

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**NITRIFICATION ACTIVITY IN  
NEW ZEALAND SOILS AND THE VARIABLE  
EFFECTIVENESS OF DICYANDIAMIDE**

**A thesis presented in partial fulfilment of the requirements  
for the degree of**

**Doctor of Philosophy (PhD)**

**in**

**Soil Science**

**at Massey University, Palmerston North, New Zealand**



**Janice Rajendran**

**2011**

---

---

## *Abstract*

A perfusion technique was developed by which the rate of nitrification could be monitored as it changed over time following one or more additions of a nitrification inhibitor called dicyandiamide (DCD) in two contrasting soils - namely Manawatu Silt Loam (MSL) and Manawatu Fine Sandy Loam (MFSL). The modes of action of DCD in both soils were similar but the effectiveness of DCD varied between the two soils, with greater inhibition of nitrification in the MFSL than in the MSL when expressed as a percentage of the control soil. However when expressed in actual nitrification rates (absolute terms), greater inhibition of nitrification was obtained in the MSL as compared to MFSL. The actual reductions in nitrification rates between the two soils were almost similar, but the effect of DCD on the  $\text{NO}_3^-$ -N reduction in the MSL was slightly higher than in the MFSL. The nitrification rates in both soils gradually recover following the addition of DCD, but it didn't return to the initial levels in either soil. This ongoing inhibition effect was more obvious in the MFSL. The effect of DCD on the ammonia oxidising bacteria (AOB) populations in both soils followed a similar pattern to the nitrification activities, with an inhibition of nitrifier population in the presence of DCD and a recovery of the temporarily suppressed nitrifier populations when the DCD solution was removed from the system and was replaced with a fresh nitrogen source. Again, there was a residual effect of DCD on AOB numbers and this appeared to be greater in the MFSL than in the MSL.

---

In a separate experiment the effectiveness of DCD in the two soils was similar to that obtained in Chapter 3, in which it differed when expressed as percentage and absolute terms. DCD was more effective, with higher inhibition was obtained, in the MFSL than in the MSL when expressed as a percentage of the control. This was probably due to the differences in the rate of DCD degradation in both soils, in which DCD degraded two times slower in the MFSL than in the MSL. The effectiveness of DCD was also different between the two soils, when the same amount of DCD remained in both soils, with higher inhibition was obtained in the MSL than in the MFSL. Thus, in absolute terms DCD was more effective in the MSL.

In a further experiment it was demonstrated that soils collected from steep slopes (SS) in a hill country paddock had low nitrification rates compared to soils collected from adjacent camp sites (CS). These low nitrification rates were associated with similarly low populations of AOB in the SS soils. Of interest was the observation that the numbers of AOB and the nitrification rate in absolute terms in the SS did not increase greatly over the time, even with a plentiful supply of  $\text{NH}_4^+$  substrate from added urea and the associated higher pH. It was not clear whether the low initial population of AOB in SS resulted from low inputs of  $\text{NH}_4^+$  substrate over many years, or whether in addition there was an inhibitory effect that may have prevented a build-up of the nitrifiers. A subsequent investigation suggested that the low nitrifying SS soil may exert a small inhibiting effect when mixed with high nitrifying CS soils.

In conclusion DCD was found to vary in its effectiveness in soil types. The effectiveness of DCD in reducing  $\text{NO}_3\text{-N}$  production in grazed pasture systems is a

---

function of both its half life in the soil and also the extent of inhibition of nitrification at a given concentration of DCD in the soil.

## *Acknowledgements*

This thesis would not have been possible without the guidance and the support of several individuals who in one way or another contributed and extended their valuable assistance in the preparation and completion of this study.

I would like to thank The Agriculture and Marketing Research and Development Trust (AGMARDT) for providing me with a doctoral scholarship for the duration of my study in Massey University. I am grateful to the Department of Soil Science Massey University which made this study possible by providing me with the support and research facilities.

I am heartily thankful to my chief supervisor, Professor Emeritus Russ Tillman, for many insightful conversations during the development of the ideas in this thesis, and for helpful comments on the text. Your supervision, support and encouragement from the preliminary to the concluding level enabled me to develop an understanding of the subject. Beyond that, your cool mentoring and teaching brings cheer to each conversation. To my co-supervisor, Professor Peter Kemp, I appreciate your time and contribution during the development of the work carried out in this thesis and final review of this thesis. To Mr. Andrew Carran and Dr. Saman Bowatte thank you for your time, ideas and contributions during the initial discussions of this research.

---

I am grateful to Professors Mike Hedley and Nanthi Bolan for your guidance and support to pursue my study in Soil Science. To all the technical team of Soil Science, thank you so much for your technical assistance throughout this study. Thanks also to Michelle Tamehana and Warwick Johnson, technician of Food Science and Technology Massey University for allowing me to use the laboratory and assisting me with the HPLC for the DCD analysis. I thank Moira, Liza, Denise Brunskill and Denise Stewart for being accommodating and their continuous moral support. To Andy Wood I appreciate your time for helping me with the formatting of this thesis.

To all the past and present fellow post graduate students, Bambang, Peter, Jeya, Noveline, Uttara, Indika, Saman, Saleem, Sam, Erwin, Amandeep and Neha, my sincere thanks for your continuous moral support throughout this study and thank you for sharing your thoughts.

Special thanks also to my parents and family for all the constant moral support and encouragement when I couldn't see the light at the end of the tunnel.

Last but not least, to my husband, Peter, the love of my life. Thanks for believing in me and inspiring me to greater things. Your love, friendship and your kind gentle spirit make living with you each day a gift. I am blessed and thanks for finding me so many jokes!

Most of all, I am exceedingly grateful to God for all His abundance blessings in each day of my life and for giving me the strength in completing this study, thank you so much Dear Lord.

---



---

## *Table of Contents*

<b>Abstract.....</b>	<b>ii</b>
<b>Acknowledgements.....</b>	<b>v</b>
<b>Table of Contents .....</b>	<b>vii</b>
<b>List of Tables .....</b>	<b>xiii</b>
<b>List of Figures.....</b>	<b>xiv</b>
<b>Chapter 1 .....</b>	<b>1</b>
<b>General introduction.....</b>	<b>1</b>
1.1 The issue .....	1
1.2 The aim and thesis structure.....	4
<b>Chapter 2 .....</b>	<b>8</b>
<b>Review of literature.....</b>	<b>8</b>
2.1 Introduction .....	8
2.2 Nitrification in soil .....	11
2.2.1 Autotrophic nitrifiers.....	11
2.2.1.1 Distribution of AOB in different environments .....	15
2.2.1.1.1 Marine environments.....	16
2.2.1.1.2 Salt lakes.....	17
2.1.1.1.3 Freshwater environments .....	17
2.1.1.1.4 Waste water treatment systems (WWTS).....	18
2.1.1.1.5 Distribution of autotrophic AOB in different soils .....	19



---

2.2.1.2 Ammonia oxidising bacteria as an indicator of environmental quality.....	24
2.2.2 Heterotrophic nitrifiers.....	25
2.2.2.1 Nitrifiers involved in heterotrophic nitrification.....	25
2.2.2.2 The biochemistry of heterotrophic nitrification .....	27
2.2.3 Is nitrification in soils caused by autotrophic or heterotrophic nitrifiers? .....	28
2.3 The potential effects of nitrification.....	32
2.4 Factors that control nitrification in soils .....	34
2.4.1 Environmental factors .....	35
2.4.1.1 Soil moisture and aeration.....	35
2.4.1.2 Soil temperature .....	37
2.4.2 Chemical factors.....	38
2.4.2.1 Soil pH .....	38
2.4.2.1.1 Mechanisms of autotrophic nitrification at low pH.....	38
2.4.2.2 Organic matter and C:N ratio.....	42
2.4.3 Allelopathy .....	43
2.4.4 Population of nitrifying organisms .....	44
2.5 Methods used to estimate the numbers of nitrifiers .....	45
2.6 History and role of nitrification inhibitors .....	49
2.7 Characteristics and performance of DCD .....	52
2.7.1 Bacteriostatic or bacteriocidal effect of DCD? .....	53
2.8 Factors that influence the degradation and the effectiveness of DCD .....	55
2.8.1 Soil types .....	56
2.8.2 Soil temperature .....	57
2.9 Research on DCD in New Zealand and elsewhere .....	59
2.9.1 Effectiveness of DCD in reducing $\text{NO}_3^-$ leaching.....	59
2.9.2 Effectiveness of DCD in reducing $\text{N}_2\text{O}$ emissions .....	63

2.10 Summary of literature review.....	64
<b>Chapter 3 .....</b>	<b>67</b>
<b>Measuring soil nitrification activity over time with a perfusion technique.....</b>	<b>67</b>
3.1 Introduction .....	67
3.2 Materials and Methods.....	72
3.2.1 Soil samples .....	72
3.2.2 Soil analysis .....	72
3.2.3 Perfusion apparatus .....	73
3.2.4 Rapid determination of nitrification activity over time in three pasture soils.....	76
3.2.5 Effects of DCD and residual effects of DCD on soil nitrification rates .....	78
3.2.6 Estimation of AOB populations.....	80
3.2.6.1 MPN media .....	80
3.2.6.2 Dilution bottles.....	81
3.2.6.3 MPN procedure .....	81
3.2.6.4 Cell counts.....	82
3.2.7 Statistical analysis .....	83
3.3 Results .....	83
3.3.1 Nitrification rates in the three pasture soils.....	83
3.3.2 Numbers of AOB in the three original soils .....	89
3.3.2.1 Weekly AOB growth and the estimated counts in the three original soils .....	89
3.3.3 Effect of DCD on soil nitrification activity in MSL .....	91
3.3.3.1 Nitrification activity .....	91
3.3.3.2 AOB populations.....	97
3.3.4 Effect of DCD on soil nitrification activity in MFSL .....	99

---

3.3.4.1 Nitrification activity .....	99
3.3.4.2 AOB populations.....	106
3.4 General discussion.....	110
3.5 Conclusions .....	121
<b>Chapter 4 .....</b>	<b>123</b>

## **Decomposition of dicyandiamide (DCD) and the effect on nitrification**

### **rates – laboratory incubation study .....123**

4.1 Introduction.....	123
4.2 Materials and Method .....	125
4.2.1 Soil sampling and preparation.....	125
4.2.2 Bulk soils incubation procedure.....	125
4.2.3 Degradation of DCD and DCD analysis .....	126
4.2.4 Calculation of DCD half-life.....	126
4.2.5 Nitrification assay and analysis of mineral N .....	127
4.3 Results .....	128
4.3.1 Degradation of DCD .....	128
4.3.2 Nitrification .....	130
4.3.2.1 Nitrate concentrations in bulk soils.....	130
4.3.2.3 The effect of DCD concentration on soil nitrate formation .....	136
4.4 General Discussion.....	138
4.5 Conclusions .....	142
<b>Chapter 5 .....</b>	<b>144</b>

## **Laboratory study on nitrification in hill country and lowland soils**

### **amended with urea.....144**

5.1 Introduction.....	144
5.2 Materials and Methods.....	145
5.2.1 Sampling site and soil preparation .....	145

---

5.2.2 Soil incubation - N transformation in soils amended with urea.....	146
5.2.3 Estimation of AOB population .....	147
5.3 Results .....	147
5.3.1 Chemical properties of the three soils .....	147
5.3.2 Mineral N changes and nitrification in the presence and absence .....	148
of added urea .....	148
5.3.3 Nitrification rates in the control and urea treated soils .....	152
5.3.4 Estimation of AOB populations .....	154
5.4 General Discussion.....	155
5.5 Conclusions .....	159
<b>Chapter 6 .....</b>	<b>160</b>
<b>The effect of mixing the low and high nitrifying soils on nitrification</b>	
<b>activity .....</b>	<b>160</b>
6.1 Introduction .....	160
6.2 Materials and Methods.....	161
6.2.1 Sampling sites and soil preparation .....	161
6.2.2 Soil incubation study and soil analysis .....	162
6.3 Results .....	163
6.3.1 Chemical properties of the two soils.....	163
6.3.2 Changes in mineral-N in the mixed soils with and without addition	
of urea.....	164
6.3.3 Nitrification rates in the control soil and soil with added urea .....	169
6.4 General Discussion.....	171
6.5 Conclusions .....	173
<b>Chapter 7 .....</b>	<b>175</b>

---

<b>Overall summary and future work.....</b>	<b>175</b>
7.1 Overall summary .....	175
7.2 Future directions.....	182
<b>References .....</b>	<b>184</b>

---



---

## *List of Tables*

Table 2.1 The diverse lineages associated to $\gamma$ - and $\beta$ -subgroups of the <i>Proteobacteria</i> group described by Purkhold <i>et al.</i> (2003) on the basis of 16S rDNA and <i>amoA</i> gene sequences. Information for <i>Nitrosospira</i> clusters 9-12 was obtained from Avrahmi and Conrad (2003), based on <i>amoA</i> gene sequences. Other information provided in this table was obtained from Koops and Pommerening-Röser (2001) and Prosser (2007). ....	13
Table 2.2 The presence of autotrophic AOB in soils in relation to environmental factors and soil management. ....	20
Table 3.1 Chemical properties of the three soils studied. ....	73
Table 3.2 Treatments applied to the MSL and MFSL soils. ....	79
Table 3.3 Effectiveness of DCD (applied at the same rate; 20 kg DCD ha <sup>-1</sup> ; +DCD) in cycles 1 and 3 quantified in percentage (%) and absolute terms in the MSL and MFSL soils. ....	115
Table 4.1 First order DCD half-life and degradation rate constant in the two soils following the application of 2 rates of DCD. ....	130
Table 4.2 Effectiveness of DCD (applied at low; 20 kg DCD ha <sup>-1</sup> (+DCD) and high rates; 40 kg DCD ha <sup>-1</sup> (++DCD)) quantified in percentage (%) and absolute terms in the MSL and MFSL soils. ....	140
Table 5.1 Chemical properties of the three soils studied. ....	147
Table 5.2 AOB populations prior to and following incubation of soils for 15 days with added urea, and the average daily nitrification rate per AOB over the 15-day incubation period calculated using the average of the AOB populations at Day 0 and Day 15. ....	158

---



---

## *List of Figures*

Figure 2.1 Bacteria and enzymes involved in the nitrification process. Abbreviations: AOB, ammonia oxidising bacteria; NOB, nitrite oxidising bacteria; AMO, ammonia monooxygenase and HAO, hydroxylamine oxidoreductase.....	9
Figure 2.2 The role of nitrification inhibitors in soil. ....	50
Figure 2.3 Specific inhibition of DCD on nitrification. Modified from (Amberger 1989). ....	53
Figure 3.1 Soil perfusion apparatus (Audus 1946). The components are as follows : P, a glass tube containing soil crumbs between pads of glass wool (G); F, the solution supplied to the soil; A, a constant suction applied by a suction pump connected by flexible tubing ( $R_1$ and $R_2$ ) to the column; T, tubing carrying the solution and S, the sample taken out for analysis. ....	67
Figure 3.2 Perfusion apparatus used in this study to determine the nitrification activity in New Zealand pasture soils. ....	75
Figure 3.3 Decrease in $\text{NH}_4^+$ -N concentrations in the perfusing solutions over time (a) and increase in $\text{NO}_3^-$ -N concentrations over time (b) in three New Zealand pasture soils. Vertical bars denote the standard errors of six replicates.....	85
Figure 3.4 Mean changes in the concentrations of inorganic N over time in (a) MSL, (b) TSL and (c) MFSL during the perfusion experiment. Error bars represent the standard errors of the six replicates. Symbols represent total mineral N ( $\blacksquare$ ), $\text{NH}_4^+$ -N ( $\triangleleft$ ) and $\text{NO}_3^-$ -N ( $\diamond$ ). ....	86
Figure 3.5 Mean nitrification rates in three New Zealand pasture soils during the experimental period. Vertical bars denote the standard errors of six replicates. ....	87
Figure 3.6 Daily (a) and 4-day day moving average (b) nitrification rates in three New Zealand pasture soils during perfusion. ....	88
Figure 3.7 Estimate of AOB populations in (a) MSL, (b) MFSL and (c) TSL with time of incubation in growth medium after serial dilution. ....	89

- Figure 3.8 The estimated populations of AOB present in the original soil samples. Vertical bars in the columns indicate the range of AOB numbers obtained in the three replicates in each soil. .... 90
- Figure 3.9 Mean nitrification rate and the estimated numbers of AOB in (a) MFSL and (b) TSL and (c) MSL..... 90
- Figure 3.10 Mean concentrations of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N in MSL in (a) cycle 1, (b) cycle 2, (c) cycle 3 and (d) cycle 4, during the perfusion experiment. The soils in the perfusion apparatus in cycles 1 and 3 received either  $(\text{NH}_4)_2\text{SO}_4$  solution only (control) or  $(\text{NH}_4)_2\text{SO}_4$  solution with DCD at  $20 \text{ kg ha}^{-1}$  (+DCD), while in cycles 2 and 4, all the soils were treated with  $(\text{NH}_4)_2\text{SO}_4$  solution only (control and -DCD). Error bars represent the standard errors of the three replicates. .... 92
- Figure 3.11 Mean total mineral-N in the perfusion system during the experiment with MSL in (a) cycle 1, (b) cycle 2, (c) cycle 3 and (d) cycle 4. The soils in the perfusion apparatus in cycles 1 and 3 received either  $(\text{NH}_4)_2\text{SO}_4$  solution only (control) or  $(\text{NH}_4)_2\text{SO}_4$  solution with DCD at  $20 \text{ kg ha}^{-1}$  (+DCD), while in cycles 2 and 4, all the soils were treated with  $(\text{NH}_4)_2\text{SO}_4$  solution only (control and -DCD). Error bars represent the standard errors of the three replicates. .... 93
- Figure 3.12 Mean nitrification rates in the MSL during the 4 cycles of the experiment. The MSL soils in the perfusion apparatus in cycles 1 and 3 received either  $(\text{NH}_4)_2\text{SO}_4$  solution only (control) or  $(\text{NH}_4)_2\text{SO}_4$  solution with DCD at  $20 \text{ kg ha}^{-1}$  (+DCD), while in cycles 2 and 4, all the soils were treated with  $(\text{NH}_4)_2\text{SO}_4$  solution only (control and -DCD). Vertical bars are the standard errors for three replicates. .... 95
- Figure 3.13 Daily (a) and 4-day moving average (b) nitrification rates in MSL in (i) cycle 1; (ii) cycle 2; (iii) cycle 3 and (iv) cycle 4. The soils in the perfusion apparatus in cycles 1 and 3 received either  $(\text{NH}_4)_2\text{SO}_4$  solution only (control) or  $(\text{NH}_4)_2\text{SO}_4$  solution with DCD at  $20 \text{ kg ha}^{-1}$  (+DCD), while in cycles 2 and 4, all the soils received  $(\text{NH}_4)_2\text{SO}_4$  solution only (control and -DCD). .... 96
- Figure 3.14 Estimates of AOB populations in the MSL with time of incubation in growth media after serial dilution of extracts from soils sampled at the end of (a) cycle 1; (b) cycle 2; (c) cycle 3 and (d) cycle 4. The soils in the perfusion apparatus in cycles 1 and 3 initially received  $(\text{NH}_4)_2\text{SO}_4$  solution only (control) or  $(\text{NH}_4)_2\text{SO}_4$  solution with  $20 \text{ kg DCD ha}^{-1}$  (+DCD), while in cycles 2 and 4, all the soils were treated with  $(\text{NH}_4)_2\text{SO}_4$  solution only (control and -DCD). .... 98
- Figure 3.15 AOB populations in the perfusion columns containing MSL soil after each cycle. Control and -DCD ( $(\text{NH}_4)_2\text{SO}_4$  solution only) and + DCD ( $(\text{NH}_4)_2\text{SO}_4$  solution +  $20 \text{ kg DCD ha}^{-1}$ ). Vertical bars in the columns indicate the range of AOB numbers obtained in the 3 replicates in each treatment. .... 99



- Figure 3.16 Mean concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  in MFSL in (a) cycle 1, (b) cycle 2, (c) cycle 3 and (d) cycle 4, during the perfusion experiment. The soils in the perfusion apparatus in cycles 1 and 3 received  $(\text{NH}_4)_2\text{SO}_4$  solution only (control),  $(\text{NH}_4)_2\text{SO}_4$  solution with 20 kg DCD  $\text{ha}^{-1}$  (+DCD) and  $(\text{NH}_4)_2\text{SO}_4$  solution with 40 kg DCD  $\text{ha}^{-1}$  (++DCD), while in cycles 2 and 4, all the soils were treated with  $(\text{NH}_4)_2\text{SO}_4$  solution only (control, -DCD and --DCD). Error bars represent the range of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  concentrations in the two replicates..... 101
- Figure 3.17 Mean mineral-N in the perfusion system during the experiment with MFSL (a) cycle 1, (b) cycle 2, (c) cycle 3 and (d) cycle 4. The soils in the perfusion apparatus in cycles 1 and 3 received  $(\text{NH}_4)_2\text{SO}_4$  solution only (control),  $(\text{NH}_4)_2\text{SO}_4$  solution with 20 kg DCD  $\text{ha}^{-1}$  (+DCD) and  $(\text{NH}_4)_2\text{SO}_4$  solution with 40 kg DCD  $\text{ha}^{-1}$  (++DCD), while in cycles 2 and 4, all the soils were treated with  $(\text{NH}_4)_2\text{SO}_4$  solution only (control, -DCD and --DCD). Error bars represent the range of mineral N values obtained in the two replicates. .... 102
- Figure 3.18 Mean nitrification rates in control soil, soil receiving DCD (cycles 1 and 3) and after DCD treatment (cycles 2 and 4) in MFSL. The MFSL soils in the perfusion apparatus in cycles 1 and 3 received  $(\text{NH}_4)_2\text{SO}_4$  solution only (control) and  $(\text{NH}_4)_2\text{SO}_4$  solution with 20 kg DCD  $\text{ha}^{-1}$  (+DCD) and  $(\text{NH}_4)_2\text{SO}_4$  solution with 40 kg DCD  $\text{ha}^{-1}$  (++DCD), while in cycles 2 and 4, all the soils were treated with  $(\text{NH}_4)_2\text{SO}_4$  solution only (control, -DCD and --DCD). Vertical bars are the range of  $\text{NO}_3^-\text{-N}$  obtained in the two replicates. .... 104
- Figure 3.19 Daily (a) and 4-day moving average (b) nitrification rates in MFSL in (i) cycle 1; (ii) cycle 2; (iii) cycle 3 and (iv) cycle 4. The soils in the perfusion apparatus in cycles 1 and 3 received  $(\text{NH}_4)_2\text{SO}_4$  solution only (control) and with DCD at 20 kg  $\text{ha}^{-1}$  (+DCD) and 40 kg  $\text{ha}^{-1}$  (++DCD), while in cycles 2 and 4, all the soils were treated with  $(\text{NH}_4)_2\text{SO}_4$  solution only (control and -DCD). .... 105
- Figure 3.20 Estimates of AOB populations in the MFSL soils with time of incubation in the growth medium after serial dilution (a) cycle 1; (b) cycle 2; (c) cycle 3 and (d) cycle 4. The soils in the perfusion apparatus in cycles 1 and 3 initially received  $(\text{NH}_4)_2\text{SO}_4$  solution only (control),  $(\text{NH}_4)_2\text{SO}_4$  solution with 20 kg DCD  $\text{ha}^{-1}$  (+DCD) and  $(\text{NH}_4)_2\text{SO}_4$  solution with 40 kg DCD  $\text{ha}^{-1}$  (++DCD), while in cycles 2 and 4, all the soils were treated with  $(\text{NH}_4)_2\text{SO}_4$  solution only (control, -DCD and --DCD). .... 108
- Figure 3.21 AOB populations in the MFSL soils in the perfusion columns after each cycle. The MFSL soils in the perfusion apparatus in cycles 1 and 3 received  $(\text{NH}_4)_2\text{SO}_4$  solution only (control) and  $(\text{NH}_4)_2\text{SO}_4$  solution with 20 kg DCD  $\text{ha}^{-1}$  (+DCD) and  $(\text{NH}_4)_2\text{SO}_4$  solution with 40 kg DCD  $\text{ha}^{-1}$  (++DCD), while in cycles 2 and 4, all the soils were treated with  $(\text{NH}_4)_2\text{SO}_4$  solution only (control, -DCD and --DCD). Vertical bars in the columns indicate the range of AOB populations obtained in the 2 replicates in each treatment. .... 108

- Figure 3.22 The effect of DCD applied at the same rate ( $20 \text{ kg DCD ha}^{-1}$ ) in cycles 1 and 3 on the AOB populations in the MSL and MFSL soils. Vertical bars in the columns indicate the range of AOB numbers obtained in the 3 replicates in each treatment for MSL and 2 replicates in each treatment for MFSL..... 109
- Figure 3.23 Relationship between mean nitrification rate and the estimated numbers of AOB in each cycle in (a) MSL and (b) MFSL. .... 110
- Figure 3.24 The effect of DCD on nitrification rates (expressed as % of control) in the MSL (I) and MFSL (II and III). The soils in the perfusion apparatus in cycles 1 and 3 received  $(\text{NH}_4)_2\text{SO}_4$  solution containing DCD at  $20 \text{ kg ha}^{-1}$  (I and II) and  $40 \text{ kg ha}^{-1}$  (III), while in cycles 2 and 4, all the soils were treated with  $(\text{NH}_4)_2\text{SO}_4$  solution only. .... 114
- Figure 3.25 The effect of DCD on AOB populations (expressed as % of control) in the MSL (I) and MFSL (II and III). The soils in the perfusion apparatus in cycles 1 and 3 received  $(\text{NH}_4)_2\text{SO}_4$  solution containing DCD at  $20 \text{ kg ha}^{-1}$  (I and II) and  $40 \text{ kg ha}^{-1}$  (III), while in cycles 2 and 4, all the soils were treated with  $(\text{NH}_4)_2\text{SO}_4$  solution only. .... 114
- Figure 3.26 Hypothetical curves indicating effect of nitrapyrin (NP) on nitrification rate in a less susceptible soil (I) and a more susceptible soil (II), showing an initial drop in nitrification (a), a lag phase (b) and a recovery phase (c). Adapted from Hendrickson and Keeney (1979). .... 120
- Figure 4.1 Decomposition of DCD with time in (a) MSL and (b) MFSL after application of DCD at  $10 \text{ kg ha}^{-1}$  (+DCD;  $\blacklozenge$ ) and  $20 \text{ kg ha}^{-1}$  (++)DCD;  $\oplus$ ). Each point represents the mean of two replicate analyses of each of two separate subsamples of the incubated bulk soils. Error bars denote standard errors of the four analytical results for each point. .... 129
- Figure 4.2 Nitrate levels in (a) MSL and (b) MFSL during the initial bulk soil incubation without addition of DCD ( $\diamond$ ) and with addition of DCD at low ( $\triangle$ ; +DCD) and high ( $\square$ ; ++DCD) rates. No  $\text{NO}_3^-$ -N analyses were performed on the bulk incubating soils from from day 1 to day 28. Each point represents the mean of two replicate analyses of each of two separate subsamples of the incubated bulk soils. Error bars denote the standard errors of the four analytical results for each point. .... 131
- Figure 4.3 Assayed nitrification rates in (a) MSL and (b) MFSL after the addition of urea as affected by time after DCD application at  $10 \text{ kg ha}^{-1}$  (+DCD) and  $20 \text{ kg ha}^{-1}$  (++)DCD). Each point was the mean of two replicates analyses of the two separate subsamples of the incubated bulk soils. Error bars represents standard error of the four analytical results for each point. .... 134
- Figure 4.4 Assayed nitrification rates after the addition of urea, expressed as a percentage of control in (a) MSL and (b) MFSL with time after the addition of DCD at  $10 \text{ kg ha}^{-1}$  (+DCD) and  $20 \text{ kg ha}^{-1}$  (++)DCD). Each point was the mean of two replicates analyses of the two separate subsamples of the incubated

bulk soils. Error bars represents standard error of the four analytical results for each point. ....	135
Figure 4.5 Relationship between DCD concentration in the two soils and (a) the rate of nitrification of added urea and (b) the reduction in nitrification rates from those in the control soil without added DCD. DCD was added at 10 kg ha <sup>-1</sup> ; +DCD and 20 kg ha <sup>-1</sup> ; ++DCD. Each point was the mean of two replicates analyses of the two separate subsamples of the incubated bulk soils. Error bars represents standard errors of the four analytical results for each point.....	137
Figure 5.1 Total mineral N (■), NH <sub>4</sub> <sup>+</sup> -N (▲) and NO <sub>3</sub> <sup>-</sup> -N (◆) concentrations during incubation of control (-urea) and urea treated (+urea) soils. Error bars represent the range of inorganic N concentrations obtained between the two replicates. Note that the concentration scale in the control graphs differs from that in the + urea treatment. ....	150
Figure 5.2 Changes in pH with time of incubation in (a) control soils and (b) soils with urea addition. Vertical bars denote the range of pH obtained between the two replicates. ....	151
Figure 5.3 Nitrification rates in the three soils; (a) during the 15-day incubation period, with and without urea addition, (b) in successive 5-day periods in the control soils and (c) successive 5-day periods in the soils with urea added. ....	153
Figure 5.4 Viable population size of AOB in steep slope, SS; camp site, CS and (c) Manawatu silt loam, MSL. Vertical bars in the columns indicate range of AOB populations obtained in the 2 replicates in each treatment. ....	154
Figure 6.1 Concentrations of mineral N fractions in mixtures of camp and steep soils (ratio indicated represents proportions of CS:SS) following the addition of urea ((ii)+ urea) and without urea ((i)-urea; control) and subsequent incubation. Error bars represent the standard deviation of the four replicates. Note that the concentration scale in the control (- urea) graphs differs from that in the + urea treatment. Symbols in both (i) and (ii) represent total mineral N (■), NH <sub>4</sub> <sup>+</sup> -N (▲) and NO <sub>3</sub> <sup>-</sup> -N (◆) concentrations. ....	168
Figure 6.2 Changes in pH with time of incubation in (a) control soils and (b) soils with urea applications (ratio indicated represent proportions of CS:SS). Vertical bars denote the standard deviation of four replicates. ....	169
Figure 6.3 Nitrification rates in the mixtures of camp and steep sites soils (CS:SS) in (a) the control treatment and (b) the treatment with added urea, during the 15-day incubation. Vertical bars denote the standard deviation of four replicates. ....	170
Figure 6.4 Measured and predicted nitrification rates in soil mixtures after the addition of urea and incubation for (i) 5 days, (ii) 10 days and (iii) 15 days. Vertical bars represent the standard deviation of four replicates. ....	173