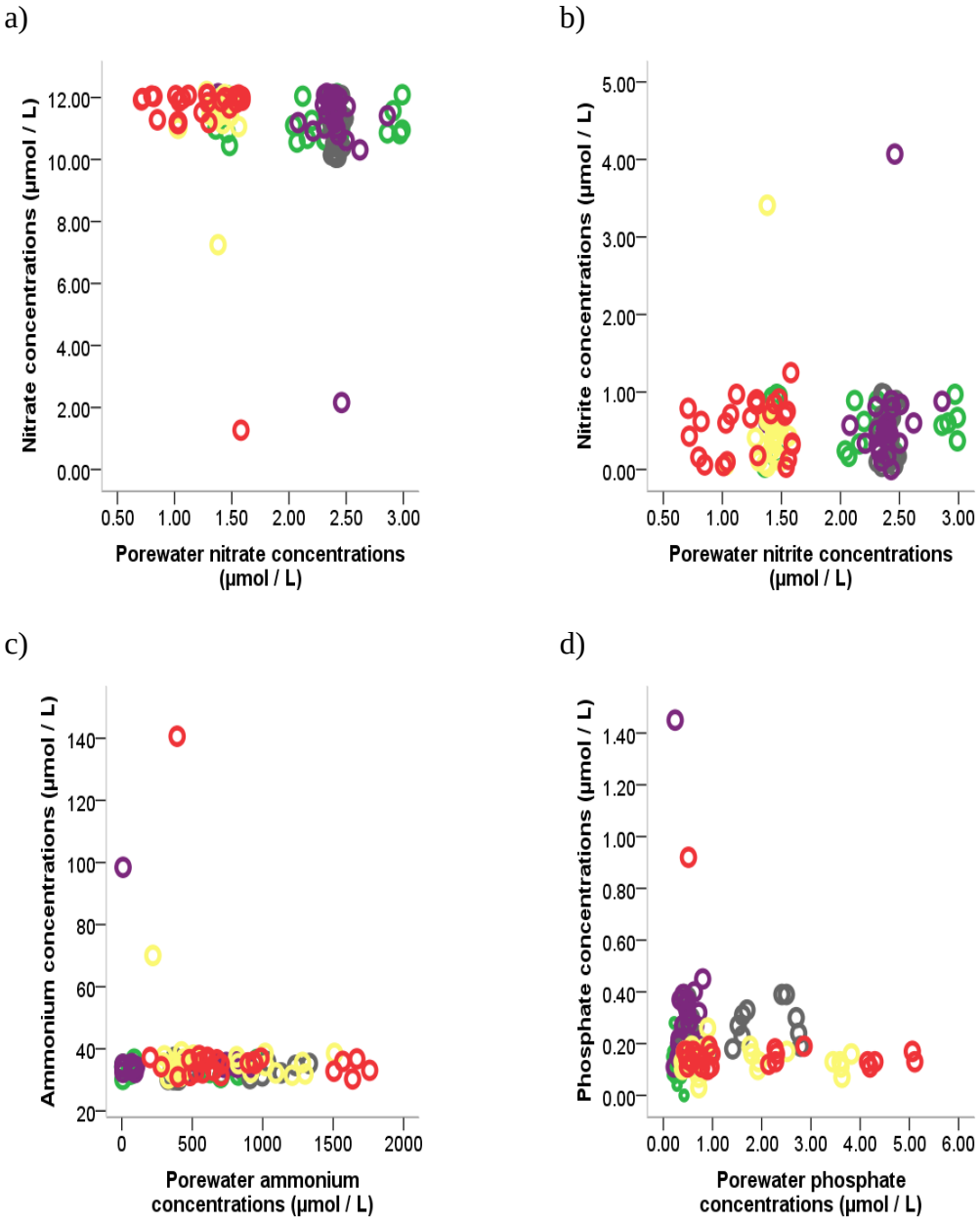


Figure 3.11 Relationship of a) nitrate and porewater nitrate, b) nitrite and porewater nitrite, c) ammonium and porewater ammonium and d) phosphate and porewater phosphate according to treatments. Red circles - anoxic treatments, yellow circles - four days without oxygen and re-aerated for 3 days (4DA), grey circles - hypoxic conditions, purple circles - 1 day without oxygen and re-aerated for 6 days (1DA), green circles - oxic treatments.

3.5.5 Effect of Different Treatments on Porewater Diffusive Fluxes in the Sediment During 7 Days of Laboratory Incubations

The porewater diffusive fluxes were calculated from the porewater concentration gradients in Figure 3.10 a –d. The calculated diffusive fluxes are all very low (Figure 3.12 a - h), because of the low porewater concentrations in the sediments (Figure 3.10 a - d). An exception was for ammonium, which has high porewater concentrations (Figure 3.10 c) and not surprisingly had the highest diffusive fluxes (Figure 3.12 e). Overall, the data variability in the diffusive fluxes as well as the non-significant diffusive fluxes limits the strength of the conclusion that can be derived from this result.

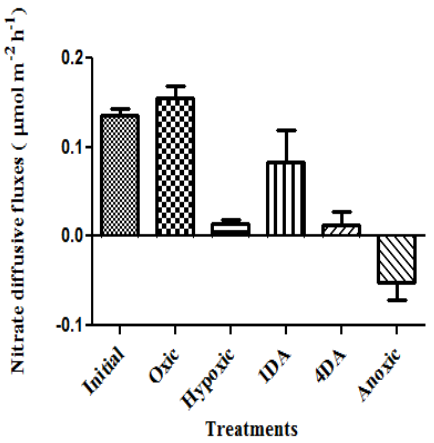
Porewater nitrate diffusive fluxes were directed into the overlying water in all except the anoxic treatments (one-way ANOVA, $p < 0.001$, Figure 3.12 a). Porewater nitrate diffusive fluxes were significantly lower in the hypoxic, 4DA and anoxic treatments than in the oxic and initial treatment (Tukey $p< 0.001$). Lower porewater nitrate diffusive fluxes in the hypoxic, 4DA and anoxic treatments could indicate decrease nitrification. This is consistent with the conversion of nitrate to ammonium from the fluxes inferred from concentration changes in the overlying water of these treatments (Figure 3.6 a – c).

Porewater nitrite, ammonium and phosphate diffusive fluxes show fluxes directed to the sediments in all treatments in Figure 3.12 c, e and g (one-way ANOVA, $p<0.001$). No significant differences were observed for porewater nitrite and ammonium diffusive fluxes between the different treatments (Tukey, $p > 0.05$; Figure 3.12 c and e).

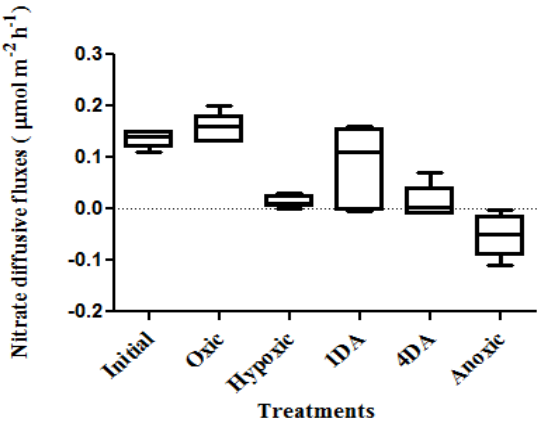
Phosphate diffusive fluxes were significantly lower in the oxic and 1DA treatments and higher in the 4DA and anoxic treatments compared to initial. Phosphate diffusive flux directed into the sediment could be the consequence of adsorption on iron (III) oxyhydroxides, which might explain the pattern in the oxic and 1DA treatments (Figure 3.12 g). Phosphate diffuses into the sediment in the 4DA treatment due to re-aeration. Re-aeration may cause phosphate binding to iron (III)

oxyhydroxides. Box plots of the diffusive fluxes (Figure 3.12 b, d, f and h) summarise the variability across the dataset.

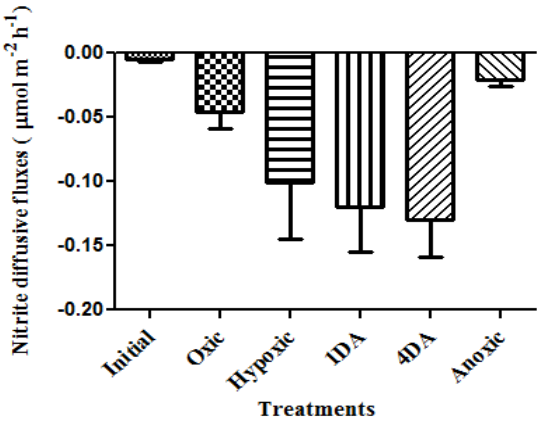
a)



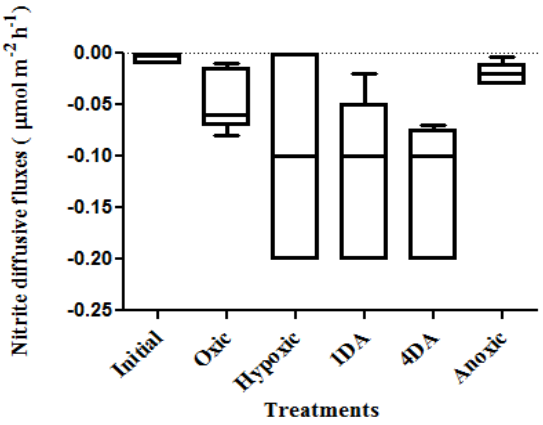
b)



c)



d)



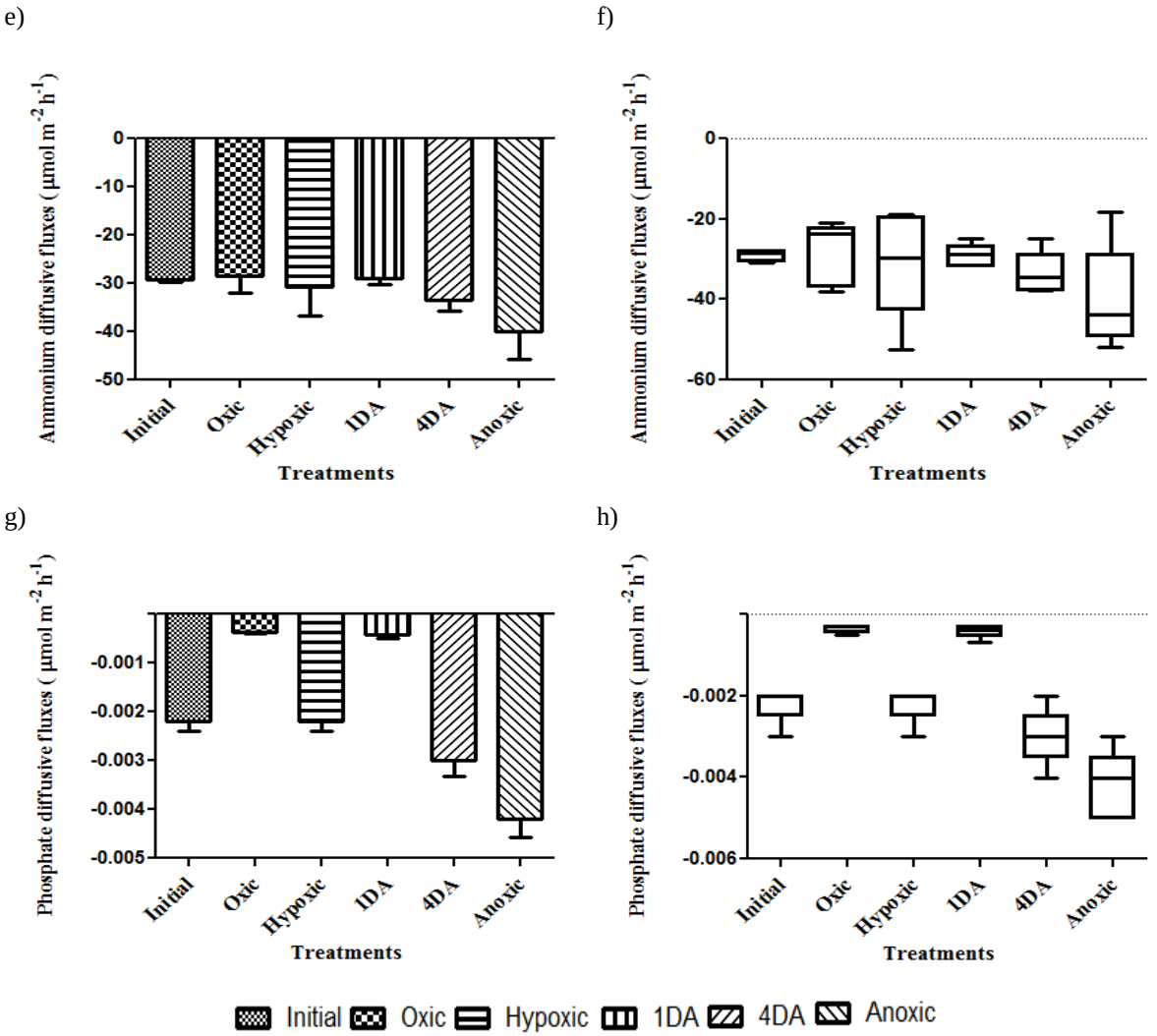


Figure 3.12 Porewater diffusive fluxes of a) nitrate, c) nitrite, e) ammonium and g) phosphate calculated from concentration gradients in the cores at the start and on day 7 of laboratory incubations from 5 different treatments, oxic, hypoxic, 1DA, 4DA and anoxic, calculated from porewater concentration gradients. b, d, f and h) Box whisker plot; central line in each box indicates median, ends of box 25th and 75th percentiles, error bars and points. Values are reported as mean ± SE, n=5.

3.5.6 Comparison between Measured Nutrient Fluxes and Calculated Diffusive Fluxes

In general, DIN fluxes were dominated by ammonium fluxes in the overlying water and ammonium diffusive fluxes in the sediments than other nitrogen species (Figure 3.6 c and 3.12 e).There was a small contribution of nitrate and nitrite diffusive fluxes to the overall DIN flux. Similarly, small fluxes were measured for phosphate in the overlying water and the porewater (Figure 3.6 d and 3.12 g).

Apparent disagreement was observed between the measured nutrient fluxes in the overlying water and the calculated porewater diffusive fluxes (Figure 3.6 a-d and 3.12 a-g). The pattern and direction of the fluxes were variable, making it difficult to interpret. The inconsistency might be influenced by biological and chemical effects dominating the treatments. The diffusive fluxes showed variable patterns, suggesting that surface processes occurring at the sediment-water interface may determine the extent and direction of the fluxes.

3.6 Enzyme Activities in the Sediment Taken at the Start and on Day 7 of Laboratory Incubations

All enzyme activities decreased significantly with depth and were influenced by treatments (two-way ANOVA, $p < 0.001$, for both depth and treatment effect, Figure 3.13 a-e). Significant interactions between treatment and depth were found for AP, CB and chitinase (two-way ANOVA, $p < 0.001$). This results from higher enzyme activities at the surface of oxic and 1DA treatments relative to those at depths compared with the initial pattern (e.g. AP, CB and chitinase activities). As for BG, activities at the sediment surface and 3 cm depths in the 4DA and anoxic treatment were significantly lower than those measured at the beginning of the incubation. In contrast, urease activities in the 4DA and anoxic treatments were significantly higher than at the start of the incubation at all depths except at 9 cm depths (Bonferroni corrected, $p < 0.001$).

AP was strongly correlated with porewater phosphate concentrations (Figure 3.14 a - b). This relationship might be due to a response to porewater phosphate concentrations or to overall bacterial activity, which in turn is controlled by the availability of oxygen. Anoxia elevates porewater phosphate concentrations as observed in the deepest part of the anoxic treatment. AP is also low near the surface of the anoxic treatment and decreased with depth. Hence, it is more likely that this significant inverse relationship is due to a response to porewater phosphate concentrations as oxygen concentration is low throughout the anoxic treatment.

BG, CB and chitinase activities were consistently high on the surface and decreased with depth (Figure 3.13 b – d). This pattern indicates the presence of labile organic matter on the surface, which supports high microbial activity. Microbial activity reduces with depth as the more labile components were broken down.

Urease activities were inversely correlated with porewater ammonium concentration (Figure 3.14 c-d). Urease activities explained 38% of the variance of porewater ammonium concentrations when fitting the relationship using simple linear regression. Figure 3.14 c-d shows that high porewater ammonium concentrations were associated with low urease activities particularly at the deepest part of the sediment. This relationship suggests that urease might be partly inhibited by porewater ammonium concentrations. Urease activities were also positively correlated with TOC as shown in Figure 3.14 e-f. This relationship suggests that bacteria with urease enzymes are enhanced by TOC. On the other hand, high urease activities at the sediment surface of anoxic treatments might be due to the death of aerobic benthic organism. The availability of organic matter is enhanced with the death of these organisms as a food source for anaerobic bacteria with urease activities. Potentially, the response of urease is associated with the influence of both porewater ammonium concentrations and TOC in the sediment.

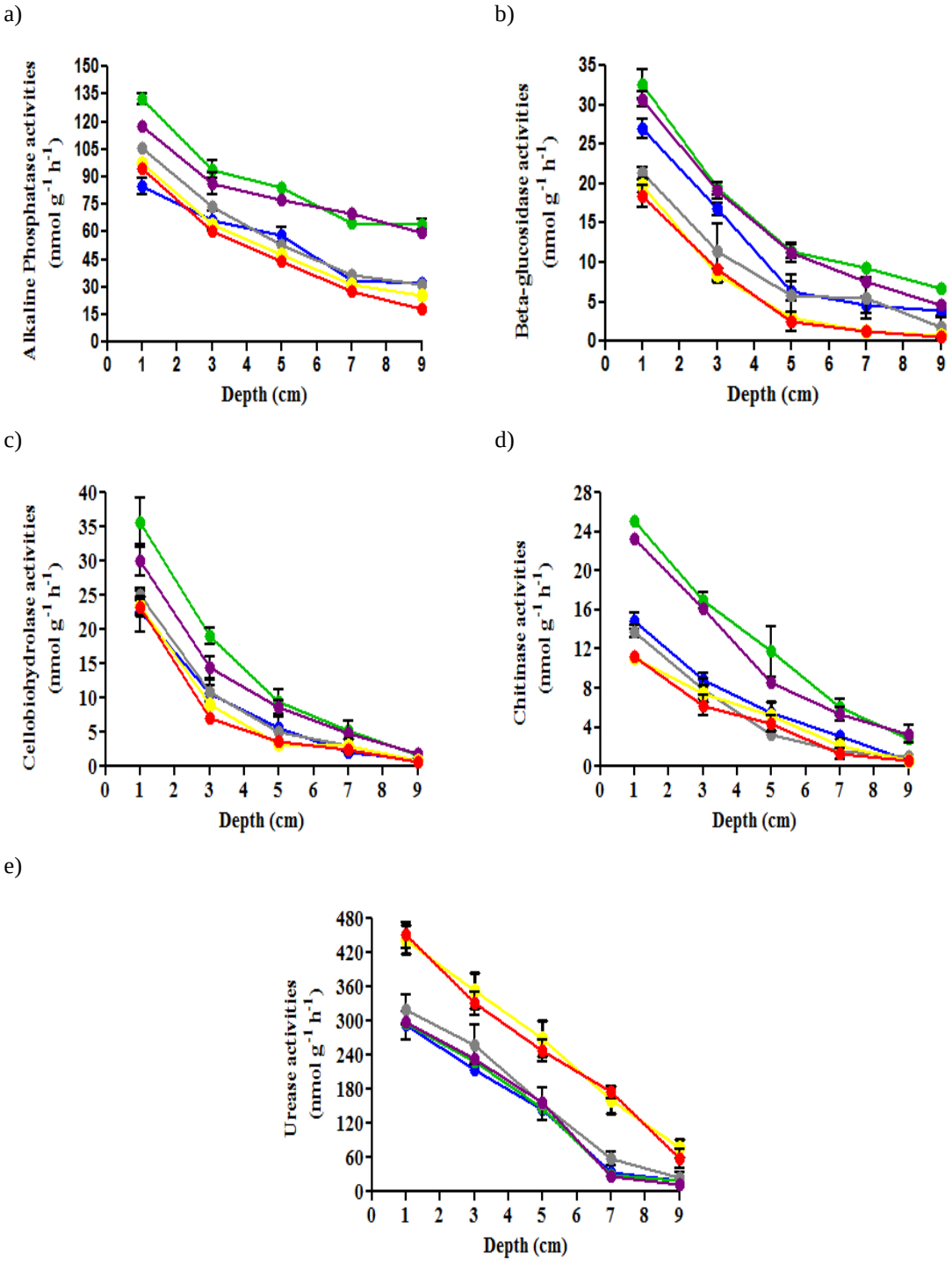


Figure 3.13 Vertical profiles of potential enzyme activities of a) alkaline phosphatase, b) cellobiohydrolase, c) beta-glucosidase, d) chitinase and e) urease activities in the sediments exposed to different oxygen concentrations taken at the start and on day 7 of laboratory incubations, reported as mean ±SE, n=5. Red circles - anoxic treatments, yellow circles - four days without oxygen and re-aerated for 3 days (4DA), blue circles - initial treatments, grey circles - hypoxic conditions, purple circles - 1 day without oxygen and re-aerated for 6 days (1DA), green circles - oxic treatments.

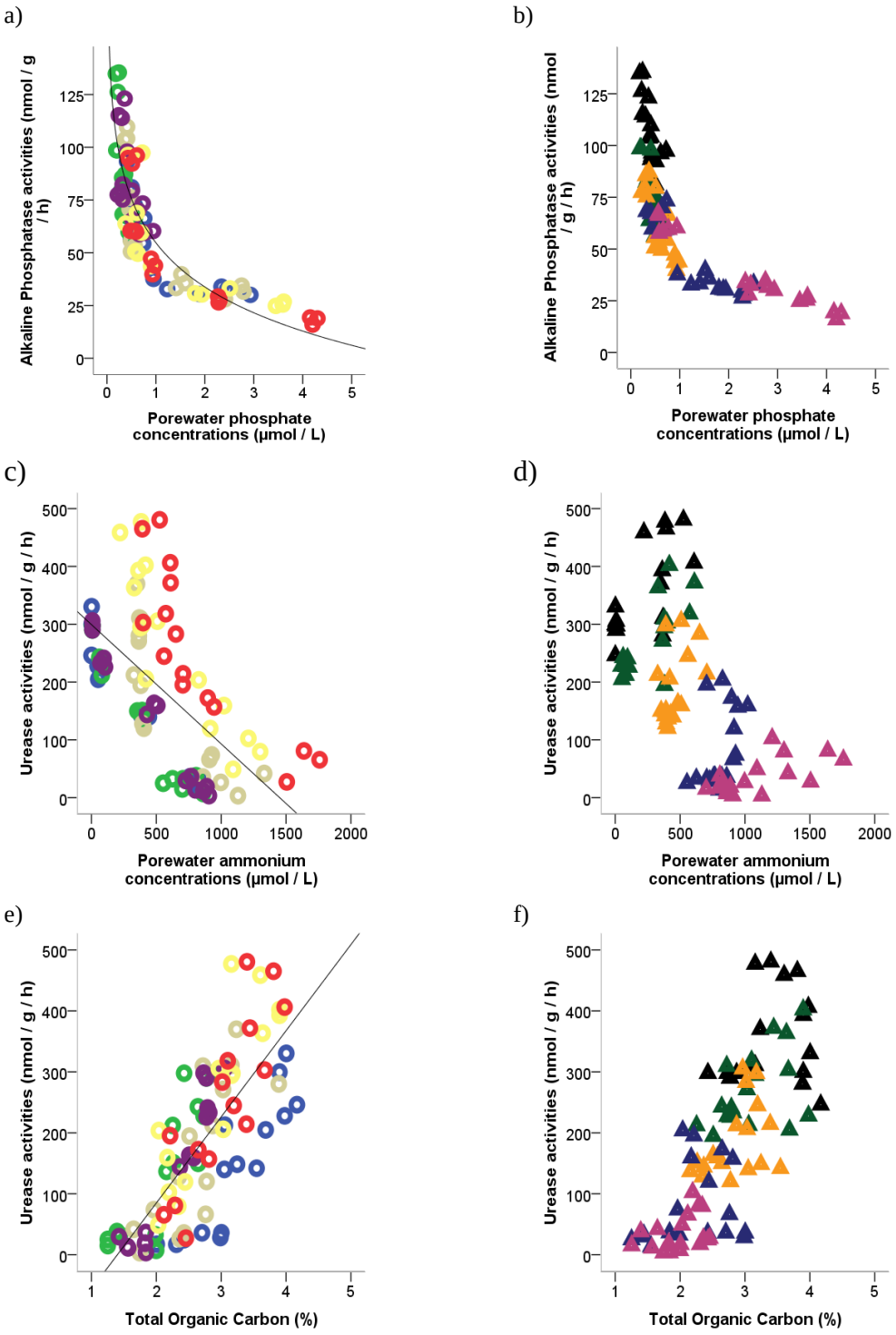


Figure 3.14 Scatterplots of AP and porewater phosphate concentrations according to different a) treatments and b) depths, urease and porewater ammonium concentrations according to different c) treatments and d) depths, and urease with TOC according to different e) treatments and f) depths, reported as mean ±SE, n=5. Red circles - anoxic treatments, yellow circles - four days without oxygen and re-aerated for 3 days (4DA), blue circles - initial treatments, grey circles - hypoxic conditions, purple circles - 1 day without oxygen and re-aerated for 6 days (1DA), green circles - oxic treatments. Black triangles - 1 cm depth, green triangles – 3 cm depth, orange triangles – 5 cm depth, blue triangles – 7 cm depth, pink triangles – 9 cm depth.

3.6.1 Interrelationships between Parameters in the Sediment

PCA was used to investigate the relationship between porosity, total organic carbon (TOC), total nitrogen (TN), porewater ammonium concentrations, alkaline phosphatase activities (AP), cellobiohydrolase activities (CB), beta-glucosidase activities (BG), chitinase and urease activities measured in the sediments. The first two components explained 86.8% (component 1 70.2%, component 2 16.6%) of the variability observed in the sediments data. Table 3.8 shows the component matrices of factor loadings for each of the original variables.

Factor 1 was strongly correlated with increasing CB, BG, chitinase, AP, porosity and urease; and decreasing porewater ammonium concentration. TOC and TN were positively correlated with both factor 1 and 2. Figure 3.15 shows a plot of factor 1 against factor 2 with data points identified by treatments and by sediment depths.

In general, both plots show the sequence of each treatments from anoxic: 4DA: initials: hypoxic: 1DA and oxic (Figure 3.15 a). The anoxic and 4DA treatments are grouped together and surprisingly initial treatments followed closely to the 4DA treatments. Oxic treatments are clustered together with 1DA treatments, implying the effect of 1 day without oxygen to the physico-chemical characteristics, porewater ammonium concentrations and enzyme activities closely resemble those in oxygenated conditions. Enzyme activities also clearly varied with depths (Figure 3.15 b).

AP, BG, CB and chitinase activities were highly correlated with one another and their activities increased with increasing oxygen concentrations (for example in Figure 3.16 a, c and e). But, urease activities showed a weaker correlation with other enzymes and inversely related to oxygen concentrations (e.g. figure 3.16 e). The oxic treatment leads to increased AP, BG and chitinase activity relative to initial values (e.g. Figure 3.16 a and c). In contrast, urease activities shifted to higher activities in the anoxic treatments relative to initial values (e.g. Figure 3.16 e). The consistent pattern for AP, BG, CB and chitinase is a shift of enzyme activities with organic

matter from the surface to increasing depth. AP, CB and chitinase activities were high at the sediment surface and generally decreased with depth, suggesting a decrease in substrate quality with depth (e.g. Figure 3.16 a-h). CB activities were weakly related to the TOC (Figure 3.16 g-h). Chitinase activities were inversely related to porewater ammonium concentrations, suggesting its function in the nitrogen cycle. Porosity decreases with depth because of sediment compaction and is correlated with other parameters largely due to the influence of depth.

Table 3.8 Component matrices for principal component analysis of sediments in the study by extracting eigenvalues of greater than 0.6 and above and suppressing factor loadings of less than 0.4.

Variable	Component	
	1	2
Cellobiohydrolase	**.917**	
Beta-glucosidase	**.895**	
Chitinase	**.888**	
Alkaline phosphatase	**.872**	-.420
Porewater ammonium concentrations	**-.865**	
Porosity	**.862**	
Urease	**.833**	
Total Nitrogen	.689	.627
Total Organic Carbon	.683	.651

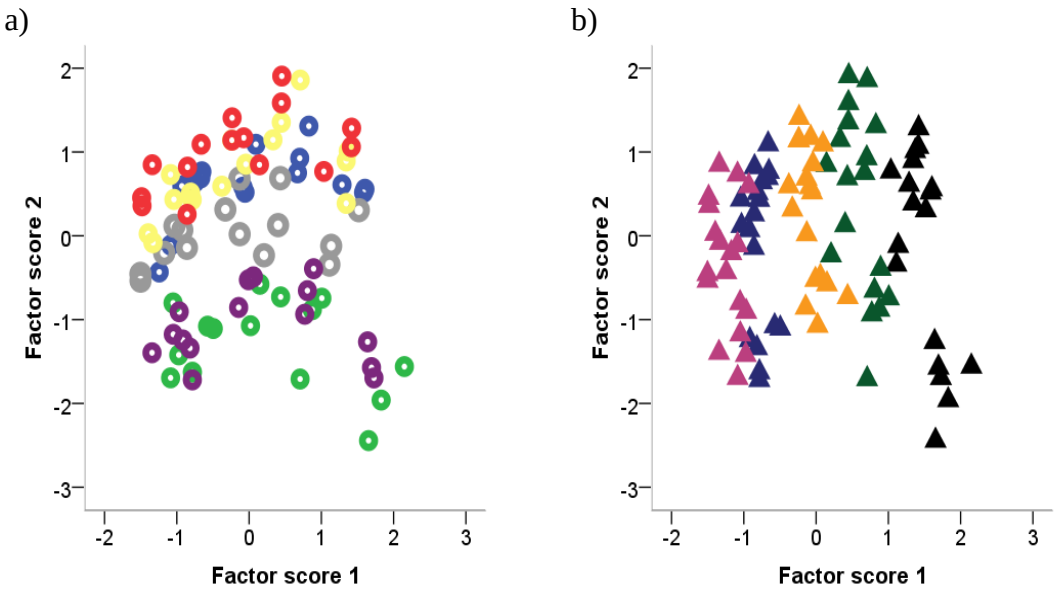
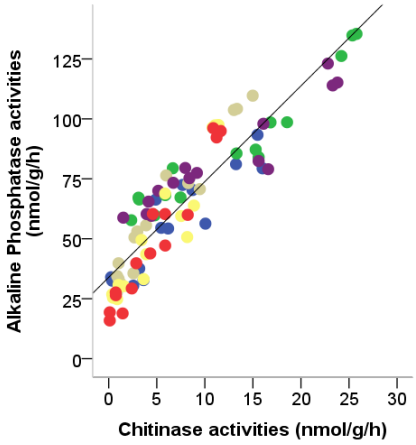
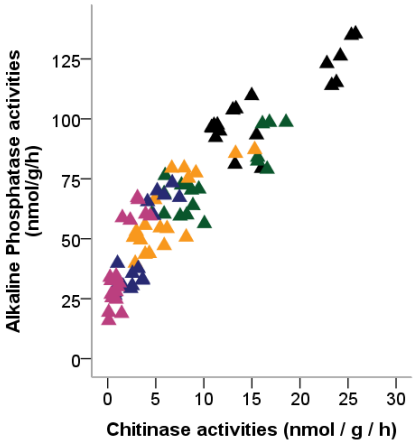


Figure 3.15 Scatterplots of the a) principal component analysis of porosity, total organic carbon, total nitrogen, porewater ammonium concentrations, alkaline phosphatase activities, cellobiohydrolase activities, beta-glucosidase activities, chitinase activities and urease activities identified by a) 5 different treatments and b) sediment depths measured. Red circles - anoxic treatments, yellow circles - four days without oxygen and re-aerated for 3 days (4DA), blue circles - initial treatments, grey circles - hypoxic conditions, purple circles - 1 day without oxygen and re-aerated for 6 days (1DA), green circles - oxic treatments. Black triangles - 1 cm depth, green triangles – 3 cm depth, orange triangles – 5 cm depth, blue triangles – 7 cm depth, pink triangles – 9 cm depth.

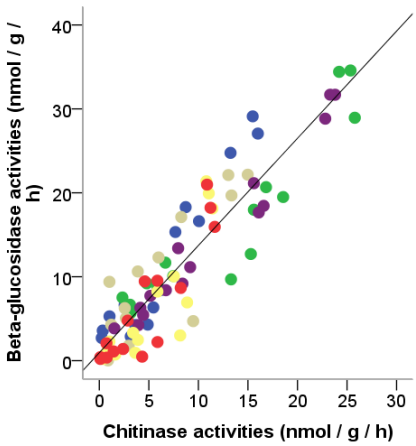
a)


Alkaline Phosphatase activities (nmol/g/h)
0
25
50
75
100
125
0 5 10 15 20 25 30
Chitinase activities (nmol/g/h)

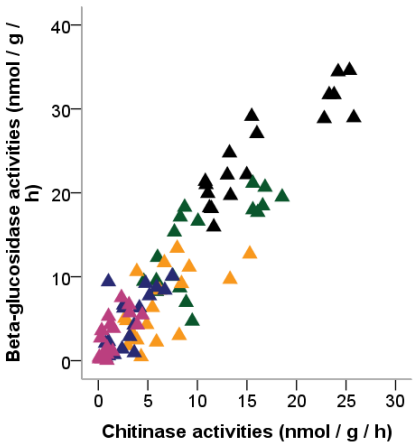
b)


Alkaline Phosphatase activities (nmol/g/h)
0
25
50
75
100
125
0 5 10 15 20 25 30
Chitinase activities (nmol / g / h)

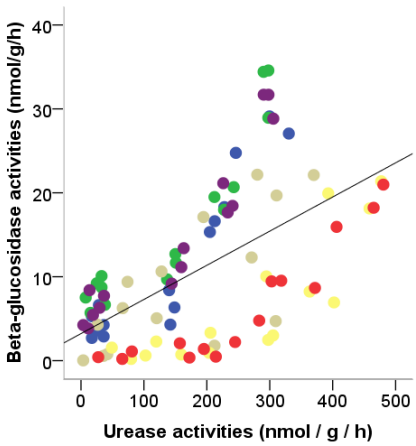
c)


Beta-glucosidase activities (nmol / g / h)
0
10
20
30
40
0 5 10 15 20 25 30
Chitinase activities (nmol / g / h)

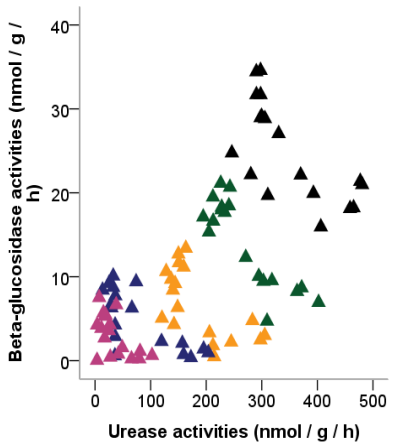
d)


Beta-glucosidase activities (nmol / g / h)
0
10
20
30
40
0 5 10 15 20 25 30
Chitinase activities (nmol / g / h)

e)


Beta-glucosidase activities (nmol/g/h)
0
10
20
30
40
0 100 200 300 400 500
Urease activities (nmol / g / h)

f)


Beta-glucosidase activities (nmol / g / h)
0
10
20
30
40
0 100 200 300 400 500
Urease activities (nmol / g / h)

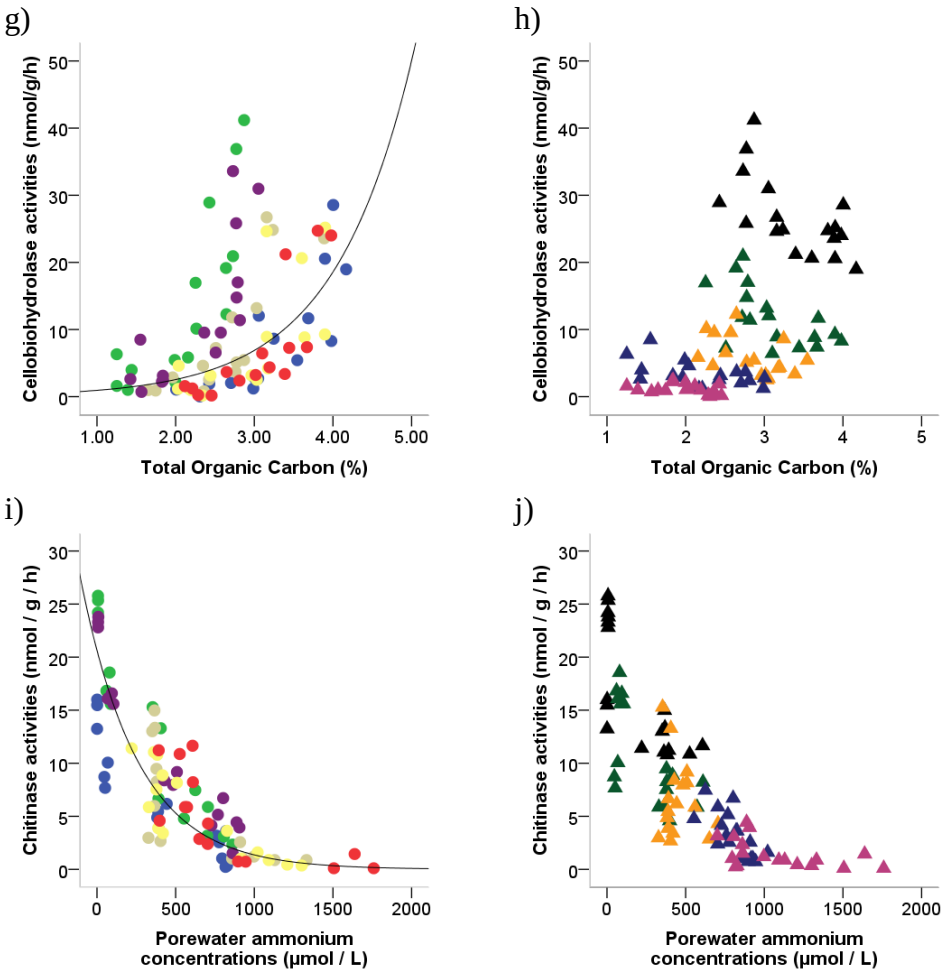


Figure 3.16 Relationship between chitinase and AP according to a) treatments and b) depth, between chitinase and BG according to c) treatments and d) depth, BG and urease according to e) treatments and f) depth, CB and TOC according to g) treatments and h) depth , chitinase and porewater ammonium concentrations according to i) treatments and j) depth. Red circles - anoxic treatments, yellow circles - four days without oxygen and re-aerated for 3 days (4DA), blue circles - initial treatments, grey circles - hypoxic conditions, purple circles - 1 day without oxygen and re-aerated for 6 days (1DA), green circles - oxic treatments. Black triangles - 1 cm depth, green triangles – 3 cm depth, orange triangles – 5 cm depth, blue triangles – 7 cm depth, pink triangles – 9 cm depth.

CHAPTER 4 DISCUSSION

4.1 Introduction

The aim of Chapter 4 is to discuss the results and interpretation of the data presented in Chapter 3. Integration of all parameters measured was done to investigate the underlying mechanisms involved in each treatment. This chapter begins with the significance and limitations of the methodology selected in Chapter 2 and continue with a general assessment of the sampling area. The discussion is then narrowed down to processes responsible for the patterns inferred from the nutrients and enzyme activities in each treatment.

4.2 Methodological Considerations

Before embarking on a thorough discussion of the data, it is worth discussing the significance and the limitations of the experimental technique selected. Laboratory incubation of sediment cores rely on careful sediment extraction and transport to laboratory (Mortimer et al., 1998). Cores are overlain with a specific volume of water and samples are taken at set intervals during the incubation period (Tengberg et al., 2003; Jenkins, 2005). Laboratory incubations of sediment cores was selected in this study as cores need to be aerated and bubbled with nitrogen to induce different levels of anoxia and hypoxia. Removal of the cores from the estuary does lead to some disturbance of the cores, but for muddy sediments which only have to be transported a short distance to the laboratory, disturbance is small.

Enhanced solute transport in excess of molecular diffusion is commonly calculated using a 1-dimensional and non-steady state mass conservation equation (Forster et al., 1995; Berg et al., 2001). Solute transport models are often used to simulate the exchange of solutes between sediment and water and solute transport within sediment inhabited by benthic macroorganisms (Matisoff and Wang, 1998). The generally accepted models are the enhanced diffusion model, the cylindrical burrow irrigation model and the nonlocal exchange model (Martin and Banta, 1992; Berg et al., 2001; Meile et al., 2005). But, the limitation of our data prevented calculation of enhanced diffusion coefficient irrigation models, which requires

information about the burrows (Meile et al., 2005). Therefore, we opted to use the difference between measured fluxes and diffusive fluxes as the irrigation fluxes and to compare the differences between oxic and oxygen depleted treatments (section 2.7.1).

Using tracers that occur in the field can be a useful technique, as it follows natural sediments and benthic communities to be studied undisturbed. This in turn may provide a more realistic picture of the processes occurring. Some previous studies have used naturally occurring isotopes, ^{234}Th, ^{7}Be, and ^{210}Pb to study downward mixing (Berg et al., 2001; Shull and Mayer, 2002). But, this is done to assess particle mixing in the sediment. *In situ* measurements are again preferable compared to laboratory incubations (Forster et al., 1999). But, these require specific and complex instrumentation and the variability between replicates can cause problems of interpretation (Bradshaw et al., 2006). Another problem is that the short half lives of some of these isotopes requires measurement to be made immediately, which was not possible in this study. Moreover, our focus on the experimental manipulation of the effects of different concentrations of oxygen would be very difficult to carry out in the field. Adding bromide to the system being studied creates an artificial set-up in which conditions are different to those found in the field, but this allowed full control of the experimental design and the use of multiple replicates and controls.

For the estimation of oxygen diffusive fluxes from the concentration gradient (section 2.8.1), the linear concentration change in the diffusive boundary layer (DBL) is commonly used because there is no need to have data on sediment porosity (Tengberg et al., 2004). However, the DBL procedure has experimental uncertainties due to the difficulty of determining the exact position of the sediment-water interface which is not always obvious. In this study, the concentration gradient was taken from the upper millimetre of the sediment and the porosity data extrapolated using polynomial curve fitting to get porosity measurement in millimetre scale (section 2.8.1).

Measurement of nutrient fluxes from sediments to the overlying water can be carried out either by *in-situ* benthic chambers, concentration gradients in sediment pore waters or through laboratory incubations of sediment cores (Hammond et al., 2004; Jenkins, 2005; Macreadie et al., 2006). However, it should be noted that any measurement of fluxes will only give the estimation of the ability of the sediment to release or take up the nutrients as they require a containment of water overlying the sediments. This therefore does not mimic the dynamic continuous physical movement of water over the mudflats, with periods of drying out and inundation of water. The chemical changes in water overlying the sediments may also alter fluxes. Despite these limitations, measurement of nutrient fluxes is useful as a basis to understand nutrient biogeochemistry. Moreover, the vast majority of studies of fluxes in the literature are of this type (e.g. Liu et al., 2003; Rasheed et al., 2006; Belias et al., 2007) facilitating comparison of the data produced in this study.

Measurements of *in-situ* benthic fluxes involve placing enclosed sediment corers on the surface of the sediments at the sampling area containing both sediment and water (Haag et al., 2005; Wild et al., 2005). The variations in nutrient concentrations in the overlying water within the chamber are measured and used to infer nutrient flux. Benthic chambers are considered the best method for estimating nutrient flux since they involve few assumptions and minimal perturbation to the sediment-water interface. However, the downside of this method is that they are time consuming and expensive to construct. They also require logistically elaborate deployments with significant occupational hazard (Jenkins, 2005).

Nutrient concentration gradients across the sediment-water interface can be obtained by sampling sediment pore waters at discrete depths (Denis and Grenz, 2003). This concentration-depth data may then be used to calculate nutrient diffusive fluxes on the basis of Fick's Law of Diffusion (Lavery et al., 2001; Liu et al., 2003). Fick's First Law is used to model sediment-water nutrient flux, assuming that diffusion is the dominant transport mechanism and to predict those fluxes (Liu et al., 2003). According to this law, flux is proportional to the slope of the concentration gradient (Jenkins, 2005). In this study, the nutrient fluxes in the overlying water and porewater diffusive fluxes in the sediment were measured (section 2.9.4 and 2.10.1)

as comparisons between the two can provide information about the relation between fluxes at the sediment-water interface and nutrient cycling within the sediment column (Clavero et al., 2000).

Microplate fluorimetric and colorimetric methods were used for measurement of enzyme activities (section 2.13.1 and 2.13.2) because they allow large number of sediment samples and enzymes to be analysed within a short time (Wirth and Wolf, 1992; Marx et al., 2001). This microplate method also enables well-known colorimetric assays to be scaled down for high-throughput analyses (Allison et al., 2007). It also allows reaction product formation to be analysed straight away in the microplate using fluorometrically labelled substrates, without the need to extract and purify the product through centrifugation and filtration prior to analysis (Saiya-Cork et al., 2002).

However, a limitation in the use of substrate proxies lies in the competition for available enzyme between the MUB substrates and any naturally occurring substrates (Liu et al., 2008). In this study, the problem was minimised by conducting assays at saturation concentrations of the fluorometric substrate (section 2.11.2). Secondly, MUB substrates are dimeric, rather than polymeric, so the extent to which their enzyme hydrolysis is representative of that of natural biopolymers is uncertain. Nevertheless, this form of enzyme activity measurement is very valuable and has generated major advances in the understanding of the functioning of enzymes in various environments (German et al., 2011; De et al., 2012) and facilitates comparison of data across environments (Arnosti, 2011).

4.3 Sediment Physico-chemical Characteristics, Nutrients and Enzyme Activities of Breydon Water, Great Yarmouth

The sampling site of Breydon Water is adjacent to the North Sea (Figure 2.3), hence the saline water. The pH readings of the overlying water in Breydon Water (section 3.2) fall within the typical range for estuarine systems of 7.6 - 8.5 (Olausson and Cato, 1980; Hinga, 2002). The porosity of the sediment decreases with depth (Table 3.1) because of compaction and precipitation of diagenetic materials (Gluyas and

Coleman, 1992). Compaction happens when water is squeezed out, leaving denser solid particles within the sediments (Zonneveld et al., 2010). Initial carbon and nitrogen content in the sediment (Table 3.1) were high as it is typical for estuarine sediment (Hansen and Kristensen, 1998). TOC content in estuaries and productive ecosystems are in the range of 1 to 4% DW in areas where there are high organic matters content in sediments (Bianchi, 2007). High accumulation of organic matter on the surface of the sediment indicated high rates of mineralisation on the surface of fine grained sediment with less penetration deeper into the sediment except that due to the presence of invertebrates (Soetart et al., 1997). The CN ratio of 8.0 to 9.3 (Table 3.1) at the start of the incubations implies a mixture of both terrigenous and marine organic matter (Rasheed, 2004). An increase of CN ratio with depth down to 5 cm (Table 3.1) reflects the faster mineralisation of nitrogen than carbon (Kristensen and Blackburn, 1987; Meyers, 1994). This is also often linked to preferential loss of marine relative to terrigenous organic matter, which is often low in nitrogen (Hedges and Keil, 1995). The oxygen penetration depth of 4 mm in this study (Figure 3.4) is typical in coastal sediment compared to the centimetre or decimetre oxygen penetration depth in deep ocean sediments (Rasmussen and Jorgensen, 1992; Glud et al., 1994; Cai and Sayles, 1996; Mouret et al., 2010).

Nitrate concentrations are higher in the overlying water than the porewater (Table 3.5). This pattern illustrates that most of the regenerated nitrate was not consumed at the sediment-water interface, but was released by nitrification (Liu et al., 2003). Nitrite concentration was low in both the overlying water and porewater, suggesting conversion to nitrate and ammonium. The increase in porewater ammonium concentrations compared to porewater nitrate and nitrite concentrations, particularly from 3 to 9 cm depth, could be due to organic matter mineralisation processes (Kristensen, 2000; Al-Rousan et al., 2004; Jantti and Hietanen, 2012). Ammonium production deeper in the sediments could be through the process of ammonification, where nitrogen is incorporated into inorganic forms via microbially mediated reactions (Dong et al., 2009). The surface of the sediment was depleted of ammonium compared to the overlying water (Table 3.5), suggesting the consumption of ammonium near the sediment surface. The process of nutrient assimilation by diatoms might explain the rapid ammonium consumption near the sediment surface

as sources of nitrogen (Rasheed, 2004). It is also possible that availability of nitrogen from ammonium allows heterotrophs to break down organic carbon in the surface layers where there is oxygen available (Galloway et al., 2004). Ammonium is the preferable inorganic nitrogen to be assimilated over nitrate and nitrite by microphytobenthos, particularly diatoms (Canfield et al., 2010). The main reason is because less redox energy is required to incorporate its nitrogen into biomolecules, primarily amino acids and the nucleotide bases (Libes, 2009). The appearance of a diffusive boundary layer (Roy et al., 2002; Kelly-Gerreyn et al., 2005) from 0.5 mm to the sediment surface (Figure 3.4) might have changed porewater ammonium concentrations dramatically near the sediment surface. It should be noted that the depth resolution of 2 cm intervals might not have captured the extensive changes at the sediment surface. Phosphate concentrations increase with depth as a response to low dissolved oxygen in deeper sediment (Conley et al., 2009). With low oxygen, Fe (III) is reduced to Fe (II) releasing phosphate ions in the deeper sediment (Belias et al., 2007).

Alkaline phosphatase activity (AP) in the sediments ranges from 32.17 to 86.40 nmol g^{-1} h^{-1} (Figure 3.13 a), within the range reported for intertidal mudflats of the German Wadden Sea by Coolen and Overmann, (2000), in the Vellar Estuary, India (20 to 138 nmol $g^{-1}h^{-1}$) (Ayyakkannu and Chandramohan, 1971) and within the range of 2 to 70 nmol g^{-1} h^{-1} in the Venice Lagoon, Italy (DeGobbis et al., 1986). AP was inversely related to porewater phosphate concentrations (Figure 3.14 a-b) potentially because autrotroph need less AP when free phosphate concentrations is high (Jones, 2002; Zhou et al., 2008; Steenbergh et al., 2011; De et al., 2012).

ß-glucosidase (BG) activities of sediments in Breydon Water, Great Yarmouth (3.9 to 27.0 nmol g^{-1} h^{-1} in Figure 3.13 b), is similar to other studies conducted in intertidal marine sediments. King, (1986) reported BG activity of 23.2 nmol g^{-1} h^{-1} in intertidal sediments. In the sediments of a eutrophic lake, a study by Mallet et al., (2004) exhibited BG activity ranging between 1.0-26.1 nmol g^{-1} h^{-1}. Mallet and Debroas, (2001) has also shown BG activity of 30.7 nmol g^{-1} h^{-1} in superficial sediments of a eutrophic lake.

To date, only one previous study has been conducted measuring cellobiohydrolase (CB) activity in lake sediments (Hakulinen et al., 2005). The majority of measurements of CB activity are in forest soils (Saiya-Cork et al., 2002; DeForest, 2009) and agricultural soils (Niemi and Vepsäläinen, 2005). So, this study is the first attempt to measure CB in estuarine sediments (Figure 3.13 c). Initial CB activities were between 1.0 and 22.7 nmol g^{-1} h^{-1} from sediments of Breydon Water, Great Yarmouth. Comparison with other systems showed CB activity was lower from this study, reflected by different sampling location and sources. Hakulinen et al., (2005) reported CB value of 410 nmol g^{-1} h^{-1} in Lake Uurainen and 60 nmol g^{-1} h^{-1}in Lake Jamijarvi, Finland. The higher values in this lake sediment reflected a forested catchment lake as the main source of cellulose degradation. A study by Sanaullah et al., (2011) in a grassland site, France reported CB activities ranging from 10 to 23 µmol g^{-1} h^{-1}, a thousand fold higher than the activities reported in this study. This is expected since grassland provides more cellulose inputs.

Chitinase activities in sediments (Figure 3.13 d) were relatively low compared with those of other enzymes. This may be due to the heavy seaweed growth, *Ulva* sp. which covered the mudflats at the time of sampling as this will input organic matter that does not contain chitin to the sediments (section 2.3). It may also be that the sediment contains other carbon sources than chitin. LeCleir and Hollibaugh, (2006) has reported chitinase activity in sediments of a salt lake, Mono Lake of 3.3-81.1 nmol g^{-1} h^{-1} and 137-874 nmol g^{-1} h^{-1} for 4°C and 20°C incubations, respectively which were contributed by high numbers of *Artemia* in the lake and the chitin rich exuvia they produce during the maturation process. Bacteria capable of chitin hydrolysis were generally rare in estuarine and marine environment (Kollner et al., 2012). This is probably related to a high availability of very diverse food substances, many of which can be metabolised more easily than chitin (Podgorska and Mudryk, 2003).

Urease activity was detected from 1 to 9 cm depth, recording the highest in the upper sediment (313 nmol g^{-1} h^{-1}) and decreasing with depth by a factor of 10 (Figure 3.13 e). The method used in detecting urease activity was adapted from the protocol commonly used in soils of forest systems (Sinsabaugh et al., 2000; Saiya-

Cork et al., 2002; Sinsabaugh et al., 2005). Urease activities were between 700 to 2960 nmol g^{-1} h^{-1} in four English river sediment; River Lathkill, River Wye (Derbyshire), River Swale and Porter Brook in Sheffield (Duddridge and Wainwright, 1982). A study by Sabil et al., (1995) on urease activity in Venice Lagoon sediment reported values of 1140 to 4400 nmol g^{-1} h^{-1} using a spectrophotometric method.

Surprisingly, the overall pattern of enzyme activities in initial treatment resembles the pattern observed in the 4DA treatment (Figure 3.15 a-b). What might be happening? Initial sampling was carried out during high oxygen saturation of 75 % which was different from the 4DA treatment. Possibly, this reflects the rapid changes of microbial communities during the early stage of sediment handling prior to transferring to the cold room. The vertical profiles of AP, CB, BG, chitinase and urease activity were consistently higher in the presence of high TOC at the sediment surface (Figure 3.14 e and Figure 3.16 g) and decreased in deeper sediments with decreasing organic matter (Figure 3.16 a-h). In the sediments, the highest enzyme activity occurred in the 0-5 cm surface layer and decreased rapidly with depth (King, 1986). High inputs of organic matter are followed by enhanced microbial activity of heterotrophic bacteria (Cavari and Hadas, 1979), inducing the synthesis of enzymes (Riadas and Pinkas, 1997).

4.4 pH in the Overlying Water and Total Organic Carbon (TOC) and Total Nitrogen (TN) in the Sediment under Five Different Treatments

The pH response showed an increase of 0.2 units, equivalent to an average difference of 4 nmoles L^{-1} hydrogen ion concentrations in the anoxic treatments relative to the oxic treatment (Figure 3.1). If we examine hydrogen ion concentrations, a big difference was observed between the anoxic and oxic treatments which might be overlooked if data are presented as pH units. Nevertheless, both these measurements showed a move towards more alkaline pH in the anoxic treatment. The more alkaline pH in anoxic condition does not cause major physiological or biogeochemical changes in the treatments and does not relate to enhanced acidification problems (Howarth et al., 2011). A decline of 0.2 units may cause physiological changes to the

calcifiers or non-calcifiers inhabiting the system (Mucci et al., 2011). It is likely that the ecological and biogeochemical consequences of anoxia are much larger than the effects of a change of 0.2 pH units.

TOC and TN showed decreasing patterns with depth (Figure 3.2 a and b). This pattern reflects reduced organic matter lability with depth (Souza, 2009). All treatments showed rapid usage in sediment TOC and TN compared to the initial condition (Figure 3.2 a and b). This implies that up to half of the organic matter is broken down in 7 days, even at depths well below the depth of oxygen penetration. During organic matter degradation, organic carbon and nitrogen are remineralised (Kristensen, 2000). This leads to a decreasing TOC and TN with depth until non-degradable fraction remains. But, degradation of this proportion of the organic matter is unlikely to occur over a timescale of only 7 days. Presumably, the rapid use of organic carbon reflects various benthic organisms and microorganisms depending on the limited organic carbon source since primary production by photoautotrophs is inhibited in the closed dark incubation.

The highest loss of TOC and TN at all depths in the sediment (1 to 9 cm depth) is in the oxic treatment at the end of the 7 day laboratory incubation (Figure 3.2 a and b). This would imply enhanced preservation of organic carbon in the anoxic relative to the oxic treatment, although the difference is quite substantial to occur within only 7 days. Lower degradation of TOC and TN in the anoxic treatments (Figure 3.2 a and b) can be related to restricted mixing (Lee, 1992) as shown by less exchange of Br in Figure 3.3 in the anoxic treatments and possibly, lack of oxygen. Presumably, the lower decrease of TOC in the anoxic treatment compared to the oxic treatment may be caused by the destruction of macrofauna and the disruption of the microbial loop via changes in bacterial grazers (Bianchi et al., 2000). In the absence of grazers who feed on bacterially derived organic matter, thus organic carbon may be less efficiently remineralised and preferentially preserved (Burdige, 2007). Bioturbation and the macrofaunas contribution to the catabolism of sedimented organic matter are also absent (Burdige, 2007).

The CN ratio in the oxic treatments at 7 cm depth (Figure 3.2 c) is closest to the Redfield ratio of 6.6, that implies labile organic carbon (Heilskov and Holmer, 2001) but this is not possible at 7 cm depth where the sediment is less likely exposed to rapid changes and the sediment have been deposited at that depth for quite some time. Higher CN ratio in the hypoxic and anoxic treatment, suggests a shift to refractory organic matter relative to the oxic treatment, but organic matter diagenesis would not happen in such a short timescale. A decrease of CN ratio suggests preferential organic matter processing of carbon compared to nitrogen, presumably due to the consumption of carbon by heterotroph and incorporation of nitrogen during degradation of organic matter (Meyers, 1997; 2003). Overall, this is a paradox and we could not point out the reason for what is happening. The TOC and TN pattern require further study, perhaps by measuring sulphate reduction rates, the microbial communities or isotopic carbon and nitrogen composition and biomarkers of carbohydrate, amino acid etc to get a better picture.

4.5 Nutrient Concentrations and Measured Nutrient Fluxes in the Overlying Water under Five Different Treatments

The present study demonstrates the importance of oxygen in regulating the nitrogen and phosphorus cycles. This section begins by explaining the possible underlying mechanisms involved in nutrient concentrations and nutrient fluxes. Secondly, section 4.6 assesses the relationship of nutrient concentrations and porewater nutrient concentrations; thirdly, section 4.7 covers the relationship of porewater nutrient concentrations and porewater diffusive fluxes, as well as nutrient fluxes and porewater diffusive fluxes; fourthly, section 4.8 explains the patterns observed in the enzyme activities and fifthly, attempts to relate between nutrients and enzyme activities. The relationships between bromide, oxygen fluxes, TOC and TN in the treatments are also discussed as these variables influence nutrient exchange and enzyme activities.

The following discusses the processes influencing nutrient concentrations and nutrient fluxes in each treatment. In the overlying water of oxic treatments, there was a loss of 2 μmol L^{-1} nitrate concentrations simultaneous with an increase of 4 μmol L^{-}

[1] nitrite concentrations (Figure 3.5 b and c). This pattern shows a gain of 2 μmol L^{-1} nitrite concentration, implying nitrate reduction to nitrite. At the same time, ammonium concentrations increase at first, then decreases after day 2. By day 7, 8 μmol L^{-1} ammonium concentration was loss, which may be partly due to ammonium oxidation (nitrification) from ammonium to nitrite (Camargo and Alonso, 2006; Bianchi, 2007; Beman et al., 2008), explaining the gain in nitrite concentrations. In terms of total nitrogen, nitrogen is lost from the overlying water. The decrease of ammonium concentrations in the overlying water (Figure 3.5 d); causes a decrease of ammonium flux (Figure 3.6 c), into the sediment. Nitrite flux was also directed into the sediment by the end of the experiment in the oxic treatment (Figure 3.6 b). The direction of the fluxes is a response of improved oxygen supply in the treatments, indicating nitrification (Rasheed et al., 2006; Belias et al., 2007).

In the hypoxic treatment, the decreasing nitrate concentrations in the overlying water was consistent with moderately increasing nitrite and ammonium concentrations during the 7 days experiment (Figure 3.5 b-d). This pattern shows that nitrate reduction to nitrite and ammonium was carried out (Camargo and Alonso, 2006; Piehler and Smyth, 2011). Simultaneous nitrite and ammonium was released into the overlying water. Nitrate flux was directed into the sediment in the hypoxic treatment (Figure 3.6 a-c). This pattern shows how the concentration of both nitrites and ammonium under hypoxia is regulated by the availability of nitrates (Belias et al., 2007). This process appears to be an important sink of nitrate in coastal and estuarine sediments (Kemp et al., 2009; Washbourne et al., 2011).

Our re-aeration experiment revealed contrasting responses between the 1DA and 4DA treatments. The small increase of nitrate towards the end of the experiment in the 1DA treatment (Figure 3.5 b) indicates that some nitrification from nitrite to nitrate was occurring (Figure 3.5 b-c). Re-aeration caused ammonium to be oxidised to nitrate and nitrite through nitrification (Belias et al., 2007; Canfield et al., 2010). The increase of nitrite and decrease of ammonium after day 3 in the 1DA treatment (Figure 3.5 c and d) resulted from ammonium oxidation to nitrite in an aerobic environment (Madsen, 2008). The increase of nitrite concentrations over time

(Figure 3.5 c) is consistent with the nitrite flux out into the overlying water in the 1DA treatment (Figure 3.6 b).

After re-aeration in the 4DA treatment, a slight increase in nitrite concentrations was measured in combination with low nitrate concentrations (Figure 3.5 b and c). This pattern indicates that the system does not recover quickly from anoxia. Continuously increasing ammonium concentrations were observed (Figure 3.5 d) despite the system being continuously aerated during the last 3 days of the experiment. This may result from continuing organic matter decomposition in anoxic conditions (Kemp et al., 2005; Prokopenko et al., 2011). It could also be that it takes some time for the ammonium to diffuse out of the sediments as observed from the direction of the ammonium diffusive flux into the sediment in the 4DA treatment (Figure 3.12 e). Higher nitrate flux into the sediment in the 4DA treatments on day 2 (Figure 3.6 a) is consistent with the decreasing nitrate concentrations in the overlying water (Figure 3.5 b). This pattern was probably caused by increased nitrate reduction to ammonium in the sediment as a response to oxygen depletion (Larson and Sundback, 2008; Caffrey et al., 2010). Nitrate release into the overlying water was observed in the 4DA treatment at the end of the experiment (Figure 3.6 a). This release was coupled with ammonium flux into the sediment (Figure 3.6 c) which could indicate that nitrifying bacteria were unable to meet their ammonium demand from the sediment (Soetart et al., 2006). A flux of nitrogen into the sediment would enable bacteria in the sediment to take up nitrogen from the porewater.

In the anoxic treatment, very low nitrate and nitrite concentrations were coupled with progressively increasing ammonium concentration (Figure 3.5 b-d). This pattern indicates the dominance of nitrate and nitrite reduction into ammonium (Belias et al., 2007; Jantti and Hietanen, 2012). Ammonium release to the overlying water (Figure 3.6 c) is commonly observed in anoxic condition (Neubacher et al., 2011; Hietanen et al., 2012).

In the oxic treatments, the decreasing pattern of phosphate over time may be attributed to the high removal of phosphate at the oxic sediment-water interface. At this sediment-water interface, phosphate can be bound to the surface of carbonate

grains of the sediments (Rasheed, 2004). Re-aeration after 1 day of anoxia (Figure 3.6 d) was probably sufficient for the capture of released phosphate back to the sediment surface. This was also evident from the quickly decreasing concentration of phosphate in the water column in this treatment (Figure 3.5 e). Similar sorption of phosphate from the water column to the sediment during a positive redox-shift in lab experiments has been described by Hupfer and Lewandowski, (2008). After the shift from anoxic to oxic conditions, the sediment started to oxidise and bind phosphate more efficiently (Kemp et al., 2005; Hietanen and Lukkari, 2007; Jordan et al., 2008). In anoxic conditions, phosphate is continuously released due to iron mobilisation at the sediment-water interface (Golterman, 2001; Hupfer and Lewandowski, 2008; Middelburg and Levin, 2009).

Overall, distinct patterns of nutrient response in each treatment were observed (Figure 3.7 a-b). Nitrite increases in oxic conditions as a result of ammonium oxidation to nitrite (Figure 3.9 c and e) whilst nitrate decreases through nitrate reduction to nitrite. Hypoxia caused a continuous increase of ammonium through nitrate reduction (Conley et al., 2009; Bianchi et al., 2010). Under reducing conditions, ammonium increases (Hietanen et al., 2012). The re-aeration experiment showed that lower ammonium concentrations were observed in the 1DA treatment in comparison to the hypoxic treatment (Figure 3.5 d and 3.9 e). The lower ammonium concentrations in the 1DA treatment might be due to the re-oxygenation, which leads to nitrification (Conley et al., 2007). The extensive increase of ammonium in the 4DA treatment closely resembled the anoxic treatment (Figure 3.5 d); indicating increased ammonification of organic matter in the sediment (Conley et al., 2007). The hypoxic and anoxic treatments led to an increase of phosphate concentration in the overlying water as phosphate bound to iron oxides is released when iron (III) is reduced (Hietanen and Lukkari, 2007). Low phosphate concentrations in the oxic and 1DA treatments (Figure 3.9 g) reflect phosphate adsorption or coprecipitation with iron (III) in oxic conditions (Rasheed et al., 2006).

Figure 3.8 a-b shows the relationship between dissolved oxygen and nutrient fluxes. When oxygen saturation decreases, nitrate fluxes were into the sediment, indicating that nitrate production by nitrification was low relative to nitrate removal

by processes such as denitrification (Caffrey et al., 2010). In contrast, at high oxygen saturation, nitrite fluxes were into the overlying water (Figure 3.9 c), reflecting oxidation of ammonium to nitrite. Large release of ammonium and phosphate to the overlying water in low oxygen condition (Figure 3.9 e and g) indicates that the sediments in short term anoxic treatments act as a source of ammonium and phosphate to the system, processes that would exacerbate eutrophication problems in the field (Kemp et al., 2009).

4.6 Nutrient Concentrations and Porewater Nutrient Concentrations in the Sediment under Five Different Treatments

A framework to integrate all the parameters measured in this study is proposed in Figure 4.1 a-e. This section refers to the framework to link between nutrient and porewater nutrient concentrations in each treatment. As the overlying water consisted of nutrient free artificial seawater at the start of the laboratory incubations, the nutrients measured in the overlying water were clearly released from the sediment to the overlying water (Figure 3.5 b-e and Figure 3.11 a-d). Extensive exchange of overlying water and porewater in the sediment is shown by the vertical profile of porewater bromide concentrations (Figure 3.3). The fluxes of bromide was measured as a tracer of overlying water and porewater exchange on the assumption that lack of oxygen will cause the activity of benthic infauna to cease and thereby limit exchange to that accomplished by molecular diffusion.

The present study indicates that the process responsible for enhanced water column and porewater exchange is irrigation. In the oxic treatment, bromide irrigation flux was 937 μmol m^{-2} h^{-1} compared to the lower bromide diffusive flux of 537 μmol m^{-2} h^{-1} (Table 3.3). Vertical profiles of bromide also showed the deepest bromide exchange up to 9 cm depth in the oxic treatment (Figure 3.3). The higher irrigation flux in the oxic treatment, suggests that biologically driven sediment irrigation occurs when the burrows of benthic infauna, which are filled with overlying seawater exchange solutes with porewaters; are flushed frequently due to the infaunas activities. During the field sampling and porewater extraction from the sediment of this study, the ragworm, *Nereis diversicolor* were observed. So, most

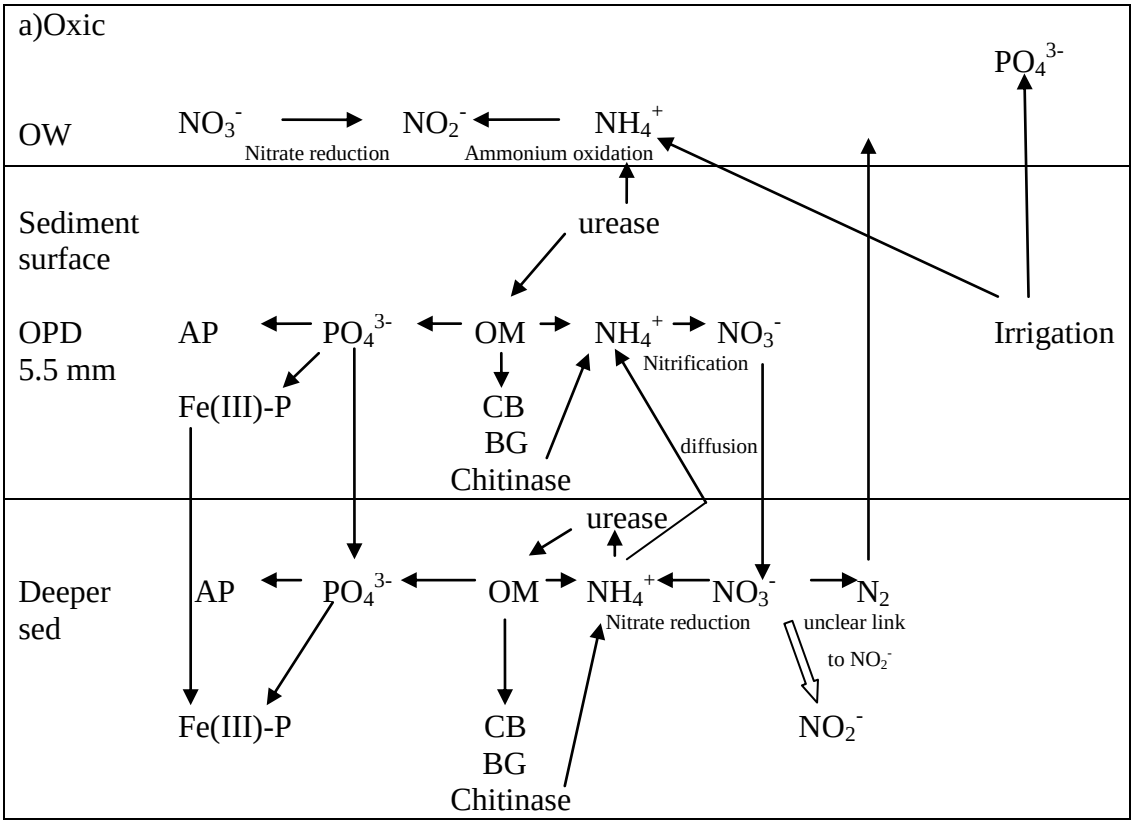
probably higher irrigation was related to the presence of this polychaete. But, further study is needed on the exact number of the worms to get a concrete picture. Hedman et al, (2011) have reported that irrigation caused by the movement of benthic invertebrates can cause physical transport of bromide enhancing overlying water exchange with porewater.

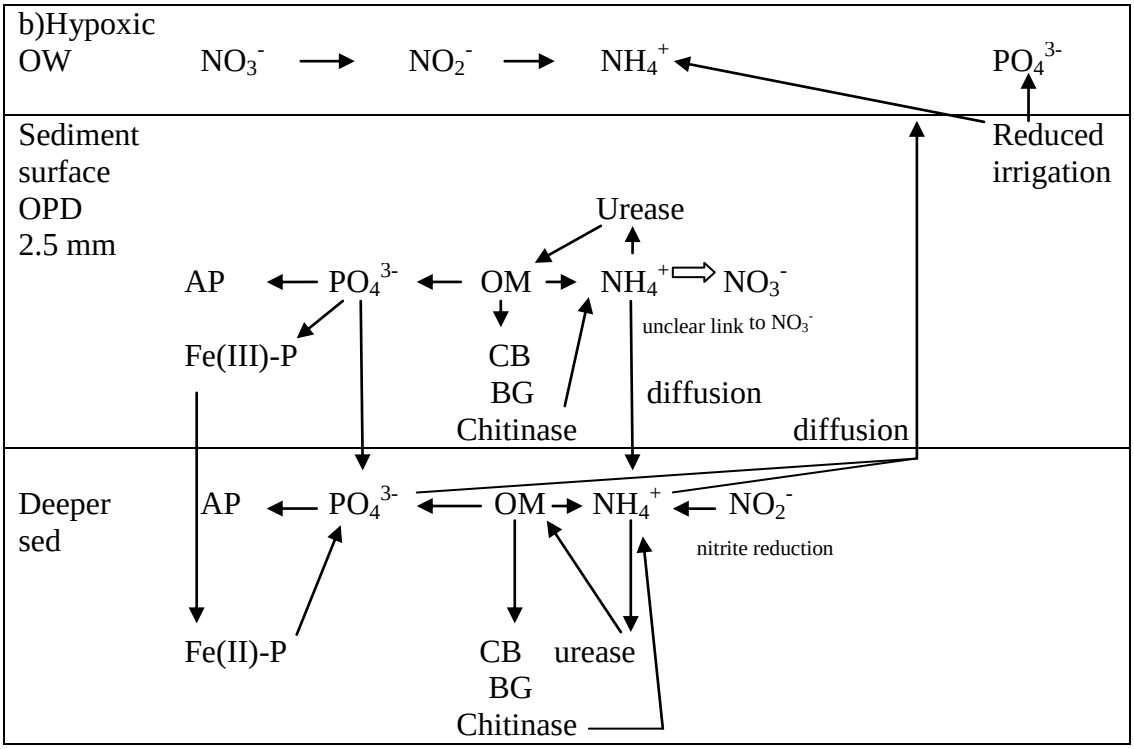
Extensive irrigation between the overlying water and porewater can have an effect to the diagenetic reactions by transporting oxygen deeper (Green et al., 2002; Quintana et al., 2007). This is consistent with oxygen penetration depth (OPD) in the oxic treatments increasing from 4 mm to 5.5 mm after 7 days of laboratory incubations (Figure 3.4). A significantly higher diffusive oxygen flux in the oxic treatment (Table 3.4) could be related to bacterial activity in the sediment (Rowe et al., 2008), using oxygen for organic matter degradation and for oxidation of reduced compounds (Giles et al., 2007; Mouret et al., 2010). Figure 4.1 a shows the link between irrigation and OPD in the oxic treatment.

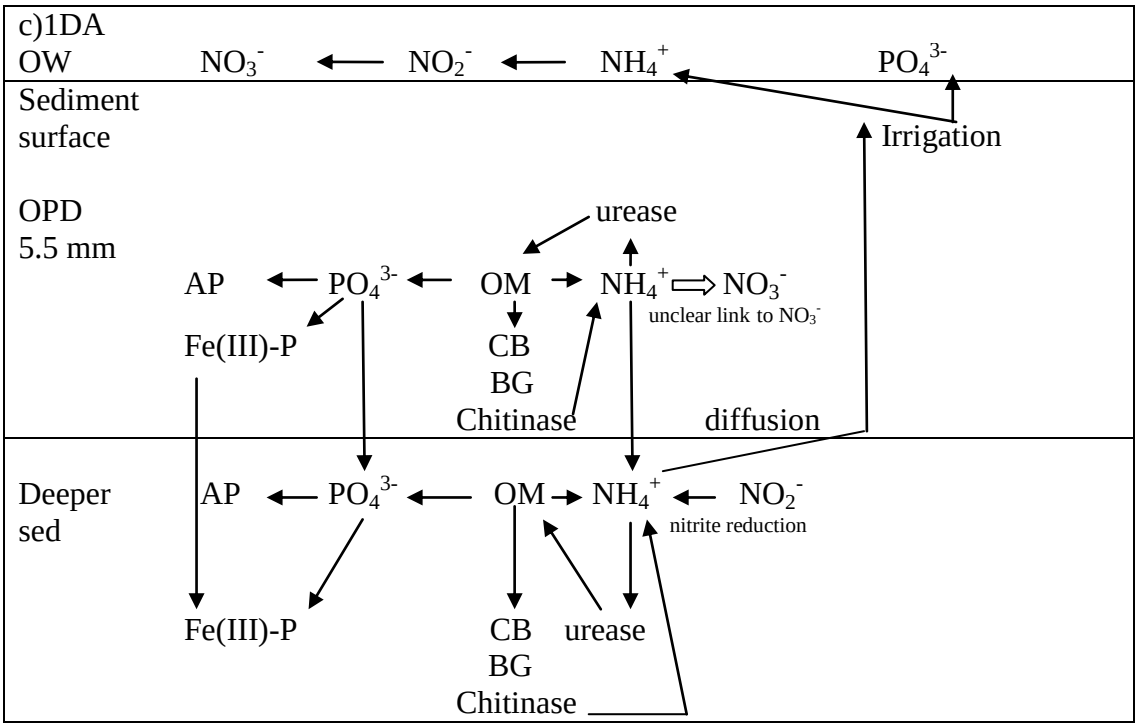
Nitrification occurs in the oxidised sediment surface (Lavery et al., 2001) as shown in the upper sediment surface of oxic sediments (Figure 4.1 a). Porewater ammonium profiles show increasing concentration with depth as it is produced from the degradation of organic matter (Mortimer et al., 1998). Below the oxic zone, porewater nitrate decreases with depth. This decrease indicates nitrate conversion into porewater ammonium concentrations through the process of nitrate reduction in the deeper sediment (Figure 4.1 a). Porewater ammonium in the deeper sediment diffuses upward and was consumed near to the sediment surface. Porewater nitrate in the deeper sediment might undergo denitrification and be released as nitrogen gas from the sediment. Nitrate generated in nitrification is reduced in surrounding anaerobic zones via denitrification to gaseous nitrogen (Kemp et al., 2009). But, there is an unclear relationship of nitrate to nitrite in the porewater of the sediment (Figure 3.10 a, Figure 3.11 a-b and Figure 4.1 a). Where has nitrite come from in the porewater? Nitrite is the intermediate in both nitrification and denitrification and therefore fluxes are difficult to interpret, since these processes are often closely coupled in marine sediments (Dong et al., 2009). Irrigation released ammonium to the overlying water (Figure 4.1 a). Ammonium release from the sediment is

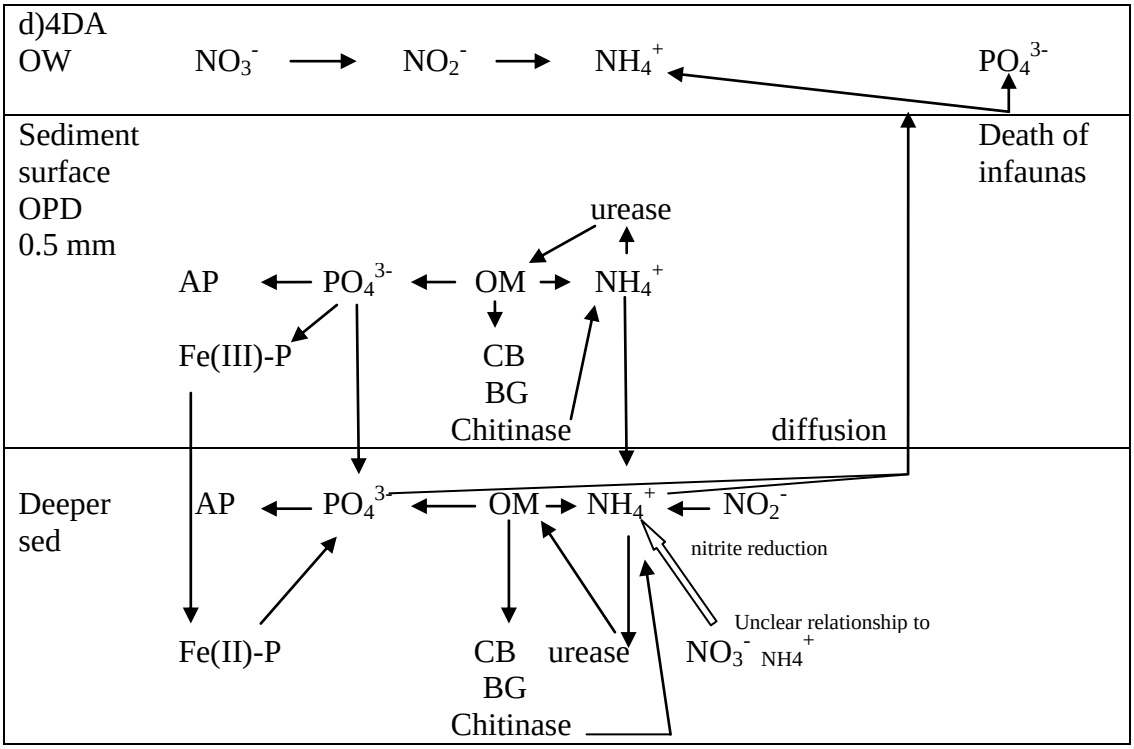
enhanced by bioirrigation and excretion by macrofauna (Mortimer et al., 1998). Ammonium is then oxidised to nitrite; and nitrate also reduces to nitrite in the overlying water of oxic treatment, accumulating nitrite in the overlying water (Figure 4.1 a).

In the oxic treatment, phosphate is released to the sediments from organic matter mineralisation (Figure 4.1 a). Phosphate is then bound to iron (III) (Denis and Grenz, 2003), causing low concentrations of phosphate in the sediment porewater and the overlying water (Figure 3.11 d). Irrigation at the sediment-water interface may release phosphate to the overlying water (Figure 4.1 a).




b)Hypoxic
OW
NO_3^-
NO_2^-
NH_4^+
PO_4^{3-}
Reduced
irrigation
Sediment
surface
OPD
2.5 mm
Urease
AP
PO_4^{3-}
OM
NH_4^+
NO_3^-
unclear link to NO_3^-
Fe(III)-P
CB
BG
Chitinase
diffusion
diffusion
Deeper
sed
AP
PO_4^{3-}
OM
NH_4^+
NO_2^-
nitrite reduction
Fe(II)-P
CB
BG
Chitinase
urease


c)1DA
OW
NO_3^-
NO_2^-
NH_4^+
PO_4^{3-}
Sediment
surface
Irrigation
OPD
5.5 mm
urease
AP
PO_4^{3-}
OM
NH_4^+
NO_3^-
unclear link to NO_3^-
Fe(III)-P
CB
BG
Chitinase
diffusion
Deeper
sed
AP
PO_4^{3-}
OM
NH_4^+
NO_2^-
nitrite reduction
Fe(III)-P
CB
BG
Chitinase
urease



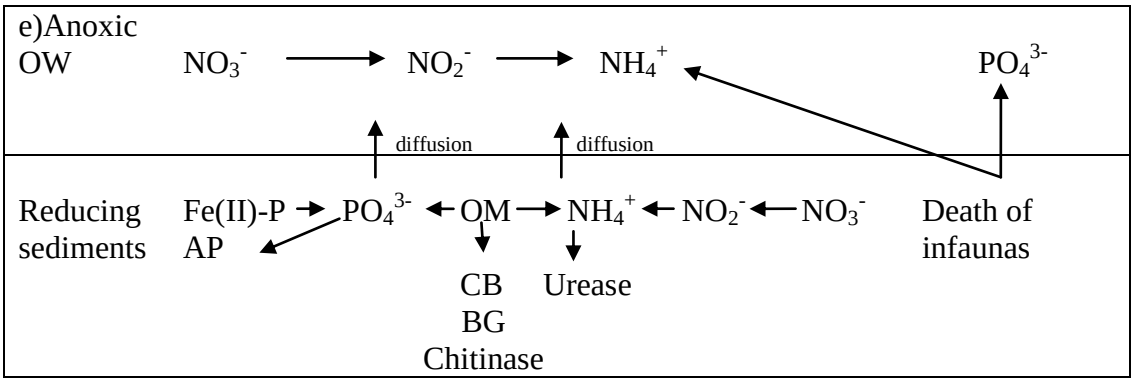


Figure 4.1 Framework of biogeochemical processes in the sediments under a) oxic, b) hypoxic, c) 1DA, d) 4DA and e) anoxic treatments. The schematic highlights the different response of nutrients and enzyme activities in each treatment. The response is also supported by bromide fluxes, oxygen penetration depth and fluxes and total organic carbon and total nitrogen in each treatment. OW- overlying water, NO_3^- - nitrate, NO_2^- - nitrite, NH_4^+ - ammonium, PO_4^{3-} - phosphate, OPD- oxygen penetration depth, Deeper sed- deeper sediment, Fe (III)-P – iron (III)-phosphorus, Fe(II)-P – iron (II)-phosphorus, AP- alkaline phosphatase activity OM- organic matter, CB- cellobiohydrolase, BG- beta-glucosidase, ⟶ clear relationship, unclear relationship ⇨.

Oxygen penetrated less than 2.5 mm depth into the sediment in the hypoxic treatment, corresponding to low oxygen diffusive fluxes (Table 3.4 and Figure 4.1 b). Both the penetration of oxygen into the sediment and consumption of oxygen within the sediment decrease in hypoxic conditions (Neubacher et al., 2011). The decrease is explained by a reduction in the concentration gradient and the narrowing of the oxic layer (Diaz and Rosenberg, 2008). Ammonium is released to the sediment through organic matter mineralisation (Figure 4.1 b). Organic matter degradation produces porewater nutrients, ammonium and phosphate (Clavero et al., 2000). As the OPD is reduced, ammonium diffuses down into the deeper sediment (Figure 4.1 b). Nitrite reduction to ammonium occurs in the deeper sediment, and ammonium is then released to the overlying water through diffusion. There is an unclear link of nitrate to ammonium in the sediment as nitrate profiles were almost constant with depth (Figure 3.10 a and c; Figure 4.1 b).

Higher bromide diffusive fluxes of 889 μmol m^{-2} h^{-1} was measured compared to the sediment irrigation fluxes of 417 μmol m^{-2} h^{-1} in the hypoxic treatment (Table 3.3). Possibly, reduced irrigation during hypoxia releases ammonium into the overlying water in this treatment (Figure 4.1 b). In oxygen depleted treatments such as hypoxia; molecular diffusion dominates, influenced by the reduction of macrofauna (Foster et al., 1995). Burrowing and irrigation of macrofauna are suppressed following hypoxia (Rasmussen and Jorgensen, 1992). Nitrate is also reduced to nitrite and ammonium in the overlying water (Figure 4.1 b). Hypoxia suppresses nitrification and caused nitrate to be reduced to ammonium (Kemp et al., 2009). The overall framework implies that nitrogen is retained in the form of ammonium in the hypoxic treatment (Figure 4.1 b). The release of phosphate into the overlying water is through reduced irrigation (Figure 4.1 b) and Fe(III) reduction to Fe (II) (Emeis et al., 2000; Belias et al., 2007), releasing in turn phosphate ions into the overlying water and the porewater of hypoxic treatment (Figure 3.11 d and Figure 4.1 b).

In spite of the aeration supplied throughout the remaining 6 days of the experiment to the overlying water in the 1DA treatments, there is unclear evidence of nitrification in the surface sediments (Figure 3.10 a-c and Figure 4.1 c). There is also

unclear relationship between nitrate to nitrite and ammonium in the porewater (Figure 3.10 a-c and Figure 4.1 c). Increasing ammonium concentration was observed in the deeper sediment through nitrite reduction to ammonium (Figure 4.1 c). Despite the continuous air supply, nitrite reduction to ammonium is still occurring because full oxygenation of the sediment is not taking place (Belias et al., 2007). Consistently, vertical oxygen profiles in the 1DA treatment (Figure 3.4) only reached 5.5 mm depth in the sediment (Figure 4.1 c), causing the buildup of inhibitory metabolites such as ammonium below this depth. The sediment surface is generally oxidised only within the upper 3 mm for fine- grained sediment (Revsbech et al., 1980; Glud, 2008). Changes of oxygen concentration to the water above the sediments may alter the penetration depth of oxygen within the sediment (Rasmussen and Jorgensen 1992). With less oxygen penetration in the sediment, nitrification rates are slow, increasing ammonium concentration (Kemp et al., 2009). Ammonium is released to the overlying water through diffusion from the deeper sediment and irrigation from the sediment surface (Figure 4.1 c).

Total bromide incubation fluxes were 1.8 times the bromide diffusive fluxes (Table 3.3), showing the influence of irrigation in this treatment. Bromide irrigation in the 1DA treatment (611 μmol m^{-2} h^{-1}) is also higher than in the hypoxic treatment (417 μmol m^{-2} h^{-1} in Table 3.3). Consistent with this, higher diffusive oxygen flux and deeper oxygen penetration depth was also observed in the 1DA treatment relative to that in the hypoxic treatment (Figure 3.4 and Table 3.4). In the 1DA treatment, as oxygen concentration was increased (Table 3.4), anaerobic metabolites could be chemically re-oxidised causing an increase in diffusive oxygen flux (Murrell and Lehrter, 2010). Presumably, benthic infauna present in the cores were able to withstand 24 hours without oxygen and the benthic community compensated for a lack of oxygen by pumping more overlying water through the sediment (Foster et al., 1995). Gamenick et al. (1996) has found that 50% of *Nereis diversicolor* adult and juvenile can survive up to 60 hours without oxygen. This result suggests that brief anoxic events up to 24 hours can be tolerated by benthic infauna.

In the 1DA treatment, ammonium released from irrigation and diffusion from the sediment is oxidised to nitrite and nitrate through nitrification in the overlying

water (Figure 4.1 c). Low nitrite concentration relative to that in the nitrate concentration (Figure 3.11 a and b) also point towards nitrification. In the presence of oxygen, ammonium tends to be oxidised to nitrate (Kemp et al., 2009). Overall, nitrate is retained in the 1DA treatment. Phosphate concentration is low in both the overlying water and porewater in the 1DA treatment (Figure 3.11 d) due to the formation of iron (III)-phosphate complex (Rasheed 2004; Kemp et al., 2009). This formation in the sediment inhibits the transport of phosphate into the overlying water in the 1DA treatment (Figure 4.1 c).

After 3 days of aeration, the oxygen concentration did not seem to recover in the 4DA treatments (Figure 3.5 a). Ammonium accumulates in the porewater from nitrite reduction to ammonium in the deeper sediment and is released to the overlying water through diffusion (Figure 4.1 d). With less oxygen, less water exchange between the overlying water and sediment (Figure 3.3) thus, less bromide going into the sediments (Martin and Banta, 1992). The shape and smoothness of the bromide profiles indicated that diffusion or diffusive-like transport process (Berg et al., 2001) was the dominant process in the sediment of 4DA treatment. This is not surprising if all the infauna are dead, releasing ammonium in the treatment. However, there is an unclear relationship of nitrate to nitrite and ammonium in the porewater with increasing depth of the sediment (Figure 3.10 a-c and Figure 4.1 d). Figure 3.11 a-c suggests nitrate and nitrite reduction to ammonium in the porewater and overlying water. Overall, nitrogen is stored as ammonium in the 4DA treatment (Figure 4.1 d). Oxidised iron minerals in the sediment are reduced with low oxygen conditions releasing bound phosphate (Conley et al., 2007). The different response between the 1DA and 4DA treatment points out that the different duration of anoxic exposure may act as an important factor in determining the response of nitrogen and phosphorus cycle (Conley et al., 2007; Katsev et al., 2007; Riedel et al., 2008; Middelburg and Levin, 2009).

In the anoxic treatment, porewater nitrate and nitrite are reduced to ammonium in the sediment (Figure 3.11 a-c and Figure 4.1 d). Anoxic conditions favour ammonium release from the sediment by anaerobic breakdown of organic matter rather than the release of nitrate and nitrite (Kristensen, 2000; Middelburg and

Levin, 2009). Ammonium is also released to the overlying water in anoxic conditions through diffusion (Figure 4.1 e). In the absence of dissolved oxygen, phosphate also accumulates in the sediment (Figure 3.11 d and Figure 4.1 e). In anoxic condition, phosphate is released during iron mobilisation at the sediment-water interface (Golterman, 2001; Hupfer and Lewandowski, 2008; Middelburg and Levin, 2009). Studies have shown that anoxic condition results in the release of phosphate (Rozan et al., 2002; Skoog and Arias-Esquivel, 2009; Ozaki et al., 2011) from the sediment to the porewater and the overlying water. Irrigation flux is low relative to diffusive flux in the anoxic treatment (Table 3.3). The lowest bromide exchange as expected is shown in the anoxic treatments, which are primarily driven by molecular diffusion (Libes, 2009). With the absence of infauna which irrigates the sediment through burrows, no oxygen penetration was observed in the anoxic treatments (Figure 3.4), thereby supporting the dominance of molecular diffusion within this treatment (Forster et al., 1995; Glud et al., 1996). In accordance, the lowest oxygen diffusive flux was also measured in the anoxic treatments (Table 3.4). The absence of oxygen rapidly suppresses the oxygen penetration depth into the sediment (Abell et al., 2011).

4.7 Porewater Nutrient Concentrations and Porewater Diffusive Fluxes; and Nutrient Fluxes and Porewater Diffusive Fluxes in the Sediment under Five Different Treatments

This section begins with the relationship between porewater nutrient concentrations and porewater diffusive fluxes in each of the treatments. This is followed by the discussion about nutrient fluxes and porewater diffusive fluxes. Decreased porewater nitrate and increased porewater ammonium concentrations in the oxic treatment indicate the occurrence of nitrate reduction into ammonium with increasing depth in the sediment (Figure 3.10 a and c). But, where has nitrite come from in the porewater? Vertical porewater nitrite with depth (Figure 3.10 b) remains relatively the same that limits our interpretation. Looking at the diffusive fluxes in the oxic treatment, simultaneous porewater nitrate flux into the overlying water (Figure 3.12 a), and the flux of nitrite and ammonium into the sediments was measured (Figure 3.12 a, c and e). These fluxes suggest that nitrification is occurring in the oxidised

sediment surface layer (Lavery et al., 2001). However, comparison between the porewater nutrient concentrations and porewater diffusive fluxes in the oxic treatment shows an inconsistency between these 2 parameters. Porewater nutrient concentrations suggest nitrate reduction to ammonium whereas porewater diffusive fluxes indicate nitrification.

In the case of 1DA treatment, fairly constant vertical profiles of porewater nitrate concentrations with depth were observed (Figure 3.10 a), that limits our interpretation. However, nitrite reduction to ammonium is clearly observed at 9 cm depth (Figure 3.10 b). A linear increase in ammonium concentrations over space is related to the degradation of organic matter (Denis and Grenz, 2003). Porewater nitrate diffusive fluxes show a similar pattern to the oxic treatment (Figure 3.12 a). The pattern might be due to nitrification in the 1DA treatment. Nitrite diffusive flux into the sediment simultaneous with nitrate diffusive flux directed into the overlying water is indicative of nitrite consumption by nitrification (Mortimer et al., 1998). Again, an inconsistency is observed between porewater nutrient concentrations and porewater diffusive fluxes. The occurrence of nitrification suggested by the porewater diffusive fluxes is not consistent with the pattern of nitrite reduction to ammonium observed from the vertical porewater nutrient concentrations in the 1DA treatment.

In the hypoxic, 4DA and anoxic treatments, the majority of porewater DIN is in the form of ammonium throughout the core with low porewater nitrate and nitrite concentrations (Figure 3.10 a-c). The reduction of porewater nitrite to ammonium occurred in the hypoxic and 4DA treatments (Figure 3.10 b and c). In the anoxic treatment, porewater nitrate and nitrite are reduced to ammonium, demonstrating anaerobic degradation of organic matter (Mortimer et al., 1998; Rasheed et al., 2006; Middelburg and Levin, 2009). Under low oxygen conditions, ammonium is released (Abell et al., 2011).

Looking at the porewater diffusive fluxes in the hypoxic, 4DA and anoxic treatment, porewater nitrate diffusive fluxes were directed to the overlying water in the hypoxic and 4DA treatments but were into the sediments in the anoxic treatment

(Figure 3.12 a). On the other hand, the fluxes of nitrite and ammonium were into the sediment (Figure 3.12 c and e). This pattern indicates decreased nitrification (Denis and Grenz, 2003) in the hypoxic and 4DA treatments. In the anoxic treatment, nitrate uptake into the sediment was observed, suggesting denitrification (Mortimer et al., 1998). But, the diffusive fluxes of nitrite and ammonium in the anoxic treatment were into the sediments, suggesting nitrification. One possible explanation is that ammonium uptake in these 3 treatments might reflect ammonium consumption by bacteria living at/near the sediment water interface (Maksymowska-Brossard and Piekarek-Jankowska, 2001). Core incubation was carried out in the dark, which would inhibit ammonium uptake by benthic photosynthetic autotrophs (Lerat et al., 1990).

Phosphate concentration increased extensively in particular in the 4DA and anoxic treatments (Figure 3.10 d). This pattern suggests reduction of ferric oxyhydroxide phases and release of iron-bound phosphorus to the porewater under low oxygen condition (Steenbergh et al., 2011). All phosphate diffusive fluxes were into the sediment (Figure 3.12 g) and although significant differences were measured between treatments, all diffusive fluxes are too small to reflect major changes in the sediment.

The variable pattern of the porewater diffusive flux might be due to the dominance of non-physical processes such as biological and chemical retardation processes controlling the fluxes; for example, the formation of iron (III)-phosphate complexes, nitrification and denitrification (Clavero et al., 2000; Lavery et al., 2001). All of these retardation processes may occur on timescales comparable with the 7 days of our flux experiment.

The variable pattern of porewater diffusive flux also leads to lack of consistencies between the measured nutrient fluxes and the calculated porewater diffusive fluxes. This indicates that Fick's 1st Law for diffusive fluxes calculation should be used with caution.

4.8 Enzyme Activities in the Sediment under Five Different Treatments and the Relationship of Enzyme Activities and Other Parameters in the Sediment

The present study shows that AP, BG, CB, chitinase and urease profiles followed a similar decreasing trend moving down core from the surface of the sediment (Figure 3.13 a-e). The trend with depth was particularly marked in the CB activities, decreasing to near zero at 9 cm depth (Figure 3.13 c). It would be expected that the highest concentrations of both total and labile organic matter would occur at the sediment surface. This influx of fresh organic matter stimulates microbial activity in surface sediment (Boetius et al., 2000), leading to a corresponding increase in enzymatic decomposition rates (Souza, 2009). As a result, enzyme activities are highest in the surface or near surface sediments and decrease with depth (Meyer-Reil, 1986; Bowman et al., 2003; Liu et al., 2008). With increasing sediment depth, the quantity and quality of decomposable organic matter is reduced, and the remaining material becomes more and more resistant to microbial attack (Fabiano and Danovaro, 1998; Hill et al., 2006).

Factors that influenced enzyme activities in this study are shown in Figure 4.2. Each factor will be discussed in turn. This section also refers to the proposed framework in Figure 4.1 a-e to relate between enzymes and nutrients. Increasing AP, CB and chitinase activities were observed in the oxic treatments relative to that in the initial treatments (Figure 3.13 a, c and d). The inverse relationship of AP to porewater phosphate concentrations showed that AP is high when porewater phosphate concentration is low (Figure 3.14 a-b). There are two possible mechanisms that may cause this. Firstly, high phosphate concentration is known to act as an inhibitor of AP, based on the catalysis of AP to produce phosphate from organic phosphorus (section 1.6.1). Secondly, at high phosphate concentrations, there is a reduced requirement for the microorganisms to produce AP (Newman and Reddy, 1993; Zhou et al., 2008). In this study, increasing phosphate concentrations were observed in the deepest layers of anoxic sediment and presumably anaerobic microorganisms are not experiencing phosphate limitation, so the expression of AP was repressed (Steenbergh et al., 2011). When phosphate concentration are low in

the water, microorganisms or plankton may produce phosphatase to hydrolyse organic phosphorus (Newman and Reddy, 1993; Takano et al., 2006; Deng et al., 2009; Chen et al., 2011). Phosphate is immobilised by co-precipitation of Fe (III) in oxic condition (Figure 4.1 a), so AP must be released to allow microorganisms to overcome the resulting lower phosphate concentrations. AP is lower in the hypoxic, 4DA and anoxic treatments (Figure 3.14 a-b; Figure 4.1 c-e) that may result from AP inhibition in the presence of phosphate concentrations (Jones, 2002) as well as by inhibition of the reduced metal from Fe (III) to Fe (II) in the sediment (Jordan et al., 2008).

CB and chitinase activities increased in the oxic treatments relative to the anoxic treatments (Figure 3.13 c and d). There are two possible explanations for CB and chitinase activities in this study. Firstly, anoxia inhibits the activity of aerobic benthic organisms using chitin and cellulose as carbon sources. Secondly, a number of aerobic benthic organisms are killed in the anoxic treatment, so alternative carbon sources are available apart from CB and chitin. BG catalyses cellobiose to glucose (under section 1.6.3), which could be an alternative carbon source in the treatment. But, anoxia also reduces BG activity (Figure 3.13 b). Therefore, the patterns observed in CB, chitinase and BG activities might imply that anoxia inhibits the activity of aerobic benthic organisms using chitin, cellulose and BG as carbon sources (Figure 4.2).

Additionally, weak relationship between CB and TOC (Figure 3.16 g-h), suggests that other factors than TOC contributes to its activity. Chitinase activities were inversely correlated to porewater ammonium concentrations (Figure 3.16 i-j; Figure 4.1 and Figure 4.2), suggesting the relationship of chitinase in the nitrogen cycle. Chitinase activity is linked to the acquisition of nitrogen (Hill et al., 2012). Higher BG activities in the oxic treatment, suggests labile carbon acquisition as glucose is a very labile carbon source (Hill et al., 2012).

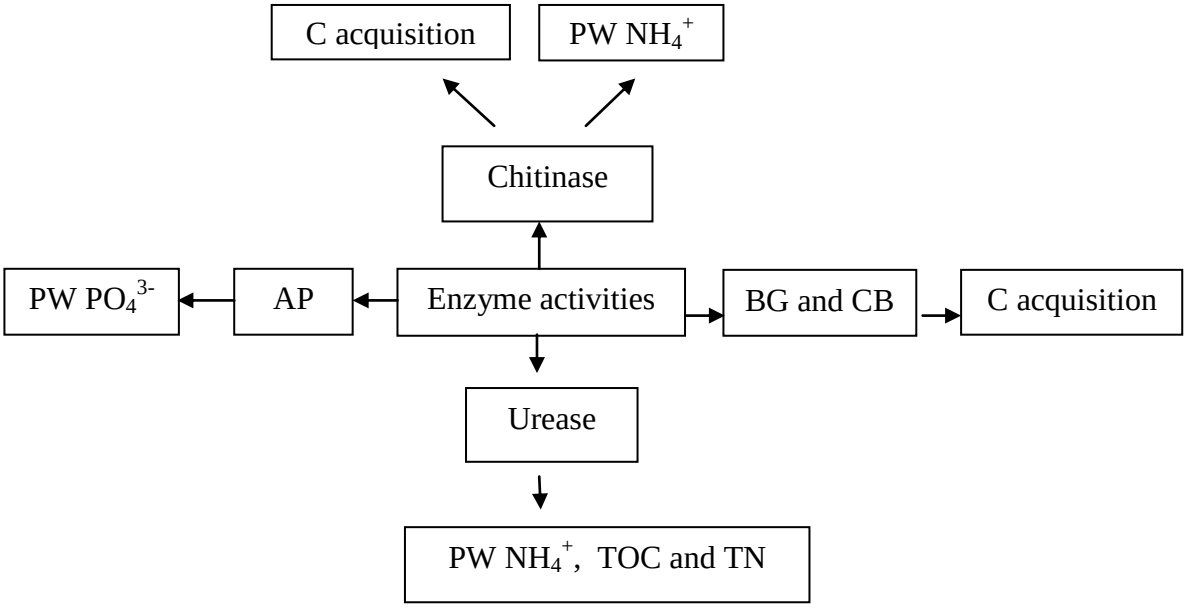


Figure 4.2 Factors affecting enzyme activities in the sediment of this study. C- carbon, PW NH_4^+ - porewater ammonium, BG- ß-glucosidase, CB- cellobiosidase, TOC- total organic carbon, TN- total nitrogen, AP- alkaline phosphatase, PW PO_4^{3-} - porewater phosphate.

Interestingly, urease activities significantly increased in the anoxic treatments relative to the oxic treatments at all depths from 1 to 7 cm depths in the sediment (Figure 3.13 e). What could produce such high urease activities? There was an inverse relationship between urease activities with porewater ammonium concentrations (Figure 3.14 c-d), reflecting a repression of urease activity by porewater ammonium concentrations. Solomon et al., (2010) have reported that urease activity was repressed by ammonium concentration in Chesapeake Bay due to less demand for ammonium via urease pathway. At the same time, high urease activities were positively related with total organic carbon (Figure 3.14 e-f) and total nitrogen, implying that organic carbon and nitrogen stimulates the activity of bacteria with urease enzymes (Figure 4.2). The positive correlation between TOC and TN content and urease activities indicates urease dependence on polymeric substrate content (Souza, 2009).

Similar to the anoxic treatments, urease activity was enhanced in the hypoxic and 4DA treatments. Possibly, the facultative and obligate anaerobic bacteria living in the sediment may have produced more urea in the sediment (Satoh, 1980) which

might exceed the high ammonium concentrations in the hypoxic and 4DA treatments. Urea can also increase through organism excretion and from release of the sediments (Lund and Blackburn, 1989; Therkildsen et al., 1996). Urease activity was not affected in the oxic and 1DA treatments. This is consistent with Zantua and Bremner, (1977) and Bremner and Mulvaney, (1978) who reported that oxygen had little, if any effect on urease activities in soil.

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Overall, the response in the sediment of 4DA treatments followed closely to that in the anoxic treatment whereby 1DA treatments resembled oxic treatments (Figure 3.15 a). This pattern implies the importance of the duration of anoxia in regulating the nutrients and enzyme activities in estuarine sediment. The influence of depth profiles were prominent in all enzyme activities (Figure 3.15 b), most activities were highest on the surface, suggesting labile organic matter and less degraded with increasing depth (Kahkonen and Hakulinen, 2012). Enzyme activities were positively related to one another except urease (Figure 3.16 a, c and e). This indicates the close associations of AP, CB, BG and chitinase are related to the acquisition of carbon, nitrogen and phosphorus in fine-grained sediment (Hill et al., 2012). The fact that CB and chitinase activities were higher in the surface of the sediment (2 cm) than deeper layers (7-9 cm) in Figure 3.16 h and j indicates that there was highly active cycling of carbon and nitrogen in the surface sediment.

4.9 Conclusions

There were clear responses of nutrient concentrations in the overlying water to the oxic, hypoxic, 1DA, 4DA and anoxic treatments. These must reflect the combined effect of nutrient exchanges with the sediment and, for nitrogen, transformation between chemical forms. Concentrations of nutrients in the porewater were less straight forward to interpret. This leads to an apparent disagreement between measured nutrient fluxes in the overlying water and the calculated porewater diffusive fluxes. This is influenced by chemical and biological processes occurring within the sediment that control the release of nutrients. Overall, looking at the fluxes estimated from nutrient concentrations in the overlying water (Figure 3.6 a-d), the cores act as sinks for nitrate, minor sources for nitrite and phosphate and major

sources of ammonium in the overlying water. Examining porewater concentrations alone would give the opposite conclusions, indicating that porewater is a source for nitrate and a sink for nitrite, ammonium and phosphate (Figure 3.12 a, c, e and g). Referring to each treatment, nitrite accumulates in the oxic treatment (Figure 4.1 a). Nitrate is retained in the overlying water of 1DA treatment (Figure 4.1 c). Ammonium is retained in the hypoxic, 4DA and anoxic treatment (Figure 4.1 b, d and e), exacerbating eutrophication problems in oxygen depleted treatments. Phosphate is released into the overlying water in low oxygen conditions. In oxygenated conditions, phosphate is directed into the sediments, driven by the redox chemistry of iron.

Higher enzyme activities were found in the surface sediment with potential easier degradable organic material. Deeper sediment has less degraded material. The response of AP was related to porewater phosphate concentrations (Figure 4.2); and chitinase was inversely related to porewater ammonium concentrations (Figure 4.2). Weak relationships of AP, CB, BG and chitinase activities to total organic carbon and total nitrogen in the sediment suggest other sources affecting these enzymes. In contrast, higher urease activities in the hypoxic, 4DA and anoxic treatments were probably influenced by both the porewater ammonium concentrations and total organic carbon in the sediment (Figure 4.2). Nevertheless, clear differences were observed in the vertical profiles of the enzyme activities. Therefore, enzyme activities can be proposed as indicator of ecological effects in the sediment in comparison to porewater nutrient concentration. Enzyme activities in the sediment are less affected to the 1 day of anoxia than the 7 days hypoxia. Based on the robustness of the system to the 1DA treatment, the take home message is that the short term anoxia of 1 day is less likely to give a negative impact to the biogeochemical functioning of the estuarine sediment. The effect of hypoxia on a longer term should be considered to determine its effect to the environmental quality.

CHAPTER 5

SUMMARY AND FUTURE RECOMMENDATIONS

5.1 Introduction

This chapter summarises the key findings that answer the scientific questions posed at the start of this research in Section 1.7. Selection of the experimental setup and timing of sampling events throughout this research were also included. This chapter also considers the limitations, remaining issues and future research priorities.

The research conducted for this thesis set out to evaluate the oxygen concentration at which harmful effects start to occur, the rates that the system responds to different levels of oxygen saturation in the overlying water and the recovery times of system processes when re-aeration are restored. The aims were achieved through the study of nutrient fluxes in the overlying water and enzyme activities in the sediment. The motivation of this thesis was triggered by the escalating growth of hypoxia (oxygen depletion to 25% saturation) and anoxia (absence of oxygen) in shallow waters and estuaries associated with human induced eutrophication. This led to the questions of:

1) Does short term hypoxia and anoxia affect pH in the overlying water and vertical profiles of total organic carbon and total nitrogen in the sediments?
2) Are differences in oxygen saturation in the overlying water reflected by changes in bromide solute transport between sediment-water interfaces and the oxygen consumption in the sediments?
3) How does oxygen affect nutrient cycling, which in turn influences nutrient fluxes?
4) Does the duration of hypoxia and anoxia influence the nitrogen and phosphorus transformations in the estuarine sediment-water system?
5) What factors affect enzyme activities in sediments?

5.2 Summary of Findings

This section provides answers to each research question posed in Section 1.7.

1) Does short term hypoxia and anoxia affect pH in the overlying water and vertical profiles of total organic carbon and total nitrogen in the sediments?

The finding in this study indicates that short term anoxia elevates pH slightly compared with oxic treatments, equivalent to a decrease of hydrogen ion concentrations or alkalinity (section 3.2.1). Presenting the pH changes in terms of hydrogen ion concentrations showed an average difference of 4 nmoles L^{-1} between oxic and anoxic treatments. This is an average difference of 0.2 pH. The actual magnitude of the increase may seem small, but pH is based on a logarithmic scale, so even a change of 0.2 units makes a substantial difference to hydrogen ion concentrations. But, since the anoxic treatment increases pH by 0.2 units, anoxia does not markedly enhance acidification problems. Only if the development of hypoxia is associated with a release of carbon dioxide, which can reduce the pH levels by 0.2 units (Mucci et al., 2011) will the physiological and biological activities of benthic organism would be affected (section 4.4).

In terms of total organic carbon (TOC) and total nitrogen (TN) distribution in the sediment, the reduction of TOC was 1% in the oxic treatment, 0.5% in the hypoxic treatment and 0.1% in the anoxic treatment relative to the initial treatments (Figure 3.2 a). Minor differences of TN were observed between treatments, less than 0.1% (Figure 3.2 b). The obvious pattern of vertical profiles of TOC and TN, suggests the decreasing substrate availability with depth (Kristensen, 2000). The smaller loss of TOC in the anoxic treatment in our study relative to the oxic treatment suggests preservation of organic carbon at low oxygen concentrations, but the loss is not feasible within a timescale of 7 days. CN ratio in the oxic and 4DA treatments at 7 cm depth (Figure 3.2 c) shows the closest value to the Redfield ratio of 6.6 and suggests labile carbon, but this is not possible at 7 cm depth where the sediment is highly unlikely to be exposed to rapid changes. The TOC and TN pattern is inconclusive and requires further study (section 4.4).

2) Are differences in oxygen saturation in the overlying water reflected by changes in bromide solute transport between sediment-water interfaces and the oxygen consumption in the sediments?

Higher solute transport between sediment and overlying water in the oxic treatments using bromide as a tracer, implies that water movement is controlled by irrigation fluxes (Table 3.3) whereas in the hypoxic and anoxic treatments, it is dominated by diffusive fluxes. Under Table 3.4, highest oxygen diffusive fluxes in the oxic treatment; reflect bacterial activity in the upper milimetres of the sediments relative to the anoxic treatments.

3) How does oxygen affect nutrient cycling, which in turn influences nutrient fluxes?

The nitrogen and phosphorus cycles are strongly regulated by oxygen. As mentioned in section 3.5.2, nitrate concentrations are high when dissolved oxygen saturation is high and started decreasing when dissolved oxygen saturations were low. The largest nitrate flux into the sediment occurred when the dissolved oxygen was low (section 3.5.2). In oxygenated condition, nitrate is the thermodynamically stable form of nitrogen (Thamdrup and Dalsgaard, 2008).

Nitrite accumulates in oxic conditions, but in anoxic conditions this is reduced further to ammonium (Figure 3.9 c and e). Nitrite concentrations increase in the overlying water and ammonium concentration increases initially in the oxic treatment because the volume of overlying water is small relative to the quantities being released from the sediment in the cores during incubations. At low oxygen saturation, ammonium and phosphate concentrations increase, corresponding to ammonium and phosphate effluxes to the overlying water but were low at high oxygen saturation (Figure 3.9 e and g). Ammonium is inevitably being produced from both nitrate and nitrite reduction in the absence of oxygen (Middelburg and Levin, 2009). When supplied with re-aeration, most of ammonium were partly oxidised to nitrite before being converted into nitrate concentrations. With oxygen, phosphate remains relatively low, showing an uptake into the sediment (Figure 3.9

g). This pattern reflects a response of phosphate immobilisation as Fe (III) dominates in oxic condition (Paytan and McLaughlin, 2007). The consumption of oxygen and the predominance of hypoxia and the extensive anoxic treatments, in turn released phosphate concentrations into the overlying water. Re-aeration was sufficient to maintain phosphate at low concentrations in the 1DA treatment and reduces phosphate concentrations in the 4DA treatment (Figure 3.9 g).

Nutrient concentrations in the porewater were more variable and are hard to interpret (Figure 3.10). Nevertheless, clear increasing ammonium and phosphate concentrations were measured with depth, as a response of low oxygen concentrations (Kristensen, 2000).

4) Does the duration of hypoxia and anoxia influence the nitrogen and phosphorus transformations in the estuarine sediment-water system?

This study demonstrates that the duration of hypoxia and anoxia are the key factors determining the response on estuarine system. The system was surprisingly robust to short term anoxia, the 1DA treatment showed a very similar response to the effect in oxic treatments (Figure 3.15 a). Apparently, the effect of a brief episode of anoxia lasting up to 24 hours did not cause a severe impact to the system and effects were largely reversible, with the system recovering to normal condition within the 7 days of treatment. The effects observed in the 1DA treatment were less marked than in continuous hypoxia of 7 days. This was evidenced particularly by a smaller ammonium concentration increase in the 1DA treatment than that in the hypoxic treatment (Figure 3.5 d). Both hypoxia and longer duration (4 days) anoxia followed by re-aeration caused substantial negative effects on the system, with subsequent biogeochemical effects. Ammonium and phosphate effluxes were shown to dominate the nutrient fluxes in the 4DA treatment (Figure 3.6 c and d). In a system without oxygen (anoxia), molecular diffusion controls the responses of nitrogen and phosphorus dynamic in the system (Quintana et al., 2007; Hedman et al., 2011). Ammonium and phosphate dominate in the overlying water and porewater, exacerbating nutrient problems. So, sediment biogeochemistry has some resilience

towards short term anoxic events, but more prolonged hypoxic or anoxic events that are continued for only a few days can have major effects on ecosystem function.

5) What factors affect enzyme activities in the sediments?

Increasing alkaline phosphatase (AP), cellobiohydrolase (CB), beta-glucosidase (BG) and chitinase activity were observed in the oxic treatments relative to that in the initial treatment, but urease showed the opposite pattern (section 3.6). The response of AP depends on the phosphorus requirement of microorganism (section 4.8). In the oxic treatments, phosphorus solubility is limited by coprecipitation with iron III (Middelburg and Levin, 2009; Chen et al., 2011). Therefore AP are synthesised to overcome phosphate limitations. Vertical profiles of CB and chitinase activities were highest on the surface and decrease with depth, due to shifts of organic matter input and quality as it goes deeper (Fabiano and Danovaro, 1998; Zonneveld et al., 2010). CB, BG and chitinase activities are lowest in the anoxic treatment, possibly because anoxia inhibits the activity of aerobic organisms using these enzymes as a carbon source (section 4.8). Increasing urease activity in the absence of oxygen may be related to elevated urea concentration from excretion by bacteria and death of organisms in anoxic condition that mitigates the repressive effect of high ammonium concentration on urease (Ruan et al. 2009; Mortensen et al. 2011).

5.3 Selection of Experimental Setup and Research Timeline

This section explains the decision to carry out the experiment as described in section 2. 4 and reflects on the research timeline. To address the aims and research questions outlined, oxygen concentrations were manipulated in 5 different treatments, oxic (96% saturation in the overlying water), hypoxia (25% saturation), 1DA (24 hours without oxygen followed by re-aeration of up to 6 days), 4DA (4 days without oxygen and subsequent re-aeration for 3 days) and anoxia (0% saturation). Although short term hypoxia and anoxia are generally confined to the warmer summer months, its occurrence and intensity tends to vary on daily to weekly time scales in estuarine system associated with periodic fluctuations in sunlight and tides as well as rain and wind events (Wenner et al., 2004; Taylor et al., 2009). Hence, the

significance of evaluating short term effect of anoxia within 1 day to 7 days. Although the sampling area selected for this study (Breydon Water) was not naturally hypoxic, the location was chosen with practicality and sample integrity in mind. Considering the cost of the gas mixtures ordered and the time consuming process of trying to set up the experiments and maintaining the oxygen concentrations, this study was focused on the effect of episodic anoxia, which normally occurs within 7 days. This study also test the effect of different duration of episodic anoxia; 1 day anoxia and 4 days anoxia and the re-aeration effects to the sediment biogeochemistry. The effects of the treatments to nutrients and enzyme activities were substantial, that it is reasonable to run the experiment for just 7 days.

Throughout this research, three sampling events were conducted, the first one (around Great Yarmouth) in August 2008 to survey the sampling location for the sediments and to test out the method of analysing nutrients using microplate analysis. But, problem was encountered with the nutrient analysis and no results were derived. I intercalated in February 2009 to October 2009 due to medical conditions and returned to Malaysia throughout the intercalation period. A second sampling campaign was carried out in November 2009 to test out the gas setup and preliminary experiment of using oxygen minielectrode, bromide tracer experiment and nutrient analysis using the autoanalyser but, the minielectrode did not function, and gas setup did not work. A third sampling was carried out in June 2010, and the data for this study were presented in Chapter 3 under the results section. Only one sampling event and one laboratory manipulation was carried out for this research mainly due to time and funding restrictions.

5.4 Recommendation for Future Research

This section explains the contribution and the limitations of this thesis. Future studies are also proposed. This experimental setup or laboratory simulation provides a guideline for nutrients and enzymes regulations applicable to estuarine system showing signs of short term (days to a week) oxygen depletion. Despite of that, the limitation of this study lies largely on the inability to reflect measurement exactly to the field situation. Ideally, all measurements are preferred to be carried out in the

field but, this is not practically possible in the intertidal without substantially larger logistic resources. Even if it were practically possible to set up the incubations *in situ*, there will be complications because the water changes rapidly and in the case of Breydon Water, the sediments are exposed to the air for up to 2 days during periods of neap tides (section 2.3). Therefore, a realistic assessment of the consequences of short term hypoxia and anoxia could be achieved by conducting seasonal field sampling for a year taking into account the effect of tides in intertidal sediments.

Good quality data were produced by minimising the effect of variability (Figure 3.5) by using field sediment and nutrient free artificial seawater. However, by using nutrient free artificial seawater, this eliminates *in-situ* production of the water. To overcome this, *in-situ* seawater could be included as a control as recommended by NICE protocol handbook (Dalsgaard et al., 2000). This study used artificial seawater because water taken from shallow areas of estuaries may have been exposed to large diurnal changes in light, temperature, nutrients, dissolved oxygen that could affect the experimental results of this study (section 2.4).

Clear patterns of lower hydrogen ion concentrations in the anoxic treatment were shown, corresponding to higher pH in anoxic treatments relative to lower pH in oxic treatment (section 3.2.1). A consistent pattern of rapid usage of TOC and TN in the surface relative to deeper depths was also found (section 3.2.3). Future studies should consider measuring carbon dioxide, sulphide concentrations and alkalinity in the overlying water and sediments, which might provide a more detailed picture of what is happening.

The negative consequences of short term hypoxia and anoxia on the nitrogen and phosphorus cycles were clearly shown in an experiment that runs for only one week (section 3.5.1). But, since hypoxia and anoxia in the field often occurs as repeated events of different durations, future studies should consider the resilience of benthic organisms, nutrient fluxes and enzyme activities to repeated perturbations.

A clear and consistent pattern of enzyme activities between treatments (Figure 3.13) were found. This suggests that enzyme activities could be used as a

reliable indicator of ecological effects in sediment in place of nutrient fluxes. Incorporation of DNA based techniques would also be useful to allow more exact identification of the aerobic and anaerobic microorganism responsible and the sources of these enzymes in terms of reaching a better management of estuarine ecosystem. For example, by identifying chitin decomposing bacteria for chitinase activity and measuring urea to better understand the pattern observed for urease activity.

This study provides an interesting approach to determine what processes govern the sediment-water interface by using bromide as a tracer (section 3.3). But, the number of worms in each core should be included to further understand what is happening in each treatment. The worms were not counted with the aim to prevent disruption of the vertical sediment profiles of nutrient concentration and a range of biogeochemical processes in the sediment when counting the animals at the same time. A further study should consider using the conservative approach by adding an increasing number of individual to intact sediment cores to assess the effect of anoxia on biogeochemical processes at different densities of the polychaete worm in estuaries.

So, overall, the research between the link of oxygen and sediment biogeochemistry is very important from the perspective of ecosystem functions by providing ecotechnology measures using oxygen to overcome estuarine ecosystem imbalance. A preliminary threshold of the severity of short term effects was also examined. In this viewpoint, the research questions explored in this thesis may offer beneficial values in terms of ecosystem management. Hypoxia and anoxia up to 7 days alter the oxygen penetration depth, bromide fluxes and enhanced sediment effluxes of ammonium and phosphate but short term anoxia up to 24 hours has only mild biogeochemical effect. Therefore, the duration of hypoxic and anoxic events on sediment biogeochemistry should be incorporated as an important factor influencing estuarine ecosystem studies if we are to further our understanding and predictive capabilities of the response of estuarine ecosystem to perturbations such as eutrophication or climate change.

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