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UNIVERSITY OF ALBERTA

DENITRIFICATION AND NITROUS OXIDE EMISSION IN THAWING SOIL

BY

JOHN W. A. LAIDLAW

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE**

IN

SOIL FERTILITY

DEPARTMENT OF SOIL SCIENCE

EDMONTON, ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled DENITRIFICATION AND NITROUS OXIDE EMISSION IN THAWING SOIL submitted by JOHN W. A. LAIDLAW in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in SOIL SCIENCE.

M. Nyborg
M. Nyborg (Supervisor)

W. B. McGill

R. César Izaurralde
R. C. Izaurralde

John Ashworth
J. Ashworth

J.P. Tewari

Date: October, 1, 1993

This thesis is dedicated to my family.

ABSTRACT

The research was to identify the time of denitrification loss, and specifically, N_2O emission in relation to soil thawing. The hypothesis was that thawing soil emitted N_2O in the field or in the laboratory, and further, that denitrification (as measured by the ^{15}N mass balance technique) occurs at soil thaw.

Denitrification and N_2O flux were monitored in the field during two springs and one summer, followed by two laboratory experiments. Cylinders were placed in the field in the fall of 1988 and 57 kg N ha^{-1} of ^{15}N labeled KNO_3 added to the soil. Soil ^{15}N mass balance technique showed 23 kg N ha^{-1} of the added N had been lost by April 29, 1989. Daily gas trapping showed 3.5 kg N_2O-N ha^{-1} was emitted in April, mostly during an 11-day period of soil thaw. The N_2O flux was monitored throughout the growing season, but the April flux was greater than the accumulated flux in the summer. In 1990, ^{15}N labeled KNO_3 was applied at the outset of the thaw at a rate of 100 kg N ha^{-1}. The ^{15}N mass balance technique showed 32.7 kg N ha^{-1} was lost by May while there was a flux of 16.3 kg N_2O-N ha^{-1} from added-N during a 10-day thawing period in April.

The first laboratory experiment included three soils. Treatments were N-added (^{15}N-KNO_3 at 80 kg ha^{-1}), gamma irradiation, and frozen and non-frozen soil. The incubation was carried out for 88 h with gas sampling every 8 h to analyze for N_2O and CO_2. Denitrification occurred during the thawing of frozen soils with 14 to 23 % of added ^{15}N denitrified. N_2O-N constituted less than 3 % of the total gaseous N produced. Denitrification and N_2O flux from irradiated soils were not different from non-irradiated soils, suggesting the activity of radiation-resistant enzymes or the loss of sterility. Little difference was found between the denitrification rates of non-

frozen and frozen soils, suggesting that freezing neither enhances nor inhibits denitrification at cool temperatures.

In a second laboratory experiment, denitrification and N_2O flux were measured during thaw of frozen, manure amended soil samples. During a 66 h thaw period, treatments with manure emitted 0.97 kg N_2O-N ha^{-1} and non-manure treatments emitted 0.25 kg N_2O-N ha^{-1}. Soil ^{15}N mass balance indicated that 39% of added KNO_3-N (80 kg ha^{-1}) was denitrified in manure treated soil and 20% was lost from the non-manure treatments. The addition of manure significantly increased denitrification of added NO_3^- but appeared to decrease the proportion of N_2O emitted from the thawing soil.

In all, this work indicates that denitrification and N_2O emission occur during the soil thaw in north central Alberta.

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CHAPTER 1

DENITRIFICATION AND NITROUS OXIDE EMISSIONS FROM SOIL IN PERSPECTIVE

1.1 INTRODUCTION

This thesis is only one of several aimed at quantifying nitrogen loss and denitrification from Alberta soils, but it is the first to include both measurement of nitrous oxide (N_2O) flux and ^{15}N mass balance, specifically as the soil thaws. The initial research on the loss of fall-applied nitrogen from Alberta soils began in the early 1970's with an emphasis on economics. Leitch and Nyborg (1972) found that plots receiving fall applications of nitrogen were often out yielded by plots fertilized in the spring. Malhi (1978) and Malhi and Nyborg (1983) found, using ^{15}N-enriched fertilizers, that substantial and irrecoverable losses of fall-applied nitrate occurred over winter or in early spring. Denitrification losses in the field were apparently dependent on soil saturation (Heaney et al., 1992). Malhi et al. (1990) determined that soil moisture and temperature in early spring after snow melt in north-central Alberta may be high enough to result in substantial losses of nitrate (NO_3^-). Recently, with the growing awareness of environmental issues, the emphasis on nitrogen loss and N_2O evolution from soils has moved away from an economic focus.

1.2 NITROGEN OXIDES AND THE ENVIRONMENT

Growing concerns over the possible adverse effects of agriculture on the environment have led to interest and funding for research in nutrient cycling and the role of nitrogen in the global environment. The use of inorganic and organic nitrogen fertilizers has been identified as contributing nitrogen oxides (NO, NO_2, and N_2O) to the atmosphere (Crutzen and Ehhalt, 1977). Nitrogen oxides act as greenhouse gases

(Wang et al., 1976) by trapping radiated energy from the earth. Increased levels of greenhouse gases, such as N_2O, can potentially result in global warming. Nitrous oxide (N_2O) is estimated to contribute about 4% of the activity of greenhouse gases after carbon dioxide, methane and chlorofluorocarbons (CFC's) (Alberta Environment, 1990). Nitrogen oxides also contribute to the destruction of stratospheric ozone (O_3) (Crutzen and Ehhalt, 1977) and the production of tropospheric, or ground level, O_3 (Conrad, 1990; Crutzen, 1979) (Appendix 1). Tropospheric O_3 contributes to the greenhouse effect (Ramanathan et al., 1987; Isaksen, 1988) and has deleterious effects on health (Hinrichsen, 1985; McKee, 1990; Friedman, 1988, U.S. EPA, 1988, Lippmann, 1989).

Nitrogen oxides are part of the natural cycling of nitrogen and are products of denitrification, the microbial reduction of nitrates; and nitrification, the oxidation of ammonia (Paul and Clark, 1989). Nitrogen gases can also be released from soil by non-biological means through chemodenitrification (Paul and Clark, 1989). Over the past millennia, the destruction and formation of the gaseous components of the atmosphere have been held in equilibrium. The formation of ozone is constantly counterbalanced by its catalytic destruction in the atmosphere.

Atmospheric nitrogen is in equilibrium between release from the soil through denitrification and by N-fixation or by wet and dry deposition. Estimates of the global fluxes of nitrogen into and out of the terrestrial biosphere are listed in Table 1.1. The main processes by which N-gases are emitted into the atmosphere are through denitrification (40 to 350 Tg N yr^{-1}) and ammonia volatilization (36 to 250 Tg N yr^{-1}). In comparison, the human elements of 'Industrial fixation' and 'Fossil fuel burning' are approximately 10 times less. However, the effect of man's added activity could threaten the atmospheric equilibrium resulting in a decrease in stratospheric ozone and an increase in global temperatures.

Table 1.1 Global fluxes of nitrogen into and out of the terrestrial biosphere.†

Inputs and Outputs	Process Rate
	Tg N yr-1
Inputs	
Wet and dry deposition (NH_3/NH_4^+)	90-200
Wet and dry deposition (NO_x)	30-80
Wet and dry deposition (Organic N)	10-100
Atmospheric fixation	0.5-30
Biological fixation	100-200
Industrial fixation (fertilizers)	60
Outputs	
Ammonia volatilization	36-250
Denitrification ($N_2 + N_2O$)	40-350
Biogenic NO_x production	1-15
Fossil fuel burning (NO_x)	10-20
Fires (NO_x)	10-20
Leaching and runoff (inorganic)	5-20
Leaching and runoff (organic)	5-20

†Data compiled from Delwiche, 1977; Söderlund and Rosswall, 1982; Crutzen, 1983; Galbally and Roy, 1983; and Rosswall, 1983 *all in* Haynes, 1986.

1.3 DENITRIFICATION

Denitrification is the dissimilatory reduction of NO_3^- to nitrite (NO_2^-) and then principally to N_2O and N_2. In the absence of O_2, N-oxides act as terminal electron acceptors for respiratory electron transport (Tiedje, 1988). This process is often called enzymatic denitrification or dissimilatory denitrification. The latter term should not be confused with the dissimilatory reduction of nitrate to ammonium (NH_4^+) that is accomplished by certain microorganisms under highly reduced conditions. Another process found in green plants, cyanobacteria, bacteria and fungi called assimilatory reduction involves the reduction of NO_3^- to NH_4^+ for the biosynthesis of amino acids and protein. Under anaerobic conditions, when O_2 is not available as an electron acceptor, electron transport branches off from *b*-type cytochromes to the several forms of oxidized nitrogen as the terminal acceptor (Paul and Clark, 1989). Specific reductases are involved at each acceptor level. The pathway of N-oxide reduction is usually represented as:

$$2NO_3^- \rightarrow \quad 2NO_2^- \rightarrow \quad 2[NO] \rightarrow \quad N_2O \quad \rightarrow \quad N_2$$

The enzymes responsible in the pathway are NO_3^- reductase, NO_2^- reductase, NO reductase and N_2O reductase. Many denitrifying bacteria possess all the reductase enzymes, however, some contain some or only one of the reductases. About 20 genera of denitrifying bacteria have been reported (Payne, 1973; 1981; Focht and Verstraete, 1977; Firestone, 1982; Knowles, 1982). Most are chemoheterotrophs which obtain energy from organic substrates. Some denitrifiers grow as chemolithotrophs (John and Whatley, 1975; Thauer et al., 1977), are photosynthetic (Satoh, 1977; Sawada et al., 1978), or fix N_2 (Eskew et al., 1977; Neyra and van Berkum, 1977; Neyra et al., 1977; Scott and Scott, 1978). Nitrous oxide and N_2 are usually produced in varying ratios depending on the organisms involved, substrate, environmental conditions, and on the time elapsed since the onset of denitrifying activity (Paul and Clark, 1989).

Nitrite is unstable in soil and can be reduced to other oxides of nitrogen and N_2 through non-enzymatic pathways in a process called chemodenitrification (Cady and Bartholomew, 1963; Reuss and Smith, 1965; Bulla et al, 1970; Nelson and Bremner, 1970). The reduction of NO_3^- to NO_2^- is a biological process requiring NO_3^- reductase, so chemodenitrification can be limited by a lack of biological reduction of NO_3^-. The reduction of NO_3^- to NO_2^-, however, is not strictly a biological occurrence. Cawse and Cornfield (1977) showed that the chemical reduction of NO_3- to NO_2^- occurs during gamma irradiation of soil.

1.4 OBJECTIVES

The purpose of this thesis was to identify and quantify denitrification and N_2O emissions in some soils of north central Alberta during the soil thaw. The thesis explores using N_2O measurements as an indicator of denitrification activity in soil. Nitrogen added to soils in the experiments was labeled with ^{15}N so that the loss of added N could be determined by mass spectrometry. Analysis of mineral N (NO_3^- and NH_4^+) in soil was also used to determine movement and loss of N from treatments. Concerns of possible contribution by agricultural N-fertilizers to stratospheric O_3 depletion, tropospheric O_3 formation, and the greenhouse effect give broader implications to the thesis.

Chapter 2 includes three experiments that were conducted in the field near Ellerslie, Alberta. Chapters 3 and 4 describe experiments conducted in the laboratory. The first laboratory experiment involved warming frozen and non-frozen soils. One treatment involved the use of gamma irradiation to sterilize soil. The second laboratory experiment, described in Chapter 4, included thawing soil amended with cattle manure.

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CHAPTER 2

DENITRIFICATION AND NITROUS OXIDE EMISSIONS FROM SOIL DURING SPRING THAW[1]

2.1 INTRODUCTION

North central Alberta soils have often shown marked loss of mineral nitrogen in the early spring (Heaney et al., 1992; Nyborg et al., 1990a; Malhi and Nyborg, 1986; Malhi and Nyborg, 1983) and even during winter (Heaney and Nyborg, 1988). Apparently, the losses were not caused by leaching (Heaney et al., 1992) but instead by denitrification (Nyborg et al., 1990b). Substantial N_2O emissions occurred from time to time from spring to fall in Saskatchewan (Aulakh et al., 1982), but measurements were not taken during the thaw. Simulated field conditions with three soil samples taken from southern Alberta did not show measurable N emissions at 2 to 3°C (Cho et al., 1979). However, research elsewhere has shown substantial N_2O flux rates during thawing of soil in the field (Christensen and Tiedje, 1990; Cates and Keeney, 1987; Goodroad and Keeney, 1984a; Goodroad and Keeney, 1984b).

Several factors could contribute to denitrification while soil thaws. During the soil thaw, surface soil is underlain with frozen subsoil which impedes drainage and creates saturated conditions (Ferguson et al., 1964; Sartz, 1969). The saturated conditions create an anaerobic environment that favors reduction of NO_3^- by denitrifiers. It has been hypothesized and observed that N_2O is produced during the winter in warmer subsoil and is physically released as the soil thaws (Goodroad and Keeney, 1984a). Chemical reactions reducing NO_2^- to N_2O and N_2 (chemodenitrification) can also occur on freezing of moist soil (Christianson and Cho,

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[1] Part of this chapter has been printed in the 27 Annual Alberta Soil Science Workshop Proceedings, 1990: 203-212.

1983). Soil N loss also occurs at the soil surface where freezing may enhance organic matter availability as a substrate for denitrifiers (McGarity, 1962 and Christensen and Christensen, 1991). Nitrification in non-frozen subsoil is also a possible source of N_2O release during the soil thaw (Bremner and Blackmer, 1978).

Our hypotheses were that in the field: (1) denitrification measured as a loss of ^{15}N labeled NO_3^- occurs during the spring thaw, and (2) N_2O is a large component of the emitted N gases when the soil is thawing.

2.2 MATERIALS AND METHODS

2.2.1 Introduction

Three field experiments were conducted on an Eluviated Black Chernozem of the Malmo series located near Ellerslie, Alberta. The experiments were laid out within a 20 m by 30 m site. A description of the soil is in Table 2.1.

Table 2.1: Characteristics of the Malmo Series

Depth	Texture	Bulk Density	pH	Total C	Total N
cm		Mg m^{-3}		dag kg^{-1}	dag kg^{-1}
0-15	L	1.03	6.2	5.75	0.51
.15-30	CL	1.05	6.2	3.06	0.35
30-45	L	1.43	6.1	0.86	0.08

Denitrification was monitored in two experiments in 1989, during the spring (March 28 to April 29) and summer (June 20 to August 30), and in a third study in the spring of 1990 (March 28 to April 29). The two spring studies involved the use of lysimeters with two treatments, 'N added' and 'control', each replicated four times. The summer experiment included four replicates of fallowed no-till and tilled plots with and without fertilizer N additions.

2.2.2 Spring 1989 and 1990

Both ^{15}N mass balance and N_2O flux were measured during the two spring experiments. The 1989 spring experiment was initiated in the fall of 1988 on barley stubble. Sixteen bottomless metallic cylinders (diameter of 40 cm and a height of 22 cm) were placed in the soil to a depth of 19 cm on November 3, 1988. Four cylinders received 57 kg N ha^{-1} as ^{15}N labeled KNO_3 (98% atom abundance) and four other cylinders were designated as controls. The remaining eight cylinders were fallowed in 1989 without treatment until the initiation of the 1990 spring experiment. Four cylinders were fertilized with 100 kg N ha^{-1} as ^{15}N-KNO_3 (4.63 % abundance) on March 26, 1990, just prior to thaw, and the four remaining cylinders were controls. Thus, we had two conditions, an experiment on stubble in 1989 and one on fallow in 1990. Mass balance involved the addition ^{15}N-labeled KNO_3 to soil. The ^{15}N-labeled KNO_3 was incorporated into the 0-15 cm depth. After a period of time, the soil was sampled at different depths and analyzed by mass spectrometry to detect the ^{15}N isotope. The added ^{15}N that can not be accounted for was assumed to have been lost from the soil by either leaching or gaseous emissions through denitrification. Both experiments were randomized complete blocks.

2.2.3 Summer, 1989

The plots used during the summer (June 20 to August 30) were adjacent to the spring thaw lysimeters and were each 2.5 m wide and 7.5 m long. Treatments were no till and tilled plots with and without N added. The tilled-plots were disced twice during the study period and the no-tilled plots were maintained as chemical fallow. Nitrogen was added to '+ N' treatments by broadcasting 100 kg N ha^{-1} as urea.

Nitrous oxide flux was monitored every seven days throughout the summer and mass balance was not used.

2.2.4 Nitrous Oxide Trapping and Analysis

Gas samples were collected before and after vented soil covers were placed over the soil for 30 or 60 minutes. The soil covers used were the same as those described by Hutchinson and Mosier (1981). The effect of inserting soil covers into the soil was tested in a separate laboratory experiment (Appendix 22). Gas samples were collected by first mixing air in the headspace by pumping the plunger of a 30 mL syringe several times and then removing 24 mL of air from the headspace. The air sample was then placed in an evacuated 25 mL Vacutainer© test tube with 1 mL silica gel desiccant (0.65 to 1.23 mm). The gas samples were analyzed for N_2O using a Perkin Elmer, Sigma 3 gas chromatograph equipped with a ^{63}Ni electron capture detector at an operating temperature of 300°C with $Ar:CH_4$ (95:5 ratio) as a carrier gas at a flow rate of 65 ml min^{-1}. A 1.8 m column of Poropak Q (50/80 mesh) was used for separation of N_2O. Ambient air N_2O concentration was subtracted from the N_2O trapped from the cores to give emitted N_2O per trapping period. The correction for concentration gradient inside the trap suggested by Hutchinson and Mosier (1981) was not used. The N_2O fluxes were calculated as g N_2O-N emitted ha^{-1} h^{-1} (Appendix 2 and 3). Daily and cumulated spring N_2O emissions were estimated from the hourly fluxes (Appendix 4 and 5).

Differences in accumulated and daily nitrous oxide emissions among treatments in each of the three experiments were tested using Duncan's multiple range test (Appendix 8).

2.2.5 Soil Analyses

Soil from 0-15 cm, 15-30 cm, 30-45 cm, 45-60 cm and 60-75 cm depths was sampled on May 15, 1989 and May 7, 1990 for the 1989 and 1990 spring experiments, respectively, for N mass balance and determination of bulk density. The upper 15 cm of soil was removed from the cylinders and thoroughly mixed and sub sampled. Soil samples from lower depths were taken using a tube-type core sampler (diameter of 2.0 or 3.2 cm). Soil samples from the cylinders were placed in trays and air dried at 22°C. The soil was analyzed for ^{15}N using a NA 1500 Carlo Erba N Auto analyzer coupled to a VG Isogas mass spectrometer for determination soil N mass balance. The soil was also analyzed for mineral NO_3^- and NH_4^+ by extraction with 2 N KCl and using a colorometric method (Technicon Industrial Systems, 1973a and 1973b) (Appendix 6).

2.3 RESULTS AND DISCUSSION

2.3.1 Mass Balance

The ^{15}N balance indicated that by May 15, 23 kg N ha^{-1} of the fall added ^{15}N-KNO_3, was not accounted for in the soil (Table 2.2). During 1990, 32.7 kg ha^{-1} of added N had denitrified during the spring thaw. The distribution of soil ^{15}N recovery in both years appears in Appendix 7. The lost ^{15}N was apparently not leached from the soil profile. Lateral movement may have occurred, though other research on the same soil profile does not support this (Malhi and Nyborg, 1983; Heaney et al., 1992). Since the labeled fertilizer was added in November, 1988, the spring, 1989, N loss may have occurred outside of the March 27 to April 29 period of N_2O measurement. The same problem did not exist in 1990 since the labeled-N was added only 2 days prior to N_2O monitoring and 40 days prior to soil sampling. Therefore, added-N not accounted for by mass balance was assumed lost to denitrification.

The added-N lost from soil during the thaw was greater than annual N loss recorded by Aulakh et al. (1984) and Aulakh et al. (1991) for Chernozems in Saskatchewan. Denitrification in our work was an order of magnitude greater than determinations under warmer temperatures and higher rates of applied N in the mid-west (Hutchinson and Mosier, 1979; Mosier et al., 1986; Mosier and Hutchinson, 1981; Cates and Keeney, 1987).

Table 2.2. Mineral N and recovery of ^{15}N-labeled N at termination of experiments, and cumulated N_2O-N emissions in the springs of 1989 and 1990.

Year	Treatment	Mineral N †		^{15}N Recovery	Emitted Added N	Emitted N_2O-N §	N_2O ‡ Emitted Added N
		NH_4^+-N	NO_3^--N				
		kg ha^{-1}		%	kg ha^{-1}	kg ha^{-1}	%
1989	+N	92 ±15	90 ±11	59.5	23.0	3.50	9.4
	Control	89 ±13	43 ±17	NA	NA	1.34	NA
1990	+N	31 ±9	148 ±31	67.3	32.7	16.31	31.4
	Control	32 ±9	74 ±19	NA	NA	6.03	NA

† Data represents 0 to 75 cm.

‡ Determined by dividing the difference of the +N and control 'Emitted N_2O-N' by 'Emitted Added N' and multiplying by 100 to get percent.

§ N_2O emissions for 33 days in 1989 and 38 days in 1990.

2.3.2 Nitrous Oxide

The average concentration of N_2O in the ambient air during April 1989 was 0.31 mL m^{-3} and 0.29 mL m^{-3} in April of 1990. Daily values ranged from 0.1 to 1.0 mL m^{-3} in 1989 and 0.1 to 0.8 mL m^{-3} in 1990. These values are similar to those reported by Bremner and Hauck (1982).

In 1989, N_2O concentrations in the soil covers placed over frozen soil was somewhat greater than ambient air values (Figure 2.1). There was a sharp increase in flux as the soil thawed and became saturated, from April 2 to April 10. The maximum N_2O flux from the +N treatment was 16.9 g N_2O-N ha^{-1} h^{-1} on April 10. The substantial N_2O emissions continued until April 16 with the remainder of the month recording fluxes of 2.4 g N_2O-N ha^{-1} h^{-1} or less. The total N_2O-N emitted during the one month spring thaw period (33 days) was estimated at 3.50 kg ha^{-1} from the +N treatment and 1.34 kg ha^{-1} from the control. Of the 57 kg N ha^{-1} added, N_2O represented an estimated 9.4 % of the 23.0 kg N lost (Table 2.2).

Cumulative N_2O-N emissions between June 20 and August 30 in the summer experiment were estimated at 0.11 kg ha^{-1} to 0.46 kg ha^{-1} with coefficients of variability ranging from 6 % to 68 % (Table 2.3). No differences were found between treatments. N_2O concentrations under the soil covers were greater than ambient air in at least one treatment on 7 of the 11 days when measurements were taken. Negative flux was found on 5 days on the no till, no N treatment and on 1 or 2 days on the other treatments. The total precipitation during the summer was 191 mm with only three rainfall events recording more than 20 mm. July was the wettest month with 109 mm but N_2O flux did not appear to increase with rainfall.

Table 2.3 Cumulated nitrous oxide emissions during the summer, 1989.†

Treatment	**Emitted N_2O**	**Coefficient of Variance**
	kg N ha^{-1}	%
No till, no N	0.11	68
No till, +N	0.20	6
Tilled, no N	0.46	70
Tilled, +N	0.13	59

†Emissions were monitored from June 20 to August 31.

In spring, 1990, the first N_2O flux measurement of 26.6 g N_2O-N ha^{-1} h^{-1} from the +N treatment was made on March 28 (day 87) after soil thaw had already commenced and saturated conditions existed. The maximum flux recorded from the +N treatment was 87.2 g N_2O-N ha^{-1} h^{-1} on April 3. Values remained high until April 6, after which no flux values greater than 1.6 g N_2O-N ha^{-1} h^{-1} were recorded (Figure 2.2). Total N_2O-N emissions were estimated as 16.3 kg ha^{-1} for the +N treatment and 6.0 kg ha^{-1} for the control (Table 2.2). The maximum N_2O flux from both treatments was 4.5 times greater than in 1989. The large increase in N_2O emissions in 1990 over 1989 were attributed to the effects of increased soil NO_3^- (Table 2.2 and Table 6.12) due to summer fallow.

Nitrous oxide fluxes determined in the field during spring thaw by others are generally lower than those found in the +N treatments of 1989 and 1990 (Table 2.4). However, Christensen and Tiedje (1990) measured a similar average emission of N_2O in an acid soil as the 1990, +N treatment but recorded a maximum daily flux more than twice as large. Their soil was heavily fertilized and very acid and thus was prone to large emissions of N_2O. Nitrogen amended plots in Wisconsin (Goodroad and Keeney, 1984a; Cates and Keeney, 1987) had maximum and average N_2O fluxes similar to or less than the 1989 and 1990 control treatments (Table 2.4).

The large flux of N_2O during the spring thaw appeared as the single large event that occured during a period of about 10 days (days 96 to 106 in 1989 and days 87 to 97 in 1990). This flux event did not recur despite the availability of NO_3^- and soil moisture. In spring, 1990 , the heavy rainfall on days 113 and 114 (Figure 2.2) did not result in increased N_2O emissions. The second freeze-thaw event in 1990 (Figure 2.2) was not accompanied by N_2O flux. Perhaps a recovery period after a large flux event may be required. Possibly, the natural procession of microbial populations and the availability of nutrients and removal of waste products were involved.

Table 2.4 Comparisons with other published nitrous oxide emissions during the spring thaw.

Source	Location and Site Description	Average N_2O Flux During Thaw	Largest N_2O Flux During Thaw	Annual N_2O Flux
		($g N ha^{-1} d^{-1}$)	($g N ha^{-1} d^{-1}$)	($kg N ha^{-1} y^{-1}$)
1989 N_2O	Control treatment	41	111	1.5
	+N treatment	106	406	3.7
1990 N_2O	Control treatment	159	683	na[§]
	+N treatment	429	2093	na
Goodroad and Keeney (1984a)	Wisconsin, pH 4.7, manured	116[†]	152	2.1[‡]
Cates and Keeney (1987)	Wisconsin, pH 6.7, manured, fertilized, loam	24	47	5.2
Christensen and Tiedje (1990)	Wisconsin, fallow, pH 3.8, sandy loam	486	5300	na

[†] Average of three values determined on 2, 10, and 17 April.
[‡] Data for the same site in a different year (Goodroad and Keeney, 1984b).
[§] Not available.

2.3.3 A Provincial Perspective on Nitrogen Oxide Emissions

Alberta's annual anthropogenic production of nitrogen oxides (N_2O + NO_x's) is estimated at 434 kilotons or 23% of the national average annual emissions (Alberta Environment, 1992; Anonymous, 1990). The great majority of the N-oxides are produced by the energy and transportation sectors. The contribution of N-oxides from cultivated soils in Canada has not been estimated. In Alberta, there are about 5.6 million hectares of cultivated black and gray soils (Anonymous, 1976). Assuming N_2O emissions from the control treatments in the spring and summer, 1989 experiments represent average annual N_2O emissions from black and gray soils (1.5 kg N_2O-N $ha^{-1}y^{-1}$), we estimate 8.4 kt of N_2O-N are emitted from cultivated northern Alberta soils (Appendix 9). This is equivalent to 2 % of Alberta's anthropogenic N_2O

and NO_x emissions. This estimate is conservative since only N_2O soil emissions were considered (not NO or NO_2) and fall application of N-fertilizer, summer fallowing and perhaps other management practices would result in larger emissions of N-oxides.

2.4 CONCLUSIONS

Our hypothesis that denitrification occurs during the spring thaw was accepted based on the results of the spring, 1989 and 1990 experiments. The hypothesis that N_2O is a major component of the emitted N-gases in the field when soil is thawing was also shown to be correct in both years.

In this study denitrification and N_2O flux appeared the most active during the spring thaw and very slow throughout the rest of the year. Soil moisture status is the main constraint on denitrification since it affects the activity of anaerobic denitrifying bacteria and the availability of substrates. The Malmo series drained rapidly so saturated conditions generally occurred only during the spring when subsurface soil was frozen and consequently, drainage was impeded. The large increase in the flux of N_2O during the thaw in the second spring (1990) over that in 1989 was attributed to the increased native soil nitrate concentration produced during previous year of fallow. The apparent proportion of N_2O in emitted N gases also increased the second spring. This increased proportion of N_2O was attributed to the increased soil NO_3^- concentration concomitant on summer fallowing.

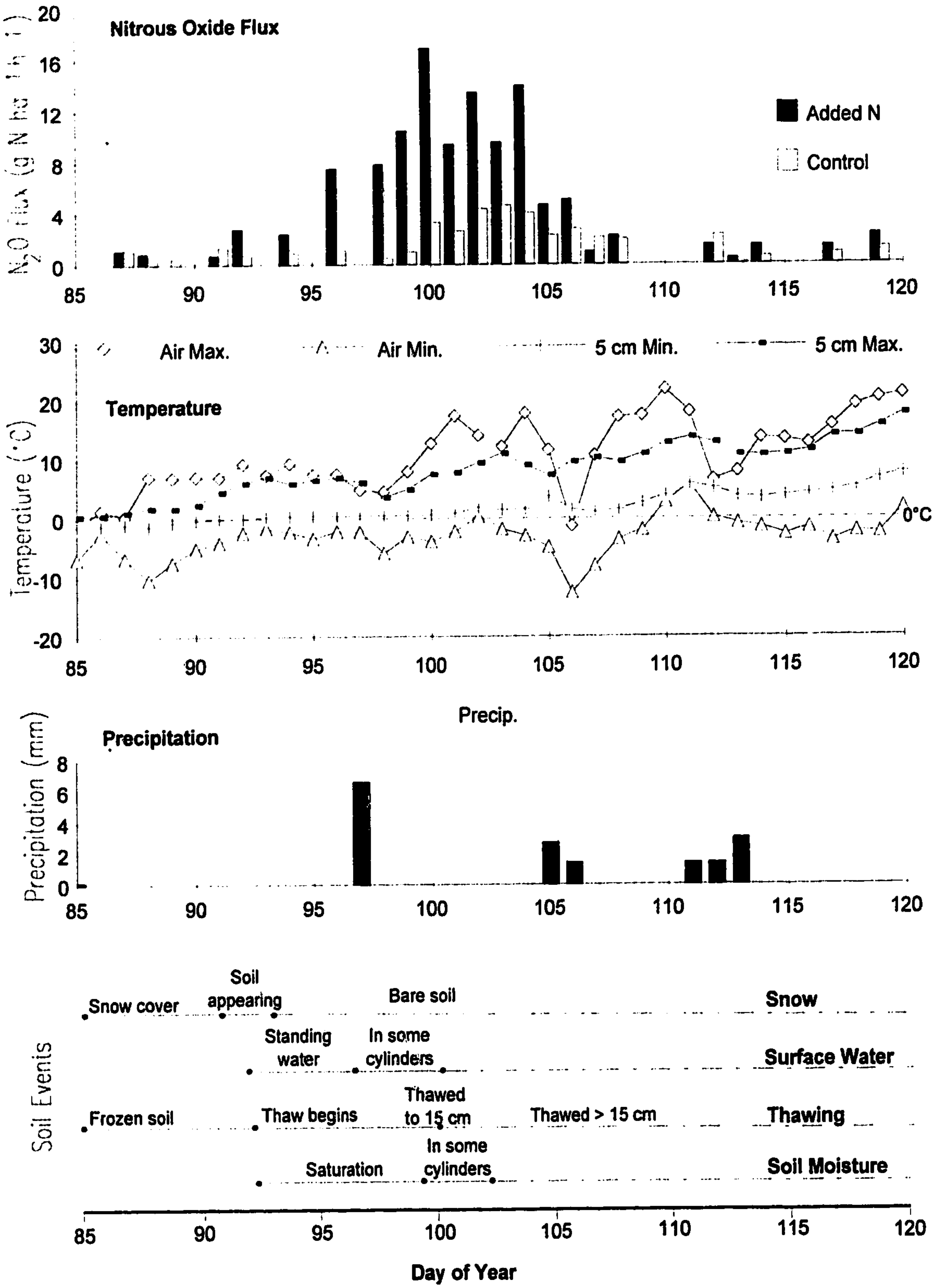

Figure 2.1 Nitrous oxide flux, temperature, precipitation and soil events during the spring of 1989.

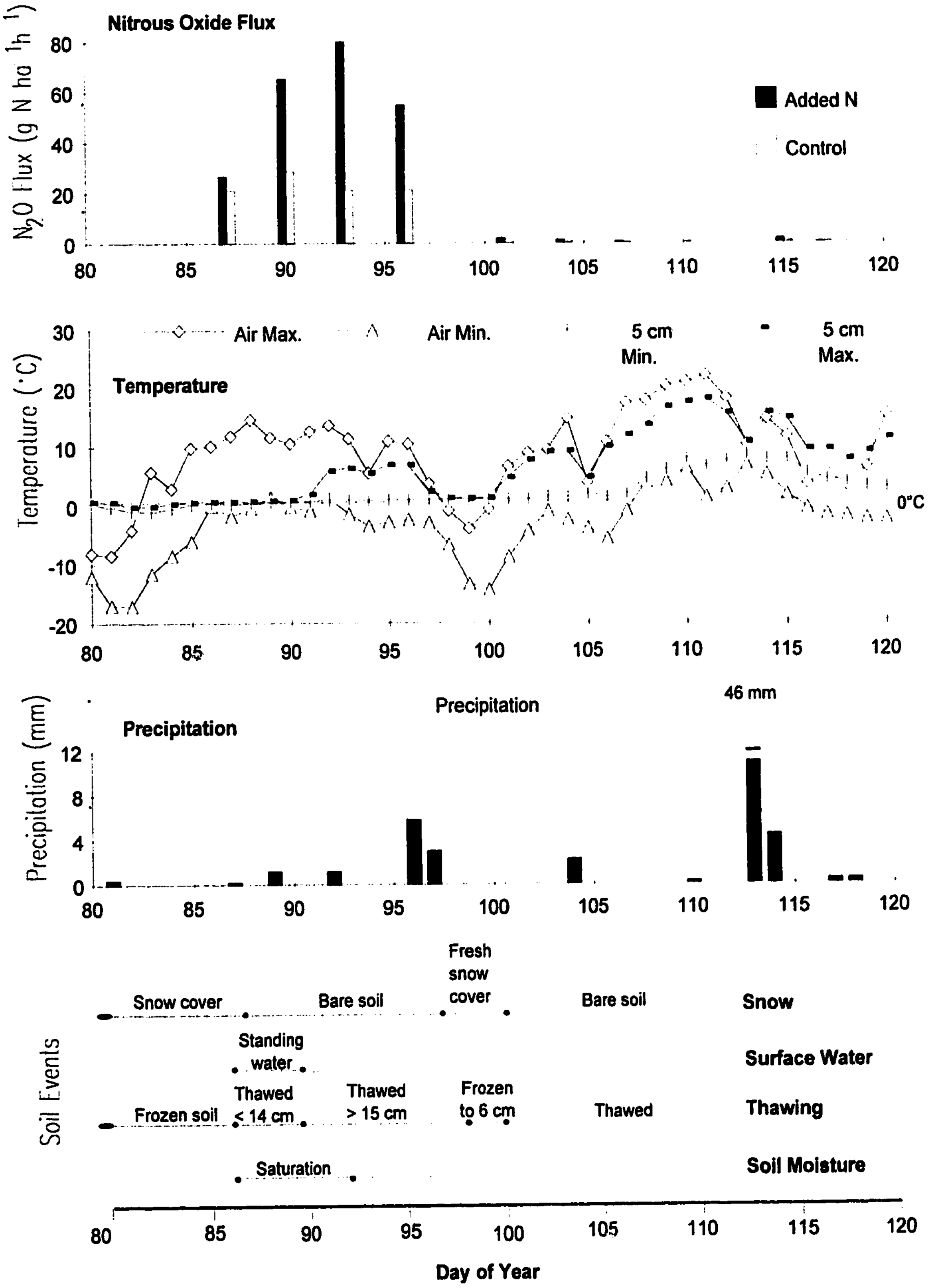

Figure 2.2 Nitrous oxide flux, temperature, precipitation and soil events during the spring of 1990.

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CHAPTER 3

DENITRIFICATION AND N_2O FLUX FROM FROZEN AND NON-FROZEN SOIL AT COLD TEMPERATURES[1]

3.1 INTRODUCTION

Soils of north central Alberta exhibit significant denitrification activity during the spring thaw (Heaney et al., 1992; Nyborg et al., 1990; Malhi and Nyborg, 1986) and apparently substantial gaseous losses of soil N to the atmosphere. Several causes of soil N loss during soil thaw and cold temperatures have been suggested. Soil freeze/thaw increases the carbon availability from detritus or microorganisms killed by freezing (Christensen and Tiedje, 1990; Christensen and Christensen, 1991). Soil thaw can permit the physical release of subsurface produced N gas (Goodroad and Keeney, 1984b). Also, the action of extra cellular denitrifying enzymes (Smith and Parsons, 1985) and chemodenitrification (Christianson and Cho, 1983) mediate N loss during soil thaw. The action of psychrophilic denitrifiers has also been suggested (Dorland and Beauchamp, 1991) and they may explain why soils from different climates respond differently to laboratory temperatures (Gamble et al., 1977; Powlson, 1988).

Nitrous oxide is usually the second greatest product of denitrification, after N_2. The ratio of N_2O to N_2 varies greatly (Gilliam et al., 1978) and is mainly affected by organic C, pH, NO_3^-, temperature, water and redox potential. The proportion of N_2O in gaseous N emissions has been shown to increase with acidity (Firestone et al. 1980; Koskinen and Keeney, 1982) and represented 100% of the N flux of an acid soil used by Christensen and Tiedje (1990). Keeney et al. (1979) found the ratio of N_2O to N_2 increased as the soil temperature was lowered. Wetter conditions resulted

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in greater N_2O production in work by Drury et al. (1992). Soil redox potential is related to many of the soil parameters which affect the N_2O to N_2 ratio. Some confusion may come from considering the effects of any one variable such as pH or temperature separately without considering the role of redox.

Because of the importance of N oxides to the environmental issues of stratospheric ozone depletion and climate warming, we conducted a laboratory experiment to assess the potential for N loss by denitrification in selected Alberta soils during the thaw. Our hypotheses were: thawing of frozen soil released more N_2O and more denitrification-evolved N gases than did non-frozen soil, and that irradiation of soil stopped the generation of emitted N_2O or all denitrification N gases. We tested those hypotheses by incubation of pre-frozen and non-frozen soils at low temperatures and incubation of γ-irradiated soil, with the measurement of N_2O flux and ^{15}N labeled N mass balance.

3.2 MATERIALS AND METHODS

Soil samples were taken from the cultivated horizon of three north-central Alberta soils with varying pH, C and texture (Table 3.1). Soils were collected during the fall of 1991, sieved through a 1 cm mesh, dried at room temperature and stored until commencement of the experiment.

Table 3.1 Soil Properties[†]

Soil Series	Classification (U.S.)	Texture	pH	Total C	Total N	Mineral N	
						NO_3^--N	NH_4^+-N
				- - -dag kg^{-1}- - -		- - -mg kg^{-1}- - -	
Malmo	Typic Cryoboroll	SiCL	5.8	6.03	0.57	23.8	4.9
Breton	Typic Cryoboralf	CL	6.3	1.23	0.13	7.6	3.0
Josephine	Typic Cryaquent	CL	4.4	3.65	0.30	1.5	12.6

[†] Values prior to initiation of experiments.

Studies on zero-time ^{15}N recovery from soil were conducted to assess denitrification losses by difference. For the three soils, the average zero-time recovery was 96.6 %, with a range from 95.5 to 97.8 % (Appendix 21). The upper limit of the 95% confidence interval around mean recovery values reached 100 % for 2 soils (Malmo and Josephine) and was within 0.9 % of 100 in the other one (Breton). Therefore, for simplicity, the labeled ^{15}N recoveries in the experiment were taken as 100 percent.

We measured soil N loss of ^{15}N labeled KNO$_3$ and the flux of N$_2$O emissions from frozen and non-frozen soils during an 88 h incubation at 5°C. The experiment consisted of three soils, six treatments (non-frozen and frozen soil, γ-irradiated and non-irradiated frozen soil, each with and without N addition) and four replications and the design was randomized complete block. The γ-irradiated treatments received 34 kGy of gamma irradiation with Co^{60} (Appendix 10) at the Cross Cancer Institute in Edmonton before starting the incubation. The sterility of irradiated soil was confirmed by plating the soil on soil extract agar (for bacteria) and rose bengal agar (for fungi) as well as placing soil in nutrient broth. Aseptic technique was used in the handling of γ-irradiated soil treatments until the start of incubation. Air-dry soil was placed in glass jars (20 cm tall; 9 cm in diam.) to a height of 10 cm and was saturated with cold sterile distilled water (5°C). Fertilized treatments received 80 kg N ha^{-1} as ^{15}N-labeled KNO$_3$ (76.7 atom % abundance) added to the surface of the soil prior to water saturation. Plexiglass lids fitted with rubber septums and removable #12 rubber bungs were attached to the jars with silicone caulking. Jars to be frozen were then placed in styro-foam containers and maintained at -15°C (±3°C) for 14 to 21 days. Non-frozen soils were saturated at the start of the incubation with cold water (5°C). Overhead lights (35 watts) with foil lined shades were placed above each jar to speed downward thaw of the soil and thus simulate spring thaw conditions. The air temperature 10 cm above the soil surface was maintained at 15°C. Depth of soil thaw and surface temperatures were monitored throughout the incubation (Figure 3.1.a.).

Gas samples were collected from the head space at the start of each eight hour period. Samples were taken 1.5 or 2 h after the jars were sealed, but otherwise the jars were left open. The gas samples were collected by inserting the needle of a 30 cc syringe, pumping the plunger several times, and then slowly removing 24 mL of air from the head space. The air samples were stored in evacuated 25 mL Vacutainer© test tubes containing 1 g silica gel desiccant. The samples were analyzed for N_2O using a Perkin Elmer, Sigma 3 gas chromatograph with ^{63}Ni electron capture detector and a Porapak Q column. Gas concentration values (Appendix 11 and 12) were corrected for decreasing concentration gradient in the head space during trapping by multiplication by a factor of 1.47 as determined in a subsequent experiment (Appendix 13) using the method described by Hutchinson and Mosier (1981) (Appendix 14). Nitrous oxide data were transformed to base ten logarithms for statistical analyses (Appendix 18). The concentration of CO_2 (Appendix 15) in the air samples was analyzed with He as a carrier gas (flow rate of 75 mL min^{-1}) using a Hewlett Packard, 5890 Series II gas chromatograph fitted with a thermal conductivity detector (TCD) at 140°C. After incubation, the soil was emptied into trays and quickly dried at 22°C. The soil was analyzed for ^{15}N using a Carlo Erba N Auto analyzer coupled to a VG Isogas mass spectrometer for determination of soil N mass balance of the added ^{15}N-labeled N (Appendix 16). Non-recovered ^{15}N was assumed to represent the combination of denitrification and chemodenitrification. Mineral NO_3^- and NH_4^+ were analyzed by MgO steam distillation (Bremner, 1965) (Appendix 17).

3.3 RESULTS AND DISCUSSION

Losses of added-N, as determined by ^{15}N mass balance, ranged from 13 to 23 kg ha^{-1} after 88 h of incubation in both frozen and non-frozen soils (Table 3.2). There was no difference in ^{15}N loss among soils or frozen and non-frozen treatments. Also, ^{15}N recovery from γ-irradiated treatments was not different from non-irradiated treatments.

Low emissions of N_2O were found from all soils and treatments and comprised only 0.3 to 2.6 % of total denitrification (Table 3.2). The Malmo sample had the greatest N_2O flux followed by Breton and then Josephine. In both the non-frozen and frozen soils, N_2O emissions did not differ between the N added and no-N treatments. The added NO_3^- might have increased microbial activity resulting in a greater demand of N-oxides as electron acceptors. This would result in greater reduction of N_2O to N_2. Christensen and Christensen (1991) and Christensen and Tiedje (1990) found that freezing of soil markedly enhanced N_2O emission during thaw, but our soils did not behave in this manner. Nitrous oxide flux remained relatively constant in all treatments throughout the incubation and increased slightly after thaw was complete (Figures 3.1.b., 3.2.a., 3.3.a.). The surface temperature of non-frozen soils (Figure 3.1.a.) was about 2°C warmer than frozen soils and that may have contributed to increased N_2O in the Malmo and Breton series.

The low ratio of N_2O to N_2 in our work does not agree with other reports involving pH and NO_3^- concentration. "Brief and vigorous" N_2O emissions were found by Christensen and Tiedje (1990) from an extremely acid soil (pH 3.8), and they assumed that only N_2O was emitted. The Josephine soil (pH 4.4), however, had a low ratio of N_2O to N_2. Increasing NO_3^- concentrations usually cause an increase in the proportion of N_2O as a product of denitrification (Firestone et al., 1980; Firestone et al., 1979; Letey et al., 1980; and Vermes and Myrold, 1992), but in our work N_2O flux did not increase with NO_3^--amendment of soils. Nitric oxide was not accounted for, but there is a possibility NO was dominant to N_2O (Galbally and Roy, 1978).

Table 3.2. Soil and gaseous nitrogen loss during 88 hours.

Soil Series	F[†]	N[‡]	I[§]	^{15}N Recovery	Non-Recovered Added N	Cumulative Emitted N2O-N	$\frac{N_2O}{N_2O+N_2}$[¥]
				%	kg ha^{-1}	kg ha^{-1}	%
Malmo	+	+	-	82.5	14.0	0.18	1.3
Malmo	+	-	-	NA[£]	NA	0.21	NA
Malmo	-	+	-	80.3	15.8	0.41	2.6
Malmo	-	-	-	NA	NA	0.45	NA
Malmo	+	+	+	80.1	15.9	0.10	0.6
Malmo	+	-	+	NA	NA	0.17	NA
Breton	+	+	-	77.7	17.8	0.12	0.7
Breton	+	-	-	NA	NA	0.11	NA
Breton	-	+	-	74.0	20.8	0.16	0.8
Breton	-	-	-	NA	NA	0.22	NA
Breton	+	+	+	79.6	16.3	0.03	0.1
Breton	+	-	+	NA	NA	0.03	NA
Josephine	+	+	-	71.6	22.7	0.06	0.3
Josephine	+	-	-	NA	NA	0.03	NA
Josephine	-	+	-	83.5	13.2	0.05	0.4
Josephine	-	-	-	NA	NA	0.10	NA
Josephine	+	+	+	73.4	21.3	0.04	0.2
Josephine	+	-	+	NA	NA	0.04	NA

[†] Frozen (+) versus Not Frozen (-).
[‡] ^{15}N-KNO_3 added (+) or not added (-)
[§] γ-Irradiated (+) versus Not Irradiated (-).
[¥] Total N loss (N_2O+N_2) was assumed to be equal to 'Total Added N Emitted'. The ratio was multiplied by 100 to give percent.
[£] Not Applicable.

The loss of added-N from γ-irradiated treatments suggests a large proportion of N loss was non-biological (chemodenitrification of NO_2^-). During irradiation, Van

Cleemput and Baert (1972) determined NO_3^- could be chemically reduced to NO_2^-. This would provide the NO_2^- necessary for chemodenitrification to proceed. As well, Goodroad and Keeney (1984b) treated slowly warming frozen soil cores with chloroform ($CHCl_3$), and found nearly all the released N_2O appeared as a result of decreased N_2O solubility as temperature increased from 1 to 10°C. In their work, greater N_2O was released from non-treated than $CHCl_3$-treated cores and was attributed to biological activity. Also, N_2O could have been released from lysed microbial cells (Zablotowicz and Focht, 1979). The emission of CO_2 from irradiated treatments suggests that soils did not remain sterile for the duration of the incubation. For this reason, caution was taken in interpretation of the data from γ-irradiated treatments. However, Van Cleemput and Baert (1972) measured CO_2 emissions from irradiated soil and attributed its production to resistance of respiratory enzymes. According to Skou (1962) a completely sterilized soil still respires. Likewise, nitrogen loss in γ-irradiated soils could be caused by the activity of radiation-resistant denitrifying enzymes (Lensi et al., 1991; Lensi and Chalamet, 1982; Cawse and White, 1969; Van Cleemput and Patrick, 1974) (Appendix 10). Ammonium did not increase in irradiated treatments, as it had in non-irradiated soils, suggesting microorganisms involved in dissimilatory reduction of NO_3^- to NH_4^+ had been killed by γ-irradiation.

Losses of added N in our experiment were similar to those found in field investigations in Alberta (Heaney et al., 1992; Nyborg et al., 1990; Chapter 2). However, N_2O fluxes were approximately two times greater during spring thaw in the field (Chapter 2) than fluxes found in this laboratory experiment. The difference in the apparent proportions of N_2O in emitted gases between the field and the laboratory could be explained by warmer surface temperatures in the field where the solubility of

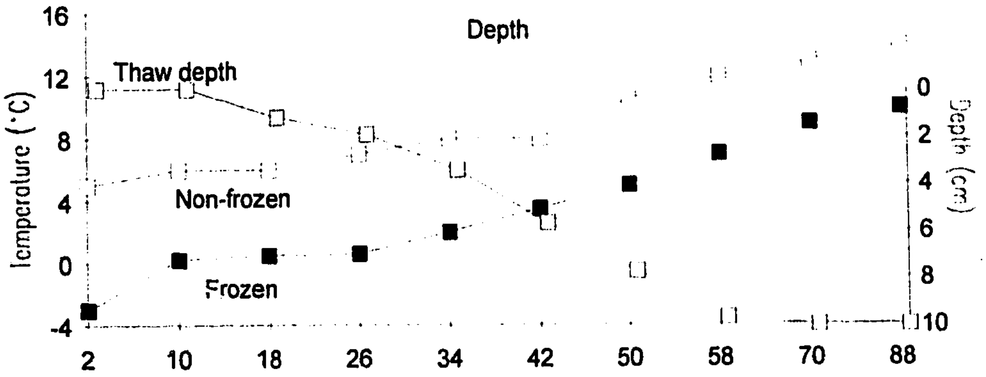
N_2O would decrease . Also, the increase in NH_4^+ during incubation suggested dissimilatory reduction of NO_3^- had occurred. Dissimilatory reduction of NO_3^- to NH_4^+ occurs only under highly reduced conditions (Paul and Clark, 1989) which would also result in the further reduction of N-oxides.

3.4 CONCLUSIONS

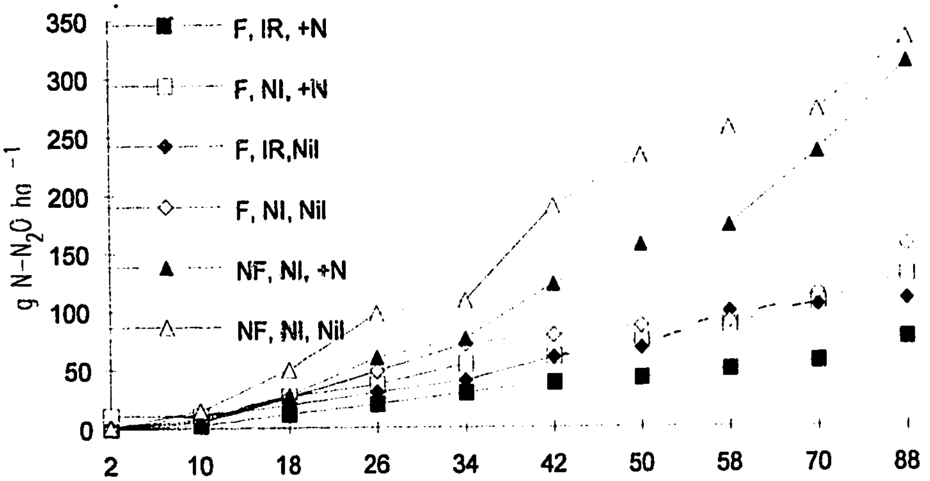
Our hypothesis that thawing, frozen soil would release more N_2O and more denitrification-evolved N gases than non-frozen soil was found to be false. The second hypothesis that irradiation of soil would stop the generation of emitted N_2O or all denitrification N gases also appeared to be false.

Substantial denitrification losses occurred from the ^{15}N-labeled KNO_3 additions to thawing and non-frozen soils. Denitrification losses after 88 h ranged from 13 to 23 kg N ha^{-1} among three very different soils. However, N_2O emissions were a small proportion of the denitrification losses (<3%) of any of the soils. Whether the soil was pre-frozen, or not, had little influence on denitrification or N_2O emission.

a. Temperature and Thaw.



b. Nitrous oxide.



c. Carbon dioxide.

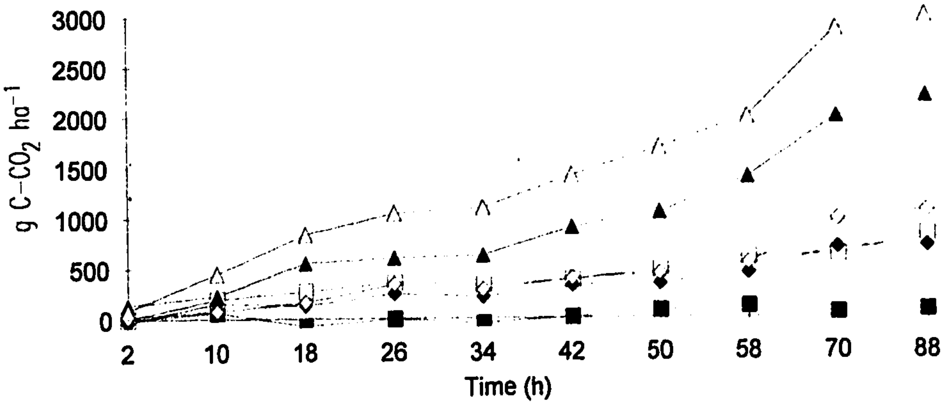
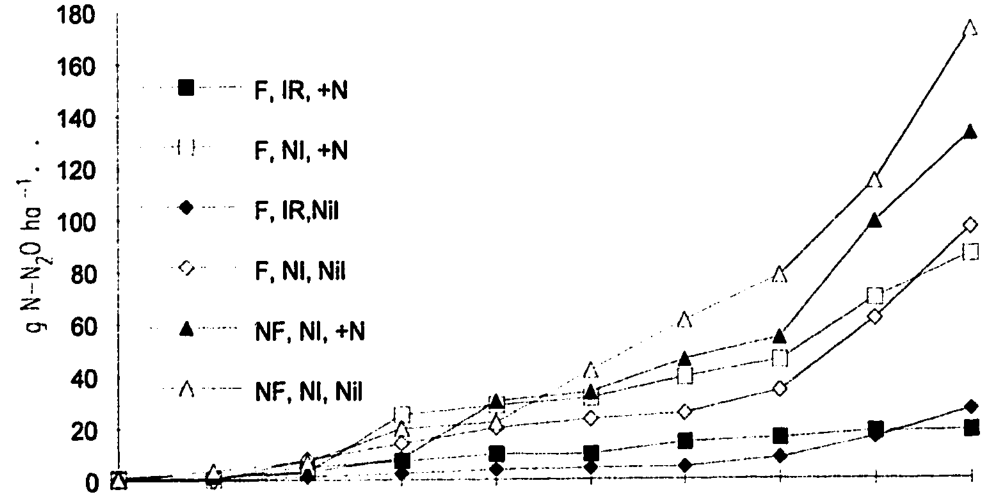


Figure 3.1 (a.) Soil temperature and thaw depth, (b.) cumulative nitrous oxide flux, and (c.) cumulative carbon dioxide emissions for the Malmo series. F=frozen; NF=not frozen; IR=irradiated; NI=not irradiated; +N=80 kg N; Nil=no N added.

a. Nitrous oxide



b. Carbon dioxide

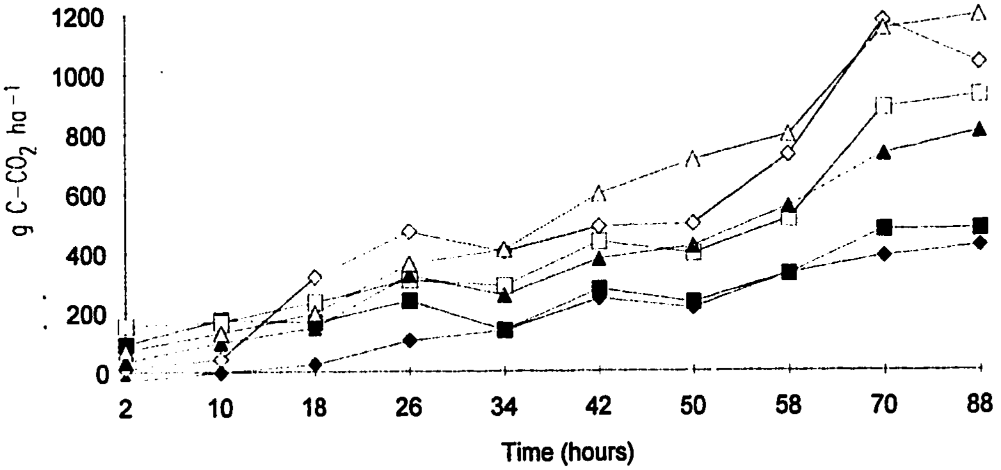
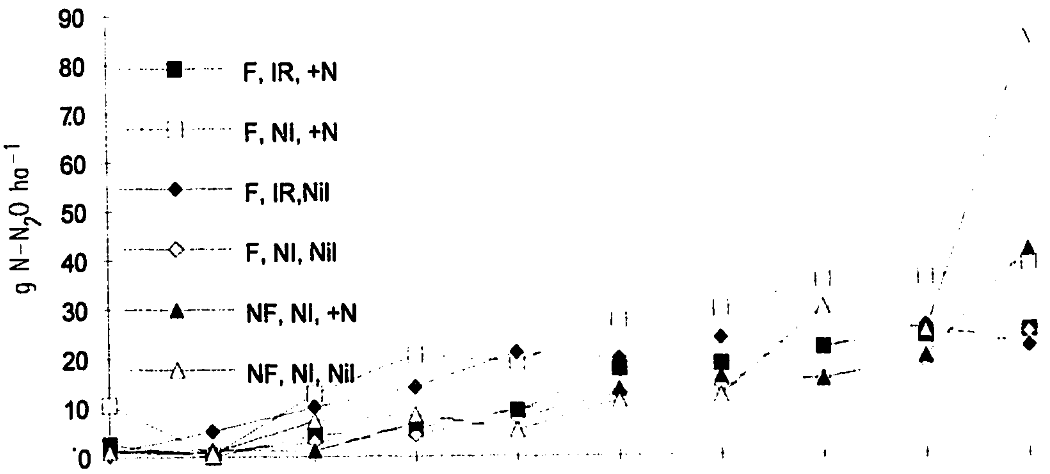


Figure 3.2 (a.) Cumulative nitrous oxide flux, and (b.) cumulative carbon dioxide emissions for the Breton series. F=frozen; NF=not frozen; IR=irradiated; NI=not irradiated; +N=80 kg N; Nil=no N added. Temperature and thaw is found in Figure 3.1.a.

a. Nitrous oxide



b. Carbon dioxide

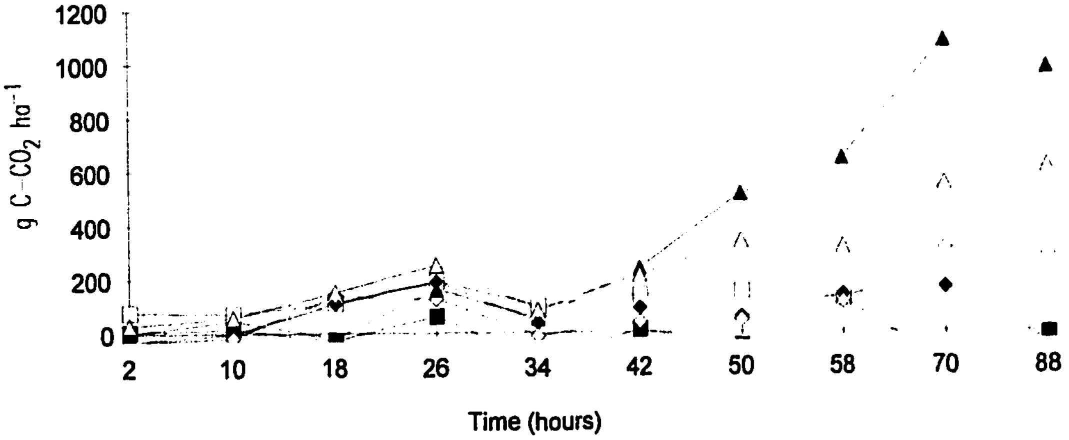


Figure 3.3 (b.) Cumulative nitrous oxide flux, and (b.) cumulative carbon dioxide emissions for the Josephine series. F=frozen; NF=not frozen; IR=irradiated; NI=not irradiated; +N=80 kg N; Nil=no N added. Temperature and thaw is found in Figure 3.1.a.

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CHAPTER 4

DENITRIFICATION DURING THE THAW OF MANURE AMENDED SOILS[1]

4.1 INTRODUCTION

Environmental concerns and cost of inorganic fertilizers have created a renewed interest in the use of manure as a fertilizer source. Manure additions to soil, however, lead to large fluxes of nitrogen oxides (Rolston et al., 1978; Magg, 1989) from agricultural soils. In north central Alberta, the greatest denitrification activity in soil occurs during the spring thaw (Heaney et al., 1992; Malhi et al., 1990; Chapter 2) resulting in substantial fluxes of N_2O (Nyborg et al., 1990). Our hypothesis was that manure addition would increase denitrification and N_2O emissions from an agricultural soil in Alberta during the thaw. We monitored N_2O and CO_2 emissions during thaw of manured and non-manured soils and determined ^{15}N mass balance at the end of incubation. This experiment was similar to the one in Chapter 3. However, soils in this experiment were frozen at -5°C rather than -15°C. Also, the soil samples in this experiment were taken from the field in the fall, and kept cool and moist before the start of incubation. The changes in preparation of incubation soils, we hoped, would result in results more representative of denitrification in the field.

4.2 METHODS AND MATERIALS

Samples of the Ap horizon of an Orthic Black Chernozem (Angus Ridge Series) were collected south of Josephburg, Alberta in October, 1992 (Table 4.1). Soil samples were taken from a manure-treated plot (75 Mg ha^{-1} (oven dry)) and a fertilized plot (100 kg N ha^{-1} and 29 kg P ha^{-1}, spring applied). The manure and no

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[1] Part of this chapter has been published in the Proceedings of the 1993 Alberta Soil Science Workshop.

manure soil samples were placed in separate pails, capped, and transported to the laboratory for overnight storage at 3°C. The following morning, the samples were passed through a 1 cm mesh and sub sampled for determination of moisture content. The fresh, cold soil was placed in glass jars (20 cm tall; 9 cm in diam.) to a height of 10 cm. An additional 16 Mg ha^{-1} (oven dry) decomposed manure was mixed into the manure treated soil. The soil was brought to saturation with distilled water and ^{15}N labeled KNO_3 (5.42 atom % abundance) was added at a rate of 100 kg N ha^{-1} to +N treated soils. Plexiglas® lids fitted with septums and removable #12 rubber bungs were attached to the jars using silicone caulking. Jars to be frozen were placed in styrofoam containers and maintained at -5°C for 13 days. During incubation, frozen samples were kept at 5°C. Overhead lights (35 watts) with foil-lined shades were placed above each jar to encourage downward thaw and thus simulate spring thaw conditions. The air temperature 10 cm above the soil surface was maintained at 15°C. The depth of soil thaw and the soil surface (0.5 cm) temperature were monitored throughout the incubation period.

Table 4.1 Characteristics of the Angus Ridge series.[†]

Treatment	Moisture	Texture	pH	Bulk Density	Total C	Total N	NO_3-N	NH_4-N
	kg kg^{-1}			Mg m^{-3}	- - -dag kg^{-1}- - -		- - -mg kg^{-1}- - -	
Manure[‡]	0.28	SiL	7.8	0.70	6.6	0.67	0.6	3.8
Control	0.17	SiL	6.5	0.95	3.6	0.33	0.0	2.1

[†] Represents condition of soil in jars prior to saturation, addition of KNO_3 and freezing.
[‡] Includes manure supplement added prior to freezing.

Gas samples from the head space were collected for each eight hour period. Samples were taken 1.5 or 2 h after the jars were sealed, but otherwise left open. The gas samples were collected by inserting a needle of a 30 cc syringe and pumping the plunger several times, and then slowly removing 24 mL of air from the head space. The gas samples were stored in evacuated 25 mL Vacutainer© test tubes containing 1 mL silica gel desiccant. The samples were analyzed for N_2O using a Perkin Elmer, Sigma 3 gas chromatograph with ^{63}Ni electron capture detector with a Poropak Q column. Gas concentration values were corrected for decreasing concentration gradient in the head space during trapping by multiplying by a factor of 1.47 as determined in a subsequent experiment (Appendix 13) using the method described by Hutchinson and Mosier (1981). Statistical analyses included analysis of variance (anova) and least significant difference (LSD) (Appendix 21). CO_2 was analyzed using a Hewlett Packard, 5890 Series II gas chromatograph fitted with a thermal conductivity detector (TCD).

After 66 hours of incubation, the soil from each jar was thinly spread in aluminum trays and quickly dried at 22°C. The samples were analyzed for NH_4^+ and NO_3^- by MgO steam distillation (Bremner, 1965) and for ^{15}N using a NA 1500 Carlo Erba N Auto Analyzer coupled to a VG Isogas mass spectrometer for determination of soil N mass balance (Hauck, 1982)(Appendix 20).

4.3 RESULTS AND DISCUSSION

Soil ^{15}N mass balance in manure and non-manure treatments indicated that 39 and 20 kg ha^{-1} of added N, respectively (Table 4.2). During the 66-hour incubation, the majority of the soil volume remained frozen and the soil surface temperature remained below 5°C excepting the last 16 h (Figure 4.1.a. and 4.1.b.). The rate of denitrification of added NO_3^--N in the manured soil was 12.7 kg ha^{-1} d^{-1} while the non-manured soil averaged 5.8 kg ha^{-1} d^{-1}. Those emissions were approximately 100 times greater than those reported by Cates and Keeney (1987) during thaw of manured soil in the field (Table 2.4). They were also consistent with the large losses of NO_3^--N recorded for north-central Alberta soils, when those soils were cold and saturated in the laboratory (Malhi et al., 1990). Our values of denitrification loss from thawing soils were 10 to 100 times greater than the N losses reported by others during soil thaw (Christensen and Tiedje, 1990; Dorland and Beauchamp, 1991).

The estimated flux of N_2O-N from N-amended soil was 0.40 kg ha^{-1} day^{-1} from the manured soils and 0.15 kg ha^{-1} day^{-1} from non-manured soils. Goodroad and Keeney (1984) reported N_2O-N fluxes of 0.05 to 0.15 kg ha^{-1} d^{-1} in manure-amended plots during soil thaw in early to mid-April. The larger flux of N_2O from manured treatments over non-manure treatments was attributed to the greater supply of organic C and concomitant microbial activity. The N_2O flux was relatively constant between 18 and 58 h and decreased after 58 h in manure and non-manure treated soils (Figure 4.1.c.). Our time of greatest N_2O production after initiation of incubation was similar to the peaking after 1 to 3 days as found by Letey et al.(1980) and Rolston et al.(1978) in non-frozen soils.

Table 4.2 Soil Nitrogen at Termination of Thaw.

Treatment	NO_3^--N	NH_4^+-N	Total N	^{15}N Recovery
	- - -mg kg^{-1}- - -		dag kg^{-1}	%
Manure + ^{15}N[§]	79.7 ±3.2 A[†]	8.2 ±0.8 A	0.57 ±0.04 A	60.9 ± 6.1 A
Manure	0.0 ±0.0 B	7.1 ±0.6 B	0.58 ±0.01 A	NA[‡]
No Man.+ ^{15}N	75.9 ±6.5 A	6.5 ±0.2 B	0.32 ±0.01 B	80.0 ±11.3 B
Control	0.0 ±0.0 B	3.8 ±1.1 C	0.30 ±0.01 B	NA

[†] Values following ± represent the standard deviation. Treatments with different letters are significantly different ($p<0.05$) using Duncan's multiple range test for variability.

[‡] Not Applicable

[§] ^{15}N-labeled NO_3^--N was added at a rate of 114.3 mg kg^{-1}soil.

The N_2O emissions represented less than 3 % of the added-N denitrified from the manured soil and 2 % from non-manured soil. In our work, the percent of N_2O-N to total N lost from soil during denitrification under cold temperatures was considerably lower than those reported by Keeney et al. (1979), Lensi and Chalamet (1982), Denmead et al. (1979), Christensen and Christensen (1991) and Christensen and Tiedje (1990). Explanations for the lower N_2O to N_2 ratio in our work included (1) the presence of denitrifiers able to efficiently utilize N_2O and, (2) more highly reduced conditions. Keeney et al. (1979) and Cates and Keeney (1987) concluded the N_2O to N_2 ratio increases with lowered temperature by suppression of N_2O reductase activity. However, N_2 evolution did not appear inhibited in our soil by cold temperatures. In contrast, Letey et al. (1980) suggested the extended time period for gas entrapment in cold water allows time for the production and activity of N_2O reductases. We hypothesize that N_2O-reducing denitrifiers able to function efficiently under cold temperatures are present in our soils. An increase in NH_4^+ (Appendix 19) during incubation was likely a result of dissimilatory reduction of nitrate which occurs under highly reduced conditions. Reduced conditions would create a greater demand for N-oxides as electron acceptors resulting in lower N_2O emissions.

Nitrous oxide emissions of 0.25 and 0.97 kg N_2O-N ha^{-1} occurred from the control and non-N, manure treatments, respectively, during the incubation. The concentration of NO_3^--N was only 0 and 0.6 mg kg^{-1} in the control and manure treatments at the start of incubation. Therefore, immobilized N must have been mineralized to NO_3^- during the incubation to supply NO_3^- for denitrification on the control.

The flux of CO_2 from the manure and non-manure treatments was much the same with 1.1 kg CO_2-C ha^{-1} emitted during the incubation period (Figure 4.1d).

Because CO_2 emissions were not greater in the manured soils, we suspect methane (CH_4) was a significant product during soil thaw. Also, the activity of autotrophs may have reduce CO_2 emissions. Future work should include an assay for CH_4 to accompany N_2O and CO_2 measurements to provide more comprehensive information on the production of greenhouse gases during the soil thaw.

Table 4.3 Nitrous oxide emissions for manure and non-manure amended soils during thaw.

Treatment	Cumulative Emitted N_2O-N[†]	Non-recovered Added N[‡]	$\frac{N_2O}{N_2O+N_2}$[§]
	kg ha^{-1}	kg N ha^{-1}	%
Manure + ^{15}N	1.10 (24) A[¥]	39.1	2.8
Manure	0.97 (13) A		
No Man. + ^{15}N	0.42 (9) B	20.0	2.1
Control	0.25 (16) B		

[†] Includes ^{15}N-labeled and non-labeled N_2O-N from the fertilizer and the soil.

[‡] Added ^{15}N not recovered after 66 h.

[§] Values calculated by dividing N_2O emitted by +N treatments by the 'Emitted Added N'. Values were multiplied by 100 to get percent.

[¥] Values in brackets represent coefficients of variance in percent. Treatments with different letters are significantly different ($p<0.05$) using Duncan's multiple range test for variability..

4.4 CONCLUSIONS

Substantial loss of added-N occurred during the soil thaw when temperature at 1 cm below the soil surface was less than 5°C and much of the soil volume was frozen. The loss of added N from the manure-amended treatment was twice that of the non-manure treatment. Nitrous oxide represented 2.1% and 2.8% of the added ^{15}N denitrified in the non-manure and manure treatments, respectively. Since a large quantity of N was denitrified, N_2O emissions, although a small percent of the N-gases emitted, may contribute significantly to the greenhouse effect and ozone depletion. The large N loss is also of economic importance to farmers. The addition of manure doubled the loss of added N during soil thaw. Therefore, it is suggested that manure additions be carried out in the spring after the soil thaw.

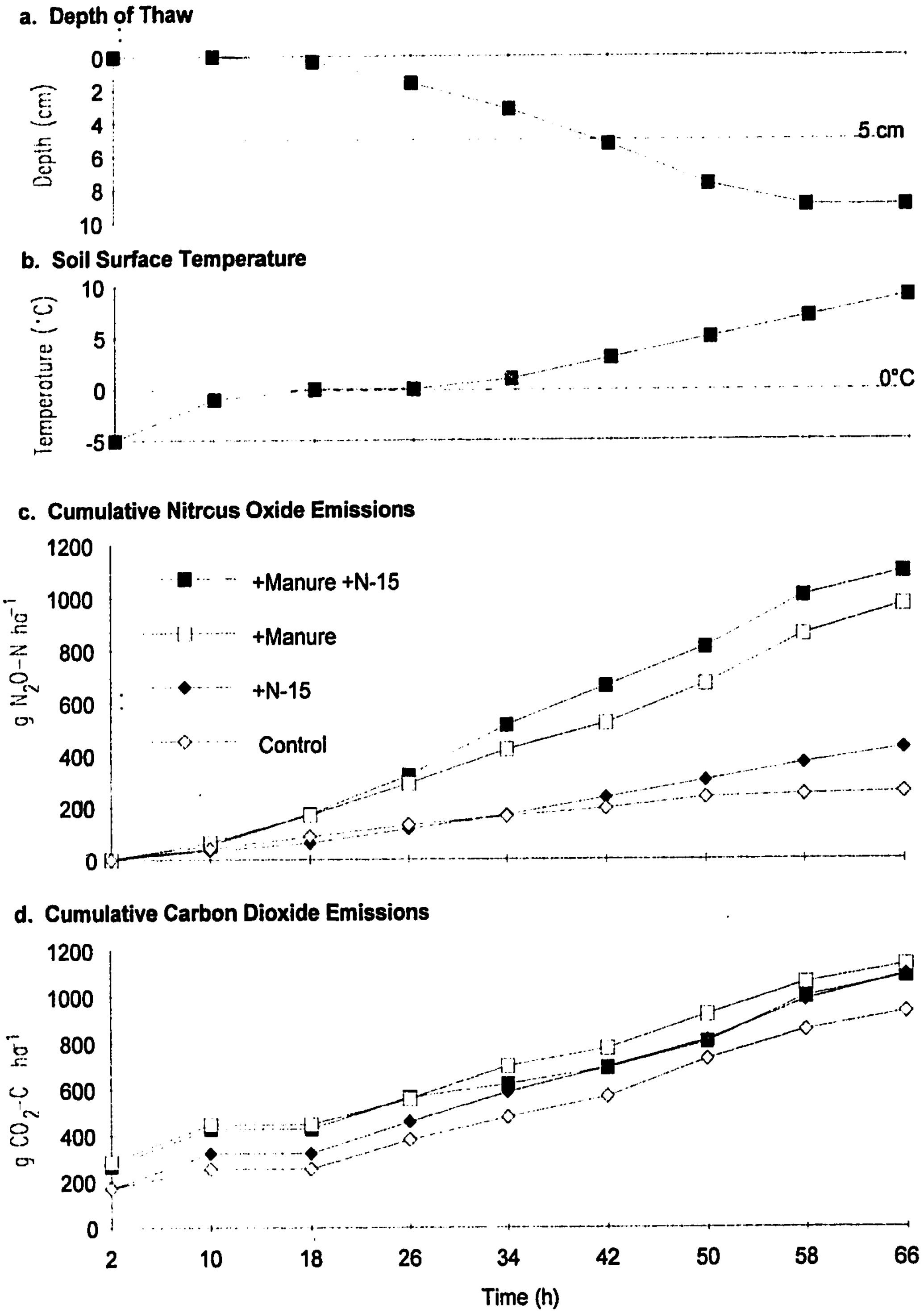

Figure 4.1 : **(a) Cumulative nitrous oxide flux, (b) cumulative carbon dioxide emissions, (c) depth of soil thaw, and (d) surface temperature changes during 66 hours of soil thaw at 5°C.**

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CHAPTER 5
SYNTHESIS

Large losses of nitrogen by denitrification and N_2O emissions took place as soil thawed in the field in northern Alberta. This is a unique finding. As much as 40% of added-N was denitrified during soil thawing in both the field and laboratory. The nitrous oxide fluxes and the proportion of N_2O of N denitrified was lower in the laboratory than in the field. The contrasting proportions of N_2O might be explained by increased solubility of N_2O in sun-warmed water in surface soils in the field and by highly reduced conditions in laboratory soils.

Nitrous oxide emissions during spring thaw from summer fallowed soil (Chapter 2) were greater than any of the few reports (except one) found in the literature of N_2O emitted during spring thaw (Table 2.3). The second spring, following summer fallow, N_2O emissions were 4.5 times greater than the previous year on stubble. In Chapter 4, the addition of manure to soil resulted in a doubling in the loss of added N and 2.5 to 4 times larger emissions of N_2O than non manured soil. This research shows summer fallow and addition of manure can dramatically affect the emission of N_2O from thawing soil.

Losses of added N in field and laboratory experiments were somewhat the same (15 to 40% denitrified), but the N_2O emissions represented less than 3% of added N denitrified in the laboratory and 9 to 30 % of added N lost in the field (Chapters 2, 3 and 4). An explanation for this apparent disparity between field and laboratory emissions can be found in redox theory. Redox potentials were not measured, however, there was evidence that soils in the laboratory were under highly reduced conditions. The increases of NH_4^+ content of soil indicated NO_3^- was undergoing dissimilatory reduction to NH_4^+. This process is carried out by strict anaerobes (eg. *Clostridium* and *Wolinella*) and occurs only under highly reduced conditions (Paul and Clark, 1989). Highly reduced conditions would result in a greater demand for N-oxides as electron acceptors by denitrifying anaerobes. This would result in a tendency for N_2O to be reduced to N_2. Conversely, under less reduced conditions where NO_3^- and NO_2^- (and other oxides) satisfy microbial metabolic demands, N_2O would be in less demand as an electron acceptor. This would lead to emissions of N_2O from soil. In any case, N_2O emission in the laboratory was a poor indicator of denitrification.

In many instances, the results of the laboratory experiments contradict much of the current literature. The low pH soil (Chapter 3) did not exhibit increased proportion of N_2O to N_2 in emitted gases. Also, increased concentration of NO_3^- did not affect the proportion of N_2O to N_2 in emitted gases (Chapter 3) although the effect was evident in the field (Chapter 2). Denitrification from soil during thaw and cold conditions was greater than values reported elsewhere (Christensen and Christensen, 1991; Christensen and Tiedje, 1990). Emission of N_2O was not enhanced during thaw over non-frozen soils as determined by Christensen and Tiedje (1990). The apparent disparity between some of our results in the laboratory and the literature could be explained by redox potential.

The loss of added N and the N_2O emissions from γ-irradiated soils (Chapter 3) were perplexing. Although irradiated soils may have been recolonized, our results still demand some explanation. We had thought the irradiated treatments would show us the extent of chemodenitrification compared to denitrification during thaw. There were, however, difficulties inherent in the use of γ-irradiation. There is evidence that denitrifying enzymes persist in soil after γ-irradiation (Van Cleemput and Baert, 1972; Van Cleemput and Patrick, 1974; Lensi et al., 1991). However, denitrifying enzymes are associated with cytochromes in the electron transport system and would require the oxidation of NADP in order to reduce nitrate. This does not support the hypothesis that denitrifying enzymes can function without the living cell. This leads to more questions about whether denitrifying enzymes can function without living cells and act in ways yet undiscovered. Another explanation for N_2O emissions from the no N treatment is γ-irradiation caused the reduction of NO_3^- to NO_2^-. Irradiation was shown by Van Cleemput and Patrick (1974) to cause NO_2^- accumulation in soil. The NO_2^- could then be reduced through chemodenitrification. Future work with γ-irradiation should involve improved aseptic technique and determination of NO_2^- before and after irradiation.

We estimated that 8.5 kt N_2O-N y^{-1}, equivalent to 2% of Alberta's anthropogenic emissions, are emitted from cultivated black and gray soils in Alberta (Chapter 2). Fall addition of N, summer fallowing and manure amendments would result in larger emissions (2 to 3 times greater) (Chapters 2 and 4). In Table 1.1, terrestrial (180 million ha) denitrification losses were estimated at 40 to 350 Tg N y^{-1}. This equates to denitrification emissions of 0.2 to 1.9 kg N ha^{-1} ($N_2 + N_2O$) per year. The N_2O production from our non fertilized, soil in stubble was 1.5 kg N ha^{-1} y^{-1} and combined N_2 and N_2O emissions was approximately of 15 kg N ha^{-1} y^{-1} (assuming $N_2O \approx 10$ % of emitted gas). This means, per hectare, nitrogen emissions from north central Alberta soils would be about 10 times greater than the estimated global average. The extent and magnitude of denitrification and N_2O emissions have not been determined in northern soils, particularly during thaw. This unique research shows that substantial losses of nitrogen and fluxes of N_2O do occur during the soil thaw.

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CHAPTER 6

APPENDICES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Miscellaneous

APPENDIX 1
DESTRUCTION AND PRODUCTION OF OZONE BY NITROGEN OXIDES

The reactive forms of nitrogen in the production or destruction of ozone (O_3) are nitric oxide (NO) and nitrogen dioxide (NO_2). The NO and NO_2 are in photochemical equilibrium and are called NO_x. NO_x is extremely reactive and has only a short lifetime within the troposphere so it is unable to reach the stratosphere. N_2O, unlike NO_x, has a long lifetime (150 years) and, after transportation through the troposphere to the stratosphere, it is photochemically decomposed to NO_x (Crutzen and Ehhalt, 1977; Conrad, 1990).

Reactions in the Stratosphere (Crutzen and Ehhalt, 1977; Conrad, 1990):

1. Formation of ozone:

O_2 + solar radiation $\rightarrow 2\ O$ ($\lambda < 240$ nm)
$O + O_2 + M$ $\rightarrow O_3 + M(2x)$ (M= stabilizing third body (N_2 or O_2))
Net: $3\ O_2$ $\rightarrow 2\ O_3$

2. Formation of NO_x from N_2O:

O_3 + solar uv $\rightarrow O(^1D) + O_2$ ($\lambda < 310$ nm)
$O(^1D) + M$ $\rightarrow O\ M$ (normal fate of $O(^1D)$, deactivation to less reactive, ground state O)
$O(^1D) + N_2O$ $\rightarrow 2\ NO$ (main production of stratospheric NO)

3. Catalytic destruction of ozone:

$NO + O_3$ $\rightarrow O_2 + NO_2$
O3 + hv $\rightarrow O_2 + O$ ($\lambda < 1140$ nm)
$NO_2 + O$ $\rightarrow O_2 + NO$
Net: $2\ O_3$ + hv $\rightarrow 3\ O_2$

Reactions in the Troposphere:

Formation and destruction of ozone:

$NO + O_3$ $\rightarrow NO_2 + O_2$
NO_2 + hv $\rightarrow NO + O$ ($\lambda < 420$ nm)
$O_2 + O + M$ $\rightarrow M + O_3$ (M= N_2 or O_2)

NO reacts within minutes with O_3 to form NO_2 which photolyzes rapidly, eventually reforming O_3. The net destruction or formation of O_3 in the troposphere depends on many other reactions. Net production or destruction of tropospheric O_3 during the decomposition of CO, CH_4, and nonmethane hydrocarbons is dependent on the concentration of NO. For example, in the destruction of CO (to CO_2), net loss of O_3 occurs if [NO] is less than 5 parts per trillion, volume per volume (pptv), while a net increase in O_3 occurs if [NO] is greater than 5 pptv (Conrad, 1990; Crutzen 1979). Presently, tropospheric O_3 concentrations seem to be increasing (Logan, 1985; Penkett, 1988) The increase of O_3 in the troposphere is in contrast to its decrease in the stratosphere (Crutzen, 1979; Cicerone, 1987).

NO_x is finally transformed into HNO_3,

$$NO_2 + OH + M \quad \rightarrow M + HNO_3$$

which is deposited to the ground by wet (acid rain) or dry deposition (Logan, 1983).

In summary, N_2O emission affects stratospheric O_3 and NO_x emission affects tropospheric O_3.

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APPENDIX 2
FIELD EXPERIMENT N_2O CALCULATIONS

Conversion of N_2O ppm h^{-1} to g N-N_2O ha^{-1} h^{-1}.

Given:

Surface Area = 130 cm^2
Head Space Volume = 1.118 L
N = 28 g mol^{-1}
N_2O = 44 g mol^{-1}
1 mol of gas = 22.4 L
PPM(v/v) h^{-1} = Flux -Ambient
1 hectare = 10^8 cm
PPM = $1/10^6$

g N_2O-N ha^{-1} h^{-1} =

$$\frac{PPM(v/v)N_2O\cdot h^{-1}}{10^6}\times\frac{1.964g\cdot N_2O}{L}\times\frac{28g\cdot N\cdot mol^{-1}}{44g\cdot N_2O\cdot mol^{-1}}\times\frac{1.118L}{130cm}\times\frac{10^8cm^2}{ha}=1.075\times PPM(v/v)hr^{-1}$$

Note: Ambient N_2O concentrations should be subtracted from trapped emitted N_2O concentrations prior to using this conversion.

APPENDIX 3
1989 NITROUS OXIDE DATA

Table 6.1 Nitrous oxide emissions during Spring, 1989.

	N-Added			*Control*		
Day of Year	N_2O-N Flux	Standard Deviation	Coefficient of Variance	N_2O-N Flux	Standard Deviation	Coefficient of Variance
	g ha^{-1}hr^{-1}		%	g ha^{-1}hr^{-1}		%
87	1.12	0.75	67	1.21	0.19	16
88	0.88	0.07	8	0.55	0.06	10
89	-0.22	0.14	62	0.48	0.15	31
91	0.73	0.84	115	1.34	1.04	77
92	2.80	3.49	125	0.72	0.60	83
94	2.42	3.16	130	0.86	0.51	59
96	7.52	8.56	114	1.13	0.61	54
98	7.84	9.00	115	0.50	0.85	170
99	10.46	4.24	41	0.97	1.32	136
100	16.90	4.32	26	3.31	2.19	66
101	9.40	3.30	35	2.62	2.05	78
102	13.47	6.63	49	4.36	4.00	92
103	9.59	3.11	32	4.64	2.91	63
104	13.99	17.25	123	4.06	2.47	61
105	4.66	2.79	60	2.29	2.42	106
106	5.07	5.55	110	2.80	1.22	44
107	1.00	1.05	106	2.12	2.23	105
108	2.22	0.81	36	2.00	0.87	44
112	1.49	0.31	21	2.28	1.79	79
113	0.45	0.71	165	0.14	0.78	578
114	1.47	0.55	37	0.60	0.79	132
117	1.43	0.61	43	0.85	0.40	46
119	2.35	1.05	44	1.28	0.19	15

Note: Day 87 is March 23 and Day 119 is April 29.

Table 6.2 Nitrous oxide emissions during Spring, 1990.

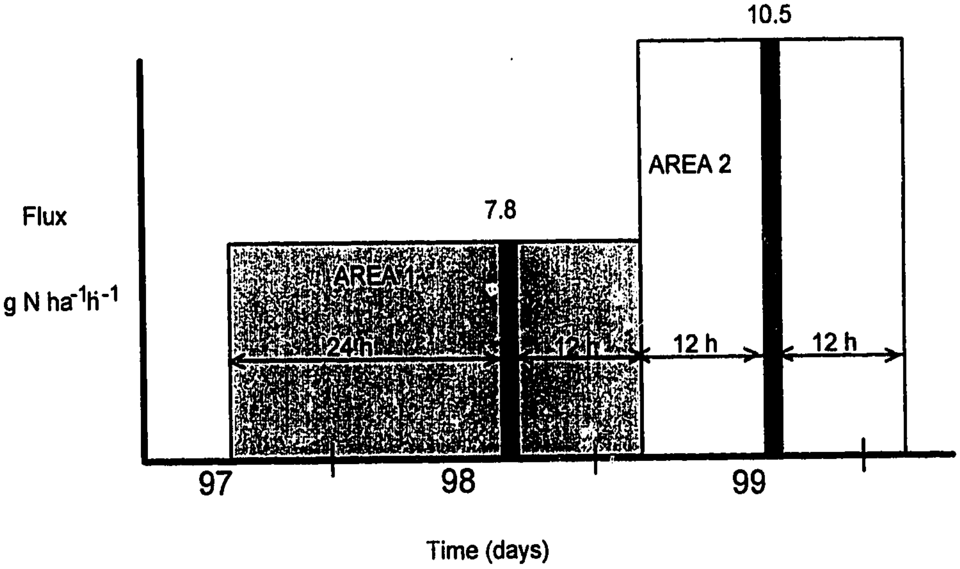
	N-Added			*Control*		
Day of Year	**N_2O-N Flux**	**Standard Deviation**	**Coefficient of Variance**	**N_2O-N Flux**	**Standard Deviation**	**Coefficient of Variance**
	g $ha^{-1}hr^{-1}$		%	g $ha^{-1}hr^{-1}$		%
87	26.63	13.61	51	20.81	10.66	51
90	65.25	51.25	79	28.45	8.58	30
93	87.19	69.18	38	21.17	19.91	94
96	54.89	21.39	59	21.04	23.08	110
99	-0.12	0.07	53	-0.17	0.08	45
101	2.01	1.06	40	1.03	0.43	41
104	1.23	0.49	107	0.58	0.22	37
107	0.40	0.43	365	0.16	0.17	106
110	0.03	0.11	85	0.05	0.16	294
115	1.60	1.36	106	0.67	0.30	45
117	0.18	0.19	39	0.09	0.11	123
124	0.39	0.15		0.25	0.08	33

Note: Day 87 is March 28 and Day 124 is May 4.

APPENDIX 4
CALCULATION OF TOTAL N_2O EMISSIONS

The N_2O fluxes measured each day or every other day were used to estimate emissions for the entire spring. The hourly fluxes we assumed to be constant throughout a given day.

Figure 6.1 Example of estimate for N_2O emissions during two days.



AREA 1 = 36 h x 7.8 g N_2O-N ha^{-1} h^{-1} = 282 g N_2O-N

AREA 2 = 24 h x 10.5 g N_2O-N ha^{-1} h^{-1} = 250 g N_2O-N

Note: No sample was taken on day 97 but there was on day 96, therefore, the fluxes on days 96 and 98 each represented 12 h of day 97.

APPENDIX 5
DAILY N_2O EMISSIONS

Table 6.3 Cumulated nitrous oxide emissions during 33 days in spring, 1989.

		Treatment	
		N-Added	Control
Day	Time Period	N_2O-N Emissions	N_2O-N Emissions
	hours	kg period^{-1}	kg period^{-1}
87	24	0.03	0.03
88	24	0.02	0.01
89	36	0.00	0.2
91	36	0.03	0.05
92	36	0.10	0.03
94	48	0.12	0.04
96	48	0.36	0.05
98	36	0.28	0.02
99	24	0.25	0.02
100	24	0.41	0.08
101	24	0.23	0.06
102	24	0.3[illegible]	0.11
103	24	0.23	0.11
104	24	0.34	0.10
105	24	0.11	0.06
106	24	0.12	0.07
107	24	0.02	0.05
108	60	0.13	0.12
112	72	0.11	0.16
113	24	0.01	0.00
114	48	0.07	0.03
117	60	0.09	0.05
119	60	0.14	0.08
TOTAL		3.50	1.34

Table 6.4 Cumulated nitrous oxide emissions during 38 days in spring, 1990

		Treatment	
		N-Added	**Control**
Day	Time Period	N_2O-N Emissions	N_2O-N Emissions
	hours	kg period^{-1}	kg period^{-1}
87	36	0.96	0.75
90	72	4.70	2.05
93	72	6.28	1.53
96	72	3.95	1.52
99	60	0.00	0.00
101	60	0.12	0.06
104	72	0.09	0.04
107	72	0.03	0.01
110	96	0.00	0.01
115	84	0.14	0.06
117	108	0.02	0.01
124	84	0.03	0.02
TOTAL		16.31	6.03

APPENDIX 6
AMMONIA AND NITRATE ANALYSIS

Ammonia (NH_4^+) analysis using the Autoanalyzer.
The automated procedure for the determination of ammonia in water utilizes the Berthilot Reaction in which the formation of a green colored compound believed to be closely related to indophenol occurs when the solution of an ammonium salt is added to sodium phenoxide. A solution of potassium sodium tartrate (Rochelle Salt) is added to the sample stream to eliminate the precipitation of the hydroxides of heavy metals which may be present.[1,2,3,4,5]

Nitrate and nitrite analysis in soil extracts using the Autoanlyzer.
The automated procedure for the determination of nitrate and nitrite utilizes the procedure whereby nitrate is reduced to nitrite by a copper-cadmium reductor column.[6,7]. The nitrite ion then reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-naphthylethylenediamine dihydrochloride to form a reddish-purple azo dye. Soil extract samples were manually prepared with a solution of 2N potassium chloride.

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APPENDIX 7
MASS BALANCE VALUES FOR 1989 AND 1990

Equation for the calculation of soil ^{15}N mass balance:

$$\%\text{Recovery} = \frac{[\%\text{N soil}] \times [\text{kg soil ha}^{-1}] \times [\%^{15}\text{N abund. soil} - \%\text{natural }^{15}\text{N}]}{[\text{Fertilizer Rate}] \times [\%^{15}\text{N atom excess of fertilizer} - 0.3663\%]}$$

Note: Fertilizer was applied in both years as ^{15}N-labeled KNO_3.

1989 Mass Balance Results

Rate = 56.75 kg N ha^{-1}; Atom abundance = 98 %

Table 6.5 Average mass balance results for spring, 1989.

Depth	Bulk Density	Total N	Abund. Natural	Abund. Soil	Excess	Total N	Recovery
(cm)	(Mg m^{-3})	(%)	(%)	(%)	(%)	(kg N ha^{-1})	(% N)
0 to 15	1.03	0.514	0.36769	0.68289	0.31519	8072.29	46.0
15 to 30	1.10	0.356	0.36760	0.47050	0.10290	5963.79	10.3
30 to 45	1.22	0.115	0.36762	0.42197	0.05434	2128.88	2.0
45 to 60	1.50	0.072	0.36690	0.39072	0.02382	1645.92	0.7
60 to 75	1.47	0.064	0.36670	0.38572	0.01902	1433.78	0.5
						SUM	59.5

The added ^{15}N recovered from 0 to 75 cm depth after being in the soil between November 3, 1988 and May 15, 1989 was 59.5 % (33.8 kg N ha^{-1}) indicating a loss of 40.4 % (23.0 kg N ha^{-1}) of added N during winter and spring.

Example calculation for 1989, 0 to 15 cm sample:

$$\%\text{Recovery} = \frac{[0.514\ \%][1030\ \text{kg m}^{-3} \times .15\ \text{m} \times 10^{4}\ \text{m}^{2}\ \text{ha}^{-1}][0.68289 - 0.36769]}{[56.75\ \text{kg N ha}^{-1}][98\ \% - 0.3663\ \%]}$$

$$= 45.96\ \%$$

Note: Values in the table and example calculation are averages from 4 replicates. The value for % recovery may appear incorrect from manual calculation due to rounding.

1990 Mass Balance Results

Rate = 100.0 kg N ha^{-1}; Atom abundance = 4.6337 %

Table 6.6 Average mass balance results for spring, 1990.

Depth	Bulk Density	Total N	Abund. Natural	Abund. Soil	Total N	Recovery
(cm)	(Mg m^{-3})	(%)	(%)	(%)	(kg N ha^{-1})	(% N)
0 to 15	1.00	0.513	0.368333	0.402323	7800.69	57.1
15 to 30	1.04	0.390	0.368447	0.375063	6149.90	8.8
30 to 45	1.36	0.123	0.368190	0.370017	2533.70	1.0
45 to 60	1.68	0.063	0.368300	0.368687	1644.04	0.1
60 to 75	1.70	0.060	0.367747	0.368467	1539.44	0.2
					SUM	**67.3**

The total fertilizer ^{15}N recovered from 0 to 75 cm depth after being in the soil between March 26, 1990 and May 7, 1990 was 67.3 % (67.3 kg N ha^{-1}) indicating a loss of 32.7 % (32.7 kg N ha^{-1}) of added N during the spring.

APPENDIX 8
STATISTICS FOR 1989 AND 1990 N_2O EMISSIONS

Table 6.7 F-Values for 1989 and 1990 Field N_2O Emissions.

Comparison	F-Value	Significance
1989		
Control vs N-Added	20.5	0.01
N-Added vs Ambient	24.9	0.01
Control vs Ambient	35.9	0.01
1990		
Control vs N-Added	4.7	0.05
N-Added vs Ambient	9.7	0.01
Control vs Ambient	8.9	0.01

Fluxes ($p<0.10$) of N_2O using Duncan's Multilple Range Test occurred for 13 and 10 of 23 measurements for the N-added and the control treatments, respectively, during the 1989 spring thaw. The N-added treatment had significantly greater fluxes of N_2O than the control on 9 of the 23 days where N_2O was collected.

During the spring of 1990, N_2O emissions were significantly greater than ambient air N_2O ($p<0.10$) on 9 of 12 days measurements were made for the N-added treament and 7 of 12 measurements for the control.

APPENDIX 9
CALCULATION OF N_2O PRODUCTION IN ALBERTA

Assume:	Area of Alberta	66×10^6 ha
	Area of cultivated Black and Grey soils in Alberta	5.6×10^6 ha
	Nitrogen-oxide emissions in Alberta ($N_2O + NO_x$)	434 kt
	N_2O emissions per year	1.5 kg ha^{-1} y^{-1}

Calculations:

Nitrous oxide emissions from black and grey soil in Alberta:

$$N_2O\text{-}N\ flux = 1.5\ kg\ ha^{-1}\ y^{-1}\ \times\ 5.6 \times 10^6\ ha = 8.4\ kt\ y^{-1}$$

Emissions of N_2O-N y^{-1} from Black and Grey soils were estimated to be <u>8.4 kt y^{-1}</u>.

$$Soil\ N_2O\ over\ anthropogenic\ emissions = \frac{8.4\ \text{kt}\ \text{y}^{-1}}{434\ \text{kt}^{-1}\text{y}^{-1}} \times 100\% = 2\ \%$$

Emissions of N_2O-N y^{-1} from Black and Grey soils represented 2 % of anthropogenic N-oxide emissions in Alberta.

Other calculation:
Area of cultivated black and grey soils in Alberta:

$$Area\ of\ Black\ and\ Grey\ soils = \frac{5.6 \times 10^6\ ha}{66 \times 10^6\ ha} \times 100\% = 8.5\%$$

Cultivated Black and Grey soils in Alberta cover <u>8.5%</u> of the land area.

APPENDIX 10
LITERATURE REVIEW OF SOIL IRRADIATION

Units: Gamma irradiation provides a means of partial or complete sterilization of soil, depending on dose applied, without an increase in temperature. Energy imparted by gamma irradiation is referred to as "radiation absorbed dose", or "rad", corresponding to the deposition of 100 ergs/g of absorbing material (Natl. Bur. Standards, 1956). A unit used in more recent publications, measures irradiation in the units kGy. A 'Gy', short for 'gray', is the SI unit for absorbed dose and is equivalent to 1 Joule per kilogram (J kg^{-1}) (UNSCEAR, 1982). Since 1 erg = 10^{-7} Joules, a rad is equivalent to 10^{-2} J kg^{-1} or 10^{-2} Gy (1 rad = 10^{-2} Gy or 1 rad = 10^{-5} kGy or 1 Mrad = 10 kGy).

Function: Gamma irradiation induces a lethal effect on microorganisms through direct action by creating injuries to the living structure and prohibiting subsequent multiplication. The non-proliferating cells have been found to maintain some metabolic functions (Lensi et al. 1991; Cawse, 1975).

Soil Properties: Moisture status of the soil has been shown to have no effect on the efficiency of the sterilization lending that the choice of sterilizing dry or wet soil is determined by changes in the physico-chemical properties or the intensity of residual enzyme activities in the soil after irradiation. Aggregate stability was not affected by irradiation (25 kGy = 2.5 Mrad)(Lensi et al., 1991; Griffiths and Burns,1968). Soil pH has also been found not to be affected by irradiation regardless of moisture status (Lensi et al., 1991). Soluble organic carbon increases after irradiation (Lensi et al. 1991; Cawse and Mableson, 1972). Possible causes are identified as lysis of cells killed by gamma rays (Bowen and Cawse, 1965) and soil organic matter degradation (McLaren, 1969) releasing organic acids (Skipper and Weterman, 1973; Powlson and Jenkinson, 1976). Lower levels of soluble carbon were found in moist irradiated soils and is explained by the transformation of organic C to CO_2 by peroxides (Lensi et al. 1991).

NH_4^+ and NO_3^- : Wet irradiated samples generally have large increases in NH_4^+ and both wet and dry samples have a decrease in NO_2^- immediately after irradiation (Lensi et al.,1991). This is interpreted as a direct effect of irradiation through peroxide production (Salonius et al. 1967). NH_4^+ might be released through the deamination of nitrogenous organic compounds and could be enhanced by the proteolysis of damaged microbial cells. In Lensi et al. (1991), increasing NH_4^+ content during incubation following the sterilizing treatment suggested that enzymes (proteases and deaminases) were still active. NO_3^- dissimilatory reduction to NH_4^+ (Tiedje et al., 1982) may take place in saturated samples but Lensi et al. (1991) recorded stable NO_3^- levels due to low moisture levels. Cawse (1975) reported that

the NO_3^- level increased for irradiation doses up to 5 kGy (4 Mrad) and decreased for doses greater than 5 kGy. Two hypotheses may explain the small changes observed in NO_3^- and NO_2^- in Lensi et al. (1991): gamma irradiation (25 kGy) inhibited nitrifying enzyme activity as suggested by Ramsay and Bawden (1983); or nitrifying enzymes remained functional but NO_3^- and NO_2^- production was compensated by the activity of radioresistant denitrifying enzymes. It is more likely that the nitrifying enzymatic system was strongly affected by gamma rays in Lensi et al. (1991) since favorable denitrification conditions did not exist.

Enzyme Activity: The potential for denitrifying enzymes to remain active after irradiation was shown by Lensi et al. (1991). Cawse and White (1969) and Van Cleemput and Patrick (1974) also demonstrated that intracellular enzymes like denitrifying enzymes may be radioresistant and would be active after irradiation. Air-drying soil before irradiation with 0.8 Mrad decreased the subsequent accumulation of nitrite under moist conditions (Cawse and Cornfield, 1971). Of ten soils tested, only soils containing carbonate were found to have increased nitrite levels 24 hrs after irradiation (0.5-0.8 Mrad) (Cawse and White, 1969). The accumulation and persistence of nitrite was poor in soils with $pH<7$ and was not improved by the addition of calcium carbonate before irradiation (Cawse and Cornfield, 1971) suggesting the absense of enzymes capable of reducing nitrate after irradiation.

Soil Preparation: Lensi et al. (1991) suggests that soil be irradiated in a dry state to reduce enzyme activity and the subsequent accumulation of ammonia and nitrite. It is also suggested that the dose be 4 Mrad or greater to minimize NO_3^- increases in the soil. The effect of freezing on enzymes in irradiated soil has not be studied, although, it would be reasonable to assume that an incubation period of several weeks under subzero conditions would reduce enzyme activity.

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APPENDIX 11

CALCULATION OF N_2O EMISSIONS IN CHAPTER 3

A. Conversion of ppm N_2O hr^{-1} to g N_2O-N ha^{-1} h^{-1}:

Assume:	Soil Depth	10 cm
	Headspace Volume	0.554 L
	Headspace Area	55.4 cm^2
	N_2	28 g mol^{-1}
	N_2O	44 g mol^{-1}
	1 mol gas (STP)	22.4 L
	N_2O	ppm (v/v)

Calculation:

$$\text{g } N_2O\text{-N ha}^{-1}\text{ h}^{-1} = \frac{\text{ppm(v/v) } N_2O \text{ h}^{-1}}{10^6} \text{ x } \frac{44 \text{ g } N_2O \text{ mol}^{-1}}{22.4 \text{ L mol}^{-1}} \text{ x } \frac{28 \text{ g N mol}^{-1}}{44 \text{ g } N_2O \text{ mol}^{-1}} \text{ x}$$

$$\frac{0.554 \text{ L}}{55.4 \text{ cm}^2} \text{ x } \frac{10^8 \text{ cm}^2}{\text{ha}} = \underline{1.250} \text{ x ppm(v/v) } N_2O \text{ h}^{-1}$$

B. Conversion of ppm N_2O hr^{-1} to µg N_2O-N g $soil^{-1}$ h^{-1}:

Assume:	*Series*	*Bulk Density (Mg m^{-3})*	*g soil jar^{-1} (g)*
	Malmo	1.00	554
	Breton	1.14	680
	Josephine	0.93	515

Example:

$$\text{ug } N_2O\text{-N g soil}^{-1} = \frac{\text{ppm(v/v) } N_2O \text{ h}^{-1}}{10^6} \text{ x } \frac{44 \text{ g } N_2O \text{ mol}^{-1}}{22.4 \text{ L mol}^{-1}} \text{ x } \frac{28 \text{ g N mol}^{-1}}{44 \text{ g } N_2O \text{ mol}^{-1}} \text{ x}$$

$$\frac{0.554 \text{ L}}{554 \text{ g soil}} \text{ x } \frac{10^6 \text{ µg}}{\text{g}} = 0.00125 \text{ x ppm(v/v) } N_2O \text{ h}^{-1}$$

Malmo series: µg N_2O-N g $soil^{-1}$ = $\underline{0.00125}$ x ppm(v/v) N_2O h^{-1}

Breton series: µg N_2O-N g $soil^{-1}$ = $\underline{0.001018}$ x ppm(v/v) N_2O h^{-1}

Josephine series: µg N_2O-N g $soil^{-1}$ = $\underline{0.001345}$ x ppm(v/v) N_2O h^{-1}

APPENDIX 12
CHAPTER 3 N_2O EMISSIONS

Table 6.8 Nitrous oxide emissions after 88 hours.

---Treatment---				-------Block-------						
S†	F‡	^{15}N§	I¥	1	2	3	4	Mean	STD	CV
				------g N_2O-N ha^{-1}------						%
M	+	+	+	69.9	43.9	189.7	97.3	100.2	64	63
M	+	+	-	108.4	16.4	234.1	369.7	182.2	154	84
M	+	-	+	201.6	16.8	148.1	300.4	166.7	118	71
M	+	-	-	148.6	10.8	334.5	330.3	206.1	156	76
M	-	+	-	264.1	12.6	676.0	695.4	412.0	332	81
M	-	-	-	440.7	25.0	706.7	628.9	450.3	305	68
B	+	+	+	29.2	11.8	21.9	59.3	30.6	20	67
B	+	+	-	53.2	87.4	183.5	137.9	115.5	57	50
B	+	-	+	8.4	0.0	66.7	53.5	32.2	33	102
B	+	-	-	40.9	10.0	277.3	115.1	110.8	119	108
B	-	+	-	28.7	12.0	342.9	245.8	157.3	163	104
B	-	-	-	124.6	9.8	400.0	349.7	221.0	185	84
J	+	+	+	48.4	5.6	41.3	68.2	40.8	26	64
J	+	+	-	69.7	47.7	57.5	56.1	57.8	9	16
J	+	-	+	18.9	10.6	7.1	118.6	38.8	53	138
J	+	-	-	19.4	8.4	69.4	22.2	29.9	27	91
J	-	+	-	17.8	11.5	115.1	52.7	49.3	47	96
J	-	-	-	103.3	10.7	238.6	42.2	98.7	101	102

†Soil(S) designations are: M=Malmo; B=Breton; J=Josephine.

‡Frozen(F) soils are marked as '+' and non-frozen soils are designated as '-'.

§Treatments with ^{15}N-KNO_3(^{15}N) added at 80 kg ha^{-1} are marked by '+'.

¥Gamma irradiated(I) treatments are indicated by '+'.

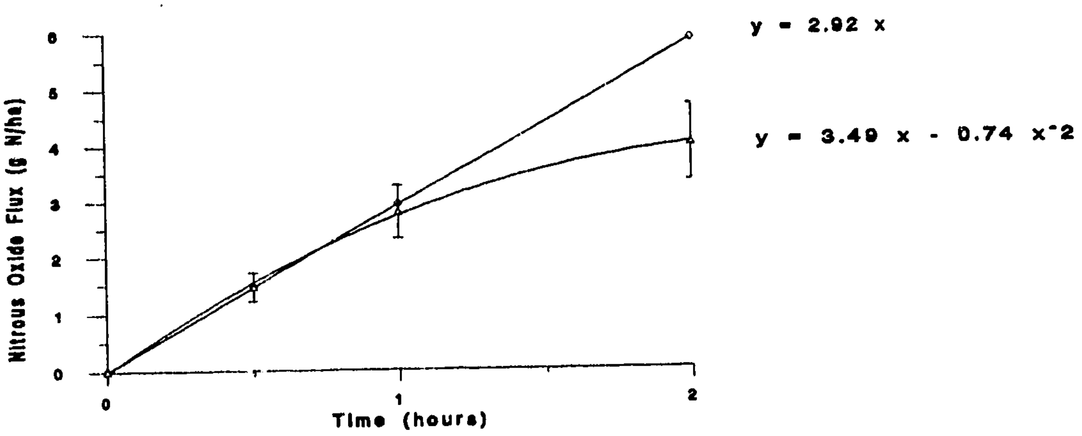
APPENDIX 13
CORRECTION FOR DECREASING CONCENTRATION GRADIENT

Air samples were taken at the start and at the end of the trapping period of 1.5 or 2.0 h. During the trapping period, the rate of N_2O flux from the soil could decrease due to the decreasing N_2O concentration gradient between the headspace air and the soil. Hutchinson and Mosier (1981) include a detailed discussion on correcting for decreasing concentration gradient. The correction method used by Hutchinson and Mosier (1981) could not be used in this research since headspace air was not sampled midway through the trapping period. The laboratory N_2O values were adjusted on the basis of values derived from the N_2O flux measured on a Malmo soil sample during thaw at 0, 0.5, 1 and 2 h (Table 6.9).

Table 6.9 N_2O values for calculation of correction term.

Time	N_2O	N_2O-N Flux	Standard Deviation	Coefficient of Variance
hours	ppm (v/v)	g ha^{-1}	g ha^{-1}	%
0.0	0.34	0.00	0.02	5
0.5	1.51	1.46	0.29	20
1.0	2.34	2.78	0.64	23
2.0	3.53	3.98	1.19	30

Figure 6.2 Correction term for Malmo Series.



The slope of the linear segment derived from the first 0.5 h flux is 2.9. Assuming flux is constant over 2 h, an emission of 4.0 g N_2O-N ha^{-1} would be estimated as 5.8 g N_2O-N ha^{-1} or 3.4 g N_2O-N ha^{-1} h^{-1} after accounting for the decreasing concentration gradient. The flux, after 2 h of trapping, can be corrected by multiplying the flux by 1.47 (5.84 - 3.98=1.86; 1.86/3.98=1.47). This correction method is not perfect, however, it is the best estimate that can be made with the data collected. Future work should follow the procedure outlined by Hutchinson and Mosier (1981).

APPENDIX 14
THE HUTCHINSON AND MOSIER CORRECTION CALCULATION

The preferred method of correcting for decreasing concentration gradient involves measuring N_2O concentrations under covers at 3 times during the trapping period. The first measurement should be made at t=0 with the next two taken at equal intervals after t=0 (e.g. t=15 min. and t=30 min.). The final equation derived by Hutchinson and Mosier (1981) is as follows:

$$f_0 = \frac{V(C_1-C_0)^2}{At_1(2C_1-C_2-C_0)} \ln\left(\frac{C_1-C_0}{C_2-C_1}\right), \text{ if } \frac{C_1-C_0}{C_2-C_1} > 1$$

Where f_0 is the N_2O flux, V is the internal volume of the soil cover, C_0 is the N_2O concentration at t=0, C_1 and C_2 are the first and second N_2O concentrations after C_0, respectively, A is the surface area under the cover, and t is the time interval between C_0, C_1 and C_2 extraction times.

Using the data in Table 6.9, the flux (f_0) using $t_0=0$, $t_1=0.5$ and $t_2=1.0$ h would be calculated as:

$$f_0 = \frac{554 \text{ cm}^3 (1.510-0.344)^2}{55.4 \text{ cm}^2 \times 0.5\text{h}\,(2(1.510) - 2.565 - 0.344)} \ln\left(\frac{1.510 - 0.344}{2.565 - 1.510}\right) = 24.6 \text{ mL cm}^{-2}\text{ h}^{-1}$$

to convert to g N_2O-N ha^{-1} h^{-1}:

$$24.6 \text{ mL cm}^{-2}\text{ h}^{-1} \times \frac{1}{10^6 \text{ ppm}(v/v)} \times \frac{44 \text{ g N}_2\text{O mol}^{-1}}{22.4 \text{ L mol}^{-1}} \times \frac{28 \text{ g N mol}^{-1}}{44 \text{ g N}_2\text{O mol}^{-1}} \times \frac{10^8 \text{ cm}^2}{\text{ha}}$$

$= 24.6 \times 0.125 = 3.1$ g N_2O-N ha^{-1} h^{-1}

REFERENCE

Hutchinson, G.L. and **A.R. Mosier**. 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. Soil Sci. Soc. Am. J. 45:311-316.

APPENDIX 15
CALCULATION OF CARBON DIOXIDE EMISSIONS

Conversion of ppm CO_2 hr^{-1} to g CO_2-C ha^{-1} h^{-1}:

Assume:	Soil Depth	10 cm
	Headspace Volume	0.554 L
	Headspace Area	55.4 cm^2
	C	12 g mol^{-1}
	CO_2	46 g mol^{-1}
	1 mol gas (STP)	22.4 L
	CO_2	ppm (v/v)

Equation:

$$\text{g } CO_2\text{-C ha}^{-1}\text{ h}^{-1} = \frac{\text{ppm(v/v) } CO_2 \text{ h}^{-1}}{10^6} \times \frac{46 \text{ g } CO_2 \text{ mol}^{-1}}{22.4 \text{ L mol}^{-1}} \times \frac{12 \text{ g N mol}^{-1}}{46 \text{ g } CO_2 \text{ mol}^{-1}} \times$$

$$\frac{0.554 \text{ L}}{55.4 \text{ cm}^2} \times \frac{10^8 \text{ cm}^2}{\text{ha}} = 1.204 \times \text{ppm(v/v) } CO_2 \text{ h}^{-1}$$

APPENDIX 16
CALCULATION OF ^{15}N RECOVERY AND MASS BALANCE

Equation for kg N ha^{-1}:

Assume:	Rate	80.0 kg N ha^{-1}
	Atom abundance	76.7 %
	Soil Depth	10 cm
	KNO_3	0.306 g jar^{-1}

$$\%Recovery = \frac{[\%N\ soil]x[kg\ soil\ ha^{-1}x\ 10cm]x[\%^{15}N\ ab.\ soil-\%nat.\ ^{15}N]}{[Fertilzer\ Rate]\ x\ [76.7\% - 0.3663\%]}$$

Equation using µg N g $soil^{-1}$:

Assume:	Malmo	554 g soil jar^{-1}
	Breton	680 g soil jar^{-1}
	Josephine	515 g soil jar^{-1}
	N-added	0.04432 g jar^{-1}

$$\%Recovery = \frac{[\%N\ soil]\ x\ [g\ soil\ jar^{-1}]\ x\ [\%^{15}N\ abund.\ soil-\%natural\ ^{15}N]}{[Fertilzer\ N\ jar^{-1}]\ x\ [76.7\% - 0.3663\%]}$$

Table 6.10 Mass balance results for Chapter 3.[†]

Treatment	Bulk Density	Total N	Abund. Soil	Excess	Recovery	Standard Deviation	Coefficient of Variance
	Mg m^{-3}	%	%	%	% N	% N	%
M I F[‡]	1.00	0.58050	1.21222	0.84290	79.89	16.62	21
M NI F	1.00	0.57725	1.24159	0.87228	82.41	7.47	9
M NI NF	1.00	0.57625	1.21992	0.85060	80.23	10.61	13
B I F	1.23	0.13075	3.39331	3.02400	79.75	13.09	16
B NI F	1.23	0.12750	3.39282	3.02351	77.85	11.94	15
B NI NF	1.23	0.13775	3.03622	2.66691	73.82	4.16	6
J I F	0.93	0.30175	1.97244	1.60287	73.73	6.84	9
J NI F	0.93	0.30325	1.92052	1.55096	71.60	15.01	21
J NI NF	0.93	0.30625	2.15889	1.78933	83.44	5.79	7

[†] Values represent the mean of four replicates.

[‡] M=Malmo; B=Breton; J=Josephine; I=Irradiated; NI=Not Irradiated; F=Frozen; NF=Not Frozen.

APPENDIX 17
MINERAL N DISTILLATION

Extraction: Ammonia and nitrate were extracted from the soil with 2M KCl (100 mL per 10 g soil). The 2M KCl solution was made by dissolving 1500 g of reagent-grade KCl in 10 liters of water. Ten grams of soil was placed in a 250 mL bottle and 100 mL of 2M KCl added. The solution was capped and shaken an a mechanical shaker for 1 hour. The supernatant liquid was drained off and filtered (Whatman #42 filter paper) and stored in a refrigerator (3°C).

NO_3^--N and NH_4^+-N Determination: Determination of NO_3^--N and NH_4^+-N followed the method described by Keeney and Nelson (1982). The method uses MgO as the alkaline reagent and Devarda's alloy as the reductant. The MgO method of determining NH_4^+ is based on finding that NH_3^+-N in solutions containing glutamine and other alkali-labile organic N compounds can be determined quantitatively from the NH_3^--N liberated by steam distillation of these solutions with a small amount of MgO for 2 to 3 minutes. Nitrate is liberated by steam distillation by adding Devarda's alloy after destruction of NO_2^- with sulfamic acid and removal of NH_4^+ by steam distillation with MgO.

Titration: The NO_3^--N and NH_4^+-N solutions were titrated using 2% (w/v) solution of boric acid (H_3BO_3) indicator solution.

REFERENCE

Keeney, D.R. and **D.W. Nelson.** 1982. Nitrogen-Inorganic Forms. *In* Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. Agronomy Monograph no. 9 (2nd Edition). ASA-SSSA Madison, WI. pp.643-653.

Table 6.11 Mineral nitrogen for Malmo, Breton, and Josephine soils in Chapter 3.

--- Treatment ---				------NH_4^+-N-----			------NO_3^--N-----		
S†	F‡	^{15}N§	I¥	Mean	STD	CV	Mean	STD	CV
				mg kg^{-1}		%			%
M*			-	4.89	0.06	1.19	23.81	3.20	13.43
M*			+	7.98	0.79	9.94	24.02	0.16	0.68
M	+	+	+	9.64	2.57	26.61	71.35	15.22	21.33
M	+	+	-	10.47	3.53	33.77	59.69	10.07	16.87
M	+	-	+	9.62	3.05	31.72	8.60	5.59	65.00
M	+	-	-	13.10	1.85	14.15	9.99	5.14	51.42
M	-	+	-	22.17	24.90	112.34	50.38	27.61	54.80
M	-	-	-	7.09	6.01	84.70	4.85	3.45	71.12
B*			-	3.04	0.35	11.47	7.57	0.70	9.22
B*			+	3.48	0.45	13.01	4.88	4.26	87.19
B	+	+	+	4.32	1.60	37.15	51.80	9.54	18.42
B	+	+	-	5.22	1.74	33.39	52.08	5.53	10.61
B	+	-	+	7.28	0.96	13.13	3.21	2.62	81.81
B	+	-	-	7.05	1.47	20.83	0.95	0.32	33.68
B	-	+	-	7.20	4.23	58.78	50.42	2.81	5.57
B	-	-	-	4.75	1.52	32.07	0.45	0.62	137.27
J*			-	12.58	3.14	24.96	4.52	0.00	0.00
J*			+	7.24	6.29	86.97	1.54	2.18	141.97
J	+	+	+	10.57	2.13	20.11	62.84	3.63	5.78
J	+	+	-	9.83	1.05	10.68	55.52	10.21	18.40
J	+	-	+	12.79	2.12	16.55	0.95	0.79	83.25
J	+	-	-	11.41	1.86	16.28	1.62	2.29	140.83
J	-	+	-	6.46	7.48	115.86	51.04	31.72	62.16
J	-	-	-	11.49	2.38	20.72	17.54	33.55	191.26

†Soil(S) designations are: M=Malmo; B=Breton; J=Josephine.

‡Frozen(F) soils are marked as '+' and non-frozen soils are designated as '-'.

§Treatments with ^{15}N-KNO_3(^{15}N) added at 80 kg ha^{-1} are marked by '+'.

¥Gamma irradiated(I) treatments are indicated by '+'.

*Treatments were not incubated, only irradiated or not irradiated.

Note: To convert mg N kg soil^{-1} to kg N ha^{-1}, multiply M by 1.00, B by 1.23 and J by 0.93.

Table 6.12 Changes in mineral nitrogen during freezing and thaw.

S†	F‡	^{15}N§	I¥	NO_3^--N¶			Loss of Added NO_3^--N	Loss of Added ^{15}N	NH_4^+¶		
				Before	After	Change			Before	After	Change
				kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
M	+	+	-	103.8	59.7	-44.1	30.3	14	4.9	10.5	5.6
M	+	-	-	23.8	10.0	-13.8			4.9	13.1	8.2
M	-	+	-	103.8	50.4	-53.4	34.5	15.8	4.9	22.2	17.3
M	-	-	-	23.8	4.9	-18.9			4.9	7.1	2.2
M	+	+	+	104.0	71.3	-32.7	17.3	15.9	8.0	9.7	1.7
M	+	-	+	24.0	8.6	-15.4			8.0	9.6	1.6
B	+	+		87.6	64.1	-23.5	15.4	17.8	3.7	6.4	2.7
B	+	-	-	9.3	1.2	-8.1			3.7	8.6	4.9
B	-	+	-	87.6	62.0	-25.6	16.8	20.8	3.7	8.8	5.1
B	-	-	-	9.3	0.5	-8.8			3.7	5.8	2.1
B	+	+	+	84.9	63.7	-21.2	19.1	16.3	4.3	5.3	1.0
B	+	-	+	6.0	4.0	-2.0			4.3	9.0	4.7
J	+	+	-	84.5	51.6	-32.9	30.2	22.7	11.7	9.1	-2.6
J	+	-	-	4.2	1.5	-2.6			11.7	10.6	-1.1
J	-	+	-	84.5	47.5	-37.0	24.9	13.2	11.7	6.0	-5.7
J	-	-	-	4.2	16.3	12.1			11.7	10.7	-1.0
J	+	+	+	81.5	58.4	-23.1	22.6	21.3	6.7	9.7	3.0
J	+	-	+	1.4	0.9	-0.5			6.7	11.9	5.2

†Soil(S) designations are: M=Malmo; B=Breton; J=Josephine.

‡Frozen(F) soils are marked as '+' and non-frozen soils are designated as '-'.

§Treatments with ^{15}N-KNO$_3$(^{15}N) added at 80 kg ha^{-1} are marked by '+'.

¥Gamma irradiated(I) treatments are indicated by '+'.

*Treatments were not incubated, only irradiated or not irradiated.

¶Values 'Before' and 'After' incubation followed by the 'Change' from start to end.

APPENDIX 18
CHAPTER 3 STATISTICS

Table 6.13 ANOVA of Log transformed N_2O data as dependent variable.

Source	DF	Sum of Squares	Mean Square	F Value	Significance p>F
Treatment	20	20.23	1.01	9.80	0.0001
Error	51	5.27	0.10		
Total	71	25.50			

R-Square = 0.79 C.V. = 17.81

Table 6.14 T-groupings of Log transformed N_2O data.

Comparison	Treatment	Mean	T-Grouping
Nitrogen	+N	1.83	A
	no-N	1.78	A
Treatment	Frozen/Irradiated	2.00	A
	Frozen/Not Irrad.	1.84	A
	Not Froz./Not Irrad.	1.57	B

Table 6.15 ANOVA of carbon dioxide emissions.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance p>F
Treatment	17	32930516	1937089	4.42	0.0001
Error	36	15791723	438659		
Total	53	48722238			

Table 6.16 ANOVA of Log transformed ^{15}N recovery data.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance p>F
Treatment	11	0.04436	0.004032	1.13	0.380
Error	24	0.08541	0.003559		
Total	35	0.12976			

R-Square = 0.34 C.V. = 3.16

Table 6.17 T-groupings of Log transformed ^{15}N data.

Comparison	Treatment	Mean	T-Grouping
Soil	Malmo	1.90	A
	Breton	1.88	A
	Josephine	1.88	A
Treatment	Frozen/Irradiated	1.90	A
	Frozen/Not Irrad.	1.88	A
	Not Froz./Not Irrad.	1.88	A

APPENDIX 19
CHANGES IN MINERAL NITROGEN

Table 6.18 Change in mineral nitrogen for manured and non-manured soils in Chapter 4.

Treatment	NO_3^--N (initial)	NO_3^--N (final)	Change in NO_3^--N	NH_4^+-N (initial)	NH_4^+-N (final)	Change in NH_4^+-N
	mg kg^{-1}	mg kg^{-1}		mg kg^{-1}	mg kg^{-1}	
Manure+^{15}N	100.6	79.7	-20.9	3.8	8.2	4.6
Manure†	0.6	0.0	-0.6	3.8	7.1	3.3
No Man.+^{15}N	100.0	75.9	-24.1	2.1	6.5	4.4
Control‡	0.0	0.0	0.0	2.1	3.8	1.7

†Bulk density of Manure treatment is 0.70 Mg m^{-3}.
‡Bulk density of the Control is 0.95 Mg m^{-3}.

Observations:

No ^{15}N added treatments:

1. NH_4^+-N increased 3.3 mg N kg soil^{-1} and 1.7 mg N kg soil^{-1} in the manure and control treatments, respectively, during the 66 h incubation.
2. NO_3^--N decreased 0.6 mg N kg soil^{-1} in the manured soil during the 66 h incubation.

^{15}N added treatments:

1. NH_4^+-N increased 4.4 mg kg soil^{-1} in both the manure and control treatments during the 66 h incubation.
3. NO_3^--N decreased 20.9 and 24.1 mg kg soil^{-1} (14.6 and 22.9 kg ha^{-1}, respectively†) in the manured and non-manured soils, respectively, during the 66 h incubation.
4. Mass balance results showed 39.1 and 20 kg ha^{-1} of added NO_3^--N was lost from the manured and non-manured soils, respectively. Therefore, analysis of the NO_3^--N in the manured, +N treatment showed only half the loss indicated by mass balance.

†*Conversion of data to kg N ha^{-1}:*

$$\text{kg N ha}^{-1} = \frac{\text{mg N}}{\text{kg soil}} \times \frac{\text{Mg}}{\text{m}^3} \times \frac{10^3\ \text{kg}}{\text{Mg}} \times \frac{10^4\ \text{m}^2}{\text{ha}} \times 0.10\ \text{m} \times \frac{\text{kg}}{10^6\ \text{mg}}$$

APPENDIX 20
STATISTICS FOR CHAPTER 4

Table 6.19 ANOVA and LSD of nitrous oxide emissions from manured and non-manured treatments in Chapter 4.

Source	DF	Sum of Squares	Mean Square	F Value	Significance p>F
Treatment	6	4417748	736191	14.01	0.0004
Error	9	47305	52556		
Total	15	4890153			

R-Square= 0.90 C.V.= 21.91

Source	DF	Sum of Squares	Mean Square	F Value	Significance p>F
Block	3	113302	37767	0.72	0.5655
Manure	1	4115826	4115826	78.31	0.0001
+N	1	183826	183826	3.50	0.0943
Manure+N	1	4192	4192	0.08	0.7840

APPENDIX 21
RECOVERABILITY OF ADDED ^{15}N FROM SOIL

The determination of soil ^{15}N mass balance requires the assumption that all of the ^{15}N in a soil sample is recovered or detected by mass spectrometry. The recoverability of added ^{15}N from soil was tested in four different soils. Soils from the cultivated horizons of the Malmo, Breton, Josephine and Josephburg soil series were air dried and passed through a 2 mm mesh. The air dry soils were sub sampled and oven dried to determine moisture content. Each soil, 200 g (oven dry basis), was placed in aluminum trays. Nitrogen was added to the soils at a rate of 100 μg N g soil^{-1}. Labeled ^{15}N-KNO_3 (5.4123 % atom abundance), 0.144 g, was dissolved in 15 mL warm water, spread over the soil evenly, and the beakers rinsed three times with 10 mL H_2O and the washings were added to the soil. The soil and solution were mixed thoroughly with a spatula and left to dry. The dry soil was mixed and passed through a 2 mm mesh and mixed thoroughly. A sub sample was ground to a fine powder in a ball mill and analyzed using mass spectrometry.

Table 6.20 Summary of ^{15}N recovery from four soils.

Soil Series	N	^{15}N Recovery	Standard Deviation	Area of 95% Confidence (T-Test)
		%		%
Malmo	6	97.8	2.4	±2.4
Josephburg	12	96.4	7.7	±4.8
Breton	14	95.5	6.3	±3.6
Josephine	6	96.4	3.6	±3.7

Table 6.21 Recovery of ^{15}N from Malmo soil.

Date	Sample†	Total N	Abundance	Background Abundance	Excess	Recovery
1993		%	%	%	%	%
April 19	1-1	0.555	0.45609	0.36923	0.08686	95.43
	1-2	0.562	0.45601	0.36923	0.08678	96.55
	1-3	0.563	0.45545	0.36923	0.08622	96.10
April 28	2-1	0.585	0.4561	0.3688	0.0873	101.10
	2-2	0.631	0.4465	0.3688	0.0777	97.06
	2-3	0.624	0.4502	0.3688	0.0814	100.55
Average		0.587	0.4534	0.3690	0.0844	**97.80**
Stdev.		0.033	0.0041	0.0002	0.0039	**2.41**

†The first number represents the batch and the second number the replicate.

Table 6.22 Recovery of ^{15}N from Josephine soil.

Date	Sample†	Total N	Abundance	Background Abundance	Excess	Recovery
1993		%	%	%	%	%
April 19	1-1	0.312	0.51966	0.36803	0.1563	93.65
	1-2	0.304	0.52104	0.36803	0.15301	92.08
	1-3	0.301	0.52974	0.36803	0.16171	96.36
April 28	2-1	0.299	0.5288	0.3682	0.1606	95.06
	2-2	0.315	0.5279	0.3682	0.1597	99.59
	2-3	0.332	0.5226	0.3682	0.1544	101.48
Average		0.311	0.5250	0.3681	0.1576	**96.37**
Stdev.		0.012	0.0044	0.0009	0.0036	**3.58**

†The first number represents the batch and the second number the replicate.

Table 6.23 Recovery of ^{15}N from Breton soil.

Date	Sample†	Total N	Abundance	Background Abundance	Excess	Recovery
1993		%	%	%	%	%
April 19	1-2	0.137	0.68976	0.36909	0.32067	86.97
	1-3	0.130	0.72113	0.36909	0.3504	90.60
April 22	2-1	0.131	0.73452	0.36921	0.36531	94.74
	2-2	0.139	0.76060	0.36921	0.39139	107.70
	2-3	0.127	0.73066	0.36921	0.36145	90.87
	2-4	0.130	0.71474	0.36921	0.34553	88.92
	2-5	0.128	0.76810	0.36921	0.39889	101.08
	2-6	0.128	0.76145	0.36921	0.39224	99.39
	3-1	0.139	0.7013	0.3690	0.3323	91.44
	3-3	0.129	0.7165	0.3690	0.3475	88.74
	3-5	0.132	0.7570	0.3690	0.3880	101.39
April 28	2-1	0.139	0.63940	0.36885	0.27055	74.45
	2-2	0.138	0.68405	0.36885	0.31520	86.12
	2-3	0.145	0.70710	0.36885	0.33825	97.09
Average		0.174	0.68769	0.36910	0.30826	**95.46**
Stdev.		0.079	0.09173	0.0001	0.10105	**6.27**

†The first number represents the batch and the second number the replicate.

Table 6.24 Recovery of ^{15}N from Josephburg soil.

Date	Sample[†]	Total N	Abundance	Background Abundance	Excess	Recovery
1993		%	%	%	%	%
April 19	1-1	0.342	0.49434	0.36921	0.12513	84.72
	1-2	0.334	0.52596	0.36921	0.15675	103.64
April 22	2-1	0.317	0.52624	0.36883	0.15741	98.78
	2-2	0.324	0.52616	0.36883	0.15733	100.91
	2-3	0.326	0.52721	0.36883	0.15838	102.21
	2-4	0.318	0.51766	0.36883	0.14883	93.69
	2-5	0.317	0.51851	0.36883	0.14968	93.93
	2-6	0.315	0.50881	0.36883	0.13998	87.29
	3-1	0.332	0.5252	0.3689	0.1563	102.73
	3-3	0.344	0.5233	0.3689	0.1544	105.15
	3-5	0.361	0.5092	0.3689	0.1403	100.27
April 28	2-1	0.331	0.4965	0.3691	0.1274	83.48
Average		0.330	0.5166	0.3689	0.1477	**96.40**
Stdev.		0.014	0.0118	0.0002	0.0118	**7.65**

[†] The first number represents the batch and the second number the replicate.

APPENDIX 22
COVER PLACEMENT EXPERIMENT

INTRODUCTION

In this laboratory experiment we set out to discover whether inserting soil covers into the soil affected N_2O flux. Two treatments were used: the 'in' treatment, where the lip of the soil cover (3cm) was inserted into the soil during gas trapping and the 'on' treatment, where the lip of soil cover was placed on the soil. The hypothesis was that inserting the cover would result in increased N_2O flux.

METHODS AND MATERIALS

Moist soil from the Ap of the Malmo series was placed into eight 2.4 L pails (1.8 kg soil (oven dry basis) per pail). Nitrogen was added to each pail as KNO_3 (100 kg ha^{-1}). The soils were brought up to saturation with deionized water and incubated at room temperature (22°C) for three days. Gas samples were collected using soil covers 24 (Day 1) and 48 h (Day 2) after the start of the incubation. Collection of gas from the head space occurred 0.5, 5, 15, 30, 60 and 90 min. after placing or inserting covers. Gas sampling and analysis followed the same procedure described Chapter 2.

RESULTS AND CONCLUSIONS

Nitrous oxide emission was greater from soils when the lip of the soil cover was placed 'in' the soil (Table 6.25). The flux decreased in latter samples from the 'in' treatment but remained constant for the 'on' treatment (Figures 6.3 and 6.4). Inserting the cover caused increased emissions of N_2O.

Table 6.25 Average N_2O data from 'Hutchinson' covers placed 'on' and 'in' the soil.

TRT	TIME	DAY ONE N_2O-N Flux	DAY ONE Standard Deviation	DAY ONE C.V.	DAY TWO N_2O-N Flux	DAY TWO Standard Deviation	DAY TWO C.V.
	min.	g ha^{-1}h^{-1}		(%)	g N ha^{-1}		(%)
ON	0.5	65.15	35.65	55	43.31	6.27	14
	5	18.76	1.16	6	24.96	3.46	14
	15	13.96	3.51	25	22.90	5.29	23
	30	12.71	5.77	45	18.22	3.53	19
	60	11.25	2.62	23	17.10	2.78	16
	90	10.67	3.55	33	16.49	2.88	17
IN	0.5	113.10	51.89	46	180.63	127.83	71
	5	66.26	26.54	40	51.42	19.04	37
	15	46.01	11.40	25	41.78	7.68	18
	30	34.80	11.69	34	36.17	12.31	34
	60	24.87	4.50	18	29.06	8.77	30
	90	20.84	5.63	27	24.16	5.37	22

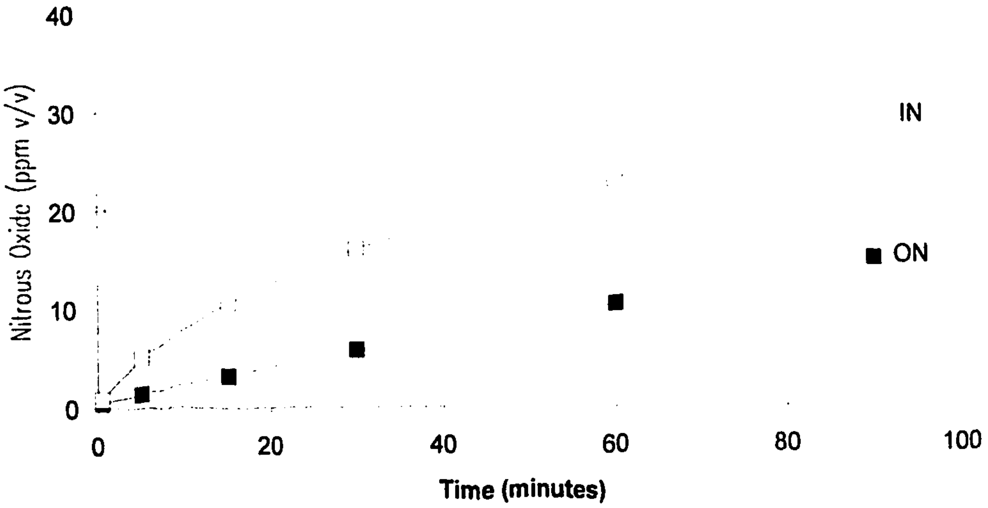


Figure 6.3.. Change in nitrous oxide concentration under soil covers on Day 1.

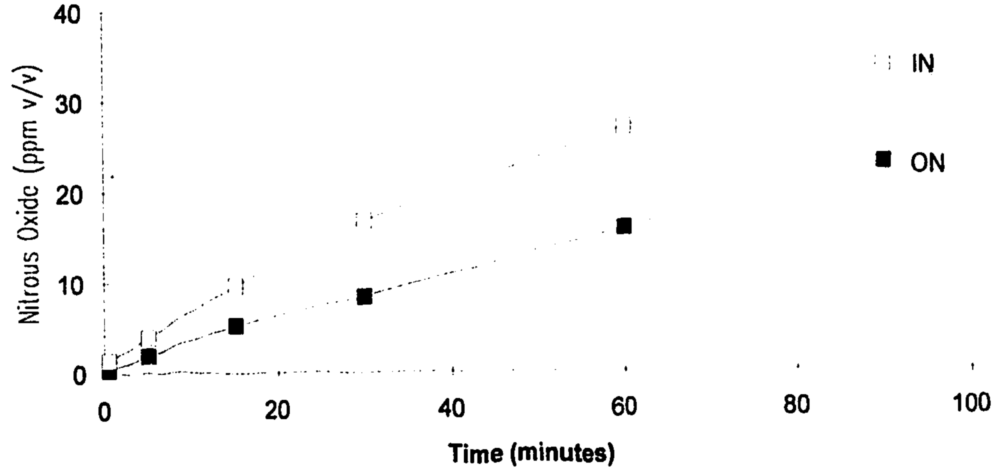


Figure 6.4.· Change in nitrous oxide concentration under soil covers on Day 2.