Seasonal variation in the emission of greenhouse gases and biogeochemical cycling of nutrients in a farm dam of south-eastern Australia

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Thesis summary

Farm dams are integral part of Australian agriculture and they contain a substantial fraction of inland fresh water on the Australian continent. Storage of water can alter the physical, chemical and biological processes occurring within the dam and subsequently, any downstream receiving water body. However, limited research has been directed into understanding the factors controlling biogeochemical processes in the dam and the effect of the dam on downstream water quality. In this thesis, a dam (11.8 ML) with a catchment area of 1.3 km^2 on a dairy farm at Poowong East (ca. 130 km south-east of Melbourne, VIC) was studied to investigate: (1) the seasonal variations in nutrient concentrations and greenhouse gas (N_2O and CH_4) emissions, (2) the role of aquatic sediments in the emission of the greenhouse gases, and (3) the biogeochemical cycling of nitrogen and phosphorus in the dam.

Fluxes of N_2O, CH_4 and bioavailable inorganic nutrients (NH_4^+, NO_x and FRP) between the sediment and the water column at 3 different sites within the dam were determined over a period of 18 months (from July 2010 to December 2011). Factors controlling the gas and nutrient fluxes (i.e. temperature, dissolved oxygen, nutrient availability and faunal abundance) were analysed. The physicochemical characteristics of the creek upstream and downstream of the dam were also measured throughout the sampling period. This study also involved sediment core incubations, deployment of equilibrium dialysis samplers (pore water peepers) and planar optode experiments.

The dam was found to be a major source of N_2O during periods when both NO_x and O_2 concentrations were high in the water column. Sedimentary CH_4 flux increased gradually during summer when the overlying water was very close to, or completely, anoxic. The production rate of CH_4 was almost 3 times greater in the deeper site (3.5 metres) than in the

shallow site (1 metre) and the highest fluxes were associated with the bottom water temperature maxima.

The dam was a net source of NH_4^+ but a net sink for NO_x and FRP. The internal load of NH_4^+ was almost 3 times higher than the external load on an annual basis. Conversely, the dam removed about 14% and 5% of the annual external NO_x and FRP loads, respectively. There was a significant seasonal variation in inorganic nutrient fluxes in the dam. The diffusive flux of nutrients across the sediment-water interface, particularly during summer months, emphasized the role of the bottom sediment as an alternative source of bioavailable nutrients into the water column.

This study found that benthic macrofauna (here both *Chironomidae* and *Oligochaeta*) did not impose any significant effect on *in situ* N_2O production but the results suggested a perhaps important involvement in the cycling of biogenic CH_4. *Chironomidae* larvae significantly increased sediment oxygen consumption rates and decreased the NH_4^+ efflux. *Chironomidae* larvae also appreciably increased the water column NO_x-derived denitrification (D_w) as well as the coupled nitrification-denitrification (D_n) rates in the sediment.

Overall, this study produced three key findings:

(1) Farm dams can be a major source of N_2O and CH_4 depending on the season and nutrient availability.

(2) Seasonal variation of nutrient processing within the dam resulted in significant effects on the downstream water quality. In most sampling months, the outflowing water failed to meet the state and regional water quality standards particularly in terms of turbidity, dissolved oxygen and different forms of nutrients.

(3) Long term monitoring of the seasonal variation of the nutrient processing in farm dams is essential to predict the influence of such dams on the downstream water bodies and to assess the effectiveness of any management actions.

General declaration

In accordance with Monash University Doctorate Regulation 17/ Doctor of Philosophy and Master of Philosophy (MPhil) regulations, the following declarations are made:

I hereby declare that this thesis is composed of my original work and contains no material which has been accepted for the award of any other degree at any university or equivalent institution and that, to the best of my knowledge, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

The ideas, development and writing up of all the chapters in the thesis were the principal responsibility of myself, the candidate working under the supervision of Assoc. Prof. Mike Grace and Dr. Perran Cook.

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Chapter One

General Introduction

1.1 Overview of the research project

Headwater streams in agricultural catchments are often impounded for temporary or permanent water storage. These water storage ponds are commonly known as "farm dams" (Lewis 2002). In Australia, farm dams also refer to private dams that are used to intercept catchment runoff or overland flow (SKM 2012). These dams are generally used to supply drinking water for stock, for irrigation of crops and other household activities or simply for fishing and aesthetics (Brainwood *et al.* 2004). Brainwood and Burgin (2009) reported there are more than half a million farm dams in Australia of which the majority are in Victoria, the south eastern part of the country. Hence, these dams represent a major fresh water resource in Australia and particularly in Victoria. Despite their importance in sustaining and increasing agricultural activities, these dams can potentially impact downstream water bodies by effects including modification of stream flow, changes to water temperature, dissolved oxygen (DO) and suspended matter and by alteration of nutrient (N and P) dynamics (Schreider *et al.* 2002; Brainwood *et al.* 2004; Lowe *et al.* 2005; Fairchild and Velinsky 2006). All of these processes can vary widely within and between ecosystems and it is necessary to understand the site specific processes dominating nutrient biogeochemistry for effective nutrient management.

Biogeochemical processes within standing water bodies such as lakes greatly depend on the surrounding land use type (e.g. agricultural *versus* pristine). Different land use and agricultural practices have the potential to influence in-pond biogeochemical processes by determining the nutrients and organic carbon transported to the impoundment via connected streams, groundwater and surface runoff (Huttunen *et al.* 2003). For example, constructed wetlands and ponds connected to agricultural areas and sewage treatment plants are different from natural lakes and wetlands because of the direct disposal of sewage and agricultural

runoff into the former impoundments. Consequently, these impoundments often experience severe nutrient loading and as a result can suffer from intense eutrophication.

Eutrophication is characterized by excessive plant and algal growth due to the increased availability of nutrients, such as nitrogen (N) and phosphorus (P) in aquatic ecosystems. Algal blooms are a common manifestation of eutrophication and are a well documented cause of aquatic system degradation (Bricker *et al.* 1999; Hoeger *et al.* 2004). The development of an algal bloom requires both sunlight and an excess of nutrients (Redfield 1958). Algal blooms can cause depletion of dissolved oxygen in many ways e.g. directly via respiration and indirectly via limiting photosynthesis by blocking sunlight from the water surface (Boulton and Brock 1999). Moreover, at the time of bloom collapse the phytoplankton cellular detritus settles to the bottom of the aquatic system where it is the source of organic carbon for prolific bacterial respiration. Such respiration consumes dissolved oxygen and may cause hypolimnetic anoxia, particularly under stratified conditions (Boyd *et al.* 1975). Furthermore, decaying algae (e.g. Cyanobacteria) can also release toxins into the water which are harmful to the aquatic fauna (Christoffersen 1996). Thus, algal blooms driven by high bioavailable nutrient concentrations, adversely affect rivers, lakes, and estuaries worldwide and are one of the most significant aquatic ecosystem management problems (Bricker *et al.* 1999). In Australia, a severe cyanobacterial bloom was observed in late 1991, extending for a distance of 1000 km in the Darling River in New South Wales and caused the state government to declare a State of Emergency (Bowling and Baker 1996).

It has been estimated that fresh water algal blooms cost the Australian community about AUD $180-240 million per annum (Davis and Koop 2006). Adding the blooms in estuaries and coastal waters (e.g. the Gippsland Lakes in eastern Victoria) which affect fisheries, aquaculture and tourism, the annual cost is much higher (Hallegraeff 1992; Stephens *et al.*

2004). Fundamental to effective eutrophication management is an understanding of how rivers, lakes, estuaries and the coastal ocean are coupled with nutrient export from their watersheds (Howarth *et al.* 2003). Not only do such ponds and impoundments provide this hydrologic coupling, they also play a central role in altering the concentrations and forms of nutrients exported downstream (Howarth *et al.* 2003).

N_2O and CH_4 are potent greenhouse gases. They not only contribute to global warming but also have the potential to destroy the atmospheric ozone layer (Le Mer and Roger 2001; de Bie *et al.* 2002; Blackwell *et al.* 2010). The global warming potentials (GWP) of CH_4 and N_2O are 25 and 298 times greater than CO_2 (over a 100 year time horizon) with respective life times of 12 and 114 years in the atmosphere (Forster *et al.* 2007). Atmospheric concentrations of both of these gases have increased remarkably in the last century compared to the preindustrial era (prior to the industrial revolution in the mid 19^{th} century) due to increased anthropogenic and agricultural activities (Alley *et al.* 2007). Alley *et al.* (2007) reported that global atmospheric concentrations of CH_4 and N_2O increased from preindustrial values of about 715 ppb to 1774 ppb and from 270 ppb to 319 ppb by 2005 respectively. According to the national inventory report for Australia, in 2009 the agricultural sector contributed about 57% (3.1 Mt) and 73% (0.063 Mt) of total CH_4 and N_2O emissions respectively, and agricultural emissions accounted for 15.5% (84.7 Mt) of the total national net GHG emissions (Department of Climate Change and Energy Efficiency, 2011). It is evident from the report that Australian agriculture makes a significant contribution to total Australian GHG emissions, particularly in terms of CH_4 and N_2O, but the information is not detailed enough to evaluate the specific agricultural sources of N_2O and CH_4.

Hence, the focus of the study will be investigating the temporal variation of green house gas (N_2O and CH_4) emissions and processing of nutrients (N and P) in a farm dam in an

agricultural catchment in south eastern Victoria and examining the factors controlling these processes. The study site (details of the study site are presented in chapter 2, section 2.1) is at Poowong East in the Lang Lang catchment. The Lang Lang River flows into Westernport Bay. The study site includes a dairy farm (230 cows) and surrounding streams together with a dam on this property. The dam provides water for irrigation, dairy shed washing and household activities.

1.2. Nutrient (N, P and C) cycling in aquatic ecosystems

Biogeochemical cycling generally refers to a set of interacting processes involving the element of interest, including physical transport and storage, changes in form (organic vs inorganic, solute vs particulate) and chemical transformations in elemental speciation (oxidation state, complexation) and association (adsorption). Headwater streams can influence larger downstream ecosystems such as rivers, estuaries and even coastal waters through longitudinal linkage of nutrients, suspended solids and contaminants (Alexander *et al.* 2000; Meyer *et al.* 2001). During transition from uplands to oceans through lotic networks, biogeochemical processes in streams are altered. The River Continuum concept shows how various characteristics of a stream change with stream order (Vannote *et al.* 1980; Sedell *et al.* 1989). Damming of the stream can cause additional changes by altering water residence time and velocity of running water. Longer water residence time enables nutrients to undergo multiple cycles of uptake, biological, chemical and physical storage and remineralization. Damming also reduces water velocity which can result in settling of larger particles from the water column. Traditionally, aquatic sediments have simply been viewed as either a source or sink of nutrients to the overlying water column (Sondergaard *et al.* 2003). A better description of the role of sediments in nutrient cycling is that nutrients within

the aquatic sediments are in a dynamic equilibrium with nutrients in the overlying water column (Sondergaard *et al.* 2003). The prevailing environmental conditions (such as dissolved oxygen, temperature, redox conditions and pH) and often the concentration gradient of the nutrient across the sediment-water interface will determine whether nutrient input or output from the sediments dominates.

1.2.1. Cycling of N and P

1.2.1a. N cycling

Nitrogen and phosphorus occur in a variety of interchangeable chemical forms. Nitrogen is the most abundant element in the atmosphere and is also an essential element to all biota as a building block of proteins and nucleic acids such as DNA and RNA. Atmospheric nitrogen is dominated by gaseous N_2 but most organisms cannot use nitrogen in this 'inert' form. Some algae i.e. cyanobacteria and leguminous plants having N fixing bacterial symbiont can 'fix' atmospheric N_2 and convert it into ammonium (NH_4^+). NH_4^+ may then be converted into nitrate (NO_3^-) in the presence of dissolved oxygen by the nitrification process which occurs through a series of intermediate steps in which N_2O is liberated as a side reaction (Jorgensen *et al.* 1984). This reaction sequence is represented by equation 1.1:

$$NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-, \quad NO_2^- \rightarrow N_2O \qquad \text{Eq. 1.1}$$

Nitrogen compounds, in the form of dissolved NH_4^+ and NO_3^-, are taken up or assimilated - by plants for the formation of plant proteins (Fig 1.1). There are some species of blue green algae, more correctly called cyanobacteria, including *Anabaena sp.* which have the ability to directly utilize dissolved nitrogen in the water column rather than relying on bioavailable

forms such as NH_4^+, NO_3^- and NO_2^-. Thus these species have the ability to outcompete other algae when nitrogen concentrations drop to very low levels (McClain *et al.* 1998).

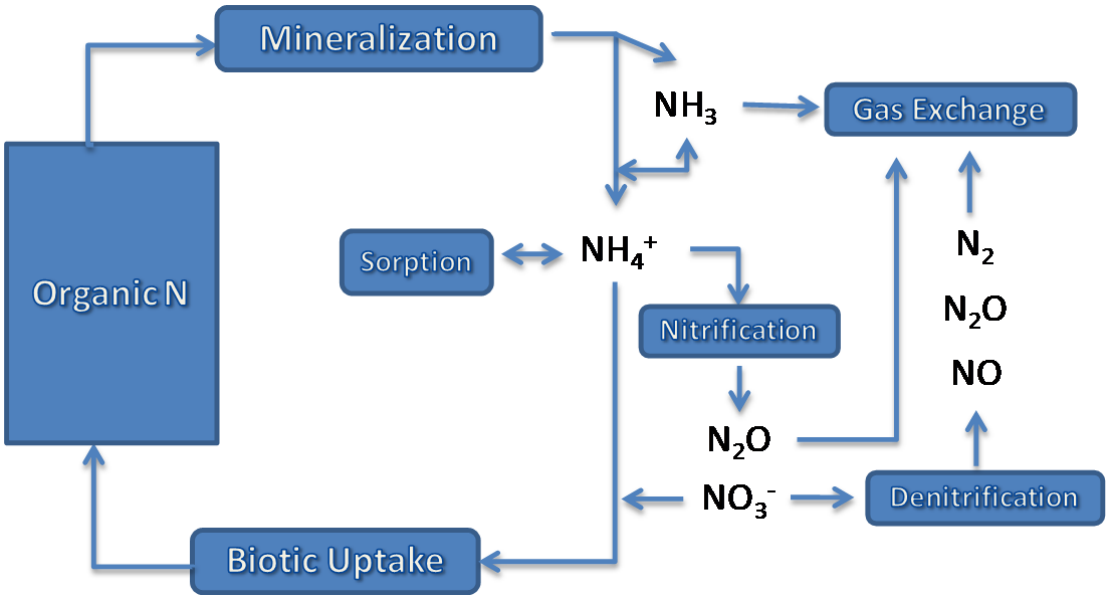

Figure 1.1. Generalized N cycling (adopted and modified from McClain *et al.* (1998))

When plants and animals die or when animals emit wastes the nitrogen in the organic matter is broken down by bacteria to NH_4^+ in the mineralization process. NH_3 and NH_4^+ are in very rapid equilibrium where the dominant species is determined by solution pH (Equation 1.2) (Freney *et al.* 1983). The pK_a value of NH_4^+ is 9.3 which indicates that the protonated form i.e. NH_4^+ dominates at pH < 9.3 whereas the deprotonated form i.e. NH_3 dominates at pH > 9.3 (Byrne 2002).

$$H_2O + NH_4^+ \leftrightarrow H_3O^+ + NH_3 \qquad \text{Eq. 1.2}$$

NO_3^- can be used up by the biota via assimilatory reduction (Wetzel 1983). Under anaerobic conditions NO_3^- typically undergoes denitrification, a process performed by a consortium of microorganisms commonly known as denitrifying bacteria, according to Equation 1.3:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \text{Eq. 1.3}$$

The denitrifiers use nitrate as an electron acceptor instead of oxygen during anaerobic respiration, resulting in free nitrogen gas as the end-product. The gases NO (nitrogen oxide) and N_2O are intermediates as well as by products of denitrification (Butcher *et al.* 1992).

1.2.1b. P cycling

Phosphorus is also a key element for all forms of life. It is a major component of cellular membranes in phospholipids and also in DNA and RNA. It also assists in the strengthening of bones through calcium phosphate salts (Riess 1952). The phosphorus cycle differs from the other major biogeochemical cycles in that it does not include a gas phase component under the temperatures and pressures typically found in aquatic systems. However, several phosphines (gases) are generated under highly reducing conditions e.g. in organic rich wetlands and bayous (D**é**vai *et al.* 1999). Phosphorus typically originates from sedimentary rock. Phosphate is removed from rocks via weathering and then distributed throughout both soil and water. It also enters waterways by fertilizer runoff, natural mineral deposits, sewage leaching and wastes from different point and diffuse sources. Phosphorus occurs in the aquatic environment as organic and inorganic phosphates. The inorganic form, commonly known as orthophosphate (as PO_4^{3-} and the protonated analogues HPO_4^{2-} and $H_2PO_4^-$), is the form required by plants. Once incorporated by plants, it is then converted to organic compounds (most commonly as phosphate esters) through a variety of cellular processes.

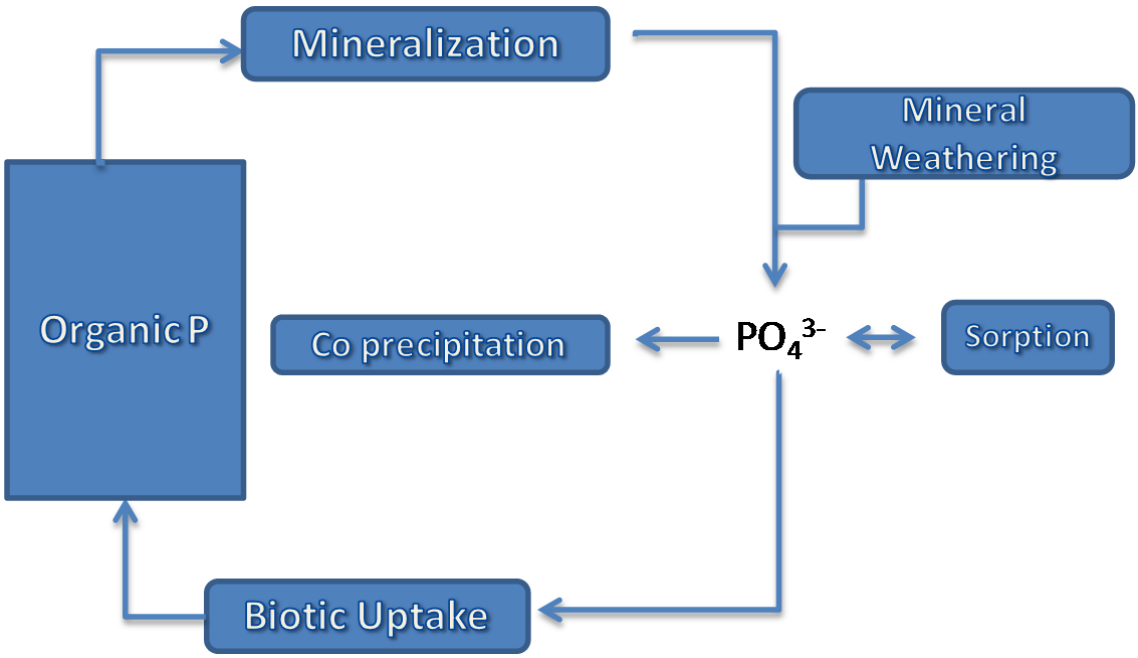

Figure 1.2. Generalized P cycling (adopted and modified from McClain *et al.* (1998))

After death of a plant or animal, decomposition and mineralization reactions convert organic-P back into the inorganic form (Fig 1.2). However, a number of additional equilibrium reactions such as adsorption-desorption reactions with surfaces of iron (FeO_x), aluminum oxides (AlO_x) and clay mineral particles, and co-precipitation with Fe (III), Fe(II), Ca and Al can make PO_4^{3-} biologically unavailable to organisms on the time scales needed for cellular growth (McClain *et al.* 1998).

1.2.2. Production and mineralization of organic matter (carbon cycling)

In the aquatic environment, photosynthesis and respiration are two important processes affecting the production and mineralization of organic matter. Photosynthesis (P) is the biochemical process by which photosynthetic organisms use sunlight energy to drive the synthesis of organic compounds. On the other hand, respiration (R) is the oxidation of

organic carbon to CO_2 and one of the key steps in the mineralization of organic matter. Mineralization is the breakdown of complex organic matter (e.g. plant and animal detritus) into simple recyclable forms (CO_2, phosphate, NH_4^+ etc). Heterotrophs (bacteria, protozoa and fungi) rely on organic carbon to obtain the energy for their cellular metabolism and growth from respiration. The respiration process consumes oxygen. When the available O_2 pool is exhausted a number of other oxidants become important i.e. NO_3^-, MnO_x, FeO_x, SO_4^{2-} and CO_2. Once anaerobic conditions are established, denitrification of NO_3^- is followed by metal (Mn then Fe) and sulfate reduction as shown in the following equations (Eq 1.4 – 1.8). These reactions usually take place in sequence and are commonly known as the 'redox cascade':

$$CH_2O + O_2 \rightarrow CO_2 + H_2O \qquad \text{Eq. 1.4}$$

$$CH_2O + (4/5)\,NO_3^- + H^+ \rightarrow CO_2 + (2/5)N_2 + (7/2)\,H_2O \qquad \text{Eq. 1.5}$$

$$CH_2O + MnO_2 + 4H^+ \rightarrow CO_2 + 2Mn^{2+} + 3H_2O \qquad \text{Eq. 1.6}$$

$$CH_2O + 2FeO_2H + 8H^+ \rightarrow CO_2 + 4Fe^{2+} + 7H_2O \qquad \text{Eq. 1.7}$$

$$CH_2O + (1/2)SO_4^{2-} + H^+ \rightarrow CO_2 + (1/2)H_2S + H_2S \qquad \text{Eq. 1.8}$$

where 'CH_2O' represents organic matter.

Using algae as an exemplar primary producer, equations 1.9 and 1.10 show how nutrients are utilised during photosynthesis (PS) and liberated by heterotrophs through respiration. The difference between two equations is the source of nitrogen (NO_3^- or NH_4^+) used by the algae. Nutrients will be used by other autotrophs in different ratios or stoichiometries to algae, but the form of the equations will remain the same (Stumm and Morgan, 1996).

$$106\,CO_2 + 16\,NH_4^+ + H_2PO_4^- + 116\,H_2O \underset{R}{\overset{PS}{\rightleftharpoons}} \text{Protoplasm} + 106\,O_2 + 15\,H^+ \qquad \text{Eq. 1.9}$$

$$106\,CO_2 + 16\,NO_3^- + H_2PO_4^- + 122\,H_2O + 17\,H^+ \underset{R}{\rightleftharpoons} \text{Protoplasm} + 138\,O_2 \qquad \text{Eq. 1.10}$$

The C:N:P ratios in these two equations (i.e 106:16:1), also known as the Redfield ratio (Redfield 1958; Atkinson and Smith 1983), indicate the causal relationship between nutrients and metabolism (primary production and respiration). Provided other conditions for photosynthesis are met (e.g. sufficient photosythetically active radiation (PAR)) these equations also indicate why elevated nutrient concentrations can result in excessive plant growth.

In the absence of other terminal electron acceptors (according to the redox cascade), organic matter is degraded via methanogenesis (Le Mar and Roger 2001). Methanogenesis can be inhibited in the presence of sulfate reducing bacteria (SRB) (Lovley and Klug 1983). However, under low sulfate concentrations (< 0.3 mM) methane producing archaea (MPA) becomes dominant in anoxic sediments (Kuivila *et al.* 1989; Grace *et al.* 2010). Under anoxic conditions, methanogens (CH_4 producing microorganisms) use inorganic carbon (CO_2) as the terminal electron acceptor for their respiration and produce CH_4 , as shown in Equation 1.11. Another pathway for CH_4 production is acetate fermentation (Eq 1.12) (Kuivila *et al.* 1989). The methanogens involved in the production of CH_4 by CO_2 reduction and acetate fermentation are known commonly as hydrotrophic and acetotrophic methanogens respectively. However, oxic conditions inhibit the growth of methanogens and also facilitate oxidation of CH_4 by the methanotrophs (CH_4 oxidizing bacteria) represented by equation 1.13.

$$CO_2 + 2H_2 \longrightarrow CH_4 + O_2 \qquad \text{Eq. 1.11}$$

$$CH_3COOH \longrightarrow CH_4 + CO_2 \qquad \text{Eq. 1.12}$$

$$CH_4 + O_2 \longrightarrow CO_2 + 2H_2 \qquad \text{Eq. 1.13}$$

The above discussion demonstrates the importance of various biogeochemical processes (for example microbial decomposition, degradation and dissolution) in transforming different nutrient species in aquatic ecosystems (Grenz *et al.* 2010). Both the thermodynamics and

rates of these biogeochemical transformations are often controlled by a number of physicochemical factors including temperature, pH, redox conditions, dissolved oxygen (DO) concentration and the analyte concentration gradient across the sediment-water interface (Chowdhury and Bakri 2006; Spears *et al.* 2008; Biswas *et al.* 2009; Zhang *et al.* 2010; Ozkundakci *et al.* 2011; Roy *et al.* 2012). The impact of these processes on nutrient transformations can vary widely both spatially and temporally within as well as between ecosystems.

1.3. Thesis Outline

The major aim of this thesis is to investigate the nutrient biogeochemistry of a small dam in a headwater catchment of south eastern Australia. More specifically, this study will improve understanding of the contribution of farm dams to greenhouse gas (N_2O and CH_4) emissions, the role of dam sediments in processing bioavailable nutrients (nitrate, ammonium and orthophosphate), the factors controlling these processes and the overall impact of such a dam on downstream water quality. This study is presented in three experimental chapters and will address the following key questions and associated hypotheses:

Key Questions and Hypotheses:

- How do N_2O and CH_4 emissions from the dam vary spatially within the dam and temporally (over months and weather events) and what are the factors controlling the emission? (Chapter 3). It was hypothesised that N_2O emission would be greater at the inlet site than at the outlet site within the dam whereas CH_4 emission would be the opposite. It was also hypothesised that higher nutrient input during high flow conditions in winter may facilitate N_2O emission and high temperature and low flow conditions in summer may promote CH_4 emission from the dam.

- How and when does the dam act as a source or sink of bioavailable nutrients and what are the factors influencing the relevant processes? (Chapter 4). It was hypothesised that the dam would be a source of nutrients in winter and a sink in summer, and that dissolved oxygen, temperature and water residence time would be the dominant factors influencing the processes.

- What is the impact of the dam on downstream water quality? (Chapter 5). It was hypothesised that irrespective of the season, the dam would discharge poor quality water into the downstream water body.

In addition, Chapter 2 describes the research site, the individual sampling locations and the general experimental procedures. This chapter also outlines the standard protocols maintained for sample collection, preservation and analysis. Chapter 6 describes the summary of the dissertation and possible future research directions.

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Chapter Two

Materials and General Methods

2.1 Study site

This study was conducted at a dairy farm dam in the Poowong East headwater catchment (PEC) of the Lang Lang River, which is approximately 130 km southeast of Melbourne CBD (Central Business District), near the town of Poowong East (Fig 2.1.a). According to the Melbourne Water's Index of River Condition (IRC, an overall integrated measure of the environmental river conditions including hydrology, physical form, streamside zone, water quality and aquatic life) the present state of the Lang Lang River (upper) is very poor (http://www.melbournewater.com.au, last accessed on 9th August 2012). The Lang Lang River flows in to the Westernport Bay. The climate of the PEC is humid and cool temperate with a wet winter, an early spring and dry summer. The mean annual (1981-2010) rainfall is approximately 956 mm, temperature is 18.9 ^{0}C (max) to 9.7 ^{0}C (min) and relative humidity is 80% in the morning and 66% in the afternoon (data collected from Wonthaggi weather station (088127, about 30 km away from the site) of the Australian Bureau of Meteorology). About 80% of the land use by area within the PEC is pasture and other dryland agricultural activities (Russell Adams, personal communication). The sub catchment has many dams of varying size and volume. The dam (≈ 12 ML) selected for this study is located at the southern end of the farm (188 ha) and is a part of a headwater stream (draining the upper catchment of the dam) which flows south to north through the dam and the farm (Fig 2.1.a and 2.1.b). The dam was well fenced to prevent animal access and surrounded by riparian vegetation of shrubs and native gum trees. The emergent reed, Typha was found only along the south-eastern side of the dam (Fig 2.1.b). Three sites within the dam were chosen for sampling: close to the inlet "dam south", in the middle "dam middle" and close to the outlet "dam north" which will hereafter be reported as DS, DM and DN respectively. Moreover, stream sampling sites at the inlet and outlet of the dam will be reported as dam upper (DU) and dam lower (DL) respectively (Fig 2.1.a).

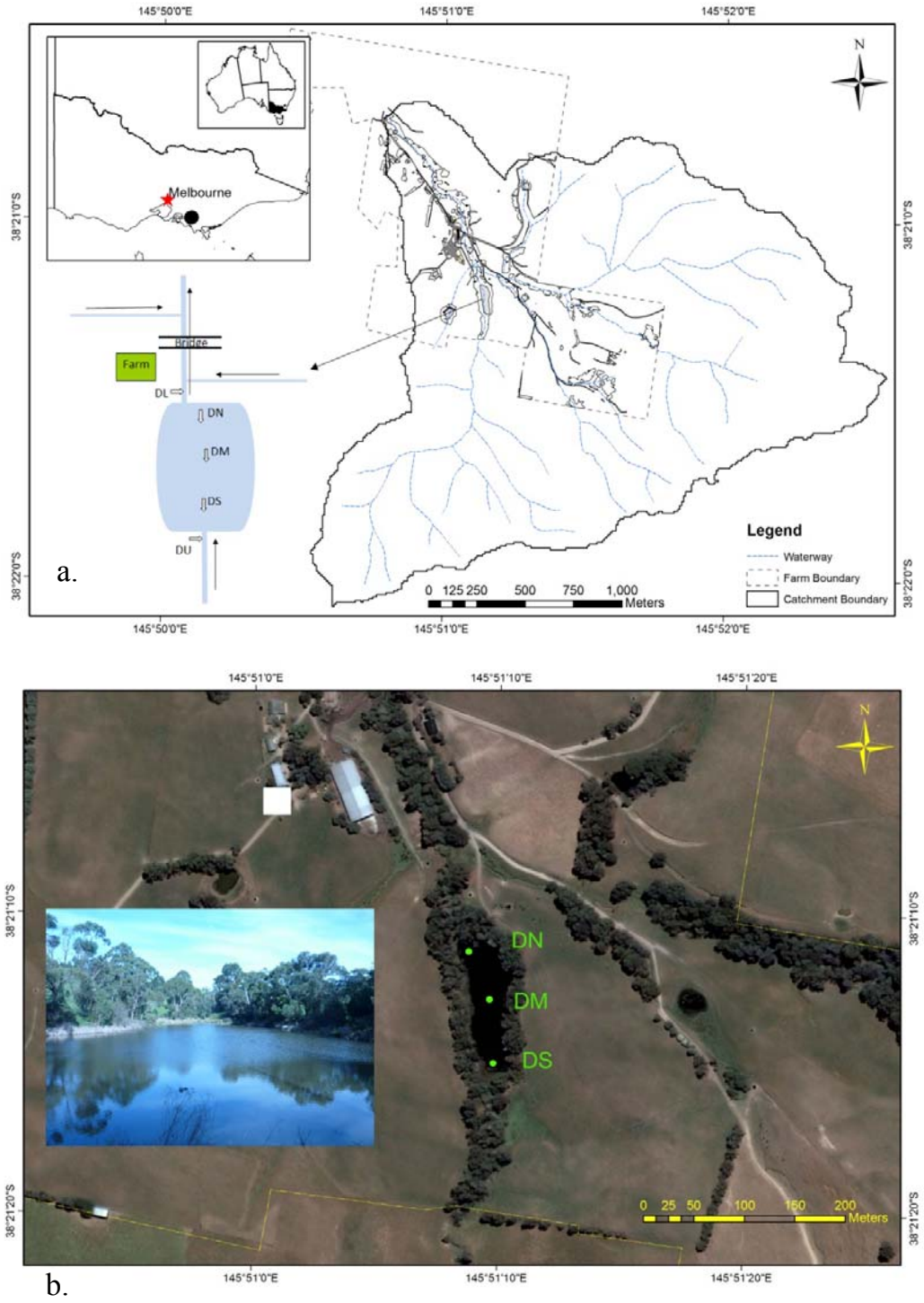

Figure 2.1a. PEC catchment boundary and stream network, farm boundary, dam and associated streams with flow direction (⟶) and sampling points (⇩) (Map obtained and modified from Russell Adams, personal communication); b. Aerial and pictorial (inset, from north to south) view of the farm dam. Green dots represent approximate sampling locations

2.2 Methods

2.2.1 Experimental

2.2.1a Sediment core incubation

Sediment cores were collected from three sites (DS, DM and DN) within the dam to study the seasonal variation of sediment-water solute (dissolved nutrients, organic carbon and green house gases) exchange and to measure sediment porosity and benthic macrofaunal abundance. Every month 12 undisturbed sediment cores (4 replicates from each site) were collected by cylindrical plastic cores (height 28.5 cm and diameter 6.7 cm) with the aid of a sediment corer (Large bore sediment corer, Aquatic Research Instruments, www.aquaticresearch.com). Each core collected contained approximately 0.15 m of sediment and the rest was the overlying water. Once collected the cores were capped at both ends with rubber bungs, placed on ice in a temperature insulated container (Esky) and the empty spaces were filled up with bubble wrap to minimize disturbance during transport. The cores were brought back to the laboratory within 2 hours of collection, placed in an incubation tank and kept in darkness at in situ bottom water temperature of the dam (see section 2.2.3 below). The temperature of the incubation tank was regulated by an aquarium chiller (DBE-200 - Arctica titanium chiller, JBJ-USA). The cores were allowed to settle for at least 12 hours before further treatment (Grace *et al.* 2010). A magnetic stirrer (suspended from a metal bracket inside the core) was carefully placed in each of the cores, approximately 5 cm above the sediment surface and set to rotate at a rate of approximately 30 rpm to prevent sediment disturbance (Fig 2.2). Each of the cores was sealed with an air tight lid with the provision of taps for water recirculation and ports for dissolved oxygen measurement and sampling. Dissolved oxygen (DO) was measured using a calibrated multi O_2 probe (HACH HQ40d). During incubation, the overlying water column O_2 saturation in the cores was maintained according to the measured O_2 state in the hypolimnion of the dam at the time of sampling.

Reservoir water (collected *in situ* and stored in a plastic container) was recirculated through each core using Tygon tubing (Masterflex, 06401-16, Illinois, USA) for at least 12 hours for acclimation of the sediment-water environment by a 12 channel peristaltic pump (Ismatec, Glattbrugg-Zürich, Switzerland) (Grace *et al.* 2010). Different levels of oxygen saturation (according to the *in situ* concentrations) in the overlying water of the cores were achieved either by aeration (for 100%) or purging with an air-N_2 gas mixture (for intermediate concentrations) or only N_2 gas (for 0%) into the reservoir water and recirculated until equilibrium was established in the cores.

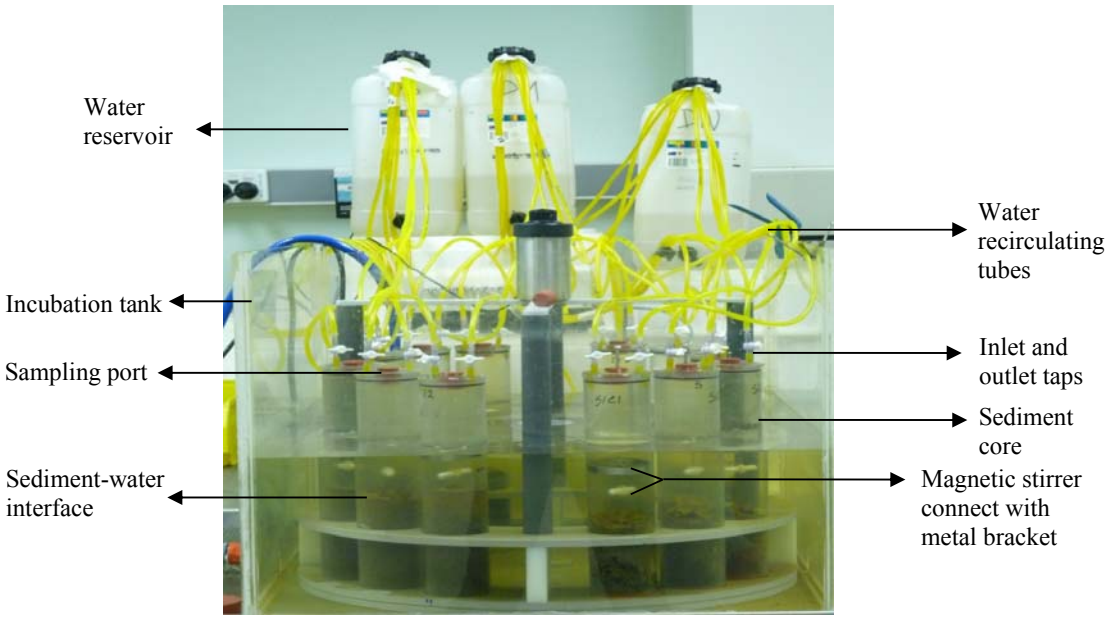

Figure 2.2. Experimental setup of sediment core incubation techniques

2.2.1.b Equilibrium dialysis samplers (Peepers)

Equilibrium dialysis samplers, commonly known as "pore water peepers", are multi-chamber probes, originally designed by Hesslein (1976). The peepers are generally deployed into the sediments for two weeks to allow establishment of chemical equilibrium between the

sediment pore water and water inside the probe chambers (Carignan *et al.* 1985). The process enables various analytes (i.e. dissolved nutrients and metal ions) from the interstitial water to enter into the chambers via diffusion and provides information on the vertical concentration profile of the analytes concerned. Each of the peepers used in this project had 3 PMMA (Poly methyl methacrylate, Perspex) plates (1 base and 2 covers) including 36 chambers arranged vertically and spaced 1 cm apart (Fig. 2.3). Prior to the deployment, the peepers were assembled in the lab under ultrapure water including 0.2 µm pore size polysulfone filter paper on both sides of the base plate which corresponded to approximately 3.5 mL of water in each chamber (Fig 2.3). The peepers were placed in a polyvinyl chloride (PVC) container filled with ultra pure water and bubbled with N_2 gas continuously for two weeks for complete deoxygenation (Grace *et al.* 2010). After two weeks the containers were sealed to prevent oxygen diffusing back into them and the peepers were taken to the field site for deployment.

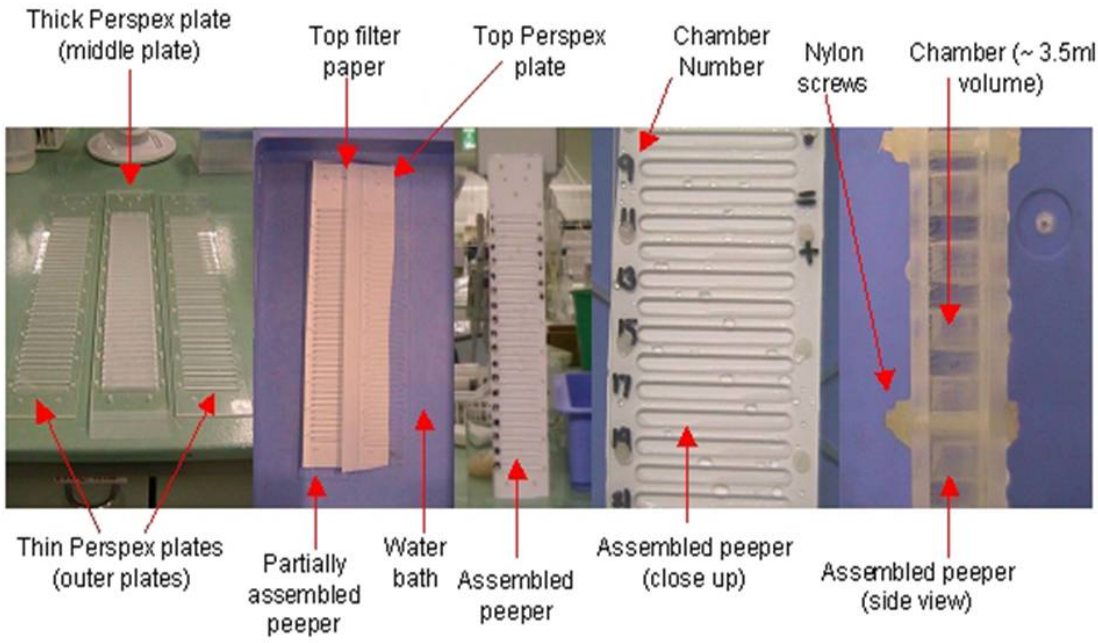

Figure 2.3. Setup and assembly of pore water peepers (picture taken from Lansdown 2004)

Water samples of varying depth from the water column (up to 10 cm from sediment-water interface) and the sediment pore water (up to 10 cm below the sediment-water interface) were collected by deploying 3 peepers (about 50 cm apart from each other) at the DS, DM and DN sites of the dam respectively. An inflatable boat (Zodiac) was used to reach the sampling sites and a custom made device was used to deploy the peepers into the sediment. At each site, one side of a thin nylon rope was tied to each peeper and the other end was commonly tied to a floating buoy for easy location and retrieval. The peepers were deployed on two occasions (9/3/2011 and 10/8/2011) and retrieved after two weeks of deployment every time. From each site, two peepers were sampled (on site) for Fe (II), filterable reactive phosphorus (FRP) and dissolved inorganic nitrogen (NH_4^+ & NO_3^-) and one peeper was sampled (on site) for dissolved organic carbon (DOC).

2.2.1c Planar Optode

Oxygen is a key component in controlling benthic microbial ecology and biogeochemical processes (Oguri *et al.* 2006). Quantification of the O_2 concentration profile across the sediment-water interface (SWI) thus provides useful information on the biogeochemistry of benthic ecosystems. Common techniques for such studies include sediment core incubations and microelectrodes and microoptodes (Glud *et al.* 1996; Oguri *et al.* 2006). Sediment core incubation gives an overall quantification of total O_2 exchange rate at the SWI whereas the other two techniques (microelectrode and microoptode) enable more detailed study of the sediment O_2 distribution with a high temporal and spatial resolution (Rasmussen and JÃrgensen 1992). However, both micrelectrodes and microoptodes provide only one dimensional measurements of O_2 concentration which often makes it difficult to explain spatiotemporal variability of O_2 distribution in heterogeneous benthic environments (Oguri *et*

al. 2006). The introduction of two dimensional planar optodes for studying O_2 dynamics in complex aquatic systems helps to resolve this problem (Glud *et al.* 1996; Glud *et al.* 2001; Larsen *et al.* 2011). The technique is based on the ability of O_2 to quench fluorescence from an excited immobilized fluorophore (Glud *et al.* 1996). The excitation is generally obtained by illumination with LEDs (Light Emitting Diodes). Once excited, the optode fluoresces and the emission intensity decreases due to the presence of O_2, which quenches the fluorescence. The fluorescence intensity can then be imaged by a CCD (Charge-Coupled Device) camera. There are various methods e.g. intensity based measurements (Glud *et al.* 1996), lifetime based O_2 sensitive luminophore measurements (Holst *et al.* 1998) and ratiometric (intensity ratio between two emission images) measurements (Song *et al.* 1997; Hulth *et al.* 2002) available for planar optode experiments. In this study, a simple and high resolution colour ratiometric approach developed by Larsen *et al.* (2011) was used to study the sediment oxygen dynamics and bioturbation activity of benthic macro fauna. The O_2 sensitive optode sensor (70 mm × 55 mm) was prepared by Dr. Perran Cook and Mr. Adam Kessler at the Water Studies Centre, Monash University, Victoria, Australia. Excitation of the sensor was achieved by commercially available high power blue LEDs (λ_{max} = 445nm, LXHL-LR3C, Luxeon) and a ratiometric method was used to analyse the results, as described by Larsen *et al.* (2011) using a Canon Rebel 500D DSLR (Digital single-lens reflex) camera and a combination of the freeware image analysis program "ImageJ" (http://rsbweb.nih.gov/ij/) and custom-designed hardware automation software (Peter Ellis and Adam Kessler, Monash University) prepared in Labview (v. 8.0, National Instruments). Inlet and outlet holes were drilled into the lid of an airtight polycarbonate food container (height 160 mm, width 95 mm and length 100 mm) to allow for water recirculation (to and from the reservoir) and sampling (Fig 2.5). The planar optode (70 mm × 55 mm) was attached along one inside surface of the container with adhesive tape as shown in the Fig 2.4.

Sediment from three intact cores was transferred to the rectangular container in layers, so as to best preserve the sediment layering. Whilst some mixing is unavoidable, the sediment was packed tightly and allowed to equilibrate for 24 hours before experiments were commenced. Homogeneous profiles observed on the optode indicated that this equilibrium had been reached.

Intact sediment cores collected from the dam were transferred carefully into the container with minimum disturbance. A calibrated needle type O_2 microsensor (ZB7-534-207, 50 µm, Pyroscience) was also inserted into the vessel for continuous monitoring of the water column oxygen concentration. To achieve different levels of oxygen saturation (i.e. 100, 50, 25 and 0 percent O_2 sat) in the overlying water of the experimental container, the reservoir water was either aerated (for 100%) or purged with air-N_2 gas mixture (for intermediate levels) or only N_2 gas (for 0%) and recirculated until a stable O_2 reading was established in the container.

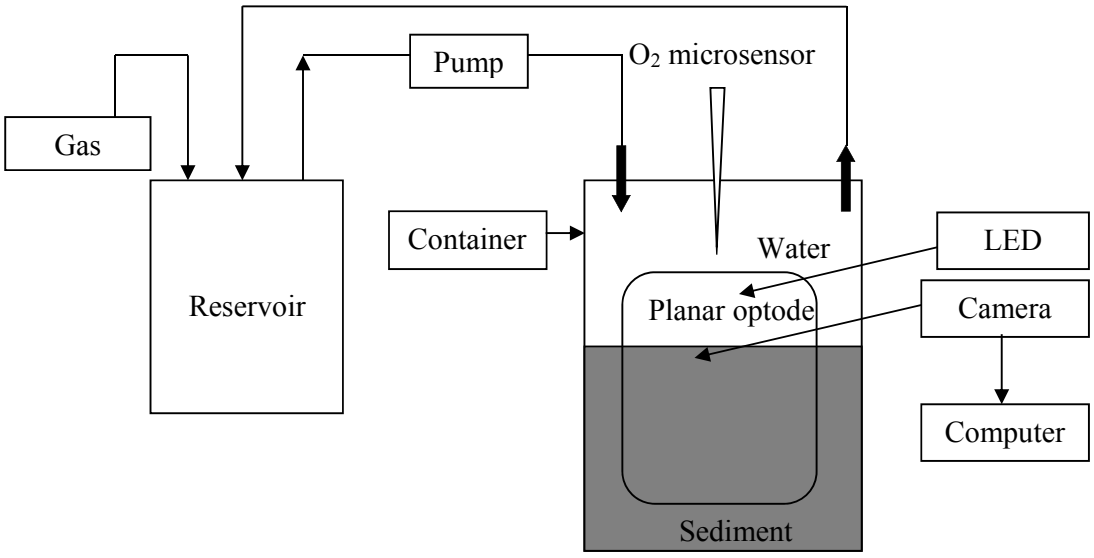

Figure 2.4. Simplified diagram of the planer optode experimental set up

Images of the optode were recorded every 30 minutes (average of 10 rapid consecutive images taken every 5 seconds) for 12 hours once equilibrium was established at each oxygen saturation level. To calculate Sediment Oxygen Consumption (SOC) rates (except for 0% O_2 saturation), the system was incubated for 3-6 hours (gas addition and water recirculation was completely stopped during the incubation period) to allow the dissolved oxygen in the overlying water column to drop by at least 20% (Dalsgaard *et al.* 2000).

2.2.2 Sampling, preservation and analysis

2.2.2.a Water samples

All sample bottles and glassware were cleaned (5% Extran®), acid soaked (overnight in 10% HCl) and rinsed three times with ultrapure water (Milli-Q Academic, EMD Millipore Corporation, Billerica, MA, USA) before sampling. Water samples for nutrients were collected in high density polyethylene (HDPE) bottles and organic carbon samples were collected in amber borosilicate glass jars. Prior to use, these glass jars were wrapped in aluminium foil and baked in a muffle furnace at about 520 ^{0}C for an hour to remove any residual carbon. Baked aluminium foil was also placed between the plastic cap and the jar to avoid contamination. Samples for dissolved nutrients were filtered through 0.2 µm-pore sized cellulose acetate syringe filters (Advantec) and dissolved organic carbon samples were filtered through glass fibre filter (grade GF-75, Advantec) papers whereas total nutrients (TP and TN) and organic carbon (TOC) samples were collected unfiltered. Water samples were filtered using disposable 30 mL polyethylene syringes (Terumo®, Tokyo, Japan). Water samples for dissolved gases (N_2O and CH_4) were collected (unfiltered) in 12 mL gas tight exetainers (Labco, High Wycombe, UK). Field blank samples using ultrapure water were

also collected on every sampling occasion and treated in a similar manner as the samples to detect any contamination occurring during sampling procedures.

Water samples from the streams were first collected in a pre-washed (cleaned, acid soaked and rinsed) bucket and then sub-sampled from the bucket for different analytes into designated bottles as mentioned earlier. From the dam, the surface water samples were collected directly from the site and bottom water was first collected by a Niskin bottle (General Oceanics Inc. North Miami, Florida), emptied into a bucked and then sub-sampled in a similar manner to the stream samples. Water samples from the sediment core incubations and optode experiments were collected in the laboratory during the incubation at different O_2 concentration levels. Once the overlying water column O_2 concentration was stabilized (O_2 saturation was measured using a calibrated multi O_2 probe (HACH HQ40d) for the cores and a needle type O_2 microsensor for the optode experiment), the cores were incubated for 4-8 hours (3-6 hours for the optode experiment) and during the incubation period water samples were collected (3-5 times) by 30 mL disposable syringes for dissolved gas and nutrient analysis. Each time 30 mL of water was then replaced from the reservoir and reservoir water was sampled for any corresponding concentration corrections. From the peepers, the desired volume of pore water for each analysis was collected using a calibrated auto pipette and the samples were dispensed in sampling vials containing relevant preservative solutions (Table 2.1).

In every instance, all of the field samples were kept on ice during transportation and brought back to the laboratory within 3-4 hours of collection. The samples were either frozen (dissolved nutrients) or refrigerated (total nutrients and total and dissolved organic carbon) until analysis (Gardolinski *et al.* 2001; APHA 2005). Total and dissolved organic carbon samples (TOC and DOC) were acidified (pH < 2) by adding 1-2 drops of concentrated HCl in

the field (APHA 2005). Gas samples were preserved by adding 1 drop of 50% $ZnCl_2$ (w/v) into the sampling vials both in the field and in the laboratory (Dalsgaard *et al.* 2000). Sampling volume, preservation and storage procedures for the peepers samples are presented in Table 2.1.

Table 2.1 Sample volume, preservative solution and storage procedure used for peeper sampling

Analyte	Sample volume (mL)	Preservative solution	Storage technique
NO_x and NH_4	1	Ultrapure water (9 mL)	Frozen
FRP	1	0.202M H_2SO_4 (9 mL)	Frozen
DOC	3	0.5% HCl (2 mL)	Refrigerated
Iron (II)	0.5	Buffered phenanthroline solution (4.5 mL)	Refrigerated in the dark

All samples were analysed within one month of collection following standard procedures of analysis described in the methods of the NATA (Australian National Association of Testing Authorities) accredited Water Studies Centre analytical laboratory and APHA 2005. Quality control procedures used in this research study included analysis of duplicates, standard checks, use of standard reference materials and analysis of laboratory and field blanks to assure the reliability of obtained data. Analytical grade chemicals were used in all of the chemical analysis performed in this research project. A summary of the chemical methods used to analyse water samples for different analytes is presented in Table 2.2.

Table 2.2 Summary of general analytical methods and method references. * FIA = Flow injection analysis (Lachat QuickChem 8000, Colorado, USA)

Analyte	Method reference	Method of analysis	Principle of detection
Ammonium-N (NH_3 + NH_4^+ ammonium) (NH_4-N)	Method 4500-NH_3-G (APHA 2005)	Spectrophotometric determination by FIA*	NH_4^+ in the sample reacts with hypochlorite to form monochloramine which further reacts with phenol, nitroprusside and excess hypochlorite to form indophenols blue. Quantification of NH_4^+ concentration is achieved by measurement of the sample absorbance at 630 nm.
Nitrogen oxides-N (NO_3^- + NO_2^-) (NO_x-N)	Method 4500-NO_3^- I (APHA 2005)	Spectrophotometric determination by FIA*	NO_x in the sample is reduced to nitrite using a cadmium column. The nitrite is then mixed with sulphanilamide and N-(1-napthyl)-ethylene diamine dihydrochloride to form the final product: purple azo dye and measured colourimetrically at 520 nm absorbance.
Filterable reactive phosphorus (PO_4^{3-}-P) (FRP)	Method 4500-P G (APHA 2005)	Spectrophotometric determination by FIA*	FRP in the sample is converted to a blue complex by reacting with ammonium molybdate, antimonyl potassium tartrate and ascorbic acid. The absorbance is measured at 880 nm.
Total nitrogen and phosphorus (TN and TP)	Method 4500-P J (APHA 2005)	Persulfate digestion and Spectrophotometric determination by FIA*	Samples were first digested according to the alkaline persulphate method (APHA 2005) and autoclaved for about 45 minutes at 15 lb pressure and 121 ^{0}C temperature to convert all N and P to NO_x and FRP. The resulting samples were then analysed as per NO_x and FRP above.
Total and dissolved organic carbon (TOC and DOC)	Method 5310 B (APHA 2005)	High temperature oxidation	Quantification of organic carbon concentration is based on peak area in the infrared spectrum. Sample is injected into a catalyst chamber under high temperature (i.e. 720 ^{0}C) with the carrier gas (high purity O_2) and combusted. The combustion process oxidizes the organic carbon present in the sample to CO_2 which is detected on an infrared detector.
Iron (II)	Grace *et al.* (1997), Lansdown (2004)	Spectrophotometric determination using a Cecil Ce1010 U-V Spectrophotometer	The Fe(II) in the sample instantly forms an intense orange coloured $Fe(o\text{-}phen)_3^{2+}$ complex with the addition of buffered phenanthroline solution. The colour intensity of the solution is directly proportional to the amount of iron (II) present. Quantification of Fe(II) concentration is achieved by measurement of the sample absorbance at 510 nm.

2.2.2.b Benthic macrofauna

Benthic macrofauna were collected from sediment cores. After finishing each incubation experiment, each of the cores were sieved through a 1-mm sieve to collect *in situ* benthic invertebrate species. The animals were collected in glass jars, preserved in 70% ethanol and later identified (Gooderham and Tsyrlin 2002) and counted under a dissecting microscope (Fig 2.5).

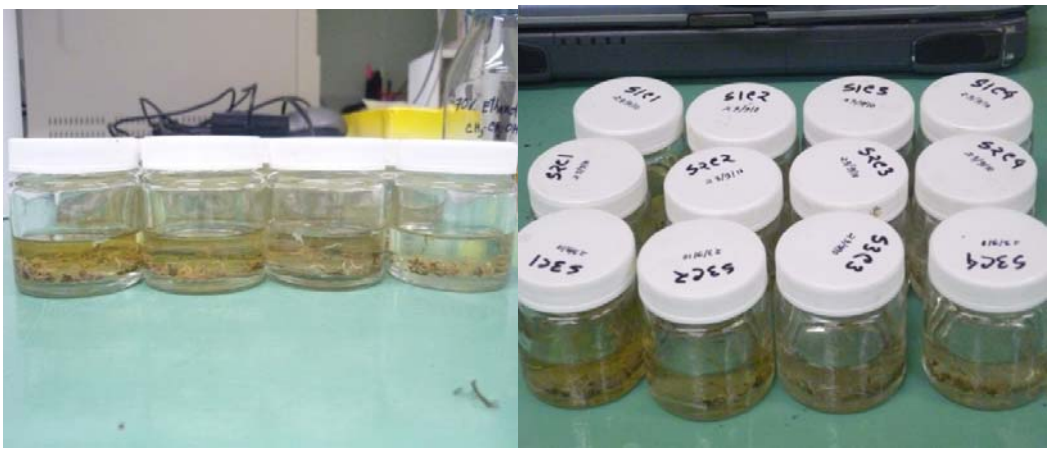

Figure 2.5. Collection and preservation of benthic macrofauna

2.2.3 Water quality measurements

The *in situ* physicochemical water quality variables pH, electrical conductivity (EC), turbidity, dissolved oxygen (DO) concentration and water temperature were measured by a calibrated Horiba U10 multi-probe (Kyoto, Japan) water quality sonde. Prior to each field trip, the instrument was calibrated for every parameter in the laboratory and calibration was done within 12 hours of field use. An approximately 10-15 minutes stabilization period was allowed for the instrument to equilibrate before taking any reading and then 3-5 replicate measurements were taken for each parameter. Table 2.3 illustrates the calibration methods applied for different parameters according to the manufacturer's instructions.

Table 2.3 Standard calibration procedures for the Horiba U-10 multi-probe

Parameter	Calibration process
pH	pH 4.01 and 6.86 with phthalate and phosphate buffer respectively
Electrical Conductivity (mS/cm)	0 and 0.718 mS/cm with air and 0.005 M KCl solution respectively
Turbidity (NTU*)	0 and 400 NTU with ultrapure water and diluted hydrazine sulphate/hexamethylenetetramine solution respectively
DO (% sat)	0 and 100% saturation by adding Na_2SO_3 and air bubbling of water
Temperature (^{0}C)	Checked against pre-calibrated thermometer

*NTU = Nephelometric Turbidity Units

2.2.4 Statistical analysis

The statistical software, SPSS version 20 (IBM® SPSS® Statistics 20) and Microsoft Office Excel 2007 were used for various data analysis including one-way and repeated measures analysis of variance (ANOVA), Pearson's product-moment correlation coefficient and simple linear regressions. When required, some data were log-transformed to more closely approximate a normal distribution.

2.3 References

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Chapter Three

Factors affecting nitrous oxide and methane emissions from an agricultural farm dam in south-eastern Australia

3.1 Introduction

N_2O and CH_4 are two of the primary greenhouse gases in the earth's atmosphere. The global warming potentials (GWP) of CH_4 and N_2O are 25 and 298 times greater than CO_2 (100 year time horizon) (Forster *et al*. 2007). The concentration of both of these gases in the atmosphere has increased markedly in recent times compared to the preindustrial era due to increased anthropogenic, predominantly agricultural, activities (Alley *et al.* 2007).

Production and release of these biogenic trace gases involves several microbial processes and anoxic conditions. CH_4 is produced during anaerobic mineralization of organic matter by methanogenic bacteria; this CH_4 can subsequently be consumed via oxidation by methanotrophic bacteria in oxic environments (Chapter 1, section 1.2.2) (Le Mer and Roger 2001). A well mixed and oxygenated water column can thus act as a protective layer against diffusive flux of CH_4 to the atmosphere. In contrast, well-developed water column anoxia during stratification has the potential to increase atmospheric emission of CH_4 (Liikanen and Martikainen 2003). Ebullition or bubble formation is likely the most significant mechanism of CH_4 emission from fresh water ecosystems because the resultant gas bubbles largely avoid oxidation and uptake by aquatic flora and fauna (Bastviken *et al.* 2003). Production of N_2O involves both oxic and anoxic processes (Chapter 1, section 1.2.1) i.e. N_2O is an intermediate in both the oxidation of NH_4^+ (nitrification) and dissimilatory reduction of NO_3^- to N_2 (denitrification) under hypoxic conditions mediated by nitrifying and denitrifying bacteria respectively (Roy and Knowles 1994; Meyer *et al.* 2008; Deemer *et al.* 2011). Forster *et al.* (2007) reported that natural sources like wetlands and lakes are responsible for one third and 55% of total global CH_4 and N_2O emissions respectively. A clear understanding of the processes and factors associated with aquatic CH_4 and N_2O dynamics is essential for better and effective management.

Production of N_2O and CH_4 often exhibit seasonal patterns, mainly governed by O_2, temperature, NO_3^- and organic carbon supply (Mengis *et al.* 1997; Wang *et al.* 2006). Elevated temperature and depleted O_2 concentrations favour net CH_4 production by increasing microbial activity and decreasing CH_4 oxidation (Liikanen *et al.* 2002). Increased production of N_2O is also observed under hypoxic (Ritchie and Nicholas 1972; Goreau *et al.* 1980) and oxic (Liikanen *et al.* 2002) conditions within a moderate temperature range (6-16 ^0C). In the absence of O_2, other electron acceptors (e.g. NO_3^-, SO_4^{2-}) can greatly influence anoxic mineralization of organic C (Hunt *et al.* 2007) and has the ability to suppress CH_4 production (Dodla *et al.* 2009) and increase N_2O emission. When NO_3^- is present, degradation of organic C is led by NO_3^- reduction (denitrification) rather than via methanogenesis in fresh water sediments (Capone and Kiene 1988). Moreover, high NO_3^- concentrations can significantly increase N_2O production during denitrification (Martikainen *et al.* 2002). Under anaerobic conditions, microbial oxidation of CH_4 coupled with denitrification is also observed (Raghoebarsing *et al.* 2006). Water depth is another prominent factor in sediment gas dynamics. Due to different physical, chemical and biological compositions of littoral (near bank), shallow (within the range of effective light penetration) and profundal (below the range of effective light penetration) zones, their influence on gas emission also varies. Profundal zones are shown to emit more CH_4 than littoral and shallow zones in fresh water lakes (Liikanen *et al.* 2003). This was attributed to the higher C and N content in the deep profundal sediments than in the shallow and littoral sediments (Liikanen *et al.* 2003).

Benthic macrofaunal activity i.e. bioturbation and bioirrigation, can appreciably alter the physical and chemical structure of the sediment and the distribution of microbial populations (Krantzberg 1985). Despite their influence on sediment reworking, fewer studies are available on fresh water macrofauna compared to those from the coastal marine environment

(Fenchel 1996). Chironomids (order: *diptera*) and Oligochaetes (subclass: *oligochaeta*) are often the most abundant faunal species found in fresh water lakes and ponds. Chironomid larvae, due to their distinctive burrow structure and feeding activity, have drawn the most research attention compared to other species. Chironomid larvae increase the availability of O_2 and NO_3^- deep into their burrows and surrounding pore water through irrigation of their burrow, thus promoting coupled nitrification-denitrification which in turn, increases sedimentary N_2O production (Stief and De Beer 2006; Stief and Schramm 2010). Stief *et al.* (2009) demonstrated that filter-feeding *Chironomus plumosus* larvae directly emitted N_2O and ascribed this to the activity of denitrifying bacteria present in their anoxic gut. Their changed grazing and burrowing activity in response to altered food sources and O_2 condition is also reported (Altmann *et al.* 2004; Stief *et al.* 2005). Oligochaetes, on the other hand, have shown to be less effective than Chironomids in reworking sediment (Svensson *et al.* 2001). In a recent study, Jones and Grey (2011) found Chironomid larvae transferred CH_4 to the aquatic food web due to their grazing on methane oxidizing bacteria (MOB) and consequently these larvae have the potential to act as significant source of biogenic CH_4.

Fresh water ecosystems can thus effectively act as both the source and sink for a particular greenhouse gas based on localized variations in a range of natural factors. Additionally, several human-induced factors, such as disposal of sewage and agricultural runoff into these ecosystems, further enhance trace gas emission to the atmosphere. Constructed wetlands and ponds connected to agricultural areas and sewage treatment plants are different from natural lakes and wetlands in that the former often receive – or are designed to receive - high concentrations of nutrients and organic carbon. Consequently these constructed wetlands and ponds may potentially be an important source of GHG (Lal and Pimentel 2008; Soumis *et al.* 2004). Huttunen *et al.* (2003), in their study on the effect of catchment disturbance on aquatic greenhouse gas emission, demonstrated the highest (12 mmol/m^2/day) CH_4 flux were from

eutrophic lakes within agricultural catchments. They explained that excess nutrient loading in such lakes increased autochthonous primary production, increasing sediment O_2 consumption and anaerobic decomposition in the sediment leading to increased CH_4 release from lakes to the atmosphere. In a study of GHG emission from nitrogen removal ponds in southern Sweden, Stadmark and Leonardson (2005) reported emission rates as high as in lakes in temperate regions. In a recent study on the carbon budget and GHG emission from an agricultural reservoir in the US Midwest, Jacinthe *et al.* (2012) demonstrated that over a period of 4 years, the overall GHG emission (2.82 g CO_2/m^2/day) entirely compensated for the organic C burial (2.61 g CO_2/m^2/day) of the reservoir. They pointed out that further enhancement of GHG fluxes can easily shift the reservoir C balance from net sink to source.

Biogeochemical processes in lakes greatly depend on the surrounding catchment. Land use including agricultural practice (crop, pasture) will influence the source and quantity of organic C and nutrients transported via streams, ground water and surface runoff to the impoundment (Huttunen *et al.* 2003). Australia is the third largest dairy producing country in the world and dairy is the largest agricultural industry in the state of Victoria (http://www.dpi.vic.gov.au/agriculture). Farm dams are an essential component of the dairy industry. They are commonly used as a water supply for irrigation, stock water and household requirements. These dams often experience intense nutrient inputs and suffer from severe eutrophication and O_2 depletion which in turn may favour seasonal CH_4 and N_2O production. Page and Dalal (2011) reported gas flux of CH_4 (70 g/m^2/year) and N_2O (0.02-0.12 g/m^2/year) from Australian freshwater wetlands and mangrove systems, in good agreement with worldwide average rates (79.5 g/m^2/year and 0.07 g/m^2/year for CH_4 and N_2O respectively) for similar systems. In another study, Sherman *et al.* (2001) demonstrated that Australian water storage reservoirs are a source of GHG, emitting 220-760 mg CH_4 and 21-168 mg CO_2/m^2/day, but their observations were limited to autumn and winter. Australian

temporary and permanent wetlands were also reported as a major source of CH_4 (about 10% of Australia's total annual CH_4 emission) to the atmosphere (Dalal *et al.* 2008). To date, there is limited information on GHG emissions from varied wetland systems including lakes, reservoirs, ponds and streams within Australia (Page and Dalal 2011; Deutscher *et al.* 2010; Dalal *et al.* 2008) and no data for emissions from farm dams. Hence, this study was conducted to address i) seasonal variability of N_2O and CH_4 gas flux from a farm dam; ii) identification of environmental regulatory factors affecting the gas flux; and iii) the effect of benthic macrofauna on GHG flux.

3.2. Materials and methods

3.2.1. Study site and sampling

Sediment cores were collected every month (except February, September and November 2011) from December 2010 to December 2011 for monthly flux measurements, and in late November 2011 for optode experiments (see below), from a dairy farm dam ($38^0$21'05.64"S, $145^0$51'05.67"E, elevation 161 m). The site is located approximately 130 km south east of the Melbourne CBD, Victoria, Australia. Details of the study site and sampling locations are presented in Chapter 2, section 2.1. Sediment samples were collected from 3 sites within the dam (chapter 2, Fig 2.1). Dam Middle (DM) and Dam North (DN) represent the deeper (3-3.5 metre) sites and Dam South (DS) represents a shallow (1-1.5 metre) site. During each sediment collection trip, water quality parameters were measured using a calibrated (Chapter 2, section 2.2.3) Horiba U10 multiprobe water quality checker and *in situ* surface and bottom water samples were also collected each occasion from all sites for dissolved gases and nutrients measurements. All gas samples were collected in 12 mL gas tight exetainers (Labco, High Wycombe, UK) and dissolved nutrient samples were filtered through 0.20 µm-

pore sized cellulose acetate filter units (Advantec). Samples for gas analysis were preserved by adding $ZnCl_2$ (100 µL, 50% w/v) and nutrient samples were kept frozen until analysis to prevent microbial activity.

3.2.2. Experimental setup

3.2.2a. Planar Optode

The preparation and experimental set up of the planar optode is described in chapter 2, section 2.2.1c. In brief, sediment cores were collected from DS and were transferred into a customized airtight plastic container (height 160 mm, width 95 mm and length 100mm). The container has previously been set up (Chapter 2, Fig 2.5) with inlet and outlet for water recirculation (to and from the reservoir) and sampling. A transparent planar optode (Chapter 2, section 2.2.1c) was attached along one side of the container (Chapter 2, Fig 2.5). The experiment was run at successively decreasing oxygen concentrations and the water collected from the core was replaced with the reservoir water. A calibrated needle-type O_2 microsensor (50 µm, Pyroscience) was also inserted into the vessel for continuous monitoring of the overlying water column oxygen saturation. The incubation experiment was run at 5 different levels of O_2 saturation (100, 50 25, 12.5 and 0 % saturation). Different levels of oxygen saturation in the overlying water of the container were achieved either by aeration or purging with an air-N_2 gas mixture into the reservoir water and recirculated until equilibrium was established in the container (Chapter 2, section 2.2.1c). During each incubation, water samples for dissolved nutrients i.e. ammonium (NH_4^+), nitrate (NO_x) and the greenhouse gases nitrous oxide (N_2O) and methane (CH_4) were collected every hour (for determination of flux rates) and replaced with the reservoir water.

3.2.2b. Monthly core incubation

The experimental set up of the cores and incubation techniques were performed as described in chapter 2 section 2.2.1a (Fig 2.3). Every month, 12 undisturbed sediment cores (4 replicates from each site) were collected in cylindrical plastic cores (height 0.285 m and diameter 0.067 m) and brought back to the laboratory within 2 hours of collection. On each occasion, the overlying water column O_2 saturation was maintained (Chapter 2, section 2.2.1a) according to the O_2 state in the hypolimnion of the dam at the time of sampling (Table 3.1). Reservoir water (collected *in situ*) was recirculated for at least 12 hours for acclimation by a 12 channel peristaltic pump (Ismatec, Glattbrugg-Zürich, Switzerland). The cores were incubated for 4-8 hours and during the incubation period water samples were collected (4-5 times) by 30 mL disposable syringes for gas and nutrient analysis. Each time, this 30 mL of water was replaced from the reservoir. Reservoir water was sampled for concentration corrections. Dissolved Oxygen (DO) was measured by a calibrated oxygen probe (HACH HQ40d) at each time of sampling to calculate sediment oxygen consumption (SOC) rates. Sampling for denitrification rate estimation (Dalsgaard *et al.* 2000) was carried out the next day, followed by sieving (1 mm sieve) all of the sediments to collect *in situ* benthic invertebrate species. The sediment denitrification rate was determined using the Isotope Pairing Technique (IPT) described by Nielsen (1992). The animals were preserved in 70% ethanol and later counted and identified (Gooderham and Tsyrlin 2002) under a dissecting microscope (Chapter 2, section 2.2.2b).

3.2.3. Chemical analysis

Dissolved gas concentrations were determined after collection using a headspace equilibration technique (Jones and Simon 1980). Concentrations of gases in the headspace

were analysed by gas chromatography (Varian 3300 and HP 5710A) equipped with flame ionisation and electron capture detectors for CH_4 and N_2O respectively (Dalsgaard *et al.* 2000). Dissolved nutrients (NH_4^+ and NO_x) were analysed spectrophotometrically using a Lachat QuickChem 8000 Flow Injection Analyser (Chapter 2, Table 2.2). The IPT involved the addition of $^{15}NO_3^-$ tracer to the overlying water of the cores and the determination by isotope-ratio mass spectrometry (IRMS) of $^{15}NO_3^-$ –labelled gases sampled in the exetainers by an ANCA GSL2 elemental analyser interfaced to a Hydra 20-22 continuous-flow isotope ratio mass-spectrometer (Sercon Ltd., UK).

3.3. Results

3.3.1. General observations

During the sampling period, the average depth at the shallow (DS) and deeper (DM and DN) sites of the dam were 1.4 (±0.2) m and 3.2 (±0.3) m and the hypolimnion was anoxic during that period, particularly at the deeper sites (Table 3.1).

Table 3.1. Monthly variation of water quality parameters above (0.5 m) the sediment-water interface at different sites

	Shallow				Deep							
	DS				DM				DN			
Month	Temp (^{0}C)	DO (% Sat)	Turb (NTU)	pH	Temp (^{0}C)	DO (% Sat)	Turb (NTU)	pH	Temp (^{0}C)	DO (% Sat)	Turb (NTU)	pH
Dec 2010	17	58	22	7.2	15	16	41	6.9	15	2	44	6.9
Jan 2011	21	0	25	7.2	15	0	43	6.8	15	0	44	6.9
Mar 2011	17	0	14	7.0	16	0	12	6.6	16	0	12	6.6
Apr 2011	15	59	11	7.1	15	0	26	6.8	15	0	53	6.8
May 2011	12	57	8	7.2	11	45	8	7.4	11	46	7	7.4
Jun 2011	8	78	65	7.1	8	74	73	7.1	8	74	92	7.2
July 2011	9	81	140	7.0	9	70	142	7.2	9	63	152	7.2
Aug 2011	11	99	30	7.0	10	52	15	6.7	10	42	13	6.7
Oct 2011	15	62	23	7.3	14	11	21	7.1	14	12	20	7.1
Dec 2011	15	65	15	7.0	15	49	20	7.0	15	19	28	6.9

In July 2011, water column turbidity along with dissolved NH_4^+ and NO_x concentrations throughout the dam were the highest (Table 3.1, Fig 3.1a & b). The bottom water pH varied from 6.6 -7.4 during the sampling period (Table 3.1).

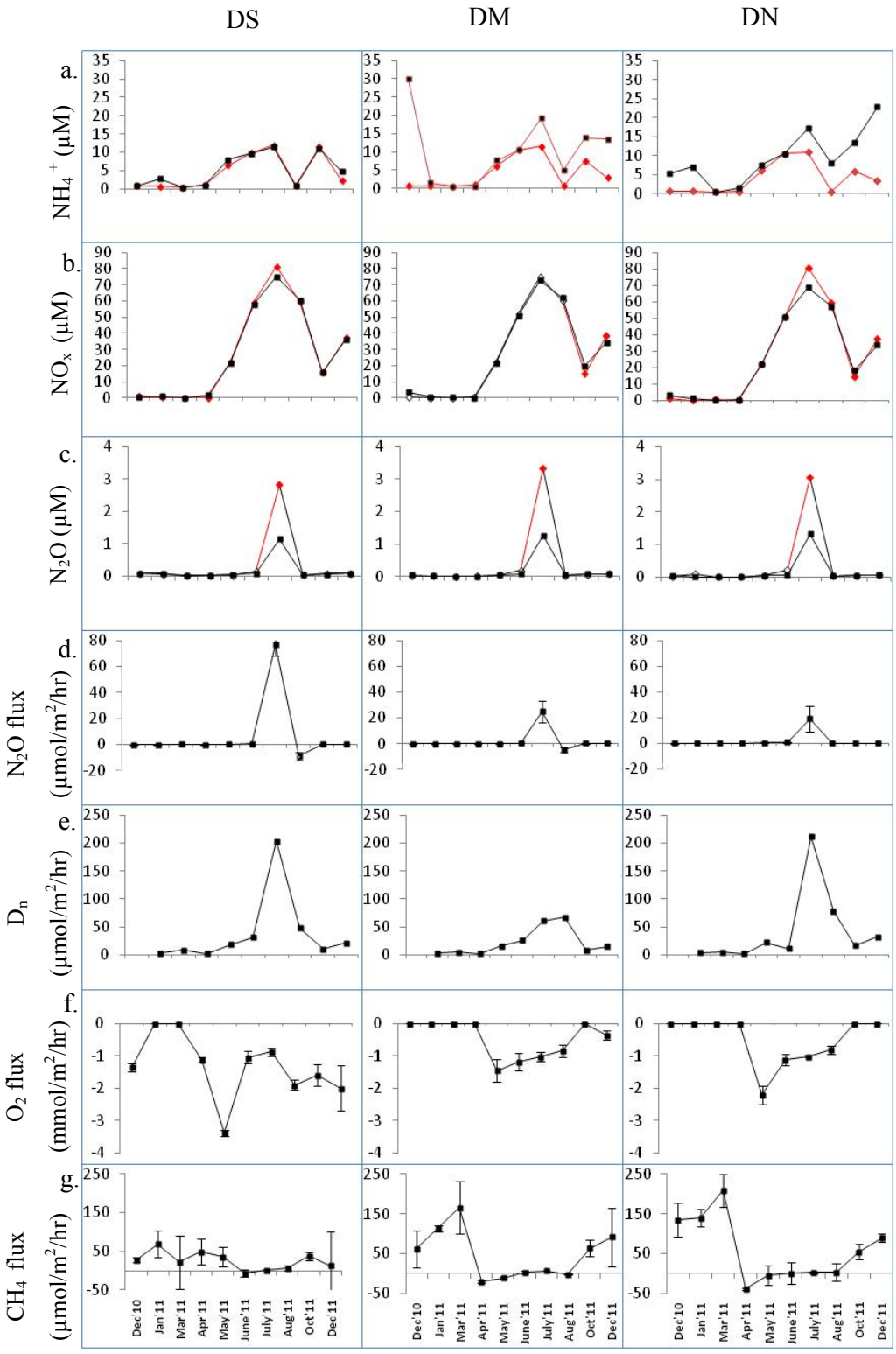

Figure 3.1. Monthly variation of dissolved surface (-◆-) and bottom (-■-) water NH_4^+ (a), NO_x (b) and N_2O (c) concentrations, sedimentary diffusive flux of N_2O (d), denitrification rate D_n (e), SOC (f) and CH_4 flux (g) from DS, DM and DN. Error bars represent ± SE.

3.3.2. Optode experiment

The NO_x concentration in the overlying water in the experimental core varied between 23-30% of the initial concentration (0.26 mg/L) having the lowest concentration (0.18 mg/L) at 0% oxygen saturation The N_2O fluxes at different overlying O_2 saturation varied significantly, reaching the highest rate at 25% (2 ± 1 μmol/m^2/hr) and the lowest (0.03 ± 0.01 μmol/m^2/hr) at 0% O_2 saturation (Table 3.2). The release of CH_4 from the sediment was significantly and negatively correlated with O_2 saturation in the overlying water column (R^2 = 0.68, $p < 0.05$, n = 6). O_2 penetration depth was the highest (1.6 ± 0.1 mm) at the maximum oxygen saturation (Table 3.2) and was significantly correlated with the overlying water column O_2 concentrations (R^2 = 0.78, $p < 0.001$, n = 16). O_2 penetration depth was negatively correlated to sedimentary NO_x consumption rates and CH_4 efflux (Fig 3.2 and Fig 3.3 respectively). The variations of nutrient concentrations at different O_2 levels were within 30% of the initial concentration. Abundance of Chironomid larva was 1474 ind/m^2 and there were no Oligocheate worms observed in the optode experiment.

Table 3.2. O_2 penetration depth ± (SD) and diffusive fluxes of SOC, N_2O and CH_4 ± (SE) at different O_2 saturation concentrations in the water column

DO (% Sat)	O_2 penetration Depth (mm)	SOC (mmol/m^2/hr)	N_2O (μmol/m^2/hr)	CH_4 (μmol/m^2/hr)
100	1.7 ± 0.1	-2.1 ± 0.2	0.3 ± 0.2	49 ± 12
50	1.5 ± 0.2	-1.5 ± 0.8	0.1 ± 0.1	69 ± 14
25	0.6 ± 0.1	-1.9 ± 0.4	1.9 ± 0.6	80 ± 40
12.5	0.5 ±0.2	-0.4 ± 0.2	0.99 ± 0.03	129 ± 5
0	0.0 ± 0.0	0.0 ± 0.0	0.03 ± 0.01	102 ± 4

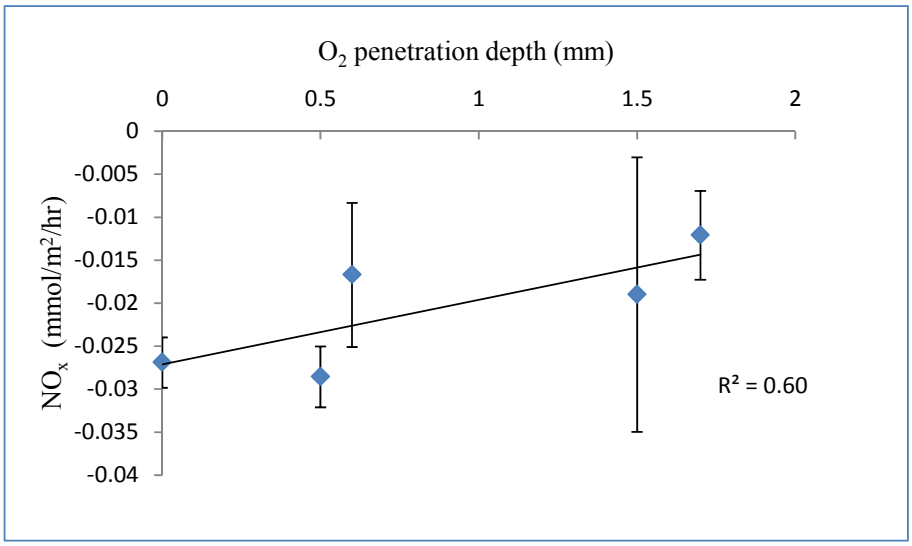

Figure 3.2. Relationship between O_2 penetration depth and sedimentary NO_x consumption rate

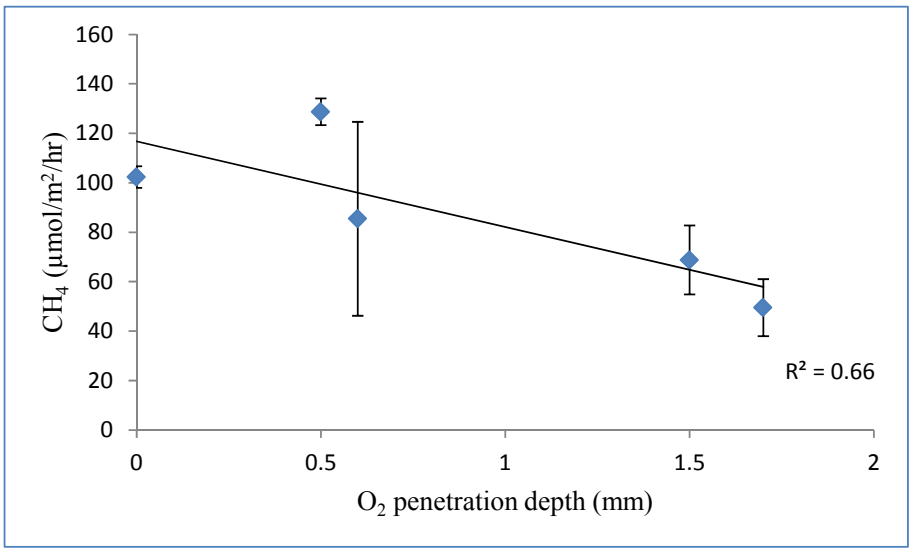

Figure 3.3. Correlation between sedimentary CH_4 flux and O_2 penetration depth

3.3.3. Core incubation

N_2O production/consumption rates from the core incubations, over the whole experimental period, varied from -9 (±3) to 0.5 (±0.1) μmol/m^2/hr and -5 (±2) to 0.8 (±0.3) μmol/m^2/hr in the shallow and deeper sediments respectively, with the exception of a distinctive peak (77 ± 9, 25 ± 9 and 19 ± 10 μmol/m^2/hr in DS, DM and DN respectively) in July 2011 for all sites (Fig 3.1d). *In situ* water column N_2O concentrations (Fig 3.1c) closely correlated with the flux rates reaching the highest in the same month (July 2011). In July 2011, the average bottom water N_2O concentration (1.5 ± 0.6 μM) was lower than that of the surface water (3 ± 2 μM).

Bottom water % DO saturation varied between 0-100% in the shallow region but the highest value observed in the deeper region was 74% (Table 3.1). Sediment Oxygen Consumption (SOC) rates varied linearly (R^2 = 0.56, p = 0.004, n = 30) with overlying water column oxygen saturation. The highest SOC was observed in May 2011 with values -3.4 (±0.1), -1.5 (±0.3) and -2.2 (±0.3) mmol/m^2/hr in DS, DM and DN respectively just after the period of anoxia (Fig 3.1f). During the July sampling, the highest sedimentary denitrification rates were found: 211, 61 and 203 μmol/m^2/hr at DS, DM and DN respectively (Fig. 3.1e). However, no significant relationships between either N_2O production or denitrification and environmental variables other *in situ* environmental variables i.e. temperature, %DO saturation and pH were identified.

The sedimentary CH_4 fluxes varied greatly among the sites throughout the sampling period (Fig 3.1g). CH_4 generation increased gradually during the summer when the overlying water was completely or very close to (< 16 % sat) anoxic conditions (Table 3.1). The highest flux at DS was 70 (±40) μmol/m^2/hr whereas 170 (±70) and 210 (±40) μmol/m^2/hr were observed

at DM and DN respectively (Fig 3.1g). The highest fluxes observed were associated with the bottom water temperature maxima (21 and 15 ^{0}C in shallow and deeper sites accordingly, Table 3.1). CH_4 production correlated negatively ($R^2 = 0.31$, $p < 0.01$, $n = 30$) with the % DO saturation in the overlying water at all sites (Fig 3.4). The average surface water CH_4 concentration at all sites was found to be 0.34 ± 0.18 µM.

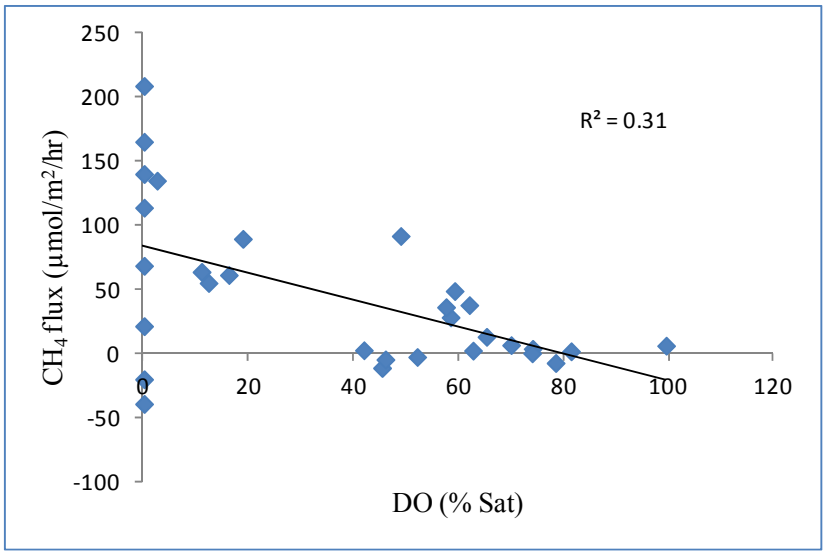

Figure 3.4. Correlation between bottom water % O_2 saturation and CH_4 flux. n = 30.

3.3.4. Benthic macrofauna

Two different benthic macrofauna, Chironomids and Oligochaetes, were identified during the sampling period. They were classified as larvae (*Chironomidae*) and worms (*Oligochaeta*). The prolonged anoxic conditions from Dec 2010 to Apr 2011 suppressed the abundance of Chironomids and Oligochaetes at the deeper sites to a greater extent than at the shallow site. Upon the water column overturn in May 2011, and the resultant hypolimnetic reoxygenation, the overall faunal abundance started increasing and reached the highest density in May 2011: 7000 ± 3000 and 15000 ± 8000 ind/m^2 for Chironomids and Oligochaetes respectively at DS

(Fig 3.5a). No significant correlation was observed between monthly GHG fluxes and faunal abundance.

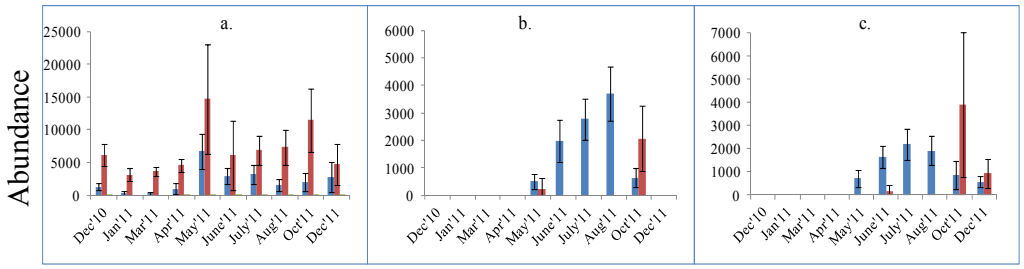

Figure 3.5. Monthly variation of Chironomids ( ) and Oligochaetes ( ) abundance ± SD at a) DS, b) DM and c) DN. Note the different Y-axis range in each figure.

3.4. Discussion

3.4.1. Gas Fluxes

3.4.1a. N_2O flux

The optode experiment showed that low O_2 concentrations favour N_2O production. This observation is consistent with other studies on factors controlling N_2O production (de Bie *et al.* 2002; Jørgensen *et al*. 1984). Low O_2 concentrations enhance denitrification activity which is one of the two major pathways for N_2O production (Jørgensen *et al*. 1984). With low O_2 availability in the overlying water column, O_2 penetration depth in the sediment decreases (Table 3.2) which reduces the distance NO_x (both NO_3^- and NO_2^-) has to diffuse from the overlying water into the anoxic zone of the sediment. Under such conditions, denitrifying bacteria consume NO_x as an electron acceptor for their metabolic activity and reduce NO_x to N_2 via denitrification which contributes to the production of N_2O (Chapter 1, Fig 1.1). The gradual decrease in the N_2O flux after reaching the peak (1.9 ± 0.6 µmol/m^2/hr)

at 25% O_2 saturation with further decreasing O_2 level was possibly due to the decreased NO_x concentration in the water column (Table 3.2).

Monthly sedimentary N_2O production along with the water column N_2O concentration were negligible (< 0.8 μmol/m^2/hr and <0.17 μM respectively), except for the peak in July 2011. The highest production of N_2O in the sediment (77 μmol/m^2/hr) and the highest concentration in the water column (3.3 μM) were observed at about 60-80% O_2 saturation. Nitrification in fresh water sediments is generally O_2 limited (Sweerts *et al.* 1991) and denitrification depends on either sedimentary NO_x produced by nitrification or NO_x diffused from the overlying water column. Thus, it is apparent that O_2 is the key driver of sediment N_2O generation (Liikanen and Martikainen 2003) and the availability of O_2 is necessary for N_2O release (Jensen *et al.* 1993; Clark and Rosswall 1981). The anomalous peak in N_2O production in July 2011 coincided with the highest sedimentary denitrification rate and water column NO_x and NH_4^+ concentration maxima. The high concentrations of dissolved nutrients most likely originated from material washed out of the upper catchment during the intense rainfall (53 mm, data collected from a weather station set up within the farm boundary) during the July 2011 sampling. The water column turbidity also reached the highest value (145 ± 6 NTU) at the same time. Greater NO_x availability is probably the reason for enhanced sedimentary N_2O production via incomplete denitrification (Chapter 1, Eq. 1.2) (Blackmer and Bremner 1978).

The highest bottom water (0.5 m above the sediment-water interface) N_2O concentrations were found at the same time (July 2011) and were similar at DS, DM and DN (1.2 ± 0.6 μM), even though the sediment production rates differed between sites. This similarity is attributed

to the thorough mixing of the water column within the dam resulting from high rainfall events. Similar values of turbidity (140, 142 and 152 NTU in the overlying water column at DS, DM and DN respectively, Table 3.1) and similar concentration of NH_4^+ and NO_x in the surface and bottom water throughout the sites (i.e. DS, DM and DN) also supported the mixed water column condition at the same time. In July 2011, surface water (0.1 m below the air-water interface) N_2O concentrations were almost two times greater than that of the bottom water, most likely due to water column nitrification. This conclusion is supported by a number of estuarine studies, where water column nitrification has been attributed to the nitrifiers attached to tidal-flood introduced, suspended particulate matter (Helder and De Vries 1983; Somville 1978). Owens (1986), in his study of the water column nitrification in Tamar river estuary, south-west England, demonstrated that the highest nitrification in the water column always coincided with the turbidity maximum, which in turn was associated with the highest abundance of NH_4^+ oxidising bacteria. In a study of intertidal salt marsh and mudflats of river estuaries, Kenny *et al.* (2004) found a significant positive correlation between N_2O production and flood water NO_3^- concentration and sediment NH_4^+ concentration. They pointed out that various processes such as water column nitrification and coupled nitrification-denitrification in the sediment possibly occurred at the same time resulting in higher N_2O production.

A water depth dependence of sediment N_2O production was also observed in this study. The production rate was almost three times higher at the shallow site than the deeper sites (Fig 3.1d). This observation is in a good agreement with the findings of Martikainen *et al.* (2002) and Wang *et al.* (2006). Variability in sediment structure and the presence of benthic flora and fauna might be some of the reasons contributing to the difference as suggested by Martikainen *et al.* (2002) and Wang *et al.* (2006), but further study is required to elucidate the factors.

In this study, the relative abundance of the benthic animals (both Chironomids and Oligocheates) was higher in the shallow site than the deeper sites in July 2011 when highest N_2O fluxes were measured at all sites. No significant relationship was found between N_2O production and temperature but the highest production was observed at a hypolimnetic temperature of 9 ^{0}C when % DO saturation in the overlying water varied between 60-80% among sites. This result shows that the low temperature favours N_2O production when O_2 is available, as described by Liikanen *et al.* (2002). At low temperature, O_2 availability in the overlying water column increases O_2 penetration depth into the sediments resulting in increased coupled nitrification-denitrification (Liikanen *et al.* 2002). Consequently, N_2O production rate in the sediment environment increases as N_2O is produced both through nitrification and denitrification processes (Chapter 1, Fig 1.1).

3.4.1b. CH_4 flux

In this study, availability of O_2 in the overlying water column appeared to be an important factor controlling sedimentary CH_4 generation as well as N_2O production. Both the optode study and the monthly core incubations yielded the highest CH_4 flux when the hypolimnion had very low or no O_2. This observation is consistent with other studies which demonstrated O_2 as a key variable influencing CH_4 production in fresh water ecosystems (Liikanen *et al.* 2002; Huttunen *et al.* 2003; Allen *et al.* 2011). It is believed that O_2 availability inhibits methane production (Liikanen and Martikainen 2003) by decreasing bacterial (methanogenesis) activity and/or accelerates oxidation of produced CH_4 by CH_4 oxidizing bacteria (methanotrophy) (Sweerts *et al.* 1991; Kiene 1991). In a laboratory microcosm experiment on eutrophic lake sediment, Liikanen *et al.* (2003) observed a positive flux of CH_4 despite a well oxygenated overlying water column and explained that the thickness of the aerobic sediment layer was insufficient to oxidize all of the CH_4 produced in the anoxic sediment layer. The flux data from the optode experiment agrees well with that observation.

In this experiment, the production rate of CH_4 in the sediment was negatively correlated to the O_2 penetration depth at varied overlying O_2 saturations.

Monthly core incubation data were more dynamic and revealed strong seasonal variability in sedimentary CH_4 production. At DS, a positive flux of CH_4 was observed until May 2011 when the hypolimnion became reoxygenated after water column overturn. The production of CH_4 under such conditions was most likely due to the continuous supply of fresh organic matter from the inflow of the connected stream draining the upper catchment (after a rainfall event of 22 mm). But the inflow was not strong enough to drive allochthonus input to the other two sites which experienced longer anoxia than DS (Table 3.1). Casper (1992) described increased CH_4 production with increasing lake eutrophication in fresh water sediments. A positive correlation between CH_4 production and availability of fresh organic matter to the sediment was also reported by Kelly and Chynoweth (1981). Sedimentary CH_4 flux at DM and DN increased gradually during the period of stratification and was almost 4 times greater than that at DS until Mar 2011. In April and May 2011 negative fluxes (consumption) of CH_4 were observed at the deeper sites. It is possible that due to the prolonged anoxia and lack of external C sources, CH_4 is being used as an alternative source of C for anaerobic metabolism processes such as denitrification (Raghoebarsing *et al.* 2006). Bastviken *et al.* (2003) demonstrated that CH_4 was a significant source of C for pelagic food webs in an extensive microbial study of a Swedish lake. In eutrophic lakes during stratification, an oxic-anoxic interface can typically develop at the thermocline above the sediment-water interface. In this common circumstance, CH_4 oxidation can be profound in the presence of methane oxidizing bacteria (MOB). The MOB can then potentially be consumed by benthic and pelagic zooplankton as a food source (Jones and Grey 2011). The sediment CH_4 flux was insignificant at all sites during the well mixed water column period

(June-August 2011). The water column NO_x concentration increased at the same time. In this study, a weak, but statistically significant, negative trend between overlying water NO_x concentration and sedimentary CH_4 flux ($R^2 = 0.25$, $p < 0.01$, $n = 30$) was observed. Production of CH_4 can be inhibited by NO_3^- (Stadmark and Leonardson 2005; Liikanen *et al.* 2002; Le Mer and Roger 2001). Methanogenesis usually takes place under low redox potential ($E_h < 200$ mV) but the presence of NO_3^- increases the redox potential and thus restricts CH_4 production (Le Mer and Roger 2001). Dodla *et al.* (2009) also found a negative effect of NO_3^- concentration on CH_4 production in a prolonged microcosm study of a fresh water marsh soil and explained the fact that the inhibitory effect was due to the increased toxicity on methanogens produced by the derivatives (NO_2, NO, N_2O) of NO_3^- reduction process under anaerobic conditions in the sediment.

During the period of stratification (Dec 2010 – Mar 2011) the surface water CH_4 concentrations were about 21, 53 and 60 times lower than that of the bottom water at DS, DM and DN respectively (Fig 3.6).

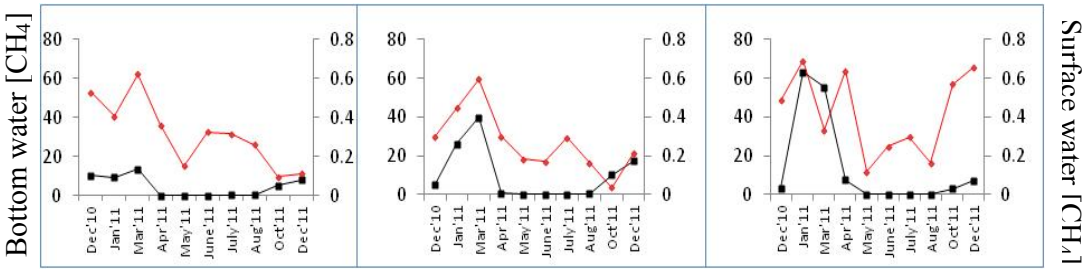

Figure 3.6. Monthly variation of surface (—■—) and bottom (—■—) water CH_4 concentration at DS, DM and DN.

The diminished surface water CH_4 concentrations were most probably a consequence of the oxidation of diffused CH_4 from the bottom layer at the oxic/anoxic boundary in the stratified water column (Stadmark and Leonardson 2005; Liikanen 2002; Kiene 1991). Under the well-

mixed water column (June –Aug 2011) resulting from the autumn lake overturn (May 2011), the concentration difference became negligible with an average of 0.27 ± 0.06 μM and 0.25 ± 0.05 μM in the bottom and surface water respectively throughout the sites. This was most likely due to the low sedimentary production as well as the shift in the oxic/anoxic boundary layer from the water column down to the sediment-water interface where most of the diffused CH_4 was oxidized. CH_4 oxidation can be an important pathway to offset atmospheric CH_4 emission in freshwater environments. Boon and Lee (1997) demonstrated that in sediments of a floodplain wetland in south-eastern Australia, the rates of CH_4 oxidations were almost an order of magnitude greater than the sedimentary CH_4 production rates. They credited methanotrophic bacteria for the reduction of net CH_4 flux to the atmosphere via CH_4 oxidation process. In a review, Whalen (2005) also reported 20 to 40% reduction in diffusive CH_4 flux in wetland soil caused by the oxidation of CH_4 in the rhizosphere and in the surficial oxic soil layer. However, Studies have shown that ebullition is an important process for CH_4 to escape from the sediment to the atmosphere (Huttunen *et al.* 1999) and that the ebullition rate is closely related to the production of CH_4 (Kiene 1991). This pathway of CH_4 emission is important in that it avoids loss by oxidation and/or consumption in the surface sediment and water column and thus enables emission of CH_4 directly to the atmosphere. Casper (1992) observed 96% CH_4 emission by ebullition from a small hypertrophic lake in northern England and explained that this was due to the low solubility of CH_4 compared to other dissolved gases (CO_2). In another study, Liikanen *et al.* (2003) reported ebullition was the major pathway of CH_4 emission from a shallow eutrophic lake (Lake Kevätön in Finland) contributing up to 84% of total CH_4 flux. Bubble formation was observed in the experimental cores and also *in situ* while sampling, particularly during the period of stratification but measurement and analysis of ebullition was not done in the present study. Measurement of ebullition has traditionally been done by capturing bubbles by deploying chamber or funnels

at the sediment-water or air-water interface (Bastviken *et al.* 2004; Duchemin, 1995). The major advantage of the technique is the simplicity of data capture. In contrast, the main disadvantages include, this only covers very small area (< 1 m^2) thus provides limited information regarding the spatial variability of ebullition and the time required for incubation (> 3 h) (DelSontro *et al.* 2011; Grinham *et al.* 2011). Several studies have demonstrated that a hydroacoustic (sonar) technique can be employed for the precise identification of the ebullition zone in the water bodies enabling real time quantification of ebullition volume and fluxes (Ostrovsky *et al.* 2008; DelSontro *et al.* 2011). However, the main limitations of the hydroacoustic technique include the requirement for a minimum water depth of at least 10 m and that it cannot discriminate the volume ratio of CH_4 in the bubbles which can vary significantly as demonstrated by Casper *et al.* (2000). To overcome the limitations, recently Grinham *et al.* (2011) developed a novel method using a survey grade Optical Methane Detector (OMD) combined with an Autonomous Surface Vessel (ASV). They demonstrated that depending on the wind speed, the OMD method covered about 7800 m^2 in 2.5 h and was very effective in determining CH_4 ebullition rates at fine spatiotemporal scale and quantifying atmospheric CH_4 emission from water bodies.

In this study, average summertime CH_4 production was about 2.5 ± 1.7 mmol/m^2/day. Assuming 84% CH_4 (based on percentage reported by Liikanen *et al.*, 2003) production is by ebullition, it was estimated that 13 mmol/m^2/day would be yielded from ebullition. Moreover, Liikanen *et al.* (2003) demonstrated that under hypolimnetic anoxia during summer stratification, sedimentary CH_4 flux was similar to the *in situ* atmospheric emission from the air-water interface and credited ebullition to explain this fact. Assuming ebullition is the primary pathway of CH_4 emission and that the ebullition rate is similar to the sedimentary production (Kiene 1991), estimation of atmospheric CH_4 emission (by ebullition) in this study (13 mmol/m^2/day) falls within the range of other studies conducted in fresh water

ecosystems in temperate and boreal regions. Based on the above assumption, annual CH_4 and N_2O emission (considering an average emission rate of 3.7 μmol/m^2/hr from the study period) were found to be 76 and 1.4 g/m^2/year respectively which are higher than for Australian (3.1 g CH_4/m^2/year and 0.02-0.12 g N_2O/m^2/year) and worldwide (2.7 g CH_4/m^2/year and 0.07 g N_2O/m^2/year) mangrove wetlands systems (Page and Dalal 2011) (Table 3.3). The estimated CH_4 emission was similar to the Australian floodplain wetland (70 g/m^2/year) and about 5 times higher than worldwide saltmarsh (14 g/m^2/year) system emissions (Page and Dalal 2011). In a recent comparison of CH_4 emissions from different aquatic systems, Ortiz-Llorente and Alvarez-Cobelas (2012) presented emission rates (3-43 gm/m^2/year) from stratifying lakes around the world, which is lower than the emission (76 gm/m^2/year) from the stratified farm dam observed in this study (Table 3.3).

Table 3.3. Comparison of N_2O and CH_4 emission rates. Values are overall means or seasonal means from individual studies

Source	N_2O	CH_4	Reference
	g/m^2/year	g/m^2/year	
Farm dam, Victoria, Australia	1.4	76	This study
Mangrove Wetlands, Australia	0.02 - 0.12	3	Page and Dalal (2011)
Mangrove Wetlands, Worldwide	0.07	2.7	Page and Dalal (2011)
Lakes Kevätön, Finland	0.12	46	Liikanen and Martikainen (2003)
Lakes Postilampi, Finland		21 - 28	Huttunen *et al.* (2003)
Lake Lokka, Finland	0.3 - 2.1	17 - 70	Huttunen (2002)
Stratifying lakes, worldwide		3 - 43	Ortiz-Llorente and Alvarez-Cobelas (2012)
Peatland, Michigan, USA		67 - 77	Shannon and White (1994)
Riparian Marshes		28	Altor and Mitsch (2006)
Lake Morris, North America		16	Bastviken *et al*. (2004)
Taihu Lake, China	0.13 - 3.7		Wang *et al.* (2006)
Lake Lacamas, USA	0.07 - 0.82		Deemer *et al.* (2011)

Due to limited information on the surface area of Victorian farm dams, the volume to surface area ratio of these dams was assumed to be the same as the studied dam. Hence, considering the average CH_4 and N_2O emission rates of 76 and 1.43 g/m^2/year (from this study) respectively along with the recent estimation of the total farm dam water volume within

Victoria (1291600 ML), it is estimated that farm dams throughout Victoria could contribute about 984 Gg CO_2e-CH_4 (CO_2 equivalents as CH_4) and 220 Gg CO_2e-N_2O (CO_2 equivalents as N_2O) to the atmosphere every year (CO_2e-CH_4 and CO_2e-N_2O are calculated based on the GWP of the respective gases compared to CO_2 for a 100 year time horizon; (Forster *et al*. 2007)) . This is approximately 10% CO_2e-CH_4 and 7% CO_2e-N_2O of the emissions from agricultural systems in Victoria (based on the National Greenhouse Gas Inventory, 2010)

3.4.2. Faunal abundance

The abundance of benthic larvae and worms varied greatly throughout the sampling period. Both of these species were less abundant at the deeper sites than at the shallow site, most probably due to prolonged anoxia and lack of fresh food sources (detrital organic matter settling down through the water column to the sediment surface; Jónasson, 2004). Faunal contribution towards nitrification, denitrification and consequently N_2O emission has extensively been studied (Altmann *et al.* 2004; Svensson *et al.* 2001; Drake and Horn 2006; Svensson 1998). In a laboratory microcosm study, larval abundance has been shown to increase N_2O production due to incomplete gut denitrification (Stief *et al.* 2009). Svensson *et al.* (2001) demonstrated a positive correlation between worm (Oligochaetes) biomass and sediment nitrification and denitrification. However they also reported that at similar biomass, larvae (Chironomids) are more effective in transporting NO_x into deeper sediments than worms (Oligochaetes) due to different grazing and burrowing behaviour. In the present work neither the larvae nor the worms showed a relationship between density and N_2O production (Fig 3.7a). An extremely weak but statistically significant negative correlation between larval abundance and sedimentary CH_4 production, however, indicated decreased CH_4 production at higher larval abundance ($R^2 = 0.18$, $p < 0.05$, $n = 30$, Fig 3.7b). This observation supports the findings of Jones and Grey (2011), who reported larvae (Chironomid) contributed to

transport of biogenic CH_4 in freshwater food webs due to their distinct grazing behaviour on methane-oxidising bacteria (MOB). It has been shown that CH_4 oxidation rate and MOB abundance were higher within Chironomid larval burrows in flooded rice paddies than in the surrounding soil environment (Kajan and Frenzel 1999). Thus Chironomid burrows can provide localised hotspots of MOB production where the MOB can then be exploited by the larvae as a food source (Jones and Grey, 2011).

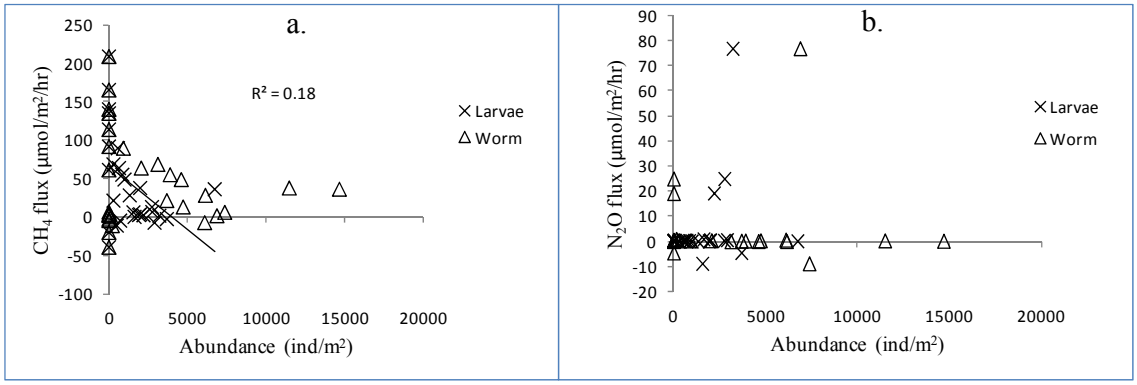

Figure 3.7. Correlation between animal abundance and sedimentary fluxes of: a) CH_4 and b). N_2O

3.5. Conclusion

Farm dams are hydrologically dynamic, aquatic systems, where stochastic rainfall events and seasonal stratification result in complex biogeochemistry, which is also influenced by benthic invertebrate density. Such impoundments are an integral part of most agricultural systems but have been largely ignored in GHG dynamics studies worldwide and particularly in Australia. The present study is the first attempt to quantify GHG emission from one of the several thousand farm dams in Victoria, Australia. This study found that farm dams can be a major source of N_2O when high NO_x (72 ± 3 µM) and NH_4^+ (16 ± 4 µM) concentrations in the water column coincide with oxic water column conditions (71 ± 9 %DO sat). A depth

dependence of sediment N_2O production was also observed in this study. No significant relationship was found between N_2O production and temperature but the highest production was observed at a hypolimnetic temperature of 9 ^{0}C when % DO saturation in the overlying water varied between 60-80% among sites. The production rate was almost three times higher at the shallow site (76 ± 8 μmol/m^2/hr, 1 m depth) than the deeper sites (22 ± 13 μmol/m^2/hr, 3.5 m depth). In contrast, the highest flux of CH_4 from the sediment was observed during the summer stratified period and at the deeper sites. The highest flux of CH_4 was associated with the bottom water temperature maximum (16 ^{0}C). The production rate of CH_4 was 3 times higher in the deeper site (210 ± 40 μmol/m^2/hr) than in the shallow site (70 ± 40 μmol/m^2/hr). According to both field sampling and laboratory experiments, sedimentary CH_4 flux was highly dependent on hypolimnetic oxic conditions. Prolonged anoxia favoured increased CH_4 production. In general, in this study farm dams were found to be a potential source of GHG contributing about 10% CO_2e-CH_4 and 7% CO_2e-N_2O of the state's (Victoria's) GHG emission from the agricultural sector. Benthic macrofauna did not have any significant effect on *in situ* N_2O production, but their involvement in cycling biogenic CH_4 within the system may be significant (due to their distinctive grazing activity on MOB) and this question warrants further research.

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Chapter Four

Factors controlling bioavailable nutrient fluxes across the sediment-water interface in an agricultural farm dam in south-eastern Australia

4.1. Introduction

Internal nutrient loading from sediment is considered an important source of bioavailable nutrients to freshwater and marine ecosystems (Qu *et al.* 2003; Nowlin *et al.* 2005; Thornton *et al.* 2007; Grenz *et al.* 2010; Green *et al.* 2012). Various biogeochemical activities (for example microbial decomposition, degradation and dissolution) at and below the sediment surface play a vital role in processing deposited material and ultimately contribute bioavailable products, particularly N and P species, to the water column (Grenz *et al.* 2010). Production and transformation of the nutrients are often controlled by physicochemical factors such as temperature, pH, redox condition, dissolved oxygen (DO) concentration, nutrient concentration gradient, sediment resuspension, presence of benthic algae, bioturbation and bioirrigation (Chowdhury and Bakri 2006; Spears *et al.* 2008; Biswas *et al.* 2009; Zhang *et al.* 2010; Ozkundakci *et al.* 2011; Roy *et al.* 2012). All of these processes can vary widely within and between ecosystems and it is therefore necessary to understand the site or system specific processes controlling chemistry of N and P to enable more effective nutrient management.

Microbially mediated degradation of organic matter results in the formation of NH_4^+ in the surface sediment layer, increasing the concentration of this species in the sediment pore water (Chowdhury and Bakri 2006). Under hypolimnetic anoxia - predominantly driven by stratified conditions - a significant proportion of sediment-derived NH_4^+ is susceptible to being released into the water column. This internal loading can substantially increase the risk of algal blooms even if the external input is minimal (Burger *et al.* 2007). In contrast, under oxic bottom water conditions, sedimentary NH_4^+ can be converted to NO_x via nitrification (Chapter 1, Equation 1.1) thus decreasing NH_4^+ efflux (Spears *et al.* 2008). The resultant NO_x can either be denitrified in an anoxic location within the sediment environment or will diffuse out to the water column (Chapter 1, Fig 1.1).

Denitrification generally occurs just below the oxic-anoxic interface in the sediment, utilising NO_x that has diffused from the water column and/or is produced within the oxic sediment environment via nitrification (Thornton *et al.* 2007). Transportation of NO_x from the overlying water column into the sediment mainly depends on the concentration gradient between the two regions. However, the diffusion can be restricted by an increase in the depth of O_2 penetration in the sediment (Rysgaard *et al.* 1995). Rysgaard *et al.* (1995) demonstrated a positive correlation between water column NO_x and denitrification using NO_x derived from diffusion (D_w) in a Danish estuarine sediment. They have also reported decreased sedimentary coupled nitrification-denitrification (D_n) activity under low concentrations of water column NO_x and NH_4^+ in summer and attributed this outcome to the presence of benthic microalgae competing with nitrifying and denitrifying bacteria for bioavailable nutrients. Qu *et al.* (2003) in their study of Lake Illawarra, Australia, also showed that macroalgal mats acted as a filter, reducing benthic nutrient flux to the water column.

Phosphorus dynamics in the freshwater environment is more widely studied than N for several reasons. For example, P management is much more easier and cost effective than N because of the more complicated nature of N dynamics, which has multiple oxidation states and important gas phase components, and N_2 fixation can provide algae with labile nitrogen which can make nitrogen control ineffective (Burger *et al.* 2007; Lewis and Wurtsbaugh 2008; Schindler *et al.* 2008). Factors controlling the exchange of P (mainly as filterable reactive phosphorus, FRP, which is commonly equated with orthophosphate, but see Dodds (2003a) who highlights that other forms of phosphorus also contribute to FRP) across the sediment-water interface (SWI) include desorption and dissolution of metal-bound P based on redox conditions (Moore and Reddy 1994), organic matter breakdown by microbial mineralization, steep concentration gradients (Pettersson 1998; Chowdhury and Bakri 2006) and bioirrigation and/or bioturbation (Rysgaard *et al.* 1995; Biswas *et al.* 2009). Varying

organic matter content of the sediment, along with spatially heterogeneous sediment characteristics, also influence P flux rates (Søndergaard *et al.* 2003; Nowlin *et al.* 2005). Redox potential and DO are the two most important factors controlling sediment P (as FRP) uptake and/or release mechanisms (Jensen and Andersen 1992; Søndergaard *et al.* 2003; Al Bakri and Chowdhury 2006). It is well established that during summer stratification, under elevated temperature and substantial organic matter loading, increased biological activity significantly decreases hypolimnetic DO and pH and eventually sediment redox potential (Nowlin *et al.* 2005). Under such conditions, insoluble Fe(III) is reduced to soluble Fe(II) and consequently Fe(III)-bound P (FRP) returns to the sediment pore water and then diffuse into the water column (Rozan *et al.* 2002; Grace *et al.* 2010; Carey and Rydin 2011). On the other hand, with a well mixed water column, an oxygenated hypolimnion promotes P binding to Fe(III) oxyhydroxides in the sediment. Despite Fe(III) acting as a sink for P, sedimentary FRP flux under well oxygenated water column has been documented (Krivtsov *et al.* 2001). This flux was attributed to the higher decomposition of organic matter under aerobic conditions producing localised reducing conditions resulting in metal bound FRP release (Jorgensen and Revsbech 1985). However, it has also been reported that the flux of P to the water column under oxic conditions is due to the extremely high external P loading overwhelming the sediment sorption capacity (Søndergaard *et al.* 2001; Jensen and Andersen 1992). It is also well established that availability of NO_x in the water column inhibits FRP release by increasing the redox potential of the surface sediment, preventing any substantial Fe(III) reduction (Hemond and Lin 2010). Benthic algae can be another factor affecting FRP concentrations and fluxes in bottom waters. A number of studies have shown that presence of epipelic algae (cyanobacteria and eukaryotic algae) decreased the concentration (from the water column and sediment) and sedimentary fluxes of FRP via a combination of oxic

precipitation with Fe(III) and direct sequestration by the benthic algal community (Woodruff *et al.* 1999; Dodds 2003b; Spears *et al.* 2008).

As noted in Chapter 3, macrofauna which rework the sediment, can influence the nutrient flux dynamics at the SWI through bioturbation and bioirrigation activities. Several studies have demonstrated the importance of such macrobiological activity in processing benthic fluxes of N and P in both marine and freshwater ecosystems (Rysgaard *et al.* 1995; Søndergaard *et al.* 2003; Karlson *et al.* 2007; Biswas *et al.* 2009; Zhang *et al.* 2010). Chironomid larvae and Oligochaete worms are common species in eutrophic fresh water environments. They are commonly classified as gallery-diffusers (larvae) and upward-conveyors (worms) based on their sediment reworking activities (Zhang *et al.* 2010; Søndergaard *et al.* 2003). In a study of the impact of different benthic animals on P dynamics, Zhang *et al.* (2010) demonstrated Fe(II) and FRP concentrations in pore water were significantly decreased by larval (Chironomid) burrowing action whereas worms (*Tubificid*) didn't show such an effect. They also found increased O_2 penetration depth in the sediment arising from larvae rather than from worms and attributed this fact to the larval bioirrigation practice. In another study, Lewandowski *et al.* (2007) showed Chironomid larvae decreased pore water Fe(II), FRP and NH_4^+ concentrations and they observed a shift in the release pathway from the sediment surface to the burrow walls. The change in the release pathway was credited to the oxygenated burrow wall of Chironomid larvae, deep in the otherwise anoxic sediment, facilitating loss of NH_4^+ via nitrification and co-precipitation of FRP with oxidised Fe(III). In contrast, it has also been shown that larval activity can potentially increase sediment P release, particularly in Fe-deficient sediments (Phillips *et al.* 1994).

In Australia, the study of benthic, biogeochemical nutrient cycling has mainly focussed on riverine and coastal environments (Davis and Koop 2006) whereas inland water bodies such as lakes, reservoirs and farm dams have largely been overlooked. Recently, Grace *et al.* (2010) highlighted in their study of a polymictic lake in the Royal Botanic Gardens, Melbourne, the significant contribution of sediment FRP release (0.1-0.2 mmol/m^2/day) to the annual formation of a springtime toxic algal bloom and stressed that effective bloom management may require a >100 fold attenuation in water column P concentration. Al Bakri and Chowdhury (2006) in their study of a major water supply reservoir in New South Wales, obtained the highest FRP (0.2 mmol/m^2/day) and NH_4^+ (1.6 mmol/m^2/day) fluxes under summertime anoxic conditions and they demonstrated that temperature and DO were the prime factors controlling the flux. Farm dams (see definition in chapter 1, section 1.1) are integral parts of Australian agriculture, and cumulatively comprise a major portion of inland water bodies (Chapter 1, section 1.1). These impoundments often experience high nutrient and organic matter loading (for example from fertilizer and animal manure) during high rainfall and runoff events (Adams et al. 2014) and are susceptible to seasonal stratification and hypolimnetic anoxia under summer low flow conditions (Nowlin *et al.* 2005). Thus, detailed information is required on the dynamics of biogeochemical processes in these impoundments controlling the release of bioavailable nutrients to downstream receiving water bodies to facilitate more effective management practices.

This chapter presents results from a 12 month investigation of dissolved inorganic nutrient flux measurements (NO_x, NH_4^+ and FRP) across the sediment-water interface, and the factors influencing these flux rates, in a farm dam in a headwater, agricultural catchment in south-eastern Australia. Two approaches were taken to study the seasonal variation of nutrient fluxes and the factors controlling their uptake and/or release: i) monthly incubation of sediment cores in the laboratory to estimate sedimentary inorganic nutrient flux, and ii)

deployment of *in situ* dialysis samplers (pore water peepers) to study the temporal variation of vertical distribution of inorganic nutrient concentrations throughout the water column and surface layers of sediment.

4.2. Materials and methods

Detailed description of the sampling site, locations and procedures is presented in Chapter 2, sections 2.1 and 2.2. In brief, sediment cores were collected monthly from 3 sites (DS, DM and DN, Fig 2.1) within the dam and incubated in the laboratory for nutrient flux (Chapter 2, Fig 2.3) and faunal abundance measurements over the period Sep 2010 to Dec 2011 (except Nov 2010 and Feb, Sep and Nov 2011). Cores were incubated at the same temperature and %DO saturation as measured in the bottom water at the time of core collection (Chapter 2, section 2.2.1a). In addition, denitrification rates were estimated using these cores from Jan 2011 to Dec 2011 (except Feb, Sep and Nov 2011). Water column (surface and bottom) samples for dissolved nutrients (NO_x, NH_4^+ and FRP) were collected every month from Sep 2010 to Dec 2011 (except Nov 2010 and Feb, Sep and Nov 2011). At the same time, water quality variables (i.e. temperature, DO, pH and turbidity) were also recorded using a calibrated Horiba U10 multiprobe (Chapter 2, section 2.2.3). In the cores, fluxes of nutrients from the sediment to the water column, sediment O_2 consumption rates (SOC) and sediment denitrification rates were calculated using equations 4.1-4.6 as described in Dalsgaard *et al.* (2000):

$$Flux = \frac{\alpha \times V}{A} \times 10{,}000 \qquad \text{Eq. 4.1}$$

where $\boldsymbol{\alpha}$ = slope of the linear regression of concentration (µmol/L for nutrients and mmol/L for DO) against time (hr); V = volume of water in the sediment core (L), A = sediment surface area in the core (cm^2). The denitrification rate was calculated from mass spectrometric analysis of the isotopic composition of N_2, more specifically from ratios of

$^{29}N_2$ and $^{30}N_2$ to total N_2. Finally, denitrification rates were estimated from the production of ^{15}N isotopes (Nielsen 1992):

$$D_{15} = P(^{29}N_2) + 2P\ (^{30}N_2) \qquad \text{Eq. 4.2}$$

$$D_{14} = \lfloor P(^{29}N_2) / 2P\ (^{30}N_2) \rfloor \times D_{15} \qquad \text{Eq. 4.3}$$

where D_{15} and D_{14} are the rates of denitrification of $^{15}NO_3^-$ and $^{14}NO_3^-$ respectively and P is the production rate of the respective paired N_2.

The proportion of D_{14} that was derived from water column NO_3^- (D_w) was calculated using the following equation (Dalsgaard *et al.* 2000):

$$D_w = \lfloor (^{14}NO_3)_w / (^{15}NO_3)_w \rfloor \times D_{15} \qquad \text{Eq. 4.4}$$

where $(^{14}NO_3)_w$ and $(^{15}NO_3)_w$ are the concentrations in the water column. Finally *in situ* coupled nitrification-denitrification (D_n) was calculated as:

$$D_n = D_{14} - D_w \qquad \text{Eq. 4.5}$$

And total denitrification (D) as:

$$D = D_w + D_n \qquad \text{Eq. 4.6}$$

Denitrification efficiency (DE) measured as the percentage of inorganic nitrogen (DIN=NO_x + NH_4^+) released from the sediment as N_2 gas during organic matter breakdown (Eq 4.7, as described in Eyer and Ferguson 2002):

$$DE = (N_2\text{-}N/(DIN + N_2\text{-}N) \times 100\%) \qquad \text{Eq. 4.7}$$

In situ dialysis samplers (pore water peepers) were deployed in Mar 2011 and Aug 2011 and sampled for NO_x, NH_4^+, FRP and Fe(II) from replicate peepers on both occasions (details of peeper preparation and deployment are given in Chapter 2, section 2.2.1b). All samples were preserved (see chapter 2.2.2a and Table 2.1) and analysed (chapter 2, Table 2.2) following standard procedures. The methods for measuring burrowing activity of Chironomid larvae and *in situ* O_2 images during the planar optode experiment are described in chapter 2, section 2.2.1c.

The denitrification rate calculation involved the addition of $^{15}NO_3^-$ tracer to the overlying water of the cores and the determination by isotope-ratio mass spectrometry (IRMS) of ^{15}N – labelled gases sampled in gas-tight vials by an ANCA GSL2 elemental analyser interfaced to a Hydra 20-22 continuous-flow isotope ratio mass-spectrometer (Sercon Ltd., UK). Statistical analysis involved ANOVA, the non-parametric Mann-Whitney *U* test, the student's *t*-test, multiple and simple linear regressions performed using Microsoft Office Excel 2007 and the statistical software SPSS version 20 (IBM® SPSS® Statistics 20) (Chapter 2, section 2.2.4).

4.3. Results

4.3.1. Variation in water column nutrient concentrations and physicochemical parameters

Spatial and temporal patterns were observed in temperature, %DO saturation, turbidity, pH, and dissolved inorganic nutrient concentrations (NO_x, NH_4^+ and FRP) in the surface and the bottom of the water column within the dam. Table 4.1 represents the monthly variation of mean dissolved inorganic nutrient concentrations (mean values obtained from the three sites, DN, DM and DS). At the surface, mean monthly NO_x concentration ranged from 0 - 86 (±3) µM compared with 0 - 72 (±3) µM at the bottom. NH_4^+ had a minimum concentration of 0.4

(±0.1) and a maximum of 11.4 (±0.5) μM at the surface and 0.4 (±0.1) - 15 (±4) μM at the bottom. As shown in Fig 4.1a, the surface water NO_x concentration was highest (86 ± 3 μM) in spring (Sep 2010 and Oct 2010), decreased to below detection (0.1 μM) during summer and early autumn (Dec 2010-Apr 2011) and gradually increased to 79 (±4) μM in winter (May 2011- Aug 2011). Fig 4.1a also shows that bottom water NO_x concentration followed the same temporal trend as the surface water.

Table 4.1. Monthly variation of mean ± 1SD surface and bottom water nutrient (dissolved) concentrations across the sampling sites. n = 3.

Month	NO_X µM		NH_4^+ µM		FRP µM	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
Sep 2010	58 ± 2		2.8 ± 0.3		0.8 ±0.3	
Oct 2010	86 ± 3	56 ± 12	5.2 ± 0.3	12 ± 5	1.0 ± 0.2	0.4 ± 0.1
Dec 2010	1.1 ± 0.3	2 ± 2	0.8 ± 0.1	12 ± 16	0.7 ± 0.2	0.8 ± 0.9
Jan 2011	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.1	4 ± 3	0.6 ± 0.0	19 ± 8
Mar 2011	0.3 ± 0.3	0.2 ± 0.3	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.0	24 ± 7
Apr 2011	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.3	1.0 ± 0.5	0.9 ± 0.1	19± 16
May 2011	22 ± 0	22 ± 0	6 ± 0	8 ± 0	0.4 ± 0.0	0.4 ± 0.1
Jun 2011	54 ± 4	53 ± 4	10 ± 0	10 ± 0	1 ± 0	0.6 ± 0.0
July 2011	79 ± 3	72 ± 3	11 ± 0	14 ± 3	2 ± 0	0.7 ± 0.0
Aug 2011	59 ± 0	60 ± 2	0.7 ± 0.2	5 ± 4	1 ± 0	0.4 ± 0.0
Oct 2011	15 ± 1	18 ± 2	8 ± 3	15 ± 4	1 ± 0	0.5 ± 0.1
Dec 2011	38 ± 0	35 ± 1	3 ± 1	14 ± 9	0.4 ± 0.1	0.2 ± 0.0

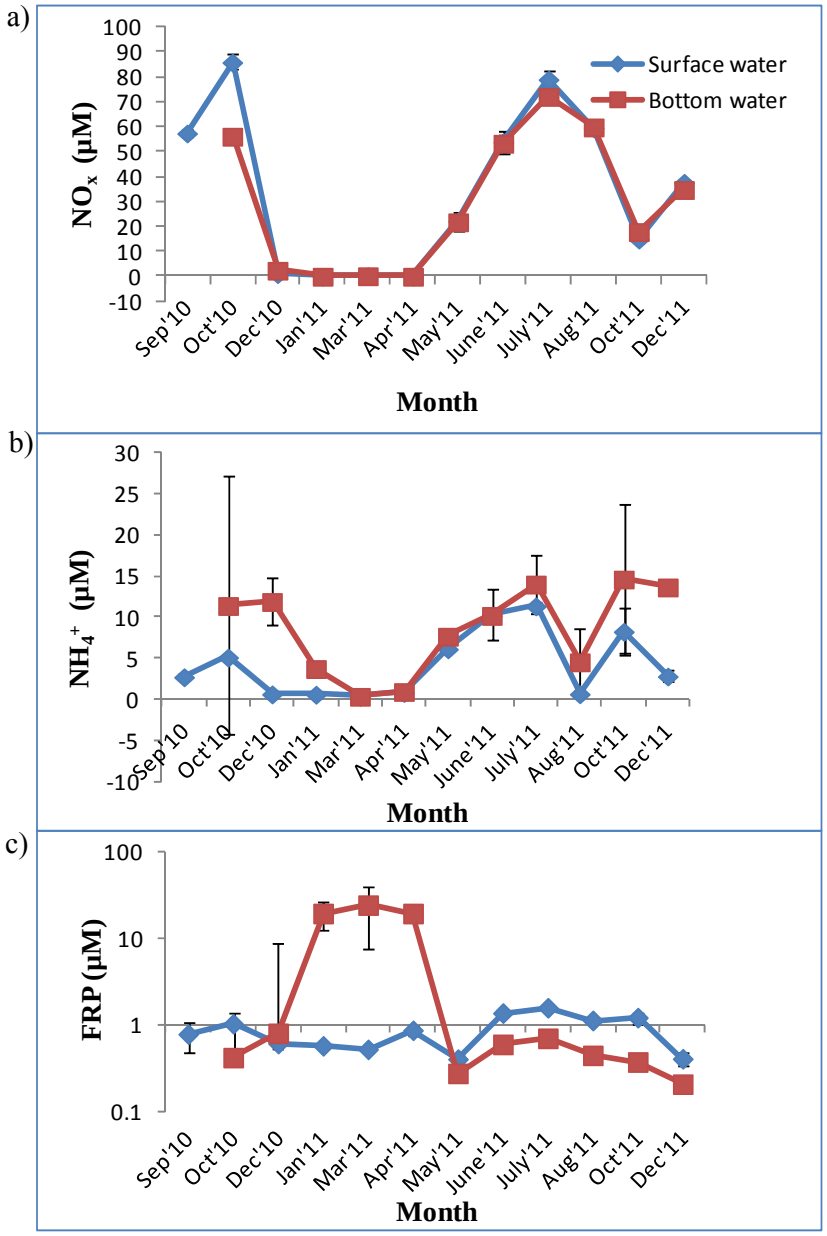

Figure 4.1. Temporal variation of mean ± 1 SD in surface and bottom water: a) NO_x, b) NH_4^+ and c) FRP concentrations across the sampling sites. n = 3

Bottom water NH_4^+ concentrations were higher than the surface water except for the months from Mar 2011 to Jun 2011 (Fig 4.1b). The highest concentrations of NH_4^+ were observed in late spring (Oct 2010-Dec 2010) and mid-winter (Aug 2011) in both the surface and bottom waters (Table 4.1). Surface water mean monthly concentrations of FRP ranged from 0.4

(±0.1) to 1.6 (±0.1) μM with higher values in winter and lower values in summer. Bottom water FRP concentrations were generally lower (0.2 ± 0.0 to 0.8 ± 0.9 μM) except for the months from Jan 2011 to Apr 2011 when the concentration values ranged from 19 (±8) to 24 (±7) μM.

Fig 4.2 illustrates the monthly variation of temperature, DO saturation and turbidity in both surface and bottom waters within the dam over the period Sep 2010 to Dec 2011. Between Sep 2010 and Apr 2011, surface and bottom water column temperature varied from 10.7 (±0.4) to 24.3 (±0.0) ^{0}C with moderate to strong thermal stratification (Fig 4.2a). During this period, the temperature difference between the epilimnion and the hypolimnion varied from 2-8 ^{0}C. For the remaining months (May 2011 to Aug 2011), the water column was mostly isothermal where the temperature ranged from 8.6 (±0.3) to 13.0 (±0.2) ^{0}C. The highest (24.3 ± 0.0 ^{0}C) and the lowest (8.6 ± 0.3 ^{0}C) temperatures were recorded in Jan 2011 and Jun 2011 respectively in the epilimnion of the dam. In the epilimnion, mean monthly DO saturation varied from 59.8 (±0.1) to 107 (±2) % compared with 0 to 75 (±3) % in the hypolimnion. During the stratified period (Sep 2010 to Apr 2011), the difference in %DO saturation across the thermocline increased from 34 % (Sep 2010) to 107 % (Jan 2011) and then gradually decreased to 10 % in May 2011, before the water column became completely mixed in Jun 2011 (Fig 4.2b).

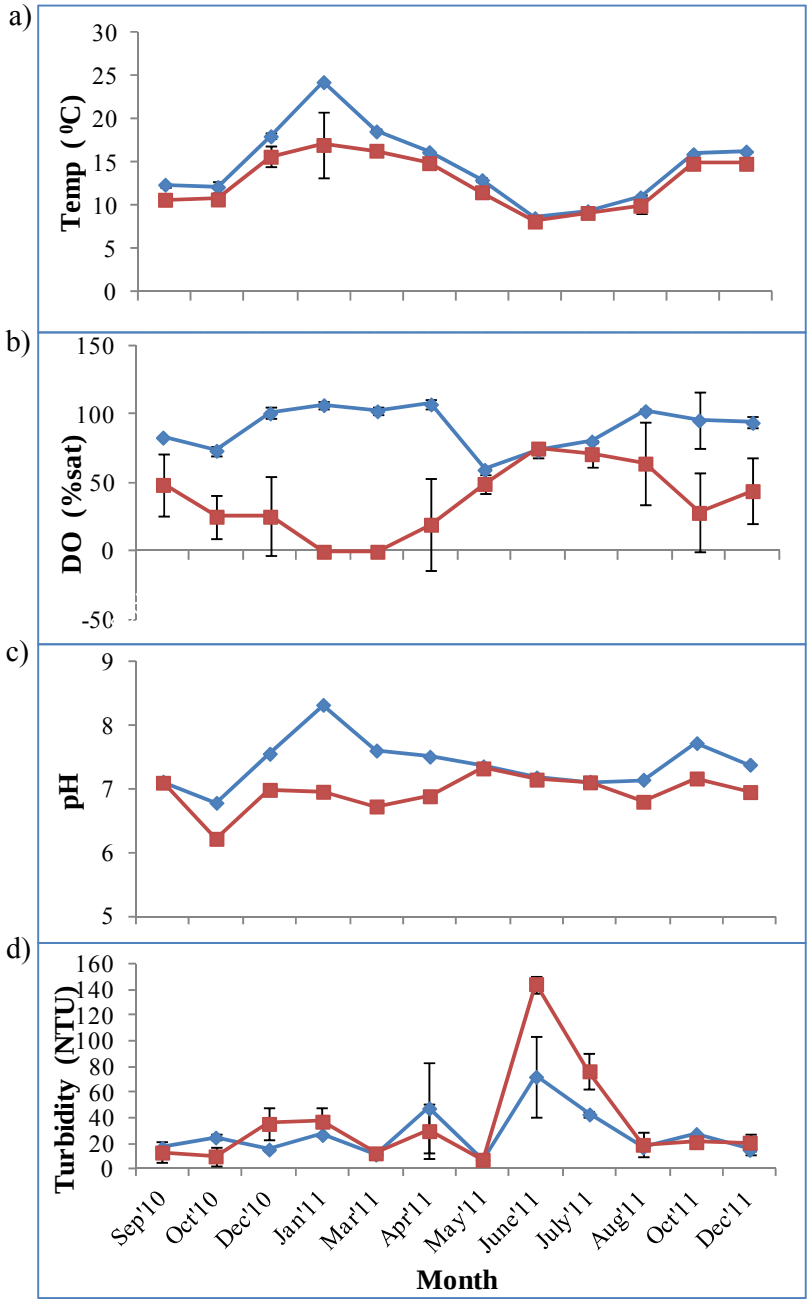

Figure 4.2. Monthly variation of: a) Temperature, b) Dissolved Oxygen Saturation, c) pH and d) Turbidity at the surface (—■—) and bottom (—■—) water column. Error bars reflect ± 1 standard deviation among sites, n = 3. For pH, error bars are too small to be seen

During the months corresponding to stratification, the bottom water pH was 0.6 – 1.4 lower than the surface water whereas the pH was consistently about 7.0 throughout the water

column in the months after water column mixing (Fig 4.2c). The highest value (8.3 ± 0.5) of pH was observed in Jan 2011. Water column turbidity varied at the surface from 8 (±1) to 70 (±30) NTU and at the bottom from 7.7 (±0.6) to 145 (±6) NTU (Fig 4.2d).

4.3.2. Sediment core incubation

The nutrient flux rates from the sediment cores incubated in the laboratory under conditions that best simulated real (*in situ*) conditions (temperature and % DO saturation) varied considerably and are presented in Table 4.2. In general there was uptake of NO_x from the overlying water by the sediment in cores from all three sites on all 12 sampling occasions, with the exception of the efflux (56 ± 3 μmol/m^2/hr) at DS in July 2011.

Table 4.2. Monthly variation of average benthic nutrient flux (μmol/m^2/hr) and SOC (mmol/m^2/hr) across all three sites, obtained from monthly core incubations. Error bars represent ± 1 SD, n = 3. A negative flux means net movement from the water column to the sediment.

Month	SOC	NO_x	NH_4^+	FRP
Sep 2010	-1 ± 0	-60 ± 40	-40 ± 60	-1 ± 0
Oct 2010	-0.1 ± 0.0	-280 ± 70	170 ± 80	-1 ± 0
Dec 2010	-0.5 ± 0.0	-105 ± 5	220 ± 20	4 ± 1
Jan 2011	0 ± 0	-2 ± 1	160 ± 20	6 ± 1
Mar 2011	0 ± 0	-12 ± 6	290 ± 20	15 ± 2
Apr 2011	-0.4 ± 0.0	0 ± 0	210 ± 20	5 ± 1
May 2011	-2 ± 0	-90 ± 10	140 ± 40	-60 ± 10
Jun 2011	-1 ± 0	-70 ± 40	-1 ± 13	-0 ± 1
July 2011	-1 ± 0	10 ± 20	30 ± 10	2 ± 1
Aug 2011	-1 ± 0	-80 ± 10	40 ± 10	-1 ± 1
Oct 2011	-0.5 ± 0.1	-40 ± 40	230 ± 40	-2 ± 1
Dec 2011	-0.8 ± 0.3	-70 ± 30	50 ± 20	0 ± 2

The NO_x flux varied from -179 (±6) to 56 (±3), from -200 (±40) to 4 (±30) and from -470 (±160) to 0 μmol/m^2/hr at DS, DM and DN respectively (Fig 4.3.a). The highest NO_x uptake

rates were measured in Oct 2010 at all sites when the hypolimnion was suboxic (26 ± 16 % O_2 saturation) with the highest uptake at DN (-466 ± 160 μmol/m^2/hr, Fig 4.2b and Fig 4.3a).

NH_4^+ fluxes were mostly positive (ammonia release to the water column) and were similar between sites except in Sep 2010 and Jul 2011 when net uptake was observed at the deeper sites (DM and DN, Fig 4.3b). The highest effluxes of NH_4^+ were measured in Mar 2011 and Oct 2011 with the highest rate of 320 (±30) μmol/m^2/hr at DS in Mar 2011. Generally, the fluxes were lower during winter (Jun 2011 – Aug 2011), corresponding to the period of a well-mixed water column (Fig 4.2a). FRP fluxes from the sediment into the water column were observed at all sites from Dec 2010 to Mar 2011 and ranged from 1.1 (±1.0) to 23 (±2) μmol/m^2/hr during that period (Fig 4.3c). Bottom water DO saturation and sediment oxygen consumption (SOC) were at the minimum during the same time (Fig 4.2b and 4.3d). A distinctive peak in sediment FRP uptake (ranging from -29.0 (±0.5) to -85 (±4) μmol/m^2/hr throughout the sites) was observed upon mixing of the water column in May 2011. SOC also peaked at the same time, with fluxes of -1.5 (±0.3) to -3.4 (±0.1) mmol/m^2/hr (Fig 4.3d).

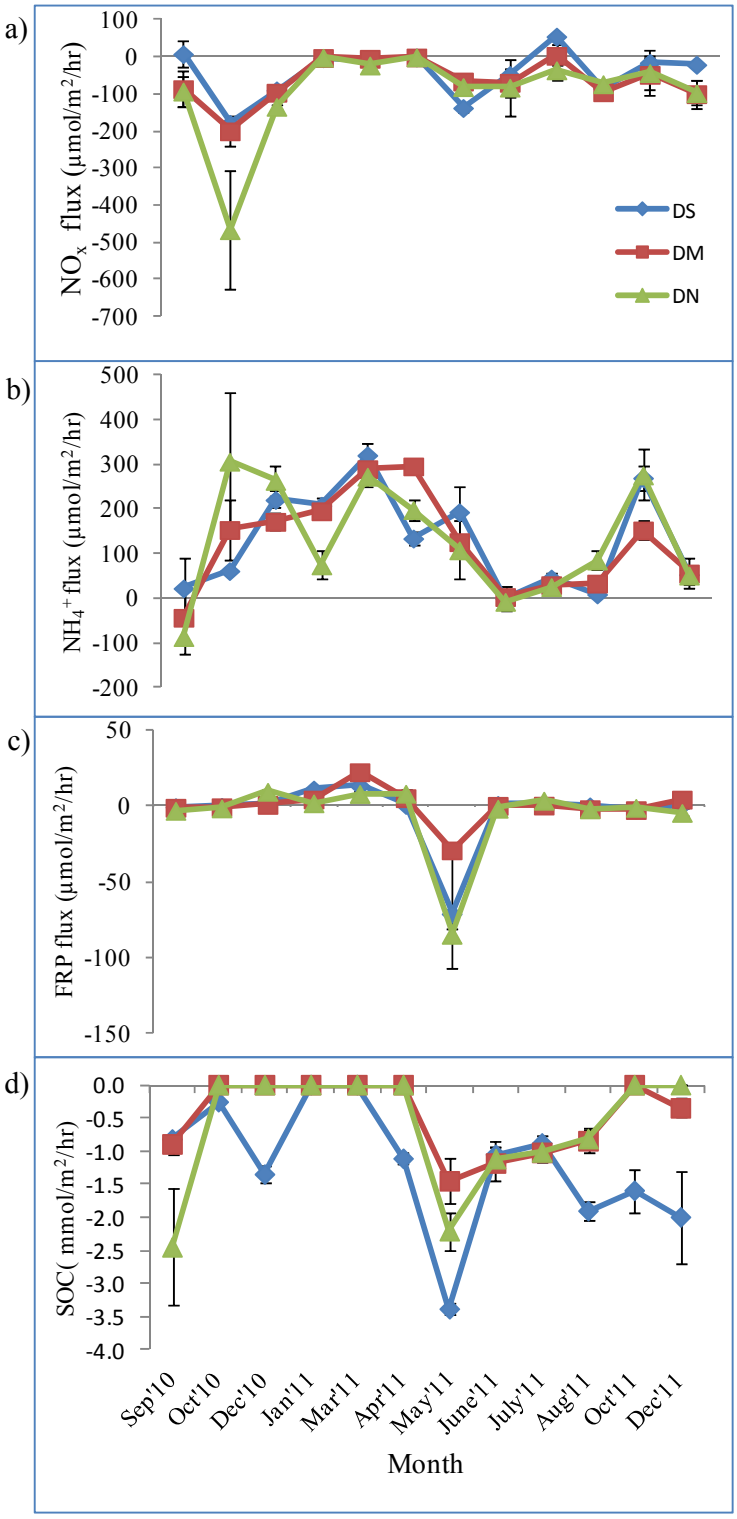

Figure 4.3. Seasonal variation of benthic nutrient fluxes at DS, DM and DN from monthly core incubation. Error bars represent ± 1 SE, n = 4

The mean monthly flux rates across the three sites are presented in Table 4.2. The monthly NH_4^+ fluxes were significantly positively correlated ($R^2 = 0.54$, $p = 0.006$, $n = 12$) with the bottom water temperature and negatively correlated ($R^2 = 0.66$, $p = 0.001$, $n = 12$) with %DO saturation. In contrast, sedimentary flux of NO_x and FRP did not show any relationship with these two parameters.

Monthly variation was observed in sediment denitrification rates both from the water column derived NO_x (D_w) and coupled nitrification-denitrification (D_n) (Fig 4.4). In general, total denitrification ($D_w + D_n$) was highest in late winter (Jul 2011 and Aug 2011) and lowest in summer and autumn (Jan 2011 to Apr 2011). Both D_w and D_n were significantly correlated ($R^2 = 0.76$, $p = 0.002$ and $R^2 = 0.65$, $p = 0.008$, $n = 9$ for D_w and D_n respectively) with water column NO_x concentrations. The average rates of D_w across all three sites were lower (0.2-0.7 μmol $N/m^2/hr$) from Jan 2011 to Apr 2011, started increasing from May 2011, reached a maximum of 83 (±11) μmol $N/m^2/hr$ in Aug 2011 and then decreased to 28 (±13) μmol $N/m^2/hr$ in Dec 2011 (Fig 4.4). D_n rates showed a similar temporal trend, reaching a maximum value (160 ± 80 μmol $N/m^2/hr$) in Aug 2011 and a minimum (1.6 ±0.1 μmol $N/m^2/hr$) in Apr 2011. Overall, the relative annual contributions of D_w and D_n to total sedimentary denitrification were 40% and 60% respectively. DE ranged from 2-90%, showing higher values in winter and lower in summer (Fig 4.5). DE was correlated positively to water column NO_x and %DO saturation ($R^2 = 0.86$ and 0.71 for NO_x and %DO sat respectively, $p = 0.01$, $n = 9$) and negatively to sedimentary NH_4^+ flux ($R^2 = 0.60$, $p = 0.01$, $n = 9$) (Table 4.3).

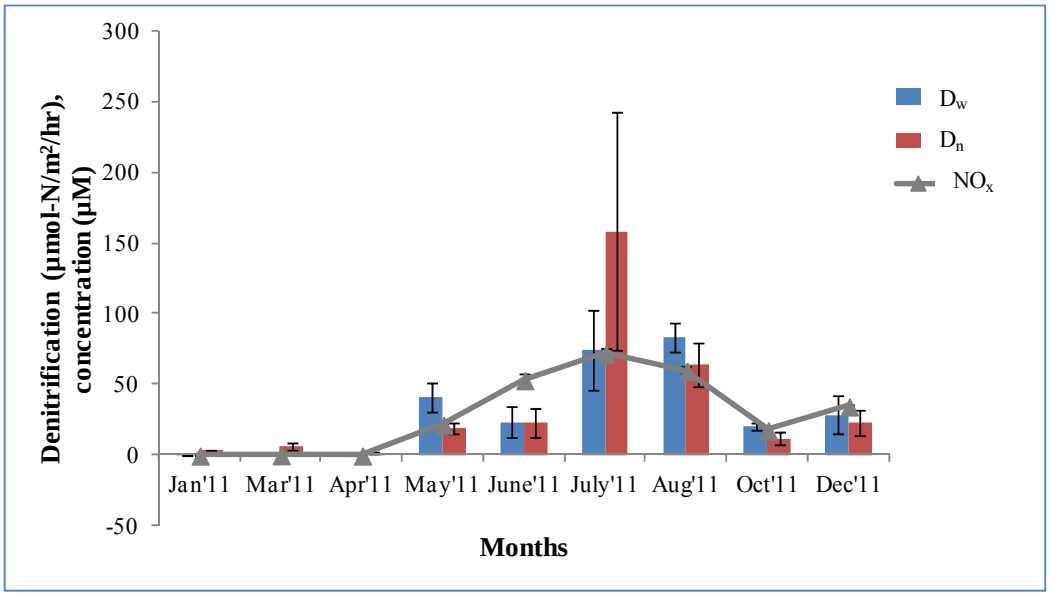

Figure 4.4. Temporal variation in denitrification rates (D_w and D_n) and bottom water NO_x concentration. Data obtained from monthly core incubations. Error bars represent ± 1 SD among sites, n = 3

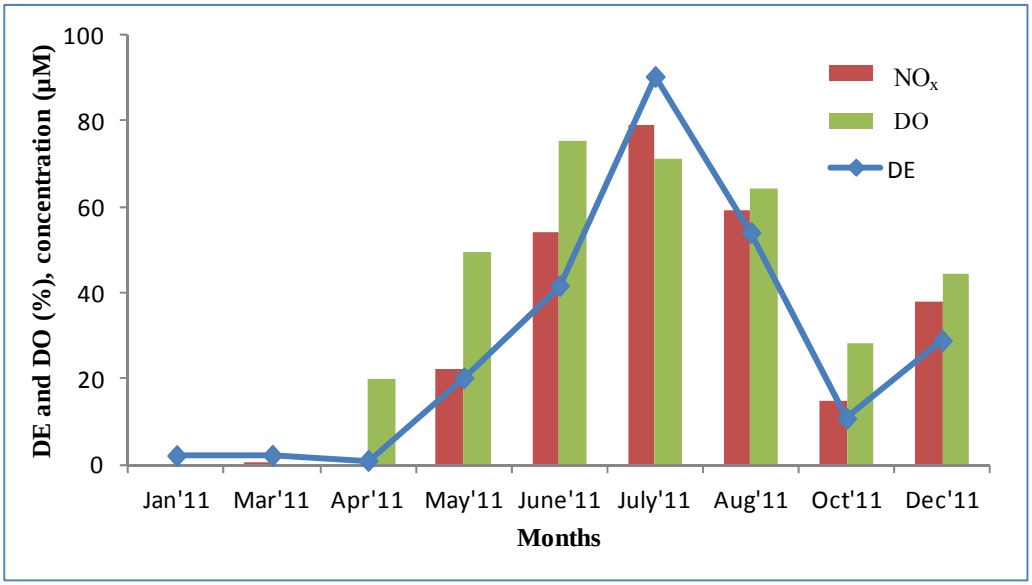

Figure 4.5. Temporal variation in denitrification efficiency (DE) and bottom water dissolved oxygen (DO) and NO_x concentration.

Table 4.3. Correlation coefficients between sedimentary nutrient fluxes and water quality variables in the study area (n = 12). Significant correlations are highlighted in bold

	NH_4^+ flux	NO_x flux	FRP flux	DE	Temp	DO	Turb	pH	$[NH_4^+]$	$[NO_x]$	[FRP]
NH_4^+ flux	1										
NO_x flux	-.011	1									
FRP flux	.109	.200	1								
DE	**-.776****	-.100	-.016	1							
Temperature	**.737****	.295	.272	**-.816****	1						
DO	**-.815****	-.049	-.302	**.840****	**-.877****	1					
Turbidity	-.424	.224	.187	.456	-.470	.524	1				
pH	-.116	.208	.389	-.181	.012	.007	.184	1			
$[NH_4^+]$	-.358	-.311	-.111	.524	-.324	.470	.230	-.187	1		
$[NO_x]$	**-.749****	-.296	.160	**.930****	**-.818****	**.741****	.428	.095	.471	1	
[FRP]	-.585	.145	.192	**.806****	**-.737****	**.622***	**.700***	.217	.249	**.761****	1

*P < 0.05 and **P < 0.01

The seasonal variation in larvae and worm numbers in the cores was shown in chapter 3, Fig 3.3. Average larval abundance throughout the three sites was significantly positively correlated ($R^2 = 0.84$, $p < 0.001$, $n = 9$) with bottom water %DO saturation whereas worm abundance did not show any such correlation ($R^2 = 0.05$, $p = 0.54$, $n = 9$). Figure 4.6a illustrates that sediment O_2 consumption (SOC) rate increased with increasing larval abundance ($R^2 = 0.72$, $p = 0.003$, $n = 9$) whereas sedimentary NH_4^+ flux decreased with increasing larval abundance ($R^2 = 0.50$, $p = 0.03$, $n = 9$). On the other hand, a positive relationship was observed between larval abundance and D_w ($R^2 = 0.72$, $p = 0.003$, $n = 9$) and D_n ($R^2 = 0.43$, $p = 0.05$, $n = 9$) (Fig 4.6b). It is important to note that multiple linear regression (MLR) analysis performed on the data set of Fig 4.6 resulted in statistically insignificant relationship ($R^2 = 0.90$ and $p = 0.07$, 0.38, 0.51 and 0.25 for the variables SOC, NH_4^+ flux, D_w and D_n respectively) among faunal abundance and SOC, sedimentary NH_4^+ flux and denitrification.

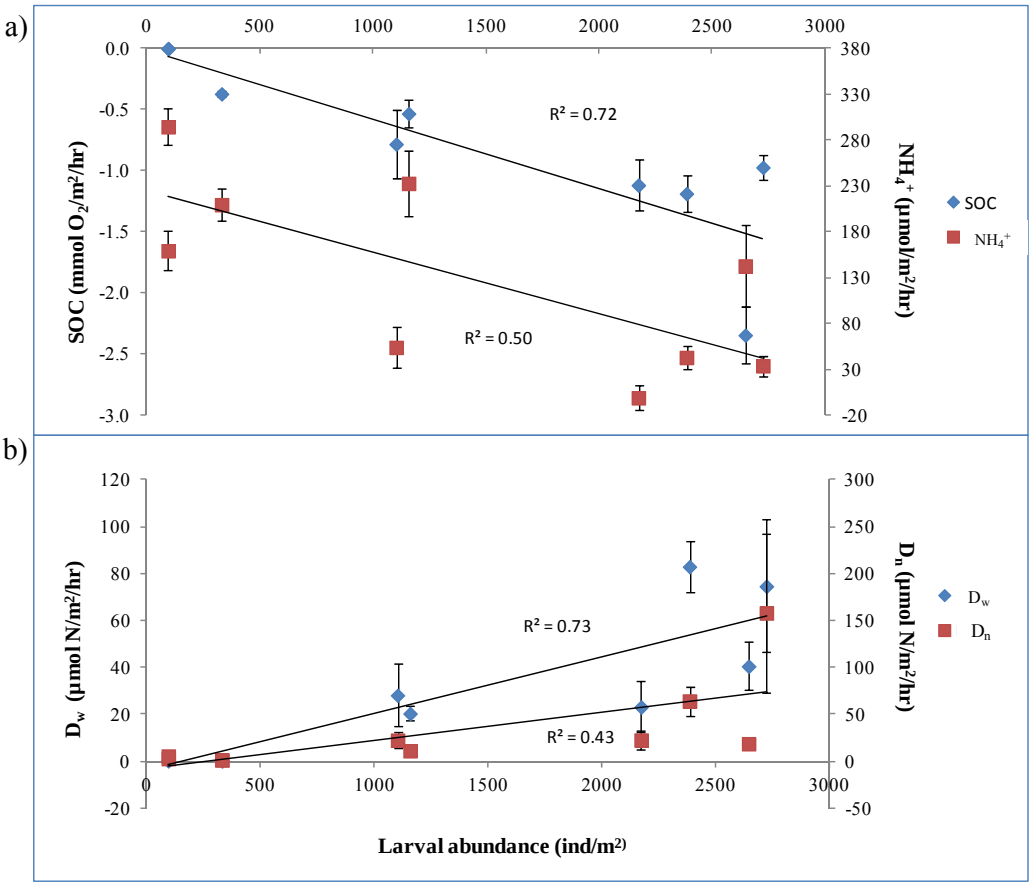

Fig. 4.6. Larval density versus SOC and NH_4^+ flux (upper panel) and denitrification rates (lower panel). Error bars represent ± SD among sites, n = 3. Regression lines (with R^2) are plotted for each comparison.

4.3.3. Pore water peepers

The vertical distributions of nutrient concentrations in the water column (up to 10 cm above the SWI) and in the pore water (up to 10 cm into the sediment from the SWI) are shown in Fig 4.7 from the summer (Mar 2011) and winter (Aug 2011) peeper deployments. The profiles are each the mean of 3 peepers per site across all three sites. In summer, the NO_x concentration was below the detection limit (< 0.07 µM, method 4500-NO_3^- I in APHA 2005) in both the water column and the sediment pore water, whereas in the winter sampling, the

water column NO_x concentration varied between 0.4 (±0.8) µM and 20 (±10) µM across the sampling sites. A sharp increase in NO_x was observed from just above the SWI during the winter sampling (Fig 4.7a).

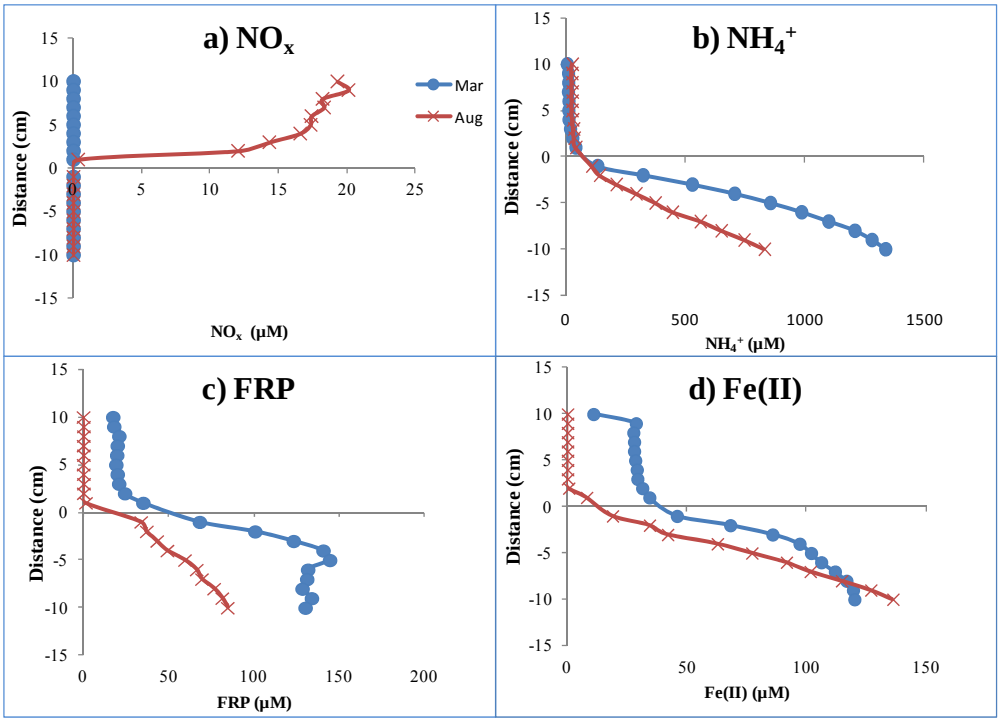

Figure 4.7. Vertical concentration profiles of different analytes in March and August 2011. Plots represent average concentrations for multiple peepers from the three dam sites. Error bars are not shown for clarity but % relative error typically ranged from 10-76% for all data points. Positive values represent distances above the sediment-water interface (distance = 0).

During Aug 2011, pore water NO_x concentrations were below the detection limit. NH_4^+ concentrations (Fig 4.7b) in the sediment pore water were significantly higher than those in the water column on both occasions ($p < 0.05$, $n = 20$, non-parametric Mann-Whitney *U* test). The pore water NH_4^+ concentrations ranged from 130 (±70) µM to 1340 (±170) µM and from 110 (±50) µM to 800 (±400) µM during the Mar 2011 and Aug 2011 deployments respectively. The NH_4^+ concentration profile showed a steep concentration gradient and increased almost linearly with sediment depth ($R^2 = 0.97$ and 0.99 in Mar and Aug 2011) on

both occasions. The sediment pore water NH_4^+ concentrations were found to be significantly higher in Mar 2011 than Aug 2011 ($p < 0.05$, $n = 10$, non-parametric Mann-Whitney *U* test).

FRP concentrations in the water column ranged from 17 (±5) µM to 35 (±18) µM during the summer deployment but were below the detection limit (< 0.03 µM, method 4500-P G in (APHA 2005)) during winter (Fig 4.7c). The pore water FRP concentrations were significantly higher than in the water column ($p < 0.05$, $n = 20$, non-parametric Mann-Whitney *U* test) and increased linearly with sediment depth on both occasions (Fig 4.7c). Like NH_4^+ and FRP, Fe(II) concentrations also increased nearly linearly with sediment depth (Fig. 4.7d, $R^2 = 0.86$ and 0.99 in Mar and Aug 2011 respectively). Average water column Fe(II) concentrations were significantly higher ($p < 0.05$, $n = 10$, non-parametric Mann-Whitney *U* test) in Mar 2011 than in Aug 2011. Linear correlations were found between FRP and Fe(II) in both the Mar 2011 and Aug 2011 deployments (Fig 4.8).

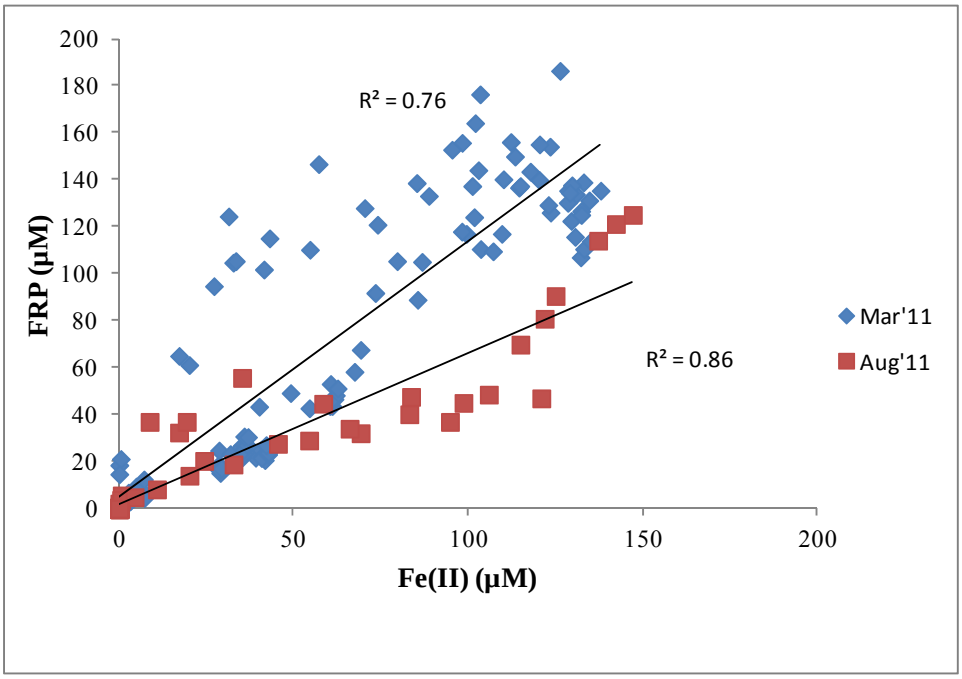

Figure 4.8. Correlation between FRP and Fe(II) concentrations both in the sediment pore water and overlying water column. Data obtained from peeper profiles, n = 120

According to the peeper results, the average molar ratios of DIN ($NH_4^+ + NO_x$) to FRP in the water column (up to 10 cm from the sediment-water interface) were 356 ± 333 and 0.6 ± 0.3, n = 10 in Aug and Mar 2011 respectively. Hence, the water column DIN:FRP ratio was found to be lower than Redfield ratio of 16:1 (see chapter 1, section 1.2.2 for further explanation) in Mar 2011, whereas the ratio was higher than 16:1 during the Aug 2011 sampling (Fig 4.9) (Redfield 1958).

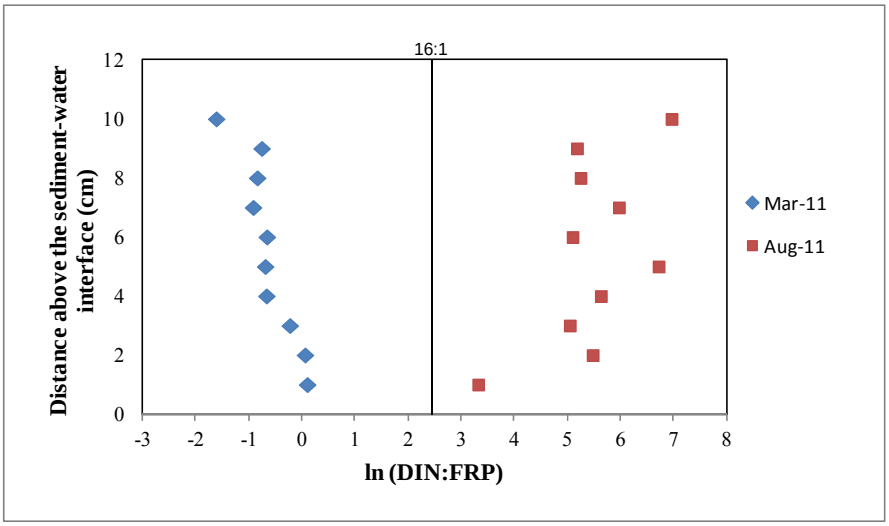

Figure 4.9. Seasonal variation of water column DIN:FRP ratio. Data on x-axis are log-transformed to allow a better pictorial representation on this graph. Zero on the y-axis represents the sediment-water interface

4.3.4. Burrowing activity of Chironomid larvae

Bioirrigation and the solute transport mechanism of Chironomid larva under an oxygenated water column is shown in Fig 4.10. Chironomid larvae introduced oxygenated water deep into the otherwise anoxic sediment through their pumping activities. The larvae also flushed out the deoxygenated water from inside the burrow structure into the overlying water column.

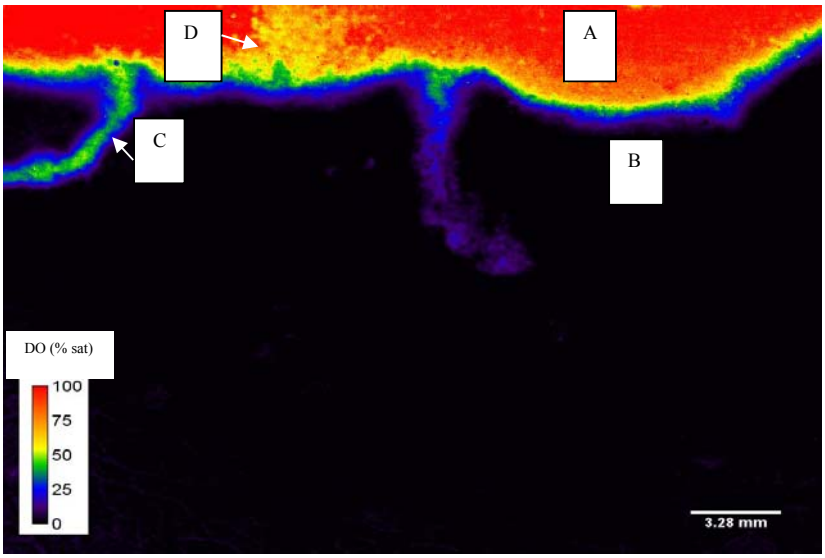

Figure 4.10. Burrowing activity of Chironomid larvae and in situ O_2 images measured during the planar optode experiment. A) Water column. B) Sediment column. C) Burrow structure transporting oxygenated water deeper into the sediment and D) Burrow ventilation by Chironomid larvae

4.4. Discussion

4.4.1. Variability of temperature and DO

In this study, seasonally-dependent differences in temperature and DO saturation were observed between the surface and bottom waters of the dam. The water column was thermally stratified during the summer and autumn followed by mixing in winter. Under the stratified conditions recorded in the dam over the period Dec 2010 – Mar 2011, O_2 resupply to the bottom water from the atmosphere was inhibited and organic matter breakdown in the surface and sub-surface sediment layers rapidly consumed available O_2, leading to hypolimnetic anoxia (Gao and Song 2008). The much higher DO saturation (101 – 107 % DO saturation) in the surface water in summer was most probably due to the photosynthetic activity of phytoplankton (Brainwood *et al.* 2004). Higher values of pH (7.6 - 8.3) at the same time can also be attributed to photosynthetic activity because during photosynthesis, phytoplankton take up dissolved inorganic carbon (e.g. CO_2 and HCO_3) for their growth and

cell maintenance, thus increasing the pH (Chapter 1, equation 1.3 and 1.4) (Brainwood *et al.* 2004; Fairchild and Velinsky 2006).

4.4.2 Nutrient Flux Rates

Seasonal variation in inorganic nutrient flux between the sediment and the water column was also evident from the monthly core incubation results. Monthly measurements of NO_x fluxes suggested the dam was a net sink for NO_x-N (Details of the annual load calculations and results are presented in chapter 5, section 5.2.1 and Table 5.3). Sedimentary NO_x uptake was highest under hypolimnetic hypoxia, reflecting NO_x acting as an alternative electron acceptor for microbial metabolism (Nowlin *et al.* 2005). Insignificant NO_x fluxes during summer months were associated with low availability of NO_x in the overlying water column (Table 4.1) and also due to lack of sedimentary nitrification under anoxic conditions, as nitrification requires O_2. Peeper results from the Mar 2011 deployment also support this observation, showing that NO_x was absent from both the water column and the sediment pore water. During the Aug 2011 deployment, absence of NO_x in the sediment pore water can be attributed to the higher sedimentary denitrification rate (148 μmol/m^2/hr) rapidly removing NO_x from the sediment pore water (Al Bakri and Chowdhury 2006; Burger *et al.* 2007). Denitrification of NO_x from the water column (D_w) and coupled nitrification–denitrification (D_n) within the sediment both played an important role in nitrogen removal from the dam (D_w and D_n accounted for about 40% and 60% of the total annual denitrification, respectively). Rysgaard *et al.* (1995) in their study on a shallow estuary on the east coast of Denmark reported an approximately 50% contribution of D_w to the total annual denitrification and highlighted the importance of D_w in removing NO_x from the system. This study also indicates that D_w can be an important NO_x removal process in farm dams that receive significant

nutrient inputs. In this study, both the D_w and D_n activities were highest in winter when NO_x was present in the water column and the flux of NO_x was generally directed into the sediment. David *et al.* (2006) in their study of a reservoir in an agricultural landscape of upper-western USA reported denitrification rates of 62-225 g-N/m^2/yr which were much higher than the rates (0.4-28 g-N/m^2/yr) observed in this study. The variation in rates was most likely due to limited water retention time in this Australian farm dam because longer retention time facilitates greater interaction and nutrient cycling between sediment and overlying water column (Saunders and Kalff 2001, David *et al.* 2006). The DE in the farm dam was also associated with the higher concentration of water column NO_x and dissolved oxygen. A statistically significant negative correlation between sedimentary NH_4^+ flux and DE demonstrated that under oxic conditions sedimentary release of NH_4^+ was suppressed by coupled nitrification-denitrification activity in the sediment environment. Higher DO saturation in the bottom water increases the O_2 penetration depth into the sediment (chapter 3, Table 3.2), which in turn facilitates increased organic matter mineralization and denitrification activity in the sediment (Rysgaard *et al.* 1995). The higher DE (50-90%) in winter observed in this study was in agreement with the observations of Eyre and Ferguson (2009) who demonstrated that denitrification can remove in excess of 60% of the inorganic nitrogen released from the sediment. However, it is crucial that oxic water column conditions are maintained. Under hypoxia the link between nitrogen removal (via coupled nitrification-denitrification) and mineralisation breaks down, resulting in decreased denitrification and net release of NH_4^+ from the sediment into the water column (Kemp et al. 2005; Rysgaard *et al.* 1994).

Sediment-water exchange of NH_4^+ also varied between seasons. NH_4^+ flux from the sediment into the overlying water was negligible during winter months under high DO (70 ± 14 % DO

saturation) and low temperature (9.1 ± 0.4 ^{0}C) conditions at the SWI, which were consistent with the findings of Moore and Reddy (1994) and Qu *et al.* (2003). In contrast, significant release (170 ± 80 to 290 ± 20 μmol/m^2/hr) of NH_4^+ was measured in the summer under opposing (hypolimnetic) conditions, i.e. low DO (9 ± 10 % DO saturation) and high temperature (16 ± 2 ^{0}C). A positive correlation between bottom water temperature and NH_4^+ flux and negative correlation between overlying water DO saturation and NH_4^+ flux indicated that high temperature and low DO promoted sedimentary release of NH_4^+ into the water column (Table 4.3). Similar results were also reported by Liikanen *et al.* (2002), who explained that increased microbial degradation of organic matter at elevated temperature and decreased nitrification activity under low O_2 concentration promoted NH_4^+ efflux from the sediment.

In this study, benthic macrofauna also seemed to have a significant effect on sedimentary DIN and O_2 dynamics, as was observed by Kristensen *et al.* (1991), Karlson *et al.* (2007) and Lewandowski *et al.* (2007). Even though the average pore water (averaged over the 10 cm depth from the SWI) NH_4^+ concentration was much higher than that in the overlying water column (60 and 15 times in March 2011 and August 2011, respectively), a negative correlation ($R^2 = 0.50$, $p = 0.03$, $n = 9$) between larval abundance and NH_4^+ flux suggested that Chironomid larvae reduced the NH_4^+ efflux into the water column. The presence of benthic animals can enhance oxygen penetration depth by burrowing and ventilation activities extending the oxygenated area of the sediment-water interface (Wang *et al.* 2001; Biswas *et al.* 2009; Zhang *et al.* 2010). The increased O_2 availability within an otherwise anoxic sediment environment facilitates nitrification which in turn reduces NH_4^+ efflux. Several studies have demonstrated that benthic macrofauna can potentially increase sedimentary denitrification rates (Pelegri *et al.* 1994; Rysgaard *et al.* 1995; Karlson *et al.* 2007). In this study, both D_w and D_n increased with increasing Chironomid density indicating

that Chironomid larvae promoted sedimentary denitrification activity. This can be attributed to the bioirrigation activity of Chironomid larvae (Rysgaard *et al.* 1995; Aller 2001; Zhang *et al.* 2010). To supply oxygen and remove metabolites, Chironomid larvae actively flush their burrows with overlying water. This pumping mechanism increases the transport of electron acceptors (i.e. O_2 and NO_x) from the water column into the sediment thus creating greater areas of sediment containing O_2 for nitrification, while higher NO_x supply to the suboxic sediment zone will enhance denitrification (Jiang *et al.* 2010).

The seasonal dependence of sedimentary FRP fluxes was in agreement with other studies which revealed FRP efflux from the sediment in summer and influx/uptake in winter months (Al Bakri and Chowdhury 2006; Miao *et al.* 2006; Spears *et al.* 2007; Spears *et al.* 2008). Higher FRP concentrations in the sediment pore water than in the overlying water column during both peeper deployments suggested a positive flux of FRP from the sediment into the water column. Such a flux (14 ± 2 μmol/m^2/hr, Table 4.2) was observed during the hypolimnetic anoxia that prevailed during summertime. However, in winter, with a mixed, oxygenated water column, a much lower FRP efflux and even sedimentary uptake were measured (Table 4.2). According to the peeper results, the average water column FRP concentration was 70 times higher in Mar 2011 (22 ± 5 μM) than in Aug 2011 (0.03 ± 0.09 μM) and the difference was statistically significant ($p < 0.001$), indicating increased accumulation of sediment-released FRP in the water column under anaerobic conditions. Release of sediment-bound P is also associated with Fe biogeochemistry. In the sediment, when DO and NO_x are depleted, Fe(III) acts as an alternative electron acceptor for microbial metabolism and thus is reduced to soluble Fe(II), which in turn releases Fe bound P into the pore water (Villar *et al.* 1999; Grace *et al.* 2010; Ozkundakci *et al.* 2011; Roy *et al.* 2012). A linear correlation (both in the water column and in the sediment pore water) between Fe(II) and FRP concentrations ($R^2 = 0.76$, $n = 120$, $p < 0.001$ and 0.90, $n = 100$, $p < 0.001$ in Mar

2011 and Aug 2011 respectively), observed during both peeper deployments, demonstrated that P release from solid Fe(III) oxyhydroxides upon iron reduction is a very important P release mechanism in this dam.

Despite the high pore water FRP measured in the peepers (Aug 2011), this did not translate into high water column FRP as previously described for the summertime measurements. This was most likely due to the well-oxygenated bottom water conditions. Under such conditions, a 'cap' of Fe(III) oxyhydroxides can be formed in the surface sediment layer; this cap provides a large sorptive capacity for FRP, thereby intercepting the phosphorus diffusing up through the sediments before it can reach the overlying water (Søndergaard *et al.* 2003; Grace *et al.* 2010; Green *et al.* 2012). Benthic fauna may also lower FRP flux by enhancing the surface area of oxygenated sediment via burrows and activities such as bioirrigation and bioturbation, which in turn increases the Fe(III) 'cap' (Fig 4.9). Zhang *et al.* (2010) in a study of the effects of different benthic macrofauna on P dynamics across the sediment-water interface, showed that Chironomid larvae substantially decreased pore water Fe(II) and FRP concentrations by increasing O_2 penetration depth deeper into the otherwise anoxic sediment column. In May 2011, a combination of a well mixed water column with the consequent reintroduction of oxygenated bottom water after prolonged hypolimnetic anoxia and increased larval abundance (Chapter 3, Fig. 3.3) enlarging the surface area of the Fe 'cap' was the most likely cause for the highest (-61 ± 14 µmol/m^2/hr) sediment uptake of FRP (by adsorption onto the Fe(III) oxyhydroxide surfaces). For the remaining sampling months (Jun 2011 to Dec 2011) average FRP fluxes were insignificant throughout the sites and ranged from -1.8 (±0.6) µmol/m^2/hr to 1.8 (±0.8) µmol/m^2/hr (Table 4.2).

A substantial shift in the bottom water DIN:FRP ratio occurred when moving from summer (Mar 2011) to winter (Aug 2011). The higher value (~356:1) of DIN:FRP in winter was due

to the higher concentration of NO_x (60 ± 2 μM) compared to the FRP (0.4 ± 0.0 μM) in the bottom water (Table 4.1). During this period, the well oxygenated water column facilitated P sedimentation and also inhibited water column accumulation of P from the sediment environment due to the formation of the Fe 'cap' in the surficial sediment (Moore and Reddy 1994; Søndergaard *et al.* 2003). In contrast, during the summer deployment (Mar 2011) under anoxic bottom water conditions, very low NO_x concentration (0.3 ± 0.3 μM) compared to FRP (24 ± 7 μM) indicated increased P accumulation in the bottom water due to reductive dissolution of Fe. Excess bioavailable P in combination with low DIN, particularly in summer months, has the potential to favour dominance of cyanobacteria within phytoplankton communities (Downing *et al.* 2001). Lewis and Wurtsbaugh (2008) also mentioned in their review that a low N:P ratio along with high P concentration in freshwater lakes can substantially alter phytoplankton composition to N-fixing cyanobacteria. One important reason that P often controls cyanobacterial blooms in freshwater ecosystems is that many cyanobacteria have the ability to fix N from the atmosphere (Paerl 2008; O'Neil *et al.* 2012). In a freshwater lake in Victoria, Grace *et al.* (2010) observed a significant increase in a cyanobacterial population (*Anabaena*) in summer after the springtime increase in the water column FRP concentration. They reported mean water column bioavailable N and P concentrations of 18 μM and 10 μM respectively with a molar DIN:FRP ratio of 1.8:1, which was used to explain the prevalence of the N-fixing *Anabaena* species. In the farm dam, mean DIN and FRP concentrations were found to be 0.6 ± 0.5 μM and 21 ± 20 μM respectively during Jan 2011 to Apr 2011 indicating highly N limited conditions (DIN:FRP = 0.03:1) and the potential for significant N-fixing cyanobacterial growth.

4.4.3. Comparison with other studies

Sedimentary nutrient flux rates from inland lentic fresh water ecosystems have been measured in a limited number of Australian studies (Davis and Koop 2006). Table 4.4 lists nutrient flux rates in such water bodies within Australia and worldwide. Benthic nutrient fluxes in the farm dam (this study) were found to be substantially higher than an urban lake in Victoria (Grace *et al.* 2010) and water supply reservoirs in New South Wales (Al Bakri and Chowdhury 2006). Nitrate (NO_x) release rates (around 0.2 mmol/m^2/day) were similar to those in Lake Cataouatche, USA (Miao *et al.* 2006) under aerobic conditions in the sediment-water column. However, under bottom water hypoxic conditions sedimentary uptake (-7 ± 2 mmol/m^2/day) of NO_x was within the rate range reported for sediment in Lake Sempach, Switzerland (-2.4 to -11.1 mmol/m^2/day) (Höhener and Gächter 1994), but much higher than those reported in other studies (Table 4.4). The greater NO_x uptake rates were probably due to high biogeochemical activity i.e. assimilation (biological uptake) and dissimilation (denitrification) by bacteria and benthic microalgae in the sediment environment removing NO_x from the water column (Miao *et al.* 2006; Zilius *et al.* 2012).

When comparing NH_4^+ flux rates from this study to similar studies done elsewhere (Table 4.4), the average NH_4^+ release rates (7.0 ± 0.4 mmol/m^2/day) under anaerobic condition in the farm dam were higher than the fluxes from many other sites cited in the literature (Table 4.4), but were substantially lower than the flux rates (19 – 157 mmol/m^2/day) reported for sediment in Lake Rotorua, New Zealand (Burger *et al.* 2007). The greater rates of sediment NH_4^+ release in Lake Rotorua were attributed to high organic matter supply to the sediment which might also be the case in farm dams that have high organic matter input during high rainfall and runoff events (Adams et al. 2014). In another study, Beutel *et al.* (2008) reported high rates (3-4 mmol/m^2/day) of NH_4^+ release from Deer Lake sediment, USA. They explained that the use of NH_4^+-free make-up water during the core incubation experiment

was the probable cause of high NH_4^+ efflux due to an increased concentration gradient between sediment pore water and chamber water. However, in the present study *in situ* bottom water from the farm dam was collected on each sampling occasion and used as replacement water during core incubation to minimize such an effect (Chapter 2, section 2.2.1a).

The average FRP flux rates of 0.34 ± 0.06 mmol/m^2/day under hypolimnetic anoxia from the farm dam were within the typical range (0.01 – 0.7 mmol/m^2/day) measured in fresh water systems (Søndergaard *et al.* 2003; Burger *et al.* 2007; Spears *et al.* 2007). Sedimentary release rates under anaerobic conditions reported in this study were similar to those estimated from Suma Park Reservoir, Australia (0.16 mmol/m^2/day), Loch Leven Lake, Scotland (0.34 mmol/m^2/day) and Lake Acton, USA (0.29 mmol/m^2/day), but were substantially lower than those measured from Lake Rotorua, New Zealand (2.68 mmol/m^2/day, Table 4.4). Burger *et al.* (2007) explained that the high release rates of FRP from Lake Rotorua were due to a high P sedimentation rate facilitating regeneration of bioavailable P under high temperature and anoxic bottom water conditions, particularly at the deeper sites (~20 m). Sediment P uptake has been observed in other studies and generally occur under aerobic conditions (i.e. -0.01 mmol/m^2/day in Lake Cataouatche (Miao *et al.* 2006), and -0.03 mmol/m^2/day in Loch Leven (Spears *et al.* 2008). The comparatively higher P uptake rates (-1.5 ± 0.3 mmol/m^2/day) measured in this study occurred upon mixing and reoxygenation of bottom water column after prolonged anoxia (Fig 4.2a & b and Fig 4.3c). This was most likely due to adsorption of P on newly oxidised Fe(III) oxyhydroxides (Grace *et al.* 2010).

Table 4.4. Comparison of sedimentary dissolved nutrient fluxes (mmol/m^2/day). Values in parentheses denote ±1 SD. Negative values indicate sediment uptake

Source	Method	NO_x	NH_4^+	FRP	Reference
Farm dam, Victoria, Australia	Core incubation	-7(2) to 0.2(0.4)	-0.8 (1.3) to 7.0(0.4)	-1.5(0.3) to 0.34(0.06)	This study
Ornamental Lake, RBG, Victoria, Australia	Core incubation	0.06 ± 0.03#	0.21 (0.01) to 0.25 (0.08)*	0.10 ± 0.03*	Grace *et al.* (2010)
Suma Park Reservoit, NSW, Australia	Core incubation	-0.004 to -0.01	0.05 to 1.45	0.002 to 0.16	Bakri and Chowdhury (2006)
Chaffey Dam, NSW, Australia	Benthic chamber	-0.02 to -0.04	0.02 to 0.24	0.001 - 0.03	Sherman (2000) in Chowdhury and Bakri (2006)
Loch Leven, Scotland	Core incubation		-0.57 to 1.57	-0.03 to 0.34	Spears *et al.* (2008)
Lake Cataouatche, Louisina, USA	Core incubation	-0.02 to 0.15	-0.001 to 0.1	-0.01 to 0.10	Miao *et al.* (2006)
Lake Rotorua, North Island, New Zealand	Benthic chamber		19 to 157	0.07 to 2.68	Burger *et al.* (2000)
Lake Acton, Ohio, USA	Core incubation		0.95 to 1.39	0.03 to 0.29	Nowlin *et al.* (2005)
Deer Lake, Washington, USA	Core incubation		3 to 4#	0.03 0.06*	Beutel *et al.* (2008)
Lake Sempach, Switzerland	Benthic chamber	-2.4 to 11.1	1.1 to 16.1		Höhener and Gächter (1994)
Lake Eucha, Oklahama, USA	Core incubation			0.03 to 0.14	Haggard *et al.* (2005)
Beaver Reservoir, Arkansas, USA	Core incubation			<0.01 to 0.06	Sen *et al.* (2007)

= Incubation under oxic conditions only; * = Incubation under anoxic conditions only

It is important to note that some variability in the flux rates presented in the comparison table (Table 4.4) might also be due to the difference in sampling techniques i.e. *in situ* benthic chambers and laboratory core incubations. The core incubation technique has advantages over benthic chamber deployments due mainly to its simplicity and cost effectiveness. However, core incubation results can be influenced by several artifacts e.g. variation in macrofaunal irrigation rates and pore water profiles due to altered temperature and pressure conditions (Hammond *et al*. 2004). Miller-Way *et al.* (1994) in their methodological comparison study found no differences in sediment oxygen consumption and nutrient flux rates between the two techniques. In another study, Hammond *et al.* (2004) observed decreased nitrate uptake rates in core incubations compared to benthic chambers and explained that this was due to reduced denitrification activity in the incubated cores. However, variations in the phosphate fluxes were insignificant between the two techniques at most of the sites in their study. They, however, concluded that despite some discrepancies, results for the two methods were identical.

4.5. Conclusion

The seasonal variation of surface and bottom water temperature and DO clearly indicated monomictic behaviour in the dam, with a single mixing event occurring in May. The seasonal cycling of temperature, and particularly DO concentrations, greatly influenced the water column concentrations of bioavailable nutrients (NO_x, NH_4^+ and FRP) and the fluxes of these into and out of the surficial sediments. The dam sediment acted as a sink of NO_x, particularly under hypoxia in the bottom waters. The water column NO_x concentration was positively correlated with both the water column NO_x-derived denitrification (D_w) and coupled nitrification-denitrification (D_n), which contributed nearly equally to overall denitrification

(40% and 60% respectively). In contrast to NO_x, the dam sediment acted as a net source of NH_4^+ to the water column with higher release rates in summer and lower in winter months.

Seasonal variation of sedimentary FRP flux was also observed. The dam was found to be a net source of FRP in summer under hypolimnetic anoxia and as a sink upon mixing of the water column and the increasingly oxic bottom water in winter. A linear relationship between Fe(II) and FRP concentration both in the water column and in the sediment pore water revealed the importance of Fe chemistry as a key factor controlling bioavailable P cycling at the sediment-water interface. Benthic macrofauna, particularly Chironomid larvae, had a considerable effect on sediment DIN ($NH_4^+ + NO_x$) and O_2 dynamics. Larvae substantially increased SOC and NO_x uptake rates, but reduced the release rate of NH_4^+. Larval abundance was also found to have significant control over sedimentary denitrification processes. Both the D_w and D_n rates increased with increasing density of larvae, due to their bioirrigation activity.

According to the molar ratio of DIN and FRP, the water column of the dam was P limited in winter and N limited in summer. This shift in the water column N:P ratio from an average of ~356:1 (mol/mol) in winter to ~0.6:1 in summer can greatly enhance favourable conditions for cyanobacterial growth, which often dominate systems with N:P ratios <15 (Paerl 2008). The diffusive flux of nutrients across the sediment-water interface, particularly during summer months underpinned the role of the bottom sediment as an alternative source of bioavailable nutrients to fuel algal blooms. This study has demonstrated that farm dams can be extremely biogeochemically active and the activity is highly seasonally-dependent. However, further investigation, including algal bioassays together with nutrient analysis, are required for better prediction of nutrient limiting conditions in such dams.

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Chapter Five

Potential of a farm dam to improve stream water quality in an agricultural catchment in south-eastern Australia

5.1. Introduction

In many regions of the world, a major proportion of headwater streams are impounded by constructed dams or ponds for purposes including water supply for irrigation, household and other farming activities (Bosch 2008; Brainwood and Burgin 2009). These impoundments within a catchment have the potential to significantly alter the physicochemical properties of receiving waters. Water quality is influenced by various processes occurring within the reservoir such as reduced water velocity and increased hydraulic retention time (HRT) inducing thermal stratification, change in dissolved oxygen (DO) conditions, suspended solids, and biogeochemical activity. These processes are often subject to variation due to weather and climate conditions (Maxted *et al.* 2005; Fairchild and Velinsky 2006). Small shallow ponds are expected to therefore show seasonal variation in how they alter downstream water chemistry (Fairchild and Velinsky 2006).

Several studies have shown that damming rivers to create water storage results in significant seasonal variation of nutrient dynamics, suspended particulate matter (SPM) and also plant and animal species composition (McKergow *et al.* 2003; Bosch and Allan 2008; Wilhelm and Adrian 2008). These impoundments often act as a major sink for phosphorus (P) and to a lesser extent for nitrogen (N), predominantly depending on season and hydraulic retention time (Sherman *et al.* 2001; Avilés and Niell 2007; Bosch and Allan 2008). Ejsmont-Karabin *et al.* (1993) found higher P retention at longer HRT; Stanley and Doyle (2002) attributed this mainly to trapping of suspended sediments and subsequent storage by the impoundments. Decreased velocity and thus increased residence time of water allows sediment-bound P to settle out of the water column and ultimately become buried and unavailable. In a review on N and P biogeochemistry in Australian catchments, Harris (2001) reported that dissolved organic nitrogen (DON) and total Kjeldahl nitrogen (organic N and NH_4^+) were consumed and dissolved inorganic nitrogen (DIN) was exported from Australian freshwater lakes and

reservoirs. Removal of N from the water column is mainly governed by sediment-water interactions (Bosch 2008). DIN can permanently be removed from the water column by denitrification (reduction of NO_x to N_2 gas) or by transformation to particulate forms via assimilation during phytoplankton growth (Chapter 1, section 1.2.1). The removal of N differs from P in that P normally does not have any gaseous form. The removal rates and ultimately the concentrations of N and P of the water column often determine the extent of nutrient limitation to phytoplankton growth.

According to the Redfield ratio, algal growth is N limited when the (molar) N:P ratio is below 16:1 whereas a value above 16:1 indicates potential P limitation (Redfield 1958). Though DIN:FRP incorporates readily available inorganic nutrients, Dodds (2003) argued that TN:TP is a better measure to predict trophic state and nutrient limitation of aquatic systems. He explained that the balance between uptake and remineralization rates of inorganic nutrients determines actual concentrations at a particular time. As inorganic nutrients are readily available to biota and turn over rapidly, according to Dodds (2003), DIN:FRP is a weak substitute for TP:TN to predict trophic status. Harris (2001) pointed out that regulation of most Australian rivers and hence the long water retention time resulted in potential N limitation and recurrent incidence of N-fixing cyanobacterial blooms in Australian freshwater and coastal ecosystems. In a number of studies it has also been shown that in freshwater systems N limitation is as common as P limitation (Elser et al. 1990; Elser et al. 2007; Lewis et al. 2008). Recently, Muhid and Burford (2012) found in their algal bioassay study across Wivenhoe Reservoir, Queensland, Australia, that during the stratified summer months, the reservoir was co-limited by both N and P, rather than N or P alone which contrasted with *in situ* water column N:P ratios and nutrient concentrations. Therefore they stressed the need for nutrient enrichment bioassays over nutrient analysis alone as a better indicator of nutrient limitation in aquatic ecosystems.

Organic carbon (dissolved and particulate) is an important source of energy for aquatic food webs and also as a source of organic pollution (Wetzel 1984; Findlay and Sinsabaugh 1999). It also has the potential to alter optical properties of water bodies by limiting light availability to primary producers (Findlay and Sinsabaugh 1999). Organic carbon is often divided into two broad categories, labile and refractory, based on their biological significance (Wetzel 1975). The labile fraction of organic carbon is easily degradable and available to microbial metabolism whereas the refractory component is comprised of more complex compounds and is more resistant to microbial degradation (Wetzel 1975). Bioaccessability and metabolism of the organic carbon often depends on the molecular size of the organic matter (Wetzel 1984). Dissolved organic carbon (DOC) has received the most attention due to its bioavailability, influence in the cycling of trace elements and also as a control on the biological activity of some components i.e. bacteria and phytoplankton (Salonen *et al.* 1992; Findlay and Sinsabaugh 1999). Alterations to the various forms of organic carbon in impoundments are more likely, mainly due to the longer water residence time compared to river systems.

In Australia, dams are an essential part of most farming business, providing necessary water supply for irrigation and stock. Farm dam development has been an integral part of agricultural expansion (Lewis and Harrison 2002). Baillie (2008) reported that there are millions of farm dams in Australia, indicating the importance of these impoundments in Australian agricultural systems. The number has increased significantly in recent times due to prolonged drought and altered climate conditions (Sinclair Knight Merz, 2012). According to a recent survey, in Victoria (south-eastern state of Australia) there are about 455,000 farm dams existing in 18 different land use categories, of which the highest percentage are designated “Grazing modified pastures” and “Rural residential” (Sinclair Knight Merz, 2012). They have also reported that between 2005 and 2009/10; over 75% of new dams (around 35,000) were constructed in these two land use categories. While providing useful

support for farming processes, these dams have the potential to significantly affect stream flow and other environmental variables (temperature, dissolved oxygen, turbidity and nutrients) for downstream receiving waters. However, despite their increased numbers in Australia, little research has been performed to quantify what impact these farm dams are having on downstream water quality.

So far, most of the research conducted on Australian farm dams has been focused on the downstream hydrologic impacts of these impoundments (Schreider *et al.* 2002; Nathan *et al.* 2005; Brainwood and Burgin 2009; Nathan and Lowe 2012). In a review on ecological impacts of damming, water diversions and river management on Australian floodplain wetlands; Kingsford (2000) highlighted that upstream river management, including construction of dams, can adversely affect large areas of floodplain wetlands in Australia due to reduced flooding with the eventual risk that the wetlands will turn into terrestrial ecosystems. In another study, Nathan *et al.* (2005) reported that farm dams reduced around 4.5 ML/km^2 of mean annual stream flow across Victoria. According to that report, the total farm dam volume in Victoria was around 870,000 ML, 60% of which was stored by farm dams larger than 5 ML, even though these large dams made up only 7% of the total number of Victorian farm dams. The majority of Australian rivers are affected by the construction of dams and reservoirs in the upper catchment resulting in reduced downstream water flow, disconnection of river channel and floodplain and altered lowland in-stream environments (Davis and Koop 2006). Brainwood and Burgin (2009) demonstrated that Australian farm dams are a sustainable reservoir of biodiversity within the landscape and stressed the need for proper management practice to optimize hydrodynamics of farm dams as functioning ecosystems. In a recent Australian study, Burford *et al.* (2012) showed that a subtropical southeast Queensland reservoir, having input from two different rivers (dammed and unregulated), retained about 60% of total incoming P over a period of six years whereas N

retention was negligible. They also demonstrated that the major portion of the P load came from the unregulated river and pointed out the importance of upstream catchment river management to reduce P loss from terrestrial ecosystems. Adams *et al.* (2014) estimated that in 2010, the dam reported in this study removed about 67% and 5% of incoming TP and TN respectively and also observed net export of TN during winter months. However, the role of farm dams as processors of the forms and concentrations of nutrients and other water quality variables is not well understood, both in Australia and worldwide, and requires further research to address the potential of such impoundments to modify stream water chemistry.

Consequently, measurement and interpretation of temporal variation in nutrient loads and water quality from farm dams is a significant knowledge gap. Understanding the modification of stream water quality by such impoundments is crucial due to their large number in Australian catchments and to provide insights to underpin better management practices to improve water quality in the future.

The primary purpose of this study was to investigate the seasonal behaviour of a farm dam in a headwater agricultural catchment in temperate south-eastern Australia to improve knowledge on the impact of farm dams on stream water chemistry. Hence, this study aimed to investigate: i) temporal changes in nutrient and organic carbon dynamics within the farm dam, ii) the factors controlling these biogeochemical processes, and iii) the overall impact of such an impoundment on downstream water quality.

5.2. Methods

5.2.1. Sampling and analysis

A detailed description of the sampling site is presented in chapter 2, section 2.1. The dam was sampled once every month from June 2010 to March 2011 for temperature, pH, turbidity, dissolved oxygen (DO) and total and dissolved nutrients (N and P) and organic carbon. A mass balance approach (inflow vs outflow measurements) was undertaken to evaluate the role of the dam in processing nutrients and other physicochemical variables. Samples from the water column were collected from the inflow (DU, Fig 2.1.a), about 10 m upstream and the outflow (DL), about 5 m downstream of the dam. Water samples were collected directly from the stream into a pre-cleaned bucket and later sub-sampled for various analytes. Total nutrients (TP and TN) samples were collected in 50 mL pre-washed HDPE bottles and samples for dissolved nutrients (NH_4^+, NO_x and FRP) were filtered through 0.20 µm pore-sized cellulose acetate filter units (Advantec) before collection (APHA. 2005). Total and dissolved organic carbon (TOC and DOC) samples were collected into pre-washed (see chapter 2, section 2.2.2 for details) 60 mL amber glass bottles and baked aluminium foil was used in capping to avoid contamination. DOC samples were filtered through glass fibre filters (grade GF-75, Advantec). Organic carbon samples were preserved by acidification (1-2 drops of conc. HCl); nutrient samples were kept frozen until analysis to prevent any microbial activity. Physicochemical variables (pH, conductivity, turbidity, dissolved oxygen and temperature) were measured *in situ* with a pre-calibrated Horiba U10 multiprobe water quality data sonde on each occasion. Temperature and DO concentration in the surface and 'bottom' (approximately 0.5 m above the sediment surface) water of the dam were also recorded every month to evaluate thermal stratification and oxic status.

Water discharge at the outlet (DL) was estimated as the residual term in the water balance calculation of the dam (Adams *et al.*, unpublished data). Dam volume was calculated by

multiplying dam surface area (D_{SA}) and mean depth (D_{MD}). The dam surface area (D_{SA}) was calculated once (when the dam was full and overflowed water) by field surveying and assumed to be constant based on the field observations during those periods of overflow (May 2010-June 2011) at the outlet (DL). To obtain average depth (D_{MD}), depth transects were measured manually from a boat at various locations within the dam every month within the sampling period. Hydraulic retention time was calculated by dividing the dam volume by discharge (Fairchild and Velinsky 2006). The percent retention efficiency of nutrients was calculated as the percent removal for each parameter:

$$\% \text{ Retention efficiency} = ((\text{Input-output})/\text{input}) \times 100 \qquad \text{Eq. 5.1}$$

where input and output are the influent and effluent concentrations in µmol/L. It was assumed that the inflow volume equals the outflow (Cromar and Fallowfield 1997; Fairchild and Velinsky 2006). Percent retention has widely been used as a tool to compare different water quality parameters (nutrients, total suspended solids and turbidity) varying in concentrations among sites (Cromar and Fallowfield 1997; Paul 2003; Fairchild and Velinsky 2006; Li *et al.* 2008). It is important to note that nutrient retention by the dam equates to the removal of nutrients from the water travelling between DU and DL.

N:P ratios are expressed on a molar basis. Various forms of nutrients and organic carbon were analysed following respective standard procedures described in chapter 2 (Table 2.2) and (APHA, 2005). The following water chemistry variables were derived from measured values:

Particulate and dissolved organic nitrogen (PNnDON)

$$= \text{total nitrogen (TN)} - \text{dissolved inorganic nitrogen (DIN)} \qquad \text{Eq. 5.2}$$

Particulate and dissolved organic phosphorus (PPnDOP)

$$= \text{total phosphorus (TP)} - \text{filterable reactive phosphorus (FRP)} \qquad \text{Eq. 5.3}$$

Particulate organic carbon (POC)

= total organic carbon (TOC) – dissolved organic carbon (DOC) Eq. 5.4

The flux of nutrients (NH_4^+, NO_x and FRP) from the sediment to the overlying water was obtained from monthly core incubations (Chapter 4, section 4.2). A mean flux value from monthly core incubations from 3 different sites within the dam was calculated to represent the total flux for each of the nutrients for that particular month. External load (nutrient concentration × flow) and internal load (nutrient flux rate × sediment surface area) of dissolved inorganic nutrients were calculated from monthly data obtained from DU and average sedimentary nutrient fluxes respectively for the period of Jun 2010 – Mar 2011. Sediment surface area was assumed to be the same as the dam surface area. Data for a missing month was calculated as an average of the previous and following months. The % relative contribution of the dam was calculated as:

% relative contribution = (Internal load / (External load + Internal load)) ×100 Eq. 5.5

A Campbell Scientific automatic weather station (AWS) was installed at the site on a ridgeline to record meteorological data (rainfall, air temperature and solar radiation). A calibrated DO logger (D-opto logger, ZEBRA-TECH LTD, New Zealand) was deployed in the middle of the dam (suspended from a buoy about 5 cm below the water surface) to log hourly DO readings throughout the sampling period.

5.2.2. Data treatment

Monthly observations of water quality variables were compared with rainfall, flow and HRT. Figure 5.1 shows that at DU, after a rainfall event (August 2010), it takes ~ 24 hours for the system to return to baseflow conditions, including nutrient concentrations and flow. Hence,

December 2010 was excluded from the data set used for analysis due to a rainfall (> 1 mm) event in the 24 hours prior to sampling. All other sampling dates were during baseflow conditions and it was assumed that inflowing nutrients and other water chemistry variables were constant at the time of sampling and representative of baseflow conditions.

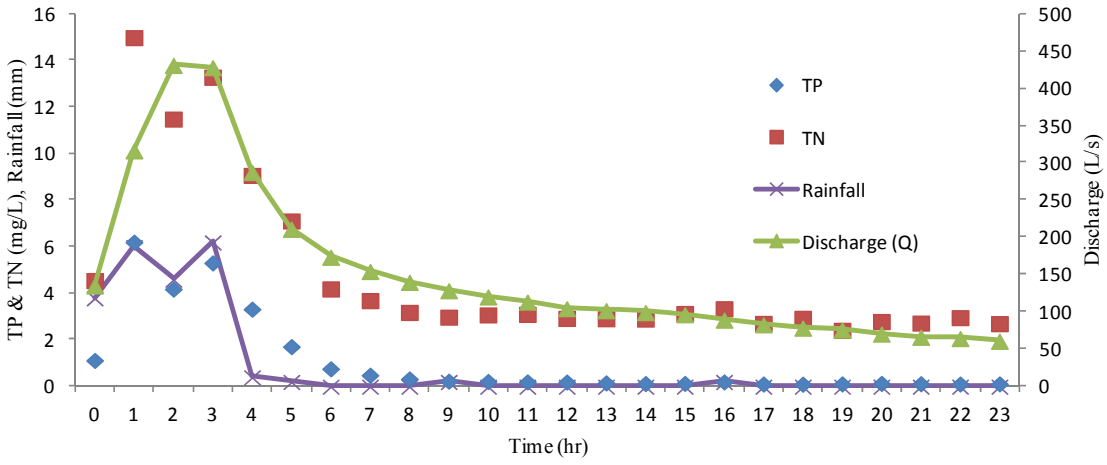

Figure 5.1. Effect of rainfall on nutrient concentrations. Secondary axis represents discharge. Data presented from 1st and 2nd Aug 2010 sampling

5.2.3. Statistical Analysis

All statistical analysis was performed using the software SPSS 20 (IBM® SPSS® Statistics 20). The variable HRT was log-transformed to obtain a normal distribution. The coefficient of correlation between physicochemical variables and nutrient concentrations was calculated by the Pearson correlation test. One way analysis of variance (one way ANOVA) and repeated measures analysis of variance (RM ANOVA) were used to explore the spatiotemporal effect on water quality variables. Statistical significance was set at $P < 0.05$. Interrelationships between parameters were tested by multiple and simple linear regression analysis.

5.3. Results

5.3.1. Patterns in physicochemical behaviour

Based on the evaluation of within-dam temperature and dissolved oxygen concentrations in the surface and bottom waters, the dam was characterized as monomictic i.e. well mixed in the winter and stratified in the summer with an autumn overturn (Fig 5.2). Changes in values of the *in situ* measured water quality parameters, temp, pH, turbidity and dissolved oxygen (DO), at DU and DL are presented in Fig 5.3. Mean temperature was not significantly different between sites while seasonal variation at both sites was significant ($p = 0.001$, $n = 18$). No other variables (pH, turbidity and DO) were found to be significantly different spatially or temporally. Mean temperature values were the lowest in winter (Jun-Aug 2010), started increasing in the spring (Sept-Nov 2010) and reached the peak in summer (Dec 2010-Feb 2011). The dam considerably influenced the temperature regime particularly in the summer months, which is evident from the variability of temperature difference between sites (Fig 5.3a). The temperature of the outflowing water was higher in the summer than the inflowing water. Thus in the summer, the dam was a net source of heat to the downstream water body. The variation of pH was not significant.

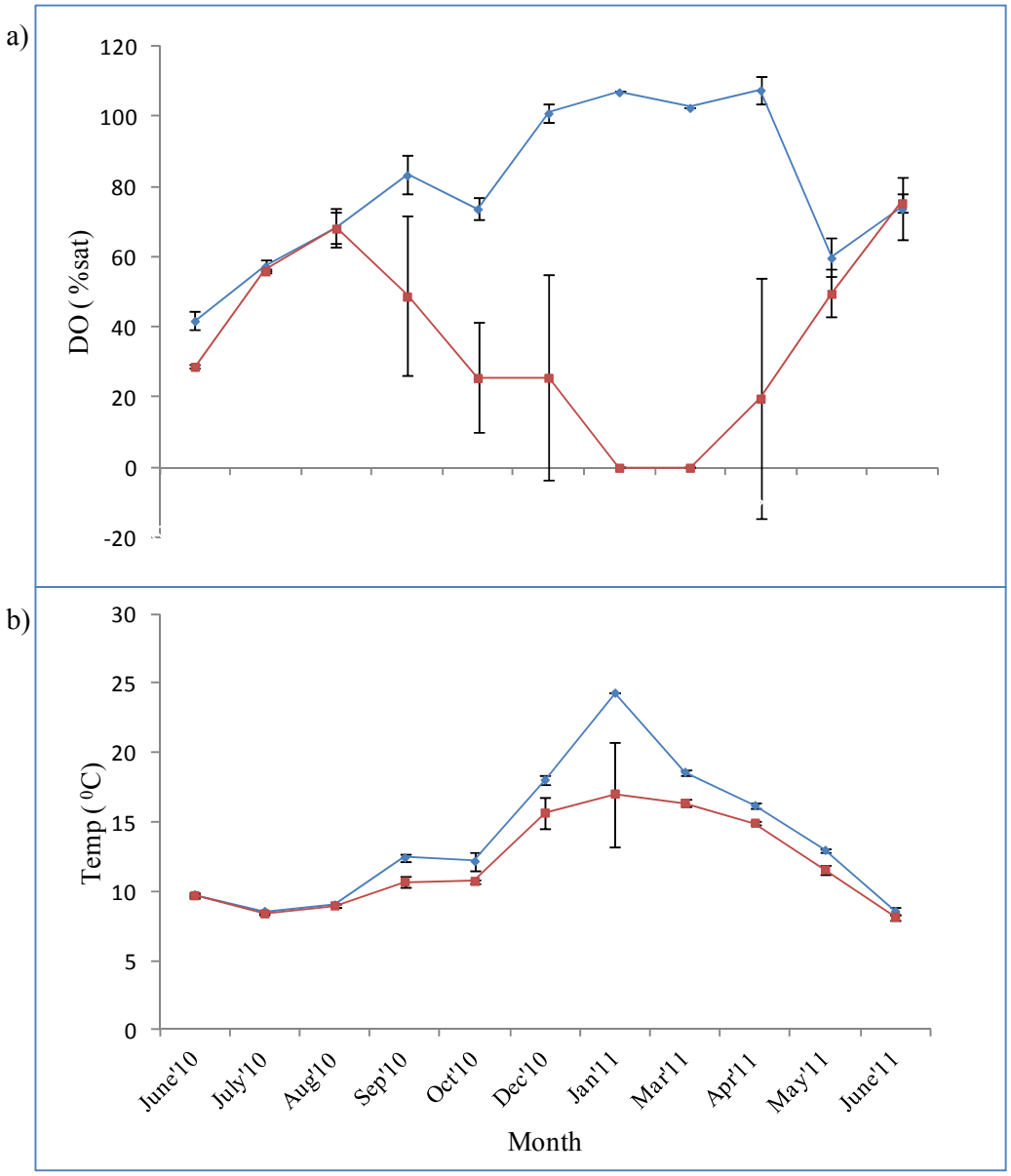

Figure 5.2. Monthly variation of a) temperature and b) dissolved oxygen (mean ± SD, n = 3) at the surface (—■—) and bottom (—■—) water within the dam from 3 sites

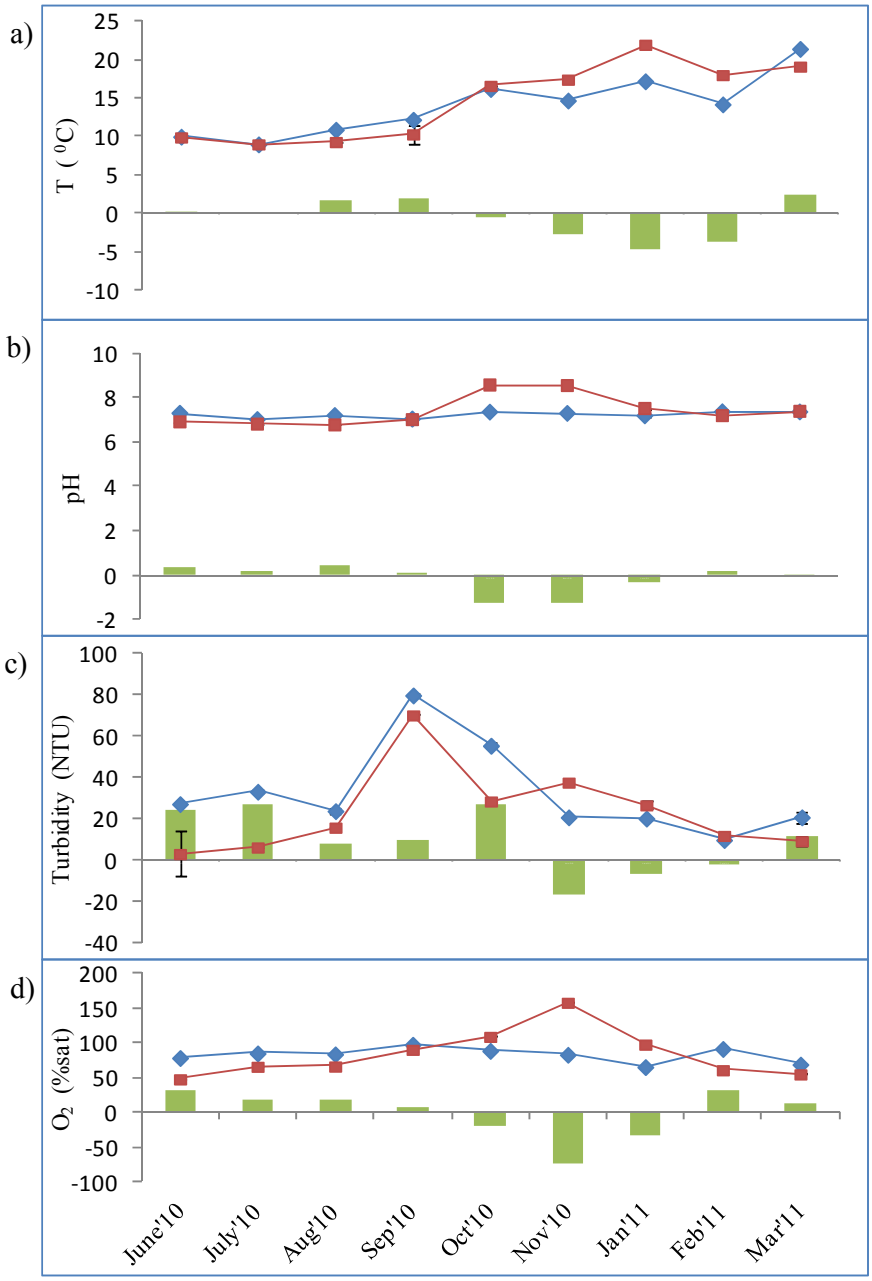

Figure 5.3. Monthly variation of water quality variables (mean ± SD, n = 5) at DU (-■-) and DL (-■-). The bar represents the difference (DU-DL). Negative values indicate higher values at DL. Error bars are too small to be seen.

The pH value ranged between 7.0 - 7.4 and 6.8 - 8.6 at DU and DL sites respectively throughout the sampling time (Fig 5.3b). The range of values recorded for turbidity was relatively wide, from 10 to 80 NTU at DU and 3 to 70 NTU at DL (Fig 5.3c). Decreased turbidity was recorded at DL compared to DU on each of the sampling dates except Nov 2010, Jan 2011 and Feb 2011 when net export was observed (Fig 5.3c). The largest decrease was 88%, occurring in June 2010 while the lowest was 12% in Sep 2010. Dissolved oxygen concentration was less variable at the inflow than at the outflow averaging 83 (±10) % saturation at DL (Fig 5.3d). The monthly mean % DO saturation was always lower at DL than DU except in Oct 2010, Nov 2010 and Jan 2011, when the outflow was supersaturated with oxygen (Fig 5.3d).

5.3.2. Variability in nutrient concentrations

5.3.2a. At the inlet and outlet of the dam

Monthly variability in the concentrations of different forms of nutrients is shown in Table 5.1. TN in the inflowing water at DU ranged from 47 to 152 µM. The higher concentrations were observed in winter (126 ± 15 µM) compared to summer (57 ± 11 µM). The NO_x concentration varied between 10-80 µM and its % contribution to TN ranged from 21 – 65%. The NH_4^+ concentration ranged between 2-12 µM and its % contribution to TN was generally insignificant (1-12%), especially when compared to NO_x, in the inflowing water throughout the 9 month sampling period (Fig 5.4a). Other forms of N e.g. particulate and dissolved organic nitrogen (PNnDON) made up the rest of the incoming TN and averaged 47 (±13) %. From 78 µM to 207 µM of TN was present in the outflow water at DL during the sampling period. At DL, the NO_x concentration ranged from 0 to 149 µM while NH_4^+ varied between 1-9 µM. At DL, the % contributions of NO_x, NH_4^+ and PNnDON to TN varied between 0-

74%, 1-5% and 22-99% respectively depending on the month (Fig 5.4a). The net change in the N forms in the dam was dominated by removal of NO_x from Oct 2010 to Mar 2011 during which period NH_4^+ was also removed (Fig 5.4a). The dam was a net source of PNnDON throughout the whole sampling period with the highest value (89 µM) observed in Nov 2010.

Total phosphorus (TP) at DU in the inflow ranged from 1.2 to 4.0 µM (Table 5.1). Total particulate and dissolved organic phosphorus (PPnDOP) typically made up about 80% of the TP and filterable reactive phosphorus (FRP) made up the rest (Fig 5.4b). Concentrations of the different forms of P were very consistent in the inflowing water irrespective of the season while seasonal variation in concentration was observed in the outflowing water at DL (Fig 5.5). At DL, average FRP concentrations were found to be 0.36 (±0.08) µM and 0.5 (±0.2) µM in winter and summer respectively and PPnDOP concentrations were 1.81 (±0.11) µM and 3.46 (±0.72) µM during the same period. A statistically significant positive correlation ($p = 0.01$, $n = 18$) between P (TP and PPnDOP) and turbidity was observed (Table 5.2).

The TOC concentration ranged from 0.5-1.3 mM and 0.5-1.7 mM at DU and DL respectively throughout the sampling period (Table 5.1). The distribution of organic carbon between DOC and POC showed a dominance of DOC at both sites (Fig 5.4c). The contribution of DOC to TOC ranged from 76 to 99% at DU and from 59 to 99% at DL over the sampling period. A gradual increase in POC concentration was observed both in the inflowing and outflowing water from Sep 2010 to Feb 2011 with higher values at DL (Fig 5.4c). Concentrations of all forms of organic carbon (TOC, DOC and POC) were higher in the outflowing water than in the inflowing water except in July and August 2010.

Table 5.1. Monthly variation of nutrient concentrations (µM) at DU (inlet) and DL (outlet)

Months	Site	TN	NH_4	NO_3	PNnDON	TP	FRP	PPnDOP	TOC	DOC	POC
June 2010	DU	126	4	55	68	3.16	0.38	2.78	820	737	83
	DL	171	9	79	84	2.16	0.32	1.84	919	902	17
July 2010	DU	140	2	79	58	3.14	0.39	2.75	676	636	40
	DL	207	4	143	60	2.23	0.39	1.85	623	613	10
Aug 2010	DU	110	3	71	36	1.68	0.48	1.20	515	508	7
	DL	201	8	149	44	2.12	0.42	1.70	514	510	3
Sep 2010	DU	152	3	80	69	4.00	0.62	3.39	729	699	31
	DL	169	5	95	70	4.37	0.57	3.79	942	744	198
Oct 2010	DU	71	4	33	33	2.74	0.49	2.26	813	704	109
	DL	84	1	24	59	2.27	0.49	1.78	1096	821	275
Nov 2010	DU	56	3	36	16	1.73	0.64	1.09	517	455	62
	DL	118	2	27	89	4.59	0.44	4.16	652	530	122
Jan 2011	DU	47	3	10	34	2.21	0.59	1.62	1258	953	305
	DL	88	3	2	84	4.50	0.53	3.97	1686	988	698
Feb 2011	DU	68	3	26	39	1.18	0.40	0.78	954	858	96
	DL	90	2	6	81	4.58	0.33	4.25	1536	921	615
Mar 2011	DU	103	12	42	49	2.03	0.54	1.48	683	661	22
	DL	78	1	0	77	1.91	0.77	1.14	804	769	35

Table 5.2. Correlation coefficients between water quality variables in the study area (n = 24). Significant correlations are highlighted in bold

	DO	pH	Turbidity	Temp	TP	TN	NH_4^+	NO_x	FRP	PNnDON	PPnDOP	TOC	DOC	POC	TN:TP	DIN:FRP	Dis	ln HRT
DO	1**																	
pH	**.726****	1																
Turbidity	**.488***	.049	1															
Temperature	.179	**.530***	-.091	1														
TP	**.536***	.178	**.633****	.105	1													
TN	-.133	-.461	.094	**-.705****	.220	1												
NH_4^+	-.374	-.351	-.169	-.070	-.247	.368	1											
NO_x	-.197	**-.569***	.090	**-.819****	-.053	**.902****	.400	1										
FRP	.333	.351	.403	.451	.281	-.283	-.277	-.350	1									
PNnDON	.160	.188	.050	.117	**.650****	.343	-.134	-.092	.127	1								
PPnDOP	**.530***	.164	**.592****	.058	**.993****	.267	-.218	-.014	.183	**.670****	1							
TOC	-.060	.166	-.081	**.525***	.341	-.375	-.281	**-.579***	-.051	.398	.349	1						
DOC	-.252	.041	-.137	.383	.139	-.305	-.144	**-.506***	-.063	.388	.150	**.890****	1					
POC	.103	.239	-.024	**.557***	.449	-.374	-.347	**-.547***	-.033	.344	.453	**.932****	**.665****	1				
TN:TP	-.419	**-.481***	-.419	**-.632****	**-.480***	**.722****	**.500***	**.803****	-.462	-.102	-.427	**-.515***	-.345	**-.570***	1			
DIN:FRP	-.318	**-.563***	-.151	**-.784****	-.154	**.895****	**.489***	**.936****	**-.521***	.013	-.092	**-.487***	-.353	**-.518***	**.886****	1		
Discharge	.016	-.311	.429	**-.651****	.083	**.649****	.161	**.723****	-.067	-.057	.093	-.451	-.448	-.384	**.489***	**.563***	1	
ln HRT	**-.806****	.307	-.427	**.688***	-.309	-.554	.378	**-.734***	.414	-.232	-.340	**.740***	.651	**.772***	.353	**-.668***	**-.724***	1

*P < 0.05 and **P < 0.01

Dis = Discharge and ln HRT = (natural) log transformed value of Hydraulic Retention Time

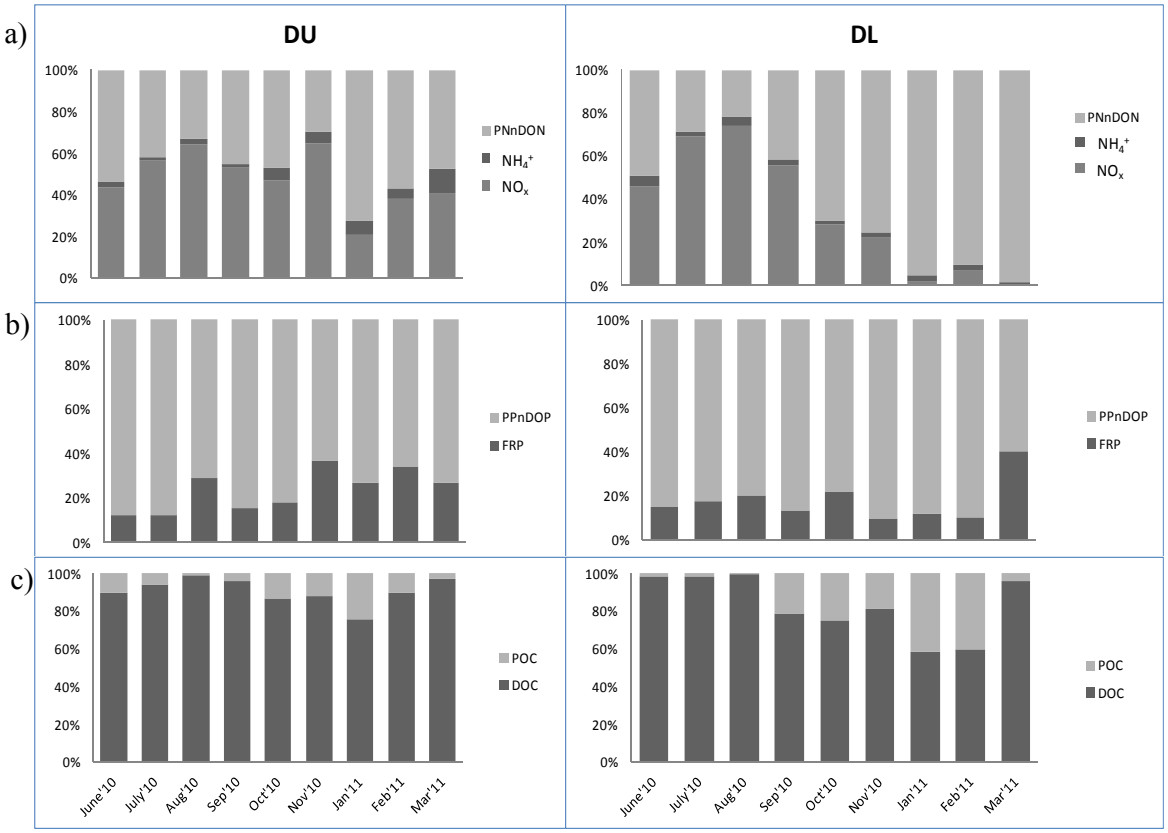

Figure 5.4. Temporal variation in % contributions of different forms to total concentrations of: a) N, b) P, and c) organic carbon at DU (left panel) and DL (right panel)

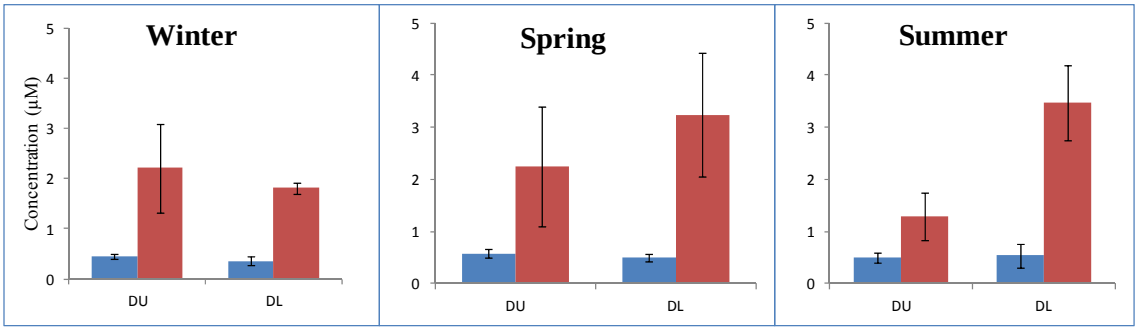

Figure 5.5. Seasonal variation in FRP ( ) and PPnDOP ( ) concentrations (µM) ± SD at DU and DL

5.3.2b. Within the dam (sediment-water exchange)

The contribution of dissolved N species (NO_x and NH_4^+) from the sediments to the hypolimnion is shown in Fig 5.6. Release of NH_4^+ from the sediment was higher during

periods of high temperature and low oxygen (Fig 5.3 and 5.6). Sedimentary uptake of NO_x was observed throughout the sampling period, with the highest demand in June 2010 and Oct 2010 (Fig 5.6) when the bottom water O_2 saturation was 29% and 26% respectively. Fig 5.6 also indicates temporal variation of sedimentary contribution of FRP to the water column. The highest release of FRP from the sediment, which was associated with net export at DL, was observed in March 2011.

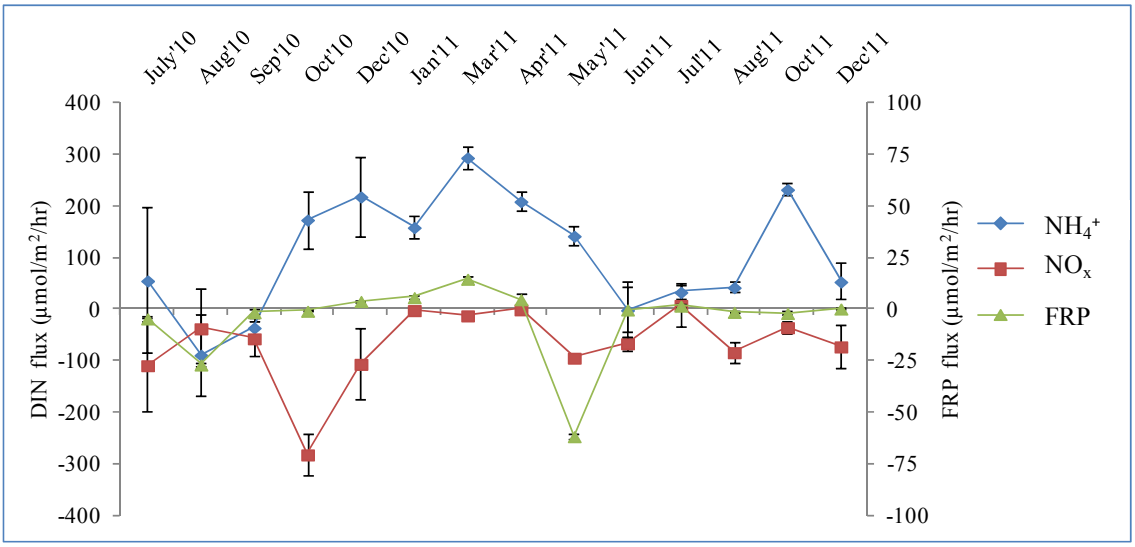

Figure 5.6. Variation of sedimentary flux (µmol/m^2/hr) ± SE (n=4) of dissolved nutrient species. Secondary vertical axis represents FRP. Negative values indicate uptake. Data obtained from monthly core incubations (Chapter 4, section 4.3.2)

5.3.3. Nutrient retention efficiency

The nutrient removal efficiency fluctuated greatly during the 9 months sampling period (Fig 5.7) but no statistically significant seasonal variation was found ($p > 0.05$). However, high fluctuations were observed in the various forms of N removal by the dam (Fig 5.7a). Higher

removal for NH_4^+ and NO_x appeared in spring and summer (Oct 2010 – Mar 2011), while in winter, net export was observed. In most sampling months the concentrations of TN and PNnDON were higher at the outlet than the inlet indicating the dam was a net source of these forms of N to the downstream water body. The retention efficiency of TP and PPnDOP was higher in early winter (June – July 2010) and then decreased (with some fluctuation) over the remainder of the sampling period (Fig 5.7b). A statistically significant positive correlations between temperature and retention efficiency of NO_x ($R^2 = 0.87$, $p < 0.01$, $n = 9$) and NH_4^+ ($R^2 = 0.76$, $p < 0.01$, $n = 9$) were observed (Fig 5.8). However, multiple linear regression between retention efficiency of NO_x and NH_4^+ and temperature resulted in statistically insignificant correlation ($R^2 = 0.66$, $p = 0.13$ and 0.84 for temperature and dissolved oxygen respectively).

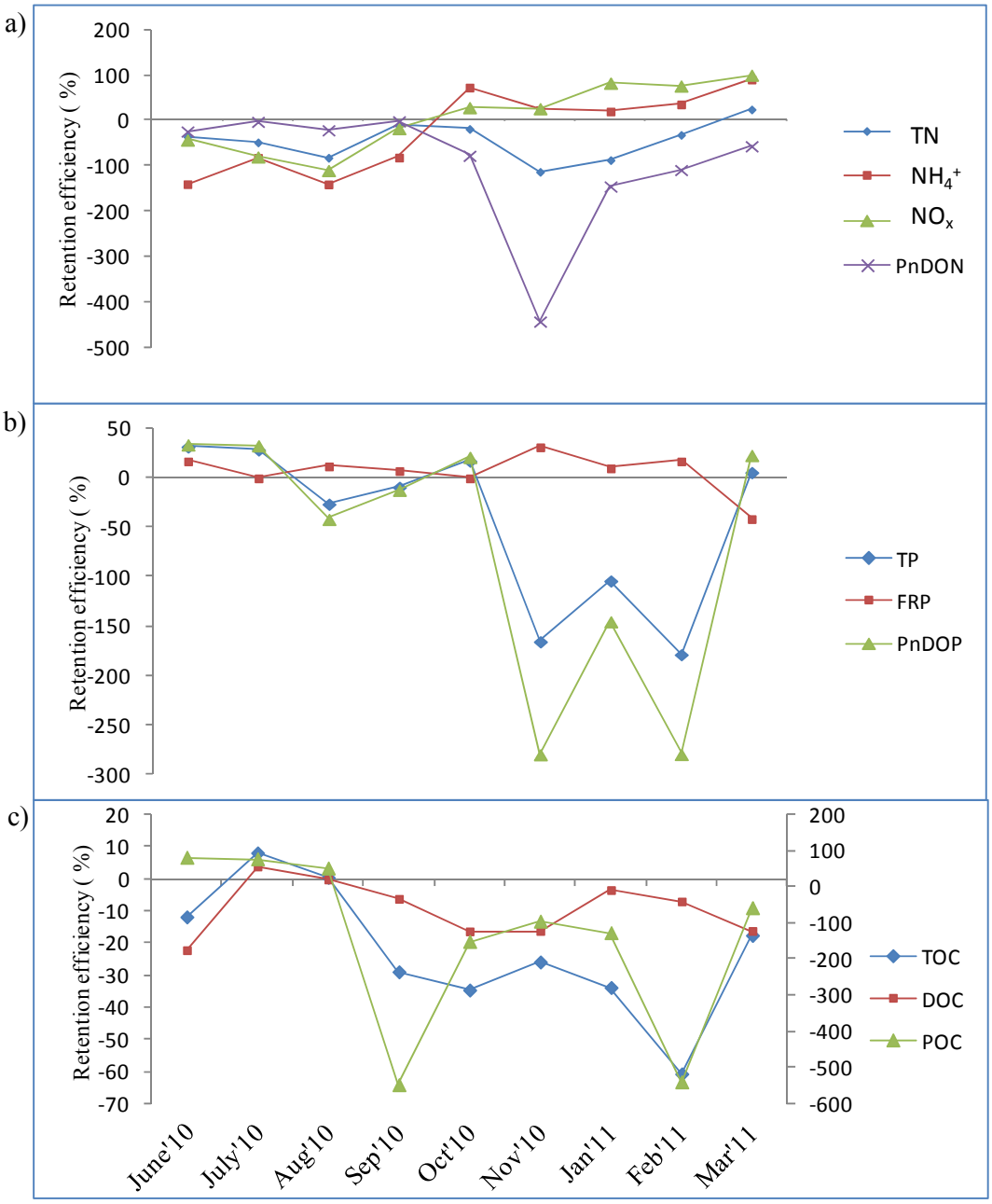

Figure 5.7. Monthly variation of retention efficiency (%) of various forms of a) nitrogen, b) phosphorus and c) organic carbon. Negative values indicate export. The secondary y-axis in c represents retention efficiency of POC

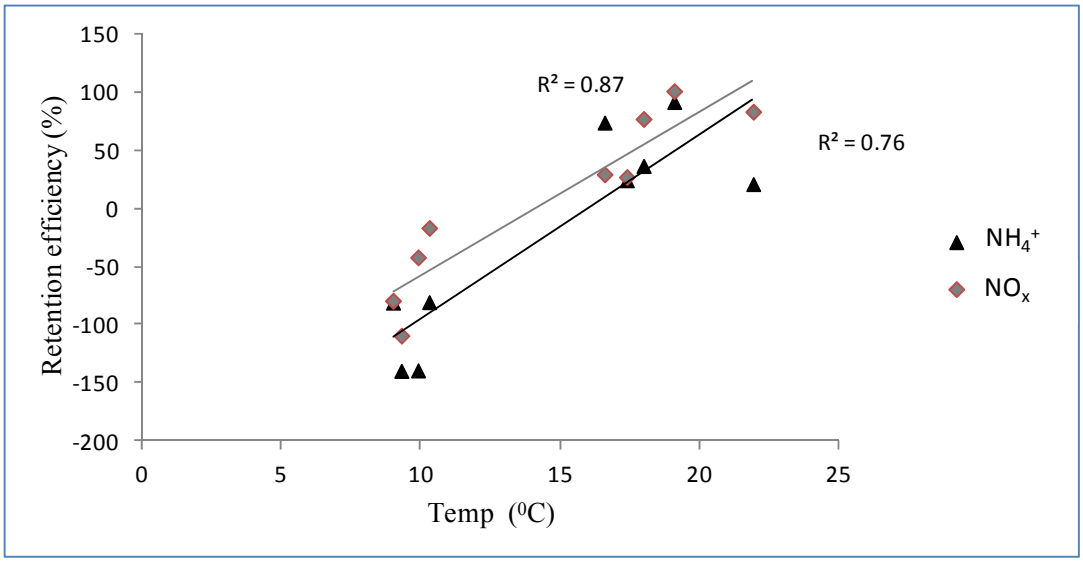

Figure 5.8. Correlation between temperature and % Retention efficiency of NO_x and NH_4^+

The highest FRP removal (31%) was observed in Nov 2010 whereas net export (41%) was observed in Mar 2011. Retention of TOC and DOC was only positive (removal from the water) in late winter (July - Aug 2010) whereas POC was removed throughout the winter months (June - Aug 2010). Retention of all forms of organic carbon (TOC, DOC and POC) then decreased resulting in downstream export with fluctuations (Fig 5.7c). Retention efficiency of TOC was lower than DOC while POC showed the most negative values.

5.3.4. N:P ratios

Both TN:TP and DIN:FRP ratios showed seasonal variation at both sites. At DU and DL respectively, the TN:TP ratio ranged from 21-66:1 and 20-93:1 while the DIN:FRP ratio ranged from 22-199:1 and 1-380:1 (Fig 5.9). These ratios were highest in winter and gradually decreased to the lowest values in summer. According to both ratios, the inflowing water at DU indicated probable P limitation throughout the sampling period, whereas at DL,

TN:TP suggested P limitation but DIN:FRP showed N limitation in summer months (Fig 5.9b).

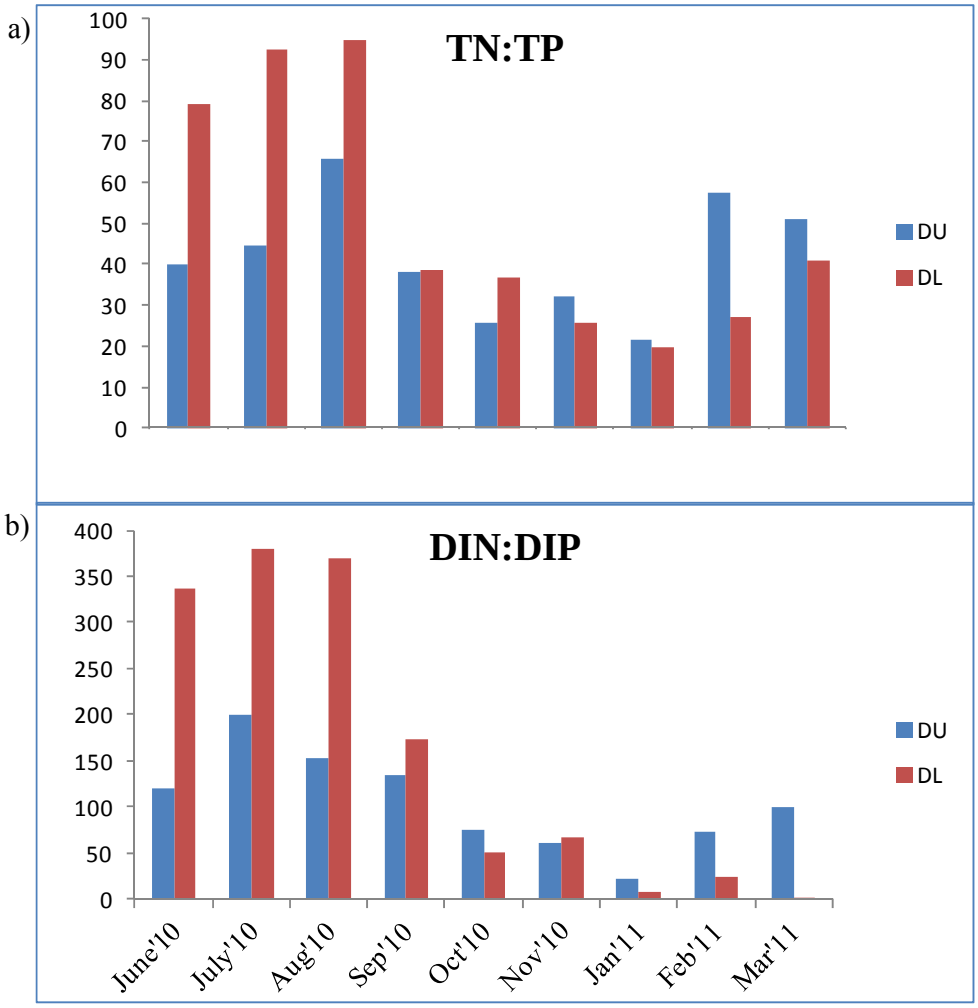

Figure 5.9. Temporal variation of molar N:P ratios at DU and DL

5.3.5. Hydraulic Retention Time (HRT)

Fig 5.10 shows the monthly variation in hydraulic retention time (HRT) and discharge. A statistically significant negative correlation was observed between HRT and: DO ($p = 0.01$, $n = 9$), NO_x ($p = 0.02$, $n = 9$) and DIN:FRP ($p = 0.05$, $n = 9$), whereas significant positive correlation was found with temperature ($p = 0.04$, $n = 18$), TOC ($p = 0.02$, $n = 9$) and POC ($p = 0.02$, $n = 9$) (Table 5.2). A statistically significant nonlinear (polynomial) relationship ($R^2 = 0.80$, $p = 0.01$, $n = 9$) was found between NO_x retention efficiency and HRT. Retention efficiency of all other forms of nutrients (N, P and C) was not significantly correlated to HRT.

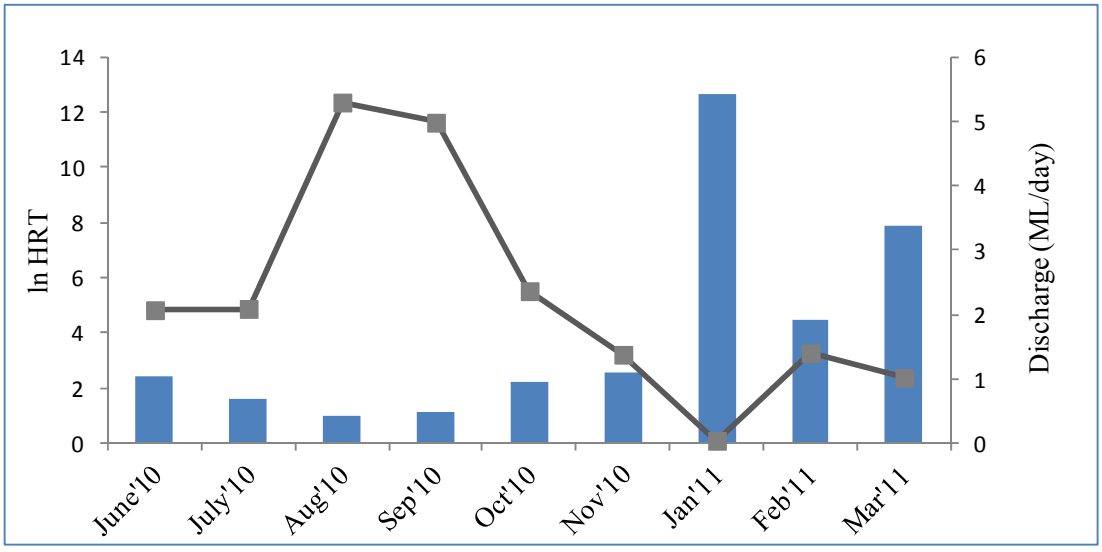

Figure 5.10. Temporal variation of hydraulic retention time (log-transformed, bar chart) and water discharge (line chart) at DL

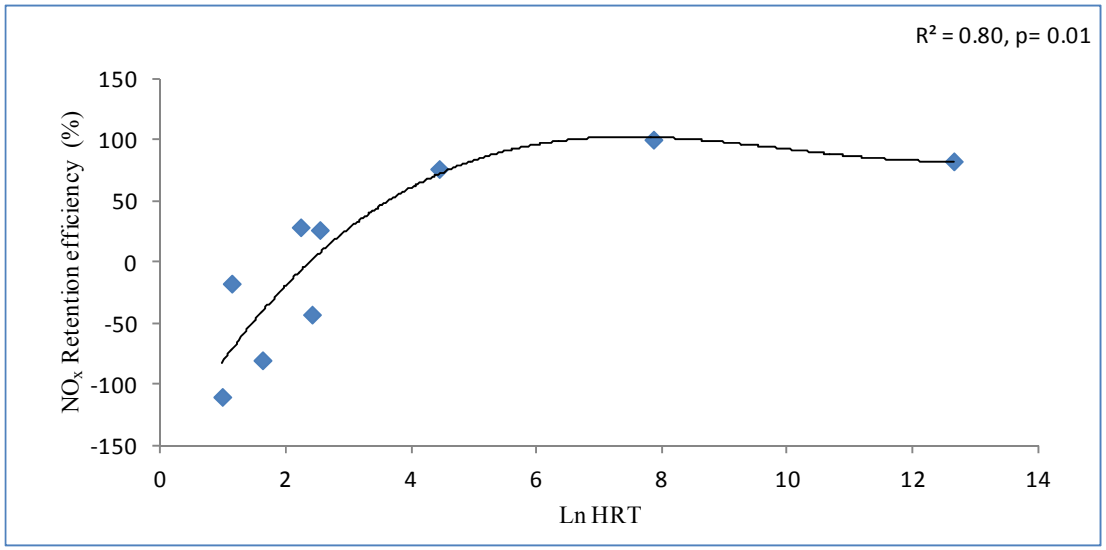

Figure 5.11. Relationship between ln HRT and NO_x retention efficiency

5.4. Discussion

5.4.1. Spatiotemporal variation in physicochemical parameters

In winter (mean air temperature 7.6 ± 1.6 ^{0}C, n = 92), the absence of thermal stratification indicated a well mixed water column within the dam. While in summer (mean air temperature 16.4 ± 3.7 ^{0}C, n = 90), with warm weather, longer HRT and thermal stratification, the surface layer of the dam heated up quickly, and delivered warmer water to the downstream water body. The dam increased the temperature of outflowing water by 1-5 ^{0}C during summer months which agrees well with the findings of other studies in North America and New Zealand (Lessard and Hayes 2003; Maxted *et al.* 2005). For example, Maxted *et al.* (2005), reported that small dams in rural catchments of Auckland region in New Zealand increased downstream water temperature by 3.1-6.6 ^{0}C during summer, which had a detrimental effect on downstream macroinvertebrate communities. Alexander (1998) also showed that a significant increase in stream temperature affected invertebrate communities below two beaver ponds in Canada. In a review of temperature effects on

phytoplankton growth and respiration, Robarts and Zohary (1987) reported that photosynthetic capacity, specific respiration and growth rate of various phytoplankton were all dependent on temperature with optimum values close to 25 ^{0}C or higher. Paerl and Paul (2012) demonstrated that at temperatures of 25 ^{0}C or higher, growth rates of eukaryotic phytoplankton decreased while growth rates of cyanobacteria reached the maximum and continued to remain high. A similar trend was also reported in temperate freshwater systems where cyanobacterial growth tends to dominate phytoplankton communities during the warmest periods (O'Neil *et al.* 2012). In this study, significant positive correlations between temperature and retention efficiency of NO_x were observed. It was also evident from the results that both NO_x and NH_4^+ were removed from the system above 15 ^{0}C. Increased removal of NO_x and NH_4^+ at elevated temperature can be attributed to assimilation via photosynthesis and/or denitrification during the summer period.

Turbidity at the outlet (DL) was lower than the inlet (DU) in most sampling months. The highest values of turbidity both at the inlet and outlet were observed in late spring. This can be attributed firstly to the fact that turbidity is caused by the presence of particulate matter i.e. clay, silt and colloidal particles in water during high flow and runoff events and secondly to spring time phytoplankton growth (Henley *et al.* 2000; Järvenpää and Lindström 2004). In summer months (Nov 2010 - Feb 2011) higher values of turbidity at the outlet than the inlet were most probably due to higher densities of phytoplankton within the dam. This is consistent with the higher values of % DO saturation observed at DL than at DU in late spring and summer (Oct 2010 – Jan 2011). The increased DO saturation at DL coincided with increased pH values during the same period and these were positively correlated. The concurrent elevation of both DO and pH values was most probably due to the photosynthetic

activity of phytoplankton, a process involving removal of inorganic bioavailable nutrients (CO_2, DIN and FRP) from the water column while increasing the DO saturation (Fairchild and Velinsky 2006; Avilés and Niell 2007). In a study of temporal variation in water quality of farm dams in NSW, Australia, Brainwood *et al.* (2004) also observed higher values of DO and pH during the summer than during autumn due to higher photosynthesis and diurnal peaks in the late afternoon just after the period of maximum photosynthetic activity. In this current study which incorporated continuous hourly DO and temperature just below the water surface, the highest DO saturation was observed in the late afternoon, supporting those observations of Brainwood *et al.* (2004) (Fig 5.12). Supersaturated values of DO (122 ± 32 %sat) during late spring and summer months were most likely due to photosynthetic activity aided by elevated temperature and solar radiation (Fig 5.13).

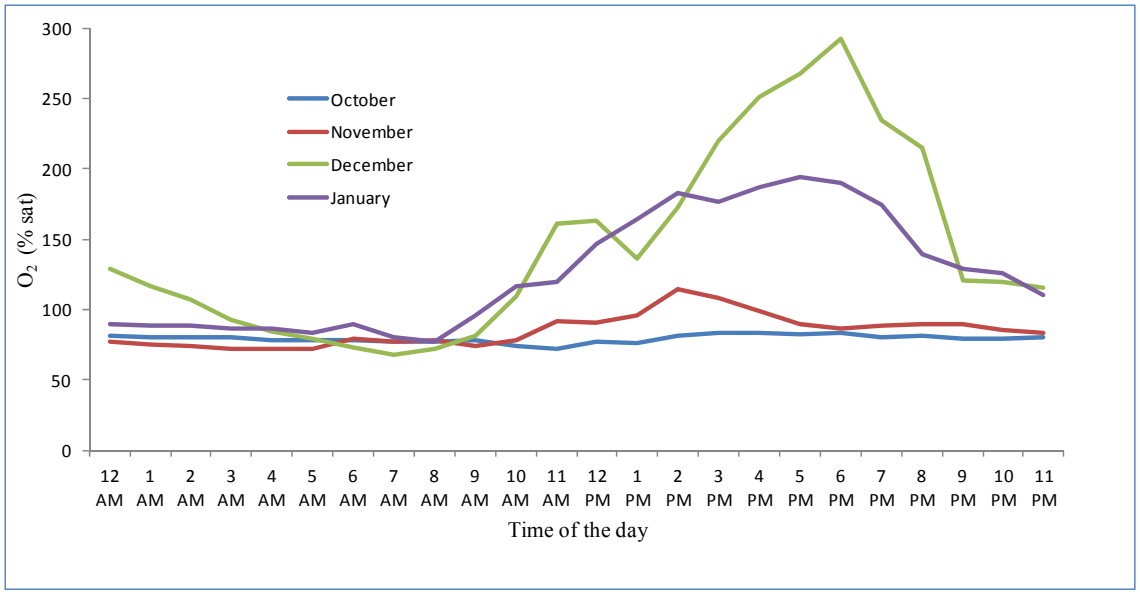

Fig 5.12. Daily variation of dissolved oxygen (% saturation) within the dam (surface layer) in different months

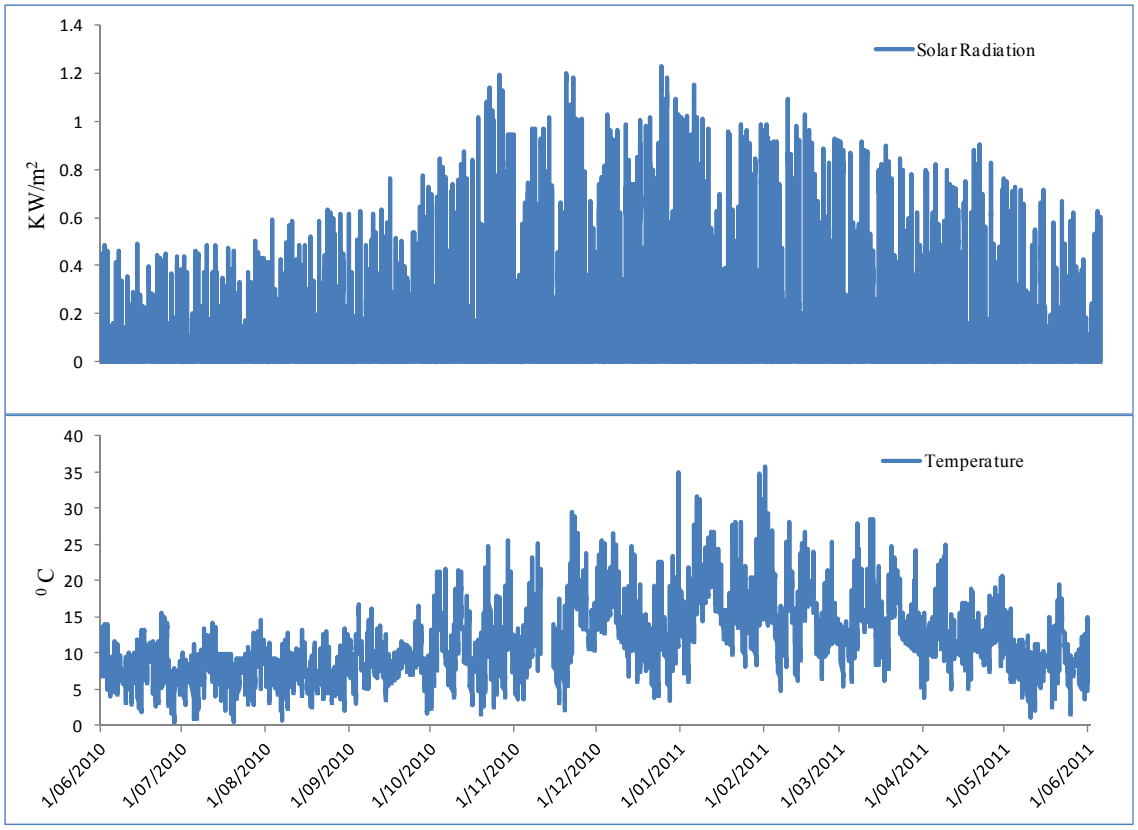

Figure 5.13. Seasonal variation of solar radiation and air temperature at the study site

The extremely high values (~ 290 %DO sat) in Dec 2010 was almost certainly due to the very close proximity of the sensor to the water surface, hence it is most likely that these extremely high %DO readings were just in the top few cms of the water column. Moreover, though the algal biomass was not measured in this study, the presence of algae at the surface water of the dam was visually observed during this period. This might be another reason contributing to the higher %DO values due to algal photosynthetic activity (Fairchild and Velinsky 2006).The lower %DO values (~ 200 %DO sat) in Jan 2011 could be attributed to higher wind speed, 1.8 ± 1.5 m/sec (n = 743) compared to 1.1 ± 1.3 m/sec (n = 743) in Dec 2010; causing more active mixing of the near-surface layer with slightly deeper water.

5.4.2. Effect of the dam on nutrient processing

The effect of the dam on nutrient processing is evident from the difference in concentrations and % contribution of various forms of nutrients upstream and downstream. N:P ratios in the outflowing water also reflected the seasonal variation in individual retention of N and P within the dam. Primary production in the dam was potentially P limited during winter (but also limited by low temperature and decreased solar radiation) when both the TN:TP (40-95:1) and DIN:FRP (121-380:1) ratios were found higher at DU and DL than the Redfield ratio of 16:1. However, higher N:P ratios were observed at DL than DU due to higher retention efficiency of all forms of P than N (all forms). During winter, the observed NO_x concentration was almost two times higher at DL (123 ± 39 µM) than DU (68 ± 12 µM). The increased value of NO_x was most probably due to nitrification as a result of well mixed oxygenated water column and NH_4^+ availability (Chapter 4, section 4.3.3, Owens 1986; Bosch *et al.* 2009). However, input from other sources i.e. rainfall and runoff from gullies and farm tracks during high rainfall events may have also contributed. Adams *et al.* (2014) demonstrated that during high rainfall events, track runoff contained higher NO_x (maximum 1500 µM) and NH_4^+ (maximum 121 µM) concentrations than concurrent stream samples (maximum 129 µM and 23 µM of NO_x and NH_4^+ respectively) due to the presence of solid and liquid animal excreta (Edwards *et al.* 2008).

The much lower DIN:FRP ratios in the outflowing water at DL (11 ± 12:1, n = 3) compared to DU (65 ± 40:1, n = 3) during the summer period is attributed to increased consumption of DIN (via assimilation and/or denitrification) when HRTs were long (Eyre 1997). NO_x can be removed permanently via denitrification whereas assimilation of DIN (NO_x and NH_4^+) by primary producers can convert dissolved inorganic nitrogen to particulate form (Fairchild and Velinsky 2006; Bosch 2008). Bosch *et al.* (2009) reported higher NO_x removal during

summer and autumn months due to the prevailing conditions (i.e. higher temperature, lower oxygen saturation) favouring biological assimilation and denitrification. Similar results have been found in a large reservoir in an agricultural area of Illinois (Wall *et al.* 2005). Higher retention of inorganic N than FRP and an increase in FRP concentration from sedimentary contributions (Chapter 4, section 4.3.2) during summer months, resulted in considerably lower DIN: FRP ratios at DL indicating a shift away from P limiting conditions. This finding agrees well with the observations of Harris (2001), who demonstrated potential N limitation of Australian freshwater systems due to longer water retention time particularly in the summer months. Higher retention of FRP within the dam in summer (20 ± 11 %) than winter (9 ± 8 %) was associated with the highest export (235 ± 77 %) of PPnDOP downstream which was most probably due to the summer time higher phytoplankton growth. Paul (2003) found similar results in pre-dams (dams located immediately upstream of reservoirs to improve water quality of the inflowing water) of a south-eastern German reservoir. He reported FRP removal as a function of phytoplankton growth during summer months and emphasized the dependency of nutrient removal on the water residence time. Within the farm dam, production of organic particulate phosphorus and sedimentary contribution from remineralisation along with external P input into the system during this time exceeded overall P removal rates, thus resulting in net export of P from the dam.

It is highly likely that during summer low flow conditions (0.82 ± 0.70 ML/day), when external loading is at a minimum, the main source of bioavailable nutrients supporting water column productivity is the mineralization of sediment organic matter (McKee *et al.* 2000). Results (Chapter 4, section 4.3.2) from sediment flux measurements for the different forms of nutrients strongly support this hypothesis. It is evident from sedimentary nutrient fluxes that sedimentary release of NH_4^+ and FRP gradually increased during the summer months reaching a peak in March 2011 when the bottom water was completely anoxic. In another

study in the same farm dam, Adams *et al.* (2014) reported that the dam was a net sink of P except for the period in March 2011 when the dam exported P. In the present study, the lowest DIN:FRP ratio (1:1) was observed at DL in March 2011 when sedimentary release of FRP was the highest (14.8 μmol/m^2/hr, Chapter 4, Table 4.2), DIN (NO_x and NH_4^+) removal within the dam was almost 100% and eventually the dam exported FRP to the downstream water body. Increased accumulation of hypolimnetic FRP can be attributed to prolonged hypolimnetic anoxia favouring release of sediment bound phosphorus to the overlying water column (Wilhelm and Adrian 2008; Grace *et al.* 2010). The dam sediment was a net source of NH_4^+ contributing about 86 kg-N/yr and the internal load of NH_4^+ was about 3 times higher than the external load (from DU) over the course of a full year (Table 5.3). However, overall (on an annual basis) the dam removed about 14% and 5% of external NO_x and FRP load respectively thus acting as a sink for the respective nutrients (Table 5.3).

Table 5.3. Comparison of annual external (from DU) and internal (sedimentary contribution) dissolved inorganic nutrient loading to the farm dam. +values indicate addition and –values indicate removal.

	External load	Internal load	%Relative contribution (internal)
Nitrogen (kg-N/yr)			
NH_4^+	32	86	73
NO_x	526	-63	-14
Phosphorus (kg-P/yr)			
FRP	10	-0.5	-5

In temperate lakes and reservoirs, planktonic biomass is generally lower in winter than summer mainly due to lower temperature, reduced sunlight hours and intensity, and reduced availability of autochthonous particulate and dissolved organic substrates (Oliver and Ganf

2000; Wetzel 2001, Davis and Koop 2006). Thus increased POC concentration at DL during spring and summer was most probably due to planktonic POC production (Cadée 1984) as phytoplankton POC can be 50% of total POC in aquatic systems (Wetzel 2001; Rukminasari and Redden 2012). Statistically significant positive correlations were observed between POC and DOC ($r = 0.67$, $p = 0.01$, $n = 18$) and between HRT and total and particulate organic carbon ($r = 0.74$ and 0.77, $p = 0.05$, $n = 18$, for TOC and POC respectively) in this study (Table 5.2) which agree well with Briggs *et al.* (1993) who found positive correlations between DOC and POC concentrations and phytoplankton productivity in two wetlands in south-eastern NSW, Australia. Cromar and Fallowfield (1997) also demonstrated positive relationships between HRT, nutrient removal and algal biomass concentration in small Scottish glasshouse ponds. They explained that longer HRT and higher C loading at elevated temperature and solar irradiance facilitated total biomass production and consequently dissolved nutrient removal. DOC and POC are closely interlinked by biological activity in the aquatic environment. DOC is derived from breakdown of POC and POC is mainly comprised of living organisms (e.g. bacteria and phytoplankton) and detrital components of those living organisms (Smith *et al.* 1992; Fisher *et al.* 1998). Therefore, heterotrophic microbial community can also utilise DOC to contribute to the overall POC (Hanamachi *et al.* 2008).

5.4.3. Effect of the dam on downstream water quality

Table 5.4 shows a comparison of state and regional water quality standards with the results obtained from the outflow of the dam. According to ANZECC/ARMCANZ (2000) for slightly disturbed ecosystems of upland rivers (>150 m altitude) in south-eastern Australia, and the Victorian Government SEPP (State Environmental Protection Policy, 2003; http://www.epa.vic.gov.au/media/Publications/905.pdf) for the Westernport regional river

and stream water quality, the dam discharged high turbidity water in 6 out of the 9 sampling months. The 75th percentile value (29 NTU) was higher than both of the guidelines. Overall, the 75th percentile pH of the outflowing water was within the acceptable limit of the guidelines whereas values in late spring were higher than the maximum permissible limits (7.5 – 7.7). Observed DO saturation values were mostly lower than (75th percentile = 62% O_2 saturation) and exceeded once (159% O_2 saturation) in late spring the standard guidelines. All TP concentrations (lowest = 59 µg P/L, 75th percentile = 135 µg P/L,) were higher than both the recommended guideline values (20 µg/L and 45 µg/L, maximum and 75th percentile respectively for ANZECC/ARMCANZ and SEPP). Outflowing FRP concentrations were mostly less than the ANZECC/ARMCANZ trigger value of 15 µg P/L, with slightly higher values in summer (up to 24 µg P/L). All through the sampling period, the most severe guideline exceedance was observed in N concentrations at the dam outlet. The lowest TN value (1192 µg N/L) observed in summer was 2-5 times higher than the ANZECC/ARMCANZ and SEPP guidelines and these TN values were even higher (up to 2900 µg N/L) during winter. Dramatic seasonal variation in NO_x concentrations was observed. The NO_x concentration in the outflowing water was about 2 orders of magnitude higher than the guideline value of 15 µg N/L in winter and gradually decreased to zero in late summer. NH_4^+ was an order of magnitude higher in winter and 2-3 fold higher in summer months than the guideline value (13 µg N/L). In the latest Australian National Water Quality Assessment (Sinclair Knight Merz, 2011), where compliance was based on ANZECC/ARMCANZ (2000), water quality in south Gippsland region was rated as "good" in terms of physicochemical variables (turbidity, conductivity and pH), "fair" for TN and "very poor" in terms of TP. It was also pointed out in that report that the lack of monitoring frequency and sufficient data hindered the assessment of actual status of the physicochemical variables and TN. Comparing the measurements from this study with the report of Sinclair

Knight Merz (2011), it was found that N was a much bigger issue than P in terms of water quality in this region.

Table 5.4. Comparison of default trigger values of key water quality variables for Victorian upland rivers (by ANZECC/ARMCANZ 2000) and Westernport regional rivers and streams (by Victorian Gov. SEPP 2003) with the observations (at DL) in this study.

Variables	ANZECC/ARMCANZ 2000	SEPP 2003	This study
Turbidity (NTU)	2 - 25	$\leq 10^{*}$	3 - 70 (29^{*})
pH	6.5 - 7.5	≤ 6.4 to $\geq 7.7^{\wedge}$	6.8 - 8.6 (7.7^{*})
DO (%sat)	90 - 110	≤ 85 to 110^{+}	49 - 159 ($62^{\#}$)
TP (µg P/L)	20	45^{*}	59 - 142 (135^{*})
FRP (µg P/L)	15		8 - 24 (16^{*})
TN (µg N/L)	250	600^{*}	1192 - 2900 (2396^{*})
NO_x (µg N/L)	15		0 - 2080 (1493^{*})
NH_4^+ (µg N/L)	13		16 - 120 (66^{*})

* = 75th percentile; ^ = 25th to 75th percentile; + = 25th to highest; # = 25thpercentile.

5.5. Conclusion

Seasonal variation was observed in water quality variables including temperature, pH, turbidity, DO and different forms of nutrients in the inflow and outflow of the dam. Temporal variation in nutrient processing within the dam had significant implications for downstream water quality. In most of the sampling months, the outflowing water failed to meet the state and regional water quality standards. The dam acted as a source of DIN (mainly NO_x) in winter and as a sink for the same in spring and summer while exporting TN and PNnDON throughout the sampling period. FRP was removed with higher efficiency in summer than winter except after the period of long stratification and hypolimnetic anoxia in Mar 2011 when there was a net export due to release of sediment-bound FRP. The dam contributed about 86 kg-N/yr of NH_4^+-N which was about 3 times higher than the annual external NH_4^+ input. The dam was found to be a net sink of NO_x, particularly under hypolimnetic hypoxic conditions removing about 14% of external NO_x input on an annual basis. Overall, the dam also acted as a net sink of FRP and removed about 5% of external FRP load. TP and PPnDOP, along with all forms of organic carbon (TOC, DOC and POC), were exported downstream all through spring and summer months when conditions (longer HRT, higher temperature and solar radiation and nutrient availability) favoured biological assimilation. The seasonal change observed for downstream N was dominated by DIN in winter (57-74% of TN) and PNnDON in summer (70-99% of TN) whereas P and C in the system was mostly PPnDOP (60-91% of TP) and DOC (59-99% of TOC) respectively, irrespective of the season. HRT was found to play an important role in controlling nutrient removal. Shorter HRT was effective for P reduction while longer HRT facilitated N, in particular NO_x, reduction within the dam. This work has clearly demonstrated that in spite of the general belief that they act as nutrient sinks (Avilés and Niell 2007), farm dams can be a major source of nutrient pollution and therefore have the potential to alter adversely the water quality of

the outflowing water. This work has also showed that monitoring seasonal variation in nutrient processing is essential to predict the overall influence on downstream water bodies and to assess the effectiveness of management actions. The impact of such an individual dam on downstream water quality may be small but the collective impacts of many individuals may be substantial and therefore warrants further research.

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Chapter Six

Conclusions and recommendations for future work

6.1. Summary and conclusion

This research was the first to investigate the effect of a farm dam in an agricultural catchment of south eastern Australia on Greenhouse Gas dynamics and nutrient retention, processing and export to the downstream water body. The following conceptual diagram (Fig 6.1) shows the important processes occurring within the farm dam ecosystem during the two very distinct periods of the year. It also identifies the important chemical species studied throughout the thesis. The diagrams 6.1a and 6.1b illustrate the various processes occurring under winter (June 2010 – Aug 2010) mixed and summer (Dec 2010 – Feb 2011) stratified water column conditions respectively within the dam.

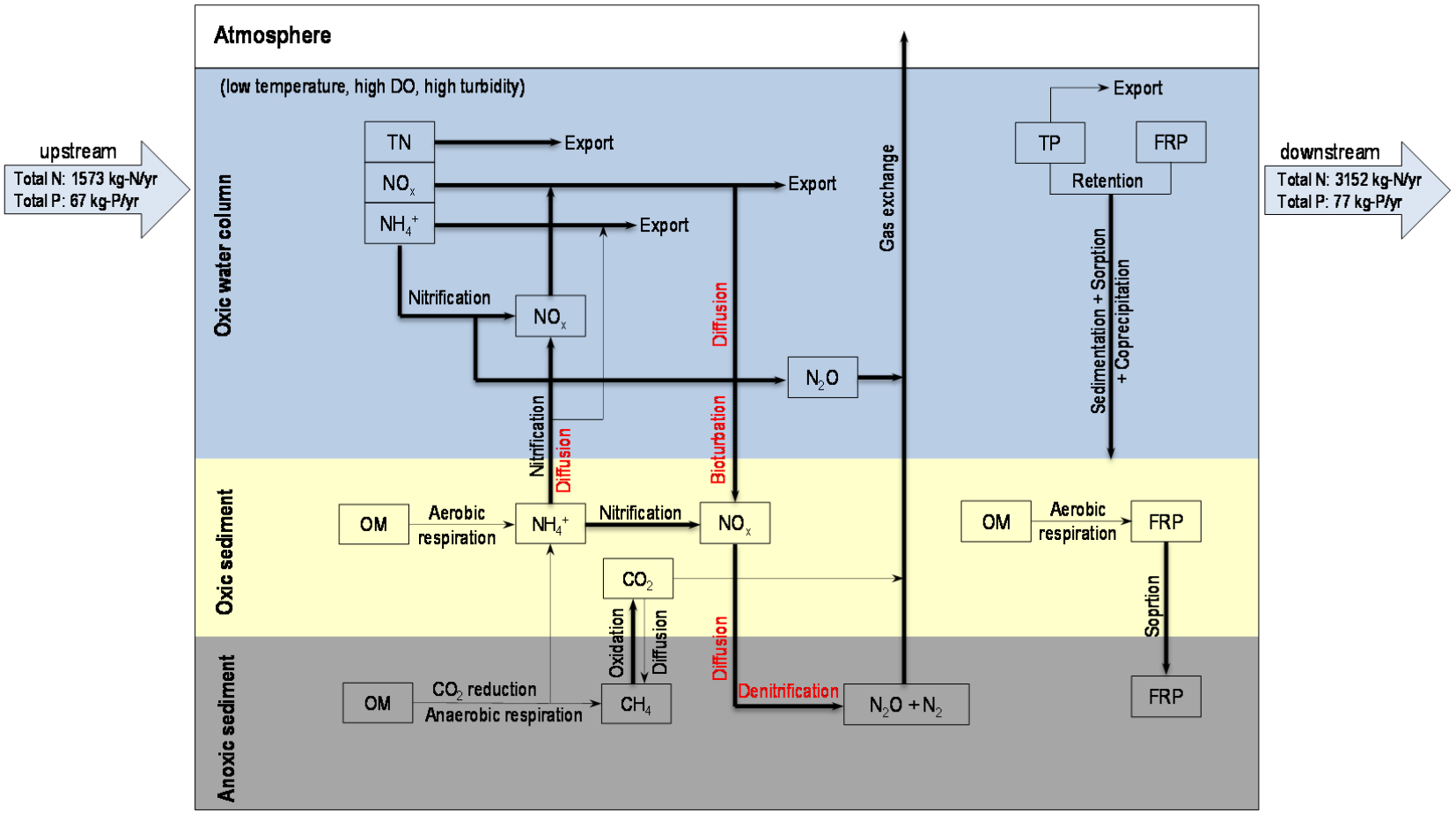

Figure 6.1a. Diagrammatic summary of key processes occurring within the dam in a) winter mixed and b) summer stratified water column conditions. Red text highlights the significant correlations between processes and thicker lines represent higher magnitude fluxes.

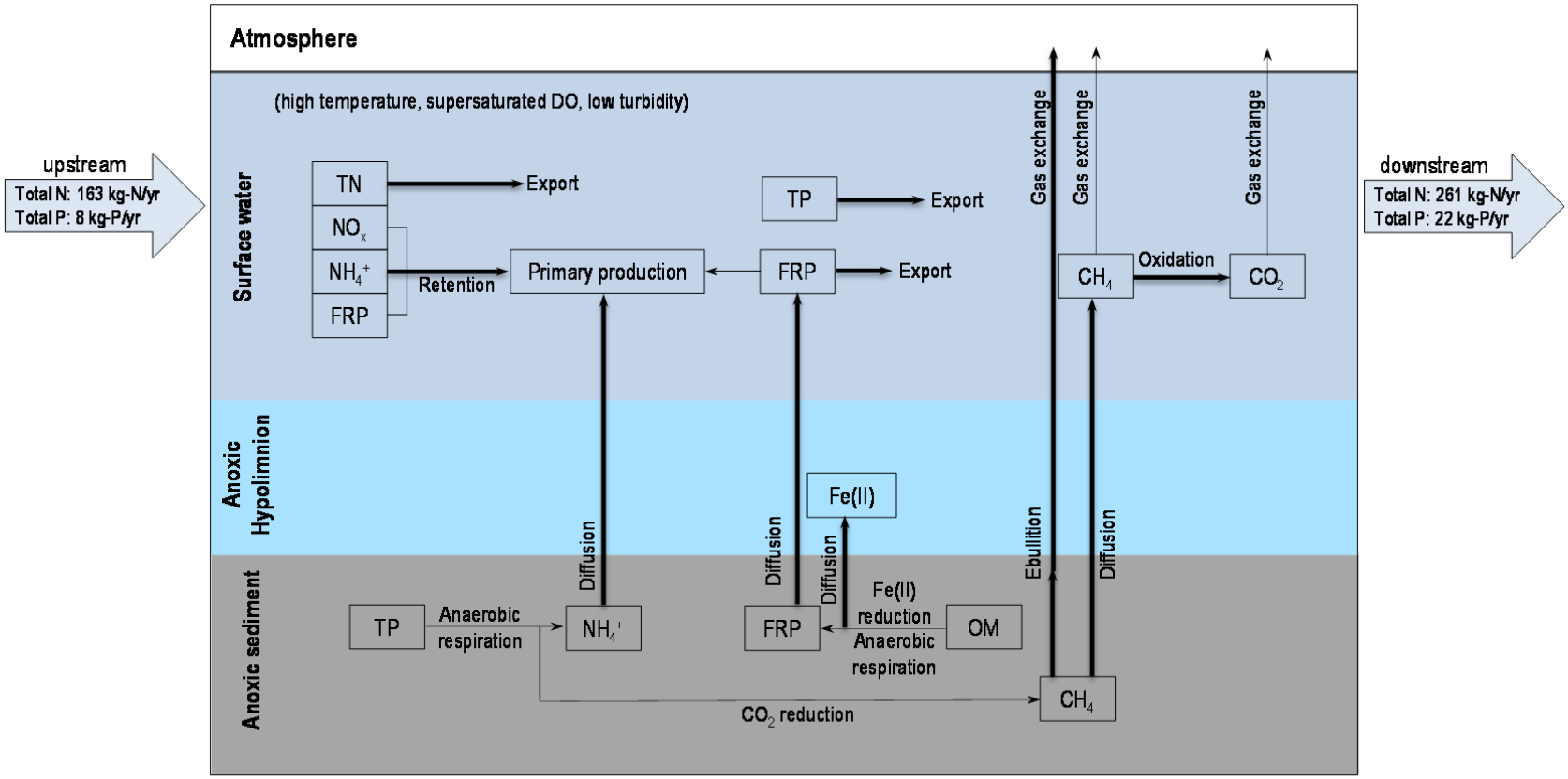

Figure 6.1b. Summer stratified water column conditions

6.2 N_2O and CH_4 gas dynamics

In this study, spatiotemporal variability in sediment release rates of both N_2O and CH_4 was observed. The highest N_2O flux was observed under a high flow condition with greater nutrient loading from the upper catchment and was associated with the highest sediment denitrification. Sediment N_2O efflux was found to be 3 times higher at the shallow site than that at the deeper sites (Chapter 3, section 3.4.1a). Similar results were observed in other studies by Martikainen *et al.* (2002) and Wang *et al.* (2006), who suggested that the variability in sediment structure and the presence of benthic flora and/or fauna were controlling the difference in the production rate of N_2O. In this study, a greater density of benthic animals (i.e. Chironomids and Oligochaetes) was observed in the shallow site than in the deeper sites at the time of highest sediment N_2O production. No significant correlation between temperature and N_2O production was observed in the present study. However, the highest sediment N_2O flux was observed at low bottom water temperature (≈ 9 °C) and oxic (60-80 %DO saturation) bottom water conditions, in agreement with the findings of Liikanen *et al.* (2002). They described that at low temperature, high O_2 concentration in the overlying water increases O_2 penetration depth into the sediment, which facilitates increased coupled nitrification-denitrification activity, eventually increasing the N_2O production rates.

In contrast, flux rates of CH_4 were higher under summer low flow and a stratified water column. CH_4 release rates were negatively correlated to overlying water % DO saturation and NO_x concentrations (Chapter 3, section 3.4.1b). Overall, NO_x availability and a well-oxygenated water column appeared to be the most influential factors inhibiting the flux of CH_4 from the sediment environment in winter. In contrast, during summer the hypolimnion was often anoxic and NO_x absent, and this promoted increased CH_4 release. A large proportion of the CH_4 diffusing into the water column was lost, mainly via oxidation to CO_2, which decreased the CH_4 concentration in the oxic surface water column. It is recognised that

sediment CH_4 production is often closely related to the rate of bubble formation/ebullition under anoxic conditions (Kiene 1991). Ebullition can be an important pathway for atmospheric CH_4 emission, avoiding the loss via oxidation in the surface water (Casper *et al.* 2000; Huttunen *et al.* 1999). This suggests that ebullition rates should be considered together with diffusive rates to better account for total CH_4 emission from aquatic ecosystems.

Like N_2O, sediment CH_4 flux rates were found to be sensitive to water column depth in this study. During summertime stratification, sedimentary CH_4 fluxes were almost 4 times higher at the deeper sites than at the shallow site. Moreover, sediment uptake of CH_4 was eventually observed at the deeper sites after prolonged stratification and prior to mixing of the water column. This is attributed to the lack of bioavailable C in the deeper sites, where CH_4 was used as an alternative energy source to support anoxic metabolism processes (Bastviken *et al.* 2003; Raghoebarsing *et al.* 2006).

The abundance of benthic macrofauna - in this location, larvae (Chironomids) and worms (Oligochaetes) - was significantly correlated to bottom water % DO saturation. They were also more abundant at the shallow site than the deeper sites. In laboratory microcosm studies, Stief *et al.* (2009) showed that the presence of benthic macrofauna, particularly Chironomid larvae, increased the production of N_2O and this was attributed to the incomplete gut denitrification of the animals. Interestingly, in this study sediment N_2O release was not sensitive to larvae and worms. However, a weak but significant negative correlation between Chironomid larval density and CH_4 flux supported the findings of Jones and Grey (2011), who reported Chironomid larvae as a medium of transporting biogenic CH_4 in freshwater food webs (Chapter 3, section 3.4.2).

6.3 Nutrient budgets and downstream export

In winter, under high flow and a mixed water column, net export of all forms of N, particularly DIN, was observed (Table 6.1). The well-oxygenated water column facilitated nitrification of NH_4^+ diffusing from the sediment, which then contributed to the overall N export (3000 ± 2000 kg-N/yr) which was about 2 times higher than the upstream input (1600 ± 700 kg-N/yr) (Table 6.1). Sediment denitrification rates were also higher in winter than summer and positively correlated to water column NO_x concentrations (Chapter 4, section 4.3.2). During winter, net N loss via denitrification was 100 ± 70 kg-N/yr and was almost 7 times higher than that in summer (10 ± 20 kg-N/yr) (Table 6.1).

Table 6.1 Seasonal variation of nutrient input (inflow), output (outflow) and denitrification ± SD

	Upstream import		**Loss via denitrification**		**Downstream export**	
	Winter	Summer	Winter	Summer	Winter	Summer
Nitrogen (kg-N/yr)						
TN	1600±700	200±200	100±70	10±20	3000±2000	300±300
NH_4^+	40±30	9±10			120±90	6±8
NO_x	900±600	60±80			2000±2000	20±20
Phosphorus (kg-P/yr)						
TP	67±12	8±7			80±40	20±30
FRP	13±9	3±3			14±10	2±3

The main mechanisms of nitrogen removal in lakes and reservoirs include sedimentation, uptake by vegetation and denitrification, of which denitrification is the most important process as it permanently removes nitrogen from the system (Seitzinger 1988; Fleischer *et al.* 1994). Therefore, it is necessary to consider the factors affecting denitrification efficiency in a given environment to achieve optimum nitrogen trapping. Denitrification often increases with increased nitrogen, particularly NO_x loading (Janson *et al.* 1994; Harrison *et al.* 2009) which was in agreement with the observation in this study. Along with the NO_x input, oxic

water column condition also play an important role in increasing the DE in the farm dam. Despite the highest DE in winter, overall downstream nitrogen export was much higher than net import due mainly to shorter water retention time (David *et al.* 2006). Therefore, maintaining an oxic water column along with longer residence time throughout the year can greatly reduce downstream nitrogen export.

A well-oxygenated (%DO > 60%) water column during winter facilitated retention of external (upstream contribution) P via sorption within the dam and also inhibited release of sediment bound P into the water column due to the formation of the Fe(III) oxyhydroxide "cap" in the oxygenated surface sediment. However, during high flow events the dam exported P to the downstream water body (Chapter 5, section 5.3.3). Overall, the dam exported 80 ± 40 kg-P/yr whereas the upstream contribution was 67 ± 12 kg-P/yr (Table 6.1).

Under low inflow and a stratified water column during summer, the external nutrient input was limited and the dam acted as a net sink of DIN and FRP while exporting TN and TP to the downstream reach (Table 6.1). The bottom water was anoxic during the period Dec 2010 – Apr 2011 and sediment NH_4^+ and FRP flux rates gradually increased (Chapter 4, section 4.3.2). Prolonged hypolimnetic anoxia further increased sediment P release via Fe reduction and eventually, net export of FRP to the downstream water body was observed. The water column of the dam was found to be N limited under such conditions. Overall, the internal loading (sedimentary contribution of 86 kg-N/yr) of NH_4^+ from the dam was about 3 times higher than the external input (32 kg-N/yr) and the dam removed about 14% and 5% of external NO_x and FRP loading over the course of a full year (Chapter 5, section 5.4.2).

In several studies faunal abundance has been shown to have substantial effect on increasing sediment O_2 penetration depth, increasing sediment denitrification rates and decreasing sediment P release (Karlson *et al.* 2007; Biswas *et al.* 2009; Zhang *et al.* 2010). In this

research, bioturbation by Chironomid larval abundance showed a significant positive effect on sediment O_2 and NO_x uptake and consequently on denitrification rates. The Chironomids were also found to reduce sediment NH_4^+ efflux (Chapter 4, section 4.4.2).

Hence, the findings from this study play an important role in improving understanding of the impact of a farm dam on seasonal variability of GHG emission and nutrient dynamics and the key factors influencing the processes. The results presented in this work have identified the following major points:

- Farm dams can be a significant source of N_2O and CH_4 to the atmosphere, but the flux rates are dependent on the season, water column depth and nutrient availability.

- In times of high external nutrient input, internal loading remains at a minimum and the dam acts as a net source of N, particularly NO_x and NH_4^+ and a sink of P. In contrast, when nutrient inputs are low and water column conditions are conducive (i.e. high temperature and low % DO saturation), sediment release rates of bioavailable nutrients increase significantly. Prolonged hypolimnetic anoxia favours sediment bound P release via Fe reduction and eventually facilitates downstream FRP export.

- Benthic macrofauna, particularly Chironomid larvae, play an important role in altering benthic nutrient chemistry due to their distinctive bioirrigation and bioturbation activity and should not be ignored while studying aquatic nutrient dynamics.

- Comparing the measurements from this study with the reports of other state and national water quality guideline for slightly disturbed ecosystems of upland rivers (>150 m altitude) in south-eastern Australia, it was found that the outflowing water from the dam regularly failed to meet the state and regional water quality standards and N was a much bigger issue (much higher ratio of measured concentration to guideline concentration) than P in terms of water quality in this region.

6.4 Recommendations for future work

There is limited information on the biogeochemistry of N and P in farm dams around the world and particularly in Australia. It is difficult to extrapolate the results from this project due to the study being carried out in one dam over the period of two years. The temporal scale of the current study is comparable to that of other published literature. However, there is a need for shorter (diurnal) and longer (extended years) term investigations in order to understand GHG and nutrient dynamics in response to day and night and to climate change respectively in farm dams. Future work to investigate GHG and nutrient dynamics in farm dams should incorporate multiple dams within and between catchments of different farming activities (i.e. crop and pasture) and climate (i.e. temperate, subtropical, tropical and Mediterranean) zones. These future steps are necessary to gain greater confidence in the generality of the findings of the present study and to determine whether the outcomes of this research are broadly generalizable to other regions. Phytoplankton can be an important source and/or sink of nutrients. Strong positive relationships between nutrient enrichment and algal biomass have been observed in many lakes and reservoirs around the world (Hecky and Kilham 1988; Smith 1998). In contrast, at the time of death and decay, decomposition of phytoplankton (predominantly through heterotrophic bacteria) recycles the nutrients back into the water column (Deborah *et al.* 1994). Therefore, it is also necessary to measure phytoplankton nutrient fluxes along with sedimentary flux for a better understanding of nutrient dynamics in the studied system.

This project focused only on the sedimentary release of N_2O and CH_4. However sediment flux of CO_2 should also be included in the future for a better understanding of overall GHG release. While the current research provides useful information regarding the sedimentary

release of GHGs, holistic understanding of emission requires concurrent investigation of atmospheric emission of these gases from the surface water. It is important to note that ebullition can be a significant pathway of CH_4 release and should not be ignored for overall estimation of a GHG budget. In this study, spatial variability of N_2O and CH_4 release from sediment was observed. The variability in sediment structure, water depth and the presence of benthic flora and fauna might be some of the reasons but further investigation is required to identify the exact cause. Hence, these steps need to be considered for the future study in farm dams and other aquatic environments for a better understanding of nutrient and GHG dynamics in these ecosystems and to facilitate more targeted and effective management approaches.

6.5 References

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Appendix 1: Raw Thesis Data

The CD-ROM attached contains the raw data collected and analysed in the preceding thesis.