THE ABILITY OF NITRIFICATION INHIBITORS TO DECREASE DENITRIFICATION RATES IN DAIRY FARM SOILS

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ABSTRACT

Increasing pressure is being placed on the dairy industry to reduce nitrogen losses from soil. Nitrification inhibitors are a management strategy that could be implemented on dairy farms to help reduce losses of nitrogen. Nitrification inhibitors work by temporarily inhibiting the microbial conversion of soil ammonium to nitrate. Past trials have indicated that nitrification inhibitors can increase grass production and decrease nitrate leaching; however, little is known about the long-term effects on other soil nitrogen processes such as denitrification. Denitrification rates in soils can be limited by the availability of substrate (carbon and nitrate) and by insufficient anaerobic microsites.

The objective of this thesis was to establish whether the nitrification inhibitor, dicyandiamide (DCD), could decrease denitrification rates in dairy farm soils by limiting nitrate availability. A field trial was established at Dexcel's research farm near Hamilton, New Zealand on a Typic Orthic Allophanic Soil. Twenty replicated field plots were established in a paddock, ten plots acted as controls and ten plots had DCD applied to the soil once a month at a rate of 30 kg ha^{-1} yr^{-1}. Denitrification rates were measured using the acetylene inhibition technique on intact soil cores. Ammonium and nitrate concentration, soil carbon availability, denitrifying enzyme activity and soil pH were measured from soil samples collected monthly.

Two further field experiments and one laboratory experiment were undertaken. The distribution of denitrifying enzyme activity with soil depth was measured to ensure that the depth to which denitrification was sampled (15 cm) in the field experiment was sufficient. DCD degradation in the field during 20 days was measured to establish how long the effects of DCD might last. A laboratory study investigated whether DCD would decrease denitrifying enzyme activity in soil, when soil conditions were optimized for denitrification.

More than 80% of the denitrifying enzyme activity occurred in the top 15 cm of the soil profile, indicating that the depth to which samples were collected was sufficient. There was no significant decrease in denitrification rates in the field experiment when DCD was added. Nitrification was partially inhibited as shown by a significant increase in soil ammonium (+14%) and a significant decline in soil nitrate (-17%) in the DCD-amended soils compared to the control soils. However, the decline in soil nitrate was not great enough for nitrate to limit denitrification. Nitrate concentrations were consistently greater than 5 mg NO_3^- kg^{-1} soil (the proposed threshold for declines in denitrification). The laboratory study supported the field study with DCD having no effect on denitrifying enzyme activity and nitrate concentrations remaining above 5 mg NO_3^- kg^{-1} soil. So while DCD reduced nitrification rates and the formation of nitrate, denitrification rates were not limited by nitrate availability. DCD was completely degraded in the soil 19 days after DCD application, with a half-life of 2.9 days, which may be a reason for the minor inhibition of nitrification. Denitrifying enzyme activity, carbon availability and soil pH were all unaffected by the application of DCD.

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TABLE OF CONTENTS

LIST OF FIGURES

Chapter 5

LIST OF TABLES

Chapter 1
General Introduction

1.1 INTRODUCTION

Nitrogen is necessary for life on Earth and of all the major chemical elements it has the greatest total abundance in the Earth's hydrosphere, biosphere and atmosphere. A large amount of nitrogen (99%) is not available to most living organisms (Galloway *et al.,* 2003). For a variety of reasons, humans have intervened in natural processes through widespread cultivation of legumes, combustion of fossil fuels and the Haber-Bosch process, which has greatly increased the availability of reactive nitrogen. An increase in reactive nitrogen has lead to an accumulation of nitrogen in the environment at all spatial scales, and inputs of nitrogen into the environment are greater than returns of nitrogen to the atmosphere (Galloway *et al.,* 2003). The increases in reactive (available) nitrogen have been beneficial to sustain a large proportion of the world's population; however there have also been detrimental effects on the environment (Galloway *et al.,* 2003).

The dairy industry in New Zealand is under scrutiny regarding the environmental sustainability of current dairy farm management practices (PCE, 2004). Dairy farms are often under intensive grazing management, producing large quantities of animal excreta, and often rely on nitrogen fertiliser. The soils in dairy farming systems may become saturated with nitrogen (Schipper *et al.,* 2004) and nitrogen, from animal excreta and fertiliser application, in excess of plant needs, can be leached into the groundwater (mostly as nitrate) or lost to the atmosphere (as nitrous oxide).

Nitrate leaching from agricultural land is an environmental concern worldwide. Increasing levels of nitrate in ground and surface waterways are leading to contamination and eutrophication of aquatic systems (Di and Cameron, 2002b). In New Zealand nitrogen fertiliser, applied to balance plant needs, can have little direct impact on nitrate leaching (Cameron *et al.,* 2002). But animals grazing on pasture concentrate the nitrogen and excrete as much as 80% of the nitrogen ingested, mostly

as urine (Van der hoek, 1998). The nitrogen excreted in a patch of cow urine is equivalent to approximately 1000 kg N ha^{-1}, and the soil/plant system is unable to cope with such high inputs of nitrogen and hence leaching and atmospheric loss of nitrogen occurs (Di and Cameron, 2002b).

Nitrous oxide contributes to global warming by its actions as a greenhouse gas and is also involved in the destruction of stratospheric ozone (McTaggart *et al.,* 1997). Nitrous oxide is particularly problematic as the warming potential of 1 kg of nitrous oxide is nearly 300 times greater than 1 kg of carbon dioxide, over a 100-year period (Smith *et al.,* 2003). Soils have been identified as a source of nitrous oxide accounting for approximately two-thirds of nitrous oxide emissions to the atmosphere (Smith *et al.,* 2003). In New Zealand total nitrous oxide emissions make up about 20% of New Zealand's total greenhouse gas inventory (Di and Cameron, 2002b). Nitrous oxide emissions from agricultural soils are the second largest source of emissions from the agricultural sector and there has been a 30% increase in nitrous oxide emission levels since 1990 (Brown and Petrie, 2003). New Zealand's ratification of the Kyoto Protocol has legally bound New Zealand to limit emissions of greenhouse gases, including nitrous oxide to 1990 emission levels (de Klein *et al.*, 2003).

Nitrous oxide is mainly produced in the soil by two contrasting microbial processes; nitrification and denitrification. Nitrification (the oxidation of ammonium to nitrate) requires an aerobic environment, while denitrification (the reduction of nitrate to dinitrogen gas) requires anaerobic conditions (Dobbie and Smith, 2003; Merino *et al.*, 2001). The process of denitrification not only results in the production of nitrous oxide, it also represents a potential mechanism of loss of plant available nitrogen (Barton *et al.,* 1999).

Increasing pressure is being placed on the dairy industry to avoid any adverse impacts on the environment and to meet tighter environmental standards (PCE, 2004). Tools need to be developed to protect the environment while also ensuring that the dairy

industry's productivity is not compromised. Nitrification inhibitors are a technology that could contribute to both productivity and environmental goals. Nitrification inhibitors work by temporarily inhibiting the microbial conversion of soil ammonium to nitrate. Ammonium is more readily absorbed to the soil than nitrate, allowing greater opportunity for ammonium to be utilized by plants or immobilized into the soil organic matter, rather than being leached or lost to the atmosphere (Di and Cameron, 2005). Past trials have indicated that nitrification inhibitors can increase grass production and decrease nitrate leaching (Williamson *et al.,* 1998); however, little is known about the long-term effects on soil nitrogen processes. Nitrification inhibitors could decrease denitrification by reducing the amount of nitrate substrate available for denitrification (McTaggart *et al.,* 1997), but this needs to be tested.

1.2 OBJECTIVES OF THIS STUDY

The main objective of this study was to establish if the nitrification inhibitor, dicyandiamide (DCD), decreases the denitrification rate from dairy farm soils by limiting nitrate availability.

Specifically, the objectives of the study were to:

1. To investigate the effectiveness of DCD at controlling denitrification rates in a typical dairy farming environment by limiting nitrate availability;
2. To investigate the effectiveness of DCD at influencing the denitrifying enzyme activity in a laboratory environment where conditions for denitrification are optimized;
3. To determine the rate of DCD loss in the soil.

This thesis is structured by firstly reviewing the literature on nitrification inhibitors, specifically their ability to influence denitrification rates. The analytical methods used in the experiments are outlined in Chapter 3. The ability of nitrification inhibitors to decrease denitrification rates in the field is discussed in Chapter 4 and the effect of nitrification inhibitors on denitrifying enzyme activity is discussed in Chapter 5. Chapter 6 is a general discussion and concluding chapter.

Chapter 2

Literature Review

2.1 INTRODUCTION

2.1.1 Overview of the nitrogen problem

There is growing worldwide concern over the effects of nitrogen in our environment. Nitrogen is widely dispersed by atmospheric and hydrologic transport processes and is responsible for a number of environmental problems (Galloway *et al.,* 2003). Agriculture is largely dependent on the use of nitrogen. In all agricultural systems, nitrogen is an essential element for plant growth, crop and/or animal productivity and farm profitability (Erisman *et al.,* 1998). The benefits of increasing use of nitrogen on our farmlands are offset by the environmental degradation that can result from excess nitrogen polluting nitrogen-limited systems.

The number of dairy cows in New Zealand has been increasing over the last 15 – 20 years. The total dairy cattle numbers in June 2006 reached 5.2 million, an increase of 21% since 1995 (Statistics New Zealand, 2006). In New Zealand, dairy cows are grazed outside all year round on pasture which predominantly relies on nitrogen fixation by clover (Ledgard *et al.,* 1998). However, in the last 15 years there was been a 4-fold increase in the use of nitrogen fertiliser as dairy farming has become more intensive (de Klein and Ledgard, 2005).

Dairy farming is one of the most intensive pastoral land management systems and a pressing environmental issue confronting dairy farming is the widespread concern about the loss of nitrogen originating from agricultural land (Di and Cameron, 2004a). The excess nitrogen in the soil system results in nitrogen leaching causing nutrient enrichment of our waterways (Ministry for Environment, 1997). Concern is also being raised over the loss of nitrogen to the atmosphere as gases (ammonia (NH_3), dinitrogen (N_2) and nitrous oxide (N_2O)), with the latter being a potent greenhouse gas. The concern over the potential losses of nitrogen from the soil

system is not solely an environmental issue; it also has economic implications, as there is a reduction in nitrogen-use efficiency (McTaggart *et al.*, 1997).

2.1.2 Tools to manage nitrogen

Effective tools need to be developed that minimize the loss of nitrogen. In New Zealand mitigation strategies for reducing nitrogen losses from agricultural land have focused on animal manipulation and grazing management (Ledgard and Menneer, 2005). Animal manipulation strategies can involve; feeding stock low-nitrogen feed supplements and the use of plants with high tannin levels that have the potential to cause more nitrogen to be excreted in manure relative to urine (Ledgard and Menneer, 2005).

Grazing management strategies can involve, appropriate timing and application rates of nitrogen fertilisers and strategic grazing. Strategic grazing involves restricting grazing during the winter months, when nitrogen loss is the greatest, through the use of housing sheds, feedpads and standoffs. Nitrogen loss from the soil can also be managed through soil manipulation by the use of chemicals known as nitrification inhibitors (Ledgard and Menneer, 2005).

Nitrification inhibitors work by actively managing soil nitrogen, by temporarily inhibiting the nitrification process. Nitrification inhibitors restrict the microbial conversion of soil ammonium to nitrate reducing nitrate accumulation in the soil (Di and Cameron, 2002b). The greater amount of ammonium in the soil extends the time that plants are able to take up extra mineral nitrogen from the soil. Consequently, nitrification inhibitors can reduce the concentration of nitrate in the soil and lead to a decrease in nitrate leaching and gaseous losses through the process of denitrification. A large number of studies have researched the ability of nitrification inhibitors at influencing nitrate leaching and nitrous oxide emissions, but limited studies have investigated the effect of nitrification inhibitors on denitrification.

2.1.3 Denitrification

Denitrification is the reduction of nitrogen oxides (nitrate (NO_3^-) and nitrite (NO_2^-)) to the gases (nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2)) (Groffman *et al.*, 2006). Denitrification plays both beneficial and detrimental roles in the environment. Denitrification is one of the major pathways by which nitrogen is returned to the atmosphere, but it is also responsible for the production of the greenhouse gas, nitrous oxide.

2.1.4 Structure of literature review

The first section is devoted to the role of nitrogen in agriculture; it will discuss the soil nitrogen cycle and the benefits and potential consequences of excess nitrogen. The following section will go over the role of nitrification inhibitors in managing soil nitrogen and will discuss the effectiveness of nitrification inhibitors, with a strong focus on their influence on denitrification. The last section will discuss the process of denitrification; this will include the consequences of denitrification, factors controlling denitrification, measurement procedures for denitrification and rates of denitrification.

2.2 NITROGEN

2.2.1 Nitrogen cycle

The nitrogen cycle is characterised by a huge reservoir of nonreactive nitrogen and small amounts of reactive nitrogen (Erisman *et al.,* 1998). The majority of nitrogen is nonreactive and thus, not directly available to plants. Nitrogen enters the soil system through biological nitrogen fixation of nonreactive nitrogen by legumes, and via the use of nitrogen fertiliser and animal excreta/manure (Figure 2.1).

Nitrogen in agricultural soils is present in two primary forms: inorganic-N and organic-N. Inorganic-N is readily available for plant uptake and includes the nitrogen forms of ammonium, nitrate, and nitrite and the gases nitric oxide, nitrous oxide, dinitrogen and ammonia. Organic-N is associated with soil organisms and plant material. Organic-N is held in the soil organic matter and is where the majority of soil nitrogen (95%) is stored, but in the organic-N form it is generally unavailable to plants (Xu *et al.,* 2003). Through the process of mineralization soil organisms convert organic-N into inorganic-N. Soil microorganisms can then take up some of the inorganic-N produced via immobilization (Moritsuka *et al.,* 2003). Nitrogen can be lost from the soil system by plant and animal uptake; nitrate leaching; denitrification and ammonia volatilization (Figure 2.1).

2.2.2 Benefits of Nitrogen

Nitrogen is an essential nutrient for plants, crops and animals; it can lead to greater pasture growth and crop yields and is involved in the production of animal tissue, milk, eggs and wool (Van der Hoek, 1998). A large proportion of the world population is sustained today due to the role of nitrogen in the environment. Nitrogen has become more readily available through cultivation-induced biological nitrogen fixation and through the use of synthetic fertilisers (Galloway *et al.,* 2003). The greater availability of nitrogen has allowed farmers to cultivate previously less productive soils and to intensify production.

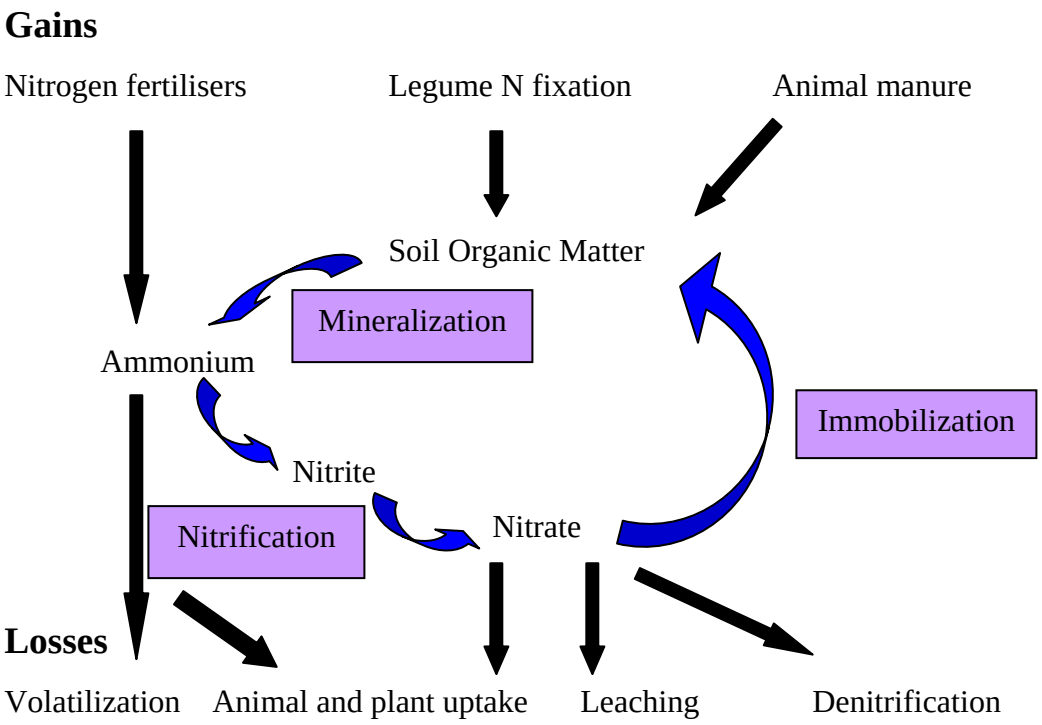

Figure 2.1 The nitrogen cycle in agricultural systems (after McLaren and Cameron, 1996).

2.2.3 Problems of Nitrogen

Nitrogen in a pastoral environment is not always utilized efficiently. Plant uptake of fertiliser nitrogen seldom exceeds 50% of the nitrogen applied (Mosier *et al.,* 2002). Furthermore, animals do not fully utilize the nitrogen they ingest. On average only about 10.5% of the nitrogen present in grass, silage and other feedstuff is converted into milk, meat, eggs or wool. The remaining nitrogen is excreted in manure and urine (Van der Hoek, 1998). A large proportion of the excess nitrogen in the environment originating from fertiliser nitrogen and animal excreta is lost from the plant/soil system through (1) gaseous losses to the atmosphere and (2) nitrate leaching (Mosier *et al.,* 2002).

2.2.3.1 Gaseous losses

Three important nitrogen gases are emitted from the plant/soil system: ammonia, nitric oxide and nitrous oxide. Ammonia is emitted to the atmosphere predominantly by volatilization from soils following the application of animal waste and synthetic fertiliser (Olivier *et al.,* 1998). Ammonia has a short atmospheric lifetime of only a few hours to a few days. Ammonia influences the pH of aerosols and rainfall, which can lead to eutrophication of ecosystems and soil acidification, due to the enhanced deposition of ammonia (Olivier *et al.,* 1998).

Nitric oxide is produced during denitrification. Nitric oxide is also a short-lived gas with an atmospheric lifetime of 1-10 days. Nitric oxide contributes to the generation of ozone in the troposphere and also contributes to acidification (Olivier *et al.,* 1998). Nitric oxide can also adversely affect human blood pressure and memory (Olivier *et al.,* 1998).

The microbial processes, nitrification and denitrification are principal producers of nitrous oxide (Akiyama *et al.,* 2000). Nitrous oxide is an important greenhouse gas contributing to global warming by absorbing terrestrial thermal radiation (de Klein *et al.,* 2003). Nitrous oxide is a long-lived greenhouse gas, with a mean average lifetime of 120 years and the radiative force of nitrous oxide is about 300 times that of carbon dioxide (Olivier *et al.,* 1998). Nitrous oxide is also a major source of stratospheric nitric oxide that contributes to ozone depletion (Olivier *et al.,* 1998; Shoji *et al.,* 2001).

Soils are the major source of nitrous oxide, contributing to about 65% of the total global nitrous oxide emissions (Pathak and Nedwell, 2000). In New Zealand, nitrous oxide emissions from agricultural soils account for 34.9% of all greenhouse gas emissions made by the agricultural sector (Brown and Petrie, 2003). Over 50% of our total nitrous oxide emissions in New Zealand originate from animal excreta-N, which is deposited during grazing (de Klein *et al.,* (2003) and emissions of nitrous

oxide from soils increase with nitrogen fertiliser application (Pathak and Nedwell, 2000; Akiyama *et al.*, 2000).

2.2.3.2 Nitrate leaching

Nitrate leaching from agricultural land and the contamination of ground and surface waterways is an area of environmental concern in many countries around the world (Ledgard *et al.,* 1997). Excess nitrogen in surface waters and in groundwater causes eutrophication of our rivers, lakes and estuaries. Eutrophication of waterways can result in algal blooms, excessive growth of nuisance aquatic plants and fish poisoning (Di and Cameron, 2004a; 2005). Nitrate leaching is not just an environmental issue; high nitrate levels in groundwater used for drinking are harmful to both livestock and human health. The Ministry of Health in New Zealand has placed limits on the acceptable levels of nitrate allowed in drinking water; the limit is 11.3 mg N L^{-1}.

It has been shown (Ledgard *et al.,* 1998) that with increasing fertiliser application there can be a significant increase in nitrate leaching, with approximately a 4-fold increase in nitrate leached when fertiliser application increases from 0 kg N ha^{-1} yr^{-1} to 400 kg N ha^{-1} yr^{-1}. However Silva *et al.,* (1999) showed that the amount of nitrate leached from applied nitrogen fertiliser and waste effluent irrigation to pasture are relatively low if application rates are reasonable.

Therefore the dominant source of nitrate leaching in grazed pasture systems in New Zealand is from animal urine patches. The nitrogen loading rate under a cow urine patch can be the equivalent of 1000 kg N ha^{-1}, much more than the plant/soil system can utilize (Haynes and Williams, 1993). The excess nitrogen in the soil from cow urine patches is nitrified through to nitrate and after three to five weeks, nitrate is the major form of nitrogen present in a urine patch (Haynes and Williams, 1993). Cow urine patches are particularly problematic in the late autumn, winter and early spring period in New Zealand, as this is when the soil is likely to be saturated already from excess rainfall and therefore nitrate can be leached even more readily through the soil profile (Di and Cameron, 2005).

2.3 NITRIFICATION INHIBITORS

2.3.1 Theory behind nitrification inhibitors

Nitrogen is dynamic in the soil and is always being transformed. Ammonium in the soil is derived from mineralization of organic matter and the addition of ammonium-based fertilisers. Ammonium is also derived from the hydrolysis of urine and urea fertiliser (Edmeades, 2004). Ammonium is positively charged and retained by negatively charged cation exchange sites on soil clays and organic matter (Di and Cameron, 2005). The ammonium that is not utilized is nitrified through to nitrate.

Nitrification is an oxidation reaction that occurs mainly by the action of specific nitrifying bacteria. Nitrification occurs in two steps, the first step is mediated mainly by *Nitrosomonas* bacteria, which convert ammonium to nitrite, and the second step is carried out by *Nitrobacter* species, which convert nitrite to nitrate. Nitrate is negatively charged and poorly held by the soil, because soils have a net negative charge. Therefore nitrate in solution is subject to nitrogen leaching and atmospheric loss via denitrification (Engels and Marschner, 1995).

Nitrification inhibitors work by interfering with the action of *Nitrosomonas* bacteria, inhibiting the conversion of ammonium to nitrite, the first step in nitrification (Figure 2.2) (Zacherl and Amberger, 1990). Nitrification inhibitors function by delaying bacterial oxidation of the ammonium ion, this is done by limiting the activity and population of the *Nitrosomonas* bacteria (Dinnes *et al.,* 2001; Irigoyen *et al.,* 2003).

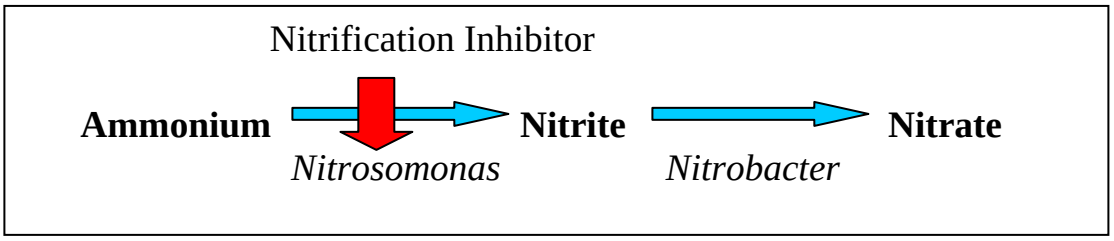

Figure 2.2 Nitrification inhibitors slow the rate of conversion of ammonium to nitrite by interfering with the action of *Nitrosomonas* bacteria.

By blocking nitrification these inhibitors are proposed to have several environmental benefits. Nitrification inhibitors could reduce emissions of nitrous oxide, directly by reducing nitrification and indirectly by reducing the availability of nitrate for denitrification (Malla *et al.,* 2005). The reduced nitrate pool can also lead to a reduction in nitrate leaching (Williamson *et al.,* 1996). Nitrogen in the soil stays in the ammonium form, and can be immobilized into soil organic matter or the plants can use the ammonium for growth and as a result there is potential for increased production (Di and Cameron, 2005).

2.3.2 History of nitrification inhibitors

Nitrification inhibitors are not a new technology; references to them can be found as far back as 1908. Northern Hemisphere countries, particularly Europe have been using nitrification inhibitors for decades with the aim of increasing the efficiency of nitrogen fertilisers (Di and Cameron, 2002b). Early on the importance of nitrification inhibitors in agriculture was recognized. Rodgers stated in 1986 that the "Agricultural usage of nitrification inhibitors will be expected to become more routine on many farms, due to increased fertiliser use and legislation limiting nitrate levels in groundwater".

The development of the nitrification inhibitor N-Serve in 1962 [2-chloro-6(trichloromethyl) pyridine] (chemical name for nitrapyrin) sparked the emergence of nitrification inhibitors as a group of agrichemicals (Prasad and Power, 1995; Zerulla *et al.,* 2001). Research in the 1960's was mainly confined to laboratory-based studies and it wasn't until the late 1960's and 1970's that field-based trials were established (Prasad and Power, 1995). Since the 1960's a large number of chemicals have been reported to have nitrification inhibiting properties (Table 2.1). Only three of the nitrification inhibitors listed in Table 2.1 have gained importance on a global scale for practical use; Nitrapyrin in the United States, DCD in Europe and more recently the development of DMPP in Europe (Zerulla *et al.,* 2001). The majority of the other nitrification inhibitors (listed in Table 2.1 and those not listed); despite having excellent nitrification inhibiting properties have failed under practical conditions to show any commercial benefit (Zerulla *et al.,* 2001). Alongside the development of

specific chemicals as nitrification inhibitors, a number of natural products have been found to have nitrification inhibiting properties. In many South Asian countries the use of specific chemicals as nitrification inhibitors has not been widely adopted, due to their high cost and non-availability (Malla *et al.,* 2005). This has created a need to identify locally available and cheaper materials that have nitrification inhibiting properties. The use of natural products like those from "neem" and "karanja" has been widely evaluated in South Asia for their ability to act as nitrification inhibitors (Prasad and Power, 1995; Majumdar, 2002; Malla *et al.*, 2005). Again these products have had mixed success. Majumdar (2002) reported that karanja was a more potent nitrification inhibitor than DCD, mitigating total N_2O-N emission by 92-96%, compared with DCD 60-71% reduction. However neem has been reported to only reduce total N_2O-N emissions by 9% in wheat (Majumdar *et al.*, 2002).

Table 2.1 Summary of the main nitrification inhibitors that have been developed and widely tested after Prasad and Power (1995).

Abbreviated Name or Trade name	**Chemical Name**	**Reported as nitrification inhibitors**
Nitrapyrin	2-chloro-6-(trichloromethyl)pyridine	1962
AM	2-amino-4-chloro-6-methylpyrimidine	1965
ST	2-sulfanil-amido thiazole	1968
Terrazole/Dwell	5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole	1976
DCD	Dicyandiamide	1978
TU	Thiourea	-
MBT	2-mercaptobenzothiazole	1986
C_2H_2	Acetylene	1981
DMPP	3,4-dimethylpryazole phosphate	1999

Use of nitrification inhibitors in New Zealand has been relatively recent. Compared to other countries, New Zealand animals are grazed outdoors all year round and less fertiliser is applied. It has been proven that losses of nitrate from applied fertiliser nitrogen, or farm dairy effluent, are relatively small compared to the large leaching losses from urine patches (Di and Cameron, 2002a). It is a challenge to manage the losses of nitrogen from animal urine patches as they are scattered throughout the field in an irregular pattern and the urine is concentrated in a small area (Di and Cameron, 2004a). The application of nitrification inhibitors to urine patches is a new problem. Internationally, nitrification inhibitors have not been widely used to reduce nitrate leaching and nitrous oxide emissions from urine patches (Di and Cameron, 2002b). In New Zealand, the majority of the research on nitrification inhibitors has been based on the inhibitor dicyandiamide.

2.3.3 Dicyandiamide

The nitrification inhibitor, dicyandiamide (DCD) is the most extensively used inhibitor in New Zealand and is most commonly used in agriculture (Williamson *et al.*, 1996). The chemical formula of DCD is $NH_2C(:NH).NH.CN$ (Figure 2.3; ACROS Organics). Dicyandiamide was the nitrification inhibitor used in my research experiment.

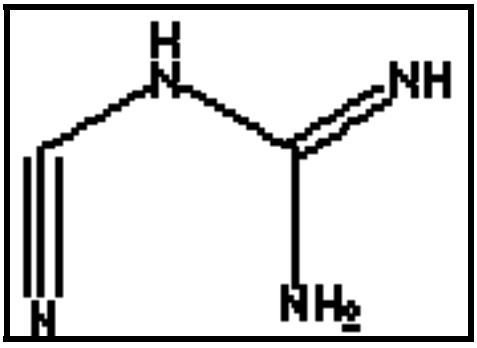

Figure 2.3 Chemical structure of dicyandiamide (ACROS Organics).

DCD is a convenient inhibitor as it is nonvolatile and is chemically and physically stable. These properties allow DCD to be effectively formulated with a variety of fertilisers (Di and Cameron, 2002b; Gioacchini *et al.*, 2002). DCD is made up of

65% nitrogen, and can be regarded as a slow release nitrogen fertiliser (Di and Cameron, 2002b). DCD works by inhibiting the first stage of nitrification, the oxidation of ammonium to nitrite, by acting on the bacteria *Nitrosomonas* sp. and rendering the bacteria's enzymes ineffective (Merino *et al.*, 2001). DCD acts through a bacteriostatic effect (McTaggart *et al.*, 1997; Merino *et al.*, 2001), not a bactericide, and does not affect other heterotrophs that are responsible for much of the soils biological activity (Di and Cameron, 2002b; Zacherl and Amberger, 1990). DCD is naturally broken down in the soil to non-toxic products (Merino *et al.*, 2001).

The limitations of DCD are that high application rates are often needed for sufficient nitrification inhibition to occur (15 – 30 kg DCD ha^{-1} yr^{-1}) and this can make application of DCD for large-scale use non-economic (Zerulla *et al.*, 2001). DCD is relatively water soluble and intensive rainfall could lead to the translocation of DCD down the soil profile, limiting the efficiency of DCD (Williamson *et al.*, 1996; Zerulla *et al.*, 2001). DCD can also be rapidly degraded in warm soils (Williamson *et al.*, 1996) and therefore the effectiveness of DCD can decline rapidly with increasing temperature (Irigoyen *et al.*, 2003). Di and Cameron (2004a) showed that at a soil temperature of 8ºC, the half-life of DCD was 111-116 days, while at a soil temperature of 20ºC the half-life of DCD was 18-25 days. DCD has also been reported to have deleterious effects on clover growth (Hatch *et al.*, 2005; Macadam *et al.*, 2003) and may cause phytotoxicity problems, which could have implications for the marketability of leaf vegetables (Zerulla *et al.*, 2001).

DCD has most commonly been applied at rates of 10 - 30 kg ha^{-1} (Merino *et al.*, 2001; Cookson and Cornforth, 2002; Macadam *et al.*, 2003). Di and Cameron (2005) reported that DCD applied at a rate of 5 kg ha^{-1} was not high enough to provide the desired benefits, but at an application rate of 10 kg ha^{-1} was sufficient to see reductions in nitrate leached. Di and Cameron have also recommended that in New Zealand DCD be applied twice yearly (May and August) to best achieve a reduction in nitrate leaching (Di and Cameron, 2004b).

2.3.4 Effectiveness of nitrification inhibitors

Research results on nitrification inhibitors have been variable. The majority of the research has indicated that nitrification inhibitors offer potential benefits at reducing nitrate leaching and losses of nitrous oxide to the atmosphere, and as a result greater efficiency in nitrogen use, which will result in increased plant growth (Di and Cameron, 2002b, 2004b; Majumdar, 2002; McTaggart *et al.,* 1997; Shogi *et al.*, 2001; Weiske *et al.*, 2001; Williamson *et al.,* 1996). But these results have not been consistent, many studies have shown that there have been no benefits from the use of nitrification inhibitors, in terms of reduced nitrate leaching and gaseous losses and there has been no increase in plant growth (Fox and Bandel, 1989; Malzer *et al.*, 1989; Davies and Williams, 1995; Gioacchini *et al.*, 2002). This variability in results is not unexpected, as the effectiveness of nitrification inhibitors varies greatly depending on soil type, soil temperature, moisture content, form of nitrogen, soil pH, and soil organic carbon. Therefore the effectiveness of nitrification inhibitors in the field is difficult to predict (Kpomblekou-A and Killorn, 1996; Davies and Williams, 1995).

2.3.4.1 Nitrification inhibitors and nitrate leaching

Studies have examined the ability of nitrification inhibitors to reduce nitrate leaching with mixed results reported (Table 2.2). Studies have shown that the main source of nitrate leaching is from cow urine patches (Di and Cameron, 2002b). Di and Cameron (2002b) showed DCD applied as a solution and as a fine particle suspension (Di and Cameron, 2005) decreased nitrate leaching from cow urine. Francis (1995) also reported a reduction in nitrate leaching when DCD was applied to soil.

But not all results have been so positive. In New Zealand dairy farm effluent is often applied to pasture. Williamson *et al.,* (1998) studied the ability of DCD to reduce nitrate leaching in pasture that had been irrigated with dairy farm effluent and only reported a 18% reduction in the cumulative amount of total nitrogen leached. Williamson *et al.,* (1998) concluded that under high effluent N-loadings, even with the use of DCD, groundwater quality was comprised. Davies and Williams (1995)

also concluded that DCD gave no significant reduction in the amount of nitrate leached. Davies and Williams (1995) tested the ability of DCD to reduce nitrate leaching from two high-risk leaching agricultural practices; (1) ploughing-in pasture and (2) autumn application of inorganic-N fertiliser. Gioacchini *et al.,* (2002) also reported that DCD was not effective at reducing nitrate leaching in a lysimeter study; more soil-derived nitrogen was lost through leaching in the presence of DCD relative to their control treatment.

Table 2.2 The effectiveness of the nitrification inhibitor, DCD at controlling nitrate leaching.

Description of study	Nitrate Leaching	Reference
Lysimeter study testing the ability of DCD using undisturbed monoliths.	18% reduction	Williamson *et al.*, 1998
Lysimeter study measuring the ability of DCD (solution form) at controlling losses from dairy cow urine patches.	59% annual reduction	Di and Cameron, 2002b
Lysimeter study measuring the ability of DCD (fine particle suspension) at controlling losses from dairy cow urine patches.	68% reduction	Di and Cameron, 2005

2.3.4.2 Nitrification inhibitors and nitrous oxide emissions

Nitrification inhibitors have also been shown to reduce nitrous oxide emissions. By inhibiting the conversion of ammonium to nitrate, nitrification inhibitors directly reduce the loss of nitrous oxide from nitrification and indirectly from denitrification (Pathak and Nedwell, 2000). Excellent results have been produced in terms of reduction in nitrous oxide emissions (Table 2.3). Hatch *et al.,* (2005) found DCD to be effective in lowering nitrous oxide emissions during both the nitrification and denitrification phases. Di and Cameron (2002b) reported DCD to reduce nitrous oxide emissions by 82%. McTaggart *et al.,* (1997) and Dobbie and Smith (2003) reported emissions of nitrous oxide being reduced when DCD was applied with urea

and ammonium sulphate. However, smaller reductions have been reported, Kumar *et al.*, (2000) applied DCD with urea and ammonium sulphate and reported only 11% and 26% reductions in nitrous oxide emissions respectively.

Table 2.3 The effectiveness of the nitrification inhibitor, DCD at controlling nitrous oxide emissions.

Description of study	**Nitrous oxide emissions**	**Reference**
Study assessing the effectiveness of DCD at reducing N_2O emission following the application of NH_4^+ or NH_4^+ forming fertilisers in the field.	58-78% reduction when applied with urea 41-65% reduction when applied with $(NH_4)_2SO_4$	McTaggart *et al.*, 1997
Short-term field study (37 days) looking at N_2O emissions. from urine.	74% reduction	Williamson and Jarvis 1997
Nitrous oxide emissions from different fertilisers and its mitigation by DCD in irrigated rice in the field.	11% reduction when applied with urea 26% reduction when applied with $(NH_4)_2SO_4$	Kumar *et al.*, 2000
3-year field experiment looking at how DCD effects N_2O emissions	26% reduction over 3 years	Weiske *et al.*, 2001
Lysimeter study measuring the ability of DCD (solution form) at controlling losses from dairy cow urine patches.	82% reduction	Di and Cameron, 2002b
Laboratory experiment looking at DCD effect on gaseous emissions of soil amended with slurry.	10 to 20-fold reduction	Hatch *et al.*, 2005

2.3.4.3 Nitrification inhibitors and denitrification

Through inhibiting nitrification, nitrification inhibitors can reduce the nitrate pool in the soil, potentially leading to a reduction in denitrification rates. However, to date mixed results have been found when it comes to the ability of nitrification inhibitors to influence denitrification.

Bremner and Yeomans (1986) conducted a study looking at 28 nitrification inhibitors and their ability to reduce denitrification rates. Only one nitrification inhibitor (potassium azide) reduced denitrification when applied at a rate of 10 µg g^{-1} soil. Two other nitrification inhibitors decreased denitrification when applied at a rate of 50 µg g^{-1} soil and the rest of the nitrification inhibitors, including DCD, had no appreciable effect on denitrification. Vallejo *et al.,* (2001) showed that although DCD inhibited the oxidation of ammonium to nitrate, a subsequent decrease in denitrification rates was not observed. Vallejo *et al.,* (2001) concluded that DCD, reduces nitrous oxide emissions, but it does not clearly affect denitrification rates.

Research has shown that when DCD is applied with a carbon source, a decrease in denitrification rates can occur as the effectiveness of DCD on denitrification rates can be dependent on the quantity of available carbon (Table 2.4; Thompson, 1989; Pain; *et al.,* 1989; Merino *et al.,* 2001). Thompson (1989) and Pain *et al.,* (1989) found that DCD was more effective at reducing denitrification rates as greater amounts of carbon, in the form of cattle slurry, were added to the soil with DCD. Pain *et al.,* (1989) also showed that when DCD was applied to slurry, the greatest reduction in nitrogen lost through denitrification occurred at the highest DCD and slurry application rates. As the rate of DCD and slurry application decreased, so did the level of denitrification reductions. Merino *et al.,* (2001) also found that when DCD was applied to slurry it was effective at reducing nitrous oxide production from both nitrification and denitrification. But when DCD was applied to mineral fertilised soil, no reduction in nitrous oxide emissions was observed (Merino *et al.,* 2001).

Not all research has supported the ability of nitrification inhibitors to control denitrification when a carbon source has been added. Bremner and Yeomans (1986) added an organic carbon source (as mannitol) to promote denitrification of nitrate by soil microorganisms. They found seven nitrification inhibitors (potassium azide, sulfathiazole, potassium ethylxanthate, sodium isopropylxanthe, 4-nitrobenzotrichloride, sodium thiocarbonate and phenylmercuric acetate) did inhibit denitrification when the inhibitor was applied at rate of 50 µg g^{-1} soil to soil amended with mannitol. But 21 nitrification inhibitors, including DCD, had no effect on denitrification. Calderon *et al.,* (2005) studied the ability of the nitrification inhibitor, nitrapyrin, at affecting the timing and amounts of denitrification and nitrous oxide fluxes in manured soils under conditions favourable to denitrification. Calderon *et al.,* (2005) established that nitrapyrin did not affect cumulative denitrification, but in some of the soils tested, a delay in denitrification was observed when nitrapyrin was added to the soil.

Table 2.4 The effectiveness of the nitrification inhibitor, DCD at controlling denitrification rates.

Description of study	**Denitrification rates**	**Reference**
The ability of DCD to cause a reduction in denitrification in slurry treated soil.	37% reduction at slurry application rate 40 t ha^{-1} 90% reduction at slurry application rate 80 t ha^{-1}	Thompson 1989
The ability of DCD to reduce gaseous losses of N from cattle slurry applied to grassland	70% reduction at application rate of 25 kg ha^{-1} 55% reduction at application rate of 20 kg ha^{-1} 30% reduction at application rate of 15 kg ha^{-1}	Pain *et al.,* 1989
Nitrification and denitrification derived N_2O production from a grassland soil under application of DCD.	78.67% reduction in nitrous oxide production by denitrification.	Merino *et al.,* 2001

2.3.4.4 Nitrification inhibitors and pasture/plant growth

The treatment of soils with nitrification inhibitors can provide agronomic benefits of increased yield; this is due to the increased ammonium retention in the soil and therefore greater availability of nitrogen to plants. Williamson *et al.,* (1998) reported greater dry matter yields in pasture. This study was conducted using lysimeters that received DCD-amended effluent relative to effluent-irrigated lysimeters. A 33% increase in dry matter yield has also been obtained with the application of DCD as both a fine particle suspension and as a straight solution (Di and Cameron, 2005).

Francis (1995) reported that DCD had no effect on wheat yield and Gioacchini *et al.,* (2002) also found that DCD was unable to increase the grain or plant yield of wheat. It was found that although DCD held more fertiliser-derived nitrogen in the ammonium form, there was both a reduced mobility of ammonium and there was also a preferential immobilization of ammonium by soil microbes (Gioacchini, 2002). Fox and Bandel (1989) reported that DCD had no significant beneficial effect on turf clipping yields and corn grain yield, but they did report greater yields of wheat when applying DCD with fertiliser.

2.3.5 Nitrification inhibitors and soil N cycling

The effects of nitrification inhibitors on the soil nitrogen cycle have been studied (Giacchini *et al.*, 2002; Cookson and Cornforth, 2002). It is important to understand the fate of nitrogen that is retained in the soil through the use of nitrification inhibitors (Williamson *et al.,* 1996). Better knowledge of the possible effects of inhibitors on the soil nitrogen cycle is required to increase crop productivity and reduce the losses of nitrogen.

1. Volatilization

Nitrification inhibitors have the potential to cause a greater loss of ammonia. This could occur through either the accumulation of ammonium in the soil or the associated rise in soil pH that will also result in greater levels of ammonium present (Prasad and Power, 1995; Cookson and Cornforth, 2002; Irigoyen *et al.,* 2003).

Gioacchini *et al.,* (2002) reported that the application of the nitrification inhibitor (DCD) with the urease inhibitor (N-(n-butyl) resulted in a significant increase in volatilization losses with respect to a urea and urease inhibitor treatment. It was concluded that the application of the nitrification inhibitor with a urease inhibitor reduced the efficiency of the urease inhibitor (Gioacchini *et al.,* 2002). However, Prasad and Power (1995) reported that by incorporating a nitrification inhibitor with a nitrogen fertiliser, volatilization losses could be reduced.

2. Mineralization

Gioacchini *et al.,* (2002) reported a priming effect with extra mineralization of soil nitrogen after the application of DCD. It was suggested that by maintaining more ammonium in the soil, a priming effect resulted in an increase in the rate of soil organic matter mineralization and this led to release of soil organic nitrogen (Gioacchini *et al.,* 2002). However, Francis (1995) reported that net nitrogen mineralization in ploughed pasture was not affected by the application of DCD to pastures.

3. Immobilization

Nitrification inhibitors have been reported to favour immobilization (Prasad and Power, 1995; Gioacchini *et al.,* 2002). Gioacchini *et al.,* (2002) stated that DCD potentially stimulates soil microbial activity by maintaining more nitrogen in the ammonium form, which is then preferentially immobilized by soil microbes. When a carbon source is readily available to microbes and autotrophic growth has been restricted through the application of DCD, considerable hetertrophic microbial growth occurs. It has been assumed that heterotrophic microbes are more competitive than autotrophic microbes for ammonium, and hence a greater immobilization of ammonium can occur when conditions are optimized for hetertrophic microbes (Tietema and Wessel, 1992; Cookson and Cornforth, 2002).

2.4 DENITRIFICATION

The ability of nitrification inhibitors to influence nitrate leaching and nitrous oxide emissions has been well studied. But the influence of nitrification inhibitors on the process of denitrification is not so well understood and the results to date have been variable. This section of the literature review discusses the process of denitrification.

2.4.1 The denitrification process

Denitrification is the reduction of nitrogen oxides (nitrate (NO_3^-) and nitrite (NO_2^-)) to the gases (nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2)) (Groffman *et al.,* 2006). The denitrification pathway is irreversible and follows a series of steps (equation 2.1.). A taxonomically diverse group of bacteria carry out the process of denitrification and a specific reductase enzyme is required to activate each step along the denitrification pathway (Mosier *et al.,*¸ 2002). The emission ratio of nitrous oxide to dinitrogen, is affected by soil moisture, nitrate concentration, soil pH, available carbon and soil temperature (Barnard and Leadley, 2005; Ullah *et al.,* 2005). A larger fraction of nitrous oxide is usually produced when the nitrate concentration is high relative to available carbon supply and when the soil pH and moisture content are low (Blackmer and Bremner, 1978; Weir *et al.,* 1993; Mosier *et al.,* 2002). The production of dinitrogen is dominant in more anoxic sites, such as flooded soils (Mosier *et al.,* 2002).

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2 \quad \text{(equation 2.1)}$$

(nitrate) (nitrite) (nitric oxide) (nitrous oxide) (dinitrogen)

Large losses of nitrogen can occur through biological denitrification, but chemical denitrification can also play a role (Mosier *et al.,* 2002). Chemical denitrification occurs when nitrifying or denitrifying microorganisms produce nitrite, and chemical reactions convert the nitrite through to gaseous nitrogen compounds, predominantly nitric oxide, but also dinitrogen and nitrous oxide (Tiedje, 1994). Chemical denitrification is not considered a major process on a global scale (Tiedje, 1994).

Biological denitrification is the dominant form of denitrification in most soil environments (Mosier *et al.,* 2002). Biological denitrification includes both non-respiratory and respiratory denitrification. Non-respiratory denitrification involves numerous types of organisms such as bacteria, fungi and algae producing nitrous oxide. Respiratory denitrification, often just referred to as denitrification, involves heterotrophic bacteria oxidizing organic compounds to gain energy, while using nitrate or nitrite as an electron acceptor (Mosier *et al.,* 2002). The majority of denitrification is carried out by respiratory denitrifiers. Nearly all respiratory denitrifiers would prefer to use oxygen as their electron receptor, but they have the capability of using nitrate and nitrite as an electron receptor when anaerobic conditions exist (Tiedje *et al.,* 1989).

Denitrification has enormous spatial and temporal variability. The spatial variability of denitrification arises due to "hot spots" of denitrifier activity. Hotspots of denitrification result from the irregular distribution of available carbon in the soil, as established by Parkin (1987). Temporal variations in denitrification rates occur due to variations in soil temperature, both seasonally and daily and through variations in nitrogen fertiliser application, irrigation, rainfall and animal grazing (Luo *et al.,* 2000). Measurements of denitrification are hence variable with typical coefficients of variations exceeding 100% (Hénault and Germon, 2000).

2.4.2 Consequences of denitrification

Denitrification is an important part of the nitrogen cycle as it is the main process that converts fixed nitrogen back to the atmosphere (Tiedje *et al.,* 1989). Historically the rate of removal of nitrogen was in balance with the inputs of nitrogen. But with increased fertiliser use the rates of removal of nitrogen via denitrification are small in comparison to the rates at which reactive nitrogen enters the environment. This has led to a large increase in the amount of reactive nitrogen accumulating in the environment at all spatial scales (Galloway *et al.,* 2003).

The process of denitrification can have both negative impacts on the environment and the economics of farming. Historically the major concern with excess denitrification

has been the loss of nitrogen from crops/pasture. The focus of denitrification research has been attempts to reduce losses of plant-available nitrogen (Tiedje *et al.*, 1989). From an environmental view-point denitrification also contributes to nitrous oxide production, which is a greenhouse gas and contributes to ozone depletion (section 2.2.3.1).

2.4.3 Factors controlling denitrification

Soil denitrifying microorganisms are responsible for carrying out denitrification and are generally widely distributed (Tiedje *et al.*, 1989). Hence, denitrification is not limited by the presence of denitrifying microorganisms; denitrification is controlled by factors that affect the growth and activity of microorganisms. These factors can be divided into proximate factors; oxygen availability, nitrate concentration, carbon concentration, soil temperature and soil pH; and distal factors; rainfall, organic matter and soil texture/structure. These factors all vary irregularly and substantially in time and space and also interact with each other (Hénault and Germon, 2000).

2.4.3.1 Proximate Factors

1. Oxygen availability

As denitrification is an anaerobic process, the primary factor controlling denitrification is oxygen availability (Hofstra and Bouwman, 2005). The oxygen availability within the soil is primarily controlled by the soil water content and rate of oxygen consumption (Mosier *et al.*, 2002). Denitrifying microbes have the capability of using either oxygen or nitrate as the electron acceptor but they will only reduce nitrate when oxygen is unavailable (Barton *et al.*, 1999). High soil moisture contents (> 60%) create anoxic conditions as oxygen diffusion into the soil is restricted (Bollmann and Conrad, 1998) and hence denitrification is promoted (Dalal *et al.*, 2003). At 80-90% water–filled pore space the dominant form of gaseous nitrogen loss is nitrogen gas (Dalal *et al.*, 2003). Depending on the soil texture, threshold values exist for soil water content and above these values denitrification occurs (Table 2.5). In a nitrogen-fertilised grassland soil, oxygen availability will be the dominant factor controlling denitrification, as nitrate and carbon would be at concentrations adequate for denitrification to occur (Barton *et al.*, 1999).

Table 2.5 Threshold water-filled pore space values above which denitrification rates increase (after Barton *et al.,* 1999).

Soil texture	Water-filled porosity (%)
Sand	> 82
Sandy loam	> 74
Loam	62 - 83
Clay loam	50 - 74

2. Nitrate concentration

Soil nitrate is required as the electron acceptor for denitrifying microbes (Hofstra and Bouwman, 2005). The availability of nitrate to denitrifying microbes depends on the rate of nitrate production, nitrate transport and nitrate consumption by other organisms (Barton *et al.*, 1999). In nitrogen-fertilised grasslands it is unlikely that nitrate would be a controlling factor in denitrification as nitrate levels within the soil are well above the threshold level, which would limit denitrification (Barton *et al.*, 1999). The threshold values of soil nitrate for denitrification range from 2 – 5 mg NO_3^- kg^{-1} soil depending on soil texture (Barton *et al.*, 1999). For a loam soil, Ryden (1983) established a threshold value of 5 mg NO_3^- kg^{-1} soil.

3. Carbon concentration

Soil carbon serves as an electron donor (Hofstra and Bouwman, 2005) and carbon availability can regulate microbial biomass and activity (Dendooven *et al.,* 1996). Carbon can also decrease oxygen concentration following respiration by other microbes. Generally, denitrification will increase with increasing carbon availability in the soil (Dendooven *et al.*, 1996) and the ratio of nitrous oxide to dinitrogen will decrease with increasing carbon availability (Weir *et al.*, 1993). The composition of the organic material as well as the amount plays an important part in denitrification activity. In nitrogen-fertilised grasslands the availability of soil carbon can limit denitrification (Barton *et al.*, 1999).

4. Soil temperature

Temperature is an important regulator of all biochemical processes (Groffman and Tiedje, 1991). As temperature increases (within a certain range) the denitrification rate will also increase (Dalal *et al.,* 2003). A 10-fold increase in denitrification rates from non-irrigated plots as the temperature increased from 10 – 20°C and a 3-fold increase in irrigated plots were reported by de Klein and Van Logtestijin (1996). The lower limiting soil temperature for denitrification in the field has been estimated to range from 4 – 6°C (Ruz-Jerez *et al.,* 1994).

5. Soil pH

Soil pH is considered one of the major factors controlling denitrification and particularly the production of nitrous oxide from soils (Hall *et al.,* 1998). Studies have shown that the rate of denitrification increases with increasing soil pH (Focht and Verstraete, 1977; Hall *et al.,* 1998). As the soil pH increases the formation of dinitrogen is dominant over nitrous oxide production (Focht and Verstraete, 1977; Simek *et al.,* 2002; Simek and Cooper, 2002).

2.4.3.2 Distal Factors

Physical and biological soil factors "distal factors" influence denitrification by controlling the proximate factors described previously. Rainfall and irrigation increase the soil moisture content and decrease oxygen availability. Soil organic matter provides sources of carbon and energy for denitrifying organisms (Dalal *et al.,* 2003) and provides anaerobic microsites (Parkin, 1987). Soil texture can influence the oxygen availability by creating anoxic spaces within the soil (Bollmann and Conrad, 1998). Fine-textured soils with their smaller pores can create more anoxic microsites at lower soil moisture contents than coarse-textured soils (Bollmann and Conrad, 1998). Soil structural damage through compaction can create conditions favourable to denitrification, particularly on soils with high water contents, as this may lead to anaerobic sites within the soil (Luo *et al.,* 1999). Menneer *et al.,* (2005) showed that animal treading alone can lead to an increase in denitrification.

2.4.4 Measurement of denitrification

Of all the biogeochemical processes, denitrification is arguably one of the most difficult to measure in the field (Tiedje *et al.,* 1989). The inability to measure the final product of denitrification, nitrogen gas, due to its high background concentration in the environment and the fact that denitrification is spatially and temporally variable creates difficulties in the measurement process (Tiedje *et al.,* 1989; Groffman *et al.,* 2006). However, a number of methods have been used to measure denitrification at field scales. Extensive reviews of the techniques available to measure denitrification have been made (e.g. Groffman *et al.,* 2006; Tiedje *et al.,* 1989). The following section will just focus on two of the techniques available; nitrogen isotope tracer methods and the acetylene inhibition technique.

2.4.4.1 Nitrogen isotope tracer methods

Several different methods based on the use of ^{15}N have been applied to measure denitrification rate in soils, including; mass balances, isotope fractionation, isotope dilution and direct measurement of ^{15}N labelled gases. Direct measurement of ^{15}N labelled gases has been one of the most important advances in nitrogen isotope tracer methods and is the most popular (Groffman *et al.,* 2006).

One limitation of the nitrogen isotope tracer method is the laborious procedures and expensive instrumentation required (Groffman *et al.,* 2006). Another major disadvantage of the ^{15}N method is that gaseous nitrogen loss may not only be due to denitrification, but also volatilization losses need to be accounted for (Tiedje *et al.,* 1989). However, the use of ^{15}N is one of the only approaches that allows quantification of all the rates of the nitrogen cycle processes as they interact naturally (Tiedje *et al.,* 1989).

2.4.4.2 Acetylene inhibition technique

The acetylene inhibition technique is the most widely used method for determining denitrification (Groffman *et al.,* 2006), and was the method used in this study. The acetylene method allows for a large number of samples to be collected over a short

period of time, which is important due to the large spatial and temporal variability associated with denitrification. Acetylene works by inhibiting the reduction of nitrous oxide to dinitrogen gas during denitrification and thus allows total denitrification to be measured by the accumulation of nitrous oxide (Abbasi and Adams, 2000). The ability of acetylene to enable denitrifiers to accumulate nitrous oxide, by blocking the final step of denitrification was first noted in 1973 by Fedorova *et al.,* (Tiedje *et al.,* 1989). This discovery led to a large increase in studies on denitrification and led to a greater understanding of the process of denitrification (Tiedje *et al.,* 1989). Like any measurement procedure the acetylene inhibition technique has both a number of advantages and disadvantages.

The advantages of the acetylene inhibition technique that have allowed denitrification to be more widely studied include:

1. The use of the natural nitrate substrate pool.
2. The large number of samples that may be analysed. Reducing the statistical concerns over the spatial and temporal distribution of denitrification.
3. The relatively low cost of the method.
4. Versatility of the method allowing lab, field measurements and studies at remote sites (Tiedje *et al.*, 1989; Groffman *et al.*, 2006).

There are also a number of disadvantages with using the acetylene inhibition technique that have led to concern over the validity of the denitrification rates estimated through this method including:

1. Acetylene can affect other processes within the soil e.g. nitrification.
2. The technique can fail if not enough acetylene is present.
3. Contaminants in the acetylene may affect denitrifiers.
4. Inaccurate results will occur due to a number of physical aspects e.g. dispersal of the acetylene, recovery of nitrous oxide and the significant water solubility of nitrous oxide (Tiedje *et al.,* 1989; Groffman *et al.*, 2006).

The majority of the disadvantages can be overcome with care and appropriate design. The only disadvantage that may not be overcome is the effect of acetylene on the process of nitrification. Tiedje *et al.*, (1989) found that this was only a concern in soils where the nitrate concentration was low, but this can lead to an underestimation of denitrification in these systems (Groffman *et al.*, 2006). Overall Groffman *et al.*, (2006) stated that the majority of denitrification rates estimated in terrestrial systems are based on the acetylene inhibition technique and these results have appeared relatively robust. Groffman *et al.*, (2006) also stated that the acetylene inhibition technique is appropriate to use in soils with high nitrate concentrations, such as fertilised systems. Denitrification rates measured by two acetylene techniques (soil cores and chamber techniques) were in agreement in well-drained soils (Ryden *et al.*, 1987) and these techniques have also compared well against ^{15}N methods (Barton, 1998).

2.4.4.3 Variations of the acetylene inhibition technique

There are three different variations of the acetylene inhibition technique for use in either lab or field studies. These techniques are briefly described below, including the advantages and disadvantages of each.

1. Soil Cores: Static cores

The most widely used acetylene inhibition technique has been the static core method and was the method used in this study. The static core method involves extracting intact soil cores from the field and placing them in a jar that can be sealed (Barton, 1998). The acetylene is then injected into the headspace of a sealed core and the nitrous oxide production is measured over time (Groffman *et al.*, 2006). This method has become the most widely used in a range of ecosystems because of its simplicity and the large numbers of cores that can be collected and analysed to account for the spatial and temporal variability of denitrification (Groffman *et al.*, 2006).

2. Soil Cores: Gas phase recirculation cores

The idea behind this system is that acetylene distribution and nitrous oxide recovery from intact soil cores will occur faster and with a greater accuracy if there is mass flow through the soil macropores (Tiedje *et al.,* 1989). Soil, air and acetylene are continuously recirculated through the soil core and a gas chromatograph sampling loop. The gas chromatograph continuously measures the nitrous oxide produced and a denitrification rate can be obtained within two hours (Tiedje *et al.,* 1989). This system has a number of advantages including; (1) the natural soil structure is preserved, (2) the measurement procedure is rapid and (3) soil cores can be re-used for measurements of other soil properties. The main disadvantage of this system is that the equipment required is moderately complex and expensive and only a small number of samples can be analysed at one time (Tiedje *et al.,* 1989).

3. In situ measurements with soil chambers

A major disadvantage of the soil core method is that the soil is disturbed, this problem led to the development of the soil chamber method for measuring denitrification (Tiedje *et al.,* 1989). The soil chamber method involves placing covers over the soil surface and measuring the nitrous oxide produced either by the accumulation of nitrous oxide in the chamber or allowing acetylene to flow through the chamber and measuring nitrous oxide in the exit air stream (Tiedje *et al.,* 1989). The main advantage of the soil chamber technique is that it allows *in situ* field measurements to be made and involves minimal disturbance to the soil (Ryden *et al.,* 1987; Tiedje *et al.,* 1989). There are however a number of disadvantages associated with this technique; (1) the effect of repeated exposure of the soil to acetylene; acetylene is a broad-spectrum inhibitor that can affect a large number of processes in the soil (Klemedtsson and Mosier, 1994), (2) in wet soil with low air-filled porosities acetylene diffusion may be restricted (Ryden *et al.,* 1987), (3) a large number of measurements will need to be made to overcome the spatial variability of denitrification and (4) chamber measurements can be expensive and time consuming (Tiedje *et al.,* 1989).

2.4.5 Rates of denitrification in agricultural soils

The greatest rates of denitrification will occur when soils are warm, wet and soil nitrate and carbon are readily available (Luo *et al.,* 2000; Mosier *et al.,* 2002). Barton *et al.,* (1999) carried out an extensive review of denitrification rates in agricultural soils worldwide and found a geometric mean rate of 13 kg N ha^{-1} yr^{-1}. Annual denitrification rates in agricultural systems are variable with rates reported in the literature between 0 to 239 kg N ha^{-1} yr^{-1} (Barton *et al.,* 1999). The largest reported loss of denitrification (239 kg N ha^{-1} yr^{-1}) was from an agricultural soil that received irrigation of wastewater (liquid cattle manure) at a rate of 643 kg N ha^{-1} yr^{-1} (Lowrance *et al.,* 1998). Fertilised agricultural soils tend to have a greater annual denitrification rate than unfertilised soils (Table 2.6) (Ledgard *et al.,* 1998; Hofstra and Bouwman, 2005). The application of inorganic nitrogen fertiliser has been shown to lead to a greater annual denitrification rate than the application of organic fertilisers (Estavillo *et al.,* 1994). In New Zealand a number of studies have researched denitrification rates from agricultural systems (Table 2.6).

Table 2.6 Denitrification rates reported in New Zealand agricultural systems.

Agricultural System	Rate (kg N/ha)	Soil Texture	Method	Reference
Sheep pasture		Fine sandy loam	C_2H_2 block	Ruz-Jerez *et*
Unfertilised	3.4		soil cores	*al.,* 1994
Fertilised	19.3			
Dairy pasture				
N fert. 0 kg N ha^{-1}	7	Silt loam	C_2H_2 block	Ledgard
225 kg N ha^{-1}	11		soil cores	*et al.,* 1996
360 kg N ha^{-1}	14			
Intact soil cores	2.9	Clay	^{15}N ratio	Clough and
with synthetic	20.2	Peat	technique	Ledgard, 1997
urine applied	13.1	Sandy loam		
	5.9	Silty loam		
Dairy pasture				
N fert. 0 kg N ha^{-1}	2.4	Silt loam	C_2H_2 block	Bailey,
200 kg N ha^{-1}	6.0		soil cores	1997
400 kg N ha^{-1}	12.4			
Dairy pasture		Silt loam	C_2H_2 block	Ledgard
N fert. 0 kg N ha^{-1}	5		soil cores	*et al.,*
200 kg N ha^{-1}	17			1998
400 kg N ha^{-1}	25			
Dairy pasture	4.5	Silt loam	C_2H_2 block	Luo *et al.,*
legume-based			soil cores	2000

2.5 SUMMARY AND CONCLUSIONS

Nitrification inhibitors work by interfering with the action of *Nitrosomonas* bacteria, inhibiting the conversion of soil ammonium to nitrite, resulting in a reduction in the nitrate pool within the soil. Nitrification inhibitors offer environmental benefits through a reduction in nitrous oxide emissions and by reducing the availability of nitrate, a reduction in nitrate leaching and denitrification. However, studies to date have reported mixed results about the effectiveness of nitrification inhibitors. A number of studies have reported that the nitrification inhibitor, DCD can reduce nitrate leaching and nitrous oxide emissions. However, mixed results have been reported about the effectiveness of DCD on reducing denitrification rates. Bremner and Yeomans (1986) and Vallejo *et al.,* (2001) reported that DCD did not decrease denitrification in incubation experiments, while in contrast Thompson (1989) and Pain *et al.,* (1989) reported DCD to reduce denitrification in soil where cattle slurry had been applied. Whether DCD can decrease denitrification rates from legume-based pastoral agricultural situations, where nitrogen fertiliser and animal urine patches are the major source of nitrogen, has not yet been determined.

Chapter 3
Analytical Methods

3.1. INTRODUCTION

This chapter outlines the analytical methods used in the field and laboratory experiment.

3.2. IN SITU DENITRIFICATION RATES

Field measurements of in situ denitrification rates were made using the static soil core incubation system, using the acetylene inhibition technique as described by Ryden *et al.,* (1987). Acetylene stops the conversion of nitrous oxide to dinitrogen gas by denitrifiers (Abbasi and Adams, 2000). As the final step of nitrous oxide to dinitrogen is blocked, nitrous oxide accumulates and represents the total production of nitrous oxide and dinitrogen from denitrification (Tiedje *et al.,* 1989). Although there are concerns over the acetylene inhibition technique, the technique allows for a large number of samples to be taken quickly. The acetylene inhibition technique accounts for the large degree of spatial and temporal variability associated with denitrification in grazed pasture.

The static soil core incubation system works by intact soil cores being removed and placed into a glass preserving jar. The soil cores are made out of PVC pipe with holes evenly spaced down the shaft of the pipe to allow gas exchange. The preserving jars are sealed with lids fitted with a septum stopper. A volume of acetylene, equivalent to 10% (v/v) headspace (120 ml) was injected into the jars through the septum stopper. The syringe was pumped three times to ensure that acetylene was thoroughly mixed through the jar. The jars were then placed in a temperature-controlled room. The temperature was set to the soil temperature at the time of sampling. Gas samples (22ml) were taken from the jars 30 minutes, 3 hours, 6 hours and 24 hours after the addition of acetylene and injected into vacutainers for subsequent analysis. Samples were analysed using a Philips gas chromatograph,

fitted with an electron capture detector at an operating temperature of 350ºC. Gases were separated using a porous packed column at 80ºC and at an injector port temperature of 120ºC. Total denitrification rates were calculated taking into account the solubility of nitrous oxide in the soil water using the temperature-dependent Bunsen absorption coefficients (Tiedje, 1994).

3.3 KCL EXTRACTABLE NITRATE AND AMMONIUM

The nitrate content of the soil was determined because the nitrate concentration in the soil is a controlling factor of denitrification. The ammonium content was important as ammonium is converted through to nitrate and hence may provide a further indication of the amount of nitrate available for denitrification. Nitrification inhibitors can alter the concentration of nitrate and ammonium available in the soil. Soil nitrate and ammonium were extracted by shaking a 10 g soil sample with 100 ml of 2M potassium chloride for one hour and filtering through Advantec 5C filter paper into extraction bottles. The samples were then frozen until subsequent analysis on an autoanalyser (Blakemore *et al.,* 1987).

3.4 SOIL MICROBIAL BIOMASS

Estimates of microbial biomass are difficult to obtain directly due to the minute size of the microbial organisms. Indirect methods have to be used to obtain estimates of the carbon content of the microbial biomass (West *et al.,* 1986). A conversion factor is then used to convert to biomass carbon (West *et al.,* 1986). Substrate-induced respiration was the technique used to determine soil microbial biomass (Anderson and Domsch, 1978; West *et al.,* 1986). This is a rapid estimation of the amount of carbon held in living microorganisms within the soil sample. The initial respiration response of soil is recorded before any growth of the existing soil micro-flora can occur.

A 1 g dry weight equivalent soil sample was weighed out into a McCartney bottle to which glucose was added. The volume and concentration of glucose added was adjusted to ensure a total solution volume of 2 ml at a concentration of 30 mg glucose

ml^{-1} (Sparling *et al.,* 1990). Analysis of carbon dioxide produced was done on the Infra red gas analyser, one hour after the soil sample was incubated at 25ºC and then again after four hours of incubation. The SIR-rates were calculated through to biomass carbon using the formula: µg Cg^{-1} soil = μl CO_2 g^{-1} soil h^{-1} x 50 (Sparling *et al.,* 1990).

3.5 SOIL CARBON AVAILABILITY

The amount of available carbon present in the soil can be indicated by soil microbial respiration. Soil microbial respiration is a process that reflects the potential activity of the soil microbial population (Anderson, 1982). Soil respiration can fluctuate depending on temperature, water content and disturbance of the soil (Brookes, 1995). The availability of carbon in the soil is a controlling factor for denitrification. To determine soil carbon availability, 35 g of soil was weighed into a 1.8 L glass preserving jar. The jar was sealed with lids fitted with a septum stopper. The soil was then incubated for seven days at 25ºC after which the accumulation of carbon dioxide in the headspace gases of the preserving jar were measured on an Infra red gas analyser as an indication of soil respiration (Sparling and Zhu, 1993).

3.6 DENITRIFYING ENZYME ACTIVITY

Denitrifying enzyme activity (DEA) provides an indirect assessment of the size of the denitrifying population at the time of sampling (Smith and Tiedje, 1979). DEA is based on the principal that if conditions are optimized for enzyme catalysed reactions, the reaction rate will be proportional to the enzyme concentration in the soil (Tiedje *et al.,* 1989). To optimize conditions for the catalysed reaction, soil was saturated with carbon and nitrate under anaerobic conditions. To measure DEA (Smith and Tiedje, 1979; Tiedje, 1994), soil (10 g) was weighed into a 100 ml Schott bottle. A 20 ml glucose nitrate solution (0.2 g glucose and 0.1 g KNO_3, dissolved in 1 litre water) including chloramphenicol (0.125 g), to prevent protein synthesis, was added to each Schott bottle. The bottles were sealed with lids fitted with rubber septums and flushed for two minutes with nitrogen gas. Acetylene was added (10 ml) to inhibit the conversion of nitrous oxide through to dinitrogen gas. The samples were

incubated shaking at 25ºC before 5 ml of headspace was removed at 15 and 75 minute intervals and stored in a 3 ml evacuated vacutainer until analysis. The samples were analysed using a Philips gas chromatograph (section 3.2).

3.7 TOTAL CARBON AND TOTAL NITROGEN

The total carbon and nitrogen content of the soil samples were determined by dry combustion on air-dry, finely ground soils using a Laboratory Equipment Corporation (LECO) TruSpec Carbon/Nitrogen Determinator, using software version 1.6x (LECO Corporation, 2006).

3.8 SOIL pH

Soil pH is a measure of the activity of (H^+) ions in the soil solution. Many soil chemical and biological reactions, including denitrification are controlled by the pH of the soil solution. Soil (4 g) was mixed with 10 ml of distilled water, left overnight and soil pH determined using a calibrated pH electrode (Blakemore *et al.*, 1987).

3.9 MOISTURE CONTENT

Soil moisture content was determined gravimetrically on each sampling occasion, from the weight loss of a sub-sample dried over-night at 105ºC (Blakemore *et al.*, 1987).

3.10 TIME DOMAIN REFLECTOMETERS

The moisture content of the soil was continuously measured using Time Domain Reflectometers (TDR) (Campbell Scientific Inc.) located at the field site (Figure 3.1). The in-situ volumetric soil moisture content was indirectly measured using CS 625 water content reflectometers. The water content information is derived through the electrical properties of soils, using the probes sensitivity to the dielectric constant of the medium surrounding the probe rods (Campbell Scientific Inc.). Rainfall at the site was measured using a tipping bucket. A CR200 data logger collected the soil moisture content and rainfall data every 10 seconds and then logged to final storage with an average figure every 30 minutes. The data was downloaded from the CR200

data logger by connecting the data logger to a computer with compatible software and downloading the data directly. The advantages of TDR are that it is highly accurate, there is minimal calibration requirements and measurements are simple to obtain and can be continuously made (Jones *et al.*, 2002).

Figure 3.1 Time Domain Reflectometer set up at the field site at Dexcel's Scott Farm to measure soil moisture content and rainfall.

Chapter 4

Do nitrification inhibitors decrease denitrification rates in dairy farm soils?

4.1 INTRODUCTION

Nitrogen is an essential element for plant growth and agriculture is largely dependent on the use of nitrogen (Erisman *et al.,* 1998). However, soils in dairy farming systems often become saturated with nitrogen (Schipper *et al.,* 2004). Nitrogen entering the soil system from animal excreta is well in excess of plant requirements and can be leached into the groundwater (mostly as nitrate) or lost to the atmosphere (as nitrous oxide a nitrogen gas). Nitrous oxide is a potent greenhouse gas and can be produced through the process of nitrification and denitrification (Barnard and Leadley, 2005). The dairy industry in New Zealand is under scrutiny regarding the environmental sustainability of current dairy farm management practices (PCE, 2004). Nitrification inhibitors are a management strategy that could be implemented on dairy farms to help reduce losses of nitrogen. Nitrification inhibitors work by actively managing soil nitrogen, by temporarily inhibiting nitrification. By inhibiting nitrification the nitrate pool in the soil is reduced and nitrate leaching minimized (Di and Cameron, 2002b). By reducing nitrate concentration, nitrification inhibitors may also indirectly affect other microbial processes such as denitrification rates. Denitrification is controlled by a number of factors, including nitrate concentration (Hénault and Germon 2000).

The aim of this field trial was to establish if the nitrification inhibitor, dicyandiamide (DCD), could reduce denitrification rates by limiting nitrate availability, in soils subjected to standard dairy farming practices. The field trial was located at Dexcel's Scott Research Farm near Hamilton (Figure 4.1).

Twenty replicated field plots were established in a paddock, ten plots acted as controls and ten plots had DCD applied to the soil once a month at a rate of 30 kg ha^{-1} yr^{-1}. Five days after the application of DCD, denitrification rates, ammonium and

nitrate concentration, soil carbon availability, denitrifying enzyme activity (DEA) and soil pH was measured from soil samples collected from each plot. This main experiment was supplemented with two further experiments. I determined the distribution of denitrification activity with depth to ensure the sampling depth used to measure denitrification rates was sufficient to capture most of the denitrifying activity. Four times during the year soil samples were collected at three depths down the soil profile (0 – 15, 15 – 30, 30 – 45 cm). The soil samples were then analysed for DEA, soil microbial biomass, soil carbon availability, total carbon and total nitrogen content. Finally, I measured degradation of DCD with time to determine how long the effects of DCD might last. After DCD application, soil samples were taken from DCD-amended plots each day for six days and then every second day for a further twelve days. Soil samples were analysed to determine DCD concentration within the soil with time.

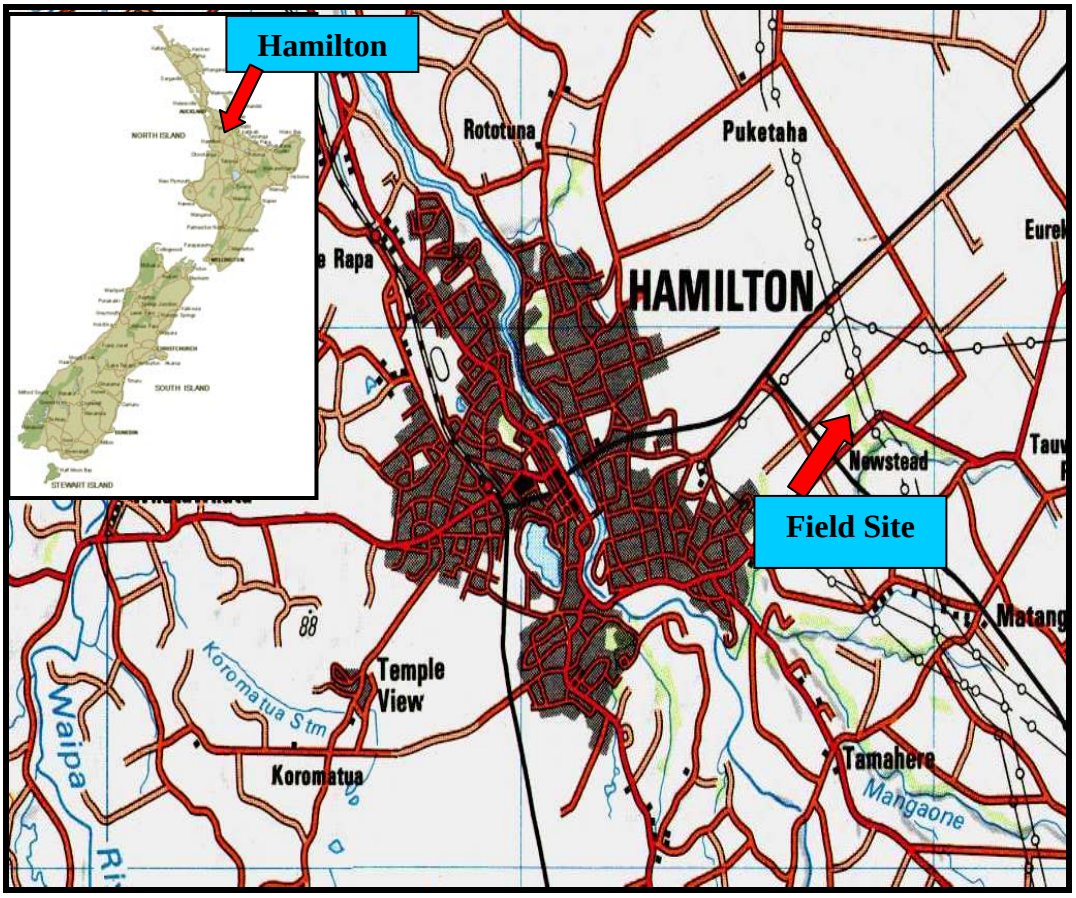

Figure 4.1 Location of field study within Dexcel's Scott farm, Hamilton.

4.2. STUDY SITE

4.2.1 RED Trial

The field trial was established in part of the Resource Efficient Dairying (RED) Trial at Dexcel (Figure 4.1 and 4.2). The aim of the RED Trial was to measure the economic and environmental effects of different feed inputs and management processes on a dairy farm. The RED Trial will provide data on the environmental consequences of intensive dairying and will address the industry's requirements to improve milk solids production. The trial began in 2001 and the initial design was based on six farm systems with feed inputs that vary from 17.5 to 40.5 t DM ha^{-1} yr^{-1}, and with stocking rates varying from three to seven cows ha^{-1} (Table 4.1; Jensen *et al.*, 2005).

Table 4.1 RED Trial farm treatments from 2001 till June 2006. Indicating type of treatment, amount of nitrogen applied and supplements feed, total dry matter over one-year period and the stocking rate (Jensen *et al.*, 2005).

Farmlet	**Treatment**	**Total Dry Matter (t ha^{-1} yr^{-1})**	**Stocking rate (cows ha^{-1})**
A	Control – 200 kg N ha^{-1} yr^{-1}	17.5	3.0
B	Stand-off – 200 kg N ha^{-1} yr^{-1}	17.5	3.0
C	Low input – zero N	15.0	2.3
D	Supplement – maize silage (5t DM ha^{-1} yr^{-1})	22.5	3.8
E	Supplement – maize silage and irrigation	30.5	5.3
F	Supplement – maize silage irrigation and soya meal	40.5	7.0

In June 2006 the RED Trial under-went a number of changes. Two new prototype farmlets were introduced to replace three of the original farmlet treatments. The two new prototype farms introduced were a Super Productivity farmlet and a Tight Nitrogen farmlet (Table 4.2).

Table 4.2 RED Trial farm treatments as of June 2006. Indicating type of treatment, amount nitrogen applied and supplements feed, total dry matter over one-year period and the stocking rate (Jensen *et al.*, 2005).

Farmlet	**Treatment**	**Total Dry Matter (t ha^{-1} yr^{-1})**	**Stocking rate (cows ha^{-1})**
A	Control – 200 kg N ha^{-1} yr^{-1}	17.5	3.0
B	Tight Nitrogen Farm – 200 kg N ha^{-1} yr^{-1}	17.5	3.0
C	Low input – zero N	15.0	2.3
D	Supplement – maize silage	22.5	3.8
F	Super Productivity Farm	40.5	5.0

My field experiment was established in paddock C34a of the farmlet A trial, which was the control treatment (Figure 4.2). The paddock was about 0.5 hectares and was periodically grazed by cows at a stocking rate of three cows per hectare. This paddock was subject to standard farming practice but without irrigation and the control treatment was not going to change during this study.

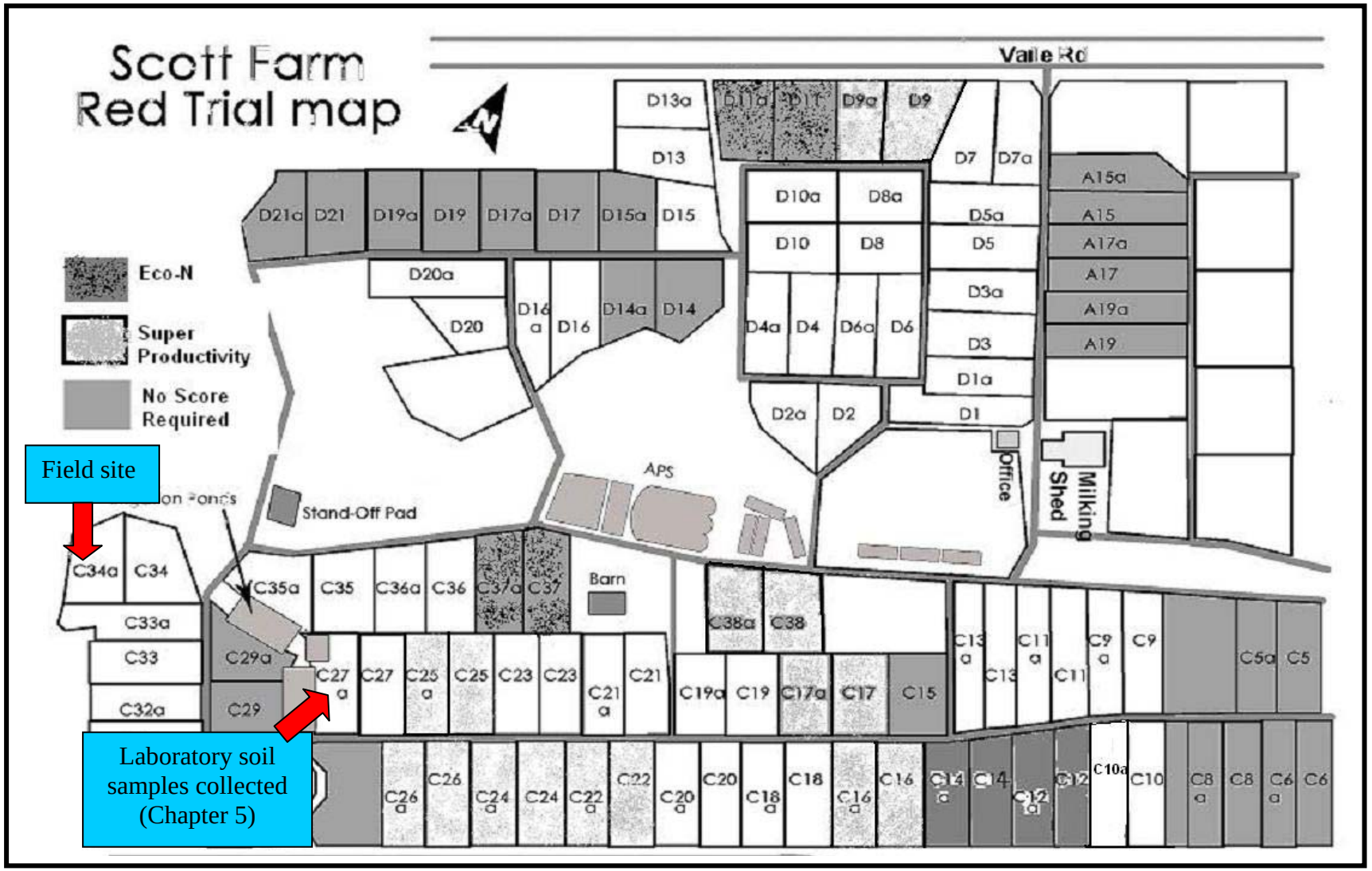

Figure 4.2 Map of Dexcel's Scott Farm RED Trial indicating the location of my field site, paddock C34a and location of where soil samples were collected for the laboratory experiment, paddock C27a (Chapter 5).

4.2.2 Climate and vegetation

The Waikato Region has a temperate climate with a mean annual rainfall of 1250 mm and mean summer temperature of 23.8ºC and a mean winter temperature of 13.6ºC (Environment Waikato, 2006). The pasture at the field site was dominated by ryegrass (*Lolium perenne* L), paspalum (*paspalem distichum* L) and white clover (*Trifolium repens* L).

4.2.3 Soil Characteristics

The soils at the study site were Horotiu silt loam (Typic Orthic Allophanic Soil; Hewitt, 1998). The Horotiu series was formed from alluvium deposited as low linear ridges by the ancient Waikato River system. The Horotiu series soils have medium to low soil dry bulk densities, moderate permeability, and high phosphate retention (Singleton, 1991). Physical and chemical properties of the Horotiu silt loam were measured at the field site (Table 4.3).

Table 4.3 Physical and chemical properties of the Horotiu soil (0 – 15 cm) at the field site. (Data supplied by Landcare Research; Palmerston North and Hamilton).

Property	**Measurement**	**Property**	**Measurement**
Total Carbon	8.17%	pH (in water)	5.3
Total Nitrogen	0.75%	Bulk Density	0.82 g/cm^3
Total Phosphorus	2413 mg/kg	Olsen P	18.5mg/kg
Dissolved organic carbon	917 µg C/g soil	Hot water extractable carbon	2388 µg C/g soil
56 day aerobic mineralisable N	53 mg/kg	Anaerobic mineralisable N	62 µg N/g soil
Extractable NH_4^+	1.2 mg/kg	Extractable NO_3^-	9.4 mg/kg

4.3 EXPERIMENTAL DESIGN

4.3.1 Experiment 1: Effect of DCD on denitrification rates

Twenty replicated field plots were established in a randomized plot design. Each plot measured 4 m x 4 m and had a 0.5 m-guard strip between the plots (Figure 4.4). Each plot was randomly assigned one of two treatments and there were ten replications of each treatment. Treatment one acted as a control, as no nitrification inhibitor was applied. Treatment two had the nitrification inhibitor applied to the soil at a rate equivalent to 30 kg ha^{-1} yr^{-1} as an aqueous solution (48 g DCD per 16 m^2 plot per year). DCD application to the assigned plots occurred either three days after grazing of the paddocks or during periods of longer grazing rotation (> 30 days) application of DCD occurred approximately once every three weeks (Table 4.4). The application of DCD after grazing of the paddock ensured that the pasture was short enough that the application of DCD reached the soil surface (Di and Cameron, 2005).

Table 4.4 Timetable indicating the day cows grazed the field trial, and the days application of DCD and sampling occurred.

Sampling Month	Cows Grazed	DCD application	Sampling
January	-	6th Jan	12th Jan
February	22nd Jan	26th Jan	1st Feb
March	19th Feb	23rd Feb	1st March
April	-	-	-
May	22nd April	26th April	2nd May
June	-	26th May	1st June
July	26th June	30th June	4th July
August	-	26th July	1st Aug
September	1st Sep	5th Sep	11th Sep
October	24th Sep	28th Sep	4th Oct
November	30th Oct	3rd Nov	9th Nov
December	21st Nov	5th Nov	1st Dec

Three days after every grazing event or once every three weeks, DCD (4.8 g) was applied to each plot. The DCD was mixed with five litres of water and the aqueous solution was sprayed onto the plots using a "knap-sack sprayer" to ensure even coverage of the plot (Figure 4.3). The control plots did not receive any supplementary water irrigation, as the amount of water applied to the DCD plots was minimal, equivalent to 3 mm of rainfall over the year.

Figure 4.3 Application of DCD to assigned plots (photo: Lisa Watkins)

Five days after the application of DCD about 100 g of soil from each plot was collected using a soil corer (0 - 15 cm). In the laboratory the soil samples were passed through a 4 mm sieve. This soil was then analysed for a variety of soil biochemical parameters (methods given in Chapter 3);

1. Ammonium and nitrate concentration
2. Soil carbon availability
3. Denitrifying enzyme activity
4. Soil pH
5. Soil moisture content

Soils not used immediately were stored at 4°C. All soil biochemical measurements were carried out within two days of field sampling.

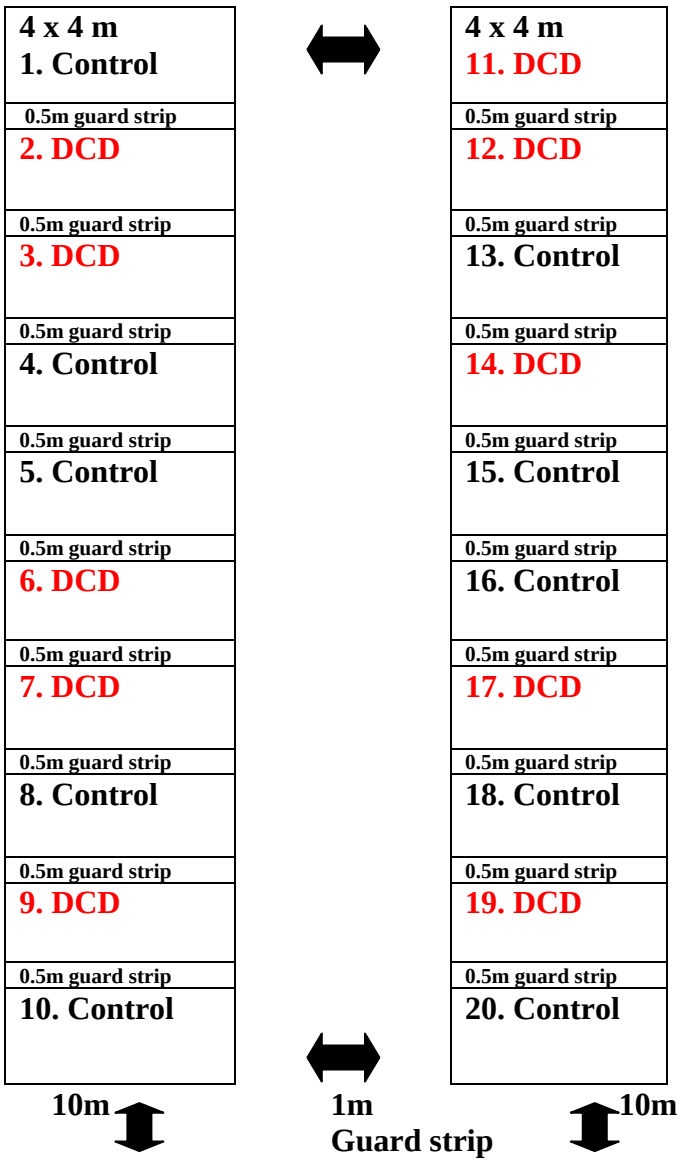

Figure 4.4 Field plot design, paddock C34a. The 20 plots were in two rows of 10 plots. A 0.5 m guard strip was present between plots and 1 m guard strip was present between rows. Half the plots had DCD applied (4.8 g DCD) after every grazing event.

Annual denitrification rates from each plot were determined using the acetylene inhibition technique on soil cores (section 3.2; Ryden *et al.*, 1987). Four minimally disturbed soil cores (16 cm in length and 3.2 cm in diameter) were removed from each plot five days after DCD application and placed into a 1.8 L glass-preserving jar (Figure 4.5). The jars were then sealed and the gas acetylene was injected into each jar. Gas samples were removed after acetylene had been injected, at times 0, 3, 6 and 24 hours, for subsequent analysis.

Figure 4.5 Field sampling to determine denitrification rates using the acetylene inhibition technique on intact soil cores (photos: Jacinta Parenzee).

4.3.2 Experiment 2: Changes in soil biochemical properties with depth

The majority of research on denitrification rates has focused on the top 0 - 20 cm of soil (Tiedje *et al.,* 1989), it is argued that beyond this depth soil organic matter and nitrifying and denitrifying bacteria are insufficient to support denitrification (Barton *et al.,* 1998). Inorganic and organic materials that enter the soil from plants and animals provide energy sources and nutrients for microorganisms and this material is largely deposited directly onto the soil surface (Luo *et al.,* 1998).

To confirm that denitrification activity was greatest in the top 0 - 20 cm of the soil, an experiment looking at distribution of soil biochemical properties with depth was undertaken. Soil samples were collected from eight sites adjacent to the DCD trial, four sampling times during the year (autumn, winter, spring and summer). At each site, soil samples were taken using a soil sampling tube (Figure 4.6). The eight sampling sites were located around the perimeter of the field trial plots. Two soil cores were taken at each site and the soil divided into three depths (0 - 15 cm, 15 - 30 cm and 30 - 45 cm). The two soil cores from each site were combined to ensure there was enough soil for the laboratory experiments. On return to the laboratory, the soil was passed through a 4 mm sieve and analysed for a variety of soil biochemical parameters (methods given in Chapter 3);

1. Soil microbial biomass
2. Soil carbon availability
3. Denitrifying enzyme activity
4. Total carbon and total nitrogen
5. Soil moisture content

Soils not used immediately were stored at 4°C. All soil biochemical measurements were carried out within two days of field sampling.

Figure 4.6 Soil sampler used to sample at different depths down the soil profile and to collect soil samples to measure the rate of DCD degradation

4.3.3 Experiment 3: Rate of DCD loss in the soil

It was important to establish the degradation of DCD to determine how long the effects of DCD application might last. The rate of degradation within the soil has been shown to be principally controlled by soil temperature (Rajbanshi *et al.*, 1992; Di and Cameron, 2004a).

Twelve months after the trial commenced (December 2006), soil samples were collected from four DCD plots (3, 9, 14, and 17; Figure 4.4) every day for the first six days following DCD application and then every second day for a further twelve days. Soil samples were collected to a depth of 10 cm using a soil sampler (Figure 4.6). Soil samples were bulked from plots 3 and 9 and plots 14 and 17 to give two replications per day.

The soil samples were passed through a 4 mm sieve and moisture content determined (section 3.9); the remaining soil was stored at 4°C overnight. A 20 g oven-dry equivalent soil sample was weighed into a 250 ml centrifuge bottle and extracted with 100 ml distilled water and placed on an end-over-end shaker for one hour. The sample was then centrifuged at 14,500 rpm for five minutes at a temperature of 20°C. The extract (10 – 15 ml) was filtered through Whatman #42 paper into an extraction bottle and frozen until subsequent analysis. Analysis for DCD was undertaken using the method of Schwarzer and Haselwandter (1996), using Shimadzu high performance liquid chromatograph (HPLC), with an Aminex organic acid column HPX-87H 300 x 7.80 mm, at AgResearch, Hamilton.

4.4 DATA ANALYSIS

Analysis of variance (ANOVA) was carried out using the statistical package, Statistica version 7.1 (2006). ANOVA was performed on; denitrification rates, ammonium and nitrate concentrations, DEA, carbon availability and pH, to determine whether there were significant differences ($p < 0.05$) between control plots and DCD-amended plots. Denitrification rates, DEA, and ammonium and nitrate concentrations were log-transformed prior to analysis, while carbon availability and soil pH values were squared prior to analysis to normalise the data. The following soil properties were investigated for their relationship with denitrification using a general linear regression model; water-filled pore space (WFPS), DEA, soil ammonium and nitrate concentration, carbon availability and soil pH.

4.5 RESULTS

4.5.1 Site climate data

Soil temperature and rainfall showed a strong seasonal trend. As expected, soil temperature, at a depth of 10 cm and measured at 8.30 am on the day of field sampling, was lowest in the winter months and highest in the summer months. The wettest months were May, August and January and the driest months were February and September. Soil moisture content was only slightly lower during the summer months and was fairly consistent for the rest of the year (Figure 4.7).

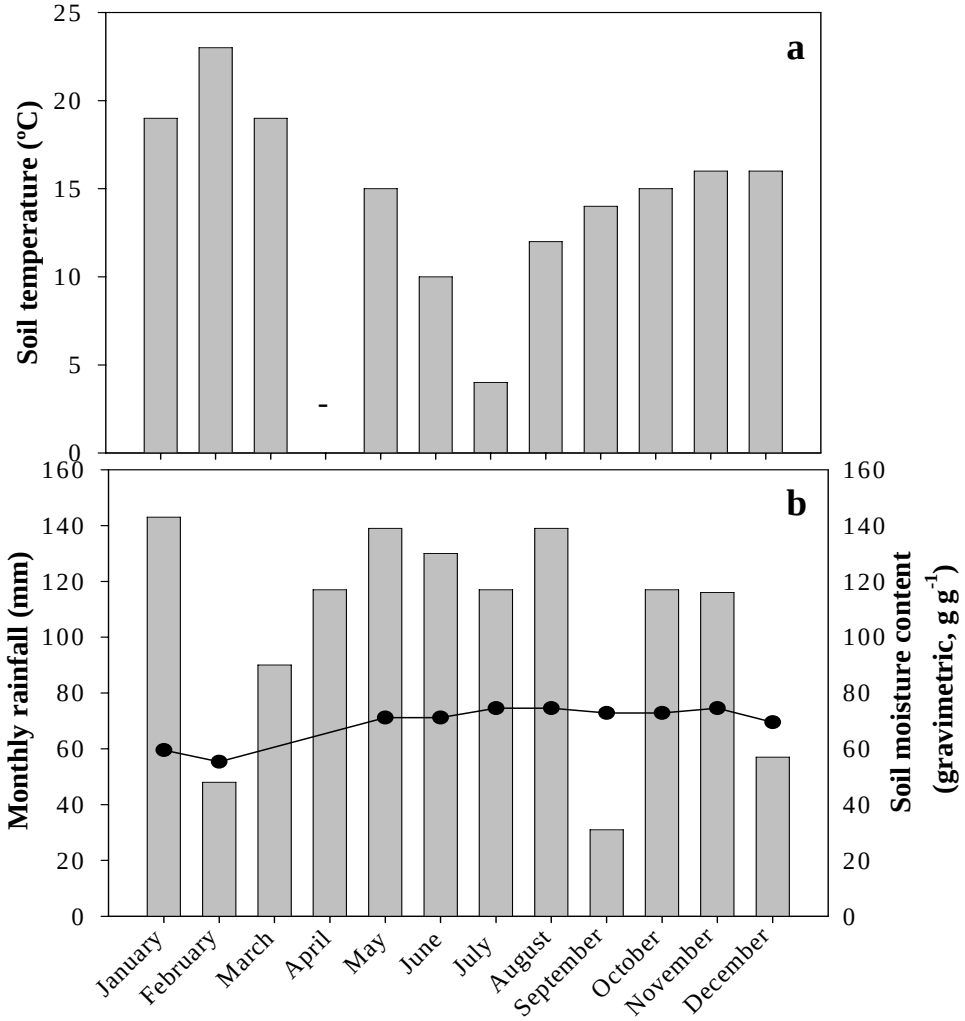

Figure 4.7 Soil temperature (a), rainfall and moisture content (b) as measured at the field site, bars represent rainfall and line represents moisture content, note no measurement of soil temperature was taken for April.

4.5.2 Experiment 1: Effect of DCD on soil denitrification rates

A range of soil biochemical parameters were measured throughout the one-year field study (Table 4.5). Median values for denitrification in control soils and DCD-amended soils were very similar. Soil ammonium concentrations were higher and soil nitrate concentrations were lower in the DCD-amended soils compared to the control soils. Denitrifying enzyme activity, carbon availability and soil pH showed similar values for both control and DCD-amended soils. Appendix A contains the raw data for each variable measured.

Table 4.5 Summary statistics for soil properties in control soils and the DCD-amended soils (n = 110).

Soil parameter	**Mean** (standard deviation)	**Median**	**Mean** (standard deviation)	**Median**
	Control soils		**DCD-amended soils**	
Denitrification rates ($kg\ N\ ha^{-1}\ yr^{-1}$)	14 (37.4)	5.9	28 (86.7)	5.1
Soil ammonium ($\mu g\ N\ g^{-1}$ soil)	15.9 (22.4)	6.2	22.0 (25.7)	8.0
Soil nitrate ($\mu g\ N\ g^{-1}$ soil)	27.7 (23.8)	21.1	23.0 (21.7)	15.3
Denitrifying enzyme activity ($ng\ N\ g^{-1}$ soil h^{-1})	311 (402)	237	368 (500)	247
Carbon availability ($\mu g\ CO_2\text{-}C g^{-1} h^{-1}$)	4.2 (1.5)	4.4	4.5 (1.6)	4.5
Soil pH	6.0 (0.4)	6.1	6.0 (0.4)	6.1

4.5.2.1 Effect of DCD on denitrification rates

There was no evidence that DCD inhibited denitrification as there was no significant difference between control soils and DCD-amended soils (Figure 4.8). Denitrification rates showed a marked seasonal effect. The highest denitrification rates were found in winter and spring and the lowest denitrification rates were found in summer and autumn (Figure 4.8).

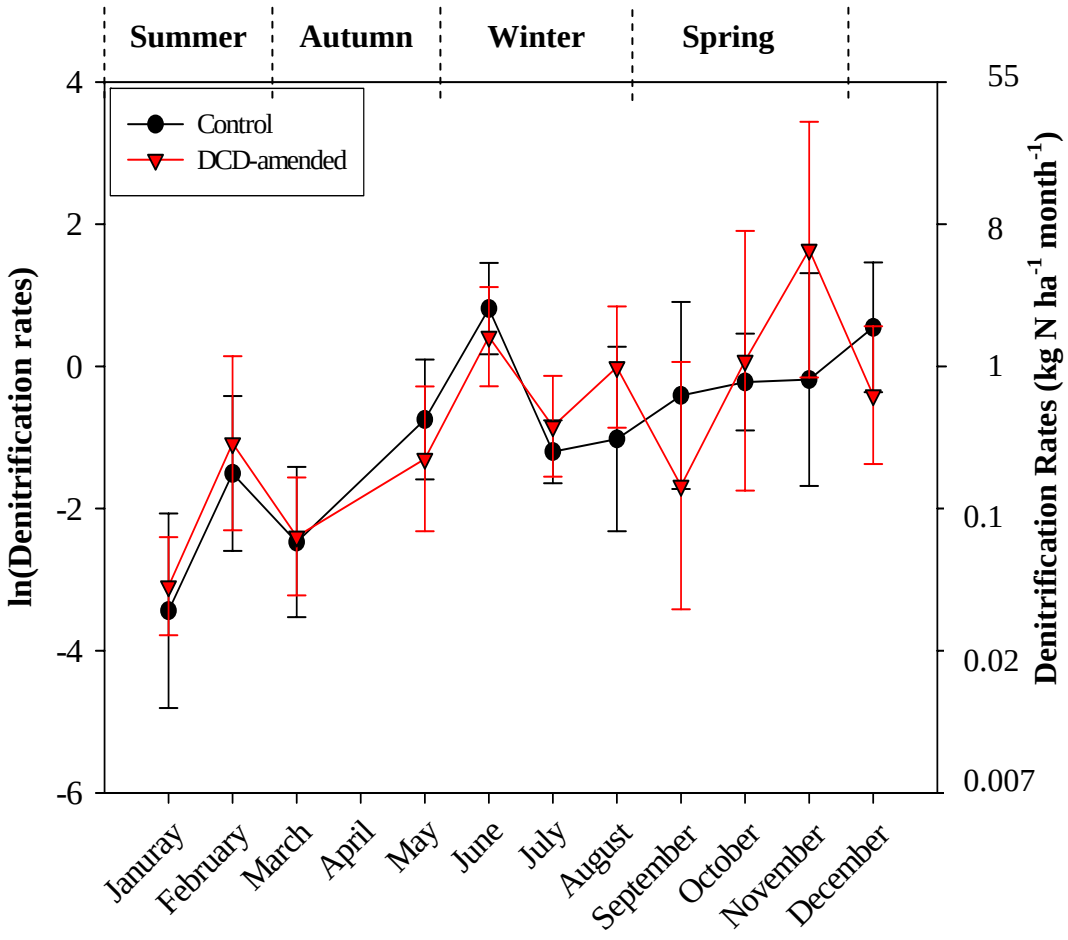

Figure 4.8 Denitrification rates in DCD-amended soils and control soils over a one-year period. Left y-axis is the natural-log of denitrification rates and the right axis is denitrification rates in kg N ha^{-1} $month^{-1}$, error bars indicate 1 standard deviation, (each point n =10).

The DCD-amended soil had a higher mean denitrification rate of 28 kg N ha^{-1} yr^{-1} (although not significantly different from controls) with a much greater range of values than the control plots. The maximum rate measured was 1.38 kg N ha^{-1} day^{-1} during one sampling and four other denitrification rates above 0.99 kg N ha^{-1} day^{-1}. The control soils had a mean denitrification rate of 14 kg N ha^{-1} yr^{-1}. However, when comparing the denitrification rates of the DCD and control soils on a box plot (Figure 4.9), both treatments had similar median values and similar first quartiles, but the third quartile was greater in the DCD-amended soils, due to the greater range of data values.

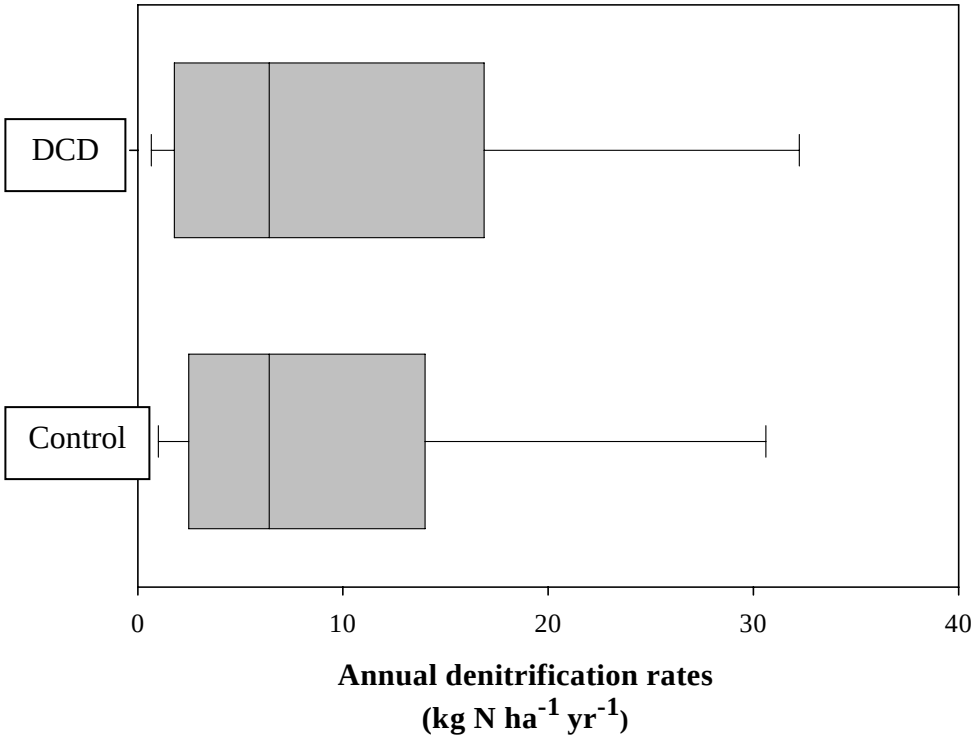

Figure 4.9 Box plot comparing annual denitrification rates for DCD-amended plots and control plots. The centre vertical line marks the median, the edges of the box mark first and third quartiles and the horizontal lines indicate the range of values that fall within 1.5 (midrange) of the hinges.

As with denitrification rates, there was no difference in DEA between the DCD-amended soils and the control soils (Figure 4.10). DEA showed a seasonal trend with the highest concentration of activity reported in the summer months and the lowest concentration of activity reported in the winter months.

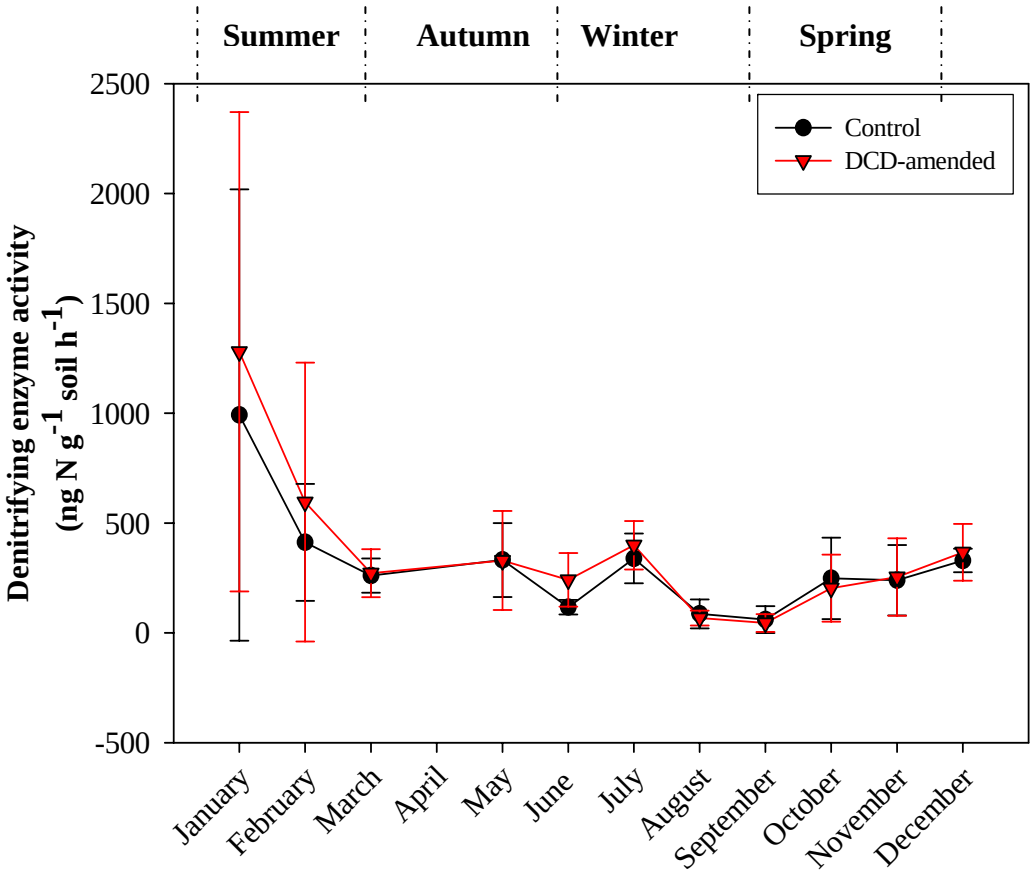

Figure 4.10 Soil DEA in DCD-amended soils and control soils, error bars indicate 1 standard deviation (each point n = 10).

4.5.2.2 Effect of DCD on ammonium and nitrate concentration

DCD had an effect on both ammonium and nitrate concentrations, indicating that nitrification was inhibited in the DCD-amended soils. While there was considerable variability, ammonium concentrations were greater ($p < 0.01$) while nitrate concentrations were less ($p < 0.001$) in the DCD-amended soils compared to the control soils (Figure 4.11).

A strong seasonal effect was observed for both ammonium and nitrate concentrations. Ammonium concentration increased during the winter months and then decreased during the spring months as nitrification increased (Figure 4.11). Nitrate concentration decreased markedly in autumn and then gradually began to rise again in spring (Figure 4.11).

Nitrate concentration is a controlling factor for denitrification. Once nitrate concentrations fall below a certain threshold denitrification becomes nitrate limited. The nitrate threshold that is considered to be limiting for denitrification has been reported for loam soils to be 5 mg NO_3^- kg^{-1} soil, indicated by the dashed horizontal line (Figure 4.11; Ryden, 1983). At each sampling occasion, except for the month of June, nitrate concentration within the soil is consistently above this threshold for both control and DCD-amended soils.

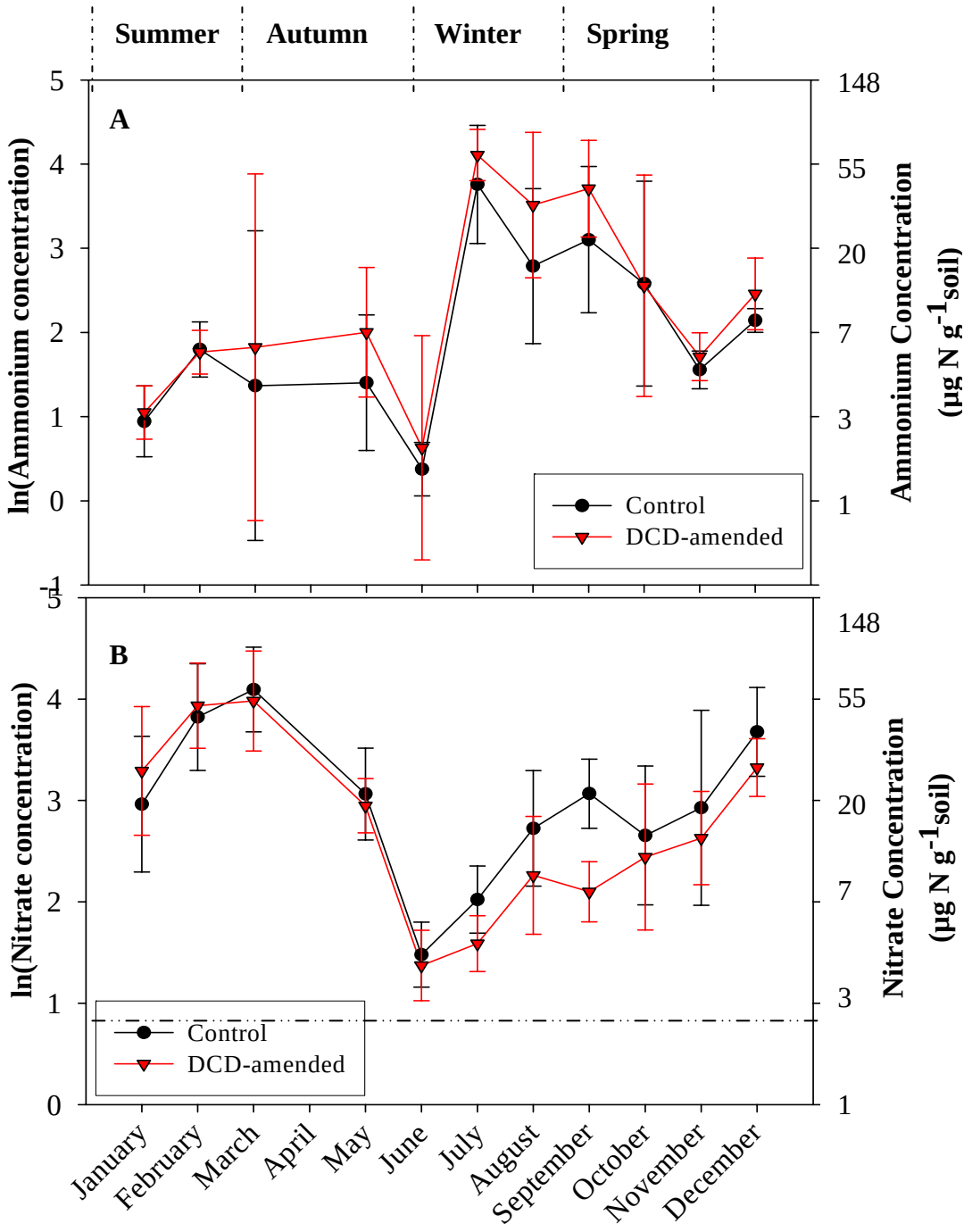

Figure 4.11 Soil ammonium and nitrate concentration in DCD-amended soils and control soils. Left y-axis is the natural-log of concentration and the right axis is data in µg N g^{-1} soil, error bars indicate 1 standard deviation (each point n = 10). On graph B the dashed horizontal line represents the nitrate threshold that is considered to be limiting for denitrification (Ryden, 1983).

4.5.2.3 Effect of DCD on soil carbon availability

DCD had no effect on soil carbon availability (Figure 4.12). Soil carbon availability was slightly higher in the DCD plots on 9 out of the 11 sampling occasions, but the difference was not significant. No seasonal trend was observed.

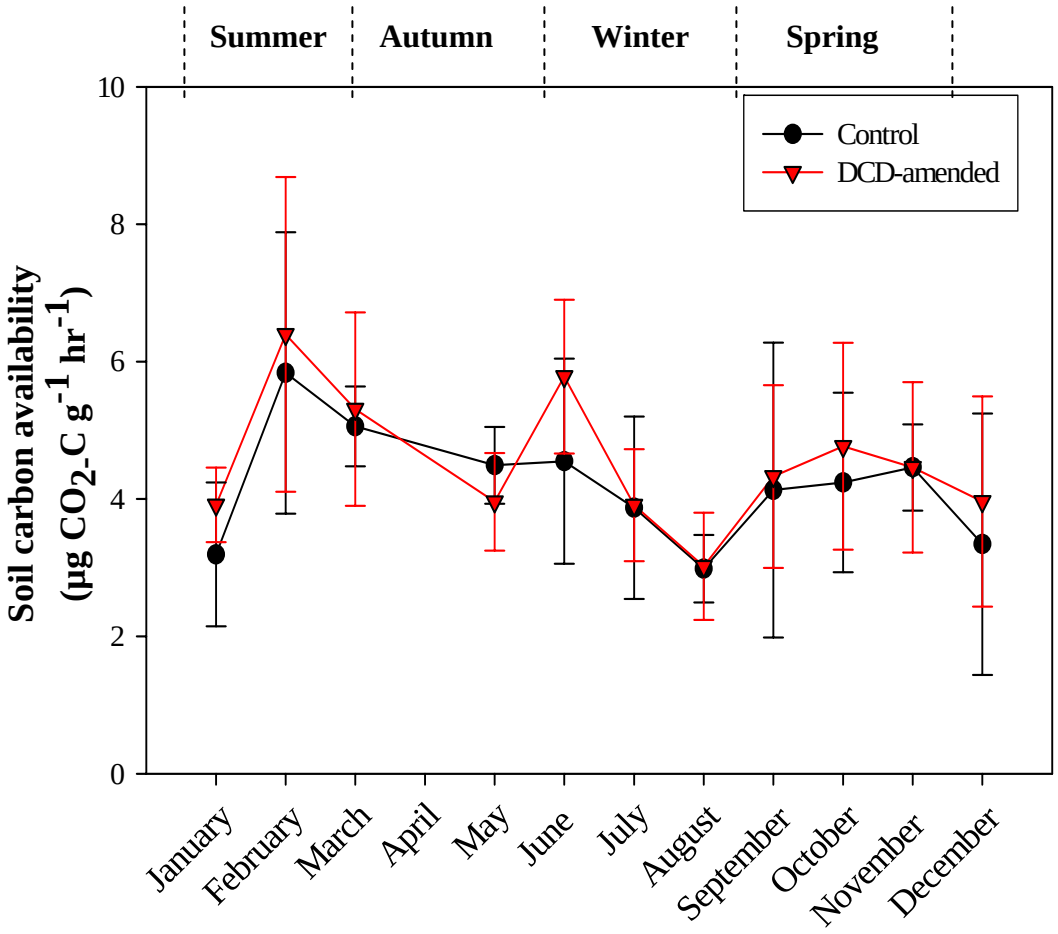

Figure 4.12 Soil carbon availability in DCD-amended soils and controls soils, error bars indicate 1 standard deviation (each point n = 10).

4.5.2.4 Effect of DCD on soil pH

DCD had no effect on soil pH with no significant difference found between DCD-amended soils and control soils (Figure 4.13).

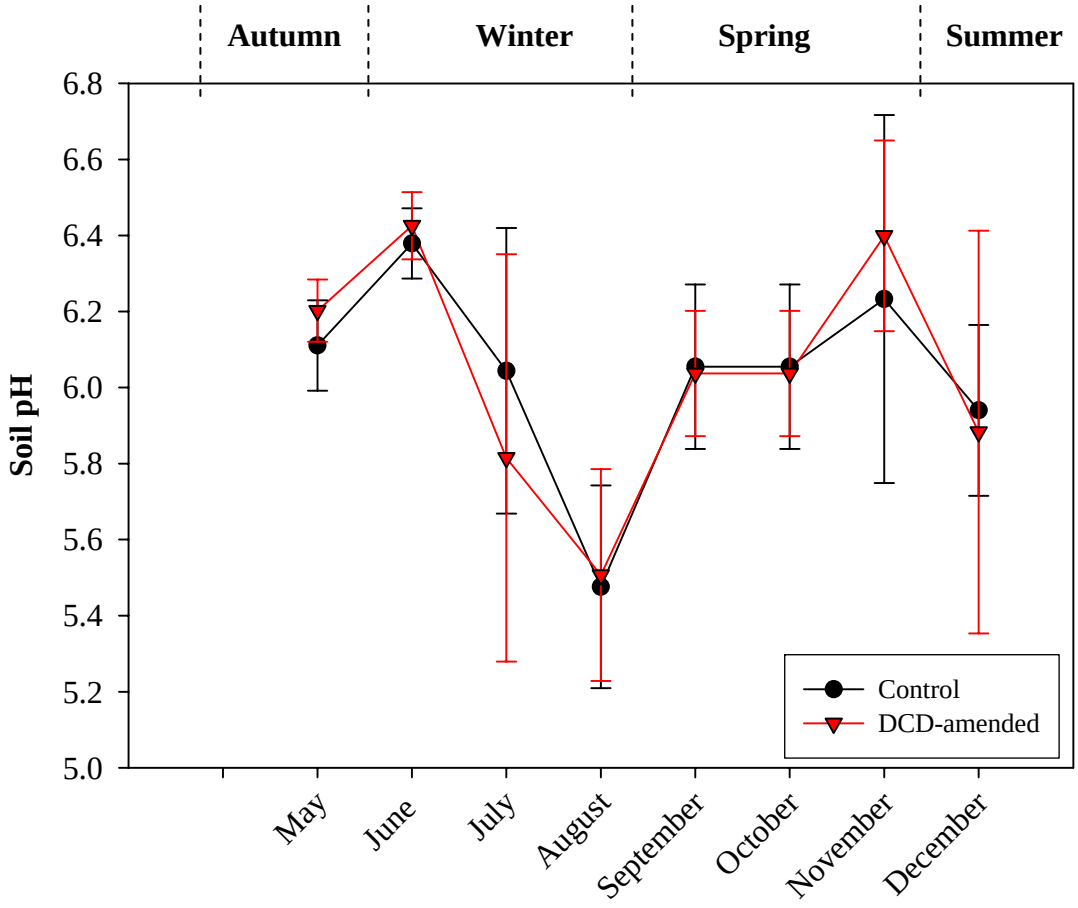

Figure 4.13 Soil pH in DCD-amended soils and control soils, error bars indicate 1 standard deviation, (each point n = 10), note measuring of soil pH did not begin till May.

4.5.2.5 Relationship of denitrification rates to selected soil properties

The following soil properties were investigated for their relationship with denitrification: water-filled pore space (WFPS), DEA, soil ammonium, soil nitrate, carbon availability and soil pH. A linear regression model was trialled with denitrification as the dependent variable and the variables stated above as the explanatory variables. None of the variables explained observed changes in denitrification rates.

Although the linear regression model gave no relationship between denitrification rates and WFPS, a threshold value over which denitrification rates increased was approximately between (55 – 60%; Figure 4.14). This value corresponds to a volumetric water content of 41 – 44 g H_20 g^{-1} dry soil. As WFPS increased above the 60% threshold denitrification rates increased.

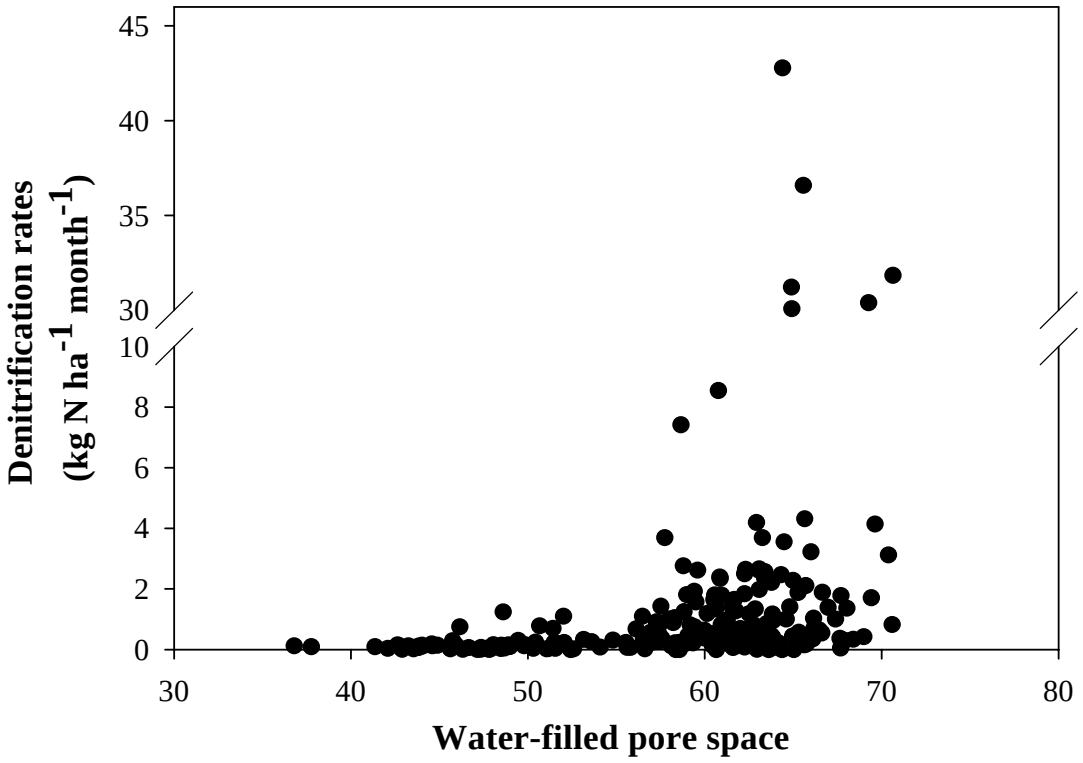

Figure 4.14 The relationship between denitrification rates and the water-filled pore space.

4.5.3 Experiment 2: Changes in soil biochemical properties with depth

Soil biochemical properties were measured at three depths down the soil profile. The DEA, soil microbial biomass and carbon availability were greatest in the top 0 – 15 cm of the soil profile, with 80%, 60% and 61% of total activity occurring within this zone respectively (Table 4.6). Soil total carbon and total nitrogen was also greatest in the top 0 – 15 cm of the soil profile, with a carbon to nitrogen ratio of 10:1. Appendix B contains the raw data for each variable measured.

Table 4.6 Summary statistics for changes in soil properties with depth.

Soil parameter	**n**	**Mean** (standard deviation)	**Median**
Denitrifying enzyme activity (ng N g^{-1} h^{-1})			
0 – 15 cm	32	32.0 (28.0)	20
15 – 30 cm		5.6 (3.4)	6.0
30 – 45 cm		2.2 (2.1)	1.3
Soil microbial biomass (µg C g^{-1} soil)			
0 – 15 cm	32	564 (138)	576
15 – 30 cm		248 (96)	261
30 – 45 cm		125 (41)	118
Soil carbon availability (µg CO_2- C g^{-1} h^{-1})			
0 – 15 cm	32	1.9 (0.7)	1.8
15 – 30 cm		0.8 (0.3)	0.6
30 – 45 cm		0.4 (0.6)	0.4
Total Carbon (%)	2		
0 – 15 cm		7.3 (0.1)	7.3
15 – 30 cm		3.6 (1.0)	3.6
30 – 45 cm		1.5 (0.1)	1.4
Total Nitrogen(%)	2		
0 – 15 cm		0.7 (0.0)	0.7
15 – 30 cm		0.4 (0.8)	0.4
30 – 45 cm		0.2 (0.0)	0.2

4.5.4 Experiment 3: Rate of DCD loss in the soil

The DCD concentration in the soil was measured during December (average soil temperature 16°C at soil depth of 10 cm) to establish the rate of DCD degradation. The amount of DCD applied to each plot was 3.7 µg DCD g^{-1} soil, to a soil depth 10 cm. DCD concentration degraded rapidly within the first two days after application and then the rate of degradation slowed (Figure 4.15). One day after DCD application, 2.83 µg DCD g^{-1} soil (76%) had been lost. No DCD was present in the soil 19 days after DCD application. The half-life of DCD at an initial concentration of 3.7 µg DCD g^{-1} soil was 2.9 days (calculated from the exponential decay equation $y = ae^{-bx}$). Appendix C contains the raw data for the rate of DCD loss in the soil

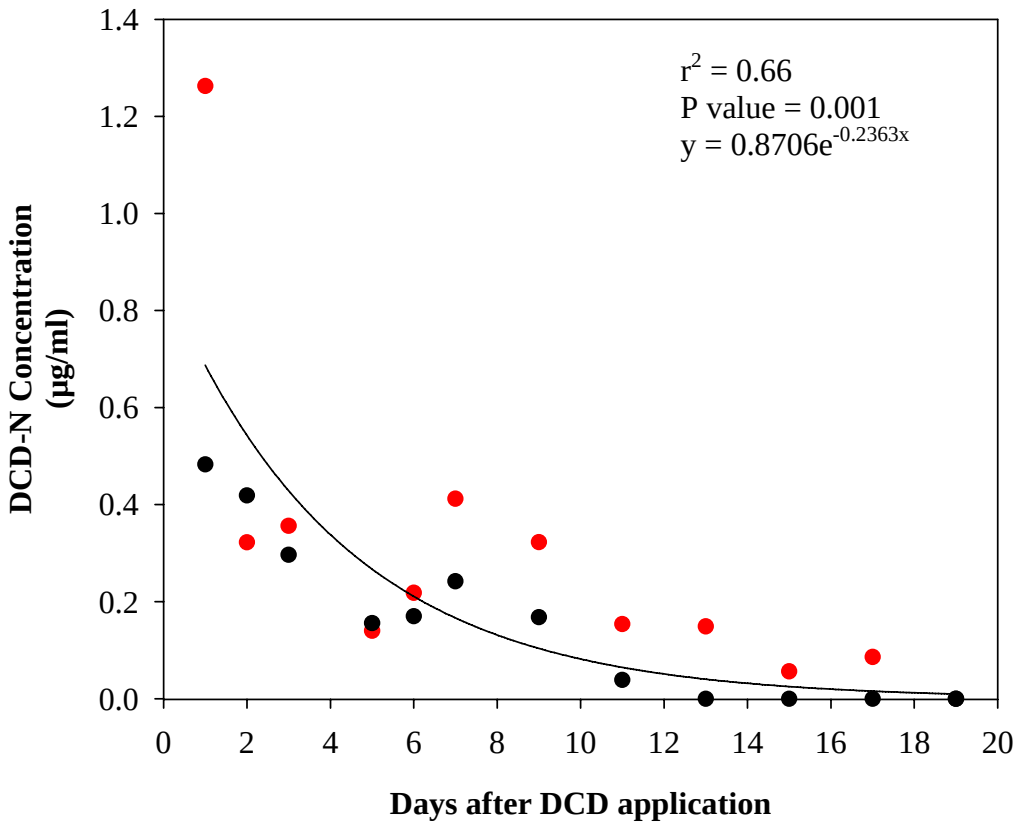

Figure 4.15 Degradation of DCD after application to dairy farm soils during the month of December fitted with an exponential decay curve ($y = ae^{-bx}$), red dots represent replicate one and black dots represent replicate two.

4.6 DISCUSSION

4.6.1 Experiment 1: Effect of DCD on denitrification rates

My hypothesis was that the nitrification inhibitor, DCD, would reduce denitrification rates by limiting nitrate availability in dairy farm soils. However, the application of DCD did not decrease denitrification rates (Figure 4.8). This was most likely because nitrate concentrations was consistently above 5 mg NO_3^- kg^{-1} soil, which is considered to be the threshold for denitrification (Ryden, 1983). However, the application of DCD did inhibit nitrification, predominantly in the winter months. An increase in ammonium concentrations ($p < 0.01$) and a decrease in nitrate concentration ($p < 0.001$) in the DCD-amended soils were found towards the end of the year (Figure 4.11). Although the differences in ammonium and nitrate concentrations were significant, the magnitude of difference was small. In the DCD-amended soil, ammonium concentration increased by 14% and nitrate concentration decreased by 17% compared to the control soils. Ryden (1983) reported a value of 5 mg NO_3^- kg^{-1} soil to be a threshold value for a loam soil, above which *in situ* denitrification rates increased. Throughout the year, nitrate concentrations in both the control soils and the DCD-amended soil were above the 5 mg NO_3^- kg^{-1} soil threshold (except for the month of June; Figure 4.11). Another reason why nitrate did not limit denitrification may have been due to DCD being rapidly degrading in the soil and therefore reducing the effectiveness of DCD, further discussion in section 4.6.4.

Previous research on whether nitrification inhibitors decrease denitrification rates has provided mixed results. Bremner and Yeomans (1986) reported that DCD applied at a rate of 10 µg g^{-1} soil resulted in no appreciable change in denitrification rates compared to the control. When they applied DCD at 50 µg g^{-1} soil, they measured a small increase in denitrification. Vallejo *et al.*, (2001) similarly showed in an incubation experiment that although DCD inhibited nitrification, there was no decrease in denitrification rates. Calderon *et al.*, (2005), although working with the nitrification inhibitor nitrapyrin, found that nitrapyrin did not affect cumulative denitrification, but in some of the soils tested they noted that nitrapyrin delayed the

onset of denitrification. In contrast, some research has reported that the application of DCD can result in a decrease in denitrification rates when applied to slurry treated soil (Pain *et al.,* 1989; Thompson, 1989). They argued that the effectiveness of DCD at reducing denitrification rates was dependent on the quantity of available carbon in the soil. With increasing concentrations of carbon, denitrification becomes increasingly dependent on soil nitrate concentration (Thompson, 1989).

The high nitrate concentrations found in the soil throughout the experiment may render nitrification less important for the adequate supply of nitrate to denitifiers (Calderon *et al.,* 2005). Calderon *et al.,* (2005) stated that, with high initial nitrate availability in the soil, nitrification becomes less important at ensuring that denitrifying microbes have an adequate supply of nitrate to be denitrified. However, it is possible that soils with low initial nitrate concentrations, nitrification inhibitors may be able to reduce denitrification losses by influencing the nitrate pool available to denitrifying microbes (Calderon *et al.,* 2005; Vallejo *et al.,* 2001).

There have been some mixed results reported in terms of the ability of DCD to inhibit nitrification. A number of studies have shown that DCD inhibited the oxidation of ammonium through to nitrite, thereby resulting in lower nitrate concentrations within the soil. Vallejo *et al.,* (2001), in an incubation experiment, found an 18-fold increase in ammonium concentration in a sandy loam soil 10 days after DCD application compared to the control. The inhibition of nitrification resulted in low nitrate concentrations (5 - 10 mg NO_3^-) observed in the soil for up to 60 days. Di and Cameron (2004a) found that the application of DCD to soil with urea and urine increased ammonium concentrations and decreased nitrate concentrations. Williamson *et al.,* (1996) also found, in soils amended with DCD and effluent, that ammonium was greater in the DCD-amended soils and remained greater for 99 days with the ratio of nitrate to ammonium always much lower in DCD-amended soils. However, not all previous research has shown DCD inhibits nitrification. McTaggart *et al.,* (1997) reported, in a field study, that DCD did not cause a delay in the disappearance of soil ammonium. In the presence of DCD, immobilization of

ammonium increased and therefore McTaggart *et al.*, (1997) suggested that immobilization of ammonium may be masking any reduction in the rate of nitrification occurring after the application of DCD. In a lysimeter study, Davies and Williams (1995) also reported DCD to be having no inhibitory effective on nitrification.

DCD also had no appreciable effect on DEA (Figure 4.10), as DCD did not decrease nitrate concentrations or denitrification it would be unlikely to decrease DEA. No other studies have directly reported the effect of DCD on DEA. The inability of DCD to influence denitrifying activity in the soil, explains one of the reasons why denitrification rates were not affected from the application of DCD as the denitrifying population was not suppressed.

In this experiment, DCD had no effect on carbon availability and soil pH (Figure 4.12 and 4.13). In a field study McTaggart *et al.*, (1997) found DCD had no significant effect on soil pH, as did Davies and Williams (1995) in a lysimeter study. No other studies were found to have directly measured the impact of DCD on soil carbon availability.

4.6.2 Denitrification rates

In New Zealand literature, annual denitrification rates for dairy farm soils have been reported to vary with nitrogen fertiliser input (Table 4.7). In general, the greater the nitrogen fertiliser input, the greater the denitrification rate. No fertiliser was applied to my field trial site throughout the duration of the experiment, but previously (> 1 year ago) fertiliser was applied at 200 kg N ha^{-1} yr^{-1}. The mean annual denitrification rate for the control soils (14 kg N ha^{-1} yr^{-1}) was close to the value Barton *et al.*, (1999) reported of 13 kg N ha^{-1} yr^{-1}, which was compiled from a range of studies measuring mean annual denitrification rates in agricultural soils. Denitrification rates were however, higher than previous studies of unfertilised soils in New Zealand (Table 4.7). The mean annual denitrification rate in the DCD-amended soils (28 kg N ha^{-1} yr^{-1}) was higher than the control soils, but not significantly different, and was also higher than reported annual averages for soils receiving no fertiliser.

Table 4.7 Comparing denitrification rates with fertiliser input in dairy farm systems in New Zealand.

Nitrogen fertiliser applied	Denitrification rate (kg N ha^{-1} yr^{-1})	Reference
0 kg N ha^{-1} yr^{-1}	7	Ledgard *et al.*, 1996
	2.4	Bailey, 1997
	5	Ledgard *et al.*, 1997
200 kg N ha^{-1} yr^{-1}	11	Ledgard *et al.*, 1996
	6	Bailey, 1997
	17	Ledgard *et al.*, 1997
400 kg N ha^{-1} yr^{-1}	14	Ledgard *et al.*, 1996
	12.4	Bailey, 1997
	25	Ledgard *et al.*, 1997

Denitrification rates in well drained soils are principally controlled by oxygen, nitrate concentration and availability of carbon and to a lesser extent soil temperature (de Klein and Van Logtestijn, 1996). A linear regression model found that the variables, WFPS, ammonium and nitrate concentration, carbon availability, pH and DEA were not explanatory variables for the rates of denitrification observed in this study. Vallejo *et al.*, (2001) also found no significant linear correlation between denitrification rates and nitrate concentration; they did however see a marked dependence of denitrification losses on WFPS. The relationship between denitrification and soil nitrate concentration was tested by Thompson (1989) on data sets where different carbon additions had been made to the soil. Where high amounts of carbon were added to the soil the analysis showed a strong curvi-linear relationship, but where low carbon additions were made, linear regression suggested that soil nitrate had no influence on denitrification. Thompson (1989) concluded that

the influence of soil nitrate on denitrification appears to increase with the supply of available carbon substrate. Carbon and denitrification should be correlated when respiration significantly reduces the oxygen supply in soils, this often occurs when soils are wet and oxygen diffusion is slow (Groffman and Tiedje, 1991). Soil drainage class and texture were not considered in my model as they were constant but Groffman and Tiedje (1989) showed that up to 80% of the variations in denitrification rates could be explained at a landscape scale by these two variables.

Soil water content is normally the most important factor controlling denitrification rates in well drained soils. With increasing soil moisture content, air in soil pores is replaced with water, which leads to a reduction in oxygen availability. A reduction in oxygen availability leads to the onset of anaerobic conditions which is one of the requirements for denitrification to occur (Ruz-Jerez *et al.,* 1994). The critical WFPS for the Horotiu soil in my experiment was between 55 – 60% (Figure 4.14). This value corresponds to a volumetric water content of 41 - 44 g H_20 g^{-1} dry soil. When the WFPS was lower than 55%, denitrification could only occur in the presence of anoxic microsites within soil aggregates (Vallejo *et al.,* 2001).

The critical WFPS determined in my study was lower than previous researchers have reported for loam soils, 83% reported by de Klein and Van Logtestijn, (1996) and 62% reported by Ryden (1983). Vallejo *et al.,* (2001) stated that denitrification rates are often negligible at a critical WFPS of < 65% and a review by Barton *et al.,* (1999) found only two studies where critical WFPS was less than 60%. WFPS varies with soil texture and in general critical WFPS threshold for denitrification will decrease as soil texture becomes finer (de Klein and Van Logtestijn, 1996). Sandy soils tend to have the highest critical WFPS (82%; de Klein and Van Logtestijn, 1996), followed by loam soils (62 – 83%) and clay soils will have the lowest (50 – 74%; Barton *et al.*, 1999).

4.6.3 Experiment 2: Changes in soil biochemical properties with depth

Debate arises about the depth at which soil cores should be sampled to get an accurate measurement of denitrification activity. In the field experiment, soil cores to measure denitrification were taken to a depth of 15 cm. A sampling depth of 15 cm appears to be sufficient to capture most of the denitrifying activity, as measurements of DEA found that 80% occurred in the top 15 cm of the soil profile and that DEA decreased markedly with soil depth (Table 4.5). Similar decreases in DEA with depth have been observed (Luo *et al.,* 1998; Barton *et al.*, 1998). Soil microbial biomass (60%), carbon availability (61%) and total nitrogen and carbon were also greatest in the top 0 - 15 cm of the soil profile (Table 4.5). It is generally widely accepted that microbial activity is greater in the upper parts of the soil profile (Speir *et al.*, 1984).

4.6.4 Experiment 3: Rate of DCD loss in the soil

A marked decline (76%) in DCD concentration was measured one day after DCD application (section 4.5.4). DCD is relatively water soluble (Williamson *et al.,* 1996; Zerulla *et al.,* 2001) and translocation of DCD down the soil profile could occur. Total rainfall during the first two days after DCD application was only 3 mm and so it is unlikely that sufficient leaching occurred and the rapid decline in DCD concentration was predominantly due to microbial degradation.

At higher temperatures DCD will degrade more rapidly in the soil than at lower temperatures (Rajbanshi *et al.*, 1992; Williamson *et al.,* 1996; Di and Cameron, 2004a). During the time that soil samples were taken for this experiment, soil temperature averaged around 16°C and degradation of DCD was rapid. The total amount of DCD applied to each plot was 3.7 µg DCD g^{-1} soil, with complete degradation of DCD within 19 days, and a half-life of 2.9 days. The total time of degradation measured in my field experiment was slower than that reported by Rajbanshi *et al.,* (1992), despite a higher DCD application rate used by Rajbanshi *et al.*, (1992). Rajbanshi *et al.,* (1992), in a laboratory experiment found that the total degradation time for 6.7 µg DCD g^{-1} dry soil was 12 days at 10°C and 6 days at 20°C. However, Rajbanshi calculated similar half-life (2.9 days) when DCD was applied at

a rate of 6.7 µg DCD g^{-1} soil, at a temperature of 20ºC. Di and Cameron (2004a) clearly showed the effect of temperature on DCD degradation. At 8°C, DCD had a half-life of 111 - 116 days and at 20ºC the half-life of DCD was reduced to 18 – 25 days, when DCD was applied at application rates of 7.5 kg ha^{-1} or 15 kg ha^{-1} (Di and Cameron, 2004a).

Soil type and application rate of DCD are also important factors in determining the rate of degradation of DCD in the soil. Soil type can influence the capacity for microbial biodegradation of DCD (Williamson *et al.,* 1996). At higher application rates of DCD the degradation rate is slower. Williamson *et al.,* (1996) in a laboratory experiment applied 60 µg DCD g^{-1} soil and calculated DCD half-life of 39 days at 22ºC. Rajbanshi *et al.,* (1992) also showed that with increasing DCD application rate half-life time of DCD increased. A DCD half-life of 2.9 days and 11.5 days were reported for application rates of 6.7 µg DCD g^{-1} dry soil and 33.3 µg DCD g^{-1} dry soil respectively.

In this experiment, DCD had been applied to the soil approximately monthly for a year and it is possible that repeated applications of an inhibitor would reduce its effectiveness. Repeated applications of the inhibitor may result in microbial populations utilizing the inhibitor as a substrate for growth, which could increase the rate of its degradation (Rodgers, 1986). Repeated applications may also result in the development of inhibitor-resistant strains of nitrifying bacteria (Rodgers, 1986). However, previous studies have generally found that the efficiency of DCD was not affected by repeated application. Rodgers (1986) found that after four annual applications of DCD there was little effect on either the rate of DCD decomposition or the ability of the DCD to inhibit nitrification. McTaggart *et al.,* (1997) similarly reported that the effectiveness of DCD was not diminished by the repeated application (twice annually) of DCD over a two year field trial.

4.7 CONCLUSION

My hypothesis was that the nitrification inhibitor, DCD would reduce denitrification rates by limiting nitrate availability in dairy farm soils. DCD did not decrease denitrification rates or change denitrifying enzyme activity. Nitrification was partially inhibited as shown by a rise in soil ammonium concentrations and a decline in soil nitrate concentrations in the DCD-amended soils compared to the control soils. However, the reduction in soil nitrate was not great enough to limit nitrate availability to denitrifiers and hence no reduction in denitrification rates was observed. One possible reason for the small differences in nitrate and ammonium concentration was that DCD was rapidly degraded in the soil. DCD was completely degraded 19 days after DCD application at an average soil temperature of 16°C. Soil carbon availability and soil pH were unaffected by the application of DCD. Soil denitrifying enzyme activity, microbial biomass and carbon availability are greatest in the top 0 – 15 cm of the soil, which indicates the depth to which denitrification was sampled in the field was sufficient.

Chapter 5

Impact of DCD on denitrifying enzyme activity

5.1 INTRODUCTION

The uneven distribution of cow urine patches with high nitrogen loads in grazed pasture systems, leads to high spatial distribution of nitrogen cycling. Furthermore there is large spatial variability exhibited by soil denitrification rates due to "hot-spots" of organic carbon material in the soil (Parkin, 1987). It may be difficult to determine in a field environment the ability of nitrification inhibitors to decrease denitrification rates, as the large spatial variability of denitrification may mean the effect of DCD is not observed. The lack of effect of nitrification inhibitors on denitrification rates shown in the field experiment (Chapter 4) may be due to conditions not being optimal for denitrification to occur.

A laboratory study was undertaken to test whether dicyandiamide (DCD) would decrease denitrification, when conditions were optimal for denitrification. There are three main controlling factors of denitrification, oxygen availability, nitrate concentration and carbon concentration. To optimize conditions for denitrification an adequate supply of nitrate and carbon was added either by applying urea fertiliser or cow urine to incubated soil samples. Urea and cow urine are the two most common nitrogen sources entering a farming system and are rapidly converted to nitrate by soil microbes. Oxygen content was limited by maintaining the water-filled pore space (WFPS) of the soil above 80%, which is well above the critical WFPS that limits denitrification, but the soil was not saturated, as that would inhibit nitrification.

To measure the effect of DCD on denitrification rates changes in denitrifying enzyme activity (DEA) were measured. DEA is an indicator of the size of the denitrifying population within the soil, which is a reflection of previous conditions for denitrification to occur (Tiedje *et al.,* 1989). Hence, DEA can be used as a means of estimating denitrification rates (Luo *et al.,* 1996).

The aim of this laboratory experiment was to determine whether DCD could reduce the soil DEA by limiting nitrate formation via nitrification, in an environment where conditions for denitrification had been optimized.

5.2 EXPERIMENTAL DESIGN

Twenty kilograms of soil (Horotiu silt loam) was collected from paddock C27a (Chapter 4, Figure 4.1) at Dexcel's RED trial low input system (farmlet C), which has had no fertiliser applied since 2001. The soil was passed through a 4 mm sieve into one large tray.

Two kilograms of soil from the large tray was then placed into six smaller labelled trays, where each tray represented one treatment (Table 5.1). The moisture content of the soil in each tray was adjusted to 80% WFPS by adding distilled water and maintained at 80% throughout the experiment. I determined the amount of water to add to achieve a WFPS of 80% by multiplying the mass of dry soil by the target gravimetric water content and taking away from this the mass of water within the soil sample. The target gravimetric water content was determined from the volumetric water content equivalent to 80% WFPS. Total porosity of the soil multiplied by 80% WFPS gave the desired volumetric water content (Linn and Doran, 1984). The six trays were placed into a temperature-controlled room, set at 20ºC, and left to pre-incubate for three weeks, to allow effects of sampling and sieving to subside.

After the soil had been pre-incubated for three weeks DCD, urea or urine were applied to the soils using a hand-held sprayer. The experiment consisted of three treatments and associated controls (Table 5.1). Urea was applied at a rate of 0.116 g urea kg^{-1} soil (equivalent to 40 kg N ha^{-1}) and dairy cow urine at rate of 75 mls urine kg^{-1} soil (equivalent to 1000 kg N ha^{-1}). DCD was applied to the soil three times over the duration of the experiment at a total loading of 23.08 mg DCD kg^{-1} soil (equivalent to 30 kg N ha^{-1}). The soil was then mixed to ensure that the treatment applied had equal contact with all the soil.

Table 5.1 Description of treatments used in the laboratory experiment.

Treatment	Description	Amendments
A) Control		
1	Control without DCD	150 mls of water
2	Control with DCD	0.0154 g of DCD and 150 ml water
B) Urea amended soil		
3	Soil + Urea	0.232 g of urea in 150 ml water
4	Soil + Urea + DCD	0.0154 g of DCD and 0.232 g of urea dissolved in 150 mls of water
C) Urine amended soil		
5	Soil + Urine	150 mls of urine
6	Soil + Urine + DCD	0.0154 g of DCD and 150 mls urine

A subsample of the soil (200 g) was transferred from each tray into a labelled, 620 ml, preserving jar. The preserving jars were randomly placed in the temperature-controlled room (20ºC) and left for five days. The jars were sealed to maintain moisture content, but the lids were removed once every three days for two hours, to ensure the maintenance of aerobic headspace in the soil so that nitrification could still occur. After 5, 34 and 62 days three randomly selected jars from each treatment were removed for soil biochemical analysis. The soil biochemical measurements made were:

1. Denitrifying enzyme activity
2. Ammonium and nitrate concentration
3. Carbon availability
4. Microbial biomass
5. soil pH

Analytical methods are described in Chapter 3. There were three laboratory replications of each measurement.

DCD has a half-life of 18-25 days at temperatures of 20ºC (Di and Cameron, 2004a). To ensure DCD was present throughout the incubation, 34 and 62 days after the initial treatment of the soil, DCD was reapplied to treatments 2, 4 and 6. The soil in the preserving jars for treatments 2, 4 and 6 were placed back into trays with DCD reapplied and mixed through the soil again. The amount of DCD applied was adjusted to take into account the soil previously removed for analysis. After DCD was applied, 200 g of soil was transferred back into the preserving jars and returned to the incubator. Five days after each DCD application, three randomly selected jars were removed for soil biochemical analysis.

5.3 DATA ANALYSIS

Statistical analysis of the soil biochemical parameters measured was undertaken using two way analysis of variance (ANOVA) using the statistical package Statistica version 7.1 (2006). ANOVA compared each treatment individually against their associated controls to determine whether DCD had affected DEA, ammonium and nitrate concentration, carbon availability, microbial biomass or soil pH. ANOVA was also used to determine whether there were differences ($p < 0.05$) between control soils, urea-amended soils and urine-amended soils and to establish whether soil biochemical parameters varied with time.

5.4 RESULTS

5.4.1 Denitrifying enzyme activity

DEA was unaffected by the application of DCD in all treatments (Figure 5.1). Application of DCD to control soils appeared to show a trend of decreasing DEA, but this was not significant (Figure 5.1 a). DEA was greatest ($p < 0.001$) in the urine-amended soils compared to the other two treatments (control and urea-amended). The increase in DEA in the urine-amended soil was most apparent five days after the application of urine (Figure 5.1 c). DEA for the urea and urine-amended soils decreased with time ($p < 0.01$) (Figure 5.1 b and c). However, there were no changes in DEA in the control soils with time (Figure 5.1 a). Appendix D contains the raw data for each variable.

5.4.2 Changes in selected soil properties

5.4.2.1 Ammonium and nitrate concentrations

In the urine-amended soil, DCD addition led to an increase ($p < 0.001$) in ammonium concentrations compared to the soil without DCD (Figure 5.2 c). The urea-amended soil also had a higher ammonium concentration when DCD was added compared to the soil without DCD, but this was only significant ($p < 0.001$) at day 5, and by day 32 the difference was negligible (Figure 5.2 b). In the urine-amended soil, DCD application resulted in lower nitrate concentrations ($p < 0.05$; Figure 5.3 c). However, there were no significant differences in nitrate concentration with or without DCD application in the urea-amended soil (Figure 5.3 b). There were no significant differences in ammonium and nitrate concentrations in the control soils with or without DCD application (Figure 5.2 a and 5.3 a).

Ammonium and nitrate concentrations were higher in the urine-amended soil compared to the urea-amended and control soils ($p < 0.001$) (Figure 5.2 and 5.3). In the urea and urine-amended soils, ammonium concentrations decreased during the 62 day incubation whereas there was a small increase in ammonium concentrations in the control soil. In all treatments soil nitrate concentration increased over time ($p < 0.001$) presumably as ammonium was nitrified (Figure 5.3).

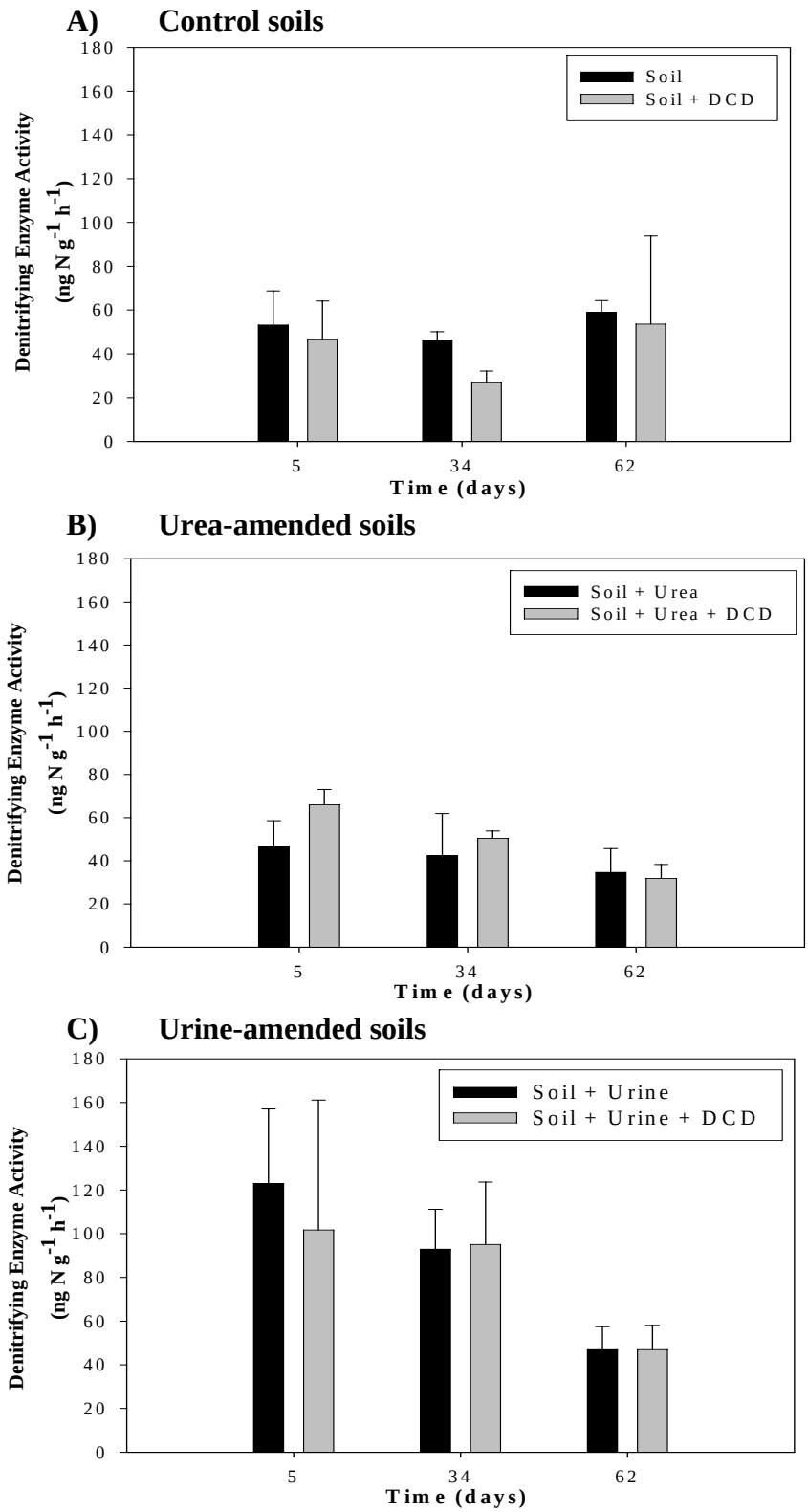

Figure 5.1 DEA for a) control soils, b) urea-amended soils, c) urine-amended soils, error bars are 1 standard deviation (n = 3).

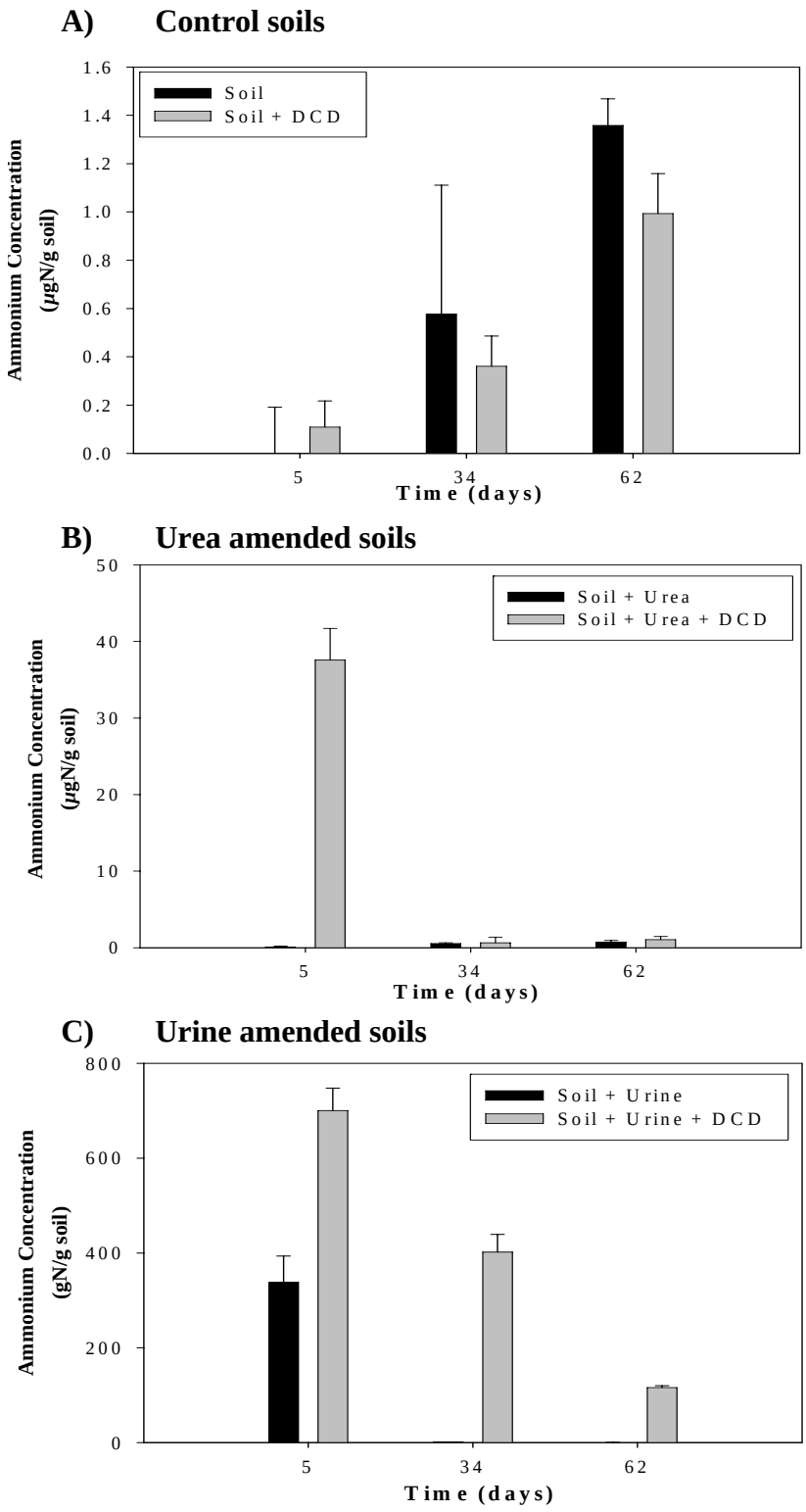

Figure 5.2 Ammonium concentrations for a) control soils, b) urea-amended soils, c) urine-amended soils, error bars are 1 standard deviation (n = 3) note differences in scale of y-axis.

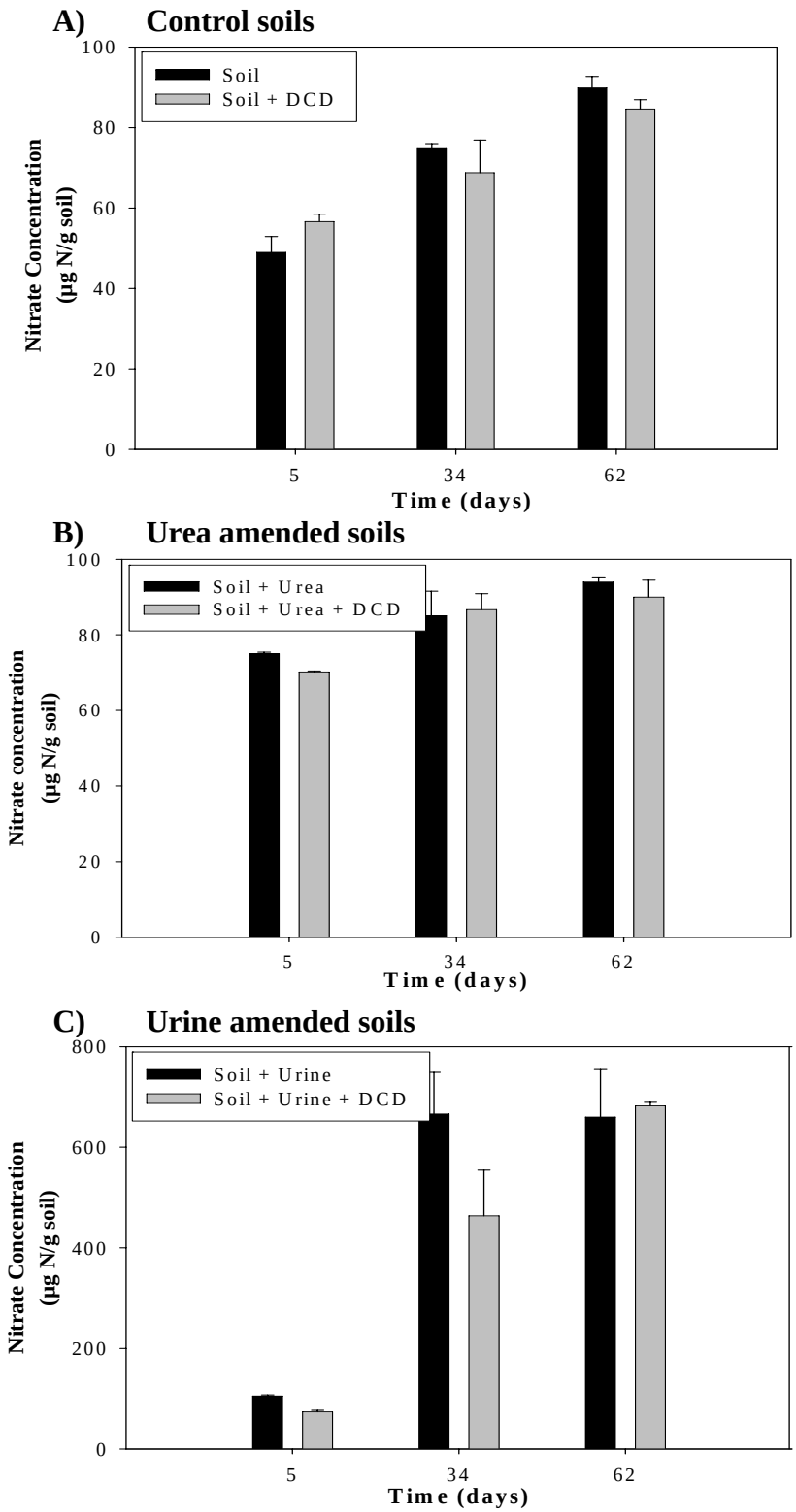

Figure 5.3 Nitrate concentrations for a) control soils, b) urea-amended soils, c) urine-amended soils, error bars are 1 standard deviation (n = 3) note differences in scale of y-axis.

5.4.2.2 Soil microbial biomass

Soil microbial biomass was unaffected by the application of DCD in all treatments (Figure 5.4). The urine-amended soil had a greater ($p < 0.001$) soil microbial biomass than the urea-amended and control soil 5 days after the application of urine, but by day 34 the difference was negligible (Figure 5.4).

In the urea-amended and control soils there were no significant changes in soil microbial biomass over the duration of the experiment (Figure 5.4 a and b). In the urine-amended soil, soil microbial biomass decreased ($p < 0.05$) between day's 5 and 34.

5.4.2.3 Carbon availability

Carbon availability was unaffected by the application of DCD in all treatments and there were no significant differences in carbon availability between the treatments (Figure 5.5). The urine-amended soil showed a clear decrease in carbon availability over the 62 days ($p < 0.05$). However, the urea-amended and control soils did not show declines in carbon availability (Figure 5.5).

5.4.2.4 Soil pH

In the urea-amended soil, pH increased ($p < 0.001$) with the application of DCD compared to the soil with no DCD applied, by a pH of 0.4 (Figure 5.6, b). There was no difference in soil pH due to the addition of DCD in urine-amended and control soils. Generally the soil pH decreased for all treatments between day's 5 and 34 and then increased between day's 34 and 62 (Figure 5.6).

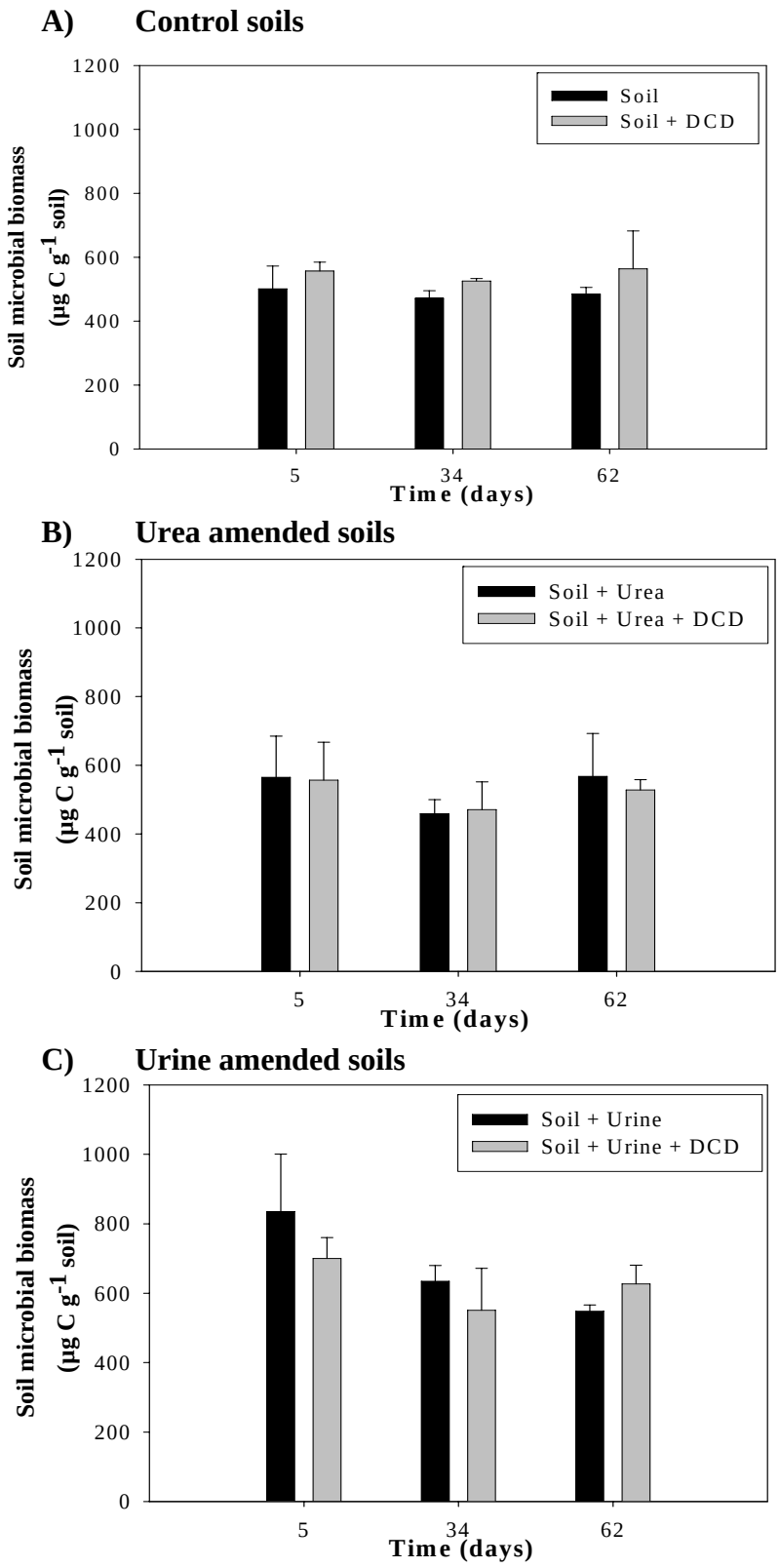

Figure 5.4 Soil microbial biomass for a) control soils, b) urea-amended soils, c) urine-amended soils, error bars are 1 standard deviation (n = 3).

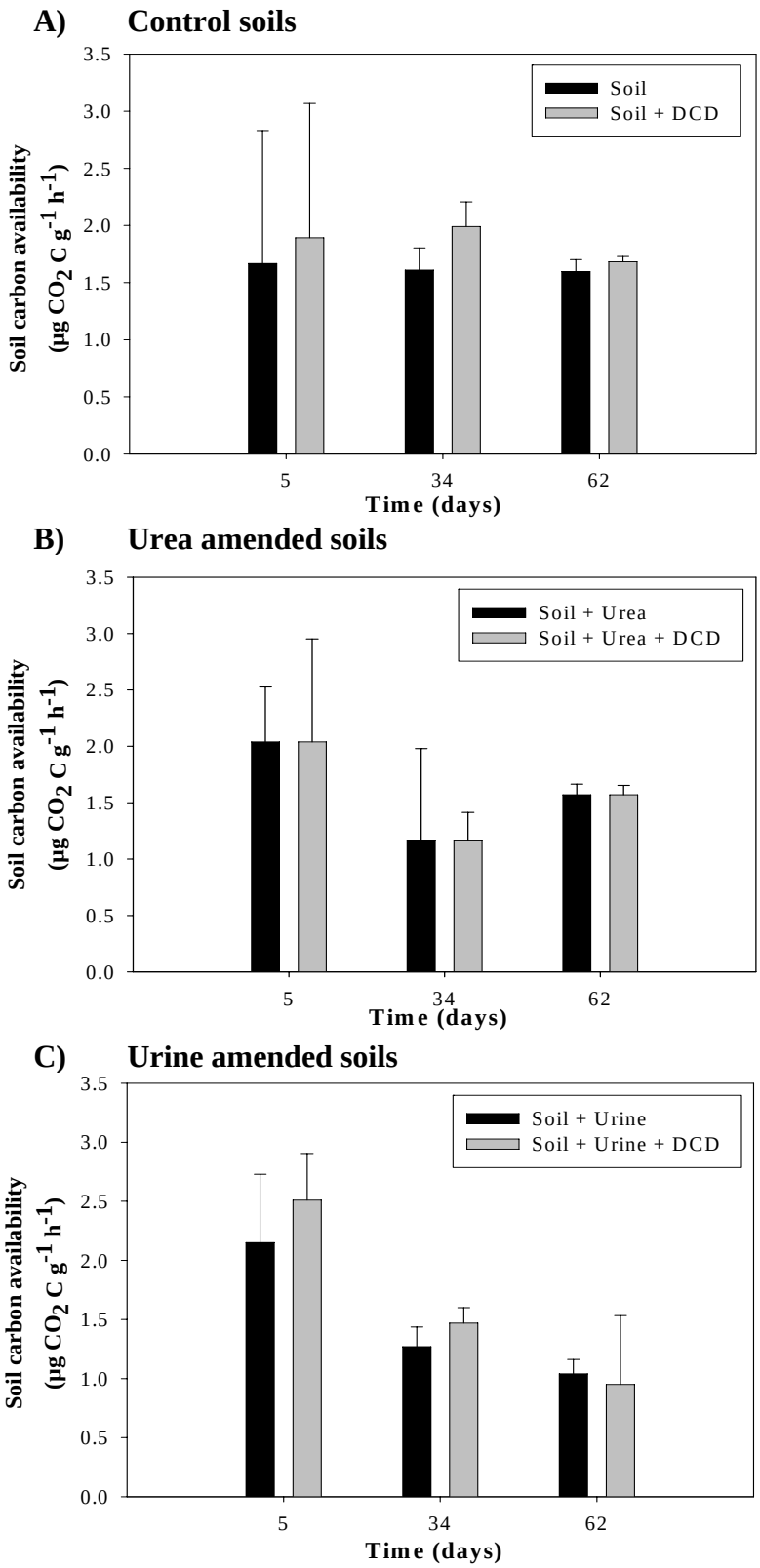

Figure 5.5 Soil carbon availability for a) control soils, b) urea-amended soils, c) urine-amended soils, error bars are 1 standard deviation (n = 3).

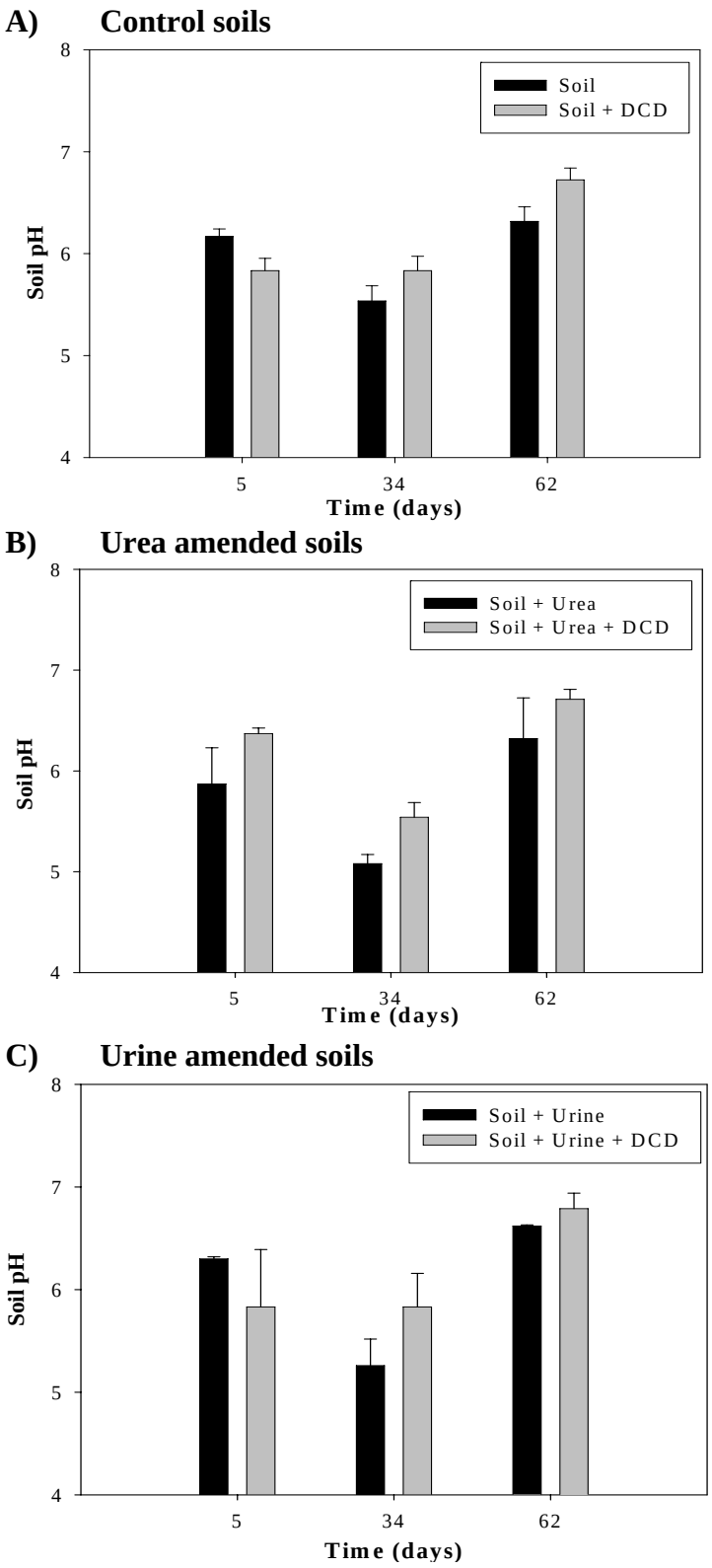

Figure 5.6 Soil pH for a) control soils, b) urea-amended soils, c) urine-amended soils, error bars are 1 standard deviation (n = 3).

5.5 DISCUSSION

5.5.1 Impact of DCD on the soil denitrifying enzyme activity

The aim of this laboratory experiment was to determine whether DCD could reduce the soil DEA by limiting nitrate formation via nitrification, in an environment where conditions for denitrification had been optimized. I found that DCD had no effect on the soil DEA, in any of the treatments (Figure 5.1). DEA can indicate the size of the denitrifying population, which is a reflection of suitability of previous conditions for denitrification (Tiedje *et al.,* 1989). The effect of nitrification inhibitors on the DEA has not been previously reported. However, previous research has suggested that the application of nitrification inhibitors to soil will not always have an effect on denitrification. While not measuring DEA, Bremner and Yeomans (1986) tested 28 nitrification inhibitors in laboratory studies and found that 21 of the nitrification inhibitors, including DCD (10 µg g^{-1} soil) had no appreciable effect on denitrification, but when DCD was applied at 50 µg g^{-1} soil denitrification was actually enhanced. Similarly Vallejo *et al.,* (2001) found that DCD applied with urea did not inhibit denitrification, which was measured using a soil core incubation system in the presence of acetylene.

Previous work has demonstrated that DCD inhibits denitrification when applied with carbon. The effectiveness of DCD on denitrification rates can be dependent on the quantity of available carbon (Thompson, 1989; Pain *et al.,* 1989). Thompson (1989) found that DCD was more effective at reducing denitrification rates as greater amounts of carbon were added to the soil with DCD. When carbon was applied to the soil at a rate of 1720 kg C ha^{-1}, in the form of cattle slurry, denitrification rates were considerably reduced by DCD, whereas when no extra carbon was added DCD had no apparent effect on denitrification. Similarly Pain *et al.,* (1989) found that DCD had the greatest inhibition effect on denitrification when slurry was applied to the soil at a rate of 25 kg N ha^{-1} and that decreased as the amount of slurry applied was reduced. Thompson (1989) suggested as carbon substrate increased, denitrification became increasingly dependent on nitrate concentrations formed from

nitrification. However in contrast, Bremner and Yeomans (1986) found that when soil was amended with DCD, and 4.5 mg carbon, as mannitol, denitrification rates were enhanced.

The application of urea or urine to the soil would have led to a small increase in the amount of carbon present in the soil and hence the effectiveness of DCD in the soil amended with urea and urine might be expected to be slightly enhanced. However, the extra carbon supplied to the soil from urea (urea contains 20% carbon) and urine (urine contains 2% urea) in this experiment was small and much less than what Thompson (1989) and Pain *et al.,* (1989) applied.

The soil amended with urine did however, result in a much higher DEA (Figure 5.1 c) and soil microbial biomass (Figure 5.4 c) compared to the other two treatments. The increase in the microbial population was probably caused by the urine supplying an additional source of soluble carbon and nitrate for the microbes to utilize. Luo *et al.*, (1999) reported an increase in the DEA during the winter months after cows grazed the pasture, due to nitrogen and carbon additions from urine and manure. The addition of a carbon substrate stimulates the activity of denitrifying bacteria (Limmer and Steele 1982).

5.5.2 Impact of DCD on soil ammonium and nitrate concentrations

DCD increased ammonium concentrations within the soil. The effect of DCD on nitrification was particularly apparent in urea and urine-amended soils (Figure 5.2). Many other laboratory studies have shown increased ammonium and decreased nitrate concentrations in soils amended with DCD (Vallejo *et al.*, 2001; Merino *et al.*, 2001), but there are fewer studies where DCD was added with urea or urine. Dobbie and Smith (2003) found that the application of urea with DCD resulted in much lower nitrate concentrations in the soil compared to the urea only treatment, however, similar nitrate concentrations were found in the unfertilized control over a 35 day period. Di and Cameron (2004a) reported that the application of DCD increased ammonium concentrations and decreased nitrate concentrations when applied with urine or urea. Di and Cameron (2004a) found that at higher temperature (20ºC)

ammonium concentrations decreased with time more rapidly than at 8ºC. Irigoyen *et al.*, (2003) also showed that the effectiveness of DCD with ammonium sulfate was strongly temperature dependent at holding nitrogen in the ammonium form.

DCD showed the greatest nitrification inhibiting properties when the soil was amended with urine, but it was unclear why this was. Ammonium concentrations were greater throughout the duration of the experiment and nitrate concentrations lower until day 62 in the DCD treated soil compared to the control soils (Figure 5.2 c and 5.3 c).

In the urea-amended soil, DCD inhibited nitrification for the first five days shown by a much greater concentration of ammonium present in the DCD treated soil, but over time the difference became negligible (Figure 5.2 b). While ammonium concentrations were elevated in the urea-amended soils there was no difference in nitrate concentrations (Figure 5.3 b). The lack of effect of DCD on nitrate concentrations was in contrast to a number of previous studies. Thompson (1989) found that the addition of DCD resulted in lower soil nitrate concentrations throughout a 13 week field trial. Calderon *et al.*, (2005) reported that the nitrification inhibitor, nitrapyrin, applied with manure increased ammonium and decreased nitrate concentrations for a 10-week period. Di and Cameron (2004a) also found that DCD inhibited nitrification, but it was observed that nitrate concentration increased more slowly in DCD treated soils at a temperature of 8ºC, and at 20ºC the difference in nitrate concentration between the DCD and non-DCD treated soils was smaller. The lack of obvious inhibition of nitrification in my study may have been due to relatively high incubation temperatures 20ºC and possible rapid degradation of DCD.

In contrast to urine and urea-amended soils there was no effect of DCD on ammonium and nitrate concentrations in the control soils, although both nitrogen mineralization and nitrification occurred (Figure 5.2 a and 5.3 a). The lack of effect of DCD in the control soils may have been due to lower available nitrogen and carbon concentrations and high incubation temperatures.

DEA was not suppressed by DCD addition because nitrate concentrations remained high with or without DCD application. The threshold value of nitrate which will limit denitrification for a loam soil has been reported to be 5 μg N g^{-1} soil (Ryden, 1983). In all treatments for the duration of the experiment, nitrate concentrations remained well above this threshold. Nitrate concentration increased as more ammonium was nitrified in all three treatments (Figure 5.3). In the control and urea-amended soils, nitrification was rapid. Five days after initial treatments, a greater concentration of nitrate compared to ammonium was present, regardless of whether or not DCD was applied. However in the urine-amended soil, five days after the application of the initial treatments, a greater concentration of ammonium compared to nitrate was present. The rate of nitrate accumulation in urine varies, depending on soil and environmental conditions, particularly soil temperature (Haynes and Williams, 1995). Holland and During (1977) reported that under New Zealand field conditions nitrification of urine N was not noticeable until seven days after urination. As our sampling occurred five days after the application of urine and DCD, it is possible that nitrification of ammonium in the soil treated with urine had not yet begun.

5.5.3 Impact of DCD on soil microbial biomass and carbon availability

The soil microbial biomass average 564 μg C g^{-1} soil and was generally lower than what would typically be expected under grazed pasture. Haynes and Williams (1993) reported a large microbial biomass under improved pasture of around 1200 μg C g^{-1} soil. Sparling *et al.,* (2001) reported for a Horotiu soil, a microbial biomass C of 1590 μg g^{-1} soil.

It is important to determine whether application of DCD will adversely affect the growth of the general microbial population within the soil, as ideally DCD will just be specific to the bacteria responsible for nitrification (Di and Cameron, 2004a). In this study soil microbial biomass was not altered by the application of DCD (Figure 5.4). Similarly, Williamson *et al.,* (1998) and Di and Cameron (2004a) found that the microbial biomass was unaffected through the application of DCD. The lack of effect of DCD on microbial biomass was likely because DCD is a bacteriostatic not a

bactericidal compound, that specifically only inhibits the activity of the *Nitrosomonas europaea* (Amberger, 1989), which are responsible for the first step in the process of nitrification and the nitrifying organisms only make up a small proportion of total microbial biomass.

Potentially the application of DCD to the soil could lead to an increase in the amount of carbon available within the soil, as DCD contains 28.6% carbon. The increase in carbon availability could increase soil microbial biomass. However, the results from this study show that carbon availability was not affected by the application of DCD (Figure 5.5) and DCD did not support an increase in the soil microbial biomass.

5.5.4 Impact of DCD on soil pH

Generally addition of DCD did not have an affect on soil pH. However, the urea-amended soil treated with DCD had a small, yet significantly higher soil pH than the soil without DCD (Figure 5.6 b). It is unclear why DCD would increase soil pH. In contrast, a field experiment carried out by McTaggart *et al.*, (1997) found that the addition of DCD to soil fertilised with urea did not alter the soil pH.

Between days 5 and 34 a decrease in soil pH was observed for all treatments. A decline in soil pH can be associated with the occurrence of nitrification, as nitrification is a major source of soil acidification (Davies and Williams, 1995). The subsequent rises in soil pH for all treatments between days 34 and 62 may be associated with decreased nitrification occurring.

5.6 CONLUSION

There was no evidence from my laboratory experiment that DCD suppressed soil denitrifying enzyme activity, and this is in agreement with the field experiment (Chapter 4). Nitrate concentrations remained high in the DCD amended soils and were unlikely to limit denitrification. DCD decreased ammonium concentrations, but only when urea or urine were mixed in with the soil. DCD addition to urine-amended soils resulted in a longer lasting inhibition on nitrification, with greater ammonium concentration measured for up to 62 days when DCD was added and a lower nitrate concentration for up to 34 days when DCD was added. In the urea-amended soils, nitrification was only inhibited at day 5 and by day 34 there was no difference in ammonium and nitrate concentrations with or without DCD addition. In the control soils, nitrification was not inhibited by DCD. Soil microbial biomass, microbial respiration and soil pH were unaffected by the application of DCD regardless of whether urea or urine was mixed in with the soil.

Chapter 6
General Discussion and Conclusions

6.1 GENERAL DISCUSSION

Nitrification inhibitors work by slowing the conversion of soil ammonium through to soil nitrate by inhibiting nitrification (Zacherl and Amberger, 1990). The inhibition of nitrification can directly lead to a reduction in nitrous oxide emissions and reduce the availability of nitrate for nitrate leaching and denitrification (Malla *et al.*, 2005). The aim of this thesis was to determine whether DCD could decrease denitrification rates by limiting nitrate availability on dairy farm soils.

To establish whether DCD will decrease denitrification rates by limiting nitrate availability, a field trial was established at Dexcel's Scott farm. The field trial showed that the application of DCD, when applied to dairy farm soils (Horotiu silt loam), did not decrease denitrification rates or the denitrifying enzyme activity in the soil, as nitrate availability was not limiting. The lack of effect of DCD on denitrification has been shown by previous studies (Bremner and Yeomans, 1986; Vallejo *et al.*, 2001), but in contrast other studies have shown DCD to inhibit denitrification (Thompson, 1989; Pain *et al.*, 1989). However, nitrification was inhibited in the field trial. Effective inhibition of nitrification by DCD is characterized by an increase in soil ammonium concentration and an associated decline in soil nitrate concentration, when compared to the control treatments (Davies and Williams, 1995). Although the decrease in nitrate concentrations was significant, it was not great enough for nitrate to limit denitrification. Vallejo *et al.*, (2001) also observed that DCD inhibited nitrification but no follow-through effect on a reduction in denitrification rates due to the decreased nitrate pool was observed.

Rapid degradation of DCD occurred in the soil, which may be one reason DCD had limited inhibition on nitrification. The DCD was completely degraded 19 days after application, at an average soil temperature of 16ºC, giving a half-life of 2.9 days at a

DCD application rate of 3.7 µg DCD g^{-1} soil. The rapid loss of DCD within the soil cannot be explained by leaching, as rainfall was minimal during the study period, thus microbial degradation of DCD was likely the dominant loss mechanism.

Denitrification is difficult to measure in the field as two of the proximal factors controlling denitrification are highly spatially variable in pastures. Cow urine patches contribute high nitrogen loads and are unevenly distributed in pasture. Availability of carbon is also irregularly distributed and can result in "hot spots" of denitrifier activity (Parkin, 1987). To establish, in a more controlled environment, in which to determine the effect of DCD on denitrification, a laboratory experiment was conducted. The laboratory experiment was designed to ensure that conditions were optimal for denitrification and denitrifying enzyme activity (DEA) was measured to gain an indication of the potential for denitrification to occur (Tiedje *et al.,* 1989). The results from the laboratory experiment supported the field experiment showing that the application of DCD did not suppress DEA within the soil. Again nitrification was inhibited when DCD was applied, but only when the soil was amended with urine or urea, however, as in the field experiment the decrease in nitrate concentration was not large enough to cause nitrate concentration to be limiting for denitrification.

In both the laboratory experiment and the field experiment DCD generally had no measurable effect on soil carbon availability and soil pH. No previous research was found that reported the effect of DCD on soil carbon availability. However, previous research has shown that DCD does not have an effect on soil pH (Davies and Williams, 1995; McTaggart *et al.,* 1997). Soil microbial biomass was measured in the laboratory experiment and it was found that microbial biomass was not affected by the application of DCD. Williamson *et al.,* (1998) and Di and Cameron (2004a) also reported DCD to have no impact on microbial biomass.

It has been suggested that the effectiveness of DCD at reducing denitrification rates may be enhanced when applied to the soil with a carbon source. Thompson (1989) found that DCD was more effective at reducing denitrification rates as greater

amounts of carbon, in the form of slurry were added to the soil with DCD. With increasing concentrations of carbon, denitrification becomes increasingly dependent on soil nitrate concentration (Thompson, 1989). Denitrification rates may be reduced if DCD can inhibit nitrification sufficiently causing soil nitrate concentration to be reduced below the threshold (5 μg NO_3^- g^{-1} soil) that is considered limiting for denitrification (Ryden, 1983).

The annual denitrification rate for the control soils was 14 kg N ha^{-1} yr^{-1} (37.4) and for the DCD-amended soils was 28 kg N ha^{-1} yr^{-1} (86.7), because of the variability associated with these values the difference was not significant. These rates were similar to those reported by Barton *et al.,* (1999) in a review of annual denitrification rates in agricultural soils, with an average rate of 13 kg N ha^{-1} yr^{-1}. A linear regression model showed that denitrification rates were not correlated with nitrate concentration, carbon availability or soil pH. However, a threshold critical WFPS was observed of between 55 – 60%, above which denitrification rates increased. The threshold observed in this study was much lower than de Klein and Van Logtestijn (1996) reported (83% threshold) and slightly lower than what Ryden (1983) reported (62% threshold) for a loam soil.

The majority of research on denitrification has focused on the top 0 – 20 cm of the soil profile (Tiedje *et al.,* 1989). Beyond this depth it is thought that soil nitrifying and denitrifying bacteria are insufficient to support denitrification activity (Barton *et al.,* 1998). To determine denitrification rates in the field trail, soil samples were collected to a depth of 15 cm. DEA was measured down the soil profile to ensure that the samples collected were where the majority of DEA was occurring. DEA was greatest in the top 0 – 15 cm of the soil profile accounting for 80% of DEA of the whole soil profile. Soil microbial biomass, soil carbon availability and total carbon and nitrogen were also greatest in the top 0 – 15 cm of the soil profile. Therefore the sampling depth of 0 – 15 cm, as used in the field study, was sufficient.

Overall the results of this study suggest that DCD does not decrease denitrification rates in soils with high nitrate concentrations. In contrast to a number of studies (Di

and Cameron, 2004a; Williamson *et al*., 1996), DCD failed to inhibit nitrification effectively, possibly due to rapid degradation of DCD. Previous laboratory and lysimeter studies have shown a concomitant DCD-related rise in soil ammonium concentration and decline in soil nitrate concentrations (Di and Cameron, 2004a). However, in this study although nitrification was significantly inhibited, the magnitude of rise in ammonium concentrations and decline in nitrate concentrations was small. The inability of DCD to cause large changes in nitrate and ammonium concentrations in the soil questions the benefits of nitrification inhibitors in Horotiu soils in the Waikato.

6.2 CONCLUSIONS

The following conclusions can be drawn for this study:

1. DCD did not decrease denitrification rates or denitrifying enzyme activity in dairy farm soils by limiting nitrate availability. This was likely due to minimal inhibition of nitrification. The reduction in nitrate concentrations was not large enough to cause nitrate to limit denitrification.

2. One possible reason for lack of inhibitory effect was that DCD was rapidly degraded, within 19 days, when applied at a rate of 3.7 μg DCD g^1 dry soil at an average soil temperature of 16ºC.

3. DCD had no measurable effect on soil biochemical parameters such as; soil pH, carbon availability and soil microbial biomass.

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Appendix A

Do nitrification inhibitors decrease denitrification in dairy farm soils

January 12th Sampling

Plots	Moisture Contents (gravimetric)	Carbon Availability ($ugCO_2$-$Cg^{-1}h^{-1}$)	Extractable Ammonium (µgN/g soil)	Extractable Nitrate (µgN/g soil)	Denitrifying Enzyme activity (nmolN/g/h)	Denitrification Rates (nmolN/g/h)
NON DCD PLOTS						
1	64.66	2.90	3.67	21.08	39.90	0.0029
4	57.38	3.81	3.33	19.14	25.23	0.0220
5	62.51	3.68	6.00	59.39	28.56	0.0123
8	60.80	1.25	2.39	28.75	10.92	0.0026
10	53.80	2.14	1.8	46.53	38.82	0.0008
13	59.98	3.93	2.60	17.28	83.37	0.0026
15	57.25	4.92	2.80	10.78	262.21	0.0042
16	59.40	3.12	2.28	41.63	103.83	0.0057
18	59.35	2.50	1.64	9.75	37.99	0.0007
20	54.61	3.69	1.42	21.83	77.52	0.0014
DCD PLOTS						
2	63.26	4.10	2.39	21.90	55.48	0.0182
3	58.52	4.05	4.50	13.13	39.07	0.0046
6	61.06	3.74	2.60	12.94	24.86	0.0042
7	65.96	4.68	4.64	17.59	36.33	0.0014
9	59.78	4.13	3.55	40.06	36.78	0.0032
11	63.10	2.94	3.24	70.27	258.81	0.0027
12	52.78	3.09	2.90	32.85	167.47	0.0034
14	59.83	4.27	1.96	63.06	53.44	0.0029
17	57.22	3.81	2.17	36.69	80.94	0.0016
19	60.17	4.32	1.96	14.10	160.76	0.0039

February 1st Sampling

Plots	Moisture Contents (gravimetric)	Carbon Availability ($ugCO_2$-$Cg^{-1}h^{-1}$)	Extractable Ammonium (µgN/g soil)	Extractable Nitrate (µgN/g soil)	Denitrifying Enzyme activity (nmolN/g/h)	Denitrification Rates (nmolN/g/h)
NON DCD PLOTS						
1	72.09	6.13	5.31	34.04	8.16	0.0666
4	64.58	7.52	8.36	102.79	19.98	0.0178
5	67.83	5.55	6.03	34.97	38.00	0.0064
8	68.74	7.41	8.07	75.19	45.44	0.0236
10	64.50	8.99	7.61	74.36	23.12	0.0537
13	67.23	5.88	4.75	29.19	27.85	0.0201
15	64.50	5.20	6.01	53.38	68.92	0.0109
16	64.17	6.09	4.84	50.07	40.42	0.0020
18	61.44	4.07	9.08	43.57	11.26	0.0080
20	57.89	1.51	3.12	17.19	11.19	0.0576
DCD PLOTS						
2	66.68	5.09	5.71	40.16	37.19	0.0258
3	65.29	9.22	5.49	25.83	15.13	0.0179
6	70.84	7.70	6.59	34.43	60.36	0.0846
7	63.55	6.02	7.82	52.06	154.76	0.0605
9	65.74	9.67	5.49	44.49	13.25	0.0000
11	70.91	7.52	5.19	83.16	24.59	0.0241
12	62.03	7.13	4.83	42.96	4.74	0.0232
14	65.24	5.14	8.26	99.33	76.07	0.0851
17	61.33	2.33	7.38	77.74	22.38	0.0014
19	55.21	5.94	0.00	15.30	22.57	0.0117

March 1st Sampling

Plots	Moisture Contents (gravimetric)	Carbon Availability ($ugCO_2$-$Cg^{-1}h^{-1}$)	Extractable Ammonium (µgN/g soil)	Extractable Nitrate (µgN/g soil)	Denitrifying Enzyme activity (nmolN/g/h)	Denitrification Rates (nmolN/g/h)
NON DCD PLOTS						
1	62.44	4.78	3.08	94.93	26.22	0.0101
4	53.69	5.95	0.00	84.82	12.62	0.0050
5	59.14	5.33	0.32	55.92	24.76	0.0004
8	57.59	4.56	0.00	51.19	16.94	0.0074
10	47.36	5.19	0.00	30.37	13.34	0.0096
13	55.23	5.46	5.07	71.86	17.37	0.0096
15	54.24	4.72	15.18	64.63	20.26	0.0103
16	55.90	4.31	85.95	104.43	26.86	0.0150
18	56.30	4.43	53.87	56.09	13.73	0.0122
20	46.15	5.82	2.51	31.80	14.14	0.0106
DCD PLOTS						
2	58.11	4.41	24.23	66.77	31.11	0.0010
3	54.96	2.30	76.17	75.75	17.30	0.0050
6	60.28	5.61	17.92	57.36	31.16	0.0135
7	54.95	4.96	26.58	66.57	20.53	0.0098
9	55.94	5.60	0.00	34.85	10.60	0.0102
11	60.84	4.70	1.70	77.34	10.43	0.0117
12	51.88	5.73	80.78	67.53	13.15	0.0074
14	53.61	6.02	0.21	58.90	24.04	0.0099
17	53.47	7.80	3.27	61.63	13.28	0.0128
19	55.21	5.94	0.00	15.30	22.57	0.011

May 4th Sampling

Plots	Moisture Contents (gravimetric)	Carbon Availability ($ugCO_2$-$Cg^{-1}h^{-1}$)	Extractable Ammonium (µgN/g soil)	Extractable Nitrate (µgN/g soil)	Denitrifying Enzyme activity (nmolN/g/h)	Denitrification Rates (nmolN/g/h)	pH
NON DCD PLOTS							
1	79.65	4.52	1.67	16.95	7.86	0.0656	6.11
4	71.42	3.66	9.05	45.86	26.54	0.0443	6.15
5	76.57	4.08	1.45	10.87	12.22	0.0464	6.12
8	74.06	4.28	2.31	29.75	28.86	0.0356	6.25
10	71.83	5.83	3.70	26.05	33.51	0.0704	6.13
13	76.68	4.65	5.98	17.23	43.71	0.0218	6.33
15	77.26	4.47	3.07	18.31	17.05	0.0047	6.09
16	76.34	4.36	3.61	13.02	30.42	0.0486	5.97
18	75.42	4.32	21.35	36.46	7.52	0.0920	6.02
20	72.21	4.74	4.66	20.69	29.27	0.0285	5.94
DCD PLOTS							
2	75.79	5.08	29.32	31.84	8.65	0.0108	6.31
3	75.68	4.29	15.54	14.52	18.15	0.0412	6.30
6	80.17	3.87	4.27	21.82	54.88	0.0056	6.24
7	71.55	2.46	3.05	15.97	39.73	0.0187	6.23
9	73.70	3.96	14.76	23.94	10.13	0.0136	6.15
11	77.05	4.40	9.10	16.16	29.17	0.0485	-
12	71.82	4.16	6.91	14.36	38.01	0.0505	6.17
14	73.16	-	7.67	16.31	10.39	0.0170	6.19
17	69.98	3.82	4.12	17.55	9.31	0.0055	6.19
19	74.63	3.59	2.85	24.72	16.89	0.1209	6.04

June 1st Sampling

Plots	Moisture Contents (gravimetric)	Carbon Availability ($ugCO_2$-$Cg^{-1}h^{-1}$)	Extractable Ammonium (µgN/g soil)	Extractable Nitrate (µgN/g soil)	Denitrifying Enzyme activity (nmolN/g/h)	Denitrification Rates (nmolN/g/h)	pH
NON DCD PLOTS							
1	80.87	4.38	1.46	6.00	12.00	0.2830	6.45
4	78.40	1.81	2.43	7.82	6.15	0.094	6.33
5	82.97	5.61	1.90	5.58	8.51	0.0824	6.25
8	79.12	1.89	1.35	3.08	9.22	0.1580	6.23
10	77.26	5.80	0.00	4.26	9.35	0.1093	6.44
13	76.32	4.67	1.35	2.85	4.28	0.1907	6.43
15	87.32	5.71	1.90	3.97	6.59	0.3295	6.45
16	84.92	5.28	1.57	4.61	11.40	0.1416	6.30
18	76.22	5.44	1.45	3.17	7.68	0.6803	6.30
20	74.50	4.90	0.81	4.67	8.33	0.1532	6.48
DCD PLOTS							
2	78.09	4.54	0.05	6.09	4.74	0.1465	6.40
3	80.05	5.97	2.97	3.29	17.92	0.0932	6.41
6	85.33	5.88	2.33	5.70	33.31	0.1086	6.58
7	78.15	4.85	1.89	5.23	14.32	0.2105	6.48
9	81.51	5.04	2.87	3.84	19.61	0.0352	6.42
11	79.09	7.43	2.54	4.15	21.75	0.2090	6.48
12	82.34	7.90	8.39	4.17	16.44	0.3434	6.45
14	77.46	5.77	2.75	4.47	26.80	0.1009	6.41
17	79.99	4.76	3.40	2.43	9.56	0.1760	6.40
19	78.96	5.67	1.35	2.10	8.05	0.0481	6.23

July 4th Sampling

Plots	Moisture Contents (gravimetric)	Carbon Availability ($ugCO_2$-$Cg^{-1}h^{-1}$)	Extractable Ammonium (µgN/g soil)	Extractable Nitrate (µgN/g soil)	Denitrifying Enzyme activity (nmolN/g/h)	Denitrification Rates (nmolN/g/h)	pH
NON DCD PLOTS							
1	78.19	0.62	50.89	5.28	23.50	0.0420	5.18
4	78.17	5.85	9.49	10.67	24.02	0.0226	6.11
5	80.45	4.27	76.28	9.72	34.85	0.0171	6.05
8	78.22	3.39	75.58	9.81	37.01	0.0509	6.19
10	74.66	3.82	62.44	4.84	37.01	0.0278	6.50
13	76.97	4.10	61.28	5.38	21.69	0.0150	5.63
15	79.53	4.44	36.49	10.26	16.69	0.0212	6.11
16	84.85	4.29	40.14	10.31	16.90	0.0285	6.14
18	72.92	3.56	78.32	5.47	18.51	0.0130	6.20
20	73.44	4.38	17.39	7.51	15.46	0.0177	6.33
DCD PLOTS							
2	80.12	4.21	79.93	4.00	18.86	0.0743	5.06
3	78.24	3.65	39.89	3.99	31.73	0.0349	5.83
6	88.55	4.78	66.40	5.88	27.16	0.0631	6.27
7	76.62	4.33	42.31	6.89	33.16	0.0628	6.27
9	78.89	4.51	53.19	5.07	32.39	0.0326	6.26
11	81.48	4.50	106.20	4.00	41.36	0.0341	5.01
12	72.87	3.45	65.12	5.47	37.40	0.0093	5.15
14	75.23	3.63	50.75	6.77	19.48	0.0492	5.89
17	75.18	1.95	53.54	5.59	21.44	0.0291	6.18
19	77.58	4.10	77.37	2.91	21.78	0.0105	6.23

August 1st Sampling

Plots	Moisture Contents (gravimetric)	Carbon Availability ($ugCO_2$-$Cg^{-1}h^{-1}$)	Extractable Ammonium (µgN/g soil)	Extractable Nitrate (µgN/g soil)	Denitrifying Enzyme activity (nmolN/g/h)	Denitrification Rates (nmolN/g/h)	pH
NON DCD PLOTS							
1	79.93	2.83	15.50	28.73	4.19	0.0369	5.05
4	69.76	2.39	1.55	6.85	4.08	0.0056	5.73
5	76.84	3.63	29.13	30.80	8.06	0.0756	5.63
8	73.12	2.37	16.37	21.14	5.09	0.0808	5.02
10	70.40	3.51	20.07	8.88	18.27	0.0521	5.53
13	71.89	2.58	51.83	30.34	2.80	0.0409	5.59
15	74.53	2.72	22.83	14.40	5.46	0.0582	5.30
16	78.35	3.09	11.38	12.51	3.13	0.0182	5.50
18	74.22	3.05	17.99	7.73	2.36	0.0627	5.64
20	71.00	3.68	22.97	14.14	8.49	0.0014	5.77
DCD PLOTS							
2	74.42	4.03	78.81	21.60	6.22	0.1364	5.18
3	71.99	2.49	45.29	6.97	7.65	0.0207	5.30
6	78.13	3.70	14.82	8.41	7.79	0.2023	5.45
7	73.01	3.59	51.56	7.83	7.02	0.0689	5.86
9	76.01	3.71	69.67	9.15	4.78	0.1173	5.36
11	75.96	2.95	47.50	18.94	4.04	0.1390	5.83
12	72.14	2.88	39.94	17.05	1.76	0.1130	5.80
14	72.42	2.65	45.95	10.30	4.63	0.2842	5.08
17	71.61	1.37	4.34	3.00	0.65	0.0429	5.54
19	73.45	2.83	25.28	7.41	3.80	0.0000	5.67

September 11th Sampling

Plots	Moisture Contents (gravimetric)	Carbon Availability ($ugCO_2$-$Cg^{-1}h^{-1}$)	Extractable Ammonium (µgN/g soil)	Extractable Nitrate (µgN/g soil)	Denitrifying Enzyme activity (nmolN/g/h)	Denitrification Rates (nmolN/g/h)	pH
NON DCD PLOTS							
1	84.52	5.70	48.73	23.30	0.69	0.0751	6.11
4	78.90	6.65	5.70	31.48	1.90	0.1990	6.54
5	87.07	6.08	18.39	29.05	2.73	0.1362	6.06
8	79.50	5.05	10.15	27.32	1.28	0.2049	6.22
10	73.21	3.76	65.71	10.62	9.85	0.0000	6.08
13	80.67	4.42	13.54	26.25	7.20	0.1967	5.98
15	79.03	4.50	59.19	24.75	0.20	0.0119	6.05
16	78.09	0.50	58.02	14.35	2.19	0.0066	5.78
18	69.66	0.34	12.08	18.83	13.34	0.0181	5.89
20	74.42	4.29	16.30	19.26	3.69	0.0173	5.84
DCD PLOTS							
2	78.96	5.61	69.84	8.83	1.68	0.0011	6.03
3	79.80	4.81	62.92	10.11	1.82	0.0000	6.07
6	81.57	5.11	53.89	8.90	6.79	0.0000	6.02
7	77.35	6.29	61.62	5.79	1.49	0.0116	6.35
9	77.21	4.62	23.98	5.79	1.84	0.0088	6.07
11	79.62	3.96	35.54	13.17	7.99	0.0078	6.12
12	76.07	3.84	42.76	11.69	7.11	0.0000	5.74
14	73.74	3.30	80.06	5.77	0.88	0.2200	6.00
17	79.81	4.22	16.19	7.26	2.35	0.0215	6.13
19	77.24	1.50	18.35	7.67	0.08	0.0513	5.84

October 4th Sampling

Plots	Moisture Contents (gravimetric)	Carbon Availability ($ugCO_2$-$Cg^{-1}h^{-1}$)	Extractable Ammonium (µgN/g soil)	Extractable Nitrate (µgN/g soil)	Denitrifying Enzyme activity (nmolN/g/h)	Denitrification Rates (nmolN/g/h)	pH
NON DCD PLOTS							
1	83.59	4.70	16.60	21.74	30.74	0.1459	6.11
4	80.06	4.36	7.92	34.07	5.21	0.0314	6.54
5	82.99	4.74	109.35	17.70	5.44	0.0534	6.06
8	81.87	5.23	4.05	8.11	33.43	0.1452	6.22
10	73.96	4.79	4.48	14.94	27.02	0.0106	6.08
13	76.42	4.73	71.74	7.99	32.03	0.0631	5.98
15	81.28	5.04	3.65	7.35	1.24	0.1091	6.05
16	83.36	4.28	5.07	5.07	9.95	0.0505	5.78
18	76.81	0.70	18.21	18.32	26.62	0.0536	5.84
20	72.77	3.84	25.54	39.05	26.62	0.0778	5.84
DCD PLOTS							
2	83.21	4.04	7.77	16.96	5.92	0.0435	6.03
3	82.32	6.09	8.61	7.75	10.07	0.0118	6.07
6	84.88	5.31	5.94	6.17	2.60	0.0042	6.02
7	77.77	1.61	52.49	13.35	9.37	0.0535	6.35
9	79.34	4.85	4.37	7.30	12.18	0.2846	6.07
11	84.00	5.68	2.78	18.22	34.86	0.1074	6.12
12	78.72	4.58	2.76	5.05	25.63	0.0625	5.74
14	76.45	4.53	49.47	54.23	27.74	0.1384	6.00
17	76.35	3.76	77.14	15.88	5.27	0.1808	6.13
19	80.77	7.20	47.05	6.42	11.68	3.2980	5.84

November 9th Sampling

Plots	Moisture Contents (gravimetric)	Carbon Availability ($ugCO_2$-$Cg^{-1}h^{-1}$)	Extractable Ammonium (µgN/g soil)	Extractable Nitrate (µgN/g soil)	Denitrifying Enzyme activity (nmolN/g/h)	Denitrification Rates (nmolN/g/h)	pH
NON DCD PLOTS							
1	88.28	4.16	4.98	71.22	45.50	0.2489	5.50
4	77.68	3.42	3.80	68.99	13.41	0.0217	6.28
5	86.53	5.03	6.59	38.42	23.27	0.0336	5.30
8	83.55	3.97	4.43	9.91	6.94	0.0435	6.48
10	81.43	5.62	5.39	27.25	14.52	2.3961	6.97
13	81.93	4.74	4.59	31.74	13.73	0.0453	6.35
15	82.91	4.04	3.29	5.78	22.74	0.0275	6.30
16	85.19	4.38	3.82	10.01	15.22	0.0242	6.31
18	76.10	4.35	5.30	7.60	7.45	0.0216	6.42
20	77.34	4.89	6.27	7.27	8.38	0.1311	6.42
DCD PLOTS							
2	85.79	5.14	5.23	10.89	9.87	0.0274	6.18
3	81.52	1.09	5.89	15.24	45.70	0.1820	6.34
6	86.87	4.92	4.68	8.76	15.93	2.4216	6.48
7	81.40	4.47	9.50	15.11	8.64	2.4869	6.52
9	82.24	4.05	3.28	5.78	36.66	2.9145	6.45
11	88.60	5.31	5.20	15.35	15.41	2.5360	5.80
12	79.51	4.72	5.98	10.94	12.94	0.0434	6.57
14	79.12	4.80	4.74	21.28	13.38	0.2118	6.47
17	84.42	5.20	5.52	23.97	9.46	0.1680	6.70
19	80.73	4.92	7.45	23.96	13.35	0.0000	6.48

December 1st Sampling

Plots	Moisture Contents (gravimetric)	Carbon Availability ($ugCO_2$-$Cg^{-1}h^{-1}$)	Extractable Ammonium (µgN/g soil)	Extractable Nitrate (µgN/g soil)	Denitrifying Enzyme activity (nmolN/g/h)	Denitrification Rates (nmolN/g/h)	pH
NON DCD PLOTS							
1	82.79	0.74	11.11	47.29	25.05	0.2486	5.92
4	78.92	2.67	7.60	40.27	22.23	0.3234	6.11
5	78.91	4.47	6.76	56.98	28.02	0.0309	5.83
8	78.83	0.24	8.70	28.39	22.06	0.1033	5.89
10	74.74	1.52	8.45	100.71	21.32	0.2019	6.13
13	76.04	5.33	8.14	42.34	22.10	0.1032	6.61
15	81.03	5.27	10.17	36.64	21.22	0.0774	5.39
16	79.50	4.12	8.17	21.80	20.59	0.1823	6.07
18	78.01	4.56	8.05	25.27	20.44	0.0416	6.01
20	73.56	4.52	8.87	34.97	32.15	0.5714	5.89
DCD PLOTS							
2	77.20	4.48	31.02	31.54	21.82	0.1091	5.76
3	76.70	4.52	11.64	21.22	24.37	0.0301	5.89
6	82.46	5.42	11.87	20.62	24.56	0.0135	6.40
7	77.34	4.17	7.93	20.39	25.63	0.0485	6.08
9	79.28	4.40	8.06	32.06	19.57	0.0087	6.03
11	60.97	2.78	17.75	29.64	44.31	0.2252	5.82
12	73.79	4.29	9.53	41.80	20.26	0.0962	5.89
14	73.96	4.02	8.77	41.65	41.84	0.1398	4.50
17	75.91	0.15	8.78	28.76	21.95	0.1272	6.34
19	77.38	5.40	13.32	20.44	17.43	0.0414	6.12

Appendix B

Changes in soil biochemical properties with depth

Different Depths Sampling

Sampling date	Depth sampled	Moisture Content (gravimetric)	Carbon Availability (µg CO_{2-} C g^{-1} h^{-1})	Denitrifying enzyme activity (nmolN g^{-1} h^{-1})	Microbial biomass (µg C g^{-1} soil)
Autumn	0 – 15	60.74	2.72	1.91	694.42
	15 – 30	57.11	1.22	0.54	336.12
	30 – 45	48.43	0.81	0.07	168.91
Winter	0 – 15	70.03	1.11	0.94	576.32
	15 – 30	66.81	0.54	0.11	261.32
	30 – 45	61.66	0.36	0.07	118.07
Spring	0 – 15	64.50	1.68	0.95	419.89
	15 – 30	62.99	1.88	5.23	145.63
	30 – 45	51.58	0.23	0.12	87.13
Summer	0 – 15	64.44	1.88	5.23	598.60
	15 – 30	57.63	0.73	0.66	237.90
	30 – 45	50.73	0.37	0.38	120.40

Total Carbon and Total Nitrogen

Sampling date	**Depth sampled** (cm)	**Total Carbon** (%)	**Total Nitrogen** (%)
Rep 1	0 – 15	7.03	0.70
	15 – 30	4.31	0.40
	30 – 45	1.41	0.17
Rep 2	0 – 15 cm	7.63	0.70
	15 – 30 cm	2.90	0.29
	30 – 45 cm	1.30	0.16

Appendix C

Rate of DCD loss in the soil

Sampling Day	Rep	DCD-N concentration µgN/ml
1	1	1.26
1	2	0.48
2	1	0.32
2	2	0.42
3	1	0.36
3	2	0.30
5	1	0.14
5	2	0.16
6	1	0.22
6	2	0.17
7	1	0.41
7	2	0.24
9	1	0.32
9	2	0.17
11	1	0.15
11	2	0.04
13	1	0.15
13	2	0.04
15	1	0.06
15	2	0.00
17	1	0.00
17	2	0.00
19	1	0.00
19	2	0.00

Appendix D

Impact of DCD on denitrifying enzyme activity

Treatment	Moisture Content (gravimetric)	Carbon Availability ($\mu gCO_2\text{-}Cg^{-1}\ h^{-1}$)	Extractable Ammonium ($\mu gN\ g^{-1}$ soil)	Extractable Nitrate ($\mu gN\ g^{-1}$ soil)	Denitrifying Enzyme activity ($nmolN\ g^{-1}\ h^{-1}$)	Microbial Biomass ($\mu g\ C\ g^{-1}$ soil)	pH
Day 5							
Control	89.62	1.67	0.00	48.99	3.80	501.23	6.17
Control + DCD	88.03	1.89	0.11	56.63	3.34	556.93	5.83
Urea	83.72	2.04	0.11	75.01	3.31	564.88	5.87
Urea + DCD	82.48	1.23	37.56	70.20	4.71	556.93	6.37
Urine	86.17	2.15	337.90	105.38	8.78	835.39	6.30
Urine + DCD	83.27	2.51	700.27	74.00	7.26	700.14	5.83
Day 34							
Control	76.74	1.61	0.58	74.99	3.30	471.35	5.54
Control + DCD	83.42	1.99	0.36	68.77	1.93	525.67	5.83
Urea	84.61	1.17	0.54	85.02	3.02	458.60	5.08
Urea + DCD	78.40	1.29	0.65	86.68	3.60	470.85	5.54
Urine	81.18	1.27	1.01	666.33	6.63	634.07	5.25
Urine + DCD	84.40	1.47	401.84	463.64	6.79	551.08	5.83
Day 62							
Control	86.24	1.60	1.36	89.83	4.21	484.87	6.32
Control + DCD	82.99	1.68	0.99	84.62	3.83	563.71	6.72
Urea	82.65	1.57	0.74	93.97	2.48	567.65	6.32
Urea + DCD	80.19	1.58	1.06	89.98	2.28	528.23	6.71
Urine	83.62	1.04	0.63	660.22	3.35	547.94	6.62
Urine + DCD	84.08	0.95	116.20	682.32	3.35	625.78	6.79