Insight into the Nitrogen Cycling in the North Sea

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By

Alida Rosina Rosales Villa

School of Environmental Sciences
Faculty of Sciences
University of East Anglia

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Abstract

Fixed nitrogen (N) is an important element which may limit marine primary production. Nitrogen inputs to coastal waters have increased putting pressure on this ecosystem. Supply and removal of N depends on a series of N-cycling processes including canonical denitrification and anammox which remove N, while dissimilatory nitrate reduction to ammonium (DNRA) recycles N allowing it to remain available for primary producers. The sediments in the coastal zone are a key site for these processes but the environmental factors regulating them are still poor understood and the fluxes poorly quantified.

This study investigated sedimentary N-cycling at 5 sites in the open North Sea in August 2013, and at one station in the Wash estuary in May, June, September and October 2013 using ^{15}N tracer techniques, pore water studies and direct sediment flux measurements. All sites had relatively low sedimentary organic carbon content (<5%). The results of this study showed temporal variation in the Wash, and spatial variation in the North Sea and the tracer studies provided valuable new information about the sedimentary nitrogen cycle. At all sites the main process contributing to total N_2 production was denitrification (>95%) with on average 80% associated with coupled nitrification-denitrification. The average rate of denitrification was higher in the North Sea (7.62 µmol $m^{-2}h^{-1}$) than in the Wash (4.4 µmol $m^{-2}h^{-1}$). Anammox was not detected at the Wash sites and contributed only 6.6% to total N_2 production at the North Sea sites. DNRA was observed during three of the months studied at the Wash sites but only at one North Sea site and, where measurable, was responsible for between 6.5 and 30% of nitrate reduction. Temperature was identified as an important control on the oxygen penetration depth. The results indicate that denitrification is a major sink for nitrate in the North Sea.

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Chapter 1. Introduction

Nutrients are elements that all organisms need for organic matter synthesis and are involved in the processes of all living organisms (Parsons, 1975; Gruber, 2008; Gruber and Galloway *et al*., 2008). Nutrient inputs play an important role in marine ecosystems because they support primary production. Among the most important elements required by the organisms, nitrogen (N) is of major importance as a metabolite, and the concentration of fixed N is considered limiting for primary production (Howarth, 1988; Day *et al.,* 2012).

There are many chemical forms of N in nature (Table 1.1) and it can be found as gas (e.g. dinitrogen, nitrous oxide, ammonia), and in nongaseous forms. The main nongaseous inorganic fixed forms of dissolved N are nitrite (NO_2^-), nitrate (NO_3^-), and ammonium (NH_4^+). Organic N encompasses detritus or particulate organic nitrogen (PON) and dissolved organic nitrogen (DON). All of these forms are also grouped as non-reactive N, and reactive N (Nr) (Rabalais, 2002; Brandes *et al.,* 2007). In marine ecosystems, N is present mainly in five forms: dinitrogen (N_2), nitrate (NO_3^-), ammonium (NH_4^+), particulate N, particularly in the organic form (PON), and dissolved organic N (DON) (McCarthy, 1998; Brandes *et al.,* 2007; Glibert, 2006). From these forms, dissolved N_2 and nitrous oxide (N_2O) are considered non-available, while NO_2^-, NO_3^-, NH_4^+, and DON are considered available fixed N for species involved in primary productivity (Glibert, 2006; Gruber and Galloway *et al*., 2008), and from these NH_4^+ and NO_3^- are the most important constituents of the N pool in estuaries and coastal zones (48% and 31%; 45% and 34% respectively) (Berman and Bronk, 2003).

Although the supply and removal of fixed marine N depends on a series of processes, all involved in the N-cycling (Fig. 2.1), since a couple of decades ago, worldwide anthropogenic activities have increased the amount of N of shallow coastal areas (Howarth *et al.,* 2000; Howarth *et al.,* 2002). The anthropogenic inputs are associated with agriculture, aquaculture, urbanization, coastal development, industrial expansion as well as discharge of human waste, animal production, and the combustion of fossil fuels resulting in nitrogen oxides (NOx) (Nixon, 1995; Galloway *et al*., 2004; Seitzinger *et al*, 2006;). It is worthwhile noticing that from these activities, agriculture, combustion of fossil fuels, and the production of fertilizers increased the production of N from 1860 to 2000, from 15 Tg yr^{-1} to 165 Tg yr^{-1} (Galloway *et al.,* 2003). As a result of these human activities, coasts throughout the world have elevated concentrations of N (Cloern, 2001).

Even though nutrients are important for both growth of organisms and for marine ecosystems, the nutrient over-enrichment generally triggers ecological changes (Howarth *et al.,* 2000): habitat destruction, shifts in species composition (Karlson, *et al.,* 2002; Glibert, 2006) and distribution ranges, invasion of non-native species and changes in food web efficiency (Cloern, 2001). It is particularly problematic when excessive nutrients loading create low oxygen or hypoxic conditions (<4 mg l^{-1}, ~ 125 μmol l^{-1}) inasmuch as, under these conditions, nutrients

cycle (Paerl, *et al.,* 1998; Rabalais and Turner, 2001; Diaz and Rosenberg, 2008; Cartensen *et al*., 2014), particularly N processes can be strongly affected (Conley *et al.,* 2011. See section 2.3.2).

Table 1.1 Different forms of N in nature. Nr is reactive nitrogen.

Forms of N in nature	Non-Nr	Nr
Gaseous		
• Inorganic reduced forms		NH_3, NH_4^+
• Inorganic oxidized forms		N_2O, NO_2, NO
• Non-reactive form of N	N_2	
Particulate		
• Alive		plankton
• Detritus		
- Organic (PON)		Organic matter in decomposition (tripton)
Dissolved		
• Gaseous	N_2	
- Inorganic oxidized forms		N_2O
- Inorganic reduced forms		NH_4^+
• Non-Gaseous		
- Inorganic oxidized forms		NO_2^-, NO_3^-
- Organic reduced forms		RNHx (amines, proteins, amino acids, urea)

1.1 The North Sea

The North Sea, as many coastal areas, is highly productive and provide valuable services that range from recreation to food supply (Jickells, 1998), but also, like many marine environments around the world, its coasts have been densely populated. Also the highly industrialized countries along its coast have increased the anthropogenic contributions of N (Weatherhead, 2015). Particularly in the North Sea, as a result of the use of fertilizer in agriculture, riverine N inputs increased from 202 to 918 kt N y^{-1} from 1950 to 1980 (Colijn *et al.,* 2002). The high loads of nutrients into the North Sea aroused concern between the countries of Europe due to the possible eutrophication of the maritime area and its nuisance effects in the marine environment. Thus, in order to battle the eutrophication, the Convention for the Protection of the Marine Environment (OSPAR) was established in 1992 and entered in force in 1998. The main goal of the OSPAR was to reduce the inputs of N by 50% with the purpose of achieving and maintaining a healthy marine environment (OSPAR, 2003). Even though recent studies showed that the figures of total N riverine inputs started to decrease (Fig. 1.1a; OSPAR, 2013), the figures for NO_3^- concentrations seems to be more erratic, with no significant reduction, and it still remains over the established value required to achieve a 50% reduction of the riverine inputs (Fig. 1.1b; Weatherhead, 2015).

Nutrient over enrichment is restricted to coastal areas, which are influenced by riverine discharges. However, although these areas represent between 5 to 10% of the North Sea area, and there are no higher concentrations of nutrients offshore (Beukema, 1986; Mc Quatter-Gollop *et al*., 2007) the effect of high loads of N into the North Sea have been linked to increases in productivity, changes on phytoplankton and microzooplankton structure, and to an increase of higher trophic levels (Colijn *et al*., 2002; OSPAR, 2009). Additionally, because N over enrichment stimulates organic matter production and consequently high organic matter fluxes to the bottom waters (Conley *et al.,* 2009), N over enrichment has also been linked to hypoxia in the German Bay, mainly because of the loads of fertilizers through riverine discharges (Diaz and Rosenberg, 2008), and along the eutrophic European mainland coasts, from Belgium to Norway (OSPAR, 2009). Hypoxia is currently not widespread in the North Sea, however, during summer most of the area shows a bottom water oxygen saturation lower than 75% (Fig 1.2a, Queste *et al.,* 2012). Also, low oxygen bottom water concentrations (65 to 206 μmol O_2 l^{-1}) have been observed in offshore waters at Oyster Grounds, North Dogger, and the central North Sea (Fig. 1.2b, Weston *et al.*, 2008; Greenwood *et al.,* 2010; Queste *et al.,* 2012). Although the low concentrations of O_2 is considered to be the result of natural processes (Weston *et al.,* 2008; Greenwood *et al.,* 2010; Queste *et al.,* 2012), under such events the N-cycling may be strongly altered (Conley *et al.,* 2011).

The role of O_2 in the processes of the N-cycling in the North Sea, was discussed by Neubacher *et al.* (2011; 2013) who found that while short periods of hypoxia reduced the oxygen penetration depth (OPD) by 50%, it increased denitrification rates, while anammox rates were

constant. However, under long periods of hypoxia, both processes increased, and N_2 production increased by up to 72%. It is important to keep in mind the effect of hypoxia on late summer, because it has been predicted that in 80 years, the North Sea, namely the Oyster Grounds, will be reduced by 24 μM its bottom water O_2 concentrations due to the climate change (Meire *et al.*, 2013). While, it seems that hypoxia may help to control the excess of N, others studies have found contrasting results that suggest that long periods of hypoxia may trigger the dissimilatory nitrate reduction to ammonium (DNRA). This process, unlike denitrification and anammox will release N to the water column, and may enhance a vicious cycle of eutrophication by recycling and releasing bioavailable N as NH_4^+, instead of removing the excess of N. (Jäntti and Hietanen, 2012).

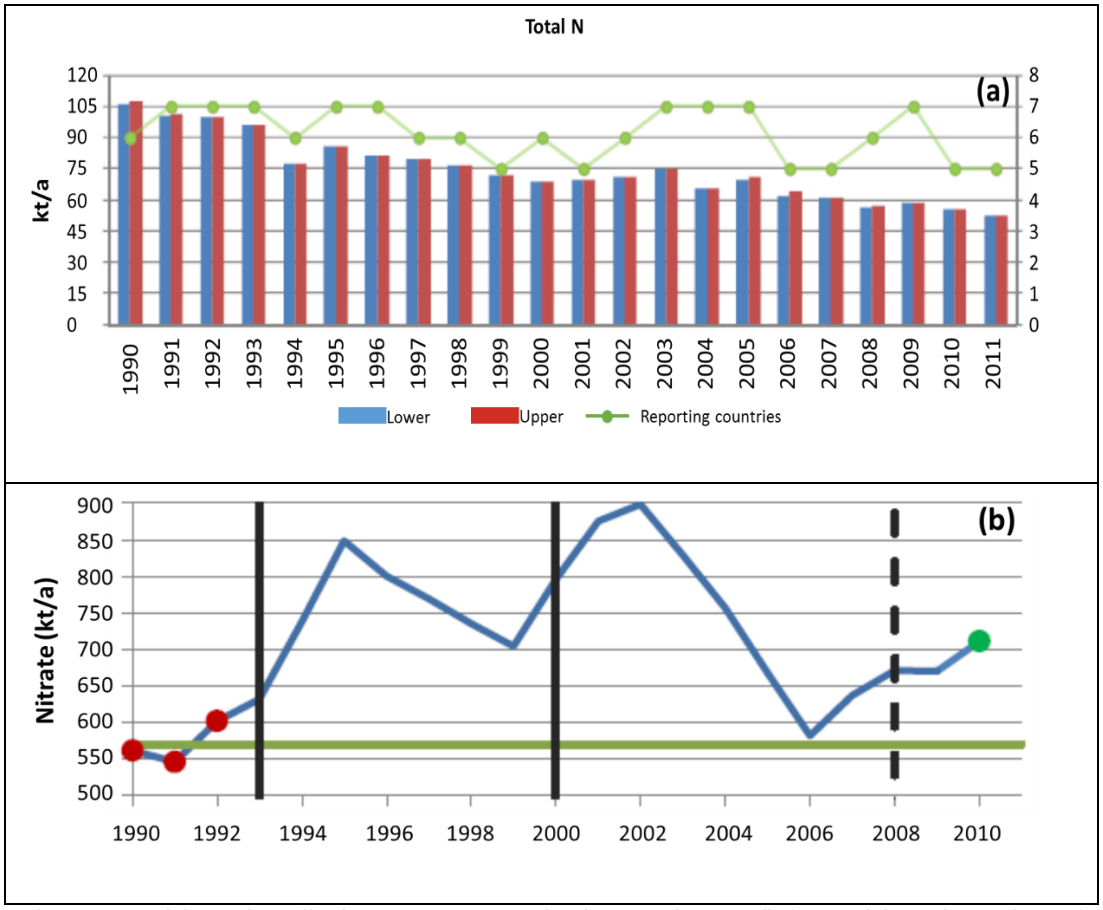


Figure 1.1. (a)Total N discharges per year in the North Sea (left y axis) and number of countries that reported the discharges (right y axis). Taken from (OSPAR, 2013).
(b) Riverine input of NO_3^- (blue line). Green line is the baseline which indicates the nutrients status prior to implementation of the Nitrates Directive (ND) and the Urban Waste-Water Treatment Directive (UWWTD). Black lines represent the start of the implementation action of UWWTD and ND (1993), and implementation action of WFD (2000). Black dotted line indicates implementation of Marine Strategy Framework Directive (2008). Reds dots mean no data available for Denmark and green dots mean no data available for Belgium. Taken from Weatherhead (2015).

The N cycle involves denitrification and anammox, biological processes that remove bioavailable N by transforming fixed nitrogen into gaseous N (N_2, or N_2O), which is not available for most primary producers (Howart, 1988), and thus affecting ecosystems and biological cycles at different scales (Codispoti and Richards, 1976; Nixon et al, 1981; Seitzinger, 1988, Revsbech and Sorensen, 1990). On a local scale, when the systems are low in N, denitrification and anammox may limit primary production, either by decreasing N concentrations or by reducing the N:P ratio. Thus in systems that receive considerable amounts of N, denitrification might help to control the degree of eutrophication (Seitzinger *et al*., 2006). On the other hand, dissimilatory nitrate reduction to ammonium (DNRA), conserves N as NH_4^+ in the ecosystems (Rysgaard *et al.,* 1996; An and Gardner, 2002; Rütting *et al.,* 2011). These processes will be discussed in detail in chapter 2.

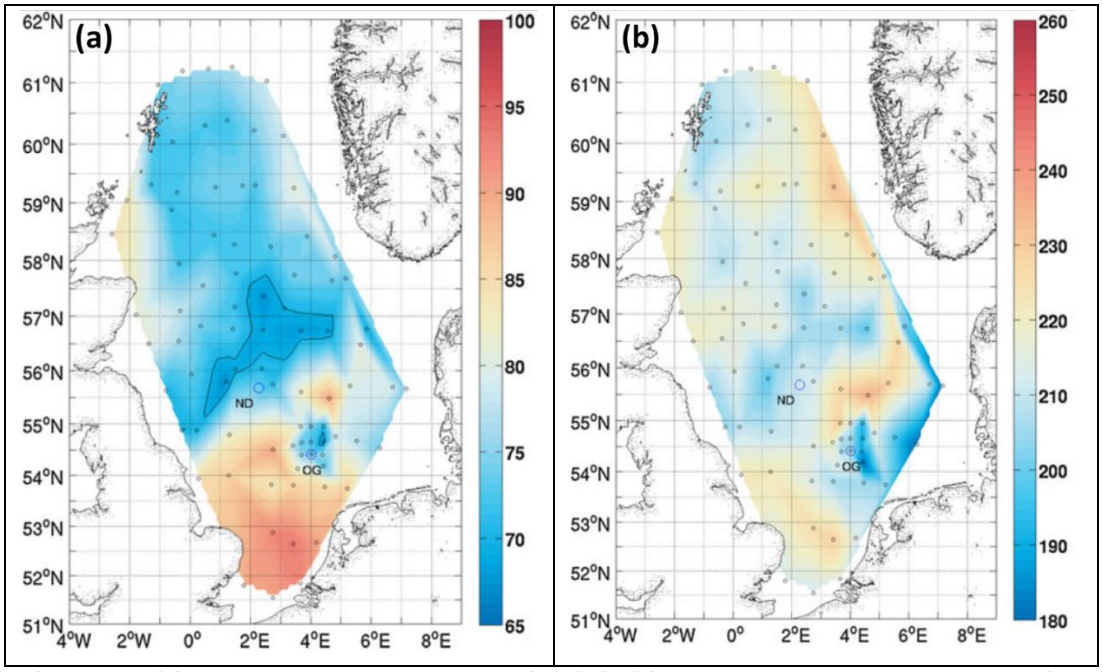


Figure 1.2. (a) Bottom water oxygen saturation (%). (b) Bottom water oxygen concentration (μmol l^{-1}). Taken from Queste *et al.* (2012).

Most of the studies about denitrification and anammox in the North Sea have been carried out on estuaries such as the Thames (Trimmer *et al.,* 2003; Trimmer 2006; Engström *et al.,* 2005), intertidal zones such as the Great Ouse River (Trimmer *et al.*, 1998), and in the Wadden Sea (Lohse *et al.,* 1993; Deek *et al.*, 2012). There are also studies on the deep waters of the Skagerrak and shallow water of Aarhus Bay, and German Bight (Lohse *et al.,* 1993; Thamdrup and Dalsgaard, 2000; Thamdrup and Dalsgaard, 2002). Studies in offshore waters of the North Sea have been carried out mainly in the central North Sea, namely in areas such as the Dogger Bank, Oyster Grounds, and Frisian Front (Neubacher *et al.,* 2011; Neubacher *et al.,* 2013, Lohse *et al.,* 1993). From those studies, it has emerged that the denitrification rates

range between 0 to 130 μmol N m^{-2} h^{-1}, and that for some places denitrification rates is an important pathway for N sink. For example, it has been estimated that up to 14% (16 kt N y^{-1}) of the annual Elbe river N load are removed from the north of the Wadden Sea (Deek *et al.,* 2012), while it has been estimated that about 23% of the total N inputs (8870 ± 4860 kT N y^{-1}) are removed by denitrification from the North Sea (Brion *et al.,* 2004).

Others studies have been focused on the availability of NO_3^-, and organic matter (Dalsgaard and Thamdrup, 2002; Nichols and Trimmer, 2009). Regarding the availability of NO_3^- and organic matter on the processes of the N-cycling, Nicholls and Trimmer (2009) found that anammox was positively correlated with both NO_3^- concentrations in the overlying water and with the content of organic matter in sediments. On the other hand, Dalsgaard *et al.* (2005), observed that although anammox may have higher rates in shallow waters, its relative importance to total N_2 production increases with depth where the availability of organic matter tends to decrease.

In coastal zones, the N-cycling is under the effect of diverse physical, chemical, and biological environmental factors such as substrate availability, oxygen concentration, organic matter supply and temperature (Seitzinger, 1988; Rysgaard *et al.,* 1994; Dalsgaard and Thamdrup, 2002). In spite of the increased number of studies focused on understanding the effect of the environmental factors on the N-cycling, there are still contrasting results, and it is not yet very well understood what is triggering that one of the processes of the N-cycling predominates over others. On the other hand, the complex characteristics in the environment affecting the processes involved in the N cycle might make it difficult to understand what are the factors driving the processes of the N-cycling that control the abundance of the fixed N.

It is important to understand what factors favoured one process over another because, although denitrification and anammox have the same impact on the N-cycling by removing N from the marine environment, the impacts on the C-cycling have different implication in the environment (Babbin and Ward, 2013). Denitrification release greenhouse gases (N_2O and CO_2) to the atmosphere, while anammox removes CO_2 and so far it is not known that during the process any greenhouse gas is released (Koeve and Kähler, 2010; Babbin *et al.,* 2013). DNRA, in turn may worsen eutrophic environmental conditions by recycling N instead of removing it. Therefore, understanding the biogeochemical processes of the N-cycling in the water column and particularly in the sediments, is of fundamental importance in marine ecosystems because of its potential to produce available N to control the rate of primary production (Ward, 1996) and consequently food web dynamics (Bashkin, 2002), or to produce a vicious cycle of eutrophication (Jäntti and Hietanen, 2012; Marchant *et al.*, 2014). Additionally, understanding the processes of the N-cycling will help to understand if the marine sediment is a sink or source of N.

1.2 Aims and objectives

The focus of this study was to investigate the main differences in the processes of the N-cycling in two different sites: (a) an intertidal area of The Wash and (b) deep sites of the North Sea. Additionally, the temporal (Wash), and spatial (North Sea) variation of the processes of the N-cycling was investigated.

The aims were the following:

- Investigate differences in the processes of the N-cycling in two sites: The Wash and the North Sea.
- Determine the predominant process of the N-cycling, both in The Wash and in the North Sea.
- Determine the effect of the variable oxygen penetration depth (OPD) on the processes of the N-cycling

1.3 Area of Study

1.3.1 The North Sea.

The North Sea is a marginal sea of the Atlantic Ocean located on the continental shelf of north-west Europe, it covers an area of about 750,000 km^2 making a volume of about 94,000 km^3. It opens into the Atlantic Ocean to the north, and through the English Channel to the south-west. Towards the East it communicates with the Baltic Sea through the Skagerrak (Fig. 1.3a). Its bottom depth is less than 30 m in the south and increases gradually toward the north to about 200 m, reaching more than 700m at the Norwegian Trench (Otto *et al*., 1990; Turrel and Bannister, 2003; Ducrotoy and Elliot, 2008).

The salinity of the North Sea is highly variable in areas close to rivers discharges, and the Baltic Sea (Rodhe *et al*., 2004). Areas of the southern North Sea, influenced by local fresh water (e.g. Thames, Rhine and Elbe estuaries), show the highest variability in salinity, which ranges from close to fresh water in the upper estuary, to very similar to the open sea at the mouth, while the northern Kattegat keep salinity as low as 10. Apart from that areas, beyond estuaries, the salinity of the North Sea, ranges between 32 and 34.5 (Otto *et al*., 1990).

The North Sea surface average temperatures range from 6°C (winter) to 17°C (summer).

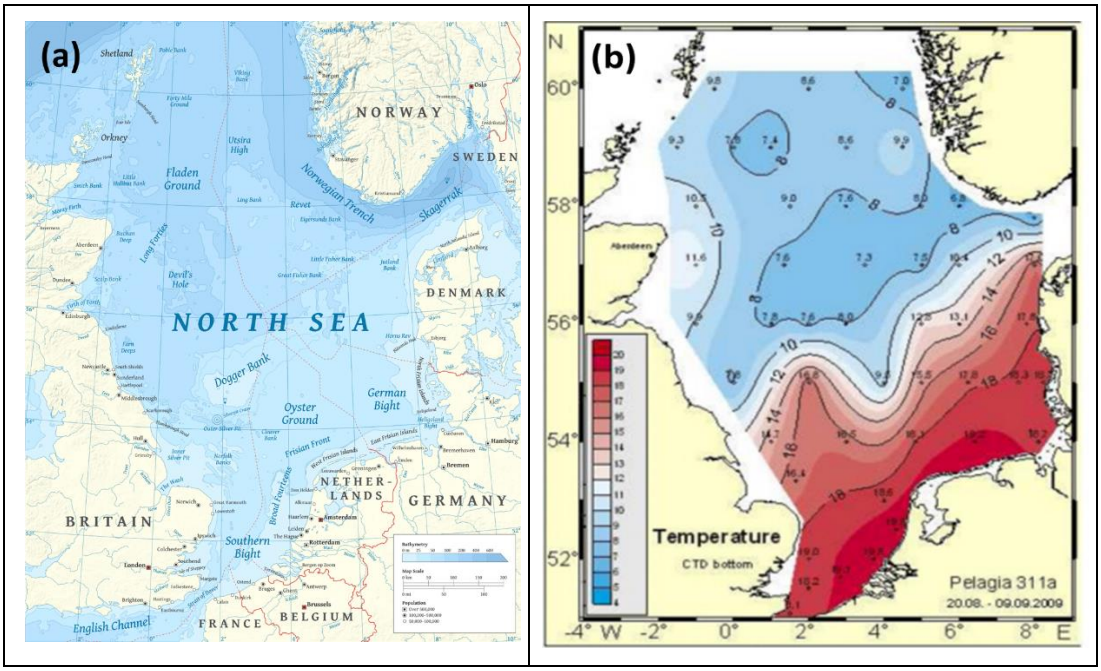


Figure 1.3. (a) The North Sea showing the bathymetry (https://en.wikipedia.org/wiki/File:North_Sea_map-en.png).
(b) bottom water temperature during summer (http://www.bsh.de/en/Marine_data/Observations/MURSYS_reporting_system/index.jsp).

The North Sea can be subdivided in two contrasting areas: the shallow southern North Sea, and the deeper northern North Sea (Howarth, 2001; Blass *et al*., 2001). The areas are separated by the isobath of 50 m that runs across the North Sea from the Flamborough Head on the English coast to Skagen on the northern tip of Denmark. The shallow parts of the southern North Sea and the Chanel are well-mixed through the year, while the deeper northern part becomes stratified (Reid *et al*., 1988). As heat inputs increase in the late spring, a thermocline is established and the North Sea separated into a southern part, characterized by higher temperatures, less dense, and well-mixed surface layer; and a stratified northern part where the temperatures remains low at the bottom (Fig. 1.3b). The depth of the thermocline varies both temporarily and spatially, increasing up to 50 m from May to September in the northern North Sea and up to 20 m in the western Channel (Ducrotoy *et al*., 2000).

1.3.2 The Wash

The Wash is located on the northwest margin of East Anglia (Fig. 1.4) on the east coast of England, and is mainly a shallow rectangular embayment that opens up into the southern North Sea. It has an area of about 666 km^2 making the Wash, the largest estuary in the UK and the second largest area of intertidal mud and sand flat which in total represent one third (225 km^2) of its area. The maximum depth is 50 m, but in general it has a mean depth of 10 m (Murby, 1997). The tidal regime of the Wash is semi-diurnal regime, it ranges from 3.5 to 6.5 m (Tsompanoglou *et al*., 2010). The rivers Witham, Welland, Nene, and Great Ouse, drain into the embayment of The Wash, with a total catchment area of around 6420 km^2, that makes a total flux of 32 $m^3\ s^{-1}$ (Nedwell *et al.,* 2002; Jickells *et al.,* 2014). Despite of the large inputs of fresh water, marine water dominates its physical characteristics, with mean salinity of 31, although in some particular areas (the Nene mouth) the salinity may be as low as 9. The temperature ranges between 4°C (February) to 22°C (July-August) with a mean temperature of ~13°C (Stringer, 2014). The annual fluvial load of total N is >150 mmol N y-1 (Nedwell *et al*., 2002).

Figure 1.4. The Wash location, showing rivers that drain the south and east midlands (https://en.wikipedia.org/wiki/The_Wash).

1.4 Thesis outline and structure

The chapters of this thesis are presented with their own section of methods, results and discussion

Chapter 2: The N cycle

Based-on literature review, this chapter provides a theoretical framework of reference under which it is possible to have a general approach about the N-cycling. Thus, in this chapter are described the processes of the N-cycling as well as their importance in marine environments. Additionally, it is described how the process of the N-cycling may be affected by factors such as temperature oxygen penetration depth (OPD), organic matter or nutrient availability.

Chapter 3: Rates of denitrification, anammox and DNRA

The purpose of this chapter was to determine the main processes of the N-cycling that take place in the sediments of The Wash and the North Sea. Therefore, this chapter provides rates of anammox denitrification and DNRA. Additionally, there is also a description of the spatial or temporal variation of these variables. It also includes a discussion about the possible causes that may have triggered or enhance one or another process.

Chapter 4: Oxygen penetration depth and effect on N-cycling process

In this chapter the OPD from two sampling sites: The Wash and the North Sea, will be compared and its possible effect in the processes of the N-cycling will be discussed. Also, the possible effect of some of the factors such as temperature and carbon supply on the availability of O_2, and hence in the OPD will be discussed.

Chapter 5: Nutrient fluxes

In order to better understand the processes described in chapter 3 and 4, this chapter provide an overview about how the sediment, might respond to the processes that sediments undergo. Hence, this chapter relates to possible processes that affect the shape of the vertical profile and fluxes of ammonium and nitrate, and how these variations may impact or be related with the processes of the N-cycling.

Chapter 6: Conclusions

In this chapter are summarized the main findings of this study. Additionally, built on the findings, it is suggested to carry out further works in order to improve our knowledge and understanding of the N-cycling.

Chapter 2. The N cycle

2.1 Background

The N cycle (Fig. 2.1) could be described as complex biological and chemical reactions by which N is converted between its different forms (Hall, 2009; Brandes *et al*., 2007). Through these processes N is also moved to different reservoirs on earth such as air (N_2), via photosynthesis or bacterial N uptake; N goes to living organisms as PON, or can be found dissolved in water either as inorganic or organic N (DIN and DON).

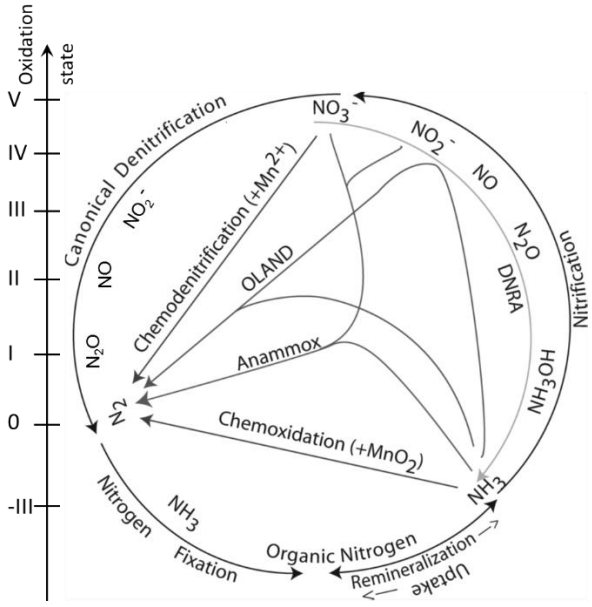


Figure 2.1. Diagram of N cycle (modified from Brandes *et al.*, 2007).

The different forms of nitrogen are interconnected by biological and chemical reactions, which can involve either assimilatory or dissimilatory processes (Zehr and Kudela, 2011). Both processes have different functions, thus, while assimilation involve the acquisition of inorganic N, such as NO_3^- or NH_4^+, and its conversion into organic forms required for growth and reproduction of organisms, dissimilatory transformations consist in producing and decomposing compounds available in the environment to produce energy needed for growing, motion and maintenance of organisms (Thamdrup and Dalsgaard, 2008). It has been estimated that through assimilatory processes about 30% of N released as NH_4^+ from mineralization can be removed in coastal sediments (Thamdrup and Dalsgaard, 2008). Dissimilatory processes, however, may not only remove N through denitrification, but also conserve it as NH_4^+ in the ecosystems through DNRA (Rütting *et al.,* 2011).

There are many processes involved in the N-cycling, but according to Thamdrup and Dalsgaard (2008), the balance between N_2 fixation, and the loss of fixed N to N_2 are the main

pathways that control the fixed N abundance. N_2 becomes bioavailable via fixation into biologically available NH_4^+ and thereby N_2 fixation is the main source of fixed N in the ocean. The process is carried out by photoautotrophic prokaryotes known as diazotrophs belonging to the groups of Archaea and Bacteria (Gruber, 2008: Lam *et al*., 2009; Zehr and Kudela, 2011). Once N_2 is available it is incorporated into organic matter and remains in marine ecosystems as PON such as the N assimilated into algal or microbial biomass (Burgin and Hamilton, 2007; Brandes *et al*., 2007; Lam *et al*., 2009). Virtually all the organic matter formed in the surface waters is respired or assimilated to form new biomass (Rullkötter, 2006). However, on shallow coastal waters, the coupling between pelagic and benthic compartments is tighter, so that about 25 to 50% of the organic matter produced by phytoplankton sinks to the benthos (Jorgensen, 1996; Glud, 2008). Particularly the flux of organic carbon in the North Sea, varies from 50 to 185 mg C m^{-2} d^{-1}, which represents between 20% and 35% of the primary production (Davies and Payne, 1984).

The sinking particles of organic matter deposited on the seabed undergo several complex biogeochemical reactions and it may either become buried or be mineralized, leading to nutrient regeneration (NO_3^-, NH_4^+ or N_2) through which PON is returned back to NO_3^- (Middelburg *et al.,* 1996; Hensen *et al.,* 2006). Organic matter degradation in marine sediments is driven by the redox potential of the oxidant, hence, the higher the potential of the oxidant, the higher the free energy yield for respiration (LaRowe and Van Cappellen*,* 2011). Thus, according to Froelich *et al*. (1979), the oxidants used in organic matter mineralization follow the sequence described below (equation 2.1) that corresponds to a gradual decrease in the production of energy per mole of organic carbon oxidized (Table 2.1; Jorgensen, 2006; LaRowe and Van Cappellen, 2011). Consequently, O_2 and NO_3^- are the most favorable electron acceptors in this sequence of oxidants. The sequence in which the oxidants appear in equation 2, indicates that they are utilized by bacteria in the order of decreasing energy per mol of carbon oxidized

$$O_2 \rightarrow NO_3^- \rightarrow Mn(IV) \rightarrow Fe\,(III) \rightarrow SO_4^{2-} \rightarrow CO_2 \quad (2.1)$$

Within the oxic sediments, organic matter is respired or mineralized and turned back into inorganic forms such as NH_4^+. The NH_4^+ released by deamination is oxidized to NO_3^- by aerobic nitrifying bacteria. During the nitrification, NH_4^+ is first oxidized to NO_2^- in a two steps process (equations 2.2, 2.3, and 2.4) with hydroxylamine as byproduct. Then, NO_2^- is oxidized to NO_3^-. The best known organisms mediating this process are *Nitrosomonas* and *Nitrobacter*, the former converts NH_4^+ into NO_2^-, while the second converts NO_2^- into NO_3^- (Hensen *et al.,* 2006; Gruber, 2008).

Table 2.1. Sequence of organic matter degradation with different electron acceptors.

Redox process	Electron acceptor	Oxidation Reaction	Free energy yield ΔG^0 (kJ mol^{-1})
Aerobic respiration	O_2	$(CH_2O)_{106}\,(NH_3)_{16}(H_3PO_4) + 138O_2 \rightarrow 106CO_2 + 16HNO_3 + 122H_2O + H_3PO_4$	-29.9
Nitrate reduction	NO_3^-	$(CH_2O)_{106}\,(NH_3)_{16}(H_3PO_4) + 84HNO_3 \rightarrow 42.4N_2 + 106CO_2 + 16NH_3 + 148.4\,H_2O$	-28.4
Manganese reduction	Mn-oxides	$(CH_2O)_{106}\,(NH_3)_{16}(H_3PO_4) + 236MnO_2 \rightarrow 236Mn^{2+} + 106CO_2 + 8N_2 + 366\,H_2O + H_3PO_4$	-23.3
Iron reduction	Fe-oxides	$(CH_2O)_{106}\,(NH_3)_{16}(H_3PO_4) + 212\,Fe_2O + 48\,H^+ \rightarrow 424Fe^{2+} + 106CO_2 + 16\,NH_3 + 530H_2O + H_3PO_4$	-10.1
Sulfate reduction	SO_4^-	$(CH_2O)_{106}\,(NH_3)_{16}(H_3PO_4) + 53SO_4 \rightarrow 53\,S^{2-} + 106CO_2 + 16NH_3 + 106\,H_2O + H_3PO_4$	-5.9
Methane reduction	Methane fermentation	$(CH_2O)_{106}\,(NH_3)_{16}(H_3PO_4) + 53SO_4 \rightarrow 53\,CH_4 + 53CO_2 + 16NH_3 + H_3PO_4$	-5.6

$2NH_3 + O_2 \rightarrow 2NH_2OH$ (2.2)

$2NH_2OH \rightarrow 2NO_2 + H_2O$ (2.3)

$NO_2^- + {}^1/_2\, O_2 + NO_3^-$ (2.4)

These inorganic forms of nitrogen can be assimilated again and enhance organic matter production, or can be lost from the microbial community through canonical denitrification (hereinafter called denitrification) process by being converted into gaseous end products. Denitrification is a process whereby NO_3^- is reduced to N_2 with N_2O as byproduct (equations 2.5, 2.6, 2.7). This process takes place below the O_2 penetration depth, where oxygen starts to be depleted, and NO_3^- is used as electron acceptor. There are two main sources of NO_3^- reaching the anoxic sediments. One is the NO_3^- produced by the nitrification in the sediments, and the other one is the NO_3^- from the overlying bottom water which reaches the anoxic sediment through bioturbation, bioirrigation, or diffusion (Hensel *et al.,* 2008).

$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$ (2.5)

$NO_2^- + 6H^+ + 4e^- \rightarrow N_2O + 3H_2O$ (2.6)

$N_2O + 2H^+ \rightarrow N_2 + 3H_2O$ (2.7)

It seems that under this model of N cycling the balance between N_2-fixation and denitrification, determines the bioavailability of N. However, it is widely recognized that anaerobic ammonium oxidation (anammox) has an important role in N-removal from the marine environment (Thamdrup and Dalsgaard, 2002: Thamdrup *et al.,* 2006). Anammox is a process mediated by bacteria belonging to *Planctomycetes*, which fix CO_2 using NO_2^- as electron acceptor. Unlike denitrification, N_2 formation by anammox involves the oxidation of NH_4^+ by NO_2^- under anaerobic conditions and the process encompasses hydroxylamine and hydrazine formation (van Degraaf, 1995; 1997). Apart from these processes that lead the removal of NO_3^- from the overlaying water, there is another process that does not result in N_2 formation. This is the dissimilatory nitrate reduction to ammonium (DNRA) which differs from the denitrification and anammox processes because it conserves N within the sediment. Eventually, NH_4^+ can be oxidized to NO_3^- and thus returned back to be assimilated into PON. DNRA is carried out by either fermentative bacteria that use C as electron donor or by chemolithoautotrophic organisms which use NO_3^- to oxidize inorganic substrates such as sulphide (equation 2.8 represents fermentative DNRA, and 2.9 represents autotrophic DNRA). The process entails the reduction of NO_3^- to NH_4^+ (Burgin and Hamilton, 2007; Giblin *et al.,* 2013).

$$CH_2O + 0.5NO_3^- + 0.5H_2O \rightarrow NH_4^+ + HCO_3^- \quad (2.8)$$

$$HS^- + NO_3^- + H^+ + H_2O \rightarrow SO_4^{2-} + NH_4^+ \quad (2.9)$$

2.2 Processes of the N cycling

2.2.1 Nitrogen fixation

Nitrogen fixation is a process in which N_2 gas is converted to fixed N species. N_2 gas is a molecule composed of two atoms of N, linked by a very strong triple bond. The general reaction for N_2 fixation can be represented in the following reaction:

$$N_2 + 8H^+ + 8e^- + 16\ ATP \rightarrow 2NH_3 + H_2 + 16\ ADP + 16\ P_i \quad (2.10)$$

Where *pi* is the orthophosphate ion.

The N_2 fixation can be achieved through a series of mechanisms that can be abiotic or biotic. The abiotic processes can be the result of atmospheric fixation by lightning flashes from thunderstorm, cosmic rays, and ultraviolet light. Another abiotic process is a chemical N_2 fixation, which involves an industrial production of fertilizers through the Haber-Bosch method, and nowadays, there is an average fertilizer input of more than 25 Kg N ha^{-1} y^{-1} (Erisman *et al.,* 2008; Galloway *et al*., 2008).

While the chemical N_2 fixation involves the burning of fossil fuels to obtain the high temperatures and pressures needed to reduce the N_2 molecule and convert it to ammonia, the biotic process involves organisms that derive the energy from the conversion of carbohydrates to reduce molecular N_2. But, the extremely stable triple bond of the N_2 molecule demands an equivalent of 16 ATP (800 kJ) per mole of N_2 to reduce (Postgate, 1982; Karl *et al.,* 2002; Dixon and Kahn, 2004; Dekas *et al.,* 2009). This huge amount of energy demanded to convert N_2 to NH_3, makes the biological N_2-fixation a process mediated by a select, but taxonomically diverse group of microbes that includes both autotrophs and heterotrophs (Herbert, 1999; Karl *et al.,* 2002; Zehr, 2011). The group is best represented in oligotrophic tropical and subtropical waters by *Trichodesmium spp., a* non-heterocystous cyanobacteria (Capone, 1988; Sohm *et al.,* 2011).

The N_2 fixation process plays a very important role in aquatic ecosystems by increasing the amount of bioavailable new N and affecting the N dynamic by adding up to 3% of the total riverine loading of N to the budget of a system (Piehler *et al*., 2002). Nitrogen fixation rates are variable and represent from 1% in oligotrophic lakes and marine sediments to 82% in eutrophic lakes (Howarth *et al.,* 1988).

Until recently oceanic N_2 fixation had been attributed to cyanobacteria *Trichodesmium*, however, new molecular techniques have provided new findings of potential N_2 fixers. The new findings showed that bacterioplankton was able to fix N_2 at rates similar to that of *Trichodesmium* (47 mmol N m^{-2} day^{-1}; Falcón *et al.,* 2004).

Regarding the estuaries, just few estuaries such as the Baltic Sea (Wallström et al, 1992; Moisander *et al.,* 2003; Stal *et al.,* 2003), the Peel-Harvey estuary (Huber, 1986) and the Neuse River (Piehler *et al.,* 2002) have been considered to have important N_2 fixation rates (0.93 to 128 mol N m^{-2} yr^{-1}). However, these rates have just been recorded when salinities are < 10 -12 (Howarth and Marino, 2006).

The conspicuous variation of N_2 fixation between ecosystems (from 0 to 80% of total nitrogen input to various systems) could be due to differences in the biogeochemical controls, such as nutrient concentration and loads, N:P loading ratios, and the presence of trace metals (Howarth *et al.,* 1988), as well as physicochemical parameters, such as light, temperature pH, and oxygen (Capone, 1988). For example, Horne and Goldman (1972) found that the heterocysts formation by cyanobacteria were suppressed at 0.14 – 0.16 μM of NO_3^- and 1.4 – 12 of μM NH_4^+. So this substances control rates of nitrogen fixation, because they suppress the synthesis of the nitrogenase enzyme (Howarth, 1988). On other hand it is thought that the tendency toward lowers rates of N_2 fixation in estuaries and coastal seas is maybe due in part to a lower availability in seawater of one or more trace elements required for N_2 fixation. Both molybdenum (Mo) and iron (Fe) are essential components of nitrogenase, but the requirements of Mo in N_2 fixation is not absolute (Howarth, 1988) inasmuch as alternative nitrogenase based on vanadium has been isolated from *Azotobacter chrooccocum* (Robson *et al.,* 1986). In marine ecosystems, Mo can be more limiting even though its concentration could be higher; Its lower availability is because sulfate can inhibit the Mo assimilation (Howath, 1988).

2.2.2 Nitrification

Nitrification represents the autotrophic biological oxidation of ammonia with oxygen to nitrite followed by further oxidation to nitrate (equations 2.11 and 2.12).

$$2NH_4^+ + 3O_2 + 1.5O_2 \rightarrow 2NO_2 + H_2O + 4H^+ \quad (2.11)$$

$$NO_2^- + 0.5\, O_2 \rightarrow NO_3^- \quad (2.12)$$

The end products of nitrification can subsequently be reduced to N_2 through the denitrification process (Jenkins and Kemp, 1984). Nitrification is particularly important in estuarine sediments where it is coupled to denitrification.

Coupled nitrification-denitrification has been used to explain the occurrence of fixed N removal in oxic environments. It can also be used to explain constant or even increasing NO_3^- concentrations in regions where denitrification is thought to be occurring (Codispoti and Christensen, 1985). However, both processes should be occurring at different depths of the sediment because, while nitrification is an aerobic process, denitrification is an anaerobic one.

2.2.3 Denitrification

Denitrification is a heterotrophic process that involves a form of anaerobic microbial respiration which consists of organic matter oxidation using nitrate and/or nitrite as electron acceptor (Herbert, 1999; Childs *et al.*, 2002) (equation 2.13).

$$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 94.4HNO_3 \rightarrow 106CO_2 + 55.2\,N_2 + H_3PO_4 + 177.2\,H_2O \quad (2.13)$$

In this process NO_3^- and NO_2^- are reduced to N_2 through a series of intermediate N compounds such as nitrogen monoxide (NO) and N_2O (Groffman *et al*., 2006). Then N_2 recirculates into the N cycle through N_2-fixation to come back to ammonium (Newton, 2006). Denitrifying bacteria are facultative anaerobes, capable of using either the nitrogen oxides or oxygen in respiration (Dalsgaard and Bak, 1994; Rich *et al.,* 2003). The bacteria switch to denitrification under suboxic (< 2 mg O_2 l^{-1}) or anoxic conditions.

Denitrification provides an important sink in the global N budget and therefore is considered to play an important role in removing the available N from natural and human altered ecosystems (Rysgaard *et al*., 1995; Seitzinger *et al.,* 2006). At a global scale the denitrification may control the amount of fixed N in the world ocean and in turn regulates primary production and the production of CO_2 (Altabet *et al.,* 2002). At a local and regional scale denitrification removes fixed N that otherwise would be available for primary production. In low N systems denitrification contributes to N limitation, decreasing N concentration and by reducing the N:P ratio of the recycled N. However, in systems highly enriched with N from anthropogenic sources, denitrification is capable of removing significant quantities of NO_3^- from the water column reducing the export of N and thus eutrophication (Nedwell and Walker, 1995; Seitzinger, 1988; Seitzinger *et al*., 2006). For example, in estuarine and coastal waters, denitrification rates range between 0 to 1,067 μmol N m^{-2} h^{-1}, with the highest rates reported for sediment from eutrophic areas such as Tama and Tejo estuaries, where denitrification rates are 594 and 1,067 μmol N m^{-2} h^{-1} respectively (Seitzinger, 1988).

So far it is not totally understood the influence of environmental factors on the processes regulating denitrification. However, in general it is said that what controls denitrification are NO_3^- availability, oxygen concentration and organic matter (Seitzinger *et al*., 1991; Seitzinger *et al*. 2006). The main sources for denitrification come from the NO_3^- diffusing into the sediments from the water column and from the nitrate produced into the sediments via nitrification of NH_4^+ released from benthic oxidation of organic matter (Seitzinger, 1988; Nielsen, 1992; Rysgaard *et al*., 1995; Steingruber *et al*., 2001). Hereafter, when the source for denitrification be the NO_3^- diffusing from the water column, denitrification will be called Dw, while if the source of NO_3^- is through nitrification, it will be called either Dn, or coupled nitrification-denitrification process.

Where the source of NO_3^- used for denitrification come from seems to depends on the eutrophic state of the system, which at the same time is related with oxygen dynamic and organic matter loading into the coastal areas (Dong *et al.,* 2006). Organic matter is one of the most important variables controlling denitrification. It is especially important in coastal waters because about 25 to 50% of the primary production reaches the sediment (Jorgensen, 1996; Glud, 2008). Organic matter can increase the rates of sediment metabolism and O_2 demands (Glud, 2008; Elridge and Morse, 2008; Jickells and Weston, 2011) in such a way that when the dissolved oxygen drops below 2 mg l^{-1} O_2 diffusion into the sediment decreases (Neubacher *et al.,* 2011). When oxygen penetration into the sediment is reduced, NO_3^- produced in the sediment by nitrification is also reduced and thereby reducing coupled nitrification-denitrification rates (Cornwell *et al.,* 1999), but total denitrification increases (Neubacher *et al.,* 2011). The fact that denitrification depends on suboxic conditions (2 mg l^{-1} O_2; Cornwell *et al*., 1999, Howarth, 1988, Seitzinger *et al*., 2006) and NO_3^- (Nielsen *et al*., 1990), while nitrification requires O_2 restrict the coupled nitrification-denitrification to be carried out only if the scales of interaction between oxic and anoxic conditions is small enough in space (< cm) to allow a tight coupling between nitrification and the coupled nitrification-denitrification processes (Seitzinger *et al*., 2006). In other words, the OPD, should allow nitrification in sediment and at the same time should allow that the NO_3^- from nitrification diffuses to the anoxic zone. An increased O_2 penetration into the sediment will enhance coupled nitrification-denitrification rates, but will prevent the NO_3^- diffusion from the overlying water due to an increased diffusive path length between the sediment surface and anoxic sediment horizons (Rysgaard *et al.,* 1995; Cornwell, 1999).

On the other hand, despite the fact that low concentration of O_2 can indirectly inhibit Dn and decrease nitrification, increased denitrification rates have been observed in eutrophic coastal systems. Some studies have reported that estuaries with high concentration of NO_3^- have higher denitrification rates associated with N_2 and N_2O production (Pelegri, *et al.,* 1994; Kana *et al*., 1998; Dong *et al*., 2006; Seitzinger *et al*., 1993, Seitzinger *et al*., 2006). This is possible because in eutrophic systems, N loading is high (Nixon and Buckley, 2002, Howarth *et al*.,

2000, Howarth and Marino, 2006; Dong *et al*., 2006) and turn into available sources of NO_3^- for denitrification which uses NO_3^- diffusing from the overlying water (Cornwell *et al.,* 1999).

Denitrification can also be affected by benthic microalgae and by burrowing infauna. Both can affect the oxygen and nitrogen dynamics within the sediment. It was mentioned before that O_2 penetration into the sediment prevents the diffusion of NO_3^- from the overlaying water, but enhances nitrification and thereby the coupled nitrification-denitrification. Such effect was observed during the day, as a result of benthic microalgae oxygen production (Risgaard-Petersen *et al*., 1994; Rysgaard *et al*., 1995), but the photosynthetic activity of benthic microalgae can also reduce the activity of coupled nitrification-denitrification when NO_3^- and NH_4^+ concentrations are low. This is because of the competition for inorganic N between nitrifiers and benthic microalgae, which is particularly intense during periods of illumination (Henriksen and Kemp, 1988). Moreover, benthic microalgae can assimilate NO_3^- and NH_4^+ at high rates for up to 60 h after sediment has darkened, all of which reduce nitrification and thereby the coupled nitrification-denitrification (Rysgaard *et al*., 1995). However, when high concentrations of NO_3^- and NH_4^+ are present in the overlaying water, the competition between benthic microalgae and nitrifiers for the NH_4^+ is reduced inasmuch as there is enough source of N for the benthic assimilatory demand, so the oxygen production by benthic microalgae, stimulates the nitrification-denitrification (Rysgaard *et al.,* 1995).

The benthic animal activities and consequently the bioturbation profoundly affect the structure and geochemistry of marine sediments (Smith and Rabouille, 2002). Near shore sediments support large populations of burrowing macrofauna which are able of building burrows within the upper 10 to 100 cm of the sediment column creating a relatively more oxidized sediment compartment (Koretsky *et al.,* 2002). It is well documented that in high fluxes of organic matter, as is usual in eutrophic estuaries, organic loading and oxygen stress cause a reduction in the abundance, body size and burrowing depths of the macrobenthos reducing the penetration depth of oxygen into the sediment (Smith and Rabouille, 2002).

Besides of the supply of NO_3^-, organic matter and oxygen concentration, denitrification also depends on temperature, (Seitzinger, 1988; Seitzinger *et al*., 2006). Studies considering the effect of temperature on denitrification rates have been controversial, some of these studies have shown increasing rates with increasing temperatures, while others have not shown statistical differences in the rate of NO_3^- uptake. This controversial result could be due to the difficulty of considering just the effect of temperature because as it changes, other factors such as nitrification rate and oxygen concentration, could also be affected (Seitzinger, 1988).

2.2.4 Anammox

Anaerobic ammonium oxidation (anammox) is a recently unveiled process of the N-cycle that involves the loss of reactive N through the anaerobic oxidation of NH_4^+ to N_2 using NO_2^- as electron acceptor to obtain the energy necessary to fix CO_2 (Mulder *et al.,* 1995; Van de Graaf *et al.,* 1995; Strous *et al.,* 1999a; Güven *et al.,* 2005; Penton *et al.,* 2006) (equation 2.14).

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 0.26\ NO_3^- + 1.02\ N_2 + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (2.14)$$

The anammox process was first uncovered in wastewater (Mulder *et al.,* 1995) and it was demonstrated that through this process it was possible to remove NH_4^+ under ananerobic conditions (Mulder *et al.,* 1995; Van De Graaf *et al.,* 1995; Strous *et al.*, 1999a; Kuenen and Jetten, 2001), but it was not until this process was first recorded in natural marine environments (Thamdrup and Dalsgaard, 2002) that its role in the nitrogen cycle was noticed. Nowadays, it is recognized that anammox has an important role in the marine nitrogen cycle as a process able to contribute more than 50% to the removal of fixed nitrogen forms (Thamdrup and Dalsgaard, 2002; Galán *et al.,* 2009; Jensen *et al.,* 2008). Since then, research groups around the world have generated more than 700 publications (Zhu *et al.,* 2010) trying to elucidate biodiversity, metabolism, distribution, and controls on the anammox process.

The process is carried out by a specialized group of anaerobic chemolitothrophic coccoid bacteria belonging to the order of *Planctomycetales* (Shivaraman and Shivaraman, 2003; Amano *et al.,* 2007; van Niftrik, 2004). This group not only is able to oxidize NH_4^+ with NO_2^- (equation 2.14), but their versatile metabolism allows them to use some organic compounds as electron donors to reduce NO_2^- to N_2. Anammox bacteria grow slowly, with doubling times of about 9 to 11 days (Strous *et al*., 1999b), but the mixotrophic condition gives anammox bacteria an advantage, because they can increase their growth rate or yield (Güven *et al.,* 2005) and can contend with heterotrophic denitrifiers (Kartal *et al.,* 2007a, 2007b; Strous *et al.,* 2006). Additionally, it has been shown that anammox bacteria are able to reduce NO_3^- to NO_2^- to produce N_2 (Güven *et al.,* 2005). Moreover, it is also believed that anammox bacteria can reduce NO_3^- into NH_4^+ and then reducing it to N_2 by using NO_2^- (Fig. 1.4; Simon, 2002; Güven *et al.,* 2005; Kartal *et al.,* 2007a; 2007b). The last process has been shown to occur even in the absence of dissimilatory nitrate reducers which represent another advantage because this strategy allows anammox bacteria to survive even under NH_4^+ limited conditions, since anammox bacteria do not depend on the NO_2^- or NH_4^+ production by other organisms (Kartal *et al.,* 2007a. 2007b).

Although, both denitrification and anammox form N_2 as the end product, the processes have quite different pathways. Denitrification is an anaerobic process where NO_3^- acts as the

terminal electron to oxidize organic matter, and its N_2 end product is the result of the combination of two molecules of NO_3^- (Burgin and Hamilton, 2007; equation 2.13). Anammox (equation 2.14, Fig. 2.2) produces N_2 through a one-to-one coupling of NH_4^+ and NO_2^- (Van De Graaf etal. 1995). Moreover, the process, involves the production of hydrazine (N_2H_4) as the result of NH_4^+ oxidation, and hydroxilamine (NH_2OH) as the product of NO_2^- reduction (Shalk *et al.,* 1998; Jetten *et al.,* 1999; Van De Graaf, 1997).

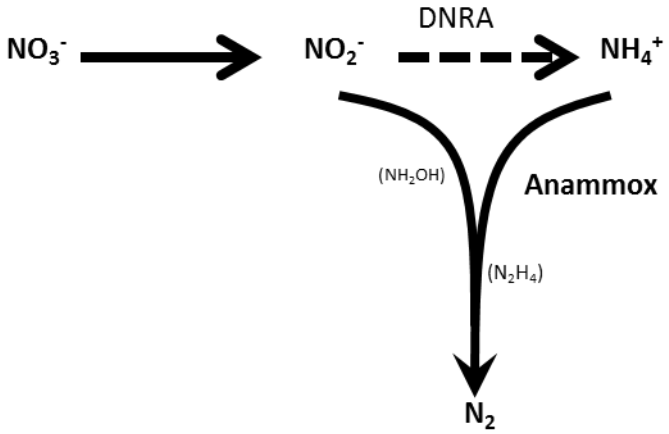


Figure 2.2. Possible route for nitrate reduction by anammox bacteria (modified from Kartal *et al.,* 2007 and Shalk *et al.,* 1998).

2.2.5 OLAND

Oxygen-limited autotrophic nitrification-denitrification (OLAND) is a process for autotrophic ammonium removal consisting of two steps: aerobic nitrification of NH_4^+ to NO_2^- or NO_3^- with oxygen as electron acceptor (equation 2.15), and an anaerobic denitrification of NO_2^- or NO_3^- to gaseous N_2 with NH_4^+ (equation 2.16) as the electron donor (Kuai and Verstraete, 1998).

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+ \quad (2.15)$$

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad (2.16)$$

The reaction is carried out by ammonium oxidizer, not by nitrite oxidizers and unlike the anammox, the NO_2 consumed in the second reaction of OLAND is produced during the first step of the reaction, whereas anammox consumed ambient NO_2 and/or NO_2 produced as an intermediate of heterotrophic denitrification (Kuai and Verstraete, 1998).

2.2.6 Manganese and chemo-denitrification

Another alternative pathway for both nitrification and denitrification involve redox metals as catalysts (equations 2.17 and 2.18). Manganese oxides are considered as strong environment oxidants for organic matter decomposition (Luther and Popp, 2002), which has an important implication in the N cycle providing alternative pathways for N_2 production or the nitrification process (Fig. 2.3; Aller, 1990, Murray, 1995; Hulth, 1999; Luther *et al*., 1997; Luther and Popp, 2002; Newton, 2006).

The N_2 production process involves two reactions: the anaerobic oxidation of NH_4^+ by manganese dioxide (MnO_2), and the reduction of NO_3^- by Mn^{2+} in anaerobic conditions (Aller, 1990; Murray *et al.,* 1995; Schulz, *et al.,*1994).

$$3MnO_2 + 2NH_4^+ + 4\,H^+ \rightarrow 3Mn^{2+} + N_2 + 6H_2O \quad (2.17)$$

$$5Mn^{2+} + 2NO_3^- + 4H_2O \rightarrow 5MnO_{2(solid)} + N_2 + 8H^+ \quad (2.18)$$

The nitrification process mediated by Mn involves the oxidation of NH_4^+ by MnO_2 in anaerobic sediments (equation 2.19).

$$4MnO_2 + NH_4^+ + 6H^+ \rightarrow 4Mn^{2+} + NO_3^- + 5H_2O \quad (2.19)$$

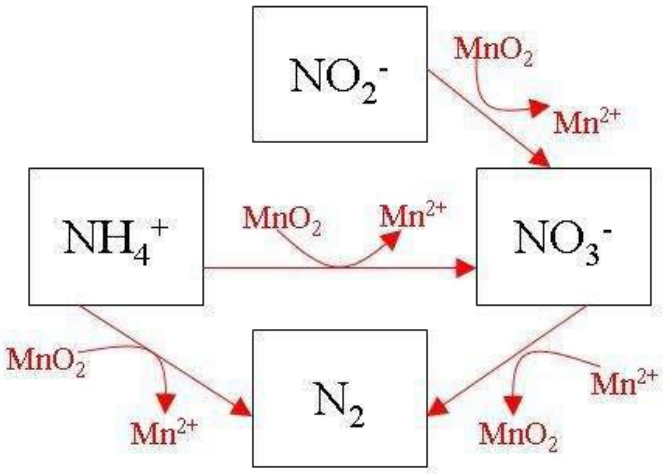


Figure 2.3. Schematic diagram of proposed Mn-catalyzed reactions involved in nitrogen cycling. All reactions are labeled with the corresponding reduction or oxidation of Mn species. All catalyzed reactions couple to Mn reduction except denitrification from NO_3^- (taken from Newton, 2006).

Although the coupling between the Mn cycle and the N cycle has been cited as an alternative pathway for either fixed N removal and N_2 production, or for nitrification processes, the role of Mn in the N cycle is still controversial because, while some authors say that in manganese rich sediments N_2 production can account up to 90% (example: Luther and Popp, 2002; Engström *et al*., 2005), others had not found evidence of the role of the Mn in the N_2 production process (example: Thamdrup and Dalsgaard, 2000). In a recent study Bartlett *et al.* (2008) proposed a new reaction taking place in the sediments. Through this reaction the anoxic nitrification of NH_4^+ released from organic matter is coupled to the Mn reduction. Then, the NO_3^- released can produce N_2 either via denitrification or coupled to Mn oxidation (Fig. 2.4). According to the authors, anoxic nitrification would not be significant in sediments rich in Mn, if Mn oxides are not redistributed in such a way that they reach the anoxic layer where it is possible to oxidize NH_4^+ to NO_3^-. This hypothesis was supported because during the experiments the authors observed accumulation of NO_3^-, NO_2^-, and N_2, and a decrease in NH_4^+ concentration and the increase of Mn^{2+} concentration.

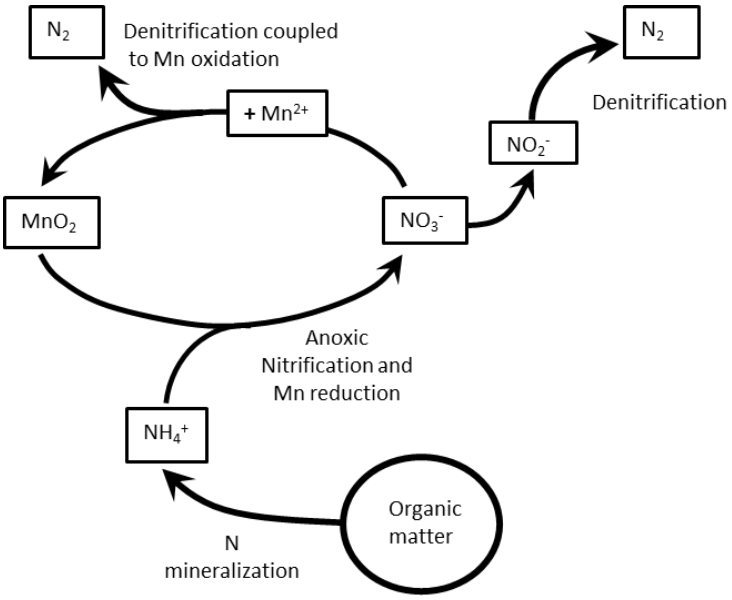


Figure 2.4. Reaction for anoxic nitrification (Modified from Bartlett *et al.,* 2008).

2.2.7 DNRA

The dissimilatory nitrate reduction to ammonium (DNRA) is also known as NO_3^- ammonification and respiratory reduction of NO_3^- to NH_4^+. There are two recognized DNRA pathways. One is a fermentative pathway through which NO_3^- is reduced to NO_2^- followed by NO_2^- reduction to NH_4^+, while the other one is a process that couples the reduction of NO_3^- to the oxidation of reduced sulfur forms (An and Gardner, 2002; Burgin and Hamilton, 2007; Mohan and Cole, 2007).The distribution of denitrifying or NO_3^- ammonifying bacteria in the estuarine sediments is going to be determined by certain physico-chemical parameters (Jorgensen, 1989) as well as by the oxidation level of the electron donor (King and Nedwell, 1985; Samuelsson, 1985). Hereafter, bacteria that carry out denitrification and DNRA will be called denitrifiers and ammonifiers respectively.

Both DNRA and denitrification can develop under similar environmental conditions, such as anoxia, available nitrate and organic substrate (Burgin and Hamilton, 2007), but, the fermentative DNRA is favored in NO_3^- limited environments, rich in labile carbon (Burgin and Hamilton, 2007). Under this scenario, organisms that use electron acceptor more efficiently are favored. DNRA transfers eight electrons per mole of NO_3^- while denitrification only transfers five electrons per mol of NO_3^- reduced, so DNRA is favored under high labile carbon availability (Burgin and Hamilton, 2007). On the other hand, in reduced sediment receiving high concentration of NO_3^-, the redox potential is changing because of the oxidation capacity of NO_3^-. In such environments denitrifiers are favored relative to NO_3^- ammonifiers. However, environments with lower NO_3^- concentrations, low redox conditions and increasing sulfate (SO_4^{2-}) reduction stimulate DNRA (Jorgensen, 1989). Other reason to increase DNRA in such kind of environments is that the K_m (inverse of substrate affinity) of ammonifiers is higher (100 to 500 uM NO_3^-) than denitrifiers (5 to 10 uM NO_3^-) and it makes them more competitive under NO_3^- free conditions (Jorgensen, 1989).

The pathway linked to sulfur oxidation is a chemolithoautotrophic process that couples the reduction of NO_3^- to oxidation of sulfur forms (Brunet and Garcia, 1996; Otte *et al.,* 1999). The pathway involves the reduction of either NO_3^- to NH_4^+ as a form of DNRA or to N_2 as a form of denitrification (Zopfi, 2001). Also, it is thought that the enzymes that sustain respiratory denitrification are inhibited in the presence of sulfide (Jorgensen, 1989; Burgin and Hamilton, 2007).

2.3 Factors influencing N transformations

Under the model of the N-cycling, the balance between N_2-fixation and denitrification, determines the bioavailability of N and therefore the N cycle is of fundamental importance for sustaining life in the sea (Ward, 1996; Seitzinger *et al*., 2006; Gruber, 2008). However, within the sediment, the N-cycling is controlled mainly by microorganisms which depending on the redox conditions, carry out oxidative or reductive processes that at the same time can lead to several potential end products of NO_3^- (Burgin and Hamilton, 2007) such as: N_2, nitric oxide (NO), nitrous oxide (N_2O) or NH_4^+. Because the redox conditions in the pore water of the sediments are controlled by physical, chemical and biological factors, the N-cycling is under the influence of salinity, pH, O_2 concentrations, substrate availability (DOC, NO_3^-, NO_2^-, NH_4^+) and temperature (Seitzinger, 1988; Rysgaard *et al.,* 1994; Daalsgaard, 2002; Joye and Anderson, 2008). Therefore, the influence of each factor and the complex characteristics in the environment affecting on the processes involved in the N cycle will allow one of these to predominate and to control the abundance of fixed N.

2.3.1 Salinity

The salinity regime of coastal systems varies in response to some factors such as circulation, tidal and wind mixing, or fresh water discharges and these changes in salinity influence the N cycle (Marks, 2010). It could be said that salinity affects the benthic nitrogen cycle in two main ways that have as a consequence a decrease in the nitrification and consequently a decrease in the coupled nitrification-denitrification process (Boatman and Murray, 1982; Seitzinger, 1998; Gardner *et al*., 1991; Rysgaard *et al*., 1999). It has been demonstrated that salinity favours the ion pair formation between NH_4^+ and seawater anions (mainly Na^+ and Mg^+; Boatman and Murray, 1982). This mechanism triggers a series of related processes. First, NH_4^+ reduces both the polarity and its adsorption to the sediment. Consequently, the timing of the N release is altered hence NH_4^+ flux out of the sediment increases. Finally, as a result of this, no NH_4^+ is available for nitrification in the sediments and thus the coupled nitrification-denitrification and the denitrification processes are affected (Gardner *et al*., 1991). Another way by which salinity affects the processes of the N cycle is through the physiological activity of microorganisms that mediate the nitrification and denitrification processes (Rysgaard *et al.,* 1999). There is some evidence supporting that in estuarine water, the concentration of sulphate (SO_4^{2-}) is higher than in freshwater (10-20 uM to 30 mM). The magnitude of the concentration is important because under anaerobic conditions SO_4^{2-} is reduced to sulphide (Joye and Hollibaugh, 1995), which has been demonstrated to inhibit nitrifying and denitrifying bacteria (An and Gardner, 2002). Others studies supporting these findings are Giblin *et al*. (2010) and Weston *et al*. (2010) who found that under salinity of 8 psu of the overlying water, NH_4^+ flux directed from the sediment to the water column, is up to two to four times higher (3.8

to 4.9 mmol m^{-2} day^{-1}) than at salinity of 0 psu. Similarly, Giblin *et al*. (2010) noticed that the coupled nitrification-denitrification was the dominant process under low salinity and total denitrification was related negatively to salinity, although no statistical significance was found. In contrast DNRA rates were related positively with salinity. Besides, unlike salt marsh sediment, fresh marsh sediment under the same treatments have showed high denitrification rates at 0 psu (373 mg N_2O-N Kg^{-1} d^{-1}) and lowest at 35 psu (99.7 mg N_2O-N Kg^{-1} d^{-1}; Marks, 2010). This may suggest that the different microorganisms present different tolerance to salinity changes.

Despite of some evidence supporting that salinity exerts a physiological effect on denitrifying and nitrifying bacteria, others studies have found no effect on the processes. For example, Marks (2010) showed that denitrification rates of salt marsh sediment, under two short-term (2 days) and different regimen of salinity (0 and 35) decrease at higher salinity during phase 1 (24 h) from 18.1 to 14.1 mg N_2O-N Kg^{-1} d^{-1}. This result is consistent with the findings mentioned above. However, during phase 2 (24 to 48 h) highest denitrification rates were observed at 35 psu (155 mg N_2O-N Kg^{-1} d^{-1}) and the lowest at 0 psu (8 mg N_2O-N Kg^{-1} d^{-1}). In contrast, during phase 1 (24 h) of a long-term experiment (11 days), sediment from the same sites under three different regimen of salinity (0, 15, and 35), showed low denitrification rates at low salinity (0 psu; 1.09 mg N_2O-N Kg^{-1} d^{-1}), whereas the highest was observed at 35 psu (59.5 mg N_2O-N Kg^{-1} d^{-1}). Nonetheless, during the second phase (3 to 11 days) the denitrification rates rebound as in the short-term experiment, but sediment at salinity of 0 still have the lowest, while sediment at 35 psu showed denitrification as high as 615 mg N_2O-N Kg^{-1} d^{-1}. Similarly, Magalhaes *et al*. (2005) found no significant difference in denitrification rates in three different salinity treatments (0, 15, 30) which showed that denitrification rates ranged between 6 to 7 nmol N_2 g wet weight h^{-1}. Additionally, they found an inverse relation between NO_3^- concentration measured at time zero and salinity. According to the authors, this suggests that NO_3^- concentration might be diluted by the intrusion of high salinity water and thus denitrification rates decrease because of NO_3^- concentration decrease, rather than an increase in salinity.

2.3.2 Oxygen

Benthic oxygen concentration plays an important role not only for benthic communities, but because it regulates many biogeochemical process that take place in the sediment such as the N cycle (Cai and Sayles, 1996; Glud, 2008). This process is regulated mainly by the concentration of oxygen which might have either negative or positive effect on them. Regarding the former, O_2 has an inhibitory effect on N-fixation, anammox, and DNRA, but unlike these, nitrification of ammonium requires O_2. Unlike the other processes, ammonification can be found both in anoxic and oxic conditions, and denitrification occurs under anaerobic conditions, since denitrifiers bacteria are able to respire in anaerobic conditions using NO_3^- as electron acceptor. Thus, these processes are not considerably affected by O_2 (Joye and Anderson, 2008). Thus oxygen availability impacts the N cycle by determining the activity of microbial assemblages present in the soil (Marks, 2010).

The concentration of O_2 in the pore water of the sediments is regulated by biological and physical process such as sedimentation rates of organic matter, bottom water O_2 concentration, biological activity, light and sediment permeability (Glud, 2008). Physical properties of the sediments such as porosity are strongly linked to permeability, which describes how easy a fluid flow through a porous medium (Breitzke, 2006). High permeability sediments enhance fluid exchange between sediment and the overlying water, while in sediments characterized by low permeability, biological process play an important role in pore fluid transport (Joye and Anderson, 2008). When sediments are light-exposed, O_2 is produced by biological processes such as benthic primary production. The released O_2 might be transported either by advective or diffusive processes into the sediment and thereby modifies the vertical distribution of oxygen over scales of microns or centimetres. On the other hand, bioturbation due to tubes or burrows constructed by macrobenthos inhabiting the sediments, modify the distribution of oxygen over scales of centimetres to tens of centimetres (Koretsky *et al.,* 2002; Joye and Anderson, 2008). Since macrobenthos may either irrigate with O_2 rich overlying water or introduce organic matter, their activity alters sedimentary solute profiles, reacting rates distribution and finally, because of the introduction of O_2, the area of oxic-anoxic boundaries increases. As a result of that there is a change in the oxidation-reduction reactions and thus the N cycle is altered (Aller, 1988). In general, very well oxygenated burrows represent sites of nitrification (Aller, 1988) which may favour the coupled nitrification-denitrification process. On the other hand, high inputs of organic matter into deeper sediment layers stimulate mineralization and results in anoxia (Hensen, *et al.,* 2006).

It has also been demonstrated that under hypoxia (<4 ml O_2 L^{-1}) the penetration depth and oxygen consumption decreases by 50% (Rysgaard, *et al.,* 1994; Neubacher *et al.,* 2011). Under this condition denitrification increased by 32%, but anammox remained constant (Neubacher *et al.,* 2011). Similarly, nitrification decreases and thereby the main source of NO_3^- for denitrification is the overlying water. This has also been observed at night when the

concentration of O_2 is reduced by high O_2 consumption in the sediment. Therefore, the oxic zone is also reduced and then again the main source for denitrifcation is the NO_3^- from the overlying water (Rysgaard, *et al.,* 1994).

Although anoxic conditions are required for denitrification, reducing conditions may also favour DNRA (Rysgaard *et al.,* 1996; An and Gardner, 2002). Moreover, variation in O_2 concentration may be also responsible for sulphide increases if anoxic conditions predominate. Since sulphide is toxic for some nitrifier and denitrifier bacteria, both nitrification and denitrification processes may be constrained (An and Gardner, 2002). The ecological consequence of the change of the predominance of processes leads to N retention, since the previous conditions may favour DNRA rather than denitrification, which results in fluxes of NH_4^+ out of the sediments (Rysgaard, *et al.,* 1996; Gardner *et al*., 2006; Dalsgaard and Thamdrup, 2002; Rysgaard, *et al.,* 2004; Joye and Anderson, 2008). Similar results were showed by Dong *et al*. (2011) who observed high fluxes of NH_4^+ under anoxic conditions.

Moving onto the common assumption that anammox is an anaerobic process, recent evidence proved that anammox bacteria are able to tolerate up to 13 μmol l^{-1} O_2. Although anammox rates decrease to about 70%, 50%, and 2% at 3.5, 8, and 13.5 μmol l^{-1} O_2 (Jensen *et al.,* 2008), it is clear that the activity of anammox bacteria is not inhibited at oxygen concentrations above of 1 μmol l^{-1} O_2 as it was first asserted (Strous *et al*., 1997). Moreover, it has also been demonstrated that the presence of sulfide at steep gradients of O_2, such as detected in the Mariager Fjord has an inhibitory effect on the anammox process, but not on the denitrification process (Jensen *et al*., 2008). Similarly, the anammox process has been found to be flexible with respect to temperature (Dalsgaard and Thamdrup, 2002). Studies carried out in waste water system treatments showed that the temperature where anammox can take place ranged between 20 and 43˚ C, having its optimum about 37˚ C (Strous *et al*., 1999b). In contrast, natural environments with temperatures as low as 4 to 6 ˚ C, such as the sediments at the Skagerrak, have shown that anammox accounts up to 67% of the total N_2 production (Thamdrup and Dalsgaard, 2002).

2.3.3 Substrate availability

Organic matter content in sediment plays an important role in N-cycling because through its oxidation N is mineralized (Seitzinger, 1998) and thus supplies the NH_4^+ required not only for the coupled nitrification-denitrification process, but also for anammox. Similarly, the substrate NO_3^- in the overlying water has been found to be proportional to D_w (Nielsen, *et al.,* 1990). For example, Rysgaard *et al.* (1996) showed that the highest rates of D_w (0.277 mmol N m^{-2} d^{-1}) occurred during January when the highest concentrations of NO_3^- (25.5 μM) are recorded, while the lowest rates (0.001 mmol N m^{-2} d^{-1}) were observed during June with lowest

concentration of NO_3^- (0.32 μM). Furthermore, Dong *et al*. (2000) also showed higher rates of Dw (530 – 1140 μmol N m^{-2} h^{-1}) at sites of the Colne estuary where both organic matter content (2-3%) and concentrations of NO_3^- (76 to 1171 μM) were high. However, although there is a relationship between the concentration of NO_3^- in the overlying water and high rates of D_w, different rates of denitrification have been observed. This suggests that another factor may be determining the potential capacity for denitrification. In the study of Dong *et al*. (2000) for instance, different rates of D_w were observed under the same NO_3^- concentration (100 μM NO_3^-) in the overlying water. It was suggested that the differences could be due to the different organic matter content at each site. The study of Rysgaard *et al*. (1996) showed contrasting results because for one locality, the difference in NO_3^- was significant between June (1.2 μM NO_3^-) and September (4.9 μM NO_3^-), with higher D_w rates during September (0.44 mmol m^{-2} d^{-1}) than during June (0.09 mmol m^{-2} d^{-1}). However, the total denitrification was higher during June (1.1 mmol m^{-2} d^{-1}) despite the 4 times higher NO_3^- concentration recorded in September. In this case it seems that anoxic conditions during September inhibited the coupled nitrification-denitrification process and thus the total denitrification decreased, whereas during June the nitrification-denitrification was significant.

In contrast to the denitrification that in general shows high rates in shallow rich organic sediment, anammox rates are lower, despite the high NH_4^+ availability (Thamdrup and Dalsgaard, 2002; Dalsgaard *et al*., 2005; Trimmer and Nicholls, 2009). It seems that low rates of anammox are regulated by NO_3^- and NO_2^- availability. Given that O_2 and NO_3^- are the most favorable electron acceptors in the sequence of oxidants, when O_2 is consumed, the demand for NO_3^- increases and thus the NO_2^- liberated during NO_3^- reduction is not available for anammox (Dalsgaard *et al*., 2005). Moreover, unlike denitrification that is enhanced by high NO_3^- concentrations in the water column, anammox seems to be supported by NO_2^- from denitrification in sediments (Trimmer and Nicholls, 2009).

On the other hand, given that high organic carbon content in sediments consumes O_2 during mineralization, O_2 penetration depth into the sediment may be altered. Since the oxidation of NH_4^+ is important for the coupled nitrification-denitrification, the total denitrification (D_w + D_n) may decrease (Rysgaard *et al.,* 1995). Similarly, high O_2 demand may cause anoxic conditions and as it was highlighted before, anoxic conditions favour the reduction of sulphate, but denitrification is inhibited. Moreover, anoxic conditions may enhance DNRA (Rysgaard *et al.,* 1996).

The importance of anammox is not limited to sediments, the process has also been observed in the water column where its contribution to the total N_2 production ranges between 19 to 35% (Dalsgaard *et al*., 2003; Kuypers *et al*., 2003). However, regarding the controls on anammox in the water column, it seems that it is not yet clear what the main controls are. Some studies claim that low concentrations of NH_4^+ in the anoxic waters at the Golfo Dulce

limits the rate of anammox (Dalsgaard *et al.,* 2003). On the other hand, other studies have found that NO_2^- rather than NH_4^+ is limiting the anammox process in the suboxic zones, and thus, an internal nitrification process has to be supplying anammox with substrate (Jensen, *et al.,* 2008). In the first case, it is suggested that the mixture of surface waters supplies the NO_3^- for the oxidation of NH_4^+ which at the same time is produced by the remineralization of sinking organic matter (Dalsgaard, *et al.,* 2003). This result agrees with the findings off northern Chile where it is also possible that the activity of anammox is related to the availability of NH_4^+ (Galán *et al.,* 2009).

Similarly, the factors controlling the anammox process in sediments are controversial. On the one hand, some evidence shows that anammox has a positive strong correlation with the organic carbon in sediments and its implicit reactivity (Thamdrup and Dalsgaard, 2002; Trimmer *et al.,* 2003; Meyer *et al.*, 2005; Nicholls and Trimmer, 2009), but on the other hand, the importance of the contribution of anammox to N_2 production has been found to be inversely related to remineralized solute production (NH_4^+, and dissolved inorganic carbon; Engström *et al.,* 2005). This has also been found in deep water sediments where content of organic matter and then the mineralization is lower than in shallower water sediments (Dalsgaard, *et al.,* 2005). Considering the first case, it was argued that NO_2^- supplied to the suboxic sediment layer relies on the rate of NO_3^- reduction, which at the same time depends on the reactivity of the organic matter in the sediment. Regarding the second case, the absolute rates of anammox have been found 3 to 4 times lower than in shallower water sediments, but its contribution to total N_2 production seems to be significant (Dalsgaard *et al.,* 2005). From this, it can be seen that the dependence of anammox bacteria on the organic carbon content in the sediments is not clear, and thus other processes may play an important role in controlling the anammox activity. For example, the highest absolute rates of anammox (3.5 μmol $l^{-1}h^{-1}$) and the highest relative contribution of anammox to total N_2 production (40-79%) were found in sediments with high concentrations of Mn (250 – 325 μmol g dry sed^{-1}), in the Skagerrak. However, the lowest rates of anammox (0.91 – 1.2 μmol $l^{-1}h^{-1}$) and contribution to total N_2 production (4 – 7%) were not found in sediments with low concentration of manganese, but in sediments with high concentrations of NH_4^+ (Engström *et al.,* 2005).

2.3.4 External inputs of N

The availability of biologically useable forms of N in marine environments plays a major role in controlling primary productivity since N is considered to limit primary production in marine environments (Paasche 1988; Herbert, 1999; Capone, 2000; Paerl and Piehler, 2008). Although the abundance of the fixed forms of N is controlled by the processes involved in the N cycle, internal remineralization is not the only input of N. It is also supplied from external sources via river, groundwater, and direct atmospheric deposition (Herbert, 1999; Galloway *et al*., 2003; Paerl and Piehler, 2008; Jickells and Weston, 2011) and often derived from human activities. External inputs can explain huge variation in concentrations of DIN and total nitrogen (TN) that can represent about the 70% of the annual variation of TN in estuaries (Boynton, *et al*., 1982; Conley *et al*., 2000; Boynton *et al.,* 2008). Additionally, external sources of N might compensate N losses via the denitrification process (Paerl and Piehler, 2008).

Land-based N inputs from outside the coastal zones have been estimated (Fig. 2.5), and on a global scale, rivers seem to be the main input of N (65 Tg N yr^{-1}), followed by atmospheric deposition (8 Tg N yr^{-1}) and ground water discharges (4Tg N yr^{-1}) (Seitzinger and Harrison, 2008). Rivers have an important role transporting nutrients into the coastal zones and beyond, its importance lies in the fact that rivers export the largest fraction (25%) of their watershed total N inputs (230 Tg N yr^{-1} to 270 Tg Nyr^{-1}; Galloway *et al*., 2004; Seitzinger and Harrison, 2008). Unlike rivers and groundwater, atmospheric deposition is not focused at a point, but dispersed through the coastal zones (Jickells and Weston, 2011). Atmospheric nutrients enter the water column by direct atmospheric deposition (dry), composed of DIN (dissolved inorganic nitrogen) and DON (dissolved organic nitrogen) and through rainwater (wet deposition) that includes mainly ammonia (Baker *et al*., 2007; Elliot *et al*., 2009; Violaki *et al*., 2010). The magnitude of the atmospheric deposition of N varies widely with fluxes ranging between 0.9 mmol N m^{-2} yr^{-1} (Alaska) to 92 mmol N m^{-2} yr^{-1} (Chesapeak Bay; Jickells and Weston, 2011). Atmospheric deposition not only contributes directly to coastal waters through wet and dry deposition, but also contributes to groundwater and fluvial N fluxes (Seitzinger and Harrison, 2008; Howarth *et al*., 2000; Jickells and Weston, 2011). For example, it has been estimated that the contribution of the oxidized nitrogen (NOx), originated from fossil fuel combustion represent between 24% to 80% of the nitrogen flux of the main rivers of New England and 25% of the Mississipi River (Howarth *et al*., 2011). In terms of the percentage of the N inputs from rivers, direct atmospheric deposition accounts between 5% to 40% for estuaries and coastal embayments, while its contribution to continental shelves is about 17% of the TN inputs from land-based sources (8 Tg N yr^{-1}) (Seitzinger and Harrison, 2008).

In spite of groundwater discharges, globally only represent about 10% of river discharges, some regional groundwater inputs can become important. This was highlighted by Jickells and Weston (2011) for the Yutacán Peninsula in Mexico. This karstic region is characterized by a highly permeable limestone plateau, and high rates of water recharges. Because of this

characteristic, the Yucatan Peninsula is devoid of rivers, and groundwater is the main pathway of N to the coast. The N flux for this region through groundwater is estimated to be 2.4 TN km^{-1} yr^{-1}. This amount represents only about 6% of the flux of a similar place (Puako, Hawaii-Kona, 42,286 T N km^{-1} yr^{-1}), however a high concentration of NO_3^- (286.6 uM) has been found in the places of discharge in Yucatán (Hernandez-Terrones *et al*., 2011).

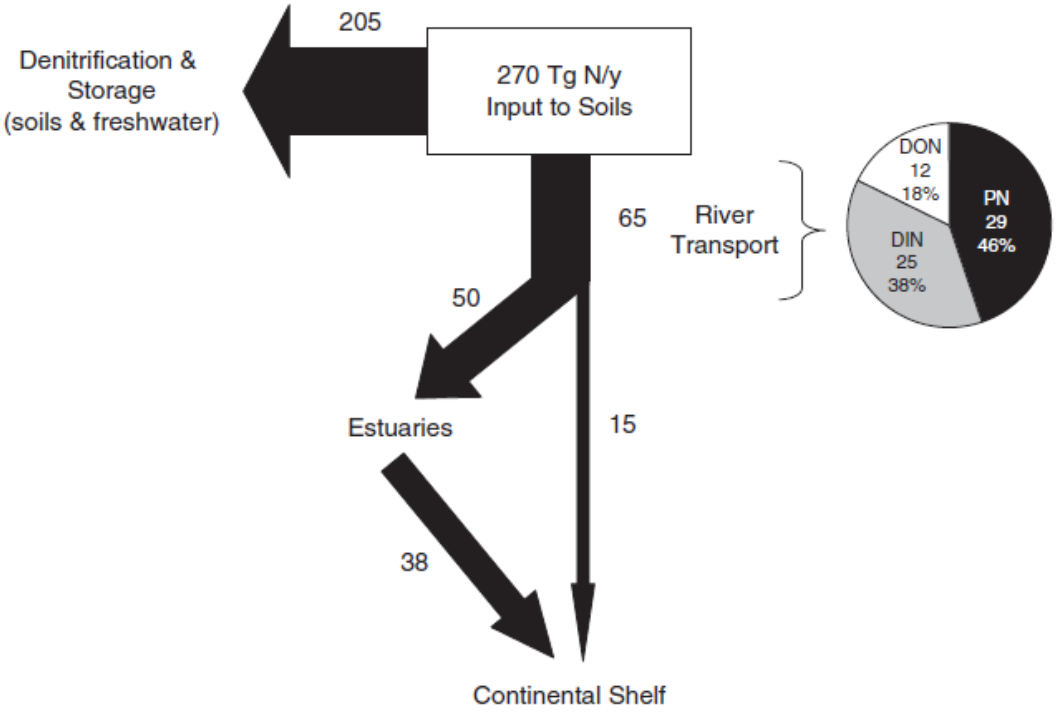


Figure 2.5. Land base N river inputs to the coastal zone (taken from Seitzinger and Harrison, 2008).

Although nitrogen is important in coastal marine systems because it controls primary production, excessive nitrogen loading is the main agent of coastal eutrophication. During the past century human activity has altered the balance between N inputs and N losses (Table 2.2). Over the last 4 decades, human population has increased more than 70%, (Galloway *et al.,* 2008) and currently about 75% of the world's population lives close to the coast. Consequently, coastal marine systems have been subjected to extensive development activity which has led to direct induced changes, such as habitat alteration or eutrophication. The population growth has brought industrial and agriculture expansion, wastewater, and rural and industrial discharge. All of these have not just increased concentrations of phosphorus and nitrogen, but have also affected benthic metabolism and nutrient cycling (Galloway *et al.,* 2008; Paerl and Piehler, 2008). The expansion of agriculture represents a special problem because it widely uses synthetic fertilizer which has increased about 120%, producing 187 Tg

N y^{-1} (Galloway, *et al.,* 2008) from which, 70 million tons per year is transported to coastal waters (Howarth *et al*., 2002).

If there is excessive load of N, the ecosystems' response results in a fast growing algae, followed by an increase in the supply rate of organic matter. In turn, benthic microbial metabolism is stimulated and causes important changes in the chemistry of the sediments (Cloern, 2001). This is particularly problematic when the organic matter consumption creates low oxygen or hypoxic conditions (4 mg L^{-1} O_2) because, under these conditions, N cycle might be altered (Paerl, *et al.,* 1998; Rabalais and Turner, 2001; Boesch, 2002; Paerl *et al*., 2002; Boyer *et al*., 2002; Rabalais, 2002; Seitzinger and Lee, 2010; Galloway, 2004, 2008, Boyer *et al.,* 2006; Boyer and Howart, 2008; Seitzinger and Harrison, 2008). Moreover, different forms of N supplied from external sources depend on the human activities. For example, the changes in chemical composition of N due to intensive livestock operation and agricultural activities in coastal watersheds, combined with hypoxia and anoxia, leads to increase NH_4^+ concentrations. Whereas, urban lands favors NO_3^- concentration increases (Paerl and Piehler, 2008).

Table 2.2. Rate of increase of main sources of Nr production. From Galloway *et al.* (2003)

Main sources of Nr	1860 (Tg N yr-1)	1910 (Tg N yr-1)	2000 (Tg N yr-1)
Biological N fixation	15		33
Combustion of fossil fuels	1		25
Haber-Bosch process		0	100

Another human activity affecting the N-cycling is trawling. It is considered the most destructive of them, though the ecological significance is not very well understood (Duplisea *et al.,* 2001; Dernie *et al.,* 2003), in general it can have implications for eutrophication and biogeochemical cycling (Kaiser *et al.,* 2002). Among the most important biogeochemical implication of trawling, resuspension is the alteration of the content of sedimentary organic matter decay, fluxes of nutrients, and a change in infaunal benthic metabolism (Pilskaln *et al.,* 1998; Duplisea *et al.,* 2001; Dounas *et al.,* 2007). Studies conducted for more than 2 decades have showed that trawling is a mechanism of sediment resuspension (Churchill, 1989) and as was mentioned before, its effects have implication in the organic matter structure (Pilskaln *et al.,* 1998; Mayer *et al.,* 1991). For example, it has been estimated that suspended organic matter can reach more than 10 m above the sediment surface and reach concentration in the range of 100 to 500 mg L^{-1}, which is exposed to oxic conditions (Churchill, 1989). Consequently, mineralization is accelerated in the water column and thus, more N is available for primary

production. However, the processes that normally take place in the sediment such as denitrification or anammox are altered (Wainright and Hopkins, 1997). Likewise, it has been proved that organic matter shows a conspicuous decrease in concentration in sediment after trawling (Mayer *et al.,* 1991; Bhagirathan *et al.,* 2010). On the other hand, the biologically recyclable organic matter from the surface can be buried deep in sediments (Mayer *et al*., 1991; Pilskaln *et al*., 1998) and thus alter the rate and magnitude of nutrients regeneration (Dounas *et al.,* 2007). Moreover, since trawling causes high mortality of macrofauna (5 to 65%), there would be an excess of organic matter available to be recycled and thus the potential N release derived from recycling of killed organism could range between 2 – 68 mmol N m^{-2}. Apart from the effect on organic matter, because of sediment resuspension by trawling, NO_3^- and NH_4^+ are released up to 20 and 45 times greater (234 μmol m^{-2} h^{-1} of NO_3^- and 475 μmol m^{-2} h^{-1}) than the undisturbed sediment (Duplisea *et al.,* 2001). In the same way, during the resuspension of only 1 mm layer of sediments, significant concentration of NH_4^+ are removed from the sediment (Fanning, *et al*., 1982) and thus denitrification by coupled nitrification-denitrification (Dn) and anammox processes can be altered. Finally, nitrifier and denitrifier bacteria are resuspended which could cause a decrease in the number of the organisms, thus, affecting nutrient regeneration in marine sediments (Pilskaln *et al*., 1998). Furthermore, resuspension by trawling provoke sediment erosion with implications in the depth of the redox potential discontinuity (RPD). This was highlighted by Dellapenna *et al.,* (2006) who proved that the reduction in the depth of RPD before and after trawling ranges between 0.25 – 1.7 cm which result in variations of diffusivity of chemicals.

Chapter 3. Rates of denitrification, anammox and DNRA

3.1 Introduction

This chapter presents the production of N_2 either by denitrification, anammox, or both in the North Sea, as well as the production of NH_4^+ through dissimilatory NO_3^- reduction to ammonium (DNRA). It also includes sampling and handling methods, techniques used to measure N_2 and NH_4^+ production from NO_3^- reduction, as well as equations to calculate both N_2 and NH_4^+. The sampling sites for this survey are the North Sea, and The Wash. The sampling at The Wash is limited to one station called Mare Tail, where a total of four sampling campaigns were carried out during May, June, September, and October 2013 (Fig. 3.1). In contrast there were five North Sea sampling stations, visited on one sampling trip during August 2013 (Fig. 3.1).

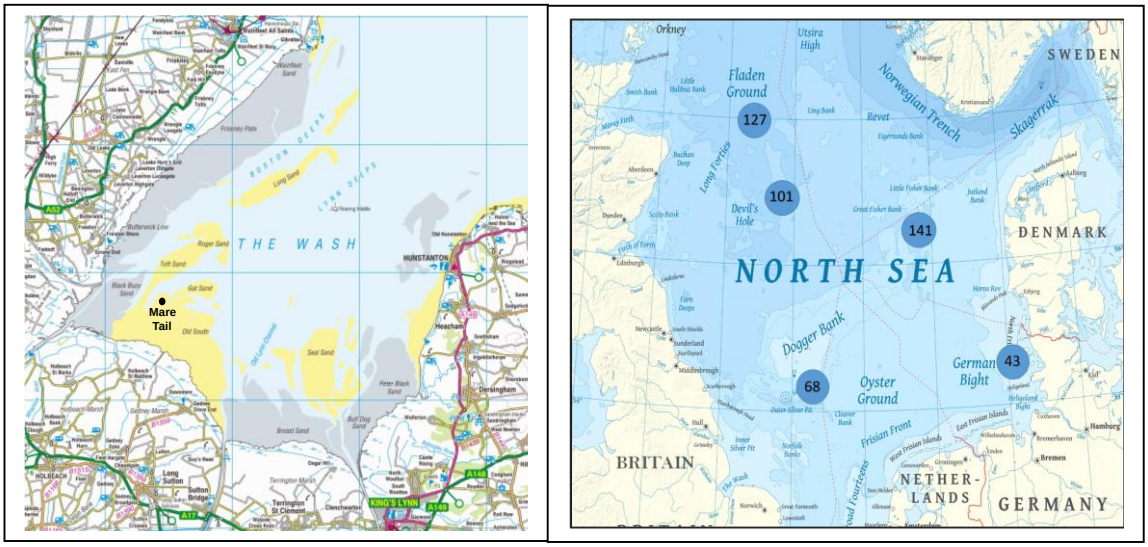


Figure 3.1. Sampling sites: The Wash (left, http:// upload.wikimedia.org/wikipedia/ commons/c/c8/ Ordnance_Survey_1-250000_-_TF.jpg) and The North Sea (right), showing the sampling sites in blue circles.

3.2. Sediment sampling and handling

The sampling in The Wash was carried out during low tide when the sediment was exposed. Access to the Mare Tail station was achieved on board of the fisheries research RV Three Counties from EIFCA (Eastern Inshore Fisheries Conservation Authority), and by walking. Once close to the sampling station, people were brought even closer to shore on board of a rubber boat; from that point, the site was reached by wading ashore and walking. There, 30 samples of intact sediment for measurements of N_2 and NH_4^+ production were collected by hand in 300 mm plexiglas tubes (6 cm i.d.), the bottom of each core was sealed with rubber stoppers at the moment of extraction of the tubes from the sediment. The column of sediment in all cores was about 15 cm height, with an overlying volume of water of about 420 ml. After the sampling, the samples were placed in buckets filled with site water in order to keep them at the ambient temperature, then were transported back to the UEA laboratory facilities (~ 3 h). In the lab the samples (without the top rubber stopper) were placed in 200 L containers in a room at *in situ* temperature and left overnight.

In the North Sea, the sampling was carried out on board of the RV "Cefas Endeavour", using a NIOZ cylindrical box corer (31 cm i.d., Netherlands Institute for Research). The column of sediment in the box corer ranged between 40 cm to 50 cm, and was overlaid with about 15 L to 25 L of the original bottom water. Subsamples with a column of sediment of about 15 cm, were taken by hand from the box core with 300 mm plexiglass tubes (6 cm i.d.). The bottom of each core was sealed with rubber stoppers at the moment the tubes were extracted from the sediment. After taking about 6 subsamples the box corer was redeployed until completing a total of 30 subsamples at each station. All the experiments carried out in the North Sea were completed aboard the RV "Cefas Endeavour".

3.3 Denitrification, anammox, and DNRA

In order to determine processes of the nitrogen cycle, namely, denitrification, ananmox, and DNRA, the isotope pairing technique (IPT) was applied (Nielsen, 1992) in sediment samples collected both in The Wash and the North Sea. For all sampling campaigns and locations two types of experiments were conducted (a) a time-series experiment using intact sediment cores; and (b) an end point anaerobic sediment slurry. The time-series experiment in intact sediment cores was designed to determine rates of denitrification, anammox, and DNRA, while the aim of the anaerobic sediment slurries experiment was to confirm the presence of anammox. The general approach of the methods carried out during this survey were based on other studies on anammox, denitrification, and DNRA, (e.g. Nielsen, 1992; Nielsen and

Glud, 1996; Risgaard-Petersen and Rysgaard, 1995; Dalsgaard *et al.*, 2000; Thamdrup and Dalsgaard, 2002; Risgaard-Petersen *et al.*, 2003; Trimmer *et al*., 2003; Trimmer and Nicholls, 2009). See section 3.4 for more details.

3.4 Principle of the isotope pairing technique (IPT)

Ideal conditions for microbial NO_3^- reduction are found in anoxic sediments, where NO_3^- supplies O_2 in the process of organic matter degradation (Koike and Sorensen, 1988). Through denitrification processes such as denitrification or anammox, fixed N is transformed and recycled to the atmosphere as N_2 (Seitzinger, 1987). The fact that N is the most abundant pure element of the Earth and that the natural abundance of the isotope ^{14}N is 99.634%, as well as its naturally high concentration in seawater make it difficult to observe small additions of $^{28}N_2$ from natural denitrification processes. However, the IPT developed by Nielsen (1992) allowed determining rates of denitrification, and alternative calculations procedures allowed determining N_2 production when denitrification and anammox coexist (Risgaard-Petersen *et al*., 2003).

Denitrification processes occurring in anoxic sediments have different sources of NO_3^-, one is the NO_3^- in the water column overlying the sediments (Dw) and the other is the result of nitrification ocurring within the sediment (Dn).

The IPT relies on the use of the strongly enriched ^{15}N stable isotope of N, so that, by enriching with $^{15}NO_3^-$ (>98%) the water overlying the sediment in an intact sediment cores experiment, it is possible calculate the formation genuine N_2 (D_{14}), or said in others words, the production of unlabeled $^{28}N_2$ as it would occur without the addition of $^{15}NO_3^-$ (Nielsen, 1992). IPT states that:

$$D_{14} = 2p^{28}N_2 + p^{29}N_2 \quad (3.1)$$

However, because $^{28}N_2$ is not detectable due to the high atmospheric background, D_{14} is estimated indirectly from the denitrification rate of $^{15}NO_3^-$ (D_{15}) as follows:

$$D_{14} = \frac{p^{29}N_2}{p^{30}N_2} \times D_{15} \quad (3.2)$$

where, $p^{30}N_2$ and $p^{29}N_2$, are the production rates of $^{30}N_2$ and $^{29}N_2$, $D_{15} = 2 \times p^{30}N_2 + p^{29}N_2$.

Calculating D_{14} is possible provided that the following assumptions be fulfilled (Nielsen and Glud, 1996): (1) The addition of $^{15}NO_3^-$ does not affect the rate of denitrification of the natural $^{14}NO_3^-$, and therefore nor the production of $^{28}N_2$, (2) the three isotopic N_2 species produced are binomially distributed (Hauck *et al*., 1958), and (3) there is a complete and uniform mixing of the $^{15}NO_3^-$ and $^{14}NO_3^-$ through the NOx reduction zone, so that the ratio between $^{15}NO_3^-$ and $^{14}NO_3^-$ is constant.

3.5 The IPT when denitrification and anammox coincide

According with Risgaard-Petersen *et al*. (2003), when anammox and denitrification coincide, it is not possible to determine the genuine N_2 production from the previous equations. What happens when anammox and denitrification coincide is depicted figure 3.2.

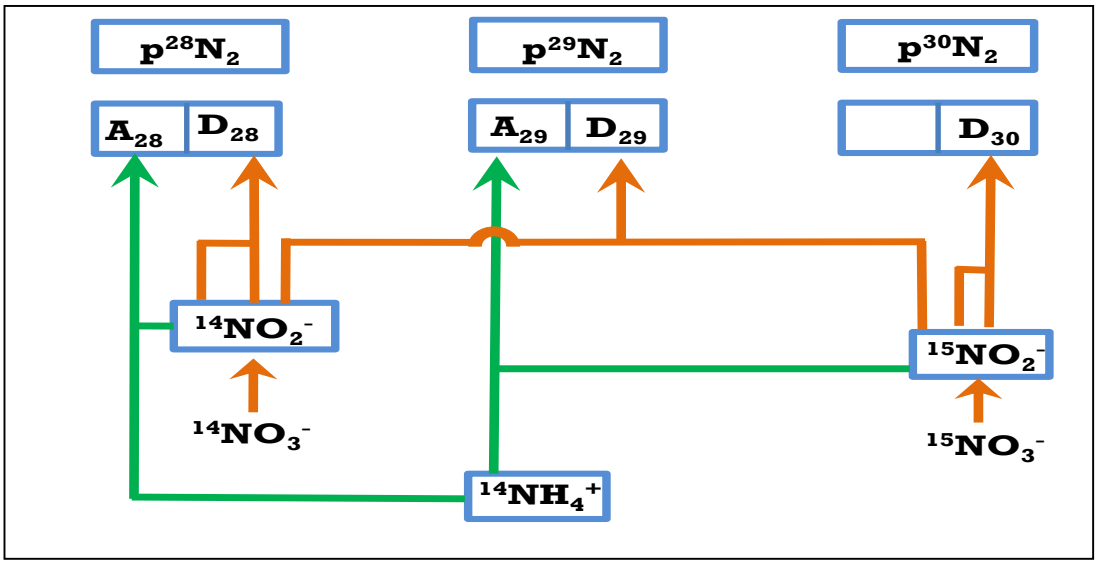


Fig. 3.2. Illustration of the isotopic distribution of N species when denitrification and anammox coincide. *A*, refers to anammox; *D*, refers to denitrification; 28, 29, and 30 indicate the isotopic species of N; and *p* indicates the production of either of the isotopic species of N_2. Green arrows indicate the anammox process, and orange arrows represent denitrification. Modified from Risgaard-Petersen *et al*. (2003).

According with Risgaard-Petersen *et al*. (2003), both anammox and denitrification will contribute to the production of $^{28}N_2$ and $^{29}N_2$, but only denitrification will produce $^{30}N_2$. Unlike denitrification, the addition of $^{15}NO_3^-$, will increase the rate of oxidation of the $^{14}NH_4^+$ present in the sediments, so that, $^{29}N_2$ will increase as more $^{15}NO_3^-$ is added. However, $^{28}N_2$ produced also by anammox will remain constant as illustrated in equations 3.3 and 3.4.

$$^{14}NH_4^+ + {}^{15}NO_2 \rightarrow {}^{29}N_2 \quad (3.3)$$

$^{14}NH_4^+ + {}^{14}NO_2 \rightarrow {}^{28}N_2$ (3.4)

Thus, the two reaction are independent and it violates both (1) the assumption of independence between the added $^{15}NO_3^-$ and the production of $^{28}N_2$, and (2) the assumption of binomial distribution. Besides, as $^{29}N_2$ is correlated with the amount of $^{15}NO_3^-$ added, genuine N_2 production will be overestimated when Nielsen equation be used.

The equations proposed for Risgaard-Petersen *et al*. (2003) are the following:

$D_{14} = 2D_{28} + D_{29} + 2A_{28}$ (3.5)

Which expressed from measurable parameters: $p^{29}N_2$, $p^{30}N_2$ and r_{14} it becomes:

$D_{14} = r_{14} \times [p^{29}N_2 + p^{30}N_2 \times (1 - r_{14})]$ (3.6)

Where, D_{28} is the production of $^{28}N_2$ from denitrification, D_{29} is the production rate of $^{29}N_2$ from denitrification, A_{28} is the production of $^{28}N_2$ from anammox, r_{14} is the ratio between $^{14}NO_x^-$ and $^{15}NO_x^-$ in the NO_x^- reduction zone, $p^{30}N_2$ and $p^{29}N_2$, are the production rates of $^{30}N_2$ and $^{29}N_2$.

The parameters in the equation can be directly measured in an isotope ratio mass spectrometer (IRMS), with the exception of r_{14}. Three methods were proposed in order to know r_{14}. More details about methods 1 and 2 will be found in Risgaard-Petersen *et al*. (2003) and details for method 3 will be found in Trimmer *et al*. (2006).

Method 1 – Determining the potential contribution of anammox and denitrification in anoxic sediments (e.g. Thamdrup and Dalsgaard, 2002) is possible to know the contribution of anammox (ra) and therefore r_{14} as follows:

$ra = \frac{p^{29}N_2 - 2r_{14} \times p^{30}N_2}{p^{29}N_2 + p^{30}N_2 \times (1 - r_{14})}$ (3.7)

$r_{14} = \frac{(1 - ra) \times R_{29} - ra}{(2 - ra)}$ (3.8)

Where, *ra* is anammox contribution to N_2 production, R_{29} is the ratio between $p^{29}N_2$ and $p^{30}N_2$.

Calculating r_{14} from method 1 reduces the value of D_{14} -overestimated when is calculated with the original IPT. However, there is still a dependency on the $^{15}NO_3^-$ concentration in the water

column and D_{14} (Trimmer *et al*., 2006). Although, $^{29}N_2$ from anammox can be corrected and thus the correlation between D_{14} and the increasing concentrations of $^{15}NO_3^-$ should not be seen, the fact that the original IPT includes $^{29}N_2$ production (equation 3.7) to calculate D_{14} implies that the slurry method underestimates the actual contribution of anammox to total N_2 production (Trimmer *et al*., 2006).

Method 2 – r_{14} can be estimated by carried out multiple $^{15}NO_x$ incubations based on the $^{29}N_2$ and $^{30}N_2$ production rates measured in two sets of parallel intact sediment core experiments.

$$r_{14} = \frac{p^{29}N_2^{(1)} - V \times p^{29}N_2^{(2)}}{2 \times (p^{30}N_2^{(1)} - V^2\, p^{30}N_2^{(2)})} \quad (3.9)$$

$$V = \frac{[^{15}NO_3^-]_{(1)}}{[^{15}NO_3^-]_{(2)}} = \frac{p^{29}N_2^{(1)} + 2 \times p^{30}N_2^{(1)}}{p^{29}N_2^{(2)} + 2 \times p^{30}N_2^{(2)})} \quad (3.10)$$

Where, *V* is the ratio between the concentrations of $^{15}NO_3^-$ in the water column of the two incubations.

Method 2 assumes equal contribution from anammox to total N_2 production (ra) for each core, therefore sediment should have minimal heterogeneity, otherwise, the value of V produces negative values of D_{14}. On the other hand, when heterogeneity is eliminated by sieving the sediment to calculate r_{14}, a dependency on the $^{15}NO_3^-$ concentration in the water column and the true D_{14} is still observed (Trimmer *et al*., 2006). However, no significant dependency between D_{14} and the concentration of NO_3^- is observed when V from sieved sediments is used in equation of method 1 to calculate r_{14}. Although, by sieving the sediment, the in situ redox conditions of sediment is altered.

Method 3 – r_{14} can be determined from the produced isotopic N_2O species: $^{45}N_2O$ and $^{46}N_2O$ during the incubation with ^{15}N-labelling. It is assumed that denitrification is the only source of N_2 in a ^{15}N-NO_3^- labelling experiment.

$$r_{14} = \frac{p^{45}N_2O}{2 \times p^{46}N_2O} \quad (3.11)$$

Where, $p^{45}N_2O$ and $p^{46}N_20$ are the isotopic production of N_2O during the incubation.

The method is based on the assumption that only denitrification produces N_2O in a $^{15}NO_3^-$ sediment core experiment, and that the $^{44}N_2O$ and $^{45}N_2O$ production is distributed binomially which reflects the ratio of $^{14}NO_3^-$ and $^{15}NO_3^-$ undergoing dissimilatory reduction (r_{14}). The value of r_{14} is lower when it is calculated from $^{15}N_2O$ than when it is calculated from the production

of $^{29}N_2$ and $^{30}N_2$ (methods 1 and 2). This is because the addition of $^{15}NO_3^-$ induces oxidation of NH_4^+ and consequently produces $^{29}N_2$ from anammox, but does not affect the $^{15}N_2O$ produced by denitrification. This can be confirmed when no relationship is observed between D_{14} and increasing concentrations of NO_3^-. Besides, method 3 enables direct measurements of r_{14} because it is calculated from the $^{15}N_2$ production in each core where the experiment is carried out. Unlike methods 1 and 2 which estimate r_{14} from two different experiments, the fact that r_{14} can be measured in each core, reduces the variation around D_{14} because in the other methods r_{14} is estimated on the assumption that the contribution of anammox to total N_2 production is constant irrespectively of the heterogeneity of the sediment.

The IPT method as well as the IPT modified to estimate rates of denitrification when anammox is present allows to know if the source of NO_3^- is the water column or the nitrification process carried out in sediment.

According with Trimmer *et al*. (2006) the slurry method underestimate anammox rates.

3.6 Incubation experiments

3.6.1 Time series experiments

Intact sediment experiments consisted of incubations of sediment samples collected in the core tubes. In brief, after collecting the samples, the intact sediment cores were left to equilibrate over night for about 12-14 h in a 200 L tank in a room at in situ temperature. Each core was aerated through individual pieces of silicon tubing connected to a distributor and aquarium pump. After the equilibration period, 18 cores were enriched with $^{15}NO_3^-$ (by adding 800 μL of 25 mM $Na^{15}NO_3$ [98 ^{15}N atom%] Sigma-Aldrich) to get a final concentration of ~50 μmol L in the overlying water, which represented a concentration approximately 50% above the ambient concentration. Six cores were not enriched and left as reference for natural abundance of N_2. The cores were then pre incubated with the purpose of allowing $^{15}NO_3^-$ to reach the nitrate reduction zone. The timing was based on previous studies carried out in the North Sea (Neubacher *et al*., 2011; 2013), so in general it was decided to pre-incubate the samples for 30 to 60 minutes.

After the preincubation a set of 3 cores, with and without $^{15}NO_3^-$ addition, were sacrificed and taken as time zero (T_0) measurements. In order to determine the ratio of $^{15}NO_3^-$ and $^{14}NO_3^-$ (r_{14}) as well as to determine the concentration of inorganic nutrients (NH_4^+, NO_2^-, PO_4^-, and Si), 5 ml of water sample was collected from the overlying water with a 20 ml syringe from all

the cores before and after addition of $^{15}NO_3^-$. The samples were filtered with syringe filters (0.2 μm Ministar PlusTM; Sartorious, UK), and kept frozen at -20°C until analysis.
At T_0, after having enriched the samples and as soon as water sample for nutrients was taken, the overlying water was gently slurrified by mixing the sediment and the overlying water in the core with a glass rod. Afterwards, samples of the slurries were collected and carefully poured into 12 mL gas-tight vials (Exetainer, Labco) for isotopic analysis of N_2, N_2O production. Additionally, another 5 mL of the slurry sample were placed in 15 mL polypropylene tubes to calculate DNRA rates through the $^{15}NH_4^+$ production. The processes of denitrification and anammox were stopped by adding 100 μL formaldehyde (38% w/v) to the gas-tight vials, then the vials were sealed and stored upside down at ambient temperature until analysis. Samples for DNRA and inorganic nutrients were kept frozen for further analysis of $^{15}NH_4^+$ and NH_4^+, NO_2^-, PO_4^-, and Si. Formaldehyde was used to stop the process in the time-series experiments, because, measurements of $^{15}N\text{-}N_2O$ were going to be carried out, and according to previous studies, it seems that $ZnCl_2$ might interfere with such measurements (Neubacher, 2011).

The other 18 cores were carefully sealed with rubber stoppers, avoiding that bubbles of air were created at the moment of sealing the cores. After that, the samples were incubated in the dark and a set of three cores were sacrificed every hour for 5 h. At the end of each 5 h incubation intervals the cores were sampled as above.

A 3-cm teflon-coated magnet was placed 5 cm above the sediment surface in each core before they were sealed. Then, a set of 6 cores were arranged around a large external magnet that provided the motion of rotation (60 r.p.m.) for the small magnets. Consequently, during the incubation, the magnet rotation allowed a homogeneous mixing in the water column overlying the sediment during the incubation (Rysgaard *et al*., 1996).

3.6.2 End-point slurry experiments

Sediment samples for the end-point slurry experiment to confirm the presence of anammox were subsampled from cores by extruding a 3-cm thick slice (both oxic and anoxic layers were included). Then, sediment was homogenized and 18 subsamples of 2 mL were placed into 12-mL gas-tight vials (Exetainer; Labco), then the vials were filled with in-situ seawater, previously degassed with oxygen free nitrogen (OFN). Vials were closed and incubated in the dark at in situ temperature for at least 24 h to eliminate all residual NO_3^-, NO_2^- and O_2 present in the samples. Samples of *in situ* seawater were collected before and after the preincubation in order to measure the concentration of $NO_3^- + NO_2^-$, and thus confirm that it had been

removed from the samples. After pre-incubation, a deoxygenated solution of both $^{15}NH_4^+$ plus $^{14}NO_3^-$ were added by injecting 50 μL of $^{15}NH_4Cl$ (120 mM; [98 ^{15}N atom%] Sigma-Aldrich) and 50 μL of $Na^{14}NO_3$ (25 mM) through the vial septa, to a final concentration of 500 nmol cm^{-3} of $^{15}NH_4^+$ and 100 nmol cm^{-3} for $^{14}NO_3^-$. Immediately after addition, 3 vials amended and 3 unamended were sacrificed by adding 100 μL of $ZnCl_2$ (7M). Samples were stored upside down at ambient temperature. The other 6 vials were placed in a rotating wheel and incubated for 24 h in a room at the in situ temperature. After incubation, the remaining vials were sacrificed and treated as above.

3.7 Sample analysis

Two types of measurements were carried out in order to know the rates of denitrification, anammox and DNRA: (1) isotopic distribution of ^{15}N of N_2, and (2) isotopic distribution of ^{15}N in the N_2O. Measurements to determine the distribution of ^{15}N in N_2 were carried out in the laboratory of Biology of the University of Southern Denmark, and measurements to determine the distribution of ^{15}N in N_2O were carried out in the School of Biological and Chemical Sciences of the Queen Mary University of London

N_2 and N_2O extractions – In order to extract and measure the isotopic composition of the N_2 produced during the incubation period, a headspace was created by introducing a hypodermic syringe through the septa and withdrawing water sample. At the same time, a needle in line with an analytical-grade He (99.9995%) flow, was also introduced into the vial. The line that supplies He had a 3-way stopcock with a luer connection, so that He enters the vial when water sample is being withdrawn (Dalsgaard *et al*., 2003; Fig. 3.3). For the isotopic composition of N_2, 1 mL headspace was created, while a headspace of 4 mL was created for the measurement ^{15}N isotopic composition of N_2O. After that, the exetainer sample vials were shaken vigorously and stored upside-down to allow the gases in the water phase and the headspace to reach the equilibrium (Trimmer and Nicholls, 2009).

N_2 analysis– The measurements of nitrogen isotopes of N_2 were carried out using a custom-made gas chromatograph (GC) coupled to a Thermo Delta V Plus isotope ratio mass spectrometer (IRMS) with a ConFlo III interface. To determine ^{15}N in the N_2, 100 μl from the headspace were injected manually to the injection port of the gas chromatograph coupled to the IRMS, the sample was analysed as detailed below.

The GC consisted of a manual injection port connected to a combined chemical Ascarite trap (10 mm x 200 mm) containing magnesium perchlorate ($Mg(ClO_4)_2$), and Ascarite (NaOH) on

a silica substrate to remove both H_2O, and CO_2. This is followed by a Porapak R chromatographic column (3 m x 0.45 mm), and a quartz reduction reactor (15 mm x 300 mm) packed with high purity Cu (and kept at 600° C). The chromatographic column separates N_2, O_2 and CO_2, then O_2 is removed in the reactor. Finally, before the sample enters the IRMS, it passes through a second $Mg(ClO_4)_2$ trap (Anhydrone) to remove only water (Carter and Barwik, 2011; Dalsgaard *et al*., 2012).

The ConFlo III is the inlet system for the IRMS and allows the coupling between the GC and the IRMS via an open split arrangement that limits the gas flow entering the ions source.
In the IRMS the sample gas is ionised, then the ions are deflected according to their mass to charge ratio, then detected in Faraday cups, such that, when a beam of ions strike its wall it produces a current in proportion to their abundance. The signal received is then amplified and sent to a computer, where is converted into ion intensities (Hoffman and Stroobant, 2007). For N_2 gas the ions of m/z (mass to charge ratio) 28, 29 and 30 were measured.

N_2O Analysis – In order to find the concentration range of the N_2O samples, 5, 10 and 15 μL of sample from the headspace were subsampled using a gas-tight syringe (Vi Precision Sampling) and injected into air filled gas-tight vials (Exetainer, 12 mL, Labco Ltd., UK). All the gas sample from this vial was swept, using a two-way needle and analytical grade He (25 mL/min), to a trace gas preconcentrator (Cryo-Focussing; Precon, Thermo-Finnigan). The N_2O gas is purified by passing it through a column containing Ascarite and $Mg(ClO_4)_2$, where the gas is dried and scrubbed of most of the CO_2 before being cryo-focused twice using liquid nitrogen cooled traps. Then, the N_2O gas sample is injected into a GC for a final separation of N_2O from CO_2 on a Poraplot Q capillary column. Finally, the purified N_2O gas sample passes to the CF-IRMS via the ConFlo III interface (Thermo-Fisher), where the ions of m/z 44 45 and 46 are determined.

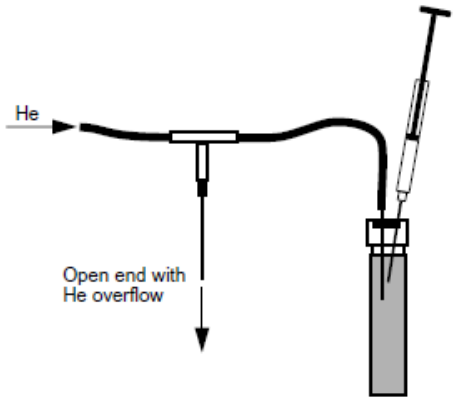


Figure 3.3. Creating a headspace in a gas-tight vial. Taken from Dalsgaard *et al*. (2000).

Dissimilatory nitrate reduction to ammonium (DNRA) – In this survey, the general approach was the same as described above in order to determine denitrification and anammox, but unlike those, the production of $^{15}NH_4^+$ was tracked over time after enriching with $^{15}N\text{-}NO_3^-$ the overlying water of an intact sediment core.

Conversion of NH_4^+ to N_2 – In order to measure rates of DNRA, the produced NH_4^+ had to be converted to N_2. The method followed was based on the combined microdiffusion-hypobromite oxidation method (Risgaard-Petersen *et al*., 1995). The method can detect concentrations as little as 2 nmol NH_4^+ with high precision as described below.

In brief, a volume of 2 mL was subsampled from the defrosted slurry samples from the time-series incubations. This sub-sample was placed into a 6 mL gas-tight vial, then, the headspace was flushed for 2 min using He gas by introducing two needles, one that serves as a vent, and the other for He (Fig. 3.4). After the flushing, 50 μL of hypobromite were added through the septum using a Hamilton syringe, in order to convert the NH_4^+ to N_2 gas. The samples were placed on a shaker for 24 h at 20°C.

After the incubation period, 500 μL of gas sample was extracted from the headspace and injected through the injection port of the GC coupled to the IRMS. The isotopic measurements were done as described above for determining the isotopic composition of N_2.

Figure 3.4. Procedure for flushing the headspace of the vials to measure DNRA before converting NH_4^+ to N_2.

3.8 ^{15}N isotopic composition of gas and rates of denitrification, anammox and DNRA

To determine the rates of N_2 production either by denitrification or anammox, or the rate of DNRA it is necessary to know the fluxes of $^{29}N_2$ and $^{30}N_2$. These fluxes can be determined from the linear fitting slope of the production of $^{29}N_2$ and $^{30}N_2$, the sediment surface area in the core, and a conversion factor ($1x10^4$; to convert cm^{-2} into m^{-2}). In turn, the production of $^{29}N_2$ and $^{30}N_2$ in the sample can be determined as explained in section 3.8.6. Finally, in order to know the number of moles of $^{29}N_2$ and $^{30}N_2$ it was necessary to produce a calibration curve, based on a standard gas, so that knowing the volume of gas injected and the area of the peak of mass 28 for N_2 (or 44 for N_2O) it was possible to know the number of moles of gas in the samples. In the following sections is described the process and the calculation to determine the production of $^{29}N_2$ and $^{30}N_2$.

3.8.1 Calibration

Accuracy, precision and sensitivity of the method was determined by preparing the following set of standards: 1, 2, 3, 5, 7 and 10, μL of oxygen free nitrogen (OFN 99.998% N). In brief, a 12 mL gas-tight vial was flushed and filled with OFN by introducing a needle connected to the OFN tank into the gas-tight vial through the septa of the vial. At the same time a needle that served as a vent was also inserted. The gas tight vial was then flushed for two minutes before removing the needles. In order to keep the gas inside the vial at atmospheric pressure, the vial was placed in a bowl full with tap water, then a needle was introduced through the septa to allow the excess of gas escape without any intrusion of air. Afterwards, from this vial, sub-samples of 3, 5, 7 and 10 μL were injected into the injection port of the GC on-line with the IRMS.

3.8.2 Time drift correction

Theoretically the response of the mass spectrometer should not change when injecting a standard (same material and same amount) at different times within a batch. In practice various factors could vary that would give a non-linear response (i.e. not constant) and this is called time drift, effects of which we have to correct for. The time drift of the IRMS was corrected by doing the following: (i) plotting the data (n = 16) of the ratio of the areas $^{30}N_2/^{28}N_2$ of the standard corresponding to 10 μL injections of OFN against to the time, (ii) the equation of the trend line that best fit the data (polynomial order 2) was used to correct ratio of the areas

$^{30}N_2/^{28}N_2$ of the injections of different volumes (3, 5, 7, 10 μL) of the standard. The plot of the corrected data more or less follows a horizontal line (Fig. 3.5).

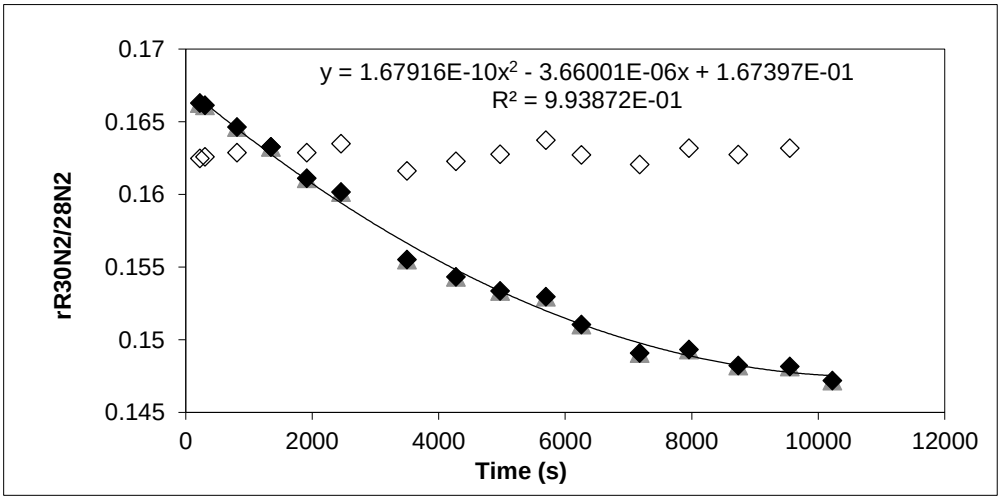


Figure 3.5. Example of a plot to get the equation to correct the time drift of the ratio of the area $^{30}N_2/^{28}N_2$ for a volume of 10 μL of the standard. Black diamonds are data of the ratio of the areas $^{30}N_2/^{28}N_2$ against the retention time. White diamonds represent the data after correction.

3.8.3 Amount effect correction

In theory the isotopic ratio of a gas should be constant regardless of the amount of sample this ratio was measured on, but in practice the response of the mass spectrometer is not always linear (i.e. ratio is not constant irrespective of the sample amount). Due to this, the measured ratio of the different volumes (3, 5, 7, 10 μL) of the standard injected was corrected as follows: (i) plotting the ratio $^{30}N_2/^{28}N_2$, after the time drift correction was applied, of all the standard injections (3, 5, 7, 10 μL) against to the area of $^{28}N_2$, (ii) the equation of the trend line that best fit the data (polynomial order 2) was used to correct the ratio $^{30}N_2/^{28}N_2$ of the standard. After this correction the plot of the corrected ratio data more or less follows a horizontal line (Fig. 3.6).

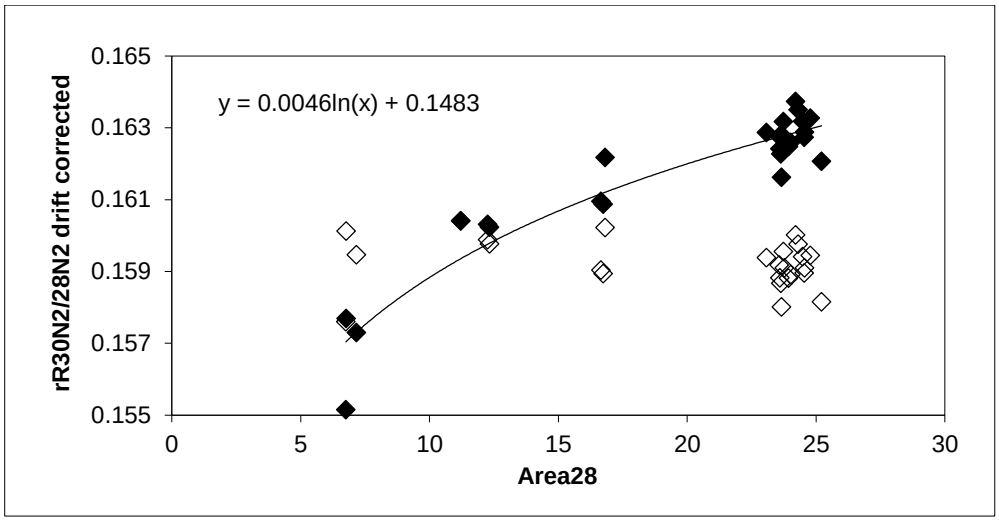


Fig. 3.6. Example of a plot to get the equation to correct the ratio of $^{30}N_2/^{28}N_2$ for the different volume (3, 5, 7, and 10 μL) of the standard. Black diamonds are data of the drift corrected ratios $^{30}N_2/^{28}N_2$ against the area of $^{28}N_2$. White diamonds are the corrected data.

3.8.4 Calculating the number of moles of N in the standard

The number of moles in the volume of the standard was calculated using the equation of state of an ideal gas:

$$n = \frac{P \times V}{R \times T} \quad (3.12)$$

Where
n = number of moles of N
P = Partial pressure of the gas (atm)
V = Volume of the sample (μL) injected
R = gas constant (0.082057837 L x atm/K x mol)
T = temperature of the gas in Kelvin degrees

Calibration curve – The calibration curve was obtained by plotting the area of $^{28}N_2$ against the number of moles (n) in the standard (Fig.3.7). Then, the slope of the calibration curve was used to calculate the number of moles of N_2 in the samples. The $^{28}N_2$ area was used in these

calculations because, although the gas produced in these experiments was ^{15}N enriched, the species of nitrogen with mass 28 ($^{14}N^{14}N$) is still the most abundant isotopic species of the nitrogen gas and is representative of the overall amount of gas produced.

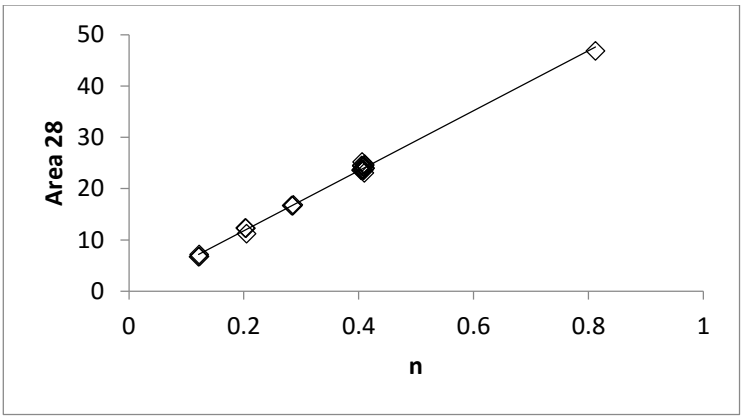


Fig. 3.7. Example of the calibration curve. Area of the standard vs number of moles (n) of $^{28}N_2$ in 3, 5, 7, and 10 μL of the standard.

3.8.5 Concentration of N_2 in the sample

Total concentration of N_2 nmol μl^{-1}- was calculated as follow:

$$[^{28}N_2] = \frac{^{28}A/b}{V_{sam-inj}} \times \left(\frac{V_{sam-hs}}{V_{sam}} + BC\right) \quad (3.13)$$

Where: $[^{28}N_2]$ is the concentration of N_2 in the exetainer (nmol μl^{-1}), ^{28}A is the beam area of $^{28}N_2$, $V_{sam\text{-}inj}$ is the volume (100 μl) of the sample injected to the IRMS, $V_{sam\text{-}hs}$ is the volume of the headspace (2 ml) from where the sample injected to the IRMS was taken, V_{sam} is the volume of the sample (10.3 ml), and BC is the Bunsen coefficient (0.01252).

3.8.6 Calculating the production of $^{29}N_2$ and $^{30}N_2$

The production of $^{29}N_2$ and $^{30}N_2$ can be calculated as follows:

$$p^x N_2 = Excess\ p^x N_2 \times [^{28}N_2] \quad (3.14)$$

Where p^xN_2 is either the production of $^{29}N_2$ or $^{30}N_2$, and $[^{28}N_2]$ is the total concentration of N_2. The excess production of $^{29}N_2$ or $^{30}N_2$ can be calculated from the ratios $^{29}N_2/^{28}N_2$ and $^{30}N_2/^{28}N_2$ by subtracting the background (area drift).

3.8.7 Percent of recovery of $^{15}NH_4^+$

Accuracy, precision and sensitivity of the method for DNRA was determined by preparing the following set of standards: 0, 5 10, and 15 μM of $^{14}NH_4^+$ with 1% of $^{15}NH_4^+$. Three replicates of each concentration were prepared by placing 2 mL of the standards solutions in a 6-mL gas-tight vial. Afterwards, the standards were treated as above and the measurements of ^{15}N abundance in NH_4^+ was determined by measuring the ^{15}N composition of the converted N_2. The standard errors of three replicates of each concentrations shows a high precision with standard error < 0.017 (n = 3). The mean percent recovery of $^{15}NH_4^+$ was >90% (Table 3.1). The samples were corrected with the correction factor obtained from the equation of the standard calibration curve (Fig. 3.8).

Table 3.1. Expected vs measured ^{15}N abundance in 2 mL of water containing 0, 5, 10, and 15 ml of $^{14}NH_4^+$ with 1% of $^{15}NH_4^+$. The standard deviation of 3 replicates is shown in parenthesis.

$[NH_4^+]$ μM	Expected ^{15}N (μM)	Measured ^{15}N (μM)	Recovery (%)
0	0	0.001 (0.000)	100.1
5	0.05	0.048 (0.004)	96
10	0.1	0.094 (0.007)	94
15	0.15	0.131 (0.017)	87.3

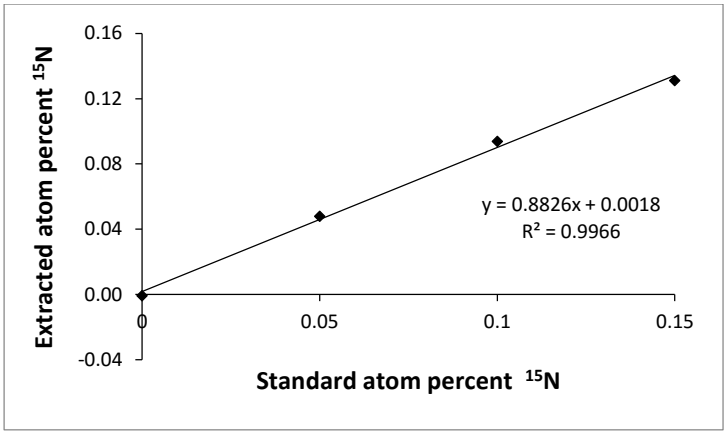


Figure 3.8 Calibration curve of the standards from 2 mL of 0, 5, 10, and 15 μM of $^{14}NH_4^+$ with 1% of $^{15}NH_4^+$.

3.9 Calculation of denitrification rates, anammox and DNRA

The total ambient N_2 gas production was calculated using both the equations of Nielsen (1992), and the revised formulation of the isotope-pairing technique of Risgaard-Petersen *et al*. (2003). The equation of the isotope pairing technique (IPT) of Nielsen (1992) quantifies the production of ^{14}N from the production of $^{29}N_2$ and $^{30}N_2$ after previous addition of $^{15}NO_3^-$. The production of $^{29}N_2$ and $^{30}N_2$ were measured by the IRMS and later used in Nielsen equation as follows:

Nielsen equation

$$D_{14} = \frac{p^{29}N_2}{2 \times p^{30}N_2} \times D_{15} \quad (3.15)$$

$$D_{15} = 2 \times p^{30}N_2 + p^{29}N_2 \quad (3.16)$$

Risgaard-Petersen *et al*. (2003) equation

$$D_{14} = 2\ r_{14}\ [p^{29}N_2 + p^{30}N_2\ (1 - r_{14})] \quad (3.17)$$

$$A_{14} = 2\ r_{14}\ [p^{29}N_2 - 2\ r_{14}\ p^{30}N_2] \quad (3.18)$$

r_{14} was calculated as in Trimmer *et al*. (2006) as follows:

$$r_{14} = \frac{p^{45}N_2O}{2p^{46}N_2O} \quad (3.19)$$

$$D = D_{14} - A_{14} \quad (3.20)$$

$$D_{14w} = D_{14} \frac{r_{14w}}{r_{14}} \quad (3.21)$$

$$D_{14n} = D_{14} - D_{14w} \quad (3.22)$$

Where:

- D_{14} = rate of denitrification from unlabelled NO_3^-.
- D_{15} = denitrification rate of labelled NO_3^- added to the overlying water.
- D = denitrification rate
- r_{14} is the ratio of $^{14}NO_3^-$ to $^{15}NO_3^-$ in the nitrate reduction zone.
- $p^{29}N_2$ and $p^{30}N_2$, are the production rates (μmol m^{-2} h^{-1}) of ^{15}N-labeled N_2 gas produced either as $^{29}N_2$ or $^{30}N_2$ during the incubations.
- A_{14} is the production of N_2 due to anammox.
- $p^{45}N_2O$ and $p^{46}N_2O$, are the measured amount of ^{15}N-labeled N_2O gas produced either as $^{45}N_2O$ or $^{46}N_2O$ during the incubations.

The equation of Nielsen (1992) was modified by Risgaard-Petersen *et al*. (2003) when anammox coexists, but if anammox was not present, Nielsen equation was used to calculate D_{14}. The source of NO_3^- for denitrification can come either from the overlying water of the sediment or from the nitrification process. Differentiation between the sources of NO_3^- for denitrification was calculated according to Nielsen and Glud (1996) as follows:

$$D_w = (D_{15}) \times {[^{14}NO_3^-]}/{[^{15}NO_3^-]} \quad (3.23)$$

$$D_n = D_{14} - D_w \quad (3.24)$$

Where:

- D_w is the denitrification of unlabelled NO_3^- diffusing from the overlying water.
- $[^{14}NO_3^-]$ is the concentration in the overlying water measured in water samples taken before addition of $^{15}NO_3^-$.
- $[^{15}NO_3^-]$ is the concentration in the overlying water measured as the concentration increase in samples taken after addition of $^{15}NO_3^-$.
- D_n is the denitrification of NO_3^- produced by the coupled nitrification-denitrification process within the sediments.

The in situ rate of DNRA was calculated using the relative rates of D_{14} and D_{15} calculated from the IPT equations as in Risgaard-Petersen and Rysgaard (1995):

$$DNRA = p^{15}NH_4 \times ({}^{D_{14}}/_{D_{15}}) \quad (3.25)$$

Where:

- $p^{15}NH_4$ is the production rate of $^{15}NH_4$ (μmol m^{-2} h^{-1})

The in situ rates of DNRA of NO_3^- supplied from the water column, and the DNRA coupled to nitrification were also estimated. The in situ rates were calculated as follows:

$$DNRA_w = p^{15}NH_4^+ \times (\frac{^{14}NO_3^-}{^{15}NO_3^-}) \quad (3.26)$$

$$DNRA_n = DNRA_{total} - DNRA_w \quad (3.27)$$

Where:

- $p^{15}NH_4^+$ is the production rate of $^{15}NH_4^+$
- ^{15}N atom% NO_3^- is the ^{15}N atom% of NO_3^- in the water column.

Rates of anmmox in slurry experiments were estimated from $^{29}N_2$ production according to Thamdrup and Dalsgaard (2002):

$$A_{total} = p^{29}N_2 \times F_A^{-1} \quad (3.28)$$

Where:

- $F_A = {}^{[^{15}NH_4^+]}/_{[NH_{4\,total}^+]}$
- $\frac{[^{15}NH_4^+]}{[NH_{4\,total}^+]}$ represents the fraction of ^{15}N in the total soluble NH_4^+, determined by the difference from the non-enriched reference samples.

3.10 Results and Discussion

Two types of experiments were carried out for anammox. One experiment, merely to confirm the presence of anammox and determine anammox potentials, consisted of anoxic sediment slurry incubations. The other experiment, consisted of intact sediment core incubation, and it was carried out in order to measure denitrification, anammox, and DNRA rates. First, the results from the anoxic sediment slurry incubations are described. Afterwards, the result from the intact sediment core incubations are described. These are followed by a section to justify the use of Nielsen equation (1992).

3.10.1 Anoxic sediment slurry incubations

The $p^{29}N_2$ in the experiment carried out to confirm anammox, ranged between 6.12 x10 $^{-5}$ and 1.57 x 10^{-3} μmol N cm^{-3} d^{-1} in wet sediment in The Wash and between 8.4 x10 $^{-4}$ and 1.70 x 10^{-2} μmol N cm^{-3} d^{-1} in wet sediment in the North Sea (Table 3.2). The slope was very low (Fig. 3.9), so that in order to evaluate if the production of $^{29}N_2$ had had a significant increase during the incubation time, a t-test to evaluate that the slope was different from zero was carried out. The production of $^{29}N_2$ was different from zero in all the stations of the North Sea. In The Wash, May, and October, the slopes were significantly different from zero ($p<0.05$), while June did not show a slope different from zero. During September, the chemicals used to amend the vials for the anammox confirmation experiment were inadvertently mixed up, so that, the added combination was $^{14}NH_4^+$ plus $^{15}NO_3^-$, instead of $^{15}NH_4^+$ plus $^{14}NO_3^-$ (see section 3.6.2). In this case it was possible to calculate both denitrification and anammox as in Thamdrup and Dalsgaard (2002), however, anammox was not detectable.

The potential rate of anammox in the slurry experiment was calculated as in Thamdrup and Dalsgaard (2002). Anammox ranged between 3.89 x 10^{-3} and 0.73 nmol N cm^{-3} h^{-1} in wet sediment (Table 3.2). In the North Sea the potential for the anammox reaction, estimated from anoxic homogenized sediment, were greater than in The Wash and ranged from 0.04 to 0.73 nmol N cm^{-3} h^{-1} in wet sediment, while the rates of anammox in The Wash ranged from 0.0 to 0.06 nmol N cm^{-3} h^{-1} in wet sediment (Fig. 3.10; Table 3.2). The highest and lowest potential for anammox in The Wash were found during May and June respectively. In the North Sea the highest and lowest potential were found at station 127 and station 43 (Table 3.2).
There are no previous measurements for anammox potential for The Wash, but the values for anammox potentials found for the North Sea in this study agree with previous values reported for the North Sea by Trimmer *et al*. (2003) in the Thames, and by Bale *et al*. (2014) in the Oyster Grounds, Dogger Bank, Frisian Front, and Dutch Coast.

The anammox potentials reported by Trimmer *et al*. (2003) ranged between 0 and ~ 10 nmol ml^{-1} h^{-1} in wet sediment, with a trend to decrease along a gradient from high to low organic silt-clay content. In this study, the maximum anammox potential rates were found during October and November with a maximum contribution to N_2 formation of 8%. On the other hand, the survey carried out by Bale *et al*. (2014) showed that anammox was more active in the Oyster Grounds, where it ranged from ~ 0.5 to 3.0 nmol N cm^{-3} h^{-1}, with a maximum contribution to N_2 formation during February (29%) and a mean overall contribution during the year of ~ 18 % (± 8). The reported values during August (that was the month when the present study was carried out in the North Sea) were ~2.2 nmol N cm^{-3} h^{-1}. In the same study carried out by Bale *et al*. (2014), however, the potential for anammox reaction was not found in Frisian Front and it was < 1 nmol N cm^{-3} h^{-1} for Dogger Bank and the Dutch Coast.

3.10.2 Anammox confirmation in anoxic sediment slurry

Anammox was confirmed through anoxic sediment slurry experiment in The Wash and in the North Sea. However, in none of the station of The Wash, and only in stations 43, and 101 in the North Sea anammox was detected, accounted for 2% and 6.6 % respectively. It is hard to understand what is the cause of this discrepancy, based only on rates of anammox measurements and NO_3^- concentrations of the overlying water of the sediment cores. However, one reason might be the depth distribution of anammox in sediments. Schmid *et al*. (2007) determined the depth distribution of anammox cells in marine sediments and found that the percentage of anammox cells to the total prokaryotic population varied from 0% to 8.6%, and in general the abundance of anammox bacteria increases with sediment depth for sediments of the Golfo Dulce (Costa Rica) and Gullsmarsfjorden (Sweden). Particularly for the Frisian Front (North Sea) the authors found that the percentage of anammox in the upper 1 centimetre was 3.3% and increased to 7% between 1-1.5 cm depth. Interestingly in the study of Schmid *et al*. (2007), NO_3^- concentrations decreased from ~37 μM in the surface to < 5 μM at 1-1.5 cm depth. If the drop in NO_3^- concentration is due to denitrification, it might be possible that anammox bacteria have thrived at 1-1.5 cm depth due to the availability of NO_2^- formed as a by-product of denitrification. Hence, it is possible that NO_2^- was not available neither in The Wash nor at stations 68, 127 and 141 in the North Sea, to allow anammox bacteria to thrive and be detected in intact sediment cores. In anoxic slurry experiments the sediments are mixed and over enriched, which may enhance the anammox reaction.

Table 3.2. Anammox potential determined in anoxic sediment slurry. Total N_2 production (D_{14}), denitrification (DNTR) and anammox rates, anammox contribution to total N_2 production (ra), determined from intact sediment cores at The Wash and the North Sea.

Site	Anammox potential (nmol cm^{-3} h^{-1})*	D_{14} (μM m^{-2} h^{-1})	DNTR (μM m^{-2} h^{-1})	Anammox (μM m^{-2} h^{-1})	ra (%)	Dw (%)	Dn (%)	DNRA (μM m^{-2} h^{-1})	$[NO_3^-]$ (μM)
The Wash									
May	0.06	6.9 ± 2.3	6.9 ± 2.3	0.00	0.00	20.5	79.5	0.0	
June	0.004	8.1 ± 3.7	8.07 ± 3.7	0.00	0.00	64.5	35.5	3.3	7.5 ± 0.14
Sept	0.02	0.42 ± 0.5	0.42 ± 0.5	0.00	0.00	100.0	0.00	0.16	0.74 ± 0.15
Oct	0.02	2.9 ± 1.2	2.92 ± 1.2	0.00	0.00	32.9	67.1	0.19	3.02 ± 0.97
The North Sea									
Stn 43	0.04	9.5	9.31	0.19	2.01	12.7	87.3	0	2.34 ± 0.59
Stn 68	0.16	7.7 ± 2.5	7.65 ± 2.5	0.00	0.00	11.5	88.5	1.3	1.96 ± 0.12
Stn 101	0.25	2.9	2.73	0.19	6.59	12.0	88.0	0	2.99 ± 0.67
Stn 127	0.73	7.8 ± 0.86	7.81 ± 0.86	0.00	0.00	18.79	81.2	0	5.99 ± 0.22
Stn 141	0.22	10.6	0	0.00	0.00	13.63	86.4	0	2.33 ± 0.38

Dw is denitrification of NO_3^- diffusing from the overlying water.
Dn is denitrification of NO_3^- produced by nitrification.
* in wet sediment.

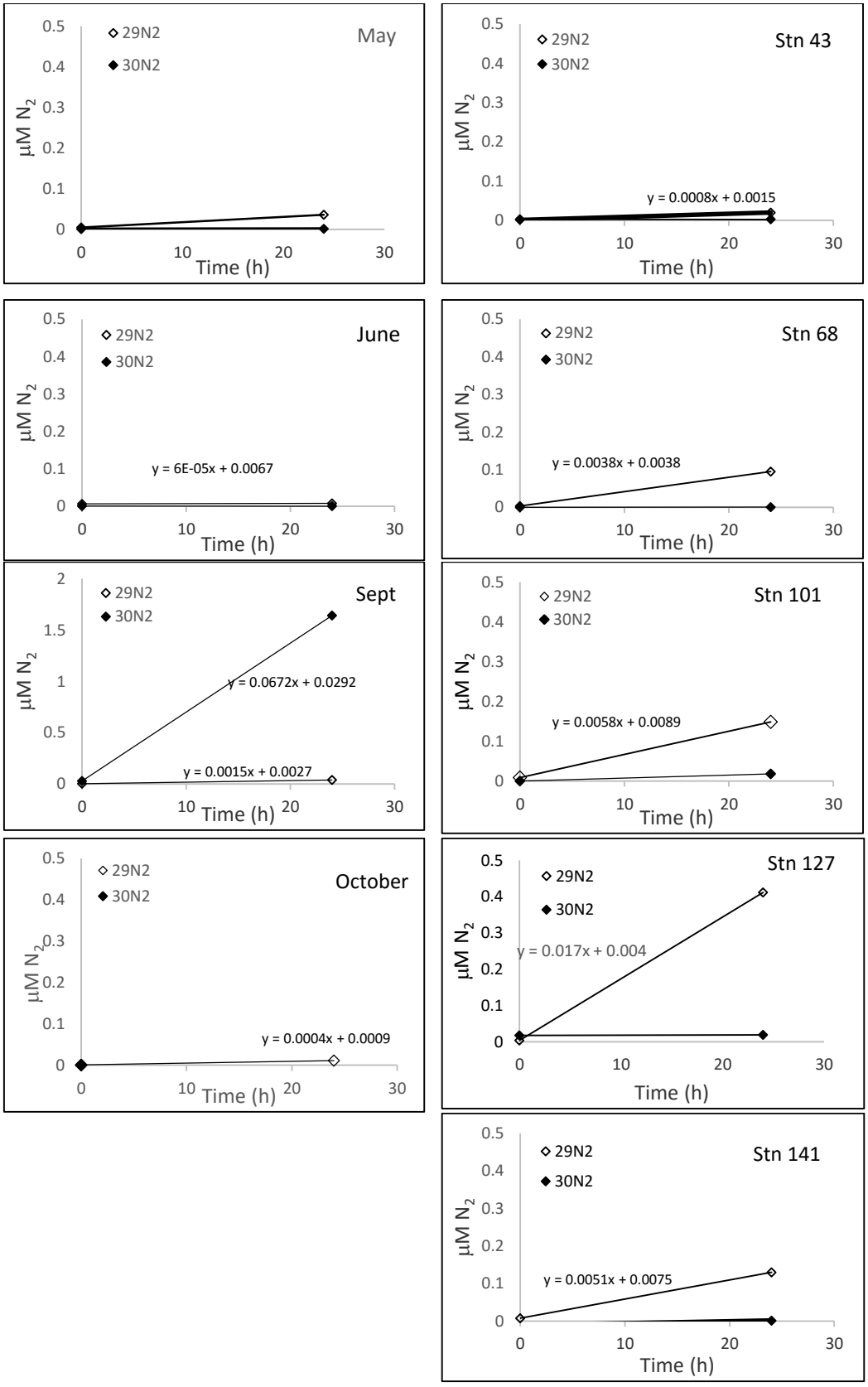


Fig. 3.9. Production of $^{29}N_2$ from anoxic sediment slurry incubation carried out for anammox confirmation in The Wash (right hand side panels) and in the Norht Sea (left hand side panels). The slurry experiment was ammended with $^{15}NH_4^+$ and $^{14}NO_3^-$.

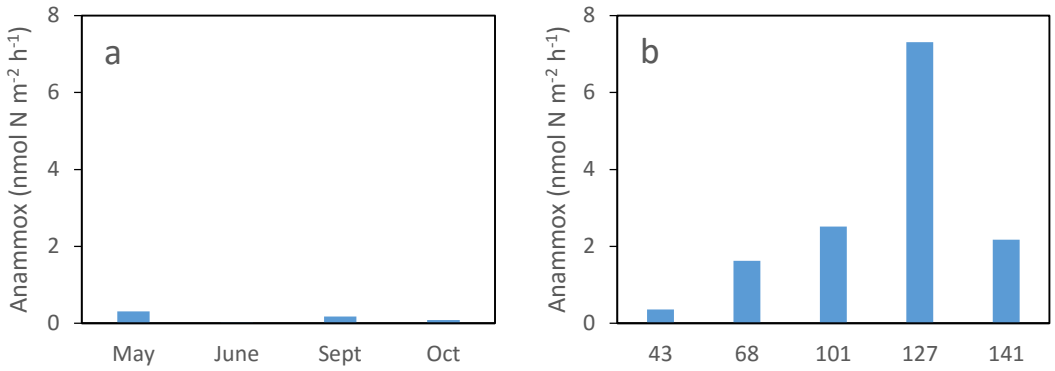


Fig. 3.10. Production of N_2 (D_{14}) from anammox measured in anoxic sediment slurry incubations, in The Wash (a) and during August in the North Sea (b).

3.10.3 Intact sediment core incubations

Even though the potential for the anammox reaction was confirmed, the values for anammox rates are low and in general did not contribute to N_2 production. This could be confirmed when the equations of Risgaard-Petersen *et al*. (2003) and Trimmer *et al*. (2006) were applied to the data from the intact sediment cores. Anammox could not be measured in The Wash, and it was only possible to detect rates of anammox in two stations of the North Sea: 43 and 101, with rates of N_2 production of 2.81 μmol m^{-2} h^{-1} and 1.47 μmol m^{-2} h^{-1} respectively (Fig. 3.11), with relative contribution of anammox to N_2 production, ranging between 2% for station 43, and 6.6% for station 101. Although, the relative contribution of anammox was higher in the station 101, it is worth noting that this station has the lowest rate of denitrification (3.95 μmol m^{-2} h^{-1}) in the North Sea (Fig. 3.11).

Other recent studies in the North Sea have determined anammox rates from intact sediment core experiments. One of the studies (Trimmer *et al*., 2006) carried out at two stations in the Thames estuary: Gravesend, and Southend, found high rates of anammox (48.94 μmol m^{-2} h^{-1}) in Gravesend, with a total N_2 contribution of about 21%. However, they did not find anammox in Southend (Trimmer *et al*., 2006). Another study carried out at three sites in the North Sea: Sean Gas, Oyster Grounds, and North Dogger, found the mean highest rates of anammox (3 μmol m^{-2} h^{-1}) in Oyster Ground, and equal rates of anammox (1 μmol m^{-2} h^{-1}) in Sean Gas, and North Dogger. The respective anammox contribution to the total N_2 production was: 18%, 20%, and 13% (Neubacher *et al*., 2011).

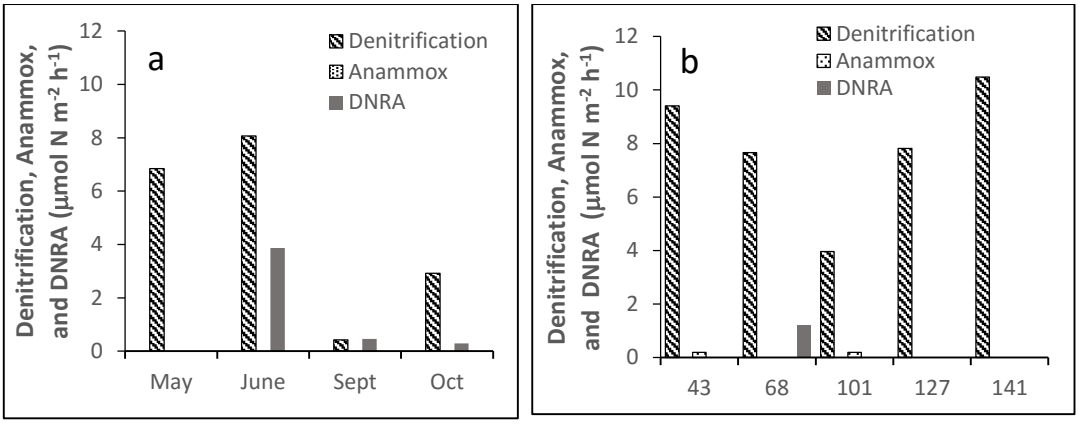


Fig. 3.11. Rates of denitrification, anammox, and DNRA, measured in intact sediment cores, in The Wash (a) and during August in the North Sea (b).

Both the anammox rates, and its contribution to total N_2 production determined in this study were lower than the ones found by Trimmer *et al*. (2006) and Neubacher *et al*. (2011), although, neither anammox was not detectable at Southened (Trimmer *et al*., 2006). Regarding the study of Neubacher *et al*. (2011), although they found anammox at the three stations, on some visits, they did not find anammox during May at Sean Gas, and February and April at North Dogger. In this study, the sampling was carried out during August in the North Sea, and anammox was found only in two of the five stations sampled. In The Wash, in none of the months was possible measure anammox activity, by using Risgaard-Petersen *et al*. (2003) equation and Trimmer *et al*. (2006) equation.

Although, some estuaries have shown that anammox has a contribution as high as > 20% (Trimmer *et al*., 2006; Rich *et al*., 2008), in general it is expected that in estuaries the relative importance of anammox to be < 5 % (Risgaard-Petersen *et al*., 2003), and it tends to increase with depth, although the absolute rates of anammox diminish (Thamdrup and Dalsgaard, 2002; Dalsgaard *et al*., 2005). The findings in The Wash of the present study, agrees with the expectations for estuaries.

Previous studies in the North Sea have been carried out in the southern North Sea in areas around the Dogger Bank, Frisian Front, Oyster Grounds and Dutch Coast (Neubacher *et al*., 2011, 2013; Bale *et al*., 2014). In contrast, the present study presents data from both the southern (stations 43, and 68) and the northern (stations 101,127 and 141) North Sea. It is also worthwhile to highlight that the present study is the first one in which a survey in the northern North Sea was carried out.

Even when anammox was correlated with depth, from the two stations with the deepest depth (110 m, stations 101; 116 m, station 127), in only one (station 101), the anammox process was observed, although, the relative contribution to total N_2 production was ~6.6 %. A

contrasting result was also observed by Trimmer *et al*. (2013), who found that in the stations S4 (176 m depth) and S6 (384 m depth) at the Skagerrak, where previous studies (Thamdrup and Dalsgaard, 2002; Dalsgaard, *et al*., 2005) had found that anammox contribution accounted for > 60%, was absent at the time that the survey was carried out.

3.10.4 Absence of anammox

The fact that anmmox was not detected in some of the stations might be due to one of the following causes.

Firstly, in slurries amended with $^{15}NH_4^+$ plus $^{14}NO_3^-$, the production of ^{15}N-N_2 by anammox bacteria, might be due to either of the following reactions (Risgaard-Petersen *et al*., 2004):

$$^{15}NH_4^+ + {}^{14}NO_2^- \rightarrow {}^{29}N_2 + 2H_2O \quad (3.29)$$

$$5\,{}^{15}NH_4^+ + 3\,{}^{14}NO_3^- \rightarrow 3\,{}^{29}N_2 + {}^{30}N_2 + 9H_2O \quad (3.30)$$

Most of the studies (Risgaard-Petersen *et al*., 2004; Thamdrup and Dalsgaard, 2002; Engström *et al*., 2005; Engström *et al*., 2009) have shown that only the reaction described by equation 3.29 is taking place. Under this premise then, the presence or absence of anammox may be linked to the availability of NO_2^- (Risgaard-Petersen *et al*., 2004; Dalsgaard *et al*., 2005). This implies that anammox bacteria rely on the NO_3^- reduction in the suboxic zone of the sediment, so that, if NO_3^- concentration is low, the production of NO_2^- may not be enough to support anammox bacteria (Risgaard-Petersen *et al*., 2004). The fact that NO_3^- concentrations in the overlying water, both in The Wash and in the North Sea were mainly below 3 μM (Table 3.2), may have limited the NO_2^- production by NO_3^- reduction to a level that was not enough to support anammox. Besides, the fact that NO_3^- reducers are limited under 2-3 μM NO_3^- (Dalsgaard and Bak, 1994) might explained the absence of anammox contribution to the total N_2 production.

Secondly, it has been suggested that anammox bacteria are able to carry out the process of DNRA by reducing NO_3^- to NH_4^+ (Kartal *et al*., 2007b), thus, producing the NH_4^+ that they require. Then NH_4^+ could combine with NO_2^- via the anammox process (Kartal *et al*., 2007b). Under such scenario, because the overlying water in the intact sediment core experiment was enriched with $^{15}NO_3^-$, the reduction of NO_3^- by DNRA would produce $^{15}NH_4^+$. Once $^{15}NH_4^+$ is combined with $^{15}NO_2^-$ produced from $^{15}NO_3^-$ reduction, the final product of anammox would be $^{30}N_2$. The ITP equations assume that anammox will produce only $^{29}N_2$ through reaction one (equation 3.15), and hence, anammox would not be detected by using the ITP equations. Therefore, anammox contribution might be underestimated, while denitrification might be overestimated. However, if anammox bacteria were carrying out DNRA this would mean that

both anammox bacteria and denitrifiers would compete for NO_3^-, and thus even when NH_4^+ were available for anammox, NO_2^- would be limiting the anammox process since less NO_3^- would be available for denitrification and consequently for NO_2^- production. In this manner, anammox would not be taking place, and would confirm why anammox was not detected.

Finally, the low concentrations of NO_3^- in most of the stations may have limited the NO_2^- required for anammox, and on the other hand, the high affinity for NO_3^- (0.5 μM; Dalsgaard and Bak, 1994) of DNRA bacteria may have outcompeted anammox bacteria and thus anammox was not detected. Interestingly, unlike other studies (Trimmer *et al*., 2003; Rysgaard *et al*., 1996) in this study, in the stations where DNRA was detected, anammox was absent which may confirm the hypothesis that DNRA bacteria may outcompete anammox bacteria under low concentrations of NO_3^-.

In The Wash, other factors, such as the biogeochemical processes that occur within the sediments of the estuaries, may have played an important role in controlling the anammox process. Biogeochemical processes in estuaries are continually changing and the intertidal areas are even more variable. In these areas, apart from the many factors that may regulate the biogeochemical processes that are taking place in sediments (i.e infauna, benthic respiration rates or organic matter content), the spring and neap cycle and the freshwater flow from the tributary river affect the magnitude of the variation of the interstitial nutrients (Malcom and Syvier, 1997). For example, a study carried out at the intertidal areas of the Great Ouse, in The Wash, showed that during the time that it takes the tide to go out (~1 h), the concentration of NO_3^- in the interstitial water of the sediment increased from 65 μmol L^{-1} to 200 μmol L^{-1} (Malcom and Sivyer, 1997). Additionally, Syvier (1999) also found that the sediments in The Wash are mainly a source of NH_4^+, in which case it may be that anammox is not limited by NH_4^+, but it may be limited by the availability of NO_2^-.

Some studies have found that the contribution to total N_2 production was strongly correlated with high concentrations of NO_3^- and anammox bacteria (Thamdrup and Dalsgaard, 2002; Risgaard-Petersen *et al*., 2005; Hou *et al*., 2012; Hou *et al*., 2013), suggesting that anammox might be favoured by higher concentrations of NO_3^-. However, other studies have not observed this correlation. For example, low activity for anammox bacteria was observed in shallow waters with organic rich sediments and high NO_3^- availability (Dalsgaard *et al*., 2005). In the study of Dalsgaard *et al*. (2005), it was suggested that although high concentration of organic matter and NO_3^- were available, anammox was not stimulated because higher concentrations of organic matter create a high demand for NO_3^- and NO_2^- such that only a small fraction of NO_3^- is available to be reduced to NO_2^-, which is necessary for the anammox reaction. On the other hand, the amount of CO_2 fixed per mol of NH_4^+, and the doubling time (7 to 20 days) of anammox bacteria are very low (Strous *et al*., 1999b; Güven *et al*., 2005; Jetten *et al*., 2009). These characteristics, plus the highly variable conditions in The Wash,

may have affected the anammox bacteria community in such a way that do not allow them to maintain an active enzymatic system that allows them to thrive (Risgaard-Petersen *et al*., 2005). It is worthwhile to notice that, although the samples in The Wash were collected in May, June, September and October, there was only one sampling site in the intertidal area of The Wash. The fact that in this study the presence of anammox was not observed does not mean that this process is not taking place in other months or in other sites of The Wash. Thus, in order to better understand this process in The Wash more studies should be carried out, including sampling at more stations within the estuary.

3.10.5 Nielsen equation

According to Risgaard-Petersen *et al*. (2003), the isotope pairing technique (Nielsen, 1992) overestimates D_{14} when anammox is present. In the Nielsen's equation (1992), D_{14}, the indigenous rate of denitrification, which represents the denitrification rate of unlabelled NO_3^-, is calculated from D_{15} (equation 3.2). In turn, D_{15} represents the denitrification of the $^{15}NO_3^-$ added, which produces both $^{29}N_2$ and $^{30}N_2$. Given that anammox bacteria obtain their energy for growth from the conversion of NH_4^+ and NO_2^- into N_2 (Strous *et al*., 1998; equation 3.3), the $^{15}NO_3^-$ added to the overlying water of the sediment in the time-series experiment will produces $^{29}N_2$ ($^{14}N^{15}N$).; from ^{15}N from the reduced $^{15}NO_3^-$ to $^{15}NO_2^-$, and ^{14}N from the NH_4^+ present in the sediment as the result of organic matter mineralization. Consequently, if anammox is present, both denitrification and anammox will produce $^{29}N_2$, and then, D_{14} would be overestimated by including the $^{29}N_2$ produced by anammox. Therefore, the equation proposed by Nielsen (1992) cannot be used to calculate D_{14}, if anammox and denitrification coincide (Risgaard-Petersen *et al*., 2003). In this study if the anammox process was not detected or was <2%, Nielsen's equation (1992) was used to calculate denitrification rates.

Based on the results obtained in the intact sediment core experiment, it was assumed that the production of $^{29}N_2$ observed was solely the result of the reduction of $^{15}NO_3^-$ by denitrification in all the sampling campaigns in The Wash, and in the stations 68 and 127 of the North Sea. By assuming this, denitrification rates were calculated with Nielsen (1992) equations, and the equations of Risgaard-Petersen *et al*. (2003) and Trimmer *et al*. (2006) were used only in the stations were anammox was detected.

3.10.6 Denitrification

Rates of total N_2 production calculated from intact sediment cores ranged from 1.10 to 10.83 μmol m^{-2} h^{-1}, in The Wash, and from 2.49 μmol m^{-2} h^{-1} to 10.48 μmol m^{-2} h^{-1} in the North Sea (Table 3.2). A trend between the N_2 production and depth was not observed for the North Sea. Also, no temporal trend of N_2 production from May to October was observed in The Wash.

In The Wash, the rates of denitrification during May and June were noteably higher than during September and October, with maximum rates of denitrification during June, and the minimum during September (Fig. 3.11a). On the other hand, in the North Sea station 101 had the lowest rate of denitrification (2.49 μmol m^{-2} h^{-1}), while in all the other stations the rate of denitrification was > 6.5 μmol m^{-2} h^{-1}, with the maximum denitrification rate peaking at station 141 (Fig. 3.11b).

Previous studies in The Wash were carried out along a stretch of the Great Ouse River, on the intertidal flats on the edge of The Wash. (Trimmer *et al*., 1998). In their study, the denitrification rates were determined by using the acetylene blockage method, and results showed that in the most seaward station, the denitrification rates ranged between 8 μmol m^{-2} h^{-1}, and 88 μmol m^{-2} h^{-1}, with the highest concentration in May (88 μmol m^{-2} h^{-1}), and the lowest in July (4 μmol m^{-2} h^{-1}). In general, excluding May, the denitrification rates (4 μmol m^{-2} h^{-1} to 9 μmol m^{-2} h^{-1})
observed by Trimmer *et al*. (1998) coincide with the ones reported in this study (1.10 μmol m^{-2} h^{-1} to 10.83 μmol m^{-2} h^{-1}).

In another study by Sivyer (1999), carried out at three sites within The Wash embayment (the Great Ouse estuary), the rates of denitrification were estimated from interstitial water profiles who observed that the denitrification rates were proportional to the NO_3^- in the overlying water in two sites, suggesting that denitrification is mainly driven by the NO_3^- supplied from the overlying water. The rates estimated ranged between 80 μmol m^{-2} h^{-1} to 350 μmol m^{-2} h^{-1}. At the third site of Sivyer's (1999) study a correlation between denitrification and NO_3^- concentration in the overlaying water was not found, therefore, it was suggested that at this site denitrification was tightly coupled to nitrification. The rates of denitrification at this site ranged from 36 to 70 μmol m^{-2} h^{-1}.

A study also carried out in intertidal areas of the North Sea at two sites of the Thames recorded denitrification rates that ranged from 0 μmol m^{-2} h^{-1} at Southend to 192 μmol m^{-2} h^{-1} at Gravesend (Trimmer *et al*., 2006). The highest rate at the Gravesend agrees well with the denitrification rates observed by Sivyer (1999), but are much higher than the ones reported in the present study.

Table 3.3 Rates of total N_2 production (D_{14}), denitrification, anammox (μmol N m^{-2} d^{-1}), and contribution of anammox (ra) to total N_2 production in the North Sea.

Locations	Method	D_{14}	Denitrification	Anammox	ra	Source
					(%)	
St Lawrence Estuary	ISCE	16.8	11.3	5.5	33	Crowe *et al*. (2012)
Gullmarsfjorden			6.08	6.64	48	Trimmer *et al*. (2006)
Thames Southend			na	na	0	Trimmer *et al*. (2006)
Thames Gravesend			192.9	48.94	21	Trimmer *et al*. (2006)
Smeerenburgfjiorden (Svalbard archipielago)	ISCE	12.71 - 13.33	12.04 - 12.25	0.63 - 1.08	5 - 8	Gihring *et al*. (2010)
Kongsfjorden (Svalbard archipielago)	ISCE	1.17 - 2.5	1.42	0.42	23	Gihring *et al*. (2010)
Randers Fjord	ISCE	14.83	13.96	0.88	5.9	Risgaard-Petersen *et al*. (2005)
Norsminde Fjord	ISCE	7.96	7.96	0	0	Risgaard-Petersen *et al*. (2004)
Sylt (Waden Sea)	ISCE	0.27 -3.32	0.25 -2.86	0.016 - 0.0.48	6 - 14	Canion *et al*. (2014)
Ymerbukta (Svalbard)	ISCE	0.41	0.38	0.027	6.5	Canion *et al*. (2014)
Skagerrak S4	ISCE	13.25	13.25	0	0	Trimmer *et al*. (2013)
Skagerrak S6	ISCE	9.57	9.57	0	0	Trimmer *et al*. (2013)
Skagerrak S8	ISCE	2.62	0.61	2.01	77	Trimmer *et al*. (2013)
Skagerrak S9	ISCE	1.07	0.3	0.77	72	Trimmer *et al*. (2013)
North Sea (Sean Gas)	ISCE	4	3	1	20	Neubacher *et al*. (2011)
North Sea (Oyster Ground)	ISCI	14	11	3	18	Neubacher *et al*. (2011)
North Sea (North Dogger)	ISCI	8	7	1	13	Neubacher *et al*. (2011)
North Sea (Oyster Ground)	MABT		0.5 - 0.8	na	na	Lohse *et al*. (1993)
North Sea (Weiss Bank)	MABT		0.7 - 0.9	na	na	Lohse *et al*. (1993)

North Sea (Tail End)	MABT		0.0 - 0.1	na	na	Lohse *et al*. 1993)
North Sea (Esbjierg)	MABT[b]		0.4 - 3.8	na	na	Lohse *et al*. (1993)
North Sea (Helgoland Bight)	MABT[b]		1.9 - 8.2	na	na	Lohse *et al*. (1993)
North Sea (Elbe Rinne)	MABT[b]		0.2 - 1.3	na	na	Lohse *et al*. (1993)
North Sea (Frisian Front)	MABT[b]		0.7 - 1	na	na	Lohse *et al*. (1993)
North Sea (Sean Gas)	ASSI			0.75 - 3.0[a]	2 - 20	Bale *et al*. (2014)
North Sea (Oyster Ground)	ASSI			0.49 - 0.80[a]	10 - 26	Bale *et al*. (2014)
North Sea (North Dogger)	ASSI			0.25 - 0.58[a]	6 - 18	Bale *et al*. (2014)
Norht Sea (Frisian Front)	ASSI			0	0	Bale *et al*. (2014)
North Sea (Stn 43)	IPT	9.5	9.31	0.19	2	This study
North Sea (Stn 68)	IPT	7.7	7.7	0	0	This study
North Sea (Stn 101)	IPT	2.9	2.7	0.19	6.6	This study
North Sea (Stn 127)	IPT	7.8	7.8	0	0	This study
North Sea (Stn 141)	IPT	10.6	10.6	0	0	This study
The Wash (May)	IPT	6.9	6.9	0	0	This study
The Wash (May)	IPT	8.1	8.1	0	0	This study
The Wash (Sept)	IPT	0.42	0.42	0	0	This study
The Wash (Oct)	IPT	2.9	2.9	0	0	This study

ISCE: intact sediment core experiment), ASSI: Anoxic sediment slurry experiment, MABT (modified acetylene block technique).

Rates of denitrification observed in the North Sea, determined either by using the acetylene blockage method (Lohse *et al*., 1993) or by applying the corrected IPT (Neubacher *et al*., 2011) range between 0 to 11 μmol m^{-2} h^{-1} (Table 3.3). The rates of the denitrification of the present study are in the range of the rates previously reported.

The partition of total N_2 production into denitrification of NO_3^- produced by nitrification (Dn) in the sediments, and denitrification of NO_3^- diffusing from the overlying water (Dw) indicate that Dn was responsible for more than 80% of the total N_2 produced in all the stations of the North Sea, while in The Wash, it varied from 34% to 90% (Table 3.2). In The Wash the higher percentages of Dn were observed during May and October, while during June and September the N_2 production due to Dn accounted for 34% and 54% respectively.

3.10.7 Dissimilatory nitrate reduction to ammonium (DNRA)

The production of NH_4^+ by dissimilatory nitrate reduction was detected during June (3.3 μm m^{-2} h^{-1}), September (0.16 μM N m^{-2} h^{-1}), and October (0.19 μM N m^{-2} h^{-1}) in The Wash, but only at station 68 (1.3 μM N m^{-2} h^{-1}) in the North Sea (Fig. 3.11b). A study carried out by Kerry-Gerreyn *et al*. (2001) along the Great Ouse, and at the intertidal areas of The Wash, showed that DNRA accounted for 7% to 58% of the total NH_4^+ produced. The authors highlighted that DNRA was tightly related to the NO_3^- concentration in the water column as well as to temperatures ranging between 15 – 22°C and suggested that under extended warm summer, DNRA may contribute to eutrophication. In The Wash the NO_3^- concentrations in the water column were not as high as the recorded ones for the Great Ouse, however, the temperatures of the months where DNRA was detected, ranged between 16°C – 19.8°C (Table 3.2) which agrees with the study of Kerry-Gerreyn *et al*. (2001), although in The Wash DNRA rates were lower than DNRA rates previously estimated, DNRA accounted for 6.5 to 40% of NO_3^- reduction which agreed with the findings of Kelly-Gerreyn *et al*. (2001).

In a recent study, carried out in the southeastern part of the North Sea (Wadden Sea) the rates of DNRA were < 1 mmol N m^{-3} h^{-1} which represented between 10-20% of NO_3^- consumption and accounted for 15% of total NO_3^- reduction (Marchant *et al*., 2014).

According with Tiedje *et al*. (1982) the partitioning of NO_3^- between denitrification and DNRA is a function of carbon electron acceptor (NO_3^-) so that in extreme anoxic conditions where electron acceptors are limited, DNRA may be favoured by the fact that in the DNRA reaction (equation 3.31) 8 electrons are transferred per mol of NO_3^-, while in denitrification reaction only 5 electrons are transferred (equation 3.32).

$$NO_3^- + 4H_2 + 2H^+ \rightarrow NH_4^+ + 3H_2O \quad (3.31)$$

$$2NO_3^- + 5H_2 + 2H^+ \rightarrow N_2 + 6H_2O \quad (3.32)$$

Station 68 is close to the Wadden Sea, and in this area the degradable carbon varies from 8 μmol org-C m^{-3} in January-February to 250 μmol org-C m^{-3} during May-July (Beer *et al*., 2005). This high carbon concentration may enhance high carbon to electron acceptor-donor ratios, thus it may favoured the DNRA reaction at station 68.

Although, DNRA has been observed in the Baltic Sea (Jäntti *et al*., 2012;) estuaries of the North Sea, the Great Ouse and intertidal area of The Wash (Kerry-Gerreyn *et al*., 2001), and Wadden Sea (Marchant *et al*., 2014), so far there are no reports of DNRA on sediments offshore the North Sea, and although the rate observed at station 68 (1.3 μM m^{-2} h^{-1}) is lower than the rates observed for those places, the total NO_3^- reduction rates due to DNRA accounted for around 17% at station 68.

3.10.8 Summary

Denitrification, anammox and DNRA compete for nitrous oxides in sediments. If from these pathways, it is considered the energy released per mole of either NO_3^- or NO_2^- reduced during their respective reactions, then, DNRA would dominate over the other two process because it delivers slightly more energy (Porubsky *et al*., 2009; Table 3.4.).

Table 3.4. Energy delivered from denitrification, anammox and DNRA processes (Porubsky *et al*., 2009).

Process	Equation	Energy (kJ $mole^{-1}$ NOx)
Denitrification	$2NO_3^- + 5H_2 + 2H^+ \rightarrow N_2 + 6H_2O$	~560
Anammox	$NO_2^- + NH_4^+ \rightarrow N_2 + 2H_2O$	~358
DNRA	$NO_3^- + 4H_2 + 2H^+ \rightarrow NH_4^+ + 3H_2O$	~600

In the present study, denitrification dominated over anammox and DNRA both at The Wash and the North Sea. In The Wash, anammox was not detected and DNRA accounted for 6 to 40% of the NO_3^- reduction. In the North Sea, anammox was detected, although its contribution

was <7%. On the other hand, DNRA was found only at station 68 where accounted for 17% of the NO_3^- reduced. This results are in line with the observations of Trimmer *et al*. (2003) and Nicholls and Trimmer (2009) who found that denitrification dominated over anammox in the Thames estuary and in nine estuaries of the southeast coast of England. In the Colne estuary, Dong *et al*. (2009) also found that denitrification dominated over anammox and DNRA. Similarly, the results from the North Sea, agreed with the observations of Neubacher *et al*. (2011), which also found that denitrification dominated over anammox, with the average contribution of anammox ranged from 13 to 20%.

Some studies highlight that the efficiency and dominance of denitrification, anammox and DNRA, depend on factors such as: organic carbon (Babbin *et al*., 2014), C:N ratios (Tiedje, 1988; Porubsky *et al*., 2009), sulfide concentrations (Rysgaard *et al*., 1996; Ann and Gardner, 2002; Jäntti and Hietanen, 2012), temperature (Kelly-Gerreyn *et al*., 2001) and NO_3^- concentrations (Koop-Jakobsen and Giblin, 2010;). Also, the importance of anammox has been correlated to depth, although at the same time it has been recognized that organic carbon decreased with depth (Dalsgaard *et al*., 2005; Engstöm *et al*., 2005). However, the effect of the factor is contrasting and therefore not quite clear yet.

In a study carried out by Burgin and Hamilton (2007), the authors highlight the importance of organic carbon as a main factor controlling the pathway to N_2 or NH_4^+ production. The authors also emphasize the influence of other factors, so that the presence of sulfide and C:N ratio will setup the conditions that favor the process previously mentioned. In agreement with their hypothetical summary, denitrification would be favored at both high and low carbon. Under high carbon conditions, denitrification would be favored in not sulfidic sediments and low C:N ratios, while not sulfidic sediments and high C:N ratios would favor DNRA. Under this high C conditions, DNRA is also favored when sulfide is present in the sediments. On the other hand, low carbon conditions and high C:N ratios would favor denitrification, while anammox would be favored under low C:N ratios (Figure 3.12).

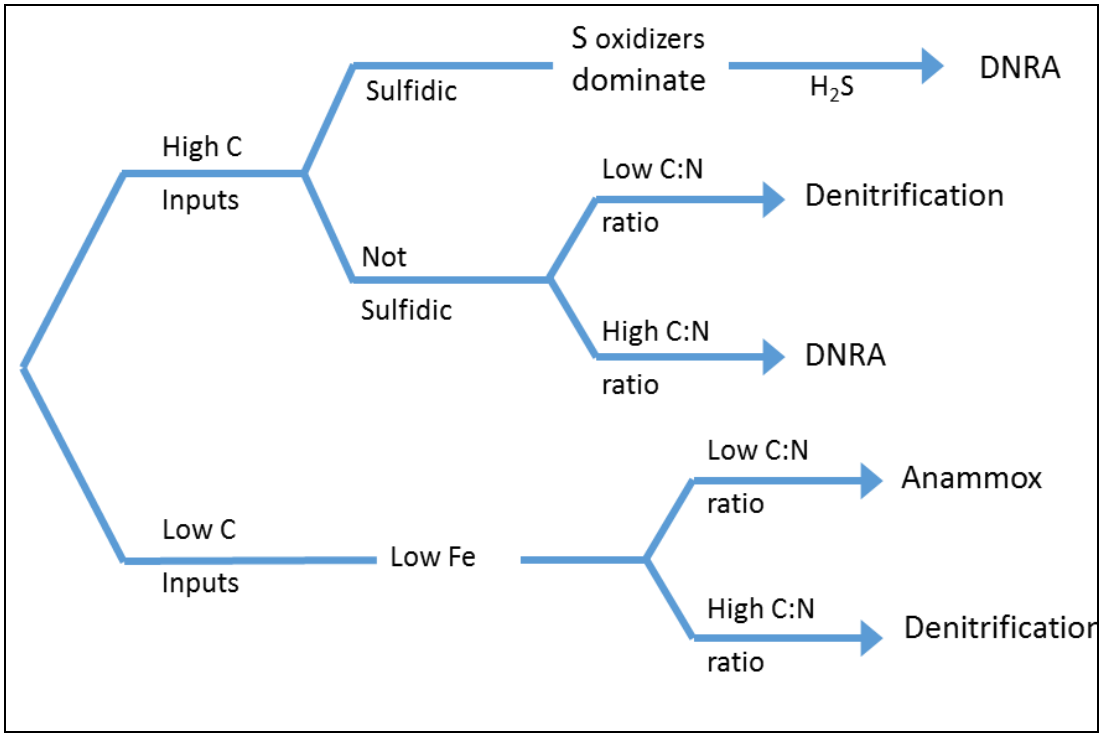


Figure 3.12. Possible pathways of NO_3^- reduction. S means sulfur, from the different forms of S, only the free forms (H_2S and S_2^-) inhibits denitrification. Modified from Burgin and Hamilton (2007).

In the present study, the organic carbon content measured in sediment ranged between 0.05 and 0.48% (Table 4.2), which is low compared with other studies with up to 7.7% (Trimmer *et al.*, 2000). Thus, in agreement with the hypothetical model of Burgin and Hamilton (2007), anammox should be favored. In the same way, DNRA would be found if C:N ratios were high. However, as it was mentioned before, anammox was not detected in The Wash, which average organic carbon content in sediment (0.093%) was lower than in the North Sea (0.244%; Table 4.2). Nevertheless, DNRA was found at low C:N ratio in The Wash (4.2 to 9.3) and in the North Sea (~3). The result of anammox does not agree with the findings of the studies in sediments from the Skagerrak and the Bay of Aarhus (Dalsgaard *et al*., 2005) and Skagerrak, Kattegat and Long Islan Sound, USA (Engström *et al*., 2005) where a negative correlation between the relative importance of anammox and organic carbon was observed. Both studies also observed that the organic carbon content in sediments decreases with depth and although denitrification and anammox decreased accordingly, the relative importance of anammox increased up to 79% with depth. Thus, it was expected to find a higher relative importance of anammox with depth at the stations of the North Sea. In contrast with this result, anammox was only observed at stations 43 (28 m) and 101 (110 m) and although the contribution of anammox to total N_2 production was higher at the deepest station, it was < 7%. Therefore, in this study anammox did not dominated over denitrification with depth neither was a function of organic carbon content in sediment.

As mentioned before DNRA was expected to be favored under high C:N ratios (Tiedje, 1988, Burging and Hamilton, 2007; Decleyre *et al*., 2015) but the findings of DNRA under low and high C:N ratios in The Wash (4.2 to 9.3) and the North Sea (~3) as well as under low organic carbon content in sediment are in contrast with Burgin and Hamilton (2007), do not agree with the previous studies, but were in agreement with Jantti and Hietanen (2012) who also found DNRA at low carbon content in sediment. Decleyre *et al*. (2015) found that when the C:N ratio is >7.7 the pathways of NO_3^- reduction become dominated by DNRA. However, the results of the present study, first showed that DNRA was not the dominant process and secondly, that DNRA occurred either at low or high C:N ratios. Similarly, denitrification and anammox was observed either at low or high C:N ratios, indicated that at least for the present study C:N ratios did not drive these processes.

Sulfide is another factor commonly considered to hamper denitrification and anammox, but that favor DNRA (Rysgaard *et al*., 1996; Ann and Gardner, 2002; Jäntti and Hietanen, 2012), although, studies focus on the effect on this factor over the denitrification anammox and DNRA are still controversial. For example, a study carried out by Senga *et al*. (2006) showed that under concentrations of 1- 5 mg l^{-1} of hydrogen sulfide, denitrification and DNRA accounted for ~75% and ~20% of N_2 and NH_4^+ production respectively. An increase of hydrogen sulfide from 5 – 10 mg l^{-1} resulted in a notoriously decrease of denitrification (10% of N_2 production) and increased of DNRA (2.5% - 15% of NH_4^+). But an increase of hydrogen sulfide > 20 mg l^{-1} reduced drastically DNRA and consequently the NH_4^+ formation, whereas accumulation of NO_3^- and N_2O increased. Thus, although DNRA seems to be favored by the presence of hydrogen sulfide, it will be also inhibited at higher concentrations. Contrasting with the previous studies, in a study carried out in sediment highly variable in sulfide and carbon content, Behrendt *et al*. (2013), found that denitrification was always dominant independently of the concentration of organic carbon, sulfide and iron. Although the highest rates of DNRA were found at higher concentrations of sulfide, DNRA only accounted for ~ 9% of the NO_3^- reduced. Additionally, Dong *et al*. (2011) found that in tropical estuaries, DNRA dominated over denitrification, but the higher rates of DNRA might have been due to the higher affinity of NO_3^- by the nitrate ammonifiers rather than to the inhibition of denitrification by the presence of sulfide.

In the present study sulfide was not measured, but the sediment was brown color and there was not the rotten egg smell characteristic of sediment with sulfide, therefore based on these characteristics, it was only possible to speculate that sulfide was not present and hence did not have any effect neither on the anammox or denitrification processes.

In the present study NO_3^- concentrations ranged between 0.8 and 7.5 µM, the highest concentration was observed at The Wash were the concentration ranged between 0.8 to 7.5 µM, while at the North Sea stations NO3- concentrations ranged between 2 and 6 µM (Table 3.2). NO_3^- is also considered to control denitrification, anammox and DNRA, but likewise for

the other factors, the results of studies focused on the NO_3^- effect on the processes are contrasting. Few studies focused on anammox (Rich *et al*., 2008, Trimmer and Nicholls, 2009; Brin *et al*., 2014), observed that the contribution of anammox (10 – 22%) correlated with higher concentrations of NO_3^- (70 – 170 µM) which in turn was higher in the fresh portion of the Chesapeakebay (USA) than in the mesohaline or seaward sites (0.4 – 0.5 µM) where anammox contribution ranged from 1 – 3% (Rich *et al*., 2012). On the other hand, the study of Trimmer and Nicholls (2009) showed that on the shelf of the Atlantic continental slope anammox contribution remain constant despite of NO_3^- concentrations increased up to 20.4 µM. In a more recent study carried out in estuarine and shelf sediments of New England, denitrification rates correlated with oxygen consumption and temperature, while potential anammox rates correlated with pore-water NO_3^- concentrations in the shelf sediment, but not in estuarine sediments (Brin *et al*., 2014).

Similarly, the effect of NO_3^- on DNRA are contrasting. On the one hand, some studies have found a correlation between DNRA and high NO_3^- concentrations (Kelly-Gerreyn *et al*., 2001; Koop-Jakobsen and Giblin, 2010), on the other hand, there are other studies that found DNRA even under free NO_3^- conditions (Kamp *et al*., 2011). In addition to these contrasting results, the fact that not only prokaryotes (Bacteria and Archaea), but some eukaryotes are able to reduce NO_3^- either to N_2 (foraminifera; Risgaard-Petersen *et al*., 2006) or NH_4^+ (diatoms), made it more difficult to understand the effect of NO_3^- on DNRA. With regard to the last point, it is documented that diatoms are able to store up to 274 mM of intracellular NO_3^-, so that when diatom experienced dark and anoxic conditions they can survive by reducing the stored NO_3^- to NH_4^+ even with no NO_3^- present in the environment (Kamp *et al*., 2011).

In the present study, NO_3^-correlated positively with denitrification in The Wash, although no correlation was observed in the North Sea (Fig. 3.13a). Likewise, no correlation was observed between DNRA and NO_3^- (Fig. 3.13b). Finally, independently of the concentration of NO_3^- in The Wash, anammox was not detected and although anammox was only present at two stations of the North Sea, it was not observed a correspondence between the rate or anammox importance and NO_3^- concentrations. Thus, in agreement with the results of this study neither NO_3^- nor the C:N ratio are considered to control denitrification, anammox or DNRA. Other effects such as OPD and oxygen consumption might have influenced also the processes, but these factors will be analized in chapter 4.

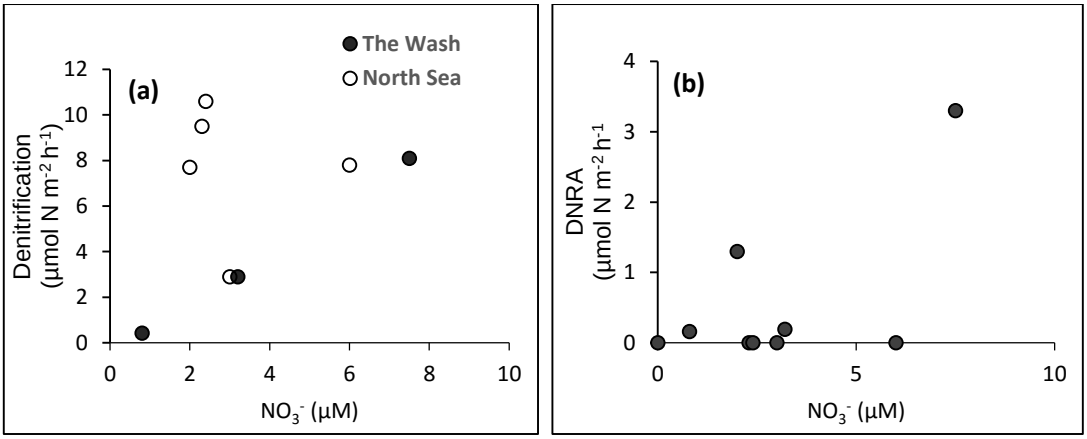


Figure. 3.13. Denitrification and DNRA as a function of NO_3^-. (a) Data of denitrification are plotted for The Wash and the North Sea (b) Data of DNRA are plotted together for The Wash and the North Sea.

Chapter 4: Oxygen penetration depth and its influence on the N cycle

4.1 Introduction

Dissolved oxygen (DO) plays an important role in marine sediment pore water, not only for benthic communities, but also because oxygen regulates many biogeochemical process that take place in the sediments (Cai and Sayles, 1996; Glud, 2008). The extent of the oxygen penetration in sediments is known as the oxygen penetration depth (OPD), that is defined as the depth at which oxygen diffusing from the overlying water into the sediment has been depleted and correspond to the point of anoxia (Katsev *et al*., 2007). The OPD defines the thickness of the oxic zone in sediments and thus, regulates the depth of redox reactions in sediments, such as the processes of the N cycle (Table 2.1 in chapter 2)**.**

The processes of the N-cycling are regulated mainly by the concentration of oxygen, for instance, nitrification oxidizes ammonium released during organic matter mineralization with O_2, into nitrite followed by the oxidation into nitrate. The end products of nitrification can subsequently be reduced to N_2 either through the denitrification or anammox process under anoxic conditions (Jenkins and Kemp, 1984). Unlike denitrification and anammox, that are considered to be the main processes of N lost in marine sediments (Seitizinger, 1988), dissimilatory NO_3^- reduction to NH_4^+ (DNRA), recycles NO_3^- to NH_4^+, and accordingly, preserves fixed N in the environment (Dale *et al*., 2011).

Nitrification is particularly important in estuarine sediments where it is coupled to denitrification or anammox. The N_2, the product of denitrification, and anammox, may diffuse toward the surface of the sediments where nitrification takes place, the NO_3^- produced may diffuse deeper into the sediment towards the nitrate reduction zone. Both nitrification, denitrification/anammox should occur at different depth of the sediment because, while nitrification is an aerobic process, denitrification is a nitrate reduction process that is carried out when O_2 concentrations are < 2 μM. Therefore, the OPD impacts the N cycle by determining the activity of microbial assemblage present in the sediment (Marks, 2010) and by separating different environments where either aerobic or anaerobic metabolisms predominate (Cai and Sayles, 1996; Katsev *et al*., 2007). Consequently, the OPD controls the depth distribution of the redox reaction in the sediments, and the process that predominates, which is key when evaluating the processes of the N cycle (Glud, 2008) because the role of the sediments as a sink or source of fixed N and its impact in the water column, will depend on the pathways occurring in the sediment (Ward, 1996; Dale *et al*., 2011).

Anoxia, which defines the OPD, strictly means total absence of oxygen (International Union of Pure and Applied Chemistry, 1993; U.S. Environmental Protection Agency, 2009), however, because of limitations of measurements by standards methods, anoxia had been taken as <1 μmol O_2 l^{-1}, albeit, with the development of a new sensor (STOX) capable of measuring ultra-

low O_2 concentrations, the limit has been moved to <10 nmol O_2 l^{-1} (Canfield and Thamdrup, 2009). In this study, the OPD was considered to be the depth at which the oxygen concentration reached a value < of 0.99 μmol O_2 l^{-1}.

In this chapter the OPD from two sampling sites: The Wash and the North Sea, will be compared and its possible effect in the processes of the N-cycling will be discussed. Also, the possible effect of some of the factors such as temperature and carbon supply on the availability of O_2, and hence in the OPD will be discussed.

4.2 Methods

4.2.1 Sediment sampling

Sediment for intact sediment core experiments collected in The Wash were transported back to the laboratory, and placed in a tank topped up with *in situ* water. The cores were left in the tank overnight to equilibrate. The water in the tank was kept at *in situ* temperature, in the dark and the overlying water of each individual core was aerated (Fig. 4.1). Profiles of O_2, were measured the next day. Profiles of O_2 for intact sediment cores in the North Sea were measured immediately after collecting the sample. The measurements for oxygen profiles were carried out in the dark in order to avoid oxygen production by benthic microalgae.

Figure 4.1. Cores collected at the sampling sites, submerged in a tank topped with *in situ* water. Each core was aerated individually overnight. Cores with grey tops are magnets.

4.2 .2 Sediment characteristics

4.2.2.1 Porosity

At each sampling site of the North Sea and The Wash, 3 replicates sediment cores were taken (30 cm long, 6 cm diameter) in order to estimate porosity. Each core was sectioned in slices of 0,5 cm for the first centimetre, and then every 1 cm until 10 cm deep in the sediment. The samples were kept frozen at -16°C until analysis.

Porosity was determined based on weight and volume measurements of sediment slices. In brief, samples of sediment were unfrozen, and each slice of wet sediment was weighed. Afterwards, the samples were dried at 60°C, then the dried samples were weighed again. The calculation of the volume of sediment was based on the radio (r = 3 cm) of the cores and the thickness of the layer of sediment (h, either 0.5 cm or 1 cm). Sediment volume, bulk density, water content and porosity were calculated as follows:

$$Volume\ of\ wet\ sediment\ = \pi \times r^2 \times h \quad (4.1)$$

Where, r, is the ratio of the diameter of the corer, h is the width of each slice of sediment (0.5 cm or 1 cm), and π is the constant of the circle's circumference to its diameter equals to 3.1416.

$$Bulk\ density\ (g\ cm^{-3}) = \left[\frac{(Weight\ wet\ sediment)}{(Volume\ of\ wet\ sediment)}\right] \quad (4.2)$$

$$Sediment\ water\ content\ = \frac{(Weight\ wet\ sediment) - (Weight\ dry\ sediment)}{(Weight\ wet\ sediment)} \quad (4.3)$$

$$Porosity\ = (Bulk\ sediment\ density) \times (Sediment\ water\ content) \quad (4.4)$$

4.2.2.2 Particle size analysis determination

The particle size analysis was carried out with a Mastersizer 2000-G laser diffraction particle size analyser (Malvern Instruments Ltd) designed for measuring a broad size distribution that range between 0.02 μm to 2000 μm (Malvern specification). The analysis was performed by dispersing approximately 2 g of the dry sample in water (25 ml). A 3 cm magnet was placed into the beaker containing the sample in order to stir and kept the sediment homogeneously resuspended while the sample was taken to be placed into the tank of the particle size analyzer.

Principle of the particle size analyzer – The equipment Mastersize Hidro 2000 G, operates with an ultrasonic probe dispersion, and a stirrer that kept resuspended large and dense

material once the sample is into the tank. From the tank, the sample is pumped through the measure cell where the particles are irradiated by a laser beam, the light adsorbed by the particles is measured as obscuration and represents the amount of sample added to the dispersant liquid.

The particle size analyzer measurements are based on a distribution pattern generated by the scattered light of all individual particles when they are irradiated with the laser beam (Fig. 4.2). The distribution pattern changes with the angle at which the light is scattered by the particles, as the angle is inversely proportional to size of the particles, small particle will scatter large angles, and large particles will scatter the light at small angles. The data are then analysed by the Malvern Mastersize program, which is based on the Mie theory which uses the optical properties (refractive index and absorption) of the particles to calculate the size distribution (Storti and Balsamo, 2010; Malvern, 2007).

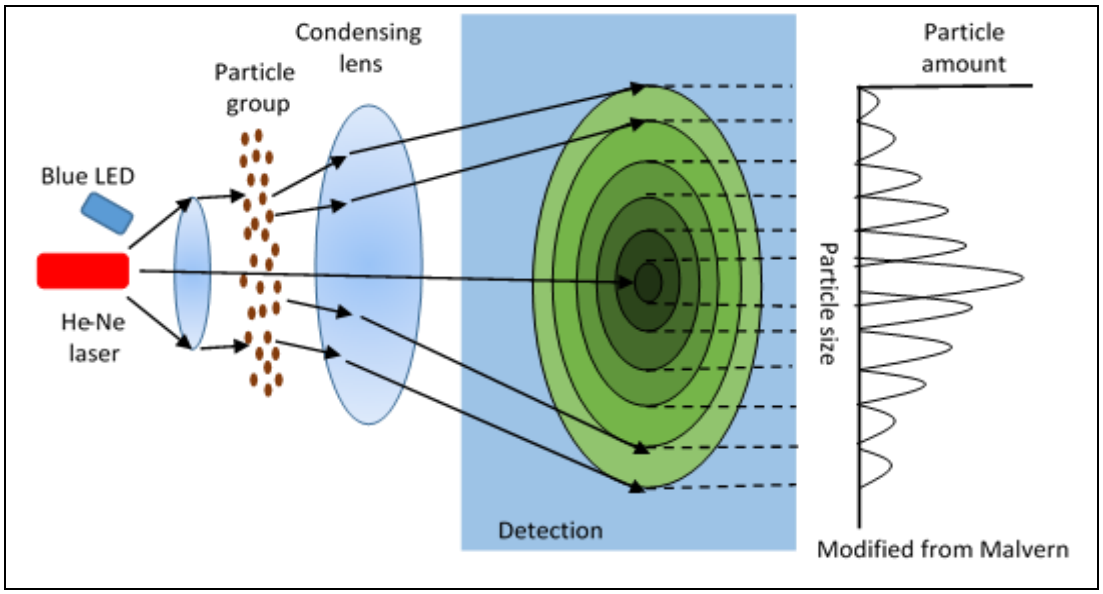


Figure 4.2. Representation of an optical system of a Laser Diffraction Particle Size Analyser (Modified from Malvern, 2007).

4.2.3 Measurement of oxygen pore water profiles

Vertical oxygen concentration profiles were measured using two different diameter size of oxygen microsensor: 100 μm or 500 μm (OX-100, OX-500, Unisense, AS, Denmark). The microsensor was connected to a 4-channel microsensor amplifier (Microsensor Multimeter, Unisense AS, Denmark), and fitted either to a manual microprofiling system, or fitted to a motorized microprofiling system (Unisense As, Denmark). In The Wash the profiles were measured at a resolution of 200 μm. However, because the cores used to do oxygen profiles on board of a research cruise experience constant vibrations that can damage the sensors, a

more robust sensor (500 μm) was used for the North Sea, with resulting reduction in resolution to 500 μm.

Calibration – The O_2 microsensor was calibrated with 0 and 100% O_2 saturated water. In order to get a zero reading, an anoxic 0.1 M solution was prepared with NaOH, and sodium ascorbate, the 100% reading was achieved by vigorous bubbling with air the water contained in a Unisense calibration chamber for at least 5 minutes before the measuring (Unisense, 2012).

4.2.4 Oxygen consumption rates

Oxygen consumption rates, and O_2 fluxes through the sediment were estimated from the profile of O_2 concentration measurements in the sediments by using the numerical model PROFILE (Berg *et al*., 1998). The model is based on the following one-dimensional mass conservation equation:

$$\frac{d}{dx}(\emptyset D_s + D_B)\frac{dC}{dx} + \emptyset \propto (C_0 - C) + R = 0 \quad (4.5)$$

where C is the pore water concentration, C_0 is the bottom water concentration, x is the depth, Φ is the porosity, Ds is the molecular diffusivity, D_B is biodiffusivity, α, is the irrigation coefficient, and R is the rate of oxygen consumption/production. In this study it was assumed that D_B and α were zero, then, these parameters of the model were set to zero, Ds was obtained from the tables of Unisense, by taking into account salinity and temperature of the sediment samples.

The model identifies changes in the slope of the O_2 to the depth, and thereby identifies particular zones within the sediment to describe the variation of oxygen production/consumption by finding the best square fits to the measures of O_2 profiles in the sediment. Finally, the fits are compared through a statistical F-test in order to prove if the addition of zones result in a better fit. Once the best fit is found, and R^2 and p values are evaluated at a level of significance of 0.01, the final number of zones is chosen, and consequently the fluxes, and production/consumption for each zone, as well as the depth integrated production/consumption are determined (Berg *et al*., 1998).

4.2.5 Carbon and nitrogen content in sediments

In order to determine C and N content in sediment, the unfrozen samples were dried, then about 1.5 grams were placed into 75 ml beakers and 15 ml of sulfurous acid (6%) was added. Subsequently, the sampled were dried at 40°C and re weighed again. From the dried acidified sediment, 4.5 mg were weighed and folded into 8 x 5 mm tin capsules (Elemental microanalysis, LTD). Additionally, a set of five sulphanilamide standards, references and

blanks were prepared. The sulphanilamide standards were prepared by weighing sulphanilamide between the range of 0.01 to 1.5 mg. Reference materials were also prepared using both sulphanilamide and acetanilide by folding tin capsules containing between 1.0 – 1.1 mg of sulphanilamide, and 0.3 – 0.5 mg of acetanilide respectively. These were measured after every 12 samples. The blanks were prepared by folding the empty tin capsules. The samples were analysed with an Elemental Analyser CHNS (Carlo Erba EA 1108) elemental analyser.

Principle of the CHNS – The Elemental Analyser analysis is based on the dynamic flash combustion method. During the process, the sample previously placed on a carrousel is dropped into a quartz tube, where it is combusted in an exothermic reaction at high temperature (904°C) under high purity oxygen conditions (99.9995%). During the combustion process, carbon is converted to carbon dioxide (CO_2), hydrogen to H_2O, and nitrogen to N_2. In order to assure the complete oxidation and removal of any byproducts (e.g. sulfur, phosphorous), the products of the combustion of the sample are carried by an inert gas (He) through an oxidation zone packed with high purity copper (II) heated at 600°C, then the products of the combustion of the sample are driven to the reduction column packed with copper wire. In the reduction zone nitrogen oxides and sulphuric anhydride are reduced. Finally, the sample is taken to a chromatographic column where CO_2 and N_2 are separated. The detection of the gases is carried out by a thermal conductivity detector (TCD) that heats up and change its resistance when a product is separated and thermal conductivity of the effluent is reduced. This change on the resistance is detected by an electrical circuit (Fig. 4.3). The concentration of the components of the sample are determined by the ratio of the analyte peak area to all the standards (CHNS Manual Instrumentation; Thompson, 2008).

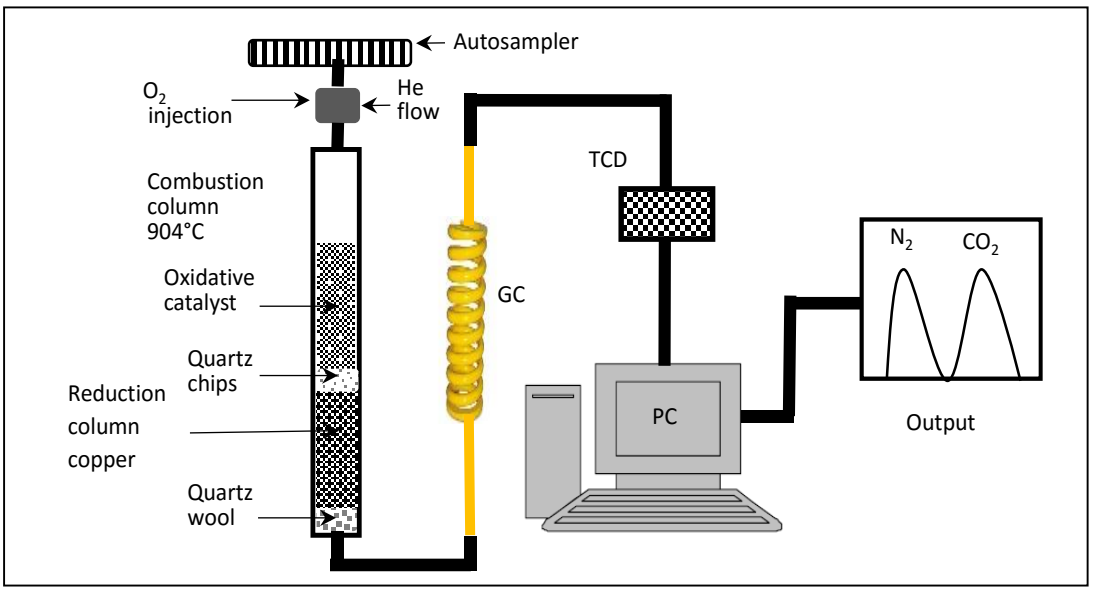


Figure 4.3. Schematic of the CHN Analyser. Modified from Thompson (2008) and from the University of Santa Cruz website (http://es.ucsc.edu/~silab/ea.inst.php)

4.3 Results

4.3.1 Oxygen pore water profiles in intact sediment cores

The OPD in The Wash ranged from 2 mm to 3.6 mm (Fig. 4.4), with the minimum recorded in June (2 mm) and maximum in October (3.6 mm). When the data from The Wash are plotted taking the mean during spring it was observed a temporal trend which slightly increased from spring to autumn (Fig. 4.4).

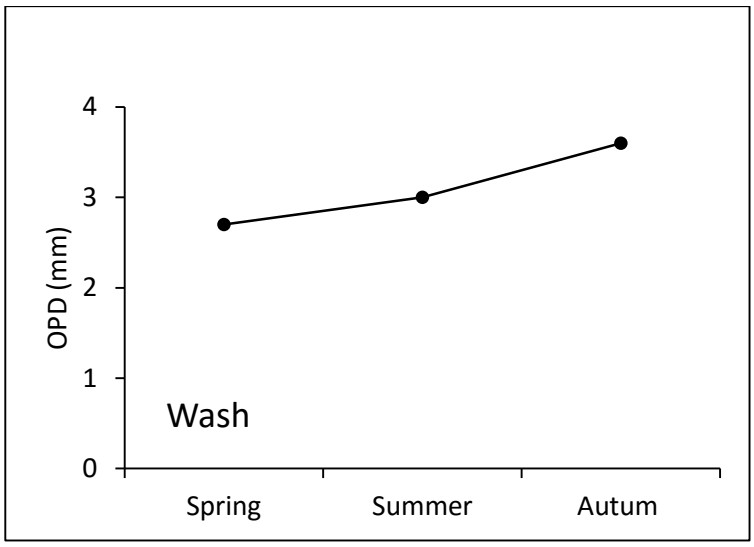


Figure 4.4. Spatial variation of the OPD at The Wash

The OPD in the North Sea was significantly higher than in The Wash ($p<0.003$), ranged from 6 mm to 8 mm (Fig. 4.5), with the minimum OPD at the stations 43 and 101, and the maximum OPD at station 141 (Table 4.1). The OPD did not show any spatial trend related to geography or water depth, nor was there a relationship between the OPD and the grain size or C content of the sediments.

4.3.2 Sediment Oxygen consumption

Oxygen consumption rates were calculated with the numerical model PROFILE of Berg *et al*. (1998) using the data of oxygen profiles measured with the microelectrodes. The results of the model from The Wash and the North Sea, are summarized in Figure 4.6 (note the

difference in scale for O_2 consumption), and table 4.1. The output of the model suggests that the oxygen consumptions within the sediments was not homogeneous (Fig. 4.6), but varied with depth both in The Wash and in the North Sea. In The Wash, three different zones of consumption were estimated during May, June and October, and only two oxygen consumption zones in September (Fig. 4.6). During May and June, the zones with maximum oxygen consumption was deeper in the sediment (zone two), while the maximum oxygen consumption for September and October was at the surface of the sediment. The flux across the surface of the sediments ranged from -1.1 to -4.5 mmol m^{-2} d^{-1}. Although during May and June (Spring) the maximum fluxes were observed, there is not a clear temporal trend because the flux during September and October were similar (1.1 and 1.1 mmol m^{-2} d^{-1} respectively).

In the North Sea, the model suggests only two zones of oxygen consumption rates, the exception was the station 127, that had 3 zones and similar to some of the stations at The Wash, with the maximum oxygen consumption deeper in the sediment at zone 2. The zone 2 of the station 127 was also the zone with highest O_2 consumption (-223 μmol m^{-2} h^{-1}). The zones of oxygen maximum consumption for the other stations was at the surface of the sediment (Fig.4.6), from these zones, in the station 68 the maximum O_2 consumption was observed at the surface (-111 μmol m^{-2} h^{-1}).

The benthic O_2 consumption, ranged from -1.2 to -5.8 mmol m^{-2} d^{-1}, with the maximum rate of consumption at station 127 and the minimum at the station 43 (Table 4.1). There was a clear correlation between O_2 consumption and water column depth, with the fluxes increasing as the water column depth increased (Fig. 4.7).

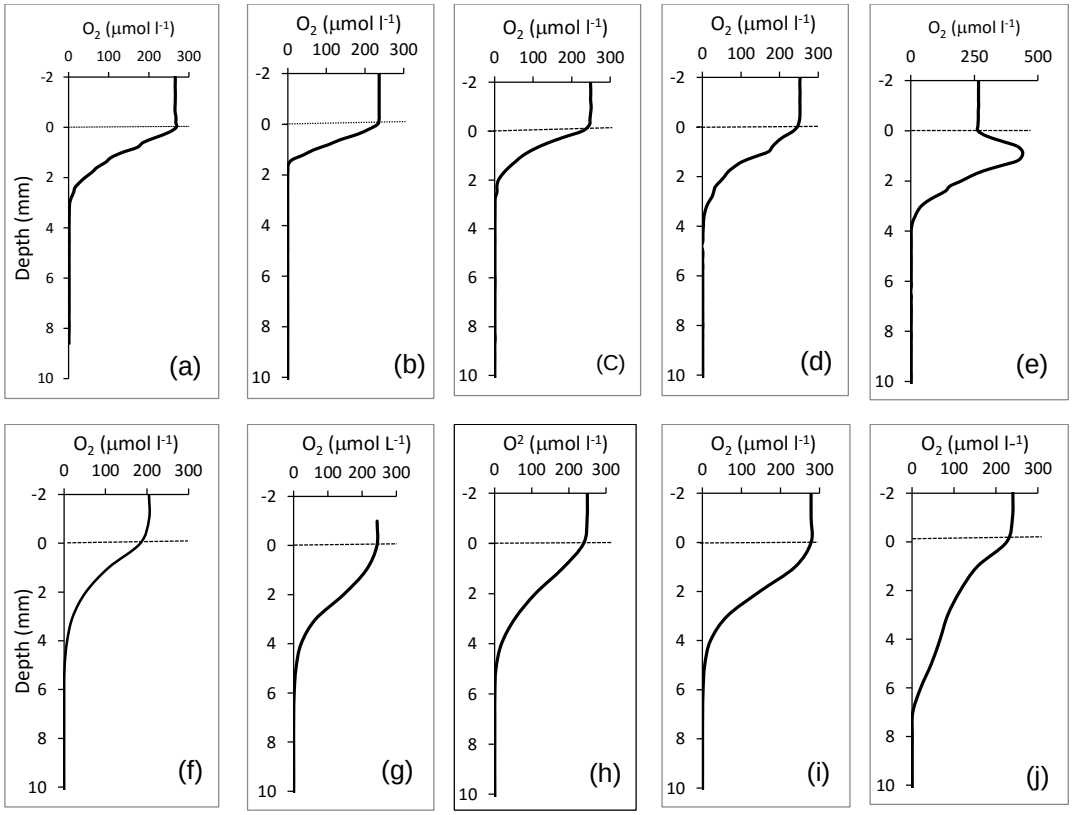
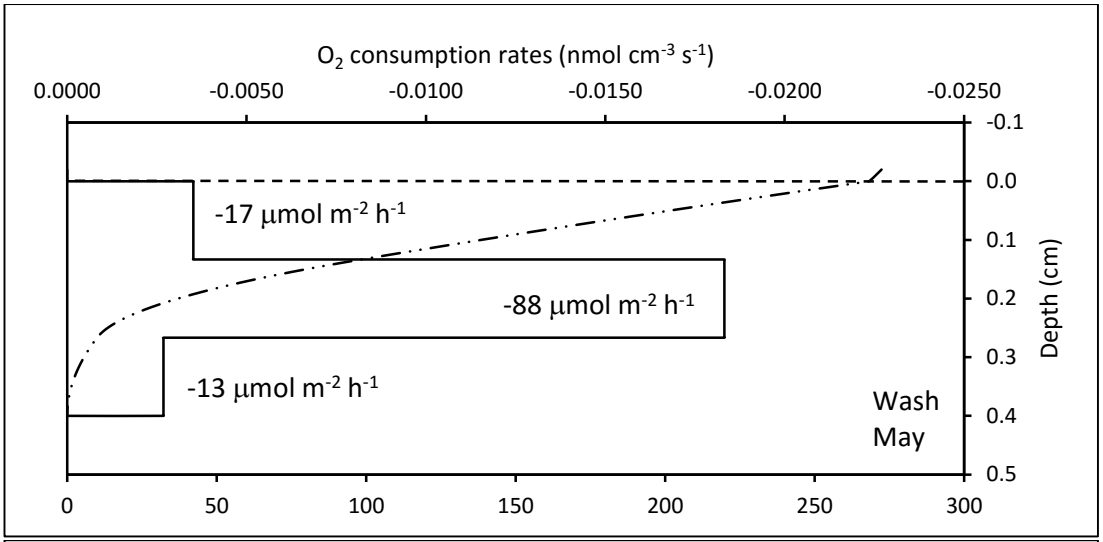
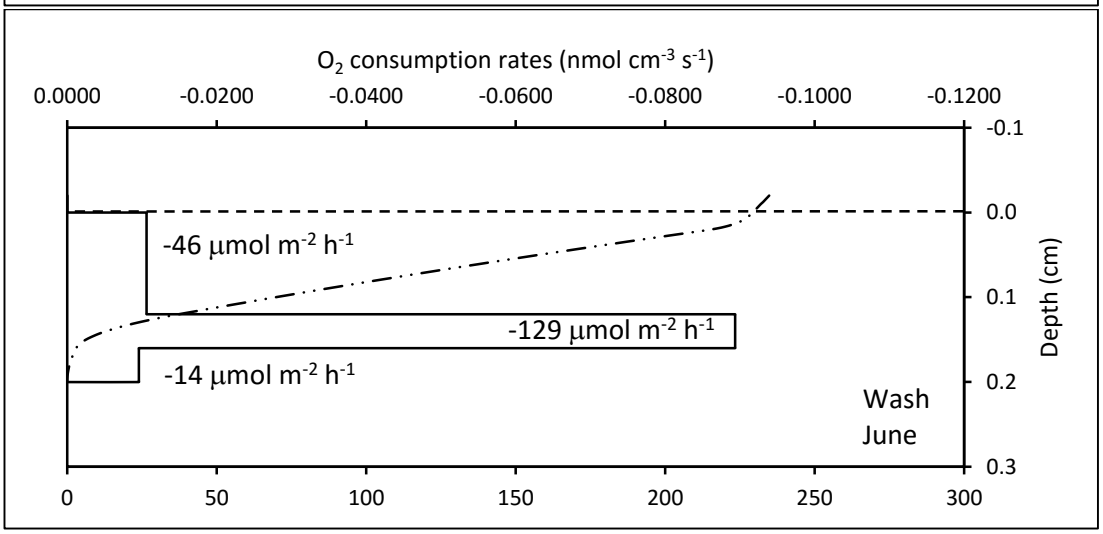


Figure 4.5. Pore water profiles of O_2 in The Wash during May (a), June (b), September (c) and October (d), and at the North Sea at stations: 43 (f), 68 (g), 101 (h), 127 (i) and 141 (j). Profiles of oxygen in sediments exposed to light (e).

Table 4.1. OPD, O_2 concentration, O_2 consumption (O_2 Con), temperature, porosity, rates of denitrification (DNTR), Dw, Dn, anammox and DNRA measured in intact sediment cores. The units of O_2 concentration are in µM, O_2 flux is in mmol m^{-2} d^{-1}, DNTR, Dw, Dn, and DNRA are µmol m^{-2} h^{-1}. The percentage of the contribution to the total N_2 production of Dw and Dn is in brackets.

Site	OPD (mm)	[O_2]	O_2 Con	Depth	Temp	Porosity	DNTR	Dw	Dn	Anam	DNRA
Wash											
May	3.4	265.08	-2.8		12.1	0.37 (0.05)	6.9	1.29 (20.5)	5.01 (79.5)	0	0
June	2	236.66	-4.5		16.2	0.53 (0.02)	8.1	5.20 (64.5)	2.89 (35.5)	0	3.3
Sept	3	247.33	-1.5		19.8	0.35 (0.03)	0.42	0.42 (100.0)	0 (0.00)	0	0.16
Oct	3.6	250.18	-1.1		20.6	0.37 (0.06)	2.9	0.95 (32.9)	1.95 (67.1)	0	0.19
North Sea											
43	6	205.06	-1.2	28	17.8	0.41 (0.07)	9.31	1.18 (12.7)	8.13 (87.3)	0.19	0
68	7	243.25	-2.9	60	12.9	0.40 (0.09)	7.65	0.88 (11.5)	6.77 (88.5)	0	1.3
101	6	239.93	-4.3	110	8	0.56 (0.08)	2.73	0.33 (12.0)	2.4 (88.0)	0.19	0
127	7	278.28	-5.8	116	9	0.53 (0.12)	7.81	1.47 (18.8)	6.34 (81.2)	0	0
141	8	249.27	-2.6	65	7	0.39 (0.06)	10.6	1.44 (13.6)	9.16 (86.4)	0	0





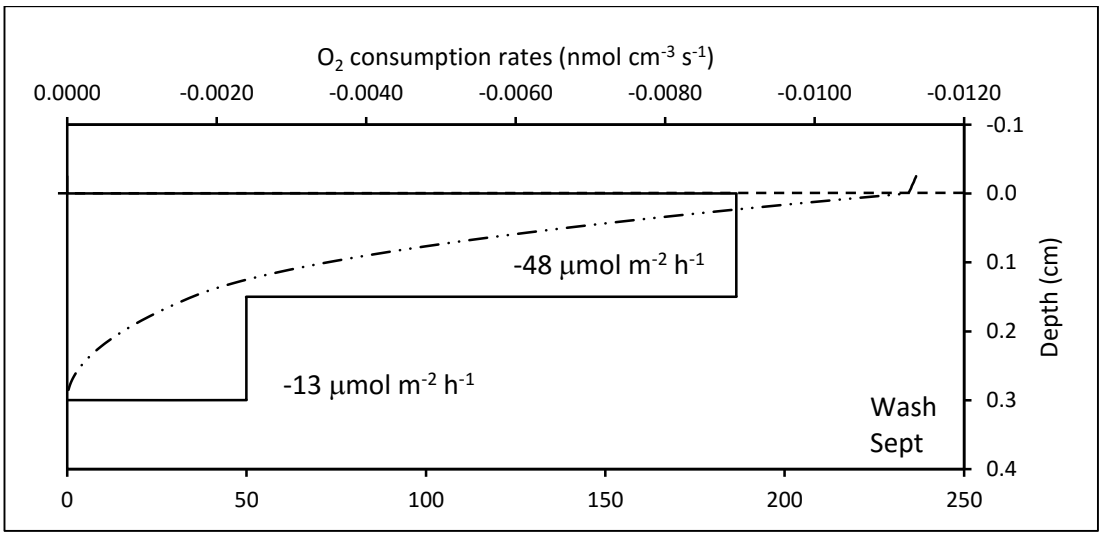
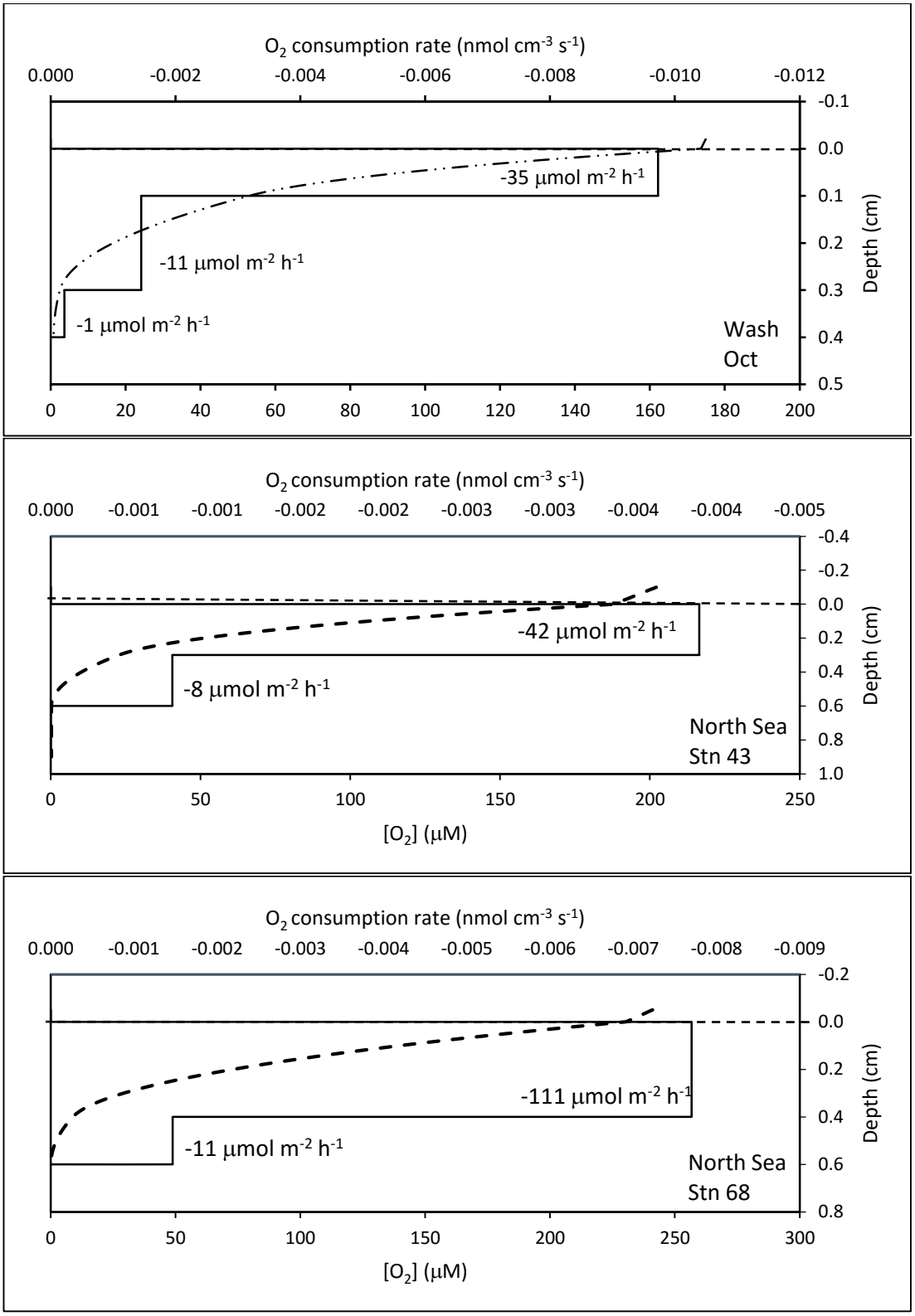


Figure 4.6. Output of the model PROFILE (Berg *et al*., 1998) indicates oxygen consumption rates per unit of volume of sediment (top x axis) and per depth integrated at each zone (boxes), for The Wash: May, June, September, and October, and for the North Sea: stations 43, 68, 101, 127 and 141. The dashed line is the best-fitting calculated profile for O_2 concentration (bottom x axis).



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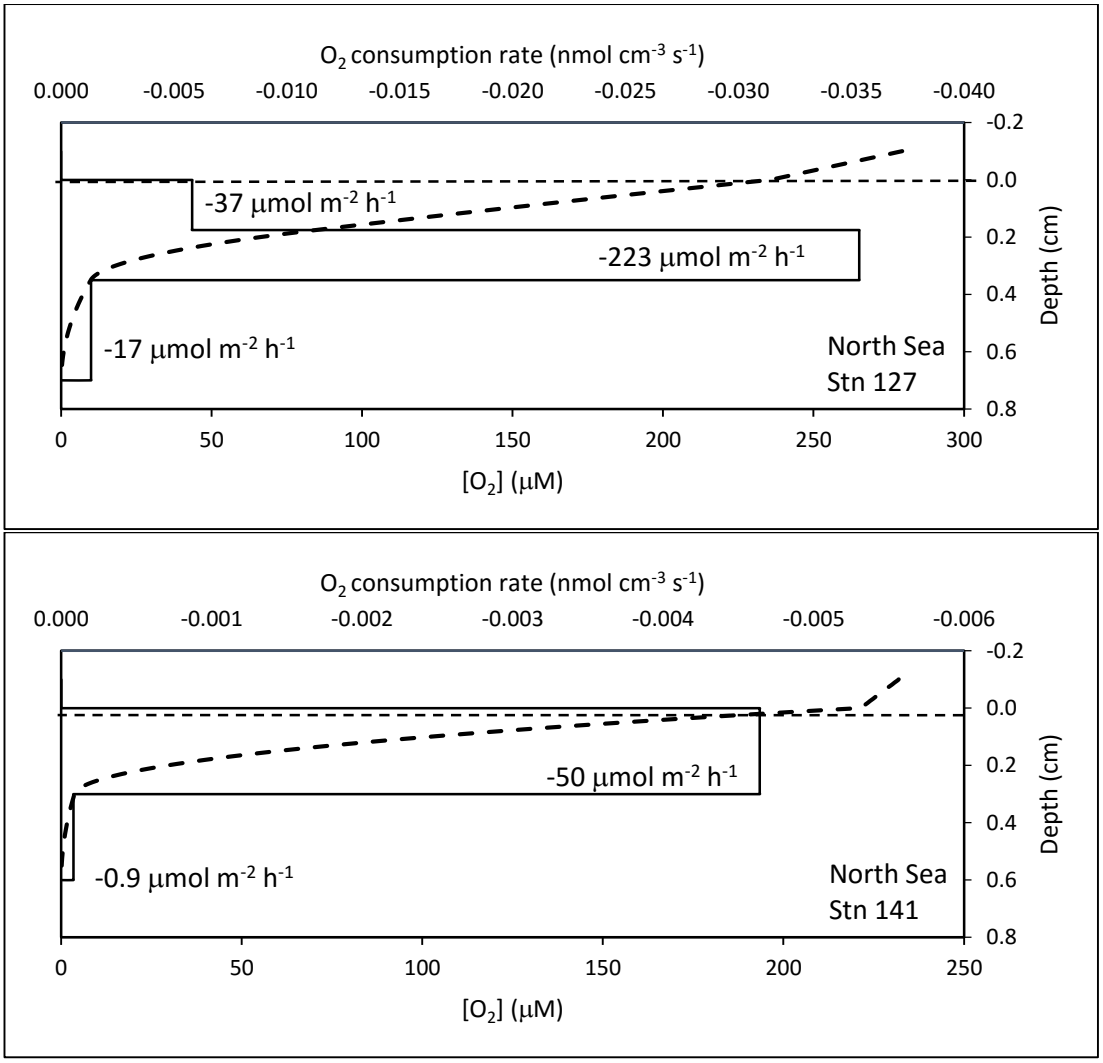


Figure 4.6. Output of the model PROFILE (Berg *et al*., 1998) indicates oxygen consumption rates per unit of volume of sediment (top x axis) and per depth integrated at each zone (boxes), for The Wash: May, June, September, and October, and for the North Sea: stations 43, 68, 101, 127 and 141. The dashed line is the best-fitting calculated profile for O_2 concentration (bottom x axis).

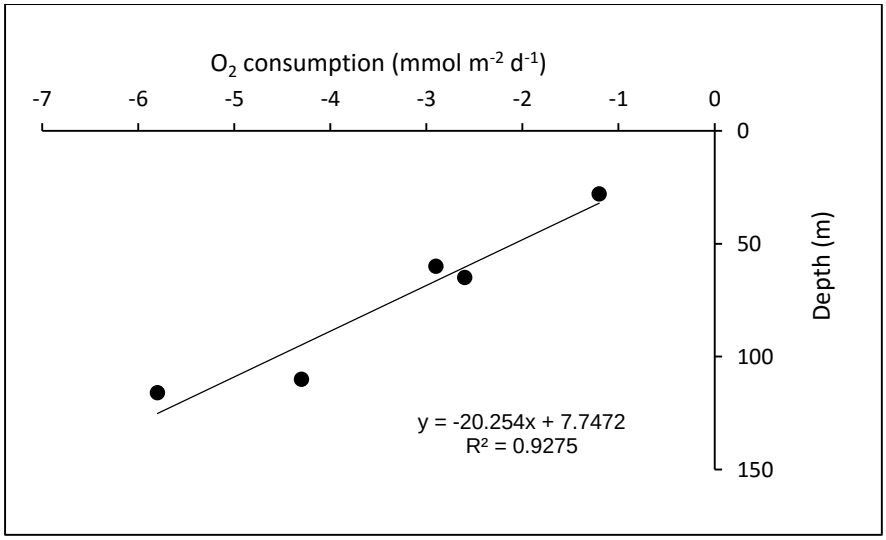


Figure 4.7. Benthic O_2 consumption as a function of depth in the North Sea samples.

4.3.3 Organic C and N content in sediments

The content of C and N in sediments of the North Sea ranged between 0.04 - 0.54% for C, and 0.01 - 0.06% for N (Table 4.2). The vertical distribution of N in the sediments was practically constant, with values lower than 0.07% in all the stations. The vertical distribution of C had little variability at stations 43 and 68 as well as spatially. The C increased from 0.04 (Stn 43) to 0.54% (Stn 127), with the greatest variation in the vertical dimension observed at station 127 (Fig. 4.8). In The Wash, the C and N content in sediments was <0.2%. Like the North Sea, the vertical distribution of N was homogeneous, with values of N content in the sediment <0.03% (Fig. 4.8). The vertical distribution of C content in the sediment decreased with depth during June, September, and October. June showed the greatest C content at 10 cm depth (0.15%).

C/N ratios in the North Sea varied from 2.2 to 8.1. The C/N ratios were particularly low at stations 43 (1.6 to 2.7) and 68 (2.6 to 3.1), while at stations 101, 127, and 141, the C/N ratios ranged between 7.5 and 8.5 (Table 4.2). Other than station 101, where the vertical distribution remained constant with depth, the others stations of the North Sea tend to have slightly higher C/N ratios at 10 cm depth (Fig. 4.9). On the other hand, the C/N ratios in The Wash ranged between 3.6 and 11.4, the lowest C/N ratios were observed during June (3.6 to 5.5), and the highest during September (8.1 to 8.4). During October, the vertical distribution of the C/N ratios was almost constant along with depth below the first centimetre (Fig. 4.9). The vertical distribution for May, June, and September varied with depth. May and June showed a similar trend with the lowest C/N ratios at the surface and the maximum at 10 cm depth. On the contrary, September had the lowest C/N ratios at the surface (0 cm) and the lowest at 10 cm depth.

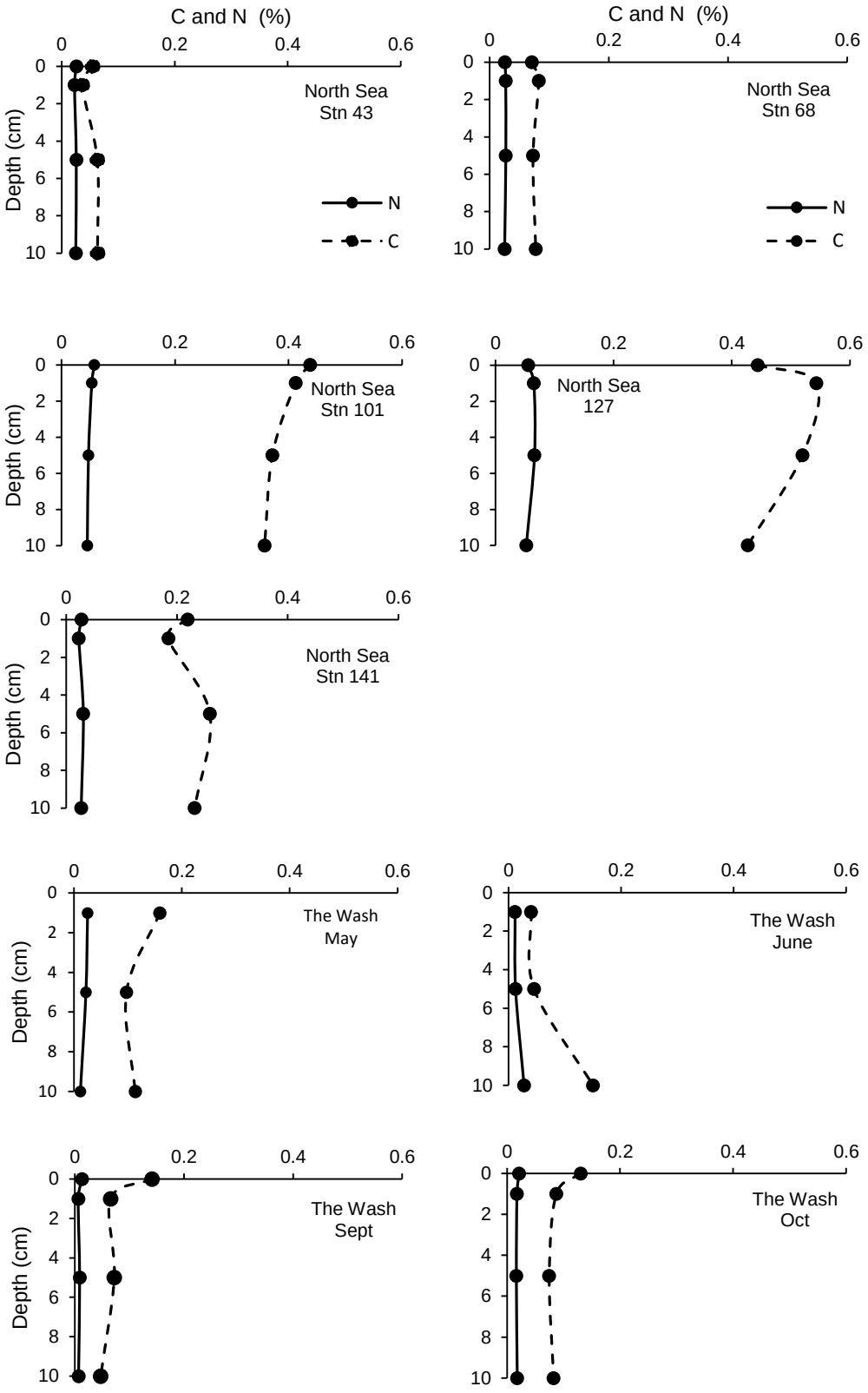


Figure 4.8. Vertical distribution of C and N content in sediments at The Wash and the North Sea.

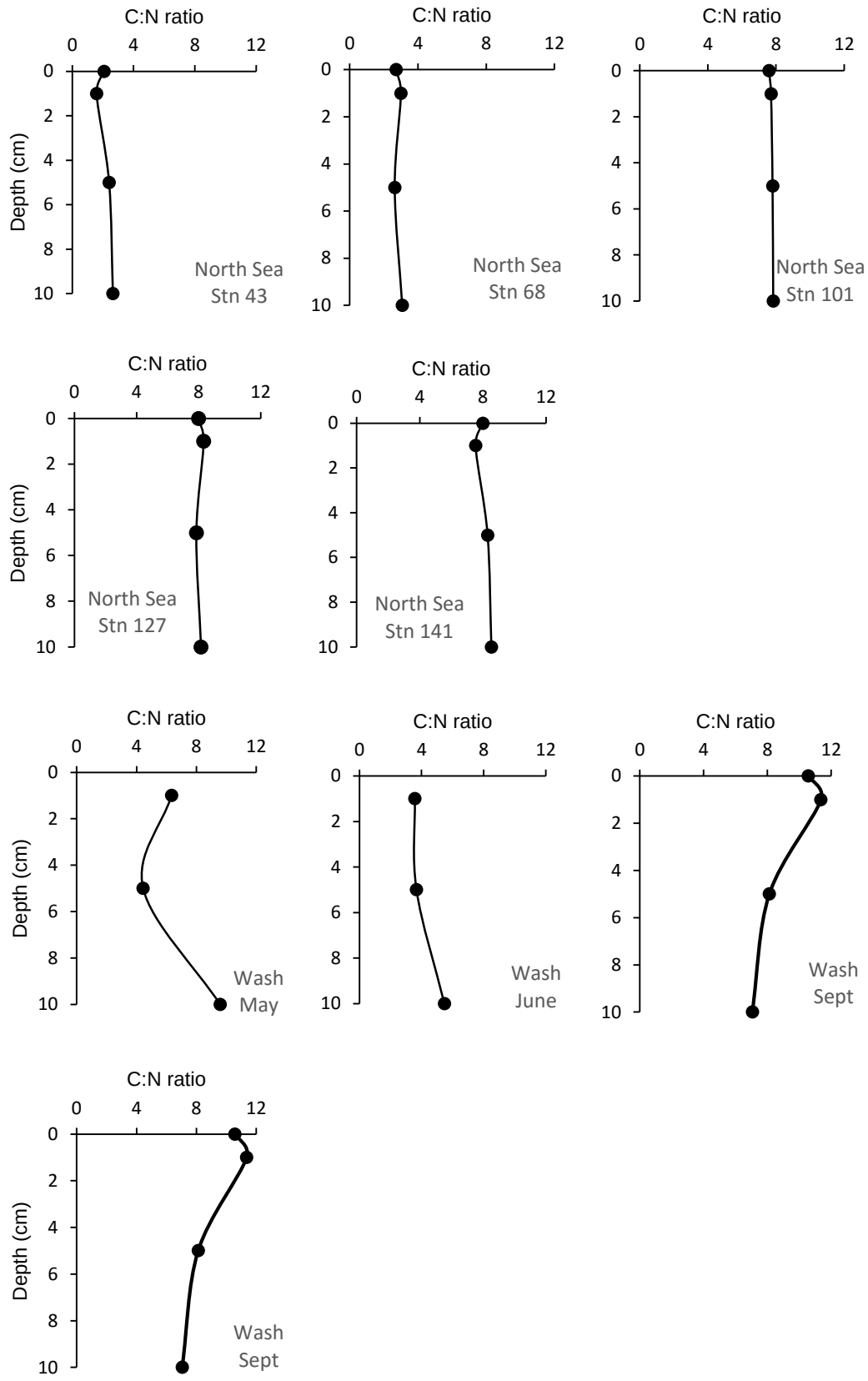


Figure 4.9. Vertical distribution of C:N ratios in sediments at The Wash and the North Sea.

Table 4.2. Average C and N content in sediment, and average C/N ratios. Values in brackets indicate the standard deviation.

Station	N (% dry sed)	C (% dry sed)	C/N	Average grain size (μm)	Sand (%)	Mud (%)	Sand: Mud ratio	
North Sea								
43	0.02 (0.00)	0.05 (0.02)	2.2 (0.60)	90	95	5	18	Fine Sand
68	0.03 (0.00)	0.08 (0.02)	2.9 (0.52)	141	92	8	12	Sand
101	0.05 (0.01)	0.39 (0.05)	7.7 (0.19)	83	79	21	4	Muddy Sand
127	0.06 (0.01)	0.48 (0.08)	8.1 (0.33)	76	90	10	9	Muddy Sand
141	0.03 (0.01)	0.22 (0.09)	8.1 (0.96)	92	89	11	8	Muddy Sand
Wash								
May	0.02 (0.01)	0.12 (0.05)	6.8 (1.15)	165	95	5	15	Sand
June	0.02 (0.01)	0.08 (0.05)	4.2 (0.91)	157	96	4	23	Sand
Sept	0.01 (0.00)	0.08 (0.04)	9.3 (1.96)	141	90	10	9	Muddy Sand
Oct	0.02 (0.00)	0.09 (0.04)	4.9 (1.29)	148	94	6	17	Sand

4.3.4 Porosity

The porosity of the sediments from the Wash and the North Sea varied temporarily in the Wash and spatially at the North Sea. In the Wash, the mean sediment porosity varied from 0.35 to 0.53, with the minimum porosity observed in September and the maximum in June (Table 4.1). The porosity of the sediments also varied vertically within the sediment. The percentage of variation to the mean ranged between 3 to 10%, the minimum vertical variation of the porosity was observed in May and the maximum in September (Fig. 4.10).

At the North Sea the mean porosity ranged from 0.40 to 0.56. The highest porosity was observed at the station 101, and the minimum at the station 141 (Table 4.1). The percentage of vertical variation was higher than in the Wash, and ranged between 15 to 24%, the maximum vertical variation around the mean was observed at stations 68 and 127 and the minimum at station 101 (Fig. 4.10). In the North Sea the porosity at all the stations was higher in the top 1 cm of the sediment core.

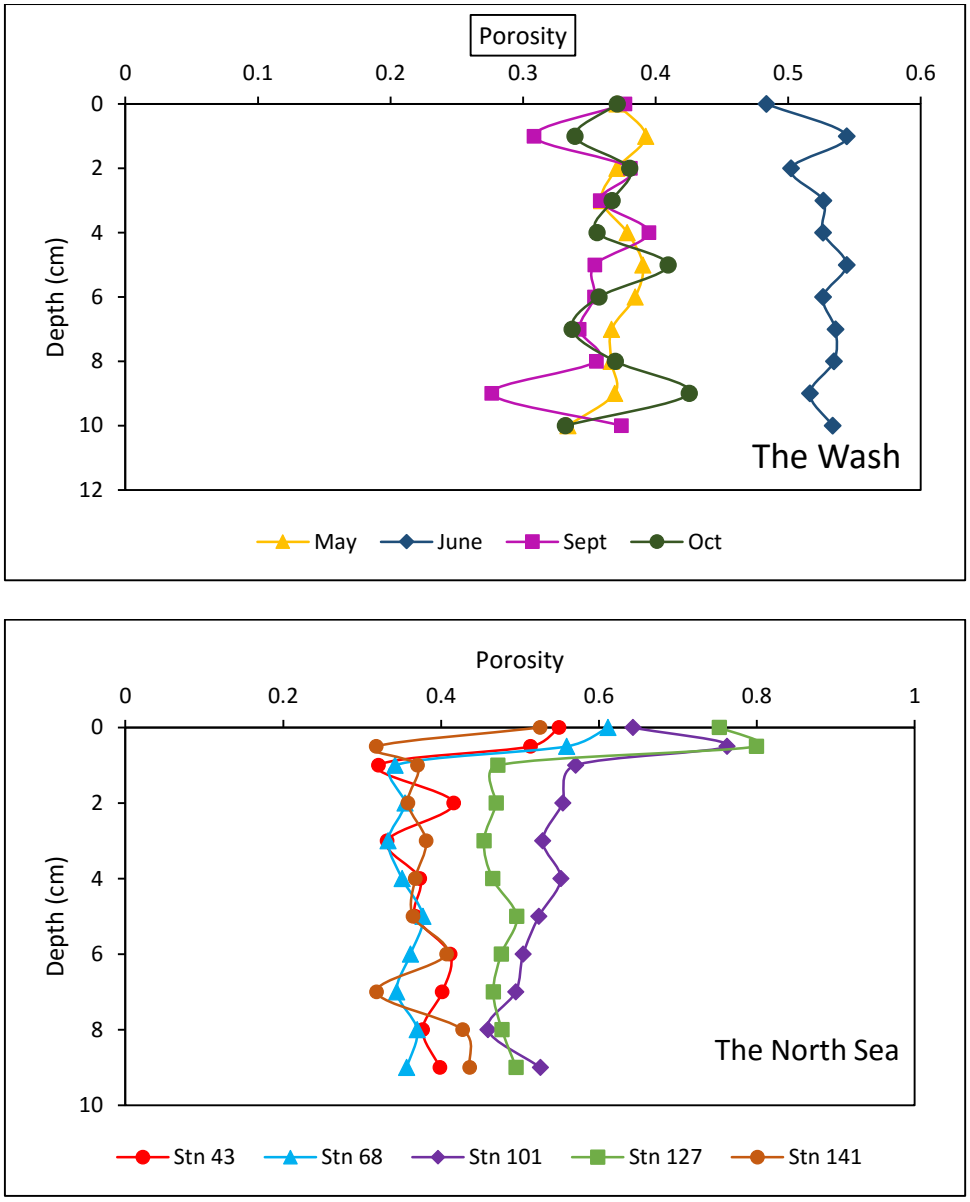


Figure 4.10. Porosity profiles at the Wash and the North Sea.

4.4 Discussion

Changes in oxygen concentration within the sediment affect the biogeochemical reactions of the N cycling, such as nitrification, denitrification or DNRA, so that, depending on the extent of the OPD, sediments can act as a source or a sink of N (Bonaglia *et al*., 2014). Regarding the importance of the OPD on the processes of the N-cycling, in this section the factors affecting the OPD, will be discussed first, then the effect of the OPD on the N-cycling.

4.4.1 Factors affecting the OPD

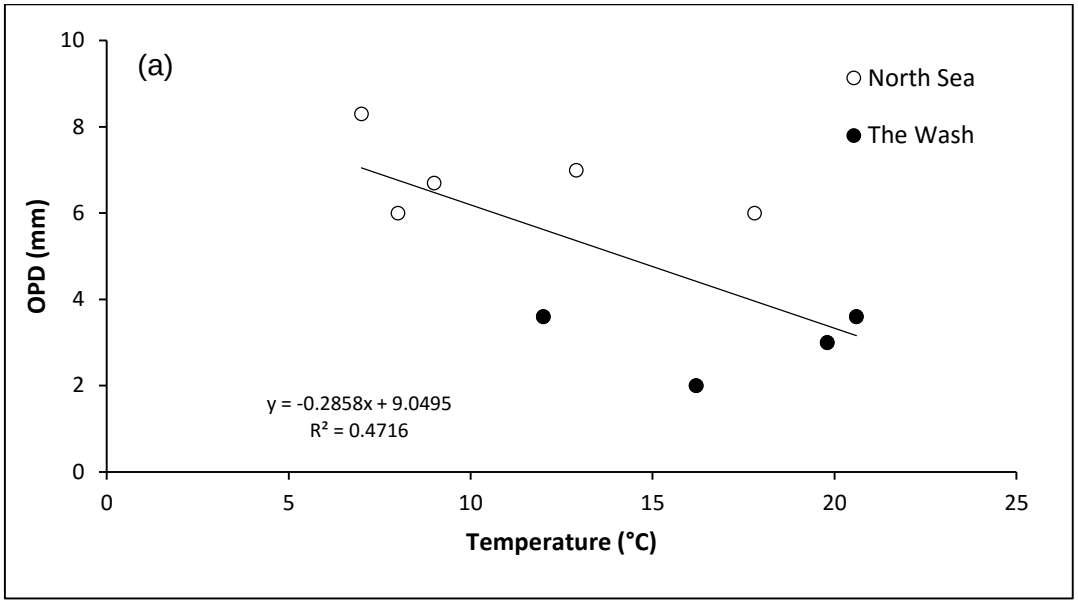
4.4.1.1 Temperature

The OPD of the North Sea was significant deeper than the OPD in the Wash. In the Wash, the temperature observed, ranged between 12 to 21°C, but only May showed a temperature <16°C, on the other hand, in the North Sea, the temperature ranged between 7 to 17.8°C, but only at one station (43), the temperature was > 16°C, while the average temperature for the other four stations was ~9°C (Table 4.1).

There are many factors that affect the OPD such as: temperature, organic matter supply, light, bioturbation, and the diffusive boundary layer (Kristensen, 2000, Risgaard-Petersen *et al*., 2005; Glud *et al*., 2007). However, between the many factor affecting the OPD, temperature is a key factor, because it not only affects the oxygen consumption rate, but also it affects the solubility of oxygen in such a way that an increase of 10°C, reduces the solubility of oxygen by 17 – 23% (Kristensen, 2000). Consequently, as the temperature rise the bacterial and chemical oxygen consumption rate increases and the concentration at 100% saturation falls, and accordingly, the OPD also decreases (Kristensen, 2000). A clear effect of temperature on the OPD based on a case study in the lake Vilhelmsborg and Norsminde Fjiord (Kattegat), was highlited by Kristensen (2000), who reported that the OPD was 2 times higher during winter (2°C) than during the summer (22°C).

In the present study, when the OPD and temperature data from the Wash and the North Sea were combined a negative relation was found ($p < 0.05$) between the variables (Fig. 4.11a). This finding was also observed when data from other studies carried out in the North Sea were plotted (Fig.4.11b), both slopes are similar, which suggests a regionally consistent relationship at least. The data used for the plot were carried out in the following places of the North Sea: Wadden Sea (Kieskamp *et al*., 1991), Sean Gas Field, Oyster Ground, North Dogger Bank (Neubacher *et al*., 2011), Elbe estuary (Deek *et al*., 2013).

Apart from temperature, it has also been demonstrated that under hypoxia in the overlying water (<91.4 μmol O_2 l^{-1}), the OPD decreases by 50% (Rysgaard, *et al*., 1994; Neubacher *et al*., 2011). However, The Wash, and the North Sea showed O_2 concentration in the overlying water >200 μmol O_2 l^{-1}, that is well above the hypoxia conditions.



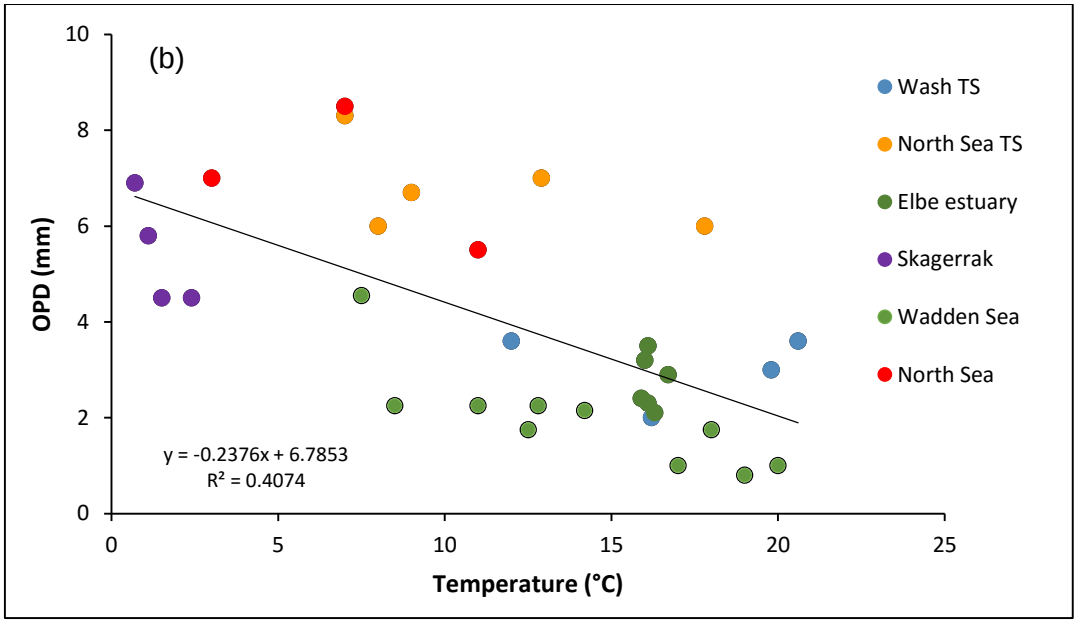


Figure 4.11. Relation between temperature and OPD in the North Sea with data from the Wash and the North Sea of this study (a) and with pool of data from different places of the North Sea (b). Data of this study are indicated as TS in graph b.

OPD and Light – Additionally to the effect of temperature, light also affect the OPD, because it stimulates photosynthesis by benthic algae and hence, O_2 production. When sediments are exposed to light, high rates of O_2 production from microbenthic algae primary production, might increase the OPD (Revsbech *et al*., 1980; Revsbech and Jorgensen, 1986). Thus, the effect of the temperature on the OPD will vary depending on, whether the sediments are exposed to light or not. Although a higher temperature may also increase the number of respiring organisms, the effect of higher temperature on the OPD, when combines with light is more obvious than when sediments are kept in dark. This effect was shown by Revsbech *et al*. (1980). In that study, sediments collected from the Randers Fjord estuary (Kattegat) during the summer and kept at in situ temperature of 18°C, showed an OPD of 5.5 mm when exposed to light, but once the light was turned off, the OPD decreased to ~1.5 mm. However, in sediments kept at 10°C, OPD was 7 mm when they were exposed to light, but ~ 5 mm once the light was turned off. Hence, the change in the OPD when sediments at low temperature (10°C) were exposed to light, was 50% less, than when sediments at higher temperature (18°) were exposed to light. It is worth noting that the OPD at low temperature (10°C), was deeper both in the dark (~5 mm) and in light (7 mm), than the OPD observed in sediments in dark (~1.5 mm) and in light (5.5 mm) at higher temperature (18°C).

Although, in the present study, the experiments were carried out in the dark in order to compare them with the deeper samples from the North Sea, which were collected at depth well below at which light penetrates, the effect of the light on the OPD could be confirmed in one of the experiments carried out during May at the Wash. In this experiment the profiles of oxygen were measured after the sediment were exposed to light. The mean OPD of three profiles of sediments exposed to light was ~25% deeper than when were measured on the sediment core before exposure to light. In the core exposed to light, the OPD increased from ~ 3.4 mm to 4.9 mm (Fig. 4.5e). In the Wash, an intertidal area, the effect of the light on the OPD, will be an additional complication on the OPD variability, and this variability may have an effect on the processes of the N-cycling.

4.4.1.2 Porosity

Sediment may be defined as unconsolidated granular particles conformed by organic or inorganic material deposited in the sea floor (Breitzke, 2006). Porosity is a physical characteristic of the sediment and measures the void spaces of the sediment to total volume of the sediment. This property of the sediments is important because allows water to penetrate into sediment, where, pore water solutes such as N and O_2 are consumed or produced and consequently their concentration change in scale of mm across the depth (Meile and Cappellen, 2003).

According with Cai and Sayles (1996), under steady state conditions, the OPD may be predicted by the following equations:

$$Z_0 = 2\emptyset D_s \frac{[O_2]_{bw}}{F_{O_{2(z=0)}}} \quad (4.6)$$

$$F_{O_{2(z=0)}} = Z_0 \times k_0 \quad (4.7)$$

Where, Φ is porosity, Z_0 is the OPD in cm, Ds is pore water diffusivity of oxygen (cm^{-2} s^{-1}), $[O_2]_{bw}$ is bottom water oxygen concentration, F_0 is oxygen flux into the sediment at the surface of the sediment, and K_0 is depth-independent oxygen consumption rate (µmol cm^{-3} s^{-1}).

Regarding Ds, it depends on porosity in such a way that when porosity increases, Ds also increases. Thus a change in porosity would be reflected in the OPD. However, this simple assumption seems to be more complicated because according to Cai and Sayles (1996) equation it follows that the OPD is also affected by the ratio of bottom water O_2 concentration, and O_2 flux into the sediment. Then, there is not a simple relation between the porosity and the OPD. This can be observed, both in the Wash and in the North Sea where no direct relation was observed between the OPD and the porosity (Fig. 4.12a). Thus, the lack of a direct relation between the porosity and OPD, highlights the importance of the other factor that are also affecting the OPD.

Besides, Ds and O_2 concentration, are in turn affected by the temperature, but, while the temperature reduces the solubility of O_2, and therefore the O_2 concentration, for a given salinity, Ds increases as the temperature increases (Ramsing and Gundersen). For example, for the range of temperatures for the Wash (12°C to 20°C), and the North Sea (7°C to 18°C), and assuming a salinity of 35 for both sampling sites, Ds would have ranged between 1.5613 x 10^{-5} cm^2 s^{-2} to 1.9569 x 10^{-5} cm^2 s^{-1}, in the Wash and between 1.3359 x 10^{-5} cm^2 s^{-1} and 1.8538 x 10^{-5} cm^2 s^{-1} in the North Sea which represents an increase of Ds of about 1.3. If additionally, the effect of porosity on Ds and the OPD is added, it would be difficult to find a direct relationship between the OPD and the porosity.

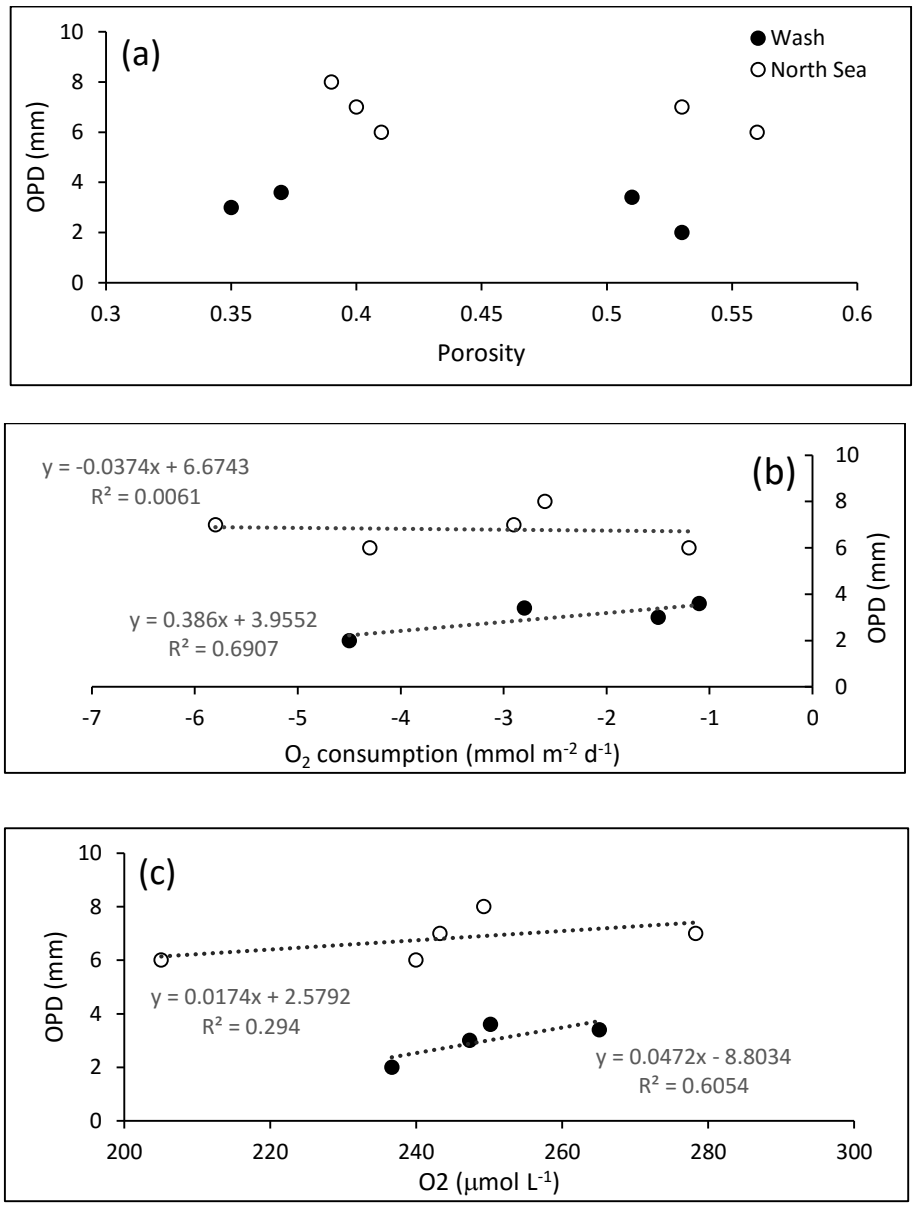


Figure 4.12. OPD vs porosity (a), O_2 flux (b), and O_2 concentration (c) in the bottom water for the Wash (black filled circles), and for the North Sea (open circles).

4.4.1.3 Organic matter

Organic matter (OM) in the marine environment can be derived from primary producers that encompasses different species of phytoplankton, or it can have an alloctonous origin, for example OM is transported from land by rivers (Zonneveld *et al*., 2010). The organic matter content in sediment plays an important role in N-cycling because through its oxidation, nutrients such as N and C are mineralized (Seitzinger, 1998). C:N ratios also may indicate the origin of the organic matter (Escobar-Briones and Garcia-Villalobos, 2009).In the present study, the percentage of organic C and N, as well as the C:N ratios in sediments at the Wash and the North Sea were in the range observed in other studies (Trimmer *et al*., 1998; Bristow *et al*., 2013; Neubacher *et al*., 2011).

The range of the C content in sediments (0.05% - 0.48% in dry sediment) observed in the present study is in the range of C (0.03 to 3.3%,) observed for most sediments of the North Sea (Trimmer *et al*., 1998; Bristow *et al*., 2013; Neubacher *et al*., 2011). The sinking flux of C in the North Sea have been estimated to range between 50 mg C m^{-2} d^{-1} and 185 mg C m^{-2} d^{-1}, which represents around 20% and 35% of primary production (Davies and Payne, 1984). However, assuming that 90% of the organic C is decomposed before reaching the sediments (Canfield *et al*., 2005), only between 5 and 18.5 mg C m^{-2} d^{-1} (2 to 3.5% of primary production) might be reaching the sediments. If these values are compared for example, with the sinking rates of C (244. 8 mg C m^{-2} d^{-1}) and N (51.4 mg N m^{-2} d^{-1}) of the German Bight (Oehler *et al*., 2015), where the organic C reaching the sediments is around 33%, or with the rates of organic C (140 mg C m^{-2} d^{-1}) in the Belgian coast, it is evident that the fluxes to the sediment, and therefore the C and N content in sediments of the North Sea are low. According to Painting (2010) low C content in sediments of the North Sea may be due to low levels of the spring bloom which in turn are associated with higher light extinction rates. Additionally, it should be considered that in the North Sea, phytoplankton blooms occur during spring (April-May) and autumn (October) and the sampling was carried out during August.

The scarceness of studies carried out at the Wash related with this topic made it difficult to compare and discuss in deep the result observed at the Wash.

C:N ratios – C and N are two important components of organic matter. According to Redfield (1934), the global C:N ratio is ~ 6.6, although it can be greatly variable (Martiny *et al*., 2014), with C:N ratios ranging from 3 - > 20 (Sampei and Matsumoto, 2001; Trimmer *et al*., 1998; Bristow *et al*., 2013) . Changes in C:N ratios depend on many factors such as terrestrial inputs, the stoichiometry of plankton communities (Martiny *et al*., 2014), or different microbial degradation carried out by aerobic and anaerobic process (Lehman *et al*., 2002).

This last point implies that different fraction of the organic matter is degraded at different rates because of selective degradation of N-containing compounds (Jorgensen, *et al*., 1990). When there is a selective degradation of N, the C:N ratio tend to increase. Regarding the

stoichiometry of plankton, the C:N ratios varies in function of the primary compounds that form the organic matter of the organisms. Thus, C:N ratios of plankton which main compounds are proteins rich in N, range between 4-10 (Rullkötter, 2011). In turn, from these values C:N ratios between 5 to 6 may correspond to a community of Phytoplankton and zooplankton, while freshly deposited organic matter may have C:N ratios between 6 to 9 (Sampei and Matsumoto, 2001). On the other hand, C:N ratios of terrestrial plants which are rich in cellulose, are >15 (Rullkötter, 2011).

Previous studies observed C:N ratios in the Wash ranging between 5 to 22 (Trimmer *et al*., 1998; Bristow *et al*., 2013) and between 6.6 to 9.7 in the North Sea (Neubacher *et al*., 2011). Unlike the findings in the Wash, in this study the C:N ratios (4.2 and 9.3) were lower, being indicative of a phytoplanktonic origin rather than terrestrial (Table 4.2). This result is also confirmed by Bristow *et al*. (2013) who found that alga was the major constituent of PON during the spring and summer in the Great Ouse and intertidal flats of the Wash. It is worthwhile to notice that the sampling of the present study did not include the month of August that was the month when Bristow *et al*. (2013) observed the highest C:N ratios.

In the North Sea, except for stations 43 and 68, which C:N ratios were abnormally low (2.2 and 2.9 respectively), C:N ratio (7.7 to 8.1) indicated that the main source of C was phytoplankton (Table 4.2). The percentage of carbon content in sediment at all the stations of the North Sea was < 1%. It has been observed that when organic C content is < 1%, the presence of inorganic N causes a decrease in the C:N (Sampei and Matsumoto, 2001). It happens because during the organic matter oxidation the NH_4^+ released may be being adsorbed to the sediment, while the CO_2 is lost (Müller, 1977). For example NH_4^+ absorbed to clay mineral in sediments may account for 25 - 40% of the total N causing the C:N ratios to decrease to values unusually low (<4, Müller, 1977).

In order to evaluate the effect of inorganic N on the C:N ratio, the carbon (%) versus N (%) of stations 43 and 68 were plotted. The point in the X-axis reached by the line of the correlation trend is considered to be the percentage of inorganic N adsorbed by the sediment as NH_4^+. (Sampei and Matsumoto, 2001). The inorganic N percentage adsorbed to the sediment were ~0.013% and 0.010% respectively (Fig. 4.13). Considering that the effect of inorganic N on the C:N ratios is intensified under low (<1%) organic C content in sediment, the adsorbed N in sediments could have been the reason of the lowest C:N ratios (2.2 and 2.9) observed at stations 43 and 68.

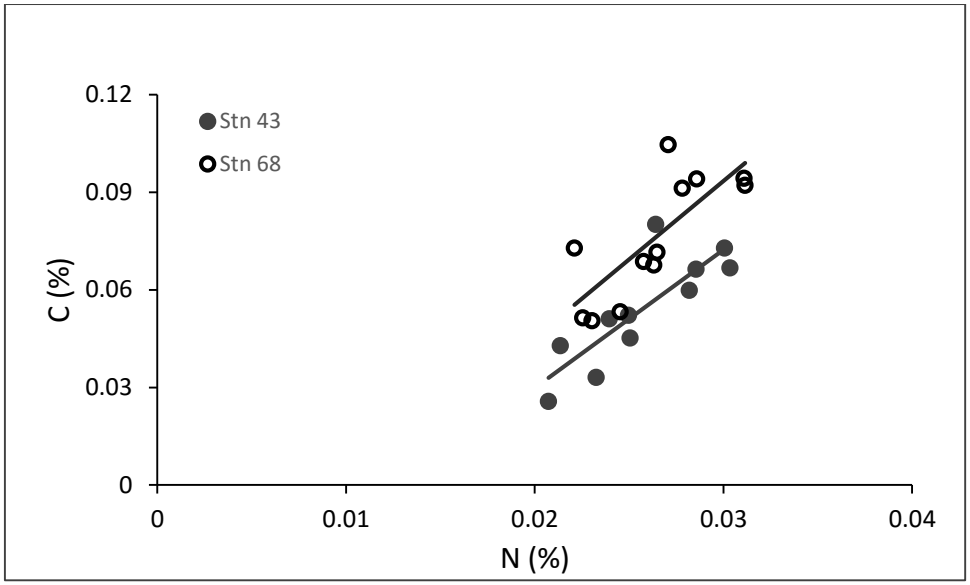


Figure 4.13. C (%) content in sediment versus N (%) content of sediment.

4.4.2 Oxygen consumption

Oxygen consumption may reflect the labile organic matter content in marine sediments, but is also linked to benthic community structure and bottom water temperature. Regarding the organic matter in sediment, and its variation with depth, O_2 consumption can be as high as 200 mmol m^{-2} d^{-1} in coastal waters with high OM fluxes to the sediments or as low as 0.1 mmol m^{-2} d^{-1} in the open ocean (Canfield *et al*., 2005). Southern North Sea coastal areas, namely the German Bight are characterized by containing high organic C content (1.9%), with O_2 fluxes ranging between -3 to -55 mmol m^{-2} d^{-1} (Oehler *et al*., 2015). Considering that O_2 consumption is coupled to labile organic matter, then it may be possible that the lower O_2 fluxes observed at the Wash (-1.1 to -4.8 mmol m^{-2} d^{-1}) and at the North Sea (-1.2 to -5.8 mmol m^{-2} d^{-1}) were mainly due to the low organic C content in sediment. Nevertheless, the relationship between O_2 fluxes and organic C content was clear in the North Sea, but it was not clear at the Wash (Fig. 4.14)

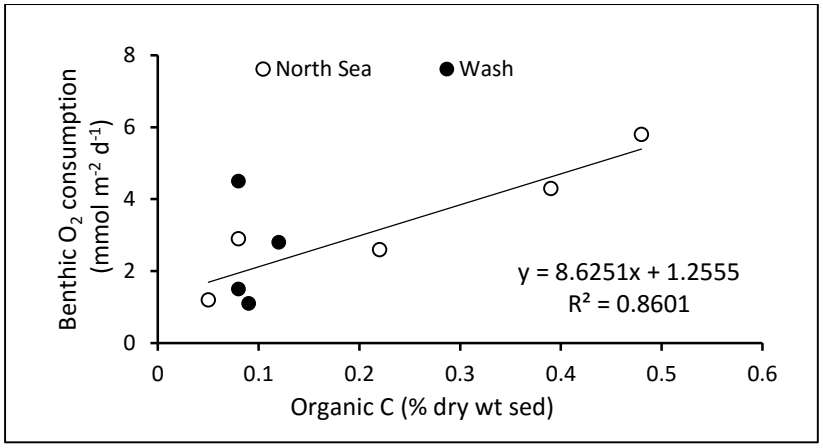


Figure 4.14. Benthic O_2 consumption rates as a function of organic C content.

The lack of relationship at the Wash may be explained by the fact that the O_2 fluxes do not depends only on organic matter, but it also depends on the variation of biomass and activity of benthic macrofauna which in turn may have an important impact on benthic O_2 consumption. Bioirrigation, for example may have an important impact by inducing transport of reduced solutes to the oxic layers of the sediment, leaving just a small portion of O_2 for mineralization (Oehler *et al*., 2015). As it will be explained later in the following paragraphs, NH_4^+ oxidation may have contributed to O_2 consumption.

In the sediments, O_2 consumption decreases in deeper layers, being the consumption of the few millimeters, mainly due to aerobic respiration, while any increase observed deeper may be due to reoxidation of reduced inorganic metabolites such as NH_4^+, ferrous iron, sulphide or methane (Canfield *et al*., 2005). Taking into account that coupled nitrification-denitrification rates accounted for more than 60% at most of the stations, then it may be possible that the increase in O_2 consumption deeper in the sediment in some stations, have been due to NH_4^+ oxidation. Although, only during May and June at the Wash and at station 127, O_2 consumption increased deeper in the sediments. In the Wash both, May and June had the highest nitrification-denitrifcation rates, accounting for around 80% in May and 35% in June. In June the increase of O_2 consumption observed between 0.11 mm and 0.16 mm was higher (129 μmol m^{-2} h^{-1}) than during May (88, μmol m^{-2} h^{-1}) even when in June, the denitrification coupled to nitrification accounted for only 35%, however, during June DNRA was observed (3.3 μM m^{-2} h^{-1}), and accounted for up to 40% of the total NO_3^- reduction, so that, the oxidation of NH_4^+ released during DNRA may have contributed to consume more O_2.

It results more complicated to explain the increase in the O_2 consumption observed at the stations 127 at the North Sea because denitrification coupled to nitrification accounted for >80% in all the stations (Table 3.2 in chapter 3). However, it was observed the presence of worms in the sediments, thus, it may be possible that other reduced solutes apart from NH_4^+,

have been transported to the oxic layers as a result of the activities of the organisms present in the sediments. Once in the oxic zone, the reduced metabolites may have been reoxidized, and thus increased the O_2 consumption in deeper layers of the station 127.

4.4.3 Organic matter, O_2 consumption, and OPD

One of the factors that has been suggested to be highly related to the OPD is the organic matter supply. Most of the organic matter formed in the surface of the water column is respired or assimilated to form new biomass (Rullkötter, 2006), and in shallow coastal waters, where the coupling between pelagic and benthic compartments is tighter, about 25 to 50% of the organic matter produced by phytoplankton sinks to the benthos (Jorgensen, 1996; Glud, 2008). The sinking particles of organic matter deposited on the seabed undergo several complex biogeochemical reactions that quickly consume O_2 (Cai and Sayles, 1996), consequently, in rich-organic sediments, the rates of oxygen respiration might be as high as 10 μM s^{-1} (Revsbech and Jorgensen, 1986). As a consequence of this high oxygen consumption due to mineralization of labile organic matter deposited in sediments, the extent of the OPD may be from few mm (Glud, 2008) to 10 cm on the continental margin (Cai and Sayles, 1996). On the contrary, in deep sediments, the OPD tends to increase because of either low sedimentation rates of organic carbon, or low water surface productivity (Glud, 2008; Fisher *et al*., 2009). Accordingly, the O_2 consumption is low, oxygen respiration tends to be as low as 2 μM y^{-1} (Revsbech and Jorgensen, 1986), and the OPD tends to be as deep as >10 m in oligotrophic marine environments (Fisher *et al*., 2009). Oxygen consumption reduces the O_2 transported into sediments affecting the OPD (Canfield *et al*., 2005), thus, the OPD may reflect changes in mineralization rates pathways and organic C supply to the sediments (Li *et al*., 2012), suggesting that organic C content in sediments may control the OPD. Following the rationale, organic C content in the North Sea would have been lower than the Wash because the OPD was deeper in the North Sea. Nevertheless, in general the C content was higher in the North Sea.

According to Moll, (1997) and Skogen and Moll (2000), the largest primary production in the North Sea occur in the coastal zones of Netherlands (>300 g C m^{-2} y^{-1}), the German Bight (>250 g C g C m^{-2} y^{-1}) and Dogger Bank (>200 g C g C m^{-2} y^{-1}), and the lowest production is in the central northern North Sea (90 and 100 g C m^{-2} y^{-1}). Considering these values then, stations 101, 127 and 141 should have lower C content than stations 43 and 68 which are in the more productive area of the North Sea, but once again contrary to what it was expected stations 101, 127 and 141 had higher organic C content. On the other hand, even when organic C content in sediments was higher in the North Sea, the OPD was deeper. Thus, in the present study, no relationship between OPD and organic C content (Fig. 4.15a), depth (Fig. 4.15b), or benthic O_2 consumption (Fig. 4.15c) was observed neither at the Wash nor at the North Sea. There was a relationship between organic C content and depth (Fig. 4.15d).

Other factors that were not considered in this study could have affected the OPD. One of this factors is the diffusive boundary layer (DBL) which is defined as a thin film (0.2 – 1.2 mm) of water that covers the sediments (Roy *et al*., 2002) and it has been observed that variations in its thickness may affect the diffusion between the sediment and the overlying water (Jorgensen and Revsbech, 1985; Roy *et al*., 2002; Wang *et al*., 2012). For example, in a study carried out in an intertidal mudflat, Wang *et al*. (2012) found that the diffusion flux varied from 15.4 to 53.6 mmol m^{-2} d^{-1} with a 0.25 mm variation on the thickness of the DBL (0.10 – 0.35 mm) during a tidal period and this could affect OPD.

Something that complicates even more the understanding of the role of each factor on the OPD is the temperature. Since it not only affects the dynamic viscosity that in turn affect the thickness of the DBL, but it also affects metabolic processes and respiration. For example, according to Jorgensen and Revsbech (1985), an increase of 20°C would cause a reduction of 31% of the thickness of the DBL, an increase of 73% of O_2 coefficient diffusion, and a decrease of 35% on the O_2 solubility. Additionally, temperature will also increase the O_2 respiration up to 300-800%.

Given the amount of factors affecting the OPD, is was not possible to conclude what factor apart from the temperature, are key to understand the OPD variability and consequently the effect of its variability on the processes of the N-cycling.

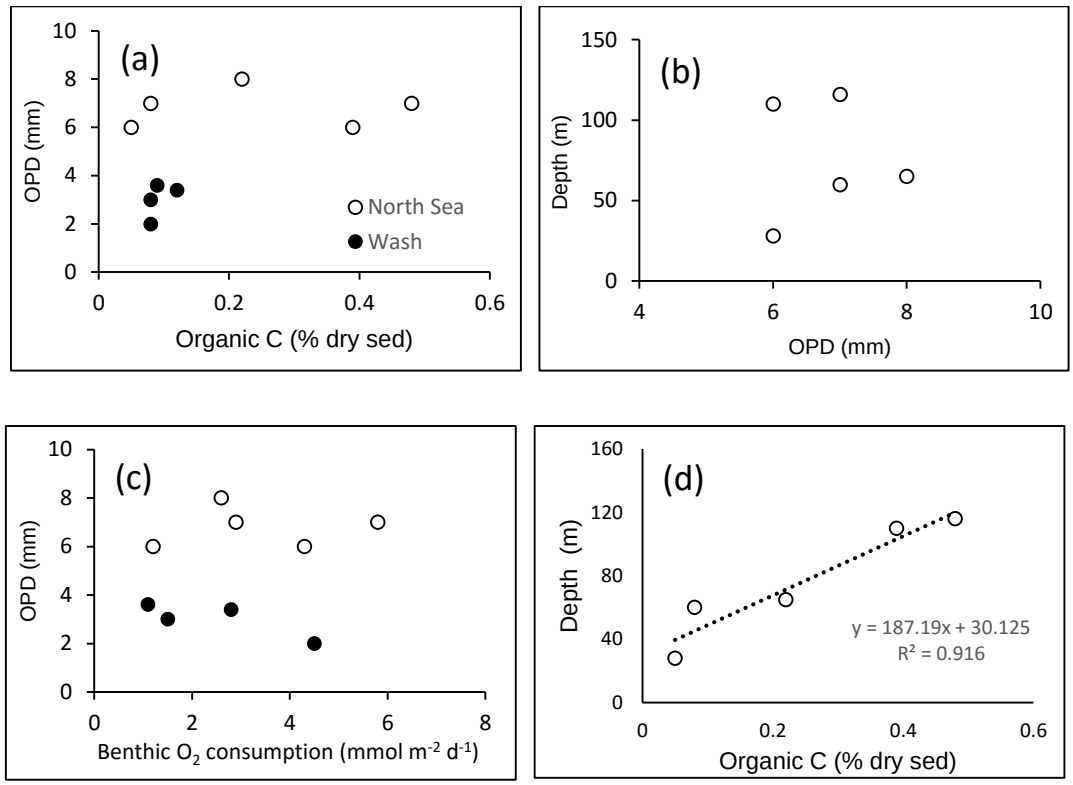
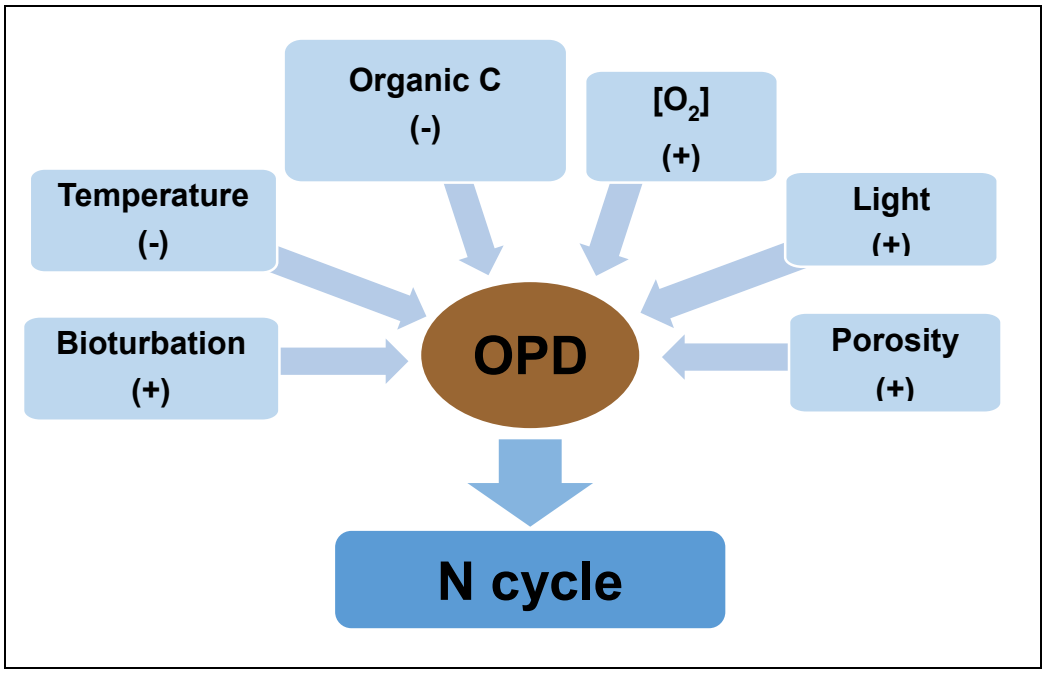


Figure 4.15. OPD as a function of organic C content in sediments (a), depth (b), and benthic O_2 consumption (c). Organic C as a function of depth (d).

4.4.4 Summary of factors affecting the OPD

A summary of factors affecting the OPD is showed in Fig. 4.16. From those factors, bioirrigation was not included in the current study. In the light of what was discussed in previous sections, it seems to be clear that from the data of the current study it was not possible to observe any correlation between the OPD and the following factors: organic carbon content in sediment, bottom water oxygen concentration and porosity. Nevertheless, a negative correlation between OPD and temperature was observed.



4.16. Summary of factors that affect the OPD.

Although it was not an objective of the current study to evaluate the effect of light on the OPD, an increase of ~25% on the OPD was observed when on May, the sediment was exposed accidentally to light. Light might have stimulated oxygen production through the microphytobenthos present in the sediment, enhancing thus, the OPD. The station at The Wash is an intertidal area that during the low tide is exposed to light, therefore in the natural environment, the OPD might have subject to variation of light. In the North Sea the maximum mean depth of the photic zone at which the photosynthetic available radiation is 1%, is estimated to be ~11 m (Capuzzo *et al*., 2015). The depth of the shallowest station (28 m) of the North Sea is well bellow of the penetration of the light, therefore, in the natural environment, variation in the OPD in the North Sea might not be due to light. Apart from the incident during May, all the oxygen profiles (including May) either from the Wash or from the

North Sea, were carried out under dark conditions, so that changes on the OPD are due to other factors than the light.

Coming back to the effect of temperature on the OPD, a negative correlation between the OPD and temperature (Fig. 4.11a), was observed when the data from the North Sea and The Wash were plotted together. The correlation was confirmed when data from other studies carried out in the North Sea were plotted (Fig.4.11b). Considering the effect that the temperature has on the OPD, then, the deepest OPD observed in the North Sea (6.8 mm) compared with the one observed in The Wash (3.7 mm) is in agreement with what it was expected, inasmuch as, the temperature at the North Sea (~10 C) were lower than in The Wash (~17 C). The fact that the solubility of oxygen decreases with temperature (Kristensen, 2000) might have been the reason of the negative correlation observed.

The organic matter content in sediment is also considered to have an effect on the OPD, some studies carried out in laboratory observed that an increase of the organic matter content is accompanied with a rapid response of respiration rate in sediment and consequently the OPD my decrease to < 1 mm (Slomp *et al*., 1993). In the North Sea, the organic carbon content in sediment (0.35%) was higher than in The Wash (0.09%), thus, following with the rationale, the North Sea would have shown a higher oxygen consumption and the OPD would have been smaller than in The Wash. Nevertheless, the results of the current study showed that the OPD at the North Sea was deeper than in The Wash, and when organic carbon were plotted against the OPD no correlation was observed (Fig. 4.15a). The effect of organic carbon content on the OPD, could have been observed if the temperature would have kept constant. In the current study there were variations in temperature and also the difference of temperature between the North Sea and The Wash was ~7 ^{0}C, so that, the effect of organic carbon content in sediment could have been masked by the effect that temperature has also on the organic matter (Kristensen, 2000), and hence, the lack of correlation between organic matter content in sediment and the OPD.

Additionally, the difference of temperature between the North Sea and The Wash may be the reason of higher organic matter content in the North Sea. Firstly, temperature affects organic matter decomposition (Banta *et al*., 1995), in such a way that when there is an increase on the temperature, the organic matter decomposition increases at the same time that oxygen is consumed (Slomp *et al*., 1993), accordingly OPD decreases. Secondly, organic matter decomposition also depends on the quality and lability (Hedges and Keil, 1995). Quality and lability, in turn are related with the elemental composition (C:N:P) of the organic matter (d'Annunzio *et al*., 2008; Güsewell and Gessner 2009) and depends on the content of protein, cellulose or lignin (Fenchel *et al*., 2012). Thus, plankton formed by N-rich proteins (Rullkötter, 2011), are considered to be of high quality and more labile organic matter (Hedges and Keil, 1995) than terrestrial vascular plants rich in cellulose and lignin (Rullkötter, 2011).

According to the C:N of The Wash (4 – 9), organic matter content in sediment can be considered as high quality, labile fresh organic matter, therefore because of the higher temperatures in The Wash, organic matter could have been more susceptible to decomposition than in the North Sea, consuming more oxygen and therefore both the OPD and the organic matter content decreased. Although in the North Sea the C:N ratios (2 - 8) can also be considered labile, the lower temperature may have limited the organic matter decomposition and thus have preserved it. Hence, less oxygen consumption and less decomposition contributed to deeper OPD and higher organic carbon content in sediment in the North Sea.

The results of this study did not show any evident correlation between the porosity and the OPD (Fig. 4.12a). The fact that porosity is the volume of void spaces of the sediment to the total volume of the sediment, does not mean that porosity itself determine the penetration of oxygen into the sediment. Firstly, as it was previously mentioned, the OPD is not only a function of porosity and the oxygen solubility, but OPD, also is a function of the ratio of bottom water oxygen concentration to the oxygen flux into the sediment at the surface of the sediment (mentioned in section 4.3.1.2). The fact that the OPD is also a function of the coefficient diffusion of oxygen (Ds), bottom water oxygen concentration and the flux of oxygen at the sediment surface (Cai and Sayles, 1996; Equation 4.2) implies that in the relation proposed by Cai and Syles (1996; see section 4.3.1.2), the effect of the porosity may be masked by the other factors which in turn are affected by the temperature. As it was previously mentioned, the temperature affects the solubility of oxygen, thus an increase on the temperature will increase the Ds, but on the other hand it will decrease the oxygen solubility and then the bottom water concentration. Secondly, the bottom water oxygen concentration is affected by the oxygen consumption which in turn depends on the organic matter content. Furthermore, bioturbation caused by organisms inhabiting the sediment may transport organic matter vertically, flush their burrows with rich-oxygen bottom water (Kristensen *et al*., 2012). Thus affecting the OPD. The effect of bioturbation on the OPD was not an objective in the current study, however, some studies (Aller and Aller, 1986; Meyers *et al*., 1987; Volkenborn *et al*., 2007; Kristensen *et al*., 2012) have observed that burrowing and flushing with oxygen-rich water, destabilize the sediment and cause changes in redox conditions of the sediment (Bertics and Ziebis, 2009) which in turn could affect the OPD (Volkenborn *et al*., 2007; Bertics and Ziebis, 2009; Ouellette *et al*., 2004).

It is clear that temperature has an important influence on the effect that each factor has on the OPD which complicate even more the complex net of relationship between the factors affecting the OPD.

In summary, the lack of correlation between other factors than temperature and light, seems to be due to the complex interactions between factors affecting the OPD. The temperature is influencing all the other factors, because of that, it is probable that temperature be responsible

for most of the variation on the OPD, and hence that it has been possible to observe a correlation between these two variables.

4.4.5 Organic carbon and processes of the N-cycling

It has been showed a positive relationship between the organic C content in sediments and denitrification. This relationship has been showed to be present at the Dundrum Bay in the Irish Sea (Trimmer and Nichols, 2009), at the Skagerrat and Aarhus Bay (Thamdrup and Dalsgaard, 2002), and at the Skagerrak and Kattegat (Engström *et al*., 2005). In the present study there seems to be a relationship between total N_2 production and the organic carbon content, expressed as percentage (org C % dry wt), however, the relationship is not significant ($p > 0.05$) either for the Wash or for the North Sea. The trend observed for the North Sea was inverse, meaning that if there were a significant relationship between the organic carbon content in sediments and total N_2 production, then the total N_2 production would be higher under low organic carbon content in sediments, the reverse of the results of previous studies (Fig. 4.17). The findings of the present study, however, agreed with a study carried out by Jäntti and Hietanen (2012) in the Gulf of Finland and the Baltic Sea. The authors did not find a relationship between C and denitrification rates. Besides, like in Jäntti and Hietanen (2012), in the present study the DNRA process was observed, although, the rates of DNRA founded by the authors previously mentioned were much higher (0.5 to 44 μmol N m^{-2} h^{-1}) than the rates observed in the present study (0.16 to 3.3 μmol N m^{-2} h^{-1}).

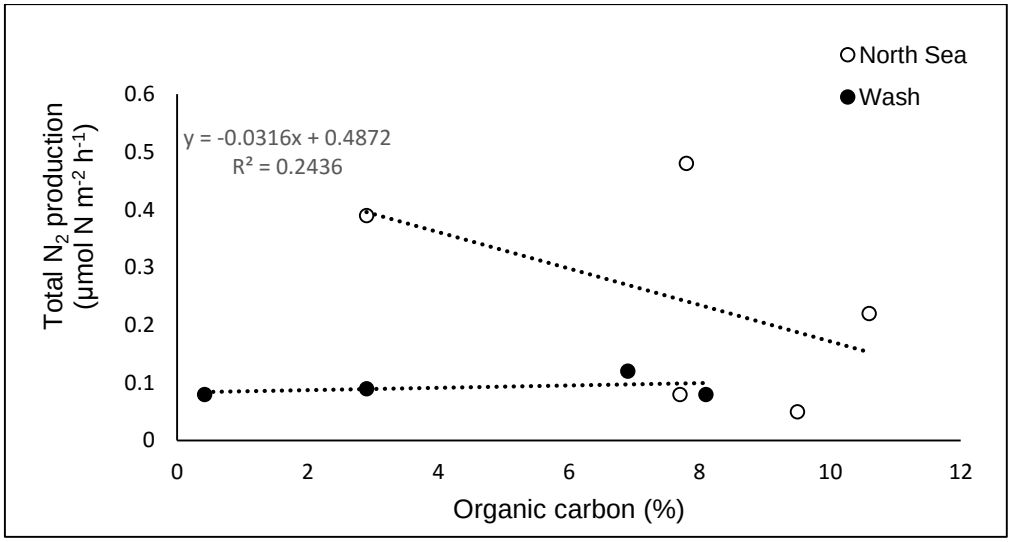


Figure 4.17. Total production of N_2 as a function of organic carbon C (% dry wt) in sediments.

Organic C has been also associated with high denitrification rates, through the ratio between NO_3^- and O_2 concentrations in bottom water. Because of the high rates of O_2 consumption in coastal water with high inputs of organic matter, the ratio NO_3^-/O_2 tend to be high and enhance anaerobic process such as denitrification (Canfield, 1994).

Studies carried out in batch reactors have found a maximum efficiency of simultaneous nitrification denitrification, and N removal at C:N ratios as high as 20 (Feng *et al*., 2011). While, low C/N ratios of 2.5 - 3.0 have shown low denitrification rates, although this did not affect the NO_3^- reduction, but it enhanced accumulation of NO_2^-. In another study carried out by Babbin *et al*. (2014), it was suggested that the stoichiometry of organic matter may favour either denitrification or anammox in a such way that when the sediment C:N ratio is high, denitrification is favoured and under lower C:N ratios, anammox may have important contributions to total N_2 production. In the study of Babbin *et al*. (2014) it was demonstrated that by modifying the C:N ratios of the particulate organic matter there are different contribution of anammox to total N_2 production. According to the findings of Feng *et al*. (2011) and Babbin *et al*. (2014), and considering that in the present study the stations 43 and 68 had low C:N ratio (2.2 and 2.9 respectively) it would be expected, firstly that the rates of total N_2 production had been lower than in the stations with higher C:N ratio. Secondly that anammox would have been favoured in stations 43 and 68. Nevertheless, neither lower rates of total N_2 production nor anammox was observed at these stations. On the contrary, the total N_2 production rates of these two stations were comparable with stations were higher C:N ratios were observed. For example, the total N_2 production at stations 127 and 141 was 7.8 and 10.6 $\mu M\ m^{-2}\ h^{-1}$, both stations with C:N ratios of 8.1 (Table 4.2),while the total N_2 production at stations 43 and 68 was 9.5 and 7.7 $\mu M\ m^{-2}\ h^{-1}$ respectively (Table 3.2 in chapter 3). On the other hand, rates of total N_2 production as low as 0.42 $\mu M\ m^{-2}\ h^{-1}$, and 2.9 $\mu M\ m^{-2}\ h^{-1}$ were observed during May at the Wash (C:N ratio of 9.3), and at the station 101 at the North Sea (C:N ratio of 7.7). Similarly, it was not evident that lower C:N ratios favoured anammox. This process was present only at station 43, although the anammox contribution was just 2%, and no anammox was detected at station 68. Contrary to what was expected following the rationales of Feng *et al*. (2011) and Babbin (2014), anammox contributed with 6.6% to total N_2 at the station 101 where the C:N ratio (7.7) was higher than in stations 43 and 68.

The fact that in the present study neither C nor the C:N ratio showed a relationship with the denitrification rates suggested that these factors do not influence denitrification either in the Wash not in the North Sea. The processes of the N-cycling may be therefore influenced by the interaction of other factors rather than only by OM availability and its C:N ratio. Thus, more studies should be carried out in order to better understand the factors influencing the partitioning between the different processes of the N-cycling.

It is worth noting here that in this study it was assumed that the exchange between the sediments and the overlying water was diffusive. Although this mechanism of transport

between the sediment water-interface is one of the most important in aquatic environments (Huettel *et al*., 2003), the transfer of solutes may be strongly affected by other processes such as bioturbation or bioirrigation. Aller (1988) for example, found that the burrows created by the fauna inhabited the sediment maybe irrigated with O_2 rich overlying water, so that the oxygen diffuses through the walls of the burrows creating gradients that may affect the biogeochemistry of the sediments and thus the fluxes of O_2 and the OPD.

4.4.6 OPD and processes of the N-cycling

As it was mentioned in section 2.3.2, the concentration of O_2 in the pore water control the distribution of the many biogeochemical process that take place in the sediment, such as the N cycle (Cai and Sayles, 1996; Glud, 2008). Thus, the extent of the OPD will affect the process of the N cycle significantly. Studies of denitrification have shown that variation of the OPD may have affected on the rates of nitrification and denitrification (Rysgaard *et al*., 1993; Rysgaard *et al*., 1995; An and Joye, 2001; Neubacher *et al*., 2011). For example, Risgaard-Petersen *et al*. (1994) observed that the extent OPD zone is reduced at night when the concentration of O_2 is reduced by high O_2 consumption in the sediment. The reduction of the OPD facilitated the diffusion of NO_3^- from the overlying water to the pore water anoxyc zone of the sediment and enhance Dw. On the other hand, during the day the microphytes inhabitanting the sediment surface produce O_2 via photosynthesis. The resulting rise of O_2 in the overlying water of the sediment at the sediment surface, increases the diffusion of O_2 into the sediment and thus the OPD increases. While this increase in the OPD may enhance nitrification, and thus may also boost Dn, Dw decreases because by increasing the OPD, the supply of nitrate to the denitrification zone decreases (Christensen *et al*., 1990; Rysgaard-Petersen *et al*., 1994; An and Joye, 2001).

Other studies showed that when the water column is under hypoxic conditions the OPD decreases by 50% (Rysgaard, *et al*., 1994; Neubacher *et al*., 2011) and such reduction enhanced the D_{14} by 32%, but nitrification decreases, and thus the main source for denitrification was NO_3^- from the water column. Although under hypoxic conditions a reduction of the OPD may favoured denitrification, reducing conditions may also favour DNRA (Rysgaard *et al*., 1996; An and Gardner, 2002). As the O_2 concentration is one of the factors that determine the OPD, if the water overlying the sediments is persistently hypoxic, the oxidized zone where nitrification take place may be lost (Jäntti and Hietanien, 2012). Moreover, the reduced sediment layer, where H_2S tends to accumulate may increase and be closer to the denitrification zone (Jäntti and Hietanien, 2012). Since sulphide is toxic for some nitrifier and denitrifier bacteria, both nitrification and denitrification processes may be constrained (An and Gardner, 2002). Consequently, the reduction of the OPD may enhance DNRA rather than denitrification, which results in fluxes of NH_4^+ out of the sediments

(Rysgaard, *et al*., 1996; Gardener *et al*., 2006; Dalsgaard and Thamdrup, 2002; Rysgaard, *et al*., 2004; Joye and Anderson, 2008). Similar results were showed by Dong *et al*. (2011) who observed high fluxes of NH_4^+ under anoxic conditions.

In the present study no relationship between the OPD and D_{14} was found in the North Sea and this was also the case in the study by Neubacher *et al*. (2011; Fig. 4.18). However, the data from The Wash showed a relationship between the OPD and total denitrification ($p > 0.05$; Fig. 4.18a), although it should be mentioned that the number of data is very small. No relationship was observed neither in The Wash nor in the North Sea, between the OPD and Dn, or between the OPD and Dw (Fig. 4.19a and 4.19b). Here, it is important to highlight that in the study of Risgaard-Petersen *et al*. (1994) there was a clear effect of the light on the OPD (Table 4.3). However, when plotting denitrification as a function of the OPD from their data, no clear relationship between variables was seen. For example, the data from the dark did not show a relationship between Dn and the OPD, or between Dw and OPD (Fig. 4.19c). Nevertheless, a strong relationship ($p< 0.05$) was observed between the variables when plotting the data from the experiment in the light (Fig. 19d). This results suggest that the OPD itself cannot explain all the variation of the processes of the N cycle. For example, in a study carried out by Rysgaard *et al*. (1996) NH_4^+ was only released to the overlying water in the dark. Thus, even if the OPD increases when the experiment take place in the light, Dn is not going to take place because NH_4^+ will limit the nitrification process and hence Dn. Similar results to the ones found by Rysgaard *et al*. (1996) under dark conditions, were found in the present study. No relationship was observed when Dn and Dw were plotted as a function of the OPD (Fig. 4.19c and 4.19d).

Another notable feature is the fact that for the same range of the OPD (4 – 6 mm) the rates in the study of Risgaard-Petersen *et al*. (1994) are much higher than the ones observed in this study for the Wash. This again confirm that the OPD is not the only factor influencing the processes of the N cycle.

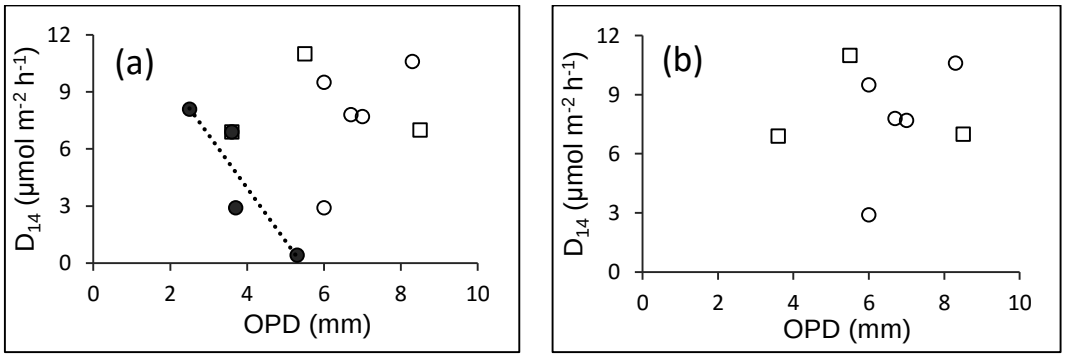


Figure 4.18 Total denitrification (D_{14}) as function of the OPD (a) in the North Sea (this study, open circles), The Wash (this study, black circles) and the southern North Sea (squares, taken from Neubacher, *et al*., 2011). (b) The North Sea (this study open circles) and Neubacher's data (open squares).

Table 4.3. Rates of total denitrification of NO_3^- supplied from the water column (Dw) and from coupled nitrification-denitrification (Dn) and OPD. Data from this study, Risgaard-Petersen *et al*. (1994). 1 and 2 in the column of site, indicate types of experiments.

Site	OPD (mm)		Dw μmol N m^{-2} h^{-1}		Dn μmol N m^{-2} h^{-1}	
	Dark	Light	Dark	Light	Dark	Light
Vilhelmsborg So (1)	2.5	4.7	48	18	9	19
Vilhelmsborg So (2)	1.9	4.9	103	36	4	11
Norsninde Fjiord (1)	2.1	3.9	199	96	18	35
Norsninde Fjiord (2)	2.5	4.4	180	65	15	24

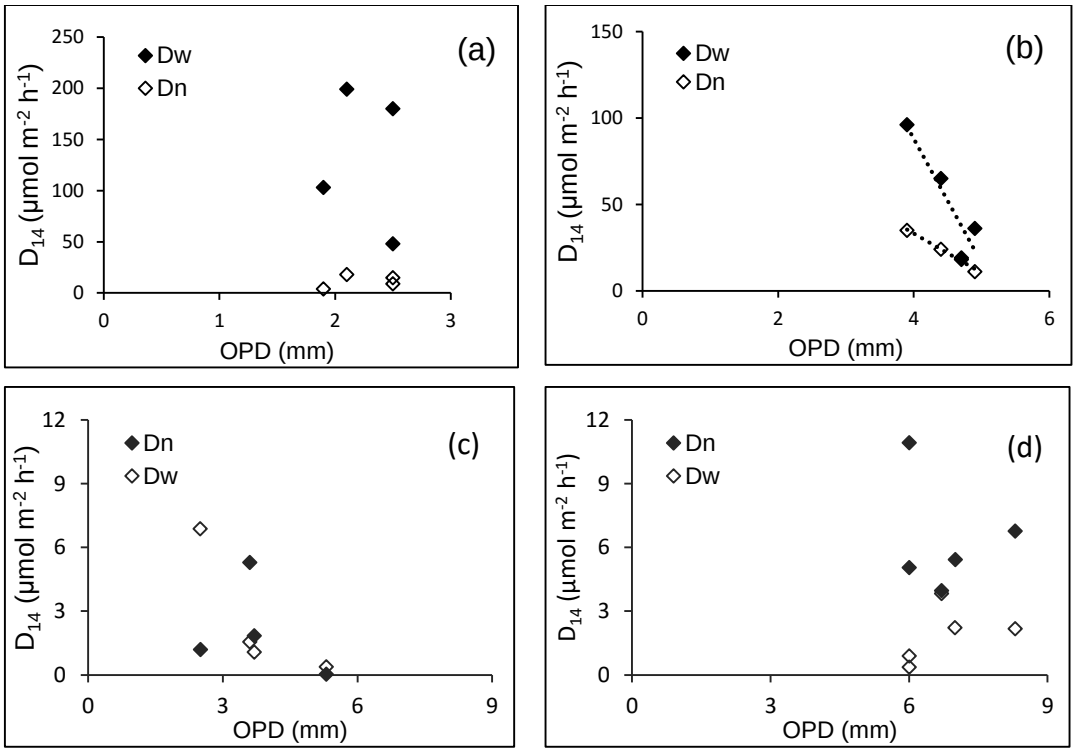


Figure 4.19. Denitrification, (Dw and Dn) as a function of the OPD from two experiments carried out at dark (a) and light (b). Data from Risgaard-Petersen (1994). Dn and Dw as a function of the OPD under dark conditions in The Wash (c) and in the North Sea (d).

Chapter 5. Nutrient fluxes and pore water profiles at the North Sea

5.1 Introduction

The analysis of sediment pore water and determination of nutrient exchange from and to the sediment-water interface is useful to better understand the biogeochemical process that take place in the sediments (Rysgaard *et al*., 1996). For example, denitrification in the sediments may be supported by NO_3^- diffusing into the sediment from the overlying water or by NO_3^- produced within the sediments. In the first case, the diffusion of NO_3^- either into or out from the sediments is mainly controlled by the concentration gradient between the overlying water and the sediments. On the other hand, nitrification will mainly depend on the availability of NH_4^+ in sediments and the presence of O_2. All of this processes may in theory be deduced from the measurements and interpretation of vertical profiles of nutrients and their fluxes.

In order to better understand the processes described in chapter 3 and 4, this chapter provide an overview about how the sediment nitrogen fluxes might respond to the processes within the sediments. Hence, this chapter analyse pore water profiles of nutrients and relates them to possible processes that affect the shape of the vertical profile and the fluxes of ammonium and nitrate flux measured, and how these variations may impact or be related with the processes of the N-cycling. The chapter also reports direct fluxes estimates of nutrients from intact sediment cores.

5.2 Methods

Determination of pore water concentration and water nutrient exchange were carried out only in the North Sea at stations 43, 68, 101, and 127.

5.2.1 Pore water sampling

Pore water sampling was carried out on a box core sediment sample (without removing the sediment from the core) as described in Sivyer (1995). In brief, the sampling was carried out with a sipper (Fig. 5.1) which consists of a set of probes, which in turn are made up of a porous plastic filter ring (15 mm high x 27 mm o.d. x 20 mm i.d.) connected to a vacuum chamber through a tubing. In order to give support to the plastic filter ring, each plastic filter ring was attached to hollow tubes with different lengths. The interstitial water was sucked from the sediment by inserting the probes into the sediment through a guide plate placed on the surface of the sediment. The interstitial water was sampled each centimeter in the first 5 centimeters, then every 2.5 cm until a 10 cm depth, and finally at 14, 17 and 20 cm depth. The interstitial water of each probe was collected individually in 11.5 ml test tubes. After collection, the

sample was filtered through 0.2 mm disposable syringe filters (Minisart Plus, Sartorius, UK Ltd), placed into 20 ml plastic pots, and preserved with mercuric chloride until analysis.

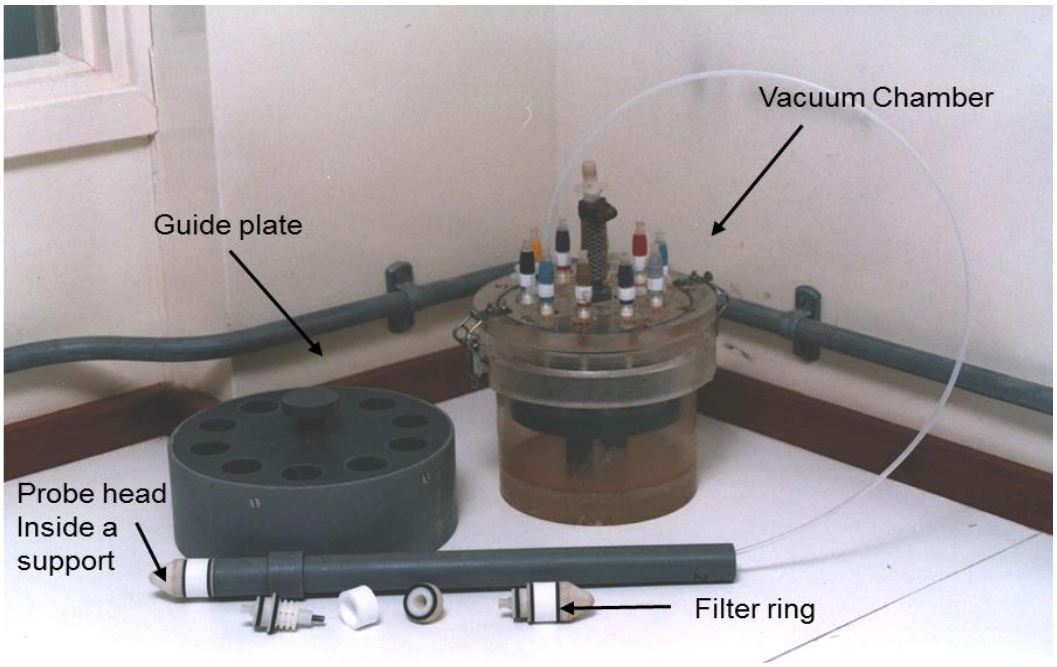


Figure 5.1. Sipper for pore water sampling (Taken from Sivyer, 1995)

5.2.2 Water nutrient exchange incubation experiment

In brief, 3 vertical sediment cores were collected at each station of the North Sea in 30 cm long x 10 cm i.d. Plexiglas tubes. The samples of sediment were subsampled from a NIOZ cylindrical box corer (for more details about the box corer see section 3.2). Every core was filled up with the water overlying the sediments collected with the box core, to a final volume of about 750 cm^3. Three more cores containing only bottom water were collected to be used as controls. Afterwards, the cores were placed in a water bath kept at the same temperature as the water at the sampling site and the core were left to re-equilibrate for a period of two hours. Next, the overlying water of each core was subsampled roughly every hour for the first five hours and then, two more subsamples were collected from the overlying water at 12 and 24 hours after the initial sampling (t=0). The samples were filtered immediately after collection through a 0.2 mm disposable syringe filter (Minisart Plus, Sartorius, UK Ltd), placed into 20 ml plastic pots, and preserved with mercuric chloride until analysis. Over the course of the experiment the volume of the overlying water of the cores decreases due to sample removal, therefore the sample concentrations were corrected for the volume extracted from the overlying water.

5.2.3 Benthic nutrient flux

The corrected concentrations from the nutrient exchange experiment (controls and sediment cores) were plotted against time (Fig. 5.2a to Fig. 5.2e), and the flux calculated from the slope (k) of the linear portion of the graph, then the fluxes were converted from µmol cm^{-2} h^{-1} to µmol m^{-2} h^{-1} as follows (Baric *et al*., 2002):

$$J = k * A^{-1} * F \quad (5.1)$$

Where A is the sediment surface area (78.54 cm^2), and F is the conversion factor ($1x10^4$) to convert cm^2 to m^2.

Finally, the fluxes from the sediment cores were corrected by subtracting the fluxes of the controls. As discussed below the fluxes estimated from the direct benthic exchange were used in the later estimates rather than fluxes from the pore water gradients. The fluxes are shown in section 5.3.1 (Fig. 5.3)

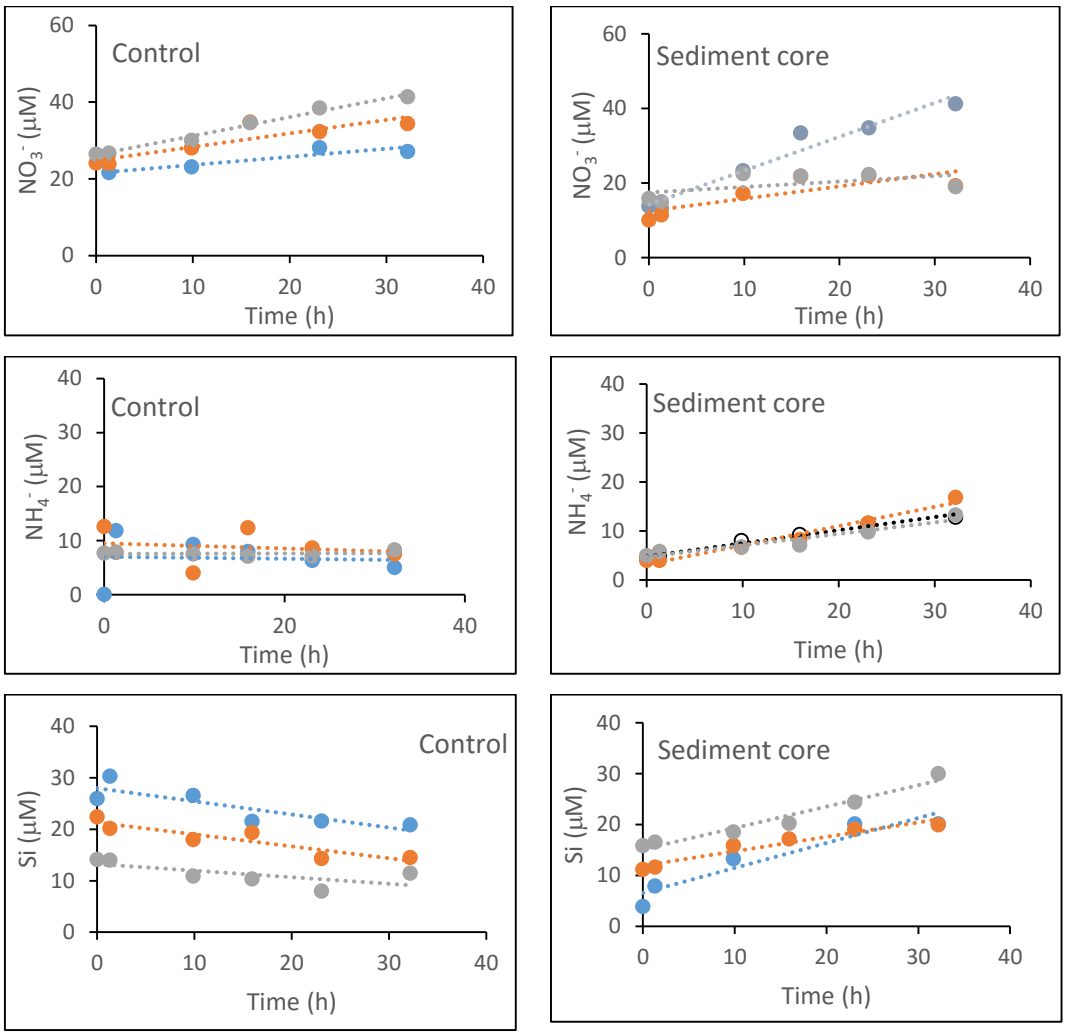


Figure 5.2a. The graphs illustrate the trend of NO_3^-, NH_4^+, and Si concentrations observed at station 43. Each graph shows the trend of the three replicates from the controls and from the sediment cores from the water nutrient exchange experiments.

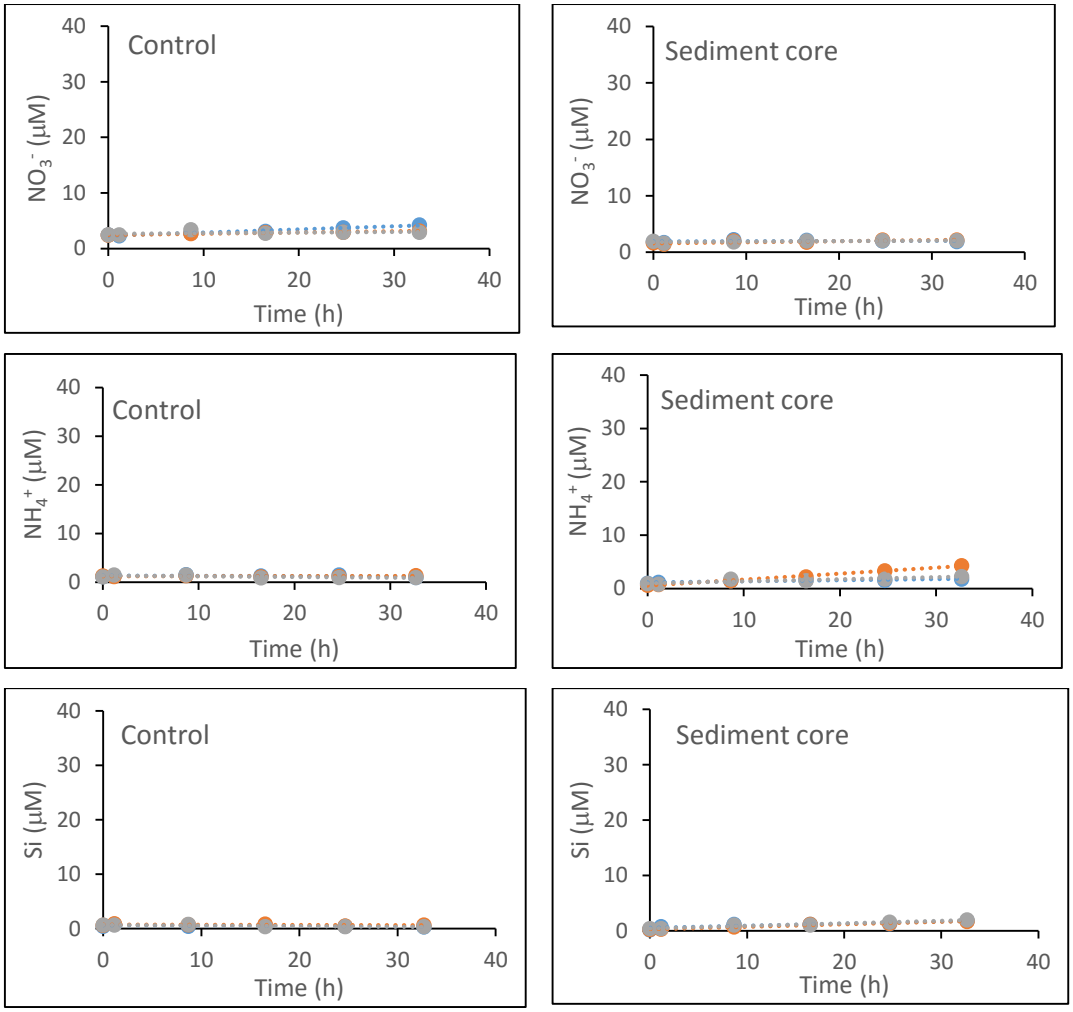


Figure 5.2b. The graphs illustrate the trend of NO_3^-, NH_4^+, and Si concentrations observed at station 68. Each graph shows the trend of the three replicates from the controls and from the sediment cores from the water nutrient exchange experiments.

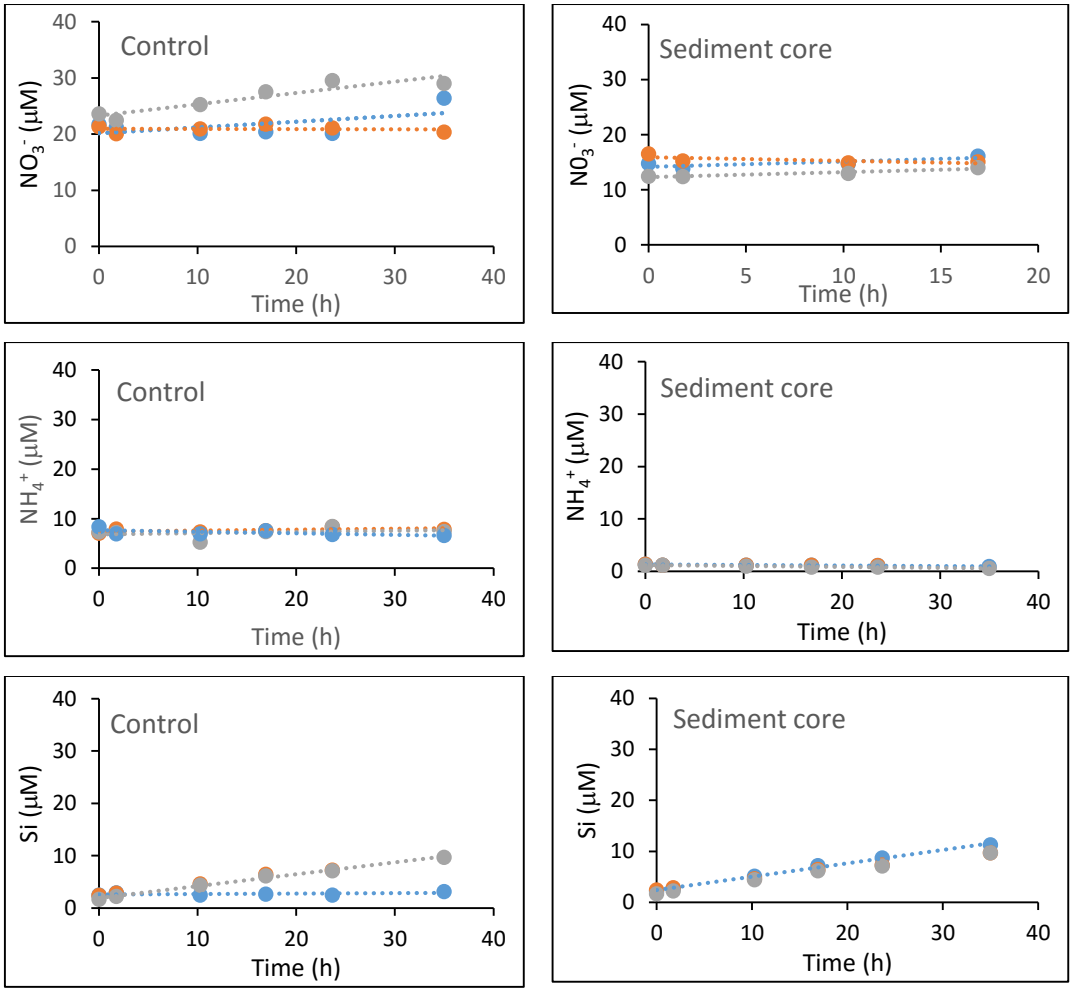


Figure 5.2c. The graphs illustrate the trend of NO_3^-, NH_4^+, and Si concentrations observed at station 101. Each graph shows the trend of the three replicates from the controls and from the sediment cores from the water nutrient exchange experiments.

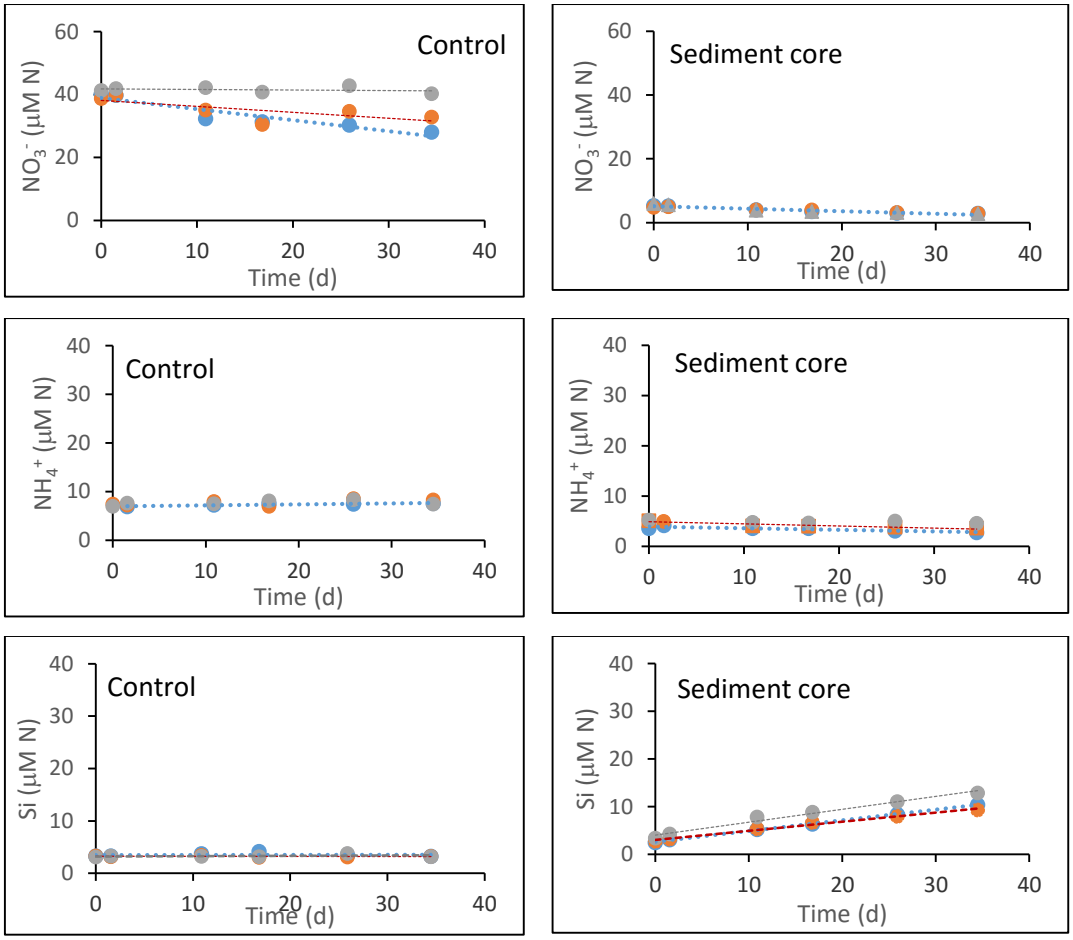


Figure 5.2d. The graphs illustrate the trend of NO_3^-, NH_4^+, and Si concentrations observed at station 127. Each graph shows the trend of the three replicates from the controls and from the sediment cores from the water nutrient exchange experiments.

5.2.4 Denitrification efficiency, nitrification and mineralization rates in sediments

The flux measurements (5.2.3) allow estimation of other key processes of the N cycle such as nitrification and mineralization. Rates of nitrification and mineralization were calculated using equations in Gihring *et al*. (2010) which in turn was based on the equation of Rysgaard *et al*. (1994):

$$Nitrification = NO_{3\,flux}^{-} + NO_{2flux}^{-} + Annamox \times 0.5 + pD_{14} \quad (5.2)$$

$$Mineralization = NO_{3\,flux}^{-} + NO_{2flux}^{-} + NH_{4\,efflux}^{+} + pD_{14} \quad (5.3)$$

Where: NO_3^-, NO_2^-, NH_4^+ flux are the fluxes of NO_3^-, NH_4^+, and NO_2^- determined by the benthic nutrient flux experiment (5.2.3), anammox and pD_{14} correspond to the rates of anammox and denitrification estimated in the intact sediment core experiment with the ITP technique modified by Rysgaard *et al*. (2004; Chapter 3, Table 3.2).

Sediment denitrification efficiency (DE) represents the percentage of inorganic N released from the sediment as N_2 (Eyre and Ferguson, 2009). It was calculated as the sum of N fluxes lost as N_2 through denitrification during remineralization of organic matter by the following equation (Eyre and Ferguson, 2009; Pérez-Villalona *et al*., 2015):

$$DE = N_2\_N/(N_2\_N + NO_3^- + NH_4^+) \times 100 \quad (5.4)$$

Where, N_2_N is the N lost as N gas through denitrification, and NO_3^- and NH_4^+ are the fluxes estimated through the water nutrient exchange experiment.

5.2.5 Nutrients analysis

The nutrients analysis were carried out in the Analytical Instrument Laboratory facilities of the University of East Anglia. Analysis for NO_x^- (NO_3^- + NO_2^-), NH_4^+, and Si, were determined by colorimetric methods. The protocols and standards preparation were based on the methods described by Grasshoff *et al*. (1983). Additionally, certified reference materials (CRM) were analysed as a sample in order to determine the accuracy of analysis. The standard deviation of the CRMS used through the analysis (~4) was < 10%. A Continuous Flow Analyzer (Skalar San++) was used to determine the concentrations on nutrients. The range and minimum detection limits are shown in the table 5.1 (Skalar San++ Instrumentation Manual)

Table 5.1. Ranges and detection limits of the Continuous Flow Analyzer Skalar San++

	NO_3^- + NO_2^-	NH_4^+
Ranges	0.07 – 35.7 μM N	0.07 – 71.4 μM N
Minimum detection limit	0.009 μM N	0.005 μM N
Relative standard deviation (%)	0.43%	0.35%

5.3 Results

5.3.1 Fluxes measurements

Because the marine biogeochemical cycle of silicate depends mainly on the weathering of rocks, and in marine sediments (Bernard *et al*., 2010) its concentrations are not considerably affected by other biogeochemical process. Thus, silicate normally increases with depth in marine sediments, consequently there is a constant supply to the water overlying the sediments (Vanderborght *et al*., 1977). In this study, although with variable fluxes between the stations, there was also a constant flux of silicate from the sediment to the overlying water (Fig. 5.3). By contrast, measured fluxes of NH_4^+ and NO_3^- were directed both into and out from the sediments reflecting the more complex sedimentary biogeochemistry of N. This is also illustrated by the error bar based on the average rates from the three replicates core. Sediments from stations 43 and 127 were sources for NO_3^-, with the magnitude of flux ranged between 27 and 50 μmol m^{-2} h^{-1} (Table 5.2), while sediments from stations 68 and 101 were a sink for NO_3^- with magnitude of flux ranging between -35 and -8 μmol m^{-2} h^{-1} respectively (Table 5.2). On the other hand, stations 43 and 68 were sources for NH_4^+, and stations 101 and 127 were sink (Fig. 5.3). The magnitude of the fluxes of NH_4^+ out from the sediment at stations 43 and 68 (36 and 41 μmol m^{-2} h^{-1}) were >5 fold higher than the fluxes directed into the sediments at stations 101 and 127 (~9 μmol m^{-2} h^{-1}, Fig. 5.3).

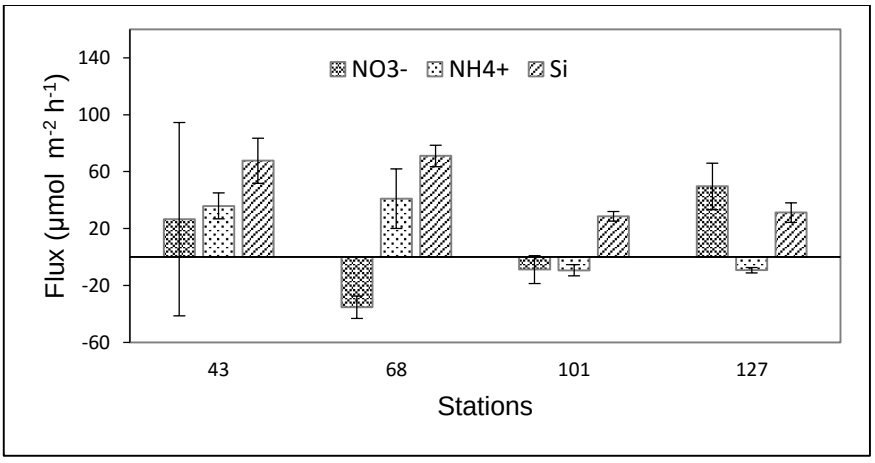


Figure 5.3. Fluxes of NO_3^-, NH_4^+, and silicate (Si) measured in intact sediment cores. Negative values indicate fluxes into the sediments. Positive values indicate fluxes out of the sediment.

The fluxes of NO_3^- and NH_4^+ out from the sediments at station 43, indicated that sediments act as a net source of N. Likewise, stations 68 and 127 were a net source of N to the water column. However, the magnitude of the fluxes out from the sediment was much smaller at station 68 (6 μmol m^{-2} h^{-1}) than at stations 127 (41 μmol m^{-2} h^{-1}). The net source of N at station

68 was as NH_4^+, whereas, at station 127 was as NO_3^-. Sediments at station 101 were sink (~-9 µmol m^{-2} h^{-1}) of both NO_3^- and NH_4^+.

5.3.2 Vertical nutrient concentration in pore water

NO_3^- concentrations in pore water varied both vertically and spatially. With the exception of station 127, the vertical distribution of NO_3^- varied between 14 to 30 µmol N l^{-1} with concentrations at 20 cm ranging between 15 and 32 µmol N l^{-1}. The minimum and maximum concentrations were observed at stations 43 and 127 respectively. Although all the stations showed sub-surface peaks at different depths, no systematic vertical pattern was observed for all the stations. The highest mean concentration of NO_3^- were observed at station 127 which mean concentrations was 37.5±9 µM, while the lowest was observed at station 43 with a mean concentration of NO_3^- of ~20 µM (Table 5.2).

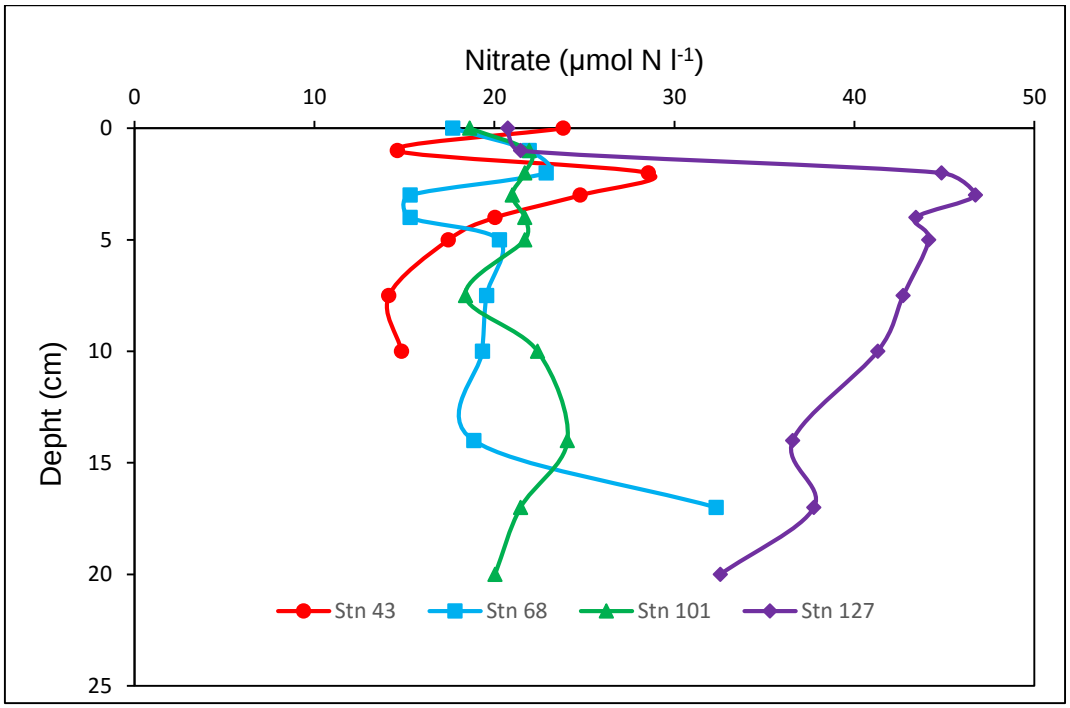


Figure 5.4. Pore water profile of NO_3^- at the North Sea.

Table. 5.2. Minimum (Min) and maximum (Max), mean and standard deviation of nutrients concentrations, of the vertical distribution in sediment pore water of the North Sea.

North Sea	NO_3^- (µM)				NH_4^+ (µM)			
Station	Min	Max	Mean	Std dev	Min	Max	Mean	Std dev
43	14.1	28.5	19.8	5.4	4.3	6.5	5.3	0.8
68	15.3	32.3	20.3	4.9	5.5	28.5	12.2	7.3
101	18.4	24.1	21.2	1.6	4.1	8.3	6.7	1.3
127	20.7	46.7	37.5	9.1	6.7	9.3	8.1	0.9

NH_4^+ concentrations in pore water showed less variation both vertically and spatially than NO_3^- (Fig. 5.5). With the exception of station 127 which had an unusual sub surface peak of NH_4^+ (~29 µmol N l^{-1}) at 2 cm depth of the sediment, the vertical NH_4^+ concentration in pore water ranged between 3.6 and 11 µM (Table 5.3). The pattern of the vertical distribution of NH_4^+ showed a slightly trend to increase below 5 cm depth, but then, decreases again to a similar concentration to the one observed at the surface (Fig. 5.5). The average concentration ranged between 5 and 12 µM, with the minimum and maximum average at stations 43 and 68 respectively (Table 5.3). The highest concentration of NH_4^+ was observed at station 68. This station showed an unusual peak at 2 cm depth, but then the concentration decreases with depth and fluctuated in the same range of concentration that the remaining stations (Fig. 5.5).

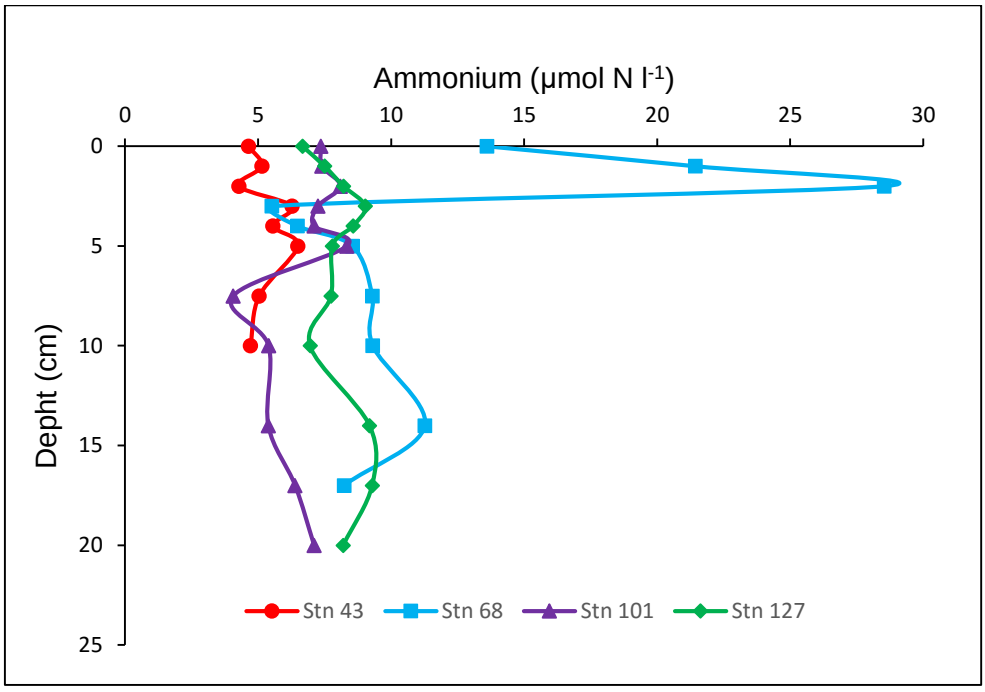


Figure 5.5. Pore water profile of NH_4^+ at the North Sea.

With the exception of the station 68, the NO_3^- concentration of the water overlying the sediments was higher than pore water of the sediment surface (Table 5.3) suggesting that, unlike station 68, stations 43, 101 and 127 stations were sink of NO_3^-. On the other hand, the concentration of NH_4^+ in the overlying water was higher than the top pore water concentration only at the station 43. The direction of the flux of NH_4^+ was not clear because the concentrations in the overlying water and the pore water of the sediment surface was almost the same.

Table 5.3. Concentration of NO_3^- and NH_4^+ on the overlying water of the sediments and at the sediment surface of the pore water profile.

Station	NO_3^- (μM)		NH_4^+ (μM)	
	Overlying water	Surface sediment	Overlying water	Surface sediment
43	25.4	23.8	10.2	4.6
68	2.43	17.7	1.24	13.6
101	22.2	18.6	7.6	7.4
127	40	20.7	7.3	6.8

5.4 Discussion

The spatial and temporal analysis of the vertical distribution of nutrients in the sediment pore water is fundamental to understand the processes undergoing in the sediment (Magni and Montani, 2006). The processes at the sediment-water interface regulate the dynamic of nutrients exchange and thus for example the fluxes of NH_4^+ or NO_3^- may also regulate the water column primary production (Longhi *et al*., 2013). As was mentioned (section 2.1), NH_4^+ is released during organic matter mineralization, in the oxic layers of the sediment, NH_4^+ can in turn be oxidized with O_2 into NO_3^-. The products of nitrification can subsequently be removed from the sediment pore water by denitrification and be converted to N_2, or be recycled into NH_4^+ through NO_3^- ammonification. (see section 4.1). Additionally, NH_4^+ and NO_2^- can also be removed through the anammox process. Within the oxic zone of the sediments where organic matter mineralization release NH_4^+, there can be an increase of NO_3^- because of NH_4^+ oxidation (Emerson and Hedges, 2006). According to the sequence of metabolic pathways as electron acceptors utilized during organic matter mineralization (Froelich *et al*., 1979), once the O_2 has been depleted deeper in the sediment, NO_3^- would be utilized as an electron acceptor. Thus, NO_3^- concentration in sediments starts to decrease with depth, because is being reduced to N_2 through denitrification or anammox. Then, after the denitrification zone, N from organic matter will remain as NH_4^+, which in turn starts to increase with sediment depth. The typical pattern derived from that process is shown in Fig. 5.6a.

Although nitrification is frequently associated with O_2, recent studies (Mortimer *et al*., 2004; Anschutz *et al*., 2005; Bartlett *et al*., 2008; Oliveira-Fernandes *et al*., 2015) have demonstrated that it also occurs under anoxic conditions. The oxidation of NH_4^+ is then carried out by Mn-oxides which act as a terminal electron acceptor (equation 5.4). The profile of NO_3^- under this condition shows unusual peaks under anoxic layers of the sediment (Fig. 5.6b)

$$8MnO_2 + 2NH_4^+ + 12H^+ \rightarrow 8Mn^{2+} + 2NO_3^- + 10H_2O \qquad (5.4)$$

Besides those processes, particle reworking and burrow ventilation caused by bioturbation destabilize the sediment affecting the physical and chemical properties of the sediment by introducing organic matter and oxygen deeper into the sediment. As a result, bioturbation may drive biogeochemical transformation of the sediments (Volkenborn *et al*., 2007; Kristensen *et al*., 2012) and create a situation where simple vertical gradients of properties are disrupted. Furthermore, in sandy sediments where advection is the main transport of solutes, the flow through the interstitial space can be deflected by the sea bed topography. The deflection of the flow creates gradients that can force water up to 10 cm deep into the sediment (Huettel *et al*., 1996). These advective processes affect the diagenesis of the sediments, the fluxes, and increase the OPD. The increasing of the OPD for example results on nitrification and therefore in accumulation of NO_3^- within the sediments (Huettel *et al*., 1998).

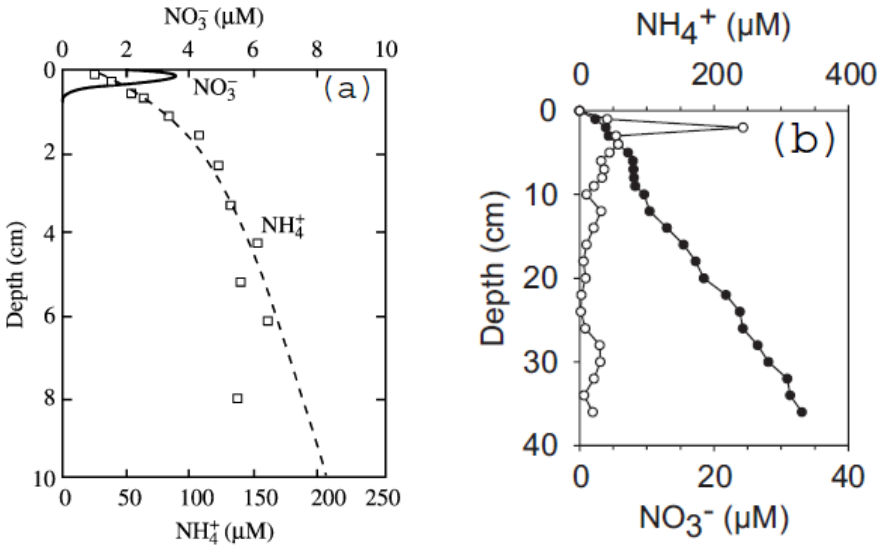


Figure 5.6. (a) Pore water profiles of NO_3^- and NH_4^+ from sediments near-shore waters of Denmark. Taken from Emerson and Hedges (2006). (b) Pore water profile of NO_3^-(empty circles) showing an unusual sub-surface peaks of NO_3^- in anoxic layer of the sediment. Taken from Bartlett *et al*. (2007).

In the present study, the pore water profile shape of NH_4^+ was characterized firstly by a maximum peak of NH_4^+ observed near-surface and secondly, with the exception of the station 43, the concentration of NH_4^+ decreased again after the near-surface peak. This trend was also observed by Engström *et al*. (2009), in pore waters of deep sediments (>2000 m), and was attributed to a cell lysis of microbes and meifoauna, because of a decompression. Even though the maximum sampling depth (~116 m) was not so deep, it could be possible that this has also happened with the samples of the present study. Another cause of these peaks might be DNRA. Through this process NO_3^- is reduced to NH_4^+ and increases its concentration in the sediment pore water. However, although the DNRA processes was observed at the North Sea, it could not be the reason of the near-surface peaks observed in all the stations. Firstly, because although the DNRA process was detected at station 68 and matched the maximum peak concentration of NH_4^+, this process was only observed at this station. Secondly, assuming that all the NO_3^- had been reduced either to N_2 or to NH_4^+, then, NH_4^+ would have been only 16% of the NO_3^- reduced.

The classic profile of NH_4^+ in pore water (as in Emerson and Hedges, 2006, Fig. 5.6a) is a trend from lower concentration at the surface to higher concentration deeper into the sediments. This pattern was observed by Lohse *et al*. (1993) at stations of the southern North Sea. The pore water concentration of NH_4^+ observed by these authors ranged from 50 µM (at 0.5 cm) to 60 µM (at ~15 cm) at the offshore stations, while concentrations as high as 2800 µM were observed at 9 cm depth at stations near the German coast. Although in the present

study, the concentrations of NH_4^+ slightly increased with depth up to a maximum of ~28μM, it still was much lower than the concentrations observed by Lohse *et al*. (1993). In the present study, low concentrations of NH_4^+ might suggest that anammox process might have been responsible for NH_4^+ removal. However, anammox was only detected at stations 43 and 101, and its contribution to total N_2 production was <6%. Alternatively, the lower NH_4^+ can reflect low organic carbon and hence a low source of N.

The typical vertical profile shape of NO_3^- in the sediment pore water (Fig. 5.6a) is in general the result of NO_3^- consumption below the oxic layer, where it is used as terminal electron acceptor by heterotrophic bacteria (Mortimer *et al*., 2004). As a consequence of that, the vertical profile of NO_3^- is characterized by high concentration at the surface of the sediments, followed by a decrease with depth. However, in this study, although the concentration of NO_3^- showed a slightly trend to reduce after the near-surface peak, the concentrations at deeper depths were still higher than the ones observed at the surface. The range of the mean concentration of NO_3^- (20 μM to 38 μM) was in the same range of a study carried out by Lohse *et al*. (1993), nevertheless, the profiles observed by the authors previously mentioned, followed the typical profile for NO_3^- in sediment pore water. Thus, the NO_3^- concentration in that study reached concentrations around zero between 25 mm and 60 mm depth. Unlike these profiles, in the present study the concentration of NO_3^- remained >10 μM. These relatively high NO_3^- concentrations below 10 cm depth coincided with low concentrations of NH_4^+ and could be the result of other processes other than only nitrification and denitrification.

One process that might lead to low NH_4^+ concentration is an anoxic nitrification reaction (Mortimer *et al*., 2004; Anshutz *et al*., 2005; Bartlett *et al*., 2008; Oliveira-Fernandes *et al*., 2015). This process involves the oxidation of NH_4^+ to NO_3^- by Mn-oxides and has been observed in Loch Duich on the west coast of Scotland (Mortimer *et al*., 2008), in the Humber estuary in the north-east England (Bartlett *et al*., 2008), and at the Archon Bay, southwest France (Oliveira-Fernandes *et al*., 2015). Basically, these studies have demonstrated that in experiments amended with Mn-oxides and NH_4^+, NO_3^- increases as NH_4^+ decreases. Although, the anoxic nitrification might explain the lower concentrations of NH_4^+ and high concentrations of NO_3^- at deeper depths in the ssediment, the hypothesis is difficult to prove because Mn was not measured in the present study.

The maximum OPD observed at the North Sea was 8 mm, which in principle means that below that depth sediment are anoxic, and NO_3^- should decrease. It also may be possible that high NO_3^- concentration in pore water reflects low organic carbon and hence low rates of anammox and denitrification that are not consuming all the NO_3^-. Alternatively, the presence of macroinvertebrates such as worms may ventilate the sediment and modify significantly the biogeochemistry of the sediments. One of the many effects that bioturbation have on the biogeochemistry of the sediment is the fact that may deepen the OPD by pumping rich oxygen water into the burrows. The effect of ventilation through the burrows was observed by

Delefosse, *et al*. (2015) who found how this processes was present at the bottom of a 25- cm long burrow. The O_2, may diffuse through the burrows walls into anoxic zones (Koretsky *et al*., 2002) and there might oxidize NH_4^+.

Furthermore, in addition to these possible explanations of the dynamics of NO_3^- and NH_4^+ in the sediment pore water, it should be noted that denitrification coupled to nitrification accounted for >80% of N_2 production (Chapter 1). The NO_3^- produced by nitrification in the upper oxygenated sediment layer, can diffuse to the overlying water, or to deeper anoxic sediments were it can be reduced to N_2 (Lohse *et al*., 1993). Accordingly, if NO_3^- formed by nitrification diffuses within the sediment, then might have supported high concentrations of NO_3^- in the sediment pore water. Therefore, nitrification could explain why the mean concentration of NO_3^- remained >20 μM in deeper sediments.

The vertical distribution of nutrients helps to understand the processes that take place in the sediments, but, the numerous processes influencing the shape profiles of the vertical distribution of nutrients might make it difficult to recognize how the sediments regulate the dynamic of these solutes (Longhi *et al*., 2013). On the other hand, insufficient vertical resolution of the nutrients profiles may cause uncertainty when nutrient pore water concentrations are used to determine fluxes (Schulz, 2006). Additionally, to this uncertainty, sipper sampler could cause a convective motion in the sediment so that sample concentration in the sediment may be not representative (Grigg *et al*., 1999). All of these causes are likely to be more important in coastal systems with high organic flux and stronger currents than offshore.

In the present study the pore water resolution through the first 5 cm was 1 cm, but, because of the shape profile, it was not possible to get an accurate gradient of concentration to determine the fluxes of their exchange with the overlying water with these data. Thus, a more direct experiment to estimate fluxes that measures DIN over time, was carried out in intact sediment cores to measure water nutrient exchange. For example, the decrease of NH_4^+ and accumulation of NO_2^- or NO_3^- with time, in the water overlying the sediments, indicates net nitrification in the sediments. If consumption of NO_3^- within the sediments exceeds the NO_3^- production there will be a reduction of NO_3^- in the overlying water which could be caused either by denitrification or anammox (Ward, 2011). Thus, whatever the process that the sediments undergo, by measuring the DIN concentration in the water overlying the sediment, it is possible to know the net flux either directed into or out from the sediments. Although the method provides no information on where reactions are taking place in the sediment, because of the relative simplicity of this method, the fluxes of NO_3^- and NH_4^+ were determined using the data of these experiments. Subsequently, the fluxes were used to calculate nitrification, mineralization and denitrification efficiency (DE).

Rates of nitrification, ranged from 12 to 57 μmol m^{-2} h^{-1}. The lowest rates of nitrification occurred at station 101 and the highest at station 127. Mineralization rates ranged between 21 and 79 μmol m^{-2} h^{-1} with the maximum rate at station 68 and the minimum at station 101. The DE ranged from 9 to 16% of the remineralized N (Table 5.4). The lowest and highest DE occurred at stations 43 and 127 respectively.

Table 5.4. Rates of nitrification, mineralization and denitrification efficiency (DE). Units are in μmol m^{-2} h^{-1}.

Station	Nitrification	Mineralization	DE (%)
43	36	79	9
68	43	84	16
101	12	21	14
127	57	67	11

The stations 68 and 101, where fluxes of NO_3^- (Fig. 5.3) were directed into the sediments, showed the highest DE (14 and 16% respectively). While stations 43 and 127 with fluxes of NO_3^- directed out of the sediments (Fig. 5.3), showed the lowest DE (9 and 11%),. The station 101, which was a net sink of N, showed the lowest rate of mineralization (21 μmol m^{-2} h^{-1}). This implies that no much NH_4^+ was available for nitrification and could be the reason of the lowest fluxes of NO_3^- and NH_4^+ observed. It is important to highlight that in the North Sea >80% of the NO_3^- reduced to N_2 is due to the coupled nitrification-denitrification process, therefore, it is possible that low mineralization rates with low NH_4^+ available to be oxidized to NO_3^- have limited and be the cause of the low denitrification rate (2.9 μmol m^{-2} h^{-1}) at this station.

The fact that the nitrification rates were higher than N_2 production, suggests that, with the exception of station 101, NO_3^- did not limit denitrification. The rates of nitrification at stations 43, 68 and 101 were around 50% of the N mineralized, whereas at station 127 nitrification was 86%. These percentages imply that >50% of the N mineralized was available as NH_4^+ either in the water column or within the sediment. In agreement with the percentage of nitrification and independently of the direction of the fluxes, at stations 43, 68 and 101, roughly 50% of the flux of N were as NO_3^- and 50% as NH_4^+, while at station 127 the flux of NO_3^- was higher than the flux of NH_4^+ (Fig. 5.3).

The reliance on nitrification on the availability of NH_4^+ was roughly confirmed by the correlation between nitrification and mineralization (Fig. 5.7a). Similarly, it was observed a correlation between nitrification and total N_2 production (Fig. 5.7b), thus supporting that in the North Sea

>80% of the NO_3^- reduced to N_2 is due to the coupled nitrification-denitrification process. However, despite this fact no correlation was observed between nitrification and DE (Fig. 5.7c).

Likewise, even though, no correlation was observed between denitrification rates and carbon content in sediment, it is worthwhile to note that mineralization rates and denitrification showed a strong correlation (Fig. 5.7d). In part this may be due also to the fact that coupled nitrification-denitrifcation was the main source of NO_3^- for denitrification in all the stations in the North Sea.

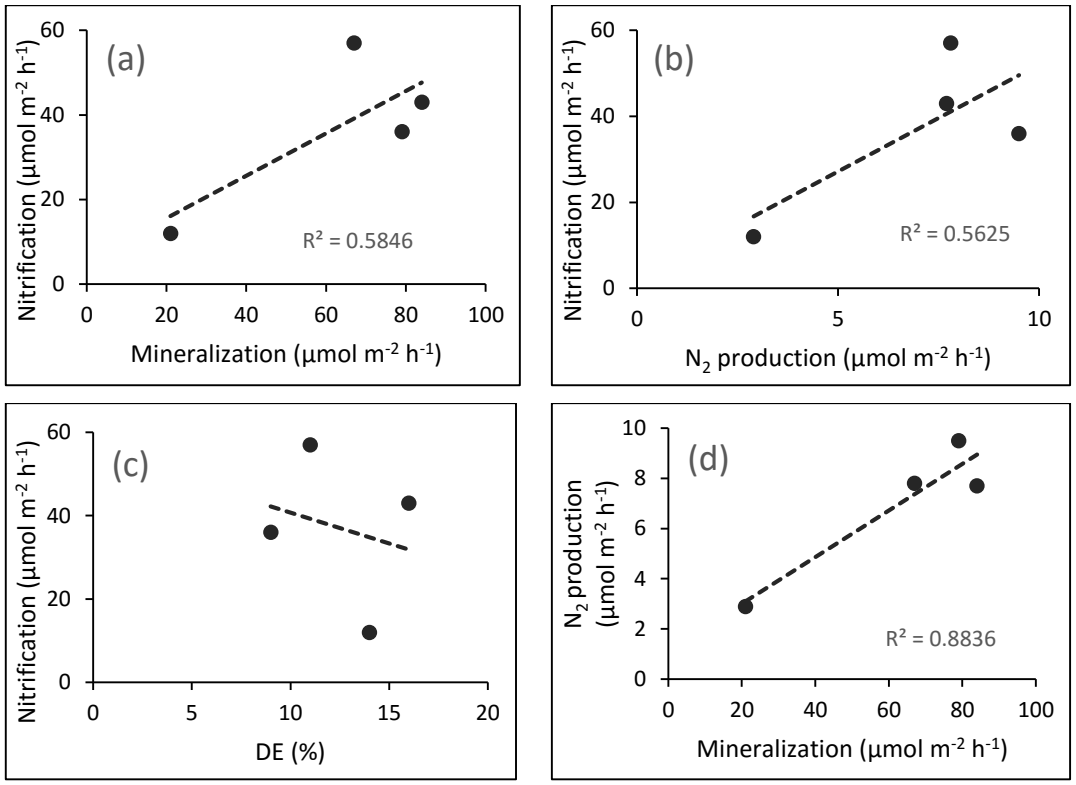
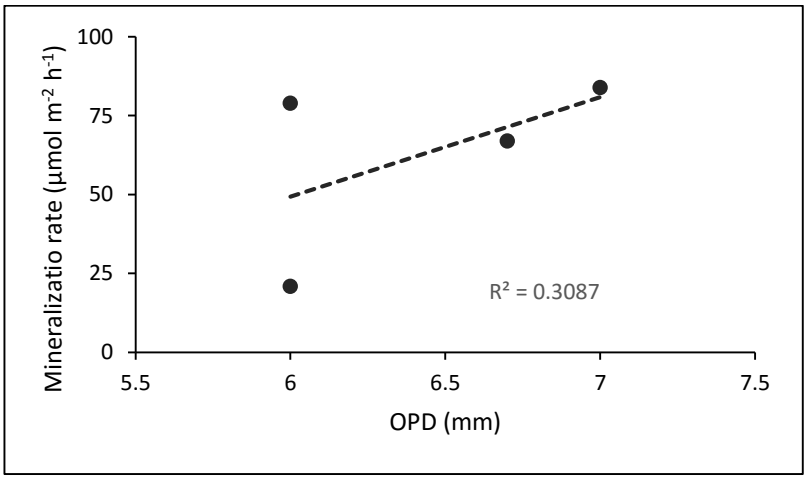


Figure 5.7. Nitrification as a function of mineralization rate (a), and N_2 production (b), and DE (c). N_2 production as a function of Mineralization (d). Data are from the North Sea, stations 43, 68, 101 and 127.

It is important to note that even when at station 101, the rate of mineralization was the lowest, the percentage of carbon content in sediment was one of the highest (0.39%) and a C:N ratio of 7.7, which indicates a labile organic matter. The station 127 which is comparable with station 101 in carbon content in sediments and in temperature, had however, a mineralization rate 3 fold higher than in station 101. Here, the difference of the OPD observed between stations 101 (6 mm) and 127 (6.7 mm) could have affected the mineralization rate. It is known that the OPD causes changes in the redox conditions of the sediment (Cai and Sayles, 1995; Glud, 2008) and that depending on its extent, it may enhance mineralization (Aller *et al*., 1982). In

this study is was not observed a clear correlation between the OPD and mineralization (Fig. 5.8), however, although inconsistencies such as the one mentioned between stations 101 and 127, cannot be satisfactorily explained only with the results of this study, it cannot be ruled out that the OPD had caused an increase of the metabolic zone where mineralization takes place within the sediment, thus enhancing the mineralization downwards in the sediment at station 127.



5.8. Relation between temperature and the OPD in the North Sea (stations: 43, 68, 101 and 127).

The variability between the cores, namely the cores where the nutrient exchange incubation experiments took place, was high indicating considerable spatial variability in the N cycle (Fig. 5.3). As it was mentioned before bioturbation was evident by the presence of worms and marine sea pens (Fig. 5.9) and might be responsible of this variability as well.

Figure 5.9. Image of a marine sea pen inhabiting the sediments of some of the cores were the experiments were carried out.

The rates of directly measured N exchange (Fig. 5.3) are relatively large compared to the rates of denitrification observed in chapter 3 in most of the stations (43, 68 and 1017). This suggests that sediments of these stations are source rather than a sink of N. This results are in line with the DE, the percentage of inorganic N released from the sediment as N_2. DE was relatively low (<20%), thus allowing the sediment to behave as a source of N.

In a study carried out by Eyre and Ferguson (2009), showed that DE was negatively correlated with carbon loading rates. Based on this correlation the authors proposed a trophic scheme (Table 5.5). Here, it is noteworthy that even when the over enrichment of the North Sea is considered to be restricted to coastal areas, according with the findings of Eyre and Ferguson (2009), the North Sea would be an eutrophic system because DE <18%.

Table 5.5. Trophic scheme proposed by Eyre and Ferguson (2009).

Trophic type	Organic carbon loading ($g\ C\ m^{-2}\ y^{-1}$)	Median DE (%)
Oligotrophic	<200	68
Mesotrophic	200-400	40
Eutrophic	400-600	18
Hypertrophic	>600	8

Chapter 6. Conclusion

6.1. Concluding remarks

In this thesis the data from the experiments carried out with intact sediment cores to determine rates of denitrification, anammox and DNRA suggested that denitrification was the main process of N_2 production at both the Wash and the North Sea. The results also showed that main source of the total N_2 produced was driven by coupled nitrification denitrification, which in general accounted for ~80% both at the Wash and the North Sea.

No clear trends were observed temporally at the Wash sites or spatially at the North Sea sites, although the data set is limited. On average the rates of denitrification at the Wash sites were lower than the rates at the North Sea sites. Anammox was not detected at the Wash site, and in the North Sea anmmox was detected only at two stations, but its contribution was < 6%. The DNRA process was observed in three of the four stations of the Wash, and assuming that all the NO_3^- had been reduced either to N_2 or NH_4^+, DNRA accounted between 6 to 29% of the NO_3^- reduction, and accordingly between 6 and 29% of NO_3^- is being returned back to the overlying waters as NH_4^+. The DNRA process was detected only at one of the station in the North Sea, and accounted for ~15% of the NO_3^- returned back as NH_4^+. These results suggest that denitrification is the dominant route for nitrate removal in the sediments, although the other mechanisms of DNRA and anammox can be important.

The possible effects of temperature and organic carbon on the OPD were analysed as well as the effect of the variation of the OPD on the process of the N cycle. In general, the OPD was deeper at the North Sea than in the Wash. Variation of the OPD did not show any relation with organic carbon. However, its depth variation shows a negative relationship with temperature. One motivation for this study was to see if the OPD (which can be measured relatively easily) could be used to estimate sediment denitrification and anammox, but the results here suggest this association is not simple. In the present study no relationship between the OPD and total N_2 production (D_{14}) was found at the North Sea sites, although a relationship was observed at the Wash sites. However, by comparing N_2 production as a function of the OPD with other studies it could be seen that for the same range of values of OPD there are rates of denitrification up to one order of magnitude greater found at other sites, therefore the OPD itself cannot be used as a key factor to indicate changes on the processes of the N_2 cycle. Carbon content in sediment was relatively low in the sites investigated here (and typical of the open North Sea), and did not show a relationship with the denitrification process and neither was a relationship observed with the OPD.

The studies of pore water nitrogen chemistry suggest that the pore waters in these low organic carbon and bioturbated sediments do not show simple smooth gradients with depth. It was difficult to recognize through the pore water profiles how the sediment regulates the

dynamic of NO_3^- and NH_4^+ probably due to the presence and activity of organisms inhabiting the sediments.

The direct measurements of nutrient fluxes from intact sediment cores yielded rather variable fluxes and patterns but in general agree in magnitude or direction with those derived from the isotope tracer techniques. Some inconsistencies between nutrient fluxes and intact sediment cores may have been caused by a no homogeneous distribution of the organisms inhabiting the sediments so that some of the cores may have been under the effect of bioturbation.

As it was mentioned before (section 1.1), high loads of N are discharged into the North Sea (Colijn *et al*., 2002). For example, the riverine N discharges into the North Sea is 76 Gmol N y^{-1} (Pätsch and Kühn, 2008). Thus denitrification in sediments may play an important role as a sink of N in the North Sea and by alleviating the possible eutrophication impact of this input. A summary of the rates of denitrification estimated for the North Sea in a study carried out by Brion *et al*. (2004) indicates a wide range of estimates of denitrification rates which range between 0.9 to 219 mmol m^{-2} y^{-1} (Table 6.1). The data presented in the table are derived from different methods ranging from models to a few direct measurements by the acetylene block method. In a more recent compilation of rates of denitrification, Pätsch and Kühn (2008) used basically the same data and estimated a total North Sea denitrification rate of 118 x 10^9 mol N y^{-1} which roughly is in the range of denitrification estimated by Lohse with the IPT technique (Table 6.1).

The mean rate of denitrification of the present study based on average of all the rates of all the sampling sites (Table 3.2) was around 68 mmol m^{-2} y^{-1}, which is of the same order of magnitude of most of the estimates in Table 6.1 .Scaling up this estimate to the whole North Sea area (750,000 km^2) yields an estimate of the rate of denitrification for the whole North Sea of 51 x 10^9 mol N y^{-1} which represent around 66% removal of total riverine N loading of the North Sea, comparable but a little lower than that of Brion (2004) and Pätsch and Kühn (2008). This illustrates that denitrification is a very important nitrogen sink within the open North Sea and the model of Pätsch and Kühn (2008) suggests even higher rates in near coastal regions richer in organic matter. As noted earlier nitrogen loads into the North Sea have increased due to human activity and it is therefore important to quantify these nitrogen loss processes and understand their environmental controls if society wants to effectively manage the region.

Table 6.1. Denitrification rates (mmol m^{-2} y^{-1}) measurements estimated for the North Sea (Table taken from Brion, *et al*., 2004).

Author	DNIT rate	Area	Method and period
Lohse *et al*. (1993)	0.9–71.8	Southern North Sea	Acetylene block – August and February
Seitzinger and Giblin (1996)	219	North Sea	Extrapolation from PP values – Annual cycle
Kieskamp *et al*. (1991	110	Wadden Sea	Acetylene block – Annual cycle
Law and Owens (1990)	3.5–109.5	North Sea	Acetylene block – July
van Raaphorst *et al*. (1992)	7–101.6	Southern North Sea	Acetylene block – Annual cycle
Lohse *et al*. (1995)	85.8–115.6	Central North Sea	Isotope pairing technique – July
Hydes *et al*. (1999)	255	Southern North Sea	Budget approach – Annual
Smith *et al*. (1997)	130	Southern North Sea	Budget approach – Annual
van Raaphorst *et al*. (1990)	55.2–85.8	Dogger Bank	Pore-water concentrations + model – Summer

6.2. Further work

Based on the results and in order to identify the factors that may favour one or other process of the N-cycling it is suggested to carry out a systematic study of the processes of denitrification, anammox and DNRA. So far, most of the studies have based their surveys on variation of nutrients, O_2 and temperature. However, building on the results of this study a key to better understand the processes of the N-cycling is to study also the effect of the bioturbation of organisms inhabiting the sediments as part of isotope tracing experiments.

The measurements of NO_3^- concentrations in the pore water with sensor of high resolutions will help to interpret the variation of denitrification rates as well as to get better estimates of nutrient fluxes.

The study of the processes of the N-cycling will be improved if it is supported by the genetic analysis of the microorganisms present in the sediment which are involved in the nitrogen cycle.

This study demonstrated the presence of DNRA in the North Sea and the Wash, however, it was not possible to identify the factors that might have triggered this process. Thus, further work is necessary to identify the key variables that enhance the process. Experiments in laboratory where variables such as temperature, organic matter, NO_3^- and NH_4^+ concentrations can be controlled would help to identify key variables.

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