1961

Nitrate reduction in soils

John Thomas Moraghan

Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Biochemistry Commons

Recommended Citation

https://lib.dr.iastate.edu/rtd/2410

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
MORAGHAN, John Thomas. NITRATE REDUCTION IN SOILS.

Iowa State University of Science and Technology
Ph.D., 1961
Chemistry, biological

University Microfilms, Inc., Ann Arbor, Michigan
NITRATE REDUCTION IN SOILS

by

John Thomas Moraghan

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major Subject: Soil Fertility

Approved: Signature was redacted for privacy.

In/Charge of Major Work Signature was redacted for privacy.

Head of Major Department Signature was redacted for privacy.

Dean/of Graduate College

Iowa State University Of Science and Technology Ames, Iowa

1961
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENT</td>
<td>iii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>REVIEW OF LITERATURE</td>
<td>3</td>
</tr>
<tr>
<td>Historical</td>
<td>3</td>
</tr>
<tr>
<td>Terminology</td>
<td>3</td>
</tr>
<tr>
<td>Biochemical Pathway of Denitrification</td>
<td>5</td>
</tr>
<tr>
<td>Physical Factors and Denitrification in Soils</td>
<td>7</td>
</tr>
<tr>
<td>Ammonification under Water-logged Conditions</td>
<td>9</td>
</tr>
<tr>
<td>Ammonia Formation through Nitrate Respiration</td>
<td>11</td>
</tr>
<tr>
<td>Ammonia Formation through Chemical Reduction</td>
<td>14</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>16</td>
</tr>
<tr>
<td>Soils and Incubation Procedures</td>
<td>16</td>
</tr>
<tr>
<td>Analytical Methods</td>
<td>18</td>
</tr>
<tr>
<td>Statistical Analyses</td>
<td>22</td>
</tr>
<tr>
<td>Incubation Experiments</td>
<td>23</td>
</tr>
<tr>
<td>Tracer Nitrogen Experiments</td>
<td>30</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>36</td>
</tr>
<tr>
<td>Incubation Experiments</td>
<td>36</td>
</tr>
<tr>
<td>Tracer Nitrogen Experiments</td>
<td>65</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSIONS</td>
<td>71</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>76</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>81</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENT

The author wishes to express sincere thanks to Dr. John Pesek for his stimulating counsel in the planning of the author's research and course programme, and for his helpful advice in the preparation of this manuscript.

The author is indebted to the staff of the Iowa State University Department of Agronomy for the generous help and treatment that he has received during his residence in Ames. In particular, the writer wishes to thank Dr. Lloyd Frederick and Dr. J. M. Bremner for having given freely of their time in discussions associated with the work.

Sincere gratitude is expressed to The Texas Company for providing a financial grant to support the writer's research assistantship. The author is especially appreciative of the sustained interest shown in the work by the head agronomist of The Texas Company, Dr. C. Loyal Swanson.
INTRODUCTION

Ammonification of organic matter and subsequent nitrification of the ammonia result in nitrate production in soil. According to Russell (1950), most soils contain 2 to 20 p.p.m. of nitrate. Generally, in fallow soils nitrate accumulates during the spring and summer, but by early winter the levels are relatively low. Nitrate can also be present in soil as the result of application of nitrogenous fertilizers. The increased industrial production, through chemical fixation, of relatively low-cost ammonia has led to the consumption of larger amounts of nitrogen fertilizer during the past decade. Concurrently with the increased use of nitrogen fertilizer, research in the areas of soil microbiology and soil fertility has been intensified to determine the fate of the applied fertilizer. The use of tracer nitrogen in research has resulted in valuable gains to our knowledge of nitrogen losses from soils. Reduction of nitrate to nitrogen gas and nitrogen oxides is a process with which most research workers in soils are now well acquainted.

A review of literature indicated that the biological reduction of nitrate to ammonia, involving the substitution of nitrate for oxygen as a hydrogen acceptor, is a well-demonstrated process. However, in studies of nitrate in soils under anaerobic conditions little attention has been paid to this phase of nitrate reduction. Several limited
studies have shown that nitrate reduction to ammonia occurs in oxygen-deficient soils, but compared to denitrification nitrogen losses the ammonia accumulation is small. Nitrate reduction to ammonium results in the formation of a product which may under certain conditions be retained by the soil. Since such a reduction has little effect on availability of nitrogen to plants, investigations into the occurrence and mechanism of this ammonia formation are justified.

The objectives of the studies in this dissertation are: to investigate the importance of reduction of nitrate to ammonia under water-logged conditions in a range of soils, and in the event of soils being found in which ammonia formation from nitrate was appreciable, to investigate the mechanism of the formation.
REVIEW OF LITERATURE

Historical

During the latter half of the nineteenth century, European workers displayed considerable interest in the microbial reduction of nitrate to nitrite, nitrous oxide, nitrogen and ammonia. Schónbein (1868) reported that various groups of microorganisms were capable of reducing nitrate to nitrite. Gayon and Dupetit (1882) were among the first to indicate that nitrogen and nitrous oxide could be derived microbiologically from nitrate. The importance of anaerobic conditions and an organic source of energy for denitrification was demonstrated by Deherain and Maquenne (1882). Marchal (1894) isolated from soil a facultative anaerobe, *Bacillus mycoides*, which under anaerobic conditions reduced nitrate to ammonia.

Terminology

The term denitrification has been used to describe the microbial processes through which nitrate is reduced to gaseous products, such as nitrous oxide or nitrogen, with a resulting decrease in the overall nitrogen content of the organism's immediate environment (Fry, 1955). Verhoeven (1956, p. 64) to overcome the unsatisfactory situation in regard to nomenclature of biological nitrate reduction
systems suggested that three different types of nitrate reduction exist:

1. Nitrate assimilation, in which nitrate is reduced only for the building up of cell protein.

2. Incidental dissimilatory nitrate reduction, in which nitrate acts as a non-essential hydrogen acceptor.

3. True dissimilatory nitrate reduction, in which nitrate acts, at least under certain conditions, as the essential hydrogen acceptor which enables the organism to grow.

Delwiche (1956, p. 239) questioned the value of the term incidental dissimilatory nitrate reduction. Evidence was presented to show that *Escherica coli* which was classified as an example of an incidental dissimilatory nitrate reducer in the reduction of nitrate used the nitrate as an obligatory hydrogen acceptor. A number of other facultative anaerobes were found to be in the same category.

Nason and Takahashi (1958, p. 208) have proposed the term nitrate respiration to describe the biological processes by which nitrate acts as a terminal electron acceptor in place of oxygen under anaerobic or partially anaerobic conditions. Denitrification is a type of nitrate respiration whereby nitrate is reduced biologically to the lower oxidation states of gaseous nitrogen and nitrous oxide. Nitrate assimilation is used by these workers in the Verhoeven sense.
Biochemical Pathway of Denitrification

Nason and Takahashi (1958, p. 215) in reviewing the literature, presented a diagrammatic biochemical pathway of denitrification as follows:

\[
\begin{align*}
\text{NO}_3^- & \rightarrow \text{NO}_2^- \\
\text{NO}_2^- & \rightarrow \text{NO} \\
(\text{H NO}) & \rightarrow \text{H}_2\text{N}_2\text{O}_2 \\
\text{H}_2\text{N}_2\text{O}_2 & \rightarrow \text{N}_2\text{O} \\
\text{NO}_2 \cdot \text{NH}_2 & \rightarrow \text{N}_2
\end{align*}
\]

The unanswered problems which Delwiche (1956, p. 246) discussed still remain. The mechanism of reduction of nitrite, the roles of nitrous oxide and hyponitrite as intermediates, and the place of nitric oxide in the denitrification scheme are as yet unresolved.

The observation by Iwasaki and Mori (1958), that a washed suspension of denitrifying bacteria formed nitrogen from nitrite and hydroxylamine in the presence of lactate, but in the absence of the hydrogen donor formed nitrous
oxide, has been the cause of much speculation. Supplementary experiments indicated that the reactions were enzyme controlled. From the point of view of inorganic chemistry these results are interesting. Audrieth (1930) reported that hydroxylammonium salts and sodium nitrite react rapidly in aqueous solution at room temperature with the formation of nitrous oxide. The reaction was further studied by Bothner-By and Friedman (1952) using tracer nitrogen and tracer oxygen. In neutral solutions it appeared that most of the nitrous oxide was produced by dehydration of a symmetrical intermediate which they thought was probably hyponoritrous acid. However, in acid media a competitive dehydration of an unsymmetrical intermediate played an important role. The evidence strongly indicated that any mechanism involving nitrooxyl as an intermediate was untenable.

Empirical denitrification studies (Wijler and Delwiche, 1954, Nommik, 1956 and Cady, 1960) in soils have tended to show that nitrous oxide first accumulates, and that this can under certain conditions be further reduced to nitrogen. The last author further indicated that nitric oxide is not on the main pathway of reduction of nitrate to nitrogen gas. He postulates an equilibrium existing between nitric oxide and nitrite, such that nitrous oxide is produced from nitric oxide with nitrite as an intermediate.
Physical Factors and Denitrification in Soils

In denitrification studies in soils, excess moisture has often been employed to induce anaerobiosis. Anaerobic conditions are assumed to exist in the soil matrix since the diffusion coefficient of oxygen in air is ten thousand times as great as that in water. Cady (1960, p. 29) questioned whether moisture levels above the moisture equivalent have no effect on denitrification apart from that on oxygen diffusion. He found that the rate of formation of the sequential denitrification products of tracer nitrogen applied to soil was affected by the moisture level of the soil. The higher the moisture level, the greater was the rate of formation of nitrogen gas. The anomaly may be associated with nitrous oxide solubility. At high moisture levels there is a larger amount of nitrous oxide dissolved, and it is possible that this dissolved nitrous oxide is the form used by the denitrifying organisms. Nitrous oxide has an appreciable solubility in cold water, and the Henry's Law Constant is \(0.210 \times 10^7\) at \(30^\circ\text{C}\). (Hodgman, 1953, p. 1611).

Where excess moisture is used to induce anaerobiosis, it is possible that ammonium-N can be lost from the soil through denitrification. Pearsall (1950) showed that in a wet soil an oxidized zone, adjacent to the atmosphere, exists above the reducing zone. In the oxidizing zone ammonia can be oxidized to nitrate and nitrite through
biological nitrification, and these anions can in turn diffuse into the reducing zone where reduction to gaseous nitrogen compounds may occur. Factors which influence nitrogen loss by this mechanism possibly include pH, exchange capacity, water content and partial pressure of oxygen above the liquid layer. Shioiri and Tanada (1954) added ammonium sulfate to flooded soils and after 24 days found that an appreciable amount of nitrogen gas had been evolved. A mechanism similar to this may explain why Bremner and Shaw (1958b) found that denitrification losses from incubated flooded soils exceeded the amount of nitrate-N added. According to Abichandani and Patnaik (1958) ammonium-N fertilizer losses are less when the fertilizers are applied below the surface. The literature on this important aspect of denitrification has been reviewed by Greene (1960).

The effect of oxygen on denitrification has been the cause of much controversy. After reviewing the literature Delwiche (1956, p. 241) concluded:

...that denitrification takes place only when the oxygen available to cells is in sufficiently short supply that nitrate can effectively compete as a hydrogen acceptor in a system which is primarily geared to aerobic oxidation.

Broadbent (1951) and Wagner and Smith (1958) found evidence of gaseous nitrogen losses from soils under apparently aerobic conditions. Cady (1960, p. 44) showed that it is not necessary for the oxygen concentration to be zero for
gaseous loss of nitrogen to occur. Most of the gaseous loss of nitrogen occurred in the aerobic system when the need for oxygen by the microbial population was relatively high.

Denitrification is very pH dependent. According to Nommik (1956) the optimum soil pH for denitrification is between 7 and 8 and denitrification is greatly reduced at pH 5 and below.

Denitrification is an active process over an appreciable temperature range. At 10°C, denitrification can result in a significant loss of nitrogen; Bremner and Shaw (1958b) reported that between 25 and 60°C, the rate of denitrification was extremely rapid with little variation within this temperature range. Sampling at shorter time intervals, Nommik (1956) found the optimal soil temperature for denitrification to be 65°C.

Ammonification under Water-logged Conditions

It seems appropriate here to review the effect of waterlogging on ammonification of soil organic nitrogen. Harmsen and Van Schreven (1955) after reviewing the literature considered that ammonification was almost entirely normal in water-logged anaerobic soils used for growing rice. Shapiro (1958) by extrapolating rice yield curves under flooded and non-flooded conditions found that flooding increased the availability of soil phosphorus, but had no effect on the
availability of soil nitrogen.

Immobilization of inorganic soil nitrogen is a concomitant process with mineralization, and the net effect, ammonification, is determined by the energy level and the nitrogen content of the system (Jansson, 1958). Ponnamperuma (1955), arguing from the fact that water-logging would affect the aerobic fungal population, suspected that ammonification and immobilization of nitrogen under anaerobic conditions, apart from being more pH sensitive, should be retarded. In this connection Janssen and Metzger (1928) found that after 10 weeks the ammonium- plus nitrate-N-content in a well drained soil was 33 per cent greater than that in a water-logged soil. The relative intensities of the mineralization and immobilization processes may also be affected by anaerobiosis. Acharya (1935) observed that during the decomposition of straw both the nitrogen factor and the nitrogen equivalent were higher under aerobic conditions than under anaerobic conditions.

A variation in oxygen content between water-logged and non-water-logged soils is not the only difference which occurs during the course of an incubation experiment. The pH of a water-logged system increases with time, but, due to the accumulation of the anion nitrate, the reverse is the case under aerobic conditions. The divalent forms of iron and manganese are seldom encountered in an aerobic soil
system. However, in a water-logged soil these ions, through microbial reduction, may accumulate.

Ammonia was volatilized when Willis and Sturgis (1945) incubated water-logged soils. High organic matter contents and high pH values of the soils favored this loss. In ammonification studies care must be taken in using ammonium-N as an index of ammonification. Under certain conditions ammonia can be lost through volatilization, while the previous discussion on an oxidized layer above a reduced layer allows for the eventual loss of ammonium-N as either nitrogen or nitrous oxide.

Ammonia Formation through Nitrate Respiration

The organism isolated by Marchal (1894), which produced ammonia when incubated with nitrate under anaerobic conditions, appeared to use the nitrate as an essential hydrogen acceptor. Subsequently, ammonia was regarded as an important product of anaerobic nitrate reduction in soil. Waksman (1927) discussed this aspect of nitrate reduction very extensively.

In a biochemical study of nitrate reduction Stephenson and Stickland (1931) showed that organisms which reduced nitrate and possessed the enzyme hydrogenase were able to use molecular hydrogen as a hydrogen donor in the reduction of nitrate. Woods (1938) observed that washed suspensions of
Clostridium welchii catalyzed the reduction, by molecular hydrogen of nitrate, nitrite and hydroxylamine to ammonia. The volume of hydrogen taken up was also measured, and the uptake was in agreement with the following equations:

\[ \text{HNO}_3 + 4 \text{H}_2 \rightarrow \text{NH}_3 + 3 \text{H}_2\text{O} \]  
(1)

\[ \text{HNO}_2 + 3 \text{H}_2 \rightarrow \text{NH}_3 + 2 \text{H}_2\text{O} \]  
(2)

\[ \text{NH}_2\text{OH} + \text{H}_2 \rightarrow \text{NH}_3 + \text{H}_2\text{O} \]  
(3)

The data were strongly indicative of nitrite and hydroxylamine serving as intermediates. Nitrite was the initial product formed from nitrate reduction, and this tended to accumulate until the nitrate had disappeared. After the nitrate had substantially disappeared, the nitrite was utilized, and the hydrogen uptake was then in accordance with Equation 2. Strains of Bacterium coli, capable of the same form of reduction, were also found. The author, in conclusion, stated:

It would seem that the reduction of nitrate to ammonia is more general than supposed, and that the reduction must be seriously considered in assessing the oxidation of ammonia to nitrate by other microorganisms in the general circulation of nitrogen in nature.

It now appears that Woods (1938) may have placed over-importance on his results. Kessler (1959) used the technique employed by this worker in studies of nitrate reduction with algae. In the absence of oxygen the rate of
nitrate reduction was extremely low. Apparently endogenous fermentation was not able to provide an adequate supply of hydrogen donors. The green alga Ankistrodesmus braunii possesses the enzyme hydrogenase, and the above author found that this organism was able to use hydrogen activated by the enzyme for the reduction of nitrate and nitrite under anaerobic conditions. Thus, it would seem that in the work of Woods (1938) ammonium formation as the result of nitrate reduction was not due to nitrate respiration.

Salle (1948) discussed the relatively common occurrence of microorganisms capable of reducing nitrate and nitrite under an anaerobic or partially anaerobic environment. The writer stated that the pH of the medium is of importance in determining the direction of the reaction. In an acid environment reduction to ammonia was considered more likely.

Verhoeven (1956, p. 67) showed that certain Bacillus species under "moderate" aeration reduced nitrate to ammonia, but under anaerobic conditions reduction to either nitrogen or nitrous oxide was more likely.

Wallihan (1937) interpreted certain of his results to indicate that under water-logged conditions nitrate was reduced possibly to ammonium, and this under aerobic conditions was re-oxidized microbiologically to nitrate. The evidence for such a conclusion seemed debatable. Using California soils, which in some cases had been supplemented
with an external energy source, Broadbent and Stojanovic (1952) reported that the loss of tagged nitrate by reduction to ammonia amounted to only 0 to 6%. Nommik (1956) questioned whether the ammonia, reportedly formed under anaerobic conditions, was a consequence of deamination of nitrogenous organic compounds. He found that considerable amounts of tagged nitrate were reduced to ammonia only under strictly anaerobic conditions. Decreasing the acidity of an originally acid soil resulted in the decrease of the percentage of nitrogen gas evolved and in a concomitant increase in ammonia. The writer considered that the ammonia formed by nitrate reduction had not been the result of organic cycling. Bremner and Shaw (1958b) in their study of general aspects of denitrification found that ammonia production, which was apparently formed from nitrate, in waterlogged soils incubated with nitrate and glucose was greater in moderately acid than in slightly alkaline soils.

Ammonia Formation through Chemical Reduction

Bremner and Shaw (1955) reported that ferrous hydroxide in the presence of nitrate can interfere with the quantitative recovery of ammonia when alkalis are used to liberate the ammonia from solution. Under these conditions reduction of nitrate to ammonia is possible. The authors were unable to show that acid potassium chloride extracts of soils
contained sufficient ferrous ion to interfere with the de-
termination of ammonia in the presence of nitrate. However,
this method of interference should be considered when ammo-
nia is determined in extracts obtained from incubated water-
logged soil samples. Investigations by Szabo and Bartha
(1957) indicated that only ferrous hydroxide of a certain
structure can reduce nitrate. The activity of the ferrous
hydroxide is pronounced at pH 6 to 8. The active centers of
the hydroxide are destroyed by excess of base, and they be-
come active again only at a very high concentration of alka-
li. The Hungarian workers also reported that either copper
or silver catalyzes the reduction of nitrate by inactive
ferrous hydroxide. A fine silver or copper sol forming in
the mixture participates in the transfer of electrons from
inactive ferrous hydroxide to nitrate.

The biological reduction of Fe$^{+++}$ ions to Fe$^{++}$ ions in
water-logged soils is a well known phenomenon, but the re-
duction in water-logged soils containing appreciable amounts
of nitrate has not been extensively studied. Mortimer
(1941) investigated the reduction of nitrate and Fe$^{+++}$ ions
in lake-bed muds under anaerobic conditions. After 51 days
all the nitrate had disappeared and only then did Fe$^{++}$ ions
commence to accumulate. The presence of nitrate appeared to
maintain the potential of the system at a level above that
required for the reduction of Fe$^{+++}$ ions.
MATERIALS AND METHODS

This chapter contains a discussion of the soils, incubation procedures and analytical methods used in the investigations. A description of the plans of the experiments is also given, and these have been grouped into two main sections, "Incubation Experiments" and "Tracer Nitrogen Experiments". The former section is devoted to inorganic nitrogen changes which occur in time under water-logged conditions, and of prime importance was the study of reduction of nitrate- to ammonium-N. In the "Tracer Nitrogen Experiments" labelled nitrate-N was used to determine the relative importance of denitrification and nitrate respiration with ammonium formation in the soils studied.

Soils and Incubation Procedures

The descriptions of the soils used in this investigation together with other pertinent information are given in Table 1. After collection from the field, all soils were air-dried, ground and stored in stoppered jars. Preliminary experiments were conducted on soils which had been ground sufficiently fine in a rotary steel-disc grinder to pass an 0.5 mm. sieve. All results reported in this dissertation with soils 1 to 6, however, were obtained on soil samples hand ground to the above specification in a mortar. The results with soils 7 and 8 were obtained with the
Table 1. Description of soils used in investigations of nitrate reduction in soils under water-logged conditions

<table>
<thead>
<tr>
<th>Soil</th>
<th>Location</th>
<th>Soil type</th>
<th>Soil depth inches</th>
<th>Immediate past history</th>
<th>pH</th>
<th>%O</th>
<th>%N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Agronomy Farm</td>
<td>Clarion loam</td>
<td>0-3</td>
<td>Corn</td>
<td>6.1</td>
<td>2.21</td>
<td>0.19</td>
</tr>
<tr>
<td>2</td>
<td>Agronomy Farm Transition</td>
<td>Transition between Webster</td>
<td>0-3</td>
<td>Alfalfa</td>
<td>6.4</td>
<td>2.83</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>silty clay</td>
<td>silty clay and Nicollet loam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Wadena, Iowa</td>
<td>Fayette silt loam</td>
<td>0-3</td>
<td>Kentucky bluegrass sod</td>
<td>6.6</td>
<td>2.31</td>
<td>0.21</td>
</tr>
<tr>
<td>4</td>
<td>Clarion-Webster</td>
<td>Webster silty clay loam</td>
<td>0-3</td>
<td>Fescue sod</td>
<td>6.4</td>
<td>3.92</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Experiment Farm Kanawha,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Agronomy Farm</td>
<td>Glencoe silt loam</td>
<td>8-15</td>
<td>Mixed grass sod</td>
<td>7.5</td>
<td>2.56</td>
<td>0.23</td>
</tr>
<tr>
<td>6</td>
<td>Clarion-Webster</td>
<td>Webster silty clay loam</td>
<td>0-3</td>
<td>Corn</td>
<td>6.4</td>
<td>3.76</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Experiment Farm Kanawha,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Osceola County, Michigan</td>
<td>Kalkaska sand (B&lt;sub&gt;H&lt;/sub&gt;)</td>
<td>13-15</td>
<td>Red maple, bracken</td>
<td>5.2</td>
<td>0.63</td>
<td>0.04</td>
</tr>
<tr>
<td>8</td>
<td>Georgia</td>
<td>Cecil sandy loam</td>
<td>0-3</td>
<td>Mixed native grasses</td>
<td>5.8</td>
<td>0.34</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The Iowa soils are described by Simonson et al. (1952). Soil 7 was collected by Dr. E. P. Whiteside, Michigan State University, E. Lansing, Michigan, and was described as a podzol. Soil 8 was collected by Dr. Joel Giddens, University of Georgia, Athens, Georgia, and was described as a red-yellow podzolic.
mechanically ground samples.

The techniques for all incubation experiments except those involving $^{15}N$ were essentially similar. Five grams of soil on an oven-dry weight basis were added to 2 dram glass vials. Four milliliters of water were sufficient to cause water-logging of all soils, but the depth of the water layer above the soil surface varied among soils. The nitrogen water-logged treatments were added in solution in the 4 ml. of water. The tops of the vials were covered with thin sheets of polyethylene plastic which were fastened to the vials with Scotch tape. The incubations were conducted in a constant temperature room maintained at approximately 78°F.

Analytical Methods

**Inorganic nitrogen**

In incubation experiments not involving the use of $^{15}N$, the soil-containing vials were placed into 250 ml. Erlenmeyer flasks containing 50 ml. 1 M NaCl. After the soil suspensions were shaken on a wrist-type shaking device for 60 minutes, the soluble constituents were then removed by filtering. Analyses for ammonium-, nitrite- and nitrate-N were performed on aliquots of the filtrates within 6 hours of filtering. With several minor modifications ammonium- and nitrate-N were estimated by the method of Bremner and
Shaw (1955a). However, unsatisfactory results were obtained with the indicator mixture recommended by these workers. It was found that an indicator mixture consisting of 0.0132 g. brom-cresol green and 0.0066 g. methyl red in the boric acid mixture gave a better titration end point. The vendor of the indicators used here was Hartman-Leddon Co., Philadelphia. Initially a 20% titanous sulfate solution supplied by the Lamotte Chemical Products Co., Baltimore, was used to reduce nitrate to ammonium. With this product, however, the recoveries of standard nitrate were only approximately 60%. An attempt was made using a zinc amalgam to reduce Ti$^{++++}$ ions in the solution to Ti$^{+++}$ ions. Use of this product still did not result in quantitative recoveries of the nitrate standard. Successful quantitative reduction of nitrate to ammonium was obtained only when a 15% (weight to volume) solution of titanous sulphate supplied by The British Drug Houses Ltd., Poole, England, was employed. The reason for the poor recovery of nitrate with the American product is not known. Quantitative yields of nitrate- and ammonium-N were obtained with diffusion times set at 36 and 24 hours respectively.

Ammonia volatilized during incubation was estimated by enclosing a vial with the water-logged soil in a sealed container together with a vial of dilute sulfuric acid (approx. 10 N). At the appropriate sampling date the acid vial was
removed, and a 1 ml. aliquot of the sulfuric acid was analyzed for ammonium-N by the microdiffusion method. Potassium hydroxide (approx. 6 N) instead of magnesium oxide was used to liberate ammonia within the diffusion dishes.

Nitrite was estimated by the method of Shinn (1941). This method was modified by employing sodium nitrite as a standard instead of sulfanilamide. Ammonium sulfamate was thus not used to remove excess nitrite. Nitrite was standardized by the method described by Vogel (1951, p. 285). Solutions of standardized nitrite were always used within 24 hours of preparation.

**Total soil nitrogen**

The macro-Kjeldahl method of Bremner and Shaw (1958a) was used to determine total soil nitrogen. Potassium permanganate oxidized nitrite to nitrate, and nitrate was subsequently reduced to ammonium by a pre-digestion with reduced iron and sulfuric acid. In the Kjeldahl digestion 10 g. $\text{K}_2\text{SO}_4$, 1 g. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.1 g. Se and 30 ml. $\text{H}_2\text{SO}_4$ were added to the flasks prior to heating.

**Soil pH**

Soil pH determinations were made with the glass electrode on soil suspensions prepared by mixing 1 part of soil with 2.5 parts of distilled water.
Mass spectrometric analyses

Solutions containing approximately 1 mg. ammonium-N in 2 ml. of solution acidified with sulfuric acid were prepared. The solutions were never greater than 0.1 normal with respect to sulfuric acid. The techniques used for the preparation of the gas samples and the methods used in the calculation of the results were essentially those of Rittenberg (1946). However, the sodium hypobromite was diluted prior to use with an equal volume of 0.1% potassium iodide. Three milliliters of this solution were added to one arm of a Rittenberg tube, while to the other arm 2 ml. of the test ammonium sulfate solution were added. The use of a potassium iodide-sodium hypobromite solution was suggested by Sims and Cocking (1958) to eliminate the catalytic breakdown of sodium hypobromite to sodium bromide and oxygen by Cu$^{++}$ ions. The Rittenberg tubes were evacuated in an apparatus similar to that described by Sprinson and Rittenberg (1949). A manifold with 6 outlets allowed 6 Rittenberg tubes to be evacuated at any one time. The evacuations were performed by a combination of oil diffusion and mechanical pumps connected in series. After evacuation the solutions in the tubes were mixed and the nitrogen gas samples were collected and analyzed in a model 21-620 mass spectrometer manufactured by the Consolidated Engineering Corporation, Pasadena, California. Mass change peaks of 28, 29 and 32
were measured. The 32 peak was assumed to result from atmospheric oxygen contamination. The contribution of nitrogen from the air to the 28 peak was obtained by multiplying the 32 peak height by 5.5. The mass/charge peak 40 was found to be extremely small and was not used in corrections associated with air contamination.

Soil moisture tension

The moisture retained by water-logged soils 1 to 8 when a positive pressure of 0.3 atmosphere was applied was determined by the method of Neilson (1958, p. 23). The moisture retained in the soils at the above pressure can be said to be under a tension of 0.3 atmosphere.

Statistical Analyses

Although the statistical design of a soil incubation study in which the effect of various treatments on a particular property is measured appears superficially to be a "split plot", the fixed nature of the time periods precludes the handling of the experiment in this way. In the incubation experiments of this investigation each soil treatment at each sampling time was regarded as a distinct statistical treatment. The procedure suggested by Kempthorne (1952, p. 154) of comparing range and mean of treatments to determine the transformation, if any, required to give homogeneity of error variance was used. If the range of a treatment
was approximately independent of the treatment mean, homogeneity of error variance was assumed.

The standard errors in actual or transformed units of the mean soil ammonium-N values obtained in the various experiments and the standard error of the mean denitrification nitrogen losses are given in Appendix Table 20.

In the experiments in which different levels of nitrate- and nitrite-N were applied as soil treatments, because of heterogeneity of error variance and limited data, standard errors of the mean soil nitrate- and nitrite-N values at the various samplings were not computed.

Incubation Experiments

These experiments were established to study the inorganic nitrogen changes which occur in water-logged soils in the presence and absence of added nitrate. The experiments were conducted in three stages. The first stage involved a preliminary study of the effect of applied nitrate on the inorganic nitrogen changes in eight water-logged soils. Experiments in the second stage were conducted to determine if the production of larger amounts of ammonium in the presence of nitrate was the result of nitrate respiration. The importance of chemical reduction of nitrate to ammonium in soils was investigated in the final stages of this work.
Inorganic nitrogen changes in water-logged soils

This experiment was conducted to study the inorganic-N changes which occur in water-logged soils at different levels of nitrate. Specifically, it was desired to compare the ammonium production resulting from these different nitrate treatments. If nitrate respiration resulting in ammonium formation occurs to any appreciable extent in the tested soils, the ammonium contents for the nitrate series would be expected to be higher than those for the corresponding control treatments. The four treatments in Table 2 were applied to 5 g. samples of soils 1 to 8. Potassium nitrate was the source of nitrogen employed.

Table 2. Weight of nitrate and moisture treatment applied to 5 g. samples of soils 1 to 8 for studying inorganic changes in water-logged soils

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight of ( \text{NO}_3^- \text{ N} ), g.</th>
<th>Soil moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>W</td>
</tr>
<tr>
<td>B</td>
<td>0.001</td>
<td>W</td>
</tr>
<tr>
<td>C</td>
<td>0.005</td>
<td>W</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>NW</td>
</tr>
</tbody>
</table>

\(^a0.001\) g. \( \text{N} \) is equivalent to 200 parts of nitrogen per million parts of oven-dried soil.

\(^b\)W indicates water-logged soil samples. NW indicates 0.3 atmosphere soil moisture tension.
Two replicates of each treatment were analyzed for soil ammonium-, nitrite- and nitrate-N at 0, 4, 8, 20, 30 and 40 days. The effects of the treatments on soil pH and ammonia volatilization were determined on separate samples.

**Biological reduction of nitrate to ammonium in soils**

On incubating soils 7 and 8 under water-logged conditions larger amounts of ammonium were produced in the presence of nitrate than in its absence. To determine if the ammonium was produced through nitrate respiration, the effects of autoclaving of soils, varying soil moisture, and nitrite addition on ammonium formation in the presence of nitrate were investigated in the following experiments.

**Effect of soil moisture level on ammonium production**

The influence of aeration on the formation of ammonium in the presence of nitrate was investigated in incubation studies which included treatments with the soil moisture tension maintained at 0.3 atmosphere. The assumption was made that at this soil moisture air could diffuse freely through the soil matrix. The five treatments applied to soils 7 and 8 are given in Table 3. Ammonium- and nitrate-N were supplied in the forms of ammonium sulfate and potassium nitrate respectively. Two replicates of each treatment applied to soils 7 and 8 were analyzed for ammonium-, nitrite- and nitrate-N at 0, 4, and 8 days. The ammonium sulfate
Table 3. Nitrogen and soil moisture treatments applied to 5 g. samples of soils 7 and 8 in a study of biological reduction of nitrate to ammonium

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nitrogen applied</th>
<th>Soil moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>NW</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>W</td>
</tr>
<tr>
<td>3</td>
<td>0.00025</td>
<td>NH₄⁺</td>
</tr>
<tr>
<td>4</td>
<td>0.001</td>
<td>NO₃⁻</td>
</tr>
<tr>
<td>5</td>
<td>0.001</td>
<td>NO₃⁻</td>
</tr>
</tbody>
</table>

NW indicates soil moisture tension maintained at 0.3 atmosphere. W indicates water-logging of soil by addition of 4 ml. of water.

treatment was included in order to observe the extent of nitrification during the experimental period.

Effect of nitrite on ammonium production. Meiklejohn (1940) observed that the ability of certain species of de-nitrifying bacteria to reduce nitrate in acid cultures was affected when the nitrite-N content exceeded 14 mg. per liter. Bremner and Shaw (1958b) showed that in acid soils nitrite-N in excess of 200 p.p.m. inhibited denitrification. The purpose of this investigation was to determine whether nitrite affected the formation of ammonium in soils 7 and 8.
The six treatments applied to 5 g. samples of the two soils are given in Table 4. The nitrite- and nitrate-N were applied as sodium nitrite and potassium nitrate respectively.

Table 4. Nitrogen treatments applied to 5 g. samples of soils 7 and 8 in experiments studying the effect of nitrite on ammonium production under waterlogged conditions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nitrogen applied</th>
<th>g. N</th>
<th>Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.001</td>
<td>NO₃</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.001</td>
<td>NO₂</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.005</td>
<td>NO₂</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.001</td>
<td>NO₂</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>NO₃</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.001</td>
<td>NO₃</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>NO₂</td>
<td></td>
</tr>
</tbody>
</table>

At 0, 2, 4 and 8 days two replicates of each treatment applied to soils 7 and 8 were analyzed for nitrate-, nitrite- and ammonium-N. Determinations for soil pH were made on duplicate sets of treatments at each of the above time periods.
Effect on ammonium production of autoclaving soils

If the ammonium-N was derived from nitrate-N through biological means, the autoclaving of soils 7 and 8 prior to waterlogging and incubating them in the presence of nitrate should inhibit or severely reduce ammonium formation. Seventy-five gram samples of soils 7 and 8 in beakers enclosed in wrapping paper were placed in an autoclave, and steam at 15 pounds per square inch pressure was applied for 60 minutes. This procedure was repeated on successive days. Autoclaved samples (5 g.) of both soils were incubated under waterlogged conditions in the presence and absence of 0.001 g. nitrate-N supplied as potassium nitrate. At 0, 2, 4, and 8 days two replicates of each treatment were analyzed for nitrate-, nitrite- and ammonium-N.

Chemical reduction of nitrate to ammonium

Ammonium-N levels above those found in the check treatment accumulated when nitrite was added to soils 7 and 8. The accumulation occurred even in the zero-time analyses, and this suggested that the reduction was accomplished by chemical rather than biological means. The studies discussed here were conducted to investigate the possibility that more highly oxidized forms of nitrogen were reduced chemically to ammonium in soils 7 and 8.
Reduction of nitrate to ammonium by ferrous hydroxide

As reported in the Review of Literature, in certain pH ranges ferrous hydroxide causes the reduction of nitrate to ammonium. The following experiment with soils 7 and 8 was established to determine if such a reaction during the analytical procedures resulted in higher values of ammonium being found in the presence of nitrate. Soils 7 and 8 (5 g. samples) were incubated for 4 days under water-logged conditions in the presence and absence of 0.001 g. nitrate-N. Each treatment was replicated twice. At the end of the incubation period ammonium was estimated in two ways. In method 1 ammonium analyses were conducted on aliquots of the soil extract, while in method 2 the test aliquots were pretreated with acidified potassium permanganate. With this latter treatment any Fe^{++} ions present were oxidized to Fe^{+++} ions.

Reduction of nitrate to ammonium by metallic iron

In an acid medium containing iron activated hydrogen produced on the iron particles can reduce nitrate to ammonium. Metallic iron is not normally found in soils, but the possibility existed that iron contamination occurred during grinding, and that this iron was responsible for the nitrate reduction observed in soils 7 and 8. To test this hypothesis para- and ferro-magnetic substances were removed from soils 7 and 8 by use of a bar magnet. The two soils
contained approximately 7 and 5 mg. of magnetic material, respectively, in each 5 g. of oven-dried soil. Soils from which magnetic substances were removed will be subsequently described as "de-magnetized soils". Five-gram samples of the "de-magnetized" soils were incubated for 4 days under water-logged conditions in the presence and absence of 0.001 g. nitrate-N. The remaining portions of the "de-magnetized" soils were again passed through the mechanical grinder, and 5 g. samples of the reground soils 7 and 8 were also incubated for 4 days in the presence and absence of 0.001 g. nitrate-N. Each treatment was replicated twice. Ammonium analyses were conducted on the individual replicates at the end of the incubation period.

Tracer Nitrogen Experiments

The finding that the ammonium-N contents of soils 1 to 6 incubated under water-logged conditions were not higher with nitrate treatments does not necessarily mean that nitrate respiration resulting in ammonium formation has not occurred. A detrimental salt effect of the potassium nitrate on ammonification may obscure increases in ammonium due to nitrate respiration. Nitrate disappeared in water-logged incubations of soils 1 to 6, but the relative importance of denitrification, immobilization of nitrogen by microorganisms, and nitrate respiration resulting in
ammonium formation in explaining the disappearance cannot be ascertained from the previous experiments. The purpose of the experiments described below was to obtain a solution to this problem by use of N\textsuperscript{15}. The determinations of the amounts of applied nitrate lost from the system and reduced to ammonium were conducted separately.

Denitrification losses of applied nitrate

Five gram samples of soils 1 to 6 of Table 1 were added to 800 ml. Kjeldahl flasks. Added to each container were 4 ml. of a solution containing enough nitrogen-labeled potassium nitrate (99.2\% N\textsuperscript{15}) to supply the quantity of nitrogen per sample indicated in Table 5.

Table 5. Weight of nitrate-N (99.2\% N\textsuperscript{15}) added in 4 ml. water to 5 g. samples of soils 1 to 6 contained in 800 ml. Kjeldahl flasks for denitrification studies

<table>
<thead>
<tr>
<th>Soil</th>
<th>NO\textsubscript{3} - N g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>0.001</td>
</tr>
<tr>
<td>3</td>
<td>0.0005</td>
</tr>
<tr>
<td>4</td>
<td>0.001</td>
</tr>
<tr>
<td>5</td>
<td>0.0005</td>
</tr>
<tr>
<td>6</td>
<td>0.001</td>
</tr>
</tbody>
</table>
A smaller amount of nitrate-N was added to soils 3 and 5 because previous experiments had indicated that at the higher rate of application a marked excess of nitrate would still be present after 20 days incubation. Each soil treatment was replicated three times. The tops of the Kjeldahl flasks were covered with thin polyethylene plastic strips which were attached to the containers with Scotch tape. At the completion of 20 days incubation at a temperature of 78°F., Kjeldahl total nitrogen determinations modified to include both nitrate- and nitrite-N were made on each soil. The atom percent excess N\textsuperscript{15} in the total nitrogen of each soil was determined by mass spectrographic analyses on nitrogen gas samples prepared from the ammonium-N of the distillates. The differences in the atom percent excess N\textsuperscript{15} values between the beginning and end of the experiment permitted the calculation of losses of nitrate-N from the soils. To determine the amount of applied nitrate remaining at the end of 20 days the above experiment was repeated except for the use of non-tagged potassium nitrate. The weights of the non-tagged potassium nitrate used were corrected for the lower atomic weight of the contained nitrogen. At the end of the 20-day period nitrate- and nitrite-N determinations were made on sodium chloride extracts of the soils.
Ammonium-N derived from applied nitrate

The purpose of this experiment was to determine, by the use of isotopic nitrogen, the importance of biological nitrate reduction to ammonium during water-logged incubations of certain Iowa soils. The soils employed in this study were soils 1, 2, 3, 4 and 6 of Table 1. Soil 5 was not included because of the low amount of ammonium-N that was found to accumulate on incubation. Approximately 1 mg. ammonium-N is required to perform a mass spectrographic analysis, and the 5 g. weight of soil used in the previous experiments would not be adequate to provide this amount. The oven-dry weights of the soils used in this study are given in Table 6. The soils were placed into individual 500 ml. Erlenmeyer flasks, and 0.001 g. KN03-N (99.2% N15) dissolved

<table>
<thead>
<tr>
<th>Soil</th>
<th>Weight of soil g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
</tr>
</tbody>
</table>
in 4 ml. of water was added for each 5 g. of soil. The tops of the flasks were covered similarly to those in the previous experiment, and the flasks were then incubated at 78°F. for 20 days. Each treatment was replicated twice. After 20 days, exchangeable and water soluble inorganic nitrogen were extracted with 1 N NaCl by shaking the mixtures for 60 minutes. After filtering, analyses for nitrate-, nitrite- and ammonium-N were made on aliquots of the filtrates. Ammonium-N was also estimated on aliquots which had been treated with acidified potassium permanganate to oxidize any Fe++ ions to Fe+++ ions. The second procedure served as a check on possible chemical reduction of nitrate to ammonium by ferrous hydroxide within the diffusion dishes. The portions of the filtrates not employed in the above analyses were used for the mass spectrographic determinations of the atom percent excess N^{15} in the soil ammonium fractions. Potassium sulfide (0.2 g.) was added to these residual filtrates to precipitate Fe++ ions as ferrous sulfide, and after shaking the insoluble sulfides were removed by filtration. The latter filtrates were made alkaline with excess sodium hydroxide, and the ammonium-N was distilled into 25 ml. 0.01 N-H_2SO_4. The distillates were evaporated on a steam plate to a volume in which 2 ml. of solution contained approximately 1 mg. N. The atom percent N^{15} of the ammonium-N was determined on the resulting samples.
RESULTS AND DISCUSSION

The presentation of the results of the incubation experiments and the tracer nitrogen experiments is given in the same order as they were described in the section on materials and methods. This arrangement permits an orderly reference back to the procedures used to obtain the results presented in this section.

Incubation Experiments

Inorganic nitrogen changes in water-logged soils

The effects of incubation period, nitrate treatment and soil moisture on the ammonium-, nitrite- and nitrate-N contents of soils 1 to 8 are given in Table 7. The addition of nitrate affected the ammonium-N accumulation of all soils incubated under water-logged conditions. In soils 7 and 8 the ammonium-N accumulation for the control treatment was substantially less than when either 0.001 g. or 0.005 g. nitrate-N was applied. With the latter two nitrate treatments the ammonium-N contents for corresponding time periods were similar. Nitrite-N accumulated at intermediate samplings when soils 7 and 8 were treated with nitrate. In contrast to ammonium-N, nitrite-N accumulation was affected by the level of nitrate application. The effects of different rates of nitrate application on nitrite- and ammonium-N accumulations are graphically illustrated in Figures 1 and
Table 7. Influence of incubation period, nitrate treatment and soil moisture content on the inorganic-N content of 5 g. of soils 1 to 8 as p.p.m. \( \text{NH}_4^- \), \( \text{NO}_2^- \) and \( \text{NO}_3^- \) N

<table>
<thead>
<tr>
<th>Incubation period (days)</th>
<th>Treatment A</th>
<th>Treatment B</th>
<th>Treatment C</th>
<th>Treatment D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{NH}_4^- )</td>
<td>( \text{NO}_2^- )</td>
<td>( \text{NO}_3^- )</td>
<td>( \text{NH}_4^- )</td>
</tr>
<tr>
<td>Soil 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>8</td>
<td>31</td>
<td>0</td>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>20</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>51</td>
</tr>
<tr>
<td>30</td>
<td>64</td>
<td>0</td>
<td>4</td>
<td>53</td>
</tr>
<tr>
<td>40</td>
<td>71</td>
<td>0</td>
<td>0</td>
<td>61</td>
</tr>
<tr>
<td>Soil 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>0</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td>8</td>
<td>41</td>
<td>0</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td>20</td>
<td>83</td>
<td>0</td>
<td>0</td>
<td>47</td>
</tr>
<tr>
<td>30</td>
<td>93</td>
<td>0</td>
<td>5</td>
<td>56</td>
</tr>
<tr>
<td>40</td>
<td>94</td>
<td>0</td>
<td>1</td>
<td>62</td>
</tr>
</tbody>
</table>

\(^a\) Treatment A = 0 g. \( \text{NO}_3^- \) N, water-logged soil (control).
Treatment B = 0.001 g. \( \text{NO}_3^- \) N, water-logged soil.
Treatment C = 0.005 g. \( \text{NO}_3^- \) N, water-logged soil.
Treatment D = 0 g. \( \text{NO}_3^- \) N, 0.3 atmosphere soil moisture tension.
<table>
<thead>
<tr>
<th>Incubation period (days)</th>
<th>(\text{NH}_4^-)</th>
<th>(\text{NO}_2^-)</th>
<th>(\text{NO}_3^-)</th>
<th>(\text{NH}_4^-)</th>
<th>(\text{NO}_2^-)</th>
<th>(\text{NO}_3^-)</th>
<th>(\text{NH}_4^-)</th>
<th>(\text{NO}_2^-)</th>
<th>(\text{NO}_3^-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>9</td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>977</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td>0</td>
<td>0</td>
<td>60</td>
<td>1.3</td>
<td>6</td>
<td>50</td>
<td>26.1</td>
<td>667</td>
</tr>
<tr>
<td>8</td>
<td>77</td>
<td>0</td>
<td>0</td>
<td>79</td>
<td>0</td>
<td>7</td>
<td>78</td>
<td>2.1</td>
<td>575</td>
</tr>
<tr>
<td>20</td>
<td>119</td>
<td>0</td>
<td>0</td>
<td>127</td>
<td>0</td>
<td>0</td>
<td>103</td>
<td>0.1</td>
<td>407</td>
</tr>
<tr>
<td>30</td>
<td>158</td>
<td>0</td>
<td>0</td>
<td>150</td>
<td>0</td>
<td>0</td>
<td>84</td>
<td>0</td>
<td>336</td>
</tr>
<tr>
<td>40</td>
<td>153</td>
<td>0</td>
<td>0</td>
<td>131</td>
<td>0</td>
<td>0</td>
<td>53</td>
<td>0</td>
<td>220</td>
</tr>
<tr>
<td>Soil 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>194</td>
<td>7</td>
<td>0</td>
<td>973</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>0</td>
<td>1</td>
<td>18</td>
<td>0.1</td>
<td>94</td>
<td>19</td>
<td>8.9</td>
<td>830</td>
</tr>
<tr>
<td>8</td>
<td>27</td>
<td>0</td>
<td>1</td>
<td>18</td>
<td>0</td>
<td>57</td>
<td>15</td>
<td>0.1</td>
<td>770</td>
</tr>
<tr>
<td>20</td>
<td>46</td>
<td>0</td>
<td>0</td>
<td>23</td>
<td>0</td>
<td>3</td>
<td>19</td>
<td>0</td>
<td>737</td>
</tr>
<tr>
<td>30</td>
<td>53</td>
<td>0</td>
<td>0</td>
<td>35</td>
<td>0</td>
<td>4</td>
<td>14</td>
<td>0</td>
<td>704</td>
</tr>
<tr>
<td>40</td>
<td>55</td>
<td>0</td>
<td>0</td>
<td>34</td>
<td>0</td>
<td>2</td>
<td>10</td>
<td>0</td>
<td>679</td>
</tr>
<tr>
<td>Soil 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>198</td>
<td>2</td>
<td>0</td>
<td>982</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>0</td>
<td>4</td>
<td>10</td>
<td>20.8</td>
<td>146</td>
<td>10</td>
<td>30.2</td>
<td>915</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>0</td>
<td>5</td>
<td>8.6</td>
<td>134</td>
<td>8</td>
<td>16.4</td>
<td>917</td>
<td>7</td>
</tr>
<tr>
<td>20</td>
<td>17</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>110</td>
<td>10</td>
<td>0</td>
<td>879</td>
</tr>
<tr>
<td>30</td>
<td>17</td>
<td>0</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>114</td>
<td>4</td>
<td>0</td>
<td>880</td>
</tr>
<tr>
<td>40</td>
<td>16</td>
<td>0</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>102</td>
<td>5</td>
<td>0</td>
<td>880</td>
</tr>
</tbody>
</table>
Table 7 (Continued).

<table>
<thead>
<tr>
<th>Incubation period (days)</th>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>NH&lt;sub&gt;4&lt;/sub&gt;-N</th>
<th>NO&lt;sub&gt;2&lt;/sub&gt;-N</th>
<th>NO&lt;sub&gt;3&lt;/sub&gt;-N</th>
<th>NH&lt;sub&gt;4&lt;/sub&gt;-N</th>
<th>NO&lt;sub&gt;2&lt;/sub&gt;-N</th>
<th>NO&lt;sub&gt;3&lt;/sub&gt;-N</th>
<th>NH&lt;sub&gt;4&lt;/sub&gt;-N</th>
<th>NO&lt;sub&gt;2&lt;/sub&gt;-N</th>
<th>NO&lt;sub&gt;3&lt;/sub&gt;-N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>G</td>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>0</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>205</td>
<td>3</td>
<td>0</td>
<td>981</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>145</td>
<td>6</td>
<td>0</td>
<td>921</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>113</td>
<td>6</td>
<td>0</td>
<td>921</td>
<td>16</td>
</tr>
<tr>
<td>20</td>
<td>25</td>
<td>0</td>
<td>3</td>
<td>12</td>
<td>0</td>
<td>81</td>
<td>6</td>
<td>0</td>
<td>859</td>
<td>8</td>
</tr>
<tr>
<td>30</td>
<td>27</td>
<td>0</td>
<td>1</td>
<td>15</td>
<td>0</td>
<td>57</td>
<td>5</td>
<td>0</td>
<td>836</td>
<td>5</td>
</tr>
<tr>
<td>40</td>
<td>26</td>
<td>0</td>
<td>1</td>
<td>13</td>
<td>0</td>
<td>34</td>
<td>7</td>
<td>0</td>
<td>814</td>
<td>5</td>
</tr>
<tr>
<td>Soil 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>10</td>
<td>0</td>
<td>193</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>48</td>
<td>0.5</td>
<td>151</td>
<td>45</td>
<td>3.2</td>
<td>910</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>55</td>
<td>0</td>
<td>114</td>
<td>50</td>
<td>0</td>
<td>895</td>
<td>6</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>59</td>
<td>0</td>
<td>105</td>
<td>56</td>
<td>0</td>
<td>880</td>
<td>5</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>61</td>
<td>0</td>
<td>103</td>
<td>55</td>
<td>0</td>
<td>875</td>
<td>5</td>
</tr>
<tr>
<td>40</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>64</td>
<td>0</td>
<td>99</td>
<td>55</td>
<td>0</td>
<td>870</td>
<td>6</td>
</tr>
<tr>
<td>Soil 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>7</td>
<td>0</td>
<td>202</td>
<td>5</td>
<td>0</td>
<td>980</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>0</td>
<td>2</td>
<td>49</td>
<td>2.4</td>
<td>130</td>
<td>45</td>
<td>12.9</td>
<td>900</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>0</td>
<td>2</td>
<td>61</td>
<td>0.9</td>
<td>90</td>
<td>60</td>
<td>10.3</td>
<td>860</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>62</td>
<td>0</td>
<td>85</td>
<td>65</td>
<td>0.1</td>
<td>850</td>
<td>2</td>
</tr>
<tr>
<td>30</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>67</td>
<td>0</td>
<td>86</td>
<td>68</td>
<td>0</td>
<td>854</td>
<td>1</td>
</tr>
<tr>
<td>40</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>68</td>
<td>0</td>
<td>87</td>
<td>67</td>
<td>0</td>
<td>848</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>NH<sub>4</sub>-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N represent nitrogen compounds in the soil.
2. No ammonia (Table 8) was volatilized from either soil during the experimental period. The differences in the effects of rates of nitrate application on the patterns of ammonium and nitrite formation are difficult to explain biologically, so the possibility of chemical reduction of nitrate to ammonium must be considered.

Compared to the water-logged control treatment, the addition of nitrate to soils 1 to 6 prior to incubation tended to decrease soil-ammonium-N, especially at the end of the longer incubations. The effect was most pronounced with the 0.005 g. nitrate-N application. The ammonium-N decreases observed in the nitrate-treated soils 1 to 6 may have resulted from nitrification, as described by Pearsall (1950), of ammonium in the oxygenated layer adjacent to the water surface. To be nitrified in this manner the ammonium-N must be in the soil solution. In those water-logged soils treated with potassium nitrate, the ammonium-N of the solution phase can be increased at the expense of exchangeable ammonium in several ways:

1. The added K$^+$ ions can replace NH$_4^+$ ions of the soil colloidal complex.

2. Also on incubation of the water-logged soils denitrification may occur. Associated with denitrification there is a decrease in total soil acidity. A decrease in acidity favors ammonia and ammonium hydroxide formation as
Figure 1. Influence of time of incubation under water-logged conditions and two rates of nitrate-N (NO$_3$-N) application on the ammonium-N (NH$_4$-N) and nitrite-N (NO$_2$-N) contents of 5 g. of soil 7 (Kalkaska sand)
DAYS OF INCUBATION

N (ppm.)

+ + NH₄-N, 0.005 g. NO₃-N
O - NH₄-N, 0.001 g. NO₃-N
+ - NO₂-N, 0.005 g. NO₃-N
O - NO₂-N, 0.001 g. NO₃-N

0 4 8 12 16 20 24 28 32 36 40
Figure 2. Influence of time of incubation under water-logged conditions and two rates of nitrate-N ($\text{NO}_3^-\text{N}$) application on the ammonium-N ($\text{NH}_4^-\text{N}$) and nitrite-N ($\text{NO}_2^-\text{N}$) contents of 5 g. of soil 8 (Cecil sandy loam)
Table 8. Influence of incubation period and nitrate treatment on the cumulative ammonia, as p.p.m. ammonia-N, volatilized from 5 g. of water-logged soils 1 to 8

<table>
<thead>
<tr>
<th>Treatment g. NO₃-N</th>
<th>Incubation period, days</th>
<th>Soil 1</th>
<th>Soil 2</th>
<th>Soil 3</th>
<th>Soil 4</th>
<th>Soil 5</th>
<th>Soil 6</th>
<th>Soil 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>20</td>
<td>30</td>
<td>40</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>-</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0.005</td>
<td>-</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>-</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0.005</td>
<td>-</td>
<td>4</td>
<td>15</td>
<td>31</td>
<td>39</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>-</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0.005</td>
<td>-</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>-</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0.005</td>
<td>-</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>-</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0.005</td>
<td>-</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0.005</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>
illustrated by the following equation:

\[ \text{NH}_4^+ + \text{OH}^- \rightleftharpoons \text{NH}_4\text{OH} \rightleftharpoons \text{NH}_3 + \text{H}_2\text{O} \]  

(4)

The ammonium of the soil solution could diffuse into the oxygenated surface and be either nitrified or, depending upon the solution pH, lost as ammonia gas. The data contained in Table 8 indicate that ammonia volatilization was marked only in soil 3.

The effect of the water-logged treatments on the ammonium-N of the water above the soil surfaces was later made (Appendix Table 19). Very little ammonium-N was in the water layers of soils with the control treatments. Most ammonium was found at the 4- and 8-day samplings of the water layers above soils treated with the highest level of nitrate.

The largest amounts of nitrite-N were found in those soils treated with 0.005 g. nitrate-N. In no case was
nitrite found to be present at the 30-day sampling. In soils 3 and 5 nitrite accumulated above the levels described by Ponnamperuma (1955, p. 39) as being usual for nitrate-treated water-logged soils.

The effects of nitrate treatment and incubation time on the pH values of the eight soils are given in Table 9. Soil pH in all cases was affected both by treatment and time of incubation. The addition of potassium nitrate to soils resulted in lower pH values. Black (1957, p. 135) ascribes the effect of potassium chloride reducing the soil pH to the cation of the salt replacing H\(^+\) ions of the exchange complex and to a reduction in junction potential if such a junction potential is present. Reduction of nitrate- to ammonium-N together with denitrification losses of nitrate would partially explain pH increases with time for the nitrate-treated soils.

A comparison of the soils incubated under water-logged conditions and under 0.3 atmosphere soil moisture tension, respectively, shows that inorganic nitrogen accumulation was generally greater in the water-logged series. The data provide no information as to possible denitrification losses of mineralized nitrogen at the lower soil moisture.
Table 9. Effect of nitrate treatment on pH values of 5 g. of soils 1 to 8 incubated under water-logged conditions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incubation period, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>g. NO₃-N</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Soil 1</td>
</tr>
<tr>
<td>0</td>
<td>6.1</td>
</tr>
<tr>
<td>0.001</td>
<td>5.5</td>
</tr>
<tr>
<td>0.005</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 9 (Continued).

<table>
<thead>
<tr>
<th>Treatment g. NO$_3$-N</th>
<th>Incubation period, days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Soil 8</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5.8</td>
</tr>
<tr>
<td>0.001</td>
<td>5.1</td>
</tr>
<tr>
<td>0.005</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Biological reduction of nitrate to ammonium in soils

Large amounts of ammonium-N accumulated on incubation of soils 7 and 8 under water-logged conditions in the presence of nitrate. Discrepancies in the pattern of nitrite- and ammonium-N production, however, did not support the hypothesis of biological reduction of nitrate to ammonium. The experiments in this section were established to investigate further the above hypothesis.

Effect of soil moisture level on ammonium production

The effects of varying soil moisture and differing nitrogen treatment on the inorganic-N contents of soils 7 and 8 are given in Table 10. At 0.3 atmosphere soil moisture tension there was little reduction of nitrate to ammonium. Nitrate decreases at this soil moisture level were small compared to those in the corresponding water-logged nitrate series. Figures 3 and 4 show the influence of time and moisture
Table 10. The effects of varying soil moisture level\textsuperscript{a} and differing nitrogen treatment on the inorganic-N content of 5 g. of soils 7 and 8 as p.p.m. NH\textsubscript{4}\textsuperscript{+}, NO\textsubscript{2}\textsuperscript{-} and NO\textsubscript{3}\textsuperscript{-} N

<table>
<thead>
<tr>
<th>Nitrogen treatment</th>
<th>Soil moisture</th>
<th>Incubation period, days</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>g.N</td>
<td>Form</td>
<td>NH\textsubscript{4}\textsuperscript{+}</td>
<td>NO\textsubscript{2}\textsuperscript{-}</td>
<td>NO\textsubscript{3}\textsuperscript{-}</td>
<td>NH\textsubscript{4}\textsuperscript{+}</td>
<td>NO\textsubscript{2}\textsuperscript{-}</td>
</tr>
<tr>
<td>0</td>
<td>NW</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>W</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>0.00025</td>
<td>NH\textsubscript{4}\textsuperscript{+} NW</td>
<td>49</td>
<td>0</td>
<td>4</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>NO\textsubscript{3}\textsuperscript{-} NW</td>
<td>10</td>
<td>0</td>
<td>194</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>NO\textsubscript{3}\textsuperscript{-} W</td>
<td>10</td>
<td>0</td>
<td>192</td>
<td>32</td>
<td>0.2</td>
</tr>
<tr>
<td>0</td>
<td>NW</td>
<td>6</td>
<td>0</td>
<td>8</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>W</td>
<td>6</td>
<td>0</td>
<td>8</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>0.00025</td>
<td>NH\textsubscript{4}\textsuperscript{+} NW</td>
<td>51</td>
<td>0</td>
<td>10</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>NO\textsubscript{3}\textsuperscript{-} NW</td>
<td>5</td>
<td>0</td>
<td>204</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>NO\textsubscript{3}\textsuperscript{-} W</td>
<td>5</td>
<td>0</td>
<td>205</td>
<td>42</td>
<td>2.6</td>
</tr>
</tbody>
</table>

\textsuperscript{a}NW indicates soil moisture tension maintained at 0.3 atmosphere. W indicates water-logging of soil by addition of 14 ml. of water.
Figure 3. Influence of time of incubation and two soil moisture levels on the ammonium-N (NH$_4^-$-N) content of 5 g. of soil 7 (Kalkaska sand) treated with 0.001 g. nitrate-N.
Figure 4. Influence of time of incubation and two soil moisture levels on the ammonium-N (NH₄-N) content of 5 g. of soil 8 (Cecil sandy loam) treated with 0.001 g. nitrate-N
level on the ammonium-N contents of soils 7 and 9 treated with 0.001 g. nitrate-N. The stability of the applied ammonium in these soils at 0.3 atmosphere soil moisture tension indicates that nitrification during the incubation period was of little importance in either soil. This suggests that the lower contents of ammonium-N in the presence of nitrate at the lower soil moisture level were not due to the nitrification process. The results with one anomaly are similar to those that would be expected for biological nitrate reduction. At the initial sampling with soil 7 the ammonium-N content was greater in both of the nitrate treatments than in the check. Since from the time of the addition of the nitrate to the soils until the commencement of the ammonium analyses there was at the most only a 3-hour delay, a biological explanation for the increase in ammonium seems doubtful.

**Effect of nitrite on ammonium production** The results of the study under water-logged conditions in which the influence of incubation period and nitrite treatment on the inorganic-N contents of soils 7 and 8 were studied are given in Table 11. In soils 7 and 8 treated with nitrate, 0.005 g. nitrite-N inhibited ammonium production. The rate of ammonium accumulation in soil 8 in the presence of nitrate was reduced by 0.001 g. nitrite-N. These effects are illustrated graphically in Figures 5 and 6.
Table 11. Influence of incubation period and nitrogen treatment on the inorganic-N contents of 5 g. of soils 7 and 8 under water-logged conditions as p.p.m. \( \text{NH}_4^- \), \( \text{NO}_2^- \) and \( \text{NO}_3^- \) N

<table>
<thead>
<tr>
<th>Incubation period, days</th>
<th>Treatment 0</th>
<th>Treatment 2</th>
<th>Treatment 4</th>
<th>Treatment 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{NH}_4^- )</td>
<td>( \text{NO}_2^- )</td>
<td>( \text{NO}_3^- )</td>
<td>( \text{NH}_4^- )</td>
</tr>
<tr>
<td>Soil 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>0</td>
<td>186</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>168</td>
<td>2</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>936</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>162</td>
<td>197</td>
<td>29</td>
</tr>
<tr>
<td>6</td>
<td>17</td>
<td>929</td>
<td>205</td>
<td>14</td>
</tr>
<tr>
<td>Soil 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0</td>
<td>200</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>189</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>981</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>191</td>
<td>197</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>990</td>
<td>204</td>
<td>9</td>
</tr>
</tbody>
</table>

\(^a\)Treatment 1 = 0, treatment 2 = 0.001 g. \( \text{NO}_3^- \) N, treatment 3 = 0.001 g. \( \text{NO}_2^- \) N, treatment 4 = 0.005 g. \( \text{NO}_2^- \) N, treatment 5 = 0.001 g. \( \text{NO}_3^- \) N + 0.001 g. \( \text{NO}_2^- \) N, treatment 6 = 0.001 g. \( \text{NO}_3^- \) N + 0.005 g. \( \text{NO}_2^- \) N.
Figure 5. Influence of time of incubation and nitrite treatment (NO₂-N) on the ammonium-N (NH₄-N) content of 5 g. of soil 7 (Kalkaska sand) water-logged and treated with nitrate-N (NO₃-N)
Figure 6. Influence of time of incubation and nitrite treatment ($\text{NO}_2^-\text{N}$) on the ammonium-$\text{N}$ ($\text{NH}_4^-\text{N}$) content of 5 g. of soil 8 (Cecil sandy loam) water-logged and treated with nitrate-$\text{N}$ ($\text{NO}_3^-\text{N}$)
Quantitative recoveries of nitrite-N were not obtained when nitrite was added to soils 7 and 8. Both soils 7 and 8 are acidic, and decomposition of nitrous acid probably explains these discrepancies. Equations 5 and 6 are given by Barnett and Wilson (1931, p. 351) to explain the decomposition:

\[ 2 \text{HNO}_2 \leftrightarrow \text{NO} + \text{NO}_2 + \text{H}_2\text{O} \]  

\[ \text{H}_2\text{O} + 2 \text{NO}_2 \leftrightarrow \text{HNO}_3 + \text{HNO}_2 \]  

The reactions are generally represented by the single equation:

\[ 3 \text{HNO}_2 \leftrightarrow \text{HNO}_3 + 2 \text{NO} + \text{H}_2\text{O} \]  

The more acid nature of soil 7 possibly accounts for the greater losses of nitrite obtained with that soil.

Again with one anomaly, the data appeared to support the hypothesis of biological reduction of nitrate to ammonium. The anomaly is in the zero sampling. Especially with soil 7 the addition of nitrite results in higher amounts of ammonium. This occurs even at the higher rate of nitrite application. The sodium nitrite employed contained no measurable amount of ammonium.

The effects of incubation time and nitrogen treatment on soil pH are given in Table 12. Sodium nitrite had less effect on the soil pH values than potassium nitrate did.
Table 12. Influence of incubation period and nitrogen treatment on the pH values of 5 g. of soils 7 and 8 under water-logged conditions

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Incubation period, days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Soil 7</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.2</td>
</tr>
<tr>
<td>2</td>
<td>4.5</td>
</tr>
<tr>
<td>3</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>4.9</td>
</tr>
<tr>
<td>5</td>
<td>4.6</td>
</tr>
<tr>
<td>6</td>
<td>4.8</td>
</tr>
<tr>
<td>Soil 8</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.8</td>
</tr>
<tr>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td>3</td>
<td>5.4</td>
</tr>
<tr>
<td>4</td>
<td>5.4</td>
</tr>
<tr>
<td>5</td>
<td>5.2</td>
</tr>
<tr>
<td>6</td>
<td>5.1</td>
</tr>
</tbody>
</table>

aTreatment 1 = 0, treatment 2 = 0.001 g. NO₃⁻-N, treatment 3 = 0.001 g. NO₂⁻-N, treatment 4 = 0.005 g. NO₂⁻-N, treatment 5 = 0.001 g. NO₃⁻-N + 0.001 g. NO₂⁻-N, treatment 6 = 0.001 g. NO₃⁻-N + 0.005 g. NO₂⁻-N.

This is probably the result of two reactions involving nitrous acid. The decomposition of nitrous acid described earlier results in a lower concentration of H⁺ ions. In addition, the dissociation constant of the reaction, 
\[ \text{HNO}_2 \rightarrow \text{H}^+ + \text{NO}_2^- \], is \( 4 \times 10^{-4} \) at \( 18^\circ \text{C} \). (Hodgman, 1953,
The addition of nitrite would shift this equilibrium to the left with a resultant decrease in $H^+$ ion concentration. Both of these reactions would tend to counterbalance any increase in $H^+$ ion concentration due to exchange reactions involving sodium.

**Effect on ammonium production of autoclaving soils**

The results of the study involving the effects of time of incubation and nitrate treatment on the inorganic-$N$ contents of autoclaved soils 7 and 8 are given in Table 13. Autoclaving of both soils resulted in increases in the soil ammonium-$N$ contents. Nitrate treatments had little effect on the ammonium-$N$ contents in soil 7. With soil 8 the ammonium-$N$ values at the 2, 4 and 8 day samplings were greater in the presence of nitrate. However, the levels are much lower than those found previously. Nitrite was found only in the latter soil. The results do not conflict with the biological nitrate reduction hypothesis advanced to explain the formation of ammonium in the presence of nitrate under water-logged conditions. Ineffective sterilization could explain the slight reduction of nitrate found in soil 8.

**Chemical reduction of nitrate to ammonium**

The results of the previous experiments with soils 7 and 8 did not conclusively indicate that ammonium accumulation in the presence of nitrate was the result of biological
Table 13. Influence of incubation time on the inorganic-N contents, as p.p.m. NH$_4^-$, NO$_2^-$ and NO$_3^-$ of 5 g. of autoclaved soils 7 and 8 under waterlogged conditions in the presence and absence of nitrate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incubation period, days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>NO$_3^-$ N</td>
<td>NH$_4^-$</td>
</tr>
<tr>
<td>Soil 7</td>
<td></td>
</tr>
<tr>
<td>0 16 0 1</td>
<td>19 0 0 0</td>
</tr>
<tr>
<td>0.001 17 0 194</td>
<td>21 0 193</td>
</tr>
<tr>
<td>Soil 8</td>
<td></td>
</tr>
<tr>
<td>0 14 0 8</td>
<td>15 0 7 7</td>
</tr>
<tr>
<td>0.001 15 0 203</td>
<td>21 1.3 199</td>
</tr>
</tbody>
</table>
processes. In this section the results of experiments established to study the possibility of chemical reduction of nitrate to ammonium in both of these soils are given.

Reduction of nitrate to ammonium by ferrous hydroxide

The ammonium-N contents of soils 7 and 8 after 4 days of incubation are given in Table 14. The ammonium-N levels were higher where nitrate had been applied, but for corresponding treatments methods 1 and 2 gave similar results. Hence, it is concluded that chemical reduction of nitrate by ferrous hydroxide during the analytical procedures was not responsible for the higher ammonium yields in the presence of nitrate.

Table 14. Effect of method of analysis\(^a\) and nitrate treatment on the estimation of the ammonium-N contents of 5 g. of soils 7 and 8 after a 4-day incubation period under water-logged conditions

<table>
<thead>
<tr>
<th>Treatment, g. NO(_3)-N</th>
<th>NH(_4)-N, p.p.m.</th>
<th>Method 1</th>
<th>Method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>0.001</td>
<td>35</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Soil 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td>43</td>
<td>42</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Method 1 as described under analytical methods did not provide for oxidation of Fe\(^{++}\) ions; method 2 also described involved the pre-treatment of the test aliquot with acidified KMnO\(_4\) to oxidize Fe\(^{++}\) ions before estimation of the ammonium by the microdiffusion method.
Reduction of nitrate to ammonium by metallic iron

The effects of mechanical grinding on the ammonium-N contents of the "de-magnetized" soils 7 and 8 are given in Table 15. If the magnetic substances were removed from the soil prior to incubation, the ammonium-N content after the 4-day incubation period was little affected by nitrate treatment. However, if these soils were again passed through the grinder the ammonium content was again influenced by nitrate treatment. The results indicate that substances acquired during grinding were responsible for the nitrate reduction which was found with soils 7 and 8. It is therefore concluded that the nitrate reduction which was extensively studied in soils 7 and 8 was the result of chemical rather than biological processes. It seems probable

Table 15. Effect of mechanical grinding on the ammonium-N contents of "de-magnetized" soils 7 and 8 after a 4-day incubation period under water-logged conditions in the presence and absence of added nitrate

<table>
<thead>
<tr>
<th>Treatment, g. NO₃-N</th>
<th>NH₄-N, p.p.m.</th>
<th>Soil 7</th>
<th>Soil 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>0.001</td>
<td></td>
<td>12</td>
<td>35</td>
</tr>
</tbody>
</table>

a- indicates "de-magnetized" soil.
b+ indicates "de-magnetized" reground soil.
that activated hydrogen produced on the surfaces of the metal particles reduced nitrate to ammonium. On a unit weight basis it was found that contamination from the grinders was three times more effective in reducing nitrate than was 100-mesh hydrogen-reduced iron.

Acid attack of metals has been extensively investigated in corrosion studies. Starkey and Wight (1945) state that electrolytic cells form over the surface of the metal and the reactions at the electrodes can be expressed as follows:

\[
\begin{align*}
\text{Fe} & \rightarrow \text{Fe}^{2+} + 2 \text{e} \quad \text{anodic reaction} \\
2\text{H}^+ + 2 \text{e} & \rightarrow 2\text{H} \quad \text{cathodic reaction}
\end{align*}
\]

According to Speller (1951, p. 188) hydrochloric acid will attack steel with the evolution of hydrogen if the pH is less than 4, but with buffered solutions hydrogen may be released at pH 6. This author states that total acidity rather than pH determines the susceptibility of steel to attack.

The reaction between nitric acid and iron is complex. Evans (1944) reports that ammonia and nitrous acid are formed when dilute nitric acid reacts with iron. Reduction of nitric acid by activated hydrogen produced at the cathode results in ammonia formation, and the author postulated the following cathode reactions to explain the formation of nitrous acid:
\[
\begin{align*}
\text{HNO}_3 + \text{H}^+ + \text{e} &= \text{NO}_2 + \text{H}_2\text{O} \\
\text{NO}_2 + \text{H}^+ + \text{e} &= \text{HNO}_2
\end{align*}
\]

This worker also indicated that in reactions with nitric acid the introduction of carbon into iron favors ammonia production. A test showed that the addition of dilute hydrochloric acid to the magnetic material removed from the soils resulted in acetylene formation, so the contamination must have contained some carbon. This possibly explains why the contamination from the grinder was more effective in reducing nitrate to ammonium than was laboratory hydrogen-reduced iron.

The results of the incubation experiments with soils 7 and 8 involving soil moisture levels, autoclaving and addition of nitrite to the soils can be explained on the corrosion of metal hypothesis. Whitman et al. (1924) postulated that at low \( \text{H}^+ \) ion contents in the presence of oxygen the cathode reaction is probably given by the reaction:

\[
\frac{1}{2} \text{O}_2 + \text{H}_2\text{O} + 2 \text{e} = 2(\text{OH}^-).
\]

This worker showed that hydrogen gas can be evolved from solutions of lower acidity when the oxygen content of the solution is lowered. The reaction involving oxygen probably explains why little reduction of nitrate to ammonia occurred when the soil moisture tension was 0.3 atmosphere.
The reduced soil moisture tension resulted in soils with higher contents of oxygen. The effect of nitrite in inhibiting nitrate production is most probably explained by the decreases in \( H^+ \) ion content resulting from nitrous acid decomposition and non-dissociation of a portion of the nitrous acid. Decreases in \( H^+ \) ion concentration would decrease the potential difference between the cathodic and anodic areas on the metal surfaces and reduce metal corrosion. Autoclaving of both soils also reduced nitrate reduction, and this probably resulted from the formation of protective films around the iron particles. Speller (1951, p. 249) reported that steam reacts with iron at high temperature with the production of magnetite and hydrogen. In steam boilers only small amounts of hydrogen are evolved at temperatures of 585°F, but with finely divided particles the reaction should be more pronounced.

**Tracer Nitrogen Experiments**

Previous experiments showed that nitrate disappeared from soils 1 to 6 which were incubated under water-logged conditions. The results of the following experiments with labelled nitrate-N indicate the relative importance of denitrification, immobilization of nitrate by microorganisms, and nitrate respiration with ammonium formation in accounting for the disappearance of nitrate from the six soils.
Denitrification losses of applied nitrate

The denitrification losses of applied labelled nitrate-N from soils 1 to 6 are given in Table 16. Appreciable losses of nitrate-N have occurred without the addition of an external organic energy source for the heterotrophs responsible for denitrification. The oxidizable carbon requirements of these organisms were provided by constituents of the soil organic matter.

The auxiliary experiment with non-tagged nitrate-N indicated that no nitrite-N was present in any soil at the end of 20 days. Also, apart from soils 5 and 6, in which

Table 16. Losses of applied labelled nitrate-N\(^a\) from 5 g. samples of six Iowa soils incubated for 20 days under water-logged conditions

<table>
<thead>
<tr>
<th>Soil</th>
<th>Loss of NO(_3)-N</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.p.m.(^b)</td>
<td>129</td>
<td>159</td>
<td>185</td>
<td>163</td>
<td>46</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Percent of applied NO(_3)-N</td>
<td>69</td>
<td>85</td>
<td>94</td>
<td>88</td>
<td>49</td>
<td>48</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Before incubation 187 p.p.m. NO\(_3\)-N were applied to soils 1 to 4, and 94 p.p.m. NO\(_3\)-N were applied to soils 5 and 6.

\(^b\) p.p.m. N refer to the number of parts of nitrogen per million parts of oven-dried soil. For consistency in presentation all results are expressed in terms of an atomic weight of 14 for nitrogen.
8 and 11 p.p.m., respectively, remained, nitrate-N had disappeared from all soils.

The earlier incubation experiments showed that little ammonification of organic matter occurred in soils 5 and 6 incubated under water-logged conditions. The present study shows that denitrification is restricted in both of these soils. Soils 4 and 6 were obtained from the Clarion-Webster Experiment Farm, Kanawha. Soil 4 which was obtained from a sodded area was appreciably more active both in regards to ammonification and denitrification than was the soil from the adjacent cultivated area. The latter soil is slightly lower in both organic nitrogen and carbon. This comparison may conceivably indicate that ammonification and denitrification in these two soils are not related to the total organic matter, but rather to an active fraction of the organic matter. The denitrifying organisms active in soil 4 may even have obtained most of their energy materials required for growth from the relatively fresh decomposing plant material fraction of the soil organic matter.

The relationship, for soils 1 to 6, between loss of nitrate-N and percent soil carbon is given in Table 17. Different pH values and microbial populations of the soils used in this study probably partly account for the poor relationship found.
Table 17. Influence of percent soil carbon on the amount of applied labelled nitrate-N\(^a\) lost from 5 g. of soils 1 to 6 after incubation for 20 days under anaerobic conditions

<table>
<thead>
<tr>
<th>Soil</th>
<th>% Soil C.</th>
<th>NO(_3)-N loss, p.p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.21</td>
<td>129</td>
</tr>
<tr>
<td>2</td>
<td>2.83</td>
<td>159</td>
</tr>
<tr>
<td>3</td>
<td>2.31</td>
<td>185</td>
</tr>
<tr>
<td>4</td>
<td>3.92</td>
<td>163</td>
</tr>
<tr>
<td>5</td>
<td>2.56</td>
<td>46</td>
</tr>
<tr>
<td>6</td>
<td>3.76</td>
<td>45</td>
</tr>
</tbody>
</table>

\(^a\)Before incubation 187 p.p.m. NO\(_3\)-N were applied to soils 1 to 4 and 94 p.p.m. NO\(_3\)-N were applied to soils 5 and 6. For consistency in presentation, all results are expressed in terms of an atomic weight of 14 for nitrogen.

Ammonium-N derived from applied nitrate

The amounts of applied nitrate-N reduced to ammonium-N in soils 1, 2, 3, 4 and 6 are given in Table 18. A maximum of 1.6% of the applied nitrate-N was found as ammonium-N after incubation for 20 days under water-logged conditions. The ammonium-N produced from the applied nitrate could have arisen either through nitrate respiration or ammonification of immobilized nitrate-N. Irrespective of the means, nitrate respiration resulting in ammonia formation is
Table 18. The inorganic-N contents and amounts of ammonium-N (NH$_4^-$-N) derived from applied labelled nitrate-N (187 p.p.m. N) before and after incubation of five Iowa soils under water-logged conditions

<table>
<thead>
<tr>
<th>Soil</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time Days</td>
<td>Time Days</td>
<td>Time Days</td>
<td>Time Days</td>
<td>Time Days</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>NH$_4^-$-N, p.p.m. (Method 1)$^a$</td>
<td>8</td>
<td>49</td>
<td>10</td>
<td>48</td>
<td>9</td>
</tr>
<tr>
<td>NH$_4^-$-N, p.p.m. (Method 2)$^b$</td>
<td>7</td>
<td>48</td>
<td>8</td>
<td>48</td>
<td>10</td>
</tr>
<tr>
<td>NO$_2^-$-N, p.p.m.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NO$_3^-$-N, p.p.m.</td>
<td>190</td>
<td>2</td>
<td>191</td>
<td>0</td>
<td>190</td>
</tr>
<tr>
<td>NH$_4^-$-N derived from applied NO$_3^-$-N, p.p.m.</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Percent NO$_3^-$-N reduced to NH$_4^-$-N</td>
<td>-</td>
<td>1.6</td>
<td>-</td>
<td>1.1</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$p.p.m. N refers to the number of parts of nitrogen per million parts of air-dried soil. For consistency in presentation all results are expressed in terms of an atomic weight of 14 for nitrogen.

$^b$Method 1 as described under analytical methods did not provide for oxidation of Fe$^{++}$ ions; method 2 also described involved the prior treatment of the test aliquot with acidified potassium permanganate.
seemingly unimportant in the soils studied. All the tagged ammonium-N which was formed during the 20-day incubation may not have accumulated. Ammonium-N which diffused into the oxygenated layer adjacent to the air may have been nitrified. On diffusion into the anaerobic part of the soil water system nitrate-N produced in this way would again be subject to loss through denitrification.

Precautions were taken to prevent chemical reduction of nitrate to ammonium by ferrous hydroxide during the distillation processes involved in the analyses. The results in Table 18 indicate that the precautions were not necessary. Approximately identical results were obtained whether or not Fe$^{++}$ ions were oxidized to Fe$^{+++}$ ions in the aliquots analyzed.

The results of the previous denitrification study using labelled nitrate-N indicated that portions of the applied nitrogen remained in the soil in forms other than nitrate- or nitrite-N. The present study suggests that this unaccounted-for nitrogen is not in the ammonium form. Possibly the unaccounted nitrogen had been immobilized into organic forms by microorganisms.
SUMMARY AND CONCLUSIONS

This investigation was undertaken to study nitrate reduction to ammonium in water-logged soils. Eight soils (soils 1 to 8 of Table 1), which varied in physical and chemical composition, were used in the investigation. The work was essentially carried out in two stages. The first stage was concerned with inorganic nitrogen changes in water-logged soils, while in the second stage labelled nitrate-N was used to determine the relative importance of denitrification and nitrate reduction to ammonium in soils 1 to 6.

In the inorganic nitrogen studies 5 g. samples of soils 1 to 8 were incubated under water-logged conditions in the presence and absence of added nitrate (0.001 and 0.005 g. NO$_3^-$-N), and at 0, 4, 8, 20, 30 and 40 days analyses for ammonium-, nitrite- and nitrate-N were made. Upon incubation, soil nitrate-N decreased in all cases. Nitrite-N accumulated at intermediate samplings in soils treated with nitrate, but by the 30-day sampling none was found in any soil. Of particular interest was the effect of level of nitrate application on the cumulative ammonium-N production. In this regard soils 1 to 6 reacted differently to soils 7 and 8. In the first-named soils at the 8-day and subsequent samplings ammonium accumulations were less in the nitrate-treated soils. The 0.005 g. nitrate-N treatment suppressed
the ammonium accumulation to a greater extent than did the 0.001 g. nitrate-N treatment. Appreciable losses of vola-
tilized ammonia were found to occur only in soil 3 treated
with 0.005 g. nitrate-N. Since the depressing effect of
nitrate was not marked at the 4-day sampling, a detrimental
effect of salt on ammonification is not suspected as being
the cause. The hypothesis is advanced that in nitrate-
treated water-logged soils a larger portion of the ammonium
produced through ammonification is nitrified in the oxygen-
ated water layer adjacent to the air. To be nitrified in
this manner the ammonium-N must be in the soil solution.
Analyses of water layers above soils 1 to 6 showed that at
the 4-, 8- and, to a lesser extent, 20-day samplings the
ammonium-N contents were highest in the soils treated with
0.005 g. nitrate-N and least in the control-treated soils.
Since less of the soil ammonium was in the solution phase
in the control-treated soils, it would be expected that any
surface layer nitrification would also be less in these
soils. The results of the inorganic-N incubation studies
with soils 1 to 6 gave no indication that any applied ni-
trate was reduced to ammonium under water-logged conditions.

On incubation of soils 7 and 8 under water-logged con-
ditions, considerably more ammonium-N was produced when the
soils were pre-treated with either 0.001 or 0.005 g. ni-
trate-N. The increase in soil ammonium over that found in
the control-treated soils was found to be the result of chemical rather than biological reduction of nitrate. Passage of these two soils through a steel-disc grinder had resulted in contamination by steel particles. By use of a bar magnet 7 and 5 mg. of magnetic material were removed from 5 g. samples of soils 7 and 8, respectively. The ferro-magnetic portion of the magnetic material was shown to affect the stability of nitrate in both soils. Activated hydrogen produced on the surfaces of the steel particles under certain conditions was able to reduce nitrate to ammonium. Before the cause of the reduction was ascertained the effects of various soil treatments on the ammonium accumulation were studied and it was found that:

1. Water-logging favored nitrate reduction to ammonium, but in both soils maintained at 0.3 atmosphere soil moisture tension, little nitrate reduction occurred.

2. Nitrite-N (0.005 g.) effectively inhibited nitrate reduction under water-logged conditions in the two soils (5 g.).

3. Autoclaving of the soils before incubation was also found to reduce nitrate-reduction in the two soils.

The 0.3 atmosphere soil moisture tension effect was subsequently explained by postulating that at the $H^+$ ion concentration of the two soils oxygen reduction in preference to nitrate reduction occurred. The addition of nitrite
was found to reduce the soil $H^+$ ion concentration. It appears that at the lower $H^+$ ion concentrations in the presence of excess nitrite sufficient activated hydrogen was not produced on the steel particles to enable nitrate reduction to proceed. Protective oxide films possibly were produced on the steel particles during autoclaving, and these would be expected to hinder nitrate reduction. The results obtained with soils 7 and 8 indicate that extreme care should be taken in the preparation of soil samples for inorganic-N studies.

In the investigation's second stage, in which labelled nitrate-N was used, denitrification was shown to occur readily in soils 1 to 6, but nitrate reduction to ammonium was of little importance in any of the soils. A maximum of 1.6% of the applied nitrate-N was reduced to ammonium. The relative unimportance of this form of nitrate reduction would appear to be due to either or both of the following reasons:

1. The absence of soil organisms capable of reducing nitrate to ammonium by nitrate respiration.

2. The inability of the soil organisms capable of reducing nitrate to ammonium in nitrate respiration to compete under the experimental conditions with the denitrifying and other heterotrophic organisms of the soil microbial population for the factors essential for growth.

No evidence was found to indicate that nitrate was
reduced chemically to ammonium by ferrous hydroxide during the distillation processes involved in the analytical procedures. The problem of whether Fe\textsuperscript{+++} ions had been reduced to Fe\textsuperscript{++} ions in water-logged soils in the presence of nitrate ions was not studied.

Little relationship was found between the total carbon contents and the denitrification losses in the soils studied. A comparison of the denitrification losses of the two soils from the Clarion-Webster Experiment Farm would seem to suggest that in those two soils the bulk of the soil organic matter was a poor source of oxidizable carbon for the denitrifying organisms.

In soils 1 to 6 portions of the applied nitrate-N remained in the soils after incubation for 20 days under water-logged conditions in forms other than nitrate-, nitrite- or ammonium-N. It is suggested that the unaccounted-for nitrogen has been immobilized into organic forms by micro-organisms.
BIBLIOGRAPHY


Cady, F. B. 1960. Gaseous nitrogen products resulting from
Raleigh, North Carolina, North Carolina State College Li-
brary.

Dehérain, [P.P.] and Maquenne, [L.]. 1882. Sur la reduc-
Sci. 95: 691-693.

Delwiche, C. D. 1956. Denitrification. In McElroy,
William D. and Glass, B., eds. A symposium on inorganic
nitrogen metabolism. pp. 233-255. Baltimore, Md., The
Johns Hopkins Press.


Fry, B. A. 1955. The nitrogen metabolism of micro-organ-

Gayon, [U.] and Dupetit, [G.]. 1882. Sur la fermentation

(London) 186: 511-513.

Harmsen, G. W. and Van Schreven, D. A. 1955. Mineraliza-
tion of organic nitrogen in soil. Advances in Agronomy 7:
299-398.

Hodgman, Charles D., ed. 1953. Handbook of chemistry and
physics. 35th ed. Cleveland, Ohio, Chemical Rubber
Publishing Co.

Iwasaki, H. and Mori, T. 1958. Studies on denitrifica-
tion. III. Enzymatic gas production by the reaction of
nitrite with hydroxylamine. J. Biochem. (Tokyo) 45: 133-
140.

Janssen, G. and Metzger, W. H. 1928. Transformation of

Jansson, Sven L. 1958. Tracer studies on nitrogen trans-
formations in soil with special attention to mineralization-
immobilization relationships. Kungl. Lantbrukshögskol.

Kempthorne, Oscar. 1952. The design and analysis of experi-
ments. New York, N. Y., John Wiley and Sons, Inc.


APPENDIX
Table 19. Effect of time of incubation and nitrate treatment on the ammonium-N contents (p.p.m. N) of the water layers above 5 g. samples of soils 1 to 6 contained in vials

<table>
<thead>
<tr>
<th>Treatment NO₃-N, g.</th>
<th>Incubation period, days</th>
<th>4</th>
<th>8</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soil 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>1.2</td>
<td>0.8</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.005</td>
<td>2.6</td>
<td>4.5</td>
<td>3.2</td>
<td>2.9</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td><strong>Soil 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>0.4</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.005</td>
<td>1.6</td>
<td>0.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Soil 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>1.4</td>
<td>0.4</td>
<td>0.2</td>
<td>0.5</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>0.005</td>
<td>5.6</td>
<td>3.8</td>
<td>1.3</td>
<td>1.7</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td><strong>Soil 4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.005</td>
<td>0.5</td>
<td>0.7</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Soil 5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.005</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Soil 6</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.005</td>
<td>0.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 20. Standard errors (S.E.) and the degrees of freedom (d.f.) on which the standard errors are based on mean quantitative estimates of certain soil properties examined in the study of "Nitrate Reduction in Soils"

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Table</th>
<th>Soil</th>
<th>S.E.</th>
<th>d.f.</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.p.m. N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>7</td>
<td>1</td>
<td>1.3</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1.5</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0.48</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>1.6</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>1.5</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>1.4</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>1.9</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>1.7</td>
<td>24</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>10</td>
<td>7</td>
<td>1.6</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>1.2</td>
<td>14</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>11</td>
<td>7</td>
<td>2.0</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>1.9</td>
<td>24</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>13</td>
<td>7</td>
<td>0.8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>1.4</td>
<td>8</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>14</td>
<td>7</td>
<td>1.2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>1.1</td>
<td>4</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>15</td>
<td>7</td>
<td>1.3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>1.1</td>
<td>4</td>
</tr>
<tr>
<td>Loss of NO$_3^-$</td>
<td>16</td>
<td>1 to 6</td>
<td>3.2</td>
<td>12</td>
</tr>
</tbody>
</table>

*Text table to which standard errors and degrees of freedom apply.

*Square root transformation was used to obtain a homogeneous error variance. The S.E. is given in the transformed units.