

DENITRIFICATION OF UREA AND
SODIUM NITRITE IN SOME MANITOBA SOILS

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by

Carlyle Bruce Christianson

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CARLYLE BRUCE CHRISTIANSON

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ABSTRACT

The kinds and amounts of nitrogen gas lost during the oxidation of urea were determined. One thousand (1000) ppm-N as urea labelled with 52.4% ^{15}N was uniformly mixed with soil to approximate the concentration of urea - N near the pellet site or fertilizer band. With Wellwood soil, gaseous losses as N_2O and N_2 amounted to approximately 25% of the added N at pH 6.1 in 2 months. Samples of this soil were shown to accumulate NO_2^- . The appearance of NO_2^- coincided with the appearance of gaseous N products in the soil atmosphere. Gaseous production declined as NO_2^- oxidized to NO_3^- . The majority of the N_2 gas (which accounted for 40% of the N evolved) resulted from a van Slyke-type reaction in which one atom of N came from a soil source and one came from the fertilizer. When the pH of the Wellwood soil was increased to 7.6 by the addition of 10% CaCO_3 , the accumulation of NO_2^- occurred to a greater extent and lasted for the duration of the study. Liming slightly increased losses of nitrogenous gases. The rate of gaseous evolution slowly declined with time even though NO_2^- persisted for the duration of the study. In identical experiments, very little gaseous loss occurred from a Neuenberg soil (pH 7.1) even when the pH was lowered to 6.0 or raised to 8.0.

Nitrate applied as $\text{Ca}(\text{NO}_3)_2$ to the Wellwood and Neuenberg soils was stable during aerobic incubation.

The rate of gaseous evolution tended to decrease with increased soil

pH and increased with increasing initial NO_2^- concentration when the Wellwood soil was treated with varying concentrations of NaNO_2 . This trend was especially evident in the case of van Slyke-type N_2 evolution. Further work showed sterilization of soil had little effect on the rate of van Slyke-type N_2 evolution although it greatly decreased the rate of N_2O formation. This indicates the evolution of van Slyke-type N_2 was a chemical process while the production of N_2O was, for the most part, a biological reaction.

Solution studies were performed using the amino acid glycine and NO_2^- between pHs 2 and 8 inclusive. The van Slyke reaction did not occur at any significant rate at pH values in excess of 5.0

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INTRODUCTION

Agricultural scientists are very concerned with improving the plant utilization of applied nitrogen fertilizers. Many studies have been carried out in order to increase the efficiency of fertilizer added to the soil since plant recoveries from applied nitrogen are usually only about 50% of that added. One method of increasing the efficiency of nitrogen fertilizers is to decrease losses by denitrification.

One much-studied fertilizer is urea. This product has several characteristics, which have resulted in its increased use in recent years. However, on occasion, recoveries have been found to low with this product, especially under conditions where nitrite has been found to accumulate.

Gaseous losses of nitrogen as N_2O and N_2 have been recorded by many workers where nitrite accumulates. Factors affecting the rate of loss and the exact mechanism of loss are in doubt. Recent reports have also implicated N_2O formed from these products in the depletion of the ozone layer of the stratosphere.

Many theories have been put forward to explain the mechanism of formation of N_2 . One theory suggests N_2 is formed by a van Slyke process where one atom of evolved N_2 comes from the soil N and one comes from nitrite. This theory is generally not accepted as occurring in most agriculturally important soils. Instead, the preponderance of work seems to show N_2 as being a reduction product of N_2O or indicates N_2 is formed via a transitory association of NO_2^- with phenols or carbonyl groups in the soil organic matter. To further elucidate the mechanism

involved in the formation of N_2 in fertilizer-treated soils a study was undertaken with Wellwood soil using urea as the nitrogen source. The work reported in this study involved incubations of soil and urea under aerobic conditions and in this respect was similar to conditions usually found in the field. The concentrations of nitrogen fertilizer used were quite high for, in the field, high concentrations of N occur in the soil adjacent to the fertilizer granule.

Losses of gaseous N were studied using ^{15}N tracer techniques developed in this laboratory. The effect of biological activity, soil pH and nitrite concentration on denitrification were studied.

LITERATURE REVIEW

Organic Nitrogen

At least 90% of the total nitrogen in soils is found in organic combination, (Bremner, 1965b). This nitrogen is quite stable in soils and is only slowly available to plants. Neither the reasons for this stability nor the forms of organic nitrogen are well understood.

Using acid hydrolysis methods, it has been determined that up to 40% of the nitrogen is in the form of amino acids, 10% as hexosamines and 1% as nucleic acids. These compounds themselves are not inherently stable in soils and it is only after microbial decomposition and excretion that resistance to further destruction is conferred upon the product. Several of the theories proposed to account for this stability assume a reaction between partially decomposed lignins, phenols, or other ring structures and amino compounds (Gottlieb and Hendricks 1946, Bennett 1949).

It has also been postulated by Stevenson (1960), and Bremner (1965b) that polymers of glycosamines (termed melanoidins) formed by reactions of soil carbohydrates with amino structures are responsible for some of the nitrogen stability in soils.

Since no single theory can account for all forms of soil nitrogen, it is probable that stabilization is the result of several mechanisms acting on different components at one time.

Inorganic Nitrogen

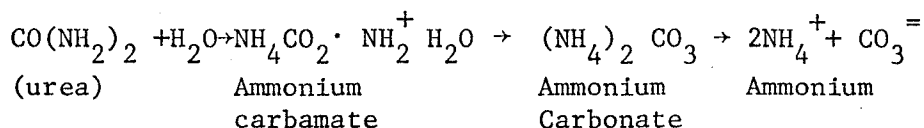
Inorganic forms make up less than 10% of the soil nitrogen but are the most important agriculturally. It is only as ammonium, nitrite, or nitrate, that plants can assimilate nitrogen. Ammonium and nitrate tend to be the predominant forms of nitrogen in soils. Nitrite is rarely present in large amounts except in cases to be described later.

There exists in soil an interchange between organic and inorganic forms of nitrogen as evidenced by the work of Jansson et al. (1955). They were able to show that through the action of decomposers organic nitrogen enters an inorganic pool - a process termed mineralization. This mineralized nitrogen mixes freely and is available to microbes for protein synthesis. The rate at which this inorganic pool is depleted depends on the C/N ratio of the decomposing substrate. If the ratio is greater than 30:1 nitrogen becomes rate limiting to microbial growth and cells must utilize the inorganic nitrogen for amino acid production. In such a case, the amount of inorganic nitrogen available to plants is diminished and that assimilated by the soil or microflora is

transformed into sparingly available organic forms. This process is termed immobilization. At C/N ratios between 20 and 30, immobilization may or may not occur, depending on the environmental conditions of the soil. If however, the ratio in the decaying tissues is less than 20:1, nitrogen is present in excess of microbial needs and will be released by the decomposers in the form of ammonia. Thus there will occur a net increase in plant-available nitrogen through mineralization.

Urea Hydrolysis

Urea is an organic compound which is deaminated by the enzyme urease to ammonia via the following pathway, (Court et al. 1964).



This process, termed ammonification by Bartholomew (1965), goes to completion without any accumulation of the intermediates. Further oxidation of NH_4^+ to NO_2^- and NO_3^- occurs via the nitrification pathway.

Ammonification of urea requires the presence and activity of the enzyme urease. The enzyme is produced chiefly by the urobacteria (Wallace and Smith 1954), although it is also found in many other microorganisms as well. It is also found in the residues of dead plants. Urease is quite stable in dry soil and its activity will not decrease significantly with prolonged storage (Gould et al. 1973).

Although adsorption may decrease urease activity, adsorbed urease

is much more stable than the free enzyme (Zantua and Bremner, 1976, 1977) Pinck and Allison (1951) suggested adsorption onto soil colloids protected the enzyme. Burns et al. (1972) feel the urease is incorporated into soil organic matter. If this were the case, small molecules such as urea and water could move through pores in the organic matter to the enzyme site and NH_4^+ could move out. Proteolytic enzymes are too large to get in close enough to destroy the urease. This would explain why soil urease is not as vulnerable as free urease to proteolysis. The characteristic urease level of a soil is determined by the degree of protection that soil can provide.

Urease in the soil may be either directly associated with soil microorganisms or adsorbed by soil colloids (Chin and Kroontje, 1963), a situation which raises the K_m of the enzyme and lowers its activity (Paulson and Kurtz, 1970). This could be due to a change in enzyme caused by colloidal adsorption which results in a decreased affinity for urea (Gould et al. 1973).

The reaction rate of urea hydrolysis has been shown to follow Michaelis-Menton kinetics and to be first order with respect to concentration of added urea when urea levels are low (Court et al. 1964, Fisher and Parks, 1958, Overrein and Moe, 1967). Simpson and Melsted (1963), as well as Laidler and Hoare (1949), found that saturation of the enzyme complex will occur as concentration of urea becomes high; the upper limit varying with the soil urease level and temperature (Overrein and Moe, 1967). As saturation levels are approached, the hydrolysis reaction becomes zero order and

may even become negative. This shift may be partially explained by end product inhibition for, if excess ammonium is added to the soil, urea hydrolysis slows (Laidler and Hoare 1949). Although urea may be absorbed by soil colloids (Broadbent *et al.* 1958, Said 1972) and soil urease amounts vary with soil type (Vasilenko, 1962), the levels of soil urease are usually sufficiently high to allow complete hydrolysis of urea in less than a week. Rarely does urea reside in the soil for longer periods.

As microbial tissue is the most important source of soil urease, physical and chemical conditions of the soil (moisture, temperature, pH, etc.) which optimize the microbial growth also optimize urease production and ultimately urea hydrolysis rate (Chin and Kroontje 1962).

The activity of soil urease with respect to temperature may be described by the Arrhenius equation.

$$\log k = \frac{-E_a}{2.303 RT} + \log A$$

k-rate constant
 T-temp °K
 E_a-activation energy
 R-gas constant
 A-a constant

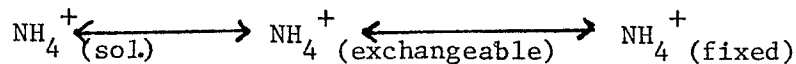
It is evident that the rate constant increases with a rise in temperature. Using an Arrhenius plot Gould *et al.* (1973) were able to show that hydrolysis rate increased linearly between 2° and 45°C. Similar trends have been reported by other workers (Fisher and Parks 1958, Simpson and Melsted 1963, Overrein and Moe 1967). The activation energy determined by Gould *et al.* (1973) was 9.8 kcal/mole.

Although urease is active over a wide pH range, the optimum pH is around neutrality (Vasilenko 1962).

The effect of moisture content on urea hydrolysis is not pronounced except in cases of very low moisture content (Volk 1966, Gould et al. 1973, Simpson and Melsted 1963, Vasilinko, 1962). Volk found only 7% hydrolysis in six days with air-dry soil; 98% with the same soil at 67% of field capacity. Gould et al. (1973), showed a slight decrease in hydrolysis rate as moisture content rose above field capacity, possibly due to decreased oxygen diffusion and therefore decreased microbial activity.

It has been shown that increased soil organic carbon levels will lead to increased rates of hydrolysis (Simpson and Melsted 1963, Gibson 1930, Gould et al. 1973, Conrad 1940, Zantua and Bremner 1976). Moe (1967) was able to increase urease activity by 40% upon mulching soils. High amounts of organic carbon will promote microbial growth and thereby increase urease content of the soil in question.

Ammonium entering the soil system, be it by desorption of fixed ammonia, mineralization or fertilizer application becomes part of a soil-ammonium equilibrium. Three forms of ammonium participate in this equilibrium; fixed, exchangeable, and soluble.



Addition or removal of one form of ammonium will therefore result in an equilibrium shift and change the relative amounts of other ammonium forms.

Another equilibrium is that between the ammonium ion and ammonia. Below pH 8, ammonium is the dominant form. As pH rises to 10, the

amount of free ammonia produced increases (Fig. 1).

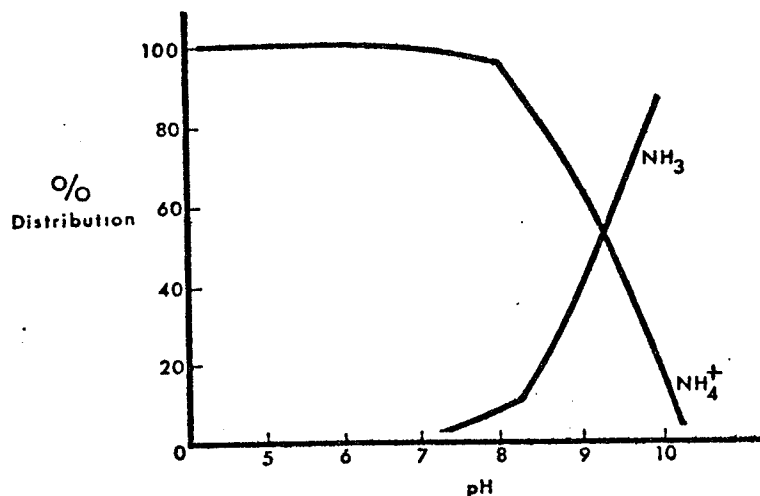
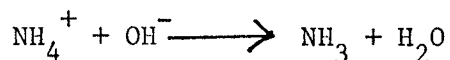


Figure 1. Percent distribution of ammonium and ammonia in aqueous systems as a function of pH (Bates and Barber 1950).

Volatilization

Ammonia volatilization is an important mechanism of nitrogen loss under specific conditions of pH, temperature and concentration. Losses as high as 59% of the added nitrogen have been reported (Volk, 1959).

The following reaction which describes the loss of NH_3 illustrates the importance of pH (Wahhab *et al.* 1960).



Although some gaseous loss will occur at pHs as low as 5.0 (Filimonov and Strel'nikova 1974) losses do not become significant until the pH approaches neutrality. Losses continue to increase with increasing pH (Volk 1959, Filimonov and Strel'nikova 1974, Earnst and Massey 1960).

Upon application of urea, volatilization from poorly buffered slightly acid soils may be due to the rise in pH induced by hydrolysis of urea to ammonium carbonate (Earnst and Massey 1960, Moe 1967). Increasing pH by liming also results in an increased volatile loss (Volk 1959). Earnst and Massey (1960) have proffered the following theories in an attempt to explain the increased loss with increased pH;

- 1) calcium saturation of the soil exchange complex is increased with increasing pH, leaving fewer sites for ammonium adsorption on the colloid,
- 2) increased OH^- activity pushes the reaction described by Wahhab et al. (1960) to the right, resulting in increased ammonia loss.

Urea in air-dry soil is quite stable due primarily to the inhibition of hydrolysis (Fenn and Escarzaga 1976). However, as soil moisture content increases, volatile ammonia losses increase also, the maximum being reached at a point halfway between air dry status and field capacity (Volk 1959). As moisture increases above this point, volatilization rates decrease. This could be because:

- 1) more moisture allows more NH_3 to become dissolved throughout,
- 2) high moisture results in a high nitrification rate, thereby lowering ammonium levels in the soil (Prasad 1976).,
- 3) As water content increases, the free pore volume decreases, thus limiting diffusion of gaseous ammonia from the soil (Gasser 1964).

Drying the soil from field capacity increases NH_3 losses by increasing the concentration of NH_4^+ in the soil water (Earnst and Massey 1960).

High temperature also promotes volatile losses (Earnst and Massey 1960, Prasad 1976). At low temperature, nitrification can more easily keep up with hydrolysis and so NH_3 accumulation is less likely to occur (Volk 1959). At higher temperatures, hydrolysis is completed quickly and a high concentration of ammonium builds up in the soil with an attendant increase in volatile loss.

Since ammonia is usually in its cationic form in soil, the negatively charged colloids of the soil will hold NH_4^+ and prevent its volatilization. Soils with a cation exchange capacity less than 10 m. eq. per 100 grams are incapable of preventing volatile loss. However, with increasing cation exchange capacity, losses decrease (Filimonov and Strel'nikova 1974). The addition of organic matter will result in an increase in exchange capacity and it is for this reason that incorporation of organic residues will decrease ammonia volatilization (Mortland 1958, Gasser 1964).

It has been shown by many workers that volatilization rates are inversely proportional to the depth at which urea is placed in the soil (Gasser 1964, Earnst and Massey 1960, Overreïn and Moe 1967). Earnst and Massey (1960) were able to show a 75% decrease in volatilization when urea was covered to a depth of 3.5 cm.

Nitrification

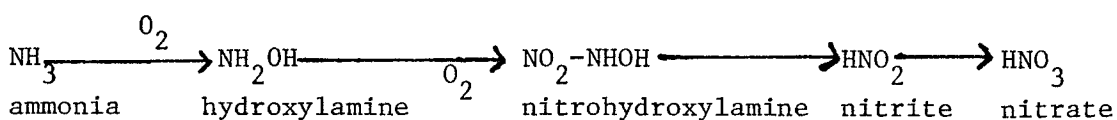
Nitrification is defined by Alexander (1965) as "the biological conversion of nitrogen in organic or inorganic compounds from a reduced to a more oxidized state". This is a very important process in soils for several reasons. Among these is the fact that oxidized nitrogen compounds are subject to losses via leaching and denitrification. Of importance also is the fact that over a period of years nitrification tends to result in acidification of the profile, particularly in poorly buffered soils.

Although some heterotrophs possess the ability to carry out nitrification, they can not use the reaction as a sole source of energy (Hirsch et al. 1961). Nitrosomonas spp. and Nitrobacter spp. which oxidize NH_4^+ to NO_2^- and NO_2^- to NO_3^- respectively, meet all their energy requirements by means of these exothermic reactions. Nitrosomonas spp., characterized by N. europaea, is a rod-shaped organism 0.9-1.0 x 1.1-1.8 μ and is sometimes flagellated with one or two polar flagella. An example of Nitrobacter spp. is N. winogradskyi, a short, gram negative, non-motile rod measuring 0.6 - 0.8 x 1.0 - 1.2 μ (Alexander 1965). Eight other nitrogen autotrophs have been reported, although the taxonomic validity of two is open to question (Buchanan and Gibbons 1974).

Both the oxidation of NH_4^+ to NO_2^- and NO_2^- to NO_3^- are exothermic, the enthalpy being -65.0 to -84.0 kcal/mole and -16.5 to -20.0 kcal/mole respectively (Gibbs and Schiff 1960). It is evident, therefore, that Nitrosomonas spp. get potentially more energy per mole of substrate than Nitrobacter spp. Nitrite concentration is usually rate limiting to growth of Nitrobacter spp. and as a result, rarely accumulates under natural conditions.

Nitrite accumulation does occur however when free NH_3 levels are high. This condition results when ammonia or ammonia-yielding fertilizers are added to soils of high pH. Free ammonia, which is found only at pHs in excess of 7.2 (Morrill and Dawson 1967, Aleem and Alexander 1960) inhibits the Nitrobacter spp. thereby stopping the oxidation of NO_2^- .

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



Only NO_2^- and NO_3^- are found in soil, the other intermediates are not detected. Hydroxylamine and nitrohydroxylamine could occur intracellularly (Alexander 1965). During this oxidation, the valency of N changes from -3 in NH_3 to +5 in NO_3^- .

As nitrification is, by definition, strictly biological, environmental factors which affect the growth rates of Nitrosomonas spp. and Nitrobacter spp. will affect nitrification rate as well.

Nitrification patterns are of four basic types, the occurrence of each governed primarily by pH (Morrill and Dawson 1967, Dancer et al. 1973). The patterns observed were:

- 1) Type I Ammonium rapidly oxidized to NO_2^- which accumulates above pH 7.3.
- 2) Type II Ammonium and NO_2^- oxidized to NO_3^- -pH 6 - 7.3.
- 3) Type III Ammonium oxidized to NO_3^- without appearance of NO_2^- -pH 5.5 - 5.9.

4) Type IV No ammonium oxidation - pH less than 5.4.

Nitrobacter spp. grow well in neutral or slightly acid soils and under normal conditions can oxidize NO_2^- as soon as it is made available. If, however, the pH rises above neutrality, Nitrobacter spp. activity is limited and a transient accumulation of NO_2^- may occur (Type II) or NO_2^- oxidation may cease completely (Type I).

Aleem and Alexander (1960) demonstrated that with increased pH, the ratio of nonionized to ionized ammonia increases. This results in a suppression of O_2 uptake by Nitrobacter spp. and thereby lowers its activity. Thus NO_2^- oxidation is slowed and an accumulation of this oxide of nitrogen occurs. Work done by Tyler and Broadbent (1960) has shown that the presence of the NO_2^- ion itself will also inhibit its own oxidation by Nitrobacter spp. and thereby promote NO_2^- stability. This inhibition decreased with increasing pH. Nitrosomonas spp. are not as susceptible to inhibition by either ammonia or nitrite.

The optimum pH of Nitrosomonas spp. is in the alkaline range between 7.6 and 8.2 (Morrill and Dawson 1967, Hofman and Lees 1953) and activity is still significant at pH 9.0. This is contrasted with a pH optimum of 6.2 - 7.0 for Nitrobacter spp. Addition of urea to even acid soils will result in local alkaline sites around the pellet (Clark, et al. 1960, Soulides and Clark 1958). Thus addition of urea has two effects on the bacterium's continued growth;

- 1) it raises the pH as it is hydrolysed which in itself is deleterious to the bacterium's continued growth.

- 2) the ratio of NH_4^+ to NH_3 decreases as pH rises which is another factor limiting NO_2^- oxidizers.

The effect becomes more pronounced as concentration of added urea increases (Hauck and Stevenson 1965, Aleem and Alexander 1960, Wetselaar et al. 1972). Thus a high concentration of ammonia-N in combination with high pH, both conditions occurring at a pellet site, will result in NO_2^- accumulation. Hauck and Stevenson (1965) and Bezdicek et al. (1971) have shown that with increasing pellet size, higher local concentrations of urea result and NO_2^- accumulation occurs.

As a rule, biological reaction rates rise with increasing temperature. Such is the case with nitrification where rates have been reported to increase over the range of $2^\circ - 40^\circ\text{C}$ (Justice and Smith 1962, Kowalenko and Cameron 1976). At temperatures above 40°C denaturation of enzymes occurs and nitrification ceases. Justice and Smith (1962) showed Nitrobacter spp. to be more sensitive to low temperature than Nitrosomonas spp. causing a slight increase in NO_2^- concentration in soils incubated with ammonium sources at 2°C . Work by Broadbent and Tyler (1962) shows similar trends. Anderson (1962) however, found no accumulation of NO_2^- at such low temperatures though the overall nitrification rate was depressed greatly.

Nitrification has been shown to decrease with decreasing soil moisture content between field capacity and the air-dry state (McGill 1971, Justice and Smith 1962 and Reichman et al. 1966). Alexander (1965) suggests the optimum moisture content of a soil for nitrification to proceed is between 50 and 75% of moisture holding capacity. Further

increases in soil moisture result in decreased O_2 diffusion into the soil, thereby diminishing nitrifier activity. The lowered O_2 tension will allow the growth of anaerobic denitrifiers with a concomitant loss of N_2 via denitrification.

Nitrification has been shown to take place on colloidal surfaces (Lees and Quastel 1946). As moisture levels are lowered, the concentration of added NH_4^+ in the water film surrounding the colloid increases. As mentioned previously, NH_4^+ inhibits Nitrobacter spp. with the consequence that at low moisture contents added NH_4^+ may nitrify only as far as NO_2^- and then accumulate (Justice and Smith 1962).

Denitrification

The denitrification process can be defined as "the gaseous loss of nitrogen either by biological or chemical mechanisms but exclusive of ammonia volatilization", (Broadbent and Clark 1965). As mentioned in the above definition, there are two basic types of denitrification - chemical and biological - each of which will be discussed.

A. Chemical

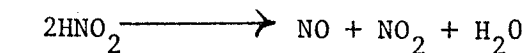
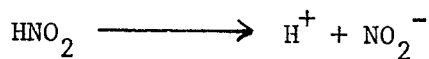
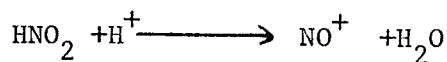
Addition of NH_4^+ or NH_4^+ -yielding fertilizers such as urea have, in many cases resulted in losses of the added N. These losses have been found to occur in apparently well-aerated soils, especially when accumulations of NO_2^- have occurred (Jones and Hedlin 1970, Nelson and Bremner 1970, Bollag et al. 1973). Losses have been significant even if the soils in question have been sterilized, confirming the importance of chemo-denitrification (Cady and Bartholomew 1963, Reuss and Smith 1965,

Van Cleemput 1974). It has been postulated by Jansson and Clark (1952) and Greenland (1962) that nitrogen is nitrified in aerobic portions of the soil profile and carried to anaerobic pockets or centres of soil granules where it is subsequently denitrified. However, similar amounts of NO_3^- -N added to the aerobic soil are quite stable, a fact which is not consistent with the anaerobic site theory. If anaerobic sites were responsible for denitrification, losses of the added NO_3^- -N would occur at similar rates as NH_4^+ -N losses.

Broadbent and Clark (1965) suggest four pathways of nitrogen loss via chemo-denitrification.

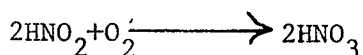
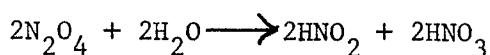
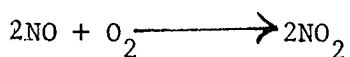
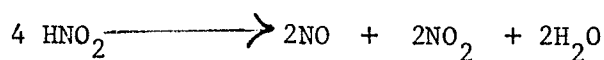
a) Decomposition of nitrous acid.

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



Nitric oxide is present only in anaerobic systems (Smith and Clark 1960, Reuss and Smith 1965) for, in the presence of O_2 , it is rapidly oxidized to NO_2 . Nitric oxide has also been shown to occur when NO_3^- -N is added to acidic soils under anaerobic conditions (Wijler and Delwiche 1954, Nommik 1956 Cady and Bartholomew 1960, 1963). Cady and Bartholomew (1963) concluded that this was due to the self-decomposition of nitrite formed during the

reduction of nitrate. Nitrogen dioxide is very soluble in water and in most cases is trapped by soil moisture before it leaves the soil system (Broadbent and Clark 1965, Broadbent and Stevenson 1966). The oxidation of NO and its subsequent hydration are represented as follows (Nelson and Bremner 1970).



It is evident that the presence of the HNO_2 molecule is obligatory before this disproportionation reaction can commence. Its presence in soil depends greatly on pH for, with a K of 6.0×10^{-4} , only 1.6% NO_2^- -N exists in the HNO_2 form at pH 5.

To test the hypothesis that NO and NO_2 are oxidized to NO_3^- , Nelson and Bremner (1970), injected the gases into moist soil samples and then analysed the samples for nitrate content. An increase in nitrate level was recorded. In a further experiment, vials of alkaline KMnO_4 were put in the centre of sealed vessels containing soil samples to which nitrite had been added. Alkaline KMnO_4 will absorb NO and NO_2 and thereby lower the amount of nitrate produced if NO and NO_2 are intermediates in the conversion of nitrite to nitrate. Nitrate production was lower in samples incubated with the KMnO_4 than in controls where KMnO_4 was absent.