

LABORATORY STUDIES ON THE BEHAVIOUR OF MINERAL NITROGEN  
IN THREE MANITOBA SOIL MATERIALS

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by

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## ABSTRACT

A study was undertaken of the behaviour of mineral nitrogen in three Manitoba soil materials under laboratory conditions. The aim was to trace and understand the changes undergone by mineral nitrogen during incubation, especially those changes related to losses of applied fertilizer nitrogen by chemical denitrification involving nitrite.

A preliminary evaluation of the extraction-distillation procedure (16) of estimating mineral-N revealed that recoveries of applied ammonium- and nitrate-N were satisfactory on all three soils. Nitrite recoveries were more complete and reproducible using the Griess-Illosvay procedure (13). An interaction between ammonium and nitrite ions occurred when extracts of Wellwood and Holland were stored at room temperature, leading to an equivalent loss of N from each ion.

Twelve-day incubations with 50 ppm-N revealed losses of mineral-N which could be predominantly ascribed to ammonium fixation. The nitrogen mineralized during incubation was reduced in the presence of fertilizer, especially ammonium sulphate, in Wellwood and Holland. Salt effects are a possible explanation for this. The nitrification rate was increased in the calcareous Lakeland in the presence of phosphate, whilst net mineralized-N was reduced. This could reflect an increase in microbial activity with phosphate, leading to enhanced immobilization. Incubations for fifteen weeks showed large discrepancies in the recovery of N from ammonium sulphate, urea, sodium nitrite, and potassium nitrate. Mineralization and immobilization balances appeared mainly to account for this observation.

Incubations with higher rates of fertilizer revealed a marked tendency for nitrite accumulation in urea-treated Wellwood, with a much

smaller tendency in Lakeland and Holland. This effect was more marked at the higher rate of application. Ammonium sulphate did not lead to marked nitrite buildup, the difference between the 200 and 800 ppm-N levels being very small. Nitrobacter and Nitrosomonas activities per se appear to be important determinants in this process. Losses of mineral-N were incurred in Wellwood and Holland, their magnitude being higher with the initially most acid soil material. Chemical denitrification was a probable mechanism of loss. Thus losses generally increased with higher fertilizer concentrations, possibly associated with increased nitrite turnover and high initial ammonium concentration. Also, losses frequently increased with time of incubation, a feature possibly related to the development of acidity with nitrification.

Investigations using three techniques to demonstrate the interaction of soil and nitrite showed that nitrite is an important avenue of N loss. The losses obtained confirmed the apparent pH relationship noted above in that nitrite-N disappearance increased with decreasing pH. This was not a direct pH effect, however, but was mediated through various pH-dependant factors, prominent among which was organic matter. The level of exchangeable ammonium was also influential.

The study revealed that losses of fertilizer-N can occur under conditions of good aeration, concomitant with active nitrification.

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## CHAPTER ONE

### INTRODUCTION.

Nitrogen is commonly the most important fertilizer element applied to crops. This manifests itself in a number of ways, thus (a) crops have a large demand for nitrogen, and respond quickly to its application, (b) agricultural soils generally are able to supply a larger percentage of the crop's need for P, K, and other essential nutrients than they are of nitrogen, and (c) the total financial outlay on nitrogen fertilizer is generally greater than on all other nutrients combined. Hence, the efficiency of utilization of applied nitrogen is of prime importance from the agronomic and strictly scientific points of view. Relevant questions that are being asked concern the fate of nitrogen applied to soils (9), particularly in view of the demonstration that significant volatile losses of nitrogen occur under aerobic conditions where ammonia volatilization and biological denitrification are minimal.

The present study is an attempt to understand the behaviour of applied nitrogen (as influenced biologically) in relation to deficits of mineral nitrogen in three soil materials covering a range of pH, especially as these could be accounted for by losses of nitrogen from the soil system. Changes in applied fertilizer nitrogen were studied under well-defined laboratory conditions. The role of aerobic chemical denitrification as a mechanism of loss was given particular attention. The study resolved itself into fairly well-defined subsections which underline the rationale of the approach to the problem. These were:

(a) A brief evaluation of the procedures used to estimate mineral nitrogen. This was necessary since information was lacking on the recoveries

of applied ammonium-, nitrate-, and nitrite-nitrogen in the soil materials used, as estimated by the extraction-distillation procedure of Bremner and Keeney (16). Nitrite occupied a central part in the investigation, so that it was desirable to compare nitrite recoveries as estimated above with those from an independent method.

(b) The determination of mineral nitrogen changes during short-term incubation of soil materials treated with 50 ppm ammonium sulphate and urea-nitrogen. Changes were followed by periodic measurement of exchangeable ammonium-, nitrate-, and nitrite-nitrogen. This level was chosen since it approximates the level of 100 lbs per acre often used in field applications.

(c) Incubations for fifteen weeks were made under the same conditions as the above experiments, to determine whether the same trends and agents of mineral nitrogen deficits operated over an extended period of time.

(d) Mineral nitrogen deficits were investigated with higher rates of fertilization, on the premise that this would lead to an accumulation of nitrite. Some of the factors in nitrite accumulation were studied, together with the relationship of nitrite to nitrogen losses. The concentrations used were comparable to concentrations resulting from the band application of fertilizers.

(e) The direct role of nitrite in mineral nitrogen deficits was studied by subjecting soil samples to various treatments which demonstrated more clearly the instability of added nitrite. Two of these techniques, namely autoclaving and air-drying of treated samples, were further used to study some of the factors involved in chemical denitrification.

## CHAPTER TWO

### REVIEW OF LITERATURE

#### INTRODUCTION

Allison (7,9) has reviewed the many nitrogen balance experiments in which the credit side, namely applied fertilizer N, N from rainwater, and soil N were balanced against debit N in the form of plant uptake and leaching. It was found that overall deficiencies occurred with the passage of time on cropped and uncropped soils, indicating a gaseous loss of N. This amounted to about 15% in lysimeter experiments and 0-47% in greenhouse experiments (9). In long-term field experiments where leaching losses were not determined, unrecovered N frequently ranged between 50 and 75% of that applied (7). Most of this was generally lost probably by leaching, but gaseous losses appeared to be nearly as large in some cases.

Net loss of N, both gaseous and soluble, partly explain why plant recoveries of fertilizer N are so low. Viets (111), in a study of irrigated and dryland soils of western United States, estimated that crops on average recovered only 7-50% of applied N. These low recoveries may be explained by (a) loss of nitrogen from the system, and (b) chemical and biological conversion of applied N into unavailable forms (11, 23, 68). These facts have become more apparent in recent years with the enormous increase in fertilizer N use, a practice which has also intensified those efforts directed towards understanding the various channels of loss.

This review will attempt to cover the various channels by which N may be lost from soils as a gas. In this context, denitrification is defined as the chemical or biological reduction of nitrite and/or nitrate to gaseous forms. It is pointed out that this is not the definition given

in the Glossary of Terms approved by the Soil Science Society of America<sup>1</sup> which reserves this term for biological denitrification only. Since the thesis concerns itself with conditions of loss which would be difficult to explain on a microbiological basis alone, special attention is given to the occurrence and precipitating circumstances of chemical denitrification. Account is also taken of the role of ammonia volatilization in gaseous N losses.

#### BIOLOGICAL DENITRIFICATION

This process is carried out by a number of species of facultatively anaerobic bacteria (29). Nitrates and nitrites are used instead of oxygen as electron acceptors when the latter becomes limiting. The enzyme systems involved have several features in common, and appear to differ basically in the final linkage to the electron acceptor. This possibly explains why both systems compete with each other for the electrons produced from metabolic processes. Since the terminal electron acceptor system generally has a greater affinity for oxygen, it is normally used in preference to nitrate. Skerman and MacRae (100) showed that nitrate reduction by Pseudomonas denitrificans was inhibited by very low levels of oxygen. The dependence of denitrification upon oxygen status has been shown in soils by many workers (10, 24, 28, 69, 119). Pichinoty (87) found evidence also for the suppression of nitrate, nitrite, and nitrous oxide reductase biosynthesis. Some organisms such as Denitrobacter licheniformis (29) possess a highly active nitrate reductase system whose action is suppressed only by a fairly high aeration of cultures.

Campbell and Lees (29) point out that anaerobic conditions do not lead to nitrite accumulation from the reduction of nitrate. Nitrite instead is simultaneously reduced to nitrous oxide and nitrogen gas. Wijler

<sup>1</sup>Soil Sci. Soc. Am. Proc. 26: 307.

and Delwiche (119) showed that almost 100% of the added nitrate was reduced to nitrous oxide and nitrogen gas under carbohydrate-induced anaerobiosis. The sequence nitrate → nitrite → nitrous oxide → nitrogen gas has been established by many workers on a range of soils (5, 28, 40, 80, 98). Where complete anaerobiosis occurred, as in waterlogging, Wijler and Delwiche (119) showed that nitrogen gas was the main product of reduction. Under partial anaerobiosis, however, some of the intermediate nitrous oxide escaped resorption by the soil and accumulated in the atmosphere above. Woldendorp (121) showed that nitrate reduction did not yield significant increases in ammonium. Where the latter did accumulate under waterlogged conditions, less than 1%N came from nitrate-N, the remainder being the product of ammonification. Campbell and Lees (29) point out that mild anaerobiosis in bacterial cultures leads to ammonium accumulation, whereas complete anaerobiosis gives nitrous acid and nitrogen gas. It would appear that ammonium accumulation from nitrate in cultures indicates a demand for cellular N and is the result of normal assimilatory nitrate reduction. Loss of gaseous N, however, indicates a need for an electron acceptor in the absence of oxygen. The factors governing these two relationships are little understood, especially in soils (29).

#### Role of oxygen.

Some confusion appears in the literature as to the role of oxygen in the inhibition of biological denitrification. Skerman and McGarity (100) found that the threshold level of oxygen concentration which inhibited denitrification in Pseudomonas denitrificans was very low. Meiklejohn (77), devoting a paper to aerobic denitrification, reported denitrification by P. denitrificans and P. fluorescens in aerated cultures. Her results were

supported by the work of Marshall et al. (71) on the same species, and by Korsakova (66) on Achromobacter siccum and P. aeruginosum.

It is possible that no real contradictions occur in the foregoing observations, and that the explanation lies in the use of the words "aerobic" and "anaerobic" (21). Whereas the oxygen tensions in their experiments remained high extra-cellularly, Marshall et al. (71) conceded that limited diffusion into the cell could occur. Under conditions where the biological demand for oxygen is high, as in the presence of a rapidly decomposing substrate, the micro-environment of the bacteria may become essentially anaerobic whilst the soil or solution medium is well-aerated by ordinary standards (17). Thus the word "aerobic" can be applied to the medium as a whole, whilst "anaerobic" describes more accurately the oxygen environment of the bacterial cell which suffers an inadequate diffusion rate.

Instances have also been recorded where the reduction of nitrite, but not nitrate, can occur aerobically. Kefauver and Allison (64) showed that Bacterium denitrificans reduced nitrite in an abundance of oxygen, whilst Skerman et al. (99) reported that reduction of nitrite by Achromobacter liquefaciens was essentially independent of oxygen concentration.

In bacterial cultures which contained nitrifying and denitrifying organisms, both nitrification and denitrification have been observed to occur (9). Here, the explanation would presumably be that the autotrophs depleted available oxygen and enabled the denitrifiers to thrive. These experiments indicate that a balance of organisms is possible when the terminal respiration of one group utilises the metabolic product of another. This observation could explain the simultaneous reduction of oxygen and nitrate noted by Kefauver and Allison (64) and Cady and Bartholomew (28).



### Denitrification in apparently aerobic soils.

Rapid and complete denitrification in submerged soils has been reported by many workers (17, 18, 40, 62, 123). Much of the work which reports consistent losses of nitrogen in field, greenhouse, and laboratory, however, has been done with soils at or slightly below field capacity for the majority of the time. These moisture levels are common for agricultural soils (7), and, under these conditions, soil macropores would be empty and accessible to oxygen, so that the medium as a whole would be considered aerobic. It is concluded from the work with bacterial cultures that, if conditions in a vigorously aerated medium can lead to denitrification, then the nature of soils might certainly be expected to be conducive to losses of nitrogen.

Role of oxygen. The importance of oxygen supply has been demonstrated in the works of Allison et al. (10), Broadbent (19), Broadbent and Stojanovic (24), Cady and Bartholomew (28), Carter and Allison (32), and McGarity et al. (75). The extent of denitrification varied inversely with the partial pressure of oxygen in the air surrounding the soil, this relationship generally holding for the wide range of 0.1% to 19.0% oxygen by volume. The conditions of aeration employed were either a static mixture of gases above the sample, or a stream of gases passing through a container and over, but not necessarily through, the soil.

These experiments indicated the potential denitrifying capacity of soils in contact with adequate oxygen. When the technique employed ensured that the gaseous atmosphere actually passed through the soil, aerobic losses were markedly reduced. Carter and Allison (32), and Bremner and Shaw (18), demonstrated this by sucking air through a column of soil enclosed in a glass cylinder. Both authors concluded that where adequate

aeration was thus assured, biological denitrification losses were negligible under atmospheric oxygen. These conditions of aeration, however, would not be expected to prevail in field soils, where gaseous diffusion to and from the atmosphere is the main mechanism of movement.

Soil factors which have been found to lay critical emphasis on the rate of replenishment of oxygen are texture, structure, and presence of easily decomposable organic matter (21). The small pores of fine-textured soils are prone to the development of anaerobic conditions even though many of the larger pores are filled with air. Nommik (80) emphasized the role of structure when he made the observation that denitrification decreased with increasing size of aggregates. However, beyond a certain size limit, the rate of denitrification increased due to what was attributed to be the longer time for oxygen to diffuse into the centre of large aggregates. The role of carbonaceous material in promoting nitrogen losses has already been referred to (p. 6). Suffice it to say here that a number of workers (10, 24, 31) have supplemented this work with apparently aerobic soils, and found that easily decomposable organic materials promoted denitrification at both high and low oxygen levels. Cady and Bartholomew (28) used the concept of "biological pressure" to discuss the effect of added materials on nitrogen losses. Presumably, at high biological pressure, the demand for an alternative electron acceptor to oxygen is high due to very active intermediary metabolism and to a rapid uptake of available oxygen. The influence of texture, structure, and carbonaceous material suggest that the rate of oxygen supply to soil microorganisms is more important than the partial pressure of oxygen in determining whether denitrification will occur in apparently aerobic soils.

Coincidence of nitrification and denitrification. Because of the general lack of homogeneity in soils at the microbe level, the probability exists that nitrification and denitrification can occur simultaneously, so that a demonstration of the former does not necessarily rule out the latter. As early as 1917, Russell and Richards (95) found that loss of nitrogen occurred from farmyard manure under fluctuating aerobic and anaerobic conditions, but none occurred when either aeration status was constant. It was postulated that nitrate formed in the aerobic zone was denitrified when it diffused into anaerobic regions. It was noticed in the data of Bremner and Shaw (18) that the evolved nitrogen accounted for more than that nitrogen which was originally present as nitrate and nitrite. This phenomenon occurred on two saturated soil samples which could have contained dissolved or trapped oxygen initially.

Jansson and Clark (59) reported the overall nitrification of ammonium in their organically amended samples, but total nitrogen figures indicated a nitrogen deficit even when ammonia volatilisation was accounted for. Added ammonium and nitrate gave comparable deficits. Their conclusion that a micromosaic of aerobic and anaerobic spots could account for simultaneous nitrification and denitrification has been supported by the work of Arnold (12), Greenland (56), Lowenstein et al. (67), and Meek and McKenzie (76). The possibility of greater losses in soils where nitrification is active was suggested by Arnold (12), who observed an evolution of nitrous oxide from field soils when ammonium was added. Patrick and Wyatt (85) found alternate submergence and drying led to greater losses than continuous submergence. In the latter case, only that nitrate and nitrite present before submergence was denitrified, whilst the introduction of an aerobic phase led to an additional accumulation of nitrate which was subsequently

reduced in the anaerobic phase.

Effect of plants. In the field, plant roots can yield microregions conducive to denitrification. Thus, Woldendorp (120) observed increasing losses of nitrogen in the rhizosphere as the result of (a) uptake of oxygen by the roots, and (b) oxygen deficits produced by rhizosphere organisms feeding on root excretions.

### Summary

Biological denitrification is characteristically a process which occurs under conditions of oxygen deficiency. This may be produced by an individual or combined effect of low oxygen partial pressure and slow diffusion. Its occurrence in submerged soils is understandable. However, it is now recognized to be an important process in aerobic soils, primarily due to the mosaic of microhabitats which affect the ease of availability of oxygen. Allison (9) suggested that biological denitrification is second only to leaching as the cause of net losses of nitrogen from soils.

### CHEMICAL DENITRIFICATION

Broadbent and Clark (21) note that biological denitrification does not adequately explain all losses of gaseous nitrogen from soils. Various workers have recorded that nitrate nitrogen added to well-aerated fallow soil could be quantitatively recovered after a period of incubation, whilst added ammonium on similar plots showed a net gaseous deficit not accounted by volatilisation. These losses were especially pronounced when nitrite accumulated, amounting to more than 50% of added ammonium (37). In contrast to biological denitrification, this process can occur under fully aerobic conditions, with or without the occurrence of anaerobic microhabitats, and with sterilized or unsterilized soils if nitrite is present (92). Since it seems to be associated with nitrite, it may not be a strictly chemical

process in so far as the accumulation of the latter may be the result of biological ammonium oxidation (72).

There is extensive evidence that chemical denitrification occurs in soils (7, 9, 37, 39, 54, 102, 104, 110, 116, 119). In the following discussion emphasis will be placed on those processes which have been found to be feasible in general agricultural soils.

#### Role of nitrite.

In view of the known instability of nitrite (19), it was suggested as early as 1914 by Temple (108) that the low level of this ion in acid soils could be related to possible gaseous nitrogen losses. Robinson (93) made a similar statement. It is quite possible, however, that nitrite accumulated little in these experiments because of the relatively faster rate of biological oxidation to nitrate. The soils subjected to these observations received no nitrogen amendments, so that there would be little tendency for nitrite to accumulate aerobically during nitrification (3).

Dhar (41) drew attention to the role of nitrite in chemical losses of gaseous N. He suggested that the N lost was the result of an oxidation process (presumably biological) followed by photochemical and catalytic decomposition of the nitrite produced. Different kinds of soil materials were active as catalysts in promoting N loss. Wahab and Uddin (116, 117) produced evidence to indicate that the soils in Dhar's experiments did not act catalytically, and that the observed effect was due to the varying instability of nitrite at the different pHs of the soil materials used.

In pot experiments with acid sandy soils, Gerretsen and de Hoop (54) found losses up to 74% of N added as ammonium sulphate. These losses were not due to ammonia volatilization, and biological denitrification was

ruled out since added nitrate was fully recoverable. A biological agency was necessary, however, since volatile losses stopped with pasteurization. This was interpreted to indicate that the nitrite formed biologically was decomposed chemically. This work indicates that, under the appropriate conditions, nitrite accumulation is not a necessary prerequisite of chemical losses, since only traces were detected in these experiments. Soil pH appeared to be an important factor, since losses were considerably reduced if pH was made greater than 5.5 through additions of calcium carbonate.

Wagner and Smith (114) observed a direct correlation between nitrite accumulation and N losses when a range of commonly used N carriers were applied. The maximum of nitrous and nitric oxides evolved coincided with maximum nitrite. Losses were noted from various carriers in proportion to their nitrite-accumulating tendency. Thus, on one soil, urea gave 85% loss, aqua ammonia 24.7% and ammonium sulphate 0%. That biological denitrification was also occurring was indicated by losses from added nitrate (24.7%) on another soil, compared to a 46.3% loss from ammonium sulphate.

Soulides and Clark (104) also noticed the relationship between nitrite and losses of mineral N. On grassland soils and their intertilled counterparts, losses were indicated from urea-treated soils whenever nitrite accumulated. Grassland soils were shown to have a slower nitrification rate than their intertilled equivalents, so that ammonium persisted and consequently had a more prolonged effect on the oxidation of nitrite to nitrate (see next major section). It was found that deficits were invariably greater on these soils. In a subsequent paper, Clark and Beard (38) confirmed the role of nitrite in total N deficits, and in a further extension of this study, Clark et al. (39) examined 41 soils, and found

chemical losses ranging from 2 to more than 40% of applied urea-N. Mineral-N deficits greater than 25% were recorded for poorly buffered soils which accumulated nitrite during incubation. These were either initially acid in reaction, or achieved acidity as the result of nitrification. When potassium nitrite was added, deficits were directly linked to initial soil pH. The latter did not change as the result of nitrification, so that initially alkaline soils were found to remain so and give very little N losses.

The importance of soil pH, buffering, and nitrifying capacities are stressed by Clark et al. (39), as suggested above. Well-buffered acid soils or alkaline soils did not lead to mineral nitrogen deficits since nitrite did not accumulate in the former, whilst pH remained alkaline in the latter. When urea was added, maximum losses occurred at an initial and final soil pH of 7.3 and 6.3 respectively (average values). Larger deficits were obtained for soils in the range 5.5 - 6.5 than 4.5 - 5.5, an observation which could reflect the chemical oxidation of nitrite to nitrate at lower pH (90). Soils of low nitrifying capacities, especially grassland soils, showed a tendency for greater mineral-N deficits attributable to the greater persistency of nitrite. Similar findings were reported by Carter and Allison (31) using ammonium sulphate.

Tyler and Broadbent (110) suggested that pH is not the sole factor governing N losses from nitrites. Losses were obtained in their soils even after liming. At pH 7.2 and 6.9 for two different soils, losses were 20% and 30% respectively of the 200 ppm nitrite-N added. Losses from the unlimed soils were higher, and chemical oxidation of nitrite was observed in unlimed, but not limed, soils. Sterilization had very little effect on N changes in three out of four soils. The authors reiterated earlier suggestions that low nitrite levels, especially in acid soils, could be the

result of instability leading to gaseous losses. This suggestion was made, however, with the present high rate of fertilization in mind.

Mechanisms of chemical denitrification.

Several mechanisms have been proposed to account for the chemical losses of nitrogen gas from nitrites. Some of these have been found to be of only minor significance.

Van Slyke-type reaction. The true Van Slyke reaction involves the formation of nitrogen gas from nitrous acid and amino acids (9). One atom of nitrogen is donated from each of the reagents to give a molecule of gas. It is generally accepted to be insignificant in soils for the reasons: (a) a pH of 5.0 or lower is required for appreciable reaction, and (b) free amino acid concentrations in soils are too low.

Van Slyke-type reactions between nitrous acid and the amino groups of urea, or even the ammonium ion itself, have also been proposed. These are also discounted on the basis of pH and/or concentration of reactants required (102, 96). At pH 4.1, Smith and Clark (102) found that reaction of nitrous acid and ammonium sulphate proceeded at about one-third the rate of nitrous acid and alanine reaction. In both cases, concentration of sodium nitrite added was very high at 61,700 ppm of solution.

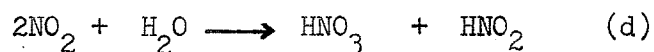
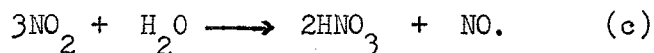
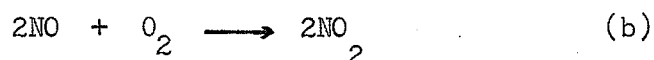
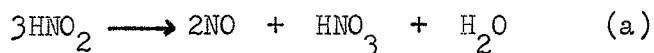
Allison (8) suggested that nitrous acid can interact with ammonium to form ammonium nitrite, which simultaneously decomposes to nitrogen gas:  $\text{NH}_3 + \text{HNO}_2 \longrightarrow \text{NH}_4\text{NO}_2 \longrightarrow \text{N}_2 + 2\text{H}_2\text{O}$ . Ammonium nitrite could also arise from ionic species, so that this theory could account for losses in acidic and alkaline media. Support for this has come from the work of Wahab and Uddin (116, 117) who noted significant losses of gaseous N on air-drying soils (pH greater than 8.0) in the presence of nitrite and ammonium



concentrations greater than 25 ppm. According to these authors, losses by interaction of ammonium and nitrite were ten times those from nitrite alone. Concentration effects were noted, especially under the influence of ammonium which gave an optimum effect at a five to one ratio of ammonium to nitrite. Differences between soils in their promotion of gaseous losses were due to pH alone, since variations disappeared when buffered solutions were used for the ammonium and nitrite solutions. Dhar (41) found evidence for the photochemical stimulation of ammonium nitrite decomposition. Ewing and Bauer (48), studying the theoretical interaction between nitrous acid and ammonium on a kinetic basis, concluded that soils likely to show this reaction significantly are (a) soils with large amounts of exchangeable ammonium, (b) acidic soils, and (c) dry but not desiccated soils.

Whilst Allison and a few other workers are strongly in favour of a role for ammonium nitrite in chemical denitrification, many other workers are equally against it. Smith and Clark (102) treated soil systems with ammonium sulphate and potassium nitrite, discovering that the N lost came mainly from nitrite N. In acid media, it is known that nitrite has a stronger tendency to decompose than to react with ammonium (21). Reuss and Smith (92) discovered no relationship between N losses from potassium nitrite and the amount of ammonium in the system.

Chemical decomposition of nitrous acid. The decomposition of ammonium nitrite does not explain the frequent occurrence of nitrous oxide in the gaseous products associated with nitrite decomposition (8, 54, 69, 92, 114, 122). In acid soils, the decomposition of nitrous acid has been suggested to account for this phenomenon in the manner shown:

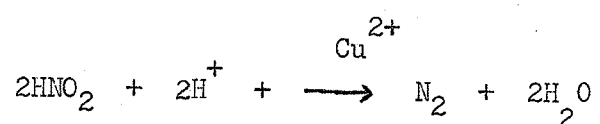


Generally, the reaction seems of little potential significance. Thus, Gerretsen and de Hoop (54) reported an acidity of at least pH 5.0 to be necessary for appreciable decomposition. Reaction (a) would have little tendency to occur in neutral or alkaline soils. In aerated acid soils, the chemical oxidation of nitric oxide would be rapid (b-d). The nitrogen-dioxide formed would itself be adsorbed readily, and oxidised to nitrous and nitric acids. This is confirmed by the observation of Smith and Clark (102) that nitrite had a greater tendency to be oxidised to nitrate in acid soils. Also, these authors could only detect gaseous nitrogen in the decomposition products.

Reactions of nitrous acid with other soil constituents. The certain amount of confusion apparent in the aforementioned mechanisms of loss could possibly be the result of other reaction mechanisms not considered in these experiments. Thus, losses of nitrous oxide are not accounted for by these mechanisms, nor are losses in neutral and alkaline soils. Evidence has recently emerged that organic matter is an important component of the system which affects instability of nitrite (39, 104). At a given pH, Clark and Beard (38) showed that the presence of soil led to greater instability of nitrite. This effect was considerably reduced after hydrogen peroxide destruction of the organic matter. Stevenson and Swaby

(106) found an interaction between nitrous acid and humic acid, fulvic acid, lignins, and polyphenols to give nitrous oxide and nitrogen gas. Reuss and Smith (92) suggested that a pH dependent Van-Slyke-type reaction could occur between nitrite and labile amino groups of organic matter.

Other components of soils have also been suggested to play a role. Wullstein and Gilmour (122) showed that sodium chloride extraction of soil reduced volatile losses of N from nitrite. Copper and manganese were implicated in a reaction of the type.



Addition of the extract to pure sand caused it to loose 63% of the nitrite-N added to it. Losses occurred on all soils, which ranged in pH from 3.9 to 6.9. Chao and Bartholomew (33) showed that nitrite decomposition in an acid medium in the presence of aluminium saturated bentonite and ferrous ions led to gaseous losses directly proportional to the two cations. Catalytic effects of soil cations were therefore suggested in this and the above works.

#### Summary.

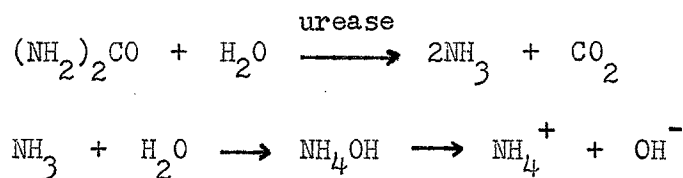
Allison (9) concludes that the present available information does not justify a positive conclusion as to what soil constituents are involved in reaction with nitrites. In most cases, nitrogen gas seems to be the end-product, although nitrous oxide and small amounts of nitric oxide and nitrogen dioxide have also been detected. Generally, large and rapid losses of nitrogen by chemical means are encountered only under special conditions of very heavy fertilization, usually with urea or ammonia, that bring about an accumulation of nitrite. Moderate amounts of nitrogen applied in bands

could give these conditions. Soil drying has also been found to give large losses of nitrogen in the presence of nitrite (38, 70, 116). This is probably the result of increased concentration of the reactants (48). Clark and Beard (38) discovered that losses on air drying were comparable to those from moist soils over a period of 1-2 weeks, and that the mechanisms involved were essentially the same.

On the whole, little work has been done to utilize a range of fertilizer concentrations simulating the concentration gradient across a band in the soil. Temporary and drastic lowering of pH in poorly buffered soils, together with an accumulation of nitrite, could result in large losses as mentioned. Losses could be a regular feature of certain types of soils under systems of fertilization where nitrification is active.

#### NITRITE ACCUMULATION IN SOILS

From the previous discussion, it is apparent that processes in soils leading to nitrite accumulation, especially if these are accompanied by lowering of pH, are important in chemical denitrification. Fraps and Sterges (49), and Kelley (65), found that nitrite accumulated when (a) high levels of ammonium sulphate were applied, and (b) the pH of the soil was raised by calcium carbonate additions. Chapman and Liebig (35) confirmed these observations, and further stated that urea had a greater tendency to produce nitrite than ammonium sulphate. This would be attributable to the release of ammonia and its subsequent ionisation to give the base ammonium hydroxide. Ammonium sulphate, on the other hand would have no tendency to



raise the pH of a soil. Martin et al. (72) found a threshold pH value of  $7.7 \pm 0.1$  above which nitrite accumulated. They hypothesized that microorganisms were unable to oxidise nitrite at alkaline pH. In their extensive work, however, Broadbent et al. (27) were unable to confirm the presence of a threshold pH, demonstrating that nitrification occurred even at pHs greater than 9. The authors emphasized the difficulty in predicting nitrite accumulation.

Stojanovic and Alexander (107) showed that high ammonium concentrations had no effect on the ammonium oxidation rate, but appeared to inhibit the oxidation of nitrite through the effect of free ammonia, rather than pH. This was further confirmed by Aleem et al. (2) using bacterial culture solutions. Aleem and Alexander (1) obtained nitrite oxidations at pH greater than 7.7 in the absence of added ammonium, but at pH 9.5, 1.4 ppm ammonium-N caused marked suppression of nitrite oxidation. Free non-ionized ammonia is favoured above pH 7.0, and Warren (117) showed that the percentages of non-ionised ammonia in solution at pH 6, 7, 8 and 9 are 0.1, 1.0, 10.0 and 50.0% respectively. In contrast to the ammonium ion, ammonia crosses the cellular boundaries of bacteria freely. Low concentrations are not toxic to Nitrobacter spp because of internal detoxifying mechanisms leading to the formation of non-toxic urea, glutamate and asparagine. At higher concentrations, however, the cell is unable to cope with the ammonia. In perfusion experiments, Stojanovic and Alexander (107) observed that nitrite accumulated only when ammonium was detectable in the perfusate. When the latter was depleted by oxidation to nitrite, Nitrobacter activity was resumed.

Soil factors likely to affect nitrite accumulation are therefore those which affect the concentration of nonionized ammonia in the soil

solution. Among these are:

1. Cation exchange capacity, which determines the total amount of ammonium in the soil solution. This seems to be especially important where discrete nitrogen sources are employed. Smith (101) found that lowering the cation exchange capacity of a soil, by additions of sand, led to greater accumulation of nitrite from alfalfa particles. With high exchange capacity, steeper concentration gradients were set up around particles, leading to greater volumes between particles where ammonium concentration was sufficiently low for nitrification to be uninhibited.

2. Soil pH, which determines the ratio of ammonia to ammonium in the soil solution.

3. Buffering capacity, which determines the pH rise when an "alkaline" fertilizer such as urea is employed.

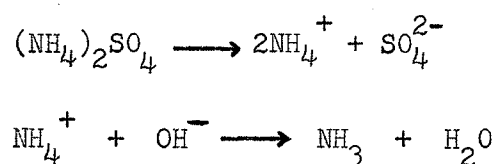
4. Factors of fertilizer use such as type, level, and mode of application are obviously important also.

#### AMMONIA VOLATILISATION.

Allison (9) comments that losses of nitrogen from soils as ammonia are more serious than previously thought. This is mainly due to the following factors: (a) large increases in the rate of nitrogen applied, (b) use of fertilizers such as anhydrous or aqua ammonia, which have alkaline reactions, and (c) extensive use of urea which is readily hydrolyzed by the enzyme urease to ammonia.

Numerous workers (73, 109, 112, 113) have shown that surface application of ammonia and urea to grass-covered or bare soils gave losses of nitrogen in the range 15-30% applied-N. In addition, large losses of ammonia were obtained when ammonium sulphate was applied to the surface of calcareous soils (53, 73, 109). Jewitt (60) found that large losses from

ammonium sulphate were confined to soils in the pH range 8-10. When applied in solution, adsorption of the ammonium ion occurred at the surface, and subsequent evaporation of the water was accompanied by volatilization. Steenbjerg (105) obtained a 60% loss of N from ammonium sulphate in a soil of pH 8.0 in 4 weeks. Wahab et al. (115) postulated ammonia volatilization even from slightly acid soils due to the equilibria:



The relationship of free ammonia to pH has previously been discussed (118).

The pH of both soil and fertilizer, then, is one of the main factors in ammonia volatilization. Plessidu and Kroontje (88) found that this factor alone explained the correlation between theoretical and measured losses from ammonium sulphate in acid and alkaline soils. Other workers, notably Ernst and Massey (47), Chao and Kroontje (34), and Parr and Papendick (84) found that increases in volatilization occurred with increases in temperature, concentration of ammonium, and decreases in moisture and cation exchange capacity. An important physical factor is depth of application. Jackson and Chang (58), and Steenbjerg (105), found that covering the fertilizer with two to six inches of soil almost completely eliminated volatile losses. Losses in the field would not therefore be appreciable unless the ammonia or ammonium used is excessive, or the soils have very low retention capacities due to their dryness or coarse texture (34). Generally, agricultural soils have high retention capacities for ammonium. Mortland (79) attributed this to extensive chemisorption of ammonium to clay under acid conditions, and of ammonia to organic matter under alkaline conditions.

## CHAPTER THREE

### MATERIALS AND METHODS

#### SOIL MATERIAL

The term soil material is used in this study in preference to that of soil. The use of the term is meant to emphasize the fact that the subsequent investigations are of a laboratory nature only, carried out on a substrate removed from its natural environment, ground, air-dried and representative of only one horizon. Due note has been taken of the concept of a soil existing as a unique body in the field, undisturbed, and with its horizons intact.

#### Choice of materials.

In choosing soil materials, it was born in mind that pH and buffering capacity would probably be some of the most important chemical factors in both nitrite accumulation and its subsequent chemical instability. Ammonia toxicity to Nitrobacter spp. was shown by Warren (117) to be strongly pH dependent, whilst Allison (9) has reviewed and emphasized the possible interactions between  $\text{NO}_2^-$  and pH in processes heading to chemical losses of nitrogen. Consequently, it seemed desirable to choose three soils covering the range from acidity through neutrality to alkalinity.

In addition, it was deemed an advantage if a reasonable constancy could be attained in factors such as organic matter and total N, cation exchange capacity, texture, and exchangeable cations. This would aid in the interpretation of the soils' comparative behaviour. Also, by limiting some of the variability in physical and chemical characteristics, biological and chemical processes which might contribute to the proposed phenomena would be more capable of direct understanding. Since many other inorganic



and biological variables were not thus compensated for, the narrow choice of materials still seemed amply justified. This selection did mean that the choice of pH was limited to mildly acid and alkaline values, since at the extremes, large variation would have occurred in exchangeable bases.

#### Physical and chemical characteristics.

Soil materials chosen to fulfil the preceding requirements were taken from soils of the Lakeland, Wellwood, and Holland associations. The latter are described briefly in the next subsection. The materials used were bulk samples of Ap horizons<sup>1</sup> allowed to air-dry on the bench prior to grinding into less than 2mm particles. Careful mixing was performed by hand prior to subsampling. Routine chemical and physical characterisation, together with all experimental work, was performed on less than 2 mm air-dried subsamples unless otherwise stated.

The results of routine analyses by the Soil Survey Laboratory, University of Manitoba, are shown in Table 1. Factors which were very constant for the three materials were % organic nitrogen and cation exchange capacity. No great variation existed in texture and % organic matter. The sum of exchangeable cations of Lakeland soil material was higher by 12.5 meq/100 gms than cation exchange capacity due to the high carbonate content. The latter would have contributed  $\text{Ca}^{2+}$  and possibly  $\text{Mg}^{2+}$  as contaminants in the analytical procedure. The material was predominantly  $\text{Mg}^{2+}$  and/or  $\text{Ca}^{2+}$  saturated, as judged from the amounts of the remaining cations. Real values for exchangeable cations were therefore much closer to those for Wellwood and Holland soil materials.

<sup>1</sup> See report on the Sixth Meeting of the National Soil Survey Committee of Canada, 1965, Laval University, Quebec, for definitions.

TABLE 1  
 CHEMICAL AND PHYSICAL CHARACTERISTICS OF LAKELAND,  
 WELLWOOD, AND HOLLAND SOIL MATERIALS

CHARACTERISTICS	LAKELAND	WELLWOOD	HOLLAND
Textural class	VFSL	VFSL	VFSL
%S	49.4	56.0	52.3
%Si	22.7	27.5	24.4
%C	27.9	15.6	23.3
% Organic Matter	4.8	6.1	4.8
% Organic Nitrogen	.28	.30	.29
% CaCO <sub>3</sub> Equiv.	19.6	.3	.4
Ca <sup>2+</sup> , meq/100gm.	27.5	20.0	19.8
Mg <sup>2+</sup> , "	8.0	2.9	4.4
Na <sup>+</sup> , "	0.2	0.2	0.1
K <sup>+</sup> , "	0.5	1.1	0.6
H <sup>+</sup> , "	0.0	0.1	2.0
Cat. Exch. Cap.	23.7	23.4	25.2
pH of sat. paste	8.0	7.1	6.2
Buffering capacity*	0.6	1.0	2.3

\*Expressed as pH increase in 10 gms. of soil material per meq. added NaOH.

### Field Information

A brief sketch of some pertinent field information is included in an attempt to place the soil materials in a broader context. It was not intended that direct extrapolation be made from laboratory experiments to the field situation. However, it was in the latter environment that the soil materials existed as an integral part of a soil individual, and it is here that materials transported to the laboratory are ultimately real. Hence, a little emphasis should be placed on where they came from, and what significance their parent soils have in the field.

Lakeland Association This is a chernozemic<sup>1</sup> soil association, generally characterized by an imperfectly drained, gleyed calcareous rego black<sup>2</sup> profile developed on strongly calcareous deltaic deposits. Due to the high carbonate content, the solum seldom exceeds 10 inches in thickness, and cultivation often brings the C horizon to the surface (89). The soils are moderately good agriculturally, with moderate to low fertility, fair organic matter reserve, and moderate to low water holding capacity. Free lime limits their fertility through repression of phosphate solubility. They are largely used for grain, and cover an area of approximately 273,000 acres in the West Lake, Grandview, Fisher and Teulon, and Swan River Map Sheet Areas (43, 44, 45, 89).

Wellwood Association This is dominantly characterized by a chernozemic, orthic black<sup>3</sup> profile developed in medium textured lacustrine deposits generally overlying stratified sand (42). They are naturally fertile, with fair to good organic matter reserve, favourable reaction, and fair to good water holding capacity. The soils are subject to drought in

<sup>1,2,3</sup> See Report on the Sixth Meeting of the National Soil Survey Committee of Canada, 1965, Laval University, Quebec, for definitions.

extremely dry seasons because of the low water holding capacity of the sand substrate. Grain growing is the dominant enterprise on an area that covers approximately 86,000 acres in the West Lake and Carberry Map Sheet Areas (42,43).

Holland Association The dominant soil profile is a chernozemic orthic black, developed in medium textured lacustrine deposits and usually well drained. Member soils are generally highly fertile, with good reserves of organic matter and a neutral reaction. The profile has moderately good to good water holding capacity. Grain growing is the main enterprise on the Association's 23,300 acres, found in the Carberry Map Sheet Area (42).

#### EXPERIMENTAL METHODS AND MATERIALS

##### Extraction of exchangeable ammonium, nitrate, and nitrite.

Potassium chloride solution (2N) has been found to have practical advantages (16) without affecting the ammonium extracted. Nitrate and nitrite are not adsorbed on the exchange complex, and are therefore extractable by most aqueous reagents. Extraction by 2N KCl was therefore adopted for all three forms of N.

Advantages and disadvantages of 2N KCl extraction It has been found (15) that extraction by 2N KCl caused no change in most cases to the relative proportions of ions extracted, either through chemical or biological agencies. This was in contrast to acid extractants such as 10% NaCl (pH 2.0 - 2.5) and  $N K_2SO_4 + N H_2SO_4$  which led to release of ammonium by chemical attack on organic matter. The latter reactants are also unsuitable for estimations of nitrites, since the ion decomposes chemically as a direct function of pH (15). These considerations imply that extractions by 2N KCl lead to results whose comparison between workers is more meaningful. When

allied to steam distillation, the extraction procedure may be used to estimate all three forms of N. Finally, in cases where extracts could not be analysed immediately, analyses after 4 months' storage at 2°C gave identical values to those estimated immediately after extraction (16), in contrast to acid extractants.

Recoveries by Bremner and Keeney (16) ranged from 84.5 - 96.6% ( $\text{NH}_4^+$ ), 99.4 - 100.6% ( $\text{NO}_3^-$ ), and 93.0 - 99.1% ( $\text{NO}_2^-$ ) for a range of soil pH. Low recoveries for exchangeable ammonium were attributed to ammonium fixation on most soil materials, supplemented by ammonia volatilization on alkaline, calcareous soils. Less than 100% recoveries for nitrite were indicated to be the result of chemical decomposition by soil constituents. These recoveries were obtained on N added before KCl solution. When the latter preceded N addition, recoveries averaged 100% for all forms of N. This observation emphasized the fact that deviations from 100% recovery were generally the result of soil material, rather than extractant, influence.

Because less than 100% recoveries were encountered, it was desirable to test the method on the present soil materials for possible exchangeable ammonium and nitrite loss. Results for various levels and combinations of mineral-N<sup>1</sup> are presented and discussed in the next chapter.

Method of extraction The method of equilibrium extraction described by Bremner and Keeney (16) was adopted. A 10 gm sample or subsample of soil material was shaken for 1 hour at room temperature in a 250 ml. Erlenmeyer flask. The appropriate 100 ml volume of extractant was delivered from a Pyrex 100 ml automatic pipette. Whatman #1 filter paper was of sufficient fineness to give a clear filtrate. Where analyses on the

<sup>1</sup>Mineral-N = exchangeable  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, or  $\text{NO}_2^-$ -N or any combination of these.

latter could not be performed immediately, the solution was stored in a cold room at 2°C (c.f. Chapter Four). Blank extractions, composed of 100 ml 2N KCl + vol water added to 10 gms air-dried soil material, were performed simultaneously. At frequent intervals, the extraction routine was also repeated with extractant + standard N solution to give the same N-concentration as that initially in the soil materials. Generally, these last two processes were carried out in triplicate.

#### Analysis of KCl extract for mineral N.

Estimation of exchangeable ammonium, nitrate, and nitrite may be carried out on the soil extract by steam distillation. However, for reasons outlined in the following chapter, exchangeable ammonium- and nitrate-N only were estimated by distillation. Nitrite was estimated by a colorimetric procedure.

Estimation of exchangeable ammonium and nitrate-N. This method is described by Bremner and Keeney (16). The principle involved was steam distillation of the extract in the presence of magnesium oxide. The ammonium which distilled over was collected in a mixed indicator/boric acid solution, and estimated by titration with standard  $H_2SO_4$ . In the estimation of nitrate, sulfamic acid was added to destroy nitrite prior to distillation in the presence of magnesium oxide and Devarda's alloy. The latter reduced nitrate to ammonium, which then distilled over with indigenous ammonium. The apparatus employed was similar to that described by Bremner (15) with the exception that the side-arm was found to be unsuitable for addition of sulfamic acid and alloy following exchangeable ammonium distillation. Hence, the sulfamic acid was added directly to the distillation flask. After five minutes to ensure complete reaction, first

magnesium oxide and then Devarda's alloy was added. Immediately thereafter the flask was attached to the apparatus. Losses of N were incurred when alloy was added to the hot solution after the initial ammonium distillation. Direct estimations of nitrate-N were not therefore feasible, so that a separate distillation was made for exchangeable ammonium- and nitrate-N combined.

In every batch of soil materials analysed, aliquots of extracts were also included from a blank and a standard solution containing a known amount of  $(\text{NH}_4)_2\text{SO}_4$ . Distillation of the standard gave a value for N per ml titrant, from which the N concentration in the soil material was calculated.

Estimation of nitrite-N The modified Griess-Ilosvay (14) procedure was followed. The instrument employed was the Coleman Junior Spectrophotometer, for which maximum absorption was found experimentally to occur at 524 mu. This was adopted instead of 520 mu as recommended in the reference.

#### Estimation of pH.

For pH, the procedure outlined in Jackson (57) for a 1:2 ratio of soil:  $0.1\text{N CaCl}_2$  was adopted. It was thought that the repeatability of this method compared to soil and water mixtures would be an advantage.

#### Laboratory incubation procedure.

This was similar to the procedure used by Carnie (30) with the added refinement that soil materials were well-disturbed once the equilibration of the added solution had occurred. This was an attempt to promote optimum nitrification by ensuring that aeration was not limiting. Physical analyses revealed that field capacities ranged from 35 to 40% on an air-dry

basis. The water and solution added brought the soil materials to 25% moisture, air dry basis, a level which therefore represented 63 - 72% of their field capacities. Temperature was thermostatically controlled at 20°C. These conditions of aeration, moisture, and temperature were believed to be close to optimum conditions for nitrification (70, 94).

Further details of procedures are given in the appropriate chapters.



## CHAPTER FOUR

### EVALUATION OF THE EXTRACTION-DISTILLATION PROCEDURE OF ESTIMATING MINERAL NITROGEN

#### INTRODUCTION

The aim of this work was to extend the study of Bremner and Keeney (16) to the estimation of mineral-N in the soil materials used in this investigation. Information was required on the distillation procedure's accuracy and repeatability. Bremner and Keeney (16) showed that ammonium-N recovery ranged from 79.8 to 96.6% in their soils, whilst nitrite ranged from 93.0 to 99.1%. Ammonium fixation was suggested to occur, and nitrite was postulated to undergo decomposition and volatilization by soil constituents.

Lakeland had almost twice the calcium carbonate content of a calcareous soil investigated previously (16), giving more probability of ammonia volatilization during extraction. Also, variabilities to be expected in the estimation of known quantities of mineral-N added to soil materials were unknown. This was especially important for nitrite values. Carnie (30) obtained nitrite-N of up to 38 ppm in incubations of Lakeland with 50 ppm urea-N. The unexpectedly high nitrite made comparisons with an independent method of estimation desirable. Knowledge was also required of the stability of KCl extracts containing a wide range of mineral-N. Whereas the stability of treated soil extracts has been studied for concentrations up to 50 ppm ammonium and nitrate-N, and 25 ppm nitrite-N (16), information on higher levels was desirable in this study.

#### EXPERIMENTAL PROCEDURES

Solutions of ammonium sulphate, potassium nitrate, and sodium

nitrite were prepared such that 2.5 mls added to 10 gms of soil material gave N concentrations of 50 and 200 ppm on an air-dry basis. The N-carriers were added individually, and as a combination of all three to give total concentrations of 150 and 600 ppm mineral-N. Potassium chloride extractions were performed immediately on five replicates of treated and untreated samples, together with 2.5 mls of the added solutions. Three replicates were analysed immediately, the remaining two being split for storage at room temperature and 2°C. Analyses on the latter were performed at weekly intervals.

Analysis for ammonium, nitrate, and nitrite have been discussed previously. An additional analysis was done for total mineral-N in a separate distillation. This is described by Bremner and Keeney (16) and involves distillation of the extract with magnesium oxide and Devarda's alloy. The various mineral-N fractions estimated by extraction-distillation could, by subtraction, give values for ammonium-, nitrate-, and nitrite-N. Comparisons were made between colorimetric and extraction-distillation estimates of nitrite-N in recovery tests, and in incubations with 50 ppm urea and ammonium sulphate-N.

## RESULTS AND DISCUSSION.

### Recoveries of mineral nitrogen.

Recoveries of ammonium-, nitrate-, and nitrite-N are shown in Table 2. Ammonium-N figures were consistently less than a hundred at both levels and modes of addition. It appeared that ammonium fixation, rather than ammonia volatilization, operated to give less than theoretical recoveries. Thus, recoveries were (a) uniform for the three soil materials, in spite of their pH-range, and (b) better at the higher level of

TABLE 2

RECOVERIES\* OF AMMONIUM-, NITRATE-, AND NITRITE-N ADDED TO  
THREE SOIL MATERIALS, (%)

SOIL MATERIAL	MODE OF ADDITION	50 PPM ADDED-N				200 PPM ADDED-N			
		Distillation			Colour	Distillation			Colour
		NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>
Lakeland	Single Min-N Species	89.1	96.5	85.8	98.4	92.6	98.7	99.6	99.0
Wellwood		90.5	98.9	94.4	98.4	92.5	100.1	100.0	97.6
Holland		90.7	97.4	93.5	99.1	94.6	98.2	97.6	98.5
Mean recoveries		90.1	97.6	91.2	98.6	93.2	99.0	99.1	98.4
Lakeland	Combined Min-N Species	87.8	98.3	99.0	98.3	94.2	103.0	116.7	99.1
Wellwood		91.5	100.2	96.9	98.0	94.1	102.1	85.7	98.0
Holland		90.9	98.6	99.4	96.6	93.9	102.0	94.1	98.5
Mean recoveries		90.1	99.0	98.4	97.6	94.1	102.4	98.8	98.5
Overall means		90.1	98.3	94.8	98.1	93.7	100.7	98.9	98.5

\*Recoveries are means of triplicates, expressed as  $\frac{X-Y}{Z} \times 100$ ,  
where X = N content of treated soil material  
Y = " " " untreated " "  
Z = " " " same volume of solution as applied to  
soil.

application. The last observation is consistent with the findings of Allison et al. (11) that fixation from ammonium sulphate solution was proportionately less at higher concentrations. If ammonia volatilization was the dominant process, constant proportional losses could be expected for different ammonium concentrations (34). Allison et al. (11), working with calcareous soils, showed that this method of estimating fixed ammonium was valid, the ammonium lost from the solution being found in the soil after leaching away exchangeable ammonium. Recovery data therefore showed that an immediate loss of 5 and 12 ppm-N was to be expected from the application of 50 and 200 ppm ammonium-N respectively.

Recoveries of nitrate were close to one hundred percent. This is in agreement with the results of Bremner and Keeney (16). The range encountered was 96.5 to 103.0%, with a tendency for recoveries to be slightly higher in combined solutions than when nitrate was added alone. Most of the random variation was undoubtedly the result of experimental errors.

Recoveries of nitrite-N by extraction-distillation ranged from 85.7 to 116.7% compared to 96.6 - 99.1% by colourimetry. The discrepancy with colourimetric values suggest that the variability encountered in the distillation procedure was inherent in the method. Bremner and Keeney discounted any oxidation of nitrite to nitrate during extraction. This was tested by comparing the nitrate-N of control samples with nitrite-treated samples, with results shown in Table 3. Some oxidation to nitrate did occur during extraction. This accounted for slightly less than complete recoveries of nitrite-N, and also the slight increase in nitrate-N recovered at 150 and 600 ppm-N addition. Less than complete recoveries for nitrite was therefore the result of oxidation, rather than reduction to volatile products

TABLE 3  
 OXIDATION\* OF 200 PPM NITRITE-N TO NITRATE DURING  
 EXTRACTION

SOIL MATERIAL	NITRATE-N (ppm)			INCREASE AS % $\text{NO}_2^-$ -N
	CONTROL SOIL MATERIALS	$\text{NO}_2^-$ -TREATED SOIL MATERIALS	INCREASE OVER CONTROL	
LAKELAND	5.1	7.9	2.8	1.4
WELLWOOD	12.6	14.9	2.3	1.2
HOLLAND	17.8	21.8	3.0	1.5

\*Evaluated as increase in nitrate-N of nitrite-treated samples over values of control soils.

as suggested by Bremner and Keeney (16) for their soils.

Nitrite data obtained during incubation of Lakeland with 50 ppm urea- and ammonium sulphate-N are shown in Table 4. Results with Wellwood and Holland are not included because nitrite was low throughout. In all cases, values by the colourimetric method were less than 1 ppm-N, and these are also not given. When estimated by extraction-distillation, Lakeland showed a pattern of increase and subsequent decrease in nitrite with urea, analogous to the results of Carnie (30). Since nitrite as indicated colourimetrically was negligible, it would again seem that total mineral-N data by extraction-distillation were questionable, particularly for urea-treated Lakeland. In this case, however, the pattern obtained suggested that some unknown component was being estimated. It is concluded that nitrite did not, in fact, accumulate; and that the sudden apparent loss of mineral-N obtained by Carnie (30) was due to an abnormally high estimate for total mineral-N at the previous sampling date. It is not known how the discrepancy in the two methods of estimating nitrite could arise, nor why it was particularly marked in urea-treated Lakeland.

#### Variability in mean data

In arriving at the final figure for mineral-N, a number of variabilities are inherent in the calculation. If triplicate analyses for blanks, controls, treatments, and standard solutions are made, each mean obtained will carry its own range of variability. When this is expressed in the conventional manner of a  $\pm$  range, the final figure for the variability in the measurement of added-N is the sum of the variabilities of the individual components in the calculation.

Expressed in this manner, the following average variabilities

TABLE 4

EXTRACTION-DISTILLATION ESTIMATION OF NITRITE-N IN CONTROL, UREA, AND  
 AMMONIUM SULPHATE-TREATED SAMPLES\* OF LAKELAND SOIL MATERIAL  
 INCUBATED FOR TWELVE DAYS (PPM-N)

INCUBATION PERIOD (days)	CONTROL	$(\text{NH}_2)_2\text{CO}$	$(\text{NH}_4)_2\text{SO}_4$
0	1	2	2
2	3	5	6
4	2	13	0
8	0	20	5
12	0	11	4

\*Samples were treated with 50 ppm-N, data are to nearest whole number.

(as % of estimated N) for the forty-eight values in Table 2 were obtained:

ammonium-N	.....	+ -	1.2
nitrate-N	.....	+ -	2.5
nitrite-N	.....	+ -	3.0
nitrite-N (col)	.....	+ -	0.6

These data express the maximum "uncertainty" in quoted data when these are the means of triplicate analyses. Within experimental error, percent variation was similar for 50 or 200 ppm-N estimations of a particular ion, and no differences were apparent between soils or single and combined species addition. The variabilities shown above were therefore inherent in the method.

The variability in ammonium- and nitrate-N values was acceptable for subsequent work, whilst the colourimetric method was better adapted to the precise study of nitrite where small changes were anticipated. Only one source of comparison with the above data could be found in the literature. Data released by Nuttall and McKee (82) indicated a similar variability in ammonium- and nitrate-N estimated by extraction-distillation.

#### Stability of extracts

Extracts of soil materials treated with 50, 150, 200, and 600 ppm-N remained stable for four weeks in most cases. The only instances of instability were recorded for Wellwood and Holland extracts at room temperature and the higher level of combined-N additions. These data are shown in Table 5, which also includes Lakeland for comparison. Nitrate was stable in Wellwood and Holland whereas ammonium and nitrite concentrations gradually decreased. These data do not agree with those of Bremner and Keeney (16), who showed that nitrate and nitrite, but not ammonium, were unstable in



TABLE 5

MINERAL NITROGEN\* OF STORED EXTRACTS OF SOIL MATERIALS TREATED WITH  
 200 PPM EACH OF AMMONIUM-, NITRATE-, AND NITRITE-N  
 AND STORED AT ROOM TEMPERATURE (ppm)

STORAGE TIME (weeks)	LAKELAND			WELLWOOD			HOLLAND		
	NH <sub>4</sub> <sup>-</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	NO <sub>2</sub> <sup>-</sup> -N	NH <sub>4</sub> <sup>-</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	NO <sub>2</sub> <sup>-</sup> -N	NH <sub>4</sub> <sup>-</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	NO <sub>2</sub> <sup>-</sup> -N
0	210	210	200	230	230	200	210	220	200
1	210	210	200	220	220	200	210	220	200
2	220	210	200	220	220	190	200	210	190
3	210	210	200	210	230	180	180	210	170
4	220	210	200	200	230	170	170	220	160
N changes	+10	0	0	-30	0	-30	-40	0	-40

\*Data are expressed to two significant places.

extracts of soils pretreated with 50 ppm ammonium and nitrate-N and 25 ppm nitrite-N and stored at 25°C. The results did agree with previous work (16) where extracts were stored at the lower temperature. Microbial assimilation could account for Bremner and Keeney's observations. In the present study, fungal growth appeared towards the end of the storage period at room temperature. With a longer time interval, it is possible that this would have accounted for the loss of some nitrate at all N levels.

The loss of ammonium and nitrite-N under the above conditions could be the result of interaction of the two ions to give gaseous N losses, possibly through the intermediate formation of ammonium nitrite (8). This would be consistent with the observations that (1) loss of ammonium-N was equivalent to that of nitrite-N, and (2) losses increased with the acidities of the extract, which averaged 7.7 (Lakeland), 7.4 (Wellwood), and 6.9 (Holland). The latter were in turn related to the acidities of the soil materials (Table 1).

#### SUMMARY AND CONCLUSIONS

(1) Recoveries of ammonium-N added to Lakeland, Wellwood, and Holland soil materials were less than one hundred percent. The deficit appeared to be due to ammonium fixation, this being responsible for an immediate loss of 5 and 12 ppm-N from solutions of 50 and 200 ppm applied ammonium-N, respectively. Nitrate recoveries were close to one hundred percent. Nitrite behaved erratically in the extraction-distillation procedure, whereas colourimetric values were uniformly slightly less than one hundred percent due to oxidation to nitrate.

(2) Nitrite formed during incubation was overestimated in urea-treated Lakeland by the extraction-distillation procedure, the trend

obtained suggesting that an unknown component was being estimated.

(3) Variabilities obtained for each N value indicated that the extraction-distillation procedure was acceptable for convenient estimation of ammonium- and nitrate-N, whilst the colourimetric procedure was far superior for the estimation of nitrite-N.

(4) Extracts were stable for four weeks at concentrations of up to 600 ppm-N of ammonium-, nitrate-, and nitrite-N alone or in combination when stored at 2°C. Some instability, apparently chemical, occurred in Wellwood and Holland soil extracts at the 600 ppm-N combined species level when stored at room temperature.

(5) Analyses for ammonium and nitrate-N were made in subsequent work by the extraction-distillation procedure, whilst nitrite-N was estimated colourimetrically. Extracts, if kept for more than a day, were stored at 2°C. The instability of nitrite- and ammonium-N in some soil extracts was born in mind as a possible aid in interpreting data.

## CHAPTER FIVE

### PRELIMINARY EXPERIMENTS

#### INTRODUCTION

Preliminary experiments were conducted to gain information on the potential of Lakeland, Wellwood, and Holland soil materials to show chemical denitrification. Aspects of this problem which were studied, involved: (a) comparisons with similar soil materials studied by Carnie (30), (b) recovery of fertilizer-N compared to control samples, (c) nitrite accumulation, and (d) microbiological activities, as expressed in urea hydrolysis, mineralization, and nitrification rates.

Morrill and Dawson (78) classified soils into four categories on the basis of pH and microbial activities. Class I, pH greater than 7.3, accumulated nitrite and had a highly active Nitrosomonas population; Class II, pH 6.0 - 7.3, had a high nitrification rate similar to Class I, but did not accumulate nitrite because of a very short Nitrobacter lag period; Class III, mostly in the pH range 5.5 - 5.9, showed slow nitrification rates in which Nitrosomonas spp. were characterized by low counts and long generation times; Class IV, pH usually less than 5.4, showed no nitrification in the incubation period used. Carnie (30) found losses of mineral-N from phosphate-amended Lakeland soil material. Ammonia volatilization was suggested to be an important avenue of loss. The rate of release of ammonia from urea is a possible factor in determining nitrite accumulation. When this is rapid, previous workers (cf. Literature Review) have shown an inhibition of Nitrobacter activity due to the presence of unionised ammonia at alkaline pHs.

## MATERIALS AND METHODS

Wellwood soil material came from the same bulk sample as used by Carnie. Lakeland, however, came from another source but was similar in physical and chemical characteristics to that previously studied, as shown below.

	Carnie, 1966	Jones, 1967
Texture	SIC	VFSCL
% org. matter	8.0	4.8
%CaCO <sub>3</sub> equiv.	28.5	19.6
pH	8.0	7.8
Exch. NH <sub>4</sub> -N, ppm	3.1	6.0
NO <sub>3</sub> -N, ppm	9.0	7.5

Incubation procedures have been described in Chapter Three. Control samples were included in which 12 ml of distilled water was added to 50 gms of air-dried soil material. Half the treated samples contained sufficient N as urea and ammonium sulphate in 12 ml of solution to give 50 ppm on an air-dry basis. The other half contained, in addition, 0.75 mgms P/12 ml solution as dipotassium phosphate. The latter repeated the treatments of Carnie (30). Triplicate samples were taken for analyses at 0, 2, 4, 8, and 12 days of incubation. Extractions were performed immediately after subsampling, followed by storage at 2°C until analyses for ammonium-, nitrate-, and nitrite-N was convenient.

Examination of pH was made on a separate subsample as described previously.

## RESULTS AND DISCUSSION

The data from incubations in the absence of added phosphate are shown in Tables 6, 7, and 8. Phosphate had no effect on N in Wellwood and Holland incubations, whereas the effects observed in Lakeland seemed to

TABLE 6

MINERAL NITROGEN CHANGES IN LAKELAND DURING INCUBATION WITH  
WATER, AMMONIUM SULPHATE, AND UREA SOLUTIONS

INCUBATION PERIOD (days)	TREATMENT	pH	CONCENTRATION (ppm air-dried basis)			
			NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	NO <sub>2</sub> <sup>-</sup> -N	Total-N
0		7.8	10	5	tr	15
2	H <sub>2</sub> O (Control)	7.8	11	9	0	20
4		7.9	5	14	0	19
8		7.9	3	20	tr	23
12		7.9	1	25	0	26
0			7.8	52	5	tr
2	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> solution (50ppm-N)	7.8	56	7	0	63
4		7.8	48	14	0	62
8		7.8	25	42	0	67
12		7.8	7	65	0	72
0			7.8	19	2	tr
2	(NH <sub>2</sub> ) <sub>2</sub> CO solution (50ppm-N)	7.8	52	9	tr	61
4		7.8	48	11	0	59
8		7.7	36	29	0	65
12		7.8	3	67	0	70

TABLE 7

MINERAL NITROGEN CHANGES IN WELLWOOD DURING INCUBATION WITH  
WATER, AMMONIUM SULPHATE, AND UREA SOLUTIONS

INCUBATION PERIOD (days)	TREATMENT	pH	CONCENTRATION (ppm air-dried basis)			
			$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	$\text{NO}_2^-\text{-N}$	Total-N
0		7.2	26	8	0	34
2	$\text{H}_2\text{O}$ (Control)	7.1	42	17	0	59
4		7.1	39	24	0	63
8		7.0	26	42	0	68
12		7.0	22	60	0	82
0			7.3	71	9	tr
2	$(\text{NH}_2)_2\text{SO}_4$ solution (50ppm-N)	7.2	85	22	0	107
4		7.2	83	26	tr	109
8		7.1	52	57	0	109
12		7.0	3	123	0	126
0			7.2	38	14	0
2	$(\text{NH}_2)_2\text{CO}$ solution (50ppm-N)	7.2	86	22	0	108
4		7.2	82	27	0	109
8		7.0	29	82	tr	111
12		7.0	1	123	0	124

TABLE 8  
 MINERAL NITROGEN CHANGES IN HOLLAND DURING INCUBATION WITH  
 WATER, AMMONIUM SULPHATE, AND UREA SOLUTIONS

INCUBATION PERIOD (days)	TREATMENT	pH	CONCENTRATION (ppm air-dried basis)			
			$\text{NH}_4^+$ -N	$\text{NO}_3^-$ -N	$\text{NO}_2^-$ -N	Total-N
0		6.2	9	12	0	21
2	$\text{H}_2\text{O}$ (Control)	6.1	11	16	0	27
4		6.2	10	16	0	26
8		6.1	7	25	tr	32
12		6.0	11	31	0	42
0			6.2	52	12	0
2	$(\text{NH}_4)_2\text{SO}_4$ solution (50ppm-N)	6.2	49	18	0	67
4		6.2	52	16	0	68
8		6.1	49	24	0	73
12		6.0	46	32	0	78
0			6.2	10	12	0
2	$(\text{NH}_2)_2\text{CO}$ solution (50ppm-N)	6.3	50	18	tr	68
4		6.1	53	21	tr	74
8		6.2	45	28	0	73
12		6.0	43	40	0	83



merit a separate discussion, given later.

Mineralization and nitrification occurred in most cases, as shown by increases in total mineral-N and the oxidation of ammonium to nitrate, respectively. Ammonium sulphate-treated Holland was an exception, since no more nitrate was produced than in the control. The maximum nitrite recorded in all cases amounted to only a trace which could not be accurately measured. Lakeland was constant in pH, whilst Wellwood and Holland decreased slightly with nitrification. The largest decrease of 0.3 pH units was recorded for ammonium sulphate-treated Wellwood.

#### Recovery of fertilizer nitrogen.

Fertilizer recovery may be calculated by expressing the difference between treatment and control total mineral-N as a percentage of the applied-N. This is not an entirely satisfactory way of calculation since the N carrier may affect net mineralization by (a) inducing an increased mineralization of organic matter through the priming effect noted by Broadbent (20), and (b) inducing the biological assimilation of mineral-N, as noted by Broadbent and Tyler (26). Some impression of the effectiveness of fertilizer-N may, however, be gained from such data.

Recoveries, based on the data of Tables 6, 7, and 8 are shown in Table 9. Extractions of control soils (Chapter Three) revealed that, where variation between air-dry subsamples existed, most of this occurred in nitrate-N. In Table 9, three columns compare recoveries which were corrected for initial differences in nitrate with uncorrected data. Corrected figures were considerably different for urea-treated Lakeland and Wellwood samples, and slightly less different for ammonium sulphate-treated Wellwood. Hence different meanings could be associated with the data. Such correction made

TABLE 9

RECOVERY\* OF FERTILIZER NITROGEN AT VARIOUS INCUBATION PERIODS  
(percent)

SAMPLING TIMES (days)	LAKELAND		WELLWOOD		HOLLAND	
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	(NH <sub>2</sub> ) <sub>2</sub> CO	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	(NH <sub>2</sub> ) <sub>2</sub> CO	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	(NH <sub>2</sub> ) <sub>2</sub> CO
0	85	-	90 (93)	-	89	-
2	84	85 (80)	93 (96)	87 (98)	82	84
4	85	84 (80)	91 (93)	83 (94)	87	94
8	87	89 (84)	75 (78)	74 (86)	82	82
12	88	89 (85)	85 (88)	84 (96)	73	83
Means	86	87 (82)	87 (90)	82 (94)	83	85

\*Expressed as  $\frac{X-Y}{50} \times 100$ , where X = total mineral-N of treated samples  
Y = total mineral-N of control samples  
50 = added N (ppm)

Figures outside brackets are corrected for subsample variations in initial nitrate-N. Bracketted figures are uncorrected.

little difference to the remaining values.

Since recoveries were generally markedly less than 100%, treated soil materials under these conditions lost some of their potential N-supplying power. An approximate 10% loss of ammonium by fixation would account for a large part of this reduction. Many workers have found that fixed ammonium (estimated as in Chapter Three) is largely unavailable for nitrification (11, 68, 81). Availability ranged from ten percent (six weeks' incubation) to less than twenty-five percent (five months' incubation). Nommik (68) showed that the oxidation of clay-fixed ammonium depends on the nature of the clay, and the amount fixed.

Recoveries were still lower than if ammonium fixation alone was operating. This process occurs rapidly in soils (11) and would not influence net mineralization after potential fixation under a given set of conditions was satisfied (81). This phenomenon would therefore not account for reduced net mineralization in the presence of N-carrier, an observation made for Wellwood and Holland from the data in Tables 7 and 8. Net mineralization, measured by the difference between initial and final total mineral-N, was clearly reduced in urea- and ammonium sulphate-treated Wellwood and Holland respectively. Smaller reductions were observed for the other two treatments on these soil materials.

The extent of net mineralization can be indirectly reduced through biological immobilization, denitrification, or ammonia volatilization. Direct effects can be manifested by salts, especially on acid soils (61). There was no clear evidence of any of these pathways occurring, although some presumptive evidence might be proposed for biological denitrification in Wellwood samples and salt effects (osmotic effects) in Holland. In the

former, two low recoveries of 75.2 and 74.4% were found for ammonium sulphate and urea treatments, respectively, after 8 days' incubation (Table 9). Figure 1 (based on data of Tables 6, 7, and 8) shows that nitrification was proceeding rapidly at this time, making possible biological denitrification through lowered oxygen tensions. In Holland, recoveries were lower with ammonium sulphate. This could be associated with salt effects, promoted by the sulphate ion, which reduced net mineralization. Urea, being hydrolyzed to ammonium which was adsorbed on the exchange complex, would have a smaller salt effect depending on nitrification to the nitrate ion. Salt effects on nitrification were suggested by higher nitrate for the urea treatment, without an increase in pH which could stimulate the activities of nitrifiers (46).

Lowered recoveries through chemical denitrification were improbable. Nitrite did not accumulate, and pH in all cases was greater than six. The conducive factors of high nitrite and slightly acid pH (30) or active nitrification at low pH (54) were not encountered in this experiment.

#### Microbiological activity.

Examination of Tables 6, 7, and 8 reveals that exchangeable ammonium values for urea- and ammonium sulphate-treated samples were not significantly different at two days. This indicated that urea hydrolysis was rapid, a finding which is substantiated by other workers (22,55). On this basis, urea was in fact found to be completely hydrolyzed in one day in a separate incubation. Carnie (30) also showed complete hydrolysis of 50 ppm urea-N in two days using similar soils and conditions. Differences between initial exchangeable ammonium of control- and urea-treated samples show that hydrolysis began immediately on addition of urea to the soil material (36). Table 10 shows the percent urea hydrolyzed up to the filtration stage after

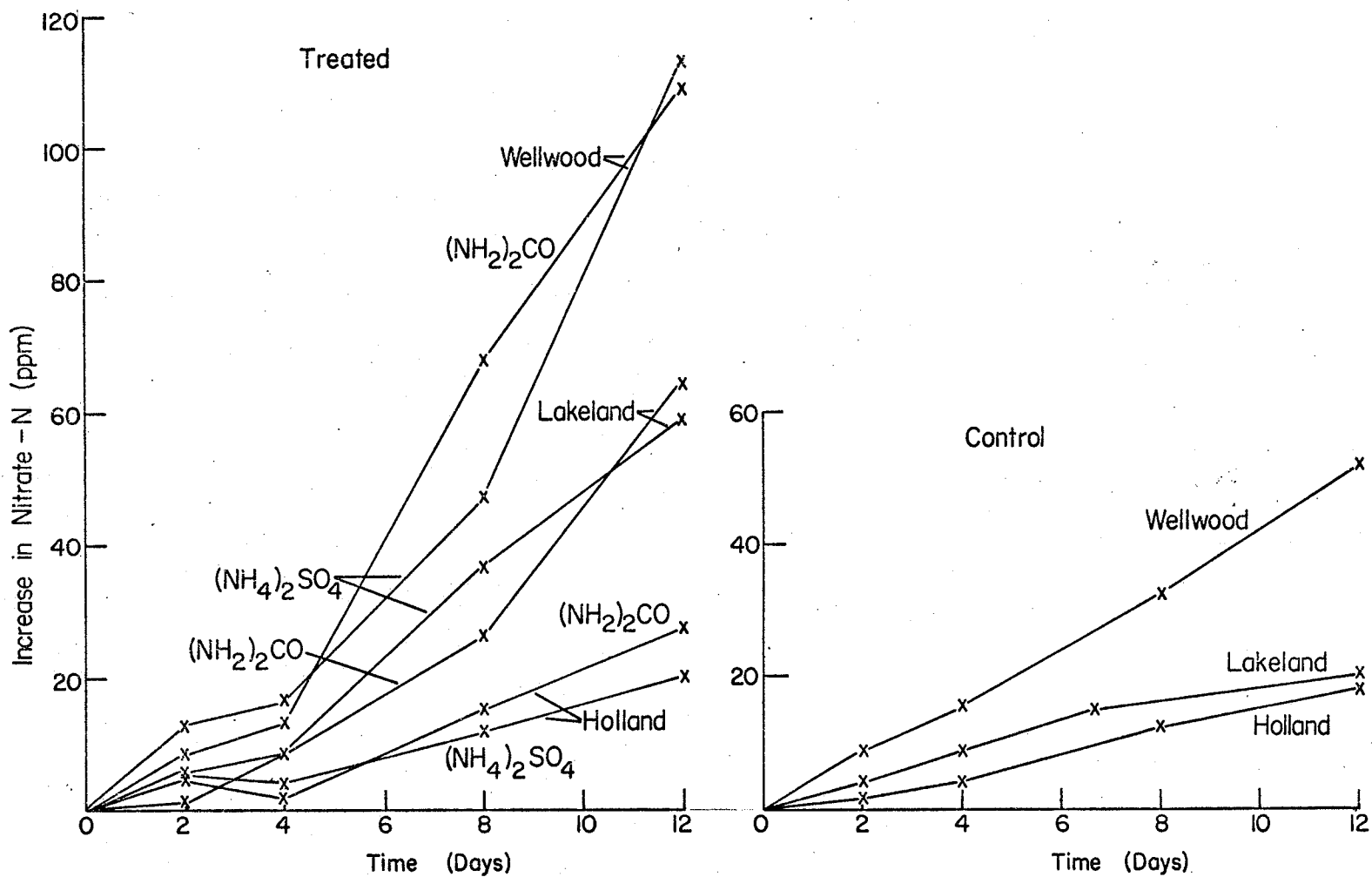


Figure 1. Nitrate accumulation in treated and control soil materials.

TABLE 10

## UREA HYDROLYSIS (percent)

SOIL MATERIAL	EXCH. $\text{NH}_4^+$ -N AT 0 TIME (ppm)		INCREASE OF $\text{NH}_4^+$ -N OVER CONTROL	UREA HYDROLYSIS (%)
	Control	Urea-treat.		
LAKELAND	9.8	19.1	9.3	18.6
WELLWOOD	25.9	37.7	11.8	23.6
HOLLAND	8.9	10.3	1.4	2.8

KCl extraction. The assumption was made that urea-N hydrolyzed gave a corresponding increase in exchangeable ammonium, as shown by Keeney and Bremner (63). Percent hydrolysis determined by these authors under similar conditions ranged from 24.5 (pH 7.0) to 3.5 (pH 7.5). Hydrolysis was the result of microbial activity prior to and during the extraction process, and did not occur in the clear filtrate. In the present study, the samples were exposed to urea for the same length of time prior to, during, and after extraction, so that their urea-hydrolysing potential were of the order Wellwood > Lakeland > Holland. Holland was relatively inactive at 2.8%, a feature suggested from the work of Gibson (55) to be the result of acidity.

Nitrification rates are illustrated graphically in Figure 1 (p.51). Treated soil materials were easily distinguished from each other, and nitrification rates were obtained in the order Wellwood > Lakeland > Holland. Lakeland and Wellwood showed a lag phase of about four days before nitrification appreciably increased, whereas Holland was fairly uniform. Lag phases were not obtained in control samples, the overall nitrification rate being similar to the initial phase of treated samples. The essential order for control samples was the same as the treated, with Lakeland and Holland showing less divergence. It would seem that fertilizer stimulated the nitrifying population after about four days, as also noted by Broadbent and Tyler (25,) but had no appreciable effect on rates prior to this time.

Mineralization rates were of the order Wellwood > Holland > Lakeland, as may be calculated from Tables 6, 7, and 8. The rate of nitrification in Holland was the same for ammonium sulphate as for control samples, indicating that mineralization from organic matter was the controlling process.

Fraps and Sterges (49) similarly observed that many soils did not nitrify added ammonium sulphate, although they usually produced nitrate from organic matter. Low pH and low initial population of nitrifiers were found by Frederick (51) to account for this phenomenon. Sabey et al. (97) cautioned that some reports of low nitrification rates were the result of too short an incubation period, such that this was within the lag phase for microbial proliferation. Within the lag phase, it is noted that nitrification rates for all treatments are essentially no different from the controls, indicating that initial and mineralized ammonium were the chief sources of nitrate. A preferential use of initial ammonium- and organic-N would therefore seem to exist in the first few days of incubation for all soils and treatments.

Delay periods and maximum rates of nitrification are shown in Table 11. These may be used as parameters to describe nitrification, according to the findings of Sabey et al. (97). The delay period is characteristic of both the initial population and the conditions governing the proliferation of nitrifiers. Maximum rates reflect soil factors which govern the activities of microorganisms (51, 97). This is not normally affected by altering the delay period, as through inoculation, but depends on overall factors such as pH, amount of substrate and energy materials. In Table 11, it is shown that fertilizers increased the delay period on Lakeland and Wellwood. The delay was longer on the alkaline soil with "alkaline" fertilizer (urea) and shorter for the neutral soil. Within the relatively large sampling times used, maximum rates were fairly constant for urea and ammonium sulphate on any one soil. This indicated that the two fertilizers affected the initial proliferation of organisms to different extents,



TABLE 11

PARAMETERS OF NITRIFICATION IN LAKELAND, WELLWOOD,  
AND HOLLANDSOIL MATERIALS

SOIL MATERIAL	DELAY PERIODS (days)			MAXIMUM RATES (ppm/2 days)		
	$(\text{NH}_4)_2\text{SO}_4$	$(\text{NH}_2)_2\text{CO}$	Control	$(\text{NH}_4)_2\text{SO}_4$	$(\text{NH}_2)_2\text{CO}$	Control
LAKELAND	3	5	0	12	15	2
WELLWOOD	5	3	0	33	27	4
HOLLAND	0	0	0	2	2	2

depending upon initial soil pH, but had similar effects on their eventual numbers and activities. Maximum nitrification rates of fertilized Lakeland were 7-9 times the control, and 6-8 times for Wellwood. The increased rate with fertilizer in the latter was especially noticeable as reflected in exchangeable ammonium values compared to the control (Table 7).

The absence of nitrite indicated that Nitrobacter spp. were more active than Nitrosomonas. A separate incubation with 50 ppm nitrite-N showed a 42.5% oxidation to nitrate in Holland in twelve days, compared to about 20% oxidation of ammonium from urea, as revealed in Table 8. It is possible that nitrite exhibited some bacteriostatic effects which caused some lowering of oxidation rate when added thus (110). Hence, this oxidation rate may underestimate the potential turnover of nitrite when it does not markedly accumulate. In any case, this datum indicates that nitrification rates in Holland may be severely limited by ammonium oxidation. In Lakeland and Wellwood, complete oxidation of nitrite was observed in this time.

Holland could be classified according to the scheme of Morrill and Dawson (78) as a Class III soil. Lakeland and Wellwood defied classification on the basis of preceding information. Thus, Lakeland was of high pH yet did not accumulate nitrite even with urea, whilst Wellwood might be expected to enter Class II on the basis of its pH and high nitrification rate. However, lag phases based on ammonium sulphate nitrification would assign Lakeland and Wellwood to Classes II and I respectively. Probably fertilizer concentration was not high enough to inhibit Nitrobacter activities, and perhaps promote the long lag periods (8-10 days) noted by the above workers. The classification scheme was developed using 280 ppm ammonium sulphate-N in perfusion liquids.

Effect of phosphate on mineral nitrogen in Lakeland.

Mineral-N changes for Lakeland in the presence and absence of added phosphate are shown diagrammatically in Figures 2a and 2b. Considerable differences in nitrification rates occurred with and without phosphate. Calculations of delay periods and maximum rates (from the graph) give respective estimates of one-and-a-half days (+P), four days (-P); and 15 ppm-N/2 days (with or without P), for nitrate formation from urea. The delay in ammonium oxidation was also markedly reduced in the presence of phosphate. Nitrate was formed at a maximum of 12 ppm-N/2 days in ammonium sulphate treatments, and delay periods were half a day and two days with and without phosphate respectively. Phosphate therefore caused a marked reduction in delay period without affecting maximum rate, an indication that it increased the rate of microbial proliferation (49, 97). Since phosphate deficiency is known to limit plant growth in the field (Chapter Three, p.26) it is possible that microbial activities were restricted through low availability, and that phosphate addition caused an initial stimulation. Little work has been done in calcareous soils on such interactions between major nutrients, usually nitrate formation in non-calcareous soils was not affected by phosphate (91).

Consistently lower total mineral-N values were obtained with phosphate. At the end of the incubation period, differences of 5.5 and 19.0 ppm-N existed for urea and ammonium sulphate treatments respectively (from Figures 2a and 2b) compared to non-phosphate treatments. Carnie (30) also found a greater net loss of mineral-N with ammonium sulphate-P treatments than with urea-P under similar conditions. Ammonia volatilization is not a plausible explanation for this since (a) greater losses might be expected

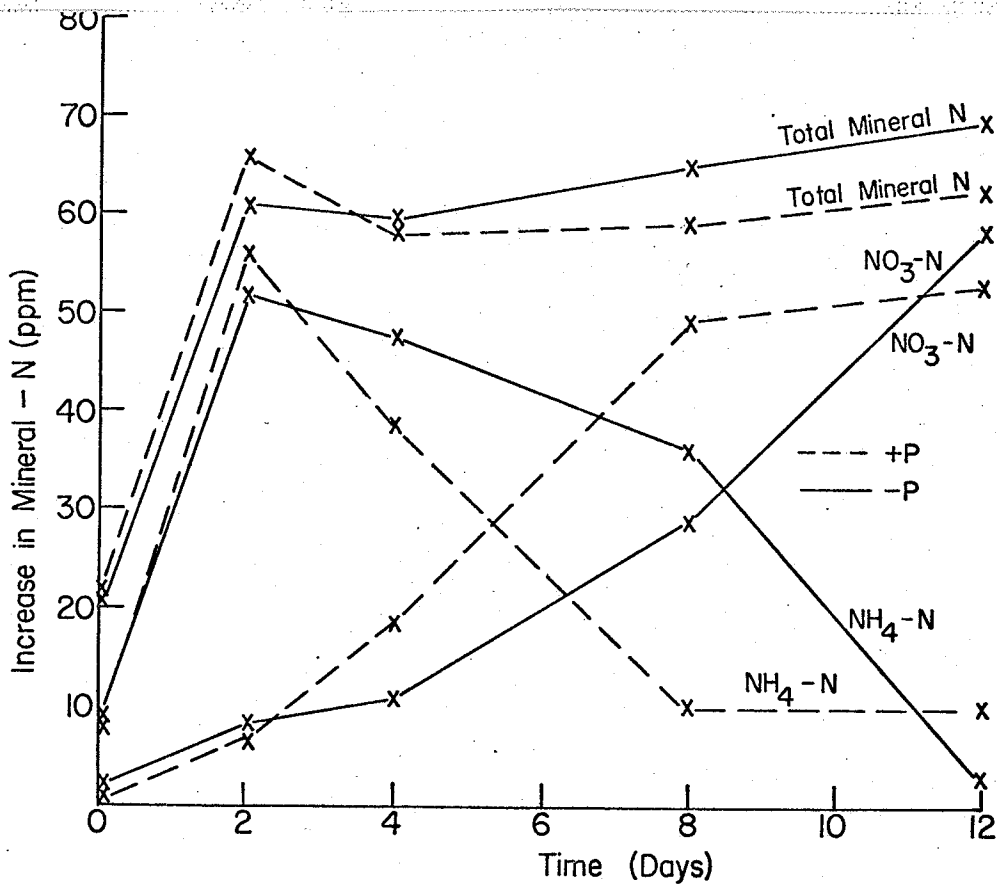


Figure 2a. Mineral nitrogen changes in urea-treated Lakeland soil material.

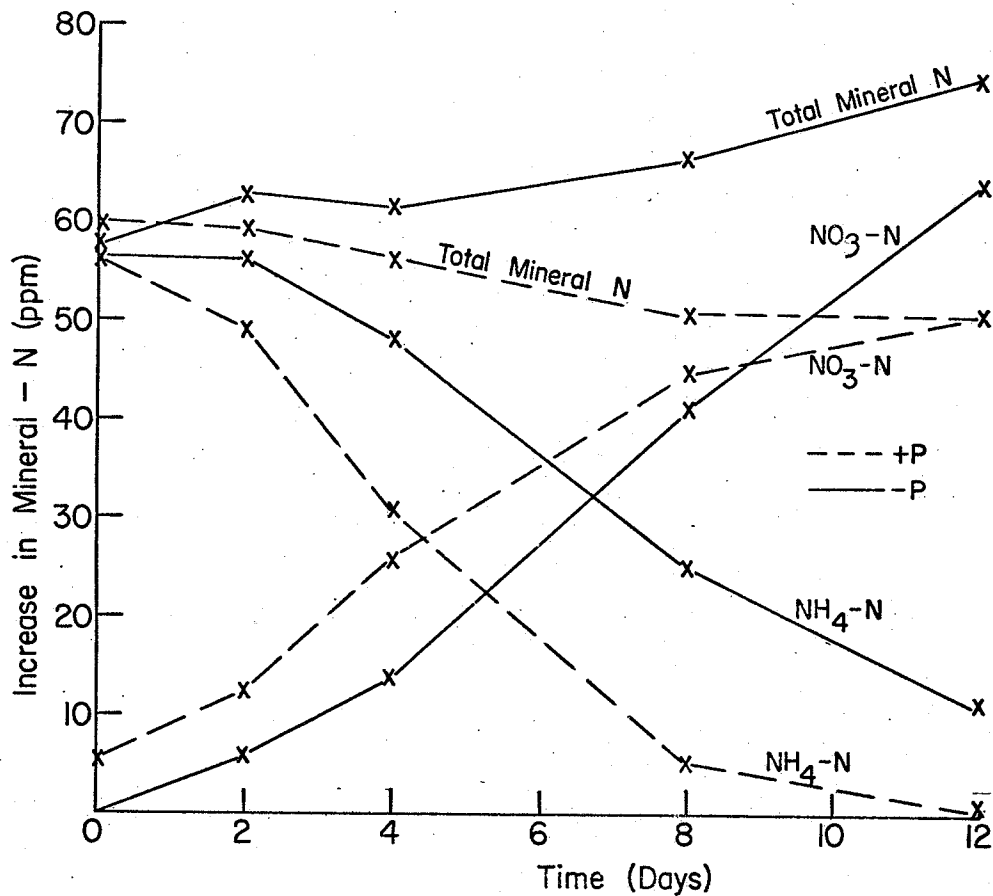


Figure 2b. Mineral nitrogen changes in ammonium sulphate-treated Lakeland soil material.

with urea, and (b) phosphate cannot be expected to promote such a loss. A more acceptable hypothesis is that phosphate, through generally increasing microbial activity, also promoted biological immobilization and caused a decrease in net mineralization. That added N was not necessary to show this effect, is shown in Table 12 which compares control with and without phosphate. Total mineral-N (after the first sampling) and net mineralized-N decreased in the presence of phosphate. Biological immobilization could likewise account for differences in the overall decrease of ammonium-N compared to increase in nitrate-N. In phosphate treatments, ammonium decrease was 56.0 ppm-N, nitrate increase was 45.1 ppm-N with ammonium sulphate, indicating a net loss of mineral-N. Without phosphate, this was reversed, corresponding figures being 44.0 and 64.0 ppm-N, indicating that net mineralization occurred. Similar relative tendencies were found where urea was the N- source, as may be calculated from Figure 2a, although phosphate caused a smaller effect.

#### SUMMARY AND CONCLUSIONS

- (1) Nitrite did not accumulate in any soil or treatment, possibly because the applied ammonium concentration was insufficiently high to inhibit Nitrobacter activities. Oxidation of 50 ppm nitrite-N was quantitative in Lakeland and Wellwood in 12 days, and more than twice as fast as ammonium oxidation in Holland.
- (2) Recovery of fertilizer-N, based on comparisons with control-N, averaged less than one hundred per cent for all treatments. The major portion of mineral-N loss could be ascribed to initial fixation of ammonium without subsequent nitrification. Recoveries, however, were generally less than the ninety percent

TABLE 12

TOTAL MINERAL NITROGEN IN LAKELAND INCUBATED WITH AND WITHOUT  
ADDED PHOSPHATE

INCUBATION PERIOD (days)	CONTROL +P (ppm)	CONTROL -P (ppm)	INCREASE OF -P OVER +P
0	14	14	0
2	12	20	8
4	18	19	1
8	19	23	4
12	19	27	8
Means	16	20	4
Net mineralized N	5	13	8

predicated on this basis. Corrected recoveries were no less for urea-treated Lakeland than for other samples, indicating that ammonium volatilization may not have been appreciable on this soil material. Urea-treated Wellwood showed lower average recovery than the ammonium-sulphate treatment, so that ammonia volatilization may have occurred during rapid urea hydrolysis. In addition, some biological denitrification may have occurred for both treatments at the time of maximum nitrification and lowered oxygen tension. Salt effects may have operated on Holland, where urea gave higher average recovery than ammonium sulphate. Chemical denitrification under these conditions was considered improbable, due to either low nitrite, high pH, or slow nitrification.

(3) Urea hydrolysis rates occurred in the order Wellwood > Lakeland > Holland; nitrification rates were similarly associated. Mineralization rates were of the order Wellwood > Holland > Lakeland. Wellwood was therefore, consistently more active in N turnover, as considered from these view-points. Nitrite accumulation in Lakeland and Wellwood could not be predicted from these and pH data on the basis of Morrill and Dawson's (78) scheme. It would seem that Holland would have little potential since ammonium oxidation was considerably slower than oxidation of nitrite; also pH, at about 6.2, would keep ammonium predominantly as the ionic form (120).

(4) No evidence was found of ammonia volatilization as an agent of mineral-N loss in phosphate-amended Lakeland. Observations

made here agreed with previous work (30) in that a reduction in mineral-N occurred compared to phosphate-unamended samples. However, this is probably due to increased biological immobilization in the former case. Phosphate was shown to increase nitrification rates and decrease net mineralization, especially in ammonium sulphate treatments. This was inferred to indicate that phosphate increased biological activity on a calcareous soil which could be phosphate-deficient.



## CHAPTER SIX

### LONG TERM INCUBATION EXPERIMENT

#### INTRODUCTION.

Ammonium sulphate-and urea-treated Holland soil material nitrified very little in twelve days' incubation. Also, net mineralization from organic matter was generally reduced with fertilizer, showing a potential loss of mineral-N. It was therefore desirable to conduct a long-term experiment to study mineral-N turnover. The factors operating to reduce fertilizer recovery would have, it was hoped, an extended cumulative effect which would be more measureable than in a short-term experiment.

The range of N-carriers was broadened to include potassium nitrate and sodium nitrite. Hence, comparisons could be made between controls and fertilizers which underwent complete nitrification (urea and ammonium sulphate), sodium nitrite which potentially could lead to losses by chemical denitrification, and potassium nitrate which would not undergo nitrification. The level of N and conditions of incubation were similar to the previous experiments. At 50 ppm, the added N corresponded approximately to levels of fertilization (100 lbs. per acre) in common use in field soils.

#### EXPERIMENTAL PROCEDURE.

The only modification to previous techniques was the periodic checking carried out for constant moisture contents of the samples. This was done by weighing the jar and soil material, adding the requisite amount of water to give 25% moisture, air-dry basis. Duplicate jars were well-aerated every four days, and subsamples were taken at 0, 3, 6, and 15 weeks.

#### RESULTS AND DISCUSSION

Results are presented in Table 13. Lakeland maintained a uniform

TABLE 13

MINERAL NITROGEN CHANGES IN LAKELAND, WELLWOOD, AND HOLLAND  
DURING INCUBATION FOR FIFTEEN WEEKS (ppm-N)\*

SOIL MATERIAL	TREATMENT	SAMPLING PERIOD														
		Three weeks					Six weeks					Fifteen weeks				
		NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	Total	pH	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	Total	pH	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	Total	pH
LAKELAND	H <sub>2</sub> O	3	30	0	33	7.8	1	33	0	34	7.8	1	37	0	38	7.8
	NaNO <sub>2</sub>	8	59	0	67	7.8	2	73	0	75	7.8	0	92	0	92	7.8
	KNO <sub>3</sub>	3	63	0	66	7.8	1	70	0	71	7.8	1	60	0	61	7.8
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	3	61	0	64	7.8	0	68	0	68	7.8	1	86	0	87	7.8
	(NH <sub>2</sub> ) <sub>2</sub> CO	4	58	0	62	7.8	0	65	0	65	7.8	5	82	0	87	7.8
WELLWOOD	H <sub>2</sub> O	4	67	0	71	7.0	3	67	0	70	7.0	2	89	0	91	7.0
	NaNO <sub>2</sub>	9	96	2	107	7.0	2	102	0	104	7.0	1	134	0	135	7.0
	KNO <sub>3</sub>	2	98	0	100	7.0	0	96	0	96	7.0	2	109	0	110	7.0
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	2	117	0	119	6.8	4	111	0	115	6.8	2	104	0	106	6.8
	(NH <sub>2</sub> ) <sub>2</sub> CO	6	100	0	106	6.9	5	105	0	110	6.9	5	123	0	128	6.9
HOLLAND	H <sub>2</sub> O	5	34	0	39	6.0	0	40	0	40	6.0	0	52	0	52	6.0
	NaNO <sub>2</sub>	36	21	21	78	6.0	28	50	1	79	6.0	0	92	0	92	6.0
	KNO <sub>3</sub>	6	68	0	74	6.0	0	79	0	79	6.0	1	87	0	88	6.0
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	5	65	0	70	5.9	1	69	0	70	5.8	5	70	0	75	5.8
	(NH <sub>2</sub> ) <sub>2</sub> CO	4	65	0	69	5.9	2	73	0	75	5.9	3	76	0	79	5.9

\*Total mineral-N initially in untreated samples was 12, 28, and 16 ppm in Lakeland, Wellwood, and Holland respectively. Similarly, pH was 7.8, 7.2, and 6.2, respectively. Nitrogen was added at the rate of 50 ppm.

pH throughout, whilst Wellwood and Holland showed a small decrease in control, sodium nitrite, and potassium nitrate treatments, with a slightly larger decrease in ammonium sulphate and urea treatments. The data are consistent with the view that acidity is the result of acidic anions applied in the fertilizer and formed as the result of nitrification (6). Ammonium sulphate was most acid producing due to nitrate and sulphate ions, urea gave rise to nitrate ions only, whilst nitrite and nitrate did not change the hydrogen ion activity and hence showed similar acidities to control samples.

Table 13 reveals that ammonium sulphate and urea were completely nitrified in Holland during three weeks' incubation. This confirms the suggestion, made as the result of preliminary experiments, that the absence of a lag period in short-term incubation was due to the incubation period being the shorter of the two. In the present experiment, an increase in nitrification rate must have occurred somewhere between twelve days and three weeks, so that the initially slow oxidation of ammonium to nitrate was probably more the result of limited bacterial numbers than their low activities. Similar tendencies for acid soils have been observed by Broadbent et al. (27) and Sabey et al. (97).

Treatment with nitrite caused an accumulation of ammonium. This was particularly clear in Holland, where ammonium concentration at three weeks was about six times higher than that of other treatments. This was apparently caused by an inhibition of the oxidation of ammonium released from organic matter, rather than reduction of nitrite to ammonium, since oxidising conditions permitted nitrification for all remaining soils and treatments. Inhibition of ammonium oxidation persisted in Holland for six weeks, when nitrite had almost completely disappeared.

In addition, whereas nitrite was completely oxidized in Lakeland and Wellwood, 21 ppm-N was still present in Holland after three weeks. The preliminary experiments showed faster oxidation of nitrite than ammonium in Holland during twelve days' incubation, but this was apparently reversed as the time of contact with nitrite increased. Nitrite therefore inhibited Nitrosomonas and Nitrobacter activities in Holland. Other workers have reported that nitrite inhibited Nitrosomonas activities in alkaline and acid soils, whereas the inhibition of Nitrobacter was much more marked in acid soils (78, 110). This could lead to the higher persistency of nitrite in acid soils, and hence the greater probability of chemical denitrification through nitrite instability.

Evidence for chemical denitrification was difficult to adduce from the data of Table 13. Throughout the incubation period, total mineral-N of nitrite treatments were, if anything, higher than totals for nitrate, ammonium sulphate, and urea treatments. Only for Holland at three weeks was there any evidence of mineral-N loss. The application of 50 ppm nitrite-N resulted in the formation of 12 ppm nitrate-N, with a corresponding reduction of 29 ppm in nitrite-N. A total net loss of 17 ppm-N (or 34% initial -N) was therefore indicated, assuming no reduction of nitrite to ammonium. In the subsequent three week period, nitrification from ammonium obscured any further losses from nitrate, since increase in nitrate was about twice the nitrite decrease. A similar increase in nitrate in Lakeland and Wellwood made analagous inferences of N loss impossible.

The gross effectiveness of various N carriers are illustrated by the percent recovery data of Table 14. An extremely wide range of recoveries was obtained at fifteen weeks, from 37% for ammonium sulphate-treated Wellwood to 136% for nitrite-treated Lakeland. Recoveries were highest

TABLE 14

## RECOVERY OF FERTILIZER NITROGEN (percent)

SOIL MATERIAL	TREATMENT	SAMPLING PERIOD		
		Three weeks	Six weeks	Fifteen weeks
LAKELAND	NaNO <sub>2</sub>	86	102	136
	KNO <sub>3</sub>	86	89	61
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	81	84	124
	(NH <sub>2</sub> ) <sub>2</sub> CO	77	78	125
	Means	82 ± 3*	88 ± 7	111 ± 26
WELLWOOD	NaNO <sub>2</sub>	87	88	109
	KNO <sub>3</sub>	79	66	51
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	118	114	37
	(NH <sub>2</sub> ) <sub>2</sub> CO	93	99	92
	Means	94 ± 12	92 ± 15	72 ± 26
HOLLAND	NaNO <sub>2</sub>	87	97	101
	KNO <sub>3</sub>	85	99	89
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	75	74	57
	(NH <sub>2</sub> ) <sub>2</sub> CO	75	88	69
	Means	80 ± 6	89 ± 9	79 ± 16

\* Range values are average mean deviations.

for nitrite at the end of the incubation period, prompting the suggestion that mineralization may have been enhanced by this ion. Its known bacteriostatic effect (110) may promote breakdown of organic matter after a certain time interval, analogous to the effects of chemical sterilants. Differences in recoveries of various N carriers generally increased with time, as indicated by the range values of Table 14.

Mineralization data are illustrated diagrammatically in Figures 3 and 4. The highest rate of net mineralization occurred in the first three weeks. During this time, all soil materials with the exception of nitrite-treated Holland had completely nitrified the added N. Hence maximum biological oxygen demand probably occurred in this period. The findings of other workers (14, 83) on rewetting air-dried soils support this, so that biological denitrification was potentially greatest in the first three weeks. Mineral-N formed in nitrate-treated samples was always less than that formed in control soils during this period. In Wellwood, the shape of the nitrite curve (Figure 4) suggests that biological denitrification was over in six weeks, leaving the sample to mineralize at the same rate as the control after this time.

Biological and chemical denitrification are unlikely explanations for the wide range of recoveries obtained after three to six weeks. Maximum oxygen demand was over, whilst the complete nitrification of ammonium fertilizers would ensure a low nitrite turnover. More likely explanations are the residual effects of fertilizers on mineralization and assimilation processes.

There are numerous ways by which the N carriers introduced different factors into the incubations, some of which may be enumerated: (1)

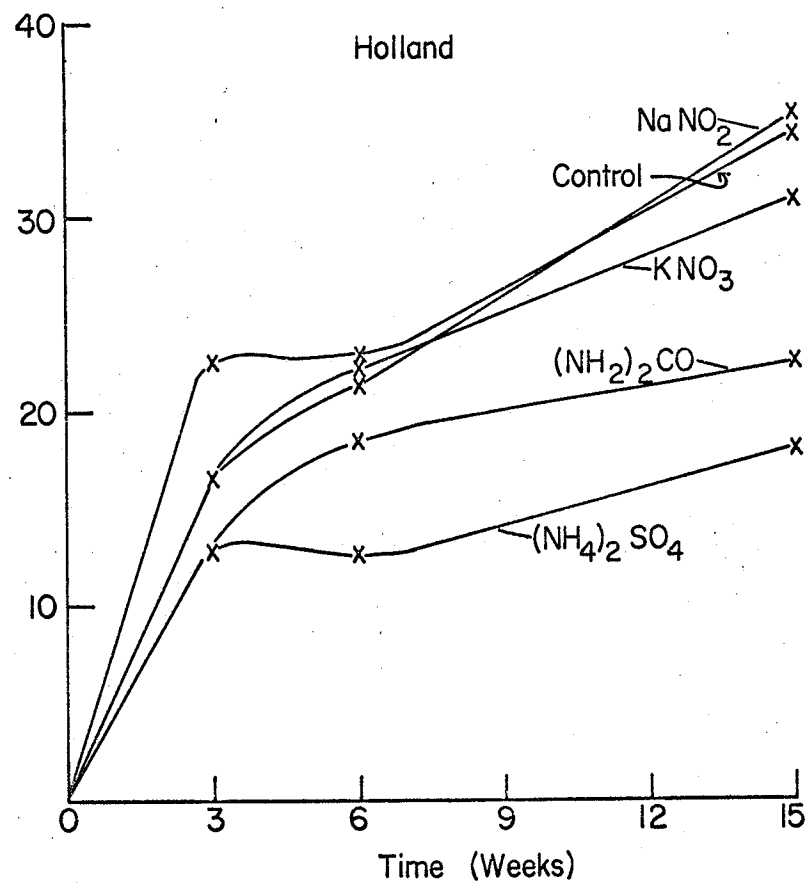
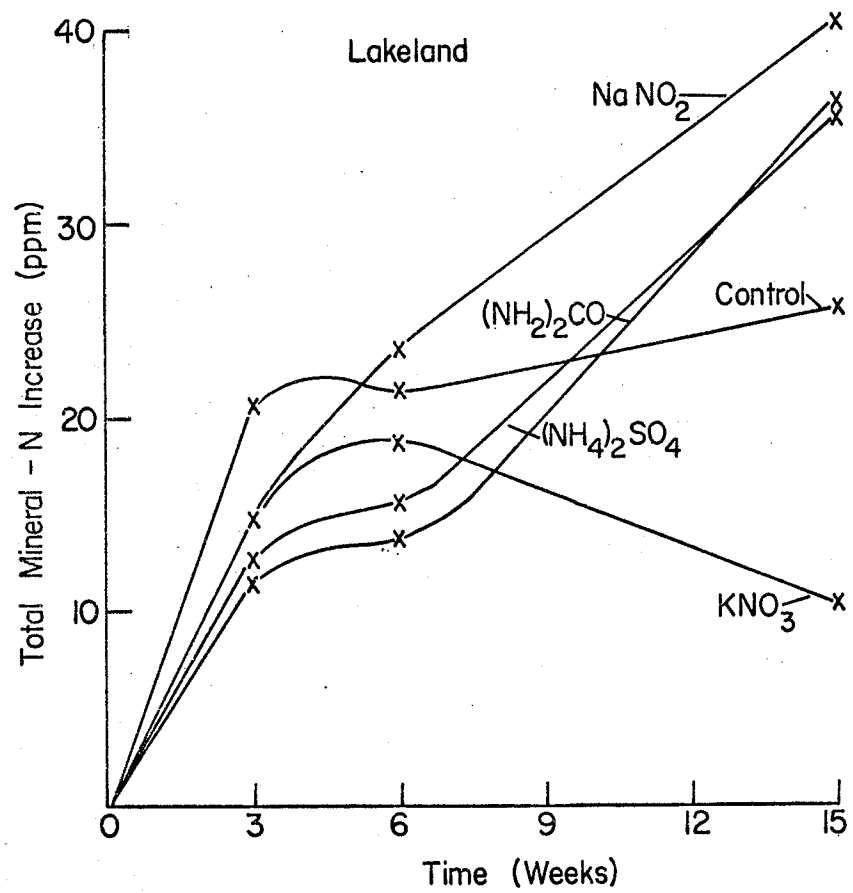


Figure 3. Mineralization of soil N in Lakeland and Holland after various treatments.

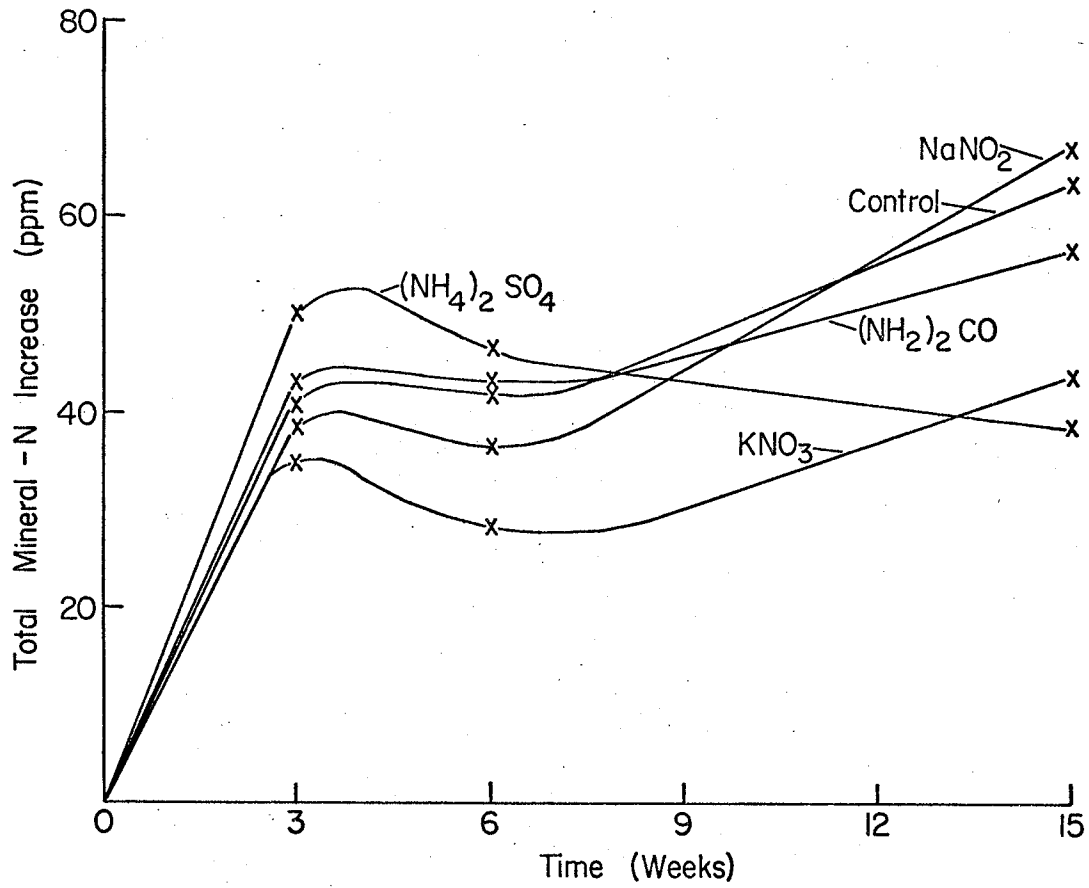


Figure 4. Mineralization of soil nitrogen in Wellwood after various treatments.



stimulation of various fractions of the microbial population, (2) long-term effects on pH, and (3) the residual effect of various ions such as  $\text{SO}_4^{2-}$ ,  $\text{K}^+$ , and  $\text{Na}^+$ . It is not known how these factors might act or interact to give some of the long-term effects on mineral-N indicated in Figures 3 and 4.

#### SUMMARY AND CONCLUSIONS

(1) Long-term incubation of Lakeland, Wellwood, and Holland soil materials led to large differences in the final level of mineral-N with nitrite, nitrate, ammonium-sulphate, and urea as N carriers. These differences appeared to be mainly the result of various mineralization and immobilization balances.

(2) A loss of N amounting to 34% applied nitrite-N could have occurred in Holland, but not in Lakeland and Wellwood soil materials. After fifteen weeks, total mineral-N was highest on nitrite-treated samples, suggesting that sodium nitrite stimulated mineralization from organic matter. Such enhanced mineralization would obscure any losses of N as based upon comparisons of total mineral-N data.

(3) Nitrite reduced the activity of Nitrosomonas in the three soil materials, but particularly in the acid Holland. In the latter, Nitrobacter activities were also inhibited, leading to the greater persistency of nitrite in a soil which was already slow to nitrify added ammonium from urea and ammonium sulphate. Acidic conditions, in addition to promoting nitrite instability, might also be expected to promote mineral-N loss through increasing nitrite persistence.

(4) It is concluded from this and preliminary experiments that there is little danger of chemical denitrification in Lakeland and Wellwood

when 50 ppm fertilizer-N is uniformly applied. Holland indicated a potential for such a loss, but Lakeland and Wellwood oxidised 50 ppm nitrite-N too rapidly for potential losses to be manifested. Biological denitrification, however, could be appreciable on the active Wellwood in the initial stages of incubation.

## CHAPTER SEVEN

### STUDIES ON NITRITE ACCUMULATION

#### INTRODUCTION

The data and conclusions presented in Chapters Five and Six show that nitrite did not accumulate during nitrification of 50 ppm-N added as ammonium sulphate and urea. On the basis of work by previous authors (35, 65, 72) it was reasoned that higher fertilizer concentrations were necessary to cause an inhibition of nitrite oxidation.

Two experiments were conducted to test this. In the first, a short duration incubation of Lakeland and Wellwood was made with 100 and 150 ppm urea- and ammonium sulphate-N. Holland was not included because of its slow nitrification rate. The second experiment entailed incubation of Lakeland, Wellwood and Holland soil materials with 200, 400 and 800 ppm urea- and ammonium sulphate-N. The aim was (1) to evaluate the concentration of applied-N at which nitrite accumulation became significant, (2) to study some of the factors responsible for nitrite accumulation, and (3) to establish whether nitrite buildup was related to mineral-N losses through chemical denitrification.

#### MATERIALS AND METHODS

Experimental procedures for incubation were similar to those previously described. In the short-term experiment, duplicate samples were collected at 0, 2, 4, 8, and 12 days. In the second experiment, duplicate sampling was made at 0, 1, 2, and 4 weeks. Mineral-N and pH analyses were made as previously described.

## RESULTS AND DISCUSSION

Incubations with 100 and 150 ppm-N

Data from the incubation experiment at 100 and 150 ppm-N are not shown in detail, since only two new features emerged concerning net mineralization rate and urea hydrolysis. Table 15 shows that nitrite did not accumulate substantially, although small increases appeared at higher N levels. After twelve days' incubation only urea-treated Lakeland (100 ppm-N) completely nitrified the original plus applied N. Net mineralized-N decreased at higher concentrations, especially in Lakeland. Ammonium sulphate at 150 ppm promoted a sharp reduction in mineralized-N for Wellwood, analagous to the previous effect noted at 50 ppm-N with Holland, (c.f. Chapter Five, p.50). Salt effects are suspected to account for these results. Urea also promoted an increase in pH on this soil material which lasted for the first four days of incubation, but this declined to give similar values to ammonium sulphate as nitrification progressed.

Figure 5 illustrates diagrammatically the different urea hydrolysis rates, determined as in Chapter Five (p.50), as related to applied urea-N level. Data from 200, 400, and 800 ppm-N incubations are also included. At the concentrations used, urea hydrolysis in Wellwood was a function of applied N level, so that urease activity was apparently not saturated by 800 ppm urea-N. In Lakeland, however, no further hydrolysis occurred beyond 100 ppm-N. These results show that the previously noted differences in urea hydrolysing rates were accentuated at higher concentrations.

Nitrite accumulation with 200, 400, and 800 ppm-N.

Mineral-N and pH data from incubations at 200, 400 and 800 ppm-N are shown in Tables 16, 17 and 18. At 200 ppm, only Wellwood produced any

TABLE 15

SELECTED DATA\* ON NITRIFICATION DURING  
TWELVE DAYS' INCUBATION (ppm)

SOIL MATERIAL	FORM ADDED N	LEVEL (ppm)	MAX. NO <sub>2</sub> <sup>-</sup> -N	NET <sup>+</sup>		MAX. pH	FINAL NH <sub>4</sub> -N
				MINERALIZED N	INITIAL-FINAL pH		
LAKELAND	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0	0.0	12	7.8 - 7.8	7.9	1
		50	0.0	14	7.8 - 7.8	7.8	7
		100	0.0	7	7.8 - 7.7	7.8	56
		150	0.8	2	7.8 - 7.8	7.8	29
	(NH <sub>2</sub> ) <sub>2</sub> CO	0	0.0	12	7.8 - 7.8	7.9	1
		50	0.0	12	7.8 - 7.8	7.8	3
		100	0.0	10	7.8 - 7.8	7.8	3
		150	2.6	-7	7.8 - 7.8	7.8	58
WELLWOOD	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0	0.0	49	7.2 - 7.0	7.2	22
		50	0.0	46	7.3 - 7.0	7.3	3
		100	0.9	34	7.2 - 6.7	7.2	33
		150	1.3	18	7.3 - 6.6	7.3	32
	(NH <sub>2</sub> ) <sub>2</sub> CO	0	0.0	49	7.2 - 7.0	7.2	22
		50	0.0	43	7.2 - 7.0	7.2	1
		100	0.0	32	7.4 - 6.9	7.4	37
		150	3.2	33	7.5 - 6.7	7.6	61

\* 0 and 50 ppm data are means of triplicates, others are means of duplicates.

<sup>+</sup> Final total mineral-N minus initial total mineral-N

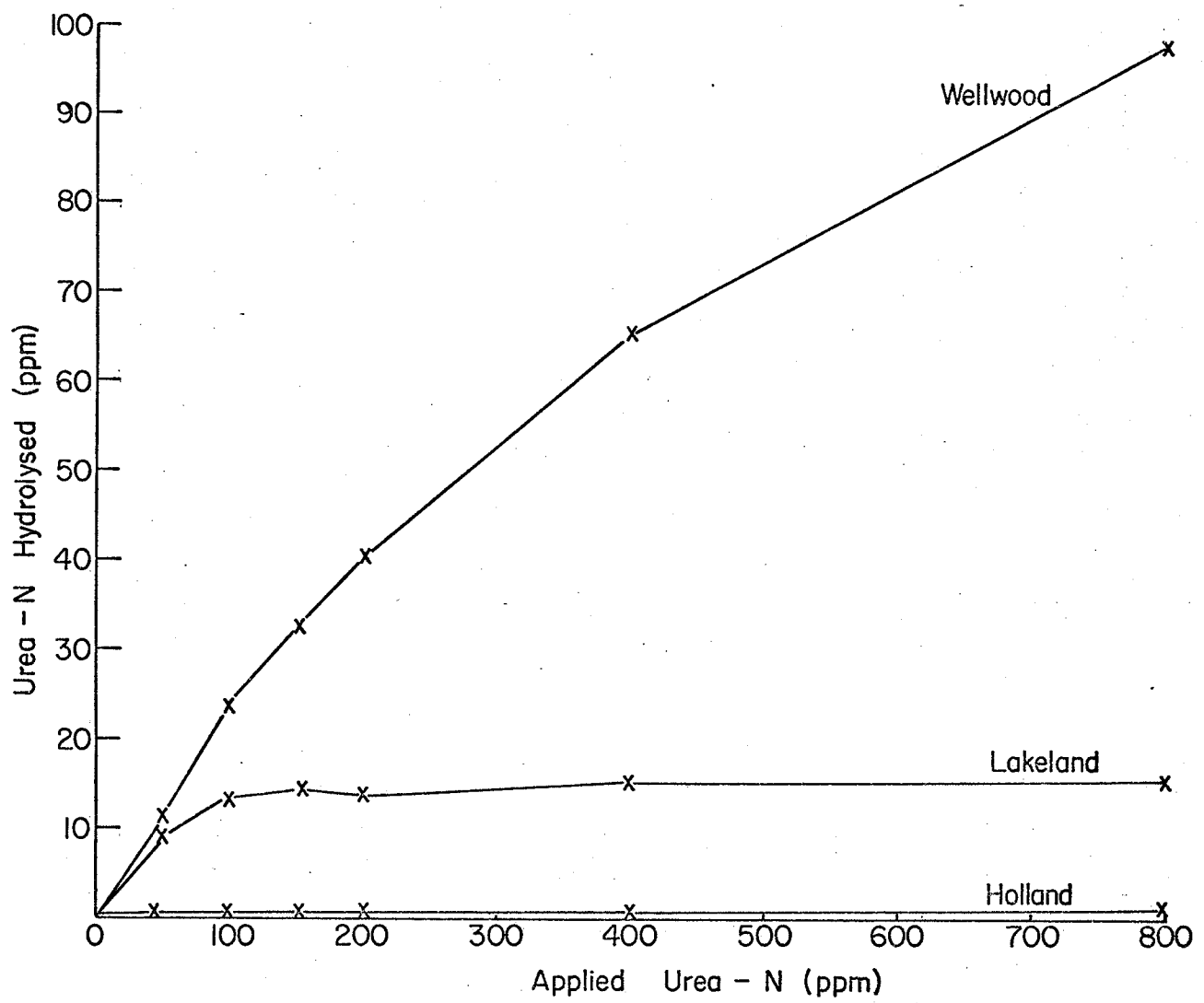


Figure 5. Urea-N hydrolysed during extraction as a function of applied-N level.

TABLE 16

MINERAL NITROGEN LEVELS AND pH\* IN LAKELAND INCUBATED WITH 200, 400,  
AND 800 PPM-N AS AMMONIUM SULPHATE  
AND UREA

TIME (weeks)	LEVEL N	AMMONIUM SULPHATE					UREA				
		pH	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	NO <sub>2</sub> <sup>-</sup> -N	Tot-N	pH	NH <sub>4</sub> <sup>-</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	NO <sub>2</sub> <sup>-</sup> -N	Tot-N
	200	7.8	206	6	0	212	7.9	15	5	0	20
0	400	7.8	407	5	0	412	7.8	15	6	0	21
	800	7.8	806	5	0	811	7.8	15	4	0	19
.....											
	200	7.9	179	33	4	216	8.3	180	41	3	224
1	400	7.9	352	51	5	408	8.4	340	41	7	388
	800	7.9	722	103	4	829	8.5	729	103	7	839
.....											
	200	7.8	148	83	2	233	8.3	112	125	2	238
2	400	7.8	297	113	3	414	8.3	252	155	4	411
	800	7.9	705	136	3	844	8.4	677	136	25	838
.....											
	200	7.3	5	238	1	244	7.3	7	233	1	241
4	400	7.1	0	444	1	445	7.1	6	431	1	438
	800	6.8	158	664	2	824	6.9	0	860	1	861

\*Data are means of duplicates, N expressed as ppm air-dry basis.

TABLE 17

MINERAL NITROGEN LEVELS AND pH\* IN WELLWOOD INCUBATED WITH 200, 400  
AND 800 PPM-N AS AMMONIUM SULPHATE  
AND UREA

TIME (weeks)	LEVEL N	AMMONIUM SULPHATE				UREA					
		pH	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	NO <sub>2</sub> <sup>-</sup> -N	Tot-N	pH	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	NO <sub>2</sub> <sup>-</sup> -N	Tot-N
0	200	7.2	234	6	0	240	7.4	68	7	0	75
	400	7.2	436	7	0	443	7.5	93	6	0	99
	800	7.2	834	6	0	840	7.7	120	7	0	127
.....											
1	200	7.3	226	35	10	271	7.9	204	39	17	260
	400	7.3	373	32	11	416	8.0	368	28	20	416
	800	7.3	809	18	11	838	8.2	754	50	21	825
.....											
2	200	6.8	85	162	2	249	7.5	47	207	6	260
	400	6.7	300	108	2	410	7.5	187	103	149	439
	800	6.7	702	103	2	807	7.7	502	52	231	785
.....											
4	200	5.5	3	252	0	255	5.9	3	258	1	262
	400	4.7	36	349	0	385	5.0	14	192	206	412
	800	4.2	388	432	1	821	6.2	254	70	437	761

\*Data are means of duplicates, N expressed as ppm air-dry basis.



TABLE 18

MINERAL NITROGEN LEVELS AND pH\* IN HOLLAND INCUBATED WITH 200, 400,  
AND 800 PPM-N AS AMMONIUM SULPHATE  
AND UREA

TIME (weeks)	LEVEL N	AMMONIUM SULPHATE				UREA					
		pH	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	NO <sub>2</sub> <sup>-</sup> -N	Tot-N	pH	NH <sub>4</sub> <sup>-</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	NO <sub>2</sub> <sup>-</sup> -N	Tot-N
0	200	6.2	208	13	0	221	6.1	11	13	0	24
	400	6.2	408	12	0	420	6.2	12	13	0	25
	800	6.2	810	14	0	824	6.1	11	14	0	25
1	200	6.6	178	17	2	197	7.3	170	32	2	204
	400	6.6	349	21	2	372	8.0	318	40	3	361
	800	6.6	746	36	1	783	8.4	750	13	7	770
2	200	6.5	145	43	1	189	7.2	152	66	2	220
	400	6.6	320	49	1	370	7.7	289	102	3	394
	800	6.5	728	33	2	763	8.3	647	87	15	749
4	200	5.4	84	105	0	189	5.2	30	191	0	221
	400	5.6	298	75	0	373	4.8	84	309	0	393
	800	5.9	669	45	0	714	5.3	305	432	0	737

\*Data are means of duplicates, N expressed as ppm air-dry basis.

significant nitrite, this being a maximum of 10 and 17 ppm-N with ammonium sulphate and urea, respectively. At 400 ppm, nitrite was generally higher, especially in urea-treated Wellwood. Urea-N at 800 ppm produced maxima of 25 and 15 ppm nitrite-N in Lakeland and Holland respectively, Wellwood being vastly different in giving 400 ppm-N which increased up to four weeks. The two most important factors which appeared to influence nitrite accumulation were those pertaining to pH and soil material. For convenience, nitrite accumulation is discussed under these two broad headings.

Influence of pH. The influence of pH was manifested in both type and level of N-carrier. Large differences in nitrite were obtained between urea and ammonium sulphate on Wellwood (Table 17) with similar but smaller differences on Lakeland and Holland (Table 16 and 18). Markedly higher pH was obtained with urea treatments, especially during the first two weeks before nitrification became active enough to promote acidity. In Holland, urea had nitrified at two to ten times the rate for ammonium sulphate in four weeks, accounting for the lower pH at this time. Wellwood differed from Lakeland and Holland after two weeks in that nitrite continued to accumulate with 400 and 800 ppm urea-N, even though the pH at the 400 ppm level was markedly acid.

The rate of ammonium oxidation was consistently higher with urea than ammonium sulphate, particularly in the Holland soil material. The slow ammonium oxidation in ammonium sulphate treated Holland could be due to its lower initial pH and consequent slow proliferation of Nitrosomonas (c.f. Chapter Six, p.65). The fact that the already alkaline Lakeland also showed this differential oxidation rate suggests that salt effects may have operated in conjunction with the response of nitrifying organisms to higher pH.

Optimum pHs are regarded to be higher for Nitrosomonas spp. than Nitrobacter (4), so that an increase in pH may favour the oxidation of ammonium to nitrite and partly account for nitrite accumulation. In Lakeland and Holland, where nitrite did not markedly accumulate, the faster ammonium oxidation with urea was reflected in a higher nitrification rate compared to ammonium sulphate. In Wellwood, however, the urea nitrification rate was higher only at 200 ppm-N, since nitrite accumulated at 400 and 800 ppm -N. This gave a lower nitrification rate than with ammonium sulphate, even though the ammonium oxidation rate was much higher.

At a given pH, higher concentrations of ammonium will lead to higher amounts of unionized ammonia in solution. Urea, in addition, raises the pH and the ammonia to ammonium ratio. Higher concentrations of urea might therefore be expected to promote more nitrite accumulation than the same increment in ammonium sulphate concentration. In fact, increases in ammonium sulphate concentration had no effect on nitrite accumulation. Urea, in contrast, always promoted more nitrite at the highest level of application. Wellwood contained 6, 150 and 230 ppm nitrite-N after two weeks with 200, 400, and 800 ppm urea-N respectively. Under these experimental conditions, concentrations of urea-N greater than 200 ppm caused significant nitrite accumulation in Wellwood, whereas in Lakeland and Holland concentrations of 800 ppm-N produced only a small buildup. There were no indications that ammonium sulphate applications greater than 800 ppm-N would promote significant nitrite buildup in any soil material.

Influence of soil material. Important differences in nitrite accumulation existed between soil materials, which were not directly related to pH. According to many workers (35, 65, 74, 78, 101), the higher pH of urea

treatments at 0 and 1 week was more favourable for nitrite accumulation in Lakeland than in Wellwood soil material. Neither the threshold pH value of  $7.7 \pm 0.1$  suggested by Martin et al. (72) nor the initial-pH categories of Morrill and Dawson (cf. Chapter Five, p. 42) were useful in predicting the observed nitrite accumulation. This was most clearly shown with ammonium sulphate where pH values of Lakeland and Wellwood were above and below 7.7 respectively after one week, but Wellwood accumulated twice as much nitrite. The pH of 800 ppm-N urea treated samples was 8.4 and 7.7 in Lakeland and Wellwood after two weeks respectively, yet approximately ten times as much nitrite accumulated in Wellwood whilst three times as much nitrate was formed in Lakeland. It must be concluded with Broadbent et al. (27) that pH is not the sole factor which determines nitrite accumulation.

Morrill and Dawson (78) demonstrated that rapid oxidation of nitrite could occur in the presence of high ammonium levels at a pH higher than 7.8 if Nitrobacter spp. had time to generate. The higher buffering capacity (Table 1) and slower urea hydrolysis rate of Lakeland (Figure 5) would combine to produce less inhibition of Nitrobacter spp. than in Wellwood during the growth initiation period. Other factors which probably enhanced this effect were (1) the high net mineralization rate of Wellwood, contributing ammonium to enhance the inhibition of Nitrobacter spp. (Table 15), and (2) the relative increase in the oxidation rate of ammonium in urea-treated samples compared to ammonium sulphate treatments, Wellwood showing bigger differences than Lakeland and hence a more stimulated Nitrosomonas population (Tables 16 and 17).

There was a marked similarity in the pH trends of urea-treated Wellwood and Holland (Tables 17 and 18). That there was no such similarity

in nitrite accumulation may be attributed to their different Nitrosomonas activities. Holland was slow to oxidise ammonium, particularly as shown in two-week data, whereas Wellwood had a shorter lag-period and higher maximum rate, as shown in Figure 6. Lakeland and Holland had similar lag-periods of about one-and-a-half weeks with 800 ppm urea-N. The lag-period of less than one week with Wellwood emphasizes the importance of Nitrosomonas activities in nitrite accumulation, particularly as expressed during the initial phase of incubation. The longer is the lag-period, then the more time do Nitrobacter spp. have for generation.

Figures 7 and 8 indicate the activities of Nitrosomonas and Nitrobacter spp. in Wellwood under different rates of urea application. Nitrosomonas activities were not greatly affected by ammonium concentration in the first two weeks. In the two to four week period, however, activity declined at the 200 and 400 ppm rates, probably as the result of limiting ammonium concentration. At 800 ppm-N, the observed decline could not be due to limiting ammonium concentration since 250 ppm ammonium-N were still present at four weeks. The relatively high nitrite produced at this level could be inhibiting ammonium oxidation in the same manner as observed earlier in incubations with 50 ppm nitrite-N (cf. Chapter Six, p.65). If so, an even greater effect would be exerted on Nitrobacter activities, particularly with the development of acidity from nitrite formation (110). Figure 8 shows that nitrate formation was rapid in the first week at the highest level of urea application, and thereafter nitrate increased only slowly as nitrite buildup occurred. It is therefore suggested that, where large concentrations of urea were applied, an inhibition of Nitrobacter spp. was caused by unionized ammonia in the initial stages of incubation. As time

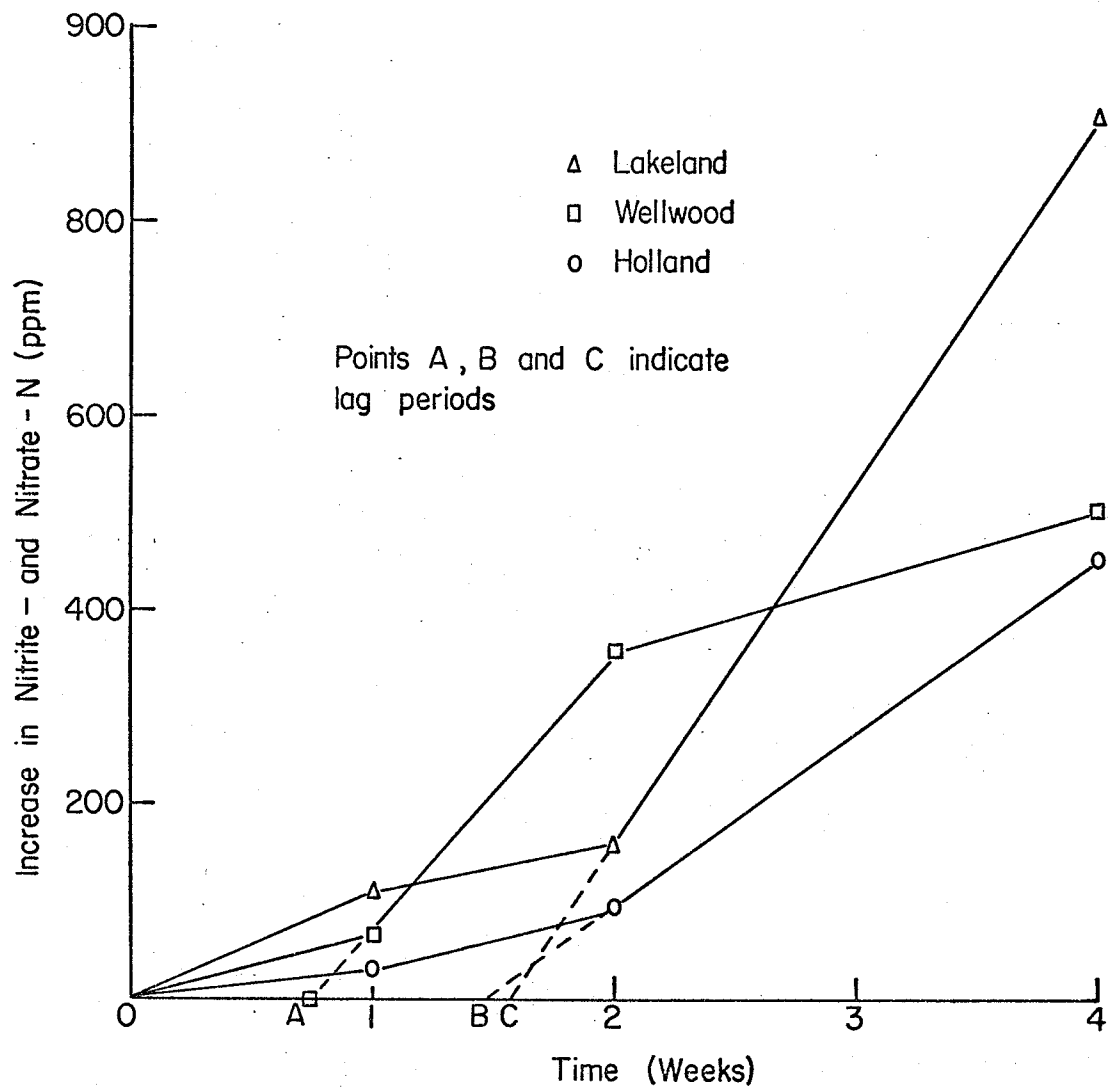


Figure 6. Increase in nitrite- and nitrate-N (indicating Nitrosomonas activities) during incubation with 800 ppm urea-N.

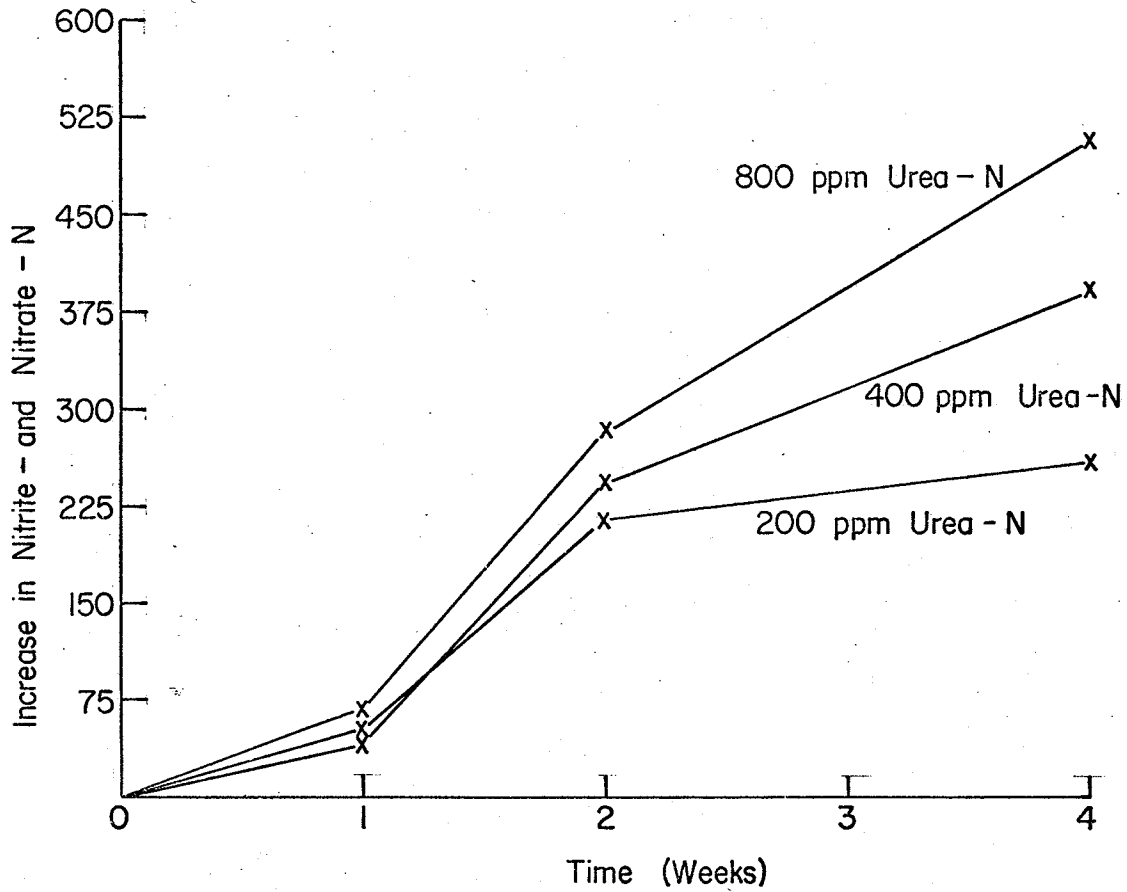


Figure 7. Nitrite and nitrate-N formed in Wellwood at three levels of urea-N.

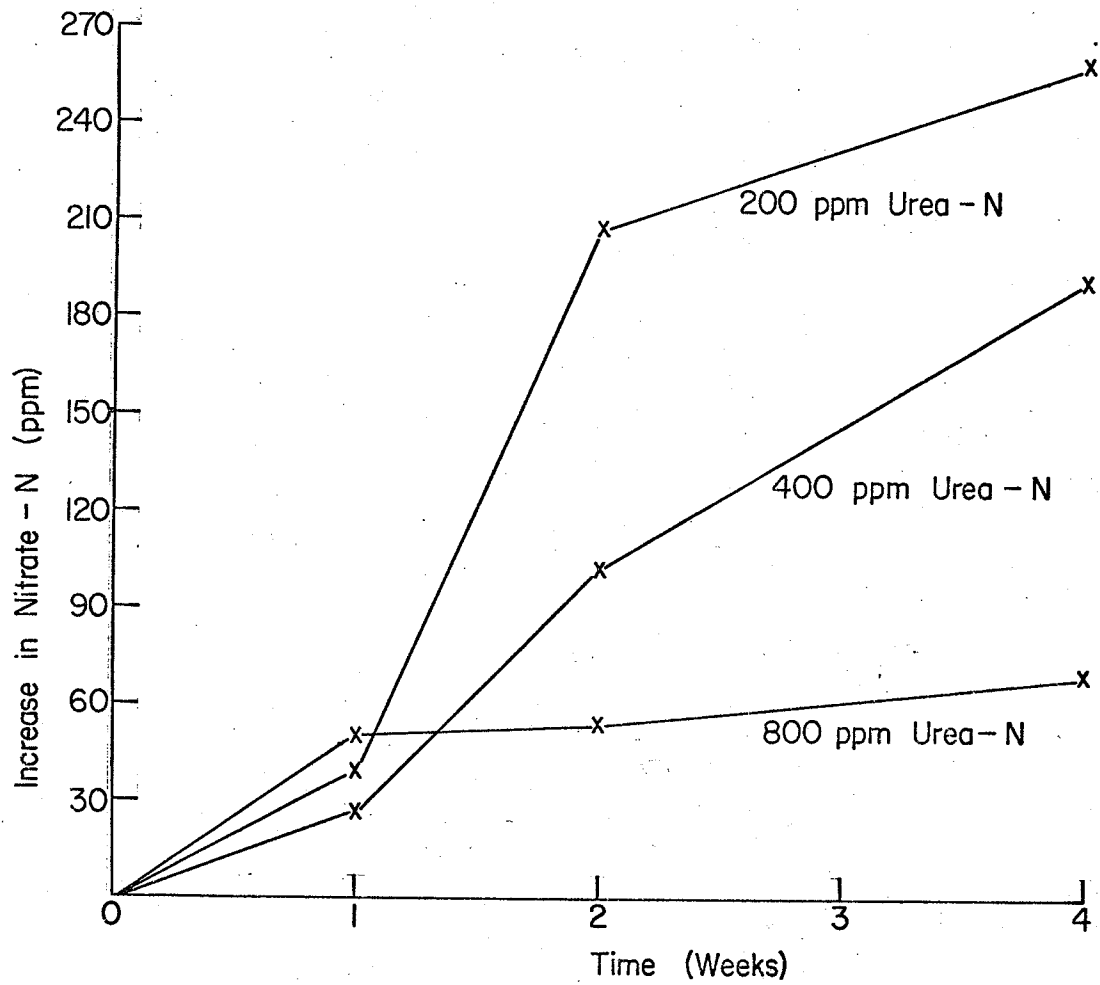


Figure 8. Nitrate-N formed in Wellwood at three levels of urea-N.



proceeded, however, a combination of high nitrite and lower pH supplemented this inhibition and prolonged the persistency of nitrite down to acid pHs. This mechanism would also account for the accumulation of nitrite at acid pH. It is relevant to note that nitrite added to Holland soil material retarded the formation of nitrate, as well as the oxidation of mineralized ammonium, at a pH of 6.2 (Chapter Six, p.66).

Since solution ammonium, in association with pH, has a direct bearing on Nitrobacter inhibition, it was felt that water extractions of the soil materials would have yielded useful information in the interpretation of data. It was suspected that the high carbonate content of Lakeland had some influence on solution ammonium, possibly through a common ion effect on the dissociation of ammonium bicarbonate. This might be born in mind for future work.

#### Mineral nitrogen losses

Table 19 shows the mineral-N gains and losses for the three soil materials treated with ammonium sulphate and urea. The calculation was made using total mineral-N at 0 time as a reference. Since mineralization from organic matter was inhibited in the presence of fertilizer, particularly with ammonium sulphate (Table 15), the view was taken that losses were more certainly expressed as reduction in the initial total-N, rather than assuming that mineralization of control samples was paralleled in the treatments. Mineralization in treated samples, if it occurred, would lead to an underestimation of losses.

Gains of mineral-N, indicative of net mineralization, were obtained on Lakeland and Wellwood samples, the latter at the lowest rates of application only. Gains were not as high as those obtained at lower

TABLE 19

## GAINS AND LOSSES\* OF MINERAL NITROGEN

SOIL MATERIAL	TIME (weeks)	$(\text{NH}_4)_2\text{SO}_4$ ADDED ppm-N			$(\text{NH}_2)_2\text{CO}^+$ ADDED ppm-N			MEANS FOR BOTH TREATS.	INITIAL pH OF CONTROL SOIL
		200	400	800	200	400	800		
LAKELAND	1	-4	-4	+18	+12	-24	+28	+18	7.8
	2	+11	+2	+33	+26	-1	+27		
	4	+32	+33	+13	+29	+26	+60		
	Means	+13	+10	+21	+22	0	+38		
	Overall means	+15			+20				
.....									
WELLWOOD	1	+31	-27	-2	+20	-27	-15	-15	7.2
	2	+9	-33	-33	+20	-4	-55		
	4	+15	-58	-19	+22	-31	-79		
	Means	+18	-39	-18	+21	-21	-50		
	Overall means	-13			-17				
.....									
HOLLAND	1	-24	-48	-41	-17	-59	-54	-44	6.2
	2	-32	-50	-61	-1	-26	-75		
	4	-32	-47	-110	0	-27	-87		
	Means	-29	-48	-71	-6	-37	-72		
	Overall means	-49			-38				

\*Expressed as the difference between initial total mineral-N and total mineral-N after incubation.

†Assuming that initial mineral-N was the same as for the ammonium sulphate treatment.

fertilizer rates (Table 15 and Figures 3 and 4), indicating either increased losses or reduced mineralization at higher rates.

Losses of mineral-N were obtained at the 400 and 800 ppm rate on Wellwood and for all treatments on Holland. Larger losses were obtained with urea than with ammonium sulphate on Wellwood, possibly due to the higher nitrite which accumulated with the former. In Holland, losses with urea were less than with ammonium sulphate, acidity being higher in the latter for the first two weeks. Average losses were lower with high initial pH and buffering capacity. This is consistent with the operation of chemical denitrification, since nitrite formed as an intermediate during nitrification is increasingly unstable as pH decreases. The data indicate that losses through chemical denitrification were of the order Holland > Wellwood > Lakeland.

Ammonia volatilization did not contribute markedly towards N losses. Highest losses would be expected at the highest pH and urea level (60, 88), yet there was no consistent difference between ammonium sulphate and urea at this level. Contrary to the operation of this mechanism, losses frequently increased progressively with nitrification and consequently pH decline. This was clearly seen for urea-treated Wellwood (800 ppm-N) and ammonium sulphate-treated Wellwood and Holland at 400 and 800 ppm-N respectively.

Only at the highest level of urea application in Wellwood was there any association between mineral-N losses and nitrite accumulation. Figure 9 shows that mineral-N declined steadily whilst nitrite-N increased. This treatment lost about three times as much N as ammonium sulphate. The N was lost at a greater rate at lower nitrite concentrations, whilst

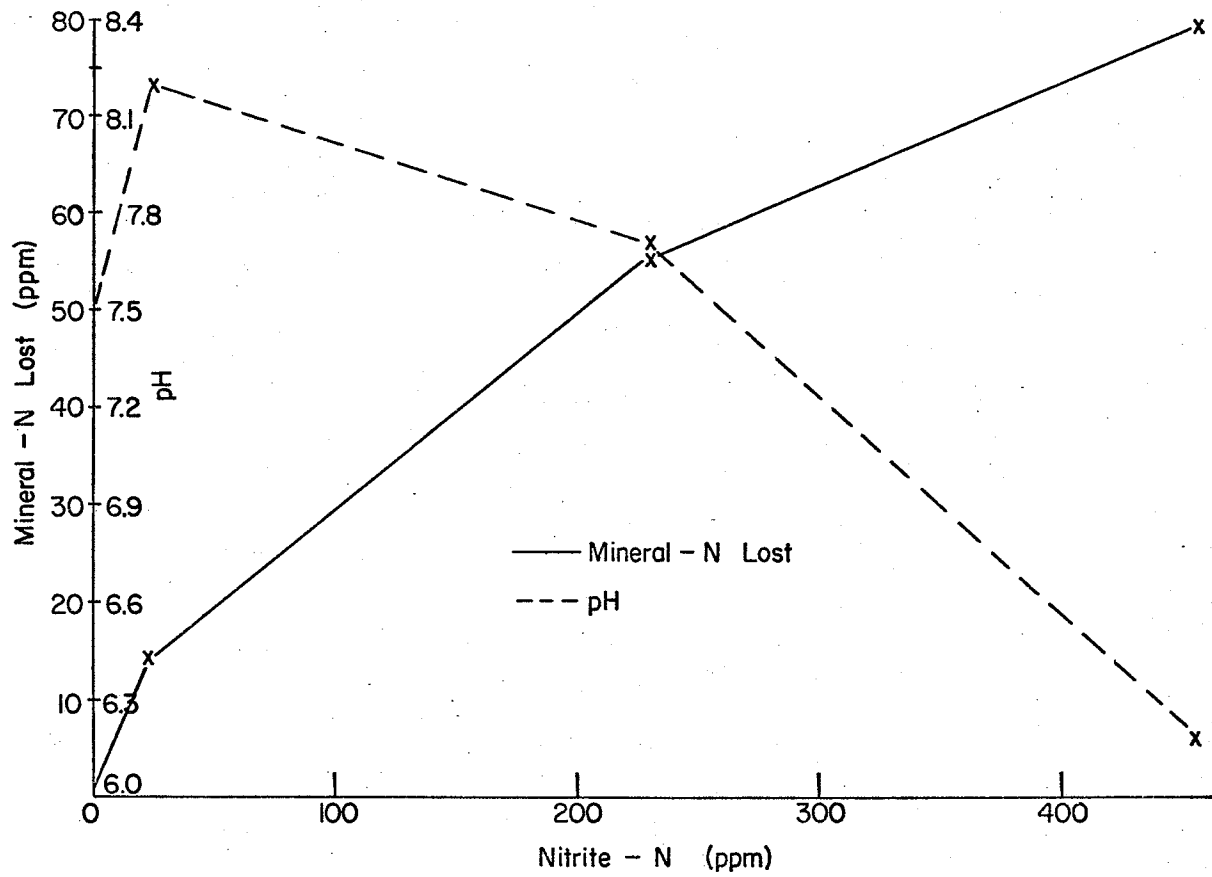


Figure 9. Mineral-N loss and pH of 800 ppm urea-N treated Wellwood as related to nitrite accumulation.

acidity increased most between two and four weeks. The simple pH-dependent decomposition of nitrite does not adequately explain the shape of the curve. The work of Wahab and Uddin (116, 117) suggests a possible explanation in that the interaction between nitrite and ammonium leading to gaseous N losses was optimal at an ammonium to nitrite ratio of five to one. This was approached more closely during the initial stages of incubation. An interaction between ammonium and nitrite has previously been found in KCl extracts of Wellwood soil material (Chapter Four, p.40).

The high initial loss in other incubations might also be thus explained. Gerretsen and de Hoop (54) showed that nitrite accumulation was not necessary for chemical denitrification to occur, provided that nitrite was formed biologically. Tables 16, 17 and 18 show that some nitrate was formed in the first week in all cases, entailing the concomitant formation of more nitrite than was indicated by the small accumulations.

Except for Lakeland and ammonium sulphate treated Wellwood, losses of mineral-N increased with increasing fertilizer rates. Band application of fertilizer to field soils of these associations could therefore show enhanced losses close to the source. Where about 100 lbs N per acre (50 ppm) were applied, experiments indicated little loss by denitrification, whereas the same overall concentration applied in a band may give losses of 0.2 to 13% of applied N (as calculated from Table 19) when local soil concentration reaches 800 ppm-N.

#### SUMMARY AND CONCLUSIONS

(1) Nitrite accumulation was of the order Wellwood > Lakeland > Holland, with Wellwood showing by far the greatest buildup. Initial pH and pH attained during incubation were not useful in predicting this

trend. However, nitrification, mineralization, and urea hydrolysis rates in conjunction with pH and buffering capacities could be used to explain the reverse of the predicted trend with Lakeland and Wellwood. Holland, because of its low pH and slow nitrification rate, had little potential to accumulate nitrite.

(2) Urea promoted the accumulation of more nitrite than ammonium sulphate for the three soil materials. It was also nitrified more rapidly, with the difference between the ammonium sulphate rate increasing with the initial acidity of the soil material. Nitrosomonas activity was enhanced with urea, a fact which would contribute to nitrite accumulation.

(3) Increasing levels of urea were more conducive to nitrite accumulation, in contrast to ammonium sulphate where a clear concentration effect was not shown. Urea-N at 400 ppm caused a large buildup in Wellwood after one week, whereas applications of 800 ppm-N gave no significant buildups in Lakeland and Holland. With 800 ppm urea-N in Wellwood, nitrate formation was strongly inhibited down to pH 6.2. The high level of nitrite probably accounted for this, as well as for the inhibition of ammonium oxidation at non-limiting concentrations of the substrate.

(4) Mineralization of organic matter gave increases in mineral-N over initial values in Lakeland and in Wellwood at 200 ppm ammonium sulphate- and urea-N. Losses were obtained at the higher applications on Wellwood on for all levels on Holland. These ranged from 0.2 to 13% of applied N. Averages for all treatments and levels related the soil materials in the order Holland > Wellwood > Lakeland for N lost, suggesting an overall dependancy on initial pH and buffering capacities comensurate with the operation of chemical denitrification.

(5) Wellwood, with 800 ppm urea-N, was the only case where mineral-N losses increased with increasing nitrite concentration. The shape of the N-loss/nitrite curve in relation to pH change suggest that simple chemical decomposition of nitrite was not the only mechanism involved.

(6) The data indicate that chemical denitrification is an important mechanism of loss at higher fertilizer rates, and that nitrite accumulation is a conducive but not necessary prerequisite. A general conclusion drawn from the data is that band applications of ammonium sulphate and urea will lead to higher losses at high local concentrations of fertilizer.

## CHAPTER EIGHT

### STUDIES ON NITRITE INSTABILITY

#### INTRODUCTION

Data presented in Chapter Seven indicated the need for a more direct study of mineral-N losses in relation to pH and the presence of nitrite and ammonium. A series of experiments were therefore conducted in which the soil material was treated with a known amount of nitrite, and mineral-N changes were followed under conditions in which nitrification was minimal. These involved (1) incubation of autoclaved samples with nitrite, (2) the autoclaving of soil materials and buffered solution of similar pH with nitrite, and (3) air-drying of samples in the presence of nitrite. The use of the last two techniques was intended to accelerate the decomposition of nitrite, allowing a more convenient study of some of the factors involved over a short period of time. Of interest throughout was the relative tendency of Lakeland, Wellwood, and Holland soil materials to undergo aerobic, chemical denitrification. This was investigated with a view to further understanding the conclusions reached in Chapter Seven.

#### MATERIALS AND METHODS

Samples were autoclaved at  $120^{\circ}\text{C}$  and 15 lbs/sq. in. pressure for thirty minutes. In the incubation experiment, this was performed on 50 gms air-dried material contained in a 500 ml jar. Sodium nitrite solution, distilled water, pipette, and rubber stoppers were sterilized simultaneously. Nitrite, which did not undergo any breakdown in the time used, was subsequently added in 12.5 ml of solution to give a concentration of 50 ppm, air-dry basis. Control samples were treated with the same volume of distilled water. Samples did not increase in moisture content during



sterilization, so that final concentrations of nitrite and moisture contents were similar to those for the long-term incubation experiment (Chapter 5). Duplicate subsamples were collected for mineral-N analyses at 0, 2, 5, and 8 weeks. Periodic aeration and moisture checks were performed as in Chapter 5.

Buffered solutions were made using the proportions shown. These were used to make up one litre solutions containing sodium nitrite which would have given 50 ppm nitrite-N if applied to 10 gms of soil material in 2.5 ml of solution. The pH of the final solution is shown in parenthesis.

pH 5.6 50 ml 0.1 M  $\text{KH}_2\text{PO}_4$  + 36.6 ml 0.1 M NaOH (5.45)

pH 7.0 50 ml 0.1 M  $\text{KH}_2\text{PO}_4$  + 29.1 ml 0.1 M NaOH (6.85)

pH 7.8 50 ml 0.1 M  $\text{KH}_2\text{PO}_4$  + 44.5 ml 0.1 M NaOH (8.0 )

Duplicate 10 gm samples of soil materials treated with 2.5 ml of unbuffered nitrite solution, and 2.5 ml of buffered solution, were sterilized in 125 ml Erlenmyer flasks for thirty minutes. Analyses were then performed for ammonium-, nitrite-, and nitrate-N as for previous experiments.

Air-drying of nitrite-treated samples was performed on the laboratory bench, using a small fan to accelerate the process. Ten-gram samples were used, contained in a 125 ml Erlenmyer flask. These were periodically weighed to test for completeness of drying. It was found that 48 hours was usually sufficient. A further discussion of methods is given under individual headings.

## RESULTS AND DISCUSSION

### Incubation of sterilized samples with nitrite.

Results showing the changes in mineral-N of sterilized soil materials incubated with 50 ppm nitrite-N are shown in Table 20. In all cases,

TABLE 20

MINERAL NITROGEN OF STERILIZED SAMPLES INCUBATED WITH 50 PPM  
ADDED NITRITE

SOIL MATERIAL	INCUBATION PERIOD (wks)	ADDED NITRITE				LOSS*	pH
		$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	$\text{NO}_2^-\text{-N}$	$\text{NO}_2^-\text{-N}+\text{NO}_3^-\text{-N}$	$\text{NO}_2^-\text{-N}$ (%)	
LAKELAND	0	32	11	50.0	61	0	7.7
	2	30	12	46.4	58	7.2	7.7
	5	31	11	44.7	56	10.8	7.7
	8	24	11	28.3	39	43.4	7.7
WELLWOOD	0	46	10	50.0	60	0	7.1
	2	54	10	42.1	52	15.8	7.1
	5	78	11	36.0	47	28.0	7.1
	8	99	11	34.8	45	30.4	7.1
HOLLAND	0	16	13	50.0	63	0	6.2
	2	19	13	36.1	49	27.8	6.2
	5	18	21	29.4	51	26.8	6.2
	8	20	25	17.0	42	42.4	6.2

\*Expressed as % of 50 ppm applied  $\text{NO}_2\text{-N}$ , assuming no oxidation of ammonium to nitrate, and taking increased nitrate to indicate chemical oxidation of nitrite, which was not therefore lost from the system.

nitrite-N decreased with incubation, the greatest reduction being shown in Holland (pH 6.2) and least in Wellwood (pH 7.1). No special precautions were taken to maintain sterility beyond those employed in the initial autoclaving. Sterility was not tested for at the various sampling times. Incomplete sterility was indicated in Wellwood by an extensive increase in exchangeable ammonium with time, a smaller increase also being recorded for Holland. However, nitrate was stable in Lakeland and Wellwood, so that nitrification did not occur. The persistence of ammonification but not nitrification is consistent with the fact that ammonifiers are far more abundant in nature (3) and hence more liable to contaminate the sterilized samples.

Holland contrasted with Lakeland and Wellwood in showing an increase in nitrate with incubation. However, control incubations without nitrite did not show this increase, as shown in Table 21. Exchangeable ammonium increased in all cases in the controls, but again biological oxidation to nitrate apparently did not occur. Also, negligible quantities of nitrite were detected. In Holland, the increase in nitrate with added nitrite suggests that chemical oxidation may have taken place. This is consistent with the results of Puri *et al.* (90) who found nitrite oxidation more likely to occur as percent base saturation decreased.

Losses of N from nitrite, assuming the chemical oxidation of nitrite to nitrate in Holland, are shown in Table 20. For the first five weeks, loss of nitrite-N decreased with increasing soil pH i.e. Holland > Wellwood > Lakeland. The high loss of N from Lakeland at eight weeks was inconsistent with its high pH and previous behaviour. A separate experiment to test the validity of the data in Table 20, particularly regarding Lakeland soil material, was therefore conducted. The results of this incub-

TABLE 21

CHANGES\* IN MINERAL NITROGEN OF STERILIZED CONTROL  
 SAMPLES AFTER INCUBATION FOR EIGHT WEEKS (ppm)

SOIL MATERIAL	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	$\text{NO}_2^-\text{-N}$
LAKELAND	+42	0	0
WELLWOOD	+13	+2	0
HOLLAND	+62	0	0

\*Changes refer to increases or decreases in mineral-N relative to the initial starting values.

ation, in which sterilized containers were not opened for eight weeks, are shown in Table 22. The sterility of the samples, and confirmation for the aerial contamination of samples recorded in Tables 20 and 21, are indicated by the stable ammonium contents of all soils. Losses of nitrite-N were again related to pH, these being generally smaller than losses from opened jars. A higher proportion of nitrate was formed in Holland than for opened jars (Tables 20 and 22). These data indicate that any evolved oxides of nitrogen formed from nitrite may have equilibrated more completely with the sample in closed jars, instead of disturbing the equilibrium by being lost to the atmosphere. In Lakeland and Wellwood, two base saturated soil materials, this was reflected in lower losses only; whilst the slightly unsaturated Holland also produced more nitrate presumably because some of the gaseous nitrogen oxides could be intermediates in the chemical oxidation of nitrite to nitrate.

The data of Tables 20, 21, and 22 strongly implicate nitrite in aerobic denitrification, and suggest that chemical denitrification was at least partly responsible for the losses observed at high fertilizer concentrations (cf. Chapter Seven, p.89). In both experiments, the apparent relationship of losses to increasingly acid pH was manifest, making biological denitrification and ammonia volatilization unlikely processes. In addition, this experiment shows that exposure of the sample to the atmosphere, and hence the ease of gaseous interchange, are important factors which influence nitrite-N loss. That losses of N still occurred with closed containers suggests that an inert volatile product, possibly nitrogen gas, was produced which did not enter into equilibrium reactions once formed. In acid soil materials, the equilibrium of gaseous oxides may be further

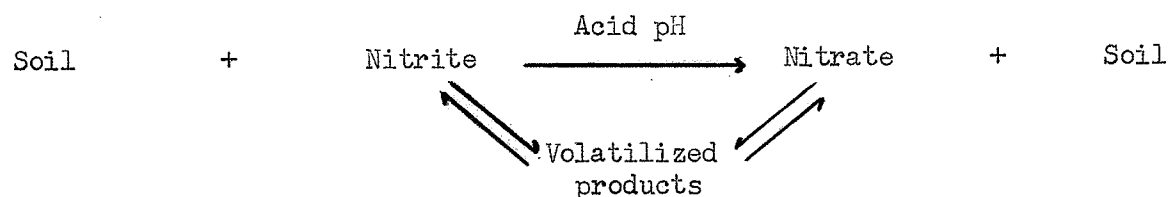
TABLE 22

MINERAL NITROGEN CHANGES AND LOSSES OF NITRITE-N\* FROM UNOPENED  
JARS AFTER EIGHT WEEKS' INCUBATION

SOIL MATERIAL	CHANGE IN NH <sub>4</sub> <sup>+</sup> -N	CHANGE IN NO <sub>3</sub> <sup>-</sup> -N	CHANGE IN NO <sub>2</sub> <sup>-</sup> -N	LOSS OF NO <sub>2</sub> <sup>-</sup> -N (%)
LAKELAND	+2.1	0	-5.0	10.0
WELLWOOD	+2	-1	-11.2	22.4
HOLLAND	0	+15	-29.2	28.0

\* Applied at 50 ppm-N.

affected by the tendency to form nitrate i.e., reduction and oxidation of nitrite would appear to be competitive processes. This was also suggested in the work of Clark et al. (39). A simplified form of the possible interactions involving nitrite might be made thus:



#### Autoclaving of sample plus nitrite.

Data from the autoclaving of nitrite with soil samples and buffered solutions are shown in Table 23. Distilled water treatments are included for comparison. Ammonium and nitrate-N levels were similar in water- and nitrite-treated samples of Lakeland and Wellwood. Holland showed a small increase of 3 ppm nitrate-N which may be attributed to nitrite oxidation. Nitrite was lost extensively from Wellwood and Holland, the relationship of N loss to pH being clear as in the previous experiment. Thus, autoclaving accelerated the decomposition process compared to incubation, but resulted in the same relative trends.

Only the pH 5.5 buffered solution showed any nitrite instability. The nitrite which disappeared was not recovered as nitrate, indicating a loss from the system. However, the loss was about one-seventh that from Holland, which was higher in pH by about 0.8 units. Estimations of pH after autoclaving showed that the buffers had not broken down, but retained their original pH values.

The data indicate that, although nitrite instability is related to pH, the instability is more marked in soil than in buffer solutions. The

TABLE 23

MINERAL-N LEVELS OF BUFFERED SOLUTIONS AND SOIL MATERIALS  
AFTER AUTOCLAVING WITH 50 PPM NITRITE-N (ppm)

SOIL MATERIALS	TREATMENTS	$\text{NH}_4^+$ -N	$\text{NO}_3^-$ -N	$\text{NO}_2^-$ -N	LOSS* OF $\text{NO}_2^-$ -N (%)
LAKELAND	Nitrite solution	21	7	44.1	11.8
WELLWOOD		46	9	29.4	41.2
HOLLAND		20	14	1.6	91.6
LAKELAND	Distilled water	22	7	0	-
WELLWOOD		47	9	1	-
HOLLAND		20	12	1	-
.....					
Buffered solution	pH 8.0	-	-	50.0	0.0
"	" " 6.9	-	-	50.0	0.0
"	" " 5.5	-	-	43.4	13.2

\*cf. Table 20.



evidence supports the work of others (38, 39, 92, 110, 122) who have shown that soil confers instability on nitrite, apparently through the operation of a pH-dependant factor.

Exchangeable ammonium did not contribute to nitrite instability, since it did not decline in the presence of nitrite as judged from comparisons with the water treatment (Table 23). This could be the result of an insufficiently high ammonium level, so the experiment was repeated with 250 ppm ammonium-N in addition to the 50 ppm nitrite-N previously used. This gave an ammonium to nitrite ratio close to 5 : 1 as quoted by Wahab and Uddin (116) for optimum interaction. Results for Lakeland and Wellwood are shown below. Considerable amounts of ammonium were lost, presumably by ammonia volatilization. Recoveries of nitrite were less than without ammonium addition (cf. Table 23), the greatest difference occurring on Wellwood. Ammonium at this level therefore stimulated the loss of N from nitrite, the effect being related to the pH of the soil material. This agrees with the

INTERACTION OF AMMONIUM AND NITRITE WHEN AUTOCLAVED WITH 250 PPM

AMMONIUM-N AND 50 PPM NITRITE-N.

Soil Material	Mineral-N levels after autoclaving*			% loss N from NO <sub>2</sub> -
	NH <sub>4</sub> -N	NO <sub>3</sub> -N	NO <sub>2</sub> -N	
LAKELAND	175	7	38.4	23.2
WELLWOOD	186	8	15.7	68.6

\*Means of duplicates.

findings of Wahab and Uddin (116) and lends support to the contention that initial losses of mineral-N in incubations with high ammonium levels may have been due to chemical denitrification (Chapter Seven, p. 91). At the pH of the soil materials (7.7 and 7.1 for Lakeland and Wellwood, respectively,

after autoclaving) a Van-Slyke type interaction was unlikely (8, 102). The interaction of ammonium and nitrite to form ammonium nitrite was more probable (8, 116). Wahab and Uddin (117) found interaction leading to N-losses even at pH 10.1, in the presence of ammonium- and nitrite-N concentrations each greater than 50 ppm.

Air-drying of sample plus nitrite.

Data indicating nitrogen loss from soil materials air-dried in the presence of nitrite are shown in Table 24. Nitrite was lost from the system for all three soil materials. As in previous experiments, the lost N increased with soil acidity, from an average of 4% with Lakeland to 32% with Holland, Wellwood being intermediate at 13%. The nitrogen lost was proportional to the nitrite present. However, when expressed as a percentage of applied nitrite-N, losses increased up to a concentration of 200 ppm nitrite-N, afterwards decreasing to give about half the maximum percentage loss at 1600 ppm-N. This effect was most clearly seen on Wellwood and Holland, and indicated the development of limiting soil conditions at very high nitrite concentrations. The initial increase in percent loss was puzzling. Of possible relevance may be the fact that many soils have been found to fix nitrite in small quantities on air drying (52). Since this nitrite is fixed very tenaciously, competition would exist for the nitrite available for volatilization, particularly in acid soils where fixation is greatest. Table 24 shows that the greatest increase in N-loss between 50 and 200 ppm nitrite-N occurred in the acid Holland. The fulfillment of the fixation capacity at increased nitrite concentrations would release proportionally more nitrite for volatilization, giving the observed increase in percent loss.

TABLE 24

MINERAL NITROGEN LEVELS AFTER AIR-DRYING SOIL MATERIALS IN THE PRESENCE  
OF VARIOUS NITRITE LEVELS (ppm)

SOIL MATERIAL	LEVEL NO <sub>2</sub> -N APPLIED	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	NO <sub>2</sub> <sup>-</sup> -N	NO <sub>2</sub> -N LOST	% NO <sub>2</sub> -N LOST
LAKELAND	0	28	8	5.2	-	-
	50	29	7	48.1	1.9	3.8
	100	30	6	95.7	4.3	4.3
	150	31	7	142.8	7.2	4.8
	200	32	8	189.4	10.6	5.3
	400	32	7	383.0	17.0	4.3
	800	33	8	773.8	26.2	3.3
	1600	31	8	1558.4	41.6	2.6
WELLWOOD	0	40	5	1.3	-	-
	50	40	4	43.2	6.8	13.6
	100	38	5	86.0	14.0	14.0
	150	36	4	128.1	21.9	14.6
	200	36	3	167.3	32.7	16.4
	400	33	5	348.0	52.0	13.0
	800	33	6	726.6	71.4	9.0
	1600	33	5	1485.0	115.0	7.2
HOLLAND	0	15	6	0.8	-	-
	50	15	7	35.1	14.9	29.8
	100	12	13	65.7	34.3	34.3
	150	12	11	97.9	52.1	34.8
	200	11	9	121.0	79.0	39.5
	400	10	10	288.0	112.0	28.0
	800	9	10	597.0	203.0	25.4
	1600	7	10	1257.0	343.0	21.5

The data show that nitrate did not increase on air-drying, in contrast to incubations with sterilized materials. This probably reflected the forced drying employed, which removed more quickly any volatilized oxides of nitrogen and prevented their equilibration with the sample. It would seem that free interchange of the products of chemical denitrification with the atmosphere is an important factor in determining losses from acid soils.

Loss of nitrite-N was accompanied by decreases in exchangeable ammonium in Wellwood and Holland. Since autoclaving of nitrite and ammonium-amended samples indicated an interaction between the two ions resulting in higher nitrite losses, it was desirable to investigate the effect of ammonium during the air-drying process. Samples treated with 50 and 200 ppm nitrite-N, the latter included because of its higher percent loss, were therefore air-dried in the presence of 250 and 1000 ppm ammonium-N as ammonium sulphate. This gave approximately ammonium to nitrite ratios of 5 : 1 as before. Results, summarized in Table 25, show that losses of nitrite-N did increase with higher ammonium levels, as compared to the data of Table 24. The effect of soil acidity was again demonstrated by the order of increased loss i.e., Holland > Wellwood > Lakeland. Clark and Beard (38) failed to show any effect of added ammonium at ratios of ammonium to nitrite up to 2.5 : 1 and pH 5.5 when nitrite and soil material were incubated at 80°C for one hour. Their losses were constant at about 30% nitrite-N.

Clark and Beard (38) reported close similarities in nitrite-N losses from air-drying with those from incubation of soil materials for two weeks at 28°C. The present work differs in the sterility and 20°C temperature of incubated samples, but comparisons between two week data of Table 20 with data of Table 24 lend support this statement. It is significant also that

TABLE 25

LOSS OF NITROGEN FROM NITRITE IN THE PRESENCE OF ADDED  
AMMONIUM (percent)

SOIL MATERIAL	50 PPM $\text{NO}_2^-$ -N 250 PPM $\text{NH}_4^-$ -N	200 PPM $\text{NO}_2^-$ -N 1000 PPM $\text{NH}_4^-$ -N
LAKELAND	4.1 (3.8)	5.9 (5.3)
WELLWOOD	16.9 (13.6)	23.4 (16.4)
HOLLAND	39.6 (29.8)	52.1 (39.5)

Data in brackets are for treatments without added ammonium, taken from Table 24.

the loss of nitrite-N deduced for Holland (34%) in three weeks (cf. Chapter Six, p.66) was proportionally close to the loss from sterilized and air-dried samples. Air-drying would appear to be a useful tool in accelerating nitrite-N losses, allowing a more convenient study of the factors involved. Autoclaving in the presence of nitrite resulted in almost complete destruction in Holland in a short time, thus making it a less sensitive tool for study.

Hydrogen peroxide treatment and air-drying. Clark and Beard (38) investigated the effect of organic matter upon nitrite loss by comparing mineral-N deficits of buffered nitrite-treated soil materials with peroxidized samples, after incubation for one hour at 75°C. It was decided to investigate the effects of organic matter using air-drying as an inducement to N-loss. Ten grams of soil materials were repeatedly treated at room temperature for four hours with 50% hydrogen peroxide. The samples were then rinsed with 10 N KCl solution for half an hour, since peroxide treatment gave large increases in exchangeable ammonium. Finally, samples were rinsed with distilled water for fifteen minutes, and air-dried. A second subsample was given parallel treatment with distilled water. All samples were then treated with 200 ppm nitrite-N, contained in the three buffer solutions previously described, and air-dried. Results for nitrite-N losses are shown in Table 26.

At a given pH, nitrite losses were greatly reduced in Wellwood and Holland after destruction of organic matter, confirming Clark and Beard's findings on the role of the latter in nitrite losses. The influence of organic matter appeared to be related to pH, larger discrepancies between peroxide and water treatments being recorded at lower soil pH.

TABLE 26

NITRITE-N\* LOST FROM PEROXIDE-TREATED AND CONTROL  
 SAMPLES DURING AIR-DRYING

SOIL MATERIAL	TREATMENT	pH	NO <sub>2</sub> -N LOST (%)
LAKELAND	peroxide	8.0	0.0
	control	8.0	0.0
WELLWOOD	peroxide	6.9	0.9
	control	6.9	5.4
HOLLAND	peroxide	5.5	3.6
	control	5.5	25.6

\*Nitrite was added at the 200 ppm-N rate.

Water treatment and KCl rinsing reduced the nitrite-N loss, as indicated in Tables 24 and 26. A factor which contributed to nitrite instability was therefore leached from the soil materials, giving lower losses in spite of the slight decrease in pH in the buffers. Water extraction alone, without buffering or potassium chloride rinsing, was responsible for a substantial lowering of nitrite reactivity, as indicated in Table 27. The effect was greatest on Holland and least on Wellwood. Clark and Beard (38) failed to remove any nitrite-instability factors in the leachate. Wullstein and Gilmour (122) however, believe that an important factor in nitrite instability is removed by sodium chloride extraction. Aluminium, copper, manganese, and ferrous ions have been implicated in the process (33, 122). These could be removed by KCl solution but probably not by water extraction. Water did give a coloured extract of Lakeland and Wellwood possibly due to dissolved organic matter. Air drying has been found to result in dismutation of organic matter, giving increases in amino acids, hexosamines and other small molecules (86), which could induce nitrite instability, particularly with the second drying. Some of these may be removed on water extraction, to lower the reactivity of nitrite. This hypothesis would explain the relative trends in nitrite-N loss, which increased with the treatments KCl extraction - H<sub>2</sub>O extraction - untreated samples, shown in Table 27. This hypothesis needs clarifying by much more rigorous experimental work.

Hydrogen peroxide treatment resulted in increases in acidity, probably through the incomplete oxidation of organic matter to carboxylic acids. This afforded an opportunity to study the manipulated effect of pH on chemical denitrification. Bulk samples of the three soil materials were



TABLE 27

## INSTABILITY OF NITRITE IN LEACHED AND CONTROL SOIL MATERIALS

SOIL MATERIAL	pH	LOSS OF 200 PPM NITRITE-N (%)		
		KCl	H <sub>2</sub> O	Control
LAKELAND	7.8	0.0	0.0	2.5
WELLWOOD	7.2	2.8	4.0	7.5
HOLLAND	6.2	23.2	26.0	34.2

treated with hydrogen peroxide followed by leaching with potassium chloride and water as before. Various combinations of ten grams of treated and untreated air-dried samples were then studied for decomposition of nitrite-N. Results are shown in Table 28 and Figure 12. An excellent correlation coefficient ( $P \ll 0.001$ ) was obtained between percent nitrite-N lost and pH, showing that the overall controlling factor in N-loss was acidity. This agrees with the findings of Clark and Beard (38) that "degree of acidity was of overriding importance among the experimental variables employed".

#### SUMMARY AND CONCLUSIONS

(1) The three techniques used to study the instability of nitrite in soil materials, namely incubation of sterilized samples with nitrite, autoclaving of soil plus nitrite, and air-drying of soil plus nitrite, demonstrated that the presence of this ion is conducive to losses of mineral-N by aerobic chemical denitrification. The same comparative trends of N-loss were obtained on the three soils, albeit to different extents with individual methods, the order being Holland > Wellwood > Lakeland. Air-drying gave comparable losses to incubation at two weeks, losses by autoclaving were much higher probably due to the high temperature employed.

(2) Acidity was the single most important factor which governed nitrite instability. Air-drying of mixed samples whose pH was altered by hydrogen peroxide treatment gave a straight-line plot of percent loss against pH for 200 ppm applied nitrite-N. However, autoclaving of buffered nitrite solutions covering approximately the same pH range as soil materials gave greatly reduced losses compared to soils. The purely chemical, pH-dependant breakdown of nitrite in solution was responsible for the loss of only 13.2% nitrite-N (applied at 50 ppm-N) at pH 5.5, compared to a 91.6% loss

TABLE 28

NITRITE-N LOST FROM VARIOUS COMBINATIONS OF HYDROGEN PEROXIDE-TREATED,  
KCl-LEACHED, AND CONTROL SOIL MATERIALS

SOIL MATERIAL	TREATMENT*	pH	NO <sub>2</sub> -N LOST (%)
LAKELAND	control	7.6	2.4
	KCl leached	7.6	0.0
	1:1 KCl/H <sub>2</sub> O <sub>2</sub>	7.4	0.0
	H <sub>2</sub> O <sub>2</sub>	7.3	9.8
.....			
WELLWOOD	control	7.3	7.7
	KCl leached	7.3	3.6
	1:1 KCl/H <sub>2</sub> O <sub>2</sub>	6.5	26.4
	H <sub>2</sub> O <sub>2</sub>	5.1	83.9
.....			
HOLLAND	control	6.3	34.4
	KCl leached	6.4	25.9
	1:1 KCl/H <sub>2</sub> O <sub>2</sub>	5.8	39.6
	H <sub>2</sub> O <sub>2</sub>	4.7	97.0

\*10 gms. of soil material were used. In the 1:1 KCl/H<sub>2</sub>O<sub>2</sub> treatment, 5 gms each of potassium chloride leached and hydrogen peroxide-treated samples were used, after careful mixing.

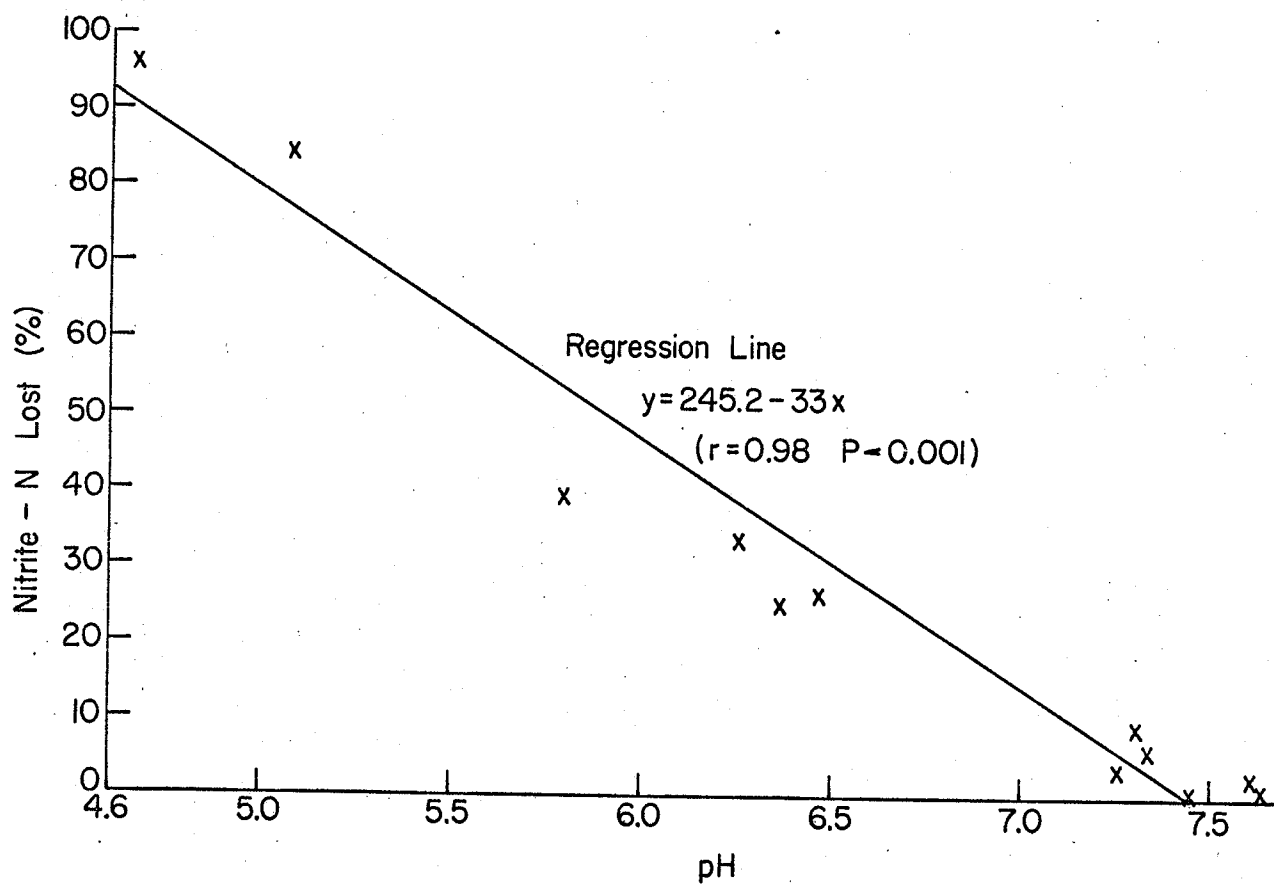


Figure 10. Relationship between nitrite - N lost (%) and pH.

at a soil pH of 6.2 (for Holland). Thus, under the experimental conditions used, pH operated indirectly through the agency of various pH-dependant factors.

(3) Organic matter was the most significant pH-dependant factor. Removal of this material with hydrogen peroxide, followed by air-drying of nitrite-treated samples buffered approximately to original soil pH, almost completely eliminated N loss in all cases. Organic matter was therefore of greatest importance in the acidic Holland, followed in order by Wellwood and Lakeland. The process of autoclaving led to an increase in the extract colour probably due to dissolved organic matter. The presence of the latter may have contributed to the high losses obtained by this method.

(4) Addition of ammonium sulphate, giving an ammonium to nitrite ratio of five to one, stimulated nitrite-N loss by both autoclaving and air-drying, the effect being enhanced at increased soil acidity. Agents which were removed from the soil materials by KCl and H<sub>2</sub>O leaching also appeared to be involved in the pH-dependant promotion of nitrite instability. Incubation of Holland revealed that another factor, namely the chemical oxidation of nitrite to nitrate at acid pH, can be competitive with the reductive processes of N loss. This effect was enhanced in closed containers in which gaseous oxides of N could equilibrate more completely with the sample and undergo hydrolysis to nitric acid (cf. Chapter Two, p.16).

(5) Other environmental factors which seem important on the basis of this work are the extent of atmospheric gaseous interchange (not related to nitrate formation), temperature (as revealed by autoclaving), and drying, the latter probably being an effect of increased reactant concentration as well as dismutation of organic matter.

## CHAPTER NINE

### SUMMARY AND CONCLUSIONS

The aim of this study was to determine the behaviour of fertilizer nitrogen in three soil materials covering a range of pH. In view of the emphasis being placed on the efficiency of fertilizer utilization, mineral nitrogen deficits were studied, and chemical denitrification involving nitrite was singled out as a possible agency for nitrogen loss. As important aspects of the latter, nitrite accumulation with a range of urea and ammonium sulphate levels, and nitrite instability under conditions of minimum biological activity, were investigated.

At the onset of the study, it was necessary to evaluate the extraction-distillation procedure (16) of estimating mineral nitrogen, and to compare this method of estimating nitrite with a colourimetric method. Recoveries of 50 and 200 ppm ammonium-N added as ammonium sulphate were less than complete due to ammonium fixation. Recovery of nitrite by extraction-distillation was erratic, that by colourimetry was slightly less than a hundred percent on all soils. Extracts of Wellwood and Holland containing 200 ppm each of ammonium-, nitrite-, and nitrate-N were unstable with respect to the former two when stored at room temperature. A pH-dependant interaction between ammonium and nitrite was indicated.

The following points emerged from the subsequent investigation:

- (1) Ammonium fixation was the main reason for incomplete recovery of 50 ppm fertilizer nitrogen in twelve day incubations, as judged by comparison with control soils. However, recoveries were generally lower than predicted on this basis, especially in urea-treated Wellwood and ammonium sulphate-treated Holland. Biological denitrification probably occurred

in Wellwood, supported by the finding that low recovery of nitrate was obtained in fifteen weeks' incubation. Ammonium sulphate probably exerted a salt effect on the mineralization of organic matter in Holland. The latter showed a long lag period in nitrification of added fertilizer.

(2) Differences in recovery of fertilizer nitrogen were greatly accentuated in fifteen-week incubations, with 50 ppm sodium nitrite-, potassium nitrate-, urea-, and ammonium sulphate-N. This was probably the result of many factors, but predominantly seemed to be due to various mineralization and immobilization balances after three to six weeks. Recoveries with nitrite were the highest, and chemical denitrification could only be surmised to occur in Holland. Biological denitrification from nitrate appeared to occur in all soils, judging by less than complete recoveries in comparison with control treatments.

(3) Incubations with 100 and 150 ppm urea- and ammonium sulphate-N did not result in significant nitrite accumulation. Net mineralization was reduced in Lakeland in proportion to the level of nitrogen applied, and in Wellwood at 200 ppm ammonium sulphate-N. During incubation with higher rates of fertilizer, nitrite accumulation was appreciable in urea-treated Wellwood, (pH 7.1) and much smaller but significant in Lakeland (pH 8.0) and Holland (pH 6.2). Ammonium sulphate produced only small accumulations of nitrite at application rates up to 800 ppm-N in all soils. The factors affecting nitrite accumulation were as much related to the biological turnover of nitrogen as they were to the classical factor of soil reaction. It was indicated that the significant feature of accumulation is the balance between Nitrosomonas and Nitrobacter activities.

(4) Accumulated nitrite was not directly associated with mineral

nitrogen losses except in urea-treated Wellwood, where losses at the 800 ppm-N rate were three times those from ammonium sulphate. However, the occurrence of chemical denitrification during nitrification was indicated by higher losses with decreasing pH and buffering capacities.

(5) Chemical denitrification involving nitrite was directly related to various pH-dependant factors characteristic of the three soil materials. Losses of nitrite nitrogen generally occurred in the order Holland > Wellwood > Lakeland. The main pH-dependant factor was organic matter; exchangeable ammonium and a leacheable agent(s) were also involved. In the acid Holland, competition appeared to exist between reductive processes leading to nitrogen loss, and acid-catalysed oxidative processes leading to nitrate formation.

The study has shown that, in the main, fertilizer application to soil materials incubated under laboratory conditions results in less mineral nitrogen than expected from the sum of the nitrogen applied and the mineral -N formed in untreated samples. This phenomenon is more marked with increasing acidity of the soil material. At low rates of fertilizer application, no losses of nitrogen per se were obtained except in the calcareous soil treated with phosphate and ammonium sulphate. Ammonium fixation, and various mineralization and immobilization balances appear to be the main factors influencing recovery of fertilizer N at these rates. However, as the rate of fertilization is increased, chemical denitrification seems to be a significant mechanism leading to actual losses of nitrogen, an effect which is enhanced by acidity. The work clearly demonstrates a need to estimate unequivocally any gases that may be evolved from fertilizer nitrogen. This would be possible only by utilizing tagged N sources, measuring the isotope



in the atmosphere above the sample. The different nitrification and mineralization rates noted with urea and ammonium sulphate might also be studied further, especially with a view to elucidating possible salt effects on these processes.

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