The potential for N pollution swapping from riparian buffer strips and an instream wetland

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Declaration

I hereby declare that this thesis is my own work, except where otherwise stated, and has not been submitted for any other degree or qualification at any other institution. The work described is my own, with information derived from others thoroughly acknowledged.

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Abstract

Diffuse agricultural pollution is a major contributor to poor water quality in many parts of the world. Consequently agri-environment policy promotes the use of riparian buffer strips and/or denitrifying wetlands to intercept and remove diffuse NO_3^--N pollution. However, these methods have the potential to cause ‘pollution swapping’: the exchange of one form of pollution as a result of measures implemented to reduce another. Thus the benefits of intercepting NO_3^--N could be offset by enhanced emissions of the potent greenhouse gases, nitrous oxide (N_2O) and methane (CH_4), from buffer strips and wetlands.

This research aimed to: (1) quantify the direct N_2O emissions from an irrigated buffer strip (IBS), using nitrate-rich agricultural drainage water, compared to a non-irrigated control (CP); (2) improve the understanding of N_2O production and consumption within soils using controlled soil monolith experiments; (3) assess the effectiveness of a small (60 m^2) instream wetland at intercepting and removing diffuse NO_3^--N pollution, and quantify pollution swapping in the form of CH_4 and N_2O emissions; (4) assess the production of CH_4 and N_2O within the sediment, and their emissions as well as inorganic-N concentrations in the overlying water column in response to temperature and turbulence, using intact wetland sediment and membrane inlet mass spectrometry (MIMS). The research focused on mitigating diffuse NO_3^--N pollution from grazed pasture at a farm in north-east England.

Annual N_2O-N emissions from the IBS and CP were not statistically different ($P > 0.05$): 509 and 263 g N_2O-N ha^{-1}, respectively, in 2007 and 375 and 500 g N_2O-N ha^{-1} $year^{-1}$, respectively, in 2008. Irrigation of the IBS increased spatial variability in flux and generated hotspots of denitrification compared to the CP. However, these changes were short-lived. Direct N_2O emission factors (EF_1) calculated using the available NO_3^--loading data (September 2007 - December 2008) for the IBS were lower (c.0.1%) than those calculated for the CP assuming N input from biological N fixation only (<1.9%). Soil monolith experiments under a variety of irrigation and

NO_3^--N loading regimes confirmed low direct and indirect (of dissolved N_2O-N in leachate) emissions (<3.1 and <2.3% of applied NO_3^--N emitted as N_2O-N, respectively), similar to the IPCC default emission factors. However, N loss in leachate was high, up to 82% of added NO_3^--N with concentrations reaching 24 mg NO_3^--N L^{-1}. Therefore even though no pollution swapping occurred the high leachate losses indicate irrigation of buffer strips are not effective mitigation methods.

Monitoring for 2 years of the instream wetland that received median NO_3^--N concentrations of c. 6 mg N L^{-1}, but up to c. 20 mg N L^{-1}, showed it to be ineffective at intercepting diffuse NO_3^- pollution: likely a result of the relatively high discharge and short water residence time, as well as the direct input of NO_3^--N to the wetland from secondary sources: field drains and/or overland flow. The wetland was a net source of NH_4^+-N in both 2007 and 2008, and a net sink of NO_3^--N in 2007 only.

Annual wetland CH_4 and N_2O emissions were 713 and 237 mg CH_4 m^{-2} $year^{-1}$, and 3.5 and 1.9 mg N_2O-N m^{-2} $year^{-1}$, for 2007 and 2008, respectively and were highly variable between seasons. N pollution swapping was minimal from either direct or indirect emissions, but CH_4 emissions were found to be of greater importance at a net cost of ~ £600 ha^{-1} over the study period (2007 to 2008), compared to N_2O emissions (~ £60 ha^{-1}) and low NO_3^--N interception savings (~ £24 ha^{-1}). Incubation experiments suggest that spatially variable microsites of nitrifying, denitrifying or methanogenic activity and CH_4 oxidation occur within the wetland sediment. Therefore off-line, larger wetland systems offer the best prospects of enhanced NO_3^--N interception and potentially reduced CH_4 emissions by maintaining shallow water depths (increased CH_4 oxidation) and long residence times (increased opportunity for denitrification), within the wetland or wetland cells.

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Contents

Chapter 1 - Introduction

1.1 Global Nitrogen Enrichment

Global population and economic growth are driving the need for increased food production and with it crop production and fertiliser use. However, the exponential production and use of synthetic fertilisers worldwide over the last century has greatly perturbed the global N cycle and led to a variety of environmental problems such as eutrophication and global acidification and production of the greenhouse gas nitrous oxide (N_2O) (Gruber and Galloway, 2008; Rockstrom *et al.*, 2009). According to Rockstrom et al, (2009) the planetary boundary i.e. the safe operating space for humanity in relation to the N-cycle, has been exceeded, with agriculture the major contributor of this pollution. Moreover, the potential global consequences of interactions between the N-cycle and other biogeochemical cycles such as the carbon cycle in relation to climate change are still unknown (Gruber and Galloway, 2008). With the global population expected to reach 9 billion people by 2050, a 70% increase in agriculture productivity will be necessary to support them (FAO, 2010). Sustainable, efficient and climate-smart agriculture is of significant importance when addressing rising global food demands and climate change. Several critical issues related to food security, water supply and bioenergy will require integrated land and water management balanced with economic development on the global scale (FAO, 2010).

The development of the Haber-Bosch process in the early twentieth century provided a solution to the shortage of fixed N (Smil, 2004). This process utilises high temperatures and very high pressures, H_2 and N_2 to produce ammonia (NH_3), the primary ingredient in artificial fertilisers. Arguably, without this process modern-day agricultural yields, food production and population growth would not be possible (Smil, 1999; Jenkinson, 2001; Erisman *et al.,* 2007) (Figure 1.1). In the past twenty years the two major sources of anthropogenic N: that associated with food production (i.e. fertiliser use) and that associated with fossil-fuel combustion, are estimated at more than 160 Tg N per year, exceeding that supplied by biological nitrogen fixation (BNF) on land (110 Tg N per year) and in the ocean (140 Tg N per year), (Gruber and Galloway, 2008).

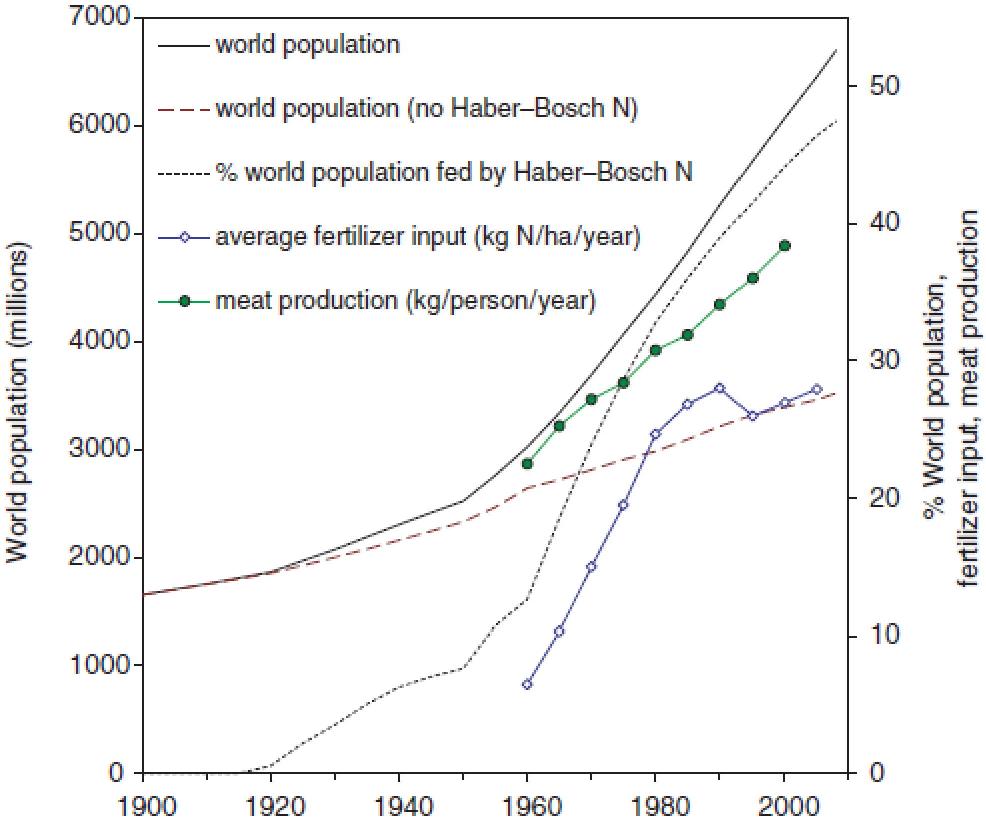


Figure 1.1: Trends in population growth and N use over the last century as estimated by Erisman *et al*, (2011).

Nitrogen (N) is a key component of all living organisms but it is also commonly found to be the limiting nutrient for increased primary production in many ecosystems, both aquatic and terrestrial (Vitousek *et al.*, 1997; Galloway *et al.*, 2008). Di-nitrogen gas (N_2) is the most abundant gas in the atmosphere, comprising 78% by volume. However, due to its triply bonded atoms, it remains largely unavailable to organisms prior to being converted into nitrogenous compounds by microbial processes (Schlesinger 1997, Vitousek *et al.*, 1997; Butterbach-Bahl *et al.*, 2011). Reactive nitrogen (Nr) is the term used to describe any nitrogen compound that is radiatively, chemically or biologically active in either organic or inorganic form (UNESCO and SCOPE, 2007; Galloway *et al.*, 2008). Inorganic forms of Nr include ammonia (NH_3), ammonium (NH_4^+), nitric oxide (NO), nitric acid (HNO_3), nitrous oxide (N_2O) and nitrate (NO_3^-); the organic Nr compounds include urea, amines, proteins and nucleic acids (UNESCO and SCOPE, 2007).

Additions of Nr greatly perturb the natural cycling of N, (Galloway, 1998; Rockstrom *et al.*, 2009) and lead to multiple pathways of environmental pollution: atmospheric, aquatic, marine and terrestrial (Jenkinson, 2001; Rockstrom *et al.*, 2009). Since 1960 Nr in the terrestrial environment has doubled, with more than half of all the synthetic fertiliser ever used on the planet used since 1985 (Figure 1.2), (Howarth *et al.*, 2002; Millennium Ecosystem assessment, 2005). Cultivated systems (at least 30% of land area in cultivation) covered 24% of the terrestrial surface by the year 2000 (Cassman *et al.*, 2005).

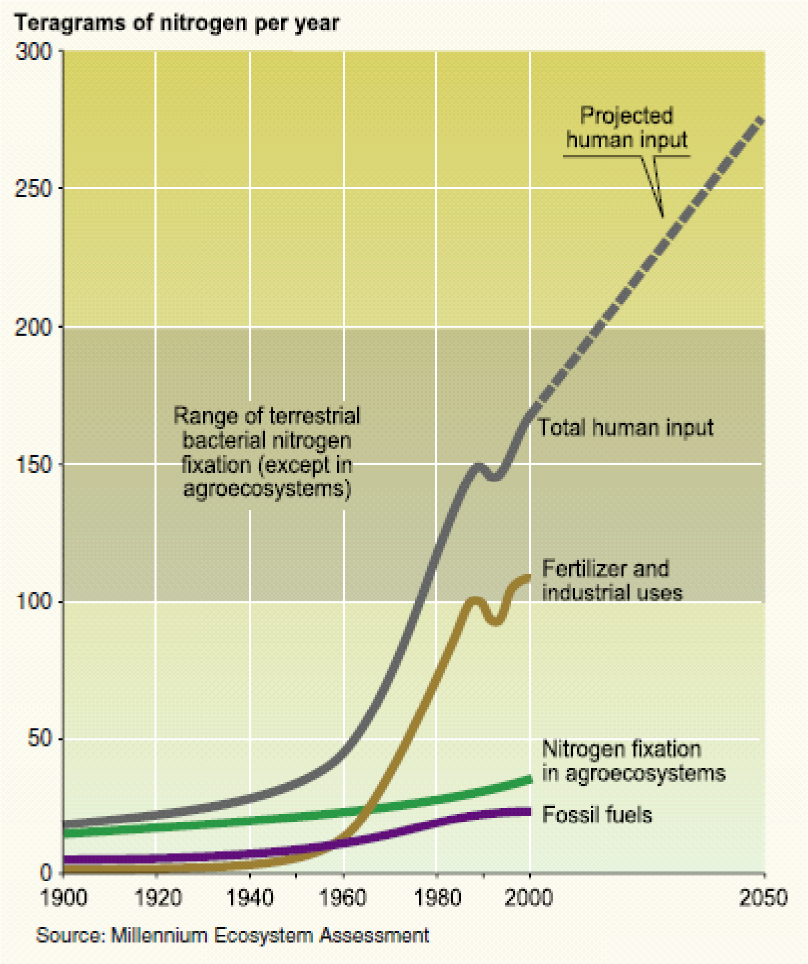


Figure 1.2: The global trend in Nr creation through human induced processes with projected increases of total N by human activities until 2050, from the Millennium Ecosystem Assessment, 2005.

Undoubtedly the use of synthetic fertilisers will rise concomitantly with world population as a result of increased food demand. Indeed since 1970 the world population and Nr creation have increased by 78% and 120% respectively (Galloway *et al.*, 2008). The costs of increased cultivation and fertiliser use are environmental and socio-economic. World N fertiliser demand is estimated to reach 111.6 million tonnes by 2014 at an annual growth rate of 1.8% (FAO, 2010). Europe is estimated to be consuming approximately 13% of global N fertiliser. Mineral N fertilisers are currently applied to 135.1 million ha of farmland in Europe (Fertilisers Europe, 2010). European N consumption is set to increase annually by 1.9% from 2010 to 2014 (FAO, 2010).Global distribution of fertiliser usage as well as the distribution and extent of alteration to nutrient cycling varies greatly from region to region. In regions of intensive agriculture, such as Europe, atmospheric N deposition has increased greatly: by as much as 10-fold or more (FAO, 2010). Moreover the global distribution of Nr is dominated by atmospheric transport (as NO_x and NH_3) and subsequent deposition (as NO_y and NH_x); from 1860 to 1995 this pool increased from c. 34 Tg N per year to 100 Tg N per year (Figure 1.3).

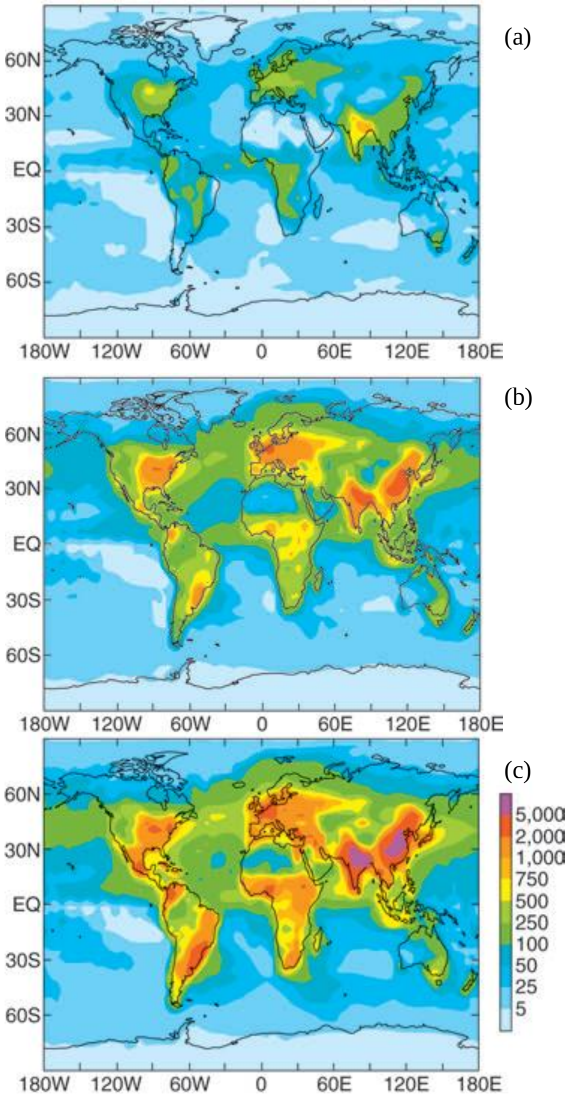


Figure 1.3: Estimated Total Inorganic Nitrogen Deposition, Wet and Dry, in 1860 (a), early 1990s (b), and projected for 2050 (c) (mg N m^{-2} yr^{-1} meter per year) (Galloway, 2004)

However, commercial trade of N-containing goods also plays a role in global Nr distributions. The efficient transport of NO_x and NH_3 result in increased deposition of N of up to 10 kg N ha^{-1} per year in some places, exceeding critical loads and causing detrimental impacts on the receiving ecosystems. Although even relatively small atmospheric N deposition to pristine environments c. 0.5 kg N ha^{-1} per year or less can cause adverse effects (Galloway *et al.,* 2008). In areas of intensive agriculture and high synthetic fertiliser use high rates of N deposition occur, such as in Western Europe (Millennium Ecosystem Assessment, 2005).

1.2 The problem of 'N pollution'

N pollution is complex and difficult to mitigate compared with other forms of pollution because of its high mobility through all sectors of the biosphere: atmospheric, aquatic/marine and terrestrial (Galloway *et al.,* 2003). Moreover, each N atom can be converted to any other form of N and can therefore 'cascade' through the environment causing multiple problems: ecological, environmental and human health effects (Vitousek *et al.,* 1997; Rabalais, 2001; Galloway *et al.,* 2003, Townsend *et al.,* 2003). A measure of the impact of human activities on the N cycle can be seen through the N flux in rivers; in Southwestern Europe N fluxes in rivers to coastal oceans have increased c. 3.7-fold since the industrial/agricultural revolutions (Howarth *et al.,* 1996). Agriculture dominates the N inputs into the Baltic and North Seas (Howarth *et al.,* 1996). However, human wastewater flows are estimated to contribute to 25% of the riverine N flux in Western Europe (Howarth *et al.,* 1996). The net anthropogenic input of Nr throughout Europe is 3700 kg N km^2 yr^{-1}: 5 times the background of natural fixation (Durand *et al.,* 2011: Voss *et al.,* 2011).

In Western Europe N pollution is generally increasing, as it has since the 1950s (Howarth *et al.,* 1996). The total flux of nitrogen discharge into European coastal waters has been estimated at 4760 kton N yr^{-1}: 22 % of anthropogenic inputs to European watersheds (Sutton *et al.,* 2011) (Figure 1.4). Non-point source or diffuse pollution is a more complex issue, due to the multiple sources, that can travel over long distances and involve chemical transformations as these compounds move through different media - atmosphere, terrestrial and aquatic. Moreover this type of pollution is extremely difficult to monitor or treat at the source; therefore mitigation measures are employed to reduce the contaminant load either before it reaches water bodies (such as improved management practices or introduction of buffer strips) or after it reaches water bodies (such as instream denitrifying wetlands) (Kay *et al.*, 2009; Dawson and Smith, 2010; Novak and Fiorelli, 2010).

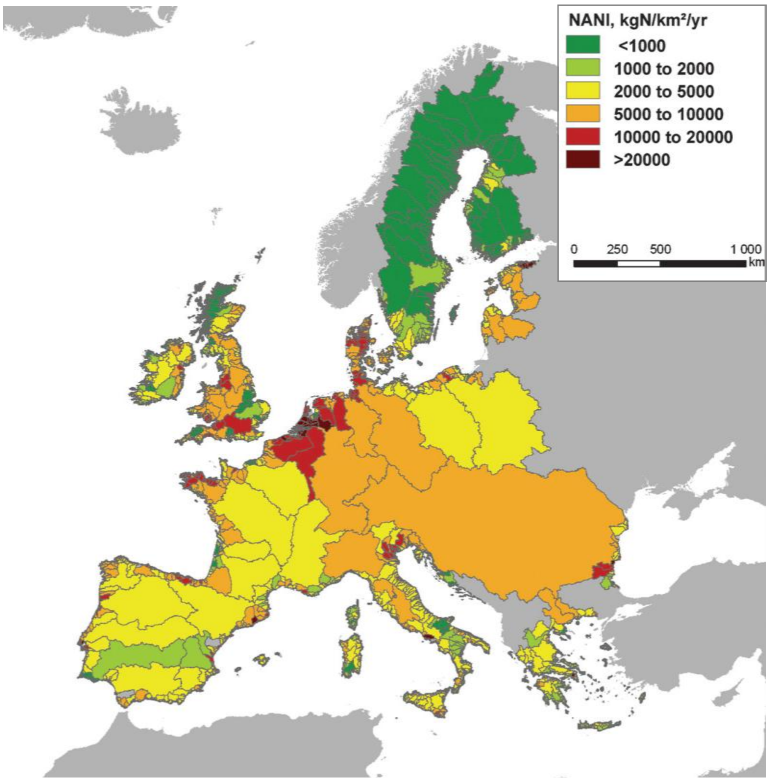


Figure 1.4: The basin averaged net anthropogenic nitrogen inputs to European watersheds (Leip *et al.*, 2011)

N excesses occur when the fertiliser applied exceeds the need of the crop and is therefore available for leaching to surface or groundwaters (Howarth *et al.*, 2002). Variability in leaching from fields exists, with greatest nutrient losses in areas of high rainfall, particularly in wet years (Cuttle and Scholefield, 1995; Jarvis, 2000). Improved management techniques to include reductions in fertiliser use or controlling the timing and amount of fertilisation to match the needs of the crops would most likely reduce the pool of Nr available for leaching, (Kay *et al.*, 2009)

Mitsch and Jørgensen, (2004) argue that riparian zones or artificial wetlands could reduce the flux of NO_3^--N to surface waters by intercepting leached N, whilst Peterson *et al.*, (2001) suggest that small natural streams can also be extremely effective sinks. However, both these interceptors rely primarily on the process of denitrification; which can produce the harmless N_2 gas or the long-lived greenhouse gas N_2O (Vitousek *et al*, 1997). Therefore there is a need to design wetlands that minimise the production of N_2O (Howarth *et al.*, 2002). Composting animal wastes increases ammonia volatilisation and subsequent deposition. Riverine transport of dissolved inorganic nitrogen (DIN) has increased substantially from c. 2-3 million tons per year in the pre-industrial period to 15 million tons today, with order-of-magnitude increases in drainage basins that are heavily populated or supporting extensive industrial agriculture (Vorosmarthy *et al.,*, 2005). Aquatic ecosystems are natural filters, removing on average approximately 80% of the N load. However, this value varies widely (Howarth *et al.*, 1996; Seitzinger *et al.*, 2002; Galloway, 2004).

Diffuse pollution is largely comprised of suspended solids, nutrients, faecal pathogens and toxic compounds, dependent on the catchment scale land use, geologic and geographic conditions. This research presented in this thesis focuses on diffuse NO_3^- pollution from agriculture: where land use and management, livestock management, atmospheric deposition, sheet runoff or seepage from farmyard hard standings and fields as well as minor or intermittent point sources from field drains all contribute to the load of diffuse pollution reaching water bodies. The diffuse nature of this type of pollution leads to highly temporal changes in the severity of the pollution, usually linked to meteorological events (Campbell *et al.*, 2004). As a result of diffuse NO_3^- pollution, potable water courses can be contaminated, leading to high costs of treatment prior to human consumption (Pretty *et al.*, 2002) (see Section 1.8 for a fuller discussion on costs). NO_3^- influx to N limited environments has the potential to cause eutrophication or nutrient enrichment of water bodies, most visibly by stimulating algal growth but also by reducing biodiversity or numbers of aquatic organisms (Dodds *et al.*, 2009; de Klein and Monaghan, 2011; Sutton *et al.*, 2011).This issue of eutrophication will be discussed more fully in section 1.3.1.

NO_3^- pollution has been defined as water bodies that exceed or have the potential to exceed the drinking water quality standard (WQS) of 50 mg NO_3^- per litre (World Health Organisation, 2011). It is important to note that water bodies can be polluted without exceeding this WQS, as particularly for N limiting environments, even a seemingly small increase in available N can affect the biological integrity of a water body by affecting the composition, numbers or well-being of aquatic life. N losses associated with farming practises are varied in time and space as a result of seasonal farming activities, farming type (arable or pastoral and species variations) in addition to the effects of climate, soil type and hydrological conditions (McNiell *et al.,* 2005). Leaching losses are affected by the quality and quantity of N inputs as well as the type (organic or inorganic), timing and composition, in relation to the efficiency of use of these inputs by plants (McNiell *et al.,* 2005). N applications in excess of plant demand leads to greater N leaching, with greater leaching generally occurring during the non-growing season, after fertiliser or slurry applications and from intensively managed grasslands (Cuttle and Scholefield, 1995). Furthermore leaching losses associated with dung and urine from grazing animals can be high, particularly from urine as urine-N is rapidly hydrolysed to inorganic-N forms (NH_4^+ and NO_3^-) whereas dung contains more organic N forms that are less rapidly mineralised (Cuttle and Scholefield, 1995).

To tackle diffuse pollution both the root causes as well as the symptoms need to be addressed. For diffuse pollution, best management practices (BMPs) are the regulatory and practical means of controlling water quality and encompass two broad categories: procedures and structures (Campbell *et al.,* 2004). BMPs are the methods, measures and practices to manage diffuse pollution to achieve an expected level of control over the pollution (Novotny and Olem, 1994). BMPs cover a wide range of measures such as education of the problem and solutions available, practices, technologies, processes, setting criteria, operating methods or other alternatives. Nevertheless, all these measures need to be economically achievable, effective and sustainable in reducing diffuse pollution (D'Arcy *et al.,* 2000). Diffuse pollution can be tackled at the source (i.e. fertiliser and manure applications/loadings, stocking densities) and secondly by preventing the delivery of the NO_3^- to the aqueous environment (i.e. through the implementation of riparian buffer zones or denitrifying wetlands to intercept and attenuate diffuse pollution) (Kay *et al.,* 2009; Dawson and Smith, 2010; Novak and Firoelli, 2010).

1.3 Environmental impacts of N enrichment

Diffuse N pollution from agriculture is a consequence of increased synthetic fertiliser and manure applications that exceed plant N demands combined with poor farming practices.

NO_3^- losses occur post-harvest as soil microbes' breakdown these organic residues into inorganic forms of reactive N that are washed out of soils and into the aquatic environment or released into the atmosphere (Cuttle and Scholefield, 1995; McNeill *et al.*, 2005). Moreover areas adjacent to intensive livestock farm areas can be subject to high rates of atmospheric deposition of NH_3 (Erisman *et al.,* 2007).

1.3.1 Eutrophication

In regions where nutrients (both N and phosphate (P)) flow in excess from terrestrial to aquatic environments an enrichment or eutrophication occurs. P is regarded as the limiting nutrient in aquatic environments, however N can be co-limiting and therefore the control of both N and P are required to prevent eutrophication as well as restore affected areas (Voss *et al.,* 2011; Durand *et al.,* 2011). Degradation of aquatic ecosystems occurs when a threshold of nutrient loading is exceeded, resulting in eutrophication and hypoxia (OSPAR, 2001; Ferreira *et al.,* 2007). The intensification of agriculture and the increased use of fertilisers have greatly increased the supply of nutrients from the terrestrial to the aquatic environment (both P and N). The resultant eutrophication produces undesirable ecological changes: reduced biodiversity and loss of habitat, as well as increased toxic algal blooms and pathogens which are harmful to all forms of life, including human health (Ferreira *et al.,* 2007).

Nitrogen (N) is essential to living organisms and its availability plays a crucial role in the organisation and functioning of all ecosystems (Vitousek *et al.*, 1997). Too little and systems are stressed but too much results in accelerated growth and eutrophic environments (Galloway, 1998). N is lost from agriculture as NO_3^- primarily through agricultural runoff and as NH_4^+ by volatilisation from the soil. Diffuse N pollution has been identified as one of the most important causal factors of eutrophication in coastal and estuarine environments where N is the limiting nutrient for primary production (Howarth *et al*., 2000). Eutrophication is a global problem than creates many hypoxic bottom waters in coastal environments (European Environment Agency, 2001; Ferreira *et al.*, 2011). Sources of nutrient enrichment to coastal locations across the world are the result of increased human populations, high population densities in coastal locations, the intensification of agriculture and point sources pollution from industry (Rabalais, 2001).

The enhanced supply of nutrients is manifest in the prolific growth of algae, called ‘algal blooms’ (Ferreira *et al.,* 2011). Microbial decomposition of algae and faecal pellets from grazing zooplankton creates a high oxygen demand that often results in bottom water hypoxia, where dissolved oxygen concentrations drop to 2 mg l^{-1} or less (Neretin, 2006). These hypoxic zones are often called ‘dead zones’ as the low oxygen environment is very stressful to marine life. Mobile marine species can move away to more oxygenated waters, but non-mobile species may die due to the lack of oxygen, resulting in a reduced biomass and benthic diversity, ultimately altering species composition and affecting the structure and food web of the whole ecosystem. Some algal blooms release harmful toxins that may poison fish or humans and as a result cause lost revenue to the commercial fishing industry and the closure of recreational waters (Anderson *et al.,* 2000; HELCOM, 2010).

1.3.2 Human health

Historically NO_3^- in drinking water was thought to cause methaemoglobinaemia or ‘blue-baby’ syndrome in infants. This anaemic condition was thought to occur when ingested NO_3^- was reduced to NO_2^- in an infant’s stomach by microbes, then NO_2^- in the bloodstream converted haemoglobin to methaemoglobin, reducing its capacity to carry oxygen and ultimately causing brain damage or infant death (Wolfe and Patz, 2002). This view is now challenged by medical researchers (Benjamin *et al*., 1994: Dykhuizen *et al*., 1996; Benjamin *et al*., 1997) who suggest that NO_3^- is beneficial to human health and that methaemoglobinaemia is caused by bacteria. Nevertheless, nitrate and nitrites are sited as risk factors for methaemoglobinaemia in infants by the World Health Organisation (WHO), and as such ‘safe’ limits for nitrate or nitrite in potable water sources have been set at not exceeding 50 mg NO_3^- L^{-1} and 3 mg NO_2^- L^{-1}, respectively (World Health Organisation, 2011).

Benjamin et al. (1994), (1997 and Benjamin, 2000) suggested that NO_3^- undergoes a series of reactions in the human body that ultimately makes it an important antimicrobial agent. The human body concentrates NO_3^- in saliva, up to ten times the concentration in blood plasma, where it is rapidly reduced to NO_2^- by bacteria in the mouth, followed by acidification to nitrous acid on entering the stomach (Benjamin, 2000). Nitrous acid is an effective antimicrobial agent that has been shown to be effective at eliminating the organisms responsible for gastroenteritis in humans at typical human stomach concentrations (Benjamin, 1994; Dykhuizen *et al.*, 1996; Benjamin, 2000). Studies have found little evidence to implicate NO_3^- as the prime cause of methaemoglobinaemia; rather, it is bacterial pollution of drinking water that is most likely responsible, with enhanced nitric acid production as a defensive reaction against bacterial gastroenteritis (Avery, 1999; L'hirondel and L'hirondel, 2001).

Other studies of the behaviour of NO_3^- in the human body have led to concerns over the possible link between NO_3^- and gastric cancer through the process of nitrosation (Al-Sa'doni and Ferro, 2000). Many N compounds are effective nitrosating agents (nitric oxide donors) including nitrous acid. Nitric oxide is a free radical and very reactive. The addition of a nitric oxide group to another compound is 'nitrosation' to produce nitroso compounds, two of which are of medical interest N-nitrosamines and S-nitrosothiols. N-nitrosamines are carcinogenic and are cited in the link between NO_3^- and gastric cancer in humans, although no causal link has been proven (Pretty *et al.*, 2000, Al-Sa'doni and Ferro, 2000). S-nitrosothiols are effective nitrosating agents that have therapeutic properties and are used in the treatment of cardiovascular disorders (Al-Sa'doni and Ferro, 2000).

Despite all the advancements in knowledge of NO_3^- behaviour in the human body there still remains a great deal of uncertainty concerning its potential benefits or hazards. Until medical professionals can prove the benefits of NO_3^- to human health, UK water companies must continue to spend large amounts of money every year to meet the water quality standard.

1.4 Managing N in the agro-ecosystem: the UK perspective

Western Europe has for decades been a hotspot of fluvial N flux with UK rivers presenting some of the highest NO_3^--N concentrations of the region (Worrall *et al.*, 2012). Total N inputs in the EU agriculture for 2000 were estimated as 2.0, 0.8, 11.3 and 10.4 billion kg for atmospheric deposition, biological N_2 fixation, N fertiliser and N excretion (Oenema *et al.*, 2009). Less than half the total N input by fertilisers and manure in crop production is effectively utilised (Galloway *et al.*, 2003; Galloway *et al.*, 2008). Moreover, for the period 2004-2007, 15% of EU 27 drinking water monitoring stations exceeded the NO_3^- standard of

50 mg per litre, with concentrations in a further 6%, including in central England, ranging between 40 and 50 mg per litre (World Health Organisation, 2011).

The UK has approximately 16.9 Mha of land utilised for agriculture and the nitrogen use efficiency (NUE) is c. 48% (Oenema *et al.*, 2009). The poor use efficiency combined with the high N loadings result in surplus N within the terrestrial environment. The high mobility of reactive N species means that this surplus flows easily into the aquatic environment, resulting in the aforementioned human health and environmental impacts (Section 1.3). Several European Union (EU) directives and UK policies address N losses from agriculture. Initially NO_3^- concentrations were controlled in potable water sources through the EU Drinking Water Directive first ratified in 1980 and in its current revised form from 1998 (98/83/EC). However, more recently the EU Nitrates Directive and Water Framework Directives target NO_3^- in all surface and ground water bodies at the risk of exceeding the water quality standard of 50 mg NO_3^- L^{-1}.

1.4.1 The EU Nitrates Directive

The control of NO_3^- pollution from agriculture is addressed directly in the Nitrates Directive (91/676/EC) which aims to not only control and/or reduce water pollution at the source and also to improve water quality by the implementation of Good Farming Practices. The Directive requires member states to identify and monitor both the NO_3^- concentrations and trophic state of each water body. The purpose is not only to protect human health from high NO_3^- concentrations but also to prevent eutrophication and the subsequent effects on aquatic life, thereby expanding the definition of controlled water bodies to all surface and groundwaters, not just potable water sources.

The UK has implemented the Nitrates Directive in discrete Nitrate Vulnerable Zones (NVZs) for all farmland located in regions where the concentration of nitrate in surface or groundwaters exceeds the water quality standard or has the potential to exceed this limit. Currently the UK has designated as NVZs approximately 62%, 4% and 14.2% of the land areas of England, Wales and Scotland, respectively (EA, 2011a and 2011b) (Figure 1.5). The whole of Northern Ireland has been declared a NVZ under the Nitrates Directive, despite 99% of sampled water bodies having < 20 mg NO_3^- per litre; designation in this region was due to significant proportions of NO_3^- in freshwater originating from agricultural sources and the high instances of eutrophication in many freshwater rivers and lakes in the region (NIEA, 2009).

Within NVZs, Action Programmes to promote Codes of Good Agricultural Practice must be adhered to. For farmland situated outside NVZs, farmers are encouraged to implement these actions on a voluntary basis. Codes of Good Agricultural Practice include the timings and amounts of fertiliser applications and closed periods, as well as limiting the conditions of fertiliser application to reduce the potential for leaching (NIEA, 2006; The Scottish Government, 2011). Moreover, livestock manure storage conditions and capacity are defined and all farmers must keep detailed records spanning five years on cropping, livestock numbers, fertiliser/manure usage and storage. These records will be inspected annually by the enforcing body for each region.

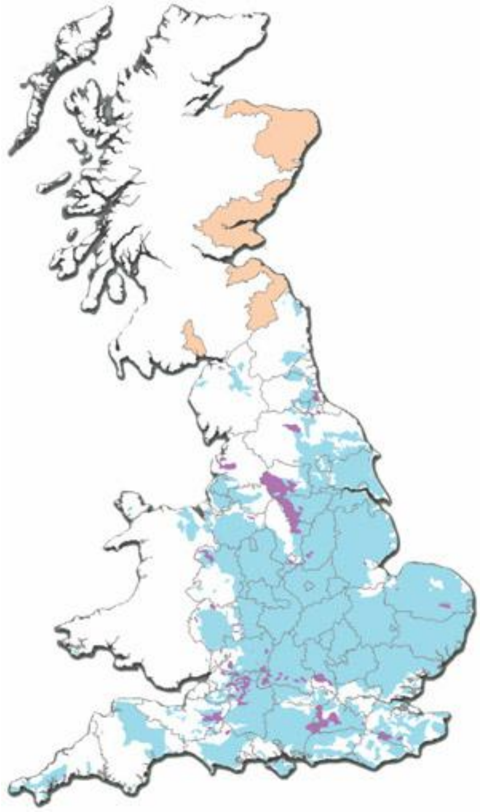


Figure 1.5: NVZs within Great Britain. The purple shaded areas are those with deferred slurry storage areas until 2013 (Courtesy of The Agricultural Mortgage Corporation plc, 2011).

The regulations that implement the Nitrates Directive in the UK are known as the Nitrate Pollution Prevention Regulations 2008 (as amended) in England and came into force on 1st January 2009, with some parts being phased in over subsequent years (EA, 2011c). Similar regulations exist within each region of the UK tailored to the specific problems associated within each region; i.e. in Northern Ireland the NVZs are targeting eutrophication of fresh and marine waters, whereas NVZs in Scotland are largely for groundwaters (NIEA, 2006 and The Scottish Government, 2008).

1.4.2 The EU Water Framework Directive

The Water Framework Directive (WFD) (2000/60/EC) is the operational tool that sets the objectives for surface and groundwater protection. However, the WFD utilises existing regulations and directives to achieve its targets, including the Nitrates Directive. It aims to harmonise the approach to pollution control and streamlining legislation, and it will eventually replace the Nitrates Directive. It is an innovative and ambitious piece of EU legislation that uses a combined approach of source controls that utilise the available technology as well as setting quality standards to form a management framework for all European surface and ground waters. It adopts a risk-based approach in developing a list of priority substances for action at EU level.

The principal aim of the WFD is to protect, enhance and restore all surface and groundwater bodies and aim to achieve ‘good ecological and chemical status’ by 2015 (Defra, 2006). The WFD extends to at least 1 nautical mile beyond the coastal baseline for biological quality elements and to 12 nautical miles for the physiochemical quality elements that include nutrient pollution (Tett *et al.*, 2003). Management plans are to be at the River Basin level and therefore cross administrative and political boundaries. In addition, European governments must work together to reduce pollution to coastal environments.

In the UK the transposition and implementation of the WFD was devolved to each of the constituent countries: England, Scotland, Wales and Northern Ireland, with their respective Environment Agencies undertaking the implementation and monitoring of this directive through River Basin Management Plans set at the River Basin level (NIEA, 2009; SEPA, 2009 and EA, 2011d). The WFD UK TAG organisation provides technical assistance to the

UK and Ireland for consistent implementation of the WFD and where necessary developing a coordinated approach for river basins crossing national boundaries (UKTAG, 2011).

In the construction of the River Basin Management Plan (RBMP), an assessment of ecological, quantitative and chemical status, along with any protected area objectives, must be considered, with the costs transferred to consumers through either drinking water costs or wastewater charges. This aims to promote sustainable use of water and reflect the true costs of abstraction, distribution and treatment of potable water. Public participation will ensure greater transparency of the quality standards set and drinking water costs applied in addition to contributing to RBMPs. The first reviews on the success of the RBMP (2009 to 2012), are expected in 2012; despite the WFD setting the target of achieving ‘good ecological and chemical status’ by 2015 it is recognised that this may take considerably longer to be achieved within the UK, potentially decades (SEPA, 2009; EA, 2011e).

Northern Ireland, being designated as a total territory under the Nitrates Directive, gives an example of how the Nitrates Directive is being used to help the country comply with the demands of the Water Framework Directive (NIEA, 2009) and is in line with the European Commission’s guideline that NVZs should be designated when agriculture is shown to contribute to a least 20% of the total NO_3 loadings (Jordan and Smith, 2005).

1.4.3 Financial incentives for farmers

In 2005 the CAP reform introduced the Single Payment Scheme (SPS) - a new grant scheme that aims to decouple grant payments from production and reward farmers that adopt management practices to maintain land in “Good Agricultural and Environmental Condition” (GAEC), and comply with Statutory Management Requirements (SMRs) (i.e. the Nitrates, Groundwater and Sewage Sludge Directives), collectively known as “Cross Compliance” (EA, 2006; Defra, 2005; Netregs, 2011). The implementation of buffer strips between 2 and 6 m wide; or even 12 m wide, adjacent to water courses is promoted by all agri-environmental schemes within the different countries of the UK and forms part of the way farmers receive financial aid via the SPS. To meet the cross-compliance conditions in England all farmers must establish a 2-m wide buffer strip along the edge of rivers and streams on their land (EA, 2006).

Farmer compliance with the action programme measures is ensured through farm visits and inspection of farm records. The Nitrates Directive is one of the SMRs which farmers are required to comply with as a condition of receiving the Single Farm Payment (Scottish Executive, 2005a). The Single Payment Scheme (SPS) (EC Council Regulation 1782/2003) replaced most existing crop and livestock payments from January 2005. Within England three flat rates per hectare of farmland apply: €40.82, 233.95 and 289.94, dependent on farm and farming community type i.e. for upland severely disadvantaged areas (SDA) moorland, upland SDA other than moorland and non-SDA, respectively (RPA, 2011). Farmers who maintain their land in 'Good Agricultural and Environmental Condition' (GAEC) and comply with 'Statutory Management Requirements' (SMR's) are eligible to receive these payments as part of the SPS's cross-compliance (RPA, 2011).

The monitoring, enforcing and Cross Compliance are devolved regionally within the UK (Scottish Executive, 2005a; Scottish Executive, 2005b; DARD, 2010; Welsh Government, 2011; RPA, 2011). Cross Compliance allows farmers to apply for direct payment in the form of grants under the Common Agricultural Policy (CAP); these include the Single Payment Scheme (SPS) and Rural Development Schemes. Cross compliance reflects existing EU and UK law and includes a variety of requirements; the environment, animal welfare as well as human, plant and animal health. Failure to meet Cross Compliance standards would result in a financial penalty to the farmer based on the extent, intent, permanence, severity and repetition of the breach; multiple failures would result in cumulative penalties being applied, and this establishes a strong incentive to achieve the outlined standards across all areas of Cross Compliance.

1.4.4 Agri-environment schemes

There are many agri-environment schemes within the UK aimed at protecting natural resources, conserving wildlife, and maintaining or enhancing the landscape quality, as well as managing flood risk (NetRegs, 2011). The Catchment Sensitive Farming (CSF) programme in England is a proactive approach to tackling all forms of agricultural pollution to aquatic environments that encourages farmers to join voluntary schemes that aim to deliver simple yet effective environmental management, in return for farm payments. Farmers in priority catchments within England can apply for up to £8,000 per farm holding from the Capital Grant Scheme, part of CSF, to improve or install facilities that will reduce diffuse water pollution from agriculture. Different schemes exist in England, Scotland, Northern Ireland and Wales, as part of each country's "Rural Development Programme" running from 2007 - 2013; but they all aim to financially reward farmers or land managers for reducing pollution and improving the environment. Within England the major agri-environment scheme is Environmental Stewardship that rewards good environmental management (Natural England, 2011).

Environmental Stewardship

Environmental Stewardship is open to all farmers and is divided into three main categories: Entry Level Stewardship (ELS), which also includes an Uplands Entry Level Stewardship (Uplands ELS); secondly an Organic Entry Level Stewardship (OELS); and thirdly Higher Level Stewardships (HLS). Agreements are made on a voluntary basis between farmers or land owners for a five-year term (a 10-year or greater agreement exists for HLS schemes).

All farmers are eligible to join the ELS or OELS if they are not receiving Organic Farming Scheme aid, with each agreement lasting for five years (Natural England, 2011a). The scheme rewards management that goes beyond the SPS requirement of maintaining land in GAEC. Currently almost 60% of England agricultural land is in ELS. It is a 'whole farm' scheme that offers payments at the flat rate of £30 ha^{-1} yr^{-1} or £8 ha^{-1} yr^{-1} for land with 15 ha or more within Less Favoured Areas (LFAs) for ELS, £60 ha^{-1} yr^{-1} for OELS, with organic conversion aid payments of £175 per hectare for the first two years or £600 ha^{-1} yr^{-1} for fruit orchards for the first three years (Cuttle *et al*., 2007; Natural England, 2010a). Upland ELS receive the standard payment rate of £62 ha^{-1} yr^{-1} or £23 ha^{-1} yr^{-1} for land with 15 ha or more above the Moorland line with Upland OELS receiving £92 ha^{-1} yr^{-1}.

The ELS offers over 65 options under the scheme that include a two- to six-metre-wide buffer strip (either in-field or riparian) aimed at reducing nutrient leaching by slowing and filtering runoff, as well as trapping pollutants (Natural England, 2010a; Natural England, 2011). Buffer strip options within ELS must not overlap with the Cross Compliance requirement of not cultivating land within 2 m of a hedgerow or watercourse or 6 m from any watercourse on land covered by the Upland ELS compulsory requirement (Natural England, 2010a). Any ELS buffer strip option would then start where the uncultivated land ends.

Buffer strips can be field-edge, in-field and/or adjacent to watercourses; the width options are 2, 4 or 6 m in intensive grasslands or on cultivated land; a further option of a 12-24 m buffer strip for water courses on cultivated land is also available (Natural England, 2011). Restrictions on grazing and cutting of the buffer strip area, as well as other management criteria such as not applying, fertilisers, manures or herbicides. The buffer strips should also not be used for regular vehicle access in order to meet the full criteria of the ELS (Natural England, 2010a). Further financial rewards can be gained under the HLS options by enhancing buffer strips either floristically or by planting hedgerow trees (Natural England, 2010b).

Higher Level Stewardship (HLS) is available to farmers in high priority areas and includes more advanced options to tackle diffuse pollution such as the establishment of artificial wetlands (Cuttle *et al.,* 2007). Wetlands will be used to capture runoff and sediment from fields or farm hard-standings, intercept pollutant delivery, reduce nitrate leaching, provide a buffering zone and potentially clean up polluted water. There are a variety of wetland options available under the HLS: ponds (< or > than 100 m^2), reed beds, fens, lowland raised bogs, coastal and flood plain grazing marsh; a management plan is normally required and a 20-year agreement may be offered for some wetland options (Natural England, 2010b). Environmental Stewardship schemes now cover nearly 70% of England agricultural land (5.5 million ha of land encompassed by 43,000 Environmental Stewardship agreements).

Although both buffer strips and wetlands have multiple benefits - not least a reduction in diffuse pollution and increased biodiversity and habitat for wildlife - there are potential drawbacks in the form of pollution swapping. The interception and reduction of diffuse NO_3^- pollution via denitrification may result in emissions of the greenhouse gas N_2O: swapping a water pollution problem for an air pollution problem (Cuttle *et al.,* 2007). Soils and fertiliser use are the source of two thirds of N_2O emissions in the UK (EA, 2006). The interception of nutrients by these methods can increase emissions of the greenhouse gases N_2O and CH_4 (Hefting *et al*., 2003). Although EU policy generally addresses NO_3^- pollution of water, little attention has been paid to the potential problem of ‘pollution swapping’ and its likely impact on climate change (Reay *et al*., 2003). All agri-environment schemes within the UK promote the use of buffer strips and wetlands with the added incentive of financial reward to farmers.

1.5 N processes in the biosphere

In a natural undisturbed environment plants obtain N from two sources: N fixation and or N mineralisation. In many regions of the world today applications of synthetic N, produced by the Haber-Bosch process, exceed those produced through natural fixation pathways. Inorganic forms of nitrogen are very reactive and mobile. Practically all N in the soil is organic N, the 1-2% of soil N that is inorganic or in ‘mineral’ form (NH_4^+-N or NO_3^--N), is the most available to plants but causes most of the environmental problems (Galloway, 2004). The effects of N on the environment are specific to individual compounds but any reactive N species can be converted to any other N species through the processes of nitrification (Equation 1.1) and denitrification (Equation 1.2), provided the environmental conditions are appropriate (Galloway, 1998). Moreover, many of the reactions associated with the N transformations from one species to another require a C source, making the N and C cycles closely linked.

$$NH_3 \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^- \qquad \text{Equation 1.1}$$

$$2NO_3^- \Leftrightarrow 2NO_2^- \Leftrightarrow 2NO \Leftrightarrow N_2O \Leftrightarrow N_2 \qquad \text{Equation 1.2}$$

Soil microbes recycle plant material and release nutrients into the soil, gaining energy in the process. However, this natural process of decomposition can become a problem when the soil is left bare for part of the year without an overlying protective vegetated cover, resulting in nutrient leaching (Cuttle and Scholefield, 1995; McNeill *et al.,* 2005).

1.5.1 Inorganic N species

N mineralization is the breakdown of organic N by bacteria or fungi into inorganic, plant usable forms of N such as NH_4^+ or NO_3^- carried out by soil microorganisms (Killham, 1994). The reverse reaction is known as immobilisation and is carried out by soil microorganisms as well as soil fauna and plants. Both reactions occur simultaneously and the amount of Nr available in the soil pool is determined by the rate of these reactions (Killham, 1994). NO_3^- and NO_2 are oxidised forms of N and are readily soluble in water; however NO_2^- is relatively unstable and is therefore commonly not found in high concentrations. NO_3^- is commonly found in soil and is one of the main forms used by plants to obtain N (McNeill *et al.,* 2005). Additionally NO_3^- is the form of inorganic N that is lost via leaching as it is highly soluble and therefore mobile within soils (Cuttle and Scholefield, 2005; McNeill *et al.,* 2005). NO_3^- is also responsible for causing eutrophication in aquatic environments when levels are in excess of requirements (Durand *et al.,* 2011).

NH_4^+ ions are formed from organic nitrogen during the breakdown of organic material. NH_4^+ is also used by plants as an N source and is commonly added directly to land as synthetic fertiliser (in the form of ammonium salts or as urea, which rapidly hydrolyses to NH_4^+) (Di and Cameron, 2002; Nieder and Benbi, 2008). Volatilisation of NH_3 from NH_4^+ salts and urea causes significant losses to the atmosphere, and when re-deposited it can cause acidification of soils and water, and in systems naturally low in N can cause a loss of bio-diversity (van Vuuren *et al.,* 2011). Excessive concentrations of NH_4^+ in aquatic environments can kill fish (Alonso and Carmargo, 2003).

1.5.2 N fixation

The conversion or 'fixing' of atmospheric N_2 gas to biologically available forms that contain C, H or O, by autotrophic and heterotrophic bacteria (diazotrophs), some plants and cyanobacteria, is known as Biological N Fixation (BNF) (Equation 1.3) (Erisman *et al.,* 2011). This process of can occur symbiotically or symbiotically.

$$2N_2 + 3H_2 \rightarrow 2NH_3 \qquad \textit{Equation 1.3}$$

Free-living N_2 fixing bacteria (non-symbiotic bacteria) require an energy source either chemical or photosynthetic to convert N_2 in to biologically available forms. This pathway of N_2 fixation is thought to contribute little (< 5 kg N ha^{-1} yr^{-1}) to N_2 fixation in agricultural systems (Nieder and Benbi, 2008). Diazotrophs (symbiotic bacteria) capable of forming a symbiotic relationship with plants by living in the rhizosphere or within the plants themselves; usually as nodules mainly on the roots, gain their energy from the plants in return for supplying fixed N in the form of NH_4^+; examples include clover and soybean (Nieder and Benbi, 2008). Symbiotic N_2 fixation is an important integral part of modern agriculture to increase yields however the rate of fixation depends on bacteria species, temperature, pH and soil mineral content (Nieder and Benbi, 2008

Total global N_2 fixation on land is difficult to estimate and large regional differences will occur. However, Galloway, (2004) estimated it at c. 140 Tg N yr^{-1}. A secondary pathway of N fixation occurs in the atmosphere, where N_2 and O_2 ionised by lightning combine to form NO; globally this accounts for c. 3-5.4 Tg N yr^{-1} (Nieder and Benbi, 2008). NO is subsequently oxidised to NO_2 and then HNO_3 which can fall as either dry or wet deposition.

1.5.3 Ammonification

Readily decomposable organic N compounds such as proteins and nucleic acids are converted by heterotrophic bacteria and fungi into ammonium (NH_4^+) or ammonia (NH_3), via the process of ammonification (Equation 1.4). The optimum pH for ammonification ranges from pH 6.0 to 8.0 (Aciego Pietri and Brookes, 2008).

$$Organic\ N \rightarrow NH_4^+ + OH^- \qquad \text{Equation 1.4}$$

NH_3 is a gas released by NH_4^+ volatisation of manure. NH_4^+ is retained in the soil where it can follow several pathways; oxidation to NO_2^- or NO_3^-, held as an exchangeable cation on negatively charged particles such as clay, or assimilated by plants (immobilised). NH_4^+ is in equilibrium with NH_3, therefore high application rates of NH_4^+ containing fertilisers will result in increased NH_4^+ volatilisation to NH_3 gas. Ammonification (also known as mineralisation) is always coupled with immobilisation; the reverse reaction where microbes, soil fauna or plants assimilate inorganic-N forms and transform them to organic N forms (Schlesinger, 1997; Nieder and Benbi, 2008).

The uptake of NH_4^+ can be restricted by the present of negatively charged soil particles (such as clay), due to the charge attraction between them: this may lead to the preferential build-up of NH_4^+ in soils and/or the preferential usage of NO_3^- by plants (Killham, 1994). Plants will release acidic (NO_3^-) or alkali (NH_4^+) substances to maintain a charge balance within soils:

therefore immobilisation can have an important effect on soil pH (Killham, 1994: Schlesinger, 1997).

1.5.4 Nitrification

NH_4^+ is readily oxidised to NO_3^- via the process of nitrification (equation 3) by free-living or symbiotic N-fixing bacteria. Chemoautotrophic bacteria oxidise NH_4^+ in two stages. Both stages produce energy and the bacteria use CO_2 as a carbon source (Wrage *et al.,* 2001). Firstly *Nitrosomonas* genera convert NH_4^+ to nitrite (NO_2^-) (Equation 1.5) (Mendum *et al.*, 1999; Killham, 1994; Nieder and Benbi, 2008).

$$2NH_4^+ + 2O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O + energy \qquad \text{Equation 1.5}$$

Secondly *Nitrobacter* genera convert NO_2^- to NO_3^- (Equation 1.6).

$$2NO_2^- + O_2 \rightarrow 2NO_3^- + energy \qquad \text{Equation 1.6}$$

Nitrification facilitated by fungi is less well understood and is known as heterotrophic nitrification; this process can nitrify organic N without it first being mineralised into NH_4^+. The products of this type of nitrification are the same as autotrophic nitrification; however, the C source is organic C rather than CO_2 (Wrage *et al.,* 2001).

Nitrification is inhibited by low temperature, low pH and poorly oxygenated soils. Under low oxygen conditions the intermediate products of nitrification, NO or N_2O, can be produced rather than NO_3^- (Wrage *et al.,* 2001). Within soils O_2 supply is moderated by soil water content, water logged soils could therefore inhibit nitrification due to a lack of O_2 (Nieder and Benbi, 2008). Within wetlands nitrification is largely confined to the water column or a narrow oxygenated sediment layer at the sediment water interface (Nieder and Benbi, 2008). Nitrification is low a low temperatures with *Nitrobacter* more sensitive than *Nitrosomonas*; therefore potentially leading to a build-up of NO_2^- in the soil (Nieder and Benbi, 2008). Likewise *Nitrobacter* is more sensitive to changes in pH resulting in NO_2^- build-up in alkaline soils (Nieder and Benbi, 2008). The optimum range of pH for nitrification is between 7-8 (Nieder and Benbi, 2008).

1.5.5 Denitrification

In anaerobic conditions or oxygen-limited soils NO_3^- (or NO_2^-) are the substrates used as an electron acceptor in a series of redox reactions known as denitrification carried out by a large variety of bacteria (facultative anaerobes or heterotrophs) and catalysed at each step by a variety of enzymes (Equation 1.7). During this process the nitrogenous oxides are reduced and organic carbon is oxidised (Nieder and Benbi, 2008).

$$NO_3^- \xrightarrow{\text{nitrate reductase}} NO_2^- \xrightarrow{\text{nitrite reductase}} NO \xrightarrow{\text{nitric oxide reductase}} N_2O \xrightarrow{\text{nitrous oxide reductase}} N_2$$

Equation 1.7

Denitrification requires low oxygen levels as well as both an N and C source (Sánchez-Martín *et al.,* 2008). Under anaerobic conditions complete denitrification to N_2 gas occurs (Equation 1.8), effectively removing N from further interactions with the environment.

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O \qquad \text{Equation 1.8}$$

This denitrification process ultimately produces the unreactive N_2 gas through a series of reactive intermediaries ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$). However, for the denitrification process to progress through each stage, the prevalent conditions need to be optimal. The prevailing conditions that favour denitrification; i.e. the presence of low or no oxygen; is commonly achieved by high soil water contents (Nieder and Benbi, 2008). This in turn limits diffusion of the reaction products and favours their consumption by denitrifiers rather than release to the atmosphere (Davidson, 1991). Further adding to the complexity of N cycling is that the enzyme nitrous oxide reductase is highly sensitive to both O_2 and pH, at low pH, and in the presence of O_2 it is inhibited, resulting in N_2O as the final denitrification product under these conditions and its subsequent release into the atmosphere (Wrage *et al.,* 2001), (Equation 1.9).

$$4(CH_2O) + 4NO_3^- \rightarrow 4HCO_3^- + 2N_2O + 2H_2O \qquad \textit{Equation 1.9}$$

Denitrification, just like nitrification, requires a C source (organic C), illustrating the tight coupling of both the C and N cycles (Sánchez-Martín *et al.*, 2008). Moreover, the relative importance of these two processes can affect soil pH. When denitrification is dominant, for each NO_3^- molecule that is reduced one proton is consumed, resulting in an increase of pH. During nitrification two protons are produced for every NO_3^- molecule formed, resulting in decreased pH (Hefting *et al*, 2006).

1.5.6 Nitrifier-denitrification

Autotrophic NH_3-oxidisers carry out the process of nitrifier-denitrification in soils (Wrage *et al.*, 2001). The process involves the oxidation of NH_3 to NO_2, followed by the reduction of NO_2, to N_2O to N_2 without the production of NO_3 (Equation 1.10) (Wrage *et al.*, 2004).

$$NH_3 \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \textit{Equation 1.10}$$

Potential soil conditions where this pathway may be favoured over nitrification or denitrification are low oxygen and low organic matter, high NO_3^- concentrations and warm temperatures (Wrage *et al.*, 2001; Nieder and Benbi, 2008). This process requires a carbon source in the form of CO_2. Research suggests this process could contribute as much as 30% of the total N_2O production from soils (Wrage *et al.*, 2001).

All major brands of nitrogenous fertilisers contain either NH_4^+ or NO_3^- or both, with the exception of urea, and therefore supply available substrate for nitrification, nitrifier-denitrification and denitrification. The high heterogeneity of soils allows for these processes to occur simultaneously almost side-by-side, creating a very complex situation for environmental monitoring and remediation of pollution. Moreover each of the processes described require C either in the form of organic C or CO_2, illustrating the tight coupling of both the C and N cycle, and therefore large perturbations in one cycle would suggest likewise for the other.

1.6 Mitigation of diffuse N pollution: Wetlands and Buffer Zones/Strips

Riparian areas represent the transitional area between terrestrial and aquatic environments and as a result play an important role in intercepting diffuse pollution from agricultural run-off (Jordan *et al*., 2000; Sabater *et al*., 2003; Hefting *et al*., 2003). Field-edge buffer strips and natural or constructed wetlands are two remediation strategies that have great potential for mitigating diffuse NO_3^- pollution whilst improving water quality and enhancing biodiversity (Jordan *et al*., 2000; Mitsch *et al*., 2005).

In buffer strips lateral movement in the form of surface runoff or shallow groundwater flow in agricultural regions is typically high in N, P and C. (DeSimone *et al.,* 2010). The main processes of NO_3^- reduction in buffer strips are denitrification, microbial immobilisation and plant uptake, which operate under a variety of climatic, hydrological and environmental conditions (Sabater *et al*., 2003; Hefting *et al.,* 2006). The removal methods can operate either simultaneously or alone and under a variety of different environmental conditions (Sabater *et al*., 2003). Therefore the relative removal efficiencies vary greatly both spatially and temporally. Denitrification permanently removes NO_3^- from the biosphere to the atmosphere as either N_2 or N_2O gases, whereas plant uptake only temporally removes NO_3^- (Hernandez and Mitsch, 2007). Extensive studies in riparian areas have found denitrification to be the principal removal mechanism for NO_3^- (Lowrance, 1992; Pinay *et al.*, 1993; Hanson *et al.,* 1994; Jordan *et al.,* 1998; Pinay *et al.*, 2000; Watts and Seitzinger, 2000).

Buffer strips have been shown to be useful remediation strategies against diffuse NO_3^- pollution (Lowrance *et al*., 1997; Jacinthe *et al*., 1998; Sabater *et al*., 2003; Mitsch *et al*., 2005); however, the degree of N retention in riparian areas differs widely between areas. Hefting *et al.* (2006) reported that a Dutch riparian grassland buffer strip receiving 15 g NO_3^--N m^{-2} yr^{-1} was able to remove c. 63% of the NO_3^- load. Other studies have found N retention by riparian buffer strips to range from between 50 and 90% to less than 30% (Lowrance *et al,*. 1997). Moreover, high seasonality in removal efficiencies exists, with greater removal of NO_3^- noted both for periods of high and low surface runoff (Burt *et al.*, 2002; Clement *et al.*, 2003; Maitre *et al.*, 2003). The large variation in N removal is in part due to the high spatial variability of denitrification associated with small scale variations in soil characteristics, NO_3^- or C concentrations and oxygenation state. However, the buffer strip width, the buffer area in relation to the field area, hydrology, soil/vegetation types as well as runoff conditions, all affect the removal efficiencies of buffer zones (Groffman *et al.*, 2000; Sabater *et al.*, 2003; Hefting *et al.*, 2006; Stevens and Quinton, 2009).

Wetlands likewise have received considerable attention as a way of intercepting diffuse pollution, trapping sediment, improving water quality, and increasing biodiversity and habitat (Stevens and Quinton, 2009; Montreuil *et al.*, 2011). There exist a variety of wetland types such as reed beds, ponds or marshes. Moreover wetland design varies widely and is site specific. The effectiveness of wetlands in intercepting diffuse NO_3^- pollution is highly variable, based on wetland type, vegetation, slope of wetland, hydraulic conductivity, retention time, water column depth, soil type, and temperature, as well as influent concentration of NO_3^- (Stevens and Quinton, 2009). Denitrification is the main pathway of NO_3^- removal in wetlands; however, additional NO_3^- removal also occurs through plant uptake or immobilisation (Poe *et al.*, 2003). The dependence of NO_3^- removal on microbial processes lends to the common observation of seasonality in NO_3^- removal, due to seasonal temperature changes and reduced rates of microbial processes at lower temperatures (Raisin and Mitchell, 1995; Braskerud, 2002). Stevens and Quinton (2009) found a worldwide average annual NO_3^- removal efficiency from constructed wetlands draining catchments containing combination crops of 26%.

The relative influence of N input, water residence time, soil-water interactions, vegetation type and the complex interactions of biogeochemical processes (both aerobic and anaerobic) all affect the removal capacity of NO_3^- (Lowrance *et al*., 1997; Jacinthe *et al*., 1998; Sabater *et al*., 2003). However general patterns can be observed, for example, increases in the soil nitrate concentration or low soil oxygen concentrations increase denitrification (Hefting *et al*., 2003). Average removal efficiencies of buffer strips have been reported as greater than 1.5 g NO_3^--N m^{-2} d^{-1} whereas wetlands can remove up to 3.5 g NO_3^--N m^{-2} d^{-1} (Mitsch *et al*., 2005).

Microbial processes (denitrification and nitrification) have the potential to produce the powerful greenhouse gas N_2O as either an end- or by-product, suggesting the potential for high indirect N_2O emissions during NO_3^- mitigation (Hefting *et al*., 2003). N_2O production depends on: soil NO_3^- concentration, temperature, organic matter availability, pH, oxygen availability and microbial population dynamics (van Cleemput, 1998). These controlling factors are highly variable over space and time and therefore so are N_2O emissions (Skiba and Smith, 2000). Generally denitrification is the dominant process of N_2O production from soils in wetter environments. Greater than 5% of the end-product of denitrification is nitrous oxide, compared to less than 1% of N_2O from nitrification (Lipschultz *et al*., 1981). However, under aerobic conditions nitrification can be the dominant process of N_2O production (Wrage *et al*., 2001).

Due to the high dependence of microbially mediated processes to remove the diffuse NO_3^- within buffer zones, hydrology plays an important role (Stevens and Quinton, 2009). Soil type, texture, depth and organic matter content also affect the water movement and denitrifying capacity of buffer zones (Pinay *et al.,* 2000). Generally speaking, a wider buffer zone will increase the removal of NO_3^- due to increased residence times and therefore opportunities for microbial processes to take place or plant assimilation. However, artificial field drainage systems can cause NO_3^- rich drainage waters to by-pass the buffer zones and enter the aquatic environment (Lowrance *et al.,* 1984; Groffman *et al.,* 2000; Sabater *et al.,* 2003; Hefting *et al.,* 2006; Stevens and Quinton, 2009).

Despite the potential for NO_3^- removal in riparian zones, Groffman *et al.* (2000) suggest that riparian zones are hotspots of N transformations and, as a result, N_2O emissions. However, Groffman *et al,* (2000) further state that the location of N_2O production could be altered – but not necessarily the total N_2O production; emissions will only increase if the N_2O:N_2 ratio is altered during denitrification. N_2O emissions from riparian zones range between 0.1 and 5.3 kg N_2O-N ha^{-1} yr^{-1} (Weller *et al*., 1994; Augustin *et al*., 1996; Groffman *et al*., 1998; Skiba *et al*., 1998; Sozanska *et al*., 2002).

1.7 Greenhouse gases and climate change

1.7.1 Pollution swapping

Pollution swapping can be defined as the exchange of one form of pollution as a result of measures implemented to reduce another (Stevens and Quinton, 2009). In this case the use of riparian buffer zones or wetlands to reduce diffuse NO_3^- pollution, results in increased N_2O and/or CH_4 emissions (Stevens and Quinton, 2009). Both wetlands and buffer strips have the potential to ‘swap’ pollution from NO_3^- water pollution to increased greenhouse gas emissions; CO_2, CH_4 and N_2O (Reay *et al.*, 2004; Stevens and Quinton, 2009).

CH_4 is produced by anaerobic decomposition of organic material (Hernández and Mitsch, 2007). Natural wetlands are the greatest global source of CH_4 emissions, estimated to contribute to 110 x 10^{12} g CH_4 yr^{-1} (Denmen *et al.*, 2007). N_2O is produced by either nitrification or denitrification and can be emitted from both wetlands and buffer strips. Wetlands are generally C sinks however they can become net CO_2 sources under dry conditions when soil respiration and decomposition rates increase (Gorham, 1991). For the purpose of this research only N_2O and CH_4 will be considered further. The increased land coverage of buffer strips and wetlands as a result of mitigation strategies to remediate diffuse N pollution could become an important indirect source of greenhouse gas emissions that needs further investigation (Hefting *et al.*, 2003; Reay *et al.*, 2003; Quinton and Stevens, 2009).

1.7.2 Nitrous oxide

Nitrous oxide is an important greenhouse gas with a global warming potential 298 times greater than carbon dioxide on a mass basis over a 100 year time horizon and an atmospheric life-span of 160 years (Lashof and Ahui, 1990; Zumft., 1997; IPPC, 2007; Solomon *et al.*, 2007). It absorbs outgoing radiant heat from the Earth at infrared wavelengths that are not captured by the other major greenhouse gases (H_2O, CO_2, and CH4) (Vitousek *et al.*, 1997). N_2O is produced by denitrification, nitrifier-denitrification and nitrification in soils and sediments. Enhanced available N by fertilisation or deposition generally increases these emissions. Moreover N_2O is responsible for stratospheric ozone depletion (Ravishankara *et al.*, 2009). During nitrification, N_2O is principally produced through ammonia oxidation (Wrage *et al.*, 2001). In contrast to nitrification N_2O is produced as a regular intermediate of denitrification in the reduction of NO_2^- to N_2 (Section 1.6.6, Equation 7) (Wrage *et al.*, 2001). Greater proportions of N_2O are produced when NO_3^- concentrations are high, where metabolisable organic carbon is available, and at low pH as nitrous oxide reductase is inhibited (Wrage *et al.*, 2001). During nitrifier-denitrification autotrophic nitrifiers oxidise NH_3 to NO_2^- which is then reduced through a series of intermediates that include N_2O, to N_2

(Wrage *et al*., 2001). It is estimated that up to 30% of N_2O emissions from soils could be created through this pathway (Webster and Hopkins, 1996). Therefore soil transformations of mineral N by nitrification, denitrification and nitrifier denitrification can all contribute to N_2O production (Wrage *et al*., 2001).

1.7.3 Methane

Since pre-industrial times (1750), CH_4 concentrations have increased from c. 715 ppb to 1774 ppb in 2005 resulting in an increased radiative forcing of 0.48 W m^{-2} (IPCC, 2007). The atmospheric concentration of methane is increasing annually at a rate of 0.8 % and currently constitutes some 20 to 39% of total anthropogenic climate forcing by greenhouse gases (IPCC, 2007). CH_4 is produced during the breakdown of organic material under anaerobic conditions by methanogen microbes (Hernández and Mitsch, 2007). CH_4 has a lifespan of 8.4 years and a global warming potential 25 times greater than CO_2 over a 100 year time scale (Solomon *et al.,* 2007). Wetlands, rice paddys, marshes and swamps are all known sources of CH_4, emissions (Sha *et al.,* 2011), with over 40 % of annual CH_4 emissions originating from natural and agricultural wetlands (Ström *et al.,* 2007). However, CH_4 emissions from constructed wetlands vary greatly, both temporally and spatially with ranges from 0 to 240 g CH_4-C m^{-2} d^{-1} (Sha *et al.,* 2011). Nevertheless, Mander et al, (2005), suggest that even if all domestic water were to be treated by wetlands, its share in the global emissions budget would be less than 1%.

CH_4 emissions follow three pathways; diffusion through wetland sediment, ebullition from soil porewater and diffusion through aerenchyma of plants (Lai, 2009). However, CH_4 as it passes into aerobic sediment or soils can be oxidised by methanotrophic bacteria (Lai, 2009). The oxygenation state of the sediment, affected by water table height, as well as temperature, can exhibit a large degree of control over wetland emissions (Bubier and Moore, 1994). A comprehensive literature review carried out by Saarnio *et al.,* (2009) estimated total CH_4 emissions from European wetlands and watercourses at approximately 5.22 Tg yr-1 with wetlands estimated to contribute 3.60 Tg CH_4 yr^{-1} of this (ranging from 0 - 68 g CH_4–C m^{-2} yr^{-1}).

1.8 *Estimated costs of nitrate pollution*

Evaluating the cost of NO_3^- pollution is difficult as although the cost of purifying drinking water or reduced recreational or amenity value of water bodies, can be estimated many other impacts, such as eutrophication or loss of biodiversity, have no direct monetary value (Pretty *et al.,* 2003). Furthermore the degree of nutrient loading to generate eutrophication is not uniform across all aquatic ecosystems but is dependent on localised biological, chemical and hydrological factors (Pretty *et al.,* 2003; Dodds *et al.,* 2009). Brink *et al.,* 2011 estimate the total annual cost of pollution as a result of Nr in Europe as $95.2 to 435 billion, equivalent to $204 - 1020 per capita. The greatest costs (75%) were associated with gaseous emissions (NO_x, N_2O) and ammonia particles that resulted in adverse health impacts and air pollution (Brink *et al.,* 2011). Some effects of increased nutrients into freshwater ecosystems are shown below in Figure 1.6.

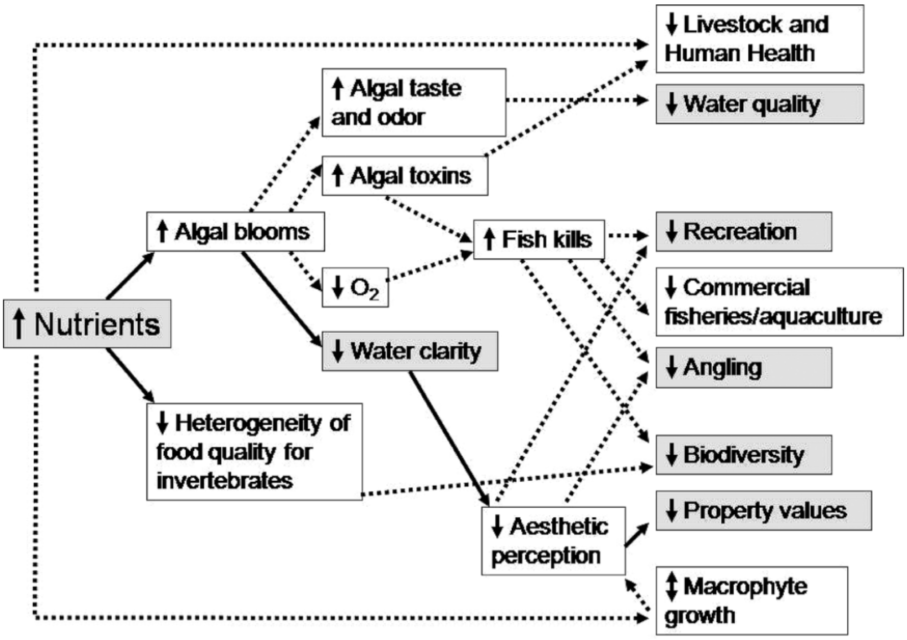


Figure 1.6: Some effects of increased nutrients to freshwater ecosystem goods and services, (Dodds *et al.,* 2009).

The U.K is predicted to spend $434 M over the next twenty years to meet the NO_3^- standard for potable water (Pretty *et al.*, 2003). Furthermore the cost of eutrophication in England and Wales has been estimated at $117 to 178 million per year, this includes reduced values of waterside dwellings or water bodies for abstraction, reduced recreational and amenity value, losses for tourism, economic costs to fisheries and aquaculture in addition to drinking water treatment costs, cleanup costs of waterways, and health costs to humans and animals. Pretty et al, (2003) estimated an annual capital cost of NO_3^- removal by water companies of c. $31 million per year. Brink et al, (2011) estimate the ecosystem impacts for Europe associated with N-runoff at c. $7 to 27/kg N and the cost of NO_3^- in drinking water at $0 to 5/kg N. In addition to the direct costs of increased NO_3^- in the environment, indirect costs in the form of increased N_2O emissions from farmland or increased N_2O and CH_4 emissions from wetlands, constructed to intercept and remove NO_3^- pollution need to be taken into consideration. Stern (2006) estimates the social costs of carbon on a 'business as usual' trajectory at $25-30 per tonne, this means that for a tonne of CH_4 and N_2O the costs are $575-690 and $7400-8880 respectively. Inferred social cost of N_2O emissions associated with the greenhouse gas balance is c. $7 to 20/kg N (Brink *et al.*, 2011).

1.9 Thesis Overview

This research investigated the potential for pollution swapping, i.e. the exchange of one form of pollution (NO_3^- water pollution) for another (N_2O and/or CH_4 air pollution), as a result of the implementation of two mitigation methods: riparian buffer strips and a denitrifying wetland and was accomplished through both field and laboratory studies.

The main study aims were to:

(1) Quantify direct *insitu* N_2O emissions from an irrigated buffer strip (IBS), using agricultural drainage water with high NO_3^- loadings, compared to a non-irrigated control (CP), using the paired plot method. Complimentary laboratory soil monolith experiments designed to mimic *insitu* conditions aimed to assess indirect N_2O emissions and leaching losses, as well as improve the understanding of N_2O production and consumption within agricultural soils using within soil profile Accurel® probes.

(2) Assess the effectiveness of an instream wetland for intercepting diffuse NO_3^- pollution and any associated pollution swapping in the form of CH_4 or N_2O emissions. In addition intact wetland sediment cores in laboratory incubation experiments were conducted using membrane inlet mass spectrometry to investigate the effects of water temperature and turbulence on the production and emission of greenhouse gases and inorganic-N concentrations in the overlying water column.

In situ field experiments, both the riparian buffer strip (Chapter 3) and wetland (Chapter 5) experiments were conducted within the same field system at Nafferton Farm, a research farm operated by Newcastle University (see Chapter 2.1 for further details): Chapter's 4 and 6 represent the laboratory component of these two field experiments, respectively.

The thesis is comprised of 7 chapters in total, with Chapters 3 to 6 comprising the 4 main results chapters from field and laboratory experiments. Following the description of general methodology for the research in Chapter 2, Chapter 3 details the spatial and temporal effects on direct N_2O emissions from irrigation of a riparian buffer strip compared to a non-irrigated control. This paired plot experiment was carried out at Nafferton Farm by the use of static flux chambers (weekly measurements) and automated gas flux chambers (daily measurements) between October 2006 and June 2009. Chapter 4 is complimentary work that investigated N_2O production and consumption within the soil profile both *in situ* for a short 8 week study period within the IBS at Nafferton Farm and in extensive laboratory studies in which *in situ* temperature and irrigation conditions were mimicked for a series of experiments using intact soil cores taken from adjacent to the IBS.

Collectively Chapters 3 and 4 allow the extent of pollution swapping (if any) through direct and indirect N_2O emissions and leachate losses as a result of irrigation with NO_3^--rich agricultural drainage water to be assessed. They also allow insight into the origin of N_2O emissions within the soil in response to key drivers (temperature, NO_3^--N additions and WFPS).

Chapter 5 assesses the effectiveness of a small instream wetland to intercept and remove diffuse NO_3^- pollution in addition to quantifying greenhouse gas emissions. Daily emissions were measured (where possible) by 3 automated gas flux chambers. Likewise daily NO_3^- concentrations at the wetland inflow were measured from bulked water samples collected by an automatic sampler. Furthermore, fortnightly water samples taken on a longitudinal transect was used to quantify the N interception of the wetland. A cost-benefit analysis was performed to establish the cost of greenhouse gas emissions in relation to any savings generated through NO_3^- interception and removal.

Chapter 6 examines the effect of water temperature and turbulence on greenhouse gas emissions and inorganic-N concentrations in the overlying water column from incubation experiments using intact wetland sediment cores. Additionally the production and concentration of greenhouse gases within the sediment was measured using Membrane Inlet Mass Spectrometry at two depths selected within each core to target different redox conditions. This research aided the understanding of the controlling factors of greenhouse gas emissions and therefore potential ways to reduce them within the wetland system.

Together the findings of Chapters 5 and 6 allow the effectiveness of the instream wetland to be assessed and critically examined in order to recommend suitable wetland design and optimal conditions leading to enhanced N removal whilst reducing greenhouse gas emissions.

The findings of the field and laboratory experiments for both the riparian buffer strip (Chapters 3 and 4), and the wetland (Chapters 5 and 6) will help inform policy decisions with respect to their design, function and implementation within the UK. This is discussed in the relevant chapters and synthesised in the Conclusions (Chapter 7).

Chapter 2: Materials and Methods

This chapter provides a description of the study sites in addition to details of generic methods utilised in more than one chapter of this thesis. Comprehensive descriptions of manual and automated flux chambers for the riparian buffer strips (Chapter 3) and the instream wetland (Chapter 5) are given. Specific methodologies are given in the relevant chapters.

2.1 Site description

Nafferton Farm is a 294 ha working farm employing both conventional (c. 52%) and organic (c. 48%) farming practices. It lies approximately 24 km west of Newcastle-upon-Tyne, north-east England, latitude 54°51’23.65″ N, longitude 007°36’03.92″ E at an altitude of 100 m above sea-level and within a designated Nitrate Vulnerable Zone (NZ06). Nafferton Farm is operated as a teaching facility as well as a commercial enterprise by the University of Newcastle’s School of Agriculture, Food and Rural Development. The farm is rented from the Allende Estate on a full repairing lease. It is designated as a level-2 site under the ‘NitroEurope’ project, (www.nitroeurope.eu), contributing through determining annual N fluxes through the use of low-cost techniques.

The farming practises used at Nafferton Farm are typical of the U.K. and Europe making the findings of these studies relevant to other farms in the U.K. and Europe. The principal issue is diffuse pollution; polluted runoff from farmyard hard standings and leached NO_3^--N from fertilised fields contaminate surface or ground waters with NO_3^--N concentrations above designated critical values, these areas are known as ‘nitrate vulnerable zones’ and as such are subject to environmental controls and protocols for good farming practises as laid out by Defra.

UK/EU policy addressing diffuse pollution suggests the use of riparian areas to remediate this pollution. This research has investigated the utility of an instream wetland and an irrigated riparian buffer strip at N interception and the trade off of by air pollution i.e. ‘pollution swapping’. The instream wetland and riparian buffer strip field experiments were carried out within the organic section of the farm in the field known as the ‘Gilchrist Strip’ (Figures 2.1 (a) and (b)).

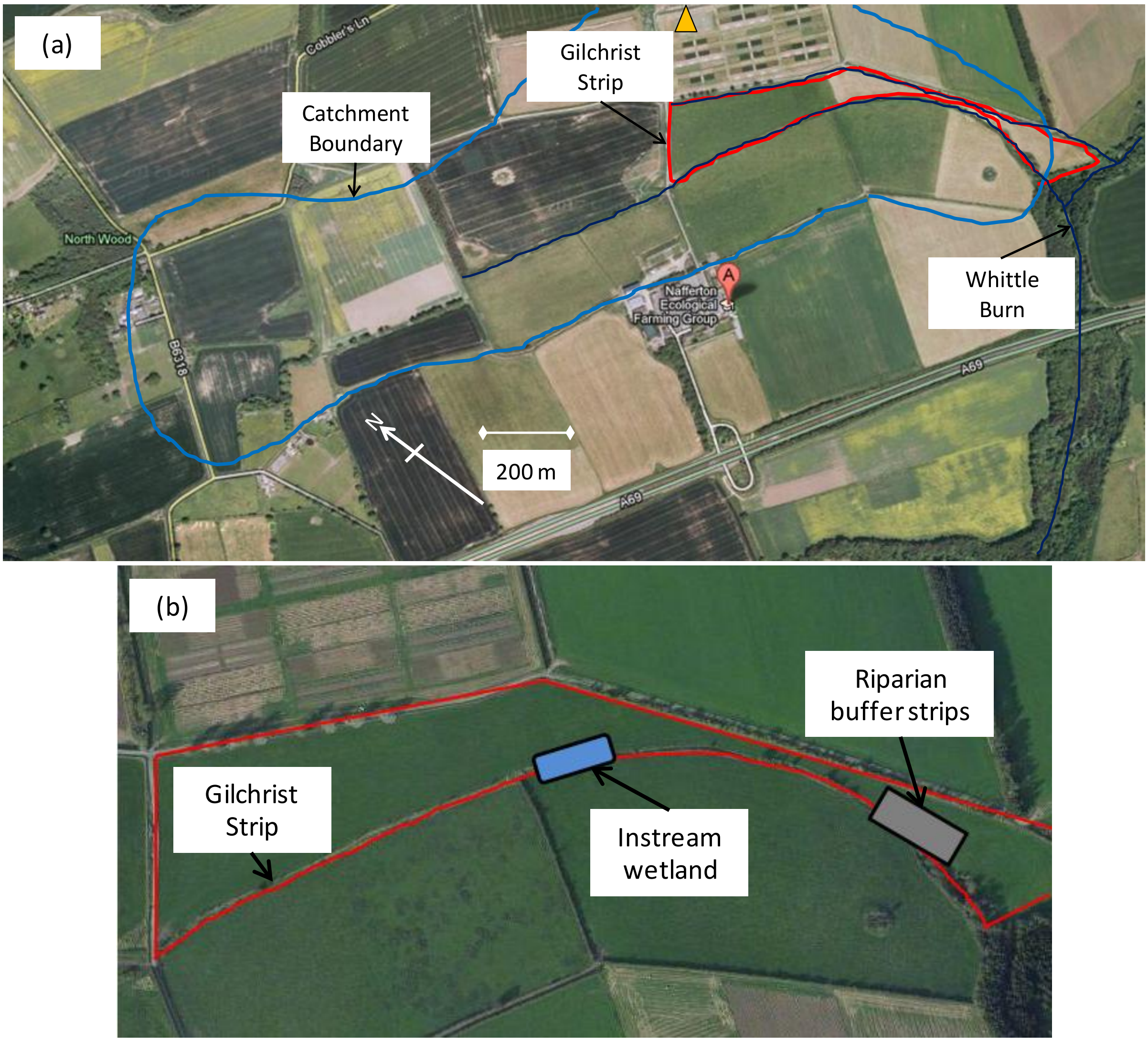


Figure 2.1: (a) Map of Nafferton Farm showing the catchment area of the drainage ditch (the light blue line) and the location of farm buildings and hardstandings (as labelled). The field site location is contained within the Gilchrist Strip outlined in red; the drainage ditches, tributaries of the Whittle Burn are marked (dark blue lines). The location of the on-site meteorological site is indicated by the orange triangle. (b) Enlarged map of the Gilchrist Strip, the location of the instream wetland and riparian buffer strips have been identified. (©2008 NAVTEQ Multimap)

The Gilchrist strip is a gently-sloping c. 15° organic clover/grass sward grazed by Friesian dairy cattle and spring lambing grassland flock of Mule ewes. The white clover mixture used at Nafferton farm was medium leaved varieties to suit cattle rather than sheep grazing. The advantage of a mixed grass/clover ley is that the clover can supply nitrogen through N-fixation and increase grass growth. Animals were excluded from the sample area for the duration of the study. Livestock stocking density within the Gilchrist Strip were organic cattle at 2.44 LSU/ha and sheep at 1.67 LSU/ha of grassland.

A clean grazing policy is operated at the farm so that alternate grazing of cattle and sheep takes place. Slurry and composted farmyard manure was applied to grass swards during early spring. Additionally an annual grass cut was taken from the Gilchrist Strip during early summer 2007. The field is drained by field ditches at the field edge and old clay pipe field drains of 30 – 40 mm diameter within the field. Irrigation water was taken from the adjacent ditch that drains a natural catchment of 0.96 km^2, and subsequently drains into the Whittle Burn.

An automated on site meteorological station (see Figure 2.1 for location) measured rainfall, air temperature, humidity, soil temperature, radiation, wind speed and wind direction on an hourly basis. Mean annual rainfall was 541 and 741mm yr^{-1} in 2007 and 2008, respectively. Mean monthly air temperature ranged from a high of 15.9°C during the summer of 2008 to a low of 2.38°C in January 2009, with a mean annual air temperature of 9°C.

During September 2008 a very large rainfall event with over 115 mm of rainfall falling in just three days is estimated to have a return period of 200 years. This is based on the Met Office's calculated return period at greater than 200 years for this rainfall event in Morpeth: 20 miles north of Nafferton Farm, which experienced similar rainfall (Met Office, 2008).

2.2 Flux chambers

Manual and automated chambers were used in the riparian buffer strips (Chapter 3). Automated gas flux chambers were used in the riparian buffer strips and the wetland (Chapter 3 and 5). Sampling at the field site was carried out by Edinburgh University staff and students (Robert Howard and Willena Boukelia) and Newcastle University staff and students (Jennine Jonczyk and Peter Dawber), on alternating sampling weeks. Additional assistance was received from Rebecca McKenzie (CEH Edinburgh PhD student) on specific field visits.

2.2.1 Manual static chamber design

Static chambers were distributed within the plots to provide a representative sample of the study area. Chambers comprised UPVC tubing of 20 cm long and 40 cm diameter with a UPVC flange of 50 cm width sealed onto the top end of the pipe, (Clayton *et al.,* 1994) (Figure 2.2). The bottom end of the pipe was tapered to allow easy insertion into the ground. A metal cutting ring with the same diameter as the chamber was used to make a circular slot of 5 cm depth into the topsoil. The chamber was then pressed firmly into the slot and the soil around the chamber was tapped down using the handle end of a sledgehammer to create a seal between the soil and the chamber edge. The location of chambers within sample areas was randomly assigned.

Figure 2.2: Static chamber design used for weekly flux measurements, (Chapter 3)

The chamber lid was constructed from a flat, opaque square of aluminium with a sample port drilled into the centre. The lid was sealed with a ring of foam rubber draft excluder glued to the underside of the lid in a circle exactly matching the diameter of the chamber ring additionally round plastic cable clips (RS Components Ltd, Northants, UK) were used to clip the metal lid to the plastic flange edge of the underlying chamber. The inlet tube was comprised of the barrel of a 1 ml plastic hypodermic syringe fitted with a three-way stopcock with luer fittings (Edwards Life Sciences Ltd, Berkshire, UK). The sample port was lined with rubber to form a tight fitting seal with the inlet tube. The inlet tube was inserted through the port and extended into the centre of the chamber. After installation the mean height of each chamber from the soil surface to the lid was measured and recorded from a mean of 20 measurements and used to calculated chamber volume. The chamber bases remained *in situ* for the duration of the study period however the chamber lids were fitted only for the duration of the sampling time. Chambers were closed for approximately 40 minutes before sampling.

Samples were collected using a jumbo syringe (1 litre SGE Jumbo Syringe, SGE Analytical Science Pty Ltd) and flushing a sealed 20 ml vial with 100 ml of sample, five times the vial volume. This method was employed rather than pre-evacuating vials as it ensured vials were always at ambient pressure and therefore less likely to leak. Laboratory tests conducted by Mr Robert Howard (Edinburgh University) have shown that flushing a vial with this volume of sample (100 ml) is adequate. The sample is introduced into the vial via a hypodermic needle inserted through the septum; a second needle is also inserted to act as the outlet for the flushed sample. The static chamber, syringe and sample vial are connected to one another by means of a three-way tap and polyethylene tubing. An initial 50 ml of sample is used to flush residual air from the tubing before a sample volume of 100 ml is flushed into the vial, after which the two needles are removed simultaneously.

Samples were stored in the glass vials until analysis by electron capture detector gas chromatography (Hewlett Packard 5890 series II gas chromatograph (Agilent Technologies, Stockport, UK), within ten days of sample collection. Additionally six ambient air samples were taken at the field site using the above method and used in the construction of the calibration curve for determining N_2O concentrations. N_2O concentrations in parts per million (ppm) are then converted into flux measurements (μg N_2O-N m^{-2} h^{-1}) (See section 2.2.6).

2.2.2 Automated chamber design for use in the grassland plots

Grassland automated chambers were constructed of a galvanised steel frame, 70 cm by 70 cm in area, 30 cm depth and a 2 cm-wide inward facing flange with a 3 mm thick sheet of galvanised steel for the lid (Smith and Dobbie, 2001). The chamber is inserted into the soil to a depth of 5cm and the soil outside the chamber is tamped down against the chamber edge to form a seal. The lid was fitted with rubber draught excluder that made a tight seal with the flange of the chamber below it. The chamber is linked to a battery powered control unit and an external sampling box containing 0.25 L evacuated Cali-5 Bond foil bags (Calibrated Instruments Inc, NY USA), (Figure 2.3).

Figure 2.3: Automated gas flux sampler with external sampling box showing the Cali-5 Bond foil bags (bottom right) and power control unit (top right), (Chapter 3).

The external control unit with the same mechanism as in Smith and Dobbie, (2001), was set to take one sample daily. However this system differed in that samples are stored in evacuated Cali-5 Bond foil bags until analysis by ECD-Gas Chromatography (Ball *et al.*, 2007). The lid was attached by two metal bars on pistons that were controlled by the settings programmed into the control box. Daily samples were taken from the closed chamber at noon. Samples remained in the 0.25 l evacuated Cali-5 Bond foil bags until analysis within 30 days of collection. Ambus *et al*, (2010) has shown that gas samples were unaffected after 175 days of storage.

2.2.3 Automated chamber design for use in the wetland

Daily nitrous oxide and methane fluxes were measured using automated gas flux chambers, 80 cm x 80 cm in area, and made of 0.3 cm thick sheets of transparent perspex, fitted to a steel frame. It was originally used to measure methane emissions from a rice field (Marik *et al.*, 2002). There chambers were positioned within the denitrifying wetland on stainless steel stilts so that the bottom of the chamber rested above the sediment surface but below the water surface, in order that the water acted as an airtight seal for the chamber. The height of the chamber therefore varied with water depth which was determined using a pressure sensor (PDCR 1830, Campbell Scientific).

The chambers remained *in situ* for the duration of the experiment. The lid (an 80 x 80 cm square sheet of perspex) was attached to pistons that lower and close the lid according to pre-programmed instructions for 60 minutes when air samples were taken from the closed chamber and stored in 0.25 l evacuated Cali-5 Bond foil bags (Calibrated Instruments Ltd, NY, USA) (Figure 2.4).

Figure 2.4: Automated gas flux chamber within the wetland site (Chapter 5).

2.2.4 N_2O analysis by Electron Capture Detector Gas Chromatography

Headspace N_2O was analysed using an Agilent 6890 GC fitted with a 1.8-m Hayesep Q column and electron capture detector (ECD). Ten per cent methane/argon was used as the carrier gas with column and detector temperatures of 70°C and 390°C, respectively. Samples collected manually from the static flux chambers into glass vials were directly placed onto an auto-sampling tray attached to the ECD-GC, controlled by a computer program.

Autosampler samples stored in foil bags were transferred into glass vials using a hand held pump. The hand pump is connected to the foil bag by a polyethylene tube attached to a three-way stopcock; the sealed vial is connected to the hand pump via a hypodermic syringe. The vials are first evacuated then the connection between the vial and the foil bag is opened via the stopcock for a period of 20 seconds. This procedure is repeated 3 times to ensure an uncontaminated sample is transferred from the foil bags to the vials. Nitrous oxide standards of 1.1 and 10.2 ppm, were transferred from gas cylinders into foil bags and transferred into vials as outlined previously. These standards as well as an ambient air sample collected in the field and assigned a value of 0.31 ppm: based on the current global average atmospheric concentration (IPCC, 2007), were analysed before and after field samples, and were used to produce a linear regression from which chamber N_2O concentrations could be calculated.

2.2.5 CH_4 analysis by Flame Ionising Detector Gas Chromatography

CH_4 was analysed using Hewlett Packard 5890 series II gas chromatograph with a flame ionisation detector (FID). Nitrogen was used as the carrier gas with column and detector temperatures of 70°C and 250°C, respectively. Autosampler samples stored in foil bags were transferred into 60 ml plastic syringes. Syringes were connected to the foil bag via interconnecting tubing and a three-way stopcock. A 40 ml sample was withdrawn from the foil bag into the syringe and released to flush residual air in the stopcock and repeated three times. Then a 60 ml sample was withdrawn from the foil bag and the stopcock was closed. A glass vial was then attached to the syringe plunger and held in place by a rubber band. The plunger was depressed over pressurising the syringe and just before attaching it to the automated sampler release the stopcock. CH_4 standards of c. 1.0, 3.5 and 11 ppm were transferred from gas cylinders into foil bags and subsequently into syringes as outlined above. These standards were used to produce a linear regression from which chamber CH_4 concentrations could be calculated.

Both ECD and FID gas chromatographs use Peaksimple software to determine the peak area of the produced curve. Linear regressions determined from the standard calibrations are used to convert this raw peak area into concentrations in parts per million (ppm) using the standard regression equation.

2.2.6 Concentration to flux calculations

The soil flux calculation assumes that the increase in gas concentration over time within the chamber is linear. Chamber linearity; the linear increase of N_2O concentration with increased closure time was based on the published works that have used identical static or automated chambers for flux measurement in comparable grassland systems: Smith et al., (1998); Ball et al., (1999) and Reay et al., (2009).

Reay et al., (2009), states that linearity of increase in headspace N_2O from static flux chambers during a 45 min cover period was confirmed by 5 minute sampling intervals throughout the closure period. Smith et al., (1998), states that the N_2O concentration in the chamber headspace, for both the automated and static flux chambers, was found to be linear for closure periods of greater than 1 hour. Ball et al., (1999) determined that N_2O accumulation using identical manual and automated gas flux sampling chambers was linear for a period of up to 3 hours.

The concentration of gas in ppm was first converted into µg m^{-3} using the ideal gas law, taking into consideration field and laboratory temperature and pressure. Soil-atmosphere flux (µg m^{-3} h^{-1}) was calculated by measuring the change in concentration from measured ambient air within an enclosed chamber of known volume and after a known duration of time, where C is the concentration in µg m^{-3}, V is the volume in m^3, SA is the surface area in m^2 and T is the time in hours.

$$Flux = \frac{C_{chamber} - C_{ambient} * V_{chamber}}{SA_{chamber} * T_{closure}}$$

Equation 2.1

2.3 Soil extraction and analyses

2.3.1 Bulk density determination

Soil bulk density measurements of the two plots were carried out in June 2007 using the excavation method (Elliott *et al.*, 1999). Two large pits extending 0.5 m depth were dug adjacent to the irrigated (IBS) and control (CP) grassland plots (see Chapter 3, Figure 3.1). Using 5 cm deep straight sided plastic cylinders, four replicate samples were taken at 5 cm depth intervals. These samples were used to measure bulk density, soil texture, moisture content at field capacity, wilting point and available water using NitroEurope standard methodology (NitroEurope, 2011 unpublished).

2.3.2 Gravimetric soil water content

Gravimetric moisture contents were conducted on fortnightly bulked soil samples collected from the IBS and CP (Chapter 3) (by Mr Robert Howard and the Author), on the soil samples collected via the excavation method (Section 2.3.1) in June 2007 (by Mr Robert Howard) and on the monolith soil cores used in laboratory experiments (Chapter 4) (By the Author) using the following method.

Collected soil samples were homogenised and sub-sampled. The empty weight of the labelled oven tray is recorded (t wt), the sub-sample is placed into the tray and the combined weight recorded (t+Wwt). This process was repeated for all sampling depths and replicates. All samples are placed in an oven at 105 °C and left over the weekend to dry. The weight of each tray and dried soil sample (t+Dwt), was recorded. The gravimetric moisture content was then calculated from the Equation 2.2 below.

$$Gravimetricmoisturecontent(\%) = \frac{(t+Wwt)-t}{(t+Dwt)-t} * 100 \qquad \textit{Equation 2.2}$$

2.3.3 pF curve

Using soil samples collected by the bulk density method (Section 2.3.1); samples taken every 5 cm to a depth of 50 cm below the soil surface, from two sampling locations one adjacent to the IBS and the other adjacent to the CP. Tension splitting was at 1, 10, 30, 100 and 1500 kPa. This was carried out by Colin Crawford at the Scottish Agricultural College.

2.3.4 Total Carbon and Nitrogen

Total carbon and total nitrogen were measured from bulked soil samples for each soil layer using the combustion method. Air dried soil was passed through a 2 millimetre sieve and then ball milled into a fine talc before being passed through a Carlo Erba NA2500 CHN analyzer to determine total C and N. The analysis was carried out by Ann Mennim, Edinburgh University after sample preparation by the author. Total-C is considered to be organic-C in this work since the soils are non-calcareous and have not been limed. Organic soil N was determined by subtracting the available soil N measured as described above from the total N content.

2.3.5 Soil particle size

Using the samples collected by excavation in June 2007 soil particle size analysis was determined by working out the proportion of each particle size class i.e. from coarse sand to clay. First soil samples were weighed then the organic matter is destroyed using hydrogen peroxide and the samples are re-weighed. Then the soil is sieved by progressively finer sieves (0.2–2 mm, 0.05-0.2 mm, 20-50 µm, 2-20 µm, <2 µm) weighing each of the sample portions for each size fraction and converting these into percentage fractions of the total sample weight. The soil was classified as a well-drained medium loam (Brickfields Association), with a depth of approximately 1 m, comprised of approximately 60% sand, 30% silt and 10% clay. Total porosities were 0.39 and 0.37 m^3 m^{-3} in the IBS and CP, respectively.

2.3.6 Soil inorganic N

An intensive study into the inorganic N content from the IBS was undertaken in August 2008. One metre soil cores were taken from the IBS using a soil column cylinder auger (Eijkelkamp Agrisearch Equipment, Netherlands). The soil column cylinder was hammered into the soil to a depth of one metre using a mechanical hammer. The column is then extracted using a jack and lever system using a metal chain tied around the column. The author acknowledges Sigrid Dengel and Robert Howard for their assistance in extracting the soil cores. The cylinder auger was a half round design with a removable cover allowing the complete soil profile to be studied and ease of sub-sampling (Figure 2.5).

Figure 2.5: A 1 m soil core extracted using a column cylinder auger.

Soil compression was less than 5 % and most of this was concentrated on the uppermost layers of the soil. Triplicate cores (approximately 100 cm long and 10 cm diameter) were extracted along transect across the width of the IBS 1 m downslope from the irrigation boom. The triplicate cores were 80 cm, 100 cm and 90 cm in length as a result of the corer impacting into harder underlying material. Duplicate samples of each of the three replicate cores were analysed for nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+) content at 10 cm intervals using the standard methodology outlined below in section 2.3.5.

2.3.7 NO_3^- and NH_4^+ determinations

Soil mineral N (NO_3^- and NH_4^+) was determined by first obtaining extracts from the soil samples using standard KCl extraction methodology, conducted by either Robert Howard, Edinburgh University or the author. Fresh or defrosted soil samples (frozen on the day on sampling at - 16 °C), are sieved through a 4 mm sieve, a subsample of 20 g is added to 100 ml of 1 M KCl solution and shaken for 2 hours. Extracts were then filtered through Whatman No 42 filter paper and presented for analysis by continuous flow colorimetry. Blank extracts were also prepared and the values subtracted from the sample concentrations.

Extracts and wetland water samples were analysed for NO_2^--N, NO_3^--N and NH_4^+-N colorimetrically with a Bran and Luebbe AutoAnalyser III continuous-flow analyser [Bran & Luebbe®, SPX corporation, USA], by John Morman or Andy Gray, Edinburgh University, using matrix-matched standards. The detection limit is 0.01 mg L^{-1}. The manufacturer's method G-102-93 rev1 and method G-109-94 rev3 were used for NH_4^+-N and NO_3^--N, respectively. NO_3^- is first reduced to nitrite using hydrazine with a copper catalyst after which it is reacted with sulphanilamide to form a pink compound measured at 520 nm. NH_4^+ is reacted with salicylate and dichloroisocyanuric acid to produce a blue compound measured at 660 nm, nitroprusside is used as a catalyst.

2.4 Statistical methods and data handling

2.4.1 Statistical methods

Statistical tests were used to determine correlations, relationships, differences or similarities within and between datasets for flux and auxiliary measurements. A significance level of $P < 0.05$ was adopted throughout, unless otherwise stated. Flux and auxiliary measurements were tested for normality using the Kolmogorov-Smirnov test. Parametric and non-parametric tests were carried out for normally and not normally distributed datasets, respectively and are detailed in the text. Statistical tests were carried out using Minitab statistical software version 15 (Minitab Inc., UK), unless otherwise stated.

2.4.2 Q_{10} co-efficient

A common measure of temperature response is the empirical Q_{10} co-efficient, which standardises temperature-related differences in reaction rates to proportional changes per 10°C rise in temperature. The Q_{10} is defined as the ratio of the rate of change as a consequence of increasing the temperature by 10°C, and was calculated using Equation 2.3 below.

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2 - T_1}}$$

Equation 2.3

Where R denotes the rate and T denotes the temperature in Celsius. Subscripts 1 and 2 denote the upper and lower limits of the rate and temperature variables.

For the purposes of this research the Q_{10} coefficients were calculated for methane and nitrous oxide production and emission rates from sediments (Chapter 6), to assess the microbial sensitivity of these biological processes to successive 10°C increases in temperature.

Chapter 3: N_2O fluxes from an irrigated and control riparian buffer strip

3.1 Introduction

Long-lived greenhouse gases - carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and halocarbons - are the most important drivers of anthropogenic climate change, generating a combined radiative forcing of + 2.63 [± 0.26] W m^{-2} (Forster *et al.*, 2007). N_2O is a powerful, long-lived greenhouse gas with a global warming potential 298 times greater than CO_2 on a per mass basis over a 100 year time horizon (Solomon *et al.*, 2007). In addition to this, N_2O is also the major source of ozone-depleting nitric oxide (NO) and nitrogen dioxide (NO_2) in the stratosphere (Forster *et al.*, 2007).

Atmospheric concentrations of N_2O have risen from 270 ± 7 ppb in pre-industrial times to around 319 ± 0.12 ppb in 2005, an increase of 18% above pre-industrial levels (Solomon *et al.*, 2007). The primary driver for this increase in atmospheric N_2O concentrations has been enhanced emissions from agricultural sources producing 6.8 Tg N_2O-N $year^{-1}$ (IPCC, 2007), the equivalent of 2.8 Gt CO_2-eq yr^{-1} (Smith *et al.*, 2007). In the UK, grasslands cover an estimated 27% of total land area and contribute approximately 35 kt N_2O annually, 6% of the total annual UK greenhouse gas emissions (Sozanska *et al*., 2002; Jones *et al*., 2007).

Major processes leading to N_2O emissions are the nitrification, denitrification and nitrifier-denitrification reactions carried out in soils by bacteria and fungi as well as archaea and some other eukaryotes (Bremner, 1997; Wrage *et al.*, 2001; Flechard *et al.*, 2007). These N_2O producing processes are complex, often occurring simultaneously in distinct microsites within the same soils (Smith, 1980; Bateman and Baggs, 2005). The largest contributor is from N additions to farmed soils and the corresponding increases in microbially-mediated N_2O production contributing 4.2 (0.6 – 14.8) Tg N_2O-N year $^{-1}$ (IPCC, 2007; Forster *et al.*, 2007; Smith *et al.*, 2007). Globally total N_2O emissions from all sources continue to increase at an approximately linear rate of 0.2 - 0.3% per year (Forster *et al.*, 2007).

Mineral N input, water residence time, soil-water interactions, vegetation type, soil structure and the complex interactions of biogeochemical processes (both aerobic and anaerobic) all affect the net emissions of N_2O (Lowrance *et al*., 1997; Jacinthe *et al*., 1998; Sabater *et al*., 2003) (see Chapter 1 Section 6 and 7). In addition, the heterogeneous nature of soils means that these processes vary over small spatial and temporal scales, thereby creating highly variable net N_2O emissions over space and time (Skiba and Smith, 2000).

Riparian areas (i.e. land adjacent to watercourses) can act as natural biofilters and buffering zones for the protection of aquatic environments from diffuse nitrate (NO_3^-) pollution in drainage waters (Hefting *et al.,* 2006). The main processes of NO_3^- interception occurring in riparian buffer strips are plant uptake and denitrification (Sabater *et al*., 2003). Generally denitrification, is the dominant process of N_2O production in riparian soils, and is associated with high NO_3^- and low oxygen concentrations in the soil (Hefting *et al*., 2003). Nitrifier denitrification, the reduction of nitrite (NO_2^-) to nitric oxide (NO), N_2O and ultimately to dinitrogen gas (N_2), may also contribute to N_2O emissions (Poth and Focht, 1985; Wolf and Russow, 2000; Wrage *et al.,* 2001). Therefore despite potentially intercepting diffuse NO_3^- pollution, riparian areas may act as strong sources of N_2O emissions to the atmosphere as a result of the incomplete denitrification of intercepted NO_3^-. This is an example of ‘pollution swapping’ (Ambus, 1998; Mosier *et al.*, 1998; Hefting *et al.*, 2003; Reay, 2004), in which a water pollution problem is replaced by a climate change problem.

The European Community’s Nitrates Directive (91/676/EEC) directly addresses diffuse NO_3^- pollution from agriculture. The UK adopted a strategy of implementing discrete Nitrate Vulnerable Zones (NVZs), rather than a whole area approach, for land that drains into waters polluted with nitrates (Defra, 2011b). Where ground and surface waters exceed or are at risk of exceeding 50 mg NO_3^- L^{-1}, NVZs have been established. Currently 62%, 14.2% and 4% of the land area in England, Scotland and Wales, respectively, is designated as NVZs (The Scottish Government, 2006; The Welsh Government, 2008; Defra, 2011c).

Within NVZs farmers must adhere to controls on maximum application rates for livestock manures and inorganic fertiliser application, as well as ‘closed periods’ when no applications can be made, which for grasslands is 15th September - 1st February, (Defra, 2011e). Within closed periods, farmyard manure must be stored in such a way as to prevent the drainage of liquid. The maximum application of total N over the whole farm area must not exceed 170 kg

ha^{-1} yr^{-1} from livestock manures (RPA, 2011). A higher total N application of up to 250 kg ha^{-1} for farms in which at least 80% of the total farm area is grassland can be applied for by individual farms, under a derogation agreed by the European Commission (Business Link, 2011). In arable cropping systems the combined organic and inorganic fertiliser applications should not exceed the maximum application rate (N_{max}) for the crop type (RPA, 2011). The implementation of this directive is devolved within each of the UK administrations (England, Wales, Scotland and Northern Ireland), and the respective environmental agencies within each country are responsible for enforcement of the regulations. Additionally all farmers are required to keep extensive records of farming activities (Defra, 2011d).

In many countries agri-environment schemes encourage the use of riparian areas for mitigation of diffuse NO_3^- pollution and as a means of improving water quality through decreased phosphate, sediment and faecal pathogen contamination of surface waters. In England and Wales Catchment Sensitive Farming (CSF) is an initiative to promote good land management to reduce diffuse pollution from farming and the associated knock-on effects to ecological environments alongside associated agri-environment schemes such as Environmental Stewardship (Defra, 2011a).

Under CSF a range of strategies, including riparian buffer strips, are recommended to land owners as a measure to protect watercourses and improve soil management. Attached to CSF is the CSF Capital Grant Scheme within priority catchments in England, where grant aid is given towards improving or installing facilities that would improve water quality through reducing diffuse pollution from agriculture, such as riparian or in-field buffer strips (Natural England, 2010). As a consequence, such strips have become increasingly widespread in the UK but the potential of such an increase in usage for elevating N_2O emissions via ‘pollution swapping’ remains unclear (Ambus, 1998).

Experimental rationale

Riparian buffer strips are actively promoted under agri-environment schemes within the U.K. However, these buffer strips have the potential to swap NO_3^- water pollution for N_2O air pollution. This research tests the effectiveness that irrigation of a riparian buffer strip has at minimising N_2O emissions compared to a non-irrigated control. It is hypothesised that irrigating a riparian buffer strip will maintain a higher percentage water-filled pore space (WFPS) compared to a non-irrigated control, resulting in more complete denitrification to N_2 and low direct N_2O emissions. Furthermore, it is hypothesised that the high NO_3^--N loading of the IBS via the irrigation water will not result in an accumulation of inorganic-N in the upper 15 cm of the soil compared to the CP as a result of increased complete denitrification to N_2 gas.

This study aimed to quantify direct N_2O emissions from an irrigated riparian buffer strip (IBS) compared to an un-irrigated control plot (CP). Statistical analysis of the flux data will be used to determine if the N_2O emissions from both plots are similar or different from one another. The hypotheses tested were as follows:

(1) Irrigation of the IBS will not increase direct emissions of N_2O-N compared to the CP.

(2) The supply of additional NO_3^--N within the irrigation water will not increase soil inorganic-N in the IBS compared with the CP.

3.2 Materials and methods

3.2.1 Site description

The site is located at the University of Newcastle-upon-Tyne’s Nafferton Farm in the Tyne Valley (54°51’23.65 N, 7°36’03.92 E), approximately 20 km west of Newcastle, northern England, which lies within a Nitrate Vulnerable Zone (NZ06). It consists of grazed pasture and arable land, with c. 48% managed by organic and the remainder by conventional farming practices. The vegetation of the experimental site is organic clover grassland. Soils are medium loams, (as identified by sampling and analysis – see Chapter 2.2), pH 5.4 and drained by clay pipe field drains of 30 – 40 mm diameter. Mean annual rainfall was 541 and 741 mm in 2007 and 2008, respectively. Mean monthly soil temperatures ranged from 2.38 in winter to 15.9°C in summer during the study period.

3.2.2 Experimental design

Although ideally replicated irrigated and control plots would be used for this study it was not possible due to logistical and resource constraints. Only two riparian plots were utilised for this research: one irrigated and one control. This ‘paired-plot’ design is commonly used in situations where multiple plots could not be used. For example, a comparable riparian buffer strip study conducted by Hefting et al, (2003), investigated N_2O emissions from one forested and one grassland riparian zone, with the eight static flux measurements from each riparian zone subdivided into three pseudoreplicates for statistical analysis.

Evidently the lack of replicated plots in this research limits the robustness of the research findings and their applicability to other grassland riparian sites. It is therefore difficult to conclusively state if observed differences or similarities between plots are due to the effect of the treatment applied or inherent differences in the plots. As such this research will concentrate on temporal trends in emissions and other measured variables as well as the temporal and spatial effects of irrigation on direct N_2O emissions. Furthermore, as it is well known that N_2O emissions are inherently highly variable both in time in space; the experimental design sought to capture this variability by intensive sampling through the use of multiple gas flux chambers within each plot.

Two experimental riparian grassland plots were located along the field edge, adjacent to a drainage ditch, on gently sloping organic clover grassland (Figure 3.1), using the paired plot design (Ambus and Christensen, 1993). One plot received treatment to simulate a buffer strip (the IBS) whilst the other served as a control (the CP). The IBS (covering 77 m^2) was irrigated with N-rich agricultural drainage water, extracted from the near-by ditch, with a mean concentration of 6.63 ± 4.74 (range 0.05 – 59.7) mg NO_3^--N l^{-1} and 0.61 ± 1.65 (range 0.00 – 15.8) mg NH_4^+-N l^{-1}. Seasonally, concentrations in the irrigation water varied by four orders of magnitude, with NO_3^--N concentrations greater during winter and summer and NH_4^+-N concentrations greater during summer and autumn (Table 3.1).

The IBS received 3.5 hours per day irrigation from November 2006 to August 2007 via a single pipe located at the top of the IBS near to chamber 1 and 2 (Figure 3.1). Because of the difficulty of problems quantifying irrigation in this period (field site managers did not provide information on the irrigation rate) and concern of uneven irrigation across the IBS from September 2007 onwards, irrigation water was supplied via an irrigation boom fitted with a water meter.

Table 3.1: Mean seasonal concentrations of NO_3^- and NH_4^+ (± one standard deviation of the mean), in irrigation water, from winter 2006 to autumn 2008.

Season	Mean NO_3^--N (mg NO_3^--N L^{-1})	Range of NO_3^--N (mg NO_3^--N L^{-1})	Mean NH_4^+-N (mg NH_4^+-N L^{-1})	Range of NH_4^+-N (mg NH_4^+-N L^{-1})
Winter (86)	16.8 ± 4.20	7.45 - 22.9	0.28 ± 0.37	0.00 - 3.21
Spring (131)	10.3 ± 6.11	2.09 - 59.7	0.12 ± 0.31	0.00 - 4.10
Summer (98)	3.54 ± 5.05	0.09 - 11.5	0.93 ± 2.03	0.00 - 15.8
Autumn (88)	3.91 ± 4.64	0.05 - 12.7	0.71 ± 1.07	0.00 - 6.08

Numbers given in brackets after each season denote the total number of samples from which the mean was calculated i.e. ‘n’.

The adjacent CP (covering 134 m^2), part of the same clover-rich grass sward, received water via rainfall only and no applied N. No organic or inorganic fertiliser applications or grazing of the plots took place during the study period; however, the surrounding field area was grazed by sheep and dairy cattle. A single cut of grass was taken from the whole area of both plots by hand at the end of July 2007. Total soil porosities are 0.39 and 0.37 m^3 m^{-3} in the IBS and CP, respectively (see Chapter 2 for method of determination).

3.2.3 Flux measurements

Static gas flux chambers (area 0.126 m^2) were used to measure weekly N_2O fluxes from October 2006 to June 2009, following the method of Clayton *et al.* (1994). Eight and six chambers were installed in the IBS and CP, respectively (see Chapter 2.2.1). On each measurement occasion duplicate headspace gas samples from each chamber were taken using a jumbo syringe (1 litre SGE Jumbo Syringe, SGE Analytical Science Pty Ltd) after 40 minutes of closure time, along with six ambient air samples. Duplicate samples for each static gas flux chamber were analysed and used as the mean for that chamber. All measurements were made between 9 am and 3 pm. Additionally an automated gas flux chamber in each plot was used to measure daily N_2O fluxes from March 2007 until June 2009; however, equipment malfunctions meant that this daily flux dataset is incomplete. Analysis of headspace N_2O was by ECD-gas chromatography, using an Agilent 6890 GC fitted with a 1.8-m Hayesep Q column and electron capture detector (see Chapter 2.2.4). Monthly average N_2O flux data are a composite of both the static and automated chamber measurement where available.

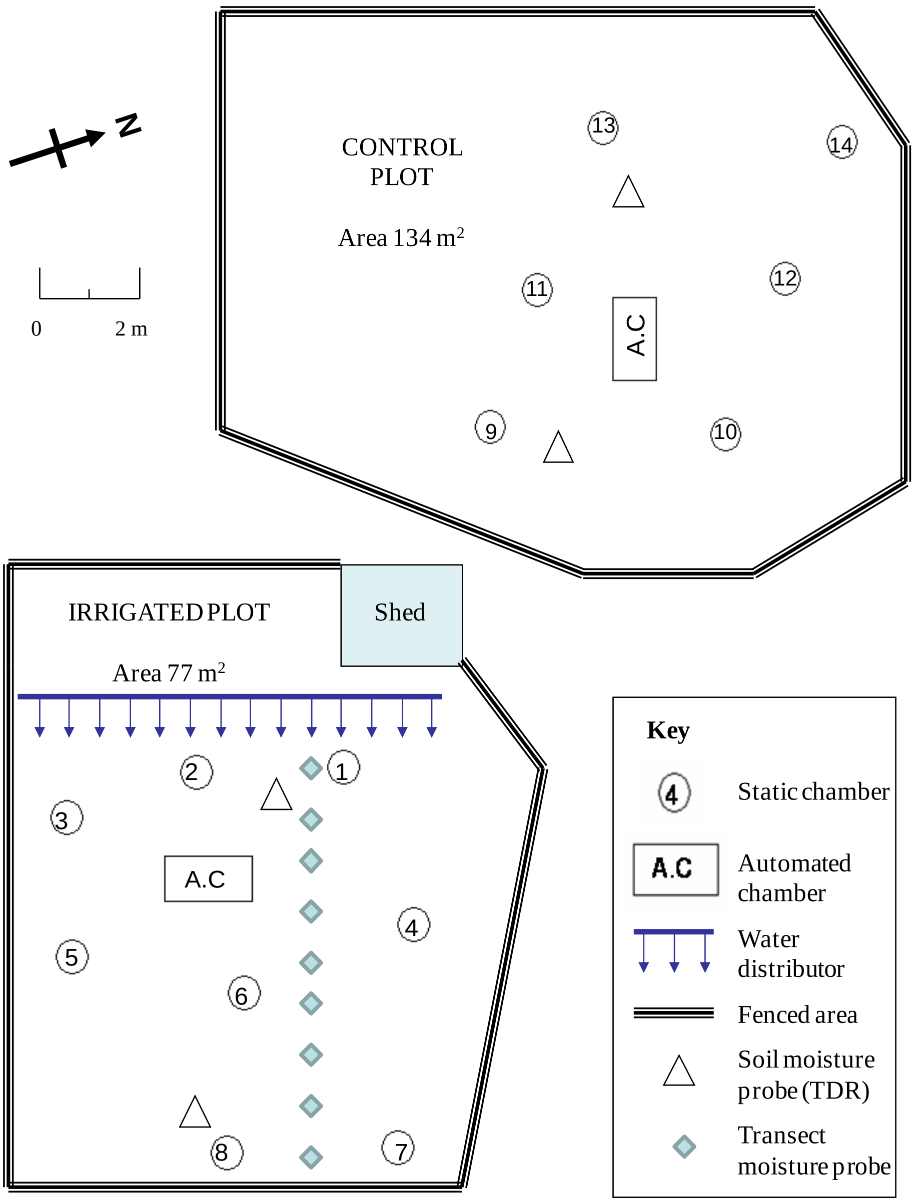


Figure 3.1: Schematic diagram showing the site design and instrumentation of the grassland plots. The drainage ditch is adjacent to both plots along the south western side of the diagram (approximately 1 and 2 m from the edges of the IBS and CP, respectively). Cattle and sheep grazing takes place to the east of the plots.

3.2.4 Auxiliary measurements

From March 2007 onwards the drainage ditch water was sampled every 8 hours within a 24-hour period, using an ISCO 6712 full-size portable sampler and bulked to produce a composite daily water sample. Sample bottles contained 2 µg $HgCl_2$ L^{-1}, to preserve sample integrity between collection and analysis (Kirkwood, 1992; Clough *et al.*, 2007). NO_3^--N and NH_4^+-N in the water samples were determined colorimetrically with a Bran and Luebbe continuous-flow analyser, using the manufacturer's method G-102-93 rev1 for NH_4^+-N and method G-109-94 rev3 for NO_3^--N (see Chapter 2.3.7).

Two dual temperature and soil moisture sensors (DL6 Soil Moisture Logger, Delta-T Devices Limited) in each plot measured average soil temperature and moisture integrated over the uppermost 10 - 30 cm of soil every 30 minutes (the location of these sensors are marked as triangles on Figure 3.1). Temperature sensors (Hobo pendant temperature loggers, Tempcon Instrumentation) located within each plot recorded soil temperature every 30 minutes in the top 5 cm of soil (one located inside and one outside the autochamber in each plot). Additionally a transect of nine soil moisture sensors (Hobo Soil Moisture Smart Sensor, Tempcon Instrumentation) measured soil moisture integrated over the uppermost 20 cm of the soil and were positioned at one metre intervals along the length of the IBS, with the first probe positioned one metre in front of the irrigating boom (marked as blue diamonds on Figure 3.1). A flow meter (Zenner® water meter ZR 07606384, ZENNER International GmbH, Germany) monitored the volume of irrigation water supplied to the irrigated plot, from September 2007 onwards. These data allowed the calculation of the total NO_3^--N input into the plot from September 2007.

Available soil NO_3^- and NH_4^+ was determined fortnightly from May 2007. On each occasion eight soil samples from each plot were taken from the upper 10 cm soil using a hand auger and bulked to produce a composite sample for each plot. Available soil NO_3^- and NH_4^+ were determined using a standard KCl extraction method (NitroEurope, 2011; see Chapter 2.2.5). NO_3^- and NH_4^+ concentrations in the filtrates were determined colorimetrically by a Bran and Luebbe continuous-flow analyser (see Chapter 2.3.7).

Aboveground biomass measurements were carried out during the peak growing season (mid-July, 2007). In each plot three replicate samples of 0.25 m^2 were cut to 10 cm above ground level and dried at 80°C. Dried samples were milled and sub-sampled for C and N analyses using a Carlo Erba NA2500 CHN analyzer. Soil pH, bulk density, particle size, stone content, total carbon and nitrogen as well as soil moisture release curves, were measured on soil samples taken adjacent to the experimental plots in June 2007, using NitroEurope standard methodology (NitroEurope, 2011; see Chapter 2 for details). An on-site automated weather station recorded hourly rainfall, air temperature, humidity, soil temperature, radiation, wind speed and wind direction (see Chapter 2.1, Figure 2.1).

Attempts were made to measure leachate within the IBS and CP using porous cups positioned at 30 cm depth, however, these failed to capture leachate and as a result no leachate data is available.

3.2.5 Statistical analysis

Initially normality tests were carried out (Kolmogorov-Smirnov), to test for normality of the data. Flux measurements were not normally distributed whereas auxiliary measurements were normally distributed therefore non-parametric or parametric tests, respectively, were carried out to determine the differences, relationships and correlations that exist within the data over various temporal and spatial scales as detailed below in the results (section 3.3). A significance level of $P < 0.05$ was adopted throughout, unless otherwise stated.

Static flux measurements are reported as the mean (± standard error) of the eight and six static chambers in the IBS and CP, respectively, unless otherwise stated. N_2O fluxes from the automated and static flux chambers from both plots, taken on corresponding sampling days, were analysed statistically for their similarity using the Mann-Whitney test ($P < 0.05$), and were found to be not statistically different. Therefore, mean monthly flux measurements (± standard error) are a composite of the static and automated chamber measurements where available.

3.3 *Results*

3.3.1 Temporal trends in N_2O flux

Total annual N_2O fluxes from the IBS and CP were 509 ± 18.8 and 263 ± 5.24 g N_2O-N ha^{-1} $year^{-1}$, respectively, in 2007 and 375 ± 16.9 and 500 ± 19.4 g N_2O-N ha^{-1} $year^{-1}$, respectively, in 2008. Mean weekly fluxes varied widely over the study period from -0.85 to 88.7 and -0.96 to 89.7 g N_2O-N ha^{-1} d^{-1} from the IBS and CP, respectively (Figure 3.2). Mean (±SE), median, maximum and minimum daily fluxes across the study period where; 8.65±1.45, 2.75, 88.7, and -0.85 g N_2O-N ha^{-1} d^{-1}, respectively, from the IBS; and 7.05±1.14, 2.67, 89.7, and -0.70 g N_2O-N ha^{-1} d^{-1}, respectively, from the CP.

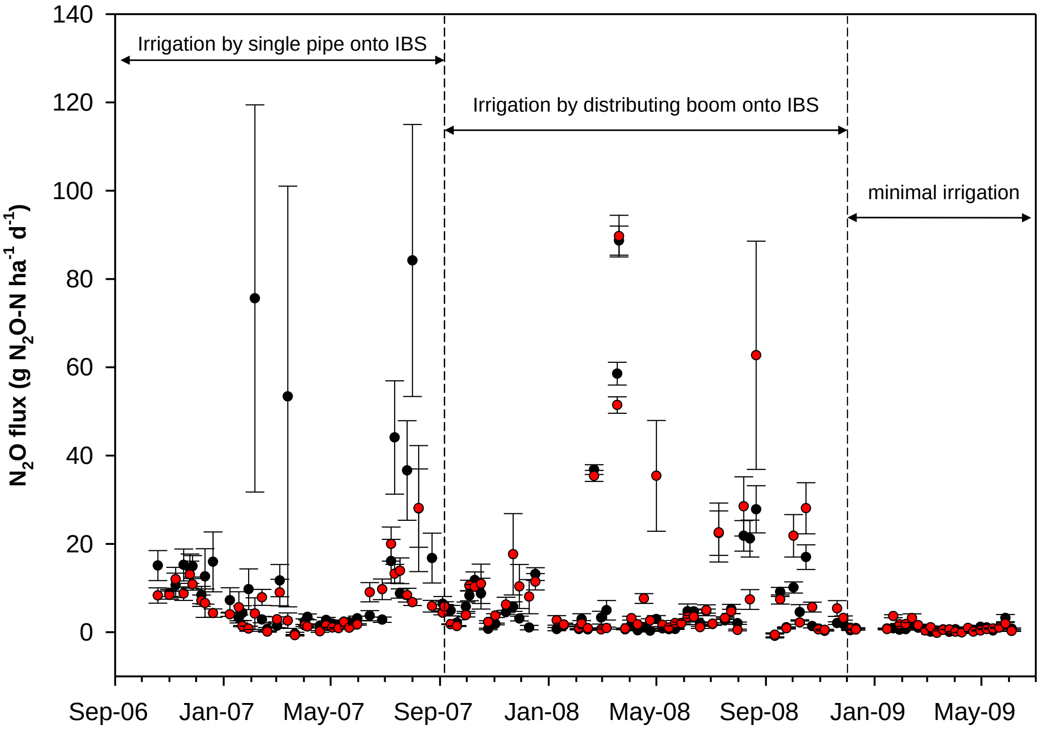


Figure 3.2 Average weekly N_2O fluxes from the IBS (black circles) and CP (red circles) plots for the duration of the field study. Error bars are the standard error of the mean for the eight and six static chambers in the IBS and control plots, respectively. Mode and duration of irrigation to the IBS are clearly labelled.

There was no statistically significant difference over the study period (P > 0.05, Mann-Whitney test, n = 119) between the measured weekly fluxes from the two grassland plots, despite the large additional amount of water and inorganic-N applied to the IBS (Figure 3.3 and Table 3.2). Furthermore, no statistical difference in N_2O flux was observed between plots for discrete sampling years 2007 (n = 44) or 2008 (n = 45); or for either the initial 6 months after irrigation began (January to June 2007: n = 22) or the final 6 months of the study period when irrigation was minimal (January to June 2009: n = 21). Therefore, irrigation of a riparian buffer strip did not generate statistically different N_2O emissions compared with a non-irrigated control.

Seasonally the greatest mean fluxes occurred during the summer months, peaking at 84.2 g N_2O-N ha^{-1} d^{-1} in the IBS in 2007 and 62.7 g N_2O-N ha^{-1} d^{-1} in the CP in 2008, when high soil temperature and WFPS occurred together. Negative fluxes are recorded on discrete sampling occasions, generally during the summer months from both the IBS and CP and after a 1 in 200 year rainfall event in mid-September 2008 (see Chapter 2.1). Seasonally the lowest observed fluxes of 7.58 and 13.1 g N_2O-N ha^{-1} d^{-1} for the IBS and CP occurred during the winter months of 2008/2009.

High mean weekly fluxes (i.e. those greater than 50 g N_2O-N ha^{-1} d^{-1}), from both the IBS and CP occurred during March and August of both 2007 and 2008. Atypically large fluxes occurred in March 2007 with 88.7 and 89.7 g N_2O-N ha^{-1} d^{-1} emitted from the IBS and CP, respectively. Most probably the result of increased substrate supply for denitrification through the slurry application to the adjacent fields during the week prior to sampling, resulting in nitrogen-enriched runoff passing over and through both field plots, as evidenced by a more than doubling in NO_3^--N concentration in the drainage ditch water from 8.13 to 19.5 mg NO_3^--N L^{-1}.

3.3.2 Temporal trends in rainfall and irrigation

Total annual rainfall for the site ranged from 541 mm in 2007 to 741 mm in 2008. The total monthly rainfall ranged from 8.40 mm in May 2008 to 158 mm in September 2008 with the summer months receiving the greatest amount of rainfall during the year: 223 mm and 276 mm in 2007 and 2008, respectively (Figure 3.3). The winter of 2008/2009 received less than half the rainfall compared to the previous two winters (Figure 3.3) and received the lowest recorded rainfall of any seasonal period.

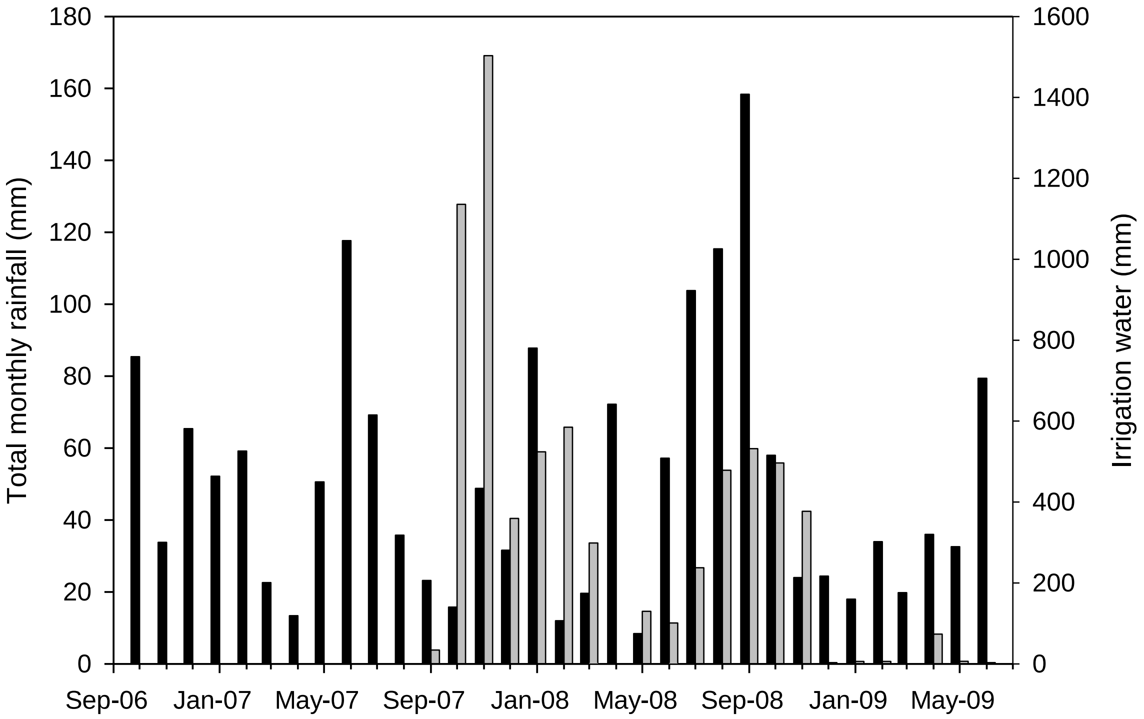


Figure 3.3 Total monthly rainfall at Nafferton farm (black bars) and total monthly depth of irrigation water (grey bars) supplied to the IBS after water meter fitted in September 2007.

The average monthly depth of irrigation water was 212 mm which is 423% greater than the average monthly rainfall received by the IBS. However, the depth of irrigation water supplied to the IBS per month ranged from 3.01 mm in December 2008 to 1503 mm in November 2007. This variability was due to inconsistent power supply for the water pump, from initially a wind turbine (November 2006 to August 2007), then by battery power (from September 2007 to November 2008) and subsequently by solar power (from December 2008 for the remainder of the study).

The IBS was irrigated initially through a single irrigation pipe for 3.5 hours a day; however, the irrigation rate was not measured. An estimate of the total volume of water added (from December 2006 to August 2007), based on a conservative rate of 1 - 2 litres per minute, would equate to a daily irrigation rate of 2.8 - 5.6 mm per day or approximately 80 - 170 mm per month. Mean monthly NO_3^--N concentrations of the irrigation water ranged from 1.23 to 13.9 mg NO_3^--N l^{-1} in 2007. However, the exact irrigation and N loading rates to the IBS prior to September 2007 are unknown.

From September 2007 onwards irrigation water was applied via an irrigating boom. Irrigation rates were measured using a water meter. The average monthly irrigation was 363 ± 400 mm, 626% greater than the average monthly rainfall received by the IBS. The installation of this water meter also allowed NO_3^--N loadings in the irrigation water to be calculated from September 2007 to the end of the study (Table 3.2).

NO_3^--N concentrations were determined from daily composite water samples taken using an automated ISCO water sampler, apart from a few occasions when this was not available (7th to 14th November 2007, 22nd to 29th November 2007, 29th January to 5th February 2009, April to May 2009). On occasions when the daily composite samples were not available mean (n = 11) water samples taken along the wetland transect (Chapter 5) were used and assumed to be representative of corresponding time period.

Despite the irrigation and enhanced NO_3^--N loadings to the IBS (equivalent to a total annual loading of c. 500 kg NO_3^--N ha^{-1} for 2008) mean weekly N_2O fluxes remained low (8.44 ± 2.30 g N_2O-N ha^{-1} d^{-1}), and not significantly different from those observed from the CP (10.34 ± 2.56 g N_2O-N ha^{-1} d^{-1}). This would suggest either complete denitrification of added NO_3^--N to N_2 or high NO_3^--N leaching losses.

Table 3.2: Monthly total NO_3^--N loadings to the IBS supplied through the irrigation water

Month-year	Total monthly irrigation (mm)	Mean monthly mass of added NO_3^--N ($kg\ NO_3^-\text{-}N\ ha^{-1}\ month^{-1}$)
Sep-07	34.2	0.93
Oct-07	1136	21.75
Nov-07	1503	39.17
Dec-07	359	21.73
Jan-08	524	50.65
Feb-08	585	87.39
Mar-08	299	142.74
Apr-08	No irrigation	-
May-08	130	65.99
Jun-08	101	4.29
Jul-08	238	8.71
Aug-08	479	25.11
Sep-08	532	35.31
Oct-08	497	55.42
Nov-08	377	38.07
Dec-08	3.01	0.63
Jan-09	6.01	2.84
Feb-09	6.01	1.85
Mar-09	No irrigation	-
Apr-09	73.6	5.80
May-09	6.38	0.67

3.3.3 Temporal trends in N_2O flux and soil temperature

Soil temperature at Nafferton Farm followed a distinct seasonal pattern with summer high temperatures and winter lows. Soil temperatures recorded across the field site using a variety of different instrumentation show a very good agreement of measured values and follow a seasonal pattern of higher temperatures during the summer months reaching a maximum of 19.9ºC, and winter lows of 0.49ºC, just above freezing (Figure 3.4).

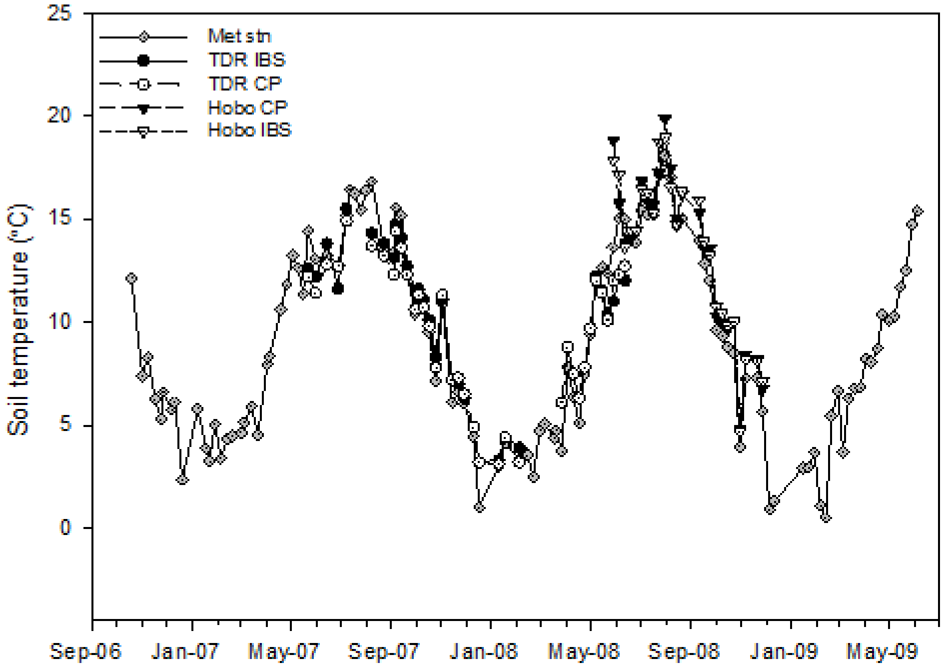


Figure 3.4 Soil temperature (°C) recorded on sampling days across the entire monitoring period using different instrumentation. Soil temperature recorded at an onsite meteorological station and by the Hobo pendant temperature loggers were measured at 5 cm depth. Measurements using TDR probes are the average soil temperature at 10 - 30 cm depth. Reported values from the TDR probes and Hobo pendant temperature loggers are an average of two probes within each plot.

Seasonally winter was the coldest (3.78 ± 0.84°C), summer the warmest (15.4 ± 0.51°C), with temperatures during spring and autumn broadly similar, 8.87 ± 3.17°C and 9.47 ± 3.21°C, respectively. Seasonal differences in N_2O flux are evident in that fluxes from both plots show an increase at times when soil temperature increases. But high fluxes were recorded under colder soil temperatures also, for example, in December 2007 mean recorded fluxes were 13.16 ± 1.44 and 11.42 ± 1.86 g N_2O-N ha^{-1} d^{-1} from the IBS and CP, respectively, despite a mean soil temperature of 1.01 ± 0.07°C on the day of sampling.

The Hobo pendant temperature loggers recorded mild minimum soil temperatures of 4°C, suggesting that denitrification could continue throughout the year even during winter months. However, the Hobo pendant temperature loggers placed inside and outside of the automated chambers in both the IBS and CP displayed a statistically significant difference in recorded temperatures, with generally higher temperatures recorded within the chamber compared to outside (paired *t*-test; IBS $P < 0.001$, $n = 24$ and CP $P < 0.05$ $n = 23$) (Figure 3.5).

Mean soil temperatures for the IBS and CP were 14.1 and 13.4°C inside and 12.5 and 13.0°C outside the chambers, respectively; in some instances the difference in temperature was greater than 6°C.

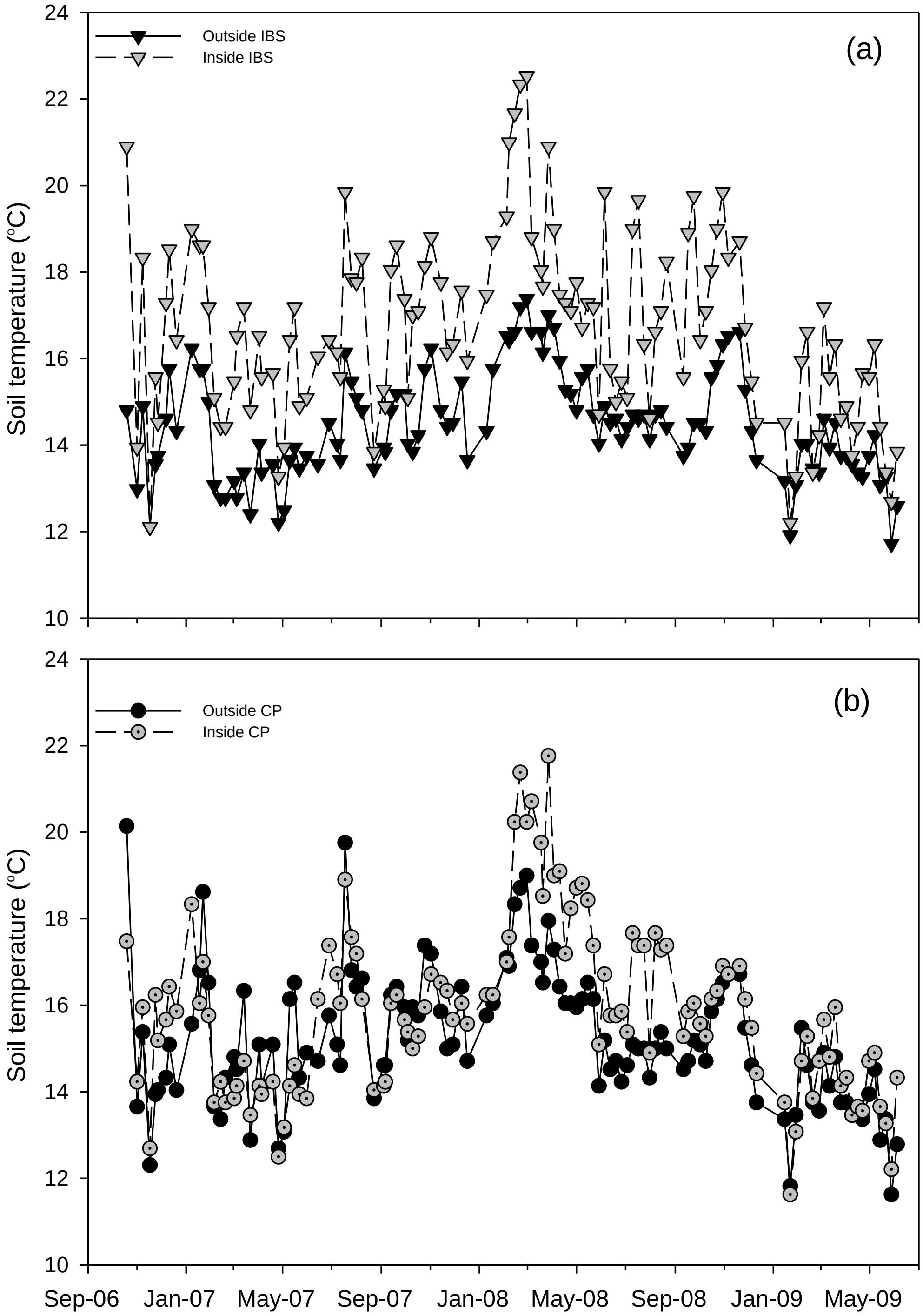


Figure 3.5 Soil temperature measurements from the Hobo pendant temperature loggers placed within and outside automated gas flux chambers in the IBS (a) and the CP (b). Data points correspond to the date and sample time of flux measurements by the automated flux chambers.

3.3.4 Temporal trends in soil inorganic N content and N_2O flux

Soil available mineral-N data (NO_3^--N and NH_4^+-N) for both plots generally varied concordantly, with lower summer concentrations compared to winter, and with higher concentrations in 2008 compared with 2007 (Figure 3.6). Soil NO_3^--N concentrations in the IBS and CP follow the same trend as the N_2O fluxes of generally lower concentrations during the growing season. Mean concentrations of NH_4^+-N from the IBS and CP, (8.15 ± 4.29 and 7.18 ± 3.75, respectively) are greater than mean NO_3^--N concentrations (6.91 ± 5.68 and 6.71 ± 4.71 mg mineral-N g^{-1} dry soil, respectively). However, the maximum soil concentrations of both NO_3^--N and NH_4^+-N from the IBS (24.1 ± 0.34 and 20.8 ± 0.49 71 mg mineral-N g^{-1} dry soil, respectively) are greater than those measured in the CP (18.1 ± 0.44 and 17.5 ± 0.04 71 mg mineral-N g^{-1} dry soil, respectively).

During the autumn/winter period (i.e. the non-growing season) of 2008/2009 soil NO_3^--N increased to the highest recorded values of 24.1 ± 0.34 and 18.1 ± 0.44 mg NO_3^--N g^{-1} dry soil in the IBS and CP, respectively (Figure 3.6) which corresponds with the lowest N_2O fluxes (Figure 3.2) and lowest rainfalls (Figure 3.3) recorded during the study period also occurring at this time. Highest concentrations of soil NH_4^+-N generally occurred during the non-growing seasons with the exception of a high concentration of 20.8 ± 0.49 and 17.5 ± 0.04 mg NH_4^+-N g^{-1} dry soil recorded on 26th July 2007 from the IBS and CP, respectively. During July 2007 the grass within the plots was cut and the high soil NH_4^+-N was most likely due to the remineralisation of the cut grass. Soil NH_4^+-N and NO_3^--N concentrations followed normal and non-normal distributions, respectively, (Anderson-Darling test): subsequent statistical tests (2-sample t-test and Mann-Whitney tests, respectively), found no statistical difference existed in inorganic-N contents between the two grassland plots ($P > 0.05$, $n = 46$). Therefore, irrigation of the IBS has not altered the underlying inorganic-N content of the soils compared to the control, confirming hypothesis two.

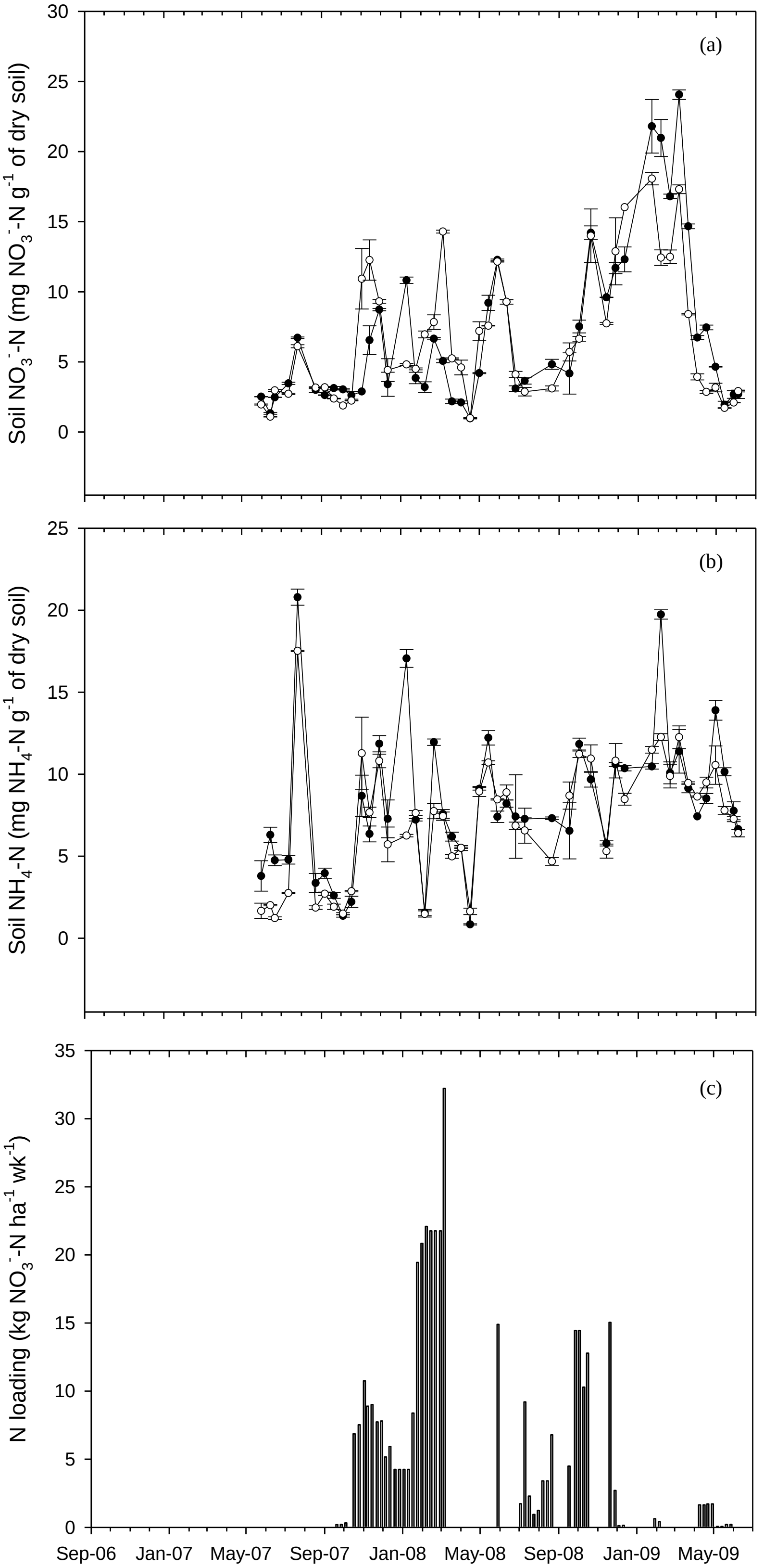


Figure 3.6: (a) Soil NO_3^--N for the IBS (black circles) and the CP (white circles) (b) soil NH_4^+-N for the IBS (black circles) and CP (white circles) and (c) weekly NO_3^--N loading via the irrigation water to the IBS.

3.3.5 Temporal and spatial trends soil water-filled pore space and related N_2O emissions

Although the WFPS dataset was limited due to equipment failures some interesting patterns were identified. Percentage WFPS in the IBS was higher compared to that in the CP when irrigation occurred (Figure 3.7).

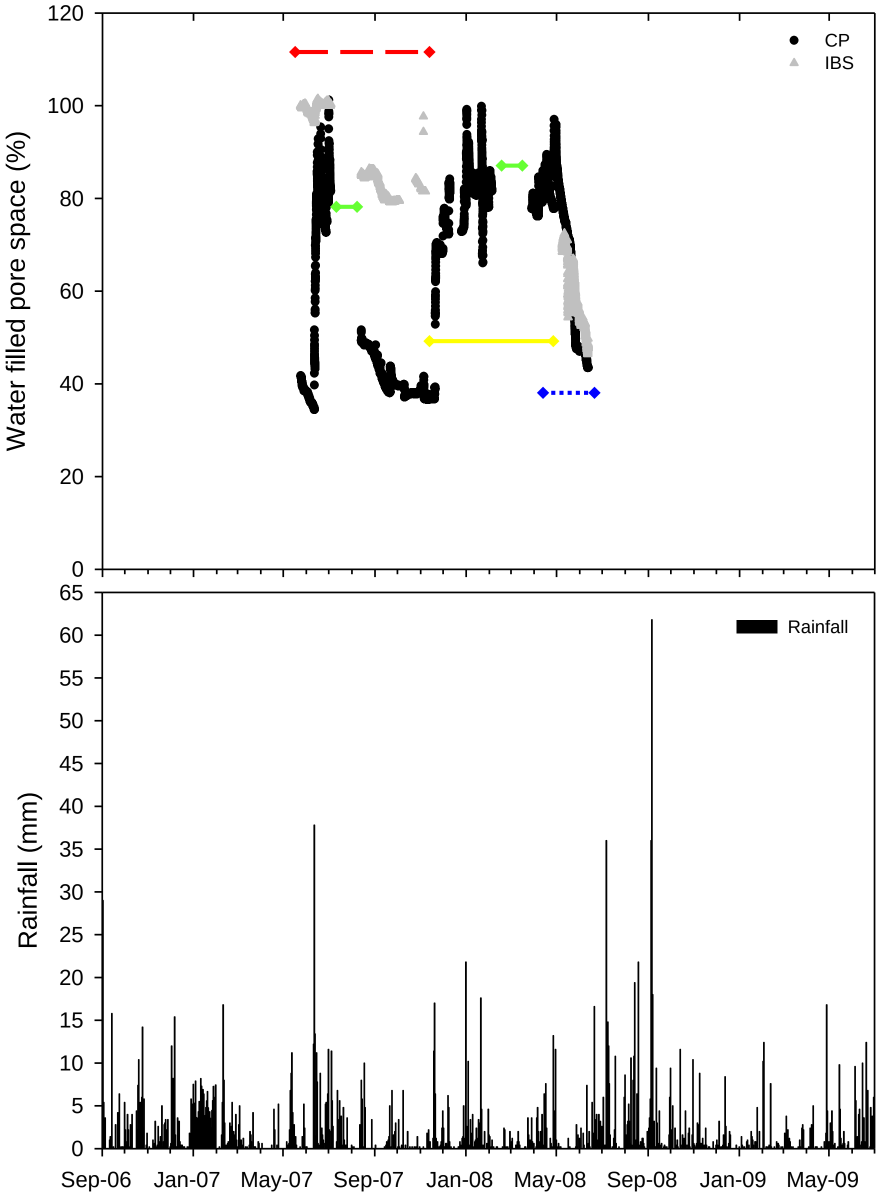


Figure 3.7 (a) Mean percentage water filled pore space (WFPS) in the IBS (grey circles) and CP (black circles), recorded every 30 minutes at two locations within each plot at 0-30 cm depth and (b) daily rainfall. The red dashed line indicates irrigation to the IBS and the blue dashed line indicates no irrigation to the IBS. Gaps in the dataset were due to equipment failures, solid green lines relate to missing data for both the IBS and the CP whereas solid yellow lines relate to missing data for the IBS only (Figure (a)).

Under irrigated conditions WFPS in the IBS varied between 85 and 100%, compared to 40 - 50% WFPS in the un-irrigated CP. After times of high rainfall, for example June 2007, WFPS in the control plot rapidly increased and then decreased. However, WFPS in the IBS responded by increasing rapidly to full capacity (100% WFPS) and maintaining a much higher percentage WFPS than the control plot until after the rainy period. Low rainfall at the end of May and during the first 10 days of June resulted in a WFPS of less than 40% in the CP, whereas irrigation of the IBS maintained WFPS at almost 100%, due to the overpowering effect of the high irrigation rate. However, the non-irrigated CP shows a marked increase in WFPS, from approximately 40% before and between 80 and 100% after the prolonged heavy rainfall event (Figure 3.8).

An intensive study into the effect of irrigation on the water content of the soil illustrates quite clearly that in areas closer to the irrigation boom there was a greater frequency of change in WFPS and a higher baseline percentage WFPS than in those furthest away from it (Figure 3.8). Therefore proximity to the irrigation boom in the IBS may have affected the spatial distribution of N_2O emissions due to the different water contents of the underlying soils.

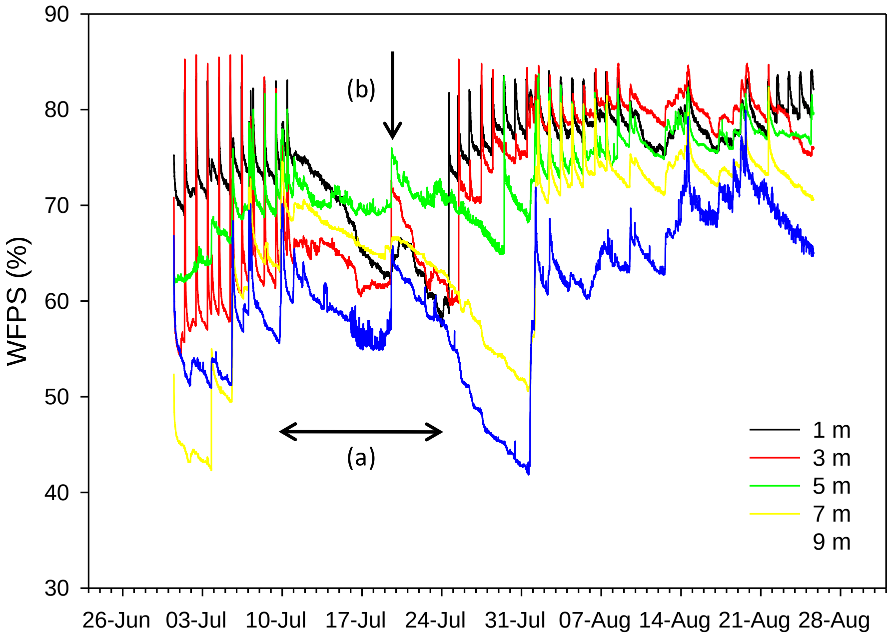


Figure 3.8 Transect of soil moisture within the IBS over an intensive study period July - August 2008. WFPS was measured at five minute intervals at 1, 3, 5, 7 and 9 m distance from the irrigation boom with one probe buried at 0-10 cm depth at each location. Arrows (a) and (b) are explained in the text below. Additionally frequent peaks denote the irrigation events.

At times of irrigation in the IBS WFPS increases rapidly over a period of 30 minutes followed by a much slower return to the baseline WFPS over a period of several hours. The frequent high peaks on Figure 3.8 illustrate these irrigation events. A break in irrigation between the 10th and 24th of July 2008 (Figure 3.8 arrow (a)) shows the effect that a rainfall event on the 19th July (Figure 3.8 arrow (b)) had on the water content of soils; there was a lower peak WFPS followed by a very gradual return to baseline conditions taking several days, as opposed to several hours after irrigation. Additionally the return to irrigation on 24th July resulted in an immediate increase in WFPS in soils within 3 m of the irrigation boom but for soils at 9 m from the irrigation boom an increase in WFPS did not occur until seven days after irrigation resumed.

3.3.6 Factors controlling N_2O fluxes

Correlation analyses were conducted to investigate the factors controlling N_2O fluxes from the plots. No measure of temperature (air or soil) could explain the observed fluxes from either the IBS or the CP. Likewise, available NO_3^--N and NH_4^+-N, known substrates for denitrification and nitrification respectively, did not have statistically significant relationships with N_2O flux ($P > 0.05$). Higher NO_3^- availability did not produce significantly higher fluxes, indicating that N_2O flux was not always limited by substrate availability.

A correlation between the observed N_2O fluxes from the IBS and CP shows a weak positive but highly significant relationship (Figure 3.9). The statistically highly significant ($P < 0.001$) correlation of fluxes from both plots suggests that the fluxes are created by the same driving factors, e.g. soil aeration, NO_3^- availability and temperature. Also, it appears that the irrigation of the IBS does not significantly alter the overall flux of N_2O compared with the control, as evidenced by the strong correlation between fluxes and may indicate potentially high leaching losses are occurring.

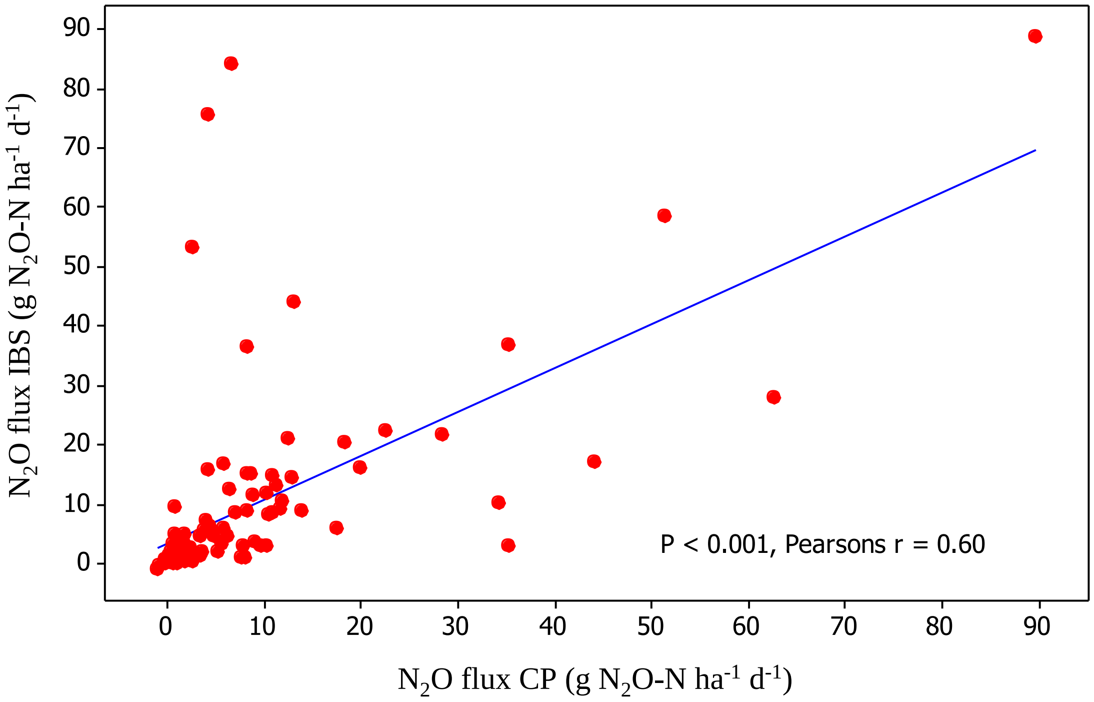


Figure 3.9 Correlation between the mean weekly N_2O flux (n = 118) from the static chambers within the IBS and CP. The blue line represents the linear regression (IBS = 0.49 + 3.1 CP).

Regressions performed using the short term intensive WFPS data from a transect of soil moisture probes within the IBS showed a strong positive relationship with the N_2O flux produced. At some locations higher WFPS (up to 80%) generated greater N_2O fluxes, with the relationships being statistically significant (Figure 3.10).

Fluxes from the chambers closest to the irrigating boom (1 m and 3 m), had weakly positive but not statistically significant relationships (P-value = 0.246 and P = 0.127 respectively, n = 24) with WFPS. These flux chambers experienced high WFPS for more prolonged periods than those further away, with the exception of chambers located at 5 m distance. Very low N_2O fluxes (< 2 g N_2O-N ha^{-1} d^{-1}) were observed at 1 m from the irrigation boom on a few occasions where WFPS ranged from 60 - 80%. Additionally, the greatest fluxes (between 20 and 40 g N_2O-N ha^{-1} d^{-1}), observed at 1 m from the irrigating boom occurred between 77 - 80% WFPS, with flux declining at WFPS greater than 80%, (< 2 g N_2O-N ha^{-1} d^{-1}). At WFPS of < 75% fluxes were < 5 g N_2O-N ha^{-1} d^{-1}.

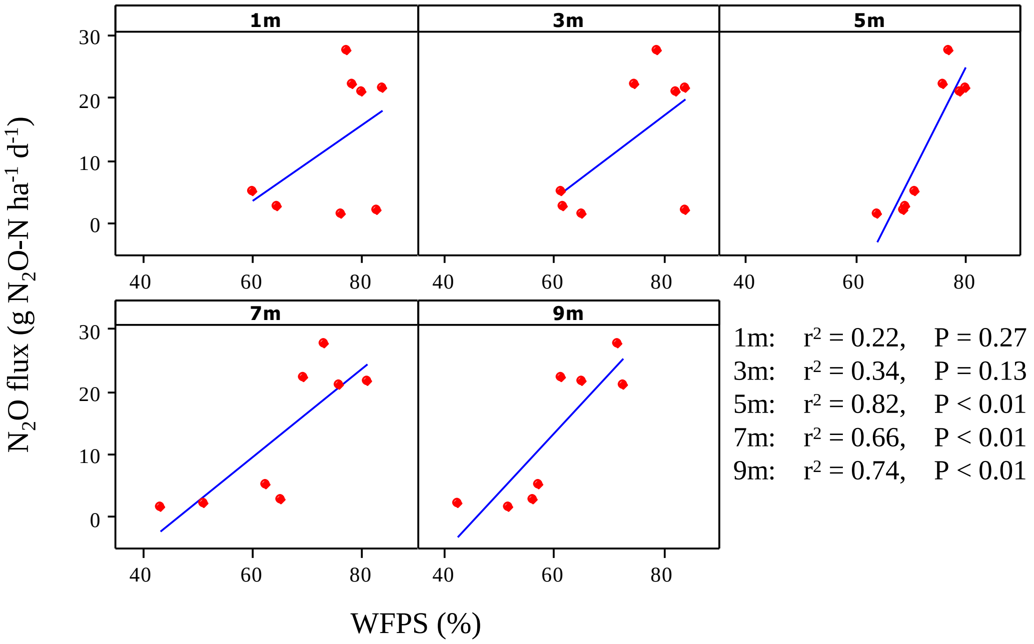


Figure 3.10 N_2O flux from the IBS versus WFPS at different distance down slope from the irrigating boom, 1 - 9 m, July-August 2008.

The strongest relationship between flux and WFPS (r^2 0.819 and P = 0.002) was from the chambers located at 5 m distance from the boom, where N_2O flux was still high despite WFPS ranging between 60 and 80%. WFPS at this location within the IBS remained higher (~ 70%) than at other locations within the IBS during times of no irrigation, suggesting a higher baseline water level.

Soils beneath chambers located further from the irrigating boom (at 7 m and 9 m) experienced a wider range of WFPS (from 40 to 80%) and had a lower baseline WFPS. Accordingly, N_2O fluxes were significantly positively related to WFPS at 7 and 9 m ($P < 0.01$, $r^2 = 0.7$, n = 24 and $P < 0.01$, $r^2 = 0.7$, n = 16, respectively).

Regressions performed for the IBS between N_2O flux and WFPS measured by the TDR probes (Figure 3.11), showed both weak positive and negative relationships which were not significant. This suggests that the mean WFPS measured by the TDR probes were unable to capture the spatial variability and differences in flux generation within the IBS. Even when the flux data was separated into chambers closest to the irrigation boom (chambers 1-3) (Figure 3.11 (b)) and those further away (chambers 4 - 8) (Figure 3.11 (c)) corresponding to the two locations of the TDR probes within the IBS, in an attempt to capture spatial variability, no significant relationships were observed.

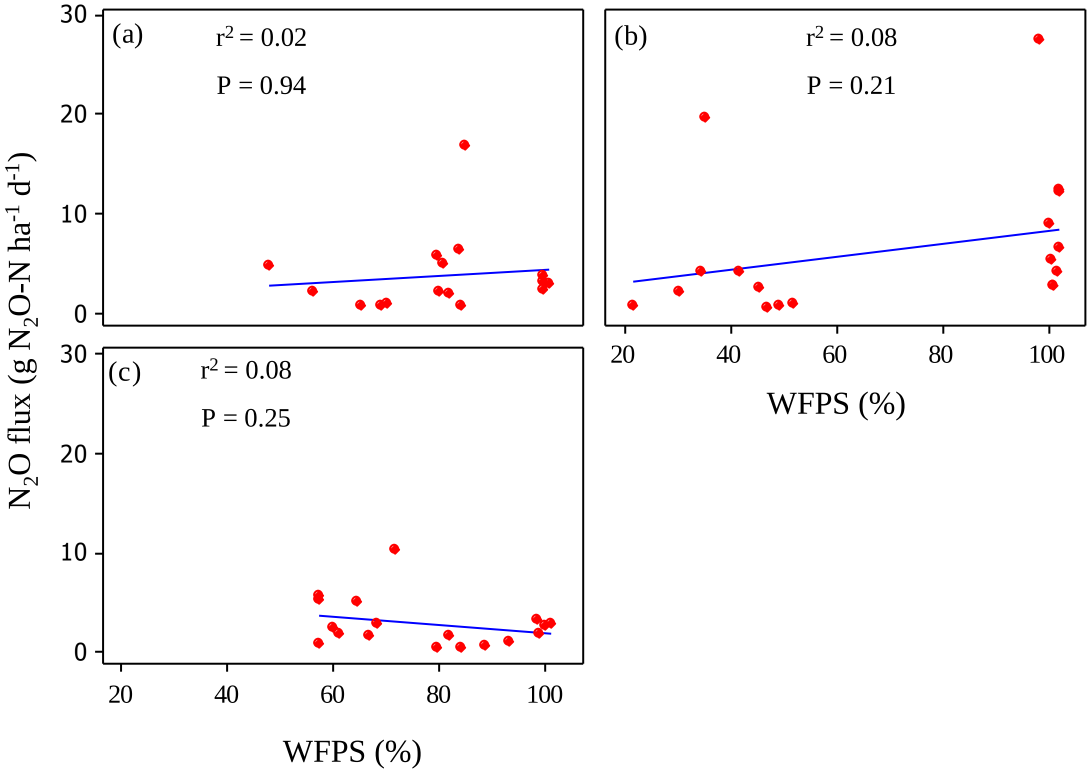


Figure 3.11 Regression relationships between (a) mean weekly N_2O flux and mean WFPS, (b) mean weekly flux from static chambers 1 -3 and WFPS measured near to these chambers at the 'top' of the IBS, (c) mean weekly flux from static chambers 4 - 8 and WFPS measured near to these chambers at the 'bottom' of the IBS, (see Figure 3.1 for positioning of TDR probes and static chambers within the IBS).

3.4 Discussion

3.4.1 Flux magnitude

The observed N_2O fluxes from Nafferton Farm are consistent in magnitude with published values of N_2O fluxes from similar grassland environments within the UK (Conen *et al.*, 2000; Dobbie and Smith, 2003; Jones *et al.*, 2005). N_2O emissions over the whole sampling period (2006 - 2009) from the IBS and CP were not significantly different from one another. Furthermore, the fluxes from both plots are significantly positively correlated, suggesting that the fluxes generated from the plots are created by the same environmental or meteorological driving factors such as temperature, substrate availability or soil aeration (Nieder and Benbi, 2008). Therefore, there is insufficient evidence to reject the null hypothesis i.e. the application of N-rich agricultural drainage water to the IBS did not significantly increase direct N_2O emissions compared to the control: confirming hypothesis one. However, the lack of replication of the treatment and control plots may have obscured inherent differences/similarities of the two grassland plots it is therefore difficult to conclusively state this finding of no significant difference in direct N_2O emissions as a result of irrigation.

The generally low N_2O fluxes from the IBS compared to the CP throughout the sampling period, despite these high NO_3^--N additions, suggest that NO_3^--N leaching may have been high. Although complete denitrification to N_2 gas at times of high WFPS may also have contributed to the low N_2O fluxes from the IBS, albeit in a minor way. Leached NO_3^--N was not measured for this study but subsequent core incubations (Chapter 4) that mimic the environmental and irrigation conditions observed at the field site have been carried with the aim of better understanding N cycling and losses within these soils.

3.4.2 The N loading effects on N_2O emissions and inorganic-N content of the soil

NO_3^- loadings received by the IBS from irrigation ranged from 0 to 32.2 kg NO_3^--N ha^{-1} wk^{-1} in 2007 and 2008. The cumulative loading of NO_3^--N received by the IBS for 2008 was c. 500 kg NO_3^--N ha^{-1}. This amount greatly exceeds the maximum annual application rate of N fertiliser (170 kg N ha^{-1} yr^{-1}) for farms within NVZs, and is double that for grassland farms given the higher N application limit of 250 kg N ha^{-1} yr^{-1} under derogation (Scottish Executive, 2005a).

The available inorganic-N pool in both plots varies concordantly during the different seasons. However, on some occasions, such as during November 2007 NO_3^--N and NH_4^+-N were greater in the CP than the IBS. Additionally, greater soil aeration occurred in the CP as evidenced by the lower WFPS (c. 40%) compared with the IBS (c. 80%). Under more oxygenated conditions, NH_4^+-N is oxidised to NO_3^--N by the process of nitrification. The higher NO_3^--N pool within the CP could result in a higher rate of nitrification compared to the

IBS. Conversely, a higher WFPS in the IBS would create suboxic or anoxic conditions within the soil under which denitrification would primarily dominate the N transformations and therefore reduce the NO_3^--N pool within the soil. However, the high N loading supplied to the IBS via the irrigation water has not altered the underlying inorganic-N content of the soils compared to the control: confirming hypothesis two. An explanation for this is enhanced leaching of the applied N from the IBS and/or that gaseous N loss via denitrification was enhanced. This hypothesis was tested in further process studies using soil cores, which are reported in Chapter 4.

3.4.3 Controls on temporal variability in emissions

The surface emissions of N gases are the net effect of production and consumption processes based on the prevailing soil conditions. It is likely, however, that site specific factors such as soil type, structure and compaction affect gas movement, be it O_2 from the atmosphere into the soil or products of microbial processes within the soil, as well as the development of anaerobic microsites (Smith, 1980; Stevens *et al,* 1997; Batemann and Baggs, 2005). Furthermore, the localised factors of soil temperature, substrate type and availability and microbial communities present, will all play a part in determining which of these processes dominate the type of N fluxes from this site.

The highest N_2O fluxes from both plots, with the exception of the slurry-induced high fluxes in March 2008, were measured during the summer months of 2007 and 2008. The CP emitted 45% less N_2O-N ha^{-1} than the IBS during the summer of 2007 and 27% more N_2O-N ha^{-1} than the IBS in the summer of 2008. This is not an unexpected finding, as summer mean monthly temperatures reach the highest of any time of the year, at 18.3 and 19.9°C for 2007 and 2008, respectively, increasing the activity of the microbial organisms responsible for nitrification and denitrification (Saad and Conrad, 1993; Abdalla *et al.,* 2010). Maag and Vinther (1996) suggest nitrification reaches maximum activity at 20°C whereas denitrification peaks at a higher temperature between 25 or 30°C (Saad and Conrad, 1993).

The predominance of either of these two microbial processes is determined by the prevailing environmental conditions, largely driven by the aerobic or anaerobic status of the soils (Wolf and Russow, 2000; Smith *et al.,* 2003; Mathieu *et al.,* 2006). Additionally, the total rainfall during the summer months was again the greatest of any season, accounting for 41.2% and 34.9% of total annual rainfall in 2007 and 2008, respectively. Higher rainfall reduces the available air filled pore space within the soils and this in turn can increase the size and number of denitrifying microsites (Smith, 1980). The redox state of these microsites will determine the N flux produced; N_2O under suboxic conditions or N_2 under strongly reducing conditions (Ambus, 1998; Dobbie and Smith, 2001). Moreover, diffusive movement of gases through the soil is limited under saturated conditions, reducing net emissions and a suppression of N fluxes from the IBS. However, the soil under warm and very wet conditions (i.e. summer conditions) may become a net N_2O sink by the downward diffusion of atmospheric N_2O into the soil as evidenced by net negative fluxes observed both in this work, from the IBS and CP, and by others (Vieten *et al.*, 2008; Vieten *et al.*, 2009; Neftel *et al.*, 2010). This phenomenon may occur more frequently as the global climate warms.

Seasonally, lowest fluxes from both plots occur in the winter months, in particular the winter of 2008/2009 when soil temperatures reached a minimum of 0.47°C and the rainfall received was 96 mm: 37% less than the previous winter. The combined effect of low temperature (decreasing microbial activity) and rainfall (reducing substrate supply and increasing soil aeration) would most likely have generated the low fluxes (Firestone *et al.*, 1979; Conen and Neftel, 2007).

Annual N_2O emissions from both the IBS and CP were not significantly different and mean weekly fluxes from both plots in 2007 were not correlated. But mean weekly fluxes in 2008 were positively correlated ($r^2 = 0.9$), although again not statistically different. This difference in behaviour of the two plots between 2007 and 2008 can be explained through a combination of environmental factors and changes to the IBS as a result of prolonged irrigation. Irrigation to the IBS was greater in 2007 than in 2008, averaging 344 and 150 mm wk^{-1} respectively, with only 6 weeks receiving no irrigation in 2007. In contrast in 2008 there was no irrigation for almost 4 months, from March to June, and intermittent irrigation for the remainder of the year, due to power supply failures. The lack of correlation between the observed fluxes from the IBS and the CP in 2007 is likely due to the difference in primary factors driving the fluxes, i.e. the CP is more greatly affected by rainfall events and soil temperature whereas percentage WFPS plays a much greater role in the IBS due to the irrigation regime. The effect of the high irrigation rates and NO_3^--N loadings on the IBS probably led to conditions more optimised for denitrification and dominated the effects that other environmental factors and temporal changes such as rainfall or temperature may have had on N_2O flux. Additionally N_2O fluxes from the IBS were very low when WFPS was at or near to 100%, due to optimal conditions for denitrification and thus the reduction of N_2O to N_2. Equally the reduced irrigation to the IBS in 2008 would account for the significant positive correlation between the fluxes from both plots, as they are both affected primarily by the same environmental factors.

Short-term (daily) temporal variations in N_2O flux are largely driven by soil temperature that undergoes a diurnal fluctuation coincident with air temperature. A recent study by Alves et al, (2012) has shown that the time most representative of the daily mean emissions was either 09:00 to 10:00 am or 21:00 to 22:00 pm. Daily sampling of the IBS and CP using the static chamber methods was conducted between 09:00 am to 15:00 pm, but mostly between 12:00 to 14:00 pm, due to the travel time required between Edinburgh and Newcastle on field sampling occasions. Furthermore, sampling using the automated system was always between 12:00 to 13:00 pm, set by the pre-defined program. Avles et al, (2012) also found that highest mean soil temperature (the greatest variable for N_2O emissions); occurred at 15:00 pm. This may suggest that the ‘daily mean’ emissions measured at the site may have overestimated the actual daily mean, had samples been taken out-with the suggested peak soil temperature time (i.e. 15:00 pm). However, as shown by Alves et al, *(*2012) irrigation also produced increased N_2O emissions. Irrigation of the IBS (at 12:00 pm) often occurred before gas sampling by either the static or automated systems; this may have skewed the dataset to higher emissions (or potentially lower if WFPS was very high, leading to a greater proportion of N_2 rather than N_2O emissions), recorded for the IBS on these occasions, than would be expected for the daily mean.

3.4.4 Controls on spatial variability in emissions

N_2O fluxes are notoriously spatially variable due to the range of biological, environmental and meteorological factors that affect the microbial processes of nitrogen cycling: primarily aerobic nitrification and anaerobic denitrification as well as substrate supply (Killham, 1994; Vitousek *et al.*, 1997; Wrage, 2001). The irrigation of the IBS has apparently created hotspots of denitrification in the soil; however these appear to change location with time. In the initial period after irrigation began flux chambers positioned closer to the irrigating boom were extremely large (> 380 g N_2O-N ha^{-1} d^{-1}), compared to a mean plot flux (53.4 g N_2O-N ha^{-1} d^{-1}). However, after several months of irrigation chambers positioned closer to the irrigating boom had much lower emissions than those further away. This may suggest that the irrigation regime had caused a priming effect in the IBS and potentially altered the soil microbial community to favour denitrification, due to the long time period that the soils experienced high WFPS. However, no microbial analysis was carried out for this research so this cannot be confirmed.

"Hotspots" of N_2O emissions exist where conditions are suboptimal for complete denitrification: depleted oxygen availability, greater than 60% but less than 100% WFPS, warm soil temperatures, and abundant available NO_3^- substrate and C source exist (Davidson, 1991; Wolfe and Russow, 2000; Ruser *et al.,* 2006). Under suboptimal conditions denitrification is incomplete and as a result N_2O gas is produced. It is also likely that both nitrification and denitrification are occurring in close proximity to one another, and diffusion of N_2O into an oxygenated pore space would prevent further reduction and thus the release of N_2O gas to the atmosphere (Smith *et al.,* 2003).

Hotspots are not limited to those created as a result of changing WFPS (as a result of either irrigation or rainfall). Rather, hotspots are also the result of localised anaerobic zones where levels of substrate allow for high respiration of microbes, leading to anoxic conditions (Smith *et al.,* 2003). These anaerobic zones are commonly associated with areas where livestock have urinated or defecated (Skiba *et al.*, 1998). However, localised areas of high nitrification in aerated soils associated with urine patches have also been found, creating aerobic hotspots for N_2O production (Carter, 1995). Van der Weerden et al. (2011) suggest that N_2O emission associated with urine patches occurs during the first ten days after application; however, increased emissions of up to 30 days were also observed. A delay in N_2O emissions associated with dung deposition is often reported (Allan *et al.*, 1996; van Groenigen *et al.*, 2004; van der Weerden *et al.*, 2011). Allen *et al.*, (1996) observed N_2O emissions associated with dung deposition up to 60 days after application, whereas van der Weerden *et al,* (2011) observed increased N_2O emissions between 125 and 173 days after dung application, although emissions during the final 1 - 2 months were low. This would suggest that dung incorporated into the soil prior to the start of the experiment (before plots were fenced and ungrazed by livestock) may represent hotspots of denitrification, such as potentially within the soil under chamber 9 in the CP. But dung effects are unlikely to affect plots after c. 6 months.

Increases in percentage WFPS within the CP apparently led to higher N_2O fluxes as denitrification most likely became the dominant microbial process - under suboxic conditions denitrification was incomplete and the intermediary product N_2O was produced. Spatial variability in fluxes also occurred in the CP, as higher N_2O fluxes were often measured at chamber 9 compared to the other chambers within the plot, especially following periods of high or persistent rainfall. This would suggest that chamber 9 was somehow more sensitive to changes in WFPS: for example after a moderate rainfall event, the N_2O flux from chamber 9 was much higher c. 80 g N_2O-N ha^{-1} d^{-1} compared to a mean of 4.0 ± 2.4 g N_2O-N ha^{-1} d^{-1} for the CP. A possible reason for this could be that a clay pipe, part of the old drainage system within the field, could lie near to chamber 9, supplying it with more nitrate-rich leachate. Alternatively, the frequently higher N_2O emissions from chamber 9 compared to the other chambers of the CP suggest that this chamber could overlie a ‘hotspot’ generated by some other mechanism, not necessarily WFPS, such as a higher level of organic material (C and N) for denitrification and therefore N_2O production (Skiba *et al.*, 1998). Hotspots of denitrification likely existed in both the IBS and the CP and were not constant over time.

The effects of long term irrigation appear to be short-lived as during 2009 the irrigation was minimal (0 - 20 mm wk^{-1}) and the fluxes generated were very low, demonstrating that the effects of long term irrigation do not continue into the next growing season. Therefore, if irrigation of a riparian buffer strip is well managed to maintain high WFPS levels and to reduce leachate losses, then enhanced denitrification can take place without generating high N_2O fluxes. The cessation of any irrigation treatment does not appear to have a lasting effect on soil N_2O fluxes, as the IBS has been shown to behave in the same way as the CP after irrigation ceased.

3.4.5 Policy implications for this mitigation method

The IPCC recommends a default direct N_2O emission factor (EF_1) for fertiliser and animal manure of 1% (uncertainty range of 0.3 - 3%) of the N applied to the soil emitted as N_2O (IPCC, 2006). Observed emissions from the control plot for 2008 correspond to an emission factor of 1.5%, assuming the N input via biological N fixation (BNF) was of the order of that determined by Vinther and Jensen (2000), in a similar environment of 10.0 - 23.5 g N m^{-2} per year (corresponding to 100 - 235 kg N ha^{-1}). In contrast, the emission factor from the irrigated plot over the same period was only 0.08%, based on the NO_3^--N addition and additional inputs from BNF.

This would suggest that either much of the added NO_3^- had been fully denitrified to N_2 or losses of NO_3^- (or indirect N_2O losses) via leaching were high. Despite the low direct emissions (i.e. no pollution swapping of increased N_2O emissions) this method of irrigating a riparian buffer strip may not be a suitable mitigation option due to potentially high leached losses of N.

3.4.6 The effect of automated gas flux chambers on soil temperature

Soil temperatures within chambers were found to be significantly higher than those outside in both the IBS and the CP, suggesting that the placement of chambers affects soil temperature in a localised way. The magnitude of mean temperature difference outside/inside the chambers is low, approximately 1°C, but on specific days there can be over 6°C difference.

Elevated temperatures increase microbial activity within the soil resulting in enhanced nitrification under oxic conditions and denitrification under suboxic/anoxic conditions, when substrates (NO_3^- and carbon) are not limiting, up to an optimum level after which biological activity is progressively inactivated (Tiedje, 1988; Saad and Conrad, 1993; Abdalla *et al.*, 2010). However, soil respiration (and therefore O_2 demand) likewise increases with increasing temperature, resulting in greater soil anaerobiosis (Smith and Arah, 1990; Zumft, 1997; Dobbie *et al.*, 1999; Smith *et al.*, 2003; Groffman *et al.*, 2006; Abdalla *et al.*, 2009; Butterbach-Bahl and Dannenmann, 2011). An increase in soil anaerobiosis would likely increase the potential for denitrification (Smith, 1980; Dobbie *et al.*, 1999; Wolf and Russow, 2000; Smith *et al.*, 2003). However, under increasingly anaerobic conditions N_2 is the endproduct of denitrification, equating to a reduction in the N_2O:N_2 ratio (Ambus, 1998; Butterbach-Bahl and Dannenmann, 2011). Therefore although temperature plays an important role in both governing the rate of microbial activity as well as soil respiration, a complex interplay of several factors collectively determines the resultant N_2O emissions (Robertson and Tiedje, 1987; Davidson, 1991; Smith and Dobbie, 2001; Bateman and Baggs, 2005).

Higher soil temperatures as discussed above have two main effects: increased microbial activity (Firestone, 1982; Maag and Vinther, 1999; Abdalla *et al.*, 2009) and increased soil respiration, leading to increased size and number of anaerobic microsites within the soil (Saad and Conrad, 1993; Groffman et al., 2006; Butterbach-Bahl and Dannenmann, 2011). Both of these factors could potentially enhance N_2O production within the soils and subsequent emissions from the areas covered by chambers, provided substrates are not limiting (Butterbach-Bahl and Dannenmann, 2011). Alternatively, if artificially higher soil temperatures are maintained within the chambers more anaerobic microsites may develop, resulting in complete denitrification to N_2 and reduced N_2O emissions.

That being said, the highest observed fluxes during March 2008 occur at mean soil temperatures of less than 5°C. These observations suggest that microbial activity is active even under low temperatures. Microbial communities in different geographical locations have been found to be adapted to the prevailing climatic conditions so that in temperate soils denitrifiers are capable of more rapidly denitrifying at lower temperatures than exotic denitrifying communities are able to (Powlson *et al.,* 1988; Dorland and Beauchamp, 1991). Therefore high temperatures do not necessarily produce high emissions, as demonstrated by the findings of this research, but increasing temperatures such as those artificially created within chambers may have created hotspots of microbial activity within the soil beneath chambers. This would suggest that the presence of chambers could enhance microbial activity and emissions of either N_2O or N_2 from these locations, compared with adjacent areas not covered by chambers. It is therefore likely that the artificially stimulated higher temperatures would have affected the resultant fluxes either positively or negatively on some occasions, dependent upon other factors such as WFPS.

This localised temperature effect is similar in both plots and it is likely that all chambers, both within this study and other studies, will be affected in a similar way and would not affect the overall findings in terms of inter-plot trends and variations. However, the magnitude of N_2O flux could be underestimated if these higher soil temperatures within the soils beneath chambers resulted in the greater production of N_2, rather than N_2O. Future studies should consider removing chambers from the field site except when carrying out measurements, to prevent any hotspot of activity being generated. Furthermore, different materials for chamber construction could be investigated to determine if any material(s) may be better suited to prevent artificially maintaining warmer soil underneath the chambers, compared to the surrounding soil not covered by a chamber.

3.5 Conclusions

Despite the lack of replication of experimental plots, some tentative conclusions can be drawn. N_2O emissions are apparently not increased as a result of irrigating a riparian grassland buffer strip. Additionally the very low direct N_2O emission factor of 0.08% in 2008 from the IBS suggest that the bulk of additional N inputs via the irrigation water were either fully denitrified or that NO_3^- leaching and indirect N_2O emissions were enhanced.

The principle driver of N_2O emissions from temperate grassland was found to be percentage WFPS, with the greatest fluxes observed from between 70 and 85% WFPS. At higher percentage WFPS more NO_3^- was likely fully denitrified to N_2 gas and below this optimal WFPS range nitrification is likely to be an increasingly important microbial process for N_2O production within the soil. However, N loss by leaching has not been quantified. The questions remaining about N_2O production within the soils as well as losses through leaching lead to the need for further study and a series of incubation experiments using the field soils which are presented in Chapter 4.

Irrigation has been shown to increase spatial variability in N_2O flux and generates hotspots of denitrification within the irrigated area that change with time. However all changes are short-lived and do not continue into the subsequent growing season. Greatest fluxes from the IBS were observed when irrigation ceased or was reduced, allowing the WFPS to decrease into the optimal range for N_2O production. This would suggest that irrigation would need to be maintained to create optimal conditions for complete denitrification; however, associated leaching losses may be high. Laboratory incubation studies (Chapter 4) aim to determine if leaching losses from these soils are high and under which seasonal conditions emissions (direct and indirect), as well as leaching losses are most severe.

In the field, this research found that chambers affect soil temperature by increasing temperatures compared to outside the chamber. However, the artefact was relatively small (c. 1°C) and affected chambers equally from both plots and is therefore unlikely to have affected the trends and differences observed for the two experimental plots.

Chapter 4: N_2O emission and subsurface N_2O concentration in field and laboratory studies using a novel technique

4.1 Introduction

Diffuse nitrate (NO_3^-) pollution is a major problem affecting riverine, coastal and ground waters across the world (Vitousek *et al.,* 1997). High NO_3^- loadings can cause eutrophication and incur substantial economic costs to water companies required to comply with drinking water NO_3^- limits (Pretty *et al.,* 2000; Howarth *et al.,* 2000; Dodds *et al.,* 2009). Dodds et al, (2009) estimated the total annual cost of eutrophication in the USA at $2.2 billion primarily through recreational losses and devaluation of waterfront properties as a result of reduced water clarity, unpleasant odour and algal blooms.

Targeting potential point sources of NO_3^- pollution from agriculture such as farmyard runoff, effluents from milking sheds as well as manure storage areas should lead to reduced losses (Dawson and Smith, 2010; Novak and Firoelli, 2010). Additionally improved management of grasslands, fertiliser use, manure storage and application as well as improved farming practises are important to reduce point and non-point sources of NO_3^- pollution (Kay *et al.,* 2009). The use of mitigation measures such as buffer strips or constructed wetlands to intercept and reduce diffuse pollution from entering receiving waters is promoted within the UK, however, ‘pollution swapping’ is a major concern (Stevens and Quinton, 2009).

Within Europe, diffuse NO_3^- pollution is being addressed through several directives aimed at improving water quality, in particular the EC Nitrates Directive [91/676/EC] and the wider Water Framework Directive [2000/60/EC]. The implementation of the Nitrates Directive requires the designation of Nitrate Vulnerable Zones (NVZs) where surface and/or groundwater NO_3^- levels exceed 50 mg NO_3^- l^{-1}, within which farmers must adhere to codes of good farming practice controlling fertilizer/manure applications. The Directive also promotes the implementation of riparian buffer strips, to intercept and reduce diffuse pollution. Microbially-mediated denitrification is the key process utilised in such buffer strips to reduce NO_3^- through a series of intermediate products to nitrogen (N_2). Incomplete denitrification has the potential to release the potent greenhouse gas nitrous oxide (N_2O), thereby swapping a water pollution issue for a climate change problem (Hefting *et al.,* 2003; Reay *et al.,* 2003; Quinton and Stevens, 2009).

N_2O is produced within the soil from the combined effects of microbially mediated nitrification and denitrification (Granli and Brockman, 1994). During nitrification, NH_4^+ is oxidised to NO_3^-, however, if oxygen is limiting nitrite (NO_2^-) is used as an electron acceptor by NH_4^+ oxidisers to produce N_2O. Additionally during anaerobic microbial denitrification NO_3^- is reduced to N_2, however, incomplete denitrification can produce the powerful greenhouse gas N_2O.

These processes are principally controlled by the physical and chemical properties of the soil microsite, such as: soil temperature, soil moisture, and oxygen availability, nitrogen substrate availability (ammonium (NH_4^+) and NO_3^-) and carbon substrate availability (Davidson, 1991; Skiba *et al.*, 1993; Maag and Vinther, 1996; Skiba *et al.*, 1998, Dobbie *et al.*, 1999; Skiba and Smith, 2000; Dobbie and Smith, 2006; Inselbacher *et al.*, 2009). However, these controls are influenced by soil properties (organic matter content, pH, bulk density, and clay content), weather, vegetation, and land-use management (fertiliser and manure application, irrigation and compaction) (Bremner, 1997; Lowrance *et al.,* 1997; Skiba and Smith, 2000; Sabater *et al*., 2003; Bateman and Baggs, 2005; Baggs, 2011).

The complex interactions of all these factors determine the dominant microbial process and the resulting gaseous emissions produced. The heterogeneity of soils produces microsites of denitrification and nitrification operating side-by-side, further increasing the complexity of nitrogen cycling within soils (van Cleemput, 1998; Skiba and Smith, 2000; Bateman and Baggs, 2005). Additionally, both NO_3^- and N_2O are highly soluble in water (van Cleemput, 1998), therefore very mobile and easily transported from the terrestrial to aquatic environments with the potential for increased water pollution or indirect N_2O emissions (Hefting *et al.,* 2003; Reay *et al.,* 2003).

4.1.1 Characteristics and benefits of the Accurel® membrane tube

This research will measure *in situ* N_2O production from within the soil profile in a field setting as well as within intact monolith cores in a laboratory setting using Accurel® probes. The key properties of the Accurel® membrane tube (PP V8/2) used for this research are given in Table 4.1.

Table 4.1: Characteristics and physical properties of the Accurel® membrane tube (PP V8/2) as given by the manufacturer.

Characteristic	**PP V8/2 HF**
Polymer type	Polypropylene
Residual oil content	≤ 500 ppm
Wall thickness (mean ± SD)	1550 ± 150 µm
Inner diameter (mean ± SD)	5500 ± 300 µm
Burst pressure (at 25°C)	8 bar
Implosion pressure (at 25°C)	2.5 bar

Accurel® is a polypropylene hydrophobic capillary membrane with a nominal pore size of 0.2 µm (Figure 4.1). This allows soil pore space gas to pass through the capillary membrane, even under water logged conditions (Gut *et al.*, 1998; Neftel *et al.*, 1998).

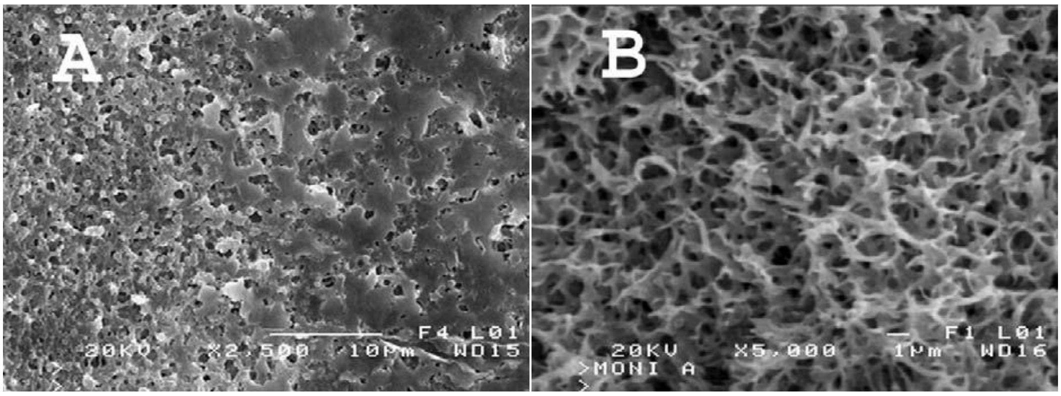


Figure 4.1 SEM image of the Accurel® PP V8/2 HF membrane illustrating the internal surface (A) and a cross-sectional view (B) (source: Barancewicz, 2012).

These Accurel® membranes are commonly used in microfiltration processes (Accurel® factsheet, Membrana). However, these membranes are chemically inert and mechanically stable making them very useful for measuring soil trace gases via a low-invasive method (Neftel *et al.*, 1998).

4.1.2 Experimental rationale

N_2O emissions from grasslands vary greatly due to the heterogeneity of soils and the complex interaction of biological processes that govern the N-cycle, principally denitrification and nitrification (Skiba and Smith, 2000; Bateman and Baggs, 2005). The selection of depth intervals to measure N_2O production is based on evidence from previous studies. For example, Smith *et al.* (1998) found that temporal relationships between diurnal cycles in temperature and emissions are indicative of N_2O originating from different depths in the soil profile. They hypothesized that peak surface emissions from soil monolith experiments originated from the upper 5-10 cm depth when they coincided with peak temperature. However; when a lag time of several hours between peak emissions and peak temperature occurred they hypothesized that emissions originated from a greater depth. The use of Accurel® probes enables the researcher a non-destructive method to sample intra-soil gas and to identify the source depth of N_2O emissions within the soil profile (Gut *et al.*, 1998; Neftel *et al.*, 1998). The probe assembly was adapted for use both in the field and in laboratory studies reported here.

The presence of the two distinct soil horizons in the uppermost 50 cm of the soil (0 to 20 and 20 to 40 cm), combined with the knowledge of the organic N content as well as the dynamic nature of soil microbial processes suggested the need for selecting regular intervals of 5 - 10 cm to capture the nature of intra-soil N_2O production. On this basis field measurements of N_2O concentration were conducted at 2-8, 10-20, 20-30, 30-40 and 40-50 cm below the ground surface using Accurel® tubing (Accurel PP V8/2 HF hydrophobic tube membrane, Membrana GmbH, Wuppertal, Germany) (Gut *et al.*, 1998).

Field measurements of N_2O concentration with depth were carried out from January to April 2009. This was followed by a series of controlled experiments using intact soil monoliths of 40 cm depth.

The laboratory experiments were in part designed to clarify a key question that remained unanswered from Chapter 3: Does leaching occur? Additionally, these experiments were devised with the aim of providing a better understanding of the processes of N_2O production and consumption within the soil profile in response to controlled conditions of the known key drivers: N application, temperature and percentage water filled pore space (WFPS).

The experiments were designed to mimic the *in situ* field conditions experienced at Nafferton Farm throughout the seasons (see Chapter 3).

The key hypotheses tested were as follows:

(1) - N_2O production within the soil profile is greater than that emitted at the soil surface.

(2) - Irrigation will produce N-loss in leachate.

(3) - Higher N application in the irrigation water will increase N_2O production within the soil profile and concurrent emissions.

4.2 Materials and methods

4.2.1 In situ N_2O profile concentrations within the IBS at Nafferton Farm

Above surface N_2O measurements were taken as the average of static chambers number 1 to 3 within the IBS (see Chapter 3.2.3 and Figure 3.1 Chapter 3). Soil profile gas samples were collected using Accurel® tubing (Accurel Systems, Membrana GmbH), a gas permeable but liquid-impermeable plastic tube (0.3 cm in diameter). Three sets of Accurel® probes were positioned in a line across the width of the IBS approximately 1 m from the irrigating boom, using a designed metal spike equal to the dimensions of the Accurel® probe assembly. Replicate 1 was near to static chamber 1; replicate 2 to static chamber 2 and replicate 3 to static chamber 3 (Figure 4.1, see also Figure 3.1 Chapter 3). All results reported are an average of triplicate measurements unless otherwise stated. The replicate Accurel® probes were positioned at five depths beneath the ground surface: 2 - 10, 10 - 20, 20 - 30, 30 - 40 and 40 - 50 cm.

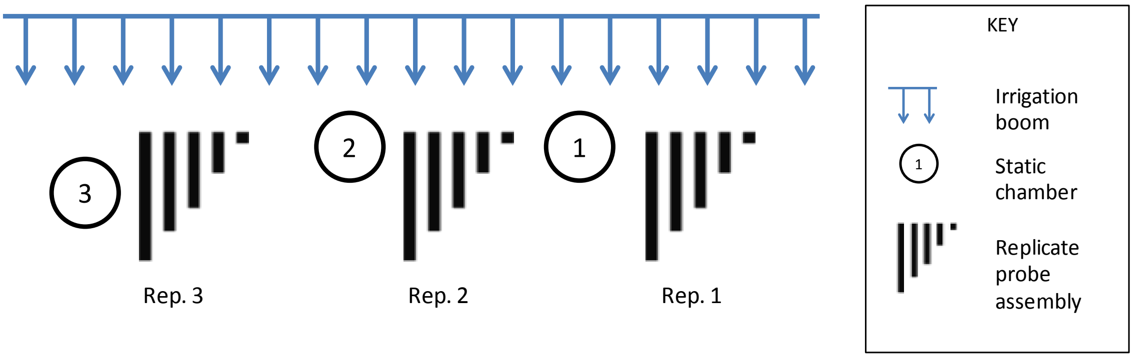


Figure 4.1: Position of the replicate Accurel® probe assemblies (labelled as Rep. 1, 2 and 3, respectively) in relation to the irrigation boom and static chambers (see key). Each assembly is comprised of five probes positioned at the specified sampling depths beneath the ground surface

The Accurel® probe assembly (hereafter referred to as the Accurel® probe) had a total length of 70 cm: 10 cm of exposed Accurel® tubing at the base of the probe with the remaining 60 cm of Accurel® tubing contained within a plastic pipe bonded together using araldite (Figure 4.2). The insertion spike had the same external diameters as both the plastic pipe and Accurel® tube component parts of the probe assembly.

The spike was inserted first into the soil until the desired depth was reached before being removed, allowing the probe assembly to easily slot into position without causing damage to the probe. The probes are gas permeable, therefore due to diffusion, gas within the probes equilibrates with the soil gas, providing a non-destructive method of measuring internal N_2O concentrations within soil profiles. For all depths the active length of Accurel® was 10 cm with the exception of the probe sampling at 2 - 10 cm depth where the active length was 8 cm. This ‘surface’ probe sampled only 2 - 10 cm beneath the soil surface to ensure that diffusion of atmospheric air into the top layer of the soil did not diffuse across the Accurel® probes creating a ‘false’ ambient N_2O concentration.

The internal diameter of the Accurel® tube is 0.3 cm; the active length plus the additional length of Accurrel® contained within the plastic pipe of the probe resulted in slight internal volume differences (Table 4.1). But the volume of air sampled from each probe was the same and therefore the total probe volume difference did not affect results.

Table 4.1: Internal probe volumes for in situ Accurel® probes

Probe depth (cm)	Total internal volume (cm^3)
0-10	27.8
10-20	33.7
20-30	45.1
30-40	56.5
40-50	67.9

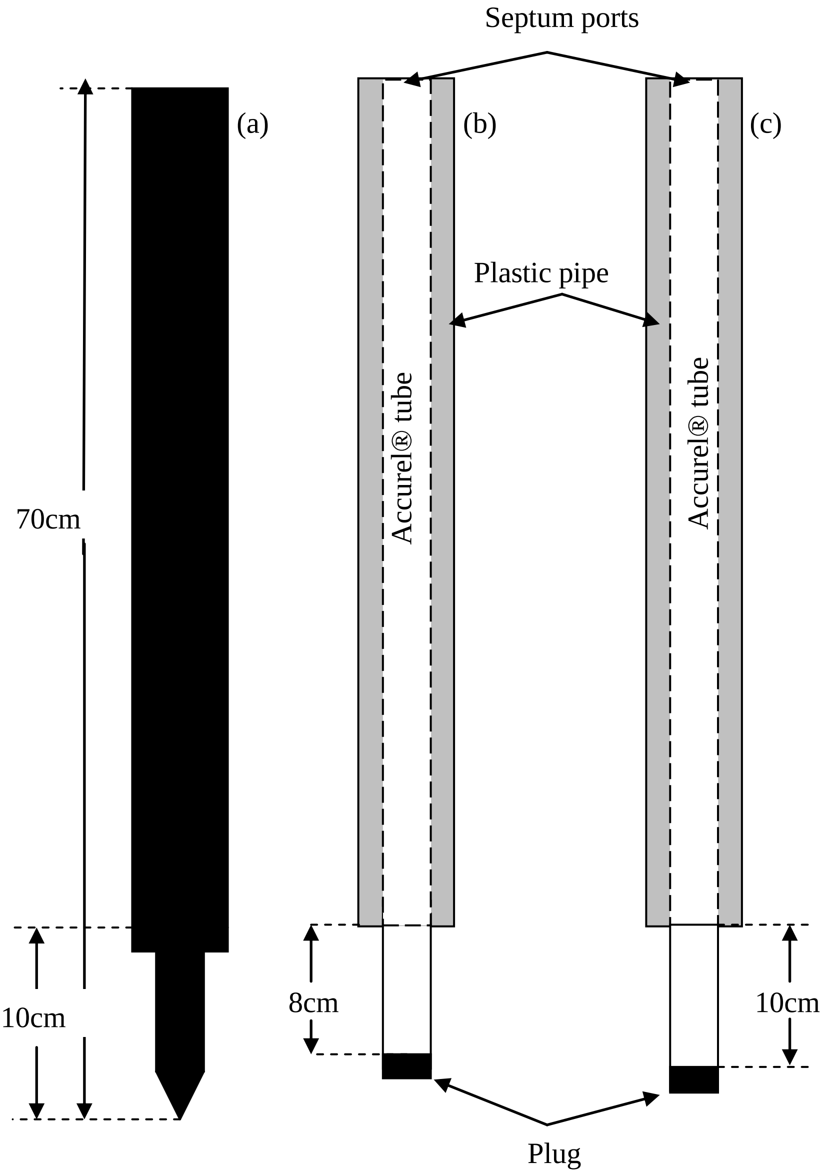


Figure 4.2: The insertion spike (a) and the Accurel® probe assembly for 2-10cm depth (b) and the probe assembly for all other depths (c). Courtesy of Mr Robert Howard modified.

Fortnightly measurements of N_2O concentrations within the soil profile were made during an eight week period from January 22nd to April 30th 2009. Air samples were taken using 5 ml gas tight glass syringes (SGE Analytical Science, Europe Ltd. UK). Using the syringe a 1 ml sample was taken from the sampling port at the end of the Accurel® tube and used to flush residual air from the syringe. Then a sample of the full volume of the syringe (5 ml plus approximately 1 ml) was taken and the syringe was locked off and transported back to the Edinburgh laboratory for analysis.

In the laboratory the syringe containing the sample was prepared by compressing the syringe to 5 ml volume and releasing the excess gas just prior to injection into a pre-evacuated glass vial (20 ml volume). The vial was then opened to the atmosphere to allow the vial to depressurise and equilibrate for approximately five seconds. Standard preparation followed an identical method and all samples were analysed on an automated ECD-gas chromatograph, using an Agilent 6890 GC fitted with a 1.8-m Hayesep Q column and electron capture detector (see Chapter 2.2.4 for further details). Standards were prepared both at the field on 22nd January 2009 and in the laboratory for comparison and found not to differ. Thereafter all standard preparation was conducted in the laboratory.

Following these field measurements of soil N_2O profiles, laboratory measurements on intact soil cores were then undertaken. The effects of irrigation, temperature and N addition on *in situ* soil profile N_2O concentrations and net emissions were investigated using triplicate intact soil cores, incubated under controlled conditions.

4.2.2 Laboratory experimental set-up

Prior to core extraction three identical UPVC pipes (UPVC commercial drainage pipe 21.5 cm external diameter by 50 cm length, Capper Plastics, Sighthill Industrial Estate, Edinburgh) were pre-drilled with five small circular holes at 10 cm intervals along the length of one side of the core which were used as sampling ports during the experiment. Three intact soil cores, 21 cm diameter by 40 cm long, were extracted on 5th June 2009 from the riparian zone immediately south of the IBS (see Figure 3.1 Chapter 3), at the field study site near Newcastle, northeast England. Firstly all vegetation was cut to just above the soil surface from the area of core extraction, due to the fact that the cores would be incubated in the dark inside upright incubators. Then a steel cutting ring fitted to the end of the UPVC pipe was driven into the soil using a hand driven wrench system to a depth of 40 cm. The soil surrounding the column was dug away to enable the core to be lifted out intact and placed onto a flat Perspex base of 25 cm diameter and placed upright in a box ready for transportation to the laboratory at Edinburgh. The procedure was repeated three times and cores were transported and stored at the laboratory within three hours of collection. The set up of the soil cores in the laboratory is shown in Figure 4.3.

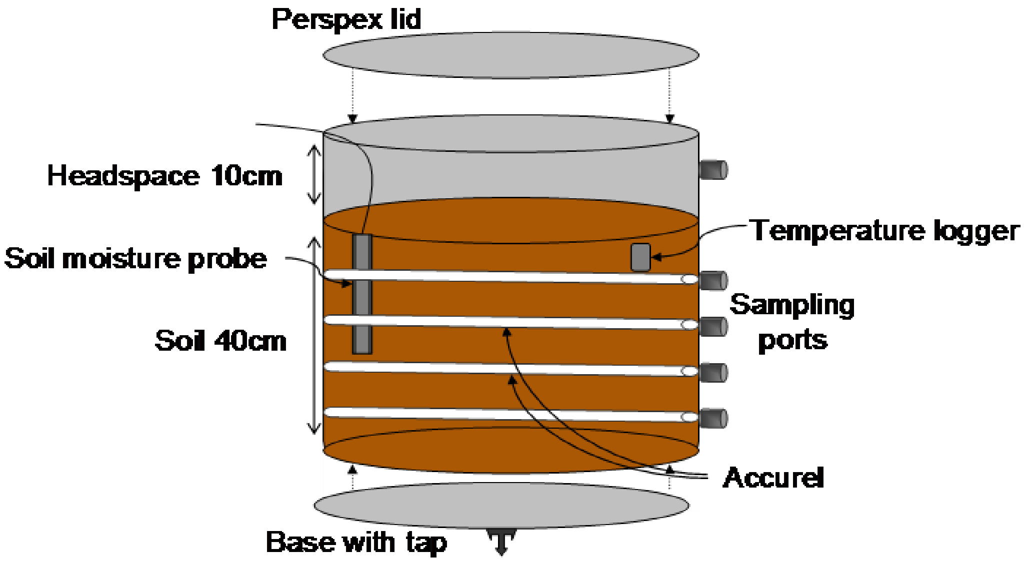


Figure 4.3: A schematic of the intact core experimental set-up

In the laboratory a horizontal cylinder of soil, exactly matching the diameter of the Accurel® tubing, was removed through the pre-drilled holes on the UPVC pipe at each of the sampling depths. Pre-cut 21 cm lengths of Accurel® tubing with an attached septum port at one end were inserted into these drilled holes and secured into place with silicone sealant. A soil moisture probe 20 cm in length (Hobo Soil Moisture Smart Sensor, Tempcon Instrumentation) and a temperature sensor 5 cm in length (Hobo pendant temperature loggers, Tempcon Instrumentation) were inserted vertically from the surface into the top of the soil core. A small notch was cut into the top edge of the UPVC pipe and the cable connecting the moisture sensor to the data logger was glued into place using a silicone based glue to allow the Perspex lid to lie flat against the top of the cylinder during headspace gas sampling and create an air-tight seal. A Perspex base with a pre-fitted three-way stopcock with luer fittings (Edwards Life Sciences) was glued into position in the centre of the base Perspex plate. The three-way tap allowed leached irrigation water to be collected from the base of the intact core.

4.3 Sample measurement and analysis

4.3.1 Headspace gas sampling and analysis

Cores were sealed by placing a weighted Perspex lid on top of the Perspex cylinder. A ring of foam rubber draft excluder was glued to the underside of the lid in a circle exactly matching the diameter of the core cylinder, to produce an airtight seal. The cores were sealed for 40 – 60 minutes prior to sampling. Headspace gas samples were taken from each core using a hand held pump directly into glass sample vials (20 ml). Vials were first flushed with headspace air three times with the fourth sample retained for analysis. Duplicate samples were taken from each core at each sampling time. All surface N_2O emissions reported are the average of two measurements unless otherwise stated. After sampling, the lids were removed and the core and incubators were flushed with air from a gas cylinder (ambient N_2O concentration of 0.31 ppm), to prevent the development of an unrepresentative atmosphere within the incubators. Samples were stored in the glass vials until analysis by gas chromatography within ten days of sample collection (see Chapter 2.2.4).

4.3.2 Within-core soil profile N_2O measurements

In situ gas collection was carried out at four depths within each soil core: 5, 15, 25 and 35 cm below the soil surface using Accurel® tubing. Pore space gases diffused into the hollow Accurel® tube (5.65 cm^3 volume) ready for sampling using gas tight glass syringes, (250 µl volume). Prior to sampling 250 µl of air sample was extracted from the Accurel® tube using the gas tight syringe to flush residual air from the syringe and prevent contamination. A subsequent sample of 250 µl was taken using a glass syringe for direct measurement by gas-chromatography by manually injecting each sample immediately into the ECD-GC. Prior to injecting the gas sample directly and manually into the gas-chromatograph the syringe plunger was depressed from 250 µl to 200 µl and released just before sample injection. Immediately afterwards the sample was injected into the column of the gas chromatograph and the sample was run for 5 minutes with the resultant peak areas recorded. This process was repeated for each depth at each core for the required number of sampling occasions on each experimental day. Additionally standard gas samples were prepared in the same way using the same gas tight syringes and manually injected into the ECD-GC.

4.3.3 Leachate sampling

Leached irrigation water was collected from the base of each core via the outlet tap into 60 ml plastic sampling containers at between 30 and 60 minutes after irrigation. Samples were frozen until analysis of NO_3^--N and NH_4^+-N determined colorimetrically with a Bran and Luebbe continuous-flow analyser, using the manufacturer's method G-102-93 rev1 for NH_4^+-N and method G-109-94 rev3 for NO_3^--N, (see Chapter 2.3.7 for details).

4.3.4 Soil physical parameters measurement (also includes chemical composition)

In this section soil physical and chemical properties are presented from four different sources: samples collected *insitu* adjacent to the IBS in March 2007 (Chapter 3) using the excavation method (see Chapter 2.3), samples collected using a 1 m soil column auger collected in August 2008 (see Chapter 2.3.6), samples collected at the same time as the soil cores from the adjacent area, and samples gained by destructive sampling of the cores.

Soil temperature was measured every 5 minutes using an *in situ* temperature sensor that integrated over the uppermost 5 cm of the soil within each core (Hobo pendant temperature logger, Tempcon Instrumentation). Soil moisture data was integrated over the top 20 cm of the core and recorded every 5 minutes using a Echo dielectric aquameter (Hobo Soil Moisture Smart Sensor, Tempcon Instrumentation). The active length of the probe was only 20 cm therefore all soil moisture measurements account for the top 20 cm of each core only. For experiments one and two, soil moisture data are unavailable due to problems with the data logging software. For experiments one and two headspace and soil profile gas samples were taken three times daily: at 09:00 (before irrigation) and subsequently at 13:00 and 17:00 (one and five hours after irrigation). However, due to time and resource constraints, sampling frequency was reduced to two daily samples (before and approximately one hour after irrigation) for all the final experiment.

Soil composition and total C and N measurements were carried out in June 2007 using the excavation method (see Chapter 2.3 for further information). A large pit extending 0.5 cm in depth was dug adjacent to the IBS and quadruplet samples were taken at 5 cm depth intervals and bulked before oven drying at 105°C and sub sampled for ball milling and analysis (Table 4.2).

Table 4.2: Soil characteristics at Nafferton farm, samples were collected from an excavation pit located just outside the fenced area of the IBS in June 2007. Values are the mean ± 1 standard deviation.

Soil depth (cm)	Total C %	Total N %	Sand %	Silt %	Clay %
0 - 10	29.5 ± 0.98	2.66 ± 0.14	61.7 ± 3.69	28.4 ± 2.85	9.90 ± 0.84
10 - 20	28.1 ± 6.09	2.48 ± 0.40	59.7 ± 2.14	29.6 ± 1.84	10.7 ± 0.29
20 - 30	14.4 ± 1.57	1.25 ± 0.19	61.6 ± 1.08	27.8 ± 0.87	10.6 ± 0.21
30 - 40	11.6 ± 0.43	0.97 ± 0.00	61.3 ± 1.05	28.4 ± 0.88	10.3 ± 0.18
40 - 50	10.1 ± 1.54	0.81 ± 0.13	63.5 ± 3.26	27.7 ± 2.61	8.78 ± 0.65

The soil is characterised as a sandy loam based on the ratio of its sand:silt:clay content. Total C and N (%) in the top 20 cm of the soil was approximately double that of the soil between 20-50 cm.

Detailed soil analysis of the inorganic available N content was carried out on three replicate cores of 1 m length, taken along a transect across the IBS approximately 1 m parallel to the irrigating boom during August 2008, to determine the organic N content within the soil (Table 4.3).

Table 4.3: Mean soil inorganic available-N concentrations within the top 1m of the soil profile.

Soil depth interval (cm)	NO_3^--N (µg g^{-1})	NH_4^+-N (µg g^{-1})
0-10	0.37 ± 0.05	3.41 ± 3.15
10-20	1.58 ± 1.53	0.75 ± 0.14
20-30	1.10 ± 0.93	0.33 ± 0.17
30-40	0.87±0.71	0.23±0.18
40-50	0.42±0.22	0.24±0.23
50-60	0.40±0.19	0.16±0.14
60-70	0.46±0.24	0.14±0.09
70-80	0.32±0.11	0.12±0.12
80-90	0.27	0.12
90-100	0.33	0.12

Data represent the mean of three replicate soil cores ± 1 standard deviation. Values from 80-90 cm depth are the average of two values. Values for 90-100 cm depth are from one measurement only.

Soil NH_4^+-N was greatest in the top 10 cm of the soil profile (3.41±3.15 µg g^{-1}) and decreased rapidly down the profile to <1.0 µg g^{-1} from 10 cm depth onwards. Soil NO_3^--N was low in the uppermost 10 cm of the soil (0.37 ± 0.05 µg g^{-1}) and then increased sharply to a mean concentration of 1.58 ± 1.53 µg g^{-1}, and subsequently decreased in concentration with increasing depth. Nitrite concentration was very low at all depths (data not shown). Regression analyses showed no statistically significant ($P > 0.05$, $n = 8$) relationship existed between the mean inorganic-N content with soil depth (0 to 80 cm). However some of the individual replicate soil cores did show a decreasing concentration of NH_4^+-N and NO_3^--N with increasing depth (Table 4.4).

Table 4.4: Statistical results (regression analyses) for soil inorganic NO_3^--N and NH_4^+-N concentrations within the top 0.8m of the soil profile from triplicate soil cores.

Nitrate			
ID	P-value	R^2 (%)	N =
Mean	P > 0.05	29.7	8
Replicate A	**P < 0.05**	71	8
Replicate B	P > 0.05	34.6	10
Replicate C	P > 0.05	39	9
Ammonium			
ID	P-value	R^2 (%)	N =
Mean	P > 0.05	47.4	8
Replicate A	**P < 0.05**	77.9	8
Replicate B	P > 0.05	32.6	10
Replicate C	**P < 0.05**	55.1	9

Soil samples from the upper 15 cm of the soil were sampled on the same day as core extraction, using a hand held auger, adjacent to where the cores were extracted. Soil samples were collected in triplicate with the litter layer removed. Available NO_3^--N and NH_4^+-N concentrations were 3.10 and 7.42 µg g^{-1} dry soil respectively at the outset of the experiment. The pH in the upper 35 cm of the soil was 5.37 ± 0.15. Mean bulk density of the upper 50 cm of the soil was 1.31 ± 0.12 g cm^{-3}.

Destructive soil sampling of the monolith cores for available-N and total C and N analysis was carried out on completion of the experiments. Four bulked samples from each core were taken using a hand held auger and divided into three soil depths: 0 - 20, 20 - 30 and 30 - 40 cm beneath the soil surface. Bulked soil samples were then analysed using standard methodology (see Chapter 2.3 for more details).

Soil water content was determined using the gravimetric method, by determining the difference in weights of wet and oven dried (at 105°C) soil, (Smith and Mullins, 2000). Soil water content is then converted into percentage WFPS to allow comparisons with those measured directly from the soil cores using the following equation:

$$WFPS = \frac{SWC * BD}{\left(1 - (BD/PD)\right)} \qquad \textit{Equation 4.1}$$

where SWC is the soil water content determined gravimetrically (g g^{-1}), BD is the bulk density (g cm^{-3}), determined using field samples (see Chapter 2.3.1), and PD is the particle density (a standard value for soils and clays of 2.65 g cm^{-3} was used; Smith and Mullins, 2000).

4.3.5 Experimental design

The experiments were designed to mimic the *in situ* field conditions experienced at Nafferton Farm throughout the seasons (see Chapter 3 and section 4.1.2 of this chapter). Each intact core was prepared in the same manner and the cores were stored in two cooled incubators (Weiss-Gallenkamp, Leicestershire, UK), due to the lack of space for all intact cores to fit in one incubator whilst also allowing sampling to take place. All cores were treated as replicates of one another.

The experiments began one week after the collection of the soil cores from the field site, to allow for the final preparations of the cores, and were conducted from June to July 2009. The three experiments conducted are summarised in table 4.5 and detailed below.

Table 4.5 Summary of laboratory experimental conditions, irrigation rate and loading and indications of available soil temperature or moisture data.

#	Name	Incubator temperature (°C)	Irrigation per day		Mass of N added (mg NO_3^--N per day per core)	Available data	
			Volume (ml)	NO_3^- (mg NO_3^- l^{-1})		Soil temperature	Soil moisture
1	Control	14	none	none	none	Yes	No
2	Summer	14	395	20	1.78	Yes	No
3	Winter	4	620	74	10.4	Yes	Yes

Experiment 1: Control (27th - 28th June 2009)

All cores were subjected to an initial control period of two days at 14°C with no irrigation. This period was used to establish the background N_2O levels of all cores, at the surface and at depth.

Experiment 2: Summer (30th June - 3rd July)

For a period of four days each core received 395 ml of a solution containing 20 mg NO_3^- l^{-1} each day at approximately 12:30 pm, which is equivalent to the average summer irrigation conditions experienced at the field site of 20 mg NO_3^- l^{-1} and 80 l m^{-2} wk^{-1}. Cores were incubated at 14°C, in line with mean summer soil temperatures measured during the field sampling period.

Experiment 3: Winter (08th - 10th July)

'Winter conditions' of soil temperature 4°C and daily irrigation of 620 ml of 74 mg NO_3^- l^{-1} $core^{-1}$ mimic average winter conditions experienced at the field site of 124 l m^{-2} wk^{-1}. Cores were irrigated at approximately 12:30 pm daily. The incubation temperature was set to 4°C, but temperature gauge inaccuracies in the incubators made it difficult to fine tune incubation temperature. Both incubators were at 10°C on day one of the experiment and 4°C on day three of the experiment. During day two of the experiment the temperature in the incubator containing cores A and B was 6°C and in the incubator containing core C it was 4°C.

4.3.6 Experimental procedure and sampling strategy

The NO_3^- irrigation solutions were made using KNO_3. Irrigation of the cores was performed by pouring the solution directly onto the surface of the core at the specified time as one application and allowed to infiltrate. Before and after each experiment one or two days were required to increase or decrease the incubator temperatures and allow the cores to equilibrate.

4.3.7 Dilution procedure for soil profile gas samples

In the winter experiment the magnitude of intra-soil N_2O concentrations produced at some of the depths required samples to be diluted before analysis on the GC. However, not every sample required dilution. The need for sample dilution was assessed on an individual basis by manually analysing each sample and subsequently re-sampling and diluting the sample if the observed peak area exceeded the calibration range.

To dilute a sample, 200 µl of air from within the soil was taken using the syringe method (see Chapter 4 Section 3) and injected into a sealed vial (22 ml) containing atmospheric air taken from outside the laboratory, and assumed to contain 0.31 ppm N_2O (see Chapter 2.2.4); this was taken into account when calculating the N_2O concentration in samples. Using the syringe 200 µl of diluted sample was then directly injected into the gas chromatograph for analysis.

4.4 Results

These results are comprised of two main parts: the *in situ* N_2O soil profile experiments conducted at Nafferton Farm (sections 4.4.1 and 4.4.2) followed by a series of laboratory experiments using intact soil cores (sections 4.4.3 to 4.4.7).

4.4.1 In situ N_2O profile concentrations at Nafferton Farm

N_2O concentrations within and between replicates varied both temporally and spatially during the *in situ* field monitoring period (Figure 4.1). Mean N_2O concentrations across all measured soil depths ranged from 0.33 to 6.51 ppm, with individual samples reaching concentrations up to 9.48 ppm. The range of concentrations observed from the probes located within the uppermost 30 cm of the soil profile were very similar and ranged from 0.33 to 3.76 ppm across the study period. The range of N_2O concentrations observed at 40 to 50 cm depth was larger: 0.46 to 6.51 ppm. Furthermore the variation in N_2O concentrations between replicate probes located at the same depth within the soil profile varied by more than 9 ppm on some occasions. However, mean N_2O concentrations at all depths except 2 to 10 cm, and 40 to 50 cm beneath the soil surface were within one standard error of the mean.

Variation in N_2O concentrations within and between soil depths

Mean concentrations of N_2O from 2 to 10 cm beneath the soil surface ranged from 0.33 ± 0.00 to 3.43 ± 0.58 ppm and decreased over time, despite increasing soil temperatures (Figure 4.4, Table 4.5). Greatest mean concentrations for this soil depth were recorded for February 5th, 3.43 ± 0.58 ppm, ten times greater than those recorded at the end of April 2009, 0.35 ± 0.03 ppm, however, a measure of N_2O concentration for this depth in January is not available.

N_2O concentrations from 10 to 20 cm beneath the surface show the greatest variability compared to all other measured depths; the range of mean N_2O concentrations was 0.48 ± 0.11 to 3.76 ± 2.87 ppm. The mean N_2O concentration over the entire period (1.94 ± 0.43 ppm) was the greatest of all sampled depths.

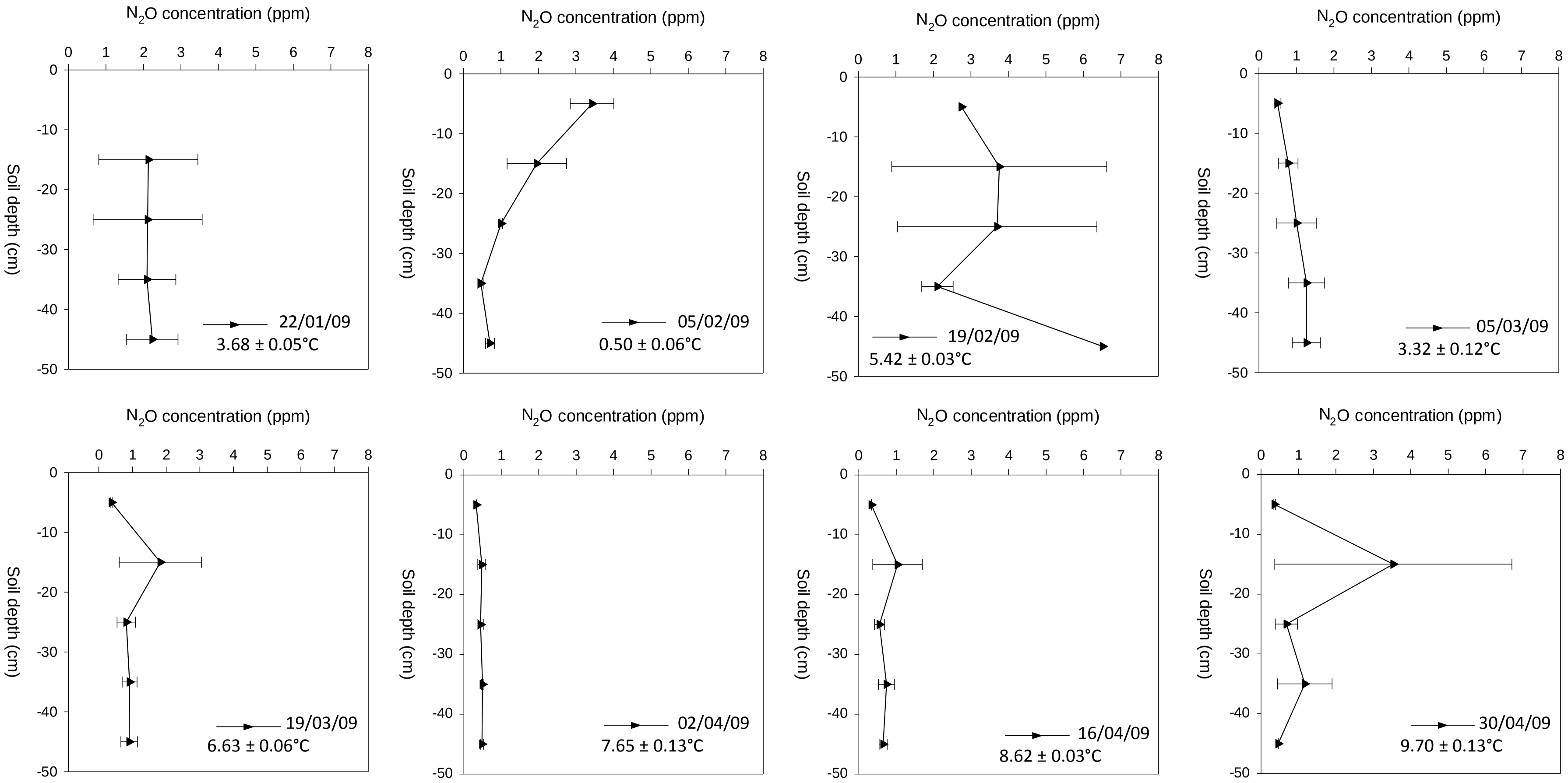


Figure 4.4: In situ N_2O soil profile concentrations, (January to April 2009), from the IBS field site at Nafferton Farm. Soil profile N_2O data are the mean of three replicate Accurel® probe samples ± one standard error of the mean, with the exception of 2 to 10 and 40 to 50 cm depth on the 19 February 2009 that are the mean of two replicates due to a syringe blockage. Sample collection date and soil temperature are as labeled. Mean soil temperature (n = 6 ± 1 standard error) was measured *in situ* on the day of sampling using a standard hand held digital thermocouple thermometer integrated over the top 10 cm of the soil

Mean field N_2O profile concentrations are not consistently greatest at any particular depth. That being said with the exception 5th February, the measured N_2O concentrations at 2 to 10 cm depth across the sampling period was always less that that recorded at greater depths. This trend of highly variable N_2O concentrations in space, (within and between replicates) and over time occurred for all the measured depths within the soil profile. For example; the greatest N_2O concentration measured in one of the replicate samples at this depth was 9.87 ppm on 30th April from the probe located adjacent to static chamber 3, compared with 0.38 and 0.35 ppm for the other two probes on the same sampling date, resulting in a mean N_2O concentration of 3.53 ± 3.17 ppm. This illustrates the spatial heterogeneity of N_2O production from soils, even over short distances. Moreover, samples from replicate probe 1 located near to static chamber 1, recorded high N_2O concentrations in January (4.77 ppm) and February 19th (9.48 ppm) despite samples from the other replicate probes recording concentrations of less than 1.15 ppm on these sampling dates.

4.4.2 Concurrent *insitu* N_2O emissions

Mean surface N_2O emissions taken over the same period using the static chamber method, (see Chapter 2.2.1) were 0.60 ± 0.50 g N_2O-N ha^{-1} d^{-1} (the mean surface emissions denotes the mean of three static chambers (numbers 1 - 3) as identified on Figure 4.1). The lowest emission of -2.74 ± 0.10 g N_2O-N ha^{-1} d^{-1} was recorded on 5th March 2008 despite N_2O concentrations in excess of 2 ppm from samples taken from 20 to 50 cm beneath the surface. Furthermore the N_2O concentration recorded from 2 to 10 cm below the surface was approximately eight times less than the two previous sampling occasions and almost twice as much as all subsequent samples. But on no other occasion were negative emissions recorded. This negative emission means that the soil on this occasion became a net sink for N_2O as it moved from the atmosphere into the soil.

On three occasions (5th February, 16th and 30th April) the weekly amount of irrigation water combined with rainfall exceeds 20 mm (28.6, 23.6 and 43.0 mm respectively), however on these occasions the N_2O concentrations are similar, within error, to those on the other sampling days. Soil temperature increases from less than 2°C to greater than 10°C during by the end of the sampling period; however, the mean N_2O concentration decreases over time.

Table 4.6: N_2O surface emissions using the static chamber method (n = 3, ± 1 standard error of the mean) and field meteorological and irrigation data.

Date for the week preceding (2009)	Mean (± 1 standard error)	Total weekly rainfall (mm week^{-1})	Weekly irrigation (mm week^{-1}).
22nd Jan	0.95 ± 0.16	5.6	-
5th Feb	0.30 ± 0.08	22.6	6.0
19th Feb	1.53 ± 0.24	0.4	-
5th Mar	-2.74 ± 0.10	5.8	-
19th Mar	0.97 ± 0.17	0.2	-
2nd Apr	0.97 ± 0.05	5.0	0.1
16th Apr	0.96 ± 0.16	5.6	18.0
30th Apr	1.84 ± 0.16	24.2	18.8

The highest surface emission; 1.84 ± 0.16 g N_2O-N ha^{-1} d^{-1} (Table 4.6), occurred on the day of highest soil temperature as well as the highest combined rainfall and irrigation application of the period, 30th April. However, beneath the soil surface at 2 to 8 cm depth mean N_2O concentration was 0.35 ± 0.03 ppm with a mean soil profile N_2O concentration of 1.24 ± 0.59 across all measured depths. However, the range of N_2O concentrations from all replicates on this date was the greatest of the measured period (0.32 - 9.87); the highest concentration from one of the replicates was recorded at 10 to 20 cm depth and coincided with highest surface emission from the overlying static chamber; 3.01 g N_2O-N ha^{-1} d^{-1}. The variability between replicates illustrates the high spatial heterogeneity of soil and therefore N_2O emissions.

But high subsurface N_2O concentrations do not necessarily mean high surface emissions. On 19th February mean surface emissions from the replicate chambers (1, 2, and 3), were broadly similar, (1.30, 1.28 and 2.01 g N_2O-N ha^{-1} d^{-1}, respectively).

However, mean soil profile concentrations differed; 6.67 ± 1.47, 1.89 ± 0.90 and 1.48 ± 0.18 ppm from replicates A, B, and C, respectively, with the greatest emissions measured from the soil with the lowest soil profile concentrations. This suggests that the existence of high N_2O concentrations within the soil profile does not equate to high emissions at the surface, rather that N_2O producing and consuming processes are both fast and dynamic within the soil profile, with the surface emission depending on the net balance of these processes.

These results illustrate the dynamic nature of N_2O production within soils both temporally and spatially. High (or low) soil profile N_2O concentrations do not necessarily produce high (or low) surface emissions. Furthermore it demonstrates that, despite low near-surface N_2O concentrations, deeper subsurface concentrations can be much greater and would merit further investigation. On this basis a series of laboratory experiments were devised to mimic the field conditions, using intact soil cores.

4.4.3 Soil profile N_2O production from triplicate incubated intact soil cores

Experiment 1: Control conditions

These experimental conditions are analogous to no irrigation or rainfall and warm spring/summer temperatures (14°C). Within the soil profile N_2O concentrations were very consistent both temporally and spatially for each replicate core. Experimental mean N_2O concentrations across all measured depths ranged from 4.23 ± 0.02 to 4.35 ± 0.03 ppm, and increased with increasing soil depth. However, mean N_2O concentrations from core C were consistently higher than those measured within the other replicate cores and not within one standard error of the mean (Figure 4.5).

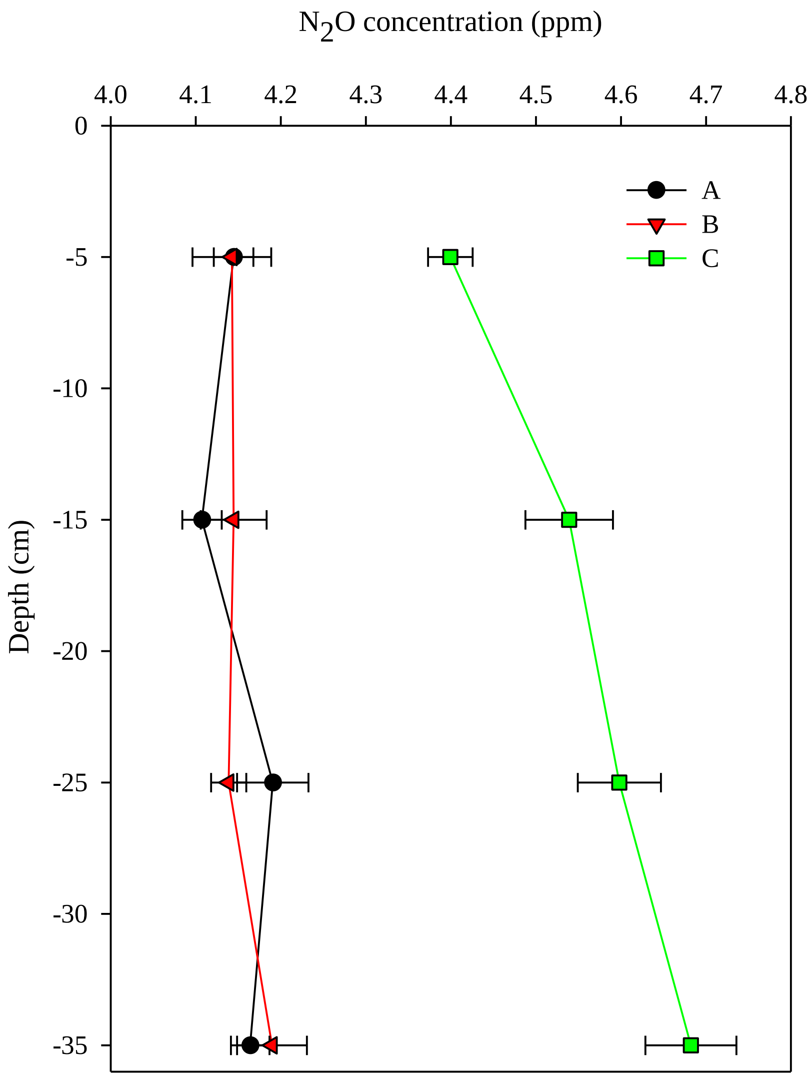


Figure 4.5: Mean experiment N_2O concentrations down the soil profile (n = 6, ± SE) for each of the replicate soil cores; A, B, and C (as labelled).

The experiment was designed to treat all cores as replicates; however, as shown in Figure 4.5, N_2O concentrations from cores A and B are within one standard error of one another; but core C appears to produce higher concentrations of N_2O. This is possibly an artefact as a result of slightly different soil temperatures within the two incubators (Table 4.7). This may demonstrate the sensitivity of microorganisms to temperature and therefore N_2O production.

Table 4.7: Mean daily soil temperature recorded in the upper 5 cm of the soil of each replicate core (mean ± SE, n = 287).

	Soil temperature (°C)		
	Replicate A	Replicate B	Replicate C
Day 1	13.6 ± 0.02	13.9 ± 0.01	15.3 ± 0.01
Day 2	14.1 ± 0.03	14.2 ± 0.02	15.4 ± 0.01

Mean soil temperatures in the uppermost 5 cm of cores A and B (within the same incubator) were broadly similar; however, those recorded from replicate core C were greater by nearly 1.5°C. This difference in soil temperature will compromise all further experimental results due to the effect of temperature on soil microbial processes (i.e. nitrification, denitrification, immobilisation, and mineralisation). Therefore, inherent differences in the inorganic and organic content of the soils will most likely exist between replicate cores. Since the experiments were carried out sequentially on the same replicate cores, this temperature anomaly would most likely have created ‘knock-on’ effects for N_2O production and emissions from the replicates; compromising the result of the two subsequent experiments.

Experiment 2: Summer conditions

These experimental conditions are analogous to mean summer temperatures (14°C), irrigation volume (80 litres per week) and NO_3^--N loadings (20 mg NO_3^- per litre); experienced at the Nafferton Farm field site.

Mean experimental N_2O concentrations show a slight suppression of N_2O production as a result of irrigation; however, measured concentrations both before and after irrigation (as well as within the soil profile) were within standard error of the mean (Figure 4.6). Again the use of two separate incubators resulted in slightly different incubation temperatures of the replicate cores: the experimental mean temperature for cores A and B were very similar: 13.6 ± 0.03, 13.8 ± 0.03, respectively, but core C located in a different incubator was approximately 1.5°C greater (15.5 ± 0.02°C). Nevertheless mean experimental N_2O concentrations within the soil profile were temporally (i.e. before and after irrigation) within standard error of the mean. Soil profile N_2O concentrations were spatially (i.e. from all depths) similar. However, N_2O concentrations at 35 cm depth were greater and not within standard error of those at 5 cm depth.

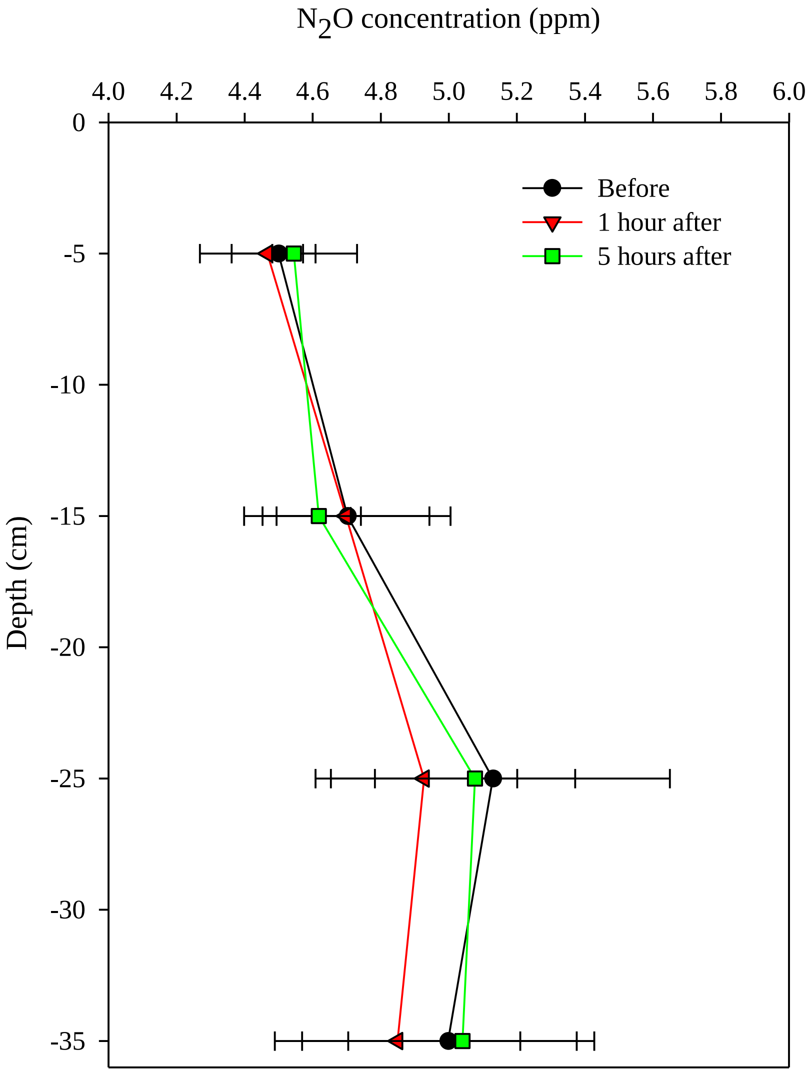


Figure 4.6: Experimental mean N_2O concentrations (n = 4, ± SE) measured before, 1 hour after, and 5 hours after, irrigation was applied (as labelled).

Mean N_2O concentrations before or after irrigation (ranging from 4.47 - 5.13 ppm) are not different from those measured in experiment 1 when cores were not irrigated and had similar soil temperatures (4.23 - 4.35 ppm). Therefore irrigation does not appear to have any effect on N_2O production within the soil.

Experiment 3: Winter conditions

These experimental conditions are analogous to mean winter temperatures (4°C), irrigation volume (124 litres per week) and NO_3^--N loadings (74 mg NO_3^- per litre); as experienced at the Nafferton Farm field site.

The daily mean soil temperature of the cores was not consistent due to incubator temperature gauge inaccuracies. Daily mean soil temperature for each core decreased by approximately 2 to 3°C during the experiment; with the greatest change in soil temperature observed for the incubator containing replicate core C (Figure 4.7).

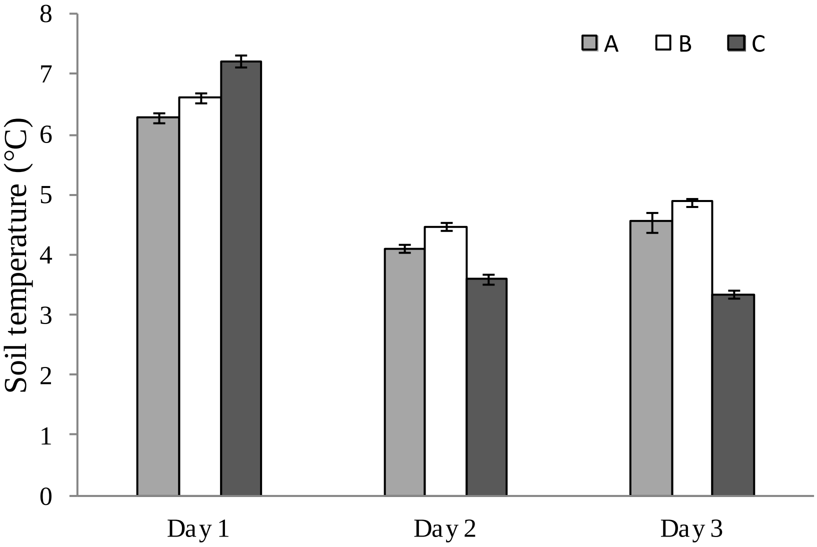


Figure 4.7: Mean daily temperature (n = 287, ± SE) measured in the uppermost 5 cm of the soil from each replicate core on subsequent experimental days (as labelled).

Mean soil profile concentrations from 5 and 15 cm depth were 4.09 ± 0.07 and 4.07 ± 0.06 ppm, respectively: within the range of those reported for the control experiment. But experimental mean N_2O concentrations at 25 and 35 cm depth were

274 ± 26.1 and 209 ± 43.0 ppm, respectively: much larger than those recorded for either of the first two experiments.

The large standard error for N_2O production at 25 and 35 cm depth is a result of very different concentrations observed for replicate B compared with replicates A or C. Soil profile N_2O from cores A and C were broadly similar to those observed in previous experiments with a mean range of concentrations from 3.86 to 4.29 ppm. However, replicate core B, despite having low mean N_2O concentrations at 5 and 15 cm depth of 4.18 ± 0.08 and 4.39 ± 0.13, respectively, had extremely high mean concentrations of 815 ± 78.3 and 617 ± 129 ppm N_2O at 25 and 35 cm depths, respectively. These high concentrations of N_2O produced at depth are not a temperature artefact, as cores A and B are together in the same incubator and only core B shows these very high N_2O concentrations. Therefore despite the colder temperature of approximately 5°C, for the winter experiment, large concentrations of N_2O are produced within the soil. N_2O production within the soil profile is greater before irrigation rather than after it but still within 1 standard error of the mean (Figure 4.8).

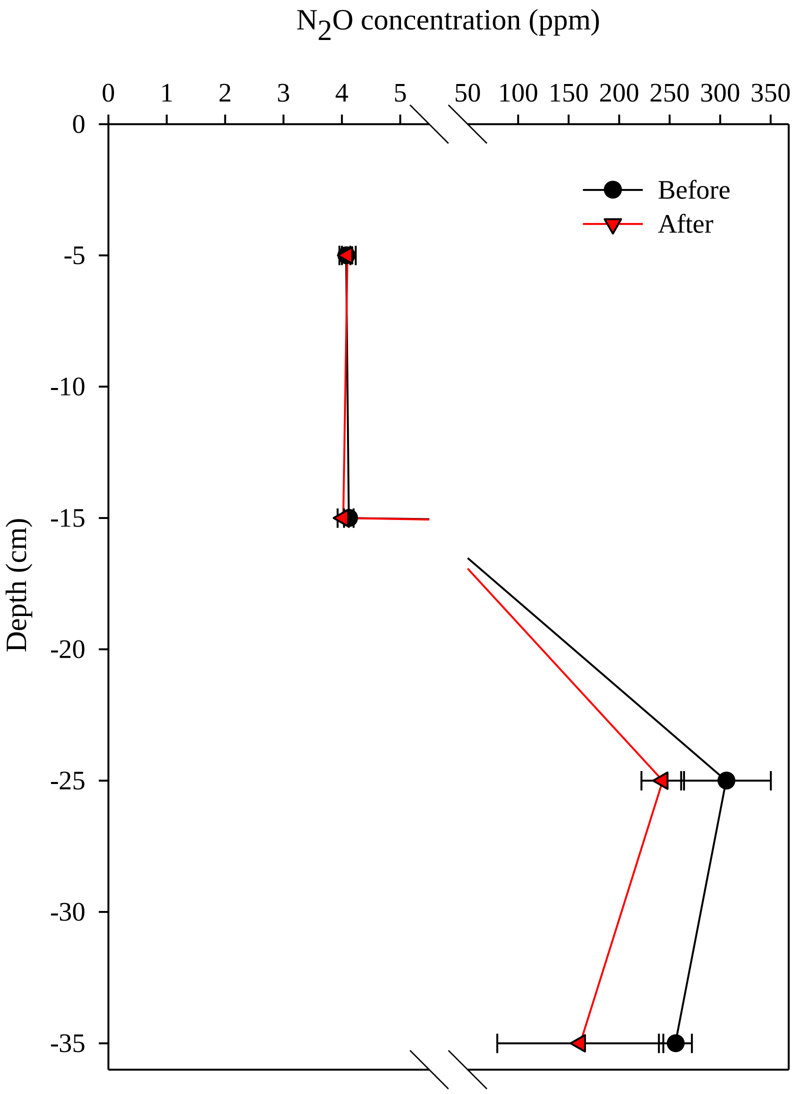


Figure 4.8 Mean nitrous oxide concentration within the soil profile before and after irrigation (as labelled) (n = 3, ± SE).

Percentage WFPS (integrated over the uppermost 20 cm of the soil), was consistently very different between cores throughout the experiment, by up to 38%, despite receiving the same irrigation treatment (data not shown). Mean WFPS increased one hour after irrigation by approximately 5% (Table 4.8).

Table 4.8: Mean WFPS (%) integrated over the top 20 cm of soil (n=3, ±SE).

	WFPS (%)
Experimental mean	53.4 ± 0.66
Mean 1 hour before irrigation	52.0 ± 2.08
Mean 1 hour after irrigation	57.7 ± 1.94

Percentage WFPS at the base of the soil cores (i.e. 20 - 40 cm depth) was not measured directly and could be different to those recorded at the surface. This may be responsible for the observed differences in N_2O at 25 and 35 cm depth. Alternatively, differences in soil contents within and between the cores particularly in inorganic-N or carbon may be the driving factors of N_2O production.

4.4.4 Direct N_2O emissions from the soil surface and emission factors

The lowest mean surface emission was from the winter experiment and the highest from the summer experiment (Table 4.9); despite the high N_2O production within the soil profile during the winter experiment. Emission factors (EFs) i.e. the proportion of NO_3^--N input that is emitted as N_2O have been calculated for the summer and winter experiments and are given below.

Table 4.9: Mean experimental surface N_2O-N emission (g N_2O-N ha^{-1} d^{-1}) (± 1 SE, n = 6 for all experiments)

Experiment	Mean surface N_2O-N emission (g N_2O-N ha^{-1} d^{-1})
Control	14.6 ± 1.14
Summer	15.7 ± 1.01
Winter	4.08 ± 1.55

Mean surface emissions from the control and summer experiments were within standard error of each other despite the very different N additions and irrigation rates. Mean surface N_2O emissions before and after irrigation and N additions (data not shown) were likewise within standard error of the mean for each experiment.

Direct emissions from the winter experiment were much lower than the summer experiment despite receiving a higher volume and loading of NO_3^--N in the irrigation water. The surface emissions generated from the laboratory experiments agree with the findings of the field experiment (Chapter 3.3.1) with seasonally higher emissions during summer than winter. Emissions recorded from the laboratory experiments are also within the range of emissions recorded at the field site for each of the seasonal conditions they represent.

4.4.5 Leached N

Leachate samples were collected at the base of each of the soil cores excluding the control, (experiment 1), for some but not all experimental days (see Table 4.10). Mean concentrations of leached N was highly temporally variable across the sampling days of each experiment. Experimental mean concentrations of leached NO_3^--N for the summer experiment were low (6.18 ± 0.13 mg NO_3^--N L^{-1}). Likewise on the first day of the winter experiment NO_3^--N concentration was also low (5.68 ± 0.72 mg NO_3^--N L^{-1}), however, on the second day of this experiment the concentration more than doubled to 13.4 ± 3.39 mg NO_3^--N L^{-1}, and increased further to 14.3 ± 3.44 mg NO_3^--N L^{-1} on the final day of the experiment. However, mean leached NO_3^--N concentrations for both experiments were within standard error of the mean. Experimental mean NH_4^+-N concentrations were low: 0.37 ± 0.06 and 0.29 ± 0.08 mg NH_4^+-N L^{-1} for the summer and winter experiments, respectively.

Table 4.10: Mean (±SE) leached NO_3^--N from each of the experiments, (numbers in brackets indicate number of available samples per day per core)

#	Experiment	Amount N applied (mg NO_3^--N per core per day)	Mean leachate concentration (mg NO_3^--N L^{-1} per core per day)	Mean leachate volume (ml per core per day)	Mass of leached N (mg NO_3^--N per core per day)	Fraction applied N in leachate
2	Summer	1.78	6.18 ± 0.13 (4)	237 ± 66.6 (1)	1.46	0.82
3	Winter	10.4	11.1 ± 2.72 (3)	414 ± 30.6 (3)	4.60	0.44

To allow ease of comparison between leached-NO_3^- concentrations, the mass of leached N and the fraction of the N applied in the leachate for each experiment were calculated (Table 4.10). It is important to note that the leached fractions reported here have been calculated over short time-scales (< 3 days); and therefore are not comparable to annual leached fractions that have been reported in the published literature: they are however useful in comparing the relative leached fractions from each experiment. The mass of leached N was greatest from the winter experiment and low from the summer experiment. But a higher fraction of leached NO_3^--N occurred from the summer experiment i.e. almost double the fraction leached from the winter experiment. However, it is important to note that the concentration of leached-N and therefore the leached fraction will be compromised by within core production and consumption processes of inorganic-N. The leachate volume equates to 59.9 and 66.8% of the added volume of irrigation water, from the summer and winter experiments, respectively. These experiments have demonstrated leachate losses in terms of both concentration and volume.

4.4.6 Dissolved N_2O in leachate

A pathway of indirect N_2O emissions is from dissolved N_2O in runoff and leachate. Mean dissolved N_2O concentrations are greatest from the winter experiment, however, high variations between replicates exist resulting in high standard error of the mean for the summer and winter experiments (Table 4.11). Mean dissolved N_2O

concentrations were very high; greatly exceeding the air equilibrium of 0.3 µg N_2O-N L^{-1} from all replicates and both experiments.

Table 4.11: Mean dissolved N_2O (µg N_2O-N L^{-1}) (n = 3, ± 1 SE).

Experiment name	Experimental mean (µg N_2O-N L^{-1})	Concentration range between replicates (µg N_2O-N L^{-1})
2. Summer	158 ± 142	1.36 to 442
3. Winter	231 ± 99.3	34.1 to 649

The range of dissolved N_2O-N concentrations between replicates was very high despite very similar (and within 1 standard error of the mean), mean leached NO_3^--N concentrations and soil profile N_2O concentrations for the summer experiment.

For the winter experiment the highest direct N_2O emissions and dissolved N_2O concentrations came from the same replicate core, (replicate core B, mean experimental direct N_2O emissions: 13.6 ± 6.10 and N_2O concentration: 628 ± 10.6 µg N_2O-N L^{-1}).Whereas, low direct emissions and dissolved N_2O concentrations in leachate were found from the other 2 replicates (A and C: 3.49 ± 1.43 and 6.53 ± 2.43 g N_2O-N ha^{-1} d^{-1} and 30.3 ± 6.31 and 34.8 ± 0.36 µg N_2O-N L^{-1}, respectively). These findings may be a reflection of differing inorganic or organic N and C contents of the replicate soil cores and the heterogeneity of soils in general.

4.4.7 Monolith Soil analysis

Destructive sampling of the monolith cores was performed after the cessation of all experiments. Despite all cores receiving the same treatment over the same length of time there exist inherent differences in inorganic N, soil moisture and carbon (C) both within and between cores. These differences may well explain the observed differences in N_2O production at the different sampling depths. Some of these differences may have been generated as a consequence of the different incubation temperatures due to temperature gauge inaccuracies of the incubators.

Soil water content

Soil water content was determined gravimetrically (Section 4.3.4, Equation 4.1), for 0-20, 20-30 and 30-40 cm depth and was used to calculated soil WFPS (Table 4.12), using soil bulk and particle densities.

Table 4.12: Mean WFPS (%) of the replicate soil cores at the difference sampling depths, calculated from soil water content (n = 3, ± SE)

Soil depth (cm)	Mean WFPS (%)
0 - 20	58.8 ± 1.87
20 - 30	72.9 ± 1.77
30 - 40	70.3 ± 3.43

Higher mean WFPS (> 70%), occurred lower in the soil profile and may account for the higher N_2O production at these depths within the soil cores. The lower WFPS (< 60%), in the upper 20 cm of the soil may suggest both nitrification and denitrification may be active and producing/consuming N_2O within the soil closer to the surface.

Inorganic- N, total N and C content of the soils

Inorganic-N (NO_3^- and NH_4^+) from the soils was found to occur in the greatest concentrations at the surface, approximately double the concentration found at depth (Table 4.13). Soil NO_3^--N and NH_4^+-N concentrations from 0 to 15 cm soil depth were 3.10 and 7.42 µg-N g^{-1} dry soil respectively at the outset of the experiment (section 4.3.4), much lower than those measured post-experiment (Table 4.13). This would suggest that N applied to the cores may have been unevenly distributed down the cores with a higher proportion of it concentrated in the top 20 cm of the soil cores. Additionally both total N and C content within the soils decreased within increasing soil depth.

Table 4.13: Mean inorganic-N (NO_3^--N and NH_4^+-N), total N (%) and C (%) content of the soil cores (n = 3 ± 1 SE), sampled post-experiment.

Soil depth interval (cm)	Mean NO_3^--N per g oven dried soil	Mean NH_4^+ -N per µg g^{-1} oven dried soil	Mean N (%)	Mean C (%)
0 - 20	21.9 ± 0.24	11.9 ± 1.58	0.30 ± 0.00	3.33 ± 0.04
20 - 30	11.7 ± 0.71	5.87 ± 0.38	0.24 ± 0.01	2.77 ± 0.15
30 - 40	9.37 ± 1.94	4.26 ± 0.87	0.17 ± 0.02	1.97 ± 0.23

4.5 Discussion

4.5.1 High N_2O production within the soil profile

Concentrations of N_2O within the soil profile varied greatly, both temporally and spatially in field and laboratory experiments; likely as a result of localised hotspots of denitrification (Robertson and Tiedje, 1987; Davidson, 1991; Bateman and Baggs, 2005). *In situ* field N_2O emissions (January to April 2009), were low: 0.22 ± 0.52 kg N_2O-N ha^{-1} $year^{-1}$, and less than the range of emissions from the laboratory experiments that had higher temperatures and/or higher irrigation and NO_3^--N loading rates (1.49 ± 0.57 to 5.75 ± 0.37 kg N_2O-N ha^{-1} $year^{-1}$). High, subsurface concentrations of N_2O exist even where surface emissions remain low: with mean subsurface N_2O concentrations up to 6.5 ppm *insitu* and up to 274 ppm during the winter experiment. The field and laboratory experiments clearly illustrate that despite low surface emissions, N_2O concentration within the soil profile can be many times greater: agreeing with hypothesis one of this research. Furthermore, the high soil profile N_2O concentrations observed both *in situ* and during experiment 1: Control (i.e. no irrigation or NO_3^--N addition), suggesting that without altering the irrigation or NO_3^- loading of soils, subsurface N_2O production can be high (van Cleemput, 1998).

The *in situ* soil profile N_2O concentrations were measured between January and April 2009 i.e. winter and early spring. Consequently the seasonally low temperature (mean ~ 5°C) may have resulted in lower soil profile concentrations of N_2O than at other times of the year when soil temperatures are higher (in excess of 20°C during the summer months). Further *in situ* measurements would be needed to quantify the seasonal range and how much higher (if at all) soil profile N_2O concentrations potentially are.

The consistently low N_2O emissions measured at the soil surface compared with the concentrations within the soil profile highlight the rapid N-cycling capacity of soils by either aerobic (i.e. nitrification to NO_3^-) or anaerobic (i.e. denitrification to N_2) microbial processes as the N_2O diffuses upwards from the site of production to the atmosphere (Conrad, 1996). The relatively low surface emissions compared to the high within soil N_2O concentrations, would suggest that either much of the N_2O produced at depth is denitrified, buffering surface emissions or that there are high leached N losses (Webster and Hopkins, 1996; Abdalla *et al.,* 2009). This suggests that pollution swapping of increased direct N_2O emissions may not be an issue of concern as previously suggested (Mosier *et al.,* 1998; Ambus, 1998; Hefting *et al.*, 2003; Reay, 2004), rather, high leachate losses are of greater importance.

One replicate core (replicate B), produced much greater N_2O within the soil profile than either of the other two replicates, particularly at 25 and 35 cm depth where N_2O production was up to 2 orders of magnitude greater. Destructive soil sampling conducted at the end of the experiments revealed that at these depths this replicate had much higher concentrations of NO_3^--N, NH_4^+-N, total C and total N. This higher substrate availability may help to explain the larger concentrations of N_2O produced at this depth.

Increased mineral-N (both NO_3^--N and NH_4^+-N) were observed post-experiment to accumulate preferentially in the upper 20 cm of the soil, suggesting nitrification is active in more aerobic conditions (mean WFPS < 60%), near to the soil surface. However, without measurement of N_2 emissions or the use of isotopes, the contributions of these microbial processes remain uncertain. Nevertheless both rapid N-cycling and high leachate losses may explain why the measured emissions observed at the field site from the IBS and CP (Chapter 3) do not differ greatly from one another despite the high NO_3^--N loadings applied to the IBS. Additionally this research demonstrates the importance of soil profile N_2O measurements in understanding N cycling and budgets.

4.5.2 Irrigation by NO_3^--N rich water results in leachate

Daily mean experimental leached NO_3^--N concentrations were variable, ranging from 5.98 ± 1.65 to 14.3 ± 3.08 mg NO_3^--N L^{-1}; exceeding the drinking water quality standard of 11.3 mg NO_3^--N L^{-1} on the final two days of the winter experiment. Interestingly, the fraction of leached N as a proportion of added N was nearly double from the summer experiment compared to the winter experiment; 82 and 44%, respectively; despite much greater volume and loading of irrigation water added during the winter experiment. It is suggested that the higher volume of irrigation water added for the winter experiment (compared to the summer experiment), may have created a higher WFPS allowing for more complete denitrification to N_2. However, the lack of measured WFPS in the first two experiments prevents confirmation of this hypothesis. The winter experiment produced a greater loss of N on a per mass basis due to the higher volume of leachate lost compared with the summer experiment. The internal production, consumption and N-cycling processes have not been quantified in the experiments, compromising the leachate fraction so that no definitive conclusions can be drawn.

Despite the drawbacks associated with the internal production and/or consumption of N being un-quantified for these experiments the laboratory results have shown these soils to be susceptible to leachate losses; confirming hypothesis two of this research. Therefore these results suggest *in situ* leaching at the Nafferton field site as a result of irrigation of the IBS (Chapter 3), would be very likely.

Mean NO_3^--N loss in leachate exceeded the drinking water quality standard for the winter experiment. Furthermore, as may be expected the high solubility of N_2O (Valiela and Bowen, 2002) resulted in very high dissolved N_2O-N concentrations: in excess of 600 µg N_2O-N L^{-1} on a single sampling occasion and by at least a factor of ten greater than the air equilibration value of 0.3 µg N_2O-N L^{-1} on all sampling occasions. This suggests that leached N losses are of greater importance than direct N_2O emissions from the surface as a result of irrigation with N-rich water.

5.4.3 Higher NO_3^--N concentrations in the irrigation water do not increase N losses

Greatest within profile N_2O concentrations were observed during the winter experiment which received the highest NO_3^--N additions and volume of irrigation water. But this experiment had the lowest direct N_2O emissions of all experiments and a lower fraction of leached NO_3^--N compared with the summer experiment. The consistently low N_2O emissions measured at the soil surface compared with the concentrations within the soil profile highlight the rapid N-cycling capacity of soils by either aerobic (i.e. nitrification to NO_3^-) or anaerobic (i.e. denitrification to N_2) microbial processes as the N_2O diffuses upwards from the site of production to the atmosphere (Conrad, 1996). This demonstrates the complexity of production, consumption and transformations processes of the biological N-cycle over relatively short temporal and spatial scales.

It is likely that these leached N_2O emissions, the net product of nitrification and denitrification, were the result of multiple sources, including the added NO_3^--N, soil inorganic-N and organic-N mineralisation (Seitzinger and Kroeze, 1998; Nevison, 2000). The NO_3^--N leachate losses measured from all experiments suggest this is of greater significance than direct or indirect N_2O emissions (i.e. pollution swapping). It is likely that high NO_3^--N leachate losses *in situ* at Nafferton Farm; comparable to that observed in the laboratory experiments i.e. in excess of 80% on occasion has occurred. However, greater rates of denitrification to N_2 may have occurred at depths greater than 40 cm or as the leachate water travels both vertically and laterally through the soil until it reaches ground water or a surface water body to discharge into. Furthermore, irrespective of the potential for this NO_3^--N to be converted to N_2O-N, and subsequently lost as direct or indirect emissions, this method of diffuse pollution interception and removal has failed.

4.6 Conclusions

This research adds to the body of knowledge by investigating the source of N_2O emissions within the soil profile and the concurrent direct or indirect N_2O emissions and NO_3^--N losses. These experiments were designed to mimic the *insitu* field conditions experienced at Nafferton Farm throughout the seasons and to aid the understanding of the key N-cycling processes within the soil profile in response to N application, temperature and WFPS.

A key finding of this study was the high sub-surface N_2O concentrations produced within the soil profile. Therefore observed N_2O surface emissions are not always representative of soil profile N_2O concentrations at greater depths. This research has shown that despite high concentrations within the soil profile direct emissions remain low. However, high NO_3^--N leachate losses suggest that although some portion of the added NO_3^--N may be completely denitrified to N_2 a greater proportion is mostly likely lost in leachate. Therefore irrigation of a riparian buffer strip with NO_3^--N rich drainage water resulted in high leachate losses without increased pollution swapping by direct or indirect N_2O emissions.

As has been discussed, higher temperatures increase the rate of microbial activity and soil respiration conditions which often increase N_2O emissions. It is likely that the very high within profile N_2O concentrations may in part be due to the experimental conditions in that the whole core effectively “heats up” as a result of incubating the cores rather than just the top layer of the soil in natural settings. However, *in situ* soil temperatures measured at the field site were up to 22°C in the top 5 cm, suggesting temperatures deeper down the soil profile may still be sufficiently high for denitrification to take place. N_2O production is known to occur even under lower temperatures (Powlson *et al*. 1988; Dorland & Beauchamp, 1991; Holten-Hartwig *et al.*, 2000), although not as high as under higher soil temperatures. Therefore the reduction in emissions from the cores under winter conditions i.e. with lower temperatures would support that the rate of microbial process is reduced as a result of lower temperatures. Nevertheless further *in situ* field experiments measuring soil profile N_2O concentrations under different climatic, seasonal and/or management practices are needed to fully understand the range of within profile N_2O concentrations.

N_2O emissions do not increase as a result of irrigating with NO_3^--N rich water therefore pollution swapping of increased N_2O-N emissions through decreased NO_3^--N is not taking place. Soils with high substrate availability (NO_3^- and C) represent areas under which high N_2O production can occur provided soil physical conditions are optimal for denitrification. The high leachate losses imply the failure of this method at intercepting or reducing diffuse NO_3^--N in the applied irrigation water.

This research also highlights the usefulness of Accurel® tubing in *insitu* or laboratory settings for direct measurement of N_2O within the soil profile. The physical properties of Accurel® tubes makes them ideally suited for gas sampling in environmental settings, particularly under waterlogged conditions. There is scope for the development of this minimally invasive technique to measure *in situ* production of environmental gases within the soil or similar media using this relatively novel Accurel® technique.

Chapter 5: Greenhouse gas emissions and nitrate interception for an instream wetland

5.1. Introduction

Wetlands have high potential for mitigating diffuse NO_3^- pollution whilst improving water quality and enhancing biodiversity (Jordan *et al*., 2000; Mitsch *et al*., 2005). The main processes of NO_3^- removal in wetlands are denitrification and plant uptake which can operate either simultaneously or alone under a variety of climatic, hydrological and environmental conditions (Sabater *et al*., 2003); therefore their relative removal efficiencies vary greatly both spatially and temporally. Denitrification permanently removes NO_3^- from the biosphere to the atmosphere as either N_2 or N_2O gases whereas plant uptake only temporarily removes NO_3^- (Hernandez and Mitsch, 2007).

The relative influence of N input, water residence time, sediment-water interactions, vegetation type, groundwater table level and the complex interactions of biogeochemical processes (both aerobic and anaerobic) all affect the removal capacity of wetlands for NO_3^- (Lowrance *et al*., 1997; Jacinthe *et al*., 1998: Sabater *et al*., 2003). However general patterns can be observed, for example, low oxygen concentrations in the wetland porewater and sediment increase denitrification and potentially N_2O production (Clément *et al.,* 2002; Hefting *et al*., 2003). Average removal efficiencies of buffer strips have been reported as greater than 1.5 g NO_3^--N m^{-2} d^{-1} whereas wetlands can remove up to 3.5 g NO_3^--N m^{-2} d^{-1} (Mitsch *et al*., 2005). The higher removal efficiencies of wetlands could be due to low oxygen content but also more available carbon sources in wetlands as substrates for microbial activity.

Wetlands have been shown to be useful remediation strategies against diffuse NO_3^- pollution (Lowrance *et al*., 1997; Jacinthe *et al*., 1998., Sabater *et al*., 2003; Mitsch *et al*., 2005; Carty *et al*., 2008; van de Weg *et al.*, 2008; Gouriveau, 2009; Mustafa *et al.*, 2009) associated with surface run-off from farm hard standings and concentrated farming activities, (Kadlec and Knight, 1996). However, the construction of wetlands has the potential to generate ‘pollution swapping’ by increasing CH_4 or N_2O emissions (Reay, 2004; Oenema *et al.,* 2009; Stevens and Quinton, 2009; del Prado *et al.,* 2010).

Microbial processes (denitrification and nitrification) produce the powerful greenhouse gas N_2O as either an end- or by-product suggesting the potential for high indirect N_2O emissions during NO_3^- mitigation (Hefting *et al*., 2003). N_2O production depends on: available NO_3^- concentration, temperature, organic matter availability, pH, oxygen availability and microbial population dynamics (van Cleemput, 1998). These controlling factors are highly variable over space and time and therefore so are N_2O emissions (Faulwetter *et al*., 2009). Generally denitrification is the dominant process of N_2O production. Greater than 5% of the gaseous end-product of denitrification is N_2O compared to less than 1% of N_2O from nitrification (Lipschultz *et al*., 1981). However, under aerobic conditions nitrification can be the dominant process of N_2O production (Wrage *et al*., 2001). N_2O emissions from wetlands are spatially highly variable; mean emissions range from 4.5 - 24.6 kg N_2O-N ha^{-1} yr^{-1} (Fey *et al.,* 1999; Johansson *et al.,* 2004; van de Weg *et al.,* 2008). Therefore using wetlands to remediate diffuse N pollution can be an important but uncertain indirect source of N_2O that needs further investigation.

Natural wetlands are also a known source of CH_4 and globally emit 100-231 $TgCH_4$ yr^{-1} directly contributing 18% of direct radiative forcing from greenhouse gases (IPCC, 2007). CH_4 is produced by the process of methanogenesis, the anaerobic microbial breakdown of organic material. Methanogens consist of two main groups that produce CH_4: either by fermentation of simple organic compounds such as acetate to produce carbon dioxide and CH_4, or through the oxidation of molecular hydrogen to water using carbon dioxide which is reduced to CH_4 (Segers, 1998). Methanogenesis through fermentation processes dominate nutrient-rich wetlands (Gauci *et al.,* 2004) and acetate (CH_3COOH) and hydrogen are thought to be the initial substrate for 95% of all CH_4 production in freshwater systems (Segers, 1998).

CH_4 reaches the atmosphere via diffusion, ebullition or by passage through aerenchymous tissue of vascular plants (Brix, 1997; Wassmann and Aulakh, 2000; Ström *et al.,* 2005; Mitsch *et al*., 2008; Sha *et al.,* 2011). Plants growing in wetlands influence CH_4 production by providing substrates for methanogenesis from root exudates and plant litter (Ström *et al,* 2005). Moreover plant root systems can provide oxic microsites as oxygen is transported from the atmosphere to the roots, leading to the oxidation of CH_4 to produce carbon dioxide (Brix, 1997; Zhu *et al.,* 2010). Therefore CH_4 emissions will also be affected by vegetation heterogeneity and could contribute to high spatial variability in wetland emissions over relatively short distances (Mitsch and Jørgensen, 2004; Laanbroek, 2010).

Estimates of CH_4 emissions from a constructed farm wetland in Scotland for 2007 and 2008 were 450 and 970 kg CH_4 ha^{-1} (Rao Pangala, 2008). Estimates of N_2O emissions from the same wetland were considerably less ranging from 0.001 to 0.005 g N_2O-N m^{-2} d^{-1} between 2006 to 2008 (Reay and Paul, 2008; van der Weg *et al.,* 2008; Rao Pangala, 2008), suggesting that CH_4 emissions from farm wetland systems may be more environmentally significant than emissions of N_2O.

The aim of this part of the research was to assess the utility of an instream denitrifying wetland in the remediation of diffuse NO_3^- pollution determined by the interception ability and perceived monetary benefits of the intercepted N. Additionally quantification of the associated greenhouse gas emissions (CH_4 and N_2O) and their relative importance will be assessed.

This research differs from conventional constructed farm wetlands (CFWs) in that it utilises an existing farm drainage ditch that has been modified to create an instream wetland. The use of an instream wetland is novel and if successful at remediating diffuse NO_3^--N pollution would result in no loss of productive farmland from the total farm area. Successful CFWs at remediating diffuse pollution have been shown to be dependent on an adequately large size to achieve long water residence times and therefore increased opportunities for removal processes to occur: it is unknown if this novel design will be as effective as conventional CFWs

The hypotheses tested were as follows:

(1) NO_3^--N interception will be low due to the small size and fast flow of water in the wetland studied, reducing the potential for denitrification to occur.

(2) The N pollution swapping effect (i.e. the emission of N_2O at the expense of intercepting NO_3^--N) will be minimal.

(3) Emissions of the greenhouse gas CH_4 will be larger and more important from the denitrifying wetland than N_2O.

The 'importance' of CH_4 and N_2O emissions will be determined based on their CO_2 equivalents and comparing perceived monetary cost in relation to savings generated by intercepted NO_3^--N.

Experimental rationale

A combination of wetland water chemistry measurements and gaseous emissions will be used to determine the ability of wetlands to remediate diffuse NO_3^- pollution and quantify the associated greenhouse gas emissions.

5.2 Materials and methods

5.2.1 Wetland construction and design

An instream wetland was constructed in June 2006 at Nafferton Farm, the site of the Earth Systems Laboratory (ESL) Initiative at the University of Newcastle (see Chapter 2.1). This denitrifying wetland formed part of a larger diffuse pollution mitigation and flood prevention scheme run at Nafferton farm by Newcastle University. The wetland, 20 m in length and 3 m wide was constructed through deepening of an existing field edge stream and introducing a series of revetments along its length (Figure 5.1). The wetland plus an additional 1.5 m either side of it was fenced from the adjacent organic grass-clover pasture, grazed by sheep and cattle.

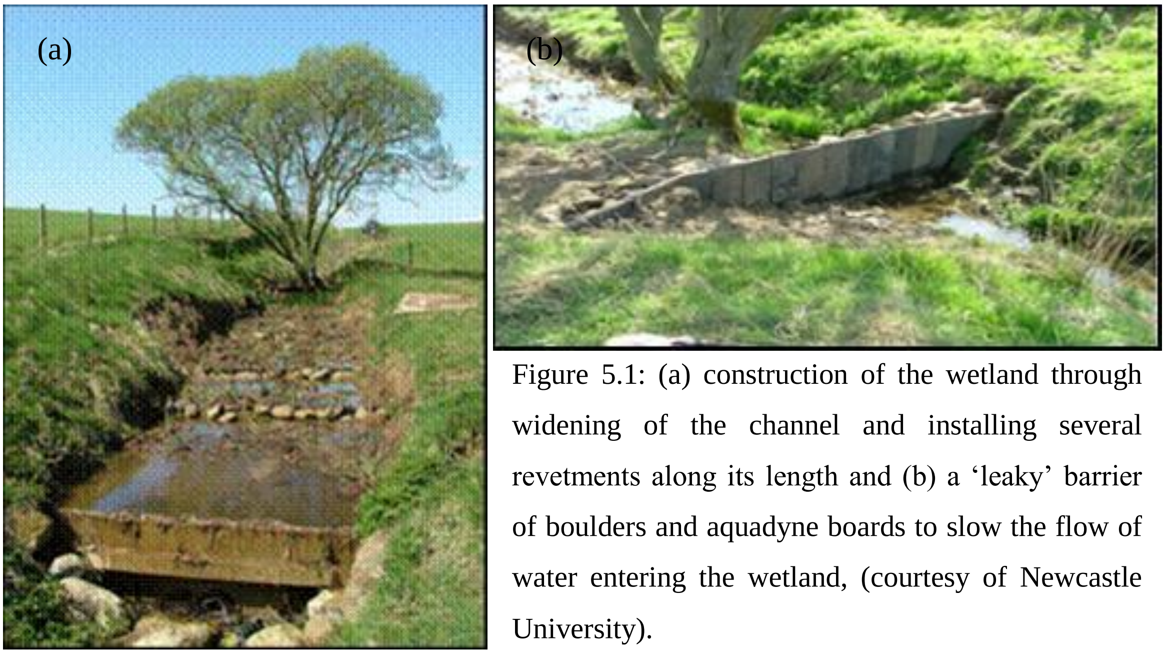


Figure 5.1: (a) construction of the wetland through widening of the channel and installing several revetments along its length and (b) a ‘leaky’ barrier of boulders and aquadyne boards to slow the flow of water entering the wetland, (courtesy of Newcastle University).

The revetments positioned at approximately 0, 7 and 12 m from the start of the wetland were comprised of woven willow branches and aquadyne (boards made of recycled plastic), which were supported by small boulders. The revetments provided multiple benefits not least by creating a physical barrier to slow the speed of water flow through the wetland leading to longer water residence times and potential for denitrification. This will support sedimentation allowing for more available carbon sources to promote denitrification. The aquadyne also encourages the formation of a bacterial biofilm that can enhance the potential denitrification capacity of the wetland.

The wetland was planted with sage grass and willow trees to increase phytoremediation and further ‘clean’ the water of other contaminants. Phytoremediation is promoted in the plant rhizopheres through the creation of a habitat for bacteria that promote degradation of contaminants. Moreover these plants provide food and habitat space for wildlife. The willow barriers flourished during the growing season and were cut and removed from the site once annually, by colleagues from the University of Newcastle, also removing some nutrients permanently from this wetland system. After allowing time for vegetation establishment, in October 2006 three automated gas flux chambers (referred to as AC1, AC2 and AC3) were installed along a longitudinal transect (Figure 5.2), with chamber AC1 closest to the wetland inlet and AC3 closest to the outlet.

Figure 5.2: View of the constructed instream wetland in May 2007 from approximately 18 m along the water sampling transect which starts beneath the tree. Two of the transparent automated gas flux chambers in the wetland and control and sample boxes along the bank are apparent.

Greenhouse gas and water chemistry sampling along transect began in January 2007 (Figure 5.3).

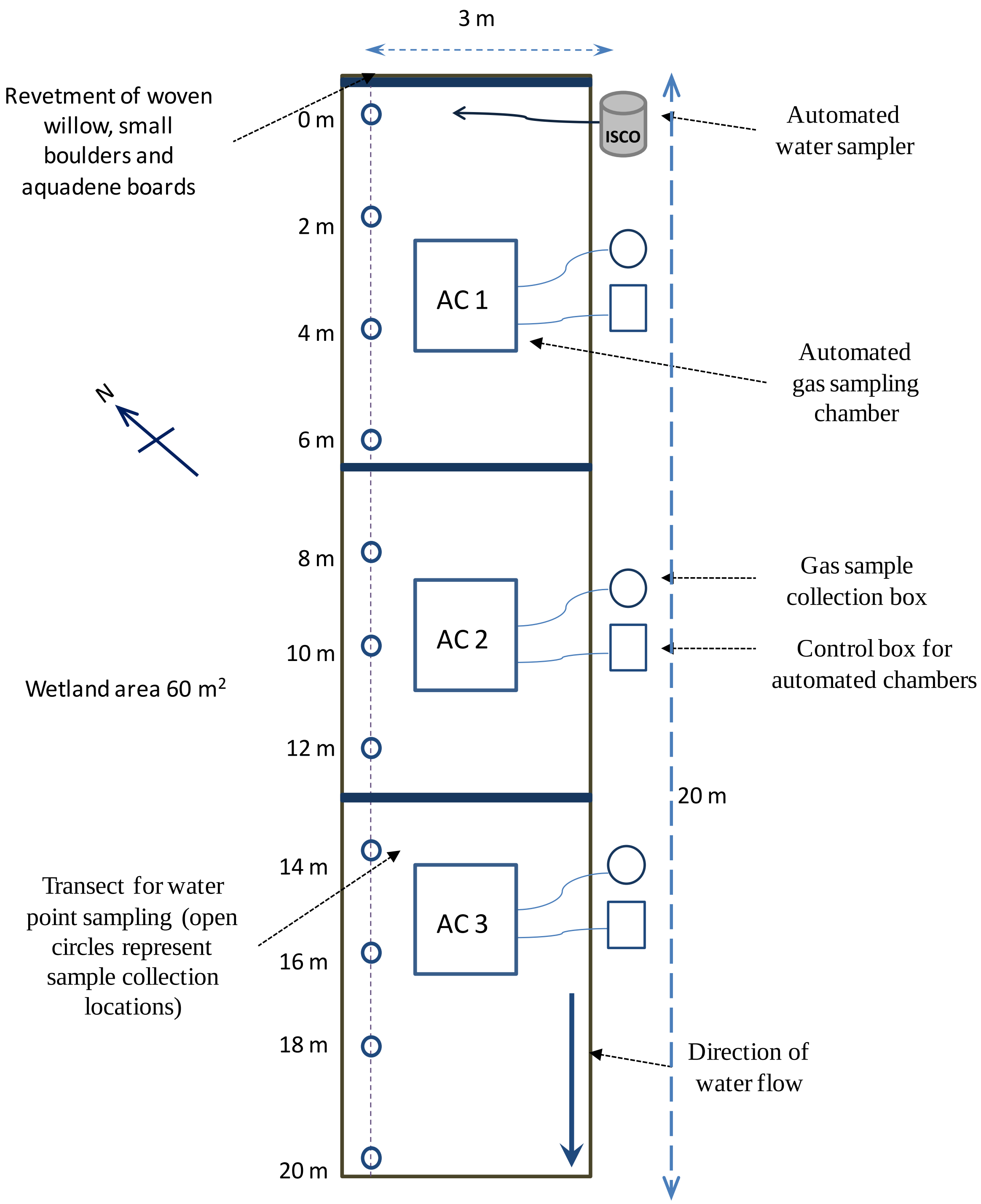


Figure 5.3: Schematic diagram of the instream wetland (not to scale) with water and gas sampling points and equipment marked.

5.2.2 Sampling strategy

Emissions of CH_4 and N_2O were determined from daily gas samples collected from three *in situ* automated gas flux chambers positioned along the length of the wetland. Concentrations of NO_3^- and NH_4^+ in the wetland water were determined from daily bulked water samples taken at the very top of the wetland (06 March 2007 - 22 January 2009). Additionally NO_3^- and NH_4^+ as well as concentrations of water N_2O were determined from point water samples collected fortnightly at 2 m intervals along the length of the wetland (11th November 2006 - 22nd January 2009).

5.2.3 Wetland gas sampling and analysis

Three in-situ automated chambers positioned along the length of the wetland at approximately 5, 10 and 15 m from the upstream end were used to collect daily gas samples. Each chamber had a square footprint, 80 cm by 80 cm, and comprised 0.3 cm thick transparent Perspex sheets fitted to a steel frame. Chambers were anchored into position using stainless steel rods that also allowed it to rest above the sediment surface but below the water surface, enabling the free movement of water within the wetland whilst also establishing an air tight seal at the base of each chamber. The height of the chambers therefore varied with water depth which was monitored using a pressure sensor (PDCR 1830, Campbell Scientific), positioned at approximately 10 m along the transect. Water depth calculations were performed using standard manufacturer's conversion protocols and used to calculate the automated chamber heights and thus greenhouse gas emissions.

The chamber lid was attached to pistons that lowered and closed it according to pre-programmed instructions for 60 minutes each day at 12:00 noon when an air sample was taken from the closed chamber and stored in the chamber set-up in 0.25 L evacuated Cali-5 Bond foil bags (Calibrated Instruments). Samples remained in the 0.25 l evacuated Cali-5 Bond foil bags until analysis within 30 days of collection (see Chapter 2.2.2).

Collected gas samples were transported back to the Edinburgh University laboratory on the day of sampling when conducted by Edinburgh University staff. Those collected by Newcastle University staff were sent by post and received within one week from the time of sampling. Daily gas sampling using the automated gas flux chambers began in January 2007 and continued until December 2008; however equipment failures resulted in some gaps in the dataset: 171 and 119 days during 2007 and 2008, respectively have no measurements available from any chamber. Data shown are the mean of three chambers where available unless otherwise stated.

Chamber air samples were analysed for N_2O using an Agilent 6890 gas chromatograph fitted with a 1.8-m Hayesep Q column and electron capture detector. Ten per cent CH_4/Ar was used as the carrier gas with column and detector temperatures of 70°C and 390°C, respectively. N_2O standards of 1.1 and 10.2 ppm, as well as an ambient air sample assigned a value of 0.31 ppm (see Chapter 2.2.4 for details) were analysed before and after chamber samples and used to produce a linear regression from which chamber N_2O concentrations could be calculated.

CH_4 in chamber air samples was determined using a HP5890 gas chromatograph with a flame ionising detector (GC-FID) and automated injection system. Standard gas mixtures of 1.0, 3.4 and 10 ppm CH_4 were prepared in an identical manner and analysed to produce a linear regression from which chamber CH_4 concentrations could be calculated.

5.2.4 Wetland water sampling and analysis

Fortnightly point water samples were taken every 2 m along a longitudinal transect of the wetland from 11 November 2006 - 22 January 2009. A 30 mL polystyrene vial (Sterilin, Fisher Scientific), and screw top lid was rinsed with sample water before collection and then immersed fully into the water to remove all the air. Vials were then transported to Edinburgh in a cool box and transferred to the refrigerator until analysis for dissolved N_2O (11th November 2006 to 29th April 2008). Samples were analysed within 48 hours of collection for N_2O using gas chromatography. A plastic 5 mL syringe was used to remove 5 mL of air from within a sealed gas vial (22 mL) containing ambient air. This air was then discarded and replaced with 5 mL of wetland water which was removed from the sample bottle and injected directly into the sealed vial using a hypodermic needle. The vial was vigorously shaken for 2 minutes and left for 30 minutes to equilibrate (Reay *et al.,* 2003). For all point water samples duplicate analyses were carried out. N_2O concentrations in the vial headspace were then analysed using an Agilent 6890 gas chromatograph fitted with an electron capture detector (17 November 2006 - 27 November 2008). Laboratory and wetland temperatures measured on the day of sample collection and analysis, respectively, was used to determine dissolved N_2O concentration. *In situ* N_2O concentrations were calculated based on the solubility of N_2O at laboratory versus *in situ* pressure and temperatures (Weiss and Price, 1980). The remaining sample water was either analysed for NO_3^- and NH_4^+ immediately, or, if the analyser was unavailable, samples were frozen until analyses could be performed.

Daily bulked water samples (24-hour composite of samples taken every 8 hours) were collected at the start of the wetland using an automated ISCO sampler (ISCO 6712 Full-size Portable Sampler, Environmental Monitoring Solutions Ltd.) from 06 March 2007 to 22 January 2009. ISCO sample bottles contained 10 ml of mercuric chloride solution (2 µg $HgCl_2$ L^{-1}) to preserve the samples and prevent N degradation between sampling and analysis (Clough *et al.,* 2003). Fortnightly, the daily bulked water samples were subsampled into labelled 30 mL polystyrene vial with screw top lids (Sterilin, Fisher Scientific) after rinsing with the sample. The remainder of the samples were transferred to a 5 L plastic container and transported to Edinburgh University for safe disposal. Insitu measurements sampled fortnightly at 0 m along transect were not statistically different ($P > 0.05$, $n = 22$, one way ANOVA) from those measured in the bulked water sample collected by the ISCO sampler and demonstrates that the mercuric chloride preservative is effective.

NO_3^--N and NH_4^+-N concentrations within all wetland water samples were determined colorimetrically with a Bran and Luebbe continuous-flow analyser, using the manufacturer's method G-102-93 rev1 for NH_4^+-N and method G-109-94 rev3 for NO_3^--N, (see Chapter 2.3.7 for details).

5.2.5 Auxiliary measurements

Auxiliary measurements of wetland physical parameters - water temperature and water depth - were taken using an *in situ* probe (Druck PDCR 1830, Campbell Scientific; Loughborough, UK), positioned approximately 10 m along the wetland (2 October - 28th November 2007 and 29 May 2008 - 21 May 2009; the gap between the two sets of dates is when the probe was not operational). An onsite meteorological station recorded hourly rainfall, air temperature, humidity, soil temperature, radiation, wind speed and wind direction.

5.2.6 Wetland interception of diffuse N

Concentration reduction efficiencies (CRE) (Equation 5.1) as well as mass removal efficiencies (MRE) (Equation 5.2) were estimated for each sampling occasion during the study period using the inlet (0 m) and outlet (20 m) concentrations of inorganic-N (NO_3^--N and NH_4^+-N).

$$CRE\ (\%) = 100 * [(C_i - C_o)/C_i] \qquad \text{(Equation 5.1)}$$

$$MRE\ (\%) = 100 * [(M_i - M_o)/M_i] = 100 * [(V_i * C_i) - (V_o * C_o)/ (V_i * C_i)] \qquad \text{(Equation 5.2)}$$

where C_i and C_o are the concentrations (mg L^{-1}) of the inlet and outlet respectively, M_i and M_o are the mass of N at the inlet and outlet respectively, and V_i and V_o are the flow volume at the inlet and outlet respectively. Additionally the mass removal of N was calculated in g N m^{-2} for each sampling occasion and for the total annual budget.

The wetland inflow volume, V_i, was estimated by calculating mean 2007 and 2008 flow from the mean water depth during the measurement period and the wetland dimensions.

$$Q = (A \times R^{2/3} \times S^{1/2})/n \qquad \text{(Equation 5.3)}$$

where Q is flow ($m^3\ s^{-1}$), A is cross-sectional channel area (m^2), R is the hydraulic radius (the cross-sectional area divided by the wetted perimeter), S is the slope of the water surface and n is Manning's roughness. A value for n of 0.345 measured in bulrush wetlands was used (WRP, 1994). The outflow was calculated by subtracting evapotranspiration from the wetland surface area from the inflow rate. Evapotranspiration was not measured, however mean values of 3.32 mm d^{-1} for spring and summer, and 1.24 mm d^{-1} for autumn and winter obtained from a reedbed in Teesside, north-east England, were used (Fermor *et al.,* 2001).

5.3 Results

5.3.1 Field site environmental parameters measured during the study period

Total measured rainfall at the site was 541 and 741 mm in 2007 and 2008, respectively. The lowest rainfall occurred during the spring months (86.6 and 100.2 mm in 2007 and 2008, respectively, March - May) and the highest rainfall occurred during the summer months of both years (222.7 and 276.4 mm, respectively, June - August) (Figure 5.4). However the autumn months (September - November) of each year showed a great difference in total rainfall, 87.8 and 240.4 mm in 2007 and 2008, respectively. This disparity is due to the very high monthly rainfall total in September 2008, including a one-in-200 year rainfall event (see Chapter 2.1).

The wetland water depth varied temporally, dependent upon localised, short-term hydrological conditions at the site. Water depth was measured in 2007 during only October and November, when monthly mean water depth was 22.4 ± 8.3 and 20.1 ±

9.9 cm, respectively, much lower than the mean water depths for the same months in 2008 of 36.3 ± 8.0 and 42.3 ± 1.3 cm, respectively. The limited water depth data available for 2007 does not allow for seasonal comparisons however the more complete data set for 2008 shows that the water level varied from 3.1 to 72.6 cm with the lowest observed water depths occurring during December (Figure 5.4).

Rainfall is not the only determining factor of wetland water level as some months with the greatest rainfall levels do not coincide with the months of highest water levels; thus evapotranspiration would likely have contributed to the observed water depth. Although evapotranspiration rates were not measured, in comparable sites within the UK, greater rates were found to occur during the spring and summer months as a result of higher temperatures and plant growth (Fermor *et al.*, 2001). This would result in lowering the water depth of the wetland during these seasons. In spring and summer 2007 and 2008 the effects of evapotranspiration (and low rainfall during the spring) lead to areas within the studied wetland drying out and exposure of sediments to the atmosphere. Exposed sediments tended to occur between 2 - 4 m along transect on the eastern side of the wetland with generally deeper water maintained between 15 - 20 m along transect between the lower two revetments. Water depth was measured at 10 m along transect only and failed to capture this spatial variability. However, at no time did the water level fall below the base of the automated gas flux chambers.

Mean wetland water temperatures (mean of all available measurements) in 2007-2008 were 9.43°C, with temperature ranging between 1.98 and 16°C. Water temperatures (recorded fortnightly at the time of water sampling), for both years were greater than 10°C from April to September with the lowest recorded temperatures occurring in February, 4.85 and 6.00°C in 2007 and 2008, respectively.

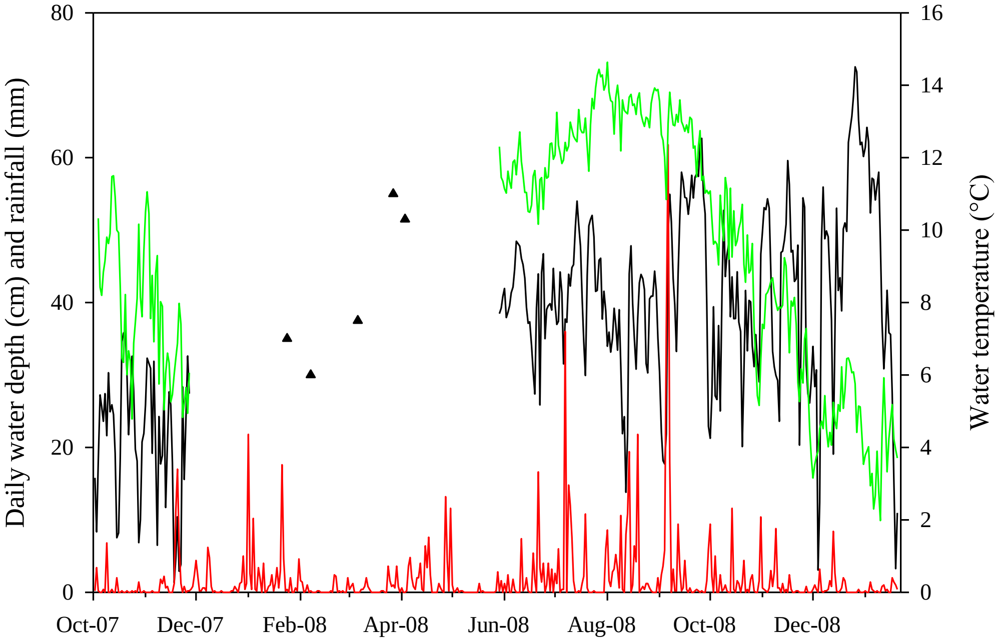


Figure 5.4: Mean daily rainfall (mm) (red line), water depth (cm) (black line) and water temperature (green line from the in situ probe and black triangles from fortnightly water temperature measurements) from October 2007 to January 2009 where data is available.

Additionally mean wetland water pH was 6.48 ± 1.11 (available data from January to October 2007, n = 130; courtesy of Newcastle University).

5.3.2 Wetland emissions of methane and nitrous oxide

Total annual CH_4 emissions were greater from the constructed wetland during 2007 than 2008: 713 ± 11.1 and 237 ± 4.9 mg CH_4 m^{-2} $year^{-1}$, respectively (mean of the three automated chambers ± one standard deviation, unless otherwise stated). Additionally total annual N_2O emissions from the wetland were also greater from 2007 than from 2008, 3.5 ± 0.8 and 1.9 ± 1.2 mg N_2O-N m^{-2} $year^{-1}$, respectively.

Seasonally the highest mean CH_4 emissions occurred during the spring months (March to May), of both years, with the greatest occurring during the spring of 2007, (Figure 5.5) with mean emissions from all chambers of 13.6 ± 16.4 and 3.73 ± 11.5

mg CH_4 m^{-2} hr^{-1}, in 2007 and 2008, respectively. However the range of emissions observed from individual chambers were high (0.1 – 69.0 mg CH_4 m^{-2} hr^{-1}) as illustrated by the high standard deviation of the mean.

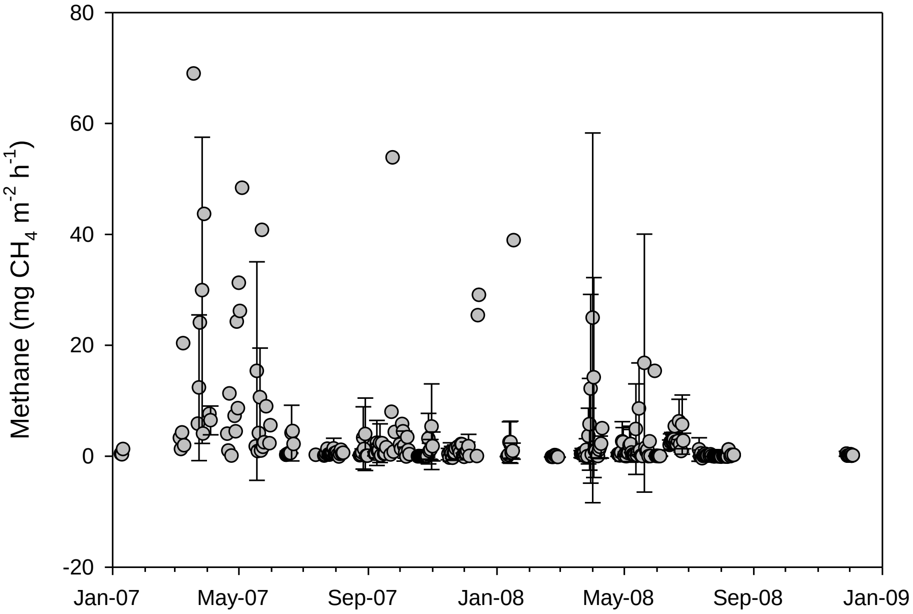


Figure 5.5: Mean daily wetland CH_4 emissions (mean ± 1 S.D. where data available).

CH_4 emissions require a carbon source and have been shown by many researchers to increase with increasing temperatures (van Hulzen *et al.,* 1999). High spring CH_4 emissions are most probably associated with the rise in water temperatures from a winter mean of 5.87 ± 0.98°C to a spring mean of 10.2 ± 1.95°C (mean data for 2007 and 2008). Moreover the very high emissions from all three chambers in 2007 compared with high emissions primarily from AC1 only in 2008 (Figure 5.6) can be explained by carbon source availability.

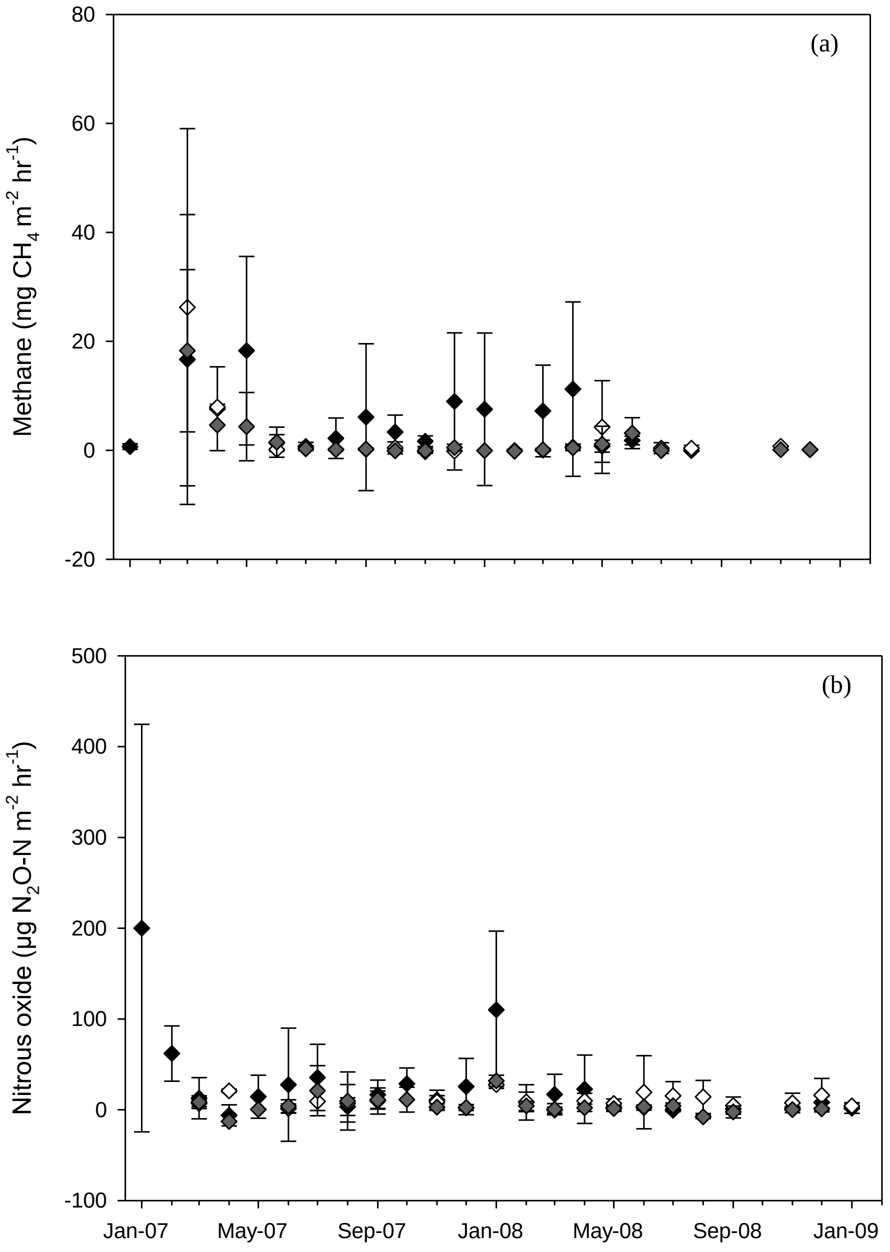


Figure 5.6: Monthly CH_4 (a) and N_2O (b) emissions from each of the automated chambers (mean ± 1 S.D. where data available), (AC 1: black diamonds, AC 2: white diamonds, AC 3: grey diamonds).

Mean water temperature increased further from spring to summer to 12.8 ± 1.32°C, however CH_4 emissions decrease by an average of 88% (mean of 2007 and 2008).

Winter emissions of N_2O in 2007 and 2008 are greater than those of any other season; 131 ± 97.7 and 39.5 ± 50.3 μg N_2O-N m^{-2} hr^{-1}, respectively. The highest concentrations of NO_3^--N generally occur in the winter months, 17.4 ± 5.15 and 14.4 ± 3.36 mg L^{-1}, in 2007 and 2008, respectively (Figure 5.7). It is most probable that a combination of the mild winter temperatures, mean 5.3 and 4.5°C in each year, respectively, in addition to the high winter mean NO_3^--N concentrations is the causal factors of the high N_2O emissions.

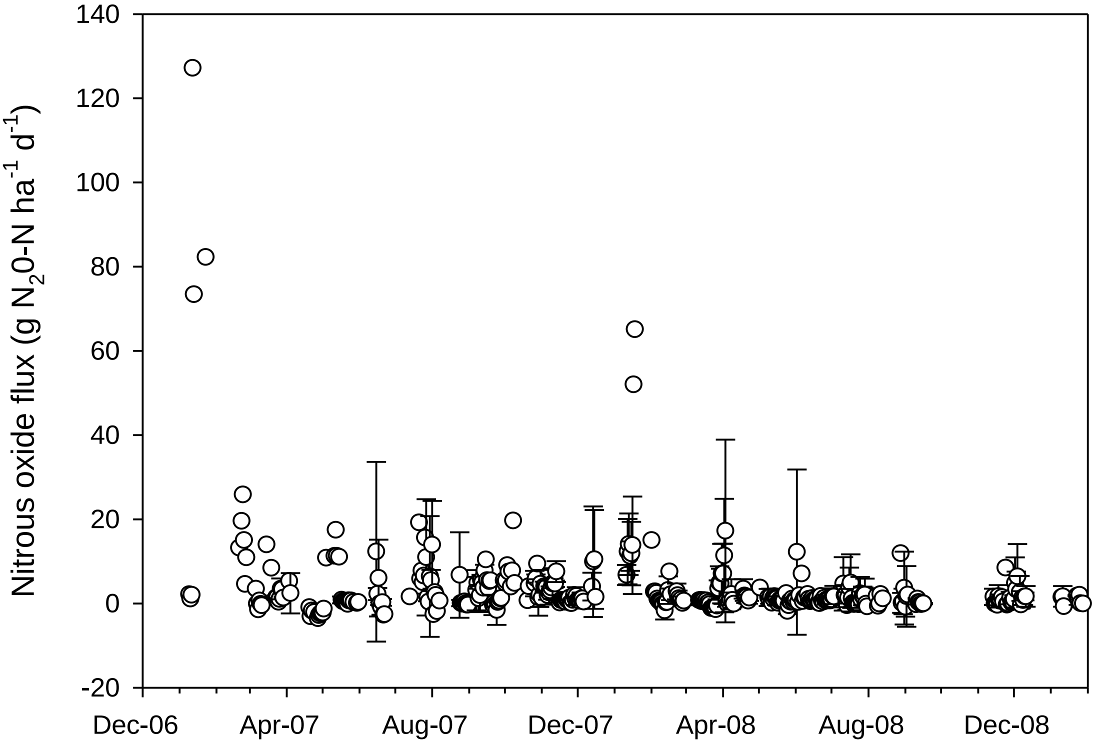


Figure 5.7: Daily N_2O emissions (mean ± 1 S.D. were data available).

The CH_4 and N_2O emissions from the three automated flux chambers within the wetland are significantly different to one another ($P < 0.001$, $n = 28$, and $P < 0.01$, $n = 144$, respectively, 1-way ANOVA) and were generally in the order AC1 > AC2 > AC3. Furthermore, AC1 is significantly different to either AC2 or AC3 ($P < 0.001$, $n= 28$, Tukey's multiple comparison test). However, fluxes from adjacent automated chambers within the wetland i.e. differences between the fluxes from AC1 and AC2, or AC2 and AC3, are not statistically different ($P > 0.10$) from one another (Tukey's multiple comparison test, n = 70 and 142, respectively).

These findings are not unexpected as the chamber positioned furthest 'upstream' in the wetland, i.e. AC1, would have higher available carbon and organic matter as a result of greater sedimentation due to the reduction in water velocity at the wetland inlet. Although sediment depth and composition were not measured, it was observed that the upper 15 m of the wetland accumulated more sediment than elsewhere. That being said a greater water depth was maintained between 15 - 20 m with lower turbidity and a deep sediment layer existed in this location. Additionally after high rainfall, scouring of the wetland channel occurred resulting in sediment removal, particularly from the uppermost 5 m of the wetland. However between 15 - 20 m along transect sediment loss was less noticeable with a relatively deeper sediment layer maintained throughout the study.

5.3.3 Dissolved N_2O concentrations in wetland water

Dissolved N_2O concentrations within the wetland ranged from 0 – 231 µg N_2O-N l^{-1} across the study period, with a mean and median of 6.73 and 3.34 µg N_2O-N l^{-1}, respectively. The highest dissolved N_2O concentration of 231 µg N_2O-N l^{-1} occurred at 18 m along the transect during December 2006. However, more than 60% of dissolved N_2O concentrations are less than 5 µg N_2O-N l^{-1} and just 2% greater than 30 µg N_2O-N l^{-1}. Nevertheless these concentrations are still much greater than the air equilibrium concentration of ~ 0.3 µg N l^{-1}; suggesting indirect N_2O emissions via degassing of the wetland water are important (Reay *et al.*, 2009). Since mean values are skewed by occasional high values, median seasonal N_2O-N concentrations along the transect are shown in Figure 5.8.

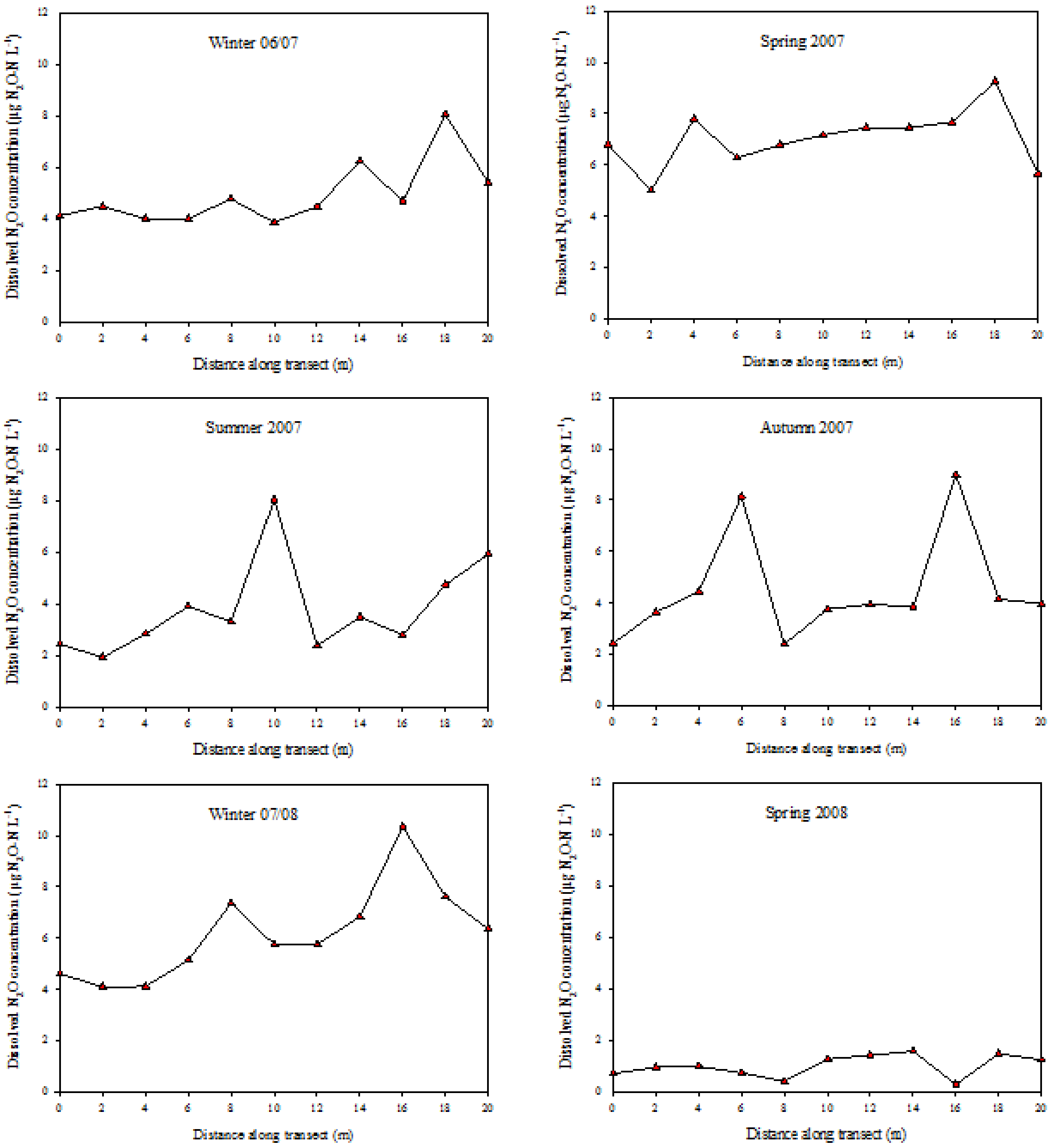


Figure 5.8: Seasonal median dissolved N$_2$O-N concentrations along transect (season/year are as indicated).

Seasonally dissolved N$_2$O concentrations do not vary greatly, with the exception of spring 2008 when concentrations were lower. The median dissolved concentrations tend to increase along the transect, with the exception of spring 2008 where concentrations are broadly similar. Slightly higher concentrations tend to occur from two locations, between 6 - 10 and 16 - 18 m along transect. The position of revetments at approximately 7 and 12 m along transect may be decreasing wetland water velocity allowing greater accumulation of N_2O in the water column. The higher N_2O concentrations may be from nitrification within the water column or from active sites of either nitrification or denitrification in the underlying sediments from these locations.

5.3.4 NO_3^--N and NH_4^+-N concentrations in wetland water

NH_4^+-N concentrations in the Isco-sampled water near the wetland inlet are generally low, with median concentrations of 0.08 and 0.05 mg NH_4^+-N l^{-1} in 2007 and 2008, respectively. Maximum concentrations of 8.45 and 8.38 mg NH_4^+-N l^{-1} were measured during October 2007 and July 2008 (Figure 5.9 (a)).

Median NO_3^--N concentrations were 4.49 and 7.64 mg l^{-1} during 2007 and 2008 respectively, whilst maximum concentrations in the two years monitored were 18.5 and 18.1 mg l^{-1}, respectively. NO_3^--N concentrations in the wetland water were generally high during the winter and spring months and decreased in summer and autumn months (Figure 5.9 (b)). Measured concentrations of NO_3^- and NH_4^+ exceed the water quality standards (11.3 mg N l^{-1} and 0.78 mg N l^{-1}, respectively) on 15 and 24% of sampled occasions, respectively.

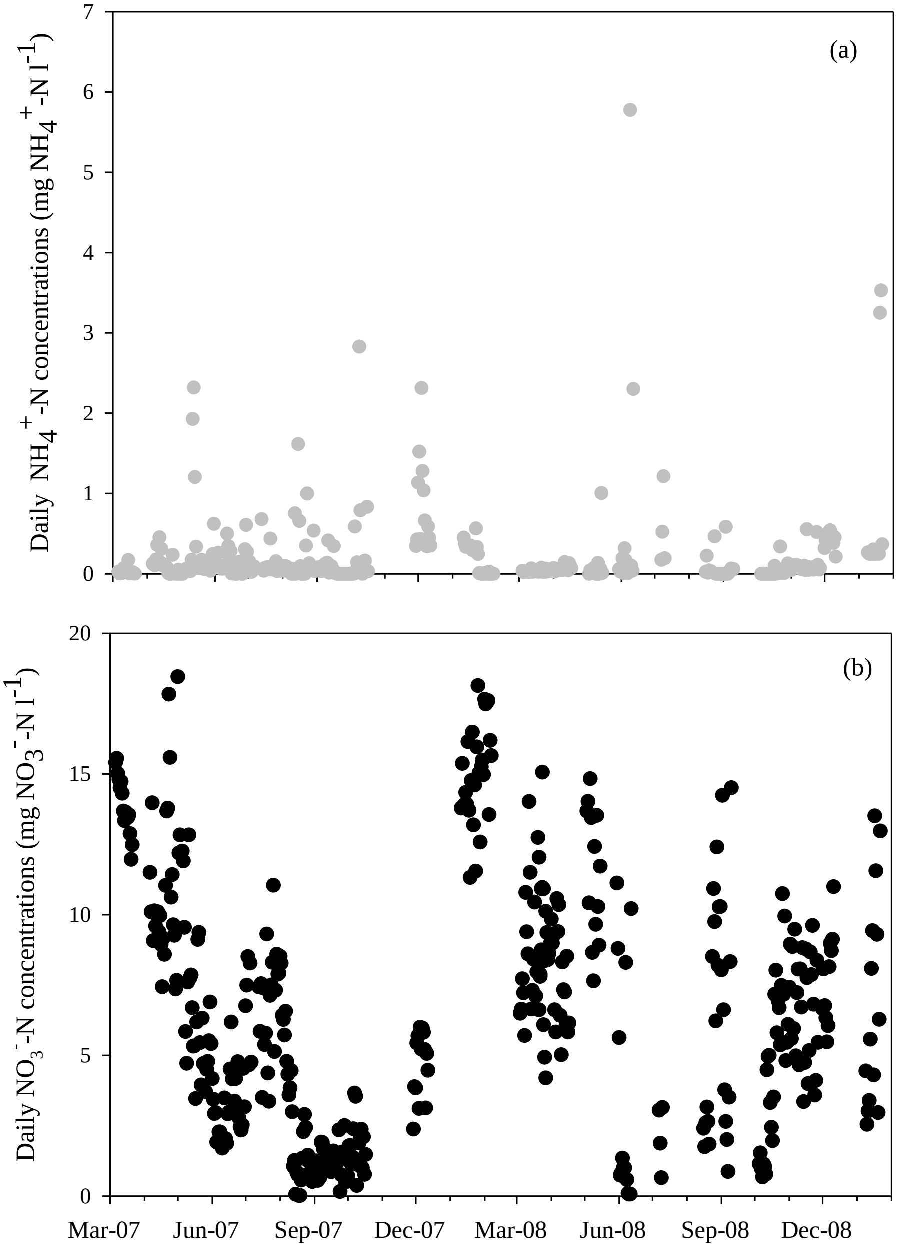


Figure 5.9 Daily NO_3^--N (a) and NH_4^+-N (b) concentrations (mg N L^{-1}), from daily bulked samples taken using the automated ISCO sampler at the start (0 m) of the wetland. Solid lines represent the maximum allowable drinking water concentration of 11.3 mg NO_3^--N l^{-1} and the European Freshwater Fish Directive (78/659/EEC) directive maximum guideline value of 0.78 mg NH_4^+-N l^{-1}. Gaps in the data set are when equipment malfunctioned. The total number of days sampled were 220 and 210 days during 2007 and 2008, respectively.

NO_3^--N concentrations measured along the transect within the wetland followed a similar seasonal pattern in both 2007/2008 of higher winter concentrations (mean 17.44 ± 3.73 and 16.24 ± 1.02 mg NO_3^--N L^{-1} in 2007 and 2008, respectively), most probably due to high rainfall and low interception within the wetland and plant uptake during these colder months (Figure 5.10). NO_3^- concentrations then decrease rapidly in late spring-early summer most probably as a result of plant growth and uptake. This is followed by a gradual rise in concentration through the autumn most probably due to high rainfall and lower evapotranspiration, conveying more NO_3^- into the wetland and a slowdown in plant uptake of NO_3^- due to lower temperatures.

NH_4^+-N concentrations were low during the spring and early summer months of both sampling years with only a few occasions exceeding the European Freshwater Fish Directive (78/659/EEC) directive maximum guideline value of 0.78 mg NH_4^+-N l^{-1}. Concentrations of NH_4^+-N several times greater than the maximum guideline value occurred during October 2007 and July 2008.

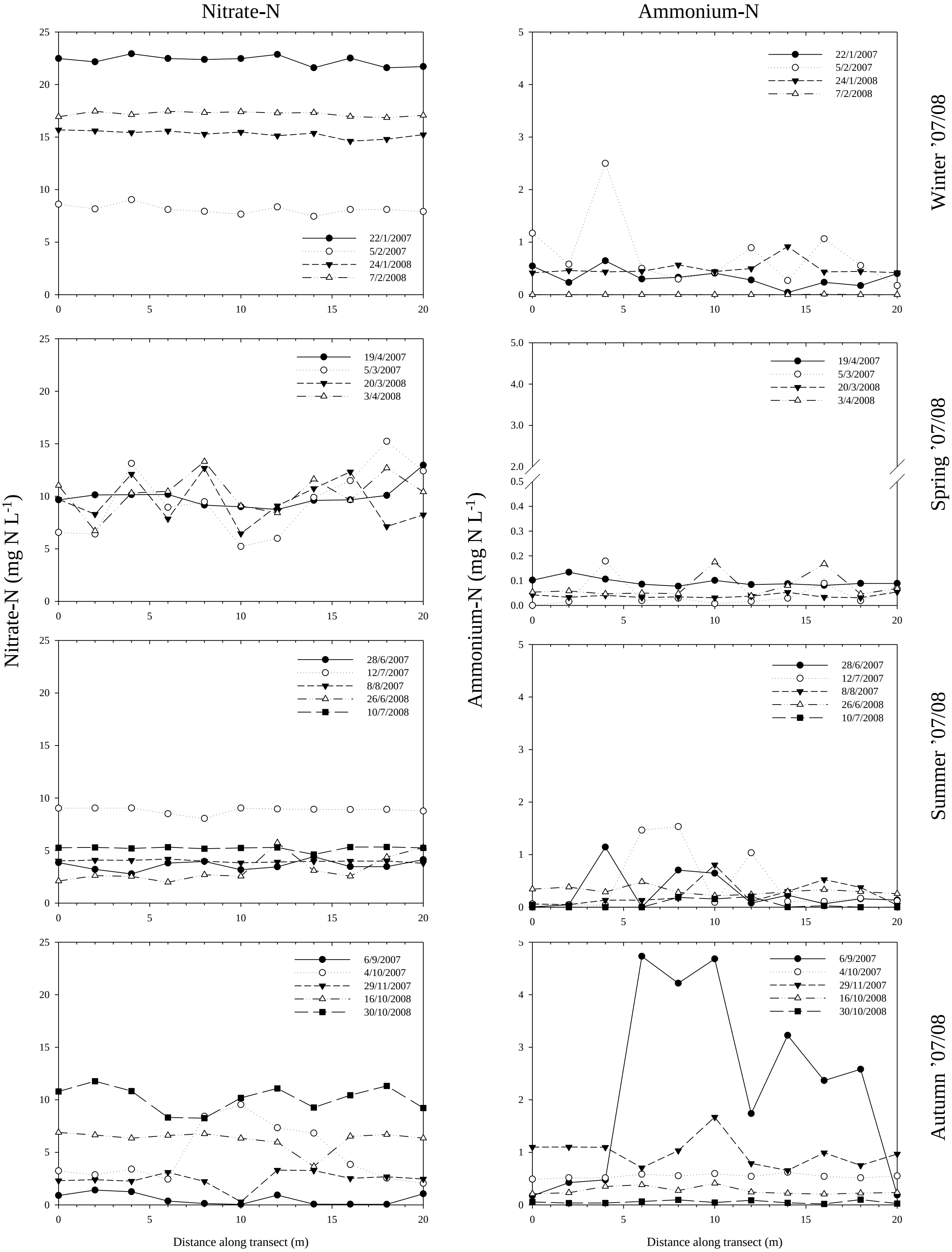


Figure 5.10 NO_3^--N and NH_4^+-N concentrations at 2 m intervals along the wetland transect, for selected dates during 2007 and 2008 for each season (as labelled).

5.3.5 Wetland interception of diffuse nitrogen

The wetland appeared to be relatively ineffective for NO_3^--N and NH_4^+-N removal at all times of the year, as concentrations along the transect do not decrease (Figure 5.10). Moreover much more variable concentrations along the transect occur during the spring and autumn months, in many cases increasing along its length, suggesting secondary sources of nitrogen input potentially from throughflow or through old field drains, such as observed after heavy rainfall (Figure 5.11). However, the reasons why these secondary sources seem to only affect spring and autumn months may be related to rainfall i.e. high rainfall prior to sampling increases the input from the secondary sources.

Figure 5.11: Picture showing an intrusion of water through the north soil bank of the wetland at approximately 10 metres along transect, taken 01-May-2008 after heavy rain, two automated flux chambers are evident in the picture foreground.

Transect nutrient data is highly variable in that mean concentrations differ markedly between sampling dates, (0.6 – 22.3 mg NO_3^--N l^{-1}), as well as along transect length varying by up to 3.3 mg NO_3^--N l^{-1}. Furthermore concentrations of NO_3^- along the wetland are seen to increase at between 4 and 12 m along the transect for 50% of sampled months, on many occasions coinciding with high rainfall months which may be associated with secondary inputs of N to the wetland as mentioned previously. However, high rainfall does not always equate to high NO_3^--N concentrations; a sampling occasion in March 2007 had high NO_3^--N concentrations (mean transect concentration of 15.5 ± 14.7 mg NO_3^--N l^{-1}) with relatively low total rainfall of 22.6 mm.

NO_3^--N and NH_4^+-N concentration reduction efficiencies (CRE) or Mass removal efficiencies (MRE) are a good way to assess the effectiveness of a wetland in removing diffuse N; these were calculated as described in section 5.5.4. However, the intrusion of a secondary source of N into the wetland would prevent possible N interception from being identified using either CRE or MRE. For this reason the data has not been presented.

The wetland has an estimated mean inflow of 103 (48.2 - 173) L s^{-1}; suggesting a low residence time within the wetland and reduced opportunity for removal of dissolved nutrients. Nevertheless in 2007 the wetland was a net sink of NO_3^--N (321 g N m^{-2} $year^{-1}$) but a net source of NH_4^+-N (- 195 g N m^{-2} $year^{-1}$). In 2008 the wetland was a net source for both NH_4^+-N and NO_3^--N, - 8995 and - 633 g N m^{-2} $year^{-1}$, respectively. This is most likely due to the increased discharge (123 L s^{-1}), and therefore lower water residence time in 2008 compared to 2007. The mass of N removal for both NH_4^+-N and NO_3^--N were very different between 2007 and 2008. Winter 2007 and spring 2008 were the largest seasonal net sinks of NH_4^+-N whereas, spring was the highest net source of NO_3^--N in 2007. However, decisive conclusions cannot be drawn due to the secondary N source along the wetland transect.

5.4 Discussion

5.4.1 CH_4 emissions: spatial and temporal variability and controlling factors

Annual CH_4 emissions were three times greater in 2007 than 2008. Seasonally the greatest emissions occurred during the spring of both years however the difference in emissions between both years is largely due to an 80 % reduction in spring emissions from 2007 to 2008. Spring represented a time of increased temperatures and therefore potentially higher rates of microbial activity. The wetland construction was completed during the summer of 2006 therefore spring 2007 represented the first occasion when higher water/sediment temperatures and available C sources co-existed with low plant growth (Segers, 1998; van Hulzen *et al.,*1999; Gauci *et al.,* 2004), therefore potentially resulting in these high emissions in spring 2007. It is suggested that as vegetation became more established during the summer of both years CH_4 emissions decreased markedly. Furthermore wetland vegetation could have led to increased sediment aeration during summer months, leading to less anaerobic conditions, suppression of methanogenesis and CH_4 emissions or enhanced methane oxidation (Laanbroek, 2010).

Higher temperatures are a known causal factor for increased CH_4 emissions, (Segers, 1998; van Hulzen *et al.,* 1999), with often an exponential relationship reported (Macdonald *et al.,* 1997). However, the relationship between mean monthly CH_4 emissions and water temperature did not fit with a logarithmic trend that is commonly found with CH_4/temperature relationships (Christensen *et al.,* 2003) (Figure 5.16).

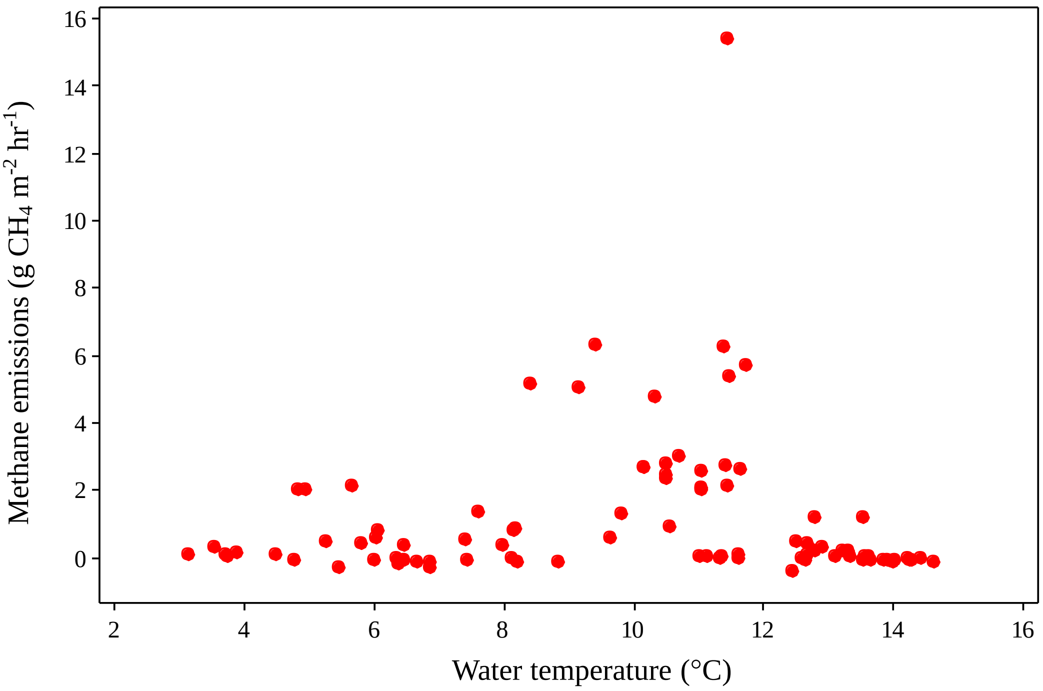


Figure 5.16 Regression plot of CH_4 emissions versus water temperature for all available data 2007 and 2008 ($r^2 = 0.001$, n = 56).

The increased emissions from winter to spring were most probably related to increased temperatures. However, emissions decreased significantly during the summer months despite increasing temperatures (r^2 = 64% and 65% for 2007 and 2008, respectively), suggesting that several factors control CH_4 emissions at this site. Increased plant growth and evapotranspiration from spring to summer may have lowered mean water depth and increased sediment oxygenation resulting in decreased methanogenesis and/or increased CH_4 oxidation (Fermor *et al.*, 2001; Zhu *et al.*, 2010).

In addition the rise in water temperatures from the winter lows of 5.5 – 6.5°C to the higher spring temperatures of approximately 10°C may have caused an increased rate of microbial activity which could have resulted in an exhausted or limited supply of organic material required for methanogenesis, limiting CH_4 production during warmer summer temperatures. Additionally, the relatively shallow water depth of less than 50 cm and the fast flowing nature of the instream wetland water could potentially have maintained a deeper oxic layer within the sediments preventing methanogenesis from occurring or from only occurring within anaerobic microsites. The effect of water turbulence on CH_4 production and emission from the wetland sediments was investigated in more detail in the subsequent laboratory experiments using membrane inlet mass spectrometry (see Chapter 6).

A primary control of CH_4 production and therefore emissions is the establishment of anoxic conditions, therefore hydrology or inundation of the wetland is important (Bartlett and Harris, 1993; Mitsch and Jørgensen, 2004). However wetland water depth and CH_4 emissions were not significantly related at this site in 2008 ($n=56$, $r^2 = 0.004$, $P>0.05$). Despite the water depth increasing by approximately 20 cm from 2007 to 2008, annual emissions decreased. This wetland experienced turbulent flow which may have maintained a deeper oxic layer preventing methanogenesis from becoming widespread but rather confining it to anaerobic microsites (Mitsch and Jørgensen, 2004). Furthermore, flow was channelized, resulting in drying out and exposure of sediment in parts of the wetland, although not in the location of automated flux chambers. This would have created spatially very different conditions within the wetland sediment i.e. aerobic in exposed sediments and anaerobic in submerged, potentially creating very different greenhouse gases (N_2O and CH_4, respectively). Mitsch and Jørgensen (2004) reported higher N_2O emissions and lower CH_4 from sites within a wetland that were exposed to air compared to flooded parts.

Net uptake (i.e. negative CH_4 emissions) were measured on some sampling occasions during the summer months of both years suggesting more aerobic conditions of the wetland sediments. Therefore wetland hydrology in relation to water depth, turbidity and flow pattern is an important controlling factor in CH_4 emissions for this wetland and other small instream or riverine wetlands (Bartlett and Harris, 1993; Mitsch and Jørgensen, 2004).

Invariably the emissions of CH_4 result from a complex interplay of various factors, such as temperature and redox status of the sediments, which vary over time and space. However the dynamic conditions experienced within the wetland of water level changes in addition to high turbulence and therefore aeration of the overlying water column have resulted in some atypical relationships of CH_4 emissions within the instream wetland compared to larger, more established wetland systems.

5.4.2 N_2O emissions and dissolved N_2O concentrations: spatial and temporal variability and controlling factors

Highest N_2O emissions from the wetland occurred during winter and early spring in both 2007 and 2008 and are most likely caused by high concentrations of NO_3^--N, mild winter temperatures and minimal plant growth resulting in higher rates of denitrification (Zhu and Sikora, 1994; Sabater *et al.*, 2003). However, annual N_2O emissions in 2007 were twice those of 2008 perhaps as the result of unlimited substrate, C source availability and warmer temperatures during early spring 2007 after the wetland was constructed in summer 2006, all of which promote denitrification potential under suitable redox conditions (Spieles and Mitsch, 1999; Kadlec *et al.,* 2005). Furthermore, after establishment the turbulent flow of this wetland would most probably have maintained an aerobic or suboxic surface layer in the sediment creating environments suited to incomplete denitrification and N_2O rather than N_2 emissions (Martin and Reddy, 1997; Hefting *et al.,* 2003).

Higher microbial activity is expected to occur with higher temperatures (Philpps and Crumpton, 1994; Fromin *et al.,* 2005) however there was no significant relationship between N_2O emission and water temperature (Figure 5.17). Higher temperatures at this site were associated with spring and summer months when optimal plant growth and therefore greatest substrate (NO_3^-) demand and competition between microbes and plants would occur: suggesting plant uptake may be a key removal pathway of N from this wetland (Sabater *et al.,* 2003; Fromin *et al.,* 2005; Randerson, 2006; Bialowiec *et al.,* 2012). Plant growth effectively removes this substrate, albeit temporarily from the N cycle (Fromin *et al.*, 2005). The lower emissions at times of increased temperature are likely a result of reduced substrate supply.

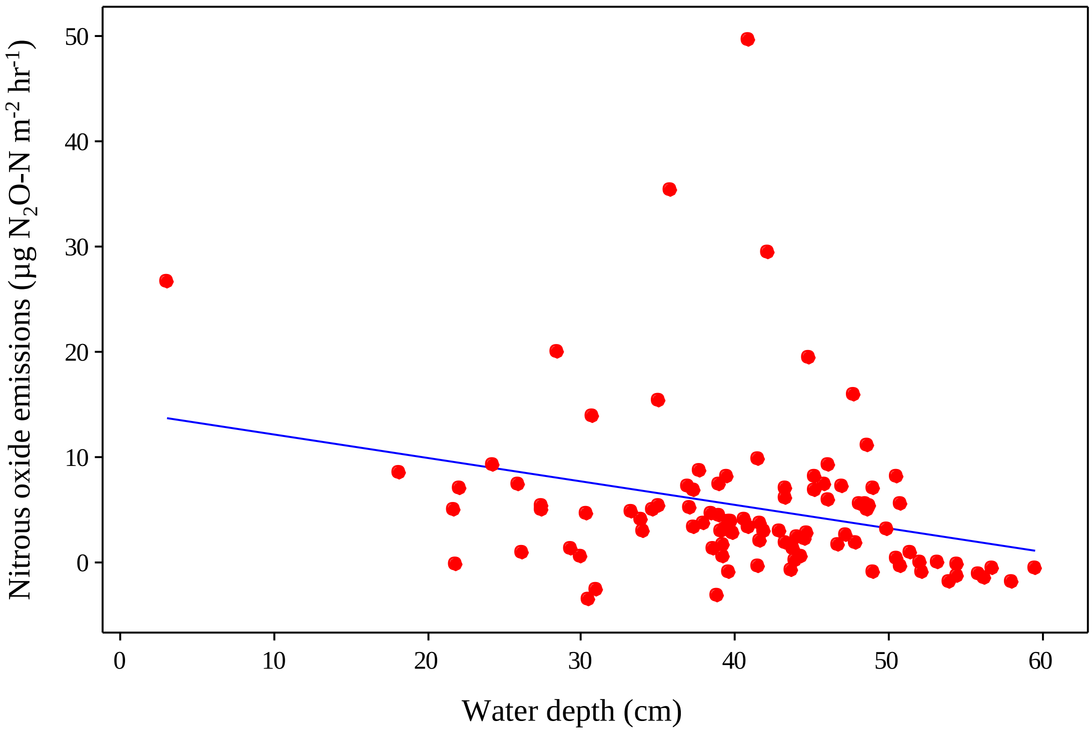


Figure 5.17: Regression plot of N_2O-N emission and wetland water temperature, for all corresponding data of 2007 and 2008

Previous studies (Hiscock *et al.*, 2002; Dong *et al.*, 2004) have found strong positive correlations between N_2O emissions and NO_3^--N concentrations however these measured systems were larger river systems or aquifers, potentially were *in situ* N_2O production via nitrification within the water column would be occurring. N_2O emissions were found to not be significantly related to either NO_3^--N or NH_4^+-N concentrations in the wetland water ($P > 0.05$), and may suggest limited *in situ* production of N_2O (Reay *et al.*, 2009). Additionally it is likely that the suggested multiple sources of water entering this wetland, contained very different concentrations of the dissolved inorganic-N species or organic-N as well as the complex N processing within the water column and sediments combined to the short length of the wetland has obscured any discernable link between dissolved N_2O and either NO_3^--N or NH_4^+-N concentrations.

Moreover linear fits produced very poor relationships explaining less than 2% of variation in N_2O emission (Figure 5.18). Therefore, although N_2O emissions generally increased with increasing NO_3^- and/or NH_4^+ concentrations the results suggest that other factors within the wetland contributed to the conversion rate of these substrates to N_2O.

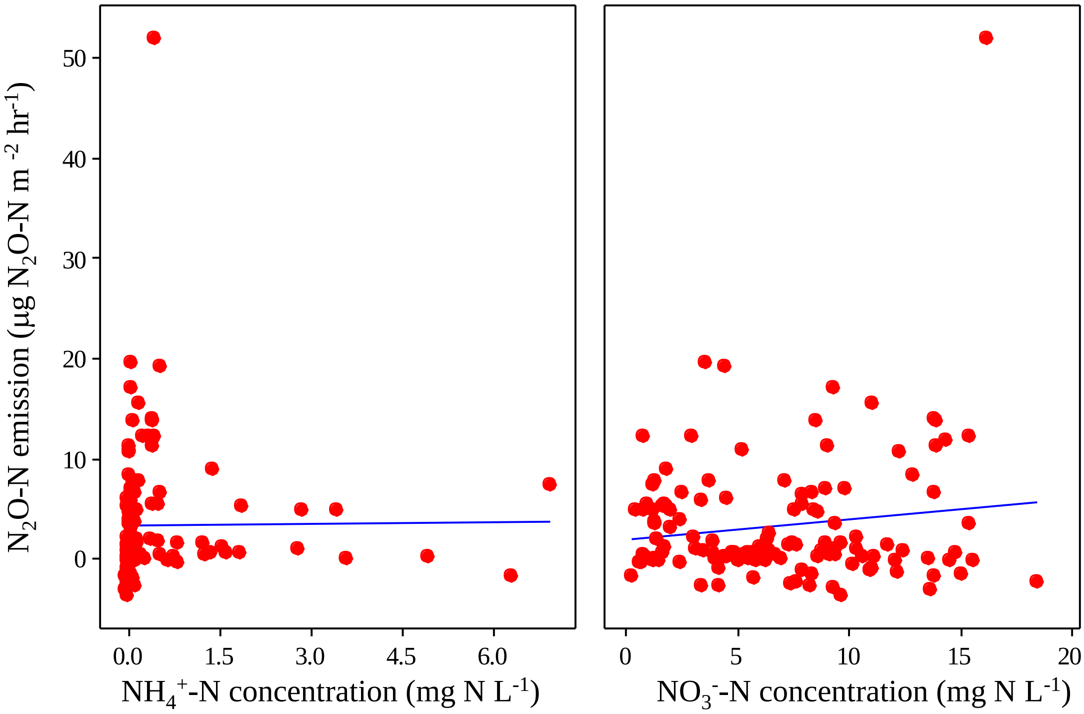


Figure 5.18: Regression of N_2O emission versus wetland water NO_3^--N and NH_4^+-N concentration for all available data of 2007 and 2008 (n = 134).

Regression analysis showed that although water depth and N_2O were significantly related ($P < 0.05$), the negative relationship was poor ($r^2 = 0.075$, n = 99, for all available data 2008) (Figure 5.19). This may be due to some other factor related to water depth, e.g. during drier periods when there is lower water depth in the wetland, the water residence time may increase allowing more time for denitrification to occur and giving this negative relationship.

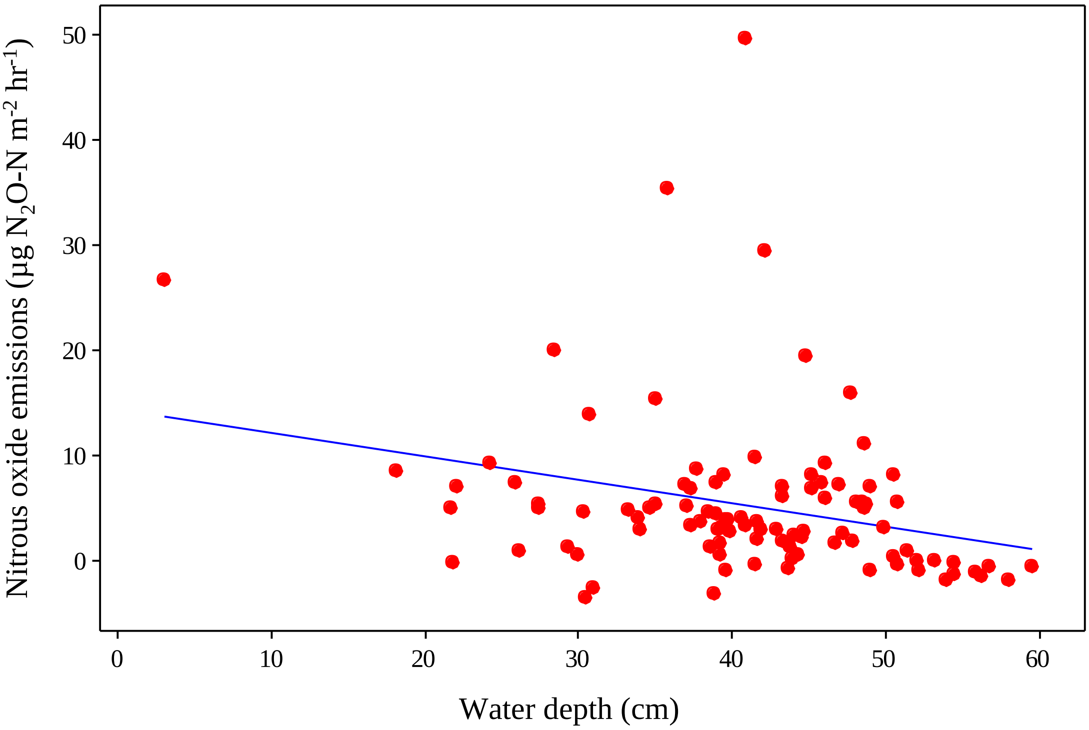


Figure 5.19: Regression plot of N_2O emission against wetland water depth for all available corresponding emission and water depth data for 2008.

Median dissolved N_2O concentrations were ten times greater than the air equilibrium value and generally increased along the wetland transect, suggesting the wetland is a net source for indirect N_2O emissions via degassing (Reay *et al.*, 2003; Reay *et al.*, 2009). Concentrations were generally within the range reported by other studies (Reay *et al.*, 2003; Reay *et al.*, 2009). However, occasionally dissolved N_2O concentrations measured from this wetland were much larger, 230 μg N L^{-1}, than those reported elsewhere from agricultural drainage water (Dowdell *et al.*, 1979; Hasegawa *et al.*, 2000; Reay *et al.*, 2003; Reay *et al.*, 2009). These large concentrations could indicate sites of high *in situ* N_2O production via either nitrification within the water column, or nitrification/denitrification within the wetland sediments.

Additionally water inputs from a secondary source were observed on 1st May 2008 coincident with a period of heavy rainfall. On this occasion mean dissolved N_2O concentrations along transect (6.68 ± 3.26 μg N L^{-1}), were 10 times greater than samples collected in the previous or subsequent sampling month (0.69 ± 1.61 and 0.18 ± 0.91 μg N L^{-1}, respectively), and may suggest that higher concentrations of dissolved N_2O may occur at times of greater rainfall. It is suggested that as leachate passes through deeper soil horizons with high intra-soil N_2O concentrations (such as those observed *in situ* in the adjacent field and in subsequent laboratory studies (Chapters 3 and 4)); the leachate becomes more concentrated in N_2O. At times of high rainfall more water is likely to reach the wetland via throughflow, therefore increasing dissolved N_2O concentrations.

No monthly, seasonal or annual correlations between dissolved N_2O concentrations or inorganic-N concentrations measured in this study were significant, perhaps because of the high flow rate in the wetland. On discrete sampling occasions (< 10 %) NO_3^--N and dissolved N_2O-N concentrations were significantly negatively related (Figure 5.20). This relationship is opposite to the expected relationship found in other studies (Reay *et al.,* 2003; Sawamoto *et al.,* 2005; Reay *et al.,* 2009). Most notably a review of dissolved NO_3^--N and N_2O-N concentrations carried out by Sawamoto *et al.,* (2005), that found positive correlations to exist for 14 datasets. Low rainfall (i.e. potentially increased water residence time and decreased water depth), occurred on the sampling occasions negative relationships were found and may explain these unusual correlations.

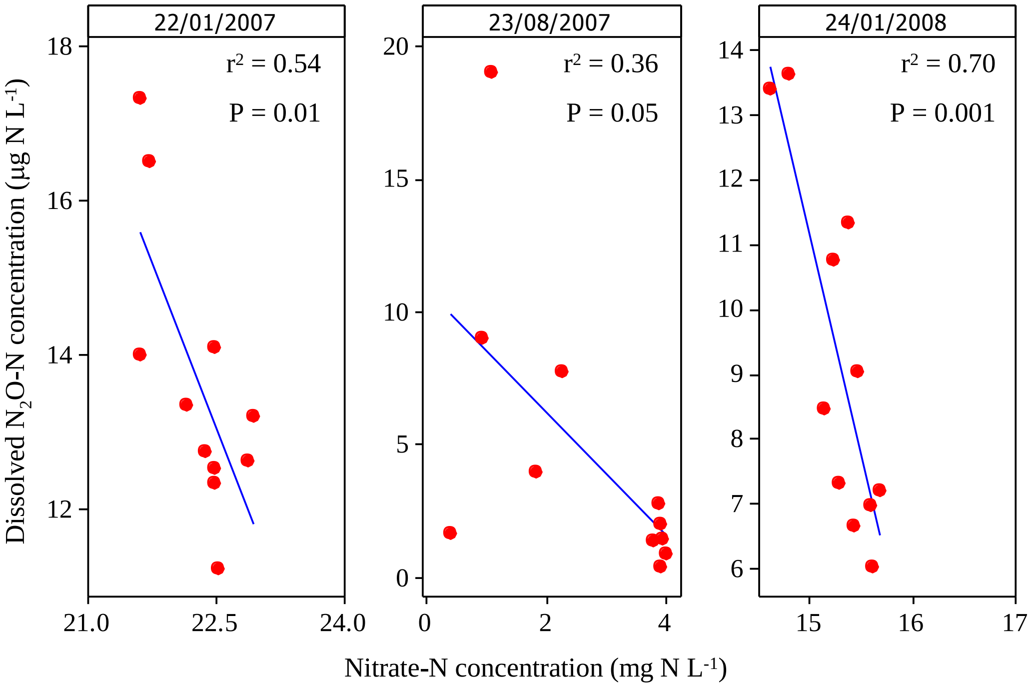


Figure 5.20: Regressions of dissolved N_2O-N and NO_3^--N concentrations within the wetland at each sample point along transect on selected sampling occasions.

5.4.3 Trends in NO_3^--N and NH_4^+-N concentrations and wetland interception of diffuse N

Concentrations of both NO_3^--N and NH_4^+-N exceeded water quality standards (11.3 and 0.78 mg N L^{-1}, respectively) on 15 and 24%, respectively of sampling occasions, however, NH_4^+-N concentrations were generally low. Seasonally the lowest NO_3^--N and NH_4^+-N concentrations occurred during late spring and summer months. It is suggested that these reduced concentrations in inorganic-N species are most likely due to enhanced plant uptake during the growing season, (Kadlec and Knight, 1996; Brix *et al.*, 1997; Fromin *et al.*, 2005; Randerson, 2006); however, this was not investigated directly by this research. Likewise highest concentrations were observed late autumn to early spring that experienced the coldest annual temperatures and may suggest a slowdown in plant uptake and therefore N removal (Moustafa *et al.*, 1996; Hernandez and Mitsch, 2007). It is hypothesised plant uptake is the main, albeit temporary, removal pathway of diffuse N within this wetland, however further work is required to confirm this (Cooke, 1994; Sabater *et al.,* 2003; Hernandez and Mitsch, 2007).

Secondary sources of water to the wetland occurred particularly during summer and autumn (i.e. seasons of high rainfall). On a number of occasion's concentrations of inorganic-N increased along wetland transect consistent with secondary water inputs from an observed field drain and/or through-flow during periods of higher rainfall. Therefore combinations of N addition and removal pathways are occurring such as intrusions of N-rich water, plant uptake and denitrification. Wetland design should therefore take account of *in situ* conditions ensuring the wetland size can buffer the effects of any intrusion source and remain an effective tool for diffuse pollution mitigation (Sabater *et al.,* 2003; Carty *et* al., 2008; Mustafa *et al.*, 2008).

The wetland has been shown to be generally ineffective in intercepting diffuse N. This is most likely due to its small area and length and unfavourable hydrological conditions, i.e. short water residence time and channelised and fast flowing water (0.09 ± 0.05 m s^{-1}), all of which lead to decreased opportunities for NO_3^--N removal by either plant uptake or denitrification (Cooke, 1994): confirming hypothesis 1. Other studies have found wetlands to be good at intercepting N from agricultural runoff (Sabater *et al.,* 2003; Hernandez and Mitsch, 2007; Carty *et* al., 2008; Mustafa *et* al., 2008; Gouriveau, 2009), however these involved much larger wetlands, with longer water residence times and lower water velocity and turbulence.

Annually the wetland was a net source for NH_4^+-N for both years and NO_3^--N in 2008 only. However, seasonally there were large differences in mass removal of inorganic-N; mass removal was greatest during late spring and early summer and lowest during the winter or early spring which may suggest that plant uptake, is a key removal pathway in this wetland (Kadlec and Knight, 1996; Spieles and Mitsch, 1999).

5.4.4 Nitrous oxide indirect emission factors

The N_2O indirect emission factors from ground water and drainage ditches (termed 'EF_{5g}' by the IPCC), were calculated by dividing the dissolved N_2O-N concentration by the dissolved NO_3^--N concentration, as described by Mosier et al. (1998), for all available transect samples. The mean indirect emission factor across the study period (January 2007 - June 2008 inclusive) was 0.76%, although this is greater than the IPCC default EF_{5g} of 0.25%, it still lies within the range. Calculated indirect emissions were highly spatially variable along the wetland transect ranging from 0.35 to 45.2% on a single sampling occasion during September 2007. Furthermore high temporal variability existed across the study period ranging from 0.001 to 45.2%. Generally emission factors were greater during 2007 than 2008 (Figure 5.21).

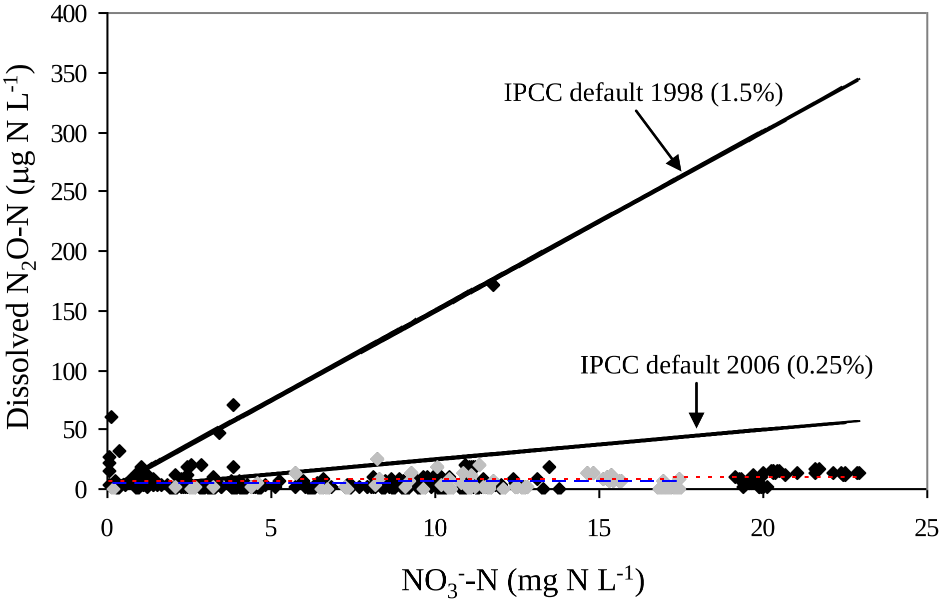


Figure 5.21: Relationship between dissolved NO_3^--N and N_2O-N concentrations in wetland water samples for 2007 (black diamonds) and 2008 (grey diamonds). The red and blue dashed lines represent first order regressions for samples collected in 2007 and 2008, respectively. The black lines represent the IPCC default regressions for the samples collected using the 1998 (Mosier et al., 1998) and revised 2006 (IPCC, 2006) IPCC default values (as labelled).

The high mean EF_{5g} for 2007 (0.9%) falls between the 2006 revised and 1998 IPCC default emission factors of 0.25 and 1.5%, respectively, however, it is within the current IPCC range, with many EF_{5g} equal to or lower than the current IPCC default value of 0.25%. Whereas, the mean indirect emission factor of 0.045% for 2008 is at the lower end of the 2006 IPCC default EF_{5g} range but similar to that found by Clough *et al.*, 2006 for a short river system (0.05%). However, the range of both dissolved NO_3^--N and N_2O-N concentrations within this wetland were much larger (one to two orders of magnitude greater) than those reported by Clough *et al.*, (2006).

Using the IPCC indirect EF for drainage water (EF_{g5}) the predicted mean EF for 2007 and 2008 based on dissolved NO_3^--N concentrations would be 0.0002 (ranging from <0.0001 to 0.0015) and 0.0002 (ranging from <0.0001 to 0.0004), respectively. This shows that the IPCC default under- and over-estimates mean indirect emissions for 2007 and 2008, respectively. However, the mean indirect EF for 2007 is skewed by a small number of large emissions, as shown by Figure 5.21, which follow the higher 1998 IPCC default emission factor of 1.5%. Nevertheless the majority of indirect emissions for 2007 are equal to or less than the 2006 IPCC default of 0.25%.

Previous authors (Reay *et al.*, 2003), have found a positive relationship, close to a 1:1 fit between the calculated and predicted indirect EF; however, statistically significant ($P < 0.05$) albeit poor negative ($r^2 < 0.1$) relationships exists between the measured and predicted indirect EF for 2007 and 2008 (Figure 5.22).

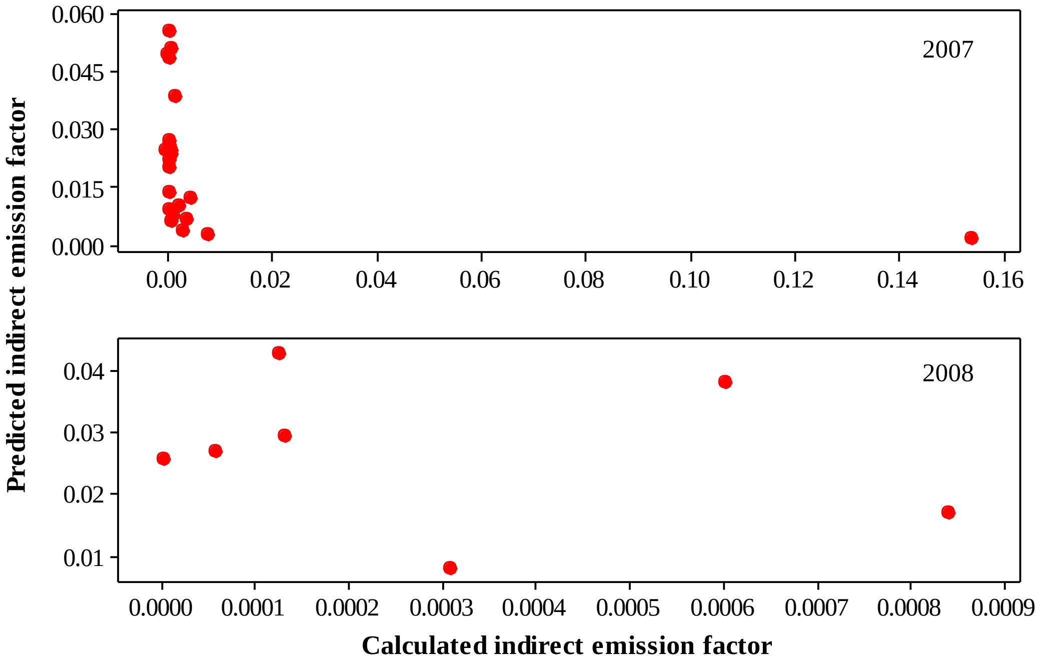


Figure 5.22: Regression plot of the predicted and actual indirect emission factors using all available data for 2007 and 2008 (as labelled).

This research has shown that high spatial and temporal variability in indirect emissions of N_2O exist within a relatively short (20 m) instream wetland and reinforce the suggestions made by previous authors that spatially intensive sampling is required when estimating indirect N_2O emissions from drainage waters (Reay *et al.*, 2003; Clough *et al.*, 2006). Furthermore the IPCC default factor is a worldwide constant, suggesting the need for at least country, if not regionally specific, EF values to quantify this indirect emission source.

5.4.5 Cost-benefit analysis and importance of greenhouse gas emissions of CH_4 and N_2O from this wetland site

The wetland receives water primarily via the small drainage ditch as well as rainfall or runoff so there are not costs associated with pumping additional water to it. Furthermore the wetland utilises natural processes such as plant uptake and denitrification to remove diffuse NO_3^--N pollution entering the wetland, therefore minimal costs will be incurred post-construction. Vegetation removal may be a method of nutrient export from this site; however removal may disrupt the wetland function or efficiency as well as incur costs for cutting, transport and disposal. Furthermore high sedimentation may result in 'clogging' of the channel which may require dredging particularly closer to the inlet location. No dredging of the wetland was performed during the 2-year sampling period; however, an annual cut back of willow was taken and transplanted to a nearby wetland (Jonczyk *pers. comm.*).

The cost-effectiveness of the wetland will depend upon the net environmental and economic benefits of its construction and maintenance as well as its effectiveness at intercepting and removing diffuse-N in addition to any pollution swapping costs incurred. The production of greenhouse gases, N_2O and CH_4, and NO_3^--N not intercepted will be compared to the net NO_3^--N interception which will determine if the wetland is causing pollution swapping and negating any perceived benefits in N interception. Such an assessment can be achieved by assigning a monetary value per kg intercepted NO_3^--N or greenhouse gas emitted.

Assigning a monetary value to environmental pollutants is a difficult problem (Pretty *et al.,* 2003; Pretty *et al.,* 2006; Brink *et al.* 2011), as it involves estimating the cost of environmental, social and economic impacts. There are many costs estimates of N pollution within the published literature. For the purpose of this research two cost estimates of NO_3^--N interception and greenhouse gas emissions will be used. NO_3^--N interception will be valued at a net gain of £0.04 kg^{-1} and £1.76 kg^{-1} removed (i.e. the mean of values c. £0.034 to 0.048 kg^{-1} and £0.00 to 3.52 kg^{-1} removed, given by Pretty, 2006 and Brink *et al.,* 2011, respectively).

The cost of greenhouse gas emissions will be evaluated using two evaluations: the value of £50 t^{-1} CO_{2e} emitted (Gouriveau, 2009 derived from Stern, 2006) and the mean CO_{2e} price of €19 as used by the European Nitrogen Assessment (ENA) as the implied abatement cost in the EU by the Kyoto Protocol (Brink *et al.,* 2011).

Evaluating N_2O and CH_4 has been based on global warming potentials of 296 and 25, respectively, equating to the costs listed in Table 5.1.

Table 5.1: Cost estimates of greenhouse gas emissions per kg

Greenhouse gas evaluation	**Cost N_2O/kg**	**Cost CH_4/kg**
$^{\#}$£16.74/tCO_{2e}	£4.96	£0.35
*£50 t^{-1} CO_{2e}	£14.80	£1.25

$^{\#}$based on the evaluation of €19/tCO_{2e} given by the implied abatement cost in the EU by the Kyoto Protocol. *Based on the evaluation given in the Stern Report, 2006.

The total cost to the environment excluding biodiversity benefits was calculated using the equation below:

Total cost to the environment (£) = (Cost of N_2O emissions + cost of CH_4 emissions) - net value of NO_3^--N interception. *(Equation 5.4)*

The annual cost and savings of greenhouse gas emissions and NO_3^--N interception per hectare are given in Table 5.2. Despite the different cost estimates used for greenhouse gas emissions or NO_3^--N interception the wetland is a net cost to the environment in both 2007 and 2008; with 2007 generating higher environmental costs largely a result of CH_4 emissions.

Table 5.2: Annual cost and savings of NO_3^--N interception and greenhouse gas production per hectare using the mean cost estimates given Pretty, 2006; Stern, 2006 and Brink *et al.*, 2011, as detailed in the text above.

Cost parameter	**Monetary value**	**2007 (£ ha^{-1})**	**2008 (£ ha^{-1})**
Value NO_3^--N intercepted (£ ha^{-1})	£0.04 kg^{-1} (Pretty, 2006)	-26.88	3.08
	£1.76 kg^{-1} (Brink *et al.*, 2011)	-7.10	0.81
Cost N_2O emissions (£ ha^{-1})	£50/tCO_{2e} (Stern, 2006)	41.59	18.17
	€19/tCO_{2e} (Brink *et al.*, 2011)	13.94	6.09
Cost CH_4 (£ ha^{-1})	£50/tCO_{2e} (Stern, 2006)	597.68	164.35
	€19/tCO_{2e} (Brink *et al.*, 2011)	168.09	46.22
Estimate of total cost per annum (£ ha^{-1})	**(Pretty, 2006 and Stern, 2006)**	**612.39**	**185.60**
	(Brink *et al.*, 2011)	**174.93**	**53.12**

*Note the cost of indirect N_2O emissions from degassing of dissolved N_2O are negligible (<<<£0.01) and have been excluded.

The total environmental cost of this wetland, based on the wetland area of 60 m^2, is low c. £1.00 to 4.00 and £0.30 to 1.00 per annum in 2007 and 2008, respectively. Additionally, the saving generated by NO_3^--N interception is low compared with the cost of greenhouse gas emissions, primarily from CH_4 emissions suggesting that this wetland is not benefiting the environment and is contributing to global warming. Therefore despite minimal costs associated with N pollution swapping the costs associated with CH_4 emissions and the contribution to global warming is greater. These findings confirm hypotheses 2 and 3.

Furthermore, the above cost benefit analysis does not take account of the construction and maintenance costs of the wetland. Construction costs have been estimated for this wetland at c. £5000 (Quinn *et al.*, 2007), including the labour and material costs associated with deepening and widening of the existing drainage channel using a small digger, fencing, revetment construction as well as vegetation costs. The small volume of sediment excavated meant it could be spread evenly around the wetland banks removing the need for costly transport or disposal. Additionally the placement of the wetland was within a drainage ditch and not part of the farmed/grazed area within the field. Therefore the farmer has not 'lost' land or earnings from the area utilised for the wetland.

N_2O emissions are just as likely to occur from the area studied, whether it is an instream wetland or a drainage ditch. Indeed mean annual N_2O emissions from within the same field and farm system from irrigated and non-irrigated grassland buffer strips were c. 3.1 ± 1.1 and 3.8 ± 1.1 kg N_2O ha^{-1} (mean of 2007 and 2008, see Chapter 3). Using the mean value from both grassland plots and over the whole study period, the grassland plots would incur an environmental cost of between £17 to 51 ha^{-1} yr^{-1} equivalent to £ 0.10 to 0.31 yr^{-1} for the area covered by the wetland: which is similar to the range of the mean cost of N_2O emissions from the wetland over the study period.

In contrast, CH_4 emissions would most probably have been much lower if the wetland had not been created, as a faster flow rate and higher water turbidity would have generated greater sediment aeration, particularly in the upper sediments, leading to minimal CH_4 emissions. The monetary cost does not account for the environmental benefits of increased habitat or biodiversity conservation. However the small size and limited species composition of this wetland (primarily sage grass and willow) may limit its value. Therefore the proportionally higher costs of CH_4 emissions would suggest this wetland site has resulted in a net cost to the environment, excluding any benefits associated with ecosystem improvement or habitat development which are suggested to be minimal.

Estimated CH_4 emissions from a constructed wetland in south-east Scotland, UK, receiving NO_3^--rich agricultural drainage water were comparable at 450 and 970 kg CH_4 ha^{-1} yr^{-1} for 2007 and 2008, respectively (Paul, 2007; Rao Pangala, 2008). This is equivalent to c. £160 to 560 and £340 to 1200 ha^{-1} yr^{-1}, in 2007 and 2008, respectively. Rao Pangala (2008) estimated N_2O emissions from the Scottish wetland site at c. £59 ha^{-1} yr^{-1}.

Emissions were slightly higher from the Scottish wetland compared to the wetland studied in this research, mostly likely due to the Scottish site having more suitable conditions for the microbial processes producing these greenhouse gases (deeper slower moving water column with long residence times) (Van de Weg, 2006; Paul, 2007; Rao Pangala, 2008; Gouriveau, 2009). The Scottish wetland however was more effective at intercepting diffuse NO_3^--N pollution with a mass reduction efficiency of 45 ± 8 % and 48 ± 23 % for NO_3^--N and NH_4^+-N, respectively (Gouriveau, 2009). Hence constructed wetlands for treatment of agricultural runoff and diffuse-N pollution must maximise nutrient interception with reduced greenhouse gas emissions to prevent pollution swapping (Reay, 2004; Oenema *et al.*, 2009; Stevens and Quinton, 2009; del Prado *et al.*, 2010), such as is the case for the wetland studied, where these emissions outweigh any improvements in water quality.

5.4.6 Suggested wetland design to improve NO_3^--N interception and reduce greenhouse gas emissions.

No universal design of a constructed farm wetland exists as site specific information is required such as; effluent type, volume and pollutant loading as well as topography, geology, soils and hydrology to assess the suitability of each farm for implementing a wetland. The scientific basis and guidelines for wetland design, operation and maintenance in a temperate climate is detailed in Carty et al, (2008) and should be referred to for detailed information.

A comprehensive site evaluation and characterisation of Nafferton Farm should be carried out by a qualified person to assess the site suitability and to estimate the costs (both construction and maintenance) of implementing a Constructed Farm Wetland (CFW) in addition to the value of land ‘lost’ by constructing a wetland. This can then be objectively related to all other practical and cost-effective best management practices to select the most suitable option for treating farmyard run-off at Nafferton Farm (Carty *et al.*, 2008).

If a farm is deemed suitable for the construction of a wetland based on suitable site characteristics (Carty *et al.,* 2008), a key first step is to determine the size (surface area) of wetland required. On a study of 13 wetland systems in Ireland Carty et al, (2008) suggest that the wetland area required to remove phosphorous to < 1 mgL^{-1} at the outlet (i.e. the limiting nutrient for freshwater systems), should have an aspect ratio of at least 1.3 times greater than the size of the farmyard area i.e. the effective yard run-off area to include yards, tracks and roofs that generate effluent. Treatment effectiveness is enhanced by increasing hydraulic residence times that can be maximized by ensuring a sufficient wetland size is constructed to buffer the farm effluent, by segmenting the wetland into a number of smaller cells (ideally a minimum of 4 cells), managing the water depth by adjusting the outflow pipe height, avoiding preferential flow and having densely vegetated cells (Carty *et al.,* 2008).

The farmyard area of Nafferton farm has been calculated at approximately 35,700 m^2 that would require a wetland area of 46,400 m^2 i.e. 1.3 times greater: this would result in four wetland cells each with an area of c. 12,000 m^2. Wetland cells with an aspect ratio of 2:1 that are square or circular are most effective at removing pollutants (CFW Manual, 2008). The resulting dimensions of each cell required at Nafferton Farm would be 160 m by 75 m, or ideally square or circular with the dimensions of 110 m by 110 m (Figure 5.23).

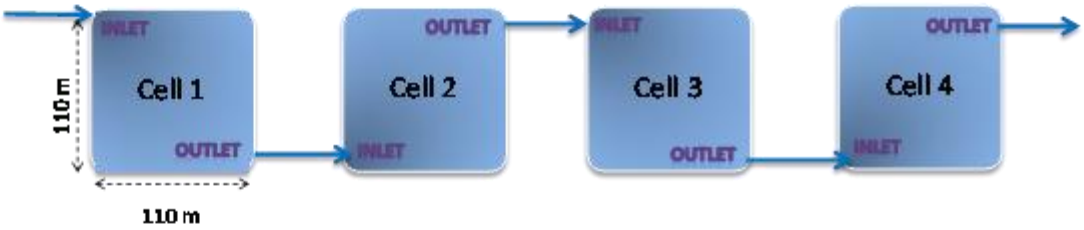


Figure 5.23: Schematic design of the proposed wetland.

The wetland cells need not be uniform in size; however, the total wetland area should equal 46,400 m^2 for Nafferton Farm with the inlet and outlet pipes located at maximum distances from each other; as shown in Figure 5.23; this will increase the water residence time within each cell and prevent any short-circuiting of the system (CFW Manual, 2008). Inlet/outlet pipes should not be over-engineered and should be of sufficient diameter to prevent clogging (approximately 15 cm), with adjustable bends in the outlet pipes of each cell will enable the water depth to be controlled (recommended at < 30 cm) (CFW Manual, 2008).

A CFW should be located down-gradient from the farmyard allowing the water to flow by gravity and removing any need for costly pumping (Carty *et al.*, 2008). The base of the wetland should lie c. 1 m above the water table to protect groundwater from contamination. Furthermore, a soil liner with a high clay content i.e. with a permeability of $< 1 \times 10^{-8}$ m s^{-1}, is required throughout the wetland to a depth of 1 m; otherwise a non-hazardous artificial plastic liner would be required adding to the construction and replacement costs (Carty *et al.*, 2008, CFW Manual, 2008). The sandy-loam soil type of Nafferton Farm suggests that an artificial liner or imported clay soils would be required at this site adding substantial costs to the wetland construction; potentially rendering this an unviable option due to the relatively high costs compared with other methods of diffuse pollution remediation available.

5.5 Conclusions

Inter-annual variability in CH_4 and N_2O emissions from the instream constructed wetland studied has been shown to be high, with emissions in 2007 much greater than those in 2008. Although emissions decreased substantially in subsequent sampling years this could be a result of short-term climatic conditions such as the greater rainfall in 2008 compared with 2007, rather than the diminishing effect of construction. Higher rainfall would most likely have had several effects - increased water volume, discharge, depth and turbidity - leading to lower residence time and opportunity for denitrification to occur, as well as increased aeration of the upper sediments and water column and decreasing methanogenesis or increasing CH_4 oxidation as it diffuses through the sediment. Indeed higher CH_4 emissions from the autochamber closest to the inlet of the wetland (0 m) is attributed to greater sedimentation rates and increased C source supply for methanogenesis. Despite the high inter-annual variability in greenhouse gas emissions, N removal was low in both sampled years.

N removal in the wetland was also highly seasonal, as reported for other constructed farm wetlands. N interception was lower in autumn and winter months probably due to increased discharge (as a result of higher rainfall and lower evapotranspiration), reduced plant growth and therefore NO_3^- uptake, and lower temperatures (and therefore microbial activity) compared with spring and summer months. This wetland has a net environmental cost, due to almost no reduction in diffuse-N pollution and its CH_4 and N_2O emissions. However other studies have found constructed farm wetlands to have higher N interception, suggesting that increased wetland area and improved site specific hydrological characteristics are required to achieve success and improve water quality.

Chapter 6: Assessment of nitrogen and trace gas fluxes in wetland sediments using Membrane Inlet Mass Spectometry

6.1 Introduction

Wetlands are natural biofilters that are a popular tool for the remediation of waste waters, for example those associated with agricultural run-off (Kadlec and Knight, 1996; Carty *et al.*, 2008). However their effectiveness is highly variable and associated with seasonal temperature changes, as well as hydraulic or nitrate (NO_3^-) loading rates (Spieles and Mitsch, 1999). In addition to nutrient interception, wetlands may provide unique habitats for wildlife and plants (Mitsch *et al.,* 2005; Mitsch *et al.,* 2009).

Plant uptake is a minor removal pathway for nitrogen (N) in wetlands, with microbial transformations - mainly denitrification - providing the major total N removal pathway (Kadlec and Knight, 1996). Wetlands can be sinks for carbon dioxide (CO_2) through organic matter sequestration however they may also be CO_2 sources, in addition to methane (CH_4) and nitrous oxide (N_2O) sources as a result of microbial processes occurring within the sediments and overlying water column (Brix *et al.*, 2001; Mander *et al.,* 2003; Song *et al.,* 2003; Hernandez and Mitsch, 2006). Respiration, methanogenesis and denitrification in anaerobic or suboxic sediments are the main processes producing CO_2, CH_4 and N_2O respectively (Zhu and Sikora, 1994; Segers, 1998; Hernandez and Mitsch, 2007; Kim *et al.,* 2009).

Wetland microbial processes are known to occur in microsites so that multiple processes can occur effectively side-by-side (Mander *et al.,* 2005). But the prevailing process will depend on the oxidation-reduction (redox) state of each microsite (Faulwetter *et al.,* 2009). It is these oxidation and reduction reactions that govern many of the biogeochemical reactions in environmental settings (Delaune and Reddy, 2005). Redox reactions are coupled oxidation-reduction reactions and as such follow a known sequence of electron donors within a range of redox potentials (Table 6.1) (Delaune and Reddy, 2005). A high redox potential is associated with the presence of O_2 and promotes microbial processes such as nitrification, as the redox potential decreases NO_3^- becomes the dominant electron acceptor. Lower redox potentials are associated with increasingly more anaerobic conditions. Furthermore available energy for the microbes decreases as the redox potential decreases and therefore microbial growth decreases also (Mitsch and Gooselink, 2000). For this reason the most energetically favourable electron acceptor will be completely utilised before microbes progress to less energetically favourable ones (Mitsch and Gooselink, 2007; Faulwetter *et al.*, 2009). Therefore from the measurement of the gaseous end-products of these reactions, the dominant microbial process can be inferred.

Table 6.1: Microbial oxidation-reduction reactions: processes and conditions.

Sediment condition	Redox condition	Process	Electron acceptor	End products
Aerobic	Oxidised	Aerobic respiration	O_2	H_2O
Anaerobic	Moderately reduced	Nitrate reduction	NO_3^-	N_2, NO_x
		Manganese reduction	Mn^{4+}	Mn^{2+}
	Reduced	Iron reduction	Fe^{3+}	Fe^{2+}
		Sulphate reduction	SO_4^{2-}	S^{2-}
	Highly reduced	Methanogenesis	CO_2	CH_4, CO_2

Adapted from Faulwetter et al. 2009 and Delaune and Reddy, 2005.

This research is primarily concerned with the fate of NO_3^- within a constructed (narrow) instream wetland receiving runoff from agricultural land (mixed arable and livestock). Riverine wetlands experience high variability in seasonal flow, temperatures, with temporally variable NO_3^- loadings. As such water residence times, and therefore contact time between water borne nutrients and wetland components (i.e. plants and microbes), may vary considerably. As a result, nutrient removal and gaseous emissions may also be temporally variable (Pinay *et al.*, 1993; Jacinthe *et al.*, 1998; Mander *et al.*, 2005; Teiter and Mander, 2005; Altor and Mitsch, 2006;

Noorvee *et al.,* 2007; Mitsch *et al.,* 2009; Nahlik and Mitsch, 2010; Nahlik and Mitsch, 2011).

The design of this wetland: narrow channel, short length with fast flowing water; resulted in short water residence times and therefore reduced opportunities for microbial processes to occur (see Chapter 5). As a result plant uptake was a major temporary removal process of NO_3^--N for this particular wetland. Due to time and resource constraints as well as the suggested changes to wetland design: suggested in Chapter 5; that should increase the microbial interactions and NO_3^--N removal the role of plant uptake for NO_3^- removal was not investigated. The primary focus of the experiments detailed in this chapter was the production and emission of greenhouse gases (N_2O and CH_4), under varying environmental conditions: temperature and turbulence; known to affect redox conditions.

Within wetland sediments greenhouse gases are formed within different redox conditions. N_2O is formed in a less reducing environment, typically closer to the sediment surface were O_2 is either limited or nearly absent, whereas CH_4 is produced in more strongly reducing environments in the complete absence of O_2 (Delaune and Reddy, 2005). The aerobic and anaerobic N cycling processes of nitrification and denitrification, respectively, are of key importance not only for the removal of NO_3^- but in the release of N_2O during these processes (Zaman *et al*., 2008). Furthermore sediment accumulation associated with a loss in water velocity as it enters the wetland creates a source of organic matter and organic carbon availability for microbial processes, with exchanges of nutrients and gases between the sediment and water column (van Hulzen *et al.,* 1999; Sabater *et al.,* 2003; Hernandez and Mitsch, 2007).

NO_3^- removal from wetlands is largely by denitrification and requires both NO_3^- and a carbon source (Knowles, 1982; Groffman, 1994, Xue *et al.,* 1999; Billore *et al.,* 1999; Kadlec *et al.,* 2005; Sirivedhin and Grey, 2006; Vymazal, 2007; Faulwetter *et* *al.,* 2009). Denitrification is well understood and reduces NO_3^- through a number of intermediary products (NO_2^-, NO_x, and N2O) to ultimately produce N_2 in anaerobic conditions (See Chapter 1.5 Equation 1.2). Another potential N removal pathway without producing N_2O is Anammox (ANaerobic AMMonium OXidation), the oxidation of ammonium (NH_4^+) with nitrite (NO_2^-) in the absence of O_2 directly to N_2 (Equation 6.1) (Jetten *et al.,* 2001).

$$NO_2^- + NH_4^+ \rightarrow N_2 + 2H_2O \qquad \text{Equation 6.1}$$

However this process is still not well understood with limited evidence for its occurrence in comparable environments, mainly marine or estuarine settings or in waste water treatment facilities with very high NO_3^- loadings (Rich *et al.,* 2008; Faulwetter *et al.,* 2009). Therefore this research will focus on denitrification as the likely dominant process of microbial NO_3^- removal.

During the processing of organic matter fermentation products - CO_2 and H - are formed which serve as substrates for methanogenesis. CH_4 production by the process of methanogenesis (by methanogenic archaea) is strictly an anaerobic process occurring under strongly reducing conditions usually in deeper layers of sediment i.e. further from the oxic layer in either the sediment or overlying water column (Equation 6.2) (Zhu *et al.,* 2010).

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad \text{Equation 6.2}$$

Atmospheric CH_4 emissions are the net effect of production, consumption, transport and release of the gas and therefore multiple microbial processes as well as physical and/or chemical processes are involved (Frenzel, 2000). A major process of consumption of CH_4 is CH_4 oxidation by methanotrophic microbes under aerobic conditions (Equation 6.3) (Oremland, 2010).

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \qquad \textit{Equation 6.3}$$

Experimental rationale

Intact sediment cores from the constructed wetland (Chapter 5) were used to investigate the effects of temperature (Experiment 1), and temperature and turbulence (Experiment 2) on wetland greenhouse gas emissions (CH_4 and N_2O). The location of production and concentration of these greenhouse gases within sediments and emission to the atmosphere as well as the concentrations of inorganic N (NO_3^- and NH_4^+) in the overlying water column were examined.

Laboratory investigations used the technique of Membrane Inlet Mass Spectrometry (MIMS) for near continuous gas measurements from the wetland sediments at two different depths within the sediment cores. Two depths were selected to provide a measurement interval of approximately 0.5 - 1.5 cm and 3.5 – 4.5 cm beneath the sediment surface. The selection of these two sediment depths were chosen to target different substrate availability (N and C) as well as differing redox conditions (O_2 availability) and as such potentially different dominant microbial metabolisms namely denitrification and methanogenesis. Furthermore the use of MIMS facilitated the measurement of N_2 in addition to N_2O. Isotopically heavy (^{15}N) in NO_3^- supplied in the overlying water column (Experiment 2 only), was also used to examine the fate of this added N (either N_2 or N_2O).

These experiments aimed to assess potential physical determinants (temperature and turbulence) of inorganic-N concentrations (in the overlying water column) and the production of greenhouse gases within the sediment, and their emissions, using intact wetland sediment cores. The key hypotheses tested were as follows:

(1) As temperature increases the production of N_2O and CH_4 within the sediment and headspace emissions will increase.

(2) CH_4 production and emissions will increase, whilst N_2O emissions and NO_3^--N concentrations will decrease, when columns are incubated with no turbulence.

(3) In the absence of plant uptake, denitrification is the principle removal mechanism of NO_3^--N in these wetland sediments (Experiment 2 the addition of $Na^{15}NO_3^-$ will allow the measurement of isotopically heavy or light N_2 production within the sediments).

The MIMS results from two experiments investigating the response of temperature, water turbulence and nitrate additions will be presented in this chapter. In addition to the headspace gas (N_2O and CH_4) results, water column nutrient results (NH_4^+-N and NO_3^--N) will be discussed. These aim to identify the processes operating in these wetland sediments as well as providing potential explanations to the findings of the field monitoring results of the constructed wetland (Chapter 5).

6.2 Materials and methods

6.2.1 Sediment core collection

Duplicate sediment cores were taken from within the wetland by pressing a metal ring of 12 cm depth and 18 cm diameter into the sediment until the top of the metal ring was level with the sediment. Carefully, using a spade and cutting around the metal ring, the sediment core was removed from the wetland, being preserved intact within the metal ring. The sediment cores were wrapped tightly in plastic to prevent moisture loss and a flat metal sheet was placed beneath the bottom of the core in the metal ring to keep the sediment within the core.

Additionally, at the time of core collection, the *in situ* water temperature in the wetland was recorded and a water sample for NO_3^--N and NH_4^+-N analysis was collected using a 60 ml plastic sample container with a screw-top lid (polystyrene 60 ml, Sterilin, Fisher Scientific). This and all subsequent samples were frozen at - 15°C until analysis for NO_3^--N and NH_4^+-N by standard methodology (see Chapter 2.3.7 for details). Using large plastic bottles 5 litres of *in situ* water was collected and placed in a cool box for transportation to the laboratory.

6.2.2 Laboratory preparation of sediment cores

The plastic wrappers were carefully cut away from the top of each core, taking care not to disturb the sediment beneath. A custom made Perspex cylinder of 19 cm diameter and 40 cm height, was placed within a water bath of width 36 cm, depth 20 cm, and length 75 cm (Haake DC10 Open Bath Immersion Circulator fitted with a Haake EK20 Immersion Cooler; Thermo Haake Intl. Karlsruhe, Germany) capable of heating or cooling water using a digital control to maintain the desired temperature to 0.02 K.

The Perspex cylinder had a custom made lid of Perspex drilled with holes for the inlet probes and mixer blade. The lid was 1.0 cm thick but had a thinner rim of 0.5 cm thickness that allowed it to slot tightly into it whilst the outer edge rested on top of the cylinder (Figure 6.1). The underside edge of the cylinder lid was fitted with rubber foam draft excluder to create an air tight seal to the cylinder.

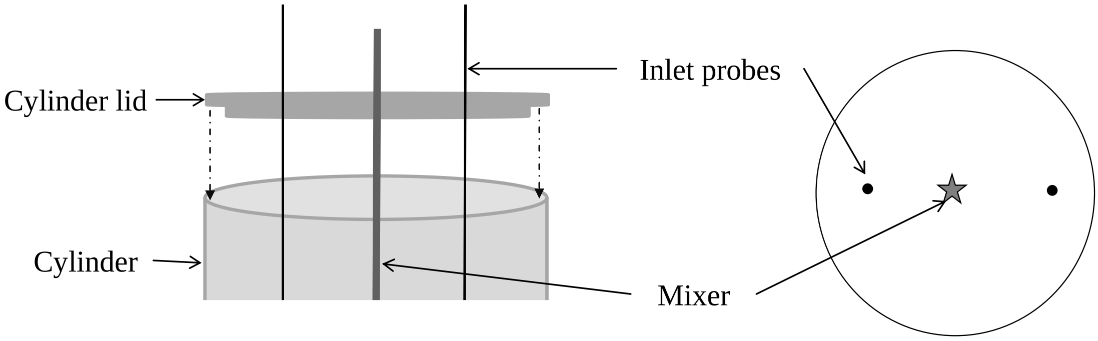


Figure 6.1: Schematic diagram illustrating the positioning of the inlet probes and mixer, as well as the design of the cylinder lid (not to scale).

Whilst keeping the core encased within the metal ring and supported on the base by the plastic wrapper each intact sediment core was lowered into a Perspex cylinder (Figure 6.2), until it rested on the cylinder base.

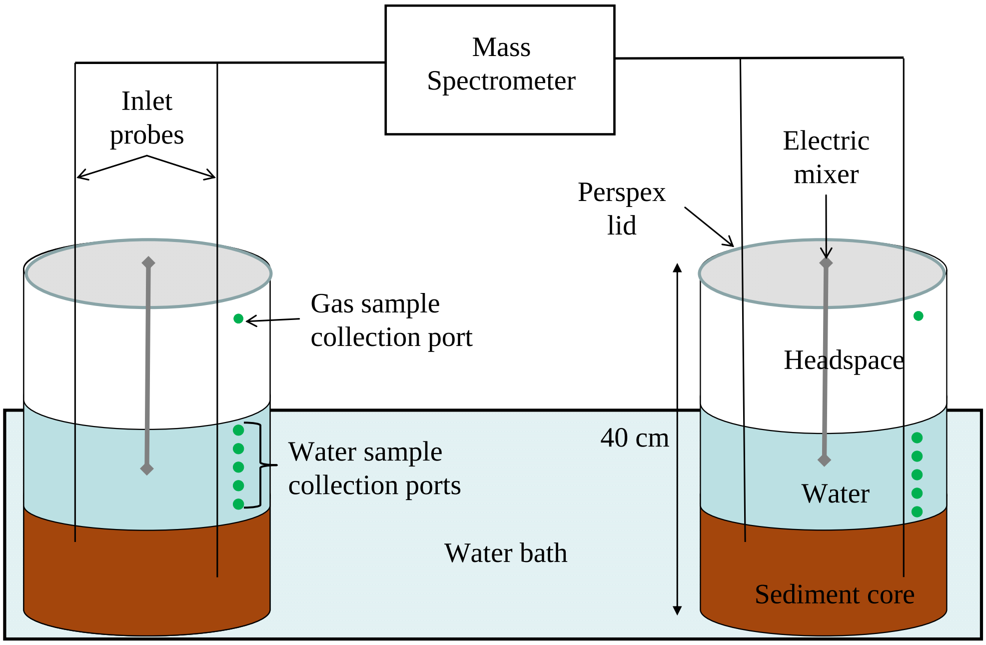


Figure 6.2: Schematic diagram illustrating the experimental set-up, key components of the experiment are clearly labelled (not to scale).

Then, using a narrow diameter plastic tube, water (either the *in situ* collected field water or an artificial water solution, for experiments one and two, respectively) was slowly introduced above the sediment into a lightweight foil tray (with multiple holes on its base and sides), placed on top of the sediment surface to between 7 - 9 cm depth, to replicate field conditions at the time of sampling. The use of the foil tray prevented disturbance to the core. The sediment cores were maintained at constant field temperature unless otherwise stated using a large water bath capable of cooling as well as warming.

Two stainless steel inlet probes were inserted through pre-drilled holes on the lid of each incubation experiment and subsequently into each sediment core to a depth designed to provide a probe measurement interval of 0.5 - 1.5 cm and 3.5 – 4.5 cm beneath the sediment surface. The selection of these two soil depths were chosen to target different environmental controls (substrate and O_2 availability) and as such potentially different dominant microbial metabolisms. Furthermore the selection of two depths within the sediment would allow for production rates at different depths to be compared.

6.2.3 Water sampling: NO_3^--N and NH_4^+-N

The overlying water (7 - 9 cm total depth) was sampled either 2 or 3 times per day every 2 cm up the profile i.e. from the sediment surface (0 cm) up to just under the water surface. This was achieved by inserting a hypodermic needle through the water collection septum ports and withdrawing 1 ml into a 5 ml plastic syringe, rinsing the syringe and discarding the water. Then a 5 ml sample is removed at each 2 cm interval up the water column (i.e. 0, 2, 4, 6 and 8 cm from the sediment-water interface) and immediately transferred into a 30 ml plastic sample bottle (polystyrene 60 ml, Sterilin, Fisher Scientific). The samples were frozen at - 15°C until analysis for NO_3^--N and NH_4^+-N using standard methodology (Chapter 2.3.7) at Edinburgh University. There was no significant change in water height over the experiments (less than 1 cm) therefore the overlying water was not topped up for either experiment.

6.2.4 Headspace gas sampling: N_2O and CH_4

Headspace gas samples were collected approximately every 90 minutes (Experiment 2 only) during daytime working hours, (samples for experiment 1 are not available). Chambers remained closed to the atmosphere throughout the experiment except for a short (10 - 15 minute) period after each sample was taken, when the headspace sample ports were opened and using a syringe, atmospheric air was flushed into the chamber headspace.

A hypodermic needle attached to a 60 ml plastic syringe and 0.25 l evacuated Cali-5 Bond foil bag (Calibrated Instruments) by a three-way stop cock (Edwards Life Sciences) and interconnecting polyethelyene tubing was used to collect headspace gas samples from the septum port near the top of the cylinder. Initially 60 ml of gas was sampled and used to flush the tubing before 120 ml of gas was withdrawn and stored in the foil bag. After sampling the septum port was removed and using the syringe ambient air was flushed into the headspace for a period of 10 minutes to prevent the formation of an artificial atmosphere within the incubating chamber. Headspace gas samples were analysed for CH_4 and N_2O using FID- and ECD-gas chromatography, respectively (see Chapter 2.2.4 and 2.2.5, respectively) at Edinburgh University within one week of the incubation experiment.

6.2.5 Membrane Inlet Mass Spectrometry (MIMS) measurements

Instantaneous and continuous dissolved gas concentrations of O_2, Ar, N_2, N_2O, CH_4 and CO_2 were measured at two known depths within the intact cores using the mass to charge (m/z) ratios listed in Table 6.2.

Table 6.2: The M/Z ratios used to measure dissolved gases.

Gas	M/Z ratio(s)
Nitrogen (N_2)	28 [29 and 30]
Argon (Ar)	40
Oxygen (O_2)	32
Methane (CH_4)	15
Nitrous oxide (N_2O)	31 [and 32]

Isotopically heavy (^{15}N) for the 50 mg $^{15}NO_3$-N per litre overlying water solution for experiment 2 (Temperature and Turbulence) only, was used to examine the proportion of added ^{15}N that is released as either N_2 or N_2O. Therefore both N_2 and N_2O were measured at the heavy and lighter atomic mass possibilities.

The m/z ratios were measured using an inlet probe fitted to a HAL series quadrupole mass spectrometer (WR12042, HPR-40, Hiden Analytical Limited, Warrington, U.K.). This technique is minimally invasive and allows for direct and continuous monitoring of several dissolved gases simultaneously. MIMS is a quadrapole mass spectrometer that has a series of inlets attached to stainless steel tubes (length 50 cm, diameter 0.2 cm). The inlet probes are hollow stainless steel tubes operated under a vacuum that are sealed at the end. A silicone gas permeable membrane covers the sealed end of the probe beneath which a series of small holes have been pre-drilled approximately 1 cm from the probe end (Figure 6.3). Therefore measurements taken at various depths are integrated over 1 cm. Sample gases pass through the membrane and into the mass spectrometer via a cold trap (crushed dry ice) and into the mass spectrometer for analysis.

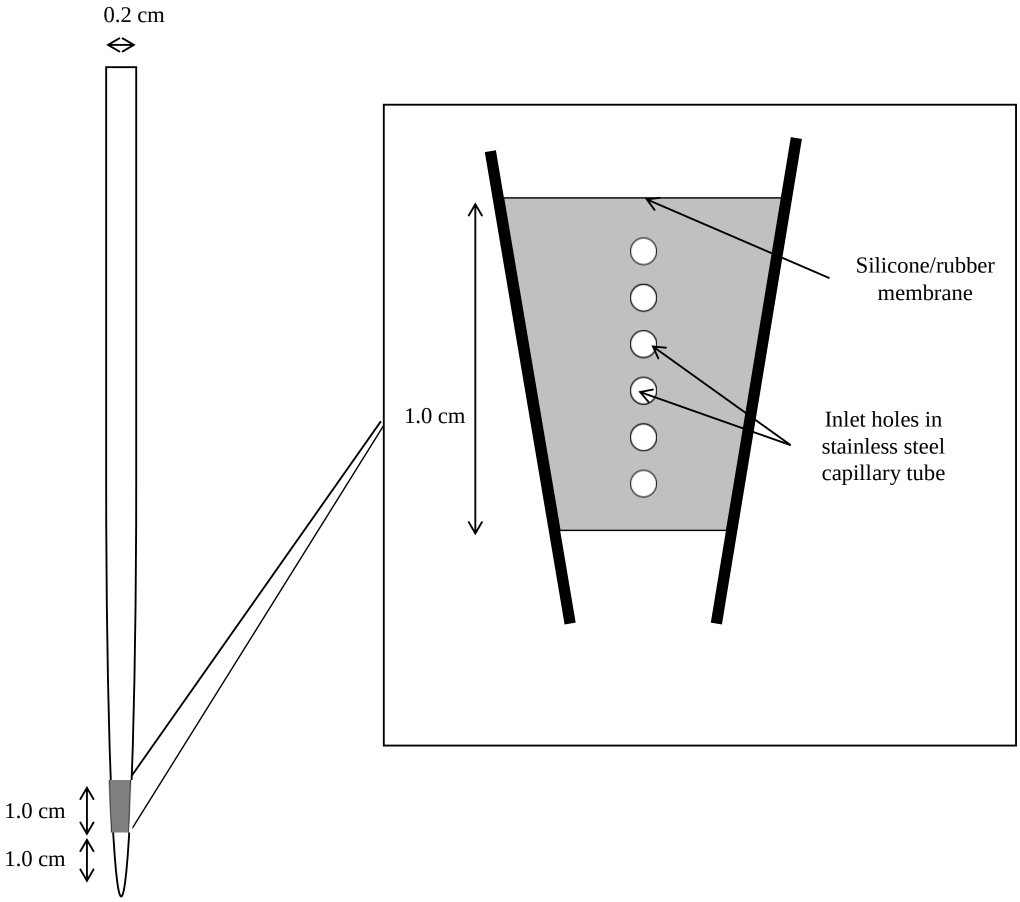


Figure 6.3: Schematic diagram of the stainless steel inlet probe showing the positioning of the membrane and inlet holes (not to scale).

The mass spectrometer is controlled by a computer program (MASsoft Version 5, HA-085-097 Issue A, Hiden Analytical Limited). The program dictates that each of the probes is sampled sequentially for a period of 5 minutes with a 30 second purge between each probe sampling time. All dissolved gases are measured simultaneously and continuously during the sample period for each probe. The program also applies correction factors for overlap with other measured gases or problems with gas cracking patterns. A malfunction in the software controlling probe switchovers prevented it being used for Experiment 2: instead sampling using MIMS was conducted by manually switching between sampling probes sequentially every 20-30 minutes during working hours. This evidently resulted in lower resolution of the sampled gases.

Corrections to measured dissolved gases

Measuring gases using molecular masses creates problems when more than one gas can be measured at a specific mass. For gases measured in this study both N_2O and N_2 can be measured at mass 28 therefore the influence of N_2O is subtracted from those recorded at mass 28 to give N_2.

6.2.6 Calibration

The measured output of MIMS is in torr. Therefore the concentration of N_2O and CH_4 gases in parts per million (ppm) were determined by measuring the response of known concentrations of the standard gas in torr and then applying the ratio of this response to the response of the sample, following a linear regression to construct the calibration line. Calibration was conducted for CH_4 and N_2O gases only.

To construct the calibration line, 9 ml purified water and magnetic stirrer (volume 1 ml) were placed in a sealed glass vial (Wheaton Industries Inc., USA) of volume 12 ml, leaving 2 ml headspace. Each probe was calibrated individually by inserting it through the septum of the vial and into the distilled water. Three to five known volumes of standard gas were injected into the vial through the septum sequentially and then the gas and liquid phases were allowed approximately 40 minutes to reach equilibrium after each injection.

6.2.7 Experimental protocol

Two intact sediment cores (known as core A and core B for identification purposes only), were collected using the above methodology from approximately 1 - 2 m from the upstream end of the wetland and prepared according to the laboratory protocol detailed above. For experiment 1 channel scouring as a result of a recent storm did not allow the collection of two cores from 1 - 2 m along transect. Therefore for experiment 1 core A the sediment core was collected at ~16 m along transect.

For an initial settlement period of approximately 17 hours both cores received no treatment and the overlying water was mixed using a steel stirring blade positioned in the water column approximately 5 cm above the sediment water interface. The water was mixed at 40 rpm and cores were maintained at *in situ* field temperature. This period was used to compare the changes (if any) in measured parameters before and after treatment as well as to assess the similarity between duplicate sediment cores. Sampling took place during daytime working hours, approximately 7.5 hours daily: due to the restriction imposed by Newcastle University of “no night time access to the laboratory”, in line with their health and safety, and insurance protocols.

Overlying water samples used to measure inorganic-N (NO_3^--N and NH_4^+-N) as well as headspace gas samples analysed for N_2O and CH_4, were taken as described in section 6.2.3 and 6.2.4, respectively. Differences in the experimental protocol and sampling frequencies for each experiment are detailed below.

Experiment 1: Temperature

Two sediment cores were collected, one at approximately 3 m and the other at 16 m along the wetland transect due to recent scouring of the wetland channel as a result of heavy rainfall and the associated high volume and flow rate of water through the system. The cores were incubated for two complete days, hereafter referred to as day 1 and day 2, after the initial settlement period (~ 17 hours duration). Four probes, two per core, were positioned at 0.5 – 1.5 cm for the shallow probes and the deeper probes at 3.5 - 4.5 cm and 3.0 – 4.0 cm depth in cores A and B respectively, to target the different regions within the soil that produce either N_2O or CH_4. The difference in depths of the deeper probes between cores was unintentional. Tension in the steel capillary tube drew the probe out of the sediment by 0.5 cm from within core B which only became apparent during dismantling of the apparatus.

Following the 17 hour settlement period, the incubation temperature was increased from the *in situ* temperature (10.5°C) in 2.5°C intervals every 2.5 hours between 08:30 am and 4:00 pm daily, equalling a 10°C rise in temperature daily. At 4:00 pm on day 2 the temperature was increased by 10°C to 40.5°C as the final incubating temperature. These temperature changes are summarised in Table 6.3.

Table 6.3: Summary of the temperature changes during the experiment

Experimental day	**Time GMT**	**Cumulative time [HH:mm]**	**Temperature (°C)**
Preliminary period	-	00:00	10.5
Day 1	08:30 am	15.48	13.0
	11:00 am	18.20	15.5
	01:30 pm	20.42	18.0
	04:00 pm	22.13	20.5
Day 2	08:30 am	39.50	23.0
	11:00 am	42.15	25.5
	01:30 pm	44.55	28.0
	04:00 pm	47.32	30.5
	06:45 pm	50.22	40.5

The overlying water column extended 9 cm above the sediment surface of the core, collected from the wetland and remained mixed in both cores for the duration of the experiment. Sampling frequency of overlying water and headspace gas samples are outlined below in Table 6.4.

Table 6.4: Summary of sampling time and strategy for headspace gas and the overlying water column.

Experimental day	Water column sampling per core*	
	Sample time (GMT)	Cumulative time (HH:mm)
Day 1	09:00 am 16:20 pm	16:15 23:35
Day 2	08:45 am 16:30 pm	40:00 47:45
Day 3	08:40 am	64:00

* 5 water samples per core (0, 2, 4, 6 and 8 cm above the sediment surface) at each sampling time. Additionally one *in situ* water sample was taken at the time of core collection. No water samples were taken from the preliminary period.

Experiment 2: Temperature and Turbulence

Sediment cores were incubated for three complete days hereafter referred to as day 1, day 2 and day 3, respectively after the initial control period. At 10:30 am on day 1 of the experiment the water mixer for core A was switched off and remained off for the duration of the experiment. This allowed the effects of water turbulence on nutrient and gaseous diffusion rates, and ultimately emissions of the greenhouse gases to the atmosphere, to be compared between the two cores. Additionally incubation temperature was increased daily at the start of each experimental day by 10°C, rising from 13°C to 33°C during the experiment (see Table 6.5 for details).

Probes were positioned at two depths within each sediment core, 0.5 – 1.5 cm and 3.5 – 4.5 cm depth. From these depths instantaneous measurements of dissolved gases were taken using MIMS at the m/z ratios discussed earlier (Table 6.1). A problem with the software program prevented the MIMS from sampling automatically. Therefore the probe was manually ‘switched-over’ by first closing one probe inlet and subsequently opening another probe’s inlet on the mass spectrometer; this is achieved without moving the probes themselves. Each probe was sampled for approximately 15 minutes before switching over to the next probe. All four probes were cyclically sampled in this manner during day-time working hours for the duration of the experiment. Hence the data series had a lower time resolution than for Experiment 1.

The overlying water solution was made using ultra high purity water and adding labelled 99 atom % ^{15}N-nitrate ($Na^{15}NO_3^-$) (Sigma - Aldrich Company Ltd, Dorset, UK, batch reference 364606) to a concentration of 50 mg $Na^{15}NO_3^-$ per litre. This concentration was selected as it was similar to the yearly mean concentrations measured from the wetland (Chapter 5). The water column was mixed at a speed of 40 rpm, unless otherwise stated. The overlying water column extended 7 cm above the sediment core surface therefore water samples were collected at 2 cm intervals between 0 (i.e. immediately above the sediment surface) – 6 cm above the sediment surface only. The sampling frequency is detailed in Table 6.5 below.

Table 6.5: Summary of experiment variables and headspace and water sampling strategies.

Experiment day	Temperature (°C)	Turbulence/core water mixing	Headspace gas sampling	Water column sampling
Preliminary period	13	Both cores mixed	1 sample per core	1 insitu wetland water sample
Day 1	13	Core B mixed Core A mixed until 10:30am then unmixed	6 samples per core 09:25am, 10:35am, 12:05pm, 13:35pm, 15:05pm & 16:35pm	3 sets* per core 09:00am, 11:45am & 16:35pm
Day 2	23	Only core B mixed	6 samples per core 09:05am, 10:30am, 12:05pm, 13:35pm, 15:00pm & 16:35pm	3 sets* per core 08:45am, 11:45am & 16:35pm
Day 3	33	Only core B mixed	5 samples per core 11:15am, 12:40pm, 14:05pm, 15:35pm & 17:10pm	3 sets* per core 08:45am, 11:45am & 16:35pm

*A set of water samples denotes 4 samples per core at each sampling time i.e. 0, 2, 4 and 6 cm within the water column.

6.3 Results

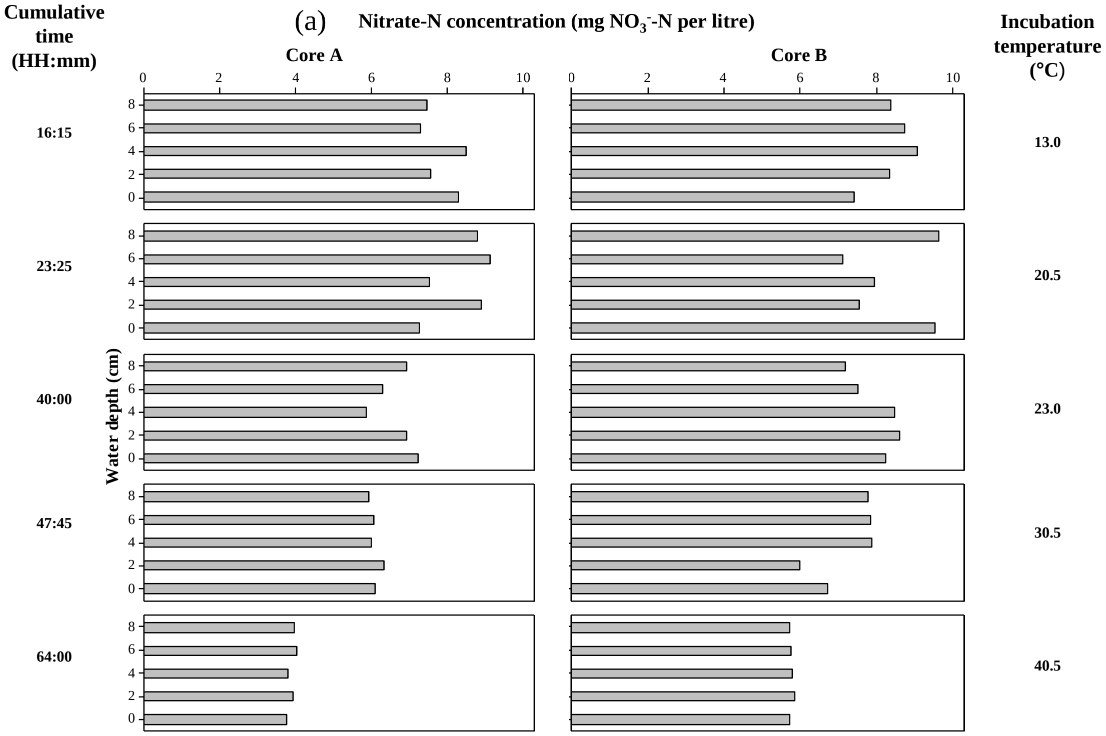
6.3.1 Inorganic-N concentrations in the overlying water columns

Experiment 1: Temperature

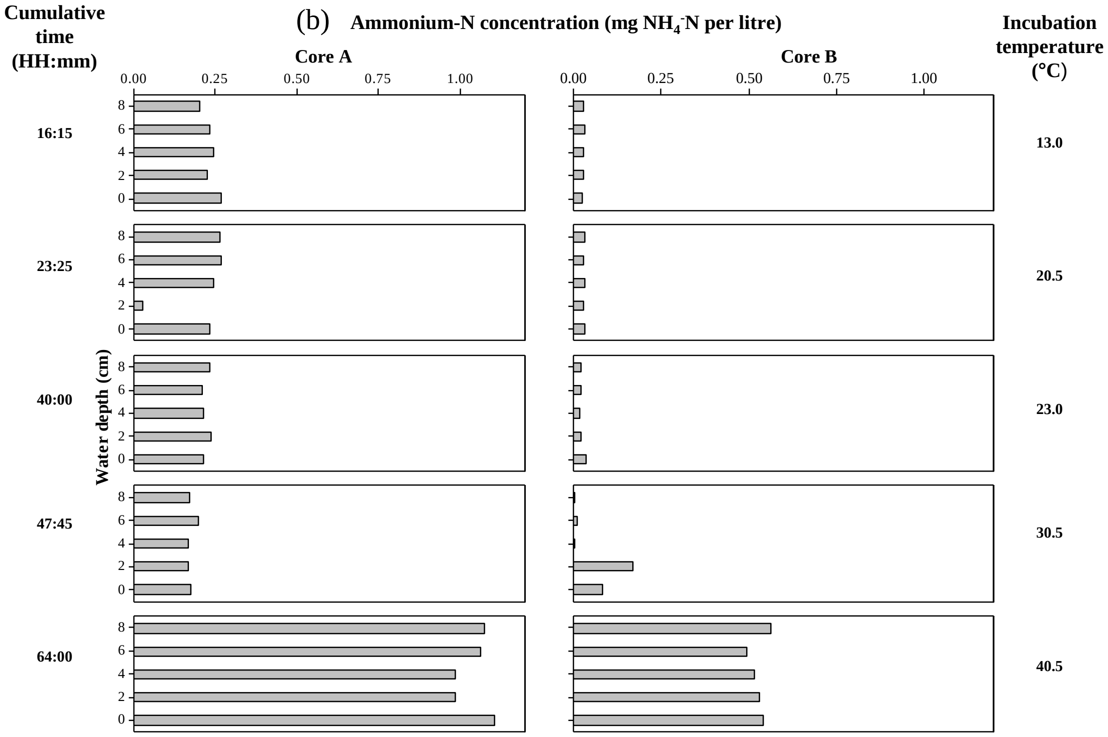
In situ NO_3^--N concentration of wetland water and therefore the initial concentration of the overlying water column for the incubation experiment was 9.80 mg NO_3^--N L^{-1}. Both incubations had mixed overlying water columns and as would be expected no stratification of NO_3^--N or NH_4^+-N occurred within either water column. *In situ* NH_4^+-N concentration was low, 0.025 mg NH_4^+-N L^{-1}. However the concentration increased within the water column of both incubations (Figure 6.4 (a)). Furthermore, NH_4^+-N concentrations from incubation A were nearly double those recorded for incubation B, despite having the same initial concentration in the overlying water.

The two sediment cores were taken from different distances along the wetland transect (3 and 16 m from the upstream end of the wetland), (see Section 6.2.7). Observationally sediments at 16 m along transect were deeper, with a deeper overlying water column and lower turbidity than those at 3 m along transect. These differences may have led to potentially very different C or N contents of these soils and/or redox conditions. However destructive sampling of the cores did not take place to confirm this.

NH_4^+-N concentrations within the water column remained broadly similar for each incubation experiment, but very different from each other, for approximately the initial 48 hours of the experiment: with the exception of samples taken at 47 hrs 45min cumulative time from 0 and 2 cm above the sediment surface of incubation B. After 60 hours the concentration of NH_4^+-N increased by an order of magnitude in both incubations. The mean concentration of NH_4^+-N from incubation A on the final sampling occasion 1.04 ± 0.05 mg NH_4^+-N L^{-1} exceeded the FFD guideline value of 0.78 mg NH_4^+-N L^{-1}.



(a) Nitrate-N concentration (mg NO_3^--N per litre)



(b) Ammonium-N concentration (mg NH_4^-N per litre)

Figure 6.4: NO_3^--N (a) and NH_4^+-N (b) concentration of the overlying water column from incubations A and B (as labelled). Also shown is the cumulative time since the experiment began and incubation temperature at each sampling time (as labelled).

Generally the concentration of NO_3^--N decreased with experimental time from both incubations (Figure 6.4 (b)). NO_3^--N concentrations from both cores are broadly similar on the first day of the experiment (i.e. 16 Hrs 15 mins and 23 Hrs 25 mins sampling occasions). However, after this time the concentration decreased more rapidly from incubation A than B.

<u>Experiment 2: Temperature and turbulence</u>

Mean NH_4^+-N concentrations in the overlying water column decreased in the mixed core but generally increased in the unmixed incubation, more than doubling in concentration during the experiment. The concentration of NO_3^--N (Figure 6.5 (a)) at 0 cm height from the sediment surface within the unmixed water column was often slightly lower than higher in the water column; this trend was not observed in the mixed water column. Suggesting there was a slightly stratified lower water column within the unmixed water column.

The initial concentration of NO_3^--N in the overlying water was 50 mg $Na^{15}NO_3^-$ L^{-1} (11.3 mg N L^{-1}), added at time zero of the experiment with zero NH_4^+-N. NO_3^--N concentrations had dropped by 38.8 and 27.8% from the unmixed and mixed incubations, respectively after 18 hours of incubation time and continued to decrease in both incubations over the three experimental days. On the final sampling occasion (~ 67 hours since the experiment began), mean NO_3^--N concentrations from the unmixed incubation were 0.52 compared to 6.63 mg NO_3^--N L^{-1} from the mixed incubation, reductions of c. 95.4 and 41.3%, respectively.

No trend of greater depletion of NH_4^+-N (Figure 6.5 (b)) from within any position within the vertical profile of the water column for either incubation was observed. NH_4^+-N generally increased on consecutive sampling days within the water column of the unmixed core. By the final day of the experiment the N concentrations within both water columns were very different with the unmixed incubation having less NO_3^--N and greater NH_4^+-N than the mixed.

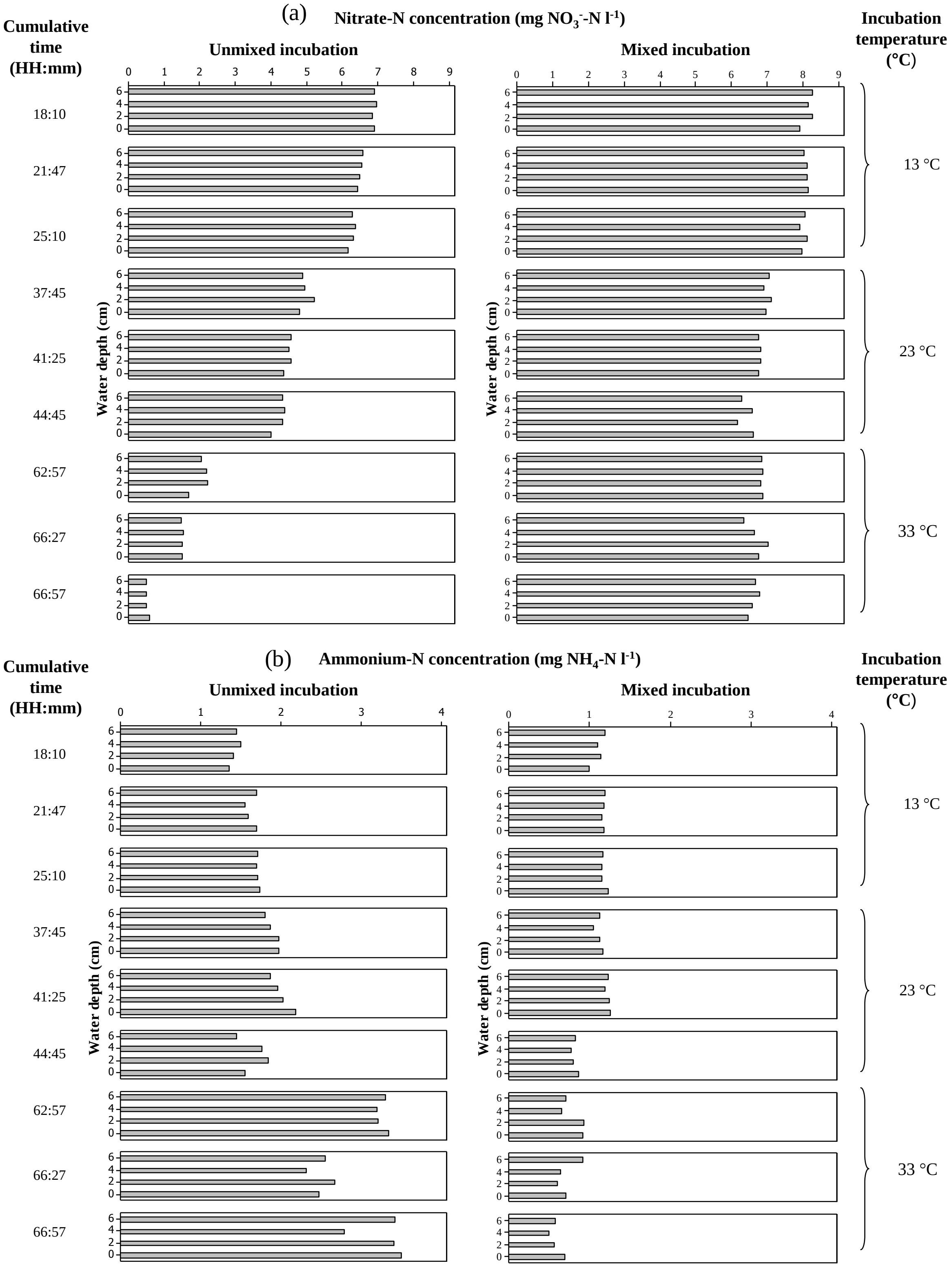


Figure 6.5: NO_3^--N (a) and NH_4^+-N (b) concentration of the overlying water column from unmixed and mixed incubations (as labelled). Also shown is the cumulative time since the experiment began and incubation temperature at each sampling time (as labelled).

6.3.2 Nitrous oxide and methane emissions

Greenhouse gas emissions are only available for the second experiment: Temperature and turbulence. It is important to consider that the long closure times could have resulted in headspace concentrations reaching an asymptote. This was not checked for; therefore if this has occurred then the flux rate will have been underestimated. Future work should ensure frequent headspace sampling and adequate closure times to ascertain the linearity of headspace gas increases.

Nitrous oxide fluxes:

Generally the unmixed incubation had greater N_2O fluxes than the mixed, with both incubations producing higher fluxes as the incubation temperature increased. Initially both incubations had a mixed overlying water column therefore for the first headspace sample both cores were experiencing identical conditions (mixed water column and 13°C); despite this the N_2O flux from both cores was very different. The unmixed incubation shows a high emission of 59.2 µg N_2O-N m^{-2} hr^{-1} compared to 14.0 µg N_2O-N m^{-2} hr^{-1} from the mixed incubation after c. 18 hours of incubation (Figure 6.6). Headspace fluxes from the unmixed incubation decline throughout this sampling day (i.e. until 21:05 cumulative time inclusive) whereas those from the mixed incubation were similarly low.

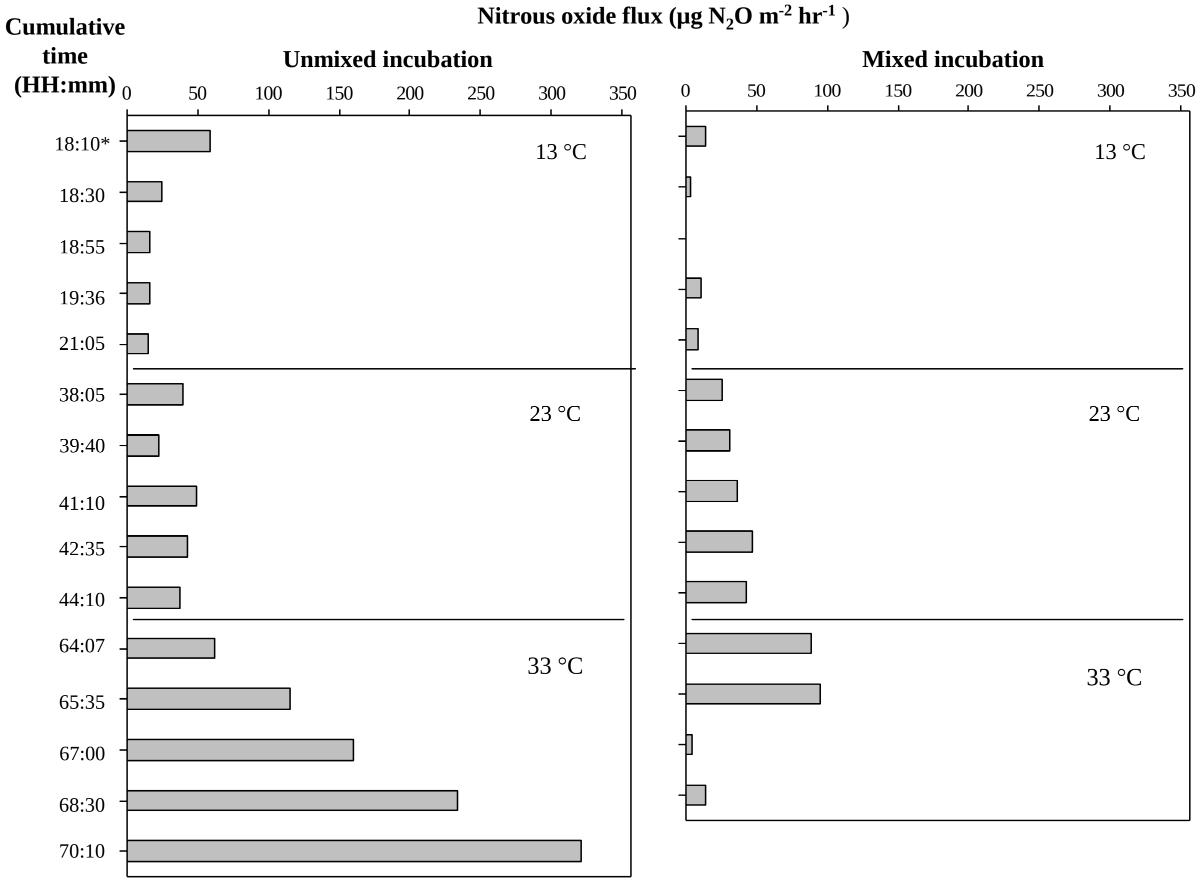


Figure 6.6: Nitrous oxide flux from the mixed and unmixed incubations (as labelled), incubation temperatures are labelled on each figure. *Note that at 18 Hrs 10 mins the unmixed core still had a mixed overlying water column i.e. both incubations were mixed at this time. Also no flux measurements are available from the mixed incubation for the following times, 18:55 and 70:10.

During the second day (from 38:05 to 44:10 cumulative time inclusive) of the experiment incubation temperature was increased to 23°C. Headspace N_2O fluxes from both incubations were broadly similar, a daily mean of 38.1 ± 9.95 (22.0 - 48.7) and 36.6 ± 8.43 (25.8 - 46.5) µg N_2O-N m^{-2} hr^{-1} for the unmixed and mixed incubations respectively. Headspace fluxes from both incubations were greater than those for day one of the experiment; the higher incubation temperature may have increased the rate of microbial activity and enhanced emissions.

The final day of the experiment (sampled between 64 Hrs 07 mins - 70 Hrs 10 mins inclusive), resulted in the greatest fluxes of the experimental period; this was also the time of greatest incubation temperature (33°C). Mean daily headspace fluxes were 179 ± 102 and 74.1 ± 47.4 µg N_2O-N m^{-2} hr^{-1} for the unmixed and mixed incubations, respectively. Headspace fluxes increased from the unmixed incubation until the end of the experiment reaching a maximum flux of 321 µg N_2O-N m^{-2} hr^{-1}

Methane fluxes:

The first CH_4 headspace flux (18:10 cumulative time, both incubations have a mixed water column) was much greater from the mixed than the unmixed incubation, 5.78 and 1.43 g CH_4 m^{-2} hr^{-1} respectively (Figure 6.7). This initial headspace flux from the mixed incubation was the greatest for the whole experiment. The CH_4 flux from the unmixed incubation varied little throughout the day with fluxes ranging from 1.25 - 2.16 g CH_4 m^{-2} hr^{-1} despite the water column being unmixed. The CH_4 flux from the mixed incubation despite the high flux early in the day decreased to < 1 g CH_4 m^{-2} hr^{-1} at the end of the first day.

The headspace CH_4 flux on the second day from the unmixed incubation decreased sharply from the first measured flux of 3.80 g CH_4 m^{-2} hr^{-1} to < 1.5 g CH_4 m^{-2} hr^{-1} for the rest of the day despite an unmixed water column and higher temperatures than the previous day. Furthermore headspace fluxes from the unmixed incubation, with the exception of the first sample on this day, were less than those from the mixed incubation. Conversely the CH_4 flux from the mixed incubation despite a lower flux at measured at 38 Hrs 05 mins of 1.18 g CH_4 m^{-2} hr^{-1}, all subsequent CH_4 fluxes were > 3.55 g CH_4 m^{-2} hr^{-1}: which are three to four times greater than those measured from the unmixed incubation.

On the final day of the experiment CH_4 fluxes from the mixed incubation were broadly similar with mean daily fluxes within one standard deviation of the mean despite the 10°C increase in temperature. Headspace CH_4 fluxes from the unmixed incubation were the greatest of any CH_4 fluxes recorded during the experiment (< 13.0 g CH_4 m^{-2} hr^{-1}).

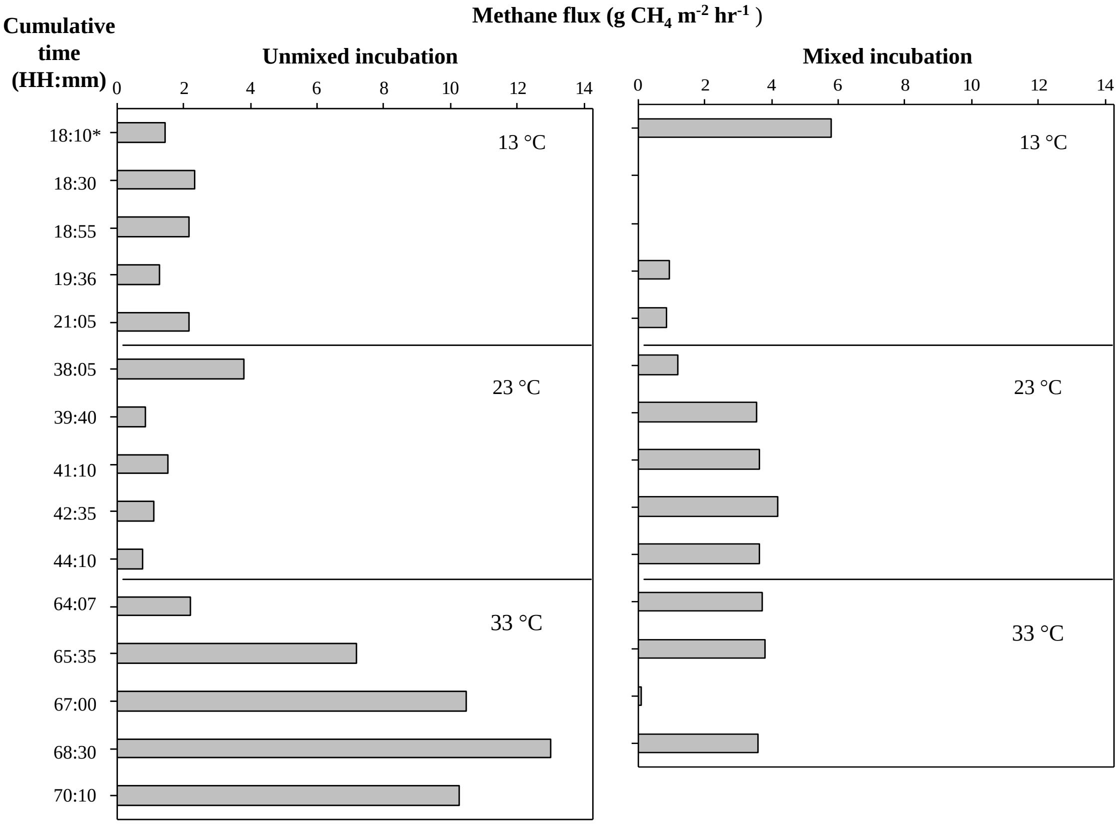


Figure 6.7: Methane flux from the mixed and unmixed incubations (as labelled), incubation temperatures are labelled on each figure. *Note that at 18 Hrs 10 mins the unmixed core still had a mixed overlying water column i.e. both incubations were mixed at this time. Also no flux measurements are available from the mixed incubation for the following times, 18:30, 18:55 and 70:10.

6.3.3 Methane and nitrous oxide production within the sediment

Methane concentrations within the sediment and response to increased temperature

Experiment 1: Temperature

CH_4 was only calibrated for the two deeper probes within each core. The change in CH_4 concentrations at 3.5 - 4.5 cm in core A and 3.0 - 4.0 cm in core B follow the same pattern; that are, relatively stable concentrations until approximately 48:00 hours into the experiment when the incubation temperature was increased to 40.5°C

(Figure 6.8). During the settlement period (i.e. 00:00 - 06:00 HH:mm), CH_4 concentrations decreased from c. 325 and 30 ppm from incubations A and B, respectively suggesting *in situ* conditions of both sediment cores were more reducing and that some limited disruption of *in situ* redox conditions has occurred during extraction.

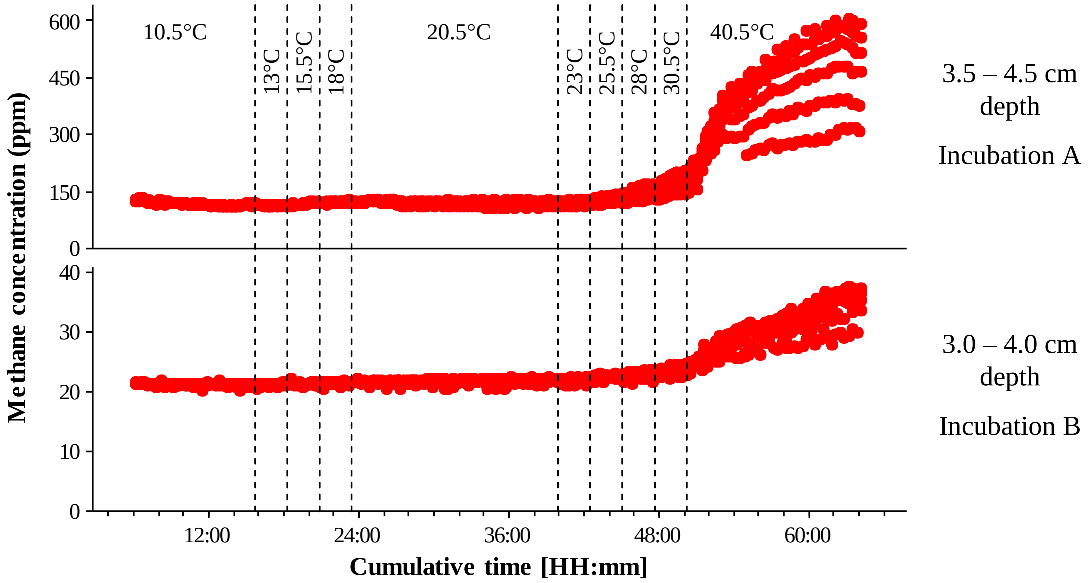


Figure 6.8: CH_4 concentrations at 3.5 - 4.5 cm in core A and 3.0 - 4.0 cm in core B as labelled. Incubation temperature increments are labelled. Note the different Y-axis scales. The six lines presumably relate to the six inlet holes in each probe that cover the 1 cm interval.

Mean concentrations for the first 48 hours were 114.6 ± 8.74 and 21.62 ± 0.59 from cores A and B, respectively: greatly exceeding atmospheric background CH_4 concentrations of c. 1.8 ppm, suggesting methanogenesis is very active at these depths within the sediment. The difference in mean concentrations from each core may be due to the different sediment depths from which the measurements were taken. Additionally the 6 separate lines on this, and subsequent graphs, are most probably from the 6 different inlet holes on each probe (see Figure 6.3). This would suggest that the MIMS has not been able to integrate over the 1 cm sampling interval. Additionally, this shows that CH_4 is increasing at different rates within the different microsites of microbial activity that each inlet samples. For the purposes of these results I discuss the separate traces produced by each probe at the same sampling time as one trace, integrating the data over the 1 cm interval.

The temperature response of CH_4 production (i.e. the change in CH_4 with time for each temperature interval), from each core was determined by calculating the Q_{10} coefficient (Table 6.8). Q_{10} is a measure that summarises the effect of rising temperature on the reaction rate, in this case methanogenesis. The Q_{10} coefficients for both cores are close to 1.0; suggesting little effect of increased temperature. Initially the calculated Q_{10} coefficients for CH_4 production within the sediment show a slight negative effect of increased temperature from both incubations. The final increase in temperature (30.5 to 40.5°C) resulted in the highest Q_{10} from each core: 17 and 9% increase in production within the selected depth of core A and B, respectively; suggesting increasing temperature may have increased CH_4 production.

Table 6.8: The response of CH_4 production to each 10°C rise in temperature.

Temperature rise (°C)	Core A (4 - 4.5 cm)	Core B (3 - 3.5 cm)
10.5 - 20.5	0.93	0.98
13.0 - 23.0	0.92	1.05
15.5 - 25.5	1.00	0.95
18.0 - 28.0	1.00	1.06
20.5 - 30.5	1.05	1.02
30.5 - 40.5	1.17	1.09

CH_4 production in cores A and B is significantly related to temperature, ($P = 0.006$ and 0.001, respectively). Indeed 72 and 77% of variance in production from cores A and B, respectively are explained by the rise in temperature. The increase of CH_4 with increasing temperature is exponential i.e. CH_4 increases logarithmically with increasing temperature (Figure 6.9). It is likely that methane oxidation or poor diffusion of CH_4 through the sediments has led to the observed low CH_4 flux despite high CH_4 production within sediments.

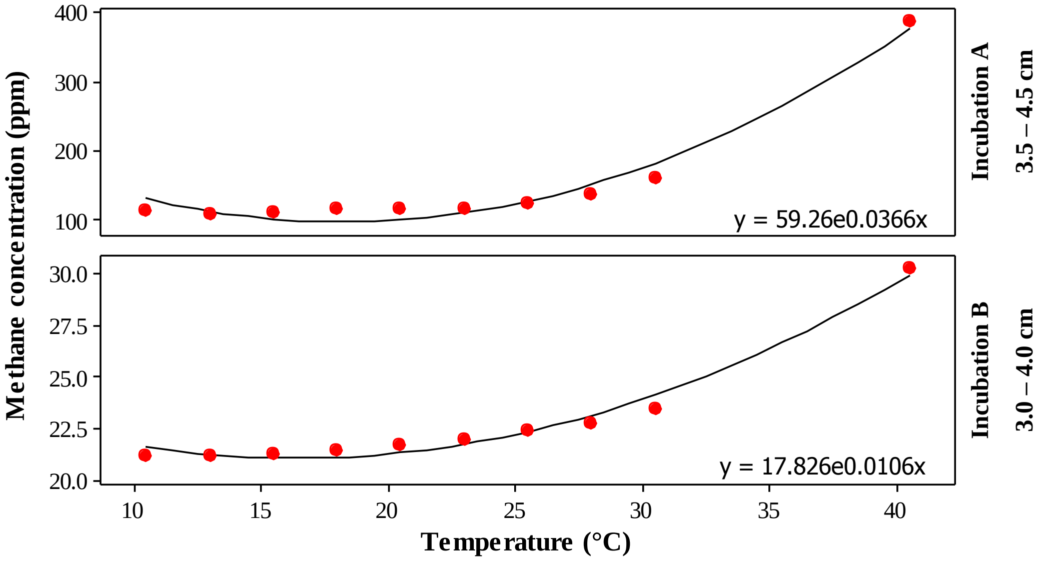


Figure 6.9: Mean CH_4 concentration at each incubation temperature from incubations A and B measured using the probes located deepest in the sediment cores (as labelled). An exponential regression has been fitted.

Experiment 2: Temperature and turbulence

Due to time limitations on use of the MIMS apparatus the concentrations of CH_4 within the cores were calibrated for both probes within the mixed incubation but only for the surface probe for the unmixed incubation. Furthermore the concentrations on the second and third days of the experiment exceeded the calibration curves (i.e. > 300 ppm). Therefore both CH_4 concentrations as well as CH_4:Ar ratios will be discussed.

The highest CH_4 concentrations were observed from the unmixed incubation at 1.0-1.5 cm depth, with concentrations exceeding 300 ppm and the range of standards used for the calibration (Figure 6.10). Potentially as the overlying water column stratifies, anoxic conditions would develop allowing methanogenesis to occur close to the sediment surface. The mixed incubation had much lower CH_4 concentrations than the unmixed at both measured depths (0.5 - 1.5 cm and 3.5 - 4.5 cm depth).

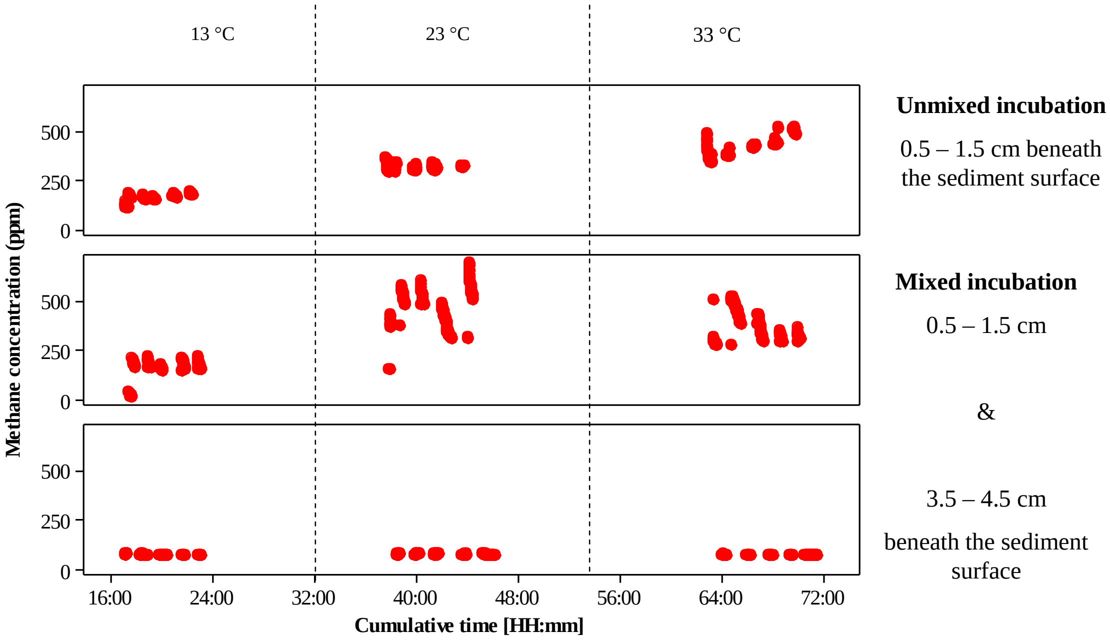


Figure 6.10: CH_4 concentration from the unmixed and mixed incubations (as labelled). Note that for the unmixed incubation at 3.5 - 4.5 cm the probe was not calibrated therefore CH_4 concentration is unavailable.

In support of the higher CH_4 concentrations closer to the sediment surface the CH_4:Ar ratios from the unmixed incubation showed little variation and only a slight increase of CH_4 with time at 3.5 - 4.5 cm depth, whereas at 0.5 - 1.5 cm depth much higher ratios were measured for the entire experiment than those at depth with the amount of CH_4 increasing with time (Figure 6.11). Therefore both cores produce greater CH_4 closer to the sediment surface than at depth.

CH_4 concentrations from the mixed incubation at depth ranged from 70.6 to 86.3 ppm across the sampling period and were much lower than those observed near the sediment surface. The CH_4 concentrations from both cores at 3.5 - 4.5 cm depth were broadly similar with lower concentrations on the first day when the incubation temperature was 13°C and more than doubling on the second day of the experiment when temperature were 23°C.

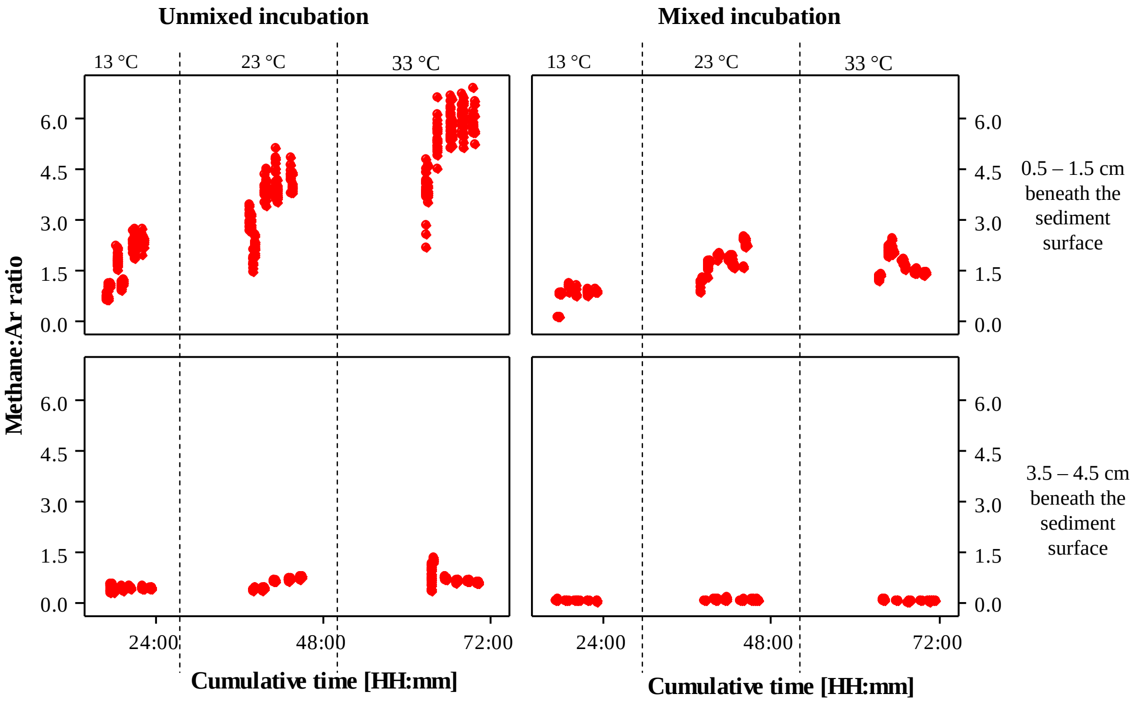


Figure 6.11: CH_4:Ar ratios measured at all depths within the mixed and unmixed incubated columns (as labelled).

As mentioned previously the concentrations on the second and third days of the experiment exceeded the calibration curves however the ratios of CH_4:Ar show CH_4 continued to rise at a high rate from the unmixed incubation at 0.5 - 1.5 cm depth. Likewise the CH_4:Ar ratio from the mixed core at 0.5 - 1.5 cm increased with experimental time but not as dramatically as from the unmixed incubation. Moreover the headspace concentrations (< 170 ppm) were much lower than those measured in the sediment at 0.5 - 1.5 cm depth suggesting CH_4 oxidation by methanotrophs was occurring. Furthermore the very low CH_4 concentrations observed from the mixed incubation and inferred from the unmixed incubation at depth suggest that the redox state of the sediments may be much lower than closer to the surface or that methanogenesis is not possible due to a lack of substrate i.e. H_2/CO_2 or acetate, at this depth.

Nitrous oxide concentrations within the sediment and response to increased temperature

Experiment 1: Temperature

N_2O concentrations were determined from all probes at all depths in the sediment. N_2O concentrations differed greatly both between the two cores and within each core at different depths (Figure 6.12).

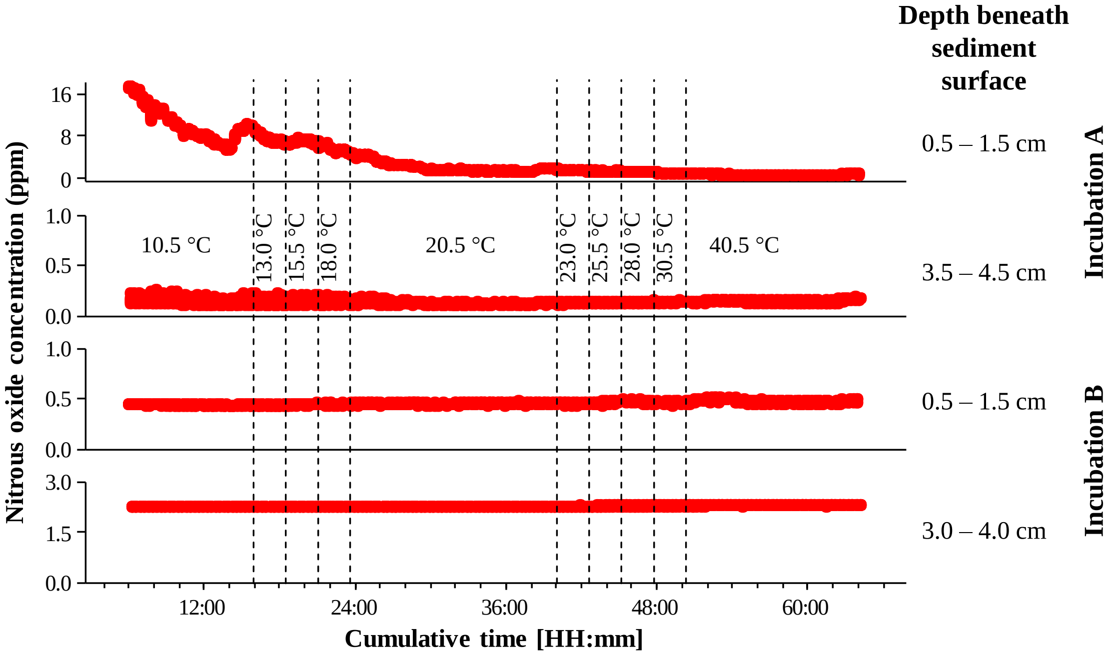


Figure 6.12: Continuous measurement of N_2O concentrations (ppm) within both incubated cores (as labelled). Incubation temperature increments are labelled. Note the different Y-axis scales.

Concentrations of N_2O from core A from 0.5 - 1.5 cm depth for the first 22 hours of the experiment exceeded the range for the detector (> 5.15 ppm). Thereafter concentrations continued to decrease albeit at a much slower rate.

The concentrations of N_2O from core B and from 3.5 - 4.5 cm depth in core A were relatively stable over the experiment duration. Mean concentration of N_2O from core B at 3.0 - 4.0 cm (2.30 ± 0.02 ppm) was much greater than those recorded either at the surface of core B (mean 0.44 ± 0.02 ppm) or at depth in core A (mean 0.12 ± 0.02 ppm). Lowest mean N_2O concentrations were from the greatest depth, 3.5 - 4.5 cm from core A.

Q_{10} coefficients were calculated of each 10°C rise in temperature and the corresponding mean N_2O production (Table 6.9). The response of N_2O production with increasing temperature was negative within core A, from both measured depths within the sediment. Temperature increase had no effect in N_2O production at 3.0 - 4.0 cm depth within core B, and a positive effect on just two occasions at 0.5 - 1.5 cm depth (Table 6.9).

Table 6.9: The Q_{10} coefficients illustrating the response of N_2O production to each 10°C rise in temperature.

Temperature rise (°C)	Core A (0.5 - 1.5cm)	Core A (3.5 - 4.5 cm)	Core B (0.5 - 1.5cm)	Core B (3.0 - 4.0 cm)
10.5 - 20.5	0.36	0.81	1.34	1.00
13.0 - 23.0	0.15	0.60	0.95	1.00
15.5 - 25.5	0.09	0.66	0.50	1.00
18.0 - 28.0	0.10	0.63	0.62	1.00
20.5 - 30.5	0.47	0.83	2.61	1.00
30.5 - 40.5	0.14	0.87	0.21	1.00

Experiment 2: Temperature and turbulence

Pulse-chase experiment using $^{15}NO_3^-$*-N in the overlying water.*

The addition of $^{15}NO_3^-$ at the beginning of experiment (at a concentration of 50 mg $Na^{15}NO_3^-$ L^{-1} in purified water using 99 atom % ^{15}N), in the overlying water column created a "pulse-chase" experiment, using the changes in both N at the lighter (^{14}N) and heavier (^{15}N) atomic weights (nitrogen 28, 29 and 30). This removes some of the ambiguity associated with the processes of N-cycling within the sediment i.e. the incorporation of this added ^{15}N into N_2 would be strong evidence for denitrification within the sediments through the production of $^{29}N_2$ or $^{30}N_2$ i.e. one ^{14}N and one ^{15}N or two ^{15}N atoms, respectively forming N_2 molecules.

N profiles within the sediment cores

Decreasing ratios of N28:N29 in sediment porewater over the sampling period at both sampling depths within the two sediment cores were observed (Figure 6.13). This decreasing ratio is the result of increasing ^{29}N within the sediment porewater as a result of the downward diffusion of the added $^{15}NO_3^-$-N and its reduction via denitrification to $^{29}N_2$ gas.

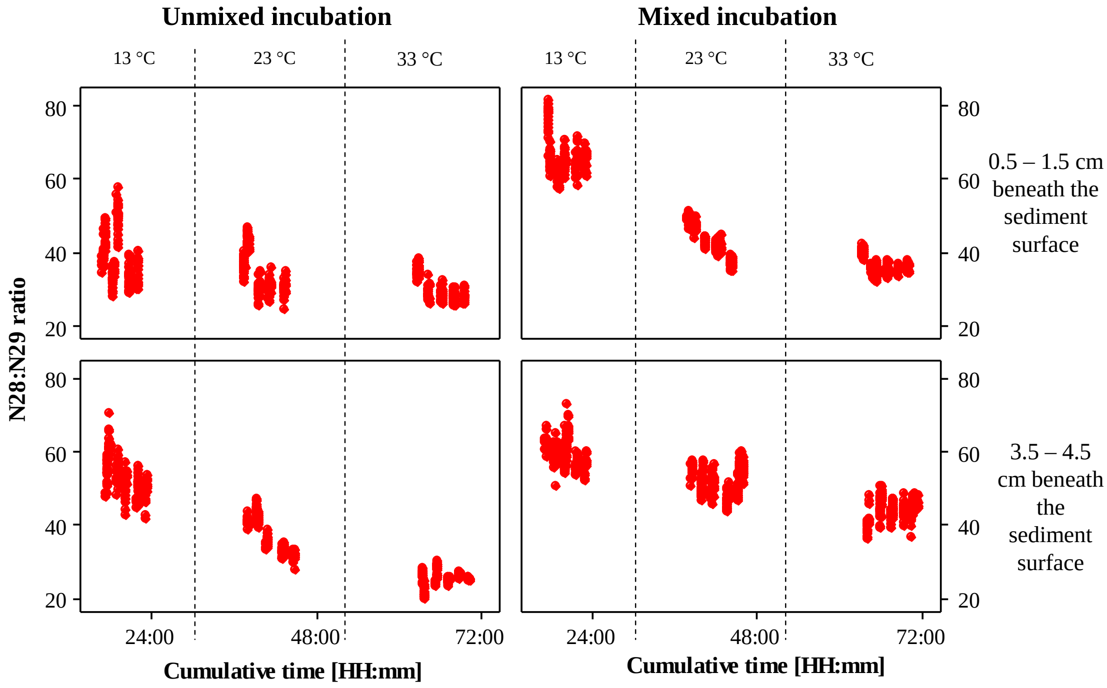


Figure 6.13: N28:N29 ratios from within the sediment of the mixed and unmixed incubations (as labelled).

Higher N28:N29 ratios were observed deeper within both cores compared with nearer to the sediment surface, most probably a result of the increased diffusion times required for ^{15}N-enriched NO_3^- to reach deeper within the cores. The ratios observed from the mixed incubation were higher than those for the unmixed incubation suggesting mixing of the overlying water facilitated better diffusion. Furthermore the ratios from both measured depths within each core decreased with experimental time suggesting that the added ^{15}N was rapidly being utilised and that the ^{15}N-NO_3^- coming in to the sediment is denitrified alongside existing pore-water NO_3^-.

The gradual change in N28:N29 ratios also suggest that pore-water NO_3^- is still relatively abundant as an exhausted or near exhausted supply of *in situ* NO_3^- would result in a marked drop in ratios associated with a switch from the *in situ* $NO_3^-$to the added nitrate via the water column. The decrease in the ratios further suggests biogenic production of N_2 via denitrification from the cores. N_2O was not detected at any depth from either core suggesting the prevailing conditions were strongly anoxic and denitrification was complete to N_2. The relative changes in both the N29:Ar and N30:Ar ratios highlight the production of N_2 gas as a result of complete denitrification of the added $^{15}N\text{-}NO_3^-$ (Figures 6.14 and 6.15)

Within the unmixed incubation at both measured depths the N29:Ar ratio increases over the 3 experimental days. The probes sampling at 0.5 - 1.5 cm depth contained higher ^{29}N (i.e. higher N29:Ar ratio). The N29:Ar ratios approximately 60 hours after $^{15}NO_3^-\text{-}N$ addition at 0.5 - 1.5 and 3.5 - 4.5 cm depth in the unmixed incubation were similar. However, those at 0.5 - 1.5 cm depth from within the mixed incubation were much greater than at 3.5 - 4.5 cm depth.

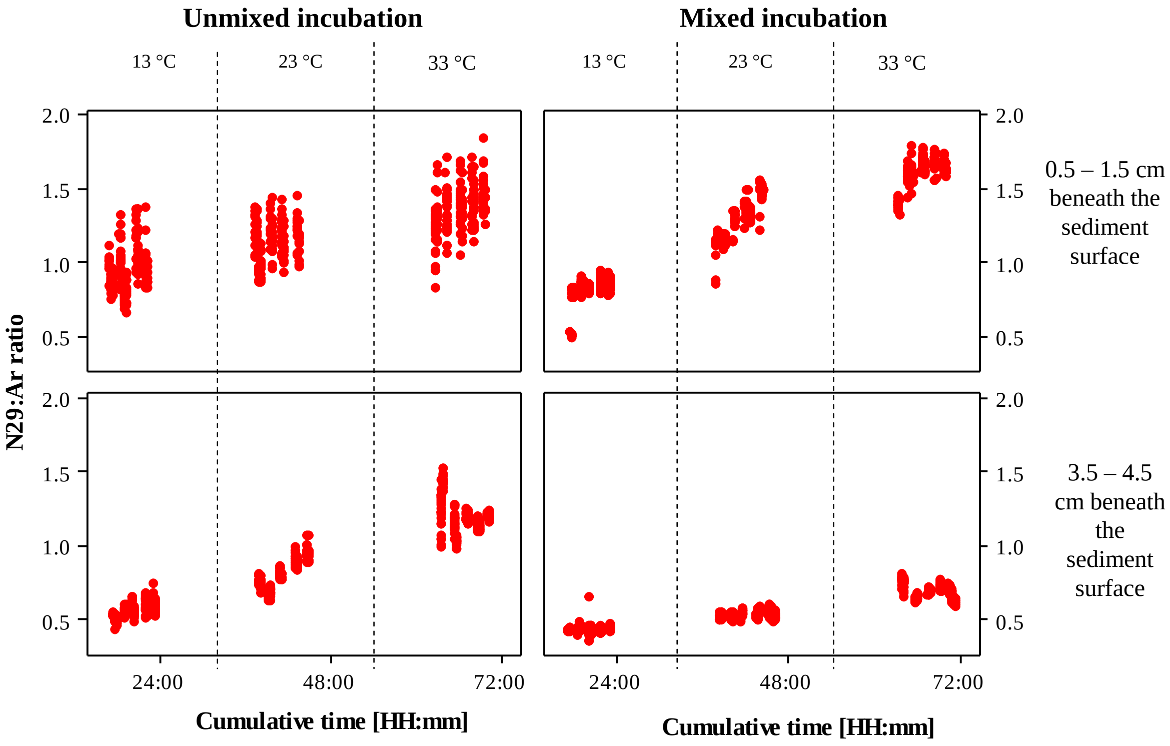


Figure 6.14: N29:Ar ratios from within the sediment of the mixed and unmixed incubations (as labelled).

The N29:Ar ratios from both depths within the mixed incubation are very different; the probe located at 0.5 - 1.5 cm depth shows a sharp increase each day with increasing temperature suggesting the potential presence of a site of denitrification at this location/depth, in contrast to the steady rise observed from the unmixed incubation, most probably due to the greater mixing taking place within the water and sediment. In contrast the probe located at depth (3.5 – 4.5 cm) increases very little compared with those recorded on previous days.

N30:Ar profiles (Figure 6.15) between unmixed and mixed incubations are quite different with ratios from the mixed being much smaller than those from the unmixed at both depths within the cores. The much lower N30:Ar ratios within the mixed incubation may suggest an alternative NO_3^- source i.e. *in situ* nitrification.

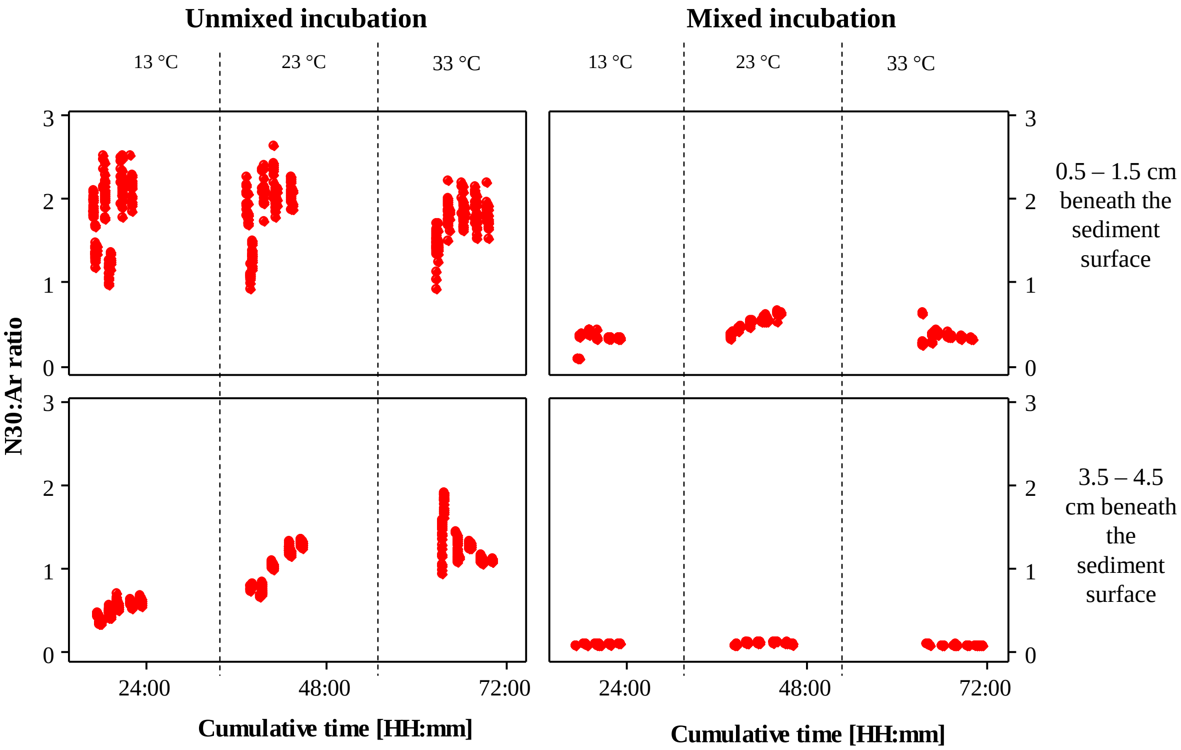


Figure 6.15: N30:Ar ratios from within the sediment of the mixed and unmixed incubations (as labelled).

Within the unmixed incubation the N30:Ar ratios were greater at 0.5 - 1.5 cm than at 3.5 - 4.5 cm depth. Probably due to the longer diffusion times to reach deeper within the sediments. Ratios within the unmixed incubation increase with experimental time at 3.5 - 4.5 cm depth whereas at 0.5 - 1.5 cm depth the ratios decrease gradually with experimental time, this reflects the near exhausted $^{15}NO_3^-$-N pool in the overlying water column.

Dissolved oxygen profiles within the sediment

A lack of dissolved oxygen (DO), would indicate anoxic conditions however the O_2:Ar ratios across the sampling period are fairly consistent. This would suggest that sediments are at least suboxic from the start of the experiments and that DO concentrations are very low (Figure 6.16). If this is the case then the much lower CH_4 concentrations at depth may be due to substrate near exhaustion at this depth prior to the start of the experiment.

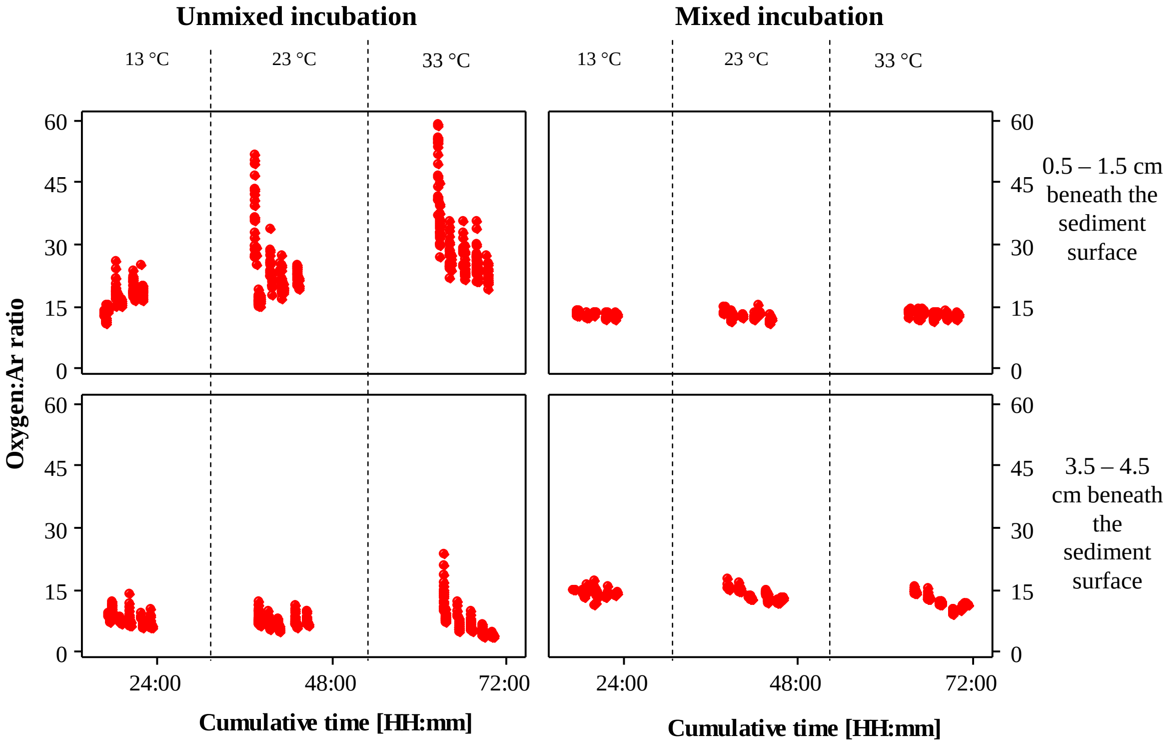


Figure 6.16: Oxygen:Argon ratios from the unmixed and mixed incubations (as labelled).

The apparently slighter higher O_2:Ar ratios from the probes at the start of sampling days two and three are likely to be an artefact of the manual operation of the MIMS system. The probes were left *in situ* for a period of approximately 16 hours between the close of sampling on the previous day and the start of sampling on the subsequent. During this inactive period diffusion of gases from the probes into the inlet tubes may have occurred. Then on the commencement of the first sampling from each probe these residual gas concentrations would have been analysed by the mass spectrometer. A longer purging time for each probe would be required for each probe when the MIMS system is used manually.

6.4 Discussion

6.4.1 N cycling, evidence for denitrification and reduced sediment aeration

Nutrients, inorganic and organic matter as well as gaseous exchanges occur between and within the sediment, water column and atmosphere of wetland environments making them spatially and temporally dynamic (van Cleemput, 1998; Hefting *et al.*, 2003; Sabater *et al.*, 2003). Denitrification has been identified as the main permanent removal pathway of NO_3^--N in many wetland environments (Lipschultz *et al.*, 1981; Hauck, 1984; Jetten *et al.*, 1998; Kadlec *et al*, 2005). Within the incubated columns of all experiments NO_3^--N concentrations decreased whilst NH_4^+-N increased, suggesting denitrification and organic matter mineralisation is occurring and that aerobic conditions most likely decreased with experimental time (Groffman, 1994; Kadlec *et al.*, 2005; Vymazal, 2007).

Diffusive exchange of NO_3^--N from the water column to the sediment and its reduction via denitrification is most likely occurring. During denitrification NO_3^--N is reduced through a series of intermediaries such as N_2O to N_2 gas. Headspace fluxes of N_2O increased from all cores with experimental time and were within the range of those observed *in situ* from the wetland, with the exception of the unmixed incubation from Experiment 2 which exceeded the range observed *in situ*. The lack of measureable N_2O within the sediments concurrent with increasing N_2O emissions may suggest that N_2O production was occurring in the water column and not within the sediments as hypothesized.

NH_4^+-N is formed via the process of ammonification from decomposed organic N compounds by bacteria (Kadlec and Knight, 1996; Vymazal, 2007). This is the substrate for nitrification but this latter process requires aerobic conditions. The increasing concentrations of NH_4^+-N within the water column (concurrent with decreasing NO_3^--N concentrations) would suggest that the sediments became progressively more reducing so that nitrification (i.e. the oxidation of NH_4^+-N to NO_3^--N) could not take place and resulted in the observed accumulation of NH_4^+-N in the water column.

Furthermore NH_4^+-N concentrations were much higher in the unmixed core of Experiment 2 than either of the cores from Experiment one, despite similar conditions. Cores for Experiment 1 were collected in May 2008 and in July 2009 for Experiment 2, and may suggest increased organic material recycling by microbes associated with the accumulation of more sediment with the increase of time (Vymazal, 1995). Potentially higher sedimentation rates and therefore organic matter availability and degradation may account for the higher NH_4^+-N concentrations in year 3 (2009) than year 2 (2008) since wetland construction. However destructive sediment sampling was not conducted at the wetland site or at the completion of the experiments to confirm this. Further work should include systematic sediment sampling and analysis for both organic and inorganic N and C.

Within the sediments gas sampling allowed the identification of potential processes to be inferred. N_2O:Ar ratios from within the sediments of Experiment 1 only (and O_2:Ar ratios) decrease and N_2:Ar ratios increase with experimental time from all incubated sediments, suggesting a shift from suboxic conditions, more favourable for N_2O emission to more reducing concentrations optimal for complete denitrification to N_2 (Vymazal, 2007). The lack of measureable N_2O-N within the sediments in

Experiment 2 lends further support that more reducing conditions had been established within the sediments of the wetland with increased time and therefore to complete denitrification to N_2.

However as mentioned previously N_2O headspace fluxes continued to increase with experimental time from both experiments. Perhaps previously aerated sediments just beneath the water surface (as evidenced by higher O_2:Ar ratios within the sediments at the start of the experiment and higher NO_3^--N concentrations within the water column) became increasingly more reducing (lower NO_3^--N and increased NH_4^+-N within the water column). This would potentially create a greater number of new suboxic denitrifying microsites near to the sediment/water interface that may account for the increase N_2O-N flux with time. Additionally the more reducing conditions may have allowed N_2O production within water column to take place.

The pulse-chase experiment using labelled $^{15}NO_3^-$-N provides strong evidence for denitrification as N_2:Ar ratios at heavier atomic masses (i.e. ^{29}N or ^{30}N) were measured i.e. complete denitrification of this added $^{15}NO_3^-$-N to N_2, with these ratios increasing with experimental time. Evidence from the pulse-chase experiment suggests the following scenario: $^{15}NO_3^-$-N in the overlying water column is diffusing into the sediments, facilitated by a turbulent water column and that this $^{15}NO_3^-$-N is denitrified alongside *in situ* porewater nitrate (gradual change in the ^{28}N:^{29}N ratio and increasing ^{29}N:Ar and ^{30}N:Ar ratios). The gradual change in the ^{28}N:^{29}N ratio also suggests that *in situ* nitrate pool is not near exhaustion and perhaps that these sediments are rich in inorganic-N.

6.4.2 Evidence for methanogenesis

CH_4 emissions increase with increasing time from all the cores Within the sediments CH_4 concentrations were found to be higher from the unmixed core that produced highest headspace fluxes CH_4 concentrations from both incubated cores (of Experiment 2); exceeded 300 ppm measured from 0.5 - 1.5 cm beneath the sediment surface on the second and third days of the experiment. Measured concentrations of CH_4 from the headspace were much lower (10 - 50 ppm) and broadly similar. However headspace fluxes from the unmixed incubation on the final day of the experiment were almost double those from the mixed. This would suggest that the unmixed incubated column may have become very reducing and therefore methanogenesis dominated microbial processes.

Observational evidence for the strongly reducing nature of the sediments was the presence of a strongly unpleasant odour emanating from both incubations but particularly the unmixed. Most probably this unpleasant smell was H_2S gas produced from sulphur reduction (Schiffman and Williams, 2005). Additionally indirect evidence that the reducing conditions within the unmixed incubation were more severe than the mixed incubation was from the movement of snails from the sediment into the water column. A large number of small snails (> 50) were observed to come out of the sediment and into the overlying water column of the unmixed incubation, whereas < 10 snails were observed in the water column of the mixed incubation. Furthermore the majority of these snails had perished within the unmixed incubation, whereas < than 5 snails within the mixed incubation had died. However NH_4^+-N (or potentially NO_2^-) toxicity may have caused the snail death (Alonso and Carmargo, 2003).

CH_4 is readily oxidised in the presence of O_2. Research has shown that the diffusive movement of CH_4 through sediments often leads to a high percentage of CH_4 oxidation by methanotrophs, making this an important removal pathway for this greenhouse gas (van Hulzen *et al.*, 1999; Zhu *et al.*, 2010). It is likely that the unmixed condition of the overlying water column has made the unmixed incubation more reducing with potentially completely anoxic sediments or a very shallow suboxic layer at the sediment/water interface. Therefore the potential for CH_4 oxidation would be reduced due to this increased soil anaerobiosis compared with the unmixed incubation. Methanogenesis is known to increase with increasing temperature, however headspace fluxes were not found to be strongly dependent on temperature suggesting that increasing CH_4 production may be the result of increasingly more reducing conditions within the sediments. However, poor diffusion and/or methane oxidation has resulted in limited loss of this CH_4 to the headspace.

CH_4:Ar ratios within the unmixed incubation were lower deeper within the sediment of both cores than nearer to the surface, additionally, CH_4 concentrations measured at greater depths within the sediment of the unmixed incubation were lower than those measured nearer to the surface for either core of experiment two ($<$ 86 ppm). This may suggest a lack of substrate availability deeper within the cores (Segers, 1998). Additionally the CH_4:Ar ratios at this deeper depth within the sediment of both cores were similar although slightly higher from the unmixed incubation. This would suggest that CH_4 concentrations within the unmixed incubation at 3.5 - 4.5 cm depth would have been lower than those produced at the surface. Deeper sediments tend to be more reducing as they are further from the atmosphere and therefore gaseous exchange of O_2 is by diffusion through the sediment is more limited (Barlett and Harris, 1993; Mitsch and Jørgensen, 2004; Laanbroek, 2010). It is likely that these sediments are strongly reducing however the lower CH_4 production may indicate a lack of substrate availability commonly H_2/CO_2 or acetate produced from organic matter decomposition by a variety of microorganisms (Segers, 1998; Gauci *et al.*, 2004).

All cores were collected from approximately between 1 - 2 m from the upstream end of the wetland except core A from Experiment 1 which was collected at approximately 16 m along the wetland. The much higher CH_4 production measured within the sediments combined with the observed high CH_4:Ar ratios during the settlement period before the experiment began would suggest this core was highly reducing *in situ* with experimental conditions creating less reducing conditions. Furthermore, this may suggest that the wetland field experiment may not have captured the potential CH_4 emissions from this location, as no automated chambers were positioned 16 - 20 m along transect. Despite the problem associated with the long headspace closure times and CH_4 accumulation within the headspace, measured

CH_4 concentrations within them sediment core exceed 300 ppm prior to the start of the experiment. At this time the incubation temperature was 10.5°C, typical of late spring/early summer temperatures from the field site. This suggests high potential for methanogenesis and CH_4 emissions from the instream wetland. However, the low measured *in situ* CH_4 fluxes may suggest high CH_4 oxidation is occurring as it diffuses through the sediment.

Automated gas flux chambers were positioned at approximately 5, 10 and 15 m from the upstream end of the wetland (see Chapter 5 for details). In the lower part (15 - 20 m) observational evidence suggests a deeper water column depth, sediment depth and reduced water turbidity. The combination of these factors would resemble conditions of the unmixed incubation for Experiment 2, which became increasingly reducing as a result of these conditions. Potentially, in the last 5 m of the wetland, higher CH_4 emissions were associated with higher CH_4 production within the sediment and the highly reducing sediments and therefore much less CH_4 oxidation would be occurring (Segers, 1998; Mitsch and Jørgensen, 2004; Lannbroek, 2010). Additionally it is hypothesized that a smaller or absent aerobic layer would exist within the surface layer of the sediment leading to lower CH_4 oxidation and thus greater net CH_4 emissions. However neither flux measurements nor sediment sampling from this location in the wetland were performed to confirm these hypothesises. Furthermore, seasonally during the drier months of the year the water level decreased and exposure of the sediments to the atmosphere occurred in some areas of the wetland particularly in the top 10 m. However sediments never became exposed between 15 - 20 m suggesting that reducing conditions could be maintained in the lower part of the wetland throughout the year and may also explain the lower CH_4 production within the sediments at the 3.5 - 4.5 cm depth i.e. the persistent anaerobic conditions have exhausted the available substrate supply.

These experiments were conducted with the absence of wetland plants. Within wetlands plants have several important roles; uptake of N and its temporary removal from the wetland system, supply of oxygen and substrates (C source) for microbial processes in the form of root exudates (Brix, 1997, Fromin *et al.,* 2005; Randerson, 2006). Furthermore plants facilitate the movement of gas between the sediment and atmosphere such as the transport of O_2 into the sediment or the transport of gaseous out of the sediment through the plant vascular system to the atmosphere such as CH_4 and therefore preventing CH_4 oxidation, enhancing CH_4 emissions (Reddy, 1989; Cooke, 1994; Brix, 1997; Spieles and Mitsch, 1999; Randerson, 2006; Borrel *et al.,* 2011). This vascular transport pathway has been estimated to account for up to 90% of CH_4 emissions in rice paddies (Holzapfel-Pschorn *et al.,* 1986; Schűltz *et al.,* 1991). Future experiments should investigate the role of plants in N removal and greenhouse gas production within wetlands.

6.4.3 Factors controlling fluxes

Due to the problem of headspace gas accumulation as a result of long closure times, only relationships between headspace flux and variables will be discussed in relation to Experiment 2. Significant ($P < 0.05$) relationships were observed between N_2O emissions and both NH_4^+-N (positive relationship) and NO_3^--N (negative relationship) concentrations within the water column from the unmixed incubation. These relationships would support denitrification as the most likely source for N_2O emissions due to the inferred redox state of these sediments. The mixed incubation however had poor and weak correlations with NH_4^+-N and NO_3^--N concentrations which may suggest that N_2O emissions were the result of both nitrification and denitrification processes.

Temperature can increase microbial activity (Knowles, 1982) such as denitrification or methanogenesis and therefore increase the production of N_2O (N_2) or CH_4. Significant positive relationships were found between N_2O flux and temperature from both the mixed and unmixed core (54 and 51%), suggesting increased microbial activity associated with increased temperatures.

Likewise CH_4 flux from the mixed incubation was positively and significantly related to temperature accounting for 49% of variance in flux. However no relationship was evident between temperature and CH_4 flux from the unmixed core. Calculated Q_{10} coefficients for two subsequent 10°C rises in temperature suggest an initial negative effect followed by a positive effect of CH_4 flux from the unmixed core. The opposite effect of temperature at these two temperature intervals was observed from the mixed core. This would suggest other factors such as substrate availability and prevailing redox conditions within the sediment profiles are playing a more dominant role in CH_4 production and therefore emissions (Davidson *et al.,* 2006). Exponential and significant relationships between CH_4 production within the sediment and temperature for both cores A and B from Experiment 1 were observed, with 72 and 77% of variance in production explained by increasing temperature. This would suggest that temperature is an important driver for methanogenesis (Segers, 1998).

CH_4 must diffuse from its strongly reducing site of production through increasingly more oxic environments to be emitted into the atmosphere. The significant positive relationship between CH_4 production and temperature combined with the lack of clear relationship between temperature and CH_4 flux would suggest that CH_4 oxidation by methanotrophs in more oxic environments is occurring (Frenzel and Rudolph, 1998; Segers, 1998; van Hulzen *et al.,* 1999). However as sediment becomes increasingly reducing, such as in the unmixed incubation of Experiment 2, CH_4 can diffuse through the sediment without being oxidised. Therefore increased temperature will have a positive effect on CH_4 emissions provided redox conditions are suitable and substrate is not limiting (Segers, 1998; van Hulzen *et al.,* 1999; Fromin *et al.,* 2005).

6.4.4 The effect of turbulence on diffusive movement of greenhouse gases and dissolved inorganic-N species

Reduced turbulence increased CH_4 emissions associated with increased anaerobiosis. Additionally the enhanced reducing conditions create different dominant inorganic-N species: NH_4^+-N compared to NO_3^--N in oxic environments. Turbulence enhances the diffusive movement between the water column and sediment, as evidenced by the enhanced downward diffusion of $^{15}NO_3^-$-N in the incubation with a mixed overlying water column.

6.4.5 Review of the MIMS technique

An apparent drift in probe response was observed on each sampling occasion particularly on the first sampling occasion of each experimental day (Experiment 2). The time between each cyclic measurement from an individual probe was approximately 1 hour and greater than 17 hours for the overnight period. It is suggested that during this inactive period diffusion of gases from the pore space through the probes and into the inlet tubes may have occurred. This residual gas may then have been analysed creating the supposed drift seen in the various measured gases. A longer purging time, particularly after long inactivity periods may be required. This drift is not seen in measured samples during experiment 1, most probably because under the automated sampling procedure probes not in use remain under vacuum.

Additionally, MIMS has produced six 'lines' or ‘traces’ corresponding to the 6 inlet holes over the 1 cm sampling interval at each sampling occasion. This would suggest that the MIMS system does not integrate the separate traces for each inlet probe.

6.5 Conclusions

These experiments have shown the complexity of inter-related processes: microbial, physical and chemical; in the production and emission of greenhouse gases from wetland sediments. N_2O emissions have been observed despite no measureable concentrations within the sediments. Conversely, high CH_4 concentrations within sediments are measured with low surface emissions. Furthermore, the use of isotopes has shown that denitrification and nitrification are likely occurring simultaneously within sediments based on the observed N gas ratios. These experiments have highlighted the merit and limitations of the MIMS technique and the need for intensive sampling at multiple depths of multiple trace gases to fully understand the inferred processes operating.

The pulse-chase experiment has shown that denitrification is a key process occurring within these sediments and most probably the principle NO_3^--N removal pathway within the sediments. Furthermore the added $^{15}NO_3^-$-N in the overlying water column is denitrified alongside porewater NO_3^-. High NH_4^+-N concentrations may suggest increased mineralisation of organic-N material and possibly lower oxygenation of the underlying sediments. Therefore high *in situ* concentrations of NH_4^+-N may indicate that the underlying sediments are strongly reducing preventing nitrification from taking place and potentially result in higher CH_4 emissions.

The lack of CH_4 emissions, despite inferred strongly reducing conditions at deeper depths within the cores of experiment 2 collected in 2009, suggest substrate supply may be limited and may explain the observed low CH_4 emissions from the wetland in subsequent years. Additionally redox state is key to creating and maintaining suitable conditions for methanogenesis. The drying out of patches of sediment within the wetland would have greatly influenced the greenhouse gas emissions produced i.e. N_2O as opposed to CH_4 in more oxygenated sediments. Turbulence therefore is an important control on creating anoxic conditions and wetland systems. More turbulent water may exhibit high N_2O emissions with low turbulent or stratified systems producing more CH_4 under more strongly reducing conditions.

Chapter 7: Conclusions and recommendations for further work

7.1 Key results

7.1.1 N_2O fluxes from an irrigated and control riparian buffer strip

This research has shown that irrigation of a riparian buffer strip with NO_3^--rich agricultural drainage water does not increase direct N_2O emissions compared with an un-irrigated control.

Irrigation was observed to create hotspots of N_2O emissions particularly from soil located closer to the irrigating boom. Irrigation was also shown to increase the spatial variability of N_2O fluxes, likely a result of uneven wetting and drying of the soils. Cessation of irrigation to the riparian buffer strip lead to priming of soils for denitrifying activity near to the irrigating boom and the greatest spatial variability in N_2O fluxes. These effects were found to be short-lived as they did not last into the next growing season.

Evidence from the riparian buffer strips (Chapter 3) suggests that un-irrigated, riparian buffer strips have the potential to generate high direct N_2O emissions compared to irrigated buffer strips over short time periods due to rainfall-driven wetting and drying cycles. Subsequent laboratory experiments found low indirect losses occurred without added NO_3^--N, suggesting direct emissions as a result of rainfall events are more important.

Both field (Chapter 3) and laboratory (Chapter 4) experiments highlight the seasonal variability in direct N_2O emissions from grassland buffer strips, with summer direct emission factors much higher (c, 1 to 3% greater) than winter emission factors. Therefore the use of seasonal emission factors may be more accurate when compiling emissions inventories.

7.1.2 Nitrous oxide emission and soil profile N_2O concentration in intact soil cores

Laboratory experiments using intact soil cores confirmed that irrigation with NO_3^--rich agricultural drainage water did not lead to high direct N_2O emissions but leaching losses were high (up to 82% of added N). The calculated mean fraction of leached N from incubation experiments (25 to 82%) falls mostly within the range of the IPCC default (10 to 80%). However, leachate samples had very high mean dissolved N_2O-N concentrations (experimental means of 94.3 ± 134 to 231 ± 297 µg N_2O-N L^{-1}). Additionally high leached N_2O-N concentrations (94.3 ± 134 µg N_2O-N L^{-1}) occurred even under simulated rainfall conditions without added NO_3^--N, suggesting the potential for high leachate losses and thus indirect N_2O emissions in un-irrigated grassland pastures. Furthermore very high dissolved N_2O-N concentrations (up to 627 ± 17.6 µg N_2O-N L^{-1} from one of the replicate cores for one of the experiments), were measured in leachate with high NO_3^--N concentrations during the experiments (11.1 mg NO_3^--N L^{-1} similar to the drinking water standard level). This emphasises the need for site specific measurement of dissolved N_2O-N concentrations when quantifying N leachate losses for N budgets.

In situ soil profile N_2O concentrations measured using Accurel® probes were high compared to those measured at the surface. This prompted laboratory studies that confirmed that observed N_2O surface emissions are not always representative of N_2O production within the soil profile. Higher concentrations of N_2O within the soil profile occurred where the greatest C and N substrates were available, soil temperatures were higher and the WFPS was c. 75 - 77%.

7.1.3 Greenhouse gas emissions and nitrate interception for an instream wetland

Although N pollution swapping from the riparian wetland is minimal from either direct or indirect emissions, this wetland is ineffective at intercepting diffuse N pollution and is a net cost to the environment due to greenhouse gas emissions. The wetland was ineffective largely as a result of its small size (60 m^2) in addition to the probable additional N inputs from a field drain. The N removal contribution of this wetland was complicated due to the existence of additional inputs of N from field drains or possibly via overland flow (although the latter was not observed) at times of high rainfall.

CH_4 emissions have proved to be of greater importance generating a net cost of ~ £600 ha^{-1} over the 2-year study period compared to ~ £ 60 ha^{-1} from N_2O emissions with minimal savings from NO_3^--N interception ~ £24 ha^{-1}. However actual costs of pollution swapping (both CH_4 and N_2O) compared with NO_3^--N interception savings over the two-year study period adjusted to the wetland area (60 m^2) were low at ~ £5 and would not greatly contribute to the national greenhouse gas budget. Nevertheless, the high construction in addition to maintenance costs renders this wetland a cost-ineffective option for reducing diffuse pollution at this field site. This is likely due to the location of the wetland instream and its small size, and therefore fast flowing water with short residence time for N removal from multiple sources.

Likewise greenhouse gas emissions from the wetland were highly seasonal, with greater N_2O flux during the mild winter and spring months, when NO_3^--N concentrations were generally high with low plant growth and therefore reduced competition for this substrate from plants. CH_4 emissions were greatest during spring months most likely as a result of increased temperatures. However the lack of significant relationships between greenhouse gas fluxes and key controlling variables, such as water temperature, are most likely a result of wetland hydrology. The wetland had channelized, turbulent flow with relatively fast flowing water, most likely creating increased aeration to the upper sediments and short residence times all of which impact both the production and sustenance of suitable redox conditions for denitrification or methanogenesis.

Wetlands for treatment of agricultural runoff or diffuse pollution must seek to maximise nutrient interception whilst reducing greenhouse gas emissions so that pollution swapping does not occur. The main requirement for increasing N interception at this site is largely longer water residence time (to allow for denitrification to occur) that could be created by a longer wetland with multiple cells. Additionally an ‘off-line’ wetland would allow greater control of the water flow and depth in addition to diverting the water to secondary wetland cells. Therefore careful management of the inflow volume to the wetland cells could maximise N removal (low flow and long residence time) whilst reducing emissions of CH_4 (shallow water depth therefore more aerated water column and underlying sediments).

7.1.4 Assessment of nitrogen and trace gas fluxes in wetland sediments using Membrane Inlet Mass Spectrometry

Incubation experiments using wetland sediment confirmed that denitrification is a dominant N removal pathway within these sediments. Denitrification is the most likely source for N_2O emissions in more reducing environments. However nitrification may contribute to N_2O emissions in more aerated sediments or within the water column.

In these experiments CH_4 emissions were low despite high production within the sediments, most likely a result of CH_4 oxidation as CH_4 diffuses through the sediments. It is likely that CH_4 oxidation plays an important role in reducing CH_4 emissions from this wetland. However high CH_4 production and emissions were also observed from a sediment core collected near to the wetland outlet, suggesting high spatial variability in greenhouse gas fluxes from within this wetland. Therefore maintaining an aerobic layer at the sediment/water interface may be a method to reduce CH_4 emissions, through enhanced CH_4 oxidation. This could potentially reduce the pollution swapping effect of constructed farm wetlands whilst intercepting diffuse N pollution. Greater sediment aeration could be achieved by controlling the water flow, i.e. by maintaining a shallower water depth or turbulent flow.

The variability in observed emissions from the instream wetland were likely due to spatially variable microsites of nitrification, denitrification or methanogenesis activity, under suitable redox conditions, with greater CH_4 emissions in regions overlying more reducing sediments, but higher N_2O emissions by either denitrification or nitrification from within more aerated sediments or the water column. Furthermore the drying out of areas of sediment within the wetland would have greatly influenced the greenhouse gas emissions produced, i.e. favouring N_2O production as opposed to CH_4, in more oxygenated sediments.

7.2 Limitations of research and further work:

7.2.1 N_2O fluxes from an irrigated and control riparian buffer strip

This part of the research was carried out using the paired plot technique and intensive sampling both spatially and temporally of direct N_2O emissions. A major uncertainty with this research is that the similarity of the two riparian plots (the IBS and CP) before the study period was not characterised, for example the precise management history (grazing, cropping, fertilisation and microtopography). Despite the close proximity and location within the same field of the two plots differences may be considerable, particularly when N_2O production occurs within microsites. Financial and spatial constraints prevented the implementation of multiple plots at Nafferton Farm. However, all future studies should aim to have a minimum of three replicated treatment and control plots to improve the robustness of research findings.

Greater replication of plots such as three or more irrigated or non-irrigated grassland plots would add robustness to the findings of this research. Additionally, testing a variety of irrigation regimes, with different irrigation volume as well as NO_3^- loading, would add greater depth of understanding to allow the optimal (if any) irrigation strategy whilst causing minimal pollution swapping or leaching, to be identified.

Furthermore, high spatial and temporal variation in fluxes from the riparian buffer strips was observed. The use of more chambers (both static and automated) within each plot would have helped to reduce this. A minimum of three automated chambers sampling at least three times per day would have increased the robustness of estimates of daily emissions and variation within the plots. Additionally the use of fast response N_2O analysers that allow many measurements in a short space of time could improve temporal resolution of N_2O emissions estimates.

Further work, that will conduct sampling once-per-day should determine the time of day that most represents the daily mean and sample at this time if possible. Furthermore seasonally the most representative sampling time may change and should be investigated for each season. Particular attention should be given to the time of irrigation with respect to the time of emission sampling. Additionally, gas sampling before and after irrigation *in situ* may enhance the understanding of the temporal variability in N_2O emissions.

A major limitation of this study was the lack of measured leachate. Unsuccessful attempts to measure leachate *in situ* was a result of very little leachate being produced. Nevertheless, as shown by the subsequent soil core experiments (Chapter 4), leachate losses were of greater importance than either direct or indirect N_2O-N losses. Potentially the applied irrigation water rapidly leached away preventing the collection of leachate. Although no pollution swapping was found to occur leachate losses are critical to the evaluation of the success or failure of this remediation method for interception of diffuse N. Further work should include leachate sampling to ascertain seasonal leaching patterns (if any) and the degree of interception of the irrigation water by the buffer strips.

Chambers located in the riparian buffer strips were found to create artificially warmer underlying soils of ~ 1 °C, and in some cases up to 6 °C, compared with adjacent soil outside the chamber area. It is recommended that chambers be removed from the study area, except when in use for sampling, and until further work can be carried out to examine this issue. Additionally future work should investigate the effects of different types of chamber materials or design on this insulating effect.

7.2.2 Nitrous oxide emission and soil profile N_2O concentration in intact soil cores

This research would benefit from detailed soil analysis before and after the experiments are conducted to determine within soil changes in the inorganic-N and carbon pools. Additionally an increased number of soil cores will reduce the error associated from inherent variability between replicates due to soil heterogeneity.

Experiments run in tandem on multiple cores rather than sequentially may remove the uncertainty that the incubation conditions (i.e. heating-up of the entire soil core) may affect microbial processes and therefore N cycling within the sediment cores. The measurement of gaseous N_2 emissions both within and from cores and the use of ^{15}N isotopes will help to untangle the key processes and sources of emissions from the various soil depths.

In situ soil temperature and WFPS measurements from within different depths of the cores would also increase the robustness of future experimental data. Ideally more extensive field studies should be conducted to measure soil profile N_2O concentrations in a wide variety of climatic conditions, soil types and irrigation strategies as well as leached N (N_2O-N and NO_3^--N) losses at high temporal resolution to capture indirect emissions as a result of shorter lived ‘hot moments’ of denitrifying activity (Groffman *et al.,* 2009). Furthermore longer duration of field experiments to capture seasonality is also required.

7.2.3 Greenhouse gas emissions and nitrate interception for an instream wetland

The use of wetland soil cores has shown that there exists potentially high spatial variability in greenhouse gas emissions. Further work should include greater measurement of fluxes *in situ* from the lower part of the instream wetland (i.e. 15 - 20 m along transect). No sediment sampling of the wetland was undertaken during the study period. Therefore future work should undertake periodic sediment sampling, at least seasonally if not on a monthly basis for analysis for inorganic-N, organic-N as well as C content. This would greatly add to the overall understanding of the possible controls on the observed emissions.

The N interception of the wetland was complicated by the multiple sources of water into the wetland. Targeted sampling occasions of short duration (several hours) with intensive water and emissions sampling along transect, during times of high rainfall and wetland discharge could help identify the location and NO_3^- concentration of these other water sources.

7.2.4 Assessment of nitrogen and trace gas fluxes in wetland sediments using Membrane Inlet Mass Spectrometry

The incubation experiments would be improved by increasing the frequency of headspace and water sampling. Gaseous emissions only allow microbial processes to be inferred. Further work should include bioassays at different depths within the sediment profile to ascertain the dominant microbes at these intervals. Measurements of NO_2^--N may aid the identification of the dominant microbial processes as well as any changes in redox state.

The use of $^{15}NO_3^-$ has enhanced understanding of N-cycling within the sediments and gaseous N (N_2 or N_2O) production (or lack thereof), within the sediment. Further studies should consider using C isotopes and the measurements of both CH_4 and CO_2 production from within the sediments as well as headspace fluxes.

Sediment sampling and analysis of inorganic and organic C and N should be performed adjacent to the site of core collection, as well as destructive sampling of the cores at the completion of the experiments. This would allow firmer conclusions to be drawn about the factors controlling the observed fluxes both *in situ* and during the experiments.

In addition to investigations into the effects of water temperature and turbulence on processes of greenhouse gas production in wetland sediment, further studies should examine other factors such as organic C supply or changes in water depth. A range of NO_3^--N, NO_2^--N and NH_4^+-N additions could be investigated also. Additionally in depth studies of kinetics and microbial community using acetylene blocking e.g. is nitrification an important source of N_2O emissions from these wetland sediments?

Furthermore analysis of wetland sediments from a variety of sites throughout the UK (particularly wetlands effective at intercepting diffuse N pollution) to compare with sediment cores taken from the instream wetland at Nafferton farm would reveal the similarities and/or differences in sediment composition, gaseous production as well as microbial populations. This would shed further light on if is the wetland design at Nafferton Farm or some other microbial/sediment composition difference that is affecting the efficiency of the wetland. This would allow a more informed decision to be made, not only with regard to improving the wetland efficiency at Nafferton, but also the wider use of wetlands as remediation strategies for tackling diffuse pollution within the UK, whilst reducing greenhouse gas emissions.

7.3 Policy implications of the research

7.3.1 Irrigation of riparian buffer strips

Although no pollution swapping of increased direct N_2O emissions occurred from irrigation of a riparian buffer strip with NO_3^--rich agricultural drainage water, this method of diffuse NO_3^--N removal was unsuccessful and would not be recommended due to the likely high leaching losses.

Further work should include the effects of different irrigation strategies, application methods and rates in relation to direct and indirect N_2O emissions as well as leachate losses, which are suggested to be of greater importance especially in more well-drained soils. Work should also be done to investigate *in situ* N_2O production at different times of the year, under different climatic environments and soil types. These investigations should aim to measure *in situ* soil temperature and WFPS at multiple corresponding soil depths and direct emissions of N_2O and N_2 as well as leachate losses to include dissolved N_2O-N and inorganic-N species (NO_3^--N, NO_2^--N and NH_4^+-N). This could be aided by the use of isotopes either labelled isotopes of N and tracking this through the system or stable environmental isotopes such as deuterium or oxygen to follow the water flow. High spatial resolution soil sampling to determine inorganic-N, organic-N as well as C contents would increase the experimental value.

7.3.2 The use of an instream wetland for diffuse N pollution interception

The ineffectiveness of the wetland at Nafferton Farm has highlighted the need for site specific considerations before wetland design or construction. Additionally this research has highlighted the importance of assessing the interception of diffuse pollutants and the trade off of greenhouse gas emissions.

At the Nafferton Farm site only NO_3^- pollution was investigated however a variety of pollutants associated with agricultural runoff (phosphorous, suspended solids, pathogens or hydrocarbons) should be investigated for each constructed farm wetland. Additionally, monitoring of greenhouse emissions would greatly add to the understanding of the total environmental cost of introducing constructed wetlands, i.e. are the trade offs (greenhouse gas emissions) too great to justify their implementation?

Obviously this would entail a certain level of knowledge and skill and would need to be carried out by competent authorities such as the Environment Agency in England and Wales or its respective counterparts in Scotland or Northern Ireland. The high cost associated with this undertaking would make it most unlikely to be carried out; but targeted assessment of large constructed wetland systems in key catchments within the UK are necessary to truly assess the effectiveness of constructed wetlands and the potential pollution swapping caused.

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