NITROUS OXIDE DISTRIBUTION IN SOIL AND SURFACE FLUX

by

Michael William Kagan

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER'S OF SCIENCE

Department of Soil Science
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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

The production of nitrous oxide (N_2O) in agroecosystems has become a topic of concern due to it's effect on global warming and ozone destruction. Estimates by Agriculture Canada of N_2O emission from soil in Manitoba's Red River valley region range from approximately 18.94 to 31.52 ng N_2O-N m^{-2} s^{-1}, and are based on N inputs (Janzen et al., 1999). No research on direct measurement of N_2O emission or profile concentration has been done to confirm these estimates. Large N_2O surface emission has been reported during spring thaw events, and cropping systems have been found to affect N_2O emission in some cases, but again no information on these phenomena is available in the literature for Manitoba. A field study examining N_2O profile concentrations and surface flux from four cropping systems during spring thaw, a study examining the effectiveness of different N_2O storage methods, and a laboratory study examining N_2O redistribution in three different textured soils were completed.

The N_2O surface emission and profile redistribution in a silty clay soil was investigated with 4 cropping systems (alfalfa, summerfallow, wheat and native grass during spring thaw. The relationship between gas concentration profiles and surface flux was also explored. Nitrous oxide surface flux and profile concentration increased during spring thaw at all sites. Significant increases occurred in the alfalfa and summerfallow cropping systems, while smaller fluxes were observed with wheat and native grass. The alfalfa cropping system had the highest surface flux of all treatments with a maximum

N_2O flux of 7.0 ng N_2O-N $m^{-2}s^{-1}$. The N_2O surface flux was relatively small in this silty clay when compared which is small when compared to Agriculture Canada's estimate of emission in this area. Increased profile N_2O accumulations appeared to drive surface flux.

There is little information in the literature reporting the efficiency of current storage methods for N_2O samples collected in the field. One of the most common methods is storage in a syringe with subsequent manual injection into a gas chromatograph for analysis. Four methods of storage are investigated: the syringe method, the vacutainer method, and autosampler both with and without a liquid diffusion barrier. Of the storage methods tested, the vacutainers proved most effective for long term storage, while the syringe method was the least effective.

Many N_2O flux studies focus on the inputs into the N_2O-forming processes and the timing of significant flux events, while little has been done specifically comparing N_2O movement through different soil textures. Thus a laboratory method was developed to compare the effects of soil texture on permeability and redistribution of N_2O in three different soils (sandy clay, clay loam and clay). The stimulation of indigenous microbial populations through addition of nitrate and carbon substrates was also examined in this investigation. The data from the soil columns was compared to a model predicting redistribution of N_2O. The method developed to measure the redistribution of N_2O in soil columns was very effective. No major differences in N_2O gas redistribution in different textured soils at air-dry moisture content were seen. However, some evidence suggests that the fine textured soils retained N_2O for a longer period of time than course textured soils.

Additional field studies into the timing of N_2O production and surface flux in soils of different textures, landscape positions, and under different cropping systems need to be completed in order to improve the estimates of Manitoba's contribution to N_2O emission. Subsurface (profile) and surface (flux) investigations of N_2O concentrations along with correlation to controlling factors of production, consumption and redistribution will lead to improved recommendations for managing N_2O emissions. Controlled laboratory investigations are an important step in understanding these factors. Future laboratory experiments should manipulate the soil physical properties of texture and moisture, as well as inputs for biological production.

ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

LIST OF TABLES

TABLE OF FIGURES

1. INTRODUCTION

Existence is all about balance. On the atomic level and molecular level, balance exists between forces of attraction and repulsion. In chemical reactions, equilibrium is reached between reactants and products. The study of thermodynamics has shown that systems tend to reach equilibrium at a point where minimum enthalpy (energy) and maximum entropy (randomness) is reached. Civilizations and cultures throughout the centuries including our own believe that a balance exists in the natural world, and it in turn depends on numerous balanced cycles of use and reuse. Nitrous oxide (N_2O) is an important compound in the nitrogen cycle. The impacts of N_2O on the atmosphere of the earth are beneficial to a point. However, if too much N_2O is produced in the N cycle globally, atmospheric effects become detrimental and a serious concern.

Nitrous oxide is among a group of gases in our atmosphere known as greenhouse gases, which provide the unique effect of sustaining temperatures suitable for the development of life on our planet (Harvey, 1991). Solar radiation passes through the atmosphere to the earth's surface. The earth re-emits much of this energy as infrared radiation. While some of this radiation continues through the atmosphere into space, some of it is reflected by the greenhouse gases and continues warming the earth. The average temperature of the earth's surface has reached a steady state balance in which life can exist, with changes in temperature occurring slowly. Fossil records of pre and post ice age show that rapid changes in global temperature cause mass extinction of organisms

unable to adapt or evolve.

Nitrous oxide is also important in regulating stratospheric ozone levels. Cell destroying ultraviolet light is filtered from our atmosphere by ozone (O_3) and oxygen (O_2). The ozone layer exists in the stratosphere at approximately 25 km above the earth's surface. Ozone is formed by the reaction of O_2 with ultraviolet (UV) light as follows (Parry et. al., 1970):

$$O_2 + \text{UV light} \rightarrow 2O$$

The highly reactive oxygen atom combines with oxygen molecules to form ozone:

$$O + O_2 \rightarrow O_3$$

Nitrous oxide can react in the stratosphere nitrogen oxides and can destroy ozone through the following process:

$$N_2O + O \rightarrow N_2 + O_2$$

$$N_2O + O \rightarrow 2NO$$

$$NO + O_3 \rightarrow NO_2^{\cdot} + O_2$$

The oxides of nitrogen produced above can react with oxygen atoms, hindering ozone production. Badr and Probert (1993) give a more complete treatment of the various reactions involving the destruction of ozone.

The reaction of nitrous oxide with UV light to form oxygen atoms contributes to the balance between destruction and creation of ozone. The reaction occurs as follows:

$$N_2O + \text{UV light} \rightarrow N_2 + O$$

Rising N_2O levels in the atmosphere will leads to increased destruction of ozone until a new equilibrium state is reached. The question is whether there will be enough

ozone at this stage to continue protecting life on earth from ultraviolet radiation.

Nitrogen compounds usually enter the atmosphere from the soil in gaseous forms. Two major biological processes that contribute to the addition of nitrogen to the atmosphere are nitrification and denitrification. Nitrification produces fuel for denitrification, which in turn produces nitrogen gas. Forms of nitrogen found in these processes are as follows:

nitrification: $NH_4^- \rightarrow NO_2^- \rightarrow NO_3^-$

denitrification: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$

In agriculture, humans are changing the form of N in the nitrogen cycle to aid in crop production. Fertilizer production utilises atmospheric N, which is fixed and added to the pool of soil N (NO_3^- or NH_4^-). Investigations have shown that this results in increased amounts of N_2O being emitted from the soil into the atmosphere (Granli and B.·ckman, 1994, Aulakh et al., 1992). Nitrous oxide emission from soil is of particular concern due to both its effects as a potent greenhouse gas and an ozone destroyer.

The purpose of this thesis is the following:

1. To review of the process controlling N_2O emission, along with current methods used to measure and model this emission.
2. To determine if a large flux of N_2O occurs in a selected Red River soil in Manitoba during spring thaw. Large surface flux of N_2O has been found in other areas in the spring (Goodroad and Keeney, 1984, van Bochov et al., 1996). It is hypothesised that this same spring emission will occur in Red River valley soils in Manitoba, that it is dependant on subsurface production, and vegetative cover may affect this emission.

3. To examine subsurface N_2O gas in soil profiles.
4. To develop a method of investigating N_2O redistribution in a soil column and estimating diffusion coefficients.
5. To investigate the effect of soil texture on N_2O redistribution and movement at air-dry moisture content.
6. In addition, methods for storage of N_2O were investigated and developed.

This work contributes to a greater understanding of the production of N_2O in the agricultural environment and understanding anthropogenic impacts on the planet that we inhabit.

2. LITERATURE REVIEW

2.1 Importance of Nitrous Oxide (N_2O) in the Environment

The emission of nitrous oxide (N_2O) from agroecosystems is important as N_2O may contribute to global warming and destruction of stratospheric ozone. Nitrous oxide, carbon dioxide (CO_2), and methane (CH_4) are all greenhouse gases. They absorb electromagnetic radiation in the infrared region and trap this thermal radiation coming from the earth's surface, increasing the earth's mean temperature. Nitrous oxide is of particular concern because on a molar basis it adsorbs about 250 times more infrared radiation than CO_2, and has an atmospheric residence time of about 130 years (IPCC, 1992), which is thirteen times that of methane. Nitrous oxide has been estimated to account for 15% of the total global warming potential (Isermann, 1994). Atmospheric concentration of this gas is currently rising at a rate of approximately 0.5 to 0.8 ppb(v/v) $year^{-1}$ (Khalil and Rasmussen, 1992). Studies of ice cores indicate that before 1700, N_2O concentration was about 285 ppb(v/v), and had remained near this level since 0 AD. The current atmospheric concentration has risen to 310 ppbv (IPCC, 1992).

Ozone (O_3) in the upper atmosphere screens out most of the sun's ultraviolet radiation. Nitrogen oxides produced from N_2O by photochemical reactions in the stratosphere destroy O_3 resulting in increased ultraviolet radiation at the earth's surface (Granli and Bockman, 1994).

2.2 Sources of N_2O

It is estimated that 9.6 to 12 % of known contributions to atmospheric N_2O result from combustion of fossil fuels and biomass (IPCC, 1992). However, it is also produced by many of the microbial reactions in the soil (Banin, 1986). In fact, soil is a major global source of N_2O. Isermann (1994) estimates soil produces about 67.5 % of atmospheric N_2O. In the soil, N_2O can be produced through both chemical and biological processes. Nitrous oxide is produced mainly as an intermediate in the processes of nitrification and denitrification (Granli and Bockman, 1994).

2.2.1 Chemical

Denitrification occurring from chemical reactions that do not involve biological organisms, is known as chemodenitrification. It can occur through oxidation of organic nitrogen (N) by nitrite (NO_2^-) to form N_2 gas (Christianson et al., 1979, Christianson and Cho, 1983). Some of these reactions will now be discussed.

An important chemodenitrification reaction which can produce nitric oxide (NO), and nitrite (NO_2^-) in the soil is the disproportion of nitrous acid. This is important in the discussion of N_2O production in that it provides substrate (NO_2^-) for denitrification and hydroxylamine (NH_2OH) formation. The reaction occurs as follows (Nelson and Bremner, 1970).

$$2HNO_2 \rightarrow NO + NO_2^- + H_2O$$

This is most likely to occur in acid soils high in organic matter content. This process may also occur in neutral soils in undisturbed microsites where high solute concentration of NO_3^- and high pH has occurred due to freeze concentration (Shapiro,

1961).

Nitrous oxide production can occur in chemodenitrification when conditions are such that hydroxylamine (NH_2OH) is produced and then proceeds through a decomposition reaction as follows (Nelson, 1982).

$$2NH_2OH \rightarrow N_2O + 2H_2 + H_2O$$

Nitrous acid can react with compounds containing free amino groups (amino acids, urea, amines, etc.) under acidic conditions to form N_2 gas (Smith and Chalk, 1980, Christianson and Cho, 1983). The reaction is often referred to as the Van Slyke reaction and proceeds as follows.

$$R{\bullet}NH_2 + HNO_2 \leftrightarrow R{\bullet}OH + N_2 + H_2O$$

Chemical oxidation of organic N by NO_2^- producing nitrogen gas (N_2) has been shown to occur in soil (Christianson et al., 1979). Nitrite has a tendency to react with soil constituents forming the nitrogen gases N_2, N_2O, NO, or NO_2 (Nelson, 1982). Other reactions of nitrite to form nitrogen gases are discussed by Nelson (1982), but are not considered to contribute significantly to chemodenitrification. Both Christianson (1981) and Nelson (1982) give a more complete explanation of the above reactions.

The amounts of N_2O produced in chemodenitrification are thought to be insignificant when compared to those produced in nitrification/denitrification reactions (Hutchinson and Davidson, 1993). However, chemodentitrification is important in that it may affect the amount and proportion of reactants (NO_2^-) in the biological processes of nitrification and denitrification.

2.2.1.1. Chemodenitrification and Low Temperature

Low temperatures can enhance chemodenitrification rates. When a liquid freezes, two phases exist: a liquid phase and a solid phase. A solute may be "frozen out" of the solid phase, and concentrated in the liquid phase (Shapiro, 1961). This increase in solute concentration lowers the freezing point of the remaining liquid. Increasing reactant concentration favors formation of product (le Chatalier's principal), which is another example of the tendency of a system to proceed towards maximum entropy and minimum enthalpy. The reactants in chemodenitrification reactions can thus be concentrated to levels that favor these reactions. Peak levels of Van Slyke type chemodenitrification were found to occur at the temperature that the soil water first freezes and are thought to be caused by freeze concentration of NO_2^- (Christianson and Cho, 1983). Soil water has been found to exist at temperatures as low as -40 ° C, which means that this reaction may be enhanced by low winter temperatures in soil (Anderson and Morgenstern 1973, *in* Christianson and Cho, 1983).

2.2.2 Biological

The two main biological reactions producing N_2O in soil are nitrification and denitrification. While biological nitrification occurs in the presence of oxygen, biological denitrification occurs when oxygen is absent. These processes and their major controlling factors are discussed in the following sections.

2.2.2.1. Nitrification.

Nitrification is the process where ammonium (NH_4^+) is biologically oxidized to NO_2^- or NO_3^- to produce energy. It can occur though both autotrophic (using CO_2 for carbon) and heterotrophic (using organic matter for carbon) processes, but the major form of nitrification in soil is chemoautotrophic (Schmidt, 1982; Killham, 1994). Chemoautotrophic denitrification is an aerobic process, carried out mainly by ammonium oxidizers (prefix of *Nitroso-*) and nitrite oxidizers (prefix of *Nitro-*). *Nitrosomonas* and *Nitrobacter* are the two more commonly known gram-negative nitrifying bacteria. Carbon dioxide is the carbon source for biosynthesis, and energy is obtained by oxidation of NH_4^+. Ammonium is obtained by mineralization of soil organic material or fertilizer. Nitrification can decrease the pH of the soil in localized areas due to the formation of hydrogen ions (Killham, 1994). Nitrification occurs in two main steps:

Step 1
Nitrosomonas:
Oxidation state:
-3 +3

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+ + \text{energy}$$

Step 2
Nitrobacter:

+3 +5

$$NO_2^- + .5O_2 \rightarrow NO_3^- + \text{energy}$$

The oxidation of NO_2^- is more rapid than the oxidation of NH_4^+, thus there is rarely a build up of NO_2^- in the soil (Nelson, 1982).

Heterotrophic nitrification can also occur, but rates appear very low when compared to autotrophs. Kuenen and Robertson (1988) found that nitrification rate could not be estimated by the accumulation of NO_3^- or NO_2^-, as in some cases there may be no accumulation at all. In fact, heterotrophic rates even may be higher than previously thought because the usual method of measuring their nitrification rate assumed an

accumulation of NO_3^- or NO_2^-.

Robertson (1989) depicts the factors controlling nitrification from the global to the microsite scale (Figure 2.1). The major controlling factors and their relevance in N_2O production will be discussed in more detail in later sections.

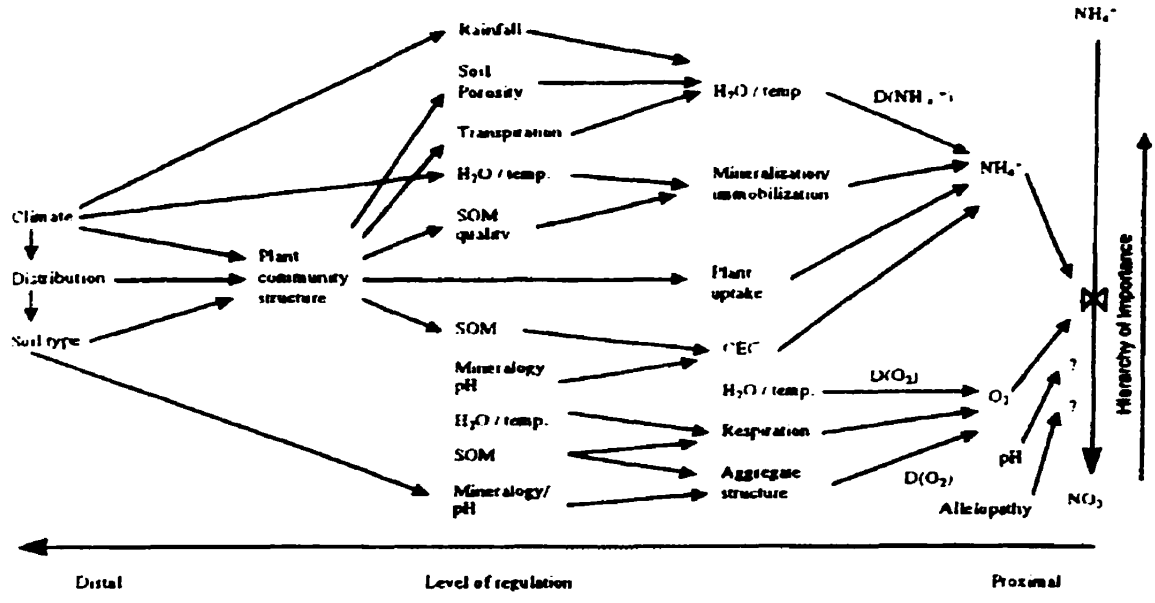

Figure 2.1 Regulation of nitrification in soil. D (NH_4^+) and D (O_2) indicate diffusion of NH_4^+ and O_2, respectively (Robertson, 1989).

Nitrite oxidizers (*Nitrobactor*) rarely produce N_2O (Goreau et al., 1980), however ammonium oxidizers (*Nitrosomonas*) may use NO_2^- as an electron acceptor when O_2 is limited. An intermediate of the ammonium oxidation reaction may be N_2O (Firestone and Davidson, 1989). Nitrite, or intermediates between NH_4^+ and NO_2^- may chemically denitrify to N_2O in acid soils.

Figure 2.1 shows the distal and proximal regulators of nitrification. Denitrification is dependent upon the products of nitrification, which are used as electron acceptors.

2.2.2.2. Denitrification.

Heterotrophic denitrification requires an organic carbon (C) source for energy and biosynthesis, a terminal electron acceptor (NO_3^-, NO_2^-, N_2O), water, anaerobic conditions, minerals, and a near neutral pH. In denitrification, NO_3^- replaces O_2 as the terminal electron acceptor in microbial respiration (Aulakh et al. 1992). This is sometimes referred to as nitrate respiration. It is carried out by facultative anaerobes, mainly heterotrophic bacteria. *Pseudomonas* and *Alcaligenes spp.* are two common microorganisms that perform this process in soil. A more compete list of known denitrifying organisms is given in Nelson (1982), and Beauchamp et al. (1989). As the organic C is oxidized to produce energy, nitrate is reduced to nitrogen in the following steps:

Oxidation state:	+5		+3		+2		+1		0
Nitrogen form:	NO_3^-	→	NO_2^-	→	NO	→	N_2O	→	N_2

The local pH of the soil may increase during denitrification because of the formation of hydroxyl ions in proportion to the amount of nitrate denitrified (Killham, 1994). Because most soils have a high buffering capacity, changes are slight.

The presence of O_2 has been shown to inhibit the synthesis of nitrate reductase, the enzyme used in denitrification. (Hochstein and Tomlinson, 1988). This is probably an evolutionary trait as less energy is consumed when using O_2 as an electron acceptor than N compounds, making it a more efficient pathway.

Denitrification can also occur in autotrophic and fermentive bacteria. Autotrophic denitrification involves the oxidization of inorganic compounds, such as iron (Fe^{2-}) and

sulfer (S^{2-}) for energy production, instead of organic carbon. This usually occurs in shallow water sediments where nitrate diffuses into zones rich in iron sulfide. A group of fermentive bacteria can carry out dissimilatory reduction of NO_3^- to NH_4^+. The main electron donor for most of this fermentive group is formate. This reduction reaction occurs primarily in carbon rich anaerobic environments (Tiedje et al., 1982 *in* Killham, 1994). It is uncertain at this time what role these bacteria may have on N_2O production at this time.

The term denitrification actually refers to the step where nitrite is reduced to gaseous nitrogen product. Nitric oxide (NO) is frequently referred as the first gaseous intermediate (Payne, 1981) although it is argued that NO may not be a true intermediate in the denitrification pathway, but merely a byproduct of the reaction (Amundson and Davidson, 1990). Nitrous oxide is definitely an intermediate product of denitrification, readily diffusing from the site of production (Payne 1981). Many soil microorganisms are able to denitrify, although a variety of pathways exist, both complete and incomplete. Intermediate products such as N_2O can accumulate and escape the denitrification pathway, depending on surrounding conditions. A summary of factors affecting denitrification from the macro to the micro scale is presented in Figure 2.2.

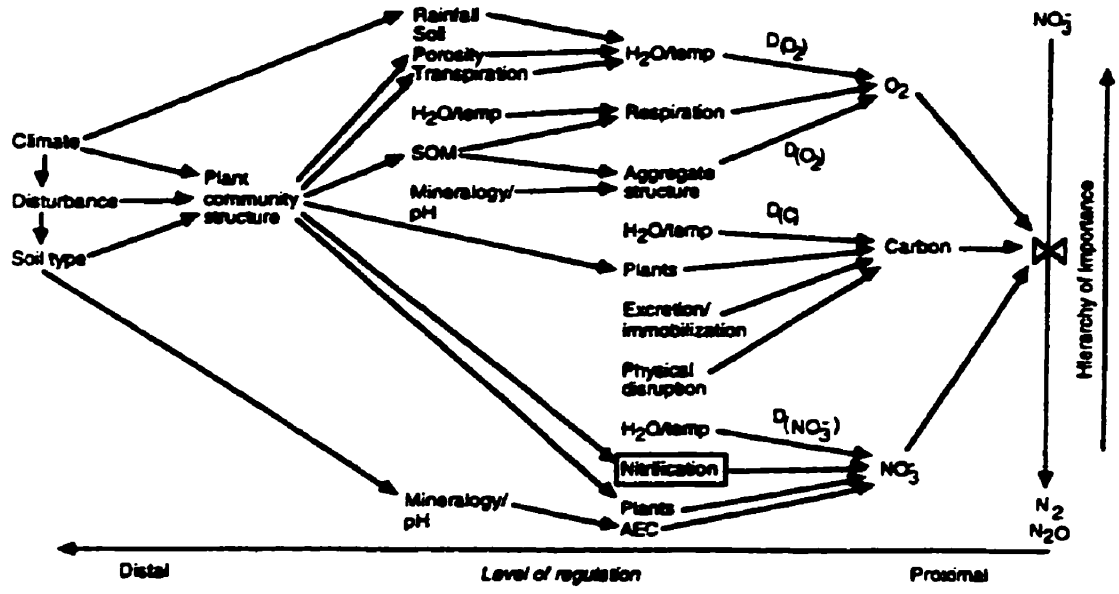

Figure 2.2 Regulation of denitrification in soil. D (O_2), D(C) and D (NO_3^-) indicate diffusion of O_2, C, and NO_3^-, respectively (Robertson, 1989).

2.2.2.3. Factors Influencing Biological Production of N_2O

Temperature, moisture, substrate (mainly C,N sources), pH, and aeration status of the soil all affect microbial viability and growth (Prescott, et al., 1993). Production of N_2O by soil microbes is influenced by these same environmental factors. The interaction between nitrification and denitrification along with possible pathways of N_2O production is well represented in Figure 2.3 (Knowles, 1978).

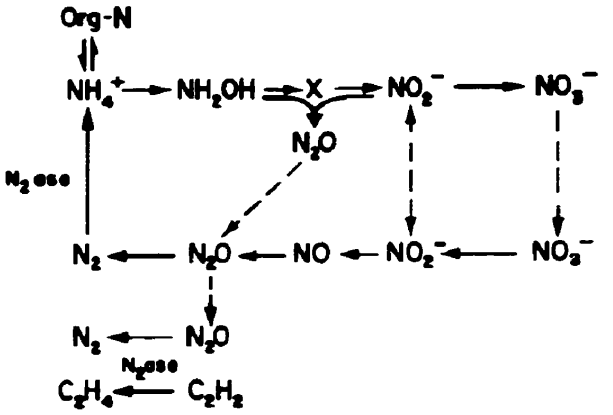

Figure 2.3. Possible interrelationships of nitrification (top), denitrification (middle) and nitrogen fixation (bottom) with N_2O production (Knowles, 1978).

2.2.2.3.a. Temperature.

Temperature affects the rate of microbial processes. In general, the lower the temperature, the slower reactions occur. Optimum temperatures are different for each type of organism, and some organisms may shift their optimum temperature to better suit their environment. Nitrifying and denitrifying microorganisms have adapted to the temperatures of their environments. Dramatic shifts in temperature, over short time frames, generally result in organism death.

Optimum temperature ranges for different nitrifying bacteria range from 20 to 40° C. Soil organisms have adapted to the optimum nitrifying temperatures of the environment that they live in (Nelson, 1982). Cold wet soils do not support nitrification. It has been found to proceed as the soil temperature rises to between 4 to 5° C, which are typical spring soil temperatures (Anderson and Boswell, 1964).

The optimum temperatures for denitrification range from 30 to 67 ° C (Granli and Bockman, 1994). The wide range is due again to bacterial adaptations to different

temperatures (Malhi et al. 1990). Dorland and Beauchamp (1990) reported that the threshold temperature for biological denitrification was as low as -2 C in unfrozen soil. Knowles (1982) reported denitrification rates increasing as temperature increased from 10 to 35 C.

The relationship between production of N_2O and temperature results from a combination of the effects on nitrification and denitrification. Nitrous oxide production from nitrification increases with increasing temperature. However, the production of N_2O decreases with increasing temperature in denitrification. The net effect is that N_2O emissions increase with increasing temperature (Granli and Bœckman, 1994).

2.2.2.3.b. Moisture.

Water is a crucial factor in many soil microbial processes. It is a source of hydrogen ion and a solvent for substrates, minerals, and gases. Water is also a reactant in hydrolysis and condensation reactions. The moisture content of the soil affects the concentration of solute inside the microbe, and may affect organism viability. Water also affects the soil environment by restricting gas exchange.

Soil water provides the O_2 and the HCO_3^- needed for nitrification to proceed. Nitrification ceases when soils become saturated (Alexander, 1977). Moderately high moisture levels (medium low suctions) enhance denitrification (Figure 2.4).

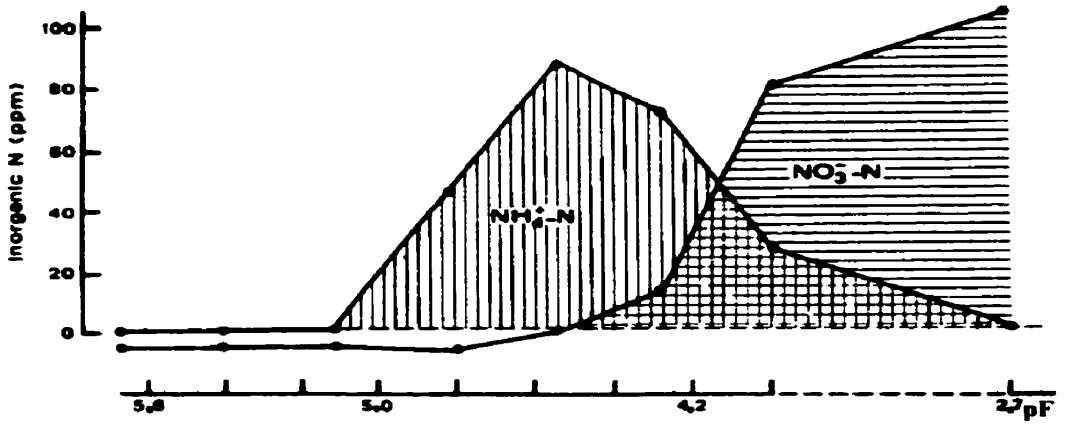

Figure 2.4 The influence of soil pF on accumulation of NH_4^+-N and NO_3^--N. (Dommergues, 1977 *in* Nelson, 1982.). pF = log (osmotic potential + matric potential) when potentials are expressed as cm of water in a column.

The effect of water content on denitrification in soil under controlled laboratory conditions has been examined in many studies (Freney et al., 1979; Bandibas et al. 1994, Myrold and Tiedje, 1985). Denitrification rate increases most markedly at water contents above 60% water filled pore space (WFP). Groffman and Tiedje (1988) used intact cores under typical field moisture regimes in denitrification studies. They found that as soils were dried from saturation to field capacity, denitrification rates decreased rapidly. As WFP decreased from 60 to 20%, denitrification rates decreased farther.

Denitrification may decrease in soils that are held at high water contents for extended periods of time due to nitrate limitation, as nitrification cannot renew the NO_3^- or NO_2^- reserves (Granli and Bockman, 1994).

Robertson and Tiedje (1987) showed denitrification can occur in aerobic regimes, implying that there may be localized anaerobic conditions.

Higher denitrification rates exist in soils that cycle between wet and dry phases than in soils that maintain constant high water contents. Groffman and Tiedje (1988)

observed a hysteresis effect during wetting and drying cycles, both in respiration and denitrification rates. When soils were dried from saturation denitrification rates decreased rapidly, with smaller decreases as WFP went from 60 to 20%. Immediately after rewetting soils from 20 to 60% WFP, denitrification rate increased much more sharply than the previous decrease, with only small increases as more water was added. This effect is probably due to substrate release, which will be discussed more detail in the substrate section.

The maximum N_2O emission generally occurs when both nitrification and denitrification can proceed, and neither reaction reaches it's respective endpoint. This phenomena usually occurs between 45 to 75% WFP (Granli and Bøckman, 1994).

The production of N_2O during nitrification may occur when NH_4^+ is converted to NO_2^- (Bremmner and Blackmer, 1978, Schmidt, 1982). Many researchers (Weier et al., 1991, Matson et al., 1990) found much more N_2O emitted from warm wet soils than cool dry ones, suggesting that moisture and temperature are key factors in N_2O production.

2.2.2.3.c. Aeration Status.

Soil water is intimately linked with soil aeration. The amount of oxygen that can diffuse into the soil is mainly dependent on the amount of air-filled pore space (Figure 2.5). Since nitrification and denitrification depend on the aeration status of the soil, most appropriate measures of soil moisture in studying these processes are either air filled pore space, or water filled pore space.

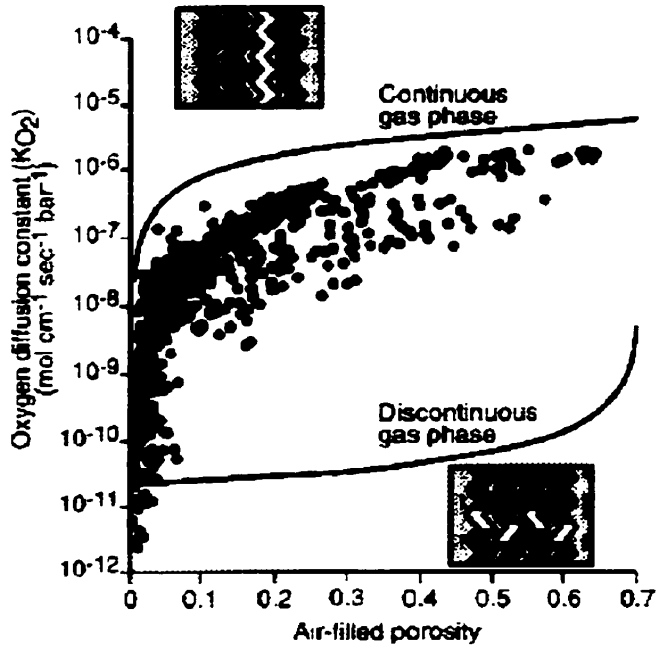

Figure 2.5 Diffusivity of O_2 as a function of soil air-filled porosity. Gaseous diffusion at any level of porosity is determined by the continuity of the enclosed air spaces which, in turn, is determined by the texture and water content of the soil media. (Focht, 1992, *cited in* Livingston, G.P., and Hutchinson, G.L. 1995).

The percentage of pore space filled with water is also well correlated with microbial activity by Linn and Doran (1984). Soil incubated in the laboratory at sixty percent WFP showed maximum aerobic microbial activity. Below 60% WFP, water content limits microbial activity. Above 60%, activity is limited by reduced aeration.

Anaerobic or partially anaerobic conditions occur when the oxygen diffusion rate is insufficient to supply the demand from microbial respiration. Reduced partial pressures of O_2 can occur in aerobic conditions as follows. When microbial activity increases, oxygen consumption rates increase as well. As a result, increased biological activity can lead to localized oxygen limited conditions if the supply of O_2 does not meet the demand. These conditions may exist under aerobic regimes in microsites in/on aggregates where oxygen diffusion is restricted (Parkin, 1987., Greenwood, 1975).

Although nitrification is an aerobic process, and denitrification is an anaerobic one, they can occur simultaneously in the field. Both of these processes have been shown to occur simultaneously under aerobic regimes (Bremner and Blackmer, 1978., Cho, 1982). Robertson and Tiedje (1987) demonstrated that both denitrifiers and nitrifiers may produce N_2O in intact soil cores with air.

Figure 2.6 displays the general relationship between nitrogen gas production and water filled pore space. As the amount of water increases, the figure shows that denitrification is more likely proceed to N_2. At high soil water content diffusion of soil gases is slow, restricting O_2 availability and allowing N_2O to completely denitrify to N_2. The position of the maxima can vary with soil type and condition.

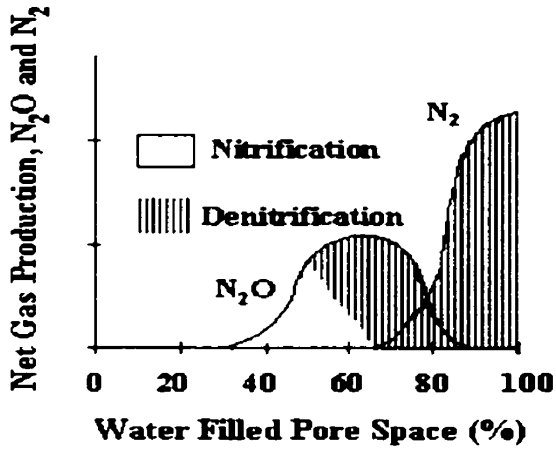

Figure 2.6. Relationship between of WFP and relative flux of N_2O and N_2. Nitrification and denitrification produce N_2O. (Davidson, 1991 *in* Granli and Bockman, 1994).

2.2.2.3.d. Substrate.

Microbial metabolism is dependent on substrate and mineral availability. Carbon and nutrient sources that stimulate microbial processes in soil include root exudates and decomposing organic matter (Aulakh et al. 1984, Prade and Trolldenier, 1988, Christensen et al. 1990.). Dissolved minerals, nutrients and microbes can be distributed with water throughout soil or deposited as the soil dries out. Rewetting of soil can increase the rate of mineralization and the availability of nutrients for the N_2O producing processes of nitrification and denitrification. Substrates for microbial process become more bioavailable after drying and rewetting or freezing and thawing soils.

Often a flush of microbial activity occurs after drying and rewetting events, due to the mineralization of carbon and nitrogen in the drying phase (Birch, 1958., Sorensen, 1974). Rapid increases in nitrification rate occur after soil is rewetted by rainfall (Chiang et. al., 1972, Figure 2.7). The flush in aerobic heterotrophic respiration reduces oxygen, making nitrate/nitrite competitive as an electron acceptor for the facultative anaerobic denitrifying heterotrophs. Increased denitrification is strongly correlated to the magnitude of mineralization (Groffman and Tiedje, 1988).

With anaerobic incubation at 30°C, Patten et al. (1980) found that air drying soils markedly increased their ability to denitrify after rewetting, and that this effect increased as drying temperature increased from 25 to 100 °C. Similar results were noted in a study of denitrification in the presence of oxygen (Kroeckel and Stolp, 1986), where denitrification rates were ten times greater in air-dried remoistened soil than in undisturbed field-moist samples.

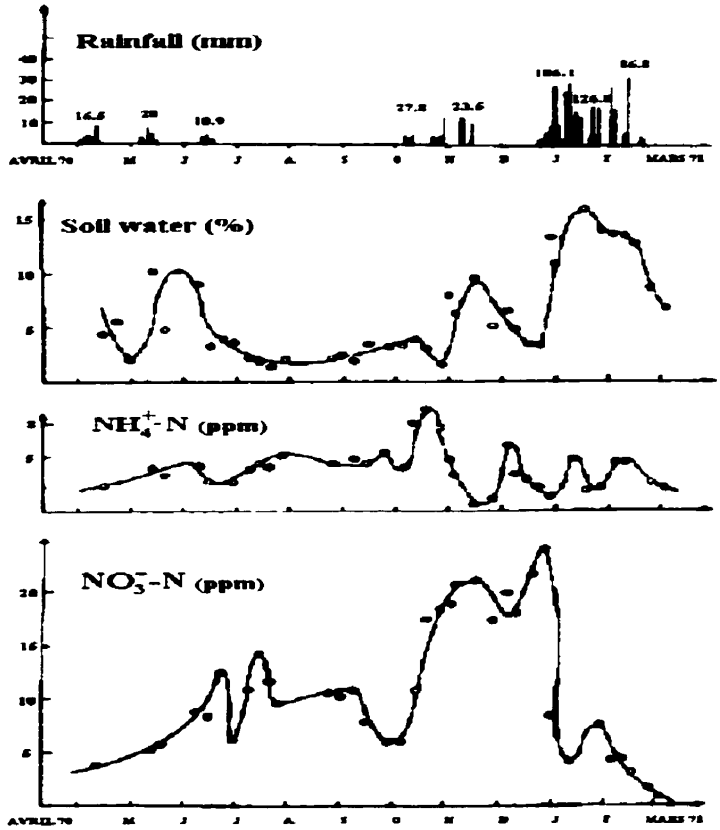

Figure 2.7 Seasonal measurements of rainfall, soil moisture and mineral nitrogen. (Chiang et al., 1972 *in* Nelson, 1982).

Water freezes at 0° C, and due to the polar nature of the molecule, expands as it becomes a solid. The expansion of the ice formed can break up soil aggregates, and may release additional nutrients to the environment (Christensen and Christensen, 1991).

The ammonium used in nitrification is produced through degradation of organic matter. Ammonia (NH_3) is produced by decomposition of proteins, amino acids, and other N-containing organics. NH_3 equilibrates with NH_4^+ in the soil, which is subsequently used in nitrification.

The availability of carbon is a major driving force of denitrification (Parkin, 1987., Christensen et al., 1990., Christensen and Christensen, 1991). Higher

denitrification rates resulting in N_2O gas emission have been found mainly in the presence of growing plants, as long as NO_3^- was not limiting (Rolston et al. 1979., Smith and Tiedje, 1979a., Mosier et al. 1990). This is thought to be a result of decaying roots and root exudates supplying energy (carbon) for denitrification.

Fertilizer nitrogen (NH_4, NO_3^-, $CO(NH_2)_2$, etc.) is added to the cultivated soils to increase crop yield. This nitrogen, once converted to NO_3^- and NO_2^-, can be used as an electron acceptor in denitrification where anaerobic conditions occur. Alternatively, high amounts of carbon can remove nitrate from solution and thereby inhibit denitrification. For example, Craswell (1977) found that addition of straw could cause increased immobilization of NO_3^-, making it unavailable for denitrification.

Rewetting and thawing soil can increase N_2O production. N_2O emissions often occur following a precipitation or irrigation event (Conrad et al. 1983., Granli and B.·ckman, 1994). This is due to the flush of microbial activity including nitrifyers and denitrifyers following the rewetting of the dry soil. The freeze/thaw action mentioned above may also increase N_2O production by increasing nutrients available for microbial processes.

When NO_3^- or NO_2^- are present in large amounts, N_2O will build up and be released from the soil. This is because NO_3^- and NO_2^- are preferred electron acceptors to N_2O in the denitrification process (Cho, 1985, Cho et. al. 1997., Firestone, et al., 1980).

2.2.2.3.e. pH.

The pH of the environment dramatically affects microbial growth and viability. Each species has adapted to a specific pH range and optimum (Prescott et al., 1990).

The majority of studies set lower limits of nitrification in soil at pH 4-8 (Nelson, 1982). Nitrification rate generally increases with pH until neutral or slightly alkaline conditions exist. Goodroad and Keeney (1984) found nitrification rates to be 80% higher at pH 6.7 then at pH 4.7. The rate of nitrification decreases dramatically below pH 6 (Alexander, 1977).

Most denitrifying organisms have a pH optimum between 6 and 8. Although denitrification is favored at higher pH, it has been found to occur at as low as 3.5 (Aulakh, et al. 1992). Decreasing pH may reduce availability of molybdenum. Since NO_3^- reductase used in denitrification contains molybdenum, synthesis of this enzyme is affected. At decreasing pH, NO_2^- formed by reduction of NO_3^- could become toxic. (Firestone, 1982). Low pH may also indirectly control denitrification by limiting carbon availability to organisms (Koskinen and Keeney, 1982).

The effect of pH on N_2O emission is complex, as previously stated N_2O evolution is a product of both denitrification and nitrification processes which occur simultaneously in the soil. Both processes flourish between pH 6 and 8. Nitrous oxide is more likely to be reduced under acidic conditions than NO_3^-. Reducing pH decreases the rate of denitrification, but favors N_2O evolution (Cho and Sakdinan, 1978; Koskinen and Keeney, 1982; Weir and Gillam, 1986). When pH is below 6.5, N_2O can make up more than half the N gas evolved from acidic soil (Alexander, 1977). There is no clear pH trend showing when N_2O is the favored product during the nitrification process (Granli and Bockman, 1994).

2.2.3 Physical Diffusion Controls

An understanding of the factors controlling evolution of N_2O into the atmosphere from production sites is necessary in order to accurately interpret N_2O measurements and form reasonable conclusions. The two main classes of factors that control the diffusion of N_2O from the soil are the physical effects of concentration and solubility, and the chemical/biological properties of N_2O production and consumption.

2.2.3.1. Concentration Gradients.

As previously stated, all systems reach equilibrium at a point where minimum enthalpy and maximum entropy is reached. Gases are no exception to this rule, and as a result tend to move from areas of higher concentration to those of lower concentration.

Fick's law describes diffusion through a gas or liquid as follows (Hillel, 1980):

$$q_d = - D \, dc/dx \qquad [2.1]$$

where: q_d = mass diffusing across a unit area per unit time
D = diffusion coefficient in area/per time
c = concentration
x = distance
dc/dx = the concentration gradient

D is a property of the matrix and in soil it depends upon pore content and tortuosity. The larger the change in concentration per unit distance, the faster the diffusion.

The value for q_d across the soil surface can be measured to determine N_2O surface flux (equation 3.1). This value can then give clues the magnitude of D, or the size of the concentration gradient.

2.2.3.2. Soil Moisture.

Diffusion of a gas through soil is not only a function of pore size and distribution, but also of pore content. Nitrous oxide, like all other gases, diffuses faster through soil air than through soil water (Hillel, 1980). Diffusion in soil air (*Ds*) is a function of air filled porosity, whereas in diffusion in soil water (*Dw*) is a function of water filled porosity. Diffusion of gases in soil water is 10,000 times less rapid than in air (Grable, 1966). The effective diffusion coefficient is a result of the influences of both *Ds* and *Dw*.

The amount of water held by a soil depends upon the soil's texture. Four main forces govern the position of water in a soil: gravitational potential, pneumatic pressure, osmotic suction, and matric suction. Two of these forces, matric suction and osmotic suction, are texture dependent and thus will be discussed in greater detail. Matric suction is the negative hydraulic pressure potential found in unsaturated soils and is due to capillary and adsorptive forces. As capillary pressure is inversely proportional to the radius of a meniscus (Hillel, 1982), smaller pores (fine textures) will have stronger capillary forces than larger ones. Fine textured soils have large cation exchange capacity (CEC), when compared to coarse textured soils. Soils with large CEC have more positively charged cations (salts) on the exchange resulting in large osmotic forces and electric double layers. This causes a relatively thick water layer to be adsorbed on a soil particle's surface (Hillel, 1982). Adsorptive forces in a coarse textured soil are generally negligible when compared to fine textured soils.

Particle surface area affects the amount of water adsorbed to the surface. A

volume of clay particles has a much larger surface area than the same volume of sand particles. This means there is more surface area available for water to adsorb to these surfaces. Monmorillonite, the type of clay found in the prairies, has the ability to swell as water is retained in the interlayer space contributing to the increased water holding capacity of fine textured soils.

The textural effect on the ability to retain water in soil has been investigated for many years. Each soil has a unique characteristic soil moisture curve, while coarse textured soils emptying at lower suctions than fine textured soils (Figure 2.8).

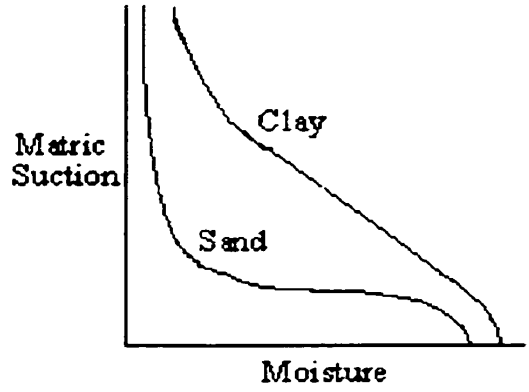

Figure 2.8 Soil moisture characteristic curve (Childs, 1940 *in* Hillel 1982).

The amount of soil water can affect the diffusion of gas through soil by changing the tortuosity of the diffusion path, and partitioning the gas between a dissolved (liquid) and free (gas) state. Soil texture should have some effect on gas diffusion due to varying water holding capacities.

2.2.3.2.a. Tortuosity Effect.

Soil water can disrupt air-filled pore continuity and have a major influence on the diffusive velocity of soil gases, including N_2O. Kristensen and Lemon (1964) discussed

some problems with permeability of O_2 through a water film even though the rest of the soil pores were open. They concluded that even a thin film of liquid could dramatically slow the rate of gas diffusion. The apparent diffusion rate of gases in soil air is thought to be approximately 2/3 that of the diffusion coefficient of gas in air due to the tortuosity of soil pores (Penman, 1940).

When a soil pore is not completely filled with water, diffusion pathways may still be affected. Thin films held together by cohesive forces may adhere to soil particles, blocking pores, thus increasing tortuosity. The smaller the pore opening, the greater the water film's ability to remain intact (Brady, 1990). Clay and humus can swell when wet, closing pores and increasing tortuosity (Hillel, 1980).

In studying the movement of gases through soil, researchers are concerned mainly with diffusion through soil air. As long as a continuous path of air filled pores exists, the movement of gases through soil air far exceeds that of the gas though liquid. However, one must keep in mind the effects of solubility on the partitioning of N_2O between the dissolved phase and the gas phase, as this will have an increasing effect on diffusion as soil water increases (2.2.3.2.b.).

2.2.3.2.b. Chromatography Effect.

As a concentration of N_2O diffuses through the soil, some of it is temporarily held in soil water, and later released. For example, as a concentration of N_2O passes a point which contains moisture, some of the gas will dissolve in the water, as the concentration gradient favors this. As the N_2O continues to diffuse though the soil air, the concentration gradient will soon favor movement of dissolved N_2O back into the soil air.

Thus the movement of N_2O though soil air can be retarded by the presence of soil water even if tortuosity is not significantly affected. This effect is termed chromatographic as a similar effect allows a gas mixture to be passed through a chromatographic column in order to separate it into component compounds based on the affinity of each compound to the filler in the column.

The greater the amount of water in a soil, the larger the fraction of N_2O dissolved in liquid and resulting retardation of N_2O movement. Nitrous oxide produced in a soil with high moisture content will diffuse much more slowly than in a low moisture soil, but diffusion will continue over a longer period of time, unless the N_2O is reduces to N_2. It seems likely that because of the higher moisture holding capacity in a fine textured soil, this effect will be more pronounced than in course textured soils.

2.2.3.3. Temperature.

Changes in soil temperature can affect concentration gradients of N_2O in soil. An increase in soil temperature will cause a decrease of the solubility of N_2O in soil water, which will then result in an increase in soil atmosphere N_2O. This N_2O will then diffuse throughout the profile areas of lower N_2O concentration.

Weiss and Price (1979) empirically describe the temperature dependent solubility of N_2O in pure water with the following equation:

$$\ln K = -64.8539 + 100.2520(100T) + 25.2049\ln(T/100) \qquad [2.2]$$

where: K = the concentration of solute (moles per solution $kg^{-1}atm^{-1}$)
T = temperature (K)

The solubility of N_2O over a range of temperatures is graphically represented in

Figure 2.9. Note that during typical soil temperature ranges of a spring thaw (-5 to 5 C), the rate of change in N_2O into soil is at a maximum.

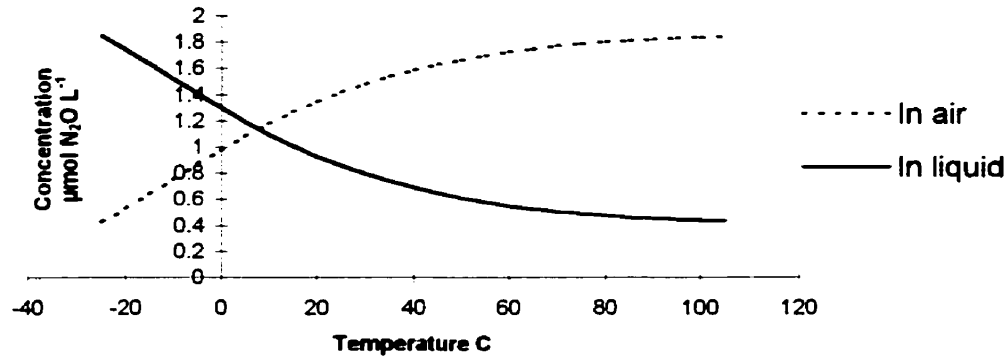

Figure 2.9 Solubility of N_2O in water at different temperatures, assuming no change in phase (Based on Wiess and Price, 1979).

When water freezes, ice crystallizes out of solution in a pure state. As a result, cooling soil water to the point that it changes phase will cause the concentration of N_2O in soil air to increase. This will increase the concentration gradient, resulting in increased diffusion.

Ice formation may present a barrier to diffusion. If the gas cannot move around an ice lens or pocket, it is trapped until the barrier is removed. As ice melts, the N_2O may then be permitted to continue it's movement to the surface.

Spring thaw is often considered to be a time of major increases in N_2O production (Section 2.5.2) and temperature effects on the physical movement of this gas should be considered when measuring flux at this time.

2.3 Sinks

The major sinks for N_2O are stratospheric photolysis, reduction to N_2 via denitrification in soil, and storage as a result of dissolution in water. As one may suspect, N_2O dissolved in soil water, aquifers, and surface waters may later be released to the atmosphere, providing further reduction has not already occurred (Ronen et al., 1988; Rice and Rogers, 1993).

2.4 Agricultural Influences on Nitrous Oxide Production

In undisturbed ecosystems, N_2O is not considered a major product because there is little NO_2^- or NO_3^- available as these N forms are rapidly immobilised by plant roots or soil microbial populations occurring in the plant rhizosphere. However, when conditions are changed, for example addition of NO_3^- or NH_4^+, or removal of plant cover, the reaction may favor the product of N_2O. Many investigations have been conducted on the influences of fertilizer and manure applications on N_2O evolution (Mckenney et al., 1980., Nyborg et al, 1997). Paul et. al. (1997) found increased denitrification below the root zone after manure application. Eichner (1990), summarizing research in this area, reports increasing N_2O with increasing fertilizer application. However, as the processes of denitrification and nitrification depend only in part on nitrogen inputs, direct correlation to this factor is difficult. Robertson (1993) proposed that the impact of applied N on global N_2O is greater than current short-term studies estimate, because most N in crop residues and harvested food will eventually become N_2O or N_2. Table 2.1 estimates agriculture's share of the production of N_2O, which includes biomass

combustion, cultivation, and recultivation, to be 36.5% of the total estimated emission. Of this 36.5%, 27% is due to soil cultivation which includes technical and biological N fixation.

Table 2.1 Estimated sources and sinks of N_2O and agriculture's share (1988/1989) (From Beauchamp, 1997 adapted from Isermann 1994).

	N_2O-N ($Tg\ yr^{-1}$)	%
Total known sources	14.8 ± > 5.1	100.0
Agriculture's share	5.4 ± > 1.0	36.5
Cultivated soils	4.0 ± > 0.8	(27.0)
Sinks		
Removal by soils	?	
Photolysis in stratosphere	10 ± 3.0	
Atmospheric Increase	3.8 ± 0.8	

2.5 Measurements of N_2O Flux/Production in Agriculture

2.5.1 Variability in N_2O Field Measurement

Soil microbial processes such as nitrification and denitrification, which may result in emissions of N_2O, are highly variable across the landscape (Corre et al., 1996., Burton and Beauchamp, 1985). In order to understand this phenomenon, one requires some understanding of the variability in two major driving forces of these microbial processes, water and carbon. The variability of N_2O emission can in part be explained by this variability in water content and substrate availability.

The amount of water held in soil depends on the soil's ability to store water, the ability of accumulated water to be removed by drainage, surface evaporation, uptake/transpiration by plants and water supplied by precipitation or irrigation.

Topography, soil texture, climate, vegetative cover and management practices all influence these factors. Water content in a field varies both spatially and temporally. Farmers either irrigate, or rely on precipitation for successful crop production; either system producing periodically higher soil water content. Burton and Beauchamp (1985) demonstrated that although there is a correlation between denitrification and water filled porosity (WFP) in a field, other factors cause variation in this process. Although two sites showed increases in denitrification at approximately 60% WFP, a third site did not show an increase until 75% WFP. This difference was attributed to variability in factors such as carbon and nitrate/nitrite conditions.

Both carbon and N are widely variable across the landscape. Carter et al. (1997) found mean soil C and N densities between sites in cool eastern agricultural soils to range from 3.1 to 13.1 and 0.36 to 1.05 kg m^{-2}, respectively. The C:N ratio between sites ranged from 8.3 to 17.1. Variation of C:N both between sites and within sites was high.

Just as carbon can vary throughout a field, it can also vary with soil depth. Rooting distribution and depth influences profile N_2O concentrations and surface flux because root exudates are a major source of microbial substrate. Smith and Tiedje (1979a) demonstrated that denitrification can decrease rapidly in the first few millimeters away from roots. The deposition by roots of energy-rich substrate deep in the soil profile drives microbial processes at that depth. Gilliam et al. (1978) measured significant denitrification rates at depths of 30 – 75 cm, which corresponded to the location of C sources. Available C and N sources can be distributed throughout the soil profile by the movement of water after precipitation events (Voltz et. al., 1976).

Soil texture can vary across the landscape and as it plays a role in soil aeration

through its influence on water retention (Section 2.2.2.3.c), it will ultimately affect N_2O emission. Textual effects on N_2O emission are investigated in this thesis.

2.5.2 Spring Thaw

High N_2O spring fluxes, particularly during rapid thaw events, have been recorded by many researchers (Goodroad and Keeney, 1984, Wagner-Riddle et. al, 1997). Christensen and Tiedje (1990) found field N_2O production to be two orders of magnitude higher during the spring thaw period than at any time during the rest of the year.

The source of N_2O fluxes in the spring have been attributed to increased rates of N_2O production (Christensen and Christensen, 1991), and reduced solubility of N_2O in soil water along with release of N_2O trapped in frozen soils (Goodroad and Keeney, 1984). Rising temperatures encourage increased biological activity. Freeze/thaw cycles release substrate that encourage denitrification reactions (Sections 2.2.2.3.d, 2.2.2.2). With increasing temperatures in the spring, many soils have a potential for high N_2O production due to high moisture content, where sufficient available carbon and nitrate levels occur.

The partitioning of N_2O between the soil atmosphere and soil solution, is a function of the soil temperature and moisture content, and this affects N_2O storage in the soil and the rate at which it diffuses through soil pores. As the temperature rises during spring thaw, less N_2O will remain dissolved in water resulting in increased gas concentrations in the profile that may cause increased flux during spring thaw. The change in temperature during the spring thaw is over a range where the slope of changing solubility is at a maximum (Section 2.2.3.3). A spring thaw production event is

investigated in this thesis (Chapter 3).

2.6 Methods Calculating N_2O Emissions in the Field

There are three common methods of measuring N_2O flux in the field: i) the measurement of gas flux at the soil surface using chambers to capture the gas (Mathais et al., 1980., Hutchinson and Mosier, 1981), ii) the use of micrometeorological methods to measure gas concentrations gradients above the soil from which surface flux is calculated (Moisier,1990., Weinhold, F. G. et al. 1994., Wagner-Riddle et al., 1996), iii) the measurement of subsurface concentration and calculation of the flux to the surface (Campbell, 1985., Burton et. al. 1997).

Some comparisons between techniques have been made in the literature. Burton et al., 1997 (Table 2.2), compared the micrometeorological technique with the soil profile concentration technique. The timing of the maximum measured flux was the same for the two techniques, and two of the three cropping treatments measured the flux is the same order of magnitude. Matthias et al. (1993) found micrometeorological techniques to yield similar results to chamber techniques during periods of large flux.

Table 2.2. Comparison of estimated flux based on in situ soil profiles and micrometeorological gradients (adapted from Burton et al., 1997).

Method		Fallow	Manure	Alfalfa
			(ng N_2O-N m^{-2} s^{-1})	
N_2O Profile	Min	-1.4	-0.3	0.5
	Max	207.8	11.2	599.0
	Mean	33.0	1.3	79.6
Micro-Met.	Min	-9.3	-6.1	-34.2
	Max	186.8	910.3	193.7
	Mean	13.7	36.1	13.3

2.6.1 Chambers

Two classifications of chambers are commonly used to measure N_2O flux from the soil surface, the closed (non-vented) and open (vented) chamber methods. Both involve covering a portion of the soil surface and measuring the change in concentration over time in the chamber. The change in concentration over time is related to the amount of gas leaving the surface over that time period. Measurements of both open and closed systems were compared by Ambus et. al. (1993), and found to agree reasonably well.

The chamber methods and associated problems have been discussed in the literature (Granli and Bœckman, 1994., Livingston and Hutchinson, 1995). Problems common to both techniques include temperature and pressure differences between the atmosphere and the closed space inside the chamber. Temperature increases inside the chamber can cause differences in temperature and pressure/concentration affecting diffusive gradients. Insulating the chamber with reflective material to prevent solar warming inside the chamber can minimize errors due to temperature differences. Vents in the closed chambers can minimize errors due to induced gradients in closed chambers (Hutchinson and Mosier, 1981). Orifices in flow through chambers can be modified producing similar effects (Granli and Bœckman, 1994., Livingston and Hutchinson, 1995).

2.6.1.1. Closed Chamber.

The closed chamber is an airtight chamber that is inserted into the ground. Gas samples are drawn from the chamber at recorded times in order to measure the increase in concentration over time.

The formula used to calculate flux (F) is (Hutchinson and Mosier, 1981):

$$F = V/A \times \Delta c/\Delta t \qquad [2.3]$$

Where: V =chamber volume
A = surface area of the soil
Δt = the change in time
Δc = the change in concentration

A major advantage in this method is that very small changes in N_2O concentration can be measured (Granli and Bockman, 1994). These chambers are rugged, easy to transport, and require no electrical power source or precision control of flow rate.

Disadvantages to the closed chamber method are related to concentration and pressure differences between the uncovered soil surface and the covered one. Change in flux due to increasing the concentration of gas inside the chamber can artificially decrease the concentration gradient between the soil and surface. Corrective equations (Jin and Jury, 1996) can be used along with short sampling times to account for these increasing concentrations inside the chambers. Adding vents can minimize differences in pressure between the atmosphere, and the inside of the chamber (Hutchinson and Mosier, 1981).

2.6.1.2. Open Chamber.

Open chambers are in many ways similar to closed chambers, except that air is forced through the chamber (Ryden et al. 1978). Flux is measured as a difference between the N_2O concentration in the incoming gas as compared to that leaving the chamber. This method has been said to mimic natural conditions better than the closed chamber method, because the atmosphere inside the chamber is most like that outside the chamber (Granli and Bøckman, 1994). The measurement assumes that equilibrium has occurred between the soil atmosphere and the chamber atmosphere. A premature measurement will result in an inaccurate flux estimate. Also, if the size of the inlet is not large in comparison to the outlet a negative pressure can occur, inducing higher advective flux measurements. Because of the increased time required for equilibration, larger temperature differences may occur between the chamber and the environment, when compared to the closed chamber techniques.

2.6.2 Micrometeorological Techniques

In this type of flux measurement, concentrations of N_2O are measured at two or more points above the soil surface. The measurement works best for approximating the flux over large uniform areas, as the dominant mechanism of gas transport is assumed to be turbulent diffusion (an eddying motion, which displaces parcels of air from one level to another). Measurements of temperature, moisture, and wind velocity (components of turbulence) are made so that the vertical flux can be calculated. Horizontal gradients are ignored, and vertical gradients are assumed to be fairly constant. An advantage of this method when compared to chamber methods is that no disturbance is made at the soil

surface. The three common methods used to calculate the flux are the flux-gradient method, the eddy correlation method, and the mass balance method. Micrometeorological techniques overcome spatial variability by integrating N_2O flux from relatively large areas.

The recent development of a Tunable Diode Laser capable of extremely sensitive and accurate measurements has improved the utility of micrometeorological methods. This instrument allows rapid, continuous measurements of N_2O over larger timeframes (Wagner-Riddle et al. 1996., Wagner-Riddle et. al, 1997).

Micrometeorological techniques generally require large uniform areas and conditions of minimal turbulence, limiting usefulness in estimating seasonal flux. Small differences in gas concentration are difficult to measure (discounting the tunable laser). However, a major disadvantage of micrometeorological methods are that they require extensive and expensive instrumentation.

2.6.3 Nitrous Oxide in the Soil Profile

In recent years, researchers have begun to measure soil atmosphere composition *in situ* through use of probes inserted to different depths in the horizon (Burton et al., 1997). The driving force behind this type of sampling is to aid in the understanding and predicting of N_2O flux from the surface. Although N_2O is consumed and produced throughout the soil profile, the depths at which production occurs is important to understand the processes that produce N_2O and their relation to the soil environment.

A number of different probe designs have been used, all with the common goal of obtaining a gas sample that accurately reveals the concentration of N_2O at that depth.

The two main types of probes found in the literature are those with a diffusion chamber consisting a dead airspace or porous cup at the depth sampled (Goodroad and Keeney, 1984., Rolston et al., 1976., Egginton and Smith, 1986., Dowdell et al. 1972), and those that draw gas directly from soil pores at that depth (Burton and Beauchamp, 1994). Both types use a very narrow gauge capillary tube to connect the depth sampled to an airtight sampling port at the surface. Problems with the probes include possible diffusion of gas from different depths along the probe, disturbance of diffusion surface due to probe insertion, mass flow from other depths when sample is drawn, and leakage of surface air through the capillary tube to the sampling depth. Careful insertion minimizes soil disturbance and gas diffusion along the probe surface. Drawing small samples at a slow rate minimizes mass flow from other depths.

Traditional methods of measuring diffusion coefficients in soils involve the removal of a soil core from the depth being investigated, or repacking a soil column (Rolston and Brown, 1977., Burton et al., 1997). As this technique involves removal of the core from the native environment, and possible core disturbance which may affect results, some researchers have attempted to calculate diffusion coefficients of soil gases using in situ profile concentrations (Jellick and Schnabel, 1986., Rolston and Brown, 1977., Lai et al. 1976). The method involves injecting a known amount and concentration of gas at a known depth, and measuring its concentration at known distances from the point of injection over a known time period. The results are then compared to an analytical or numerical diffusion model, and a diffusion coefficient calculated. Effects of production, consumption, and solubility are minimized by performing the experiment over a very short timeframe (4 mins) (Lai et al, 1976; Jellick

and Schnabel., 1986). Jellick and Schnabel (1986) found no significant difference between the in situ method and the core method. Lai et al. (1976) used this method to predict surface flux, and verified results with surface chamber techniques for CO_2 flux, and found close agreement in most measurements.

A relationship should exist between the distribution of N_2O in the profile, and flux from the surface. However, the relationship is often complicated by the fact that production and consumption can occur at different profile depths. The distribution of gas below the surface may also help in explaining some of the spatial and temporal variation in surface N_2O flux (Burton and Beauchamp, 1985., Folorunso and Rolston, 1984,. Christensen et al., 1990). Understanding the location of N_2O production/consumption may aid in the refinement of predictive relationships with soil properties and spatial and temporal estimation of N_2O flux.

2.6.4 Storage of N_2O Gas Samples

Often field measurements of N_2O concentrations occur in remote areas with no facilities to analyze gas concentrations. As a result, the gas samples need to be stored and transported to the laboratory for analysis.

Gas storage is not generally discussed in the literature. Sealed syringes (Mathais et al., 1980., Conrad et al., 1983., Mackenzie et al., 1997), bags and different glass/septum configurations (Freney et al., 1978., McKenney et al. 1980., Mummey et al., 1994) are among the most commonly used techniques. Some researchers use a molecular sieve to adsorb N_2O after water and CO_2 have been removed from the sample (Ryden et al., 1978). In all storage techniques the sample must be analyzed as soon as possible, to

minimize errors due to leakage, dilution, dissolving/diffusion. Gas storage is extremely difficult, and potentially a large source of experimental error. No information on the effectiveness of the above techniques has been found in the literature. Chapter 4 of this thesis investigates the effectiveness of three common N_2O sample storage methods.

2.7 Various Models of Nitrous Oxide Evolution

The kinetics of gaseous diffusion has long been known (Campbell, 1985., Hillel, 1982). Measurements of gas diffusion at different moisture contents have been made (Reible and Shair, 1982,, Jin and Jury, 1996). N_2O diffusion in soil is difficult to predict because of complications to the equation due to particle size, pore size and distribution, and water content. Tortuosity of continuous pore space in soil is difficult to estimate. Another problem with modeling N_2O evolution from soil is with determining the actual sources/sinks, along their magnitudes and effects on redistribution in the soil profile.

Modelling in the area of N_2O emission has focused on two main areas: gas diffusion models and denitrification models in soil.

2.7.1 Diffusion Models in the Literature

Gas diffusion in a porous medium such as soil is usually expressed as the binary diffusion coefficient of the gas in air, and some function of air-filled porosity. Campbell (1985) shows a typical expression for gas diffusivity in a porous medium as follows:

$$D = D_0\varepsilon(\phi g) \quad [2.4]$$

Where: D = effective diffusion coeficient
Do = diffusion coefficient in free air
ε = the pore space
ϕg = gas filled porosity

Several other empirical relationships have been developed. Currie (1960, 1961) fitted an empirical equation for dry granular materials of the type:

$$D/D_0 = \gamma\varepsilon^{\mu} \quad [2.5]$$

$$D=Do\ (\varepsilon_g/\varepsilon_p)^4 \text{ for wet materials} \quad [2.6]$$

Where: γ and μ are functions of the material
ε_g = the gas filled porosity
ε_p = the porosity

He later fitted data to an equation of the form:

$$\varepsilon(\phi_g) = b\phi_g^m \quad [2.7]$$

The constant m depending on the shape of the soil particles, the constant b depending on the value chosen for m (Currie 1965). Troeh et al. (1982) used the equation:

$$\varepsilon(\phi_g) = [(\phi_g - u)/1 - u)]^v \quad [2.8]$$

where u and v are empirical constants, u representing the porosity at which diffusion becomes zero. Campbell (1985) found equation 2.8 worked best at high porosity.

Collin and Rasmuson (1988) compared several models of gas diffusivity for

unsaturated porous media, and found the method proposed by Millington and Shearer (1971) gave best predictions of effective diffusivity. This equation is based on a model of interconnecting spheres and includes the probability of continuous pore space in the medium (equation 2.9).

$$Da/Da^{\circ} = (1-S)^2 \times [\varepsilon (1-S)]^{2X} \qquad [2.9]$$

Da/Da° = Diffusion coefficient compared to air
ε = porosity function
S = water-filled pore space function
x = a function of ε and S

Chapter 5 of this thesis investigates the use of Fick's law and an iterative technique to estimate diffusion in soil columns. As the diffusion coefficients are only valid for that particular column, moisture, porosity and tortuosity effects are accounted for in the general equation (equation 5.1).

2.7.2 Nitrous Oxide Flux Models

The major focus of modelling N_2O flux from soil systems has been to correlate factors influencing the rate of denitrification with N_2O production and with N_2O flux from the surface. Researchers agree that variations in soil moisture, texture, carbon and nitrogen are critical in determining N_2O emissions from soil (Li et al. 1992a., Cho et al., 1979.) Most attempts relate these factors empirically with measured outputs. Grundmann et al. (1988) investigated the relationship in the variability of field denitrification gas fluxes and water content, soil-gas diffusivity, NO_3^- concentration and water soluble organic C. Soil water was found to have the highest correlation of the tested factors, though results were not conclusive. When measured fluxes were compared

to calculated ones, it was concluded that the calculations were not adequate in accounting for small diffusivities in the soil.

First order kinetics are often used to describe the rate reaction of denitrification with respect to carbon and nitrate (Grundmann and Rolston, 1987., Grant, 1991). Grundmann and Rolston (1987) developed a moisture function dependent on the degree of soil-water. The fitted equation of this water function was used to calculate denitrification from measured values of water content, carbon, and nitrate. Results reasonably mimicked the denitrification spatial pattern and mean value.

Li et al. (1992a,b) describe other previous models which relate denitrification and N_2O production factors to N_2O emission in soil. Li used this information to form a process oriented, precipitation driven model to predict N_2O, N_2, and CO_2 from agricultural soils. It is a climatic model, utilizing submodels of thermal-hydraulic (for moisture and temperature profiles), decomposition, and denitrification. It simulated N_2O and N_2 emissions with a one day time step by combining soil thermal-hydraulic flux, aerobic decomposition, and denitrification submodels of DNDC (DeNitrification and DeComposition). Li (1992b) found similar trends in calculated vs. measured N_2O fluxes. Li (1992b) and Grundmann et al. (1987), suggested these differences were due to incorrect diffusion rates, as DNDC does not model diffusion as a gradient driven flux with diffusion coefficients, but as an empirical function based on N_2O production, soil moisture and clay content.

Cho (1982, 1985) and Cho et al. (1997a, 1997b) developed one of the most complete denitrification kinetic models which described the competition among terminal electron acceptors: oxygen, nitrate, nitrite and nitrous oxide. It integrates the basic

kinetics of N_2O production through denitrification activity in the profile to transport from the surface. Affinity of electron acceptors and their concentration determines their competitiveness. The model assumes a constant microbial activity in the soil. Cho et al. (1997b) used this model to develop five transport equations, one for each of the electron acceptors O_2, NO_3^-, NO_2^-, N_2O and N_2 to investigate the distribution of N gases, nitrate and oxygen throughout the soil profile. Moisture content, microbial activity and distribution were related to depth of oxygen penetration. Source and sink terms are based on competitive Michaelis-Menten kinetics of denitrification. Many of the observed characteristics of denitrification are successfully demonstrated in this model.

Models which reflect the complexity of gas diffusion at different moisture contents and the kinetics of denitrification assist in the explanation of why "in profile" distribution cannot directly be correlated to surface flux.

3. NITROUS OXIDE PROFILES AND SURFACE FLUX FROM FOUR CROP TYPES DURING SPRING THAW

3.1 Abstract

The emission of nitrous oxide (N_2O) in agroecosystems is a topic of concern in studies of nitrogen cycling, and the process of global climate change. Nitrous oxide is a "greenhouse gas", absorbing electromagnetic radiation in the infrared region and trapping this thermal radiation coming from the earth's surface. High N_2O fluxes in the spring, particularly during rapid thaw events, have been reported (Goodroad and Keeney, 1984., van Bochove et al., 1996). Although legumes have been found to emit more N_2O than most other crops. in the literature, no other specific trends have been noted. As specific work regarding Manitoba's contribution to agriculturally produced N_2O has not been done, this study will aid in the determination of this estimate. This study examined N_2O surface flux and soil profile concentration during a spring thaw event in four different cropping systems. The relationship between gas concentration profiles and surface flux in a silty clay soil was explored. The four cropping systems investigated were alfalfa, summerfallow, wheat, and native grass. Nitrous oxide flux increased during spring thaw at all sites. Significant increases occurred in the alfalfa and summerfallow cropping systems. In fact the alfalfa cropping system had the highest surface flux of all treatments with a maximum N_2O flux of 7.0 ng N_2O-N $m^{-2}s^{-1}$. The N_2O surface flux was small

relative to some spring flux events in the literature, but comparable to some values reported for fine textured soils. Profile concentrations based on an N_2O gradient between the 10-20 cm depths were poorly correlated ($r^2 \leq 0.182$) with surface flux measurements taken on the same day. A better correlation ($r^2 = 0.781$) was obtained in the alfalfa treatment with surface flux measurements made on the subsequent sampling period, indicating a delay in the transport of N_2O to the surface. Future work would involve similar studies with in other areas of Manitoba, as well as other cropping systems.

3.2 Introduction

The emission of nitrous oxide in agroecosystems is a topic of concern in studies of nitrogen cycling, and the process of global climate change. Nitrous oxide is a "greenhouse gas", absorbing light in the infrared region and trapping thermal radiation coming from the earth's surface. Nitrous oxide is of particular concern as, on a molar basis, it adsorbs 200–350 times more infrared radiation than CO_2 (Harvey, 1991., IPCC, 1992). Photochemical degradation of N_2O in the stratosphere produces nitrogen oxides that destroy ozone (Granli and Bockman, 1994). N_2O has an atmospheric residence time of about 130 years (IPCC, 1992), thirteen times that of methane. Concentrations of this gas are currently rising at a rate of approximately 0.5 to 0.8 ppbv yr^{-1} (Khalil and Rasmussen, 1992). Studies of ice cores indicate that before 1700, atmospheric N_2O concentration was approximately 285 ppbv, and has remained near this level since 0 AD (IPCC, 1992). The current atmospheric concentration has risen to 310 ppbv.

Significant sources of atmospheric N_2O include combustion of fossil fuels, combustion of plant biomass, and microbial reactions in the soil. Nitrous oxide in soil is produced as an intermediate in the processes of nitrification and denitrification (Davidson and Schimel, 1995). The major sinks for N_2O are stratospheric photolysis, and reduction to N_2 via denitrification. Storage as a result of dissolution in water can be considered a short-term sink.

High N_2O fluxes in the spring, particularly during rapid thaw events, have been reported (Goodroad and Keeney, 1984., van Bochove et al., 1996). Release of N_2O captured in ice, decrease in solubility, and increase in biological activity may each have

an effect on N_2O flux. N_2O trapped in ice in the fall may be released as melting occurs during thaw events (Goodroad and Keeney, 1985). The partitioning of N_2O between the soil atmosphere and soil solution is a function of soil temperature and moisture content, and this affects the storage of N_2O in the soil and the rate at which it diffuses through soil pores. As the temperature rises less N_2O will remain dissolved in water resulting in increased profile gas concentrations that may cause increased flux during spring thaw. During the spring, the soil has potential for high N_2O production due to coincidental occurrence of high water content, available carbon, and nitrate levels. Christensen and Christensen (1991) have presented evidence suggesting that the physical effect of freezing may break up aggregates, allowing previously unavailable carbon and nitrate to be released into the environment. Christensen and Tiedje (1990) found field N_2O production to be two orders of magnitude higher during the spring thaw period than at any other time during the rest of the year.

Estimation of N_2O flux from soils is complicated by the spatial and temporal heterogeneity of biological processes producing and consuming N_2O in soil (Folorunso and Rolston, 1984., Burton and Beauchamp, 1985., Christensen and Tiedje., 1990). Since the flux of N_2O is in response to a concentration gradient, logic suggests that a relationship should exist between the distribution of N_2O in the soil profile, and flux from the surface. Such a relationship is complicated by soil profile N_2O concentration being the result of combined effects of production, consumption, and storage within the soil system. The distribution of gas below the surface may also help in explaining the spatial and temporal variation in surface N_2O flux (van Bochove et al., 1996., Burton and Beauchamp, 1997., Cho et al., 1997). Understanding the location of N_2O concentrations

may aid in the refinement of predictive relationships with soil properties and spatial and temporal estimation of N_2O flux.

Microbial processes such as denitrification depend on availability of substrate (Parkin, 1987., Christensen et al., 1990., Christensen and Christensen, 1991). If it is assumed that N_2O production in soil is primarily a result of denitrification and that this process is often substrate-limited (Beauchamp et al., 1980., Beauchamp et al., 1989), rooting distribution and depth should influence profile N_2O concentrations and surface flux due to it's influence on carbon distribution. Smith and Tiedje (1979) demonstrated that denitrification can decrease rapidly in the first few millimeters away from roots. Parkin (1987) found that 85% of the denitrification occurring in a soil core was associated with a "hot-spot" resulting from the deposition of leaf material occupying only 1% of the soil mass. Different crop species have different rooting depths and rates of exudation into the rhizosphere, influencing substrate supply and distribution through the soil profile. Larger amounts of carbon may cause nitrate to be limiting so that denitrification proceeds to N_2. Perennial plant species have deeper, denser rooting systems than annual species (Russel, 1973). For this reason, different cropping treatments, with different root morphologies were chosen to investigate surface N_2O flux and profile concentrations of N_2O during spring thaw.

The objectives of this research were to; i) determine N_2O surface flux and soil profile concentration during the spring thaw in four different cropping systems, and ii) examine the relationship between gas concentration profiles and surface flux in a silty clay soil.

3.3 Materials and Methods

3.3.1 Description of the Sites

All four sites were located in a silty clay soil, (4% sand, 54% clay, 42% silt) classified as a well-drained Cumulic Regosol of the Black Lake Series, located on the campus of the University of Manitoba in Winnipeg, Manitoba. Site 1 was a 2-year-old alfalfa stand (perennial). Site 2 was a re-established perennial native grass mixture established 4 years prior to the experiment. Site 3 was a pea-barley-wheat rotation cropped to wheat in the previous season (annual). Site 4 had been in continuous summer fallow for 5 years prior to the experiment. The 16m x 5m plots were replicated four times.

3.3.2 Profile Sampling

Burton and Beauchamp (1994) describe the basic design of the soil atmosphere sampler, which was used with the following modifications. Ten ports were located at 10 cm intervals from 10 cm to 100 cm. Samples were taken through a septa attached to the sampling device instead of 2-way needles. Stainless steel pins were inserted in the sampling ports to prevent soil from plugging the apertures.

One profile sampler was installed in each replicate in the fall of 1994. Samplers were periodically checked and maintained throughout the winter. Intensive sampling began on March 15, 1995 and continued until May 10, 1995. Samples were taken approximately twice a week at 11:00 a.m. during the spring thaw period.

On each sampling occasion, 0.5 mL of gas was drawn to purge dead volume in each tube. Then a 3 mL sample was drawn and stored in 5 mL plastipak^{TM} syringe

inserted into a rubber stopper (Becton Dickenson and Co., Franklin Lakes, NJ). Only one sample was taken from each depth to minimize mass flow in the soil profile. Samples were analyzed for O_2, CO_2, and N_2O concentrations by gas chromatography using both an electron capture detector (ECD) and thermal conductivity detector (TCD) within 48 hours of collection.

Samples were injected into a Varian 3400 gas chromatograph equipped with an ECD and a sampling valve that allow simultaneous introduction into a Gow-Mac 3500 gas chromatograph equipped with a TCD, so that each sample could be analyzed for N_2O and CO_2. The sampling valve directed 0.1 mL of sample to each detector. The ECD was equipped with a Porapak Q (80/120 mesh) packed pre-column (30 cm x 0.32 cm OD) to trap and flush out water, followed by a Porapak Q packed analytical column (152 cm x 0.32 cm OD). For the TCD detector, the gas was first directed through the molecular sieve (80/120 mesh) packed column (274 cm x 0.32 cm OD) and then introduced into the sample side of the TCD. The effluent from the sample side of the detector was then passed through the packed Porapak Q column (274 cm x 0.32 cm OD) which was connected to the reference side of the detector. The ECD, with 10% methane and 90% argon carrier gas, measured N_2O while TCD, with helium as the carrier gas, measured CO_2, N_2, and O_2. Each run required approximately 7 min. Flow rate of gases for both detectors was 30 mL min^{-1}.

3.3.3 Surface Flux

Surface flux measurements were measured on the same dates as the soil profile concentrations. Surface fluxes were monitored using the vented closed soil covers as described by Hutchinson and Mosier (1981). On each sampling date, 3 mL samples were

drawn at 0, 15, 30, 45, and 60 minutes after the covers were put in place on the soil surface. The gas samples were stored in syringes by inserting the needle in a rubber stopper. Samples were transported to the laboratory and analyzed as described above.

Flux was estimated over a 60-minute period using the following equation presented by Hutchinson and Mosier (1981):

$$f = V(C_t - C_o)/At \qquad [3.1]$$

where f = surface flux (ng N_2O m^{-2} h^{-1})
C_o = concentration (ng N_2O m^{-3}) at time = 0
C_t = concentration (ng N_2O m^{-3}) at time = t
t = time (h)
A = the area of soil covered by the chamber (m^2)
V = the volume of the chamber (m^3)

Surface flux can be estimated from soil profile N_2O concentrations (Currie, 1960):

$$f = D(C_{20} - C_{10}) \qquad [3.2]$$

where f = surface flux (ng N_2O m^{-2} h^{-1})
C_{10} = concentration (ng N_2O m^{-3}) of N_2O at 10 cm
C_{20} = concentration (ng N_2O m^{-3}) of N_2O at 20 cm
D = Binary diffusion Coefficient(AFP function)
AFP function = $.9(AFP)^{2.3}/\Delta$ depth

A daily mean temperature was used in the calculation. The pressure term was taken to be standard atmospheric pressure (101.3 kPa). Water content was measured only once during the thaw period. An average of four measurements, one from each cropping treatment, was used to determine water content. Bulk density used in the formula was an average of four measurements (1.2 Mg/m^3), one from each cropping treatment.

3.3.4 Ancillary Measurements

Soil temperature was monitored at 0, 20, 40, 60, 80, and 100 cm depths using thermocouples inserted on a probe into the soil. Air temperature and precipitation were monitored throughout the sampling period using an automated weather station located on site. Soil profile was analyzed for nitrate on May 11, 20, 26 from 0 to 1 m in 15 cm intervals using the Griess-Ilosvay technique with a cadmium column to convert nitrate to nitrite (Tecator Method# 65-31/81, Topp, 1993). Gravimetric water content was determined over the same intervals on May 26.

3.4 Results and Discussion

3.4.1 Site Characteristics

Perennial grass had the highest mean levels of nitrate (NO_3^-) 10 cm from the surface (12.5 μg N g^{-1} soil) followed by wheat, alfalfa, and fallow (Figure 3.1). Mean NO_3^- in all treatments was below 4 μg g^{-1} soil at depths of 60 cm and greater. Perennial grass treatments had the highest mean NO_3^- concentration at depth. Although all treatments had mean gravimetric surface water contents near 0.40 g g^{-1} soil which decreased to 0.33 g g^{-1} soil at the 100 cm depth, the mean water content of the perennial grass plots at 10 cm and 30 cm was 0.45 g g^{-1} soil (Figure 3.1). Bulk density was relatively uniform over the top 75 cm averaging of 1.2 g cm^{-3}.

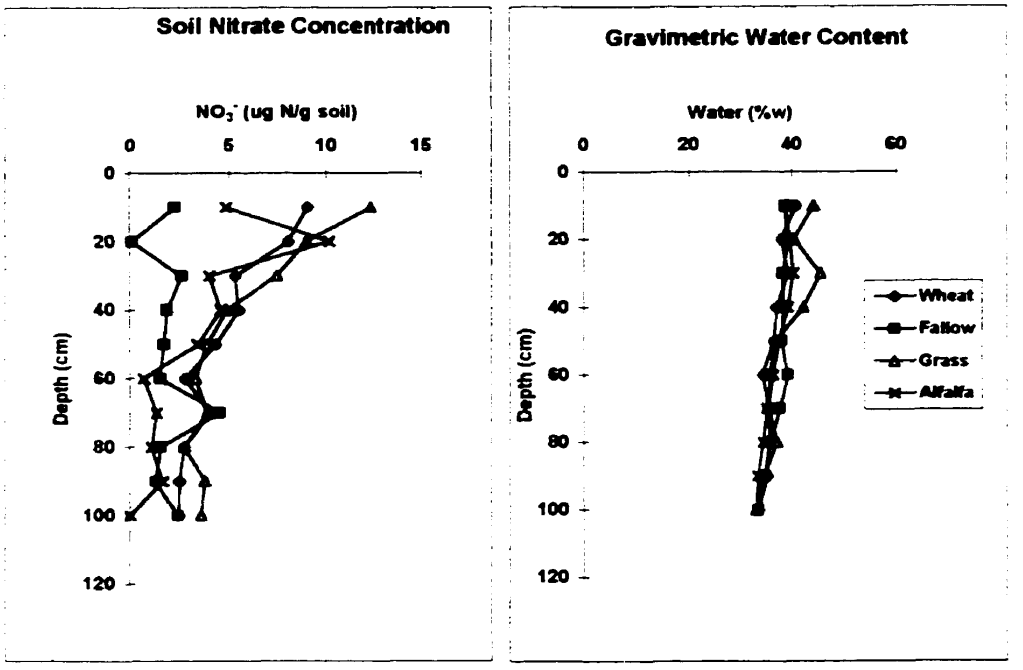

Figure 3.1 Mean NO_3^- profile concentration and gravimetric water content of all treatments completed on May 26, 1995.

Mean daily soil temperature at 5 cm was at -3 °C on February 28, and rose to a final average of 16 C on May 26. Initially, precipitation events when air temperature was above 0 °C had a large impact on soil temperature (Figure 3.2). This may be due to precipitation causing the snow cover to melt, allowing solar radiation to heat the soil. After March 26, soil temperature mirrored air temperature with less extreme ranges.

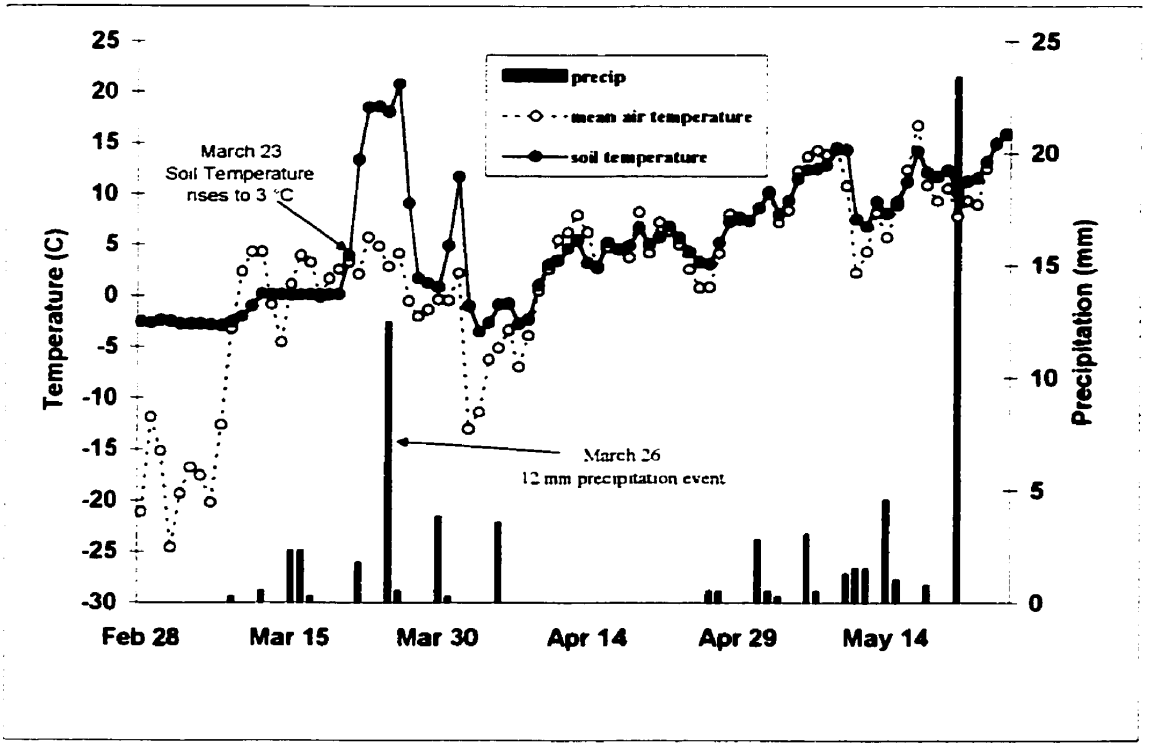

Figure 3.2 Mean air and soil (5cm) temperature and precipitation at the Plant Science Field Research Laboratory during the period March 1, 1995 to May 23, 1995.

3.4.2 N_2O Flux

Alfalfa and native grass N_2O profile concentrations are presented in Figure 3.3, while fallow and wheat N_2O profile concentrations are presented in Figure 3.4. Accumulation of N_2O in the profile (max. 8.47 μL N_2O-N L^{-1}) occurred in the sites cropped to alfalfa near the surface in March (Figure 3.3). A significant ($p \leq 0.05$) increase in N_2O surface flux occurred on March 25 for the summer fallow (6.7 ng N_2O-N m^{-2} s^{-1}) and alfalfa (7.0 ng N_2O-N m^{-2} s^{-1}) treatments (Figure 3.5). The average flux estimate for summer fallow and alfalfa was 9.7 times higher than the average over all treatments during spring thaw. The largest flux occurred consistently in alfalfa throughout the sampling period.

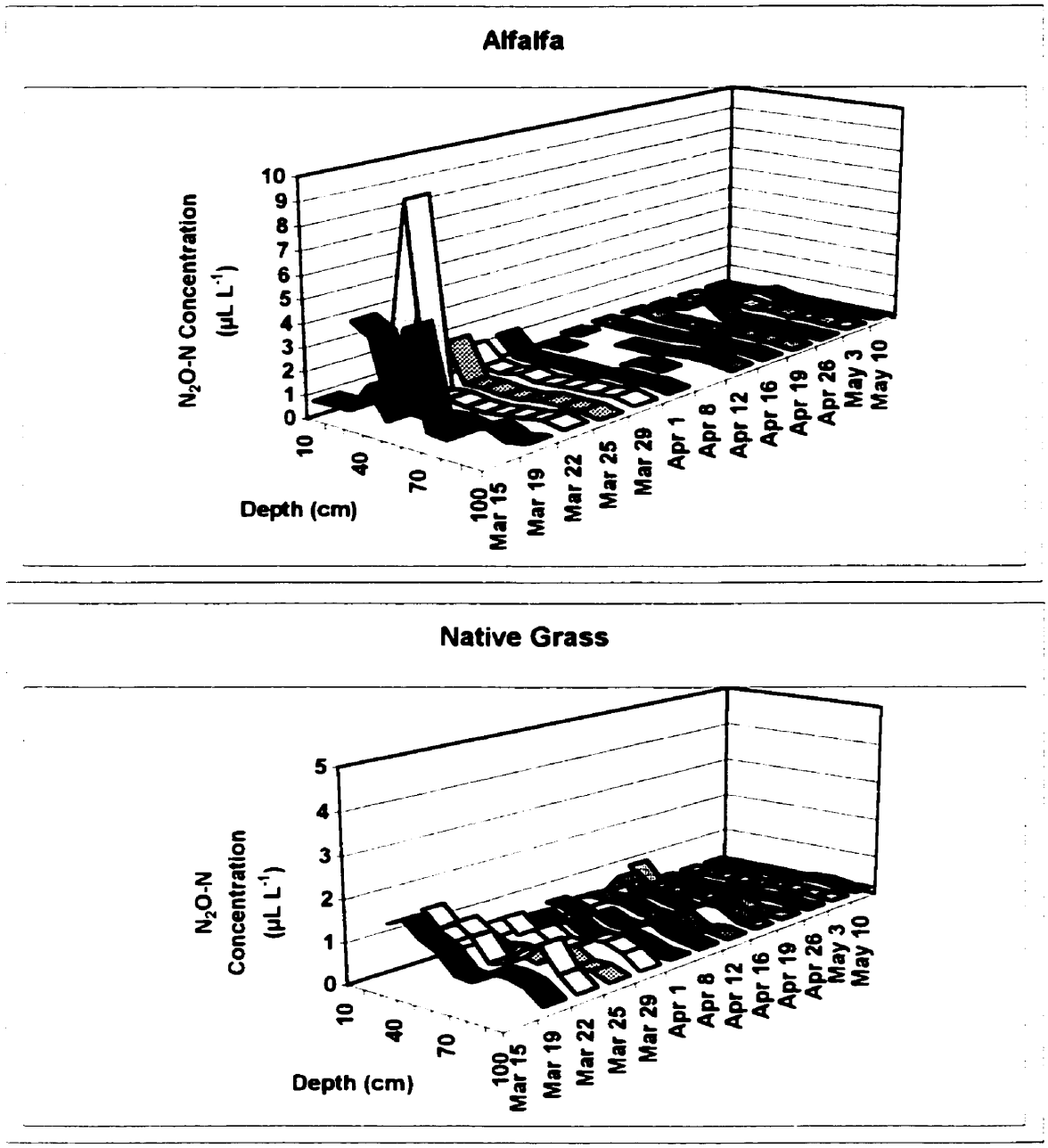

Figure 3.3 Profile N_2O concentrations in alfalfa and native grass during spring thaw 1995. Note the concentration scale is different in alfalfa than in other profiles.

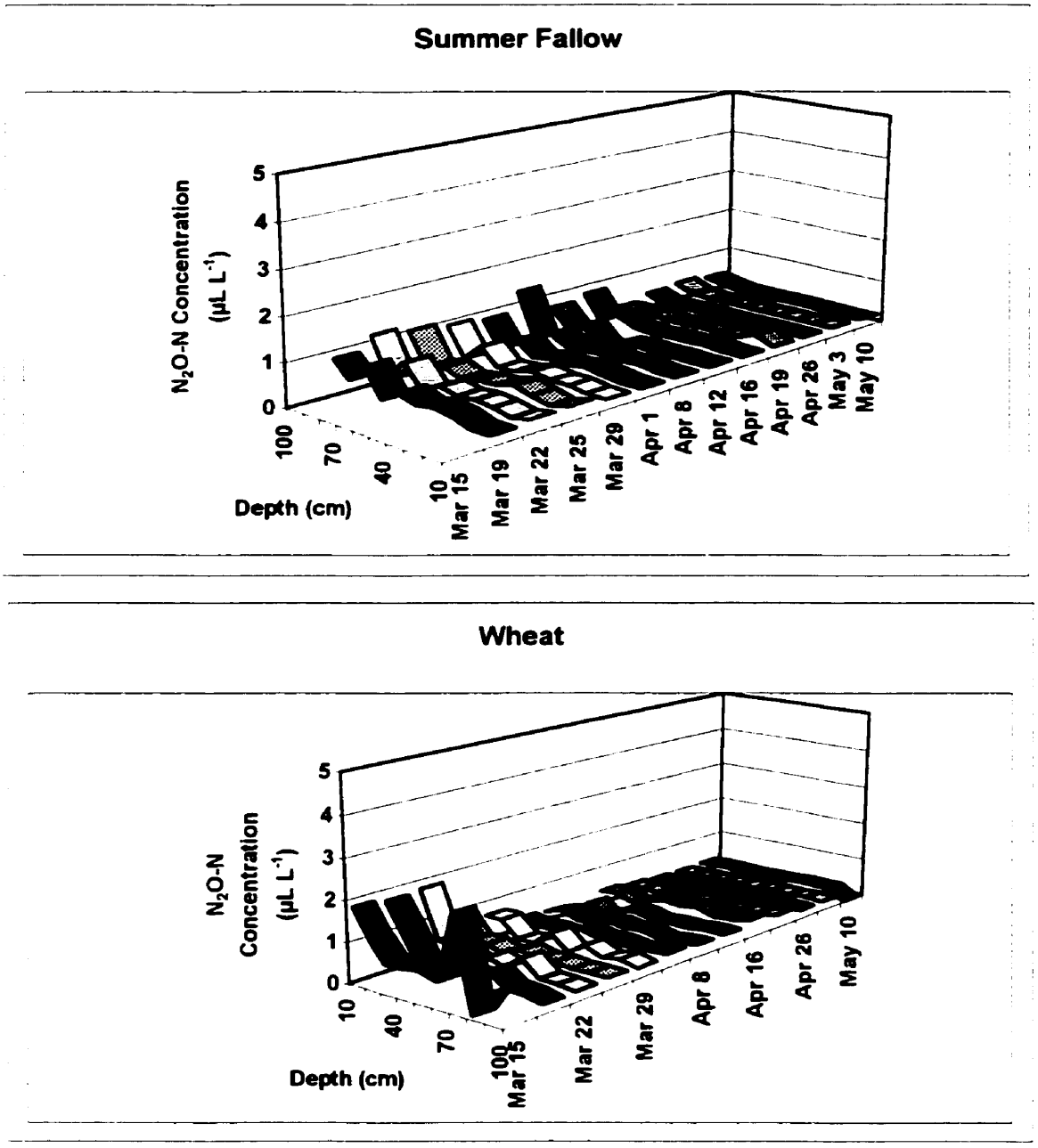

Figure 3.4 Profile N_2O concentrations in summer fallow and wheat during spring thaw 1995. Note the summerfallow depth scale is reversed.

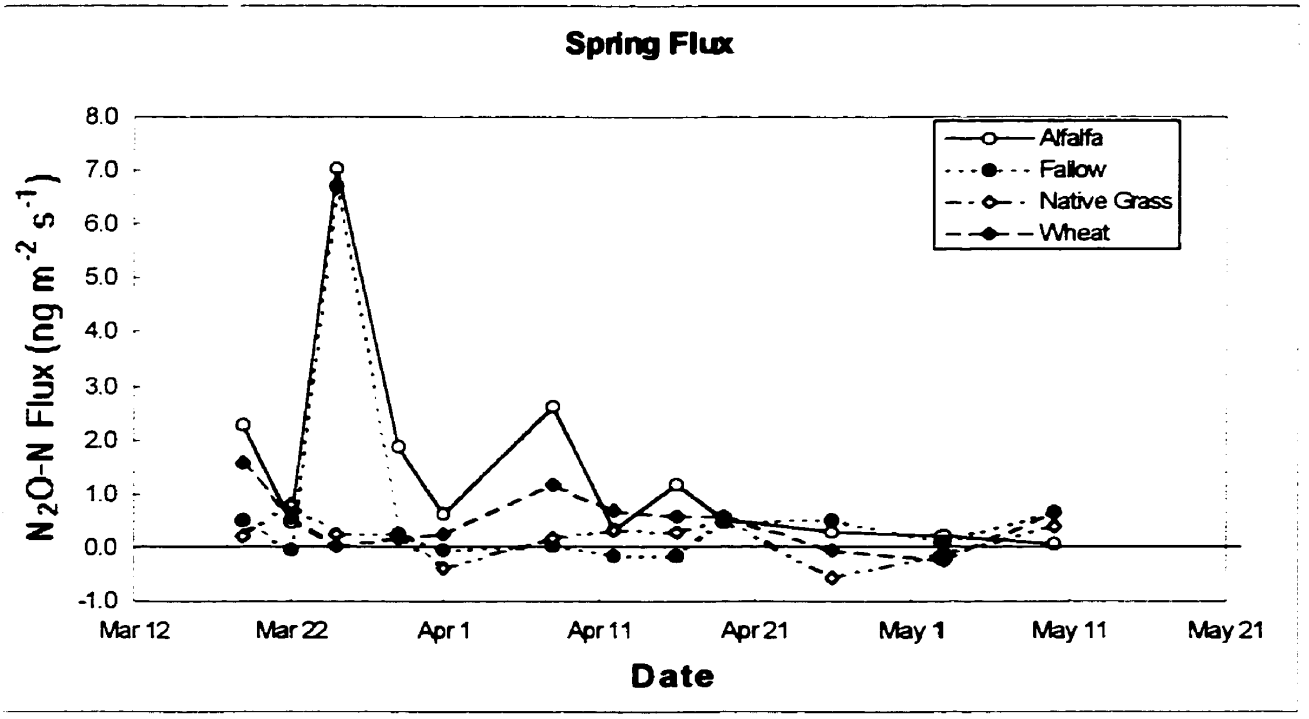

Figure 3.5 Surface flux measured using the chamber method for 4 cropping systems in the spring of 1995.

Other smaller flux increases coincided with increases in soil temperature. No large surface flux occurred in wheat or native grass. The alfalfa cropping treatment had a significantly ($p \leq 0.10$) higher N_2O surface flux than native grass (Figure 3.5). Higher soil water content and organic carbon availability likely occurred under the native grass due to the extensive root mass present in this system. Such conditions might favor higher terminal electron acceptor demand and hence complete denitrification to N_2. The increased CO_2 and decreased O_2 concentrations observed in the soil profile support this hypothesis (Figure 3.6). Alternatively, snow cover remained longer on the native grass site and may have resulted in slower warming of the soil and reduced gas exchange between the soil atmosphere and the surface. Over the entire thaw period there were no significant differences in surface flux among other cropping treatments.

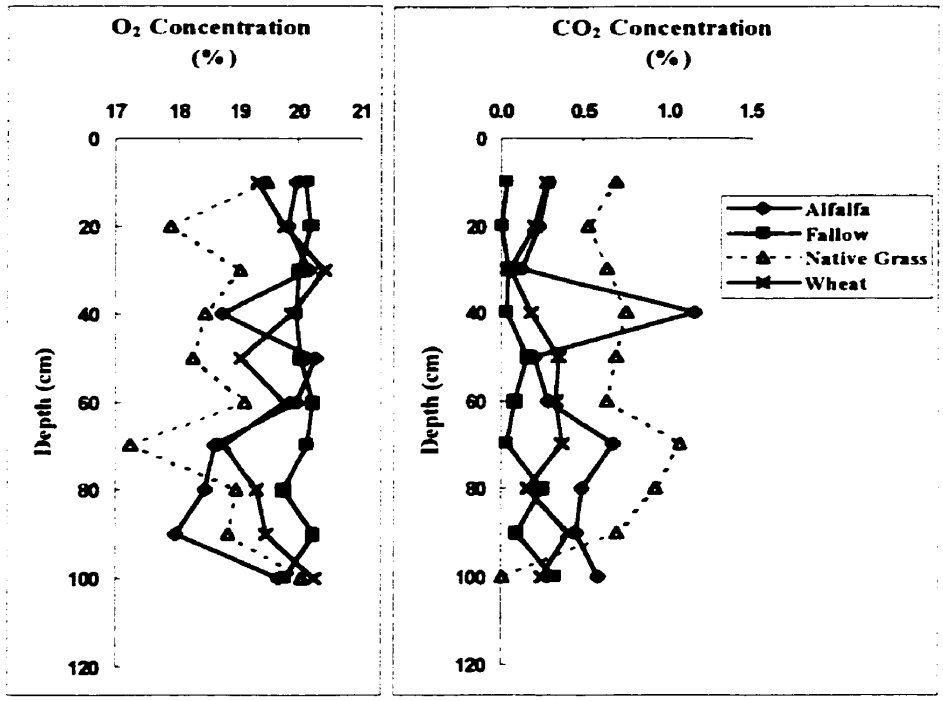

Figure 3.6 Profile O_2 and CO_2 concentrations of four cropping treatments during spring thaw 1995. Values are means of four replicates.

Although there was an increase in N_2O surface flux during spring thaw (Figure 3.4), flux was low when compared to values reported in the literature (Table 3.1). The values for fine-textured soils, such as the one considered in this study are generally lower than values reported for coarser textured soils. For example, the highest flux observed in this work was four times less than values reported for flux from a sand but of similar magnitude to values reported for clay-textured soils in Ontario and Australia (Table 3.1). Small pore size in clay, and the high water holding capacity reduce the rates of gaseous diffusion and result in reduced O_2 movement into the profile. This would also result in slower N_2O transport and loss from the soil profile. Both processes would favor the reduction of N_2O to N_2.

Table 3.1 Nitrous oxide flux reported in the literature.

Soil Texture	Max. N_2O Flux ($ng\ m^{-2}s^{-1}$)	Location	Reference	Comments (event)
Sand	11.39	Washington	Mummey et al., 1994	Addition of moisture
Loam	868.06	California	Rolston et al. 1976	Flux from fertilizer N^{15}
Fine-Loamy	18.52	California	Ryden et al., 1978	Fertilizer Application
	13.89	California	Ryden et al., 1979	Fertilizer Application
	22.22	California		
Sandy Loam	55.56	Michigan	Christensen + Tiedje, 1990	Spring Thaw
Clay	5.56	Australia	Burford + Hall, 1977	Unavailable
Clay	1.62	Australia	Freney et al., 1978	Unavailable
Clay	0.05	Ontario	Findlay and McKenney, 1979	Unavailable

Although a decrease in N_2O solubility as a result of the warming of soil water would have contributed to increased surface flux during spring thaw, increased in biological activity was also a contributing factor. High CO_2 and low O_2 profile concentrations (Figure 3.6) are indicative of higher respiration levels and thus there is a greater potential for N_2O reduction.

Following the thaw event, N_2O surface flux returned to lower levels (0.2 ng N_2O-N m^{-2} s^{-1}).

Increased surface flux was related to increased profile concentration. This was particularly notable in the alfalfa treatment, where on March 19, relatively high profile N_2O concentrations preceded the high flux observed on March 25 (Figures 3.3 and 3.5). To determine the extent of this relationship, a comparison was made between estimated flux based on the profile concentration gradient, and estimated flux rate based on surface chamber methods (Table 3.2).

Table 3.2 Nitrous oxide flux calculated from profile concentrations (Profile) and measured by chamber (Chamber) (ng N_2O-N m^{-2} s^{-1}). The correlation coefficient between methods along with a staggered correlation coefficient (profile method advanced by one sampling period) are shown.

Depth (cm)	Alfalfa Profile	Alfalfa Chamber	Fallow Profile	Fallow Chamber	Wheat Profile	Wheat Chamber	Grass Profile	Grass Chamber
19-Mar-95	-0.182	2.280	0.004	0.526	-0.272	1.582	0.079	0.212
22-Mar-95	2.635	0.463	-0.034	-0.037	-0.474	0.560	-0.152	0.810
25-Mar-95	0.640	7.038	-0.042	6.692	-0.208	0.040	0.091	0.248
29-Mar-95	-0.259	1.873	0.008	0.249	-0.001	0.184	0.089	0.263
1-Apr-95	-0.325	0.639	-0.001	-0.031	-0.027	0.237	0.083	-0.362
8-Apr-95	0.097	2.620	-0.001	0.040	0.058	1.164	-0.015	0.169
12-Apr-95	0.013	0.323	0.000	-0.146	0.133	0.706	0.066	0.313
16-Apr-95	-0.021	1.180	0.003	-0.158	0.011	0.577	0.209	0.295
19-Apr-95	0.058	0.495	0.074	0.458	0.004	0.601	-0.183	0.510
26-Apr-95	0.002	0.299	-0.004	0.509	0.004	-0.038	0.014	-0.560
3-May-95	0.007	0.206	-0.001	0.121	0.003	-0.219	-0.003	-0.106
10-May-95	0.000	0.082	0.000	0.653	0.011	0.656	0.013	0.403
Linear r^2	Alfalfa =	.003	Fallow =	.182	Wheat =	.025	Grass =	.132
Linear r^2 (profile advanced)	Alfalfa =	.787	Fallow =	.128	Wheat =	.000	Grass =	.211

3.4.3 Soil Atmospheres

The largest profile concentrations occurred consistently in alfalfa throughout the sampling period (max. 8.46 μL N_2O-N L^{-1}). Large surface accumulations (upper 60 cm) of N_2O occurred in the alfalfa in March, which is when the largest flux occurred. No large accumulations of N_2O occurred in native grass profile. Small accumulations of N_2O were distributed throughout the profile (Figure 3.3). Large accumulations of N_2O were not observed in the summer fallow profile (Figure 3.4). The O_2 profile shows little depression, suggesting low biological activity (Figure 3.6). Small accumulations of N_2O occurred deep in the summer fallow profile. Large surface flux did not occur in the wheat treatments, although there were small accumulations in the profile in March (Figure 3.6). The rooting depth of wheat is shallow and the root density is lower when compared to other treatments in this soil (Russel, 1973). Although there was a carbon source for biological activity in this treatment, carbon availability may be limited at

depth, resulting in lower N_2O production in the profile. This is consistent with higher levels of O_2, and lower levels of CO_2 in the profile.

An approximation of N_2O flux may be made based on measured N_2O concentrations and estimates diffusion coefficients based on soil characteristics. Poor linear correlation ($r^2 \leq 0.182$) was observed between the approximations of flux, based soil N_2O gradients, and measured N_2O flux (Table 3.2). Upon visual examination a temporal pattern can be detected, particularly in the alfalfa treatment. This pattern suggests that flux calculations based on soil N_2O profile concentrations precede actual flux calculations based on soil cover concentrations. If the profile calculated flux is advanced one sampling period, the correlation in the alfalfa treatment is improved. The major peak in measured alfalfa flux now coincides with the major calculated peak, giving a correlation coefficient of $r^2 = 0.787$. The temporal shift in these two estimates of flux may reflect a delay in the transport of N_2O from the soil profile.

As the thaw event passed N_2O profile concentration and surface flux returned to lower levels. This is probably a result of nitrate and carbon depletion. Eighty two percent of the total flux occurred on or before April 12 (which was at the end of the thaw period).

3.5 Conclusions

Nitrous oxide flux increased during spring thaw at all sites. Alfalfa had higher N_2O surface flux than native grass during spring thaw. In fact, alfalfa had the highest surface flux of all treatments. N_2O surface flux was small in clay soils, when compared

to values reported for courser textured soils (Table 3.1).

Surface flux calculated based on the N_2O profile concentration gradient at 10-20 cm depth was poorly correlated ($r^2 \leq 0.182$) with surface flux measurements taken on the same day. A better correlation ($r^2 = 0.787$) in the alfalfa treatment was obtained with flux measurements made on the subsequent sampling period, indicating a delay in the transport of N_2O to the surface.

It should be noted that the syringe method used to store gas samples prior to analysis in this investigation in not very effective in preventing gas loss over a 24 hour period (Chapter 5). As a result, samples taken using this method must be analyzed as soon as possible.

4. N_2O SAMPLE STORAGE

4.1 Abstract

Laboratory analysis of trace gas field samples involves storage and transport of the sample, and injection of the sample into a gas chromatograph (G.C.). Typically, N_2O is sampled in the field using a syringe, and either stored in the syringe or injected into a vial (Mathais et al., 1980., Conrad et al., 1993., Hutchinson and Mosier, 1981). There is little information in the literature about how N_2O sample storage methods compare, or about how manual injection techniques compare to automated techniques. This study investigated an autosampler vial method of N_2O storage, and compared it with more traditional methods of vacutainer and syringe storage. Two types of autosampler vials were used, one with a liquid barrier and the other without. The objectives of this study were to determine the effectiveness of each storage method over time, and to determine if silicon oil barrier is beneficial in reducing sample loss or contamination in autosampler vials. The vacutainer was found to be the best method for storage of N_2O samples of the methods investigated over a six-day period. Only 6.7 % of the original N_2O sample was lost. This method did, however, consistently have the highest daily variability in measurements. Both types of autoinjection vial systems had much less variation between replicates than other storage systems (standard deviation = 0.08 $\mu L\ L^{-1}\ N_2O$). The syringe storage method was the least effective of all the methods tested, losing 70% of

the sample within 24 hrs. Future work would involve the investigation of the possibility of using a vacucontainer vial with an autoinjection system. Different liquid barriers may be investigated, as they may be more useful in preventing sample loss. It is important to note that although some methods show promise for N_2O sample storage, the sooner samples are analysed after collection, the better.

4.2 Introduction

Laboratory analysis of trace gas field samples involves storage and transport of the sample, and injection of the sample into a gas chromatograph (G.C.). The rate of sample leakage or contamination is time dependent, therefore as the timeframe between collecting samples and analyzing them increases, storage methods need to be improved. If storage container pores can be blocked, diffusive/effusive losses will be eliminated, and longer storage times become possible. Some injection variability in regards to amount and time is expected using manual injections. Using an automated injection system can minimize injection variability.

Typically, N_2O is sampled in the field using a syringe, and either stored in the syringe or injected into a vial (Mathais et al., 1980., Conrad et al., 1983., Hutchinson and Mosier, 1981). Gas sample loss may occur due to diffusion/effusion through a barrier, dissolving within the barrier, or mass flow through small openings in the barrier. The shorter the time between sampling and analysis, the less time is available for gas loss to occur. Livingston and Hutchinson (1995) report gas loss in syringe storage to be 2% or more per day. Brooks et al (1993) tested various common storage container/septa configurations for N_2O and found great variations in leakage over a 14-day period. Literature comparing the effectiveness of different storage methods is scarce.

One problem with both the syringe and vacutainer ™ (Becton Dickinson and Co., Franklin Lakes, NJ) storage method is that they are not easily adapted to current gas chromatographic autosampler technology and thus necessitate manual analysis of samples. Manual analysis is both costly and time consuming, typically decreasing the

number of samples that can be analyzed and increasing the time the samples are stored and therefore the potential for loss. Liquid gas chromatography autosamplers, such as the Varian™ 8200 autosampler used in this study, use small sample vials sealed by a teflon/rubber septum. The potential for using this vial for gas storage was examined in this work.

Diffusion of a gas through air is 10^4 times greater than in water (Campbell, 1985). Therefore it is hypothesized that a thin film of liquid on the inside the autosampler vials will reduce the sample loss due to mass flow and diffusion through pores if the sample is stored inverted. A coating of silicon applied to the outside of the septum will further decrease gas loss by increasing the septum's thickness, enhancing resealability, blocking pores, and protecting the septum material from photooxidation during field use. This method of storage may provide a means that is amenable to automated sample analysis, because the vials can easily be used with an autosampler.

This study investigates an autosampler vial method of N_2O storage, and compares it with more traditional methods of vacutainer and syringe storage. The objectives of this study were to:

1. determine the effectiveness of each storage method over time.
2. determine if silicon oil is beneficial in reducing sample loss or contamination in autosampler vials.

4.3 Materials and Methods

Syringes, vacutainers, and autosampler vials with oil stored upright, and inverted were tested for leakage for a one week period (Figure 4.1).

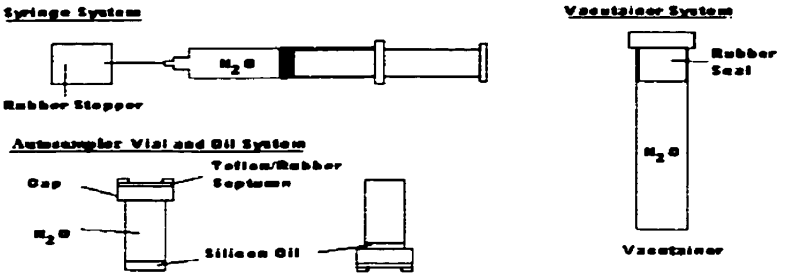

Figure 4.1 Nitrous oxide storage systems compared.

The ability of the above storage systems to prevent leakage was examined under two conditions; one with N_2O standard gas filled containers in which gas may leak out, the other with helium (He) filled containers in which N_2O may diffuse in. As the major focus of this investigation was to see how well N_2O is stored within a container, and due to equipment/time limitations, He trials contained only two replicates of each storage method. These He trials were run to see if trends of diffusion/effusion into the container would mirror the N_2O trials, although with smaller concentration gradients, it was speculated that leakage should be slower.

4.3.1 Nitrous Oxide Trials

Autosampler vials (1.5 mL; Varian™, Mississauga, ON) were sealed with screwcaps using 8 mm diameter .0254/2.286 mm thickness teflon/rubber septums (Alltech, Deerfield, IL), with the teflon side towards the inside of the container. Silicon sealant (Canadian Tire Corp, Toronto, ON) was applied liberally to the outside of the cap,

septum and glass vial assembly and allowed to dry prior to use. Silicon oil (0.3 mL dimethylpolysiloxane) was injected into each vial. Silicon oil is necessary in the upright vials to account for possible losses due to N_2O dissolving in the oil. Vials were rinsed three times and filled with He, inverted and stored overnight. Vials were then refilled to 140 mm Hg above atmospheric pressure with a standard gas containing: N_2O at 3 $\mu L\ L^{-1}$, CO_2 at 2.99 % (v/v), O_2 at 10% (v/v) and the balance as N_2.

Vacutainers (64 x 10.25 mm, red rubber stopper 11.1 mm length with r = 6.0 mm, Becton Dickinson and Co., Franklin Lakes, NJ) were rinsed three times with Hg, refilled with He, and stored overnight. Vacutainers were then refilled with a N_2O standard gas to a pressure of 140 mm Hg above atmosphere.

Three mL plastipak syringes (Becton Dickinson and Co., Franklin Lakes, NJ) were triple rinsed with He, and then filled with standard gas to atmospheric pressure. Syringes were inserted at least 1.5 cm into rubber stoppers to provide airtight seals.

The experiment was conducted over a seven-day period. Replicates of three were used in each case. Half a milliliter of gas at room temperature was injected into a Varian™ 3400 gas chromatograph fitted with a ^{63}Ni electron capture detector. A porapak Q precolumn (30.5 cm long x 0.22 cm i.d.; 80/100 mesh porapak Q – Alltech, Deerfield, IL) was used with a 6-port backflush valve to remove water from the sample. A second porapak Q column (152.4 cm long x 0.22 cm ID.; 80/100 mesh porapak Q – Alltech, Deerfield, IL) separated N_2O from the other gases. Standard curves for both manual injections and automated injections were run daily.

4.3.2 Helium Trials

Vials were prepared as in 4.2.1 except that instead of the final filling with N_2O, they were filled with helium (99% pure) to 140 mm Hg above atmospheric pressure. Vacutainers were rinsed three times, filled with He and stored overnight. Vacutainers were then refilled with helium to 140 mm Hg above atmospheric pressure. Syringes were triple rinsed with helium, and then filled with helium.

Two replicates were used in each case. The contents of the storage systems were determined by injecting 0.5 mL of gas into the gas chromatograph. Standard curves for both manual injections and automated injections were determined daily using the standard gas in order to calculate results.

4.4 Results and Discussion

4.4.1 Nitrous Oxide Trials

Storage of gas presents a difficult problem, as diffusion (concentration dependant)/effusion (pressure dependant) into other gases or in gas filled pore space is quite rapid. If any pathways for diffusion/effusion exist, temperature, pressure, and concentration can have a large affect on their rates.

Nitrous oxide can escape through very small holes in a septum. Diffusion and of N_2O through void space and punctures depends in part on molecular size. Size affects the velocity distribution of the molecule as well as the physical space a molecule can move into. Because atoms within a molecule of gas vibrate and the gas molecule itself rotates,

molecular size is difficult to determine (Parry, 1970). An approximate size based on bond lengths (Wells, 1962) would be length of 2.31 Å, and width of 1.48 Å. Due to molecular rotation, an N_2O molecule could be considered a sphere of diameter 2.31 Å. If we ignore possible charge interactions, any pore in a container 2.31 Å or larger will allow N_2O to move into it. If the pore is continuous, N_2O will escape from the container.

As most sample gas loss/contamination from a sealed container occurs due to diffusion/effusion, limiting these processes was the focus of this study. Rate of diffusion and effusion depends on temperature, concentration, and type of molecule. Graham's law states that because N_2O has the same mass as CO_2, it should have the same average molecular speed and therefore a similar rate of diffusion. Table 4.1 shows some typical gas diffusion rates including N_2O, which is similar to CO_2 in air. A value for diffusion of N_2O in liquid has not been found in the literature, but it is expected to be similar to CO_2. Thus in theory a thin liquid film inside a storage vial blocking pores should decrease the rate diffusion/effusion loss by approximately 10000 times.

Table 4.1 Some diffusion coefficients (Do) for gases in air and liquids at STP.

Gas	Medium	Do $cm^2\ s^{-1}$	Reference
N_2O	Air	0.122	Fuller et al. (1969) *in* Prichard and Currie (1982)
N_2O	Air	0.143	Prichard and Currie (1982)
O_2	Air	0.177	Campbell (1977)
CO_2	Air	0.139	Prichard and Currie (1982)
CO_2	Water	0.2×10^{-4}	Campbell (1985)

Measurements of N_2O standard taken from vacutainers compared to those taken directly from the standard tank with a concentration of 3.0 $\mu L\ L^{-1}$ N_2O did not differ significantly in a 24 hour period, indicating that N_2O is not highly soluble in the rubber septum (Figure 4.2).

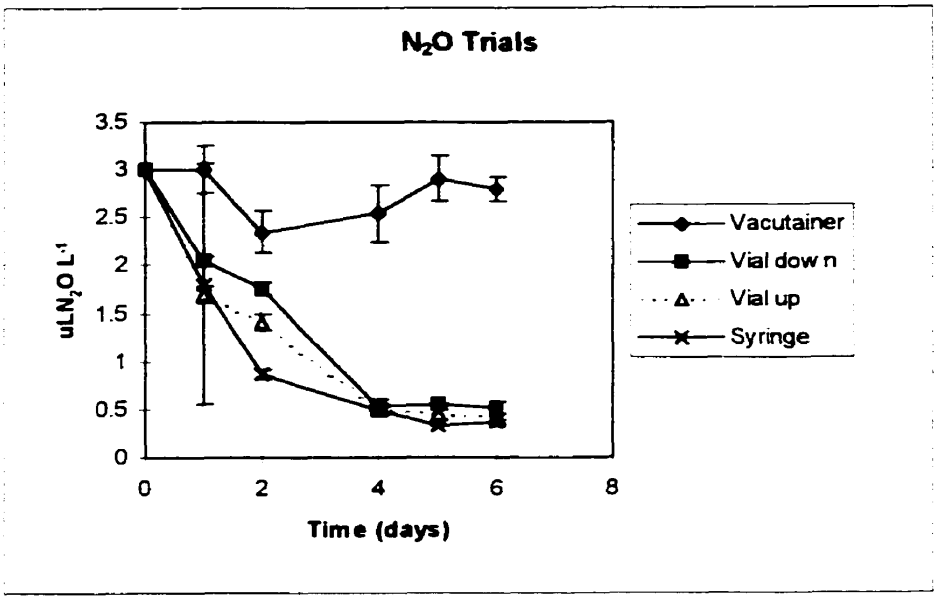

Figure 4.2 Average N_2O sample loss over a 6-day period. Error bars show standard deviation.

Both vial methods and the syringe method showed a significant loss of sample over a six-day period (alpha ≤ .05). The second day of sampling in the vacutainer method is significantly lower than the first day. On average, the N_2O concentration of the stored standard gas declined by 6.8% over a 6-day storage period when the gas was stored in vacutainers. The N_2O concentration stored in vials with added silicon oil stored in an inverted position declined by 82%. The N_2O concentration stored in vials with added silicon oil stored in an upright position declined by 86.4%. The syringes lost an average of 87.7% of the added N_2O over the six-day period. This data clearly shows that the vacutainers were superior to the other methods of storage.

One reason vacutainers performed better over a 6-day period than the other methods is probably due to the vacutianer septum's superior ability to reseal. When a gas sample is taken with a syringe and injected into a storage container, as in field sampling,

a small hole is made in the septum. The potential for the "resealing" of this hole depends upon the composition of the septum and the means by which the septum is attached to the vial. A vacutainer is sealed by a thick rubber septum that pushes against the inside walls of the glass container. In this system the hole is virtually resealed because of pressure exerted on the rubber by the glass walls, similar to, but more effective than an expanding cork in a wine bottle. This greatly reduces sample loss due to diffusion and mass flow by restricting the size of pores and holes. Autosampler vials are sealed by either crimping a cap onto the glass vial, or by screwing a cap down onto the container (Figure 4.3). The vial septum is much thinner than a vacutainer septum and is less likely to reseal from a puncture, as the pressure is downward on the lip of the lid and not inward from the walls of the container. This means that a greater potential exists for gas loss by diffusion or mass flow in an autosampler vial.

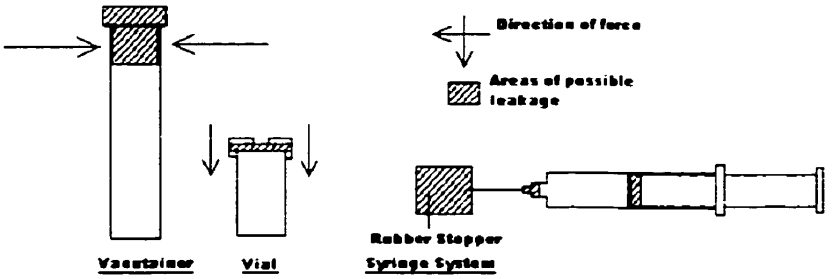

Figure 4.3 The vacutainer and vial sealing systems. The arrows show the directions of force on the seal of the container.

Field N_2O samples in this thesis were analyzed within 2 days. Therefore, percent loss as a percent of the standard gas was calculated for this timeframe. The vacutainer showed a 22% decrease in N_2O during this time. Comparing the % N_2O loss to the vacutainer, the vial down method lost 20% more, followed by the vial up at 32% and the syringe at 50%. All the samples showed a significant loss at alpha $\leq$ .05 over 6 days,

demonstrating the need to minimize sample storage time before analysis.

The autosampling vials using the automated injection showed less variability between replicates than syringes or vacutainers (Figure 4.2). The syringe sample showed the greatest variability out of all storage methods in the first day, two orders of magnitude higher than the vials. The variance in the vacutainer method was usually one order of magnitude higher. Maximum standard deviation in injection/leakage variability in N_2O storage trials for the syringe was 1.25 µl/l, the vacutainer was 0.31 µl/l, and the autoinjector vials had a variability of only 0.08 µl/l.

Some of the initial loss in the autosampler vials may be due to dissolving of N_2O into silicon oil. Choosing a better liquid barrier is difficult without running a battery of solubility tests as N_2O is a slightly polar molecule and therefore can dissolve in both polar and nonpolar solvents (Wells, 1962).

Differences were seen between the autosampler vial up and vial down methods of storage. Both the two and seven-day period, showed a significant difference at the alpha $\leq .05$ level between the inverted and non-inverted vials. The difference between the methods was most pronounced in the first few days. Inverted vials proved better at sample storage than the non-inverted.

4.4.2 Helium Trials

With ambient N_2O diffusing into containers, a trend similar to that seen in the N_2O sample loss experiment was seen (Figure 4.4). Percent gain was calculated as a percent of N_2O in ambient air. Over a six-day period, both the syringe and the vial stored in the upright position gained over 82% of the average ambient N_2O. The vial stored downwards limited the six-day N_2O gain to 74 %, while the vacutainer outperformed all the others with only a 62 % gain.

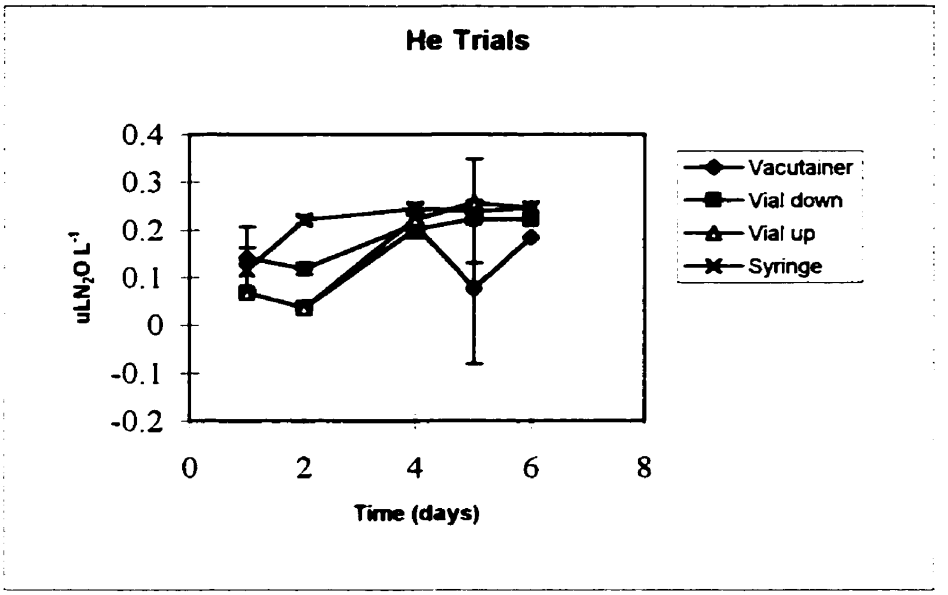

Figure 4.4 Average N_2O gain over a six-day period. Error bars show standard deviation.

The daily standard deviations of the autosampler vials were consistently much lower than the other two methods, again reaffirming that the autosampler eliminates variation in injection technique (Figure 4.4). The vacutainers had the highest total average standard deviation over six days, followed by the syringes with the autosampler vials last.

Although a liquid slows down the diffusion of gas by approximatly 10000 times, the gas can still diffuse through the liquid. Using the approximate liquid depth in the autosampling vial of 0.5 cm, and the diffusion coefficient for a gas in a liquid listed in Table 4.1 of 0.2×10^{-4} cm^2 s^{-1}, the Einstein-Smoluchowski equation (Metz, 1988) predicts a single molecule will diffuse through this distance in 1.74 hours.

In the He trials, over a two-day period, both types of vials (up and down) appear to outperform the other storage methods in preventing contamination. This is probably because it relies on an autoinjection system allowing less variation in injection technique than manual injection. Investigating sample loss/contamination at low concentrations of N_2O is difficult. At lower levels of gas concentration, small changes in N_2O level will show a high percentage of increase. For example, in the He trials, the 4-day vacutainer contamination is 70%, and this is almost 10% higher than the six-day vacutainer contamination.

Most methods showed the largest loss occurring within the first 4 days. Syringes show a rapid decrease (-0.9 μL d^{-1}) in N_2O within the first day and then a much slower decrease (-0.11 μL d^{-1}). Upright vials show a smaller decrease in the first day than the syringes losing per day, with the rate of loss decreasing to 0.2 μL d^{-1}. Inverted vials lost sample at a rate of -0.94 μL d^{-1} in the first day, with the rate of loss decreasing to -0.5 μl d^{-1}. Vacuntainers did not show consistent loss over the six-day period.

4.5 Conclusions

The vacutainer is the best method for storage of N_2O samples of the methods investigated over a six-day period. Only 6.7 % of the original N_2O sample was lost. This method did, however consistently have the highest daily variability in measurements (Figure 4.2).

For N_2O storage in a 0 to 2-day period the inverted vial with oil method performed better than the non-inverted vials and the syringe method. The syringe storage method was the least effective of all the methods tested, losing 70% of the sample within 24 hrs. Both types of vial systems had much less variation between replicates than other storage systems.

The autosampler vial down method with a liquid barrier shows some promise for N_2O sample storage, at least in the short term, and increases analysis reliability by decreasing injection variability when used with an autosampler. Trends seen in the He trials support the conclusions drawn from the N_2O trials.

It is important to note that although some methods show promise for N_2O sample storage, the sooner samples are analyzed after collection, the better.

5. NITROUS OXIDE REDISTRIBUTION AND FLUX IN THREE DIFFERENT SOIL COLUMNS

5.1 Abstract

The nitrous oxide (N_2O) forming processes of nitrification and denitrification within the soil profile are aeration dependent and directly affected by the rate of gas diffusion through soil. Thus N_2O emission can be strongly influenced by soil texture because the soil particle size interacting with soil water can affect gas diffusion pathways. Many N_2O flux studies focus on the inputs into the N_2O-forming processes and the timing of significant flux events, while little has been done specifically comparing N_2O movement through different soil textures. It is hypothesized that N_2O exhibits a longer residence time in fine textured soils when compared to medium or coarse textured soils after production events due to the effects of higher moisture contents and less continuous air-filled pore space. This means that complete denitrification of nitrogenous gases to nitrogen (N_2) is more likely in fine textured soils than in coarse textured soils, resulting in less N_2O surface emission.

In this investigation, the effect of soil texture on permeability and redistribution of N_2O in soil was examined. A method of analyzing the redistribution of N_2O in a soil column and estimation of the diffusion coefficient was developed. The movement of N_2O through columns of different textured soil was investigated, along with the

stimulation of the indigenous microbial population through addition of nitrate (NO_3^-) and carbon (C). The three soil textures investigated simultaneously were clay, clay loam, and sandy clay. The study, conducted at low moisture content, demonstrated no differences in N_2O gas redistribution in different textured soils. The methos developed effectively measures redistribution profiles of N_2O injected into soil columns. Detected profile amounts of N_2O were near ambient (atmospheric) concentrations or lower when 100 mL of solution added to a soil column either with or without nutrients, and surface flux of N_2O was not detected. This means that either the amount of N_2O produced was too small to detect, or no N_2O was produced.

Concentration profile data was fed into a computer model developed to simulate diffusion in a soil column. Diffusion coefficients calculated using the model do not show a clear textural trend. Larger amounts of water need to be added to produce significant differences in N_2O movement between soil textures. Further study would include replication of this study, the investigation of redistribution at a variety of higher moisture contents, different bulk densities, intact soil cores and other soil textures.

5.2 Introduction

The N_2O forming processes of nitrification and denitrification within the soil profile are dependent upon the degree of aeration and therefore directly affected by the rate of gas diffusion. Thus N_2O emission is strongly influenced by soil texture because both soil particle size and soil moisture affect gas diffusion pathways. Diffusion of a gas is 10^{-4} times slower through a liquid (liquid phase) than in air (gas phase), and thus the effective gas diffusion rate in soil profile is usually similar to that of continuous air filled pores until these pores are blocked (Campbell, 1985). Pores filled with water or blocked by thin films of water increase the tortuosity of pathways eventually decreasing gas diffusion rates. The amount of soil water can also affect the diffusion rate by absorbing and releasing N_2O as the gas partitions between the soil air and the soil water. This chromatography effect of soil water on N_2O diffusion is discussed in section 2.2.3.2.b. Essentially, the rate of gas diffusion through the soil will be retarded as it passes soil water and is absorbed and re-emitted by the water. Gas diffusion rates through soil are important because they will determine the retention time of N_2O in the soil. Slower diffusion means that N_2O will remain available for further reduction to N_2 by denitrification, resulting in lower N_2O surface emission.

In this chapter, a method of analysing redistribution of N_2O in a soil column and estimation of the diffusion coefficient was developed and tested. The effect of soil texture on permeability and redistribution of N_2O in soil was examined. An attempt was made to stimulate the indigenous microbial population in a soil column through addition of nitrate and carbon.

Texture affects the diffusion of N_2O through a soil column mainly through the porosity, pore size and effects of soil water. Fine textured soils have high porosity with small pores, while coarse textured have large pores with less porosity. Because different soil textures are associated with different pore space and pore size, they will have different tortuosity, even if soils are subjected to similar moisture tensions. Model simulations and experiments have shown lower O_2 in fine textured soils when compared to coarse textured soils (Figure 5.1).

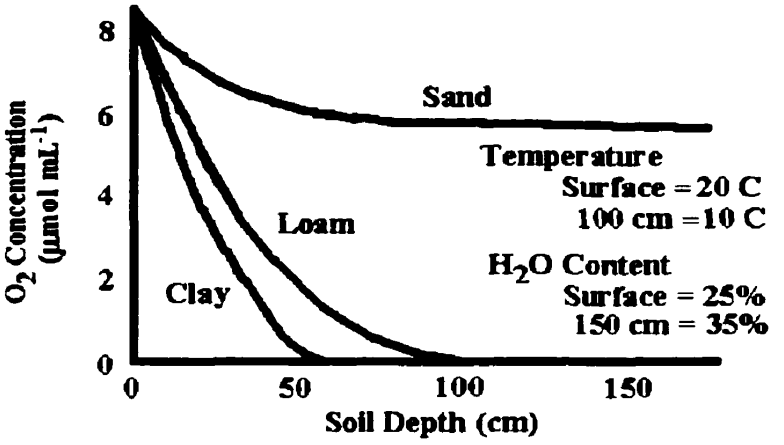

Figure 5.1 Oxygen concentrations in different soil textures. The effect is in part due to greater microbial activity in clay, but also due to decreased gas diffusion rates in clay as moisture content rises (Cho et al. 1997).

It is hypothesised that N_2O has a longer residence time in fine textured soils than in medium or coarse textured soils after a rainfall event due to the effects of higher moisture. This means that complete denitrification of NO_3^- to N_2 is more likely in fine textured soils than in coarse textured soils, as the N_2O remain available for further reduction.

When O_2 supply by diffusion from the atmosphere is insufficient to meet microbial respiration demands due to high moisture, NO_3^-, NO_2^- and N_2O may be used as

alternative electron acceptors by denitrifiers (Section 2.2.2.2). Cho et al. (1997) simulated competition of O_2, NO_3^-, NO_2^-, and N_2O for electron acceptors in denitrifiers using Michaelis-Menton kinetics and found that reduction of N_2O to N_2 occurred when O_2 and most of the NO_3^- was gone. In principal, the faster O_2 diffuses to the site of respiration and the faster N_2O can move away, the less likely the denitrification reaction will proceed to N_2. In Figure 2.6, Davidson (1991) demonstrated the effect of water filled pore space on the relative flux of N_2O and nitrogen (N_2) gas from soil, finding that as water filled pore space increased, N_2 emission increased and N_2O emission decreased. Findlay and McKenny (1979) and Freney et al. (1978) found low N_2O flux (<1.62 ng $m^{-2}s^{-1}$) in clay soils (Table 3.1). However, lower N_2O fluxes are not always found in fine textured soils because other factors may limit complete denitrification to N_2 causing high N_2O profile concentrations and resulting surface flux (Myrold and Tiedge, 1985., Bremner and Shaw, 1958).

5.3 Materials and Methods

5.3.1 Column Preparation

Three poly-vinyl chloride (PVC) columns were constructed for use as soil containers. The columns were sealed at the bottom with a plexi-glass plate using epoxy adhesive. The columns were 65.5 cm long with a 14.9 cm inner diameter (ID), with an injection port with a rubber septum at the bottom. The columns were filled to 3.0 cm with packed sand, and then soil was layered (packed) at 5 cm intervals to ensure fairly uniform bulk density throughout the column. Height of the soil in the columns was 56.5

cm. The three air dry soils used were a sandy clay from near Carberry, MB. (NE25-10-15W) a clay from near Oak Hammock Marsh, MB. (SE32-13-3E) and a clay loam from the Manitoba Zero-Till Farm, MB. (NE31-12-18W). All three soils were A horizons at air–dry moisture content (Table 5.1).

Table 5.1 Column characteristics of the soil columns prior to investigations.

Columns	Texture	Air Dry (AD) Moisture Content	Field Capacity (FC)	Bulk Density (BD)	Air Filled Pore space
		%w	%w	$g*cm^{-3}$	%
Column 1	Sandy Clay	3.1	20.0	1.14	51
Column 2	Clay Loam	3.4	28.4	1.09	53
Column 3	Clay	5.1	38.3	1.12	48

Five sampling tubes were inserted 6 cm into each column at 10.0 cm intervals from the bottom. Thin tubing (0.29 ID) connected these sampling ports to a 16 port sampling valve. Figure 5.2 shows a typical PVC column setup. A small in-line water moisture filter (containing potassium perchlorate) was placed in each tube, as water can reduce the gas chromatograph's ability to resolve the N_2O peak. This system allowed all three columns to be sampled and analyzed at five depths each, along with a standard gas, within the shortest possible time frame (2 hrs).

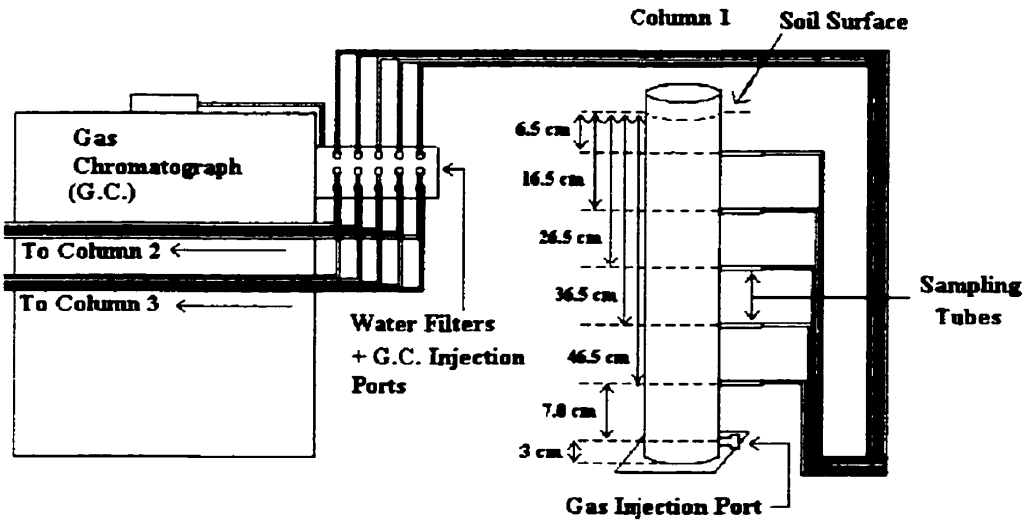

Figure 5.2 A typical soil column setup showing column sampling and G.C. injection ports. Two other columns (2 and 3) were hooked up to the remaining G.C. injection ports.

As only fifteen ports were available for gas analysis, it was decided that the three different soil textures would be analyzed simultaneously to minimize differences in temperature and atmospheric pressure which affects mass flow, thus allowing some comparison of gas diffusion between textures. Unfortunately, this made it impossible to run replicates of each soil type. However, because a sixteenth port sampled a standard gas once per hour in all investigations (3.0 μL N_2O L^{-1}), the measure of analytical resolution was determined. The standard deviation of the standard gas measurements ranged from 0.041 to 0.058 μL N_2O L^{-1} for all the experiments.

5.3.2 Gas Analysis

A sampling valve directed 0.1 mL of sample from each sampling port to a gas chromatograph (GC) equipped with an electron capture detector (ECD). The ECD was equipped with a Porapak Q (80/120 mesh) packed pre-column (30 cm x 0.16 cm ID) to

trap and flush out water, followed by a Porapak Q packed analytical column (152 cm x 0.16 cm ID.). The carrier gas consisted of 10% methane, with the balance argon. Each run required approximately 7.5 minutes. Gas flow rate through the detector was 30 mL min^{-1}. The detectin limit of the GC/ECD was 0.005 μL N_2O L^{-1}.

Concentrations of N_2O were determined by integrating chromatograph peak area. Standard curves for the N_2O concentration were determined at the start of the study, and recalibration was performed when necessary using a two-point calibration curve.

5.3.3 The Experiments

5.3.3.1. Experiment 1 - N_2O Only.

A volume of 0.1 mL of pure N_2O was injected into the bottom of each column and allowed to diffuse through the column. All ports in each column were sampled for N_2O every two hours. The experiment was run for 72 hours. Injections were synchronized so that ports in each column were sampled at identical time intervals, relative to injection time.

The N_2O concentration of in each column was plotted as a function of depth for each time interval. The diffusion coefficient through each column was calculated using the computer model discussed in 5.4.3.5. No gas concentrations were measured above the soil surface to calculate a surface flux.

5.3.3.2. Experiment 2 - H_2O Only.

Distilled H_2O (100 mL) was added to the top of each column to determine if N_2O production could be induced. Gas movement throughout the column was analyzed over a

period of 7 days, to allow for any lag in microbial production that may occur.

Once a day, gas concentrations above the soil surfaces were measured in each column by covering the columns with a plexiglass cover (sealed by vacuum grease) and measuring the trapped gas concentration for two hours. The 2 mL gas samples were taken through rubber septums in the covers at 0, 30, 60, 90 and 120 mins. These concentrations were used to estimate N_2O surface flux at the top of each column.

5.3.3.3. Experiment 3 - Glucose and Nitrate.

After drying the columns for 3 weeks, a 100 mL solution of a glucose and nitrate (100 ppm N, 1000 ppm C) was added the columns to simulate N_2O production event. Column N_2O profiles and surface flux were sampled for 7 days following the addition of the glucose and nitrate solution.

5.3.3.4. Experiment 4 - N_2O, Glucose, and Nitrate.

After drying the columns for 3 weeks, experiment 3 was repeated, along with an injection of 0.1 mL pure N_2O into the bottom of each column. Both column N_2O profile and flux data were sampled and recorded for 7 days after initiation of the experiment.

5.3.3.5. Estimation of Diffusion Coefficient.

A computer model developed with Dr. C. M. Cho describes the redistribution of N_2O within a soil column (Appendix II b). It assumes a finite amount of N_2O input with constant concentration and gas escaping at the top of the column. The distribution of N_2O in the column is calculated over time.

The value of the diffusion coefficient D can be determined by using the Crank-Nicolson (Crank and Nicolson, 1947) implicit method to solve the gaseous transport equation:

$$(\partial C/\partial t) = D(\partial^2 C/\partial x^2) \pm \varnothing \qquad [5.1]$$

where: C = concentration of N_2O
t = time
x = distance
$\varnothing$ = production/consumption term
D = diffusion coefficient

If we set the production/consumption term to zero, the Crank-Nicolson method approximates equation 5.1 by:

$$(C_{i,\,j-1} - C_{i,\,j})/t = \tfrac{1}{2}D\{(C_{i-1,\,j-1} - 2C_{i,\,j-1} + C_{i-1,\,j-1})/x^2 + (C_{i-1,\,j} - 2C_{i,\,j} + C_{i-1,\,j})/x^2\} \qquad [5.2]$$

giving:

$$-r\,C_{i-1,\,j-1} + (2+2r)\,C_{i,\,j-1} - r\,C_{i-1,\,j-1} = r\,C_{i-1,\,j} + (2-2r)\,C_{i,\,j} + r\,C_{i-1,\,j} \qquad [5.3]$$

where : $r = D\,(t/x^2)$
i = position
j = time

At any given time, i is incremented from i = 1 to i = n, totaling n grid points. There are n number of equations corresponding to equation 5.3, whereas there are n+2 grid points (ie i = 0 and i = n+1). The value of concentration (C) at any time j is determined by the left and right boundary conditions. The left side equation 5.3 contains three unknown values, while the right side of the equation contains three known values.

In our diffusion problem, the first step on the right side contains the known initial boundary conditions calculated from the N_2O injected at the bottom of the column (x = 0

cm) at $t = 0$. The rest of the concentrations in the column are calculated from these known conditions for other distances and times based on a guessed D. Once the N_2O has traveled to the top of the column, an escape coefficient must be used to account for the N_2O lost from the column.

Thus the program estimates concentration curves of N_2O diffusion through a column for different diffusion coefficients. Outputs are compared to actual data to determine the most correct diffusion coefficient. The minimum value of absolute difference between the calculated and the measured concentrations should give a correct diffusion coefficient.

5.3.3.5.a. Calculation of k, The Escape Coefficient.

In order to use N_2O redistribution model, an estimation of the escape coefficient must be made. As all we need for the model is an estimate of k, the column flux data set with the time closest to a profile measurement was used to calculate k. Diffusive flux of a gas through a medium is described by the Fickian expression (Hillel, 1982):

$$flux = -D(dc/dx) \qquad [5.4]$$

where: D = the diffusion coefficient,
dc/dx = the change in concentration over distance

This flux can be measured using the equivalent expression involving a change in concentration of N_2O in a known volume over time previously discussed in Chapter 2:

$$f = V(Ct-Co/At) \qquad [2.1]$$

Dissipative surface flux can be expressed as:

$$flux = k(c) \qquad [5.5]$$

where: k = the escape coefficient,
c = the concentration at the boundary

At the surface of the column, diffusive flux is equal to dissipative flux, otherwise accumulation of the gas occurs, and the law of mass balance does not apply. To calculate k, the concentration of N_2O throughout the column is used to estimate the concentration of N_2O at the surface by extrapolation of the best-fit polynomial:

$$y = ax^2 + bx + c \qquad [5.6]$$

where: y = concentration of N_2O in the column,
x = distance from the bottom

If we set x to be the length of the soil column, y becomes the concentration at the soil surface.

For a soil column 56.5 cm in length, the surface concentration of N_2O is calculated (equation 5) to be 0.93 $\mu L\ L^{-1}$, and the surface flux was measured, by sealing the top of the column and using equation 2.1, to be 1.25×10^{-6} $\mu L\ cm^{-1}s^{-1}$.

Using equation 4.4:

$$k(0.93) = 1.25 \times 10^{-06} \text{ and}$$
$$k = 1.34 \times 10^{-06}\ \mu L\ cm^{-2}\ s^{-1} = 1.34 \times 10^{-3} \mu L\ cm^{-2} s^{-1}$$

This is the escape coefficient used in calculating the diffusion coefficients in the columns. It should be noted that this escape coefficient really is only valid at 20 hrs. Before this time, the escape coefficient is probably higher, and after this time, it decreases.

5.4 Results and Discussion

5.4.1 Experiment 1 - N_2O Only

Gas redistribution was similar in all 3 columns (Figure 5.3). High levels of N_2O (26.13 $\mu L\ L^{-1}$) were found in the sandy clay (SC) at the 46.5 cm depth within 2.13 hours of injection. The heavier textured clay (C) and clay loam (CL) had slightly lower N_2O levels at (25.86 $\mu L\ L^{-1}$ and 25.19 $\mu L\ L^{-1}$ respectively) at 2.13 hours. These heavier textured soil continued to contain less N_2O at both the 46.5 and 36.5 cm ports for this entire run. Near the column surface at t = 0.13 hours, the picture is less clear. Generally, it can be seen that at the 16.5 cm and 6.5 cm heights for all time periods the clay retains slightly more N_2O than the sandy clay.

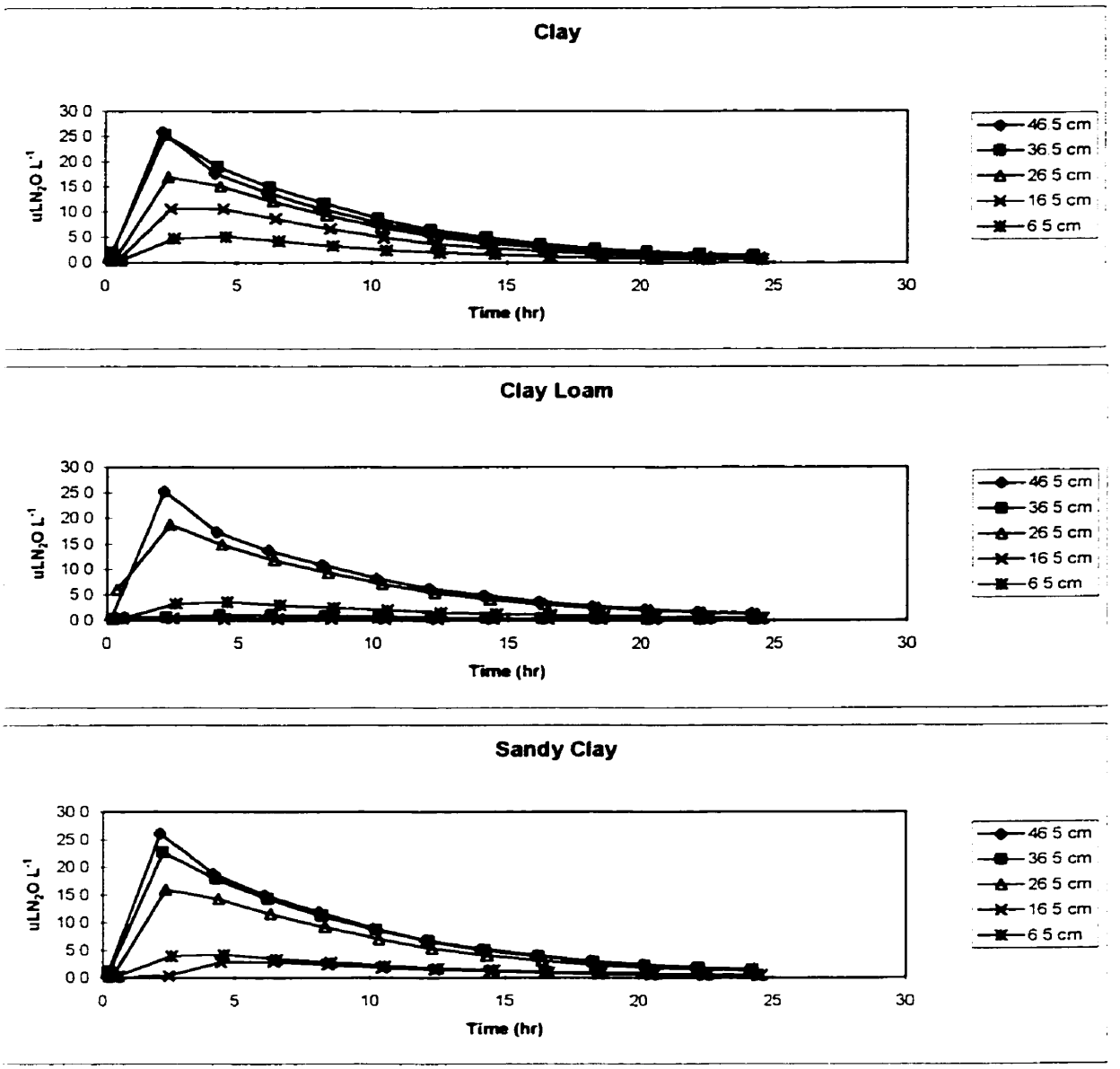

Figure 5.3 Redistribution of N_2O in soil columns of three different textures after 0.1 mL of pure N_2O was injected into the bottom of each column. The G.C./E.C.D had a standard deviation of 0.048 $\mu L\ L^{-1}$ in repeated measurements of the standard gas.

At 16.5 cm, the main peak of N_2O in the sandy clay appeared at 6.13 hours, whereas the clay N_2O peak occurred at 4.13 hours, which is opposite of what one would expect (Figure 5.4). However, closer examination shows the profile concentration of the clay was 5 times that of the sandy clay at 4.13 hours, and still 4 times that of the sandy

clay at 6.13 hours, again suggesting that a longer retention time for N_2O may occur in fine textured soil. The concentration of N_2O at 36.5 cm in the clay is at least double that of the sandy clay for the entire run.

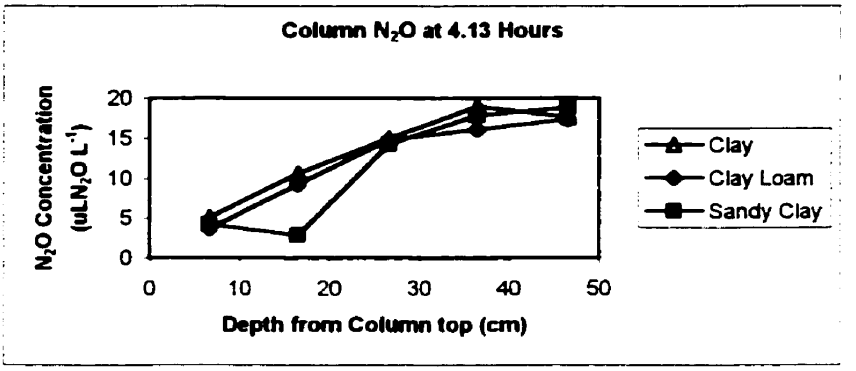

Figure 5.4 Nitrous oxide concentration differences between textures 4.13 hour after injection of 0.1 mL N_2O at the base of the column.

5.4.2 Experiment 2 - H_2O Only.

Trace amounts of N_2O were detected, but no particular redistribution pattern was noticeable when 100 mL of H_2O was added to each of the columns. There was no detectable flux from the top of the columns. This indicates that either only trace N_2O was produced due to lack of available nutrients/anoxic conditions or trace N_2O was in the column before addition of H_2O. Either amounts produced were too small to drive flux from the surface, or NO_3^- denitrified completely to N_2 before leaving the column.

Maximum profile concentrations in the clay, clay loam, and sandy clay were 0.62, 0.39 and 0.41 $\mu L\ L^{-1}$ N_2O respectively. Profile concentration was slightly higher in the clay, as shown at t = 4.13 hours (Figure 5.5).

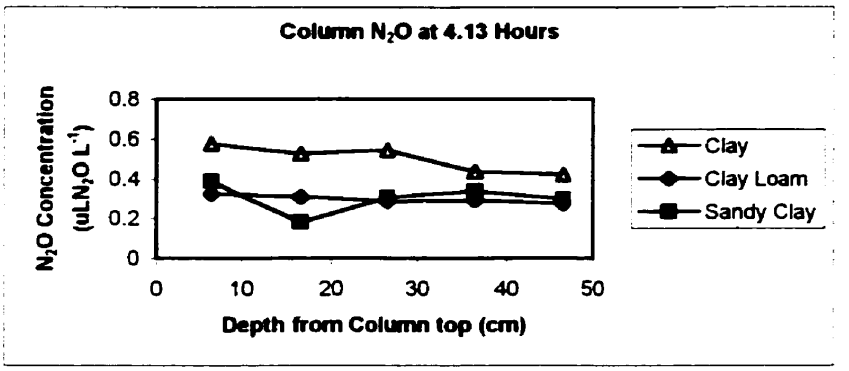

Figure 5.5 Profile concentrations of N_2O at 4.13 hours after addition of 100 mL H_2O. Although clay is highest, amounts detected are quite low, as ambient air has 0.31 μL L^{-1} N_2O.

5.4.3 Experiment 3 - Glucose and Nitrate.

As in 5.3.2, trace amounts of detectable N_2O were produced in the columns, but no particular redistribution pattern was detected after addition of glucose, water, and nitrate. There was no detectable flux from the top of the columns. This could mean that similar to experiment 2 only trace N_2O was produced due to lack of available nutrients/anoxic conditions or trace N_2O was in the column before addition of H_2O. Any N_2O produced was diluted or quickly reduced to N_2 in all columns before it could be detected as surface flux.

Maximum profile concentrations in the clay, clay loam, and sandy clay were 0.72, 0.41 and 0.44 μL L^{-1} N_2O respectively. Profile concentration was generally higher in the clay, as shown at t = 4.13 hours (Figure 5.6).

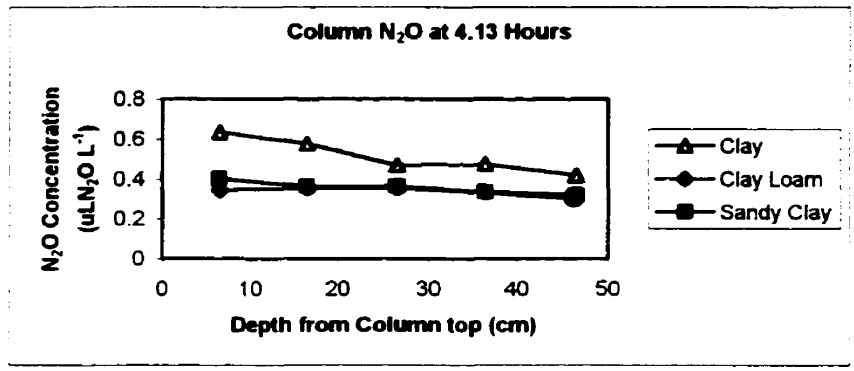

Figure 5.6 Profile concentrations of N_2O at 4.13 hours after addition of 100 mL glucose/nitrate solution. Although clay is highest, amounts detected are quite low, as ambient air has 0.31 $\mu L\ L^{-1}$ N_2O.

5.4.4 Experiment 4 - N_2O, Glucose, and Nitrate

Gas redistribution followed similar patterns in all columns, mirroring patterns found in 5.3.1 (Figure 5.7). Some evidence exists showing that fine textured soils may retain N_2O longer than coarse texture soils ones. Initially (t = 0.13 to t = 2.13 hours) the fine textured clay had less N_2O at all depths than any other column. In fact at t = 0.13 hours, depth = 6.5 cm, the sandy clay had the highest N_2O concentrations followed by the clay loam, and then the clay. As N_2O redistributed throughout the column over time (t = 4.13 to t = 12.13 hours), fine textured soils showed higher levels of N_2O than the sandy clay (Figure 5.8). From t = 14.13 hours until the experiment was complete, N_2O in the soils column was usually slightly higher in the clay, followed by the clay loam and then the sandy loam. At 26.5 cm, the peak N_2O concentration of the clay was retarded by 2 hours when compared to the peak of the sandy clay and clay loam columns, although the peaks were similar in magnitude.

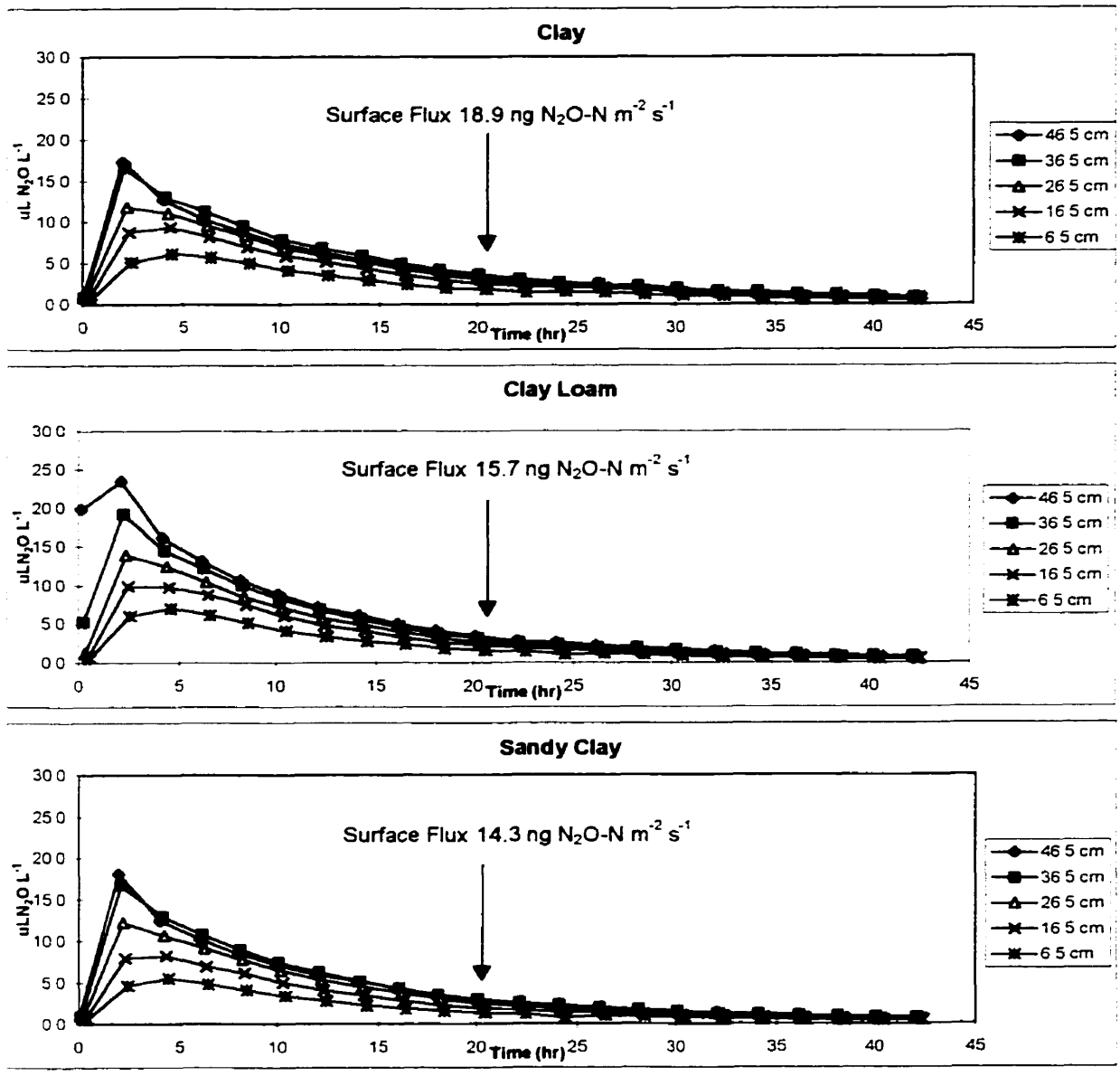

Figure 5.7 Redistribution of N_2O in soil columns of three different textures after 0.1 mL of pure N_2O was injected into the bottom of each column. Glucose and Nitrate were added to the column top at t = 0 hrs in a 100 mL solution. The surface flux. at 20 hours is indicated. The G.C./E.C.D had a standard deviation of 0.048 $\mu L\ L^{-1}$ in repeated measurements of the standard gas.

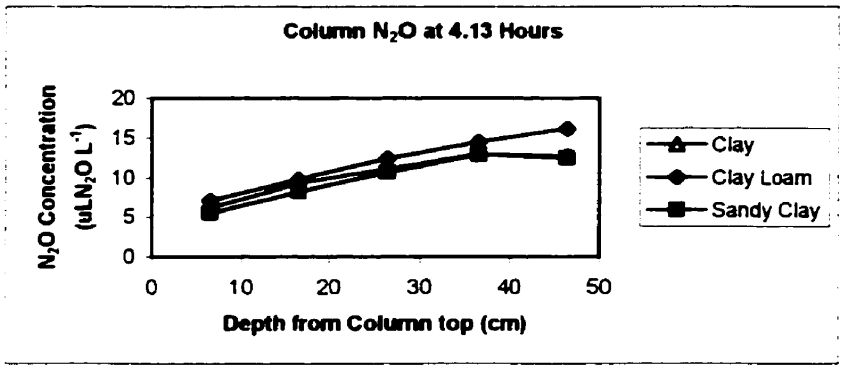

Figure 5.8 Profile concentrations of N_2O at 4.13 hours after addition of 100 mL glucose/nitrate solution to the surface and injection of 0.1 $\mu L\ L^{-1}$ of N_2O at the bottom of the columns. Clay Loam has the highest followed by the clay.

At 20 hours, the clay had the highest measured flux followed by the clay loam and then the sandy clay (Table 5.2). This again demonstrates slight retention of N_2O in the clay as the peak surface concentration in the sandy clay and clay loam have probably passed.

Table 5.2 Surface N_2O flux calculated by chamber method columns at 20 hours and measured upper profile concentrations. Of the trials measured, only experiment 4 had a detectable surface flux (N_2O, glucose, nitrate).

Texture	Upper Port Concentration ($\mu L\ L^{-1}\ N_2O$)		Flux from Covered Surface ($ng\ N_2O\text{-}N\ m^{-2}\ s^{-1}$)
	16.5 cm	6.5 cm	
Clay	2.50	1.77	18.9
Clay loam	2.28	1.56	15.7
Sandy clay	1.90	1.35	14.3

These studies shows some textural influence on N_2O redistribution in the soil. Experiment 1 and 4 both show slight retention of injected N_2O in the profiles of the fine textured soil (C,CL) when compared to coarser one (SC). Low levels of N_2O remaining in the columns for long periods of time after the peak concentrations had passed may

have contributed to masking differences between columns. Experiments 2 and 5 suggest that N_2O is more likely to remain in a fine textured soil than coarse textured soils, even though the water or glucose and nitrate solution added may not have stimulated enough N_2O production to produce a detectable surface flux or large profile concentration. Overall, no major differences were seen in N_2O profile distribution between textures.

It should be noted that this study only considered soil columns with very low water contents, and as a result, large differences in N_2O redistribution were not expected. The air dry moisture content was used so that fairly uniform moisture conditions would exist in the columns, and so that sampling ports were not plugged and instrumentation was not damaged (Table 5.1). Figure 2.5 shows that as water content is lower (air filled porosity is higher) differences in the diffusivity of gases are less than at higher water contents. At lower moisture tensions, one would expect textural differences to have an increasing effect on the diffusion of N_2O through the soil, both through blockage of pores and absorption/desorption of N_2O from soil water.

The fact that only slight changes occurred in N_2O distribution at air-dry moisture contents implies that in the field, N_2O diffusion resulting in emission will differ only when moisture suctions are medium/low. This has important implications, as these medium/high moisture contents are the exact conditions required for an N_2O production event.

5.4.5 Model Output

Differences in diffusion coefficients were low because textures had similar air filled pore space (Table 5.1).

Computer calculated diffusion coefficients were 7.60 cm^2 s^{-1} in all treatments except the clay loam N_2O, H_2O and $C_6H_{12}O_6$, which was 6.07 cm^2 s^{-1} (Table 5.3). The pattern produce by the model is similar to the measured data. However, the peaks spike at approximately 45 to 50 μL L^{-1} in the model output instead of at approximately 18 to 25 μL L^{-1} and quickly dissipate at all depths (Figures 5.9, 5.10). There is also virtually no spread in data after the first 2 hours in the model output, while the measured data shows a decreasing peak of N_2O as the depth decreases (Figures 5.4, 5.8).

The initial boundary condition used in the model is difficult to approximate numerically, as it is a variable boundary condition. Because gas diffusion is very rapid at air-dry soil moisture contents (large air-filled porosity), not only is N_2O diffusing throughout the column, but it is simultaneously diffusing throughout the sand plug. Reflection of the gas from the sides of the column may also compound the problem of approximating the initial boundary condition.

Changes in column design can minimize the importance of the boundary condition. For example, a small air space at the bottom of the column instead of a 3 cm sand plug would eliminate the problem of diffusion within the sand. Long and narrow columns, along with larger amounts of N_2O injected could also minimize the effect of the initial boundary condition. Alternatively, a constant emission of N_2O from a point source at the bottom of the column will simplify model calculations.

Approximations made such as estimating the escape coefficient and assuming an instantaneous point source and a homogeneous system also affect model accuracy, but not to the degree of the initial boundary conditions.

Table 5.3 Calculated diffusion coefficients ($cm^2\ s^{-1}$) after injection of 0.1 mL N_2O at the base of the columns.

Texture	0.1 mL N_2O Only	$H_2O + C_6H_{12}O_6 + 0.1$ mL N_2O
Sandy Clay	7.60	7.60
Clay Loam	7.60	6.07
Clay	7.60	7.60

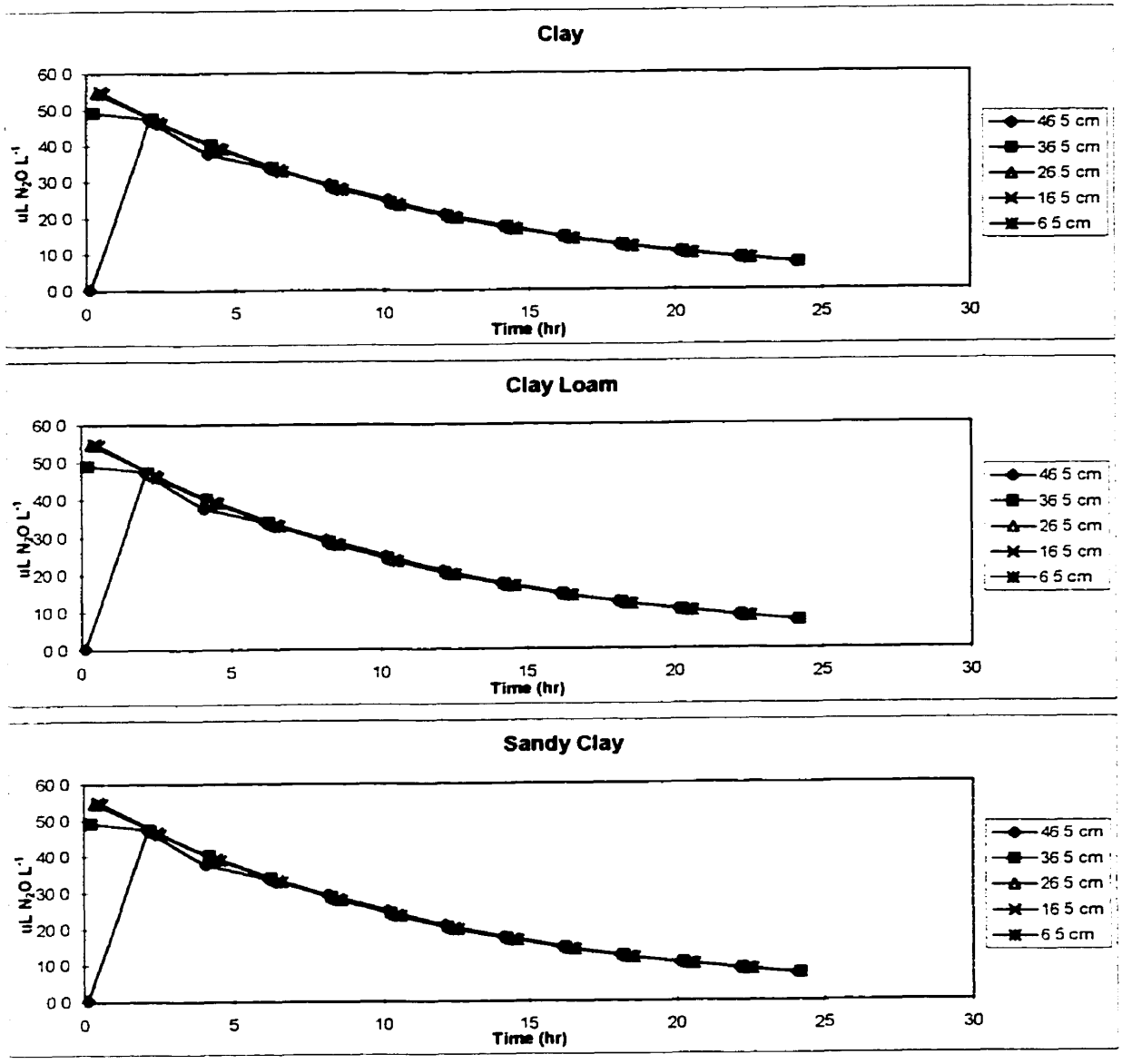

Figure 5.9 Model output of redistribution of N_2O in soil columns of three different textures after 0.1 mL of pure N_2O was injected into the bottom of each column. The diffusion coefficient in these trials was calculated to be 7.60 $cm^2\ s^{-1}$.

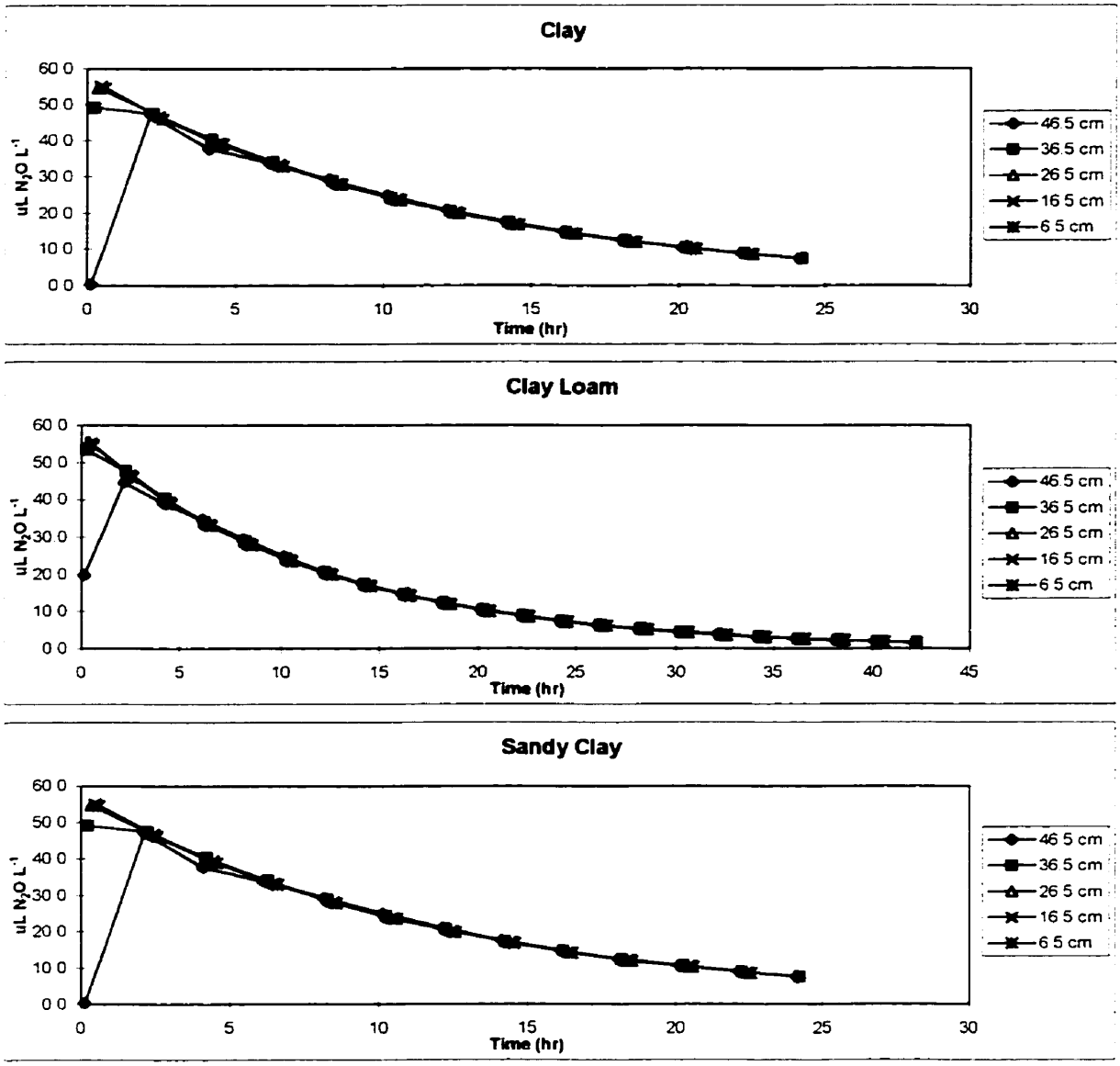

Figure 5.10 Model output of redistribution of N_2O in soil columns of three different textures after 0.1 mL of pure N_2O was injected into the bottom of each column. This models the Glucose and Nitrate solution trial. Diffusion coefficients for the clay, clay loam, and sand were 7.60, 6.07 and 7.60 $cm^2\ s^{-1}$ respectively.

5.5 Conclusions

The method developed for continuously measuring N2O redistribution in soil columns is very effective. This study suggests some differences in N_2O gas redistribution in different textured soils. Nitrous oxide injected into the clay and clay loam columns appear to redistributed more slowly than in the sandy clay. Results are not conclusive, as differences have not been shown to be statistically valid. Surface flux would have to be measured constantly to show retardation of N_2O in clay at air dry moisture content, as gas diffusion is very rapid though air filled pores.

Because detected profile amounts of N_2O were low when 100 mL of solution added to a soil column either with or without nutrients, and surface flux of N_2O was not detected, it cannot be said with certainty that N_2O was produced in any column. However, the slightly larger profile concentrations that were detected in the clay suggest that N_2O may be retained longer in fine textured soils. The maximum detectable profile concentration in the clay, clay loam and sand was 0.62, 0.39 and 0.41 $\mu L\ L^{-1}$ N_2O when 100 mL of water was added, and 0.72, 0.41 and 0.44 $\mu L\ L^{-1}$ when 100 mL of a glucose/nitrate solution was added, respectively.

Diffusion coefficients calculated using the data were higher than expected, and do not show a clear textural trend (Table 5.3). In air Prichard and Currie (1982) measured the N_2O diffusion coefficient to be 0.143 $cm^2\ s^{-1}$. In soil, the diffusion coefficient should be about 2/3 this number, or about 0.095 $cm^2\ s^{-1}$ (Penman, 1940).

This investigation developed a method of measuring N_2O concentrations in a soil

column, and comparing acquired data to a model output. Modifications in both column design and model assumptions will improve outputs.

Further study would include replication of this investigation using column designs that simplify initial boundary conditions, investigating redistribution at higher moisture contents, and different bulk densities. The model calculating diffusion coefficients can then be refined to more accurately predict diffusion coefficients.

6. GENERAL DISCUSION

Although high N_2O flux events have occurred during spring thaw periods in many locations across Canada (Goodroad and Keeney, 1984, van Bochove et al., 1996), similar events have not been measured on heavy textured soil in Manitoba. Many researchers have found heavy textured soils to produce high N_2O flux (McKenney et al., 1980, Granli and Bockman, 1994). Estimates of soil N_2O emission in Manitoba were calculated to be 57% of the Manitoba's total agricultural emission in 1996 (Janzen et al., 1999). These values are based on a percentage (approximately 1 %, source dependant) of the applied soil N values. It is assumed that that high fluxes would occur in the fine textured soils of the Red River valley because of high N inputs (about 18.94 – 31.52 ng N_2O-N $m^{-2}s^{-1}$) (Janzen et al., 1999). The silty clay soil investigated in chapter 3 had low emissions of N_2O in the spring (maximum of 7.0 ng N_2O-N $m^{-2}s^{-1}$) when compared current regional estimates and to other textures in the literature (Table 3.1). Although fine textured soils have a high potential for N_2O production when wet, they also have a high potential for retarding gas diffusion. This means that denitrification will more likely proceed to the N_2 endpoint as N_2O in the soil remains available to denitrifiers for further reduction. It has been reported that fine textured soils can produce lower fluxes than courser ones (Arah, 1991).

The influence of different cropping systems on N_2O production has been discussed in the literature (Granli and Bockman, 1994). Legumes have generally been

shown to increase N_2O emission when compared to other crops, however clear trends are difficult to determine based exclusively on cropping systems (Granli and Bockman, 1994). In the field investigation in this thesis, the alfalfa treatment had a significant increase in surface flux. Other cropping treatments had low flux, probably due to different the rooting depth and nature of root exudates. For example, natural production systems (i.e. grasslands) have extensive root systems that provide carbon, but these systems are usually NO_3^- limited, impeding the production of nitrogen gases. More information on cropping systems and their effects on N_2O production is needed in order make accurate recommendations for managing N_2O emissions.

Some studies have investigated *in situ* profile concentrations for surface flux. Burton et. al. (1997) found that although profile concentration could in some cases predict surface flux, in other cases it could not. In this thesis, increased N_2O concentrations in alfalfa soil profiles (<60 cm) occurred in March, which is when the highest surface flux was seen in this treatment. Although only small increases in N_2O concentration were observed in the lower fallow profile (>60 cm), a large surface flux occurred in March suggesting that although N_2O is produced, lack of carbon probably limits the ability of denitrifiers to reduce the gas to N_2. Small N_2O accumulations throughout the profile of other cropping systems did not reflect significant increases in surface flux. When upper profile concentrations (20 cm, 10 cm depths) were used to predict surface flux, the results were poorly correlated to flux estimated by chamber methods. Correlation improves when profile calculated flux is staggered one sampling period ahead of chamber calculated flux. This indicates a delay in N_2O reaching the surface. Differences in magnitude of flux between profile and chamber methods may in

part be attributed to production, consumption, and retardation of N_2O within the profile.

Although denitrification activity generally decreases with depth (Bailey and Beauchamp, 1973., Kahn and Moore, 1968., Cho et al., 1979), this does not always seem to be the case. Significant N_2O production has been found to occur as deep as 75 cm in soil profiles (Gilliam et al., 1978). As a result, models such as DNDC (Li et al., 1992) which use production as flux may be inappropriate in some situations The relationships between soil properties and their resulting effects on N_2O surface flux needs to be investigated farther. Attempts to model surface flux from profile concentrations are important in understanding when denitrification/nitrification potential can be related to surface flux, and when such comparisons are not appropriate.

Spatial and temporal variability found in field measurements of N_2O flux may in part be explained by the processes occurring beneath the soil surface. Most studies have focussed on different water filled pore space as a controlling factor of N_2O emission, but as pore size can affect continuous channels through which gas diffuses and the amount of water retained by the soil, texture studies are imperative in this approach. Lack of continuous pores not only affects the production of N_2O, but also the time it resides within the profile, allowing it be consumed, or slowly emitted from the surface. Measurement of subsurface concentrations *in situ*, as well as redistribution under controlled laboratory conditions in simulated profiles, are key to understanding these processes.

In order to accurately and effectively investigate the anthropogenic impacts on N_2O production in ecosystems across the country there is a definite need to develop a simple cost effective method for storage and transport of gas samples to the laboratory.

Portable G.C.s are very expensive, and often impractical. The investigation in chapter 4 into N_2O sample storage showed that a liquid barrier holds some promise in resealing puncture holes in storage vials. However, liquid diffusion barriers can only reduce diffusion through a rubber septum if the diffusion coefficient through the liquid is lower than that through the rubber. The use of an autoinjection system reduced injection variability in gas analysis. A gas storage system that combines the sampling precision of an auto-injector, along with the storage capability of vacutainers would be best.

The method developed to measure N_2O redistribution through soil columns was effective. Three column concentration profiles could be accurately monitored continually over long time periods. Calculation of the diffusion coefficient throughout the column did not demonstrate differences between textures. Modifications to the column design can minimize errors due to the approximation of the initial boundary condition in the model. Investigations at lower moisture tensions would probably yield greater differences between textures in N_2O redistribution as the moisture effects on gas movement become more pronounced.

Nitrous oxide emission and the resulting effects on the atmosphere are global problems of increasing concern. This thesis in part attempts to develop better techniques for more accurate N_2O measurements and a better understanding of the factors controlling N_2O concentrations and emissions from the soil profile. The effects of cropping system, timing (spring thaw), along with information on N_2O movement, profile distribution and surface flux provide additional information that can be used in the global effort of understanding and managing N_2O emissions in agriculture.

7. SUMMARY AND CONCLUSIONS

The purpose of this investigation was to contribute to the knowledge and understanding of the emission of N_2O and the factors controlling the fate of this agriculturally produced greenhouse gas. Specifically, three experiments were designed to gather information and these included; a spring thaw field study, a vial storage investigation and a studies investigating soil texture effects on N_2O profile redistribution and diffusion.

The main purpose of the spring thaw field study was to determine if there was an N_2O flux event during spring thaw on a typical Manitoba soil and to see if different cropping systems (native grass, fallow, alfalfa and wheat) affected the magnitude of this production event. In addition, comparisons were made between N_2O flux calculated using profile concentrations to flux estimated using the chamber method.

Flux of N_2O increased during spring thaw and cropping systems affect the production of N_2O. This study illustrated that the strongest N_2O flux event occurred in the alfalfa cropping system, where upper soil surface profiles coincided with high surface flux. Overall, the magnitude of nitrous oxide surface flux was small in this silty clay soil, when compared to literature values reported for coarser textured soils.

Timing of increased N_2O profile concentrations and N_2O surface flux generally coincided in the spring thaw study, however there seemed to be a delay between profile concentration and surface flux. A higher N_2O profile concentration usually resulted in

higher surface flux. Profile concentrations in the thaw study based on an N_2O gradient at 10-20 cm depth were poorly correlated ($r^2 \leq 0.182$) with flux measurements taken on the same day. A better correlation in the alfalfa treatment ($r^2 = 0.787$) was obtained with flux measurements made on the subsequent sampling period, indicating a delay in the transport of N_2O to the surface.

The objective of the investigation in Chapter 4 was to determine the efficiencies of current N_2O storage techniques. For relatively long sample storage times (a six-day period) the vacutainer was determined to be the best storage method for N_2O out of the four methods investigated (vacutainer, syringe, autosampler vials with and without silicon oil barriers). In fact, only 6.7 % of the original N_2O sample was lost with the vacutainer. However, this method had the highest daily variability in measurement, probably because it could not be used with the current automated injection system.

For shorter N_2O storage periods (0 to 2-day period), the autoinjection vial with silicon oil barrier out performed both the no barrier autoinjection vial and the syringe method in N_2O sample storage. Both types of autoinjection vial systems had much less variation between replicates than all other storage systems. The autosampler vial with a liquid barrier showed some promise for N_2O sample storage, at least in the short term, and increased analysis reliability by decreasing injection variability when used with an autosampler. The syringe storage method was the least effective of all the methods tested, losing 70% of the sample within 24 hrs. Syringes are currently widely used as an N_2O sampling and storage method and the results of this study question the validity of data obtained using this method.

In summary, vacutainers provide the best storage method for N_2O gas, whereas

autoinjectors eliminate injection variability. Maximum standard deviation in injection/leakage variability in N_2O storage trials for the syringe was 1.25 μL L^{-1} the vacutainer was 0.31 μL L^{-1}, and the autoinjector vials had a variability of only 0.08 μL L^{-1}. A method incorporating the vacutainers in an autoinjection system would result in low sample loss and low injection variability. This type of system would also reduce labour and allow the maximum amount of samples to be analysed in the least amount of time. It is very clear that the less time between sampling and analysis, the more accurate the results are likely to be.

One objective of the column study in Chapter 5 was to develop a method of investigating N_2O redistribution under controlled laboratory conditions. Redistribution of N_2O in soil columns was successfully measured and an attempt was made to determine the diffusion coefficient using a computer model. Although outputs were not able to determine differences between textures and diffusion coefficients were higher than expected, the method developed does provide a base for future investigations.

No significant soil textural effect was seen on the production, redistribution and ultimate emission of N_2O from air dry soil in the soil column investigation. Nitrous oxide injected into the clay and clay loam columns generally redistributed more slowly than in the sandy clay, although results need to be replicated to determine if differences are significant. There seems to be some retardation between profile concentrations and surface N_2O flux however surface flux would have to be measured constantly to show retardation of N_2O in clay, as diffusion is very rapid though air filled pores. As all soil columns were tested at air-dry moisture contents, the effect of moisture on blocking pores was similar between columns. Air filled porosity at this moisture content was similar in

all columns. This means that differences in N_2O movement between these columns was expected to be low, and this held true in the column trials. The differences should become more pronounced as moisture tension decreases (content increases).

Current literature states that N_2O surface flux can be lower in fine textured soils, when compared to coarser textured soils. The silty clay soil investigated during spring thaw to emits less N_2O than coarse textured soils in the literature, and an order of magnitude less than the estimated values of Agriculture Canada for the area (Janzen, 1999).

Although these experiments examined some of the factors that affect N_2O emission, this study merely scratches the surface of the details required to understand and manage this complicated issue. Future investigation should address the following areas of concern:

- further studies on how texture at higher moisture contents affects surface flux;
- more detailed laboratory investigations under controlled conditions with replication;
- additional field studies to investigate temporal and spatial variability;
- winter investigations (as profile temperatures can still be high enough for production at depth and winter conditions are a large portion of the calendar year in Canada); and
- integrated studies investigating the effects various agricultural inputs, soil physical properties, and vegetative cover and how they relate to N_2O profile production, consumption and emission.

8. CONTRIBUTION TO KNOWLEDGE

Current research has focused on relating N_2O emission to nitrogen inputs. While nitrogen inputs are a major controlling factor, high N inputs do not always mean there will be higher N_2O emissions. Nitrous oxide emissions are very much related to soil texture and even this is complicated by a number of factors. Fine textured soils may have high N_2O production rates, but they may emit N_2O slowly, or reduce the majority of it N_2 completely. In addition, while soil nitrate may lead to N_2O production within the profile, emission is often texture dependent.

Current models of N_2O emission such as DNDC assume surface flux reflects subsurface production, and that N inputs reflect can predict N_2O flux. Investigations here show that soil profile N_2O production in many cases is not manifested in surface emission.

This is the first time N_2O flux and Profile concentrations have been measured in Manitoba, and as a result this information can now be compared to hypothesised values in the literature. Although emissions were not extremely high, one fine textured Red River valley soil did illustrate both a significant spring thaw subsurface production as well as an emission event. This same experiment also confirmed previous research that legume systems encourage high N_2O profile concentration and flux.

Gaps in the current technology of sample storage and automated analysis were found. Efficiencies of some new storage methods were investigated and future areas of

improvement were identified.

A method for investigation of N_2O redistribution and estimation of a diffusion coefficient in a soil column was developed. These methods provide a base for future investigations into the movement of N_2O in soil.

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10. APPENDIX

10.1.1 I a. Average N_2O concentration in μL N_2O L^{-1}. Anova Results and % 6-day loss are shown.

Day	0[1]	1	2	3	4	5	6	6-day % loss[2]
Vacutainer	3	3.0	2.3a	x[3]	2.5	2.9a	2.8a	6.7
N_2O vial down	3	2.1	1.8b	1.5	0.5	0.5ab	0.5b	82
N_2O vial up	3	1.7	1.4c	1.1	0.5	0.5ab	0.4bc	86.4
Syringe	3	1.8	0.9d	x	0.5	0.3b	0.4c	87.7
Anova		NS	S		NS	S	S	

NB: [1] Time 0 value is an assumed value.
[2] The 6-day % loss is the last day compared to the standard gas.
[3] x = data not available.
Pairwise comparisons are Tukeys except day 5, which was Dunns.

10.1.2 I b. Daily variance between replicates of different N_2O storage methods.

	Total Daily Variance					
Day	1	2	4	5	6	Total avg. Variance
Syringe	1.569	0.002	0.000	0.000	0.001	0.314
Vacutainer	0.063	0.045	0.093	0.058	0.016	0.055
N_2O up	0.006	0.007	0.004	0.002	0.003	0.004
N_2O down	0.002	0.004	0.007	0.000	0.004	0.003

10.1.3 I c. Standard deviation of N_2O contamination of He samples over a 6-day period.

	0	1	2	4	5	6	Total Avg. *sd*
Vacutainer		0.200	0.001	0.012	0.026	0.158	0.397
He vial up		0.000	0.000	0.000	0.010	0.004	0.014
He vial down		0.000	0.000	0.005	0.002	0.005	0.012
Syringe		0.049	0.037	0.003	0.006	0.003	0.098

10.1.4 I d. He storage Trials. Average N_2O contamination over 6 days ($\mu L N_2O\ L^{-1}$.)

	0	1	2	4	5	6	Total Avg. S.D
Vacutainer	0.00	0.200	0.001	0.012	0.026	0.158	0.397
He vial up	0.00	0.000	0.000	0.000	0.010	0.004	0.014
He vial down	0.00	0.000	0.000	0.005	0.002	0.005	0.012
Syringe	0.00	0.049	0.037	0.003	0.006	0.003	0.098

NB: Measurements at T=0 should all be similar to a blank.
The % gain is over a six -day period are compared to air.

10.1.5 II a. Measured N_2O surface flux in chapter 5 (ng $N_2O\ m^{-2}\ s^{-1}$).

	Time (hours)			
	20.16	41.95	68.63	89.83
Sandy Clay	14.30	2.08	-0.46	2.08
Clay loam	15.68	3.23	2.54	2.08
Clay	18.91	2.08	-0.92	0.46

10.1.6 II b. Program for estimation of N_2O diffusion coefficient in a soil column.

C. M. Cho

```
#include <stdio.h>
#include <math.h>
#include <string.h>
#include <dos.h>

#define L 6
#define M 56        /* column length*/
#define N 9
#define Pi 3.1415926

typedef double LIST1[M];
typedef double LIST2[N];
typedef double LIST3[N][M];
typedef double LIST4[L][M];

LIST1 x,a,c,a1,a2,fa;
LIST2 sum;
LIST3 conc;
LIST4 data1,calc1,delta,cdelta;

int mtime[M];
double ac,k3;
void blue();

char heading1[L][10];
char heading[N][10] = { "x","T1-A","T2-A","T3-A","T4-A","T5-A","T6-A",
                        "T7-A","T8-A"};
main()
{
   double
dx,d,deltd,quant,porosity,length,radius,area,volume,t1,t2,t3,temp;
   double factor1,suma,p1,p2,q1,q2,q3,trapezoid(),tempsum[L];
   int i,j,k,m,n,no,mo,dt,row,column,dist;
   int t,time,print,count;
   void constac(),tridiagon(),outfile(),outfile0(),outfile1(),locate();
   void outfile2(),outfile3(),initialize(),readfile4(),outfile5();
   FILE *file,*file0,*file1,*file2,*file3,*file4,*file5,*fopen();
   char
*pp0,*pp1,*pp2,*pp3,*pp4,*pp5,name[20],name0[20],name1[20],name2[20];
   char name3[20],name4[20],name5[20],temp1[15];

   pp0 = ".000";
   pp1 = ".001";
   pp2 = ".002";
   pp3 = ".003";
   pp4 = "Min.";
   pp5 = ".sum";

   blue();
```

```
    printf ("\nName of File with Measured Data : ");
    scanf ("%s",name4);
    if ((file4 = fopen(name4,"r")) == NULL) {
        printf ("Too bad. The File %s cannot be opened. \n",name4);
        exit (1);
    }
    printf ("\nName of File to Store Calculated Data : ");
    scanf ("%s",name);
    if ((file = fopen(name,"w")) == NULL) {
        printf ("Too bad. The File %s cannot be opened. \n",name);
        exit (1);
    }
    strcpy(name0,name);
    strcat(name0,pp0);
    if ((file0 = fopen(name0,"w")) == NULL) {
        printf ("\n\nThe File %s cannot be opened. \n",name0);
        printf ("\nUse file name without extention.\n");
        exit (1);
    }
    strcpy(name1,name);
    strcat(name1,pp1);
    if ((file1 = fopen(name1,"w")) == NULL) {
        printf ("\n\nThe File %s cannot be opened. \n",name1);
        printf ("\nUse file name without extention.\n");
        exit (1);
    }
    strcpy(name2,name);
    strcat(name2,pp2);
    if ((file2 = fopen(name2,"w")) == NULL) {
        printf ("\n\nThe File %s cannot be opened. \n",name2);
        printf ("\nUse file name without extention.\n");
        exit (1);
    }
    strcpy(name3,name);
    strcat(name3,pp3);
    if ((file3 = fopen(name3,"w")) == NULL) {
        printf ("\n\nThe File %s cannot be opened. \n",name3);
        printf ("\nUse file name without extention.\n");
        exit (1);
    }
    strcpy(name5,name);
    strcat(name5,pp5);
    if ((file5 = fopen(name5,"w")) == NULL) {
        printf ("\n\nThe File %s cannot be opened. \n",name5);
        printf ("\nUse file name without extention.\n");
        exit (1);
    }
    blue();
    printf ("\nEnter the amount of gas injected in mL : ");
    scanf ("%lf",&quant);
    quant = 1.4*quant;   /* mL was changed to mg */
    printf ("\nEnter the probable porosity of the sand plug in %% : ");
    scanf ("%lf",&porosity);
    printf ("\nLength of the sand plug (cm) : ");
    scanf ("%lf",&length);
    dist = 10 - (int)length;  /*This is the distance of soil from port1
to sand*/
```

```
   /* this is the distance from the plug to the first port at bottom of
the column*/
   radius = 7.4;
   printf ("\nEnter dx = 1.0 and please do not use any larger value.");
   printf ("\nSince 0cm, which is 7 cm if dx = 1, 14 cm if dx = 2
etc");
   printf ("\nEnter dt and dx : ");
   scanf ("%d%lf",&dt,&dx);          /* use dx as cm unit  */
   printf ("\nEnter print-out time for intermediate result and total
time : ");
   scanf ("%d%d",&print,&time);
   printf ("\nEnter Diff. coeff. D and delta D for calculation : ");
   scanf ("%lf%lf",&d,&deltd);
   printf ("\nEnter values of gaseous escape coeff. k3 : ");
   scanf ("%lf",&k3);
   initialize();
   readfile4(file4,&no);
   area = Pi*radius*radius*0.01*porosity;          /* It is effective
area */
   volume = area*length;                           /* it is effective
vol. */
   ao = 1000000*quant/volume;       /* ppb v/v conc. of N2O in the sand
plug (check?)*/
   n = M-1;                         /* this seems to be ppm which is
what data files are */
   blue();
   fprintf (file5,"Summary on the absolute differences.\n");
   fprintf (file5,"       D ");
   for (i=1; i<L; i++)
      fprintf (file5,"%10s",heading1[i]);
   fprintf (file5,"     SUM  \n");
   for (k=0; k<10; k++) {
      blue();
      row = 7;
      column = 0;
      locate(row,column);
      printf ("Doing calculation for D = %6.4f :  Please wait.\n",d);
      suma = 0.0;
      for (m=0; m<M; m++) {
         if (m==0) {
            a1[m] = ao;
            a2[m] = ao;
         }
         else {
            a1[m] = 0.0;
            a2[m] = 0.0;
         }
         x[m] = (double)m*dx;
         conc[0][m] = x[m];
      }
      conc[0][m-1] = 0.0;
      p1 = 2.0*dx*dx/(d*dt);
      p2 = dx*dx/d;
      q1 = 2.0 + p1;
      q2 = 2.0 - p1;
      q3 = q2 + 4.0*k3/d;
      temp = 4.0*volume*dx/(d*area*dt);  /* this is the approximation
```

```
of cho's for the variable gas input..*/
      /* a plug of gas injected, then conc. decreases, NOT constant
input*/
      constac(n,a,c);
      t = 0;
      count = 0;
      mo = 1;
      j = 0;
      m = 0;
      while ((t < time) || (t < mtime[no-1] + 12)) { /*??not sure what
this is*/
       t += dt;
         row = 10;
         column = 0;
         locate (row,column);
         printf ("Processing time.");
         column = 20;
         locate (row, column);
         printf ("%d",t);
         fa[0] = (q2-temp)*a1[0] - 2.0*a1[1];
         for (i=1; i<n-1; i++)
            fa[i] = -a1[i-1] + q2*a1[i] - a1[i+1];
         fa[i] =-2.0*a1[i-1] + q3*a1[i];
         tridiagon(n,q1,temp,a,c,a2,fa);
         for (i=0; i<n; i++)
            a1[i] = a2[i];
       if (t == mtime[j]) {  /*?This is 0 min, and bottom of each
column*/
          calc1[m][j] = data1[0][j];
          m++;
          calc1[m][j] = a2[dist];
          m++;
       }
       if (t == (mtime[j] + 7 )) {     /* This 7,14,21,28 minsis
sampling time between ports.*/
          calc1[m][j] = a2[dist+10];
          m++;
       }
       if (t == (mtime[j] + 14)) {
          calc1[m][j] = a2[dist+20];  /* This is why dx must be 1, else
has caculated */
          m++;                        /* For different distance than
ports 10,20,30,40 */
                }
       if (t == (mtime[j] + 21)) {
          calc1[m][j] = a2[dist+30];
          m++;
       }
       if (t == (mtime[j] + 28)) {
          calc1[m][j] = a2[dist+40];
          m = 0;
          j++;
       }
       count += dt;
       if (count == print) {
          for (i=0; i<n; i++)
             conc[mo][i] = a2[i];
```

```
          suma = trapezoid(n,dx,a2);
          conc[mo][i] = suma;
            mo++;
             count = 0;
         }
         if (mo >= 9) {
            printf ("\nProgram terminated because it can handle up to 8
print-out");
          break;
       }
      }
      factor1 = data1[1][0]/calc1[1][0]; /* This is the points used to
calc factor. */
  /* Right now set at the 1st sampling port time 1*/
/*****************************************************************/
    /*  printf
("%10.5f\n%20.10f\n%10.5f\n\n",data1[1][0],calc1[1][0],factor1);
      for (j=0; j<no; j++) {
       for (i=1; i<L; i++)
          printf ("%14.9f",calc1[i][j]);
       printf ("\n");
      }
      printf ("\n\nPress any key to proceed. ");
      getch();     */
/*****************************************************************/
      for (i=1; i<L; i++) {
       for (j=0; j<no; j++) {
          delta[i][j] = data1[i][j] - calc1[i][j];
          cdelta[i][j] = data1[i][j] - factor1*calc1[i][j];
       }
      }
      for (j=0; j<no; j++) {
       delta[0][j] = data1[0][j];
       cdelta[0][j] = data1[0][j];
      }
      outfile (file,n+1,mo,d,print);
      outfile0
(file0,quant,porosity,length,area,volume,ao,dx,dt,d,deltd,factor1,t,pri
nt);

      outfile1 (file1,no,d);
      outfile2 (file2,no,d,tempsum);
      outfile3(file3,no,d,factor1);
      tempsum[0] = d;
      outfile5 (file5,tempsum);
      d += deltd;
   }
   row = 23;
   column = 0;
   locate(row,column);
}

void initialize()
{
   int n,m;
```

```
  for (m=0; m<M; m++) {
     mtime[m] = 0;
     for (n=0; n<L; n++) {
       data1[n][m] = 0.0;
       calc1[n][m] = 0.0;
     }
  }
  return;
}

void constac(n,a,c)
int n;
double *a,*c;
{
   int i;
   for (i=0; i<n-1; i++) {
      *(a+i) = 1.0;
      *(c+i) = 1.0;
   }
   *(a+i) = 2.0;
   *(c+i) = 1.0;
   *c = 2.0;
   return;
}

void tridiagon (n,q1,temp,a,c,xx,f1)
int n;
double q1,temp,*a,*c,*xx,*f1;
{
   LIST1 alpha,beta,y;
   int k;
   alpha[0] = -(temp+q1);
   beta[0] = *c/alpha[0];
   y[0] = f1[0]/alpha[0];
   for (k=1; k<n; k++) {
       alpha[k] = -q1 - *(a+k)*beta[k-1];
       beta[k] = *(c+k)/alpha[k];
       y[k] = (*(f1+k) - *(a+k)*y[k-1])/alpha[k];
   }
   k--;
   *(xx+k) = y[k];
   while (--k >= 0)
       *(xx+k) = y[k] - beta[k]**(xx+k+1);
   return;
}

double trapezoid(n,dx,aa)
int n;
double dx,*aa;
{
   double temp1,temp2,z;
   int i;

   temp1 = 0.0;
   temp2 = 0.0;
   for (i=1; i<n-1; i++)
      temp1 += *(aa + i);
```

```
    temp2 = 0.5*(aa[0] + aa[n-1]);
    z = (temp1 + temp2)*dx;
    return (z);
}

void outfile(file,n,mo,d,print)
FILE *file;
int n,mo,print;
double d;
{
    int i,p;

    fprintf (file,"Print-out time = %d,    D = %6.5f\n",print,d);
    for (p=0; p<mo; p++)
       fprintf (file,"%8s",heading[p]);
    fprintf (file,"\n");
    for (i=0; i<n; i++) {
       for (p=0; p<mo; p++)
       fprintf (file,"%8.2f",conc[p][i]);
       fprintf (file,"\n");
    }
    fprintf (file,"\n");
}

void
outfile0(file0,quant,porosity,length,area,volume,ao,dx,dt,d,deltd,facto
r1,t,print)
FILE *file0;
double quant,porosity,length,area,volume,ao,dx,d,deltd,factor1;
int dt;
int t,print;
{
     fprintf (file0,"Program is Thesis.C : \n");
     fprintf (file0,"Print time (min)     = %-7d  Time (min)
= %-7d \n",print,t);
     fprintf (file0,"Gas injected (mg)    = %-7.2f  Porosity
(sand,percent)= %-7.2f\n",quant,porosity);
     fprintf (file0,"Length of sand (cm) = %-7.2f  Area (cm2)
= %-6.1f\n",length,area);
     fprintf (file0,"Volume (cm3)         = %-7.1f  Ao
= %-5.1f\n",volume,ao);
     fprintf (file0,"Diff. Coeff.         = %-7.4f  delt D
= %-6.4f\n",d,deltd);
     fprintf (file0,"Esc. Coeff.          = %-7.4f  factor1
= %6.3f\n",k3,factor1);
     fprintf (file0,"dt                   = %-7d  dx
= %-7.1f\n\n",dt,dx);
     return;
}

void outfile1(file1,no,d) /*This is the first output.000*/
FILE *file1;
int no;
double d;
{
     int i,j;
```

```
    fprintf (file1,"D = %7.5f\n",d);
    for (i=0; i<L; i++)
      fprintf (file1,"%10s",heading1[i]);
    fprintf (file1,"\n");
    for (j=0; j<no; j++) {
      for (i=0; i<L; i++) {
         if (i == 0)
           fprintf (file1,"%10.0f",calc1[i][j]);
         else
           fprintf (file1,"%10.4f",calc1[i][j]); /*This in no. of
decimal places in calc. concentrations*/
      }
      fprintf (file1,"\n");
    }
    fprintf (file1,"\n");
    return;
 }

void outfile2(file2,no,d,tempsum)
FILE *file2;
int no;
double d,tempsum[L];
{
    int i,j;

    fprintf (file2,"Difference between Measured and Calculated when D =
%6.4f\n",d);
    fprintf (file2,"and Sum of absolute difference.\n");
    for (i=0; i<L; i++) {
      fprintf (file2,"%10s",heading1[i]);
      tempsum[i] = 0.0;
    }
    fprintf (file2,"\n");
    for (j=0; j<no; j++) {
      for (i=0; i<L; i++) {
         tempsum[i] += fabs(delta[i][j]);
         fprintf (file2,"%10.2f",delta[i][j]);
      }
      fprintf (file2,"\n");
    }
    tempsum[0] = 0.0;
    for (i=0; i<L; i++)
      fprintf (file2,"%10.2f",tempsum[i]); /*Decimal places of the
sum*/
    fprintf (file2,"\n\n");
    return;
 }

void outfile3(file3,no,d,factor1)
FILE *file3;
int no;
double d,factor1;
{
    int i,j;
    double tsum[L];

    fprintf (file3,"Difference between Measured and Calculated when D =
```

```
:6.4f\n",d);
    fprintf (file3,"and Factor1 =  %5.3f was Corrected for
Calculated.\n",factor1);
    for (i=0; i<L; i++) {
      fprintf (file3,"%10s",heading1[i]);
      tsum[i] = 0.0;
    }
    fprintf (file3,"\n");
    for (j=0; j<no; j++) {
      for (i=0; i<L; i++) {
         tsum[i] += fabs(cdelta[i][j]);
         fprintf (file3,"%10.2f",cdelta[i][j]);
      }
      fprintf (file3,"\n");
    }
    tsum[0] = 0.0;
    for (i=0; i<L; i++)
      fprintf (file3,"%10.2f",tsum[i]);
    fprintf (file3,"\n\n");
    return;
 }

void outfile5(file5,tempsum) /* This is the .sum file*/
FILE *file5;
double tempsum[L];
{
    int i;
    double aa;

    aa = 0.0;
    fprintf (file5,"%10.5f",tempsum[0]);   /*Decimal places of the D*/
    for (i=1; i<L; i++) {
      aa += tempsum[i];
      fprintf (file5,"%10.2f",tempsum[i]);
    }
    fprintf (file5,"%10.3f\n",aa); /* this is decimals in sum*/
    return;
 }

void readfile4(file4,no)
FILE *file4;
int *no;
{
    char temp1[10];
    int i,j;
    double temp;

    for (i=0; i<L; i++) {
      fscanf (file4,"%s",temp1);
      strcpy (heading1[i],temp1);
    }
    i = 0;
    j = 0;
    while (fscanf (file4,"%s",temp1) != EOF) {
      temp = atof(temp1);
      if ((j > 10) && (temp == 0.0))
         break;
```

```
      data1[i][j] = temp;       /* according to this, j is row no. */
      i++;
      if (i == L) {
          j++;
          i = 0;
      }
    }
    *no = j;
    for (j=0; j<*no; j++)
      mtime[j] = (int)data1[0][j];
/*****************************************************/
/* It is print out of the read-file  */
/*    for (j=0; j<*no; j++) {
      for (i=0; i<L; i++) {
         printf ("%10.3f",data1[i][j]);
         if (i==L-1)
            printf ("\n");
        }
    }
    printf ("\n Press any key to proceed : ");
    getch();  */
/*****************************************************/
}

#define BLUE 16
#define FWHITE 15
void blue()
{
   int color;
   void cls_c(int);
   color = FWHITE | BLUE;
   cls_c(color);
}

void cls_c (int color)
{
   void c_scroll(int,int,int,int,int,int,int);
   void locate(int,int);
   int row,col,wide,deep,num,f;
   row = 0;
   col = 0;
   wide = 80;
   deep = 24;
   num = 0;                                  /* No. of lines to scroll */
   f = 0x07;                                 /* function number */
   c_scroll(row,col,wide,deep,num,f,color);
   locate(row,col);
}

#include <dos.h>
#define VIDEO 0x10
void c_scroll(int row, int col, int wide, int deep, int num, int f, int
color)
{
   union REGS ireg;
   ireg.h.ah = f;
   ireg.h.al = num;
```

```
   ireg.h.ch = row;
   ireg.h.cl = col;
   ireg.h.dh = deep;
   ireg.h.dl = wide;
   ireg.h.bh = color;
   int86(VIDEO,&ireg,&ireg);
}

void locate (int row,int col)
{
    union REGS r;
    r.h.ah = 2;
    r.h.bh = 0;
    r.h.dh = row;
    r.h.dl = col;
    int86 (VIDEO,&r,&r);
}
```