Sinks of agriculturally derived nitrogen in estuarine and coastal lagoon ecosystems

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Dedication

For my grandfathers, Les and Stan.
Abstract

Increased nutrient runoff from mismanaged agricultural farming results in excess inputs of nutrients to coastal ecosystems, which can increase primary productivity causing eutrophication. Estuarine sediments provide the ecosystem service of nutrient cycling; one of the most important of which is denitrification. Denitrification is a key process of the nitrogen cycle which occurs at the interface of oxic/anoxic sediments, where bioavailable nitrate is converted to nitrogen gas and released into the atmosphere. This process filters excess nutrients, reducing bioavailable nitrogen before export to the ocean. However, the process of denitrification is still a limited area of research in New Zealand.

This thesis aims to elucidate some of the drivers of spatial and seasonal variability of denitrification predominantly focussing on a unique type of estuary, intermittently closed and open lake lagoons (ICOLLs). Firstly, the seasonality of nutrient inputs is investigated, to see what role that may play in shaping the denitrification potential of the microbial community across two contrasting ICOLLs using denitrification enzyme activity (DEA) (Chapter 2). Secondly, spatial and seasonal variability of denitrification is assessed using novel flexible chambers in situ, under pulses of labelled nitrate (K\(^{15}\)NO\(_3\)) which was identified to occur in the second chapter (Chapter 3). As well as seasonality in temperature, seasonality in the abundance of dominant macroinvertebrates also changes, and the impacts of bioturbation on denitrification was assessed in situ over a year-long seasonal study in a small ICOLL (Chapter 4). As many estuarine ecosystems are beginning to show eutrophication symptoms (such as a decrease in stable rooted macrophytes and an increase in bloom forming macro- and micro- algae), the effects of changing organic detritus on net nitrogen fluxes was examined in a low nutrient estuary, which periodically experiences blooms of algae (Chapter 5). These results are synthesised and discussed in Chapter 6.

The two studied ICOLLs (Ellesmere and Tomahawk) periodically received large inputs of nitrogen (>0.3 mg L\(^{-1}\) NO\(_3\)), which mostly occurred during spring and winter. Denitrification potential in the ICOLLs was strongly correlated with the sediment grain size, rather than near these large nutrient inflows as hypothesised. Denitrification potential was greater in sediments with higher sand content. A hierarchy of factors limited denitrification in situ. Primarily, temperature inhibited denitrification at low temperatures, occurring between 8.6 - 12°C. Following this the availability of organic
matter, shallow water depth and macroinvertebrate abundance supported increased denitrification rates. Macroinvertebrate communities supported enhanced denitrification and sediment oxygenation. *Potamopyrgus antipodarum* provided bulldozing of the upper sedimentary layers, and burrowing chironomid larvae increased the extent of the oxic/anoxic sediment interface for denitrification in deeper sediment layers.

The lability of various forms of organic carbon played an indirect role in regulating the switch between net N\(_2\) removal through denitrification, or nitrogen retention through nitrogen fixation. Cockles reduced nitrogen and sulfur enzyme activity, through changing oxygen conditions and increasing substrate supplies to denitrifying microorganisms. Minimising anthropogenic stressors such as sedimentation, managed ICOLL opening regimes, and nitrogen loading during cooler months will support positive ecosystem processes and the preservation of our unique estuarine ecosystems for future generations.
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CHAPTER ONE

General Introduction

Photo: Lake Ellesmere in situ experiments in Winter 2015.
1.1 Anthropogenic alteration of the nitrogen cycle in coastal ecosystems

Nitrogen is an essential element for life and is the fourth most abundant in the biosphere, with dinitrogen gas (N\textsubscript{2}) representing 78% of the Earth’s atmosphere (Ward, 2012). Nitrogen is a key nutrient for biological growth (Gruber, 2008), and in coastal environments, nitrate availability is important in regulating primary production (Herbert, 1999), because coastal ecosystems are often nitrogen limited (Howarth, 1988). N\textsubscript{2} is biologically unavailable to most organisms thus the distribution of N between N\textsubscript{2} and other forms of reactive nitrogen can alter the availability to organisms (Ward, 2012). The unprecedented increase in human population from the late 19\textsuperscript{th} Century has had a visible and quantifiable strain on the environment, and extreme alteration of the nitrogen cycle (Fig. 1.1). The agricultural industry has greatly expanded to support the increasing demand for food, creating large amounts of fixed nitrogen fertilizers to support this process (Fields, 2004, Galloway et al., 2004). Fossil fuel combustion to support human energy demands can emit large quantities of noxious greenhouse gases such as nitrous oxide (N\textsubscript{2}O) (Fields, 2004, Galloway et al., 2004, Townsend & Davidson, 2006). N\textsubscript{2}O is a highly efficient greenhouse gas, trapping heat emitted by Earth by absorption of infrared radiation (Vitousek et al., 1997). Nitrogen deposition to land and aquatic ecosystems have also increased due to the increased emissions of N to the atmosphere (Vitousek et al., 1997, McCrackin & Elser, 2010). Together, these industries have increased anthropogenic N production by a factor of 10 compared to the late 19\textsuperscript{th} Century (Galloway et al., 2004).

As the human requirements for food have increased, so has human’s usage of nitrogen (N) and phosphorus (P) fertilizers to increase productivity (Tilman, 1999). Over half of New Zealand’s land area is classified as farm land (Parlamentary Commissioner for the Environment, 2004), and 66% of this is dominated by sheep and beef production (Ministry of Primary Industries, 2012). In New Zealand, a 60-fold increase in the use of nitrogenous fertilizers occurred between 1961 – 2001 in line with a steady increase in irrigation (MacLeod & Moller, 2006, Ministry of Primary Industries, 2012). Excess nitrogen inputs are also coming from cattle urine, dung, and effluent sprayed onto pasture (Parlamentary Commissioner for the Environment, 2004, Howard-Williams et al., 2010). Farming practices are also adding to the pollution of the atmosphere, with 90% of New Zealand’s N\textsubscript{2}O emissions due to farming (Parlamentary Commissioner for the Environment, 2004).
Environment, 2004). The runoff of these fertilizers can have detrimental effects on downstream ecosystems, namely rivers, lakes, lowland lagoons and estuaries (McDowell & Wilcock, 2008), which has been recognised as one of the greatest environmental problems currently facing freshwaters worldwide (Dudgeon et al., 2006).

New Zealand pastoral steams are often rich in nitrates and algal biomass compared to native forest streams (Quinn et al., 1997, McDowell & Wilcock, 2008), and water quality is very poor in lowland streams in pastoral catchments, often being unsuitable for swimming (Parliamentary Commissioner for the Environment, 2004, Ministry for the Environment & Stats NZ 2017). Worldwide estuarine health is reported to be in decline, exhibiting one or more eutrophication symptoms such as elevated algal biomass (Bricker et al., 2008). The cause of eutrophication is attributed to agricultural activities, urban runoff, wastewater treatment plants and atmospheric N deposition (Bricker et al., 2008).

**Figure 1.1.** Anthropogenic alteration of the nitrogen cycle. Nitrogen is fixed from the atmosphere for fertilizer production, and the misuse of fertilizer often results in runoff into aquatic ecosystems. Excess nitrates also come from sewage and animal waste. The noxious greenhouse gas N₂O is produced through fossil fuel combustion, and emission by agriculture. Plant uptake of nitrates and the microbial conversion of nitrate to N₂ gas result in short-term removal, before fixation by cyanobacteria and bacteria, lighting or humans. Symbols courtesy of the Integration and Application Network (ian.umces.edu/symbols).
1.2 Shallow Estuarine Ecosystems

The word estuary is derived from the latin word *aestus*, meaning “of the tide” (Jackson, 2013). An estuary can be defined as “a partially enclosed coastal body of water that is either permanently or periodically open to the sea in which the aquatic ecosystem is affected by physical and chemical characteristics of both runoff from the land and inflow from the sea” (Hume *et al.*, 2007). Estuarine ecosystems encompass a range of types from almost entirely riverine influenced estuaries to those largely influenced by oceanic forces. Thus, estuaries also encompass a range of coastal ecosystem morphologies from coastal lakes and river mouths, to deep fjords. In New Zealand, estuaries have been classified into four major categories: 1. Intermittently Closed/Open Lake and Lagoon estuaries (ICOLLs), 2. Shallow, Intertidal Dominated Estuaries (SIDEs), 3. Shallow, Short Residence Time Tidal River and Tidal River with Adjoining Lagoon estuaries (SSRTREs), and 4. Deeper, Subtidal Dominated estuaries (DSDEs) (Fig. 1.2)(Robertson *et al.*, 2016).

**Figure 1.2.** Four defining estuary types dominant in New Zealand. ICOLL: Intermittently Closed/Open Lake Lagoon, SIDE: Shallow Intertidal Dominated Estuary, SSRTRE: Shallow Short Residence Time Tidal River Estuary, and DSDE: Deeper subtidal dominated estuaries (Robertson *et al.*, 2016). Lighter hatched areas denote intertidal flats.
The research presented in my thesis was predominantly conducted in ICOLLs, with one project in a SIDE, thus I will elaborate further on the characteristics of these types of estuaries types in the following paragraphs.

1.1.1 Intermittently Closed and Open Lake Lagoon (ICOLL)

Coastal lagoons are shallow brackish or marine water bodies, which are at least intermittently connected to the ocean via tidal inlets (de Wit et al., 2001, Kennish & Paerl, 2010), sometimes termed ICOLLS (Schallenberg et al., 2010) or coastal lakes (Hume et al., 2007). They generally occur on low lying coasts, parallel to the ocean (Kennish & Paerl, 2010). Worldwide there are 1477 ICOLLs identified, encompassing 3% of the estuaries globally (McSweeney et al., 2017). The freshwater inflows to ICOLLS are inadequate to sustain a permanent ocean connection, resulting in closure for extended periods of the year, with the ICOLLS becoming brackish to fresh (McSweeney et al., 2017). Under heavy rainfall events, the ICOLLS can open to the sea, causing extreme changes in salinity and water level in a short time span (hours) (Gale et al., 2006). The openings are now often managed to reduce flooding of marginal land and ICOLL openings help to flush excess nutrients (Gale et al., 2007, Schallenberg et al., 2010). Coastal lagoons are often extremely turbid environments, with wave driven sediment resuspension being common (Hume et al., 2007). Shallow lakes have been shown to predominantly occur in one of two states: (1) a clear water state in which plant life is dominated by aquatic macrophytes or (2) a turbid state in which planktonic algae dominate; the state is not always directly correlated to the nutrient status (Scheffer, 1998). The loss of macrophytes enhances turbidity due to wind-induced sediment, which further inhibits macrophytes and favours phytoplankton dominance (Scheffer et al., 1993, Hamilton & Mitchell, 1997). Macrophyte dominance is preferred in these systems, as macrophytes promote a clear water state through reducing sediment resuspension, increasing light penetration which enhances their growth, and providing habitat and a refuge from fish predation for zooplankton, which graze upon zooplankton (Scheffer et al., 1993). Unfortunately, many of the worlds coastal lakes are in degraded states, due to increasing nutrient inputs (Rabalais, 2010, Borum, 2013), and clear water ICOLLS are becoming increasingly rare.
1.1.2 Shallow, Intertidal Dominated Estuaries (SIDEs)

Shallow, intertidal dominated estuaries are the most common estuary type in New Zealand and are characterised by extensive sandflats at low tide (Robertson et al., 2016). The hydrodynamics of SIDEs are strongly driven by ocean forcing, thus the estuaries are well flushed (Hume et al., 2007). The combination of wave-induced sediment sorting and ocean forcing results in predominantly sandy habitats (Hume et al., 2007), however upper estuary tidal flats can be at risk from excess sediment and nutrient enrichment (Robertson et al., 2016). Nutrient loading to estuaries is increasing due to agricultural intensification (Heggie & Savage, 2009). Seagrass distributions in estuaries are decreasing worldwide (Short et al., 2006, Short et al., 2014), being replaced by macroalgal blooms (Cardoso et al., 2004). Seagrass decline is often due to a range of interlinking factors including increased nutrient enrichment, stimulation of macroalgal growth, and reduction of light availability (Burkholder et al., 2007).

Two of the key drivers of change in estuaries are increased loading of sediments and nutrients. Increased inputs of clays and silts smother benthic invertebrates and plants (Thrush et al., 2013). The detrimental effects of increased nutrients in coastal ecosystems has been well documented, and these effects are summarised in Figure 1.3, and outlined below. Phytoplankton blooms can have deleterious effects, such as increasing light attenuation in the waters and reducing the capacity of rooted macrophytes to grow (Cloern, 2001, Seitzinger et al., 2006, Bargu et al., 2011). Benthic microalgae can also be shaded, decreasing gross primary production (Meyercordt et al., 1999, Krause-Jensen et al., 2012). The resulting shift from macroalgae and seagrass dominated communities towards communities dominated by ephemeral macroalgae and pelagic microalgae shifts habitat quality for animals (Cloern, 2001, Borum, 2013). Shifts in the community composition of a range of species (phytoplankton, macroinvertebrates, benthic microalgae) also occurs with increasing eutrophication pressure (Pearson & Rosenberg, 1978, Andrén, 1999, Bužančić et al., 2016). As phytoplankton blooms die and decompose, oxygen is consumed rapidly from the water column by microorganisms, and hypoxia can occur in bottom waters and in sediment (Diaz & Rosenberg, 2008). The depletion of oxygen can lead to the death of fish and less tolerant benthic invertebrates (shellfish, some polychaetes) and increases the potential for the growth of toxic algae (Vitousek et al., 1997).
Estuarine habitats such as mangroves, seagrass beds and saltmarsh, are some of the most threatened habitats worldwide (Barbier et al., 2011). Reducing the abundance of these regulating habitats removes key ecosystem services they provide, such as coastal protection, erosion control, water purification, fishery habitat, and carbon sequestration (Barbier et al., 2011). As eutrophication symptoms take hold, the natural nutrient recycling capacity of the estuary may be diminished. A reduction in the amount of nitrogen directly assimilated by submerged macrophytes, increases N availability in the water column for phytoplankton or bloom forming macroalgae. Understanding the complex workings of the aquatic nitrogen cycle will help us disentangle the effects of eutrophication on the nutrient processing and cycling ecosystem services that estuaries are renowned for. Recent estimates suggest estuarine environments provide ecosystem services to the value of 28,916 US $/ha/year, with nutrient cycling a particularly important ecosystem service performed by estuaries (Costanza et al., 2014).

Figure 1.3. Schematic showing some potential effects of eutrophication on estuarine ecosystems. A healthy system is depicted on the left, and a degraded system on the right.
1.3 The aquatic nitrogen cycle

The element nitrogen can exist in more chemical forms than most other elements (Gruber, 2008), and there is a range of transformation pathways. Figure 1.4 shows how nitrogen cycles in shallow coastal sediments and overlying water, depicting the key interlinked and competing pathways for nitrogen.

![Diagram of the aquatic nitrogen cycle](image)

**Figure 1.4.** Some potential nitrogen transformations in coastal sediments. A = ammonification, DNRA = dissimilatory nitrate reduction to ammonium, PON = particulate organic nitrogen. Modified from (Stief, 2013).

1.3.1 Ammonification (A)

Particulate organic nitrogen (PON), such as dead phytoplankton cells or macrophytes, that settle onto the sediment surface are broken down over time by a range of microorganisms, releasing ammonium (NH$_4^+$), which is termed ammonification (Valklump & Martens, 1983)(Fig. 1.4). In nitrogen rich ecosystems, ammonification is a pathway adding to the eutrophication problem, by recycling organic nitrogen back into forms available to plants, setting up bottom-up controls (Kennish et al., 2014). The recycled NH$_4^+$ can be immediately assimilated, creating a detrimental positive feedback loop which creates resilience for a phytoplankton dominated system (Glibert et al., 2010). It has been reported ammonification can provide between 20 and 80% of the phytoplankton’s nitrogen requirements in shallow coastal environments (Herbert, 1999, York et al., 2010, Burkhardt et al., 2014). However, the NH$_4^+$ may be nitrified then
providing recycled nitrate for coupled nitrification-denitrification (Gilbert et al., 2003, Ferrón et al., 2009, McMillan et al., 2010). Previous studies have highlighted the variability that exists in determining the dominant nitrogen cycling pathways, which indicates further research is needed in this area (Arango et al., 2008, Ferrón et al., 2009, McMillan et al., 2010).

1.3.2 Nitrification
Nitrifying bacteria oxidise ammonium to nitrite, then nitrate, utilizing the reduction potential of ammonium to fix CO$_2$ by the Calvin cycle (Ward, 2012)(Fig. 1.4). Nitrification is carried out predominantly by autotrophic bacteria (Cebron et al., 2003) and occurs in the oxic sediment layer (Barnes & Upstill-Goddard, 2011, Stief, 2013). The initial transformation of ammonium to nitrite is extremely important in the nitrogen cycle, being the primary step between reduced ammonium and oxidised species (NO$_3^-$ and NO$_2^-$) (Helder & De Vries, 1983, Somville, 1984, Damashek et al., 2016). Spatially nitrification has been reported to be enhanced to salinity gradients leading from freshwater inflows (de Bie et al., 2002, Cebron et al., 2003) and further away from the ocean (Damashek et al., 2016). Growth optima for nitrifying bacteria (Nitrosomonas & Nitrobacter) have been reported to be between 25–35°C and 25–30°C respectively (Helder & De Vries, 1983). Nitrification and denitrification are often tightly coupled (Tomaszek & Czerwieniec, 2003, Jäntti et al., 2012), due to the close proximity of the two processes at the oxic/anoxic sediment interface (Nielson et al., 2004).

1.3.3 Denitrification
Denitrification is a respiratory microbial process predominantly carried out at the oxic/anoxic interface of sediments (Herbert, 1999, Wallenstein et al., 2006), where nitrogen oxides provide the terminal electron acceptor for respiratory electron transport (Wallenstein et al., 2006, Barnes & Upstill-Goddard, 2011)(Fig. 1.4). NO$_3^-$ can be converted in the anoxic sediment layer to biologically unavailable gaseous compounds such as dinitrogen gas (N$_2$) and the greenhouse gas, nitrous oxide (N$_2$O) by denitrifying microorganisms (Herbert, 1999, Nielsen et al., 2004, Fennel et al., 2009). N$_2$O is an intermediate product in the full denitrification pathway, and incomplete denitrification is responsible for accumulations of N$_2$O (Ward, 2012), whereas compete denitrification reduces nitrite to nitrate to N$_2$ gas (Ward, 2012). N$_2$O can also be directly consumed in the denitrification process (Xu et al., 2012), thus the global warming impact of
denitrification is dependent on whether $\text{N}_2\text{O}$ is consumed or produced (Xu et al. 2012). The ratio of $\text{N}_2\text{O}$ to $\text{N}_2$ production is influenced by the availability of $\text{NO}_3^-$ and carbon (McCrackin & Elser, 2010, Senbayram et al., 2012, Saggar et al., 2013), however the percentage of $\text{N}_2\text{O}$ produced by denitrification is still a limiting study area in estuarine sediments (Murray et al., 2015). In an unproductive boreal lake, increased carbon availability increased the $\text{N}_2\text{O}:\text{N}_2$ ratio (Myrstener et al., 2016). In agricultural sediments, when sediment nitrate concentrations were greater than 10 mM, $\text{N}_2\text{O}$ production was favoured even when labile carbon was present (Senbayram et al., 2012). Increased nitrite availability can increase nitrous oxide fluxes, reported in a eutrophic estuary (Dong et al., 2002). The combined $\text{N}_2\text{O}$ contribution of all estuarine environment types is estimated to be 0.31 Tg $\text{N}_2\text{O}-\text{N}$ yr$^{-1}$ (Murray et al., 2015), which represents less than 2% of the total anthropogenic $\text{N}_2\text{O}$ production (Denman et al., 2007, Murray et al., 2015).

Co-denitrification by fungi is another pathway that produces $\text{N}_2\text{O}$ which has recently been recognised as a major pollutant source in a urea enriched grassland ecosystem (Rex et al., 2018), utilizing two different N compounds (one N from an inorganic compound a co-metabolised organic compound) producing $\text{N}_2\text{O}$ (Phillips et al., 2016, Rex et al., 2018). Chemodenitrification (the abiotic reduction of $\text{NO}_2^-$ to $\text{N}_2\text{O}$ by $\text{Fe(II)}$ (Wankel et al., 2017) has also recently been recognized as an important source regulating $\text{N}_2\text{O}$ fluxes in redox-dynamic, organic rich coastal environments (Wankel et al., 2017). The importance of co-denitrification in aquatic ecosystems is still unknown. Accordingly, the process of denitrification is a critical component of the global nitrogen cycle, and may locally be an important buffering process (Piña-Ochoa & Álvarez-Cobelas, 2006, Fennel et al., 2009), reducing the eutrophication potential of systems with high N loads (Seitzinger et al., 2006). Under the right conditions, denitrification can potentially remove many forms of excess nitrogen in estuarine sediments when coupled to the other processes described in this chapter, supporting the removal of deposited organic material, through to reactive nitrogen species (nitrate, ammonium) (Herbert, 1999, Nielsen et al., 2004, Burgin & Hamilton, 2007, Laverman et al., 2007).

### 1.3.4 Anaerobic ammonium oxidation (anammox)

Anaerobic ammonium oxidation (anammox), is a chemolithoautotrophic process where $\text{NH}_4^+$ reduces $\text{NO}_2^-$ to $\text{N}_2$ under anaerobic conditions (Burgin & Hamilton, 2007, Devol, 2015)(Fig. 1.4). The bacteria involved in this process are thus far limited to the phylum...
Planctomycetes (Devol, 2015). The nitrite required by anammox possibly comes from the reduction of nitrate by denitrifying bacteria (Burgin & Hamilton, 2007). Unlike denitrification, anammox does not require organic carbon (Burgin & Hamilton, 2007), however they may co-exist with denitrifying bacteria which can reduce nitrate which often occur in locations with greater organic matter availability. Anammox in oceanic systems suggested to remove 1/3 to 2/3 of the sedimentary nitrogen (Dalsgaard et al., 2005). Anammox is also a direct pathway offering permanent removal of reactive nitrogen from ecosystems, however it is a much less understood pathway than denitrification (Ward, 2012).

1.3.5 Dissimilatory nitrate reduction to ammonium (DNRA)

Another direct nitrate removal pathway is dissimilatory nitrate reduction to ammonium (DNRA) (Burgin & Hamilton, 2007, Bonaglia et al., 2014)(Fig. 1.4). DNRA has been shown to be an important process competing with, or even outcompeting denitrification in shallow coastal ecosystems under certain conditions (Christensen et al., 2000, Burgin & Hamilton, 2007, Dunn et al., 2012, Giblin et al., 2013, Bonaglia et al., 2014, Nogaro & Burgin, 2014, Bernard et al., 2015, Brin et al., 2015, Hardison et al., 2015). DNRA can be the favoured process when there is high organic matter availability and low nitrate concentrations (i.e. electron acceptor limitation) (Hardison et al., 2015). DNRA bacteria reach their growth requirements at lower nitrate concentrations compared to denitrifiers (Erler et al., 2017), due to greater energy production than denitrification under conditions of high organic carbon availability compared to the availability of electron acceptors (Tiedje, 1988). If sediments are highly anoxic and sulfidic, DNRA may be enhanced relative to denitrification due to DNRA bacteria being able to oxidise reduced forms of sulphur (Murphy et al., 2016). DNRA is a less favourable pathway of nitrogen removal, as it transforms the nitrate to ammonium which remains biologically active in the system.

1.3.6 Nitrogen fixation

Nitrogen fixation is also a process occurring which can counteract the effectiveness of denitrification, by fixing atmospheric nitrogen (Anderson et al., 2014, Newell et al., 2016)(Fig. 1.4). Nitrogen fixation rates are often greater than denitrification rates in certain conditions, and often co-occur in estuarine sediments (Gardner et al., 2006, Newell et al., 2016). The ability to fix nitrogen is limited to a few groups of Bacteria and Archaea and represents a large energy expenditure (16 ATP per molecule N\textsubscript{2} reduced)
Estuarine sediments however provide anoxic conditions below the small overlying oxic layer, thus reducing the energy required to keep the sensitive nitrogenase enzyme away from oxygen (Bonaglia et al., 2014, Newell et al., 2016). Net $\text{N}_2$ fixation rates have been reported in a eutrophic estuary, however this was supported by low N levels at the time due to efficient N removal in other areas of the estuary (Rao & Charette, 2011). Although, nitrogen cycling rates have been reported to occur in ammonium concentrations as high as $200 \, \mu\text{M}$ (Knapp, 2012). N fixers can outcompete denitrifiers for poor quality organic matter sources, and conditions which support sulphate reducing bacteria may also promote N fixation (Welsh et al., 1996, Fulweiler et al., 2013).

The processes that control these nitrogen transformations in sediments are increasingly becoming a focus of nutrient management in coastal aquatic ecosystems, due to their potential influence on eutrophication. This thesis focuses on the process of denitrification, as it is an important ecosystem service and a key process in reducing the effects of anthropogenic N on the coastal marine environment.

1.2 Environmental controls on denitrification

A large amount of research has been conducted on denitrification in the last 10-20 years with advances in measurement techniques. It has been well documented that the key driving environmental factors of denitrification are sediment nitrate and organic matter supplies, sediment oxygen concentration and temperature (Piña-Ochoa & Álvarez-Cobelas, 2006, Seitzinger et al., 2006). As yet, in situ denitrification rates cannot be accurately predicted from such variables due to the interactive effects between factors. Many denitrification studies are experiments done in vitro in closed systems where enclosure effects are a major problem (Steingruber et al., 2001, Groffman et al., 2006), and this lacks the realism and inclusion of interactive effects required to elucidate key relationships and allow predictions of how denitrification in ecosystems changes in relation to environmental pressures. Below I discuss the current knowledge of the direct and indirect factors influencing denitrification rates in estuarine sediments (direct: temperature, nitrate, organic carbon, salinity, sediment particle size and microbial communities; indirect: invertebrate bioturbation and rooted macrophytes).
1.2.1 Temperature

The effects of temperature on denitrification are often unclear in some studies, due to covariance with confounding variables such as nitrate, carbon and oxygen. Thus, this review begins with temperature, and introduces the other influential variables and the interactive role temperature can play.

Denitrification rates are often increased by temperature (Veraart et al., 2011, Brin et al., 2015), when other key factors are not limiting. A number of studies focusing on temperature gradients have been carried out in the laboratory, where temperatures were carefully controlled, across a range of aquatic environments (Livingstone et al., 2000, Wang et al., 2007, Silvennoinen et al., 2008, Zhong et al., 2010, Bonnett et al., 2013). In these studies, temperature was positively related with denitrification rate (Richardson et al., 2004, Zhong et al., 2010). Temperatures below 6ºC have been shown to suppress denitrification entirely, while temperatures below 10ºC have been shown to reduce rates of denitrification (An & Gardner, 2002, Smith et al., 2003, Veraart et al., 2011). At higher temperatures ranging between 10 and 25ºC, denitrification rates increased with increasing temperature (Wang et al., 2007, Zhong et al., 2010, Barnes & Upstill-Goddard, 2011, Brin et al., 2014), when nitrate and organic carbon was available in high concentrations. Higher temperatures can also promote the creation of ammonium by mineralization (Veraart et al., 2011), providing substrate for coupled nitrification-denitrification. Some studies do however report greater denitrification rates occurring at lower temperatures (<12ºC), suggesting this is likely due to greater concentrations of limiting nutrients (nitrogen) occurring at these times (Lisa et al., 2015).

Many temperature studies are conducted using nutrient amended sediment slurries (Brin et al., 2014), whereas field studies without nutrient manipulations often do not show clear relationships with temperature. If the sediments are nutrient limited, removing the limitation would likely result in direct temperature effects on denitrification (Brin et al., 2014).

1.2.2 Oxygen

Many studies have shown microbial denitrification increases as oxygen concentration decrease (Nakajima et al., 1984, Silvennoinen et al., 2008, Chen et al., 2014). Denitrification generally occurs once oxygen concentrations drop below 0.2 mg L⁻¹ in the sediment or water column (Piña-Ochoa & Álvarez-Cobelas, 2006). Aerobic
denitrification has been shown to occur in oxygenated sediments (10% air saturation) (see review by Lloyd (1993)), however anaerobic microsites may have existed in the sediment. Fungal denitrification often dominates in the anaerobic/aerobic transition redox zone (Seo & DeLaune, 2010).

Eyre & Ferguson (2005) and Veraart et al. (2011) both reported strong relationships between temperature and respiration. Temperature directly increases respiration rates, which creates anaerobic zones within sediment particles, creating larger oxygen gradients and decreasing overall sediment oxygenation (Veraart et al., 2011). Although denitrification requires sub-oxic oxygen conditions, a decrease in the depth of oxic sediment layer can decrease overall denitrification rates, if denitrification is tightly coupled to nitrification, due to decreased nitrate supply from nitrification (Groffman et al., 2009). However, hypoxic conditions may increase denitrification in nitrate rich ecosystems driven predominantly by direct denitrification from the water column (Anderson et al., 2014). A decrease of denitrification at low temperatures may be in part due to the larger oxic sediment layer limiting nitrification then denitrification (Bonaglia et al., 2014), as illustrated by the strong relationships between denitrification and oxygen penetration depth and total oxygen uptake (which coincide with the lowest experimental temperatures) (Bonaglia et al., 2014). Thus, increased temperatures can have a negative effect on sediment oxygenation by increasing sediment oxygen consumption, reducing overall sediment oxygenation. Sediment oxygen consumption has been shown to increase linearly with denitrification rates (Eyre et al., 2013, Seitzinger et al., 1996), often driven by the deposition of organic material (Eyre et al., 2013).

The sediment oxygen penetration depth can be used to calculate the rate of gas diffusion within different sediments (Vopel et al., 2012), and sediments with greater oxygen penetration may be able to cycle nitrate faster than those with a smaller oxygen penetration, due to coupled nitrification-denitrification in the oxic sediment layer. This is supported by the findings of other studies which have shown a strong correlation between temperature and sediment respiration, and decreased sediment oxygen penetration depths at higher temperatures (Eyre & Ferguson, 2005, Veraart et al., 2011, Bonnett et al., 2013). Denitrification in waterlogged sediments is often limited by oxygen and nitrate diffusion into the sediments (Seitzinger et al., 2006) and a decrease in both nitrate supply via nitrification as well as hypoxia can inhibit coupled nitrification-denitrification (Groffman et al., 2006, Seitzinger et al., 2006, Groffman et al., 2009).
Organic matter decomposition is also strongly correlated to denitrification, due to decreasing oxygen concentrations increasing the anaerobic degradation of organic matter (Song et al., 2013), with increasing heterotrophic respiration recorded with increasing temperatures (Bonnett et al., 2013).

1.2.3 Nitrate

High nitrate loading can enhance microbial community activity and denitrification rates (Cornwell et al., 1999), and denitrifiers are able to respond to short-term changes in nitrate concentrations in the water column (<3 hours) (Kana et al., 1998). The nitrate can either originate in the water column (direct denitrification), or in the sediments from nitrification (coupled nitrification-denitrification) (Fanjul et al., 2011). Photosynthetic oxygen production stimulates the nitrification process and consequently increases the diffusion of nitrate into the bottom sediments, to fuel denitrification (Nielsen et al., 2004). The higher the concentrations of nitrate in overlying water, the greater the rate of denitrification (Nowicki et al., 1997, Pfenning & McMahon, 1997, Eyre & Ferguson, 2002, Tomaszek & Czerwieniec, 2003, Nielsen et al., 2004, Zhong et al., 2010). Often denitrification rates are highly correlated with the nitrate concentration gradients (Ogilvie et al., 1997, Dong et al., 2009, Smith et al., 2015). Sediments can be easily tested for nutrient limitations through amended sediment slurries (Pfenning & McMahon, 1997), and many studies have shown that denitrification was stimulated by nitrate amendments (Fernandes & Loka Bharathi, 2011, Pérez-Villalona et al., 2015, He et al., 2016, Teixeira et al., 2016).

Some coastal ecosystems and lakes show the greatest denitrification rates during summer, reflecting high nitrate, low redox conditions and low oxygen concentrations (Piña-Ochoa & Álvarez-Cobelas, 2006). In contrast, Zhong et al. (2010), Hasegawa & Okino (2004) and Spooner & Maher (2009) reported the greatest denitrification rate in spring, and lower rates in autumn and summer. These studies suggest that the variation in the denitrification rate was related to the higher NO$_3^-$ concentrations in the water column, which is a primary controlling factor for denitrification (Hasegawa & Okino, 2004, Piña-Ochoa & Álvarez-Cobelas, 2006). However, some ecosystems which have large nutrient supplies year-round, may show greater denitrification rates with higher temperatures, regardless of nutrient supply (Nowicki et al., 1997).
1.2.4 Organic carbon supply

Organic matter availability is one of the most important variables controlling denitrification rates in aquatic ecosystems, supporting increased sediment metabolism, oxygen demand and N remineralisation (Cornwell et al., 1999, Piña-Ochoa & Álvarez-Cobelas, 2006, Inwood et al., 2007, Dodla et al., 2008, Fulweiler et al., 2008, Loken et al., 2016). Natural organic matter is the largest reactive reservoir of reduced carbon on Earth (Hedges, 1992, Bianchi, 2011), and organic matter additions to estuarine environments can occur from both allochthonous (terrestrial and river sourced) and autochthonous (estuarine and marine) sources (Bauer & Bianchi, 2011).

A large number of studies found organic carbon to be positively correlated with denitrification rates (García et al., 1998, Livingstone et al., 2000, Teixeira et al., 2010). Previous studies have demonstrated that organic matter deposition from phytoplankton (Fulweiler et al., 2007, Fulweiler & Heiss., 2014) or fish feed (Babbin et al., 2016) drives increased denitrification fluxes. The growth and abundance of heterotrophic bacteria is correlated strongly with the C supply (Burkhardt et al., 2014), with both the quality as well as quantity being important in regulating the overall remineralization process, however studies investigating the quality or quantity are limited (Eyre et al., 2013). It is well documented that organic matter is one of the key variables driving spatial variability in denitrification (Piña-Ochoa & Álvarez-Cobelas, 2006, Seitzinger et al., 2006). The quality (C:N ratio) of organic matter has been reported to control denitrification in a range of spatial field studies (Eyre et al., 2013, Burkhardt et al., 2014, Belley et al., 2016), however limited studies have experimentally tested a range of organic matter source materials (Her & Huang, 1995, Schmidt & Clark, 2013, Grau-Martínez et al., 2015). Organic molecules of low molecular weight are suggested to be more labile thus accessible to the microbial community (Erler et al., 2017), due to bioavailability decreasing with increased complexity of organic molecules (Riekenberg et al., 2017). At lake or coastal sites near river mouths that deliver episodic loads of nitrate during floods, the loads of particulate and dissolved organic carbon supply may also peak (Bauer et al., 2013). Sites near such river mouths may therefore remove a larger proportion of nitrate compared to other locations, due to large increases in bacterial production with mixing water types (Farjalla, 2014) and inputs of dissolved organic materials (Bianchi et al., 2014).
When organic contents reach high concentrations and oxygen conditions are deplete, sulphides can accumulate in the sediments, either directly inhibiting denitrification (Plummer et al., 2015) or blocking nitrification, thereby decreasing coupled nitrification-denitrification (Joye & Hollibaugh, 1995). However, when sulphide concentrations are high, rates of DNRA can be enhanced by providing a supply of electron donors (An & Gardner, 2002, Bernard et al., 2015).

1.2.5 Salinity
Fluctuating salinity levels in estuarine environments may play a role in NH$_4^+$ availability in the sediments for coupled nitrification-denitrification. At higher salinities, NH$_4^+$ is expected to diffuse out of sediments faster, due to cation exchange site blockage by seawater cations (Boatman & Murray, 1982, Gardner et al., 1991). This can decrease denitrification fuelled through coupled nitrification-denitrification due to decreased NH$_4^+$ availability (Rysgaard et al., 1999). Studies on sediment slurries by Seo et al. (2008) have shown saline intrusions to have an inhibiting effect on denitrification potential, where decreasing the salinity from 36 to 0 could increase the denitrification potential up to 30.8%. Porewater measurements of NH$_4^+$ by Boynton & Kemp (1985) found that along a salinity gradient, NH$_4^+$ concentration in the sediments decreased sharply with increasing salinity. These results are supported by Anderson et al. (2014) and Marks et al. (2016) who found denitrification to be strongly negatively related to increased salinity. Suppression of denitrification at high salinity is likely also due to physiological stress on the denitrifying microorganisms, and extended lag times reported between salinity increases and N$_2$O production suggest that the microbial community can adapt physiologically or by a change in taxonomic composition (Marks et al., 2016). Greater abundances of key nitrogen cycling genes (nirS; nitrate reductase gene) have been shown to be greater in areas with lower salinity (Francis et al., 2013). Comparatively, pulses of freshwater have also been shown to have an inhibitory effect on denitrification (Marks et al., 2016), where extended freshwater conditions on both salt marsh and fresh marsh sediments resulted in lower denitrification potential compared with that of brackish water conditions, possibly due to inhibiting the microbial community adapted to saltier conditions.
1.2.6 Sediment particle size

Sediment particle size is known to influence denitrification capacity, where anoxic microsites are more prominent in siltier sediments than in sediments dominated by larger particles such as sand, gravel, and cobbles, due to the inhibition of oxygen diffusion through finer sediments (Solomon et al., 2009). Sediment water content (porosity) has been positively related to denitrification rates in both river and soil ecosystems (Groffman & Tiedje, 1989, García et al., 1998). Fine textured soils have a greater water content, whereas course textured soils hold a lower water content (Groffman & Tiedje, 1989). The smaller particle size in fine textured soils holds water more tightly, and anaerobic zones occur more easily, compared to the larger pores of courser sediments (Groffman & Tiedje, 1989). Soil water content has also been shown to have a positive linear effect on microbial respiration (Cook & Orchard, 2008). Smaller sediment grain sizes are also known to contain a greater content of organic matter (Vance-Harris & Ingall, 2005), providing the perfect niche for denitrifying organisms.

1.2.7 Microbial communities

Denitrifying microorganisms cover a wide range of phyla which have a variety of physiological traits (Zumft, 1997, Lee & Francis, 2017). Most denitrifiers are aerobic heterotrophic organisms which reduce carbon to nitrous oxide under anaerobic conditions (Zumft, 1997). Due to the wide abundance of groups able to denitrify, a variety of genes that play a role in denitrification are used to estimate activity of the microorganisms. The genes that code for dissimilatory nitrite reductase are often studied (nirK and nirS) as this enzyme catalyses the first step in the denitrification reduction pathway – converting nitrate to nitric oxide (Lee & Francis, 2017). Another key gene often studied is nitrous oxide reductase (nosZ) (Lisa et al., 2015). The abundance of the key genes (nirS and nosZ) is often strongly linked to denitrification rates showing their importance in controlling the magnitude of denitrification (Dong et al., 2009, Lisa et al., 2015, Smith et al., 2015). In some studies, these genes do not correlate to denitrification and explanations are because the genes are not expressed or because of low substrate availability for the enzymes (Babbin et al., 2016).

The genes responsible for nitrogen cycling are spatially variable in sediments (Zheng et al., 2015). A study showed nirK to be negatively related to sediment N, temperature and salinity, and nirS to be negatively related to sediment N and positively related to water.
column NO$_3^-$, with peak abundances in spring in line with peak NO$_3^-$ concentrations (Lee & Francis, 2017). Similarly Smith et al. (2015) and Zheng et al. (2015) found estuarine nirS abundances increased with increasing nitrate concentrations spatially. Salinity has been shown to be a strong structuring pressure for denitrifying genes, where genes are greatest in lower salinities (Francis et al., 2013, Zheng et al., 2015).

1.2.8 Invertebrate bioturbation (indirect factor)

Benthic invertebrates which interact with the sediment can play a significant role in enhancing denitrification. During the benthic infauna’s burrowing activities, they actively or passively introduce oxygenated overlying water into their burrows for respiration. This irrigation increases oxygen penetration, creating sediment oxic zones around the burrows (Fig. 1.5) (Svensson, 1997, Aller, 1998, Gilbert et al., 2003, Nizzoli et al., 2007, Stief et al., 2009, Shang et al., 2013).

![Figure 1.5](image_url)

**Figure 1.5.** Ecosystem engineering by sediment infauna, influencing benthic nitrogen cycling in aquatic ecosystems by extension of the oxic sediment zone (modified from Stief, 2013).

The oxic zones increase the extent of the sediment-water interface, and the burrows become sites of high bacterial density and high metabolic activity compared to the surrounding sediment (Svensson, 1997, Nogaro et al., 2008), due to enhancement of the resource supply to the sediment microbes (Biswas et al., 2009, Banks et al., 2013, Shang et al., 2013, Poulsen et al., 2014). The inner walls of the infauna burrows may be fairly
permeable to water and cause significant water transfer through the surface layers, thus further extending the sediment oxygenation (Aller, 2001, Olafsson & Paterson, 2004). The continuous renewal of oxygen also stimulates nitrification, mobilizing nitrate to deeper sites for denitrification (Stief, 2013). Denitrification is enhanced, through coupled nitrification-denitrification, due to the extension of the oxic layer (Carpenter & Capone, 1983, Biswas et al., 2009). These benthic organisms also play an important role in stabilizing the upper sedimentary layers, maintaining oxygen and nutrient supply to lower layers (Bartoli et al., 2009). In shallow coastal lagoon ecosystems larval chironomids (Chironomidae) are a dominant benthic invertebrate, which have been shown to greatly enhance sediment respiration (Baranov et al., 2016) and denitrification (Svensson & Leonardson, 1996).

Burrowing organisms can increase organic matter remineralization and greatly increase the flux of dissolved nitrogen species (D'Andrea & DeWitt, 2009, MacTavish et al., 2012). Surface bioturbators such as snails may directly and/or indirectly enhance microbial processes, decomposition rates and sediment oxygenation (Jones et al., 1994, Pelegrí & Blackburn, 1994, Kristensen, 2008, Dunn et al., 2009). Amphipods have previously been reported to influence benthic metabolism (Dunn et al., 2009), and increase overall mineralization processes and denitrification due to burrow creation and sediment irrigation (Pelegrí & Blackburn, 1994). Oligochaetes can stimulate denitrification (Chatarpaul et al., 1979, Pelegri & Blackburn, 1995, Nogaro & Burgin, 2014) and an experiment using oligochaete densities of 27,000 ind. m$^{-2}$ found that they stimulated water column-supported denitrification by 64% (Svensson et al., 2001).

1.2.9 Rooted macrophytes (indirect factor)

The relationship between denitrification and rooted macrophytes is highly variable, with some studies showing low denitrification rates surrounding rooted macrophytes (Sundback et al., 2000), while others show greater denitrification rates in these areas compared to bare sands or muds (Nizzoli et al., 2014). It is suggested that the variation in denitrification rates reported from rooted macrophyte studies could be methodological, where the isotope pairing technique can underestimate total denitrification due to incomplete mixing of the labelled isotope in the root layers (Eyre et al., 2016). Keeping this in mind we discuss the reported studies below.
In sediments colonized by benthic macrophytes, rates of denitrification are generally low, due to competition for nitrate and sediment oxygenation through the roots, decreasing the denitrification potential (Sundback et al., 2000, Nizzoli et al., 2014). Both filamentous macroalgae and rooted macrophytes can directly compete with denitrifiers for water column nitrate (Carriquiry et al., 2016) and a study by Olsen et al. (2017) showed that in shallow pond mesocosms, an added pulse of nitrate was predominantly assimilated by producers, bypassing the denitrifying community. However, in their research, Nizzoli et al. (2014) found that a rooted macrophyte Potamogeton pectinatus increased denitrification rates compared to areas colonized with microphytobenthos. It has been shown that aquatic plants can directly increase denitrification through deeper oxygenation of the surrounding sediments, increasing the area for nitrification (Cornwell et al., 1999, Herbert, 1999, Saunders & Kalff, 2001, Veraart et al., 2011). Macrophytes can also indirectly stimulate denitrification through enhancing sedimentation, trapping organic matter and increasing sediment metabolic rates (Cornwell et al., 1999, Groffman et al., 2009). A recent study by Reynolds et al. (2016) estimated that a 1715 ha seagrass meadow could remove up to 170 ton of nitrogen per year, along with sequestering carbon in sediment at 630 tons per year. Seagrass beds (Zostera spp.) in three Australian water bodies were reported to remove between 22-327 t N yr\(^{-1}\) through direct denitrification (Eyre et al., 2016), driven by high sediment respiration increasing availability of NH\(_4^+\) from ammonification, changing redox conditions and increasing electron donors (Eyre et al., 2013).

### 1.4 Measuring denitrification

There are several ways to measure denitrification and each method has its advantages and disadvantages (Table 1.1). Denitrification is extremely problematic to measure, due to the available methods either changing substrate conditions, disturbing the physical setting of the process, lacking sensitivity or they are time consuming and expensive (Groffman et al., 2006). Due to this, measurements of denitrification are often sparse, unreliable and can vary substantially over small spatial scales (Boyer et al., 2006), and the patchiness of denitrification over small spatial scales makes scaling up a challenge (Thrush et al., 2017). Denitrification rates in bottom sediments are difficult to measure accurately because several reactions (nitrification, denitrification, N-fixation, and nitrate reduction to ammonia) (refer to Fig. 1.4) are occurring simultaneously in an aquatic system (Tomaszek & Czerwieniec, 2003). It is very difficult to measure the main end
product (N$_2$) due to its high background concentration in the environment (Groffman et al., 2006). Of the five approaches in Table 1, I chose to utilize three approaches for various reasons, which will be discussed in detail below.
Table 1.1. An overview of the current methods available for measuring denitrification.

<table>
<thead>
<tr>
<th>Method</th>
<th>Approach</th>
<th>Application</th>
<th>Time scales</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylene Inhibition Technique</td>
<td>Blocks N₂O conversion to N₂, measure N₂O accumulation</td>
<td>Intact cores, Sediment slurries</td>
<td>1-24 hours</td>
<td>Easy, indirect measurement</td>
<td>Underestimates denitrification</td>
<td>(Groffman et al., 2006) (Steingruber et al., 2001) (Seitzinger et al., 1993) (Cornwell et al., 1999)</td>
</tr>
<tr>
<td>Isotope Pairing Technique</td>
<td>Follow labelled N species in time course</td>
<td>Intact cores, Benthic flux chambers, In situ enclosures, Flow-through systems</td>
<td>1-5 hours</td>
<td>Direct &amp; accurate measurement</td>
<td>Large number of assumptions to test</td>
<td>(Groffman et al., 2006) (Steingruber et al., 2001) (Seitzinger et al., 1993)</td>
</tr>
<tr>
<td>Direct N₂/N₂:Ar</td>
<td>Measure time course changes in gas ratios</td>
<td>Intact cores, Benthic chambers, In situ water samples</td>
<td>&lt; 12 hours</td>
<td>Measures direct denitrification end product</td>
<td>Only measures net N₂ flux Greater than 50% O₂ saturation</td>
<td>(Groffman et al., 2006) (van Luijn et al., 1996) (Poe et al., 2003) (Seitzinger et al., 1993)</td>
</tr>
<tr>
<td>Mass Balance</td>
<td>Measure all N fluxes, calculate denitrification by difference</td>
<td>Estimation using literature values, Direct in and output source measurements</td>
<td>-</td>
<td>Shows relative importance of denitrification</td>
<td>No information on spatial and temporal dynamics</td>
<td>(Groffman et al., 2006) (Cornwell et al., 1999)</td>
</tr>
<tr>
<td>Stoichiometric Approaches</td>
<td>Difference between C or O₂ based N remineralisation &amp; net DIN flux</td>
<td>Intact cores, Benthic chambers</td>
<td>-</td>
<td>Can be used for large scale (oceanic) estimates</td>
<td>Elemental ratios can change in freshwater phytoplankton</td>
<td>(Groffman et al., 2006) (Cornwell et al., 1999)</td>
</tr>
</tbody>
</table>
1.4.1 Acetylene Block Technique

The acetylene block method is a relatively easy measurement of denitrification (McCrackin & Elser, 2010), and one of the most common reported in the literature. This method uses acetylene (C$_2$H$_2$) to inhibit the final step of denitrification, the reduction of N$_2$O to N$_2$, thus measuring the end-product of denitrification as N$_2$O. The acetylene block technique is an easier method due to measuring N$_2$O which has a low atmospheric concentration and the high sensitivity of available detectors (Groffman et al., 2006). The technique allows you to amend the samples with carbon and nitrate, to look for nitrate or carbon limitation within the sediments (Pfennig & McMahon, 1997).

Denitrification enzyme activity (DEA) measures the denitrification activity of the pre-existing denitrifier population with saturated nutrients (nitrate and carbon) and anoxic conditions (Luo et al., 1996, Stevenson et al., 2011). It is only run for a few hours to measure the linear response of the community to the added nutrients (Tiedje et al., 1989, Luo et al., 1996). Chloramphenicol inhibits the production of new denitrification enzymes, thus the rate of N$_2$O production is related to the added concentrations of enzymes (carbon, nitrate) amended (Jordan et al., 2007).

The acetylene inhibition technique can lead to an underestimation of denitrification rates in systems with small or dynamic NO$_3^-$ pools due to blocking of the nitrification process by acetylene (McCrackin & Elser, 2010), especially in sediments (Groffman et al., 2006). Other problems include slow diffusion of C$_2$H$_2$ into fine sediments, the enhancement of sediment respiration by C$_2$H$_2$, the rapid decomposition of C$_2$H$_2$ by C$_2$H$_2$ degrading microbes, and the contamination of C$_2$H$_2$ with other gasses that can affect denitrification (Knowles, 1990, Groffman et al., 2006). Most importantly in sediment systems, the combination of sulphide interference and low NO$_3^-$ concentrations reduces the ability of C$_2$H$_2$ to inhibit N$_2$O reductase (Steingruber et al., 2001, Groffman et al., 2006). This technique is usually only used as a potential rate of denitrification, due to aforementioned limitations. Actual denitrification rates are notoriously difficult to measure due to the high background N$_2$ concentrations (Jordan et al., 2007). Denitrification rates will reflect the sediments conditions at the time of sediment sampling; measured denitrification rates should be considered potentials and interpreted as such.

Despite these challenges, the acetylene block method has a significant advantage in allowing large numbers of samples to be run, which facilitates measuring denitrification
on large spatial or temporal scales (Groffman et al., 2006). It is considered a useful tool to investigate the potential of sediments to denitrify under controlled conditions, and test for nutrient limitations across large spatial gradients.

### 1.4.2 Isotope Pairing Technique

The stable isotopes of nitrogen exist in two forms in nature: $^{14}$N and $^{15}$N, which occur in 99.64% and 0.4% abundance respectively (Fry, 2006). The natural balance of the isotopes in nature allows us to use the heavier isotope as a biological tracer, as it is left behind as the lighter isotopes generally react faster (Fry, 2006). $^{15}$N isotope pairing technique was developed for use in aquatic ecosystems in 1992 by Lars Nielsen (Nielsen, 1992), using stable isotope tracers based on previous work by (Hauck et al., 1958). Hauck et al. (1958) had shown that the ratios of $^{14}$N$^{15}$N and $^{15}$N$^{15}$N reflect the ratio of $^{14}$N to $^{15}$N in the source of nitrate being denitrified, thus allowing the determination of denitrification rates. Denitrification in sediments is either supported by NO$_3^-$ from the water column (D$_w$ – direct denitrification) or of NO$_3^-$ produced within the sediment by nitrification (D$_n$ – coupled nitrification-denitrification) (Steingruber et al., 2001). The technique measures denitrification in a closed system by enriching the water column with $^{15}$NO$_3^-$ and measuring the subsequent production of $^{29}$N$_2$ and $^{30}$N$_2$ in the headspace via mass spectrometry (Steingruber et al., 2001). Because the water column is enriched with $^{15}$NO$_3^-$, and NO$_3^-$ derived via nitrification is unlabelled, this method can differentiate between denitrification of water column NO$_3^-$ and coupled nitrification-denitrification occurring in the sediments (Nielsen, 1992, Steingruber et al., 2001).

The isotope pairing technique (IPT) does have a set of assumptions that must be met to ensure accurate results. Firstly, the addition of $^{15}$NO$_3^-$ should not alter the production rate of in situ nitrate (Eyre et al., 2002), which can lead to an overestimation of in situ rates. Secondly, the added label must mix homogenously with the in situ nitrate in the water column and sediments (Nielsen, 1992). The roots of aquatic macrophytes produce oxygen and coupled nitrification-denitrification cannot be measured here (Nielsen, 1992). These two assumptions can be tested by running a concentration series of additions of $^{15}$NO$_3^-$. The third assumption is that a stable nitrate gradient must be formed across the sediment layer, which is dependent on the oxygen penetration depth (OPD) which can shift seasonally (Eyre et al., 2002). This assumption is fulfilled when there is a linear production of N$_2$ gas, however when OPDs are less than 1 mm (as many
sediments are) the diffusional lag time is often less than 10 minutes and negligible in experiments longer than a couple of hours (Nielsen, 1992, Risgaard-Petersen et al., 1998). The final assumption is that anammox is not occurring (or at very low rates) in the incubated sediments. This can be tested by incubating anoxic sediment slurries with $^{15}\text{NH}_4^+$ and $^{14}\text{NO}_3^-$ to look for production of $^{29}\text{N}_2$ (Thamdrup & Dalsgaard, 2002). Water column oxygen conditions during the incubation should not change more than 20% to replicate in situ conditions (Gongol & Savage, 2016).

The isotope pairing technique has been applied in four different ways: in batch-mode assays, benthic flux chambers, enclosures and flow through systems (Steingruber et al., 2001). Batch-mode assays are the most common way of using the IPT, and a standardised procedure for estuaries has been published by (Dalsgaard, 2000). It involves adding a labelled $^{15}\text{NO}_3^-$ tracer into the overlying water of an enclosed sediment core and measuring the production rate of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ over a time period of 0.4 - 24 hours (Steingruber et al., 2001). This can either be measured as a time series experiment, measuring $^{29}\text{N}_2$ and $^{30}\text{N}_2$ over time, sacrificing cores at time intervals, or by an endpoint experiment, where all the cores are sacrificed at the same time (Dalsgaard, 2000, Steingruber et al., 2001). Time series experiments allow one to control whether the production of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ is linear with time, however the time series obtained is based on a number of sediment cores which could vary in sediment characteristics and rates (Steingruber et al., 2001). Endpoint experiments have a single denitrification rate for each core, however there is no knowledge on whether the production of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ is linear with time. Steingruber (2001) combined these two experimental approaches by running an endpoint experiment, however sampling the water column at set time intervals to ensure linear production of $\text{N}_2$, therefore overcoming the limitations of both methods.

The isotope pairing technique has also been applied in situ, both in benthic flux chambers (Nielsen & Glud, 1996) and in flexible enclosures (Risgaard-Petersen et al., 1999). The flexible enclosure added argon-saturated lake water with $^{15}\text{NO}_3^-$ into the water column. $^{29}\text{N}_2$ and $^{30}\text{N}_2$ production and loss of argon (to account for atmospheric $\text{N}_2$ loss) was measured over 24 hours by taking subsamples of the water column and the sediment using a long glass tube (Risgaard-Petersen et al., 1999). Risgaard-Petersen et al. (1999) found that laboratory measurements strongly underestimate in situ denitrification activities in shallow lakes, due to under representation of in situ transport processes in
the rigid core tubes used for lab incubations, and they attribute these transport processes to wave-induced pore water transport.

The isotope pairing technique has laborious procedures due to the assumptions that must be met, and expensive instrumentation is required for sample analysis, however it provides a direct quantification of denitrification if assumptions are met (Groffman et al., 2006), and is preferable to the acetylene inhibition technique. A major flaw identified with the method is that to date, there is no technique that provides a satisfactory simulation of the turbulent mixing conditions in shallow lakes, leading to an underestimation of denitrification rates (Risgaard-Petersen et al., 1999, Steingruber et al., 2001).

1.4.3 Direct N₂ & N₂:Ar technique

It is easier to measure N₂ in aquatic systems compared to air, due to the lower background concentrations of N₂ (~1000x greater than in air) (Groffman et al., 2006). Due to this, it is possible to measure realistic denitrification rates with a precision of 0.03-0.3% using gas chromatography or membrane inlet mass spectrometry (MIMS) (Hamersley & Howes, 2005, Groffman et al., 2006). The measured N₂ flux is a net flux of two co-occurring processes – denitrification (gross production of N₂ gas out of the sediment) and N₂ fixation (gross consumption of N₂ into the sediment) (An et al., 2001). The flux of N₂ is determined from the change in N₂ concentration, or the change in N₂:Ar, using argon concentrations at air saturation determined using solubility equations (Weiss, 1970, Groffman et al., 2006).

The N₂ flux technique is most easily utilized with intact sediment cores, in closed chambers under a N₂ free headspace (Hamersley & Howes, 2005). This technique is easy to use and is preferable to the acetylene inhibition technique (Hamersley & Howes, 2005). The disadvantages include artefacts caused by the non-equilibrium conditions created by closed chambers and low N₂ headspaces, and long pre-incubation periods (Tomaszek et al., 1997, Hamersley & Howes, 2005). The long pre-incubation periods can shift microbial communities and activities (Risgaard-Petersen et al., 1998), due to utilization of readily available organic carbon (van Luijn et al., 1996). The long pre-incubation times can also increase the risk of atmospheric contamination of N (van Luijn et al., 1996). There can also be accumulation of nitrogen species in the water column, which has been shown to shift the dominant denitrification pathway – such as reported
in Risgaard-Petersen et al. (1998). The increase in water column ammonium observed increased sediment nitrification; thus, the measured rate was heavily biased by ammonification - coupled nitrification-denitrification (Risgaard-Petersen et al., 1998). The direct N\textsubscript{2} flux technique requires minimal manipulation of the overlying water column or substrate, allowing simple and direct measurements of N\textsubscript{2} (Tomaszek et al., 1997, Patel, 2008). The N\textsubscript{2} flux technique has also been applied to an in situ chamber, measuring the accumulation of N\textsubscript{2} in a burette attached to the top of the chamber (Tomaszek et al., 1997). This technique gave similar results to concurrent in vitro N\textsubscript{2} measurements, and has the advantage of taking less time, and is a more natural approach to measuring denitrification; however, it is limited to shallow, non-tidal aquatic environments (Tomaszek et al., 1997).

The use of MIMS is a preferential method due to minimal sample preparation (Chua et al., 2016), and gases are measured directly from collected water samples (Kana et al., 1994). Hundreds of samples can be processed per day, due to the rapid measurement time (<2 minutes) and small sample required (<7 mL) (Kana et al., 1994). This method is advantageous due to directly measuring the end product of denitrification, N\textsubscript{2}, with no assumptions made about the homogeneity of isotope distributions in the sediment (Cook et al., 2006). A study has shown this method to be superior compared to the isotope pairing technique for highly bioturbated sediments, due to in-homogeneous mixing of the labelled tracer, and in oligotrophic systems with low organic matter and water column nitrate inputs (Ferguson & Eyre, 2007). It can be applied in flow-through chambers measuring the inlet and outlet N\textsubscript{2}:Ar concentrations, which allows the experiment to be run at a steady-state and the addition of perturbations to the core (Kana et al., 1998).

Artefacts that can occur when using the N\textsubscript{2}:Ar technique include oxygen interference during analysis, O\textsubscript{2} bubble formation in the cores with highly productive sediments, bubble formation if the core sample is warmed after collection, and loss of N\textsubscript{2} via N-fixation (Eyre et al., 2002). N\textsubscript{2} has a higher solubility than Ar in the bubbles which can change the ratios (Eyre et al., 2002). To reduce bubble formation in light incubations, dark incubations can be run prior to reduce oxygen concentrations (Eyre & Ferguson, 2002). During the experiment argon concentrations must remain constant (Eyre et al., 2002), and this source of error can be reduced by ensuring no bubble formations or temperature changes during incubation. If there are high N\textsubscript{2} fixation rates denitrification
can be underestimated in comparison to other techniques which directly measure denitrification, rather than net $N_2$ flux (Ferguson & Eyre, 2007).

Thus the use of MIMS to measure $N_2$ gas fluxes with $N_2$:Ar ratios may offer a superior method for measurement of net denitrification without the use of tracers or direct manipulation of the sediment core. The $N_2$:Ar technique has been compared to a variety of methods and provides comparable results (Eyre *et al.*, 2002, Bernot *et al.*, 2003). The use of isotope enrichment coupled to MIMS allows direct quantification of $N_2$ fixation, thus accurate net denitrification rates can also be determined (An *et al.*, 2001).
1.5 Thesis Rationale and Outline

Given the increasing effects nitrogen runoff from agriculture is having on some shallow coastal environments, this thesis aims to quantify one of the key processes that can mitigate eutrophication, namely denitrification. Many well flushed estuarine ecosystems in New Zealand are not showing strong eutrophication symptoms yet (Robertson et al., 2016), however the majority of coastal lagoon ecosystems draining agricultural catchments that are characterised by long residence times are extremely degraded and show severe eutrophication symptoms (Hamill et al., 2014). Denitrification is likely to be one of the key pathways reducing the effects of eutrophication through nitrate removal, however denitrification efficiency can decrease with nitrate loading (Seitzinger & Nixon, 1985). As such, it is crucial that we have a good understanding of how this process varies both spatially and seasonally.

In New Zealand there are currently only two studies that have investigated denitrification in estuaries (Gongol & Savage, 2016, Douglas et al., 2017) and one that has investigated denitrification potential in lakes (Bruesewitz et al., 2011). In the study by Gongol & Savage (2016), which measured denitrification rates across multiple sites in four estuaries, denitrification rates (IPT) were spatially variable and key drivers included sediment grain size, sediment oxygen penetration depth and water column nitrate availability. In the study by Douglas et al. (2017) estuarine sediment plots were enriched with ammonium fertilizers to test for effects of future sediment nutrient enrichment. Control plots denitrification enzyme activity (DEA) was enhanced in sediments containing greater mud content and sediment organic carbon, however denitrification potential was decreased by sediment nutrient enrichment (Douglas et al., 2017). Bruesewitz et al. (2011) reported that DEA increased with agricultural intensity, however they clearly state that further in situ work is required to test if denitrification potential then relates to actual in situ rates.

Given the limited knowledge on the ability of New Zealand estuarine ecosystems to process nitrogen via denitrification, this thesis aims to elucidate some of the key drivers of denitrification. To accomplish this, I measured both denitrification potential and nutrient limitation (using the denitrification enzyme activity; DEA), studied in situ denitrification spatial and seasonal drivers (using the isotope pairing technique), and investigated the effects of organic detritus amendments on denitrification rates as a proxy.
for the effects of eutrophication (using membrane inlet mass spectrometry; MIMS). I also used oxygen micro-profiling sensors and planar optode imaging to investigate fine-scale sediment oxygen dynamics.

Chapters are separated as publications, so some repetition of material is expected. Publication outputs of each chapter are listed below and on the chapter title page.

**CHAPTER 2: Spatial variability of denitrification potential in ICOLL ecosystems**

Parts of this section have been published as a technical report and as a scientific article, produced under a contract to Environment Canterbury to investigate nutrient dynamics within Te Waihora/Lake Ellesmere. In this chapter I led the project design, fieldwork, lab work, analysed the data and wrote the chapter. I contributed to sections in the publications listed below.


Denitrification has not been quantified in South Island ICOLL ecosystems and little is known about how they process new nitrogen. Many of the ecosystems are heavily impacted by excess nutrients and are showing eutrophication symptoms. I undertook spatial surveys of water column nutrient concentrations, sediment characteristics and sediment denitrification potential (denitrification enzyme activity) in these ICOLLs to study drivers of spatial variability in denitrification potential. It was hypothesised that sediments with high organic matter availability and located near the inflows of main tributaries would show the greatest denitrification potential, due to high supplies of new nitrate and high organic availability shaping the microbial community. The nitrate inputs into the ICOLLs was also investigated using long-term monitoring data, to investigate if seasonality in nitrate inputs occurred. Due to higher rates of runoff in winter and spring, it was hypothesised that new nitrate inputs to the ICOLLs would also be highest in winter and spring.
CHAPTER 3: A hierarchy of factors controlling denitrification rates in intermittently closed and open lake/lagoons (ICOLLs)

This chapter is a modified version of the manuscript to be submitted to Aquatic Ecology. In this chapter I designed the experiment, conducted the fieldwork and lab work, analysed the data and wrote the chapter/manuscript.

Crawshaw, J., Schallenberg, M., Savage, C., & Van Hale, R. A hierarchy of factors controlling denitrification rates in intermittently closed and open lake/lagoons (ICOLLs). In revision for submission to Aquatic Ecology.

Shallow estuarine ecosystems receive large intermittent “pulses” of dissolved nitrogen, which is bioavailable for assimilation by nuisance algal forming species. The ability of the system to remove this nitrogen through denitrification may reduce the eutrophication potential of the nitrogen load and maintain clear water ecosystems. I measured denitrification rates in situ under nitrate pulse events, designed to mimic typical pulses of nitrate in the water column reported in Chapter 2. I also tested for potential nutrient limitation using denitrification enzyme activity in the laboratory. I measured denitrification in winter and summer at a total of 9 sites across the two ICOLLs to examine how gradients in potential physico-chemical and biological drivers relate to denitrification, substrate limitation and sediment oxygenation in these ICOLLs. Finally, I developed statistical predictive models for assessing the denitrification potential of both new and recycled nitrate within these, and other similar, ICOLLs.

CHAPTER 4: Physical and biological drivers of sediment oxygenation and denitrification in a New Zealand intermittently closed and open lake lagoon

This chapter is a modified version of the manuscript published in the New Zealand Journal of Marine and Freshwater Research. In this chapter I designed the experiment, conducted the fieldwork and lab work, analysed the data and wrote the chapter/manuscript.

The rate of microbial activity is driven by temperature and availability of resources. Shallow ecosystems have large seasonal temperature fluctuations which probably influence the rate of nitrogen removal processes. The benthic infauna which inhabit these ecosystems also show large seasonality in biomass and abundance which may change the resource supplies to denitrifying microbes. The physical and biological drivers of sediment oxygenation and denitrification in a New Zealand ICOLL using novel in situ enclosures for one year. Given the limited research conducted on ICOLL ecosystems, the first objective was to investigate the influence of temperature and seasonality on denitrification, as these factors have been identified as mediators of denitrification in previous studies in estuarine ecosystems. My study sought to identify periods when, and sites where, denitrification was elevated or suppressed and to identify the dominant physical drivers of denitrification in the ICOLL. The second objective of this study was to investigate the role of macroinvertebrates (biological drivers) in supporting denitrification rates in an ICOLL ecosystem, as this has not been addressed in previous studies. Given the relatively shallow depths of the ICOLLs and the potential importance of benthic-pelagic coupling in these systems, macroinvertebrates may play an important role in oxygen and nitrogen cycling. Thus, I hypothesised that sites with high invertebrate densities would exhibit relatively greater oxygen penetration and higher denitrification rates.

CHAPTER 5: Carbon lability influences nitrogen cycling rates in temperate estuarine sediments

This chapter is a modified version of the manuscript to be submitted to Biogeochemistry. In this chapter I designed the experiment, conducted the fieldwork and lab work, analysed the data and wrote the chapter/manuscript.

*Crawshaw, J., O’Meara, T., Savage, C., Thomson, B., Baltar, F., & Thrush, S.F. Carbon lability influences nitrogen cycling rates in temperate estuarine sediments. In revision for submission to Biogeochemistry.*

The rate of denitrification is often limited by the availability of organic matter and nitrogen in estuarine ecosystems, however knowledge is limited on how different quality (lability) organic matter influences nitrogen cycling. Dominant organic sources in estuarine ecosystems is shifting with increasing nutrient inputs, and this may change the ability of estuaries to remove excess nitrogen. The rate of key microbial extracellular
enzymes can limit the availability of organic matter, and these will likely also change with organic detritus types. Large bivalves common in these estuaries may play a role in oxygenating the sediment, and their bioturbation was examined using planar optode oxygen imaging. The study aimed to investigate how various organic detrital sources influence sedimentary nitrogen processing. I hypothesised that net nitrogen removal (N₂ efflux and dissolved NO₃⁻ + NH₄⁺) would be enhanced by more labile organic matter. It was hypothesised that extracellular enzyme activity would also be increased by more labile organic material, supporting increased nitrogen removal. Large bioturbating bivalves were expected to change sedimentary oxygen conditions to be more suitable for denitrification due to increased oxygen consumption.

CHAPTER 6: Conclusions and future research directions

This chapter synthesises the key findings from Chapters 2 – 5 resulting from this research. It reviews the original contribution to knowledge in the field of estuarine sediment nitrogen cycling and provides direction for promising future research.
CHAPTER 2

Spatial variability in denitrification potential in ICOLL ecosystems

Photo: Early morning fishermen on Te Waihora/Lake Ellesmere at sunrise.

Parts of this section have been published as a Technical Report and as a scientific article, both produced under a contract to Environment Canterbury to investigate nutrient dynamics within Te Waihora/Lake Ellesmere.


2.1 Introduction

Intensifying agricultural development in catchments around coastal water bodies is leading to increased nutrient loading, causing eutrophication, particularly in New Zealand (Piña-Ochoa & Álvarez-Cobelas, 2006, Knuth & Kelly, 2011). Coastal ecosystems are generally nitrate limited (Howarth, 1988), and respond quickly to assimilate nitrogen when it becomes available, producing increases in primary producer biomass, often phytoplankton. These phytoplankton blooms can have severe effects on the receiving ecosystem, outcompeting submerged macrophytes through shading, promoting increased sediment resuspension and turbidity, thus continuing to stimulate and favour the biomass of phytoplankton (Scheffer et al., 1993, Borum, 2013) through positive feedbacks. Fortunately, uptake by phytoplankton is not the only sink for nitrate in coastal ecosystems. Denitrification is the microbially mediated ecosystem service where nitrate can be converted to nitrogen gas within the sediments through the processes of direct denitrification or coupled nitrification-denitrification (Nielson et al., 2004). The processes of removing bio-available nitrate from the system has been shown to be important in mitigating the potential effects of eutrophication under some conditions (Laverman et al., 2007, McCrackin & Elser, 2010).

Sediment denitrification is a major pathway of nitrogen removal from aquatic ecosystems (Fennel et al., 2009), and spatial variability is important to acknowledge in assessing benthic denitrification capability. Spatial analysis of denitrification has shown that there is a high degree of small scale “hotspots” of denitrification rates driven by variability in sediment characteristics (Parkin, 1987, McClain et al., 2003, Groffman et al., 2009, Thrush et al., 2017). The main direct drivers of denitrification include nitrate and organic content supply, sediment oxygenation and temperature (Tomaszek & Czerwieniec, 2003, Groffman et al., 2009). Denitrification hotspots are likely to occur near the inflows of rivers (Ogilvie et al., 1997, Smith et al., 2015), as the increased nitrate loading can enhance microbial community activity (Kana et al., 1998, Cornwell et al., 1999). Sediment particle size and porosity influence sediment oxygenation, and can control denitrification capacity, due to limiting the oxygen and nitrate diffusion to sites of denitrifiers. In fine sediment with high silt and clay content, diffusion and bioturbation will be the main nitrate transport mechanism (Piña-Ochoa & Álvarez-Cobelas, 2006), and the majority of denitrification studies have occurred in such sediments (Kessler et al., 2014). Finer sediments have smaller pore sizes than sandy sediments and the smaller
pore sizes can become anoxic more easily, promoting denitrification (Groffman & Tiedje, 1991, Solomon et al., 2009). In the less studied permeable sediments (e.g. sand), nitrate transport is dominated by advection, driven by topography pressure gradients (Kessler et al., 2014). Sandy sediments act as filtration systems, processing organic matter at high rates reducing the accumulation of fines and organics (Sokoll et al., 2016), enhanced by water movement driving organics into the porous sediments (Huettel & Rucsch, 2000). High sediment organic carbon can increase denitrification capacity by directly supplying the microbes with substrate for growth, whilst indirectly increasing oxygen consumption (Piña-Ochoa & Álvarez-Cobelas, 2006). Finer grained sediments often also contain a greater content of organic matter (Piña-Ochoa & Álvarez-Cobelas, 2006). Grain size can also affect the presence/absence of macroinvertebrates (Thrush et al., 2003), most often reported is the positive or negative correlations with percentage mud content (Thrush et al., 2003). Benthic infauna rework the sediment, increasing oxygen penetration and nitrate diffusion and mobilization at sites of denitrification (Svensson, 1997, Biswas et al., 2009, Shang et al., 2013).

Aquatic ecosystems downstream of agricultural catchments tend to be nutrient sinks. ICOLLs (Intermittently Closed and Open Lake Lagoons) are vulnerable to eutrophication due to their intermittent water replenishment and shallow depths (Eyre & Ferguson, 2002, Spooner & Maher, 2009, Schallenberg et al., 2010). However, the longer water residence times of ICOLLs compared to open estuaries may allow a greater proportion of the nitrogen load to be denitrified (Seitzinger et al., 2006), as there is greater potential for nutrient processing and particle settling before transport out of the system or sequestration (Bruesewitz et al., 2011). Due to this intermittent water replenishment, there has been increasing interest in quantifying the role that these coastal ecosystems play in nitrogen removal. New Zealand has 17 estuaries classified as ICOLLs/brackish lagoons (Hamill et al., 2014), and there is little baseline data on their sediment, infauna or nutrient conditions. Even with the limited ecological information in the literature, 11 of these systems do not meet the ICOLL national benchmarks for water quality (Hamill et al., 2014), highlighting their propensity for eutrophication.

Lake Ellesmere and Tomahawk Lagoon have been identified as sites likely to process large amounts of nitrogen due to their high eutrophication status, intermittent connection to the sea and their shallow depths (Hamill et al., 2014). I undertook spatial surveys of water column nutrient concentrations, sediment characteristics and sediment
denitrification enzyme activity in these ICOLLS to study drivers of spatial and temporal variability in denitrification.

I hypothesised that sediments with high organic matter availability and located near the inflows of main tributaries would show the greatest denitrification enzyme activity regardless of current flow rate, due to high supplies of new nitrate and high organic availability priming microbial communities. The nitrate inputs into the ICOLLS was also investigated using long-term monitoring data, to investigate if seasonality in nitrate inputs occurs. Due to higher rates of runoff in winter and spring, I hypothesised that new nitrate inputs to the ICOLLS would also be highest in winter and spring.

2.2 Methods

2.2.1 Study Locations

2.2.1.1 Lake Ellesmere (Te Waihora)

Lake Ellesmere (Te Waihora) is a large (surface area = 145 to 213 km\(^2\)) shallow (mean depth = 0.8 to 1.5 m) ICOLL located south-west of Banks Peninsula, South Island, New Zealand (Fig. 2.1A) (Schallenberg et al., 2010). It is separated from the sea by a gravel barrier bar along Kaitorete Spit (Hughey & Taylor, 2009, Jellyman, 2011), which is mechanically opened at specified water levels to prevent inundation of surrounding farmlands and to facilitate drainage of farmland (Schallenberg et al., 2010). Historically, macrophytes were common in the lagoon (primarily *Ruppia megacarpa* and *Ruppia polycarpa*) (Hearnshaw & Hughey, 2012), however macrophytes were virtually eliminated in a severe storm in 1968. Since then the mid-lake phytoplankton biomass (mean chlorophyll \(a = 88.4 \, \mu g \, L^{-1}; \, 1993-2005\) and suspended sediment concentrations (mean = 229.7 \(\mu g \, L^{-1}; \, 1993-2005\) have generally remained high (Schallenberg et al., 2010), preventing the return of the submerged macrophytes, and maintaining the lagoon in a phytoplankton-dominated state (Hearnshaw & Hughey, 2012). The catchment area of Lake Ellesmere covers approximately 2760 km\(^2\), with intensive agriculture being the predominant use of land in the catchment (Hearnshaw & Hughey, 2012), resulting in high nitrogen and phosphorus loads to the lagoon (Schallenberg et al. 2010). This opening regime is controlled by Environment Canterbury, which opens the lagoon periodically through the Kaitorete Spit using diggers and dredges (Hearnshaw & Hughey, 2012), and is usually open for < 1 month during each opening event (Schallenberg et al., 2010, Jellyman, 2011).
Figure 2.1. Site images: A - Aerial view of Lake Ellesmere with the Selwyn River inflow (right bottom corner). B - Tomahawk Lagoon No.1 (lower lagoon) on a clear calm day.

2.2.1.2 Tomahawk Lagoon

The Tomahawk Lagoons are small, shallow, hypertrophic coastal lagoons in Dunedin, Otago (Fig. 2.1B). The lower Tomahawk Lagoon (referred to here as Tomahawk Lagoon) is a small (0.19 km²), shallow, hypertrophic ICOLL in the city of Dunedin, South Island, New Zealand (Fig. 2.1B)(Hamilton & Mitchell, 1997, Drake et al., 2011, Hamill et al., 2014). It is fed by surface runoff and a stream at the northern end that drains a small agricultural catchment (area = 2.4 km²)(Mitchell, 1989, Drake et al., 2011). The lower ICOLL drains the upper brackish lake (Tomahawk Lagoon No.2) through a small narrow channel, with a catchment of 16 km² (Mitchell et al., 1988). Because of the shallow depth and the alignment of the main axis of the lagoon with the prevailing winds (S-E and N-W), sediment is frequently resuspended by the waves (Robertson, 1999). The lagoons have switched states between phytoplankton-dominated and macrophyte-dominated, alternating for periods of 1-5 years since 1963 (Mitchell et al., 1988). The ICOLL (hereafter referred to as its common name, Tomahawk Lagoon) periodically opens to the ocean, either under high rainfall or large sea events.

Sampling sites in Lake Ellesmere and Tomahawk Lagoon were distributed throughout the ICOLLs to establish the breadth of the spatial heterogeneity of various sediment properties, physicochemical properties, and denitrification potential within the ICOLLs. Due to the size differences within the two ICOLLs, 18 sites were chosen in Lake Ellesmere, and 3 sites in Tomahawk Lagoon (Fig. 2.2), to cover a representative sediment gradient in each ICOLL.
2.2.2 Sampling Dates

Snapshot sediment and physico-chemical sampling in Lake Ellesmere was carried out from the 9th – 11th April 2014. The sediment and physico-chemical sampling in Tomahawk Lagoon was conducted on the 3rd of June 2014.

2.2.3 Physicochemical Measurements

Physicochemical parameters at each site were measured including temperature, salinity, and dissolved oxygen using an YSI Professional Plus multiprobe over each survey period (Yellow Springs, Ohio, USA). Water depth was measured using a weighted rope. A water sample was taken at each site (0.5 m below surface) for analysis of dissolved and total nutrients (TP, TN, NO₂⁻+NO₃⁻, DRP, NH₄⁺) and stored on ice. For dissolved nutrients approximately 40 mL of water from each site was filtered through a Munktell glass fibre 25 mm filter (nominal pore size = 0.7 µm), into an acid washed 50 mL Falcon® tube, and frozen at -20°C until analysis. Nutrients were analysed using standard colorimetric wet chemistry, using a SANPlus segmented flow autoanalyzer (SkalarAnalytical B.V., Breda, The Netherlands). Nitrate-N & Nitrite-N were determined based on the cadmium reduction method (Morris & Riley, 1963, Wood et al., 1967). Dissolved reactive phosphorus (DRP) was measured by the reaction of ammonium molybdate and potassium antimony tartrate in an acidic medium, with diluted solutions of phosphate to form an antimony-phospho-molybdate complex (Murphy & Riley, 1963, Greenberg et al., 1992). The ammonia-N protocol was based on the Berthelot reaction (Bertholet, 1859, Solorzano, 1969). Total phosphorus (TP) and total nitrogen (TN) were measured in a simultaneous wet oxidation at 121°C, with the reaction starting at pH 9.7 and ending at pH 5-6 (Valderrama, 1981, Ebina et al., 1983). Analytical detection limits were 0.071 µM for nitrate-N, 0.93 µM for ammonium-N, 0.36 µM for total nitrogen, 0.02 µM for dissolved reactive phosphorus, and 0.06 µM for total phosphorus.
Figure 2.2. Map of New Zealand showing the two study locations, the coastline and the associated main tributaries. A: Lake Ellesmere, Canterbury, New Zealand. B: Tomahawk Lagoons, Dunedin, Otago. E1-E12 and T4-T6 indicate sample site locations, where both sediment and physico-chemical samples were taken for spatial contour mapping. Coloured circles indicate long-term nitrate sampling sites for seasonal nitrate pulse analysis.

2.2.4 Sediment Measurements
Undisturbed sediment samples were collected with a core tube and the top 1 cm of sediment was extruded for analysis of the following sediment characteristics using gravimetric determinations (Håkanson & Jansson, 1983). Surficial sediment porosity (g H$_2$O ml$^{-1}$ sediment) was calculated from the dry weight of the sample after drying 5 g of wet sediment at 50°C for 24 hours. This sediment was then combusted at 450°C for 24 hours for the calculation of organic matter as mass loss on ignition, which has been expressed as an areal concentration within fresh sediment (g dry OM m$^{-2}$). The surficial sediment grain size was determined using a laser-diffraction Malvern Mastersizer. Five
g of sediment was dried for 24 hours at 50°C. Half a g of sediment was placed into a 50 mL falcon tube and treated with 5 mL of 30% hydrogen peroxide overnight to remove organic matter. Particle sizes were expressed as percentages of the total particle numbers, segregated into the following grain size fractions: clay (0-2 µm), silt (2-63 µm) and sand (63-2000 µm).

To provide a coherent set of data, sediment organic matter as well as denitrification and invertebrate densities were all expressed on an areal basis. I calculated concentrations of organic matter and applied these to a uniform depth into the sediment of 1 cm to provide an areal unit for comparison, which I here refer to as organic matter availability. Typically, many sediment parameters are calculated and reported as contents rather than as volumetric concentrations or areal densities. Expressed as contents, sediment parameters can be strongly biased by sediment bulk density and grain size (Tolhurst et al., 2005) and, thus, contribute little information in addition to grain size to the analysis of relationships. However, organic matter availability (per g dry sediment) may reflect the nutritional value of sediments to deposit-feeding organisms. Therefore, I also used organic matter availability in our data analyses.

2.2.5 Denitrification enzyme activity
Sediment samples for the denitrification enzyme activity (DEA) were collected in April 2014 in Ellesmere and in July 2015 for Tomahawk Lagoon. Potential carbon and nitrogen limitation of denitrification was measured using the acetylene inhibition technique, as described by (Bruesewitz et al., 2011). Undisturbed sediment cores were collected, and the top 4 cm of the cores was extruded and immediately placed into Falcon® tubes. Sediment filled the tubes, which were sealed without a headspace. Sediments were immediately placed on ice until they reached the lab. In the lab sediments were brought up to room temperature. Fifteen mL of sediment was homogenized with 15 mL of unfiltered ICOLL water. Four treatments were set up to investigate possible nutrient limitations of denitrification in the sediment. These included treatments of (1) Control: sediment only (DEA), (2) sediment + 10 mg of potassium nitrate (DEA+N), (3) sediment + 12 mg of glucose (DEA+C), and (4) sediment + 10 mg of potassium nitrate and 12 mg of glucose (DEA+C+N). In addition, a sample of unfiltered ICOLL water was used to test for water column denitrification. Thirty mL of the sediment-water slurry was transferred to 45 mL glass vials and sealed with silicon septa. Anoxic conditions were created by purging the vial headspace with pure N₂. Then, 10 mL of acetylene (C₂H₂)
were added to block the conversion of N₂O to N₂, and to maintain over-pressurised conditions in the vials. The incubations were performed at room temperature (22°C). Eight mL of gas sample was collected hourly for four hours after the addition of C₂H₂. To maintain a constant pressure in the bottle, C₂H₂ was added to replace the collected gas samples. N₂O samples were analysed using a Varian CP 3800 gas chromatograph equipped with a Hayesep D column and an electron capture detector. N₂O production was calculated using the ideal gas law. This was then converted to an areal basis (m⁻²) using sediment bulk density. Sediments for Lake Ellesmere were collected in the summer, when water column nutrient concentrations were expected to be reduced, thus results must be interpreted with caution as measured denitrification rates may be underestimated.

2.2.6 Seasonal nutrient inputs
To investigate the long-term changes in nitrate concentrations in the lagoons, data were collated from various sources. The water column concentration of nitrate in Lake Ellesmere is recorded at two sites as part of Environment Canterbury’s monitoring regime, one taken from a mid-lake monitoring station, and one from the mouth of the Selwyn River (total 510 data points). Tomahawk Lagoon had limited data available on the water quality, however a substantial amount of nitrate concentration data was collected from various sources (a thesis on the Lagoon (Spencer, 2003), unpublished data sets of C. W. Burns and M. Schallenberg (Otago University), and recent community monitoring data from Tomahawk Lagoon Citizen Science Team) to provide a total of 87 measurements.

2.2.7 Data Analysis
All statistical analyses were conducted in R Studio (v. 3.4.0) or Microsoft Office 365 ProPlus. Spearman’s correlation and linear regression was used to analyse correlations between the physicochemical, sediment and denitrification enzyme activity. An α of 0.05 was used to determine statistical significance. Spatial contour plots were created in Surfer 11. I acknowledge the limitations of using a small number of data points to extrapolate across large distances, however the contour maps provide a visual comparison of a snapshot of the measured parameters spatially. Maps were produced in ArcGIS (v. 10.3.1).
2.3 Results

2.3.1 Seasonal and spatial variation of nutrient inputs

2.3.1.1 Seasonal nitrate pulses

Nitrate concentration data plotted over time shows the intermittent nature of nitrate in Lake Ellesmere (Fig. 2.3A). Examination of the data lead us to define a nitrate threshold of 0.3 mg L\(^{-1}\), above which I observed temporary pulses of nitrate. Pulses occurred almost annually, with the exception of a few years between 1993 and 2013. The pulses generally began during late autumn, with high readings continuing throughout winter and into spring and decreasing below the threshold in summer (Appendix A, Table. 7.1). Tomahawk Lagoon also experienced these pulses of nitrate (Fig. 2.3B), which appeared to occur more often in recent times due to the increase in monitoring occurring in the lagoon. The large pulses of nitrate only occurred in the winter months in Tomahawk Lagoon (Appendix A, Table. 7.1). Figure 2.4A & 2.4B shows the frequency of different magnitudes of nitrate pulses in Lake Ellesmere and Tomahawk Lagoon respectively. Nitrate most often ranged from detection levels (<0.005 mg L\(^{-1}\)) to between 0.1 and 0.2 mg L\(^{-1}\). However, there were occasional “pulses” of larger nitrate concentrations, with the greatest pulse recorded in Lake Ellesmere of 4 mg L\(^{-1}\), and 2 mg L\(^{-1}\) in Tomahawk Lagoon. In Lake Ellesmere large pulses were most often recorded in the Selwyn River, and in Tomahawk Lagoon the large pulses were most often recorded in the upper lagoon and the lagoon inflow.

2.3.1.2 Spatial variation in nutrient concentrations

The dominant inflows in the ICOLLs were predicted to input large concentrations of nutrients, creating a gradient with distance from the river mouths during high flows. Our sampling time corresponded with an extended period of reduced flow rates from the Selwyn River (average flow rates for the sampling period were 1.81 m\(^3\) s\(^{-1}\), compared to an annual average of 3.77 m\(^3\) s\(^{-1}\) from 2009 to 2014), resulting in a poorly defined nutrient gradient. Although our sampling coincided with a low flow event, the concentration of dissolved reactive phosphorus (DRP) and total nitrogen (TN) showed decreasing concentrations with distance from the Selwyn River inflow (Fig. 2.5A). The other key nutrients (nitrate, total phosphorus and ammonium) however did not show this trend. Instead, high concentrations of nitrate were located at the mouth of Hart’s Creek (site E14), where salinity was also greatly reduced (3.08)(Fig. 2.6A,E). A strong linear
relationship was exhibited between salinity and nitrate \( (r = -0.83) \) (Appendix A, Table 7.2). Along the eastern edge of the ICOLL (Greenpark Sands, site E6) there was also increased nitrate concentrations and reduced salinity. Tomahawk Lagoon had stronger nutrient gradients leading away from the main inflow at the northern end of the ICOLL than Lake Ellesmere (Fig. 2.5B). Concentrations of NO\(_3^-\) and NH\(_4^+\) were greater at the southern end of the ICOLL (Fig. 2.7E,G), where salinity was slightly higher than the northern end.
Figure 2.3. Long-term nitrate concentration data (mg L\(^{-1}\)) taken at two sites within Lake Ellesmere - Mid-lake and the Selwyn River mouth (A) and Tomahawk Lagoon – Lower Lagoon, Upper Lagoon and Inflow (B). Black dotted line indicates 0.3 mg L\(^{-1}\) marking the spike definition.
Figure 2.4. Frequency of nitrate concentration pulses (mg L$^{-1}$) from measurements taken in Lake Ellesmere (A) and Tomahawk Lagoons (B). Examination of the data lead us to define a nitrate threshold of 0.3 mg L$^{-1}$ (pulse cut-off), above which I observed temporary pulses of nitrate. Data is split into measurements taken at the two sites in Lake Ellesmere, mid-lake and at the mouth of the Selwyn River. Tomahawk Lagoon’s data is split into measurements from the lower Lagoon 1, the upper Lagoon 2, and the Lagoon 2 inflow.
Figure 2.5. Snapshot nutrient concentration data (µg L$^{-1}$) against distance from the dominant inflow (km) from the one-off spatial survey in 2014. A = Lake Ellesmere: The left y axis shows dissolved reactive phosphorus (DRP) concentrations, denoted by open circles. The right y axis shows total nitrogen (TN) concentrations, denoted by open triangles. B = Tomahawk Lagoon: The left y axis shows dissolved reactive phosphorus (DRP) and total phosphorus concentrations (TP), denoted by open circles and closed circles respectively. The right y axis shows total nitrogen (TN) concentrations, denoted by open triangles. The discharge in the Selwyn River was very low at the time of sampling. Linear regression and p-values are shown for Lake Ellesmere.
Figure 2.6. Spatial patterns of surface salinity (A), surface temperature (B), total nitrogen (C), total phosphorus (D), dissolved NO\(_2^-\)+NO\(_3^-\) (E), dissolved reactive phosphorus (F), and NH\(_4^+\) (G), across the 18 sites in Lake Ellesmere from a one-off snapshot sampling in 2014. Samples were taken from 0.5 m below the water surface.
Figure 2.7. Spatial patterns of surface salinity (A), surface temperature (B), total nitrogen (C), total phosphorus (D), dissolved nitrate\(^{-}\) (E), dissolved reactive phosphorus (F), and dissolved ammonium (G), across the 3 sites in Tomahawk Lagoon from a one-off snapshot sampling in 2014. Samples were taken from 0.5 m below the water surface.
2.3.2 Spatial variation in sediment characteristics

The sediment summary statistics for Lake Ellesmere and Tomahawk Lagoon are described in Appendix A, Table 7.3. The mean grain sizes of the sediments in Lake Ellesmere ranged from areas dominated by silt (size 2 – 63 μm), in the middle of the ICOLL (Fig. 2.8B), to sand dominant zones (size 63 – 2000 μm) (Fig. 2.8C) near the ICOLL edges and in the Greenpark Sands to the east of the Selwyn River. Silt content in the sediments ranged from 1.78 to 88.56%. Sand content ranged from 2.2 to 97.68%. The ICOLL sediment generally had a low clay content (size 0 – 2 μm) however clay content was greater in the deeper basins (lagoon range 0.54 to 12.99 %) (Fig. 2.8A). Porosity (water content) in Lake Ellesmere ranged from 0.49 to 0.88 g ml⁻¹ (Fig. 2.8D). Lake Ellesmere sediments had a low redox potential, from -20 mV down to -400 mV, showing highly reduced sediments in the middle of the ICOLL (Fig. 2.8E), and more oxidising sediments in the Greenpark Sands (around site E6). Organic matter availability within Lake Ellesmere sediments was generally low (mean 240 g m⁻² (4% LOI) with a localised site of high organic matter (745 g m⁻² (22% LOI) which appeared to be peat (personal observation) (Fig. 2.8F).

The sediments in Tomahawk Lagoon had a similar range of sediment types to Lake Ellesmere, with silt dominant areas in the middle and northern parts of the lagoon (range 7.16 – 66%) (Fig. 2.9B), and sand dominant areas near the outflow (range 27 – 92%) (Fig. 2.9C). Tomahawk Lagoon also had low clay contents which were dominant at the northern end (range 0.66 – 7.06%) (Fig. 2.9A). Tomahawk Lagoon on average had greater organic content availability across all sites compared to Lake Ellesmere (Fig. 2.9F), with a mean of 284 g m⁻² (9% loss on ignition (LOI)). Sediments in Tomahawk were more porous (greater water content) than in Lake Ellesmere (range 0.63 – 0.96 g ml⁻¹) (Fig. 2.9D). Sediments were generally less reducing than Lake Ellesmere (range from 129 down to -50 mV) (Fig. 2.9E).

The sediment characteristics were highly related to one another in both ICOLLs. In Lake Ellesmere there were significant positive relationships between both clay and silt content versus depth (r = 0.91, 0.81) (Appendix A, Table 7.2), and a negative relationship between sand and depth (r = -0.83). Sediment porosity was driven by the sediment grain size (Fig. 2.10), with higher sediment porosity (greater water content) associated with deeper, finer sediments. These sediments also had low redox potential. In Tomahawk
Lagoon the finer sediments were also very rich in available organic material, however the depth gradient was less defined.

**Figure 2.8.** Spatial variability in the sediment characteristics of Lake Ellesmere: clay % (A), silt % (B), sand % (C), porosity (D), redox potential (E) and organic matter availability (F).
Figure 2.9. Spatial variability in sediment characteristics of the lower Tomahawk Lagoon: clay % (A), silt % (B), sand % (C), porosity (D), redox potential (E) and organic matter availability (F).
Figure 2.10. The relationship between sediment grain size fractions (sand, silt, clay %) and sediment porosity 2014 survey data from Lake Ellesmere. Open circles represent clay %, closed circles represent silt % and open triangles represent sand %.

2.3.3 Spatial variation in sediment denitrification enzyme activity

Denitrification enzyme activity was highly variable across Lake Ellesmere (Fig. 2.11). N$_2$O production was below the detection limit in the water only, sediment only or carbon amended treatments, thus these are not presented in the spatial plots. Nitrate additions stimulated denitrification, ranging from 19 to 407 μmol N$_2$O m$^{-2}$ h$^{-1}$ (Fig. 2.11A). When both nitrate and carbon were amended, the N$_2$O production increased further, and showed a large variation among sites (183 to 2574 μmol N$_2$O m$^{-2}$ h$^{-1}$) (Fig. 2.11B). The addition of carbon and nitrate greatly enhanced the denitrification enzyme activity. Our spatial survey showed that hotspots of denitrification enzyme activity were located in the littoral sites, particularly those located close to the Selwyn and L2 River inflows and near Kaituna Lagoon (see Fig. 2.1 for locations) (Fig. 2.11A & 2.11B). In Tomahawk Lagoon, N$_2$O production was below the detection limit in the water only treatment. In the non-amended sediment, denitrification enzyme activity was greatest in the sandy sediments near the lagoon outflow and ranged from 355 to 843 μmol N$_2$O m$^{-2}$ h$^{-1}$ (Fig. 2.11C). In the +N treatment, denitrification enzyme activity was greatly enhanced in the silt dominant sites in the mid and northern end of the lagoon, ranging from 656 to 3674 μmol N$_2$O m$^{-2}$ h$^{-1}$ (Fig. 2.11D). In the +C+N treatment the pattern changed, and denitrification enzyme activity were enhanced greatly in the sandy sediments, however rates were reduced from the nitrate-amended
treatment in the silt dominant sites. Rates ranged from 348 to 3226 μmol N₂O m⁻² h⁻¹ (Fig. 2.11E). In the +C treatment no N₂O production was recorded.

**Figure 2.11.** Denitrification enzyme activity (μmol N₂O m⁻² h⁻¹) across the 18 Lake Ellesmere sites and 3 Tomahawk Lagoon sites. A = Lake Ellesmere sediments with nitrate amendments (+N), (B) Lake Ellesmere sediments with carbon and nitrate amendments (+C+N) Tomahawk Lagoon sediments with no amendments, (D) Tomahawk Lagoon sediments with nitrate amendments (+N) and (E) Tomahawk Lagoon sediments with carbon amendments (+C+N).

### 2.3.4 Correlations between sediment characteristics and denitrification enzyme activity

It was hypothesized nutrient loading from the main tributary (Selwyn River) would select for greater denitrification enzyme activity closer to the outflow. However, our sampling did not capture a strong nutrient gradient, and there was no relationship observed between distance from inflow and denitrification enzyme activity in Lake Ellesmere. Tomahawk Lagoon the DEA+N treatment was enhanced in the two sites closer to the inflow (Fig. 2.11D), however in the DEA and DEA+C+N treatment rates were greater with further
distance from the inflow (Figs. 2.11C,E). Pearson’s correlations were used to investigate potential relationships between sediment variables and DEA variability. In Lake Ellesmere, DEA+N was negatively correlated with depth (r = -0.50), whilst DEA+C+N was also negatively correlated with depth and clay content (r = -0.54, -0.52). Fig. 2.12A shows the negative relationship exhibited between Lake Ellesmere denitrification potential and clay content for both treatments showing N$_2$O production. In Tomahawk Lagoon the sediment grain size gradient appeared to be related to the denitrification enzyme activity. The sediment only treatment was greater in sandy sediments (Figs. 2.9, 2.11). The DEA+N treatment was greater in clay dominant, organic rich sediments (Figs. 2.9, 2.11). The DEA+C+N treatment responded similarly to the DEA+N treatment, where rates were enhanced in sandy sediments. These relationships are depicted in Fig. 2.12B with the grain size gradient reported by clay content. There appears to be a threshold around 7% clay content, where denitrification rates rapidly declined in Lake Ellesmere sediments (Fig. 2.12A). Tomahawk Lagoon does not have clay content greater than 7%, and this point is where the greatest DEA+N rates were recorded (Fig. 2.12B).
Figure 2.12. A = Denitrification potential in Lake Ellesmere in the two treatments which showed N₂O production. B = Denitrification potential in Tomahawk Lagoon with the three treatments which showed N₂O production. Open triangle represents DEA, open circles represent DEA+N, closed black circles represent DEA+C+N and closed squares represent DEA+C+N.
24 Discussion

2.4.1 The impact of nutrient inflows

Large intermittent pulses of nitrate during winter and spring have been occurring in both the ICOLLs since measurements began in the early 1970’s. Pulses of nitrate up to 4 mg L\(^{-1}\) have been recorded, which would exert a large influence on the receiving ecosystem. Interestingly, the recorded nutrient pulses into the lagoons are similar in concentration, even though Lake Ellesmere has a much larger and agriculturally dominated catchment than Tomahawk Lagoon (2760 km\(^2\) vs 16 km\(^2\)). Although Tomahawk Lagoon has a small catchment, it is steep and comprises of sheep/pig farms which possibly contribute large nutrient concentrations after rainfall events. Given the low concentrations of nitrate generally recorded in the lagoons (<0.1 mg L\(^{-1}\)), organisms that utilize nitrate (phytoplankton, bacteria, macroalgae) are expected to rapidly consume the nutrient input, as has been reported in experimental tracer studies (Olsen et al., 2017). Thus, it will be important to measure in situ denitrification rates seasonally to assess the ability of ICOLLs to remove new bioavailable nitrogen during these episodic pulse events.

I hypothesised that the river entrances would provide a new source of nutrients during high water discharges, increasing the activity of denitrifiers in sediments located near the dominant freshwater inflows (Highton et al., 2016, Marks et al., 2016), due to enhancement of the denitrifying microbial community with increased nutrient availability (Smith et al., 2015, Lee & Francis, 2017). Increased concentrations of dissolved reactive phosphorus and total nitrogen were exhibited closer to the Selwyn River inflow in Lake Ellesmere, decreasing with distance (Fig. 2.5A), indicating agricultural inputs from the river. The Selwyn River was also expected to be a large source of dissolved inorganic nitrogen (DIN), as previously reported in (Hamill & Schallenberg, 2013) (mean DIN load 646 kg d\(^{-1}\)), however this was not the case during this low flow event. Large freshwater influences were noted around the northwest and eastern edges of the lagoon (Fig. 2.6A,E), evident by lowered salinity and high nitrate concentrations, which were highly correlated. Hart’s creek (site E14) appeared to be a large source of nitrate to the ICOLL at the time of sampling, as previously reported in (Hamill & Schallenberg, 2013) (mean DIN load 651 kg d\(^{-1}\)). Large nitrate concentrations and low salinity were also reported at Greenpark Sands (site E6), where there is no obvious inflow, thus nitrate rich groundwater inputs may occur at this site, which has
been suggested to be a source of nitrate in other reports (Hamill & Schallenberg, 2013). Nitrate concentrations decreased rapidly from these two sites, suggesting rapid consumption resulting in localised impacts. Denitrification rates are often higher in lower salinity sediments (Anderson et al., 2014, Marks et al., 2016), and greater abundances of key nitrogen cycling genes have been reported at lower salinity sites (Francis et al., 2013, Zheng et al., 2015). At higher salinities, NH$_4^+$ binding to the sediment is inhibited by cation exchange blockage by seawater cations (Boatman & Murray, 1982, Gardner et al., 1991), reducing nitrogen availability for coupled nitrification-denitrification in the sediment. This may however increase availability of NH$_4^+$ in the water column, either for consumption by primary producers, or to be linked to nitrification-denitrification diffusing from the water column.

Flow rates however were enhanced at Hart’s creek compared to average flows (3.31 m$^3$ s$^{-1}$ compared to a yearly average of 2.3 m$^3$ s$^{-1}$ from 2009 to 2014), explaining the increase in nitrate in that region. In Tomahawk Lagoon there was similarly a nutrient gradient formed away from the dominant inflow, evident in the DRP, TP and TN concentrations (Fig. 2.7C,D,F). Interestingly nitrate and ammonium concentrations were increased at the southern site (T6) closest to the lagoons ocean connection (Fig. 7E,G), and greater nitrate concentrations were recorded than measured in Lake Ellesmere (ICOLL average 247 µg L$^{-1}$ vs 49 µg L$^{-1}$ respectively). This localised increase in nitrate and ammonium may suggest re-mineralization or nitrification occurring in the sediments, or alternatively allochthonous nutrient inputs from the upper lagoon inflow. Our sampling does however represent a one-off spatial survey where I have captured a low flow event, with generally low nitrate concentrations across the ICOLLS. During periods of low flows from the main river inflows, our data suggest other sites may still provide high nitrate concentrations, and that these impacts may be localised.

### 2.4.2 Sedimentary hotspots of denitrification potential

I expected denitrification enzyme activity to be correlated to the inflows, reflecting the in-lake nitrate concentrations typical of times when higher river discharge rates occur. No relationships were observed between denitrification enzyme activity and distance from inflow. As such, I examined the relationships between sediment characteristics and denitrification enzyme activity. Both ICOLLS have strong sedimentary gradients, where sediments dominated by clay and silt had higher porosity, and sand dominant sediments had higher lower porosity (Fig. 2.10). Soils literature reports that smaller particle size in
fine textured sediments holds more water, and anaerobic zones occur more easily, compared to the larger pores of courser sediments (Groffman & Tiedje, 1989). These anoxic microsites are likely to be hotspots of denitrification (García et al., 1998, Groffman et al., 2006). Denitrification enzyme activity was not correlated with the distance to the dominant inflows in Lake Ellesmere as originally expected (Appendix A, Table 7.2), instead being highly correlated with the sediment grain size gradient, with greater activity occurring in shallow, well oxygenated, sand dominant sediments when amended with nitrate and carbon (Fig. 2.13A). When sediment clay content reached greater than 7%, DEA rates were lower in Lake Ellesmere, likely due to diffusional limitations. Sediments in a New Zealand north island estuary had greatly decreased DEA and more variable activity after a 10% threshold clay content, when sediments had been enriched with a slow-release nitrogen fertilizer (Douglas et al., 2018). Greater denitrification enzyme activity has been reported in sandy sediments in past research (Jones et al., 2011). Although sandy sediments had a lower organic matter content, this may be reflective of high organic matter processing reflective of highly productive sediments (Huettel & Rusch, 2000, Sokoll et al., 2016). Sandy porous sediments can have increased denitrification due to advective pore water transport (Cardenas et al., 2008), forcing water movement across the sediment-water interface, enhancing the supply of nutrients (Santos et al., 2012), which is likely a strong factor in shallow, wind dominated ecosystems such as ICOLLS.

In contrast, Tomahawk Lagoon sediments (sediment only and DEA+N) were also related to the grain size gradient, however denitrification enzyme activity were greatest in oxygen limited, organic rich, clay dominant sediments (up to 7% clay content) (Fig. 2.13B). These sites are located closer to the main stream inflow. In the Tomahawk DEA+C+N treatment, denitrification enzyme activity was greater in the sandy, shallow sediments similar to Lake Ellesmere. Research in a northern New Zealand estuary found DEA to be enhanced with sediments containing greater organic carbon and mud content (Douglas et al., 2017). Sediment organic matter availability was greater in Tomahawk Lagoon (average 285 g m⁻² versus 240 g m⁻²) and thus may have enhanced denitrification enzyme activity in these sediments, although I acknowledge this represents a small change in organic matter content (9 % vs 4 % dry weight). Similarly, a study of DEA in New Zealand lake’s found DEA to be increased with increasing sediment organic matter and percentage catchment agriculture (Bruesewitz et al., 2011).
A positive correlation between denitrification potential and organic carbon has been found in various studies (Inwood et al., 2007, Dodla et al., 2008, Marks et al., 2016). Organic matter is one of the most important variables controlling denitrification in aquatic ecosystems, with increasing organic matter increasing rates of sediment metabolism, oxygen demand and N remineralisation (Cornwell et al., 1999, Piña-Ochoa & Álvarez-Cobelas, 2006). The organic matter in Lake Ellesmere was generally low, and the reported increase in N$_2$O production with additional carbon and nitrate suggests that the sediments are co-limited by carbon (Zhong et al., 2010, Marks et al., 2016). Patel (2008) also found carbon limitation in their system, with sediments from two different sites both having the same ability to process nitrate under carbon-enriched conditions. Sediment denitrification potential is often greatly enhanced when supplied with carbon and nitrate (Fernandes & Loka Bharathi, 2011, Jones et al., 2011, Genthner et al., 2013, Bellinger et al., 2014). Tomahawk Lagoon sediments showed a slight increase in denitrification enzyme activity with amended carbon, suggesting nitrate was available in the porewater. However, the amendment of carbon and nitrate resulted in no detectable N$_2$O production. As Tomahawk Lagoon is already rich in available carbon (mean organic matter content 9%), sediments are likely poised to remove nitrate when it is available. Seasonal episodic pulses shown in Fig. 2.2 may be periods of enhanced denitrification enzyme activity due to increased nitrate availability. The sediments of Lake Ellesmere removed nitrate when provided, however the magnitude of response depended on the sediment organic availability, redox conditions and dominant grain size.

As this is a one-off sampling, I may not have captured the seasonal variability in organic matter, and events that lead to deposition of carbon may change the importance of denitrification enzyme potential in different areas of the lake bed. I do acknowledge the small sample size in Tomahawk Lagoon (3 locations). Due to the large size differences between Lake Ellesmere and Tomahawk Lagoon, sites were chosen to cover a representative sediment gradient within budget constraints. The spatial maps are used here as a visual representation of changes in sediment and physico-chemical variables within the lagoons, and due to the large distances of extrapolation caution should be taken as no estimates of error can be provided.

2.4.3 Possible competitive nitrogen cycling pathways
Interestingly in Tomahawk Lagoon, carbon only amendments showed no detectable denitrification enzyme activity suggesting competitive interactions for the nutrients may
have occurred, such as by dissimilatory nitrate reduction to ammonia (DNRA) (Burgin & Hamilton, 2007, Giblin et al., 2013) or anaerobic ammonium oxidation (anammox) (Burgin & Hamilton, 2007, Devol, 2015). DNRA has been shown to be an important process competing with, or even outcompeting denitrification in shallow coastal ecosystems under certain conditions (Christensen et al., 2000, Burgin & Hamilton, 2007, Dunn et al., 2012, Giblin et al., 2013, Bonaglia et al., 2014, Nogaro & Burgin, 2014, Bernard et al., 2015, Brin et al., 2015, Hardison et al., 2015). This competitive process may occur in ICOLLs, where excess carbon additions to already saturated sediments could invoke a shift from denitrification to DNRA, converting nitrate to ammonium (Burgin & Hamilton, 2007, McGlathery et al., 2007), however rates of conversion by DNRA were not directly measured in our experiments. In Tomahawk Lagoon a reduction in denitrification enzyme activity was exhibited at some sites in the carbon only amendments when compared to the nitrate amended treatments, which suggest that organic carbon did not limit denitrification enzyme activity at these sites (Bellinger et al., 2014), and increased carbon additions invoked competitive interactions by alternate N cycling pathways.

Anammox can be important in ecosystems with large nitrogen inputs relative to the labile carbon supply (Burgin & Hamilton, 2007), with anammox in oceanic systems suggested to remove 1/3 to 2/3 of the sedimentary nitrogen (Dalsgaard et al., 2005). Only one study on anammox has been reported in a coastal lagoon, where it contributed only 2-4% of N cycling (Bernard et al., 2015), which suggests anammox may be an additional, albeit minor, nitrate removal pathway in ICOLL ecosystems.

Two lesser studied alternate pathways that could be removing bioavailable nitrate include codenitrification and chemodenitrification. Codenitrification may also be a source of N₂O in the denitrification enzyme assay, where two different N compounds are utilized (one inorganic and one co-metabolised organic) to produce N₂O (Phillips et al., 2016, Rex et al., 2018). Due to its relatively new discovery, there is limited published research on its importance in estuarine ecosystems. Chemodenitrification (where nitrite is converted to nitrous oxide in a chemical reaction with Fe(II)) has generally not been considered a major N removal pathway (Onley et al., 2018), however recent work has suggested it can contribute up to 10% of the N₂O yield (Wankel et al., 2017). The importance of these alternate pathways in ICOLLs warrant further research, especially under different nitrogen and carbon availabilities.
2.4.4 Sediment microbial community structure

Research carried out in parallel to this study investigated the microbial community at each of the 18 sites in Lake Ellesmere (Highton et al., 2016). The genes involved in nitrogen processing and denitrification were assessed using quantitative PCR of the nifH, nirS, nosZI and nosZII genes. Species richness decreased with a decrease in sediment sand % (Spearman ρ: -0.6966, p < 0.0001)(Highton et al., 2016). This result is consistent with the findings of Sessitsch et al. (2001), that smaller sediment size fractions host a greater diversity of microbes than larger sized particles. Smaller sediment grain size provides protection from predators being excluded from the small pore size, and the increase of surface area to volume ratio enhancing space for microbial attachment (Sessitsch et al., 2001). The sediment represents a more stable and consistent selective force in shaping the microbial community (Highton et al., 2016). In contrast, the episodic nature of the nitrate inputs to the ICOLLs (Fig. 2.2) only represents a short-term driver, which may increase enzyme activity, without altering the microbial community structure.

There was no correlation found between the genes that indicate the ability to denitrify and the denitrification enzyme activity. Microbial communities are able to respond to short-term changes in nitrate concentrations (Kana et al., 1998), and high nitrate loading can enhance microbial activity (Cornwell et al., 1999), often explaining spatial variability in nitrogen cycling gene abundance (Smith et al., 2015, Zheng et al., 2015, Lee & Francis, 2017). As I did not observe strong nitrate gradients within the ICOLLs in relation to the main tributary inflows, the sedimentary environment is likely the strongest driver of microbial abundance due to space and organic matter availability. The DEA technique blocks sediment nitrification pathways (Groffman et al., 2006), and if this is an important source of internal nitrate within the system, it would go undetected using this technique. It can lead to an underestimation of denitrification rates due to limiting the additional nitrate production pathway (Marks et al., 2016), although the addition of saturating nitrate concentrations should not limit denitrification enzyme activity. Thus, the use of other techniques which include the nitrification pathway (such as isotope pairing or membrane inlet mass spectrometry) may elucidate if this is an important pathway in this system.

Results from this study must be interpreted with caution however, and sediments for the denitrification enzyme activity were collected at different times of the year due to time
constraints. Increased nitrogen availability in winter in Tomahawk Lagoon may have led to an overestimation of denitrification enzyme activity.

Our study presents the first measurements of denitrification enzyme activity in ICOLL ecosystems in New Zealand (Highton et al., 2016). Many studies which quantify denitrification focus on a single location without considering spatial variability (Saunders & Kalff, 2001, Piña-Ochoa & Álvarez-Cobelas, 2006). Our results clearly show how spatially variable denitrification can be, and that changes in spatial variability are driven predominantly by the grain size gradient during low flow events. Sediments with higher organic carbon availability can remove nitrate when it becomes available, and sediments show large denitrification enzyme activity when amended with nitrate.

2.5 Conclusions

The ICOLLs Tomahawk Lagoon and Lake Ellesmere are influenced by large episodic nutrient pulses which occur during winter and spring, which are likely to influence the nutrient status of the ICOLL. However, nitrate concentrations were low during our experimental survey and did not create a persistent nitrate gradient. In the absence of strong water column nutrient forcing during our sediment survey, sediment gradients controlled the denitrification enzyme activity. In Lake Ellesmere, sediments were nitrate and carbon limited, and hotspots of DEA occurred in the shallow, sandy margins of the lagoon. DEA rates declined above a sediment clay content of 7%, possibly due to diffusional limitations. In Tomahawk Lagoon, sediments were rich with carbon, and hotspots of DEA were recorded in the organic rich, clay dominant sites (up to 7% clay content) in the sediment only and +N treatments. DEA+C+N was greatest in Tomahawk Lagoon also in shallow, sandy sediments. Both ICOLLs sediments appear able to remove inputs of agriculturally derived nitrogen when they occur, possibly reducing nitrate availability to phytoplankton and reducing eutrophication effects. Further studies which investigate the in situ capacity of denitrification to remove nutrient pulses will further our understanding of sedimentary denitrification.
 CHAPTER THREE

A hierarchy of factors controlling denitrification rates in intermittently closed and open coastal lakes/lagoons (ICOLLS)

Photo: The S.F. Mitchell docked at the Lake Ellesmere lake house wharf.

This chapter is a modified version of the following manuscript for submission to Aquatic Ecology: Crawshaw, J.A., Schallenberg, S., Savage, C., & Van Hale, R. A hierarchy of factors controlling denitrification rates in intermittently closed and open coastal lakes/lagoons (ICOLLS).
3.1 Abstract

Nitrogen losses from high intensity agricultural land-use fuels phytoplankton growth in many coastal ecosystems, including intermittently closed and open lakes/lagoons (ICOLLs). ICOLLs can occur in alternate stable states: clear and turbid. In aquatic receiving environments, denitrification may remove a proportion of the land-derived nitrogen load, reducing their vulnerability to eutrophication (an increase in the rate of supply of organic matter to a system). In this study, potential factors that limit denitrification (temperature, nutrient/organic matter supply, oxygen conditions, sediment type and benthic macro-invertebrates) were quantified in two eutrophic ICOLL ecosystems (clear water supporting some submerged macrophytes versus turbid phytoplankton dominated). Novel, flexible in situ enclosures and in vitro measurements were employed to determine denitrification rates in response to the addition of nitrate pulses. In situ denitrification was severely inhibited/undetectable in both ICOLLs in winter, when temperatures were between 5.1 and 8.6°C. In summer, when temperatures were above 16.7°C, denitrification rates increased with organic matter availability and in vitro denitrification rates were stimulated by the addition of glucose. Denitrification was greater in shallow, marginal sediments. Bioturbating macrofauna significantly enhanced in situ sediment oxygenation and probably transported sediment organic carbon and nitrate simultaneously to sites of denitrification at the sediment oxic-anoxic interface. As a result, macrofauna enhanced rates of coupled nitrification-denitrification, which was the dominant denitrification pathway in our study ICOLLs. Thus, a hierarchy of physico-chemical and biological factors (temperature → organic matter → water depth → bioturbation) determined the spatio-temporal dynamics of denitrification in the two study ICOLLs.
3.2 Introduction

Intermittently closed and open lake lagoons (ICOLLs) are shallow bodies of water which lie along coastlines and are intermittently connected to the ocean (de Wit et al., 2001, Kennish & Paerl, 2010, Schallenberg et al., 2010, Robertson et al., 2016). These unique ecosystems are estimated to cover up to 15.3% of the world’s microtidal coastlines (McSweeney et al., 2017). Due to their limited flushing and longer water residence times than open estuaries, ICOLLs and open lagoons are particularly vulnerable to nutrient and organic matter enrichment, leading to eutrophication (Herbert, 1999, Eyre & Ferguson, 2002, Kennish & Paerl, 2010, Schallenberg et al., 2010, Robertson et al., 2016). Prior to anthropogenic impacts, the majority of ICOLLs were likely macrophyte-dominated, with clear waters and low turbidity (Scheffer, 1998). By contrast, shallow lakes in New Zealand have been reported to be in poor condition due to human activities (Drake et al., 2011). The likelihood of a regime shift occurring in New Zealand shallow lakes (including ICOLLs) is correlated with the percentage of the catchment in pasture (Schallenberg & Sorrell 2009), and farming activities result in greater nitrogen losses from land to freshwater (McDowell & Wilcock, 2008, Gluckman, 2017) and estuarine ecosystems (Heggie & Savage, 2009). Processes which naturally remove nitrogen from the ecosystem are expected to potentially ameliorate eutrophication effects, thereby conferring ecological resistance and resilience to eutrophication. Resistance is how much external pressure an ecosystem can endure and remain in the same state (Downing et al., 2012), while resilience allows the ecosystem to maintain structure and function under disturbance, through internal reorganisation and adaptation to change (Carpenter et al., 2001). Denitrification can potentially permanently remove nitrate transferred from land to water and reduce the nitrogen supply to phytoplankton. Conversely, dense phytoplankton populations may outcompete denitrifying bacteria for nitrate and reinforce eutrophication symptoms in ICOLLs.

Denitrification rates are often highly variable, both spatially and temporally, making it challenging to accurately estimate long-term, system-wide denitrification rates (Groffman et al., 2009) and confounding whole-system and cross-system comparisons of denitrification. Denitrification has been reported to vary based on the sediment and water column characteristics, such as water temperature, porewater nitrate concentration, organic matter concentrations and dissolved oxygen concentrations (Tomaszek &
Low sediment oxygen penetration in sediments can reduce coupled nitrification-denitrification through reduced nitrate production by nitrification (Groffman et al., 2009). Nitrification is also inhibited by exposure to sulfide (Joye and Hallibaugh 1995). Invertebrate bioturbation and irrigation can play a large role in distributing oxygen, nitrate and organic matter content within the sediment and this activity can in turn stimulate or inhibit denitrification rates (Biswas et al., 2009, Nogaro & Burgin, 2014).

This study aimed to assess the capacity of two ICOLLs to remove nitrate through denitrification. I measured denitrification rates (both in situ and using denitrification enzyme activity) and oxygen penetration depth (OPD) in two eutrophic ICOLLs on the South Island of New Zealand, to determine the drivers of spatial and seasonal variation in denitrification rates and to examine the potential of denitrification to mitigate the effects of eutrophication. I measured denitrification rates in situ under nitrate pulse events, designed to mimic typical pulses of nitrate in the water column. I also tested for potential nutrient limitation using denitrification enzyme activity in the laboratory. I measured denitrification in winter and summer at a total of 9 sites across the two ICOLLs to examine how gradients in potential physico-chemical and biological drivers relate to denitrification, substrate limitation and sediment oxygenation in these ICOLLs. Finally, I developed statistical predictive models for assessing the denitrification potential of both new and recycled nitrate within these, and other similar, ICOLLs.
3.3 Methods

3.3.1 Site Locations

Te Waihora/Lake Ellesmere (here referred to as Lake Ellesmere) is a large (surface area = 145 to 213 km$^2$) shallow (mean depth = 0.8 to 1.5 m) ICOLL located in Canterbury, South Island, New Zealand (Fig. 3.1A) (Schallenberg et al.). It is separated from the sea by a gravel barrier bar along Kaitorete Spit (Hughes & Taylor, 2009, Jellyman, 2011), which is mechanically opened at specified water levels to prevent inundation of surrounding farmlands and to facilitate drainage (Schallenberg et al., 2010). Historically, macrophytes were common in the lagoon (primarily *Ruppia megacarpa* and *Ruppia polycarpa*) (Gerbeaux, 1993, Hearnshaw & Hughey, 2012), however macrophytes were virtually eliminated in a severe storm in 1968 (Hughes et al., 1974). In more recent times, the phytoplankton biomass (mean chlorophyll $a = 88.4 \mu g L^{-1}$) and suspended sediment concentrations (mean = 229.7 $\mu g L^{-1}$) have generally remained high (Schallenberg et al., 2010), inhibiting substantial re-establishment of the submerged macrophytes and maintaining the lagoon in a turbid state (Gerbeaux & Ward, 1991). The catchment of Lake Ellesmere covers approximately 2760 km$^2$, with intensive agriculture being the predominant use of land in the catchment (Hearnshaw & Hughey, 2012), resulting in high nitrogen and phosphorus loads to the lagoon (Schallenberg et al. 2010). The water column concentration of nitrate ranges from below analytical detection limits (<0.071 $\mu mol L^{-1}$) to episodic high nitrate concentrations (e.g., >21 $\mu mol L^{-1}$ with a recorded maximum 307 $\mu mol L^{-1}$)(Fig. 3.2A). Pulses of nitrate (here defined as episodes > 21 $\mu mol L^{-1}$) generally occur in late autumn through to spring, followed by generally decreasing nitrate levels, often to below detection limits, in summer.

The lower Tomahawk Lagoon (referred to here as Tomahawk Lagoon) is a small (0.19 km$^2$), shallow, hypertrophic ICOLL in the city of Dunedin, South Island, New Zealand (Fig. 3.1B)(Hamilton & Mitchell, 1997, Drake et al., 2011, Hamill et al., 2014). It is fed by surface runoff and a stream at the northern end that drains a small agricultural catchment (area = 2.4 km$^2$)(Mitchell, 1989, Drake et al., 2011). The lower lagoon receives water from the upper lagoon (also known as Tomahawk Lagoon No.2) by a small narrow channel, with a catchment of 16 km$^2$ (Mitchell et al., 1988). Because of the shallow depth and the alignment of the main axis of the lagoon with the prevailing winds (S-E and N-W), sediment is frequently re-suspended by waves (Robertson, 1999).
Tomahawk Lagoon has not been regularly studied or monitored, however intermittent measurements show nitrate concentrations range from <0.036 µM to 18 µM in the lower lagoon (J. Crawshaw, unpubl. data and A. Innes, unpubl. data) (Fig. 3.2B). The highest nitrate delivery to both ICOLLs occur in winter and spring.

### 3.3.2 In situ denitrification measurements

*In situ* rates of denitrification were measured at three sites in Lake Ellesmere during winter (August 2015) and summer (January 2016) (Table 3.1). Four additional sites were added during summer 2016 to increase the spatial coverage of the lake. *In situ* denitrification rates were measured at three sites in Tomahawk Lagoon during winter (July 2015) and summer (December 2015).

*In situ* denitrification rates were measured to test the potential of the ICOLLs to remove simulated nitrate pulses under realistic turbulent mixing conditions, across seasonal and spatial gradients. Specifically, nutrient fluxes and denitrification rates were measured in custom-built *in situ* enclosures, which isolated sediment and the overlying water of the incubation chamber (henceforth called “enclosures”) from the ambient ICOLLs (Fig. 3.3). These consisted of polyvinyl chloride bottom and top plates which held four replicate enclosures, with a flexible polyethylene tubing connecting the plates through the water column. The flexible tubing transferred turbulent energy in the lake into the enclosures providing mixing, which have been utilized in previous studies (Asmus *et al*., 1998, Risgaard-Petersen *et al*., 1999, Fanjul *et al*., 2011). Turbulence at the sediment-water interface is potentially a key driver of nitrate supply to the sediment microbial community (Risgaard-Petersen *et al*., 1999, Cook *et al*., 2006), especially in shallow, wave dominated ecosystems such as ICOLLs. The enclosure inner diameter was 6.4 cm, and the bottom plate contained core tubes which penetrated the sediment to a depth of approximately 15 cm. The top plates allowed the connection of the flexible tubing, which was adjusted so that the tops of the enclosures were submerged just below the water level at each site. The tops of the enclosures were sealed to prevent the exchange of water and gases with the ambient ICOLL. Water samples were taken from a 2-way valve on the lid of the enclosures, where the sample tubing inflow was located at the top of the flexible enclosure (~10 cm below water surface). Due to the low ambient nitrate in the ICOLLs at the time of the experiment, the background nitrate concentration was raised to 71 µmol N L⁻¹ to reflect the large nitrate pulse events which occasionally occur in the lagoon (Fig.
3.2), using NaNO$_3$. 71 µmol N L$^{-1}$ of K$^{15}$NO$_3$ was added to the enclosure, to achieve a 50% atom nitrate enrichment (Steingruber et al., 2001) (final concentration NO$_3^-$ 142 µmol N L$^{-1}$).
Figure 3.1. Map of New Zealand showing the study locations, A= Lake Ellesmere, Canterbury, New Zealand, and B = Lower Tomahawk Lagoon, Otago, New Zealand. A= Lake Ellesmere sampling sites E1, E2, E3, E6, E14 and E19. B= Tomahawk Lagoon sampling sites T4-T6.
Figure 3.2. A. Nitrate concentration data measured in Lake Ellesmere and at its main inflow (Selwyn River mouth). B. Nitrate concentration data from Lower Tomahawk Lagoon (see Fig. 3.1B) taken from site T5. Black dashed lines indicate 21 μmol L⁻¹, defining in-lake nitrate pulses (refer to Chapter 2 for the process defining in-lake nitrate pulses).
Table 3.1. Physico-chemical measurements of the ICOLLs taken at the initiation of the in situ enclosure experiments at sites in Tomahawk Lagoon (T4 to T6) and Lake Ellesmere (E1 to E3, E6, E14, E19) in summer (S) and winter (W). Hyphens indicate sites were not sampled in that season.

<table>
<thead>
<tr>
<th>Site</th>
<th>Date</th>
<th>Depth (m)</th>
<th>Temp (°C)</th>
<th>Salinity (ppt)</th>
<th>Secchi depth (m)</th>
<th>DO (µM)</th>
<th>NO$_3^-$ (µM)</th>
<th>NH$_4^+$ (µM)</th>
<th>DRP (µM)</th>
<th>TN (µM)</th>
<th>TP (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4</td>
<td>17/12/2015</td>
<td>0.66</td>
<td>17.2</td>
<td>3.5</td>
<td>6.6</td>
<td>614</td>
<td>0.42</td>
<td>18.73</td>
<td>5.14</td>
<td>0.18</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>15/07/2015</td>
<td>0.98</td>
<td>5.1</td>
<td>8.8</td>
<td>&gt;0.98</td>
<td>644</td>
<td>18.73</td>
<td>5.14</td>
<td>9.02</td>
<td>0.18</td>
<td>0.20</td>
</tr>
<tr>
<td>T5</td>
<td>17/12/2015</td>
<td>0.64</td>
<td>17.2</td>
<td>3.6</td>
<td>6.4</td>
<td>614</td>
<td>0.38</td>
<td>17.29</td>
<td>4.76</td>
<td>0.61</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>15/07/2015</td>
<td>0.93</td>
<td>5.1</td>
<td>8.9</td>
<td>&gt;0.93</td>
<td>659</td>
<td>17.29</td>
<td>4.76</td>
<td>9.10</td>
<td>0.61</td>
<td>0.20</td>
</tr>
<tr>
<td>T6</td>
<td>17/12/2015</td>
<td>0.50</td>
<td>17.4</td>
<td>3.6</td>
<td>5.6</td>
<td>614</td>
<td>0.38</td>
<td>19.99</td>
<td>5.13</td>
<td>0.24</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>15/07/2015</td>
<td>0.85</td>
<td>5.2</td>
<td>8.9</td>
<td>&gt;0.85</td>
<td>659</td>
<td>19.99</td>
<td>5.13</td>
<td>10.96</td>
<td>0.24</td>
<td>0.27</td>
</tr>
<tr>
<td>E1</td>
<td>27/01/2016</td>
<td>0.73</td>
<td>16.7</td>
<td>8</td>
<td>0.12</td>
<td>605</td>
<td>2.56</td>
<td>5.06</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>5/08/2015</td>
<td>0.90</td>
<td>16.7</td>
<td>10</td>
<td>5.8</td>
<td>627</td>
<td>0.64</td>
<td>9.61</td>
<td>4.53</td>
<td>0.13</td>
<td>0.45</td>
</tr>
<tr>
<td>E2</td>
<td>30/01/2016</td>
<td>0.50</td>
<td>16.7</td>
<td>10.5</td>
<td>0.14</td>
<td>566</td>
<td>1.07</td>
<td>6.88</td>
<td>0.24</td>
<td>0.24</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>5/08/2015</td>
<td>0.88</td>
<td>16.7</td>
<td>6</td>
<td>6.2</td>
<td>593</td>
<td>0.61</td>
<td>0.94</td>
<td>6.17</td>
<td>0.13</td>
<td>0.14</td>
</tr>
<tr>
<td>E3</td>
<td>30/01/2016</td>
<td>1.07</td>
<td>16.7</td>
<td>10</td>
<td>0.14</td>
<td>580</td>
<td>1.22</td>
<td>5.78</td>
<td>0.21</td>
<td>0.21</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>5/08/2015</td>
<td>0.38</td>
<td>16.7</td>
<td>3.5</td>
<td>0.18</td>
<td>580</td>
<td>16.37</td>
<td>5.46</td>
<td>0.44</td>
<td>0.44</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Incubations were run for 48 hours, with 50 mL of water taken with a syringe at 0, 4, 24, 30 and 48 hours to measure dissolved oxygen, dissolved nutrients and labelled N₂ gas. The dissolved oxygen concentration of water was measured using a calibrated YSI ProODO Optical Dissolved Oxygen meter (Yellow Springs, Ohio, USA), ensuring oxygen in the enclosures did not drop below 20% of the starting concentration over the experimental period. N₂ gas was extracted from the water sample by adding a 15 mL helium headspace to water samples collected in gas-tight syringes and shaking for 3 minutes. The gas sample (13 mL) was then transferred to a pre-evacuated exetainer (Labco, High Wycombe, UK), using a flexible needle extender which prevented the transfer of water. The exetainers were kept submerged in milli-Q water freshly sparged with helium gas while the needles were being removed from the septa, to ensure that air did not enter the punctured septum. To decrease the potential for air contamination, the samples were stored in 50 mL centrifuge tubes filled with degassed milli-Q water until analysis (Hamilton & Ostrom, 2007, Gongol, 2010, Gongol & Savage, 2016).

The isotopic composition of N₂ in the headspace was determined by mass spectrometry. The exetainers were sampled by gas chromatograph and isotope ratio mass spectrometer. The Lake Ellesmere 2015 summer samples were processed at Lincoln University, and the other season’s samples were analysed in the Chemistry Department of the University of Otago, following the protocol of Lewicka-Szczebak et al., (2013). The standard used for N₂ was 2.5% air mixed in helium, to match the N₂ concentration of the dissolved gas extracts in helium. Air was measured after every 8 samples to test for instrument drift. If significant drift was observed by linear regression of 30/28 ratio of air against time, then linear time-base correction was applied to air and samples. I acknowledge that these rates are likely underestimations of the total denitrification rates, due to not accounting for N₂ trapped in the sediment porewater. Methodological concerns have been raised about the use of the original isotope pairing calculations for this study. I acknowledge the modified isotope pairing calculations that account for anammox and DNRA (Risgaard-Petersen et al., 2003, Salk et al., 2017), however these were not measured in our study thus the denitrification rates may include some N₂ produced through anammox or DNRA. I however suggest that anammox is unlikely to account for a large percentage of N₂ production in estuarine sediments and a recent review paper indicates denitrification is the dominant N₂ production pathway (Damashek & Francis, 2018), as denitrification is more thermodynamically favourable when organic matter is present (Thamdrup &
DNRA can be a favoured process when organic matter availability is high and nitrate concentrations are low (Hardison et al., 2015), or when sediments are anoxic and sulfidic (Murphy et al., 2016). The studied ICOLLs however did not show signs of sediment anoxia, however seasonal organic loading may occur during phytoplankton blooms or macroalgal growth. DNRA may be a pathway to consider in further studies, however it was outside the scope of this project.

The measured $^{29}\text{N}_2/^{28}\text{N}_2$ and $^{30}\text{N}_2/^{28}\text{N}_2$ ratios were converted to molar quantities using computed concentrations of $\text{N}_2$ expected in the water column at the time of sampling using nitrogen gas solubility tables (Colt, 2012) given the temperature and salinity of the sampled water, and the volume of the enclosures (L) (Dalsgaard, 2000). The production of each species ($^{29/28}\text{N}_2$ and $^{30/28}\text{N}_2$) at each time point was calculated as the sample ratio minus the ratio at time 0. The absolute amount of $^{29/28}\text{N}_2$ and $^{30/28}\text{N}_2$ were then calculated using the following equation (Dalsgaard, 2000):

$$\text{AM}_{xx} (\mu\text{mol}) = (R_{xx} \times ^{28}\text{N}_2) \times \text{Sample Volume}$$

Where: $\text{AM}_{xx}$ is the amount of $^{xx}\text{N}_2$ present in the core structure ($\mu\text{mol}$), $R_{xx}$ is the ratio of $^{29/28}\text{N}_2$ provided by mass spectrometry, $^{28}\text{N}_2$ is the concentration of $^{28}\text{N}_2$ ($\mu\text{M}$) expected in the water calculated from nitrogen diffusion tables (Colt, 2012), and sample volume is that of the enclosure (L). The slope was calculated from the production of $\text{N}_2$ over the time course of the experiment, to integrated production over time, then divided by the surface area of the cores (0.003848 m$^2$). The production of total denitrification (produced from $^{14}\text{NO}_3^-$ and $^{15}\text{NO}_3^-$) was then calculated using the standard isotope pairing calculations (Nielsen, 1992).
Figure 3.3. The custom built *in situ* denitrification chamber. The core consists of four replicate flexible tubes made of flexible polyethylene tubing, which connect to rigid cores on the top and the bottom (a). The 4 rigid sediment cores push 15 cm into the sediment (b). The poles allow the insertion of the cores into the sediment, then are pushed into the sediment outside of the baseplate (c) to allow movement of the flexible core body supporting turbulent mixing through the flexible tubing. The rigid cores are held in a baseplate, which enables the cores to be pushed into the sediment. The cores are capped below the water and have sampling ports for nutrient and gas measurements (d). The chamber in place at Tomahawk Lagoon (e).

After gas extraction, the water samples were filtered through Microscience MS-GF 47 mm filters (nominal pore size = 0.7 µm) into 50 mL acid-washed centrifuge tubes and frozen at -18°C until analysis for nitrite + nitrate (NO$_2^-$+NO$_3^-$) and ammonium (NH$_4^+$). Nutrient fluxes were calculated as a change in nutrient concentration over time, accounting for enclosure water volume and sediment surface area (Dalsgaard, 2000).

After 48 h, the *in situ* enclosures were dismantled, the sediment cores were retrieved, a sample of homogenised surficial (top 4 cm) sediment was collected and the remaining sediment was sieved through a 500 µm mesh. All infauna retained in the sieve were preserved in 99% ethanol and were identified to species using guides by (Landcare Research, Moore, 1997, Goodeham & Tsyrlin, 2002). 200 mL of sediment was collected.
for analysis of particle size, organic matter concentration and content and porosity. Sediment samples were frozen at -20°C until these analyses were performed.

At each site water physico-chemical measurements (temperature, salinity, and dissolved oxygen) were made at the time of sampling with an YSI Professional Plus Multiprobe (Yellow Springs, Ohio, USA). A water sample was also collected at each site at the beginning of the incubations to measure the ambient total nitrogen and phosphorus (TN and TP) and dissolved inorganic nitrogen (NO$_3^-$, NH$_4^+$) concentrations.

### 3.3.3 Sediment oxygen penetration

The sediment oxic-anoxic (and denitrification) horizon (Gongol & Savage, 2016) and the sediment oxygen conditions were measured by sediment dissolved oxygen (DO) micro-profiling. For this, four sediment cores were collected from beside the *in situ* enclosures on the second day of the experiment. Clear plexiglass sediment core tubes (internal gravity corer. The depth of the sediment cored was approximately 15 cm, with overlying water of 15 cm. Cores were capped and stored cool and in the dark until being returned to the lab within five hours. For equilibration, cores were submerged and uncapped in site water circulated by a water pump maintained at approximately the *in situ* temperature and aerated using an air stone. The sediment oxygen profiles were measured within two hours of the initiation of equilibration. Down-core oxygen profiles were performed at 200 µm vertical resolution using a motorised micromanipulator and Unisense 100 µm oxygen microsensor (Unisense A/S, Denmark), which was connected to a Unisense microsensor multimeter (Unisense A/S, Denmark). Two oxygen sensors were attached and allowed to polarize for two hours. Once the reading had stabilized, a 2-point calibration curve was calculated to calibrate the probe. A 100% oxygen reading was taken from the middle of the oxygenated water column of a sediment core. The probe was then lowered into the sediment, until the oxygen reading reached a minimum stabilized reading, and this was the anoxic reading (Vopel *et al.*, 2012). Three replicate profiles positioned within the core were measured, taking care to avoid any infaunal burrows. After profiling cores were sieved, and infauna were retained for identification.

### 3.3.4 Nutrient analysis

Nutrients were analysed using colorimetric protocols, using a SANPlus segmented flow autoanalyzer (SkalarAnalytical B.V., Breda, The Netherlands), as previously reported in (Schallenberg & Burns, 1997). Analytical detection limits were 0.071 µM for nitrate-N,
0.93 μM for ammonium-N, 0.36 μM for total nitrogen, 0.02 μM for dissolved reactive phosphorus, and 0.06 μM for total phosphorus.

### 3.3.5 Sediment Characterization

Surficial sediment porosity (g H$_2$O ml$^{-1}$ sediment) was calculated from the dry weight of the sample after drying 5 g of wet sediment at 50°C for 24 hours. This sediment was then combusted at 450°C for 24 hours for the calculation of organic matter as mass loss on ignition, which was expressed as an areal concentration within fresh sediment (g dry OM m$^{-2}$). The surficial sediment grain size was determined using a laser-diffraction Malvern Mastersizer. Five g of sediment was dried for 24 hours at 50°C. Half a g of sediment was placed into a 50 mL falcon tube and treated with 5 mL of 10% hydrogen peroxide overnight to remove organic matter. Particle sizes were expressed as percentages of the total particle numbers, segregated into the following grain size fractions: clay (0-2 μm), silt (2-63 μm) and sand (63-2000 μm).

To provide a coherent set of data, sediment organic matter as well as denitrification and invertebrate densities were all expressed on an areal basis. I calculated concentrations of organic matter and applied these to a uniform depth into the sediment of 1 cm to provide an areal unit for comparison, which I here refer to as organic matter availability. Typically, many sediment parameters are calculated and reported as contents rather than as volumetric concentrations or areal densities. Expressed as contents, sediment parameters can be strongly biased by sediment bulk density and grain size (Tolhurst et al., 2005) and, thus, contribute little information in addition to grain size to the analysis of relationships. However, organic matter availability (per g dry sediment) may reflect the nutritional value of sediments to deposit-feeding organisms. Therefore, I also used organic matter availability in our data analyses.

### 3.3.6 Denitrification enzyme activity (DEA)

Sediment samples for the DEA were collected in April 2014 (autumn) in Lake Ellesmere and in July 2015 (winter) for Tomahawk Lagoon. Potential nutrient limitation of the denitrification rate of the sediment was measured using the acetylene inhibition technique, as described by (Bruesewitz et al., 2011). Fifteen mL of sediment was homogenized with 15 mL of unfiltered ICOLL water. Four treatments were set up to investigate possible nutrient limitations of denitrification in the sediment. These included treatments of (1) control: sediment only, (2) sediment + 10 mg of potassium nitrate (+N),
(3) sediment + 12 mg of glucose (+C), and (4) sediment + 10 mg of potassium nitrate and 12 mg of glucose (+N+C). Unfiltered ICOLL water was used to test for water column denitrification. 30 mL of the sediment-water slurry was transferred to 45 mL glass vials and sealed with silicon septa. Anoxic conditions were created by purging the vial headspace with pure N\(_2\). Then, 10 mL of acetylene (C\(_2\)H\(_2\)) was added to block the conversion of N\(_2\)O to N\(_2\), and to over-pressurise the vials. The incubations were performed at room temperature (22°C). Eight mL of gas sample was collected hourly for four hours after the addition of C\(_2\)H\(_2\). To maintain a constant pressure in the bottle, C\(_2\)H\(_2\) was added to replace the collected gas samples. N\(_2\)O concentrations were measured using a Varian CP 3800 gas chromatogram equipped with a Hayesep D column and an electron capture detector. N\(_2\)O production was calculated using the ideal gas law. Volume-specific production was converted to an areal basis (m\(^{-2}\)) using sediment bulk density measurements.

### 3.3.7 Data analysis

All statistical analyses were carried out in Excel, Canoco (v 4.5) or with R Studio (v.3.3.2) (R Studio Team 2015) with base R version 3.3.0 (R Core Team 2016). T-tests were used to test for significant differences in variables between ICOLLs. Multiple linear regression models were used to investigate variables related to denitrification rate. Highly correlated independent variables (Pearson r >0.5) were excluded from the analysis. Principal component analysis was conducted on the correlation matrices. For the PCA and multiple regression analyses, some variables were transformed to meet statistical assumptions.
3.4 Results

3.4.1 Water column and sediment characteristics

During our study, Tomahawk Lagoon had a substantially higher water clarity (Secchi depth ranged from >0.50 to >0.98 m, which were the site depths) than Lake Ellesmere (Secchi depth ranged from 0.11 to 0.18 m) (Table 3.1). The temperature in both lagoons during the summer survey was 17°C, reducing to 5.1°C in Tomahawk Lagoon and 8.3°C in Lake Ellesmere during winter (Table 3.1). The salinity in Tomahawk Lagoon was 3.6 and in Lake Ellesmere ranged from 3.5 to 11 across the study sites. During winter in Tomahawk Lagoon, both nitrate and ammonium concentrations in the water column were higher than in summer (Table 3.1). In contrast, there was no winter nitrate or ammonium peak in Lake Ellesmere at the time of sampling. Total phosphorus concentrations in Lake Ellesmere were always substantially higher than in Tomahawk Lagoon, owing to the high turbidity of the former. In other respects, water column nutrient concentrations showed little variation seasonally or between ICOLLs (Table 3.1).

Sediment at most sites in the ICOLLs was sandy except for sites T4 and T5 in Tomahawk Lagoon, which were dominated by silt and sites E14 and E19 in Lake Ellesmere which had substantial contents of both sand and silt (Table 3.2). Organic matter contents ranged from 0.5 to 12% whereas organic matter availability (concentration) ranged from 66 to 398 g m$^{-2}$. Sites T4 and T5 in Tomahawk Lagoon, which had the lowest sand and highest silt contents, had the highest amounts of organic matter. Porosity at the sites varied between 0.48 and 0.86 g mL$^{-1}$.

3.4.2 In situ denitrification measurements

The uptake of added nitrate in the in situ enclosures showed marked differences between the two lagoons (Fig. 3.4). In Lake Ellesmere during summer, the added nitrate pulse (~71 µM) was removed from the water column within 24 hours (~92% nitrate removal). In comparison, Tomahawk Lagoon still had high concentrations of nitrate remaining in the water column at the end of the experiment in summer (~40 µM, ~68% nitrate removal). Even in winter, nitrate was consumed rapidly within the 48-hour experiment in Lake Ellesmere, with relatively low concentrations of nitrate remaining (~15 µM, 87% nitrate removal). In Tomahawk Lagoon during winter, nitrate was also consumed, however much less than in summer (~60 µM remaining at 48-hours, 47% nitrate removal).
removal). Thus, although in winter I did not record any denitrification occurring, nitrate was still rapidly removed from the water column.

Table 3.2. Average sediment characteristics measured from four sediment cores collected from the in situ denitrification experiment for Tomahawk Lagoon and Lake Ellesmere in summer. OM = organic matter. Means and standard errors are reported.

<table>
<thead>
<tr>
<th>Site</th>
<th>Grain size %</th>
<th>OM availability (g m⁻²)</th>
<th>OM content (%)</th>
<th>Porosity (g mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clay</td>
<td>Silt</td>
<td>Sand</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>6 ± 0.04</td>
<td>89 ± 0.1</td>
<td>5 ± 0.08</td>
<td>357 ± 8</td>
</tr>
<tr>
<td>T5</td>
<td>5 ± 0.04</td>
<td>87 ± 0.7</td>
<td>8 ± 0.8</td>
<td>398 ± 5</td>
</tr>
<tr>
<td>T6</td>
<td>0 ± 0.06</td>
<td>3 ± 1</td>
<td>97 ± 1</td>
<td>81 ± 3</td>
</tr>
<tr>
<td>E1</td>
<td>4 ± 1</td>
<td>24 ± 4</td>
<td>72 ± 5.2</td>
<td>144 ± 10</td>
</tr>
<tr>
<td>E2</td>
<td>1 ± 0.3</td>
<td>7 ± 1</td>
<td>93 ± 1.3</td>
<td>124 ± 0.8</td>
</tr>
<tr>
<td>E3</td>
<td>1 ± 0.07</td>
<td>3 ± 0.62</td>
<td>96 ± 0.7</td>
<td>89 ± 4</td>
</tr>
<tr>
<td>E6</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>100 ± 0</td>
<td>66 ± 2</td>
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<tr>
<td>E14</td>
<td>3 ± 0.05</td>
<td>53 ± 2.1</td>
<td>44 ± 2.2</td>
<td>129 ± 9</td>
</tr>
<tr>
<td>E19</td>
<td>3 ± 0.03</td>
<td>62 ± 0.4</td>
<td>35 ± 0.5</td>
<td>174 ± 18</td>
</tr>
</tbody>
</table>

Figure 3.4. Mean nitrate concentrations (µmol L⁻¹) of all chamber and site replicates, measured in the in situ enclosures over time. Filled markers are from Lake Ellesmere (circle = summer; square = winter). Open markers are from Tomahawk Lagoon (diamond = summer; triangle = winter).
Nitrate consumption was significantly greater in Lake Ellesmere than in Tomahawk Lagoon (Fig. 3.5A; t-test, p<0.001), as was oxygen demand in the enclosures (Fig. 3.5B; t-test, p<0.001), and the oxygen penetration depth into the sediments (Fig. 3.5C; t-test, p<0.001). There was no significant difference between the ICOLLs in ammonium flux within the enclosures, although it was noted that some enclosures showed small net ammonium increases during the experiments (Fig. 3.5D). As nitrate was depleted from the water column at some sites by 24 hours, I used the production of labelled N₂ pairs ((²⁸+³⁰N₂) between 0 and 24 hours to calculate denitrification rates. Figure 3.6 shows a typical labelled N₂ accumulation plot over time. A comparison of the 24 hour and 48 hour denitrification rates are provided in Appendix A (Table 7.4).

In both ICOLLs, denitrification rates were severely inhibited/undetectable in winter, when temperatures were 8.6°C and lower (Fig. 3.7A). The highest rate measured during winter was 1.6 µmol N m⁻² h⁻¹ measured in Tomahawk Lagoon. In summer total denitrification rates were spatially variable, both among sites and among replicates within sites (Fig. 3.8). Mean total denitrification rates were 149 µmol N m⁻² h⁻¹ in Tomahawk Lagoon and 234 µmol N m⁻² h⁻¹ in Lake Ellesmere with high spatial variability within each lagoon. There was no significant difference in mean denitrification rates between the two lagoons (t-test, p>0.05) The site-averaged denitrification rates in summer ranged from 94 to 223 µmol N m⁻² h⁻¹ in Tomahawk Lagoon, and from 0.05 to 272 µmol N m⁻² h⁻¹ in Lake Ellesmere. An extremely high rate (4007 µmol N m⁻² h⁻¹) was recorded in one replicate enclosure in Lake Ellesmere at site E19, located at the inflow of Hart’s Creek, but I consider this to be an anomalous measurement, probably due to a very small headspace in the core.
Figure 3.5. Mean and enclosure water column nitrate flux (µmol m$^{-2}$ h$^{-1}$; A), oxygen flux (µmol m$^{-2}$ h$^{-1}$; B), sediment oxygen penetration depth (mm; C) and ammonium flux (µmol m$^{-2}$ h$^{-1}$; D) recorded in Tomahawk Lagoon and Lake Ellesmere. Error bars show 1 standard error (n=4).
Figure 3.6. Labelled $^{29}+^{30}$N$_2$ gas production ($\mu$mol m$^{-2}$) over the chamber incubation (hours) for Tomahawk Lagoon in summer.

Figure 3.7. Key univariate relationships between *in situ* denitrification rate vs water temperature (A) and organic matter availability (B, C). Includes the authors unpublished data from spring and autumn samplings in Tomahawk Lagoon. Open circles are from Tomahawk Lagoon and closed circles are from Lake Ellesmere in panel B.
Figure 3.8. Mean denitrification measurements from the *in situ* incubation chambers in summer 2015/2016 in Tomahawk Lagoon No.1 (sites T4, T5, T6) and Lake Ellesmere (sites E1, E2, E3, E6, E14, E19). An unusually high rate was recorded in one replicate at site E19 (4007 µmol N m\(^{-2}\) h\(^{-1}\)), which has been excluded from this figure. Error bars report 1 standard error (n=4).

Table 3.3. Denitrification potential (N\(_2\)O production µmol m\(^{-2}\) h\(^{-1}\)) from the denitrification enzyme assay. T4, T5, T6 = Tomahawk Lagoon sites. E1, E2, E3, E6, E14 = Lake Ellesmere sites. Sediment only = an un-amended sediment slurry; + nitrate = nitrate amended sediment slurry; + carbon = carbon amended sediment slurry; + nitrate + carbon = carbon and nitrate amended sediment slurry.

<table>
<thead>
<tr>
<th>Site</th>
<th>Sediment only</th>
<th>+ nitrate</th>
<th>+ carbon</th>
<th>+ nitrate + carbon</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4</td>
<td>355</td>
<td>2848</td>
<td>0</td>
<td>348</td>
</tr>
<tr>
<td>T5</td>
<td>635</td>
<td>3674</td>
<td>0</td>
<td>1657</td>
</tr>
<tr>
<td>T6</td>
<td>843</td>
<td>656</td>
<td>0</td>
<td>3226</td>
</tr>
<tr>
<td>E1</td>
<td>0</td>
<td>282</td>
<td>0</td>
<td>2190</td>
</tr>
<tr>
<td>E2</td>
<td>0</td>
<td>70</td>
<td>0</td>
<td>499</td>
</tr>
<tr>
<td>E3</td>
<td>0</td>
<td>350</td>
<td>0</td>
<td>2574</td>
</tr>
<tr>
<td>E6</td>
<td>0</td>
<td>191</td>
<td>0</td>
<td>2151</td>
</tr>
<tr>
<td>E14</td>
<td>0</td>
<td>199</td>
<td>0</td>
<td>1515</td>
</tr>
</tbody>
</table>
3.4.3 Potential carbon and nitrogen limitation

*In vitro* denitrification enzyme activity (DEA) was measured in summer 2014 (Chapter 2) using sediments from all the sites to test for potential carbon (glucose) and nitrogen (nitrate) limitation (Table 3.3). In the Lake Ellesmere control and carbon only treatments, N\(_2\)O production was not detectable, while nitrate addition stimulated N\(_2\)O production. Highest rates of production, however, were observed when glucose and nitrate were added, indicating primary nitrate limitation and secondary carbon limitation of denitrification (Table 3.3). In Tomahawk Lagoon, the unamended sediment showed some denitrification enzyme activity at all the sites, suggesting there was sufficient nitrate and carbon available to support denitrification. Denitrification enzyme activity in Tomahawk Lagoon sediments was further stimulated by nitrate addition at sites T4 and T5 (the silty sites), but not at site T6 (the sandy site), whereas carbon and nitrate only addition stimulated denitrification enzyme activity at sites T5 and T6. Unlike in Lake Ellesmere, the addition of carbon only resulted in a complete inhibition of N\(_2\)O production in Tomahawk Lagoon sediments.

I compared *in situ* and *in vivo* denitrification rates in summer (Fig. 3.9). In Lake Ellesmere, the DEA estimates were higher than the *in situ* measurements. In contrast, the DEA estimates in Tomahawk Lagoon were much higher than the *in situ* measurements.

3.4.4 Factors related to *in situ* denitrification rates

Temperature was a strong determinant of denitrification rate, as indicated by severe inhibition of denitrification during winter, at temperatures of 8.2 to 8.6°C in Lake Ellesmere and 5.1 to 5.2°C in Tomahawk Lagoon (Fig. 3.7A). The relationship between denitrification rate and temperature appears to be governed by a temperature threshold between 8.6 and 12°C and the summer measurements presented here were made at 16.7 to 17.4°C. Thus, further exploration of factors related to denitrification rates only relates to measurements made in summer, when temperature did not severely inhibit denitrification.

An examination of the correlation structure between variables shows that in Lake Ellesmere, a strong gradient existed in water depth, porosity and organic matter availability (Fig. 3.10A). Denitrification rate loaded strongly along this primary axis, loading positively with organic matter availability and porosity and negatively with depth. These relationships changed little with the addition of macroinvertebrate densities,
although oligochaete density loaded positively with denitrification (Fig. 3.10B). In Tomahawk Lagoon, the primary gradient was related to grain size (as indicated by sand content), organic matter availability, porosity and water depth which were all strongly correlated (Fig. 3.10C). Denitrification was also correlated to this gradient. The addition of macroinvertebrate densities showed that chironomid and amphipod (*Paracorophium excavatum*) densities also related positively and negatively to denitrification rate, respectively (Fig. 3.9D).

**Figure 3.9.** A comparison of summer denitrification rates (μmol m$^{-2}$ h$^{-1}$) from the nitrogen amended treatment in the denitrification enzyme activity (DEA) compared to the *in situ* denitrification rates spiked with nitrate in the two ICOLLs (Tomahawk Lagoon sites = T4, T5, T6. Lake Ellesmere sites = E1, E2, E3, E6, E14).
Figure 3.10. Principal components analyses for denitrification rate and sediment physico-chemical variables (left panels). Right panels also include invertebrate density variables. Percentages show the variance in the datasets explained by the primary (x) and secondary (y) axes.
When data from the two ICOLLs were combined, relationships between physico-chemical variables and denitrification rate were less clear (Fig. 3.10E). However, when macroinvertebrate densities were added to the analysis, denitrification rate loaded strongly on the first axis along with porosity, chironomid density and depth (42% of the variance explained), while sand content and *P. excavatum* density loaded strongly on the secondary axis, orthogonal to denitrification rate (26% of variance explained; Fig. 3.10F).

Univariate relationships between denitrification rate and key independent variables are shown in Fig. 3.11. It is clear from these relationships that simple univariate relationships encompassing both Lake Ellesmere and Tomahawk Lagoon sites do not exist. This is consistent with the ordinations shown in Fig. 3.10A to 3.10D. Only denitrification rates in Tomahawk Lagoon showed significant relationships with single independent variables such as organic matter concentration (positive), depth (positive), sand content (negative) and chironomid density (positive). Note that chironomid densities in Lake Ellesmere were much lower than in Tomahawk Lagoon (Fig. 3.11F). As organic matter availability appears to be a key driver of denitrification during summer in both lagoons, the dataset from this study was combined with unpublished data from additional studies to investigate possible correlations (Fig. 3.7B,C). Both ICOLLs had the same range of organic matter availability, however greater denitrification rates were recorded in Tomahawk Lagoon. A slight positive increase in denitrification with increasing organic matter availability was observed, however this is non-significant, indicating other variables may also be controlling denitrification rates in these lagoons.

Recognising that correlations between denitrification rates and independent variables in these ICOLLs are complex, I used multiple linear regression to develop multivariate models to potentially predict denitrification rates in these and other similar ICOLLs (Table 3.4). Sediment organic matter availability was the strongest and most consistent independent variable in predictive models followed by water depth, which had positive and negative partial effects on denitrification rates, respectively. The addition of macroinvertebrate densities to the models explained only small additional amounts of variance in denitrification rates (Table 3.4). Oligochaete density had a positive effect on denitrification rate in combination with organic matter availability. However, in three parameter models, macroinvertebrate densities have only non-significant effects, with amphipod and chironomid densities showing unexpected negative coefficients, probably
due to their significant collinearities with organic matter availability \( (p < 0.001) \). The strongest multiple regression models explained < 50% of the variance in denitrification rates measured in the ICOLLs. At the time of the *in situ* denitrification measurements, oxygen penetration depth (OPD) into the sediment was also measured on cores retrieved at the study sites, near to the enclosures. The mean OPD at the sites was positively correlated with mean denitrification rates measured in the enclosures in Tomahawk Lagoon \( (R^2 = 0.90, p > 0.05) \), but not in Lake Ellesmere \( (R^2 = 0.20, p > 0.05) \), where OPDs were often much deeper (Fig. 3.12). Macroinvertebrate densities were measured in the same cores, allowing a multiple regression analysis of the effects of the invertebrates on OPD. In Tomahawk Lagoon, OPD variation was best explained by the densities of chironomid larvae and oligochaetes (model \( R^2 = 0.76, p<0.01 \)), where the effect of chironomid density was positive and contributed 70% of the explanatory power of the model, and the effect of oligochaete density was negative, contributing 30% of the explanatory power. In contrast, in Lake Ellesmere, OPD was best explained by a model containing organic matter availability and polychaetes (Nereidae) density (model \( R^2 = 0.89, p<0.001 \)), where the effect of organic matter availability was negative and contributed 86% of the explanatory power of the model, and the effect of polychaetes was positive and contributed 14% of the explanatory power.

Using the developed multiple regression models and spatial data from the 2014 surveys (Chapter 2), ICOLL - wide N removal budgets (via denitrification) have been developed. I emphasise that the budgets are likely to be over-estimates of N removal via denitrification, as seasonal changes in organic matter availability, depth and oligochaete densities are not accounted for. The model assumes that denitrification remains high across 9 months of the year, with 3 months inactive due to temperatures dropping below 8ºC (where denitrification was no longer detected). It also assumes that nitrate supply is high and non-limiting, and that the sedimentary environment is split equally between the 18 sites in Lake Ellesmere and 3 sites in Tomahawk Lagoon. ICOLL - wide estimates show that Lake Ellesmere could potentially remove 6169 tonnes of N per year, and Tomahawk Lagoon could remove up to 7 tonnes of N per year. When scaled by area ICOLL - bed, Lake Ellesmere could remove 34 tonnes per km\(^2\), and Tomahawk Lagoon 39 tonnes per km\(^2\). As stated, these are extremely rough estimates and further seasonal field experimentation is required to improve on these N removal estimates, and our
ranges fall within total system budgets reported elsewhere (29 – 24,000 T N per year; (Ogilvie et al., 1997, Trimmer et al., 2000, Zaghmouri et al., 2013, Eyre et al., 2016)).
Figure 3.11. Key univariate relationships between *in situ* denitrification rate vs physicochemical variables and invertebrate densities. Filled circles are data from Lake Ellesmere. Open circles and dotted lines are data from, and significant regressions for, Tomahawk Lagoon.
Figure 3.12. Relationships between mean denitrification rate (µmol m⁻² h⁻¹) and mean oxygen penetration depth (mm) measured at each site in summer 2015 in Tomahawk Lagoon and Lake Ellesmere.

Table 3.4. Multiple regression models and partial regression coefficients predicting denitrification rate (µmol m⁻² h⁻¹) for the combined Lake Ellesmere and Tomahawk Lagoon datasets. Oligo indicates log oligochaete density (number m⁻²). *P. exc* indicates *Paracarophium excavatum* density (number m⁻²). Chirono indicates chironomid density (number m⁻²).

<table>
<thead>
<tr>
<th>Intercept</th>
<th>Organic matter (g m⁻²)</th>
<th>Depth (cm)</th>
<th>Invertebrate density</th>
<th>Model R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two parameter models</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>276</td>
<td>545(OM)**</td>
<td>-3.34(Depth)**</td>
<td></td>
<td>0.44***</td>
</tr>
<tr>
<td>-221</td>
<td>684(OM)***</td>
<td>80.1(Oligo)*</td>
<td></td>
<td>0.43**</td>
</tr>
<tr>
<td>90.1</td>
<td>577(OM)**</td>
<td>-0.012(*P. exc)*ns</td>
<td></td>
<td>0.31*</td>
</tr>
<tr>
<td>55.4</td>
<td>612(OM)ns</td>
<td>-0.001(Chirono)ns</td>
<td></td>
<td>0.26*</td>
</tr>
<tr>
<td>Three parameter models</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29.6</td>
<td>630(OM)**</td>
<td>-2.25(Depth)ns</td>
<td>50.6(Oligo)ns</td>
<td>0.49**</td>
</tr>
<tr>
<td>288</td>
<td>566(OM)**</td>
<td>-3.16(Depth)ns</td>
<td>-0.009(*P. exc)*ns</td>
<td>0.47**</td>
</tr>
<tr>
<td>287</td>
<td>833(OM)*</td>
<td>-3.73(Depth)**</td>
<td>-0.004(Chirono)ns</td>
<td>0.47**</td>
</tr>
</tbody>
</table>

*0.01 < P < 0.05
**0.001 < P < 0.01
***P < 0.001
ns indicates partial coefficient was not significant in the model
Table 3.5. Summary of published denitrification rates from aquatic environments, measured using *in situ* incubation chambers.

<table>
<thead>
<tr>
<th></th>
<th>Incubation chamber</th>
<th>Study System</th>
<th>Denitrification measurement</th>
<th>Denitrification rate (μmol m&lt;sup&gt;-2&lt;/sup&gt; h&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Final nitrate concentration (μmol L&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lake Ellesmere</strong></td>
<td>Flexible enclosure</td>
<td>Shallow eutrophic ICOLL</td>
<td>15&lt;sup&gt;N&lt;/sup&gt; Isotope</td>
<td>0 – 272</td>
<td>90 - 200</td>
</tr>
<tr>
<td><strong>Tomahawk Lagoon</strong></td>
<td>Flexible enclosure</td>
<td>Shallow eutrophic ICOLL</td>
<td>15&lt;sup&gt;N&lt;/sup&gt; Isotope</td>
<td>45 – 532</td>
<td>90 - 136</td>
</tr>
<tr>
<td><strong>Risgaard-Petersen et al. (1999)</strong></td>
<td>Flexible enclosure</td>
<td>Shallow hypertrophic lake</td>
<td>15&lt;sup&gt;N&lt;/sup&gt; Isotope</td>
<td>63 - 146</td>
<td>30 - 100</td>
</tr>
<tr>
<td><strong>Mengis et al. (1997)</strong></td>
<td>Benthic chamber</td>
<td>Eutrophic lake</td>
<td>15&lt;sup&gt;N&lt;/sup&gt; Isotope</td>
<td>179</td>
<td>230</td>
</tr>
<tr>
<td><strong>Nielsen and Glud (1996)</strong></td>
<td>Benthic chamber</td>
<td>Ocean</td>
<td>15&lt;sup&gt;N&lt;/sup&gt; Isotope</td>
<td>15 - 19</td>
<td>35-50</td>
</tr>
<tr>
<td><strong>(Smith et al. 2009)</strong></td>
<td>Incubation chamber</td>
<td>Stream</td>
<td>15&lt;sup&gt;N&lt;/sup&gt; Isotope</td>
<td>390 - 1145</td>
<td>34-1192</td>
</tr>
<tr>
<td><strong>(An and Joye 2001)</strong></td>
<td>Benthic chamber</td>
<td>Shallow estuary</td>
<td>N&lt;sub&gt;2&lt;/sub&gt; flux</td>
<td>58 - 154</td>
<td>1.6 - 23</td>
</tr>
<tr>
<td><strong>(Macreadie et al. 2006)</strong></td>
<td>Incubation chamber</td>
<td>Harbour</td>
<td>N&lt;sub&gt;2&lt;/sub&gt;:Ar</td>
<td>10 - 33</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>(Kreiling et al. 2011)</strong></td>
<td>Incubation chamber</td>
<td>Shallow lake</td>
<td>15&lt;sup&gt;N&lt;/sup&gt; flux</td>
<td>870</td>
<td>179 - 536</td>
</tr>
<tr>
<td><strong>(Tomaszek et al. 1997)</strong></td>
<td>Incubation chamber</td>
<td>Freshwater wetland</td>
<td>N&lt;sub&gt;2&lt;/sub&gt; flux</td>
<td>300 - 1200</td>
<td>0.93 - 313</td>
</tr>
</tbody>
</table>
3.5 Discussion

ICOLLs are a common estuary type along microtidal to low mesotidal coastlines globally, representing 3% of global estuaries (McSweeney et al., 2017). There are however a limited number of studies on nitrogen cycling and denitrification pathways within these estuary types e.g. (Spooner & Maher, 2009) and (Highton et al., 2016), and due to their long residence times are expected to shed large quantities of bioavailable nitrogen (Seitzinger et al., 2006). Our study of two ICOLLs which differ greatly in size and water clarity focuses on the factors driving denitrification temporally and spatially in them, attempting to elucidate a hierarchy of factors that control denitrification in both ICOLLs.

3.5.1 Temperature limitation of denitrification

Lake Ellesmere receives greater nitrogen loading in winter compared with summer due to increased rainfall and runoff (Hamill & Schallenberg, 2013) and the same is likely for Tomahawk Lagoon and I have shown that large pulses of nitrate enter these ecosystems intermittently during winter and spring (Fig. 3.2). Temperature was a key factor influencing seasonal denitrification rates in both ICOLL ecosystems, supporting findings from other systems showing the importance of temperature in regulating denitrification rate (Smith et al., 2003, Veraart et al., 2011, Nizzoli et al., 2014). Smith et al. (2003) found denitrification rates to be suppressed at temperatures below 6°C, and Veraart et al. (2011) modelled denitrification rates which were severely reduced below 10°C. In our study, winter ICOLL temperatures were c. 5°C in Tomahawk Lagoon and c. 8°C in Lake Ellesmere, and denitrification was severely suppressed to below detection levels in some cases in both ICOLLs. Thus, in our ICOLLs, the major seasonal nitrate fluxes into the ICOLLs were not converted to N₂ due to temperature limitation of denitrification in winter. At this time, competition for nitrate by denitrifiers is negligible, resulting in high nitrate availability to primary producers for uptake and growth. Colder temperatures in winter may also reduce phytoplankton productivity, but our in situ nitrate uptake measurements showed that the nitrate supplied to the water column of the enclosures was largely assimilated within 48h, even in winter (Fig. 3.4). In summer, when denitrification occurred in our enclosures and nitrate uptake was more rapid, competition between denitrification and phytoplankton uptake was feasible. Previous work by Hawes & Ward (1996) and Schallenberg et al. (2010) have shown phytoplankton in Lake Ellesmere to
be nitrate limited, thus they may be a large sink of nitrate when it becomes available in the ecosystem, however as some phytoplankton can been reported to have luxury P storages (Istvánovics et al., 1994), the N:P ratio of water column nutrients may not be an accurate representation of nutrient limitations of the plankton. Sediment oxygen penetration depths can be increased during winter due to reduced activity in the upper sedimentary layers, thus increasing the distance $^{15}$N labelled nitrate needed to diffuse to reach the sites of denitrifiers.

3.5.2 Sediment properties as drivers of spatial variation of denitrification

Sediment organic matter (content or concentration) is one of the most important variables controlling denitrification rates in aquatic ecosystems, supporting increased sediment metabolism, oxygen demand and N remineralisation (Cornwell et al., 1999, Piña-Ochoa & Álvarez-Cobelas, 2006, Inwood et al., 2007, Dodla et al., 2008, Fulweiler et al., 2008, Loken et al., 2016). When temperatures weren’t limiting denitrification, carbon supplements stimulated denitrification enzyme activity at two sites in Tomahawk Lagoon. Similarly, organic matter availability was the sediment variable most strongly correlated with in situ denitrification rates in Tomahawk Lagoon and in the combined Tomahawk Lagoon and Lake Ellesmere dataset.

In all the Tomahawk Lagoon denitrification enzyme activity sites, no N$_2$O production was detectable under the carbon only amendments, suggesting another pathway may outcompete denitrifiers for the available substrates. DNRA (dissimilatory nitrate reduction to ammonium) is a pathway identified in estuarine sediments that can compete against denitrification in certain conditions (Christensen et al., 2000, Burgin & Hamilton, 2007, Dunn et al., 2012, Giblin et al., 2013, Bonaglia et al., 2014, Nogaro & Burgin, 2014, Bernard et al., 2015, Brin et al., 2015, Hardisón et al., 2015). When carbon supply to sediments is greater than the microbial demand, DNRA can dominate over denitrification, converting nitrate to ammonium (Burgin & Hamilton, 2007, McGlathery et al., 2007). This could represent a significant pathway retaining nitrogen in the system rather than removing it through denitrification.

High nitrate loading can enhance microbial community activity and denitrification rates (Cornwell et al., 1999) and sediment microbes are able to respond to short-term changes in water column nitrate concentrations in the water column (<3 hours) (Kana et al., 1998).
While nitrate supply was expected to be saturating in our *in situ* enclosures, slow rates of diffusion and/or advection of nitrate to sites of denitrification in the sediments possibly limited the denitrification rate of new nitrate *in situ*, favouring uptake by autotrophs. Slow diffusion of nitrate may be especially important when the oxygen penetration depth was increased, such as during the cooler temperatures typical of winter months.

Water depth at the sites was another important factor correlated with denitrification rate in the study ICOLLs indicated by the PCA plots and regression models. The negative effect of depth on denitrification rate in the combined dataset may also be related to organic matter in that shallow sites must have sustained higher rates of microphytobenthic production than deep sites in Lake Ellesmere. Microphytobenthic biomass is a labile form of organic matter, and high rates of microphytobenthos production and turnover at shallow sites could enhance denitrification by fuelling denitrifying microbes with labile carbon (Hardison et al., 2013). Microphytobenthos can also increase the denitrifying microbe abundance (Decleyre et al., 2015). The microphytobenthos could also directly compete for nitrate at these sites (Cornelisen & Thomas, 2006), increasing overall nitrate removal. However, it is more likely the change in oxygen conditions in the sediment indirectly by the microphytobenthos photosynthesising activities which effects the denitrifier activity (Nizzoli et al., 2014).

The increased oxygen penetration depth that occurs in sandy sediments also means an increased area for oxic sediments, which is highly reactive compared to anoxic sediments, due to oxygen being the most favourable electron acceptor for microbial respiration (Fenchel et al., 1998, Kristensen, 2000). Shallow permeable sediments can also be highly reactive, quickly processing organic detritus as it comes available (Huettel & Rusch, 2000, Sokoll et al., 2016).

The euphotic depth in Lake Ellesmere has been estimated to be 0.31 m (Schallenberg et al. 2010) and to range between 0.3 and 0.5 m (Gerbeaux, 1989). Given that the ICOLL water level also typically varies within a range of 0.8 m (Schallenberg et al. 2010), it is possible that the very low denitrification rates measured at the deepest sites (E2 = 0.9 m and E14 = 1.07 m) reflect the fact that these sites lie beyond the typical euphotic depth range of the lake and, therefore, lack microphytobenthic primary production as a renewable source of labile organic matter and the associated changes on sediment oxygenation. Some of the shallow sites are also closest to the main tributaries which may
provide a continual source of fresh organic matter and nitrogen, priming the microbial community (Bianchi, 2011, Bianchi et al., 2014).

The denitrification rates of Lake Ellesmere varied significantly between sites, highlighting the extreme importance of spatially explicit sampling that incorporates different sediment types into denitrification experiments, rather than extrapolating from data collected from one site only, as has been criticised in previous research (Saunders & Kalff, 2001, Piña-Ochoa & Álvarez-Cobelas, 2006).

3.5.3 Sediment oxygenation supporting denitrification

The sediment oxygen penetration depth can be used to calculate the rate of gas diffusion within different sediments (Vopel et al., 2012), and sediments with greater oxygen penetration may be able to cycle nitrate faster than those with a smaller oxygen penetration, due to coupled nitrification-denitrification in the oxic sediment layer. Denitrification rates were positively related to the OPD at the sites in Tomahawk Lagoon. I measured significant increases in oxygen penetration in the presence of invertebrate burrowers in both ICOLLs, suggesting that sediment irrigation by invertebrates could also play a role in supplying nitrate to denitrifiers in these systems. Chironomid burrows were observed to oxygenate surrounding sediment, distributing dissolved oxygen well below the average oxygen penetration depth of the unbioturbated sediment, as has been previously reported (Glud et al., 2016). High bioturbation has been reported to increase coupled nitrification-denitrification (Nogaro & Burgin, 2014), and macrofaunal bioturbation was a key driver of spatial variation in both ICOLLs. Studies have also found large spatial variation around lakes and estuaries (Small et al., 2016) relating to changes in the sedimentary environment, and the location of large nutrient inputs i.e. rivers. The creation and maintenance of secretion-lined burrows by invertebrates (Olafsson & Paterson, 2004) increases the anoxic/oxic sediment area of the sediments, enhancing DO and nitrate supply to denitrifiers (Svensson, 1997, Banks et al., 2013, Shang et al., 2013, Poulsen et al., 2014), while bioturbation also redistributes organic matter from the sediment surface to deeper layers. Chironomid density was greater in Tomahawk Lagoon where they ranged between 2105 and 24,210 ind. m$^{-2}$ compared to 0 to 737 ind. m$^{-2}$ in Lake Ellesmere; however, polychaetes replaced chironomids in the more saline regions of Lake Ellesmere, likely playing a similar bioturbation role (however no in situ measurements were recorded at these sites). Nereid polychaetes are
larger than chironomids and do not have seasonal dynamics dictated by metamorphosis and hatching and may, therefore, have played a greater role in coupled nitrification-denitrification in Lake Ellesmere. Furthermore, chironomid densities in Lake Ellesmere were unusually low during our study, because of an unusually large saline intrusion that occurred in 2013, which was associated with a period of very low chironomid larvae densities in the lake, leaving a large proportion of empty burrows. Bioturbation activities of the chironomids and of nereid polychaetes in the ICOLLs increased the overall sediment DO penetration in the fine sediments to penetration depths typical of sandy sediments and potentially reduced seasonal variation in OPD in the finer sediments with lower diffusivity (i.e., those with high clay and silt contents).

3.5.4 A hierarchy of factors related to denitrification rate
Our study illustrates that a hierarchy of factors affected in situ denitrification rates in our study ICOLLs (Fig. 3.13). A conceptual model of the factors affecting denitrification was developed using the denitrification enzyme activity and in situ seasonal results, along with the multiple regression models. The primary factor was a temperature threshold that existed somewhere between 8.6 and 12°C, below which denitrification was essentially shut down, despite the presence of episodic high concentrations of nitrate in the water columns of these ICOLLs. During summer, when temperatures of 16 to 17°C allowed for denitrification, organic matter availability became an important correlate of denitrification rates, a result supported by in vitro DEAs. Organic matter availability was the most important variable in our regression models of denitrification (for Tomahawk Lagoon and the combined dataset). In our multiple regression models for the combined dataset, water depth was the next most important variable correlated with denitrification rates. The effect of water depth on denitrification rate was negative overall, possibly reflecting the inhibitory effect of increased water depth and decreasing light penetration to the ICOLL bed on the production of labile carbon in the sediments by microphytobenthos, and the microphytobenthos photosynthetic activity. In addition to these factors, macroinvertebrate densities also explained some variation, both in the OPD and in the in situ denitrification rate. The relationships with OPD were strong and positive.
(mainly with chironomids and polychaetes) whereas the relationships with denitrification rate were weaker (correlating weakly with chironomids and oligochaetes).

**Figure 3.13.** Hierarchy of factors related to denitrification in the study ICOLLs. This conceptual model assumes that the nitrate supply to denitrifying bacteria does not limit the denitrification rate.

Our study aimed to apply the isotope pairing technique under realistic turbulent mixing conditions that occur in shallow ICOLLs, rather than the typical rigid core incubations (Steingruber *et al.*, 2001), which has been experimentally tested to underestimate actual denitrification rates (Risgaard-Petersen *et al.*, 1999). Therefore, I acknowledge some of the caveats of this study. Due to the cost and maintenance required of the chambers, I was limited by the number of sites I were able to run in situ. I have only been able to capture a few seasons, thus further research to validate the model should consider conducting more experiments across a temperature gradient to identify if/where a temperature threshold might exist. Controlled laboratory experiments could also begin to tease apart the role of increasing organic matter supply and invertebrate densities on denitrification rates.
3.5.5 Denitrification as a buffer against ICOLL eutrophication

Our *in situ* denitrification rate measurements fall well within the ranges reported in other studies from aquatic ecosystems using *in situ* chambers, similar denitrification methods and similar background nitrate concentrations (Table 3.5). These studies show that while denitrification rates can be highly variable, they can sometimes be quite high, suggesting that denitrification can play an important role in regulating nitrate availability in these systems.

Nitrate can be depleted in highly eutrophic lagoons due to the extremely high phytoplankton biomass, which rapidly consumes nitrate within the lagoon when available. Thus, rates of denitrification in ICOLLs may be constrained by phytoplankton uptake and it has been shown that primary producers typically outcompete bacteria for available N (McGlathery *et al.*, 2007), consuming new nitrate before it is diffused or advected into the sediment denitrification zone. Although nitrate was depleted to low levels in the Lake Ellesmere and Tomahawk Lagoon enclosures, only a small proportion of nitrate was evolved as dinitrogen gas within 48h. It is likely that a substantial proportion of the added nitrate was consumed by phytoplankton, macroalgae, microphytobenthos and macrophytes present within the cores, limiting the eutrophication buffering potential afforded by denitrification. Olsen *et al.* (2017) showed that macrophytes and filamentous algae constitute a large nitrate uptake pathway (40 and 30% respectively), and less than 1% of the added nitrate pulse was delivered to the sediments, and less than 5% was denitrified. Although *in situ* denitrification rates in Tomahawk Lagoon were not significantly greater than in Lake Ellesmere, nitrate uptake rates from the enclosures were lower and substantial nitrate remained in the water column at the end of our experiments. Thus, greater quantities of the added nitrate pulse might have been denitrified in this lake if our experiment had run longer; however, lengthening the enclosure experiment duration could have reduced oxygen concentrations in the enclosures to levels (<20%) violating one of the assumptions of the isotope pairing method that was used to measure denitrification. This illustrates an important methodological constraint when attempting to measure *in situ* denitrification rates in closed systems over short time scales. Denitrification rates over longer time scales are likely to be different because of the many time-scale-dependent factors that influence denitrification in these systems.
Upon senescence, phytoplankton biomass can be re-mineralized to NH$_4^+$ which may be re-assimilated by autotrophs or nitrified, providing recycled nitrate substrate for denitrification (Gilbert et al., 2003). Alternatively, increased ammonium fluxes from remineralization may add to the eutrophication potential of lagoons already impacted by anthropogenic activities (Pérez-Villalona et al., 2015). The ultimate sink of N after assimilation in such ecosystems has been largely overlooked (O’Brien et al., 2012), with various studies indicating that the remineralization-nitrification-denitrification pathway is an important sink to investigate (Arango et al., 2008, Ferrón et al., 2009, McMillan et al., 2010).

A whole-ICOLL denitrification rate was calculated to scale up and estimate how much N could be removed by denitrification yearly. Scaling up our results comes with a range of caveats that need to first be discussed. Only two measurements of sediment characteristics and invertebrate densities were recorded seasonally (winter and summer) in Lake Ellesmere, thus seasonal changes in the organic matter and oligochaete densities are not accounted for in Lake Ellesmere. Tomahawk Lagoon has 5 seasons of data which are used to extrapolate seasonal changes of organic matter and oligochaete density. The spatial variability of the sediments and invertebrates was very high, with large variation exhibited within sites. The N removal rate also does not account for possible “hot moments” of denitrification when extra nutrient resources required by the denitrifiers are present. Scaling up of denitrification rates has been done using the linear regression models developed in this chapter, and using historical measurements of temperature, the spatial maps developed in Chapter 2, and the limited seasonal measurements of organic matter and oligochaete densities. The budget assumes that there is constantly high nitrate available, no ICOLL opening events, and that each ICOLL has the distinct sedimentary zones as measured in Chapter 2. Our results indicated that although the two ICOLLs have different sizes, on an aerial basis they have the capacity to shed similar quantities of nitrate (Lake Ellesmere: 34 T N km$^{-2}$, Tomahawk Lagoon: 39 T N km$^{-2}$).

### 3.6 Conclusions

Denitrification within the two study ICOLLs was controlled by a hierarchy of limiting factors. Temperature was the primary factor influencing the denitrification rates, resulting in the severe reduction of denitrification during winter, leaving large nitrate pulses available for assimilation by phytoplankton and macroalgae. Nutrient availability
(carbon and nitrate) to the sediment denitrifiers was the next most important factor limiting denitrification, as indicated by both the denitrification enzyme activity and in situ denitrification experiments. When denitrification was not limited by temperature, large spatial variability in denitrification rates was observed in the ICOLLs. Site-specific denitrification rates were positively correlated with sediment organic matter concentration, site depth and bioturbation and irrigation by burrowing macrofauna. Denitrification confers resistance and resilience to eutrophication in ICOLL ecosystems. The microbial conversion of nitrate to N₂ was substantial at some sites within the two lagoons during the summer months, and on a lagoon-wide basis this process is likely to be at least a moderate sink for nitrate in the summer time, potentially contributing to nitrogen limitation of the phytoplankton. Denitrification is unlikely important in these ICOLLs in winter due to temperature limitation. Scaled-up estimates of denitrification showed both ICOLLs have similar denitrification rates on an km² basis, however due to Lake Ellesmere’s greater size, it has a greater overall ability to shed nitrate under large pulse events.
CHAPTER 4

Physical and biological drivers of sediment oxygenation and denitrification in a New Zealand intermittently closed and open lake lagoon

Photo: Tomahawk Lagoon’s ocean connection at Tomahawk Beach.

This chapter is a modified version of the following published paper: Crawshaw, J.A., Schallenberg, M., & Savage, C. 2018. Physical and biological drivers of sediment oxygenation and denitrification in a New Zealand intermittently closed and open lake lagoon. New Zealand Journal of Marine and Freshwater Research, DOI: 10.1080/00288330.2018.1476388
4.1 Abstract

Intermittently Closed and Open Lake Lagoons (ICOLLs) are shallow estuarine ecosystems, many of which show eutrophication symptoms due to a combination of high nutrient loading from their catchments and long water residence times. The physical and biological drivers of sediment oxygenation and denitrification were examined in a New Zealand ICOLL using in situ enclosures for one year. Denitrification was seasonally and spatially variable, and higher denitrification rates were driven by organic matter availability, temperature and nitrate flux. The bulldozing invertebrates deepened oxygen penetration (18% of oxygen profiles) as indicated by multiple regression modelling. Chironomid larvae (Chironomidae) dominated the benthic community, and their tubular burrows modified oxygen penetration into the sediment (18% of profiles), potentially affecting denitrification in the ICOLL through bioirrigation. Our study shows macroinvertebrates support increased sediment oxygenation, therefore managers that use artificial opening regimes should consider the effects of elevated salinity on macroinvertebrate community composition.
4.2 Introduction

Intermittently closed and open lake lagoon systems (ICOLLs) are dynamic, shallow coastal ecosystems which have an intermittent connection with the ocean (Schallenberg et al., 2010, McSweeney et al., 2017). Worldwide there are 1477 ICOLLs and they encompass 3% of the world’s estuaries (McSweeney et al., 2017). In the absence of human intervention, the intermittent nature of the openings is due to variation in rainfall and/or oceanic forces, either of which can break open the barrier bar connecting the lagoon to the ocean (McSweeney et al., 2017). Ocean currents, waves, tides and freshwater discharge can influence the duration of opening events (Everett et al., 2007), which can range from days to months (Gale et al., 2007). Infrequent opening events result in longer mean water residence times, allowing for the accumulation of nutrients in the ICOLLs from upstream sources (Roy et al., 2001, Everett et al., 2007). Artificial openings of such systems are common and help flush excess nutrients and decrease flooding of surrounding lands, although the extent of the flushing effect of openings is context- and scale-dependent (Gale et al., 2007, Schallenberg et al., 2010, Lill et al., 2012). In New Zealand and worldwide, coastal ecosystems are undergoing eutrophication due to nutrient loading from agricultural and urban intensification around coastal water bodies (Rabalais, 2010, Borum, 2013). Symptoms of eutrophication can include algal blooms, oxygen depletion, seagrass loss, and alterations to food webs, and biogeochemical processes (Rabalais, 2010, Borum, 2013).

Shallow, productive ICOLL sediments are sites of high biogeochemical cycling (Spooner & Maher, 2009) where denitrification generally occurs (Nielsen, 1992, Steingruber et al., 2001). This important ecosystem service, which recycles nitrogen into the atmosphere in the form of nitrogen gas and thereby mitigates some of the negative effects of anthropogenic nutrient enrichment (Laverman et al., 2007, Fennel et al., 2009, McCrackin & Elser, 2010), is mediated by sediment microbes occurring primarily at the sediment oxic/anoxic interface (Herbert, 1999, Nielsen et al., 2004). Previous studies have shown that benthic denitrification rates in estuarine ecosystems are related to temperature, carbon and nitrate supply, and oxygen conditions (Tomaszek & Czerwieniec, 2003, Seitzinger et al., 2006). Anaerobic ammonium oxidation (anammox) can also remove bioavailable nitrogen from the aquatic ecosystem directly, however it is unlikely to be important in coastal ecosystems where labile carbon supply is high (Burgin
Dissimilatory nitrate reduction to ammonia (DNRA) may compete with denitrification for available nitrate and is dominant in systems with a high organic matter or sulfide availability which are required to provide an electron donor (Hardison et al., 2015, Murphy et al., 2016), and may be a pathway to consider in ICOLL ecosystems when nitrate availability is low.

The role of benthic communities in nitrogen cycling has management implications for ICOLLs since opening events can radically alter salinity in these ecosystems (Schallenberg et al., 2010) and cause shifts in faunal community composition (Lill et al., 2012). Benthic infauna may enhance denitrification through bioturbation (Svensson, 1997, Nizzoli et al., 2007, Bartoli et al., 2009, Shang et al., 2013), actively or passively transferring oxygenated overlying water into the sediments, increasing oxygen penetration depth and oxygenating the linings of the burrows (Aller, 1998, Nizzoli et al., 2007, Stief et al., 2009, Shang et al., 2013), as well as potentially delivering nitrate for denitrification at the oxic-anoxic interface. Animal burrows increase the extent of the sediment-water interface, and the burrows become sites of high bacterial density and high metabolic activity compared to the surrounding sediment (Svensson, 1997, Nogaro et al., 2008) due to enhanced supply of nitrate and carbon (Biswas et al., 2009, Banks et al., 2013, Shang et al., 2013, Poulsen et al., 2014). The enhanced supply of oxygen also stimulates microbial nitrification of ammonium in the sediment, providing recycled nitrate for denitrification (Carpenter & Capone, 1983, Biswas et al., 2009, Stief, 2013).

Although ICOLLs are likely to be key sites of denitrification, few studies have investigated denitrification in this type of estuarine environment. Spooner (2009) investigated denitrification in the deep basins of an Australian ICOLL (3.5 – 4.5 m deep) and found that the efficiency of denitrification in the sediments declined with increasing rates of microbial organic matter processing, suggesting that the overloading of sediments with organic matter hinders the efficiency of nitrogen removal via denitrification. Spooner (2009) also found that sediment oxygen consumption rates were greatest in winter and spring and that denitrification efficiency was lowest in summer, concluding that seasonal temperature variations (between 8 and 25°C) affected sediment nitrogen processing. Investigations into denitrification in a large New Zealand ICOLL, showed that the microbial conversion of nitrate to N₂ was suppressed in winter, the time when nitrate loading, and water column nitrate concentrations tended to be greatest.
Furthermore, sediment denitrification potential (nitrate amended) was spatially variable in the ICOLL during summer (Chapter 2), with the presence of nosZI genes in the sediment correlating strongly to water depth (negative) and sediment grain size (positive) (Highton et al., 2016).

Given the limited denitrification research conducted on ICOLL ecosystems, the first objective was to investigate the influence of temperature and seasonality on denitrification, which have been identified as important variables to consider in previous studies in estuarine ecosystems. My study sought to identify periods when, and sites where (physical drivers), denitrification was elevated or suppressed and to identify the dominant drivers of denitrification in the ICOLL. The second objective of this study was to investigate the role of macroinvertebrates (biological drivers) in supporting denitrification rates in a temperate, eutrophic New Zealand ICOLL, as this has not been addressed in the previous ICOLL studies described above. Given the relatively shallow depths of the ICOLLs, macroinvertebrates may have an increased role to play in oxygen and nitrogen cycling, thus I hypothesised that sites with relatively high invertebrate densities would exhibit relatively greater oxygen penetration and higher denitrification rates.

4.3 Methods

4.3.1 Study Site
The lower Tomahawk Lagoon (referred to hereafter as Tomahawk Lagoon) is a small (0.19 km²), shallow (average depth: 0.67 m), nutrient enriched ICOLL in the city of Dunedin, South Island, New Zealand (Fig. 4.1) (Hamilton & Mitchell, 1997). It is fed by surface runoff and a stream at the northern end that drains a small (2.4 km²) agricultural catchment (Mitchell, 1989, Drake et al., 2011). The lagoon is also connected to the upper lagoon (lagoon area = 0.096 km²; catchment area = 1.8 km²) by a narrow channel (Mitchell et al., 1988). During this study, Tomahawk Lagoon had relatively clear water, with some submerged macrophytes (Ruppia spp.) and macroalgae distributed patchily on the bed of the lagoon.

4.3.2 In situ denitrification measurements
In situ denitrification measurements were made across a seasonal and spatial gradient after simulation with nitrate pulses (142 µmol L⁻¹), which mimic conditions known to
intermittently occur in these types of lagoons (Chapter 2). The high nitrate pulses were used to ensure no nitrate limitation occurred during the experiment; thus, other drivers of denitrification could be examined. Therefore, these represent potential denitrification rates due to the high nitrate amendments. The background nitrate concentration was enhanced (0–19 µmol L\(^{-1}\)). Five seasonal measurements were taken during 2015: in late summer (mean water temperature: 21ºC), autumn (12ºC), winter (5ºC), spring (14ºC) and early summer (17ºC) (Table 4.1). Three sites were selected within the lagoon to encompass spatial differences in sediment organic matter content, porosity, and grain size (Table 4.2).

**Figure 4.1.** Map of New Zealand showing Tomahawk Lagoon. Enlarged insert shows Tomahawk Lagoons No.1 and No.2 proximity to the coastline, with the corresponding inflows and outflows of each lagoon. Site numbers in Tomahawk Lagoon No.1 show sites of *in situ* denitrification and collection of cores for oxygen penetration depth measurements.

Nutrient fluxes and denitrification rates were measured in novel *in situ* enclosures (Fig. 4.2), which isolated sediment and the majority of the overlying water column (hereafter referred to simply as enclosures) from the surrounding ecosystem. These consisted of a rigid bottom and top plates holding four replicate enclosures, with flexible, clear polyethylene tubing connecting the plates. Because turbulence at the sediment-water interface is potentially a key driver of nitrate supply to the sediment microbial community
(Risgaard-Petersen et al., 1999, Cook et al., 2006), I designed the enclosures with flexible tubing to improve the realism of the measurement system by allowing the transfer of turbulent energy from the lagoon into the enclosures, providing mixing during the incubation period such as reported in other in situ field enclosures (Asmus et al., 1998, Risgaard-Petersen et al., 1999, Fanjul et al., 2011). The enclosure inner diameter was 6.4 cm, and the cores of the bottom plate penetrated the sediment to a depth of 15 cm. The length of the flexible tubing was adjusted to so that the tops of the enclosures were situated a few centimetres below the water level. The enclosures were sealed to prevent atmospheric exchange, and care was taken to ensure no bubbles were trapped during enclosure insertion. Samples were taken from tubing and a two-way gas valve in the enclosure lid. The enclosures were amended with NaNO₃ to a concentration of 71 µmol N L⁻¹ to simulate a large nitrate pulse event (see above; Chapter 3). To facilitate the accurate measurement of all N₂ isotope pairs, a final enrichment of 50 atom % of ¹⁵N in the NO₃⁻ pool was achieved by additionally spiking the enclosures with a ¹⁵N labelled tracer K¹⁵NO₃ to a concentration of 71 µmol N L⁻¹, to have a total concentration of 142 µmol N L⁻¹. After both nitrate pulses were added 50 mL of water was withdrawn into a syringe and pushed back into the chamber multiple times to aid in mixing of the isotope tracer and unlabelled nitrogen. Water samples were taken before and after nitrate pulse addition to measure ambient and enriched nutrient concentrations. The experiment was run for 48 hours, to account for natural diel variation in nitrate dynamics and nitrogen gas production.
Figure 4.2. The flexible incubation chamber in place at Tomahawk Lagoon No.1.

Table 4.1. Physico-chemical measurements taken at the initiation of the *in situ* experiments in Tomahawk Lagoon across the five seasonal measurements. NO$_3$-N = nitrate-N, NH$_4$-N = ammoniacal N, DRP = dissolved reactive phosphorus, TN = total nitrogen, TP = total phosphorus. Lagoon site means and standard deviation are reported for each season (n=4). No replicate samples were collected for dissolved nutrients in autumn.

<table>
<thead>
<tr>
<th>Season</th>
<th>Date</th>
<th>Temp (°C)</th>
<th>Salinity</th>
<th>NO$_3$-N</th>
<th>NH$_4$-N</th>
<th>DRP</th>
<th>TN</th>
<th>TP (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>late summer</td>
<td>28/01/2015</td>
<td>21</td>
<td>3.8</td>
<td>1.16 ± 0.9</td>
<td>16 ± 1.7</td>
<td>1.95 ± 0.8</td>
<td>141 ± 69</td>
<td>14 ± 6</td>
</tr>
<tr>
<td>autumn</td>
<td>22/04/2015</td>
<td>12</td>
<td>3.8</td>
<td>6</td>
<td>5</td>
<td>0.03</td>
<td>67 ± 15</td>
<td>1 ± 0.01</td>
</tr>
<tr>
<td>winter</td>
<td>15/07/2015</td>
<td>5</td>
<td>2.3</td>
<td>19 ± 2</td>
<td>10 ± 2</td>
<td>0.22 ± 0.08</td>
<td>58 ± 27</td>
<td>0.86 ± 0.17</td>
</tr>
<tr>
<td>spring</td>
<td>14/10/2015</td>
<td>14</td>
<td>3.5</td>
<td>0.65 ± 0.49</td>
<td>6 ± 1</td>
<td>0.13 ± 0.03</td>
<td>41 ± 6</td>
<td>1 ± 0.28</td>
</tr>
<tr>
<td>early summer</td>
<td>17/12/2015</td>
<td>17</td>
<td>3.6</td>
<td>0.41 ± 0.1</td>
<td>5.01 ± 1.2</td>
<td>0.34 ± 0.2</td>
<td>73 ± 37</td>
<td>2 ± 0.59</td>
</tr>
</tbody>
</table>
Table 4.2. Sediment characteristics measured from sediment cores collected from the in situ denitrification experiments for Tomahawk Lagoon. Annual means and standard deviation are reported (n=5). OM indicates organic matter.

<table>
<thead>
<tr>
<th>Site</th>
<th>OM content (% dry weight)</th>
<th>OM availability (g m⁻²)</th>
<th>Porosity (g mL⁻¹)</th>
<th>Clay content (%)</th>
<th>Silt content (%)</th>
<th>Sand content (%)</th>
<th>Water depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4</td>
<td>11 ± 1.22</td>
<td>246 ± 128</td>
<td>0.9 ± 0.097</td>
<td>6 ± 1.64</td>
<td>88 ± 22</td>
<td>6 ± 2</td>
<td>0.76 ± 0.13</td>
</tr>
<tr>
<td>T5</td>
<td>9 ± 3.76</td>
<td>237 ± 135</td>
<td>0.8 ± 0.018</td>
<td>4 ± 1.61</td>
<td>65 ± 24</td>
<td>30 ± 9</td>
<td>0.75 ± 0.11</td>
</tr>
<tr>
<td>T6</td>
<td>0.64 ± 0.08</td>
<td>59 ± 33</td>
<td>0.5 ± 0.029</td>
<td>0.06 ± 0.06</td>
<td>2 ± 2</td>
<td>98 ± 2</td>
<td>0.5 ± 0.07</td>
</tr>
</tbody>
</table>

Fifty mL samples of water were extracted (after mixing by multiple charges and recharges of the syringe) from the enclosures at 0, 4, 24, 30 and 48 hours to measure dissolved oxygen, dissolved inorganic nitrogen concentrations (NO₃⁻ and NH₄⁺) and N₂ gas isotopes. The dissolved oxygen concentrations were measured at each sampling time using a calibrated YSI ProODO Optical Dissolved Oxygen meter (Yellow Springs, Ohio, USA), to confirm that the dissolved oxygen concentrations did not change more than 20% the starting value during the experiments. N₂ gas was extracted from the water sample inside the syringe by adding a 15 mL helium headspace and shaking for 3 minutes. The gas sample (13 mL) was then transferred to a pre-evacuated extainer (Labco, High Wycombe, UK), using a flexible needle extender. The extainers were kept submerged in milli-Q water (freshly sparged with helium gas while the needles were being removed from the septa) to ensure that air did not enter the punctured septum. This was achieved using degassed water-filled 50 mL centrifuge tubes to decrease the potential for air contamination (Hamilton & Ostrom, 2007, Gongol & Savage, 2016). After gas extraction, the water samples were filtered through Microscience MS-GF 47 mm filters (nominal pore size = 0.7 μm) into 50 mL acid-washed centrifuge tubes and frozen at -20°C until analysis for nitrite + nitrate (NO₂⁻+NO₃⁻) and ammonium (NH₄⁺). Nitrogen gas samples were analysed using established methods (Lewicka-Szczebak et al., 2013) at Isotrace, Department of Chemistry, University of Otago. The extainers
were placed in an autosampler connected to a gas chromatograph and a mass spectrometer. The standard used for N\textsubscript{2} was air measured at the same concentration in helium as the samples. Air was measured after every 8 samples to test for instrument drift. If significant drift was observed by linear regression of 30/28 ratio of air against time, then linear time-base correction was applied to air and samples. I acknowledge that these rates may be underestimated due to not accounting for N\textsubscript{2} trapped in the sediment porewater, however due to the high concentration of nitrate amended, the denitrification rates are more likely overestimates.

The measured \textsuperscript{29}N and \textsuperscript{30}N ratios were converted to molar quantities using computed concentrations of nitrogen expected in the water column at the time of sampling and at the measured temperature and salinity of the sampled water, using tables of nitrogen gas solubility by Colt (2012), and the volume of the enclosures (L) (Dalsgaard, 2000). The production of each ratio (\textsuperscript{29}/\textsuperscript{28}N\textsubscript{2} and \textsuperscript{30}/\textsuperscript{28}N\textsubscript{2}) at each time point was calculated as the sample ratio minus the ratio at time 0. This was used rather than the air standard due to seasonal variation in the ambient ratios in gasses dissolved in the ICOLL water. The absolute amount of \textsuperscript{29}N\textsubscript{2} and \textsuperscript{30}N\textsubscript{2} were then calculated using the equation (Dalsgaard, 2000):

\[
AM_{xx} (\mu\text{mol}) = (R_{xx} \times \textsuperscript{28}N\textsubscript{2}) \times \text{Sample Volume}
\]

where: \(AM_{xx}\) = the amount of \textsuperscript{xx}N\textsubscript{2} present in the core (\(\mu\text{mol}\)), \(R_{xx}\) = the ratio of \textsuperscript{29}/\textsuperscript{28}N provided by mass spectrometry, \(\textsuperscript{28}N\textsubscript{2}\) = the concentration of \textsuperscript{28}N\textsubscript{2} (\(\mu\text{mol L}^{-1}\)) expected in the water calculated from nitrogen diffusion tables (Colt, 2012), and sample volume = the volume of the enclosure the sample represents (L). The slope was calculated from the production of N\textsubscript{2} over the time course of the experiment, to integrate production over time, and was then divided by the surface area of the core (0.003848 m\textsuperscript{2}). The total denitrification rate of the sediment was then calculated using standard isotope pairing calculations (Nielsen, 1992). Methodological concerns have been raised about the use of the original isotope pairing calculations for this study. I acknowledge the modified isotope pairing calculations that account for anammox and DNRA (Risgaard-Petersen et al. 2003, Salk et al. 2017), however these were not measured in our study thus the denitrification rates may include some N\textsubscript{2} produced through anammox or DNRA. I however suggest that anammox is unlikely to account for a large percentage of N\textsubscript{2} production in estuarine sediments and a recent review paper indicates denitrification is
the dominant N2 production pathway (Damashek & Francis 2018), as denitrification is more thermodynamically favourable when organic matter is present (Thamdrup & Dalsgaard 2002). DNRA can be a favoured process when organic matter availability is high and nitrate concentrations are low (Hardison et al. 2015), or when sediments are anoxic and sulfidic (Murphy et al. 2016). Tomahawk Lagoon however does not currently experience sediment anoxia and organic loading is low, therefore DNRA is suggested to not be a dominant pathway. Nutrient fluxes were calculated as the change in nutrient concentration over time, accounting for enclosure water volume and sediment surface area (Dalsgaard, 2000).

At the end of the incubations the sediment cores were retrieved (15 cm depth, 6.4 cm internal diameter), an aliquot of sediment was collected, and the remaining sediment was sieved through a 500 µm mesh. All macroinvertebrates retained were preserved in 99% ethanol and were identified using guides by Gooderham & Tsyrlin (2002) and Moore (1997). Invertebrates were placed into functional bioturbation groups using categories described by Gerino et al. (2003). The surface sediment (0-2 cm) was collected for analysis of particle size, organic matter content and availability, and porosity. Sediment samples were kept frozen at -20°C until these analyses were performed. At each site, water temperature, salinity and dissolved oxygen were measured at the times of sampling with an YSI Professional Plus Multiprobe (YSI Incorporated, Yellow Springs, Ohio, USA). A water sample was also collected to measure the ambient total nitrogen and phosphorus concentrations and the dissolved inorganic nutrients, NO3-N, NH4-N, and dissolved reactive phosphorus.

4.3.3 Sediment oxygen penetration

To measure the depth of the sediment oxic/anoxic interface where denitrification occurs (Gongol & Savage, 2016), sediment dissolved oxygen micro-profiles were measured. Four sediment cores for oxygen profiling were collected from beside the in situ enclosures on the second day of the experiment. Clear plexiglass sediment core tubes (internal diameter; 6.4 cm, length; 30 cm) were used to collect sediment from each site using a gravity corer. The depth of the sediment cored was approximately 15 cm, with overlying water of 15 cm. Cores were capped and stored cool and in the dark until being returned to the laboratory within five hours. For equilibration, cores were submerged and uncapped in site water circulated by a water pump maintained at approximately the in...
situ temperature and aerated using an air stone. The sediment oxygen profiles were measured within two hours after the initiation of equilibration. Down-core oxygen profiles were performed at 200 µm vertical resolution using a motorised micromanipulator and Unisense 100 µm oxygen microsensor, which was connected to a Unisense microsensor multimeter. Two oxygen sensors were attached and allowed to polarize for two hours. Once the reading had stabilized, a 2-point calibration curve was calculated to calibrate the probe. A 100% oxygen reading was taken from the middle of the oxygenated water column of a sediment core. The probe was then lowered into the sediment, until the oxygen reading stabilized, and this was the anoxic reading (Vopel et al., 2012). Three profiles were measured within each core. After profiling, cores were sieved, and macroinvertebrates were retained for identification.

4.3.4 Nutrient analysis

Nutrients were analysed using colorimetric protocols, using a SANPlus segmented flow autoanalyzer (SkalarAnalytical B.V., Breda, The Netherlands). Nitrate & nitrite were determined based on the cadmium reduction method (Morris & Riley, 1963, Wood et al., 1967). Dissolved reactive phosphorus (DRP) was measured by the reaction of ammonium molybdate and potassium antimony tartrate in an acidic medium, with diluted solutions of phosphate to form an antimony-phospho-molybdate complex (Murphy & Riley, 1963, Greenberg et al., 1992). The measurement of ammonium was based on the Berthelot reaction (Bertholet, 1859, Solorzano, 1969). Total phosphorus (TP) and total nitrogen (TN) were measured after wet oxidation, with the reaction starting at pH 9.7 and ending at pH 5-6 (Valderrama, 1981, Ebina et al., 1983). Standards were measured at the start of each run, as well as after every 10 samples, to account for instrumental drift. In addition, at the beginning of each run of 10 samples, a duplicate sample was run. At the end of the entire run a spiked sample (laboratory fortified blank; tap water with a standard spike) was run to check recovery. Typical detection limits for our methods are 0.071 µM for nitrate, 0.93 µM for ammonium, 0.36 µM for total nitrogen, 0.02 µM for dissolved reactive phosphorus, and 0.06 µM for total phosphorus.

4.3.5 Sediment Characterization

I present sediment organic matter on an areal basis because denitrification and invertebrate densities were also measured per m² of lagoon bed, thus providing a coherent set of data for comparison. I arbitrarily chose a uniform depth into the sediment (1 cm)
over which to measure the sediment characteristics, which in effect calculates a concentration within a standard depth and area, which I call organic matter availability. In the literature, a wide variety of sediment parameters are typically calculated and reported as contents rather than as concentrations or areal densities. When expressed as contents, sediment parameters can be strongly driven by variation in bulk density and grain size (Tolhurst et al., 2005) and thus may contribute little information in addition to grain size to the analysis of relationships. However, organic matter availability (per gram of dry sediment) may reflect aspects of nutritional value of sediments to deposit-feeding organisms. So, I also used organic matter availability in our data analyses.

Sediment porosity (g H$_2$O mL$^{-1}$ sediment) was calculated from the dry weight of the sample after drying 5 g of wet sediment at 50°C for 24 hours. Sediment was combusted at 450°C for 24 hours and desiccated before calculating organic matter content as % dry weight loss on ignition (LOI) and organic matter availability (expressed as the dry mass of organic matter per unit of fresh sediment (g dry OM mL$^{-1}$), then converted to an areal basis to a depth of 1 cm (kg OM m$^{-2}$)). Sediment grain size was determined using a laser-diffraction Malvern Mastersizer after treatment with hydrogen peroxide to remove organic material. Particle sizes are expressed as percentages of the total particle numbers, segregated into the following grain size fractions: clay (0-2 µm), silt (2-63 µm) and sand (63-2000 µm).

4.3.6 Data analysis

All statistical analyses were carried out in R Studio (v.3.3.2) (R Studio Team 2015) with base R version 3.3.0 (R Core Team 2016). To test the seasonality of denitrification rates and oxygen penetration depths, nested ANOVAs were fitted to the data using a linear mixed effects model (function “lme” in the package “nlme”), with Season and Site treated as fixed effects, and Replicate treated as a random effect. Denitrification rates and oxygen penetration depth were square root transformed prior to analysis to meet normality assumptions. Post hoc effects were examined using the Multcomp package. Multiple linear regression was used to investigate predictive variables influencing oxygen penetration depth and denitrification rates across the sites and seasons. Variable selection was made using stepwise regression. Due to the high collinearity between sediment characteristics, only the best fitting variable (lowest p value) was chosen for model selection from the grain size variables (porosity, sand, silt, clay, OM%). All other
variables were included in the initial model. Collinearity and variance inflation of the resulting model was assessed using the “vif” function and anything with an inflation factor > 2 was removed from further analyses. The importance of each predictor variable to the model (R² partitioned by averaging over orders) was assessed using the R package Relaimpo (reported in text as % R²). Principal component analysis on the correlation matrix was conducted using Primer v.6 (Primer E, Quest Research Limited). Normality was assessed using draftsman plots. Variables not conforming to normality were log transformed. All data were normalized prior to producing the PCA plots.

4.4 Results

4.4.1 Seasonality in water and sediment characteristics

The water temperature of the brackish lagoon water ranged between 5 - 21ºC while salinity showed little variability between seasonal measurements (Table 4.1). Concentrations of nitrate and ammonium in the water column were higher in winter than in summer months whereas total nitrogen (TN) peaked in mid-summer and decreased in winter and spring (Table 4.1). The three study sites showed a range in sediment characteristics (Table 4.2). Sediment mean grain size ranged from the sand dominated site T6 (98% sand) to silt dominated sediments (88%) at site T4 and a mixture of silt and sand at site T5 (Table 4.2). Sediment contained some clay-size particles at sites T4 and T5, but virtually no clay was present at the sandy site T6. Sediment organic matter availability ranged from 2 to 412 g m⁻² (0.50 – 12.5% dry wt), with greatest availability in the silt-dominated sediments. Mean porosity ranged from 0.41 to 0.96 (g mL⁻¹). Invertebrate densities also varied greatly seasonally (Table 4.3), with the invertebrate community dominated by chironomid larvae (Chironomidae; 3452 – 20,471 individuals m⁻²) and mud snails (Potamopyrgus antipodarum (Grey, 1843); 1776 – 16,064 individuals m⁻²). Lower abundances of two amphipod species (Paracorophium excavatum (Thompson, 1884) and Paracalliope fluviatilis (Stebbing, 1899)) were recorded (44 – 8750 individuals m⁻²), and oligochaetes (444 – 1589 individuals m⁻²).

4.4.2 Seasonality of oxygen penetration depth, denitrification and nutrient fluxes

Oxygen penetration depth decreased during the cooler seasons at sites T4 and T6 (Fig. 4.3A), whereas no seasonality was observed at site T5. A nested ANOVA indicated that
oxygen penetration depth varied significantly among sites and seasons (Table 4.4), with late summer showing significantly shallower oxygen penetration depths compared to autumn, winter and spring respectively, and early summer compared to winter (Table 4.4).

Table 4.3. Mean densities of invertebrate species (individuals per m$^{-2}$) recorded in each season from the three sites. P. ant is the snail *Potamopyrgus antipodarum*, P. exc is the amphipod *Paracorophium excavatum*, Oligo is the worm group *Oligochaeta*, Chiro is the non-biting midge larvae family *Chironomidae*, and P. flu is the amphipod *Paracalliope fluviatilis*. Seasonal means and standard error are reported (n=12).

<table>
<thead>
<tr>
<th>Season</th>
<th>P. ant</th>
<th>P. exc</th>
<th>Oligo</th>
<th>Chiro</th>
<th>P. flu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early summer</td>
<td>1776 ± 679</td>
<td>2632 ± 5029</td>
<td>1031 ± 270</td>
<td>2763 ± 843</td>
<td>8750 ± 709</td>
</tr>
<tr>
<td>Autumn</td>
<td>16064 ± 4139</td>
<td>132 ± 54</td>
<td>1787 ± 390</td>
<td>20471 ± 3302</td>
<td>44 ± 0</td>
</tr>
<tr>
<td>Winter</td>
<td>12544 ± 2253</td>
<td>4012 ± 1442</td>
<td>444 ± 235</td>
<td>8743 ± 776</td>
<td>1370 ± 315</td>
</tr>
<tr>
<td>Spring</td>
<td>3647 ± 607</td>
<td>1972 ± 315</td>
<td>591 ± 252</td>
<td>3452 ± 616</td>
<td>326 ± 156</td>
</tr>
<tr>
<td>Late summer</td>
<td>2769 ± 686</td>
<td>1819 ± 287</td>
<td>1589 ± 512</td>
<td>17911 ± 2100</td>
<td>175 ± 126</td>
</tr>
</tbody>
</table>

I measured high variability in total denitrification rates among replicate cores at some of our sites and on certain dates (e.g., autumn at site T4) (Fig. 4.3B). The highest denitrification rates were recorded at site T4 during autumn (824 µmol m$^{-2}$ h$^{-1}$ at 12 °C), T5 in early summer (430 µmol m$^{-2}$ h$^{-1}$ at 17°C), and in late summer at site T6 (228 µmol m$^{-2}$ h$^{-1}$ at 21°C). The highest ICOLL mean denitrification rate (sites combined) was recorded during early summer (17°C) (182 µmol m$^{-2}$ h$^{-1}$), while denitrification was severely suppressed during winter (5°C) (0.4 µmol m$^{-2}$ h$^{-1}$) (Fig. 4.3B). A nested ANOVA confirmed a significant relationship between total denitrification rate and sampling event and site (Table 4.4). Post hoc Tukey tests showed that denitrification rates were significantly greater in early summer compared to all other seasons (autumn, spring, late summer and winter; Table 4.4). Post hoc tests showed that site T4 with silty, organic sediments exhibited higher total denitrification rates than site T6, which was characterized by high sand content and low organic matter availability.
The movement of nutrients between the water column and the sediment is reported as fluxes, with positive values representing an efflux out of the sediment to the water column and negative values an influx into the sediment. Oxygen flux measured in the water column in our enclosures varied significantly across the sites and seasons (Table 4.4), with site T6 showing the highest measured rates of oxygen consumption in our study during autumn and late summer (Fig. 4.4A). Oxygen flux was significantly greater in autumn compared to all seasons (early summer, spring, winter and late summer (Table 4.4)). It was also greater in late summer than in winter, spring and early summer (Table 4.4). Nitrate in the water column was rapidly consumed in all enclosures (Fig. 4.4B). While there were no significant across site differences in nitrate flux, there were significant seasonal increases in late summer at sites T5 and T6. Ammonium fluxes were much smaller and highly variable (Fig. 4.4C), showing losses from the water column in early summer and autumn at all locations (Fig. 4.4C). Early summer and autumn ammonium losses from the enclosures were greater than in winter and spring (Table 4.4), when the water column at site T5 showed significant increases in ammonium concentrations over the course of the enclosure deployments.
Table 4.4 Nested analysis of variance results for the response variables OPD (oxygen penetration depth), denitrification, O$_2$ flux, NO$_3$ flux and NH$_4$ flux. Posthoc results are shown where the relationship with site or season was significant.

<table>
<thead>
<tr>
<th>Predictor variable</th>
<th>Response variable</th>
<th>Posthoc results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OPD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td>p &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>
| Season             | p < 0.001         | • late summer < autumn (p < 0.05), winter (p < 0.001), spring (p < 0.005)  
|                    |                   | • early summer < winter (p < 0.01) |
| **Denitrification**|                   |                 |
| Site               | p < 0.0001        | • early summer > autumn, spring, late summer (p < 0.05) and winter (p < 0.05) |
| Season             | p < 0.05          |                 |
| **O$_2$ flux**     |                   |                 |
| Site               | p < 0.001         | • autumn > early summer, winter, spring (p < 0.001) and late summer (p < 0.05)  
|                    |                   | • late summer > winter (p < 0.001), spring (p < 0.01) and early summer (p < 0.01). |
| Season             | p < 0.001         |                 |
| **NO$_3$ flux**    |                   |                 |
| Site               | p > 0.05          | • late summer; T5 and T6 > T4 |
| Season             | p < 0.05          |                 |
| **NH$_4$ flux**    |                   |                 |
| Site               | p > 0.05          | • early summer > winter & spring (p < 0.05)  
|                    |                   | • autumn > winter and spring (p < 0.01) |
Figure 4.3. Mean oxygen penetration depth (mm) (A) and average total denitrification rates (µmol m⁻² h⁻¹) (B) measured *in situ* in Tomahawk Lagoon No.1, at three sites in early and late summer 17°C, 21°C, autumn 12°C, winter 5°C, and spring 14°C. Error bars are standard errors (n=4).
4.4.3 Physical drivers of oxygen penetration depth, denitrification and nutrient fluxes

Relationships among sediment oxygen penetration depth and sediment characteristics at the three sites are illustrated by a principle component analysis on the correlation matrix (PCA; Fig. 4.5A). The first two axes of the PCA explained 84.7% of the variation in the dataset. The major gradient (Axis 1) describes variation in grain size and porosity, with organic matter availability and content both correlated positively with the finer grain size fractions. Sediment oxygen penetration depth was orthogonal to this major gradient and was, therefore, not related to the sediment grain size/organic matter gradient, however it was negatively correlated with temperature. The PCA distinguishes among the three sites with site T6 being predominantly sandy, site T4 being predominantly associated with fine sediments and organic matter availability and site T5, showing some variation across the major gradient. The sandy site, T6, showed substantial variation in the oxygen penetration depth while T4 showed less variation and site T5 showed little variation in oxygen penetration depth over the study period. Stepwise multiple regression selected the best model of oxygen penetration depth which contained the physico-chemical variables temperature (% $R^2 = 0.43$; negative) and clay content (% $R^2 = 0.57$; negative) which explained 25% of the variance in oxygen penetration depth (Table 4.5).
Figure 4.4. Mean oxygen flux flux (µmol m\(^{-2}\) h\(^{-1}\)) (A), nitrate flux (µmol m\(^{-2}\) h\(^{-1}\)) (B), and ammonium flux (µmol m\(^{-2}\) h\(^{-1}\)) measured \textit{in situ} in Tomahawk Lagoon No.1, at three sites in early and late summer 17°C, 21°C, autumn 12°C, winter 5°C, and spring 14°C. Error bars are standard errors (n=4).
Figure 4.5. Principal components analysis showing the relationships between sediment characteristics and the oxygen penetration depth at the three sites (A), and between sediment characteristics, water column gas and nutrient fluxes during the enclosure denitrification experiments and measured denitrification rate (B). OPD is oxygen penetration depth. OM% is organic matter percentage. OMA is organic matter availability.
The PCA in Fig. 4.5B illustrates the correlations between denitrification rates, the physical characteristics of the sediment, and the nutrient fluxes measured in the enclosures. This PCA explained 68.1% of the variation in the dataset. Again, the major gradient reflected grain size, porosity, organic matter % and organic matter content. The secondary axis described a gradient of temperature, denitrification potential and ammonium flux within the enclosures, the latter being negatively correlated to temperature and to the measured total denitrification rates. Neither oxygen nor nitrate fluxes in the enclosures were strongly correlated to either of the two main axes of variation. This PCA shows the overwhelming importance of temperature in driving denitrification potential in this system. Added nitrate was largely taken up or transformed in all the experiments but was not strongly correlated with any of the variables. In enclosures in which temperature and denitrification were high, ammonium flux was strongly negative. Stepwise regression using the physical sediment characteristics and nutrient fluxes selected organic matter availability, temperature and nitrate flux to produce the best model of denitrification rate (model $R^2 = 0.53$, p<0.001) (Table 4.5). The relative importance of each individual predictor variable explaining denitrification rates was organic matter availability (% $R^2 = 0.49$; positive), temperature (% $R^2 = 0.039$; negative), and nitrate flux (% $R^2 = 0.12$; positive).
Table 4.5. Multiple linear regression models best explaining observed variance in the dependant variables (oxygen penetration depth, denitrification rate) before and after invertebrate inclusion in the model. % R² reports the proportional contribution of each variable to the model R². The use of (+) and (-) show the relationship direction; positive or negative. For full invertebrate names see Table 3. OPDsq is oxygen penetration depth square rooted, P. flu is Paracalliope fluviatilis, P. ant is Potamopyrgus antipodarum, Dsq is denitrification rate square rooted and OMA is organic matter availability. * indicates significance at p < 0.05, ** indicates significance at p < 0.01, and *** indicates significance at p < 0.001.

<table>
<thead>
<tr>
<th>Oxygen penetration depth Reduced Model</th>
<th>% R²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen penetration depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>0.43 (-)</td>
<td>0.0833</td>
</tr>
<tr>
<td>Clay content</td>
<td>0.57 (-)</td>
<td>0.0430*</td>
</tr>
<tr>
<td>Model R² = 0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPDsq = - (0.07<em>Temp) – (0.11</em>Clay)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oxygen penetration depth Invertebrate Full Model</th>
<th>% R²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen penetration depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. flu density</td>
<td>0.59 (+)</td>
<td>0.00286**</td>
</tr>
<tr>
<td>Clay content</td>
<td>0.21 (-)</td>
<td>0.10559</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.13 (+)</td>
<td>0.30709</td>
</tr>
<tr>
<td>P. ant density</td>
<td>0.07 (+)</td>
<td>0.29307</td>
</tr>
<tr>
<td>Model R² = 0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPDsq = (0.00093<em>P. flu) – (0.0772</em>Clay) + (0.0505<em>Temp) + (0.0000148</em>P. ant)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Denitrification Full Model</th>
<th>% R²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denitrification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OMA</td>
<td>0.49 (+)</td>
<td>1.95 e^{-4}****</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.39 (+)</td>
<td>3.31 e^{-4}****</td>
</tr>
<tr>
<td>Nitrate flux</td>
<td>0.12 (+)</td>
<td>0.00679**</td>
</tr>
<tr>
<td>Model R² = 0.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dsq = (0.035<em>OMA) + (0.0048</em>NO₃ flux) + (0.98*Temp)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.4.4 Macroinvertebrates influences on oxygen penetration depth and denitrification

The relative abundances of macroinvertebrates, and their associated functional roles, changed greatly over the five sampling events (Table 4.3). Figure 4.6 displays the
abundance of key bioturbation types across the seasons (bioirrigator = chironomid; bulldozer = *Potamopyrgus antipodarum* & *Paracalliope fluviatilis*, conveyer = *Oligochaeta*; regenerator = *Paracorophium excavatum*). The invertebrate abundance was dominated by bulldozers and bio-irrigators, which peaked in autumn, and decreased to comparatively low densities in spring.

Adding the densities of invertebrate taxa into the multiple regression models increased the amount of variance explained in oxygen penetration depth (OPD; Table 4.5). When invertebrate densities were included in stepwise multiple regression models, OPD was best explained by *Paracalliope fluviatilis* density (% $R^2 = 0.59$, positive), clay content (% $R^2 = 0.21$, negative), temperature (% $R^2 = 0.13$, positive), and *Potamopyrgus antipodarum* (% $R^2 = 0.07$, positive). (Table 4.5). The model explained 47% of the variation in OPD, with a significance of $p<0.01$. The addition of invertebrates to the denitrification model did not increase its explained variance.

![Figure 4.6](image_url)

**Figure 4.6.** The mean abundances (individuals per m$^2$) of invertebrates across the 5 seasons, grouped by functional bioturbation type (n=12). Bio-irrigator = Chironomid (*Chironomidae*), bulldozer = *Potamopyrgus antipodarum* & *Paracalliope fluviatilis* conveyer = *Oligochaeta*, regenerator = *Paracorophium excavatum*. 
Figure 4.7. Three types of sediment oxygen micro-profiles (top panel) of a typical sediment core. The dashed line in the upper panel shows the sediment surface. Replicate A shows a typical diffusive profile. Replicate B shows an atypical oxygen profile with a deepened oxic layer due to disturbance by bulldozing bioturbation, advective transport or photosynthetic production in the upper sediment layers. Replicate C shows deep sediment oxygenation, typical of the presence of deep invertebrate burrows. The bottom panels are stylised depictions of the profiles in 2D through the sediment core (modified from (Polerecky et al., 2006)). The white region is deoxygenated sediment, and the brown region is oxygenated sediment, and the green region is the overlying water column. The dashed line indicates the micro-profile pathways.
Oxygen micro profiles indicated that oxygen concentrations in the silt-dominant sediments was limited by rates of oxygen supply from the overlying water (Fig. 4.7, Rep A – 64% of profiles). However, in some areas of the sediment a local disturbance had occurred interrupting the typical steady state diffusional profile shown in Fig. 4.7A. Our multiple regression modelling indicated where the bulldozer species Paracalliope fluviatilis and Potamopyrgus antipodarum were present, there was an increase in sediment oxygenation. Other possible causes of these increased diffusional profiles that were not quantified include advective transport of oxygenated water into the sediment porewater, or photosynthetic production by microphytobenthos in the upper sediment layers. In some cores, several oxygen profiles (up to one-quarter of profiles depending on season) showed increases in oxygen in deep sediment layers, due to the presence of tube dwelling infauna such as chironomids (Fig. 4.7,C – 18% of profiles).

4.5 Discussion

My study has examined both the seasonal and spatial variability of seasonal oxygenation and denitrification measured in situ in a small temperate ICOLL. This study adds to a small body of work on nitrogen cycling in ICOLLs or coastal lagoons, and my study is the first to investigate both denitrification and oxygen penetration depths seasonally and spatially in situ in this unique type of estuary. My research has revealed the main drivers of sediment oxygen dynamics and the ability to rapidly denitrify nitrate loads to the lagoon. Seasonally, Tomahawk Lagoon experienced large temperature changes, resulting in the severe reduction of denitrification during winter (at temperatures ≤ 5ºC), the season when nitrate concentrations were greatest in the ICOLL (19 µM). Sites with fine, organically enriched sediments exhibited higher denitrification rates in the ICOLL. The macroinvertebrates oxygenated the sediments and increased the transport of organic matter, nitrate and oxygen to sites of denitrification (Gerino et al., 2003, Bartoli et al., 2009), and chironomid larvae likely enhanced denitrification rates along with increased organic matter availability in the ICOLL as reported in previous studies (Svensson, 1997, Shang et al., 2013, Stief, 2013), overcoming potential seasonal limitations in substrate supply.

Due to the large variability of ICOLL ecosystems in terms of depth, light climate and times of organic matter inputs, it can be difficult to compare denitrification rates between them. Although our cores were amended with nitrate (142 µM), denitrification rates
measured in Tomahawk Lagoon (0 – 824 µmol m⁻² h⁻¹) were within the ranges of
denitrification rates in benthic chambers reported for an Australian temperate ICOLL (12
– 356 µmol m⁻² h⁻¹) (Spooner & Maher, 2009) and a large eutrophic NZ ICOLL (0 – 272
µmol m⁻² h⁻¹)(Chapter 3). Benthic primary producers are an internally generated source
of organic matter likely providing an important labile carbon source in Tomahawk
Lagoon. In contrast, the euphotic depth of the Australian ICOLL was less than 2 m,
limiting benthic primary producers and the production/input of summer carbon (Spooner
& Maher, 2009). Large seasonal and spatial variability in denitrification rates were also
reported for a large coastal lagoon in Italy, where nitrate and ammonium availability, and
bioturbation likely played a role driving spatial and seasonal variation (Bartoli et al.,
2012). Tropical lagoons have been reported to have lower denitrification rates than
temperate ones (Eyre & Ferguson, 2002, Enrich-Prast et al., 2016). However, a heavily
impacted tropical coastal lagoon in Puerto Rico sustained high denitrification rates, equal
to and greater than our lagoon (Pérez-Villalona et al., 2015). The lagoon had high organic
matter content (16.7%), high porewater ammonium concentrations (541 µM) and high
ammonium effluxes from the sediment (770 µmol m⁻² h⁻¹) (Pérez-Villalona et al., 2015),
which may have supported these increased rates compared to our ICOLL and to other the
tropical lagoons mentioned above. Positive relationships between sediment organic
matter and denitrification have been previously reported in the literature (García et al.,
1998, Livingstone et al., 2000, Teixeira et al., 2010, Deng et al., 2015) and have been
attributed to the resource requirements of denitrifying organisms (Piña-Ochoa &
Álvarez-Cobelas, 2006). Finer grained sediment often contains more organic matter than
coarser sediment (Vance-Harris & Ingall, 2005), and this was supported in Tomahawk
Lagoon, as indicated in Fig. 4.5. By contrast, our best models predicting denitrification
rates were driven by the sediment organic availability (concentration per m⁻²),
temperature and NO₃⁻ flux (Table 4.5).

The sediment oxygen penetration depth and the sediment oxygen demand in Tomahawk
Lagoon was significantly influenced by water temperature as indicated by our regression
modelling. This supports the findings of other studies which have shown a strong
correlation between temperature and sediment respiration, and decreased sediment
oxygen penetration depths (OPD) at higher temperatures (Eyre & Ferguson, 2005,
Veraart et al., 2011, Bonnett et al., 2013). As temperatures and sediment respiration rates
increase and sediment oxygen concentrations decrease, nitrification could be inhibited
due to a reduction in sediment oxic layer thickness. Denitrification in waterlogged sediments is often limited by oxygen and nitrate diffusion into the sediments (Seitzinger et al., 2006) and a decrease in nitrate supply via nitrification from a reduced oxic layer can reduce coupled nitrification-denitrification (Groffman et al., 2006, Seitzinger et al., 2006, Groffman et al., 2009). However, when nitrate concentrations in the water column are high, a decrease in the OPD may increase direct denitrification due to the decreased distance nitrate needs to diffuse to reach sites of denitrification. Nitrate – amended denitrification rates in our experiment were severely reduced in winter. This supports research in other coastal ecosystems, which shows denitrification rates are strongly temperature dependent (Livingstone et al., 2000, Piña-Ochoa & Álvarez-Cobelas, 2006, Wang et al., 2007, Zhong et al., 2010, Bonnett et al., 2013), with temperatures below 5°C suppressing microbial activity (Song et al., 2013) whereas temperatures between 10 and 20°C stimulated denitrification (Wang et al., 2007, Zhong et al., 2010, Barnes & Upstill-Goddard, 2011). With deeper oxygen penetration depth in winter in Tomahawk Lagoon, our labelled amended nitrate may have been delayed in reaching sites of denitrification at the oxic/anoxic boundary in the sediments. However, nitrate consumption rates in the enclosures were not lower than in other seasons (Fig. 4.4B), indicating that biogeochemical processes other than denitrification (e.g., nitrate uptake by autotrophs, assimilatory nitrate reduction, etc.) could have been important in winter too.

4.5.1 Invertebrate bioturbation enhances sediment oxygen penetration

Burrowing organisms can increase organic matter remineralization and greatly increase the flux of dissolved nitrogen species (D'Andrea & DeWitt, 2009, MacTavish et al., 2012). The densities of the hyperbenthic amphipod *Paracalliope fluviatilis* and benthic snail *Potamopyrgus antipodarum* were positively correlated with sediment oxygen penetration depth in the lagoon and offset diffusional limitations imposed by the fine grain size sediment. Their active bioturbation may be working directly against the effects of temperature and clay content on reducing sediment oxygen penetration depth, supporting increased oxygenation and particle movement. Previous studies have also reported that amphipod density positively correlated with sediment oxygen and nutrient fluxes due to burrow construction and sediment irrigation (Pelegri & Blackburn, 1994, Dixon & Moore, 1997, Marsden, 2002, Dunn et al., 2009, Gongol & Savage, 2016). The
amphipod *Paracalliope fluviatilis* does not create permanent burrows such as those produced by another amphipod, *Paracorophium excavatum* (Marsden, 2002), however it does actively displace soft mud and feed on algae and detritus (Jones & Marsden, 2005), increasing sediment turnover and oxygen renewal in the upper sediment layers. The activities of both the snail *Potamopyrgus antipodarum* and *Paracalliope fluviatilis* can be defined as a bio-diffuser or bulldozer, translocating sediment particles and increasing the movement of solutes over short distances (Gerino et al., 2003). Oligochaete density was removed from the models due to its strong negative correlation to clay content. Oligochaete bioturbation is described as a conveyer, collecting fine sediments from below the sediment surface for feeding, and passing waste on the sediment surface resulting in extensive sediment mixing, which may have supported vertical oxygen transport in sandy sediments (Svensson et al., 2001, Gerino et al., 2003).

Chironomids were removed from the models due to their strong positive correlation to organic matter content (p<0.001), therefore it is not possible to distinguish between the role organic matter availability and chironomids play in increasing denitrification rates in our dataset. Denitrification rates were enhanced in areas with high organic matter content, and if organic matter availability is excluded from the modelling, chironomids became the next most important predictor of denitrification. Chironomids created oxygenated patches around their burrows evident in our oxygen micro-profiles and visually in the extruded sediment cores. The chironomids construct semi-permanent burrows which can penetrate up to 20 mm below the sediment surface, exhibiting signs of sediment oxidation (light coloured sediment) on the walls of the burrows, creating steep oxygen gradients (as shown in Fig. 4.7C) which may increase direct denitrification through nitrate delivery and/or coupled nitrification-denitrification as reported in other studies (Svensson, 1997, Shang et al., 2013, Stief, 2013). Chironomids are bio-irrigators (or gallery diffusers; Gerino et al., 2003), and actively irrigate their tubes, probably enhancing nitrate and organic material delivery to deeper sediment layers. Thus, burrowing and irrigation activities increase both the supply of substrates and oxygen to the sediment and increase the areal extent of the sediment-water interface, increasing nutrient fluxes (Meile et al., 2005). Particle reworking within these burrows can also stimulate the degradation and remineralization of organic matter (Aller, 1994), and change the abundance of nitrogen cycling bacteria in the sediments (Shen et al., 2017). Thus, macroinvertebrate activities greatly increase the supply of oxygen and likely
dissolved nitrogen species, increasing the spatial and temporal nature of denitrification in sediments (McClain et al., 2003, Groffman et al., 2009, Palta et al., 2014). Chironomid larvae are the dominant sedentary burrower in Tomahawk Lagoon and most likely greatly facilitate denitrification rates, making chironomid larvae an important species with regard to sediment oxygenation and nitrogen cycling.

4.5.2 Implications for management of ICOLLs

Our study has shown the predominant role that temperature plays in sediment oxygenation and denitrification in the temperate study ICOLL. In summer, the oxygen penetration depth was lower and denitrification rates were higher than in winter, especially in regions with high chironomid densities and organic matter content and availability. In winter, denitrification was severely inhibited; thus, nitrate loads entering the ICOLL at this time would not be denitrified and would, therefore, remain available for uptake by autotrophs. Given nitrate loading is often greater in winter than in summer in Tomahawk Lagoon (Chapter 2), management actions which reduce nitrate loading in winter will directly reduce nitrogen availability to phytoplankton and macroalgae at a time when denitrification is inhibited and does not remove nitrate loads from the system.

Management to mitigate eutrophication in ICOLLs could be further enhanced through managing carbon availability to denitrifiers. At times, macrophytes and macroalgae exist in the lagoon (Ruppia spp., Ulva spp., and occasionally detritus from Durvillea antarctica), and their decomposition and resultant remineralization may provide subsidies to carbon limited sediments, which may stimulate greater denitrification rates at certain times of the year. Waterfowl such as the black swan (Cygnus atratus) are common within the study lagoon and feed directly on submerged macrophytes and macroalgae (McKinnon & Mitchell, 1994), enhancing the cycling and availability of nitrogen, phosphorus and carbon in the ICOLL (Mitchell & Wass, 1995, Somura et al., 2015). Microphytobenthos production may also supply labile organic carbon to fuel denitrification. Improved understanding of how organic carbon supply and its lability can enhance denitrification could suggest management strategies to enhance denitrification in such systems through managing carbon inputs, both in terms of quantities and quality of the carbon sources.
At present, many ICOLLs are subject to artificial openings to flush nutrients and sediments, to facilitate drainage, prevent flooding and to allow fish passage. Barrier bar breaches can significantly increase the salinity (Crawshaw, unpublished data, Schallenberg et al., 2010, Hamill & Schallenberg, 2013) and thereby affect the invertebrate community (Schallenberg et al., 2003, Lill et al., 2012). Currently Tomahawk Lagoon supports a range of mesohaline invertebrate species. *Paracalliope fluviatilis* is considered an oligohaline species (surviving only short saline exposures up to 28 (Jones & Marsden, 2005). While it plays a key role in enhancing sediment oxygenation, prolonged artificial opening events which increase the ICOLL salinity could result in the loss of this species from the ICOLL, which could reduce the rates of denitrification during the warmer seasons. Similarly, chironomids are also generally a oligohaline - mesohaline species. Their salinity tolerance is limited, and experimental studies of *Chironomus* spp. have shown decreased survival at 2.68 ppt, and full mortality occurring at 11.9 ppt (Hassell et al., 2006). In a study of chironomid burrow irrigation, (Roskosch et al., 2010) calculated that the pumping velocity of *Chironomus plumosus* at a density of 745 individuals m\(^{-2}\) was equal to 1.3 m\(^{3}\) m\(^{-2}\) d\(^{-1}\), which would result in the entire volume of Lake Müggelsee (area 7.3 km\(^{2}\), mean depth 4.9 m) being irrigated through the burrows every 4.8 days. In comparison to our lagoon system where recorded chironomid densities were as high as 32,000 ind. m\(^{-2}\), I can infer that in shallow ICOLLs, the impact of chironomid burrow irrigation could be highly significant to denitrification rates. Hölker et al. (2015) suggested that burrowing species such as chironomid larvae play crucial roles in ecological feedbacks, conferring ecological resilience and promoting the continuation of a macrophyte-dominated stable state in shallow lakes. In conclusion, given the significant role that invertebrates play in oxygenating the sediments and likely supporting denitrification, management of openings should consider effects on macroinvertebrates during warmer seasons when denitrification rates are high, to avoid unintended consequences related to large salinity-related changes in community composition (e.g. chironomid die-offs).
CHAPTER 5

Carbon lability influences nitrogen cycling rates in temperate estuary sediments

Photo: Mangrove expansion in the upper Whangateau Harbour.

This chapter is a modified version of the manuscript for submission to Biogeochemistry:

Crawshaw, J.A., O’Meara, T., Savage, C., Thompson, B., Baltar, F., & Thrush, S. Carbon lability influences nitrogen cycling rates in temperate estuary sediments.
5.1 Foreword

This chapter is different to the ICOLL ecosystems examined in the rest of the thesis, due to a collaborative opportunity with a postdoctoral fellow at the Leigh Marine Laboratory at Auckland University during the summer of 2016-2017. The research was conducted with sediments from a mangrove containing estuary (Whangateau estuary) which was utilized due to its proximity to the laboratory and range of organic plant sources. The estuary is beginning to show signs of eutrophication stress (occasional blooms of *Ulva* spp., mangrove expansion, seagrass loss) thus provided an ideal model ecosystem to study how shifting organic detritus sources may impact the N removal capacity of the estuary.

5.2 Abstract

In aquatic ecosystems, natural processes that remove nitrogen from the biologically available pool (e.g. denitrification) have been intensively studied as an ecosystem function that removes land-derived nitrogen and reduces eutrophication. The quantity of sediment organic matter is one of the key drivers of denitrification with percent organic content positively related to rates of nitrogen removal, however few studies have investigated the influence of the quality of organic matter on nitrogen cycling in estuarine sediments. This laboratory study investigates the influence of various organic detritus sources (quality; C:N ratio) on nitrogen gas (N$_2$) and solute fluxes and extracellular enzyme activity in temperate estuarine sediments with intact benthic communities. A custom-built tank with a removable face plate was used with a planar optode film, to image sediment oxygenation. Mangrove leaf detritus increased the N$_2$ production in the sediments, while the deposition of other detrital sources and control sediments showed net N$_2$ consumption. Extracellular enzyme activity of phosphatase and β-glucosidase were similar among treatments suggesting that the detrital additions did not change microbial hydrolysis rates of phosphorus-containing organic matter. Sulfatase activity was significantly reduced in the mangrove leaves and seagrass treatments, suggesting reduced oxygen conditions limited the availability of organic sulfur compounds to sulfatase using microbes. LAPase activity was reduced in all treatments, suggesting the organic detritus provided a nitrogen supplement or limited the activity of extracellular producing microbes. An oxygen consumption threshold was found where sediments switched from net N$_2$ consumption to net N$_2$ production. Our results indicate different
detrital sources may have negative impacts on the removal of bioavailable nitrogen through denitrification.

5.3 Introduction

Nitrogen is often a limiting nutrient for primary production in the marine environment, and its availability can control the balance between an oligotrophic and eutrophic ecosystem (Howarth, 1988, Gardner et al., 2006). Anthropogenic nitrogen production has significantly increased in recent decades (Galloway et al., 2004), and the use of fertilizers to increase land productivity is having a detrimental effect on coastal ecosystems worldwide (Cloern, 2001), including New Zealand (Houlbrooke et al., 2004, McDowell & Wilcock, 2008). High nitrogen inputs can lead to eutrophication symptoms, including blooms of phytoplankton or nuisance macroalgae, lowered light and oxygen concentrations, loss of submerged aquatic vegetation and excessive deposition of carbon (Herbert, 1999, Cloern, 2001, Seitzinger et al., 2006). The removal of nitrogen through natural ecosystem processes is critical for avoiding the transition from a clear water state to a degraded ecosystem, often turbid with increased opportunistic algae. A key removal process in shallow coastal sediments is denitrification, the microbially mediated transformation of bioavailable nitrate to inert nitrogen gas (Piña-Ochoa & Álvarez-Cobelas, 2006). It removes bioavailable nitrogen from the coastal ecosystem, reducing nitrogen availability to primary producers and would accordingly reduce eutrophication.

Other processes in the nitrogen cycle can also increase the eutrophication status of an ecosystem, such as dissimilatory nitrate reduction to ammonia (DNRA) (Burgin & Hamilton, 2007), where deposited organic material can be re-mineralized producing ammonium (Stief, 2013), which can be released back into the water column and stimulate further primary production, exacerbating eutrophication symptoms (Herbert, 1999). Remineralization can be coupled to nitrification-denitrification at the oxic/anoxic sediment boundary, which removes nitrogen from the system (Jäntti et al., 2012, Stief, 2013). The balance between these interlinked processes is important to understand for management of nutrient inputs into coastal and estuarine ecosystems.

Dominant organic detritus sources in estuaries are shifting due to eutrophication, from healthy seagrass beds towards nuisance macroalgae such as *Ulva* sp. or phytoplankton
blooms and high standing stocks of microphytobenthos communities (Borum, 2013). The shift towards bloom forming algal tissue may reduce the denitrification potential of the estuary and potentially reinforce eutrophication conditions through positive feedbacks (Hardison et al., 2013). Eutrophication is not the only driver of change in coastal vegetation; in northern New Zealand, there is also widespread removal of mangroves to increase coastal access to waterways (Lundquist et al., 2014). The implication of this habitat change to denitrification potential is unknown. Mangrove clearance reduces sediment organic matter content and increases summer ammonium effluxes into the water column (Bulmer et al., 2017), suggesting changes in the nitrogen cycle that warrant further investigation.

Sediment organic matter is one of the key variables driving spatial variability in denitrification (Piña-Ochoa & Álvarez-Cobelas, 2006, Seitzinger et al., 2006), however studies investigating the quality and quantity of organic matter are limited (Fulweiler et al., 2008, Eyre et al., 2013). The quality (e.g., C:N ratio) of organic matter has been reported to control spatial variation in denitrification in field studies (Eyre et al., 2013, Burkhardt et al., 2014, Belley et al., 2016), however no studies have experimentally tested a range of organic sources to assess how lability of the carbon source influences nitrogen cycling in estuaries. The carbon to nitrogen ratio (C:N) is considered a good indicator of the lability of an organic source – the lower the ratio, the more labile and more accessible the source (Erler et al., 2017). Low molecular weight organic compounds and inorganic nutrients are readily assimilated by primary producers, while organic nutrient forms must first undergo desorption, hydrolysis, bacterial decomposition or photo-decomposition (Bushaw et al., 1996, Hendrickson et al., 2007). The microbial utilization of deposited organic matter is dependent first on the breakdown of the organic matter by extracellular or bacterial processing and can be a rate limiting step in the utilization of the nitrogen, phosphorus and carbon (Hiroki et al., 2007, Riekenberg et al., 2017). The microbial community composition can change depending on the nature of available organic matter (labile vs recalcitrant) (Ghosh & Leff, 2013) and the nutrient requirements of the organism (Middelboel et al., 1995). The activity of various bacterial extracellular enzymes responsible for the degradation of organic matter can be measured using extracellular enzyme assays, with the use of fluorogenic substrate analogues (Hoppe, 1983, Bell et al., 2013, Baltar et al., 2017), providing a quantitative measure of the rate of degradation of different organic matter sources. The bioturbation of large
macrofauna such as bivalves can support increased nitrogen cycling in estuarine sediments (Caffrey et al., 2016, Murphy et al., 2016). Bivalves (and other bioturbating macrofauna) can both enhance denitrification through changing resource supplies to denitrifiers (Smyth et al., 2013), but also provide increased habitat such as on the shell or in the gut of the organism (Stief et al., 2009, Svenningsen et al., 2012, Caffrey et al., 2016). This research suggests bivalves play a critical role in maintaining the nitrogen balance, directly by assimilating nitrogen from the water column, and indirectly by harbouring nitrifying and denitrifying microorganisms on their shells and gut, bioturbation processes reworking sediments and increasing substrate availability.

Previous research has indicated that denitrification in New Zealand coastal sediments is often carbon limited (Crawshaw et al., 2018), with the addition of carbon significantly changing the nitrogen cycling capacity of the sediment (Schallenberg & Crawshaw, 2016). However, there are limited studies on the role of changing carbon sources on nitrogen cycling in estuaries. I conducted this study using layered sediments from the Whangateau estuary, North Island, New Zealand, as it contains a diverse array of habitats and a range of carbon sources, including mangrove forests, seagrass meadows and microphytobenthic dominated sandflats. The estuary is not considered to be eutrophic (Kelly, 2009), but periodically experiences inputs of marine algae such as blooms of the sea lettuce Ulva sp. My study aimed to investigate how various organic detrital sources influence sedimentary nitrogen processing. I hypothesised that net nitrogen removal (N₂ efflux) would be enhanced by more labile organic matter. I hypothesised that extracellular enzyme activity would also be increased by more labile organic material, supporting increased nitrogen removal. Large bioturbating bivalves were expected to change sedimentary oxygen conditions to be more suitable for denitrification due to increased oxygen consumption, which was imaged using planar optode films.

5.4 Methods

5.4.1 Study Location

Sediments were collected from the northern arm of the Whangateau estuary, just outside the mangrove forest on the sand flat (36°19’S, 174°46’E). The Whangateau estuary is a shallow sandspit estuary (750 ha) located 60 km north of Auckland City (Kelly, 2009, Jones et al., 2017) on the east coast of the North Island, New Zealand. It is well flushed tidally (tidal prism 81% total estuary spring tide volume) (Jones et al., 2017), with low
dissolved nutrient levels and turbidity and high-water quality overall (Kelly, 2009). The catchment land use is mixed (native bush, plantation pine forest, pasture, urban, horticulture, viticulture) and river inputs only account for 3% of the tidal prism (Boffa Miskell, 2009).

5.4.2 Experimental design
A custom-built flow-through gas-tight tank was constructed for this experiment, which allows the insertion of a planar optode for oxygen imaging (Fig. 5.1). The tank consists of PVC sides (internal size: 37 x 27 x 4 cm) held together by large screw bolts with an o-ring seal. One side of the tank is removable to allow attachment of the planar optode film. The film was attached with double-sided adhesive film (X-film, Germany) at a height to capture the sediment and water column oxygen dynamics. At the top of the tank, there is a small hole to allow sediment input, with an o-ring sealed lid (Fig. 5.1). Water inflow is provided through tubing at the top of the tank via a peristaltic pump (flow rate: 10 ml min⁻¹). The outflow tube is positioned ~5 cm above the sediment surface.

Sediments from Whangateau were collected from specific depths (0-2, 3-5, 6-8, 9-11, 12-14 cm) using a spade. Sediments were not sieved, with the aim of achieving a natural invertebrate community within the sediments. On return to the lab, sediments were layered in the optode tank to minimise disturbance to the sediment layers, while the tank was submerged in sea water, to reduce bubble formation and facilitate sediment settling. The chamber was left open in a large tank with recirculating seawater and a bubbler overnight to allow natural biogeochemical gradients to re-establish. I acknowledge that this method may have re-oxygenated deeper sediment layers during the transportation process, and therefore influence our results.
Figure 5.1. Custom-built flow-through gas-tight tank for planar optode imaging. The front face of the tank is removable to allow attachment of the planar optode film. A removable o-ring sealed port at the top of the tank allows addition of sediment and organic detritus.

Five treatments were used in this experiment: control sediments (no amendments) (C), and sediments with microphytobenthos (MPB), sea lettuce (*Ulva* spp.) (SL), seagrass leaves (*Zostera muelleri*) (SG) and mangrove leaves (*Avicennia marina*) (ML) additions. Each treatment was replicated 4 times. MPB was extracted from sediments collected at the same site as the sediments. The top 1 cm of sediment was scraped from locations with large MPB communities (visible brown patches on the surface). This was added to a litre of sterile seawater and shaken vigorously for 1 minute to extract the MPB from the sediments (Leynaert *et al.*, 2009). After waiting for five minutes for the sediment to settle, the overlying water was collected and filtered onto a Whatman GF filter (75 mm) using a vacuum pump (Millipore). Filters were then dried at 60°C for 24 hours, and the filtrate scraped off the filter for use in the experiment. SG and ML were collected fresh from the estuary and washed in seawater, then dried at 60°C for 48 hours. SL was collected from the Mokohinau Islands (35°55'S, 175°5'E) and treated as above. Detrital additions were added to the sediment as dead ground sources since photosynthesis can produce oxygen.
bubbles, which interferes with dissolved gas measurements using the MIMS technique (see below). Moreover, this allows the different detrital sources to be added at the same quantity. The organic detritus sources were individually ground to a powder using a small coffee grinder to homogenise material. 2 g of each organic treatment was added to the overlying water in the sediment tank and allowed to settle for 1 hour before sealing the chamber. The quantity of added organic detritus was selected based on previous research of increased *Ulva* spp. amendments to estuarine sediment that did not invoke sediment anoxia (C. Savage, pers. Comms.). I acknowledge that the choice to homogenize the detritus additions will affect the applicability of our results to the real world, however it may represent the basal availability of the carbon once the outer cuticle layers have been broken down. To minimize air contamination, which could interfere with the gas measurements, chambers were sealed while submerged in seawater. The chamber was set up in a dark, temperature-controlled room (20ºC). A continuous flow of filtered (200 µm) seawater (flow rate:10 ml min⁻¹) was passed over the sediment surface using a peristaltic pump. Each tank was allowed to pre-equilibrate with the organic detritus in the dark for 12-18 hours to reach steady state (Eyre *et al.*, 2002, Scott *et al.*, 2008) before water samples were taken for dissolved nutrients (NO₂⁻-N + NO₃⁻-N, NH₄⁺-N) and dissolved gases (N₂ and O₂). Six water samples were taken in triplicate of the inflow and outflow over a 6-hour period to ensure fluxes were remaining at a steady state. The experiment was run in the dark to minimise the production of oxygen bubbles by photosynthesis, and if any bubbles larger than 1 cm accumulated in the core, the core was discarded. I acknowledge that these ecosystems are shallow, euphotic ecosystems that undergo diel light cycles, thus our results represent what would happen in the sediments during the night. Water samples for N₂ and O₂ were taken by allowing the outflow pipe to feed into a 12 mL extetainer (Labco, UK), filling from the bottom of the vial and overflowed 3 times to reduce air contamination. The tube was slowly removed to reduce any oxygen contamination, and the sample spiked with 1 µL of saturated ZnCl₂ to stop microbial processing. Samples were then stored in the dark submerged in water at 20ºC and analysed within a week. Water samples for dissolved nutrients (NO₂⁻-N + NO₃⁻-N, NH₄⁺-N) were taken in conjunction with the gas water samples, filtered through a Microscience MS-GF 47 mm filter (nominal pore size = 0.7 µm) into 50 mL acid-washed centrifuge tubes, and frozen at -20ºC until analysis.
Water samples for N$_2$ and O$_2$ analysis were measured on a Bay Instruments Membrane Inlet Mass Spectrometer (MIMS) at Leigh Marine Laboratory, following the protocols of (Kana et al., 1994). The N$_2$:Ar technique measures the balance of N$_2$ between two co-occurring processes – denitrification and N$_2$ fixation, thus the resulting N$_2$ flux is a net change in N$_2$. Production of N$_2$ is an efflux of N$_2$ out of the sediment suggesting dominance of denitrification, and N$_2$ consumption is an influx of N$_2$ into the sediment suggesting dominance of nitrogen fixation. The dissolved nutrients and gas production rates were calculated using the following equation: $\text{Flux} = \frac{(C_o - C_i)}{A} \times F$ where $C_i$ is the chamber inflow concentration (µM) and $C_o$ is the chamber outflow concentration (µM), A is the area of the sediment (m$^2$) and F is the flow rate of the chamber (L h$^{-1}$) (O’Meara et al., 2015).

Nutrients were analysed using colorimetric protocols, using a SANPlus segmented flow autoanalyzer (SkalarAnalytical B.V., Breda, The Netherlands), as previously reported in (Schallenberg & Burns, 1997). Analytical detection limits were 0.071 µM for nitrate-N + nitrite-N (henceforth reported as nitrate), and 0.93 µM for ammonium-N.

At the end of the incubation, a sample (10 g) of the top 2 cm layer of sediment was collected for analysis of organic matter, Chl a, phaeopigments, Total Nitrogen (TN) and Total Carbon (TC) content and for extracellular enzyme activity assays. The sediment was frozen until analysis. The invertebrate community was collected on a 500 µm sieve and preserved in 50% isopropyl-alcohol for identification. Macroinvertebrates were identified to family or lower where possible.

Sediment organic matter was quantified by drying 5 g of wet sediment at 50°C for 24 hours, followed by combustion at 450°C for 24 hours. Sediment organic matter was determined as the change in dry weight before and after combustion. The chla: pheophytin ratio provides a measure of the quality of organic matter (Le Guitton et al., 2015), with the greater chla: pheophytin ratios indicating freshly deposited material (Belley et al., 2016). 1 g of freeze-dried sediment was incubated with 3 mL of 90% acetone at 4°C for 24 hours (Belley et al., 2016). After acetone addition samples were briefly vortexed then sonicated in a water bath style ultrasonic cleaner for 30 seconds before refrigeration for 24 hours. After this period samples were briefly vortexed, then centrifuged at 1750 RPM for 5 minutes (Brito et al., 2009, Hannides et al., 2014). Extracts were analysed in a Shimadzu UV-1603 spectrophotometer at 630, 647, 664 and
750 nm. The sediment was then dried at 60 degrees for 24 hours and weighed to standardise pigment concentrations per gram of sediment. Chl $a$ and phaeopigments values were obtained before and after acidification with 0.5 M HCl (12 ll of HCl to 1 ml of extract), respectively (Lorenzen, 1967)(Parsons et al., 1984).

The chlorophyll $a$ concentration of the sediment (ug g DW$^{-1}$) was calculated using the following equation:

$$\text{Chl} \ a \ (\text{ug} \ \text{g} \ \text{DW}^{-1}) = \left( \frac{(11.85 \times 630) - (1.54 \times 647) - (0.08 \times 665)}{\text{acetone volume (mL)}} \right) \times (1 \times \text{sediment dry weight (g)})$$

The phaeopigment concentration of the sediment was calculated using the following equation:

$$\text{Phaeo} \ (\text{ug} \ \text{g} \ \text{DW}^{-1}) = \left( \frac{29.6 \times (665_{\text{before}a} - 665_{\text{after}a})}{\text{acetone volume (mL)}} \right) \times (1 \times \text{sediment dry weight (g)})$$

0.05 g of sediment was freeze-dried for 24 hours, and a subsample of 0.01 g was loaded into foil canisters for total carbon and total nitrogen analysis at the Department of Chemistry, University of Otago.

To assess 2D spatial patterns in sediment oxygen concentrations due to macrofauna bioturbation, three fluorescent lights were used to illuminate the planar optode sensor when photos were taken. Planar optode images were captured using a Nikon D610 camera with a Nikon AF-S Nikkor 24-85 mm 1:3.5-4.5 G zoom attachment, coupled to a Mid-PT yellow light filter, set up on a tripod 20 cm away from the tank. The camera was set to take a photo every 10 seconds collecting 15 photo blasts. The photo sets were taken at 24 and 48 hours to capture bioturbation effects of large macrofauna. Raw images were converted to Jpeg files using IrfanView 64, and then converted to false colour using ImageJ. Oxygen concentrations were assigned to the false colour image, assuming the lower sediment was 0% oxygen, and 100% oxygen at the water inflow. Issues were encountered during the experiment with the integrity of the optode films, resulting in only a few usable images, thus images from each treatment type are unable to be reported on.

5.4.3 Extracellular enzyme activity (EEA)

The hydrolysis of the fluorogenic substrate analogues 4-methylumbelliferyl (MUF)-phosphate, 4-methylumbelliferyl-ß-D-glucoside, 4-methylcoumarinyl-7-amide (MCA)-
L-leucine-7amido-4-methylcoumarin, and 4-methylumbelliferyl-sulfate potassium was measured to estimate the potential activity rates of alkaline phosphatase (APase; phosphorus cycling), β-glucosidase (BGase; carbon cycling), leucine aminopeptidase (LAPase; nitrogen cycling) and sulfatase (SULFatase; sulfur cycling) (Hoppe, 1983).

To make the sediment slurry for the extracellular enzyme activity, 3 g of wet sediment was weighed and diluted with 97 mL of sterilized (autoclaved) and filtered seawater and homogenized in a blender for one minute using a modified method from Jackson et al. (2013) and Bell et al. (2013). The sample was then centrifuged at 2500 G for 5 minutes, and the supernatant transferred into a clean centrifuge tube and frozen until analysis. Samples (300 µL) were incubated in the dark at 20ºC (average in situ temperature) for five hours, with plate readings taken at 0 and 3 hours. After sample and substrate addition, plates were incubated and measured using a Spectramax M2 spectrofluorometer (Molecular Devices, USA) at excitation and emission wavelengths of 365 and 445 nm respectively. Samples without substrate addition were used as background fluorescence blanks. The increase in fluorescence over time was transformed into hydrolysis activity using a standard curve established with different concentrations of the fluorochromes MCA and MUF (Hoppe, 1983, Bell et al., 2013, Jackson et al., 2013, Baltar et al., 2017). Extracellular enzyme activity is reported here in nmol h⁻¹ g⁻¹ DW.

5.4.4 Statistical approach

All analyses were run in R Studio (v.3.3.2) (R Studio Team 2015) with base R version 3.3.0 (R Core Team 2016). All figures were produced in Microsoft Suite 365 Pro Plus. Analysis of variance (ANOVA) was used to investigate significant differences between treatment groups, where results were deemed significant below a p-value of 0.05. Normality was assessed using QQ plots. Post hoc Tukey t-tests were then used to investigate which relationships were significant. Pearsons correlations were used to investigate linear relationships between N₂ and O₂ fluxes, and potential drivers of sediment LAPase and SULFatase activity. Step-wise linear regression was used, eliminating the least significant variable to find the best model fit.
5.5 Results

5.5.1 Detrital quality and macroinvertebrate abundances

The different organic detritus sources used in this experiment ranked from MPB as the most labile source to SG as the most refractory detrital source (C:N ratio) (Table 5.1). The organic detritus C:N ratio was significantly different between the added sources (ANOVA, \( F = (3,12) = 534, p<0.001 \)), with all sources being significantly different from each other (p<0.05). The benthic invertebrate community present across the chambers is reported in Table 5.2. The community was dominated by Neridae polychaetes, cockles (Austrovenus stuchburyi) and nut shells (Nucla hartigrava) (Table 5.2).

5.5.2 Nitrogen remineralization – NH₄⁺ and NO₃⁻ fluxes

Effluxes of ammonium were exhibited in the C, MPB and SL treatments (Fig. 5.2A). In contrast ML and SG treatments exhibited little efflux of ammonium or slight consumption of ammonium in the sediments. There was a significant difference between groups (ANOVA, \( F = (4,14) = 3.325, p<0.05 \)), and a posthoc Tukey test showed a difference in the ammonium fluxes between SG and the C treatments (p<0.05). The nitrate fluxes were highly variable and much lower than the ammonium fluxes (Fig. 5.2B), however average concentrations in the outflow remained around 3 \( \mu \text{mol L}^{-1} \). There were no significant differences in the nitrate fluxes between treatment types (ANOVA, \( F = (4,14) = 0.362, p>0.05 \); Fig. 5.2B).

Table 5.1. Organic detrital sources and treatment core sediment characteristics. Data represent sample averages and 1 standard deviation (n=3). C = control sediment (no amendments). Treatments are sediment with addition of MPB = microphytobenthos, SL = sea lettuce, SG = seagrass leaves, and ML = mangrove leaves. n.a. = not applicable.

<table>
<thead>
<tr>
<th>Organic detrital source</th>
<th>Detrital C:N mol ratio</th>
<th>Detrital δ¹³C %o</th>
<th>Sediment δ¹³C %o</th>
<th>Sediment C:N mol ratio</th>
<th>Sediment Chl a (ug g DW⁻¹)</th>
<th>Sediment OM %</th>
<th>Sediment phaeopigments (ug g DW⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>n.a.</td>
<td>n.a.</td>
<td>-22 ± 0.62</td>
<td>10 ± 0.26</td>
<td>3.7 ± 0.8</td>
<td>0.9 ± 0.17</td>
<td>0.80 ± 0.04</td>
</tr>
<tr>
<td>MPB</td>
<td>8 ± 0.85</td>
<td>-20 ± 0.17</td>
<td>-22 ± 0.33</td>
<td>10 ± 0.32</td>
<td>3.0 ± 0.1</td>
<td>0.7 ± 0.09</td>
<td>0.84 ± 0.01</td>
</tr>
<tr>
<td>SL</td>
<td>28 ± 0.25</td>
<td>-14 ± 0.22</td>
<td>-20 ± 0.67</td>
<td>14 ± 0.99</td>
<td>4.8 ± 2</td>
<td>1.2 ± 0.19</td>
<td>0.84 ± 0.01</td>
</tr>
<tr>
<td>SG</td>
<td>30 ± 0.44</td>
<td>-12 ± 0.15</td>
<td>-22 ± 0.76</td>
<td>11 ± 0.55</td>
<td>3.1 ± 1</td>
<td>1 ± 0.18</td>
<td>0.84 ± 0.01</td>
</tr>
<tr>
<td>ML</td>
<td>24 ± 0.49</td>
<td>-28 ± 0.12</td>
<td>-20 ± 0.42</td>
<td>11 ± 0.63</td>
<td>3.0 ± 0.3</td>
<td>1.3 ± 0.29</td>
<td>0.78 ± 0.13</td>
</tr>
</tbody>
</table>
Table 5.2. Macrinovertebrate abundances within the treatment cores. Data represent sample averages and 1 standard deviation (n=4). C = control sediment (no amendments). Treatments are sediment with addition of MPB = microphytobenthos, SL = sea lettuce, SG = seagrass leaves, and ML = mangrove leaves. Cockle wet weight is the wet weight of <em>Austrovenus stuchburyi</em> (shell removed) and wedge shell wet weight is the wet weight of <em>Macomona liliana</em> (shell removed).

<table>
<thead>
<tr>
<th></th>
<th>&lt;em&gt;Austrovenus stuchburyi&lt;/em&gt;</th>
<th>&lt;em&gt;Macomona liliana&lt;/em&gt;</th>
<th>&lt;em&gt;Nuclaria hartvigiana&lt;/em&gt;</th>
<th>Neridae</th>
<th>Spionidae</th>
<th>Orbinidae</th>
<th>Oligochaeta</th>
<th>Cockle Wet weight (g)</th>
<th>Wedge shell wet weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1 ± 1</td>
<td>0 ± 1</td>
<td>2 ± 2</td>
<td>8 ± 4</td>
<td>2 ± 2</td>
<td>1 ± 2</td>
<td>4 ± 1</td>
<td>1 ± 1</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>MPB</td>
<td>3 ± 1</td>
<td>0 ± 1</td>
<td>8 ± 4</td>
<td>12 ± 2</td>
<td>2 ± 2</td>
<td>0 ± 1</td>
<td>3 ± 1</td>
<td>4 ± 2</td>
<td>1 ± 2</td>
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<tr>
<td>SL</td>
<td>3 ± 3</td>
<td>1 ± 1</td>
<td>4 ± 3</td>
<td>12 ± 7</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td>5 ± 5</td>
<td>3 ± 2</td>
<td>2 ± 3</td>
</tr>
<tr>
<td>SG</td>
<td>4 ± 1</td>
<td>1 ± 1</td>
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<td>0 ± 1</td>
<td>9 ± 8</td>
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<td>3 ± 3</td>
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<tr>
<td>ML</td>
<td>5 ± 5</td>
<td>1 ± 1</td>
<td>5 ± 2</td>
<td>12 ± 3</td>
<td>10 ± 11</td>
<td>1 ± 1</td>
<td>13 ± 8</td>
<td>2 ± 2</td>
<td>1 ± 2</td>
</tr>
</tbody>
</table>
5.5.3 Nitrogen and oxygen gas transformations

Oxygen was consumed from the water column in all treatments (Fig. 5.2C). Control, MPB and SL treatments consumed oxygen at similar average rates (1053 - 1167 µmol O₂ m⁻² h⁻¹). Oxygen consumption appeared greater in the ML and SG treatments, but ML fluxes were highly variable across replicate tanks. There were no significant differences in the oxygen fluxes between treatment types (ANOVA, $F = \frac{(4,14)}{1.537} = 1.537$, $p>0.05$).

Nitrogen gas flux rates were variable across treatment types, and I report a switch between net nitrogen consumption and production occurring both in different treatments types and within the same treatment (Fig. 5.2D). Control sediments consumed N₂, with an average rate of -676 ± s.e. 139 µmol N₂ m⁻² h⁻¹. The MPB treatment was higher than the control -398 ± s.e. 272 µmol N₂ m⁻² h⁻¹. In the SL treatment, the nitrogen flux was close to balanced, with average fluxes consuming nitrogen -86 ± s.e. 72 µmol N₂ m⁻² h⁻¹. In the SG treatment, negative N₂ fluxes were higher than the control -927 ± s.e. 638 µmol N₂ m⁻² h⁻¹. The greatest change was seen in the ML treatment, which exhibited a switch from net N₂ consumption to net N₂ production (355 ± s.e. 342 µmol N₂ m⁻² h⁻¹). There were no significant differences in the nitrogen gas fluxes between treatment types (ANOVA, $F = \frac{(4,14)}{2.621} = 2.621$, $p>0.05$).
Figure 5.2. Average ammonium flux (NH$_4^+$ µmol m$^{-2}$ h$^{-1}$) (A), nitrate flux (NO$_3^-$ µmol m$^{-2}$ h$^{-1}$) (B), oxygen gas flux (O$_2$ µmol m$^{-2}$ h$^{-1}$) (C) and nitrogen gas flux (N$_2$ µmol m$^{-2}$ h$^{-1}$) (D) measured within the experimental cores for the organic detritus treatments. Error bars show standard error (n=4). C = control (no amendments), MPB = microphytobenthos, SL = sea lettuce, SG = seagrass leaves, and ML = mangrove leaves. Tukey posthoc test results (a, ab, b) are shown where significant variation was observed between groups.
5.5.4 Bacterial extracellular enzyme activity

There were no significant differences among treatments in both the phosphatase (APase) (ANOVA, $F = (4,14) = 0.578$, $p>0.05$) and β-glucosidase (BGase) activity (ANOVA, $F = (4,14) = 0.079$, $p>0.05$) (Fig. 5.3). Activity of phosphatase ranged from 416 – 477 nmol h$^{-1}$ g$^{-1}$ DW and β-glucosidase from 421 – 508 nmol h$^{-1}$ g$^{-1}$ DW. There were significant treatment differences in nitrogen and sulfur cycling extracellular enzymes. LAPase activity was significantly different between treatments (ANOVA, $F = (4,11) = 9.470$, $p<0.01$) (Fig. 5.3). LAPase activity was significantly different from control sediments in the MPB, ML and SG treatments (Tukey t-test, $p<0.05$, $p<0.01$, $p<0.001$ respectively). Activity of LAPase ranged from 0 to 463 nmol h$^{-1}$ g$^{-1}$ DW. SULFatase activity was significantly different among treatments (ANOVA, $F = (4,14) = 5.129$, $p<0.01$) (Fig. 5.3). SULFatase activity was reduced in the ML and SG treatment compared to the control sediments (Tukey t-test, $p<0.05$, $p<0.01$ respectively). Activity of SULFatase ranged from 357 – 1972 nmol h$^{-1}$ g$^{-1}$ DW.

Linear regression modelling showed LAPase activity was best explained by the wet cockle weight ($p<0.01$), organic detritus C:N ratio ($p<0.05$) and the Spionidae abundance ($p>0.05$) (model $R^2 = 0.67$, $p<0.001$). Although the Spionidae abundance was not significantly correlated, it enhanced the overall power of the model. Linear regression modelling showed SULFatase activity was driven by the organic detritus C:N ratio ($p<0.01$) and the pheophytin degradation ($p<0.05$) (model $R^2 = 0.57$, $p<0.01$).
Figure 5.3. Potential extracellular enzyme activity (nmol h\(^{-1}\) g\(^{-1}\) DW) for APase, BGase and LAPase (alkaline phosphatase, β-glucosidase and leucine aminopeptidase) and SULFatase for each of the organic detritus treatment types. Error bars show average of four replicate cores and standard error. C = control (no amendments), MPB = microphytobenthos, SL = sea lettuce, SG = seagrass leaves, and ML = mangrove leaves. Tukey posthoc test results (a, ab, b) are shown where significant variation was observed between groups.
5.5.5 Drivers of change in nitrogen flux

As nitrogen processing is strongly related to oxygen gradients in sediment, linear regression was used to investigate the relationship between N\(_2\) flux and O\(_2\) flux (Fig. 5.4). The six subsamples taken from each of the four replicate cores was used to increase the number of samples from each treatment to 24, as over the 6-hour sampling changes in oxygen consumption affected the nitrogen flux. The x-intercept indicates a shift from N\(_2\) consumption to production. The slope indicates the responsiveness of the system to subtle changes in oxygen conditions. A benthic oxygen consumption threshold occurred in the ML, SG and SL treatments, above which nitrogen fixation was dominant and below which denitrification was the dominant nitrogen process (Fig. 5.4). When treatments switched to net N\(_2\) production, benthic oxygen consumption was directly related to N\(_2\) production (Fig. 5.4). The greatest response (slope) occurred in the ML treatment (-0.27x), followed by SG (-0.22x) and SL (-0.22x).

The availability of the amended organic detritus is first dependant on the breakdown/degradation of complex organic molecules by extracellular enzymes, to release inorganic nutrients available for assimilation. LAPase and SULFatase enzymes are responsible for nitrogen and sulfur acquisition respectively, and these enzymes were both reduced in the ML and SG treatments, and in the MPB treatment for LAPase. These enzymes both showed strong negative correlations with *Austrovenus stuchburyi* wet weight and the organic detritus C:N ratios (Fig. 5.5). LAPase and SULFatase were also highly correlated with each other (Fig. 5.6), suggesting linkages between the nitrogen and sulfur cycles.

The planar optode images show the dramatic influence of bivalve bioturbation on the surrounding sedimentary matrix (Fig. 5.7). The left panel shows three images from a time lapse over 1 minute, where a cockle is bulldozing through the upper sedimentary layer, completely restructuring the upper sediment surface. In contrast, the right panel shows the siphon tube of a wedge shell penetrating up from deep in the sediments to the sediment surface, drawing down oxygenated water into the anoxic sediments, greatly enhancing the effective oxic/anoxic sediment boundary.
**Figure 5.4.** Linear relationship between oxygen consumption and nitrogen flux across the three treatment types (sea lettuce, mangrove leaves, seagrass) which showed positive nitrogen fluxes (denitrification). Grey highlighted area shows the observed oxygen consumption threshold where dominant nitrogen cycling switched from net nitrogen fixation to net denitrification. SL = sea lettuce, SG = seagrass leaves, and ML = mangrove leaves.
Figure 5.5. Two strongest univariate correlations of the extracellular enzymes SULFatase and LAPase. SULFatase against *Austrovenus stuchburyi* wet weight (g) (A), LAPase against *Austrovenus stuchburyi* wet weight (g) (B), SULFatase against organic detritus C:N ratio (C), and LAPase against organic detritus C:N ratio (D).

Figure 5.6. Linear regression of LAPase activity against SULFatase activity. The regression is significant at p<0.001.
Figure 5.7. False colour planar optode images showing the difference in bioturbation by *Austrovenus stuchburyi* and *Macomona liliana*. Left panel shows time series of a cockle bulldozing through the sediment. The white arrow shows the direction of movement of the cockle, where the sediment (light red) is pushed and flattened. Right panel shows non-disturbed sediment and oxygen gradients on day 1 (a), while the right image (b) shows a siphon hole of a wedge shell (*Macomona liliana*) drawing down oxygenated water (green) into the deoxygenated sediment (dark blue) on day 2. Scale bars are shown in the bottom right corner.
5.6 Discussion

Our study aimed to examine how different types of deposited organic detritus would influence the nitrogen cycling and extracellular enzyme activity of estuarine sediments. The C:N ratio has previously been reported to correlate with increased N$_2$ removal in field surveys (Eyre et al., 2013, Burkhardt et al., 2014, Belley et al., 2016), and can be an indicator of the quality of organic material (Erler et al., 2017). The activity of extracellular enzymes may be the limiting step in degrading more complex organic detritus (Hiroki et al., 2007, Riekenberg et al., 2017), regulating the availability of nutrients. The control estuary sediments were net NH$_4^+$ producing, however the addition of mangrove leaves and seagrass both significantly reduced sediment NH$_4^+$ production. Control sediments were net N$_2$ consuming (dominance of nitrogen fixation), and mangrove leaves invoked the greatest shift towards net N$_2$ production, however there was no significant variance in N$_2$ fluxes between treatments. LAPase activity (nitrogen cleaving enzyme) was significantly reduced in the microphytobenthos, mangrove leave and seagrass treatments, whilst SULFatase activity (sulfur cleaving enzyme) was significantly reduced in the mangrove and seagrass treatments. Nitrogen and sulfur enzyme activity were positively correlated, and the activity of both were reduced with increasing C:N ratios and cockle wet weight. The bioturbation of cockles and wedge shells were captured using a planar optode film, visualising the increased oxygenation in the sediment surrounding their burrowing activities.

5.6.1 Microbial extracellular enzyme activity

The activity of key microbial extracellular enzymes involved in organic matter hydrolysis and remineralization were compared among the organic detritus treatments as this can be rate-limiting for biogeochemical processes. More labile material is more accessible to the microbial community (Erler et al., 2017), due to bioavailability decreasing with increased complexity of organic molecules (Riekenberg et al., 2017). The presence of refractory material (greater C:N ratio) would be advantageous to sulfate reducers that fix their own nitrogen and may also suppress nitrification, both of which would decrease N$_2$ effluxes from the sediment (Fulweiler et al., 2013). The availability of the more complex molecules is thus expected to be limited by the activity of extracellular enzymes which break the complex molecules into smaller pieces available for microbial assimilation (Hiroki et al., 2007, Riekenberg et al., 2017).
β-glucosidase enzymes (BGase) are important in providing carbon through breaking the linkages between carbohydrate molecules and another group to release sugars (Dunn et al., 2014), and were expected to be a rate-limiting step in the availability of carbon to denitrifying organisms. Our study shows no significant variation among treatments in β-glucosidase activity. Alkaline phosphatase activity (APase, responsible for processing phosphorus-containing organic matter) was also not significantly different among treatments, however there is slightly increased activity in the ML and SG treatments. A study of how sources of dissolved organic matter directly influence tropical nearshore seawater extracellular enzymatic activities reports the addition of mangrove material resulted in rapid responses in the APase and BGase enzymes, and greater heterotrophic growth rates (Baltar et al., 2017). This increase in seawater APase is consistent with the phosphorus limitation, typically found in tropical seawater (Diaz et al., 2001). There are limited studies on extracellular enzyme activity in sediments, however our measured activity rates are higher than has been reported for other aquatic sediment studies. APase and BGase activity in Gulf of Mexico sediments ranged between 34 – 86 nmol g DW h\(^{-1}\) and 24 – 47 nmol g DW h\(^{-1}\) respectively (Hill et al., 2014), compared to our rates between 460 – 477 nmol g DW h\(^{-1}\) and 421 – 508 nmol g DW h\(^{-1}\). Sediments from a small stream in the USA also reported lower enzyme activity in APase and BGase (30 – 140 nmol g DW h\(^{-1}\) and 3 – 30 nmol g DW h\(^{-1}\) respectively) (Jackson et al., 2013). As I see no significant changes in APase or BGase enzyme activity among treatments, the sediments in the upper Whangateau Harbour may not be carbon or phosphorus limited (or non-responsive to our treatment types), due to close proximity to a large mangrove forest.

LAPase activity was reduced in the MPB, SG and ML treatments suggesting that nitrogen requirements were met with the addition of these substrates. Linear regression modelling suggested that the combination of C:N ratio, cockle wet weight and Spionidae abundance accounted for the most variation in enzyme activity. The nutrients supplied by the organic detritus and excreted by the cockles or the polychaetes may all have regulated the LAPase activity. Sulfatase enzyme activity was severely reduced in the ML and SG treatments, which suggests either these substrates provided sufficient sulfur compounds, or that there was a reduction in substrates available to cleave sulfur from. Lowered sulfatase activity may reflect low oxygen conditions in the sediments, as oxygen reduction affects the availability of organic sulphur compounds (such as transformation into inorganic S
compounds), thus reducing availability to sulfur using microbes. Some denitrifiers can utilize reduced sulfur compounds as an electron donor, respiring nitrate, and these have been shown to be present in estuarine sediments previously (Fulweiler et al., 2013). SULFatase activity in this study, which ranged from 357 – 1979 nmol h\(^{-1}\) g\(^{-1}\) DW, is higher than reported from a saltmarsh which ranged from 0 – 16 nmol h\(^{-1}\) g\(^{-1}\) DW (Duarte et al., 2008). Greater C:N ratios (more refractory) and greater phaeopigment concentrations resulted in lower SULFatase enzyme activity compared to control sediments, thus the more inaccessible carbon (refractory or degraded) reduced the activity of SULFatase enzymes. In my study the activity of LAPase and SULFatase were closely linked (Fig. 5.6), and the addition of certain organic detritus (ML and SG) resulted in decreased enzyme activity. LAPase and SULFatase also both showed decreased activity with increasing organic detritus C:N ratio and cockle wet weight.

5.6.2 Organic detritus quality and N cycling

Organic detritus lability was expected to influence N\(_2\) fluxes, however I could not report any significant differences between treatments in the N\(_2\) fluxes due to the high variability between treatments. In a stream ecosystem, the addition of tree detritus (red oak leaves (C:N = 49), red maple leaves (C:N = 69)) shifted the sediments from net N\(_2\) consumption to net N\(_2\) production (Stelzer et al., 2014). They attributed the change in nitrogen fluxes to direct and indirect effects of the particulate organic carbon on the microbial community, and changes in redox potential (Stelzer et al., 2014) to conditions favourable for denitrification (Tiedje, 1988). The type/mixture of sediment organic material (indicated by the δ\(^{13}\)C value) can change the slope of N\(_2\) flux vs benthic respiration in a (more so than the C:N ratio), indicating is the type or mixture of organic material undergoing remineralization that is important to enhancing net N\(_2\) flux (Eyre et al., 2013). The greatest slopes were reported in sediments with a mixture of mangrove and algal material (Eyre et al., 2013). Research from bioreactors has shown the C:N value to not be a good predictor of denitrification rates (Her & Huang, 1995, Schmidt & Clark, 2013). Instead other metrics of carbon bioavailability (% neutral detergent fibre, hemicellulose) and media surface area were predictors of denitrification rates (Schmidt & Clark, 2013), or the source of carbon used (methanol, glucose etc) determined the most efficient C:N ratio for denitrification (Her & Huang, 1995).
Sediments within the Whangateau estuary were nitrogen fixing under control conditions when the experiment was conducted (Fig. 5.2D). The occurrence of nitrogen fixation across estuarine ecosystems has recently begun to be acknowledged (Gardner et al., 2006, Eyre et al., 2011), in part due to advances in the techniques available for measuring nitrogen fixation (Fulweiler et al., 2013, Newell et al., 2016). Nitrogen fixation rates can be greater than denitrification rates in certain conditions (Newell et al., 2016), and have been reported to be higher in eutrophic estuarine sites stimulated by organic matter enrichment, due to increased efficiency of denitrification limiting nitrate availability elsewhere in the estuary (Rao & Charette, 2011). Our results indicate that under low carbon and low nitrogen concentrations the sediments are net nitrogen fixing, however once carbon has been made available and oxygen conditions decrease, the nitrogen gas balance moves further towards net nitrogen removal through denitrification. Nitrogen fixation has been reported to occur in nitrate concentrations as high as 30 µM and/or ammonium concentrations up to 200 µM, suggesting they are not important factors in suppressing nitrogen fixation (Knapp, 2012). The average nitrate concentrations in the experimental chamber outflows remained around 3 µmol L⁻¹, which suggests most of the measured nitrogen effluxes were driven by coupled nitrification-denitrification in the sediment (Eyre et al., 2013). The MIMS technique is limited by only being able to distinguish between net consumption or net production of nitrogen gas. As such I cannot directly attribute how much denitrification or nitrogen fixation is occurring, only that one is dominating the other and most likely both are co-occurring at any given time.

Coastal sediments have been reported to switch between net nitrogen gas production through denitrification and net consumption due to an increase in nitrogen fixation (Gardner et al., 2006, Fulweiler et al., 2007). An experimental approach adding organic matter in the form of freeze-dried phytoplankton found the organic material switched the dominant process from nitrogen fixation to denitrification (Fulweiler et al., 2007), which is similar to our results. Microphytobenthos additions were expected to support the greatest rates of denitrification due to the low lability, however the sediments remained net nitrogen fixing. I suggest this is due to minimal changes in oxygen concentrations and the sediments remaining well oxygenated. As our study occurred in dark conditions, the effects of changing oxygen driven by live microphytobenthos over a diurnal cycle could not be examined. Microphytobenthos may support nitrification, by enhancing the oxygen concentrations in the sediment during the day and reducing oxygen.
concentrations at night which would enhance direct denitrification. A recent study in the Whangateau harbour indicated that the microphytobenthos community play a large role in sustaining the food web (e.g. cockle populations) (Jones et al., 2017). They may play a large role in direct nitrogen assimilation from the water column, positively influencing the eutrophication status of the estuary.

Remineralization of organic matter can be a significant source of ammonium that supports nitrification-denitrification in mangrove sediments (Fernandes et al., 2016). As a result, nitrification is also coupled to denitrification in these systems since NH₄⁺ and O₂ are abundant (Fernandes et al., 2012, Gongol & Savage, 2016). Across estuarine habitats, mangrove N₂ flux was the most responsive to subtle changes in benthic oxygen consumption, while seagrass and algae beds were the least responsive (Eyre et al., 2013). Our findings support this research and suggest mangrove forests could be hotspots for nitrogen removal due to a continuous supply of organic material from the mangrove leaves, and reduced oxygen conditions favourable for denitrification. Although, this may only occur once the refractory waxy cuticle has broken down, such as in our experimental treatments. This highlights the importance of mangroves in protecting estuaries from increasing silt content and nutrient loads by trapping sediments and promoting denitrification hotspots (Fernandes et al., 2016). A study investigating the decomposition of different parts of New Zealand mangroves found that the half-life decomposition of leaves was between 63-88 days, which is much slower than tropical mangrove forests (Gladstone-Gallagher et al., 2014). Although mangroves are native to New Zealand, their natural increase and association with fine sediments has resulted in large areas of deliberate mangrove removal (Lundquist et al., 2014). After such removals, blooms of green macroalgae such as Ulva sp. were common and are suggested to be associated with nutrient release from decomposing mangrove biomass left behind (Lundquist et al., 2014). As Ulva sp. was not a highly palatable source of detritus in our experiment (likely due to the high C:N ratio at the time of sampling), our research indicates this shift of dominant plant biomass could have detrimental effects on the nitrogen removal capacity of the estuary. The Ulva sp. utilized in our study had a high C:N ratio (30), and seasonally the Ulva sp. C:N ratio is reported to range between 5 - 40 in the Tauranga Harbour (Park, 2011). Lower ratios which are commonly exhibited during winter-spring months may result in the Ulva sp. being utilized by bacteria to a greater rate than what is reported in this study.
In our study, oxygen consumption rates were greater and more variable in the ML and SG treatments, possibly enhancing conditions for denitrification. I observed an oxygen consumption threshold where net N$_2$ consumption switched to net N$_2$ production when organic detritus was present, between -500 and -1500 µmol N$_2$ m$^{-2}$ h$^{-1}$. The slope of the line was dependent on the organic detritus source, and ML drove the steepest slope and greatest N$_2$ production. However, this threshold was not observed in MPB or C treatments. If oxygen consumption had continued to increase in the microphytobenthos treatment, it may have shown a similar pattern to the other treatments, but with a lower slope. Other competitive processes for nitrate/ammonium that were not measured in this study include dissimilatory nitrate reduction to ammonium (DNRA) (Giblin et al., 2013) and anaerobic ammonium oxidation (anammox) (Devol, 2015). DNRA can be the favoured process when there is high organic matter availability and low nitrate concentrations (i.e. electron acceptor limitation) (Hardison et al., 2015). DNRA bacteria reach their growth requirements at lower nitrate concentrations compared to denitrifiers (Erler et al., 2017), due to greater energy production obtained through nitrate ammonification than denitrification under high carbon to electron acceptor conditions (Tiedje, 1988). If sediments are highly anoxic and sulfidic, DNRA may be enhanced relative to denitrification due to DNRA bacteria being able to oxidise reduced forms of sulphur (Murphy et al., 2016). DNRA is a less favourable pathway of nitrogen removal for ecosystem health, as it shifts the nitrate to ammonium which remains biologically active in the system. Anammox is suggested to be important in ecosystems with large nitrogen inputs relative to the labile carbon supply (Burgin & Hamilton, 2007). Our estuary has available carbon in the sediments, and low inputs of nitrogen. I therefore suggest anammox may not be a dominant process in estuarine ecosystems and denitrification is likely to be the dominant N$_2$ production pathway (Dalsgaard et al., 2005, Burgin & Hamilton, 2007).

5.6.3 The influence of bivalves on denitrification
LAPase and SULFase activity was reduced when cockle biomass (wet weight) was increased. Bivalves such as Macomona liliana and Austrovenus stuchburyi may play a role subsidising key nutrients (Woodin et al., 2016) that could be limiting in the presence of refractory organic detritus. The bivalves consume food particles releasing ammonia and depositing nutrient rich bio-deposits (Newell, 2004). If these bio-deposits are in the oxic sediment layer (e.g. Austrovenus stuchburyi), coupled nitrification-denitrification
can then occur (Newell, 2004, Rose et al., 2014, Turek & Hoellein, 2015), providing both a carbon and nitrogen subsidy to the denitrifiers which cannot utilize the refractory carbon quickly. This relationship is however density dependant, as high deposition of organic material by bivalves can result in anoxia and sulfide production (as reported in aquaculture farms) (Newell, 2004, Murphy et al., 2016), which inhibits coupled nitrification-denitrification. A tank experiment on clam density and denitrification rates found that the optimum nitrogen removal occurred at clam densities of 1.18 kg m\(^{-2}\) (shell on wet weight) (Shen et al., 2016), which is close to the densities (0.93 kg m\(^{-2}\)) in the seagrass addition tanks which switched from net N\(_2\) consumption to net N\(_2\) production. Further, Jones et al. (2011) found the clam Austrovenus stuchburyi enhanced sediment denitrification (denitrification enzyme activity) in sandy sediments during summer, although the relationship was not seen in muddy-sandy sediments, suggesting that the influence of bivalves on nitrogen cycling is context dependent and varies with sediment grain size. Oysters have been shown to change sediments from net nitrogen fixing to net nitrogen removal through denitrification. They change oxygen conditions and carbon availability, increasing nitrification rates and alleviating nitrogen limitation (Smyth et al., 2013). Clam density can linearly enhance denitrification rates, and the clams themselves were found to be sites of high nitrification and denitrification rates, as well as mobilising NO\(_3^-\) for denitrifiers and O\(_2\) for nitrifiers (Welsh et al., 2015). Our planar optode images show the dramatic changes caused by bivalves on the sedimentary layers (Fig. 5.7). Macomona liliana greatly enhances the area of the oxic/anoxic sediment interface into deeper layers, which likely draws down organic detritus enhancing substrate supply to denitrifiers (Turek & Hoellein, 2015). Austrovenus stuchburyi however bulldozes the upper sedimentary layers, reworking and oxygenating the sediments.

5.6.4 Implications of shifting detrital sources

As estuaries become increasingly eutrophic, the dominant primary producers shift from stable perennial macrophytes (seagrass) and emergent plants (mangroves) towards bloom forming species such as sea lettuce (Ulva spp.) or dominance of microphytobenthos/phytoplankton species (Cloern, 2001, Borum, 2013). The biogeochemistry of the sediments responds to the change in dominant primary producers. Our results indicate that under low carbon, low nitrogen conditions sediments were net nitrogen fixing. When organic detritus representing more eutrophic primary production
was introduced to the sediment (e.g. *Ulva*), ammonium continues to efflux out of the sediment, and net nitrogen gas consumption still occurs (a dominance of nitrogen fixation over denitrification). When mangrove leaves were amended, measured net ammonium consumption into the sediments and effluxes of nitrogen gas from the sediments (net denitrification). SULFatase and LAPase enzyme activity were reduced in these treatments indicating a reduction in oxygen conditions, an increase in nitrogen supply and a decrease in nitrogen fixation. Our results indicate that seagrass and mangrove ecosystems could provide a key habitat where nitrogen is removed from the ecosystem, due to sedimentary ammonium consumption and denitrification. Consequently, a shift from perennial primary producers to ephemeral bloom-forming algae associated with increasing eutrophication in temperate estuaries may limit nitrogen removal and move the ecosystem into a more eutrophic state through positive feedbacks.
5.7 Conclusions

The study found the lability of organic matter played an indirect role in regulating the switch between net nitrogen fixation and denitrification in temperate estuarine sediments. Unamended sediments were nitrogen fixing and organic detritus amendments decreased net N₂ consumption towards net N₂ production. Oxygen consumption regulated net N₂ fluxes, and a threshold occurred where sediments switched from net N₂ consumption to net N₂ production. Bivalves (*Austrovenus stuchburyi* and *Macomona liliana*) played a key role in regulating sediment oxygenation and possibly substrate supply to denitrifying organisms and decreased sediment LAPase and sulfatase extracellular enzyme activity. The different bioturbation roles of bivalves were captured by planar optode imaging, with *Austrovenus stuchburyi* bulldozing upper sedimentary layers and *Macomona liliana* drawing down oxygen into deeper sediment layers enhancing the oxic/anoxic sediment interface. The activity of extracellular enzymes phosphatase and β-glucosidase were not significantly different among organic detritus additions, suggesting sediments were not limited by carbon and phosphorus. The addition of refractory organic matter changed the activity of key extracellular enzymes which may have consequences for sediment nutrient availability under shifting organic detrital sources.
CHAPTER 6

Conclusions and future research directions

Photo: Sunrise over Te Waihora/Lake Ellesmere Winter 2015
6.1 Study rationale

Eutrophication is a major issue facing coastal ecosystems. Denitrification is a key pathway potentially reducing the eutrophication potential of nitrogen in ecosystems (Seitzinger et al., 2006). Denitrification is carried out by denitrifying micro-organisms, at the oxic/anoxic interface of sediments (Herbert, 1999). Research into denitrification has increased in the past 10-20 years with the development of robust measurement techniques, along with increasing understanding of factors which can influence denitrification rates. Temperature, oxygen, nitrate, carbon, salinity, sediment grain size, bioturbation, macrophytes and microbial communities are all key factors which can change denitrification rates (Piña-Ochoa & Álvarez-Cobelas, 2006, Seitzinger et al., 2006). These factors are extremely variable in estuarine environments, and research is still limited on spatial and seasonal variability of denitrification. Knowledge of denitrification in New Zealand coastal environments is limited to a few studies (Gongol & Savage, 2016), thus this thesis aimed to elucidate some of the key drivers of denitrification, with a strong focus on Intermittently Closed and Open Lake Lagoons (ICOLLs).

6.2 Contribution to knowledge

In New Zealand the process of denitrification is an understudied and underappreciated process. Denitrification is a key nitrogen removal process; as such, it provides an ecosystem service which helps maintain clear water aquatic ecosystems. Some of the most eutrophic coastal ecosystems in New Zealand are Intermittently Closed and Open Lake/Lagoons (Hamill et al., 2014). They provided a model ecosystem for this study, to investigate the spatial and seasonal variability of denitrification. The utilization of two ICOLLs showing different eutrophication symptoms (high algal biomass and low light penetration), allowed us to investigate how the denitrification process may change if more coastal ecosystems become eutrophic, similar to what these ICOLLs are currently experiencing.
6.2.1 Chapter 2: Spatial variability of potential denitrification rates in ICOLLs

Little is known about the spatial variability of denitrification in ICOLLs, and this chapter aimed to identify hotspots of denitrification potential across two ICOLLs and investigate the role of nitrate inputs from main tributaries in shaping the sediment denitrification activity. Two contrasting eutrophic ICOLL ecosystems were chosen, Lake Ellesmere and Tomahawk Lagoon; Lake Ellesmere had a high phytoplankton biomass and inorganic turbidity with virtually no macrophytes present, while Tomahawk Lagoon had clearer water supporting the growth of submerged macrophytes and macroalgae. Both ICOLLs received and exhibited large pulses of nitrate and, thus, have high denitrification rates because of their highly eutrophic states, long residence times and shallow depths (Hamill & Schallenberg, 2013). Denitrification enzyme activity was used to test for potential sediment denitrification nutrient limitation (nitrogen and carbon) to characterise spatial variability in denitrification potential.

The ICOLLs are generally low in background nitrate concentrations, but large episodic pulses of nitrate (2-4 mg L\(^{-1}\)) occurred during winter and spring, which could fuel phytoplankton growth (Olsen et al., 2017). However, my samplings did not coincide large nitrate pulses, and only weak nitrogen gradients in the ICOLLs were evident, close to the river inflows. Strong sedimentary gradients in organic matter and grain size occurred among sites in both ICOLLs, creating hotspots of denitrification enzyme activity. High spatial variability in denitrification enzyme activity (DEA) was not correlated with proximity to main tributaries, instead being centred on sedimentary gradients, which provide a more stable environment and nutrient source. Sediment organic matter availability was highly correlated with the sediment grain size (greater organic matter availability occurring in high porosity, silt/clay dominated sediments). Denitrification enzyme activity (DEA) was best explained by the clay content (%) of the sediments, which was chosen as a combined sediment grain size predictor due to the high co-variance of predictors with grain size. Therefore, the denitrification potential was greater in sediments with greater organic matter availability and higher clay content, where oxygen diffusion is shallow (which supported coupled nitrification-denitrification) (Fig. 6.1). It appears substrate diffusion (nitrate, carbon) may be a limiting factor in sediments where clay content reaches greater than 7%, even though these sediments hold greater concentrations of organic matter. Alternative nitrogen cycling pathways such as
dissimilatory nitrate reduction to ammonia may dominate in these sediments (Burgin & Hamilton, 2007, McGlathery et al., 2007). Sediments appear to be poised to remove nitrate when it becomes available, however the rate of nitrate conversion appears to be driven by the sedimentary properties and availability of organic carbon.

Figure 6.1. Conceptual figure describing the relationships between the measured sediment variables. The two dominant sediment types within the investigated ICOLLs – Left: a sandy sediment, composed of large particles, with low porosity (low water content) and low organic content, and right: a silt and clay dominated sediment, composed of smaller particles, with high porosity (high water content) and high organic content, bound tightly in a gelatinous, polysaccharide matrix. Greater denitrification enzyme activity was recorded with increasing silt/clay content.
6.2.2 Chapter 3: A hierarchy of factors controlling denitrification rates in intermittently closed and open lake/lagoons (ICOLLs)

Given the large pulses of nitrate which periodically occur in the ICOLLs, I aimed to determine whether substantial proportions of the nitrate pulses could be converted to \( \text{N}_2 \) gas via denitrification. I aimed to create a predictive model to assess denitrification within these and similar ICOLLs. I used the isotope pairing technique to trace the movement of labelled nitrate (\(^{15}\text{KNO}_3\)), using custom built \textit{in situ} chambers which transferred turbulence due to wave motion into the chambers, allowing water column mixing to occur.

I found that a hierarchy of factors controlled \textit{in situ} denitrification. Denitrification was inhibited below a temperature threshold which occurs somewhere between 8.6 - 12°C. Thus, in winter and early spring, when large concentrations of bioavailable nitrate often occur in the ICOLLs overloading the ecosystem, denitrification is unable to remove it, leaving inorganic nitrogen available for assimilation by phytoplankton and macroalgae. When temperatures were above the threshold, the availability of organic matter controlled denitrification rates. Denitrification rates were greater in the shallow photic regions of the ICOLLs, where labile microphytobenthos may be an important labile carbon source (Hardison \textit{et al.}, 2013) or regulator of oxygen availability supporting nitrification. In addition to this, the macroinvertebrates (predominantly chironomids and polychaetes) increased sediment oxygenation and nutrient availability (Fig. 6.2). Thus, a hierarchy of physico-chemical and biological factors (temperature \( \rightarrow \) organic matter \( \rightarrow \) water depth \( \rightarrow \) bioturbation) controlled the spatio-temporal dynamics of denitrification in the two study ICOLLs.
Figure 6.2. A schematic which illustrates the variability in nitrogen cycling during warm seasons (above 12°C; top diagram) and nitrogen cycling in winter (temperatures below 8.6°C; bottom diagram). Green squiggles are sediment organic matter. Yellow horizontal patches are denitrifying micro-organisms. Black horizontal lines show increased sediment diffusion. In winter denitrification is inhibited by low temperatures and oxygen penetration depths; nitrate remains in the water column for biological assimilation and may be also increased by ammonification and nitrification in the large oxic layer. In the warmer seasons, denitrification is enhanced in shallow marginal zones of the ICOLLs, where organic matter availability and bioturbators are high, and nitrification is coupled to denitrification.
6.2.3 Chapter 4: Macroinvertebrates enhance in situ sediment oxygenation and denitrification in a small ICOLL: implications for management

This chapter aimed to assess the role macroinvertebrates play in supporting denitrification rates and sediment oxygenation across a seasonal temperature gradient. As with the previous chapter, I used the isotope pairing technique to trace the processing of labelled nitrate (\(^{15}\)KNO\(_3\)), using custom built in situ chambers which transfer turbulence from wave motion into the chambers to facilitate realistic water column mixing experienced in ICOLL ecosystems.

Benthic macroinvertebrates redistribute dissolved oxygen and increase nitrate transport to deeper sediment layers. Seasonally, the density of the amphipod *Paracalliope fluviatilis* and that of the snail *Potamopyrgus antipodarum* significantly enhanced oxygen penetration in the sediments (Fig. 6.3). In both of these species the mode of functional bioturbation can be described as bulldozers, enhancing mixing of the upper sediment layers promoting greater sediment oxygenation (Gerino *et al.*, 2003). Tube dwelling invertebrates such as chironomid larvae are bio-irrigators (Gerino *et al.*, 2003), and they were the taxon most strongly correlated with variation in denitrification rates across Tomahawk Lagoon. Oxygen micro-profiling showed the enhancement of oxygen in deep layers which would otherwise be anoxic (Fig. 6.3). The deeper extension of the oxic/anoxic interface clearly increases denitrification rates in ICOLL sediments. Given the key role these invertebrate communities play in maintaining sediment oxygenation and enhancing denitrification rates, their biology and environmental tolerances should be considered in management practices. The ICOLLS periodically experience large changes in salinity (range 3-25 ppt; Hamill & Schallenberg, 2013), and most of these species are mesohaline or freshwater species. Increased salinity levels resulting from managed openings (e.g. to facilitate drainage) may negatively impact the benthic community (Schallenberg *et al.*, 2003, Lill *et al.*, 2012), thereby reducing the amount of denitrification occurring. Denitrifying bacteria are also negatively impacted by higher salinities (Marks *et al.*, 2016), thus compounding the negative impacts of large saline events on ICOLL nutrient processing and cycling.
Figure 6.3. Schematic illustrating the role that bulldozing and bio-irrigating functional groups have on the oxic-anoxic sediment boundary and on substrate transfer to denitrifying micro-organisms. Yellow horizontal patches are denitrifying micro-organisms.

6.2.4 Chapter 5: Carbon lability influences nitrogen cycling rates in temperate estuary sediments

This chapter aimed to investigate how shifting sources of organic detritus expected with increasing eutrophication influences sediment nitrogen cycling. A custom-built flow-through chamber with a removable front plate was used, which allowed the attachment of a planar optode film to investigate changes in sediment oxygenation by macroinvertebrates. Membrane Inlet Mass Spectrometry (MIMS) was used to measure the net change in N₂ and O₂ gases in the experimental system.

As eutrophication worsens, the dominant primary producers shift from seagrasses and mangroves, to nuisance bloom forming algae and phytoplankton (Cloern, 2001, Borum, 2013). The lability of the organic detritus source played an indirect role in regulating the switch between net nitrogen removal through denitrification, or net nitrogen retention through nitrogen fixation. Control sediments were net nitrogen fixing, and the addition of organic detritus supported increased net N₂ production. An oxygen consumption threshold was observed, where sediments switch from net N fixation to net N production. Bivalves played a key role changing oxygen conditions, and substrate supplies to
denitrifying microorganisms. LAPase and SULFatase extracellular enzyme activity (nitrogen and sulfur cycling enzymes) were significantly reduced in the microphytobenthos, mangrove and seagrass treatments for LAPase, and mangrove and seagrass for SULFatase. The organic detritus C:N ratio and cockle biomass decreased the activity of both LAPase and SULFatase, suggesting either the microbial requirements for nitrogen and sulfur were met by the organic detritus additions or excretion by the cockles. Thus, as estuarine ecosystems become more eutrophic, the dominant organic sources turn from supporting nitrogen removal, to supporting its retention within the ecosystem, however the presence of bivalves may interact and provide nutrient subsidies (Fig. 6.4).

![Figure 6.4. Schematic illustrating the net nitrogen fluxes and sulphate enzyme activity under the various carbon detrital amendments.](image)

**6.3 Management implications**

My research is the first study in New Zealand on the drivers of denitrification in ICOLLs. I have shown the importance of the sedimentary environment in shaping denitrification potential. I elucidated a hierarchy of factors which control denitrification potential and denitrification rates. I also produced multiple regression models, which may be useful in predicting hotspots of denitrification in these and similar ecosystems. The crucial role of the macroinvertebrate/bivalve community in enhancing sediment oxygenation and
turnover is clear throughout my work, and I suggest their environmental tolerances should be factored into management of these ecosystems. I have experimentally tested how the quality/lability of various carbon sources can influence nitrogen cycling, and net N\textsubscript{2} production may be lower with the removal of seagrasses and mangroves due to eutrophication symptoms.

The results of my thesis raise a number of issues that could be important for managing eutrophication in ICOLLs. Certain sedimentary areas are poised to remove bioavailable nitrogen, but it is system dependant as described in detail below. Fine sediment appears to be a key driving variable enhancing denitrification across systems up to a threshold (~7%), due to increased organic matter availability and shallower oxic/anoxic sediment boundaries. Sediment input from land has been increasing in past decades due to deforestation and land conversion to pasture (Kettner et al., 2007), and this mostly consists of fine clays and silts (Thrush et al., 2004). Terrestrial sediments can smother intertidal organisms and change sediment biogeochemistry (Norkko et al., 2002, Cummings et al., 2003). Increased sedimentation of fine sediments will reduce the denitrification capacity of marginal sediments, due to imposing diffusional limitations on solute transport from the water column (Smith et al., 2003, Solomon et al., 2009). In ecosystems that have begun showing eutrophication symptoms (increased phytoplankton densities and reduced light penetration), denitrification rates will be reduced. Competitive interactions for nitrate with phytoplankton severely reduce the amount of nitrate able to be directly denitrified (Olsen et al., 2017). Nitrate uptake due to excess phytoplankton biomass reduces the effectiveness of denitrification in removing N from the system. Consumption of nitrate by phytoplankton results in retention of nitrogen in the system, and if there is a tight benthic-pelagic coupling, nitrogen is re-mineralized as a secondary nutrient source (ammonium, NH\textsubscript{4}+) establishing bottom-up controls (Kennish et al., 2014). The produced NH\textsubscript{4}+ can be immediately assimilated, creating a detrimental positive feedback loop which creates resilience for a phytoplankton dominated system (Glibert et al., 2010). Management especially needs to target ways to reduce nitrate runoff during the wet, cooler months when denitrification is reduced/suppressed by lower temperatures. Increased eutrophication also reduces the species richness of estuarine ecosystems (Cloern, 2001), which may have negative flow-on effects to the bioturbation enhancement of denitrification. Management of artificial ICOLL openings must consider the ambient changes in salinity and the negative impacts
it can have on macroinvertebrate survival. Sea level rise will also increase saline water inputs (Yuan et al., 2013, Liu & Liu, 2014), and this has the potential to reduce denitrification in the ecosystem, and this could perhaps be managed through consideration of the artificial opening regimes that many ICOLLs are subject to. The reduction of key stable plant species such as seagrasses and mangroves will be reduced under a more eutrophic system. I have shown that changing the dominant sources of organic detritus will alter nitrogen cycling rates. Stable rooted plants such as seagrass and mangroves may support the sediment consumption of NH$_4^+$ and removal of N$_2$ gas, as well as directly consuming bioavailable nitrate. Nitrogen and sulfur extracellular enzymes are reduced in these treatments, suggesting a downregulation of activity with the nutrient requirements of the microbes met. If ecosystems become more eutrophic, bloom forming macroalgal and microalgal species enhance nitrogen retention through increasing sedimentary nitrogen fixation and ammonium production. Ensuring the continuation of vascular plant species in estuaries will support the continuation of clear water and healthy estuarine function.

### 6.4 Promising future research directions

This research has opened a range of interesting questions that would be of use to continue our exploration of denitrification as a sink of nitrogen in coastal ecosystems. There were also additional research projects I was involved in however have not been used in this PhD thesis, which are discussed briefly below.

#### 6.4.1 Additional research projects

I have conducted an additional experiment which is not presented in this thesis, due to waiting on isotope results to be analysed. The results from Chapters 3 and 4 have suggested that phytoplankton consumption of the added nitrate pulse may be a large temporary sink of nitrogen. However, on senescence, the organic nitrogen may be re-mineralized and either released back into the water column or denitrified through coupled nitrification-denitrification (Ferrón et al., 2009, Kennish et al., 2014). Organic matter remineralization has been shown to be a large source of regenerated nitrogen, potentially supplying a large percentage of phytoplankton needs (York et al., 2010, Burkhardt et al., 2014). Thus, I aimed to determine what percentage of re-mineralized nitrogen from
phytoplankton cells was denitrified, and how much was released back into the water column.

In this experiment I used isotope labelled phytoplankton to simulate a bloom senescence event and traced the loss of organic nitrogen through denitrification. Within the laboratory mesocosms experiment I had four treatments to investigate both the role of invertebrate bioturbation, and the effect of sediment organic enrichment on denitrification.

A second project I have been involved in is developing a protocol for measuring extracellular enzyme activity (EEA) in estuarine sediments as part of a large nationwide project on estuary tipping points, funded by the National Science Challenge, Sustainable Seas. The group enriched large 3m² estuary sediment plots with slow release fertilizer to represent future nitrogen loading scenarios (current, low dose and high dose), and measured how the ecosystem functions change (nutrient fluxes, net N₂ fluxes, benthic communities, organic matter usage, and extracellular enzyme activity). Within this project I managed the development of a protocol for sampling and running the assays to measure EEA. We are currently running the samples and will produce a manuscript for this research by the end of the year.

6.4.2 Future research suggestions

Here I suggest some potentially fruitful ideas for future research.

- Does short term exposure to lowered oxygen conditions (0.5 mg L⁻¹) create “hot moments” of denitrification potential? Raw data suggested when oxygen conditions were severely depleted, rates of denitrification were greatly enhanced by the short-term exposure to low oxygen.

- What are the factors that mediate carbon availability in estuarine ecosystems, and predispose it to tipping points?

- How increased levels of sediment deposition influences denitrification – small amounts of sediments may provide carbon to fuel denitrification, however after a threshold it may smother the sediment. Where does this threshold lie?

- The role of various bioturbating species in denitrification – different functional types (e.g. bulldozer, conveyer) change the sediment oxygenation and nutrient transfer in various ways. As ecosystems become eutrophic, invertebrate richness
is decreased however certain functional groups may remain. Which of these functional traits – either alone or in combination support denitrification?

- The competitive interactions between denitrifiers and phytoplankton and macrophytes – which pathway dominates the consumption of nitrate under a range of eutrophication scenarios.
- Do the nutrients produced by bivalve excrement subsidise the nutrient requirements of sediment microorganisms, reducing extracellular enzyme activity requirements? Or are the bivalves/other large macrofauna hosts of bacteria which increase extracellular activity in the sediments?
- Can the sulfatase extracellular enzyme be used to indicate sediment oxygenation in estuarine sediments?
- How does \textit{in situ} denitrification vary across a eutrophication gradient of New Zealand ICOLLs?
- The role of DNRA in shallow coastal ecosystems in New Zealand, which our research suggests could be a process directly reducing the positive effects of denitrification, and promoting retention of nitrogen in the ecosystem.
- Low temperature thresholds of denitrification in coastal ecosystems.

6.5 Overall Conclusions

Prior to this study, little was known about denitrification rates in Intermittently Closed and Open Lake Lagoons (ICOLLs), which are a type of estuarine ecosystem. Spatial variability of denitrification enzyme activity (when nitrate and carbon are supplied) was strongly driven by the gradients in sediment characteristics, specifically the sediment grain size. The spatial distribution of these characteristics in ICOLLs resulted in hotspots of denitrification in sediments with greater clay content in the ICOLLs. Further experiments \textit{in situ} showed that denitrification was able to remove some of the large nitrate pulses that episodically enter the ICOLLs, however the effectiveness of this is limited by the water temperature. In cooler months where temperatures dropped below 8.6 - 12°C denitrification was severely inhibited resulting in a larger concentration of nitrate available for biological assimilation. Rates of \textit{in situ} denitrification were
controlled by a hierarchy of factors – temperature, organic matter availability, water depth, bioturbation. The macroinvertebrates inhabiting the ICOLL sediments increased sediment oxygenation across seasonal gradients. The functional bioturbation characteristic of each species plays different roles in sediment oxygenation – ranging from bulldozing the upper layers, to drawing oxygen deep within the anoxic zone of the sediments. The bioturbating functions of these specialised invertebrate communities is important to maintaining denitrification. Stable rooted plants such as seagrasses and mangroves are important organic detrital sources to support denitrification and reduce nitrogen fixation, providing a sink of biological nitrogen. This thesis identifies key organisms which support denitrification, such as benthic invertebrate communities and stable rooted plant communities. Minimising anthropogenic stressors such as sedimentation, managed ICOLL opening regimes, and nitrogen loading during cooler months will support positive ecosystem processes and the preservation of our unique estuarine ecosystems for future generations.
Appendix A

Table 7.1. Frequency of nitrate concentration pulses (mg L\(^{-1}\)) from measurements taken between 1993 to 2013 in Lake Ellesmere, showing the seasons they occurred in. Data is split into measurements taken at the two sites, mid-lake and at the mouth of the Selwyn River.

<table>
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<th>Season</th>
<th>Occurrences</th>
<th>Season</th>
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<td>All</td>
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<tr>
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<td>0</td>
<td>-</td>
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Table 7.2. Pearson’s correlation matrix showing pair-wise relationships among the denitrification potential, water column and sediment variables for Lake Ellesmere spatial sampling in 2014.

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<th>TP</th>
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<th>DRP</th>
<th>NH₃</th>
<th>Redox</th>
<th>Clay</th>
<th>Silt</th>
<th>Sand</th>
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<td>0.15</td>
<td>0.03</td>
<td>-0.63**</td>
<td>-0.06</td>
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<td>0.54*</td>
<td>0.44</td>
<td>0.35</td>
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<tr>
<td>NH₃</td>
<td>-0.01</td>
<td>0.14</td>
<td>-0.22</td>
<td>0.19</td>
<td>0.23</td>
<td>0.34</td>
<td>0.37</td>
<td>-0.07</td>
<td>0.22</td>
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<tr>
<td>Redox</td>
<td>-0.22</td>
<td>-0.25</td>
<td>-0.01</td>
<td>-0.36</td>
<td>0.08</td>
<td>0.09</td>
<td>0.05</td>
<td>0.13</td>
<td>0.19</td>
<td>-0.12</td>
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</tr>
<tr>
<td>Clay</td>
<td>-0.39</td>
<td>-0.52*</td>
<td>0.1</td>
<td>0.91***</td>
<td>0.33</td>
<td>0.01</td>
<td>0.13</td>
<td>-0.39</td>
<td>-0.21</td>
<td>0.1</td>
<td>-0.51*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silt</td>
<td>-0.32</td>
<td>-0.34</td>
<td>0.17</td>
<td>0.81***</td>
<td>0.4</td>
<td>-0.01</td>
<td>0.11</td>
<td>-0.42</td>
<td>-0.35</td>
<td>0.22</td>
<td>-0.55*</td>
<td>0.90***</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sand</td>
<td>0.33</td>
<td>0.36</td>
<td>-0.17</td>
<td>-0.83***</td>
<td>-0.4</td>
<td>0</td>
<td>-0.11</td>
<td>0.42</td>
<td>0.34</td>
<td>-0.21</td>
<td>0.55*</td>
<td>-0.92***</td>
<td>-1.00***</td>
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<td></td>
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</tr>
<tr>
<td>Porosity</td>
<td>-0.3</td>
<td>-0.41</td>
<td>0.19</td>
<td>0.78***</td>
<td>0.25</td>
<td>0.02</td>
<td>0.14</td>
<td>-0.31</td>
<td>-0.27</td>
<td>0.05</td>
<td>-0.47</td>
<td>0.94***</td>
<td>0.89***</td>
<td>-0.90***</td>
<td></td>
<td></td>
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<tr>
<td>OM</td>
<td>-0.29</td>
<td>-0.36</td>
<td>0.24</td>
<td>0.18</td>
<td>0.13</td>
<td>0.07</td>
<td>0.08</td>
<td>-0.22</td>
<td>-0.15</td>
<td>-0.2</td>
<td>0.18</td>
<td>0.4</td>
<td>0.35</td>
<td>-0.35</td>
<td>0.61**</td>
<td></td>
</tr>
<tr>
<td>OMA</td>
<td>-0.22</td>
<td>-0.31</td>
<td>0.32</td>
<td>0.07</td>
<td>0.07</td>
<td>-0.02</td>
<td>-0.01</td>
<td>-0.24</td>
<td>-0.22</td>
<td>-0.28</td>
<td>0.14</td>
<td>0.34</td>
<td>0.33</td>
<td>-0.33</td>
<td>0.56*</td>
<td>0.97***</td>
</tr>
</tbody>
</table>
Table 7.3. Sediment characteristics of Lake Ellesmere and the Tomahawk Lagoons, in April 2014 and June 2014 respectively.

<table>
<thead>
<tr>
<th></th>
<th>Redox</th>
<th>Organic matter availability</th>
<th>Organic matter content</th>
<th>Porosity</th>
<th>Clay content</th>
<th>Silt content</th>
<th>Sand content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mV)</td>
<td>(g m(^{-2}))</td>
<td>(% dry weight)</td>
<td>(kg m(^{-2}))</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>Lake Ellesmere</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mean (n = 18)</td>
<td>-272</td>
<td>240</td>
<td>3.99</td>
<td>7</td>
<td>6.74</td>
<td>58</td>
<td>35</td>
</tr>
<tr>
<td>SD</td>
<td>28</td>
<td>133</td>
<td>5</td>
<td>1</td>
<td>0.78</td>
<td>6.93</td>
<td>7.64</td>
</tr>
<tr>
<td>Range</td>
<td>-20 - -400</td>
<td>92 - 745</td>
<td>0.69 – 22</td>
<td>5 - 9</td>
<td>0.54 - 13</td>
<td>1.78 - 89</td>
<td>2.2 - 98</td>
</tr>
<tr>
<td>Tomahawk Lagoon 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (n = 3)</td>
<td>99</td>
<td>285</td>
<td>9</td>
<td>8.3</td>
<td>4.55</td>
<td>43</td>
<td>52</td>
</tr>
<tr>
<td>SD</td>
<td>130</td>
<td>50</td>
<td>6</td>
<td>2</td>
<td>1.97</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Range</td>
<td>-50 - 192</td>
<td>232 - 331</td>
<td>2 - 13</td>
<td>6 - 10</td>
<td>0.66 – 7.06</td>
<td>7.16 – 66</td>
<td>27 – 92</td>
</tr>
</tbody>
</table>
Table 7.4. Denitrification rates calculated from the accumulation of $^{29+30}$N$_2$ gas pairs between 0-24 hours, and 24-48 hours. The 24-hour rate is reported on in the thesis, due to the non-linear accumulation of N$_2$ after 24 hours due to depletion of nitrate within the cores.

<table>
<thead>
<tr>
<th>Site</th>
<th>24-hour denitrification rate ($\mu$mol m$^{-2}$ h$^{-1}$)</th>
<th>48-hour denitrification rate ($\mu$mol m$^{-2}$ h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>4 ± 8</td>
<td>0.002 ± 0.004</td>
</tr>
<tr>
<td>E2</td>
<td>0.11 ± 0.03</td>
<td>0.004 ± 0.0004</td>
</tr>
<tr>
<td>E3</td>
<td>114 ± 107</td>
<td>221 ± 272</td>
</tr>
<tr>
<td>E6</td>
<td>98 ± 79</td>
<td>3 ± 5</td>
</tr>
<tr>
<td>E14</td>
<td>0.14 ± 0.2</td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td>E19</td>
<td>252 ± 31</td>
<td>73 ± 1106</td>
</tr>
<tr>
<td>T4</td>
<td>131 ± 49</td>
<td>153 ± 105</td>
</tr>
<tr>
<td>T5</td>
<td>223 ± 208</td>
<td>450 ± 161</td>
</tr>
<tr>
<td>T6</td>
<td>94 ± 35</td>
<td>83 ± 37</td>
</tr>
</tbody>
</table>
Figure 7.1. Single biplots of sqrt oxygen penetration depth (mm) against the four key predictors selected from modelling for the multiple regression modelling. A = Temperature (ºC), B = *P. antipodarum* (ind. m$^{-2}$), C = Chironomid density (ind. m$^{-2}$), and D = *P. fluviatillis* density (ind. m$^{-2}$).
Figure 7.2. Single biplots of key variables relating to the measured denitrification rates.

A = sqrt denitrification rate (µmol m$^{-2}$ h$^{-1}$) vs organic matter availability (g m$^{-2}$), B = sqrt denitrification rate (µmol m$^{-2}$ h$^{-1}$) vs chironomid density (ind. m$^{-2}$) and C = chironomid density (ind. m$^{-2}$) vs organic matter availability (g m$^{-2}$).


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