

THE EFFECTS OF TEMPERATURE AND CONCENTRATION
OF NITRATE UPON DENITRIFICATION IN SOME
MANITOBA SOILS

A Thesis

submitted to

The Faculty of Graduate Studies and Research
The University of Manitoba

In Partial Fulfilment
of the Requirements for the Degree

MASTER OF SCIENCE

by

Ismail Ibrahim Khdyer

September 1978

THE EFFECTS OF TEMPERATURE AND CONCENTRATION
OF NITRATE UPON DENITRIFICATION IN SOME
MANITOBA SOILS

BY

ISMAIL IBRAHIM KHDYER

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

MASTER OF SCIENCE

© 1978

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

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

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. C.M. Cho for his advice during the course of this investigation and for his assistance in the preparation of this thesis.

I also wish to thank Dr. R.A. Hedlin and Dr. N.E.R. Campbell for serving on my student advisory committee.

Grateful acknowledgement is extended to the Iraqi Ministry of Higher Education for the financial assistance throughout this study. I would also like to thank Dina, Ridha, Ian and Obi.

ABSTRACT

The effect of temperature and concentration of added NO_3^- on the rate of denitrification was investigated by studying the rate of disappearance of NO_3^- -N and production of gaseous nitrogen products under flooded conditions. Three soils differing in their initial pH (Keld - pH 5.4, Wellwood - pH 6.3, and Morton - pH 7.8) were selected for this study. Three levels of NO_3^- - 100, 150 and 200 ppm - and three incubation temperatures - 15, 25 and 35°C - were used.

Nitrate disappearance rate was found to be independent of initial NO_3^- level with the Morton soil. The rate was increased with increasing temperature. Nitrogen gas was the major gas produced and its production rate was independent of the level of NO_3^- . The temperature dependence of the zero order rate constant for N_2 production followed the Arrhenius equation.

The initial rate of NO_3^- disappearance with the Wellwood soil was independent of the initial NO_3^- concentration. Nitrous oxide was the major gas evolved during the initial period of incubation. However, further reduction of N_2O to N_2 occurred as the concentration of NO_3^- -N decreased. The maximum value of N_2O was found to be proportional to the initial concentration of NO_3^- . The amount of N_2 produced increased and its formation took place earlier as the incubation temperature was increased.

The pattern of NO_3^- disappearance and gaseous production of the Keld soil was very similar to that of the Wellwood soil. However, the maximum quantity of N_2O produced was greater with the Keld soil than with the Wellwood soil.

Liming the Keld and Wellwood soils increased the disappearance rate of NO_3^- and the production rate of N_2 . Liming decreased but did not completely suppress N_2O production.

A small accumulation of NO_2^- occurred at an early stage of incubation and this accumulation was increased with liming. There was a tendency for NH_4^+ to accumulate during the denitrification process. Up to 40 $\mu\text{g/g}$ soil of NH_4^+ -N accumulated in the system.

Oxygen consumption and CO_2 production increased with increasing temperature. The rates of O_2 consumption and the production of CO_2 were unaffected by concentration of NO_3^- . Liming the soil did not alter O_2 consumption.

The isotopic effect, if any, was found to be negligible for this investigation.

LIST OF FIGURES

FIGURES	PAGE
1. A. Incubation vessel.	43
B. Sampling apparatus	43
C. Gas sample container	44
2. Recovery of NO ₃ ⁻ -N during the incubation of added 100 ppm-N with Morton soil at various temperatures	55
3. Production of N ₂ from Morton soil (initial pH 7.8) at various temperatures (N rate = 100 ppm NO ₃ ⁻ -N).	57
4. Recovery of NO ₃ ⁻ -N during the incubation of added 150 ppm-N with Morton soil at various temperatures	60
5. Production of N ₂ from Morton soil (initial pH 7.8) at various temperatures (N rate = 150 ppm NO ₃ ⁻ -N).	61
6. Recovery of NO ₃ ⁻ -N during the incubation of added 200 ppm-N with Morton soil at various temperatures	63
7. Production of N ₂ from Morton soil (initial pH 7.8) at various temperatures (N rate = 200 ppm NO ₃ ⁻ -N).	64
8. Production of N ₂ from Morton soil (initial pH 7.8) with different initial concentration of added NO ₃ ⁻ -N at 25°C	65
9. Arrhenius plot of rate of N ₂ production (K) vs temperature of Morton soil (initial pH 7.8) at various initial concentrations of added NO ₃ ⁻ -N.	67

List of Figures Continued...

FIGURES	PAGE
10. Recovery of NO_3^- -N during the incubation of various initial concentrations of added NO_3^- in Wellwood soil at 15°C	71
11. Amounts of N_2O and N_2 during the denitrification of added NO_3^- with various initial concentrations in the Wellwood soil at 15°C	72
12. Recovery of NO_3^- -N during the incubation of various concentrations of added NO_3^- in Wellwood soil at 25°C	76
13. Amounts of N_2O and N_2 during the denitrification of added NO_3^- -N with various initial concentrations in Wellwood soil at 25°C	77
14. Recovery of NO_3^- -N during the incubation of various initial concentrations of added NO_3^- -N in Wellwood soil at 35°C	79
15. Amounts of N_2O and N_2 during the denitrification of added NO_3^- -N with various initial concentrations in the Wellwood soil at 35°C	81
16. Recovery of NO_3^- -N during the incubation of various initial concentrations of added NO_3^- -N in Keld soil at 15°C	84
17. Production of nitrous oxide from Keld soil pH 5.4, with various initial concentration, of added NO_3^- -N at 15°C	85
18. Recovery of NO_3^- -N during the incubation of various initial concentrations of added NO_3^- in Keld soil at 25°C	87

List of Figures Continued...

FIGURES	PAGE
19. Amounts of N_2O and N_2 during the denitrification of added NO_3^- with various initial concentrations in the Keld soil at $25^\circ C$	88
20. Recovery of NO_3^- -N during the incubation of various initial concentrations of added NO_3^- in the Keld soil at $35^\circ C$	90
21. Amounts of N_2O and N_2 during the denitrification of added NO_3^- with various initial concentrations in the Keld soil at $35^\circ C$	91
22. Recovery of NO_3^- -N from Keld soil at various amounts of lime added (N rate = 100 ppm NO_3^- -N)	94
23. Amounts of N_2O and N_2 during the denitrification of added NO_3^- -N (100 ppm) in the Keld soil at $25^\circ C$ as affected by liming.	95
24. Recovery of NO_3^- -N from limed and unlimed Wellwood soil (N rate = 100 ppm NO_3^- -N).	97
25. Amounts of N_2O and N_2 during the denitrification of added NO_3^- -N (100 ppm) in the Wellwood soil at $25^\circ C$ as affected by liming.	99

LIST OF TABLES

TABLE	PAGE
1. Legal location and some physical and chemical properties of the soils.	41
2. Average % ¹⁵ N of N ₂ O and N ₂ evolved from the experimental soils with varying initial NO ₃ ⁻ -N concentration	59
A-1 Amount of NO ₂ ⁻ -N in the Morton soil samples at various times of incubation under different temperatures and concentrations of added NO ₃ ⁻ -N	113
A-2 Amount of NO ₂ ⁻ -N in the Wellwood soil at various times of incubation under different temperatures and concentrations of added NO ₃ ⁻ -N	114
A-3 Amount of NO ₂ ⁻ -N in the Keld soil at various times of incubation under different temperatures and concentrations of added NO ₃ ⁻ -N	115
A-4 Amount of NH ₄ ⁺ -N in the Morton soil at various times of incubation under different temperatures and concentrations of added NO ₃ ⁻ -N	116
A-5 Amount of NH ₄ ⁺ -N in the Wellwood soil at various times of incubation under different temperatures and concentrations of added NO ₃ ⁻ -N	117

List of Tables Continued...

TABLES	PAGE
A-6 Amount of NH_4^+ -N in the Keld soil at various times of incubation under different temperatures and concentrations of added NO_3^- -N	118
A-7 The partial pressure of O_2 during incubation of Morton soil samples under different temperatures and concentrations of added NO_3^- -N	119
A-8 The partial pressure of O_2 during incubation of Wellwood soil samples under different temperatures and concentrations of added NO_3^- -N	120
A-9 The partial pressure of O_2 during incubation of Keld soil samples under different temperatures and concentrations of added NO_3^- -N	121
A-10 The partial pressure of CO_2 during incubation of Morton soil samples under different temperatures and concentrations of added NO_3^- -N	122
A-11 The partial pressure of CO_2 during incubation of Morton soil samples under different temperatures and concentrations of added NO_3^- -N	123
A-12 The partial pressure of CO_2 during incubation of Keld soil samples under different temperatures and concentrations of added NO_3^- -N.	124

List of Tables Continued...

TABLES

PAGE

A-13 Amount of NO_2^- -N in the soil samples incubated with
100 ppm NO_3^- -N at 25°C, and various amounts of CaCO_3 125

TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION.	1
II. LITERATURE REVIEW.	3
Biological Denitrification.	3
Factors affecting biological denitrification.	5
1. Effect of temperature.	5
2. Effect of pH	8
3. Effect of nitrate concentration.	11
4. Effect of organic matter	13
5. Effect of aeration	15
6. Effect of moisture	19
Chemical Denitrification.	22
1. Chemical decomposition of nitrous acid	22
2. Interaction of nitrous acid with amino acid and ammonia (Van Slyke-type reaction.	26
3. Nitrosation of organic matter.	29
Factors Affecting Chemical Denitrification.	31
1. Effect of pH	31
2. Effect of nitrite concentration.	33
3. Influence of metallic ions and soil constituents	33
Isotopic methodology in denitrification study	35
III. MATERIALS AND METHODS	40
IV. RESULTS AND DISCUSSION.	54
<u>Experiment 1.</u> Denitrification of $\text{Ca}(\text{NO}_3)_2$ in the Experimental Soils.	54
1. Morton Soil.	54
2. Wellwood Soil.	70
3. Keld Soil.	83
<u>Experiment 2.</u> Denitrification of $\text{Ca}(\text{NO}_3)_2$ in Keld and Wellwood Soils with added lime	93
1. Limed Keld Soil.	93
2. Limed Wellwood Soil.	96
V. SUMMARY AND CONCLUSIONS	100
VI. LITERATURE CITED	105
VII. APPENDIX	113

INTRODUCTION

The most important source of loss of soil nitrogen as gaseous products is denitrification, which is the biological reduction of nitrate or nitrite to gaseous nitrogen as molecular nitrogen or the oxides of nitrogen (Soil Sci. Amer. Proc. 26:307, 1965). Generally speaking the oxides of nitrogen found are nitric oxide (NO), nitrogen dioxide (NO₂), and nitrous oxide (N₂O).

Focht and Chang (1975) reviewed the history of early investigation of denitrification. According to them the production of N₂O and N₂ from nitrate was first observed by Gayon and Dupetet in 1886. These latter authors concluded that the process was biological, that it occurred in the absence of oxygen and involved the reduction of nitrate to nitrite prior to gaseous formation. Weissenberg (1902) made the first assertion that reduction of nitrate and nitrites to gaseous product was brought about by aerobic bacteria that switched over to using the oxygen in nitrate when molecular oxygen was depleted. Although he was generally correct in his assertion, nitrate per se serves as a terminal electron acceptor in lieu of oxygen during the oxidation of organic matter.

It was not until 1946, when the astronomer Adel discovered N₂O in the atmosphere, concentrated at the earth's surface, that a denitrifying process in the soil was suspected. Broadbent (1951) published the results of an investigation of denitrification in soil which indicated that denitrification is of considerable economic significance in aerated

soils in the presence of moderate amounts of organic matter and nitrate. Subsequent work by Broadbent and Stojanvic (1952), Wijler and Delwiche (1954), and Wallace and Smith (1954) has lent support to the view that a significant loss of nitrogen from soil occurs by the process of denitrification. Further support can be taken from soil nitrogen balance sheets obtained from lysimeter experiments (Allison, 1955). They frequently show substantial losses of nitrogen that are difficult to explain unless it is assumed that a considerable loss of soil nitrogen occurs by gaseous evolution.

The occurrence of N_2O in laboratory and field soils was subsequently confirmed by a number of workers (Arnold 1954; Dowdell and Smith 1974). The loss of nitrogen by the denitrification process is of great importance with respect to fertilizer economy and food production. It is also of environmental concern, since it is thought that N_2O may be responsible for decreasing the concentration of ozone in the upper atmosphere. Estimates have been made predicting a 12% reduction of ozone in 160 years from the exponentially increasing use of fertilizer (Shapley, 1977).

It is the purpose of this investigation to determine the sequential products of denitrification and to deduce possible mechanisms of the process. Several temperatures and concentrations of NO_3^- -N were used for this purpose using three Manitoba soils.

LITERATURE REVIEW

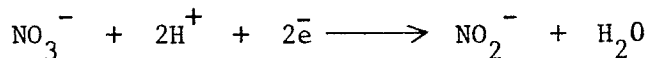
Two pathways have been found to operate in the denitrifying process. The first is biological (enzymatic) and the second is chemical (non-biological, non-enzymatic).

Biological Denitrification:

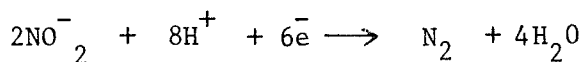
In order for enzymatic denitrification to occur, i.e., a process in which microorganisms turn to nitrate respiration in lieu of oxygen respiration, it is necessary that nitrate be present, that oxygen be absent or greatly limited in its availability, and that sufficient available substrate or energy yielding material be present to permit microbial activity. Additionally, of course, such environmental factors as moisture, temperature, reaction, etc. must be favorable for microbial activity.

Russell (1973) reported that the organisms obtain the energy they need for their vital processes through a series of chemical reactions involving the transfer of electrons from substances which serve as sources of energy to substances which may become products of respiration. If the organisms are respiring aerobically, the final electron sink is oxygen, which accepts electrons and combines with hydrogen ions to form water.

In the absence of free oxygen, a number of other substances, e.g., NO_3^- , can accept electrons and take part in the reduction reaction:



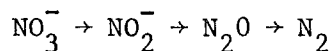
and



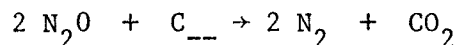
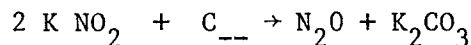
Bremner and Shaw (1958) and Valera et al. (1961) reported that the

bacteria are predominantly facultatively anaerobic in that they only use the oxygen of nitrate, nitrite or oxides of nitrogen as hydrogen acceptors in the absence of free oxygen. Bacteria differ in their ability to carry on the reduction of NO_3^- . Some denitrifying bacteria are able to carry out only part of the process, e.g. one strain has been found which reduces NO_3^- to NO_2^- , which then accumulates and inhibits the growth and denitrification by another strain (Bollag et al. 1970, 1973). Yoshida and Alexander (1970), Renner and Becker (1970), and Bollag et al. (1973) observed that some other bacteria are capable of reducing NO_3^- and NO_2^- to N_2O without production of N_2 .

It is generally accepted that nitrous oxide and N_2 are the major products and their proportions depend on environmental factors. Thus, the order of nitrate reduction has been suggested as:



In this sequence there are some questions about N_2O being a precursor to N_2 and the role of NO as an intermediate (Broadbent and Clark, 1965; Russell, 1973), although, Gayen and Dupetet (1889) did not specify intermediates other than nitrite. Beijerinck and Minkman (1910) proposed a scheme in which N_2O was included as an intermediate:



The question of whether N_2O is an obligate intermediate in nitrate reduction is not established yet. Sacks and Barker (1949) concluded, from

their experiments with Pseudomonas denitrificans by using a manometric technique, it is not a true intermediate. This conclusion was based on their observations that azide and dinitrophenol inhibited the reduction of N_2O but did not affect the reduction of nitrate to nitrogen. They also observed a lag in the utilization of N_2O by nitrate-adapted cells, which indicated that the adaptation to nitrate does not include adaptation to N_2O .

Delwiche and Bryan (1976) reported the work with the same bacterial species. They showed no lag period in N_2O reduction when lactate was used as an electron donor, nor was there any production of nitrogen from nitrite when the cells were inhibited by azide or dinitrophenol. The only gas produced during the periods of inhibition was N_2O . This was determined from gas chromatographic analysis as well as by the manometric method.

According to Russell (1973) the reduction of nitrate goes through the nitrite stage, although nitrite normally does not accumulate in the soil. Then nitrite is reduced to N_2O or to nitrogen gas. Under certain conditions it may go through the nitric oxide, NO , stage e.g., anaerobic systems. Most studies, however, have shown that NO production from soil incubated with NO_2^- originated by chemical pathway due to nitrite decomposition (Cady and Bartholomew, 1960).

Factors Affecting Biological Denitrification

1) Effect of Temperature

The effect of temperature on biochemical rate processes is exponential and adheres within limits to the classic Arrhenius equation,

$$- \frac{E_a}{RT}$$

$$K = A e$$

where K is the rate constant, A is a constant, E_a is the activation energy in cal. per mole, R is the universal gas constant in cal mole⁻¹ deg.⁻¹, and T is the temperature in Kelvin.

According to Focht (1974), T can be expressed directly in °C as follows:

$$K = c e^{\lambda T}$$

where both λ and c are constants.

Nommik (1956), and Bremner and Shaw (1958) found that the optimum temperature for biological nitrate reduction in soil was 60 to 75°C and that it ceases at 85°C. The high optimum temperature is presumably due to the exclusive predominance of thermophilic species of Bacillus (Focht and Chang, 1975).

Dawson and Murphy (1972) studied denitrification in mixed cultures by determining nitrate loss. They showed that denitrification conformed to Arrhenius kinetics from 3 to 28°C although most other studies have shown that the rate is affected proportionally more below 10-15°C than at the higher temperatures studied (Nommik, 1956; Bremner and Shaw, 1958; Bailey and Beauchamp, 1973a; Focht, 1974; and Stanford et al., 1975).

The value of Q_{10} , the ratio of the rates observed at 10°C difference, e.g. K_T/K_{T-10} , is commonly used to describe the effect of temperature upon a biological process. The lower limit of the temperature range conforming to a Q_{10} value was estimated to be 11°C, while the rate of

denitrification decreased almost ten-fold as the temperature dropped below this lower limit (Stanford, et al., 1975). Bremner and Shaw (1958) also found that the change in the rate constant was proportionally greater between 2 and 10°C than with an equal increment at higher temperature. The sharp break in the temperature curve at lower temperature is generally thought to be due to the effect of physical factors such as solubility or diffusion becoming more pronounced (Ingraham, 1962).

It is generally thought that the temperature coefficient Q_{10} , for biological reactions is equal to two or three (Focht, 1974). However, the temperature coefficient of denitrification in the soil complex has not been established. Nommik (1956) and Focht (1974), found a linear relationship between temperature and total gaseous production rate when the rate was calculated from a two-day incubation period. Q_{10} , for nitrate and nitrous oxide reduction rates, are 1.6 and 1.4 respectively. They also reported that the maximum reduction rate of NO_3^- and N_2O are 77 and 55 ppm/day respectively, with activation energy of about 8000 K cal. per mole.

Cooper and Smith (1963) and Stanford et al. (1975) on the other hand, determined the NO_3^- lost during soil incubation. They observed that the Q_{10} of nitrate reduction was approximately 2. in the range of 15 to 35°C.

Higher Q_{10} values for denitrification were observed when the rates were derived from nitrate disappearance (Bailey and Beauchamp, 1973a)

and from nitrite disappearance (Bailey and Beauchamp, 1973b).

They concluded that this is likely due to the decreased solubility of nitrous oxide and molecular nitrogen with increasing temperature.

Changes in temperature may also influence the composition of the products formed during denitrification. Although the relative proportions of nitrous oxide and molecular nitrogen produced during denitrification vary slightly, nitric oxide is detected in greater quantities at lower temperatures in soil (Nommik, 1956; Bailey and Beauchamp, 1973a). Bailey and Beauchamp (1973a) observed no reduction of nitrate and no formation of gaseous products in soil, with pH 7.3, after 22 days at 5°C. However, they did detect nitric oxide as the only gas formed when soils were incubated at 5°C with nitrite. They concluded that reduction of nitrate ceased at 5°C and that evolution of nitric oxide from nitrite was produced by chemodenitrification. Many studies, however, have shown that denitrification can occur at temperatures below 5°C where nitrate is used as substrate (Nommik, 1956; Bremner and Shaw, 1958; Dawson and Murphy, 1972).

2) Effect of pH

The effect of soil pH on denitrification is not fully understood. It is known, however, that nitrogen loss is considerably suppressed under acidic conditions. Maximum rate of nitrogen loss occurs at a slightly alkaline pH (Jansson and Clark, 1952; Valera *et al.* 1961; Renner and Becker 1970). Wijler and DeWiche (1954), Nommik (1956), and Bremner and Shaw (1958) showed that the rate of denitrification

increases linearly from pH 4, levels off between pH 7 and 8, and declines although it does not cease when the pH is raised to 9.5. They also found that neutral to slightly alkaline pH ranges not only effect faster rates of denitrification, but also the completion of reduction to N_2 .

However, many contradictory statements exist in the literature on the influence of pH on denitrification. Broadbent (1951) reported that denitrification is favoured below pH 7, with a maximum at pH 6.3. Dawson and Murphy (1972) have shown that denitrification rate was a parabolic function of pH with a peak at 7.0. The rates at pH 6.0 and pH 8.0 were approximately halved as compared to that at pH 7.0. Also it has been concluded that no correlation exists between soil pH and denitrification (Khan and Moore, 1968).

In culture media optimum pH for denitrification varies with the organism, nitrate concentration, and age of culture (Delwiche and Bryan 1976). Bollag *et al.* (1970) also reported that various microbial strains showed different responses in their denitrifying behaviour and growth to different pH values, although it was concluded that the optimum pH for nitrate reduction and organism growth was near neutral.

It has been postulated that the rate-limiting process for denitrification in acid soils is the reduction of NO_3^- , while in alkaline soils it is the reduction of nitrite (Cooper and Smith 1963). The effect of acidity on the rate of denitrification is not due to deficiency of calcium or molybdenum (Bremner and Shaw, 1958). However, by increasing the acidity, nitrite toxicity appeared to be an important ecological factor in preventing biological denitrification (Payne, 1973).

The effect of pH on the distribution of various gases that were

formed by denitrification has been reported by many investigators. Wijler and Delwiche (1954), Hauck and Melsted (1956), and Nommik (1956) noted that the ratio of N_2O to N_2 produced declined with increasing pH, and also that N_2O reduction was inhibited by acidity. Wijler and Delwiche (1954) found that below pH 7, N_2O was the major product of denitrification. Above the neutral reaction, N_2O was still produced in excess of N_2 but it was subsequently reduced to N_2 . This cannot be explained on increases in solubility of nitrous oxide since it diminished with increasing pH (Focht et al. 1975). However, Cady and Bartholomew (1960, 1961) and Cooper and Smith (1963) found that N_2O was a major product of denitrification in alkaline soils as well as in acid soils. The latter authors concluded that the speed of N_2O reduction to N_2 could explain why liming an acid soil would lead to the conclusion that N_2 is the major gas produced during denitrification in an alkaline (limed) soil, while N_2O is the major gas produced in the unlimed soil. Nitric oxide also is a gaseous product of denitrification that is usually found under acid conditions, (Wijler and Delwiche 1954; Nommik, 1956, Cady and Bartholomew 1960, Bollag et al. 1973).

Wijler and Delwiche (1954) found that a small amount of NO was produced at pH values between 6.0 and 7.0. Under more acidic conditions NO production increased and it amounted to approximately 20% of the total nitrogen evolved at pH 5.0. The question has been raised as to whether NO is formed chemically from decomposition of nitrous acid or biologically from nitrite and nitrate. Bollag et al. (1973) concluded that formation of nitric oxide in acid soils was largely chemical since sterilized soils were found to evolve as much nitric oxide as controls

upon addition of nitrite.

3) Effect of nitrate concentration

The enzyme catalyzed reactions show a distinctive feature not usually observed in non-enzymatic reactions, namely, the phenomenon of saturation with substrate. The effect of substrate concentration on the rate of the enzymatic reaction can be described as follows. At low substrate concentration, the reaction velocity is proportional to the substrate concentration and the reaction is thus first order with respect to the substrate. However, as the substrate concentration is increased, the reaction rate falls off and is no longer proportional to the substrate concentration, and the reaction is mixed order. On a further increase in substrate concentration, the rate becomes constant and independent of substrate concentration. In this range of substrate concentration, the reaction is zero-order with respect to the substrate. Then the enzyme is saturated with respect to the substrate, (Lahninger, 1970).

Although all enzyme reactions show this saturation effect, Focht (1974) reported that the kinetics for denitrification are complex, since the reduction of nitrate is coupled with the oxidation of carbonaceous substrate. A kinetic approach must involve at least a dual substrate-enzyme complex. Such equations have been developed for idealized systems by Bray and White (1966).

$$V = \frac{V_{\max} CN}{(K_c + C)(K_n + N)}$$

where V is the observed rate, V_{max} is the maximum rate, C and N are carbon and nitrogen concentrations, respectively, and K_c and K_n are

the respective Michaelis constants. Denitrification is far more complex because nitrogenous intermediates presumably have different saturation constants, they may be competitively inhibited at specific redox potentials, and the association complex of reductant and oxidant may involve more than one enzyme (Focht and Chang 1975).

Bremner and Shaw (1958) have studied the effect of varying the level of added nitrate to water-logged soil. The results showed that the loss of nitrogen after ten days, calculated as a percentage of the NO_3^- -N added, did not vary with the level of nitrate. They also showed that the rate of nitrogen loss was affected by the level of nitrate-N only when the latter was very high (4000 ppm).

Many investigations support this concept and show the rate of denitrification to be independent of nitrate concentration with a wide range of NO_3^- -N concentration (50-1000 ppm) (Wijler and Delwiche 1954; Nommik, 1956; Cooper and Smith 1963).

Cooper and Smith (1963) studied denitrification, for a wide range of variation in soil pH and soil texture, by determining the loss of nitrate. They concluded that the denitrification kinetic is zero order with respect to NO_3^- , with a maximum rate of 310-520 ppm/day depending on the soil used. However, apparent first-order kinetics were reported by Stanford *et al.* (1975) in soil, when solution concentration was lower than 32 ppm NO_3^- -N. However, Bowman and Focht (1974) showed zero-order and first-order states were merely the extreme ends of the standard Michaelis-Menton curve in describing denitrification providing the concentration of the reductant or oxidant, when held constant while the other was varied, was not limiting. Where the reaction followed Michaelis-Menton kinetics, $V_{\text{max}} = 150$ mg of N

per milliliter per day and $K_m = 170$ mg of N per milliliter for a desert soil supplemented with glucose.

4) Effect of Organic Matter

It is generally accepted that the rate of denitrification is increased with increased organic matter content (Wijler and Delwiche 1954; Myers and McGarity 1972; Stefanson 1972). This increase is greatest where glucose or some other readily oxidizable substrate is added (Stanford *et al.* 1975), and least with the addition of lignin and sawdust (Bremner and Shaw, 1958; Broadbent and Clark, 1965).

Although the stoichiometric carbon-nitrogen relationship depends upon the electrons supplied per mole of carbonaceous substrate, the rate of denitrification generally proceeds maximally where the C/N ratio is between 2 and 3 (Bremner and Shaw 1958; Bowman and Focht 1974). It seems, however, that the maximum rate of NO_3^- loss occurs when the enzyme systems involved in reduction of NO_3^- and oxidation of carbon become saturated (Bowman and Focht 1974). The latter authors reported that if available carbon in the soil is limiting, e.g., C/N ratio less than 2 to 3, the bulk of it is used for respiratory rather than assimilatory purposes. Increasing the C/N ratio by addition of methanol or other exogenous substrates beyond that needed for denitrification only increases the biological oxygen demand (Focht and Chang, 1975).

Available organic matter effects the proportion of evolved gases. Nommik (1956) showed that the proportion of N_2O to N_2 produced was in

the order of untreated > 1 percent wheat straw > 1 percent glucose. Thus, carbon which is more biodegradable, increases not only the rate of NO_3^- reduction, but increases the rate of N_2O reduction even more. Similarly, Wijler and Delwiche (1954) found N_2O and N_2 production rates to be increased when higher rates of alfalfa were added.

The addition of organic matter has an indirect effect, since it promotes the microbiological activity which may cause an oxygen stress, so that the oxides of nitrogen become alternate hydrogen acceptors. Root exudates in the rhizosphere of grasses are suspected of encouraging microbial activity (Woldendrop, 1962; Stefanson, 1972). The respiration of plant roots may help to cause an oxygen stress.

One would expect the soil to become less aerobic with depth due to the proximity of the surface soil to atmospheric oxygen. However, a study of some Alberta soils indicated that the rate of production of N_2 decreased appreciably with depth (Khan and Moore 1968). Another field study indicated that the percentage concentration of O_2 was low in the surface 30 cm, while those of CO_2 and N_2 were high (Rolston et al. 1976). This was attributed to higher microbial activity due to a high concentration of organic matter near the surface of the soil and to root respiration of the covering rye grass crop. Below 40 cm. the percentage concentration of the three gases became constant. Work with disturbed and undisturbed samples has shown that denitrification is accelerated in disturbed samples due to the redistribution of organic matter (Myers and McGarity 1972; Rolston et al. 1976).

5) Effect of Aeration

In general the factors which decrease the O_2 status promote biological denitrification since, in the absence of oxygen, NO_3^- is utilized as a terminal electron acceptor in place of oxygen (Focht 1974).

The aeration status may be expressed in terms of the oxidation-reduction potential (E_h) when dissolved oxygen concentration is low and it is difficult to measure the amount of oxygen accurately (Focht and Chang 1975). Although the NO_3^-/NO_2^- couple determined under ideal conditions is 421 mV (Latimer 1952), nitrate appears to be reduced at potentials between 300 and 350 mV in soil (Pearsall and Mortimer 1939; Patrick 1961; Meek et al. 1969; Bailey and Beauchamp 1973a), and in culture media (Kefauver and Allison 1957).

Although Broadbent (1951) and Wijler and Delwiche (1954), showed that denitrification occurred at an O_2 concentration of 4 to 5 percent, Cady and Bartholomew (1961), found that only in the presence of high levels of C and at O_2 levels <7% would an appreciable reduction of nitrate to gaseous forms occur. In a liquid medium, active reduction of added NO_3^- does not occur until the O_2 concentration is very low. It must fall to about 4×10^{-6} molar which corresponds to a concentration in equilibrium with air containing 0.3% oxygen (Russell, 1973). However, the critical factor governing denitrification is the dissolved oxygen concentration, (Focht and Chang 1975). Studies by Meek et al. (1969) showed that gaseous oxygen concentrations had no correlations whatsoever to the level of dissolved oxygen, E_h ,

or to reduction of nitrates in soils treated with animal waste.

In the soil, there will be no reduction of NO_3^- if oxygen diffuses completely through the soil core (Pilot and Patrick 1972). The oxygen diffusion rate (ODR), which is a direct indication of aeration status in the soil, is dependent directly on the temperature, the cross-sectional area of the pore space and the concentration gradient of O_2 (Taylor and Ashcroft, 1972). A decrease in pore space in soils due to a high bulk density or high water content will reduce ODR.

The texture has a close relationship to soil aeration and nitrate reduction. Pilot and Patrick (1972) found that fine-textured soils required a higher air-filled porosity than coarse textured soils to allow oxygen to diffuse completely through soil cores. Diffusion of oxygen through the core kept the redox potential at high values and prevented nitrate loss. They also expressed the oxygen status of the soil indirectly by the soil-water tension. Critical levels were between 20 and 40 cm, depending on the soil texture, above which denitrification ceased.

Small pores retain water at higher suctions than bigger ones. A fine-textured soil, therefore, has more pore space filled with water at a given suction than a coarser textured soil. The oxygen diffusion rate is slower in water than in air (Taylor and Ashcroft 1972) and the presence of many small pores filled with water increases the tortuosity of the soil. Wesseling et al. (1957) reported that oxygen diffusion in soil became critical at about 85 - 90% saturation.

A soil with good granular structure may have better aeration due to increase in pore space, but there is the possibility of anaerobic microsites within the aggregates even though the surrounding soil is aerated (Broadbent and Clark 1965; Greenwood 1961).

Large columnar structure as it is found in solodized Solonetz B horizons were found to have a high potential for denitrification if glucose was added (Myers and McGarity 1972). Increasing O_2 concentration up to 15% and decreasing water content to 18%, with 0.1% glucose added, depressed denitrification but N_2O was still detected. Without glucose little denitrification occurred even when O_2 concentration and moisture content favoured reduction.

Factors which affect the ODR also affect the diffusion of nitrogenous gases. If N_2O diffusion is slow or it has a large distance to move through the soil, it is more likely to be further reduced to N_2 especially in cases where NO_3^- or NO_2^- have been depleted (Nommik 1956; Cady and Bartholomew 1961).

Cady and Bartholomew (1961) reported that N_2O production occurred in laboratory tests involving incubation at 7-8.5% O_2 of a sandy loam treated with N^{15} labelled KNO_3 . They concluded that free O_2 , which was preferentially used over nitrate, would be even more readily used than products resulting from partial reduction of nitrate, such as N_2O .

Focht (1974) reported that, with unsaturated conditions, N_2O would represent the major portion of a gaseous mixture arising from denitrification, while under saturated conditions (not too acidic) would represent only a small percentage of the total gaseous mixture. He attributed this to an increase in the rate of N_2O reduction rather than to an increase in its rate of formation with decreasing aeration.

Most denitrification studies are carried out in closed systems

which permit measurement of evolved gases. The diffusion of gases depends on concentration gradients from the soil system to the atmosphere or vice versa. In a closed system nitrous oxide is more likely to be reduced to N_2 due to internal circulation, while in the field it is more likely to escape to the atmosphere (Roulier and Fetter, 1973).

The aeration status is important not only in influencing the rate of denitrification, but also in determining whether any intermediate products might accumulate. The observation that nitrite is reduced at about 200 mV, which is a lower potential than for nitrate (Kefauver and Allison, 1957), probably explains why nitrite is commonly observed as a transient intermediate and is not reduced until most the nitrate has disappeared in soils (Bremner and Shaw 1958; Cady and Bartholomew 1960; Cooper and Smith 1963). However, most studies with soil (Nommik, 1956, Bailey and Beauchamp 1973b) have shown that nitrite, when added as substrate, is always reduced faster than nitrate, whether the two of them are incubated together or separately, under anaerobic conditions. This is because a high nitrite concentration inhibits nitrate reduction (Payne 1973). Consequently, where nitrite exists in low concentration as a transient intermediate, redox potential would be a factor determining whether or not nitrite would be reduced.

6) Effect of moisture

Moisture content indirectly influences denitrification through its effect on the oxygen diffusion rate and creates anaerobic conditions (Bremner and Shaw, 1958; Cady and Bartholomew, 1960; Bailey and Beauchamp, 1973a).

A change in water content from 20-32% caused a large increase in nitrogenous products (Stefanson, 1972). As mentioned before, in a study involving several soils, Pilot and Patrick (1972) found the critical soil moisture tension to be dependent on texture. Below the critical soil moisture tension there was an initial rapid increase in reduction which levelled off with a further increase in moisture. Above this tension, there was little denitrification. At the critical tension erratic results were obtained and this was probably due to the formation of anaerobic microsites (Greenland, 1962).

Losses of nitrogen occur as low as 60% of water holding capacity (Bremner and Shaw 1958). This may be a direct effect of moisture on the mobility and size of bacterial population.

Following submergence, soil oxygen is rapidly consumed by the aerobic soil microflora (Macrae et al. 1968). Diffusion of oxygen into the soil is retarded, and only the surface few millimeters remain aerobic (Patrick and Delaune 1972). There was little difference between the oxygen flow through 3 mm (67% water) and 10 mm (100% water) of standing water above the soil surface (Cho, 1976). Conditions favoring the activities of denitrifying microorganisms are thereby established and the inorganic nitrogen becomes much less stable in flooded soils than in aerobic soils (Patrick and Reddy, 1976).

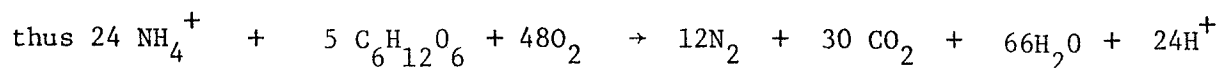
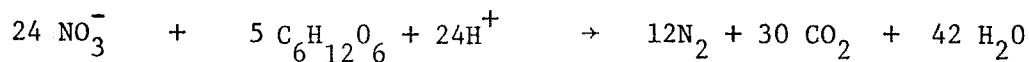
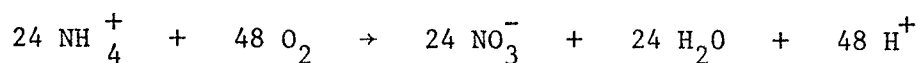
Laboratory experiments have shown that nitrate reduction after water-logging can be very rapid. The rate of denitrification was between 15 and 250 ppm N/day depending mainly on the amount of energy material (Yamane, 1957; Bremner et al. 1958; Patrick, 1961; and Patrick and Delaune 1972). Flooded soils are characterized by two distinct soil layers:

1. A surface aerobic (oxidized) layer, ranging from a few mm in thickness in a soil with high biological activity to 2 cm or more in thickness in soil with a low biological activity,
2. An underlying anerobic (reduced) soil layer (Pearsall and Mortimer 1939; Alberda, 1953; Howeler and Boulden, 1971; and Patrick and Delaune, 1972).

Inorganic nitrogen is present as nitrate in the oxidized layer and as ammonium in the reduced layer (Pearsall and Mortimer, 1939). Thus in many flooded soils, a condition exists under which both nitrification and denitrification can proceed at the same time.

Recent experiments have shown that appreciable denitrification will occur in flooded soils if both oxygen from the atmosphere and ammonium from the flooded soil are available (Broadbent and Tusneem 1971; Patrick and Gotoh 1974). The nitrogen converted from nitrate in flooded systems was derived from ammonium oxidized to nitrate in the aerobic layer. The amount of nitrogen gas produced usually greatly exceeds the amount of ammonium and nitrate present in the aerobic surface layer at any one time, (Patrick and Delaune, 1972; Patrick and Reddy 1976).

According to Patrick and Reddy (1976), removal of ammonium by nitrification in the aerobic layer creates a concentration gradient, which causes ammonium in the underlying anaerobic layer to diffuse upward to the aerobic layer where it undergoes nitrification. Nitrate formed by this process diffuses down toward the nitrate-free anaerobic layer where it is denitrified to nitrogen gas by serving as an electron acceptor in the oxidation of organic matter. They suggested the following pathway for this process.



Nitrate is an intermediate product in the overall pathway between ammonium and elemental nitrogen. Laboratory experiments utilizing N^{15} as a tracer support this mechanism. Approximately one-half of the nitrogen involved in the nitrification-denitrification process was originally present in the surface aerobic soil or water layer with the remainder diffusing up from the underlying anaerobic layer.

The limiting factor for this process, under normal conditions e.g. the pO_2 above flooded water equals 20%, is the rate of ammonium diffusion to the aerobic layer, since the diffusion coefficient for NO_3^- is about six-fold greater than ammonium diffusion (Patrick and Reddy 1976). These results are applicable for flooded soil in which no downward percolation occurs that could prevent upward diffusion of ammonia.

Chemical Denitrification

Losses of added nitrogen in sterile soils, as well as non-sterile soils when conditions are unfavourable for biological denitrification, such as high oxygen concentration, low moisture content or an acidic reaction, indicate that there are chemical mechanisms involved.

Chemo-denitrification involves loss of gaseous nitrogen to the atmosphere due to nitrite instability (Clark, 1962). Information concerning the chemo-denitrification processes, emphasize the complexity of these processes, and suggest that a whole series of reactions may be taking place. It occurs primarily in soils in which the nitrification process is sufficiently retarded to permit accumulation of nitrite as an intermediate product in the transformation of ammonium to nitrite (Clark et al. 1960; Allison, 1966; Jones and Hedlin, 1970; Bollag et al. 1973).

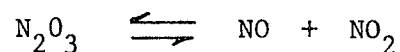
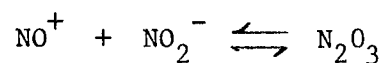
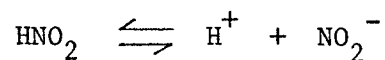
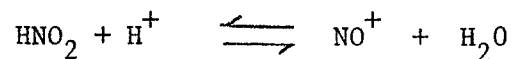
Possible pathways for denitrification are, chemical decomposition of nitrous acid, interaction of nitrous acid with amino acid and ammonia (Van Slyke-type reaction), and Nitrosation of organic matter (aromatic structure) (Broadbent and Clark, 1965; and Stevenson et al. 1970).

I. Chemical decomposition of nitrous acid

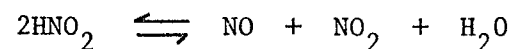
The self decomposition of nitrite-N in the soil involves the HNO_2 molecule rather than the NO_2^- ion, and as a result is highly pH dependent (Sneed and Brasted, 1956; Nelson and Bremner, 1969). Equilibria involving this acid, and some thermodynamic

properties are reported by Latimer (1952). The free energy of formation of the nitrite ion is - 8.25 K Cal/mole, and the free energy of formation of nitrous acid is - 12.82 K Cal/mole therefore the free energy of ionization is 4.570 K Cal/mole, with dissociation equilibrium constant of 4.5×10^{-4} .

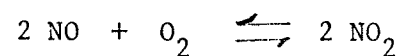
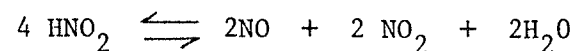
According to Nelson and Bremner (1970a) the self decomposition of nitrous acid in the anaerobic system occurs as follows:

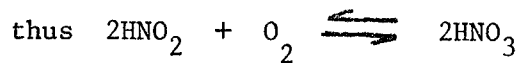
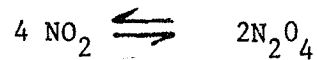


thus



Moreover, the following reaction describing the oxidation of HNO_2 to HNO_3 in an aerobic moist system has been given by Reuss and Smith (1965), Nelson and Bremner (1970a),





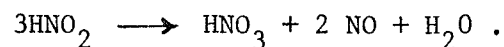
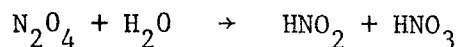
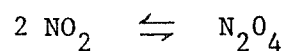
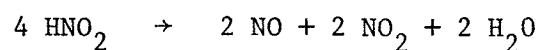
It is noted that the gases, NO, and NO₂, are both intermediate in this reaction. In an open system, these gaseous compounds might be lost from the soil (Gerretsen and DeHoop, 1957). Most of the evidence suggests that NO is likely to be rapidly oxidized to NO₂ and some nitrate is produced due to hydration of the NO₂ during diffusion of these gases through the reaction medium, (Broadbent and Clark, 1965; Broadbent and Stevenson, 1966). The amount of nitrate formed will depend mainly upon the rate of diffusion of these gases and the nature of reaction medium (Nelson and Bremner).

Indirect evidence to support this mechanism was presented by Nelson and Bremner (1970a). They showed that NO and NO₂ are converted to nitrate if the systems, e.g. an aerobic closed system, do not contain a reagent such as alkaline KMnO₄ solution that sorbs these gases. They also showed that the amounts of nitrate produced in closed systems were much larger than the amounts produced in open systems. Therefore, due to the internal circulation of these gases little loss of nitrogen occurs (Broadbent and Clark, 1965).

Reuss and Smith (1965) reported that only 3% of nitrite in a pH 3 buffer solution was converted to nitrate when helium was bubbled

through this solution. The helium had swept away the NO and NO₂ from the reaction medium, and prevented the conversion of NO₂⁻ to NO₃⁻.

Another mechanism describing self-decomposition of nitrous acid in anaerobic system was proposed by Reuss and Smith (1965) and Smith and Clark (1960). They reported that the reaction would be:



Smith et al. (1960) concluded that this reaction was a pathway for gaseous loss of nitrogen, since they found that the NO₃⁻ produced was one-half of the NO evolved when an acidic soil was incubated with nitrite. Nitric oxide was found to form when the soil was incubated with nitrate under anaerobic conditions (Wijler and Delwiche 1954; Nommik, 1956; Cady and Barthomew, 1960, 1963; Bollag et al. 1973). Its formation has been attributed to chemical decomposition of nitrite formed by microbial reduction of nitrate at pH 4.5 (Cady and Barthomew 1960) and at pH 5.0 (Bollag et al. 1973).

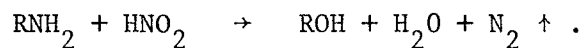
Further reactions concerning the gases produced by nitrite decomposition in soils were reported by Nelson and Bremner (1970a). They

obtained indirect evidence that NO or NO₂ was reduced chemically by soil constituents to N₂ and N₂O. They found that NO, N₂, and N₂O were detected when KMNO₄ solution was absent in the incubation vessel. However, the inclusion of KMNO₄ solution significantly reduced the amount of N₂ and N₂O. Their reasoning was confirmed by gas chromatographic studies showing that N₂ and N₂O were formed when NO was injected into a container containing 20 gm. of moist soil under a helium atmosphere.

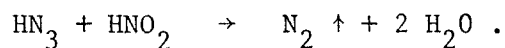
Also it is believed that NO reacts with organic components of the soil to form N₂O, CO₂ and presumably N₂ (Stevenson et al. 1970).

2. Interaction of nitrous acid with amino acid and ammonia (Van Slyke-Type reaction).

Under an atmosphere of NO and in the presence of glacial acetic acid amino acids reacted, within 5 minutes, with HNO₂ to produce N₂ (Allison, 1963). This reaction, known as the Van Slyke reaction, is



The reaction with ammonia is



Ammonia released 62% of its nitrogen in ten minutes but took 1.5 to 2 hours to react completely.

Many workers have postulated that the reaction of nitrous acid with amino acid compounds to yield molecular nitrogen occurs under certain conditions in the soil (Allison et al. 1950; Reuss and Smith, 1965; Stevenson et al. 1970). Allison et al. (1950) found that high concentrations of nitrous acid e.g. 500 ppm can react with an amino acid, such as alanine, to form nitrogen gas at pH values of 4.5 and lower. The loss of nitrogen from HNO_2 varied from 1.2% at pH 1.6 to a maximum of 5.8% at pH 3.4, and no gas was evolved at pH 5.2 or above within 5 hours. Stevenson et al. (1970) concluded that the amount of N_2 lost via the van Slyke reaction from soil and an unhydrolyzed preparation of humic acid was higher than from humic acid hydrolyzed with 6N HCl. The hydrolyzed humic acid contained fewer amino groups than the unextracted organic matter.

However, some investigators (Broadbent and Clark, 1965; Bremner and Nelson, 1968; Allison, 1963) have found that the addition of large amounts of amino acid and urea to sterilized moist soils treated with nitrite did not promote nitrite decomposition or the formation of N_2 .

The reaction of HNO_2 with ammonium has also been investigated. Gerresten and deHoop (1957) reported that at pH of 4.0 to 4.5 nitrogen gas was evolved in 6 hours from sterile buffered solutions containing substantial amounts of both ammonia and nitrite nitrogen under NO atmosphere.

Loss of N by this mechanism was greatest when the ratio of $\text{NH}_4^--\text{N}/\text{NO}_2^--\text{N}$ was 5:1 (Wahab and Uddin, 1954). Jones and Hedlin (1970) added a solution containing the same ratio (5:1) of

$\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$ to two soils which were autoclaved. They found the loss of nitrite-N occurred without an increase in $\text{NO}_3^-\text{-N}$. These losses were considerably greater than those obtained from autoclaving the same soils with added nitrite but without added $\text{NH}_4^+\text{-N}$, indicating that the ammonium ion contributes to the loss of nitrogen from nitrite. Stevenson et al. (1970) showed that significant amounts of N_2 were produced from NH_4Cl and from several amino compounds when reacted with 1M NaNO_2 in a pH 6.0 phthalate buffer for 24 hours. Furthermore, Allison (1963) reported that the greatest interaction between NH_4^+ and NO_2^- ions might be expected where the soil pH is between 5 and 6.5. In more acid soils the nitrite would probably be so unstable that there would be little opportunity for interaction with NH_4^+ .

Generally, it is accepted that NO_2^- reacts more readily with amino acid than with ammonium compounds (Stevenson, 1970; Allison et al. 1952; Smith and Clark, 1960). Allison et al. (1952) found that the reaction of nitrite with ammonium sulfate was approximately one-third as fast as its reaction with alanine. Smith and Clark (1960) found only traces to no more than 0.5% of applied nitrogen was evolved as N_2 when $(\text{NH}_4)_2\text{SO}_4$ was incubated with a substantial amount of NaNO_2 for three days in a soil with pH of 4.1. However, substituting $(\text{NH}_4)_2\text{SO}_4$ with alanine resulted in evolution of 100% of the applied nitrogen as N_2 . The latter authors have shown that under a helium-oxygen atmosphere, the rate of N_2 production was greatly reduced while the production of NO_3^- was increased. They showed that the tendency for nitrite to convert

chemically to nitrate was three to four times as strong as for it to react with the ammonium ion. This tendency was even more rapid in an aerobic soil than in solution. Stevenson *et al.* (1970) reported that significant amounts of N_2 were produced from several amino compounds and NH_4Cl in a pH 6.0 phthalate buffer in 24 hours. The recovery of amino-N as N_2 from alanine, glutamic acid, leucine, and glucosamine was nearly quantitative, whereas only 28.2% of the nitrogen from NH_4^+ was recovered as N_2 .

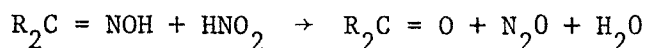
3. Nitrosation of organic matter

Many workers have postulated that organic matter plays an important role in chemical denitrification. Organic compounds contain amino and phenolic groups which may cause loss or fixation of added NO_2^- (Nelson and Bremner, 1969; Jones and Hedlin 1970; Porter, 1969). Jones and Hedlin (1970) showed that the organic portion of the soil was not responsible for N-loss from a soil with high pH value (i.e. pH 8), but it is significant in an acid soil of pH 6.2 when the soils were air dried after addition of nitrite. Nelson and Bremner (1969) found that the amount of nitrite -N self decomposed and the amount fixed or converted to N_2 on treatment of soil with nitrite at pH 5 increased, with increase in soil organic matter content. The isolated organic materials they tested (humic acid and lignin) also promoted nitrite decomposition, fixed nitrite-N and converted nitrite to N_2 and N_2O . On the other hand the soil pre-treated to remove organic matter, and the inorganic materials (quartz, sand and clay minerals)

did not promote nitrite decomposition and did not fix nitrite -N or reduce nitrite to N_2 or N_2O . These findings leave very little doubt that organic soil constituents were responsible for the evolution of N_2 and N_2O , and for the fixation of nitrite -N. Their results support previous evidence (Bremner and Nelson, 1968) that inorganic soil constituents play little, if any, part in the reactions observed on treatment of soils with nitrite.

It has been postulated that the nitrosation of the organic molecule takes place before the formation of various gases, a process which denotes the addition of nitroso group (-N=O) to an organic molecule, and is brought about by HNO_2 (Porter, 1969, Stevenson, et al. 1970). The mechanism involved in the formation of N_2 , N_2O and NO, by the reaction of NO_2^- with phenolic constituents of soil organic matter are only partially understood.

Austin (1961) pointed out that NO_2^- per se is not a direct participant in the nitrosation reaction. Nitrosation is effected by a family of species derived from NO_2^- such as the nitrous-acidium ion, H_2O^+ . NO, or the nitrosonium ion, NO^+ . Nitrosation leads to the formation of nitroso, C-N=O, and oximino, C=NOH compounds, which subsequently react with excess NO_2^- to form N_2O according to the following reaction,



Porter (1969) reported that soil organic colloids reacted with hydroxylamine to form oximes and under the most reactive condition

(pH 5.0 and 50°C) N_2O was the major gaseous product for most of the oxime compounds reacted with $NaNO_2$. Some oxime compounds produced essentially no N_2O but a considerable amount of NO. The negligible amounts of N_2O produced under less reactive conditions (i.e. 23°C and pH 6) with $NaNO_2$ suggests that the decomposition of oximes is probably not a major pathway for N_2O loss from soils.

Stevenson et al. (1970) found that, in most instances, the gas obtained by reacting NO_2^- with the lignin and humic preparation at pH 6 and 7 was NO. The production of other gases included N_2 , N_2O , and CO_2 . The ratio of N_2O to N_2 ranged from .09 to .18.

Factors affecting chemical denitrification

Among the factors that influence the degree and kind of gaseous products due to chemical denitrification are soil pH, concentration of NO_2^- , and soil constituents and metallic cations.

1) Effect of pH

In general chemical denitrification of nitrite decreases with increasing pH. At neutral or alkaline soil pH little nitrite decomposition has been observed (Reuss and Smith, 1965; Bollag et al. 1973; Nelson and Bremner, 1969; Sneed and Brasted, 1956).

As has been mentioned before, self decomposition of nitrous acid is related to the amount of nitrite-N present as the HNO_2 molecule. Sneed et al. (1956) reported that the presence of HNO_2 molecules is pH dependent,

with the equilibrium constant of 6.0×10^{-4} . At pH values of 5, 4 and 3, the amount of nitrite-N existing as HNO_2 is 1.6, 14, and 63%, respectively.

The effect of soil pH upon the chemical denitrification in soil could be an indirect one. The accumulation of nitrite in soil after the application of ammonium form of fertilizer is affected by soil pH. The presence of NH_4^+ in large quantities, which increases the pH initially, will inhibit the biological oxidation of nitrite to nitrate (Stevenson, et al. 1970; Pang et al. 1975). Clark et al. (1960) plotted the initial pH against percent Urea-N not recovered and found that the peak was located at slightly alkaline pH. When a similar plot was made for KNO_2 the peak was at about pH 6. It is generally believed that with an accumulation of NO_2^- the pH decreased and HNO_2 decomposed (Bollag et al. 1973; Nelson and Bremner 1969). Although substantial amounts of NO_2 were produced in the neutral soil there was a good negative correlation between soil pH and the amount of NO_2 formed (Nelson and Bremner 1969), with production of NO_2 and NO becoming particularly important at pH 5 (Bollag et al. 1973).

2. Effect of nitrite concentration

Increasing the amount of NaNO_2 added resulted in a proportional increase in NO evolution in a soil of pH 4.5 (Cady and Bartholomew, 1960).

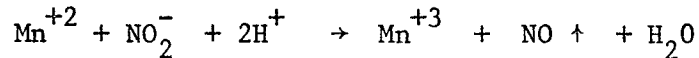
Stevenson et al. (1970) reported that N_2 gas recoveries of amino-N in pH 6.0 phthalate buffer solution after 24 hours were lower with a more dilute NaNO_2 solution. For example, 47% of the amino-N of alanine was recovered as gas using 0.5 m NaNO_2 , whereas, only 5% was obtained with 0.1 m solution. Jones and Hedlin (1970) found that the nitrogen lost was a function of the applied concentration of NO_2^- . They found that there was an initial increase in percentage of NO_2^- -N lost with increases in concentration. However, at 1600 ppm NO_2^- -N rate the percentage loss of applied NO_2^- -N in three soils with a pH range of 6.2 to 8.0 was about half that of the 200 ppm rate indicating the development of some factors limiting nitrogen loss at very high nitrite concentrations.

The amount of NO_2^- -N fixed by organic matter was not influenced by moisture content, but nitrite recovered increased with an increase in moisture and may have been due to a dilution effect (Nelson and Bremner, 1969).

3. Influence of metallic ions and soil constituents

Studies with soil and soil extracts have suggested that the reduced forms of copper, iron, manganese and certain aluminum salts were responsible for conversion of nitrite to NO (Wullstein and Gilmour

1964, 1966). The reaction, which involved a high concentration of both metal and nitrogen, was proposed as follows:



However, the work carried by Nelson and Bremner (1970b) has indicated that Fe^{+2} , Cu^+ and Sn^{+2} at pH 5.0 did not promote loss of nitrite when their concentrations were below 22, 64 and 24 ppm, respectively.

When soils known to decompose nitrite were extracted, it was found that the concentration of these metals were far below the above levels.

Evidence was obtained for a slight enhancement of Fe^{+2} in the deamination of glycine by NaNO_2 but the change was too small to be important (Stevenson et al. 1970).

It was concluded that organic matter was the agent promoting denitrification. In most studies comparing solution with a soil system, soil was found to influence the production of nitrogenous gases. Soil increased the tendency for NO_3^- to be formed by increasing the self decomposition of NO_2^- in aerobic system (Smith and Clark, 1960), and promoted the evolution of N_2 , since it is believed that significant amounts of the NO formed in the soil reactions had been converted to N_2 (Stevenson et al. 1970)

Reuss and Smith (1965) found that no N_2 was detected in the system where 20 mg. of NO_2^- -N were added to 50 g of calcium-saturated cation exchange resin buffered at pH 5.2. On the other hand N_2 was rapidly released by an acid soil at the same pH. They concluded that the N_2 released from the soils when NO_2^- was added had resulted from reaction of

NO_2^- or HNO_2 with soil constituents or from reaction that had been catalyzed by these constituents.

The pH independence of the gases evolved by various soils suggested that pH was not the only factor influencing chemical denitrification (Bulla et al. 1970).

Jones and Hedlin (1970) found that, although nitrite instability in the soils is related to pH, as shown by soil and buffer treatments, the presence of soil confers much greater instability than would be predicted from pH alone.

Bremner and Nelson (1968) found that the amount of NO_2 formed by decomposition of nitrite in soils having pH values between 5 and 7 is much larger than the amounts formed by self-decomposition of nitrite in buffer solution having the same pH values. They concluded that the soils contain regions having much lower pH values than the value obtained in determination of soil pH. Support for this explanation is provided by investigations showing that the pH at the surface of clay particles is much lower than the pH of the solution around these particles (Harter and Allrichs, 1967).

Isotopic methodology in denitrification study

In conventional denitrification studies involving the heavy isotope ^{15}N , the usual procedure has been to add the enriched ^{15}N material to soil, carry out the incubation, and then analyze the residual products. From such analysis of residual products inferences are drawn

with respect to the composition of the N_2 gas evolved (Wilson et al. 1948).

The amount of ^{15}N contained in a nitrogenous material is usually determined by the Kjeldhal procedure by converting the ammonia to N_2 by reacting with alkaline hypobromite (Rittenberg et al. 1939).

A direct analysis of the gases evolved has been applied by many investigators (Nommik, 1956, Hauck et al. 1956; Hauck et al. 1958; Cady et al. 1960; Cooper and Smith 1963; Burfort and Bremner 1975; Bremner, 1965). These investigations are carried out by using gas chromatography, infrared analysis and mass spectrometry, under anaerobic degassed or artificial atmospheres e.g. Ar, He or Ne atmosphere. The reason for the use of such atmospheres was to exclude N_2 so that any N_2 produced by the denitrification process could be measured.

The fundamental assumptions for these studies are that the behavior of ^{15}N in physical, chemical and biological processes are identical to that of ^{14}N . Therefore, these processes do not lead to variation in the relative abundance of nitrogen isotopes in soils and other natural materials. However, the validity of these basic assumptions has not been well established, since it is found that the natural abundance of ^{15}N might be different in soil than in the atmosphere.

Nier (1950) reported that in room air, the ratio $^{14}N/^{15}N$ is (273 ± 1) , thus the natural abundance of ^{15}N in atmosphere nitrogen is 0.366 atom percent. However, Cheng and Bremner (1966), Cheng et al. (1964), Owens (1960), and Chien et al. (1977) found that the atom percent

of ^{15}N of soil nitrogen is usually higher than that of atmospheric nitrogen, but it rarely exceeds 0.380.

The abundance of ^{15}N relative to ^{14}N is altered during the microbiological reduction of nitrogen compound, since the $^{14}\text{NO}_3^-$ and $^{14}\text{N O}_2^-$ species have been preferentially reduced (Wellman et al. 1968; Blackmer and Bremner 1977). Moreover, the variation in the abundance of ^{15}N in soils is small, but it cannot be attributed to analytical errors and should be considered in studies of nitrogen transformation in soils when % ^{15}N is used as a tracer at low level (Cheng et al. 1964). The higher the level of ^{15}N in the added nitrogen, the more valid the assumptions become (Blackmer and Bremner 1977).

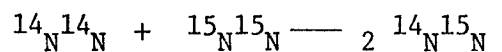
For equilibrium situations e.g. hypobromite method, by which labelled and unlabelled nitrogen atom are combined nitrogen ions of the formula $(^{14}\text{N}^{14}\text{N})^+$, $(^{14}\text{N}^{15}\text{N})^+$ and $(^{15}\text{N}^{15}\text{N})^+$ appear in the mass spectrum of N_2 gas. The relative number of ions of each type approach the ideal statistical values given by the terms of the equation

$$(p + g)^2 = p^2 + 2pg + g^2.$$

The terms on the right hand of this equation are proportional to a.m.u. 28, 29 and 30 peaks in the mass spectrometer, where p is the atom fraction of ^{14}N , g the atom fraction of ^{15}N , and $(p + g)$ is equal to unity (Hauck et al. 1958). This equation is also applied for the N_2 evolved due to the combination of nitrogen from two nitrate ions during

denitrification.

The isotopic equilibrium existing in individual samples of N_2 gas prepared by the hypobromite reaction can be described in terms of N_2 isotopic exchange reaction



Keq. = 4.

This reaction does not proceed, however, at a measurable rate at any temperature encountered in denitrification studies (Hauck et al. 1958). Therefore a mixture of two different samples of N_2 gas with different mass distribution will remain as distinct molecules and not yield an isotopic equilibrium.

The atom percent ^{15}N in N_2 gas for the equilibrium case can be calculated as follows:

$$\begin{aligned} \text{The \% } ^{15}N &= \frac{\text{NO. of } ^{15}N \text{ atoms}}{\text{NO. of } (^{14}N + ^{15}N) \text{ atoms}} 100 \\ &= 100 \frac{g}{p+g} \\ \text{Therefore \% } ^{15}N &= 100 \frac{R^1}{2 + R^1} \quad (\text{Bremner, 1965}) \\ \text{Where } R^1 &= \frac{\text{ion current at mass 29}}{\text{ion current at mass 28}} \end{aligned}$$

This equation cannot be used if the sample contains a mixture of N_2 originating from different sources such as air and labelled nitrate. Additional information such as the ratio of a.m.u. 29 to 30 is needed in order to calculate the fraction of ^{15}N in nitrogen gas (Hauck et al. 1958). Using only a.m.u. 29 and 30 due to N_2 , or similarly the a.m.u. 45 and 46 due to N_2O the % ^{15}N can be calculated as follows:

$$\% \text{ } ^{15}\text{N} = \frac{200}{R+2}$$

$$\text{where } R = \frac{\text{a.m.u. (29)}}{\text{a.m.u. (30)}}$$

If a sample contains NO the problem of assigning %¹⁵N in N₂ and NO also becomes very difficult. Nommik and Thorin (1972), for example, assumed that the %¹⁵N in N₂ derived from denitrification in the absence of atmospheric N₂ could be calculated using measured a.m.u. 28 and 29 in order to calculate %¹⁵N in the NO produced.

The use of an air atmosphere for denitrification studied has definite advantages when denitrification is studied under flooded conditions, since the atmosphere closely resembles the natural system (Cho and Sakdinan 1978).

MATERIALS AND METHODS

Soil Samples

Three soils, differing widely in point of origin, were collected from the Ap horizon (0-15 cm) of Keld, Wellwood and Morton associations, air dried and ground to pass through a 2 mm sieve. These soils were selected primarily to achieve differences in pH. The range in pH among the three was from 5.4 to 7.8. The locations of the soils and some of their chemical and physical properties are shown in Table 1.

Apparatus

The incubation vessel for soil samples consisted of B₂₄ ground glass joint-tube with an interior diameter of 2.4 cm and a length of 10.5 cm. The upper section of the incubation vessel consisted of a 2 mm stopcock to which was attached a $\frac{10}{18}$ cone ground glass joint. These two sections were connected to each other by using Corning high vacuum grease (Figure 1-A) (in order to do quantitative analysis for gases involved). The volume of the sealed container was adjusted to 42.1 ml by adding glass beads. The volume of the incubation vessel was calculated from the weight of water it held.

The gas sample containers consisted of similar glass tube, which

Table 1. Legal Location and Some Physical and Chemical Properties of the Soils.

Soil	Keld	Wellwood	Morton
Legal Location	S 15-25-20	SE 30-11-14	W 23-16-2E
Texture	VFSL	FSL	L
C.E.C. (meg/100g)	33.2	20.1	25.9
pH	5.4	6.3	7.8
Conductivity (mmho)	0.15	0.5	2.6
% Organic matter	8.1	4.3	5.9
% CaCO ₃	0.5	0.2	30.9

was topped with a right angle 2mm stopcock. To this was attached a $\text{F } 10/18$ socket glass joint (Figure 1-C). Two gas sample containers were used for each sampling, one containing solid KOH, the other containing three ml concentrated H_2SO_4 .

In order to sample the gases produced, the incubation container was vigorously shaken, and then the gas sample containers were fitted to a sampling apparatus (Figure 1-B). The gas container stopcocks were opened and the sampling apparatus was evacuated with the aid of the rotary pump to a pressure of 5 Torr, which was monitored with a Micro-McLeod gauge. The stopcock between the vacuum pump and sampling apparatus was then closed. The gas in the incubation tube was then expanded into the evacuated sample containers. After gases were transferred, the gas sample containers were removed from the vacuum line for mass spectrometric determination of a.m.u. between 28 and 46.

The gas container containing solid KOH, was shaken vigorously to remove CO_2 and moisture, while the container with H_2SO_4 was left to stand for awhile to remove the moisture.

The gas container was connected to the V.G. Micromass 602 isotopic ratio mass spectrometer and scanned for all pertinent a.m.u. between 28 and 46 inclusive.



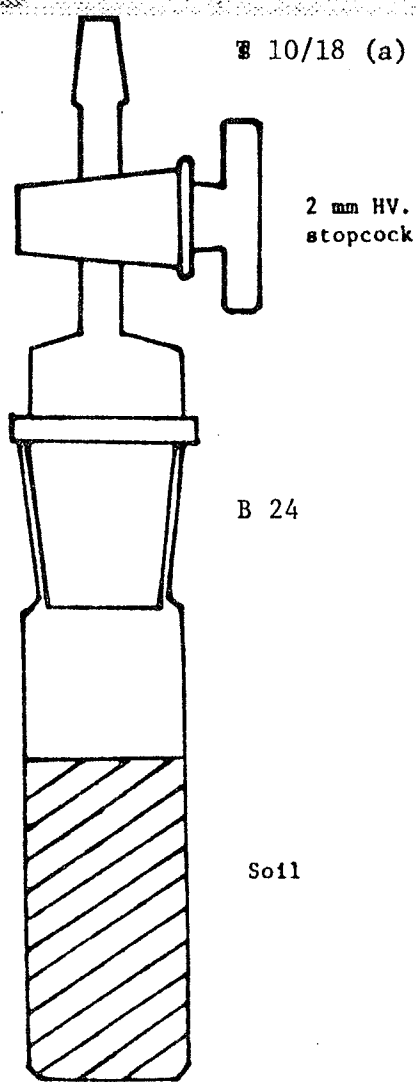
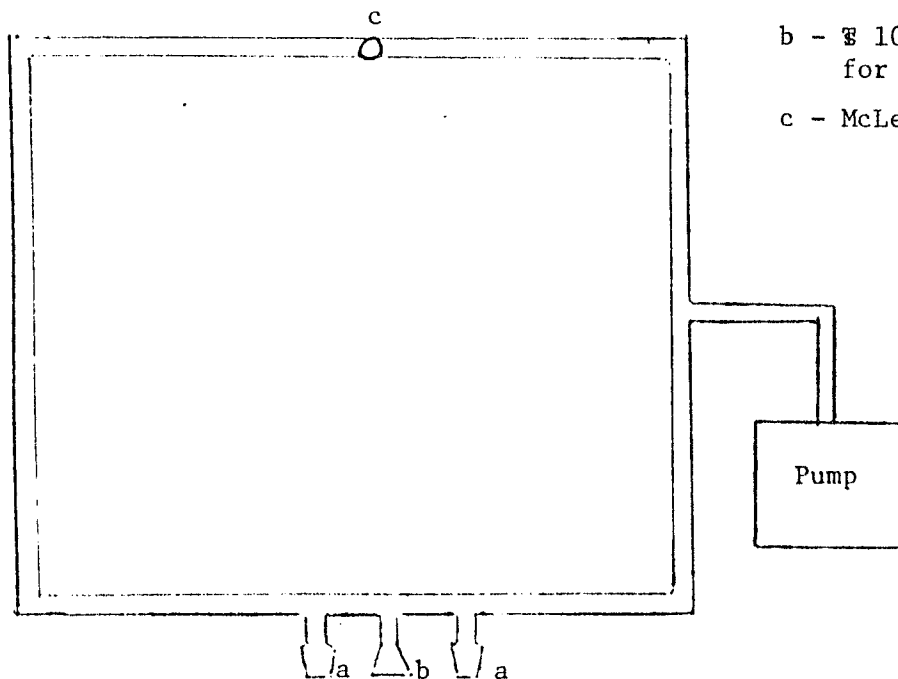


Fig. 1-A Incubation Vessel



- a - 10/18 Inner attachment for gas containers
- b - 10/18 Outer attachment for incubation vessel.
- c - McLeod Gauge

Figure 1-B Sampling Apparatus

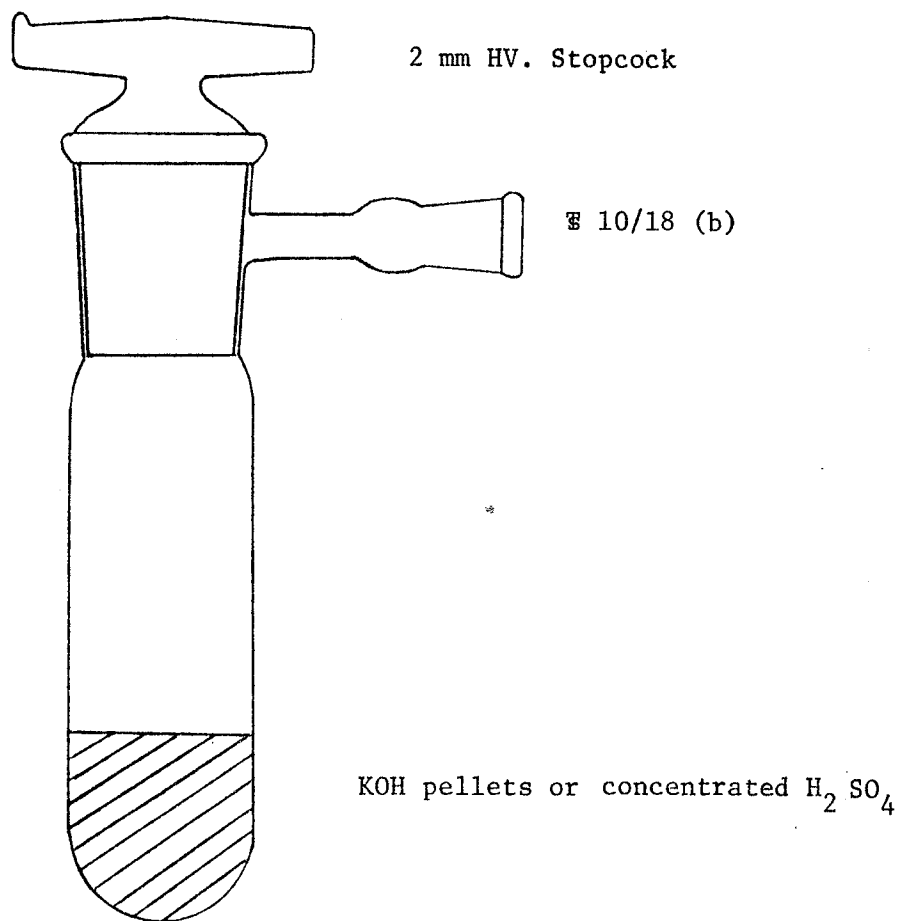


Figure (1-C) Gas Sample Container

Experiment 1. Denitrification of $\text{Ca}(\text{NO}_3)_2$ in the Experimental Soils

The experiment was carried out to determine the effect of temperature and concentration of added NO_3^- -N on the rate of denitrification for the three soils.

Samples of Keld soil -pH 5.4, Wellwood soil -pH 6.3 and Morton soil -pH 7.8 were used. Fifteen grams of the soil were incubated at 100% moisture content (oven dry basis). This flooded the soil and the depth of stagnant water above the soil surface ranged from 1.7 to 2 cm for different soils. Three different concentrations - 100, 150 and 200 ppm - corresponding to 1.50, 2.25 and 3.00 mg per 15 g of soil were used. Three incubation temperatures, namely 15, 25 and 35°C were used. Calcium nitrate enriched with N-15 at 52.3% was used.

After adding the soil and $\text{Ca}(\text{NO}_3)_2$ solution into the incubation vessel, the pressure was reduced to 650 mm Hg. by withdrawing air. This was done to accommodate the produced gases. There was a volume of approximately 25 ml available for the gas mixture. Sampling of the gases was carried out eight times in two weeks. Each treatment was duplicated.

Gas analysis was carried out mass spectrometrically by scanning from 28 to 46 a.m.u. peak heights. Quantitative estimation of the gases, N_2 , NO , O_2 , CO_2 , and N_2O were carried out as will be described later. Samples of the soil analyzed for inorganic nitrogen at the same incubation intervals as the gas analyses.

Chemical Analysis

Nitrate and Nitrite Nitrogen

Ten grams of the soil in the incubation vessel after gas sampling were mixed with 50 ml of water and stirred vigorously for 1 hour. The suspension was filtered through Whatman No. 42 filter paper. The method used to determine Nitrate-N and Nitrite-N, was a variation of that of Kamphake et al. (1967), employing a Technicon autoanalyser.

Nitrite-N initially present in the sample was determined by a conventional diazotization-coupling reaction. In order to determine the amount of nitrate-N, nitrite-N was removed from another portion of sample by reacting 0.3 ml of 3% sulfamic acid solution with 10 ml of sample for half an hour. The nitrate in the solution was quantitatively reduced with hydrazine sulfate to nitrite which was then determined by the same diazotization-coupling reaction.

Nitrate standards were run with reducing agent and nitrite standards without reducing agent. Percent transmittance was converted to optical density and plotted against concentration.

Ammonium Nitrogen

The ammonium-N was extracted by shaking 10 g of the moist soil (100% moisture) in 50 ml of 2N KCl solution for one hour. The suspension was filtered through Whatman No. 42 filter paper. Ammonium-N levels were determined with an Orion ammonia electrode coupled to a Fisher model 520 digital pH/ion meter by the method of Mertens et al. (1975).

CaCO₃

The CaCO₃ content was determined manometrically by the method of Skinner et al. (1959).

Organic Matter

The soil organic matter was determined by the potassium dichromate-conc. H₂SO₄ method described by Peech et al. (1947).

Conductivity

The electrical conductivity was measured with a Radiometer conductivity meter with a soil-water ratio of 1:1.

Soil pH Determination

Five grams of air dry soil were mixed with 20 ml of water and stirred vigorously for 15 minutes prior to pH determination. PH was determined using a Fisher model 520 digital pH/ion meter equipped with a combination glass/calomel electrode.

Experiment 2. Denitrification of $\text{Ca}(\text{NO}_3)_2$ in Keld and Wellwood

Soils with Added Lime

This experiment was carried out to evaluate the effect of pH upon denitrification.

The pH of samples of Keld soil was adjusted from an initial value of 5.4 to 6.3 and 7.3 by the addition of 4% and 12% CaCO_3 , respectively. The pH of Wellwood soil was also adjusted from its initial value, pH 6.3, to 7.3 by adding 6% CaCO_3 . To these samples was added 52.3% ^{15}N enriched $\text{Ca}(\text{NO}_3)_2$ at the rate of 100 ppm. Samples were sealed and incubated as in the previous experiment, except that the incubation was at 25°C only. Treatments were duplicated. Gas analysis and inorganic -N determination were carried out as mentioned before.

Quantitative Estimation of Gases in the Incubation Atmosphere

In order to estimate the gases produced or consumed during denitrification quantitative mass spectrometry of the incubation atmosphere was carried out using the inert gas (argon) as a reference a.m.u. The gases in the sample container were introduced into the mass spectrometer, following removal of H_2O and CO_2 with KOH. The peak height of Argon (a.m.u. 40) was chosen as a standard. Before each scan, a.m.u. 40 was selected and its measured

intensity was adjusted to a predetermined peak height by varying the inlet pressure. The peak height of a.m.u. 40 was considered to be 100 and the other measured peak heights were normalized to this figure. An air sample was identically scanned. The air contribution of each a.m.u. in the sample was deducted in order to calculate the peak height of produced or consumed gases. The peak height of CO_2 was calculated from the difference in the production of a.m.u. 44 between H_2SO_4 and KOH containers. Standard curves were developed to obtain a relationship between partial pressure and peak height by scanning known amounts of different gases mixed in varying proportions with air. For nitrogen and oxygen, the peak heights due to N_2 and O_2 in the air sample were used to calculate the partial pressure of produced and consumed values.

Fragmentation of N_2O to NO and to N_2 within a mass spectrometer was believed to occur as follows:



or



It was empirically found that fragmentation products were

$$(\text{NN})_{\text{F}} = 0.053 (\text{NNO}) \quad (3)$$

and

$$(\text{NO})_{\text{F}} = 0.177 (\text{NNO}) \quad (4)$$

where subscript (F) stands for fragmentation product. The fragmentation corrections on individual a.m.u. were carried out.

The following method of calculation was adopted to determine the peak heights of produced gases and % ^{15}N in the gases. The peaks at 45 and 46 for the KOH gas sample were considered to be N_2O only, since use of KOH in gas tubes absorbed most CO_2 present. The peak at 44 was not considered clean, since the gas sample contained O_2 resulting in CO_2 production within the mass spectrometer. The $(44)\text{N}_2\text{O}$, the peak of a.m.u. 44 due to N_2O , was calculated by using the equilibrium equation:

$$(44)\text{N}_2\text{O} = \frac{[(45)\text{N}_2\text{O}]^2}{4(46)\text{N}_2\text{O}} \quad (5)$$

$$(44)\text{N}_2\text{O} = \frac{R^2(46)\text{N}_2\text{O}}{4} \quad (6)$$

where R is equal to $\frac{(45)\text{N}_2\text{O}}{(46)\text{N}_2\text{O}}$

The total peak height due to N_2O was the sum of a peak height of $(44)\text{N}_2\text{O}$, $(45)\text{N}_2\text{O}$ and $(46)\text{N}_2\text{O}$. With the measured peak heights of a.m.u. 45 and 46, designated as $(45)\text{N}_2\text{O}$ and $(46)\text{N}_2\text{O}$, the % ^{15}N in N_2O was calculated using the following equation:

$$\% \text{ } ^{15}\text{N} (\text{N}_2\text{O}) = \frac{200}{R + 2} \quad (7)$$

The peak height of a.m.u. 31 due to fragmentation of N_2O corresponds to:

$$0.177 (46)\text{N}_2\text{O} + 0.0885 (45)\text{N}_2\text{O}$$

The value was subtracted from the measured peak height of a.m.u. 31 in order to obtain the net production of a.m.u. 31.

Next the net peak height of a.m.u. 30 due to NO was calculated using the equation

$$(30)_{\text{NO}} = \frac{(31)_{\text{NO}} \cdot 100}{\%^{15}\text{N}} - (31)_{\text{NO}} \quad (8)$$

where $(30)_{\text{NO}}$ is the net production of a.m.u. 30 due to NO and $(31)_{\text{NO}}$ is the net a.m.u. 31 due to production of NO by other than fragmentation.

In order to calculate the amount of N_2 liberated due to denitrification, the mass peaks at 29 and 30, due to N_2 were determined. The peak height of a.m.u. 30 due to fragmentation of N_2O corresponds to the sum

$$0.053 \text{ N}_2\text{O} (46) + 0.177 \text{ N}_2\text{O} (44) + 0.0885 \text{ N}_2\text{O} (45)$$

This value was subtracted from the measured peak height of a.m.u. 30 in order to obtain the net production of a.m.u. 30 due to $^{15}\text{N}^{15}\text{N}$,

$(30)_{\text{N}_2}$. The net production of $^{14}\text{N}^{15}\text{N}_1(29)_{\text{N}_2}$, was calculated by subtracting the fragmentation contribution, which is equal to 0.053

$(45)_{\text{N}_2\text{O}}$, from the measured intensity of a.m.u. 29.

It was found that the production of a.m.u. 28, as calculated by deducting the intensity of a.m.u. 28 of air from the measured intensity of a.m.u. 28 of the sample, was not reliable since $^{14}\text{N}^{14}\text{N}$ was a major component in the air while the production of $^{14}\text{N}^{14}\text{N}$ by denitrification was relatively small. Thus, only $(29)_{\text{N}_2}$ and $(30)_{\text{N}_2}$ were used to calculate the nitrogen microbially produced, that is two nitrogen

atoms derived from added NO_3^- , by using the following equation.

$$(28)_{\text{N}_2} = \frac{R^2}{4} (30)_{\text{N}_2} \quad (9)$$

$$\text{where } R = \frac{(29)_{\text{N}_2}}{(30)_{\text{N}_2}}$$

$$\text{or } = \frac{(45)_{\text{N}_2\text{O}}}{(46)_{\text{N}_2\text{O}}}$$

Thus the total peak height due to N_2 was the sum of $(28)_{\text{N}_2}$, $(29)_{\text{N}_2}$ and $(30)_{\text{N}_2}$.

The peak height of a.m.u. 28, 29 and 30 due to N_2 may be considered to have been derived from two different pathways as far as the distribution of isotopic composition is concerned. The one which has been already discussed assumed that both atoms of nitrogen molecules originated from the same source. The other pathway uses two different sources of nitrogen in order to form diatomic N_2 molecules. If labelled and unlabelled nitrogen atoms are combined due to Van Slyke reaction, the distribution of ^{15}N and ^{14}N in the resultant molecules can be described by the following expression,

$$^{14}\text{N}^{14}\text{N} : ^{14}\text{N}^{15}\text{N} : ^{15}\text{N}^{15}\text{N} = P_1 P_2 : (P_1 g_2 + P_2 g_1) : g_1 g_2,$$

where P_1 and P_2 are the atom fraction of ^{14}N in the labelled and natural N respectively, and g_1 and g_2 are the respective atom fraction of ^{15}N . Since % ^{15}N of added fertilizer is 52.3%, and the natural abundance of ^{15}N is 0.0037 the following relation should hold.

$$^{14}\text{N}^{14}\text{N} : ^{14}\text{N}^{15}\text{N} : ^{15}\text{N}^{15}\text{N} = 250.1 : 275.2 : 1.0$$

From the above expression the relative amount of mass peak 30 due to N_2 formed by Van Slyke reaction is very small. However, it is not a negligible value.

Calculation of N_2 produced due to biological and chemical denitrification which occurred simultaneously, were reported in detail (Cho and Sakdinan 1978; Christianson, 1978). In flooded samples the amount of gas produced includes both the gas in the vessel atmosphere and the gas in the liquid. The quantity dissolved in the liquid was calculated using the Henry's Law constant and the partial pressure of the gas. This quantity was added to that present in the gaseous phase.

RESULTS AND DISCUSSION

Experiment I Denitrification of $\text{Ca}(\text{NO}_3)_2$ in the Experimental Soils.1. Morton Soil

The amounts of NO_3^- -N remaining in the soil during the incubation of 100 ppm NO_3^- -N at three different temperatures are shown in Figure 2. The amount of NO_3^- -N added to the soil (15 g) corresponded to 1.5 mg. However, there was 1.86 mg-N as NO_3^- prior to the addition of labelled NO_3^- resulting in 3.36 mg-N total per incubation vessel (15 g soil).

The disappearance of NO_3^- started from the first day of incubation without any lag period. Evidently microbial activity from the first day of incubation, was sufficient so that the oxygen supply in the soil was limiting. The production of CO_2 from the first day of incubation (Table A-10), and the persistence of O_2 in the incubation atmosphere even after 14 days of incubation (Table A-7) support such speculation. The partial pressure of O_2 at day 14 of incubation was almost equal to 14% at 15°C .

The rate of disappearance of NO_3^- -N was slow at 15°C and increased with temperature. Nearly all the NO_3^- initially present in the soil disappeared within 8 days of incubation at 35°C . However, only about 30% (1 mg) and 55% (1.85 mg) of the initial NO_3^- -N disappeared at 15 and 25°C after 12 days of incubation, respectively. The rate of disappearance was nearly constant throughout the incubation period for low temperature (15°C) and amounted to $5.6 \mu\text{g N/g soil/day}$, but the rate decreased with time for higher temperature.

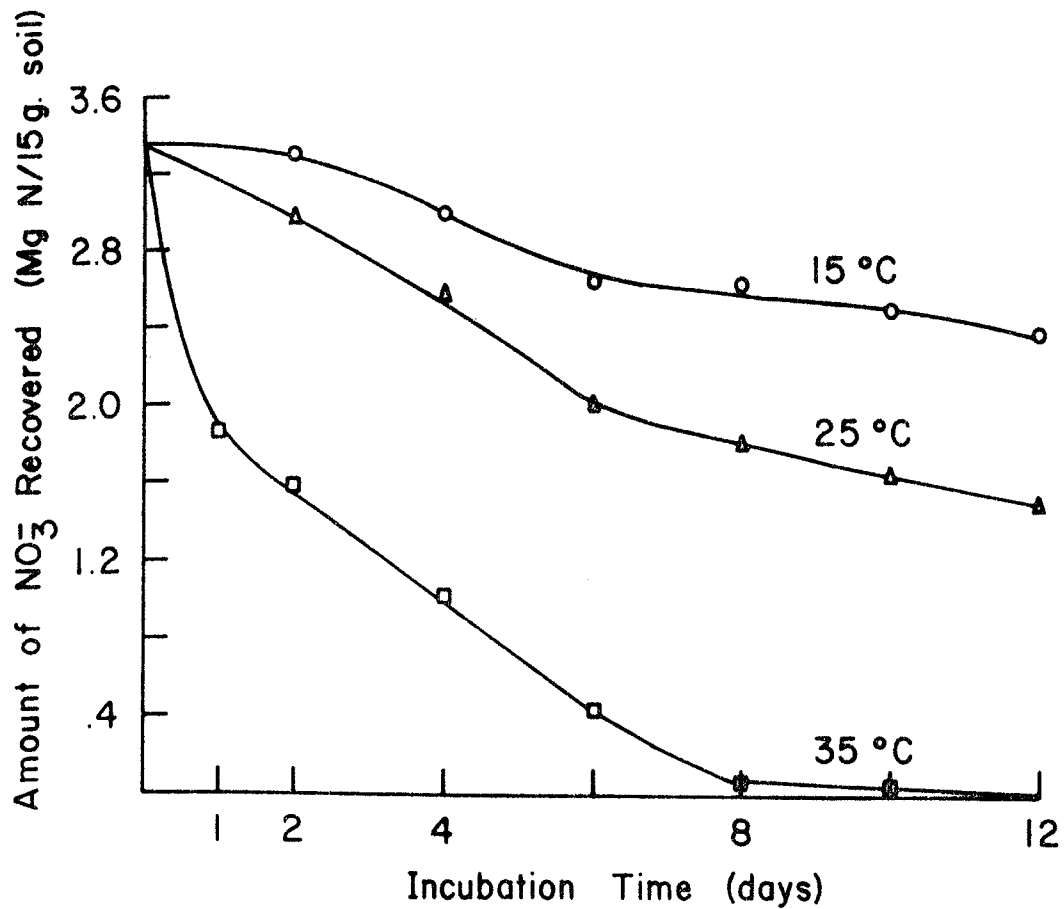


Fig.2 Recovery of NO_3^- - N during the incubation of added 100 ppm - N with Morton soil at various temperatures.

The NO_3^- -N that disappeared was recovered as N_2 gas (Figure 3). Nitrogen gas was the only form evolved during denitrification. Other gases such as N_2O and NO were not detected. Total quantity of nitrogen gas evolved during the period of incubation corresponded to approximately 32% (1.08 mg), 62% (2.08 mg) and 93% (3.13 mg) of the total NO_3^- -N initially present in the soil for incubation temperatures of 15, 25 and 35°C, respectively. The rate of N_2 production was nearly constant for 15°C incubation and amounted to 5.4 $\mu\text{g N/g soil/day}$. As the incubation temperature increased the rate of N_2 production decreased with time (Figure 3) as did the disappearance of NO_3^- -N (Figure 2). However, if the results of the first few days of incubation were disregarded, the rate of N_2 production was nearly constant as long as there was enough NO_3^- -N still remaining in the system. These rates amounted to 8.9 and 14.4 $\mu\text{g N/g soil/day}$ for the samples incubated at 25 and 35°C, respectively. Respective data for the rate of NO_3^- -N disappearance were found to be 10.0, and 17.1 $\mu\text{g N/g soil/day}$ (Figure 2). It is possible that the microbial activity at the early stage of incubation was slightly more active before reaching to a steady value. Such a speculation was also partially supported by the CO_2 production (Table A-10). A slightly higher CO_2 production rate occurred at the early stage of incubation.

The amounts of NO_3^- -N which disappeared and the N_2 gas which formed at the corresponding incubation periods matched reasonably well. This indicates that there was very little, if any, build-up of intermediates such as NO_2^- or N_2O , and little nitrate immobilization. Thus, the rate of disappearance of NO_3^- -N or the production rate of N_2 can both be used

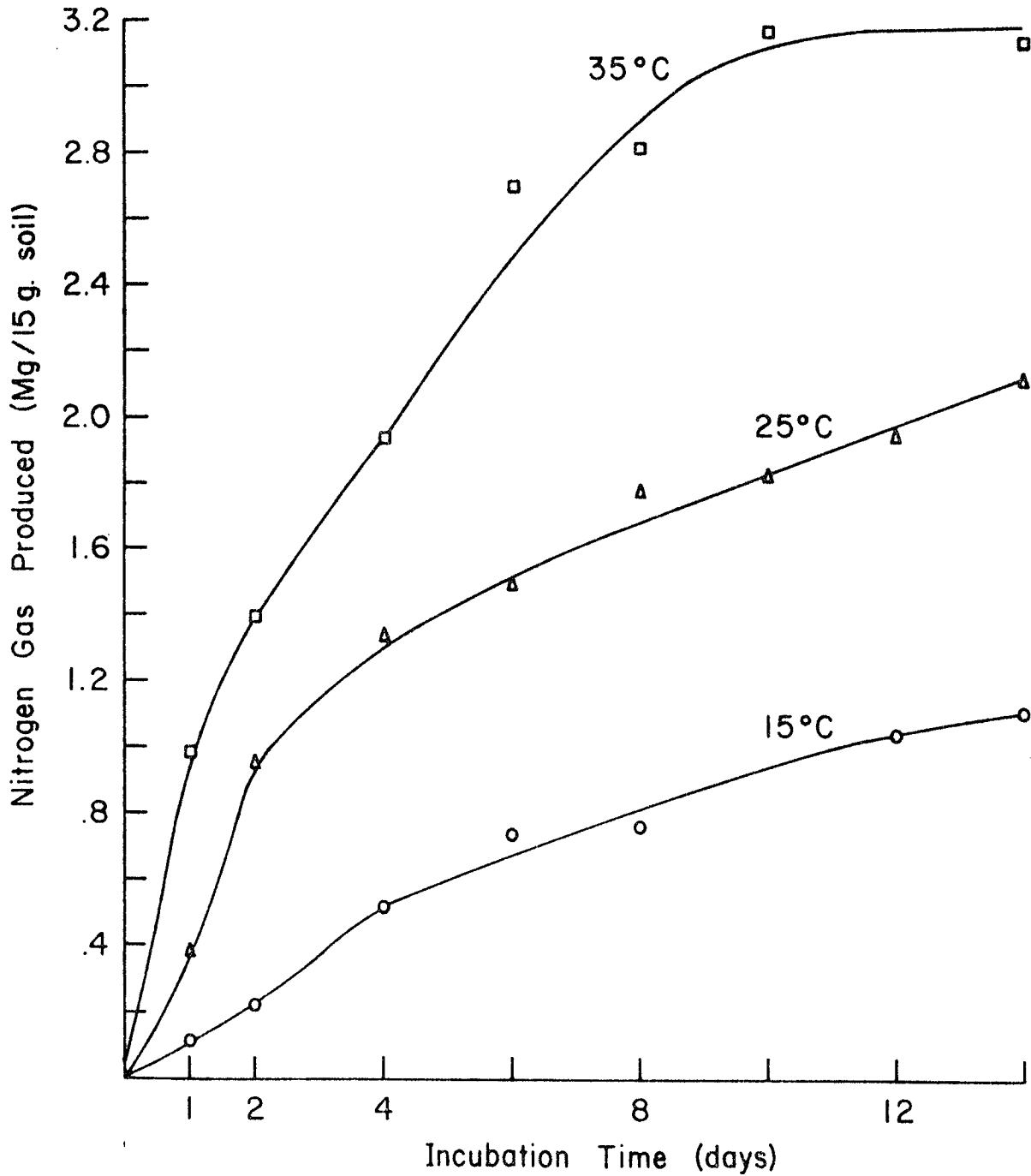


Fig.3 Production of N_2 from Morton soil (initial pH 7.8) at various temperatures (N rate = 100 ppm NO_3^- -N).

as an indication of denitrification intensity. Moreover, it seems that under flooded conditions NO_3^- is an unstable form of inorganic nitrogen, due to slow O_2 diffusion through stagnant water.

The NO_3^- which was originally in the soil and the added labelled NO_3^- seemed to behave in the same way as indicated by the results of $\%^{15}\text{N}$ in the produced N_2 (Table 2). There seemed to be no further production of NO_3^- due to mineralization as the $\%^{15}\text{N}$ in N_2 did not decrease with time.

The results obtained with 150 ppm NO_3^- -N at different temperatures are shown in Figure 4. The disappearance of NO_3^- started from the first day of incubation for samples incubated at 25 and 35°C although there was a lag period of NO_3^- disappearance at 15°C. This could be due to the low rate of O_2 consumption at low temperature which was satisfied by the dissolved O_2 in the flooded water (Table A-7). This was partially confirmed from the CO_2 production which was very low at low temperatures (Table A-10). The rate of disappearance of NO_3^- -N increased with temperature. The percentages of initial nitrate disappearance were about 18% (.75 mgN), 44% (1.8 mgN) and 90% (3.7 mgN) at the different incubation temperatures by day 14 of incubation. The rate also decreased with time at higher temperature.

The gases produced with different incubation temperatures are given in Figure 5. The major form of gas derived was N_2 . There was no time lag in gas production for 25 and 35°C, although there was a time lag for 15°C, as was observed on the nitrate reduction (Figure 4).

Table 2 Average %¹⁵N* of N₂O and N₂ evolved from the experimental soils with varying initial NO₃⁻-N concentration.

Soil	conc. NO ₃ ⁻ -N PPM	% ¹⁵ N** (X)		σ***		$\frac{\sigma}{X} \cdot 100$	
		N ₂ O-N	N ₂	N ₂ O	N ₂	N ₂ O	N ₂
Morton	100	-	23.73		1.17		4.90
	150	-	28.73		1.23		4.28
	200	-	32.57		1.23		3.77
Wellwood	100	47.88	48.39	1.21	1.69	2.53	3.49
	150	49.11	49.76	1.21	1.22	2.46	2.45
	200	49.83	50.44	0.61	1.66	1.22	3.29
Keld	100	52.54	51.76	0.58	0.99	1.10	1.91
	150	52.48	51.34	0.61	1.67	1.16	3.25
	200	52.52	51.16	0.72	2.07	1.37	4.05

* Average of Duplicates, three different temperatures and eight different days of incubation, of %¹⁵N

** %¹⁵N of added NO₃⁻ = 52.3%

*** σ standard deviation.

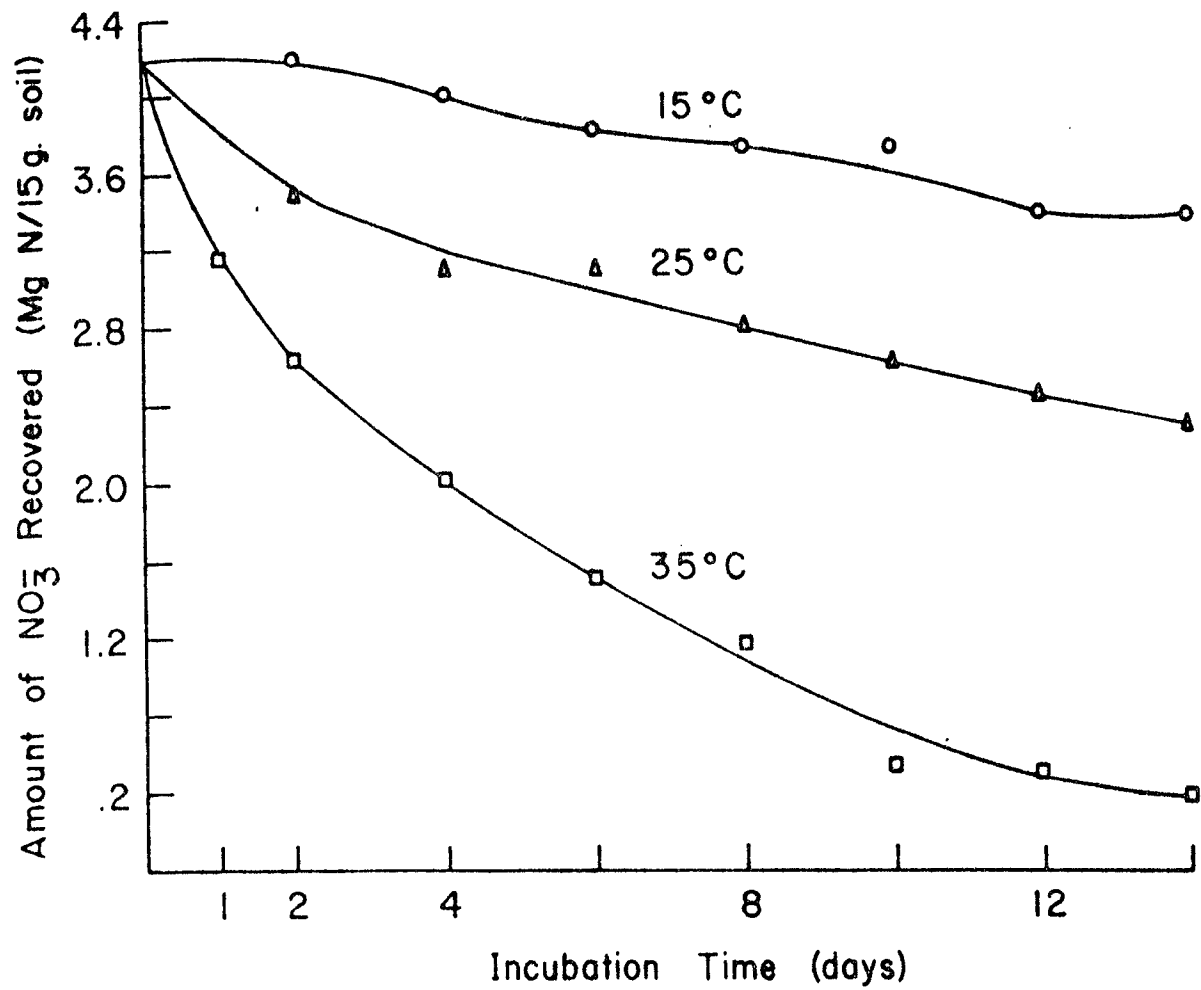


Fig. 4 Recovery of NO₃ -N during the incubation of added 150 ppm -N with Morton soil at various temperatures.

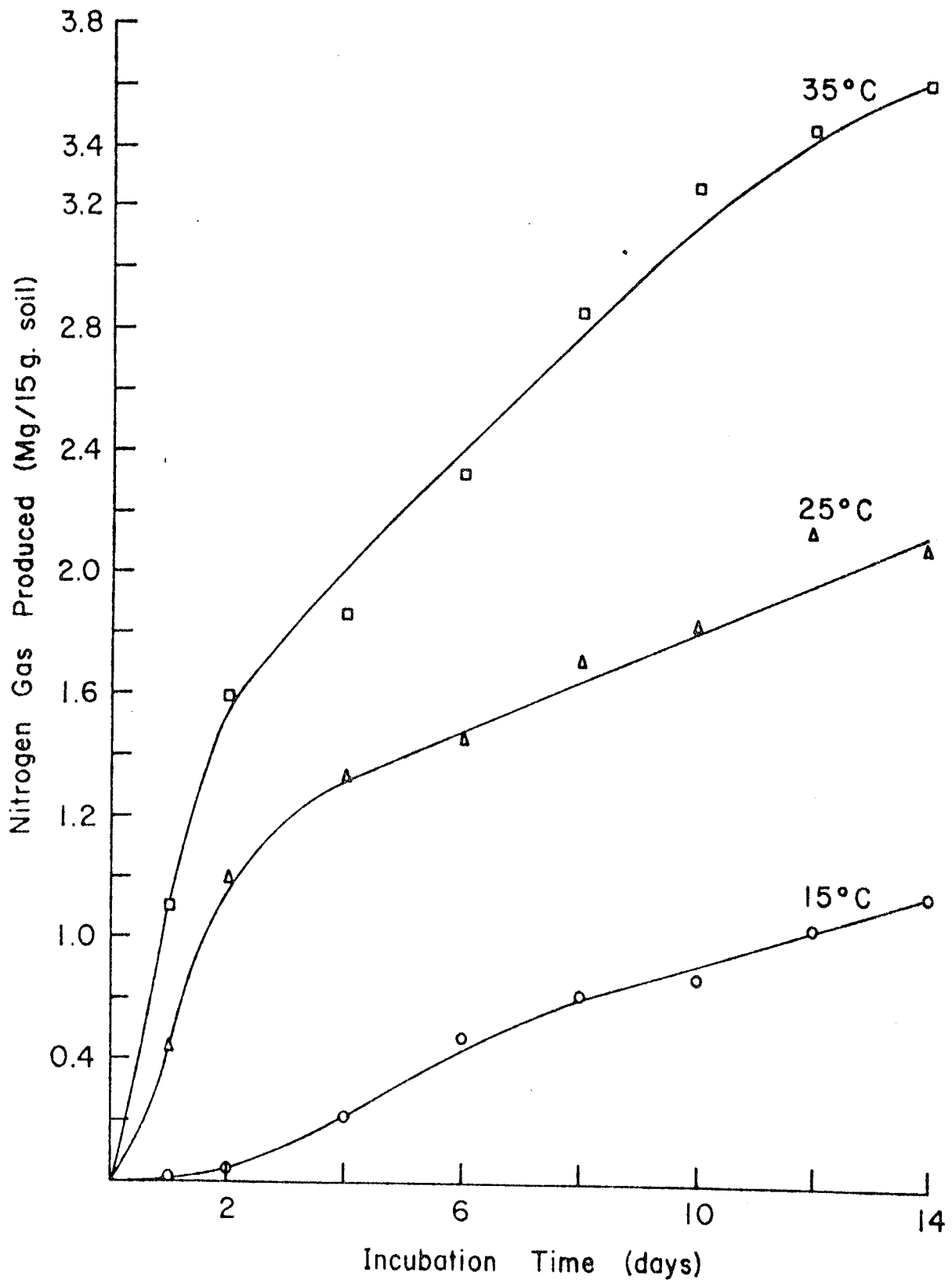


Fig.5 Production of N_2 from Morton soil (initial pH 7.8) at various temperatures (N rate = 150 ppm NO_3^- -N).

For these treatments the rates of N_2 production were nearly constant, since nitrate persisted up to the end of incubation. The rates of N_2 production after the second day of incubation were found to be 5, 7.8 and 13.3 $\mu\text{g N/g soil/day}$ for 15, 25 and 35°C incubation temperatures, respectively.

The results of O_2 consumption and CO_2 production were similar to those obtained when this soil was incubated with 100 ppm NO_3^- -N.

The disappearance of NO_3^- when 200 ppm NO_3^- -N was added was nearly identical to that obtained with samples incubated with 150 ppm NO_3^- (Figure 6). The percentages of NO_3^- -N lost were found to be about 14% (.68 mg) 42% (2.04 mg) and 70% (3.4 mg) at the end of 14 days of incubation for 15, 25 and 35°C, respectively.

The production pattern of N_2 (Figure 7) was similar to that of samples incubated with 100 and 150 ppm NO_3^- -N. Again there was no time lag in N_2 production at 25 and 35°C. Thirty six (36) and 38 percent of the N_2 production occurred during the first two days at 25 and 35°C, respectively. These results support the conclusion that steady state microbial activity was achieved soon after initiation of incubation. The rates of N_2 production from the second day to the end of incubation were found to be 4.75, 7.8 and 12.9 $\mu\text{g N/g soil/day}$ at incubation temperatures of 15, 25 and 35°C, respectively.

The results of N_2 production at 25°C (Figure 8) show that N_2 production followed the same pattern and occurred to the same magnitude irrespective of the amount of added nitrate. This suggests that the denitrification process, indicated by N_2 production, is independent

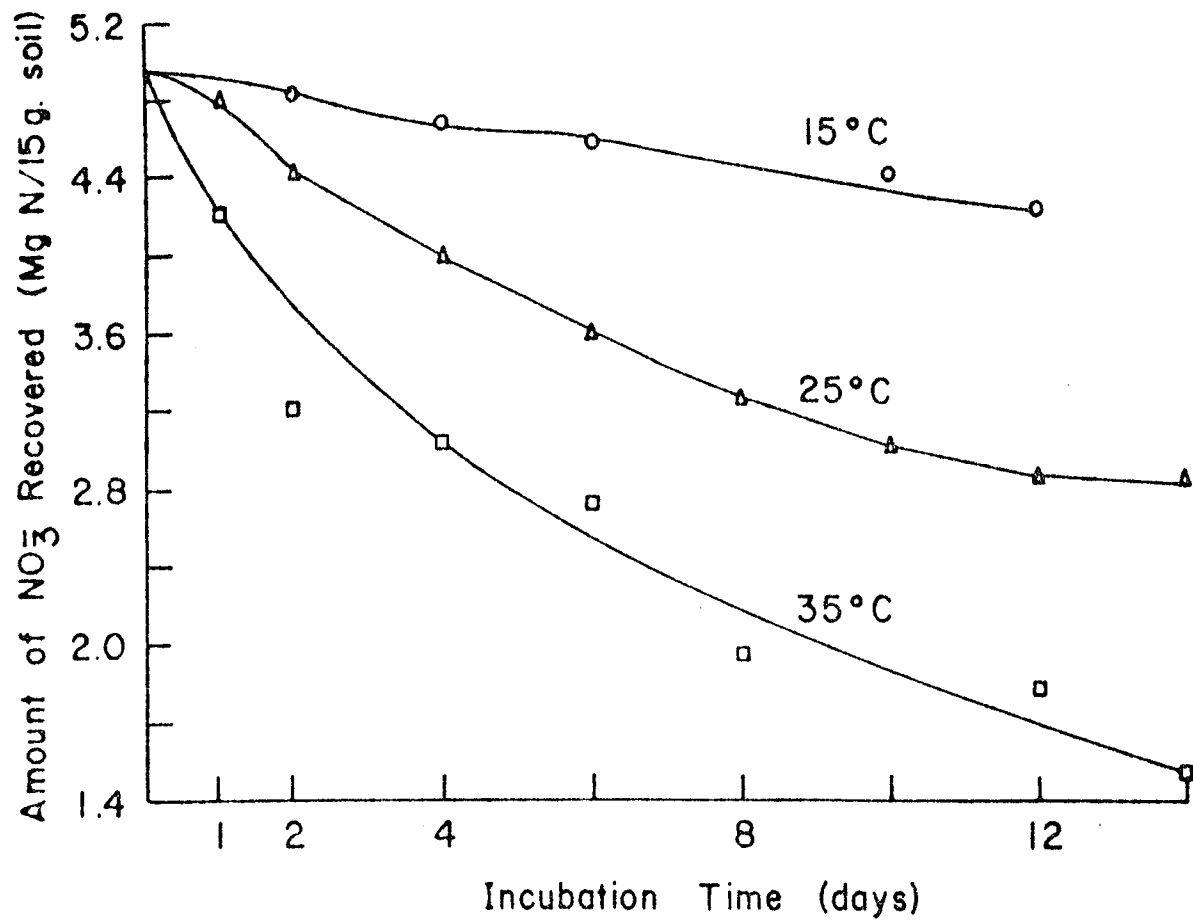


Fig.6 Recovery of NO_3^- -N during the incubation of added 200 ppm-N with Morton soil at various temperatures.

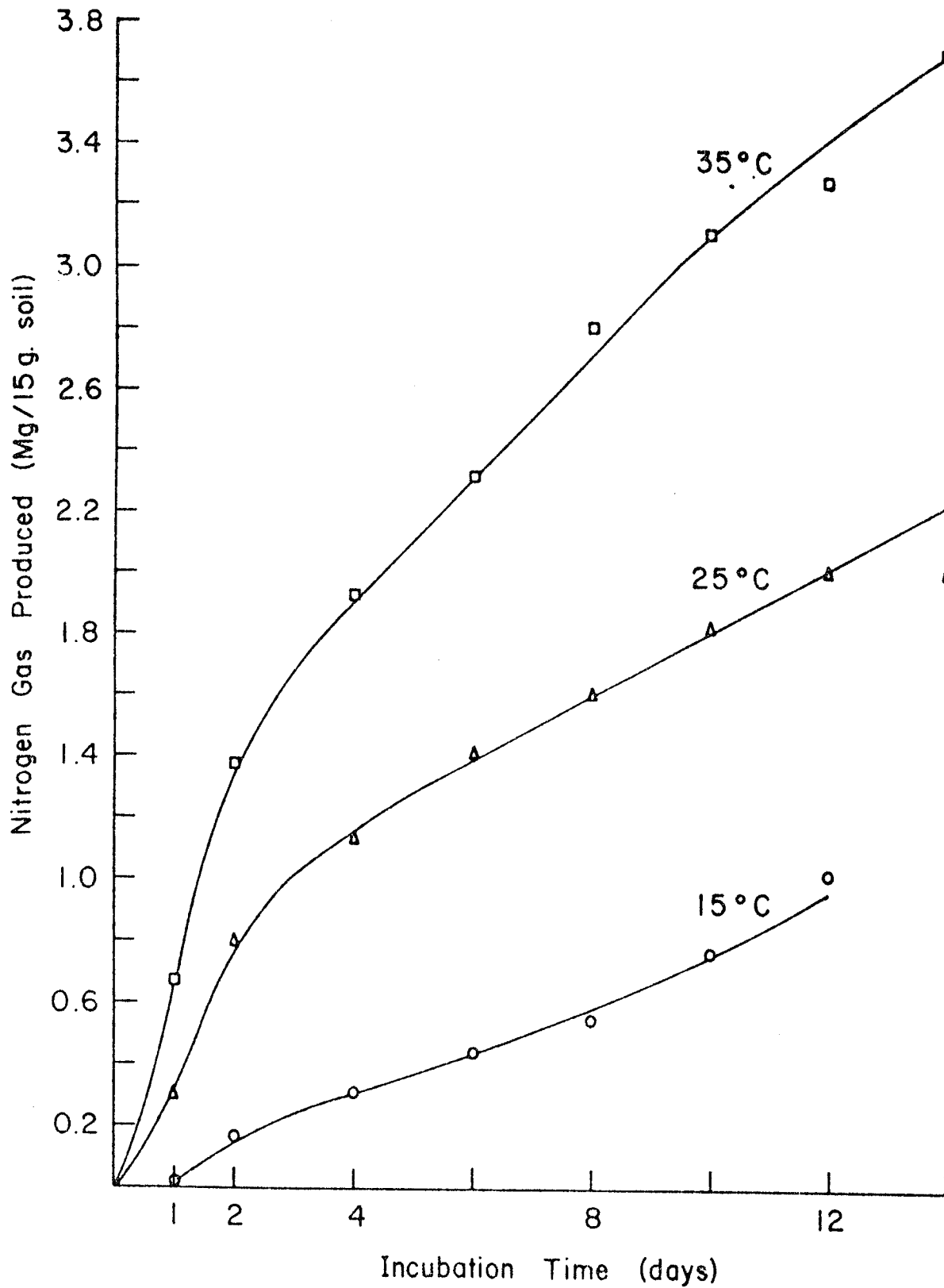


Fig.7 Production of N_2 from Morton soil (initial pH 7.8) at various temperatures (N rate = 200 ppm $NO_3^- - N$).

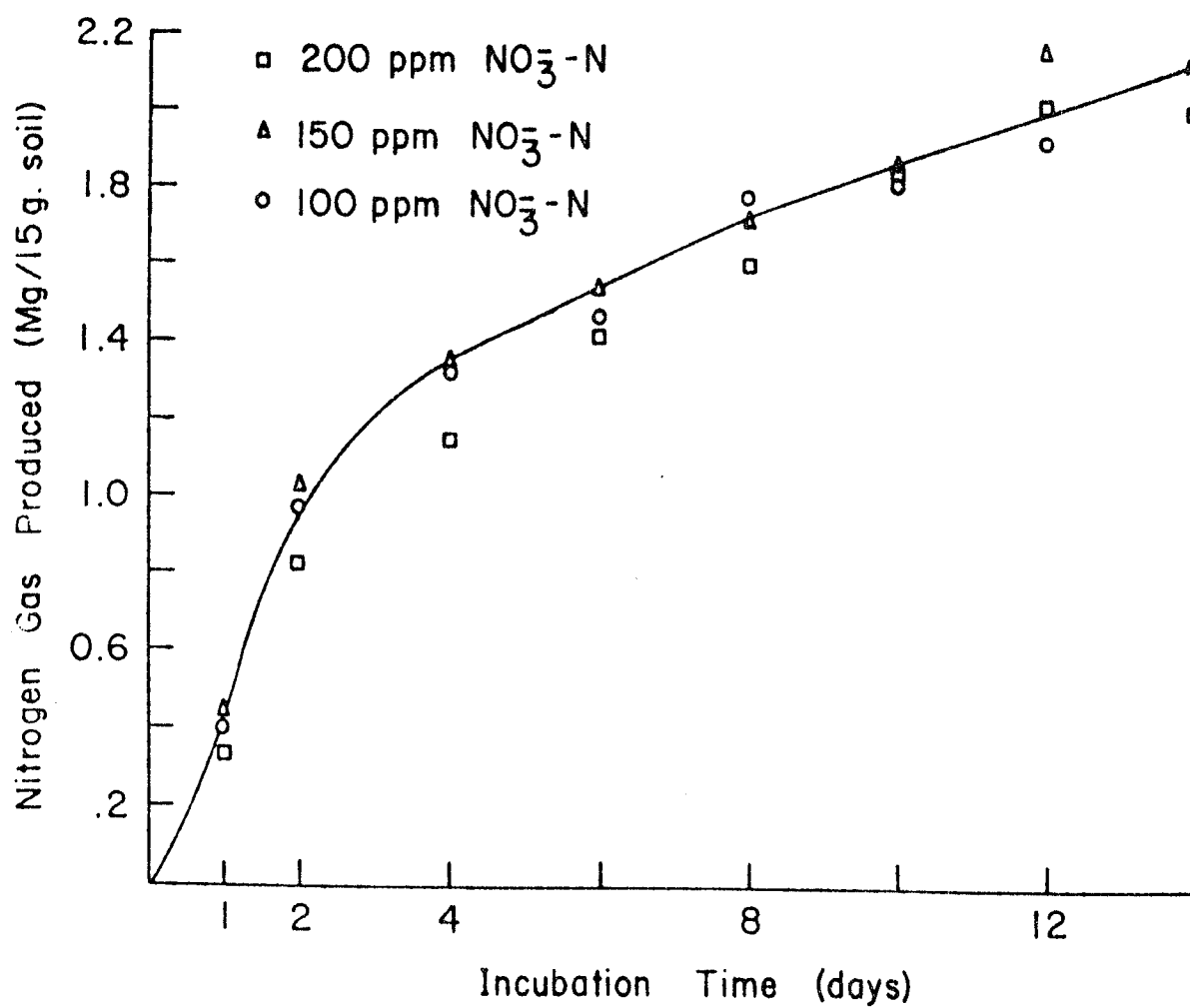


Fig. 8 Production of N₂ from Morton soil (initial pH 7.8) with different initial concentration of added NO₃⁻-N at 25°C.

of nitrate concentration (zero order kinetics) as long as nitrate is not the limiting factor for biological activity.

Figure 9 shows the effect of temperature upon rates of N_2 production. As mentioned before, the rates were calculated from the linear portion of the N_2 production curve within the period of 2-14 days. These results indicate that temperature has an exponential effect upon the rate constant and the logarithmic regression coefficient is $0.99 (\pm 0.01)$ for the three different NO_3^- concentrations. The Q_{10} for N_2 production from the soil incubated with 100 ppm NO_3^- -N is 1.62 and this is below Q_{10} of 2 that is usually accepted as an average for biological systems. The activation energy is about $8570 \text{ cal. mole}^{-1}$. Identical results for Q_{10} and activation energy were obtained from the incubation with 150 and 200 ppm NO_3^- -N. This agrees with the results of Focht (1974), who reported an activation energy of about 8000 cal/mole. The results shown above agreed with the studies of Nommik (1956), Bremner *et al.* (1958) and Focht (1974), who obtained a straight line in Arrhenius plot within the temperature range of 15 to 35°C .

There was virtually no N_2O formation from the Morton soil, regardless of the temperature or NO_3^- -N concentration. This agreed with the work of Bremner *et al.* (1958) and Hauck *et al.* (1956), who showed that no N_2O evolved from soil with pH higher than 7.

It is also of interest that no measurable amount of NO and NO_2 could be recovered in the incubation atmosphere. This is due to presence of O_2 in the system which reacts with NO to form NO_2 . The latter dissolves in the water of the system to form nitrate (Nelson

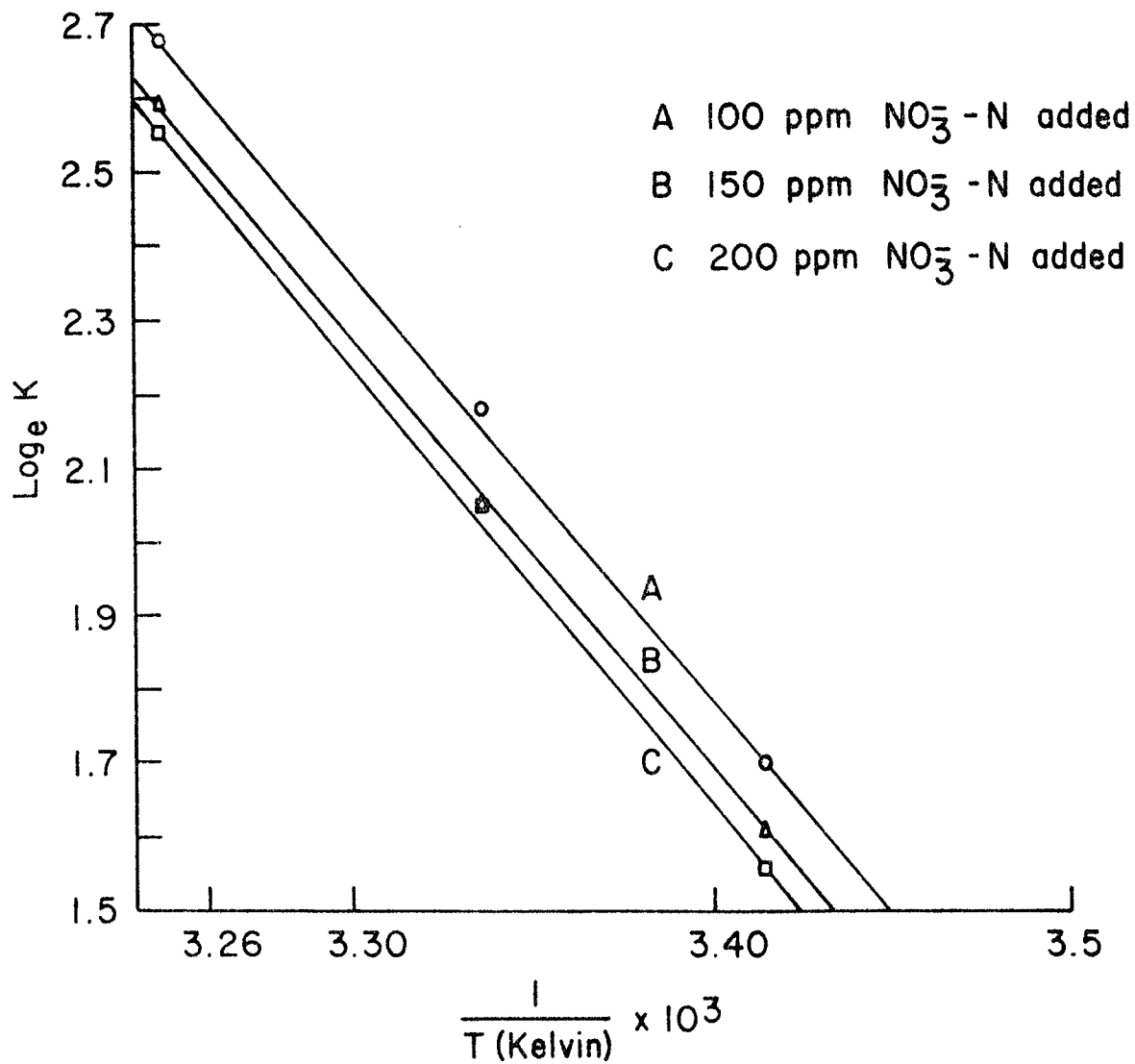


Fig.9 Arrhenius plot of rate of N_2 production (K) vs temperature of Morton soil (initial pH 7.8) at various initial concentrations of added NO_3^- -N.

et al. 1970a).

The results of isotopic composition of N_2 evolved during incubation of the Morton soil are shown in Table 2. The assumption that % ^{15}N in N_2 is not temperature dependent was confirmed. Thus, only the average values of % ^{15}N in N_2 for the three concentrations of initial NO_3^-N are listed. It is evident that the % ^{15}N in N_2 increased with an increased rate of NO_3^-N addition. This is attributed to isotopic dilution by NO_3^-N originally present in the soil. Expected values of % ^{15}N in N_2 due to this effect were calculated to be 23.66, 28.95 and 32.6% for 100, 150 and 200 ppm N respectively. Thus the results showed good agreement with the expected % ^{15}N of N_2 . Therefore, it can be concluded that N_2 was solely derived from NO_3^-N and mainly due to biological denitrification. The isotopic effect, if any, is negligible in this investigation, i.e. ^{14}N and ^{15}N behaved in the same way.

The partial pressure of O_2 in the atmosphere above the stagnant water decreased slowly during the 14-day period (Table A-7). A slightly higher consumption of O_2 occurred with higher temperatures. For example, the O_2 consumption rates, for the soil incubated with 100 ppm NO_3^-N , were .12, .195 and .225 mg O_2 /15g soil/day for incubation temperatures of 15, 25 and 35°C, respectively. This could be attributed to higher biological activity and a higher diffusion coefficient of O_2 in water at the higher temperatures. It was also noted that partial pressure of O_2 in the incubation atmosphere with different NO_3^-N concentrations varied only slightly with the change of NO_3^-N concentration. Factors that limited the O_2 consumption rate are the oxygen dissolution from gas to the liquid phase and the oxygen diffusion rate from liquid to the soil underneath. Thus,

it can be concluded that temperature is the major factor altering the rate of O_2 consumption. High temperature decreases the solubility of gases. On the other hand, it increases the diffusion coefficient, and also increases the concentration gradient due to the decrease in the thickness of the diffusion path.

The partial pressure of CO_2 in the atmosphere above the flood water is given in Table A-10. It is evident that higher production of CO_2 occurred at the higher temperatures. This can be attributed to the higher biological activity as temperature was increased.

During incubation, amount of accumulated NO_2^- -N did not exceed $15 \mu\text{g/g}$ soil for any of the treatments (Table A-1). This accumulation occurred at an early stage in the incubation. The accumulation was found to be slightly higher at the lower temperature (15°C). This would suggest that NO_2^- was utilized at a high rate and that the reduction rate of NO_2^- to N_2 was less temperature dependent than was the reduction of nitrate to nitrite.

The amount of added nitrate-N present as exchangeable NH_4^+ and as organically bound N was not investigated. However, many workers have found that within a 2-week interval conversion to these forms was negligible (Nommik, 1956; Bremner and Shaw 1958; Macrae *et al.* 1968; Christianson 1978). The amounts of KCl extractable ammonium present during incubation at different temperatures and concentrations of NO_3^- -N are shown in Table 4-A. The amount of NH_4^+ -N initially present in the soil amounted to $.38 \text{ mg } NH_4^+ \text{-N/15 g soil}$. This amount was found to increase with time and started to level off after approximately six days of incubation. These

results also showed that the nitrogen supplying capacity as NH_4^+ , under flooded condition was not related significantly to amount of NO_3^- -N added. However, it was found to increase significantly with temperature. The amounts of NH_4^+ -N for example, for soil incubated with 100 ppm NO_3^- , were .47, 0.61 and .83 mg NH_4 -N/15g soil at 15, 25 and 35°, respectively.

2. Wellwood Soil

The levels of NO_3^- -N and the incubation temperatures were the same as those used in the Morton soil. However, the pH of Wellwood soil was 6.3 as compared to 7.8 for the Morton soil (see Table 1).

The amounts of NO_3^- -N remaining in the vessel at various times of incubation at 15°C are shown in Figure 10. From the first day of incubation, nitrate nitrogen started to disappear. The decrease was almost linear with time independent of the initial nitrate concentration. Approximately 1 mg. of NO_3^- -N disappeared from each set during the 12-day period or 6 μg . NO_3^- -N/g soil/day. The rate of disappearance of NO_3^- -N from the Wellwood soil was nearly identical to that observed from the Morton soil at 15°C. The rate of disappearance was independent of the initial nitrate concentration, indicating that the reaction is zero order with respect to NO_3^- concentration. This is conceivable, since NO_3^- is a terminal electron acceptor, rather than a substrate which provides energy for reproduction of microbial population.

The composition of nitrogenous gases evolved during incubation at 15°C for different initial nitrate concentrations is shown in Figure 11. Nitrous oxide and nitrogen gas were the major gases evolved from the

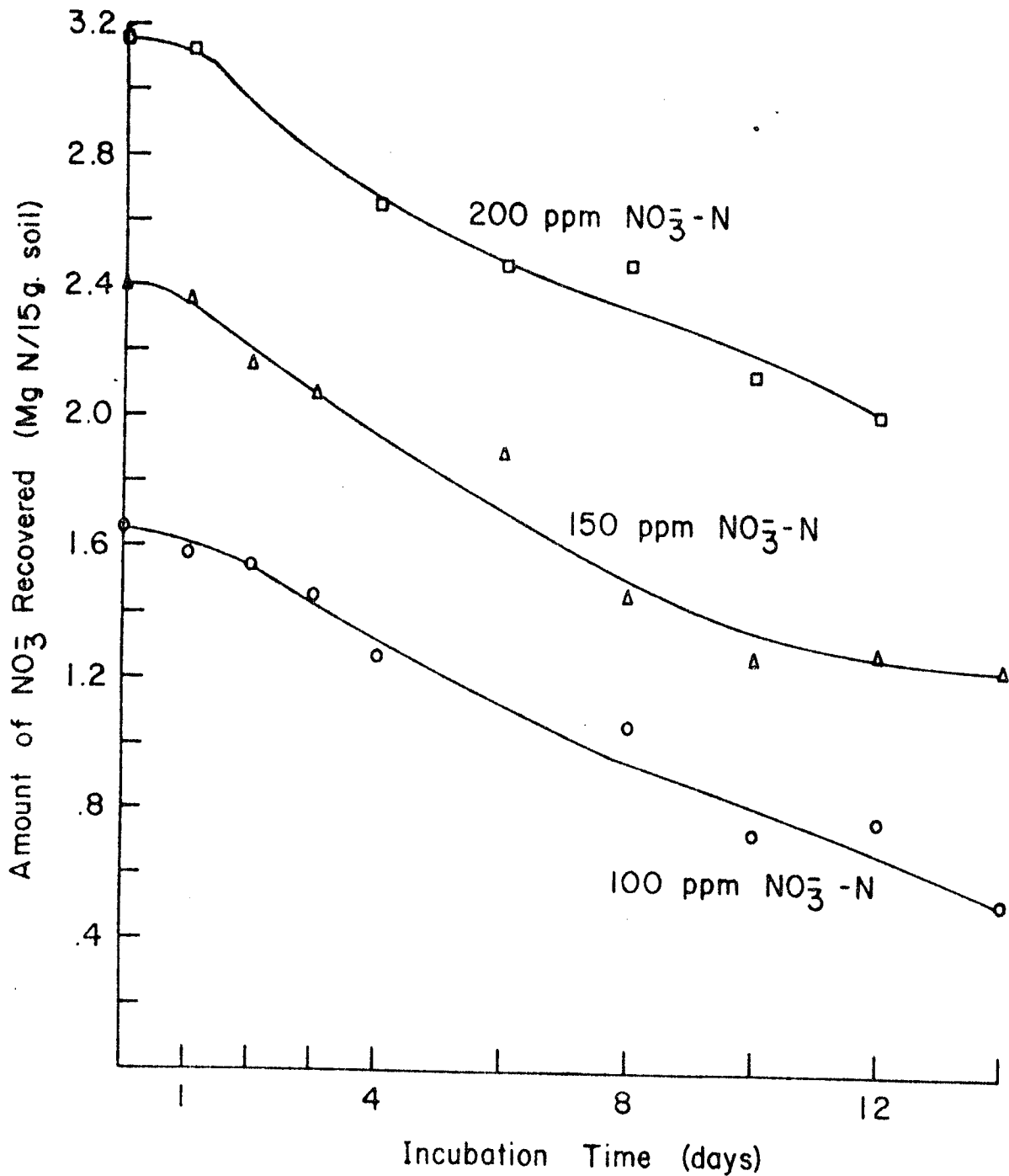


Fig.10 Recovery of NO_3^- -N during the incubation of various initial concentrations of added NO_3^- in Wellwood soil at 15°C .

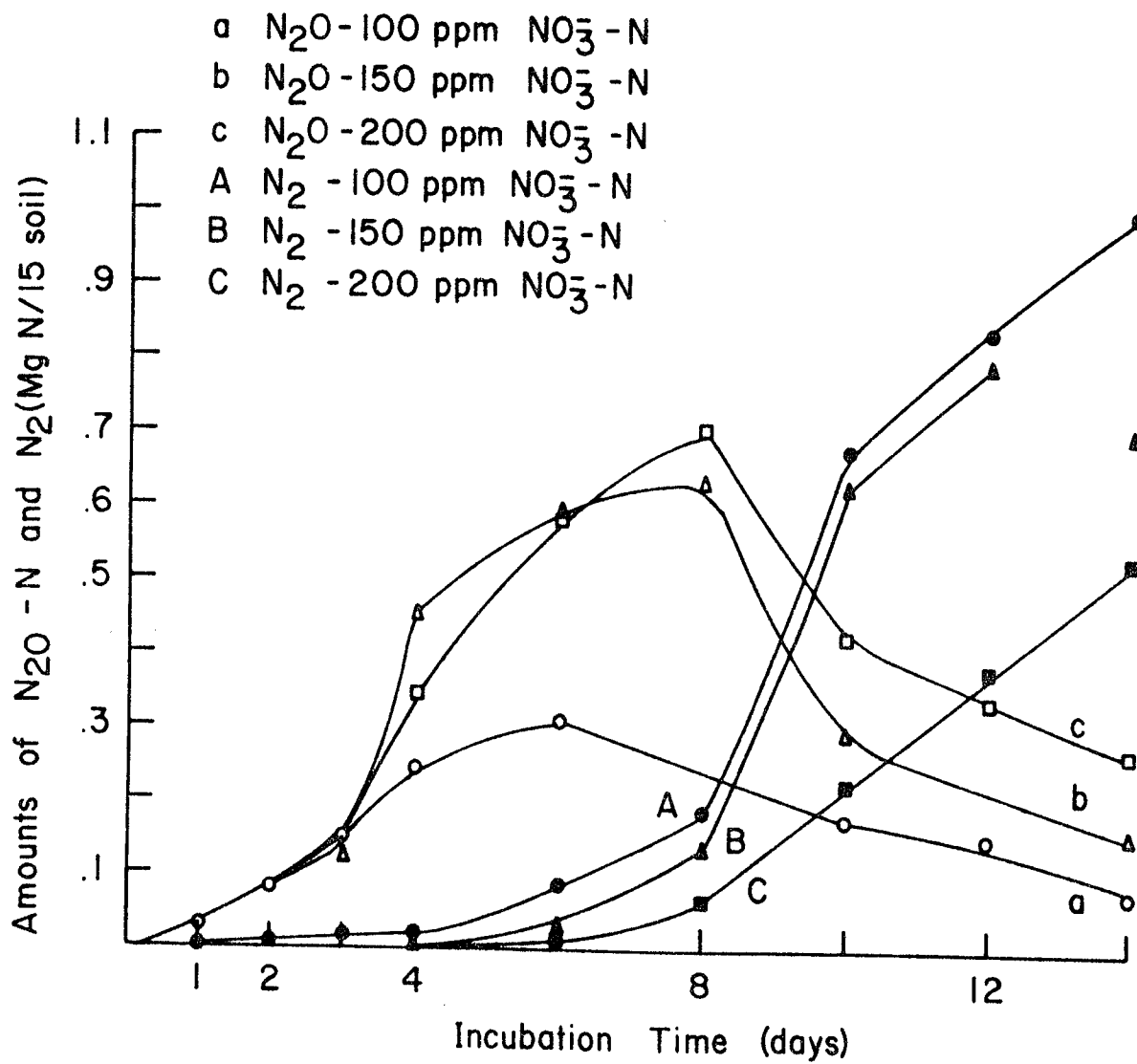


Fig. II Amounts of N_2O and N_2 during the denitrification of added NO_3^- with various initial concentrations in the Wellwood soil at $15^\circ C$.

soil. The production rates of N_2O from the soil with different concentrations of NO_3^- were identical for the first three days and equal to approximately $3.7 \mu\text{g N/g soil/day}$. This rate was slightly less than the average disappearance rate of NO_3^- -N which was approximately $6 \mu\text{g } NO_3^-$ -N/g/day for the 12 day period. With 100 ppm NO_3^- -N at day 6 N_2O amounted to $0.3 \text{ mg N/15 g soil}$ or 20% of the initial NO_3^- -N. Thereafter, N_2O content decreased slowly to 0.1 mg-N by day 14. Nitrogen gas formation was evident at day 2 although its production rate was very small until day 4. The production rate started to increase when N_2O concentration began to decline. The nitrogen lost during the incubation period as gaseous forms was 1.1 mg-N and it amounted to 67% of the N initially present as nitrate.

After day 3 the rate of N_2O production with 150 ppm NO_3^- -N was higher than with 100 ppm NO_3^- -N (Figure 11). The amount of N_2O in the incubation container reached the maximum value of $.63 \text{ mg-N}$ at day 8. This corresponds to approximately 26% of the initial NO_3^- -N. The amount of N_2O decreased to $.15 \text{ mg-N}$ at day 14. Nitrogen gas production was noticed from day 4. From day 6 it was produced in appreciable amounts. The amount of gases produced at day 12, was 0.8 mg-N and it amounted to 40% of the N initially present. This value was slightly less than the amount of NO_3^- -N (45%) that disappeared during the same period of time.

The trend of N_2O production with 200 ppm NO_3^- -N was similar to that with 150 ppm NO_3^- -N except that the maximum value at day 8, was

0.7 mg-N and this amounted to approximately 22% of the initial NO_3^- -N. After that the amount of N_2O started to decrease and reached 0.39 mg-N by day 14. Again the rate of N_2 production began to increase after day 8 and reached the maximum value of .53 mg-N at the end of the incubation.

From the results it seems that the pattern of gas evolution at 15°C was unaffected by the initial NO_3^- concentration. Nitrous oxide was the major gas generated during the initial period of incubation. With time N_2O decreased and N_2 became the predominant form. As all incubations were carried out in closed containers the intermediate product, N_2O , could not escape. Thus, the N_2O served as a terminal \bar{e} acceptor, and N_2 became the final denitrification product. When the nitrate present in the soil was increased N_2O production also increased. The value of maximum N_2O -N was found to be proportional to the initial NO_3^- concentration and amounted to approximately 25% of the initial NO_3^- -N. The time at which the maximum value of N_2O was observed, however, changed with concentration of NO_3^- . This implies that the denitrification process is not first order with respect to nitrate concentration.

Also interesting, is the effect of nitrate concentration upon N_2O reduction. Appreciable reduction of N_2O to N_2 started when the N_2O maximum was observed. Thus the formation of N_2 took place earlier when the initial NO_3^- -N concentration was lower. Also the initial rate of N_2 formation was greater with the lower NO_3^- -N

concentration. Thus, it is evident that there is a competition between N_2O and NO_3^- as \bar{e} acceptor. It seems that the competition of N_2O and NO_3^- as an \bar{e} acceptor depends upon the ratio between NO_3^- -N and N_2O -N. The ratios were approximately 3.73, 2.18 and 3.28 for 100, 150 and 200 ppm N, respectively. There was no consistent pattern in the ratio observed. This may have resulted from failure to ascertain the maximum value of N_2O by sampling every two days.

Figure 12 shows the amounts of NO_3^- -N remaining in the soil during the incubation of the three levels of NO_3^- -N at 25°C. The disappearance of NO_3^- started from the first day of incubation. The trend of NO_3^- utilization was almost linear for soil incubated with 200 ppm NO_3^- -N. With the lower concentration (e.g. 100 ppm), the rate was starting to slow down with incubation time. It is evident (Figure 12) that the initial rates of NO_3^- utilization were almost identical and amount to approximately 10 μ g/g soil/day, for six days incubation, regardless of the initial NO_3^- concentration.

The NO_3^- -N which disappeared was recovered as N_2O and N_2 as shown in Figure 13. The initial production rates of N_2O from the soil with different concentrations of NO_3^- were nearly identical for the first two days, when the rate of N_2O with 100 ppm NO_3^- -N reached its maximum, amounting to 13.0 μ g/gm soil/day. The maximum value of N_2O production with 150 ppm NO_3^- -N was found at day 4 and it amounted to .55 mg/15 gm soil. These maximum values of N_2O produced at the 100 and 150 ppm concentration amounted to approximately 25 and 23% of the initial NO_3^- -N present in the soil, respectively.

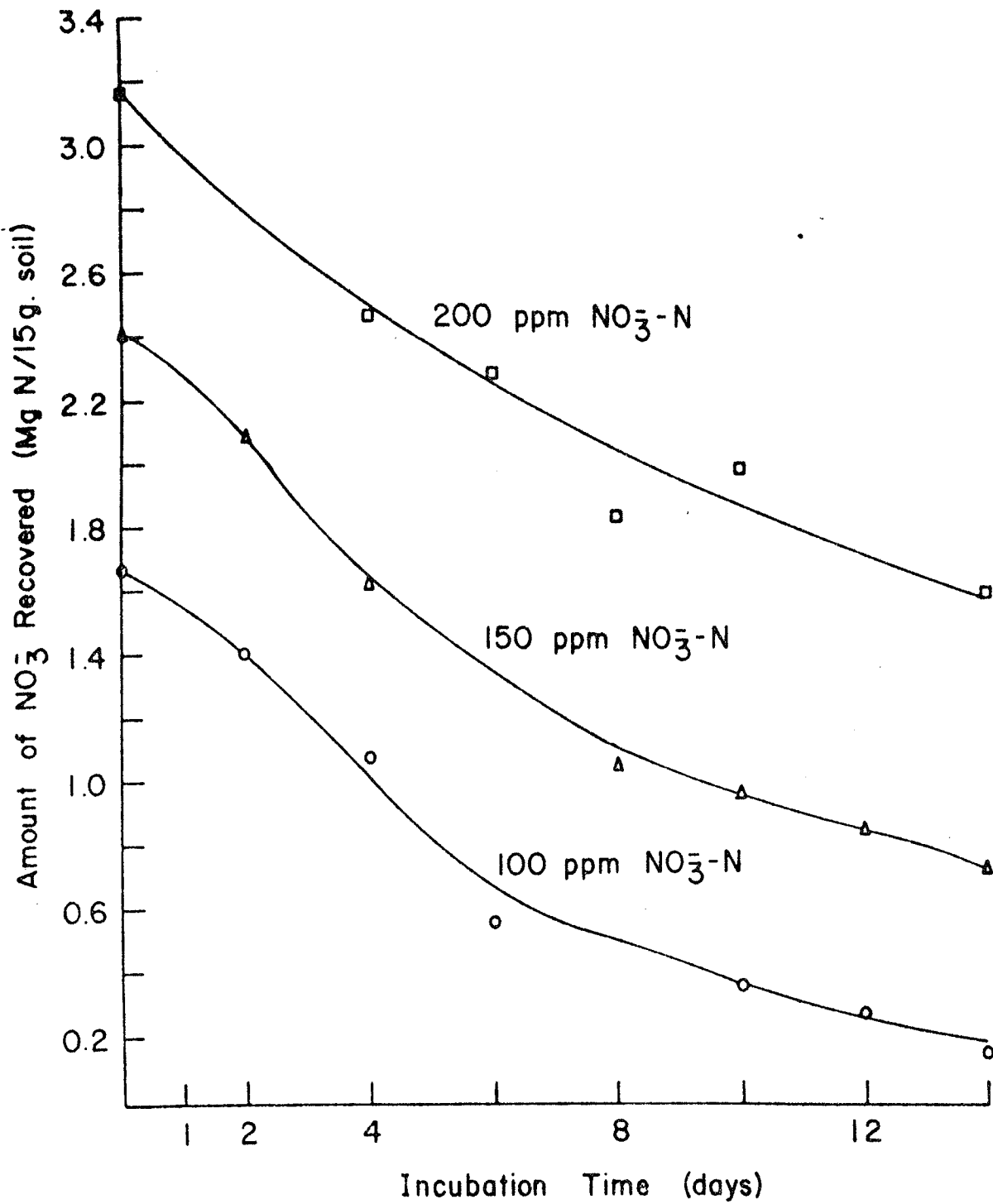


Fig.12 Recovery of NO_3^- -N during the incubation of various concentrations of added NO_3^- in Wellwood soil at 25°C.

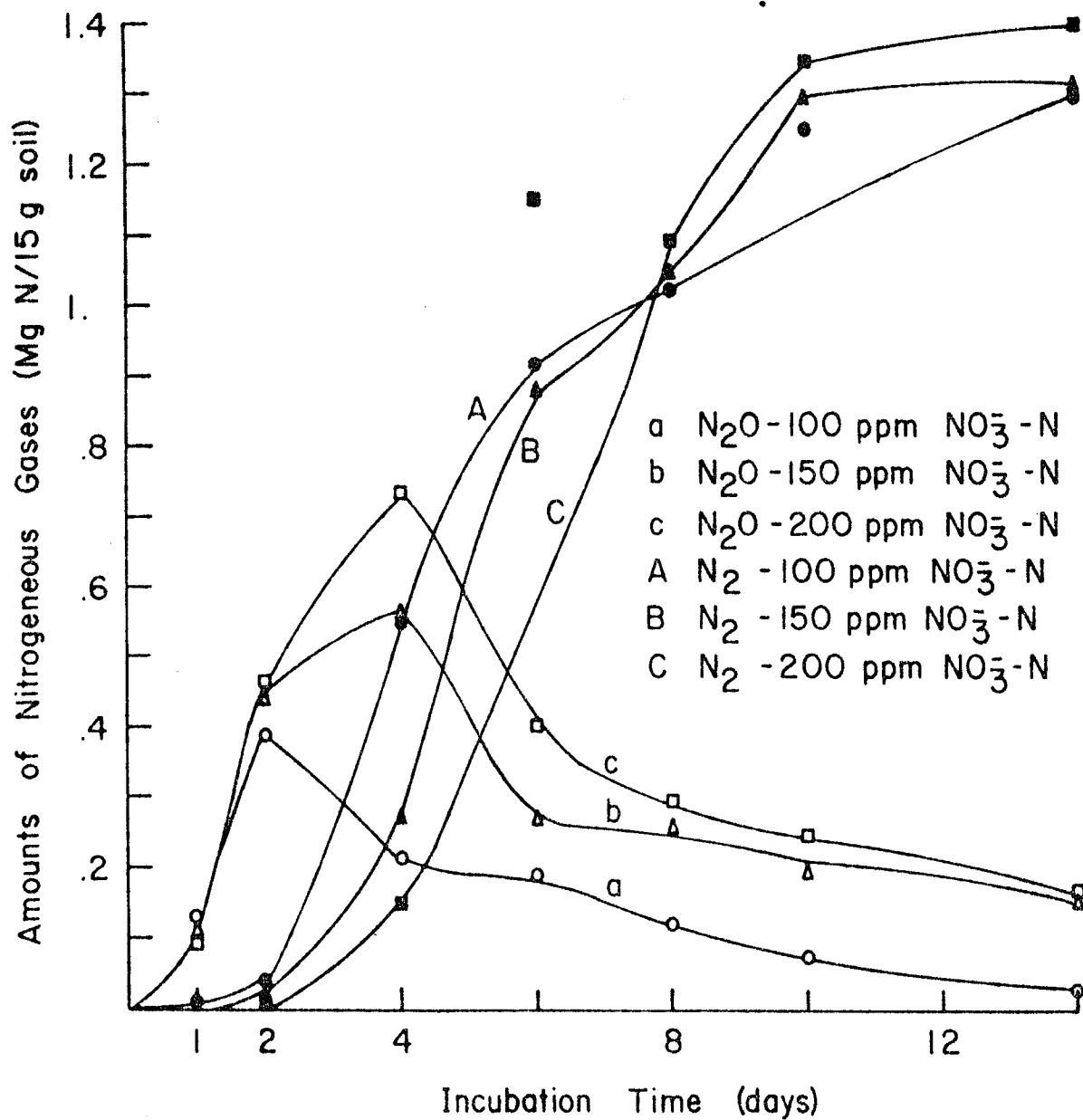


Fig.13 Amounts of N_2O and N_2 during the denitrification of added NO_3^- -N with various initial concentrations in Wellwood soil at 25°C .

For soil incubated with 200 ppm NO_3^- -N, a higher accumulation of N_2O was observed and it amounted to twice as much as the maximum concentration at 100 ppm NO_3^- -N. Thus, the maximum concentration of N_2O was directly proportional to the initial NO_3^- -N content. Consequently, the average life of N_2O was also longer with higher pool size (higher concentration) of NO_3^- .

Nitrogen gas production could be taken as a good indication of competition between the reactant, NO_3^- , and the product, N_2O (Figure 13). When the concentration of initial NO_3^- was low the reduction of N_2O to N_2 took place early in the incubation period. As the concentration of NO_3^- increased the initiation of formation of N_2 or the reduction of N_2O took place later. For example, there was 0.15 mg N_2 with the 200 ppm NO_3^- treatment and 0.55 mg N_2 with the 100 ppm NO_3^- treatment on day 4.

The amount of nitrate-N remaining at various times of incubation at 35°C with different NO_3^- -N concentrations is shown in Figure 14. The added NO_3^- -N was nearly completely lost at the end of 14 days' incubation except with 200 ppm NO_3^- -N where approximately 90% of the initial NO_3^- -N has disappeared.

Again the initial rate of NO_3^- -N disappearance was almost identical irrespective of the initial NO_3^- -N concentration, and it amounted approximately to 30 $\mu\text{g/g}$ soil/day. However, the rate started to slow down with incubation time in a manner similar to the trend of NO_3^- disappearance at 25°C (Figure 12). Similarly, the decrease in the disappearance rate

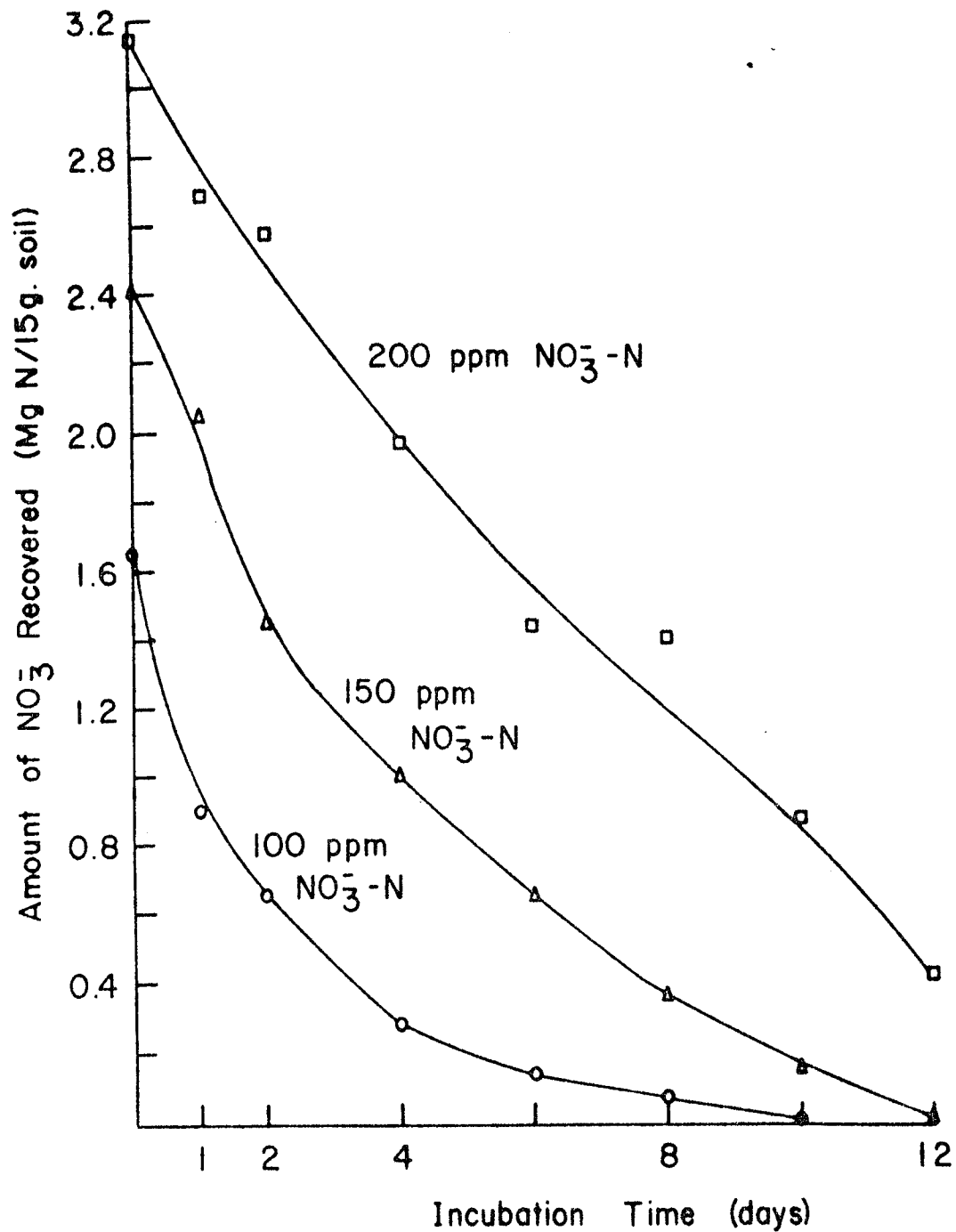


Fig.14 Recovery of NO_3^- -N during the incubation of various initial concentrations of added NO_3^- -N in Wellwood soil at 35°C .

of NO_3^- -N was small with an increase in initial NO_3^- -N. Such a dependence of the NO_3^- -N disappearance rate upon the initial NO_3^- -N could be explained on the basis of the supply of NO_3^- -N in the system and the competitive nature of the product, N_2O , with NO_3^- as \bar{e} acceptor.

If there was a small amount of competitor and a large amount of NO_3^- -N due to the slow rate of NO_3^- disappearance such as was found at 15°C (see Figure 10), the rate of NO_3^- -N disappearance during the course of the incubation was fairly constant. However, as the N_2O produced acted as an \bar{e} acceptor N_2 started to form and the rate of utilization of NO_3^- -N became slower. Thus, the decrease in the rate of NO_3^- disappearance was greater with a low initial NO_3^- concentration and a higher incubation temperature.

An additional possible reason for such a decrease in the rate may be associated with unsaturation in the enzyme- NO_3^- complex. It is known that the enzyme- NO_3^- complex becomes saturated if the concentration of NO_3^- is high so that the rate of NO_3^- -N disappearance becomes independent of NO_3^- concentration. However, the rate decreases as the NO_3^- concentration decreases due to unsaturation of the enzyme.

The pattern of gases evolved (Figure 15) is similar to that observed at 15 and 25°C . However, the initial rate of N_2O production was higher and amounted to approximately $23.4 \mu\text{g/g soil/day}$. The maximum concentration of N_2O was higher with a higher level of NO_3^- . The reduction of N_2O was almost completed by the end of the incubation period (14 days).

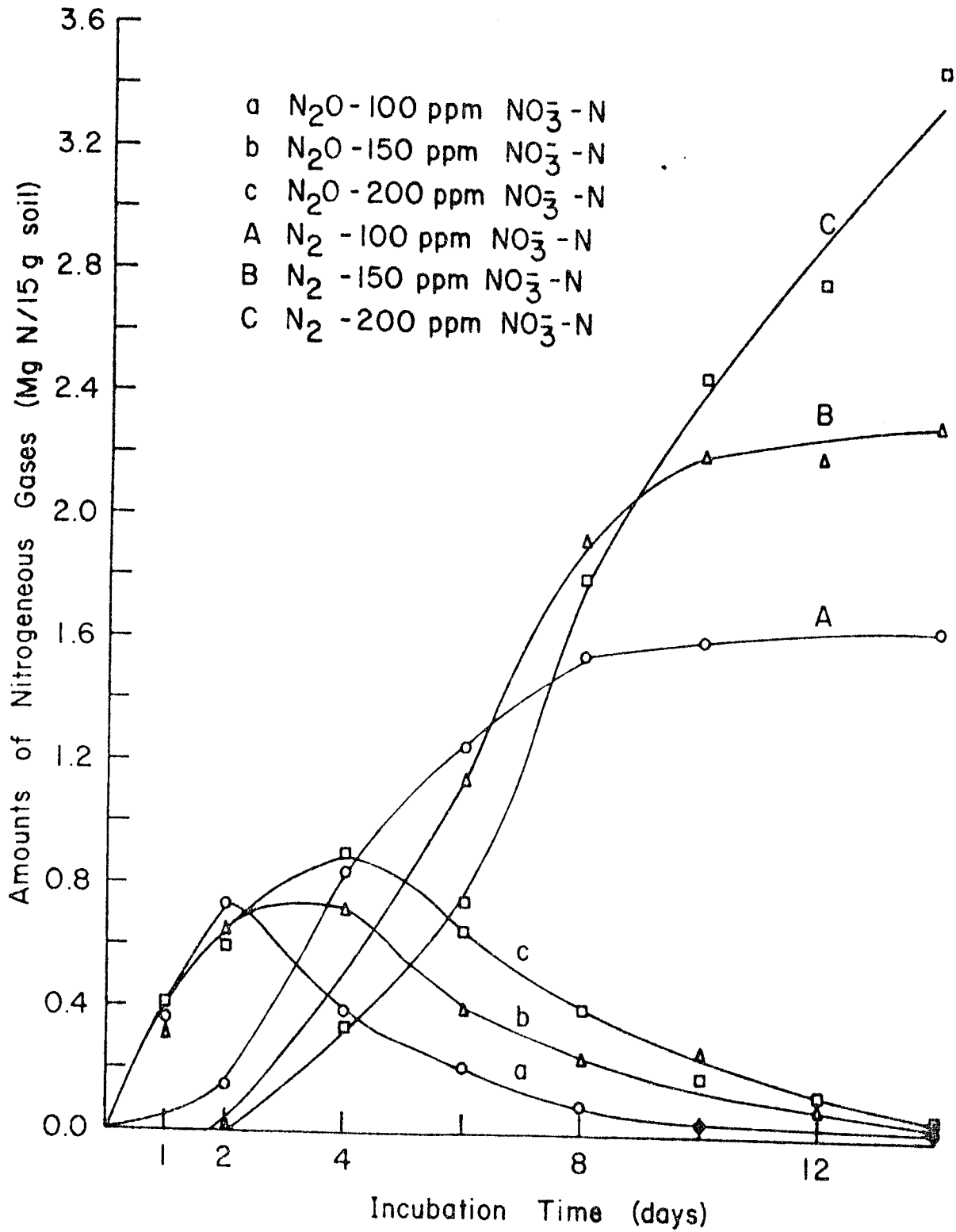


Fig.15 Amounts of N_2O and N_2 during the denitrification of added NO_3^- -N with various initial concentrations in the Wellwood soil at 35°C .

The maximum concentrations of N_2O measured were 43, 30 and 30% for 100, 150 and 200 ppm NO_3^- -N respectively. These may not be the maximum values which actually occurred since maximum levels may have occurred between sampling dates. It was concluded that temperature did not alter the pattern of gases produced. The trends of N_2O production were similar to those obtained at 15 and 25°C, although the maximum concentration and the time at which the N_2O was maximum were different. The higher temperature resulted in a higher maximum concentration and an earlier maximum in N_2O level. Nitrogen gas production also followed a similar pattern to that obtained at 15 and 25°C, except the rate of N_2 production was higher.

The results of O_2 consumption and CO_2 production are shown in Tables A-8 and A-11 respectively. Oxygen consumption was independent of initial NO_3^- -N, although the rate of consumption increased slightly with increasing temperature. A similar trend was observed with CO_2 production.

Since there was only 0.09 mg N/15g soil as NO_3^- -N in the untreated soil initially, the isotopic composition of N_2O and N_2 evolved during incubation of the Wellwood soil did not vary much from the % ^{15}N of the NO_3^- -N added (Table 2). It is evident, therefore, that the N_2O was solely derived from added NO_3^- -N.

The NH_4^- -N content, (Table A-5) as in the case of the Morton soil, increased during the time of incubation and this increase was related to temperature of incubation rather than the NO_3^- -N concentration.

The NO_2^- -N accumulation (Table A-2) was higher at the early stage of

incubation, but in no case did it exceed 10 $\mu\text{g N/g soil}$.

3. Keld Soil

The disappearance of NO_3^- did not start from the first day of incubation (Figure 16). There was a lag period of almost two days. However, 43% (1.3 mg) 57.7% (1.25 mg) and 80% (1.2 mg) of NO_3^- -N disappeared from the soil incubated with 100, 150 and 200 ppm NO_3^- -N respectively, during the 12 day incubation period. The rates of disappearance of NO_3^- -N, after the first two days, were almost identical for the different NO_3^- concentrations and amounted to 6 $\mu\text{g NO}_3^-$ -N/g/day. In other words, the denitrification process in soil under constant microbial activity is zero order within the range of 100-200 ppm NO_3^- -N. The zero order characteristics can be explained on the basis that the process is an \bar{e} accepting process. As long as energy sources of soil and the supply of \bar{e} acceptor are not limiting, the microbial activity remains nearly constant requiring \bar{e} acceptor at a constant rate.

The NO_3^- -N which disappeared was recovered as N_2O gas (Figure 17). Nitrous oxide was the only gaseous form evolved. Other gases such as N_2 , NO and NO_2 were not detected. The trends of N_2O production with different NO_3^- concentrations were almost identical and production amounted to .95 mg N_2O -N for an incubation period of 12 days. This was slightly less than the amount of NO_3^- -N that disappeared. There was a lag period in N_2O production of 2 days just as was found in NO_3^- disappearance. There was no reduction of N_2O to N_2 at 15°C.

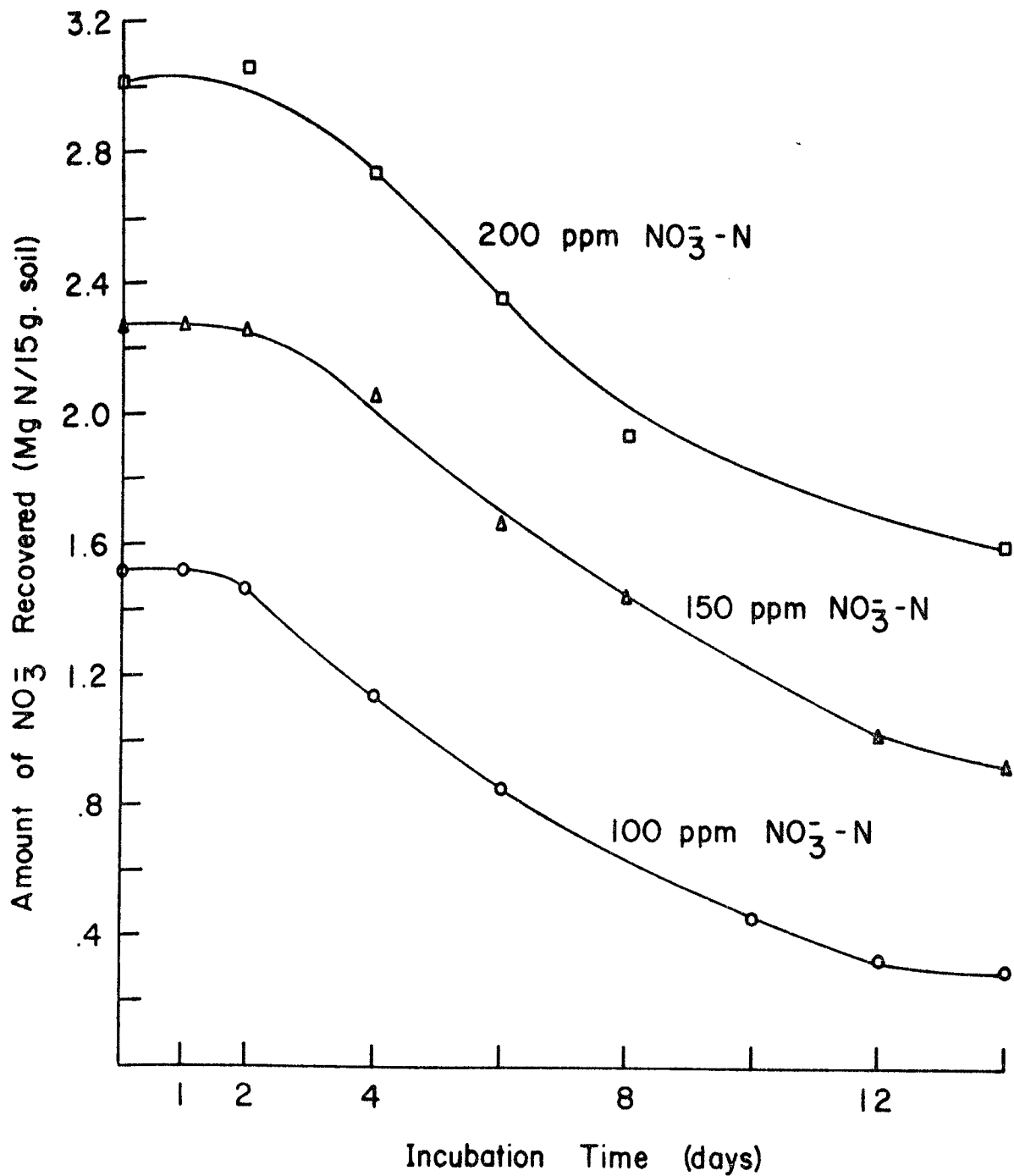


Fig.16 Recovery of NO_3^- -N during the incubation of various initial concentrations of added NO_3^- -N in Keld soil at 15 °C.

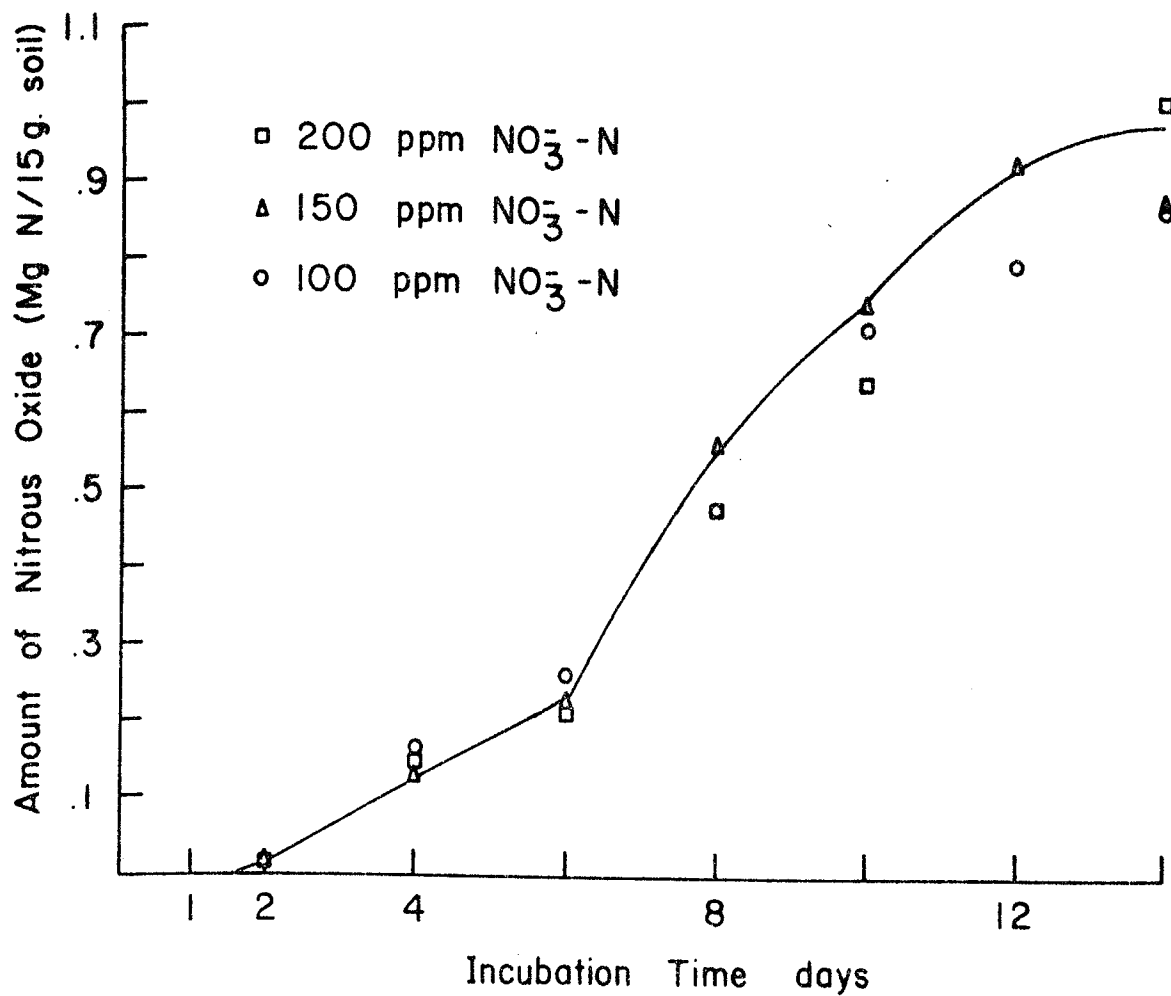


Fig.17 Production of nitrous oxide from Keld soil pH 5.4 with various initial concentrations of added NO_3^- -N at 15 °C.

There was a lag period in NO_3^- disappearance of one day at 25°C , (Figure 18) and thereafter NO_3^- -N started to decrease. As with the Wellwood soil incubated at 25°C , the trends of NO_3^- utilization were linear (except after 10 days of incubation) for Keld soil incubated with 200 ppm NO_3^- -N. However, with lower concentrations, the rate decreased with time. It is also evident (Figure 18) that the initial rates of NO_3^- utilization were almost identical and amounted to approximately 13 $\mu\text{g/g}$ soil day for soil incubated with 150 and 200 ppm NO_3^- -N. A slightly higher initial rate of NO_3^- -N utilization (18.8 $\mu\text{g/g}$ soil/day) was obtained with 100 ppm NO_3^- -N treatment.

Figure 19 shows the pattern of nitrogenous gas evolution during denitrification. Nitrous oxide and N_2 were the major gases evolved. The maximum value of N_2O amounted to 53, 51.1 and 51% of the initial NO_3^- -N for soil incubated with 100, 150 and 200 ppm N, respectively. These values are nearly twice those obtained with the Wellwood soil at the same temperature. The initial rates of N_2O formation were identical (17 μg N/g soil/day) for the different NO_3^- concentrations, suggesting that zero order kinetic was operating during denitrification. The competitive nature of NO_3^- and N_2O as \bar{e} acceptors was manifested in the production of N_2 . When the concentration of initial NO_3^- was low the reduction of N_2O to N_2 started at day 4 of the incubation. At this time N_2O had reached its maximum concentration. As the concentration of NO_3^- increased the initiation of N_2 formation or the

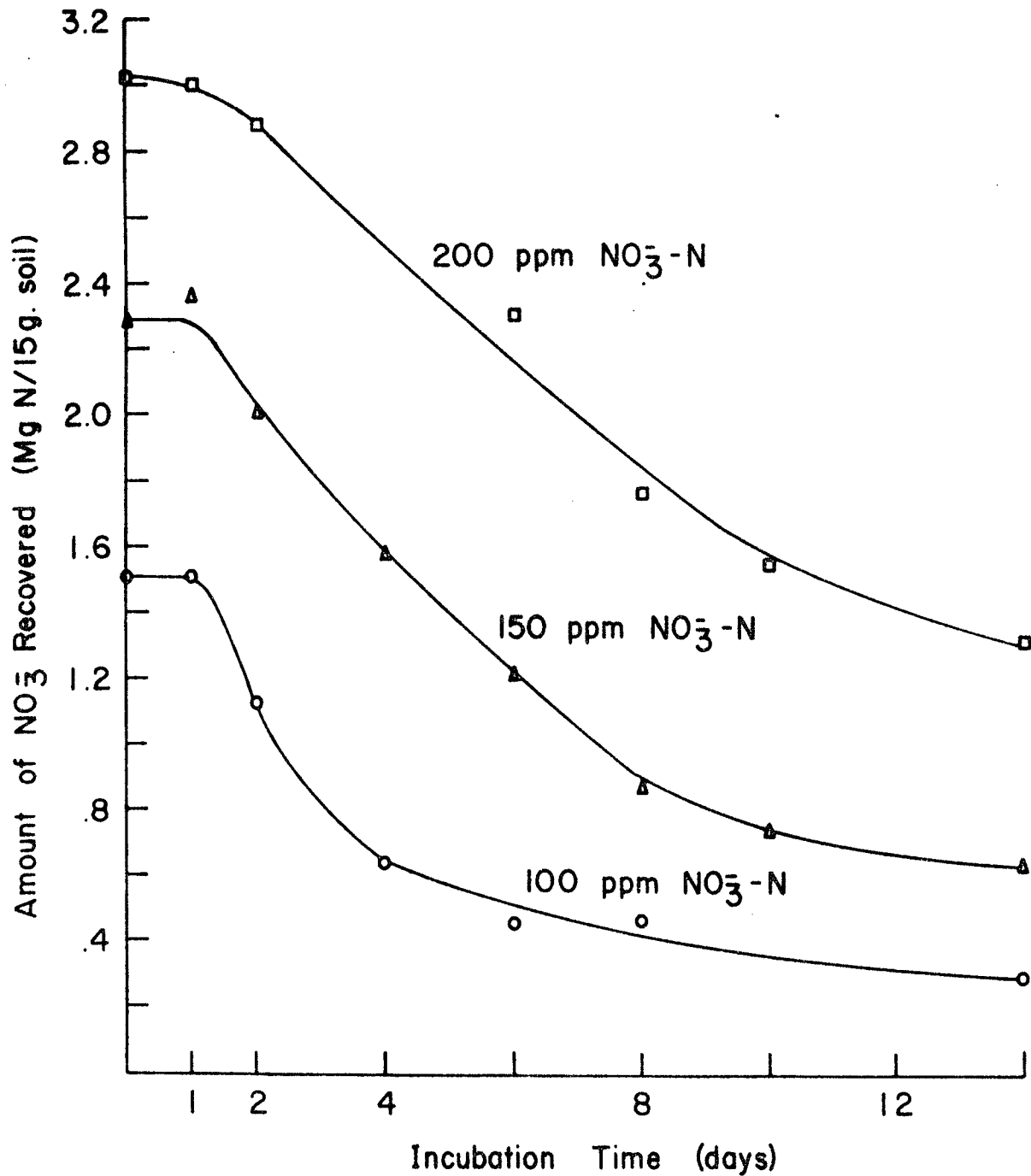


Fig.18 Recovery of $\text{NO}_3\text{-N}$ during the incubation of various initial concentrations of added $\text{NO}_3\text{-N}$ in Keld soil at 25°C .

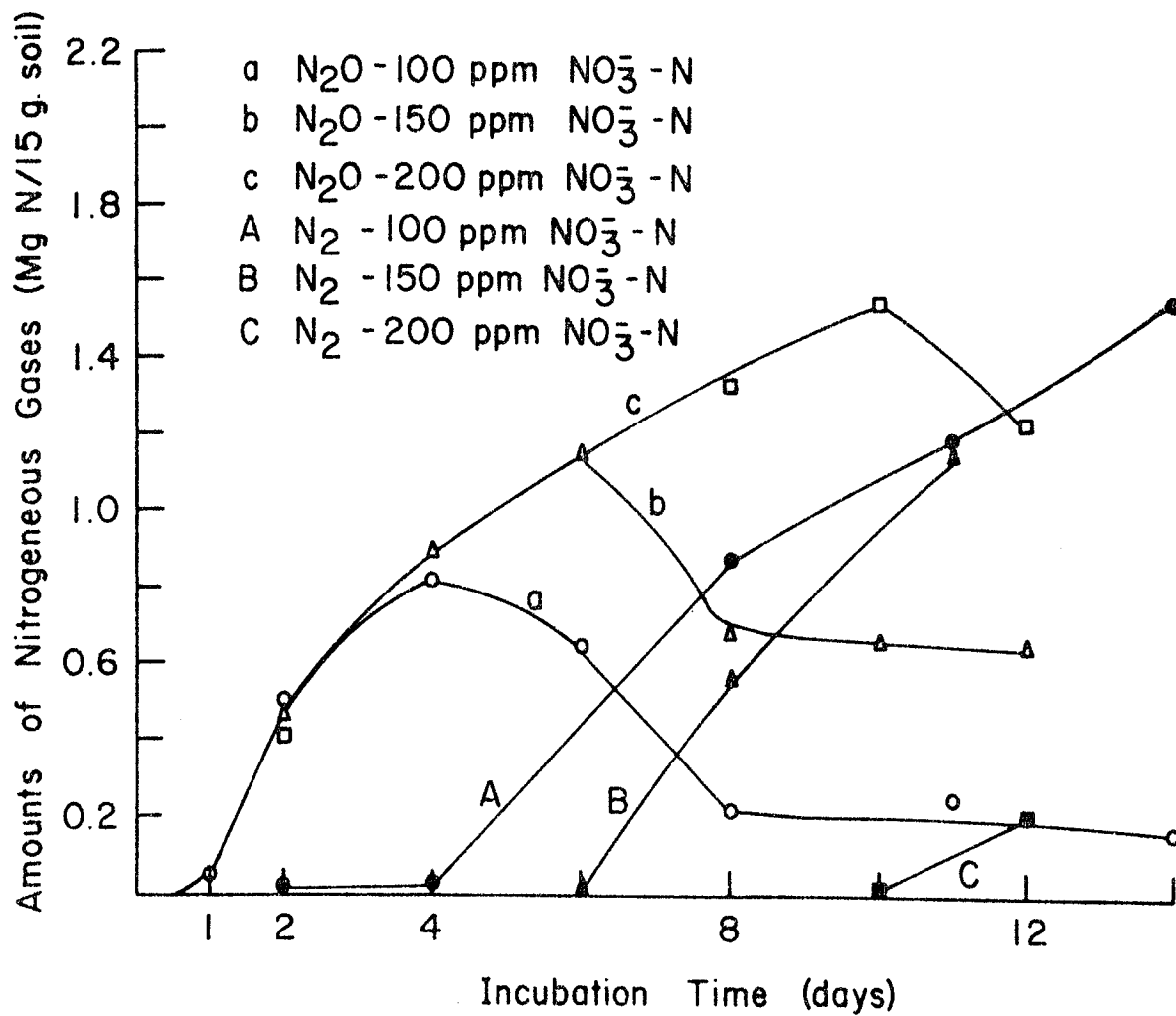


Fig.19 Amounts of N_2O and N_2 during the denitrification of added NO_3^- with various initial concentrations in the Keld soil at 25°C .

reduction of N_2O took place later. Thus at 150 and 200 ppm NO_3^- -N the maximum concentration of N_2O and formation of N_2 occurred at day 6 and day 10, respectively. The maximum concentrations of N_2O were also different with different NO_3^- concentrations. There was a lower accumulation of N_2O with the lower concentrations of NO_3^- .

The nitrate content of the soil incubated at $35^\circ C$ is shown in Figure 20. The disappearance of NO_3^- started the first day with a maximum initial rate of $33 \mu g N/g$ soil/day for soil with 150 and 200 ppm N. The rate of disappearance was slightly lower ($25 \mu g N/g$ soil/day) with soil containing 100 ppm N. The rate of NO_3^- disappearance decreased as the incubation advanced. This can be explained on the basis of available NO_3^- -N in the system and the competitive nature of the product, N_2O , with NO_3^- as e^- acceptor, since a large amount of N_2O was produced (see Figure 21). After 9 and 11 days incubation all the NO_3^- was depleted from the systems with 100 and 150 ppm N, respectively. However, 10% of initial NO_3^- -N remained in the system with 200 ppm N at the end of the incubation period (13 days).

Again N_2O and N_2 were the major gases evolved from the system (Figure 21). Initial rates of N_2O formation were identical ($25 \mu g N/g$ soil/day) for the soil incubated with 150 and 200 ppm NO_3^- -N. A lower rate was obtained with 100 ppm NO_3^- -N treatment ($13 \mu g N/g$ soil/day). This may be due to uncertainty in measuring the maximum value of N_2O production with 100 ppm NO_3^- -N. The maximum quantities

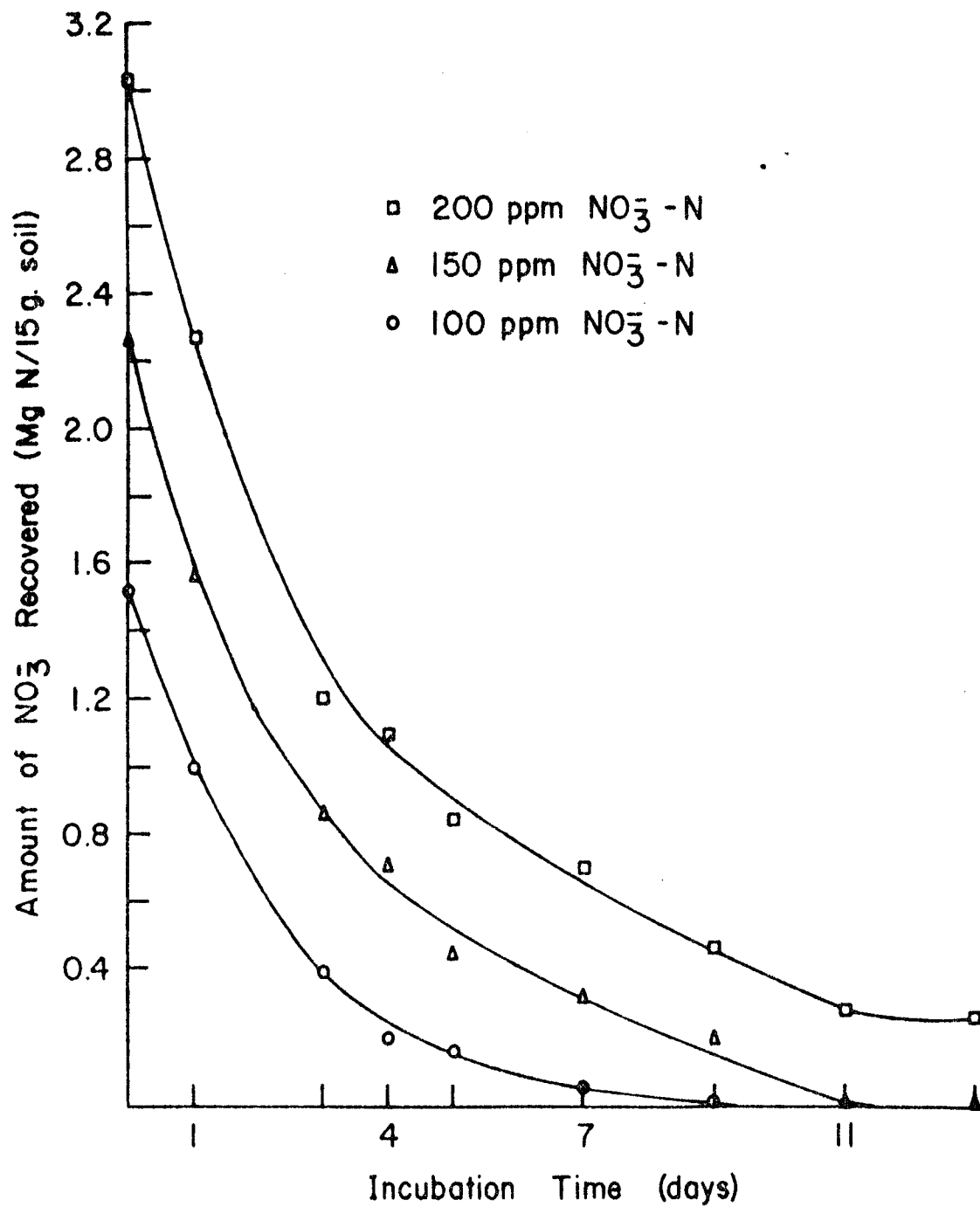


Fig.20 Recovery of NO_3^- -N during the incubation of various initial concentrations of added NO_3^- in the Keld soil at 35°C .

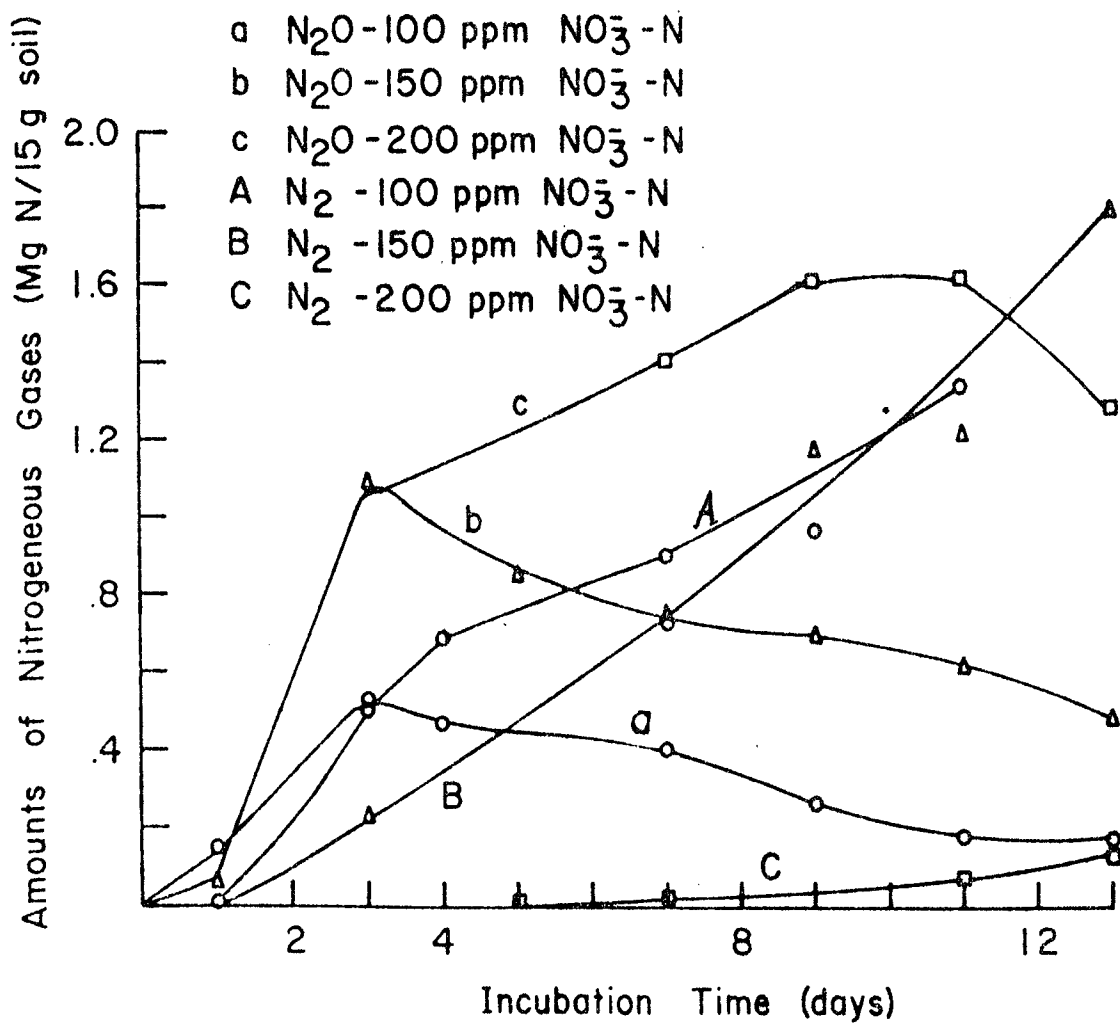


Fig. 21 Amounts of N_2O and N_2 during the denitrification of added NO_3^- with various initial concentrations in the Keld soil at $35^\circ C$.

of N_2O for soils incubated with 100, 150 and 200 ppm NO_3^- -N were 0.5, 1.1 and 1.6 mg N, respectively. These maximum values of N_2O were either equal to or less than the maximum values at $25^\circ C$. The pattern of N_2 production was slightly different from that obtained at $25^\circ C$. Nitrogen gas production started the first day of incubation with the 100 ppm NO_3^- -N treatment, and to a lesser extent with the 150 ppm. The amount of N_2 production from 200 ppm NO_3^- -N treatment was very small even at day 13.

The changing pattern of denitrification with the three temperatures indicates that the whole process is temperature dependent. The reduction of NO_3^- to N_2O and of N_2O to N_2 were both accelerated by temperature increases. Consequently the appearance and disappearance of N_2O was observed earlier with a high incubation temperature. For example, at $15^\circ C$ (Figure 17) no reduction of N_2O occurred irrespective of the NO_3^- concentration.

The consumption of O_2 (Table A-9) and the production of CO_2 (Table A-12) were similar to those of the Wellwood and Morton soils at the same temperature. Nitrite accumulation (Table A-3) and NH_4 (Table A-6) production were also identical to that of Morton and Wellwood.

Table 2 shows the isotopic composition of the gases evolved. There was only a trace of NO_3^- initially in the soil (2 $\mu g/g$ soil). Therefore it was expected that $\%^{15}N$ of the gases evolved would be nearly the same as that of the nitrate added (52.3% ^{15}N) if they were derived solely from added NO_3^- . There was good agreement between $\%^{15}N$ in the added NO_3^- and the gases evolved. The fact indicated that these gases were

solely derived from added NO_3^- and that ^{15}N and ^{14}N behaved in a similar manner. It also indicates that nitrification of ammonium, which was derived from organic matter (unlabelled) in the flooded system was negligible.

Experiment 2 - Denitrification of $\text{Ca}(\text{NO}_3)_2$ in Keld and Wellwood soils with added lime.

Limed Keld Soil

Figure 22 shows the results of NO_3^- -N content of Keld soil at various times of incubation when different amounts of lime* were added. The rates of disappearance of NO_3^- -N with 4% (initial pH 6.2) and 12% (initial pH 7.3) lime were similar and corresponded to 14.7 and 16.7 $\mu\text{g N/g soil/day}$, respectively. However, the rate of disappearance of NO_3^- -N without liming (as mentioned before) was 10 $\mu\text{g N/g soil/day}$. Thus, liming increased the disappearance rate of NO_3^- -N probably by increasing the biological activity, due to the increase in the pH.

The recovery of NO_3^- -N as nitrogenous gases is shown in Figure 23. The addition of 4 and 12% lime to the soil resulted in quite a change in the denitrification pattern of NO_3^- . The maximum concentration and the

*The liming was carried out with CaCO_3 . Hereafter the term lime refers to CaCO_3 .

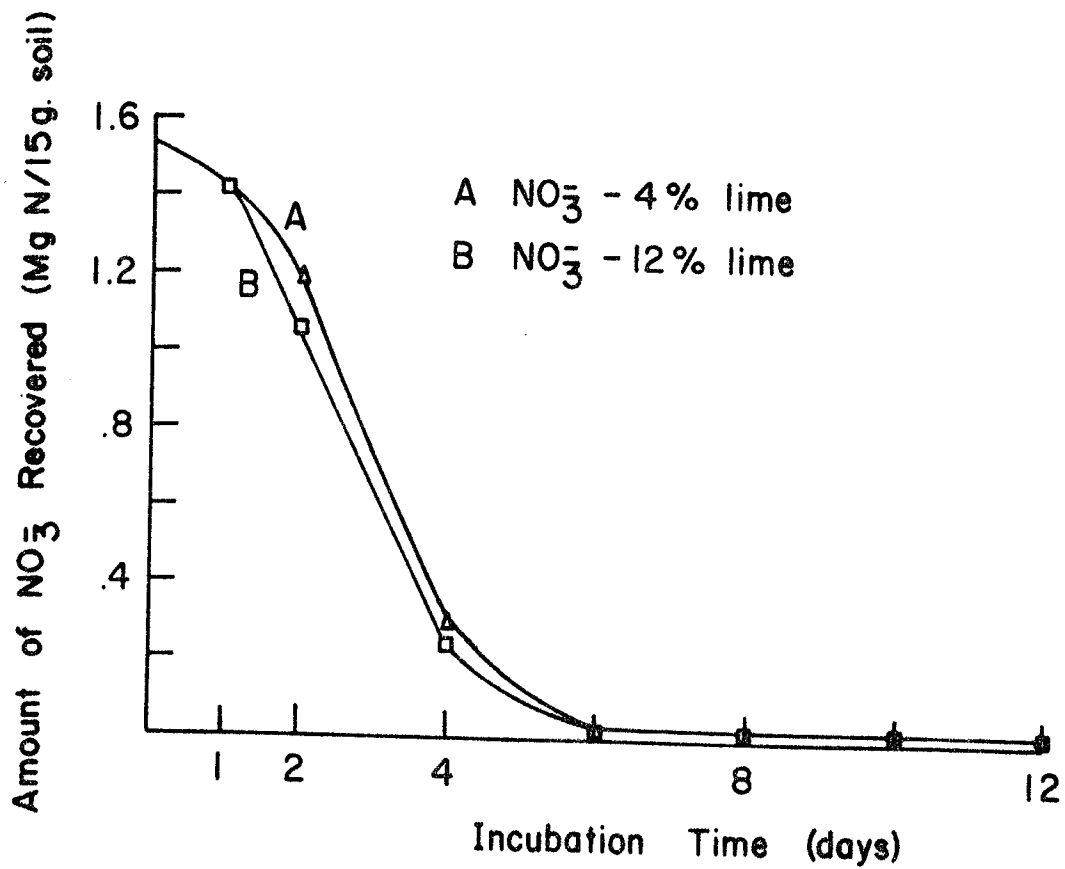


Fig.22 Recovery of NO_3^- -N from Keld soil at various amounts of lime added (N rate = 100 ppm NO_3^- -N).

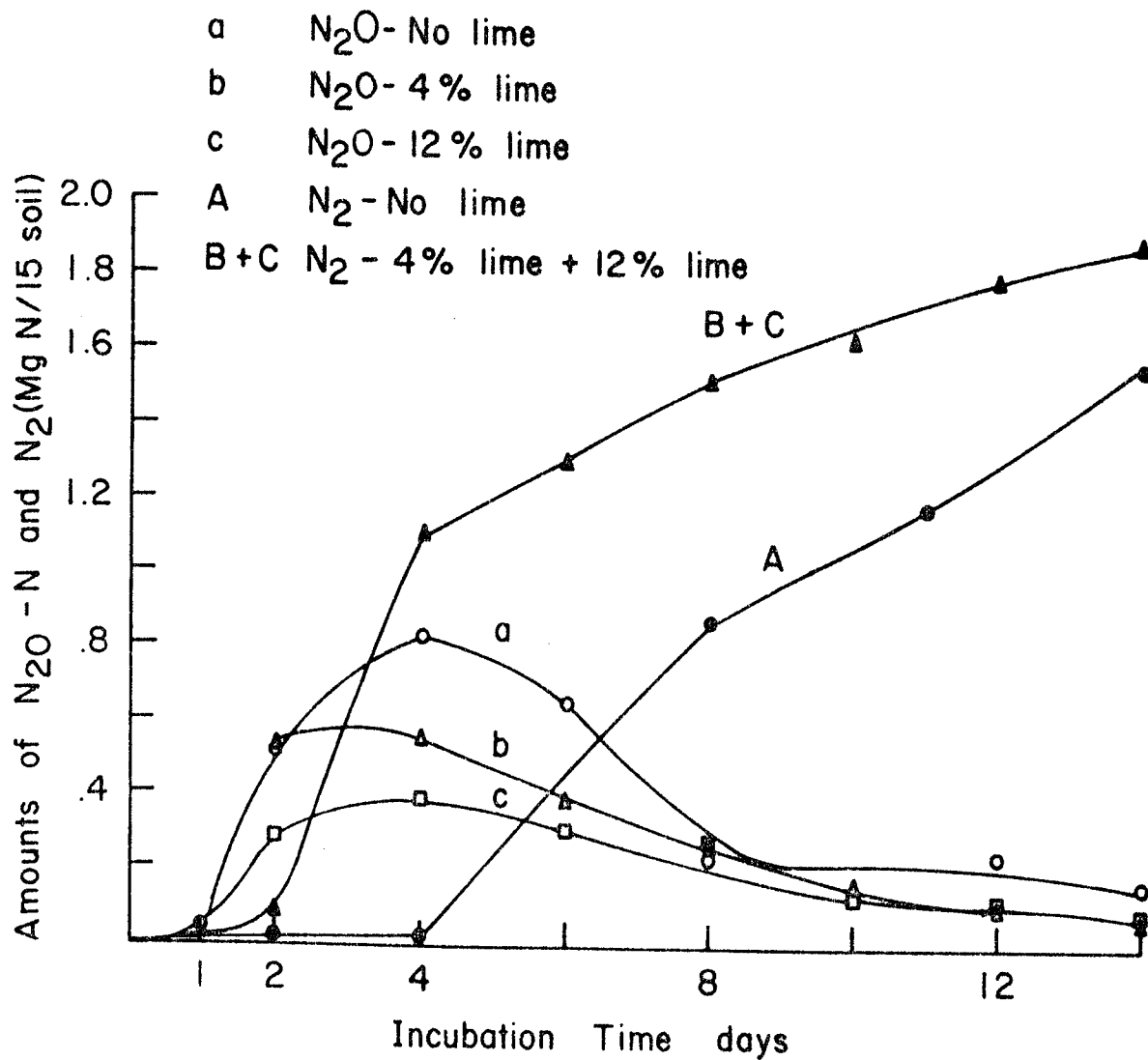


Fig.23 Amounts of N_2O and N_2 during the denitrification of added NO_3^- -N (100 ppm) in the Keld soil at $25^\circ C$ as affected by liming.

time for maximum production of N_2O were decreased by liming. The maximum quantities of N_2O formed were 0.5 and 0.4 mg N for samples incubated with 4 and 12% $CaCO_3$, respectively. The maximum quantity of N_2O produced in the unlimed samples was .8 mg N. Thus it is clear the addition of lime increased the capacity of N_2O to compete with NO_3^- as e^- acceptor. The mechanism for this is not fully understood. It is interesting that under flooded conditions liming did not completely eliminate N_2O formation even though the amount of lime added increased the pH of the Keld soil nearly to that of the Morton soil, which did not produce any N_2O during the denitrification. However, when lime was added N_2 production was initiated by the second day of incubation, while it started after the fourth day of incubation without lime (Figure 2).

The initial production of NO_2^- was increased by the addition of lime and there was quite a large accumulation of NO_2^- (44 μ g N/g soil) at the early stage of incubation (Table A-13). The average life of NO_2^- in this system was longer than that observed in the unlimed system (Table A-13). The addition of lime to the flooded system did not materially alter the O_2 consumption or CO_2 production as compared with the non-limed system (these results were not reported).

Limed Wellwood Soil

The amounts of NO_3^- -N in the limed (6% $CaCO_3$) and unlimed Wellwood soil are shown in Figure 24. The results indicate that the rate of NO_3^-

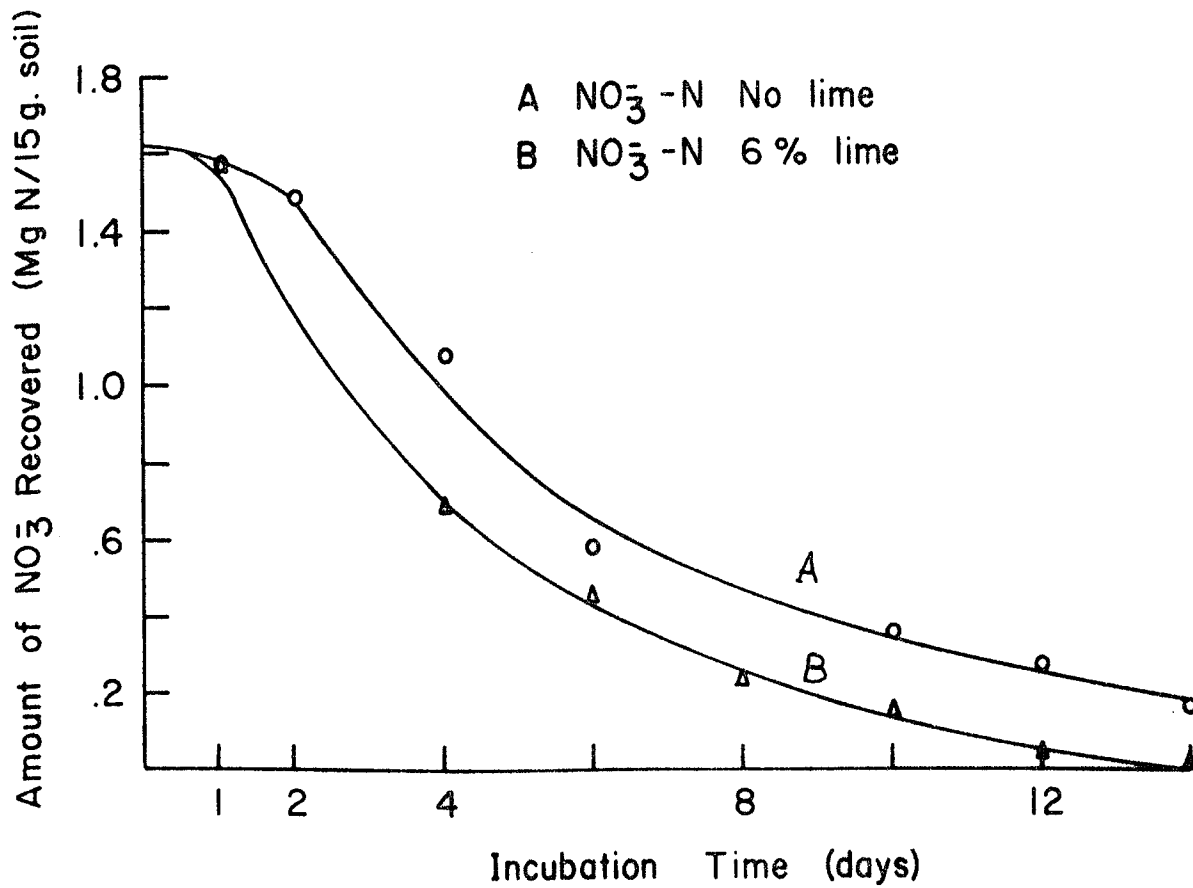


Fig. 24 Recovery of NO_3^- -N from limed and unlimed Wellwood soil (N rate = 100 ppm NO_3^- -N).

disappearance increased with liming. The amount of NO_3^- -N that disappeared within the first four days of incubation corresponded to .92 mg N and .53 mg N for limed and unlimed Wellwood, respectively. With 14 days of incubation, all the added NO_3^- disappeared from the limed soil while 90% of NO_3^- disappeared from unlimed soil.

The major gases produced from both limed and unlimed soil were N_2O and N_2 (Figure 25). The time required to attain the maximum concentration of N_2O for both soils was the same (2 days). However, the quantity of N_2O -N at the maximum was greater with the limed than without liming i.e., 0.6 mg N_2O -N for limed as compared to 0.4 mg N_2O -N for unlimed. It is not known why such an increase in the value of maximum N_2O occurred due to liming. A reverse effect, i.e., the reduction in maximum N_2O production, was observed with the Keld soil (see Figure 23).

Nitrogen gas production was increased due to liming. The amount of N_2 produced in four days was 1.0 mg-N for the limed soil compared to 0.55 mg-N for the unlimed soil. The increase in the rate of N_2 production due to liming suggests an increase in the biological activity.

Nitrite-N accumulation during incubation amounted to 21 $\mu\text{g/g}$ soil. This accumulation occurred at the early stage of incubation. Therefore, one can conclude that liming the acid and neutral soils increased the NO_2^- -N accumulation.

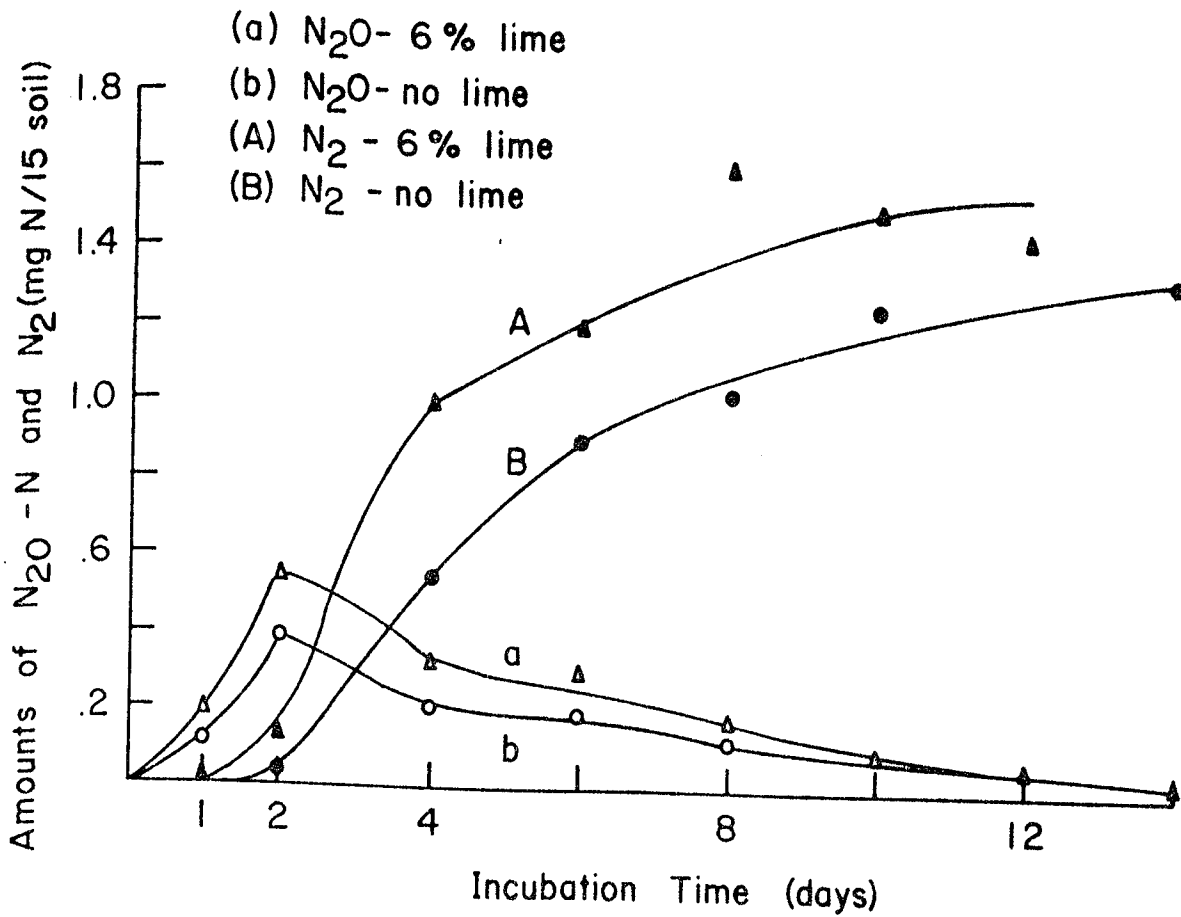


Fig. 25 Amounts of N_2O and N_2 during the denitrification of added NO_3^- -N (100 ppm) in the Wellwood soil at $25^\circ C$ as affected by liming.

SUMMARY AND CONCLUSIONS

Denitrification behavior of NO_3^- in Morton (pH - 7.8), Wellwood (pH - 6.3) and Keld (pH - 5.4) soils was investigated under flooded conditions using ^{15}N labelled $\text{Ca}(\text{NO}_3)_2$ and mass spectrometry. The rate of decrease in the added NO_3^- and the production rate of N_2O and N_2 gases were investigated in order to determine the effects of concentration of NO_3^- and temperature on denitrification. Three temperatures of incubation (15, 25 and 35°C) and three levels of NO_3^- -N (100, 150, 200 ppm N) were used. Incubation times mainly were 1, 2, 4, 6, 8, 10, 12 and 14 days.

With the Morton soil, NO_3^- disappearance followed the same pattern and occurred to the same magnitude irrespective of the levels of NO_3^- added (zero order kinetics). However, the amounts of NO_3^- lost increased with increasing temperature. There was no N_2O formation, regardless of the temperature or the NO_3^- concentration. The total quantity of nitrogen gas formed compared reasonably well with the NO_3^- -N disappearance. The effect of temperature upon N_2 formation, expressed as Q_{10} , was found to be 1.62 regardless of the level of NO_3^- , with activation energy of $8570 \text{ cal. mole}^{-1}$.

In the Wellwood soil the initial rate of NO_3^- disappearance was unaffected by NO_3^- concentration for each temperature. The rate of NO_3^- disappearance for the longest incubation time used was constant with a high concentration of NO_3^- (200 ppm N). However, the rate slowed down with incubation time at the lower concentrations of NO_3^- where incubation temperatures of 25°C and 35°C were used. The nitrogenous gases evolved

during incubation were nitrous oxide and nitrogen gas. N_2O was the major gas evolved during the initial period of incubation. Its initial rate of production was found to be unaffected by NO_3^- concentration. The maximum level of N_2O was increased with an increase in the initial NO_3^- concentration. It amounted to approximately 25% of the initial NO_3^- -N. An increase in the incubation temperature caused an increase in the production of N_2O and a decrease in the time required to reach the maximum level. As NO_3^- concentration decreased N_2O served as a terminal \bar{e} acceptor and N_2 became the final denitrification product.

Nitrogen gas production was related to the concentration of added NO_3^- . When the pool size of NO_3^- was low production of N_2 took place at an early stage of incubation. As the pool size of NO_3^- increased the formation of N_2 took place later. The amount of N_2 production increased, and its formation took place earlier with an increase in temperature.

The effect of NO_3^- concentration on the rate of disappearance of NO_3^- with Keld soil was similar to that of Wellwood. Increasing the NO_3^- concentration increased maximum N_2O production. Consequently, by increasing the pool size of NO_3^- , the amount of N_2 production decreased at the early stages of incubation.

The initial rate of NO_3^- disappearance increased with increasing temperature. As the incubation proceeded NO_3^- in the system became limiting especially at $35^\circ C$. Consequently, the competition between the product (N_2O) and reactant (NO_3^-) as \bar{e} acceptor became obvious. Thus the rate of NO_3^- disappearance decreased with the increase in

the level of N_2O .

The effect of environmental conditions on gaseous production was similar for both acid (Keld) and neutral (Wellwood) soils, but different pattern was found in the alkaline soil (Morton).

In an attempt to assess the effect of the pH on the rate of denitrification, different amounts of lime ($CaCO_3$) were added to the acid soil (Keld) and to the neutral soil (Wellwood). Liming increased the disappearance rate of NO_3^- -N, possibly due to increased biological activity. It was found, however, that under flooded conditions, liming the acid soil did not completely eliminate N_2O formation. The maximum concentration of N_2O and the time at which the maximum production occurred were decreased by the addition of lime. Therefore, the addition of lime promoted the capacity of N_2O as \bar{e} acceptor and promoted its competition with the reactant (NO_3^-).

The isotopic composition of produced N_2 and N_2O was independent of the incubation temperature and the time of incubation. The isotopic effect, if any, is negligible for this investigation since high percentage of ^{15}N was used.

Regardless of the original pH the amount of NO_2^- -N that accumulated during the incubation did not exceed 15 $\mu g/g$ soil. This accumulation occurred at an early stage of incubation and was found to be slightly higher at the lowest temperature used. Liming the soil increased NO_2^- -N accumulation to maximum values of 21 and 44 $\mu g N/g$ soil for the limed Wellwood soil and the limed Keld soil, respectively.

The KCl extractable ammonium was found to increase during incubation. This increase was not related to the amount of NO_3^- -N added, although it did increase with increasing temperature. Up to 40 $\mu\text{g/g}$ soil of NH_4^+ -N accumulated in the flooded system during 14 days of incubation.

The partial pressure of O_2 in the incubation atmosphere was affected only slightly by an increase in NO_3^- concentration. However, a higher consumption of O_2 occurred with an increase in temperature. Carbon dioxide production was related to the temperature, e.g., higher CO_2 production occurred at higher temperatures. There was no effect of NO_3^- concentration upon CO_2 production. The addition of lime to the soils did not alter the O_2 consumption or CO_2 production.

In summation, the work reported in this thesis has shown that inorganic nitrogen (NO_3^-) was quite unstable and acted as an electron acceptor under flooded conditions. The rate of initial disappearance of added NO_3^- was independent of NO_3^- concentration.

Nitrogen was the major gas produced from alkaline Morton soil. In the neutral Wellwood and acid Keld soils, however, the major gases were N_2O and N_2 . As NO_3^- was converted to N_2O the N_2O started to compete with NO_3^- as an \bar{e} acceptor and was reduced to N_2 . Thus the accumulation of N_2O was lower, or the conversion of N_2O to N_2 was favored, when NO_3^- concentration was lower. The rate of initial production of N_2O was independent of NO_3^- concentration, suggesting that the biological oxygen demand per unit weight of soil was nearly constant. The amount of N_2O which accumulated in the acid soil was higher than in the neutral soil.

When there is no accumulation of an intermediate, which competes with nitrate as the \bar{e} acceptor, the rate of disappearance of added NO_3^- or the formation of N_2 can be used as a measure of denitrification intensity.

Liming the acid soil reduced the formation of N_2O as an intermediate. Such a reduction in the accumulation was due to promotion of N_2O as an \bar{e} acceptor relative to NO_3^- .

LITERATURE CITED

- Adel, A. 1946. A possible source of atmosphere N_2O . *Science* 103: 280.
- Alberda, T.H. 1953. Growth and root development of low land rice and its relation to oxygen supply. *Plant Soil* 5: 1-28.
- Allison, F.E. 1955. The enigma of soil nitrogen balance sheets. *Advances in Agronomy* 7: 213-250.
- 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. 1965. Evaluation of incoming and outgoing processes that affect soil nitrogen. In *Soil Nitrogen*, 1st ed. American Society of Agronomy. Madison, Wisconsin. pp. 573-606.
- Allison, F.E. 1966. The fate of nitrogen applied to soil. *Adv. Agron.* 18: 219-258.
- Allison, F.E. and Doetsch, J.H. 1950. Nitrogen gas production by the reaction of nitrites with amino acid in slightly acidic media. *Soil Sci. Soc. Proc.* 14: 163-166.
- Allison, F.E., Doetsch, J.H. and Sterling, L.D. 1952. Nitrogen gas production by interaction of nitrites and amino acids. *Soil Sci.* 74: 311-314.
- Arnold, P.W. 1954. Losses of nitrous oxide from soil. *J. Soil Sci.* 5: 116-128.
- Austin, A.T. 1961. Nitrosation inorganic chemistry. *Sci. Progr.* 61: 619-640.
- Bailey, L.D. and Beauchamp, E.G. 1973a. Effects of temperature on NO_3^- and NO_2^- reduction, nitrogenous gas production and redox potential in a saturated soil. *Can. J. Soil Sci.* 53: 213-218.
- Bailey, L.D. and Beauchamp, E.G. 1973b. Effects of moisture, added NO_3^- , and macerated roots on NO_3^- transformation and redox potential in surface and subsurface soils. *Can. J. Soil Sci.* 53: 219-230.
- Beijerinck, M.W. and Minkman, D.C.J. 1910. *Zentralbl., Parasitenk., Infektionskr. Hyg., Abt.* 225, 30-63. [Cited in Focht and Chang, 1975].
- Blackmer, A.M. and Bremner, J.M. 1977. Nitrogen isotope discrimination in denitrification of nitrate in soils. *Soil Biol. Biochem.* 9: 73-77.
- Bollag, J.M., Brzymala, S. and Kardos, L.T. 1973. Biological versus chemical nitrite decomposition in soil. *Soil Sci.* 116: 44-50.

- Bollag, J.M., Orcutt, M.L., Bollag, B. 1970. Denitrification by isolated soil bacteria under various environmental conditions. *Soil Sci. Soc. Amer. Proc.* 34: 875-879.
- Bowman, R.A., Focht, D.D. 1974. The influence of glucose and nitrate concentration upon denitrification rates in sandy soils. *Soil Biol. Biochem.* 6: 297-301.
- Bray, H.G. and White, K. 1966. *Kinetics and thermodynamics in Biochemistry*. 2nd ed. Academic Press, New York.
- Bremner, J.M. 1965. Inorganic forms of nitrogen. In *Methods of Soil Analysis*. pp. 1179-1232. Monograph No. 9 Amer. Soc. Agron., Madison, Wisconsin.
- Bremner, J.M. and Nelson, D.W. 1968. *Trans. 9th Int. Congr. Soil Sci.* 495-503. [Iowa State Univ., Ames, Iowa, U.S.A.].
- Bremner, J.M., and Shaw, K. 1958. Denitrification in Soil II. Factors affecting denitrification. *J. Agr. Sci.* 51: 40-52.
- Broadbent, F.E. 1951. Denitrification in some California soils. *Soil Sci.* 72: 129-137.
- Broadbent, F.E. and Clark, F. 1965. Denitrification. In *Soil Nitrogen*. pp. 347-359. Monograph No. 10. Amer. Soc. Agron. Madison, Wisconsin.
- Broadbent, F.E. and Stevenson, F.J. 1966. Organic Matter Interaction. pp. 169-187. In *Agricultural Anhydrous Ammonia Technology and Use*. M.H. McVickal *et al.* (ed.) American Society of Agronomy, Madison, Wisconsin.
- Broadbent, F.E. and Stojanovic, B.J. 1952. The effect of partial pressure of oxygen on some soil nitrogen transformation. *Soil Sci. Soc. Amer. Proc.* 16: 359-363.
- Broadbent, F.E. and Tusneem, M.E. 1971. Losses of nitrogen from some flooded soils in tracer experiments. *Soil Sci. Soc. Amer. Proc.* 35: 922-926.
- Bulla, L.A., Gilmour, C.M. and Bollen, W.B. 1970. Nonbiological reduction of nitrite in soil. *Nature* 225: 664.
- Burford, J.R. and Bremner, J.M. 1975. Relationship between the denitrification capacities of soils and total water-soluble and readily decomposable soil organic matter. *Soil Biol. Biochem.* 7: 389-394.
- Cady, F.B. and Bartholomew, W.V. 1960. Sequential products of anaerobic denitrification in Norfolk soil material. *Soil Sci. Soc. Amer. Proc.* 24: 477-482.
- Cady, F.B. and Bartholomew, W.V. 1961. Influence of low PO_2 on denitrification products and process. *Soil Sci. Soc. Amer. Proc.* 25: 362-365.

- Cady, F.B. and Bartholomew, W.V. 1963. Investigation of nitric oxide reaction in soils. *Soil Sci. Soc. Amer. Proc.* 27: 546-549.
- Cheng, H.H. and Bremner, J.M. 1966. Determination and isotope-ratio analysis of different forms of nitrogen in soils: 2. A simplified procedure for isotope-ratio analysis of soil nitrogen. *Soil Sci. Soc. Amer. Proc.* 30:450-452.
- Cheng, H.H., Bremner, J.M. and Edwards, A.P. 1964. Variations of nitrogen-15 abundance in soils. *Science* 146: 1574-1575.
- Chien, S.H., Shearer, G. and Kohl, D.H. 1977. The nitrogen isotope effect associated with nitrate and nitrite loss from water-logged soils. *Soil Sci. Soc. Amer. Proc.* 41: 63-69.
- Christianson, C.B. 1978. Denitrification of urea and sodium nitrite in some Manitoba soils. M.Sc. Thesis submitted to the Faculty of Graduate Studies and Research, University of Manitoba, Winnipeg, Manitoba.
- Cho, C.M. 1976. Mass spectrometric investigation of denitrification. Dept. of Soil Science, University of Manitoba, Winnipeg, Manitoba.
- Cho, C.M. and Sakdinan, L. 1978. Mass spectrometric Investigation on denitrification. *Can. J. Soil Sci.* (in press).
- Clark, F.E. 1962. Losses of nitrogen accompanying nitrification. *International Soil Conference Transactions, New Zealand, 1962.* pp. 173-176.
- Clark, F.E., Beard, W.E. and Smith, D.H. 1960. Dissimilar nitrifying capacities of soils in relation to losses of applied nitrogen. *Soil Sci. Soc. Amer. Proc.* 24: 50-54.
- Cooper, G.S. and Smith, R.L. 1963. Sequence of products formed during denitrification in some diverse western soils. *Soil Sci. Soc. Amer. Proc.* 27: 659-662.
- Dawson, R.W. and Murphy, K.L. 1972. The temperature dependency of biological denitrification. *Water Res.* 6: 71-83.
- Delwiche, C.C., Bryan, A.B. 1976. Denitrification. *Ann. Rev. Microbiol.* 30: 241-262.
- Dowdell, R.J. and Smith, K.A. 1974. Field studies of the soil atmosphere. II. Occurrence of nitrous oxide. *J. Soil Sci.* 25: 231-238.
- Focht, D.D. 1974. The effect of temperature, pH and aeration on the production of nitrous oxide and gaseous nitrogen, a zero order kinetic model. *Soil Sci.* 118: 173-179.
- Focht, D.D. and Chang, A.C. 1975. Nitrification and denitrification processes related to waste water treatment. *Adv. Appl. Microbiol.* 17: 152-187.

- Gayon, U. and Dupetit, G. 1886. Mem. Soc. Sci. Phys. Nat. Bordeaux. Ser. 3, 2, 201. [Cited in Focht and Chang, 1975].
- Gerretson, F.C. and deHoop, H. 1957. Nitrogen losses during nitrification in solutions and in acid sandy soils. Can. J. Microbiol. 3: 359-380.
- Greenland, D.J. 1962. Denitrification in some tropical soils. Agr. Sci. 58: 227-233.
- Greenwood, D.J. 1961. The effect of oxygen concentration on the decomposition of organic material in soil. Plant and Soil 14: 360-376.
- Harter, R.D. and Ahlrichs, J.L. 1967. Determination of clay surface acidity by infrared spectroscopy. Soil Sci. Soc. Amer. Proc. 31: 30-33.
- Hauck, R.D. and Melsted, S.W. 1956. Some aspects of the problem of evaluating denitrification in soils. Soil. Sci. Soc. Amer. Proc. 20: 361-364.
- Hauck, R.D. Melsted, S.W. and Yankwich, P.E. 1958. Use of N-isotope distribution in nitrogen gas in the study of denitrification. Soil Sci. 86: 287-291.
- Howeler, R.H. and D.R. Bouldin. 1971. The diffusion and consumption of oxygen in submerged soils. Soil Sci. Amer. Proc. 35: 202-208.
- Ingraham, J.L. 1962. Temperature relationship. "In the bacteria, I.C. Gunsalus and R.Y. Stanier Academic Press, N.Y.," Vol. 4, pp. 265-296. [Cited in Focht, 1974].
- Jansson, S.L. and Clark, F.E. 1952. Losses of nitrogen during the decomposition of plant material in the presence of inorganic nitrogen. Soil Sci. Soc. Amer. Proc. 16: 330-334.
- Jones, R.W. and Hedlin, R.A. 1970. Nitrite instability in three Manitoba soils. Can. J. Soil Sci. 50: 339-345.
- Kamphake, L.J., Hannah, S.A. and Cohen, J.M. 1967. Automated analysis for nitrate by hydrazine reduction. Water Res. 1: 205-216.
- Kefauver, M. and Allison, F.E. 1957. Nitrite reduction by Bacterium denitrificans in relation to oxidation-reduction potential and oxygen tension. J. Bact. 73: 8-14.
- Khan, M.F.A. and Moore, A.W. 1968. Denitrification capacity of some Alberta soils. Can. J. Soil Sci. 48: 89-91.
- Lahniger, A.L. 1970. Biochemistry. Enzymes: Kinetics and inhibition. pp. 147-165. 1st ed. Worth Publishers, Inc., N.Y.
- Latimer, W.M. 1952. Oxidation potential, 2nd ed. N.Y. Prentice-Hall, Inc.
- Macrae, I.C., Ancajas, R.R. and Salandanam, S. 1968. The fate of nitrate

- nitrogen in some tropical soils following submergence. *Soil Sci.* 105: 327-334.
- Meek, B.D., Grass, L.B. and Mackenzie, A.J. 1969. Applied nitrogen losses in relation to oxygen status of soils. *Soil Sci. Soc. Amer. Proc.* 33: 575-578.
- Mertens, J.P., VanderWinkel and Massart, D.L. 1975. Determination of nitrate in water with an ammonia probe. *Analyt. Chem.* 47: 522-526.
- Myers, R.J.K. and McGarity, J.W. 1972. Denitrification in undisturbed cores from a solodized Solonetz B horizon. *Plant and Soil.* 37: 81-89.
- Nelson, D.W. and Bremner, J.M. 1969. Factors affecting chemical transformation of nitrite in soils. *Soil Biol. Biochem.* 1: 229-239.
- Nelson, D.W. and Bremner, J.M. 1970a. Gaseous products of nitrite decomposition in soil. *Soil Biol. Biochem.* 2: 203-215.
- Nelson, D.W. and Bremner, J.M. 1970b. Role of soil minerals and metallic cations in nitrite decomposition on chemodenitrification in soils. *Soil Biol. Biochem.* 2: 1-8.
- Nier, A.O. 1950. A redetermination of the relative abundances of the isotope of carbon, nitrogen, oxygen, argon and potassium. *Phys. Rev.* 77:789-793.
- Nommik, H. 1956. Investigation on denitrification in soil. *Acta. Agric. Scand.* VI. 2: 195-228.
- Nommik, H. and Thorin, J. 1972. Transformation of ¹⁵N-labelled nitrite and nitrate in forest raw humus during anaerobic incubation. *Proceedings . "Isotope and radiation in soil-plant relationship including forestry"* IAEA, Vienna, Austria, p. 369.
- Owens, L.D. 1960. Nitrogen movement and transformation in soil as evaluated by a lysimeter study utilizing isotopic nitrogen. *Soil Sci. Soc. Amer. Proc.* 24: 372-376.
- Pang, P.C. Cho, C.M. and Hedlin, R.A. 1975. Effects of nitrogen concentration on the transformation of band-applied nitrogen fertilizers. *Can. J. Soil Sci.* 55: 23-27.
- Patrick, W.H. Jr. 1961. Nitrate reduction rates in a submerged soil as affected by redox potential. *Proc. Int. Congr. Soil Sci.* 7th. 1960. pp. 494-500.
- Patrick, W.H. Jr. and Delaune, R.D. 1972. Characterization of the oxidized and reduced zones in flooded soil. *Soil Sci. Soc. Amer. Proc.* 36: 575-576.
- Patrick, W.H. Jr. and Gotoh, S. 1974. The role of oxygen in nitrogen loss from flooded soils. *Soil Sci.* 118: 78-81.
- Patrick, W.H. Jr. and Reddy, K.R. 1976. Nitrification-denitrification reaction in flooded soils and water bottoms: Dependence on oxygen

- supply and ammonium diffusion. *J. Environ. Qual.* 5: 469-471.
- Patrick, W.H. Jr. and Mahapatra, I.C. 1968. Transformation and availability to rice of nitrogen and phosphorus in water-logged soil. *Adv. Agron.* 20: 323-359.
- Payne, W.J. 1973. Reduction of Nitrogenous oxides by Microorganisms. *Bacteriol. Rev.* 37: 409-452.
- Pearsall, W.H. and Mortimer, C.H. 1939. Oxidation-Reduction potentials in water-logged soils, natural waters and muds. *J. Ecol.* 27: 483-501.
- Peech, M., Alexander, L.T., Dean, L.A. and Reed, J.F. 1947. Methods of soil analysis for fertility investigations. U.S. Dept. of Agric. Circ. 757.
- Pilot, L. and Patrick, W.H. Jr. 1972. Nitrate reduction in soils: Effect of soil moisture tension. *Soil Sci.* 114: 312-316.
- Porter, L.K. 1969. Gaseous products produced by anaerobic reaction of sodium nitrite with oxime compounds and oximes synthesized from organic matter. *Soil Sci. Amer. Proc.* 38: 696-702.
- Renner, E.D. and Becker, G.E. 1970. Production of nitric oxide and nitrous oxide during denitrification of Corynebacterium nephridii. *J. Bacteriol.* 101: 821-826.
- Reuss, J.O. and Smith, R.L. 1965. Chemical reactions of nitrites in acid soils. *Soil. Sci. Soc. Amer. Proc.* 30: 363-366.
- Rittenberg, D., Keston, A.S., Rosebury, F. and Schoenheiner, R. 1939. Protein metabolism. II. The determination of nitrogen isotopes in organic compounds. *J. Biol. Chem.* 127: 291-299.
- Rolston, D.E., Fried, M. and Goldhamer, D.A. 1976. Denitrification measured directly from nitrogen and nitrous oxide fluxes. *Soil Sci. Soc. Amer. Proc.* 40: 259-266.
- Roulier, M.H. and Fetter, N.R. 1973. Procedures for sampling and analysis of the atmosphere of field soils for trace amounts of nitrous oxide. *Agron. Abstr.* pp. 78-79.
- Russel, F.W. 1973. Soil condition and plant growth. pp. 343-349 [Longman, London].
- Sacks, L.E. and Barker, H.A. 1949. The influence of oxygen on nitrate and nitrite reduction. *J. Bact.* 58: 11-22.
- Shapley, D. 1977. Will fertilizer harm ozone as much as SST's? *Science* 195: 658.
- Skinner, S.I.M., Halstead, R.L. and Brydon, J.E. 1959. Qualitative manometric determination of calcite and dolomite in soils and limestones. *Can. J. Soil Sci.* 39: 197-204.

- Smith, D.H. and Clark, F.E. 1960. Volatile losses of nitrogen from acid or neutral soils or solutions containing nitrite and ammonium ions. *Soil Sci.* 90: 86-92.
- Sneed, M.C. and Brasted, R.C. 1956. *Comprehensive inorganic chemistry.* V.D. VanNostrand Co., Inc., Princetown, N.J.
- Stanford, G., Vanderpol, R.A. and Dzienia, S. 1975. Denitrification rates in relation to total and extractable soil carbon. *Soil Sci. Soc. Amer. Proc.* 39: 384-289.
- Stefanson, R. A. 1972. Soil denitrification in sealed soil-plant systems. 1 - Effect of plants, soil water content and soil organic matter content. *Plant Soil* 37: 113-127.
- Stevenson, F.J., Harrison, R.M., Wetsalaar, R. and Leeper, R. A. 1970. Nitrosation of soil organic matter. III. Nature of gases produced by reaction of nitrite with lignins, humic substance, and phenolic constituents under neutral and slightly acidic conditions. *Soil Sci. Soc. Amer. Proc.* 34: 430-435.
- Taylor, S.A. and Ashcroft, G.L. 1972. *Physical Edaphology* (W.H. Freeman and Co., San Francisco) pp. 533.
- Valera, C.L. and Alexander, M. 1961. Nutrition and physiology of denitrifying bacteria. *Plant Soil* 15: 268-280.
- Wahhab, A. and Uddin, F. 1954. Loss of nitrogen through reaction of ammonium and nitrite ions. *Soil Sci.* 78: 119.
- Wallace, A. and Smith, R.C. 1954. Nitrogen interchange during decomposition of orange and avocado tree residues. *Soil Sci.* 78: 231-242.
- Weissenberg, H. 1902. Uber die denitrifikation Zentr Bakteriologie. *Parnsitentk*, 11, Abt., 8: 166. [Cited in Delwiche and Bryan, 1976].
- Wellman, R.P. Cook, F.D. and Krouse, H.R. 1968. Nitrogen-15 Microbiological alteration of abundance. *Science* 161: 269-270.
- Wesseling, J. and VanWijk, W.R. 1957. Land Drainage in relation to soils and crops. *Agronomy* 7, Ch. V.: 461-504.
- Wijler, J. and Delwiche, C.C. 1954. Investigation on the denitrifying process in soil. *Plant Soil* 5: 155-169.
- Wilson, D.W., Nier, A.O.C. and Reiman, S.P. 1948. Preparation and measurement of isotopic tracers: A symposium of J.W. Edwards Co., Ann Arbor, Michigan.
- Woldendrop, J.W. 1962. The quantitative influence of the rhizosphere on denitrification. *Plant and Soil* 17: 267-270.
- Wullstein, L.H. and Gilmour, C.M. 1964. Non-enzymatic gaseous loss of nitrite from clay and soil system. *Soil Sci.* 97: 428.

Yamane, I. 1957. Nitrate reduction and denitrification in flooded soil. *Soils plant food* 3: 100-103.

Yoshida, T. and Alexander, M. 1970. Nitrous oxide formation by Nitrosomonas europea and heterotrophic microorganisms. *Soil Sci. Soc. Amer. Proc.* 34: 880-882.

APPENDIX

Table (A-1) Amount of NO_2^- -N in the Morton soil samples at various times of incubation under different temperatures and concentration of added NO_3^- -N.

Temp. C°	Conc. NO_3^- -N ppm	NO_2^- -N mg. /15g. soil							
		Incubation Time, days							
		1	2	4	6	8	10	12	14
15	100	.23	0.24	0.11	.02	.01	.01	-	-
15	150	.10	0.10	.07	.01	.01	0.01	0.01	0.01
15	200	.06	0.04	-	0.01	-	0.01	-	-
25	100	0.01	0.01	.03	0.01	0.01	0.01	0.01	-
25	150	0.01	0.02	.02	0.02	0.01	0.01	0.09	-
25	200	-	-	0.01	0.01	0.03	0.03	0.09	.08
35	100	0.02	.01	.01	0.01	0.01	0.01	0.02	-
35	150	0.02	0.02	.01	0.01	0.01	0.01	0.01	0.02
35	200	0.01	0.02	.01	0.01	0.01	0.01	.01	0.02

Table (A-2) Amount of NO_2^- -N in the Wellwood soil at various times of incubation under different temperatures and concentrations of added NO_3^- -N.

Temp. C°	Conc. NO_3^- -N ppm	NO_2^- -N							
		Incubation Time, days							
		1	2	4	6	8	10	12	14
15	100	0.03	-	.03	0.01	.01	0.01	0.01	0.01
15	150	0.04	0.01	.05	.02	.02	0.01	0.01	0.01
15	200	0.03	.047	.01	.06	.02	.01		0.01
25	100	0.04	.045	.01	0.01	0.01	0.01	.01	0.01
25	150	-	.060	.01	0.01	0.01	0.01	.01	0.01
25	200	-	.083	.01	0.01	.02	0.01	.01	0.01
35	100	0.06	.06	0.02	0.02	.01	-	-	-
35	150	0.14	0.04	0.02	0.02	0.01	-	-	-
35	200	0.11	0.05	0.02	0.02	.01	0.01	0.01	-

Table (A-3) Amount of NO_2^- -N in the Keld soil at various times of incubation under different temperatures and concentrations of added NO_3^- -N

Temp. C ^o	Conc. NO_3^- -N ppm	NO_2^- -N mg. /15 g. soil Incubation Time, days							
		1	2	4	6	8	10	12	14
15	100	-	.11	.15	.01	.02	0.01	0.05	-
15	150	0.01	.09	.17	.12	.14	0.08	0.05	-
15	200	-	0.06	.04	.12	.18	0.06	0.05	-
25	100	.06	.11	0.05	.02	0.01	0.01	-	-
25	150	-	.03	.15	.11	.05	0.05	0.02	-
25	200	-	.15	.10	.05	.07	-	-	-

Table (A-4) Amount of NH_4^+ -N in the Morton soil at various times of incubation under different temperatures and concentrations of added NO_3^- -N.

Temp. C ^o	Conc. NO_3^- -N ppm	NH_4^+ -N mg. /15 g. soil							
		Incubation Time, days							
		1	2	4	6	8	10	12	14
15	100	0.39	0.40	0.41	0.48	0.48	.48	0.47	-
15	150	0.38	0.39	0.38	0.47	0.40	.39	0.47	-
15	200	0.41	0.42	0.41	0.49	0.34	.33	0.39	-
25	100	0.41	0.48	0.55	0.60	0.65	.61	0.61	.65
25	150	0.39	0.42	0.49	0.62	0.55	.54	0.54	.53
25	200	0.39	0.43	0.51	0.59	0.57	.54	.54	.53
35	100	0.48	0.67	0.72	0.78	0.84	1.00	0.83	.87
35	150	0.47	-	0.73	0.81	0.85	1.01	0.87	.89
35	200	0.39	0.58	0.58	0.71	0.79	0.96	0.76	.79

Table (A-5) Amount of NH_4^+ -N in the Wellwood soil at various times of incubation under different temperatures and concentrations of added NO_3^- -N.

Temp. C°	Conc. NO_3^- -N ppm	NH_4^+ -N mg. /15 g. soil							
		Incubation Time, days							
		1	2	4	6	8	10	12	14
15	100	0.19	0.17	0.20	0.25	0.26	0.28	0.36	0.40
15	150	0.18	0.13	0.23	0.23	0.24	0.28	0.33	0.39
15	200	0.20	0.13	0.23	0.22	0.26	0.25	0.28	0.28
25	100	0.15	0.22	-	0.37	0.43	0.48	-	0.54
25	150	-	0.24	0.28	0.39	0.39	0.46	0.47	-
25	200	-	0.25	0.21	0.43	0.43	0.46	0.43	-
35	100	0.16	0.25	0.20	0.40	0.54	0.63	0.67	0.79
35	150	0.15	0.23	0.25	0.32	0.42	0.53	0.52	0.78
35	200	0.13	0.24	0.27	0.28	0.34	0.48	0.60	-

Table (A-6) Amount of NH_4^+ -N in the Keld soil at various times of incubation under different temperatures and concentrations of added NO_3^- -N.

Temp C°	Conc. NO_3^- -N ppm	NH_4^+ -N mg. /15 g. soil							
		Incubation Time, days							
		1	2	4	6	8	10	12	14
15	100	0.11	0.17	0.16	0.26	0.29	0.29	0.22	0.27
15	150	0.10	0.16	0.23	0.24	0.27	0.28	0.24	0.27
15	200	0.11	-	0.22	0.22	0.27	0.24	.24	0.25
25	100	0.19	0.30	0.32	0.33	0.41	0.48	-	0.41
25	150	0.18	0.19	0.28	0.34	0.37	0.48	-	0.40
25	200	0.16	0.17	0.28	0.30	0.36	0.38	0.39	0.37

Table (A-7) The partial pressure of O_2 during incubation of
Morton soil samples under different temperatures
and concentrations of added NO_3^- -N.

Temp. C°	Conc. NO_3^- -N ppm	Partial Pressure of O_2 (%)							
		Incubation Time, days							
		1	2	4	6	8	10	12	14
15	100	19.38	18.80	17.49	16.04	15.86	-	14.00	13.87
15	150	19.91	19.45	18.34	17.35	16.32	15.88	14.26	14.05
15	200	20.07	18.83	18.26	17.15	16.87	14.96	13.48	-
25	100	19.36	17.83	17.03	16.57	15.32	13.70	13.17	10.16
25	150	17.61	17.51	16.44	16.09	14.88	12.98	12.39	11.74
25	200	18.98	17.81	17.00	16.38	14.67	13.62	11.37	10.52
35	100	17.84	17.93	16.09	13.26	12.12	8.87	8.26	8.73
35	150	18.62	17.26	15.96	13.76	14.32	-	10.75	8.09
35	200	18.93	17.43	15.81	13.95	12.39	12.01	10.98	9.56

Table (A-8) The partial pressure of O_2 during incubation of Wellwood soil samples under different temperatures and concentrations of added NO_3^- -N.

Temp. C ^o	Conc. NO_3^- -N ppm	Partial Pressure of O_2 (%)							
		Time of incubation, days							
		1	2	4	6	8	10	12	14
15	100	19.99	19.29	16.58	17.90	16.94	13.02	12.78	11.75
15	150	19.06	18.47	16.06	14.33	13.92	14.46	14.46	-
15	200	18.96	18.03	14.75	14.31	12.11	13.74	13.74	-
25	100	18.31	16.68	16.12	13.81	13.84	13.17	13.17	11.51
25	150	18.85	16.60	16.60	14.11	14.64	14.08	14.08	12.42
25	200	18.88	15.66	15.5	14.69	13.19	2.23	2.23	8.5
35	100	19.04	16.98	14.54	12.45	12.01	7.39	7.39	5.56
35	150	18.59	17.62	14.87	12.12	8.78	10.39	10.39	8.92
35	200	18.59	17.59	14.98	13.59	10.95	9.42	9.42	5.11

Table (A-9) The partial pressure of O_2 during incubation of Keld soil samples under different temperatures and concentration of added NO_3^- -N.

Temp. C ^o	Conc. NO_3^- -N ppm	Partial Pressure of O_2 (%)							
		Incubation Time, days							
		1d	2d	4d	6d	8d	10d	12d	14d
15	100	19.66	19.58	18.00	17.89	17.85	16.50	16.17	15.11
15	150	19.73	19.65	18.05	17.25	17.59	16.99	15.96	16.99
15	200	20.3	19.42	17.15	17.62	17.79	17.77	14.4	-
25	100	19.07	18.48	16.97	15.45	15.72	15.15	-	14.51
25	150	19.8	18.21	16.89	16.25	16.79	16.65	-	-
25	200	19.44	18.66	15.55		16.54	14.56	13.73	-

Table (A-10) The partial pressure of CO₂ during incubation of Morton soil samples under different temperatures and concentrations of added NO₃⁻-N.

Temp. C ^o	Conc. NO ₃ ⁻ -N ppm	Partial Pressure of CO ₂ (%)							
		Incubation Time, days							
		1	2	4	6	8	10	12	14
15	100	0.15	0.26	0.41	0.49	0.61	-	1.08	1.06
15	150	0.21	0.29	0.46	0.47	0.53	0.58	0.82	0.99
15	200	0.15	0.35	0.29	0.44	0.44	0.97	0.91	-
25	100	0.45	0.64	1.39	1.73	1.76	1.69	2.45	2.60
25	150	0.85	1.27	1.69	1.55	1.91	1.94	2.25	2.31
25	200	0.23	1.06	1.39	1.48	1.82	1.55	2.18	2.55
35	100	1.69	1.67	2.35	3.92	4.36	5.74	7.34	8.03
35	150	0.94	1.66	2.26	3.26	3.57	4.98	6.36	7.5
35	200	0.88	1.53	2.72	3.23	3.73	4.48	5.02	5.95

Table (A-11) The partial pressure of CO₂ during incubation of Morton soil samples under different temperatures and concentrations of added NO₃⁻-N.

Temp. C ^o	Conc. NO ₃ ⁻ -N ppm	Partial Pressure of CO ₂ (%)							
		Incubation Time, days							
		1	2	4	6	8	10	12	14
15	100	1.20	2.13	4.48	4.80	4.70	8.79	9.56	9.99
15	150	1.55	2.55	6.09	6.53	7.68	6.74	8.67	7.27
15	200	1.60	2.90	5.27	6.33	8.09	6.62	8.26	7.68
25	100	2.69	-	-	9.88	11.10	12.77	-	14.31
25	150	2.48	5.82	7.16	10.94	11.86	12.3	12.64	14.4
25	200	2.21	6.03	7.73	10.94	11.67	13.01	12.52	15.10
35	100	4.58	7.90	11.69	15.11	18.90	24.42	-	-
35	150	4.89	6.86	11.31	150.2	21.31	20.00	-	-
35	200	5.58	6.86	10.94	12.16	18.96	22.60	24.39	-

Table (A-12) The partial pressure of CO₂ during incubation of Keld soil samples under different temperatures and concentrations of added NO₃⁻-N.

Temp. C ^o	Conc. NO ₃ ⁻ -N ppm	Partial Pressure of CO ₂ (%)							
		Incubation Time, days							
		1	2	4	6	8	10	12	14
15	100		0.85	4.60	4.66	5.39	7.21	5.59	7.68
15	150	0.71	0.67	2.00	3.72	4.31	5.16	5.25	5.16
15	200	0.55	1.08	-	3.08	3.78	4.66	5.25	7.33
25	100	3.00	4.76	5.94	-	17.83	-	-	-
25	150	5.12	4.34	11.86	12.37	11.79	16.26	-	14.90
25	200	7.92	-	9.34	11.61	11.31	13.09	12.22	-

Table (A-13) Amount of NO_2^- -N in the soil samples incubated with 100 ppm NO_3^- -N at 25°C , and various amounts of CaCO_3 .

Soil	% CaCO_3	NO_2^- - mg. N/15g. soil							
		Time of incubation							
		1	2	4	6	8	10	12	14
Keld	4	.26	.62	.10	.01	.01			
Keld	12	.28	.67	.18	.04				
Wellwood	6	132	.29	-	.04	.02			