

Chemical Denitrification in Frozen Soils

by



C. Bruce Christianson

A thesis  
presented to the University of Manitoba  
in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy  
in  
Soil Science

Winnipeg, Manitoba, 1981

(c) C. Bruce Christianson, 1981

CHEMICAL DENITRIFICATION IN FROZEN SOILS

BY

C. BRUCE CHRISTIANSON

A thesis submitted to the Faculty of Graduate Studies of  
the University of Manitoba in partial fulfillment of the requirements  
of the degree of

DOCTOR OF PHILOSOPHY

© 1981

Permission has been granted to the LIBRARY OF THE UNIVER-  
SITY OF MANITOBA to lend or sell copies of this thesis, to  
the NATIONAL LIBRARY OF CANADA to microfilm this  
thesis and to lend or sell copies of the film, and UNIVERSITY  
MICROFILMS to publish an abstract of this thesis.

The author reserves other publication rights, and neither the  
thesis nor extensive extracts from it may be printed or other-  
wise reproduced without the author's written permission.

## ACKNOWLEDGEMENTS

I wish to gratefully acknowledge the guidance and assistance of Dr. C. M. Cho under whose immediate supervision this investigation was conducted and to thank him for his valuable suggestions and helpful criticism of this manuscript.

I wish to thank the members of the thesis committee, Dr. E. C. Beauchamp of the University of Guelph, Dr. N. E. R. Campbell of the Department of Microbiology and Drs. G. J. Racz and R. A. Hedlin of the Soils Department.

I also wish to thank the staff of Soil Testing and the Department for their aid over the term of the project.

Finally, I must acknowledge the great amount of help and encouragement that Holly has provided over the course of this project.

## ABSTRACT

The effect of low temperature on chemodenitrification of nitrite in several slightly acid Manitoba soils was investigated using highly enriched  $^{15}\text{N}$  and a mass spectrometric technique which distinguished between  $\text{N}_2$  formed via a chemical reaction between  $\text{NO}_2^-$ -N and soil N and  $\text{N}_2$  formed via the reduction of  $\text{N}_2\text{O}$  biologically.

In a preliminary study, Wellwood soil (pH 6.4) was incubated at  $20^\circ\text{C}$  for three days with initial  $\text{NO}_2^-$  concentrations varying from 25 to 300 ppm-N. The rate of formation of chemically formed  $\text{N}_2$  was approximately first order with respect to initial  $\text{NO}_2^-$  concentration. The loss rate of  $\text{NO}_2^-$ -N during the three day period was approximately 3.7% of the added N per day. Biological denitrification resulted in losses of  $\text{N}_2\text{O}$  over the range of 25 to 75 ppm-N nitrite. At nitrite addition rates between 100 and 300ppm-N  $\text{N}_2\text{O}$  and  $\text{CO}_2$  production was suppressed, indicating an adverse effect on the microbial population.

The effect of soil temperature on denitrification of nitrite applied at 100 ppm-N to Wellwood soil was studied at temperatures varying from  $20^\circ$  to  $-20^\circ\text{C}$ . The rate of  $\text{N}_2$  formation decreased from 2.13 to 0.28  $\mu\text{g N/g soil/day}$  as the incubation temperature was lowered from  $20^\circ\text{C}$  to  $-1.8^\circ\text{C}$ . A dramatic increase in rate of N loss to 3.03  $\mu\text{g N/g soil/day}$  was observed as soil temperatures were decreased from  $-1.8^\circ$  to  $-3.5^\circ\text{C}$ . Decreasing the incubation temperature below  $-3.5^\circ\text{C}$  resulted in a gradual decline in rate of N loss. Data similar to that described above was ob-

tained for incubations conducted using nitrite concentrations of 50, 150, 200 and 300 ppm-N. The enhancement in rate of denitrification upon soil freezing was most likely due to freeze-concentration of nitrite into an unfrozen layer near the surface of soil colloids. This increase in nitrite concentration in the soil solution resulted in an increase in the rate of chemodenitrification. Arrhenius plots of the data above and below the discontinuity were parallel indicating that the reaction mechanism was similar over the entire temperature range.

Wellwood soil was autoclave-sterilized, treated with 100 ppm  $\text{NO}_2^-$ -N and incubated at temperatures varying from  $-16$  to  $20^\circ\text{C}$  to investigate the pathway of N loss. Results obtained with sterile soil were similar to those obtained with nonsterile soil indicating that the  $\text{N}_2$  had not been produced via a biological mechanism.

To determine the effect of soil solution nitrite concentration on  $\text{N}_2$  and  $\text{N}_2\text{O}$  production, 100 ppm  $\text{NO}_2^-$ -N (on a soil basis) was added to Wellwood soil samples which had moisture contents ranging from 11% to 50%. The incubation temperature was  $10^\circ\text{C}$ . As the moisture content was increased from 11%, the rate of  $\text{N}_2$  production decreased from  $3.73 \mu\text{g N/g soil/day}$  at 11% to  $0.18 \mu\text{g N/g soil/day}$  at 50% moisture content. Nitrous oxide production was negligible and increased only slightly when the soil became saturated.

A second experiment on the effect of moisture content was performed in which the nitrite content on a solution basis was kept constant at 800 ppm-N and the soil moisture content was varied (i.e. N content on a soil basis and average thickness of the moisture layer varied). When varying the soil solution content from 22% to 7%, little effect was noted on denitrification rates over the temperature range of  $20^\circ$  to  $0^\circ\text{C}$ .

The sample with 22% moisture showed a dramatic increase in rate of N loss while the sample with 7% moisture showed only a very slight increase. Upon freezing, the samples with 17% and 12% water were intermediate between these two levels.

A comparison of soil types at 100 ppm-N nitrite over the temperature range of  $-18^{\circ}$  to  $20^{\circ}\text{C}$  showed the effect of soil texture on the magnitude of the rise in denitrification rate that occurred upon soil freezing. It was expected that as the soil texture became more coarse, the specific surface area of soil would decrease and, with it, the amount of unfrozen water. The coarsest soil (Pineridge, a loamy fine sand) showed a very large increase in denitrification rate at  $-4^{\circ}\text{C}$ . Wellwood (a fine sandy loam) showed a less pronounced increase in denitrification rate (6.0 times that at  $-0.6^{\circ}\text{C}$ ) while Keld (a sandy clay loam) showed only a 250% increase in N loss rate upon freezing.

Denitrification rates in two other soils of higher pH were much lower than the more acid soils reported. Stockton soil (pH 6.9) showed only negligible denitrification under any conditions while nitrite was stable in Carroll soil (pH 7.1). Increasing the pH of Keld from its native value of 5.4 to 7.1 resulted in a decline in denitrification rate of approximately 30 times.

It was concluded from this project that:

1. a first order van Slyke-like reaction may occur in acidic soils containing nitrite causing denitrification of the nitrite to  $N_2$ .
2. the denitrification rate was greatly enhanced when freezing of the sample occurred due to freeze concentration of nitrite in the soil solution.
3. coarse textured soils with low surface area were more likely to exhibit an increase in N-loss upon freezing than finer samples of a similar pH.

## INTRODUCTION

Recent dramatic increases in oil and natural gas prices have resulted in higher nitrogen fertilizer prices. Plant recovery of added nitrogen fertilizer is often less than 50% of that applied, . Although losses of nitrogen can occur via denitrification, leaching, runoff and consumption by weeds, denitrification is probably the most important component of loss in Western Canada.

Fertilizer N is often applied as ammonia or ammonium-yielding products such as urea. Ammonium is subsequently oxidized by autotrophic bacteria in the soil to nitrite and nitrate. However, the oxidation of ammonium may be incomplete causing accumulation of nitrite near the pellet site under conditions of high ammonium concentration, low pH or low temperature (approximately 5°C). All three conditions can occur in soil to which fertilizer has been added in the fall. Rapid and extensive losses of nitrogen as N<sub>2</sub> gas have been shown to accompany such nitrite accumulations, most of the loss being caused by a chemical reaction of the nitrite nitrogen with soil organic nitrogen to yield N<sub>2</sub> gas (the van Slyke reaction).

Farmers in Western Canada often find it more convenient to apply fertilizer in the fall because the growing season is quite short and they are rushed for time in the spring. Many studies across the prairies have shown that the efficiency of fall applied products is often inferior to those that are spring-applied. The lower limit of biological den-



itrification has been reported as being 2° to 3°C, and hence biological denitrification over winter should not be a problem. Although one would expect chemical denitrification rates to decrease with lowered temperatures as described by the Arrhenius equation, there is no reason that this reaction should cease at 0°C. Since chemical denitrification has been linked to fertilizer losses in warm soils, a series of experiments was conducted in order to obtain information regarding the effect of freezing temperatures on rates of chemical denitrification of nitrite in soil. Parameters such as nitrite concentration, soil physical characteristics, sterilization and moisture content were examined.

## CONTENTS

ACKNOWLEDGEMENTS . . . . .	ii
ABSTRACT . . . . .	iii
INTRODUCTION . . . . .	vii

### Chapter

page

I. LITERATURE REVIEW . . . . .	1
Unfrozen Water Content . . . . .	1
Nitrification . . . . .	10
Denitrification . . . . .	12
Chemodenitrification . . . . .	13
Reactions with Soil Phenols . . . . .	14
The van Slyke Reaction . . . . .	15
Decomposition of Nitrous Acid . . . . .	17
Reaction of $\text{NHO}_2$ with Ammonia . . . . .	20
Chemodenitrification of Hydroxylamine . . . . .	20
Biological Denitrification . . . . .	21
Assumptions in Heavy Isotope Methodology . . . . .	24
II. MATERIALS AND METHODS . . . . .	26
Soils . . . . .	26
Incubation Apparatus . . . . .	28
Gas Sampling Procedure . . . . .	30
Experiment I Effect of Nitrite Concentration on Denitrification . . . . .	32
Experiment II Effect of Temperature on Chemodenitrification. Nonsterile Soil . . . . .	33
Sterile Soil . . . . .	34
Experiment III Effect of Moisture Content on Denitrification . . . . .	35
Experiment IV Role of Soil Characteristics in Influencing Denitrification Rates upon Freezing . . . . .	36
Chemical Analysis . . . . .	37
pH . . . . .	37
$\text{CaCO}_3$ . . . . .	37
Organic Matter . . . . .	37
Conductivity . . . . .	37
Cation Exchange Capacity . . . . .	38
Nitrite and Nitrate Nitrogen . . . . .	38
Gas Calculations . . . . .	39

III. RESULTS AND DISCUSSION . . . . .	49
Experiment I Effect of Nitrite Concentration on Denitrification . . . . .	49
Experiment II Effect of Temperature on Chemodenitrification . . . . .	54
Part 1 Non-sterile Soil . . . . .	54
Part 2 Sterile Soil . . . . .	65
Experiment III Effect of Moisture Content on Denitrification . . . . .	67
Effect of Dilution of Nitrite Concentration in Soil Solution upon Chemodenitrification. . . . .	67
Effect of Soil to Solution Ratio upon Chemodenitrification . . . . .	71
Experiment IV Role of Soil Characteristics on Denitrification Rates upon Freezing . . . . .	74
IV. SUMMARY AND CONCLUSIONS . . . . .	87
BIBLIOGRAPHY . . . . .	92

LIST OF TABLES

Table

	<u>page</u>
1. Subgroup designation, legal location and some physical and chemical properties of the soils used. . . . .	27
2. Normalized peak heights at a scan between a.m.u. 28 and 46 of atmosphere of Wellwood soil, 200ppm-N as NO <sub>2</sub> <sup>-</sup> . . . . .	42
3. Molecular species found between a.m.u. 28 and 46. . . . .	44
4. Nitrous oxide formation (µg N/g soil/day) as a function of temperature in Wellwood soil, 50, 100, 150, 200 and 300 ppm NO <sub>2</sub> <sup>-</sup> -N. . . . .	59
5. Effect of soil moisture on denitrification of 100 ppm-N nitrite incubated with Wellwood soil at 10°C for three days. . . . .	70
6. Calculated unfrozen water contents of three soils . . . . .	84
7. Effect of pH on Denitrification Rates in Keld Soil. . . . .	85

## LIST OF FIGURES

<u>Figure</u>	<u>page</u>
1. Unfrozen water in typical soils (1) Quartz sand (2) Sandy loam (3) Loam (4) Clay (5) Clay with montmorillonite (Nersesova and Tsytovich, 1963). . . . .	3
2. Incubation vessel used in experiments. . . . .	29
3. Gas sampling container. . . . .	31
4. Nitrogen and nitrous oxide evolution as a function of initial nitrite concentration, Wellwood soil, 20°C. . . . .	50
5. Effect of nitrite concentration on partial pressure of carbon dioxide and oxygen in the sample atmosphere, Wellwood soil, 20°C. . . . .	53
6. Effect of temperature on rate of chemodenitrification in Wellwood soil treated with 100 ppm NO <sub>2</sub> <sup>-</sup> -N. . . . .	55
7. Arrhenius plot of chemodenitrification rate versus temperature in Wellwood soil, 100 ppm NO <sub>2</sub> <sup>-</sup> -N. . . . .	58
8. Chemodenitrification rates as a function of temperature in Wellwood soil, 50, 150, 200 and 300 ppm NO <sub>2</sub> <sup>-</sup> -N. . . . .	61
9. Arrhenius plots of chemodenitrification rates as a function of temperature, 50, 150, 200 and 300 ppm NO <sub>2</sub> <sup>-</sup> -N. . . . .	62
10. Rate of N loss in Wellwood soil treated with 100 ppm-N as affected by time and temperature of incubation. . . . .	64
11. Effect of temperature on the denitrification of 200 ppm-N nitrite in autoclave-sterilized Wellwood soil. . . . .	66
12. Effect of dilution of nitrite concentration in soil solution on denitrification rate in Wellwood soil at 10°C. . . . .	68
13. Effect of soil to solution ratio and temperature on denitrification of nitrite in Wellwood soil. . . . .	73
14. Chemodenitrification of 100 ppm-N nitrite as a function of temperature in Wellwood, Keld, Pine Ridge and Stockton soils..	75

15. Moisture retention curves of Keld, Wellwood and Pine Ridge soils. . . . .	77
16. Chemodenitrification of 200 ppm-N nitrite as a function of temperature in Wellwood, Keld and Pine Ridge soils. . . . .	79
17. Chemodenitrification of 300 ppm-N nitrite as a function of temperature in Wellwood, Keld and Pine Ridge soils. . . . .	80
18. Arrhenius plots of chemodenitrification rates in Keld soil at 100, 200 and 300 ppm $\text{NO}_2^-$ -N concentrations. . . . .	82
19. Arrhenius plots of chemodenitrification rates in Pine Ridge soil at concentrations of 100, 200 and 300 ppm $\text{NO}_2^-$ -N. . . . .	83

## Chapter I

### LITERATURE REVIEW

#### 1.1 UNFROZEN WATER CONTENT

Bouyoucos (1917) was one of the earliest workers to study freezing phenomena in soils and suggested that a portion of the soil water remained unfrozen, even at low temperatures. Although pure water in any container will freeze at  $0^{\circ}\text{C}$ , the freezing point of soil water may be several degrees lower due to the effects of specific surface area and solute content on the free energy of the solution. In 1920, Freundlich showed that adsorbed water had a higher surface energy at the container-water interface than at the air-water interface. This indicated a range in the thermodynamic properties of water from the surface to bulk solution (Tyutyunov, 1973).

The surface of the colloid may be highly irregular in shape and possess different cations adsorbed on it as well as hydroxy iron and aluminum complexes. These and other surface-moderating substances affect the structure of water near them resulting in the presence of two types of water; 1) free water which is not directly affected by the soil surfaces and behaves as bulk water (this water will freeze readily). 2) bound water, water whose physical character is altered by surface and ionic effects and will not freeze as readily as will free water (Tsyto- vich, 1975).

However, an obvious distinction between bound and free water is not evident. Nor, is all bound water the same. Strongly bound water will not freeze at temperatures as low as  $-40^{\circ}\text{C}$ , while more weakly bound water will freeze at higher temperatures. There exists a gradient between free water and tightly bound water in the soil system (Nersesova and Tsytovich, 1963). The more closely associated water is with the surface, the lower is its freezing temperature.

Example curves of unfrozen water content ( $W_u$ ) versus temperature below the freezing point illustrate the changes in unfrozen water that occur as the temperature decreases (Fig 1). Between  $0$  and  $-4^{\circ}$ , the weakly bound water and free water have frozen leaving only tightly bound water in the liquid state. Tsytovich (1975) described Figure 1 in terms of three ranges;

1. the range of significant phase transition where  $W_u$  changed by more than 1% per  $1^{\circ}$  temperature depression (free water in large pores and capillaries froze).
2. the range of moderate phase transition where  $W_u$  changed by more than 0.1% but less than 1% per  $1^{\circ}$  temperature depression (freezing of loosely bound water).
3. the range of small phase transition;  $W_u$  changed by less than 0.1% per  $1^{\circ}$  (freezing of bound water).

As was evident in Figure 1 soils with greater specific surface area (clay and clay containing montmorillonite) retained more unfrozen water than coarser soils at any sub-zero temperature. Moreover, the soils with high surface area continued to lose unfrozen water below  $-4^{\circ}$  while the rate of decrease of unfrozen water in the sands was very low in this



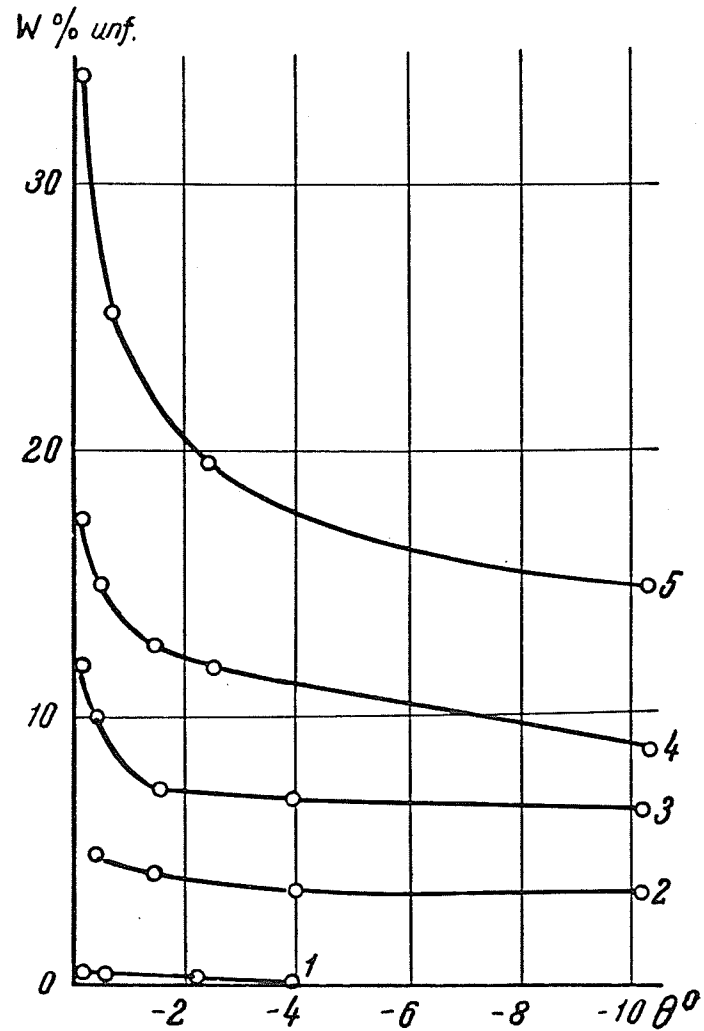


Figure 1: Unfrozen water in typical soils (1) Quartz sand (2) Sandy loam (3) Loam (4) Clay (5) Clay with montmorillonite (Nersesova and Tsytovich, 1963).

range. As temperatures reached  $-70^{\circ}\text{C}$ , all liquid water froze in all but the most highly dispersed soils (note: complete freezing was termed to be synonymous with the oven-dry soil moisture content).

The data of Figure 1 could be represented by the power curve,

$$W_u = x\theta^y \quad (2).$$

where  $W_u$  was the unfrozen water content,  $\theta$  was the temperature in  $^{\circ}\text{C}$  and  $x$  and  $y$  were parameters specific for each soil. Although equation (2) was practical for most soils, finer samples such as clays were more accurately represented by segments of two power curves (Anderson and Tice, 1971). A further refinement of the equation includes the effect of specific surface area yielding

$$\ln W_u = a + b \ln S + cSd \ln \theta \quad (3).$$

Here  $S$  was the specific surface area ( $\text{m}^2/\text{g}$ ). Using experimental data from eleven soils, the values for the coefficients were found to be  $a = 0.2168$ ,  $b = 0.5519$ ,  $c = -1.449$ , and  $d = 0.264$  (Anderson et al., 1973).

When the vapour pressure of a solution is lowered, so too is its freezing point. In considering the water-substrate interface, the degree of curvature of the interface has a direct negative effect on the vapour pressure of the soil solution in accordance with the equation

$$W_u = \frac{2G_i w}{r_i} \quad (4)$$

where  $G_{iw}$  is the interfacial tension (ice-water) and  $r_i$  is the minimum radius of the ice-water interface. In this equation,  $r_i$  is assumed to be negative on the water side of the surfaces. Thus, the greater the curvature (smaller  $r_i$ ) the larger the decrease in vapour pressure.

Free energy is related to both temperature and vapour pressure by the expression

$$G = G_o + RT \ln P/P_o \quad (5)$$

where  $G$  equals free energy,  $G_o$  is standard free energy,  $R$  is the gas constant,  $T$  degrees K,  $P$  is vapor pressure and  $P_o$  is standard vapor pressure. Since the free energy of a liquid must be equal to the free energy of a solid during a phase change, lowering the vapour pressure at a given air pressure requires a decrease in temperature of the solution before a phase change can occur, i.e. a freezing point depression must occur.

Soil is very complex physically with many small pores. In a soil composed of small particles, the menisci thus formed are very important and cause a significant vapour pressure lowering and therefore a larger unfrozen water content. Coarser soils will have relatively fewer small pores and this, in turn, results in insignificant capillary phenomena. Therefore, coarser soils have less unfrozen water at a specific temperature than fine clays.

Different clay mineral types, when frozen while wet, have different unfrozen water contents per unit surface area. Nearly all the surface of kaolinite is exposed as external surfaces while montmorillonite is

composed of only 10 to 20 percent external surface and 80 to 90 per cent internal surface. Since kaolinite contains more unfrozen water than montmorillonite with an equal surface area and at a given water content and temperature, Anderson et al. (1973) suggested that external surfaces have thicker unfrozen layers than do internal surfaces.

The freezing point of a liquid is lowered by the addition of solute in the manner described by the equation;

$$\theta = K_f C \quad (6)$$

where  $K_f$  is the molal freezing point depression constant and  $C$  is the molality of solvent A. It is evident that  $\theta$  depends on  $K_f$  (i.e. the type of solvent) and is independent of any characteristic of the solute except its concentration (Moore, 1972).

Water is an excellent solvent and soil water contains many dissolved ions. These ions are important determinants in the lowering of the freezing point of soil water. When water-solute mixtures freeze, ice crystallizes out of the solution in a pure state. This causes soluble salts to become concentrated in a melt film along colloidal surfaces. Since the vapour pressure of liquid water is higher than that of ice, ice formation will continue until the pressures of the two phases are equal (Low et al., 1968). As the temperature of the system is lowered, more ice is formed and the solute concentration rises, thereby reducing the vapour pressure and the freezing point of the remaining liquid.

Two processes are at work together in a freezing system to determine  $\theta$ ; solute concentration with freezing and freezing point depression due

to salt accumulation. The salt concentration in a soil system at a specific water content is given by the equation

$$C = \frac{1000 P_{se}}{W_u} \sum_{i=1}^n \frac{g_i}{M_i} \quad (7)$$

where C is the total concentration (meq/l),  $g_i$  is the total content of ion 'i' in the solid (mg/g solid),  $M_i$  is the equivalent weight of ion 'i' (mg/meq),  $P_{se}$  is the density of the solution in the pores (g/cc) and 'i' is an index number of the various ions in solution (Banin and Anderson, 1974).

Letting  $e = \sum_{i=1}^n (g_i/M_i)$ , (the total number of meq/g of solid) and by setting  $P_{se}$  to 1, the equation simplifies to

$$C = \frac{1000 e}{W_u} \quad (8).$$

It is evident that the concentration of salts varies inversely with  $W_u$ . With such a relation it is assumed that the solubility product of the solute is not exceeded as the sample is concentrated. However, if the concentration of solutes is high in the aqueous solution, selective rejection of solutes may lead to precipitation of  $CaCO_3$  (Hallet, 1978),  $NaCl$  (Terwillinger and Dizio, 1970) or other salts. This is usually not a serious problem in calculations involving nonsaline, noncalcareous soils.

In equation (6) it was shown that freezing point decreased as salt concentration increased. Therefore, combining equations (6) and (8), the effect of freeze concentration and moisture content on the freezing point of the system can be determined using the equation

$$\theta' = K_f e / W_u \quad (9)$$

where  $\theta'$  is the freezing point shift in degrees Kelvin. Banin and Anderson (1974) showed that  $K_f$  values for 1-1, 2-1, and 2-2 clays were 3.72, 2.79 and 1.86 respectively.

In testing the above equation, the authors found that the temperature at which a specific  $W_u$  value was obtained shifted downward as the solute content increased. This was the expected trend due to solute addition and concentration. Agreement between expected and actual  $\theta'$  determinations was good at low salt concentrations but calculated points were 10 to 20 percent lower than actual results at high salt concentrations. This was attributed to nonhomogeneous distribution of ionic solutes in the melt causing higher salt concentrations than calculated. The authors believed this to be due to diffuse double layer effects on ionic concentrations. Similar experiments using dimethyl sulfoxide (DMSO) yielded results closer to those suggested by equation (8). Dimethyl sulfoxide is a nonelectrolyte and therefore should not be absorbed, positively or negatively, by the clay surface.

Non-homogeneous salt concentrations are affected by the freezing rate of the pore solution. Unless freezing occurs at an infinitely slow rate, salts excluded from the ice do not diffuse completely into the soil solution but rather yield a gradient in salt concentration emanating from the ice surface (Hallet, 1978). This gradient may be several millimeters wide, increasing in steepness as the freezing rate increases (Burton et al., 1953).

Since the freezing point of a solution varies inversely with salt concentration, the temperature of the solution layer near the ice can

fall below the freezing temperature of the bulk solution without itself freezing. This will allow the solution immediately ahead of this layer, not having the lower freezing point of the layer, to become supercooled and freeze. Trapping of the brine layer near the ice would then occur which would ultimately freeze as the temperature of the system continued to drop (Himes et al., 1959). Freezing solutions in large containers will exhibit a laminated effect due to solute trapping with each lamination being several millimeters thick.

In a saturated soil system, similar laminations could occur (Outcalt, 1976) resulting in lenses separated by thicker sections of soil. Hallet (1978) reported seeing thin (70.5 mm) lenses in frozen and sectioned clay. This type of trapping would be more pronounced in the larger soil pores where surface and capillary effects are less important.

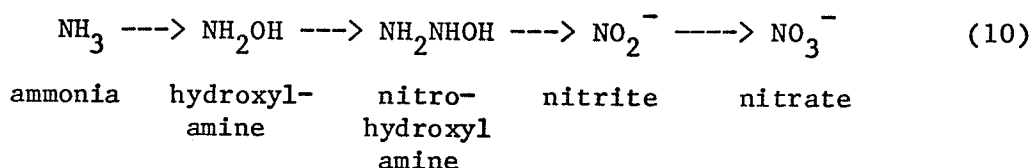
No work involving solute rejection phenomena in an unsaturated freezing system could be found in the literature.

It should be noted that the principle of solute concentration in the melt of a freezing aqueous system is the basis of freeze-concentration techniques used in analytical chemistry (Shapiro, 1961; Smith and Tasker, 1965). Freezing an aqueous system allowed greater than 99% recoveries of added solute after a concentration of as much as 20 fold. Similar results should be possible with soil solutions.

## 1.2 NITRIFICATION

Nitrification has been defined by Alexander (1965) as "the biological conversion of nitrogen in organic and inorganic compounds from a reduced to a more oxidized state."

Campbell and Lees (1967) have proposed the following pathway for nitrification;



|+-----Nitrosomonas-----|-Nitrobacter-|.

The intermediates hydroxylamine and nitrohydroxylamine have not been found in soil in a free form but could occur intracellularly (Bremner et al., 1980). Usually nitrite and nitrate are the only nitrification products in soil.

The nitrification process is biologically mediated by a few groups of bacteria and fungi but two bacterial species are most important, Nitrosomonas spp. ( $\text{NH}_3 \longrightarrow \text{NO}_2^-$ ) and Nitrobacter spp. ( $\text{NO}_2^- \longrightarrow \text{NO}_3^-$ ). The concentration of  $\text{NO}_2^-$  is usually the rate limiting factor in the growth of Nitrobacter spp. and, as a result, this ion rarely accumulates under normal soil conditions.

Complete oxidation of  $\text{NH}_3$  to  $\text{NO}_3^-$  does not always take place under all conditions and nitrite may accumulate in the soil. Several situations can give rise to this accumulation. When ammonia or ammonium yielding fertilizers are added to soil of high pH (>7.2), the high ammonia concentration will result in inhibition of the Nitrobacter spp. to a



much higher degree than the Nitrosomonas spp, thereby causing an accumulation of nitrite to occur (Morrill and Dawson, 1967). This is true where fertilizers are banded in the soil or added as pellets, especially urea. Addition of urea will result in local alkaline sites in the immediate vicinity of the pellet due to hydrolysis of urea (Soulides and Clark, 1958; Clark et al., 1960). High concentrations of ammonium and ammonia will also be found near the pellet which cause further deleterious effects to growth of Nitrobacter spp. Nitrite accumulations have been reported after addition to soil of products such as anhydrous ammonia (Nommik and Nilson, 1963; Stevenson et al., 1970), ammonium salts (Justice and Smith, 1962) and urea (Pang et al., 1977; Court et al., 1962; Hauck and Stevenson, 1965).

Nitrobacter spp. are more sensitive to low temperature than Nitrosomonas spp. (Justice and Smith, 1962; Broadbent and Tyler, 1962), a condition which could potentially result in an accumulation of nitrite in the field with fall-applied nitrogen products. Anderson (1962), however, did not show any accumulation of nitrite at low temperatures although the overall denitrification rate was lowered.

The average soil temperature at the 10cm depth in Manitoba in October is approximately 5.5°C (Reimer, 1978). Fall application of ammonia or ammonium-yielding fertilizers may result in local soil conditions of high pH and high ammonium concentration which, in addition to low temperature may result in nitrite accumulation. However, if bulk soil samples are taken in the fall,  $\text{NO}_2^-$  is rarely detected in large quantities. This does not mean that high concentrations of nitrite are not present, only that these concentrations are very local and not detected if the

soil is evenly mixed. Fertilizer N is not evenly spread throughout a soil profile but rather stays in the immediate vicinity of the original placement site of the pellet. Thus at microsites in the soil high ammonium concentrations and high pH can occur, conditions that can promote  $\text{NO}_2^-$  accumulations in the soil before freeze-up. This nitrite may be potentially available for denitrification.

### 1.3 DENITRIFICATION

Denitrification has been described as "the gaseous loss of nitrogen either by biological or chemical mechanisms but exclusive of ammonia volatilization" (Broadbent and Clark, 1965).

Work by Crutzen and Ehhalt (1977) and McElroy et al. (1977) suggested that nitrous oxide ( $\text{N}_2\text{O}$ ) (a major product of denitrification) may cause the reduction of ozone in the stratosphere. Even a small percentage loss of ozone could prove to be very serious (Crutzen and Ehhalt, 1977). This has greatly stimulated research by agricultural and environmental scientists into factors regulating the production of  $\text{N}_2\text{O}$  in the environment. One of the most important potential world sources of  $\text{N}_2\text{O}$  is that produced during denitrification of fertilizer nitrogen.

It has been reported that plant uptake of fall applied nitrogen was lower than spring applied products (Ridley, 1977) although some reports suggest the contrary (Harapiak, 1979). Possible factors affecting loss of fall-applied products include denitrification, leaching, immobilization and uptake by weeds although denitrification is believed to be the most serious. Field studies using  $^{15}\text{N}$  conducted in Manitoba by Ridley (1977) showed a lower recovery of fertilizer-N in the soil and crop after

one year with fall than with spring-applied N (50% vs 60 to 74%). Similar trends have been reported in Saskatchewan (Paul and Rennie, 1974) and Alberta (Nyborg and Leitch, 1979). Malhi (1978) showed that the amount of N present in the soil in spring increased as the time of N application in fall was delayed from October to November and suggested that the lower soil temperatures of November resulted in decreased nitrification which subsequently decreased amounts of fertilizer N which were available for denitrification. Harapiak (1979), however, found little difference between spring and fall application in terms of plant response and total N recovery in soil. This indicates that perhaps not all soils are equally susceptible to over-winter losses on the prairies.

Denitrification occurs via two basic mechanisms, both as a biologically mediated process and one due to strictly chemical reactions. Often the nitrogen compounds that react chemically in soil are produced initially by biological action on the nitrogen in the system. Thus the chemical and biological processes are not completely divorced from each other.

### 1.3.1 Chemodenitrification

Many workers have noted that losses of fertilizer-N often coincide with even transitory accumulations of nitrite during the oxidation of ammonium to nitrate (Clark et al., 1960; Hauck and Stevenson, 1965; Meek and MacKenzie, 1965). These losses have been shown to occur under conditions of apparently adequate aeration suggesting that the losses are not due to biological denitrification but occur via chemical means.

Denitrification losses in soils containing nitrite have been shown to be significant, even if the soils had previously been sterilized confirming that chemical denitrification may occur in soil (Cady and Bartholomew, 1965; van Cleemput, 1974).

Studies of chemodenitrification in soil have yielded several theories which attempt to explain the soil- $\text{NO}_2^-$  interaction. They are reviewed under separate headings.

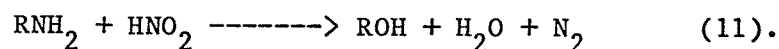
#### 1.3.1.1 Reactions with Soil Phenols

Many workers have postulated that nitrite can react with some component of the organic matter of acidic soils to yield molecular nitrogen (Clark et al., 1960; Broadbent and Tyler, 1962; Nelson and Bremner, 1970; Stevenson and Swaby, 1964). Removal of soil organic matter by means of  $\text{H}_2\text{O}_2$  treatment reduced nitrogen losses (Smith and Clark, 1960). Nelson and Bremner (1970) noted a similar cessation of  $\text{N}_2$  production from previously ignited soils. Incubation of nitrite with quartz and clay samples also resulted in no denitrification thus showing that inorganic soil constituents do not promote denitrification of nitrite. Reuss and Smith (1965) came to a similar conclusion after finding no denitrification of nitrite in a calcium-saturated exchange resin buffered to pH 5.2. Bremner (1957) suggested that lignins and other phenolic substances in the soil undergo nitrosation, a process which commences with the formation of nitrosophenols ( $>\text{C}=\text{NOH}$ ) which could be further acted upon by  $\text{HNO}_2$  to form  $\text{N}_2$  and  $\text{N}_2\text{O}$ . In such reactions both nitrogen atoms of the evolved gas would have come directly from the added nitrite. Stevenson et al. (1970) studied the reaction of nitrite with

lignins, humic substances and several phenolic substances in neutral and slightly acid solutions. They found more gas produced at pH 6.0 than pH 7.0 although significant amounts were still produced at the higher pH. The experiments were carried out anaerobically and NO was the main gas produced.

### 1.3.1.2 The van Slyke Reaction

The van Slyke reaction (equation 11) was originally proposed as the basis for an analytical method for the determination of amino acids (van Slyke, 1911)



It is a reaction in which equal proportions of amino acid N and nitrous acid N react to yield dinitrogen and proceeds to completion in the presence of glacial acetic acid and a nitric oxide (NO) atmosphere which is necessary for the reaction to occur at significant speed. Such conditions are not found in the soil profile. In addition, only a small amount of  $\text{NO}_2^-$  is present as  $\text{HNO}_2$  over pH 5. Because of the unusual soil conditions that apparently are required for this reaction, the van Slyke reaction was not considered to be of importance by most researchers (Broadbent and Clark, 1965; Allison, 1963; Clark et al., 1960).

Since the measured pH of soils is often much higher than the maximum pH permissible for such a  $\text{NO}_2^-$ -amino acid interaction, it was assumed that this reaction would not take place in normal soils. However, the assignment of a pH value to a soil is very difficult. Harter and Alhrichs (1969), by studying the infrared spectra of benzoic acid on

montmorillonite clays, were able to show that the hydrogen ion concentration on a clay surface was one hundred times that of the bulk solution (pH 7.0). Thus, reactions that may not be expected to occur due to measured neutral soil pH values may occur at the clay surface where the pH is much lower. Thus measured pH may not be as great a restriction on the van Slyke reaction as was generally supposed.

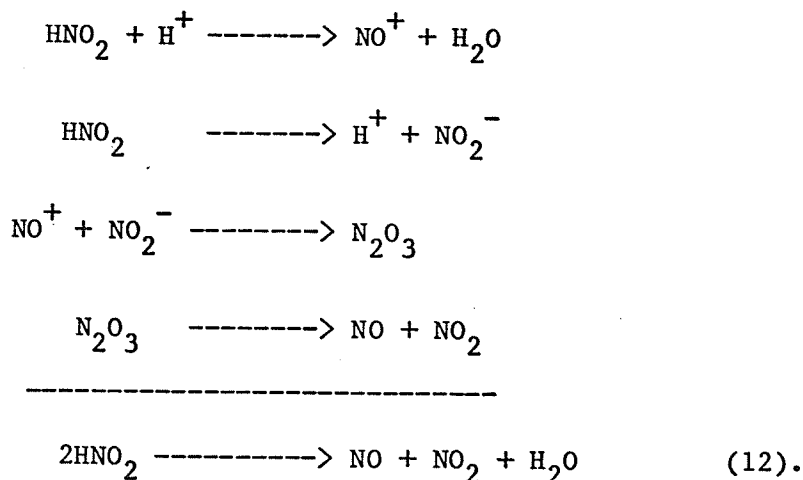
Stevenson et al. (1970) proposed that a reaction similar to the classical van Slyke reaction may occur to a limited extent. In an experiment in which nitrite and amino acids were added to phthalate buffer (pH 6.0), recovery of amino-N as evolved  $N_2$  varied from 19% with cysteine to 99.6% with glucosamine. Also hydrolysis of humic acids with 6N HCl before they were added to the nitrite solution lowered the amount of  $N_2$  produced by 50%. Soils incubated with nitrite produced still more  $N_2$  than unhydrolysed humic acids, possibly due to their high amino acid N content. These workers suggested that the amount of  $N_2$  recovered from soils treated with nitrite was directly related to soil nitrogen content indicating that the organic soil fraction was the source of the active N component.

Reuss and Smith (1965) found that the rate of  $N_2$  production from soils treated with nitrite tended to be very rapid initially and then to subsequently decrease even though nitrite was still present. They believed that some denitrification promoting component of the soil organic matter had been exhausted. They postulated that labile  $NH_2$  groups were the source of one of the N atoms of the dinitrogen evolved. Tyler and Broadbent (1960) published similar findings.

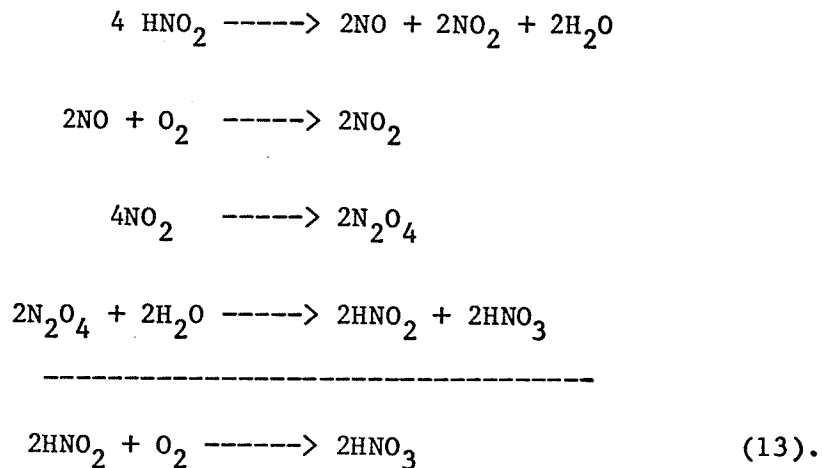
Christianson et al. (1979) and Smith and Chalk (1980) were able to show that chemodenitrification of  $\text{NO}_2^-$  occurred to a large extent via a van Slyke type of reaction. They showed that the per cent  $^{15}\text{N}$  of nitrogen gas produced from nitrite-treated soils was half that of the  $\text{NO}_2^-$  added to the system even though the per cent  $^{15}\text{N}$  of the  $\text{N}_2\text{O}$  produced at the same time was equal to that of the added  $\text{NO}_2^-$ . This could only have occurred if one atom of  $\text{N}_2$  came from the fertilizer and the other came from a soil source, presumably the organic matter. The denitrification rate was shown to increase as the soil pH decreased and the rate of  $\text{N}_2$  evolution varied directly with the concentration of nitrite added. Christianson et al. (1979) found that during the oxidation of urea to nitrate a transitory accumulation of nitrite occurred with a concomitant loss of N from the system as van Slyke-like nitrogen.

### 1.3.1.3 Decomposition of Nitrous Acid

The following reaction describing self-decomposition of nitrous acid was proposed by Nelson and Bremner (1970).



Nitric oxide is present only in anaerobic systems (Smith and Clark, 1960; Reuss and Smith, 1965) and is rapidly oxidized to  $\text{NO}_2$  in the presence of oxygen. Nitric oxide has also been shown to occur when  $\text{NO}_3^-$ -N is added to acidic soils under anaerobic conditions (Wijler and Delwiche, 1954; Nommik, 1956; Cady and Bartholomew, 1960, 1963). Cady and Bartholomew (1963) concluded that this was due to the self-decomposition of nitrite formed during the reduction of nitrate. Nitrogen dioxide is very soluble in water and in most cases is trapped by soil moisture before it leaves the soil system (Broadbent and Clark, 1965; Broadbent and Stevenson, 1966). The oxidation of NO and its subsequent hydration are represented as follows (Nelson and Bremner, 1970).



It is evident that the presence of the  $\text{HNO}_2$  molecule is obligatory before this disproportionation reaction can commence. Its presence in soil depends greatly on pH because the reaction has an equilibrium constant of  $6.0 \times 10^{-4}$  and only 1.6%  $\text{NO}_2^-$ -N exists in the  $\text{HNO}_2$  form at pH 5.



To test the hypothesis that NO and NO<sub>2</sub> were oxidized to NO<sub>3</sub><sup>-</sup>, Nelson and Bremner (1970), injected the gases into moist soil samples and then analysed the samples for nitrate. An increase in nitrate level was recorded. In a further experiment, vials of alkaline KMnO<sub>4</sub> were put in the centre of sealed vessels containing soil samples to which nitrite had been added. Alkaline KMnO<sub>4</sub> will absorb NO and NO<sub>2</sub> and thereby lower the amount of nitrate produced if NO and NO<sub>2</sub> are intermediates in the conversion of nitrite to nitrate. Nitrate production was 33% lower in samples incubated with the KMnO<sub>4</sub> than in samples without KMnO<sub>4</sub>.

Further work by Nelson and Bremner (1970) showed that NO<sub>2</sub> production was inversely related to soil pH. They were unable, however to explain the continued NO<sub>2</sub> production in neutral soils, even though the amount produced had decreased. Nitrogen dioxide production was not enhanced by addition of organic materials (humic acids or lignin) nor was it affected by soil texture (Reuss and Smith, 1965). These workers also showed that the production of this oxide of nitrogen was unaffected by soil sterilization.

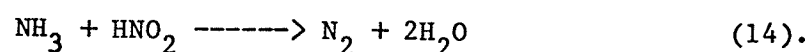
Smith and Clark (1960) and Tyler and Broadbent (1960) found very little NO<sub>2</sub> evolution from aerobic soil. This could be due to experimental methods for the KMnO<sub>4</sub> trap of Nelson and Bremner (1970) absorbed NO<sub>2</sub> quickly whereas the systems of the other authors offered a greater opportunity for absorption of the gas by the soil.

Work carried out by Wullstein and Gilmour (1964, 1966) indicated that the metallic cations Cu, Fe, Mn, and Al stimulated the evolution of NO from nitrite-treated soils. Their studies involved the utilization of very high concentrations of metal (over 5,000 ppm) and nitrogen

(10,000 ppm  $\text{NO}_2^-$ -N), conditions which are not found in soils. Studies by Nelson and Bremner (1970) failed to show any stimulation of nitrite decomposition by these metals.

#### 1.3.1.4 Reaction of $\text{NHO}_2$ with Ammonia

Gerretson and de Hoop (1957) proposed that nitrous acid reacted with free ammonia to yield molecular nitrogen via the reaction:



Gerretsen and de Hoop (1957), using sterile buffered solutions of nitrite and  $\text{NH}_4^+$ -N were able to show substantial losses of N as  $\text{N}_2$ . Smith and Clark (1960) were, however, unable to repeat these experiments. Upon addition of  $\text{NO}_2^-$ -N and  $\text{NH}_4^+$ -N solutions to soils, they were able to show  $\text{N}_2$  losses were the same whether  $\text{NH}_4^+$  was present or not. They believed the reduction of nitrite to  $\text{N}_2$  was promoted by some soil component and not by  $\text{NH}_4^+$  ions. Allison (1965) later revised his position on this reaction, concluding it to be of little importance.

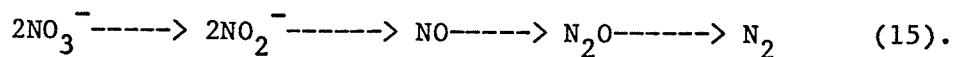
#### 1.3.1.5 Chemodenitrification of Hydroxylamine

Hydroxylamine is one of the intermediates formed during the nitrification of  $\text{NH}_4^+$  to  $\text{NO}_3^-$ . This intermediate is generally believed to be intracellular although Verstraete and Alexander (1973) reported the existence of small amounts of extracellular hydroxylamine produced during oxidation of  $\text{NH}_4^+$  in soil. Hydroxylamine undergoes chemodenitrification to  $\text{N}_2$  and  $\text{N}_2\text{O}$  in sterile soils (Bremner and Shaw, 1958) leading Bremner et al. (1980) to suggest that a lack of accumulation of the intermediate

may be due to its immediate chemodenitrification. Losses of hydroxylamine-N were highly correlated with exchangeable and oxidized manganese. This suggests that the chemical oxidation of  $\text{NH}_2\text{OH}$  to  $\text{N}_2\text{O}$  and  $\text{N}_2$  was caused by reactions of  $\text{NH}_2\text{OH}$  with Mn and Fe ( $\text{MnO}_2$ ,  $\text{Fe}_2\text{O}_3$ , etc.) (Bremner and Shaw, 1958; Bremner et al., 1980). The evidence for a significant role of  $\text{NH}_2\text{OH}$  in the total nitrogen budget in soil is however, lacking. If such a process was important  $\text{NO}_2^-$  and  $\text{NO}_3^-$  would not be produced during  $\text{NH}_4^+$  oxidation for all the N would have already escaped via  $\text{NH}_2\text{OH}$  oxidation to  $\text{N}_2\text{O}$  and  $\text{N}_2$ .

### 1.3.2 Biological Denitrification

The accepted pathway of biological denitrification is (Knowles, 1978)



Facultative anaerobic bacteria (bacteria with the ability to use either oxides of nitrogen or oxygen as hydrogen acceptors) are responsible for denitrification. Oxygen is used preferentially to nitrogen oxides and only in situations of high  $\text{O}_2$  demand is nitrate reduced.

Although complete anaerobiosis is not required, oxygen levels must be quite low before denitrification will occur (Broadbent and Clark, 1965). Nitrogen loss rates decrease by 90% if the oxygen pressure is raised from zero to five millimetres of Hg. Anaerobic sites may occur in areas where biological activity is great and oxygen diffusion is limited, thereby allowing denitrification in an "aerobic soil" (Allison et al., 1960; Broadbent and Clark, 1965). If conditions of pH, moisture and

temperature are optimal, the numbers and respiration rate of bacteria increase causing the electron donor supply to become the rate limiting step in growth. In such a case, rapid utilization of  $\text{NO}_3^-$  will occur.

During denitrification, accumulation of either  $\text{NO}_2^-$  or  $\text{N}_2\text{O}$  may occur and, if the  $\text{N}_2\text{O}$  is not retained and further denitrified in the soil, gaseous losses may result as  $\text{N}_2\text{O}$  rather than  $\text{N}_2$  (Cady and Bartholomew, 1960; Payne, 1976; Payne and Riley, 1969; Cho and Mills, 1979). This accumulation of intermediates could be due to:

1. a delay in induction of enzymes necessary for further reduction of a product until the precursor of the product is depleted i.e.  $\text{NO}_3^-$  would inhibit induction of  $\text{NO}_2^-$  reducing enzymes.
2. the precursors could repress the functioning of reduction enzymes further along in the reactive sequences (Blackmer and Bremner, 1978; Terry and Tate, 1980).
3. competition could exist between an intermediate and a precursor to act as an electron acceptor i.e.  $\text{N}_2\text{O}$  competes with  $\text{NO}_3^-$  (Cho and Sakdinan, 1978).

Payne and Riley (1969) found that the enzymes necessary for the reduction of nitrate to molecular nitrogen had all been induced in denitrifiers after growth for five hours in liquid media suggesting the first theory is not correct. Nitrate disappearance follows Michaelis-Menton kinetics whereby losses are first order at low  $\text{NO}_3^-$  concentration changing to zero order as the substrate concentration increases to saturate the enzyme complexes (Bowman and Focht, 1974).

Cho and Mills (1979) developed a kinetic formulation of denitrification which has helped explain the transitory accumulation of  $\text{NO}_2^-$  and

$N_2O$  gases that may occur. Due to competition between  $NO_3^-$  and  $N_2O$  as electron acceptors, the ratio of  $N_2O$  to  $N_2$  produced would increase as the initial concentration of  $NO_3^-$  increased. The relative amounts of  $N_2$  and  $N_2O$  in a system at any time therefore depends on initial biological demand for electron acceptors and the rate of satisfaction of this demand by  $NO_3^-$ ,  $NO_2^-$  or  $N_2O$ . Nitrous oxide has usually been reported to be the end product of denitrification in systems where the microbiological population has not been existing under ideal conditions i.e. low temperature, pH less than 6.5 while  $N_2$  is prominent under more favourable conditions (Alexander, 1977). These factors affect biological activity which, in turn cause the production of either  $N_2O$  as the final product or allow the further reduction of  $N_2O$  to  $N_2$ .

As with other biological reactions, temperature has a pronounced effect on the rate of denitrification and also affects the product ultimately formed. The rate of denitrification increases rapidly as temperature rises from  $2^\circ$  to  $25^\circ C$ . Increasing temperatures above  $25^\circ C$  has resulted in further slight increases in rate and denitrification still occurred at  $70^\circ C$  (Bremner and Shaw, 1958). A lower limit has been variously reported as being  $2^\circ C$  (Bremner and Shaw, 1958),  $2.8^\circ C$  (Cho et al., 1979), and  $5^\circ C$  (Bailey and Beauchamp, 1973). Dinitrogen production seems to predominate over  $N_2O$  production as the temperature increases (Alexander, 1977). Temperatures near the optimum value for microbial activity result in an elevated biological demand for electron acceptors which, in turn, cause the further reduction of  $N_2O$  to  $N_2$  (Cho and Mills, 1979).

Many workers have found a transitory accumulation of nitric oxide to occur. This led Payne (1976) to conclude that NO was a precursor to  $N_2O$  formation for NO decreased as  $N_2O$  levels increased. If it were a precursor of  $N_2O$ , facultative anaerobes should be able to grow using NO as a terminal electron acceptor. It does not support growth. In any case, losses of nitrogen as NO would not normally be significant. As mentioned previously, NO is rapidly oxidized by free  $O_2$  to  $NO_2$  which is very soluble in soil water (Nelson and Bremner 1970). Nitric oxide cannot leave the soil without passing through an oxygenated layer at the surface. Oxidation takes place immediately and the resulting  $NO_2$  is adsorbed by soil moisture.

#### 1.4 ASSUMPTIONS IN HEAVY ISOTOPE METHODOLOGY

Studies employing  $^{15}N$  require that two basic assumptions be made;

1. that the  $^{15}N$  content of soil is constant.
2. that light and heavy isotopes react in the same manner at the same rate.

Neither of these assumptions is strictly correct and problems can arise when working with  $^{15}N$  at near natural levels (0.366% [Nier 1950]).

Chemical and biological reactions tend to discriminate against the heavier isotope resulting in slight accumulations of  $^{15}N$  at the reaction site (Wellman et al., 1968; Delwiche and Steyn, 1970; Blackmer and Bremner, 1977; Chien et al., 1977). This discrimination is at a very low level but still makes up the basis of the so-called "delta  $^{15}N$  technique" where the difference between background and measured  $^{15}N$  contents is determined and multiplied by 1000 to give a delta  $^{15}N$  value (Karama-

nos and Rennie, 1980). Studies using this system also indicate that the natural abundance of the heavy isotope is not a constant, exact value but may vary by  $\pm 0.05\%$  (Broadbent et al., 1980).

Although both factors just mentioned can pose problems when working with low per cent  $^{15}\text{N}$  levels, they can be disregarded if the percent  $^{15}\text{N}$  is high (Blackmer and Bremner, 1977; Hauck, 1973; Edwards, 1973). In all experiments performed in this project,  $^{15}\text{N}$  levels were approximately 50%.

## Chapter II

### MATERIALS AND METHODS

#### 2.1 SOILS

Samples of soil from the Ap horizon (0-15 cm) of Keld, Wellwood, Stockton, Carroll and Pine Ridge associations were collected in the fall of 1978, air dried and ground to pass through a 2-mm sieve. Samples were stored at room temperature in cloth bags. Legal locations of the soils as well as some chemical and physical properties are listed in Table 1.

Preliminary work showed that three soils, Keld, Wellwood and Pine Ridge produced significant amounts of nitrogen gas upon incubation with nitrite. Of these, Wellwood soil was used most extensively in this project because its pH, texture and usually its rate of denitrification were intermediate between those of Keld and Pine Ridge.



TABLE 1

Subgroup designation, legal location and some physical and chemical properties of the soils used.

	Keld	Wellwood	Pine Ridge	Stockton	Carroll
Legal location	S 15-25-20W	NE 13-11-14W	SE 26-4-7E	SW 1-11-15W	SW 32-9-19W
subgroup	sombric dystric brunisol	orthic black chernozem	orthic grey luvisol	orthic black chernozem	orthic black chernozem
Texture	SCL	FSL	LFS	FSL	FSL
pH	5.6	6.4	6.5	6.9	7.1
organic matter	6.8%	4.4%	3.7%	1.8%	5.7%

## 2.2 INCUBATION APPARATUS

Soil samples were incubated in glass containers which had an interior diameter of 2.4 cm, were approximately 10.5 cm long and were sealed at one end (Figure 2). The upper portion of the unit consisted of a 2 mm stopcock to which was attached a  $\text{F } 10/18$  inner ground glass joint. The two sections were connected to each other at a B24 ground glass joint and sealed with Corning high vacuum grease. The total volume of the sealed containers with the stopcock closed was adjusted to 41.9 ml by addition of glass beads.

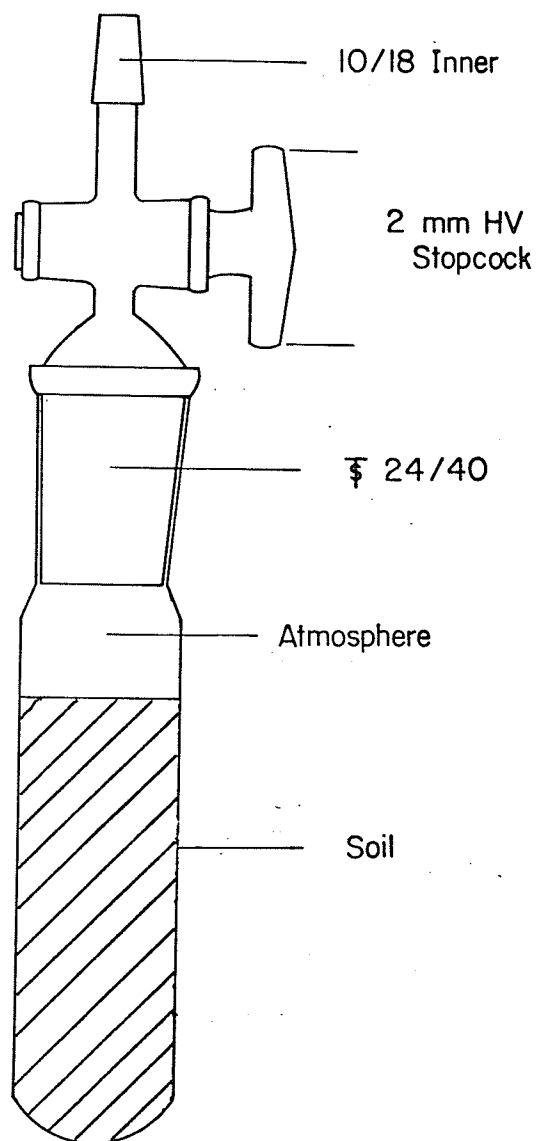


Figure 2: Incubation vessel used in experiments.

### 2.2.1 Gas Sampling Procedure

In order to ensure that the gas sample taken from the incubation vessel was representative of the soil atmosphere, the vessel was shaken vigorously before sampling. To obtain a gas sample for analysis, the sample container was attached to a sampling rack at a  $\frac{1}{8}$  10/18 outer joint. An open container with 15g of KOH pellets (Fig 3) and another containing 10 ml  $H_2SO_4$  were attached to the sampling rack at  $\frac{1}{8}$  10/18 inner joints. Both sulfuric acid and KOH acted as desiccants, removing water that could be harmful to the mass spectrometer. In addition, KOH removed carbon dioxide from the gas sample.

The apparatus was evacuated using a mechanical rotary vacuum pump to  $5 \times 10^{-3}$  Torr as measured by a McLeod gauge in the system. The sample and gas sampling tubes were then isolated by closing the stopcock of the line leading to the vacuum pump. The sample container was then opened, allowing the sample gas to expand into the gas sampling tubes. The stopcock was closed on each gas sampling tube, thereby trapping a gas sample.

After a 30 minute drying period, the gas was introduced into the Micromass mass spectrometer at a stainless steel  $\frac{1}{8}$  10/18 inner joint to permit scanning of a.m.u. 28 to 46.

All data points presented on graphs are the average of at least duplicate samples.

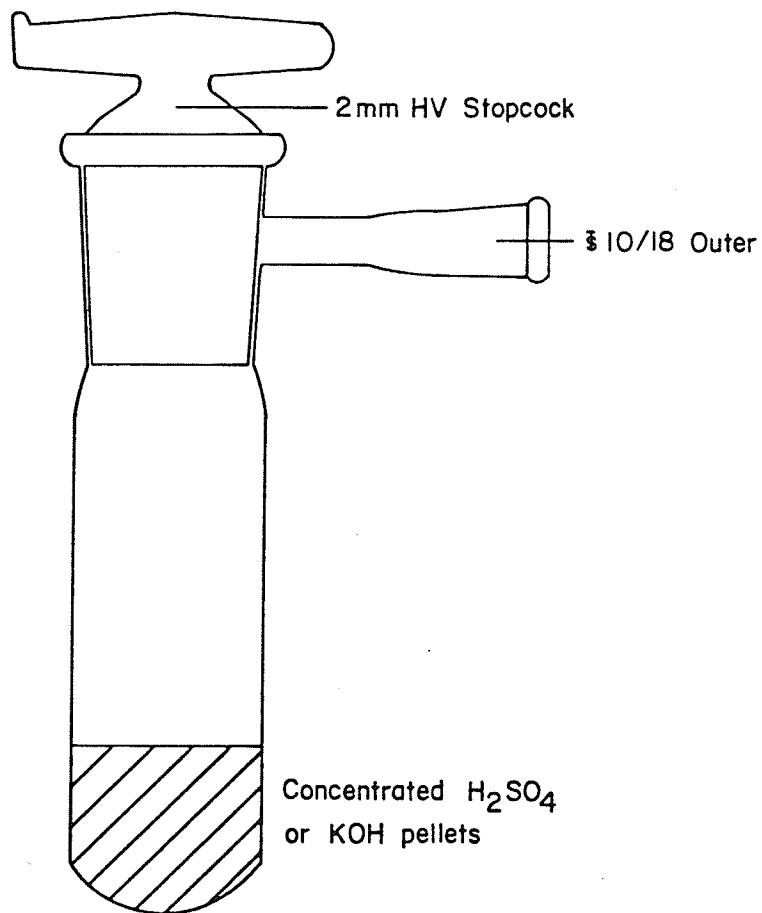


Figure 3: Gas sampling container.

### 2.3 EXPERIMENT I EFFECT OF NITRITE CONCENTRATION ON DENITRIFICATION

An experiment was designed to determine the effect of concentration of nitrite on the rate of nitrogen and nitrous oxide evolution and to determine if  $N_2$  and  $N_2O$  production were first, second or zero order with respect to nitrite added.

Nitrogen was added as 52.4%  $^{15}N$ -enriched sodium nitrite to Wellwood soil at 25, 50, 75, 100, 150, 200 and 300 ppm-N and incubated at  $20^{\circ}C$  for three days. Prior to incubation, the moisture content was adjusted to 25% which was close to the field capacity of the soil. Samples consisting of 25 g of the wet soil-nitrite mixture were placed in incubation vessels which were then sealed. Gas samples were taken as previously described and analysed with a mass spectrometer. Nitrite and nitrate were determined by Autoanalyser.

## 2.4 EXPERIMENT II EFFECT OF TEMPERATURE ON CHEMODENITRIFICATION

### 2.4.1 Nonsterile Soil

Most biological reactions cease at  $0^{\circ}\text{C}$  while nonbiological reactions do not. This experiment was designed to determine if chemodenitrification continued below the freezing point and at what rates.

In order to determine the contribution that the temperature made to the rate of  $\text{N}_2$  formation, Wellwood soil samples treated with 50, 100, 150, 200, or 300 ppm-N as  $\text{NaNO}_2$  (52.4%  $^{15}\text{N}$ ) were incubated at 25% moisture content for three days. The incubation temperatures were set for the three day period at values ranging from  $-20^{\circ}\text{C}$  to  $20^{\circ}\text{C}$ . Temperature was maintained at constant values using air circulation incubators ( $4^{\circ}$  to  $20^{\circ}\text{C}$ ) or alcohol circulator baths (Haake constant temperature circulator model FK or Lauda K-4/RD (Brinkman)). The air circulator incubators were claimed by the manufacturer to operate to an accuracy of at least  $\pm 0.5^{\circ}\text{C}$  while the alcohol circulators had a claimed accuracy of  $\pm 0.1^{\circ}\text{C}$ .

At the end of the incubation period, the soil containers were removed from the incubators and placed in a  $20^{\circ}\text{C}$  water bath for one half hour. Sample containers were then shaken vigorously and a gas sample was taken. Gas analysis was performed with the mass spectrometer and nitrate and nitrite remaining in the soil was determined.

In another study, Wellwood samples containing 100 ppm-N nitrite were incubated over time intervals varying from one to sixteen days. Two temperatures were chosen for this experiment,  $-8.3^{\circ}$  and  $10.0^{\circ}\text{C}$ . Moisture content was 25%. Gas and inorganic-N analysis was performed as described previously.

#### 2.4.2 Sterile Soil

Since denitrification may occur via biological and/or chemical processes, experiments were performed with autoclave-sterilized soils to determine if the same  $N_2$  production phenomena occurred with respect to temperature in sterile soil as with nonsterile soil.

Soil samples were autoclave-sterilized for 45 minutes at a pressure of 15 atmospheres and  $121^{\circ}C$ , allowed to cool and incubated at  $30^{\circ}C$  for one day. Soil samples were again sterilized in the same manner the next day. Glassware, spatulas, grease, etc. were also autoclaved. The  $NaNO_2$  solution was sterilized using a membrane filtration unit.

Sterile nitrite solution was added to the sterilized soil at a rate of 200 ppm-N on a soil basis. The moisture content was 25%. Samples were incubated at temperatures ranging from  $20^{\circ}C$  to  $-16^{\circ}C$ , each for a three day period. Gas samples were taken and analyzed as in previous experiments.

Colony counts were performed to give an indication of the size of the microbial population that had survived autoclaving. One gram samples were diluted to a dilution of  $10^{-3}$  in peptone water and spread-plated on T-Soy agar. The plates were incubated in the inverted position for one week in the dark at room temperature. Colony counts were then made. Although it was recognized that incubation on T-soy plates was selective for aerobic bacteria capable of utilizing that medium and that not all bacteria can use this medium, it was felt that this would give a partial indication of the survival of soil bacteria.



## 2.5 EXPERIMENT III EFFECT OF MOISTURE CONTENT ON DENITRIFICATION

This study was divided into two parts. The first part was carried out to determine the effect of a wide range of moisture contents on denitrification rates at a temperature of 10°C. Wellwood soil samples were treated at a rate of 100 ppm-N (on a soil basis) with 52.4% <sup>15</sup>N enriched sodium nitrite. Samples were wetted to moisture contents ranging from 11% to 50% and incubated for three days. Since the nitrogen content on a dry soil basis was constant, addition of excess water diluted the nitrite in the soil solution.

In the second part of the experiment, the nitrite concentration in the soil solution was kept constant while the moisture content was varied at 7, 12, 17 and 22%. This corresponded to a NO<sub>2</sub><sup>-</sup>-N content of 56, 96, 136 and 176 ppm respectively, on a soil basis. The nitrite concentration in the solution was 800 ppm-N.

In Experiment I, the thickness of the soil solution layer around the soil particles was always constant. In this study, nitrite solution concentration was the same initially, the only difference was the relative moisture content of each sample and consequently the relative amount of nitrite solution associated with the colloidal surface. The moisture contents used in this portion of the study were below the field capacity of the soil so that little free water was in the soil pores.

Samples were incubated for periods of three days at temperatures ranging from 20 to -18°C. Mass spectra were analysed with the mass spectrometer.

Gravimetric moisture contents were determined at 105°C.

## 2.6 EXPERIMENT IV ROLE OF SOIL CHARACTERISTICS IN INFLUENCING DENITRIFICATION RATES UPON FREEZING

This experiment was designed to determine if the chemodenitrification trends found in Wellwood soil were unique to that soil alone. Samples of soils of varying pH and texture were used in this comparison study.

Samples of Wellwood, Carroll, Keld, Stockton and Pine Ridge soils were moistened to 25% moisture content and incubated over the temperature range of 20°C to -20°C. Nitrogen was added to the soils at the rate of 100 ppm-N as <sup>15</sup>N-enriched NaNO<sub>2</sub>. Since Stockton and Carroll soils showed very little denitrification, they were excluded from further experiments. Further incubations were performed with Keld, Pine Ridge and Wellwood soils at nitrite concentrations of 200 and 300 ppm-N. Gas analysis was performed as before.

To correlate the chemodenitrification with soil physical characteristics, samples of Wellwood, Keld and Pine Ridge soils were placed in a pressure membrane apparatus (Soil Moisture Co., Santa Barbara, CA.) and moisture retention curves to pF 4.2 were determined.

In order to determine the effects of pH on denitrification rate, the pH of Keld soil was raised to 6.2 and 7.1 by addition of CaCO<sub>3</sub>. Samples were pre-incubated for one week to allow pH stabilization to occur and were then treated with 100 ppm-N NO<sub>2</sub><sup>-</sup>. Samples were incubated for three days at 20°C. Gas analysis was then carried out.

## 2.7 CHEMICAL ANALYSIS

### 2.7.1 pH

The soil pH was determined using a Fisher model 520 digital pH/ion meter equipped with a combination electrode. The soil-water ratio was 1:1. Samples were shaken 30 minutes prior to pH determination.

### 2.7.2 CaCO<sub>3</sub>

The CaCO<sub>3</sub> content was determined by the method of Skinner et al. (1959).

### 2.7.3 Organic Matter

The potassium dichromate-concentrated H<sub>2</sub>SO<sub>4</sub> method described by Peech et al. (1947) was used to determine soil organic matter.

### 2.7.4 Conductivity

Conductivity was measured with a Radiometer conductivity meter with a soil-water ratio of 1:4.

### 2.7.5 Cation Exchange Capacity

The cation exchange capacity was determined by the ammonium acetate method of Chapman (1965).

### 2.7.6 Nitrite and Nitrate Nitrogen

Nitrite-N and nitrate-N were determined using a 1:10 soil:water solution. The method used was a modification of that of Kamphake et al. (1967) employing a Technicon autoanalyser.

The Autoanalyser is designed to measure nitrite in solution. To measure nitrate, nitrate is first reduced to nitrite and then measured colorimetrically. In samples containing both nitrite and nitrate, optical density observed with reducing agent ( $\text{NO}_2^-$  and  $\text{NO}_3^-$ ) minus that without reducing agent ( $\text{NO}_2^-$  only) gives the amount of nitrate in the sample. It is assumed that nitrite is unaffected by the reducing agent. This was not found to be the case with the machine used in this project and measured nitrite was lower in samples containing the reducing agent. Therefore nitrite standards were run with and without reducing agent. Percent transmittance was converted to optical density and plotted against concentration.

In order to determine the amount of endogenous nitrite present during nitrate reduction, the previously measured nitrite concentration curve found without reducing agent was applied to the graph of nitrite with reducing agent. From this was determined the optical density corresponding to the endogenous nitrite. This was subtracted from the opti-

cal density of the sample with reducing agent in order to determine the optical density due to nitrate alone.

## 2.8 GAS CALCULATIONS

In order to avoid analysis problems caused by indigenous nitrogen in the system, most denitrification studies are carried out using a nitrogen-free atmosphere (Nelson and Bremner, 1970; Meek and MacKenzie, 1965; Reuss and Smith, 1965). Replacement atmospheres include helium-oxygen, argon, and argon-oxygen.

Reports by Parr et al. (1970) and Blackmer and Bremner (1977) state that alternate atmospheres do not change denitrification or biological respiration rates in soil samples. Nevertheless, utilization of a normal air atmosphere is simpler and sometimes more convenient if the analytical method of Cho and Sakdinan (1978) is employed. By allowing the level of argon in air to act as an internal standard and using a high degree of labelling, oxygen consumption and  $N_2$ ,  $N_2O$ ,  $NO$  and  $CO_2$  production can be determined.

During biological denitrification of  $NO_2^-$  or  $NO_3^-$ , two N atoms from the added fertilizer combine to form one  $N_2$  molecule. If they are allowed to recombine randomly, the distribution of the resultant molecules will be described by a binomial expansion, i.e.

$$(p+q)^2 = p^2 + 2pq + q^2 \quad (16)$$

where "p" is the atom fraction of  $^{14}\text{N}$  and "q" is the atom fraction of  $^{15}\text{N}$ . With nitrogen gas, the terms on the right hand side correspond to atomic mass units (a.m.u.) 28, 29 and 30, respectively, while with  $\text{N}_2\text{O}$  they are 44, 45 and 46 (Hauck et al., 1958). If the initial percent  $^{15}\text{N}$  of the added  $\text{NO}_2^-$  or  $\text{NO}_3^-$  is 50%, then  $p = 0.5$  and  $q = 0.5$  and equation (16) becomes

$$\begin{aligned} (p+q)^2 &= (0.5)^2 + 2(0.5)(0.5) + (0.5)^2 \\ &= 0.25 + 0.50 + 0.25 \end{aligned} \quad (17).$$

It is evident therefore, that the ratio of 28:29:30 or 44:45:46 will be 1:2:1.

When chemical denitrification of  $\text{NO}_2^-$  occurs, one atom of  $\text{NO}_2^-$ -N combines with one atom of soil N to yield  $\text{N}_2$  gas. This is not a random situation as was the previous example. If, for the sake of simplicity, soil N is assumed to be entirely made up of  $^{14}\text{N}$  and the added  $\text{NO}_2^-$  is again 50% labelled with  $^{15}\text{N}$ , then only two species of  $\text{N}_2$  gas are possible; either 28 ( $^{14}\text{N} \ ^{14}\text{N}$ ) or 29 ( $^{14}\text{N} \ ^{15}\text{N}$ ). Since half the  $\text{NO}_2^-$  is  $^{14}\text{N}$  and it combines with soil  $^{14}\text{N}$  and the other half is  $^{15}\text{N}$  which combines again with soil  $^{14}\text{N}$ , the distribution ratio of 28:29:30 will be 1:1:0.

Since nitrogen of a.m.u. 28 in an air atmosphere makes up approximately 78% of the atmosphere, small changes in the amount of a.m.u. 28 are very difficult to measure with the mass spectrometer. However, a.m.u. 29 and 30 are much rarer and a small increase here can be easily measured. The technique of Cho and Sakdinan (1978) uses  $\text{NO}_2^-$ -N and at-

omic mass measurements of 29 and 30 to determine the amounts of  $N_2$  production and whether such production was of the first or second mode. Measurable amounts of a.m.u. 29 and 30 indicate the presence of biologically formed  $N_2$  whereas an increase in 29 without an increase in 30 indicates the presence of chemically formed  $N_2$ .

The atom percent N can be calculated using the following formula:

$$\begin{aligned} \%15N &= 100 \frac{(\text{number of } 15N \text{ atoms})}{(\text{number of } 15N + 14N \text{ atoms})} \\ &= \frac{100 R'/2}{1 + R'/2} \\ &= \frac{100 R'}{R' + 2} \end{aligned} \quad (18)$$

where  $R' = (29)/(28)$ .

Using only 29 and 30, the formula becomes

$$\%15N = \frac{200}{R'' + 2} \quad (19).$$

where  $R'' = (29)/(30)$ .

Mass intensities at 44, 45 and 46 may also be used resulting in a  $R'$  value derived from 45/44 and a  $R''$  value from 45/46.

The gas sample was scanned mass spectrometrically between a.m.u. 28 and 46. Argon (mass 40) was chosen as an internal standard and before scanning the sample, the argon peak was set to a pre-arranged height by varying the inlet pressure. The peak at 40 was set to 100 and all other peaks were normalized to this figure (Table 2). An air sample was dried with KOH in order to remove  $CO_2$  and was scanned in an identical manner to determine the contribution of air to each a.m.u. scanned.

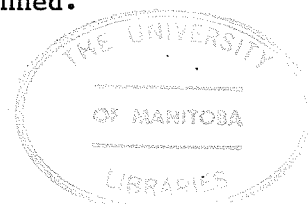


TABLE 2

Normalized peak heights at a scan between a.m.u. 28 and 46 of atmosphere of Wellwood soil, 200ppm-N as NO<sub>2</sub>.

a.m.u.	measured peak height (cm)	attenuation factor	normalized peak height
28	19.60	10 <sup>-9</sup>	3580
29	2.93	10 <sup>-11</sup>	53.4
30	0.93	10 <sup>-11</sup>	1.69
31	0.62	10 <sup>-11</sup>	1.13
32	3.61	10 <sup>-9</sup>	656.
40	5.50	10 <sup>-10</sup>	100
44	3.33	10 <sup>-11</sup>	6.05
45	4.70	10 <sup>-11</sup>	8.55
46	2.53	10 <sup>-11</sup>	4.60



Measurements of a.m.u. 28 to 46 correspond to the molecules listed in Table 3 .

Samples taken from gas containers containing  $H_2SO_4$  possessed  $CO_2$  in similar proportions to that originally in the incubation vessel. Scans of these samples were used only to determine the amount of  $CO_2$  evolved.

The probability of fragmentation of  $N_2O$  to  $NO$  and  $N_2$  in air was determined at a fixed mass spectrometric setting. The percentages of fragmentation of  $N_2O$  to  $NO$  and  $N_2O$  to  $N_2$  were found to be 17.7% and 5.3%, respectively.

Use of  $KOH$  in gas tubes removed most of the  $CO_2$  present and therefore peaks at 45 and 46 were considered to be of  $N_2O$  only. Using these peaks, the  $\%^{15}N$  in  $N_2O$  was calculated using the formula

$$\%^{15}N = \frac{200}{R + 2} \quad (20)$$

and  $R$  equals the ratio of (45) to (46) where brackets denote the measured peak height at each a.m.u.

Since  $O_2$  was present in most scans, some oxidation of carbon which was adsorbed to the mass spectrometer's gas inlet capillaries and near the ion source resulted in the formation of  $CO_2$ . The peak at 44 was not considered clean and a.m.u. 44 due to  $N_2O$  was calculated using the equilibrium equation

TABLE 3

Molecular species found between a.m.u. 28 and 46.

Mass	Molecule
28	$^{14}\text{N} \ ^{14}\text{N}, \ ^{12}\text{C} \ ^{16}\text{O}$
29	$^{14}\text{N} \ ^{15}\text{N}, \ ^{13}\text{C} \ ^{16}\text{O}$
30	$^{15}\text{N} \ ^{15}\text{N}, \ ^{14}\text{N} \ ^{16}\text{O}$
31	$^{15}\text{N} \ ^{16}\text{O}$
32	$^{16}\text{O} \ ^{16}\text{O}$
40	$^{40}\text{Ar}$
44	$^{12}\text{C} \ ^{16}\text{O} \ ^{16}\text{O}, \ ^{14}\text{N} \ ^{14}\text{N} \ ^{16}\text{O}$
45	$^{14}\text{N} \ ^{15}\text{N} \ ^{16}\text{O}, \ ^{13}\text{C} \ ^{16}\text{O} \ ^{16}\text{O}$
46	$^{15}\text{N} \ ^{15}\text{N} \ ^{16}\text{O}$

$$4 = \frac{(14N \ 15N)^2}{(14N \ 14N)(15N \ 15N)} \quad (21)$$

$$= \frac{(45)^2}{(44) (46)} \quad (22)$$

$$(44) = \frac{(45)^2}{4 (46)} \quad (23)$$

The total peak height due to  $N_2O$  was the sum of (44) $_{N_2O}$ , (45) and (46).

Using the %  $^{15}N$  determined in equation (20) and mass peak 31, the mass peak at 30 due to NO was determined as follows:

$$\left( \frac{(31)}{(30) + (31)} \right) 100 = \%15N \quad (24)$$

$$\frac{(31)100}{\%15N} - (31) = (30) \quad (25)$$

The sum of NO was divided by the sum of  $N_2O$  to determine if any NO was produced other than as a fragmentation product of  $N_2O$ . The ratio obtained should approach 0.177 if all NO were due to fragmentation of  $N_2O$ .

Since the mass intensity at 30 is the sum of  $^{14}N^{16}O$  and  $^{15}N^{15}N$ , subtraction of the calculated  $^{14}N^{16}O$  from a.m.u. 30 yields the peak height at 30 due to  $^{15}N^{15}N$ .

The mass intensities at 28, 29 and 30 are now the result of  $N_2$  formed via both a van Slyke-like process and a reduction of  $N_2O$ , hereafter designated  $N_{22}$  and  $N_{21}$  respectively.

Therefore:

$$(29)N_2 = (29)N_{21} + (29)N_{22} \quad (26)$$

and

$$(30)N_2 = (30)N_{21} + (30)N_{22} \quad (27)$$

In the above equations,  $(29)N_2$  and  $(30)N_2$  refer to the total contribution of  $N_2$  to a.m.u. 29 and 30. If it is assumed that isotopic separation is negligible during the formation at  $N_{21}$ , the distribution of  $^{15}N$  in this form of  $N_2$  should be the same as that of  $N_2O$ . Therefore :

$$(29)N_{21} = (30)N_{21} R \quad (28)$$

The distribution of a.m.u. 28, 29 and 30 in  $N_{21}$  and  $N_{22}$  can be described by the following expressions:

$$(28)N_{21}:(29)N_{21}:(30)N_{21} = p_1^2:2p_1q_1:q_1^2 \quad (29)$$

$$(28)N_{22}:(29)N_{22}:(30)N_{22} = p_1p_2:(p_1q_2 + p_2q_1):q_1q_2 \quad (30)$$

in which  $p_1 + q_1 = 1$ , and  $p_2 + q_2 = 1$ . In the above expression  $p_1$  and  $p_2$  are the fraction of  $^{14}N$  in the labelled and natural  $N$ , respectively, and  $q$  signifies  $^{15}N$  fraction. The value of  $q_2$ , natural abundance of  $^{15}N$ , as determined by the machine, Micromass 602, is 0.0037. The relationship between  $q_1$  and  $R$  is

$$q_1 = \frac{2}{2 + R} \quad (31)$$

The ratio of  $(29)N_{22}$  to  $(30)N_{22}$  becomes

$$\begin{aligned}
 \frac{(29)N_2 2}{(30)N_2 2} &= \frac{\frac{R}{2+R} (.0037) + (.9967) \frac{2}{2+R}}{\frac{2}{2+R} (.0037)} \\
 &= 269.27 + \frac{R}{2} \quad (32)
 \end{aligned}$$

Substitution of eq. (28) and (32) into eq. (26) yields

$$(29)N_2 = R(30)N_2 1 + (269.27 + R/2) (30)N_2 2 \quad (33)$$

The solution of simultaneous equations (27) and (33) yields

$$(30)N_2 1 = \frac{(269.27 + \frac{R}{2}) (30)N_2 2 - (29)N_2}{269.27 - \frac{R}{2}} \quad (34)$$

The values of  $(29)N_2 1$ ,  $(29)N_2 2$ ,  $(30)N_2 1$  and  $(30)N_2 2$  can be calculated using eq. (28), for  $(29)N_2 1$ , eq. (32), for  $(29)N_2 2$  and the relationships

$$(28)N_2 1 = R/4 (30)N_2 1 \quad (35)$$

and

$$(28)N_2 2 = \frac{R}{2 + 0.0037R} (29)N_2 2 \quad (36)$$

The sums

$$(N_2 1) = (28)N_2 1 + (29)N_2 1 + (30)N_2 1 \quad (37)$$

$$(N_2^2) = (28)N_2^2 + (29)N_2^2 + (30)N_2^2 \quad (38)$$

were used to calculate the total nitrogen production after making corrections for fragmentation of  $N_2O$ .

Standard curves were developed to obtain a relationship between partial pressure and peak height by scanning known amounts of pure  $N_2O$  mixed in varying proportions with air. For  $N_2$ , the peak heights in air scans were used to calculate the partial pressure of produced gases.

## Chapter III

### RESULTS AND DISCUSSION

#### 3.1 EXPERIMENT I EFFECT OF NITRITE CONCENTRATION ON DENITRIFICATION

Production of both  $N_2$  and  $N_2O$  occurred at most  $NO_2^-$  concentrations over the three day interval. Van Slyke-like nitrogen and  $N_2O$  were the most prominent gases and relative amounts of each varied with the initial  $NO_2^-$  values (Fig 4). The production rate of  $N_2$  was so low that it was not possible to accurately quantify it and this gas was therefore not included in Figure 4.

The rate of formation of van Slyke-like  $N_2$  increased with increasing nitrite concentration and the results were described by the quadratic equation

$$7.857 \times 10^{-5} X^2 + 1.569 \times 10^{-2} X - 0.492 = Y \quad (39)$$

where 'X' refers to initial nitrite content of the soil ( $\mu g$  N/g soil) and 'Y' indicates the amount of chemically formed  $N_2$  gas evolved ( $\mu g$  N/g soil/day).

The x-intercept of equation at 27.5  $\mu g$  N/g soil indicates that this is the concentration of  $NO_2^-$  at which the measured evolution of  $N_2$  would be expected to be zero over the three day incubation interval. This may not necessarily mean that nitrite would be stable in this soil at this concentration, only that the chemical denitrification rate would be so low that measurable amounts of  $N_2$  would not accumulate during a three day incubation period. The maximum rate of  $N_2$  evolution occurred at 300

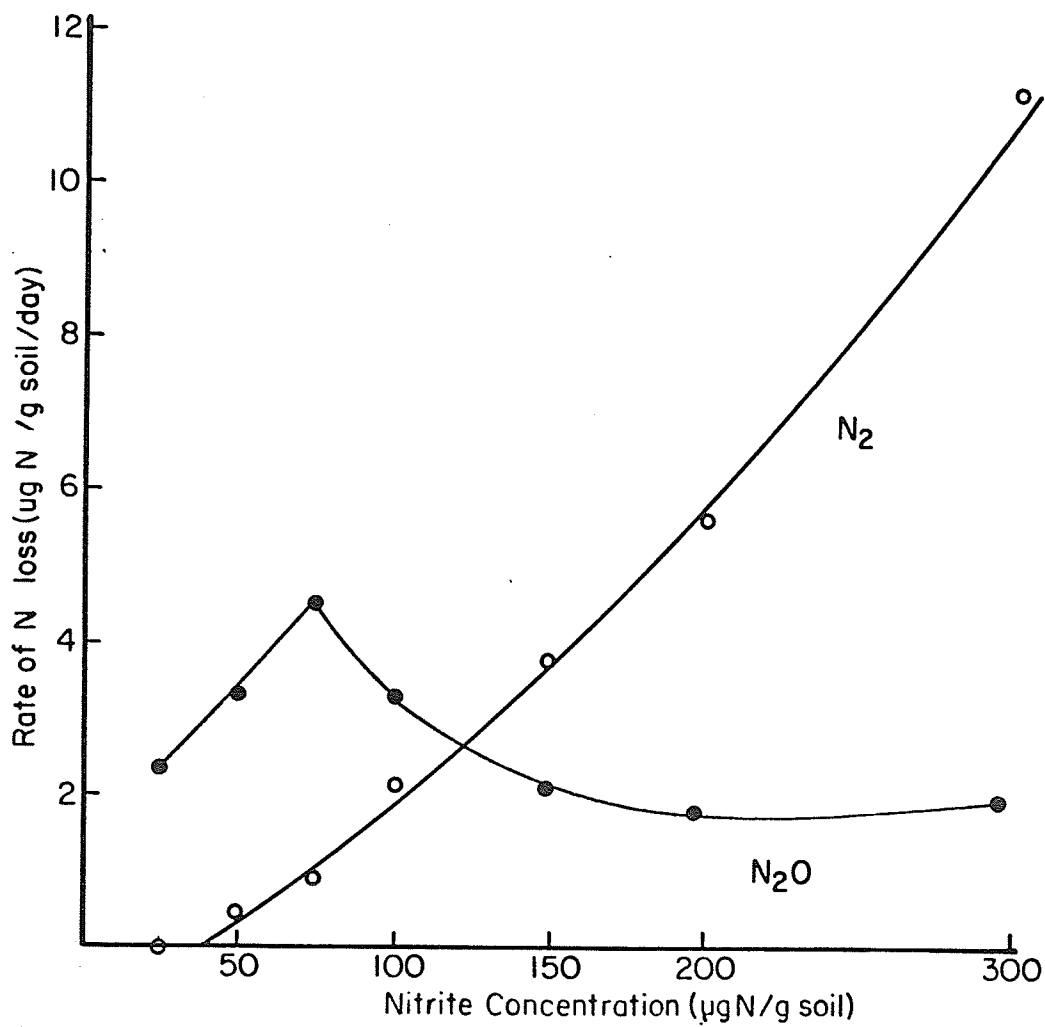


Figure 4: Nitrogen and nitrous oxide evolution as a function of initial nitrite concentration, Wellwood soil, 20°C.



ppm-N  $\text{NO}_2^-$  where the nitrogen evolved averaged 11.2  $\mu\text{g N/g soil/day}$  or 3.7% of the added N/day. Of this loss, only half came directly from the added fertilizer for one atom of the van Slyke nitrogen molecule came from the soil N itself.

It was interesting to note that the rate of  $\text{N}_2$  evolution increased in an almost direct proportion to the amount of  $\text{NO}_2^-$  initially in the system. This would be expected if a first order chemical reaction were operative. In contrast, increasing concentration would not increase the rate of  $\text{N}_2$  formation if a biological reaction were responsible for the formation of this gas. Numerous recent papers report a decrease in  $\text{N}_2$  evolution by increased initial concentration of  $\text{NO}_3^-$  (Blackmer and Bremner, 1978; Terry and Tate, 1980; Cho and Mills, 1979). Since  $\text{NO}_2^-$  is an intermediate in the reduction of  $\text{NO}_3^-$  to  $\text{N}_2$ , it would presumably have had the same effect as nitrate on  $\text{N}_2$  production. Although the presence of an approximately first order reaction mechanism is not, by itself, evidence enough to confirm that the majority of  $\text{N}_2$  evolved was produced via a chemical reaction, the production of  $\text{N}_2$  together with the distribution ratio of the isotopically labelled N (discussed in the literature review) added further evidence to the chemical nature of this reaction.

Nitrous oxide evolution increased as the nitrite concentration increased from 25 to 75 ppm-N (Fig 4) with a maximum of 4.8  $\mu\text{g N/g soil evolved/day}$ . Since the soil was incubated in a glass tube, anaerobic sites could have developed at the bottom of the tube which would account for the occurrence of biological denitrification even though oxygen was still present in the headspace above the soil. Increases in  $\text{NO}_2^-$  concentration above 75 ppm-N resulted in a decrease in  $\text{N}_2\text{O}$  production rates, possibly due to  $\text{NO}_2^-$  inhibition of the endogenous microbial population.

This is evidenced by decreased oxygen consumption and carbon dioxide evolution rates as the concentration of nitrite in the system increased (Fig 5).

Bacteria are especially sensitive to high  $\text{NO}_2^-$  concentrations when the soil pH is low (Tyler and Broadbent, 1960). Some  $\text{N}_2\text{O}$  production may have also been due to strictly chemical processes although the amounts produced in this manner would be expected to be small (Bremner et al., 1980).

Dinitrogen produced by the further reduction of  $\text{N}_2\text{O}$  was very low throughout the study (results not shown) and amounted to a maximum of only  $0.1 \mu\text{g N/g soil/day}$ . The low rate of production of this gas was probably due to a low demand for terminal electron acceptors which was satisfied by oxygen still in the system or by the production of  $\text{N}_2\text{O}$  from added nitrite.

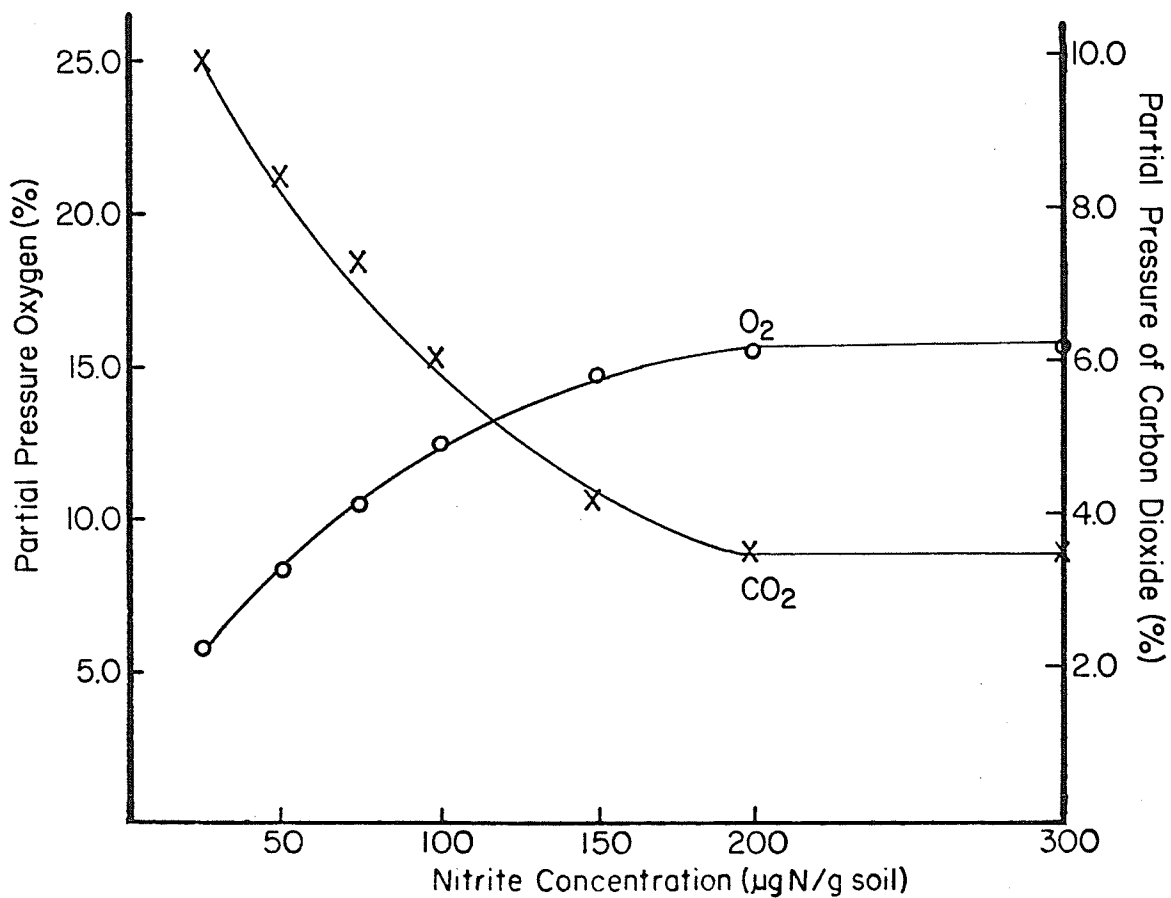


Figure 5: Effect of nitrite concentration on partial pressure of carbon dioxide and oxygen in the sample atmosphere, Wellwood soil, 20°C.

### 3.2 EXPERIMENT II EFFECT OF TEMPERATURE ON CHEMODENITRIFICATION

#### 3.2.1 Part 1 Non-sterile Soil

Arrhenius theory states that the rate of any chemical reaction should decrease as the temperature within the system decreases. The general equation relating rate and temperature is

$$V = A \exp [-Ea/RT] \quad (20)$$

where  $V$  refers to the reaction rate,  $A$  is a constant of integration called the frequency factor,  $Ea$  is the activation energy,  $R$  is the gas constant and  $T$  is temperature. This is an exponential equation and as a general rule, rate decreases between two and three fold for every  $10^{\circ}\text{C}$  drop in temperature. This relationship follows through  $0^{\circ}\text{C}$  and reaction rates only approach zero at temperatures approaching  $0^{\circ}\text{K}$  ( $-273^{\circ}\text{C}$ ). It is evident that if the Arrhenius equation holds, a plot of  $\log V$  versus  $1/T$  should give a straight line with a slope of  $-Ea/R$ . Biological reaction rates do not follow the Arrhenius theory but decrease to zero, in most cases, at  $2^{\circ}$  to  $3^{\circ}\text{C}$ . The data presented in Figure 6 show the effect of temperature on denitrification rates over the temperature range of  $20^{\circ}$  to  $-20^{\circ}\text{C}$  when 100 ppm  $\text{NO}_2^-$ -N was added to Wellwood soil.

As the incubation temperature was decreased from  $20^{\circ}\text{C}$  to  $-1.8^{\circ}\text{C}$ , the rate of  $\text{N}_2$  production decreased from 2.13  $\mu\text{g N/g soil/day}$  to 0.28  $\mu\text{g N/g soil/day}$  respectively. As expected from Arrhenius theory, the relationship between temperature and rate was curvilinear and rate did not drop to 0 at  $0^{\circ}\text{C}$  but was still measurable to  $-1.8^{\circ}\text{C}$ .

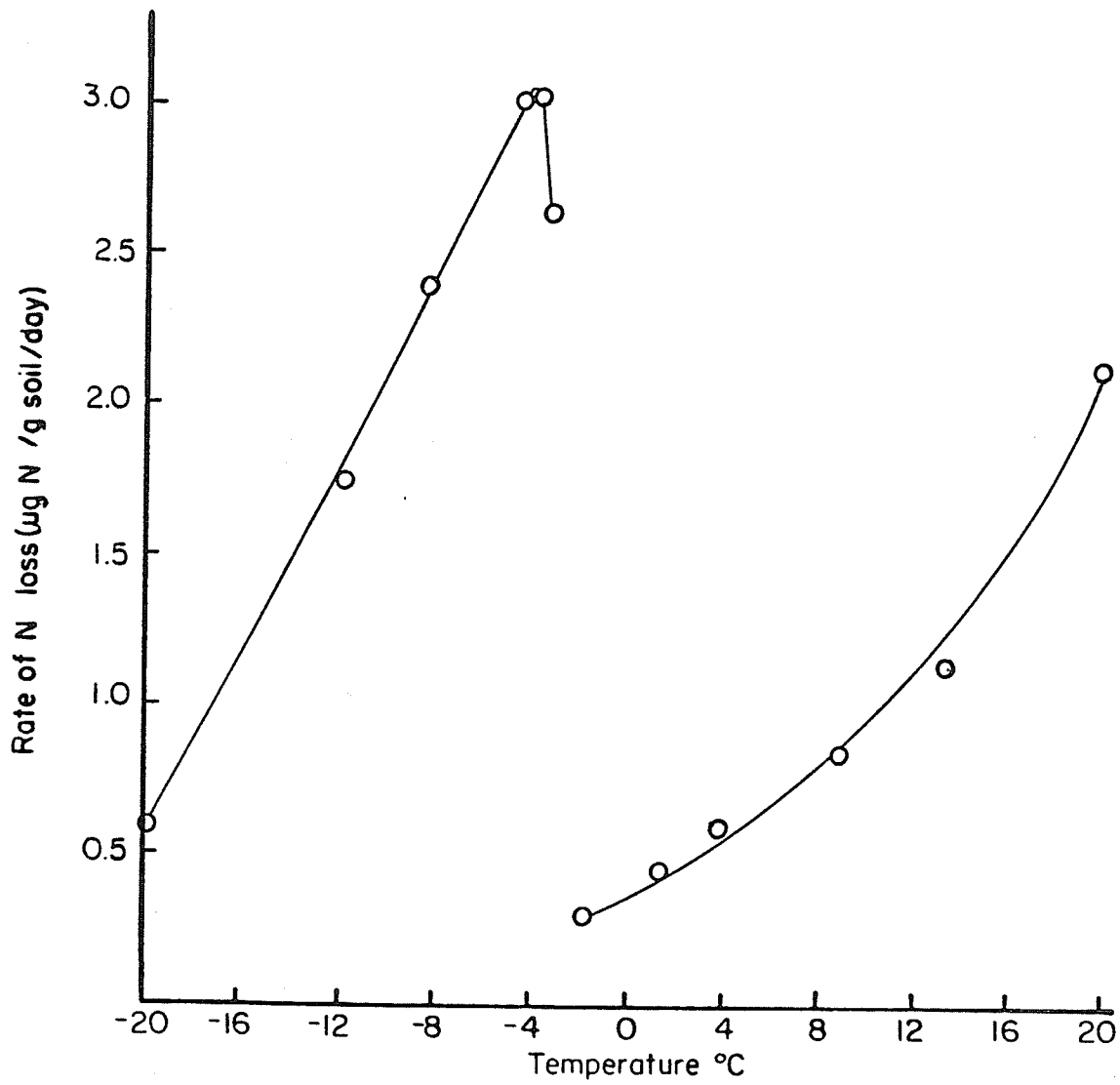


Figure 6: Effect of temperature on rate of chemodenitrification in Wellwood soil treated with 100 ppm  $\text{NO}_2^-$ -N.

Temperatures lower than  $-1.8^{\circ}\text{C}$  brought about an increase in denitrification rates. The denitrification rate reached  $3.03 \mu\text{g N/g soil/day}$  at  $-3.5^{\circ}\text{C}$ , a rate 42% higher than that at  $20^{\circ}\text{C}$  and 980% higher than that at  $-1.8^{\circ}\text{C}$ . The Arrhenius theory does not predict the occurrence of this rise in reaction rate with freezing.

A continuing decline in temperatures brought about a decrease in denitrification rate although the rate at  $-20^{\circ}\text{C}$  was still  $0.59 \mu\text{g N/g soil/day}$ , a value comparable to that at  $+4.4^{\circ}\text{C}$ .

The great enhancement of denitrification rate upon freezing of the soil may have been due to concentration of the nitrite in soil water. As discussed in the literature review, at low temperatures soil water does not freeze completely but rather remains as an unfrozen brine layer near the soil colloid. This brine layer is composed of salts excluded from the growing ice surface which is itself made up of almost pure water. In this experiment, concentration of the nitrite in the brine had probably increased which, in turn, caused a dramatic increase in  $\text{N}_2$  production rate. Since freezing of soil water usually occurs over a narrow temperature range, freeze-concentration should also occur over a short temperature span. The 980% increase in  $\text{N}_2$  evolution rates between  $-1.8^{\circ}\text{C}$  (just above freezing) and  $-3.5^{\circ}\text{C}$  (freezing almost complete) suggests that this is what occurred.

An Arrhenius plot (Fig 7) shows a high degree of linearity both above and below the freezing temperature of the system ( $r^2 = 0.99$  and  $0.96$ , respectively) and the slopes are similar ( $-6618$  and  $-6998$ ). This indicates that the same reaction appears to have been producing  $\text{N}_2$  over the entire temperature range and that the rate of the reaction varied with temperature in a similar manner both above and below the discontinuity.

This is to be expected for, although freezing should have caused a concentration of soil nitrite and therefore an increase in reaction rate, it should not have altered the actual reaction mechanism. The difference in rate at the discontinuity was due to the change in concentration of nitrite in the soil water. The slight departure from linearity around the freezing point may have been due to incomplete freezing of the soil solution which froze over a narrow range of temperatures.

Nitrous oxide production decreased steadily as the incubation temperature was lowered from  $20^{\circ}$  to  $-18^{\circ}\text{C}$  (Table 4). Production of this gas was greater at  $-3.5^{\circ}$  than at  $-18^{\circ}\text{C}$ , indicating that nonbiological formation may have occurred. If only biological processes had been responsible for this gas production, the rates should have been the same over the entire frozen portion of the experiment when biological activity would have ceased. A slight enhancement of the  $\text{N}_2\text{O}$  production rate appeared to have occurred upon soil freezing. However, the peak heights at a.m.u. 45 and 46 were very small at low temperatures and the accuracy of the  $\text{N}_2\text{O}$  determination in this temperature range was quite low.

Results with  $\text{NO}_2^-$ -N concentrations of 50, 150, 200 and 300 ppm-N, showed similar trends to those of 100 ppm-N (Fig 8). Denitrification decreased as temperature was lowered to near the freezing point. Upon freezing, an increase in denitrification rate occurred which was subsequently followed by a decrease in rate as temperature was lowered to  $-20^{\circ}\text{C}$ . As expected, the higher the initial  $\text{NO}_2^-$  concentration, the higher was the denitrification rate at any specific temperature. The maximum rate of loss at each concentration occurred at the point of freezing of the bulk of the soil solution. At  $-3.5^{\circ}\text{C}$ , the loss of 14.75

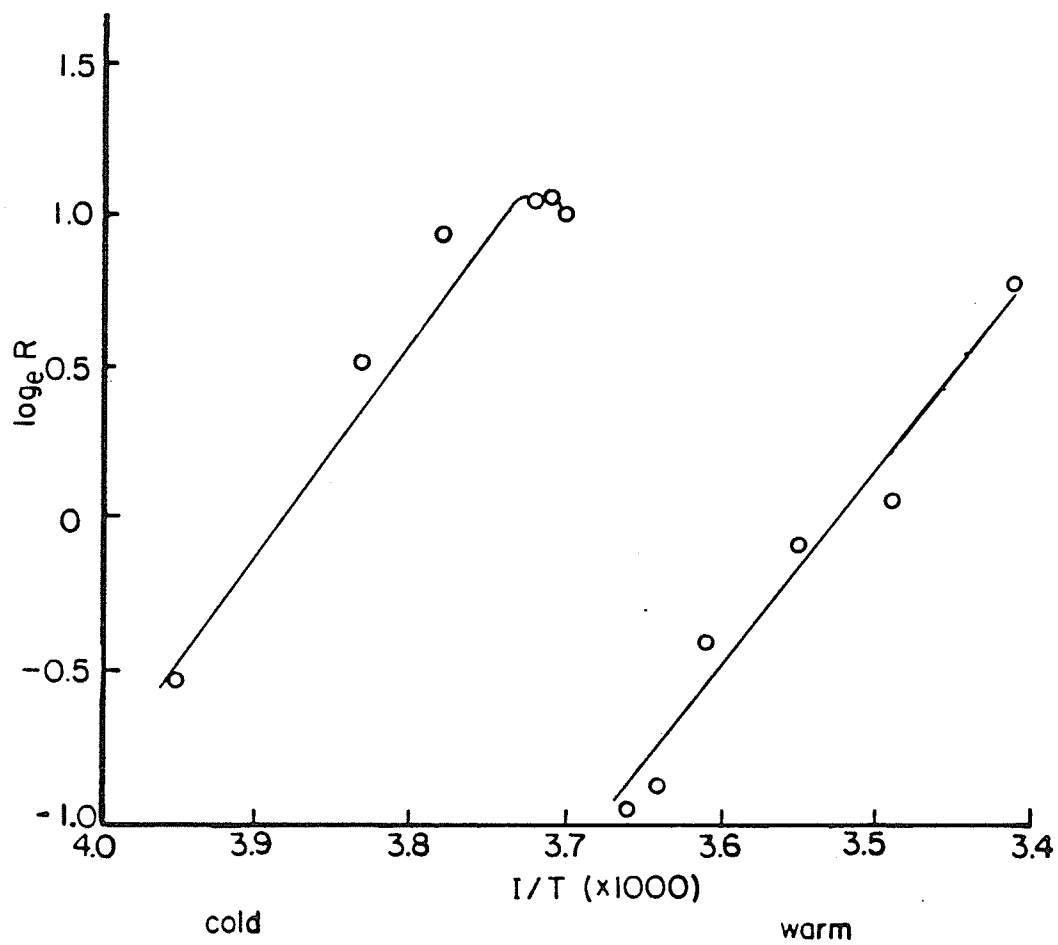


Figure 7: Arrhenius plot of chemodenitrification rate versus temperature in Wellwood soil, 100 ppm  $\text{NO}_2^-$ -N.



TABLE 4

Nitrous oxide formation ( $\mu\text{g N/g soil/day}$ ) as a function of temperature in Wellwood soil, 50, 100, 150, 200 and 300 ppm  $\text{NO}_2^-$ -N.

Temperature ( $^{\circ}\text{C}$ )	Initial Nitrite Concentration (ppm-N)				
	50	100	150	200	300
	$\text{N}_2\text{O}$ ( $\mu\text{g N/g soil/day}$ )				
20.0	3.30	3.45	2.08	1.84	1.92
13.3	1.02	1.18	1.33	1.31	1.38
10.0	0.63	----	0.88	----	0.95
6.0	----	0.67	----	0.71	----
4.4	0.48	0.55	0.69	0.60	0.63
1.4	0.33	0.33	0.33	0.40	0.40
-0.5	0.28	0.32	0.35	0.36	0.38
-1.8	0.27	0.28	0.30	0.30	0.27
-3.2	0.25	0.26	0.35	0.43	0.47
-3.5	0.25	0.28	0.33	0.42	0.58
-4.7	0.23	0.30	0.34	0.41	0.55
-8.5	0.13	0.18	0.23	0.29	0.43
-11.8	0.08	0.15	0.23	0.24	0.33
-18.1	0.05	0.10	0.15	0.16	0.20

$\mu\text{g N/g soil/day}$  at the 300 ppm-N level represents a 4.9% loss of added nitrogen from the system per day.

Arrhenius plots were linear and approximately parallel in all cases except for the samples treated with the 50 ppm-N (Fig 9). The parallel slopes indicate that the same  $\text{N}_2$ -forming reaction was occurring above and below the freezing point. With the 50 ppm-N series, lower production rates of the various gases made accurate calculation of the results more difficult than in cases where gas levels were higher. This was especially true at temperatures near  $0^\circ\text{C}$ . Competitive utilization of nitrite by denitrifying bacteria may have also contributed to some of the error in measurement.

Nitrous oxide production followed the patterns observed in Experiment I. As the nitrite concentration was increased at  $20^\circ\text{C}$ , the rate of  $\text{N}_2\text{O}$  produced increased until 100 ppm-N  $\text{NO}_2^-$  and then decreased as the nitrite concentration increased further. This trend was not evident at lower temperatures. Upon freezing a slight increase in  $\text{N}_2\text{O}$  production was evident at the higher nitrite levels. This was probably due to non-biological processes. The rate of  $\text{N}_2\text{O}$  formation increased as the nitrite concentration increased in each frozen sample but  $\text{N}_2\text{O}$  was not a major cause of nitrogen loss at low temperature over the three incubation period.

When incubations of 100 ppm  $\text{NO}_2^-$ -N in Wellwood were carried out over intervals of one to sixteen days at  $-8.3^\circ$  and  $10^\circ\text{C}$ , the total amounts of  $\text{N}_2$  produced increased over the course of the study (Fig 10). At  $-8.3^\circ$ , the  $\text{N}_2$  production increased linearly with time and the rate decreased only at day 16, probably due to utilization of easily available soil N

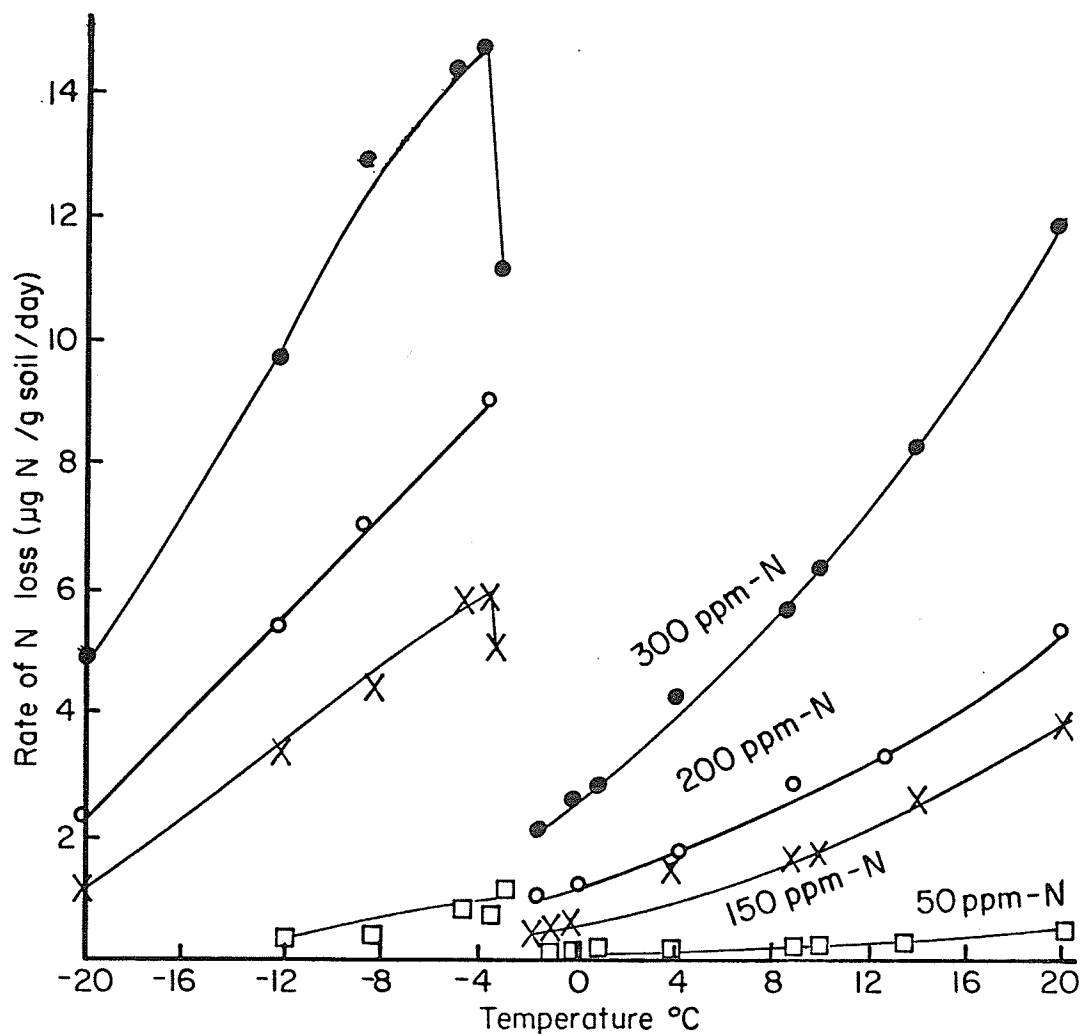


Figure 8: Chemodenitrification rates as a function of temperature in Wellwood soil, 50, 150, 200 and 300 ppm NO<sub>2</sub><sup>-</sup>-N.

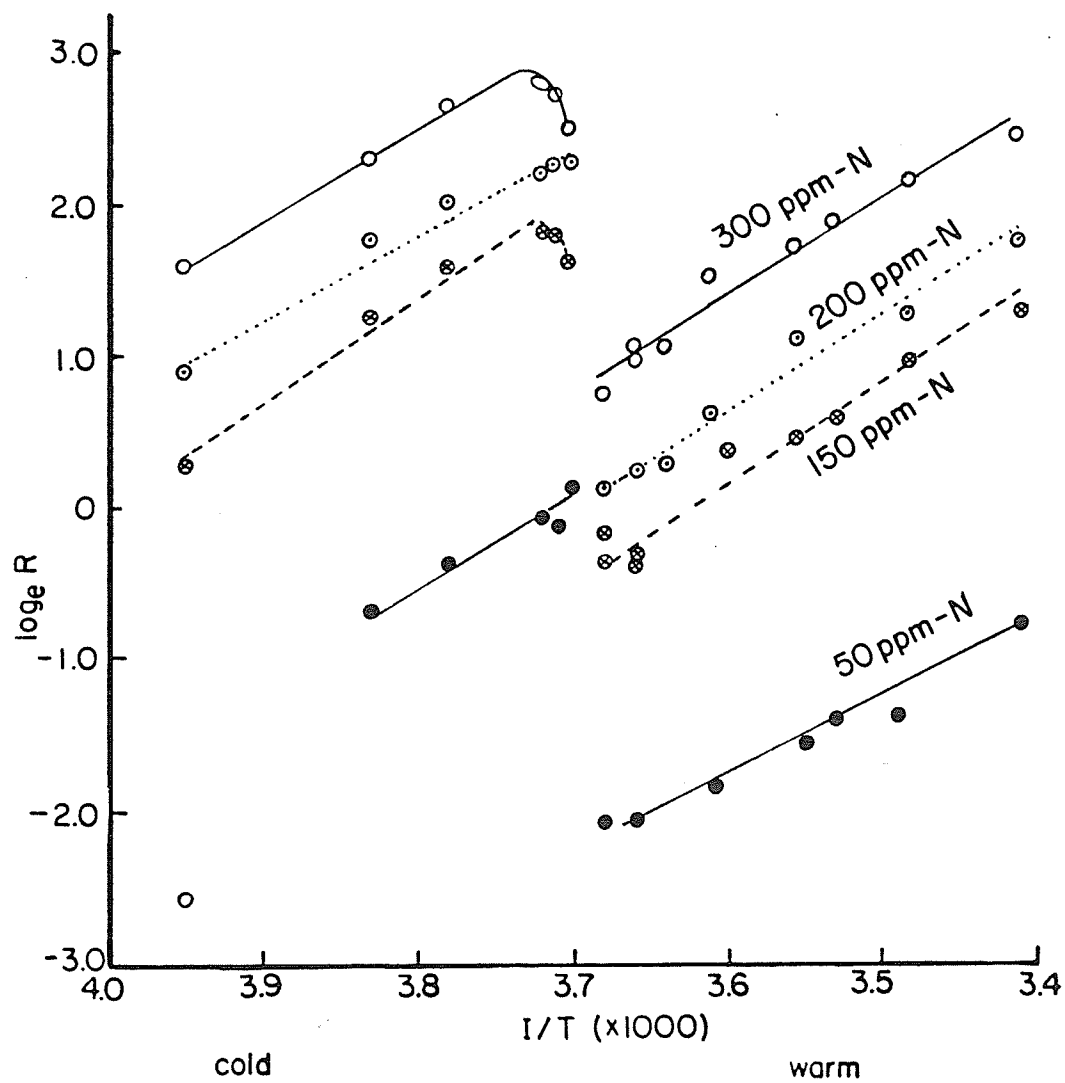


Figure 9: Arrhenius plots of chemodenitrification rates as a function of temperature, 50, 150, 200 and 300 ppm  $\text{NO}_2^-$ -N.

and to the decrease in extractable  $\text{NO}_2^-$  in the system (only 51 ppm-N was left after day 16). The chemodenitrification rate at  $10^\circ\text{C}$  was much lower than that at  $-8.3^\circ$ . Although soils in both treatments of this experiment had 100 ppm  $\text{NO}_2^-$ -N initially, freeze concentration occurred in the  $-8.3^\circ$  sample and enhanced the denitrification rate. Cumulative losses at  $10^\circ$  also increased steadily over the incubation period to a maximum of 2.9 ug N/g soil by day 16.

These results show that denitrification occurred at a steady rate as long as sufficient  $\text{NO}_2^-$  and soil N (which were responsible for the reaction) were present. The steady rate of the reaction indicates that it may be controlled, to a degree, by the rate of diffusion of nitrite to active sites in the soil. Otherwise, the rate would have declined gradually since concentration of nitrite was decreased gradually as the reaction progressed.

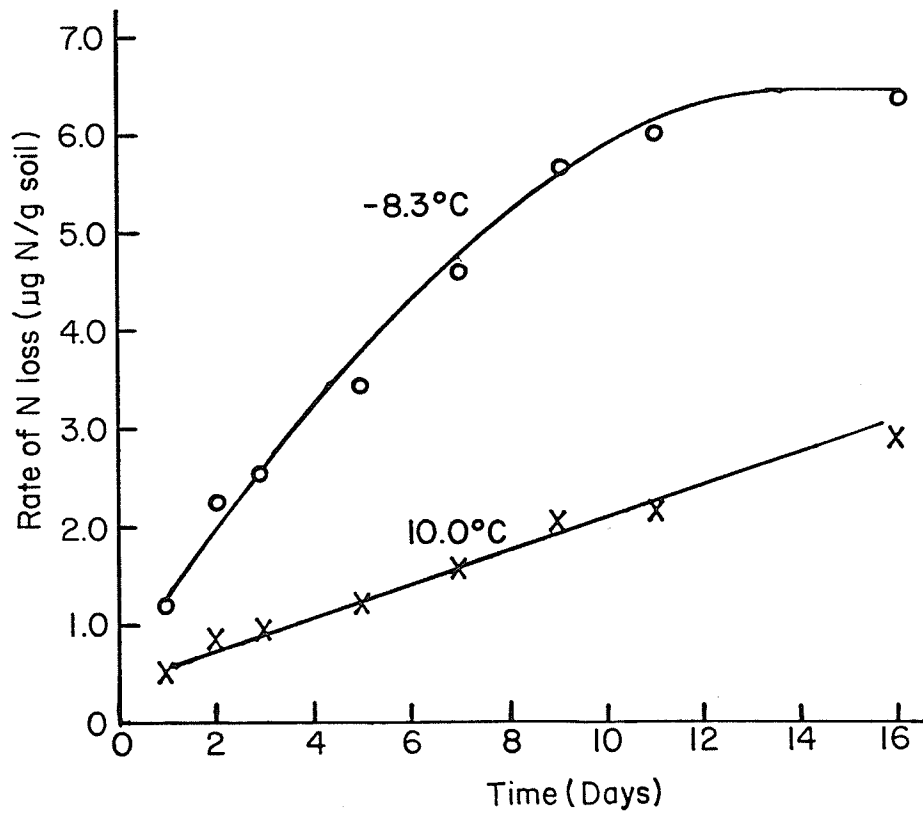


Figure 10: Rate of N loss in Wellwood soil treated with 100 ppm-N as affected by time and temperature of incubation.

### 3.2.2 Part 2 Sterile Soil

Steam sterilization had little effect on the rate of denitrification of nitrite in Wellwood soil (Fig 11). Losses of  $N_2$  were comparable, though slightly lower, than those of unsterilized soils of earlier experiments (Fig 6). The rate of loss of N decreased as temperature decreased from  $20^{\circ}$  to  $0^{\circ}C$ . A sharp increase in denitrification rate similar to that found in studies conducted with nonsterile soil, occurred at the freezing point. Lowering of temperature below the freezing point again resulted in a decrease in denitrification rate.

These results confirmed that the  $N_2$  was formed nonbiologically by a van Slyke type of reaction. The slightly lower rate of  $N_2$  production with sterile as compared to the nonsterile samples at comparable temperatures may have been due to alteration of organic matter by the rather vigorous sterilization procedures. Autoclaving twice for such periods of time may have decreased the amount of labile N available to react with the  $NO_2^-$  to produce the  $N_2$  gas.

Results of the spread plating suggest that the bacterial population was greatly suppressed. Plating the  $10^{-3}$  dilution soil yielded three to ten colonies per plate, numbers too low to be counted significantly. Colonies present may have been due to contamination from the air or survival of some spore-forming species. Since the presence of  $10^8$  bacteria per gram of soil is not unusual (Gray and Williams, 1971), the microbial population would have had to have been much greater than was measured if it were to have an important role in denitrification in this experiment.

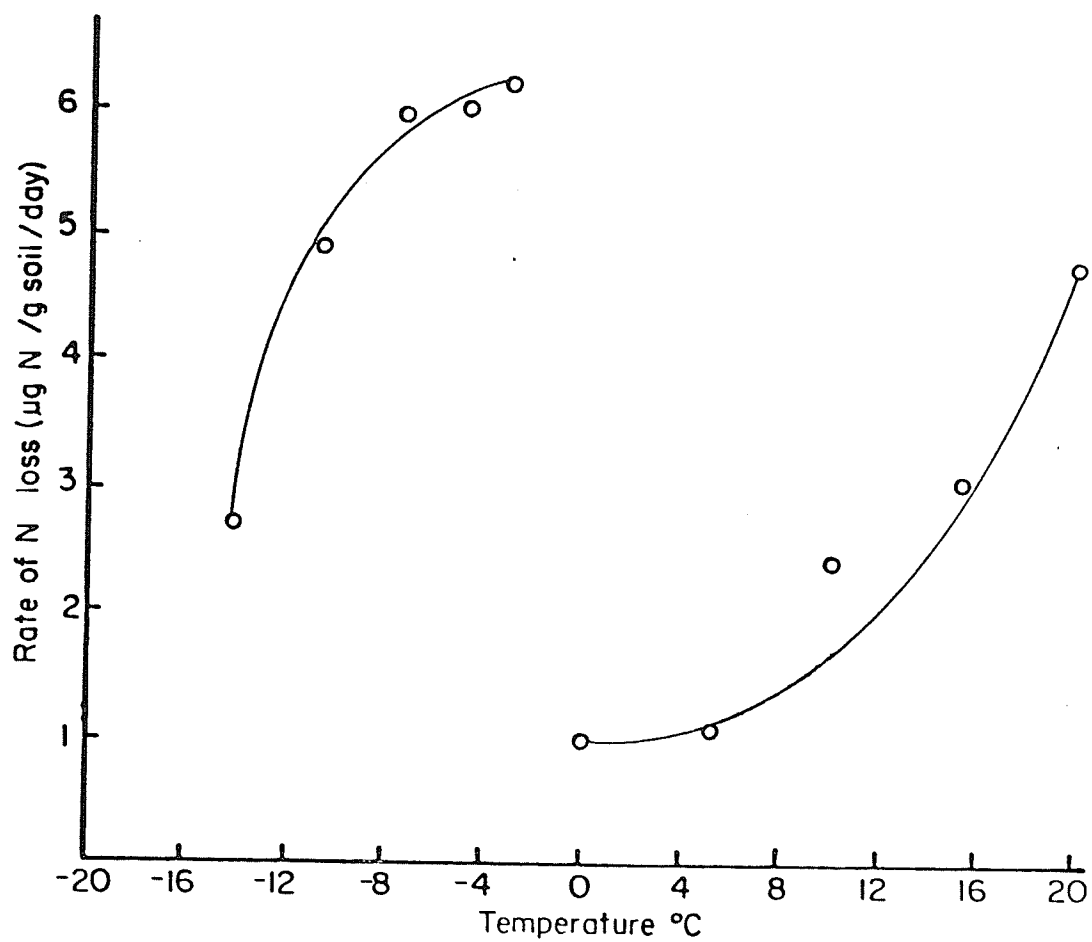


Figure 11: Effect of temperature on the denitrification of 200 ppm-N nitrite in autoclave-sterilized Wellwood soil.



### 3.3 EXPERIMENT III EFFECT OF MOISTURE CONTENT ON DENITRIFICATION

#### 3.3.1 Effect of Dilution of Nitrite Concentration in Soil Solution upon Chemodenitrification.

In the first part of this experiment, the amount of  $^{15}\text{N}$ -nitrite in the system was kept constant on a soil basis at  $1.0 \mu\text{g N/g soil}$  (100 ppm-N) and only the amount of water added to the samples was varied. This addition of varying amounts of water to a fixed amount of nitrite changed the actual concentration of the N source in the soil solution. Since concentration of nitrite and  $\text{N}_2$  production are directly related, this dilution of the nitrite would be expected to result in a decrease in denitrification rate.

At  $10^\circ\text{C}$ , the rate of  $\text{N}_2$  production was  $3.73 \mu\text{g N/g soil/day}$  at 11% moisture but it decreased to  $0.18 \mu\text{g N/g soil/day}$  at 50% moisture (Fig 12). The moisture content of 11% was slightly less than half the moisture levels in Experiment II (25%) and therefore the denitrification rate of the 100 ppm-N sample at 11% moisture should be slightly greater than that of 200 ppm-N at 25%. The rate of loss from the 200 ppm-N sample of Experiment II at  $10^\circ\text{C}$  was  $3.10 \mu\text{g N/g soil/day}$ . Similarly the rate at 100 ppm-N and 50% moisture should compare with 50 ppm-N at 25% moisture of Experiment II (0.25 vs 0.41). Thus it was demonstrated that the concentration of  $\text{NO}_2^-$  in soil solution was the parameter which controlled the rate of chemodenitrification in the Wellwood soil.

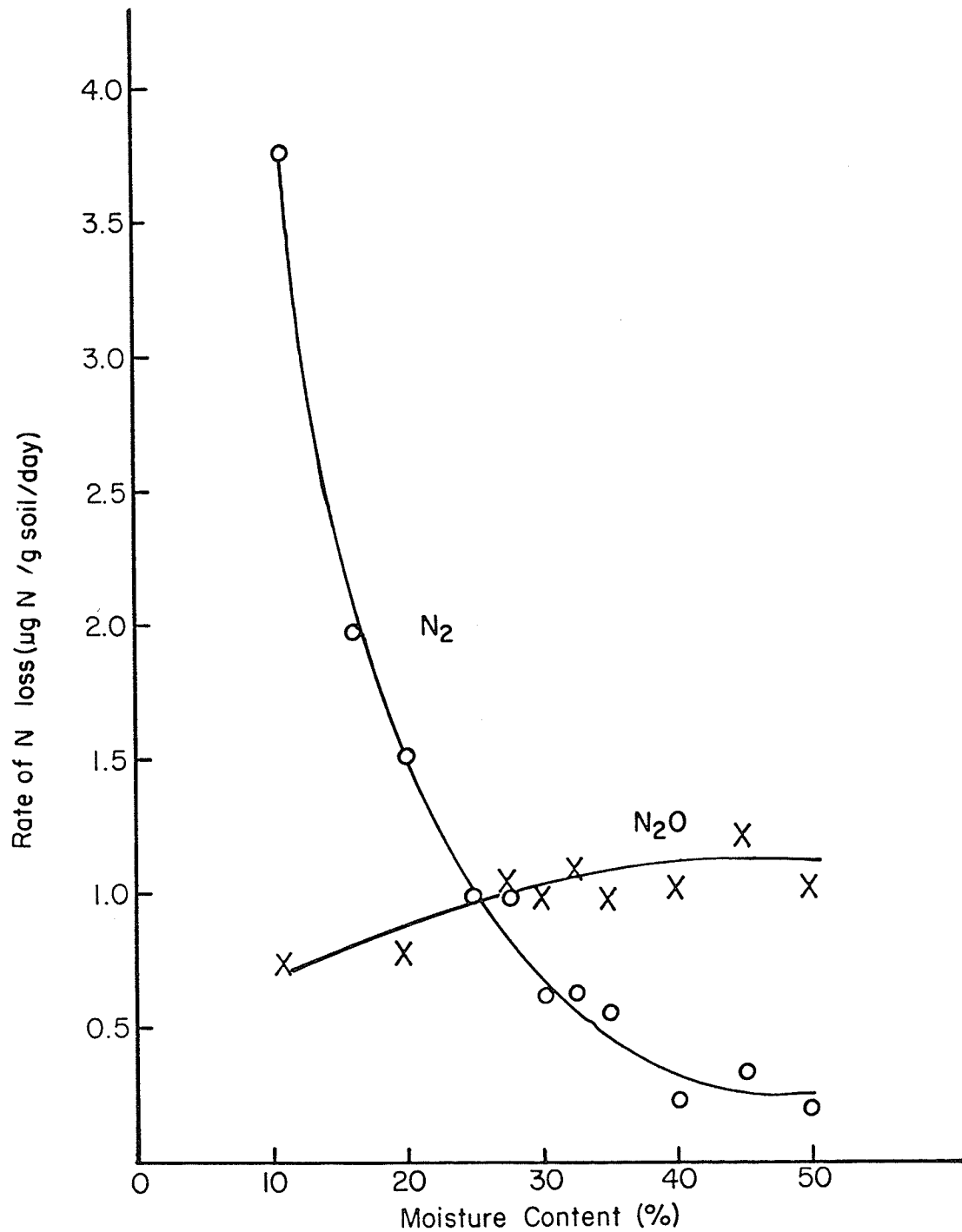


Figure 12: Effect of dilution of nitrite concentration in soil solution on denitrification rate in Wellwood soil at 10°C.

Nitrous oxide production increased slightly as the moisture content increased (Fig 12). This was probably due to increasing biological activity as the moisture increased from 11% to 25% resulting in more anoxic conditions in the soil column and therefore more denitrification. Further increases in moisture resulted in minor increases in rate of  $N_2O$  production. This was probably due to decreased oxygen transport in the soil column caused by filling of pore spaces with water.

The nitrate data in Table 5 support the speculation regarding  $N_2O$  production mentioned above. Nitrate levels were fairly constant as moisture content was increased from 11% to 20%. Once field capacity of the Wellwood soil was exceeded (moisture contents in excess of 24.6%), nitrate levels were decreased. Moisture in excess of field capacity (25% to 50%) may have caused inhibition of oxygen diffusion in the soil, allowing conditions for biological denitrification to develop.

Measured nitrite levels (on a soil basis) increased as moisture increased due to the decline in chemodenitrification rates as nitrite dilution occurred.

TABLE 5

Effect of soil moisture on denitrification of 100 ppm-N nitrite incubated with Wellwood soil at 10°C for three days.

Moisture Content ( % )	Nitrite-N (ppm)*	Nitrate-N (ppm)*
11.4	63.3	15.2
16.5	75.7	16.3
20.0	69.7	16.7
25.5	76.7	9.8
28.0	80.3	10.0
30.1	85.2	14.7
32.5	79.2	9.5
35.0	85.4	7.1
40.0	82.7	6.0
45.0	82.7	4.8
50.0	83.9	5.9

\* concentration on a soil basis

### 3.3.2 Effect of Soil to Solution Ratio upon Chemodenitrification

In the previous experiment (3.3.1), the nitrite concentration on a soil basis was kept constant and concentration of nitrite in the soil solution was varied. In this investigation, the nitrite concentration was kept constant in the soil water and the amount of nitrite solution associated with the colloidal surface was varied. This was done in order to see if denitrification rate was really independent of total nitrite content on a soil basis. Over the short interval of this experiment nitrite depletion should have been relatively low. Thus, the concentration of nitrite in the soil solution in the nonfreezing portion of the curve should have remained similar even though the amounts of solution were different.

When the incubation temperature was above freezing (Fig 13), the rate of  $N_2$  formation for all moisture contents decreased as the temperature decreased to  $0^{\circ}C$ . The amounts produced at each temperature were very similar irrespective of initial moisture level. This was probably due to the fact that the rate of denitrification was limited to some degree by the rate of diffusion of nitrite from the soil water to the active surface on the colloid. Diffusion rates are not greatly affected by the thickness of a solution layer near the colloid surface, only by the concentration of the solution. Since, in this experiment, the concentration of the solution was initially the same for each sample and would probably not have decreased significantly over the incubation interval, the rate of denitrification should have also remained constant. However, the lower rate at  $20^{\circ}C$  for the 7% moisture sample may have been due

to a partial decrease in  $\text{NO}_2^-$  concentration due to greater microbial activity as well as partial depletion of  $\text{NO}_2^-$  by  $\text{N}_2$  production. Since the initial nitrite concentration was quite low on a soil basis, a slight removal of  $\text{NO}_2^-$  would have a more pronounced effect with the 7% moisture sample than those at a higher moisture content.

Upon freezing, the denitrification rates at each moisture content increased. The increase was greatest in the sample with 22% moisture and decreased as the initial moisture content decreased (Fig 13). The sample with 7% moisture showed a negligible increase in N loss rate upon freezing which may have been due to only a small amount of the soil water actually freezing. An unfrozen water content in the range of five to six percent would have resulted in little freeze concentration and therefore a small increase in denitrification rate for this moisture treatment (7%).

When the soil samples were frozen, the original amount of nitrite solution associated with the colloid was an important factor in the denitrification rate. The amount of frozen water at any specific temperature below freezing depends very little on the initial moisture content of the sample (Tice et al., 1978). Therefore, after freezing each sample would have had approximately the same unfrozen water content, irrespective of the original soil water content. The greater the original thickness of the nitrite solution layer around the soil particles, the greater was the final concentration in the brine layer after freezing and, the greater the nitrite concentration in the brine, the higher was the rate of  $\text{N}_2$  formation at any freezing temperature.

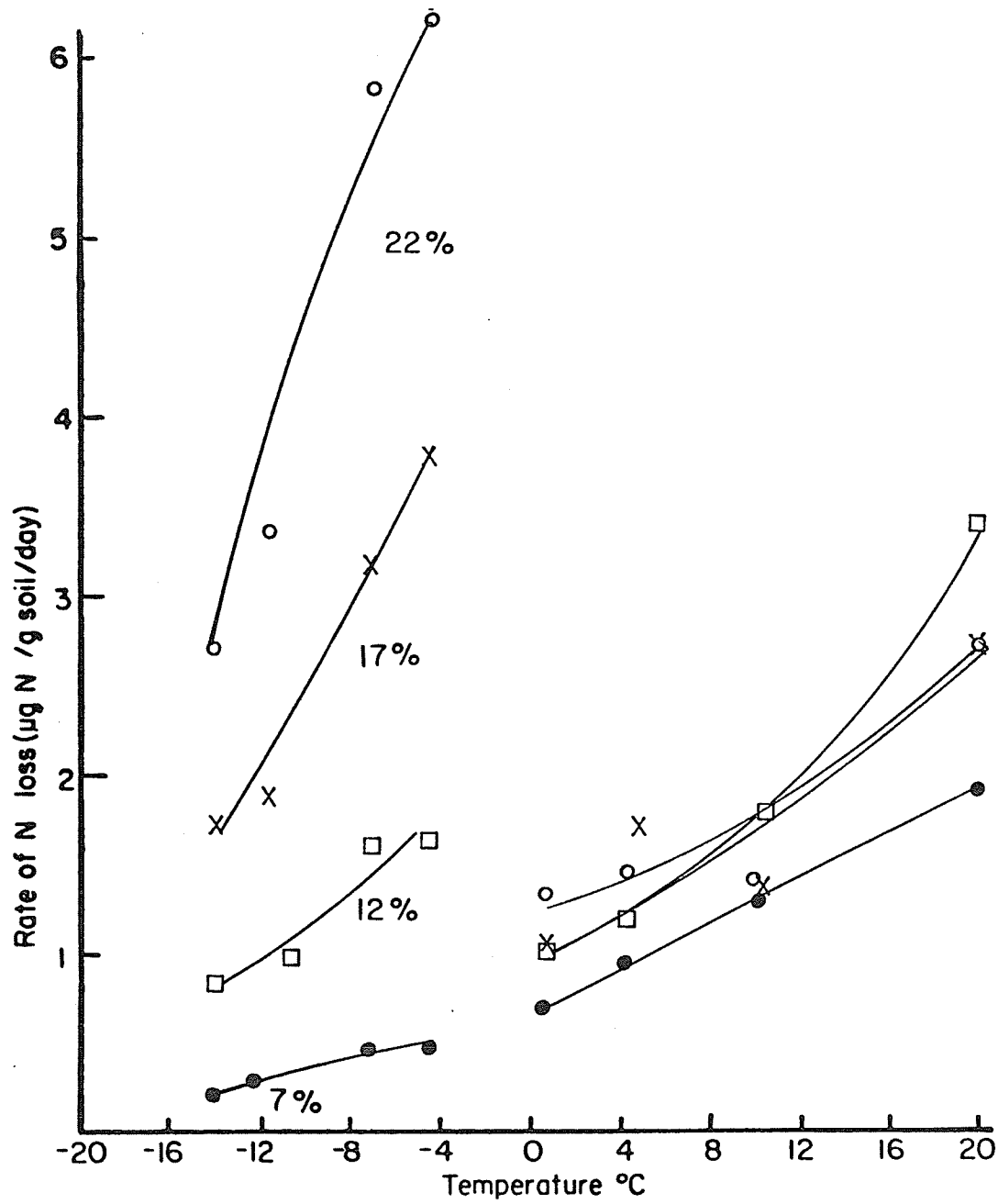


Figure 13: Effect of soil to solution ratio and temperature on denitrification of nitrite in Wellwood soil.

### 3.4 EXPERIMENT IV ROLE OF SOIL CHARACTERISTICS ON DENITRIFICATION RATES UPON FREEZING

This experiment was performed to determine if the chemodenitrification phenomenon that occurred with the Wellwood soil was unique to that soil alone. For this reason, five soils of differing pH and texture were studied at an initial nitrite concentration of 100 ppm-N.

Each soil produced a different amount of  $N_2$  gas after additions of equal amounts of nitrite (Fig 14, Carroll results not shown). The Keld sample, with its low pH and high organic matter content, produced the most  $N_2$  at temperatures above freezing with a yield of 5.2  $\mu\text{g N/g soil/day}$  at  $20^\circ\text{C}$ . The Pine Ridge sample, with the lowest organic matter content and a slightly higher pH, produced small amounts of  $N_2$  and reached a maximum rate of 0.8  $\mu\text{g N/g soil/day}$  at  $20^\circ\text{C}$ . Wellwood was intermediate between these values. In all cases, the denitrification rate decreased as the temperature decreased to  $-0.6^\circ\text{C}$ . Stockton soil produced the lowest amount of  $N_2$ , probably due to its high pH of 6.9. Carroll soil yielded no  $N_2$  production at any temperature.

Upon freezing, all soils showed an increase in rate of nitrogen loss. A drop in temperature from  $-0.6^\circ\text{C}$  to  $-4.0^\circ\text{C}$  increased the rate of loss by 250% with Keld, 670% with Wellwood and 3500% with the Pine Ridge samples. Although an increase in nitrogen loss rate was noted in the Stockton samples also, the total amounts of  $N_2$  produced were still very much lower than those of the other three soils of Figure 14. Further lowering of the incubation temperatures caused a decrease in denitrification rates in all soils. Since denitrification rates were so low in



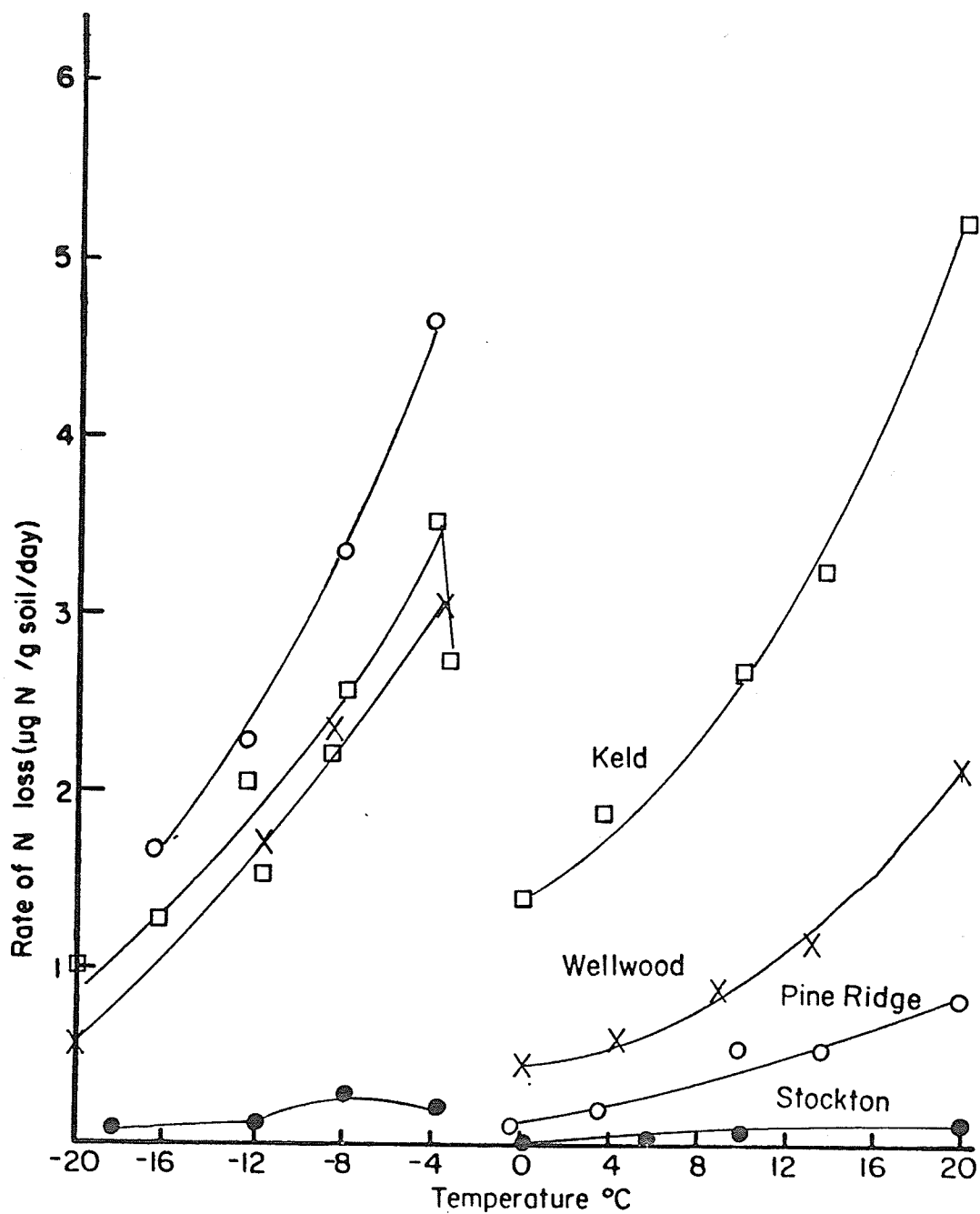


Figure 14: Chemodenitrification of 100 ppm-N nitrite as a function of temperature in Wellwood, Keld, Pine Ridge and Stockton soils.

the Stockton and Carroll soils, only Keld, Wellwood and Pine Ridge soils were used in subsequent experiments. The difference in the increases in rate of  $N_2$  loss at  $-4^{\circ}C$  appeared to be related to soil texture.

The amount of unfrozen water in a soil at any temperature depends to a very large degree on the physical characteristics of the soil in question. Moisture retention capacity is also a function of specific surface area. Since unfrozen water and moisture retention characteristics are interrelated and depend in a similar manner on surface area, a moisture retention study was carried out in an attempt to predict potential unfrozen water. Retention curves were determined for Keld, Wellwood and Pine Ridge soils.

At each suction value, the moisture retention curves indicated that Keld (sandy clay loam) held more water than the Wellwood (fine sandy loam) which held more moisture than the coarse Pine Ridge sample (loamy fine sand)(Fig 15). All three soils lost a great deal of water between pF 2.0 and 2.65, an indication of the high percentage sand in the samples. The presence of 22% clay in the Keld and 16% in Wellwood resulted in the retention of water which was removed only at high suction. The Pine Ridge samples possessed only 6% clay and therefore lost almost all the water at the relatively low suction of pF 2.65. From these moisture retention curves it was expected the Keld soil would have the most unfrozen water at any temperature and Pine Ridge the least, with Wellwood in an intermediate position.

The Keld soil with its high moisture retention capacity and clay content would be expected to have the most unfrozen water at  $-4^{\circ}C$ . This resulted in the lowest degree of freeze concentration and therefore the smallest increase in rate of loss upon freezing.

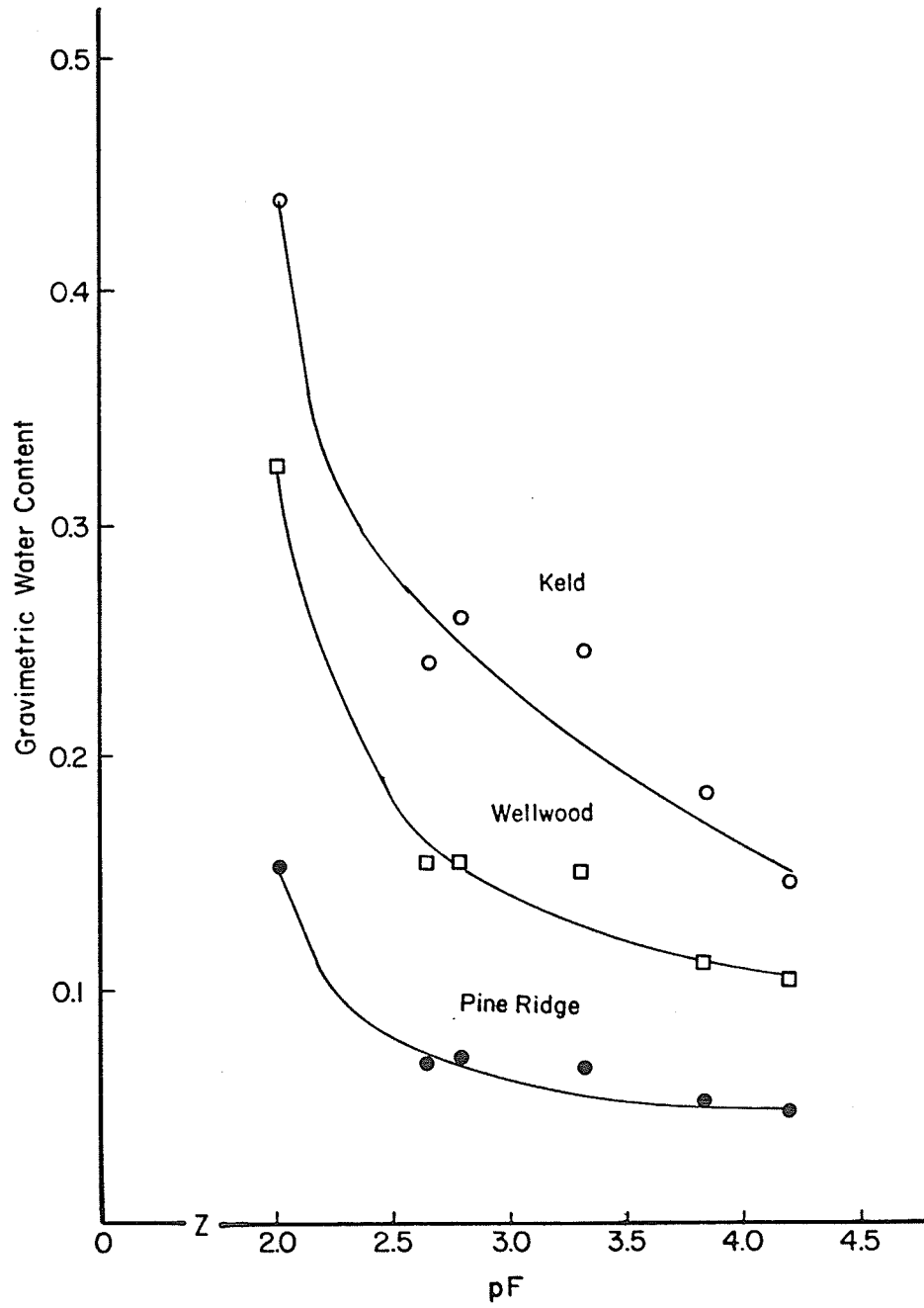


Figure 15: Moisture retention curves of Keld, Wellwood and Pine Ridge soils.

The Wellwood sample, which held water less tightly due to its lower clay content, would be expected to have less unfrozen water at each temperature as compared to Keld soil. This resulted in more freeze concentration leading to a more pronounced increase in denitrification rate upon freezing than that of Keld.

The most dramatic increase in denitrification due to freezing occurred with the Pine Ridge sample. The rise in rate of  $N_2$  loss was a direct function of the low surface area of the sample and therefore low unfrozen water content. Freezing of the moisture in Pine Ridge caused a much more pronounced freeze-concentration effect and therefore a great increase in denitrification. The rate at  $-4^{\circ}C$  was 5.8 times greater than that at  $20^{\circ}C$  with Pine Ridge soil. Measurement of  $N_2$  production from the Pine Ridge samples near  $0^{\circ}C$  was made difficult by the very low amounts of gas produced and might have been subject to error. The amounts of  $N_2$  produced at  $20^{\circ}C$  might have been low due to competition for nitrite by denitrifying bacteria. Nitrous oxide losses were high at the 100 ppm-N  $NO_2^-$  additions.

Denitrification trends of the samples treated with 100 ppm-N (Fig. 14) were repeated with the treatments at 200 and 300 ppm-N (Figs 16 and 17). The ratios between the denitrification rates at  $-0.6^{\circ}$  and  $-4.0^{\circ}C$  were not the same as those for the 100 ppm-N treatment, probably due to less pronounced errors in measurement for the 200 and 300 ppm-N treatments as discussed above. Pine Ridge continued to be the lowest producer above  $0^{\circ}C$  although production rates for all three soils were similar below  $0^{\circ}C$ .

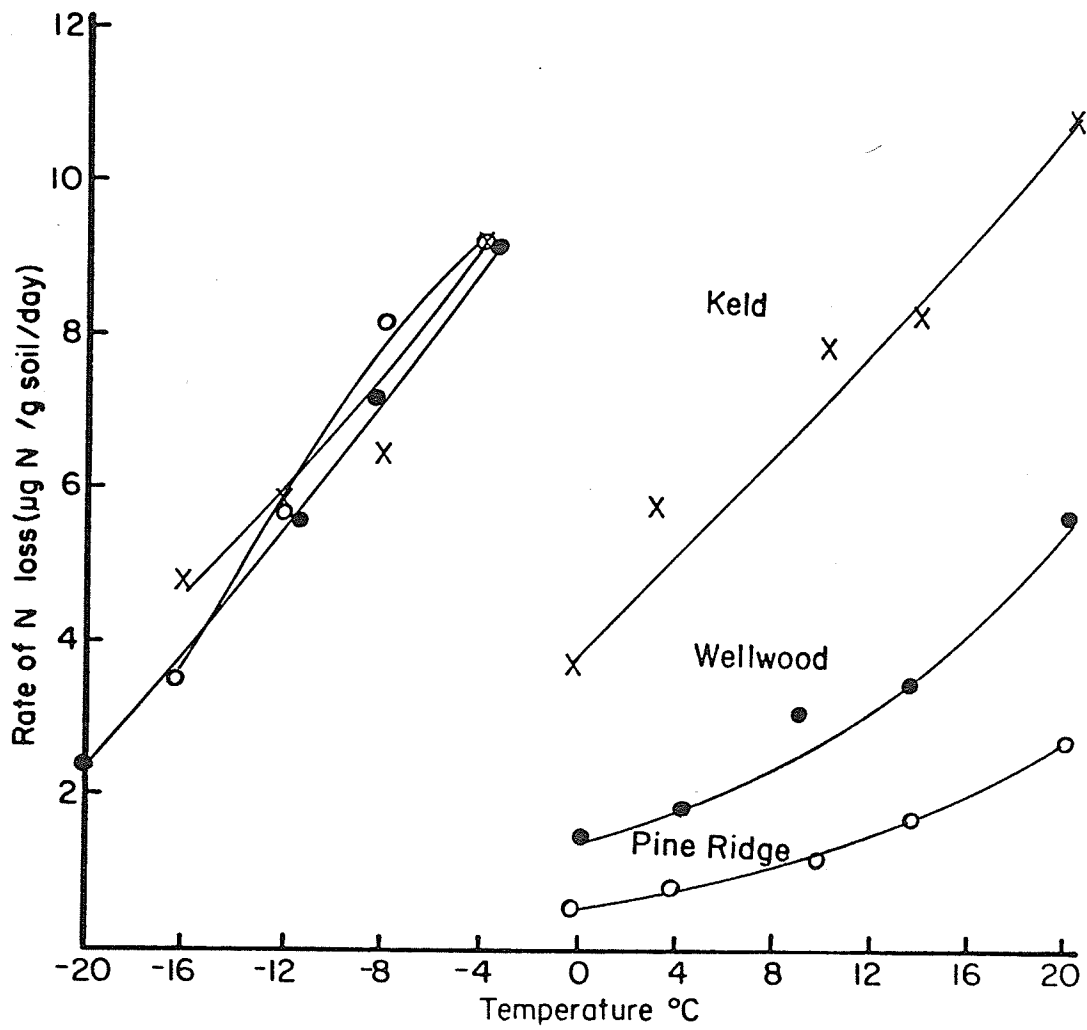


Figure 16: Chemodenitrification of 200 ppm-N nitrite as a function of temperature in Wellwood, Keld and Pine Ridge soils.

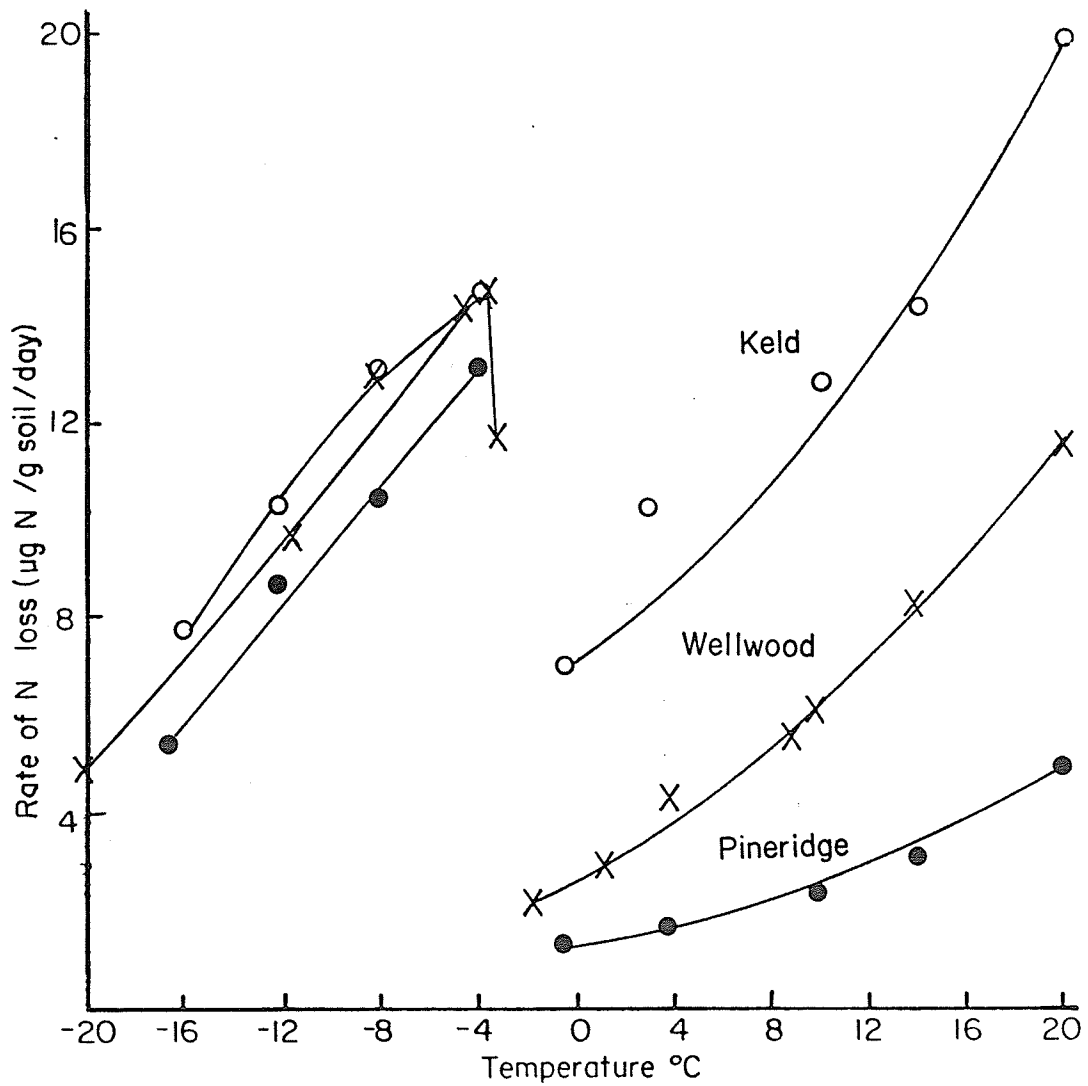


Figure 17: Chemodenitrification of 300 ppm-N nitrite as a function of temperature in Wellwood, Keld and Pine Ridge soils.

Arrhenius plots had parallel slopes at each nitrite concentration both above and below the discontinuity as did the Wellwood samples of Experiment II (Figs. 18 and 19). This indicates that the same  $N_2^-$  producing reaction was occurring both below and above freezing. The difference in slopes between soils was a function of different physical characteristics of each soil such as pH and organic matter content.

If it is assumed that very little trapping of nitrite and soluble organic matter occurred during ice formation, it is possible to estimate the amount of unfrozen water remaining in the soil. The slopes and intercepts of the Arrhenius plots were used to calculate what the rate of  $N_2$  production should have been at each temperature. Since freezing is a discontinuous function, data were extrapolated to  $0^\circ\text{C}$  from both the frozen and unfrozen sections of the graphs. The ratio of these calculated rates at  $0^\circ$ , minus a threshold rate, were compared and used to give an approximation of the unfrozen water content in the soils (Table 6).

Since the increase in rate was assumed to be due to complete freeze concentration, a doubling of the rate should have been due to a concentration of nitrite to approximately one half the original volume thereby yielding an unfrozen water content of 12.5% (25% was the original). The nitrite content in the soil water after freezing in Wellwood soil was determined using equation (39) while that for the Keld and Pine Ridge soils was determined assuming a strictly first order reaction. Considering the crudeness of the calculations, the calculated unfrozen water contents are remarkably similar within a soil type. As expected from Fig 15 the coarser soils appear to have the lowest unfrozen water content, accounting for the great rise in rate of loss of  $\text{NO}_2^-$  upon freez-

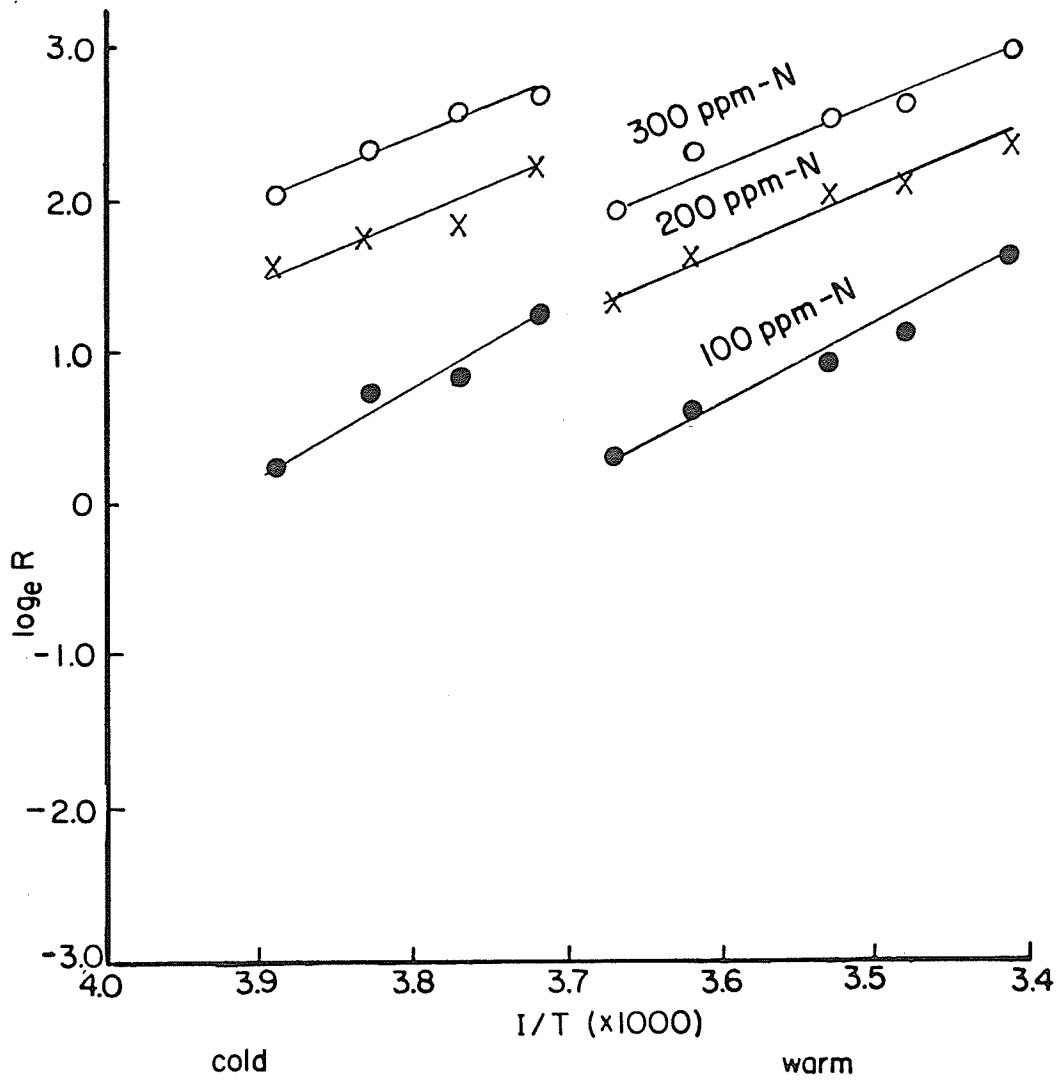


Figure 18: Arrhenius plots of chemodenitrification rates in Keld soil at 100, 200 and 300 ppm  $\text{NO}_2^-$ -N concentrations.



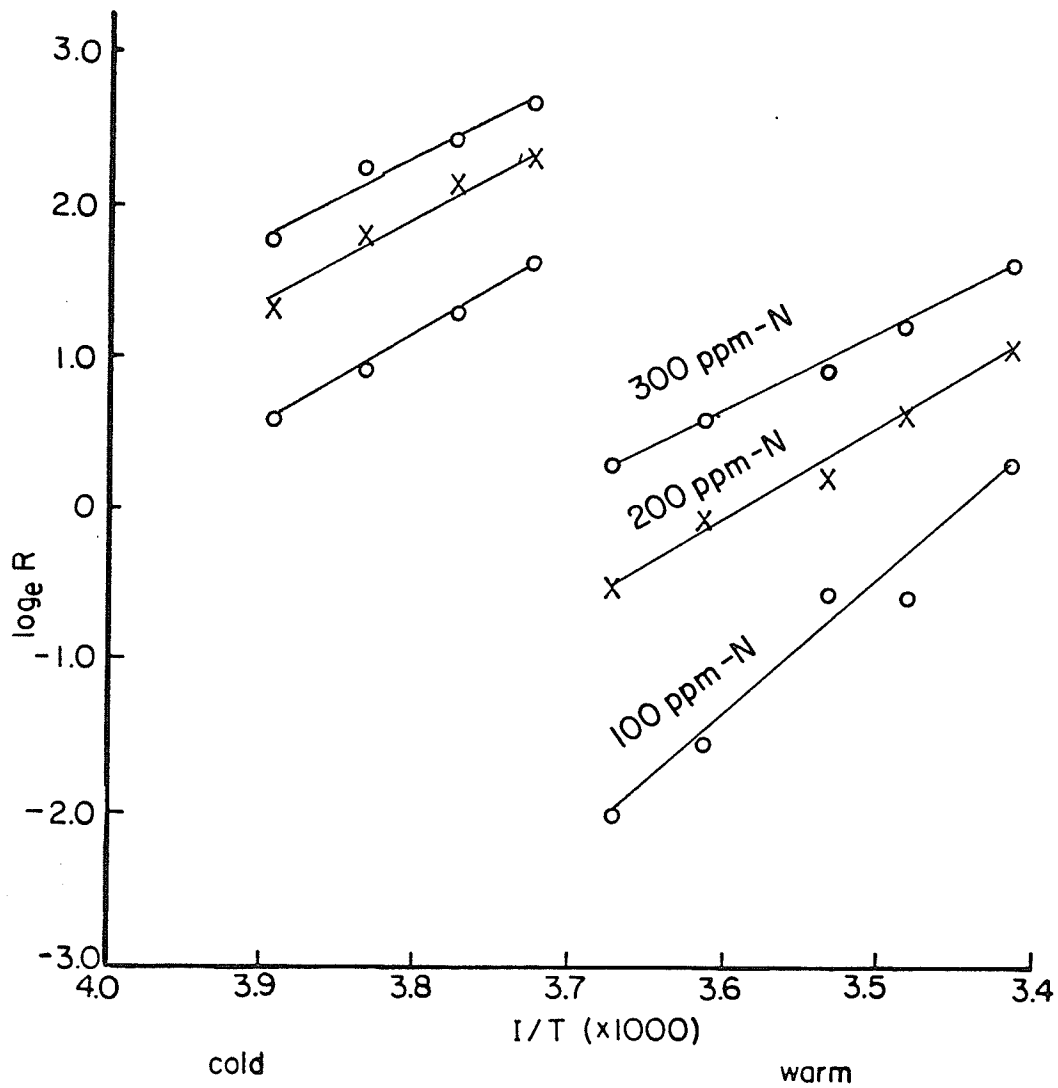


Figure 19: Arrhenius plots of chemodenitrification rates in Pine Ridge soil at concentrations of 100, 200 and 300 ppm  $\text{NO}_2^-$ -N.

TABLE 6

Calculated unfrozen water contents of three soils

nitrite*	Keld		Pine Ridge		Wellwood	
	ratio**	Wu(%)	ratio**	Wu(%)	ratio**	Wu(%)
100	1.82	13.74	10.93	2.28	5.65	4.43
150	-	-	-	-	7.05	3.55
200	2.35	10.64	14.57	1.72	6.99	3.57
300	1.99	12.55	11.29	2.21	6.75	3.70
average		12.31		2.07		3.81

\* concentration (ppm-N).

\*\* ratio of the rate between frozen and unfrozen states.  
Wu unfrozen water content.

ing. The Keld soil exhibited a much smaller increase in denitrification rate upon freezing and hence had a lower calculated unfrozen water content.

Despite the extensive losses of nitrite-N from these soils it must not be assumed that such losses will occur in all soils. When a similar experiment was performed with Stockton soil very little denitrification occurred at any temperature (Fig 14). The data points were somewhat scattered due to measurement error caused by the very low rates of  $N_2$  production. The higher pH of this soil as compared with the others used in the first part of this experiment may have been an important factor in the low denitrification rate. A fifth soil (Carroll clay loam, pH 7.1 and organic matter 5.1%) also failed to denitrify nitrite when tested in the same manner as the other soils with 100 ppm-N  $NO_2^-$  over the same temperature range.

Christianson (1978) showed that as the pH of Wellwood soil was increased, chemodenitrification rates decreased. Similar trends were recorded with Keld soil in the present study (Table 7). Increasing the pH of Keld soil did decrease denitrification greatly from 4.5  $\mu\text{g N/g soil/day}$  at pH 5.4 to 0.9  $\mu\text{g N}$  at pH 6.2 and 0.15  $\mu\text{g N/g soil/day}$  at pH 7.1, illustrating the negative effect of elevated pH on chemodenitrification of nitrite. Other factors such as amount and type of organic matter play a role in chemodenitrification but these facets were not pursued in this project.

TABLE 7

Effect of pH on Denitrification Rates in Keld Soil.

Soil pH	Denitrification Rate $\mu\text{g N/g soil/day}$
5.4	4.5
6.2	0.9
7.1	0.15

The results of Experiment IV were important for three reasons. First, they showed the great importance of relative surface area on potential changes in denitrification rate upon freezing (i.e. there was a smaller increase with soils high in clay content than those which were more sandy). Secondly, they indicated that a soil in which nitrite was quite stable at 20°C lost nitrite as  $\text{N}_2$  upon freezing (for example, very

sandy soils such a Pine Ridge). Finally, they indicated that  $\text{NO}_2^-$  was stable in some soils at all temperatures, especially in soils of high pH.

## Chapter IV

### SUMMARY AND CONCLUSIONS

The basic goal of this project was to determine the fate of nitrite in slightly acid soils as temperature was lowered from 20° through freezing to -18°C. At every temperature studied, nitrite was found to denitrify via a van Slyke-like mechanism wherein one nitrite N atom combined with one soil N atom to yield N<sub>2</sub> gas. Each molecule of N<sub>2</sub> gas thus had exactly half its atoms from the fertilizer and half from the soil N pool.

Nitrite was added to Wellwood soil at rates varying from 25 to 300 ppm-N to allow determination of the kinetics of the van Slyke-like soil N- NO<sub>2</sub><sup>-</sup> reaction. Incubation results showed an approximately first order mechanism with a threshold nitrite value of approximately 27 ppm-N required before the reaction would take place at a measureable rate in three days. The maximum rate of loss of 3.7% of the added N per day occurred with the sample treated with 300 ppm-N NO<sub>2</sub><sup>-</sup>. Nitrous oxide production in the same experiments reached a maximum of 4.8 µg N/g soil/day as the initial nitrite concentration was increased from 25 to 75 ppm-N. Further increases in soil nitrite concentrations brought about decreased N<sub>2</sub>O production rates. This was due to decreased microbial activity as evidenced by lower O<sub>2</sub> consumption and CO<sub>2</sub> production rates. The production rate of N<sub>2</sub> from N<sub>2</sub>O was low but constant. The important result of this experiment was the finding that the rate of N<sub>2</sub> production was almost directly related to NO<sub>2</sub><sup>-</sup> content in the soil solution. The fact

that the denitrification of  $\text{NO}_2^-$  to  $\text{N}_2$  did not follow Michaelis-Menten kinetics but was rather a first order reaction illustrating the chemical nature of the van Slyke  $\text{N}_2$  production mechanism.

When the incubation temperature was decreased from  $20^\circ\text{C}$  to  $-1.8^\circ\text{C}$ , the rate of  $\text{N}_2$  production decreased as was predicted by the Arrhenius equation. As incubation temperatures were lowered still further, the soil water froze and the rate of denitrification increased dramatically to rates higher than those at  $20^\circ\text{C}$ . Nitrous oxide production decreased to insignificant levels as the temperature was lowered and did not undergo the upturn that  $\text{N}_2$  production did as soil freezing occurred.

The increase in denitrification upon freezing of the soil solution was believed to have been due to freeze concentration of the nitrite. When the soil solution froze, pure water froze out leaving a highly concentrated soil nitrite solution in an unfrozen water film near the active surface of the colloids. This, in turn, had a direct positive effect on chemical denitrification rates as described in the first experiment. Further proof of the chemical nature of the van Slyke reaction was the measurable production of  $\text{N}_2$  at  $-18^\circ\text{C}$  whereas most biological reactions cease at  $2^\circ\text{C}$ . Continued production at these low temperatures ruled out the activity of even the most hardy psychrophiles. A similar experiment with 200 ppm-N nitrite and autoclave-sterilized Wellwood soil over the temperature range of  $20^\circ$  to  $-16^\circ\text{C}$  gave similar results to those of the nonsterile sample showing that soil bacteria were not directly involved in the  $\text{N}_2$  production of this study.

Arrhenius plots of Wellwood soil data at concentrations of 50 to 300 ppm-N and 25% moisture over the temperature range of  $+20^\circ$  to  $-18^\circ\text{C}$  gave

approximately parallel slopes indicating that the same reaction was responding to temperature in the same manner except at the major discontinuity near the freezing point.

When the soil moisture was decreased from 25 per cent without changing the nitrite concentration on a soil basis, denitrification rates increased. This was due to an increase in  $\text{NO}_2^-$  content in the soil water of the system. Conversely, increasing soil moisture diluted out the nitrite causing a reduction in  $\text{N}_2$  production.

Changing the soil water volume without changing the soil water  $\text{NO}_2^-$  concentration had little effect on denitrification rates from  $20^\circ$  to  $-1.8^\circ\text{C}$ . However, once the soil water had frozen, the unfrozen water content became similar in all soils regardless of the original quantity of the  $\text{NO}_2^-$  solution and amount of soil water originally present. The soils which originally had the thickest solution layer around the soil particles had the highest nitrite concentration in the unfrozen water layer. These soil samples exhibited a much higher  $\text{N}_2$  production rate than did soils which originally had a lower moisture content.

Incubation of five soils of varying pH and texture at temperatures above freezing with 100 ppm-N as  $\text{NO}_2^-$  and 25% moisture resulted in high denitrification rates for Keld, moderate rates for Wellwood and quite low rates for Pine Ridge, a reflection of soil pH and organic matter contents. The two soils with neutral pH values (Carroll and Stockton) showed little denitrification. Upon freezing, the Pine Ridge soils showed a very dramatic increase in denitrification rate due to the great degree of freeze concentration occurring in the soil. Keld showed a slight rise in denitrification rate while Wellwood was intermediate between the two.

A moisture content versus soil suction curve was constructed for each soil to give a measure of the affinity of each soil for water and indirectly, each soil's surface area. Keld soil had a higher moisture content at each suction than Wellwood which, in turn, held water more strongly than Pine Ridge. Soils with the highest surface area were expected to have the greatest unfrozen water content at any temperature which should result in a lower degree of freeze-concentration and therefore a lower rise in denitrification upon freezing.

The great change in denitrification rate for Pine Ridge upon freezing was due to its very sandy composition which allowed very little soil water to remain unfrozen. This caused a high degree of freeze concentration of nitrite and therefore subsequently, a high amount of  $N_2$  was produced. Keld had a lower sand content and from the suction data was expected to have a high unfrozen water content. This caused a lower degree of freeze-concentration which led to a less pronounced jump in rate of N-loss upon freezing. Wellwood, being intermediate in texture, was also intermediate in the degree of freeze-concentration that occurred. Similar trends were noted with 200 and 300 ppm  $-N NO_2^-$  in each of these soils.

When the pH of Keld soil was raised by the addition of carbonate, the losses recorded at pH 7.1 were only 16% of those at pH 5.4, indicating the importance of soil pH.



The following conclusions were drawn from this investigation:

1. a van Slyke-like reaction for the production of  $N_2$  was active in acid soils and was not of a biological nature.
2. the van Slyke reaction was approximately first order with respect to concentration of nitrite in the soil solution.
3. the van Slyke reaction rate was greatly enhanced when some soils were frozen due to freeze concentration of the nitrite in the unfrozen boundary layer.
4. soils with low specific surface area exhibited a more pronounced enhancement of denitrification rate upon freezing. Thus soils in which nitrite was stable at temperatures above freezing lost nitrite once freezing occurs.
5. not all soils suffered extensive  $NO_2^-$ -N losses at any temperature. Nitrite stability was associated with high measured pH.
6. production of van Slyke-like  $N_2$  may be one mechanism of over winter loss of fall-applied ammonium or ammonium yielding fertilizer products in Western Canada.

## BIBLIOGRAPHY

- Alexander, M. 1965. Nitrification. In Soil Nitrogen. Monograph No.10. Amer.Soc.Agron., Madison, Wisconsin p. 307-343.
- Alexander, M. 1977. Introduction to Soil Microbiology. John Wiley and Sons, New York.
- Allison, F.E. 1963. Losses of gaseous nitrogen from soils by chemical mechanisms involving nitrous acid and nitrites. *Soil Sci.* 96:404-409.
- Allison, F.E., J.N.Carter and L.D.Sterling. 1960. The effect of partial pressure of oxygen on denitrification in soil. *Soil Sci. Soc. Amer. Proc.* 24:283-285.
- Anderson, D.M. and A.P.Tice. 1971. Low temperature phases of interfacial water in clay-water systems. *Soil Sci. Soc. Amer. Proc.* 35:47-54.
- Anderson, D.M., A.R.Tice and A.Banin. 1973. The water-ice phase composition of clay-water systems. I. The kaolinite-water system. *Soil Sci. Soc. Amer. Proc.* 37:819-822.
- Anderson, O.E. 1962. The effect of low temperature on nitrification of ammonia in Cecil sandy loam. *Soil Sci. Soc. Amer. Proc.* 24:286-293.
- Bailey, L.D. and E.G.Beauchamp. 1973. Effects of temperature on  $\text{NO}_3^-$  and  $\text{NO}_2^-$  reduction, nitrogenous gas production and redox potential in a saturated soil. *Can. J. Soil Sci.* 53:213-218.
- Banin, A. and D.M.Anderson. 1974. Effects of salt concentration changes during freezing on the unfrozen water content of porous materials. *Water Res.* 10:124-128.
- Blackmer, A.M. and J.M.Bremner. 1977. Nitrogen isotope discrimination in denitrification of nitrate in soils. *Soil Biol. Biochem.* 9:73-77.
- Blackmer, A.M. and J.M.Bremner. 1978. Inhibitory effect of nitrite on reduction of nitrous oxide to nitrogen by soil microorganisms. *Soil Biol. Biochem.* 10:187-191.
- Bouyoucos, G.J. 1917. Classification and measurement of the different forms of water in soil by means of the dilatometer method. *Mich. Agr. Coll. Exp. Sta. Tech. Bull.* 36:43.
- Bremner, J.M. 1957. Studies on soil humic acids: II Observations on the estimation of free amino groups. Reactions of humic acid and lignin preparations with nitrous acid. *J. Agr. Sci.* 48:352-353.

- Bremner, J.M., A.M.Blackmer and S.A.Waring. 1980. Formation of nitrous oxide and dinitrogen by chemical decomposition of hydroxylamine in soils. *Soil Biol. Biochem.* 12:263-269.
- Bremner, J.M. and K.Shaw. 1958. Denitrification in soil II. Factors affecting denitrification. *J. Agr. Sci.* 51:40-52.
- Broadbent, F.E. and F.Clark. 1965. Denitrification. In Soil Nitrogen. Monograph No. 10. Amer. Soc. Agron. Madison, Wisconsin p. 347-359.
- Broadbent, F.E., R.S.Rauschkolb, K.A.Lewis and G.Y.Chang. 1980. Spatial variability of nitrogen-15 and total nitrogen in some virgin and cultivated soils. *Soil Sci. Soc. Amer. J.* 44:5524-528.
- Broadbent, F.E. and F.J.Stevenson. 1966. Organic matter interactions. In Agricultural Anhydrous Ammonia- Technology and Use. (M.H.McVickar et al. eds). American Society of Agronomy, Madison, Wisconsin p. 169-187.
- Broadbent, F.E. and K.B.Tyler. 1962. Laboratory and greenhouse investigation on nitrogen immobilization. *Soil Sci. Soc. Amer. Proc.* 26:459-462.
- Brzhan, V.S. 1959. An X-ray study of the crystalization of sorbed water. *Colloid J.* 21:621-624.
- Burton, J.A., R.C.Prim and W.P. Slichter. 1953. The distribution of solute in crystals grown from a melt. Part I, Theoretical: *Chem. Phys.* 21:1987-91.
- Cady, F.B. and W.V.Bartholomew. 1960. Sequential products of anaerobic denitrification in Norfolk soil material. *Soil Sci. Soc. Amer. Proc.* 24:477-482.
- Cady, F.B. and W.V.Bartholomew. 1963. Investigations of nitric oxide reactions in soils. *Soil Sci. Soc. Amer. Proc.* 27:546-549.
- Campbell, N.E.R. and H.Lees. 1967. The nitrogen cycle. In Soil Biochemistry. Marcel Dekker Inc. N.Y. p. 194-215.
- Chapman, H.D. 1965. Cation-exchange capacity. In Methods of Soil Analysis, Part 2. Monograph No. 9. Amer. Soc. Agron. Madison, Wisconsin. 819-901.
- Chien, S.H., G.Shearer and D.H.Kohl. 1977. The nitrogen isotope effect associated with nitrate and nitrite loss from water-logged soils. *Soil Sci. Soc. Amer. J.* 41:63-69.
- Cho, C.M. and J.G.Mills. 1979. Kinetic formulation of the denitrification process in soil. *Can. J. Soil Sci.* 59:249-257.
- Cho, C.M. and L.Sakdinan. 1978. Mass spectrometric investigation on denitrification. *Can. J. Soil Sci.* 58:443-457.

- Cho, C.M., L.Sakdinan and C.Chang. 1979. Denitrification intensity and capacity of three irrigated Alberta soils. *Soil Sci. Soc. Amer. J.* 43:945-950.
- Christianson, C.B., R.A.Hedlin and C.M.Cho. 1979. Loss of nitrogen from soil during nitrification of urea. *Can. J. Soil Sci.* 59:147-154.
- Christianson, C.B. 1978. Denitrification of urea and sodium nitrite in some Manitoba soils. M.Sc. Thesis, University of Manitoba.
- Clark, F.E., W.E.Beard and D.H.Smith. 1960. Dissimilar nitrifying capacities of soils in relation to losses of applied nitrogen. *Soil Sci. Soc. Amer. Proc.* 24:50-54.
- Conley, R.F. and A.C.Aloff. 1971. Surface acidity in kaolinite. *J. Coll. Interface Sci.* 37:186-195.
- Court, M.N., R.C.Stephen and J.S.Waid. 1962. Nitrite toxicity arising from the use of urea as a fertilizer. *Nature* 194:1263-1263.
- Crutzen, P.J. and D.H.Ehhalt. 1977. Effects of nitrogen fertilizers and combustion on the stratospheric ozone layer. *Ambio* 6:112-117.
- Delwiche, C.C. and P.L.Steyn. 1970. Nitrogen isotope fractionation in soils and microbial reactions. *Environ. Sci. Technol.* 4:929-935.
- Edwards, A.P. 1973. Isotope fractionation of  $^{15}\text{N}$  and  $^{14}\text{N}$  in microbial nitrogen transformations: a theoretical model. *J. Environ. Qual.* 2:247-252.
- Freundlich .1920. Capillary Chemistry Berlin.
- Gerretsen, F.C. and H.deHoop. 1957. Nitrogen losses during nitrification in solutions and in acid sandy soils. *Can. J. Microbiol.* 3:359-380.
- Gray, T.R.G. and S.T.Williams. 1971. Soil Micro-organisms Hafner Co. New York.
- Hallet, B. 1978. Solute redistribution in freezing ground. In Proceedings, Third International Conference on Permafrost. National Academy of Science. Washington. p. 86-91.
- Harapiak, J.T. 1979. Alberta situation-comparison of fall and spring applied nitrogen. In Proceedings of 1979 Alberta Soil Science Workshop. p. 37.
- Harter, R.D. and J.L.Ahlrichs. 1967. Determination of clay surface acidity by infrared spectroscopy. *Soil Sci. Soc. Amer. Proc.* 31:30-33.
- Hauck, R.D. 1973. Nitrogen tracers in nitrogen cycle studies, past use and future needs. *J. Environ. Qual.* 2:317-327.

- Hauck, R.D., S.W.Melsted and P.E.Yankwich. 1958. Use of N-isotope distribution in nitrogen gas in the study of denitrification. *Soil Sci.* 86:287-291.
- Hauck, R.D. and H.F.Stevenson. 1965. Nitrification of nitrogen fertilizers. Effect of nitrogen source, size and pH of the granules, and concentration. *J. Agric. Food. Chem.* 13:486-492.
- Himes, R.C., S.E.Miller, W.H.Mink and H.L.Goering. 1959. Zone freezing in demineralizing saline solutions. *Ind. Eng. Chem.* 51:1345-1348.
- Justice, J.K. and R.L.Smith. 1962. Nitrification of ammonium sulfate in a calcareous soil as influenced by combinations of moisture, temperature, and levels of added nitrogen. *Soil Sci. Soc. Amer. Proc.* 26:246-250.
- Kamphake, L.J., S.A.Hannah and J.M.Cohen. 1967. Automated analysis for nitrate by hydrazine reduction. *Water Res.* 1:205-216.
- Karamanos, R.E. and D.A.Rennie. 1980. Variations in natural  $^{15}\text{N}$  abundance as an aid in tracing fertilizer nitrogen transformations. *Soil Sci. Soc. Amer. J.* 44:57-62.
- Knowles, R. 1978. Common intermediates of nitrification and denitrification and the metabolism of nitrous oxide. In Microbiology-1978 (D. Schlessinger, ed.) American Society for Microbiology, Wash. p. 363-367.
- Low, P.F., D.M.Anderson and P.Hoekstra. 1968. Some thermodynamic relationships for soils at or below the freezing point. *Water Res.* 4:379-394.
- Malhi, S.S. 1978. Losses of mineral nitrogen over the winter in Chernozemic and Luvisolic soils. Ph. D. Thesis, University of Alberta.
- McElroy, M.B., S.C.Wofsy and Y.L.Yung. 1977. The nitrogen cycle: Perturbations due to man and their impact on atmosphere  $\text{N}_2\text{O}$  and  $\text{O}_3$ . *Philosophical Trans. Royal Soc.* 227B:159-181.
- Meek, B.D. and A.J.MacKenzie. 1965. The effect of nitrite and organic matter on aerobic gaseous losses of nitrogen from a calcareous soil. *Soil Sci. Soc. Amer. Proc.* 29:176-178.
- Moore, W.J. 1972. Physical Chemistry, 4th Edition, Prentice-Hall, Inc. New Jersey
- Morrill, L.G. and J.E.Dawson. 1967. Patterns observed for the oxidation of ammonium to nitrate by soil organisms. *Soil Sci. Soc. Amer. Proc.* 31:757-760.
- Nelson, D.W. and J.M.Bremner. 1970. Gaseous products of nitrite decomposition in soils. *Soil Biol. Biochem.* 2:203-215.

- Nersesova, Z.A. and N.A.Tsytovich. 1963. Unfrozen water in frozen soils. In International Conference on Permafrost. NRC-NAS p. 230-34.
- Nier, A.O. 1950. A redetermination of the relative abundances of the isotopes of carbon, nitrogen, oxygen, argon and potassium. *Phys. Rev.* 77:789-793.
- Nommik, H. 1956. Investigations on denitrification in soil. *Acta Agric. Scan.* 2:195-228.
- Nommik, H. and K.O.Nilsson. 1963. Nitrification and movement of anhydrous ammonia in soil. *Acta. Agr. Scan.* 13:205-219.
- Nyborg, M. and R.H.Leitch. 1979. Losses of soil and fertilizer nitrogen in Northern Alberta in Proceedings of 1979 Alberta Soil Science Workshop p. 56.
- Outcalt, S. 1976. A numerical model of ice lensing in freezing soils. In Proceedings 2nd Conference on Soil-Water Problems in Cold Regions, Calgary, Canada, p. 63-74.
- Pang, P.C., C.M.Cho and R.A.Hedlin. 1975. Effects of pH and nitrifier population on nitrification of band -applied and homogeneously mixed urea nitrogen in soils. *Can. J. Soil Sci.* 55:15-21.
- Parr, J.F., S.Smith and G.H.Willis. 1970. Soil anaerobiosis. I. Effect of selected environments and energy sources on respiratory activity of soil microorganisms. *Soil Sci.* 110:37-43.
- Paul, E.A. and D.A.Rennie. 1978. Crop utilization and fate of fertilizer nitrogen in soil. In Proceedings of 1977 Soil Fertility and Crops Workshop, Saskatchewan. p. 177.
- Payne, W.J. 1976. Denitrification. *TIBS.* 1:220-223.
- Payne, W.J. and P.S.Riley. 1969. Suppression by nitrate of enzymatic reductions of nitric oxide. *Proc. Soc. Exp. Biol. Med.* 132:258-260.
- Peech, M., L.T.Alexander, L.A.Dean and J.F.Reed. 1947. Methods of soil analysis for soil fertility investigations. U.S. Dept. Agric. Circ. 757.
- Reuss, J.O. and R.L.Smith. 1965. Chemical reactions of nitrites in acid soils. *Soil Sci. Soc. Amer. Proc.* 30:363-366.
- Ridley, A.O. 1977. Nitrogen fertilizers, time and method of placement. *Proceedings, 21st Annual Man. Soil Sci. Mtg.*, Dept. of Soil Sci., U. of Man., Winnipeg 167-188.
- Shapiro, J. 1961. Freezing-out, a safe technique for concentration of dilute solutions. *Science* 133:2063-2064.

- Skinner, S.I.M., R.L.Halstead and J.E.Brydon. 1959. Qualitative manometric determination of calcite and dolomite in soils and limestones. *Can. J. Soil. Sci.* 39:197-204.
- Smith, C.J. and P.M.Chalk. 1980. Gaseous nitrogen evolution during nitrification of ammonia fertilizer and nitrite transformations in soils. *Soil Sci. Soc. Amer. J.* 44:277-282.
- Smith, D.H. and F.E.Clark. 1960. Volatile losses of nitrogen from acid or neutral soils or solutions containing nitrite and ammonium ions. *Soil Sci.* 90:86-92.
- Smith, G.H. and M.P.Tasker. 1965. Freeze concentration of dilute solutions. *Anal. Chem. Acta.* 33:559-560.
- Soulides, D.A. and F.E.Clark. 1958. Nitrification in grassland soils. *Soil Sci. Soc. Amer. Proc.* 22:308-311.
- Stevenson, F.J., R.M.Harrison, R.Wetselaar and R.A.Leeper. 1970. Nitrosation of soil organic matter: III. Nature of gases produced by reaction of nitrite with lignins, humic substances and phenolic constituents under neutral and slightly acidic conditions. *Soil Sci. Soc. Amer. Proc.* 34:430-435.
- Stevenson, F.J. and R.J.Swaby. 1964. Nitrosation of organic matter. I. Nature of gases evolved during  $\text{HNO}_2$  treatment of lignins and humic substances. *Soil Sci. Soc. Amer. Proc.* 28:773-778.
- Terry, R.E. and R.L.Tate. 1980. The effect of nitrate on nitrous oxide reduction in organic soils and sediments. *Soil Sci. Soc. Amer. J.* 44:744-6.
- Terwilliger, J.P. and S.F.Dizio. 1970. Salt rejection phenomena in the freezing of saline solutions. *Chem. Engin. Sci.* 25:1331-1349.
- Tice, A.R., C.M. Burrows and D.M. Anderson. 1978. Determination of unfrozen water in frozen soil by pulsed nuclear magnetic resonance. In Proceedings 3rd International Conference on Permafrost. NRC, Canada p. 149-155.
- Tsyтович, N.A. 1975. The Mechanics of Frozen Ground G.K.Swinzow (ed.) McGraw-Hill, New York p. 20-52.
- Tyler, K.B. and F.E. Broadbent. 1960. Nitrite transformations in California soils. *Soil Sci. Soc. Am. Proc.* 24:279-282.
- Tyutyunov, I.S. 1973. New concepts of the nature of frozen soils. In Proceedings Second International Conference Permafrost. USSR contribution National Academy of Science. Washington, D.C. p. 303-307.
- van Cleemput, O. 1974. Nitrate and nitrite reduction in flooded gamma irradiated soil under controlled pH and redox potential conditions. *Soil Biol. Biochem.* 6:85-88.

- van Slyke, D.D. 1911. A method for quantitative determination of aliphatic amino groups. *J. Biol. Chem.* 9:185-204.
- Verstaete, W. and M.Alexander. 1973. Heterotrophic nitrification in samples of natural ecosystems. *Environ. Sci. Tech.* 7:39-42.
- Wellman, R.P., F.D.Cook and H.R. Krouse. 1968. Nitrogen-15: Microbiological alteration of abundance. *Science* 161:269-270.
- Wijler, J. and C.C.Delwiche. 1954. Investigations on the denitrifying process in soil. *Plant Soil* 5:155-169.
- Wullstein, L.H. and C.M.Gilmour. 1964. Non-enzymatic gaseous loss of nitrite from clay and soil systems. *Soil Sci.* 97:428-430.
- Wullstein, L.H. and C.M.Gilmour. 1966. Non-enzymatic formation of nitrogen gas. *Nature* 210:1150-1151.