THE TEMPERATURE RESPONSE OF

NITRATE REMOVAL IN DENITRIFICATION

BEDS

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Abstract

The addition of reactive nitrogen (N_r) to agricultural systems has helped crop production match human population growth. However, the addition of N_r comes at a cost to environment in the form of ozone destruction, habitat degradation and biodiversity loss. Denitrification beds represent an effective method for the removal of N_r from a range of wastewaters and groundwater with high nitrate (NO_3^-) concentrations. Beds are lined containers filled with a carbon (C) source to enhance denitrification: the conversion of NO_3^- to unreactive dinitrogen (N_2).

In general, the rate of NO_3^- removal in denitrification beds increases with increasing temperature. However, the temperature response of NO_3^- removal in beds is poorly constrained as other controlling factors (e.g. NO_3^- concentration and C source availability) can obscure the effect of temperature. The objective of this study was to measure the rates of NO_3^- removal in three denitrification beds as temperature changed seasonally. The beds were located in the North Island of New Zealand and were loaded with NO_3^- from wastewater from a hydroponic glasshouse (Karaka), domestic effluent from a campground (Motutere) and wastewater and domestic effluent from a research station (Newstead). Water samples were collected from wells installed along the length of each bed every month and were analysed for NO_3^- concentration by ion chromatography. Rates of NO_3^- removal were calculated using the change in NO_3^- concentration and the flow rate. The temperatures of the beds were also measured at each sampling.

Nitrate concentrations declined along the length of each denitrification bed and rates of NO_3^- removal were calculated to average 3.6, 4.3 and 1.7 g N m^{-3} day^{-1} for Karaka, Motutere and Newstead, respectively. The rates of removal increased with increasing temperature at Karaka and Motutere and the Q_{10} values (the factor by which the rate of removal increased for a 10 °C increase in

temperature) were calculated as 4.1 and 2.2 for Karaka and Motutere, respectively. The rates of NO_3^- removal and Q_{10} values were similar to those reported in previous studies of denitrification beds both in New Zealand and overseas. However, the rate of NO_3^- removal at Karaka was less than the rate of removal of 7.6 g N m^{-3} day^{-1} previously measured at Karaka in a study 5 years ago. Similarly, the temperature response at Karaka was higher than the Q_{10} of 2 reported in this previous study at Karaka. The decrease in removal and increase in Q_{10} may have been due to a decline in C source quality.

There was no evidence of an increase in the rate of NO_3^- removal with temperature at Newstead, with a Q_{10} calculated as 1.0. The denitrification bed had been recently installed and was in a start-up phase. It was likely that the pre-treatment system, in particular the nitrifying component responsible for converting ammonium (NH_4^+) in the effluent to NO_3^-, was not functioning effectively which resulted in low NO_3^- concentrations entering the bed at Newstead. Nitrate was depleted within the beds at Motutere and Newstead which indicated that the rates of removal were NO_3^- limited and that the temperature response may not have been adequately measured.

This study confirmed that the rate of NO_3^- removal increased with increasing temperature in the denitrification beds at Karaka and Motutere. The temperature response of NO_3^- removal was similar to the response reported in previous studies of denitrification beds. However, additional research is required to further constrain the range of Q_{10} values from which future denitrification beds can be designed to optimise NO_3^- removal. Whether Q_{10} values increase as wood chips age and C quality decreases also requires further investigation.

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Chapter 1 Introduction

1.1 Background

As a fundamental component of proteins, nitrogen (N) is an essential element to living organisms. The atmosphere represents a large reservoir of N in the biosphere, comprising of approximately 79% dinitrogen (N_2) (Delwiche, 1970; Robertson and Vitousek, 2009). However, the strength of the bonds which bind N_2 are such that large inputs of energy are required to break them, rendering N_2 unreactive and unavailable to the majority of living organisms (Davidson and Seitzinger, 2006; Robertson and Vitousek, 2009). Two natural processes which possess the energy required to break the bonds and convert N_2 to biologically-available ammonia (NH_3) are lightning and biological N fixation (BNF) undertaken by N_2-fixing microbes (Vitousek *et al.*, 1997; Galloway *et al.*, 2003). Subsequent processes convert NH_3 to other forms of reactive N (N_r) which include ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitric oxide (NO), nitrous oxide (N_2O) and organic N (Galloway *et al.*, 2003). However, the supply of N_r remains limited in the majority of unmanaged terrestrial ecosystems, restraining productivity and influencing structure and function (Vitousek *et al.*, 1997; Galloway *et al.*, 2003; Robertson and Vitousek, 2009).

Prior to industrial times the addition of N_r through N fixation was roughly equivalent to the loss of N_r through denitrification (Galloway *et al.*, 2003); meaning there was sufficient N_r available to maintain ecosystem productivity but insufficient N_r available to support the increase in crop productivity required by the increasing human population. As such, the addition of anthropogenic N_r to agricultural systems through the use of synthetic N fertilisers and the cultivation of N-fixing species has been fundamental in sustaining the increasing global population through increasing crop production (Robertson and Vitousek, 2009).

However, the increase in anthropogenic N_r production has exceeded the increase in population with increases of 120% and 78%, respectively, since 1970 (Galloway *et al.*, 2008).

The presence of N_r in excess of plant and animal requirements in agricultural systems is of concern due to the ability of a single nitrogen molecule to 'cascade' through the environment and impact detrimentally on the atmosphere and terrestrial and aquatic ecosystems (Galloway *et al.*, 2003). The environmental impacts are thus widespread and include ozone depletion, acidification, eutrophication, hypoxia, habitat degradation and loss of biodiversity (Vitousek *et al.*, 1997; Rabalais *et al.*, 2002; Galloway *et al.*, 2003). Management of N in agricultural systems aims to supply sufficient N_r to support crop and animal requirements without supplying N_r in excess to impact detrimentally on other ecosystems (Robertson and Vitousek, 2009). As such, a range of strategies exist which aim to improve the management of N_r at the farm scale including crop rotations to increase the N-use efficiency of crops, soil testing to predict crop N fertiliser and water requirements and watershed management to remove N before it is transported to other ecosystems (Dinnes *et al.*, 2002).

The nitrogen cascade ends with the conversion of N_r back to unreactive and biologically unavailable N_2. Heterotrophic denitrification is perhaps the most important process of N removal in terrestrial systems and is the conversion of NO_3^- to N_2 undertaken by microorganisms (Davidson and Seitzinger, 2006). Several environmental conditions are known to regulate the rate denitrification, including oxygen (O_2), NO_3^- and C concentration (Barton *et al.*, 1999; Davidson and Seitzinger, 2006). The available C concentration regulates denitrification is systems where the NO_3^- concentration is in excess, such as in agricultural systems (Barton *et al.*, 1999). Denitrifying microorganisms are facultative and use O_2 as

an electron acceptor in the oxidation of organic compounds until O_2 is depleted and replaced by NO_3^- as an electron acceptor in oxidation (Zumft, 1997). In addition, temperature is known to regulate the rate of denitrification. As is the case with the majority of biological processes, the rate of denitrification increases with increasing temperature although the exact nature of this relationship is poorly understood (Knowles, 1982; Davidson and Seitzinger, 2006). Denitrification occurs in almost all terrestrial and aquatic environments where the prerequisites of low O_2, high NO_3^- and high C concentrations are met (Seitzinger *et al.*, 2006). However, denitrification is limited in agricultural systems by the lack of available C and anaerobic microsites (Parkin, 1987).

Various strategies exist to enhance denitrification, including buffers or riparian zones, wetlands, controlled drainage systems and wastewater treatment systems (Dinnes *et al.*, 2002). Denitrifying bioreactors represent one such strategy and utilise a C source to enhance denitrification to remove NO_3^- from a range of wastewaters (Schipper *et al.*, 2010b). Designs of bioreactor differ in terms of the hydrological connection between the wastewater and the C source and can be divided into three main types: (a) denitrification walls, in which the C source is incorporated perpendicularly to groundwater flow; (b) denitrification layers, in which the C source is incorporated horizontally under tile-drained fields; and (c) denitrification beds, in which the C source is incorporated into a containerised system (Robertson and Cherry, 1995; Schipper *et al.*, 2010b).

Further research is required in various areas to ensure the improved functioning of denitrifying bioreactors, specifically in regards to the temperature dependency of denitrification. An increase in the rate of NO_3^- removal with increasing temperature has been reported in previous studies on denitrifying bioreactors (Robertson *et al.*, 2000; van Driel *et al.*, 2006; Robertson *et al.*, 2008;

Robertson and Merkley, 2009). For the purpose of comparisons, some previous studies have reported a Q_{10} value, which is the factor by which the rate of denitrification increases with a 10 °C increase in temperature (Davidson and Janssens, 2006; Davidson *et al.*, 2006). For the majority of biological processes, a Q_{10} of 2 is commonly observed (Kirschbaum, 2000). However, Q_{10} values for the rate of NO_3^- in previous studies on denitrifying bioreactors have been reported to range from 0.18 – 5.7 (Christianson *et al.*, 2012).

Denitrifying bioreactors have been and continue to be implemented across the world in a range of different environments which experience different temperature regimes. Temperature influences the rate of NO_3^- removal and further research is required to determine the nature of the influence of temperature on the performance of denitrifying bioreactors (Christianson *et al.*, 2012). The implication of a significant influence of temperature is on the design of denitrifying bioreactors, as bioreactors constructed in environments experiencing warmer regimes may be more efficient and designed smaller than those constructed in environments experiencing cooler regimes.

1.2 Objectives

The aim of this study was to further understanding of the temperature response of NO_3^- removal in denitrification beds.

The specific objective was:

- To determine the temperature response of denitrification in three field scale denitrification beds operating at Karaka, Motutere and Newstead as temperature changed seasonally.

Previous studies have reported an increase in the rate of denitrification with an increase in temperature. This study investigates three field scale denitrification

beds operating at Karaka, Motutere and Newstead to determine the temperature dependency of the rate of NO_3^- removal and takes advantage of a previous study in the denitrification bed at Karaka by Warneke *et al.* (2011a).

Warneke *et al.* (2011a) investigated the controls influencing the rate of NO_3^- removal and GHG production in the denitrification bed at Karaka and reported a Q_{10} of 2 for the temperature response of denitrification. I hypothesise that the temperature dependency of the rate of NO_3^- removal in the denitrification beds at Karaka, Motutere and Newstead will provide a Q_{10} of ~ 2.

1.3   Thesis layout

Chapter 2 provides a literature review of the N cycle, N as an environmental pollutant and N removal through denitrification. The temperature response of denitrification in denitrifying bioreactors is reviewed.

Chapter 3 provides the data and discussion on the temperature response of denitrification in three field scale denitrification beds as temperature changed seasonally. It is intended that Chapter 3 will be written up as a paper for submission to a peer reviewed journal. As such, there is some repetition of material previously covered in the abstract and also the introductory and literature review chapters.

Chapter 4 provides the conclusions drawn from the data and discussion on the temperature response of denitrification in the denitrification beds and provides recommendations for further research.

Chapter 2 Literature Review

2.1 Introduction

As a fundamental component of proteins, nitrogen (N) is essential to living organisms (Canfield *et al.*, 2010). N is abundant in the atmosphere, biosphere and hydrosphere on Earth yet the majority of N occurs as dinitrogen gas (N_2) which is unreactive and biologically unavailable to the majority of living organisms (Galloway *et al.*, 2004; Davidson and Seitzinger, 2006). As a result, biologically available N is limited in the majority of terrestrial ecosystems, restricting ecosystem productivity and influencing ecosystem dynamics (Vitousek *et al.*, 1997; Robertson and Vitousek, 2009; Canfield *et al.*, 2010). The addition of anthropogenic reactive nitrogen (N_r) to agricultural systems through fertiliser addition and biological N fixation (BNF) is one of the key reasons that crop production has been able to meet the requirements of the growing human population (Robertson and Vitousek, 2009). However, the addition of N_r in excess of crop and animal requirements in agricultural systems has had detrimental impacts on atmospheric, terrestrial and aquatic ecosystems (Dinnes *et al.*, 2002). Denitrification is process which ultimately returns N_r back to the atmosphere as unreactive and unavailable N_2 and there is considerable interest in identifying ways to utilise this process to reduce excess N_r reaching non-target ecosystems (Knowles, 1982; Dinnes *et al.*, 2002). Denitrifying bioreactors represent a strategy which is being implemented to enhance denitrification and remove N_r from a range of wastewaters (Schipper *et al.*, 2010b). However, denitrification is controlled by a number of environmental conditions, some of which require further understanding to ensure maximum N_r reduction and associated uptake of the strategy.

2.2   Structure of literature review

This literature review looks at the role of N within the environment and is divided in to three sections: (i) N cycling, (ii) N as an environmental pollutant and (iii) N removal through denitrification. The first section reviews the N cycle and the processes in which N is fixed from the atmosphere, is transformed in terrestrial and aquatic systems and is ultimately returned to the atmosphere. The influence of humans on the N cycle is reviewed. The second section reviews the beneficial and detrimental impacts on atmospheric, terrestrial and aquatic ecosystems associated with N in excess of ecosystem requirements. The third section reviews the denitrification process including the influence of environmental controls and, in particular, the influence of temperature. The methods for N removal through denitrification are briefly reviewed, with a focus on denitrifying bioreactors. Lastly, the influence of temperature on the performance of denitrifying bioreactors is reviewed.

2.3   The nitrogen cycle

Despite being abundant in the atmosphere, biosphere and hydrosphere, the majority of N on Earth is biologically unavailable to the majority of the living organisms (Galloway *et al.*, 1995; Davidson and Seitzinger, 2006). Biologically available N limits primary production and in doing so influences structure and function within an ecosystem (Vitousek and Howarth, 1991; Robertson and Vitousek, 2009; Canfield *et al.*, 2010). Understanding the cycling of N through ecosystems is imperative to ensure sustainable management of agricultural systems and to avoid adverse impact on the environment and has resulted in considerable research on the N cycle (Figure 2.1) (Robertson and Vitousek, 2009).
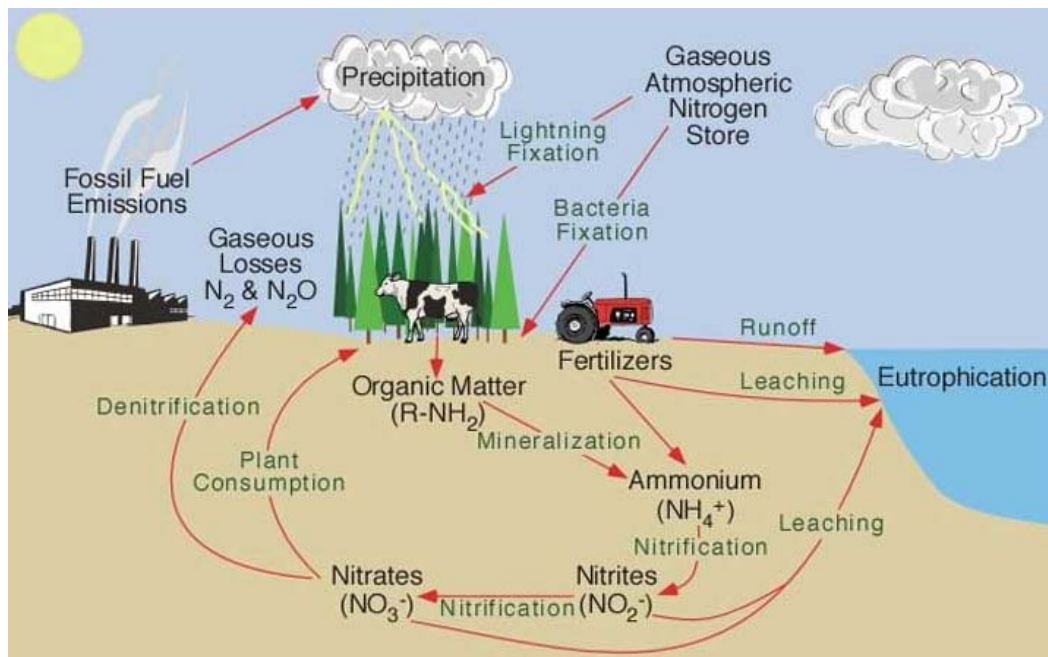
Figure 2.1: A simplified diagram of the nitrogen cycle (Image: M. Oulton, the University of Waikato).

Comprising approximately 79% of the atmosphere, N_2 represents the largest reservoir of N on Earth (Robertson and Vitousek, 2009). However, the stability provided by the strength of the triple bond binding the two N atoms renders N_2 unreactive and biologically unavailable to the majority of living organisms (Galloway *et al.*, 2004; Davidson and Seitzinger, 2006). Few processes in nature have the necessary energy required convert N_2 into reactive and biologically available forms of N, such as ammonia (NH_3) (Vitousek *et al.*, 1997). Natural processes of nitrogen fixation include BNF undertaken by microorganisms and, to a lesser extent, lightning (Vitousek *et al.*, 1997; Robertson and Vitousek, 2009). BNF undertaken by microorganisms is either symbiotic, involving microorganisms interacting with other organisms, or non-symbiotic, involving free-living microorganisms (Vitousek *et al.*, 1997). Bacteria of the genus *Rhizobium* form a symbiotic relationship with (mainly) legume species, fixing N_2 to NH_3 inside root nodules in exchange for carbohydrates (Long, 1989;

Vitousek *et al.*, 1997). In contrast, non-symbiotic cyanobacteria of the genus *Anabaena* fix N_2 to NH_3 inside specialised cells known as heterocysts during N limiting conditions in exchange for carbohydrates from photosynthetic cells (Fay, 1992). The source of fixed N, whether natural or anthropogenic, is not discriminable by organisms and as such the response of ecosystems to additions of N is the same (Robertson and Vitousek, 2009).

Assimilation is the process of uptake of NH_3 by organisms and the conversion to organically-bound N, which is unavailable to other organisms (Myrold, 2005). However, following the death of the organism or the expulsion of waste, organically-bound N is available for microorganisms to convert into biologically available ammonium (NH_4^+) and nitrate (NO_3^-) through mineralisation and nitrification, respectively (Canfield *et al.*, 2010). Whether assimilation or mineralisation dominates depends on the availability of N within the soil; with N limiting conditions being conducive to assimilation and non-limiting conditions being conducive to mineralisation (Myrold, 2005). The conversion of organic N to NH_4^+ through mineralisation is susceptible to oxidation to NO_3^- by microorganisms in a two-step process known as nitrification (Canfield *et al.*, 2010). The first step of nitrification involves the oxidation of NH_4 to nitrite (NO_2^-), generally by bacteria of the '*Nitroso-*' genera, and the second step of nitrification involves the oxidation of NO_2^- to NO_3^-, generally by bacteria of the '*Nitro-*' genera (Myrold, 2005).

There are multiple fates of NO_3^- in soil (Dinnes *et al.*, 2002); NO_3^- is biologically available but is preferentially assimilated under conditions of limited NH_4^+ and excess energy, as the assimilation of NO_3^- involves the energetic conversion to NH_4^+ which is less efficient than the assimilation of NH_4^+ directly (Myrold, 2005). The conversion of NO_3^- to NH_4^+ for assimilation is known as

assimilatory NO_3^- reduction (Canfield *et al.*, 2010). NO_3^- not assimilated is susceptible to leaching through soil, as the negatively-charged NO_3^- is repelled by the negatively-charged cation exchange capacity of soil. Leaching along with soil erosion results in a loss of N to other ecosystems, where the addition of N can impact on the cycling of N and other nutrients (Vitousek *et al.*, 1997; Smil, 1999).

In the absence of oxygen, NO_3^- is available for reduction processes including dissimilatory NO_3^- reduction to NH_4^+ (DNRA), anaerobic ammonium oxidation (anammox) and denitrification (Myrold, 2005). The process of reduction of NO_3^- to NH_4^+ for oxidisation of an electron donor as opposed to for assimilation is known as DNRA (Canfield *et al.*, 2010). Anammox is the process of reduction of NO_3^- and NH_4^+ to N_2 and, despite the importance of the process in the marine environment, is poorly understood in terrestrial ecosystems (Kuypers *et al.*, 2003; Canfield *et al.*, 2010). In contrast, denitrification is the process of reduction of NO_3^-, NO_2^-, nitric oxide (NO) and nitrous oxide (N_2O) to N_2 and is important in terrestrial ecosystems (Knowles, 1982; Myrold, 2005). The release of N_2 back to the atmosphere through denitrification represents the termination of the N cycle (Davidson and Seitzinger, 2006).

2.3.1 Human alteration of the nitrogen cycle

Prior to industrial times, the rate of natural N fixation was largely balanced by the rate of denitrification (Galloway *et al.*, 1995); N was efficiently cycled within ecosystems and prevented from accumulating within the environment (Galloway *et al.*, 2003). The productivity of many terrestrial ecosystems is still limited by biologically available N (Robertson and Vitousek, 2009; Canfield *et al.*, 2010). However, the demand for increased productivity to sustain human population growth has led to anthropogenic N fixation, the rate of which now doubles that of

natural N fixation (Smil, 1999). Although N fixation (from both natural and anthropogenic sources) remains largely balanced with denitrification (Canfield *et al.*, 2010), N is no longer efficiently cycled within ecosystems and accumulates within the environment (Rabalais, 2002; Galloway *et al.*, 2003).

The anthropogenic production of N_r is driven by agriculture and, to a lesser extent, fossil fuel energy (Vitousek *et al.*, 1997; Galloway *et al.*, 2008). In agriculture, the production of N_r involves both the Haber-Bosch process, which forms fertiliser NH_3 by reacting methane (CH_4) with N_2 under intense pressure and heat (Robertson and Vitousek, 2009), and crop cultivation, which forms organic by enhancing BNF in N-fixing crops such as legumes (Galloway *et al.*, 2003). In fossil fuel energy, the production of N_r involves the combustion of fossil fuels, which form reactive N oxides (NO_x) from N_2 and fossil N (Galloway *et al.*, 2003; Galloway *et al.*, 2008).

The anthropogenic production of N_r continues to increase annually on a global scale, with the majority of this N_r being applied to agricultural systems (Galloway *et al.*, 2008). The addition of N_r to agriculture has enabled crop production to sustain human population growth with an estimated 40% population sustained as a direct result (Smil, 1999). However, accompanying the benefits of the addition of N_r to agriculture are substantial costs (Robertson and Vitousek, 2009); N_r is easily transferred by atmospheric and hydrologic processes and a substantial proportion of added N_r is transferred to other ecosystems before it can be efficiently used within the agricultural system (Galloway *et al.*, 2003). Even N_r that is efficiently used within the agricultural system is ultimately transferred to other ecosystems, such as is generally the case with harvests (Robertson and Vitousek, 2009). Transferred N_r accumulates within the environment which is a concern as N_r has the potential to act as an environmental pollutant; a single molecule can 'cascade'

through atmospheric, terrestrial and aquatic ecosystems and impact detrimentally

on the environment (Figure 2.2) (Galloway *et al.*, 2003).
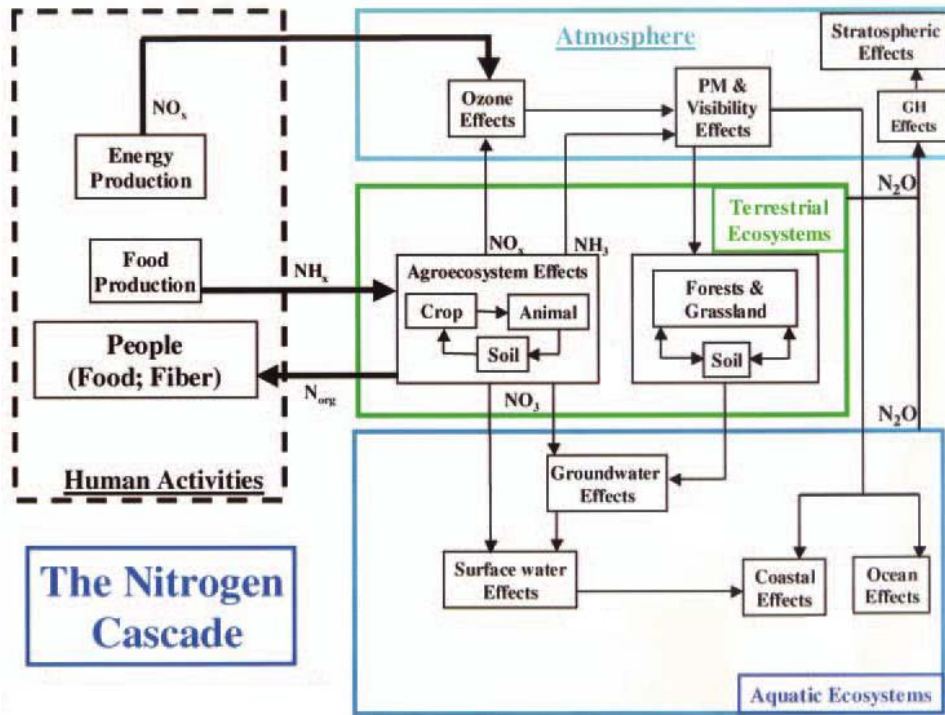
Figure 2.2: A diagram of the nitrogen cascade showing the sequential transfer of nitrogen through ecosystems and the associated effects (Modified from Galloway *et al.* (2003)).

2.4   Nitrogen as an environmental pollutant

The importance of N to ecosystems is well recognised; on an individual scale, N

is a fundamental component of amino acids (Canfield *et al.*, 2010). On an

ecosystem scale, N regulates productivity and dynamics (Robertson and Vitousek,

2009). However, N_r is limited in the majority of terrestrial and aquatic ecosystems,

with primary producers in these systems adapted to function in N-limited

conditions (Galloway *et al.*, 1995). In the short term, the addition of N_r to these

ecosystems results in an increase in productivity and organic matter (Vitousek and

Howarth, 1991). The addition of N_r is accompanied by a change in species

composition and loss of biodiversity within the ecosystem, as N-demanding

species outcompete N-fixing species (Robertson and Vitousek, 2009). Changes to the cycling of N_r within the ecosystem are compounded by changes to the cycling of carbon (C), which impact on the atmospheric carbon dioxide (CO_2) concentrations and the ecosystem response to CO_2 concentrations (Falkowski, 1997; Vitousek *et al.*, 1997). In the long term, the addition of N_r to these ecosystems results in a decreasing response in productivity as the ecosystem becomes N-saturated accompanied by an increasing loss of N_r to other ecosystems (Aber *et al.*, 1998). As such, there are three outcomes for N_r in ecosystems: accumulation within the ecosystem, loss to the atmosphere following conversion to N_2 and loss to other ecosystems (Galloway *et al.*, 2003). The N cascade (Figure 2.2) refers to the sequential transfer of N_r through ecosystems and the associated changes to the atmospheric, terrestrial and aquatic environments (Galloway, 1998; Galloway *et al.*, 2003).

2.4.1 The atmospheric environment

The atmosphere receives N_r as emissions of NO_x, NH_3 and N_2O from terrestrial and aquatic environments (Vitousek *et al.*, 1997). NO_x and NH_x (NH_3 and NH_4^+) have short residence times within the atmosphere and tend to be returned to the Earth's surface within hours to days, representing a short lag in the N cascade (Galloway *et al.*, 2003). However, internal cycling within the atmosphere can occur before NO_x and NH_x are returned to the surface. NO_x enters the atmosphere largely as an emission from the combustion of fossil fuels (Galloway *et al.*, 2008). Increased concentrations of NO_x along with volatile organic C compounds can contribute to increased concentrations of ozone and photochemical oxidants (Vitousek *et al.*, 1997; Galloway *et al.*, 2003). The majority of the NO_x within the atmosphere is converted to HNO_3, which is then either converted to an aerosol or

deposited on the Earth's surface (Galloway *et al.*, 2003). Similarly, the majority of NH_3 within the atmosphere is either converted to an aerosol or deposited on the surface (Schlesinger and Hartley, 1992).

Detrimental impacts are associated with NO_x and NH_3 in the atmosphere; increasing ozone concentrations contribute to the greenhouse potential (Galloway *et al.*, 2003). In addition, ozone has been found to have detrimental impacts on human health, with links to respiratory illness (Bell *et al.*, 2004). Aerosols contribute to radiative forcing – directly through the interaction of radiation with aerosols and indirectly through the interactions of radiation with clouds modified by aerosols – and in turn contribute to climate change (Galloway *et al.*, 2003). Aerosols contribute to fine particulate matter which decreases visibility within the atmosphere. In addition, fine particulate matter has been found to have detrimental impacts on human health with links to lung cancer and cardiopulmonary mortality (Pope *et al.*, 1995). In terms of deposition, ozone contributes to decreased productivity and NO_x (except N_2O), NH_3 and organic N_r contribute to fertilisation, eutrophication and acidification in terrestrial ecosystems (Vitousek *et al.*, 1997; Bouwman *et al.*, 2002; Galloway *et al.*, 2003)

The atmosphere receives N_2O as an emission from nitrification and denitrification from the addition of N_r to agriculture (Bouwman *et al.*, 2002). Unlike NO_x and NH_3 which have a residence time of hours to days, N_2O has a residence time of 100 years (Galloway *et al.*, 2003). Within the troposphere, N_2O is a greenhouse gas (GHG) with a global warming potential much higher than that of CO_2 and within the stratosphere, N_2O contributes to the destruction of ozone (Vitousek *et al.*, 1997; Bouwman *et al.*, 2002).

To summarise, N_r (except N_2O) has limited potential to accumulate in the atmosphere and no potential to be denitrified. However, internal cycling of N_r

within the atmosphere contributes to detrimental impacts on the atmospheric environment and atmospheric transport processes readily transfer N_r to terrestrial and aquatic ecosystems.

2.4.2 The terrestrial environment

The terrestrial environment encompasses both managed and unmanaged systems including agricultural systems, grasslands and forests. The anthropogenic production of N_r is driven by the demands of agriculture, with an estimated 75% of all anthropogenically produced N_r applied to agricultural systems (Galloway *et al.*, 2003). Agricultural systems consist of crop and animal production systems; crop systems producing grains, fruit, vegetables and fibers from inorganic N_r and animal systems producing milk and meat from the products of crop systems (Smil, 1999, 2002; Galloway *et al.*, 2003). The residence time of N_r in agricultural systems is years to decades as N_r is bound within soil organic matter (SOM) and requires mineralisation to be available for uptake by crops (Robertson and Vitousek, 2009). As N_r is bound within SOM, the majority of original N_r is unavailable for uptake for seasonal crop production (Dinnes *et al.*, 2002). Added (or new) N_r is available for uptake and as such determines crop production (Galloway *et al.*, 2003). However, when the rate of uptake does not match the rate of addition of N_r the added N_r can be lost from the agricultural system (Robertson and Vitousek, 2009).

Smil (2001) estimated that about 170 Tg of N_r is added to crop systems annually, consisting of 120 Tg of new N_r (from fertiliser and enhanced BNF) and 50 Tg of reused N_r (from residues or manure). Of the 170 Tg of N_r applied to crop systems, about 33 Tg is consumed by animal systems to produce edible protein and 16 Tg is consumed by humans. However, animal systems vary in the

efficiency by which N_r from crop systems is converted into edible protein with N-use efficiency (NUE) ranging from 40-50% in poultry systems, 35-40% in dairy systems and 15-30% in beef systems (Galloway *et al.*, 2003; Canfield *et al.*, 2010). As such, of the 33 Tg consumed by animal systems to produce edible protein, about 5 Tg is consumed by humans (Galloway *et al.*, 2003). Of the remaining 121 Tg of N_r that is not consumed by animal systems or humans, 4 Tg is recycled in the crop system through residues and manure and 117 Tg is transported to the atmospheric environment as emissions of NO_x, NH_3, N_2O and N_2 and to the aquatic environment as dissolved and particulate N_r (Vitousek *et al.*, 1997; Galloway *et al.*, 2003). The potential for denitrification exists in agricultural systems where the prerequisites of low or absent oxygen concentration, high available C concentrations high NO_3^- concentrations are met (Barton *et al.*, 1999). However, denitrification is considered counterproductive in agricultural systems despite the limited occurrence of these prerequisites (Barton *et al.*, 1999; Davidson and Seitzinger, 2006).

Unlike agricultural systems, the main source of N_r to unmanaged grassland systems is though BNF and atmospheric deposition (Galloway *et al.*, 2003). The residence time of N_r in grassland systems is decades to centuries as N_r is bound within organic matter stored underground of which the mineralisation or turnover is slow; representing a lag within the N cascade (Blair *et al.*, 1998). Grassland systems typically experience little precipitation and as such little N_r is leached to the aquatic environment (Groffman *et al.*, 1993; Galloway *et al.*, 2003). Atmospheric emissions transport N_r to the atmospheric environment, with fires causing emissions of N_r which can equal deposition of N_r to grassland systems (Blair *et al.*, 1998). However, emissions caused by fire largely consist of N_2 which, unlike N_r, is unreactive in the atmospheric environment (Kuhlbusch and Crutzen,

1995; Blair *et al.*, 1998). Similar to agricultural systems, there is the potential for denitrification in grassland systems although it is limited due to the typically well-aerated soils (Galloway *et al.*, 2003)

Similarly to grassland systems, the main source of N_r to unmanaged forest systems is though BNF and atmospheric deposition (Vitousek and Howarth, 1991). The residence time of N_r in forest systems is centuries as N_r is bound within organic matter of which the turnover is slow; representing a substantial lag within the N cascade (Galloway *et al.*, 2003). The response of forest systems to N_r depends on the degree of N saturation; forest systems where N_r is limited cycle N_r efficiently with little losses whereas systems where N_r is in excess cycle N_r inefficiently with large losses (Aber *et al.*, 1998). Losses of N_r from forest systems occur through transport to the atmospheric environment as emissions of NO and N_2O and to the aquatic environment as NO_3^- (Galloway *et al.*, 2003). Similar to both agricultural and grassland systems, the typically well-aerated soils found in forest systems limit the potential for denitrification (Robertson and Tiedje, 1984; Groffman and Tiedje, 1989).

2.4.3 The aquatic environment

The aquatic environment encompasses groundwater, surface water and coastal and marine systems. The main source of N_r to groundwater systems occurs in the form of NO_3^- from leaching from agricultural systems, although in some areas waste disposal is the main source (Dinnes *et al.*, 2002; Galloway *et al.*, 2003). The residence time of N_r in groundwater systems is variable, with low NO_3^- concentrations and high NO_3^- losses associated with some systems and high NO_3^- concentrations and low NO_3^- losses associated with other systems (Galloway *et al.*, 2003). Elevated concentrations of NO_3^- in groundwater used as drinking water

is of concern to human health. Once ingested, NO_3^- is converted to NO_2^- which reduces the capacity of the blood to carry O_2 and ultimately causes death in a disorder known as methaemoglobinemia (Vitousek *et al.*, 1997; Camargo and Alonso, 2006). Losses of N_r from groundwater occur through transportation of NO_3^- to surface water systems and as emissions of NO, N_2O and N_2 through denitrification (Blowes *et al.*, 1994; Dinnes *et al.*, 2002; Galloway *et al.*, 2003).

Surface water systems include wetlands, streams, rivers and lakes and the main sources of N_r to these systems is from the surrounding watershed, BNF and atmospheric deposition (Rabalais, 2002). The residence time of N_r in surface water systems is variable although much shorter than the residence time in terrestrial systems; representing a small lag in the N cascade (Galloway *et al.*, 2003). In the undisturbed headwaters of surface water systems N_r is present in low concentrations and is tightly cycled. However, in disturbed downstream reaches N_r can be present in concentrations which exceed the retention capacity and can be lost through transportation to downstream (coastal and marine) systems (Rabalais, 2002). In surface water systems N_r present in excess of requirements can cause acidification, eutrophication and loss of biodiversity (Rabalais, 2002; Camargo and Alonso, 2006). The potential for denitrification in surface water systems varies and, apart from the prerequisites for denitrification, depends on the residence time (Galloway *et al.*, 2003). Wetlands have relatively long residence times and large denitrification potential compared to channelized streams which have short residence times and little denitrification potential (Seitzinger *et al.*, 2006).

The main source of N_r to coastal and marine systems is from ground and surface water systems, although the main source for open marine systems is atmospheric deposition (Rabalais, 2002; Rabalais *et al.*, 2002). The productivity

of coastal and marine systems is limited by N and as such, the addition of N_r to these systems is initially met with an increase in productivity (Galloway *et al.*, 2003). Increased productivity is beneficial as it can initially result in an increase in harvestable fish. However, N_r present in excess of requirements can cause eutrophication, a loss of biodiversity and anoxic (O_2 limited) or hypoxic (O_2 absent) conditions (Rabalais *et al.*, 2002; Galloway *et al.*, 2003). Coastal areas experiencing anoxic or hypoxic conditions are referred to as 'dead zones' because of the inability to catch fish and invertebrates in such areas with low or no O_2 (Rabalais *et al.*, 2002). Coastal and marine environment represents the largest potential for denitrification, with transport of N_r to the open marine system limited due to the efficiency of denitrification in the coastal system (Galloway *et al.*, 2003; Seitzinger *et al.*, 2006).

In an ideal world, the demands of the growing human population would have no adverse impacts on the environment. However, this is not the case of the real world and there are two key areas in which interventions regarding N_r can be made; the first area involves agricultural systems, where the amount of N_r added either has to be reduced or used more efficiently to prevent N_r leaving the system. Strategies for the improved management of N_r include the timing, rates and methods of N_r fertiliser applications, crop rotations, cover crops and crop residue management and nitrification inhibitors (Dinnes *et al.*, 2002; Robertson and Vitousek, 2009).The second area involves the interface between agricultural systems and their surroundings, where the amount of N_r leaving the system can be removed or redirected away from downstream ecosystems (Dinnes *et al.*, 2002).

2.5   Nitrate removal through denitrification

The most important mechanism of permanent N removal in terrestrial systems is heterotrophic denitrification, in which microorganisms convert N_r in the form of NO_3^- to N_2 which is returned to the atmosphere, representing the termination of the N cycle (Myrold, 2005; Davidson and Seitzinger, 2006). Denitrification is a sequential reduction process, in which NO_3^- is converted to NO_2^-, NO and N_2O before being converted to N_2 (Knowles, 1982; Myrold, 2005). As such, incomplete denitrification can result in the loss of NO and N_2O which is of concern because of the role of these gases in ozone production and consumption and radiative forcing (Knowles, 1982; Davidson and Seitzinger, 2006). The rate of denitrification and level to which denitrification is completed are regulated by a number of controls.

2.5.1 Controls on denitrification

Denitrification is mediated by microorganisms, known as denitrifiers, which possess one or all of the enzymes, known as reductases, necessary to reduce NO_3^- to N_2 (Knowles, 1982). Denitrifiers are widespread within the terrestrial and aquatic ecosystems and as such, denitrifiers do not generally limit denitrification (Barton *et al.*, 1999; Seitzinger *et al.*, 2006). Instead, the activity of denitrifiers is regulated by the concentrations of O_2, NO_3^- and C which are further regulated by pH and temperature (Barton *et al.*, 1999). While the 'proximal' controls (O_2, NO_3^- and C) are important in the regulation of denitrification at a cellular scale or field scale, the 'distal' controls (pH and temperature) are important at a global scale (Barton *et al.*, 1999).

2.5.1.1 Oxygen

Denitrifiers are typically facultative aerobes and use O_2 as an electron acceptor for respiration when O_2 is present (Knowles, 1982; Davidson and Seitzinger, 2006). However, when O_2 is absent or present in low concentrations denitrifiers can instead use NO_3^- as an electron acceptor for respiration (Seitzinger et al., 2006). The activation of the reductases involved in denitrification in anaerobic conditions appears to be an indirect result of competition for electrons as opposed to a direct result of inactivation of the reductases by O_2 (Knowles, 1982). The reductases involved in the latter stages of denitrification appear to be more sensitive to the presence of O_2, which is observed in systems where O_2 is present in low concentrations as, although the rate of denitrification decreases, the fraction of N_2O produced through incomplete denitrification increases (Weier et al., 1993). The extent to which O_2 is present depends on factors such as the rate and pathways of O_2 diffusion into and within the system and the rate of O_2 consumption within the system (Knowles, 1982; Weier et al., 1993). These factors enable the existence of anaerobic microsites in which denitrification can occur in otherwise aerobic systems (Barton et al., 1999).

2.5.1.2 Nitrate

In the absence of O_2, denitrifiers use NO_3^- as an electron acceptor for respiration (Seitzinger et al., 2006). The availability of NO_3^- differs between systems, with the availability of NO_3^- in systems which do not receive large additions of N_r often limited (Barton et al., 1999). Similarly to O_2, the availability of NO_3^- depends on factors such as the rate of NO_3^- production into and diffusion within the system and the rate of NO_3^- consumption within the system (Knowles, 1982; Barton et al., 1999). Diffusion is important in systems which do not receive large

additions of N_r to ensure the availability of NO_3^- at anaerobic microsites (Barton *et al.*, 1999). In systems where NO_3^- is present in low concentrations, denitrification appears to follow first-order kinetics (Knowles, 1982; Barton *et al.*, 1999). However, in systems where NO_3^- is present in higher concentrations, the rate of diffusion of NO_3^- within the system may increase and alter the apparent kinetics (Knowles, 1982). In addition, the reductases involved in the latter stages of denitrification may be inhibited and increase the fraction of N_2O produced through incomplete denitrification (Weier *et al.*, 1993).

2.5.1.3 Carbon

Denitrifiers use C as a source of energy and as an electron donor for respiration (Davidson and Seitzinger, 2006). The availability of a C source is important for denitrification in systems where NO_3^- is present in excess of system requirements, as denitrifiers use O_2 as an electron acceptor in the oxidation of C for energy until the system becomes anaerobic and denitrifiers can instead use NO_3^- as an electron acceptor (Barton *et al.*, 1999). As such, the role of the C source is dual as it provides both anaerobic conditions and an electron for denitrification (Knowles, 1982; Barton *et al.*, 1999). Similarly to the presence of O_2, the availability of a C source may limit denitrification in systems where NO_3^- is present in excess (Barton *et al.*, 1999). In addition, the type of C source may have an influence on the reductases involved in the latter stages of denitrification, as different types of C source which support the same rate of denitrification may produce different fractions of N_2O (Knowles, 1982).

2.5.1.4 pH

Denitrifiers function at a range of pH levels, with the optimum range between pH levels 7.0 and 8.0 (Knowles, 1982). Denitrification has been observed to occur in pH levels as high as 11 in waste systems and in pH levels as low as 4 (Prakasam and Loehr, 1972; Knowles, 1982). However, at low pH levels there is a decrease in the rate of denitrification and an increase in the fraction of N_2O produced which suggests inhibition of the reductases involved in the latter stages of denitrification (Knowles, 1982).

2.5.1.5 Temperature

As is true for the majority of biological processes, the rate of denitrification increases with increasing temperature until a temperature is achieved which causes the reductases involved to denature (Davidson and Janssens, 2006). However, the nature of the temperature dependency of denitrification is not well understood (Davidson and Seitzinger, 2006).

The temperature responses of biological processes are commonly modelled using empirical functions, such as exponential and Arrhenius functions, which have been modified little since there creation in the late 19th century (Davidson et al., 2006). Both functions describe an increase in the rate of the biological process with an increase in temperature (Fang and Moncrieff, 2001). However, as a result of their empirical nature, both the exponential and Arrhenius functions erroneously assume that the response of enzymes involved in the biological process is constant at all temperatures (Davidson *et al.*, 2006). The assumption of a constant response to temperature results in the tendency of exponential and Arrhenius functions to underestimate the rate at low temperatures and overestimate the rate at high temperatures (Lloyd and Taylor, 1994).

Other functions have been developed to model biological processes, using not only the exponential and Arrhenius functions as a foundation but linear, quadratic and logarithmic functions (Lloyd and Taylor, 1994; Fang and Moncrieff, 2001). However, functions are commonly developed using an empirical approach and, despite successfully modelling the temperature response of a biological process under particular conditions, offer different explanations for the temperature dependency without a physiological foundation to the function (Fang and Moncrieff, 2001; Davidson *et al.*, 2006).

The temperature responses of biological processes are commonly compared using a Q_{10}, which is the factor by which the rate of the process increases for a 10°C increase in temperature (Davidson and Janssens, 2006; Davidson *et al.*, 2006). A Q_{10} of 2, or a doubling of the rate of the process, is observed for many biological processes over a moderate temperature range (Kirschbaum, 2000). Q_{10} values are useful for the purpose of comparisons between biological processes (Lloyd and Taylor, 1994). However, like the empirical functions from which the Q_{10} is derived, the usefulness of the Q_{10} from a physiological perspective is debated within scientific literature (Davidson and Janssens, 2006; Davidson *et al.*, 2006)

In the commonly used exponential and Arrhenius functions, the Q_{10} remains constant with temperature, despite it being widely understood that a constant response to temperature is incorrect (Fang and Moncrieff, 2001). In other functions, the Q_{10} does not remain constant and decreases with increasing temperature (Lloyd and Taylor, 1994). It is argued that the Q_{10} should decrease with increasing temperature on the grounds that with increasing temperature, there is a smaller relative increase in the amount of molecules involved in the biological process with enough energy to allow the process to occur (Davidson and Janssens,

2006). In addition, the Q_{10} is known to increase with increasing substrate complexity and be altered by substrate availability and the affinity of the enzymes involved in the biological process for the substrate (Davidson and Janssens, 2006; Davidson *et al.*, 2006).

In previous studies of denitrification in soils, the rate of NO_3^- removal has been reported to increase with increasing temperature. Q_{10} values ranging from 1.5 – 3 have been reported in previous studies for the rate of denitrification in soils over a moderate temperature range of 10 – 35 °C (Knowles, 1982). Maag *et al.* (1997) investigated the temperature response of NO_3^- removal in riparian soils adjacent to agricultural systems in Copenhagen, Denmark, and reported that seasonal changes in temperature between 5 and 15 °C would increase the rate of denitrification by a factor of 2 – 4.

2.5.2 Methods of nitrate removal through denitrification

The majority of anthropogenically produced N_r is added to agricultural systems. Of this added N_r, a substantial proportion can be lost to downwind and downstream systems in which N_r in forms such as NO_3^- can act as an environmental pollutant. In addition to interventions made in agricultural systems, interventions can be made in the interface between agricultural systems and their surroundings (Robertson and Vitousek, 2009). The potential for the removal of NO_3^- though denitrification has resulted in considerable interest in identifying ways to utilise denitrification to reduce excess NO_3^- reaching downstream ecosystems (Dinnes *et al.*, 2002). Various methods for NO_3^- removal through denitrification have been identified and include wetlands, riparian zones, controlled drainage, wastewater treatment systems and denitrifying bioreactors (Dinnes *et al.*, 2002; Robertson and Vitousek, 2009) and are discussed below.

2.5.2.1 Wetlands

Natural and constructed wetlands have been demonstrated to effectively remove NO_3^- from intercepted surface and shallow ground waters by providing the conditions conducive to denitrification (Dinnes *et al.*, 2002; Vymazal, 2007). Ingersoll and Baker (1998) investigated the ability of a laboratory wetland microcosm to remove NO_3^- at different temperatures, flow rates and C concentrations. The NO_3^- removal efficiency was found to range from 8 – < 95 % and was observed to decrease with a decrease in C concentration and an increase in flow rate. The findings of Ingersoll and Baker (1998) highlighted the importance of C in promoting denitrification and identified C as a potential limitation to denitrification in wetlands in the field environment. In addition, the flow rate was identified as a potential limitation in the field environment as the flow rate determines the residence time within the wetland and the interaction between the NO_3^- in the wastewater and C (Seitzinger *et al.*, 2006).

2.5.2.2 Riparian zones

Riparian zones have been shown to be an effective method of NO_3^- removal from intercepted surface and shallow ground waters, through both uptake by vegetation and by providing conditions which promote denitrification (Hill, 1996). Dinnes *et al.* (2002) investigated a number of studies on the NO_3^- removal in riparian zones and found that efficiencies ranged from 48 – 100 %. However, the NO_3^- removal efficiencies reported in some of the studies were attributable to processes of removal other than denitrification, including dilution and assimilation (Dinnes *et al.*, 2002). The rate of NO_3^- removal has been shown to increase in riparian zones with high water tables and high C concentrations, identifying water table height and C as potential limitations to denitrification (Dinnes *et al.*, 2002). In addition,

the slope and flow rate determine the residence time within the riparian zones and likewise have the potential to limit NO_3^- removal (Hill, 1996; Seitzinger *et al.*, 2006).

2.5.2.3 Controlled drainage

Controlled drainage has shown some promise as method of NO_3^- removal from ground waters (Woli *et al.*, 2010) and involves one of three approaches; the first consists of decreasing the discharge of the groundwater from the system, the second consists of minimising NO_3^- leaching through decreasing the infiltration depth of the soil and the third consists of maximising denitrification by decreasing the aerobic portion of the soil (Dinnes *et al.*, 2002). Decreasing the aerobic portion of the soil involves the manipulation of the water table, with shallower water table depths observed to result in greater NO_3^- removal through denitrification. However, as a result of the cost and maintenance associated with drainage control structures, the method is restricted to environments with a slope of, or less than, 1 % (Skaggs and Chescheir III, 1999; Dinnes *et al.*, 2002).

2.5.2.4 Wastewater treatment systems

Onsite wastewater treatment systems have been widely used as a method of NO_3^-, nutrient and contaminant removal from residential wastewater (Oakley *et al.*, 2010). However, wastewater treatment systems have also been widely implicated as a source of NO_3^- to ground and surface water (Robertson and Cherry, 1995; Galloway *et al.*, 2003; Oakley *et al.*, 2010). N removal occurs through sequential nitrification and denitrification and there are three general approaches for wastewater treatment; pre-anoxic systems that recirculate wastewater through an anaerobic or anoxic reactor followed by an aerobic reactor, post-anoxic systems

that circulate wastewater through an aerobic reactor followed by an anoxic reactor and simultaneous nitrification-denitrification systems that circulate wastewater through a reactor with both anoxic and aerobic zones (Oakley *et al.*, 2010). Approaches which are more passive, such as post-anoxic systems which utilise a denitrifying bioreactor, have been shown to remove NO_3^- efficiently and economically than intensive systems. However, more passive approaches require more space and as such, more intensive systems continue to be retrofitted into environments with limited space (Oakley *et al.*, 2010).

2.5.2.5 Denitrifying bioreactors

Denitrifying bioreactors represent a simple, inexpensive method for the enhanced removal of NO_3^- from groundwater and a range of wastewaters (Robertson *et al.*, 2000). Bioreactors incorporate a C source into nitrified effluent which supports denitrification in systems which would have otherwise been limited by a C source (Blowes *et al.*, 1994). A variety of liquid and solid C sources have been trialled with success for use in bioreactors, although most sources have only been trialled at the laboratory scale (Volokita *et al.*, 1996; Greenan *et al.*, 2006). Wood-particle media (sawdust and wood chip) is the C source most widely used in field scale bioreactors, largely because it is reasonably inexpensive, permeable and durable (Blowes *et al.*, 1994; Robertson *et al.*, 2008). Bioreactor designs differ based on the hydrologic connection between the groundwater or wastewater containing NO_3^- and the bioreactor containing the C source and the ratio between the two (source area: treatment area) (Schipper *et al.*, 2010b). Based on the different designs, bioreactors can be categorised as denitrification walls, denitrification layers or denitrification beds (Robertson and Cherry, 1995).

In denitrification walls, the C source is incorporated vertically into groundwater in a perpendicular direction to that of groundwater flow (Robertson and Cherry, 1995; Robertson et al., 2000). Walls can intercept either groundwater flow that is natural or groundwater flow that has been modified because of tile drainage systems (Schipper et al., 2010b). However, walls are largely restricted to sites where there are high NO_3^- concentrations such as those associated with intensive agricultural and septic systems (Schipper and Vojvodic-Vukovic, 1998). Wood chip and sawdust are the C sources most widely used in walls and are incorporated either wholly (Jaynes et al., 2008) or as a mixture with soil (Schipper and Vojvodic-Vukovic, 1998). Site characteristics including hydraulic conductivity and hydraulic retention time and C source characteristics including permeability and durability determine whether the C source is incorporated wholly or as a mixture (Robertson and Cherry, 1995; Robertson et al., 2005b).

In denitrification layers, the C source is incorporated horizontally under the soil surface (Robertson and Cherry, 1995). Layers intercept flows with high NO_3^- concentrations from tile drainage systems associated with agricultural and septic systems (Robertson et al., 2000; Schipper et al., 2010b). Sawdust is the C source most widely used in layers and is incorporated either wholly or as a mixture with soil depending on the tension saturation requirements of the site (Robertson et al., 2000). Tension ensures saturation of the layers, despite the position of the layers above the water table, which promotes anaerobic conditions which enhance denitrification (Robertson et al., 2000; van Driel et al., 2006).

In denitrification beds, the C source is incorporated into a container or trench which receives flows with high NO_3^- concentrations from a range of wastewaters (Figure 2.3) (Robertson et al., 2005a; Schipper et al., 2010b) and tile drainage systems (Blowes et al., 1994; Robertson et al., 2000). Beds can be fitted

into existing stream beds and drainage trenches, referred to as stream bed bioreactors (Robertson and Merkley, 2009), or fitted into existing stream banks, referred to as upflow bioreactors (van Driel *et al.*, 2006). Wood chip and sawdust are the C sources most widely used in beds and are incorporated either wholly or as a mixture, with upflow bioreactors using both C sources in layers because of tension saturation requirements of the site (van Driel *et al.*, 2006). The source area: treatment area is typically larger in beds in comparison to other designs because flows with high NO_3^- concentrations are essentially captured and redirected into the bed (Schipper *et al.*, 2010b).
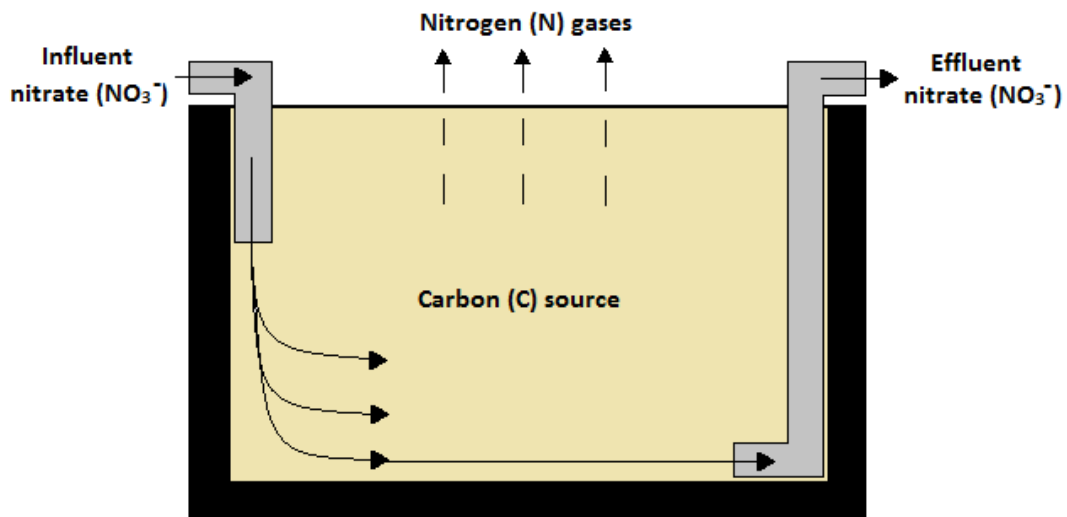
Figure 2.3: A diagram of a denitrification bed (Modified from Schipper *et al.* (2010b)).

As denitrifying bioreactors have been implemented as a method of reducing excess NO_3^- reaching downstream ecosystems, the majority of research on bioreactors has focussed on the efficiency on NO_3^- removal. In denitrification beds, NO_3^- removal rates have been reported to range from about $2 - 22$ g N m^{-3} day^{-1} (Blowes *et al.*, 1994; Robertson *et al.*, 2000; van Driel *et al.*, 2006; Schipper *et al.*, 2010a), with variations removal rates suggested to be the result of influent NO_3^- concentrations, available C source concentrations and bed temperature. In

denitrification walls NO_3^- rates have been reported to range from $0.014 - 3.6$ g N m^{-3} day^{-1}, with influent NO_3^- concentrations and wall material (as wood media is commonly in a mixture with soil which is unreactive) suggested to be the cause of the variations (Schipper *et al.*, 2010b).

The influence of O_2, NO_3^- and C concentrations on the rate of NO_3^- removal in denitrifying bioreactors is reasonably well understood. However, the temperature dependence of denitrification is not as well understood. Bioreactors have been, and continue to be implemented across the globe in a range of environments with different temperature regimes as awareness of NO_3^- as an environmental pollutant and of bioreactors as a method to remove NO_3^- spread. As such, understanding the temperature response of denitrification is important to ensure that bioreactors are designed to match predicted NO_3^- concentrations.

In perhaps the earliest study, Blowes *et al.* (1994) investigated the ability of two pilot scale bioreactors containing sand and organic C (bark, wood chip and leaf compost) to remove NO_3^- from agricultural tile drainage in Ontario, Canada. Over one year, influent NO_3^- concentrations of $3 - 6$ mg N L^{-1} were reduced to < 0.002 mg N L^{-1} within the bioreactors. The temperature of the effluent ranged from $5 - 19$ °C and the observation of NO_3^- removal in temperatures below 8 °C lead to the suggestion that bioreactors could be used to remove NO_3^- throughout the growing season Blowes *et al.* (1994).

Robertson and Cherry (1995) investigated the ability of three pilot scale bioreactors containing organic C (sawdust) to remove NO_3^- from septic system drainage in Ontario, Canada. At the Killarney and Borden sites, the bioreactors were installed as denitrification layers and at the Long Point site, the bioreactor was installed as a denitrification wall. Influent NO_3^- concentrations of 125 and 0.26 mg N L^{-1} were reduced to 1.2 and < 0.05 mg N L^{-1} within the Killarney and

Borden layers, respectively. Similarly, influent NO_3^- concentrations of 57 – 62 mg N L^{-1} were reduced to 2 – 25 mg N L^{-1} within the Long Point wall. In addition, Robertson and Cherry (1995) investigated the longevity of NO_3^- removal in bioreactors and, using mass balance calculations, suggested that bioreactors could effectively remove NO_3^- from effluent with no maintenance for a number of years.

In 2000, Robertson *et al.* investigated the long-term ability of pilot scale bioreactors to remove NO_3^- from septic system and agricultural tile drainage by revisiting the Killarney, Borden and Long Point sites (Robertson and Cherry, 1995), in addition to the previously unreported North Campus site. At the North Campus site, the bioreactor was installed as a denitrification bed containing organic C (wood mulch) to remove NO_3^- from agricultural tile drainage. Over the course of four years, the influent NO_3^- concentration of 4.8 mg N L^{-1} was reduced to 2.0 mg N L^{-1} in the North Campus bed. The NO_3^- removal in the bed was observed to be temperature dependent; ranging from about 5 mg N L^{-1} per day at temperatures of about 2 – 5 °C to about 15 – 30 mg N L^{-1} at temperatures of about 10 – 20 °C. A Q_{10} of 1.7 (Table 2.1) was calculated in this study using the linear regression fitted to the rate of NO_3^- removal and temperature (NO_3^- removal = 4.9 + 0.93T; R^2 = 0.55) between 10 – 20 °C. The linear regression fitted to the temperature dependency of NO_3^- removal did not include samplings where the NO_3^- concentration was thought to be limiting. In support of the suggestion of Robertson and Cherry (1995) regarding the longevity of the performance of bioreactors, the NO_3^- removal rate in the North Campus bed was observed to remain similar throughout the four years of the investigation.

Table 2.1: Site characteristics and temperature dependency of the rate of NO_3^- removal in previous studies of denitrifying bioreactors.

Reference	Location	Design	Size (m^3)	Influent NO_3^- concentration (g N m^{-3})	Effluent NO_3^- concentration (g N m^{-3})	Temperature range (°C)	Q_{10}
Robertson *et al.* (2000)	Ontario, Canada	Bed	2	4.8	2	2 – 20	1.7[b]
van Driel *et al.* (2006)	Ontario, Canada	Layer (lateral flow)	17	11.8	8.0	2.3 – 13	2.7[b]
	Ontario, Canada	Layer (upflow)	16	3.2	1.6	1.2 – 30	3.7[b]
Robertson *et al.* (2008)	Ontario, Canada	Wall[a]	10	9.7	5.9	6 – 22	5[b]
Robertson and Merkley (2009)	Ontario, Canada	Bed (stream)	40	4.8	1.0	3 – 14	3.2[b]
Elgood *et al.* (2010)	Ontario, Canada	Bed (stream)	40	2.8	1.3	1 – 26	2
Cameron and Schipper (2010)	Wairakei, New Zealand	Mesocosms	0.2	~150	~140	14 – 23.5	1.6
Warneke *et al.* (2011a)	Karaka, New Zealand	Bed	1320	~250	~50	15.5 – 23.7	2
Christianson *et al.* (2012)	Iowa, USA	4 x Bed	18 - 128	3.9 – 11.6	2.24 – 10.1	< 3 - > 15	0.8 – 5.7
Schmidt and Clark (2013)	Florida, USA	Mesocosms	0.03	7.5	4.6	7.9 – 24.1	4.7

[a] Columns extracted from wall
[b] Calculated in this study

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van Driel *et al.* (2006) investigated the ability of two field scale bioreactors to remove NO_3^- from agricultural tile drainage in Ontario, Canada. Two different denitrification layer configurations were investigated which consisted of alternate layers of sawdust and wood chip. In the lateral flow bioreactor, a sawdust layer overlay and underlay a wood chip layer. In the upflow bioreactor, a sawdust layer overlay a wood chip layer to promote vertical movement of effluent through the layer. The average influent NO_3^- concentrations of 11.8 and 3.2 mg N L^{-1} were reduced to 8.0 and 1.6 mg N L^{-1} in the lateral flow and upflow bioreactors, respectively. In addition, the rate of NO_3^- removal was observed to be temperature dependent for both bioreactors and ranged from about 2 – 7 mg N L^{-1} per day at temperatures of 2 – 5 °C to about 4 – 20 mg N L^{-1} at temperatures of 10 – 13 °C. Q_{10} values of 2.7 and 3.7 (Table 2.1) for the lateral and upflow bioreactors, respectively, were calculated in this study using the exponential regressions fitted to the rate of NO_3^- removal and temperature (lateral flow NO_3^- removal $= 2.9e^{0.10T}$; $R^2 = 0.25$; upflow NO_3^- removal $= 2.3e^{0.13T}$; $R^2 = 0.46$) between 3 – 13 °C. The exponential regressions fitted to the temperature dependency of NO_3^- removal did not include samplings where the NO_3^- concentration was thought to be limiting (van Driel *et al.*, 2006).

In 2008, Robertson *et al.* investigated the longevity of the performance of a bioreactor to remove NO_3^- from septic system drainage by revisiting the Long Point site (Robertson and Cherry, 1995; Robertson *et al.*, 2000). Dynamic flow tests were undertaken using cores of media extracted from the 15-year-old wall and compared to results from similar tests undertaken using fresh samples of media prior to the installation of the wall. The rates of NO_3^- removal obtained using the 15-year-old media were within 50 % of the rate obtained using the fresh samples and were observed to be temperature dependent; ranging from 0.2 – 1.1

N mg L^{-1} per day at 6 – 10 °C to 3.5 – 6.0 mg N L^{-1} per day at 20 – 22 °C. A Q_{10} of 5.0 (Table 2.1) was calculated in this study using the exponential regression fitted to the rate of NO_3^- removal and temperature (NO_3^- removal = $0.17e^{0.16T}$; R^2 = 0.96) between 12 – 22 °C. The rate of NO_3^- removal obtained using the 15-year-old media was not limited by NO_3^- concentration.

Robertson and Merkley (2009) investigated the ability of a stream bed bioreactor containing C (wood chip) to remove NO_3^- from agricultural tile drainage in Ontario, Canada. The average influent NO_3^- concentration of 4.8 mg N L^{-1} was reduced to 1.0 mg N L^{-1} in the stream bed bioreactor. The rate of NO_3^- removal was observed to be temperature dependent, with generally complete NO_3^- removal (< 0.1 mg N L^{-1}) occurring in the warmer seasons (> 10 °C) and incomplete NO_3^- removal (1 – 5 mg N L^{-1}) occurring in the colder seasons as a results of lower removal rates. A Q_{10} of 3.2 (Table 2.1) was calculated in this study using the linear regression fitted to the rate of NO_3^- removal and temperature (NO_3^- removal = 8.8 + 13.6T; R^2 = 0.46) between 4 – 14 °C. The linear regression fitted to the temperature dependency of NO_3^- removal did not include samplings where the NO_3^- concentration was thought to be limiting (Robertson and Merkley, 2009).

In 2010, Elgood *et al*. investigated the ability of a stream bed bioreactor to remove NO_3^- and produce GHG's (N_2O and methane, CH_4) by revisiting the bioreactor previously reported by Robertson and Merkley (2009). Over the course of a year, influent NO_3^- concentrations of 0.3 – 5.8 mg N L^{-1} were reduced to < 0.01 – 3.9 mg N L^{-1}. The temperature of the effluent ranged from 1 – 26 °C and, similarly to the previous study by Robertson and Merkley (2009), the rate of NO_3^- removal was observed to be temperature dependent with greater removal rates occurring in the warmer seasons and lower removal rates occurring in the colder

seasons. A Q_{10} of 2 (Table 2.1) was calculated in the study by Elgood *et al.* (2010) using a linear regression fitted to the rate of NO_3^- removal and temperature (NO_3^- removal $= 246 + 54T$; $R^2 = 0.39$) between $5 - 15$ °C. Similarly to the previous study of the bioreactor by Robertson and Merkley (2009), the linear regression fitted to the temperature dependency of NO_3^- removal did not include samplings where the NO_3^- concentration was thought to be limiting (Elgood *et al.*, 2010).

Cameron and Schipper (2010) investigated the ability of organic C (wood chip, maize cobs, wheat straw and green waste) to remove NO_3^- at two temperatures treatments (14 and 23.5 °C). Over the course of 10 - 23 months, the average rates of NO_3^- removal were 3.0 and 4.9 g N m^{-3} day^{-1} for softwood wood chips, 3.3 and 4.4 g N m^{-3} day^{-1} for hardwood wood chips, 19.8 and 15 g N m^{-3} day^{-1} for maize cobs, 5.8 and 7.8 g N m^{-3} day^{-1} for wheat straw and 7.8 and 10.5 g N m^{-3} day^{-1} for green waste for the 14 and 23.5 °C treatments. The rate of NO_3^- increased with increasing treatment temperature and was greater for the more labile C sources (maize cobs > green waste > wheat straw > wood chip). An average Q_{10} of 1.6 was calculated in the study by Cameron and Schipper (2010) using the average NO_3^- removal rates and treatment temperatures. However, the more labile C sources in the 23.5 ˚C treatment are likely to have undergone more degradation, as a result of the warmer temperature, in comparison to the C sources in the 14 ˚C treatment and were likely to support a lower NO_3^- removal rate. This may have resulted in an underestimate of the temperature response and the calculation of a lower Q_{10} value. The rate of NO_3^- removal was not limited by NO_3^- concentration.

Warneke *et al.* (2011a) investigated the ability of a denitrification bed containing organic C (wood chip and sawdust) to remove NO_3^- and produce GHG's from hydroponic glasshouse effluent in Karaka, New Zealand. Over the

course of the investigation, the average rate of NO_3^- removal was 7.6 g N m^{-3} d^{-1} and ranged from 4.6 – 11.2 g N m^{-3} d^{-1}. The temperature of the effluent ranged from 15.5 – 23.7 °C. The rate of NO_3^- removal was observed to be temperature dependent and generally increased with increasing temperature. A Q_{10} of 2 (Table 2.1) was calculated in the study by Warneke *et al.* (2011a) using an exponential regression fitted to the rate of NO_3^- removal and temperature ($y = 2.23 + 0.07x$; $R^2 = 0.91$; $p = 0.009$) between 15.5 – 23.7 °C. The rate of NO_3^- removal obtained from the bed was not limited by NO_3^- concentration.

Christianson *et al.* (2012) investigated the controls influencing the ability of four denitrification beds containing C (wood chip and mulch) to remove NO_3^- from agricultural tile drainage in Iowa. Over the course of the investigation, the average influent NO_3^- concentrations of 3.9 mg N L^{-1} in the Pekin bed, 11.6 mg N L^{-1} in the Northeast Research and Demonstration Farm (NERF) bed, 10.8 mg N L^{-1} in the Greene County bed and 8.7 mg N L^{-1} in the Hamilton County bed were reduced to 2.2, 10.1, 5.3 and 2.2 mg N L^{-1}, respectively. The temperature range from < 3 – > 15 °C and rate of NO_3^- removal was observed to increase with temperature. The Q_{10} values were calculated to range from 0.18 – 5.7 (Table 2.1) for the four beds in the study by Christianson *et al.* (2012). The rate of NO_3^- removal was not limited by NO_3^- concentration.

Schmidt and Clark (2013) investigated the controls influencing the ability of mesocosms containing organic C (sawdust) to remove NO_3^- from agricultural drainage in Alachua, Florida. The average influent NO_3^- concentration of 7.5 ± 0.73 mg N L^{-1} was reduced to 4.6 ± 3.6 mg N L^{-1} within the mesocosms. The temperature of the effluent ranged from 7.9 – 24.1 °C and the rate of NO_3^- removal was observed to be temperature dependent and generally increase with increasing temperature. A Q_{10} of 4.7 (Table 2.1) was calculated in the study by

Schmidt and Clark (2013) using the exponential regression fitted to the rate of NO_3^- and temperature ($y = 0.15e^{0.16x}$; $R^2 = 0.87$). The rate of NO_3^- removal obtained from the mesocosms was not limited by NO_3^- concentration. Schmidt and Clark (2013) suggested that the Q_{10} values of observed for the rate of NO_3^- removal which are greater than the Q_{10} value of 2 observed for other biological processes are the result of a 'synergistic response' between the rate of NO_3^- removal and processes which increase the availability of the C source with increasing temperatures.

To summarise, previous studies on denitrifying bioreactors have commonly observed the rate of NO_3^- removal to be temperature dependent and increase with increasing temperature. The Q_{10} values calculated for the temperature dependency of denitrification in previous studies of bioreactors range from 0.18 – 5.7 (Christianson *et al.*, 2012), although are more commonly observed to range from 2 – 4 (van Driel *et al.*, 2006; Robertson and Merkley, 2009; Elgood *et al.*, 2010; Warneke *et al.*, 2011a). The rate of NO_3^- removal is influenced by controls including O_2 concentration, NO_3^- concentration and C source availability and as such, there is difficulty in determining the sole influence of temperature on the rate of denitrification in bioreactors in the field environment. However, further research is required as a significant influence of temperature on the rate of NO_3^- removal may have implications of the design of future bioreactors to ensure optimum performance (Christianson *et al.*, 2012)

Chapter 3 The temperature response of nitrate removal in denitrification beds

3.1 Abstract

Denitrification beds represent an inexpensive and effective strategy for the removal of nitrate (NO_3^-) from a range of wastewaters. Beds are essentially lined containers which are filled with a carbon (C) source to support denitrification; the conversion of NO_3^- to unreactive dinitrogen (N_2). In this study, three field scale denitrification beds in New Zealand receiving NO_3^- in wastewaters from a glasshouse (Karaka), campground (Motutere) and research station (Newstead) were monitored to determine the dependence of the NO_3^- removal rate to seasonal changes in temperature. Samples of wastewater were collected from wells along each bed every month, along with measurements of temperature. Nitrate concentrations declined along the length of each bed, with average removal rates of 3.6, 4.3 and 1.7 g N m^{-3} day^{-1} for Karaka, Motutere and Newstead, respectively. The rate of NO_3^- removal increased with increasing temperature at Karaka and Motutere, with Q_{10} values of 4.1 and 2.2, respectively. The bed at Newstead had been recently installed and there was no evidence of an increase in the rate of NO_3^- removal with temperature, with a consequent Q_{10} of 1.0. Nitrate was depleted in the beds at Motutere and Newstead and indicated that the calculated rates of removal were limited by NO_3^-. The rates of removal and Q_{10} values calculated for Karaka and Motutere were similar to those reported in previous studies both in New Zealand and internationally and confirmed that temperature is a major controller of NO_3^- removal in denitrification beds. This study highlighted the need for further research to constrain the range of Q_{10} values from which design decisions can be made to optimise NO_3^- removal in denitrification beds.

3.2   Introduction

Nitrogen (N) is an essential element for living organisms and is abundant within the biosphere (Canfield *et al.*, 2010). However, the majority of N is present as dinitrogen (N_2) which is unreactive and unavailable to the majority of organisms (Davidson and Seitzinger, 2006). In most unmanaged terrestrial ecosystems, the proportion of N which is biologically available to organisms restricts productivity and influences ecosystem structure and function (Vitousek *et al.*, 1997; Robertson and Vitousek, 2009). The addition of reactive nitrogen (N_r) to agricultural systems has enabled crop production to sustain human population growth (Robertson and Vitousek, 2009). However, the addition of N_r to agricultural systems in excess of plant and animal requirements is susceptible to loss from the system (Dinnes *et al.*, 2002). Loss of N_r from agricultural systems is of concern due to the ability of N_r to 'cascade' through atmospheric, terrestrial and aquatic ecosystems where N_r can cause or contribute to a range of environmental changes (Galloway *et al.*, 2003). Initially, the addition of N_r to ecosystems can result in an increase in productivity. Over time, however, the addition of N_r can result habitat degradation and a loss of biodiversity as well as subsequent loss of N_r to downwind and downstream ecosystems (Vitousek *et al.*, 1997; Galloway *et al.*, 2003).

The cascade of N_r ends with the return of N_2 to the atmosphere. Denitrification is one of the most important processes of permanent N_r removal in terrestrial ecosystems and is the conversion of nitrate (NO_3^-) to N_2 by microorganisms in the presence of a carbon (C) source (Davidson and Seitzinger, 2006). Denitrifying microorganisms (denitrifiers) are typically facultative aerobes and oxidise a C source for energy, using oxygen (O_2) as an electron acceptor (Knowles, 1982; Davidson and Seitzinger, 2006). However, under anaerobic conditions, denitrifiers can oxidise a C source using NO_3^- as an electron accepter instead (Knowles,

1982). The importance of a C source is dual as it promotes anaerobic conditions and acts as electron donor for denitrification (Barton *et al.*, 1999).

Denitrification occurs in most terrestrial and aquatic environments in the presence of NO_3^- and C and in the absence of oxygen (Davidson and Seitzinger, 2006). However, denitrification can be limited in agricultural systems by aerobic conditions and a lack of a suitable C source and limited in wetlands and riparian zones by a loss of hydrological connection (Barton *et al.*, 1999).

Denitrifying bioreactors represent a simple, inexpensive strategy for NO_3^- removal from a range of wastewaters and agricultural tile drainage through enhanced denitrification (Schipper *et al.*, 2010b). Denitrifying bioreactors overcome the aerobic conditions and C source limitations of agricultural systems by inclusion of a C source through which wastewater is passed (Robertson and Cherry, 1995) (Section 2.5.2.5). Denitrifying bioreactors are categorised by the hydrological connection between the wastewater and the C source and the ratio of the wastewater source area to the C source (Schipper *et al.*, 2010b). Denitrification beds are a design of bioreactor where the C source is incorporated into a container or lined trench through which effluent or groundwater flows (Blowes *et al.*, 1994; Robertson *et al.*, 2000).

A wide range of NO_3^- removal rates have been reported for denitrification beds, which appears to be in part a result of varying NO_3^- concentrations and operating temperatures. The rate of denitrification is known to increase with increasing temperature yet the exact nature of the relationship is not well understood (Davidson and Seitzinger, 2006). One of the reasons for this lack of understanding is that the rate of NO_3^- removal in denitrification beds is influenced by several controls, not just temperature, which makes unravelling the sole influence of temperature field scale beds difficult. Denitrification beds are being

implemented across the world and as such further understanding of the temperature response of denitrification is required to determine whether beds constructed in cooler environments need to be of larger size to remove NO_3^- as efficiently as beds constructed in warmer environments.

Previous studies on denitrifying bioreactors have reported the rate of denitrification to increase with increasing temperature (Robertson *et al.*, 2000; van Driel *et al.*, 2006; Robertson *et al.*, 2008; Robertson and Merkley, 2009). In addition, some studies have reported the temperature response as a Q_{10} value, which represents the factor by which the rate of denitrification increases for a 10 °C increase in temperature and allows comparisons between studies (Davidson and Janssens, 2006). Elgood *et al.* (2010) reported a Q_{10} of 2 in an investigation into the rate of NO_3^- removal and greenhouse gas (GHG) production in a stream-bed bioreactor in Ontario, Canada. In a similar study, Warneke *et al.* (2011a) reported a Q_{10} of 2 in a denitrification bed in Karaka, New Zealand. In an investigation into the influence of various controls (including NO_3^- concentration and C source availability) on the rate of NO_3^- removal, Christianson *et al.* (2012) reported a Q_{10} range of $0.18 - 5.7$ in four denitrification beds in Iowa. In a similar study, Schmidt and Clark (2013) reported a Q_{10} of 4.7 in mesocosms in Florida.

This study investigated the temperature dependency of the rate of denitrification in three field scale denitrification beds in New Zealand, including the bed previously investigated by Warneke *et al.* (2011a). The rate of NO_3^- removal was determined by analysing effluent samples collected monthly from wells along the length of each bed. Temperature measurements collected during effluent sampling were combined with NO_3^- removal rates to determine the temperature response of denitrification in each bed as temperature changed

seasonally. The results from the denitrification bed at Karaka were compared to a previous study at the bed by Warneke *et al.* (2011a).

3.3   Methodology

3.3.1   Site descriptions

3.3.1.1 Karaka

This denitrification bed was installed in 2006 at a site receiving hydroponic glasshouse effluent in Karaka, North Island, New Zealand as originally described by Schipper *et al.* (2010a) (Figure 3.1). The denitrification bed was constructed by excavating a trench (141 m long x 7.8 m wide x 1.5 m deep) which was lined with plastic and backfilled with an even ratio of coarse sawdust and wood chips of the softwood *Pinus radiata*.

Figure 3.1: The denitrification bed at Karaka with glasshouses (Photo: L. Schipper).

The majority of N in the glasshouse effluent was in the form of NO_3^- (Schipper *et al.*, 2010a) and, prior to discharge, was stored in a settling pond and received no other pre-treatment. Effluent was pumped into the bed at one end through an inlet pipe (150 mm diameter) and left the bed at the other end through

four pipes (150 mm diameter) connected through T-junctions to an outlet pipe (150 mm diameter). Records of the flow rate of effluent through the bed were provided by glasshouse staff and varied daily and seasonally depending on glasshouse requirements. Effluent was discharged from the outlet pipe to a holding pond prior to irrigation onto surrounding farmland. Twelve polyvinyl chloride (PVC) pipes (50 mm diameter) were installed at even intervals (of about 12 m) along the length of the bed for sampling effluent passing through the bed which was analysed for NO_3^-. The PVC pipes were capped to prevent wood chip from entering the pipe and slotted to allow effluent to flow into the pipe (Figure 3.2 a). The PVC pipes were installed by hand or using a wooden mallet and wooded block to half the depth of the bed (Figure 3.2 b).

Figure 3.2: a) A capped and slotted PVC pipe and b) installation of a PVC pipe using a wooden mallet and wooden block (Photo: A. Keyte Beattie).

The denitrification bed at Karaka was previously sampled by Schipper *et al.* (2010a) to investigate the rate of NO_3^- removal and by Warneke *et al.* (2011a) to investigate the influence of controls on denitrification (temperature, pH and O_2 and C concentrations) on the rate of NO_3^- removal and in the bed over the course of a year.

3.3.1.2 Motutere

This denitrification bed was installed in 2007 at a site receiving municipal effluent in Motutere, North Island, New Zealand (Ewert *et al.*, 2008) (Figure 3.3). The municipal effluent was largely generated by a small campground and consisted of wastewater from kitchens and ablution blocks (Ewert *et al.*, 2008). As is common with campgrounds, the amount of effluent generated varied seasonally and was greatest in the summer and lowest in the winter. The denitrification bed was constructed by excavating a trench (28 m long x 5.6 m wide x 1m deep) which was lined with plastic and was backfilled with wood chips of *P. radiata*.

Figure 3.3: The denitrification bed at Motutere.

Prior to discharge, the effluent received pre-treatment through submerged aerated filter (SAF) tanks which converted ammonium (NH_4^+) to NO_3^- and clarifiers which separated solids from effluent (Ewert *et al.*, 2008). Effluent was pumped into the denitrification bed at one end through an inlet pipe (150 mm diameter) connected through T-junctions to six pipes (150 mm) and left the bed at the other end through six pipes (150 mm) connected through T-junctions to an

outlet pipe (150 mm). Records of the flow rate of effluent through the bed were provided by Taupo District Council staff and varied seasonally depending on campground usage. Effluent was discharged from the outlet pipe and irrigated onto surrounding land. PVC pipes (50 mm diameter) were installed at even intervals (of about 4 m) along the length of the bed to sample NO_3^- concentrations using the same method of installation as outlined in 3.3.1.1. However, initial samplings at the site showed complete NO_3^- removal had occurred by the first well and prior to subsequent samplings a further three PVC pipes were installed at even intervals (of about 1 m) between the inlet and the first 4 m to improve the detection of changes in NO_3^- concentrations.

3.3.1.3 Newstead

This denitrification bed was installed in 2013 at a site receiving municipal effluent in Newstead, North Island, New Zealand (Figure **3.4**). The municipal effluent was generated by a research station and consisted of wastewater from laboratories and ablution blocks. The denitrification bed was constructed by excavating a trench (26 m long x 10.5 m wide x 1 m deep) which was lined with plastic and backfilled with wood chips of the softwood *P. radiata*. The wood chip was overlain with geotextile mesh and planting media consisting of sand and coco-peat.

Figure 3.4: Denitrification bed at Newstead (Photo: C. Tanner).

Prior to discharge, the effluent received pre-treatment through an aerated packed bed reactor (PBR) which converted NH_4^+ to NO_3^-. Effluent entered the denitrification bed at one end through an inlet pipe connected to a perforated drainage pipe and left the other end through a perforated drainage pipe connected to an outlet pipe. Records of the flow rate of effluent through the bed were provided by research station staff and varied depending on laboratory usage. Effluent was discharged from the outlet pipe and discharged onto surrounding land. Four PVC pipes (50 mm diameter) were installed at even intervals (of about 4 m) along the length of the bed between the inlet and outlet pipes to sample NO_3^- concentrations, using the same method of installation as outlined in 3.3.1.1. The denitrification bed at Newstead was recently installed and some start-up issues were identified which affected data (3.4.3).

3.3.2   Effluent and temperature sampling

The denitrification beds were sampled at monthly intervals in 2013; at Karaka from March to November, at Motutere from April to November and at Newstead

from August to December. Effluent samples were collected from PVC pipes installed along the length of the denitrification beds (3.3.1.1) using a simple hand pump and stored in plastic tubes (50 mL) on ice for transport back to the laboratory (Figure 3.5 a). During sampling, temperature measurements were recorded from the PVC pipes installed along the length of the beds using a calibrated temperature sensor (YSI 63) which was lowered into the bottom of each pipe (about 0.75 m at Karaka and 0.5 m at Motutere and Newstead) (Figure 3.5 b). Following transport back to the laboratory, the collected effluent samples were stored at 4 ˚C, filtered within 48 hours using 0.45 μm syringe filters (Minisart) and frozen until analysis.

Figure 3.5: a) Collection of effluent samples using hand pump (Photo: R. Carter) and b) collection of temperature measurements using temperature sensor in the denitrification bed at Karaka (Photo: R. Carter).

3.3.3   Effluent and temperature analysis

Effluent samples were transported to a commercial analytical firm (Hill Laboratories, Hamilton, New Zealand) for analysis of NO_3^- using ion chromatography following standard methods (APHA, 2005). NO_3^- removal rates

(g N m^{-3} d^{-1}) were calculated as the linear decline in NO_3^- concentration along the length of the bed (ΔNO_3^-) as:

$$NO_3^- \text{ removal rate} = \Delta NO_3^- \times FR/V_{bed}$$

where FR was the flow rate of effluent through the bed (m^3 d^{-1}) and V_{bed} was the effective volume of the bed (m^3) (Warneke *et al.*, 2011b). The V_{bed} was determined by multiplying the volume of the bed by an effective porosity value of 0.7 (van Driel *et al.*, 2006).

As a result of the variability in daily FRs at Karaka and Newstead, the FR on the day of sampling was unlikely to be representative of the FRs experienced during the week, or longer, prior to the day of sampling. Consequently, an average daily FR was calculated for Karaka and Newstead by adding the daily FRs through the bed until the sum of the FRs was equal to the total volume of effluent that would saturate the effective porosity of the bed (V_{bed}). This sum of the FRs was then averaged by the number of daily FRs included in the sum to calculate an average daily FR for the treatment of sampled effluent through the bed. The FR at Motutere was less variable than those of Karaka and Newstead and the FR at the time of sampling at Motutere was determined by averaging the monthly FRs through the bed calculate an average daily FR.

Sampling at Motutere and Newstead showed complete NO_3^- removal along the length of the denitrification bed. However, complete removal suggested that the rate of denitrification was limited by NO_3^- and to accurately calculate the temperature response of denitrification it is essential that NO_3^- is non-limiting. As such, the NO_3^- removal rate was calculated from the linear decline in NO_3^- concentration at Motutere and Newstead before complete NO_3^- removal.

The temperature sensitivity of NO_3^- removal in the denitrification beds was determined by calculating a Q_{10} value. A Q_{10} value represents the factor by which the rate of NO_3^- removal increased for a 10°C increase in temperature as:

$$Q_{10} = (R_2/R_1)^{[10/(T_2 - T_1)]}$$

where R_1 is the rate of NO_3^- removal at T_1, the temperature which is 10 °C less than T_2 and R_2 is the rate NO_3^- removal at T_2, the temperature 10 °C greater than T_1 (Davidson and Janssens, 2006; Davidson *et al.*, 2006). This formula also allows the calculation of a Q_{10} value in situations where the rates of NO_3^- removal are less than 10 °C apart. As there was little variation in the temperature along the length of the bed at each sampling, the average temperature of the bed at each sampling was used to calculate the temperature response of NO_3^- removal.

The temperature response of NO_3^- removal in the Karaka denitrification bed was compared to the temperature response of NO_3^- removal in the Karaka denitrification bed previously published by Warneke *et al.* (2011a).

3.4   Results

3.4.1   Karaka

In general, there was a linear decline in NO_3^- concentration along the length of the denitrification bed at Karaka for each month of sampling as was expected (Figure 3.6). Several of the declines were not significant ($p > 0.05$) which is likely to be the result of the variability in influent NO_3^- concentrations and FRs as opposed to a lack of denitrification. Consequently, all calculated linear declines were used in determining the temperature sensitivity of NO_3^- removal.
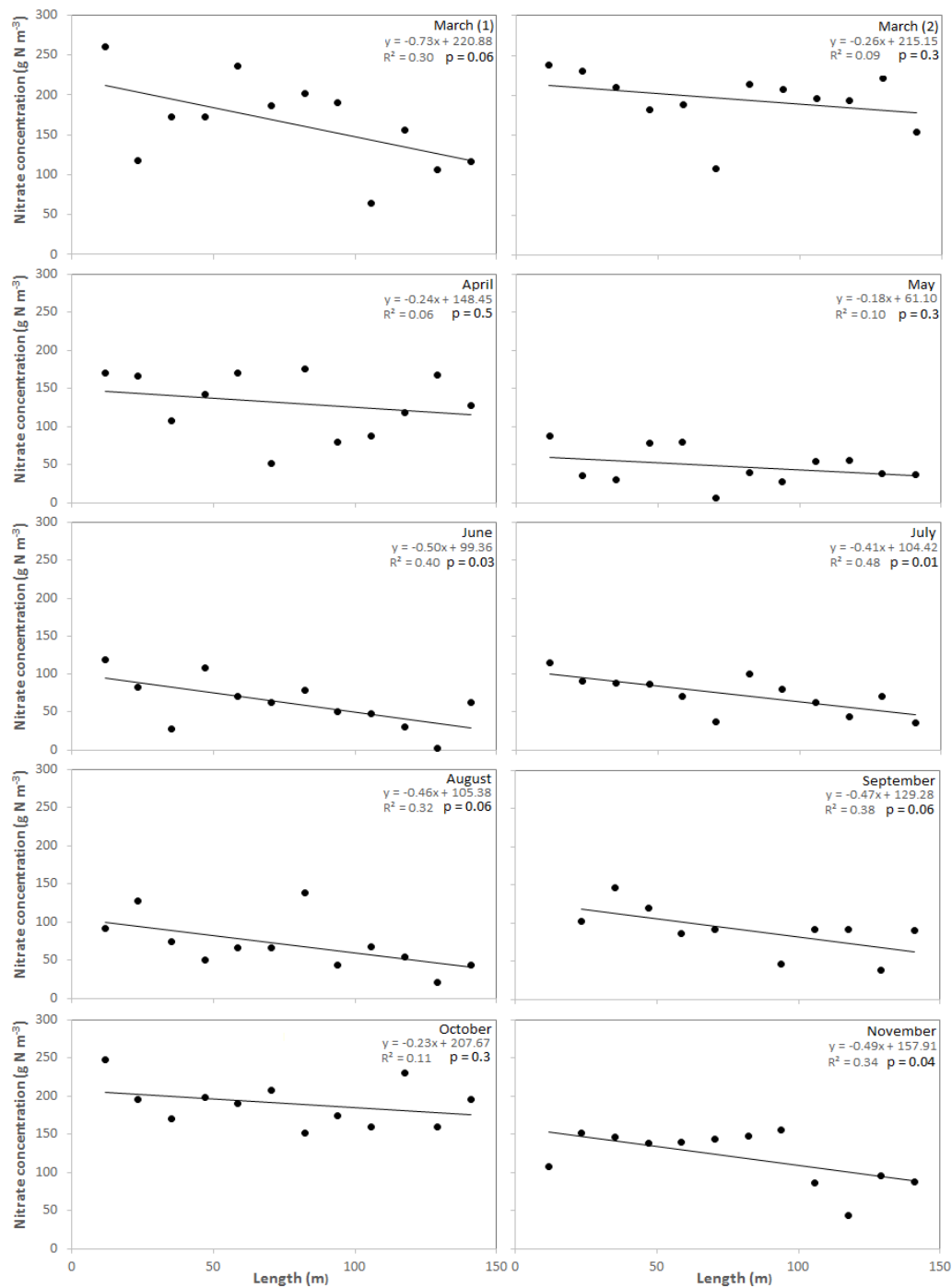
Figure 3.6: NO_3^- concentrations along the length of the denitrification bed at Karaka for each month of sampling, with two samplings in March as indicated by numbers. Linear regressions fitted.

The average rate of NO_3^- removal at Karaka was 3.6 g N m^{-3} day^{-1} and ranged from 0.9 g N m^{-3} day^{-1} in May to 11.9 g N m^{-3} day^{-1} in the first sampling in March (Table 3.1). The average temperature of the effluent in the bed at the time

of sampling was 16.9 °C and ranged from 12.0 °C in July to 21.9 °C in the first

sampling in March.

Table 3.1: Average temperature, flow rate, change in NO_3^- concentration and NO_3^- removal rate of the denitrification bed at Karaka for each month of sampling.

Month	Average temperature (°C)	Flow rate ($m^3 d^{-1}$)	Change in NO_3^- concentration (g N m^{-3})	NO_3^- removal rate (g N m^{-3} day^{-1})
March	21.9	189.5	0.7	11.9
March	20.9	229.6	0.3	5.1
April	18.7	135.0	0.2	2.7
May	15.9	58.7	0.2	0.9
June	13.2	36.9	0.5	1.6
July	12.0	44.4	0.4	1.6
August	14.1	44.3	0.5	1.7
September	15.2	63.4	0.5	2.6
October	16.7	125.0	0.2	2.5
November	20.1	124.9	0.5	5.2

The rate of NO_3^- removal in the denitrification bed at Karaka generally

increased with increasing effluent temperature (Figure **3.7**). A Q_{10} of 6.1 was

calculated using the exponential regression fitted to the rate of NO_3^- removal and

temperature for each month of sampling (NO_3^- removal = $0.12e^{0.18T}$; $R^2 = 0.71$; p

= 0.007). The first sampling in March yielded the highest NO_3^- removal rate of

11.9 g N m^{-3} per day and the highest average temperature of 21.9 °C. However,

prior to the first sampling in March there was a system blockage in the outlet of

the denitrification bed at Karaka which is likely to have resulted in abnormal

operating conditions. Based on the uncertainty of the validity of this data, a Q_{10} of

4.1 (Table 3.4) was calculated from the exponential regression fitted to the rate of

NO_3^- removal and temperature for each month of sampling except the first

sampling in March ($y = 0.22e^{0.14x}$; $R^2 = 0.61$; $p = 0.004$).
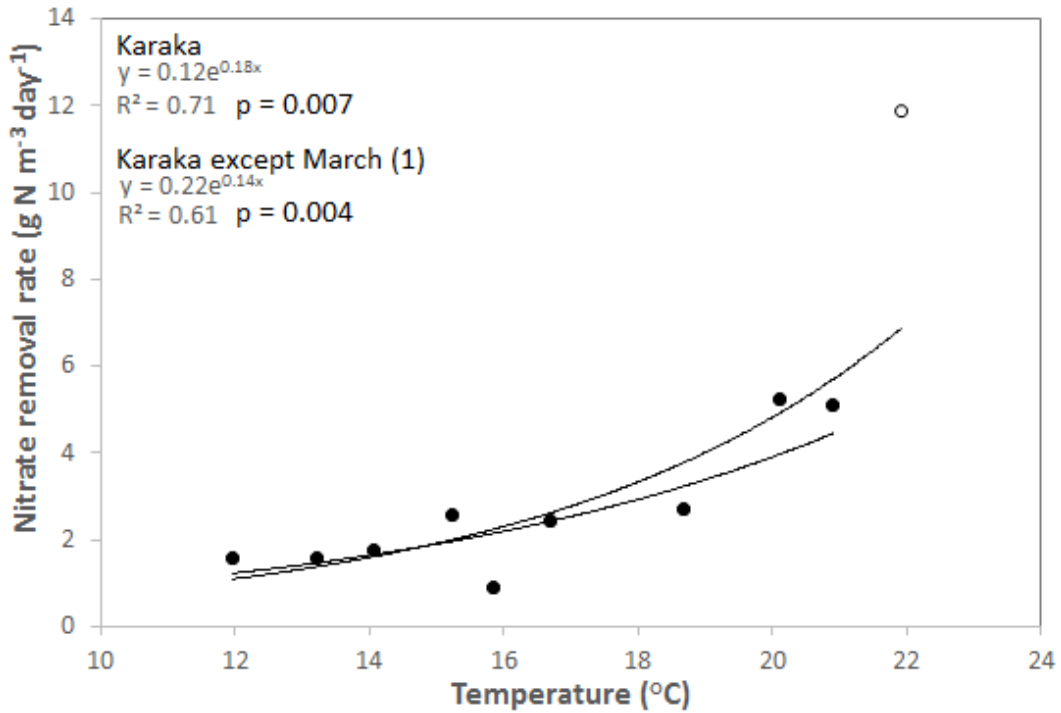
Figure 3.7: Temperature dependency of NO_3^- removal in the Karaka denitrification bed for each month of sampling. Exponential regressions fitted for each month of sampling and for each month of sampling except the first sampling in March (○).

In a previous study of the denitrification bed at Karaka by Warneke *et al.* (2011a) the rate of NO_3^- removal in the bed was strongly temperature dependent and generally increased with increasing temperature (Figure 3.8). However, prior to the sampling in December a substance (composition unknown) was applied to the glasshouse which is likely to have contaminated the denitrification bed at Karaka and resulted in abnormal operating conditions. Based on the uncertainty of the validity of this data, a Q_{10} of 2.0 (Table 3.4) was calculated using the exponential regression fitted to the rate of NO_3^- removal and temperature for each month of sampling except December (NO_3^- removal $= 2.23e^{0.07T}$; $R^2 = 0.92$; $p = 0.009$).
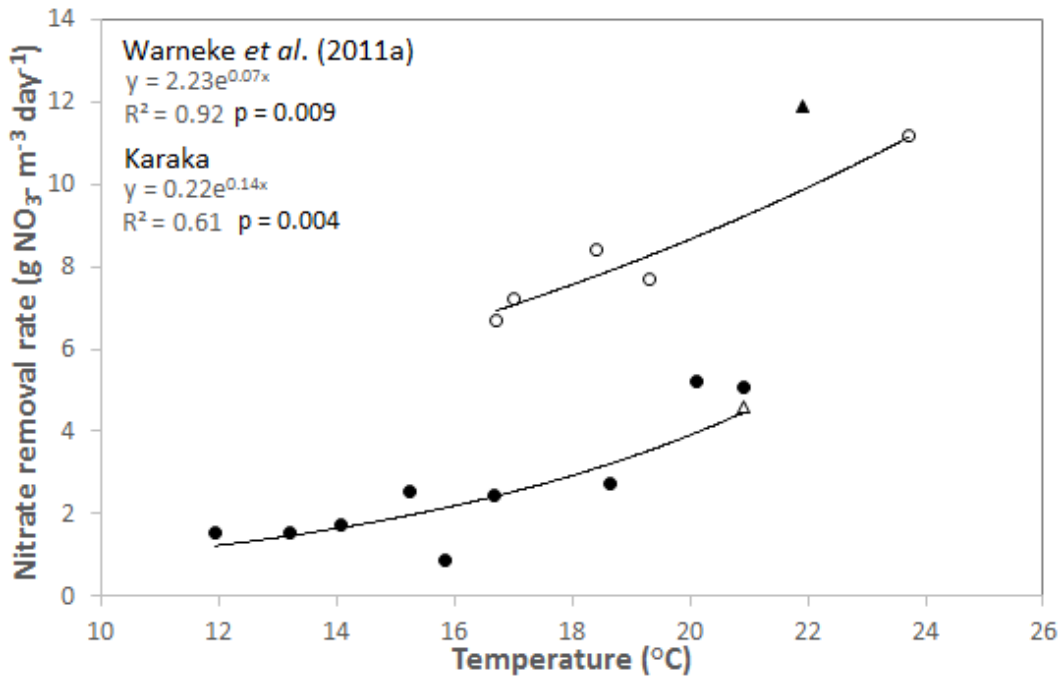
Figure 3.8: The temperature dependency of the rate of NO_3^- removal in the denitrification bed at Karaka for each month of sampling (●) and in the previous study by Warneke *et al.* (2011a) (○). Exponential regressions fitted do not include first sampling in March (▲) and, in keeping with previous study by Warneke *et al.* (2011a), do not include sampling in December (Δ).

3.4.2 Motutere

The NO_3^- concentration in the denitrification bed at Motutere declined rapidly between the inlet and the first sampling point, which meant that the calculation of NO_3^- removal rates would include a length of the bed where denitrification was limited by NO_3^- and the rate of removal would be underestimated. After the addition of a further three sampling points between the inlet and the original first sampling point, there was a strong linear decline in the NO_3^- concentration in the denitrification bed at Motutere between the inlet and the original first sampling point for each month of sampling (Figure **3.9**).

Figure 3.9: NO_3^- concentrations along the length of the denitrification bed at Motutere for each month of sampling. Linear regressions fitted do not include measurements after complete NO_3^- removal (○).

The average rate of NO_3^- removal at Motutere was 4.3 g N m^{-3} day^{-1} and ranged from 2.0 g N m^{-3} day^{-1} in August to 11.5 g N m^{-3} day^{-1} in April (Table 3.2). The average temperature of the effluent in the bed at the time of sampling was 14.1 °C and ranged from 9.4 °C in July to 19.7 °C in April.

Table 3.2: Average temperature flow rate, change in NO_3^- concentration and NO_3^- removal rate of the denitrification bed at Motutere for each month of sampling.

Month	Average temperature (°C)	Flow rate $(m^3 d^{-1})$	Change in NO_3^- concentration (g N m^{-3})	NO_3^- removal rate (g N m^{-3} day^{-1})
April	19.7	10.8	6.0	11.5
May	13.9	4.7	6.0	5.1
June	10.2	4.7	3.2	2.7
July	9.4	4.1	4.3	3.1
August	10.9	4.4	2.5	2.0
September	13.7	3.9	3.9	2.7
October	15.6	4.6	5.3	4.3
November	19.4	4.0	3.8	2.7

The rate of NO_3^- removal in the denitrification bed at Motutere generally increased with increasing effluent temperature (Figure 3.10). A Q_{10} of 2.2 (Table 3.4) was calculated using the exponential regression fitted to the rate of NO_3^- removal and temperature (NO_3^- removal $= 0.13e^{0.08T}$; $R^2 = 0.35$; $p = 0.1$). However, the rates of NO_3^- removal at temperatures above 19 °C (April and November) were very different without these samplings a Q_{10} of 2.5 was calculated.

Figure 3.10: The temperature dependency of the rate of NO_3^- removal at the denitrification bed at Motutere for each month of sampling. Exponential regression fitted.

3.4.3 Newstead

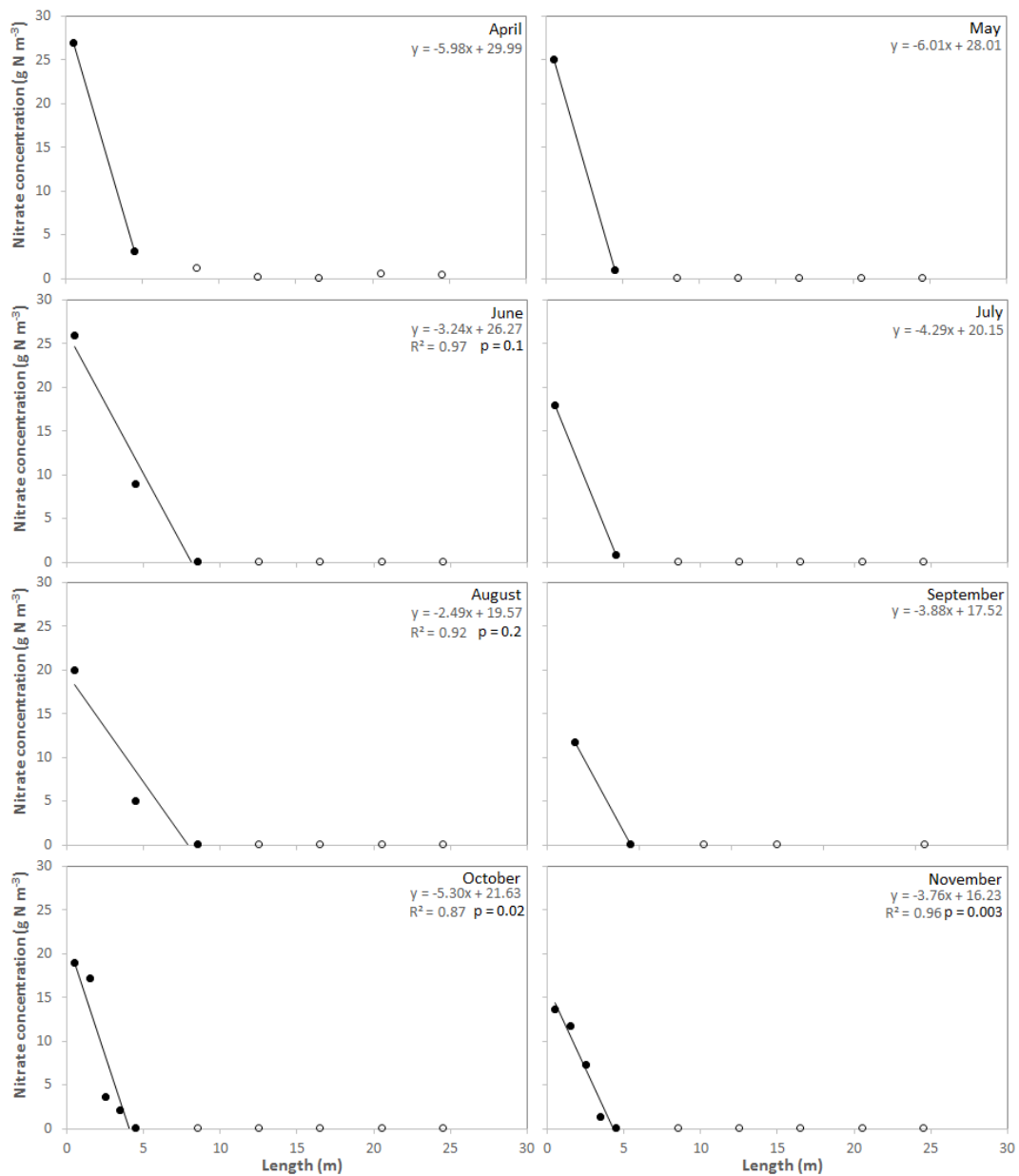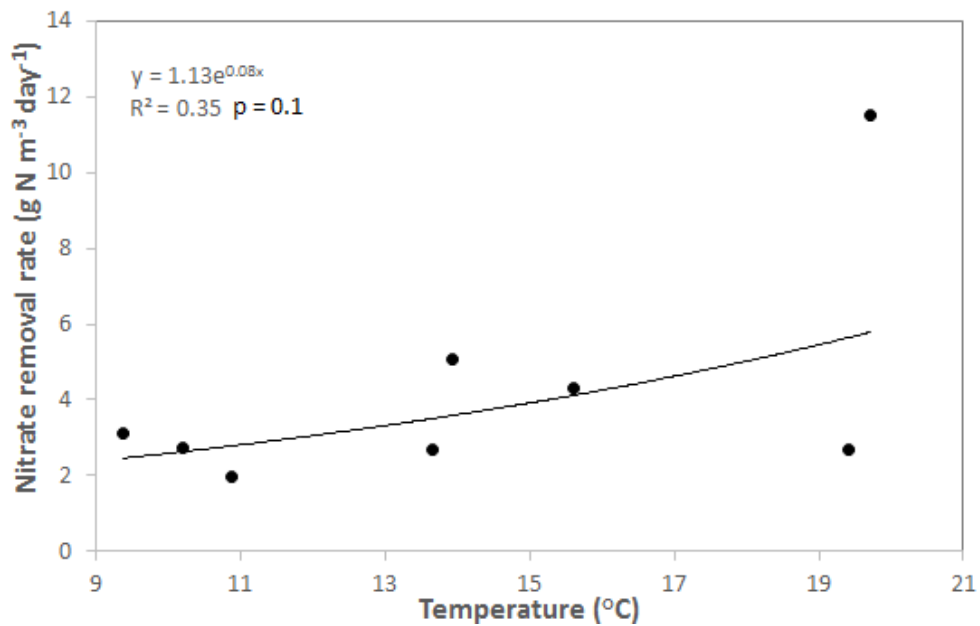The NO_3^- concentration in the denitrification bed at Newstead declined rapidly between the inlet and the first sampling point for each month of sampling (Figure 3.11). The exception was in September, when there was very little NO_3^- in the effluent entering the bed. NO_3^- concentrations were much lower than expected from the design which indicated that the pre-treatment system was not nitrifying effluent as well as expected.
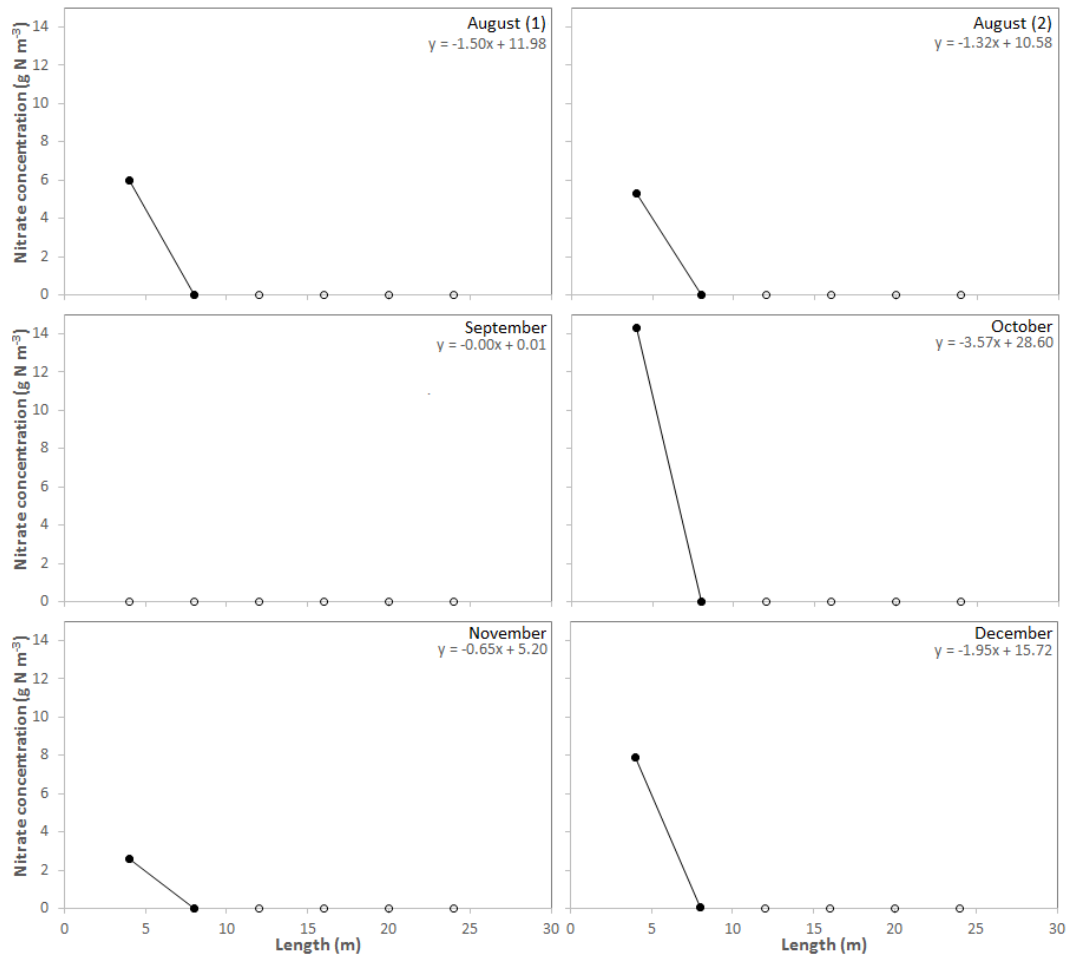
Figure 3.11: NO_3^- concentrations along the length of the denitrification bed at Newstead for each month of sampling, with two samplings in August as indicated by numbers. Linear regressions fitted do not include measurements after complete NO_3^- removal (○).

The average rate of NO_3^- removal was 1.7 g N m^{-3} day^{-1} and ranged from 0.00007 g N m^{-3} day^{-1} in September to 3.8 g N m^{-3} day^{-1} in October (Table 3.3).

The average temperature of the effluent in the bed at the time of sampling was 18.0 °C and ranged from 15.5 °C in August for the first sampling to 22.8 °C in November.

Table 3.3: Average temperature, flow rate, change in NO_3^- concentration and NO_3^- removal rate at the denitrification bed at Newstead for each month of sampling.

Month	Average temperature (°C)	Flow rate (m^3)	Change in NO_3^- concentration (g N m^{-3})	NO_3^- removal rate (g N m^{-3} day^{-1})
August (1)	15.5	12.7	1.5	1.8
August (2)	15.8	11.5	1.3	1.4
September	16.5	11.3	0.00007	0.00
October	19.2	11.2	3.6	3.8
November	22.8	21.3	0.7	1.3
December	22.8	10.92	2.0	2.0

There was no evidence that the rate of NO_3^- removal in the denitrification bed at Newstead was temperature dependent (Figure 3.12). A Q_{10} of 1.0 (Table 3.4) was calculated using the exponential regression fitted to the rate of NO_3^- removal and temperature (NO_3^- removal $= 1.88^{0.0006T}$; $R^2 = 0.00$; $p = 0.7$).
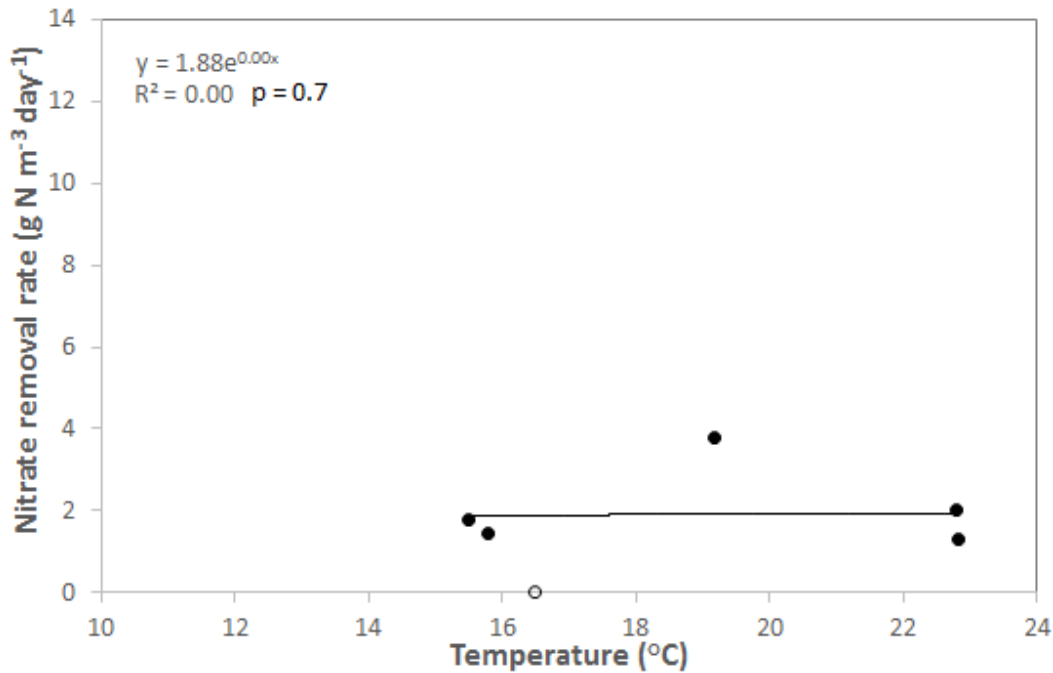
Figure 3.12: The temperature dependency of the rate of NO_3^- removal at the denitrification bed in Newstead. Exponential regression fitted does not include measurement in September (○).

3.4.4   The temperature response of the rate of nitrate removal in the denitrification beds at Karaka, Motutere and Newstead

The temperature dependency of the rate of NO_3^- removal varied both between studies in the denitrification bed at Karaka and between the denitrification beds at Karaka, Motutere and Newstead (Figure 3.13). The rate of NO_3^- removal was observed to be strongly temperature dependent in the previous study at Karaka (Warneke *et al.*, 2011a) ($R^2 = 0.92$) and moderately temperature dependent in this study at Karaka and at Motutere ($R^2 = 0.61$ and 0.35, respectively). There was no evidence that the rate of NO_3^- was temperature dependent at Newstead ($R^2 = 0.00$).
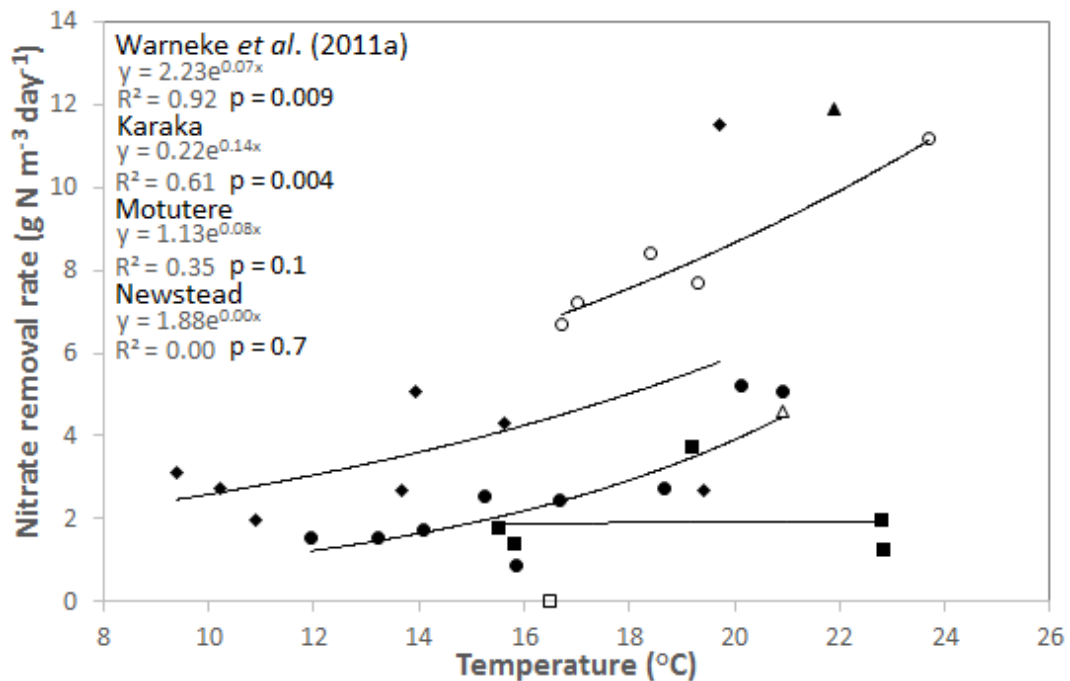
Figure 3.13: The temperature dependency of the rate of NO_3^- removal for each month of sampling in the denitrification bed at Karaka in this study (●) and in the previous study by Warneke *et al.* (2011a) (○), at Motutere (♦) and at Newstead (■). Exponential regressions fitted and do not include the first sampling in March at Karaka in this study (▲), the sampling in December in the previous study at Karaka by Warneke *et al.* (2011a) (∆) and the sampling in September at Newstead (□).

The Q_{10} values calculated from the exponential regressions of the temperature dependency of NO_3^- removal at each site varied between studies at Karaka and

between the denitrification beds at Karaka, Motutere and Newstead (Table 3.4).

The Q_{10} values ranged from 1.0 at Newstead to 4.1 at Karaka, with similar Q_{10}

values calculated in the previous study at Karaka (Warneke *et al.*, 2011a) and at

Motutere of 2 and 2.2, respectively.

Table 3.4: The Q_{10} calculated using the average NO_3^- removal rate and average temperature at Karaka in this study and the previous study by Warneke *et al.* (2011a) and at Motutere and Newstead.

Site	Influent NO_3^- concentration (g N m^{-3})	Effluent NO_3^- concentration (g N m^{-3})	Temperature range (°C)	Q_{10}
Karaka	156	95	13.2 – 21.9	4.1[a]
Karaka (Warneke *et al.*, 2011a)	~250	~50	15.5 – 23.7	2
Motutere	20	0.05	9.4 – 19.7	2.2
Newstead	7	0.01	15.5 – 22.8	1.0

[a] Calculated excluding first sampling in March

3.4.5 The temperature response of the rate of nitrate removal in denitrifying bioreactors

In previous studies of denitrifying bioreactors, the temperature dependency of the

rate of NO_3^- removal varied both between studies and sites (Table 3.5). The Q_{10}

values calculated from the regressions in each study and ranged from 0.8 to 5.7.

Table 3.5: Site characteristics and the temperature dependency of the rate of NO_3^- removal in this study in comparison to previous studies of denitrifying bioreactors.

Reference	Location	Design	Size (m³)	Influent NO_3^- concentration (g N m⁻³)	Effluent NO_3^- concentration (g N m⁻³)	Temperature range (°C)	Q_{10}
Robertson *et al.* (2000)	Ontario, Canada	Bed	2	4.8	2	2 – 20	1.7[b]
van Driel *et al.* (2006)	Ontario, Canada	Layer (lateral flow)	17	11.8	8.0	2.3 – 13	2.7[b]
	Ontario, Canada	Layer (upflow)	16	3.2	1.6	1.2 – 30	3.7[b]
Robertson *et al.* (2008)	Ontario, Canada	Wall[a]	10	9.7	5.9	6 – 22	5[b]
Robertson and Merkley (2009)	Ontario, Canada	Bed (stream)	40	4.8	1.0	3 – 14	3.2[b]
Elgood *et al.* (2010)	Ontario, Canada	Bed (stream)	40	2.8	1.3	1 – 26	2
Cameron and Schipper (2010)	Wairakei, New Zealand	Mesocosms	0.2	~150	~140	14 – 23.5	1.6
Warneke *et al.* (2011a)	Karaka, New Zealand	Bed	1320	~250	~50	15.5 – 23.7	2
Christianson *et al.* (2012)	Iowa, USA	4 x Bed	18 - 128	3.9 – 11.6	2.24 – 10.1	< 3 – < 15	0.8 – 5.7
Schmidt and Clark (2013)	Florida, USA	Mesocosms	0.03	7.5	4.6	7.9 – 24.1	4.7
This study	Karaka, New Zealand	Bed	1650	156	95	13.2 – 21.9	4.1[c]
	Motutere, New Zealand	Bed	157	20	0.05	9.4 – 19.7	2.2
	Newstead, New Zealand	Bed	273	7	0.01	15.5 – 22.8	1.0

[a] Columns extracted from wall
[b] Calculated in this study
[c] Calculated excluding first sampling in March

3.5 Discussion

3.5.1 Rates of nitrate removal

In general, the average rates of NO_3^- removal measured in the denitrification beds at Karaka, Motutere and Newstead (3.6, 4.3 and 1.7 g N m^{-3} day^{-1}, respectively) were similar to the rates of removal measured in denitrification beds in previous studies. The highest rates of NO_3^- removal (4 - 22 g N m^{-3} day^{-1}) were previously reported for a denitrification bed in Ontario, Canada (Blowes *et al.*, 1994; Robertson *et al.*, 2000). However, the majority of the rates of removal reported in other studies have been < 10 g N m^{-3} day^{-1}. For example, Robertson and Merkley (2009) and Elgood *et al.* (2010) reported NO_3^- removal rates of 3.8 g N m^{-3} day^{-1} and 0.3 - 2.5 g N m^{-3} day^{-1}, respectively, in separate studies of the same stream bed bioreactor in Ontario. Schipper *et al.* (2010a) and Warneke *et al.* (2011a) reported NO_3^- removal rates of 5 – 10 g N m^{-3} day^{-1} and 7.6 g N m^{-3} day^{-1}, respectively, in separate studies of the same denitrification bed in Karaka. Lastly, in a study of four denitrification beds in Iowa, Christianson *et al.* (2012) reported NO_3^- removal rates of 0.4 – 7.8 g N m^{-3} day^{-1}. The variation in the rates of NO_3^- removal reported in previous studies are likely the result of differences between sites and the influence of controls such as NO_3^- concentration, C source availability and operating temperature (Schipper *et al.*, 2010b).

3.5.2 The temperature response of nitrate removal

The importance of understanding the nature of the temperature response of NO_3^- removal was highlighted by Christianson *et al.* (2012), who reported that temperature was the main control influencing the rate of NO_3^- removal in four denitrification beds in Iowa. Furthermore, in a multivariate analysis Schmidt and Clark (2012) showed that temperature accounted for 50 % of the variation in the

rate of NO_3^- removal in mesocosms in Florida. In many other studies of denitrifying bioreactors, reported NO_3^- removal rates were limited by low NO_3^- concentrations (< 1 mg N L^{-1}) so that temperature effects were not always obvious. In order to disentangle the influence of temperature from other controls, the NO_3^- removal rates in the denitrification beds at Karaka, Motutere and Newstead were measured using sections of the beds that were not limited by low NO_3^- concentration (> 1 mg N L^{-1}). In general, the rate of NO_3^- removal increased with increasing temperature and the temperature response of NO_3^- removal measured in the denitrification beds at Karaka and Motutere (Q_{10} values of 4.1 and 2.2, respectively) were similar to the temperature response measured in previous studies of denitrification beds (Section 2.5.2.5; Table 3.6).

Table 3.6: Temperature dependency of the rate of NO_3^- removal in the current study in comparison to previous studies of denitrifying bioreactors.

Reference	Design	Influent NO_3^- concentration (g N m^{-3})	Effluent NO_3^- concentration (g N m^{-3})	Q_{10}
Robertson et al. (2000)	Bed	4.8	2	1.7[b]
van Driel et al. (2006)	Layer (lateral flow)	11.8	8.0	2.7[b]
	Layer (upflow)	3.2	1.6	3.7[b]
Robertson et al. (2008)	Wall[a]	9.7	5.9	5[a,b]
Robertson and Merkley (2009)	Bed (stream)	4.8	1.0	3.2[b]
Elgood et al. (2010)	Bed (stream)	2.8	1.3	2
Cameron and Schipper (2010)	Mesocosms	~150	~140	1.6
Warneke et al. (2011a)	Bed	~250	~50	2
Christianson et al. (2012)	4 x Bed	3.9 – 11.6	2.24 – 10.1	0.8 – 5.7
Schmidt and Clark (2013)	Mesocosms	7.5	4.6	4.7
This study	Bed	156	95	4.1[c]
	Bed	20	0.05	2.2
	Bed	7	0.01	1.0

[a] Columns extracted from wall
[b] Calculated in this study
[c] Calculated excluding first sampling in March

The highest and lowest temperature dependencies (Q_{10} values of 0.8 and 5.7, respectively) were reported for four denitrification beds in Iowa (Christianson *et al.*, 2012). It is noted that a Q_{10} of ~1 indicates that the rate of NO_3^- removal is not temperature dependent, which seems unlikely. However, the majority of the Q_{10} values reported for the temperature response of NO_3^- removal in previous studies are around $2 - 4$ (van Driel *et al.*, 2006; Robertson and Merkley, 2009; Elgood *et al.*, 2010; Warneke *et al.*, 2011a). For example, in the study of the denitrification bed in Ontario by Robertson *et al.* (2000), a Q_{10} value of 1.7 was calculated using the linear regression fitted to the temperature dependency of the rate of NO_3^- removal (NO_3^- removal = 4.9 + 0.93T; R^2 = 0.55) between 10 and 20 °C. In the study of the stream bed bioreactor by Robertson and Merkley (2009), a Q_{10} of 3.2 was calculated using the same method (NO_3^- removal = 8.8 + 13.6T; R^2 = 0.46) between 4 and 14 °C. In the subsequent study of the stream bed bioreactor by Elgood *et al.* (2010) a Q_{10} of 2 was reported (NO_3^- removal = 246.54 + 54T; R^2 = 0.39) between 5 and 15 °C.

In the previous study of the denitrification bed at Karaka, Warneke *et al.* (2011a) reported a Q_{10} of 2 (NO_3^- removal = $2.23e^{0.07T}$; R^2 = 0.91; p = 0.009) with an average NO_3^- removal rate of 7.6 g N m^{-3} day^{-1}. In the current study of the denitrification bed at Karaka, a much higher Q_{10} of 4.1 (NO_3^- removal = $0.22e^{0.14T}$; R^2 = 0.61; p = 0.004) was calculated with a much lower average NO_3^- removal rate of 3.6 g N m^{-3} day^{-1}. The discrepancies between the temperature responses and the rates of NO_3^- removal in two studies at Karaka may be partly due to seasonal and annual variability; however, temperature is not the only factor regulating NO_3^- removal. Nitrate removal in denitrification beds is also highly dependent on the availability of the C source (Cameron and Schipper, 2010) and the availability of the C source changes through time(Schipper and Vojvodic-

Vukovic, 1998; Robertson *et al.*, 2008; Moorman *et al.*, 2010; Robertson, 2010; Long, 2011). Wood particle media, commonly used as the C source in denitrification beds, supports NO_3^- removal for a considerable number of years but there is a well-documented decrease in removal rate with time (Robertson *et al.*, 2008; Moorman *et al.*, 2010; Robertson, 2010). This change in C availability likely contributed to the decline in NO_3^- removal from 7.6 to 3.4 g N m^{-3} day^{-1} and the change in Q_{10} through time at Karaka.

Robertson *et al.* (2008) reported that the rate of NO_3^- removal determined using sawdust from a wall that had been in operation for 15 year was around 50 % of the rate of NO_3^- removal determined using fresh sawdust. In addition, Robertson (2010) reported that the rates of NO_3^- removal determined using wood chips from denitrification beds that had been in operation for 2 and 7 years were around 50 % of the rate of NO_3^- removal determined using fresh wood chips. Robertson (2010) suggested that the reason for the decrease in the NO_3^- removal rate was the depletion of the more labile fraction of the C source and that there had been a relative increase in the more recalcitrant fraction of the C source. The denitrification bed at Karaka was installed in 2006 and rates of NO_3^- removal were initially measured in 2008/2009 (Warneke *et al.*, 2011a), five years before the current study in 2013. During this time it is likely that there was a decrease in the availability of the C source which contributed to the decline in the measured rates of NO_3^- removal in the bed at Karaka.

The temperature sensitivity of NO_3^- removal by denitrification is dependent on substrate complexity and concentration (Davidson and Janssens, 2006; Schmidt and Clark, 2013). With time, the C source in denitrification beds becomes more recalcitrant and more complex C sources are considered to have low rates of decomposition, high activation energies and, consequently, a higher

temperature sensitivity and a higher Q_{10} (Davidson and Janssens, 2006). As such, the greater Q_{10} for the NO_3^- removal rate in the current study in comparison to the previous study at Karaka by Warneke *et al.* (2011a) may be the result of the greater temperature sensitivity of the more recalcitrant fraction of aged wood chips in the bed.

In the denitrification bed at Motutere, the Q_{10} of 2.2 (NO_3^- removal = $1.13e^{0.08T}$; $R^2 = 0.35$; $p = 0.1$) and average NO_3^- removal rate of 4.3 g N m^{-3} day^{-1} were similar to the Q_{10} values and removal rates reported for previous studies of denitrification beds (Table 3.6). After the first few meters of the bed, the rate of NO_3^- removal at Motutere was likely limited by NO_3^- concentration as is frequently observed in denitrifying bioreactors (Schipper *et al.*, 2010b). Limited NO_3^- concentrations are common in studies where the NO_3^- concentration of effluent or groundwater from tile drainage is diluted prior to entering denitrification layers and stream bed bioreactors and is depleted along the length of the bioreactor (van Driel *et al.*, 2006; Robertson and Merkley, 2009).

In contrast to the denitrification beds at Karaka and Motutere, there was no evidence of an increase in the rate of NO_3^- removal with an increase in temperature at the denitrification bed at Newstead ($Q_{10} = 1.0$). Furthermore, the average NO_3^- removal rate of 1.7 g N m^{-3} day^{-1} was generally less than the rates of removal rates reported in previous studies of denitrification beds. The rate of NO_3^- removal at Newstead was almost certainly limited by NO_3^- concentration, as was observed at Motutere and in many other studies of denitrifying bioreactors (Schipper *et al.*, 2010b). Low NO_3^- concentrations would have resulted in the underestimation of the potential rate of removal. The pre-treatment system and denitrification bed at Newstead had been installed only a few month before sampling commenced. There was evidence that the denitrification bed was still in

the start-up phase as initial samplings of effluent from the wells were dark in colour, indicating leaching of dissolved C from the wood chips as effluent passed through the bed (Robertson and Cherry, 1995; Robertson *et al.*, 2005b). Furthermore, it appeared that the pre-treatment system, including the nitrification component, was not operating as planned since NO_3^- concentration entering the bed was low (average 7 g N m^{-3}).

Despite the variation in the range of Q_{10} values reported for the temperature response of NO_3^- removal in the current study and previous studies, it is recognised that temperature determines the extent to which denitrifying bioreactors can be implemented across the world (Christianson *et al.*, 2012; Schmidt and Clark, 2012). However, there is the potential to manipulate the temperature of denitrifying bioreactors. In a previous study, Cameron and Schipper (2011) investigated the influence of passive heating and different flow regimes on the rate of NO_3^- removal in denitrification beds. One of the attractions of denitrifying bioreactors is the low cost involved and as such, passive heating provides a more economically viable option in comparison to active heating. Passive heating was achieved by using dark material (black polythene and polyethylene tubing) laid on the bed surface to capture short-wave radiation emitted by the sun. Heat from long-wave radiation emitted by the dark material was then trapped using transparent material (polycarbonate sheets) laid on top of the black polythene and polyethylene tubing. Passive heating increased the bed temperature by around 3.4 °C; however, no significant difference was detected in the rate of NO_3^- removal between the passively heated and non-heating beds. It is likely that the variability in rate of NO_3^- removal obscured the increase in the rate of removal driven by temperature (Cameron and Schipper, 2011). As such, further

research on the potential for manipulating the temperature of denitrifying bioreactors to increase the rate of removal is required.

It is unlikely that the rate of NO_3^- removal and the temperature response of NO_3^- removal will remain constant with time. Comparing the results of this study at Karaka with the previous study by Warneke *et al.* (2011a), it is suggested that the rate of NO_3^- decreased with time as a result of the more recalcitrant fraction of the aged C source remaining in the bed. In addition, it is suggested that the temperature response of the rate of NO_3^- removal increase might with time as a result of the higher apparent temperature sensitivity of the more recalcitrant fraction of the C source (Figure 3.14).
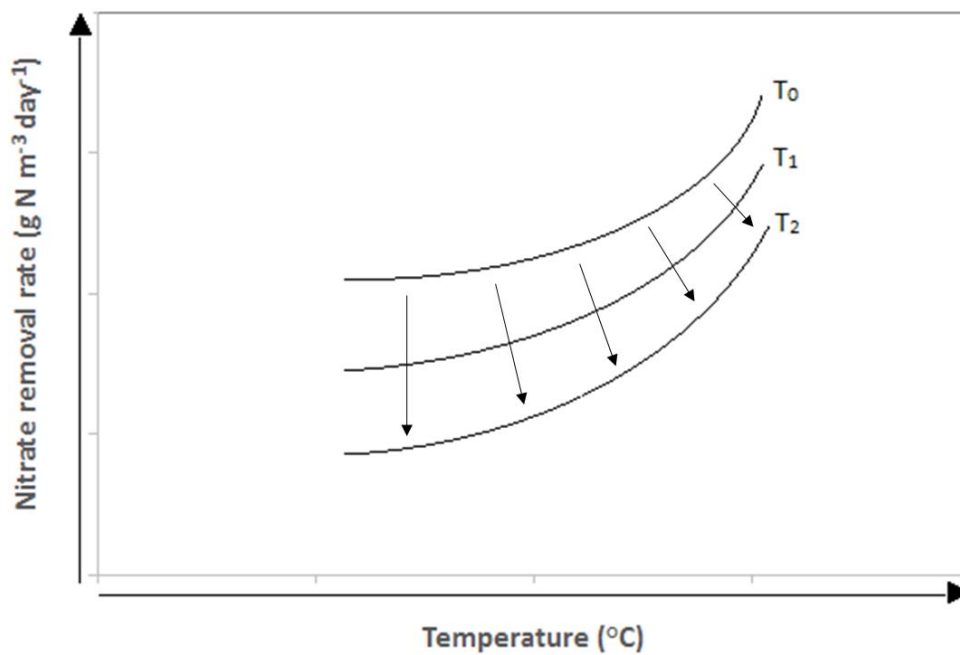
Figure 3.14: Conceptualised rate of NO_3^- removal and temperature response of NO_3^- in a denitrification bed with increasing time ($T_0 < T_1 < T_2$). Downwards arrows indicate change in rate through time, assuming NO_3 is not limiting, and curves indicate change in temperature sensitivity with time as remaining C becomes more recalcitrant (Davidson and Janssens, 2006).

Field based research is required to confirm whether the rate of NO_3^- removal decreases and whether the temperature sensitivity of NO_3^- removal increases with time. If this is confirmed, research is required to demonstrate whether the higher

apparent temperature sensitivity was the result of increasing recalcitrance of the remaining wood chips. Further research will also be required to confirm the timespan over which changes in the temperature response of NO_3^- removal occur. If the temperature dependency of NO_3^- removal does increase with time, the future design of denitrification beds will have to consider the proposed lifespan of the denitrifying bioreactor to ensure that the bioreactor is designed large enough to support NO_3^- removal for the proposed lifespan of the bioreactor.

3.5.3 Confounding influences and methodological limitations

Unravelling the influence of temperature on the rate of NO_3^- removal in denitrification beds in the field is difficult because of the variability of controls such as NO_3^- concentration and C source availability. Denitrification is assumed to follow Michelis-Menton kinetics and, as most bioreactors receive NO_3^- concentrations above the K_m value of denitrifiers (Barton *et al.*, 1999), denitrification is assumed to be zero-order (Schipper *et al.*, 2010b). Warneke *et al.* (2011a) suggested that the linear decline in NO_3^- concentration along the length of the denitrification bed at Karaka was evidence of zero-order kinetics as the effluent leaving the bed was much greater than the K_m value of denitrifiers. In addition, Warneke *et al.* (2011a) suggested that the increase in the rate of NO_3^- removal in laboratory studies of wood chip samples amended with C but not NO_3^- demonstrated that NO_3^- removal at Karaka was not limited by NO_3^- concentration. Robertson (2010) found that the rate of NO_3^- removal determined using fresh, 2- and 7-year-old wood chip samples amended with NO_3^- were zero-order until the concentration with which the samples were amended with was <1 mg N L^{-1}, after which NO_3^- removal was found to be first-order.

In the denitrification bed at Karaka, the rate of NO_3^- removal was clearly not limited by NO_3^- concentration and followed zero-order kinetics as the concentration of NO_3 in the effluent was much greater than the K_m of denitrifiers (Barton *et al.*, 1999). The lack of NO_3^- limitation meant that the denitrification bed at Karaka bed was suitable for determining *in situ* rate of NO_3^- removal and the temperature dependency of the rate of removal. However, in the denitrification beds at Motutere and Newstead, the rate of removal was limited by NO_3^- concentration and complete NO_3^- removal occurred within the first sampling points at each bed. To account for the limitation, rates of NO_3^- removal were calculated using only the lengths of each bed before complete NO_3^- removal. Even so, it was likely that the actual rates of NO_3^- removal were underestimated for the denitrification beds at Motutere and Newstead as NO_3^- concentration became limiting between sampling points (Figure 3.9; Figure 3.11).

In the denitrification bed at Newstead, there was very little NO_3^- present in the sampling in September. This low NO_3^- concentration suggested that microorganisms in the pre-treatment system, particularly in the nitrifying component which converts NH_4^+ to NO_3^- prior to discharge into the bed, had been poisoned through the use of a substance (composition unknown) in the research station. This potential poisoning was investigated in a separate study. Similarly, in the previous study at Karaka by Warneke *et al.* (2011a) a relatively low NO_3^- removal rate (4.6 g N m^{-3} day^{-1}) in comparison to the average removal rate (7.6 g N m^{-3} day^{-1}) was determined for a single sampling in December. Warneke *et al.* (2011a) suggested that microorganisms were poisoned though the use of substance (composition unknown) in the glasshouse that entered the effluent stream (Warneke *et al.*, 2011a) although this was not confirmed.

In addition to NO_3^- concentration, certainty regarding the FR and daily variations in FR were important for calculating the rate of NO_3^- removal (Robertson and Merkley, 2009). The FRs through the denitrification beds at Karaka and Newstead were variable as a result of production requirements and it was unlikely that the FR at the time of sampling was representative of the FRs experienced during the week, or longer, prior to sampling. Furthermore, daily variation in NO_3^- concentration coupled to variations in FR are not well captured on a single sampling day. The FR determines the residence time of effluent within the bed and in turn the interaction between the effluent and the C source which determines NO_3^- removal. An average daily FR was calculated for the denitrification beds at Karaka, Motutere and Newstead. The intention was this average FR would be a better representation of the general FR of effluent through the bed as opposed to the FR through the bed on the day of sampling. The rates of NO_3^- removal were calculated as:

$$NO_3^- \text{ removal rate} = \Delta NO_3^- \text{ x } FR/V_{bed}$$

and as such, the uncontrolled errors in the average daily FR calculated for each denitrification bed had an influence on rate of removal and in turn the temperature response determined for the denitrification beds at Karaka and Newstead.

Variable FRs have been reported in previous studies of denitrifying bioreactors where the FR of effluent or groundwater from tile drainage is influenced by seasonal events such as rainfall and snowmelt (van Driel *et al.*, 2006; Robertson and Merkley, 2009; Elgood *et al.*, 2010). Flow control structures allow the FR to be lowered within the bioreactor during seasonal events to increase the percentage of NO_3^- removal, though are likely to decrease overall actual NO_3^- removal rate from the effluent (Robertson and Merkley, 2009; Elgood *et al.*, 2010). Flow control structures are also likely to increase the cost associated

with bioreactors. However, it is not cost effective to design bioreactors based on infrequent peak flows (van Driel *et al.*, 2006). Designing bioreactors in association with other methods of reduced N additions or NO_3^- removal, such as wetlands, maybe an option worth further research (van Driel *et al.*, 2006; Robertson and Merkley, 2009).

In addition to the FR of effluent through the denitrification bed, effluent flow paths, particularly short-circuit flow, though the bed influence the amount of NO_3^- removal. When estimating NO_3^- removal and the influence of other controls, it is generally assumed that the flow of effluent is uniform though denitrification beds. However, rapid flow of effluent can result in short-circuiting and decreased NO_3^- removal (Cameron and Schipper, 2012) and slow flow can result in 'dead zones' and localised increases in NO_3^- removal. Slow flow in dead zones increases the retention time and the interaction between the effluent and the C source, which increases NO_3^- removal in within the zone but reduces the effective volume of the bed.

At the denitrification bed at Karaka, short-circuiting may have occurred following the system blockage in the outlet of the bed prior to the first sampling in March, as effluent was observed flowing along the surface of the bed. Additionally, a dead zone may have occurred midway along the length of the bed (around the 6th sampling point), as the NO_3^- concentration at this sampling point was consistently lower compared to the NO_3^- concentration of surrounding points (Fig. 3.6). As the denitrification bed resumed normal operating conditions in later samplings, it appeared that the flow of effluent became more uniform and the 'dead zone' ceased to occur. However, non-uniform flow of effluent may have had an influence on rate of removal and in turn the temperature response determined for the denitrification bed at Karaka. Non-uniform flow would

certainly decrease the ability to accurately estimate the decline in NO_3^- concentration along the bed and thus the rate of NO_3^- removal.

Cameron and Schipper (2011) investigated the influence of different flow regimes on the rate of NO_3^- removal in passively heated and non-heated denitrification beds. Four regimes were investigated, with the hydraulic design and short-circuit flow of each regime evaluated using tracer tests. Horizontal flow regimes were found to have higher short-circuiting in comparison to vertical flow regimes. Higher short-circuiting in horizontal flow regimes was suggested to be the result of flow across the top of the beds and was found to increase with passive heating as the result of higher buoyancy of warmer effluent. Vertical flow regimes were found to have the least short-circuit flow and were most effective in both NO_3^- removal and passive heating (Cameron and Schipper, 2011).

3.5.4 Conclusions and implications

The rates of NO_3^- removal for the denitrification beds at Karaka, Motutere and Newstead were similar to rates of NO_3^- removal reported in previous studies of denitrification beds. Interestingly, the rate of NO_3^- removal measured in the denitrification bed at Karaka was lower than the rate of removal measured in previous studies at Karaka (Schipper *et al.*, 2010a; Warneke *et al.*, 2011a). The discrepancy in the rates of NO_3^- removal was likely due to the remaining C source, from the wood chips, becoming more recalcitrant and so supporting lower rates of NO_3^- removal through time. Variation in the rates of NO_3^- removal between beds was most likely the result of differences between sites such as variation in NO_3^- concentration, available C source concentration and operating temperature (Schipper *et al.*, 2010b).

In general, the rate of NO_3^- removal increased with increasing temperature in the denitrification beds at Karaka and Motutere. However, there was no evidence of an increase in the rate of NO_3^- removal with increasing temperature in the denitrification bed at Newstead, mainly due to the NO_3^- limitation. The Q_{10} values for the temperature response of NO_3^- removal at Karaka and Motutere were similar to Q_{10} values reported in previous studies of denitrification beds. The temperature response of rate of NO_3^- removal measured for the denitrification bed at Karaka was greater than the response measured in the previous study at Karaka (Warneke *et al.*, 2011a). The discrepancy in the temperature response may have been the result of the higher temperature sensitivity of the decomposition of more recalcitrant C source in aged wood chips.

The NO_3^- concentration in the denitrification beds at Motutere and Newstead frequently limited NO_3^- removal, which may have resulted in an underestimation of the true rate and the temperature response of NO_3^- removal. The pre-treatment system and denitrification bed at Newstead had been installed just prior to sampling and were likely to be operating in the start-up phase, with the nitrifying component of the pre-treatment system likely to be the cause of low NO_3^- concentrations entering the bed. In addition, variability in the FR and pathways of the flow of effluent through the denitrification beds, particularly in the bed at Karaka, may have had an influence on the ability to accurately determine the rate and temperature response of denitrification.

This study supported previous suggestions of the importance of temperature to the rate of NO_3^- removal and to the design of denitrifying bioreactors (Christianson *et al.*, 2012; Schmidt and Clark, 2013). To ensure appropriate sizing of denitrification beds, the NO_3^- concentration and temperature of the effluent and groundwater from tile drainage require consideration. Larger

beds are required to treat effluent with higher NO_3^- concentrations or with greater flow rates, although smaller denitrification beds might be able to treat effluent in environments with warmer temperatures. The temperature sensitivity of NO_3^- removal determines how much smaller beds could be; however, the temperature sensitivity as defined by the Q_{10} values reported in previous studies are still not as well constrained as would be useful for design purposes. As such, there is a need for further research on the temperature response of NO_3^- removal in bioreactors in the field to refine the range of Q_{10} values and it is possible that better methodologies will be required. It is noted that the variation within the Q_{10} values of previous studies somewhat depends on the function used to describe the temperature response; linear functions produce very different Q_{10} values depending on the temperatures used whereas exponential functions produce similar Q_{10} values regardless. In addition, there is a need for further research on the temperature response of NO_3^- removal in bioreactors through time. An increase in the temperature response of NO_3^- removal with time will mean considering the proposed lifespan of bioreactors to ensure that the design is appropriate for the NO_3^- removal required over the lifespan.

Chapter 4 Conclusions and further research

4.1 Conclusions

Denitrifying bioreactors represent an inexpensive and effective method for the removal of NO_3^- from a range of wastewaters. The rate of denitrification is regulated by several controls including O_2 concentration, NO_3^- concentration, C source availability and temperature. As awareness of reactive nitrogen N_r as an environmental pollutant spreads, so does this method of removal to such an extent that denitrifying bioreactors are now present in many geographic locations. However, temperature changes with location and the nature of the influence of this control is poorly understood as a result of the influence of temperature being masked by the influence of other controls in the field. Improved understanding of the influence of temperature on the rate of NO_3^- removal is important for the future design of bioreactors, particularly for ensuring that bioreactors are built the right size to achieve NO_3^- removal targets. This will be important for determining the extent of the geographic location in which bioreactors will function efficiently and matching design criteria to location.

This thesis determined the temperature response of the rate of NO_3^- removal in three field scale denitrification beds in New Zealand. It was concluded that the rates of NO_3^- removal measured at the denitrification beds at Karaka, Motutere and Newstead (3.6, 4.3 and 1.7 g N m^{-3} day^{-1}, respectively) were similar to rates of removal reported for previous studies of denitrifying bioreactors. The highest rates of NO_3^- removal were reported for a denitrification bed in Ontario and ranged from $4 - 22$ g N m^{-3} day^{-1} (Blowes et $al.$, 1994; Robertson et $al.$, 2000). However, the majority of rates of NO_3^- removal reported were < 10 g N m^{-3} day^{-1} (Robertson and Merkley, 2009; Elgood et $al.$, 2010; Schipper et $al.$, 2010a; Warneke et $al.$, 2011a; Christianson et $al.$, 2012), which was consistent with the

rates in this study. The differences in the rates of removal reported in previous studies were suggested to be the result of the influence of controls such as NO_3^- concentration, C source availability and temperature.

It was concluded that the rate of NO_3^- removal generally increased with increasing temperature in the denitrification beds Karaka and Motutere (Q_{10} of 4.1 and 2.2, respectively). However, there was no evidence that the rate of NO_3^- removal increased with increasing temperature at Newstead (Q_{10} of 1.0). By comparison, the highest and lowest Q_{10} values reported for four denitrification beds in Iowa ranged from 0.8 – 5.7 (Christianson *et al.*, 2012). However, consistent with the findings of this study the majority of the Q_{10} values reported in previous studies ranged from 2 – 4 (van Driel *et al.*, 2006; Robertson and Merkley, 2009; Elgood *et al.*, 2010; Warneke *et al.*, 2011a). The differences in the temperature response were suggested to be the result of the differences in NO_3^- concentration and C source availability.

In this study of the denitrification bed at Karaka, a rate of NO_3^- removal of 3.6 g N m^{-3} day^{-1} and a Q_{10} of 4.1 were measured. In a previous study at Karaka, Warneke *et al.* (2011a) reported a rate of NO_3^- removal of 7.6 g N m^{-3} day^{-1} and a Q_{10} of 2. The difference in the rates of NO_3^- removal are suggested to be the result of the C source in the denitrification bed becoming more recalcitrant with time and supporting lower rates of denitrification while resulting in a higher apparent temperature response. In addition, variations in the FR and the occurrence of short-circuiting (Cameron and Schipper, 2012) and 'dead zones' in the bed are suggested to have an influence on the measured rate of NO_3^- removal.

In the denitrification beds at Motutere and Newstead, the rates of NO_3^- removal and the temperature response of the rate of removal were likely underestimated as a result of limiting NO_3^- concentrations in each bed. In addition,

the denitrification bed at Newstead had been recently installed and was likely to be in a start-up phase (Robertson and Cherry, 1995; Robertson *et al.*, 2005b). It is suggested that the pre-treatment system operating at the denitrification bed at Newstead may have been poisoned prior to sampling in September, similar to the poisoning reported as occurring in the denitrification bed at Karaka in a previous study (Warneke *et al.*, 2011a).

4.2   Further research

In general, the controls influencing the rate of NO_3^- removal in denitrifying bioreactors are reasonably well understood. However, further research is still required on the influence of temperature. It is widely understood that there is an increase in the rate of NO_3^- removal with increasing temperature as was supported in the findings of the current and previous studies. However, in terms of future design of bioreactors the temperature response of denitrification requires research to further refine the range of Q_{10} values from which design decisions can be made. Additionally, the potential to manipulate the temperature of bioreactors requires further research.

From the current and previous studies is it apparent that conditions within denitrifying bioreactors do not remain constant with time and that the rate of NO_3^- removal is likely to decrease through time (Robertson *et al.*, 2000; Robertson *et al.*, 2008; Robertson, 2010). Further research is required to determine the influence of variable FRs and short-circuiting and 'dead zone' behaviour on the measured rate of NO_3^- removal and how to remedy or avoid this behaviour. Previous studies have highlighted the importance of controlled flow for efficient NO_3^- removal (van Driel *et al.*, 2006; Robertson and Merkley, 2009). However, part of the attraction of bioreactors is that they are inexpensive and require little

maintenance and it is likely that controlled flow may increase both cost and maintenance requirements.

From this study it was also apparent that as the rate of NO_3^- removal decreases though time there may be an increase in the apparent temperature response of the rate of removal. Further research is required to test this suggestion and the future design of bioreactors may need to take into account both a changing rate of NO_3^- removal and changing temperature sensitivity with time. The implications of this on the design of bioreactors is that the proposed lifespan of the bioreactor would have to be considered to ensure that the bioreactor is designed appropriately to meet NO_3^- removal requirements which change with time.

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