THE EFFECT OF ALTERNATE WETTING AND DRYING
ON THE AMMONIUM AND NITRITE
NITROGEN TRANSFORMATION IN SOILS

by

Virupax C. Baligar

A thesis submitted in partial fulfillment
of the requirements for the degree
of
MASTER OF SCIENCE
in
Soil Science

Approved:

UTAH STATE UNIVERSITY
Logan, Utah
1971
ACKNOWLEDGEMENT

I express my heartfelt appreciation and gratitude to Dr. R. L. Smith for his encouragement, advice, able guidance, and support throughout my study and in writing this thesis.

I express my sincere thanks to Dr. J. J. Skujins for his helpful suggestions and constructive criticism during the investigation, also in writing this thesis. I would like to thank Dr. H. B. Peterson who very kindly agreed to work on my graduate committee. I would also like to thank Dr. R. J. Hanks and Dr. D. W. James for their helpful suggestions in the early part of this investigation.

My greatest personal indebtedness is to Miss Hilary, Ching Hwan Jang for her invaluable assistance in the laboratory in carrying out the many aspects of this investigation and in typing the rough draft copy of this thesis. Without her help this study would still be far from complete. I am thankful to Mr. Lyle Dabb for his help in chemical analysis.

I am grateful to Mrs. Ruth Cartee for typing the final thesis so nicely.

I dedicate this thesis to my brother, Dr. Laxman G. Kuratti, whose inspiration and encouragement made me to achieve this success.

Virupax C. Baligar
TABLE OF CONTENTS (Continued)

<table>
<thead>
<tr>
<th>Transformation of Added Ammonium-nitrogen During Subsequent Drying Cycles in Two Soils</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium transformation</td>
<td>35</td>
</tr>
<tr>
<td>Nitrification</td>
<td>35</td>
</tr>
<tr>
<td>Gaseous losses of nitrogen as NO and NO₂</td>
<td>41</td>
</tr>
<tr>
<td>Volatile losses of ammonia</td>
<td>42</td>
</tr>
<tr>
<td>Undetected nitrogen</td>
<td>43</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Transformation of Added Nitrite Nitrogen During Subsequent Drying Cycles in Two Soils</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonification</td>
<td>45</td>
</tr>
<tr>
<td>Nitrification</td>
<td>45</td>
</tr>
<tr>
<td>Gaseous losses of nitrogen as NO and NO₂</td>
<td>49</td>
</tr>
<tr>
<td>Undetected nitrogen</td>
<td>50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Transformation of Added Ammonium and Nitrite With Subsequent Drying Cycles in Two Soils</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium transformation</td>
<td>52</td>
</tr>
<tr>
<td>Nitrification</td>
<td>52</td>
</tr>
<tr>
<td>Gaseous losses of nitrogen as NO and NO₂</td>
<td>56</td>
</tr>
<tr>
<td>Volatile losses of ammonia</td>
<td>56</td>
</tr>
<tr>
<td>Undetected nitrogen</td>
<td>57</td>
</tr>
</tbody>
</table>

<p>| SUMMARY AND CONCLUSIONS | 58 |
| LITERATURE CITED | 60 |
| APPENDIX | 68 |
| VITA | 77 |</p>
<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Some of the physical and chemical characteristics of the soils used in this investigation</td>
<td>23</td>
</tr>
<tr>
<td>2. The effect of four wetting and drying cycles on the amount of indigenous inorganic nitrogen recovered or lost as gaseous forms in two soils maintained at 30 C</td>
<td>30</td>
</tr>
<tr>
<td>3. The effect of four wetting and drying cycles on the amount of inorganic nitrogen recovered or lost as gaseous forms in two soils maintained at 30 C when 300 ppm N is applied as ammonium sulfate</td>
<td>38</td>
</tr>
<tr>
<td>4. The effect of four wetting and drying cycles on the amount of inorganic nitrogen recovered or lost as gaseous forms in two soils maintained at 30 C when 300 ppm N is applied as sodium nitrite</td>
<td>46</td>
</tr>
<tr>
<td>5. The effect of four wetting and drying cycles on the amount of inorganic nitrogen recovered or lost as gaseous forms in two soils maintained at 30 C when supplied with 150 ppm N as ammonium sulfate and 150 ppm N as sodium nitrite</td>
<td>53</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1.</td>
<td>Apparatus and set-up for collection of NH$_3$-N, and oxides of nitrogen volatilized from the soil system.</td>
</tr>
<tr>
<td>2.</td>
<td>The effect of drying cycles on the recovery of indigenous inorganic nitrogen in Miami silt loam.</td>
</tr>
<tr>
<td>3.</td>
<td>The effect of drying cycles on the recovery of indigenous inorganic nitrogen in Yolo clay loam.</td>
</tr>
<tr>
<td>4.</td>
<td>The effect of drying cycles on the gaseous losses of nitrogen as oxides (NO and NO$_2$) from Miami silt loam.</td>
</tr>
<tr>
<td>5.</td>
<td>The effect of drying cycles on the gaseous losses of nitrogen oxides (NO and NO$_2$) from Yolo clay loam.</td>
</tr>
<tr>
<td>6.</td>
<td>The effect of drying cycles on the recovery of inorganic nitrogen in Miami silt loam applied with 300 ppm N as ammonium sulfate.</td>
</tr>
<tr>
<td>7.</td>
<td>The effect of drying cycles on the recovery of inorganic nitrogen in Yolo clay loam applied with 300 ppm N as ammonium sulfate.</td>
</tr>
<tr>
<td>8.</td>
<td>The effect of drying cycles on the volatile losses of ammonia from Yolo clay loam.</td>
</tr>
<tr>
<td>9.</td>
<td>The effect of drying cycles on the recovery of inorganic nitrogen in Miami silt loam applied with 300 ppm N as sodium nitrite.</td>
</tr>
<tr>
<td>10.</td>
<td>The effect of drying cycles on the recovery of inorganic nitrogen in Yolo clay loam applied with 300 ppm N as sodium nitrite.</td>
</tr>
<tr>
<td>11.</td>
<td>The effect of drying cycles on the recovery of inorganic nitrogen in Miami silt loam applied with 150 ppm N as ammonium sulfate and 150 ppm N as sodium nitrite.</td>
</tr>
<tr>
<td>12.</td>
<td>The effect of drying cycles on the recovery of inorganic nitrogen in Yolo clay loam applied with 150 ppm N as ammonium sulfate and 150 ppm N as sodium nitrite.</td>
</tr>
</tbody>
</table>
ABSTRACT

The Effect of Alternate Wetting and Drying on the Ammonium and Nitrite Nitrogen Transformation in Soils

by

Virupax C. Baligar, Master of Science
Utah State University, 1971

Major Professor: Dr. R. L. Smith
Department: Soils and Meteorology

Investigations were carried out to study the effect of alternate wetting and drying cycles on the mineralization, nitrification, and denitrification of soil nitrogen in two soils, in the presence or absence of added ammonium and nitrite. Soils were analyzed at the end of each drying cycle for total inorganic nitrogen, ammonium, and nitrite nitrogen. Gaseous products, i.e., ammonia, and the oxides of nitrogen (NO and NO₂) were collected in the course of drying for determination.

The drying cycles accelerated the rate of nitrogen mineralization in both soils and further increased the oxidation of applied and available ammonium. The rate of ammonium transformation was faster in Yolo clay loam soil than in Miami silt loam.

A faster rate of nitrification was also observed in Yolo clay loam. This may be because of a higher pH of the Yolo clay loam soil. The successive drying cycles had a pronounced effect on the nitrate formation in both soils.
The extent of nitrite decomposition was inversely related to soil pH. The amount of nitrogen oxides (NO and NO₂) evolved were inversely related to soil pH, but significant amounts of NO and NO₂ were evolved from even the slightly alkaline Yolo clay loam. The loss of nitrogen oxides increased with increased number of drying cycles and, further, the losses of nitrogen increased with increase in nitrite level. The majority of the nitrogen losses from these soils may be due to the direct decomposition of nitrite and possibly slight losses due to an interaction of nitrite and ammonium ions during the course of drying the soil.

A considerable amount of nitrogen remained as undetected in treatments receiving either ammonium or nitrite or both. These losses of nitrogen apparently are other than by the release of NO, NO₂, and NH₃. The undetected nitrogen loss from the soil system may be either in the form of N₂O or N₂.

No volatile losses of ammonia were recorded from the acid Miami silt loam soil. The greatest amount of applied ammonium nitrogen volatilized as ammonia in Yolo clay loam during the initial drying cycles. The rate of ammonia volatilization increased with increase in applied ammonium concentration.

(85 pages)
INTRODUCTION

Nitrifying and denitrifying organisms respond readily to alteration in the soil moisture. The optimum moisture level for greatest activity of the nitrifying organism is about one-half to two-thirds of the soil's moisture-holding capacity. Nitrate formation does not take place in air dry soil or at very low moisture level (Alexander, 1965), whereas ammonification proceeds under more stringent drouth than nitrification (Clark and Kemper, 1967). Excess water in soil restricts microbial activity, because it prevents the movement of oxygen into and through soil in sufficient quantity to meet the oxygen demands of soil organisms. Under such circumstances denitrification may occur in soil. In the absence of oxygen, but in the presence of nitrate, some facultative anaerobic bacteria in soil are capable of a nitrate respiration (Broadbent and Clark, 1965). Drying and rewetting a soil speeds up the biological activity. van Schreven (1967) observed that air dried and rewetted soils produced larger amounts of mineral nitrogen than non-treated soils.

Large fluctuation in soil moisture content have a pronounced effect on the stability of soil nitrogen. The nitrogen may be lost from soil in a gaseous form as nitric oxide (NO), nitrous oxide (N₂O), nitrogen dioxide (NO₂), nitrogen (N₂) or ammonia (NH₃). Such nitrogen losses occur quite widely in arable soils even when they are under conditions of good aeration (Allison, 1955). Several workers (Robinson, 1923; Madhok and Uddin, 1946; and Clark et al., 1960) have shown that drying of nitrite-treated soils promotes decomposition of nitrite. Johnson and Phipps (1967, 1969) recorded larger losses of added ammonia and/or
nitrite nitrogen under alternate wetting and drying cycles than under soils maintained moist. They are of the opinion that such losses of nitrogen may be either direct decomposition of nitrous acid or reaction of nitrous acid with amino group or ammonia. Drying of the soil in the field as well as in the laboratory may increase the concentration of nitrogen compounds like $\text{NH}_4^+$, $\text{NO}_3^-$, and $\text{NO}_2^-$ in soil solution and may enhance the losses of nitrogen. The losses of ammonia from soils are related to the water loss (Jewitt, 1942; Kresge and Satchell, 1960; and Volk, 1959). Upward movement of water helps the transport of ammonium ion to the soil surface, and then as the soil dries out, the ammonia at soil surface is subjected to the loss by volatilization.

In view of the above observations, the present investigation was undertaken with the following objectives:

1. To study the effect of alternate wetting and drying on the nitrification and denitrification in two soils at constant temperature.

2. To measure the effect of different initial concentrations of $\text{NH}_4^+$-N and $\text{NO}_2^-$-N on the amount of nitrogen losses in the gaseous forms as $\text{NH}_3$ and oxides of nitrogen ($\text{NO} + \text{NO}_2$).
REVIEW OF LITERATURE

The Nitrifying and Denitrifying Microbial Population

Biological oxidation of ammonia to nitrate in soil is carried out either by autotrophic or heterotrophic microorganisms. The autotrophic nitrification reaction involves a two-stage conversion (Alexander, 1965)

\[ \text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{NO}_3^- \]  \[1\]

Conversion of ammonium to nitrite is brought about by *Nitrosomonas*. The reaction can be represented by the following equation.

\[ \text{NH}_4^+ + \frac{3}{2} \text{O}_2 + \text{NO}_2^- + 2\text{H}^+ + \text{H}_2\text{O} \]  \[2\]

The conversion of nitrite to nitrate is affected largely by a second group of obligate autotrophic bacteria, *Nitrobacter*. The equation representing this reaction may be written as

\[ \text{NO}_2^- + \frac{1}{2} \text{O}_2 \rightarrow \text{NO}_3^- \]  \[3\]

Both the conversion of ammonium to nitrite and transformation of the latter to nitrate are exothermic reactions (Alexander, 1965).

In addition to the chemoautotrophic bacteria, *Aspergillus flavus* and certain other fungal strains, have been verified to be capable of nitrate formation (Eylar and Schmidt, 1959). Among the known chemoautotrophs only *Nitrosomonas* and *Nitrobacter* are encountered frequently and are major nitrifying organisms in soil. The two species recognized in *Nitrosomonas* are *N. europaea* and *N. monocella*.
Two species of Nitrobacter recognized are *N. Winogradskyi* and *N. agilis* (Breed et al., 1957).

Similar to nitrification, denitrification is a biological process and accomplished by facultative anaerobic bacteria capable of using nitrate or nitrite, in place of oxygen as a hydrogen acceptor. In the absence of oxygen but in the presence of NO₃⁻, NO₂⁻, or N₂O these bacteria are capable of carrying out respiration.

The capacity for true denitrification is limited to certain bacteria. Alexander (1961) listed that the active species are largely limited to the genera *Pseudomonas*, *Achromobacter*, *Bacillus*, and *Micrococcus*. *Pseudomonas* and *Achromobacter* are the dominant genera in soil.

**The Influence of Some Environmental Variables on Nitrification in Soil**

Nitrifying organisms respond readily to alteration in the soil moisture, pH, oxygen supply, temperature, supply of CO₂, and cultural practices. These factors may affect, alone or in combination, on the initial bacterial population or their subsequent proliferation.

**Soil properties**

Nitrification proceeds in an environment rich in organic matter, it also proceeds at high rate in organic matter poor environment. Alexander (1965) suggests that it may be due to a heterotrophic-autotrophic symbiosis or to a unique behavior of the bacteria in soil.

The rate of nitrification is closely and directly correlated with the pH, and the optimum pH for most of the ammonium oxidizing organism lies above neutrality, while that for the nitrite-oxidizers is close
to the neutral point (Morrill and Dawson, 1962). In reviewing the
effect of soil reaction on nitrifiers, Alexander (1965) stated that the
optimum pH tends to fall in the range between 7 and 9 and the activity
is favored in the more alkaline solution. Kelley (1916), Martin et al.
(1942), and Chapman and Liebig (1952) have reported that highly
alkaline conditions are not conducive to nitrite oxidizers. Morrill
and Dawson (1962) observed that during perfusion of various soils
with NH\textsuperscript{+} or NO\textsuperscript{2}\textsuperscript{-} the most favorable pH for growth of \textit{Nitrobacter} was
6.2 to 7.0 while that for \textit{Nitrosomonas} was above 7.6. The generation
time for \textit{Nitrobacter} was always below one-half that for \textit{Nitrosomonas}
at pH values below 7.2. The optimum pH for most investigated strains
of \textit{Nitrosomonas} tends to fall in the range between pH 7 and 9. Even
in slightly acid conditions, the proliferation of these bacteria is
quite markedly reduced. For \textit{Nitrobacter} strains, the optimum pH is
in the neutral to slightly alkaline range, and the activity is often
detectable from pH 5 to 10 (Alexander, 1965). Aleem and Alexander
(1960) also observed a similar inhibitory effect of pH on \textit{Nitrobacter}
\textit{agile} and they noticed only a slight inhibition in the activity of
\textit{Nitrosomonas}. An acid soil condition does not favor the accumulation
of nitrite. Lewis and Pramer (1959) reported that the growth of
\textit{Nitrosomonas europaea} increased with increasing pH in absence of NO\textsuperscript{2}\textsuperscript{-}. Toxicity of nitrite was greater at acid ranges (pH 6.0 and 6.5). They
are of the opinion that the pH influence either permeability or ioniza-
tion within the cell.

Increasing temperatures generally stimulate microbial activity
thereby favoring the oxidation of ammonium and the mineralization of
soil nitrogen. The optimum temperature for nitrifiers falls between 30 and 35 C which is somewhat lower than that of the mineralizers.

Seifert (1964) reported that the intensity of nitrification depends upon the specific surface of soil aggregates and associated with poor aeration in the internal space of large aggregates, due to retarded oxygen diffusion in aggregates of high moisture content. Seifert (1964b) further observed that the degree of nitrification and NO$_3^-$ level are inversely proportional to aggregate size.

**Soil moisture content**

As early as 1920, Greaves and Carter (1920), while studying a group of 22 soils of Cache Valley, Utah, observed that nitrification was at its maximum at 50 to 60 percent of its water holding capacity. Russel et al. (1925) found no nitrification at the hygroscopic coefficient. However, they noticed the nitrate production increased with an increase in moisture up to one and one-fourth times the moisture equivalent. Robinson (1957) observed no nitrification when the soil moisture level was just below the permanent wilting percentage, whereas ammonification of the natural soil nitrogen continued at this moisture level. Abichandni and Patnik (1958) studied the nitrification in some of the Indian soils and found active nitrification and a gradual decrease of ammonia in a soil containing 20 percent moisture during a six day incubation period at 35 C. When the moisture content of the soil was increased to 40 percent, only ammonification proceeded. However, at this moisture level the amount of nitrate that was present initially decreased until only traces remained.
Parker and Larson (1962) found a retardation of nitrification in soils with moisture content less than 50 cm (0.05 bar). A peak in nitrification rate was reported by Miller and Johnson (1964) in the 0.5 and 0.15 bar range. Further they observed nitrification at >15 bars but the rate was very slow. Ammonification took place at a fast rate both at high tension (up to dryness) and low tensions (near zero). Justice and Smith (1962) reported a delay in nitrification at 15 bars suction with consecutively faster rates of nitrification at suctions down to one bar. Reichman et al. (1966) reported that ammonification and nitrification of soil nitrogen were almost directly proportional to soil water content at suctions between 0.2 and 15 bars. Measurable ammonification and nitrification occurred at suction of 15 bars. Recently, Dubey (1968) reported that in sandy loam soil the nitrification increased as moisture tension decreased from 15 to 2 bars and decreased thereafter. Alexander (1965) stated that nitrate was not produced in air-dry soil nor was it produced at very low moisture levels, although the mineralization of nitrogen proceeded when water was present at very low amounts, the net effect being a slow rise in the ammonium concentration.

Organisms differ in the extent to which they are active in droughty soil. Not all bacterial metabolic activities are uniformly curtailed during drying out of the soil. Clark (1967) is of the opinion that ammonification can proceed under more stringent drought than can nitrification. The latter does not occur in soil at moisture content below permanent wilting point.

The nitrifying autotrophs respond readily to the alteration in soil moisture status. The optimum moisture level does not seem to be
the same for the nitrifying population in different sites (Alexander, 1965). Calder (1957), working with some African soils, observed a high nitrate appearance during drying of soil as compared to the soil maintained at steady state of moisture. Several workers (Gooding and McCalla, 1945; Birch, 1958; Birch, 1960, and van Schreven, 1967) reported that dried and rewetted soil repeatedly showed an increased rate of mineralization of soil nitrogen. Alexander (1965) states that nitrate was produced rapidly in a wet period following a prolonged drought or in a rainy season following a long dry spell. Ekpete and Cornfield (1966) studied the varying static and changing moisture contents during incubation on ammonia and nitrate levels in soil. They observed that by changing static moisture content up to 40 to 50 percent of water-holding capacity, increased NO$_3^-$ and mineral nitrogen content. With further increase in moisture, up to the water-holding capacity, both mineral and NO$_3^-$-N decreased and NH$_4^+$ accumulated. van Schreven (1967) reported that in a calcareous soil incubated at 35 and 105 C, intermittent drying and wetting stimulate the mineralization of humus N.

**Aeration**

The nitrifying autotrophs are obligate aerobes, and all the known heterotrophs are aerobic and nitrate formation depends upon adequate aeration. Amer and Bartholomew (1951) reported that the optimum partial pressure of oxygen for rapid nitrate production in soil was similar to that found in the air. High rates of aeration are not required for enrichment of active organisms. Build-up of soil carbon dioxide to a value above the normal atmospheric level is needed in
order to secure an optimum rate of nitrification (Beard and Clark, 1962). Incubation techniques that favored a rapid escape of respiratory carbon dioxide from soil depressed the rate of nitrification. Nitrification rates did not differ significantly where the atmosphere contained concentrations of oxygen in the range of 10 to 20 percent by volume. Carbon dioxide content necessary for rapid nitrification fell within the range of 0.5 to 5.0 percent by volume (Clark, 1967).

**Effect of the presence of \( \text{NH}_4^+ \) and \( \text{NO}_2^- \)**

Aleem et al. (1957) observed inhibitory effect of ammonia on the activity of *Nitrobacter agile*. Nitrite accumulated when \((\text{NH}_4)_2\text{SO}_4\) was oxidized in neutral to alkaline soils and this accumulation was increased as the concentration of ammonium sulfate perfused through the soil increased. Ammonium toxicity increased on *Nitrobacter agile* at high pH values but little effect on *Nitrosomonas europaea* was noted. The depressing influence of ammonium ion on nitrification has been reported by several workers (Stojanovic and Alexander, 1958; Justice and Smith, 1962; and Anderson and Boswell, 1964). Recently Harada and Kai (1968) observed that a high level of ammonium enhanced the sensitivity of nitrifying organism in soil and nitrate formation was inhibited more strongly than nitrite formation. Stojanovic and Alexander (1958) and Aleem and Alexander (1960) reported that the build-up and persistence of nitrite was favored by high levels of applied ammonium possibly through an inhibition of *Nitrobacter*. Harada and Kai (1968) observed similar findings and noted that nitrite remained while there was some ammonium in the medium. Nitrite disappeared after the ammonium had
vanished. Further they observed that oxidation of ammonium was delayed with the increase of ammonium in excess of 200 ppm N in the culture solution. The rate of oxidation of NO$_2^-$ to NO$_3^-$ was affected by the level of NO$_2^-$ concentration and population of NO$_2^-$ oxidizers. About 6 to 200 ppm NO$_2^-$-N were transformed rapidly, whereas at lower and higher NO$_2^-$ levels there was a slight lag phase hence longer time was required to complete the transformation. Particularly above 800 ppm the process was noticeably inhibited.

**Gaseous Losses of Nitrogen**

Gaseous losses of nitrogen from soil may take place either during nitrification, during the biological or chemical processes of denitrification or by direct ammonia volatilization.

The denitrification processes have been shown to depend upon the microflora, the level of oxygen in the media, the moisture content, the temperature, and the available energy sources for the microbes. Soil type, soil texture, and soil structure influence many of the controlling factors.

**Soil properties**

The influence of soil organic matter on nitrogen losses through denitrifying is two-fold. Organic matter, during decomposition, supplies the hydrogen donor for the denitrifying process and markedly influences the oxygen demand in the soil (Broadbent and Clark, 1965). Smith and Clark (1960) obtained indications that the rate of nitrite decomposition increases with increasing soil organic matter content. Clark and Beard (1960) showed that nitrite became more unstable in the
presence of soil than its absence at a given pH level. Such work suggests that organic reducing compounds, probably of microbial origin, accelerate nitrite decomposition (Clark, 1962). Stevenson and Swaby (1963) suggested that the losses of nitrogen via this pathway may be of significant importance in soils.

The denitrification rate is slow in acid soil and rapid in soils of high pH. The bacteria which bring about denitrification are sensitive to high hydrogen ion concentrations that is why acid soils contain a sparse denitrifying population (Alexander, 1961). Large populations of these organisms are observed above pH 5.5. Alexander (1961) is of the opinion that denitrification by microbial means is highly sensitive to acidity and losses of nitrogen in acid soils cannot be attributed directly to biological agents. Several workers (Bremner and Shaw, 1958 and Jansson and Clark, 1952) observed that an alkaline soil reaction favored denitrification. Whereas, in an acid environment, nitrite toxicity appeared to inhibit denitrification. Delwiche (1956) and Nommik (1956) further shows that the optimum pH for denitrification is between 7 and 8. He observed great reductions at pH levels below 5. Soulides and Clark (1958) and Clark et al. (1960) reported that much greater losses in near neutral soils than in soils more acidic than pH 5.5. Tyler and Broadbent (1960) also concluded that pH is not the sole factor affecting the losses of nitrogen from nitrite in soil.

Wullstein and Gilmour (1964) postulated that metallic cations play an important role in nitrite decomposition in soil. Wullstein and Gilmour (1966) further reported that both N₂ and NO were formed by the reaction of ferrous ions with nitrite under acidic conditions. A recent report of Nelson and Bremner (1970) does not completely
agree with these findings. They reported that of various metallic cations tested only ferrous cuprous and stannous ions promoted nitrite decomposition.

Soil moisture and oxygen content

High soil moisture inhibits the diffusion of oxygen (Broadbent and Clark, 1965). The decreased partial pressure of $O_2$ enhances the denitrification of added nitrate (Alexander, 1961). There is a considerable number of reports in the literature describing denitrification observed under conditions of apparently adequate oxygen supply. Early work of Meiklejohn (1940), with two species of Pseudomonas, showed denitrification in aerated cultures. In recent years several workers (Broadbent, 1951; Broadbent and Stojanovic, 1952; and Allison et al., 1960) have observed denitrification in aerated soil under a controlled atmosphere containing some oxygen.

Bremner and Shaw (1958) reported a considerable amount of nitrogen loss when soil moisture content was raised up to 450 percent of the moisture holding capacity. These results are in agreement with the earlier findings of Jansson and Clark (1952) and Nommik (1956). Many workers (Broadbent, 1951; Jansson and Clark, 1952; Allison et al., 1960; and Greenland, 1962) accept the concept that the denitrification in soil is primarily due to the moisture, reducing the availability of oxygen. Bremner and Shaw (1958) and Greenwood (1962) reported that denitrification rate increased with increase in moisture level. Cady and Bartholomew (1960) observed denitrification in sandy loam when the moisture level was increased slightly from 10 percent (field capacity) to 15 percent. Mahendrappa and Smith (1967), while studying
the effect of moisture on denitrification in acid and alkaline soils, observed that under a helium atmosphere an increase in moisture content of only 10 percent above field capacity markedly decreased the time required for complete denitrification as defined by N₂ production but had little effect on the disappearance of the nitrate.

Russell and Richards (1917) studied the alternate aerobic and anaerobic conditions on losses of nitrogen from farm yard manure. They observed that more nitrogen was lost under alternate aerobic and anaerobic conditions. McGarity et al. (1958) reported that N₂ gas was produced under anaerobic conditions while small quantities of both N₂ and N₂O were produced even under aerobic conditions. Greenland (1962) reported that the losses of nitrogen in soil were due to nitrification and denitrification proceeding simultaneously. Very recently, Johnson and Phipps (1969) have observed, a substantial nonmicrobial inorganic nitrogen transformation under alternate wetting and drying cycles at relatively high temperature (50 C). They are of the opinion that drying of the soils may increase the concentration of nitrogen compounds, NH₄⁺, NO₃⁻, and NO₂⁻, in the soil solution and may enhance the losses of nitrogen. These nitrogen losses may be due to direct decomposition of nitrous acid, or reaction of nitrous acid with amino groups, ammonia or other soil constituents.

Broadbent and Clark (1965) state that the supply of oxygen to soil organisms, rather than the partial pressure of oxygen, is important in determining whether or not denitrification occurs in soil.

Several workers (Broadbent, 1951; Broadbent and Stojanovic, 1952; and Allison et al., 1960) have observed considerable amounts of denitrification in aerated soils. Aerobic denitrification may occur more
readily in fine textured soils than in sandy ones. Small pores, which are filled with water, are particularly prone to the development of anaerobic conditions, even though many of the large pores are filled with air (Broadbent and Clark, 1965). Nommik (1956) reported that denitrification increased with increasing size of aggregates. Further, he observed that the larger the aggregate size beyond a certain limit there was an increase in the rate of denitrification which he attributed to the longer time required for oxygen to diffuse to the center of the larger aggregates. McLaren and Skujins (1967) are also of the opinion that in water saturated soil, crumbs larger than about 3 mm in radius have no oxygen at their centers. This means that micro sites, having anaerobic conditions, are ubiquitous and provides an explanation for the universality of strict anaerobes. Broadbent and Clark (1965) stated that small nitrogen losses occur within a small pocket of microbial activity or within the larger sized soil aggregates. This may account for 10 to 15 percent of the total yearly mineral nitrogen input.

**Biological denitrification**

Facultative anaerobic bacteria present in soil are capable of using nitrate in place of oxygen as a hydrogen acceptor (Broadbent and Clark, 1965). The process may be expressed as follows:

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 4\text{NO}_3^- \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} + 2\text{N}_2
\]  

(4)

Several workers (Jones, 1951; Wijler and Delwiche, 1954; and Delwiche, 1956) have observed a considerable quantity of nitrogen gas from nitrate nitrogen was evolved under anaerobic conditions.
Meiklejohn (1940) observed the reduction of $\text{NO}_3^-$ to $\text{N}_2$ by *Pseudomonas* species. Delwiche (1959) reported that *Pseudomonas denitrificans* utilized $\text{NO}_3^-$ ion as the $\text{H}^+$ acceptor in absence of oxygen. Valera and Alexander (1961), on the other hand, showed that denitrifying organisms used $\text{NO}_3^-$ as the terminal electron acceptor for growth. Denitrification even occurred under adequate oxygen supply.

Two species of *Pseudomonas*, as observed by Meiklejohn (1940), are capable of denitrifying in aerated cultures. Similar findings have been reported by Marshall et al. (1953) and Kefauver and Allison (1957).

Campbell and Lees (1967) are of the opinion that, if an organism is capable of using both nitrate and oxygen as an electron acceptor, it will normally use oxygen in preference to nitrate. Nitrate is reduced only when the oxygen tension is low. Further in their review they state that ammonia production by denitrifying bacteria under mildly anaerobic conditions, whereas under absolute anaerobic conditions, they produced nitrous oxide and nitrogen.

**Nitrous acid or nitrites**

Nitrite has been found to accumulate in soils for varying lengths of times as a transitory intermediate of the process of nitrification and denitrification. This $\text{NO}_2^-$ may be subjected to losses from soil. It is considered necessary to know under what condition $\text{NO}_2^-$ accumulation takes place in soil.

Temple (1914) and Robinson (1923) were among early workers who considered the nitrous acid decomposition in acid soil and losses of N when $\text{NO}_2^-$ was added. Temple (1914) proposed the following scheme of $\text{NO}_2^-$ transformations.
$4\text{HNO}_2 \rightarrow 2\text{NO} + 2\text{NO}_2 + 2\text{H}_2\text{O}$ \hspace{1cm} [5]

$2\text{NO}_2 \rightarrow \text{N}_2\text{O}_4$ \hspace{1cm} [6]

$\text{N}_2\text{O}_4 + \text{H}_2\text{O} \rightarrow \text{HNO}_3 + \text{HNO}_2$ \hspace{1cm} [7]


$3\text{HNO}_2 \rightarrow 2\text{NO} + \text{HNO}_3 + \text{H}_2\text{O}$ \hspace{1cm} [8]

Broadbent and Clark (1965) suggested the following reaction of nitric oxide.

$2\text{NO} + \text{O}_2 \rightarrow 2\text{NO}_2$ \hspace{1cm} [9]

This is a chemical oxidation of nitric oxide to nitrogen dioxide and this may react with water to form nitric acid.

$3\text{NO}_2 + \text{H}_2\text{O} \rightarrow 2\text{HNO}_3 + \text{NO}$ \hspace{1cm} [10]

$2\text{NO}_2 + \text{H}_2\text{O} \rightarrow \text{HNO}_3 + \text{HNO}_2$ \hspace{1cm} [11]

Allison and Doetsch (1951) and Gerretsen and de Hoop (1957) reported that acidity of at least pH 5 is necessary for appreciable decomposition of nitrous acid. The rate and extent of the decomposition were greatly accelerated by further increase in acidity.

Broadbent and Clark (1965) are of the opinion that the losses by this pathway are impossible in neutral or alkaline soil where little nitric oxide formation takes place. Further they stated that under aerated acid soil conditions where chemical oxidation of NO proceeds rapidly, it is unlikely that any considerable amount of NO would escape to the atmosphere before oxidation and hydration to HNO₃ could occur. Smith
and Clark (1960) and Reuss and Smith (1965) failed to detect the production of nitric oxide (NO) as a product of nitrite decomposition in soils under aerobic conditions, but it has been detected under anaerobic conditions (Wijler and Delwiche, 1954; Nommik, 1956; and Cady and Bartholomew, 1960) and its formation under these situations are attributed to the chemical decomposition of nitrite.

Many workers (Temple, 1914; Robinson, 1923; Madhok and Uddin, 1946; and Gerretsen and de Hoop, 1957) reported that the treatment of acidic soil with nitrite leads to the formation of NO₂ gas. Smith and Clark (1960) added nitrite to acid soils; it resulted in the production of considerable amounts of N₂ and no build-up of NO or NO₂ gases. Reuss and Smith (1965) confirmed the early work that N₂ was formed when NO₂⁻ was added to an acid soil. The formation of N₂ gas was due to a chemical rather than a microbiological action and was the result either of the reaction of HNO₂ with soil constituents or of a reaction catalyzed by soil constituents. Further they observed the evolution of gaseous oxides of nitrogen (NO + NO₂).

The amount of N₂ formed was found to increase with an increase in soil organic matter content (Smith and Clark, 1960; Reuss and Smith, 1965) and with a decrease in soil pH (Reuss and Smith, 1965). These latter workers also indicated that the amount of NO₂ gas formed similarly increased with a decrease in soil pH. Nelson and Bremner (1970) reported that the chemical decomposition of nitrite in soil showed a substantial amount of N₂ and NO₂ and small amounts of N₂O production on treatment of neutral and acidic soils with nitrite. The largest amount of N₂ was formed in an acidic soil having a high organic matter content. The amount of NO₂ produced was inversely
related to soil pH, but they observed significant amounts of NO₂ evolved from soils having pH values above 7. They represented the decomposition of nitrous acid as follows:

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

[12]

They obtained evidence that soil organic constituents are essential for the reduction of nitrite to N₂ and NO₂.

The conversion of NO₂⁻ to NO₃⁻ by chemical reaction is considerably stronger than its tendency to react with ammonia or ammonium compounds (Allison, 1963; Smith and Clark, 1960). The reaction, as given by Broadbent and Clark (1965), is shown as follows:

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

[13]

Part of nitric oxide may oxidize chemically to nitrogen dioxide and this may react with water to form nitric acid.

\[ 2\text{NO} + \text{O}_2 \rightarrow 2\text{NO}_2 \]  

[14]

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

[15]

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

[16]

The NO of Equation [15] or the HNO₂ of Equation [16] would react as in Equation [13] or Equation [14]. The sum of all this would be that no gaseous nitrogen losses would occur.
Nitrous acid reaction
with ammonia

Many workers (Gerretsen and de Hoop, 1957; Clark, 1962; Reed and Sabbe, 1963) have proposed that nitrous acid may react with ammonia and yield molecular nitrogen. The reaction may be written as follows:

\[ \text{NH}_3 + \text{HNO}_2 \rightarrow \text{N}_2 + 2\text{H}_2\text{O} \]  \hspace{1cm} [17]

Allison (1963) reported that ammonium nitrite is formed when nitrous acid reacts with ammonia and that this subsequently undergoes decomposition.

\[ \text{NH}_4^+ + \text{HNO}_2 \rightarrow \text{NH}_4\text{NO}_2 \rightarrow \text{N}_2 + 2\text{H}_2\text{O} \]  \hspace{1cm} [18]

Smith and Clark (1960) have shown the evidence that this is not likely to occur under soil conditions. Broadbent and Clark (1965) do not agree with Allison’s (1963) view. Smith and Clark (1960), in their studies, observed that in the presence of oxygen, the tendency for nitrite to convert chemically to nitrate was three to four times as strong as that for its reaction with ammonium ion. The conversion to nitrate was even more rapid in aerobic soil.

Johnson and Phipps (1967, 1968, and 1969), while studying alternate wetting and drying cycles in non-soil, clay, and a soil system, observed nitrogen losses. They have represented the mechanism of NO\textsubscript{2}^-N losses as follows:

\[ 4\text{HNO}_2 \xrightarrow{\Delta} \text{2N}_2 + 2\text{H}_2\text{O} + 3\text{O}_2 \text{ (large losses)} \]  \hspace{1cm} [19]

or

\[ 2\text{HNO}_2 \xrightarrow{\Delta} \text{N}_2\text{O} + \text{H}_2\text{O} + \text{O}_2 \text{ (large losses)} \]  \hspace{1cm} [20]

\[ 5\text{HNO}_2 \xrightarrow{\Delta} 3\text{HNO}_3 + \text{N}_2 + \text{H}_2\text{O} \text{ (moderate losses)} \]  \hspace{1cm} [21]
\[ \text{NH}_3 + \text{HNO}_2 \rightarrow \text{N}_2 + 2\text{H}_2\text{O} \text{ (slight losses)} \]  

Nitrite nitrogen loss was directly dependent on the quantity of \( \text{NH}_4^+ - \text{N} \) present. Complete loss of \( \text{NO}_2^- - \text{N} \) was noted after three drying cycles with ammonium: nitrite ratios of 1:1, 2:1, and 3:1. Nitrite loss was directly dependent on the number of drying cycles. The majority, however, was lost during the initial drying phase where approximately 90 percent of \( \text{NO}_2^- - \text{N} \) loss occurred.

**Volatile losses of ammonia**

Volatile loss of ammonia is increased by an alkaline reaction, high temperature, and the drying of soil, especially if the ammonia is at the soil surface. Martin and Chapman (1951) observed very little loss below pH 7.2. Blasco and Cornfield (1966) reported ammonia losses from six acid soils (pH 4.8 to 6.3). Ammonium movement involves diffusion in both the liquid and the gaseous phase. The relative importance of the two phases depends upon the water content of the soil (Gardner, 1965). Ernst and Massey (1960) and Baligar and Patil (1968) observed a close relationship between ammonia volatilization and initial soil moisture content of soil. The loss of ammonia from soils was found to be related to the water loss (Jewitt, 1942 and Volk, 1959). There are at least two reasons for this. The conditions which favor evaporation of water will favor volatilization of ammonia, and the upward movement of water helps to transport ammonia to the soil surface (Gardner, 1965). Drying the soil has been reported to increase ammonia volatilization (Jewitt, 1942; Martin and Chapman, 1951; Volk, 1959; and Ernst and Massey, 1960).
Johnson and Phipps (1967, 1968, and 1969) observed a large loss of ammonia with high drying temperature (50 C) coupled with cycles of alternate wetting and drying of soils. They write the transformation as follows:

\[
\text{Direct decomposition of } \text{NH}_4^+ - \text{N (larger losses)} \quad [23]
\]

\[
\text{NH}_3 + \text{HNO}_2 \rightarrow \text{N}_2 + 2\text{H}_2\text{O (small losses)} \quad [24]
\]

They further observed greatest amounts and rate of loss with the initial drying cycles; however, continued losses were observed with subsequent drying.
MATERIALS AND METHODS

Experimental

The investigation was conducted by adding ammonium sulfate and sodium nitrite alone or in combination to two different soils under varied moisture conditions and following the nitrogen interchanges in the soil or in the aerating air stream. The oxides of nitrogen and volatile ammonia were removed from the aerating air by bubbling this through suitable trapping solutions. At the end of a particular drying cycle or cycles, the soils were analysed for total inorganic N, NH$_4^+$-N, and NO$_2^-$-N. Preliminary experiments showed that nitrification and denitrification occurred in the same soil system under alternate wetting and drying conditions.

Soils used

Two soils that differ in their physical and chemical characteristics were selected for this study: Miami silt loam and Yolo clay loam. Some of the properties of these soils are given in Table 1.

Soil preparation

Soil samples were collected from 15 cm of the surface horizon, air dried, ground, passed through a 200 mesh sieve, and stored in air-tight containers. In all the treatments air dry soil to give 50 g oven dry weight was used. All soil samples were transferred to 125 ml Erlenmeyer flasks and adjusted to 0.3 bar moisture tension. The desired nitrogen
Table 1. Some of the physical and chemical characteristics of the soils used in this investigation

<table>
<thead>
<tr>
<th>Soil</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>pH</th>
<th>O.M.</th>
<th>Exchangeable</th>
<th>CEC</th>
<th>Moisture Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>meq/100g</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Miami silt loam</td>
<td>22</td>
<td>61</td>
<td>17</td>
<td>5.2</td>
<td>2.7</td>
<td>0.7  4.4</td>
<td>13.3</td>
<td>22.7  7.15</td>
</tr>
<tr>
<td>Yolo clay loam</td>
<td>27</td>
<td>45</td>
<td>28</td>
<td>7.9</td>
<td>2.1</td>
<td>1.0  8.7</td>
<td>33.9</td>
<td>24.0  10.9</td>
</tr>
</tbody>
</table>

\( ^{a}\)The Miami silt loam (Ohio) was supplied by Dr. R. B. Clark, Ohio State Experiment Station, Wooster. The Yolo clay loam (California) was supplied by Dr. F. E. Broadbent, University of California, Davis.
treatments were imposed on these samples with the moisture necessary to bring to 0.3 bar tension. The flasks were connected to an aeration system where the exiting air could be passed through two trapping solutions.

Incubation

The experiment was carried in an incubator which has thermostatically controlled temperature compartments maintained so the temperature differentials were not greater than ±1 C from the desired temperature level for all the treatments. Temperature of this incubation was 30°C and this temperature was maintained throughout the experimental period.

Treatment

The following initial concentrations of ammonium (NH$_4^+$) and nitrite (NO$_2^-$) nitrogen were used for both the soils under study.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NH$_4^+$-N (ppm)</th>
<th>NO$_2^-$-N (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>300</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Ammonium nitrogen and nitrite nitrogen were added in the form of ammonium sulfate and sodium nitrite, respectively.

Wetting and drying cycles

There were four wetting and drying cycles in this study. In each of these cycles, soil moisture was allowed to drop from 0.3 bar to 15
bars. At the end of each drying cycle the soil samples were again re-adjusted to 0.3 bar moisture tension by adding water to the system to bring the total weight of the flask plus soil back to an original weight. The drying was accelerated by removing all moisture from the incoming air by passing it through a sulfuric acid solution. It required about six to seven days for the soils to go from 0.3 to 15 bars tension.

**Apparatus Used**

A special closed system was constructed (Figure 1) to drop the soil moisture tension from 0.3 bar to 15 bars. A glass tube was sealed at the bottom of the Erlenmeyer flask to make the way for the air entering into the system. To facilitate proper distribution of the air and to prevent the loss of soil through the bottom tube, a glass wool padding sandwiched between two discs of nylon cloth was placed at the bottom of the flask. The soil samples were then placed on this padding.

The air entering the system was first passed through a 20 percent H₂SO₄ to remove the ammonia and most of the water vapor and then passed through anhydrous CaCl₂ to free it from moisture. The air output in each system was maintained at the rate of 35 to 40 ml per minute with a proper adjustment of the valves placed at the beginning of the system. The moisture-free air first passed through the incubated soil samples and was then bubbled through a 2 percent boric acid solution (50 ml) and then through the alkaline permanganate tubes. Two tubes were constructed, each with 20 ml of potassium permanganate, to trap the oxides of N volatilized from the soil sample. Before aerating, the whole system was made airtight.
Figure 1. Apparatus and set-up for collection of NH$_3$-N, and oxides of nitrogen volatilized from the soil system.
Analysis of Different Forms of Nitrogen

At the end of each drying cycle or cycles, duplicate samples of both the soils of all the treatments were harvested for the chemical analysis. The complete soil samples were extracted with 2N KCl to remove the non-gaseous nitrogen (NH$_4^+$-N, NO$_2^-$-N, and NO$_3^-$-N). Gaseous products, ammonia and the oxides of nitrogen, volatilized from the soil sample during the drying cycle or cycles were collected in 2 percent boric acid and in alkaline K-permanganate traps, respectively.

Analysis of volatile ammonia

Two percent boric acid, 50 ml, was used to trap the ammonia volatilized from the soil system. Ammonia was determined by back titration of the boric acid with standard H$_2$SO$_4$. A mixed indicator solution, as suggested by Bremner (1965), was used in the titration of boric acid with the standard H$_2$SO$_4$.

Analysis of oxides of nitrogen (NO + NO$_2$)

The alkaline K-permanganate solution was used to collect the nitric oxide and nitrogen dioxide gases swept out of the system during the course of reaction. The permanganate solutions (20 ml) held in both the tubes were analyzed at the end of each drying cycle or cycles. The solution was washed into beakers and the excess potassium permanganate solution was destroyed by adding about 20 ml oxalic acid (0.6M Na$_2$C$_2$O$_4$ in 7N H$_2$SO$_4$). The solution was made alkaline by adding 5N sodium hydroxide and then digested for 30 minutes on a hot plate. The solutions were filtered and aliquotes were used to
determine the nitrate by the phenol disulfonic acid method. A standard phenol disulfonic acid method, similar to that of Jackson (1958) was used to determine nitrate. The procedure was calibrated by adding known amounts of potassium nitrate to the alkaline permanganate trapping solution. The detailed analytical methods are included in the Appendix.

**Analysis of non-gaseous nitrogen products**

At the end of each drying cycle or cycles duplicate flasks of each treatment were extracted with 250 ml of 2N KCl solution. Samples were filtered and aliquots were analyzed for total inorganic nitrogen, $\text{NH}_4^+$-N and $\text{NO}_2^-$-N. Total inorganic nitrogen and ammonium nitrogen were determined by the micro-Kjeldahl method as modified by Bremner (1965). Nitrite nitrogen was determined by using the method as suggested by Shinn (1941).
RESULTS AND DISCUSSION

Gaseous loss of nitrogen from soil takes place either in the form of molecular nitrogen (N₂), nitrous oxide (N₂O), nitric oxide (NO), nitrogen dioxide (NO₂) or by volatile losses of ammonia (NH₃). Fluctuations in soil moisture content have a pronounced effect on the rate of nitrogen loss. Alternate wetting and drying cycles influence the transformations of the native soil as well as applied nitrogen. Drying may increase the concentration of ammonium and nitrite thereby enhancing the loss of nitrogen. Investigations were carried out to study the effect of alternate wetting and drying cycles on the mineralization, nitrification and denitrification of soil nitrogen, in two soils, in presence or absence of added ammonium and nitrite. In these investigations ammonium and nitrite were added either singly or in combination. Soils were analysed at the end of each drying cycle for total nitrogen, ammonium, and nitrite nitrogen. Gaseous products, i.e., ammonia and oxides of nitrogen (NO + NO₂) were also collected in the course of drying for determination.

Transformations of Indigenous Soil Nitrogen

The effect of four drying cycles on the mineralization of indigenous soil nitrogen in Miami silt loam and Yolo clay loam were studied in absence of applied nitrogen. The results are given in Table 2, and the data are plotted in Figures 2 and 3. The effect of drying cycles on the specific transformations of the soil nitrogen are discussed in the following sections.
Table 2. The effect of four wetting and drying cycles on the amount of indigenous inorganic nitrogen recovered or lost as gaseous forms in two soils maintained at 30°C

<table>
<thead>
<tr>
<th>Soil</th>
<th>Number of drying cycles</th>
<th>(\text{NH}_4^+\text{-N})</th>
<th>(\text{NO}_2^-\text{-N})</th>
<th>(\text{NO}_3^-\text{-N}^b)</th>
<th>Total inorganic (\text{N})</th>
<th>Oxides of nitrogen(c)</th>
<th>(\text{NH}_3\text{-N}^d)</th>
<th>Grand total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miami Silt</td>
<td>0</td>
<td>12.2</td>
<td>0.5</td>
<td>1.2</td>
<td>13.9</td>
<td>---</td>
<td>---</td>
<td>13.9</td>
</tr>
<tr>
<td>Loam</td>
<td>1</td>
<td>32.7</td>
<td>0.8</td>
<td>2.7</td>
<td>36.2</td>
<td>---</td>
<td>---</td>
<td>36.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>38.1</td>
<td>1.3</td>
<td>3.3</td>
<td>42.7</td>
<td>1.0</td>
<td>---</td>
<td>43.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>42.3</td>
<td>1.1</td>
<td>3.9</td>
<td>47.3</td>
<td>2.7</td>
<td>---</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>50.5</td>
<td>0.5</td>
<td>5.1</td>
<td>56.1</td>
<td>2.7</td>
<td>---</td>
<td>58.8</td>
</tr>
<tr>
<td>Yolo Clay</td>
<td>0</td>
<td>12.3</td>
<td>0.1</td>
<td>2.2</td>
<td>14.6</td>
<td>---</td>
<td>---</td>
<td>14.6</td>
</tr>
<tr>
<td>Loam</td>
<td>1</td>
<td>27.7</td>
<td>1.4</td>
<td>6.3</td>
<td>35.4</td>
<td>---</td>
<td>---</td>
<td>35.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>36.9</td>
<td>2.0</td>
<td>6.9</td>
<td>45.8</td>
<td>1.0</td>
<td>---</td>
<td>46.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>47.7</td>
<td>1.3</td>
<td>10.9</td>
<td>60.0</td>
<td>1.0</td>
<td>---</td>
<td>60.9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>51.6</td>
<td>1.9</td>
<td>15.0</td>
<td>68.5</td>
<td>1.0</td>
<td>---</td>
<td>69.5</td>
</tr>
</tbody>
</table>

\(^a\) In each of the drying cycles, soil moisture was allowed to drop from one-third bar to 15 bars tension.

\(^b\) \(\text{NO}_3^-\text{-N}\) is obtained by subtracting \(\text{NH}_4^+\text{N}\) and \(\text{NO}_2^-\text{-N}\) from total inorganic soil N.

\(^c\) Determined by analysis of \(\text{KMnO}_4\) solution (\(\text{NO} + \text{NO}_2\)).

\(^d\) Volatilized ammonia.
Figure 2. The effect of drying cycles on the recovery of indigenous inorganic nitrogen in Miami silt loam.
Figure 3. The effect of drying cycles on the recovery of indigenous inorganic nitrogen in Yolo clay loam.
Ammonification

It is seen from the data that in both soils there was a close relation between the number of drying cycles and the ammonium formed. In the present study in both soils each drying cycle increased the amount of ammonium formed. This finding is in an agreement with the earlier findings of Gooding and McCalla (1945) who found that drying and rewetting of a soil speeded up the biological activity, indicating a more rapid decomposition of soil organic matter. These observations were later confirmed by the findings of Cooke and Cunningham (1958), Harpsted and Brage (1958), and Soulides and Allison (1961). All these workers observed more mineral nitrogen in air dried and rewetted soils than in corresponding fresh soil. At the end of the fourth drying cycle about 50 ppm ammonium was detected in Miami silt loam and 52 ppm ammonium in Yolo clay loam. The equal amounts of ammonia recovered may be due to the relatively similar organic matter content in both soils which resulted in mineralization of ammonium (Table 1). Each drying cycle has increased the ammonium concentration as a direct result of the drying process. Birch (1959) considered that the mineralization which takes place after drying and rewetting of a soil is due to an increase of exposed organic matter surfaces and, to the high rate of decomposition and nitrogen mineralization following the moistening of a dry soil.

Nitrification

The values for nitrite-nitrogen determined at the end of each drying cycle indicated that in both soils the accumulation of nitrite
is very low (Table 2). However, somewhat more nitrite was accumulated in the Yolo clay loam than in the Miami silt loam. This may be due to the acidic soil conditions which do not favor the accumulation of nitrite (Aleem and Alexander, 1960). The chemoautotroph, *Nitrobacter*, oxidizes nitrite to nitrate at a high rate and it is difficult to detect any accumulation of nitrite in most soils. The drying cycles have no effect on the nitrite accumulation in either of the soils.

Rate of nitrate formation in both soils seemed to be related to the drying cycles, but the effect is not very pronounced. Observations of Miller and Johnson (1964) indicate that nitrification can occur at moisture levels above 15 bars, whereas ammonification was observed at a faster rate both at high tensions (up to air-dryness) and low tensions (near zero bar). In the present study both soils in each of the drying cycles were dried from 0.3 bar to 15 bars tension. In both soils under such circumstances more ammonification than nitrification was expected. Work of Justice and Smith (1962) showed that at 15 bars suction nitrification was delayed and it was faster at 1 bar suction. The data given in Table 2 indicate that drying of moist soil has an added effect on nitrification. Seifert (1969) also reported that the air drying caused successive increases in nitrate produced. The present experiment also indicates that there was comparatively higher nitrate formation in the Yolo clay loam than in the Miami silt loam. At the end of the fourth drying cycle in Yolo clay loam about 15 ppm nitrate was detected as compared to 5.1 ppm nitrate in Miami silt loam. Each successive drying has produced more nitrate in Yolo clay loam soil than in Miami silt loam. This
indicates that the slightly alkaline conditions (pH 7.9) of Yolo clay loam favored the nitrifiers (Alexander, 1965).

Gaseous losses of nitrogen as NO and NO$_2$

The data on the losses of nitrogen as oxides (NO and NO$_2$) are given in Table 2 and the results are plotted in Figures 4 and 5.

Losses of nitrogen as oxides are higher in Miami silt loam than in Yolo clay loam. However, the extent of nitrogen loss from this source in both soils was negligible.

Transformation of Added Ammonium-nitrogen During Subsequent Drying Cycles in Two Soils

The results of nitrogen transformation as affected by drying cycles, in Yolo clay loam and Miami silt loam soils with 300 ppm ammonium nitrogen added, are presented in Table 3 and plotted in Figures 6 and 7.

Ammonium transformation

Each successive drying cycle in both soils had reduced the concentration of applied ammonium nitrogen. The disappearance of ammonium was greater in the Yolo clay loam than in the Miami silt loam. About 69 percent of the applied ammonium was transformed or disappeared from Yolo clay loam soil by the end of the fourth drying cycle as compared to 55 percent loss in Miami silt loam. The higher transformation rate of the applied ammonium in the Yolo clay loam may be due to a faster rate of ammonium oxidation or to direct losses of ammonia by volatilization. The nitrifiers are favored by an alkaline reaction. Morrill and Dawson (1962) reported that the growth of *Nitrobacter* was favored
Figure 4. The effect of drying cycles on the gaseous losses of nitrogen as oxides (NO and NO$_2$) from Miami silt loam.
Figure 5. The effect of drying cycles on the gaseous losses of nitrogen oxides (NO and NO$_2$) from Yolo clay loam.
Table 3. The effect of four wetting and drying cycles on the amount of inorganic nitrogen recovered or lost as gaseous forms in two soils maintained at 30°C when 300 ppm N is applied as ammonium sulfate.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Number of Drying Cycles</th>
<th>NH$_4^+$-N</th>
<th>NO$_2^-$-N</th>
<th>NO$_3^-$-N</th>
<th>Oxides of N</th>
<th>NH$_3$-N</th>
<th>Grand Total</th>
<th>Undetected Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ppm</td>
<td>%</td>
<td>ppm</td>
<td>%</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td>Miami silt loam</td>
<td>0</td>
<td>313.0</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
<td>1.3</td>
<td>314.9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>296.9</td>
<td>-12.0</td>
<td>0.3</td>
<td>-</td>
<td>4.4</td>
<td>301.5</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>269.5</td>
<td>-28.9</td>
<td>1.1</td>
<td>-</td>
<td>10.4</td>
<td>281.0</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>235.6</td>
<td>-28.9</td>
<td>0.7</td>
<td>-</td>
<td>18.6</td>
<td>254.9</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>185.2</td>
<td>-55.1</td>
<td>1.2</td>
<td>-</td>
<td>21.6</td>
<td>208.3</td>
<td>8.8</td>
</tr>
<tr>
<td>Yolo clay loam</td>
<td>0</td>
<td>308.4</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
<td>2.2</td>
<td>310.7</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>232.9</td>
<td>-31.6</td>
<td>23.7</td>
<td>-</td>
<td>7.1</td>
<td>263.7</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>209.1</td>
<td>-42.6</td>
<td>5.1</td>
<td>-</td>
<td>13.7</td>
<td>227.9</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>163.6</td>
<td>-61.4</td>
<td>5.3</td>
<td>-</td>
<td>27.5</td>
<td>196.4</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>143.2</td>
<td>-69.5</td>
<td>1.5</td>
<td>-</td>
<td>42.8</td>
<td>187.5</td>
<td>2.9</td>
</tr>
</tbody>
</table>

a In each of the drying cycles, soil moisture was allowed to drop from one-third bar to fifteen bars tension.

b NO$_3^-$-N is obtained by total inorganic soil N = (NH$_4^+$-N + NO$_2^-$-N).

c Determined by analysis of KMnO$_4$ solution (NO + NO$_2$).

d Volatilized ammonia.
Figure 6. The effect of drying cycles on the recovery of inorganic nitrogen in Miami silt loam applied with 300 ppm N as ammonium sulfate.
Figure 7. The effect of drying cycles on the recovery of inorganic nitrogen in Yolo clay loam applied with 300 ppm N as ammonium sulfate.
by soil pH of 6.2 to 7.0, while that for Nitrosomonas was above 7.6. This may be the reason for higher nitrification rate of the applied ammonium in Yolo clay loam than in the acidic Miami silt loam. Also, in the course of drying the evaporating moisture may carry part of the free ammonia present from the soil (Gardner, 1965). These environment variables may be the main reasons for disappearance of the applied ammonium in the Yolo clay loam at all the drying cycles.

**Nitrification**

The data on Table 3 show that relatively more nitrite was accumulated in Yolo clay loam than in Miami silt loam. In the Yolo clay loam nitrite accumulated to the extent of about 24 ppm as compared to 1.2 ppm in Miami silt loam. This may be due to the higher pH value of Yolo clay loam. Investigations of Stojanovic and Alexander (1958) demonstrated that at soil pH greater than 7.0 the free ammonium is injurious to Nitrobacter and it acts as an inhibitor. Further, they observed the accumulation of nitrite in soils having high pH values. In the present study no significant build-up of nitrite could be noted in either soil. Campbell and Lees (1967) indicate that the Nitrobacter population of a soil is potentially more active than the Nitrosomonas population, and, further they stated that in well aerated soils nitrite is oxidized to nitrate as fast as it is formed. The nitrite formed in nitrification processes may be subject to losses from soil by reacting with ammonium during drying of the soil (Reaction 22). Gerretsen and de Hoop (1957), and Reed and Sabbe (1963) proposed that nitrous acid may react with ammonia in soils and yield molecular nitrogen.
Each successive drying cycle has a pronounced effect on the nitrate formation (Figures 6 and 7). These results are in agreement with the earlier opinion of Alexander (1965) who stated that a higher nitrate production in soils in the wet period following a prolonged drought and in the rainy season following a long dry spell. Both soils under study responded similarly to the drying effect. Higher amounts of nitrate were recovered at the end of each successive drying. This may be because of higher biological activities under the alternate wetting and drying condition. However, the rate of nitrate formation was faster in Yolo clay loam than in Miami silt loam. At the end of the fourth drying cycle, about 43 ppm nitrate was formed in the Yolo clay loam whereas in the Miami silt loam only about 22 ppm nitrate was found. This difference of nitrate formation in both soils could be attributed to differences of soil pH. The higher pH value of Yolo clay loam might have encouraged the activities of nitrifiers.

_Gaseous losses of nitrogen as NO and NO₂_

Released nitrogen at the end of each successive drying cycle was collected as NO and NO₂ in alkaline permanganate traps. The results obtained are given in Table 3 and the values are plotted in Figures 4 and 5.

In Miami silt loam soil a considerable amount of nitrogen as NO and NO₂ was collected at the end of all the drying cycles. About 9 ppm of NO and NO₂ was collected from the Miami silt loam at the end of the fourth drying cycle, as compared to 3 ppm from the Yolo clay loam. The relatively higher losses of nitrogen as NO and NO₂ from the Miami
silt loam may be due to a direct chemical decomposition of the nitrite formed in course of nitrification or to its chemical reaction with the added ammonium. In the present study losses of nitrogen as oxides increased with an increased number of drying cycles. The effect was more pronounced in the Miami silt loam. Nearly four times as much nitrogen was lost from the Miami silt loam as NO and NO₂ as that from the Yolo clay loam. This may be due to the effect of drying, as drying may increase the concentration of ammonium and nitrite, and thereby it may lead to a non-biological loss of nitrogen from the soil.

Volatile losses of ammonia

The effect of the drying cycles on the volatile losses of ammonia from Yolo clay loam soil are shown in Figure 8 and the values are presented in Table 3.

There was a considerable effect of the drying cycles on the extent of volatile losses of ammonia from Yolo clay loam soil when it was supplied with 300 ppm nitrogen as ammonium sulfate. At the end of the fourth drying cycle about 96 ppm ammonia was volatilized from Yolo clay loam. Early work of Jewitt (1942) and Volk (1959) demonstrated that volatile losses of ammonia were related to the water loss. Later Gardner (1965) summarized the effect of soil moisture on the loss of ammonia from soil. It was suggested that any condition which favored evaporation of water also favored volatilization of ammonia, and, in addition, the upward movement of water helped in transporting ammonia to the soil surface. In their recent report Johnson and Phipps (1969) reported large losses of ammonia under alternate wetting and drying soil conditions. No volatile losses of ammonia were detected from the
Figure 8. The effect of drying cycles on the volatile losses of ammonia from Yolo clay loam.
Miami silt loam during any of the drying cycles. The loss of ammonia from Yolo clay loam may be caused by the higher pH values of the soil. These findings are in agreement with the findings of Martin and Chapman (1951) who recorded very little losses of ammonia when the soil pH was below 7.2. Another reason for the higher losses of ammonia from Yolo clay loam may be due to the higher exchangeable calcium and sodium content (Table 1) as compared to Miami silt loam. The presence of calcium and sodium had a considerable effect on the retention of ammonium in the soil (Jackson and Chang, 1947).

Undetected nitrogen

As shown in Table 3 at the end of the experiment about 83 ppm and 157 ppm N of the added ammonium was not accounted for in Yolo clay loam and Miami silt loam, respectively. These losses of nitrogen apparently are other than by the release of NO, NO₂, and NH₃. Johnson and Phipps (1969) also detected unaccounted loss of nitrogen when the soils were subjected for alternate wetting and drying cycles. They suggested that loss of nitrogen under such conditions may be in the form of N₂. Also there are possibilities of losses as N₂ and N₂O due to the direct decomposition of HNO₂ in soil (Reactions 19 and 20).

Transformation of Added Nitrite-nitrogen During Subsequent Drying Cycles in Two Soils

The data obtained on the recovery of inorganic nitrogen in Miami silt loam and Yolo clay loam supplied with 300 ppm nitrogen as sodium nitrite under four drying cycles are given in Table 4 and shown in Figures 9 and 10.
Table 4. The effect of four wetting and drying cycles on the amount of inorganic nitrogen recovered or lost as gaseous forms in two soils maintained at 30 C when 300 ppm N is applied as sodium nitrite.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NH₄⁺-N</td>
<td>NO₂⁻-N</td>
<td>NO₃⁻-N</td>
<td>Total N in Soil</td>
<td>Oxides of N</td>
<td>NH₃-N</td>
<td>Grand Total</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recovery ppm</td>
<td>% Loss</td>
<td>Recovery ppm</td>
<td>% Loss</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td>Miami silt loam</td>
<td>0</td>
<td>11.9</td>
<td>308.8</td>
<td>1.9</td>
<td>322.6</td>
<td>32.2</td>
<td>22.0</td>
<td>137.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>22.0</td>
<td>102.4</td>
<td>-66.1</td>
<td>134.2</td>
<td>3.2</td>
<td>-18.1</td>
<td>75.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25.8</td>
<td>17.9</td>
<td>-94.5</td>
<td>57.7</td>
<td>17.9</td>
<td>-25.2</td>
<td>75.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>30.8</td>
<td>11.2</td>
<td>-96.6</td>
<td>57.4</td>
<td>18.1</td>
<td>-28.9</td>
<td>75.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>32.1</td>
<td>7.7</td>
<td>-99.9</td>
<td>55.4</td>
<td>27.7</td>
<td>-31.2</td>
<td>83.1</td>
</tr>
<tr>
<td>Yolo clay loam</td>
<td>0</td>
<td>12.3</td>
<td>300.8</td>
<td>1.4</td>
<td>314.5</td>
<td>-</td>
<td>-18.1</td>
<td>214.5</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>18.1</td>
<td>182.4</td>
<td>-39.7</td>
<td>211.7</td>
<td>3.2</td>
<td>-25.2</td>
<td>175.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25.2</td>
<td>108.8</td>
<td>-64.6</td>
<td>171.3</td>
<td>3.7</td>
<td>-31.2</td>
<td>119.9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>28.9</td>
<td>41.0</td>
<td>-86.8</td>
<td>115.5</td>
<td>4.4</td>
<td>-31.2</td>
<td>119.9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>31.2</td>
<td>13.4</td>
<td>-96.2</td>
<td>96.6</td>
<td>4.7</td>
<td>-31.2</td>
<td>119.9</td>
</tr>
</tbody>
</table>

a. In each of the drying cycles, soil moisture was allowed to drop from one-third bar to fifteen bars tension.

b. NO₃⁻-N is obtained by subtracting NH₄⁺-N and NO₂⁻-N from total inorganic N.

c. Determined by analysis of KMnO₄ solution (NO + NO₂).

d. Volatilized ammonia.
Figure 9. The effect of drying cycles on the recovery of inorganic nitrogen in Miami silt loam applied with 300 ppm N as sodium nitrite.
Figure 10. The effect of drying cycles on the recovery of inorganic nitrogen in Yolo clay loam applied with 300 ppm N as sodium nitrite.
Ammonification

In both soils the application of 300 ppm nitrite nitrogen alone resulted in less recovery of ammonium nitrogen at the end of all drying cycles than with soils incubated without any added nitrogen (Table 2 and 4). This suggested that part of the mineralized soil nitrogen might have been subjected to a chemical reaction with the added nitrite. No volatile losses of ammonia were detected in either soil. This shows that the ammonium formed from soil nitrogen may be transformed to nitrite (Reaction 2) or it might have been subjected to loss by reacting with applied nitrite nitrogen (Reaction 17). During soil drying, when the moisture tension was raised from 0.3 bar to 15 bars, there was a likelihood of ammonium reacting with nitrite. Johnson and Phipps (1969), while working with a similar situation, suggested the probable losses of soil nitrogen as \( N_2 \) when ammonium was allowed to react with nitrite. However, the early work of Smith and Clark (1960) indicated that in the presence of oxygen the tendency for nitrite oxidize to nitrate was three to four times as strong as for it to react with the ammonium ion.

Nitrification

Rate of nitrite disappearance was high in both soils. This may be due either to the oxidation of nitrite to nitrate or to direct decomposition of nitrite and subsequent losses of nitrogen from the soils. The intensity of nitrite nitrogen disappearance was high in Miami silt loam. This may be accounted for by the acid soil reaction (pH 5.2) of the Miami silt loam. These findings are in agreement with the earlier reports of Temple (1914) and Robinson (1923) and the recent findings of Nelson and Bremner (1969 and 1970). All of them reported higher
nitrite decomposition in acid soils and the rate of decomposition increased with an increase in soil acidity. In acid media, nitrite exists in the form of nitrous acid ($\text{HNO}_2$) which is more reactive than nitrite ion (Reuss and Smith, 1965). Thus, in the Miami silt loam, greater quantities of nitrite were transformed than in the Yolo clay loam with a relatively higher pH (pH 7.9). Under aerated soil conditions Allison (1963) and Smith and Clark (1960) recorded higher rate of oxidation of nitrite to nitrate than the rate of nitrite reaction with ammonium. At the end of the fourth drying cycle about 100 percent and 96 percent of the added nitrite was not recovered in the Miami silt loam and the Yolo clay loam, respectively.

A considerably larger nitrate build-up was detected in the Yolo clay loam than in the Miami silt loam (Figures 9 and 10). This may be because of the higher pH values of the Yolo clay loam which might have benefitted the nitrifying population. Morrill and Dawson (1962) reported that the optimum pH for the activity of nitrate oxidizers is close to the neutral point. While reviewing the effect of soil reaction on nitrification, Alexander (1965) stated that nitrification is faster in neutral to slightly alkaline soils. This may be the reason for the slower rate of nitrate formation in the Miami silt loam. At the end of the fourth drying cycle in the Yolo clay loam about 52 ppm nitrate was recovered as compared to 23 ppm nitrate in the Miami silt loam.

Gaseous losses of nitrogen as $\text{NO}$ and $\text{NO}_2$

The results of nitrogen lost in the form of $\text{NO}$ and $\text{NO}_2$ are given in Table 4 and the values are plotted in Figures 4 and 5.
The results show that nitrite decomposition in soils lead to the formation of NO and NO$_2$ (Reaction 12). In the Miami silt loam greater quantities of NO and NO$_2$ were determined than in the Yolo clay loam. Here again, the pH effect was evident. However, still a substantial amount of NO and NO$_2$ were formed in the Yolo clay loam. This is a contradiction to the postulations of Corbet (1934) and Gerretsen and de Hoop (1957), who suggested that above pH 5.5 nitrite decomposition may cease. The current findings are in agreement with Allison (1963) and Reuss and Smith (1965) who suggested that nitrite could be decomposed to NO at soil pH values higher than pH 5.5. Nelson and Bremner (1970) have given support for the concept of formation of NO by self-decomposition of nitrite under acidic conditions, and NO is rapidly oxidized to NO$_2$ under aerobic conditions. Further, they suggested that the amount of NO$_2$ evolved was not significantly related to soil texture or organic matter content but that there was a strong negative correlation between soil pH and the amount of NO$_2$ formed. Their studies confirmed, however, that substantial amounts of NO$_2$ can evolve from soils having pH values above six.

Undetected nitrogen

In this investigation, small amounts of added nitrite were recovered as NO and NO$_2$ (Table 4). Smith and Clark (1960) failed to detect the formation of NO and NO$_2$ in nitrite-treated soils. Instead they identified nitrogen (N$_2$) and nitrous oxide (N$_2$O) as the gaseous products. Tyler and Broadbent (1960) also identified N$_2$ as a gaseous product of nitrite decomposition in soils. Gerretsen and de Hoop (1957) and Reuss and Smith (1965) obtained much larger quantities of
$N_2$ than NO and $NO_2$. Alternatively, it can be suggested that the quantity of NO and $NO_2$ recovered from decomposing nitrite depends upon the rate of the removal of oxides from the system (Gerretsen and de Hoop, 1957) and the ability of the trap solution to absorb the oxides produced (Reuss and Smith, 1965). Other possibilities of $N_2$ formation in soil are from nitrite reacting with ammonia (Clark, 1962; Reed and Sabbe, 1963).

**Transformation of Added Ammonium and Nitrite with Subsequent Drying Cycles in Two Soils**

The different forms of inorganic nitrogen recovered from Miami silt loam and Yolo clay loam at the end of different drying cycles with nitrogen added in both the ammonium and nitrite form are given in Table 5. The data are plotted in Figures 11 and 12.

**Ammonium transformation**

The rate of the transformation of added ammonium and of ammonium formed by ammonification of soil nitrogen in the Yolo clay loam was quite high as compared to the Miami silt loam. At the end of the fourth drying cycle about 78 percent of the added ammonium was not recovered from the Yolo clay loam as compared to 62 percent from the Miami silt loam. This disappearance of ammonium from both soils may be due to the oxidation of ammonium or to direct volatilization from soil (Ernst and Massey, 1960; and Gardner, 1965). Secondly there is the likelihood of ammonium reacting with the added nitrite under the fluctuating moisture conditions, especially during drying where large losses of nitrogen may occur (Johnson and Phipps, 1967, 1968, and 1969).
Table 5. The effect of four wetting and drying cycles on the amount of inorganic nitrogen recovered or lost as gaseous forms in two soils maintained at 30°C when supplied with 150 ppm N as ammonium sulfate and 150 ppm N as sodium nitrite.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Number of Drying Cycles</th>
<th>Inorganic Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NH₄⁺-N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recovery ppm</td>
</tr>
<tr>
<td>Miami silt loam</td>
<td>0</td>
<td>162.9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>154.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>142.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>132.2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>107.8</td>
</tr>
<tr>
<td>Yolo clay loam</td>
<td>0</td>
<td>162.9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>138.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>120.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>93.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>85.1</td>
</tr>
</tbody>
</table>

a. In each of the drying cycles, soil moisture was allowed to drop from one-third bar to fifteen bars tension.

b. NO₃⁻-N is obtained by subtracting NH₄⁺-N and NO₂⁻-N from total inorganic N.

c. Determined by analysis of KMnO₄ solution (NO + NO₂)

d. Volatile ammonia.
Figure 11. The effect of drying cycles on the recovery of inorganic nitrogen in Miami silt loam applied with 150 ppm N as ammonium sulfate and 150 ppm N as sodium nitrite.
Figure 12. The effect of drying cycles on the recovery of inorganic nitrogen in Yolo clay loam applied with 150 ppm N as ammonium sulfate and 150 ppm N as sodium nitrite.
**Nitrification**

The rates of transformation of added nitrite and nitrite formed by the oxidation of ammonium in either soil was not as high as it was when nitrite was applied alone. This may be attributed to an increase in the soil pH associated with the application of the ammonium fertilizer. Slight increase in the soil pH could reduce the decomposition of nitrite in the soil. In Miami silt loam and Yolo clay loam the rate of disappearance of applied nitrite remained almost the same at the end of the fourth drying cycle.

Higher concentrations of nitrates were found in the Yolo clay loam than Miami silt loam (Table 5). At the end of the fourth drying cycle about 47 ppm nitrate-nitrogen was formed in the Yolo clay loam as compared to 21 ppm in the Miami silt loam. This can be attributed to the higher pH values of the Yolo clay loam which increased the activities of nitrifiers. It can be seen that there is relatively less ammonium remaining in the Yolo clay loam as compared to the Miami silt loam.

**Gaseous losses of nitrogen as NO and NO\textsubscript{2}**

The various quantities of NO and NO\textsubscript{2} recovered at different drying cycles from Miami silt loam soil and Yolo clay loam soil are given in Table 5 and the data are plotted in Figures 4 and 5.

The proportion of nitrite-nitrogen converted to oxides of nitrogen was found to vary inversely with the initial pH values of soils and the concentrations of the reactants. This may be the reason for a greater loss of nitrogen from the Miami silt loam. Several investigations have shown that the decomposition of nitrite is promoted by
elevated soil acidity (Robinson, 1923; Tyler and Broadbent, 1960; Reuss and Smith, 1965).

**Volatile losses of ammonia**

Losses of nitrogen in the form of ammonia were recorded at the end of each drying cycle and the values are given in Table 5 and are plotted in Figure 8.

Volatile losses of ammonia were recorded only in the Yolo clay loam soil. The highest losses were recorded in the first two drying cycles. Johnson and Phipps (1969) also observed substantial losses of ammonia in different soils subjected to alternate wetting and drying cycles (Reaction 23).

**Undetected nitrogen**

The results recorded in Table 5 demonstrate that during all drying cycles there was considerable undetected nitrogen losses in both soils. In similar studies of soil, clay, and non-soil systems, Johnson and Phipps (1967, 1968, and 1969) reported that nitrite-nitrogen loss was directly dependent on the quantity of ammonium present. They attributed these losses of nitrogen to formation of either $N_2O$ or $N_2$ (Reactions 22 and 24). This may be one of the reasons for nitrogen losses at the end of each drying cycle.
SUMMARY AND CONCLUSION

Experiments were conducted to obtain quantitative information on any gaseous losses of nitrogen from soils as NO, NO₂, and NH₃ in the course of wetting and drying cycles. Two soils having different physical and chemical properties were selected for this investigation and they were amended with various concentrations of ammonium and nitrite-nitrogen alone or in combination.

From the results of these experiments the following conclusions were drawn.

1. Wetting and drying cycles had a pronounced influence on the mineralization of soil nitrogen. Each drying cycle increased the rate of ammonification in both soils.

2. Each successive drying cycle increased the oxidation of applied and available ammonium. The drying and rewetting of soil increased the activities of nitrifying microorganisms in soil.

3. Relatively faster rate of nitrification was observed in Yolo clay loam than in Miami silt loam. This may be because of a higher pH of Yolo clay loam which favored the nitrifying organisms.

4. The rate of nitrate formation was high in both soils when treated with nitrite only.

5. Drying cycles had a direct influence on the losses of nitrogen as NO and NO₂ in both soils. The loss was much higher in Miami silt loam, probably because of its acidic reaction.
6. Rate of disappearance of applied nitrite was high in Miami silt loam. This may be due to the instability of nitrite under acid soil reaction of Miami silt loam.

7. Higher losses of nitrogen as NO and NO₂ were detected in both soils treated with nitrite alone or with nitrite and ammonium together than in the same soils treated with ammonium only.

8. Volatile losses of ammonia were recorded in Yolo clay loam only. This may be due mainly to the higher pH value of Yolo clay loam and to the higher exchangeable Ca⁺⁺ and Na⁺ content than in Miami silt loam. Rate of loss of ammonia was intensified during drying as the evaporating water may carry along with it a part of the ammonium present in the soil.

9. In both soils during the various treatments a considerable amount of nitrogen remained undetected. Such undetected nitrogen most likely may be lost from soils in the form of N₂ or N₂O.
LITERATURE CITED


APPENDIX
Nitrate Nitrogen Determination

Extraction of soil samples

1. Disconnect the Erlenmeyer flask from the apparatus.
2. Add 50 ml 2N KCl solution.
3. Place flasks on mechanical shaker for 30 minutes.
4. Pour the soil suspension after shaking onto the Buchner funnel on which 11 cm Whatman No. 42 filter paper has been moistened and sealed and seated firmly by suction. Finally, 200 ml more of the 2N KCl solution is passed through the soil in increments, the first increment being used to rinse out the Erlenmeyer flask.
5. Collect the filterates in 250 ml volumetric flasks by making the volume for the determination of NH₄, NO₂, and NO₃.

Reagents:

**Phenoldisulfonic acid (C₆H₃OH(HSO₃)₂).** Dissolve 200 g pure white phenol in 1200 ml concentrated H₂SO₄. Add 600 ml fuming sulfuric acid (15 percent free S0₃); stir well, heat for two hours on hot water bath.

**Acetic acid solution.** One part glacial acetic acid and three parts distilled water.

**Ammonium sulfamate.** 0.5 percent solution in water. Dissolve 5 g ammonium sulfamate in distilled water and dilute to 1 liter.

**Ammonium hydroxide.** One part concentrated NH₄OH to one part distilled water.

**Standard KN0₃ solution.**

Stock. Dissolve 0.722 g anhydrous KN0₃ and dilute to 1 liter with distilled water. Contains 0.1 mg N/ml.
Standard. Evaporate 50 ml stock KNO₃ solution to dryness; dissolve residue by rubbing with 2 ml phenoldisulfonic acid, dilute to 500 ml with distilled water. 1 ml = 0.01 mg N.

For preparation of standard curve use 0, 0.5, 1.0, 2.0, 3.5, 8.0, 10.0, 15.0, and 20.0 ml of standard KNO₃ solution (1 ml = 0.01 mg N).

Treat and evaporate as outlined in "Procedure."

Procedure

1. (a) Pipette 5 ml of the filtered 2NKCl extract into 100 ml tall beakers.

   (b) Pipette into extra beaker 5 ml of the 2NKCL extracting solution.

2. Add 5 drops of acetic acid; swirl to mix.

3. Add 0.5 ml of ammonium sulfamate solution; swirl to mix. (Add 1 ml to those samples having high NO₃⁻ content.)

4. Place on hot plate.

5. When warmed to 60 to 80 C, add five drops of 10 percent NaOH solution; swirl to mix.

6. Let contents evaporate to dryness.

7. Remove beakers from hot plate.

8. Add (from pipette) 2 ml of phenoldisulfonic acid to the beakers in such a way that the residue will be moistened quickly and thoroughly.

9. Rub sides of beakers with a stirring rod to bring the phenoldisulfonic acid in contact with any of the nitrates that may have spattered onto the wall on drying.

10. Let stand about 20 minutes (or longer) or until the residue is dissolved.
11. Add 34 ml of distilled water to beaker.
12. Add NH₄OH solution (1:1) to beaker until a permanent yellow color remains (14 ml). Perform this step under the fume hood.
13. Let cool. Make further dilution if color is too intense. Record final volume.
14. Mix contents by stirring with stirring rod or swirling carefully and read on electrophotometer after balancing the bridge and setting null adjustment with a blank 2NKCL solution).

**Nitrite Nitrogen Determination**

**Reagents**

**Sulfanilamide.** 0.2 percent solution in water. Dissolve 4 g p-amino-benzenesulfanilamide in distilled water in 2000 ml volumetric flask. Dilute to mark, then transfer to storage bottle. Keep tightly stoppered in the refrigerator. Prepare a new solution one or two days before required, or at least every month.

**Hydrochloric acid.** One to one dilution of the concentrated acid with distilled water.

**Coupling reagent.** Dissolve 1 g N-(1-Naphthyl)-Ethylenediamine dihydrochloride in 1 liter distilled water. Store in dark bottle.

**Sodium nitrite standard.**

1. Employing assayed NaN₃ prepare an accurately known solution containing about 0.005 mg of N per ml (Solution A).
2. Dilute 50 ml of above solution to 100 ml in volumetric flask (Solution B).
3. With a volumetric pipette measure 0.5, 1.0, 1.5, 1.8 ml of A, and 1, 2, 4 ml of B into 50 ml volumetric flasks.
4. Add 20 ml of 2NKCL extracting solution (filtered) to each.
5. Treat as outlined in steps 2 through 7 of "Procedure."
6. Prepare graph.
7. Keep standard sodium nitrite solution tightly stoppered in refrigerator.

Procedure

1. (a) Pipette 20 ml of the filtered 2NKCL soil extract into 50 ml volumetric flasks. Use smaller aliquot (2 to 5 ml) if NO₂⁻ content of sample is high. Make up to 20 ml with 2NKCL solution.
   (b) Pipette 20 ml of the 2NKCL extracting solution into an extra 50 ml volumetric flask.
   (c) Fill burettes with 50 percent HCl, 0.2 percent sulfanilamide and coupling reagent.
2. From a burette add 1 ml of 50 percent HCl.
3. From a burette add 5 ml of 0.2 percent sulfanilamide solution.
4. Wait three minutes.
   Note: Steps 2 and 3 can be performed on 10 samples within the three minutes as follows: Add the HCl and the sulfanilamide to the first sample; then set the time clock for three minutes. Then quickly add the HCl and sulfanilamide to the other nine samples in succession.
5. At the end of the three-minute interval, begin adding 1 ml of the coupling reagent to each sample, timing the additions so that each sample will have remained at three minutes after the addition of sulfanilamide.
6. Make up to volume with distilled water.

7. If the color is too dark, take smaller quantities of aliquot and develop color.

8. Read on electrophotometer, using the blank to set the null point.
   Use 525-B filter or read at 550 mμ wavelength.

Sodium Nitrite

Tests for identity

Dry about 1 g sodium nitrite to constant weight over sulfuric acid, weigh accurately in a stoppered weighing-bottle, dissolve the salt in a volumetric flask with sufficient distilled water to make 100 ml and add 10 ml of this solution, from a pipette, to a mixture of 40 ml of tenth-normal potassium permanganate, 100 ml of distilled water, and 5 ml of sulfuric acid. When adding the sodium nitrite solution, immerse the tip of the pipette beneath the surface of the permanganate mixture. Warm the liquid to 40 C, allow it to stand for five minutes, and add 25 ml of tenth-normal oxalic acid or sodium oxalate. Heat the mixture to about 80 C and titrate with tenth-normal potassium permanganate. Each ml of tenth-normal potassium permanganate is equivalent to 0.003450 g NaN0₂.

Storage

Preserve sodium nitrite in air-tight containers in refrigerator.
Analysis of Alkaline Permanganate Traps
for Oxides of Nitrogen

The oxides of nitrogen that dissolve in the traps will be oxidized to nitrate by the KMnO₄. Thus this procedure is designed to destroy the color of the KMnO₄ and permit colorimetric nitrate determination.

Reagents
1. Trapping solution of alkaline permanganate 0.2 M KMnO₄ in 2 N KOH
   Dissolve 112.2 g reagent grade KOH in about 800 ml of distilled water. Add 31.7 g KMnO₄, warm and stir until solution is complete. Transfer to 1 liter volumetric flask and make up to volume. Store in brown bottle away from direct light.
2. Oxalate sulfuric acid solution 0.6 N Na₂C₂O₄ in 7 N H₂SO₄
   Place about 1200 ml distilled water in a 2 liter beaker. Add carefully 390 ml concentrated H₂SO₄. Weigh out and add 160.6 g Na₂C₂O₄. Stir until oxalate is completely dissolved, transfer to 2 liter volumetric flask and make up to volume. Store in a brown plastic bottle; the oxalate will attack glass.
3. 5 N NaOH
   Weigh out 200 g reagent grade NaOH and dissolve in about 800 ml distilled water. Allow to cool; transfer to 1 liter volumetric flask and make to volume.

Procedure
Wash the entire contents (20 ml) of the trap into a 125 ml beaker. Avoid using excess water. A blank of unused trap solution should be included. Add 20 ml of reagent 2 (acid and oxalate). In a few minutes
the reaction should be complete and the material should be colorless. Warming on the hot plate will facilitate reaction. Incomplete reaction may be the result of insufficient acidity or insufficient oxalate.

After slight warming add 30 ml of reagent 3 (5 N NaOH) to each beaker. Precipitation will occur at this point and the base must be in sufficient quantities to make the solution alkaline. Upon standing a slight brown scum of MnO₂ should form on the surface. Failure of this to form may be due to insufficient base.

The precipitate should be digested with gentle warming for at least 0.5 hour. The material is then filtered into 250 ml volumetric flask. This filtration must be quantitative and the residue washed several times with small portions of warm water. The volume is then made to 250 ml and an aliquot (5 ml) taken for the standard phenoldisulphonic acid nitrate determination. The addition of ammonium sulphamate to destroy nitrites is unnecessary.

The blank carried through the entire procedure is used to set the zero on the spectrophotometer.

**Standard curve and calculations**

\[
\text{KNO}_3 \text{ standard} -- 200 \mu g \text{ N/ml}
\]

1.44 g KNO₃ made up to 1 liter volume.

Add 0, 1, 2, 3, . . . 8 ml of KNO₃ standard to a series of beakers containing 5 ml KMnO₄ trap solution. Carry through the procedure as outlined taking a 5 ml aliquot for nitrate determination. Carry through the standard nitrate procedure making up the colored solution to 50 ml for reading on the spectrophotometer. There will now be 0, 10, 20, . . . 80 µg N per 50 ml. Read on spectrophotometer and plot curve.
Determine from the curve the ratio \( \mu \text{g N/optical density} \) (inverse of slope if optical density is plotted on the y axis and \( \mu \text{g} \) on the x).

\[
\text{Ratio} \times \text{opt. den.} \times 50 = \text{ppm N}
\]

Ratio determined by Reuss (1963) was 128.

Notes

Do not use excess water to wash the trap solution into the beaker or for washing the precipitate as the total volume must be less than 1000 ml. The destruction of \( \text{K MnO}_4 \) by \( \text{C}_2\text{O}_4^{2-} \) requires high acidity, and incomplete reaction at this point is often the result of insufficient acidity. If the traps are to be exposed to large amounts of \( \text{CO}_2 \) evolution, it may be necessary to use 4 N KOH and/or 10 ml trapping solution per trap. If more trapping solution is used the amounts or the strengths of reagents No. 2 and 3 may be increased accordingly. If 4 N KOH is used in the trap solution, the acidity of reagent 2 should be increased to 9 N.

After the residue from the filtration is exposed to the air, it should assume a dark brown color. If it remains gray add a ml or two of NaOH to the filtrate. If any precipitate occurs, refilter.
VITA

Virupax C. Baligar

Candidate for the Degree of

Master of Science


Major Field: Soil Science

Biographical Information:

Personal Data: Born in Motebennur, Mysore State, India, June 1, 1942, son of Chanabasappa Tippanna and Veeramma C. Baligar.

Education: Attended elementary school, graduated from Karnataka High School in 1959; received the Bachelor of Science degree in 1965 with a major in agriculture; received the Master of Science degree in 1967 with a major in Agronomy, from Karnataka University, Dharwar, Mysore State, India; completed requirements for the Master of Science degree in Soil Science at Utah State University in 1971.

Professional Experience: October, 1969 to present, graduate research assistant, Department of Soils and Meteorology, Utah State University; June, 1967 to September, 1969, research assistant in University of Agricultural Science Bangalore, India; June, 1966 to April, 1967 Lecturer in Agronomy (part-time) Agricultural College, Dharwar, India.