THE UNIVERSITY OF MANITOBA

OXYGEN TRANSPORT AND DENITRIFICATION OF NITRATE DERIVED FROM UREA AND ADDED NITRATE IN A SOIL COLUMN

by

Ismail Ibrahim Khdyer

A Thesis

presented to the University of Manitoba

in partial fulfilment of the requirements for the degree

Doctor of Philosophy

Department of Soil Science

University of Manitoba

Winnipeg, Manitoba

1982

OXYGEN TRANSPORT AND DENITRIFICATION OF NITRATE DERIVED FROM UREA AND ADDED NITRATE IN A SOIL COLUMN

BY

ISMAIL IBRAHIM KHDYER

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

DOCTOR OF PHILOSOPHY

ABSTRACT

Profile samples of Wellwood soil were used to investigate soil respiration as affected by moisture and temperature. Oxygen consumption rate (OCR) increased linearly with an increase in soil moisture from the threshold value of 2.5% up to the optimum moisture content of 20% and did not change as the moisture content was increased to 30%. The OCR and CO_2 production rate were decreased with soil depth. These rates obeyed Arrhenius equation over the temperatures range from 20 to 40°C.

Soil column studies of these samples were also carried out to determine the effect of air porosity on the soil depth at which the reduction of nitrate derived from urea-N took place. The effect of nitrate concentration on the vertical distribution of various gases produced from denitrification of added nitrate in the aerobic zone was also studied. Both column studies were conducted at 20°C only. The steady state O_2 concentration in soil columns with several air porosities was measured and the O_2 diffusion coefficient in the soils was predicted at these air porosity treatments from the measurement of depth to zero O_2 concentration (x^*) which was a function of both air porosity and incubation temperature. In the column study where urea-N was mixed into soil column, the reduction of NO_3^- derived from urea and the formation of gaseous products were found to take place near x^*. The accumulation of N_2O and N_2 near x^* was found to be low, possibly because of the low rate of NO_3^- flux across the aerobic-anaerobic boundary.

In the column study with added nitrate, the isotope contents of the N_2O and N_2 showed that they were all derived from nitrate, indicating that the non-enzymatic denitrification is not taking place. The initial N_2O distribution in soil columns was unaffected by the initial concentration of NO_3^- as long as there was no major accumulation of NO_2^-. For the low concentration of NO_3^-, N_2 was produced locally from N_2O reduction. For a higher NO_3^- concentration only a small amount of N_2O was reduced locally and considerable N_2O diffused to a greater depth for further reduction to N_2.

ACKNOWLEDGMENTS

I would like express my sincere appreciation and thanks to some of the people who helped and supported me in this investigation. Thanks go to Dr C. M. Cho for his guidance during the study and for his constructive criticism of the manuscript; to Dr R. A. Hedlin for his help in the reading the manuscript and serving on the student advisory committee; to Dr C. F. Shaykewich for his help in the debugging of problems encountered in the computer-typing of the manuscript and serving on the advisory committee; to Dr R. D. Hill and E. G. Beauchamp for serving on my advisory committee; to Dr L. Loewen-Rudgers for his help in reading the manuscript; to J. G. Mills for his help in the debugging of problems encountered in the computer-typing of the manuscript.

Acknowledgment is extended to the Iraqi Ministry of Higher Education for the financial assistance throughout this study.

Special thanks go to mom and dad for their patience, help and support.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter I

INTRODUCTION

Most denitrification studies with soils have been undertaken in closed systems. Anaerobic conditions are frequently imposed by flooding the soil or by incubating the soil in an atmosphere composed of an inert gas. This provides useful information on the denitrification process but is unlike that which commonly occurs in the soil column. In the soil column, the vertical distribution of O_2 varies greatly depending upon its flux into the soil and the rate at which O_2 is used by respiration in plant roots and soil microorganisms. Such O_2 distribution throughout the soil profile provides a good indication of nitrogen transformations (nitrification and denitrification) which may occur, and thus is of great importance in terms of the nitrogen economy of soils. If the soil is in an oxidized state nitrogen tends to be nitrified with its gradual accumulation in the NO_3^- form. If the soil is in a reduced state nitrogen in the NO_3^- form tends to be converted to NO_2^- and eventually to N_2O and N_2.

In the field or in soil columns in the laboratory there is only one surface of the soil exposed to the atmosphere through which O_2 can enter. As atmospheric O_2 is transported downward through the soil, mainly by diffusion, it is consumed by respiratory activity of microbes along the diffusion path. Consequently, there exists a depth within a soil column at which all of the O_2 has been used by microorganisms. The ex-

tent of O_2 penetration into soil is thus governed by two opposing processes, the 'diffusion process' whereby O_2 moves into the soil and the 'consumption process' whereby O_2 is utilized.

The rates of diffusion and the respiratory consumption of O_2 govern the penetration of O_2, and this penetration divides the soil column into aerobic and anaerobic layers. Soil temperature, moisture content, bulk density and availability of energy for microorganisms play important roles in determining the O_2 distribution and the depth of the boundary between aerobic and anaerobic layers.

Nitrate cannot compete with O_2 as an electron acceptor. As a result, O_2 completely inhibits the reduction of NO_3^-. Consequently, NO_3^- produced from NH_4^+-yielding fertilizers occurs in the oxidized zone, while NO_3^- which diffuses into the unoxidized zone may be reduced. Thus, the determination of the depth of aerobic and anaerobic interface in a soil column is very important in determining whether nitrogen will be nitrified or denitrified.

Nitrate which crosses the boundary from the aerobic to the anaerobic layer will be denitrified provided that there is sufficient denitrifying activity within the anaerobic layer. Thus, the concentration and vertical distribution of NO_3^- within the aerobic layer which govern the flux of NO_3^- across the aerobic-anaerobic boundary will determine the magnitude of denitrification. Unlike O_2, NO_3^- can be reduced to several intermediate products, such as, NO_2^- and N_2O. These intermediates, being subject to further reduction, can compete with NO_3^- as electron acceptors. In soil, N_2O which is a gas at normal temperatures and pressures has a diffusion coefficient several thousand tines greater than that of

NO_3^-. It is also stable under aerobic conditions. Consequently, some of the N_2O produced in an anaerobic layer may escape to the surface. On the other hand, N_2O is unstable under anaerobic conditions and may be transformed into N_2.

The magnitude of denitrification, the production of N_2O and its emission from the soil into the atmosphere resulting in the loss of nitrogen applied to soil columns is not well understood. This investigation was undertaken to determine and predict the O_2 concentration profile in soil columns consisting of reconstituted field soil profiles having different moisture contents and bulk densities. In addition to determining the O_2 concentration profile, the studies reported in this investigation included determinations of nitrification of urea within the aerobic layer; denitrification of NO_3^- derived from urea near the aerobic-anaerobic interface; stability and persistence of the NO_3^- applied within the aerobic layer; and kind and amount of denitrification products as affected by the concentration of NO_3^-.

Chapter II

LITERATURE REVIEW

2.1 MOVEMENT OF GASES THROUGH SOILS

The composition of gases in the soil at any time is controlled by the relative rates of two processes operating simultaneously. The first process deals with the production of CO_2 and consumption of O_2. The second deals with the rate of interchange between soil gases and the overlying atmosphere. The composition of the gases in the soil is subject to fluctuation as a result of changes in either the rate of production of CO_2 or in the rate with which it may escape from the soil and be replaced by O_2.

Gases can move either in gaseous phase in the pores which are drained of water or in dissolved form through the liquid phase. The diffusion coefficient for gas in water is about 0.0001 times that in air (Bakker and Hidding, 1970). Therefore, soil aeration is dependent largely upon the volume fraction of air-filled pores.

Air filled porosity decreases with an increase in bulk density and with an increase in the soil water potential. The decrease in air porosity with increasing soil water potential depends on particle and aggregate sizes. Grable (1966) concluded that the air porosity decreased more in fine than in coarse-textured soils with an increase in soil water potential from -31 to -0.04 bars. Black (1957) also indicated that air porosity is usually less in fine than coarse soils at equal water

potential. Other workers presented data showing a much greater decrease in air porosity with small aggregates than with large aggregates as soil water potential increased (Baver et al., 1972; Sedgley, 1962).

Two distinct mechanisms may be involved in the interchange of gas between the soil and the atmosphere. The first is mass flow of gas to and from internal spaces in the soil, the second is gaseous diffusion.

2.1.1 Mass Flow

Mass flow can result from the penetration of wind into large soil pores or air removal due to wind suction (Russell, 1973). Mass flow can also occur due to the expansion and contraction of the soil gases as a result of changes in temperature or barometric pressure (Grable, 1966). Bouyoucos (1915) (quoted by Grable (1966)) found that temperature fluctuation may result in significant mass flow. He reported that about 30 ml of soil air plus water vapor were expelled from 1000 ml of moist soil per $10^{o}C$ increase in temperature. The same volume of air would enter the soils when they cooled. In comparison, about 18 ml of air would be expelled from soil containing no water, the difference in volume being due to water vapor. The coefficient of expansion of dry air is approximately 1/275 per degree centigrade. However, the diurnal fluctuation of soil temperature is only significant for the 0 to 20 cm depth (Baver et al., 1972; Smith, 1932) and therefore the effect of temperature on mass flow is restricted to shallow depths (Grable, 1966). Buckingham (1904) indicated that changes in barometric pressure are too small to cause appreciable gaseous transfer in soil.

Another aspect of mass flow worthy of consideration is the renewal of the soil air caused by the infiltration of rainfall and irrigation water. This results either from the displacement of air in the pores by water which is subsequently displaced again by air during drainage, or from the air dissolved in irrigation or rain water. The air enters the soil by diffusion and mass flow in a volume equal to that of the water lost from the soil (Baver et al., 1972; Grable, 1966). Water at 25°C in equilibrium with the atmosphere (20.9% partial pressure of O_2) contains about 6 ml of dissolved O_2 per liter. This is about the same amount of O_2 as is used in one hour by 1 g dry weight of active roots (Grable, 1966).

Mass flow is also an important mechanism for gas transport in soils intermittently flooded with sewage water and septic tank effluent (Lance et al., 1973; Thomas et al., 1968). For example, Lance et al. (1973) found that O_2 entering the soil by mass flow was about 0.7 times that entering by diffusion when 5-day dry periods alternated with 2-day flooding periods. Oxygen mass flow was about 0.5 times as much as diffusion during 5-day dry periods alternating with 9-day flooding periods. Therefore, the renewal of the soil air as a result of rainfall is periodic depending on the distribution of the rains.

2.1.2 Diffusion Flow

Diffusion is the molecular transfer of gases through porous media due to a partial pressure gradient. According to the kinetic theory of gases, molecules of gases are in a state of movement in all directions. Two gases will readily mix as the molecules of each gas move into the

space occupied by the other. Inasmuch as the soil air tends to contain more CO_2 and less O_2 than the atmosphere, diffusion in soil involves primarily the movement of CO_2 out of the soil into the atmosphere and the movement of O_2 from the atmosphere into the soil. An equilibrium would rarely be attained in soil due to the fact that the production and removal of CO_2 and replenishment of O_2 fluctuate continuously with changes in structure, moisture content and temperature of the soil. Thus, both the CO_2 and the O_2 concentrations in the soil air are continuously fluctuating (Russell, 1973).

The general equation of mass transport can be used to describe the O_2 transport within the soil. The equation is a statement of the law of conservation of mass. Considering a small volume element of arbitrary shape, the net rate of accumulation of mass i in the element of volume can be expressed as

$$\partial C_i/\partial t = - \nabla . J_i \pm \theta_i \qquad (1)$$

where C_i = concentration of substance (i) in (mole or g per unit volume);

t = time;

θ_i = amount of substance i produced or consumed per unit time per unit volume which is considered to be negative when consumption is occurring and positive when production of the substance is taking place;

and J_i = total mass flux expressed as mass current per unit area per unit time.

The symbol ∇ (del) is the vector differential operator representing the three-dimensional gradient in space.

Total mass flux can be obtained from dispersion theory which states that

$$J_i = - D_i \text{ grad } C_i + v\, C_i \tag{2}$$

where D_i is the dispersion coefficient of substance (i) and v is the average velocity of flowing substances as a whole. This equation states that the mass flux of an element across a fixed plane is determined by two quantities, one resulting from the dispersive flux caused by a concentration gradient and the other resulting from the convective flux. When Eq. (2) is substituted into the equation of continuity (1) and expressed in a one-dimensional form under constant average velocity the result is, the general mass transport equation (Cho, 1971)

$$\partial C_i/\partial t = (D_i \partial^2 C_i/\partial x^2) - v\, \partial C_i/\partial x \pm \theta_i \tag{3}$$

where x is the distance.

When the average velocity is equal to zero, the total mass flux from Eq. (2) is reduced to diffusive flux or

$$J_i = - D_i \text{ grad } C_i \tag{4}$$

The above equation (Fick's Law) states that the diffusive flux (dispersion coefficient becomes the diffusion coefficient) is a function of the concentration gradient and the diffusion coefficient of the medium. For gaseous movement in soil, in which the rate of mass flux due to convection is negligible, ($v\, \partial C_i/\partial x = 0$), Eq. (3) is reduced to

$$\partial C_i/\partial t = D_i \partial^2 C_i/\partial x^2 \pm \theta_i. \tag{5}$$

The diffusive transport of gases such as O_2 and CO_2 in the soil occurs mainly in the gaseous phase. Diffusion can also occur in the liquid fraction but would be much slower. The diffusion coefficient of a gas in soil (D) cannot exceed the value for diffusion in atmosphere, and because of the tortuous nature of the diffusion path in soil, D is generally less than the diffusion coefficient of a gas in air (Penman, 1940).

The solutions of this partial differential equation (5) with appropriate initial and boundary conditions, allows one to calculate the movement and distribution of gases. These solutions are mainly dependent on the rates of gaseous production or consumption (θ_i) and their diffusion coefficient (D) which, in general, decrease with soil depth (Wesseling, 1962). Therefore, these two parameters will be reviewed in detail.

2.2 THE COEFFICIENT OF DIFFUSION

The effect of temperature and pressure on the diffusion coefficient can be represented by the equation (Bakker and Hidding, 1970)

$$D_1 = D_2 (T_1/T_2)^{1.75} (P_1/P_2) \qquad (6)$$

where T is the absolute temperature (^{o}K), P is the the partial pressure and D_1 is the diffusion coefficient at T_1 and P_1.

The diffusion coefficient of O_2 is about 1.25 times that of CO_2 in both water and air (Grable, 1966). At 25°C and 101.3 kPa pressure the solubilities of O_2 and CO_2 in water are 0.039 and 1.45 g/liter, respectively. Thus, CO_2 is 37 times as soluble in water as O_2. The greater solubility of CO_2 in water increases its concentration gradient and therefore, in water it is transmitted from "inside to outside" in a hyd-

rated system at a higher rate than O_2 for equivalent distances, areas and partial pressure gradients (Grable, 1966).

The diffusion coefficient of a gas in soil is commonly described by a ratio, D/D_a, D being the coefficient of diffusion in the soil (cm^2/sec) and D_a being the coefficient of diffusion of the same gas in air at the same temperature and pressure (Penman, 1940). The ratio, D/D_a, depends on the soil only and not on the gas used for diffusion measurement (Currie, 1970; Lai et al., 1976; Penman, 1940). Therefore, the CO_2 diffusion coefficient, for example, could be obtained from the O_2 diffusion coefficient by the relationship

$$(D)_{CO_2} = (D_a)_{CO_2} \quad (D/D_a)_{O_2}. \tag{7}$$

In other words, the soil system provides the same impedance to all gases and modifies the fundamental transport constant for each component in the same way (Currie, 1970).

A good deal of research has been done on trying to find a simple relationship between the diffusion coefficient and the air porosity of soil (E). Buckingham (1904) was one of the early scientists to study the diffusion of CO_2 in soil. He derived the relationship

$$D/D_a = E^2 \qquad (0.0 < E < 0.7) \tag{8}$$

Penman (1940) proposed a general relationship for both dry and moist porous solids

$$D/D_a = (1/K_1)\, E \qquad (0.25 < E < 0.6) \tag{9}$$

where K_1 is the tortuosity factor and is equal to $2^{1/2}$. Penman's observations, however, all lie above E = 0.35, those of Buckingham above E = 0.25. Over the range stated, Penman's data fitted the linear relationship

$$D/D_a = 0.66\ E, \tag{10}$$

that is, K_1 remained constant for a number of porous materials both wet and dry. But, evaporative loss of water during experiments on wet soils could lead to erroneously high D/D_a values at low air-filled porosities. Other workers (Taylor, 1949; Van Bavel, 1952) obtained a similar relationship with the proportionality constant ranging between 0.60 and 0.67. The Penman concept has been modified to stress the importance of the size distribution of pores in the diffusion process (Marshall, 1959). The relationship of D/D_a to air porosity is expressed by the formula

$$D/D_a = (1/K_2)\ E = E^{3/2} \tag{11}$$

where $K_2 = E^{-1/2}$. However, Currie (1970) proposed a similar equation for both dry sand and wet sand as follows:

$$D/D_a = (E/E_T)^4\ (E_T)^{3/2} \tag{12}$$

where (E_T) equals total porosity. This equation reduces to the Marshall's equation (11) for a dry system $(E = E_T)$. In this equation, freedom from obstruction is considered to be a function of porosity rather than tortuosity. The higher the porosity, the greater are the chances of pore continuity. From the above equation it is clear that

when the air porosity becomes smaller than 0.43, the values of relative diffusivity (D/D_a) predicted by Marshall's equation (11) are lower than those obtained by the Penman formula (Eq. (9)). However, values predicted by Marshall's equation are higher when the air porosity is greater than 0.43.

Millington (1959) considered both mass flow and diffusive flow to be functions of the cross-sectional area available for flow and the increase in path length or tortuosity. The area available for flow is dependent upon the pore-volume distribution within the soil. The relationship for dry soil is expressed by the formula derived from particle geometry

$$D/D_a = (1/K_3)\, E = E^{4/3} \tag{13}$$

where $K_3 = 1/E^{-1/3}$. This treatment endows K_3 with characteristics of length and is an index of the number of pores per unit length. This curvilinear relationship shows close similarity with that of Marshall's equation (11). Millington also pointed out that there are deviations from the Penman formula at air porosities higher and lower than 0.29.

Other exponential power relationships have been proposed by several authors. Grable and Siemer (1968), for example, found the relative diffusivity to be equal to $(100\ E)^{3.36}\ 10^{-6}$. Lai et al. (1976) found the relative diffusivity to be equal to $(E)^{5/3}$. These differences in the values of the exponential power could be attributed to the presence of water films on the solid surface which not only reduces the air porosity, but also modifies the pore geometry and the length of gas passage.

For a narrow range of E values in which aeration becomes important, the relationship can be described best by a linear equation of the form (Bakker and Hidding, 1970)

$$D/D_a = a(E - b). \tag{14}$$

The factor b is interpreted as the volume of blocked pores. D/D_a becomes zero when E = b, provided the porosity (E) is constant. This relationship was also used by Wesseling (1962) in his study of soil aeration. Values for these factors (a and b) can be obtained from the equation

$$D/D_a = 0.9\ (E - 0.12). \tag{15}$$

Many workers (Blake and Page, 1948; Baver and Farnsworth, 1940, Taylor, 1949; Wyckhoff and Botset, 1936) have found situations in which the diffusion coefficient was practically zero for E, ranging from 0.1 to 0.15. It has also been found that soil aeration is likely to become limiting to plant growth when the air-filled porosity falls below about this range (Baver and Farnsworth, 1940), though it must be realized that it is the rate of exchange rather than simply the content of soil air that is the decisive factor.

Soil structure also influences gaseous diffusion coefficient especially at lower values of air porosity (Call, 1957; Blake and Page, 1948; Gradwell, 1961, 1965; Domby and Kohnke, 1956; Grable and Siemer, 1968). For example, Bakker and Hidding (1970) found that at equal air porosity, diffusion coefficients in samples of puddled soils were considerably lower than in samples of non-puddled soils. When E was 0.07,

D in a puddled soils was about one-tenth that in non-puddled soils. When E was 0.14, D in puddled soils was one-fourth that in non-puddled soils. However, when E was 0.2 differences became negligible. Similarly Grable and Siemer (1968) found that O_2 diffusion decreased to zero at or near air porosities of 0.1-0.12 for the smallest aggregates and the most compact soils. For large aggregates and non-compacted soil, zero values of D/D_a were obtained at air porosities of 0.18-0.29. Such observations were explained on the basis that altering aggregate size or bulk density of soil will alter its water desorption characteristics.

2.3 FACTORS AFFECTING RESPIRATION ACTIVITY

Microbial respiration is greatly influenced by temperature, moisture content and the amount of easily decomposable organic matter (Parr et al., 1963: Parr and Norman, 1964; Parr et al., 1967; Nyhan, 1976; Lance et al, 1973; Rusell, 1973). The effects of the environmental factors, temperature and moisture, will be discussed in detail.

2.3.1 Effect of Temperature

The influence of temperature on the rates of biochemical processes is exponential and is expressed by Arrhenius equation (Barrow, 1973; Dawson and Murphy, 1972)

$$V = A\, e^{-E_A/RT} \qquad (16)$$

where V = reaction rate (i.e., mole/sec), A = frequency factor (same unit as V), E_A = activation energy (joule/mole), R = universal gas constant (joule/mole/^oK), and T = absolute temperature (^oK).

The plot of ln V versus 1/T generally yields a linear relationship. According to Focht (1974), it can be expressed directly in ^{o}C as follows;

$$V = Y \; e^{Z\,T} \qquad (17)$$

where $Y = A\, e^{(-E_A/273.15R)}$

and $Z = [E_A/\{R(273.15)^2\}]$

Temperature affects chemical and biological reactions differently. Biological (enzyme) reactions are generally retarded at temperatures higher than $65^{o}C$ (Bremner and Shaw, 1958b) and lower than $5^{o}C$ (Bailey and Beauchamp, 1973a) whereas chemical reactions are not normally subject to these limitations. The rates of biological processes measured by either N_2 production or NO_3^- reduction over the temperature range from 15 to $35^{o}C$ obeyed the Arrhenius equation (Bremner and Shaw, 1958b; Khdyer, 1978; Stanford et al., 1975). A sharp break in the curve was found below 10 to $15^{o}C$ (Focht, 1974; Misra et al., 1974; Stanford et al., 1975). The latter authors suggested that this was due to temperature effects on solubility or diffusion, or to a differential influence of temperature on one of the sequential reactions. Cho et al. (1979) stated that the observed deviation from the Arrhenius relation at low temperatures was because biological activity ceased near $0^{o}C$ (273 ^{o}K), not at absolute zero ($0^{o}K$).

The value of Q_{10}, the ratio of the rates observed at temperatures differing from each other by $10^{o}C$, e.g. V_T/V_{T-10}, is also used to describe the effect of temperature upon a biological process. Biological

processes usually follow Van't Hoff's temperature rule having Q_{10} values between 2 and 3 (Stevenson, 1965). It was suggested that Q_{10} is a linear function of the reciprocal of absolute temperature when soil samples are incubated in the laboratory at various constant temperatures, and the Q_{10} calculated by this relationship holds true only for temperatures below 35 - 45°C (Nyhan, 1976). The enhanced activity of thermophilic and thermotolerant microorganisms from about 45 to 65°C results in increased Q_{10} values in some experiments (Drobnik, 1962). However, for N_2 production or NO_3^- reduction processes, Q_{10} in soil was found to be approximately two in the range 15 to 35°C (Cooper and Smith, 1963; Khdyer, 1978; Stanford et al., 1975). Focht and Chang (1975) calculated Q_{10} values from the data of various authors and found that most fell in the vicinity of two to three. However, the lower limit of the temperature range conforming to a Q_{10} of two was estimated to be 11°C in waterlogged soil. The rate of reaction decreased almost ten-fold as the temperature dropped from 11 to 5°C (Stanford et al., 1975).

A change in temperature alters the species composition of the active flora and at the same time has a direct influence on each organism within the community. Each individual microbial species and the biochemical capacities of the community as whole have temperature optima. Because the composition of the flora varies from locality to locality and is influenced in a single site by species of plant residue returned to the soil, a single optimum of microbial activity cannot be found (Alexander, 1977).

One of the procedures for preparing soil samples for biological study involves freezing. Freezing brings about changes in soil such that the

rate of release of CO_2 increases (Mack, 1963; Soulides and Allison, 1961). Mack's data suggests that the greater initial surge of CO_2 evolution from the previously frozen soils was followed by a lower eventual rate. This would account for the similar total CO_2 evolution that was obtained from frozen and non-frozen samples over prolonged periods of time. For example, the results of repeated freezing and thawing on the rate of respiration show that chilling to -1°C or freezing to -196°C resulted in a surge of biological activity upon incubation at 24°C. They showed that the initial peak of activity for the two treatments decreased with each successive chilling or freezing and the rate eventually dropped below that for the control sample.

Most research concerning temperature effects upon microbial activity has been carried out under constant temperature. However, temperatures in the soil are rarely constant as the surface soil layers undergo wide diurnal and seasonal temperature fluctuations. Some investigators have studied the effects of such fluctuations on microbial activity (Campbell and Biederbeck, 1972; 1973; Biederbeck and Campbell, 1976). In these studies the diurnal fluctuation was simulated by having in each 24-hour period 9 hours at a maximum temperature and 9 hours at a minimum temperature with two transition periods of 3 h each. They found that microbial growth was considerably greater at a constant mean temperature such as 7.5°C, than at diurnally fluctuating temperatures of 13°C during the day and 2°C at night (T13/2) (average of 7.5°C). Campbell and Biederbeck (1973) found that an upward shift in temperature after 14 days from T13/2 to T18/7 (simulating May temperatures) caused an increase in microbial population and increased the rate of N transformations. When the

incubation temperature was shifted downward from T27/16 to T18/7 (simulating September temperatures), microbial populations decreased markedly whereas ammonification and nitrification rates increased significantly, resulting in a temporary flush of mineral-N. They hypothesized that a sudden shift in soil temperature from optimal to suboptimal conditions (e.g., during early fall in the surface layer of fields soils) caused, at least initially, a 'kill' of some microbial cells. This provided an N substrate which was ammonified and nitrified by the surviving organisms. This hypothesis was supported by a 4 years field study in which it was found that at the beginning of the first cold spell each fall or following late frosts in spring there were sudden flushes in NO_3^- production.

2.3.2 Effect of Moisture

The effect of moisture content on microbial activity in soil has been the subject of many investigations. Drawing conclusions from these studies is difficult because soil moisture content is expressed in a variety of ways which cannot be compared easily, such as percentage of maximum water-holding capacity, soil water potential etc.

A high moisture content may decrease indirectly the activity of aerobes and O_2 consumption by hindering the movement of air, thus decreasing the O_2 supply and creating anaerobic conditions (Bremner and Shaw, 1958a; Bailey and Beauchamp, 1973b). For O_2 consumption to reflect accurately the respiratory activity of soil, it is necessary that the environment of the microorganism be completely aerobic. Under high moisture, CO_2 is liberated but O_2 is not consumed, and values of O_2 uptake under-estimate the extent of microbial activity. Even though extreme

care may be exercised in order to maintain aerobic conditions, O_2 may still be limiting in some microenvironments around soil particles, where the major portion of microbial activity in soil probably occurs (Stotzky, 1960).

Rates of O_2 consumption generally are highest at soil water potentials ranging from -0.5 to -0.15 bars (Wiant, 1967; Miller and Johnson, 1964). Miller and Johnson (1964) reported that the microbial activity at zero water potential (water saturated soil) exhibited 1.1 to 1.3-fold decrease relative to the maximum value. At a water potential of -3 bars a 1.1 to 1.5-fold decrease in respiration relative to maximum activity was observed. At water potential lower than -50 bars (air-dry soil) a 12 to 13.5-fold decrease in respiration occurred.

The exact water potential for the maximum evolution of CO_2 is influenced by soil aeration. Bahumick and Clark (1947) showed that some free O_2 was required for maximum production of CO_2. During 15 days incubation in which the surface to volume ratio of the soils was decreased so that aeration would be retarded, maximum production of CO_2 occurred at a water potential of -0.01 bars. Miller and Johnson (1964) found that a lag period was evident in the production of CO_2 from soils incubated at zero water potential. The lag period was related to the time required for development of a population favored by anaerobic conditions. Maximum biological activity can then be expected to take place at higher water potentials where water is available to the microorganisms but aeration is sufficient. This relationship holds true regardless of the length of the incubation period (Miller and Johnson, 1964).

The extent of CO_2 production or O_2 consumption can be estimated qualitatively from organic matter decomposition. For example, losses of tracer carbon from finely ground Blue grama grass in soil were assessed and the results used to develop a multiple regression equation which predicted percent carbon loss per hour as an exponential function of water potential, time, temperature, and the inverse of temperature (Nyhan, 1976). These data showed that variation in soil water potential influenced Blue grama decomposition patterns more than either temperature or time variations. They showed that at 10°C, when temperature was limiting microbial activity, there was a pronounced decrease in rate of carbon loss with decrease in soil water potential. If the loss rate of carbon at a water potential of -0.009 bars for Blue grama soil mixtures was given a value of 1.00 the loss rates of carbon for mixtures incubated at -0.6, -5.7 and -113 bars were 0.88, 0.32 and 0.003, respectively. This carbon loss rate-soil water potential relationship occurred for soil samples incubated at temperatures of 3, 10, 25, 40 and 50°C.

Campbell and Biederbeck (1976) studied the relationships between change in microbial population and the change in several variables such as moisture content and soil temperature using forward selection multiple regression analysis. The relationships differed among soils of different texture. For example, they found that in the heavier-textured soils, the moisture change was the most important variable and it accounted for between 40 and 80% of the microbial variability. In these soils the influence of moisture change upon microbial population was a function of maximum temperature. In the loam textured soil, relationships were more complex. Generally, the changes in microbial population

were directly related to moisture change and minimum temperature and inversely related to the initial moisture content and the maximum temperature.

Estimates of the effect of O_2 level on respiration measured by CO_2 evolution indicated that CO_2 loss was unaffected by O_2 concentration at levels as low as 2.5 percent (Parr and Reuszer, 1959). However, small changes in the concentration of CO_2 affected microbial activity in soil and hence inhibit carbon dissimilation (Nyhan, 1976).

The rate of CO_2 evolution was enhanced when soil was exposed to a cycle of drying and wetting, as compared to soils that were continuously wet (Sorenson, 1975; Stevenson, 1956; Birch, 1959, 1960). When a soil was air-dried and then rewetted there was a surge of biological activity which declined within a few days. Each successive re-wetting was accompanied by a surge of activity less than the previous one (Birch and Friend, 1961). Similarly, a higher initial respiratory activity (O_2 consumption) of the air-dried and remoistened soils as compared to fresh samples was reported (Stevenson, 1956). In the air-dried, remoistened soils there was a maximum initial rate followed by a gradual decrease approaching respiratory rate in fresh soils over a period varying from as short as 1-2 hours to as long as 1-2 weeks depending on the soil (Stevenson, 1956). Variation in the duration of the period of decrease and in the magnitude of the enhancement in biological activity brought about by drying followed by wetting could have resulted from variation in the amount of soluble nitrogenous materials (e.g. amino acid) released upon air drying (Stevenson, 1956; Mack, 1962).

2.4 SOLUTION OF THE O_2 TRANSPORT EQUATION IN SOIL

The non-steady and steady state O_2 concentration profiles must be determined in order to determine the size and location of the aerobic zone in an uniform soil column of length "L" closed at the bottom. If the initial concentration of the O_2 in the soil is C_o, the O_2 concentration at the soil surface is maintained at C_o and O_2 is being consumed in the soil at a rate (θ) grams per cubic centimeter per second, the equation of O_2 transport becomes (see Eq. (5))

$$\partial C/\partial t = D \frac{\partial^2 C}{\partial x^2} - \theta \qquad (18)$$

with initial and boundary conditions

$$\begin{aligned} C(x,t) &= C_o \quad \text{for} \quad 0<x<L \quad \text{and} \quad t = 0 \\ C(x,t) &= C_o \quad \text{for} \quad x = 0 \quad \text{and} \quad t>0 \\ \partial C(x,t)/\partial x &= 0 \quad \text{for} \quad x = L \quad \text{and} \quad t>0, \end{aligned} \qquad (19)$$

while D and θ are assumed to be constants. Papendick and Runkles (1965) have solved the problem of finding the O_2 concentration profile. More detailed applications have been reported by several workers (Grable and Siemer 1968; Kowalik et al. 1979; Kirkham and Powers, 1972).

The one-dimensional form of gaseous diffusion in a porous medium without sink or production is given by

$$\partial C/\partial t = D \left(\frac{\partial^2 C}{\partial x^2} \right). \qquad (20)$$

It is convenient to transform Eq. (18) to the form of Eq. (20) because there is a general solution for the latter (Kirkham and Powers, 1972). To achieve this transformation, Kirkham and Powers (1972) made the following substitution;

$$C(x,t) = u(x) + v(x,t), \tag{21}$$

in which the function u does not involve t while the function v involves both x and t. Substituting Eq. (21) into Eq. (18) and carrving out the differentiation gives

$$\partial v/\partial t = D\ \partial^2 v/\partial x^2 + D\ \ d^2u/dx^2 - \theta \tag{22}$$

where $du/dt = 0$.

By setting $D\ d^2u/dx^2 - \theta = 0$ (steady state condition), Eq. (22) becomes

$$\partial v/\partial t = D\ \partial^2 v/\partial x^2. \tag{23}$$

The transformation yields Eq. (23) in a homogeneous form, that is, v appears in each of the terms. By applying the method of change of variable to the boundary condition where $C(x,t) = C_o$ for $x = 0$ and $t>0$, the result is

$$C(0,t) = C_o = u(0) + v(0,t). \tag{24}$$

This equation is satisfied by choosing $v(0,t) = 0$; $u(0) = C_o$. Also, if Eq. (21) is differentiated with respect to x and $\partial C/\partial x = 0$ is substituted for $x = L$ and $t>0$, the result is

$$[\partial C/\partial x]_{x=L} = 0 = [du(0)/dx]_{x=L} + [\partial v(x,t)/\partial x]_{x=L}. \tag{25}$$

Equation (25) will be satisfied if $[du/dx]_{x=L} = 0$ and $\partial v(L,t)/\partial x = 0$, thus, Eq. (22) becomes

$$d^2u/dx^2 = \theta/D \quad \text{for} \quad 0<x<L \tag{26}$$

for the boundary condition $u(0) = C_o$, $du(L)/dx = 0$.

To obtain the value of the first term (right side) of Eq. (21), Eq. (26) may be solved as an ordinary differential equation. The value of u(x) is substituted into Eq. (21) to give

$$C(x,t) = (\theta/2D)x^2 - \theta Lx/D + C_o + v(x,t). \tag{27}$$

In this equation, the initial condition $C(x,t) = C_o$ for $0<x<L$ and $t=0$ is assumed, and then rearranged to obtain

$$v(x,0) = -(\theta/2D)x^2 + (\theta L/D)x \quad \text{for} \quad 0<x<L. \tag{28}$$

Therefore, to obtain the value of the second term of the Eq. (21), the problem can be restated as

$$\partial v/\partial t = D \, \partial^2 v/\partial x^2 \tag{29}$$

with initial conditions

$$\begin{aligned} &v(0,t) = 0 \quad \text{for } t>0 \\ &[\partial v(L,t)/\partial x] = 0 \\ &v(x,0) = -(\theta/2D)\, x^2 + (\theta L/D)\, x \quad \text{for} \quad 0<x<L. \end{aligned} \tag{30}$$

Therefore, it is possible to write the formal solution of Eq. (18) as the sum of the solution for steady state diffusion, u(x) and the solution for a case of non-steady state diffusion, v(x,t) where one surface is maintained at zero concentration, i.e., $v(0,t) = 0$, $t>0$. The method of separation of variables may be applied to obtain the solution of Eq. (29) using the boundary conditions of Eq. (30) to give;

$$v = (16\theta L^2/\pi^3 D) \sum_{n=1}^{n=\infty} [\{(e^{(-B^2Dt)}/(2n-1)^3)\}\sin(Bnx/\pi)] \tag{30a}$$

where $B = (2n - 1)\pi/2L$.

Substitution of this solution into Eq. (27) gives the desired result

$$C(x,t) = C_o - \theta x(x-2L)/2D + (16\theta L^2/\pi^3 D)\sum_{n=1}^{n=\infty} \{\sin(nBx/\pi)\, e^{(-B^2Dt)}/(2n-1)^3\} \quad (31)$$

This solution describes the O_2 concentration $C(x,t)$ when the parameters θ, D, L and C_o are known (Papendick and Runkles, 1965).

The applicability of this theoretical model was tested by comparing O_2 concentrations measured in an experiment which satisfied the boundary conditions with the concentrations computed from Eq. (31) for non-steady state conditions. The ultimate or steady-state O_2 concentration in the soil at any distance $0<x<L$ can be obtained from Eq. (31). Thus, as t goes to infinity this equation is reduced to

$$C(x,\infty) = C_o - \theta x(2L-x)/2D. \quad (32)$$

This equation gives the steady-state O_2 concentration in the soil atmosphere as a function of depth with known values of θ, D, L and C_o. Papendick and Runkles (1965) found that the relative O_2 concentration approached its ultimate value rather rapidly. The theoretical soil O_2 concentration was approximately 65 and 75 per cent of its ultimate value at the 15 and 135 cm depths, respectively, in 16 hours.

2.5 BIOLOGICAL DENITRIFICATION

Denitrification is the production of N_2 or gaseous nitrogen oxides through the reduction of NO_3^- (Wijler and Delwiche, 1954). Biological denitrification is carried out by facultative anaerobic bacteria under anoxic conditions. According to Russell (1973), the reduction of NO_3^- goes through the NO_2^- stage although NO_2^- normally does not accumulate in the soil. The NO_2^- produced is further reduced to N_2O or to N_2. Under certain conditions (in an anaerobic system) it may go through the nitric oxide (NO) stage.

It is generally accepted that N_2O and N_2 are the major products and their proportions depend on environmental factors. Thus, the steps in NO_3^- reduction have been suggested as

$NO_3^- \longrightarrow NO_2^- \longrightarrow N_2O \longrightarrow N_2$ (Cooper and Smith, 1963; Broadbent and Clark, 1965).

Although there is a generally accepted pathway for denitrification, the kinetics of this sequence of reactions is not fully understood. There are conflicting reports concerning the effect of NO_3^- concentration on the rate of denitrification. The controversy concerns the question of which kinetic rate equation applies best to NO_3^- reduction. A zero-order reaction rate would not be dependent on the NO_3^- concentration, while that of a first-order reaction would. Some workers (Bouldin et al., 1974; Stanford et al., 1975) reported the process of NO_3^- disappearance to resemble a first order reaction. However, other workers (Bowman and Focht, 1974; Focht, 1974) reported the process to be zero order at high and first order at low NO_3^- concentrations. Thus, it would be similar to the Michaelis-Menton type reaction. In a Michaelis-Menton

type reaction the rate depends directly on the amount of enzyme present when the subtrate is at a saturation level (Conn and Stumpf, 1967). When the enzyme concentration remains constant an increase in substrate results in an increase in reaction rate. But, the increase in reaction rate becomes progressively smaller with each increase in substrate concentration until a maximum constant rate is attained. At low substrate concentrations, the reaction follows apparent first-order kinetics, while at high substrate concentrations it seems to follow zero-order kinetics.

Khdyer (1978) studied the disappearance of NO_3^--N from three flooded Manitoba soils, and found that with NO_3^--N concentrations of 100 to 200 ug/g the initial rate of disappearance was independent of NO_3^- concentration. Similar findings were obtained from addition of 50 to 200 ug NO_3^--N/g soil at moisture contents of 30 and 67% (2 mm layer of stagnant water) (Cho and Sakdinan, 1978). Nicholson (1979) found that the rates of NO_3^- loss and N_2 production in an agitated lake sediment were independent of NO_3^- concentration in the range of 2.5 to 10 ug NO_3^--N/ml solution. On the other hand, rates of NO_3^- loss and N_2 production were dependent on NO_3^- concentration in the non-agitated lake sediment with the same range of added NO_3^-. He concluded that denitrification was zero-order (agitated sediment) due to the uniform distribution of NO_3^- in the sample. Similar zero-order rates of NO_3^- loss were obtained during the denitrification of stirred soil suspensions (Patrick, 1960).

Cho and Sakdinan (1978) stated that variations in initial NO_3^- concentration of soil affected the production, reduction, and accumulation of denitrification intermediates, although the rate of NO_3^- disappear-

ance was zero-order. Their experimental results showed that increased initial NO_3^- concentration increased the magnitude and duration of maximum N_2O accumulation. Similar results were obtained by Khdyer (1978) in flooded soil and by Nicholson (1979) in lake sediment. In their work N_2 was produced earlier at lower concentrations of NO_3^- than at higher NO_3^- concentrations. A similar observation was also made by Blackmer and Bremner (1978). They found that the reduction of N_2O to N_2 was inhibited by a high concentration of NO_3^-. In other words, the initial rate of molecular nitrogen formation was dependent on initial NO_3^- concentration.

Several Michaelis-Menton type equations were used to describe the disappearance of NO_3^-, the formation and disappearance of denitrification intermediates (NO_2^-, N_2O), and the production of N_2 (Cho and Mills, 1979). Some characteristic features of denitrification obtained by Cho and Sakdinan (1978), Khdyer (1978), Nicholson (1979), and Cooper and Smith (1963) agreed with those of the Michaelis-Menton model. They are (1) the independence of the rate of disappearance of NO_3^- with respect to NO_3^- concentration; (2) the constancy in the initial rate of N_2O formation irrespective of the NO_3^- concentration; (3) the delay in appearance of N_2O maximum with an increase in NO_3^- concentration; and (4) the delayed and slower rate of N_2 production due to an increase in NO_3^- concentration.

Most denitrification studies are carried out in closed systems which permit measurement of evolved gases. Such systems are quite different than that operating in the field. The diffusion of gases depends on concentration gradients from the soil system to the atmosphere or

vice versa. Nitrous oxide was observed in the absorption bands in the 7.8 μm region of the solar spectrum (Adel, 1946), but knowledge of its life cycle is still very incomplete because few measurements of its concentration are available and there is limited information of sources and sinks. Brice et al. (1977) reported evidence that the soil may act as a significant sink for N_2O. They observed a regular diurnal variation of N_2O concentration in the air at 5 meters above ground level. A minimum (0.233 μg N/ml gas) was nearly always recorded in the early hours of the morning and a maximum (0.315 μg N/ml gas) was recorded in the late afternoon or evening with an average of 0.278 μg N/ml gas. Such a diurnal variation can be explained either by some process which increases the N_2O concentration during the day, or alternatively depletes the N_2O concentration at night (Brice et al., 1977). However, Freney et al. (1978) found in both the laboratory and the field, that at soil moisture contents less than field capacity, N_2O was emitted from soils. There was no evidence that soils in the field ever absorbed N_2O.

2.6 EFFECT OF OXYGEN DIFFUSION ON THE DENITRIFICATION PROCESS UNDER FLOODED CONDITIONS

Factors which decrease the supply of O_2 to soil microbes usually promote biological denitrification since NO_3^- is utilized as a terminal electron acceptor in place of O_2. Factors which affect the O_2 diffusion rate (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 (Cady and Bartholomew, 1961).

The supply of O_2 after a soil is flooded depends upon molecular diffusion through the interstitial water (Ponnamperuma, 1972). The rate of O_2 diffusion from the flooded water is usually much slower than the rate of O_2 consumption in the flooded soil. Oxygen diffusion during incubation of soil was greatly reduced by a 2-cm layer (100% water) of stagnant water (Khdyer, 1978), and there was little difference between the O_2 flow through 3 mm than through 10 mm of standing water above the soil surface (Cho and Sakdinan, 1978).

The requirements of facultative anaerobic and true anaerobic organisms for electron acceptors upon depletion of O_2 result in the reduction of several oxidized compounds in the soil. Nitrate, NO_2^-, the higher oxides of Mn, hydrated ferric oxide or sulfate can be reduced if an energy source is available to the microorganisms. Therefore, conditions favoring the activities of denitrifying microorganisms are established and NO_3^- and NO_2^- become much less stable in flooded soil.

Flooded soils are characterized by two distinct soil layers;

1. A surface aerobic, light-coloured, reddish-brown zone which may range in thickness from a few mm to 2-3 cm (Patrick and Mahaptra, 1968); and
2. An underlying anaerobic (reduced) soil layer, dark gray or greenish-gray colour (Pearsall and Mortimer, 1939; Alberda, 1953; Howeler and Bouldin, 1971; and Patrick and Delaune, 1972).

The thickness of the oxidized zone is determined by the O_2 supply and O_2 consumption rates. In systems low in organic matter and open to the atmosphere, the depth of the surface aerobic layer may increase with time (Bouldin, 1968). The addition of readily available organic matter usu-

ally decreases the depth of the light-coloured upper zone (Engler and Patrick, 1974; and Reddy et al., 1978).

Five mathematical models for describing the diffusion and consumption of O_2 in submerged soils were reported by Bouldin (1968) and Howeler and Bouldin (1971). The first two models describe the situation where respiration by microorganisms in the oxidized zone consumes all of the O_2 which crosses the soil-water interface, and the thickness of the oxidized zone remains constant with time. As a consequence, microbial respiration in the whole oxidized zone also remains constant with time. These are referred to as steady-state models. The other three models describe situations in which no microbial respiration occurs, but O_2 is consumed by instantaneous chemical reactions at the interface between the oxidized and reduced zone. At time zero, the soil is uniformly reduced and no oxidized zone is present. Thereafter the concentration of O_2 at the soil-water interface is maintained at a constant level, and O_2 diffusion and chemical reactions occur simultaneously. These are referred to as transient-state models where reductants such as ferrous and manganous ions were considered to be non-mobile (O_2 must diffuse to the reductant for reaction to occur), mobile (as reductant reacts with O_2, concentration gradients of reductant are created resulting in the diffusion of reductant toward the interface between reduced and oxidized soil), or both mobile and non-mobile.

The best example of a chemical reductant which reacts instantaneously with O_2 is Fe^{+2}. Other examples are Mn^{+2} and S^{-2}. However, the Fe^{+2} is probably the predominant chemical reductant in most situations (Howeler and Bouldin, 1971). They reported that all of the above models have

shortcomings of various kinds, the most serious being that none considers both microbial respiration in the oxidized zone and chemical consumption at the interface between oxidized and reduced soil. Furthermore, they concluded that a model which combines both of these O_2 sinks was so complex with so many parameters involved that it was not very useful in terms of interpreting the results of their experiments. However, for the static-oxidized zone where the depth of the oxidized zone does not change with time, the following equations are used to describe the system;

$$\partial C_s / \partial t = D_s \partial^2 C_s / \partial x^2 - kC_s \qquad \text{(33, Model I)}$$

$$\partial C_s / \partial t = D_s \partial^2 C_s / \partial x^2 - \theta_s \qquad \text{(34, Model II)}$$

with the boundary condition

$$\begin{aligned} C_s &= C_s^{\circ}, \; x = 0, \; t>0 \\ C_s &= 0, \quad x>0 \quad , \; t = 0 \\ C_s &= 0, \; x = \infty \, , \; t>0 \end{aligned} \qquad (35)$$

where C_s is concentration of O_2 in soil solution at distance x below the interface; C_s° is the concentration of O_2 in solution at the interface; k is the proportionality constant relating O_2 consumption per unit volume of soil to concentration of O_2; kC_s and θ_s are first and zero-order rates of O_2 consumption in soil solution, respectively; D_s is the diffusion coefficient of O_2 in the soil solution; and t is the time.

The physical meaning of the boundary conditions corresponds to a situation where soluble O_2 is continually supplied to a soil-water interface and the soil profile does not contain O_2. In Eq. (33), Model I,

microbial respiration is proportional to the concentration of O_2 in each microvolume of soil and hence varies with distance from the soil-water interface. In Eq. (34), Model II, the microbial respiration is independent of O_2 concentration and hence independent of distance from the soil-water interface (Howeler and Bouldin, 1971).

The essential conditions for Model I are as follows:

1. The solution at the soil-water interface is maintained at a constant O_2 concentration;
2. The rate of consumption of O_2 per unit volume in an incremental volume of soil is proportional to the concentration of O_2 in solution in that incremental volume (and remains constant);
3. The diffusion coefficient of O_2 in the soil is constant; and
4. A steady-state condition exists, that is, the concentration of O_2 at any given point in the flooded-soil remains constant with time.

Under these conditions, the following equations describe the concentration of O_2 as a function of distance from the interface, the flux across the interface and the total quantity of O_2 which crosses the interface in a given time period;

$$C_s/C_s^o = 1/2 \exp\{-x (k/D_s)^{1/2}\} \operatorname{erfc} [x/2 (tD_s)^{1/2} - (kt)^{1/2}] + 1/2 \exp [x (k/D_s)^{1/2}] \operatorname{erfc} [x/2 (tD_s)^{1/2} + (kt)^{1/2}] \quad (36)$$

$$dQ/dt = C_s^o (D_s k)^{1/2} [\operatorname{erf} (kt)^{1/2} + e^{-kt}/(\pi kt)^{1/2}] \quad (37)$$

$$Q = C_s^o (D_s/k)^{1/2} (kt + 1/2) \operatorname{erf} (kt)^{1/2} + (kt/\pi)^{1/2} e^{-kt} \quad (38)$$

where $-D_s [dC_s/dx]_{x=0} = dQ/dt =$ flux of O_2 which crosses the interface, Q = quantity of O_2 which crosses the interface,

$$\text{and erfc } z = 1 - \text{erf } z = 1 - 2/(\pi)^{1/2} \int_0^z e^{-y^2}\, dy.$$

When kt is sufficiently large then erf $(kt)^{1/2} \simeq 1$, and Eqs. (36),(37) and (38) become

$$C/C_s^o = \exp\{-x\,(k/D_s)^{1/2}\} \tag{39}$$

$$dQ/dt = C_s^o\,(D_s k)^{1/2} \tag{40}$$

$$Q = C_s^o\,(D_s k)^{1/2}\,(t + 0.5/k) \tag{41}$$

Derivations of these equations from the transient-state equation were described by Danckwerts (1950). The non-steady solution is most conveniently obtained by applying the Laplace transformation. Cho (1971) reported the general solution for the non-steady state equation for convective transport of N compounds with first-order reaction kinetics for the N-transformations. This general solution can be applied to the non-convective O_2 transport by setting the average velocity of flowing system at zero. The average velocity was defined in Eq. (2).

The steady-state O_2 transport equation with constant consumption of O_2 per unit volume per unit time (Model II) can be reduced to

$$D_s\, d^2C_s/dx^2 = \theta_s \tag{42}$$

by setting $\partial C_s/\partial t = 0$.

The equations describing this system are

$$C_s = \theta_s x^2/2D_s - x(2C_s^o\theta_s/D_s)^{1/2} + C_s^o \tag{43}$$

$$dQ/dt = (2C_s^o\,\theta_s D_s)^{1/2} \tag{44}$$

$$Q = t(2C_s^{o}\theta D_s)^{1/2} \tag{45}$$

where θ_s is the rate of consumption of O_2 per unit volume of flooded soil per unit time and other symbols are the same as for Model I.

From Model I (Eq. (39)) if we define the thickness of oxidized zone, $x_{0.01}$ as the distance from the water-soil interface to where $C_s/C_s^{o} = 0.01$, $x_{0.01}$ can be calculated from

$$x_{0.01} = (-\ln 0.01)\ (D_s/k)^{1/2} \tag{46}$$

Therefore, it can be seen that reducing the value of k from $1.3\ 10^{-3}$ to $1.3\ 10^{-5}$ per sec (100 times) causes an increase in the thickness of the aerobic layer from 0.2 to 2 cm (10 times) (Bouldin, 1968).

The thickness of the aerobic layer can be defined in a similar way for Model II

$$x^{*} = (2C_s^{o}D_s/\theta_s)^{1/2} \tag{47}$$

where $x = x^{*}$ at $C_s = 0$.

These steady state models were evaluated by Howeler and Bouldin (1971) by calculation of θ_s and k, using equations for dQ/dt and x^{*}. Their data were described better by a square-root relationship between dQ/dt and C_s^{o} as predicted by Model II, than a linear relationship as predicted by Model I. They also concluded that soils with high θ_s and k values had low x^{*} values and vice versa. Soils with a high organic matter content tended to have a high microbial respiration rate and thin oxidized zone. The measured O_2 flux was fairly well correlated with organic matter content of the soil ($r = 0.64$) but was much better correlated with the content of fulvic acid ($r = 0.87$).

Many of the changes occurring in flooded systems can be explained by diffusion of both ions and molecules. Oxidation of reduced ionic species in the upper aerobic layer creates concentration gradients and mobile ions diffuse upward in response to these gradients (Patrick and Deluane, 1972).

Some authors (Patrick and Tusneem, 1972; Patrick and Gotoh, 1974) concluded that appreciable denitrification occurs in flooded soils if both O_2 from the atmosphere and NH_4^+ from the flooded soil are available. They showed that a little nitrogen loss occurred in flooded soils supplied with ammonium sulfate in an oxygen-free atmosphere. However, an increase in the O_2 content of the atmosphere to 5 or 10% resulted in formation of a thin aerobic zone which led to substantial nitrogen loss. An increase in the O_2 partial pressure from 20 to 80% increased the thickness of the aerobic layer, but did not increase the nitrogen loss appreciably (Patrick and Gotoh, 1974).

The amount of N_2 produced usually greatly exceeded the amount of NH_4^+ and NO_3^- present in the aerobic surface layer at any one time (Patrick and Deluane, 1972; Patrick and Reddy, 1976). It was proposed that NH_4^+ and NO_3^- diffused in opposite directions, up and down the soil column, respectively, in response to their concentration gradients (Patrick and Deluane, 1972). This was later verified using labelled NH_4^+-N (Patrick and Reddy, 1976). Nitrate, unlike ammonium, is not strongly adsorbed to organic or clay micelles, and hence the diffusion coefficient for NO_3^- is about six-fold greater than NH_4^+ diffusion and it is relatively free to respond to a concentration gradient (Engler et al., 1976; Patrick and Reddy, 1976). 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.

Chapter III

MATERIALS AND METHODS

3.1 SOIL SAMPLES

Eight samples from a Wellwood soil (Legal location SE 30-11-14), ranging in depth from 0 to 60 cm and increasing in pH with depth (pH 6.45-7.73) were used for all investigations. Samples from the 0 to 20 cm depth were taken at 5-cm intevals, and from 20 to 60 cm depth at 10-cm intervals. The samples were air dried and crushed to pass through a 2 mm sieve. Some of the physical and chemical properties of the soil samples are shown in Table 1. The average value of particle density for the eight samples was 2.55 g/cm^3. This value was used to estimate the air porosities of soil columns as described later.

3.2 INCUBATION STUDIES WITH CLOSED SYSTEMS

The effects of soil moisture and incubation temperature on the rates of O_2 consumption and CO_2 production were studied using closed systems (experiment 1). Incubation tubes (Fig. 1) with internal volumes of approximately 60 ml were made from Taper Standard ℧ 24/40 joints, 2 mm bore high vacuum stopcocks, and ℧ 10/18 cone joints. All stopcocks and joints were connected to each other using Corning high vacuum grease. In order to analyze quantitatively evolved gases, sets of tubes were adjusted to the same volume by the addition of glass beads.

TABLE 1

Some physical and chemical properties of Wellwood soil profile samples (Legal location SE 30-11-14).

Soil depth (cm)	$CaCO_3$ equivalent (%)	pH	Particle density (g/cm^3)	E C (mhos/cm)	Organic matter (%)
0-5	Trace	6.45	2.41	0.53	3.9
5-10	Trace	6.46	2.46	0.41	3.6
10-15	Trace	6.84	2.54	0.34	3.2
15-20	Trace	6.86	2.56	0.28	2.9
20-30	0.26	7.35	2.57	0.46	2.6
30-40	0.38	7.40	2.59	0.47	2.4
40-50	0.91	7.50	2.53	0.45	2.4
50-60	1.31	7.73	2.59	0.40	2.1

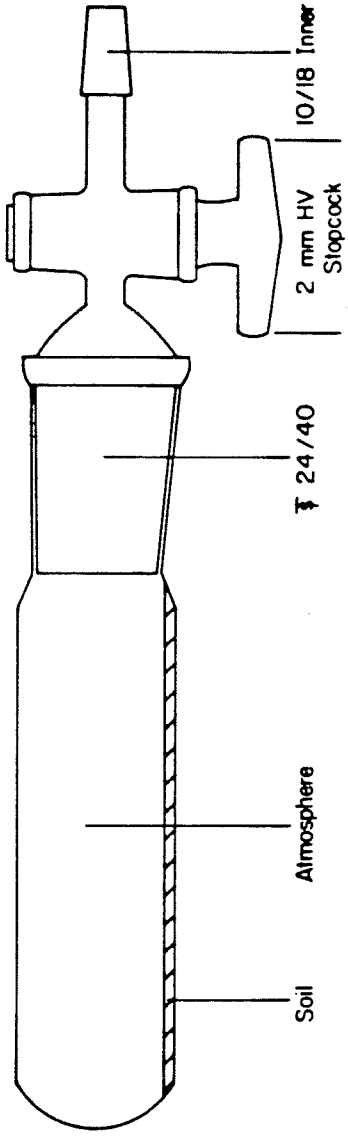

Figure 1: The incubation tube

Ten-gram samples of soil were incubated at moisture contents of 5, 10, 15, 20, 25 and 30% on dry soil weight basis with urea-N at rates of 40, 80, 120, 160, 200 and 240 µg/g oven-dried soil, respectively. These rates of urea were equivalent to 786 µg N/ml soil solution. The moistened soil sample was placed in the incubation tube and the pressure of the incubation atmosphere was lowered to 650 mm Hg (86.7 kPa) by evacuating a small amount of air. This was done to accommodate the produced gases. These soil samples were incubated at 35°C for the desired period of time in a horizontal position to maximize soil surface to soil volume ratio.

Gas sample tubes, each having about 50 ml internal volume, were constructed from right angle 2 mm stopcocks and ℨ 10/18 socket joints (Fig. 2). Two gas sample tubes were used to collect the evolved gases from each incubation tube. One gas sample tube contained about 5 g of potassium hydroxide as a water and CO_2 absorbent. The other contained 3 ml of concentrated sulfuric acid to absorb water.

After the sample had been incubated for the required time its atmosphere was sampled. The gas sample tubes were vigorously shaken after sampling and were shaken periodically for an hour to ensure maximum absorbence of moisture. The gas was introduced into a VG-Micromass 602c mass spectrometer and scanned from atomic mass unit (a.m.u.) 28 to 46. Quantitative estimation of the gases was carried out as described later. The soil samples after incubation were tranferred to an Erlenmyer flask with 50 ml 2N KCl solution and mechanically shaken for 1 hour for pH determinations. The pH values were used in the quantitative estimations of total dissolved CO_2 as described later.

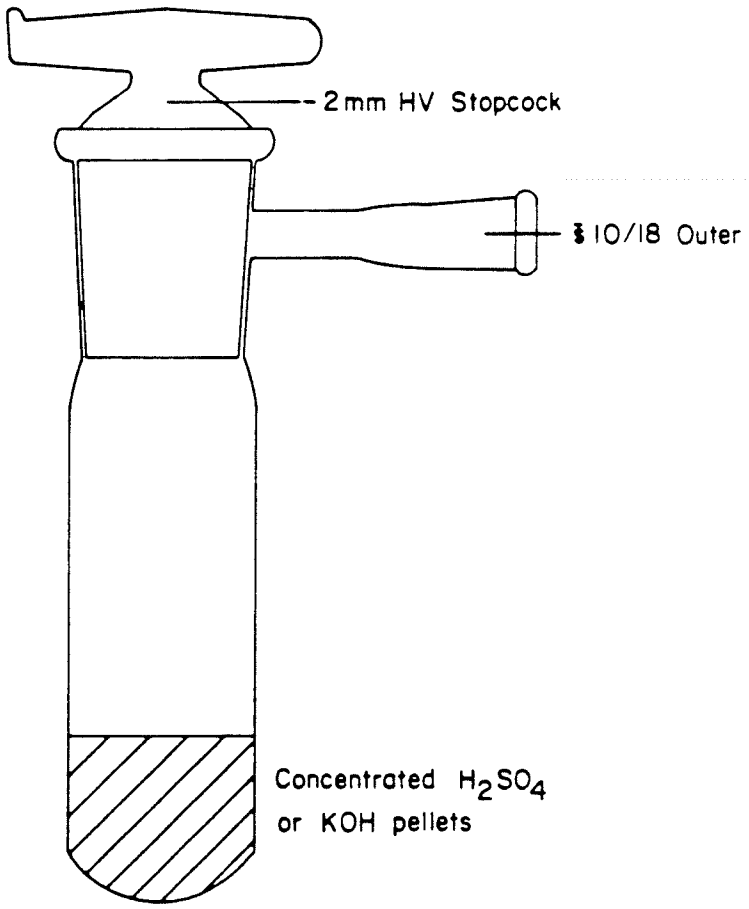

Figure 2: Gas Sample Tube.

In a similar incubation study, another set of samples was incubated at temperatures of 10, 20 and 40°C at a moisture content of 25% and an urea N concentration of 200 ug/g oven-dried soil.

3.3 COLUMN STUDIES: GENERAL PROCEDURES.

Experiment 2 concerned the influence of air porosity on gaseous diffusion and denitrification of NO_3^- derived from urea whereas experiment 3 concerned the effect of NO_3^- concentration on denitrification. Both experiments were conducted with columns designed to simulate the field conditions. The soil columns were enclosed in plastic tubes either 30 or 60 cm in length with an inner diameter of 4.4 cm. Each of the tubes had small openings covered with a rubber septum along the length of the tube for gas sampling (Fig. 3). Each of the long columns (60 cm) had gas sampling ports at 2.5, 7.5, 12.5, 17.5, 25, 35, 45, and 55 cm with an impermeable layer at 60 cm . Each short column (30 cm) had gas sampling ports at 2.5, 7.5, 12.5, 17.5, and 25 cm with an impermeable layer at 30 cm. The soil columns were filled with increments of 5 or 10 cm to reconstitute the soil profile as it was in the field. For example, the increments were 60 to 50, 50 to 40, 40 to 30, 30 to 20, 20 to 15, 15 to 10, 10 to 5 and 5 to 0 cm for the 60 cm column. As each increment of soil was added the column was compacted to provide the desired bulk density. The bulk density was estimated from the weight of soil and the volume it occupied. The bulk densities used for column studies were homogeneous throughout each column. The top of each soil column was covered with a plastic film which had several small openings to provide gaseous exchange while minimizing water loss. A small pan of water was

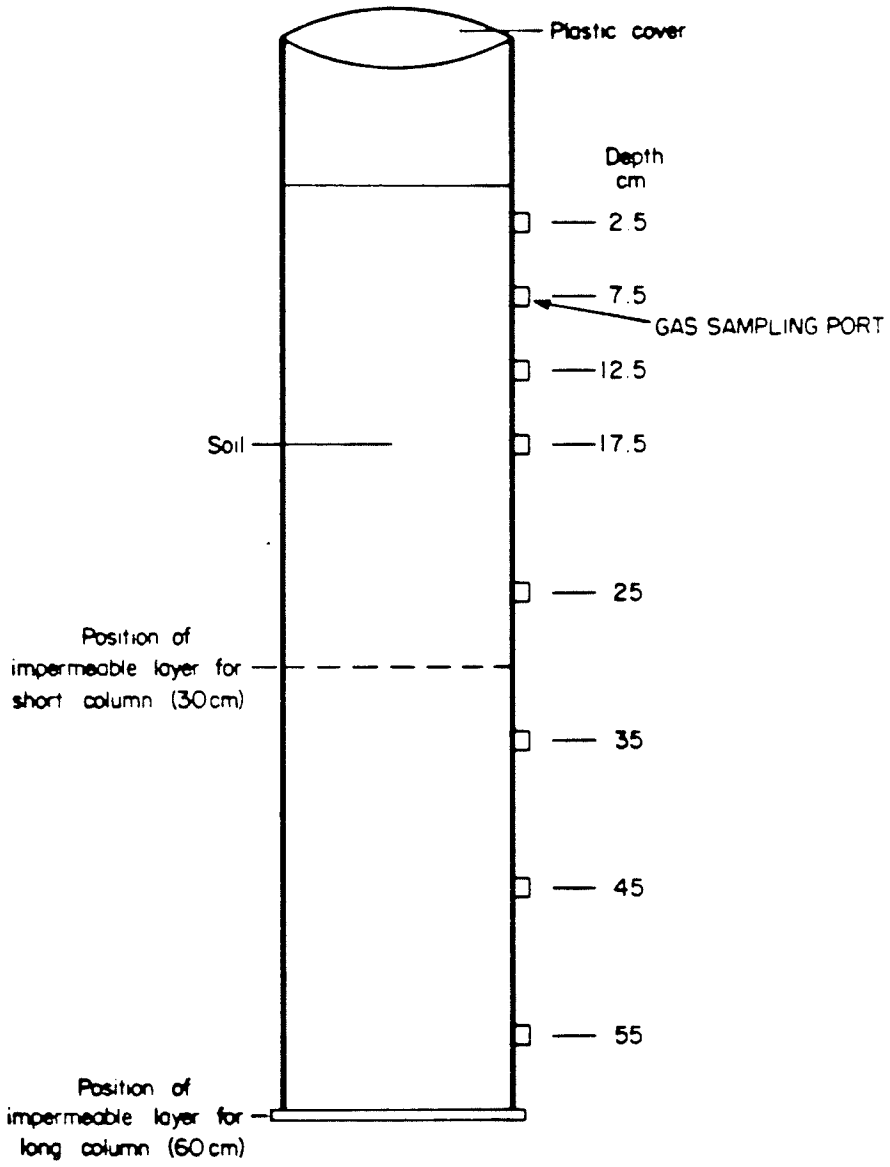

Figure 3: Schematic diagram of soil column.

placed above the soil surface to maintain the relative humidity above the soil surface near 100%.

Gas sampling involved extracting 1 ml of soil air through the rubber septum with a medical syringe and transferring the sample to a gas sample tube which contained either KOH or H_2SO_4. Air contamination during the sampling stage was minimized by using 1 ml of distilled water in the syringe and keeping the syringe in a vertical position (the needle in the bottom) to prevent direct contact between the gas sample and the air atmosphere through the needle. Transfer of the gas was carried out by using a special transferring tube. One end of the transferring tube had a rubber septum and the other was made from E 10/18 socket joint with glasswool in between. The gas sample tubes and the transfer tube were connected to a vacuum line and the gas was transferred after the system had been evacuated. The glasswool was used to absorb the water in the syringe during the gas transfer. The gas sample tube was connected to the mass spectrometer and the analysis was carried out by scanning a.m.u. from 28 to 46.

A separate soil column was constructed with several short pieces of plastic tubes in order to determine inorganic forms of nitrogen in the soil. The column was identical to the gas sampling column except that gas sampling ports were absent. After the soil column had been incubated for the required time, the soil column was sectioned into 8 layers. The contents of each layer were mixed and divided into three portions. These three portions of soil were analyzed for NO_3^- and NO_2^-, gravimetric water content and NH_4^+, respectively, as described in the physical and chemical analyses section.

3.3.1 EXPERIMENT 2: Effect of air porosity on gaseous diffusion and denitrification of nitrate derived from urea.

Variation in air porosity was accomplished by using four combinations of moisture content and bulk density. Moisture contents were 23, 25, 30 and 30% and the respective bulk densities were 1.2, 1.32, 1.32, and 1.35 g/cm^3. Percent total porosity was calculated from values of bulk density and average particle density (2.55 g/cm^3). Air porosity was calculated by subtracting the volumetric water content from the total porosity. Average values of volumetric water content for the incubation period were used in these calculations (see section 4.2.1). These four combinations gave air porosities of 25.6, 16.2, 10.5, 8.5%, respectively. All columns were incubated at 20°C with 200 µg urea-N/g oven-dried soil labelled with N-15 at 52.6 atom percent enrichment. Long columns (60 cm) were used for this treatment with the exception of 10.5% air porosity where a short column (30 cm) was used. Gas samples from various sampling ports were taken regularly for analysis as described earlier. Similarly, several columns (for the inorganic-N study) were incubated with non-labelled urea at a rate of 200 µg N/g soil and sectioned for inorganic nitrogen and moisture determinations as mentioned before. The duration of incubation was 60 days.

3.3.2 EXPERIMENT 3: Effect of nitrate concentration on denitrification in soil columns.

Three levels of N (50, 100 and 200 µg N/g soil as $NaNO_3$) in short soil columns (30 cm) were used to study the effect of NO_3^- concentration on denitrification. The nitrogen was ^{15}N labelled (52.6 %). The NO_3^- was mixed uniformly in the 0 to 20 cm layer of the soil columns. The

columns were prepared to obtain an air porosity of 10.5% (30% moisture and 1.32 g/cm^3 bulk density). A previous experiment showed that the upper 20 cm of the soil column was aerobic when air porosity was 10.5%. The columns were incubated from 12 to 22 days (depending on the concentration) at 20°C. To follow the transformation of inorganic nitrogen, sets of soil columns (10.5% air porosity) were incubated with non-labelled NO_3^- at the same concentrations as indicated above. The columns with non-labelled N were sectioned for inorganic nitrogen and moisture determinations.

3.3.3 Quantitative estimation of O_2, N_2 and N_2O in the incubation atmosphere

The method of Cho and Sakdinan (1978) was used to calculate the partial pressure of the gases in the incubation atmosphere from the mass spectrometric peak height. The gases in the sample container were introduced into the mass spectrometer following removal of water 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 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 value. An air sample without denitrification products was identically scanned. The contribution by air to each a.m.u. in the sample scan was deducted in calculating the peak height of gases produced (Cho and Sakdinan, 1978). 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. From the calculated partial pressure of O_2, N_2 and N_2O in the

incubation atmosphere, the quantity of these gases dissolved in water was calculated using Henry's Law constants. The weights of O_2, N_2 and N_2O in the incubation atmosphere and in the solution were calculated and totalled as the net gas consumption or the net gas production (Khdyer, 1978; Christenson, 1978; Nicholson, 1979).

3.3.4 Quantitative estimation of CO_2 production.

The peak height of CO_2 was calculated from the difference in the production of a.m.u. 44 between gas containers with H_2SO_4 and KOH. To estimate the CO_2 dissolved in the soil solution at any temperature, the effect of temperature on the equilibrium constants for the reactions

$$CO_2 + H_2O = H_2CO_3,$$

$$H_2CO_3 = H^+ + HCO_3^-, \text{ and} \qquad (48)$$

$$HCO_3^- = H^+ + CO_3^-$$

with their respective mass action equations

$$K_H = (H_2CO_3)/P_{CO_2},$$

$$K_1 = (H^+)\ (HCO_3^-)/(H_2CO_3), \text{ and} \qquad (49)$$

$$K_2 = (H^+)\ (CO_3^{-2})/(HCO_3^-)$$

were taken into account. In the above equations, K_H is Henry's Law constant, P_{CO_2} is the partial pressure of CO_2, the parentheses indicate the activity of each species, K_1 is the dissociation constant of H_2CO_3 and K_2 is the dissociation constant of HCO_3^-.

The basic relationship between the equilibrium constant and temperature is

$$\ln (K^{**}/K^{*}) = \Delta H^{o}/R \; (1/T_1 - 1/T_2) \qquad (50)$$

where K^{*} is the equilibrium constant of the reaction at T_1 (^{o}K), K^{**} is the equilibrium constant of the reaction at T_2, R is the universal gas constant and ΔH^{o} is the standard molar enthalpy of the reaction (j/mole) which is equal to the sum of the molar enthalpies of formation of products minus the sum of molar enthalpies of formation of reactants. The equilibrium constants at various temperatures were calculated using Eq. (50). The total dissolved CO_2 was calculated from the equation

$$\text{Total dissolved } CO_2 = CO_3^{-2} + HCO_3^{-} + H_2CO_3. \qquad (51)$$

By solving the mass action equation (49) for CO_3^{-2}, HCO_3^{-} and H_2CO_3 and substituting them into Eq. (51) the result is

$$\text{Total dissolved } CO_2 = K_H \, P_{CO_2} \{1 + K_1/(H^{+}) + K_1 \, K_2/(H^{+})^2\} \qquad (52)$$

3.4 PHYSICAL AND CHEMICAL ANALYSES

Nitrate-N and NO_2^{-}-N concentrations were determined by mixing thirty grams of moist soil with 50 ml of distilled water and stirred vigorously for 1 hour. The suspension was filtered through Whatman No. 42 filter paper and the NO_3^{-}-N and NO_2^{-}-N concentrations in the filtrate were determined in a Technicon autoanalyser using a modified procedure of Kamphake et al. (1967). Nitrite-N initially present in the sample was determined by a conventional diazotization-coupling reaction. In order to determine the amount of NO_3^{-}-N, NO_2^{-}-N was removed from another portion

of sample by reacting 0.3 ml of 3% sulfamic acid solution with 10 ml of sample for one half hour. The NO_3^- in the solution was quantitatively reduced to NO_2^- with hydrazine sulfate and the NO_2^- determined by the same diazotization-coupling reaction.

The NH_4^+ was extracted by shaking 50 g of the moist soil in 50 ml of 2N KCl solution for 1 hour. The 2N KCl extract was distilled into 25 to 50 ml of 0.1N H_2SO_4 containing methyl red indicator and the NH_4^+-N content determined with standardized 0.1N NaOH.

Organic C was determined by the Walkley-Black method as described by Allison (1965). The $CaCO_3$ content was determined by the manometric method of Skinner et al. (1959). The electrical conductivity of a 1:1 soil:water mixture measured with a Radiometer conductivity meter. Kjeldahl-N was determined by the method described by Bremner (1965). The pH of a 1:1 soil:water mixture was determined using a pH meter and Fisher glass-body combination electrode.

Chapter IV

RESULTS AND DISCUSSION

4.1 EXPERIMENT 1: EFFECT OF SOIL MOISTURE AND INCUBATION TEMPERATURE ON THE RATES OF OXYGEN CONSUMPTION AND CARBON DIOXIDE PRODUCTION IN WELLWOOD PROFILE SAMPLES (CLOSED SYSTEM)

Oxygen consumption of the surface (0-5 cm) soil samples, incubated at several moisture levels at 35°C, is shown in Fig. 4. The amount of O_2 consumed was related directly to the time of incubation allowing calculation of the O_2 consumption rate (OCR) (μmole/g soil/day). At all moisture levels the amount of O_2 consumed during the incubation period of 3 days was related to the time of incubation and could be described by zero-order kinetics with determination coefficient (r^2) values ranging from 0.96 to 0.99 (Table 2). The OCR increased with soil moisture content from 1.30 μmole/g/day at 5% moisture to a maximum value of 9.36 μmole/g/day at 20% moisture. The OCR did not increase any further as the moisture content was increased from 20 to 30% even though this increase in moisture should have resulted in increased microbial activity. Thus, it is possible that the O_2 supply became the most limiting factor for microbial activity at moisture contents greater than 20%.

The O_2 consumption rates of the remaining layers as influenced by soil moisture were also measured at 35°C (Fig 5). The results with soil samples from the 10-15 and 15-20 cm layers were combined because they were nearly identical. In the upper three layers the OCR increased linearly as the soil moisture increased up to 20%. By extrapolation it was

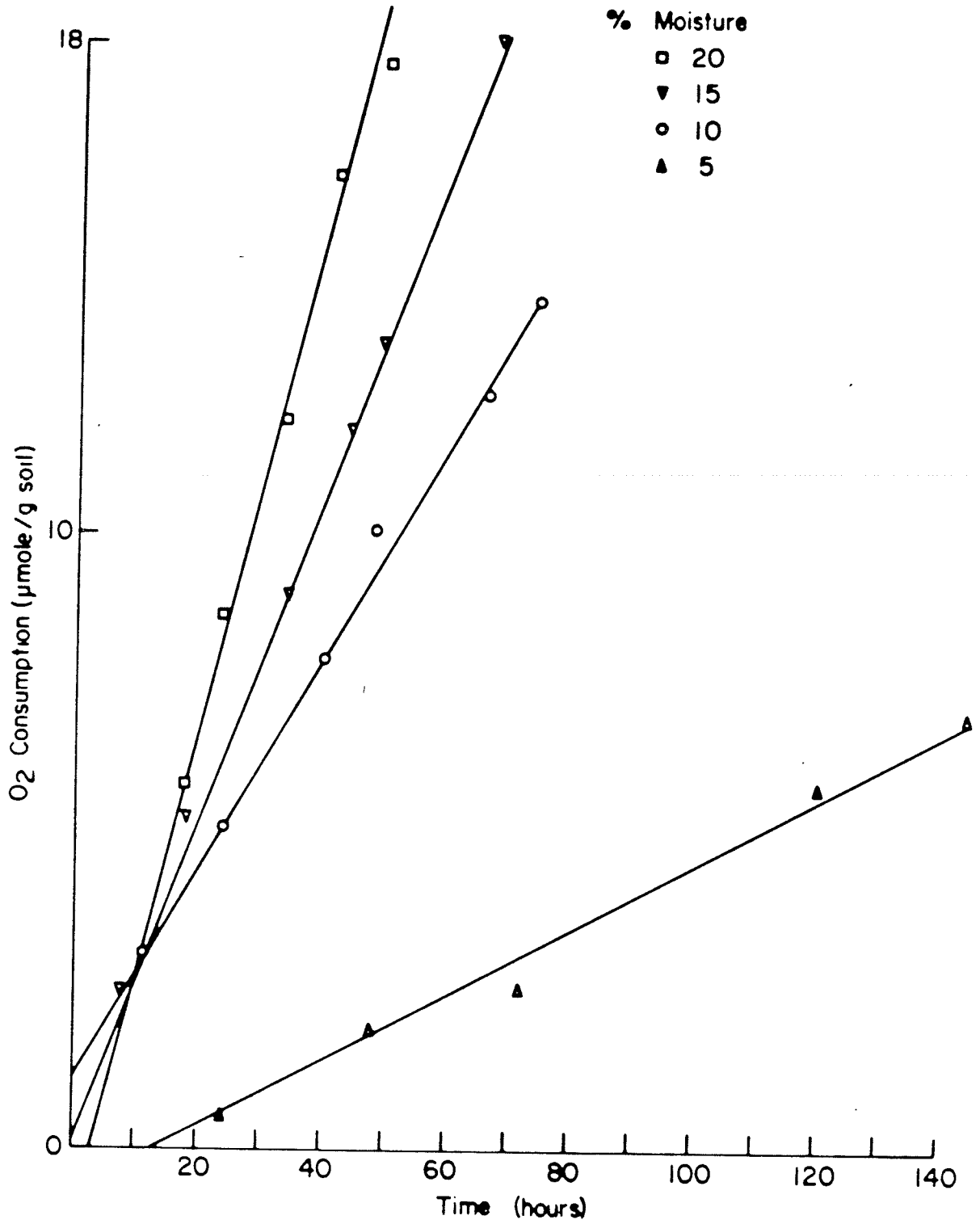

Figure 4: Oxygen consumption in the 0-5 cm layer as related to moisture content, T = 35°C.

TABLE 2

Oxygen consumption rate for surface sample of Wellwood soil (0–5 cm layer) at 35°C, as related to moisture content

Moisture content (%)	OCR (μmole/g/day)	Coefficient of determination
5	1.30	0.99
10	4.11	0.99
15	6.53	0.99
20	9.30	0.99
25	9.18	0.99
30	9.36	0.96

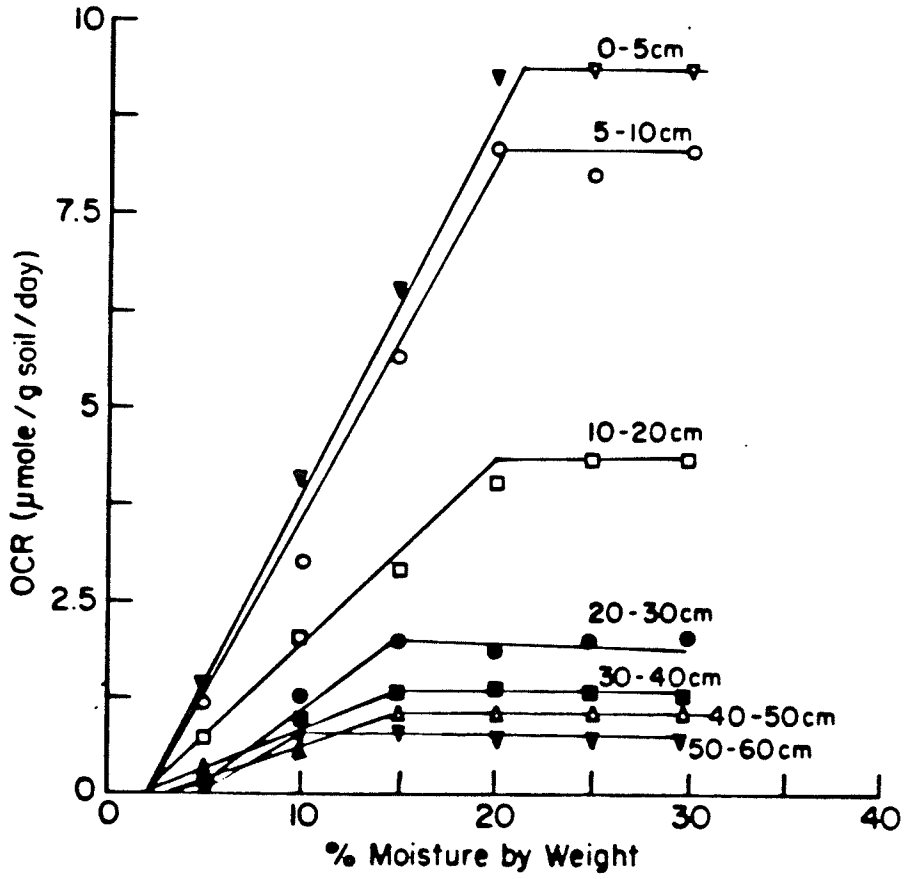

Figure 5: Oxygen consumption rates of Wellwood profile samples as related to soil moisture at 35°C.

estimated that zero OCR would occur at 2.5% moisture. The OCR did not change in these layers as moisture content was increased from 20 to 30%. The OCR increased linearly in the lower layers (20-50 cm) as soil moisture increased from the threshold value of 5% up to 15% but did not change as the soil moisture content was further increased. The maximum OCR occurred at a moisture content of 10% in the 50-60 cm layer. The maximum OCR values (i.e., between 20 and 30% moisture contents) decreased with soil depth and were 9.3, 8.4, 3.9, 2.0, 1.5, 1.1 and 1.0 μmole/g/day for samples from average depths of 2.5, 7.5, 15, 25, 35, 45 and 55 cm, respectively.

Production of CO_2 in the surface layer (0-5 cm) incubated at 35°C as influenced by moisture level is shown in Fig. 6. The amount of CO_2 produced during the first day of incubation increased from a value of 7.5 μmole/g at 5% moisture to 39 μmole/g at 25% moisture. Although there was a linear relationship between the amount of CO_2 produced and incubation time, similar to the O_2 consumption pattern, the linear regression analysis for CO_2 showed that the intercepts or the extrapolated values of CO_2 produced at zero time were greater than zero (Table 3). These extrapolated values increased with increasing soil moisture. Similar results were obtained by other workers (Miller and Johnson 1964; Cho et al., 1979). High intercept values could indicate that there was a high CO_2 production rate initially, before steady value was achieved. Several authors (Stevenson, 1956; Birch, 1959, 1960; Miller and Johnson, 1964; Sorenson, 1975) found that the microbial activity reached a maximum in the early stage of incubation, dropped slightly, and then remained constant.

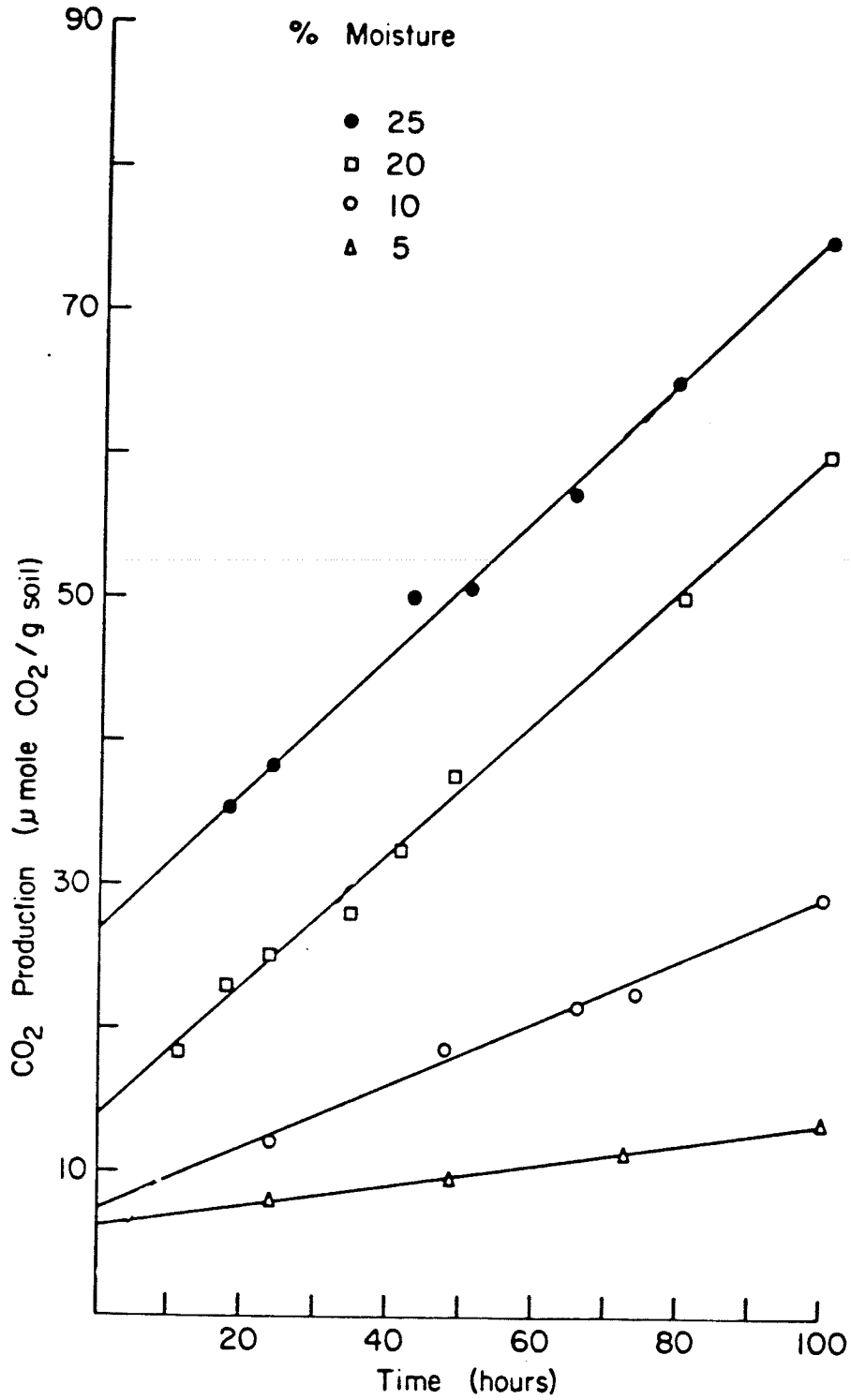

Figure 6: Carbon dioxide production by surface profile sample as related to moisture content, at 35°C.

TABLE 3

Carbon dioxide production rate for surface sample of Wellwood soil (0–5 cm layer) at 35°C, as related to moisture content

Moisture content (%)	CO_2 intercept at zero time (μmole/g soil)	Production rate (μmole/g soil/day)	Coefficient of determination (r^2)
5	6.30	1.89	0.98
10	7.83	5.11	0.98
15	7.25	10.43	0.99
20	13.01	11.75	0.96
25	24.92	13.30	0.96
30	26.28	15.84	0.92

The CO_2 production rates (R^*) of subsurface Wellwood soil samples were measured similarly. The CO_2 production rate increased sharply as the soil moisture content increased from 5 to 20% for the upper three profile samples (Fig. 7). By extrapolation it was found that the threshold moisture content for the CO_2 production in these samples was 2.5%. An increase in soil moisture content from 20 to 30% resulted in the CO_2 production rate increasing slightly in the 0-5 and 5-10 cm layers. It did not change in the 10-20 cm layer. However, except for the 40-50 cm layer the CO_2 production rate increased in the lower layers as the soil moisture level increased from 5 to 15%, but it did not change with further increases in the soil moisture.

The O_2 consumption and CO_2 production rates of the profile samples were measured at three additional incubation temperatures (10, 20 and 40°C) at 25% moisture. Semilog plots of OCR versus the average soil depth at various temperatures are shown in Fig. 8. The OCR as related to soil depth was semilog linear with a negative slope for soils which were incubated at 40, 35 and 20°C. However, for the soil samples which were incubated at 10°C, there was a deviation from linearity in the semilog plot at depths greater than 25 cm. Such a deviation at 10°C could suggest a different pattern of OCR versus soil depth.

If one considered only the linear pattern of the semilog plot, the following functional relationships between the OCR and the soil depth at 20, 35 and 40°C were obtained by regression analyses;

$$OCR_{20} = 4.598 \text{ EXP } (-0.045x)$$

$$OCR_{35} = 9.766 \text{ EXP } (-0.048x)$$

$$OCR_{40} = 12.407 \text{ EXP } (-0.047x)$$

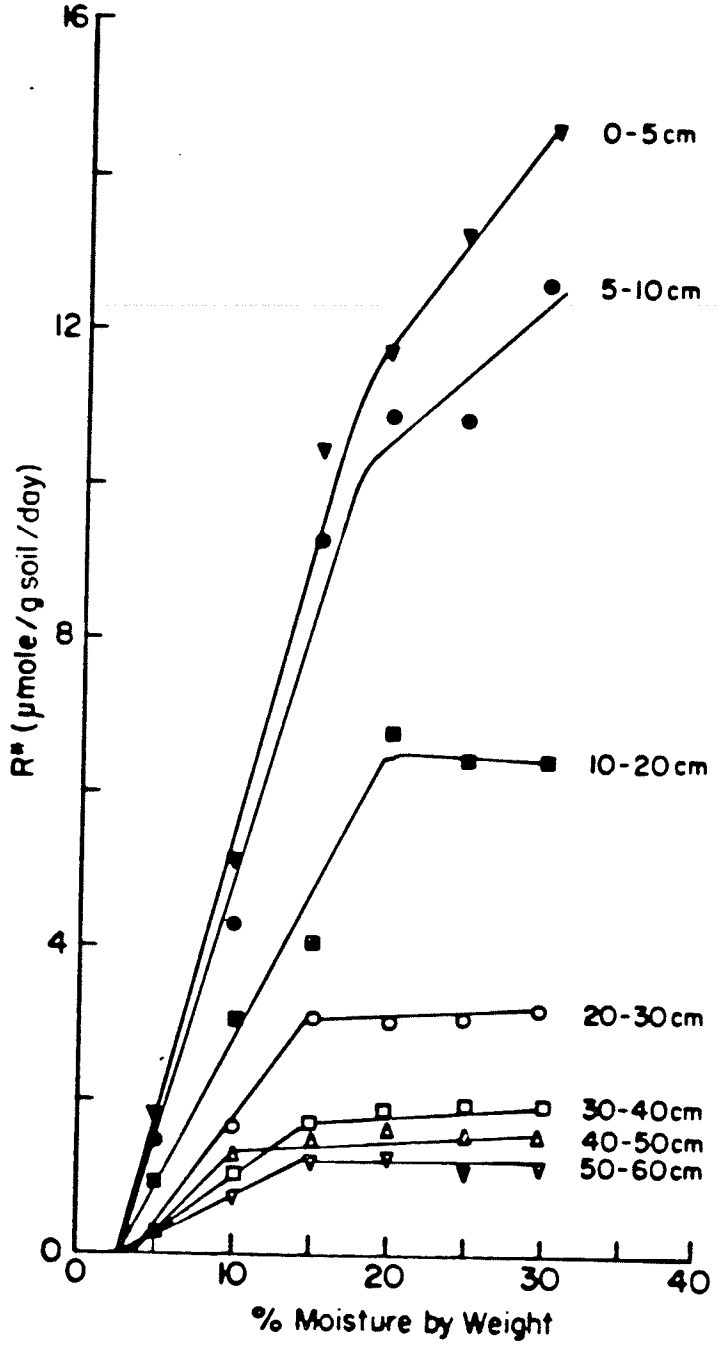

Figure 7: Carbon dioxide production rate as related to soil moisture in various sample layers at 35°C.

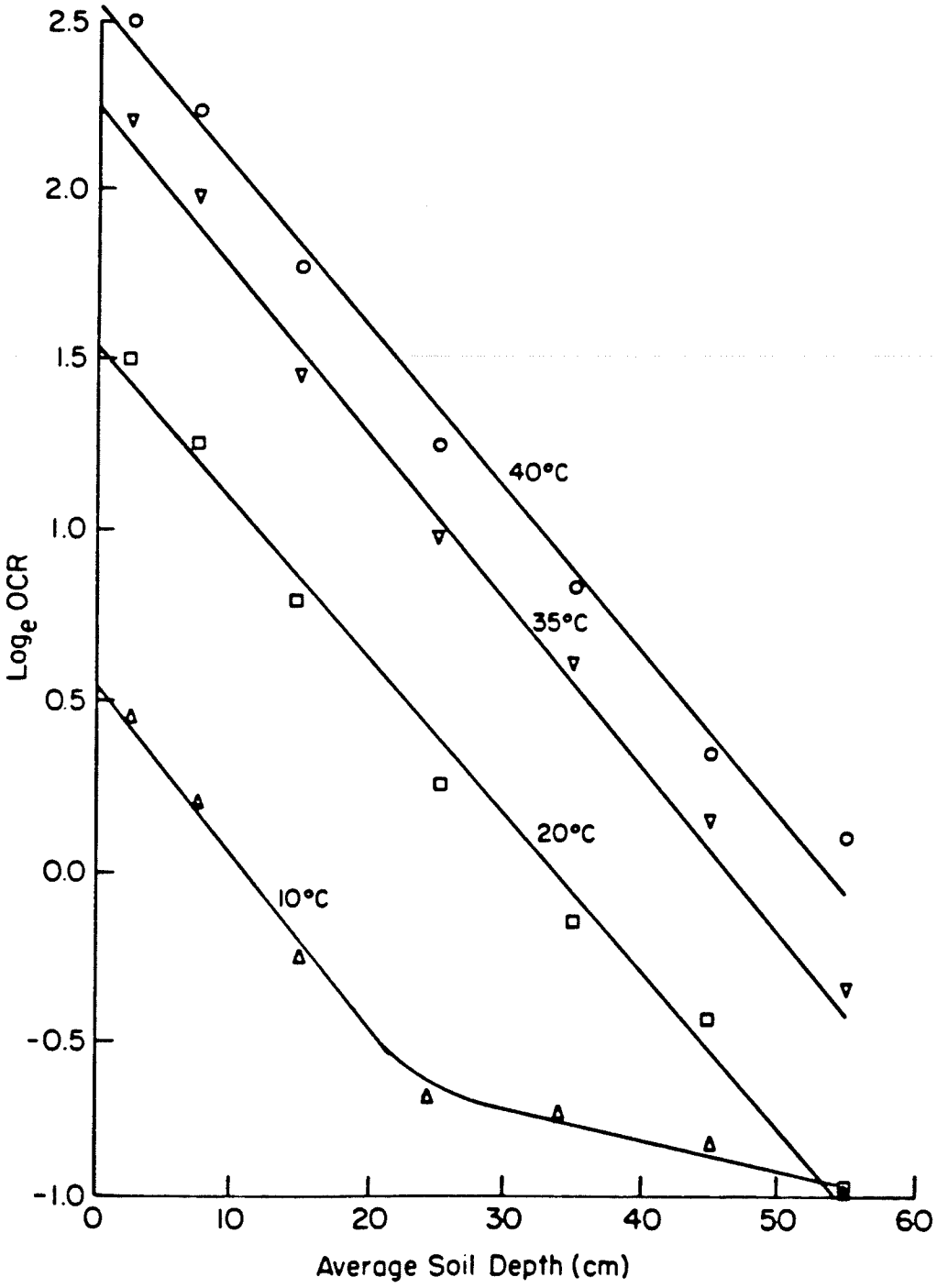

Figure 8: Semilog plot of O_2 consumption rate (R) as function of soil depth, for various temperatures at 25% moisture

where OCR_{20}, OCR_{35} and OCR_{40} are the OCR (μmole/g/day) at 20, 35 and 40°C, respectively, and x is the average depth (cm). The OCR value extrapolated to zero average depth (numerical value in front of exponential term) was the maximum OCR. This value increased as the incubation temperature increased.

The semilog plots of CO_2 production rate (μmole/g/day) versus soil depth indicate that similar to the OCR, the CO_2 production rates decreased exponentially with depth at incubation temperatures of 20, 35 and 40°C (Fig. 9). The functional relationships are described by

$$R_{20}^{*} = 9.091 \text{ EXP } (-0.053\ x)$$
$$R_{35}^{*} = 13.915 \text{ EXP } (-0.054\ x)$$
$$R_{40}^{*} = 18.596 \text{ EXP } (-0.053\ x)$$

where R_{20}^{*}, R_{35}^{*}, R_{40}^{*} stand for the CO_2 production rate at 20, 35 and 40°C, respectively. The CO_2 production rate extrapolated to zero average depth was the maximum CO_2 production rate at each temperature. This value also increased with increasing incubation temperature. The average values of slopes of the semilog plots for OCR and R^{*} versus soil depth are -0.047 and -0.053, respectively. The similarity in the slope values indicate that the change in biological activity with soil depth can be measured from the change in OCR or from the change in CO_2 production rate. Thus, it was concluded that microbial activity was responsible for biological O_2 demand and CO_2 production, and that microbial activity decreased exponentially with depth.

The molar ratios of CO_2 production rate to OCR for various incubation temperatures and moisture contents were in the range 1.3-1.6 (Table 4).

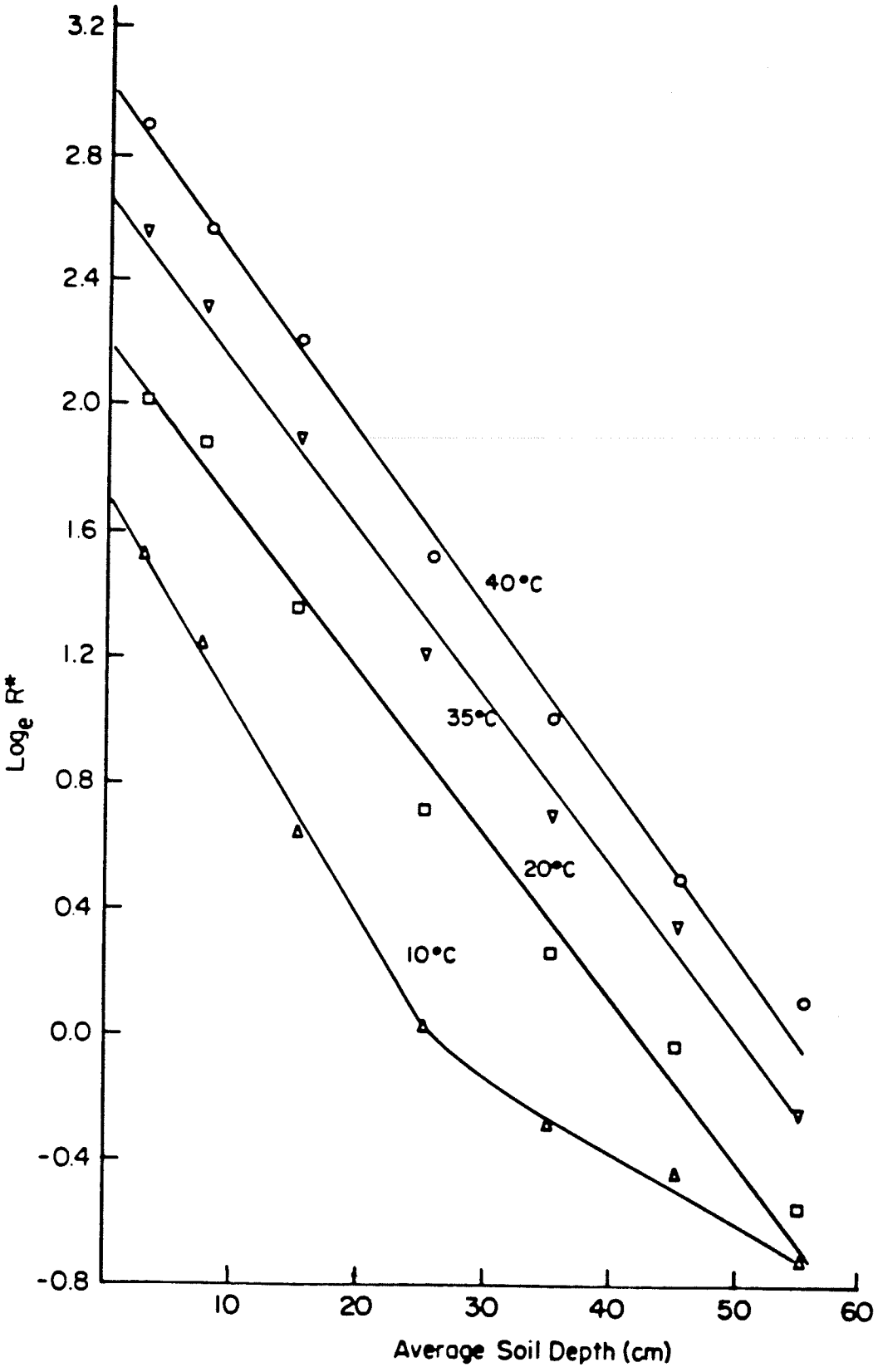

Figure 9: Semilog plot of CO_2 production rate (R^*) as function of soil depth, for various temperatures at 25% moisture.

TABLE 4

The ratio of CO_2 production rate (R^*) to O_2 consumption rate (OCR) at various incubation temperatures and moisture contents

Incubation temperature (°C)	Moisture content (%)	$\frac{R^*}{OCR}$	Variance
20	25	1.64	0.06
35	25	1.42	0.02
40	25	1.32	0.03
35	5	1.31	0.11
35	10	1.42	0.09
35	15	1.39	0.03
35	20	1.41	0.05

Each value of R^*/OCR was an average of eight profile samples. It is not known why the molar ratios were greater than the theoretical ratio of one for aerobic oxidation of soil organic carbon. Stanford et al. (1975) found that the molar ratio of glucose-C consumed to NO_3^--N reduced was 12 times the theoretical ratio of 1.5 for complete oxidation of glucose to CO_2 utilizing the nitrate as a final e^- acceptor.

The OCR values of the profile samples were plotted against temperature (Fig. 10). If it is assumed that a linear relationship existed between OCR and temperature at incubation temperatures below 20°C as was observed experimentally by Cho et al. (1979), then the threshold temperature (extrapolated value to zero OCR) can be calculated. Thus, the threshold temperature for O_2 demand was 3°C. The threshold temperature for NO_3^- reduction was reported as 2°C (Bremner and Shaw, 1958), 2.8°C (Cho et al., 1979), and 5°C (Bailey and Beauchamp, 1973a). However, the relationship between OCR and temperature at higher temperatures (>20°C) indicates that the OCR increased exponentially with increasing temperature. The natural log of OCR against the reciprocal of the absolute temperature was plotted (Fig. 11). The Arrhenius plot is a linear relationship in the temperature range of 20 to 40°C. Similarly, a number of workers (Bremner and Shaw, 1958b; Stanford et al., 1975; Misra et al., 1974; Focht, 1974; Khdyer, 1978) found that the rates of biological processes which are measured by either N_2 production or NO_3^- reduction over the temperature range of 15 to 30°C obeyed the Arrhenius equation.

The activation energies (E_A) which were calculated from the slopes of the Arrhenius plots for OCR (Fig. 11) are shown in Table 5. The average value of E_A for all profile samples was 8264 cal/mole with a standard deviation of 741 cal/mole.

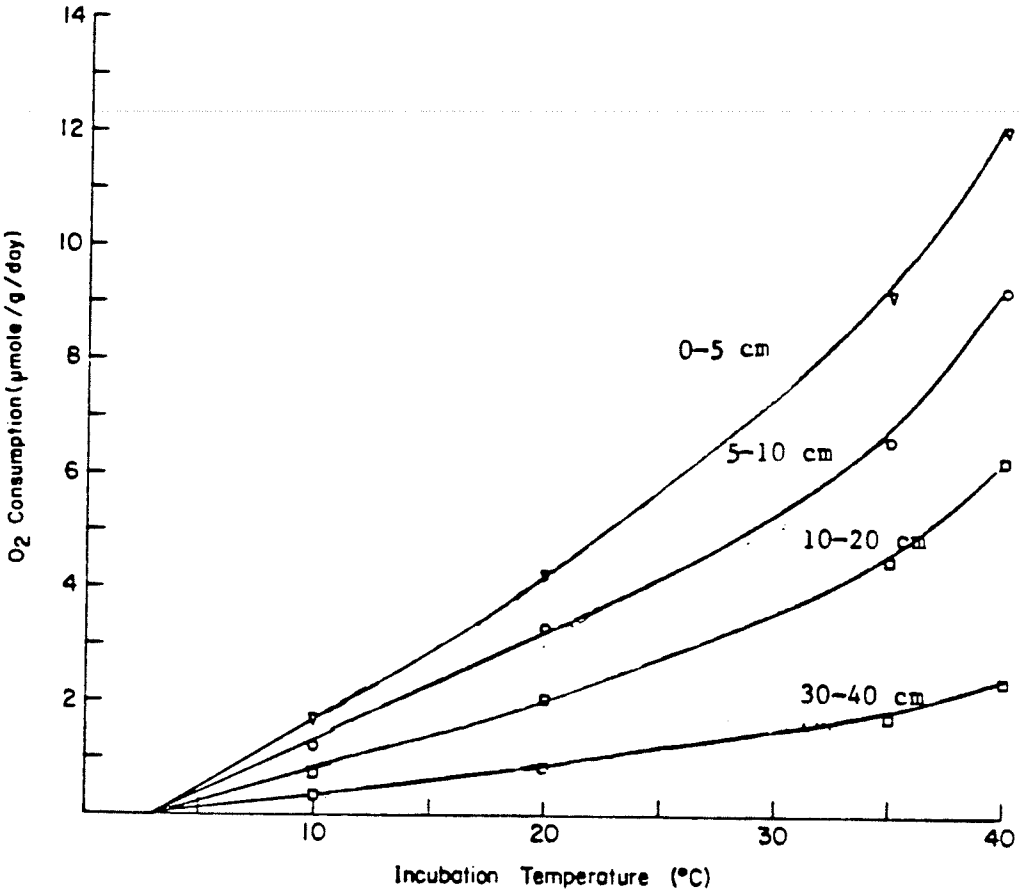

Figure 10: O_2 consumption rate by various profile samples as related to temperature at 25% moisture.

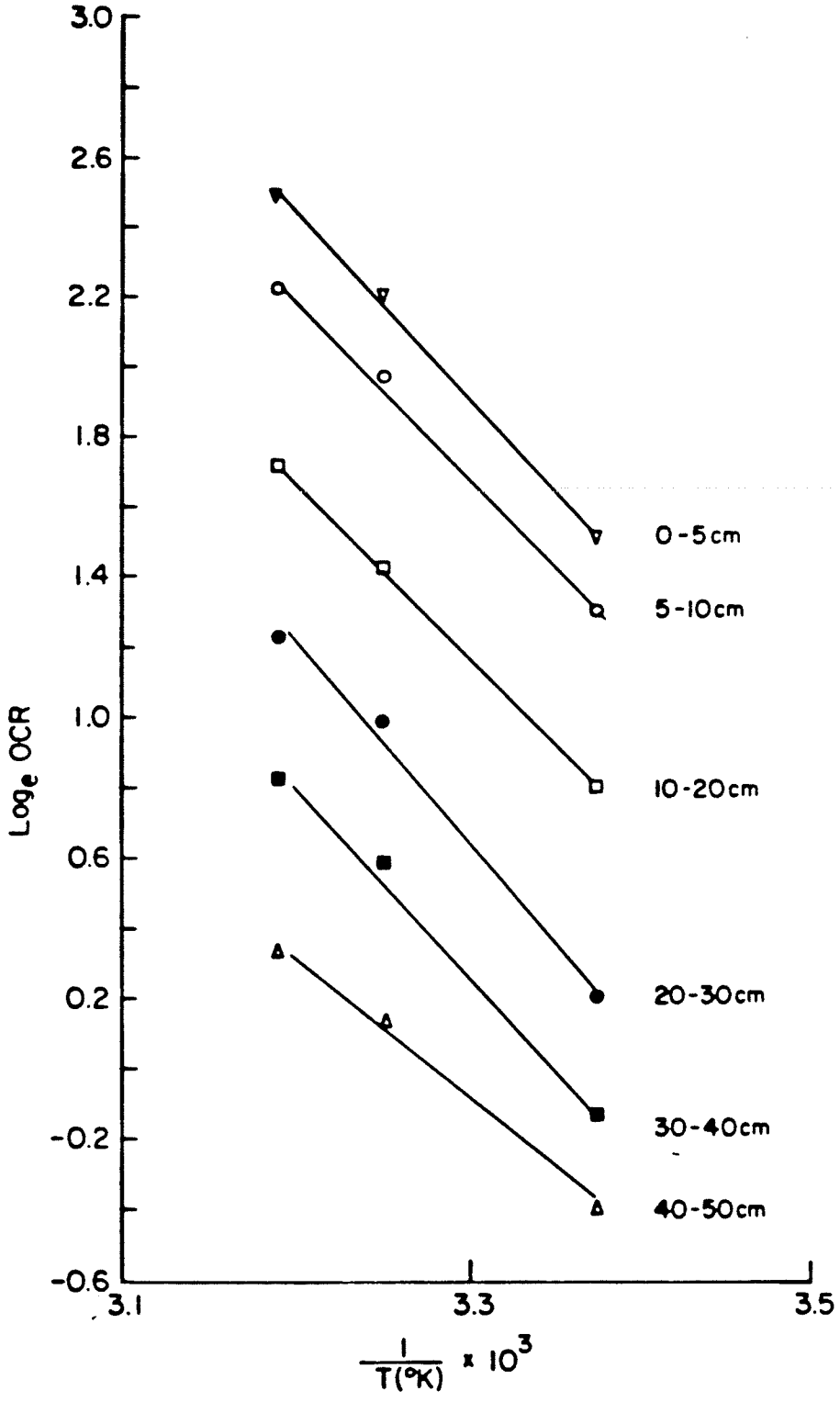

Figure 11: Arrhenius plot of O_2 consumption rate (OCR) , for various soil profile samples.

TABLE 5

The activation energy of O_2 consumption rate and coefficient of determination of Arrhenius plot for Wellwood profile samples.

Average depth (cm)	Activation energy (E_A) (Cal./mole)	Coefficient of determination (r^2)
2.5	8543	0.99
7.5	8632	0.99
15.0	8322	0.99
25.0	8479	0.99
35.0	8862	0.99
45.0	6633	0.99
55.0	8277	0.97
Average	8264	

Similar results were obtained for the Arrhenius plots of the CO_2 production rate in the same temperature range (Fig. 12). Average activation energy for the eight profile samples was 6877 cal/mole with a standard deviation of 373 cal/mole (Table 6). It was not known why there was a difference in E_A values for O_2 consumption and CO_2 production rates. However, several other workers (Focht, 1974; Khdyer, 1978; Stanford et al., 1975; Misra et al., 1974) also found some variations in E_A values for biological processes. For example, Focht (1974) and Khdyer (1978) reported an activation energy of about 8000 cal/mole and 8570 cal/mole, respectively, for the dissimilatory N_2 production process in soil. However, activation energies (10376-11431 cal/mole) were obtained using NO_3^- disappearance rates (Stanford et al., 1975; Misra et al., 1974).

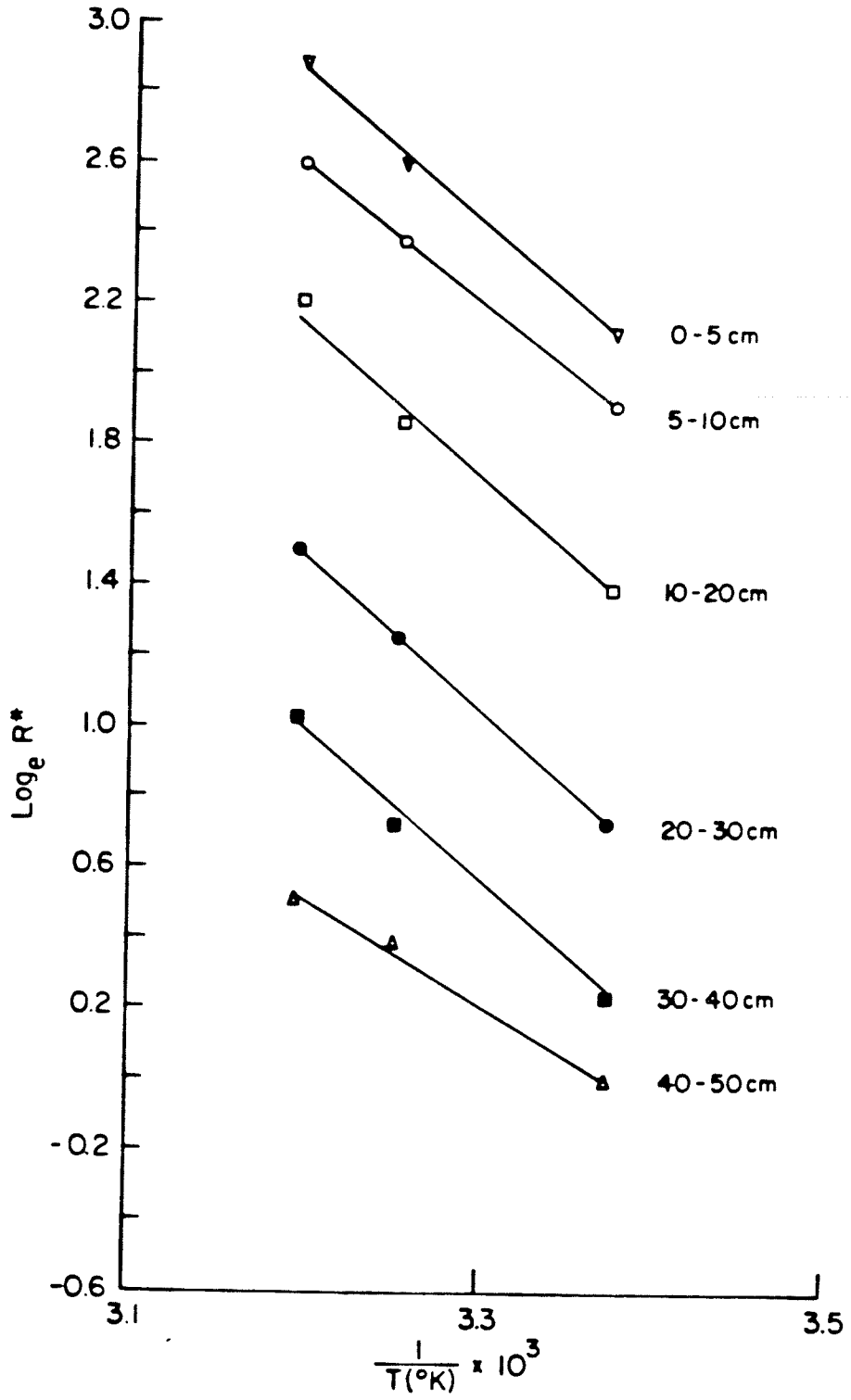

Figure 12: Arrhenius plot of CO_2 production rate (R^*), for various soil profile samples.

TABLE 6

The activation energy as calculated from CO_2 production rate for Wellwood soil profile samples.

Average depth (cm)	Activation energy (E_A) (cal./mole)	Coefficient of determination (r^2)
2.5	6710	0.98
7.5	6173	0.98
15.0	7210	0.96
25.0	7018	0.98
35.0	6879	0.96
45.0	7303	0.98
55.0	6845	0.98
Average	6877	

4.2 COLUMN STUDY (EXPERIMENT 2): EFFECT OF AIR POROSITY ON GASEOUS DIFFUSION AND DENITRIFICATION OF NITRATE DERIVED FROM UREA

4.2.1 Oxygen Transport At Various Air Porosities

The influence of incubation time upon soil moisture content at varying depths in Wellwood soil for initial moisture levels of 23, 25 and 30% and bulk densities of 1.2, 1.32 and 1.32 g/cm^3, is shown in Tables 20, 21 and 22, respectively, in the appendix. The moisture content decreased slightly with time in the surface layer (not more than 5%) most likely as a result of evaporation. The average moisture levels over the entire incubation period for all depths were taken to be 22.5, 24.0 and 28.3% for the three moisture-bulk density treatments. These average values were used to calculate the average volumetric water content and air porosity values. The respective average air porosity values were 25.6, 16.2 and 10.5%. A fourth treatment (8.5% air porosity) was obtained by increasing the bulk density to 1.35 g/cm^3 from 1.32 g/cm^3 (10.5% air porosity) without changing the moisture content.

Oxygen concentrations at varying soil depths during 8 weeks of incubation at air porosities of 16.2, 10.5 and 8.5% are shown in Tables 23, 24 and 25, respectively (see the Appendix). Although there were small increases in O_2 concentration with time, especially near the surface, O_2 concentrations after one week were not much different from the average values obtained after two months. Thus, a steady state with respect to O_2 transport through the soil was established after incubation for one week. The slight increase in O_2 concentration between one and eight weeks might have been related to an increase in O_2 diffusion coefficient due to loss of a small amount of water from the surface layer. In addi-

tion, there might have been some decrease in the biological activity with time of incubation. Profiles of average O_2 concentrations over two months at varying depths in Wellwood soil for air porosities of 16.2, 10.5 and 8.5% appear as dotted lines in Fig. 13 whereas profiles of calculated O_2 concentrations appear as solid lines. Oxygen concentrations making up the profile for 25.6% air porosity were averaged for a period of 2 weeks (Fig. 13). The 25.6% air porosity treatment was terminated after 2 weeks since the O_2 concentration did not reach zero at any depth (no denitrification). The minimum O_2 concentration of 6.5 μmole/ml of soil air space occurred in the 30-40 cm layer and O_2 concentrations were very similar at depths greater than 40 cm. In contrast to the 25.6% air porosity treatment, O_2 concentrations approached zero (<0.2 μmole/ml) at 55, 25 and 17.5 cm in depth for air porosities of 16.2, 10.5 and 8.5%, respectively.

The steady state O_2 transport equation in one-dimensional form under isothermal conditions was solved in order to predict the O_2 concentration profile under the steady state condition. The steady state equation is

$$D\, d^2C/dx^2 = OCR(x)\ \ P \tag{53}$$

where, D is the O_2 diffusion coefficient (cm^2/day), C is the O_2 concentration (μmole/ml), P is the soil bulk density (g/cm^3) and OCR(x) is O_2 consumption rate as function of soil depth (x) in μmole/g/day. The experimentally obtained relationship between OCR and average soil depth (x) can be expressed as follows (see Fig. 8);

$$OCR = A\ EXP\ (-bx) \tag{54}$$

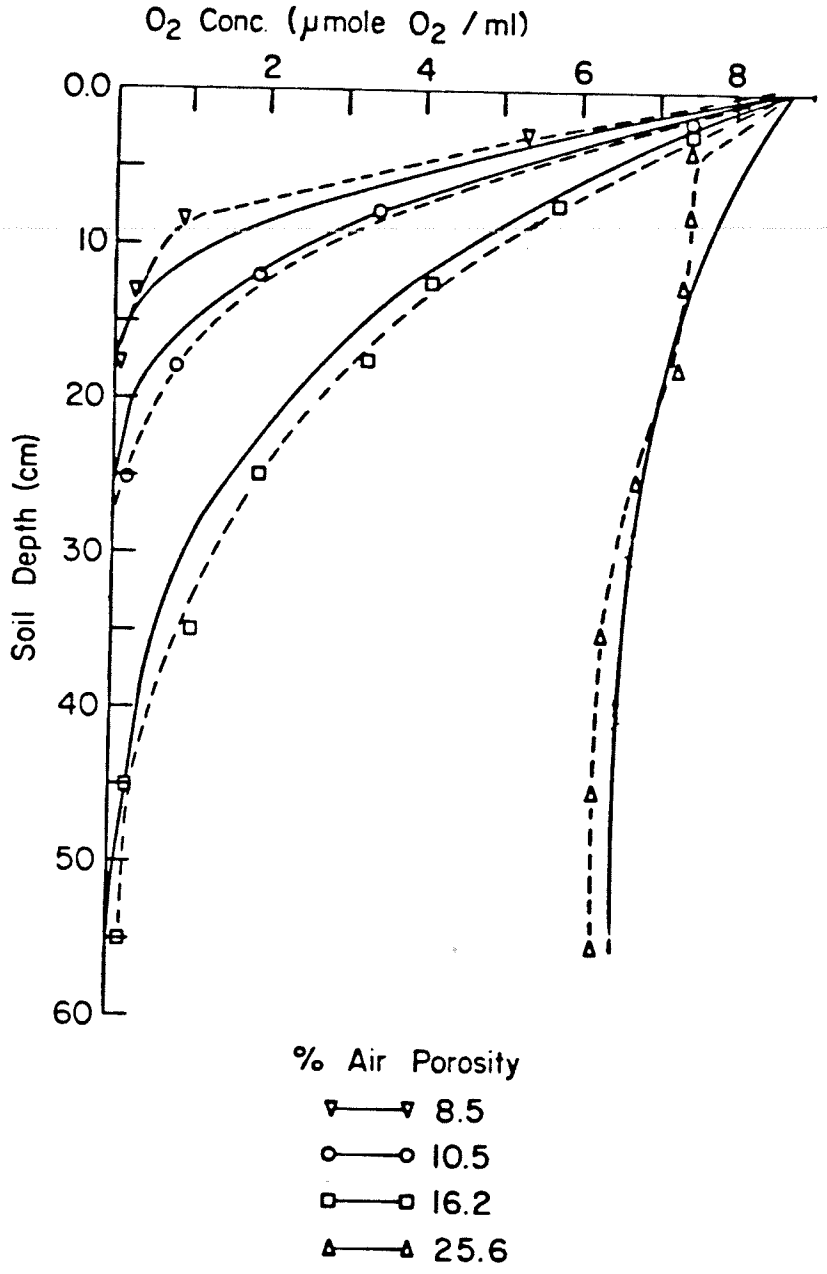

Figure 13: Measured (dotted line) and calculated (solid line) steady state O_2 concentration profiles of Wellwood soil at 20°C, for various air porosities after two months of incubation.

where A is the maximum OCR value extrapolated to zero average depth and b (1/cm) is the slope of the semilog plot of OCR as function of soil depth. The value of b was independent of soil temperature over a range of 20-40°C (see Fig. 8). If Eq. (54) is substituted into Eq. (53) and A times P assumed to equal A^* or the maximum OCR value in μmole/cm^3/day, the following equation is obtained;

$$D\, d^2C/dx^2 = A^* \text{ EXP } (-bx). \tag{55}$$

It is required to calculate C(x) in a soil column of infinite length with uniform moisture content under isothermal conditions under the following boundary conditions;

$$\begin{aligned} &C = C_o, \; x = 0 \\ &C = 0, \; x = x^* \\ &\text{and } dc/dx = 0 \quad x = x^*. \end{aligned} \tag{56}$$

The boundary conditions assume that the O_2 concentration at the surface is always kept at a constant value, C_o or the ambient O_2 concentration, 8.75 μmole/ml; that there is a depth, x^*, at which the O_2 concentration in the soil atmosphere is equal to zero; and that there is no O_2 flux and no concentration gradient of O_2 at the depth $x = x^*$. By solving the ordinary differential equation (55) under the boundary conditions (Eq. (56)), the following relationships are obtained;

$$D = \{A^*/bC_o\} \{b^{-1} - e^{-bx^*}(x^* + b^{-1})\} \tag{57}$$

and

$$C = C_o - (A^*/Db^2) + A^*/Db\{x\, e^{-bx^*} + b^{-1}\, e^{-bx}\}. \tag{58}$$

Thus, by measuring x^*, i.e. the depth of zero O_2 concentration under certain conditions, the diffusion coefficient can be estimated from Eq. (57). The steady state O_2 concentration at any depth can then be calculated by using the measured values of A^* and b and the estimated value of the diffusion coefficient. Values of x^* were used to calculate the diffusion coefficients using Eq. (57) for 8.5, 10.5 and 16.2% air porosity treatments (Table 7). The diffusion coefficient at an air porosity of 25.6% was calculated by using Eq. (58) assuming that the concentration of O_2 at 35 cm was 6.5 μmole/ml (Fig 13). Thus, the determination of diffusion coefficient was carried out by taking into account soil respiration activity under boundary conditions (Eq. (56)) that are frequently encountered in the field. However, several workers (Papendick and Runkles, 1965; Grable and Siemer, 1968) determined the O_2 diffusion coefficient from concentration measurements using the non steady-state O_2 transport equation (18). In their studies, they had to adjust the initial conditions experimentally by removing all of the gaseous O_2 from the system. Also, their work was carried out in short term experiments in which respiration activity was assumed to be negligible. This assumption may have led to erroneous results especially for soils having high biological activities.

The estimated diffusion coefficient values were used to predict O_2 concentration profiles which appear as solid lines in Fig. 13. There was good agreement between measured and calculated values except for the high air porosity (25.6%) treatment near the surface (0-10 cm).

The measured steady state O_2 concentration profiles for an air porosity of 10.5% (moisture content of 30% with bulk density equal to 1.32

TABLE 7

The depth of zero O_2 concentration and estimated diffusion coefficient as related to air porosity.

Air porosity (%)	Depth of zero O_2 concentration (cm)	Estimated diffusion coefficient (cm^2/day)
8.5	17.5	62.92
10.5	25.0	95.96
16.2	55.0	211.07
25.6	-	1380.20

g/cm^3) at temperatures of 20 and 35°C appear in Fig. 14. Values of x^* were 25 and 17.5 cm for the soil columns incubated at 20 and 35°C, respectively.

The effect of temperature on the diffusion coefficient of O_2 in the air can be represented by the equation (Bakker and Hidding, 1970)

$$D_1 = D_2\{ T_1/T_2 \}^{1.75}$$

where T equals the absolute temperature (K°) and D_1 is the diffusion coefficient at T_1. If it is assumed that the ratio of the diffusion coefficient in soil to that in air is constant at a particular air porosity and temperature, the above relationship can be extended to calculate the O_2 diffusion coefficient in the soil at any temperature (Bakker and Hidding, 1970).

The depth of zero O_2 concentration can be estimated using Eq. (57) from the values of diffusion coefficient, b and A^* at any temperature. The calculated values of x^* are shown in Table 8. With this information and using Eq. (58) the O_2 concentrations used in constructing the profiles which appear as solid lines in Fig. 14 were calculated. The calculated steady state O_2 concentration profiles suggest that the variation in x^* was more pronounced at low than at high temperatures. Increasing the temperature from 20 to 35°C, for example, caused a 70% decrease in the value of x^* whereas increasing the temperature from 35 to 40°C caused a 12% dcrease in the value of x^*.

The open system incubation studies were performed at 20°C, a temperature which was higher than the mean summer soil temperature of 12°C at 50 cm depth in the southern half of Manitoba (Mills et al., 1977). High values of x^* would be expected at low incubation temperatures. Thus,

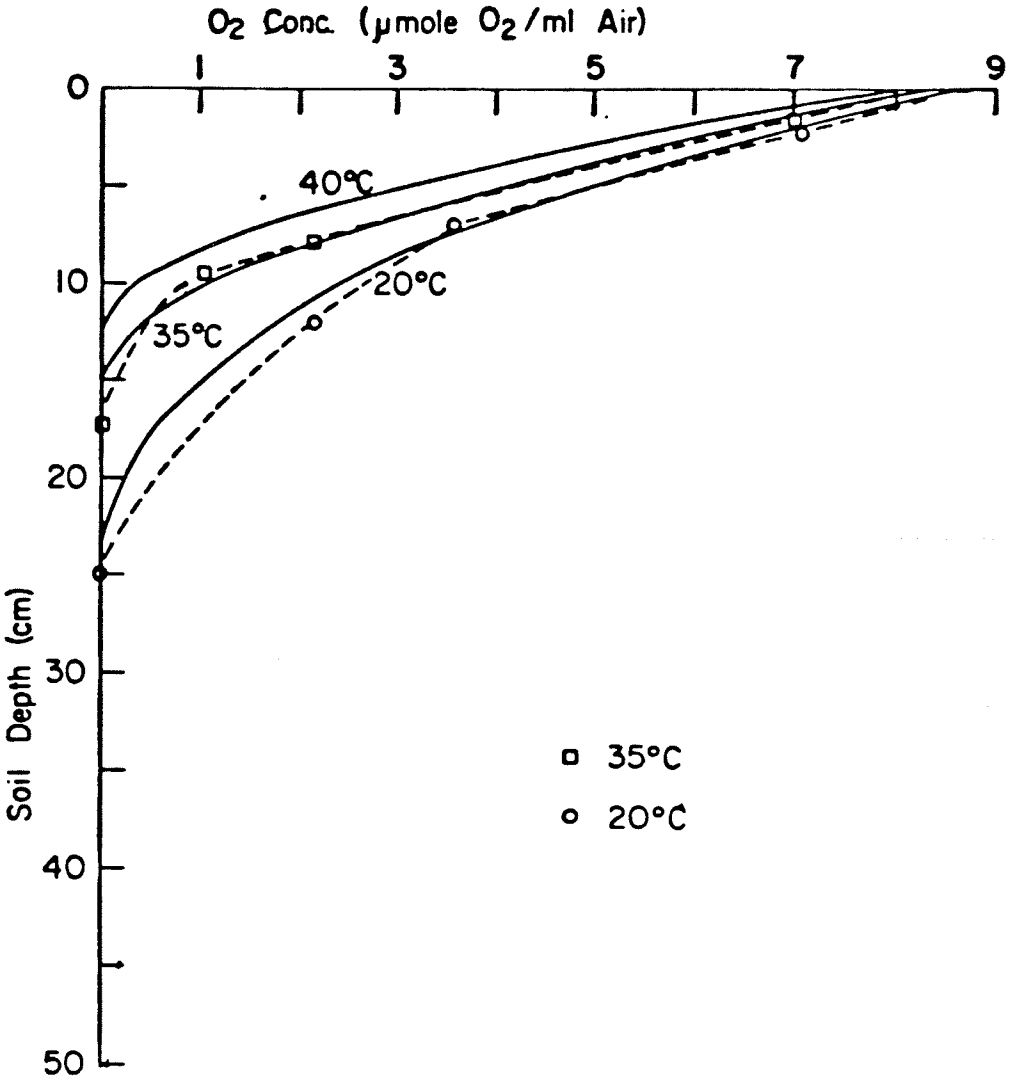

Figure 14: Measured (dotted line) and calculated (solid line) steady state O_2 concentration profiles of soil column (10.5% air porosity) incubated at various temperatures.

TABLE 8

The estimated diffusion coefficient, volumetric O_2 consumption rate and depth of zero O_2 concentration at air porosity of 10.5% as related to soil temperature.

Soil temperature	Diff. coeff. (D)	O_2 consumption rate (A)	Depth of zero O_2 concentration (x^*)
(^oC)	(cm^2/day)	(μmole/cm^3/day)	(cm)
20	95.96	6.46	25.0
35	104.71	12.89	14.7
40	107.73	16.38	13.1

using a low incubation temperature to represent field conditions would require a very long column, which would be difficult to construct and maintain at constant temperature. For this reason an incubation temperature of 20°C was used.

One can define the steady state flux of O_2 through the soil surface q_o (μmole/cm^2/day) as

$$q_o = -D\{dC/dx\}_{x=0}. \qquad (59)$$

By integrating Eq. (55) between $\{dC/dx\}_{x = 0}$ and $\{dC/dx\}_{x = x^*}$, and putting $\{dC/dx\}_{x = x^*}$ = zero, q_o can be expressed as

$$q_o = A^*/b \{ 1 - e^{-x^* b}\}. \qquad (60)$$

Values of q_o were calculated from Eq. (60) using values of b and A^* measured at various soil temperatures and moisture contents and values of x^* estimated from Eq. (57) by Newton's approximation method (Table 9). The maximum flux occurred at higher temperatures because of a higher biological O_2 demand and at higher air porosities which allowed a higher O_2 penetration (Table 9). The maximum flux of 299 μmole/cm^2/day occurred at 40°C and 25.6% air porosity. Measured rates of O_2 consumption reported in the literature range from 2 to 12 μliter O_2/g/h at 25 to 30°C, with the higher rates occurring soon after initial wetting (Miller and Johnson, 1964; Papendick and Runkles, 1966). Assuming a uniform soil 30 cm deep and an average bulk density of 1.32 g/cm^3, the O_2 consumption rates of 2 to 12 μliter/g/h correspond to q_o values of 80 to 480 μmole/cm^2/day. Respiration rates reported by other investigators are usually lower than those given above or those shown in Table 9

TABLE 9

The steady state flux of O_2 at the soil surface at various incubation temperatures and air porosities

Air porosity	Oxygen flux (q_o)		
	20°C	35°C	40°C
(%)	-------(μmole/cm^2/day)-----		
8.5	65.4	113.5	131.1
10.5	90.2	132.4	155.3
16.2	116.6	188.11	221.1
25.6	118.2	235.7	299.6

primarily because measurements were made after several wetting and drying cycles. Anderson and Kemper (1964) found that about 0.3, 0.7 and 1.0 µliter O_2/g/h were consumed after incubation of soil for several weeks at temperatures of 17, 23 and 30°C, respectively. The respective values of O_2 flux were 12, 28 and 40 µmole/cm^2/day.

4.2.2 Inorganic N Distribution.

Concentrations of NH_4^+-N at varying depths in columns of Wellwood soil (25.6% air porosity) incubated with urea at a rate of 200 µg N/g soil are shown in Table 10. Hydrolysis of urea was evident during the first week of incubation as indicated by the recovery of N as NH_4^+. In a study with relatively high urea concentration (500 µg N/g soil), Obi (1981) found that complete hydrolysis of urea occurred within 6 days in eight Manitoba soils with pH values ranging from 5.8 to 7.7. The maximum accumulation of NH_4^+ (179 µg N/g soil) occurred after 7 days of incubation in the 50-60 cm layer where nitrification was very slow. The amount of NH_4^+-N decreased with time especially in the 0-5 cm layer where all of the NH_4^+ disappeared after 20 days of incubation. After 42 days appreciable decrease in NH_4^+-N occurred even in the 40-60 cm layer.

It is evident that nitrification was more rapid in the surface layer as indicated by the high accumulation of NO_3^- (Table 11). In the 0-5 cm layer the amount of NO_3^- reached 278 µg N/g soil after 20 days of incubation. There was a further small increase in NO_3^--N between 20 and 42 days. The maximum value exceeded that which would be expected from complete nitrification of added urea (200 µg N/g soil) plus the 25 µg NO_3^--N/g soil originally present. Some of the NO_3^- at the surface may have come from mineralization of soil nitrogen. Cassman and Munns

TABLE 10

Concentration of NH_4^+ during incubation of soil columns (25.6% air porosity) with 200 µg urea-N/g soil, at 20°C.

Soil depth	7 days	20 days	42 days
(cm)	(µg NH_4^+-N/g soil)		
0-5	121.6	0.1	5.5
5-10	125.8	18.3	4.0
10-15	163.8	6.5	8.3
15-20	162.8	36.0	30.6
20-30	159.5	78.0	55.6
30-40	162.1	90.3	82.9
40-50	179.6	123.4	135.7
50-60	179.7	158.1	130.3

TABLE 11

Concentrations of NO_3^- and NO_2^- during incubation of soil columns (25.6% air porosity) with 200 µg urea-N/g soil, at 20°C.

Soil depth	7 days		20 days		42 days	
	NO_3^--N	NO_2^--N	NO_3^--N	NO_2^--N	NO_3^--N	NO_2^--N
(cm)	(µg/g soil)					
0-5	88.6	0.8	278.3	0.5	288.7	0.1
5-10	89.7	1.4	179.3	0.5	241.3	0.1
10-15	36.3	2.0	92.7	0.5	221.3	0.1
15-20	25.1	6.2	53.6	16.5	133.1	0.1
20-30	9.4	3.0	67.2	3.4	128.8	0.1
30-40	4.7	1.0	59.7	2.3	155.8	0.1
40-50	3.7	0.8	26.7	2.2	126.7	0.1
50-60	3.7	0.8	9.1	2.4	113.0	0.1

(1980) found that the estimated relative contribution of the surface layer to the total N mineralized in a 108-cm soil column was higher than the contribution from subsurface layer. Their data indicate that the total N mineralized in the 108-cm soil column were 60, 25 and 15% for 0-36, 36-72 and 72-108 cm layers, respectively, assuming uniform bulk density throughout the soil profile. Some of the NO_3^- in the 0-5 cm layer could have also come from lower layers via convective flow as water evaporated from the soil surface. Such movement of NO_3^- was likely very small since only a small amount of water evaporated from the surface (Table 20).

The maximum accumulation of NO_2^- which occurred in the 15-20 cm layer was 6.2 and 16.5 µg N/g soil after 7 and 20 days of incubation, respectively (Table 11). Such a low accumulation probably did not allow loss of N by chemical denitrification which takes place only when an appreciable amount of NO_2^- has accumulated under acidic conditions (Christianson et al., 1979; Smith and Chalk, 1980).

Ammonium concentrations at varying depths in Wellwood soil columns having 16.2% air porosity incubated with 200 µg urea-N/g soil at 20°C are shown in Table 12. Apparently most of the urea was hydrolyzed after 10 days of incubation. The amount of NH_4^+ increased with soil depth from a low value of 14 µg N/g soil in the 0-5 cm layer to 178 µg N/g soil in the 50-60 cm layer after 10 days of incubation. The amount of NH_4^+-N decreased with time in all layers but the decrease was less pronounced in the 40-50 cm and 50-60 cm layers than near the surface. The persistence of NH_4^+ below 40 cm depth could have been related to a low O_2 concentration (Fig. 13) as well as low nitrifier population.

TABLE 12

Concentration of NH_4^+ during incubation of soil columns (16.2% air porosity) with 200 µg urea-N/g soil, at 20°C.

Soil depth	10 days	20 days	30 days	52 days
(cm)	----------(µg NH_4^+-N/g soil)----------			
0-5	14.0	10.1	14.0	7.0
5-10	118.9	19.5	16.7	3.6
10-15	169.5	81.6	64.2	35.6
15-20	175.7	82.3	98.5	42.7
20-30	195.5	147.1	129.0	128.0
30-40	172.9	166.1	170.3	147.2
40-50	187.1	145.4	174.8	145.7
50-60	178.5	178.1	177.5	156.4

Nitrification was more rapid in the 0-5 cm layer as indicated by an accumulation of NO_3^- which reached 340 µg N/g soil after 20 days of incubation and remained essentially unchanged until the end of incubation (Table 13). Nitrate accumulation in the 0-5 cm layer was higher with the 16.2% air porosity (25% moisture content) than with the 25.6% air porosity (23% moisture content). This could have been related to a higher net mineralization with 16.2% air porosity. Several authors (Robinson, 1957; Reichman et al., 1966; Miller and Johnson, 1964; Stanford and Epstein, 1974; Cassman and Munns, 1980) reported that the mineralization was greatest at approximately field capacity (25% moisture content). Furthermore, Cassman and Munns (1980) found a sharp decrease in the net N mineralization between 25 and 19% moisture and then a gradual decline below this range. Low NO_3^- accumulation (<13 µg N/g soil) was observed below 40 cm (Table 13). This could have been due to a low biological activity and O_2 availability below 40 cm depth.

There was no accumulation of NO_2^- in the first 10 days of incubation (Table 13). However, NO_2^- appeared by the 20th day with maximum values in the 15-20 cm layer. Nitrite level declined between 20 and 52 days of incubation except in the 0-5 cm and 5-10 cm layers. No chemical denitrification was detected by mass spectrometry even though NO_2^- accumulation reached 83 µg N/g soil. The pH in the 15-20 cm layer was 6.86 (Table 1). An investigation using ^{15}N labelled $NaNO_2$ at a rate of 200 µg N/g soil demonstrated no tendency for chemical denitrification for some Manitoba soils which had pH values greater than 6.3 (Obi, 1981).

Ammonium became the predominant inorganic N species when air porosity was decreased to 10.5% (Table 14). The concentrations of NH_4^+ in the soil column ranged from 186 to 235 µg N/g soil after 10 days incubation.

TABLE 13

Concentrations of NO_3^- and NO_2^- during the incubation of soil columns (16.2% air porosity) with 200 µg urea-N/g soil, at 20°C.

Soil depth	10 days		20 days		30 days		52 days	
	NO_3^--N	NO_2^--N	NO_3^--N	NO_2^--N	NO_3^--N	NO_2^--N	NO_3^--N	NO_2^--N
(cm)	(µg/g soil)							
0-5	255.3	-*	339.6	0.3	344.0	-*	346.6	5.9
5-10	75.1	-	248.3	4.7	195.4	-	233.3	10.7
10-15	67.2	-	63.3	65.0	78.6	26.4	153.7	14.1
15-20	40.8	-	29.7	83.0	79.0	77.5	112.1	25.1
20-30	30.8	-	16.7	61.8	62.0	13.3	84.6	0.2
30-40	28.5	-	10.4	1.8	8.4	-	32.4	0.1
40-50	13.5	-	9.7	-	3.7	-	9.3	0.1
50-60	13.4	-	7.0	-	2.4	-	1.7	0.1

* Not detected.

TABLE 14

Concentration of NH_4^+ during incubation of soil columns (10.5% air porosity) with 200 µg urea-N/g soil, at 20°C.

Soil depth	10 days	20 days	30 days	40 days
(cm)	----------(µg NH_4^+-N/g soil)----------			
0-5	186.3	98.9	51.5	20.8
5-10	235.0	122.8	165.6	98.6
10-15	223.1	196.4	227.7	215.4
15-20	195.5	199.6	220.8	210.6
20-30	186.3	203.3	230.5	199.1

The NH_4^+-N level remained more or less unchanged during 40 days of incubation below 10 cm depth. This fact as well as the low NO_3^- and NO_2^- concentrations in the 15-30 cm layer (Table 15) indicated the reduced nature of soil at these layers. For the 0-5 cm layer the NH_4^+ was high (186 µg N/g soil) for the first 10 days of incubation and then decreased to 20.8 µg N/g soil after 40 days of incubation.

Nitrate concentrations were 69 and 15 µg N/g soil after 10 days incubation in the 0-5 and 5-10 cm layers, respectively when the air porosity was 10.5% (Table 15). Nitrate continued to accumulate after 10 days incubation and reached maximum values of 248 and 184 µg N/g soil after 40 days of incubation in these two layers, respectively. However, the amount of NO_3^- accumulation at greater depths was very small even after 40 days incubation. This could have resulted primarily from O_2 deficiency. Oxygen concentration was less than 1.3 µmole/ml below 15 cm depth in soil having 10.5% air porosity (Fig. 13). The extent of NO_3^- accumulation in the 0-5 cm layer was smaller in soil having an air porosity of 10.5% (30% moisture content) than in soil having an air porosity of 16.2% (25% moisture content) (Table 13 and 15). Net mineralization decreased as the moisture content increased from 25 to 33% at temperatures in the range of 15 to 30°C (Cassman and Munns, 1980).

The amount of NO_2^--N was very small throughout the soil column, except in the 0-5 and 5-10 cm layers after 20 days incubation and in the 5-10 cm layer after 30 days incubation (Table 15). The maximum accumulation of NO_2^- was also in the 15-20 cm layer at higher air porosities (16.2 and 25.6%). Thus, the depth of maximum nitrite accumulation was related to the air porosity and occurred close to the surface of the soil column as air porosity was decreased.

TABLE 15

Concentrations of NO_3^- and NO_2^- during incubation of soil columns (10.5% air porosity) with 200 µg urea-N/g soil, at 20°C.

Soil depth	10 days		20 days		30 days		40 days	
	NO_3^--N	NO_2^--N	NO_3^--N	NO_2^--N	NO_3^--N	NO_2^--N	NO_3^--N	NO_2^--N
(cm)	(µg/g soil)							
0-5	69.4	0.9	165.5	19.0	219.0	1.9	248.1	0.5
5-10	15.2	0.9	55.7	23.6	50.8	34.0	184.5	0.2
10-15	2.0	0.1	9.4	3.2	9.4	4.6	71.5	0.3
15-20	3.0	0.3	4.1	5.0	1.9	2.3	4.6	0.2
20-30	3.3	0.3	2.6	0.2	1.7	2.1	2.5	0.2

4.2.3 Distribution of Gaseous Nitrogen

No denitrification was observed at an air porosity of 25.6% since enough O_2 penetrated the soil column to maintain a concentration of approximately 6.5 μmole O_2/ml in the 30–60 cm layer (Fig. 13). However, at an air porosity of 16.2% denitrification occurred as indicated by N_2 accumulation (Fig. 15). Although the data are not presented in Fig. 15, a small amount of N_2 was detected during the first 3 weeks of incubation. In the fourth week there was a relatively large increase in N_2 concentration with a maximum accumulation of 0.7 μg N/g soil in the 40–50 cm and 50–60 cm layers where O_2 concentration approached zero (Fig. 13). A low N_2 accumulation could have been related to a low accumulation of NO_3^- and NO_2^- below 40 cm depth (Table 13). The accumulated N_2 would have diffused in both directions due to the concentration gradient. Diffusion downward was prevented because of the impermeable layer. Upward diffusion was noticeable as the concentration of N_2 decreased exponentially with the distance from the denitrification zone (maximum N_2 accumulation). The maximum accumulation of N_2 increased from 1.2 to 2.0 μg N/g soil after 5 and 6 weeks incubation, respectively. It decreased slightly to 1.9 μg N/g soil after 7 weeks incubation.

Distribution of N_2O in soil at 10.5% air porosity is shown in Fig. 16. After 5 weeks of incubation, there was a small N_2O accumulation with a maximum of about 1.8 μg N/g soil in the 15–20 cm layer. The N_2O concentration increased to 2.3 μg N/g soil at 7 weeks and then decreased. After 7 weeks the maximum amount of the N_2O occurred in the 10–15 cm layer.

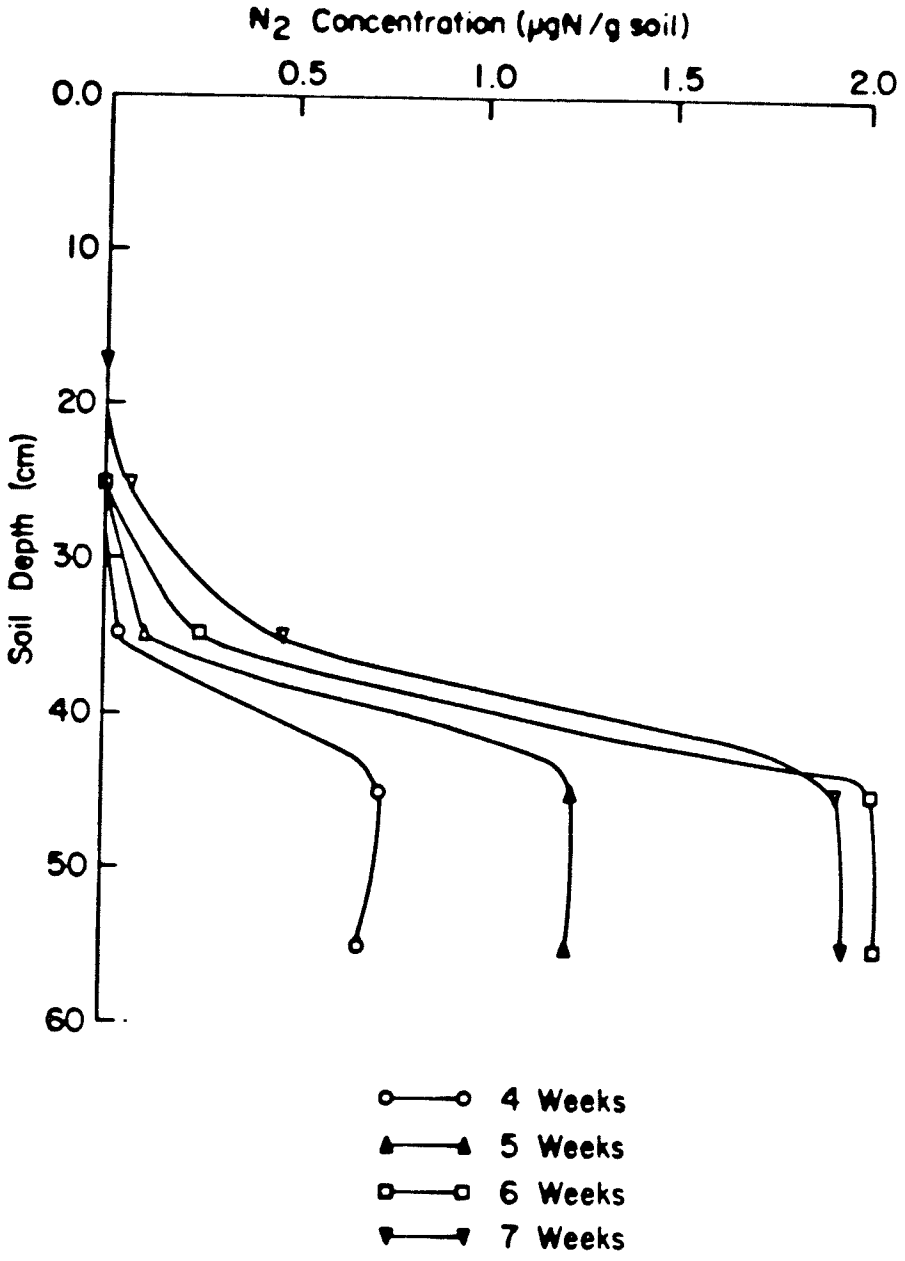

Figure 15: Concentration profiles of nitrogen gas during incubation of soil column (16.2% air porosity) with 200 μg urea-N/g soil, at 20°C.

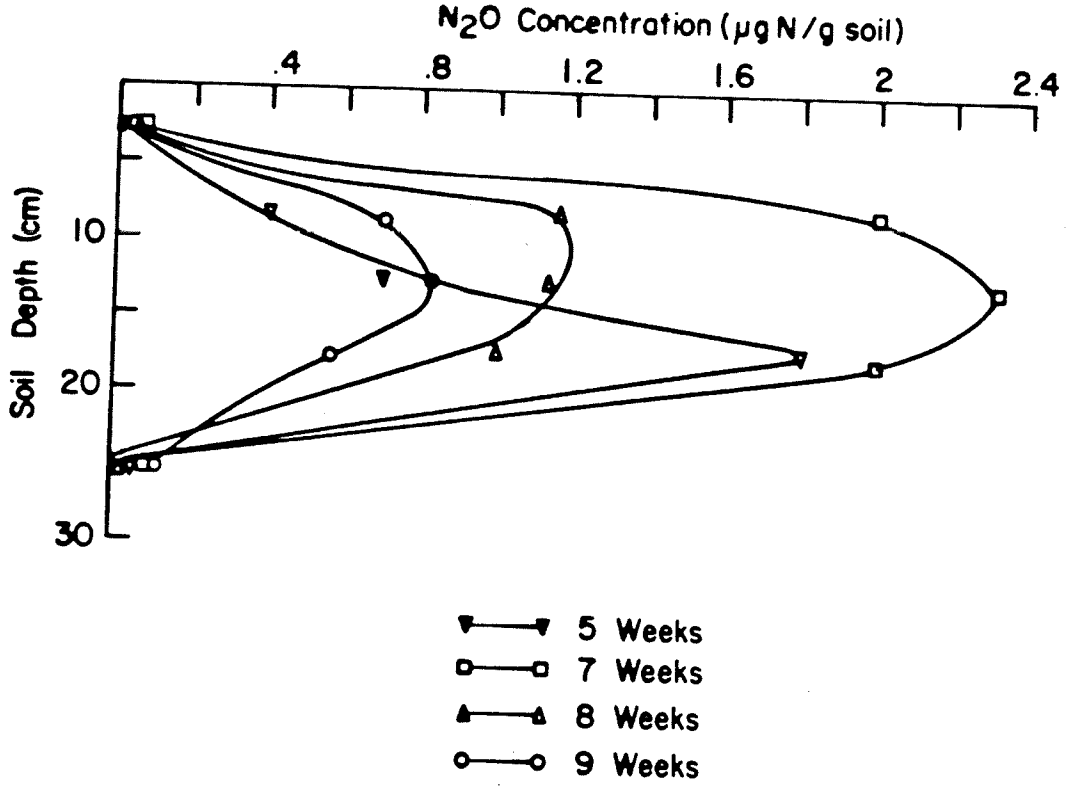

Figure 16: Concentration profiles of nitrous oxide during incubation of soil column (10.5% air porosity) with 200 µg urea-N/g soil, at 20°C.

The maximum accumulation of N_2 (0.2 µg N/g soil) in soil columns at 10.5% air porosity occurred in the 20-30 cm layer after 4 weeks incubation (Fig. 17). The maximum accumulation of N_2 increased further until the 8th week when it reached 1.7 µg N/g soil and then decreased. The rapid increase in N_2 concentration observed at the 7th week of incubation was coincidental with the accumulation of N_2O observed at the 5th and 7th weeks of incubation (Fig. 16). As was shown earlier, there was a sharp decrease in NO_3^- and NO_2^- with soil depth near and below the zone of maximum N_2O accumulation (Table 15). It is possible that N_2O became the major e^- acceptor at greater depth. This could suggest that some N_2O was diffused downward from its zone of production and reduced to N_2.

Increasing the bulk density to 1.35 g/cm^3 from 1.32 g/cm^3 without changing the moisture content caused a decrease in air porosity from 10.5 to 8.5%. It also caused a shift in the denitrification zone toward the surface. The depth of zero O_2 concentration at 8.5% air porosity was 17.5 cm as compared to 25 cm at 10.5% air porosity (Fig. 13). Nitrous oxide was not detected in the soil column at 8.5% air porosity even after 9 weeks of incubation. Nitrogen was the only gas produced via denitrification (Fig. 18). Maximum accumulation of N_2 after 4 weeks of incubation was 0.6 µg/g soil and occurred in the 10-20 cm layer. At this depth N_2 continued to accumulate and by the end of the 9th week had reached 1.2 µg N/g soil. By the 4th week of incubation, N_2 distribution was characterized by a decrease toward the surface and toward the impermeable layer at 60 cm (Fig 18). In the latter stage of incubation greater amounts of N_2 were found below 20 cm. In fact, after 9 weeks of

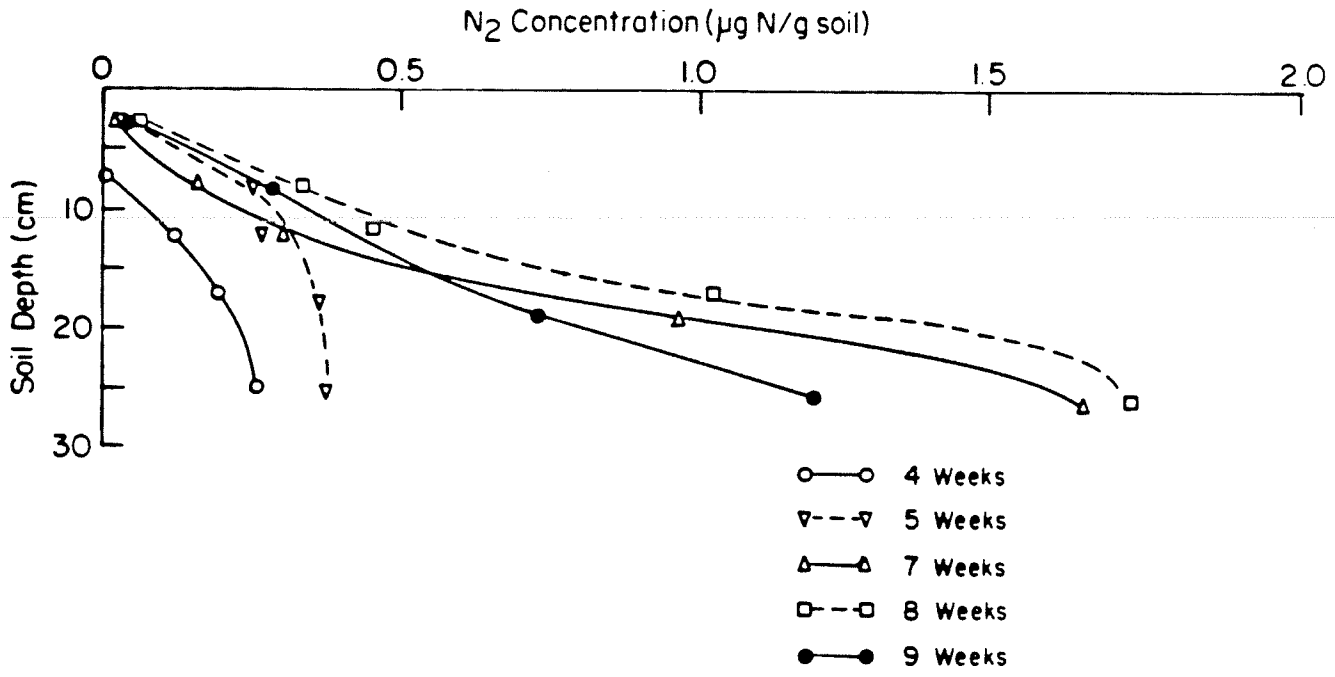

Figure 17: Concentration profiles of nitrogen gas during incubation of soil column (10.5% air porosity) with 200 µg urea-N/g soil, at 20°C.

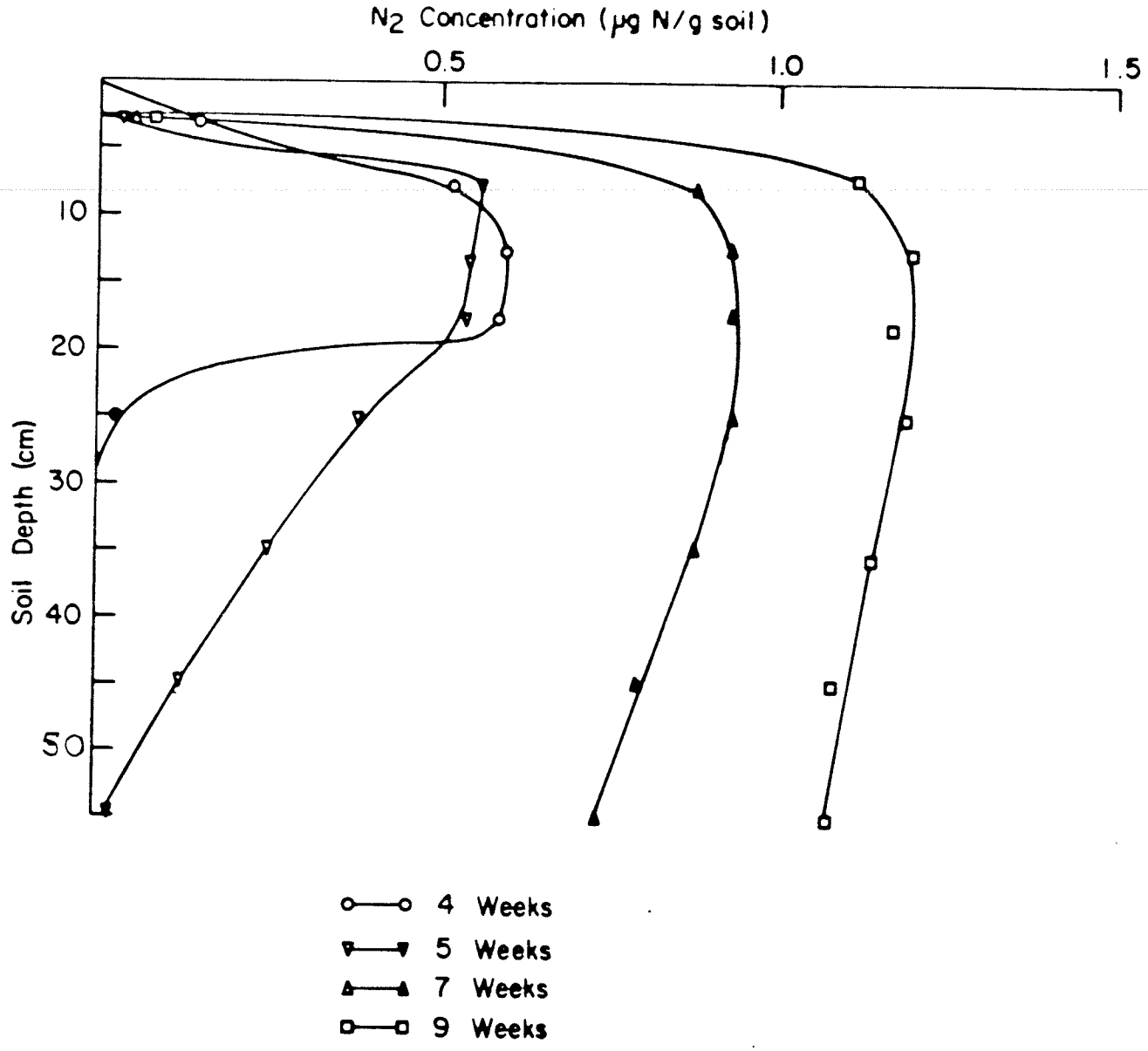

Figure 18: Concentration profiles of nitrogen gas during incubation of soil column (8.5% air porosity) with 200 µg urea-N/g soil, at 20°C.

incubation there was nearly as much N_2 in the 50-60 cm layer as in the 10-20 cm layer. Thus, the gradual accumulation of N_2 below the 20 cm depth could have been related to the contribution of N_2 via diffusion from the depth of maximum accumulation (denitrification zone). The amount of time required to accumulate N_2 below 20 cm was the greatest for the 8.5% air porosity treatment. This may have been related to the diffusion coefficient of gases in the soil. As was previously shown, the lowest gaseous diffusion coefficient was obtained in soil columns having an air porosity of 8.5% (Table 7).

The method suggested by Cho and Sakdinan (1978) was used to differentiate between nitrogen (N_2O and/or N_2) formed via a random combination of labelled ions (biological denitrification) and that formed via an association of one soil nitrogen atom and one NO_2^--N atom (chemical denitrification in which only N_2 is produced). MacGregor (1972) suggested that biological denitrification has occurred when the N-15 content in N_2O and N_2 is the same. In this study, the $\%^{15}N$ enrichment values of N_2O and N_2 at 10.5% air porosity were almost identical (Table 16). The average values over all depths were 37.3 and 36.2 for N_2O and N_2, respectively. Thus, it could be concluded that N_2 was derived solely from N_2O. The ^{15}N content of the N_2 was less than that of the urea-^{15}N (52.6%) originally added. This indicates that there was some isotopic dilution by nitrogen from other sources. Two possible sources of unlabelled N could have contributed to this isotopic dilution of ^{15}N. Firstly, the NO_3^- originally present in the soil (25.8 µg N/g soil near the surface decreasing to 0.5 µg N/g soil in the 50-60 cm layer) could have diluted $\%^{15}N$ of NO_3^- derived from urea (Table 16). Secondly, mineralization of organic-N could have diluted ^{15}N of urea derived NO_3^-.

TABLE 16

Initial concentration of NO_3^-, percentage of N-15 in N_2 and N_2O during incubation of soil columns (three levels of air porosity) with 200 µg urea-N/g soil, at 20°C.

Soil depth	NO_3^--N conc.*	8.5% Air porosity N-15 (N_2)	10.5% Air porosity N-15 (N_2O)	10.5% Air porosity N-15 (N_2)	16.2% Air porosity N-15 (N_2)
(cm)	(µg/g soil)	(%)			
0- 5	25.6	-	-	-	-
5-10	19.7	22.8	34.7	34.3	-
10-15	12.5	22.1	35.3	34.3	-
15-20	9.5	21.7	36.9	37.2	-
20-30	6.6	21.5	42.1	39.1	-
30-40	3.3	21.5	-	-	41.2
40-50	1.0	21.2	-	-	38.7
50-60	0.5	21.2	-	-	39.2
Average		21.7	37.3	36.2	39.7

* Original Concentration

In either case, maximum isotopic dilution of inorganic N derived from urea should have occurred near the surface. Therefore, one would expect that $\%^{15}N$ of N_2 derived from the denitrification zone of 10-20 cm (at an air porosity of 8.5%) have been lower than $\%^{15}N$ of N_2 derived from the 40-50 cm layer at 16.2% air porosity. The average values were, in fact, 21.7 and 39.7%, respectively.

4.3 COLUMN STUDY (EXPERIMENT 3): EFFECT OF NITRATE CONCENTRATION ON DENITRIFICATION IN SOIL COLUMNS.

4.3.1 Inorganic-N Distribution

At the end of incubation period in the 0-5 cm layer 113 and 190 µg NO_3^--N/g soil were recovered in the 50 and 100 µg NO_3^--N/g soil treatments, respectively (Table 17). These values exceeded that which was expected from 50 and 100 µg N/g soil added as $NaNO_3$ plus the 25 µg N/g soil of NO_3^- originally present in this layer. The accumulation of NO_3^- in amounts in excess of that added indicates that mineralization of organic-N took place in the 0-5 cm layer. A small amount of NO_3^- could have also moved into the 0-5 cm layer from a greater depth as water evaporated. In the 15-20 cm layer 25.4 µg N/g soil was recovered as NO_3^- in the 100 µg N/g soil treatment. However, the added NO_3^- almost completely disappeared (3.3 µg N/g soil remained) in the 50 µg N/g soil treatment in the 15-20 cm layer. In both treatments NO_2^- accumulation was small and at the end of incubation the NO_2^- concentration did not exceed 2.0 µg N/g soil.

Inorganic-N distribution where NO_3^- at the rate of 200 µg N/g soil was uniformly mixed in the 0-20 cm layer of soil columns at 10.5% air porosity and where columns were incubated for periods ranging from 3 to

TABLE 17

Concentrations of NO_3^- and NO_2^- in soil columns (10.5% air porosity) treated with 50 µg NO_3^--N/g soil (12 days of incubation) and 100 µg NO_3^--N/g soil (16 days of incubation).

Soil depth	50 µg/g soil treatment		100 µg/g soil treatment	
	NO_3^--N	NO_2^--N	NO_3^--N	NO_2^--N
(cm)	(µg/g soil)			
0-5	113.6	0.8	190.8	0.5
5-10	93.2	0.7	139.4	0.6
10-15	44.1	1.0	89.6	1.2
15-20	3.3	0.9	25.4	1.2
20-30	1.2	0.5	3.8	0.7

9 days is shown in Table 18. The level of NO_3^- near the surface (248 µg N/g soil after 9 days) exceeded the amount on NO_3^- added. This could have been due both to mineralization of organic N and to convective flow of soil water resulting from surface evaporation. In the 20-30 cm layer there were 16 µg N/g soil after 3 days incubation and the amount remained unchanged during 9 days of incubation. In the 200 µg N/g soil treatment as in the 50 and 100 µg N/g soil treatments, maximum disappearance of NO_3^- was observed in the 15-20 cm layer where the NO_3^- concentration dropped to 96 µg N/g soil after 9 days of incubation.

More NO_2^- accumulated in the soil column containing 200 µg NO_3^--N/g soil (Table 18) than in the columns containing 50 and 100 µg NO_3^--N/g soil (Table 17). The NO_2^- which was found in the 15-20 cm layer disappeared gradually. The amounts of NO_2^- in the 15-20 cm layer were 28.6, 21.1 and 13.1 µg N/g soil after 3, 5 and 9 days incubation, respectively. In the 20-25 cm layer (anaerobic conditions) the NO_2^- was present after 3 days but had almost completely disappeared by the 9th day. The pH values of the soil below 15 cm depth were greater than 6.8 (Table 1). The disapperance of NO_2^- in the anaerobic soils at a neutral and alkaline pH was related to biological activity (Keuss and Smith, 1965; Bollag et al., 1973). However, at shallower more aerobic layer (0-15 cm) NO_2^- accumulated slowly throughout the 9 days of incubation. The accumulation of small amounts of NO_2^- in 0-15 cm layer (aerobic condition) was not known, but may have been due to diffusion from the zone of maximum production (15-20 cm layer).

TABLE 18

Concentrations of NO_3^- and NO_2^- during incubation of soil columns (10.5% air porosity) with 200 µg N/g soil as $NaNO_3$, at 20°C.

Soil depth	3 days		5 days		9 days	
	NO_3^--N	NO_2^--N	NO_3^--N	NO_2^--N	NO_3^--N	NO_2^--N
(cm)	(µg/g soil)					
0-5	219.6	0.4	238.9	3.1	248.7	5.9
5-10	222.6	4.2	231.3	17.8	242.6	19.23
10-15	209.5	9.5	211.1	9.5	217.9	14.0
15-20	175.8	28.6	155.7	21.1	96.3	13.1
20-25	16.0	19.2	16.0	11.0	15.5	2.2
25-30	0.5	5.3	2.1	0.1	0.7	0.1

4.3.2 Oxygen and Carbon Dioxide Distribution.

The concentration profiles of O_2 and CO_2 obtained when 50 µg N/g soil as $NaNO_3$ were uniformly mixed in the 0-20 cm layer are shown in Fig. 19. Oxygen concentration data were averaged over 7 days of incubation (dotted line). The calculated steady state O_2 concentration profile (solid line) was obtained by using Eq. (58) at an air porosity of 10.5%. The O_2 concentration decreased with soil depth from 7.5 µmole/ml in the 0-5 cm layer to less than 1 µmole/ml in the 15-20 cm layer. Maximun accumulation of CO_2 after 1.5 days incubation was 4.5 µmole/ml and was observed in the 15-20 cm layer. The maximum accumulation of CO_2 increased with incubation duration and reached a value 20.1 µmole/ml after 3 days incubation remaining at that level for 7 days. Carbon dioxide concentration after 3 days of incubation decreased sharply toward the soil surface and toward the impermeable layer. It is obvious from Fig. 19 that the concentration of CO_2 in the 20-30 cm layer never exceeded that in the 15-20 cm layer. For example, the CO_2 accumulation after 3 days incubation was 7.0 µmole/ml in the 20-30 cm layer. This was lower than maximum accumulation of 20.1 µmole/ml in the 15-20 cm layer. However, after 7 days of incubation the CO_2 accumulation in the 20-30 cm layer had nearly doubled (13 µmole/ml). Therefore, it is possible that CO_2 accumulation in the anaerobic layer resulted primarily from diffusion of CO_2 from the zone of maximum accumulation.

Carbon dioxide production in the anaerobic zone could have resulted from NO_3^- or the intermediates of denitrification, such as, NO_2^- or N_2O, replacing the O_2 as a terminal electron acceptor in the oxidation of organic matter to CO_2 (Wijler and Delwiche, 1954). However, the rate of

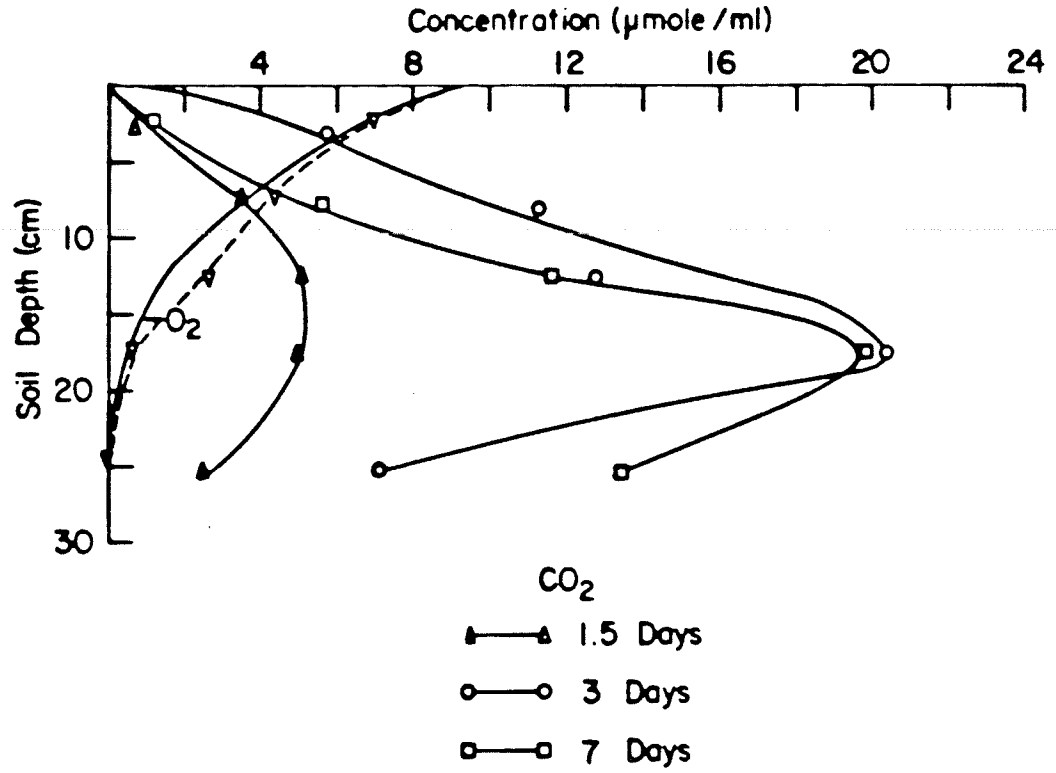

Figure 19: Concentration profiles of O_2 (measured as dotted line and calculated as solid line) and CO_2 during incubation of soil column (10.5% air porosity), at 20°C, with 50 µg NO_3^--N/g soil.

CO_2 production via this mechanism would have been smaller than the rate of CO_2 production under aerobic conditions (Cho, 1981). This may have been at least partially responsible for the lower CO_2 accumulation in the anaerobic layer (20-30 cm) than in the aerobic-anaerobic interface (15-20 cm) in this treatment.

No attempt was made to predict CO_2 distribution as a function of soil depth. The CO_2 concentration profile would have been hard to predict mathematically since at the depth of zero O_2 concentration in which the flux of O_2 would have been practically zero, the CO_2 flux would not have been zero and there would have always been a driving force (concentration gradient) causing CO_2 to diffuse below this zone .

The measured O_2 concentration profile for the soil column treated with 100 μg NO_3^--N/g soil (10.5% air porosity and 20°C) shown in Fig. 20 was constructed from O_2 concentrations which had been averaged over the period of 3-8 days. The profile of calculated steady state O_2 concentration which also appears in Fig. 20 was slightly less than the measured profile. For example, the calculated zero O_2 concentration occurred at 25 cm for 20°C and 10.5% air porosity (Table 8). However, the measured O_2 concentration was 0.5 μmole/ml in the 20-30 cm layer. Such a slight variation could have been related to the initial O_2 distribution (after 3 or 4 days) which possibly had not reached steady state. Maximum accumulation of CO_2 in the 15-20 cm layer was increased with incubation duration. An increase in CO_2 concentration of 8 μmole/ml was observed in the 20-30 cm layer during the first 3 days of incubation. The CO_2 concentration in the 20-30 cm layer increased to a value almost identical to the maximum accumulation in the 15-20 cm layer. There was

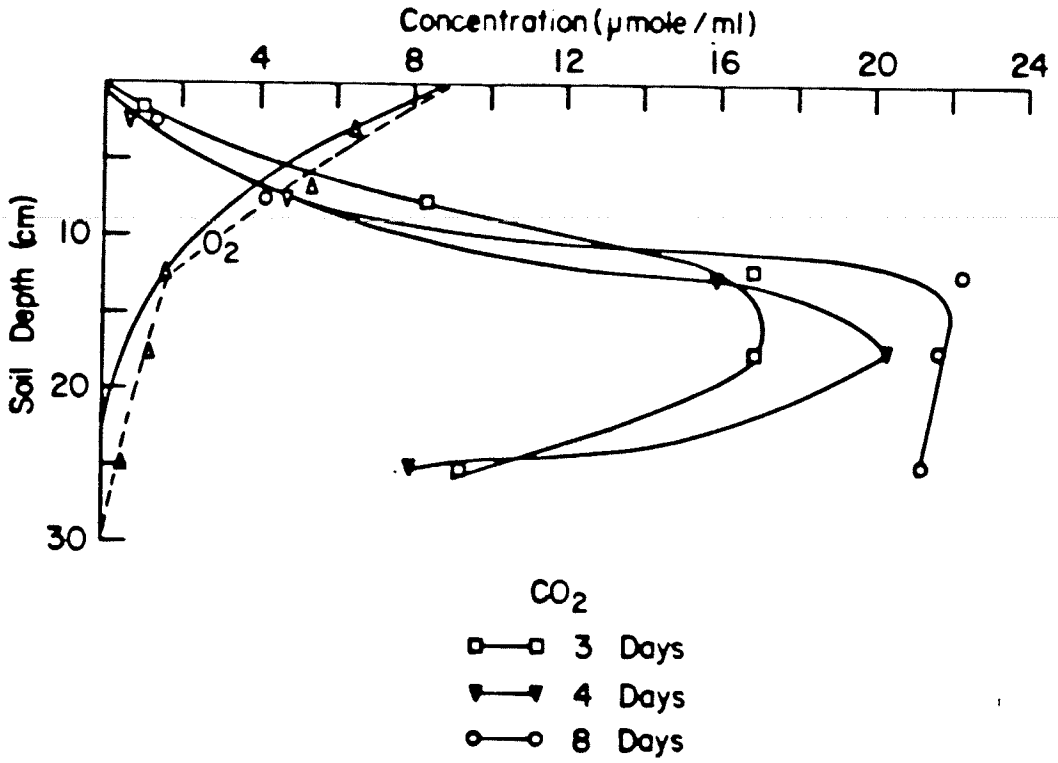

Figure 20: Concentration profiles of O_2 (measured as dotted line and calculated as solid line) and CO_2 during incubation of soil column (10.5% air porosity), at 20°C, with 100 μg NO_3^--N/g soil.

some accumulation of CO_2 below the 20 cm layer, likely resulting from a downward diffusive flux of CO_2 from the aerobic-anaerobic interface. Some CO_2 also may have been produced by anaerobic respiration with NO_3^- and/or N_2O utilized as the O_2 source.

The concentration profiles of O_2 and CO_2 in columns receiving 200 µg N/g soil as $NaNO_3$ uniformly mixed in the 0-20 cm layer are shown in Fig. 21. The air porosity of 10.5% was the same as in the 50 and 100 µg N/g soil treatments. The measured values of O_2 concentration were averaged over the period of 3-7 days. As with the 100 µg N/g soil treatment, there was a slight discrepancy between the measured O_2 profile and the calculated profile. The trend of CO_2 distribution for different incubation times was slightly different with this treatment (200 µg N/g soil) than for the 50 or 100 µg N/g soil treatment after 3 days of incubation. The maximum accumulation of CO_2 in the 200 µg N/g soil treatment was slightly lower (17 µmole/ml after 3 days in the 15-20 cm layer) than in columns receiving less NO_3^-. This value did not change much after 5 days of incubation for the 200 µg N/g soil treatment. After 7 days of incubation maximum accumulation of CO_2 shifted to a greater depth. Carbon dioxide concentration increased approximately 10 µmole/ml between 5 and 7 days incubation, with the CO_2 accumulation reaching 28 µmole/ml in the 20-30 cm layer. This shift the maximum accumulation of CO_2 to a greater depth could have resulted from some CO_2 production occurring at the greater depth due to the presence of NO_3^- and N_2O which were transported deeper due to a high concentration gradient in the 200 µg N/g soil treatment. A significant amount of NO_3^- (16 µg N/g soil) was observed only with the 200 µg N/g soil treatment in the 20-25 cm layer

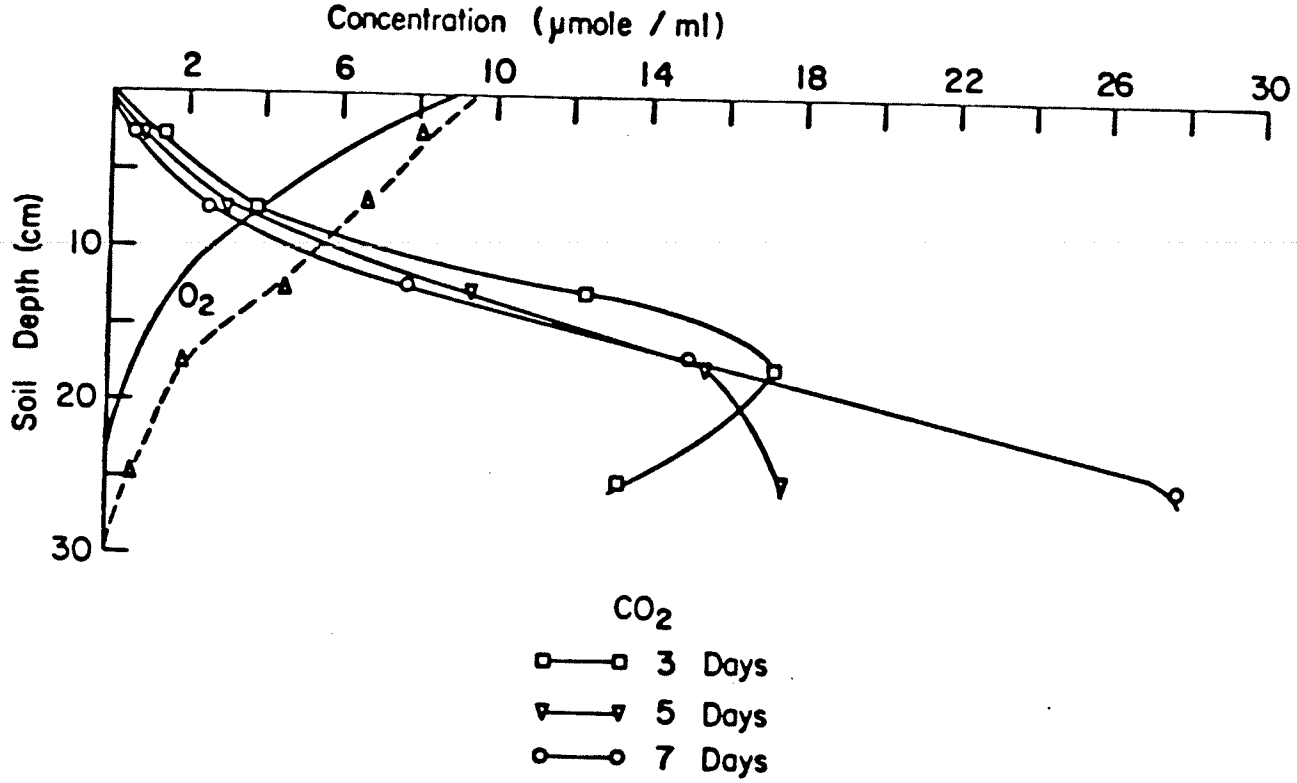

Figure 21: Concentration profiles of O_2 (measured as dotted line and calculated as solid line) and CO_2 during incubation of soil column (10.5% air porosity), at 20°C, with 200 µg NO_3^--N/g soil.

(Table 18). Thus, a greater contribution of NO_3^- to CO_2 production below the 20 cm depth was expected in the 200 than in the 50 or 100 µg N/g soil treatments.

4.3.3 Nitrous Oxide and N_2 Gas Distribution.

The maximum accumulation of N_2O in the 50 µg NO_3^--N/g soil treatment after 3 days incubation was 10.2 µg N/g soil and occurred in the 15-20 cm layer (Fig. 22). At four days of incubation the maximum accumulation of N_2O was still in the 15-20 cm layer and increased further to 20.0 µg N/g soil. However, location of maximum accumulation moved toward the soil surface after 4 days. At 7 and 9 days of incubation the maximum accumulation occurred in the 10-15 cm layer with peak values of 9.1 and 2.3 µg N/g soil, respectively. By 12 days of incubation all the N_2O had disappeared. The shift in the location of maximum accumulation toward the soil surface during disappearance of N_2O could have resulted from the low level of NO_3^- near the depth of initial maximum accumulation of N_2O (15-20 cm layer). The low level of NO_3^- near the zone of denitrification would cause N_2O to be a major e^- acceptor. Cho and Sakdinan (1978) and Khdyer (1978) found that a high rate of N_2O reduction and production of N_2 in two Manitoba soils in closed systems was related to low NO_3^- concentration. Thus, N_2O near and below the zone of denitrification was likely reduced to N_2 while that above the zone was stable due to the presence of O_2. Such a non-symmetric consumption of N_2O from the center of its production would have caused a shifting in the peak toward the soil surface.

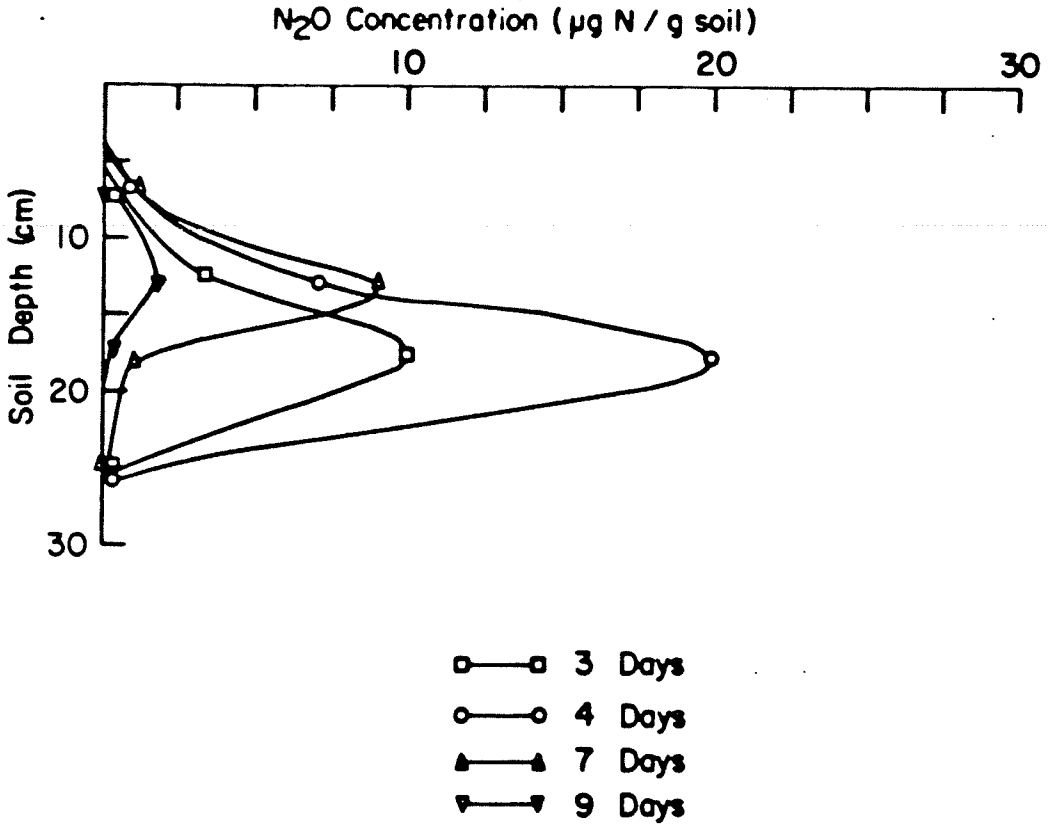

Figure 22: Concentration profiles of nitrous oxide during incubation of soil column (10.5% air porosity) at 20°C, with 50 µg NO_3^--N/g soil.

Maximum N_2 accumulation was observed at the same depth as maximum N_2O accumulation at 3 and 4 days of incubation (Fig. 23). This indicates that the N_2 was produced locally due to N_2O reduction. The conversion of N_2O to N_2 was found to be of biological cause (Cady and Bartholomew, 1960; McCarity, 1961; Bollag et al., 1973). As was shown earlier, the maximum CO_2 level was also observed at the depth of maximum N_2O accumulation. Thus, a very small contribution via N_2O reduction to CO_2 production at greater depths would have been expected with this treatment, 50 µg NO_3^--N/g soil (Fig. 19). There was no shift in the location of maximum N_2 accumulation with time. The maximum concentration of N_2 remained in the 15-20 cm layer, increasing from 15.5 µg N/g soil at 4 days to 35.2 µg N/g soil at 7 days of incubation. This increase occurred during the same stage of incubation as the rapid decrease in N_2O. During this period the N_2O maximum decreased from 20.0 µg N/g soil at 4 days of incubation to 9.1 µg N/g soil at 7 days of incubation. The maximum concentration of N_2 increased only slightly to 37 µg/g soil at 9 days of incubation.

The N_2O profiles in the soil columns receiving 100 µg NO_3^--N/g soil at 3, 4, 9 and 10 days incubation are shown in Fig. 24. The N_2O distribution at 3 and 4 days were almost identical to that in soil receiving 50 µg NO_3^--N/g soil. The maximum accumulation of N_2O occurred in the 15-20 cm layer and was 8.9 and 21.9 µg N/g soil for 3 and 4 days of incubation, respectively, almost identical to the values in the 50 µg N/g soil treatment during the same period of time (see Figs. 22 and 24). Thus, N_2O distribution in the soil column was independant of the initial concentration of NO_3^- as long as complete depletion of NO_3^- did not oc-

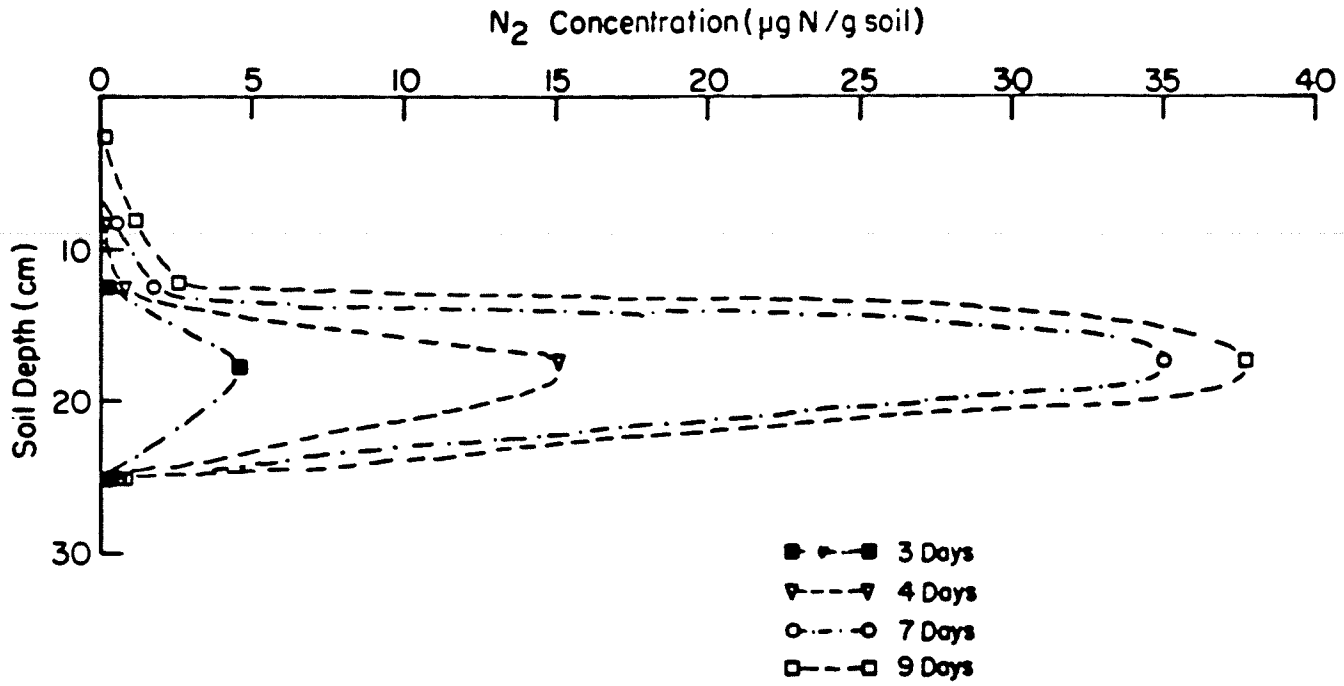

Figure 23: Concentration profiles of nitrogen gas during incubation of soil column (10.5% air porosity), at 20°C, with 50 μg NO_3^--N/g soil.

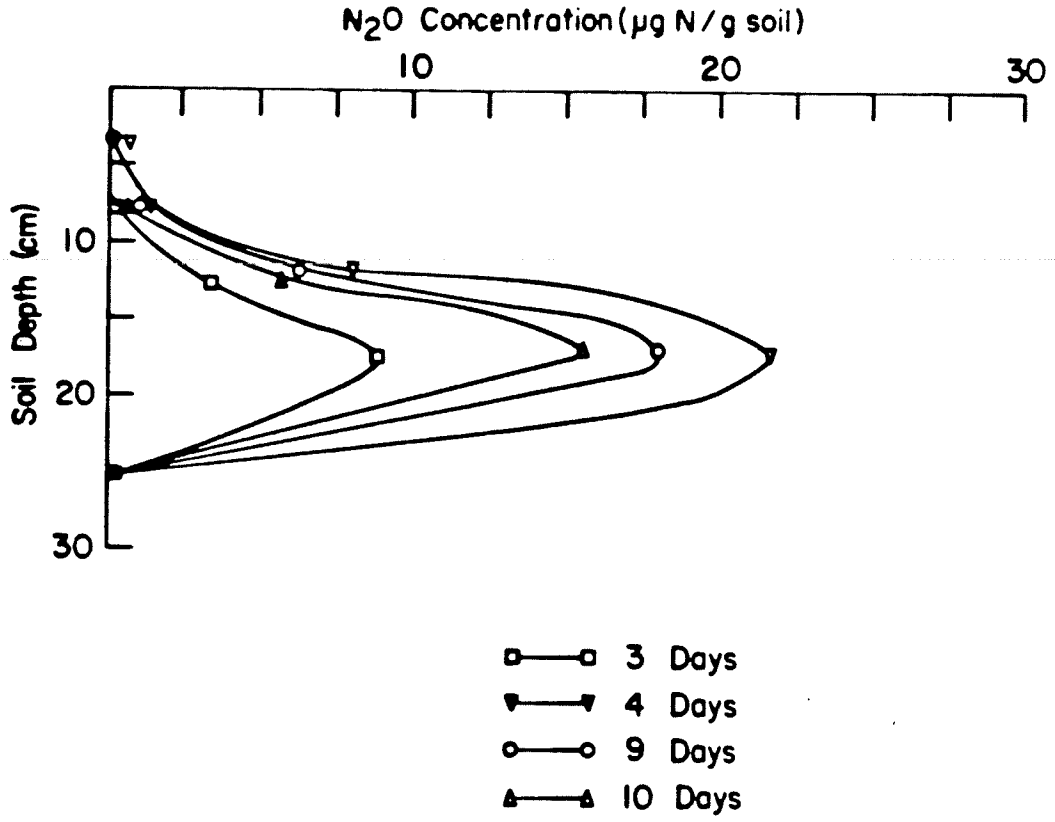

Figure 24: Concentration profiles of nitrous oxide during incubation of soil column (10.5% air porosity), at 20°C, with 100 µg NO_3^--N/g soil.

cur. However, during later stages of incubation N_2O distribution in the column receiving 100 µg NO_3^--N/g soil differed from that in soil receiving 50 µg N/g soil. In the 100 µg N/g soil treatment the maximum accumulation in the 15-20 cm layer persisted after the 4th day of incubation, decreasing only slightly from 21.9 µg N/g soil at the 4th day to 19 µg N/g soil at the 9th day. The maximum accumulations of N_2O were 16.6 and 9.7 µg N/g soil for incubation periods of 10 and 12 days, respectively (results of 12th day were not drawn in Fig. 24). There was no shift in the maximum peak toward the soil surface in the 100 µg N/g soil treatment even after there was a decrease in N_2O concentration. It was not until 16 days of incubation that all of the N_2O had disappeared.

Maximum N_2 accumulation with the 50 µg NO_3^--N/g soil treatment occurred at the same depth as maximum N_2O accumulation (15-20 cm). However, in the soil column receiving 100 µg NO_3^--N/g soil the location of the maximum concentration of N_2 remained in the 20-30 cm layer throughout the incubation (Fig. 25). The maximum accumulation of N_2 was very low (2.6 µg N/g soil) at the 3rd day of incubation but it increased with time. The increase in N_2 accumulation after the third day of incubation in the 20-30 cm layer could have been related to the N_2O accumulation which was observed during the same period of time in the 15-20 cm layer. Diffusion of N_2O downward from the depth of maximum accumulation would have led to N_2 accumulation as N_2O would have been further reduced to N_2. Nitrate and N_2O are known to compete as electron acceptors. The reduction of N_2O to N_2 in the presence of NO_3^- is governed by a number of factors, such as NO_3^- concentration and pH (Cho and Sakdinan, 1978). These authors concluded that there was a delayed and slower rate of N_2

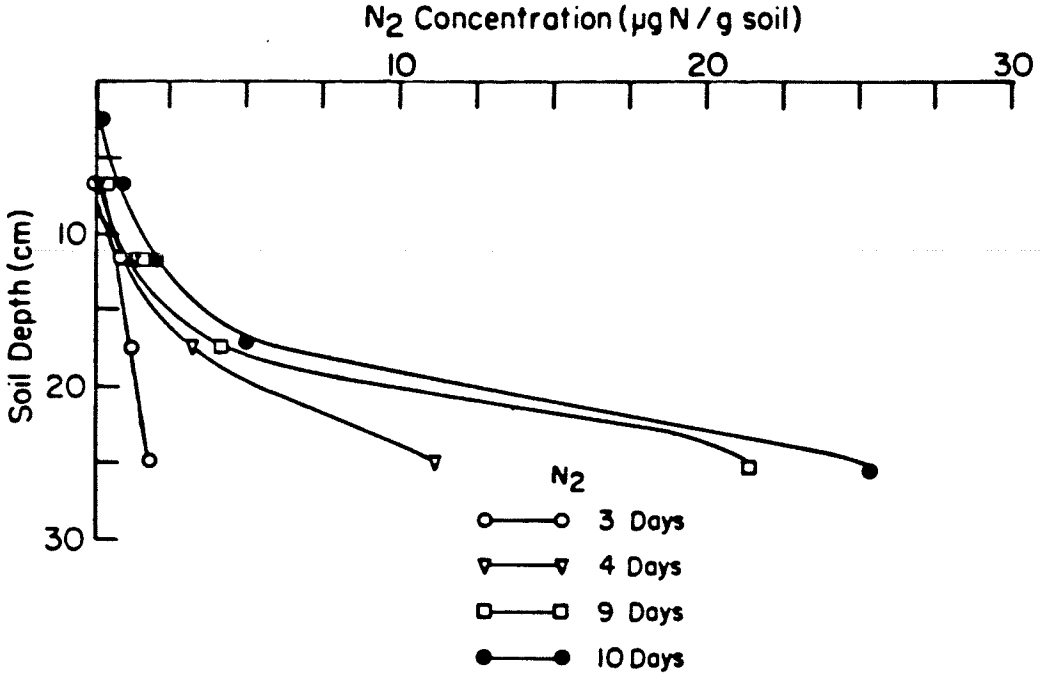

Figure 25: Concentration profiles of nitrogen gas during incubation of soil column (10.5% air porosity), at 20°c, with 100 µg NO_3^--N/g soil.

reduction with an increase in NO_3^- concentration. Thus, a relatively high NO_3^- concentration in the 15-20 cm layer with 100 µg N/g soil as compared to 50 µg N/g soil treatment at end of incubation (see Table 17) gives indirect evidence that N_2O was not immediately reduced at the site of formation. Downward diffusion of N_2O would have also led to a high CO_2 accumulation at a greater depth as N_2O was further reduced (see Fig. 20).

Concentration of N_2O at varying depths in soil column receiving 200 µg NO_3^--N/g soil after 3, 4, 6, 13 and 14 days incubation are shown in Fig. 26. At 3 days of incubation the maximum concentration of N_2O was 9.8 µg N/g soil and occurred in the 15-20 cm layer. This maximum value increased to 15.4 µg N/g soil by the next day but dropped slowly to 11.0, 10.0 and 7.7 µg N/g soil at 6, 13 and 14 days of incubation, respectively. The rate of further N_2O disappearance was slow and it was not until 22 days that N_2O completely disappeared. Although N_2O persisted for a longer period of time (22 days in the 200 µg N/g soil treatment as compared to 16 days in the 100 µg N/g soil treatment), the maximum N_2O accumulation was lower at the earlier stages in soil receiving 200 µg NO_3^--N/g soil. The lower accumulation of N_2O in the soil receiving 200 µg N/g soil may have been related to the accumulation of NO_2^- in the denitrification zone (Table 18). No such accumulation of NO_2^- took place in the 50 and 100 µg N/g soil treatments (Table 17). This observation is consistent with the report of Cho and Mills (1979), who found that the initial rate of N_2O formation, as described by competitive Michaelis-Menton type enzyme kinetics, was slower when NO_2^- accumulated. Thus, it could be suggested that the formation rate of N_2O

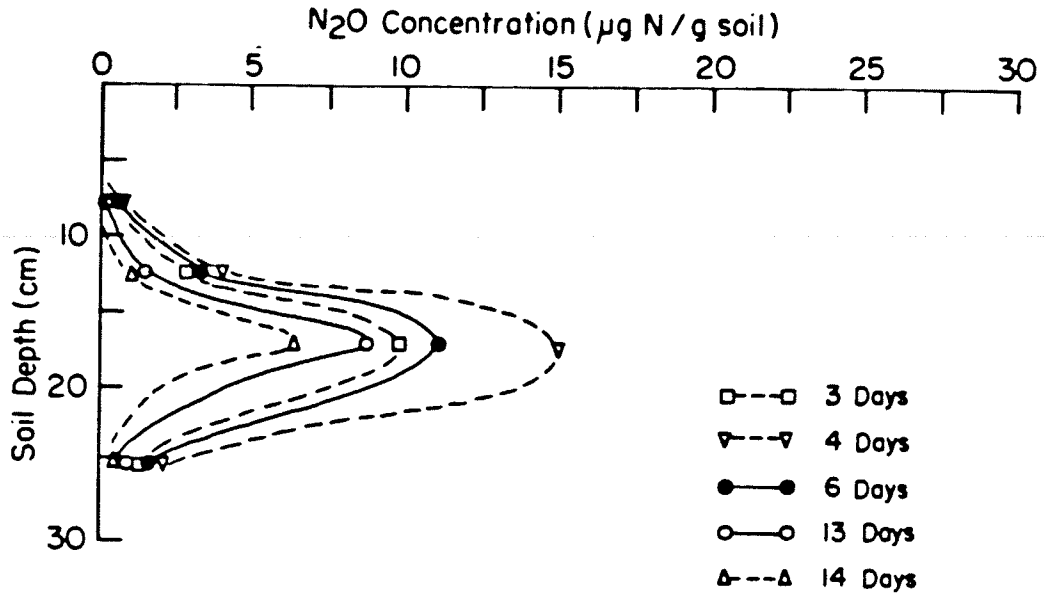

Figure 26: Concentration profiles of nitrous oxide during incubation of soil column (10.5% air porosity), at 20°C, with 200 µg NO_3^--N/g soil.

from one step reduction (NO_3^---->N_2O) without NO_2^- accumulation should be higher than the formation rate of N_2O from two steps reduction (NO_3^---->NO_2^---->N_2O) with NO_2^- accumulation.

Bailey and Beauchamp (1973a) concluded that in systems containing both NO_3^- and NO_2^-, provided there is no NO_2^--induced inhibition of denitrification, NO_3^- is preferred to NO_2^- as e^- acceptor. Furthermore, Cho and Sakdinan (1978) reported that a high concentration of NO_3^- and/or NO_2^- caused a decrease in the rate of N_2O reduction. In other words, NO_3^- and NO_2^- were preferred to N_2O as e^- acceptors. Thus, the accumulation of NO_3^- and NO_2^- in the 15-20 cm layer (Table 18) would have caused a smaller rate of N_2O reduction. The formed N_2O, thus, had a longer half life in the presence of high concentration of NO_3^- and NO_2^- and it could diffuse downward and be reduced further to N_2 as indicated by the maximum accumulation of N_2 at a greater depths in the soil column receiving 200 µg NO_3^--N/g soil (Fig. 27). The maximum accumulation of N_2 increased with time of incubation. The initial maximum N_2 accumulation (first 4 days) in the 200 µg N/g soil treatment occurred in the 20-30 cm layer and was the highest of the 3 treatments (30 µg N/g soil in the 200 µg N/g soil compared to 11 and 15 µg N/g soil in the 100 and 50 µg N/g soil treatments, respectively). Unlike the low NO_3^- treatments, the value of N_2 accumulation in the 20-30 cm layer with 200 µg N/g soil treatment was double the maximum value of N_2O accumulation in the 15-20 cm layer in the early stages of incubation. Therefore, there is a possibility that N_2 was also produced from the reduction of NO_3^- and/or NO_2^- which had accumulated in the anaerobic zone. Also, there were relatively high accumulations of NO_3^- and NO_2^- in the 20-25 cm

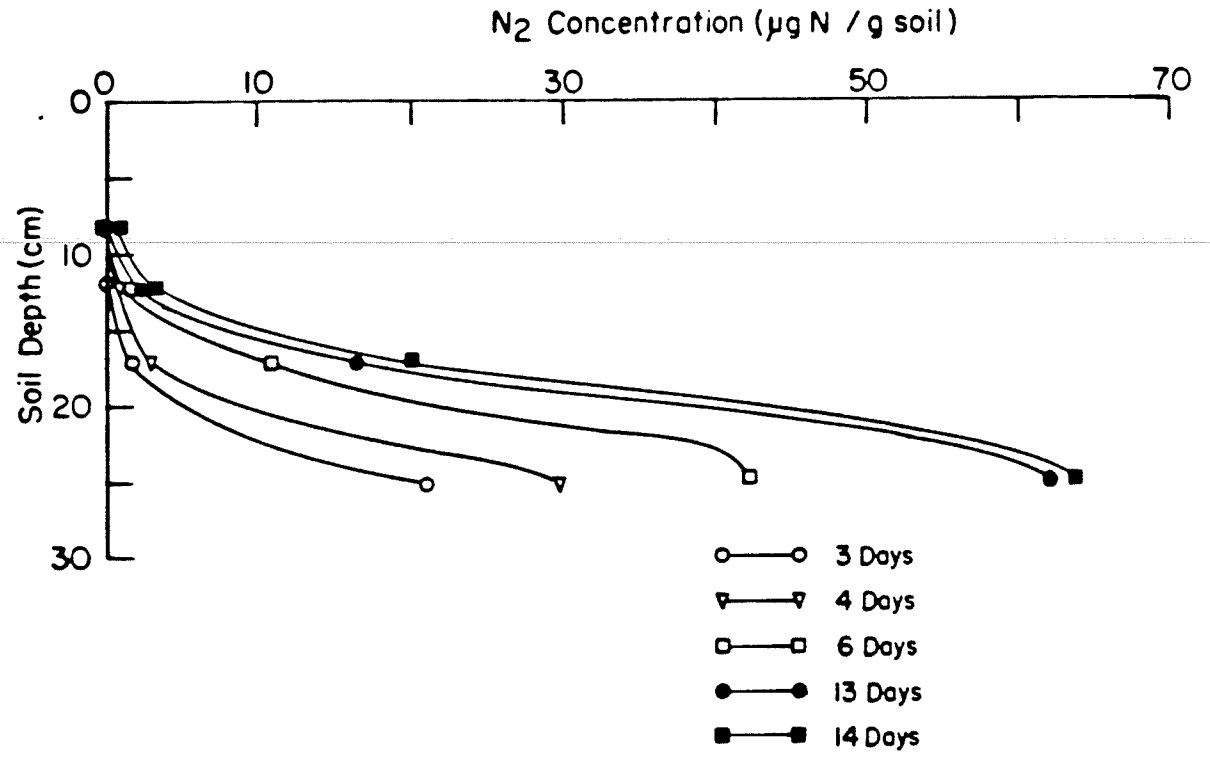

Figure 27: Concentration profiles of nitrogen gas during incubation of soil column (10.5% air porosity), at 20°C, with 200 µg NO_3^--N/g soil.

layer at 4 days of incubation in the 200 μg N/g soil treatment (Table 18). Such observations provide evidence that NO_3^-, NO_2^- or N_2O diffused deep into the anaerobic zone to produce N_2 when the addition of NO_3^- into the aerobic layer (0 to 20 cm) was high (200 μg N/g soil).

The isotopic composition of N_2O-N and N_2 evolved during incubation of NO_3^--N is shown in Table 19. The average values of $\%^{15}N$ in N_2O for 10 different incubation times and over the soil depths, were 45.8, 49.0 and 48.5% for the soil columns incubated with 50, 100 and 200 μg NO_3-N/ g soil, respectively. Comparable values for the N_2 were 46.6, 47.7 and 48.9% for the 50, 100 and 200 μg N/g soil treatments, respectively. Since the values of $\%^{15}N$ for N_2O and N_2 were almost identical and were close to $\%^{15}N$ of the added NO_3^- (52.6%), it was concluded that N_2O was solely derived from NO_3^--N and NO_2^--N and was mainly due to the biological reduction of NO_3^-. Values of the N-15 content of N_2O evolved from the 100 and 200 μg N/g soil treatment were slightly higher than those from the 50 μg N/g soil treatment especially at greater depths. All values are slightly less than the 52.6% N-15 content of the nitrogen originally added. This difference was attributed to isotopic dilution by NO_3^- originally present in the soil.

TABLE 19

The N-15 contents* of N_2O and N_2 evolved from soil columns incubated with varying amounts of added NO_3^--N.

Soil depth (cm)	Added NO_3^--N (µg/g soil)	N-15 (%)** N_2O	N-15 (%)** N_2	Standard deviation N_2O	Standard deviation N_2
5-10	50	47.1	47.2	2.0	1.4
10-15	50	45.3	46.3	1.0	1.5
15-20	50	45.0	47.1	2.3	1.0
20-25	50		45.6		2.0
Average		45.8	46.6		
5-10	100	47.7	44.0	2.5	0.2
10-15	100	49.4	47.1	0.6	1.9
15-20	100	49.9	49.7	0.3	1.5
20-25	100		49.8		0.4
Average		49.0	47.7		
5-10	200	46.3	49.00	4.9	1.0
10-15	200	48.8	45.8	1.5	2.6
15-20	200	49.8	49.6	1.3	2.6
20-25	200	48.9	51.0	1.7	1.3
Average		48.5	48.9		

* Average value of N-15% for 10 different days of incubation.

** Percent N-15 of added NO_3^- was 52.6%.

Chapter V

SUMMARY AND CONCLUSIONS

The main objective of this investigation was to determine the location and magnitude of denitrification within a reconstituted column of Wellwood soil as affected by soil moisture and nitrogeneous materials added. The stability of both nitrification products and added NO_3^- in the aerobic layer and also the transport of NO_3^- into the anaerobic layer with the formation of gaseous denitrification products were followed.

Two kinds of investigations were undertaken. Firstly, soil samples taken from varying depths up to 60 cm in a Wellwood soil were incubated in closed containers with air atmospheres for various periods of time with various moisture contents and temperatures. Oxygen consumption and CO_2 production were followed as a function of time. This provided information regarding the rate of O_2 consumption as related to soil depth and moisture content. This information was used to predict the steady-state O_2 concentration profile at different air porosities, and to estimate the apparent thickness of the surface aerobic layer as related to air porosity and temperature. Secondly, studies were conducted with columns of Wellwood soil to determine the effect of the O_2 concentration profiles on the zone of denitrification. The soil samples were packed to reconsititute the original soil as it occurred in the field. Bulk density and moisture content of the soil columns were varied to provide different air porosities (i.e. 25.6, 16.2, 10.5 and 8.5%). The N-15 en-

riched urea was mixed uniformly throughout the soil columns at a rate of 200 μg N/g soil. Additional studies were conducted with soil columns in order to determine the effect of NO_3^- concentration on the vertical distribution of various gases resulting from NO_3^- reduction. An air porosity of 10.5% was chosen resulting in an apparent surface aerobic layer 20 cm in thickness. The N-15 enriched NO_3^- (at rates of 50, 100 and 200 μg N/g soil) was mixed uniformly in the aerobic layer of soil column (0-20 cm).

The results obtained from the closed system indicated that the O_2 consumption rate (OCR) increased linearly with an increase in soil moisture content from 2.5 to 20%. For most soil samples the maximum OCR was observed at a 20% water content and did not change as the moisture content was increased to 30%. Carbon dioxide production rate followed a similar pattern for the same moisture range. The maximum OCR and CO_2 production rate (at 25% moisture) plotted as functions of soil depth indicated that these rates decreased exponentialy with soil depth at temperatures range between 20 and 40°C.

The temperature dependency of respiration as measured by the OCR and the CO_2 production rate indicated that the threshold temperature for respiration was approximately 3°C. The respiration over the temperature range from 20 to 40°C obeyed the Arrhenius equation with activation energies of 8264 ± 741 cal/mole for the O_2 consumption rate and 6877 + 373 cal/mole for the CO_2 production rate.

A steady state O_2 concentration profile was established after 7 days of incubation in the column studies. At an air porosity of 25.6% O_2 penetrated to the bottom of the soil column (60 cm). This was not the

case at lower air porosities where there was always a depth at which the O_2 concentration approached zero (x^*). The OCR as related to soil depth (closed incubation study) and x^* (column study) were used to estimate the diffusion coefficient of O_2 at specific air porosities and incubation temperatures. The values of x^* and O_2 diffusion coefficient increased with increasing air porosity. The estimated diffusion coefficient was used to predict the O_2 concentration profile. The parameter, x^* which was assumed to be governed by the capacity of soil profile for O_2 consumption was used to calculate the flux of O_2 through the soil surface (q_o). This flux (q_o) increased with increasing incubation temperature and increasing air porosity. Increasing the temperature caused a higher biological O_2 demand and increasing air porosity caused a greater O_2 penetration into the soil profile.

Inorganic- N distribution in the soil column in which urea had been uniformly mixed throughout the soil column indicated that hydrolysis of urea took place within the first week, indicating that urease activity was probably independent of O_2 concentration. The nitrogen was recovered as ammonium both above and below the zone of x^*. Persistence of NH_4^+ was observed at greater depths especially at high bulk density. Accumulation of NO_2^- was not appreciable at high (25.6%) and low (10.5%) air porosities. However, at 16.2% air porosity there was a larger accumulation of NO_2^- and it persisted for a longer time. Despite this accumulation of NO_2^-, loss of nitrogen by chemical denitrification did not occur. There was a large accumulation of NO_3^- near the soil surface at 16.2% air porosity (25% moisture content). On the other hand, only small amounts of NO_3^- and NO_2^- were found near the x^*. This together

with the persistence of ammonium near the anaerobic layer indicated that the NH_4^+ was not oxidized to NO_3^- near the reduced zone.

There was no evidence of N_2 or N_2O formation in the soil column having an air porosity of 25.6%. However, denitrification of NO_3^- derived from urea-N was evident at lower air porosities as indicated by N_2 accumulation. The depth of maximum accumulation of N_2 (zone of denitrification) was related to air porosity and was observed at shallower depths as air porosity decreased. The maximum accumulation of N_2 did not exceed 2.0 μg N/g soil for any of the porosity treatments. This could have been related to low nitrification activity near the x^* (low NO_3^- accumulation). Nitrous oxide was detected only in the column having an air porosity of 10.5% and even here it did not appear until 5 weeks of incubation.

Inorganic-N distribution in soil columns in which NO_3^- was uniformly mixed in the 0-20 cm layer indicated that very little NO_2^--N accumulated when the concentrations of applied NO_3^--N were 50 and 100 μg N/g soil. However, with 200 μg NO_3^--N/g soil, a higher accumulation of NO_2^- occurred in the 15-25 cm layer. Unlike the 50 and 100 μg N/g soil treatments a significant amount of NO_3^- from the 200 μg N/g soil mixed in the 0-20 cm layer was recovered at greater depths (20-25 cm).

Both N_2O and N_2 were produced in the NO_3^- treated soil columns. The isotope contents of these gases indicated that they were derived from NO_3^-. Maximum N_2O production was observed in the depth at which O_2 was depleted (near the x^*). The initial N_2O distribution in the soil column was unaffected by the initial concentration of NO_3^--N when there was no major NO_2^- accumulation. There was a shift in the depth of maximum N_2O accumulation toward the soil surface for the lowest NO_3^- treatment.

This shift was probably related to an asymmetric reduction and production of N_2O due to competition by NO_3^-, the concentration of which was not uniform near x^*.

Maximum accumulations of N_2O and N_2 for the 50 μg NO_3^--N/g soil treatment were observed in the same zone (near x^*) which indicates that the N_2 was produced locally by N_2O reduction. However, when 100 and 200 μg NO_3^--N/g soil were applied the maximum N_2 accumulation occurred at greater depths, indicating that only a small amount of N_2O was reduced at the zone of production, and most of the N_2O diffused downward and reduced further to N_2. Another possible cause for accumulation of N_2 at greater depths could have been the reduction of NO_3^- and NO_2^- which accumulated below the aerobic layer in the high NO_3^- treatment. As with N_2, maximum accumulation of CO_2 occurred near the aerobic-anaerobic interface (zone of maximum accumulation of N_2O) in the lowest NO_3^- treatment. However, in high NO_3^- treatments the zone of maximum CO_2 accumulation was observed at greater depths (20-30 cm), indicating that the contribution of N_2O and inorganic nitrogen (NO_3^- and NO_2^-) to CO_2 production was higher in high NO_3^- treatments.

The following conclusions can be drawn from the investigation;

1. Maximum respiration activity occurred when the moisture content of the Wellwood soil used in these investigations was near field capacity. The respiration activity decreased linearly with decreasing moisture content below field capasity.
2. Threshold temperature for respiration activity was approximately 3°C and it obeyed the Arrhenius equation over a temperatures range from 20 to 40°C.

3. Air porosity and respiration activity were the most important factors controlling the depth of the oxidized zone and O_2 flux into soil columns.

4. The O_2 diffusion coefficient in soil columns could be determined from the depth of the apparent aerobic layer for a steady-state O_2 profile.

5. Placement of NH_4^+-yielding fertilizer below the zone of aerobic-anaerobic interface decreased the rate of its oxidation to NO_3^- and NO_2^- and hence the danger of loss by denitrification. The depth of maximum accumulation of N_2 (denitrification zone) occurred near the aerobic-anaerobic interface. This zone occurred at a greater depth as air porosity was increased and as incubation temperature was decreased.

6. When NO_3^--N was uniformly mixed in the surface aerobic layer, the average position of NO_3^- reduction and N_2O formation was near the aerobic-anaerobic interface. However, because NO_3^- and N_2O likely competed as e^- acceptors, reduction of N_2O to N_2 was probably governed by the local concentration of NO_3^-. When there was a low NO_3^- concentration, the position of N_2O reduction was near the aerobic-anaerobic interface. On the other hand, when the concentration of added NO_3^- was high, N_2O reduction occurred at a greater depth. This change in the position of N_2O reduction due to a change in NO_3^- concentration affected the probable N_2O flux from the soil surface, since the majority of N_2O present above the zone of reduction was stable and tended to diffuse toward the soil surface.

7. In the lowest NO_3^- treatment the maximum CO_2 accumulation occurred in the same zone as N_2O or N_2 accumulation. As the concentration of added NO_3^- was increased, more N_2O and NO_3^- were transported deep to the anaerobic zone for further reduction. This contributed to CO_2 accumulation causing a shift in the maximum CO_2 accumulation to a greater depth.

BIBLIOGRAPHY

Adel, A. 1946. A possible source of atmosphere nitrous oxide. Science. 103: 280.

Alberda, T. H. 1953. Growth and root development of low land rice and its relation to oxygen supply. Plant and Soil. 5: 1-28.

Alexander, M. 1977. Introduction to soil microbiology. 2nd ed. pp. 251-286. Wiley,New York.

Allison, F. E. 1965. Organic carbon. In methods of Soil Analysis. part 2: Chemical and microbiological properties. (C. A. Black et al., ed.) pp. 1367-1378 Monograph No. 9. Amer. Soc. Agron., Madison, Wisconsin.

Anderson, W. B., and Kemper, W. D. 1964. Corn growth as affected by aggregate stability, soil temperature, and soil moisture. Agron. J. 56:453-456.

Bailey , L. D. and Beauchamp, E. G. 1973a. Effects of temperature on nitrate and nitrite 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 nitrate and macerated roots in nitrate transformation and redox potential in surface and subsurface soils. Can. J. Soil Sci. 53:219-230.

Bakker, J. W., and Hidding, A. P. 1970. The influence of soil structure and air content on gas diffusion in soils. Neth. J. Agric. Sci. 18: 37-48.

Barrow, G. M. 1973. Physical Chemistry. 3rd ed. pp. 457-462.McGraw-Hill Book Co., Toronto.

Baver, L. D., Gardner, W. H. and Gardner, W. R. Soil Physics. 4 th ed. Soil Aeration Ch. 6. pp 230-252.Wiley, New York.

Baver, L. D., and Farnsworth, R. B. 1940. Soil structure effects on the growth of sugar beets. Soil Sci. Soc. Amer. Proc. 5: 45-48.

Bhaumik, H. D., and Clark, F. E. 1947. Soil moisture tesion and microbial activity. Soil Sci. Soc. Amer. Proc. 12: 234-238.

Biederbeck, V. O. and Campbell, C. A. 1973. Soil microbial activity as influenced by temperature trends and fluctuations. Can. J. Soil Sci. 53: 363-376.

Birch, H. F. 1959. Further observations on humus decomposition and nitrification. Plant and Soil. 11:262-286.

Birch, H. F. 1960. The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil 12:81-96.

Birch, H. F., and Friend, M. T. 1961. Resistance of humus to decomposition. Nature 191: 731-732.

Black, C. A. 1957. Soil Plant Relationships. Wiley, New York.

Blake, G. R., and Page, J. B. 1948. Direct measurement of gaseous diffusion in soils. Soil Sci. Soc. Amer. Proc. 13: 37-42.

Blackmer, A. M., and Bremner, J. M. 1978. Inhibitory effect of nitrate on reduction of nitrous oxide to nitrogen gas by soil microorganisms. Soil Biol. Biochem. 10: 187-191.

Bollag, J. M., Brzymala, S., and Kardos, L. T. 1973. Biological versus chemical nitrite decomposition in soil. Soil Sci. 116:44-50.

Bouldin, D. R. 1968. Models for describing the diffusion of oxygen and other mobile constituents across the mud-water interface. J. Ecology 56: 77-87.

Bouldin, D. R., Johnson, R. L., Burda, C., and Kao, C. W, 1974. Losses of inorganic nitrogen from aquatic systems. J. Environ. Qual. 3: 107-114.

Bouyoucos, G. J. 1915. Michigan Agr. Coll. Expt. Sta. Tech. Bull. 22.

Bowman, R. A. and Focht, D. D. 1974. The influence of glucose and nitrate concentration upon denitrification rates in sandy soils. Soil Biol. Biochem : 6 297-301.

Bremner, J. M. 1965. Total nitrogen. In Methods of Soil Analysis. Part 2: Chemical and microbiological properties. (C. A. Black et al., ed.) pp. 1149-1179. Monograph No. 9. Amer. Soc. Agron., Madison, Wisconsin.

Bremner, J, M. and Shaw, K. 1958a. Denitrification in soil: I: Methods of investigation. J. Agr. Sci. 51: 22-39.

Bremner, J. M., and Shaw, K. 1958b. Denitrification in soil: II: Factors affecting denitrification. J. Agr. Sci. 51: 40-52.

Brice, K. A., Eggleton, A. E. J., and Penkett, S. A. 1977. An important ground surface sink for atmospheric nitrous oxide. Nature. 268: 127-129.

Broadbent, F. E. and Clark, F. 1965. In Soil Nitrogen. Ch. 9. Denitrification, (W. V. Bartholomew and Clark, F. E. ed.) pp. 344-359. Monograph No. 10 Amer. Soc. Agron. Madison, Wisconsin.

Buckingham, E. 1904. Contribution to our knowledge of the aeration of soils.U.S.A. Bur. Soils Bull. No. 25ited by Wesseling, J. 1962).

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. Influnce of low partial pressure of oxygen on denitrification process and products. Soil Sci. Soc. Amer. Proc. 25:362-365.

Call, F. 1957. Soil Fumigation. V. Diffusion of ethylene dibromide through soils. J. Sci. Food Agric. 8:143-150

Campbell, C. A. and Biederbeck, V. O. 1972. Influence of fluctuating temperatures and constant soil moistures on nitrogen changes in amended and unamended loam. Can. J. Soil Sci. 52: 323-336.

Campbell, C. A. and Biederbeck, V. O. 1976. Soil bacterial changes as affected by growing season weather conditions: a field and laboratory study. Can. J. Soil Sci. 56: 293-310.

Cassman, K. G., Munns, D. N. 1980. Nitrogen mineralization as affected by soil moisture, temperature and depth. Soil Sci. Soc. Amer. J. 44:1233-1237.

Cho, C. M. 1971. Convective transport of ammonium with nitrification in soil. Can. J. Soil Sci. 51: 339-359.

Cho, C. M. 1981. Oxygen consumption and denitrification kinetics in soil. Agronomy Abs. Soil Sci. Soc. Amer. 1981 Annual Meeting, Georgia. pp. 159.

Cho, C. M., and Mills, J. G. 1979. Kinetic formulation of the denitrification process in soil. Can. J. Soil Sci. 59:249-257.

Cho, C. M. and Sakdinan, L. 1978. Mass spectrometric investigation on denitrification. Can. J. Soil Sci. 58: 443-457.

Cho, C. M., Sakdinan, L., and Chang, C. 1979. Denitrification intensity and capacity of three irrigated Alberta soils. Soil Sci. Soc. Amer. J. 43: 945-950.

Christianson, C. B. 1978. Denitrification of urea and sodium nitrite in some Manitoba soils. M.Sc. Thesis Submitted to the Faculty of Gradute Studies and Research, University of Manitoba.

Christianson, C. B., Hedlin, R. A., and Cho, C. M. 1979. Loss of nitrogen from soil during nitrification of urea. Can. J. Soil Sci. 59:147-154.

Conn, E. E., and Stumpf, P. K. 1967. Outlines Of Biochemistry. 2nd ed. pp. 134-153. John Wiley and Sons, New York.

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.

Currie, J. A. 1960. Gaseous diffusion in porous media. Part II .Dry granular materials. Br. J. Appl. Physics. 11:318-324.

----------1970. Movement of gases in soil respiration. PP. 152-171. In Sorption and Transport Process in Soils. Monograph Soc. Chem. Ind. No. 37. Rothamsted Exp. Stn., Harpenden, England (Cited by Lai et al 1976).

Dawson, R. W. and Murphy, K. L. 1972. The temperature dependency of biological denitrification. Water Res. 6:71-83.

Danckwerts, P. U. 1950. Absorption by simultaneous diffusion and chemical reaction. Trans. Farad. Soc. 46:300-304.

Domby, C. W., and Kohnke, H. 1956. The influence of soil crust on gaseous diffusion . Soil Sci. Soc. Amer. Proc. 20: 1-5.

Drobnik, J. 1962. The effect of temperature on soil respiration. Folia Microbiol. 7: 132-140 (Cited by Nyhan, 1976).

Engler, R. M., and Patrick, W. H. , Jr. 1974. Nitrate removal from flooded water overlying flooded soils and sediments. J. Environ. Qual. 3: 409-413.

Engler, R. M., Antie, D. A. , and Patrik, W. H., Jr. 1976. Effect of dissolved oxygen on redox potential and nitrate removal in flooded swamp and marsh soils. J. Environ. Qual. 5: 230-235.

Freney, J. R., Denmead, O. T., and Simpson, J. R. 1978. Soil as a source or sink for atmospheric nitrous oxide. Nature. 273:530-532.

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. App. Microbiol. 19: 153-186.

Grable, A. R. 1966. Soil aeration and plant growth. Advance Agron. 18: 57-106.

Grable, A. R., and Siemer, E. G. 1968.Effect of bulk density, aggregate size and soil water suction on oxygen diffusion, redox potential and elongation of corn roots. Soil Sci. Soc. Amer. Proc. 32: 180-186.

Gradwell, M. W. 1961. A laboratory study of the diffusion of oxygen through pasture topsoils. N. Z. J. Res. 4:250-270.

Gradwell, M. W. 1965. Soil physical condition of winter and the growth of rye grass plants. I . The effects of the compaction and puddling. N. Z. J. Res. 8:238-269.

Howeler, R. H. and Bouldin, D. R. 1971. The diffusion and consumption of oxygen in submerged soils. Soil Sci. Soc. Amer. Proc. 35: 202-208.

Kamphake, L. J., Hannah, S. A., and Cohen, J. M. 1967. Automated analysis for nitrate by hydrazine reduction. Water Res. 1: 205-216.

Khdyer, I. I. 1978. The effects of temperature and concentration of nitrate upon denitrification in some Manitoba soils. M.Sc. Thesis submitted to Faculty of Graduate Studies and Research, University of Manitoba.

Kirkham, D., and Powers, W. L. Advanced Soil Physics. Ch. 9. Gaseous diffusion in soils. 1st ed. pp. 428-461. Wiley, New York.

Kowalik, P., Barnes, C. J., and Smiles, P. E. 1979. Oxidation of liquid animal wastes in soil . Soil Sci. Soc. Amer. J. 43:255-260.

Lance, J. C., Whisler, F. D., and Bouwer, H. 1973. Oxygen utilization in soils flooded with sewage water. J. Envion. Quality. 2: 345-350.

Lai, S. H., Tiedje,J. M., and Erikson, A. E. 1976. In situ measurment of gas diffusion coefficient in soils . Soil Sci. Soc. Amer. J. 40:3-6.

MacGregor, A. N. 1972. Gaseous losses of nitrogen from freshly wetted desert soils. Soil Sci. Soc. Amer. Proc. 36:594-596.

Mack, A. R. 1962. Low temperature research on nitrate released from soil. Nature. 193:803-804.

Mack, A. R. 1963. Biological activity and nineralization of nitrate in three soils as induced by freezing and drying. Can. J. Soil Sci. 43:316-324.

Mahendrappa, M. K., and Smith, R. L. 1967. Some effects of moisture on denitrification in acid and alkaline soil. Soil Sci. Soc. Amer. Proc. 31: 212-215.

Marshall, T. J. 1959. The diffusion of gases through porous media. J. Soil Sci. 10: 79-82.

McGarity, J. W. 1961. Denitrification studies on some South Australian soils. Plant and Soil. 1: 1-21.

Miller, R. D., and Johnson, D. D. 1964.The effect of soil moisture tension on carbon dioxide evolution, nitrification, and nitrogen nineralization. Soil Sci. Soc. Amer. Proc. 28:644-647.

Millington, J. R. 1959. Gas diffusion in porous media. Science 130 :100-102.

Mills, G. F., Tarnocai, C. and Shaykewich, C. F. 1977. Characteristics and distribution of soil temperature regimes in Manitoba, Canada. 21st Annual Manitoba Soil Sci. Meeting. p 56-77.

Misra, C. Nielson, D. R., and Biggar, J. W. 1974. Nitrogen transformation in soil during leaching:III .Nitrate reduction in soil columns. Soil Sci. Soc. Amer. Proc. 38:300-304.

Myers, R. J. K. and McGarity, J. W. 1972. Denitrification in unsaturated cores from a solodized solonetz B horizon. Plant and Soil. 37: 81-89.

Nicholson, I. K. 1979. Denitrification in lake sediment and sewage sludge. M.Sc. Thesis Submitted to the Faculty of Graduate Studies and Research, University of Manitoba.

Nyhan. J. W. 1976. Influence of temperature and water tension on the decomposition rate of carbon-14 labelled herbage. Soil Sci. 121: 288-293.

Obi, O. 1981. Isotope studies on crop utilization and soil fixation of nitrogen from calcium nitrate, ammonium sulphate, and urea in several manitoba soils. PhD. Thesis Submitted to the Faculty of Gradute Studies and Research, University of Manitoba.

Parr, J. F., and Norman, A. G. 1964. Growth and activity of soil micro-organisms in glass micro-beads. Soil Sci. 97: 361-366.

Parr, J. F., Parkinson, D., and Norman, A. G. 1963. A glass micro-beads system for the study of soil micro-organisms. Nature. 200: 1227-1228.

Parr, J. F., and Reuszer, H. W. 1959. Organic matter decomposition as influenced by oxygen level and method of application to soil. Soil Sci. Soc. Amer. Proc. 23: 214-216.

Parr,J.F.,Parkinson, D., and Norman, A. G. 1967. Growth and activity of soil microorganisms in glass microbeads. II. Oxygen uptake and direct observation. Soil Sci. 103: 303-310.

Patrick, W. H. , Jr. 1960. Nitrate reduction rates in a submerged soil as affected by redox potential. Proc. 7th Int. Congr. Soil Sci. 2: 494-500.

Patrick, W. H. , Jr., and Deluane, R. D. 1972. Charactarization of the oxidized and reduced zone in flooded soil. Soil Sci. Soc. Amer. Proc. 36:573-576.

Patrick, W. H., Jr., and Gotoh, S. 1974. The role of oxygen in nitrogen loss from flooded soil. Soil Sci. 118: 78-81.

Patrick, W. H., Jr. and Mahaptra, I. C. 1968. Transformation and availability to the ri · of nitrogen and phosphorus in waterlogged soil. Adv. Agron. 20: 323-359.

Patrick,W. H. , Jr. and Reddy, K. R. 1976. Nitrification-denitrification reaction in flooded soils and water bottoms: Dependence of oxygen supply and ammonium diffusion. J. Environ. Qual. 5:469-471.

Patrick, W. H., Jr., and Tusneem, M. E. 1972. Nitrogen loss from flooded soil. Ecology 553: 735-737.

Papendick, R. I., and Kunkles, R. J. 1965. Transient-state oxygen diffusion in soil. I. The case when rate of oxygen consumption is constant. Soil Sci. 100: 251-261.

Pearsall, W. H., and Mortimer, C. H. 1939. Oxidation- reduction potentials in water-logged soils, natural waters and muds. J. Ecol. 2:483-501.

Penman, H. L. 1940. Gas and vapor movement in the soil. I. The diffusion of vapor through porous solids. J. Agr. Sci. 30: 437-462.

Philips, R. E., Reddy, K. R., and Patrick,W. H., Jr. 1978. The role of nitrate diffusion in determining the order and rate of denitrification in flooded soil: II. Theoretical analysis and interpretation. Soil Sci. Soc. Amer. J. 42: 272-278.

Ponnamperuma, F. N. 1972. The chemistry of submerged soils. Adv. Agron. 24: 29-96.

Reddy, K. R., Patrick, W. H., jr, and Philips, R. E. 1976. Ammonium diffusion as a factor in nitrogen loss from flooded soils. Soil Sci. Soc. Amer. J. 40: 528-533.

Reddy, K. R., Patrick, W. H., Jr., and Philips, R. E. 1978. The role of nitrate diffusion in determining the order and the rate of denitrification in flooded soil: I. Experimental results. Soil Sci. Soc. Amer. J. 42: 268-272.

Reddy, K. R., Rao, P. S. C., and Patrick, W. H., Jr. 1980. Factors influencing oxygen consumption rates in flooded soils. Soil Sci. Soc. Amer. J. 44:741-744.

Reichman, G. A., Grunes, D. L., and Viets, F. G. 1966. Effect of soil moisture on ammonification and nitrification in two northern plains soils. Soil Sci. Soc. Amer. Proc. 30:363-366.

Reuss, J. O., and Smith, R. L. 1965. Chemical reaction of nitrites in acid soil. Soil Sci. Soc. Amer. Proc. 29: 267-270.

Robinson, J. B. D. 1957. The critical relationship between soil moisture content and the region of wilting point and the mineralization of natural soil nitrogen. J. Agric. Sci. 49:100-105.

Roulier, M. H., and Fetter, N. R. 1975. Procedure for sampling and analysis of the atmosphere of field soil for trace amounts of nitrous oxide. Agron. Abstr. PP. 78-79.

Russell. E. W. 1973. The chemistry of waterlogged soils. In Soil Conditions and Plant Growth. 10th ed. pp. 670-695. Longman, New York.

Sain, P., Robinson, J. B. Stammers, W. N., Kausik, N. K., and Whiteley, H. R. 1977. A laboratory study on the role of stream sediment in nitrogen loss from water. J. Environ. Qual. 6: 274-278.

Sedgley, R. H. 1962. Effect of distribution and flocculation of pore-space changes in beads of clay aggregates. Soil Sci. 94:357-365.

Skinner, S. I., Halstead, R. L., and Brydon, J. E. 1959. Qualitative nanometric determination of calcite and dolomite in soils and limestones. Can. J. Soil Sci. 39: 197-204.

Smith, A. 1932. Seasonal subsoil temperature variations. J. Agr. Res. 44: 421-428.

Smith, C. J., and Chalk, D. M. 1980. Gaseous nitrogen evolution during nitrification of ammonia fertilizer and nitrite transformations in in soils. Soil Sci. Soc. Amer. J. 44:277-282.

Sorenson, L. H. 1975. Rate of decomposition of organic matter in soil as influenced by repeated air drying-rewetting and repeated addition of organic material. Soil Biol. Biochem. 6:287-292.

Soulides, D. A., and Allison, F. E. 1961. Effect of drying and freezing soils on carbon dioxide production, available mineral nutrients, aggregation, and bacterial populations. Soil Sci. 91: 291-298.

Stanford, G., and Epstein, E. 1974. Nitrogen mineralization-water relations in soils. Soil Sci. Soc. Amer. Proc. 38:103-106.

Stanford, G., Dzienia, S. and Vandor, R. A. 1975. Effect of temperature on denitrification rate in soils. Soil Sci. Soc. Amer. Proc. 39:867-870.

Stevenson, F. J. 1965. Origin and distribution of nitrogen in soil. In Soil Nitrogen. W. V. Bartholomew and F. E. clark, ed. pp. 1-42. Monograph No. 10 Amer. Soc. Agron., Madison, Wisconsin.

Stevenson, I. L. 1956. Some observation on the microbial activity in remoistened air- dried soil. Plant and Soil 8: 170-182.

Stotzky, G. 1960. A simple method for determination of the respiratory quotient of soils. Can. J. Microbiol. 6:449-452.

Stotzky, G. 1965. Microbial Respiration. In methods of soil analysis, (C. A. Black, ed.), Agronomy 9: Part 2. Chemical and Microbiological Properties. Madison, Wisconsin: Amer. Soc. Agron. pp. 1550-1572

Taylor, S. A. 1949. Oxygen diffusion in porous media as a measurment of soil aeration. Soil Sci. Soc. Amer. Proc. 14: 55-61.

Thomas, R. E., Schwartz, W. A., and Bendixen, T. W. 1968. Pore gas composition under sewage spreading. Soil Sci. Soc. Amer. Proc. 32: 419-423.

Van Bavel, C. H. M. 1952. Gaseous diffusion and porosity in porous media. Soil Sci. 73: 91-96.

Wiant, H. V., Jr. 1967.Influnce of moisture content on soil respiration. J. For. 65: 902-903.

Wijler, J. and Delwiche, C. C. 1954. Investigation on denitrifying process in soil. Plant and Soil. 2:155-169.

Wesseling, J. 1962. Some solutions of the steady state diffusion of carbon dioxide through soils. Neth. J. Agric. Sci. 10: 109-117.

Wyckhoff, R. W., and Botset, H. G. 1936. The flow of gas-liquid mixtures through unconsolidated sand. Physics. 7: 325-345.

Appendix A

TABLE 20

Soil moisture contents during incubation of soil columns at 20°C with an initial moisture content of 23% and air porosity of 25.6%.

Soil depth (cm)	Moisture (%) 10 days	20 days	30 days
0-5	22.5	22.3	22.2
5-10	22.4	22.2	22.4
10-15	22.5	22.7	22.4
15-20	22.5	22.6	22.5
20-30	22.6	22.6	22.5
30-40	22.6	22.7	22.4
40-50	22.7	22.7	22.3
50-60	22.9	22.7	22.4

TABLE 21

Soil moisture contents during incubation of soil columns at 20°C with an initial moisture content of 25% and air porosity of to 16.2%.

Soil depth (cm)	Moisture (%)			
	20 days	30 days	42 days	52 days
0- 5	24.1	23.3	22.9	22.8
5-10	24.5	23.9	23.6	23.6
10-15	24.3	23.8	24.1	24.0
15-20	24.3	24.1	24.2	24.0
20-30	24.1	24.3	24.1	24.1
30-40	24.4	24.3	24.2	24.1
40-50	24.1	24.2	24.2	24.6
50-60	24.5	24.1	24.2	24.2

TABLE 22

Soil moisture contents during incubation of soil columns at 20°C with an initial moisture content of 30% and air porosity of to 10.5%.

Soil depth (cm)	Moisture (%)			
	20 days	30 days	42 days	52 days
0- 5	28.2	28.0	27.8	27.8
5-10	28.9	28.1	28.0	28.1
10-15	29.1	28.7	28.3	28.1
15-20	29.2	28.6	28.2	28.3
20-30	29.0	29.0	28.2	28.6

TABLE 23

Concentration of O_2 as related to soil depth during the incubation of soil columns (8.5% air porosity) with 200 µg urea-N/g soil, at 20°C.

Soil depth (cm)	O_2 concentration (µmole/ml) Time of incubation (week) 1	2	3	4	5	6	7	8	Average	Standard deviation
0-5	5.03	3.26	4.86	3.89	6.02	6.55	6.45	7.92	5.50	1.53
5-10	0.95	1.07	0.60	0.55	0.69	1.03	1.43	1.68	1.00	0.39
10-15	0.41	0.39	0.45	0.14	0.21	0.40	0.68	0.51	0.40	0.16
15-20	0.41	0.32	0.25	0.34	0.28	0.34	0.38	0.17	0.31	0.08

TABLE 24

Concentration of O_2 as related to soil depth during the incubation of soil columns (10.5% air porosity) with 200 µg urea-N/g soil, at 20°C.

Soil depth (cm)	O_2 concentration (µmole/ml) Time of incubation (week) 1	2	3	4	5	6	7	8	Average	Standard deviation
0-5	5.86	6.29	6.57	7.58	6.59	7.19	6.80	6.95	6.73	0.53
5-10	2.89	4.33	4.94	5.12	4.40	5.44	5.01	5.31	4.68	0.82
10-15	2.56	4.03	3.04	1.84	2.91	2.48	2.32	2.72	2.61	0.88
15-20	1.50	2.04	1.06	1.25	1.50	1.13	1.31	1.71	1.31	0.56
20-30	0.37	0.13	0.07	0.13	0.18	0.18	0.20	0.13	0.17	0.08

TABLE 25

Concentration of O_2 as related to soil depth during the incubation of soil columns (16.2% air porosity) with 200 µg urea-N/g soil, at 20°C.

Soil depth (cm)	O_2 concentration (µmole/ml) — Time of incubation (week) 1	2	3	4	5	6	7	8	Average	Standard deviation
0-5	5.99	7.81	6.35	7.59	7.08	7.50	7.69	7.71	7.32	0.60
5-10	5.29	5.82	4.93	5.80	6.27	6.74	5.52	6.10	5.75	0.65
10-15	3.57	3.61	4.19	4.85	4.63	4.83	4.42	4.91	4.38	0.54
15-20	2.83	2.43	3.42	3.16	3.01	3.87	2.90	3.60	3.15	0.46
20-30	2.34	1.83	3.06	2.25	2.08	2.03	2.03	2.31	2.11	0.17
30-40	1.35	0.83	2.10	1.30	1.03	0.95	1.00	1.30	1.23	0.40
40-50	0.27	0.21	0.40	0.09	0.27	0.20	0.31	0.15	0.20	0.08
50-60	0.18	0.11	0.34	0.04	0.04	0.05	0.11	0.10	0.12	0.10