POLLUTANT SWAPPING IN CONSTRUCTED AGRICULTURAL WETLANDS

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This thesis is submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

SUBMITTED SEPTEMBER 2015

DECLARATION

I HEREBY DECLARE THAT THIS THESIS IS MY OWN WORK, EXCEPT WHERE OTHERWISE STATED. THE CONTENTS HAVE NOT BEEN SUBMITTED FOR ANY OTHER QUALIFICATION AT ANY OTHER INSTITUTION BY GROUPS OR INDIVIDUALS. ALL ELEMENTS OF THE WORK ARE MY OWN, WITH ANY ADDITIONAL INFORMATION DERIVED FROM OTHERS BEING THOROUGHLY ACKNOWLEDGED.

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ABSTRACT

Diffuse agricultural pollution presents a major challenge to global water quality management, requiring the adoption of new land management practices such as constructed agricultural wetlands. These wetlands, promoted in agri-environment schemes, may effectively intercept rainfall-mobilised phosphorus (P), nitrogen (N) and carbon (C). However, wetlands may potentially facilitate 'pollutant swapping': the transfer of one form or pathway of pollution for another, as a result of mitigation efforts. Retained pollutants may be re-mobilised through solubilisation or as the greenhouse gases (GHGs): methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O). Therefore this research examines the potential for agricultural wetlands to 'swap' local improvements in water quality, for (1) increased pollution to groundwaters and (2) to the atmosphere.

GHG exchanges from an agricultural wetland (area 0.032 ha) in Cumbria, UK were monitored over an 18 month period, using floating gas chambers, ebullition traps and diffusive gas exchange models. While the wetland was a net sink of particulate C and N, mean net releases of CO_2 (2249 – 5724 mg m^{-2} d^{-1}), N_2O (0.93 – 2.04 mg m^{-2} d^{-1}) and CH_4 (169 - 456 mg m^{-2} d^{-1}) were significantly greater than those from adjacent riparian land. Wetland releases of CH_4 were most significant in terms of potential atmospheric impact compared to other

wetland GHG releases. Shallow groundwater samples extracted from a piezometer network surrounding the study site, illustrated that retained sediments acted as a source of NH_4-N and DOC to surface and local groundwaters but mitigated leaching and outward transport of NO_3-N to surface and groundwaters.

Field and laboratory microcosm experiments demonstrated that pollutant swapping of GHGs and nutrients may be increased during periods of reduced water oxygen content associated with eutrophic conditions. In wetland designs with water depths >0.5 m, anoxic conditions may perpetuate in lower water column zones and facilitate increased CH_4 and NH_4-N production and storage. Additionally, microcosm studies identified that disturbance of bottom sediments by stormflow may elicit heightened GHG and nutrient releases.

Therefore the net impact of wetland construction in catchments may need reconsiderations, with respect to the potentially detrimental effects on water and the atmosphere. However upscaling of observations suggests that wetland implementation in the UK is unlikely to significantly increase GHG budgets. Use of shallower wetlands with vegetation or inlet baffles may reduce CH_4 emissions by encouraging oxidation and protecting sediments from storm flows.

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Finally, I would like to dedicate this work to the memory of my wonderful grandmother, Betty, whose company during this journey was sorely missed.

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Chapter 1 - Introduction

1.1. The increasing environmental pressures from the intensification of agriculture

The global population has expanded substantially over the last century and is now predicted to reach 9.1 billion by 2050, driven particularly by population increases in the world's developing countries (FAO, 2009). Therefore, the global challenge to feed the planets exponentially increasing population has never been greater or more urgent, with an estimated 70 % increase in food production (from 2009 levels) required by 2050 (FAO, 2009).

The challenge of meeting this increased demand for food at minimal cost has resulted in the expansion and intensification of agricultural practices in order to maximise the crop and livestock yields from finite resources. One of the primary and potentially problematic ways this has manifested, has been the increased use of synthetic fertilisers containing inorganic nitrogen (N) in the form of nitrate (NO_3^-) and ammonium (NH_4^+), together with phosphorus (P). As of 2014, global agricultural demand for inorganic fertilisers stood at 113.1 Mt yr^{-1} and 42.7 Mt yr^{-1} for N and P respectively. However this is expected to rise further to 119.4 Mt yr^{-1} (1.4 % yr^{-1}) for N and 46.6 Mt yr^{-1} (2.2% yr^{-1}) for P by the year 2018 (FAO, 2015). Despite slower increases in population in the western hemisphere, European usage of fertilisers in relation to total global demand is still substantial, at around 13.5% of the total N and 9% of P (FAO, 2015). In addition to this use of synthetic fertilisers, the increased numbers of livestock in agricultural landscapes, together with their manures, introduce further organic sources of N, and P into the environment (Carpenter *et al.,* 1998).

The extent of the anthropogenic nutrient inputs to the environment via agricultural practices, is such that many natural processes within the earth system are now being affected (Gruber and Galloway, 2008; Gordon *et al.,* 2008; Tilman *et al.,* 2002; Bennet *et al.,* 2001). This has resulted in a growing detrimental impact on numerous elements of the environment, including increased soil loss (Montgomery 2007), eutrophication of watercourses (Carpenter *et al.,* 1998) and increases in greenhouse gas fluxes to the atmosphere (Robertson *et al.,* 2000). Because of this evidence, it is believed that the planetary boundary - the sustainable threshold of human development in relation to both global N and P – cycles, has now been exceeded, largely as a result of agricultural activity

(Steffen *et al.,* 2015). Therefore, maintaining sustainable agricultural output and economic growth, whilst limiting the detrimental impact of agricultural practices on the environment, represents a major challenge for the future (Matson *et al.,* 1997; Tilman *et al.,* 2002) and will require integration of land and water management across the globe.

1.2. Water quality reduction due to agricultural intensification

1.2.1. The need for healthy aquatic ecosystems

As an integral element of the biosphere, water quality is of paramount importance for healthy and balanced ecosystems in freshwater and marine environments, which are often seen as indicators of overall environmental quality (Defra, 2008). This is particularly due to concentrations of nutrients and their function in supporting primary producers (algae) (Smith *et al.,* 1999). Additionally, the wider environmental and economic necessity for good water quality stretches far beyond the healthy ecology of an ecosystem. For example freshwaters are abstracted for domestic or industrial use by water providers, all of which require treatment in order to meet specific quality requirements (DWI, 2009). Furthermore, there is now a growing recognition that aquatic systems provide numerous additional ecosystem services, such as tourism, leisure, game and commercial fisheries, all of which depend on healthy water resources (Pretty *et al.,* 2003)**.**

1.2.2. The problem of diffuse agricultural pollution

One of the primary threats to water quality comes in the form of increased loads of the fractions of N and P entering the aquatic environment through anthropogenic activities (EEA, 2012a). Nutrient loading of fresh waters (and subsequently coastal marine systems) has increased substantially in recent decades. For example in 2000, annual anthropogenic fluxes of terrestrial N and P into the North Sea totalled 761 Kt and 48.7 Kt respectively (EEA, 2005). The detrimental impact of this increased loading is now often associated with numerous reductions in water quality, with the potential to affect large areas of freshwater and marine aquatic ecosystems e.g. in the Baltic Sea (Boesch, *et al.,* 2008) and the Gulf of Mexico (Mitsch *et al.,* 2001).

The threat to water quality and ecosystems posed by nutrient transfer, have traditionally been associated with point source inputs to the environment (Carpenter *et al.,* 1998). Such sources may include continuous outflows from waste water treatment works and industrial activity, both of which may deliver concentrated effluents into aquatic systems at specific points in the landscape. However, recently diffuse or 'non-point' nutrient and sediment pollution from agriculture has been identified as the main driver of many water quality problems (EA, 2007; Ulen *et al.,* 2007; Wood *et al.,* 2005). These water quality issues have appeared to increase in line with the intensification of agricultural practices (Carpenter *et al.,* 1998). Such is the extent of this problem that 50-80 % of N and 25-75 % P loss into freshwater and marine ecosystems in Europe, may originate from agricultural sources (EEA, 2005). This situation is replicated in the UK, with it now estimated that 25 % of P and 60 % of NO_3 transfers to surface waters are derived from agricultural sources (Defra, 2009). Diffuse agricultural pollution also encompasses a wide range of potential pollutants including nutrients, faecal pathogens (FIOs), toxic compounds (e.g. pesticides/herbicides), fuel, heavy metals and sediment (Stevens and Quinton, 2009). The sources of these pollutants are unspecific in location and characteristics, and can include leakage from septic tanks, slurry storage, polluted runoff from farmyard and livestock areas (EA, 2007), together with direct inputs from fertiliser application, livestock defecation and transfers through field drainage (Heathwaite *et al.,* 2006).

The importance of agriculturally associated decreases in water quality has been amplified given that, in a majority of cases, the historically important 'point source' inputs are now effectively managed in the UK and Western Europe through wastewater treatment, and legal enforcement e.g. the EU Wastewater Treatment Directive (91/271/EEC) (Hunt *et al.,* 2004; Withers and Haygarth, 2007). In contrast, non-point or 'diffuse' pollution, can originate from multiple undefined sources across the landscape (EA, 2007; Ockenden *et al.,* 2014) and the mobilised pollutants may travel large distances between sources and receptors. As a result, diffuse pollution from agriculture and its impacts remain very difficult to monitor, quantify and establish specific source-receptor relationships within the environment (Edwards and Withers, 2007). Because of this, treating these water quality problems using traditional technological solutions designed for point sources is often extremely difficult, inappropriate and costly (Verhoeven *et al.,* 2006) and so requires alternative methods to management (Kay *et al.,* 2009).

1.2.3. Sources, forms and mobility of agricultural nutrients

Transfers of nutrients to watercourses are largely caused by excessive, inefficient or poor management of fertilizers and quantities applied in excess of crop requirements and high animal stocking rates (Bennett *et al.,* 2001). It is estimated that only 30-50% of applied N (Cassman *et al.,* 2002; Smil, 1999) and 45% of P (Smil, 2000) fertilisers are taken up and utilised by crops. Therefore, when nutrients are applied and not utilised immediately they are often vulnerable to mobilisation, mainly through rainfall-runoff and transportation to receiving waters (Edwards and Withers, 2008; Haygarth *et al.,* 2005). This transfer of agricultural pollutants from sources to receptors is now often viewed in terms of a source-deliver-impact continuum (Withers and Haygarth, 2007), in order to target potential problems and solutions in catchment management.

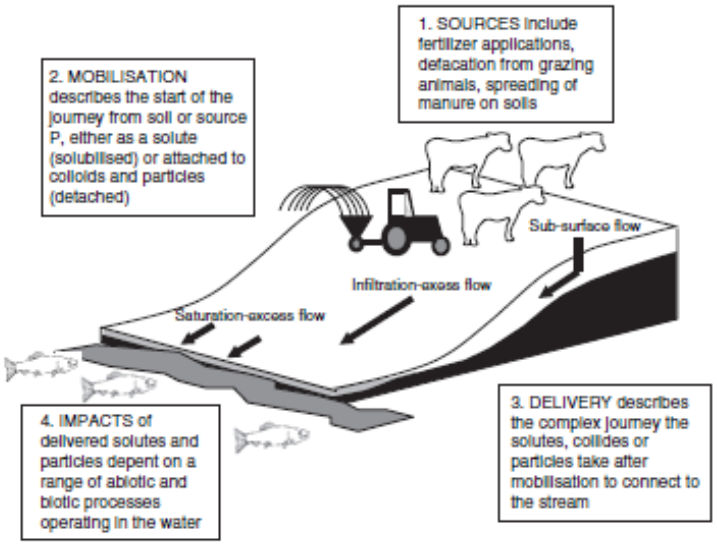

Figure 1.1 The transfer continuum of diffuse nutrient pollution (Withers and Haygarth, 2007)

When at excess quantities in agricultural soils, nutrients have variable susceptibilities to mobilisation and transport. Nitrate has a high degree of solubility and thus significant mobilisation potential via leaching from soils (EA, 2005). Furthermore, even when utilised at optimum application rates for crop uptake, nitrate may still be leached (Bhogal *et al.,* 1997). This inherent ability to be easily mobilised results in nitrate being highly problematic with respect to diffuse agricultural pollution (EA, 2005), particularly as it also permits movement vertically from the soil surface, through the unsaturated zone and into the saturated zone. Therefore, nitrate represents a particular threat to groundwater quality (Withers and Lord, 2002; EA, 2005), making it one of the most problematic contaminants (Jackson *et al.,* 2008).

NH_4-N, another key component of inorganic N fertilisers, is less mobile in the environment due to its affinity to bind with clay particles in agricultural soils (Vymazal, 2007). Its lower solubility means that it is often preferred to nitrate fertilisers due to being less vulnerable to leaching. Over time, the NH_4-N is converted to NO_3-N through microbial nitrification, where it can then be utilised by crops. This property has led to the promotion of slow release ammonium fertilisers over nitrate (Baltic Deal, 2015). However, as a source of inorganic N, the transfer of ammonium-bound to particles or through subsequent nitrification means that it is still an important potential source of N pollution.

In contrast to nitrate, P readily binds onto other materials in the environment. Even the most mobile and bioavailable form of P, orthophosphate (PO_4), has a high affinity for sorbing with clays in geological matrices and forming metal complexes with Fe and Al oxides (Holman *et al.,* 2008, 2010; Reddy *et al.,* 1999). However, although less prone to leaching, the ability to bind onto particles means that phosphorus is often mobilised in conjunction with soil erosion and rainfall-runoff, thus entering aquatic environments in a sediment bound form (Sharpley *et al* 1994). To illustrate this, phosphorus has historically been applied at high rates in agricultural systems and has accumulated in soils yearly (Carpenter *et al.,* 1998). As a result, P export to surface waters increases linearly with soil P content (Sharpley *et al*., 1996). Therefore, transfers of P to watercourses are now inextricably linked with sediment movement (Withers and Sharpley, 2008; Mainstone *et al.,* 2008). This is particularly an issue given that 70 % of sediment transferred to rivers in the UK is derived from agricultural sources (Howarth, 2011).

Because of the widespread, highly variable and intermittent nature of diffuse nutrient pollution, remedial management techniques are becoming increasingly focused on targeting the source (e.g. reduced application rates, animal stocking rates), limiting mobilisation (e.g. by using cover crops) and transfer (e.g. through the use of edge of field mitigation techniques) (Cuttle *et al.,* 2007; Newell Price *et al.,* 2011). In the case of P, land management practices also focus on the movement of sediment due to the strong association with P losses. Examples of techniques include sedimentation traps and wetlands designed to capture predominantly particulate pollutant fractions (Ockenden., *et al.,* 2014).

1.3. Negative impacts of nutrient pollution in aquatic systems

1.3.1. Eutrophication of surface waters

Excesses of (the fractions of) terrestrial N and P (particularly nitrate and phosphate) delivered into aquatic ecosystems can lead to pronounced nutrient enrichment. The primary effect of this can be the development of eutrophic conditions (Smith *et al.,* 1999), where the combination of carbon (C), P and N (i.e. the Redfield ratio) may majorly enhance the growth of the phytoplankton community, producing algal blooms. These blooms can be common in the summer months, which is the main algal growing season, when warm conditions in surface waters are ideal for rapid algal biomass increase (Parker and Maberly, 2000). The resultant stresses on the aquatic ecosystem may lead to the reduction in species diversity and even the development of 'blue-green algae' (or cyanobacteria), which can be highly toxic to fish and livestock drinking untreated water from affected watercourses (Smith *et al.,* 1999). The cost of removing harmful toxins from drinking water supplies for humans and livestock through treatment, together with additional economic costs such as through loss of tourism can be substantial (Pretty *et al.,* 2003).

The development of eutrophic algal blooms is also strongly linked to increased hypoxic or anoxic conditions in lakes and coastal regions (Diaz and Rosenberg, 1995; Rabalais *et al.,* 2010; Boesch *et al.,* 2008), where respiration of the enhanced primary producers or chemical processes consumes oxygen greater than it can be replaced in the water column (Thibodeau *et al.,* 2006; Friedrich *et al.,* 2014). This may have significant detrimental impacts on the local biota when oxygen levels are reduced below 2 mg L^{-1} (Diaz and Rosenberg, 1995; Rabalais *et al.,* 2010) and thus create dead zones in a water column.

As P is often the key limiting nutrient for primary productivity in many freshwater systems (Mainstone and Parr, 2002; Dils and Heathwaite, 2000), it is often considered a greater threat to overall water quality and thus forms the basis of Water Framework Directive (WFD) chemical water quality assessments (Defra, 2014). However, there are also instances where N can be limiting or co-limiting (Smith *et al*., 1999) in aquatic systems and so should be considered alongside P when tackling water quality issues.

1.3.2. Contamination of groundwater aquifers

While surface water quality is often the main area of concern for scientists and water authorities, groundwater pollution is potentially a much more significant and problematic issue, given that in some regions groundwater abstractions make up a large proportion of potable water resources. For example, approximately a third of UK water supply (70 % in South East England) is abstracted from groundwater sources (Shand *et al.,* 2007).

Drinking water quality is associated with nitrate (NO_3) due to its potential health impacts (EC, 2002), notably methaemoglobinaemia (blue baby syndrome) (Townsend *et al.,* 2003). Historically it was thought that the condition was caused by the reaction of NO_3 ingested by infants, decreasing the oxygen carrying capacity of haemoglobin in the blood and thus leading to brain damage (Fewtrell, 2004). Although the evidential basis for the condition is mixed, the uncertainty of the health risk is recognised, resulting in an EU and World Health Organisation drinking water nitrate limit of 50 mg NO_3 L^{-1} or 11.3 mg L^{-1} NO_3-N (EU Drinking Water Directive 98/83/EC; Heathwaite *et al.*, 1993; EA, 2007, WHO, 2011). Therefore, treating potable water carries significant cost implications to water companies and subsequently the consumers, amounting to £16.4 million yr^{-1} in the UK between 1992-1997 (Dalton and Brand-Hardy, 2003). Again, the potential for interaction with agriculturally derived nitrate and groundwater makes it particularly problematic. It is thought that the 25% of groundwater area in Europe of poor WFD status (Fig 1.2) is attributable to high NO_3 concentrations (EEA, 2012b).

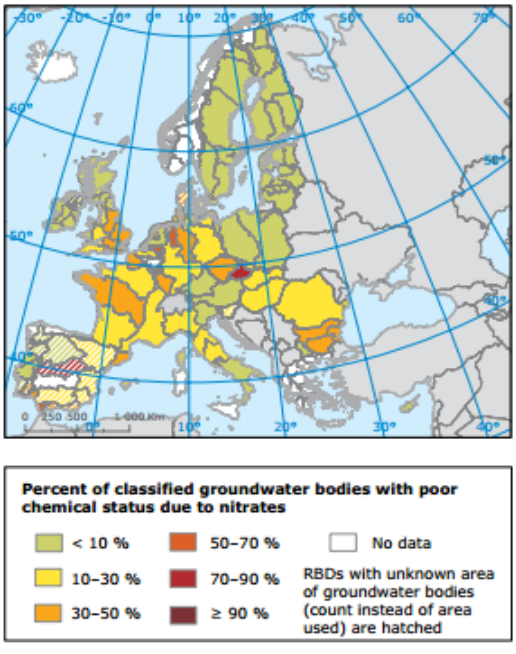

Figure 1.2
European groundwater bodies with 'poor' WFD status due to nitrate concentrations. (EEA, 2012)

Nutrients in polluted groundwaters may also contribute to surface water eutrophication problems, as they seasonally provide base flow to many surface streams and rivers (Shand *et al*., 2007; Edwards and Withers, 2008) i.e. in summer when catchment rainfall inputs are low. This may increase nutrient loadings when concentrations in water bodies may already be high, and so the chances of blooms are already often increased. Therefore, it is still important to consider groundwater as an important receptor, as well as a potential driver of surface water quality issues (Holman *et al.,* 2008).

The combined factors of health risk and ecological impact from an excess of N and P have thus warranted groundwater quality to be specifically protected under the EU Water Framework Directive and Groundwater Daughter Directive (Shand *et al.,* 2007; EA, 2006; Withers and Lord, 2002). With both surface and groundwater quality of major concern, their inextricable connection to one another and their evermore enshrined status in European legislation, the need to manage these resources within catchments is clear. Given the huge management challenge this presents, new and novel ways of reducing diffuse nutrient pollution are required.

1.3.3. Indirect greenhouse gas production from agriculture

Agricultural practices are also a major contributor to global anthropogenic greenhouse gas emissions (Fig. 1.3), emitting 5.1-6.1 Gt CO_2e equivalents in 2005. This amounted to 10-12% of total anthropogenic GHG contributions (Smith *et al.,* 2007). In the UK, agriculture is responsible for a similar proportion of total GHG fluxes, accounting for 9% of emissions (Defra, 2011).

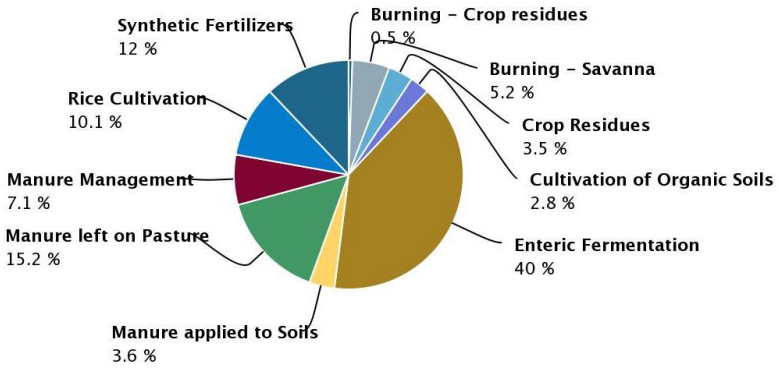

Figure 1.3 Sources of GHG emissions from global agricultural practices (FAOSTAT, 2015).

Direct GHG emissions are predominantly composed of methane (CH_4) releases via anaerobic fermentation from livestock and the handling, storage, and spreading of their manure on the landscape (FAOSTAT, 2015). The other major contributor to total emissions are direct releases of nitrous oxide (N_2O), which is produced as an intermediary substance in the incomplete denitrification of synthetic fertilisers (Defra, 2011) to N_2 under anoxic conditions in soils and sediments (Mosier *et al.,* 1998). Nitrification of inorganic N can also result in N_2O being produced, but to a much lower extent (Firestone and Davidson, 1989).

There is also increasing concern with regards to 'indirect' GHG emissions from agriculture, where in addition to direct emissions from soils, waste and livestock, nutrients leached or transferred to surface and groundwaters may also contribute to total landscape fluxes which are often overlooked in budgets. These emissions, such as N_2O production from the denitrification of nitrate (Mosier *et al.,* 1998; Reay *et al.,* 2003; 2009), may occur after fertiliser or animal waste has been transported into the aquatic environment e.g. into irrigation ditches and rivers. As a result, over 90% of N_2O emissions in river and estuary environments are thought to derive from anthropogenic inputs to aquatic systems (Seitzinger *et al.,* 2000).

This places a double emphasis on reducing the transfers of nutrients from agricultural land to receiving waters, both due to detrimental impacts on water quality and potential contribution to GHG emissions. Because of this, farming advice e.g. 'Codes of Good Agricultural Practice' (Defra, 2009) specifically include methods for farmers to reduce GHG emissions from their land. This is particularly being addressed by improving the efficiency of practices and the handling of nutrient and waste streams, i.e. utilising methods to limit the application of fertilisers (Defra, 2009). The realisation that mitigative land management strategies can play a significant role in reducing both surface/ground water and indirect GHG pollution, makes it imperative that the net impacts on water and atmosphere environments are not detrimental.

1.4. Approaches to the management of diffuse water pollution in the UK

The complex and challenging nature of diffuse agricultural pollution requires different strategies to managing water quality to those traditionally employed to tackle point source pollution problems. This has involved taking an integrated catchment based approach to

identifying and managing water quality issues (Dawson and Smith, 2010; Defra, 2013a). This thinking has highlighted the need to tackle diffuse pollution at the source rather than attempting to treat the receptor, giving rise to the increasing emphasis on land management for water quality management. In the UK, the threat of diffuse agricultural pollution, together with wider pressures on the water environment, have been addressed using a three pronged approach in an attempt to maximise the effectiveness of action. These strands are in the form of regulation, voluntary advice and action, and incentives in the form of agri-environment schemes, all of which overlap to some extent.

1.4.1. Regulation: The EU Water Framework Directive, Nitrates Directive and Cross Compliance

EU Water Framework Directive

The primary tool for protecting surface and groundwater quality is regulation through the Water Framework Directive (WFD) (2000/60/EC), an overarching piece of innovative legislation that sets a foundation for achieving specific EU-wide water quality standards. The primary aim of the WFD is to achieve 'good chemical and ecological status' in all surface and groundwaters by 2015, and 2027 at the latest (EA, 2009b). It utilises specific chemical and ecological water quality standards to form the basis of a management framework (Defra, 2014; EA, 2008). By using the all-encompassing WFD to utilise and harmonise both existing and new legislation, such as the 1991 EU Nitrates Directive (91/676/EC) and the Groundwater Daughter Directive (2006/60/EC), more effective pollution control is hoped to be achieved. As of 2009, only 26 % of water bodies in England were at 'good status' (Defra, 2011b) and it is currently recognised that it is unlikely that the stringent targets set by the WFD will be met by 2015 or even by 2027, using the technology currently available (EA, 2009). As a result the UK may incur severe financial penalties (Howarth, 2011).

In the UK, the implementation of the WFD is devolved to the environment agencies of the constituent nations. However, in all cases, a catchment based approach to pollution issues is at the heart of the directive, utilising River Basin Management Plans (RBMPS) as the operation elements for chemical and ecological water quality assessment and control (EA, 2006). It is recognised that a wide range of human activity in the landscape will impact on water resources, involving numerous land owners, interest groups and both public and private organisations, and so stakeholder participation is key to the function of each RBMP

(Defra, 2013a; EA, 2006). Therefore management strategies must balance hydrological, environmental, social and economic priorities accordingly (EA, 2006). These plans are continuously reviewed and refined on 6 year cycles to account for the continual changes in the needs of catchments and stakeholders.

The EU Nitrates Directive

Running alongside and in addition to the WFD is the Nitrates Directive (91/676/EC), newly enforced in England as the Nitrate Pollution Prevention Regulations 2015 (Defra, 2015a). As with the WFD, this scheme aims to monitor and manage water quality with respect to specific standards. Unlike the WFD, the Nitrates Directive is focused on controlling and reducing the concentration of NO_3 in surface and groundwaters, specifically recognising its potential implications for health and as a driver of eutrophication. In the UK, this has been achieved with the designation of Nitrate Vulnerable Zones (NVZs), areas where their waters are already eutrophied, are exceeding or are in danger of exceeding the drinking water quality standard for nitrate (11.5 NO_3-N mg L^{-1} or 50 NO_3 mg L^{-1} (EA, 2005) regardless of if they are a potable water source. As of 2013, 61 % of land in England, 14.2 % in Scotland and 2.3 % of land in Wales was designated as being in an NVZ (Scottish Government, 2014; Welsh Government, 2015). Additionally, the entirety of Northern Ireland was covered under the 'Nitrates Action Programme' in 2007 (NIEA, 2015). The area of land in England, Wales and Scotland within NVZs is continually reviewed every four years (Defra, 2015a).

In order to comply with the directive, all farms which are located within an NVZ must adhere to the Codes of Good Agricultural Practice (CoGAP) (Defra, 2009) and are inspected annually by the devolved regulatory body e.g. the Environment Agency in England. These codes include strict limitations on the rates and timing of fertiliser/organic manure application, as well as closed periods when risk of nutrient mobilisation may be highest (i.e. winter). Additionally, farmers must keep records of their farm activities such as fertiliser or manure applications for 5 years and meet strict requirements on the quality and capacity of animal waste storage (Defra, 2013b). In addition, the NAP guidelines adopted by Northern Ireland extend the focus of these guidelines from predominantly N management, to also specifically include P in planning (NIEA, 2015).

'Cross Compliance'

The final regulatory element in the UK is through 'Cross Compliance'. The reformation of the EU Common Agricultural Policy (CAP) in 2005 uncoupled agricultural subsidies from production (EA, 2007), instead requiring farmers and land managers to keep land in 'Good Agricultural and Environmental Condition' (GAEC) in order to receive payments (Defra, 2015b) and meet broader EU requirements of environmental practices and animal welfare. The most recent incarnation of this is the Basic Payment Scheme (BPS), a successor to the single payment scheme (SPS) (Defra, 2015b). The seven broad GAEC practices include the establishment of riparian buffer strips, maintenance of soil organic matter and regulation of substances harmful to groundwater (Defra, 2015b). In addition, farmers must comply with Statutory Management Requirements (SMRs) including the reduction of water pollution in NVZs (SMR1), strict use of pesticides (SMR 10) and the welfare of livestock (Defra, 2015b). Unlike the SPS, 30 % of BPS also requires the adoption of 'Greening' measures, such as crop diversification, reinstatement of permanent grassland and ecological focus areas (EFAs) on arable land over 15 ha (Defra, 2015b).

Adherence to the cross compliance regulations is ensured through random visits to 1 % of farms claiming BPS, by representatives from the Rural Payments Agency or Animal and Plant Health Agency (Defra, 2015b). Farmers are expected to produce records and demonstrate the suitability of their practices e.g. fertilizer storage, field activities and inspection of water abstraction licences. Failure to meet the specific requirements of the farmers' contract results in reductions in payments, in line with the severity of the offence, whether breaches are through negligence or intentional, and the repetition of previous offences. Ultimately, up to a 100 % fine and ban from future BPS payments (Defra, 2015b) may be imposed. However, while regulation of land management through this financial mechanism seems effective in theory, it is very difficult to fully monitor, particularly with the increasingly limited resources of the authorities responsible.

1.4.2. Guidance: Catchment Sensitive Farming, Codes of Good Agricultural Practice, Fertiliser Manual

The second strand of management comes from advice and voluntary action on the part of farmers and land managers. Farmers are encouraged to maintain the Code of Good Agricultural Practice (CoGAP) (Defra, 2009) which are mandatory in NVZs, and include advice

for a range of issues (not just water pollution) from nutrient management plans to the storage and separation of farmyard water.

Additionally, farmers can make use of the RB209 Fertiliser Manual (Defra, 2010), which provides a broad range of guidance and good practices in relation to the use of inorganic fertilisers and animal manures. This includes appropriate rates and timings of application in different farm types, fertiliser selection, management plans and ways to ensure accurate application. This may help to reduce unwanted excesses and mismanagement of nutrient applications.

Lastly, farmers in England and Wales within 'priority catchments' which are particularly vulnerable to diffuse agricultural pollution, are able to sign up to the Catchment Sensitive Farming (CSF) scheme. This is a rural development initiative run between Defra, the Environment Agency and Natural England. The CSF scheme provides voluntary guidance and training, primarily through one to one visits by appointed Catchment Sensitive Farming Officers (Natural England, 2013) to identify potential issues relating to water and nutrient management and improve them. CSF also provides training through workshops, demonstrations and farm events on nutrient, water, and pesticide management (Natural England, 2013).

1.4.3. Financial incentives: Agri-Environment schemes

Financial incentives in the form of agri-environment schemes are the final strand to agricultural land use for environmental and water quality management in the UK. They heavily cross over with regulation through cross compliance. Through these voluntary schemes, farmers are awarded continuous or single capital payments to undertake specific environmental or rural development, which go beyond the requirements for the BPS. Currently (2015), voluntary agri-environment schemes have undergone a radical change in England and Wales, replacing several focused incentive schemes with a single broad one. The Countryside Stewardship Scheme (Natural England 2015a), is the successor to the Entry and Higher Level Stewardship schemes as well as capital grants under the Catchment Sensitive Farming programme. It is divided into 244 land management options spread over three tiers, mid, higher (Natural England 2015b) and water capital grants (Natural England 2015c).

Mid-tier stewardship gives 120 options which are focussed on reducing diffuse water pollution from agriculture and improving farmland for wildlife. Higher tier stewardship has access to the full suite of options and goes above and beyond the requirements in mid-tier, though requires considerably more planning assistance. Both tiers are now competitively awarded to farmers based on a scoring system. Capital grants are available on a limited number of options in both mid and upper tiers, and provide single payments for the construction of certain features. Capital grants are targeted and only available in priority catchments through the Catchment Sensitive Farming Programme (Defra, 2015c). One of the newest additions to the list of mitigative options are the construction of agricultural wetlands (referred to in Countryside Stewardship guidelines as Ponds and Sediment Traps RP7) (Defra, 2015c), a mitigation tool designed to trap mobilised nutrients and particularly sediment-bound fractions, prior to delivery into a valuable receiving water. Further guidance on the construction of these simple and multi-role systems is offered by the Wildfowl and WetlandsTrust (Mackenzie and McIlwraith, 2015).

Although uptake of the new agri-environmental format is not yet known, the wide uptake of mitigation features and management under the preceding ELS and HLS schemes suggests that the numbers of mitigation features in the landscape will increase substantially in the near future.

1.5. Mitigation of diffuse nutrient pollution using constructed wetlands/sediment traps

Constructed wetlands for the mitigation of diffuse pollution utilise existing technologies adapted from their original applications in order to make them more suitable for use in an agricultural environment, while preserving the key functionality. This also helps to reduce costs and simplify operation, in order to maximise their uptake potential by farmers or land managers. This has transformed constructed wetlands from a heavily engineered and highly regulated wastewater treatment method, to a simple, basically engineered and cheap land management technique that can be implemented on a wide scale in the landscape.

1.5.1. Previous successes of treatment wetlands to reduce micro-point nutrient pollution

Natural wetlands are hotspots for carbon and nutrient cycling in the environment, with numerous aerobic and anaerobic biogeochemical exchanges transforming, immobilising or removing key nutrients. Because of the nutrient cycling and storage potential of natural wetlands, over the previous decades they have been adopted for human use in treating wastewaters with high nutrient and pollutant loadings (Vymazal, 2011). This is because constructed wetlands can be specifically designed for certain tasks and as such may utilise a number of different designs to optimise the removal of target pollutants. The principal forms of wetland are Vertical Flow (*VF*), Horizontal Subsurface Flow (*HSSF*) and Free Water Surface (*FWS*) wetlands (Kadlec and Wallace, 2009). Because of this adaptability, these 'treatment wetlands' have been used to treat wastewaters from a wide variety of sources including effluent from aquaculture, mining and industry (Tilley *et al.,* 2002; Verhoeven *et al.,* 2006; Zhang *et al.,* 2010). However, they have been most commonly employed in the effective treatment of small quantities of municipal wastewater (Cooper *et al.,* 1996; Kadlec and Wallace, 2009; Johansson *et al.,* 2003, 2004; Søvik and Kløve, 2007; Mander *et al.,* 2005a,b). This is sometimes performed as the primary method of wastewater treatment, though more commonly as a secondary or 'polishing' phase following primary contaminant removal and before release into the environment (Søvik and Kløve, 2007).

All analogous wetland systems, whether natural or used for nutrient treatment purposes, rely on the principal that they can sustain a higher level of biological, physical and chemical activity than other ecosystems (Kadlec and Wallace, 2009; Stefanakis and Tsihrintzis, 2009). As such nutrients, together with sediment and other potentially harmful substances (including pesticides, hydrocarbons and metals) are retained and used in generating biomass (mainly vegetation in the form of reed beds) or sediment (Stefanakis and Tsihrintzis, 2009; Kadlec and Wallace, 2009). Therefore nutrients and particulates are principally removed through sedimentation under gravity and filtration through the vegetation network, which together retain most suspended sediment and organic matter (Vymazal *et al.,* 1998; Cooper *et al.,* 1996).

Because of the affinity for phosphorus to sorb onto particles or precipitate in metal complexes, it is also mainly removed in wetlands though sedimentation as well as via plant uptake (Dunne and Reddy, 2005; Reddy *et al.,* 1999; Braskerud, 2002b). N removal in a

wetland is more complex, and may undergo several changes in a wetland system. In anaerobic wetland zones, organic N can be mineralised into inorganic N in the form of NH_4-N which is then nitrified aerobically to NO_3-N (Saeed and Sun, 2012). NO_3-N may also undergo dissimilatory reduction to NH_4-N in anaerobic carbon rich (Kadlec and Wallace, 2009) and NO_3-N limited environments (Laanbroek, 1990). Both NO_3-N and NH_4-N may be utilised in generating plant biomass (Saeed and Sun, 2012). However, the primary N removal process (90%) Xue *et al.,* 1999) is microbial anaerobic denitrification of NO_3-N to N_2 or N_2O gas (Bachand and Horne, 2000; Reddy & Patrick, 1984; Sun *et al.,* 2003; Vymazal *et al.,* 1998).

Efficiencies of pollutant removal from effluent vary greatly. Retention or removal of nutrients may depend on a variety of biological, hydrological and physicochemical influencing factors including effluent retention time, soil composition, temperature, oxygen availability, redox potential and pH (Reddy & Patrick, 1984; Stumm & Morgan, 1996; Vymazal *et al.,* 1998).

1.5.2. Adaptation of constructed wetlands for amelioration of agricultural runoff

Following the successful use of treatment wetlands to mitigate point source effluents and their long term capacity for retaining nutrients (Verhoeven et al., 2006), they have since been adapted into tools to mitigate diffuse agricultural pollution from farmland, while providing additional ecosystem services (Mackenzie and McIlwraith, 2015; Carty *et al.,* 2008; Vymazal, 2011). Constructed agricultural wetlands (also termed farm constructed wetlands, nitrogen farming wetlands and field wetlands) can now be found across temperate agricultural landscapes (Braskerud, 2002; Braskerud *et al.,* 2005; Diaz *et al.,* 2012; Fleisher *et al.,* 1994; Johannesson *et al.,* 2011; Milhollon *et al.,* 2009), although they have been used most prolifically in the Scandinavian region, in large scale wetland implementation programmes (Hoffmann and Baattrup-Pedersen, 2007; Thiere *et al.,* 2011). Additionally, smaller wetland designs (0.025-0.1% catchment area) have been tested in the UK (Ockenden *et al.,* 2012). These wetland systems are much smaller than their European counterparts in order to conform to the style of agriculture in the UK. The small size of these wetlands enables them to be placed in unused or unproductive areas of land, thereby minimising their impact on farm yields and maximising their uptake appeal.

The 'source' term in the pollutant transfer conceptual model has been tackled by both 'treatment wetlands', which act as an end-of-pipe tool for the treatment of polluted

wastewater, and 'in field' land management options such as cover cropping or reduced fertilizer application rates (Cuttle *et al.,* 2007). While both aim to minimise pollutant concentrations before release into the environment, agricultural wetlands aim to mitigate diffuse agricultural pollution by disrupting the transfer pathway. They do this by intercepting incidental losses of rainfall-runoff mobilised pollutants and sediment, prior to them reaching the main watercourse, placing an emphasis on removing the particulate or sediment bound fractions of nutrients by gravitational settling in a water column (Carty *et al.,* 2008). In many respects, they fulfil a similar function to buffer strips, however in addition, they can be located to intercept outfalls from field drainage, irrigation ditches/streams and farmyard runoff - thereby increasing the potential for capturing mobilised pollutants. Also, in-field source targeted mitigation measures may have highly variable success rates (Deasy *et al.,* 2009) and cannot be necessarily relied upon as the sole method of mitigating pollutant mobilisation and loss. In this respect, farm wetlands function as a fail-safe for when other methods fail (Ockenden *et al.,* 2014).

Agricultural wetlands may take a variety of forms including ponds, reed beds and ditches, with most designs being site specific. However, the construction is inherently simple, so they may be installed and maintained with minimal time and financial cost to farmers - differing considerably from treatment wetlands (Kadlec and Wallace, 2009). Many designs feature permanent water columns of approximately 0.5 m depth to allow settling of particulate material (Carty *et al.,* 2008), although in some cases deeper sediment traps of 1.5 m depth can be included where necessary (Mackenzie and McIlwraith, 2015; Ockenden *et al.,* 2012). Other aspects of wetland design may be dictated by the type of diffuse pollution to be intercepted. For example, those specifically designed to handle concentrated effluents from livestock handling areas, termed four and five star systems by the Wildfowl and Wetlands Trust construction guide (Mackenzie and McIlwraith, 2015), require impermeable liners to separate them from the surrounding geology (Carty *et al.,* 2008). However this also results in increased construction complexity and cost. In contrast, lower complexity one to three star wetlands dealing with field-derived runoff, may lack lining materials in order to minimise costs. For example, 10 different unlined wetland designs were trialled across three substrate types (clay, sand and silt) during the Mitigating Options for Phosphorus and Sediment (MOPS2) project (Ockenden *et al.,* 2012). Agricultural wetlands also lack features which regulate the timing or volumes of discharge in or out of the wetland system.

The performance of agricultural wetlands are therefore highly variable. In addition to factors affecting processes in parallel treatment wetlands, efficiencies of agricultural wetlands are heavily affected by the high variability, loading and timing of hydrological and chemical deliveries and thus residence times (Stevens and Quinton, 2009). Therefore, agricultural wetlands are often less effective than their more heavily engineered counterparts (Jordan *et al.,* 2003). Nutrient removal efficiencies can vary greatly but are generally moderate at 35% total phosphorous (TP) and 26 % of NO_3 (Stevens and Quinton, 2009), while a more recent systematic review of wetlands treating agricultural wastewaters (Newman *et al.,* 2015) reported removal efficiencies of 81 % total phosphorus and 15.4 % (median) for nitrate. Because of this, wetlands appear to represent a promising tool for mitigating diffuse agricultural pollution.

1.6. The need for an integrated view of water quality management: Pollutant swapping

Pollutant swapping is a relatively new concept and one which complicates water quality management through the use of mitigative features. Pollutant swapping is the terminology given to the phenomenon where in an attempt to reduce one or a suite of pollutants, an increase in the quantity or availability of others may occur (Stevens and Quinton, 2009). This includes potential loss of pollutants via another transfer pathway to that originally targeted by the original mitigation option design. For example, while a pollutant in its particulate fraction such as sediment bound P may be transferred by predominantly surface pathways and thus retained within a wetland, transformation into the dissolved phase (e.g. SRP) may 'swap' the dominant transfer mechanisms to those in the subsurface. Furthermore, complex biogeochemical cycling may permit the release of reaction products or intermediaries to the atmosphere. Thus 'swapping' can refer to exchanges of pollutants, chemical forms, physical fractions and transfer mechanisms. As such, there is now concern that mitigation options may be a source of pollutant exchange within already vulnerable ecosystems. Therefore, there is a danger that we swap a local scale surface water quality issue for increased groundwater pollution and contribute to the global problem of increased GHG emissions.

This presents a huge challenge for catchment management in the UK, given that at the present time, there is no accounting for linkages between pollutants and resulting prioritising action (Surridge, *et al.,* 2011). A recent study by Stevens and Quinton (2009)

highlighted swapping behaviour in several commonly practised mitigation techniques, suggesting that the issue may affect many of the current actions to combat diffuse pollution, particularly due to the promotion of many mitigative tools in the afore mentioned agri-environment schemes. An example of this, is that during a recent review of the potential for wetlands to mitigate diffuse nutrient pollution (Newman *et al.,* 2015), while recommending the use of agricultural wetland systems, there was no consideration for the potential of pollutant swapping with groundwater or atmospheric pathways. With respect to the already significant water quality issues in both surface and groundwaters, and also agricultural greenhouse gas release, this area of swapping is in urgent need of investigation.

1.7. Pollutant swapping of nutrients to local ground and surface water in analogous wetland systems

Consideration of pollutant swapping between a constructed wetland and groundwater has received very little attention in the literature (Brauer *et al.,* 2015). However, swapping of dissolved nutrients with a groundwater system or increased leaching of nutrients has previously been identified in analogous mitigation options such as riparian buffer zones (Spruill, 2000; Stutter *et al.,* 2009). As constructed wetlands and riparian buffers share many key biogeochemical processes which enable their success as mitigative devices, it can be hypothesised that there may also be potential for constructed wetlands in agricultural systems to be sources of pollutant swapping with ground/surface water. The numerous processes potentially occurring within constructed agricultural wetlands and impacting on the export of substances can be visualised through a conceptual model (Fig1.4).

Material trapped within wetland sediments is not necessarily immobilised, and may be subject to re-release at later points in time. Evidence supporting this issue stems from some studies noting the seasonal release of nutrients and especially P as a result of vegetation decay, where bio-available nutrients are released back into the water column (Vymazal *et al.,* 1998). Wetland sediments also promote the generation of anaerobic and reducing conditions, under which it has been noted that formerly particle-bound P may be potentially re-mobilised, through the reduction of Fe (III) to Fe (II) (Braskerud *et al.,* 2003; Surridge *et al.,* 2007). As such there are concerns that constructed wetlands may potentially become sources of P in the future (Braskerud *et al.,* 2005). It has also been found that in some cases aquatic NO_3 concentrations may be increased after flowing through riparian and constructed

wetlands (Newman *et al.,* 2015) due to the aerobic nitrification of trapped N. Under anaerobic conditions N may also be preferentially mineralised into NH_4 (Vymazal, 2007) and so could potentially be exported from sediments in this form. The interaction between trapped substances and physicochemical conditions affecting wetland nutrient cycling in the surface and groundwater, are therefore key to understanding the potential for nutrients to be removed or exported and remobilised. However, unlike engineered treatment wetlands, the low complexity of agricultural wetlands results in them having a greatly reduced ability to control these conditions, which may therefore vary greatly both spatially and temporally.

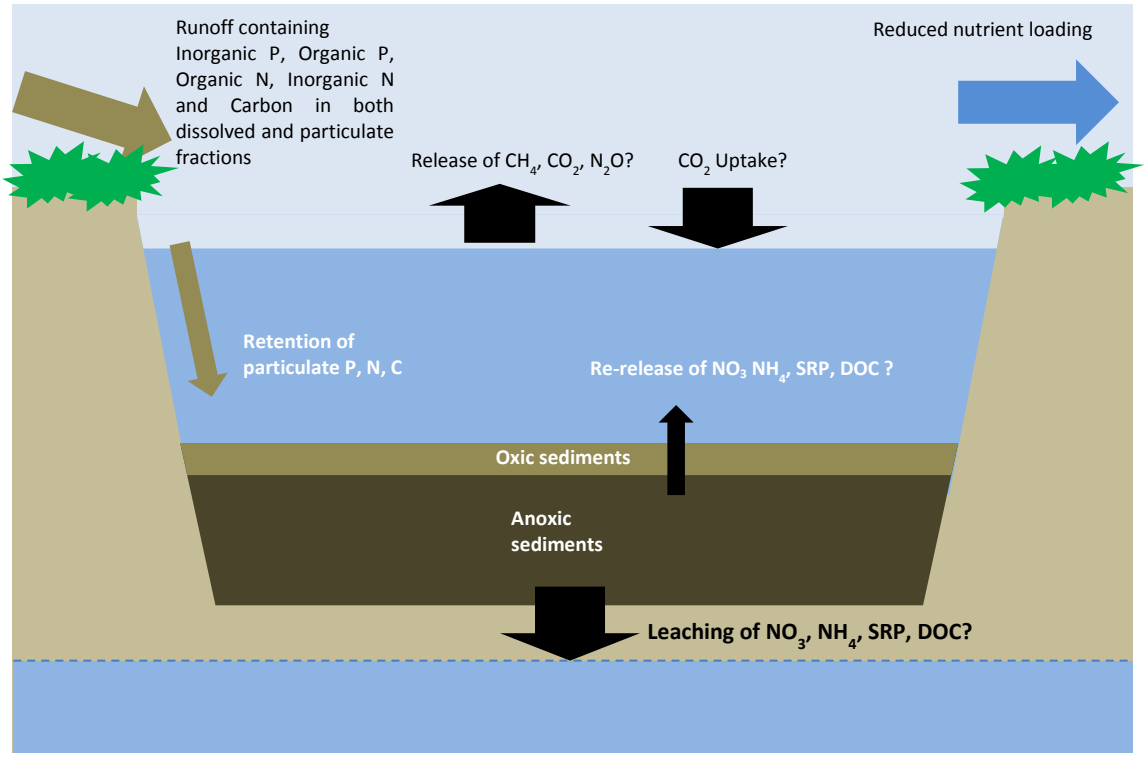

Fig. 1.4 A simplified and generalised conceptual model of the processes which commonly occur within a constructed wetland and where this may lead to the occurrence of pollutant swapping

In order to simplify construction and to minimise cost, the lack of lining using bentonite clay or synthetic membranes (Kadlec and Wallace, 2009) means that agricultural wetlands are not isolated from the surrounding groundwater. Seepage pathways between unlined wetlands and local groundwater have (intentionally) facilitated lateral nitrate transfer with surrounding groundwater (Larson *et al.,* 2000), and so it is feasible for dissolved fractions of nutrients to be transferred through advective or dispersive mechanisms. In order to

understand the dynamics of these mechanisms, an appreciation of the hydrogeological relationship between the wetland shallow groundwater in the uppermost regions of the underlying aquifer needs to be established (Surridge *et al.,* 2007).

Negative hydraulic gradients between the wetland and groundwater i.e. a recharge relationship could promote the outward movement of water and dissolved contaminants to lower piezometric surfaces, possibly even leading to 'mounding' of the water table beneath a wetland (Kadlec and Wallace, 2009). However, the hydrological relationship between surface and groundwater is unlikely to be unidirectional as flow regimes may be highly temporally variable through the year, thus favouring outward pollutant swapping during one period while hindering it in another. The inherently event-driven nature of agricultural wetlands (Ockenden *et al.,* 2012) means that temporal variations in relative water levels and supply of nutrients may also be important factors affecting the types and rates of biogeochemical cycling and the efficiency of substance retention and export. For example, high loadings of nutrients delivered during storm events to a wetland in Norway were largely unmitigated, due to the shortened retention time of storm waters, resulting in inadequate opportunities for biogeochemical cycling (Braskerud, 2001). Given that a complete understanding of agricultural wetland behaviour has yet to be fully constrained (Reinhardt *et al.,* 2005), the potentially wide use of these installations in the agricultural landscape, and the implications for groundwater and surface water swapping, the threat from export of aquatic nutrients from constructed agricultural wetlands must be clarified.

1.8. Potential pollutant swapping of greenhouse gases from constructed agricultural wetlands

Agricultural wetlands share many biogeochemical processes with parallel natural and engineered systems currently in the environment, such as natural wetlands, ponds and lakes, in addition to engineered treatment wetlands. In many cases within these systems (detailed below), the internal biogeochemical turnover of substances has generated potentially significant fluxes of greenhouse gas to the atmosphere. Due to the parallels with agricultural wetlands, GHG emissions must also be included in the conceptual model of potential wetland pollutant swapping (Fig 1.4).

Establishing the potential for constructed agricultural wetlands to act as sources of GHGs is crucial at a time of increasing concerns regarding CO_2, CH_4 and N_2O concentrations in the atmosphere. Moreover, the number of wetlands in the landscape may increase significantly as a result of agri-environmental incentives and advice schemes such as Countryside Stewardship (Natural England, 2015a), potentially increasing net GHG budgets. Numerous factors may potentially control the production and transfer of CO_2, CH_4 and N_2O within wetland environments and so an appreciation of these mechanisms is essential when considering their potential for pollutant swapping.

1.8.1. Agricultural wetlands as potential methane sources

Methane (CH_4) is a greenhouse gas, the atmospheric concentration of which has increased from 722 ppb (1750) to 1803 ppb in 2011 (IPCC, 2013) due mainly to increasing emissions from anthropogenic activity, such as agriculture. With a lifetime of 12.4 years and a global warming potential (GWP) of 34 times that of CO_2 (100 year time horizon with climate carbon feedbacks), (IPCC, 2013), CH_4 currently accounts for 20-40 % of anthropogenic climate forcing (Houghton *et al.,* 2001). Therefore, CH_4 emissions are highly significant due to their potential impact on climate change and potentially represent an important offset of terrestrial sinks (Bastviken, 2011). As such, constraining the uncertainty with regards to the magnitude of fluxes and additional sources is essential.

CH_4 is microbially produced during the breakdown of organic carbon under anaerobic conditions by methanogens (Conrad, 1996) and is a common feature of wetland and waterlogged systems where these conditions manifest. Natural wetlands are a globally significant store of terrestrial carbon (Kayranli *et al.,* 2010; Whitting and Chanton, 2001), containing 15 x 10^{14}kg of C (Schlesinger, 1991). Therefore, they represent the largest natural source of CH_4 to the atmosphere (Cicerone and Oremland, 1988) emitting 177–284 Tg CH_4 yr^{-1} (IPCC, 2013). In addition, lakes and rivers contribute a further 8-73 Tg CH_4 yr^{-1} (IPCC, 2013).

Anthropogenic activity has added to this already huge natural source, particularly by the growing of rice in flooded paddy fields (Cicerone and Oremland, 1988; Sha *et al.,*2011). More recently, the parallel organic rich and waterlogged characteristics of constructed wetlands designed to treat domestic and industrial waste water have prompted investigations of the potential CH_4 emissions to the atmosphere (Johansson *et al.,* 2003, 2004; Mander., *et al.,* 2005, 2008; Søvik and Kløve, 2007; Stom *et al.,* 2007; Tanner *et al.,*1997; VanderZaag, *et al.,* 2010). Evidence from these engineered systems strongly suggests that they function as

localised but also spatially and temporally variable hotspots for CH_4 release. Additionally, transfers from FWS wetlands with similar designs to agricultural wetlands, may be particularly high in relation to the material captured within them (Mander *et al.,* 2014a).

While these treatment wetlands have received substantial research attention, relatively little has focused on CH_4 (or GHG) fluxes from agricultural wetlands. However, a limited number of studies conducted mainly in Scandinavia (Søvik *et al.,* 2006; Stadmark and Leonardson, 2005), the USA (Groh *et al.,* 2015) and additional reviews of simply engineered constructed riverine wetlands (Mander *et al.,* 2014b; Sha *et al.,* 2011), all suggest the potential for substantial but highly variable releases of CH_4. Interestingly, Mander *et al.* (2005) posited that all the worlds' domestic wastewater could be treated by wetlands for only a 1 % increase in trace gas emissions. However, Mander *et al.* (2014b) later warned that the rapidly increasing numbers of FWS and riverine constructed wetlands meant they needed to be managed in ways to constrain their emissions. This would suggest that, despite earlier assertions, CH_4 emissions from less heavily regulated installations such as agricultural wetlands could become issues in the future. Furthermore, the release of CH_4 from wetland environments is in stark contrast to the mainly drained characteristics of agricultural soils, which can be atmospheric sinks for CH_4 (Conrad, 1996; Topp and Pattey, 1997; Mosier *et al.,* 1991). As agricultural wetland construction is targeted at the conversion of unproductive edge of field sites (Ockenden *et al.,* 2012), this therefore implies that potential CH_4 sinks may be converted into potential sources.

CH_4 production in flooded sediment environments is regulated by numerous environmental factors which may vary substantially. Firstly, methanogenesis requires a plentiful supply of available carbon from organic matter as well as substrates such as H_2 (Roy and Conrad, 1999), while rates of microbial activity and CH_4 production are also affected by temperature (Bartlett and Harriss, 1993; Crill *et al.,* 1991; Kiene, 1991; Schutz *et al.,*1989). Thus CH_4 emissions may vary seasonally (Duan *et al.,* 2005; Stadmark and Leonardson, 2005).

As methanogenesis is an anaerobic process, the presence of O_2 in sediments inhibits organic matter degradation to CH_4 (Conrad, 1996) by action as a preferential electron acceptor. In addition, the movement of CH_4 through aerobic sediments or water permits oxidation by methanotrophic bacterial communities (King *et al.,* 1990), reducing net CH_4 release. However, in riverine wetland systems with similar designs to agricultural wetlands, where a water column is maintained above sediments and the ingress of O_2 limited, CH_4 fluxes may be increased (Altor and Mitsch, 2008). Similarly to O_2, N fractions and reaction products such

as NO_3-N, may also act as suppressant to CH_4 production by acting as redox buffers or through toxic effects on methanogens (Achtnich *et al.,* 1995; Boon and Mitchell, 1995; Clarens *et al.,* 1998; Kluber and Conrad 1998a,b; Roy and Conrad, 1999; Stadmark and Leonardson, 2005). Therefore, there may be a potential for N rich agricultural wetland systems to inhibit wetland CH_4 production (Stadmark and Leonardson, 2005; 2007).

CH_4 may be swapped from aquatic environments to the atmosphere via three pathways: bubble ebullition, diffusion of dissolved gas and diffusion via rooted macrophytes (Bastviken*., et al.,* 2004; Chanton and Whiting, 1995; Macintyre *et al.,* 1995; Schutz *et al.,* 1989). Bubble ebullition (the formation of gas bubbles in sediments) is often the dominant transfer pathway (Chanton and Whiting, 1995; Bastviken *et al*., 2004; Huttenen *et al*., 2003; Casper *et al.,* 2000) as the rapid movement of bubbles through the water column minimises oxidation opportunities (Bastviken *et al.,* 2004; Chanton and Whiting, 1995).

In contrast, CH_4 transported by molecular diffusion from sediments to the atmosphere via the water column may be subject to oxidation prior to emission (Bastviken *et al.,* 2004; Casper *et al.,* 2000; Harrits and Hanson, 1980; Rudd and Hamilton, 1978). In deeper lake environments, large water column depths means this may oxidise a large proportion of diffusive CH_4 fluxes (Rudd and Hamilton, 1978). Water column oxidation may be reduced in deep lake environments during periods of summer stratification (Bastviken *et al.,* 2004) when anaerobic conditions develop in bottom waters as a result of organic matter decomposition (Reddy *et al.,* 2005), such as during eutrophic conditions. This permits dissolved CH_4 storage in a water column which, when mixed on the breakdown of stratification, can produce large pulses of CH_4 (Fernandez *et al.,*2014; Michmerhuizen *et al.,*1996; Rudd and Hamilton, 1978; Strayer and Tiedje, 1978) in what is termed 'storage flux' (Bastviken *et al.,* 2004). However, the potential for oxidation and stratification mechanisms to effect pollutant swapping in the small wetland water column <2 m (Ockenden *et al.,* 2012) of a wetland are uncertain.

Pulses of increased gas release via these pathways are often elicited by perturbation to sediment via water currents (Bussmann, 2005; Chanton and Whiting, 1995; Christensen *et al.,* 2003; Joyce and Jewell, 2003; Murase *et al.,* 2005), reductions in air and water pressure (Casper *et al.,* 2000; Devol *et al.,* 1988, 1990) and wind shear (Keller and Stallard, 1994). The effects of these mechanisms to elicit CH_4 emissions from a constructed agricultural wetland are unknown. However, these wetlands are intended to intercept rainfall-runoff mobilised pollutants, and so are likely to be subject to variable flow regimes (Jordan *et al.,* 2003;

Ockenden *et al.,* 2012), intense storm inputs and variable water levels, suggesting that there may be potential to influence pollutant swapping activity.

1.8.2. Agricultural wetlands as potential nitrous oxide sources

Nitrous oxide (N_2O) is a major greenhouse gas due to having high global warming potential of 298 times that of CO_2, and long atmospheric lifetime of 121 years (IPCC, 2013), while additionally being linked to atmospheric ozone depletion (Ravishankara *et al.,* 2009; Solomon, 1999). Atmospheric N_2O has increased due to anthropogenic activity, from 271 ppb in 1750 to 324 ppb in 2011 (IPCC, 2013), therefore limiting its further increase is essential. N_2O is produced naturally in soils and sediments through multiple processes within N biogeochemistry: as an intermediary substance during the anaerobic denitrification of organic N to N_2 (equation 1.1) in low oxygen conditions with plentiful C and N supplies. N_2O production via denitrification can be promoted by suboptimal conditions (e.g. redox and pH) (Knowles, 1982) as this inhibits full denitrification to N_2. In particular, intermittent introduction of O_2 can stimulate increased N_2O production (Maltais-Landry *et al.,* 2009; Mander *et al.,* 2011) despite it being an anaerobic process.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \textit{Equation 1.1}$$

Nitrous oxide may also be produced as an alternative intermediary substance during aerobic nitrification of NH_4^+ to NO_3^- (Dundee and Hopkins, 2001) (equation 1.2), triggered by suboptimal oxygen conditions Wrage *et al.,* 2001:

$$NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^- \quad \begin{matrix} \nearrow N_2O \\ \searrow NO \end{matrix} \qquad \textit{Equation 1.2}$$

However, as less than 1 % of reaction product in nitrification is N_2O, denitrification is often the main formation process (Firestone and Davidson, 1989). Nitrifier-denitrification (Wrange *et al.,* 2001) can also be a source of N_2O in environments with low oxygen and organic matter but abundant NO_3 (equation 1.3). The process describes the oxidation of NH_4 to NO_2, which is then reduced to N_2 without producing NO_3.

$$NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \textit{Equation 1.3}$$

Anthropogenic activity has predominantly increased the emissions of N_2O through increasing the availability of N and particularly NO_3 through the application of fertilisers in agriculture. Because of this, the high concentrations of NO_3 (as it is energetically favourable for denitrifiers to utilise NO_3 rather than N_2O (Verhoeven *et al.,* 2006) in agricultural drainage waters are often associated with elevated fluxes of N_2O (Reay *et al.,* 2003; 2004; 2009). Additionally, mitigative land management options, receiving runoff with high NO_3 loadings and suitable carbon availability, such as buffer strips, have also been observed to emit substantial quantities of N_2O (Hefting *et al.,* 2003; Teiter and Mander, 2005; Uusi-kamppa *et al.,* 2000).

The growing demand for alternative methods for municipal and industrial wastewater treatment has prompted the increased use of engineered treatment wetlands, which may receive large quantities of carbon and N-based nutrients. The abundance of source material held within an anaerobic environment makes these systems ideal for intense rates of nitrification-denitrification processes (Knowles, 1982; Vymazal, 2007). Therefore, as observed with CH_4, constructed treatment wetlands have also been found to emit substantial but variable fluxes of N_2O (Fey *et al.,* 1999; Johannson *et al.,* 2003; Mander *et al.*, 2005a, 2008; Sovik *et al.,* 2006; Sovik and Klove , 2007; Stom *et al., 2007*; Teiter and Mander, 2005; Uggetti *et al.,* 2012; VanderZaag *et al.,* 2010). However, the treatment potential of these systems is thought to outweigh the gaseous emissions they release (Mander *et al.,* 2005; Mander *et al.,* 2011).

Despite this evidence illustrating that parallel agricultural waters, buffer strips and treatment wetlands may function as N_2O sources, very little in situ evidence has been gathered on N_2O releases from agricultural wetland systems. However, that research which is available, shows substantial variability in fluxes (Bedard *et al*., 2006; Stadmark and Leonardson, 2005; Sovik *et al.,* 2006). As these systems are placed in agricultural landscapes with variable degrees of N and NO_3 enrichment, further in situ observations should be made.

1.8.3. Agricultural wetlands as potential carbon dioxide sources

Carbon dioxide (CO_2) is the most abundant greenhouse gas in the atmosphere, which as a result of anthropogenic activity has increased from 278 ppm in 1750 to 390.5 ppm in 2011 (IPCC, 2013). CO_2 is produced through the aerobic decomposition of organic matter and

oxidation of CH_4 and, as a result, is emitted in all aerobic environments where there is abundant organic material.

Pollutant swapping of CO_2 emissions in constructed wetland systems has only been considered in a few cases (Liikanen *et al.,* 2006; Mander *et al.,* 2003, 2005, 2008; Søvik *et al.,* 2006; Teiter and Mander, 2005; VanderZaag *et al.,* 2010). As many treatment and riverine wetlands are vegetated, it is generally assumed that these systems sequester some or all CO_2 they emit into plant biomass (Mander *et al.,* 2008; Mitsch and Gosselink, 2007). However, as noted by Mander *et al.,* (2011), sequestered carbon may be easily re-emitted when in situ conditions shift.

Meanwhile, agricultural wetlands may maintain permanent or semi-permanent water columns which may not be planted with macrophytes communities from the outset. Therefore, they may share more similarities with pond, lake and reservoir systems in the environment, which are generally considered to be net sources of CO_2 to the atmosphere (Maberly *et al.,* 2012; St. Louis *et al.,* 2000). This is particularly the case as, similarly to agricultural wetlands, such environments are sinks for catchment organic matter (Duarte and Prarie, 2005; Sobek *et al.,* 2003) and so the amounts of CO_2 they produce may rely on the productivity of the catchments they serve (Maberly *et al.,* 2012). In contrast, the primary productivity of photosynthetic organisms in eutrophic lakes has also been posited as a potential mechanism to sequester atmospheric CO_2 (Balmer and Dowling, 2011; Pacheco *et al.,* 2013). As such, the nutrient enriched waters of agricultural wetlands may potentially support periods of eutrophic conditions and CO_2 uptake as well as emission.

Because of the numerous variable factors affecting aquatic systems, the contribution to CO_2 budgets is still relatively poorly constrained (Maberly, 1996; Transvik *et al.,* 2009) and uncertain (Raymond *et al.,* 2013). This uncertainty is compounded due to the fact that while a bulk of research has focused on large lake systems, 90 % of lakes have a surface area < 0.01 km^2 (Downing *et al.,* 2006) with a cumulative worldwide surface area of 0.8 million km^2. Therefore these systems are heavily underrepresented in local, regional and national budgets (Holgerson, 2015). As agricultural wetlands are analogous to these small inland waters, observations of CO_2 emissions (plus other GHGs and substances) would provide valuable information not only for potential impacts of constructed wetland installation, but help to constrain uncertainties regarding a much broader range of aquatic systems in the landscape.

1.9. Aims of Research

Constructed agricultural wetlands may share many parallels with engineered and natural environments. However, while key mechanisms driving pollutant swapping in these systems are relatively well understood, some key processes may differ in agricultural wetlands as a result of their unique physical, chemical and biological factors.

This study aims to assess the potential for pollutant swapping in constructed wetlands designed to mitigate agricultural sediment and nutrient pollution. The thesis evaluates how biogeochemical overturning of retained wetland material may lead to increased transfers of greenhouse gases to the atmosphere and leaching of solubilised nutrients to groundwater. This may imply that the net impacts of mitigative wetlands in agricultural catchments may require reconsideration. The specific objectives of this thesis are therefore to:

i) Evaluate the potential for field–scale constructed agricultural wetlands to act as hotspots of greenhouse gas in the landscape, quantifying the fluxes of CH_4, CO_2 and N_2O.

ii) Evaluate the presence and effect of physical and biogeochemical mechanisms observed in other parallel environments on pollutant swapping potential e.g. effects of ephemeral disturbance events, stratification, eutrophic overturning and O_2 variation.

iii) Assess the potential for agricultural wetlands to act as sources of pollutants to local groundwater systems.

1.10. Thesis Overview

The thesis combines field based and laboratory elements of research in order to address the objectives. The structure of the thesis is as follows:

Chapter 2: Details a 12 month field-scale assessment of the potential for agricultural wetlands to act as sources of solubilised nutrients (SRP, NO_3-N, NH_4-N and DOC) to surrounding shallow groundwater environments, utilising hydrochemical, physicochemical and hydrological techniques.

Chapter 3: Utilises an 18-month field study to evaluate whether the conversion of unproductive riparian land to an agricultural wetland significantly increases the fluxes of CH_4, CO_2 and N_2O to the atmosphere. The chapter also considers the implications for annual GHG fluxes on net wetland sediment C and N retention, gas transport processes and potential physical and chemical factors influencing gas flux rates.

Chapter 4: Details a 24-hour field experiment assessing the potential for eutrophically induced diurnal water column stratification to create anoxic/hypoxic conditions, and the effects this may have on GHG and nutrient release. Also building on observations in chapter 3, this is supplemented with two microcosm experiments replicating the impact of diurnal and week-long variations in O_2 concentration on sediment GHG and dissolved nutrient (SRP, NO_3-N, NH_4-N and DOC) release.

Chapter 5: Evaluates two sediment microcosm experiments replicating the effects of stormflow induced sediment disturbance on GHG and dissolved nutrient (SRP, NO_3-N, NH_4-N and DOC) fluxes. The microcosm also builds on observations in chapter 3 and 4, examining GHG and nutrient release may be affected by anoxic or oxic antecedent conditions.

Chapter 6: Summarises and discusses the key findings of previous chapters, and the implications of the research for constructed wetland usage. In addition, emissions estimates from chapter 3 and the literature are upscaled in order to estimate the potential cumulative impact of wetlands to the national level under various uptake scenarios.

Chapter 2 Pollutant swapping of nutrients between an agricultural wetland and local groundwater system

2.1 Introduction

Constructed agricultural wetlands are a simple, low cost and effective tool for mitigating diffuse water pollution from agriculture (Braskerud, 2002a,b; Braskerud *et al.,* 2005; Carty *et al.,* 2008; Ockenden *et al.,* 2012, 14; Diaz *et al.,* 2012). The potential for widespread use of these features is promoted through agri-environment schemes such as Countryside Stewardship (Natural England, 2015a), particularly within landscapes already vulnerable to nutrient pollution. Therefore, it is imperative that these wetlands do no exacerbate pollutant transfers. However, the intrinsically simple design of the wetlands, i.e. lacking lining materials such as impermeable synthetic or clay membranes as in treatment wetlands (Kadlec and Wallace, 2009), means that they are potentially connected to the surrounding local groundwater system. As a result, there may be potential for seepage pathways to permit pollutant swapping of nutrients and other pollutants derived from surface water, or retained sediments within the wetlands, directly into the local groundwater (Larson *et al.,* 2000; Dzakpasu *et al.,* 2012).

Despite the potential for this form of pollutant swapping with groundwater, as agricultural wetlands are a relatively new land management technique, studies examining the actual occurrence of pollutant exchange with groundwater in field-scale wetlands are very limited (Brauer *et al.,* 2015). However, export of nutrients from other mitigation features, such as riparian buffer zones/wetlands, to groundwater systems has previously been identified (Spruill, 2000; Uusi-Kamppa *et al.,* 2000). Given the many parallels between the biogeochemical processes operating in these systems and within agricultural wetlands, it can be hypothesised that there may also be potential for constructed wetlands in agricultural systems to facilitate pollutant swapping with groundwater.

The potential for pollutant swapping is driven by multiple biogeochemical processes, which may act upon substances within the wetland. Already solubilised nutrients e.g. nitrate (NO_3-N), ammonium (NH_4-N) and soluble reactive phosphorus (SRP) may be transferred to groundwater through advective or dispersive mechanisms in interstitial water (EA, 2005). Organic N in trapped sediments may also be mineralised through nitrification to NO_3-N (aerobic) and ammonification to NH_4-N, which may also be produced via dissimilatory

reduction of NO_3-N (anaerobic) (Vymazal, 2007; Saeed and Sun, 2012), and made available for aquatic transfer. Particle-bound phosphorus, may also undergo physical and chemical changes though dissolution in reducing conditions, to become remobilised (Braskerud *et al.,* 2003; Surridge *et al* 2007). These complex biogeochemical processes are controlled by a variety of physicochemical influencing factors including temperature, oxygen availability, redox potential and pH (Reddy & Patrick, 1984; Stumm & Morgan, 1996; Vymazal *et al.,* 1998). As such, observation of these parameters is essential for determining the nature of nutrient cycling and transfer between the wetland and groundwater systems.

Furthermore, to fully understand the dynamics of this form of pollutant swapping, the hydrogeological relationship between the wetland and the groundwater in the uppermost aquifer must be established (Surridge *et al.,* 2007). This is because the relative response of surface versus groundwater, to catchment inputs will govern the direction of pollutant flux between the wetland and groundwater. Ultimately this may determine the potential for pollutant export from the wetland. This is particularly true in constructed agricultural wetlands that are inherently event driven systems and experience a wide range and rapid fluctuation of water levels (Ockenden *et al.,* 2012). Therefore, in order to assess the potential for pollutant swapping with groundwater from an agricultural wetland, a multi-method hydrochemical and hydrogeological approach is required.

2.2 Aim

To assess the potential for pollutants trapped within agricultural wetland waters and sediments, to act as sources of pollution to local groundwater systems.

Hypotheses to be tested:

1. The constructed wetland will be a source of pollutants in local groundwater. Concentrations of pollutants in the shallow groundwater system will be similar to those in the surface water of the wetland.
2. The agricultural wetland will have a strong hydrogeological relationship to the surrounding shallow groundwater system.
3. Physicochemical conditions in the wetland and shallow groundwater will increase the potential for pollutant swapping.

2.3 Methods

2.3.1 Study site

The study site was located on a farm in the Eden Valley of Cumbria, UK (NY 495388; 54° 44' 30''N, 02° 47'09''W) as part of the 'Mitigation options for Phosphorus and Sediment 2' (MOPS2) project (Ockenden *et al.,* 2012), in which three field edge agricultural wetlands were installed to mitigate water quality issues. The catchment (Fig. 2.1) is dominated by an intense mixed farming system containing both pastoral (improved grassland for beef-cattle and sheep) and arable (rotation of potato, winter wheat, oilseed rape) land uses. The wetland drains an area of approximately 30 ha out of 50 ha in this farm system, although receives water from field drains incoming from outside this topographical area. As part of this runoff, septic tank effluent from a nearby cattle farm was also included until amendments were made in February 2012.

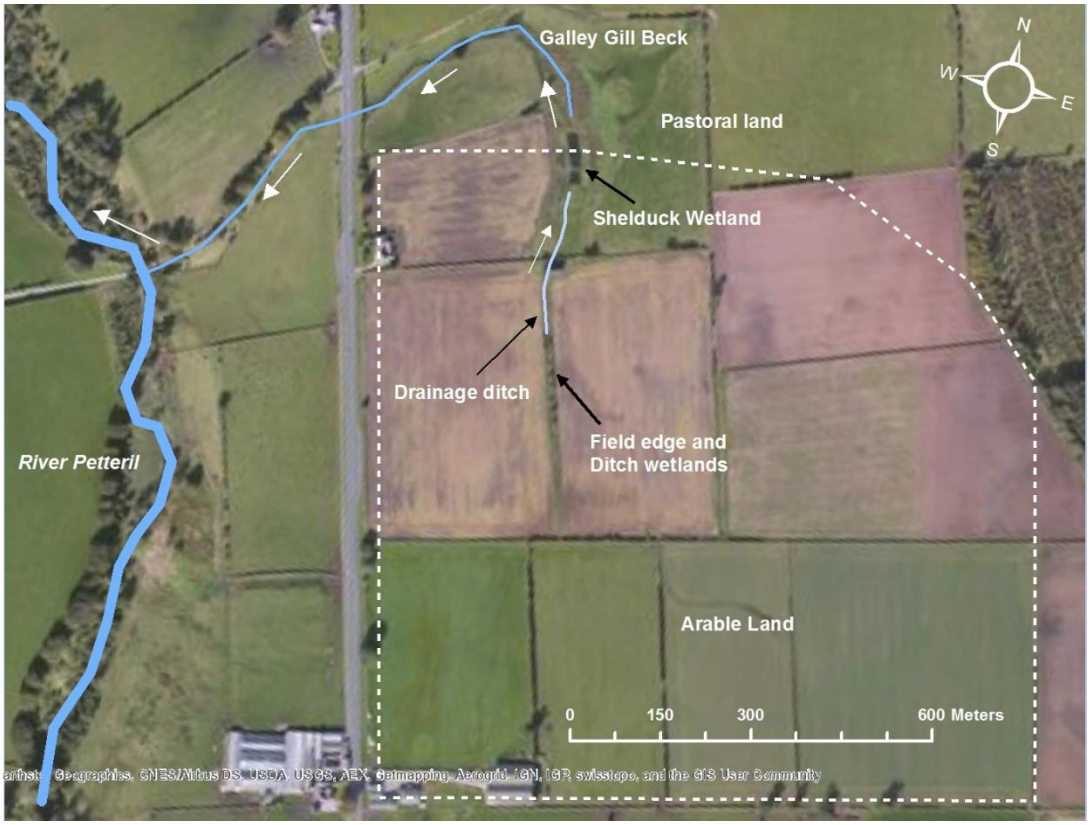

Figure 2.1 The Shelduck constructed wetland depicted within its catchment (dotted line). Land to the North and immediately East and South is intensive, mixed stock grassland. Arable areas of land higher up the catchment to the South are also shown together with the position of the two smaller wetlands which occupy the catchment. The area immediately around the main wetland is not accessible by livestock. The receiving waters of Galley Gill Beck and the River Petteril are also shown, white arrows indicate water flow direction.

The sub-catchment drains into Galley Gill Beck, a tributary of the River Petteril, which was recently upgraded from being 'poor' to 'moderate' Water Framework Directive (EU, 2000) status (Environment Agency, 2013). The Petteril is itself a tributary of the River Eden, which is used for water abstraction and is an important salmon and trout fishery (ERT, 2007). Mean rainfall for the area is 890 mm per annum. The catchment soils are from the Wick 1 Association (Jarvis *et al*., 1984), a Eutric Cambisol. The area has poorly sorted Quaternary deposits with high levels of sand lenses. These deposits are underlain by Permian Penrith sandstone, which is considered to be a major aquifer used for public supply (Allen *et al.,* 2010). The aquifer is also categorised as being at 'poor' status, due to elevated NO_3 and PO_4 concentrations, particularly from diffuse agricultural sources (EA, 2009a).

This study centred on the 'Shelduck' wetland (Fig 2.2, 2.5), the largest (0.1 % catchment area) and last in a series of 3 constructed in 2009/10. The wetland is of the free water surface (FWS) type with a double cell design. Water firstly enters into a deep sedimentation cell (deep cell) (8 m x 8 mx 1.5 m) (Fig 2.3) before flowing downstream into larger shallow cell (shallow cell) (8 m x 32 m x 0.5 m) (Fig 2.4). The shallow depth is to encourage vegetation (though not vegetated at the time of this study, as the macrophytes originally planted at the time of wetland installation did not survive). This gave a total wetland area of approximately 320 m^2. To minimalize costs, the wetland was unlined, with excavations made directly into the sandy Quaternary deposits. The wetland was fed by a field drain originating from an open drainage ditch into which the aforementioned field drains/septic runoff flowed (Fig 2.1). Due to this, the flow into the wetland was intermittent in nature. Also, backwater effects from low topography were apparent in all wetland flumes.

Figure 2.2 The Shellduck wetland looking North West (downstream) with the deep cell in the foreground and shallow cell in the distance.

Figure 2.3 The deep cell of the wetland looking West. The inlet flume is visible on the left and well WA visible in the centre of the picture.

Figure 2.4 The shallow cell of the wetland looking South (upstream). The outlet flume is visible in the bottom of the picture, with the in-wetland wells in the centre and the middle flume visible in the distance.

The Shelduck wetland was selected as a study site for several reasons. Firstly the wetland had been established for long enough to have stabilised after excavation and to have allowed a sufficient build-up of sediment to provide a substrate for likely greenhouse gas production. However the wetland was not of an age where maintenance sediment removal would be necessary during the observation period. The two-cell design would also permit observation of the impact of different wetland depths on gas and nutrient fluxes within the same site. Use of an unlined wetland built into highly permeable soils also provided potentially ideal conditions for outward transfer of pollutants to local groundwaters, allowing assessment of pollutant swapping in a 'high risk' scenario.

2.3.2 Surface and groundwater monitoring infrastructure

The wetland (Fig. 2.5) was surrounded by a shallow piezometer network (n = 11) consisting of 2 m and 4 m PVC tubes (32 mm o.d.), which were placed approximately 1 m out from the edge of the wetland excavation. The 4 m piezometers were fitted with sample lines constructed from plastic tubing (5 mm o.d.) at 1, 2 and 3 m below the ground surface. Each piezometer was vertically isolated from other depths using bentonite clay seals and capped with geotextile to prevent blockages. A further three piezometers - WA, WB and WC were located in the centre of the deep and shallow wetland cells, with sample lines extending to 0.5 m (approximately equal to 2 m perimeter well depth) and 1 m below the wetland base. The three in-wetland piezometers were each fitted with a Troll 500 (In-Situ. Inc.) non-vented groundwater logger to record hydraulic head at hourly intervals. A Baro Troll (In-Situ Inc.) barometric logger for air pressure corrections was also installed on site. Manual electronic dip-meter readings were made in both 2 m and 4 m perimeter piezometers each month. All piezometer locations were surveyed with a Trimble S6 Total Station (Trimble Navigation Ltd., Sunnyvale, California, USA) and then referenced to an arbitrary datum 10 m below the head of W1Pond level was recorded using a calibrated pressure transducer located in the middle flume (FM) due to this being most representative of overall wetland water level, which changed towards the wetland outlet due to backwater effects. Accurate measurements of water flow through the wetland could not be made due to the backwater effect distorting the stage-discharge relationship within gauging structures. As the wetland had a single inlet, changes in wetland water level within the flumes were used to indicate periods of water input to the system. Similarly, malfunction of the on-site rain gauge resulted in questionable quality of data, which therefore is not included.

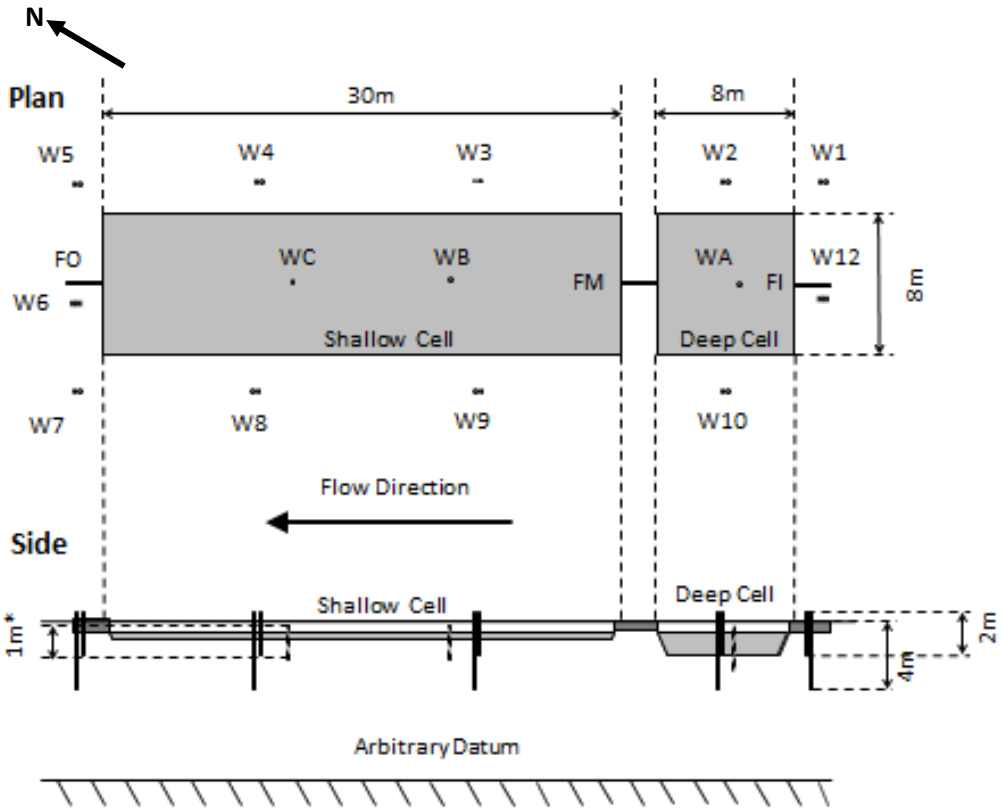

Figure 2.5 Plan and side profile view of the Shelduck wetland (shaded area). Plan view depicts the dimensions and layout of the well network including in-wetland piezometers. Side view illustrates the subsurface infrastructure and relative depths of deep and shallow ponds.* denotes that the in-wetland piezometers extend to 1 m below the respective pond base. i.e. WA extends 1 m lower that WB and WC. Inlet flume (FI), middle flume (FM) and outlet flume (FO) are also shown. All infrastructure is shown relative to an arbitrary datum located 10 m below the top of W1.

2.3.3 Groundwater sampling

Monthly water samples were collected from the piezometers for 12 months between July 2012 and July 2013, from both perimeter and in-wetland locations. Groundwater was extracted by attaching a 60 ml plastic syringe to a piezometer (Fig. 2.6) before drawing back and locking the plunger in place. The syringe then filled under the negative pressure over a period of several hours. Syringes were then removed and the samples passed through a 0.45 µm Nalgene® filter on site. The filtrate was then decanted into 60 ml centrifuge tubes before being placed in a cool box. Wetland water from the surface and sediment-water interface of each cell were collected on the same day. Groundwater samples from the parent aquifer were taken from the Brownrigg borehole and supplied via personal communication with the Environment Agency. Sampling methodology is not known.

Figure 2.6 Fitting of sampling syringe with a locking plunger to one of the nested piezometers around the wetland perimeter. Figure also shows additional sampling inlets at 0.5 m intervals which were not used during monitoring. Syringes were left in place until a sufficient quantity of sample was collected.

2.3.4 Physicochemical monitoring

Physicochemical parameters (dissolved oxygen, conductivity, temperature, redox potential, pH and total dissolved solids) were assessed on the same sampling occasions as groundwater collection using a HANNA H19828 multiparameter probe. The multiparameter probe was calibrated prior to each sampling occasion. Conductivity, temperature, dissolved oxygen and total dissolved solids electrodes were calibrated using the manufacturer's recommended calibration solution. The pH/ redox electrode was additionally calibrated using a three point calibration, with buffer solutions of pH 4, 7 and 10. Wells were prepared, by first purging all of the existing water within them using a peristaltic pump (Williams Ltd, UK). Simultaneously, argon (denser than air) was piped into well from a gas cylinder, in order to minimise oxygen ingress from the atmosphere (Surridge *et al.,* 2012). The well was then capped and allowed to refill. Physicochemical measurements were then made with a flow cell, set up in a closed loop system within the well, driven by the peristaltic pump. The equipment was purged using oxygen free N_2 prior to insertion.

2.3.5 Nutrient analysis

After being stored overnight at <5°C, filtered samples were analysed for orthophosphate (PO_4-P) to determine soluble reactive phosphorus (SRP). Analyses were carried out in accordance with US EPA method 365.1 for the determination of phosphorus by semi-automated colorimetry (O'Dell, 1993). The method utilised classical colorimetric chemistry (Murphy and Riley, 1962), whereby the phosphate reacts with acidic molybdate in the presence of antimony to form an antimony phospho-molybdate complex which is reduced by ascorbic acid to an intensely blue complex: phosphomolybdenum blue. The absorbance of

this complex is measured spectrophotometrically at 880 nm. This method was deemed as the most suitable method for the sample type and analytical instrument (AQ2+ discrete analyser, Seal Analytical, West Sussex, UK). The limit of detection for the method was 0.005 mg L^{-1}. Ammonia-N (NH_4-N) (HMSO, 1981) was also measured on the AQ2+ using classical colorimetric methods. Ammonia reacts with hypochlorite ions (from sodium dichloroisocyanurate) and salicylate in the presence of sodium nitroprusside, at a pH of approximately 12.6, to form a blue-green reaction product (thought to be related to indophenol blue). This is measured spectrophotometrically at 670 nm. The limit of detection for the method was 0.02 mg L^{-1}. Nitrate-N (NO_3-N) was measured by ion chromatography using a Dionex ICS2500 (Dionex Ltd., Camberley, UK). The limit of detection for the method was 0.023 mg L^{-1}. Dissolved organic carbon (DOC) was measured using a Thermalox analyser (Analytical Sciences Ltd., Cambridge, UK). The Thermalox measured DOC by catalytic thermal oxidation utilising a combustion and detection method described by BS ENV 1484 (BS ENV, 1997; Bartram and Ballance, 1996). All analysis was undertaken by the author.

2.3.6 Quality control

Numerous quality control measures were utilised during nutrient analyses. Calibration curves were derived using standards mixed from accredited reference solutions and performed automatically by the apparatus or by the author. Each sample batch included analytical blanks and two accredited working reference solutions at the beginning and end of each run. Additionally, a drift control made from a 50 % dilution of highest calibration standard (0.5 mg L^{-1}) was inserted every 10 samples, together with a triplicate measurement of one random sample. Four field blanks were also taken on each sampling occasion and the analyte concentrations subtracted from those in samples. Values below the limit of detection were substituted with a value equivalent to LOD/ 2 (USEPA, 2000).

2.3.7 Statistical analysis

Statistical analyses were conducted using SPSS® v 22.0 (SPSS Inc., Chicago IL, USA). Data was tested for normality and homogeneity of variance using Shapiro-Wilk and Levenes tests respectively. Although spatial heterogeneity of the superficial geological deposits was acknowledged, overall vertical changes in nutrient concentrations over space and time were anticipated to be greater, where nutrient transfer from the wetland/sediments to surrounding groundwater was occurring. Therefore, one-way ANOVA was used to assess differences between sample depths across all wells over the observation period. Where non-

normality was found, Log_{10} +1, Ln+1 or squared transforms were used. If non-normality persisted, nonparametric Kruskal-Wallis tests were used in place of ANOVA and supplemented with Dunn-Bonferroni non parametric post-hoc tests. Vertical differences in the piezometers 0.5 and 1 m below the wetland cells were assessed using unpaired t tests or Mann Whitney tests. Differences between physicochemical variables in 2 and 4 m wells across the observation period were assessed using unpaired tests. Relationships between substances or physicochemical variables were determined using Spearman's Rank order correlation. In all statistical tests, $p \leq 0.05$ was used to determine statistical significance. Outlying values were retained as they may be indicative of key wetland biogeochemical processes, particularly in such variable substrate.

2.3.8 Spatial distribution of groundwater data

The spatial distribution of groundwater nutrient concentrations at respective wells over the 12 months was depicted using in ArcGis 10.2. Interpolated concentration surfaces were not deemed suitable given the small size of the site, so the distribution of mean concentrations at 2 m depth, including in-wetland piezometers, is reported.

2.4 Results

2.4.1 Nutrient concentrations in groundwater and surface water

NO_3-N concentration (Table 2.1) in the surface water was generally below 10 mg L^{-1} although concentrations varied substantially over the 12 months. Highest concentrations were recorded during the autumn and winter, and concentrations around the limit of detection (0.023 mg L^{-1}) occurred during the summer months. NO_3-N within groundwater was generally below limit of detection at all depths, with the exception of piezometers in the south east corner of the site, where higher concentrations were recorded closer to the ground surface (Fig. 2.7). Kruskal Wallis testing with Dunn-Bonferroni post-hoc tests established significant differences in mean NO_3-N concentration with depth in the perimeter wells (X^2 (2) = 23.999, *p<0.001)*. The mean concentration at 1 m (0.46 mg L^{-1}) was significantly higher than that at 2 m (0.12 mg L^{-1}) (Dunn-Bonferroni, *p<0.05*) and 3 m (0.02 mg L^{-1}) (Dunn-Bonferroni, *p<0.001*)

Mean concentrations directly beneath the wetland, were lower than in the perimeter wells, although at times concentrations up to 0.41 mg L^{-1} were observed. No significant changes in

concentration with depth were apparent. All concentrations were substantially lower than those present in the parent aquifer (EA Personal communication, 2011).

Concentrations of NH_4-N (Table 2.1, Fig 2.7) were relatively low in the surface water of the wetland, with mean concentrations below 1 mg L^{-1}. However, concentrations in the deep cell were higher than those from the shallow cell. Mean NH_4-N was much higher in the groundwater, although wide ranges of concentrations were observed over the 12 month period. Kruskal Wallis testing with Dunn-Bonferroni post-hoc tests showed that concentrations changed significantly with depth in the perimeter wells (X^2 (2) = 31.036, *p<0.001*). In contrast to NO_3-N, it was apparent that NH_4-N increased down the vertical concentration profile, with the concentration at 3 m (2.52 mg L^{-1}) being significantly greater than at 2m (1.71 mg L^{-1}) (Dunn-Bonferroni, *p<0.05*) and at 1 m (1.59 mg L^{-1})(Dunn-Bonferroni, *p<0.05*). Mann-Whitney testing confirmed that NH_4-N concentrations in wells extending directly below the wetland cells decreased with depth (*p<0.001*), being significantly higher at 0.5 m (2.21 mg L^{-1}) than at 1 m (1.01 mg L^{-1}). NH_4-N concentrations were substantially greater in groundwater than in the surface water through the study.

Groundwater concentrations of SRP were highly variable (Table 2.1, Fig 2.7), ranging from below the limit of detection to similar concentrations as those observed in the surface water. Mean groundwater concentrations were low at all depths, although Kruskal Wallis testing with Dunn-Bonferroni post-hoc tests illustrated significant differences between depths (X^2 (2) = 24.932, *p<0.001*). Concentrations at 3 m (0.022 mg L^{-1}) were significantly less (than those at 1 m (0.042 mg L^{-1}) (Dunn-Bonferroni, *p<0.05*) and 2 m (0.064 mg L^{-1}) (Dunn-Bonferroni, *p<0.001*). Concentrations at 0.5 m (0.017 mg L^{-1}) and 1 m (0.036 mg L^{-1}) directly below the wetland were also low and did not display any significant difference with depth.

DOC was present at around 10 mg L^{-1} in wetland surface water throughout the year. Mean groundwater concentrations at 1 m depth were twice this (22.015 mg L^{-1}), although it was found that there was a great deal of spatial variation between sample points. Kruskal Wallis testing with Dunn-Bonferroni post-hoc tests revealed that DOC concentrations differed significantly at all depths (X^2 (2) = 118.692, *p<0.001*). Significant decreases in concentration were observed between 1 m (22.02 mg L^{-1}) and 2 m (12.95 mg L^{-1}) (Dunn-Bonferroni, *p<0.001*), and also between 2 and 3 m (6.45 mg L^{-1}) (Dunn-Bonferroni, *p<0.001*). Concentrations directly below the wetlands were similar to those in the surrounding groundwater. Mann-Whitney testing confirmed that DOC concentrations 0.5 m (8.69 mg L^{-1})

directly below the wetland were significantly lower than those at 1 m (13.60 mg L^{-1}) (*p<0.001).*

Table 2.1 Mean nutrient concentrations at the sediment-water interface and groundwater from the across observation period. Mean concentrations from 11 perimeter wells are grouped into 1, 2 and 3 m depths. In wetland piezometers are grouped in 0.5 and 1m depths below the wetland bottoms. Differences in superscript letters denote significant differences identified through Dunn-Bonferroni post-hoc testing. Deep and shallow cell bottom water nutrient concentrations are also displayed. 'Brownrigg' represents samples from the parent aquifer.

Chemical mg L^{-1}		**Mean**	**Min - Max**
DOC	**1m**	22.02 ± 1.62^{A}	1.52 – 90.63
	2m	13.07 ± 0.73^{B}	1.97 – 38.72
	3m	6.45 ± 0.37^{C}	1.36 – 18.30
	W 0.5m	8.69 ± 0.75^{A}	2.74 – 23.40
	W 1m	13.60 ± 1.99^{B}	3.34 – 70.52
	Deep Cell	10.02 ± 0.85	6.91 – 17.32
	Shallow Cell	12.19 ± 2.24	6.88 – 34.12
NH_4-N	**1m**	1.59 ± 0.14^{A}	0.01 – 8.29
	2m	1.72 ± 0.14^{AB}	0.01 – 7.35
	3m	2.52 ± 0.15^{C}	0.09 – 8.18
	W 0.5m	2.22 ± 0.22^{A}	0.00 - 5.46
	W 1m	1.02 ±± 0.17^{B}	0.00 - 4.74
	Deep Cell	0.75 ± 0.29	0.00 – 3.36
	Shallow Cell	0.19 ± 0.06	0.00 – 0.69
Brownrigg Borehole		<LOD	
NO_3-N	**1m**	0.45 ± 0.10^{A}	0.012 – 6.52
	2m	0.12 ± 0.04^{B}	0.012 – 3.92
	3m	0.02 ± 0.00^{BC}	0.012– 0.28
	W 0.5m	0.04 ± 0.01	0.012 – 0.39
	W 1m	0.04 ± 0.01	0.012 - 0.42
	Deep Cell	5.18 ± 0.89^{A}	0.012- 9.39
	Shallow Cell	4.90 ± 0.90^{A}	0.012 – 8.75
Brownrigg Borehole		14.1 ± 1.4	
SRP	**1m**	0.04 ± 0.00^{A}	0.00 – 0.29
	2m	0.06 ± 0.00^{AB}	0.00 – 0.34
	3m	0.02 ± 0.00^{C}	0.00– 0.16
	W 0.5m	0.02 ± 0.00	0.00 – 0.09
	W 1.0 m	0.04 ± 0.01	0.00 – 0.33
	Deep Cell	0.13 ± 0.02	0.003 – 0.23
	Shallow Cell	0.11 ± 0.01	0.003 – 0.17
Brownrigg Borehole		<LOD	

2.4.2 Spatial patterns in nutrient concentration

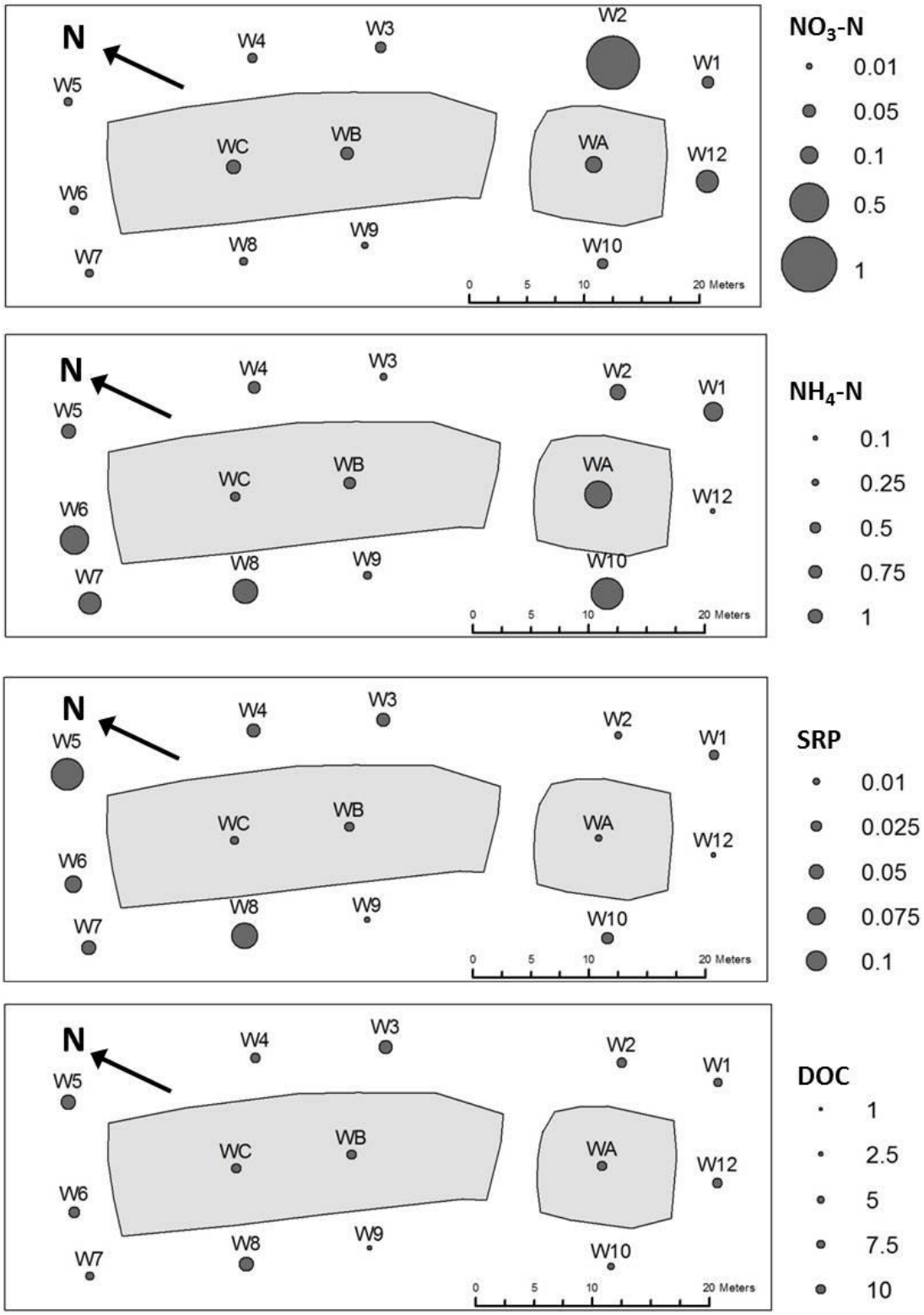

Figure 2.7 Spatial differences in dissolved nutrient concentrations at 2 m depth below the ground surface across the wetland site. Symbols are proportional to concentration (mg L^{-1}). Shaded regions represent the extent of the water surface area within the wetland cells.

Nutrients exhibited a great deal of spatial variation (Fig. 2.7). The concentration of NO_3-N was generally low. However, the greatest concentrations of 0.93 and 0.17 mg L^{-1} were present at wells 2 and 12 respectively, far exceeding all other points. NH_4-N displayed a greater level of spatial variation. A mean concentration of 3.63 mg L^{-1} was observed directly beneath the deep cell, however concentrations of a similar magnitude were also recorded in several perimeter wells across the site. Similarly, concentrations of DOC were high, although showed no consistent spatial pattern, with those beneath the wetland being of a similar magnitude to those in several perimeter wells. Concentrations of SRP were generally low in the groundwater system. The highest concentrations were observed in the perimeter wells rather than those in the wetland, with hotspots at wells 5 and 8.

2.4.3 Physicochemical conditions

Key physicochemical conditions (Table 2.2) were relatively stable both temporally and spatially. Dissolved oxygen was present at negligible concentrations in both 2 and 4 m piezometers with no significant changes with depth. Redox potential was strongly negative in 2 m and 4 m piezometers sets and displayed a wide range of values over time. However, there were no significant changes between depths. Mean temperature was marginally lower in the 4 m wells although the range of values was also smaller than that that in the upper groundwater. The pH of the groundwater varied substantially over time in both well sets. It was found that the pH was significantly higher in the 4m wells (t test, *p<0.005*).

Table 2.2 Mean and range of physicochemical variables measured in 2 and 4m perimeter wells. Differences in superscript letters denote significant differences between groups.

Physicochemical parameter		Mean	Min - Max
Redox (mV)	**2m**	-169.4 ± 12.79^{A}	-445.1 – 136.1
	4m	-178.97 ± 12.86^{A}	-435 – 35.8
DO (mg L^{-1})	**2m**	0.158 ± 0.04^{A}	0.00 – 2.72
	4m	0.123 ± 0.04^{A}	0.00 – 3.13
Temperature (°C)	**2m**	9.49 ± 0.31^{A}	4.45 – 19.88
	4m	9.44 ± 0.23^{A}	5.21 – 15.00
pH	**2m**	6.85 ± 0.06^{A}	5.98 – 9.15
	4m	7.04 ± 0.06^{B}	5.90 – 9.61

Physicochemical conditions in the surface water of the wetland (Chapter 3, Fig. 3.) were highly variable over the observation period and little affinity with the values observed in the local groundwater. Surface water physicochemical conditions displayed particular temporal patterns which appeared to coincide with the incidence of algal blooms in the wetland cells. During eutrophic algal blooms in spring and summer, dissolved oxygen concentrations were often at supersaturated levels at the water surface, while anoxic conditions were observed at the base of the water column, particularly in the deep cell. pH also varied substantially in both wetland cells, although exhibited elevated and stable values during March-April and June-July, when algal blooms were often observed. Temporal changes in redox potential did not display any consistent temporal pattern in either wetland cell.

2.4.4 Inter-variable relationships

Correlation analysis (Table 2.3) revealed significant positive correlations between concentrations of SRP and DOC at all depths in the perimeter wells, with a stronger correlation (r_s=0.65, *p<0.01*) observed at 2 m. This strong correlation with DOC was evident with NH_4-N at 1 m depth (r_s=0.45, *p<0.01*). NO_3-N was also negatively correlated with NH_4-N at 1 m (r_s= -0.42, *p<0.01*), 3 m (r_s= -0.17, *p<0.05*) and overall (r_s= -0.28, *p<0.01*). Correlation analysis with physicochemical variables revealed only two significant relationships, between redox potential and SRP (r_s= -0.25, *p<0.01*) and DOC (r_s=0.21, *p<0.05*).

Table 2.3 Spearman's Correlation coefficients between SRP, NO_3-N and NH_4-N and DOC in the perimeter wells around the wetland. Correlation between NH_4-N and NO_3-N is also displayed. Correlations between physicochemical variables in 2 m wells and nutrients in 2 m piezometers are also included. * denotes significance at the 0.05 level, ** significance at the 0.01 level.

		SRP	NO_3-N	NH_4-N	DOC
DOC	**1m**	**0.43****	-0.241	**0.45****	NA
	2m	**0.65****	-0.07	-0.104	NA
	3m	**0.40****	-0.01	0.166	NA
	All	**0.35****	0.06	**-0.12***	NA
NO_3-N	**1m**	NA	NA	**-0.42****	NA
	2m	NA	NA	-0.15	NA
	3m	NA	NA	**-0.17***	NA
	All	NA	NA	**-0.28****	NA
	Redox	**-0.25****	-0.12	-0.03	**-0.21***
	DO	-0.14	0.15	0.00	0.09
	Temperature	0.01	0.04	-0.01	-0.15
	pH	0.05	0.001	0.11	-0.07

2.4.5 Hydrogeological conditions

High frequency surface and groundwater water level data (Fig. 2.8), illustrated that head in both the wetland and groundwater were highest during the period between September 2012 (9.19 m above AD) and February 2013, and lowest in July 2013 (8.35 m above AD). The difference between peak storm levels and lowest summer levels was 0.8 m. Water depth could also vary substantially over short time periods, with the greatest increase being 0.61 m in less than 24 hours on 18/05/15 (Fig 2.8 inset).

There was generally a small negative hydraulic gradient of approximately 0.02 m/m between the wetland and the local groundwater (data not shown) driving advection from the wetland into groundwater, even during periods of high groundwater level. It was also apparent that this gradient was maintained even during storm conditions (Fig 2.8 inset). Furthermore, surface and groundwater head data illustrated that the groundwater directly beneath the wetland displayed an instantaneous response to surface water level change, with no lag-time observed between peak surface and groundwater levels.

Hydrogeological behaviour was highly complex across the study area over the observation period. Although dip meter measurements (data not shown) indicated that the shallow groundwater head varied substantially across the wetland and perimeter wells, there did not appear to be any consistent horizontal gradient. Similarly, the vertical gradient between 2 m and 4 m varied between positive and negative in adjacent wells. Generally, wells tended to display a positive gradient towards the groundwater surface during the late autumn-spring, with these gradients becoming much less significant towards the summer months.

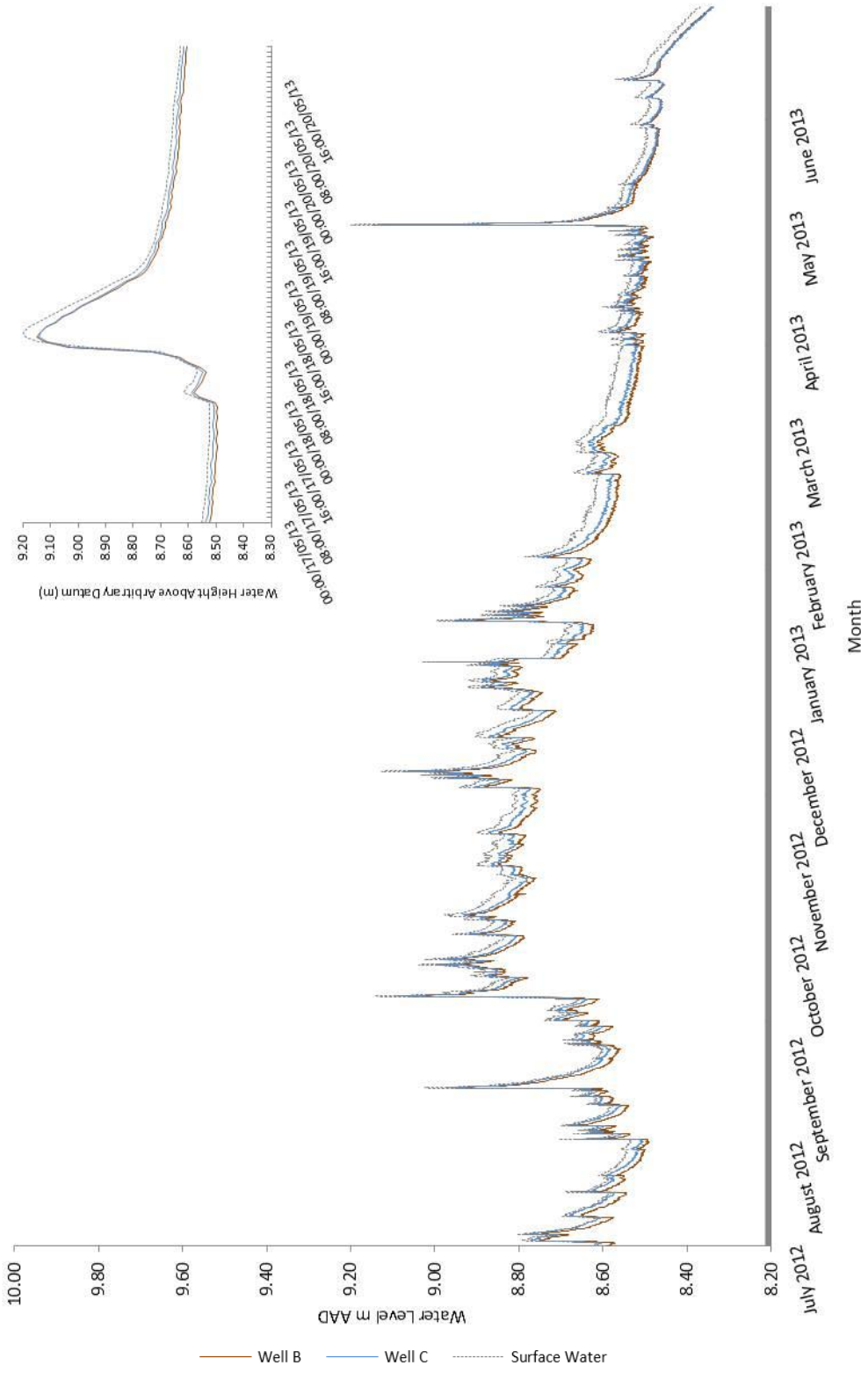

Figure 2.8 Hourly surface and groundwater level (above arbitrary datum) from July 2012-July 2013. Surface water level is derived from calibrated pressure transducer data taken from the middle flume due to this being most representatives for the site. Inset hydrograph shows surface and groundwater head change during a storm event.

2.5 Discussion

2.5.1 Mechanisms of pollutant transport

Groundwater transport processes are a key component of potential pollutant swapping in agricultural wetlands, particularly as pollutants may be transferred to local groundwater from the surface water, retained sediments, or from areas of in-situ production in superficial deposits. Hydraulic gradients between the surface water in the wetland and groundwater beneath, may have potentially promoted outward advective transfer of pollutants. Heightened NH_4-N concentrations in groundwater suggested this may have occurred. However, spatial differences in pollutant concentrations, pointed to a complex system of transport and in situ production/ transformation, which may have been influencing the quantities of observed pollutants.

The high frequency ground and surface water head measurements illustrated that there was a small but consistently negative hydraulic gradient between the wetland and the groundwater directly beneath. This was present even during the winter months when groundwater levels were high and indicated that a recharge relationship was continuously in operation throughout the study period, with the wetland potentially recharging the local groundwater system. Furthermore, the instantaneous response of the shallow groundwater to inputs from surface water, and near identical head to the surface water in the ponds, illustrated that the hydraulic connectivity of the wetland was very high. This may have been related to the permeable superficial deposits beneath the wetland. While the hydraulic conductivities of the sands, gravels and clays making up the deposits was not assessed in this case, substantial K values of around $10^{-6} – 10^{-4}$ cm s^{-1} (Fetter, 1999) may be likely in such deposits, and permit free transfer of water and pollutants. In catchment scenarios with differing underlying superficial geology such as finer silts or clays, permeability may be somewhat lower.

The net movement of water from wetland to surrounding porewater may be expected to promote advective transport of dissolved material, as has been identified in similar wetland systems (Larson *et al.,* 2000) and through the movement of deep groundwater and pollutants towards unbuffered surface streams (Spruill, 2000). Manual measurements of groundwater head in the perimeter piezometers illustrated the highly variable nature of the groundwater head distribution across the site, at times exhibiting positive gradients between deeper groundwater or surface water in the wetland. This indicated that the hydrogeology of

the site was highly complex. This was anticipated, given our prior knowledge of the variable nature of the superficial deposits in the study area. Over 75 % of the River Eden catchment is overlain with a complex mix of superficial Quaternary deposits containing sands, gravels, silts and clay lenses. The arrangement of these deposits may result in development of their own piezometric levels, potentially creating a complex mix of perched water tables above the parent sandstone aquifer (Allen *et al.,* 2010). The presence of such hydrogeological features implies that wetland-groundwater exchanges may be variable over small spatial scales.

Based on the observed and generally negative hydraulic gradient between the wetland and shallow groundwater, it was expected that there would be clear evidence for advective transfer of mobile pollutants. While elevated NH_4-N concentrations beneath the wetland appeared to show this, the lack of significantly higher concentrations of other target pollutants in the surrounding groundwater, suggested that the quantities observed may not be solely influenced by transport mechanisms. This was also evident at points when advective transfer may have been substantial, such as during large changes in surface/groundwater head during storm events, which could advect pollutants into groundwater e.g. May 2013 (Fig 2.8). Sampling of groundwater immediately after the event did not reveal any significant evidence for this. Similarly, the contrasting vertical patterns of some pollutants i.e. decreases in DOC and SRP with depth coupled with increases in NH_4-N, suggested that the effects of diffusion and dispersion (Fetter, 1999) mechanisms may also have been masked by other processes.

The lack of some target pollutants in the local groundwater and presence of others, or inverse vertical concentration profiles, implies that pollutant transfer may have been undergoing in situ transformations and interactions with the substances and conditions within the porewater. Moreover, some substances may be influenced differently to others. For example, NO_3-N may be highly susceptible to in situ denitrification or reduction, while NH_4-N is unable to be extensively nitrified in anaerobic conditions (Vymazal, 2007). The product of these transformations is a distortion of the signal observed in our monitoring network although, as will be discussed, it still potentially suggests pollutant swapping was occurring.

There was also a lack of similarity between concentrations of nutrients in the shallow groundwater, and those in the surface water or the parent aquifer. This suggested that pollutants in the local groundwater were not likely to be derived from either of these sources. This lack of connectivity with the aquifer was anticipated, given the significant

distance between the perched system here and the main groundwater level (approx. 60 m depth). As such, the pollutants observed in the local groundwater system are more likely to be derived from material in the wetland sediment or, as will be discussed, inherited from the surrounding shallow superficial deposits.

2.5.2 Nitrate and ammonium transfer, production and removal in sediments and groundwater

Agricultural wetlands are potential sources of NO_3-N, NH_4-N and DOC. However, swapping of NO_3-N from the wetland to surrounding groundwater aquifer, was potentially limited by removal through denitrification and reduction to ammonia in the local groundwater and retained sediments (Patrick and Reddy, 1976). In contrast, preferential ammonification of organic N, and nitrate reduction to ammonium increased the potential for swapping of NH_4-N to groundwater.

The mean surface water concentrations of NO_3-N during the study were in the range of those reported in other agricultural wetland systems (Braskerud, 2002a; Kovacic *et al.*, 2000). The background concentration in the parent aquifer (14.1 mg L^{-1}) was also of a similar magnitude. Despite this, shallow groundwater concentrations of NO_3-N across the wetland site (Fig. 2.7) and at all sampled depths (Table 2.1) were negligible (with spatial exceptions) even with the presence of hydraulic gradients supporting advection of wetland surface water into the shallow groundwater system. This suggested that NO_3-N was being removed from the system and its production suppressed by biogeochemical processes (Brauer *et al.,* 2015).

Further evidence to suggest that NO_3-N could be transferred to local groundwater but was being actively removed, is illustrated by the prominence of high concentrations in the groundwater at the south-eastern corner of the site (Fig 2.7). In this case, the high concentrations observed can be attributed to the development of a preferential flow pathway across the riparian zone surface. This was caused by extreme water logging of the surrounding field and bursting of a field drain from autumn 2012 onwards, causing NO_3-N rich water (grab sample data not shown) to flow around the outside of the wetland. The resulting elevated concentrations in the piezometers nearest to this zone, together with decreases in the concentrations with depth, illustrated that NO_3-N was being gradually removed. Furthermore, the marginally elevated NO_3-N concentrations directly below the wetland cells (Fig. 2.7) may be indicative of NO_3-N being transferred from surface waters via

advection but not being completely denitrified. Alternatively, nitrate in these locations could be produced at aerobic microsites within sediments.

In contrast to NO_3-N, the observed concentrations of NH_4-N in shallow groundwater were substantially higher than those in either the surface water of the wetland or the parent aquifer. Substantial concentrations of NH_4-N were present at WA (and to a lesser extent WB and WC), directly below the deep wetland cells. This was coupled with a significant decrease in NH_4-N concentration between 0.5 m and 1 m away from the wetland bottom, suggesting the presence of a concentration gradient. Therefore, export of NO_3-N from the wetland to local groundwater may have been taking place. Furthermore, similarly high concentrations of NH_4-N at 2 m below ground level in several piezometers surrounding the deep cell, may suggest that there was some vertical and lateral movement of ammonium to local groundwater. Export of NH_4–N from retained sediments is further supported by greater concentrations at the sediment-water interface than at the surface (Fig 3. Chapter 3). This implied the production and transfer of NH_4-N from the wetland sediments back into the water column and therefore suggests that pollutant swapping back to surface waters, as well as groundwater, may also occur in agricultural wetlands. Increases in release of NH_4-N during periods of algal bloom induced anoxia suggest that this may be a key contributor to incidents of pollutant swapping, as will be further discussed in chapters 3 and 4.

The strongly anoxic conditions which perpetuated in the groundwater below and surrounding the wetland, and at times at the base of the wetland water column, favoured NO_3-N removal (Patrick and Reddy, 1976). Denitrification was likely to be the dominant biogeochemical process with respect to NO_3-N, this being the main NO_3 removal pathway in many analogous wetland systems (Bachand and Horne, 2000a; Fisher and Acreman, 2004; Xue *et al*, 1999; Peterjohn and Correll, 1984), and being a reductive process, is initiated once oxygen has been exhausted (Reddy and Patrick, 1984, Vymazal *et al* 1998). Furthermore, physicochemical analysis illustrated that ideal conditions were present in the groundwater for denitrification to occur. Redox potentials were below 300 mV (Inglett *et al*, 2005) throughout the observation window and pH was at the optimum level (6.0-8.5) (Reddy and Patrick, 1984). Sampling also indicated that there was a plentiful supply of organic carbon in the subsurface (Table 2.1), which is essential for effective denitrification to occur (Taylor and Townsend, 2010). The origin of this DOC may have been from the glacial sands and gravels in the superficial deposits, however it is more likely that it was also derived from material retained in the wetland sediments. In this case, the agricultural wetland may have been functioning as both a potential source of NO_3-N and DOC to groundwater. However, the

stimulation of microbial denitrification by the organic carbon supply may have effectively prevented NO_3 from reaching deeper groundwater.

Conversely, the conditions in the sediments and groundwater may have promoted the production of NH_4-N. Faulwetter *et al.,* 2009). The elevated NH_4-N concentrations in local groundwater in comparison to that in both surface and deep groundwater, illustrated that it was most likely being produced in the wetland through the mineralisation of organic N in retained organic sediments (Reddy and D'Angelo, 1997). This may be preferentially transformed into inorganic N in the form of NH_4-N (Reddy and Patrick, 1984; Vymazal *et al.,* 1998) rather than NO_3-N, the ammonification process being aerobic yet still able to take place at a reduced rate under the anaerobic conditions observed in the groundwater (Reddy and Patrick, 1984). Anaerobic conditions may have also resulted in NO_3-N being removed from groundwater by dissimilatory reduction to NH_4-N (Vymazal, 2007) as, unlike denitrification, this process is not NO_3-N limited and so may occur even when porewater concentrations are low (Laanbroek, 1990). Thus, should NO_3 have been produced at aerobic microsites within sediments, its removal may have been almost instantaneous. The effect of these two processes is likely to have caused the observed negative correlation between NO_3-N and NH_4-N concentrations. This illustrates that while nutrient turnover in agricultural wetlands may reduce the risk for swapping of NO_3-N, this may be transferred to increased swapping of NH_4-N.

Results also exhibited a substantial degree of spatial variability between sampling points, both laterally and vertically (Fig 2.4; Table 2.1), suggesting differences in the rates of NH_4-N production. Typically the ammonification rate is controlled by the C/N ratio (Vymazal, 2007), pH (6.5-8.5), temperature (production rate doubling every 10°C), redox potential and oxygen level (Reddy and D'Angelo, 1997; Reddy and Patrick, 1984). However, poor correlation with physicochemical variables and DOC (used as a proxy for organic matter) was observed. While this may imply that these variables were not influencing NH_4-N production, in situ nutrient turnover between the wetland sediments and sampling points may have occurred. This is particularly the case given the know variability of the superficial deposits and potential presence of nitrifying zones, preferential flow pathways and microsites of organic rich sediments providing secondary sources of organic C and N. Because of this, a much more detailed investigation of in-situ nutrient cycling would be required to establish the finer-scale biogeochemical interactions.

2.5.3 Evidence of phosphorus transfer

Low groundwater concentrations and spatial heterogeneity of SRP indicated that despite a hydraulic gradient acting from the wetland into the shallow groundwater system, the agricultural wetland did not appear to be leaching soluble phosphorus into the groundwater. The low level and variability of observed concentrations under reduced conditions indicated potential precipitation or readsorption of released P, and influence of the biogeochemistry of superficial deposits in the shallow aquifer.

Groundwater concentrations of SRP, including those directly beneath the wetland were much lower than those observed in the surface water. This indicated that advective losses from the wetland did not appear to be occurring, assuming there had been no additional in situ overturning of P. Also, despite SRP concentrations in the parent aquifer also being very low, previous observations of groundwater flow and concentration patterns of other nutrients indicated that this was also not a viable source. The lack of evidence for SRP loss to the groundwater was surprising, given that the mean P content of the trapped sediment was substantial at 3265 mg P kg^{-1} (additional sediment nutrient content data provided by M. Ockenden, data not presented). Subsurface physicochemical conditions were also ideal to facilitate dissolution of bound P. This mechanism releases iron (Fe^{3+}) bound P under reducing conditions. This is because as O_2 is depleted in a reducing system, facultative microorganisms switch to using ferric iron as an electron acceptor, allowing P to be released. (Dunne and Reddy, 2005; Moore and Reddy, 1994; Reddy *et al.,* 1999; Stumm and Morgan, 1996). The anoxic reducing conditions and ideal pH levels (Dunne and Reddy, 2005; Moore and Reddy, 1994) in the groundwater created optimum conditions for high P solubility. However, despite a negative correlation between redox potential and SRP, no clear relationships with physicochemical conditions were observed and concentrations remained low. This was also surprising given the lack of redox buffering caused by the absence of NO_3-N (Christensen *et al.,* 2000; Surridge *et al*., 2007) which can inhibit P release under these conditions. However, it is also possible that dissolution of bound P was occurring, but the increased quantities of SRP and Fe^{2+} released during dissolution may have precipitated as reduced iron-phosphorus minerals e.g. Vivianite (Heiberg *et al.,* 2010). Additionally, P may also be re-adsorped back onto re-oxidised Fe^{2+} during NO_3- reduction (Weber *et al.,*2001; 2006).

Although low concentrations of SRP may have suggested the potential immobilisation of released P, it is likely that the concentrations observed in the shallow groundwater were derived from organic material in the wetland. This hypothesis is supported by the strong

positive correlations between SRP and DOC at all depths, combined with the matching vertical reductions in concentration outward from the wetland. The clear correlation between DOC and SRP could also be indicative of SRP being produced by in situ mineralisation of organic P. This may be influenced greatly by factors other than the variables measured, such as the microbial processes and available electron acceptors (D'Angelo and Reddy; 1994a, 1994b; Gale *et al.,* 1992). An alternative cause for the observed correlation between SRP and DOC, is that under the reducing conditions observed in the shallow groundwater, the dissolution of organic matter from the wetland was contributing to the increase of SRP, through competition for binding sites on the soil material (Morris and Hesterberg, 2010; Patrick and Khalid, 1974). As DOC increases, it can out-compete phosphate, resulting in an increase in the level of SRP. This could possibly be linked to the contrasting correlations between both SRP and DOC and redox potential in the 2 m piezometers.

As with other monitored determinands, a high degree of spatial heterogeneity in SRP concentrations (Fig 2.7) was evident across the site, with higher concentrations observed in some perimeter wells rather than below the wetland. This suggested the concentrations observed may also be influenced by the effects of the characteristics of the superficial deposits during transport in the subsurface region. For example organic rich sediments within the superficial deposits/ sediments may provide additional influences on P turnover. Therefore, it is likely that while P release from wetland sediments may have been supressed, the concentrations of SRP observed were likely to be derived from the retained sediments. However, it is also likely that the biogeochemistry of the surrounding superficial deposits may have a significant impact on the swapping potential of P.

2.6 Conclusions

Because of their simple design, constructed agricultural wetlands have significant potential to act as pollutant sources to local ground and surface waters. However the complex interactions of internal biogeochemistry and hydrological connectivity may significantly influence this potential.

Constructed agricultural wetlands are potential sources of pollutants to both surface and groundwater environments although, due to wetland biogeochemistry, in some situations some pollutants may present a greater risk than others. Wetlands may represent a

significant transfer point for NO_3-N into groundwaters however, due to anaerobic conditions and a plentiful supply of organic carbon from retained sediments, its removal via denitrification or reduction is likely to prevent movement to deeper groundwater. In contrast, these same conditions may lead to the increased production and export of NH_4-N into both local groundwater and back into surface waters, suggesting that reductions in TN or NO_3-N may be simply swapped to NH_4-N. The retention of P in wetland sediments appears to be more complex. While wetlands generate conditions which may promote the re-release of particle bound P from sediments, evidence suggests that this may be also potentially inhibited by subsequent re-absorption or precipitation in iron-phosphorus minerals within the wetland environment.

The overturning in N, P and C in retained sediments or shallow groundwater may be highly variable and dependent on numerous factors from the micro to site scale. In particular, the interaction of wetland derived nutrients with aspects of the biogeochemistry or composition of the surrounding superficial deposits may enhance or limit pollutant swapping. As a result, the relationships observed here may differ in other agricultural catchments and so it should not be assumed that specific pollutants do not pose a threat.

This factor should always be a primary consideration, as the unlined design of agricultural wetlands permits a potentially high level of interaction with surrounding groundwater and suggests that the exchange of pollutants from the surface water to groundwater, or from retained sediments to surface/groundwater may occur freely. However, this may be highly variable across individual wetlands with less permeable underlying geology, or hydrological regimes based on groundwater return flow to the surface. Such factors may need careful consideration when siting agricultural wetlands in the landscape, particularly where there is potential for substantial interaction with water in the parent aquifer. For example, where the main water table is close to the surface or is connected via fractures in the local geology.

Lastly, the reducing and anoxic conditions observed in the surrounding groundwater and sediments both potentially promoted and inhibited the transfer of dissolved nutrients in the system. However, the overarching biogeochemistry of these processes may also have implications for the production of greenhouse gases such as CH_4, CO_2 and N_2O, as will be addressed in the following chapters.

Chapter 3 – The potential for greenhouse gas transfers from a constructed agricultural wetland

3.1. Introduction

Natural wetlands are globally significant reservoirs of carbon and nutrients (Whitting and Chanton, 2001), storing an estimated 15 x 10^{14} kg of carbon (Schlesinger, 1991). However, due to containing both aerobic and anaerobic zones of organic matter decomposition (Kayranli *et al.,* 2010), they are also significant emitters of the greenhouse gases (GHGs), carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) to the atmosphere (Bartlett and Harriss, 1993). In particular, natural wetlands account for the flux of some 177 - 284 Tg CH_4 yr^{-1} to the atmosphere (Ciais *et al.,* 2013). The carbon and nutrient storage properties of natural wetlands have led to engineered systems being developed to treat point source effluents. Though these 'treatment wetlands' deliver water quality improvements, as in natural wetlands, transfers of CO_2, CH_4 and N_2O to the atmosphere can be substantial (Mander *et al.,* 2005, 2008; Søvik *et al.,* 2006; Søvik and Kløve, 2007). Creation of wetland systems may therefore have important implications for carbon/nutrient cycling and climate function. However, the net balance between sequestration of material and export through greenhouse gas fluxes is still poorly understood. Constraining this uncertainty is a key challenge, which must be met in order to fully understand the mechanisms of carbon transfer and climate change, particularly through local scale measurements of highly variable wetland environments (Ortiz-Llorente *et al.,* 2012).

Following the effectiveness of treatment wetlands in reducing nutrient loading from point sources, constructed agricultural wetlands are now being considered a land management tool to alleviate diffuse water pollution by nutrients exported from agricultural land (Ockenden *et al*., 2012; Diaz *et al.,* 2012; Johannesson *et al.,* 2011; Milhollon *et al.,* 2009). Wetland installations are converted from unproductive farmland and utilise similar biogeochemical processes to parallel natural and engineered wetlands. Therefore based on evidence from these systems, it can be inferred that agricultural wetlands may improve catchment water quality, but the flooded sediment environments may exacerbate global emissions of GHGs to the atmosphere. However, key features of agricultural wetlands set them apart from treatment and natural wetland examples, with unknown impacts on GHG releases. Unlike treatment wetlands, the intermittent nature of rainfall event-driven inputs means that the volume of runoff and the degree of nutrient loading they receive is highly variable throughout the year (Jordan *et al.,* 2003). As a result, the supply of nutrients that

either promote or inhibit gas production may vary greatly over time. This is particularly the case with nitrate (NO_3-N) enriched agricultural landscapes due to its inhibitory role on CH_4 production as an electron acceptor (Achtnich *et al.,* 1995; Stadmark and Leonardson, 2005). Conversely, NO_3-N loading may increase emissions of N_2O (Reay, *et al.,* 2003). Meanwhile the transfer pathways of GHG evasion influences the potential for subsequent physical and chemical interactions in the environment e.g. oxidation (Bastviken, *et al.,* 2008). To date, only a handful of studies have considered emissions from (mainly Scandinavian) agricultural wetlands, reporting substantial but variable fluxes of CH_4 and CO_2 and N_2O along with strong influences of season and nutrient supply (Søvik *et al.,* 2006; Stadmark and Leonardson, 2005). However the diversity of agricultural landscapes, climate and wetland designs means that further evidence is required in order to make robust judgements on the net impacts of wetland construction. Here we seek to examine whether conversion of agricultural land to wetlands facilitates pollutant swapping by significantly increasing greenhouse gas transfers to the atmosphere.

3.2. Aim

Aim: Assess the potential for installation of constructed agricultural wetlands to facilitate pollutant swapping of GHGs to the atmosphere and nutrients into surface waters.

Hypotheses to be tested:

1. Agricultural wetland creation will significantly increase emissions of CO_2, CH_4 and N_2O compared to those occurring in previous land use i.e. unproductive riparian farmland.

2. CH_4 emissions will be dominated by ebullition while both CO_2 and N_2O will be transferred by molecular diffusion.

3. Emission or uptake of GHGs from agricultural wetland will be affected by the oxygen and nutrient properties of the water column.

3.3. Methods

3.3.1. Study site

The 18 month field investigation was focused on the 'Shelduck' agricultural wetland based in Cumbria, UK. A full description of the study site is available in Chapter 2.

3.3.2. Gas flux chamber measurements

Total (ebullitive and diffusive) fluxes of CO_2, CH_4 and N_2O from the wetland were measured using flux chambers and supplemented with both diffusive flux models and ebullition traps in order to assess the individual contribution of specific transfer pathways.

Primarily, total gas fluxes were measured using between four and eight floating transparent acrylic chambers (Fig. 3.1), volume 255 L (0.91 x 0.78 x 0.36 m). Chambers were launched from the bank, pressure allowed to equilibrate and pulled into position without disturbing the sediment/water. Each chamber was fitted with a HOBO pendant temperature data logger and a small electric fan to homogenise the internal air. Samples were extracted utilising a recirculating flow through mechanism, with each chamber remotely connected in a sample loop with a pump (5 L min^{-1}). Samples were extracted through a butyl rubber septum built into the pump with a 10 ml syringe, which was flushed three times with sample before removal and placement into an evacuated a 3.6 ml exetainer (Labco, UK Ltd.). Samples were taken at 10 minute intervals between 0 and 30 minutes, in order to reduce chamber placement time and associated effects (Livingston and Hutchinson, 1995; Parkin *et al.,* 2010).

Due to the proportionately larger area of the shallow cell, 3 -6 chambers were deployed across this area while 1-2 were placed in the deep cell during each monitoring period. Chambers were deployed in approximately the same locations and at a similar time of day, at intervals of two weeks or one month, between July 2012 and December 2013. Parallel net GHG fluxes were also made at an undisturbed riparian area adjacent to the wetland, in order to act as a control with which to compare wetland GHG fluxes. Measurements were made using between four and eight gas flux chambers, identical to those used on the wetland, with the addition of airtight skirts affixed to the chamber exterior (Fig 3.1). Chambers were deployed in approximately the same locations in the riparian area across all sample

occasions. Soil temperature and moisture were also monitored on each occasion. Using an ML3 Theta Probe Soil Moisture Sensor (Delta T, Cambridge, UK) and electronic soil thermometer.

Figure 3.1 Acrylic Flux chambers deployed on the agricultural wetland and riparian zone with remote sampler loop. Riparian chamber fitted with weighted airtight skirts.

Gas samples were analysed within one month of collection using a PerkinElmer Autosystem XL gas chromatograph (GC) fitted with a flame ionisation detector and electron capture detector. Three mixed calibration standards (500 ppm CO_2, 1 ppm CH_4, 1 ppm N_2O; 1000

ppm CO_2, 3 ppm CH_4, 2 ppm N_2O; 4000 ppm CO_2, 10 ppm CH_4 and 0.4 ppm N_2O) were used (BOC, UK). Calibration standards were used to form a calibration curve via linear regression, from which GC outputs were converted into concentrations of gas in air (ppm). GHG fluxes (μL trace gas m^{-2} min^{-1}) were estimated through linear regression of change in gas concentrations within flux chambers over time (Parkin *et al.,* 2010). Data was only accepted where significant ($p<0.05$), which corresponded to an $R^2 > 0.73$ (n=4; Zar, 1984). 'Positive' fluxes describe gas emission while 'negative' fluxes describe uptake. Fluxes were then converted from a volumetric basis to a mass basis using the ideal gas law (equation 3.1):

$$Pv = nRT \qquad \textit{Equation 3.1}$$

Where P = pressure, V = volume, n = the number of moles of gas, R = the gas law constant (0.08206), and T = temperature, taking in to consideration field temperature and pressure.

3.3.3. Ebullitive gas emissions

Ebullitive gas emissions (Jan-Dec 2013) were captured using bubble traps (Huttenen *et al.,* 2001). Traps were constructed from inverted plastic funnels (diameter 180 mm) fitted with 60 ml syringes and three way stopcocks. Traps were held in wire frames in groups of three, which were suspended 30 cm below the water surface. Two frames were deployed in each wetland cell and allowed to drift for 24 hours. Samples were extracted in situ using a 10 ml syringe and stored as described above. Volumes of captured gas were recorded. Traps were deployed monthly. Samples were analysed as in 3.3.2, with additional calibration standards of 10 % and 50 % CH_4 (CK Special Gases, UK) for high CH_4. Volumetric concentrations of samples were then calculated using the ideal gas law.

3.3.4. Diffusive gas emissions

Diffusive emissions (Jan-Dec 2013) of CH_4, CO_2 and N_2O were calculated using local wind speed, surface water gas concentration and water temperature which were fed into a Turbulent Boundary Layer (TBL) model as described by Liss and Slater (1974) (equation 3.2):

$$F = k\,(C_w - C_{eq}) \qquad \textit{Equation 3.2}$$

Where *F* is the air-water flux of a dissolved gas, *k* is the transfer velocity of a gas, C_w is the molar mass of dissolved gas in the near-surface water, C_{eq} is the molar concentration of gas in the surface water that is in equilibrium with the air.

k was estimated using the Schmidt number (Sc) of the target gases using the relationship of Liss and Merlivat (1976) (equation 3.3).

$$k = k_{600} \left(\frac{sc}{600}\right)^{n} \qquad \textit{Equation 3.3}$$

Where Sc is the Schmidt number of a gas at surface water temperature (Wanninkhof, 1992), *n* is -0.667 for U_{10}< 3.7 ms^{-1} and -0.5 forU_{10}> 3.7 ms^{-1}. K_{600} is the gas transfer velocity which was calculated using two published relationships between wind speed and diffusive gas exchange.

By Cole and Caraco (1998) (equation 3.4):

$$K_{600}\,(\text{cm hr}^{-1}) = 2.07 + 0.215 \,.\, U_{10}^{1.7} \qquad \textit{Equation 3.4}$$

And by Crusius and Wanninkhof (2003) (equations 3.5-3.6):

$$\text{If wind is <3.7} \quad K_{600}\,(\text{cm hr}^{-1}) = 0.72.\, U_{10} \qquad \textit{Equation 3.5}$$

$$\text{If wind is <3.7 } K_{600}\,(\text{cm hr}^{-1}) = 4.33.\, U_{10} - 13.3 \qquad \textit{Equation 3.6}$$

Where U_{10} is the wind speed at 3 m above the water surface normalised to speed at 10 m. The most appropriate relationship to use for each gas was determined by the similarity between time series of chamber and calculated fluxes (Appendix I, Fig. 8.1), considering temporal patterns and magnitude of flux rates. The relationship of Crusius and Wanninkhof was used for CO_2, with that of Cole and Caraco, (2003) used for CH_4 and N_2O.

Wind speed (U_{10}) was monitored on site using a logging anemometer (Fig. 3.2) located approximately mid-way between the wetland inlet and outlet. Anemometer height was 3 m above the water surface due to the nature of the wetland excavation. Wind speed at 3 m was normalised to that at 10 m height using a ratio of $U_{10} = U_3 \times 1.22$ (Meteorological Office, 2000). Air temperature was recorded on each sample occasion using an electronic thermometer.

Figure 3.2 Logging anemometer installed at the centre of the wetland site. The shallow cell is visible in the background, with the anemometer situated approximately 3 m above the water surface.

3.3.5. Dissolved nutrients and gases in the water column

A single water sample was taken at both the surface and sediment-water interface in the centre of each wetland cell using a sample line and peristaltic pump (Williamson, UK). A low flow rate was used so as to not cause degassing during collection (comparison with measurements from a Van Dorn bottle showed no significant differences in the techniques. See Chapter 4 for further details). Samples were decanted into 250 ml HDPE bottles and those for nutrient analysis into 60 ml centrifuge tubes. Samples were immediately transferred to a cool box, then stored at <5 °C Samples were analysed for SRP, NH_4-N, NO_3-N and DOC within 24 hours using the methods described in Chapter 2, including quality control.

Dissolved gases were analysed the same day as collection using a modified headspace technique (Reay *et al.*, 2003; Sobek *et al.,* 2003). Samples were brought to room temperature, decanted into 60 ml Wheaton bottles and sealed. 30 ml was then replaced with air (with known quantities of CH_4, CO_2 and N_2O). Samples were agitated vigorously for 2

minutes and then left to equilibrate for at least 20 minutes, before 5 ml was extracted and injected into an evacuated exetainer. Samples were analysed as in section 3.3.2. GC outputs were then converted into dissolved gas concentration in water using Henry's law, the values for which were calculated according to values in Wilhelm *et al.,* (1970).

3.3.6. Antecedent and physicochemical measurements

Physicochemical variables (temperature, dissolved oxygen, pH, conductivity, redox potential) were measured in situ at the same time as water samples were taken, using a Hanna H19828 water quality meter, calibrated as described in Chapter 2. Surface water level was taken from a calibrated pressure transducer in the middle flume. Air pressure and temperature were continuously logged using a Troll (In-Situ Inc.) barometric logger.

3.3.7. Statistical analyses and data processing

Daily GHG fluxes using chamber, diffusive and ebullitive methods were calculated using mean values from January–December 2013, with weighting applied to account for differences in time between observations. Seasonally averaged fluxes were calculated using chamber observations from the whole 18 months which were split: June to August as summer (water temperatures > 14.5 °C), September to November as autumn, December-February as winter and March to May as spring.

All data was tested for normality and homogeneity of variance using Shapiro-Wilk and Levenes tests respectively. Differences between seasonal gas fluxes and dissolved gas concentrations were assessed in each wetland cell using one way ANOVA, with seasons as between group factors and supplemented with Tukeys HSD post-hoc tests. Differences in GHG fluxes, concentrations, physicochemical variables and nutrients between wetland cells were assessed using paired t tests (i.e. paired in time), as were differences between conditions at the surface and bottom of the water column.

Where normality or homogeneity of variance was violated, $\log_{10,}$ and square root transforms were applied. Where violations persisted, Kruskal-Wallis tests were used in place of one-way ANOVA, with supplementary Dunn-Bonferroni adjusted post-hoc tests when significant differences were found. Paired t tests were substituted with Wilcoxon tests. Relationships between variables were assessed using Spearman's Rank Order Correlation, due to normality not being achieved.

3.4. Results

3.4.1. Antecedent conditions in the wetland

Water temperature (Fig 3.3 A-B) in the wetland ranged between 2 °C in winter and 26 °C in summer with significantly higher temperatures in the shallow cell (t test, *p<0.001*). Temperature was greater at the wetland surface than that at the sediment water interface in both deep (Wilcoxon, *p<0.05*) and shallow cells (Wilcoxon, *p=<0.05*) over the experiment. The pH (Fig 3.3 C-D), ranged between 6.6 and 10.7, after an anomalously low value (4.3) was removed, following identification using Grubbs test for outliers. Both wetland cells displayed pH values above 7.0 on 23 out of 25 sampling occasions. pH was similar at the surface and bottom of deep (Wilcoxon *p>0.05)* and shallow cells (Wilcoxon, *p>0.05*) . The shallow cell exhibited significantly higher pH than the deep cell (Wilcoxon *p<0.05*). Dissolved O_2 (DO) concentration (Fig 3.3 E-F) varied greatly. The water column in either cell was rarely fully oxygenated, on average being around 6 mg L^{-1}. During autumn and winter 2013, this dropped to around 3 mg L^{-1}. Both peak (supersaturation) and lowest DO levels were observed during the summer, where an oxycline briefly formed in the deep cell. Over the experiment DO was significantly higher at the surface than sediment water interface (Wilcoxon, *p<0.05*) in the deep zone only, particularly during summer. Redox potential (Fig 3.3 G-H) was low, and became predominantly negative after October 2012, with negativity increasing throughout the study period and being significantly lower in the deep cell (Wilcoxon *P<0.05*). No significant differences in redox potential in surface and bottom water were detected in either cell.

3.4.2. Dissolved nutrients

Nutrient concentrations in the water column varied substantially over the experiment (Fig 3.4 A-H). NO_3-N concentration was moderate, peaking during autumn/winter while being below the limit of detection (0.023 mg L^{-1}) in both wetland cells during June and July 2013. NO_3-N concentrations were significantly higher in the deep cell (Wilcoxon, *p<0.001*). NH_4-N concentration was also significantly higher in the deep cell than the shallow cell (Wilcoxon *p*<0.05), where peak concentrations were observed in July. Bottom water concentrations of NH_4-N were greater than at the surface in the deep cell only (Wilcoxon *p<0.05*). SRP concentrations were significantly higher in the deep cell (Wilcoxon *p<0.001*). No differences in surface and bottom water SRP were observed. DOC concentration was high throughout the study period although displayed a great deal of variability. DOC concentration in surface

and bottom water did not significantly differ during regular sampling. Concentrations in the shallow cell were significantly higher (Wilcoxon, *p<0.05*) over the study.

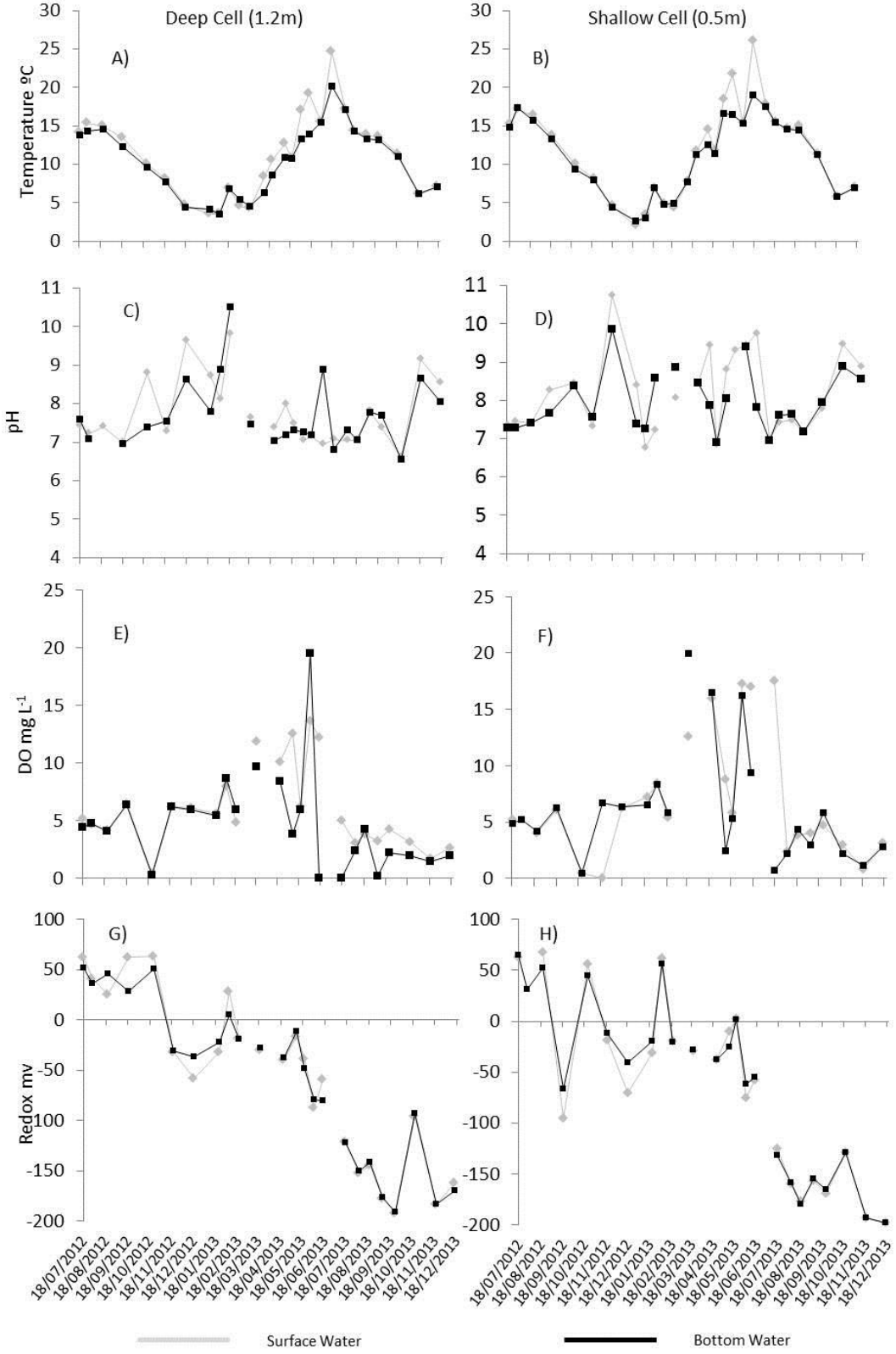

Figure 3.3 Physicochemical parameters of surface and bottom water in the deep and shallow cells of the wetland from July 2012- July 2013. Gaps in line indicate no data collected**.**

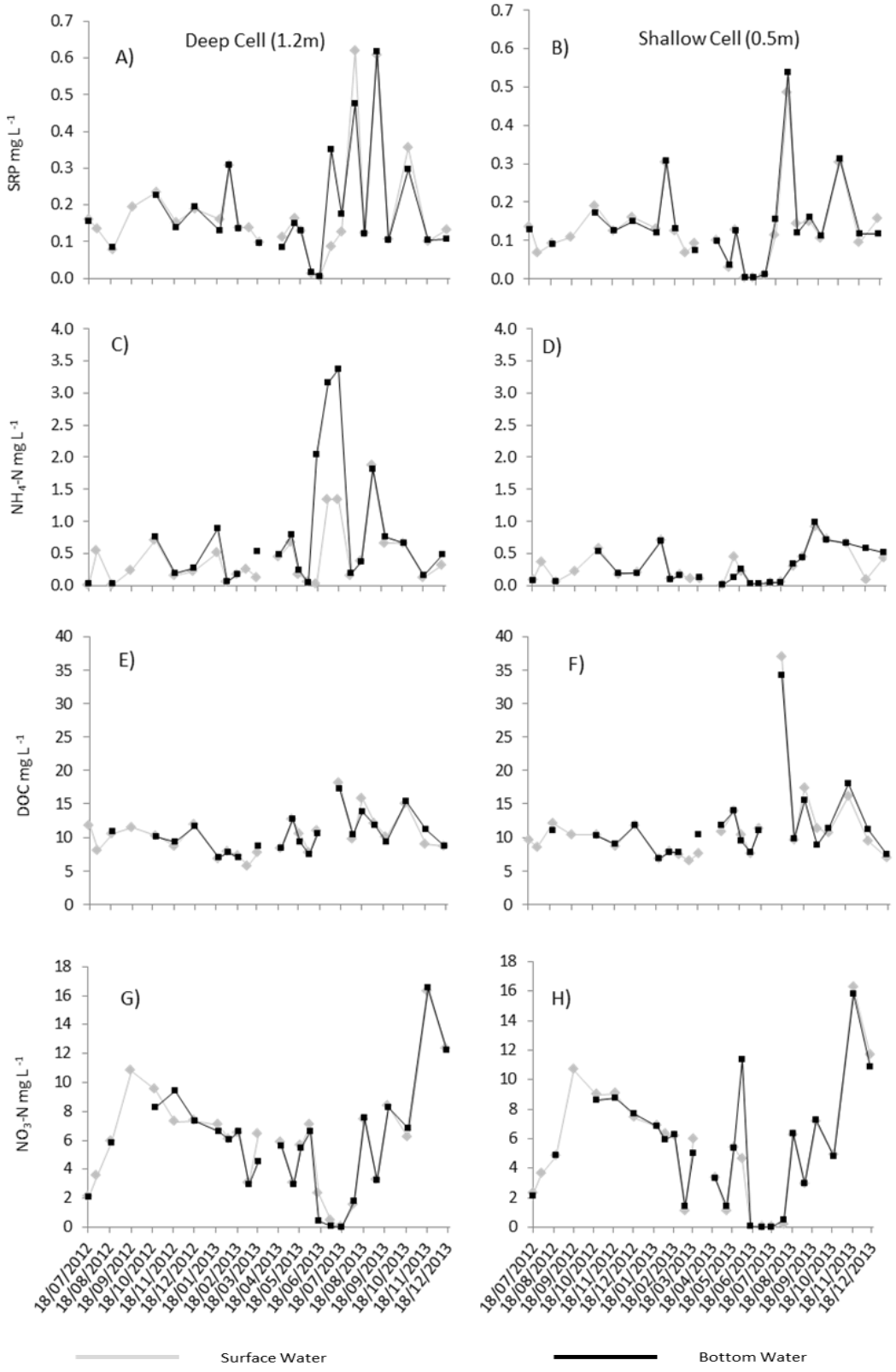

Figure 3.4 Dissolved nutrient concentrations in surface and bottom water of the deep and shallow cells of the wetland from July 2012- July 2013. Gaps in lines indicate no data.

3.4.3. Dissolved gas in the water column

Dissolved CH_4, (0.3-627 µg L^{-1}) (Fig 3.5) was supersaturated with respect to atmospheric equilibrium in both wetland cells throughout the study. One-way ANOVA revealed no significant seasonal variations in the deep ($F(3,44) = 0.745$, $p = 0.531$) or shallow cells ($F(3,44) = 2.515$, $p = 0.071$). CH_4 concentration was generally higher closer to the sediments than at the surface, although not significantly in deep or shallow cells. CH_4 concentrations were similar in both cells.

Dissolved CO_2 (Fig. 3.5) (0.10 – 31.9 mg L^{-1}) varied greatly, with particularly low concentrations observed in parts of spring and summer. One way ANOVA with Tukey post hoc test confirmed that concentrations were significantly lower in summer than in winter in the deep cell ($F(3,46) = 4.356$, $p = 0.009$, Tukey, *p<0.05*). Kruskal-Wallis with Dunn-Bonferroni post hoc tests revealed the same situation in the shallow cell, with summer CO_2 concentrations being significantly lower than during winter (X^2 (3) =10.534, *p<0.05;* Dunn-Bonferroni *p<0.*05). Concentrations were higher in the deep cell than shallow cell over the study (Wilcoxon *p<0.001*). Concentrations in the bottom water of the deep cell were significantly higher than at the surface (Wilcoxon, $p<0.001$), while concentrations of CO_2 were similar in the shallow cell (*p>0.0.05*).

Concentrations of dissolved N_2O in the water column (Fig 3.5) were low but variable (0.23-22.2 µg L^{-1}). Although generally supersaturated compared to atmospheric concentrations, both cells experienced a period of low summer concentrations from June-August before recovering in late summer/early autumn. This was illustrated by Kruskal-Wallis with additional Dunn-Bonferroni post hoc tests (X^2 (3) =13.339, *p<0.05,* Dunn-Bonferroni , *p<0.05*) which confirmed summer concentrations in the deep cell were lower than in autumn and winter . In the shallow cell, a one way ANOVA with Tukey post hoc test ($F(3,44) = 4.046$, *p < 0.05,* Tukey, *p<0.05*) found only summer concentrations were lower than those in winter. Concentrations of N_2O were higher closer to the sediment water interface in both deep (Wilcoxon, *p<0.05*) and shallow (*p<0.05*) cells. Concentrations were higher in the deep cell over the study period (Wilcoxon, *p<0.001*).

Detailed depth surveys (Fig. 3.6 A-H) of dissolved gases across the area of the wetland (undertaken twice during June and July 2013) during the summer showed that there were no significant differences between the gas profiles or physicochemical conditions within the horizontal extent of each cell. As with regular sampling, large differences between surface

and bottom water concentrations were only observed in the deep cell, with highest concentrations of CH_4, N_2O and CO_2 being found in the bottom water where the lowest levels of O_2 saturation were observed.

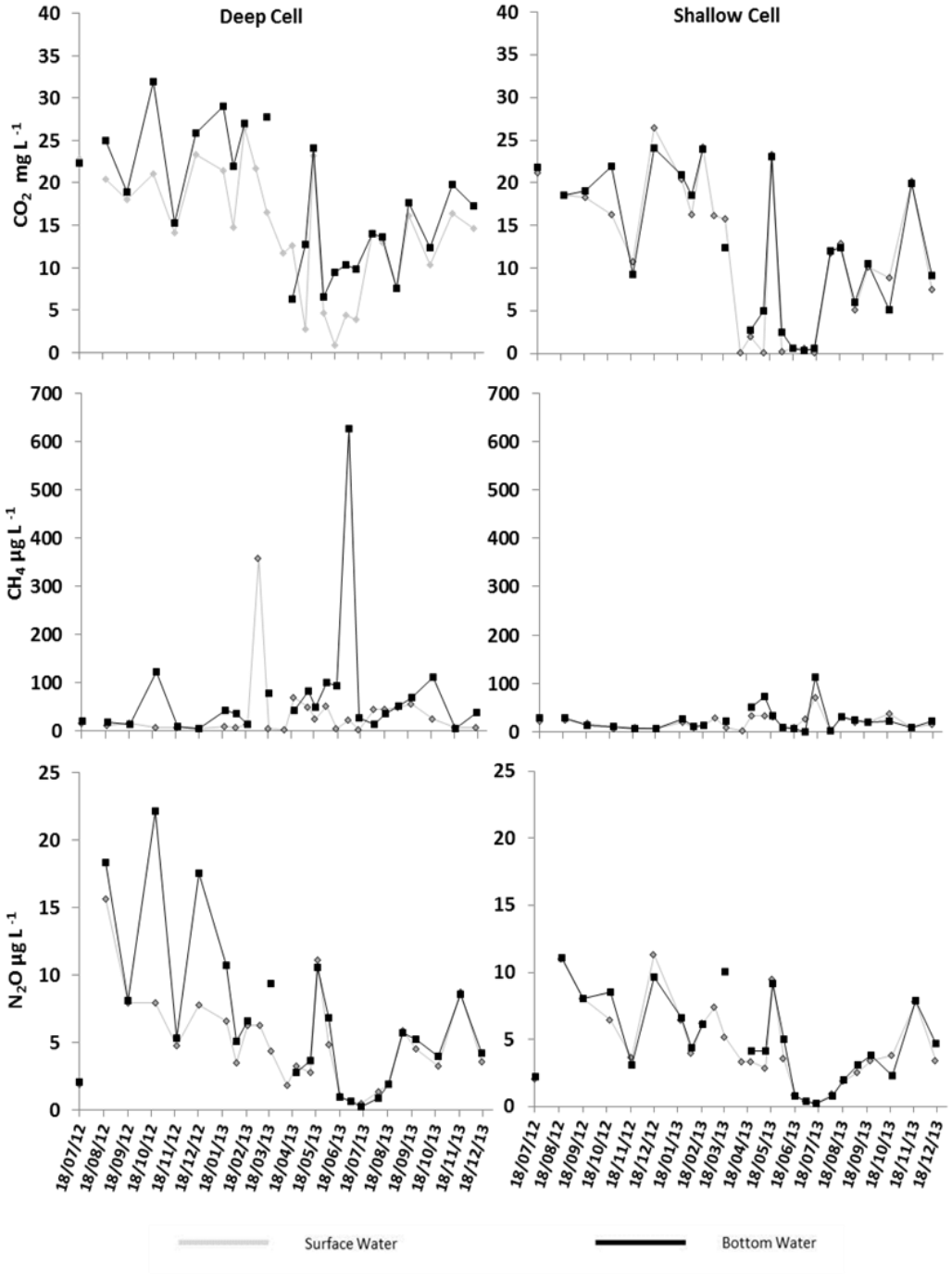

Figure 3.5 Dissolved concentrations of CH_4, CO_2 and N_2O at the surface and bottom of deep and shallow wetland cells. Gaps indicate no data collected.

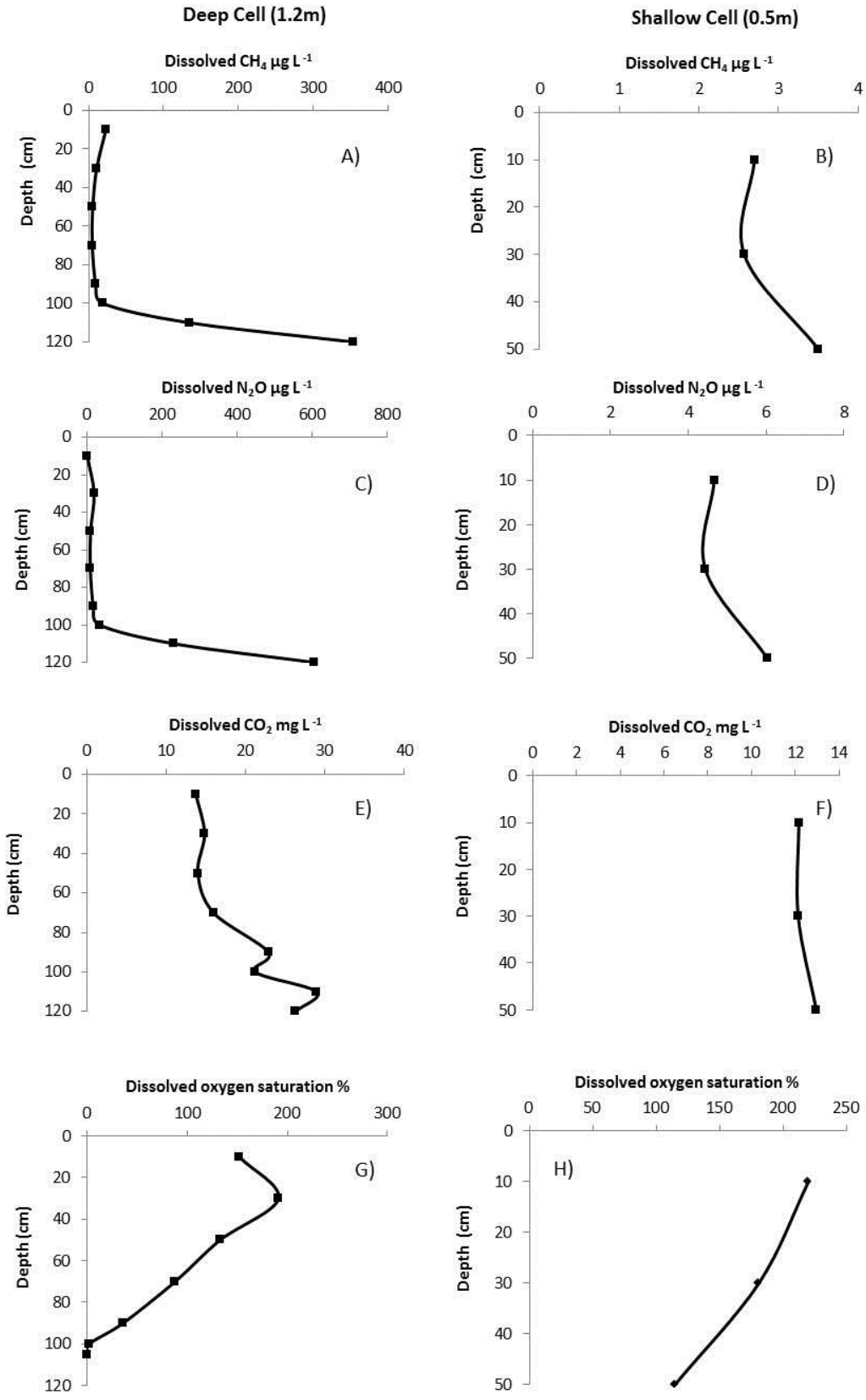

Figure 3.6 Dissolved concentrations of CH_4, CO_2 and N_2O and O_2 saturation through the water column of deep and shallow cells of the wetland zones in June 2013. Variables and wetland cells use different x and y scales.

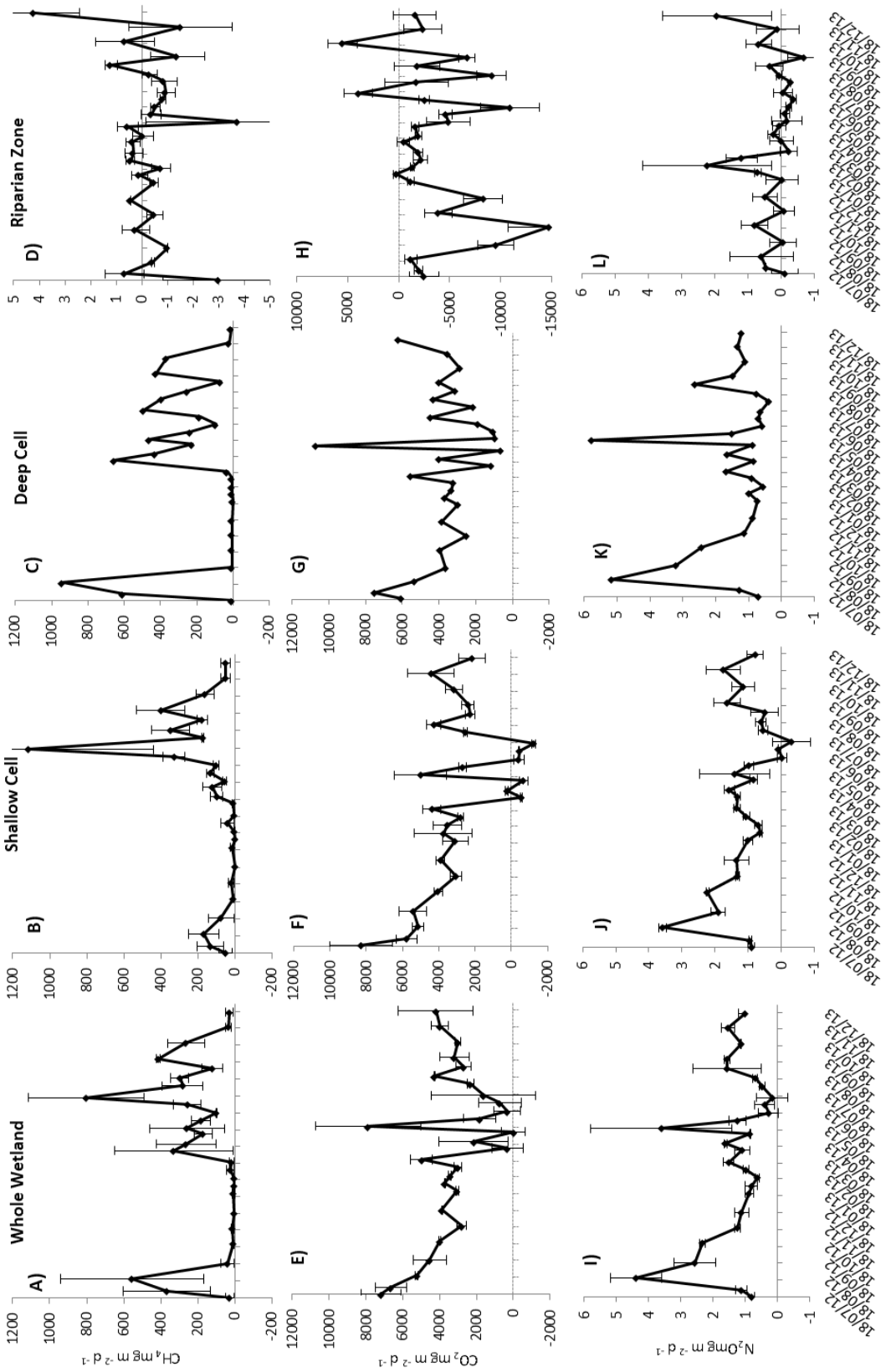

Figure 3.7 Fluxes of CH_4, CO_2 and N_2O captured in the deep, shallow and riparian zones using flux chambers (*n*=27). Whole wetland fluxes are the mean of deep and shallow emissions. Error bars represent standard error. Deep zone measurements were often made from single chamber deployments so lack error bars. Y axes on riparian plots are lower than those for the wetland.

3.4.4. Gas flux chamber measurements

Parallel estimates of gas flux using the TBL and ebullition capture techniques, produced over-estimates of mean daily fluxes when compared to the chamber-based measurements (see section 3.6. and tables 3.1-3.3). Variability between methods is accepted as common issue in gas flux studies (Duchemin *et al.,* 1999; St. Louis *et al.,* 2000).

3.4.5. Wetland gas fluxes

Positive CH_4 fluxes to the atmosphere (1.31-1115 mg m^{-2} d^{-1}) (Fig 3.7, Table 3.1) emanated from the wetland throughout the study. Mean flux was 191 ± 10 mg m^{-2} d^{-1} for the whole wetland. Emissions from the deep cell, 212 ± 11 mg m^{-2} d^{-1} were significantly higher than the corresponding observations from the shallow cell, 169 ± 12 mg m^{-2} d^{-1} (Wilcoxon, p<0.05). One-way ANOVA with Tukey post hoc tests established that CH_4 fluxes in winter were lower than during the rest of the year across the entire wetland (F(2,23) = 10.216, p < 0.001; Tukey *p<0.05*), and in both the deep (F(3,23) = 6.797, p <0.05; Tukey, *p<0.05*) and shallow cells (F(3,23) = 8.742, p <0.001; Tukey, *p <0.05*). Peak CH_4 emissions were observed between April–September. Methane emissions were characterised by occasional very low fluxes which did not fit in with seasonal patterns.

CO_2 fluxes (-1231 - 10752 mg m^{-2} d^{-1}) (Fig 3.7, Table 3.2) were approximately two orders of magnitude higher than those for CH_4, and two to three above those for N_2O, with mean fluxes of 2845 ± 87 mg m^{-2} d^{-1} across the entire wetland. Mean deep cell fluxes, 3521 ± 106 mg m^{-2} d^{-1}, were significantly higher (Wilcoxon, p<0.001) than those from the shallow cell, (2249 ± 97 mg m^{-2} d^{-1}). Kruskal Wallis tests showed that no particular seasonality across the wetland (X^2 (3) =1.589, *p>0.05)* or in either deep (X^2 (3) =0.448, *p>0.05)* or shallow (X^2 (3) =1.168, *p>0.05)* cells. However, negative or very low fluxes were observed in both zones during parts of spring and summer.

N_2O fluxes (-0.31-5.78 mg m^{-2} d^{-1}) (Fig 3.7, table 3.3) were the lowest monitored with a mean flux of 1.1 ± 0.03 mg m^{-2} d^{-1} from the entire wetland. Deep zone emissions, 1.25 ± 0.05 mg m^{-2} d^{-1} were significantly higher (Wilcoxon, *p<0.001*) than those from the shallow zone, 0.93 ± 0.03 mg m^{-2} d^{-1}. Kruskal Wallis tests highlighted significant seasonality across the wetland (X^2 (3) =10.435, *p<0.05)* and also in the deep (X^2 (3) =0.8.078, *p<0.05)* and shallow (X^2 (3) =8.589, *p<0.05)* cells. The period of low N_2O emission and in some cases uptake during the summer months, was detected by Dunn-Bonferroni post hoc tests when examining fluxes from the

entire wetland (Dunn-Bonferroni, *p<0.05*). However lower summer fluxes in individual wetland cells were not observed at a significant level.

Table 3.1 Seasonal and annual daily mean CH_4 fluxes from deep cell, shallow cell and whole wetland. Lower and upper estimates of annual mean daily GHG flux, use flux chamber (lower) and combined bubble trap and TBL model (upper) methods respectively. Emissions from the riparian area are also presented.

	CH_4 emission mg m^{-2} d^{-1}				
Cell	**Annual**	**Spring**	**Summer**	**Autumn**	**Winter**
Deep (lower	212	299	355	126	4
(Upper)	456				
Shallow (lower)	169	56	284	128	15
(Upper)	237				
Mean (lower)	191	177	319	127	9
(Upper)	361				
Riparian	-0.05	-0.35	-0.72	-0.32	0.02

Table 3.2 Seasonal and annual daily mean CO_2 fluxes from deep, shallow and whole wetland zones. Lower and upper estimates of annual mean daily flux use flux chamber (lower) and combined bubble trap and TBL model (upper) methods respectively. Emissions from the riparian area are also presented.

	CO_2 emission mg m^{-2} d^{-1}				
Cell	**Annual**	**Spring**	**Summer**	**Autumn**	**Winter**
Deep (lower)	3521	4232	3750	3374	4020
(Upper)	5724				
Shallow (Lower)	2249	1876	2959	3541	3294
(Upper)	2982				
Mean (Lower)	2885	3054	3354	3457	3657
(Upper)	4352				
Riparian	-1964	-2151	-3401	-4796	-1771

Table 3.3 Seasonal and annual daily mean N_2O fluxes from deep, shallow and whole wetland zones. Lower and upper estimates of annual mean daily flux use flux chamber (lower) and combined bubble trap and TBL model (upper) methods respectively. Emissions from the riparian area are also presented.

	N_2O emission mg m^{-2} d^{-1}				
Cell	**Annual**	**Spring**	**Summer**	**Autumn**	**Winter**
Deep (Lower)	1.25	1.95	1.30	1.90	0.88
(Upper)	2.04				
Shallow (Lower)	0.93	1.25	0.81	1.50	0.89
(Upper)	1.83				
Mean (lower)	1.09	1.60	1.06	1.70	0.88
(upper)	1.94				
Riparian	0.30	0.17	-0.01	0.15	0.68

3.4.6. Riparian gas fluxes

Riparian fluxes of all GHGs (Tables 3.1-3, Fig 3.7) were low. Riparian CH_4 was significantly less than those emanating from the wetland (Wilcoxon, *p<0.001*). Fluxes, (-3.74 – 4.19 mg m^{-2} d^{-1}) were predominantly negative, with an annual daily mean of -0.05 ± 0.08 mg m^{-2} d^{-1}. Kruskal-Wallis tests showed no seasonal differences (X^2 (3) =3.262, *p>0.05),* although when positive fluxes did occur, it was often during extreme soil wetting (Appendix II Fig 8.2).

Riparian N_2O fluxes, -0.68 – 2.23 mg m^{-2} d^{-1} were also lower than from the wetland (Wilcoxon, *p<0.001*) , though were mainly positive over the year with a mean emission rate of 0.3 ± 0.04 mg m^{-2} d^{-1}. Kruskal Wallis tests showed no significant seasonal variations (X^2 (3) = 4.275, *p>0.05)*, although as in the wetland, the smallest positive fluxes were observed during summer.

Riparian CO_2 fluxes -10943 – 5531 mg m^{-2} d^{-1} were lower than those from the wetland (Wilcoxon, *p<0.000*) and were dominated by negative fluxes throughout the study period with an annual mean of -1964.15 ± 197 mg m^{-2} d^{-1}. Kruskal-Wallis tests showed no significant seasonal differences, (X^2(3)=4.026, *p>0.05)*. Increasingly negative fluxes were observed particularly when vegetation was well developed. Smaller negative fluxes were recorded from February to April 2013. Positive CO_2 flux was only observed on two occasions during July and October 2013 although no explanation could be found as to why this was the case.

3.5. Impact of constructed wetland creation

3.5.1. Global warming potential

The relative climatological impact of each GHG emitted from the wetland to the atmosphere, was obtained by equating mean fluxes to CH_4 and N_2O to CO_2 Global Warming Potential (GWP) equivalents and accounting for cell surface area (Table 3.4). This was performed by first multiplying lower and upper estimates of daily GHG flux by the 100-year global warming factor for each gas.(34-CH_4 and 298-N_2O, IPCC, 2013), in order to express fluxes in common units (g CO_2e d^{-1} m^{-2}). Flux rates from deep and shallow cells were then multiplied by the respective cell surface areas and then combined to produce a lower and upper estimate of the total daily wetland emissions for each GHG. Gross wetland GHG emissions were calculated by combining flux estimates from CH_4, CO_2 and N_2O. Riparian CO_2e emissions were calculated using the same method, using same surface area as the wetland (320 m^2) to allow direct comparisons of wetland and riparian emissions. Additionally, net CO_2e emissions from the wetland were calculated by subtracting riparian emissions (or uptake) from gross wetland emissions. This was to allow a greater appreciation of the impact of wetland construction i.e. by potentially removing an area of GHG uptake, as well as creating a potential source of emissions in the landscape.

Annual mean daily contributions from CH_4, 1934-3054 g CO_2e d^{-1} were highest followed by CO_2, 801-1130 g d^{-1}. N_2O emissions, 95-179 g d^{-1} had the smallest contribution. CH_4 impact was the most important during spring, autumn, and particularly summer. Only during the winter did CO_2 impact exceed CH_4. The net effect of converting the 320 m^2 of unproductive land to wetland showed that CH_4 had the greatest impact, 1935-3053 g d^{-1}. Net impact from CO_2 increased substantially to 1430-1758 g d^{-1}. As the riparian area N_2O emissions were much similar to those emitted from the wetland, the net effect was minimal, 66-150 g d^{-1}.

3.5.2. Carbon and nitrogen losses

The loss of C and N from the wetland via GHGs was calculated using upper and lower estimates of mean annual daily fluxes of CO_2-C, CH_4-C, N_2O-N (Table 3.5) and cell area. Rate of C transferred to the atmosphere via CO_2 and CH_4 was 95-137 kg yr^{-1}. CO_2 was the main transfer pathway for carbon, accounting for 82-84% of the total due to a greater mass of CO_2 emitted.

Table 3.4 GWP (g CO_2e d^{-1}) equivalents of wetland and riparian gas emissions using annual and seasonal mean emission rates. GWP calculated using the factors 34-CH_4 and 298-N_2O.

CO_2e equivalents of GHG fluxes from the wetland and riparian zone																
		CH_4 CO_2e g d^{-1}					CO_2 g d^{-1}					N_2O CO_2e g d^{-1}				
	Cell of wetland	Annual	Spring	Summer	Autumn	Winter	Annual	Spring	Summer	Autumn	Winter	Annual	Spring	Summer	Autumn	Winter
Deep Cell	lower	462	650	772	274	8	225	271	240	216	257	24	37	25	36	17
	upper	993					366					39				
Shallow Cell	lower	1472	488	2472	1118	130	576	480	757	906	843	71	95	62	115	68
	upper	2061					763					140				
Deep +Shallow	lower	1934	1138	3244	1392	138	801	751	997	1122	1101	95	133	87	151	84
	upper	3054					1130					179				
Riparian		-0.6	-3.8	-7.9	-3.5	0.2	-629	-688	-1088	-1535	-567	28	16	-1	14	65
Net Emissions	lower	1935	1139	3244	1393	139	1430	1439	2086	2657	1729	66	104	58	122	56
	upper	3055					1758									
Gross wetland Emissions		2830 – 4363 CO_2e g d^{-1}														
Net emissions from land conversion		3431 – 4963 CO_2e g d^{-1}														
GWP equated fluxes calculated using 100 year forcing potentials (IPCC, 2013) CH_4= 34, N_2O =298. Fluxes calculated for individual areas of the wetland																

Table 3.5 Masses of carbon and nitrogen transferred via gas emission on a daily and yearly basis. Net retention of total carbon and total nitrogen are also given. Retention figures are from derived from Ockenden *et al.* (2014)

	Carbon/Nitrogen loss pathway		
	CH_4-C	**CO_2-C**	**N_2O-N**
C/N lost kg yr^{-1} Lower	15.5	79.8	0.04
Upper	24.5	112.6	0.07
Total C/N lost via GHG lower	95.3		0.04
Upper	137.1		0.07
Sedimentation rate kg y^{-1}	820		56
Net retention rate kg yr^{-1}	**683-725**		**55.9**

CH_4 accounted for 16-18 % of the total carbon transferred. Annual retention of C and N in the sediment was 820 and 56 kg yr^{-1} respectively (Ockenden *et al.*, 2014). Accounting for loss of C though CO_2-C and CH_4-C, the net annual retention rate of carbon in the sediments was 683-725 kg C yr^{-1}. N_2O-N accounted for losses of 0.04-0.07 kg yr^{-1} of N. Wetland retention of N was 56 kg N yr^{-1}. In all cases, contributions of CO_2, CH_4 and N_2O to C and N transfer were greater from the shallow cell compared to the deep cell.

3.6. Pathways of gas flux

3.6.1. Comparison between chamber measurements and modelled diffusive flux

The TBL model predictions of diffusive CH_4 flux (using the relationship of Cole and Caraco, 1998) between January and December 2013, showed poor agreement with chamber estimates (Appendix I, Fig 8.1), indicated by low Spearman correlation coefficients from both deep (r_s= 0.33, *p=0.176*) and shallow (r_s= 0.50, *p=0.034*) cells. Predicted fluxes were on average 0.5 - 6% of those observed in summer and autumn. Agreement only improved during winter when fluxes were low. Mean daily diffusive flux was 17.7 mg m^{-2} d^{-1} for the entire wetland, and 17.9 and 17.6 mg m^{-2} d^{-1} for the deep and shallow zones respectively. This was lower than the mean flux chamber estimates for the corresponding areas (191, 212 and 169 mg m^{-2} d^{-1}).

Predictions for CO_2 used the relationship of Crusius and Wanninkof, (2003) and yielded better agreement with chamber estimates from the shallow cell (Appendix I, Fig 8.1) (r_s= 0.81, *p<0.001*) once outlying points were removed. Agreement in the deep cell was lower

(r_s= 0.58, *p=0.012*) but still significant. Agreement improved from summer 2013 until the end of the experiment. Wind speed was also higher at some points of over prediction e.g. 5/02/13, 22/04/13. Mean daily calculated flux was 4338 mg m^{-2} d^{-1} for the entire wetland, and 5701 and 2976 mg m^{-2} d^{-1} for the deep and shallow cells respectively. This was higher than mean flux chamber estimates in the corresponding areas (2885, 3521 and 2249 mg m^{-2} d^{-1}).

Spearman correlation analysis showed significant agreement between the model (using the power relationship of Cole and Caraco, 1998) and chamber fluxes of N_2O in both the deep (r_s= 0.69, *p=0.001*) and shallow cells (r_s= 0.71, *p=0.001*)(Appendix I, Fig 8.1). During the winter and spring, the model tended to over-predict N_2O flux by approximately a factor of 2 in the deep cell and 1.5 in the shallow cell. Agreement improved during the summer when flux chamber measurements were lower, before reverting to over-prediction in the autumn. Mean daily calculated flux was 1.93 mg m^{-2} d^{-1} for the entire wetland, and 2.04 and 1.83 mg m^{-2} d^{-1} for the deep and shallow zones respectively. This was higher than mean flux chamber estimates in the corresponding areas (1.1, 1.25 and 0.93 mg m^{-2} d^{-1}).

3.6.2. Temporal and spatial variation in bubble ebullition

The volumes of bubbles varied greatly (0-664 ml m^{-2} d^{-2}) throughout the year, with an annual average of 125 ml m^{-2} d^{-1} across the wetland. Mean rates from the deep cell (171 ml m^{-2} d^{-1}) were larger than those from shallow cell (87 ml m^{-2} d^{-1}), though not significantly (t-test, *p=0.091*) due to a period in winter where deep cell ebullition was zero. Bubble production (Fig. 3.8) peaked during July and showed lowest activity during the winter. Uncharacteristically low volumes of bubble production were also noted at points during the summer months (June 2013) in both wetland wells.

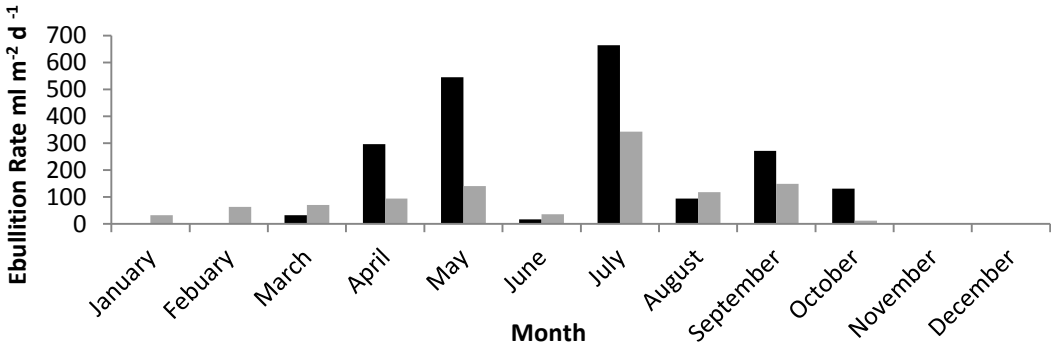

Figure 3.8 Volumes of ebullitive gas captured by bubble traps in deep and shallow zones for 24 hrs from January-December 2013. Black and grey bars denote ebullition from deep and shallow cells respectively.

3.6.3. Composition of gas bubbles and ebullitive gas fluxes

The composition of gas bubbles varied considerably between samples collected from the same site and time. When averaged across all collection sites and times, bubble compositions were 35% CH_4, 0.6% CO_2 and 0.2% N_2O, with the composition percentages being approximately equal in deep and shallow wetland cells. CH_4 was the main constituent in all samples, varying between 5-68% and generally increasing in line with bubble production rate when observing mean composition within each month. No trends were observed in the composition of CO_2 or N_2O, with the exception of mean N_2O increased to around 1% during the autumn. Bubble samples also contained unknown quantities of O_2 and N_2.

Due to an incomplete composition sample set and sample variability, mean gas compositions between April-September were used to estimate ebullitive gas fluxes in spring and summer (Table 3.6). Compositions from October 2013 were used to estimate fluxes during the rest of the year. Entire wetland, deep and shallow cell ebullitive emissions of CH_4 were 344, 438 and 219 mg m^{-2} d^{-1} respectively. Fluxes from the deep cell were higher than from the shallow cell except during winter. Spearman correlation analysis revealed a reasonable agreement between bubble CH_4 flux and chamber fluxes from the deep cell, exhibiting an almost log-linear relationship (r_s= 0.97, *p=0.000*) given by over predicting fluxes when periods of bubble activity were high. There was a positive linear relationship (r_s= 0.63, *p=0.029*) between chamber and bubble fluxes in the shallow cell with little over-prediction. Ebullitive CO_2 fluxes across the wetland, in the deep and shallow cell were much smaller at 13.3 mg m^{-2} d^{-1}, 23.4 mg m^{-2} d^{-1} and 6.5 mg m^{-2} d^{-1} respectively. Comparison with chamber fluxes showed very little relationship, ebullitive emissions being negligible in comparison with those observed using the chambers. Ebullitive fluxes of N_2O from whole, 0.0021 mg m^{-2} d^{-1}, deep 0.0021mg m^{-2} d^{-1} and shallow 0.0017 mg m^{-2} d^{-1}cells, were much lower than emissions measured by chambers and showed little relationship in correlation analysis.

3.6.4. Contribution of individual transfer pathways to total gas emissions

Mean diffusive emissions of CO_2 (Table 3.6) were greater than chamber derived estimates from the deep and shallow cells by 62 % and 32 % respectively. In contrast, ebullitive CO_2 releases made up <1 % of chamber estimates in both cells. Diffusive emissions of N_2O were

63 % (deep) and 97 % (shallow) greater than the chamber estimates. Ebullitive releases made up <1 %. Diffusive CH_4 emissions rates were only 8.4 % (deep) and 10.4 % (shallow) of the chamber estimates. Meanwhile, ebullition derived fluxes of CH_4 were 106 % and 30 % higher than chamber estimates in deep and shallow cells respectively.

The sum of ebullitive and diffusive pathways were combined to form an upper estimate of emissions, with respect to those derived from flux chambers. Estimates from these two pathways were also scaled to equal 100 % of emissions, permitting their relative contribution to this upper estimate to be calculated. Ebullitive pathway contributions to CH_4, CO_2 and N_2O flux were 96 %, 0.4 % and 0.1 % in the deep cell and 93 %, 0.2 % and 0.1 % in the shallow cell. Diffusive pathway contributions to CH_4, CO_2 and N_2O flux were 3.9 %, 99.9 % and 99.6 % in the deep cell and 7.4 %, 99.9 % CO_2 and 99.8 % N_2O in the shallow cell. The proportions of these contributions changed throughout the year, particularly in the case of methane. Ebullitive methane contributed to most emissions from the deep zone, but had no contribution to winter fluxes (zero bubble rate). In the shallow pond, bubbling continued through winter 2012, though not during November and December 2013.

Table 3.6 Mean fluxes of CH_4, CO_2 and N_2O via respective flux pathways to the atmosphere. Fluxes are averages of a years data from individual wetland cells and the wetland as a whole.

	CH_4 mg m^{-2} d^{-1}			CO_2 mg m^{-2} d^{-1}			N_2O mg m^{-2} d^{-1}		
	Whole	Deep	Shallow	Whole	Deep	Shallow	Whole	Deep	Shallow
Chamber	191	212	169	2885	3521	2248.89	1.09	1.25	0.93
Ebullition	344	438	219	13.31	23.4	6.5	<1	<1	<1
Diffusion	17.8	17.9	17.6	4338	5701	2975	1.94	2.04	1.83
Diffusion +Ebullition	361	456	237	4351	5725	2975	1.94	2.04	1.83

3.7. Controls on greenhouse gas fluxes

3.7.1. CH_4 fluxes

Correlations between physicochemical variables and flux estimates from chambers, ebullition and diffusive releases revealed numerous potential controls on CH_4 fluxes (table 3.7). CH_4 fluxes in both cells captured in the chambers and via ebullition, generally exhibited very similar relationships with variables such as water temperature and water level. Conversely, diffusive fluxes did not appear to be effected. Only fluxes from the shallow cell displayed any relationships with the dissolved oxygen or nitrate concentration of the bottom water.

Table 3.7 Spearman's rank order coefficients for CH_4 transfers and physicochemical/nutrient conditions in the bottom waters of respective wetland cells.

	Chamber Deep	Chamber Shallow	Ebullition Deep	Ebullition Shallow	Diffusive Deep	Diffusive Shallow
Air Pressure	0.22	0.23	0.31	**0.66***	NA	NA
Water depth	**-0.59****	**-0.60****	**-0.73****	**-0.77****	NA	NA
Wind speed	0.29	0.28	0.46	-0.20	**0.49***	**0.75****
Water Temperature	**0.59****	**0.82****	**0.67***	**0.73****	0.15	0.27
[DO]	-0.27	-0.38	0.05	0.08	0.23	-0.21
RP	-0.257	-0.36	-0.15	0.039	0.05	0.16
pH	**-0.63****	-0.120	**-0.74****	**-0.61***	-0.22	0.02
[NO_3]	-0.28	**-0.42***	-0.46	-0.47	0.10	**-0.48***
[DOC]	0.39	**0.47***	0.42	0.45	0.19	**0.59***
[CH_4] surface	0.16	**0.53****	0.27	0.51	**0.77****	**0.93****
[CH_4] bottom	0.27	0.24	0.19	0.48	0.31	**0.57****

*significant at 0.05 level ** significant at the 0.01 level

Chamber Deep/Shallow *n*=27. Ebullition Deep/shallow and Diffusive Deep/Shallow, *n*=20

3.7.2. CO_2 fluxes

CO_2 fluxes from the deep and shallow cells exhibited contrasting behaviour with respect to controlling factors (Table 3.8). With the exception of dissolved CO_2, estimates from both chamber and diffusive methods only showed significant relationships within the shallow cell. Due to the negligible size of ebullitive emissions, they were omitted.

Table 3.8 Spearman's rank order coefficients for CO_2 transfers and physicochemical/nutrient conditions in the surface waters of respective wetland zones.

	Chamber Deep	**Chamber Shallow**	**Diffusive Deep**	**Diffusive Shallow**
Wind speed	0.1	-0.07	**0.62****	-0.08
Water Temperature	-0.09	-0.24	-0.14	-0.37
[DO]	-0.28	-0.33	-0.39	-0.46
RP	0.18	0.24	-0.09	0.0
pH	0.21	**-0.46***	0.02	**-0.64****
[DOC]	0.04	-0.04	0.15	0.09
[CO2] SURFACE	**0.48***	**0.84****	0.29	**0.76****
[CO2] BOTTOM	0.38	**0.79****	0.32	**0.75****

*significant at 0.05 level. ** significant at the 0.01 level
Chamber Deep/Shallow *n*=27. Ebullition Deep/shallow and Diffusive Deep/Shallow, *n*=20

3.7.3. N_2O Emissions

N_2O fluxes (Table 3.9) captured in flux chambers and diffusive techniques were significantly correlated with dissolved N_2O at both the surface and sediment water interface of the wetland. Additionally, chamber fluxes were positively correlated with NO_3 concentrations. Negative correlations with water temperature were also observed will all but chamber flux estimates in the deep cell. The ebullitive pathway was considered negligible and so omitted.

Table 3.9 Spearman's rank order coefficients for N_2O transfers and physicochemical/nutrient conditions in the bottom waters of respective wetland zones.

	Chamber Deep	**Chamber shallow**	**Diffusive Deep**	**Diffusive Shallow**
Wind speed	0.09	-0.19	0.08	0.19
Water Temperature	-0.23	**-0.44***	**-0.53***	**-0.66****
[DO]	-0.21	0.12	0.25	0.23
RP	0.01	0.15	0.08	0.41
pH	-0.14	0.11	0.27	0.06
[NO_3]	**0.49***	**0.59****	0.39	0.41
[NH_4]	-0.03	0.30	-0.40	0.02
[DOC]	-0.11	0.08	-0.26	-0.11
[N_2O] SURFACE	**0.59****	**0.76****	**0.73****	**0.77****
[N_2O] BOTTOM	**0.58****	**0.73****	**0.59***	**0.69****

*significant at 0.05 level. ** significant at the 0.01 level. Chamber Deep/Shallow *n*=27.
Ebullition Deep/shallow and Diffusive Deep/Shallow, *n*=20

3.8. Discussion

3.8.1. Do agricultural wetlands increase GHG fluxes when compared with undisturbed land?

Mean wetland emissions of CH_4, CO_2 and N_2O were significantly higher than those emanating from the undisturbed riparian area, on an area for area basis. This confirms that installation of an agricultural wetland increases GHG emission above those occurring naturally. Mean CH_4 flux (-0.05 ± 0.08 mg m^{-2} d^{-1}) from the undisturbed area was lower than anticipated, given the boggy organic rich nature of the soils. The low rates of CH_4 emission/ periods of uptake, suggested that any CH_4 produced may have been oxidised in the upper soil layers (Le Mer and Roger., 2001). Positive fluxes were only observed during episodes of near total soil saturation or waterlogging (Appendix II, Fig. 8.2) suggesting this oxidation mechanism was halted when pore spaces were totally saturated or alternatively, that production exceeded oxidation. The saturated soil also created optimum conditions for N_2O production, although fluxes (0.3 ± 0.04 mg m^{-2} d^{-1}) were significantly lower than those from the wetland. It is possible that the low emissions were caused by a low supply of available NO_3 as the area was not subject to fertilizer applications. Increased emissions during winter and spring may be associated with surface water originating from improved grassland, periodically inundating this zone. This had NO_3-N concentrations of 2-3 mg L^{-1} (data not shown) which may have temporarily permitted higher N_2O production. In contrast to the wetland, the riparian area was a strong sink of atmospheric CO_2 (-1964 mg m^{-2} d^{-1}), largely due to chamber measurements including vegetation. Strong uptake in summer, late spring and autumn and winter was contrasted with negligible fluxes from February to April 2013. This may be due to a mild winter delaying senescence of vegetation and very wet conditions reducing CO_2 release from the soils. When senescence finally occurred during a cold period with snow cover, uptake and emission of CO_2 became almost negligible.

3.8.2. Which GHG released is most significant in terms of potential climatological impact?

Methane was the most significant greenhouse gas emitted in terms of potential impact on climate. Combined transfers of GHGs from the agricultural wetland were equivalent to 2.83 – 4.36 kg CO_2 per day (Table 3.4). CH_4 was the most significant gas in terms of impact, accounting for 68-70 % of emissions due to high GWP and flux rates. On a seasonal basis, CH_4

was also the most significant, particularly during summer and spring when fluxes of CO_2 (26-28 %) fluctuated between uptake and emission. Only during the winter, when CH_4 emissions effectively ceased, did CO_2 become a more significant emitter. During all times of year, the fluxes of N_2O, contributed the least (3-4 %) to the climatological impact of the wetland. As such, CH_4 can be considered as the most climatologically significant GHG being emitted from the wetland, followed by CO_2 and lastly N_2O. Other evidence from preceding wetland studies (e.g. VanderZaag, *et al.,* 2010) suggests that CH_4 would also be a dominant gas were the wetlands to be installed more widely in the landscape. The exception to this may be where particularly high levels of NO_3-N were present in runoff from agricultural land, leading to enhanced N_2O emissions (Verhoeven *et al.,* 2006).

As the unproductive riparian area was a sink of CO_2 and CH_4 from the atmosphere, the conversion of this land into an agricultural wetland and GHG source (Table 3.4) meant the net effect of wetland creation was even more significant than if solely considering wetland emissions. This was particularly due to the heavily vegetated unproductive area exhibiting such intense uptake of CO_2 for most of the year. The loss of this uptake therefore increased the net CO_2 equivalent emissions from the wetland conversion considerably (13-21 %). Due to the riparian fluxes of CH_4 and N_2O being very low, the impact on net emission increase is much smaller. However, even when considering the net effect of land conversion, gas emissions from the wetland were still dominated by CH_4 on an annual basis, further emphasising the significance of the flux rates were observed.

3.8.3. What are the implications for carbon and nitrogen losses via GHG release?

Although the wetland was a net source of greenhouse gases to the atmosphere, it still functioned as a sink of carbon and nitrogen within the agricultural catchment. Rates of annual carbon loss from the wetland (Table 3.5) (95-137 kg C yr^{-1} or 0.26-0.38 kg C d^{-1}) via GHG emissions were much lower than the 6.2 kg C d^{-1} from Priests Pot, a natural shallow eutrophic lake (area 1 ha) also located within an agriculturally dominated catchment in Cumbria (Casper *et al.,* 2000). This however was an old system many times the size of the wetland, although illustrates that the outward carbon flux from the study wetland was high. Due to high rates of C retention in sediment, the wetland was still a sink of carbon, capturing a net mass of 694-725 kg C yr^{-1}. Annual carbon losses were 11-17 % of that retained, mainly via CO_2 (80-113 kg) due to higher mean flux rates. CH_4 fluxes accounted for 16-25 kg of the carbon lost, representing 1.8-2.9 % of the carbon retained in the wetland. This percentage of

losses is around a factor of 10 lower than values reported in Scandinavian treatment wetlands (Søvik *et al.,* 2006, Søvik and Kløve, 2007). This is likely to be due to the high sedimentation rate, (and thus also TC) in the study wetland, particularly due to the sandy erosion-prone soils within the catchment. Other agricultural wetlands monitored in the MOPS2 project exhibited lower rates of sediment and carbon retention (Ockenden *et al.,* 2014). It is therefore likely that the ratio of carbon losses to retention would be higher in these systems. However, it is probable that GHG emissions would be lower than our observations due to smaller quantities of source material. The wetland was also subject to periods of C uptake and so, due to the nature of this relationship, and the variable delivery and entrainment of C, the balance between C emission and retention may shift considerably over time. However this would require further investigation. The annual loss of N via N_2O-N emission (0.04-0.07 kg N yr^{-1}) was considerably lower than that of carbon. These losses are insignificant in comparison to the 56 kg yr^{-1} N retained within the sediments (Ockenden *et al.,* 2014), placing N emission via N_2O N as <0.01 % of retained mass. This ratio between emission and retention is in the same range as values observed in several other wetland examples, both of FWS (Søvik et al., 2006, Søvik and Kløve, 2007, VanderZaag *et al.,* 2010) and SSF types (Søvik et al., 2006, VanderZaag *et al.*, 2010) dealing with a variety of wastewater inputs.

3.8.4. How do GHG fluxes from the study wetland compare with analogous systems?

CH_4 fluxes

The agricultural wetland was a net source of methane to the atmosphere throughout the study, exhibiting a wide range of flux rates (1.31-1115 mg m^{-2} d^{-1}). This year round emission of CH_4 was expected, as the anoxic conditions which lead to CH_4 production generally perpetuate year-round within the bottom sediments, despite the physicochemical conditions of the overlying water column being highly variable (Casper *et al.,* 2000).

Mean fluxes across the whole wetland (190 – 361 mg m^{-2} d^{-1} and 212 – 456 mg m^{-2} d^{-1} and 169 – 236 mg m^{-2} d^{-1} from the deep and shallow cells respectively) and range of observed fluxes were similar to those in the limited studies conducted on other FWS wetlands draining agricultural catchments (Table 3.10) (Søvik *et al.,* 2006; Stadmark and Leonardson, 2005). This was despite these systems being larger and more established than the study wetland,

which has been identified as a contributor to greater emissions (Liikanen *et al.*, 2006). Observations made by Stadmark and Leonardson (2005), where wetland sites received field drainage, and were of similar depth and antecedent conditions, displayed a similar magnitude, range and seasonality of CH_4 fluxes. This suggested that the observations regarding greenhouse gas emissions from agricultural wetlands in continental Europe may be more broadly applicable than first posited. However, the high degree of variation in summer CH_4 fluxes in this study was not mirrored in other agricultural wetland systems, which were often characterised by a singular peak during the summer growing season. The significantly lower CH_4 fluxes (close to 0) observed during winter and spring in this study, were in agreement, with those observed in Stadmark and Leonardson (2005). In the case of Hovi, a 0.6 ha shallow vegetated lake receiving agricultural runoff in Finland, (Søvik *et al.,* 2006) summer emissions were lower than this study.

Observations were also within the range (Table 3.10) of those recorded in treatment wetlands dealing with municipal wastewater (Mander *et al.,* 2005a; Teiter and Mander, 2005; Søvik *et al.,* 2006; Søvik and Kløve, 2007; Johannson *et al.,* 2004), peat mining runoff (Liikanen *et al.,* 2006) and dairy/agricultural wastewater (Tanner *et al.,* 1997, VanderZaag *et al.,* 2010). The similarity with these observations was surprising, as engineered systems generally receive a higher loading of organic matter and nutrients than the study site, and so would be expected to have a higher potential for CH_4 fluxes. This may be because many of the comparable study sites, while in temperate climates, are based in Scandinavia or at higher latitudes, and as such may experience a lower level of productivity associated with lower annual temperatures when compared to the relatively mild conditions in this study. Therefore despite lower loading in the study wetland, other constraints on productivity may not be as strong.

Fluxes of CH_4 during winter (4-15 mg m^{-2} d^{-1}) were unexpectedly lower in the study wetland than from FWS systems which could experience lower winter temperatures (Søvik *et al.,* 2006). This could be attributed to the lack of labile carbon being made available through vegetative decay in winter due to the study wetland lacking any macrophyte community, unlike in Hovi (Søvik *et al.,* 2006). However, given the wetland had a large supply of carbon in the sediments (Ockenden *et al.,*2014), it was more likely to be because an ice layer did not develop on the study wetland, as in northern wetlands and lakes (Huttenen *et al.,* 2003; Stadmark and Leonardson 2005; Søvik and Kløve, 2007). Although there were short periods of ice cover in the study wetland during winter/spring, a prolonged period of total ice cover

does not generally occur in the UK. As such, oxygen could still diffuse down to the bottom sediments rather than anoxic conditions being generated under an ice cap, as shown by moderate DO levels in both cells during winter (Fig. 3.3).

Table 3.10 Emissions (mean or range) of CH_4 reported in analogous treatment wetlands. FWS- Free water surface, HSSF-Horizontal subsurface flow, SSF-Subsurface flow.

Type	Source	Location	mg m^{-2} CH_4 d^{-1}	Study
FWS	Agricultural runoff	Cumbria, UK	1.31 - 1115	This Study
FWS	Agricultural runoff	Sweden	14-1296	Stadmark and Leonardson, 2005
FWS	Agricultural runoff	Finland	38 (Summer) 46 (Winter)	Søvik *et al.,*2006
HSSF	Agricultural wastewater	New Zealand	45-526	Tanner *et al.,* 1997
FWS	Agricultural wastewater	Canada	260	VanderZaag *et al.,* 2010
SSF	Agricultural wastewater	Canada	136	VanderZaag *et al.,*2010
HSSF	Municipal Wastewater	Estonia	-0.041 - 2796	Mander *et al.,* 2005a
FWS	Municipal Wastewater	Norway	-1.6 - 2546	Søvik and Kløve, 2007
FWS	Municipal Wastewater	Sweden	-375 - 1739	Johannson *et al.,* 2004
SSF	Peat mining wastewater	Finland	140-200 (mean at 1 and 10 yrs	Liikanen *et al.,* 2006, Søvik *et al.,* 2006
SSF	Municipal wastewater sludge	Spain	10-5400	Uggetti *et al.,* 2012

Table 3.11 Emissions (mean or range) of CH_4 reported in analogous natural lake or reservoir systems.

Type	mg m^{-2} CH_4 d^{-1}	Study
Hypereutrophic lake, UK	204	Casper *et al.,* 2000
Northern lakes USA	26	Smith and Lewis, 1992
Boreal Lakes	1.1-120	Repo *et al.,*2007
Temperate Lake	1.3-202	Fernandez *et al.,* 2014
Finnish Reservoirs	1.6-192	Huttenen et al 2003
Mid-Latitude Reservoir	200	Beaulieu *et al.,* 2014
Temperate Reservoirs	10-80	St. Louis *et al.,* 2000

Observed fluxes exceeded the mean estimates for CH_4 emission in lakes/ reservoirs (Table 3.11), such as 26 mg m^{-2} d^{-1} in Smith and Lewis (1992), and those of temperate reservoirs (St. Louis, 2000) and northern lakes (Repo *et al.,* 2007). However, the lower estimate (flux chamber) of emissions (191 mg m^{-2} d^{-1}), was similar to higher productivity systems, such as the 204 mg m^{-2} d^{-1} observed on Priests Pot (Casper *et al.,* 2000), a small eutrophic lake in the

UK Lake District. There were also similarities with mean fluxes from a reservoir with an agriculturally dominated catchment (200 mg m^{-2} d^{-1}) made by Beaulieu *et al.* (2014) and those with substantial proportion of their catchments used for agriculture in Finland (Huttenen *et al.,* 2003). While estimates of CH_4 flux from such deeper lake/reservoir systems are prone to under-estimation (Beaulieu, *et al.,* 2014), the similarities of these values with our lower flux estimate, suggests that the CH_4 emissions observed in this study are exceptionally high. This may be caused by the relatively high degree of sedimentation the wetland receives in relation to its size, together with other physical differences which permit higher levels of productivity and lower levels of CH_4 removal (oxidation) than other larger systems. Unlike many larger and deeper lakes or reservoir systems, there was no evidence in the results of secondary high flux periods from turnover induced methane release during the autumn months (Fernandez *et al*., 2014; Bastviken *et al.,* 2004), due to the system being too small to facilitate development of a sustained hypolimnion and anoxic zone. However the potential for short term stratification and overturning may be significant, as will be discussed in Chapter 4.

CO_2

Emissions of CO_2 were the highest of all GHGs monitored, although fluxes included both uptake and emission during the study period (-1231 - 10752 mg CO_2 d^{-1}). Mean annual flux was 2885-4041 mg m^{-2} d^{-1} from the entire wetland and 3521-5454 mg m^{-2} d^{-1} and 2249-2633 mg m^{-2} d^{-1} from deep and shallow cells respectively. Though having a wider variation than some other systems (Table 3.12), these emissions were relatively similar to a range of wetland and lake environments. Mean summer emissions were close to values reported from treatment wetlands in Scandinavia (Søvik *et al.,* 2006) although, conversely during the winter, the study wetland displayed a higher mean CO_2 flux than many contemporary engineered and natural systems. This lack of agreement may be largely due to emissions from more northerly wetlands being reduced in winter, when productivity is lower due to low temperatures and thus results in lower microbial activity.

Table 3.12 Emissions (mean or range) of CO_2 reported in natural lake systems. FWS- Free water surface, HSSF-Horizontal subsurface flow, SSF-Subsurface flow.

Type	Source	Location	mg m^{-2} CH_4 d^{-1}	Study
FWS	Agricultural runoff	Cumbria, UK	-1231 - 10752	This Study
FWS	Agricultural (Hovi)	Finland	4397(Summer) 769 (Winter)	Søvik *et al.,*2006
Reservoirs		Finland	-79-3212	Huttenen *et al.,* 2003
Boreal lake		Siberia	-70-3100	Repo *et al.,* 2007
Hypereutrophic lake		Cumbria, UK	1760	Casper *et al.,* 2000
Temperate Lakes		Temperate regions	750-3100	St. Louis *et al.,* 2000

N_2O emissions

Emissions of N_2O were lowest of all the three gases under investigation, with a range of -0.31-5.78 mg m^{-2} d^{-1}. Mean daily fluxes of 1.1-1.67 mg m^{-2} d^{-1} were observed for the whole wetland and 1.25-1.8 and 0.93-1.55 mg m^{-2} d^{-1} for the deep and shallow zones respectively. These emissions fall within the range of those observed for wetlands treating municipal wastewater of the FWS (Table 3.13) (Søvik and Kløve, 2007; Johansson *et al.,* 2003; Søvik *et al.,* 2006; VanderZaag *et al.,* 2010), and SSF types (Søvik *et al.,* 2006; Teiter and Mander, 2005; VanderZaag *et al.,* 2010) and highly loaded agriculturally dominated environments (Reay *et al.,* 2003; Wilcock and Sorrell, 2008; Hefting *et al.,* 2003). This indicated that the emissions measured were significant. However, the upper range of emissions in other systems far exceeded our observations. Generally, higher emissions in other studies were reported during the summer period with lower emissions during the winter. Although low winter N_2O emissions in our study were also observed, the lowest emissions actually came during the summer months, including occasions of uptake. While this behaviour was not exclusive to our study, it was unusual in such systems and indicated a complex biogeochemical relationship.

Table 3.13 Emissions (mean or range) of N_2O reported in analogous treatment wetlands. FWS- Free water surface, HSSF-Horizontal subsurface flow, SSF-Subsurface flow.

Type	Source	Location	mg m^{-2} CH_4 d^{-1}	Study
FWS	Agricultural Runoff	Cumbria, UK	-0.31-5.78	This study
FWS	Agricultural Runoff	Finland	1.25 summer 0.28 winter	Søvik et al., 2006
FWS	Municipal Wastewater	Norway	-1.53 - 345	Søvik and Kløve, 2007
HSSF	Municipal Wastewater	Estonia	0.024-196	Mander et al., 2005a
FWS	Agricultural wastewater	Canada	22.8	VanderZaag *et al.,* 2010
SSF	Agricultural wastewater	Canada	13.1	VanderZaag *et al.,* 2010
HSSF	Municipal Wastewater	Germany	0.9-11.28	Fey *et al.,* 1999
FWS	Municipal Wastewater	Sweden	-8.4-42.9	Johannson et al., 2003
SSF	Municipal Wastewater sludge	Spain	20-950	Uggetti *et al.,* 2012
Riparian Buffer (grass)	Agricultural Runoff	Netherlands	0.67-1.25	Hefting et al., 2003
Irrigation ditch	Agricultural Runoff	Scotland, UK	~24	Reay *et al.,* 2003
Agricultural stream	Agricultural Runoff	New Zealand	0-105	Wilcock and Sorrell, 2008

3.8.5. What were the dominant pathways of GHG evasion to the atmosphere?

Methane transfer pathways

Bubble ebullition was the main transfer pathway for CH_4 between the wetland sediments and the atmosphere, although diffusive emissions still contributed to overall fluxes. The poor agreement of CH_4 flux estimates using the chamber and TBL techniques suggested a failure of the model, or an overestimation of CH_4 fluxes using the chambers. Overestimation of gas fluxes by chamber methods in comparison to modelled techniques have been noted previously (Duchemin *et al*., 1999; Matthews *et al*., 2003), where unintentional disturbance or heating of the water surface may artificially enhance fluxes. Conversely, TBL estimates rely on wind speed, which when very low can lead to underestimation, as other drivers of turbulence such as precipitation start to have an increased impact (Cole and Caraco, 1998). However, failure of the model was unlikely as the same wind-flux relationships had yielded accurate predictions of flux elsewhere (Fernandez *et al.,* 2014, Repo *et al.,* 2007). Furthermore, predicted diffusive fluxes from the study wetland were strongly correlated with the concentration of dissolved CH_4 in the surface water, which suggested that they were accurate and that transfer via the diffusive pathway was low.

The significant correlations observed between chamber and ebullition measurements suggested ebullitive CH_4 transfer was primarily responsible for fluxes captured in the chambers and was thus the main methane transfer pathway. This was evident from temporal changes in bubble release matching changes in flux chamber estimates. Instances where ebullitive estimates exceeded those from the flux chambers estimates were mainly evident in the growing season, during which a majority of ebullitive releases were recorded, as has been observed elsewhere (Mattson and likens, 1990; Sorrel and Boon, 1992). This was likely to have been caused by the spatial heterogeneity of bubble release, coupled with the relatively longer (24hr) observation window used to trap samples.

The proportion of CH_4 transfer via diffusion or ebullition changed over the year, with bubbling dominating during spring-autumn, diffusion during winter. Bubble ebullition can also account for a large proportion of net CH_4 flux in lake and estuary environments (Chanton and Whiting, 1995; Bastviken *et al*., 2008; Huttenen *et al*., 2003; Casper *et al.,* 2000), due to the low solubility of methane in freshwaters (Yamamoto *et al.,* 1976). While deep lake systems generate high hydrostatic pressures which keep CH_4 in solution within the sediment pore water, shallower water columns lack this mechanism, permitting bubble formation and release (Joyce and Jewell, 2003). This relationship was evident in the study wetland due to the relatively high proportion of CH_4 within the bubbles, 5-68 % compared to dissolved methane in the water column. This was somewhat lower than the 90 % reported elsewhere (Martens and Chanton, 1989), although in the region of values reported by Huttenen *et al.* (2001) (55 %) and Repo *et al.* (2007) (38 % in fresh bubbles). Nevertheless ebullitive gas releases still made up 96 and 93 % of CH_4 flux in the deep and shallow cells respectively. These correspond with values reported in other lake environments e.g. 96 % by Casper *et al.* (2000) and 98 % by Miller and Oremland (1988). Although in the case of this study, mean diffusive emissions only contributed 4 %-7 % of emissions, dominance by the ebullition pathway is not universal in lake systems, with other studies reporting a large proportion of emissions being explained by dissolved CH_4 (Smith and Lewis, 1982; Stadmark and Leonardson, 2005). With this in mind, it should be noted that throughout the year the proportion of the contributions from each pathway changed considerably. As bubble release ceased in the deep zone during winter, emissions were exclusively by diffusion, thus explaining the improved agreement between the chamber measurements and predicted diffusive fluxes. Conversely, although diffusive fluxes accounted for a higher percentage of emissions in the shallow area, as ebullition was observed during winter (Fig. 3.9) the

dominance of diffusive emissions at this time was not as great. During the summer, most CH_4 fluxes were due to ebullition.

Figure 3.9 Gas bubbles trapped beneath ice in part of the shallow area of the constructed wetland during winter 2013.

A further two important transfer pathways were not present in the wetland system and so not considered in estimation of respective contributions. Firstly, as there was no rooted macrophyte community in the agricultural wetland, vegetative transfer of CH_4 could not have occurred. This has been observed in rice paddies (Schutz *et al.,* 1989), as well as natural wetland systems (Chanton *et al.,* 1992) and found to form the dominant transfer pathway, over ebullitive and diffusive emissions.

Also, despite some evidence of stratification in the water column (Fig 3.6), unlike deeper lake and reservoir systems the wetland did not appear to develop a prolonged hypolimnion, in which dissolved methane could be stored without being oxidised (Bastviken *et al.,* 2008; Casper *et al.,* 2000; Fernandez *et al.,* 2014; Rudd *et al.,* 1976). In these cases, during breakdown of stratification in the autumn the stored CH_4 is mixed into the upper water layers and can give rise to large diffusive fluxes to the atmosphere (Fernandez *et al.,* 2014). While this seasonally significant mechanism for diffusive fluxes was not observed in our sampling, as will be discussed in Chapter 4, temporally detailed investigation of the wetland suggested that turnover during diurnal cycles may facilitate significantly higher diffusive emissions of CH_4.

CO_2 transfer pathways

CO_2 fluxes from the wetland were dominated by diffusive transfers. This was demonstrated by the strong correlation between chamber and diffusive flux estimates, with only a small over-prediction of chamber CO_2 estimates from the shallow cell of the wetland. Significant correlation between dissolved CO_2 and both chamber and TBL estimates from the shallow zone also illustrated that diffusive transfers were the drivers of fluxes captured in the chambers. Estimated diffusive flux from the deep zone displayed poorer positive correlation and a 62 % over prediction of mean flux compared with the chamber method, suggesting that the model did not work as well. Although chamber estimates from the deep cell were significantly correlated with dissolved CO_2, TBL estimates were not, instead being positively related to wind speed. This suggested that the over-prediction of the TBL model may be due to over-estimation of the effect of wind turbulence driving gas transfer. This would make sense, as the positioning of the wetland, small size of the deep cell and its steep vegetated sides (Fig 3.10) shielded the water surface from the wind, unlike in the shallow zone which was more exposed. As the logging anemometer was positioned on the bank top (3 m above water surface), it is likely that it was exposed to more wind than the water surface. Greater overestimation during high wind speeds also supports this theory. As estimates of fluxes from the chambers did not utilise wind speed, this suggests that CO_2 concentrations were the driver of emissions. The high solubility of CO_2 meant that dissolved mean concentrations at the wetland surface were over 600 times higher than those of CH_4 over the course of the study (apart from periods of uptake). As such, molecular diffusion acted as the dominant transfer mechanism. Conversely, ebullitive CO_2 emissions were almost non-existent in both zones, with mean bubble compositions of only 0.6 %. This agrees with values of 0.02-0.57 % reported by Huttenen *et al.* (2001) and 3 % by Casper *et al.* (2000). The resultant contribution of only 1 % to chamber fluxes and 0.04-0.05 % of the upper emissions estimates illustrates that CO_2 transfer via ebullition is negligible in agricultural wetlands.

Figure 3.10 Sheltered location of the deep cell with steep vegetated banks.

N_2O transfer pathways

N_2O transfer from the wetland to the atmosphere was entirely by molecular diffusion. Due to high solubility in water, as in the case of CO_2, N_2O emissions are typically dominated by diffusive transfer. The TBL estimates, though showing strong positive correlation with flux chamber observations, seldom agreed with the emission rates. This has been noted in other studies where air-water transfer models have been employed such as in Reay *et al.* (2003). Under and over-estimation was observed, possibly attributable to the very low concentrations and fluxes of N_2O, and as such amplification of any inaccuracy. Despite this, the mean diffusive flux rate was substantially higher than that from the chambers. It is likely that the reason for this, as was previously posited, was that the wind speed used may have been higher than that acting on the water, due to shielding by the topography or vegetation. It may also be the case that as the chamber blocked the wind, the diffusive transfer of N_2O was limited. Also, some studies have hypothesised that due to the high constituent volume of N_2 noted in various bubble samples, N_2O may also be transferred by the ebullitive pathway (Gao *et al.,* 2013). However, the bubbles in this study contained a very low proportion of N_2O and as such contributed less than 0.2 % of the total emissions. It is even possible that the small quantity observed may have originated from the atmosphere or entered the sample during transfer and analysis.

3.8.6. What are the controls on observed gas fluxes in constructed agricultural wetlands?

Physical triggers of GHG release from bottom sediments

Increased CH_4 flux was driven by temperature increases as well as reductions in wetland water level, although air pressure reduction and storm-flow induced sediment disturbance may also have influenced pulses of ebullition.

The strong seasonality of CH_4 fluxes and positive correlations between chamber/ebullitive observations and temperature at the sediment water interface, illustrated that increasing temperature was driving greater methane production. This agrees with studies in natural and engineered systems, which reported significantly higher CH_4 emissions during summer or periods of warmer temperatures (Sorrell and Boon, 1992; Søvik *et al.,* 2006; Stadmark and Leonardson, 2005, 2007; VanderZaag *et al.,* 2010). Temperature is a key driver in the rates of many microbial processes, particularly methanogenesis (Schutz *et al.,* 1989; Conrad, 1996) and may also physically increase bubble ebullition by causing trapped gas to expand (Chanton *et al.,* 1989; Martens and Chanton, 1989). Temperature was therefore likely to be the key control of methane fluxes. The stronger relationship observed between fluxes and temperature in the shallow zone was likely to be because the shallower water column facilitated quicker responses of the sediment temperature to air temperature. The lack of correlation between diffusive GHG emissions/concentrations and temperature suggested other factors were influencing fluxes to a greater extent i.e. uptake in the water column.

Reductions in hydrostatic head (air and water pressure) acting on bubbles of gas within sediments has facilitated increased ebullitive gas fluxes from lakes, rivers and estuaries (Keller and Stallard., 1994; Chanton and Whiting, 1995; Martens and Chanton, 1989; Bartlett *et al.,* 1988; Smith and Lewis; Casper *et al*., 2000; Devol et al., 1988, 1990). Temporally variable inputs meant the wetland water level varied by up to 0.8 m over the study (Chapter 2, Fig.2.8). While short term changes in depth were smaller, variability was greater than many natural or engineered systems, increasing the potential to trigger ebullitive pulses. Ebullitive and chamber estimates were strongly negatively correlated with water levels, with stronger correlations in the shallow cell possibly due to less pressure exerted by the water column. This is in agreement with studies where bubble release has varied with tides and periodic flooding (Keller and Stallard, 1994; Martens & Chanton, 1989). Air pressure did not

appear to significantly influence ebullitive or chamber flux estimates, contradicting observations of significant increases (1.2-10x rate) in ebullition with lowered (1-3 %) atmospheric pressure (Mattson and Likens, 1990; Casper *et al.,* 2000). However, these observations were made under deeper and relatively stable water level conditions. While the low depth of water (and thus pressure) could mean that atmospheric pressure had a large influence, it appeared that this was not the case and the effect of the water column still dominated.

The summer was characterised by low water level and high rates of CH_4 bubbling, though interspaced with low methane/bubble fluxes, suggesting the presence of additional controlling mechanisms. Low fluxes were observed on 17/06/2013, when water level was low, water temperature was high and air pressure was 1002 mbar. These conditions should not have inhibited bubble release, although in the preceding week it was found that the site had experienced a large drop in pressure from 1011 to 987 mbar (1%) and a period of slightly warmer weather. This may have triggered a large ebullition event prior to the sampling visit and thus temporarily depleted the quantity of bubbles available for release, resulting in the low fluxes observed. This scenario may have repeated on 19/08/14, where reduced bubble production followed a preceding low pressure event. Therefore, while hydrostatic pressure associated with water depth dominated, under certain conditions atmospheric pressure may act as a trigger for bubble release. This illustrates how our instantaneous estimates of gas fluxes may be influenced by antecedent conditions when particular conditions such as low water level are present.

Sediment disturbance via storm flow (Fig 3.11) may have also increased GHG fluxes. Inputs via runoff resulted in intense but variable flow events (Jordan *et al.,* 2003), which flushed through the system. Near-bottom currents have been associated with sediment disturbance and pulses of gas and nutrient release in deeper lake systems (Joyce and Jewell, 2003; Busmann *et al.,* 2005; Murase *et al.,* 2005), while flow induced turbulence in the water column increased emissions from agricultural streams (Wilcock and Sorrell, 2008). The lack of vegetation in the wetland at the time of study possibly enhanced sediment vulnerability to disturbance, as in other aquatic systems rooted vegetation protects sediments by stabilising the water column (Chimney *al.,* 2006).

Figure 3.11 Storm-flow entering the agricultural wetland.

Given the variable nature of large storm events, only one was captured during gas sampling on 20/05/13 when water level increased by 0.6 m. Rapid flow and spikes in CH_4 (and CO_2 and N_2O) emissions were observed during the falling limb of the event suggesting disturbance had facilitated gas release and water column mixing. Although nutrient and dissolved gas concentrations did not mirror the spike, it is possible that storm water diluted the observed peak. Instantaneous groundwater exchanges (Chapter 2, Fig 2.8) during storm events may also add to disturbance of sediments and therefore contribute to bubble/dissolved gas release. Due to the highly variable temporal nature of events and potential differences in antecedent conditions, greater temporal and spatial resolution of measurements are required in order to elucidate these mechanisms. These uncertainties will be re-examined in greater detail in Chapter 5.

3.8.7. Production and consumption of GHGs in wetland sediments

Emissions or dissolved fractions of CH_4 and CO_2 from the deep cell were significantly higher than those from the shallow cell, possibly due to greater quantities of material available for decomposition. Accumulation of a larger mass of sediment in the deep cell (Ockenden *et al.,* 2014) meant a greater quantity of carbon was available. Under anaerobic conditions organic carbon is essential for the production of the necessary substrates for methanogenesis such as H_2 (Achtnich *et al.,* 1995; Kluber and Conrad, 1998a). Similarly, under aerobic conditions higher quantities of allochthonous organic carbon in the aquatic system are associated with

increased emissions of CO_2 (Sobek *et al.,* 2003). The quality of organic matter impacts on the rate of methanogenesis (Søvik *et al.,* 2006) with newer material increasing production (Mathews *et al.,* 2005; Jones and Simon, 1980). Given that the wetland was <10 years old and continuously received organic matter, the amount of bioavailable carbon was probably high, particularly around the inlet. The limited evidence of relationships between fluxes (chamber and diffusive) of CH_4 and CO_2 with aquatic DOC concentrations, suggested that emissions were not related to the quantity of material in the water column as in other, high productivity lake systems (Sobek *et al.,* 2003). Instead, the higher concentrations of dissolved gases at the base of the water columns suggested that sediments were the source.

Emissions of CH_4, CO_2 and N_2O displayed no significant relationships with oxygen at the sediment-water interface. Methane production may take place deeper in the sediment profile, while N_2O was limited by NO_3-N and CO_2 fluxes controlled by uptake processes. The presence of competing preferential terminal electron acceptors for organic matter decomposition can limit production of CH_4 (Achtnich *et al.,* 1995). The chain of substances utilised in these reactions are consumed in order of their redox potential (Stumm and Morgan, 1996), firstly O_2 (aerobic decomposition), followed by NO_3, SO_4 and CO_2, the latter leading to the anaerobic production of CH_4. Oxygen inhibits methanogenesis (Liikanen *et al.,* 2002a) and production in sediments. Conditions at the sediment water interface were temporally and spatially variable, particularly during algal blooms in summer, when anaerobic conditions in the bottom of the deep cell contrasted with supersaturation of O_2 down to the sediment water interface in the shallow cell (anaerobic conditions only occurring on 15/07/13). Despite detailed sampling revealing increasing concentrations of dissolved CH_4 and N_2O in the anaerobic zone, no significant correlations were observed with any GHG and either O_2 or redox potential, suggesting that they were not the primary drivers of emissions in this instance.

For methane this may be due to production being predominantly located deeper in the sediment profile (Bazhin, 2003) where oxygen demand is high and anoxic conditions perpetuate year-round (Casper *et al.,* 2000). An aerobic water column may only influence the uppermost region of sediments (Liikanen *et al.,* 2002b) which was likely to be region of CO_2 production through aerobic decomposition. Some of the CO_2 may also be produced through the oxidation of CH_4 in the oxidising layer at the surface of the sediment (King *et al.,* 1990). Therefore, the higher methane emissions in the deep cell, may also contribute to the higher CO_2 fluxes observed. Negative relationships between diffusive and chamber CH_4 flux

and NO_3-N, although only significant in the shallow cell, indicated that NO_3-N may have been inhibiting CH_4 production. This may have been through competition (as an electron acceptor) or toxic effects (Achtnich et al., 1995; Kluber and Conrad, 1998a, b; Roy and Conrad, 1999) as in other agricultural wetlands (Stadmark and Leonardson 2005, 2007). However, the seasonal change in both nitrate and temperature means that it is difficult to establish whether this relationship is coincidental in this case.

The denitrifying nature of the wetland sediments (Knowles, 1982; Vymazal, 2007) and strong positive correlation between NO_3-N and N_2O indicated that, as in other nutrient rich systems (Stadmark and Leonardson, 2005; Søvik and Kløve, 2007; Søvik *et al.,* 2006; Johannson *et al.,* 2003, Reay *et al.,* 2003), NO_3-N was essential for N_2O production. This relationship was most apparent during the summer when both N_2O emissions/concentrations and NO_3 were lowest both wetland cells, although for different reasons. Anaerobic conditions at the sediment water interface of the deep cell may have resulted in ammonification of organic N trapped within the sediments (Patrick and Reddy, 1976), increasing ammonium concentration from 15/06/13 to 17/07/4. This was in agreement with summer observations made by Stadmark and Leonardson (2005). Additionally NO_3-N may have also been reduced to NH_4-N (Saeed and Sun, 2012). As such, the quantity of NO_3 available for use in denitrification was reduced, although a sufficient quantity was available to permit small positive fluxes, and increased concentrations of N_2O in the anoxic hypolimnion.

N_2O uptake from the atmosphere and low NO_3 concentration in the shallow cell during the same period matched consumption observed during the summer by Johannsson *et al. (*2003) and showed the same dependence on NO_3-N, although no spike in NH_4-N concentrations was observed. This was possibly due to the aerobic conditions at the sediment-water interface producing NO_3-N (nitrification) which may have been instantaneously consumed deeper in the sediments (Patrick and Reddy, 1976). However, as N_2O may be also generated during nitrification (Søvik *et al.,* 2006; Søvik and Kløve, 2007; Mosier *et al.,* 1998; Dundee and Hopkins, 2001), this does not agree with the uptake observed. Another possibility for N_2O consumption (Johannson *et al.,* 2003), is that N_2O may be consumed by denitrifying bacteria. This may occur during complete denitrification to N_2, where, in the absence of all other alternatives, N_2O is utilised to complete the denitrification process (Firestone and Davidson, 1989). As such, any N_2O produced in the deeper anoxic sediments of the shallow pond was then consumed due to the lack of available NO_3-N.

Uninhibited N_2O production was only observed at the end of summer/early autumn (Johannson *et al.,* 2003; Fey *et al.,* 1999) when inputs from runoff had sufficiently increased NO_3 concentrations in the water column. After this time, the close relationship between NO_3 and N_2O broke down. It is likely that this was due to reduced winter temperatures affecting the activity of bacteria (Søvik *et al.,* 2006; Knowles, 1982; Kadlec and Reddy, 2001) to the extent that nitrate was no longer limiting. The significantly higher emissions of N_2O from the inlet cell across the study agreed with similar observations in treatment wetlands (Søvik *et al.,* 2006; Johannson *et al.,* 2003). However, these systems also included areas with trickling filters or zones which remained aerobic, thereby promoting nitrification (Søvik and Kløve, 2007) and ensuring a continued supply of NO_3-N.

Production and consumption of GHGs in the water column

CH_4 oxidation was the main uptake pathway for dissolved CH_4 in the water column, but had limited impact on ebullitive releases. Diffusive methane oxidation occurred but the impacts are complex. Photosynthetic uptake/release was the primary control of CO_2 fluxes and that present in the wetland water column.

Oxidation in a water column represents the main uptake route for CH_4 in aquatic systems (Casper *et al.,* 2000; Bastviken *et al.,* 2008; Harrits and Hanson, 1980); Utsumi *et al.,* 1998). However, the impact of oxidation on methane emitted via ebullition is minimal, as bubbles largely bypass the oxidation mechanism (Chanton and Whiting, 1995; Bastviken *et al.,* 2004), due to rapid transit though the upper layer of sediment and water column. This is coupled with minimal dissolution of around 15 % of bubble volume (Martens and Klump, 1980). As water depth was low, this bypassing mechanism is particularly important in the wetland given ebullition was the main transfer pathway for CH_4, meaning releases were largely impervious to any microbial uptake.

During the eutrophic blooms (Fig 3.12), oxygen was depleted in the bottom of the deep pond while the upper water column (whole water column in the shallow area) was super saturated with oxygen. While changes in CH_4 concentrations were not significant at a seasonal level, during this period water column sampling found that methane was higher at the bottom of the deep cell than at the surface. This agrees with observations in similar agricultural wetlands (Stadmark and Leonardson, 2005) and lake systems (Casper *et al.,* 2000; Bastviken

et al., 2008; Harrits and Hanson, 1980) and indicates that oxidation of released CH_4 was occurring in the water column after it left the anaerobic zone. Despite the very shallow water column, due to the proportionally large oxygenated: deoxygenated depth of water, most of the dissolved CH_4 was oxidised prior to emission at the wetland surface, thus resulting in the small diffusive fluxes and poor correlations between TBL estimates and other variables (except dissolved CH_4 concentrations). However, on the one occasion (15/07/13) where anaerobic conditions were observed at the bottom of the shallow cell, a significant pulse of diffusive emissions was observed though not mirrored in the deep cell. It is likely that this was due to reduced oxidation at the sediment water interface coupled with a shorter distance to travel in oxic water, thus minimising CH_4 uptake. This highlights the importance of diffusive gas transfers when antecedent conditions in the water column may limit uptake. Such conditions where only observed once, although may occur much more often during eutrophic episodes and diurnal cycling. The implications this and changes in water column O_2 has on GHG fluxes will be addressed in chapter 4.

Figure 3.12 Algal bloom in the shallow zone of the wetland pictured with green water on 18/06/13.

While increased oxygen in the water column may be expected to heighten CO_2 fluxes through increasing oxidation of CH_4 or respiration, negative relationships with O_2 and pH were indicative of control by photosynthetic activity (Talling 1976; Maberly, 1996). Oxygen supersaturation and high pH at the water surface during spring and summer (April-July 2013) accompanied the water turning green (Fig. 3.12), increases in pH (over 9 in the shallow cell) and negative (shallow) or low (deep) fluxes and CO_2 concentrations. This indicated that algal uptake of atmospheric CO_2 was occurring, as is common in nutrient-rich high productivity systems (Repo *et al.,* 2007; Huttenen *et al.,* 2003; Talling, 1976; Emerson, 1975; Maberly,

1996) including in some agricultural wetlands (Stadmark and Leonardson, 2005; Søvik *et al.,* 2006). The photosynthetic demand for inorganic carbon (Talling, 1976; Maberly, 1996) increases with algal biomass, and when the rate of conversion of inorganic carbon via phytoplankton outpaced the production or diffusion of CO_2 from the sediments, the shallow cell shifted below equilibrium with the atmosphere. This is also supported by the anoxic bottom water retaining high CO_2 concentrations through most of the episodes, indicating that CO_2 was still being produced from the sediments or by algal respiration (where light penetration was low), but being consumed at a greater rate than it could reach the water surface. The negative fluxes were only replaced by low emissions in the deep zone, as the higher rate of CO_2 production kept pace with uptake, thus permitting low but positive net emissions and maintaining a lower pH than in the shallow cell. The period of low/negative fluxes from both zones were interrupted by a single spike of CO_2 coupled with a drop in pH to 6.8), returning to pre-bloom levels on 20/05/13. This singular peak coincided with the intense rainfall runoff event noted previously.

While the wetland never developed a true hypolimnion during the study, the period of warmer, calmer, drier weather in the month preceding this event had allowed spring bloom conditions to perpetuate and lead to the surface water becoming disconnected from the supply of carbon. The action of this storm caused sufficient flow in both wetland zones to remix the water column and both release stored CO_2 from sediments, and flush out some of the phytoplankton rich water. After this event, bloom conditions returned and CO_2 uptake resumed until autumn. The effect of eutrophic episodes in nutrient rich lakes has been recognised as an important sink in freshwater CO_2 budgets (Balmer and Dowling, 2011; Pacheco *et al*., 2013) and may represent a significant offset in the net greenhouse gas balance for the wetland. However, as both net uptake and emission (Maberly, 1996) or emission only (Casper *et al.,* 2000) have also been observed within single growing seasons, the uptake observed in our results may not be a permanent feature year after year.

3.9. Conclusions

Observations have illustrated that the conversion of unproductive agricultural land into agricultural wetlands results in a significant increase in emissions of greenhouse gases to the atmosphere under the climatic conditions of the United Kingdom. Results have shown that despite the relatively young age and small size of the study wetland, emissions of CH_4, CO_2 and N_2O were comparable to or higher than fluxes from more established engineered and natural systems, dealing with high nutrient loadings and levels of stored carbon. However, despite being a significant source of greenhouse gases to the atmosphere, the wetlands still acted as a sink of carbon and nitrogen in the environment due to very high sediment retention rates. While CO_2 was the main gas emitted and responsible for the greatest amount of carbon lost, CH_4 was the most significant gas released from the wetland in terms of global warming potential. Furthermore, it was the only gas for which the wetland acted as a continuous source throughout the study. N_2O was the least significant gas released.

The mechanistic controls of greenhouse gas production appear to be far more complex and unpredictable than in analogous natural and engineered systems, in several cases showing little agreement with physicochemical relationships observed elsewhere. As the most climatologically significant gas emitted, CH_4 transfer was predominantly elicited via ebullition and influenced by water level, temperature and C supply, although the dominating factor may vary with antecedent conditions. CO_2 emissions were influenced by carbon supply and photosynthetic uptake and N_2O mainly by the supply of NO_3. However, it was also found that the design, small size and nature of inputs resulted in a complex system of production and consumption, aspects of which require a higher temporal resolution of observations. The observations regarding the impact of oxygen level on diffusive gas fluxes in the bottom waters requires further investigation in order to elucidate this relationship. This is also the case when considering the potential impact of physical forcing disturbance of gas fluxes caused by wind shear and storm flows.

Chapter 4 – Impacts of O_2 and summer overturning on greenhouse gas releases from sediments in a eutrophic agricultural wetland

4.1. Introduction

Constructed agricultural wetlands may contribute to the globally important issue of increasing GHG concentrations by potentially acting as hotspots for the pollutant swapping of CH_4, CO_2 and N_2O to the atmosphere, as demonstrated by field observations in Chapter 3. Wetland biogeochemistry is influenced by the O_2 concentration of the water column and sediment; in aerobic conditions, organic carbon is oxidised to CO_2 (Liikanen *et al.,* 2002b) whilst methanogenesis anaerobically produces CH_4 (Conrad, 1996; Segers, 1998). Additionally, organic N can be aerobically nitrified to NO_3-N whilst anaerobically, denitrification produces N_2O, and both ammonification and dissimilatory reduction produce NH_4-N (Vymazal, 2007). Anoxic conditions also increase the potential for dissolution of mineral-bound P compounds (Genkai-Kato and Carpenter*,* 2005; Moore and Reddy, 1994). Because these mechanisms may influence potential pollutant swapping from agricultural wetlands, it is essential to consider the processes affecting O_2 content in the water column/porewater, and understand the subsequent impacts on wetland biogeochemistry.

In many nutrient-enriched water bodies, O_2 content may be particularly spatially and temporally variable, as a result of eutrophic algal blooms during the spring/summer months. O_2 release and CO_2 uptake by phytoplankton during photosynthesis (Drever, 2002) may generate O_2 enriched, highly oxidising conditions. This contrasts with O_2 consumption/CO_2 production via algal respiration in darkness (Reddy *et al.,* 2005). Aerobic decomposition of autochthonous carbon deposited by enlarged phytoplankton stocks potentially generates hypoxic or anoxic regions at the base of a water column (Friedrich *et al.,* 2014; Thibodeau *et al.,* 2006). These anoxic conditions may increase the proportion of CH_4 produced by sediments (Liikanen *et al.,* 2002b), whilst increased inputs of organic carbon via algal deposition may also enhance the release of CH_4 (West *et al.,*2012). Field observations in Chapter 3 found that, as in many enclosed aquatic environments (Friedrich *et al.,* 2014), agricultural wetlands may develop eutrophic conditions. Therefore the processes outlined above may significantly affect pollutant swapping potential.

Water column anoxia and increased GHG release may also occur in the seasonal stratification of lake environments (Casper *et al.,* 2000; Fernandez *et al.,* 2014), during which the development of a hypolimnion in the lower water layers may isolate them from an oxygen supply at the surface. This can result in the water column storage of CH_4 and reduced compounds, over weeks to months without it being oxidised (Bastviken *et al.,* 2004). When stratification breaks down through convective overturning, large quantities of dissolved CH_4 and other compounds are convected and released, resulting in substantial seasonal increases in diffusive emissions (Fernandez *et al.,* 2014; Michmerhuizen *et al.,* 1996; Rudd and Hamilton, 1978; Strayer and Tiedje, 1978) i.e. storage flux (Bastviken *et al.,* 2004). While field observations in Chapter 3 showed that the shallow depth of agricultural wetlands (<2 m) do not permit season-length stratification, diurnal temperature and O_2 stratification have occurred in wastewater retention ponds as shallow as 0.35 m (Bokil and Agrawal, 1977; Gu and Stefan, 1995). As such, there may be potential for eutrophic agricultural wetlands to experience diurnal variations in the release of GHGs and nutrients during water column turnover, despite studies of analogous wetlands generally assuming diurnal variations in GHG fluxes were not significant (Stadmark and Leonardson, 2005; Tanner *et al*., 1997).

Therefore, although stratification/overturning and eutrophic O_2 depletion have been investigated in deeper lake systems, these processes have been overlooked in agricultural wetlands and analogous shallow pond environments. Investigating the role of these mechanisms on GHG and nutrient release will increase our understanding not only of pollutant swapping in agricultural wetlands, but also the large numbers of small freshwater ponds and lakes currently neglected in GHG budgets (Holgerson *et al.,* 2015).

4.2. Aim

The experiment aims to determine how variations in wetland water column O_2 content, as a result of eutrophication and stratification/turnover, affect potential GHG and nutrient fluxes from wetland sediments.

Hypotheses to be tested:

1. The water column of the agricultural wetland will stratify and reach anoxic turnover during a diurnal cycle in summer conditions.
2. Reductions in oxygen within the water column will increase the release of CH_4, N_2O, SRP and NH_4-N from wetland sediments, while CO_2 and NO_3-N release will decrease during anoxic periods.

4.3. Methods

4.3.1. Field Experiment: Diurnal water column sampling

The water columns of the two wetland cells were monitored over a period of 24 hours in order to examine the development and breakdown of thermal stratification and the impact this had on GHG, nutrient and physicochemical characteristics.

Diurnal field sampling was conducted on 9th July 2013 during a period of calm and warm weather, in order to capture the conditions in the wetland when stratification and an algal bloom were likely to develop. The water column was sampled every 4 hours for 24 hours, commencing at 10:00 am (7 time points). Observations were made from a dinghy, slowly manoeuvred so as to not disturb the water column or sediment. The centre of each wetland cell was used as the nominal sampling point for the entire experiment. The depths of water columns were measured and an appropriate range of vertical sample points selected. In the deep cell, water samples were extracted at 0.05, 0.5 and 1 m depth, with physicochemical parameters sampled at 0.05, 0.1, 0.3, 0.5, 0.7, 0.9, 1.0 and 1.1 m. The shallow cell was sampled at 0.05 and 0.5 m, with physicochemical sampling at 0.05, 0.1, 0.3, and 0.5 m depth.

Wetland water was sampled using a peristaltic pump (Williamson, UK), with a sample line held at the required depth using a pole. A low flow rate was used so as to not cause degassing of the sample. Measurements using the pump were tested against those from a Van Dorn bottle at the study site on an occasion prior to the experiment. During testing samples were taken from the wetland surface and the sediment water interface in four areas of the deep cell (n=8) using both methods. No significant differences in dissolved GHG concentrations were found between techniques.

During diurnal sampling water samples were decanted into 125 ml HDPE bottles, which had been pre-spiked with 6 ml of H_2SO_4 (20% v/v) to stop microbial activity. Samples for nutrient analysis were decanted into 60 ml centrifuge tubes and were immediately transferred to a cool box for field storage, followed by cold storage at <5°C post experiment and analysed within 24 hours. Temperature, dissolved oxygen, pH, conductivity, redox potential and total dissolved solids were measured in situ at the same time as samples were taken using a Hanna H19828 water quality meter, which was calibrated using the method described in Chapter 2.

4.3.2. Microcosm Experiment: Core sampling and water collection

Sediment microcosms were utilised to further observe the impact of water column oxygen concentration on GHG and nutrient release from wetland sediments, under diurnal and prolonged regimes. Sediment cores (n=6) were collected at the end of summer 2013 from across the deep and shallow cells of the wetland (Fig 4.1). Sample locations were selected by allowing the dingy to drift before randomly around a wetland cell before deploying the sampler over the side. Sampling was performed using a Jenkin sediment sampler, fitted with an acrylic tube (ø 95 mm, length 506 mm). After capturing a sediment core and overlying water column, the sample tube was removed from the corer as a single unit. The depth of the sediment core was adjusted to 100 mm, excess sediment discarded and then the tube sealed with polypropylene caps and silicone sealant in order to form the body of the microcosm, with the sediment retained in situ. Cores were kept vertical, placed in a cool box and transported into cold storage (5 °C) within 4 hours of collection. On the same day that cores were sampled, 2000 L of wetland water were collected. This was then blended, passed through a 100 µm sieve to remove sediment and then stored in two connected 1000 litre HDPE hoppers. These were left open to the atmosphere, but kept in the dark to discourage algal growth. In order to disrupt stratification and ensure homogeneity of the water supply, each hopper was fitted with a submersible pond pump (rated at 1000 L hr^{-1}) which transferred water between hoppers continuously.

Figure 4.1 Jenkin sediment sampler with sample tube loaded and primed.

4.3.3. Microcosm experimental setup

The microcosm experiments utilised 9 sediment cores (Fig. 4.2): 3 containing deep cell sediment, 3 with shallow cell sediment and 3 blanks containing no sediment. In order to minimise sample disturbance and interaction with the air, sediments were retained in the original sample tubes. This then formed the basis of the microcosms (Fig. 4.3). Inflow from the supply feed to the microcosm was controlled via adjustable valves on the inlet at the top of the column. The outlet was positioned 20 mm above the sediment water interface. Outflow from each microcosm was continuously passed through a sealed flow cell fitted into a closed loop with a multiparameter probe, to enable physicochemical measurements without disturbing the microcosm. Ebullitive fluxes were not the target of this experiment and so were not included in observations. The outlet was also fitted with a 3-way valve, which could be used to periodically divert flow for sample extraction into 60 ml Wheaton bottles and 125 ml HDPE bottles.

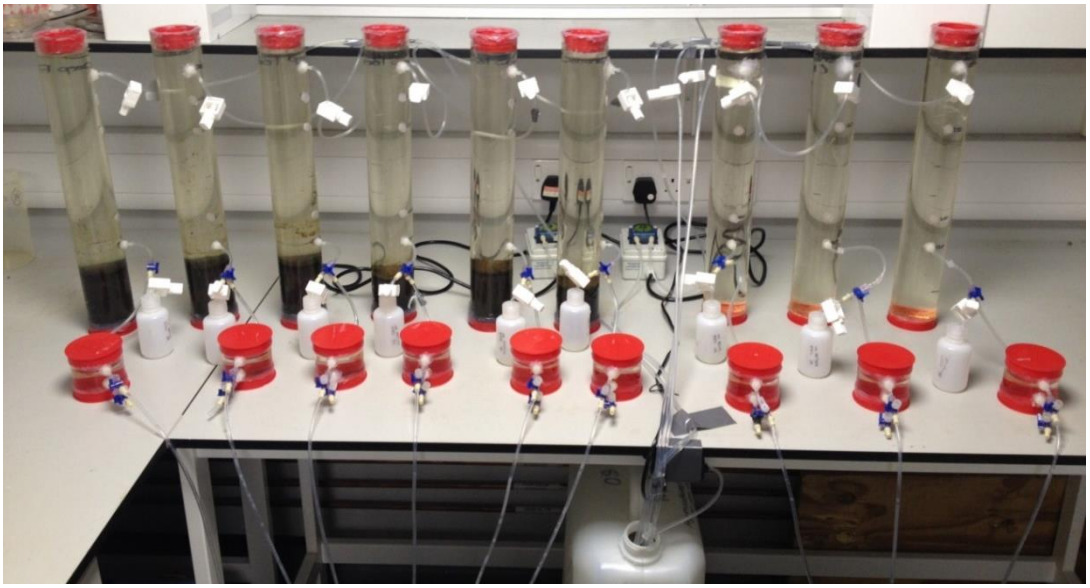

Figure 4.2 The microcosm experimental setup for prolonged and diurnal anoxic turnovers with (left to right) the 3 wetland deep cell cores, 3 wetland shallow cell cores and 3 blank cores with no sediment. Flow cells for each column are in the foreground.

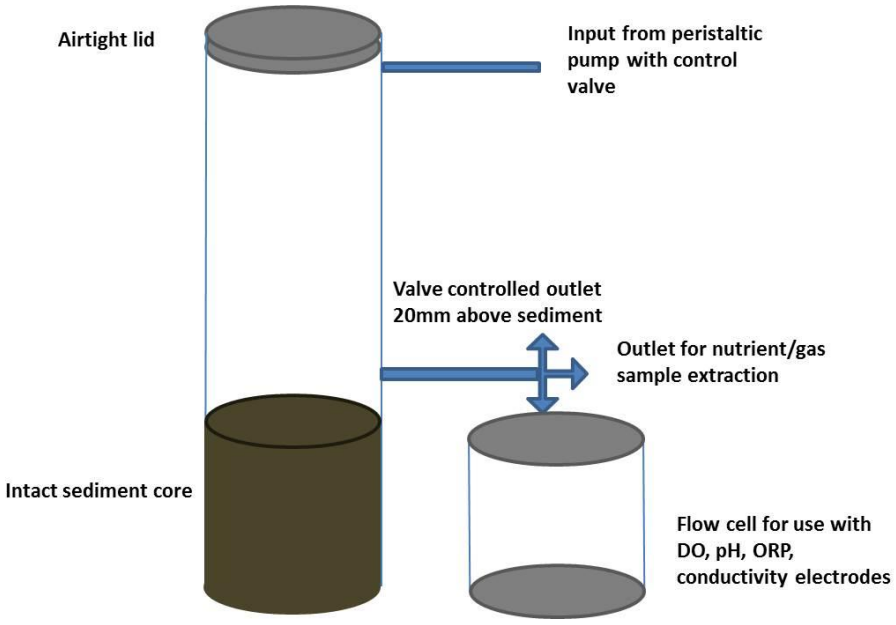

Figure 4.3 Basic design of the sediment microcosm (not to scale). Pump apparatus and monitoring devices not shown for clarity.

The continuous top to bottom flow of water in the microcosm was intended to prevent stratification. Collected wetland water from storage tanks was decanted into a 25 L polypropylene container and continuously pumped into the cores with a peristaltic pump at a rate of 0.16 L hr^{-1}. Under anoxic flow, intended to replicate a water column depleted in O_2 (~1 mg L^{-1} O_2), the supply water had been purged of oxygen in batches beforehand, by being sparged with N_2. Health and safety considerations prevented continuous purging throughout the experiment. Any headspace in the container was filled with a cap of argon (denser than air) to limit ingress of O_2 over time. Under oxic conditions, intended to replicate the oxygenated water column when an algal bloom was present, the supply water (~10 mg L^{-1} O_2) was continuously sparged with lab air using aquarium air pumps.

4.3.4. Microcosm: Experimental procedure (prolonged oxic/anoxic conditions)

All microcosms underwent a 12 hour pre-incubation at mean in situ summer temperatures at the base of the water column (15.5 °C), during which the microcosms were kept in the dark. Oxic water was then fed into the microcosms for 7 days, after which the supply water was switched to anoxic flow for 7 days. Water samples for dissolved gas and nutrient analysis, plus a physicochemical assessment of outflow were collected every 2-3 days. Flow rates in

each core were measured at each sampling point. The fluxes of dissolved gases and nutrients were calculated by multiplying the concentration differences between the blank cores and those with sediment, with individual core flow rates and sediment surface areas. Sediment oxygen consumption (SOC) was calculated using the same method, using the differences in oxygen concentrations during the aerobic phase only. The respiratory quotient (RQ); the ratio between the amount of CO_2 produced and oxygen consumed was calculated from the molar ratio of SOC to CO_2 flux.

4.3.5. Microcosm: Experimental procedure (diurnal turnover)

Immediately after the prolonged anoxia experiment ended, the diurnal experiment was initiated. This was so that the microcosm start point was effectively mimicking the anoxic turnover conditions observed at dawn during diurnal field sampling. The experiment was initiated by a switch to oxic flow, which was maintained for 12 hours (oxygenation of the water column during daylight) followed by 12 hours anoxic flow (inducement of nocturnal anoxic conditions). This full cycle was repeated a second time so that after 48 hrs, the cores had been subject to 2 complete diurnal cycles. Samples were collected and physicochemical analysis performed in the manner described above, every 6 hours. Microcosms were kept in the dark. A diurnal lighting regime was not followed during the experiment, as periodic sampling and maintenance of the microcosms required the laboratory to be lit. This was deemed to reduce the validity of simulated dark periods and so was not adopted.

4.3.6. Dissolved gas analysis

Dissolved CH_4, CO_2 (as TIC) and N_2O were analysed using a headspace method (Reay *et al.,* 2003; Sobek *et al.,* 2003). Samples were allowed to come to room temperature and then decanted into 60ml Wheaton bottles and sealed. 30ml was then replaced with zero grade N_2 (field samples used lab air with known quantities of CH_4, CO_2 and N_2O). Samples were agitated vigorously for 2 minutes and then left to equilibrate for 30 minutes, before 5 ml was extracted and injected into an evacuated 3.6 ml exetainer (labco, UK). Samples were analysed using a PerkinElmer Autosystem XL gas chromatograph fitted with a flame ionisation detector and electron capture detector, using 2 mixed standards of 1000ppm CO_2, 3ppm CH_4, 2ppm N_2O and 4000ppm CO_2, 10ppm CH_4 and 0.4 ppm N_2O (BOC, UK) in order to form a calibration curve. GC outputs were then converted into concentrations of gas in air

and then converted into dissolved gas concentration in the water sample using Henrys law, the values for which were calculated according to those in Wilhelm (1970).

4.3.7. Dissolved nutrient analysis

Field and microcosm samples were analysed for SRP, NO_3-N, NH_4-N and DOC using the methods (including quality control) as described in Chapter 2. Diurnal experiment samples were not analysed for DOC for practical reasons.

4.3.8. Statistical analysis

Data from sediment subsets and sample times were tested for normality using Shapiro-Wilk tests. When the variables displayed significantly skewed distributions and/or a high level of variance, $Log_{10}+1$ transformations were applied. Repeated measures tests could not be used due to assumptions being violated. Therefore, comparisons between fluxes or characteristics of sediment replicates were made between the start and end of each experimental phase or using all measurements in a phase. Differences between mean gas/nutrient fluxes from sediments samples from the same cell observed under contrasting oxygen conditions, were assessed using paired t tests. Differences between deep cell and shallow cell sediments were made using unpaired t tests. Where normality or homogeneity of variance was violated, Wilcoxon signed rank (paired) and Mann-Whitney U (unpaired) tests were used. Correlation between variables was assessed using Spearman's Rank Order Correlation due to the large variance and skewed distribution of data across all times and sediment types.

4.4. Field Results

4.4.1. Diurnal field observations: In situ variation in oxygen and physicochemical conditions in the water column

The field experiment was conducted in July 2013, on one of the warmest days of the year. The wetland was experiencing an intense algal bloom, although an estimate of the microbial biomass in the water column was not made. Both the deep and shallow cells of the wetland exhibited pronounced diurnal changes in physicochemical conditions over the 24 hour observation period. In the deep cell, the water column was thermally stratified (Fig. 4.4a) during the daylight hours, which then started to break down during darkness (22:00). An anoxic hypolimnion (Fig. 4.5) was present at 1 m depth in the deep wetland cell throughout the measurement period. Above this layer, the water became increasingly saturated with O_2, moving to super-saturation from 0.5 m to the surface. Throughout the day O_2 saturation increased, and spread down through the water column, peaking at 17.2 mg L^{-1} O_2 at 0.5 m depth at 18:00. Despite this, the hypolimnion was not reached or disturbed. After this time, O_2 decreased in-line with available daylight. From 22:00 as darkness fell, the anoxic hypolimnion began to rise in the water column, from 1 m to 0.9 m depth. Water layers above this became progressively hypoxic with depth, trending towards anoxic conditions. By 06:00 the entire water column had fallen below 10 mg L^{-1} O_2 (100 % oxygen saturation). The anoxic hypolimnion was still present at 0.9 m depth at 10:00 after 24 hours.

In the shallow cell, no anoxic hypolimnion was evident at the start of the experiment (Fig 4.5), with O_2 content being lowest (4.93 mg L^{-1}) at the sediment-water interface (0.5 m depth). By 18:00 the entire water column was supersaturated with O_2 (>10 mg L^{-1}). As darkness fell, O_2 rapidly declined, the base of the water column nearing anoxia (0.29 mg L^{-1}) at 02:00. This spread upwards so that by dawn, O_2 in the entire water column was at less than 6 mg L^{-1}, with near anoxic conditions in the bottom water. Although O_2 concentration at the surface was lower in the shallow cell than the deep cell, re-oxygenation during daylight occurred quickly. After 24 hours, the wetland had begun to supersaturate with O_2 once more.

The pH did not vary greatly temporally over the 24 hours (data not shown), although the highest values (9<) were observed in the shallow cell. In the deep cell, mean values decreased with depth away from the surface (8.3), being lowest at the sediment water interface (6.8).

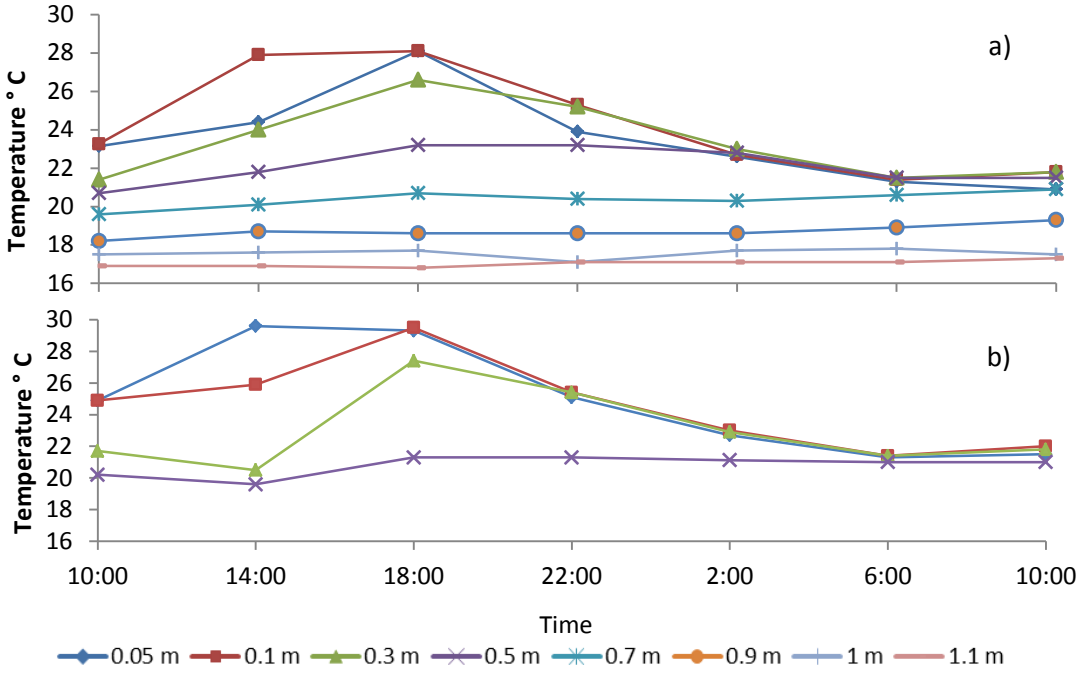

Figure 4.4 Temperature of specific water layers in the deep (a) and shallow (b) wetland cells over a 24 hour cycle in July 2013.

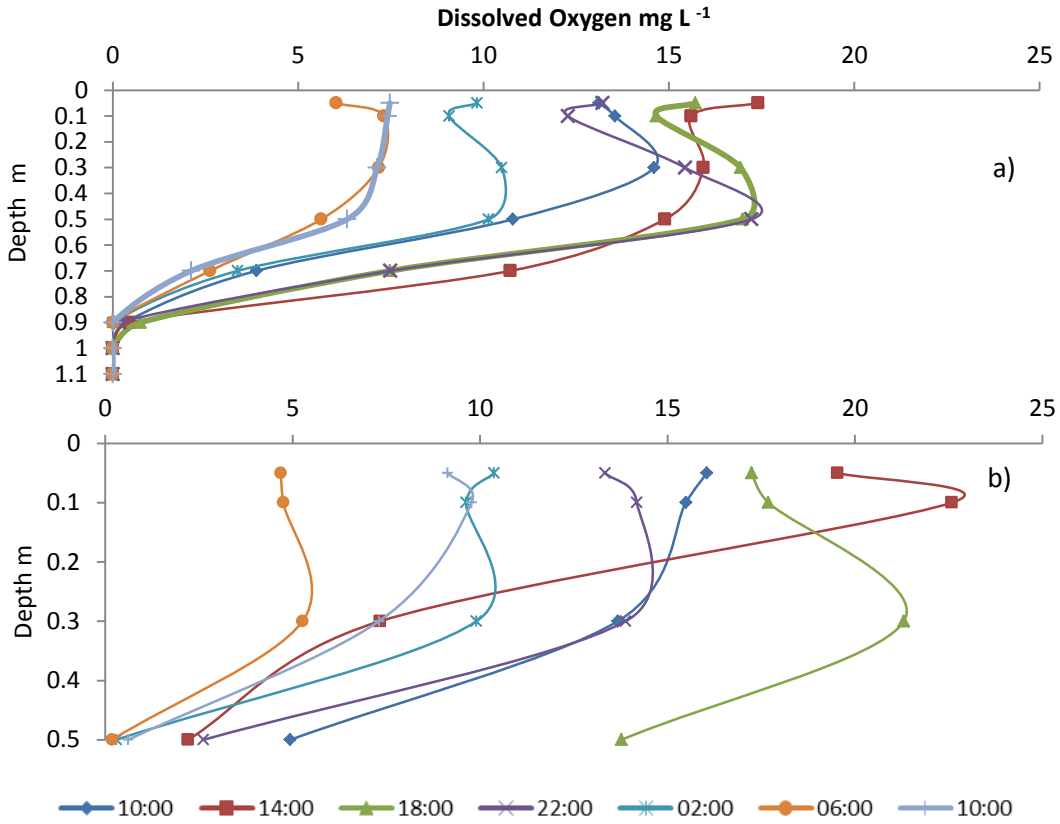

Figure 4.5 Dissolved oxygen profiles in the deep (a) and shallow (b) cells of the wetland, during sampling times throughout the 24hr field experiment. NB. Depth scales differ.

4.4.2. Diurnal field observations: Concentrations of dissolved CH_4, CO_2 and N_2O

The concentration of dissolved CH_4 was consistently highest close to the sediment-water interface in both wetland cells over the entire 24 hr period (Figure 4.6 a-b). In the deep cell, CH_4 concentration did not change greatly in this zone over the 24 hrs. In contrast, the CH_4 concentration at the sediment water interface of the shallow cell, exhibited a diurnal pattern. During the initial 12 hours of daylight (10:00 to 22:00), CH_4 concentrations were low and relatively stable. After several hours of darkness (02:00) concentrations increased rapidly, peaking at 06:00 before decreasing once more. CH_4 concentration decreased towards the surface of both cells, although in the deep cell, concentrations at the water surface were slightly higher than at 0.5 m throughout the 24 hours. While surface concentrations remained relatively stable in the deep cell, those at the water surface in the shallow cell gradually increased over the course of 24 hrs in both daylight and darkness (with the exception of an anomalous initial value).

As with CH_4, total CO_2 concentrations (Fig 4.6 c-d) were greatest close to the sediment water interface of both cells throughout the 24 hr period. Temporal patterns followed those exhibited by CH_4 in same regions of the respective cells, being stable in the deep cell but showing a pronounced diurnal pattern in the shallow cell. CO_2 concentration decreased with depth in the water column of both cells. In the shallow cell, the temporal concentration change followed the same pattern as concentrations at the sediment water interface, decreasing during the day and peaking at dawn. In contrast, there was little difference between CO_2 concentration at 0.5 m and 0 m in the deep cell.

N_2O concentrations (Fig 4.6 e-f) were extremely low in both wetland cells. Differences between concentrations at individual depths were not apparent in the deep cell, with peak levels being observed in all 3 sampled zones at different times of day. In the shallow cell a diurnal pattern was not visible, although the concentration at the surface was consistently higher than that at the sediment water interface.

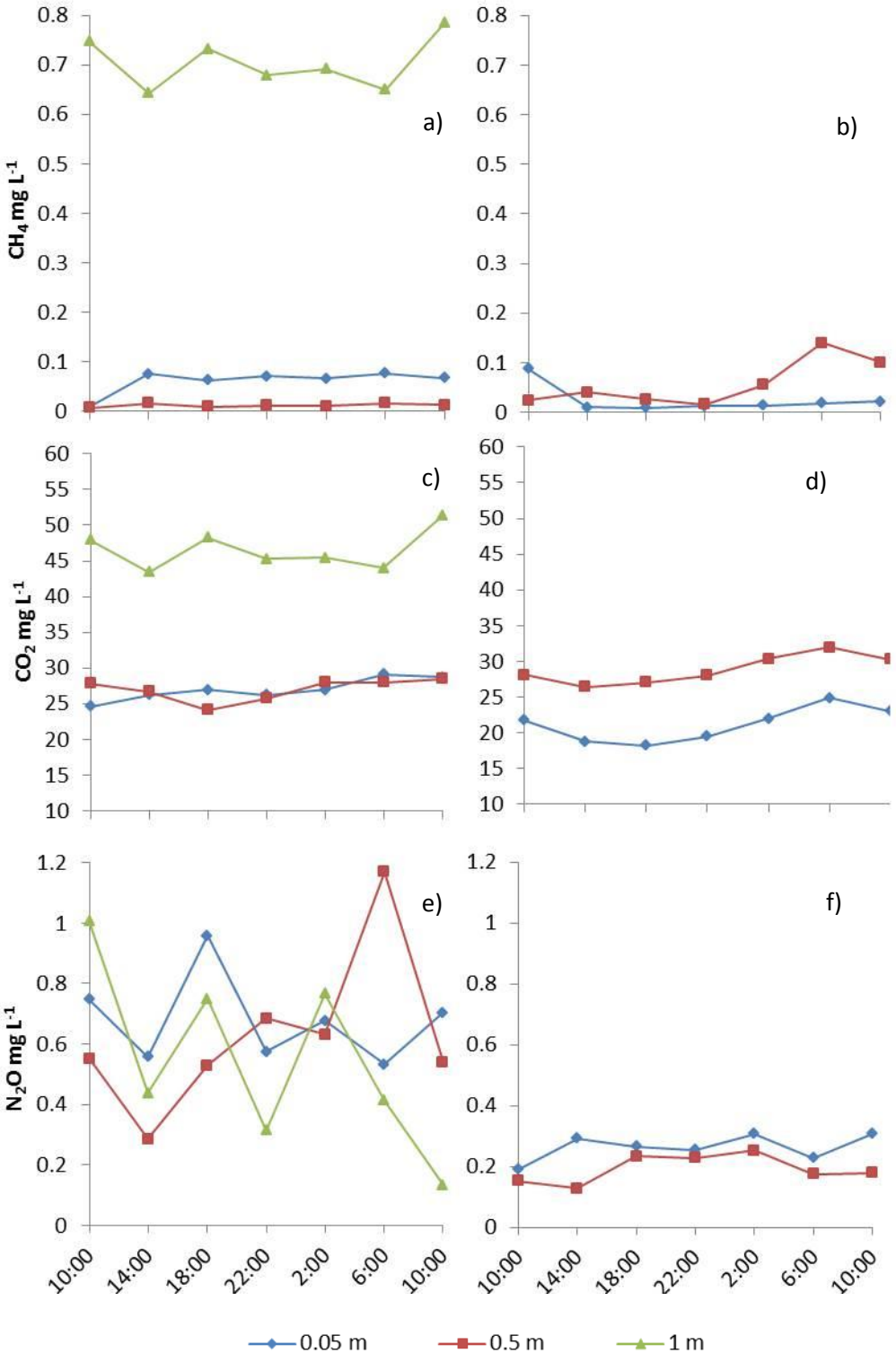

Figure 4.6 Concentrations of CH_4 (a-b) CO_2 (TIC) (c-d) and N_2O (e-f) in the water columns of deep and shallow wetland cells over the 24hr experimental period.

4.4.3. Diurnal field observations: Concentrations of dissolved nutrients

Concentrations of NO_3-N were below the limit of detection in both cells throughout the 24 hour experiment (data not shown). NH_4-N was also below the limit of detection in the shallow cell throughout the 24 hours. This was also the case at 0 m and 0.5 m in the deep cell, although at 0.5 m concentrations above the LOD were recorded at 06:00 and 10:00. NH_4-N concentrations were consistently highest at the sediment-water interface at all times (Fig 4.7a). SRP concentrations (Fig 4.7b-c) were highest at the sediment-water interface in both wetland cells. Although no temporal pattern was apparent in the bottom water of the deep cell, both the bottom and surface water in the shallow cell exhibited a diurnal cycle, with concentrations increasing as darkness fell, peaking at dawn and stating to decrease after 24 hrs.

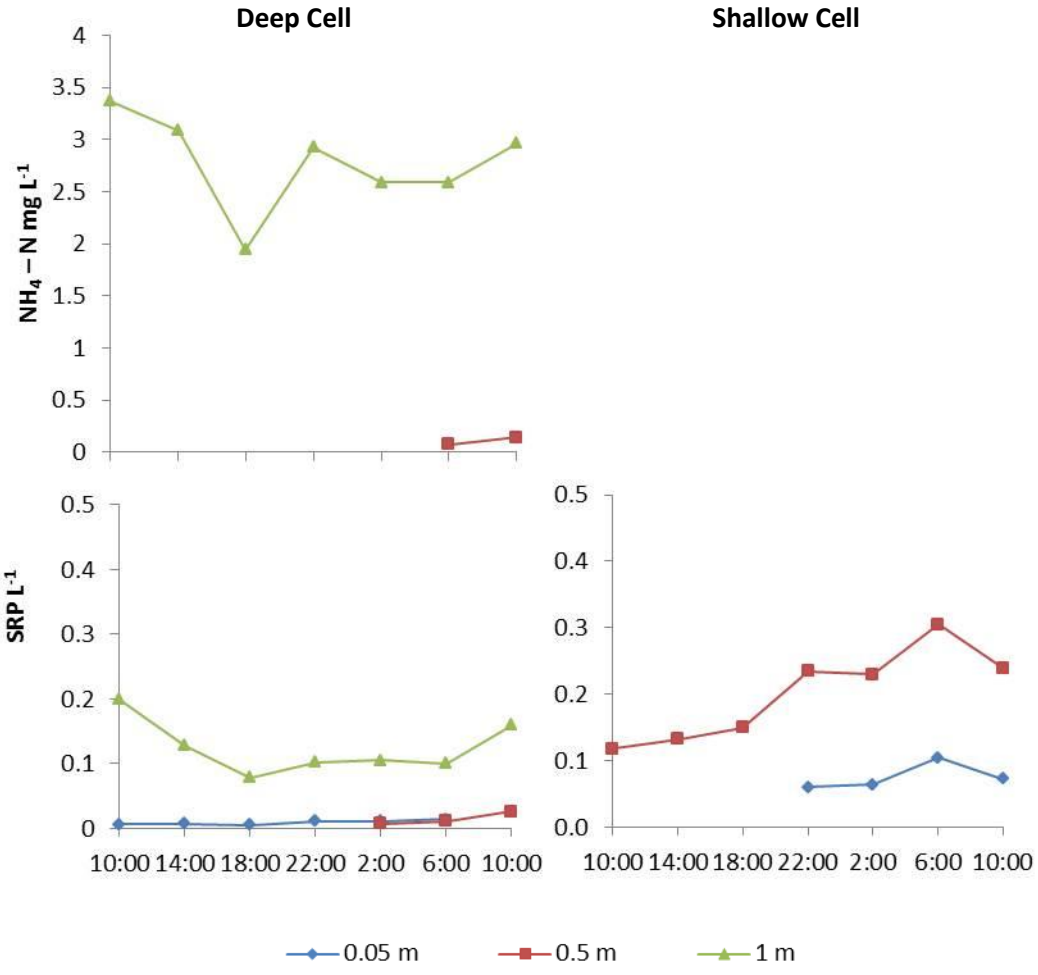

Figure 4.7 Concentrations of NH_4-N (a) and SRP (b-c) in the water columns of deep and shallow wetland cells over the 24hr experimental period. NH_4-N in the shallow cell and NO_3- N in both cells are not depicted due to concentrations being below the limit of detection throughout the experiment.

4.4.4. Correlation of O_2, GHG and nutrient concentrations

When data from all samples collected in each cell over the 24 hours were examined (n=21/n=14), Spearman's Rank Order correlations (Table 4.1) revealed a significant negative interaction between concentrations of O_2 and most GHGs and nutrients in the water columns of the wetland cells. Similar levels of correlation were apparent in both cells. Correlation analysis could not be performed with NO_3-N concentrations, as values were consistently below the limit of detection (0.023 mg L^{-1}).

Table 4.1 Spearman's rank order correlation coefficients between water column O_2 content and dissolved gases and nutrients. * denotes significance at 0.05, ** significance at 0.01 level. Correlations with NO_3-N were not made due to concentrations being below the limit of detection throughout the experiment.

Variable	Deep Cell O_2 (n=21)	Shallow Cell O_2 (n= 14)
CH_4	**-0.72****	**-0.66***
CO_2	**-0.93****	**-0.88****
N_2O	0.05	0.52
NO_3-N	NA	NA
NH_4-N	**-0.87****	NA
SRP	-0.88**	-0.87*

4.5. Microcosm results: Variations in dissolved greenhouse gases and nutrients in response to diurnal oxygen depletion

4.5.1. O_2 conditions within the microcosms

The O_2 concentration of the supply water was switched every 12 hours to mimic diurnal shifts. The large headspace: sediment ratio in the microcosms meant that the 12 hour oxic/anoxic switchovers did not produce totally oxic and anoxic conditions in the microcosm, but did increase and decrease the O_2 concentration (Fig. 4.8).

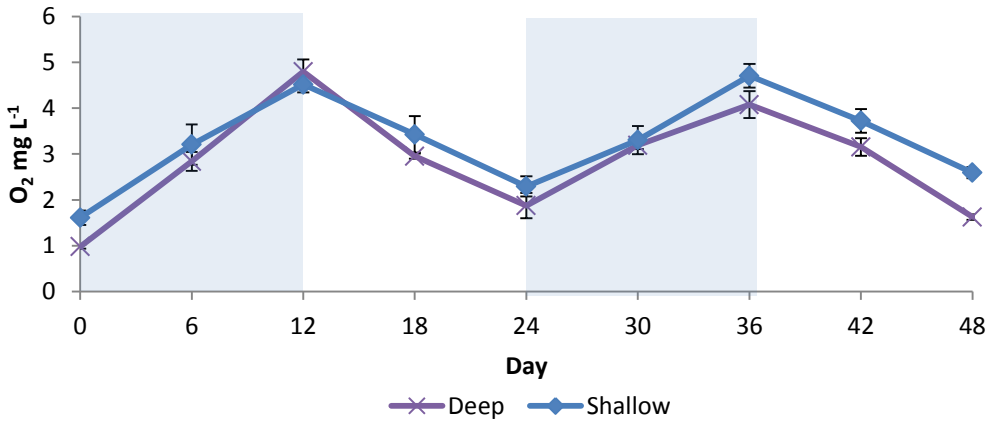

Figure 4.8 Mean (n=3) O_2 concentrations in the headspace of deep and shallow sediment microcosms across two simulated diurnal cycles. Shaded regions represent oxic water flow. Error bars represent standard error.

4.5.2. Variation of gas flux rates

Both deep and shallow cell sediments displayed a change in the flux rates of CH_4 (Fig. 4.9 a-b) between simulated diurnal O_2 states. However, a lag effect between phase switches was apparent making statistical comparisons inappropriate.

Mean CH_4 concentrations in the blank microcosms (Appendix III Fig. 8.3) followed a similar temporal pattern to those containing sediment. However, concentrations were consistently below those in deep and shallow sediment microcosms, thus positive CH_4 fluxes were observed throughout the experiment. At 0 hrs the CH_4 flux rate in all sediment cores was high, due to the anoxic starting conditions. Initiation of the 1st 12 hr oxic cycle (simulating daylight) decreased CH_4 flux rates in both deep (a) and shallow cell cores (b). After 12 hours the 1st anoxic phase (simulating nightfall) was initiated, however CH_4 flux continued to decrease for a further 6 hours (18 hrs). By the end of the anoxic phase (24 hrs) CH_4

concentrations had increased in both cell sediments. However, after this point the temporal patterns in the core subsets diverged. Following the initiation of the 2nd oxic phase (24 hrs), CH_4 flux rate in the deep cell cores continued to rise during the entire phase (24-36 hrs). As with the initial oxic/ anoxic switchover, a lagged decrease in flux rate was recorded at 42 hrs during the middle of the final anoxic period, followed by flux rates starting to climb again at the end of the experiment. In contrast, after 24 hrs flux rates in the shallow cell cores followed the change in oxygen phase closely, being lowest at the end of the 2nd oxic phase and then increasing and finally peaking at the termination of the final anoxic period.

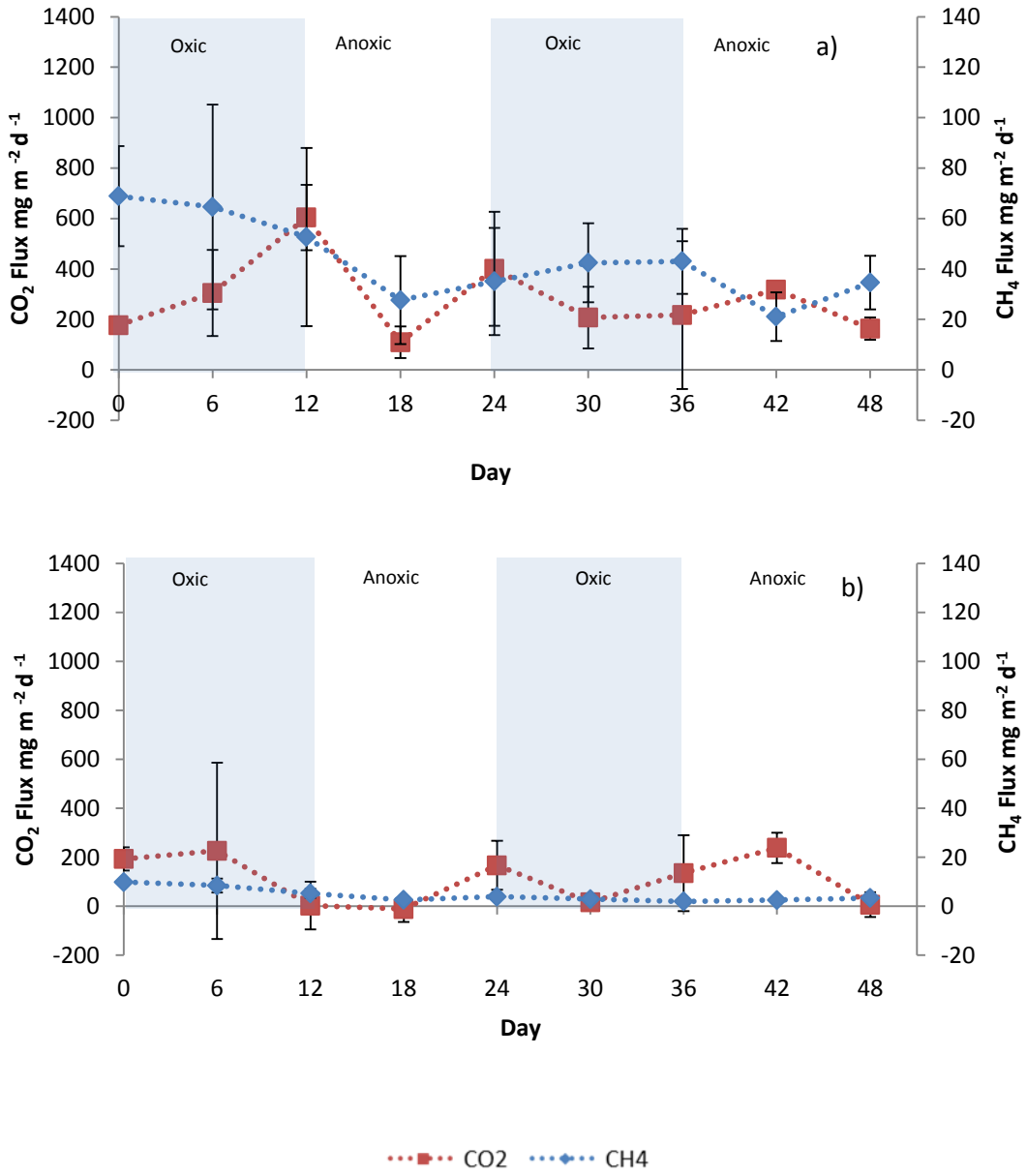

Figure 4.9 Fluxes of dissolved CO_2 and CH_4 from deep (a) and shallow (b) sediments over the simulated oxic-anoxic phases of two diurnal cycles. Error bars represent standard error of the mean. Shaded regions represent oxic phases of the experiment.

CO_2 concentrations in the blank microcosms (Appendix III, Fig. 8.3) were always less than those containing deep and shallow cell sediment, resulting in positive fluxes throughout the 48 hours (Fig 4.9 a-b). CO_2 flux was initially below 200 mg m^{-2} d^{-1} in both deep and shallow sediment microcosms due to the anoxic starting conditions. After commencement of the first oxic phase, CO_2 increased rapidly, peaking at 604 mg m^{-2} d^{-1} in the deep cores after 12 hrs. CO_2 in the shallow cores initially increased from 0-6 hrs before decreasing prior to the end of the first oxic phase. Following the initiation of the first anoxic phase (12hrs), CO_2 flux in the deep cell microcosms initially dropped to 109 mg m^{-2} d^{-1} before starting to climb again at the end of the phase. Rates in the shallow cores followed a similar pattern with lower flux rates. The temporal pattern in both cores, through the following aerobic phase (24-36 hrs) did not show an increase of CO_2 flux as would have been expected. An increase was only observed mid-way through the final anoxic phase (42 hrs) before decreasing once more at the end of the experiment. Temporal changes in CO_2 concentrations within the blank microcosms were initially similar to those observed with those containing sediment. Although after 6 hrs, patterns deviated, being characterised by a gradual increase in concentrations up to 36 hrs.

Mean N_2O concentrations in the blank microcosms (Appendix III Fig 8.3) were consistently below those with sediment, resulting in positive flux rates. N_2O fluxes (data not shown) were however, extremely low from all sediments over the entire experiment. Both deep and shallow cells exhibited increased fluxes during the initial switch to oxic flow (0-12 hrs). However behaviour then diverged, with shallow cell sediments exhibiting a similar undulating pattern which was inconsistent with the diurnal phase changes. Deep cell fluxes continued to increase over time. Blank microcosm concentrations initially displayed slight increases during the first aerobic phase, followed by decreases during the first anoxic phase.

4.5.3. Dissolved nutrient fluxes

Dissolved nutrient fluxes were highly variable over the series of diurnal switchovers (Appendix IV Fig 8.5 a-b). No consistent temporal patterns in the fluxes of any of the monitored determinands were evident over time, with variables switching between uptake and release within the same oxygen phase. NO_3-N uptake was observed in both cell sediments until 36 hrs, after which fluxes varied between positive and negative.

Nutrient concentrations in the blank microcosms (Appendix III, Fig 8.4) were stable throughout the experiment with the exception of NH_4-N, which increased from below the limit of detection to a peak of 0.037 mg L^{-1} at 24 hrs. This coincided with a small increase in sediment core NH_4-N fluxes, although concentrations remained substantially lower. Sharp

reductions in DOC concentration at 12 and 42 hrs were also observed in the blank microcosms. However this was not mirrored by those with sediment, resulting in the peaks in deep and shallow cell sediment fluxes at these times.

4.5.4. Relationship between O_2 and dissolved GHG/nutrient fluxes

Spearman's Rank Order correlations (Table 4.2) revealed few significant interactions between variables in both deep and shallow core subsets. Contrasting positive and negative correlations between O_2 and CH_4, NO_3-N and NH_4-N were observed.

Table 4.2 Spearman's rank order correlation coefficients between dissolved greenhouse gases and nutrients and dissolved oxygen content in the headspace of the diurnal sediment microcosm. * denotes significance at 0.05, ** significance at 0.01 level

Variable	Deep cell sediment O_2 n=27	Shallow cell sediment O_2 n=27	All sediments O_2 n=54
CH_4	-0.17	-0.43*	-0.21*
CO_2	0.18	-0.17	-0.11
N_2O	0.00	-0.27	-0.07
NO_3-N	0.59**	0.11	0.36**
NH_4-N	-0.51**	-0.17	-0.37*
SRP	0.18	0.29	0.07
DOC	0.24	-0.02	0.27

4.6. Microcosm results: Variations in dissolved greenhouse gases and nutrients in response to prolonged oxygen depletion

4.6.1. Conditions in the microcosms

During the prolonged seven-day anoxic phase, water fed into the microcosms was below 10 % O_2 saturation (1 mg L^{-1}). The large water column above each sediment core meant that it was difficult to achieve fully anoxic conditions (Table 4.3). During the oxic phase water fed into the microcosms was always above 70 % O_2 saturation (7 mg L^{-1}), however the sediment oxygen consumption (SOC) of the sediment cores regulated this down to 5.2 and 5.9 mg L^{-1} in the deep and shallow cell cores respectively. The SOC was significantly higher (t test, *p=0.048*) in the deep cell sediments.

Table 4.3 Sediment and water column physicochemical properties in the microcosms in the oxic and anoxic phases.

Sediment	Phase	O_2 mg L^{-1}	SOC mg L^1 d^{-1}	RQ	Redox mV
Deep	Oxic	5.2	1081	1.4	-154
	Anoxic	1.3	-	-	-150
Shallow	Oxic	5.9	362	1.3	-158
	Anoxic	2.2	-	-	-162
Blank	Oxic	6.3	-	-	-
	Anoxic	2.0	-	-	-

4.6.2. Variation in gas flux rates

Following the stabilisation period with oxygenated water, CH_4 flux across all cores decreased significantly between the start (0) and end (7 days) of the oxygenated phase (t test, *p*=0.003), although individual deep and shallow cell core subsets did not show significant decreases. Following the switch to anoxic water, sediment CH_4 release increased (Fig 4.10). Considering both deep and shallow cell sediment microcosms , significantly higher flux rates of CH_4 were observed at the end of the anoxic phase (day 14) when compared to the end of the oxygenated phase (t test, *p*<0.001). CH_4 concentration in the blank microcosms (Appendix V Fig 8.6) remained stable throughout the oxic phase though displayed increased variation during the anoxic phase. Concentrations were a factor of 10-100 lower than those observed in sediment microcosms.

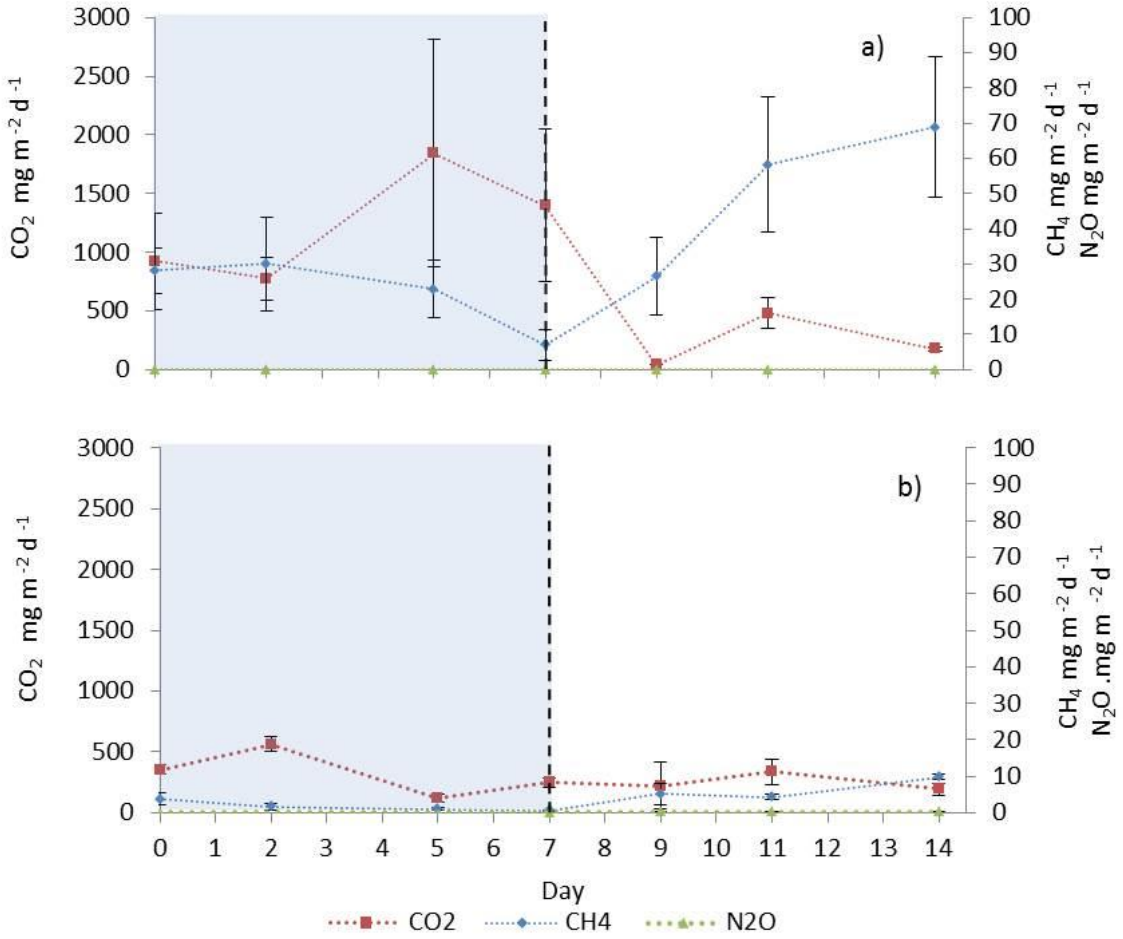

Figure 4.10 Temporal variations in dissolved mean (n=3) gas fluxes emanating from deep cell (a) and shallow cell (b) sediments under oxic and anoxic conditions. Shaded zones represent periods of oxic inputs. Error bars represent the standard error of the mean.

CO_2 fluxes displayed greater variability than CH_4 fluxes over the course of the experiment, in both deep and shallow cell core subsets. While CO_2 fluxes from the deep sediments (Fig, 4.10 a) increased between day 0 and day 7, the large flux variability and low number of replicates meant significance could not be detected. No trends were apparent in the shallow cores (Fig, 4.10b). Following the switch to anoxic water, neither deep nor shallow core subsets showed significant decreases in CO_2 flux. However, significant decreases in flux between day 7 and day 14 (*t test* p=0.049) were detected when considering all sediment cores together. CO_2 concentrations in the blank microcosms (Appendix V, Fig 8.6) were stable throughout the oxic phase. On entering anoxic conditions, concentrations briefly increased at day 9 before decreasing until the end of the experiment. Concentrations were consistently lower than those observed in the microcosms containing sediment, resulting in positive flux rates.

Fluxes of N_2O were extremely low from all cores (Fig 4.10 a-b). Initially, neither deep nor shallow cell core subsets showed any significant decrease in fluxes between day 0 and day 7, although when grouped together a significant decrease in fluxes (t test, p=0.003) was observed. In contrast, N_2O flux rates increased significantly between the start and end of the anoxic phase in both deep (0.03 – 0.15 mg m^{-2} d^{-1}) (t test, p=0.041) and shallow cell sediments (0.02 – 0.17 mg m^{-2} d^{-1}) (t test, p=0.004). N_2O concentrations in the blank microcosms (Appendix V, Fig. 8.6) were consistently lower those in the microcosms containing sediment, resulting in positive fluxes throughout the experiment. Concentrations were stable during the oxic phase and decreased from 0.8 to 0.6 μg L^{-1} after entering the anoxic phase.

All gas flux measurements made under oxic conditions were compared with those made under anoxic conditions, to ascertain which produced the highest flux rates (Fig 4.11).CH_4 fluxes (Fig. 4.11a) (from combined deep and shallow core subsets) were significantly higher (t test, p=0.005) during the anoxic phase, although fluxes from individual deep and shallow core subsets (Fig 4.11a) did not significantly change between oxygen states. During both oxic and anoxic phases, CH_4 fluxes from deep cell sediment cores were significantly higher (t test, p=0.02) than those from the shallow cell.

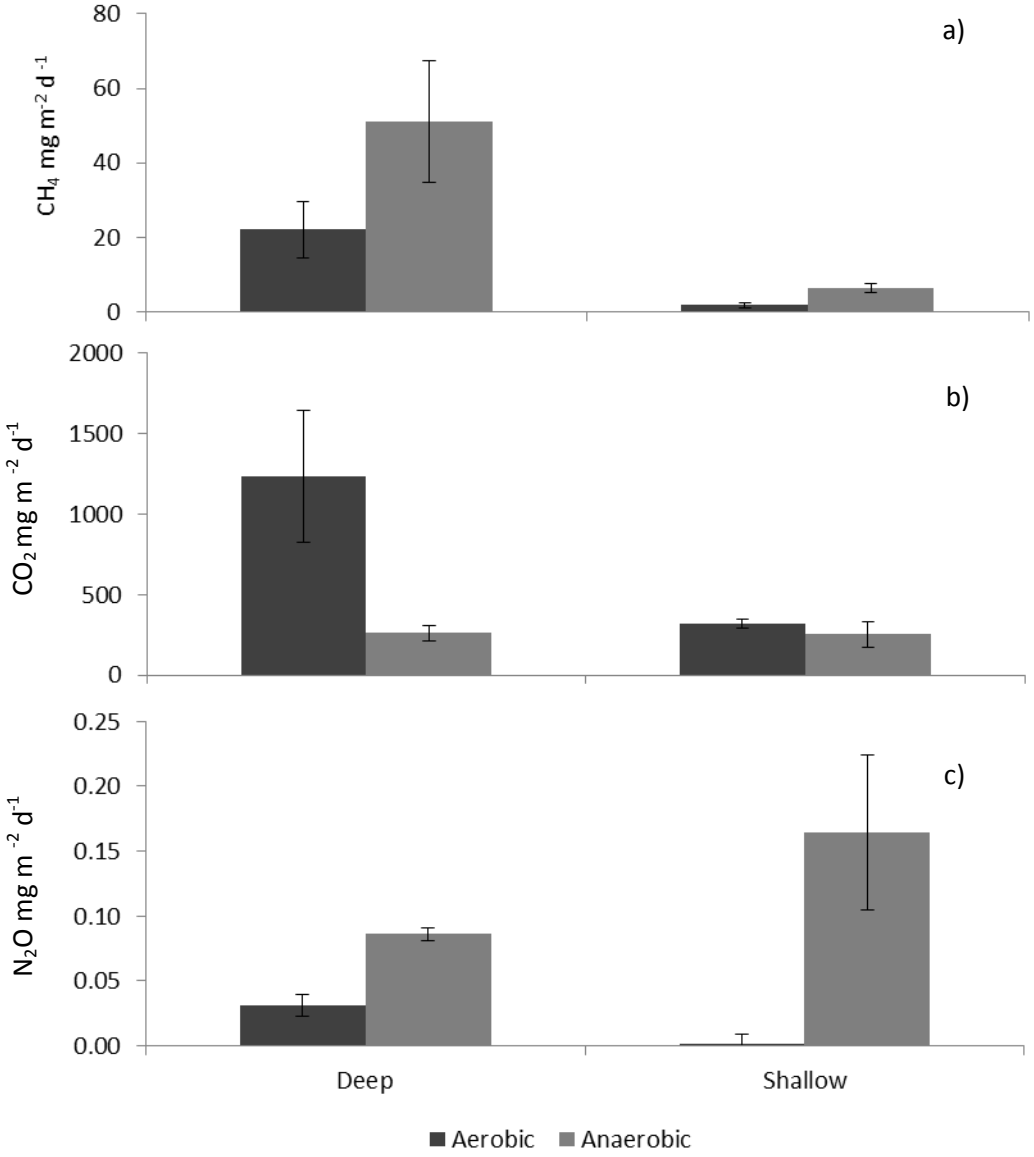

Figure 4.11 Mean dissolved greenhouse gas fluxes from deep and shallow sediment cores during aerobic and anaerobic water column conditions. Error bars represent standard error of the mean.

CO_2 fluxes (Fig 4.11b) from the aerobic deep cell sediment cores were significantly higher (t test, p=0.015) than during the aerobic phase. No difference between phases was observed in the shallow sediments. The deep cell sediment cores produced higher fluxes than those from the shallow cell during the oxic phase (t test, p=0.036). Emissions were equal under anoxic conditions.

N_2O fluxes (Fig 4.11c) from combined deep and shallow core subsets (t test p=0.033) and deep cores alone (t test, p=0.008) were significantly higher during the anoxic phase. No significant differences between deep and shallow sediment core fluxes could be detected.

4.6.3. Carbon mineralisation and RQ

Carbon mineralisation in the deep cell microcosms was significantly higher (t test, p=0.041) during the aerobic phase, although this was not mirrored in the shallow sediment cores. Deep cell cores showed significantly higher rates of carbon mineralisation than the shallow cell cores during the aerobic phase (t test, p=0.032). No differences were apparent between sediments during the anaerobic phase. Respiratory quotients of deep and shallow sediments (Table 4.3) were not found to be significantly different.

4.6.4. Nutrient fluxes

Fluxes of nutrients (Fig 4.12) from the deep and shallow cell sediments exhibited high levels of variability between replicates over the course of the experiment, and as such there were few significant differences. NO_3-N removal was observed in all cores during both oxic and anoxic phases, with greater uptake during the anoxic phase. However, this was only significant in shallow cell cores (t test, p=0.034). NO_3-N uptake was generally greater in the deep cell cores, although not at a significant level. NH_4-N was released by all sediments throughout the experiment although no significant increases in release were detected after switching to anoxic water. Deep cell sediments released significantly more NH_4 than shallow cell sediments during both oxic (t test, p=0.001) and anoxic (t test, p=0.018) phases. Sediments were taking in DOC throughout the experiment. Uptake was greater in the oxic phase and in the deeper sediments, although due to core variability this was not at a significant level. SRP flux was significantly lower (t test, p=0.023) in the deep sediment cores during the aerobic phase, due to a switch from uptake to release during the anoxic phase. Uptake in the shallow cell sediments reduced following switchover, but not at a significant level. Uptake and release from the deep cell cores could not be confirmed as significant due to sample variability. Nutrient concentrations in the blank microcosms (Appendix V, Fig. 8.7) were stable throughout both oxic and anoxic phases. NH_4-N was at or below the limit of detection throughout the experiment.

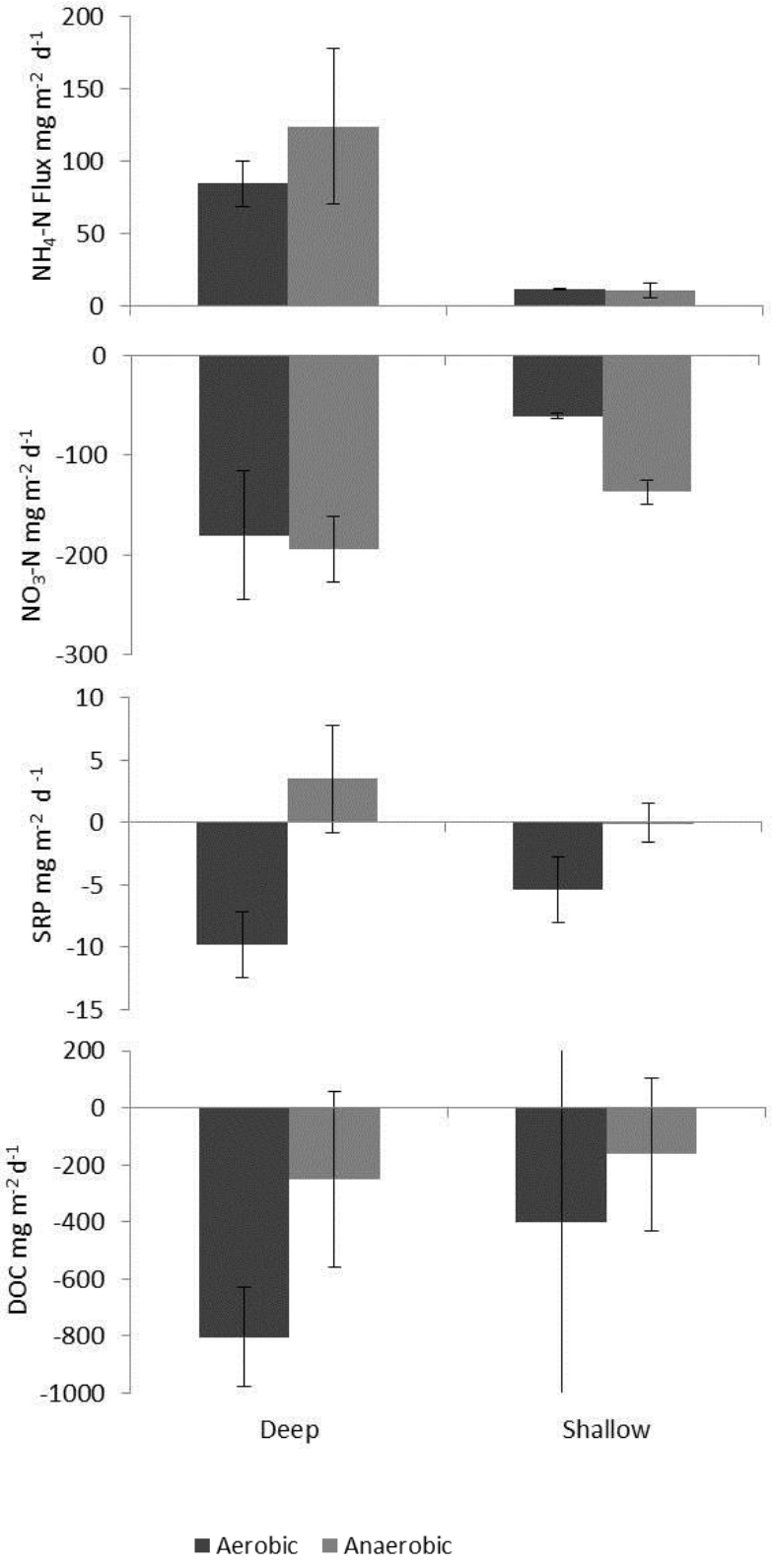

Figure 4.12 Mean dissolved nutrient fluxes from deep and shallow sediment cores during aerobic and anaerobic water column conditions. Error bars represent standard error of the mean.

4.6.5. Impacts of O_2 on gas and nutrient fluxes

Correlations of dissolved substances with O_2 concentrations in microcosms (Table 4.4) were similar in both deep and shallow core subsets. Strong negative correlations with most determinands, and particularly CH_4 and N_2O were detected. Only NO_3-N showed no correlation with O_2.

Table 4.4 Spearman's rank order correlation coefficients between dissolved greenhouse gases and nutrients and microcosm oxygen content. * denotes significance at 0.05, ** significance at 0.01 level.

Variable	Deep cell sediment O_2 n=21	Shallow cell sediment O_2 n=21	All sediments O_2 n=42
CH_4	**-0.44***	**-0.76****	**-0.63****
CO_2	**0.46***	0.07	0.19
N_2O	**-0.47***	**-0.74****	**-0.59****
NO_3-N	-0.06	0.38	0.23
NH_4-N	-0.09	-0.04	**-0.31***
SRP	-0.28	-0.29	**-0.32***
DOC	**-0.42***	-0.12	-0.21

4.7. Discussion: Stratification and diurnal turnover in a eutrophic agricultural wetland

In situ observations revealed that despite the shallow depth of the agricultural wetland, temperature and O_2 stratification, and partial overturning of the water column occurred in both 1.5 m and 0.5 m cells during warm summer weather when eutrophic conditions were present.

Deep cell stratification was more pronounced due to the greater depth of water, with a 10° C decrease between the surface and the sediment-water interface at peak stratification (Fig 4.4). The oxycline in the water column, and particularly the much lower O_2 levels below 0.9 m, illustrated that O_2 stratification was also occurring, with an anoxic hypolimnion forming around 1 m (Fig 4.5). This contrasted with supersaturated O_2 concentrations in the upper portion of the water column, driven by photosynthetic production (Drever, 2002; Reddy *et al.,* 2005). The homogenisation of temperature in the upper water layers, and similar pattern in O_2 concentrations as photosynthesis reduced during darkness, indicated that stratification was breaking down. This mirrors the similar mechanism of seasonal overturning observed in larger lake systems (Casper *et al.,* 2000; Fernandez *et al.,* 2014; Rudd and Hamilton, 1978).

Despite the homogenisation of the water column, temperature and O_2 concentration in the anoxic hypolimnion remained stable and below that of the mixed epilimnion for the full 24 hours, indicating that the thermal stratification had not fully broken down before strengthening again. However, the lack of photosynthetic O_2 inputs/ increased respiration at nightfall is likely to have decreased the depth at which total anoxia was observed from 1 m to 0.9 m at 22:00 to 10:00. The major decrease in O_2 concentration at 0.7 m depth at the same time indicated that the anoxic zone was still increasing in size before re-stratification. Therefore, although overturning was starting to take effect, the shallow depth of the water column and few hours of darkness, limited the extent to which this occurred before re-stratification. The results did reveal, however, that the nocturnal increase in the size of the anoxic zone of the wetland does perpetuate well into the following day.

The 10° C difference between surface and bottom water temperatures in the shallow cell also suggested stratification took place. However, an anoxic hypolimnion was not present as in the deep cell, the water column having moderate to supersaturated O_2 concentrations down to the sediment-water interface during daylight (Carlton and Wetzel, 1988; Reddy *et al.,* 2005). This was most likely due to sufficient sunlight reaching the algal community throughout its entire depth, thus resulting in uninhibited photosynthetic O_2 production.

As temperatures fell during the evening, stratification broke down as in the deep cell. However, the shallower depth meant that the water column briefly became fully thermally homogenised at 06:00 (Fig 4.4b). While an anoxic hypolimnion was not present in the shallow cell, the appearance of a hypoxic zone at the sediment-water interface, and a pronounced decrease in the oxygen concentrations throughout the water column during the darkest period of night (02:00), suggested turnover was occurring and strengthening. The decrease of O_2 content below that at the equivalent depth in the deeper cell implied that this part of the wetland was closer to reaching full overturning and anoxia. However, as noted previously, the return of daylight and switch from photosynthetic respiration to O_2 production reversed this mechanism (Reddy *et al.,* 2005).

Therefore summer eutrophic algal blooms, together with warm weather, may lead to stratification even in very shallow aquatic systems. Moreover, the nocturnal breakdown and overturning of this stratification is also evident, although the greater depth of a water column may actually inhibit the turnover mechanism in this instance. The greatest inhibitor of the turnover mechanism was the very short period of darkness limiting the thermal stratification and degree of respiratory oxygen depletion in the water column. This inhibitor may therefore weaken both earlier and later in the year, when algal blooms are still present in the wetland but the period of darkness is longer.

4.8. Discussion: Impacts of O_2 variation and overturning on water column GHG concentrations

4.8.1. CH_4

Field and microcosm simulations illustrated that water column O_2 content may be key to the release of CH_4 from wetlands, through regulating oxidation in the water column. Although oxidation limits CH_4, strengthening of diurnal anoxia/hypoxia during overturning may permit greater quantities in the water column. Storage of CH_4 in perpetuating anoxic zones also increases the potential for periodic pulses of diffusive emissions.

Consistently low surface water CH_4 concentrations in the deep cell suggested that the incomplete overturning of the wetland water column may have limited CH_4 being mixed from deeper storage zones, as would be expected during full overturning (Bartosiewicz *et al.,* 2015; Fernandez *et al.,* 2014; Rudd and Hamilton, 1978). However, it is also possible that

transfer was occurring, yet CH_4 was being oxidised at similar rates. This may be because although there were reductions in surface O_2 content during darkness, it is possible that as there was over 0.5 m of epilimnion which remained well oxygenated, slowly diffusing CH_4 was oxidised before reaching the sampling point at the water surface (Rudd and Hamilton, 1978). This hypothesis is supported by the pronounced, negatively correlated diurnal variations of bottom water O_2 and CH_4 concentrations at the base of the shallow cell, in which stratification fully broke down. The increase in CH_4 at the sediment-water interface as diurnal hypoxic conditions set in, yet only low diurnal CH_4 increase at the oxygenated surface (Fig. 4.6b), suggested that even in the small distance between the sediment and the atmosphere, CH_4 oxidation may have occurred. This in-situ oxidation during turnover is also the fate of large amounts of CH_4 released in seasonal lake overturning (Rudd and Hamilton, 1978; Stayer and Tiedje, 1978) and a significant limiter of seasonal diffusive emissions. However, whilst potentially showing strong oxidation may negate CH_4 release during overturning, the small diurnal increases in shallow cell surface concentrations, also suggested the potential for evasion or reduction of oxidation. This may have been due to stronger mixing through thermal homogenisation and a shorter travel distance between the sediment and the water surface than in the deep cell.

Both microcosm experiments illustrated the importance of potential water column oxidation of CH_4 in controlling dissolved releases, with negative correlations between CH_4 and O_2, combined with relatively small decreases in the O_2 content of the water column increasing net CH_4 flux when sustained over several hours. This suggested that should a longer period of darkness permit the strengthening of the water column anoxia/hypoxia observed in the field, the reduced rates of oxidation could potentially permit greater evasion of CH_4 to the water surface. The CH_4 flux regulation by oxidation in the water column is highly important, given that as in this experiment, sediment CH_4 production rates appeared to be largely independent of water column conditions. This is suggested by the continual emission of CH_4 in the microcosms and field observations (Chapter 3), when under an oxic water column (also containing NO_3-N). As O_2 ingress is normally limited to the oxidising zone uppermost region of sediments (Liikanen *et al.,* 2002a,b), it is therefore likely that CH_4 production may be focused deeper in the sediment profile in more strongly reducing sediments (Bazhin, 2003; King *et al.,* 1990; Liikanen *et al.,* 2002b; Bartlett and Harris, 1993**).**

The elevated CH_4 concentrations which persisted in the constant anoxic zone at the base of the deep cell (Fig.4.6), were likely to have been facilitated by lower rates of oxidation via O_2 in the water column throughout the diurnal cycle. The contrasting distribution of CH_4 and O_2

concentrations with depth was therefore responsible for the significantly negative correlations detected, whilst implying the decrease in O_2 concentrations at 0.7 m may have also resulted in lower rates of oxidation and increases in CH_4 concentration (although not sampled). The observations from the long term and diurnal simulation microcosms, where full anoxia was not reached, also suggest that the drop in O_2 concentrations may have significantly increased CH_4 flux rates.

Field observations suggested that the potential perpetuation of anoxia in deeper zones of an agricultural wetland, beyond the length of diurnal cycles, may lead to a build-up of dissolved CH_4 in a temporary hypolimnion close to the sediment water interface (Bastviken *et al.,* 2004), despite water less than a meter above being highly oxygenated and mixed diurnally. In addition, significant increases in observed dissolved CH_4 release over time from sediment cores under prolonged anoxic conditions implied that CH_4 production from sediments could become greater the longer they are inundated with anoxic water. This may suggest that low oxygen conditions permitting dissolved CH_4 storage in the bottom of wetlands could also increase the production rates of CH_4.

The storage of CH_4 in the perpetuating anoxic zone is a seasonal feature of larger high productivity lake systems and is coupled with periodic releases of storage flux (Bastviken *et al.,* 2004; Beaulieu *et al.,* 2014; Fernandez *et al.,* 2014; Strayer and Tiedje, 1978). Although substantial release into the upper water column did not appear to occur in this instance, mixing due to a full breakdown of stratification could happen multiple times during the summer season, such as during cooler weather or in a storm event. In this case, this layer of concentrated CH_4 may be introduced into the surface water and lead to higher diffusive releases of CH_4 (or CO_2 through oxidation). The spike in fluxes observed during May 2013 (Chapter 3) may have being partially driven by this mechanism.

4.8.2. CO_2

CO_2 was the main route for carbon mineralisation from the sediments in both in situ and microcosm observations, but appeared to be effectively controlled in the field by photosynthetic uptake. This suggests eutrophic agricultural wetlands may be daytime sinks and night time sources of CO_2 to the atmosphere.

Unlike observations during routine summer sampling (Chapter 3.4), daytime surface water concentrations of CO_2 were relatively high (Fig 4.6). This was due to the samples being

artefacts as a result of preservation via acidification, and thus including all inorganic CO_2 species (TIC). The diurnal variation in these concentrations at the wetland surface, still illustrated shifts between organic and inorganic C through uptake and production of CO_2 by the phytoplankton community (Maberly, 1996). Decreases in CO_2 during daylight were a product of photosynthetic uptake, coupled with associated O_2 production. In contrast, the increase in water column CO_2 concentrations during nightfall illustrated the respiratory production of CO_2. Aerobic respiration of phytoplankton and microbes degrading organic matter, facilitate CO_2 production and O_2 removal (Friedrich *et al.,* 2014), resulting in the strong negative correlation between CO_2 and O_2 at the wetland surface.

The diurnal pattern in CO_2 concentrations at the bottom of the shallow cell, contrasted with the much higher and relatively stable concentrations at the bottom of the deep cell, probably caused by the combination of depth and algal bloom intensity. Therefore, as light could not penetrate to the lower water layers in the deep cell, self-shading reduced potential for photosynthetic uptake (Bokil and Agrawal, 1977). This is supported by the diurnal pattern still being present at 0.5 m in the deep cell contrasting with perpetually high concentrations of CO_2 at the bottom of the water column. The reduction in pH with increasing depth was indicative that photosynthesis was being inhibited and respiratory processes dominating (Maberly, 1996; Rebsdorf *et al.,* 1991).

The lack of photosynthetic uptake in the microcosm experiments meant that in contrast to in situ measurements, increases in CO_2 fluxes were observed where oxygenated water columns facilitated aerobic decomposition of organic matter. In agreement with flux chamber and TBL modelled emissions (Chapter 3), higher flux rates when compared to CH_4 revealed that CO_2 production may have been the main pathway of organic carbon mineralisation, both during oxic and anoxic conditions. Significantly more carbon was mineralised under oxic conditions, potentially as a result of increased aerobic decomposition (Liikanen *et al.,* 2002b). The differences between CO_2 fluxes under diurnal turnover and oxic and anoxic conditions were less evident, as the pattern observed appeared to be confusing. This may be due to increases in CO_2 flux potentially being simultaneously driven by increased aerobic respiration and CH_4 oxidation during aerobic/anaerobic switchovers (Fig 4.9). However, the fraction of CO_2 produced by these two mechanisms would require additional investigation. The significantly higher fluxes of CO_2 from the deep cell sediments supported previous conclusions that there was a greater quantity of suitable substrates for GHG production (Chapter 3).

Aerobic CO_2 production was the main route for O_2 consumption by the sediment, as although RQ values (ratio of CO_2 produced to O_2 consumed) were similar in both deep and shallow cell sediments, the significantly higher rate of O_2 consumption from the deep sediment was coupled with significantly larger CO_2 fluxes. While having a similar RQ value, the much lower rates of oxygen consumption in the shallow cell were mirrored by lower associated CO_2 fluxes. However, the mean RQ values of 1.3 and 1.4 were more than one, thus indicating that more CO_2 was produced than oxygen consumed in aerobic respiration of organic matter. Therefore, as also observed in Scandinavian eutrophic lake sediments (Liikanen *et al.,* 2002b), CO_2 may have also been released by anaerobic processes rather than solely by aerobic decomposition (Richey *et al.,* 1988). This was also evident in the in situ observations, where higher quantities of CO_2 were found within the fully anoxic zone of the deep cell. As such, it may be the case that microbial communities in the sediments can adapt to decomposing organic matter in less favourable conditions.

The contrasting results of the in situ and microcosm observations therefore indicate that two mechanisms were active in the wetland. Firstly the highly aerobic conditions, such as those which reached to the sediment water interface in the shallow cell, facilitated greater mineralisation of carbon from the sediments via CO_2 production. However, this was not exclusively an aerobic process. Conversely, the photosynthetic activity which facilitated the high degree of oxygenation, was consuming CO_2 in the water column which, as observed in the field, could lead to under saturation with respect to atmosphere. Therefore photosynthetic activity, while promoting increased aerobic decomposition was effectively buffering these releases during daylight. This supports results and discussion in Chapter 3 (Balmer and Downing *et al.,* 2011; Pacheco *et al.,* 2013), which suggests that the eutrophic state of many nutrient enriched inland freshwaters could also act as a significant limiter to CO_2 emissions. However, as this mechanism clearly reverses diurnally, strong uptake of CO_2 during the day does not necessarily meant that eutrophic small pond systems are net carbon sinks in the summer. This would require further investigation.

4.8.3. N_2O

N_2O production during a summer eutrophic episode was insignificant when compared with CO_2 and CH_4 and appeared to be limited by the supply of NO_3-N available for denitrification to occur.

Extremely low in situ N_2O concentrations observed throughout diurnal sampling were typical of the behaviour of the wetland during the summer months. The lack of a clear temporal

pattern in either cell over the 24 hours, suggested that factors other than O_2 concentration may have been controlling production. However, given that it has been established (Chapter 3) that NO_3-N limitation (Knowles, 1982) is likely to be a key curb on N_2O production during the summer months, it is possible that production was too low for the impacts of other controlling factors to be fully observed.

This reasoning is supported by positive N_2O fluxes in the sediment microcosms, attributed to the supply water which contained 5-6 mg L^{-1} NO_3-N. The uptake of NO_3-N and release of N_2O by sediments throughout microcosm experimentation suggests that denitrifyers may have been utilising the NO_3-N. Without NO_3-N limitation, anoxic conditions increased fluxes during the prolonged microcosm experiment. The negative correlation between O_2 and N_2O concentrations in the microcosm would suggest that O_2 could limit production when NO_3-N was not limiting (Firestone and Davidson, 1989).

4.9. Discussion: Impacts of O_2 variation on nutrient releases

Observations indicated that oxygen depletion and stratification may facilitate the release and storage of NH_4-N and SRP from the wetland sediments. Algal uptake may potentially limit losses to the water column.

Increased concentrations of NH_4-N and SRP observed closer to the sediment water interface of both wetland cells during the diurnal cycle, indicated that nutrient release was occurring. The wetland sediments appeared to be strongly denitrifying, as illustrated by the lack of NO_3-N in the surface water of the wetland cells, together with the strong uptake observed during both oxic and anoxic phases of the microcosm experiments. As noted, this lack of in situ NO_3-N was the probable cause of low N_2O concentrations in the water column.

In contrast, although NH_4-N was also at extremely low concentrations throughout the shallow cell and in the upper portion of the deep cell, higher concentrations were observed in the anoxic zone of the deep cell. This, together with strong negative correlations with O_2 in field and microcosm observations, suggests that the anoxic conditions at the sediment water interface were responsible for the increased production of NH_4-N (Liikanen *et al.,* 2002b, 2003) and that nitrification of released NH_4-N may have been supressed in the storage zone. However, positive NH_4-N fluxes in both oxic and anoxic conditions in the microcosms indicated that organic N in the sediments was potentially being ammonified to NH_4-N, rather than NO_3-N, or being reduced from NO_3-N, (Saeed and Sun, 2012; Vymazal, 2007), even with

the presence of oxygen in the overlying water. This suggested that production was also continually occurring deeper in the sediment profile and diffusing upwards into the water column (Patrick and Reddy, 1976).

SRP exhibited the same in situ pattern with depth as NH_4-N, together with significant negative correlations with oxygen in the microcosms and field results. The switch from SRP uptake to release during the switch from oxic to anoxic flow during the prolonged microcosm experiment, therefore suggested that, in contrast to in situ observations in Chapter 2, O_2 depletion may facilitate release of mineral-bound P from bottom sediments (Dunne and Reddy, 2005; Moore and Reddy, 1994). However, the similar concentration profiles of SRP and NH_4-N, notably the increasing concentrations with depth, may have also been caused by decreasing uptake via phytoplankton with increasing depth (Drever, 2002). If this was the case, algal uptake may limit the pollutant swapping of nutrients, although increased phytoplankton stocks may enhance anoxia/release of CO_2 and CH_4.

4.10. Conclusions

As examples of small, nutrient-rich inland waters, constructed agricultural wetlands are susceptible to the effects of eutrophic algal blooms. In situ and microcosm observations demonstrated that as in larger lake systems, shallower ponds may potentially stratify and overturn diurnally, creating dynamic oxic and anoxic microenvironments which may both promote and inhibit potential pollutant swapping of GHGs and nutrients. Previous assumptions that GHG fluxes do not vary diurnally in comparable agricultural wetlands (Stadmark and Leonardson, 2005; Tanner *et al.,* 1997), are thus not appropriate in the conditions observed.

Substantial release of CH_4 during stratification-overturning, may be limited by oxidation in the water column. Therefore factors reducing oxidation opportunities, such as hypoxic water layers, shorter retention times in oxic water and rapid mixing may increase the potential for diffusive releases. Such variations may be highly dependent on the extent and rate at which anoxic turnover can occur during darkness before re-stratification and re-oxygenation take effect. Meanwhile, the perpetuation of anoxic zones in deeper wetland/ponds may permit accumulation and potentially increased fluxes of CH_4, CO_2, SRP and NH_4-N from sediments. The implications of this are that when this layer is mixed, pulses of GHGs and reduced

compounds may be released. As such, shallower wetland designs may discourage the build-up of these layers.

Additionally, whilst CO_2 may be the main route for carbon losses from retained sediments, in summer photosynthetic uptake may buffer potential diffusive gas releases, even when well oxygenated sediments emit large amounts of CO_2 into the water column. This indicated that the eutrophic nature of shallow wetland/pond systems can lead to them acting as carbon sinks at points in the diurnal cycle. However, strong nocturnal releases may considerably offset this sink. Shallower wetland designs may also increase the proportion CO_2 uptake in the water column.

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Chapter 5 – Impact of sediment disturbance by storm events on GHG releases in an agricultural wetland

5.1. Introduction

The design and function of constructed agricultural wetlands - to intercept and capture pollutants mobilised by rainfall-runoff events means that they are subjected to variable flow regimes (Jordan *et al*., 2003; Ockenden *et al.,* 2012), with sporadic bursts of intense flows. Unlike many natural lake systems, agricultural wetlands in the UK are very small, shallow (<2 m depth) and, in the case of the study wetland, un-vegetated. This small physical size, together with narrow culverted inlets, means that storm flows entering the wetland are highly focussed and lack the buffering offered by a deeper water column, as would be the case in larger systems. Instead, inputs flow directly over material previously trapped and accumulated within the wetland. Therefore, flow events (or any perturbation to the wetland surface) may result in not only the delivery of nutrients and organic material essential for GHG production, but also facilitate significant mixing of the wetland water column and disturbance of bottom sediments. This mechanism is also likely to be shared with many other shallow pond/lake systems in agricultural landscapes which are fed by storm runoff.

The effects of disturbance from water currents and wind shear on bottom sediments have been associated with pulses of increased ebullitive and diffusive GHGs and nutrient releases in numerous aquatic environments (Bussmann, 2005; Murase *et al.,* 2005; Joyce and Jewell, 2003; Christiansen *et al.,* 1997; Spagnoli and Bergamini, 1997). While ebullitive releases of accumulated gas may have little interaction with the water column between leaving the sediment and reaching the atmosphere (Chanton and Whiting, 1995), slower rates of diffusive gas transfers through sediments and water columns permit much greater interaction and oxidation (Bastviken *et al.,* 2002).

Although agricultural wetlands experience storm flows throughout the year (Ockenden *et al.,* 2012; This study), sediments are perhaps most susceptible to disturbance during the summer, when gas production in the sediment is likely to be high due to the warm temperatures encouraging microbial activity (Knowles, 1982; Segers, 1998). Furthermore, reductions in wetland water column depth during the summer may result in a reduced ability to absorb the physical energy of sporadic but intense storm flows entering the wetland. This

may increase the vulnerability of bottom sediments to disturbance. Field observations (Chapter 3) suggested that this phenomenon may occur in the study wetland, which at several points during the summer was subject to intense storm events in the middle of periods of calm, warm weather (Fig 2.8). However, the highly variable and intermittent nature of these events meant that it is difficult to observe the effects on the sediment and gas fluxes in situ.

Additionally, observations from Chapters 3, 4 and the literature (Liikanen *et al.,* 2002ab, 2003) have illustrated that the anaerobic and aerobic conditions created as a by-product of summer eutrophic algal blooms can have a profound impact on dissolved gas and nutrient fluxes, with anaerobic conditions lowering CH_4 oxidation and aerobic conditions favouring CO_2 release. As such, this variation in antecedent conditions may not only influence the availability of dissolved gases and nutrients for release via sediment disturbance, but also the ability for the wetland to buffer these releases.

The unknown potential for sediment disturbance to increase pollutant swapping of dissolved GHGs and nutrients within agricultural wetlands, and the effects of antecedent O_2 conditions on this mechanism therefore require further investigation.

5.2. Aim

To determine the potential for storm flow-induced sediment disturbance to facilitate the release of dissolved greenhouse gases and nutrients in an agricultural wetland under oxic and anoxic antecedent conditions.

Hypotheses to be tested

1. Disturbance of wetland sediment via mixing of the overlying water column will increase fluxes of dissolved CH_4, CO_2, N_2O and nutrients in an anoxic water column.

2. Disturbance of wetland sediment via mixing of the overlying water column will increase fluxes of dissolved CH_4, CO_2, N_2O and nutrients in an oxic water column.

5.3. Methods

5.3.1. Core sampling and water collection

Sediment cores (n=12) were collected at the end of the summer from random points in both the deep (n=6) and shallow (n=6) cells of the wetland (Fig 5.1). Sampling was performed using a Jenkin sediment sampler, using the method described in Chapter 4. Following the retrieval of sediment samples, sediment depth was adjusted to 100 mm in each sample tube and the excess discarded. Samples tubes were capped and transported to cold storage, the sample remaining in situ and the tube forming the body of the microcosm. Collection and treatment of water is also described in the previous chapter.

Figure 5.1 Retrieval of the Jenkin mud sampler with a sediment core and overlying water column in the sample tube

5.3.2. Microcosm experiment design

Two experiments were conducted in order to assess the effects of sediment disturbance via water column agitation on GHG and nutrient fluxes, under oxic and anoxic conditions. This was achieved by comparing microcosms with unstirred water columns (unstirred), to those featuring intense movement of the water column through stirring (stirred), thus inducing disturbance of the bottom sediments. The experiment was first conducted using anoxic antecedent conditions and then repeated after 1 month using oxic antecedent conditions

The experiments utilised 15 microcosms (Fig. 5.2): 6 unstirred cores, 6 stirred cores and 3 blank cores containing no sediment. Stirred and unstirred microcosm groups each contained two subsets of 3 microcosms containing sediment from deep and shallow cells. Microcosm

construction (Fig. 5.3) was identical to that described in Chapter 4, with inputs to the microcosm controlled via adjustable valves on the inlet and outlets. The outlet was fitted with a 3-way valve which could be used to divert flow for sample extraction and continuously passed water through a flow cell in order to permit physicochemical measurements. The caps on the 6 stirred microcosms were each fitted with a 6 V electric motor, which was connected to a plastic driveshaft and paddle inside the microcosm through an airtight and watertight seal in the lid. The plastic paddle was positioned 50 mm above the sediment-water interface to ensure sediment resuspension occurred. The continuous flow of water from the top to the bottom of the microcosm was intended to limit stratification. Supply water was continuously pumped into the cores from a 25 L polypropylene container with a peristaltic pump at a rate of 0.16 L hr^{-1}. Under anoxic conditions (supply ~1 mg L^{-1} O_2), the supply water had been purged of oxygen beforehand by being sparged intensely with N_2. Health and safety considerations prevented this from being continuous throughout the experiment. Any headspace in the container was filled with a cap of argon (denser than air) to limit exchange of O_2 over time. Under oxic conditions, the supply water (~10 mg L^{-1} O_2) was continuously sparged using dual aquarium air pumps fitted with air stones.

Figure 5.2 The stirring microcosm experimental setup with (left to right) 6 stirred microcosms from the deep (3) and shallow (3) cells, 6 unstirred microcosms with no disturbance from deep (3) and shallow (3) cells and 3 blanks with no sediment.

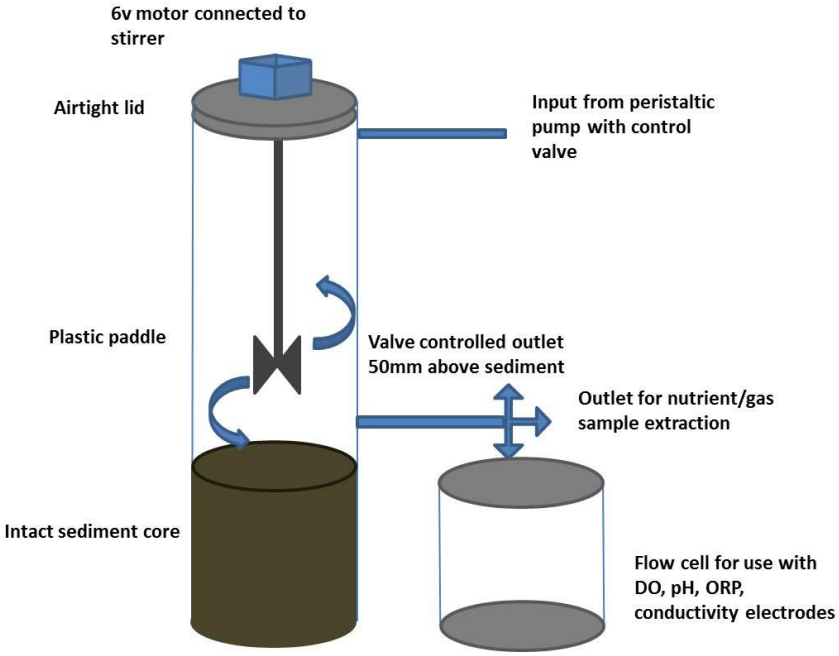

Figure 5.3 Basic design of the sediment microcosm with electric stirrer attached (not to scale. Pump apparatus and monitoring devices not shown for clarity).

5.3.3. Experimental procedure

The first experiment (experiment 1) was conducted using anoxic antecedent conditions. All 15 microcosms were subjected to a 12 hour pre-incubation phase at in situ summer temperatures at the base of the water column (15.5° C), during which the microcosms were kept in the dark. During experiment 1, the cores were fed anoxic water during the pre-incubation. The experiment (in both water column scenarios) was initiated by switching the supply water to oxygenated flow and commencement of water column mixing in the 6 stirred cores, which were stirred for 3 hours to simulate a storm event moving through the wetland (Fig 5.4). After 3 hours, stirring was stopped and the sediment allowed to settle out. Measurements were made every hour between 0-6 hours, then at 8 and 10 hours after the start of the experiment.

Water samples (60ml) for dissolved gas and nutrient analysis were collected in HDPE sample bottles by diverting outflow from the flow cells. The flow cells were then fitted into a closed loop with the multiparameter probe for physicochemical measurements. Ebullitive fluxes were not the target of this experiment and so were not included in observations. Flow rates

in each microcosm were measured at each sampling point. The fluxes of dissolved gases and nutrients were calculated from the concentration differences between the blank and sediment microcosms, flow rates and sediment surface area. After the end of the first experiment, the water column in each microcosm was replaced and the cores incubated in the dark at in situ temperatures for 1 month to allow conditions to stabilise before re-use in the second experiment (experiment 2). In experiment 2, using oxic antecedent conditions, an identical experimental procedure was employed, with the exception of using well oxygenated water during the pre-incubation phase.

Figure 5. 4 Stirred sediment microcosms at (a) the end of the pre incubation phase and (b) during the simulated sediment disturbance. Different levels of sediment in suspension illustrate the heterogeneity of the sediments.

5.3.4. Dissolved gas analysis

Dissolved gases were analysed as described in greater detail in Chapter 4 using a headspace method (Reay *et al.,* 2003; Sobek *et al.,* 2003). 60ml Wheaton bottles were filled with sample, 30ml of which was replaced with zero grade N_2. Samples were agitated vigorously for 2 minutes and left to equilibrate for 30 minutes before 5 ml was extracted and stored in an evacuated exetainer. Samples were analysed using a PerkinElmer Autosystem XL gas chromatograph, fitted with a flame ionisation detector and electron capture detector, with 2 mixed standards of 1000 ppm CO_2, 3ppm CH_4, 2 ppm N_2O and 4000 ppm CO_2, 10 ppm CH_4 and 0.4 ppm N_2O. GC outputs were converted into dissolved gas concentrations using Henrys law and the values of Wilhelm (1970).

5.3.5. Dissolved nutrient analysis

Samples were filtered using a 0.45 µm filter (Nalgene) and stored at 4 °C prior to analysis, which was conducted within 24 hours. SRP, NO_3-N, NH_4-N and DOC were analysed using the methods described in Chapter 2, including quality control procedures.

5.3.6. Statistical analysis

Data for most measured variables displayed significantly skewed distributions and a high degree of variance over the course of the experiment. Although $\text{Log}_{10}+1$ transformations were applied repeated measures tests could not be used due to assumptions being violated.

Differences between unstirred and stirred microcosms were assessed by comparing means from specific stirred and unstirred microcosm subsets at individual time points during the experiment. For example at T1, unstirred deep cell microcosms were compared with stirred deep cell microcosms and unstirred shallow cell microcosms were compared with stirred shallow cell microcosms. Differences between replicate groups were considered at 0, 1, 2, 3, 4 and 10 hrs, with each time point being examined independently of all others. Differences in microcosm characteristics between time points were not tested. Unpaired t-tests were used to compare replicate microcosm subsets. Where normality or homogeneity of variance was violated, Mann-Whitney U tests were used.

5.4. Results: Sediment disturbance in an anoxic water column (experiment 1)

5.4.1. Physicochemical conditions in the anoxic microcosms

As the microcosms were designed to observe how sediment disturbance could affect gas and nutrient release in a scenario when a low O_2 concentrations were present in the water column, initial concentrations at the end of the pre-incubation were low though not totally anoxic (mean 1.2-2.3 mg L^{-1}). On starting the experiment the O_2 concentrations (Fig 5.5) were slightly higher in the stirred microcosms at 1 hour however, after this point, the concentration in the unstirred cores surpassed that in the stirred cores and consistently increased over time. The redox potential and pH (not shown) did not display any significant changes between sediment or treatment types over the course of experiment. pH varied between 7 and 9 over the experiment.

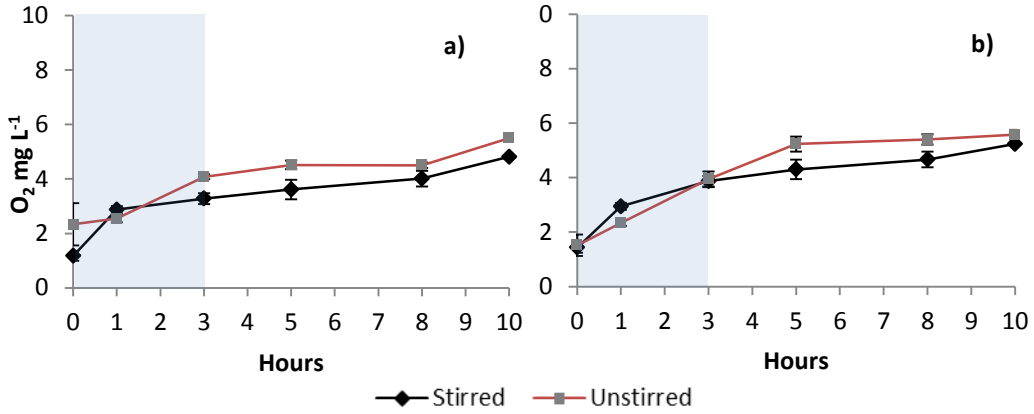

Figure 5.5 Mean dissolved oxygen concentrations in the microcosms (n=3) from the deep (a) and shallow (b) cells during the anoxic water column experiment. Error bars represent standard error. Shaded regions represent the simulated storm period.

5.4.2. Initial conditions in the anoxic microcosms

At the end of the anoxic pre-incubation phase, concentrations of dissolved CH_4, CO_2 and N_2O were greater in the microcosms containing deep and shallow cell sediment, than in either the blank microcosms or the storm water (Table 5.1). The only exceptions to this were very low (0.051 µg L^{-1}) N_2O concentrations observed in the shallow stirred sediments.

Concentrations of NO_3-N and SRP, while being of similar magnitudes in both stormwater and blank microcosms, were substantially greater than those in all sediment microcosms. In contrast, initial concentrations of NH_4-N and DOC, appeared to be higher in the sediment microcosms than either the blanks or storm water.

Table 5.1 Concentrations of dissolved greenhouse gases and nutrients in stormwater and both blank and sediment microcosms the end of the anoxic pre-incubation phase of experiment 1. Standard errors of replicate groups are displayed

Substance	Storm water (n=1)	Blank (n=3)	Deep Cell Stirred (n=3)	Deep Cell Unstirred (n=3)	Shallow Cell Stirred (n=3)	Shallow Cell Unstirred (n=3)
CO_2 (mg L^{-1})	1.14	1.37 ±	3.35 ± 0.04	2.73 ± 0.44	2.60 ± 0.33	2.24 ± 0.13
CH_4 (mg L^{-1})	0.002	0.003 ±	0.33 ± 0.04	0.32 ± 0.06	0.12 ± 0.04	0.08 ± 0.02
N_2O (µg L^{-1})	0.31	0.27 ±	0.32 ± 0.00	0.57 ± 0.30	0.051 ± 0.02	0.50 ± 0.29
NO_3-N (mg L^{-1})	6.16	6.00 ± 0.04	3.86 ± 0.00	4.80 ± 0.26	4.72 ± 0.19	5.19 ± 0.06
NH_4-N (mg L^{-1})	0.01	0.01 ± 0.00	0.72 ± 0.04	0.86 ± 0.07	0.15 ± 0.03	0.09± 0.03
DOC (mg L^{-1})	7.88	10.16 ± 0.34	12.09 ± 2.18	10.12 ± 0.97	9.08 ± 0.75	9.76 ± 1.34
SRP (mg L^{-1})	0.47	0.46 ± 0.00	0.37 ± 0.01	0.36 ± 0.00	0.36 ± 0.00	0.39 ± 0.01

5.4.3. Impact of sediment disturbance on GHG fluxes

Initial CH_4 flux rates (Fig 5.6 a-b) at 0 hours were high in all microcosms at the end of the pre incubation. Flux rates (312-372 mg m^{-2} d^{-1}) from the deep cell sediments were higher than those from the shallow cell sediments (92-133 mg m^{-2} d^{-1}), in keeping with previous observations in Chapters 3 and 4. There were no significant differences between the unstirred or stirred microcosms for any dissolved gases at this time. On commencement of stirring, both unstirred and stirred microcosms exhibited a reduction in CH_4 flux rates. There was no significant difference in flux rates between the stirred and unstirred sets of deep and shallow cell sediments during disturbance or after the simulated storm flow ceased at 3 hrs. However, the rate of reduction in flux rate (angle of the curve) appeared steeper in the stirred microcosms, particularly those from the deep cell. After stirring had ceased, flux rates in the stirred deep sediment microcosms recovered slightly before resuming the downward trend observed in all other groups. After 10 hrs at the end of the experiment, neither of the stirred microcosms containing sediment from deep or shallow cells were significantly higher than the respective unstirred microcosms. CH_4 concentration in the blank microcosms

(Appendix VI Fig 8.8) was lower than in those containing sediment throughout the experiment, resulting in the positive fluxes observed. After an initial sharp decrease from 3.2 to 1.7 μg L^{-1} following the switch to oxic storm water, concentrations displayed a gradual decline until the end of the experiment.

Initial CO_2 flux rates (Fig 5.6 c-d) were substantial in all sediment microcosms after the pre incubation, with the greatest fluxes observed from the deep cell sediments. There were initially no significant differences between stirred and unstirred cores. As stirring commenced, CO_2 flux rates in stirred and unstirred microcosms remained similar up to 1hr from both deep and shallow cell sediments. After 2 hrs of stirring, the flux rates were significantly higher in the stirred microcosms (Mann-Whitney, p=0.006). This significant difference continued after the simulated storm ended at 3 hrs (Mann-Whitney, p= 0.013). CO_2 flux rates in stirred deep cell microcosms continued to climb after stirring had ceased, peaking at 5 hrs (4608 mg m^{-2} d^{-1}) before declining. The corresponding shallow cell microcosms showed peak CO_2 release at the end of the stirring phase (1827 mg m^{-2} d^{-1}), after which fluxes declined. At the end of the experiment at 10 hrs, stirred and unstirred microcosms of the deep cell sediment were no longer significantly different due to an increase in unstirred microcosm flux rates. Shallow cell sediments microcosms were still significantly different (t test, p=0.015). Initial CO_2 concentrations in the blank microcosms (Table 5.1, Appendix VI Fig 8.8) were approximately half of those containing sediment, resulting in positive fluxes. Concentrations remained stable until an increase to 2.93 mg L^{-1} at 6 hours, after which concentrations gradually declined.

Initial N_2O flux rates (Fig 5.6 e-f) were low in all cores and those from the shallow cell stirred microcosms were slightly negative. On stirring commencing, there were no significant differences in stirred or unstirred microcosm flux rates. No major changes in N_2O release from any replicate groups were apparent until the end of the stirring phase. At this time, unstirred microcosm fluxes from both sediments increased and were momentarily greater than those from stirred microcosms. However, from 4-8n hrs stirred microcosm flux rates increased to form a delayed peak, while unstirred microcosms fluxes remained low, turning to uptake in the case of shallow sediments. No significant differences between stirred and unstirred microcosms were seen at any point for both deep and shall cell sediments. Initial N_2O concentrations in the blank microcosms (Table 5.1, Appendix VI Fig 8.8) were extremely low (<1 μg L^{-1}) in all microcosms and stormwater. Concentrations remained at a similar level throughout the experiment.

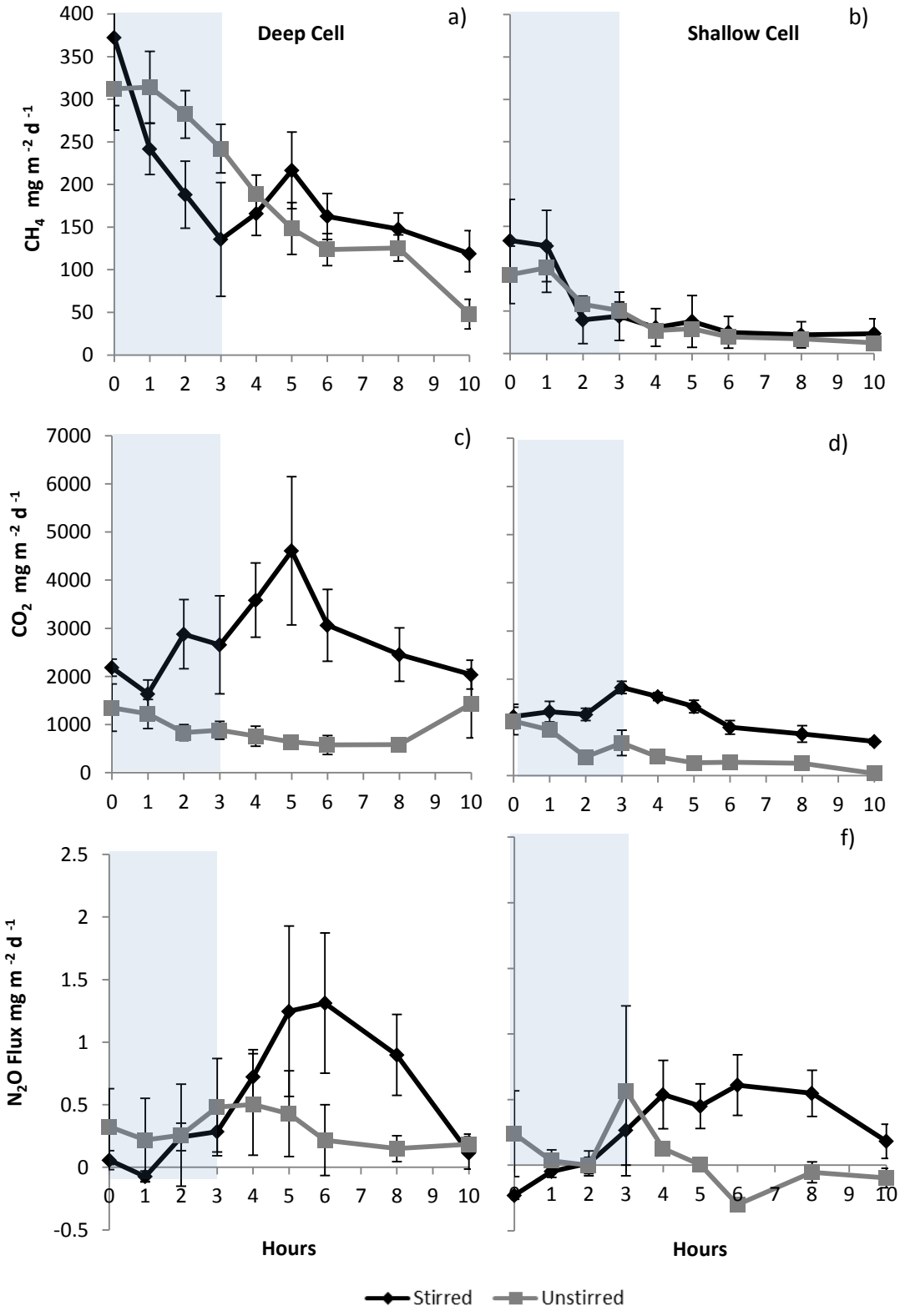

Figure 5.6 Mean (n=3) fluxes of dissolved greenhouse gases in deep and shallow, stirred and unstirred microcosms under anoxic conditions. Error bars represent standard error. Shaded regions represent the simulated storm event.

5.4.4. Impact of sediment disturbance on dissolved nutrient releases

Fluxes of all nutrients changed considerably over the course of the experiment in both stirred and unstirred microcosms and were characterised by substantial degrees of variability between microcosms. At the end of the pre incubation phase, fluxes of NO_3-N (Fig 5.7 a-b) were negative in all microcosms, with uptake being more pronounced in those which were stirred. Stirring of the microcosms did not appear to produce any significant effects, with negative fluxes in both stirred and unstirred microcosms reducing over the 10 hour experiment. NO_3-N concentration in the blank microcosms (Appendix VII Fig 8.9) was stable throughout the experiment, being consistently greater than those containing sediments.

NH_4-N fluxes (Fig 5.7 c-d) were initially similar in stirred and unstirred microcosms of the respective deep and shallow sediment subsets. On commencement of stirring, flux rates in the unstirred microcosms gradually declined, while stirred microcosms decreased rapidly from 775 to 389 mg m^{-2} d^{-1} (deep) and 148 to 97 mg m^{-2} d^{-1} (shallow) until the stirring stopped at 3 hours. Significant differences between stirred and unstirred microcosms were observed in deep sediments at 1, 2 and 3hrs (Mann-Whitney, *p= 0.05*). Significant differences in the shallow cell sediments were only observed at 2 hrs (Mann-Whitney, *p= 0.05*. Flux rates in stirred sediments from both cells then rapidly climbed after 3 hours, increasing above the rates in the unstirred microcosms and peaking at 915 mg m^{-2} d^{-1} (deep) and 44 mg m^{-2} d^{-1} (shallow) at 5 hours . Emissions then declined towards the end of the experiment to reach the same magnitude as the unstirred microcosms. NH_4-N concentrations in the blank microcosms (Appendix VII Fig 8.9) were at the limit of detection at the start of the experiment (Table 5.1). Concentrations varied over the 10 hour experiment, though remained at extremely low levels.

Fluxes of SRP (Fig. 5.7 e-f) were initially similar in respective deep and shallow sediments and all microcosms exhibited strongly negative fluxes at the end of the pre-incubation. After the experiment started, flux rates in the unstirred microcosms showed a steady positive trend over the 10 hours. Rates in both sets of stirred microcosms rapidly declined until after the storm event (4-5 hours). Significantly lower fluxes from the stirred microcosms were detected after 2 hours of stirring (t test, $p<0.001$) and were still significantly different in the shallow sediments at the end of the experiment (Mann-Whitney, $p=0.05$). Concentrations in the blank microcosms (Appendix VII Fig 8.9) remained stable across the experiment. Fluxes of DOC (not shown) did not produce any discernible pattern, with rates of uptake and

release varying considerably over time. DOC concentrations in the blank microcosms (Appendix VII Fig 8.9) were stable over the experiment.

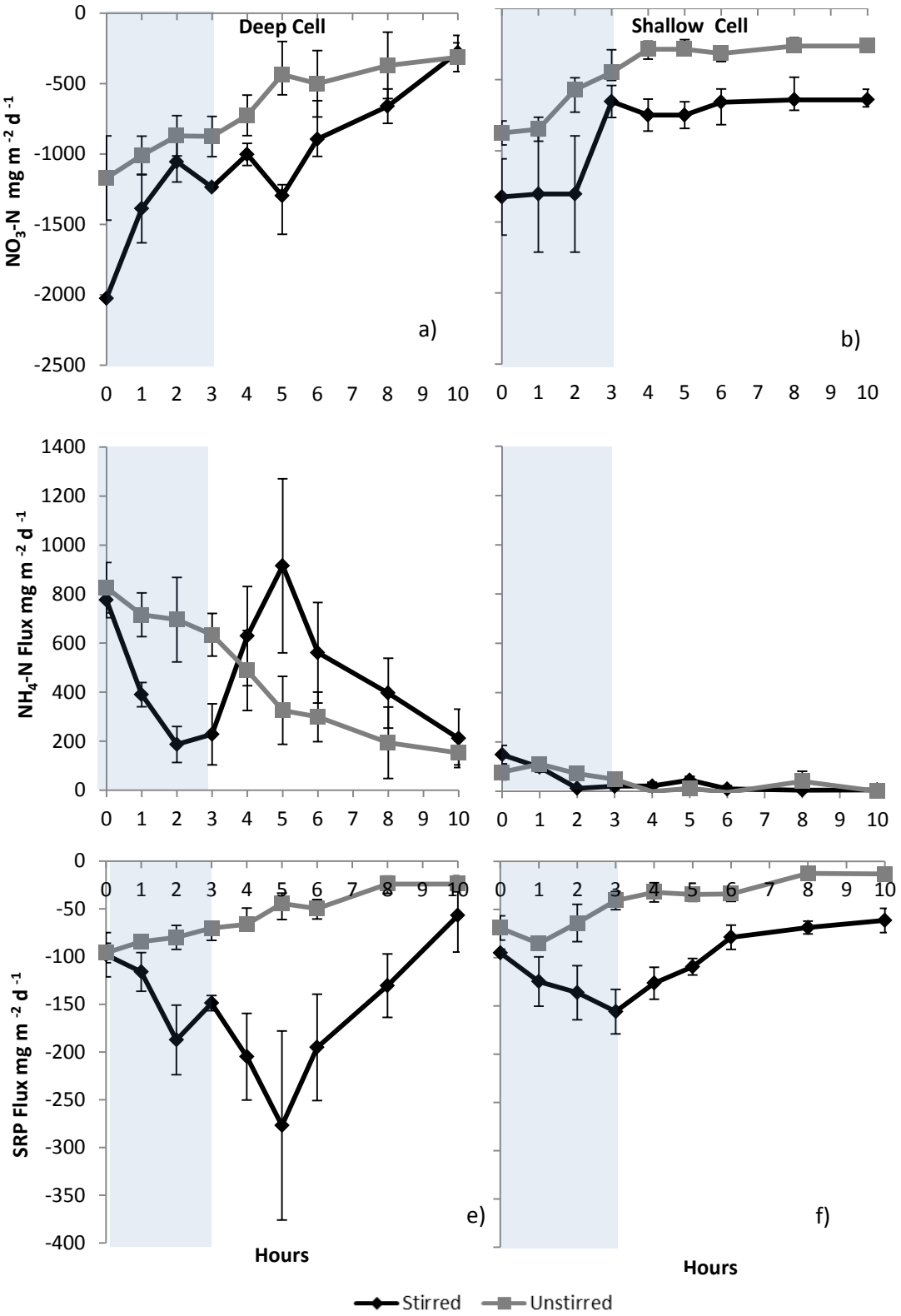

Figure 5.7 Fluxes of dissolved nutrients in deep and shallow, stirred and unstirred microcosms under anoxic conditions. Black lines denote the stirred core fluxes. Error bars represent standard error. Shaded regions represent periods of simulated storm flow.

5.5. Sediment disturbance in an oxygenated water column (experiment 2)

5.5.1. Physicochemical conditions oxygenated microcosms

As the microcosms were designed to observe gas and nutrient release when the water column was oxygenated, the initial O_2 concentrations in the microcosms (Fig 5.8) at the end of the pre-incubation were high (mean 6.7-7.9 mg L^{-1}), although not at saturation due to consumption by the sediments. On starting the experiment, the O_2 concentrations were slightly higher in the unstirred microcosms up to 1 hour, however from 2 hours onwards, the differences in concentration between the unstirred and stirred microcosms became greater, particularly in the deep cell sediments. By the end of the experiment, the concentration difference in the shallow cell sediments had narrowed slightly. The redox potential and pH (data not shown) did not display any significant changes between sediment or treatment types over the experiment.

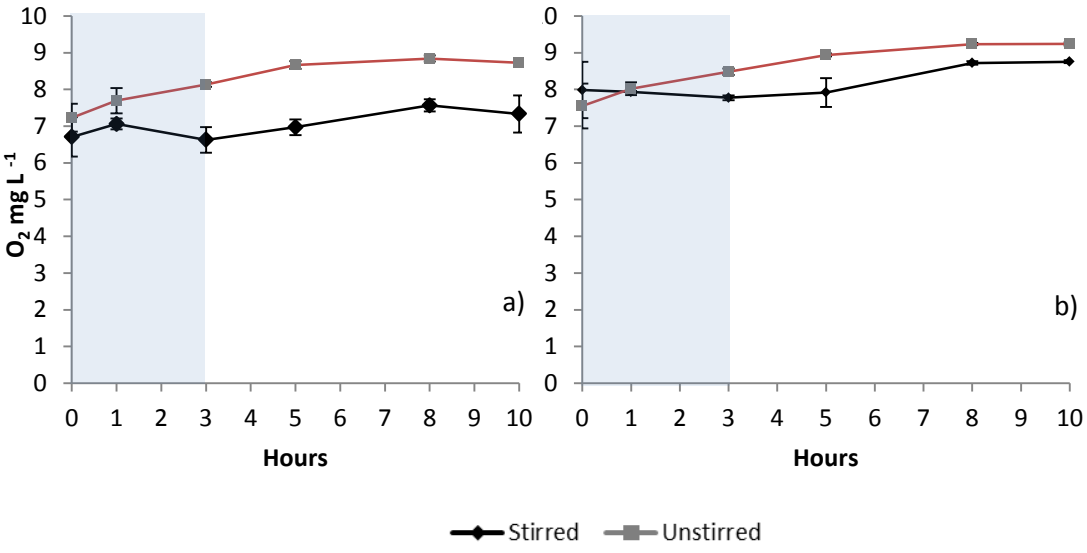

Figure 5.8 Mean (n=3) dissolved oxygen concentrations in microcosms containing sediments from the deep (a) and shallow (b) wetland cells in oxic conditions. Error bars represent standard error. Shaded regions represent periods of simulated storm flow.

5.5.2.Initial conditions in the oxic microcosms

At the end of the oxic pre-incubation phase, concentrations of dissolved CH_4 and CO_2 were similar in the microcosms containing deep and shallow cell sediment with both the blank

microcosms and oxic storm water (Table 5.2). N_2O concentrations in sediment microcosms were over twice those in both the blank microcosms and in the storm water.

Concentrations of NO_3-N were slightly higher in the storm water than those in the microcosms. NH_4-N, SRP and DOC were similar in the sediment microcosms and storm water, although DOC concentrations in the blank microcosms were somewhat higher. NH_4-N was at the limit of detection.

Table 5.2 Concentrations of dissolved greenhouse gases and nutrients in storm water and both blank and sediment microcosms the end of the oxic pre-incubation phase of experiment 2. Standard errors of replicate groups are displayed

Substance	Storm water (n=1)	Blank (n=3)	Deep Cell Stirred (n=3)	Deep Cell Unstirred (n=3)	Shallow Cell Stirred (n=3)	Shallow Cell Unstirred (n=3)
CO_2 (mg L^{-1})	2.81	3.27 ± 0.00	4.70 ± 0.52	4.28 ± 1.17	2.96 ± 0.16	2.64 ± 0.45
CH_4 (mg L^{-1})	0.001	0.001 ± 0.00	0.002 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.002 ± 0.00
N_2O (µg L^{-1})	0.59	0.73 ± 0.15	4.25 ± 0.84	2.27 ± 0.73	1.619 ± 0.33	1.30 ± 0.34
NO_3-N (mg L^{-1})	6.20	5.93 ± 0.06	6.10 ± 0.05	5.96 ±0.06	5.76 ± 0.12	5.65 ± 0.15
NH_4-N (mg L^{-1})	0.01	0.01 ± 0.00	0.09 ± 0.04	0.08 ± 0.05	0.01 ± 0.00	0.01 ± 0.00
DOC (mg L^{-1})	7.03	10.72 ± 2.45	7.35 ± 0.15	7.37 ± 0.15	6.81 ± 0.11	6.99 ± 0.07
SRP (mg L^{-1})	0.472	0.41 ± 0.01	0.423 ± 0.00	0.40 ± 0.02	0.42 ± 0.01	0.41 ± 0.00

5.5.3. Impact of sediment disturbance on GHG fluxes

Initial fluxes of CH_4 (Fig 5.9 a-b) were close to 0 in all microcosms, and approximately 1-2 orders of magnitude lower than in experiment 1. There were no significant differences between flux rates from stirred or unstirred microcosms at the end of the pre incubation. On starting the experiment and initiating sediment disturbance, the CH_4 flux increased from both stirred deep (8.3 mg m^{-2} d^{-1}) and shallow (1.6 mg m^{-2} d^{-1}) microcosms so that after 1 hour, rates were significantly higher than in the unstirred deep (Mann-Whitney, p=0.05) and shallow cell microcosms (Mann-Whitney, p=0.05) respectively. After this point, the behaviour of the deep and shallow cell sediments began to contrast. Fluxes from the deep sediments started to decline before stirring had ceased, though remained significantly higher than the unstirred microcosms after 2 hours (t test, p=0.05). After stirring had finished, stirred deep cell sediments continued to decline until 5 hours before starting to increase again, maintaining a higher rate of flux than the unstirred microcosms. Meanwhile fluxes from the stirred shallow sediments, also being significantly higher than the unstirred

microcosms after 2 hours (t test, p=0.029) continued to climb until peaking at 5 hours (6.7 mg m^{-2} d^{-1}) before declining towards the rates from the unstirred microcosms. Overall, stirred microcosm flux rates were still significantly higher (t test, p=0.011) than the unstirred at 4 hours, although due to sample variability, individual differences in the respective deep and shallow groups were not significant. At 10 hours, CH_4 flux rates in all stirred microcosms were no longer significantly higher than the unstirred microcosms. Mean CH_4 concentration in the blank microcosms (Table 5.2; Appendix VIII Fig 8.10) were initially similar to those in the sediment microcosms (approx. 1 µg L^{-1}) Concentrations remained constant until 4 hours, after which small variations occurred for the remainder of the experiment.

Initial CO_2 flux rates (1292-1330 mg m^{-2} d^{-1}) (Fig 5.9 c-d) from the deep microcosms were of a similar magnitude to those during the anoxic water column experiment, while the shallow sediments displayed negative CO_2 fluxes. There were no significant differences between stirred and unstirred sediment microcosms. On commencement of stirring, CO_2 release increased in all the stirred cores, although CO_2 fluxes only became significantly higher than unstirred microcosms containing deep sediments after 3 hours (t test p=0.007) (2798 mg m^{-2} d^{-1}) and shallow sediments (t test p=0.01) (786 mg m^{-2} d^{-1}) after 2 hours. Stirred microcosm fluxes from deep (t test p=0.038) and shallow (t test p=0.003) sediments were still significantly higher than unstirred microcosms after 4 hours. CO_2 flux remained higher in the stirred microcosms for the remainder of the experiment, although by 10 hours were only significantly higher in the stirred cores from the shallow cell (t test p=0.013). Mean CO_2 concentration in the blank microcosms (3.2 mg L^{-1}) (Table 5.2; Appendix VIII Fig 8.10) were similar to those in the sediment microcosms and storm water. Shallow cell sediment concentrations were slightly lower than those in the blank microcosms, resulting in initial negative fluxes. Blank CO_2 concentrations remained stable reducing slightly over the course of the experiment to reach a final concentration of 2.21 mg L^{-1}.

N_2O fluxes (Fig 5.9 e-f) were very low, at less than 3.5 mg m^{-2} d^{-1} and 1 mg m^{-2} d^{-1} in deep and shallow cells respectively, although initial rates were higher than those at the same time in the anoxic experiment. The high degree of inter-core variability meant that flux rates from the stirred microcosms were not significantly different to the unstirred groups at any point over the course of the experiment. However, a small peak in flux rates was detected in both sets of stirred cores in the 3 hours after sediment disturbance had ended. N_2O concentrations in the blank microcosms (Appendix VIII Fig 8.10) were consistently below 1 µg L^{-1} throughout the experiment. No temporal variations were observed, with the exception of a decrease in concentrations between 4 and 6 hours.

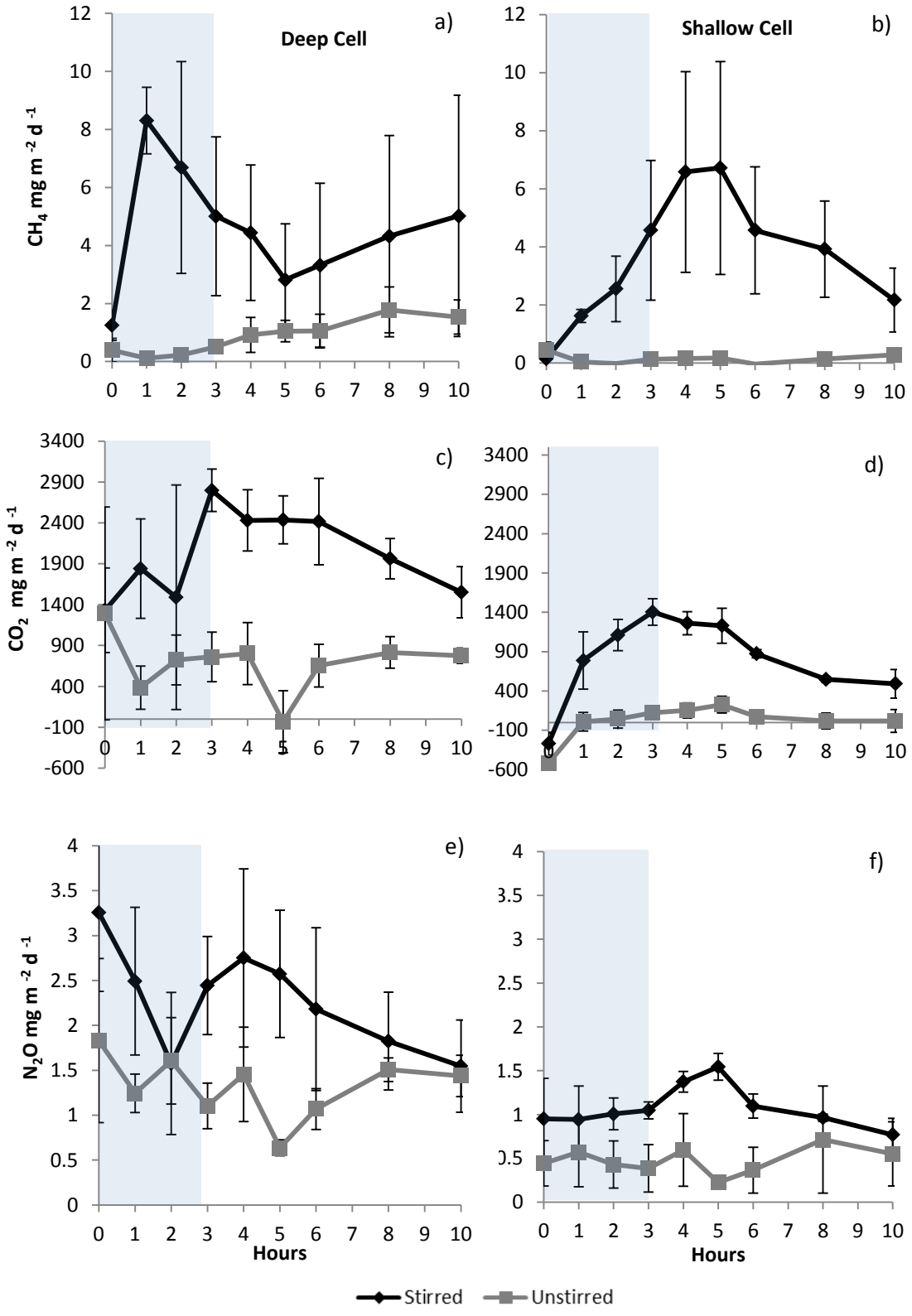

Figure 5.9 Fluxes of dissolved greenhouse gases in deep and shallow, stirred and unstirred microcosms under oxygenated conditions. Black lines denote the stirred core fluxes. Error bars represent standard error of the mean. Shaded regions represent simulated storm conditions.

5.5.4. Impact of sediment disturbance on nutrient fluxes

Nutrient fluxes in all microcosms showed a substantial degree of variability during the experiment. NO_3-N flux (Fig 5.10 a-b) was initially positive from the deep cell sediment microcosms, in contrast to uptake as seen in experiment 1. Shallow cell microcosms initially displayed negative NO_3-N fluxes. Fluxes from deep unstirred and stirred microcosms were similar and both displayed the same flux pattern. The sediments of the shallow cell maintained negative fluxes from the end of the pre-incubation to the end of the experiment. Again, the same temporal pattern in fluxes was observed. NO_3-N concentrations in the blank microcosms (Appendix IX Fig 8.11) remained at approximately 6 mg L^{-1} throughout the experiment, showing no temporal patterns.

Initial flux rates of NH_4-N in the oxygenated deep sediments (84 mg m^{-2} d^{-1}) (Fig 5.10c) were around 8 times lower than during experiment 1. Shallow cell sediment fluxes (0.0 mg m^{-2} d^{-1}) were negligible. Once the experiment started, the unstirred microcosm flux rates decreased. Deep cell stirred sediments followed a similar pattern to that seen in experiment 1, though were only significantly greater than the unstirred microcosms at 4 hours (Mann-Whitney, *p=0.05*) when fluxes peaked (103 mg m^{-2} d^{-1}). After 4 hours, flux rates gradually declined to the level in the unstirred microcosms. No differences could be detected in the shallow sediments. NH_4-N concentrations in the blank microcosms (Table 5.2; Appendix IX Fig 8.11) were below the limit of detection throughout the experiment.

SRP fluxes (Fig 5.10e-f) in all microcosm groups were approximately neutral at the end of the pre-incubation. There was a significant response to stirring in both deep and shallow sediments, with fluxes switching to uptake after an hour (Mann-Whitney, *p= 0.*05) and were still significantly lower than the unstirred microcosms at the end of the experiment (Mann-Whitney, *p=0.05*). Rates of uptake were generally greater in the deep stirred sediments, peaking at -202 and -98 mg m^{-2} d^{-1} in deep and shallow microcosms respectively. SRP concentrations in the blank microcosms (Table 5.2; Appendix IX Fig 8.11) were initially similar to those containing sediments. Concentrations remained stable at approximately 0.4. mg L^{-1} throughout the experiment.

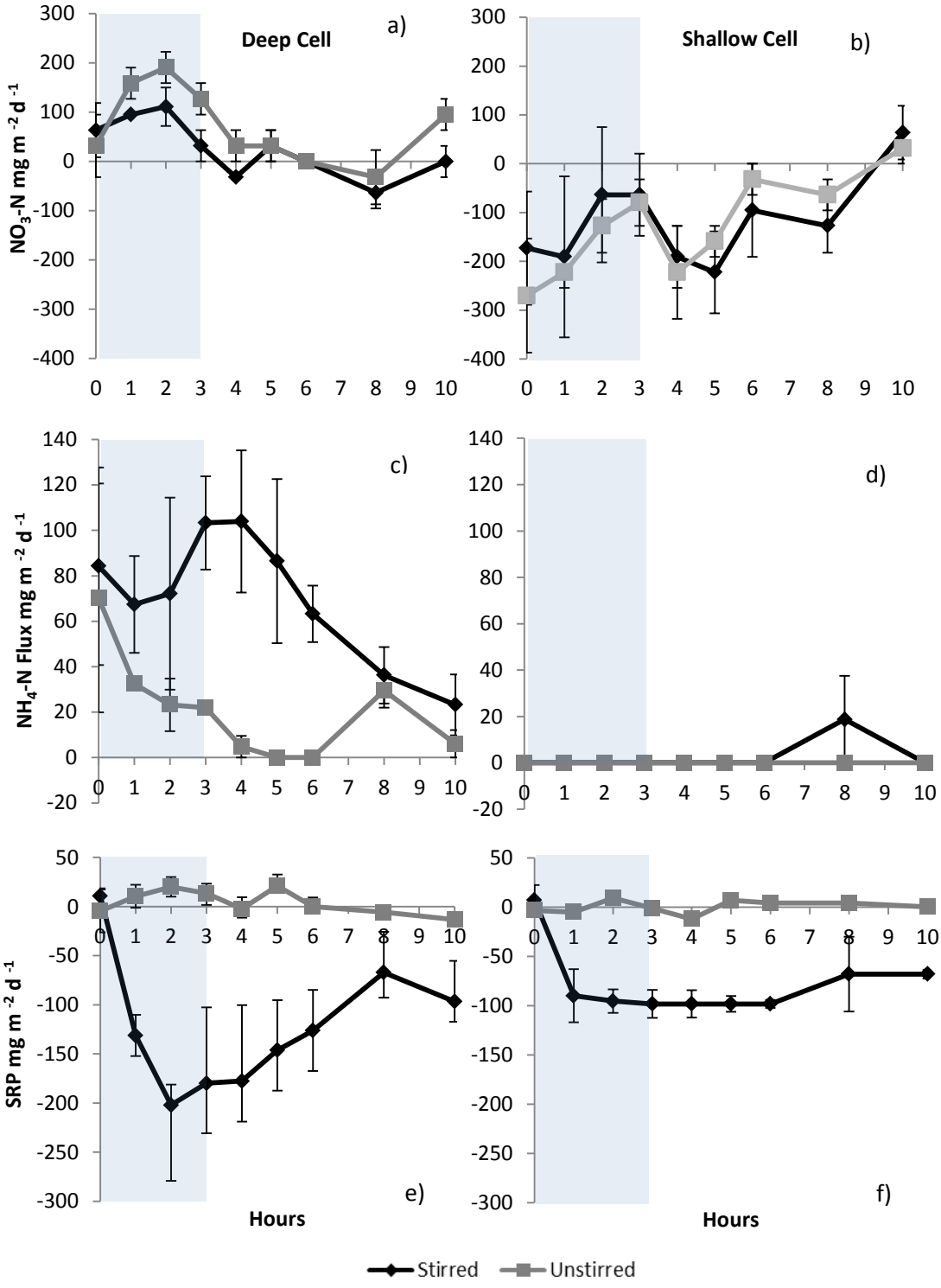

Figure 5. 10 Fluxes of dissolved nutrients in the in deep and shallow, stirred and unstirred microcosms under oxygenated conditions. Black lines denote the stirred core fluxes. Error bars represent standard error of the mean. Shaded regions represent simulated storm conditions.

5.6. Discussion

5.6.1. Effects of sediment disturbance on dissolved GHG release in an anoxic water column

Microcosm simulations showed that in contrast to the original hypotheses, disturbance of wetland sediments under anoxic/hypoxic conditions did not produce large increases in net CH_4 fluxes. However, potential oxidation buffering by an increasingly oxygenated water column may swap CH_4 emissions to significantly increased CO_2 releases.

Microcosm CH_4 concentrations of around 0.3 mg L^{-1} (Table 5.1) at the end of the pre-incubation phase were similar to those observed near the anoxic sediment-water interface during diurnal field sampling (Chapter 4). This indicated that the hypothesised 'worst case' conditions for pollutant swapping – potential for increased release of CH_4 release or reduction in rates of oxidation during hypolimnetic anoxia (Beaulieu *et al.,* 2014; Casper *et al.,* 2000; This study), had increased CH_4 abundance prior to sediment disturbance. High initial CO_2 fluxes also suggested that some CH_4 may have been oxidised by O_2 contained within the microcosms. Alternatively, CO_2 may have been produced anaerobically alongside CH_4 within the sediments, as observed in chapter 4 (Liikanen *et al.,* 2002b; Richey *et al.,* 1988).

Despite the initial conditions, disturbance failed to produce the expected pulse of CH_4 from sediment of either wetland cell (Fig. 5.6 a-b). Although the decline in CH_4 flux rates in the unstirred microcosms was anticipated due to the effects of CH_4 oxidation by aerobic supply water, the similar decrease also observed in the stirred microcosms was in contrast to expectations. While this may suggest sediment disturbance decreased the diffusive release of CH_4, the relationship with dilution and mixing should be considered. As in reality, the 'storm water' had a higher O_2 content and lower dissolved gas concentration (Table 5.1) than that already present in the microcosms. Therefore, all microcosms may have experienced ongoing dilution of the CH_4 which had been trapped in the water column, as well as oxidation. This suggests that although dissolved CH_4 was probably being released from the sediments, the effects of dilution and oxidation may have been buffering fluxes and oxidising CH_4 at a faster rate. Although there were no statistically significant differences between stirred and unstirred microcosm fluxes during mixing, the slightly steeper gradient of CH_4 flux rate decline, particularly in the stirred microcosms of the deep cell (Fig. 5.6a) , suggested that disturbance was having an additional impact on CH_4 fluxes. It is possible that this

difference was caused by the increased mixing of the water column, facilitating greater opportunity for oxidation of CH_4 by methanotrophs (Rudd and Hamilton, 1978; Utsumi *et al.,* 1998). This reasoning is supported by the secondary peak (216 and 29 mg m^{-2} d^{-1}) in fluxes at 2 hours after the disturbance had ended. This peak, present in both stirred sediment microcosm groups (more pronounced in the deep set) suggested that after the mixing of oxygenated water through the microcosm had ceased, CH_4 release exceeded the buffering capacity of the water. It may also be the case that stirring had encouraged microbial activity deep within the sediment (Busmann, 2005), resulting in the secondary pulse of release. The decline in flux rates after this pulse were likely to be as a result of the dilution from the supply water and ongoing oxidation.

While sediment disturbance and water column mixing may result in increased oxidation of CH_4, it is also likely that in situ, the increased physical movement of water from in such a shallow water column may still facilitate higher diffusive emissions. This is suggested by substantial evasion of diffusive CH_4 during breakdown of stratification in many lake and reservoir systems (Bastviken *et al.,* 2004,; Casper *et al.,* 2000; Fernandez *et al.,* 2014; Michmerhuizen *et al.,* 1996).

In contrast to CH_4, the significantly increased fluxes of CO_2 observed in both stirred sediment types from 2 hours onward (Fig.5.6 c-d), clearly illustrated that disturbance increases rates of CO_2 release. The rise in CO_2 flux until the end of the simulated storm event (3 hrs), followed by a gradual decline afterwards, contrasted with the continuous downward trend of the unstirred cores (due to dilution). This implied that the increased flux rate was due to in situ production as opposed to being introduced from the supply water, which contained lower CO_2 concentrations than the sediment microcosms (Table 5.1). While some of this CO_2 was likely to have been produced through CH_4 oxidation, the lack of a corresponding significant reduction in CH_4 fluxes in the stirred microcosms coupled with the significant increase in CO_2 flux rates, suggested that most of the CO_2 produced was likely to be from aerobic respiration of organic matter in the sediment (Liikanen *et al.,* 2002b).

As stirring mixed the water column, the introduction of oxygenated water into the surface of the sediment (Christiansen *et al.,* 1997) may have permitted greater aerobic decomposition and thus production of CO_2. Evidence to support this source of increased fluxes, is provided by slightly lower O_2 concentrations observed in the stirred microcosms as the experiment progressed (Fig. 5.8). This suggests that more O_2 was being consumed in stirred microcosms, through the production of CO_2 (through oxidation of organic matter or CH_4) and reduced

compounds (Christiansen *et al.,* 1997; Laima *et al.,* 1998). The greater CO_2 flux increases during mixing, and lower rates of decline after mixing observed in the deep cell microcosms were in line with previous observations (Chapter 3 and 4) of the sedimentation cell yielding higher quantities of greenhouse gases, most likely as a result of more abundant organic carbon or substrates. However full investigation of this factor would require observation of substrate dynamics in situ.

The similar pattern of increases in N_2O flux in the stirred microcosms (Fig.5.6 e-f), while not being significant due to low flux rates and sediment variability was not replicated in the unstirred microcosms and thus indicated that disturbance may have increased net N_2O release from sediments. Increases in the flux rates of N_2O were not apparent during the period when the cores were undergoing disturbance, instead increasing in the 2-3 hours after the event ended. A lack of increase in N_2O fluxes alongside decreases in NH_4-N suggested that nitrification was not the source of N_2O, instead denitrification being more likely (Saeed and Sun, 2012). During the pre-incubation, while the water column contained the NO_3-N necessary for denitrification to take place, its penetration into the sediment may not have been great enough to facilitate N_2O production (Patrick and Reddy, 1976). On disturbance of the sediment, increased amounts of NO_3-N may have become available to denitrifiers, resulting in a period of heightened N_2O production. Additionally, the introduction of O_2 during mixing may have interrupted full denitrification to N_2, resulting in a pulse of N_2O production (Maltais-Landry *et al.,* 2009). Once the vigorous mixing and dilution had ceased (4 hrs onwards), this increase may have appeared in a more pronounced form. Increases in N_2O with O_2 may also occur due to composition of the microbial community, with some species preferring less anaerobic conditions (Dundee and Hopkins, 2001). However as no increases in flux rates were reported during oxic water column conditions (Chapter 4) this is unlikely in this case. After flux rates had peaked (6 hours) the effects of lower concentrations of N_2O in the supply water caused these flux rates to decrease again.

5.6.2. Effects of sediment disturbance on nutrient fluxes in an anoxic water column

Sediment disturbance in an anoxic water column may increase the fluxes of NH_4-N, but also increase the potential for biogeochemical conversion or binding of SRP in oxygenated storm water.

Initially high flux rates of NH_4-N showed that the anaerobic conditions had resulted in substantial ammonification of N in the sediments or dissimilatory reduction of NO_3-N (Saeed and Sun, 2012). On initiation of the storm event with the oxygenated flow, both unstirred and stirred microcosms exhibited the effects of nutrient dilution from the storm water (Table 5.1), manifesting as an increase or decrease in flux rates (Fig. 5.7). The pronounced reduction in stirred deep cell sediment flux rates of NH_4-N from 775 to 227 mg m^{-2} d^{-1} between 0 and 3 hours, contrasted with the gradual decline observed in the unstirred microcosms as it was diluted by lower storm water NH_4-N concentrations. This would suggest the action of stirring was mixing the water column with the incoming water to a greater extent than the unstirred microcosms. However after stirring and the high rate of mixing had finished, a sudden pulse of fluxes at 4 hrs was observed in both deep and shallow stirred cores (which at this point were still well mixed and being fed low concentrations of NH_4-N). This therefore suggests that the sediment was releasing NH_4-N into the water column, the gradual decline of fluxes towards the unstirred microcosm rates being the product of the continuing dilution.

The storm event may have increased NH_4-N release into the water column, although initially dilution and mixing was masking or buffering the effect. An inverted temporal pattern in the NO_3-N and NH_4-N fluxes, also seen in field observations (Chapter 3 and 4), was initially replicated in the microcosms (Fig. 5.7) This may suggest that the initial decrease in NO_3-N sediment uptake rates during mixing may have been facilitated by nitrification of organic N exposed to oxygenated water, or nitrification of NH_4-N released into the aerobic water column (Vymazal, 2007). Additionally, flux rates may have appeared to increase due to the marginally higher NO_3-N concentrations in the supply water than in the microcosm (Table 5.1).

As concentrations of SRP were similar in the storm water to that in the microcosms (Table 5.1), the initial decline in SRP flux rates (Fig 5.7e-f) observed in both stirred microcosm sets up until 1-2 hours after the disturbance event had ended could not have occurred through dilution. It is possible that as the stirring brought sediment into suspension, it interacted with the higher concentration of SRP in the supply water. In these increasingly oxygenated conditions, the dissolved P could have been adsorbed onto any Fe (Reddy *et al.,* 2005) that may have been present in the sediments and brought into suspension. As such, this may have immobilised the P and resulted in uptake. When stirring stopped, the suspended sediment settled out and the oxidising layer at the sediment surface re-sealed (Bussman, 2005), this interaction gradually ended. The continued input of higher concentrations of SRP in the supply water then gradually brought the flux rates back towards neutrality.

5.6.3. Effects of sediment disturbance on GHG fluxes in an oxygenated water column

Simulated sediment disturbance by a storm event entering the agricultural wetland with a well oxygenated water column resulted in low but significant increases in releases of CH_4 and CO_2. However, CH_4 fluxes may be largely oxidised rapidly in the water column.

Sediments produced a considerably different response to when under an anoxic water column in experiment 1. The rates of CH_4 flux observed at the end of in the pre-incubation (Fig. 5.9 a-b) were 1-2 orders of magnitude lower (1.25 and 0.14 mg m^{-2} d^{-1}) than those at the end of the anoxic pre incubation. These reduced initial fluxes could be attributed to rapid rates of CH_4 oxidation in both the water column and the upper layers of sediments, facilitated by oxygenated conditions (King *et al.,* 1990). Thus removal of CH_4 was substantial prior to the experiment commencing. The minimal difference between CH_4 concentrations in the supply and microcosm water also meant that the effects of dilution on fluxes were less pronounced. In contrast to experiment 1, on initiation of mixing, stirred microcosms displayed significantly increased rates (8.3 and 1.6 mg m^{-2} d^{-1}) of CH_4 flux in comparison to the unstirred microcosms. At 1 hour after stirring had started, deep and shallow cell fluxes had increased by a factor of 6 and 11 respectively. This was similar to increases reported in Bussman (2005) who also noted an approximately similar pattern in fluxes over time. These results therefore confirm that sediment disturbance facilitates increased release of dissolved CH_4 from wetland sediments to the water column.

In the stirred deep cell microcosms, peak CH_4 flux rates were reached after 1 hour but, although the storm event continued for a further 2 hours, fluxes rapidly declined. This therefore suggested that while CH_4 oxidation could not initially buffer all of the dissolved CH_4 released by sediment disturbance (resulting in the spike in fluxes), after this point oxidation removed the gas released. As the amount of gas released from the sediment initially was low due to the oxygenated antecedent conditions, this removal was rapid. The increase in mean CH_4 fluxes after disturbance had ended (6 hrs) was not anticipated, as CH_4 flux rates should have continued to decrease as a result of dilution/oxidation. The pattern observed could be attributed to an increase in fluxes in one of the microcosms. The reason for this is unknown, although a possibility is that it could be related to the heterogeneity of the sediment or that, as in Bussmann (2005), disturbance of sediment could have increased anaerobic decomposition by stimulating microbial activity. As such, after 10 hours the flux rates were

higher than in the unstirred cores, though due to variability not significantly so. Excluding this from estimates, results in flux rates returning to slightly below those observed in the unstirred microcosms at 10 hours.

The stirred microcosms containing shallow cell sediment, also displayed significantly increased CH_4 fluxes (Fig.5.9b), although peak rates (6.7 mg m^{-2} d^{-1}) were not reached until 2 hours after the disturbance event had ended. After this time, flux rates gradually decreased although still had not returned to the unstirred flux rates after 10 hours. Differences in the flux rates observed from deep and shallow microcosms were small, and so solid conclusions as to the variations in stirring response under aerobic conditions cannot be drawn using limited microcosm replicates.

The rates of CO_2 flux (1840 mg m^{-2} d^{-1}) (Fig. 5.9c) in the deep cell cores were similar at the end of the pre-incubations in experiment 1 (2182 mg m^{-2} d^{-1}). However, it is likely that when the water column was fully oxygenated, these fluxes were a mainly a product of the aerobic decomposition of organic matter together with some low amounts of CH_4 oxidation (due to the low CH_4 concentrations). The initial uptake of CO_2 in the shallow microcosms was caused by the initial CO_2 concentrations being lower than those in the blank microcosms. The reason for this could not be explained, although as in other microcosms and field observations (Chapters 3 and 4), shallow sediment emissions (both stirred and unstirred) were lower than those from the deep cell.

On commencement of the simulated storm, CO_2 fluxes increased, peaking at 2798 and 1404 mg m^{-2} d^{-1} in deep and shallow sediment microcosms respectively. Although rates in both experimental groups peaked at the end of the storm event, neither displayed a rapid decrease in CO_2 flux rates afterwards. This suggested that as in the pre incubation, most of the CO_2 being produced was as a result of aerobic decomposition of the organic matter rather than CH_4 oxidation. Further evidence of this comes from the lower O_2 content in both sets of stirred microcosms once stirring had started and quantities of produced CO_2 exceeding that which could be attributable to the oxidation of the low concentrations of CH_4. Gradual decrease in CO_2 flux rates in the hours following end of mixing were likely to be a product of the gradual dilution of the water column by the inflowing storm water (Table 5.2), coupled with a decline in CO_2 flux rates as organic material settled and anaerobic conditions in the sediment re-established, (Christiansen *et al.,* 1997; Bussman, 2005) thus limiting interaction between the aerobic water column and the buried sediment. In reality, as the oxygenated water column in this experiment may be a product of eutrophic wetland

conditions, CO_2 fluxes may be largely buffered through photosynthetic uptake during daylight (Balmer and Downing, 2011), as observed in Chapter 4. However if occurring at night this buffering mechanism would be absent.

N_2O fluxes were extremely low and varied substantially in both stirred and unstirred cores. Surprisingly, pre-incubation flux rates were higher than those in the anoxic experiment, when the opposite was expected. There were no consistent significant differences in flux rates from stirred and unstirred microcosms. Although a small non-significant increase in fluxes was detected after the end of the stirred phase in both stirred subsets, it is possible that this may be attributable to the slight increase in blank microcosm N_2O concentrations observed in the same period. However the very low rate of emissions makes it harder to draw conclusions.

5.6.4. Effects of sediment disturbance on nutrient fluxes in an oxygenated water column

When subject to a storm event in an oxygenated water column, wetland sediments appeared to have a reduced potential to release NH_4-N and SRP than when in anoxic conditions.

The oxygenated pre-incubation had created less than optimal conditions for ammonification of organic N or NH_4-N in the sediment (Vymazal, 2007), illustrated by reduced fluxes of NH_4-N (84 and 0 mg m^{-2} d^{-1}) (Fig.5.10 a-b) from deep and shallow cell sediments, in comparison with those observed in experiment 1 (Fig.5.7a-b). As such, rapid rates of NO_3-N uptake observed in experiment 1 were replaced by low rates of NO_3 release/uptake (Fig.5.10c-d). Surprisingly, initial SRP flux was positive, in contrast to the uptake observed in experiment 1.

NH_4-N fluxes from the stirred deep cell microcosms increased to a low peak of 113 mg m^{-2} d^{-1} an hour after disturbance had ended. This contrasted with the simultaneous decrease in flux rates of the deep unstirred cores, and implied that the disturbance of some of the lower anoxic sediment layers may have released NH_4-N despite antecedent conditions potentially limiting production in the sediment. Declining fluxes after the peak may have been facilitated by nitrification or dilution by the low NH_4-N concentrations in the storm water (Table 5.2). Initial increases in NO_3-N in the deep sediment microcosms at the start of the storm suggested nitrification of NH_4-N may have been occurring (Faulwetter *et al.,* 2009) with rates decreasing as water column NH_4-N declined. The lack of response from shallow cell

microcosm may have been a result of the low initial NH_4-N build-up in the sediment and thus reduced availability for release.

SRP showed a pronounced response to disturbance in both sediment types, moving to uptake within the first hour (Fig.5.10 e-f) while unstirred microcosms remained stable. This contrasts with negligible changes in SRP fluxes observed during resuspension by Christiansen *et al.* (1997), who cite sediment oxygenation as a limiter for SRP release. Therefore, as hypothesised in section 5.6.2, it appears that in this experiment the oxygenated conditions may have promoted the adsorption of P onto any Fe while in suspension, resulting in a precipitation and binding of SRP (Reddy *et al.,* 1999). The gradual decrease in uptake after disturbance ended suggests that over time the sediment surface was re-sealing, together with potential development of a boundary layer increasingly limiting O_2 transfer into the sediments (Christiansen *et al.,* 1997). This strengthening anoxia may reduce SRP uptake. Analysis of Fe compounds in the sediment/water column would be necessary to confirm this mechanism.

5.7. Conclusions

The two microcosm experiments demonstrated that disturbance of wetland sediments by storm events have the potential to increase the pollutant swapping of dissolved greenhouse gases and nutrients into the water column. However, in support of observations from Chapter 4, the magnitude of releases and the degree to which the water column can buffer them may be driven by the antecedent concentrations of O_2. The risk of disturbance-induced pulses of GHGs and nutrients may increase in anoxic or hypoxic water columns, for example as a result of eutrophic algal blooms. Low oxygen conditions at the sediment water interface may increase the in-sediment production, storage and initial availability of CH_4, N_2O and NH_4-N for rapid mixing into the water column during storm flow. In contrast, well oxygenated overlying water may reduce the initial availability of these substances.

In contrast to the original hypotheses, although anoxic conditions may increase CH_4 availability, potentially heightened oxidation rates by oxygenated stormwater may act as a buffering mechanism, neutralising the 'pulse' of released CH_4. However, the physical movement of CH_4 enriched water to the surface by storm flow may still potentially facilitate increased CH_4 fluxes as potentially observed in Chapter 3, although further work would be

needed to clarify this. CH_4 pulses in oxygenated water columns may appear more pronounced, but insignificant compared to those from anoxic conditions.

Meanwhile, sediment disturbance may greatly increase diffusive fluxes of CO_2 under both tested scenarios, by potentially stimulating aerobic respiration of organic matter. In addition, while oxidation may buffer CH_4 releases, these may be simply swapped to heightened CO_2 fluxes, particularly where CH_4 has built up under anoxic conditions. Well oxygenated antecedent conditions therefore reduce this additional source of fluxes. Chapters 3 and 4 suggested that in addition to oxygenating the water column, photosynthetic CO_2 uptake by the phytoplankton may potentially buffer CO_2 fluxes, although this would depend on disturbance occurring during daylight and presence of algal blooms.

The impacts of sediment disturbances on nutrient fluxes is complex. Under both tested scenarios, NH_4-N may be released into the water column by sediment disturbance, although in anoxic conditions, the wetland biogeochemistry results in a greater quantity being available for release. In contrast, disturbance may promote the immobilisation of P from the water column by promoting interaction with Fe in sediments. However, this relationship would require additional investigation.

Chapter 6 – Risks of pollutant swapping from constructed agricultural wetlands and implications at the national scale

The original aim of this research was to determine the potential for constructed agricultural wetlands to facilitate the pollutant swapping of greenhouse gases to the atmosphere and nutrients to ground/surface waters. The key research findings relating to this overall aim are now summarised and examined, together with the potential implications for the use of agricultural wetlands as future mitigation options within the landscape.

6.1 Constructed agricultural wetlands are hotspots for pollutant swapping at the local scale

In situ observations (Chapters 2 and 3) made on a field-scale agricultural wetland confirmed that they may be potential pollutant swapping hotspots. Monitoring of wetland and riparian areas undertaken in Chapter 3 indicated that conversion of unproductive agricultural land into constructed wetlands may significantly increase net emissions of CH_4, CO_2 and N_2O to the atmosphere, throughout an annual cycle. Increased emissions of CH_4 relative to the riparian area formed the main component of wetland CO_2e emissions, due to the relatively large rates of gas release (Chapter 3) and high GWP potential. Similarly, the in situ observations of hydrochemical pollutants in the local groundwater surrounding the wetland examined in Chapter 2 identified that biogeochemical turnover of retained sediments may potentially allow the mobilisation, leaching and advective transfer of dissolved nutrients, particularly NH_4-N, to groundwater systems.

The data collected in this study therefore extends the past focus on constructed wetlands treating point sources and confirms that despite being net nutrient sinks and showing promise as land management tools (Braskerud, 2002; Braskerud *et al.,* 2005; Ockenden *et al.,* 2012; Diaz *et al.,* 2012), GHG emissions and groundwater exchanges can be enhanced from constructed wetlands in agricultural catchments. Thus their use may need reconsideration particularly in terms of potentially detrimental effects on the atmosphere and water of these land management options.

6.2 Mechanisms influencing the potential for pollution swapping in agricultural wetlands

The potential risks of pollutant swapping can be influenced by a wide range of interacting mechanisms which depend on physical, geological, hydrological and hydrochemical factors. Therefore, a broad understanding of how these temporally and spatially variable relationships may affect the risk of pollutant swapping from agricultural wetlands within differing catchments is necessary. Key findings from field and microcosm experimentation can be built into the original conceptual model (Fig 6.1) and are discussed in sections A-F.

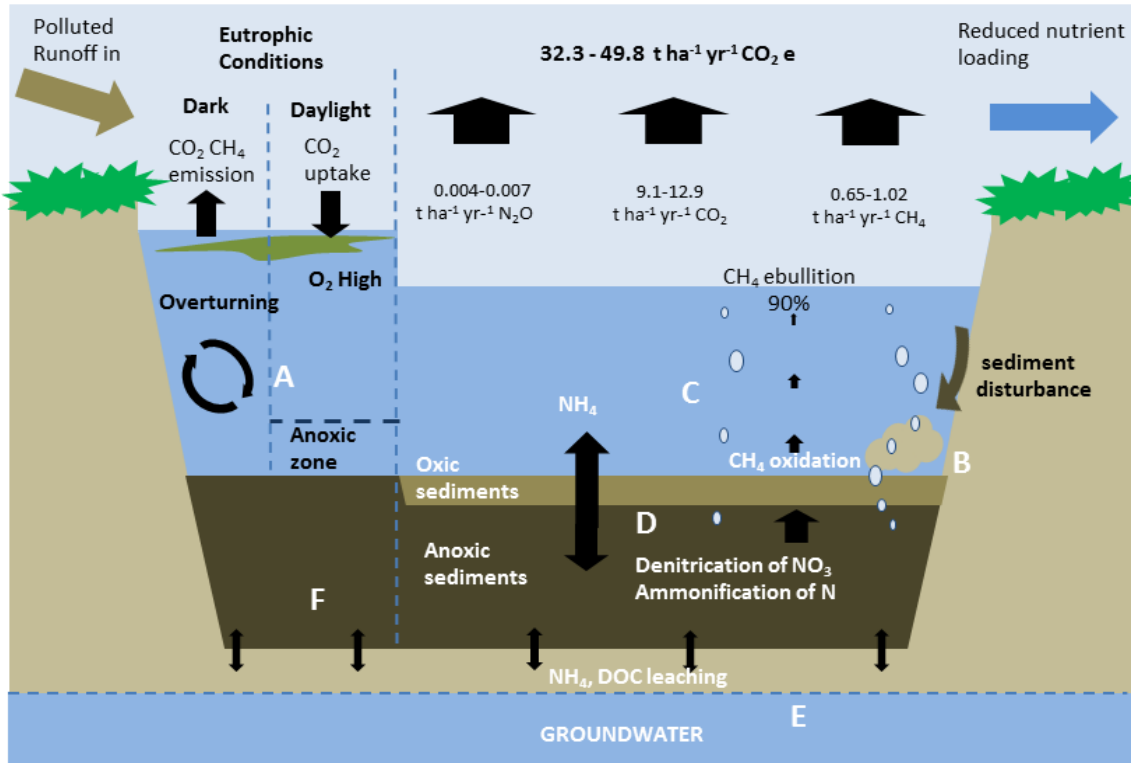

Figure 6.1 Conceptual model of key factors influencing pollutant swapping between an agricultural wetland and both the atmosphere and the local groundwater system. Eutrophic conditions segment illustrates how water column conditions change during an algal bloom. Wetland shown is a schematic only. Key mechanisms A-F are discussed in the text below.

A) Stratification and overturning of eutrophic wetlands enhance the potential risks of CH_4, CO_2 and NH_4-N export

Field observations detailed in Chapters 3 and 4 demonstrated that pollutant swapping of GHGs and NH_4-N to the atmosphere and surface water of agricultural wetlands, may increase diurnally under anoxic/hypoxic conditions driven by eutrophic and stratified water columns.

Chapter 4 demonstrated that the inherently nutrient enriched nature of agricultural wetlands may produce summer algal blooms, while thermal stratification of water columns of only 0.5 m depth may occur. During daylight, photosynthetic activity may reduce the risk of GHG transfers to the atmosphere through consuming dissolved CO_2 and producing O_2, additionally resulting in rapid oxidation of diffusive CH_4 fluxes. As observed in Chapters 3 and 4, this is particularly the case in a shallower water column where oxygenation can extend to the sediment-water interface. In this respect, shallower wetland designs may maximise oxidation of uppermost sediments and reduce the potential for GHG releases.

However, field and microcosm experimentation in Chapter 4 also revealed that diffusive releases of CO_2 and CH_4 can increase substantially during the nocturnal breakdown of stratification. This is due to phytoplanktonic respiration releasing CO_2 and reduced CH_4 oxidation as anoxic/hypoxic conditions develop at the base of the water column. Evidence also suggests that an increase of only 0.2 - 0.5 m in constructed wetland water depth may permit this anoxic zone to perpetuate over numerous diurnal cycles. This may create a storage area for dissolved CH_4 and CO_2 with minimal oxidation, as well as promoting NH_4-N and SRP release from bed sediments to surface water. The balance between diurnal release of C as CO_2 and CH_4, and daytime photosynthetic uptake of C during these episodes is highly complex and uncertain. Wetland depth, presence of vegetation (Chimney *et al.,* 2006), time of year and variations in climate may all be factors influencing turnover mechanisms.

B) GHG and dissolved nutrient release may increase during stormflows

Disturbance of bed sediments and water columns by periodic storm flows may temporarily increase GHG emissions from an agricultural wetland**,** as demonstrated by sediment microcosms in Chapter 5. The disturbance mobilises trapped CH_4, N_2O and NH_4-N, while introducing O_2 to sediments and stimulating aerobic respiration, thus increasing fluxes of CO_2. Variations in antecedent conditions evaluated in Chapter 5, suggested that the presence of an anoxic/hypoxic water column, such as those identified in Chapters 3 and 4, may increase the potential for GHG and NH_4-N release. This may be because increased rates of in-sediment anaerobic production result in greater stored quantities of gas and NH_4-N relative to those under aerobic conditions. Meanwhile, as also supported by evidence in Chapter 4, oxygenated overlying water columns may have the potential to buffer such releases via oxidation and nitrification processes. Therefore, where storm flows interact with eutrophic wetland conditions, there may be significantly increased potential for pollutant swapping. In

addition, in situ observations in Chapter 3 suggested that ebullitive releases of CH_4 may be triggered by storm events entering a wetland.

The potential for such stormflow-mediated gas and nutrient releases may vary greatly with the rate of microbial activity in the wetland and magnitude of storm flows. Intense storm flows may increase the degree of disturbance, and be influenced by the responsiveness of the storm hydrograph to rainfall inputs under varying catchment land management practices and rainfall intensities. This may also suggest the potential effects of climate change may increase risk of storm-mediated pulses of swapping in the future. Additional catchment characteristics, such as the composition of sediments, may be considerations when assessing the potential for these events to elicit heightened gas fluxes.

Inclusion of vegetation or baffles to reduce the intensity of stormflows into wetland designs may be one method of reducing the potential for increased pollutant swapping through resuspension (Braskerud, 2001). However, while helping to stabilise the water column (Chimney, *et al.,* 2006), both additions would increase capital costs to the farmer and potentially elevate sedimentation rates around the wetland inlets, increasing the need for maintenance.

C) Ebullitive wetland CH_4 emissions minimise the potential for oxidation

As the largest component of wetland CO_2e emissions, 93-96 % CH_4 is emitted via ebullitive releases from bed sediments, as demonstrated by field observations in Chapter 3. This permits rapid transfer of CH_4 through the water column, thereby minimising the opportunities for oxidation, even in well oxygenated water. Moreover, in situ monitoring in Chapter 3 suggested that highly CH_4 oxidising conditions at the sediment-water interface may not significantly reduce ebullitive CH_4 production which may therefore be focused deeper in the sediment profile. Release of ebullitive gas fluxes appears to be highly spatially and temporally complex, although may be triggered by reductions in hydrostatic pressure from shallow water levels or low atmospheric pressure. This contrasts with the relatively small proportion of CH_4 transfers through diffusive mechanisms which, due to a longer residence time in the water column, may be highly regulated by oxidation (Chapters 4 and 5) and partial pressures of CH_4 in the atmosphere.

D) Anaerobic conditions in wetlands can both promote and inhibit pollutant export

In situ monitoring of a field scale wetland, including high spatial resolution water column sampling (Chapter 3) revealed that O_2 concentrations may vary substantially in wetland bottom waters, with implications for release of GHGs. The shallow water column in agricultural wetlands often maintains aerobic respiration in oxygenated conditions near the uppermost sediments, increasing the potential for carbon loss via CO_2, as confirmed in microcosm experimentation under aerobic water (Chapter 4 and 5). In contrast, loads of biodegradable carbon may also consume all the dissolved O_2 in wetland bottom water and permit the rapid establishment of anaerobic conditions, especially below the uppermost sediments. This may result in substantial production of CH_4 and N_2O (Chapter 3).

Continuous releases of ebullitive and diffusive CH_4 emissions under aerobic conditions in field (Chapter 3) and microcosm experiments (Chapters 4 and 5), were generally combined with poor relationships with NO_3-N. This contrasts with observations of CH_4 inhibition by NO_3-N in agricultural wetlands by Stadmark and Leonardson, (2005), who asserted that such a mechanism may limit CH_4 losses in agricultural catchments with high NO_3-N loadings. This may suggest that firstly CH_4 production may be focused deep in the sediment profile at the study wetland, and secondly that redox buffering from NO_3-N in the water column or upper sediment may not have a significant inhibiting impact on CH_4 production, when at moderate concentrations. Evidence of redox buffering by NO_3-N against Fe reduction reported in other temperate catchments (Surridge *et al.,* 2007) suggests that this inhibitory mechanism varies spatially.

The anoxic conditions within wetland sediments and groundwater also have the potential to mitigate NO_3-N leaching, but promote NH_4-N production and transfer. As demonstrated in Chapter 2, negligible concentrations of NO_3-N in the anoxic porewater, suggested agricultural wetlands may mitigate NO_3-N leaching to lower groundwater (Brauer *et al.,* 2015) through intense denitrification. Additionally, Chapter 3 suggested that low NO_3-N availability may also limit the production of N_2O in anoxic sediments. However, leaching from higher NO_3-N loadings may potentially still occur or increase N_2O emissions. In contrast, Chapter 2 also suggested that anoxic porewaters favour ammonification of trapped organic N or reduction of NO_3-N, thus making them a potential source of NH_4-N to both groundwaters and surface waters (Chapters 3, 4 and 5).

The high GWP and release rate of CH_4 when compared to undisturbed riparian fluxes (Chapter 3) showed that it is the primary GHG of concern from agricultural wetlands, while

NH_4-N represents the greatest aquatic risk due to potential for subsequent nitrification or volatilisation to NH_3 (Vymazal, 2007). This suggests that additional management may be required to reduce swapping potential (Mander *et al.,* 2014b). Reducing CH_4 fluxes from wetlands by promoting oxygen penetration into the sediment may be achieved via colonising them with vegetation (Armstrong, 1978; Mander *et al.,* 2014), which may also stimulate nitrification and biological uptake of NH_4-N. However, the vascular stems of emergent macrophytes are also recognised as a major CH_4 transport pathway in aquatic systems (Shutz *et al.,* 1989) and so could also increase pollutant swapping. Other management options include the use of gypsum and ochre as redox buffers to inhibit methanogenesis, as demonstrated in field scale agricultural wetlands and microcosm experiments (Pangala *et al.,* 2010). However, planting macrophytes in a wetland during construction or periodic addition of redox buffers may incur considerable capital and time costs to land owners, possibly impacting on uptake.

E) Hydrogeological connectivity and in situ conditions

In situ monitoring in Chapter 2 illustrated that the hydrogeological interaction between an unlined agricultural wetland and shallow groundwater may be very high. Therefore, the risk that physical transport processes may facilitate pollutant swapping to groundwater systems is substantial. Whether a wetland is recharging or receiving seasonal base flow from groundwater (Shand *et al*., 2007) may impact on this risk, particularly in catchments where the water table may be close to the ground surface. Therefore, use of wetlands in areas with known high connectivity with the parent aquifer, particularly where nutrient levels may be already elevated, should be considered carefully. Meanwhile the physical, nutrient and mineral characteristics of the sediments and surrounding deposits may also be equally important in regulating aquatic pollutant swapping. This is because they may influence the processes and transformations of potential pollutants as they undergo transfer from surface water/ sediments to local groundwater. For example, as found in agricultural soils (Jordan *et al.,* 2004) the presence of Fe compounds in sediments may promote the binding and precipitation of released P (Heiberg *et al.,* 2010).The use of impermeable membranes to line wetlands would stop potential exchanges with groundwater (although not transfers back into surface water), however such action increases the capital cost of installation (Carty *et al.,* 2008).

F) The potential for pollutant swapping from agricultural wetlands may increase with the quantity of retained sediments

As noted in Chapter 3, the deep sedimentation cell of the wetland contained larger quantities of sediment and produced higher flux rates of CO_2 CH_4 and N_2O than the shallow cell. Similarly, concentrations of leached NH_4-N (Chapter 2) were highest around the deep cell of the wetland. This suggested that the greater abundance of organic C and N retained in the deep cell, may have potentially provided larger quantities of the substrates necessary for biogeochemical processes such as methanogenesis or ammonification. This may therefore imply that the more material agricultural wetlands trap, the greater the quantity of substrates potentially available for transformation and remobilisation through pollutant swapping. This relationship may be an inherent feature of agricultural wetlands. Because of their function as sediment traps, agricultural wetlands can potentially receive and store large amounts of eroded material relative to their small size, resulting in substantially larger GHG emissions than surrounding riparian land, to the extent that fluxes are comparable with much larger and established aquatic systems (Chapter 3).

The quantity and composition of sediments accumulated in wetlands are dependent on numerous catchment characteristics. These may include soil composition, soil texture, slope angle and livestock densities (Ockenden *et al.,* 2014; Ulen *et al.,* 2010; Johannesson *et al.,* 2015). For example, annual sediment capture rates ranged between 0.02 and 23 t yr^{-1} across 10 wetlands located in sandy, clay and silt dominated catchments in the MOPS2 programme (Ockenden *et al.,* 2014). In addition, the impact of future climate change on rainfall patterns has the potential to introduce further temporal and spatial variability to catchment soil erosion rates (Sholtz *et al.,* 2008). Wetlands in catchments with high soil erosion rates or C and N rich soils, could capture greater quantities of sediment and substrates, and thus have higher potential to swap pollutants via GHGs or leaching. However, these catchments would also be likely to be locations for greater improvements in surface water quality, and for the wetlands to function as larger gross sinks of particulate material.

The potential for future pollutant swapping in agricultural wetlands over coming years-decades is uncertain. However, under the assumption that greater accumulation of organic bed material heightens the potential for pollutant swapping, the risk of this occurring may increase with time, as the mass of sediments increases in an agricultural wetland. For example, significant increases in gas flux rates over 10 years were observed from a wetland treating peat mine runoff (Liikanen *et al.,* 2006). However, guidelines also state that

agricultural wetlands may be dredged to maintain them, and the sediment potentially returned to the surrounding agricultural land (Mackenzie and McIlwraith, 2015; Natural England, 2015c). This may 'reset the clock' on the potential gas and groundwater transfers from a wetland. However, while this may temporarily reduce the risk of pollutant swapping as a result, the fate of the sediment material once removed is unknown. It is possible that moving from an anaerobic, reduced environment within a wetland to a well oxygenated environment on the land surface could trigger substantial carbon oxidation, resulting in increased CO_2 emissions from the land. Similarly, the potential for nitrification of stored NH_4–N could result in significant leaching of NO_2, NO_3 or increased emissions of N_2O. Additionally, evidence from Chapters 3 and 5 also suggested that that the physical removal of sediments for maintenance may release accumulated gas in bubble or dissolved form from the sediment. As such, sediment removal during maintenance may itself act as a pollutant swapping mechanism.

6.3 Potential impact of constructed agricultural wetland creation on greenhouse gas emissions and nutrient trapping at the national scale

As demonstrated in Chapter 3, constructed agricultural wetlands are likely to be net sources of GHGs to the atmosphere. Individually, the small surface area of these wetlands may limit their potential to impact on catchment or national scale GHG fluxes, while maintaining their function as sediment and nutrient traps. However, the potential increase in the numbers of these wetlands (and thus surface area) in the agricultural landscape, could mean that cumulatively they may have a much more significant impact on net GHG budgets.

Unlike wetland creation schemes in countries such as Sweden, where targets for total created wetland area are set (12000 ha Baltic Compass, 2012), installation of agricultural wetlands in England and Wales is dependent on the decisions of individual land owners and managers. Here, wetlands are implemented on a case by case basis through competitive and targeted government capital grants (£10 m^{-2}), awarded through the Countryside Stewardship Scheme (Natural England, 2015a). As agricultural wetlands were not specific mitigation options in previous UK agri-environent schemes (ELS and HLS), and due to very recent shifts in UK agri-environment policy, the potential cumulative surface area which may be created in the landscape through Countryside Stewardship is unknown. Therefore, the impact these wetlands may have on GHG fluxes versus carbon and nutrient capture is uncertain.

6.3.1 Upscaling wetland GHG emissions to the national scale using mitigation option uptake and analogous agricultural ponds

To assess the potential cumulative impact of national-scale agricultural wetland creation on GHG emissions (Table 6.1) and nutrient trapping (Table 6.2), observations from the field-scale case study (Chapter 3) were upscaled. This was done by considering seven scenarios with differing areas of wetlands in the landscape, together with various rates of GHG release and nutrient trapping. These upscaling scenarios were estimated using two methods:

i) The potential numbers and area of wetlands were calculated based on projected uptake rates as a mitigation option. The ADAS Farmscoper tool (ADAS, 2015) was used to estimate the projected percentage of the 145,100 farms in England and Wales (Defra, 2012) which may select agricultural wetlands (RP7 wetlands and sediments traps) as a mitigation option through Countryside Stewardship. Scenarios considered uptake rates of 2 and 5 % of farms, equating to 2902 and 7255 wetlands, assuming a single wetland per farm holding. Additionally, an arbitrary 10 % uptake rate (14,510 wetlands) was included to assess the effect of two wetlands per holding. Uptake of option RP8 (Constructed Wetlands) were not included here, due to their specific role in treating farmyard wastewater, which makes them closer to 'treatment wetlands'. Wetland surface area was calculated by multiplying the number of wetlands by the surface area of a single wetland. Two wetland sizes were considered to account for variations between designs. A wetland of 0.0025 ha (25 m^2) represented the smaller wetland option in the Countryside Stewardship guidelines (Natural England, 2015c), whilst a wetland of 0.032 ha (320 m^2) as used in this study (Ockenden *et al.,*2012) represented a large wetland size.

ii) The second approach considered that agricultural wetlands are analogous to a range of existing natural, semi natural and artificial ponds within the landscape. The 2007 Countryside Survey (Williams *et al.,* 2010) revealed that pond sizes are heavily skewed towards area of 0.0025-0.04 ha, thus being similar in size to proposed wetland systems in the UK. Therefore, the impact of these existing systems may be useful in estimating that from wetlands. The Countryside Survey was first used to estimate the cumulative number (281,300) and area (13,739 – 45,068 ha) of all ponds in England and Wales. Additionally, scenarios considered ponds which are more likely to be functioning as agricultural wetlands, including only those which received agricultural field drainage. The Countryside Survey estimated 3 % (412 – 1352 ha) of ponds in England and Wales featured field drains, although suggested this was a major underestimate. Therefore, as about 10-20 % of English ponds in the CSS had arable or

improved grassland within 100m, the effects of 15 % (2061 – 6760 ha) ponds receiving agricultural runoff was also considered. A final scenario considered the impact of maximum projected wetland uptake (10 %), together with the maximum proportion of agricultural ponds (15 %).

Cumulative GHG emissions (Kt yr^{-1}) in all scenarios were calculated by multiplying wetland/pond area by the upper and lower mean CO_2e emission rates observed in the field study (Chapter 3). As these estimates were high compared with other wetland/lake systems, lower emissions rates from similar agricultural wetlands in Sweden (Stadmark and Leonardson, 2005) were also considered. Additionally, it could also be argued that the proportion of wetland CO_2e emissions derived from CO_2 would occur even if the source material was not trapped in a wetland. Hence CO_2e emissions both with and without CO_2 fluxes were included. The rates from Stadmark and Leonardson do not include CO_2 and so the low CO_2e flux estimates are identical both with and without CO_2. The annual rate of total C, P and N capture was estimated using a range of trapping rates from a variety of catchment soil types (clay, sand, silt) in the MOPS2 programme (Ockenden *et al.,* 2014). These were calculated on a mass trapped per wetland/pond basis.

Table 6.1 Potential annual CO_2e gas fluxes from the projected area of constructed agricultural wetlands and analogous small inland ponds in England and Wales.

	CO_2e emissions (Kt yr^{-1}) from potential wetland areas and existing ponds in England and Wales						
Gas flux estimate (CO_2e kg ha^{-1} yr^{-1})	**Projected wetland area (RP7) England and Wales**			**Total ponds in landscape**	**Agricultural ponds (3% total)**	**Agricultural ponds (15% total)**	**10% RP7 uptake + 15% Agricultural ponds**
	2 % uptake (7 – 93 ha)	**5 % uptake (18 – 232 ha)**	**10 % uptake (36 – 464 ha)**	**13,739 – 45,068 ha**	**412 – 1352 ha**	**2061 – 6760 ha**	**7224 ha**
Low							
CH_4+N_2O (5202)	0.038 – 0.48	0.094 – 1.2	0.19 – 2.42	71.5 - 234	2.14 – 7	10.7 – 35.2	37.6
Mid							
All GHG (32,282)	0.23 – 2.99	0.58 – 7.5	1.17 – 15	443 - 1455	13.3 – 43.6	66.5 – 218.2	233.2
CH_4+N_2O (23,145)	0.16 – 2.15	0.42 – 5.4	0.84 – 10.7	318 - 1043	9.5 – 31.3	47.69 – 156.5	167.2
High							
All GHG (49,761)	0.36 – 4.62	0.90 – 11.5	1.80 – 23	684 - 2243	20.5 – 67.2	102.6 – 336.4	359.5
CH_4+N_2O (36,874)	0.26 – 3.42	0.67 – 8.5	1.34 – 17.1	506 - 1662	15.2 – 49.8	75.9 – 249.3	266.4

Table 6.2 Potential TC, TP and TN trapping rates (t yr $^{-1}$) are based on the range of trapping rates observed in 10 wetlands of the MOPS2 programme (Ockenden *et al.,* 2014)

	Trapping rates of Carbon and total nutrients (tonnes yr^{-1}) from potential wetland areas and existing ponds in England and Wales						
Material trapped per wetland (kg yr^{-1})	**Projected wetland area (RP7) England and Wales**			**Total ponds in landscape**	**Agricultural ponds (3% total)**	**Agricultural ponds (15% total)**	**10% RP7 uptake + 15% Agricultural ponds**
	2 % uptake	**5 % uptake**	**10 % uptake**				
Total Carbon							
1	3	7	14.5	281	8.4	**42.2**	**56.7**
10	29	72	145	2813	84	**422**	**567**
100	290	726	1451	28,130	844	**4212**	**5671**
1000	2902	7255	14,510	281,300	8439	**42,195**	**56,705**
Total Phosphorus/ Total Nitrogen							
1	3	7.3	14.5	281	8.4	**42.2**	**56.7**
10	29	72.6	145	2813	84.4	**422**	**567**
50	145	363	726	14,065	422	**2110**	**2835**

6.3.2 Magnitude of potential wetland emissions and carbon/nutrient capture

Upscaling (Table 6.1) using projected mitigation option uptake yielded a wide range of emissions, releasing up to 4.62 Kt CO_2e yr^{-1} (2 % uptake) – 11.5 Kt CO_2e yr^{-1} (5 % uptake). This demonstrated that the cumulative effect of agricultural wetland creation had the potential to contribute large quantities of greenhouse gas to the atmosphere. However, even under the projected maximal scenario of 10 % uptake (5 % uptake with two wetlands per farm), the potential emissions of 23 Kt CO_2e yr^{-1} were only 0.06 % of the current 37.96 M t CO_2e yr^{-1} GHG emissions from agriculture in England and Wales (NAEI, 2015). This suggests that in this context, the national impact of agricultural wetland creation on GHG emissions may be relatively small in comparison to other agricultural sources. The relatively low magnitude of GHG emissions compared to anthropogenic budgets echoes observations from nitrogen farming wetlands in Scandinavia (Stadmark and Leonardson, 2005; Thiere *et al.,* 2011), where large scale wetland creation only accounted for a predicted 0.04 % increase in CH_4 emissions. Thus the impact of national agricultural wetland creation on GHG fluxes may in fact be relatively minor.

The potential for proportionally large contributions from wetlands to agricultural GHG budgets may be limited because of the relatively small size of wetland designs, meaning the

total land area they may cover is low, even assuming a high rate of uptake. Furthermore, as option RP7 is a 'targeted' and competitive measure (Natural England, 2015a,c), it could be hypothesised that rates of uptake may be even lower than envisaged in the scenarios described in Table 6.1. Variation in GHG estimates also illustrated that assuming larger wetland designs could substantially increase the cumulative rates of GHG flux. This would suggest that smaller wetlands closer to 0.0025 ha in area may have less potential to increase landscape GHG emissions than larger designs, while still potentially capturing pollutants. Additionally, variations in emissions estimates illustrated that assumed flux rates can have a large impact on the magnitude of predicted cumulative GHG emissions. This reinforces the importance of robust gas flux estimates that can be applied across a range of wetland types and catchments. The large emission estimates even with CO_2 excluded, further illustrated the potential importance of agricultural wetlands as potent sources of CH_4 and the potential climatological forcing affect this may have (Mander *et al.,* 2014b).

Upscaling emissions estimates using current numbers of analogous agricultural ponds (Table 6.1) illustrated that if there were significantly greater numbers of wetlands created in the landscape, the impact on GHG emissions may be more substantial. Potential GHG contributions were much greater than those from uptake-based estimates, ranging from 2.14 – 67.2 Kt CO_2e yr^{-1} with 3 % of ponds, to 10.7 – 336.4 Kt CO_2e yr^{-1} with 15 %. Due to similar cumulative surface areas, the lowest estimates of emissions at 3% agricultural ponds e.g. 20.5 Kt CO_2e yr^{-1}, were very near to the highest estimates using wetland uptake. Not only does this illustrate how wetland emissions may substantially increase with higher numbers in the landscape, but also demonstrates the potential impact of existing analogous pond systems on gas fluxes, despite such small freshwater systems being currently underrepresented in GHG budgets (Holgerson *et al.,* 2015).

The estimates of emissions including all ponds in the landscape (684-2243 Kt CO_2e yr^{-1}), while likely being an overestimate in terms of flux rates, also shows the potential significance of GHG fluxes from inland waters. Moreover, these estimates do not include features such drainage ditches and scrapes. As Davies *et al.* (2008) found during a survey of lowland water bodies, although ponds were numerous, the total surface area of ditches was substantially higher. As such, the actual number of potentially analogous water bodies could be greater than posited here.

Because of the close parallels between agricultural pond and wetland systems, the effects of future national scale wetland introduction would therefore be in addition to current pond

fluxes. Combining estimates of projected wetland creation, assuming 10% uptake of wetlands (or two per farm) and 15 % of national ponds receiving field drainage, national emissions from these small inland waters may be as high as 360 Kt CO_2e yr^{-1}. This equates to 0.95 % of GHG emissions from agriculture in England and Wales and suggests that, although future wetland creation alone may not significantly influence national GHG emissions, together with existing water bodies they represent a more significant component of the agricultural GHG budget and may require careful management.

Nevertheless, agricultural wetlands may still provide substantial surface water quality improvements relative to the potentially small increases in agricultural GHG emissions. Estimates showed that with the lowest assumed retention rates, wetland creation could capture 3 tonnes of TC and also of TP and TN yr^{-1}in England and Wales. The more realistic capture rates would likely be much higher than this, thus this lower figure is likely a major underestimate of trapping potential. Conversely the highest capture rates, particularly those for C, are likely to be mainly applicable to soils with high erosion rates, although show the massive potential for agricultural wetland systems as carbon and nutrient stores. Additionally, sediment capture in the large numbers of agricultural ponds means that up to 56,705 tons of TC, and 2835 tons of TP and TN could also be captured in these agriculturally placed systems, thereby representing a potentially massive store in the landscape. However, it is important to note from our previous reasoning, that the very large quantity of captured material in this study system was likely to be a driver of the magnitude of emissions. Therefore, greater improvements in water quality may be coupled with greater risks of higher emission rates or groundwater leaching and GHG emissions.

6.4 Recommendations for further work

6.4.1 Replication of observations across a wide range of catchments

This study was conducted on a single agricultural wetland in an intensively farmed catchment. While the catchment size and land uses were similar to many areas in the UK and Europe, the wide range of variable factors potentially influencing the magnitude of pollutant swapping to both the atmosphere and groundwaters, suggests the need for additional observations. This could be undertaken in agricultural wetland systems as well as analogous agricultural ponds, scrapes, drainage channels and unaccounted wet patches in the

landscape. Observations would be particularly informative if undertaken in catchments with a range of nutrient/sediment loadings and farming intensities or land management practices. In particular, high NO_3-N loadings may provide insight into the mitigative effect of CH_4 emissions. A wider range of observations would also verify the impacts of differing wetland designs/ degrees of vegetation on the potential for pollutant swapping, ultimately increasing the robustness of estimated flux rates and conclusions.

6.4.2 Increased temporal resolution of field and laboratory observations

Field observations revealed several mechanisms such as storms, low air pressure and overturning events, which influenced gas fluxes on timescales outside the observation window of standard in situ measurements. Although laboratory simulations of these events were made, higher temporal resolution of field and laboratory observations in a range of constructed wetlands/sediments, would improve the ability to incorporate these mechanisms into mean flux estimates.

Both future in situ or laboratory simulations of sediment disturbance and wetland overturning would benefit from the ability to make instantaneous measurements of GHGs or nutrients. Such a capability may be possible through the use of membrane inlet mass spectroscopy (MIMS), which has shown potential for use in aquatic environments, including monitoring of GHG dynamics (Lloyd *et al.,* 2002; Schluter *et al.,* 2008; Williams *et al.,* 2010). This would potentially enable the in situ observation of the extremely small temporal changes in biogeochemical processes.

In addition, laboratory simulations using a variety of wetland sediments could include investigations of in-sediment physicochemical and hydrochemical conditions. This would improve the robustness of conclusions with respect to the effects of redox buffer penetration e.g. O_2, NO_3-N, SO_4 and identify the primary zones of production for respective GHGs.

6.4.3 Rates of CH_4 oxidation and C balance during wetland overturning

The contrasting field observations of daylight CO_2 uptake and emission during darkness implies that wetlands may become net sinks of atmospheric C at certain times of diurnal/seasonal cycles. This may have potentially substantial implications for the role of small inland waters in landscape carbon cycling. Therefore further investigation of this

balance is necessary, establishing net C release through CO_2 and CH_4, uptake through photosynthesis and potential re-release through decomposition of algal organic matter.

Additionally, the rates of CH_4 oxidation in the wetland water column and upper sediments, particularly during overturning and disturbance events may potentially impact on the net climate forcing potential of wetland/ pond systems. Determining the true production rate of CH_4, together with the fraction oxidised to CO_2 would help to constrain this uncertainty and role in C balances. Oxidation of water column CH_4 has been studied extensively in deep lake environments e.g. using C labelling (Bastviken *et al.,* 2002, 2008), while the MIMS technique may also be employed.

6.4.4 Future pollutant swapping potential in agricultural wetlands

The longer term risk from pollutant swapping in agricultural wetlands, potentially due to increased accumulation of organic matter, C and N over time, is uncertain. This is compounded by the lack of 'aged' wetlands in the UK, which could be used to verify the potential for increases in GHG fluxes. As wetlands are still relatively new in the landscape, constructed ponds in agricultural catchments with known construction dates could be used as a substitute to gain some insight into potential swapping increases. Additionally the swapping risk from the action of wetland sediment removal (e.g. for maintenance), and the fate of that material in the environment is also unknown. In situ observations and laboratory column experiments could be utilised to verify the potential for gas and nutrient swapping from removed sediments once applied to agricultural land e.g. via leaching.

6.4.5 Additional pollutants

This study also only accounted for the fluxes of the three main GHGs to the atmosphere and nutrient leaching to groundwater. However agricultural wetlands may also accumulate other substances such as heavy metals, pesticides and faecal indicator organisms (Stevens and Quinton, 2009). The potential for movement or transformation of these substances to groundwater could also represent risks from unlined agricultural wetlands, particularly those which may receive runoff contaminated with septic drains or livestock shed runoff (e.g. Tanner *et al*., 1997). An examination for a wider suite of pollutants and their potential for remobilisation from wetland surface water and/or sediments would be necessary, as well as a greater appreciation for the composition and interaction with surrounding superficial deposits.

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References

Appendix I

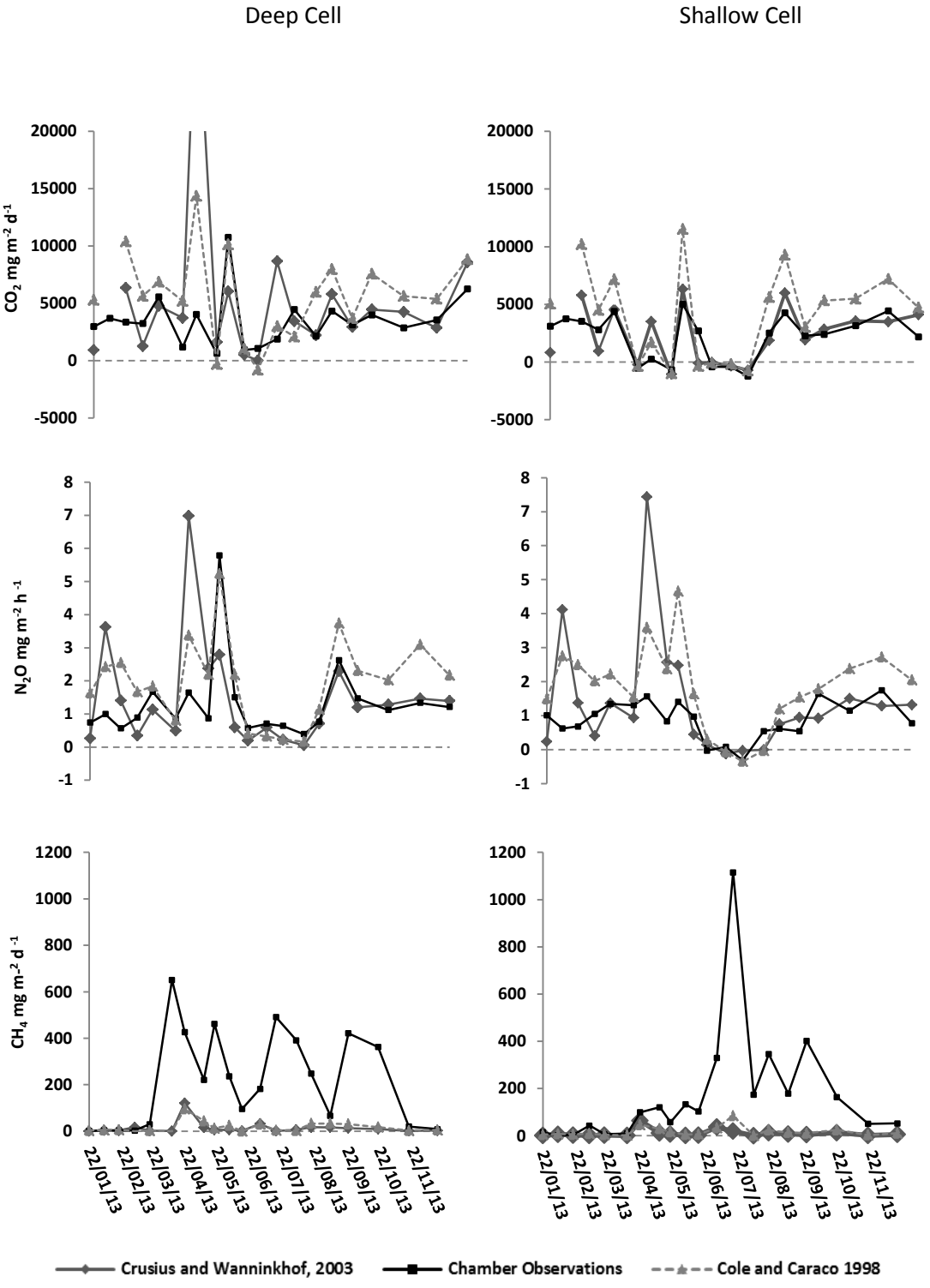

Figure 8.1. Time series of observed chamber GHG fluxes in deep and shallow wetland cells, compared with estimated diffusive fluxs using the relationships of Crusius and Wanninkhof (1998) and Cole and Caraco (2003). Cursisus and Wanninkhof was selected for CO_2 due to similar magnitudes of flux. Cole and Caraco was deemed most appropriate for estimating diffusive N_2O and CH_4. Fluxes show little similarity with CH_4 fluxes from chamber measurements due to the dominance of ebullitive releases.

Appendix II

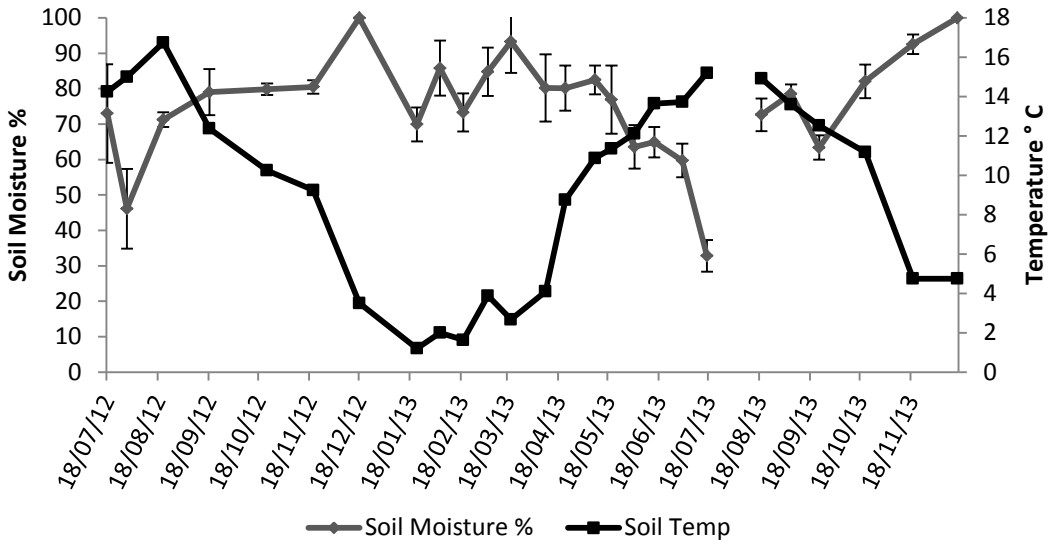

Figure 8.2. Soil moisture content (%) and temperature in the riparian zone adjacent the the constructed agricultual wetland. Soils were close to or at full saturation for much of the 18 month observation period.

Appendix III

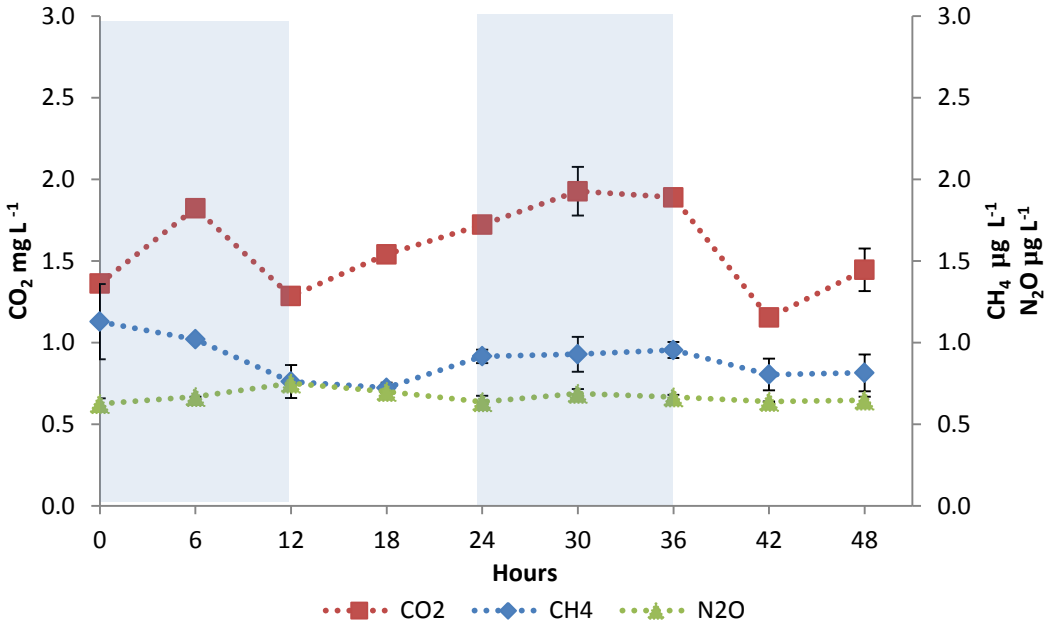

Figure 8.3 Mean dissolved GHG concentrations in the blank microcosms (*n=3*) (no sediment) over the course of 12 hour oxic and anoxic water column conditions. Error bars represent standard error. Shaded regions represent oxic conditions.

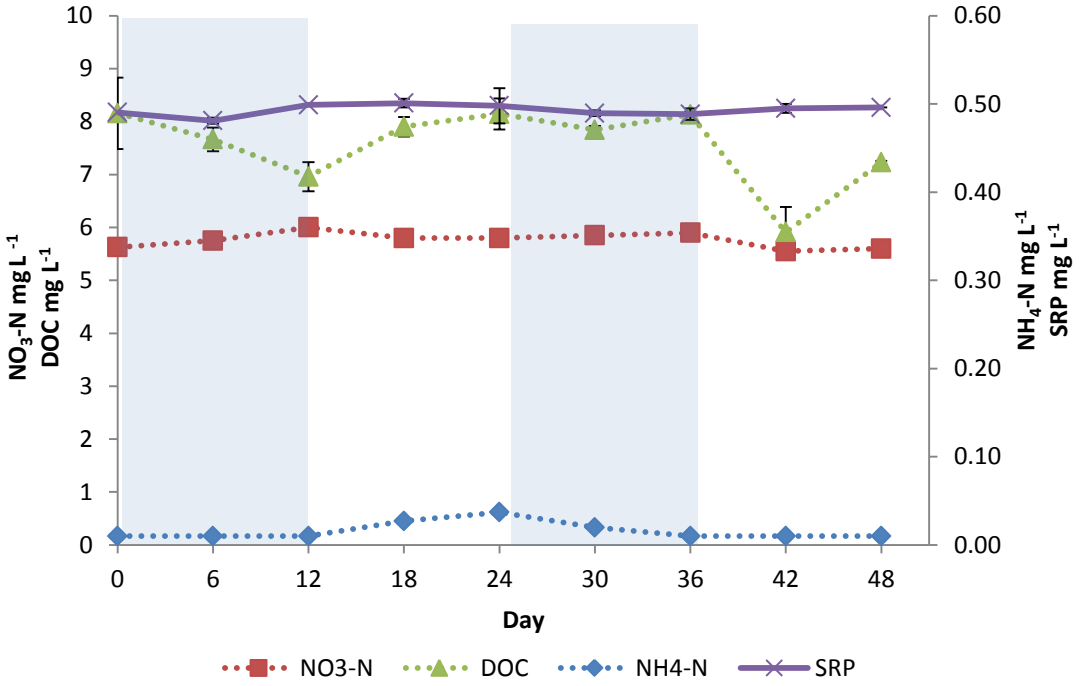

Figure 8.4 Mean dissolved nutrient concentrations in the blank microcosms (*n=3*) (no sediment) over the course of 12 hour oxic and anoxic water column conditions. Error bars represent standard error. Shaded regions represent oxic conditions.

Appendix IV

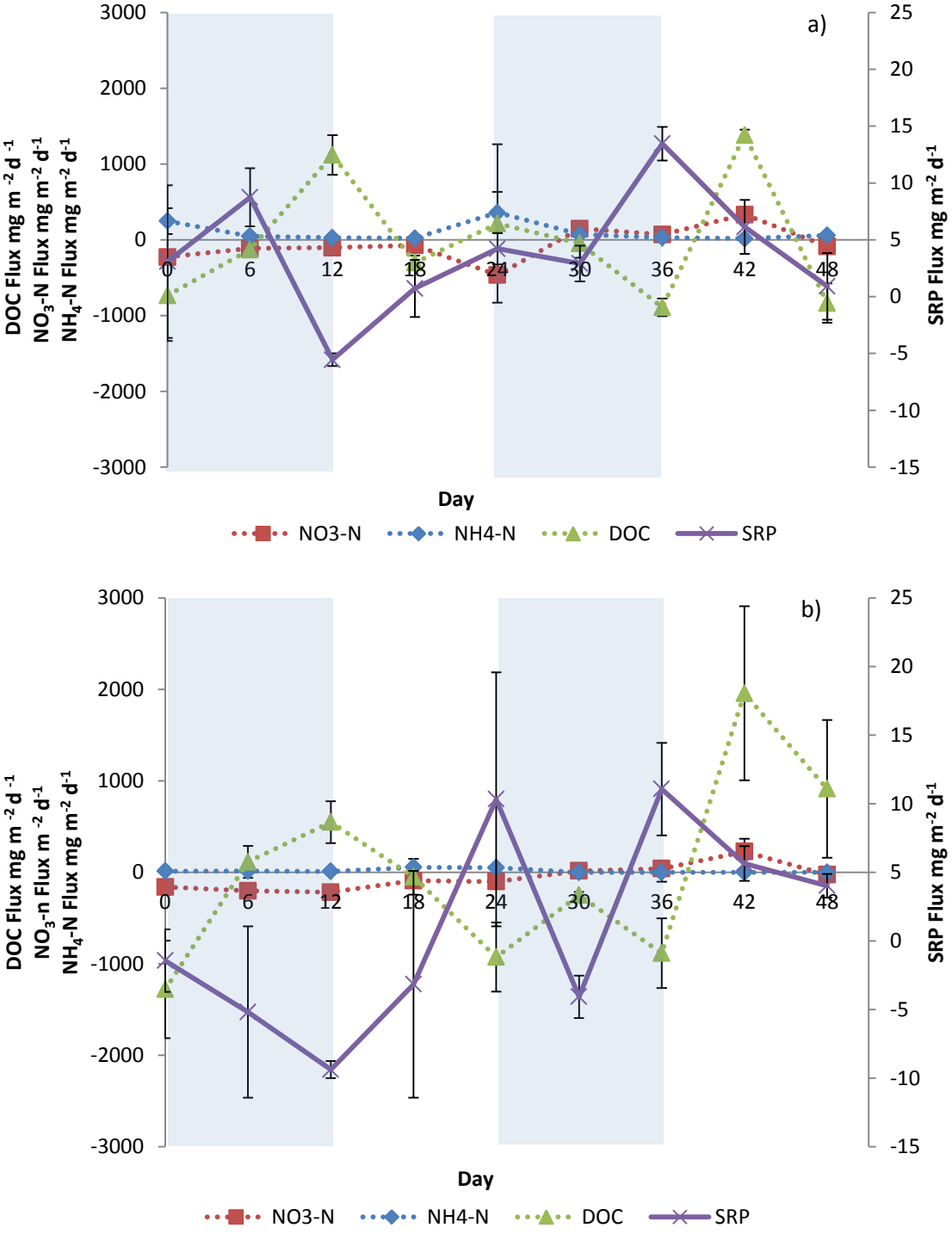

Figure 8.5 Mean dissolved nutrient fluxes in the deep cell (a) and shallow cell (b) sediment microcosms (*n=3*) over the course of 12 hour oxic and anoxic water column conditions. Error bars represent standard error. Shaded regions represent oxic conditions.

Appendix V

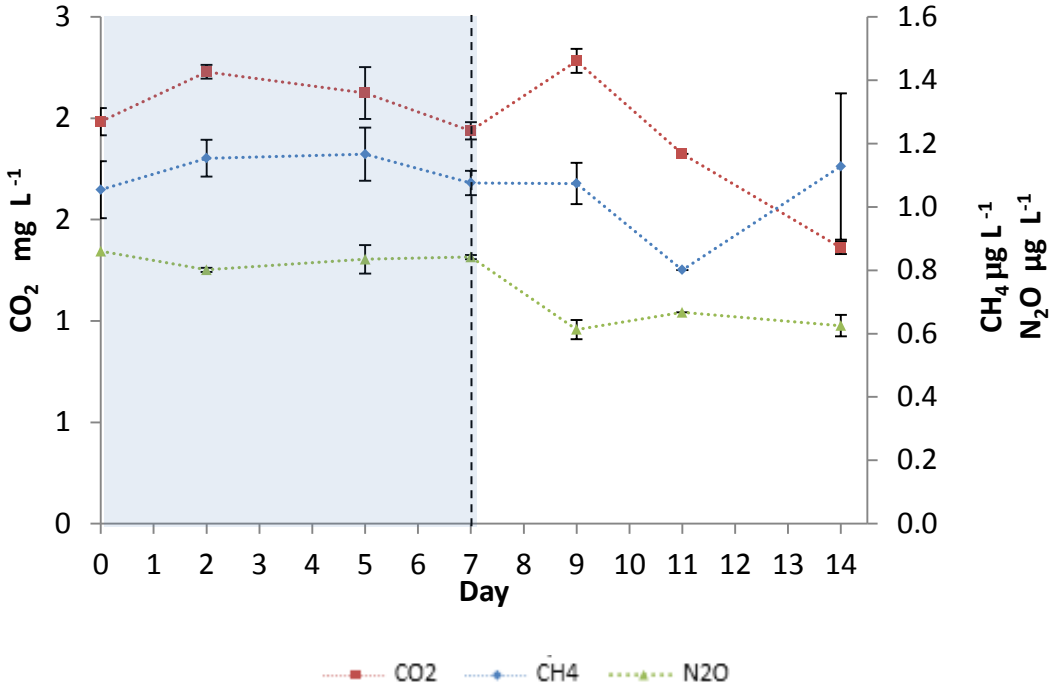

Figure 8.6 Mean dissolved gas concentrations in the blank microcosms (*n=3*) (no sediment) over the course of prolonged oxic and anoxic water column conditions. Error bars represent standard error. Shaded regions represent oxic conditions.

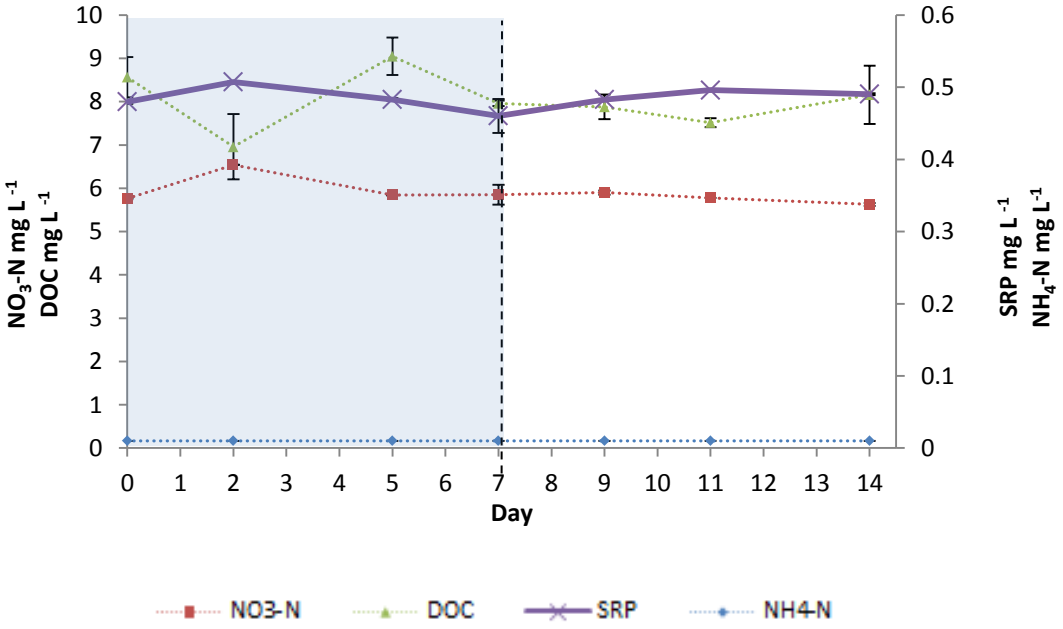

Figure 8.7 Mean dissolved nutrient concentrations in the blank microcosms (*n=3*) (no sediment) over the course of prolonged oxic and anoxic water column conditions. Error bars represent standard error. Shaded regions represent oxic conditions.

Appendix VI

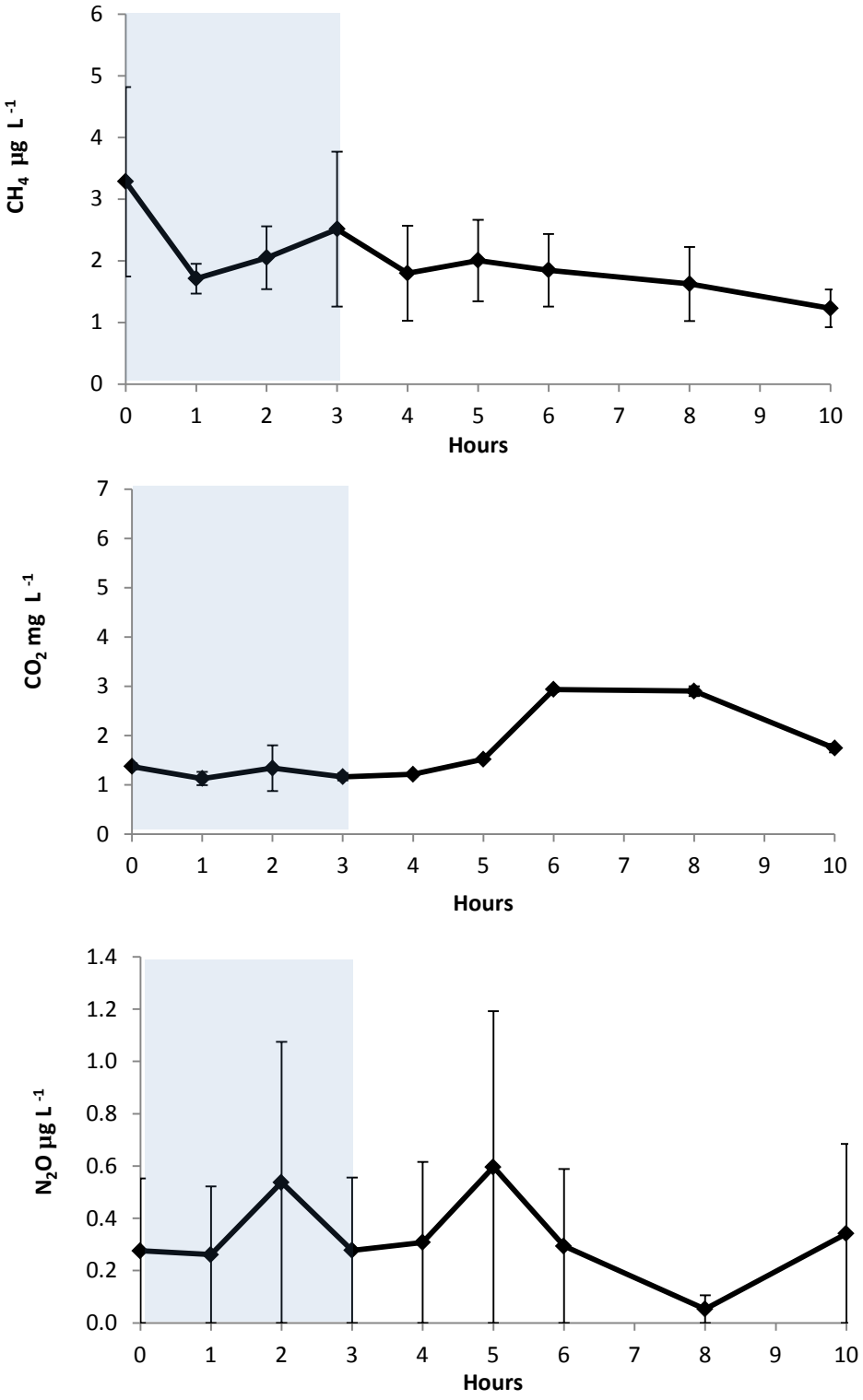

Figure 8.8 Mean dissolved GHG concentrations in the blank microcosms (*n=3*) (no sediment) over the anoxic sediment disturbance experiment. Error bars represent standard error. Shaded regions represent the simulated storm period.

Appendix VII

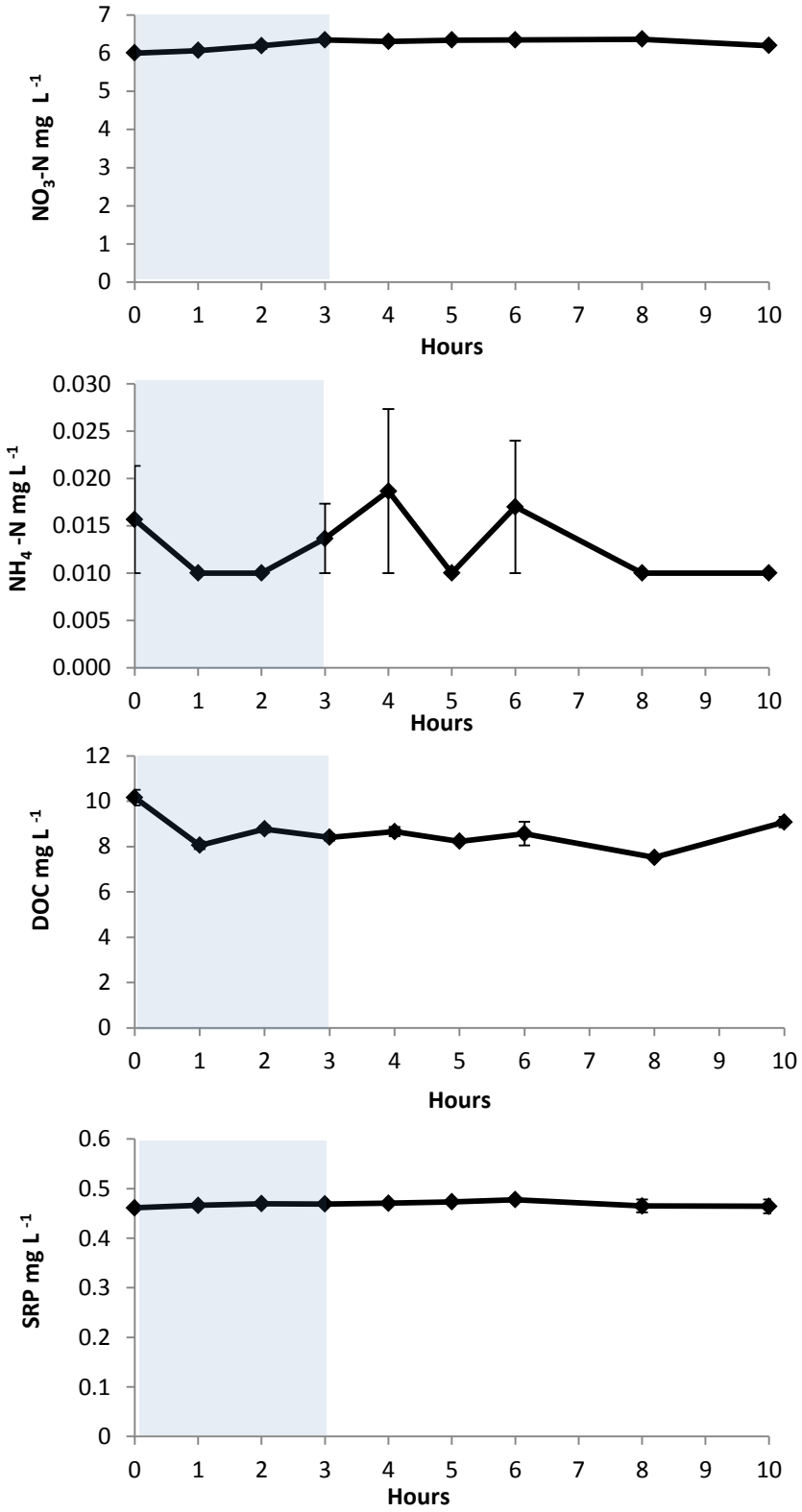

Figure 8.9 Mean dissolved nutrient concentrations in the blank microcosms (*n=3*) (no sediment) over the anoxic sediment disturbance experiment. Error bars represent standard error. Shaded regions represent periods of simulated storm flow.

Appendix VIII

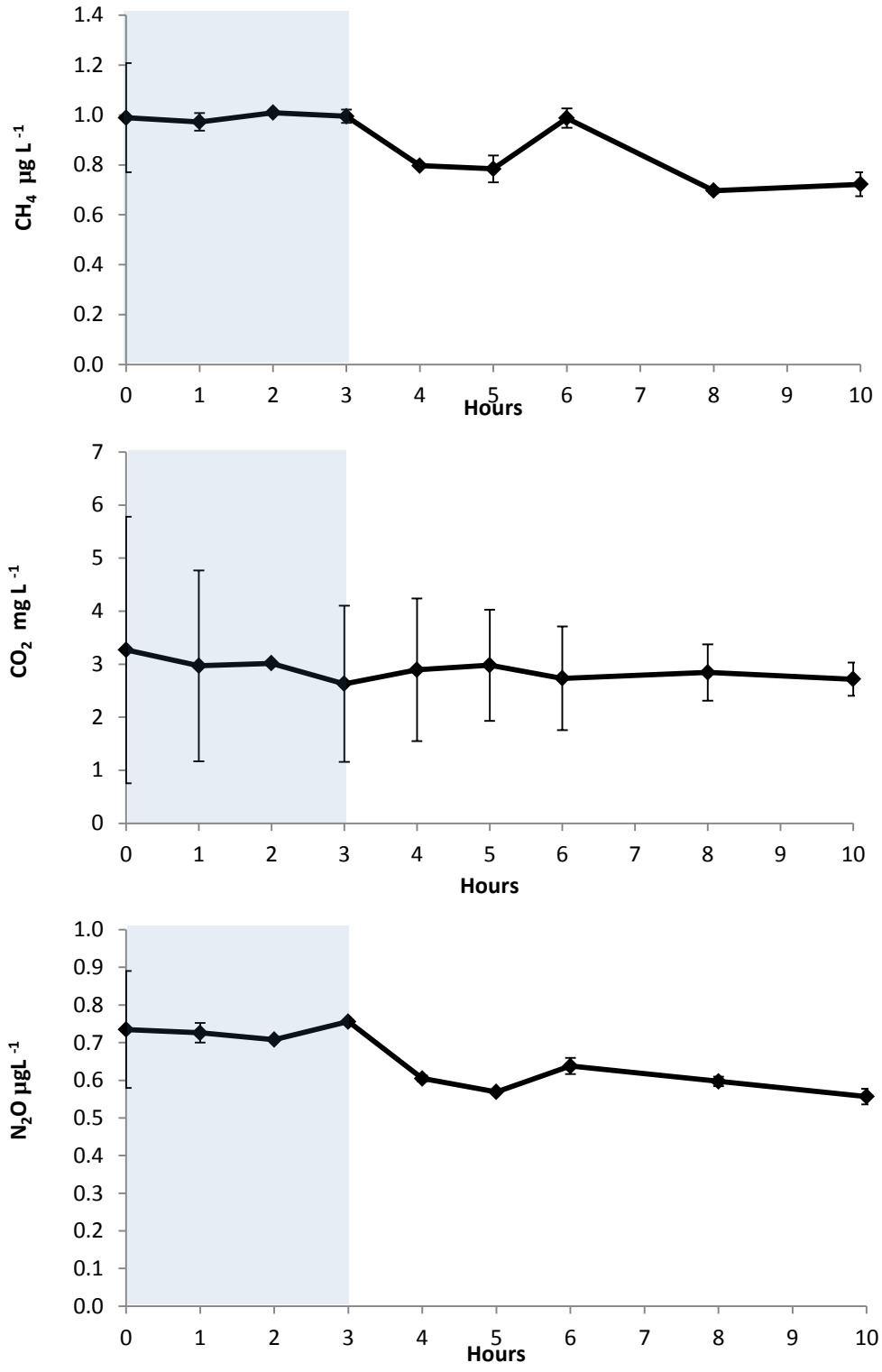

Figure 8.10 Mean dissolved GHG concentrations in the blank microcosms (*n=3*) (no sediment) over the oxic sediment disturbance experiment. Error bars represent standard error. Shaded regions represent the period of the simulated storm.

Appendix IX

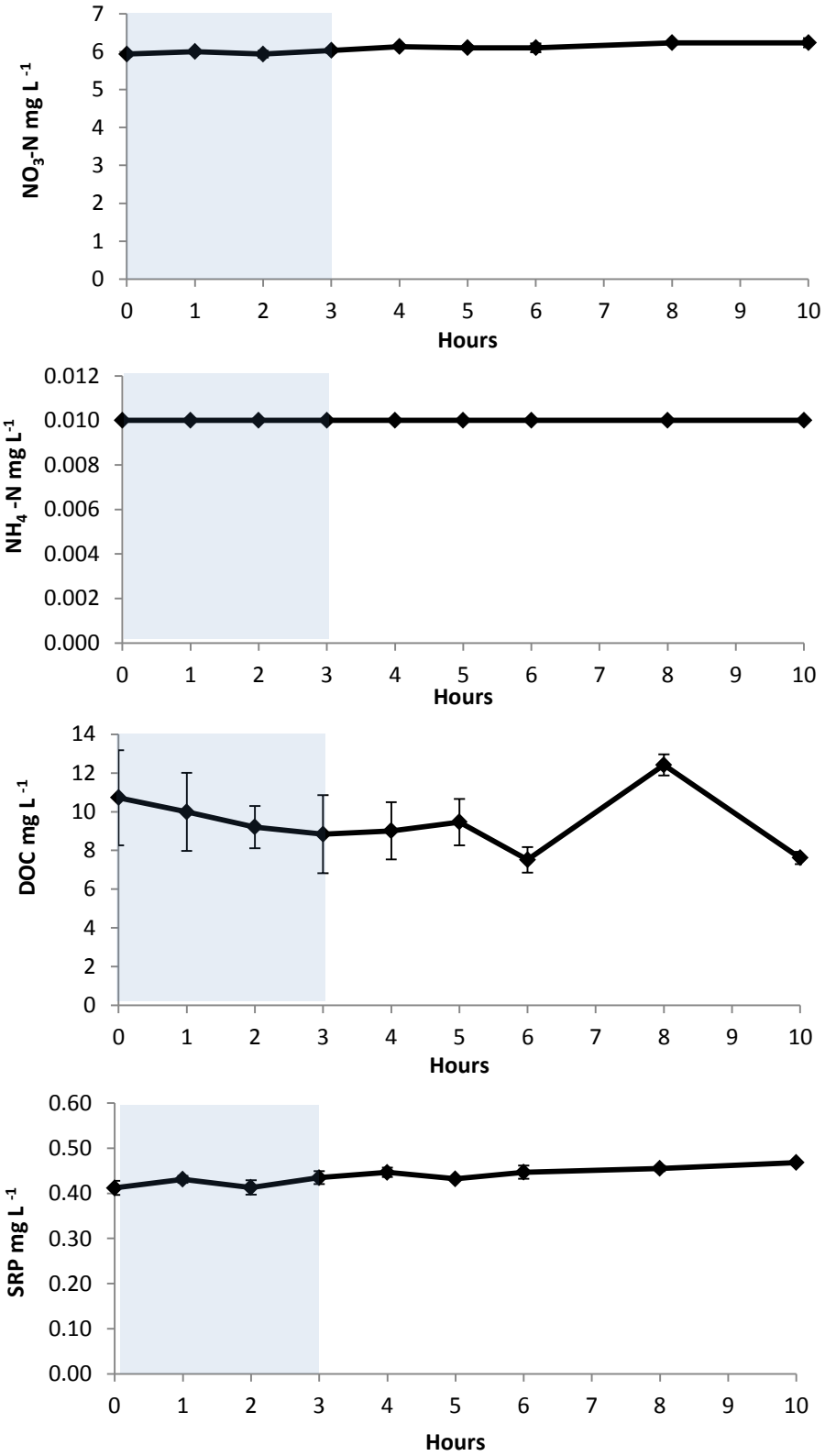

Figure 8.11 Mean dissolved nutrient concentrations in the blank microcosms (*n=3*) (no sediment) over the oxic sediment disturbance experiment. Error bars represent standard error. Shaded regions represent the period of simulated storm flow.