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Nature of Non-Biological Decomposition of Nitrite in Acid Media

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NATURE OF NON-BIOLOGICAL DECOMPOSITION OF NITRITE IN ACID MEDIA

by

Mukkatira Kariappa Mahendrappa

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

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in

Soil Chemistry

Approved:

[redacted]

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Mahendrappa

Mukkatira Kariappa Mahendrappa

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INTRODUCTION

Curiosity is the cause of investigations, and the thirst for knowledge originates from it. Many times at the end of investigations, the number of questions raised are greater than those for which answers are sought. Therefore, all the information gathered through scientific investigations may not be of value for immediate application in practical life. Nevertheless, no research can be considered less important than the other because the integration of several scientific findings considered less important at the time of investigation may reveal something of very high significance. Similarly, the study of various transformation reactions that the added fertilizers undergo may not be of value to the one whose main objective is to increase production, though it is of great value for the efficient use of fertilizers. Information on the reactions of each intermediate compound produced during the transformations of ammonical fertilizers may be useful in reducing or preventing the loss of N that could arise through some of the reactions. Such knowledge will be useful for the economical use of fertilizers.

Nitrite is an intermediate compound produced in nitrification, denitrification, and nitrate reduction processes. Chemistry of nitrite in alkaline solutions is well understood, while its fate in acid soils and solutions has eluded the attention of most workers. This is probably due to the fact that under acid conditions nitrite does not appear in large quantities contrary to its behavior in the alkaline region.

However, the existence of nitrite in smaller concentrations under acid conditions suggests the possibility that it may be rapidly transformed to other forms of N and may lead to the loss of added N. The rates of such reactions and losses may be higher in acid than in alkaline soils.

In acid solutions nitrite also exists as un-ionized nitrous acid; the concentration of the latter form increases at higher acidities. In the following pages these two terms, nitrite and nitrous acid, will be used interchangeably. In acid soils, nitrous acid undergoes several changes such as its fixation by various soil components, chemical conversion to other N compounds, and loss of N either as N_2 or in the form of oxides of nitrogen. Most of these reactions may probably result in reduced nitrogen availability to the crops.

In this investigation some of the factors influencing nitrite transformations, such as non-biological nitrification and loss of N in different forms, were studied. Validity of some of the proposed nitrite decomposition pathways were verified, and conditions favoring individual reactions were studied in various soil and resin systems.

REVIEW OF LITERATURE

Waksman (1952) has presented the history of the foundation of soil microbiology, and the materials in this paragraph are taken directly from his book. According to Waksman (1952), in a study of the putrification of sewage water, Pasteur made the passing suggestion in 1862 that nitrification was due to bacterial action. In 1877 Shloesing and Munz demonstrated the biological nature of the nitrification process, and within a couple of years in 1879, Winogradsky was successful in isolating the nitrifying bacteria.

An earlier concept that nitrification is purely a chemical process was not completely disproved by the postulation of Pasteur followed by the demonstration of the biological nature of the nitrification process by Schloesing and Munz. Intensive research carried out on the biological nitrification has revealed, to some extent, the importance of the non-biological nature of the process. Thus, the research has advanced to a point where scientists are attempting to explain nitrification, proven to be a bacterial process a century ago, in terms of still unknown chemical reactions. In the following few pages, while presenting the facts known about the chemical nitrification process, it will also be shown that such transformations are accompanied by the loss of nitrogen.

Most of the work cited here deals with aerobic nitrite transformation reaction. Except when specified, therefore, all the reactions mentioned refer to the reactions that occur under aerobic conditions. Generally elemental nitrogen cannot be directly reabsorbed by the system, although non-symbiotic nitrogen fixing bacteria exist in the soil. Thus most of the N_2 produced during various N transformation reactions will be released to the atmosphere. With this point in view, the term loss of N_2 is used to denote the evolution of nitrogen through non-biological nitrite decomposition.

Nitrification

Evidences of chemical nitrification

Following the isolation of nitrifying bacteria by Winogradsky, Munro (1886) investigated the nutritional requirements of these bacteria. To his surprise, ammonium chloride added to river water passed through two Swedish filters was nitrified, although the rate was slower than that observed in unfiltered samples. Swedish filters are said to remove bacteria from the solution. Ashby (1907) found ferric hydrate to promote nitrification by bacteria. Ferric hydrate was supplied in quantity much greater than that required for the bacteria. Thus, it was probably hastening chemical reactions.

When the study of nitrification was shifted from bacterial cultures to soil systems, discrepancies were noted with regard to the biological nitrification. Nitrification was observed in some soils that were considered too much acidic to promote the growth and action of bacteria. Hall (1907) and Stephenson (1920) suggested that nitrification does not occur in acid soils and the nitrification observed to a small extent in such soils is due to the presence or the formation of small pockets in the soil which are basic in reaction. Dhar and Bhattacharya (1933) have presented evidences of photochemical conversion of ammonical nitrogen to nitrite nitrogen. Sun's rays and ultraviolet rays are said to affect such changes in the presence of photosensitizers such as various metals present in the soil. Thus, the first part of nitrification is shown to be non-biological at least in the regions where the sunlight is abundant during most parts of the year.

Temple (1914) was the pioneer in finding out the chemical nature of nitrification in soils. When nitrification of ammonium sulfate was studied in soil sterilized and innoculated with nitrite building bacteria, a part of the nitrogen was usually recovered as nitrate. Thus, nitrate was obtained without the action of nitrate building bacteria. When $NaNO_2$ was added to both acid soils and solutions, nitrogen peroxide produced was identified by smell and nitrate was found in the solution. Temple (1914) proposed the following reaction as a probable pathway of nitrite decomposition.

$$3HNO_2 \rightarrow 2NO + H^+ + NO_3^- + H_2O \quad . \quad . \quad . \quad . \quad . \qquad (1)$$

Temple (1914) also found that the tendency for the nitrite to be transformed to nitrate is greater in soil than in solution.

In the following years most workers have failed to recognize and accept Temple's findings. Fred and Grant (1916) claimed that even in acid soils nitrification is a result of microbial action and liming is useful in hastening the process. Robinson (1923) investigated the usefulness of $NaNO_2$ as a nitrogenous fertilizer. He observed that more nitrite was lost in the form of various oxides of nitrogen in acid soils than from neutral or alkaline soils. No nitrate was observed in acid soils. Olendskii (1931) found very similar results when $NaNO_2$ was added to soil acidified to various pH values by adding hydrochloric acid.

In the early 1930's Barrit (1933) and Corbet (1934) independently obtained evidences for chemical nitrification. In bacterial cultures when the pH dropped to 5.5, nitrification ceased, followed by the disappearance of nitrous acid and the formation of nitric acid with a concomitant decrease in pH to 4.5. Therefore, Barrit (1933) suggested that nitrate was produced through chemical conversion of nitrite since at pH 4.5 to 5.5 the acidity is too strong for the bacteria to operate. He further confirmed these results by adding $NaNO_2$ to acidified solutions. Corbet (1934) obtained similar results in sterilized solutions. Both these workers used reaction (1) to explain their results but made no reference to Temple (1914). In acid soils within a period of two days from 15 to 44 ppm nitrate was recovered by Fraps and Sterges (1939) who claimed this was probably due to the non-biological decomposition of nitrous acid to nitric acid, since two days were considered too short a period for the bacteria to be operative. They found no quantitative agreement between the losses of N observed, the acidity developed, and reaction (1) of Temple (1914). Thus, an additional reaction was suggested as

$$2HNO_2 \rightleftharpoons N_2O_3 + H_2O \quad . \; . \; . \; . \; . \; . \; . \; . \quad (2)$$

Allison and Doetsch (1951) suggested that NO produced during nitrite decomposition in moist acid soils would be oxidized chemically to nitric acid. Incidentally, this explains how nitrate can be formed from nitrite in soils so acid that nitrobacter cannot live. Very recently Reuss and Smith (1965) have observed non-biological nitrification both in soil and resin systems.

From the above review of literature, it is clear that non-biological nitrification takes place in acid soils. Side by side the microbial nitrification also occurs in soils.

Factors affecting non-biological nitrification

Similar to other chemical reactions, non-biological conversion of nitrite to nitrate would definitely be influenced by temperature. In addition, the reaction of the medium and the concentration of reactants and products would determine the nature and the completeness of the process. If the reactions of nitrite are catalyzed by some soil component, the amount of catalyst may also limit the reaction.

Effect of temperature. Rossi (1935) investigated the effect of temperature on chemical conversion of nitrite to nitrate in acid media. The reactions were studied in closed systems at extremely high temperatures at which no bacteria would be operative. The rate of nitrification was reported to increase with temperature from 30 C to 110 C. No Q_{10} values are reported in this report.

As against Rossi's findings, Corbet (1934) reported lesser nitrate recovery at higher temperatures. He found greater loss of nitrite nitrogen and the loss is said to have occurred in the form of NO. Gray and Yoffe (1955) also have predicted such results (see Mechanism of Nitrification, page 11). Anderson and Boswell (1964) found that the discrepancy between the N applied and that recovered at low temperature was seldom greater than 10 percent, but at high temperature losses frequently exceeded 20 percent. Since gas phase reactions are also involved in nitrous acid decomposition, the results of Rossi (1935) would be applicable only to closed systems while in open systems there may not be a linear relationship between the temperature and the rate of chemical nitrification.

Effect of pH. Nitrous acid being more reactive than nitrite ion, it assumes importance in the process of non-biological nitrification. It is the chemical species involved in the chemical conversion of nitrite to nitrate N (Reuss and Smith, 1965). When any salt of nitrite is added to acid media, nitrite ion combines with hydrogen ion to form nitrous acid as

$$H^+ + NO_2^- \rightleftharpoons HNO_2 \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (3)$$

In a given solution both the species exist and the proportion of each species present depends on the reaction of the medium. The dissociation constant of nitrous acid being 6×10^{-4} at 25 C (Gray and Yoffe, 1958) at pH 5, 4, and 3, respectively, 1.6 percent, 14 percent, and 63 percent of the added nitrite exists as nitrous acid. This clearly indicates the extreme pH dependence of the non-biological nitrification process. Nevertheless, many workers have explained their results based on the effects of pH on nitrite decomposition. Indirect reports indicating the pH effects on non-biological nitrification can be grouped into two classes. In one case the loss of various forms of nitrogen is used as indicative of the extent of reaction and in the other the quantities of nitrate formed are directly determined. Since the pH effects on non-biological nitrification have not been investigated, almost all the workers cited below have inferred the pH effects on the basis of N losses observed.

Robinson (1923) found very rapid decomposition of nitrites and higher losses of N in acid soils as against gradual and smaller losses of N in alkaline soils. Surprisingly enough, he recovered no nitrate in the solutions. Similar results were obtained by Olendskii (1931) who observed decomposition of nitrite in soils acidified with HCl, with subsequent escape of oxides of nitrogen. With higher acidities, larger quantities of nitrite were decomposed. It is stated that for best results the acids have to be in complete contact with the soil particles.

Corbet (1934) reported that nitrite decomposition is catalyzed by hydrogen ion; therefore, the higher the acidity, the greater the loss due to nitrite decomposition. He also recovered slightly larger quantities of nitrate in the solution with pH 5.08 than in the medium with pH 5.23. In the following year Corbet (1935) reported that below pH 5.0, the chemical conversion of nitrite to nitrate is very rapid while above pH 5.5 the process may cease. Corbet's (1934) concept of hydrogen being the catalyst for nitrite decomposition can be better explained on the basis that at higher acidities the proportions of nitrite existing as nitrous acid (HNO_2) are large. Nitrous acid being the reactive species (Reuss and Smith, 1965), the reaction would proceed to greater extent at higher acidities. Gerretsen and DeHoop (1957) agree with Corbet (1935) with regard to the pH limit at which losses of N in the form of oxides of N (NO and NO_2) would stop. These workers recovered much higher quantities of oxides of N than others (Clark and Smith, 1960; Tyler and Broadbent, 1958).

Clark et al. (1960) observed loss of N during the study of dissimilar nitrifying capacities of various soils. They observed negligible N losses in alkaline soils, whereas in acid soils nitrogen deficits of the order of 50 percent of the applied N were noted. Larger deficits were measured for soils in the pH range of 5.5 to 6.5 than for those in the range of 4.5 to 5.5. They suggested that possibly this reflects a more rapid conversion of nitrite to nitrate in the more acid soils according to reaction (1). Similarly Brandt et al. (1964) observed the tendency for the mineral deficits to increase during the later stages of nitrification when pH steadily decreased with time. Such losses are thought by them to have occurred through chemical decomposition of nitrite to various oxides of N.

Reuss and Smith (1965) investigated the reactions of nitrite in different acid and alkaline soils. They observed larger quantities of both nitrate N and oxides of N formed from the nitrite added to soils with lower pH. In comparing nitrite decomposition in different soils, it has to be remembered that certain soil components catalyze nitrite decomposition (Wulstein and Gilmour, 1964; Cady and Bartholomew, 1964), and the quantity of such catalysts present in the soil should be considered.

Deficits of mineral N reported above could have been observed due to causes other than the loss of oxides of N. Turtschin (1936) claimed that nitrous acid also combined to a small extent with the organic matter of the soil and these organic compounds probably undergo nitrification with the production of nitrate. Thus, some of the deficits reported might have resulted from nitrite fixation by organic matter. Such fixation of nitrite by organic matter is reported to be greater at higher acidities (Führ and Bremner, 1964a, 1964b).

Concentration effects on nitrite decomposition. There has been no work done on the effects of nitrite concentration on either its decomposition rate or on the nature of products formed. Corbet (1934, 1935b) studied the degree of completeness of nitrite disappearance in samples treated with different levels of reactant. Only in the samples receiving the highest reactant concentration (300 ppm NO_2^--N) did all the added nitrite disappear. This is contrary to the theory of Bray (1932), according to which both the vapor pressures of reactants and products determine the rate and completeness of the reaction. (For details see the section on Results and Discussion). Wagner and Smith (1958) determined various forms of N lost in urea treated acid soils. Oxides of N were assumed to have been produced through nitrite decomposition. They observed higher losses of N in the form of NO and N_2O in soils with 94 ppm nitrite N as compared to that in soils with only 12 ppm nitrite N. Allison and Doetsch (1951) determined the quantity of N_2 produced through the reaction between nitrous acid and ammonia in the system. Losses in the form of N_2 were found to increase with the concentrations of nitrite added to acid soil.

The influence of nitrite concentration on either the fixation of nitrite by organic matter (Führ and Bremner, 1964a, 1964b) or on the retention and transformation of the oxides of N by the clay colloids (Mortland, 1965) in the soil has not been investigated. Both these processes could bring about deficits in the balance sheet for nitrogen.

Mechanism of chemical nitrification

The chemistry of N transformations has drawn the attention of chemists since the latter part of the 17th century. In the introduction to his talk, Gray (1958) mentioned that in 1670 William Clark named the nitrogen dioxide fumes given off from acid solution treated with nitrite as the "flying dragon." In 1777 Priestly prepared this gas and identified and established the formula. Thus, it was known that nitrite decomposes in acid solutions yielding oxides of N. Van Slyke (1911) created NO atmosphere in closed systems using the principle that nitric oxide is given off when nitrite is added to acid solutions. By then it was known that in the presence of oxygen nitric oxide is readily converted to nitrogen dioxide. For this reason, Van Slyke (1910) evacuated his apparatus free of air before adding nitrite to acid solution.

Yost and Russell (1944) reported that copper (Cu) catalyzes nitrite decomposition to nitric oxide according to the reaction

$$Cu + 2HNO_2 \rightarrow Cu^{++} + 2NO + H_2O \quad . \quad . \quad . \quad . \quad . \quad (4)$$

Wulstein and Gilmour (1964) found iron (Fe) and manganese (Mn) to catalyze nitrite decomposition as shown in reaction (4). Similarly, Chao and Bartholomew (1964) found aluminum (Al) and iron to catalyze such reactions. Thus, the formation of nitric oxide and nitrogen dioxide during nitrite decomposition in acid solutions was proven. And the process is found to be hastened in soils containing various transition metals which catalyze the reaction.

Another school of thought (Yost and Russell, 1944) suggested nitrogen sesquioxide (N_2O_3) as the immediate compound produced by decomposing nitrite, according to reaction (2). Further, at room temperature this compound can give rise to equal quantities of NO and NO_2 (Bray, 1932) according to the reaction

$$N_2O_3 \rightleftharpoons NO + NO_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

As mentioned earlier (Bray, 1932; Yost and Russell, 1944; Gray, 1958), NO produced in this manner will be converted to NO_2 in the presence of air as

$$NO \xrightarrow{O_2} NO_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

Thus, the reactions of nitrous acid up to its decomposition to NO and NO_2 are well understood. Further reactions of NO and NO_2 are more complex. Nitrogen dioxide is said to exist in equilibrium within its dimer form (Latimer, 1952; Gray, 1958; Gray and Yoffe, 1955) as

$$2NO_2 \rightleftarrows N_2O_4 \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (7)$$

In general it is accepted (Latimer, 1952; Gray and Yoffe, 1955; Sneed and Brasted, 1958) that N_2O_4 dissolves in water yielding equal quantities of nitrous and nitric acid in accordance with the reaction

$$N_2O_4 + H_2O \rightleftarrows HNO_2 + H^+ + NO_3^- \quad . \; . \; . \; . \; . \; . \quad (8)$$

Bray (1932) reported that reaction (8) is the rate determining step in the chemical conversion of nitrous acid to nitric acid. Gray and Yoffe (1955) and Sneed and Brasted (1958) have presented the sum of reactions (2) through reaction (8) in a sequence to show non-biological nitrification as

$$4HNO_2 \rightleftarrows 2N_2O_3 + 2H_2O \quad . \; . \; . \; . \; . \; . \; . \; . \; . \quad (2)$$

$$2N_2O_3 \rightleftarrows 2NO + 2NO_2 \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (5)$$

$$2NO_2 \rightleftarrows N_2O_4 \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (7)$$

$$N_2O_4 + H_2O \rightleftarrows HNO_2 + H^+ + NO_3^- \quad . \; . \; . \; . \; . \; . \quad (8)$$

and the sum of reactions (2), (5), (7), and (8) yields reaction (1) which was proposed by Temple in 1914. It has to be noted here that NO produced through reaction (5) is assumed to be either lost in gaseous form as observed by Temple (1914) or the system is anaerobic wherein reaction (6) does not occur.

Gray (1958) has reported a different pathway for the formation of nitrate from the oxides of N. Such a reaction is said to occur in the gaseous phase as

$$3NO_2 + H_2O \longrightarrow NO + 2H^+ + 2NO_3^- \quad . \; . \; . \; . \; . \; . \quad (9)$$

This reaction is extremely temperature dependent (Gray and Yoffe, 1955; Gray, 1958) and above 300° K the reverse reaction is favored.

If for some reason reaction (8) does not occur rapidly, reaction

(9) would be the major pathway of nitrate formation. This is true if the temperature is favorable for the reaction (9).

Now if reaction (6) is assumed to take place under aerobic conditions, the reaction sequence would be

$$2HNO_2 \rightleftharpoons N_2O_3 + H_2O \quad . \; . \; . \; . \; . \; . \; . \; . \quad (2)$$

$$N_2O_3 \rightleftharpoons NO + NO_2 \quad . \; . \; . \; . \; . \; . \; . \; . \; . \quad (5)$$

$$NO + \tfrac{1}{2}O_2 \rightleftharpoons NO_2 \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (6)$$

$$2NO_2 \rightleftharpoons N_2O_4 \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (7)$$

$$N_2O_4 + H_2O \rightarrow HNO_2 + HNO_3 \quad . \; . \; . \; . \; . \; . \quad (8)$$

and the sum of the above five reactions yields

$$HNO_2 \xrightarrow{\frac{1}{2}O_2} HNO_3 \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (10)$$

Thus, all the decomposing nitrite is transformed to nitrate while according to reaction (1) only one-third the number of moles of HNO_2 decomposed would be converted to nitrate.

The above review of literature indicates that non-biological oxidation of nitrite to nitrate definitely occurs both in acid soils and solutions. Part of the reactions involved in such oxidation processes is well understood, while the nature of the rest is yet to be known. The importance of the proposed reactions under different situations with regard to various temperature and pH effects is unknown.

Production of Elemental Nitrogen

Earlier it was demonstrated that in acid soils and solutions part of the added nitrite can be lost as elemental N and thus cause N deficits. Such losses in the form of N_2 are shown to result from the reactions of nitrous acid with either ammonia (Clark and Smith, 1960; Gerretsen and DeHoop, 1957) or with the various components of organic matter (Stevenson and Swaby, 1964; Austin, 1961), other than α-amino acids, which also produce N_2 when they react with HNO_2 (Gerretsen and DeHoop, 1957; Van Slyke, 1911). Allison (1963) and Carter and Allison (1960) claim that under field conditions Van Slyke reaction is not the important pathway of N_2 producing reaction since the acid conditions in the fields are not strong enough to promote such reactions.

Tyler and Broadbent (1960) have observed N_2 production in acid soils treated with nitrite N. Mass spectrometric analysis has confirmed such results. Ewing and Baur (1958) studied the reactions between nitrous acid and alanine and observed N_2 in check samples to which only $NaNO_2$ was added. Thorne (1961) studied the nitrous acid decomposition in closed systems and recorded N_2 production in acid solutions treated with nitrite. These studies indicate the possibilities of N_2 being produced through non-biological self degradation of nitrite in acid solutions and soils.

The nature of reactions involved in the self degradation of nitrous acid to yield N_2 is obscure. In the following pages, possible intermediate compounds that could arise during nitrite decomposition are discussed. Some of the mechanisms of reactions between the possible intermediate compounds and nitrous acid are also considered.

Probable intermediate compounds of nitrite decomposition and their role in producing N_2

The discovery of N_2 production during nitrous acid decomposition being recent, the mechanisms and intermediates involved are obscure. In most of the references cited below, only the biological oxidation of ammonia to nitrite is considered. It is assumed that most of the reactions are reversible, and the intermediates produced in the forward reactions also probably appear during the reverse reactions. If this is a valid assumption, it can be said that the intermediate compounds produced in the process of ammonia oxidation to nitrite may also be formed during the decomposition of nitrous acid. In addition, the fact that both oxidative and reductive processes are involved in nitrite decomposition adds support to the assumption.

Hydroxylamine (NH_2OH), hyponitrous acid ($H_2N_2O_2$), and hyponitric acid ($H_2N_2O_3$) are the three intermediate compounds suspected to be formed in the interconversions of ammonia and nitrite N. Mumford (1914) observed hydroxylamine salts and salts of nitrous and hyponitrous acid during the oxidation of ammonia. Loss of N invariably encountered during the study was attributed, in part, to the evolution of N_2 through complex interactions between these various intermediate compounds. Corbet (1934, 1935a) obtained somewhat identical results, but he observed only hyponitrous acid as an intermediate compound in the system. According to Corbet (1934), hydroxylamine has only an ephemeral existence, and it is converted to hyponitrous acid as fast as it is formed. In addition, it is stated that in the presence of hydroxylamine, loss of N as N_2 due to the interaction of nitrous acid and hydroxylamine was expected to be greater than that observed. Lees (1948) also obtained data that supported the production of hyponitrous acid during ammonia oxidation. However, Martin et al. (1942) presented thermodynamic data favoring the formation of both hydroxylamine and hyponitrous acid. But thermodynamically, the formation of hydroxylamine is not spontaneous (Martin et al., 1942).

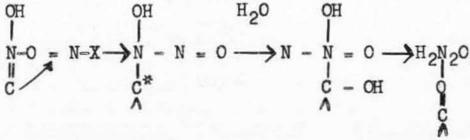
Thorne (1961) is the only person who suggested hyponitric acid as a probable intermediate produced in nitrite decomposition reactions.

Emele'us and Anderson (1938) claimed that this acid is highly unstable and decomposes instantly in water in accordance with the reactions

$$H_2N_2O_3 \rightleftarrows 2NO + H_2O \quad . \ . \ . \ . \ . \ . \ . \ . \ . \quad (11)$$

$$2H_2N_2O_3 \rightleftarrows 2HNO_2 + H_2N_2O_2 \quad . \ . \ . \ . \ . \ . \ . \quad (12)$$

Thus, the formation of $H_2N_2O_2$ is favored more than that of $H_2N_2O_3$. Austin (1961) has proposed a mechanism for the production of hyponitrous acid through the nitrosation of various organic compounds with nitrous acid. Such a proposed reaction is as follows:



. . . (13)

Thus, there are possibilities for various intermediate compounds to occur during nitrite decomposition. Also these compounds can either react with each other or decompose to yield N_2.

Mechanism of N_2 formation during nitrite decomposition

Formation of elemental nitrogen (N_2) as the end product of nitrous acid decomposition can result from either the sequential breakdown of the intermediate compounds or due to the interaction of nitrous acid with the intermediate compounds. The latter type of reaction is supported by most workers while there are a few evidences to show the sequential reduction reactions of nitrous acid that lead to the formation of elemental N.

Formation of N_2 by the interaction of various intermediate compounds is supported by the fact that nitrogen is also oxidized to nitrate during the process.

Thorne has proposed the formation of N_2 and NO_3^- according to the reaction

$$5HNO_2 \quad N_2 + 3H^+ + 3NO_3^- + H_2O \; . \; . \; . \; . \; . \; . \quad (14)$$

The interaction of hyponitrous and hyponitric acid is suggested to be the reaction responsible for N_2 production by Thorne as

$$H_2N_2O_3 + H_2N_2O_2 \quad N_2 + H^+ + NO_3^- + H_2O \; . \; . \; . \; . \; . \quad (15)$$

Alternatively, reaction (14) can be obtained through another reaction sequence as

$$4HNO_2 \quad 2HNO + 2HNO_3 \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (16)$$

$$2HNO \quad H_2N_2O_2 \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (17)$$

$$H_2N_2O_2 + HNO_2 \quad N_2 + H^+ + NO_3^- + H_2O \; . \; . \; . \; . \quad (18)$$

Here the formation of hyponitrous acid from 2HNO molecules is plausible as proposed by Corbet (1935b).

In general, the review of literature presented here indicates that there is ample scope for investigations on various reactions involved in nitrous acid decomposition. The nature of nitrification reactions is yet to be understood while the reactions that lead to the formation of N_2 through the self degradation of nitrous acid is almost unknown.

THEME AND OBJECTIVES OF INVESTIGATION

An extensive review of literature on the reactions of nitrite in acid media clearly indicates that most of the processes affected by biological agents such as bacteria also occur in nature due to purely chemical and thermodynamic forces. Some of the processes lead to the conversion of nitrite to nitrate N, which is more easily assimilated by plants than the former. At the same time, reactions of nitrite in acid media result in the loss of N in the forms of various oxides of N and also in its elemental form.

At the present time, the use of nitrogenous fertilizers to increase crop yields is steadily increasing. For the efficient and economical use of various fertilizers, attention has been focused on the controlled availability fertilizers which in theory release the nutrients at a slower rate in order that the required quantities of nutrients are made available to the crop plants throughout the growing season. N-serve (2-chloro-6 /trichloro-methyl/ pyridine) is one such commercial compound used to control the availability of N fertilizer. N-serve is used to reduce the nitrification rate so that all the added N is not lost through leaching after being converted to nitrate. Such chemicals are said to control biotic activities.

On the other hand, if most reactions are chemically feasible to occur without the action of biological agents, the advantages of certain controlled availability fertilizers will be outweighed. Thus, the knowledge of the nature and magnitude of non-biological N transformations can be useful for the more efficient use of controlled availability fertilizers. Scientific information on such reactions may be quite useful in pre-determining the time and rate of applications of fertilizers to minimize the losses of N. It may also be of value in choosing the proper form of fertilizer for each soil condition.

With the above viewpoints in mind, the present investigation was undertaken to study the following.

1. To study the effects of pH and concentration of the added nitrite on non-biological nitrification of nitrite in soil and resin systems.

2. To verify some of the proposed pathways of nitrite decomposition.

3. To determine the environmental conditions wherein each of the proposed reactions would be the major pathway of nitrite decomposition.

4. To determine the pH and nitrite concentration effects on the loss of nitrite in the elemental form.

5. To obtain indirect evidences for the occurrence of certain intermediate compounds produced during the formation of N_2 from nitrite.

6. To determine the possible mechanisms for the conversion of nitrite to N_2.

MATERIALS AND METHODS

Experimental

The present investigation mostly consisted of adding nitrite in the form of sodium nitrite to various acid soils and resin systems and of quantitatively determining various products of nitrite decomposition. In a separate series of experiments, the nature and mechanism of N_2 production from the added nitrite were investigated. In all other respects, the procedures used were common to both experiments.

Materials

Six acid soils ranging in pH values from 4.6 to 6.1 were used in this investigation. Some of the chemical and physical characteristics of the soils used are presented in Table 1. For the experiments with resin system, Dowex-50W-X8, 20 to 50 mesh spherical cation exchange resin was used. All the systems, adjusted to about 0.3 bar moisture tension, were incubated in constant temperature cabinets maintained at 30 C.

Treatment

Soil and resin systems received different treatments depending upon the purpose of the experiments.

Experiment on non-biological nitrification. The main purpose of these experiments was to investigate the effect of pH and nitrite concentration on the process of non-biological nitrification. In this set of experiments, Miami silt loam, Olympic silty clay loam, and Dayton silty clay loam were treated with 75, 150, and 300 ppm nitrite N

Table 1. Some of the physical and chemical characteristics of the soils used in this investigation

Soil	pH	Fe	Organic matter	N	Moisture content 0.3 bar	 Air dry
	paste	ppm[a]	percent	percent	percent	percent
Olympic silty clay loam	4.6	520	6.98	---	34.0	3.12
Dayton silty clay loam	4.9	1459	3.76	---	30.2	2.00
Clermont silt loam	5.2	669	1.72	0.083	25.7	0.68
Miami silt loam	5.8	525	2.01	0.109	24.2	1.24
Logan Canyon silty clay loam	5.8	1169	4.04	0.179	28.4	2.02
Walla Walla (Ore.) silt loam	6.1	--	1.90	0.094	21.9	1.23

[a] Fe was determined in 1 percent EDTA extract of soil.

supplied from sodium nitrite ($NaNO_2$). Miami silt loam was also studied after adjusting to different pH values by adding the required quantities of sulfuric acid. The treatments were arranged in a 3 x 4 factorial design with two factors. In a resin system adjusted to pH 4.0, decomposition of only 300 ppm nitrite N was investigated. In all cases, experiments were conducted with and without the alkaline permanganate trap solutions within the flasks. These trap solutions absorbed oxides of N produced in the flasks.

Experiments on the nature of N_2 producing reactions. Alkaline permanganate trap solutions were not used in any of these experiments. Various treatments given to different systems include the following:

(a) Study of N_2 production in nitrite treated systems with and without the alcohol was conducted by adding 5 ml of 95 percent ethyl alcohol to the soil and resin systems. Alcohol treated samples received 2.5 percent more moisture in order to compensate for the alcohol vapor that was lost from the system during air evacuation process described later.

(b) Influence of 250, 500, and 1,000 ppm iron (Fe) added as $FeSO_4 \cdot 7H_2O$ was investigated in Miami silt loam adjusted to pH 4.6.

(c) Toxic effects of excess intermediate and/or the end product on N_2 producing reactions was investigated by adding 150 ppm nitrate N and about 10 mg nitric oxide N to the system prior to the addition of nitrite.

(d) Prior to the addition of nitrite to the samples, soils were incubated with nitric oxide under aerobic conditions for various lengths of time. In each of the above experiments, a randomized block design was used.

Preparation of sample

Soil system. Each soil used was obtained from different states through different persons. Soils thus obtained were air dried, ground, passed through 2 mm sieve, and stored in huge tin cans. Air dry moisture was determined, and it was taken into account while weighing enough soil to give 100 g oven-dry weight per each sample. To the soil weighed into waxed paper cups, 10 percent less the water content required to bring about 0.3 bar moisture tension was added, allowed to stand for about 10 minutes, and then stirred well with a spatula. After thorough mixing of water and soil, the sample was transferred to 250 ml suction flasks. Within the suction flasks, two plastic vials were placed. One of them contained 5 ml of 0.2 M $KMnO_4$ in 2 N KOH solution (traps) to absorb NO and NO_2 produced. Due to the alkaline nature of the traps, CO_2 produced was also absorbed. To the other vial 10 ml $NaNO_2$ solution containing the required quantity of nitrite N was added. Only one vial with nitrite solution was placed inside the flask whenever the experiment was conducted without the trap.

In the initial stages of investigation, the non-biological nature of nitrite decomposition reactions was verified by working with sterile soils. In this case air-dry soil was weighed directly into the suction flasks and sterilized by autoclaving at 121 C for 30 minutes. Before sterilization, the openings of the suction flasks were closed with cotton plugs. After sterilization water was added to the soil and stirred with a sterile spatula. Sterile $NaNO_2$ solution was used, and every care was exercised to have a sterile system for incubation.

In the event of acidifying Miami silt loam, sulfuric acid was added to the soil along with water. A calibration curve was obtained to determine the quantities of sulfuric acid that are required to adjust the pH of the soil to values of 3.6, 4.0, 4.5, and 5.0. The required quantity of the acid was added to each sample and mixed thoroughly in paper cups.

Resin system. In some of the experiments Dowex 50W-X8 cation exchange resin was used instead of soil. The resin was saturated with calcium (Ca^{++}) and then adjusted to the required pH by adding fresh hydrogen (H^{+}) saturated resin in small increments. The resin was then soaked in potassium phthalate buffer solution with pH similar to that of the resin. Excess moisture from the resin was removed with the help of aspirators by sucking through porcelain aeration stones.

One hundred grams of resin thus prepared were weighed directly into the suction flasks. Two plastic vials were placed within the flasks for the same purpose as explained earlier.

Replacement of atmospheric air within the flasks and incubation technique

The gas within the flask has to be evacuated free of N_2 in order to determine the quantity of N_2 evolved from the added nitrite. For this 250 ml suction flasks were used. The flasks were sealed air tight with rubber stoppers in which a hole had been bored in the middle. The holes were fitted with soft rubber serum caps on either end with a silicone disc between them. This permitted both evacuation of air and subsequent sampling of gases with hypodermic syringe without contaminating the air within the flasks with atmospheric N_2 (Reuss and Smith, 1965).

Flasks containing the sample and two separate plastic vials having trap and nitrite solutions were connected to a manifold by means of rubber tubing. This manifold was connected to a vacuum pump, mercury manometer, and to helium and oxygen tanks separately. Following the evacuation of the air from the flasks and the whole manifold by the suction pump, flasks were filled with helium. Each time the suction pump was allowed to operate for 10 minutes before stopping the suction, and flasks were filled with helium.

This procedure was repeated three or four times. After the final evacuation, the flasks were filled with a pre-determined quantity of oxygen along with helium in order to have 20 percent oxygen and 80 percent helium within the flasks. At this stage, a negligible quantity of N_2 present in the flasks was determined, and the value was subtracted from the readings obtained later. The final pressure within the flasks was adjusted to about 850 to 900 mm Hg. This positive pressure within the flasks prevented the contamination of gas within the flask by atmospheric N_2, and it facilitated sampling of gases by the hypodermic syringe.

After the N_2 concentration within the flasks was reduced to a negligible level, nitrite solution in the plastic vial was poured into the sample by tilting the suction flask. At the same time care was taken to prevent either spilling or contaminating the trap solution. Addition of nitrite solution in this manner after flushing the samples free of N_2 prevented the nitrite reactions that could have occurred during the process of air evacuation as observed by Reuss and Smith (1965).

Of the 15 samples used in each of the nitrification experiments, ten of them received nitrite and the rest were used as check samples. Samples were incubated at 30 C for 12 hours. Two treated and one check sample were analyzed after 1, 3, 5, 9, and 12 hours of reaction time.

In the study of the evolution of elemental N, the number of samples varied with the number of treatments. Three replicate samples were used in each experiment for the investigation. Periodic analysis of only the gases was carried out.

Analytical

Both gaseous and non-gaseous nitrogen compounds produced as a result of nitrite decomposition in acid media were identified and determined quantitatively. Of the gaseous products N_2 and N_2O were determined by gas chromatograph and the oxides of N absorbed in trap solutions were analyzed for nitrate. Nitrite and nitrate were the two non-gaseous products determined colorimetrically after extraction from soil samples and resin media.

Analysis of gaseous products

Analysis of elemental N(N_2). Beckman GC-2 gas chromatograph with dual column attachment in combination with a Bristol recorder was used for N_2 analysis. A six-foot molecular sieve (13X) column was used for the separation of N_2 and O_2 in the sample. A fourteen-inch charcoal column was used for N_2O determinations. Analysis of the gas samples was carried out at 40 C with helium as a carrier gas flowing at the rate of 0.33 liters per minute and with the filament current of 260 milliamperes.

One milliliter (ml) gas samples from the incubation flasks were withdrawn by means of a hypodermic syringe and injected into the gas chromatograph for N_2 analysis. Peak heights that appeared on the recorder chart were used as the indicative of the quantity of N_2 in the sample. Separate calibration curves prepared for each column were used for calculating the amount of N_2 in the sample. Using flask volumes determined separately and the ideal gas law, the quantities of N_2 present in the flasks were calculated.

Analysis of oxides of N ($NO + NO_2$). All of the oxides of N produced during nitrite decomposition were assumed to have been absorbed by the trap solution. Oxides of N absorbed are oxidized within the traps by potassium permanganate to nitrate N. After the flasks were opened for soil analysis, the trap solutions were analyzed. The complete trap solutions were transferred to tall digestion beakers, and the plastic vials washed with water. This rinse water was added to the beakers to recover completely the N in the trap solutions. Then the color of $KMnO_4$ was destroyed by adding the required quantities of acid oxalate (0.6 M sodium oxalate in 7 N sulfuric acid), and the solution was made alkaline again with NaOH. This solution was analyzed for nitrate N by the phenoldisulfonic acid method (Jackson, 1958). Detailed methods of this procedure are presented in the Appendix.

Analysis of non-gaseous N products

Nitrite and nitrate were the two non-gaseous N compounds determined. Ten gram samples were extracted with 100 ml of saturated calcium hydroxide ($Ca[OH]_2$) solution and the aliquots were analyzed for nitrite by sulfanilamide methods (Shinn, 1941) and nitrate by phenol disulfonic acid (Jackson, 1958). Stepwise procedures followed in the determination of nitrite and nitrate N are described in detail by Mahendrappa (1963).

RESULTS AND DISCUSSION

There were two main objectives of this investigation, namely, to study nitrite decomposition in acid media with regard to the nature of reactions involved in the process of non-biological nitrification and to investigate the mechanisms of nitrite decomposition reactions that lead to the evolution of N_2. Accordingly, the results of this study are presented in two separate sections for the clarity of the findings on these two aspects of nitrite decomposition.

Experiments on Non-biological Nitrification

Olympic, Dayton, and Miami soils were used for this study. In one experiment cation resin adjusted to pH 4.0 and treated with 300 ppm nitrite N were studied. In this set of experiments, the samples were analyzed for both gaseous and non-gaseous decomposition products of three levels of nitrite N added.

Nitrite disappearance

The patterns of nitrite transformations observed in three acid soils and a cation resin system treated with 75, 150, and 300 ppm nitrite N are presented respectively in figures 1, 2, and 3. During the first few hours of reaction time, the most rapid nitrite transformation occurred in all the media regardless of the pH and concentration of N added. Subsequent hourly increments of nitrite disappearance decreased steadily in all the media. Such a steady decrease in the rate of nitrite breakdown may be caused by the buildup of some of the

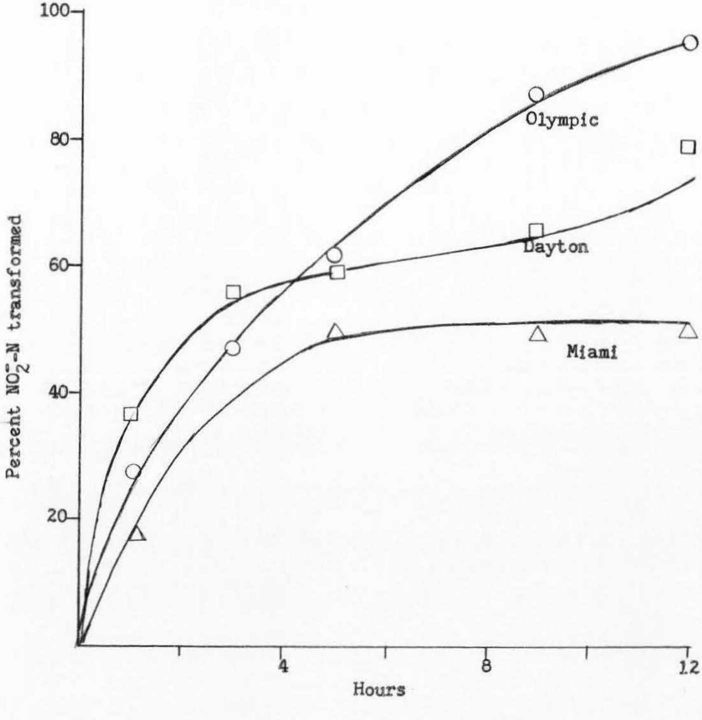


Figure 1. Pattern of nitrite transformations in different soils treated with 75 ppm NO_2^--N and incubated for various lengths of time.

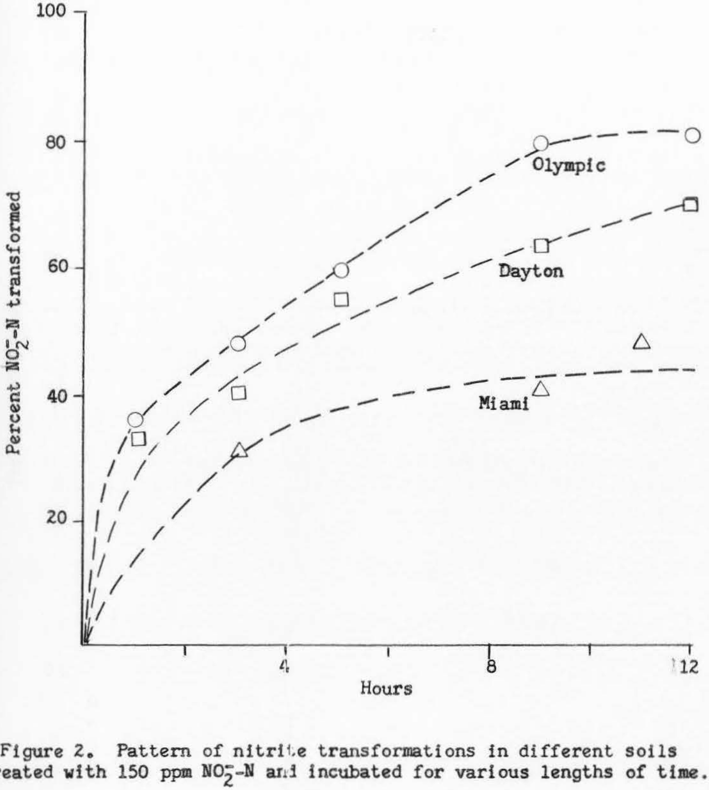


Figure 2. Pattern of nitrite transformations in different soils treated with 150 ppm NO_2^--N and incubated for various lengths of time.

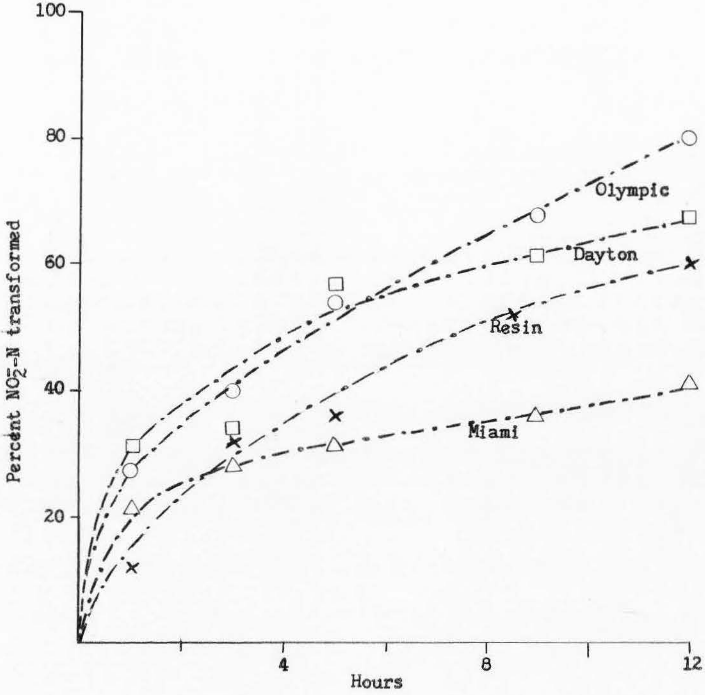


Figure 3. Pattern of nitrite transformation in various media treated with 300 ppm NO_2^--N and incubated for various lengths of time.

intermediate or end products. Bray (1932) presented evidences in support of earlier work that nitrous acid decomposition according to reaction (1) follows the relation

$$\frac{-d(HNO_2)}{dt} = k \frac{(PHNO_2)^4}{(PNO)^2} \quad . \ . \ . \ . \ . \ . \ . \ . \quad (19)$$

According to this equation, buildup of the pressure of NO slows down the reaction. In addition, the vapor pressure of nitrous acid is directly dependent on its concentration in the solution (Abel, 1931; Bray, 1932). Thus, in this study the vapor pressure of nitrous acid probably decreased with time because of the decrease in its concentration. Such a decrease in the vapor pressure of reactant (HNO_2) and a subsequent buildup of product (PNO) naturally results in the slowing down of the reaction. Such relations hold true in pure solutions, but in soil systems reactions are more complicated.

In this study the quantities of nitrite N decomposed at each sampling time were greater in the samples receiving the higher nitrite level. Until about 5 hours of reaction time, the rate of nitrite decomposition showed linear relationships with the added nitrite concentration. However, at the end of 12 hours, the decrease in the rate of nitrite disappearance was greater in the samples treated with higher levels of nitrite. This indicates that the process of non-biological nitrite decomposition in soil is not as simple as that explained by Bray (1932). Figures 1, 2, and 3 show that higher percentages of added nitrite decomposed in samples receiving smaller doses of N. If the process was an equilibrium reaction, the same percentage of the reactant must have been transformed. Under field conditions in acid soils where nitrite may appear in concentrations of the order studied here, most N transformations can occur without the biological activity.

Among the various acid soils studied, Olympic silty clay loam showed maximum nitrite transformation and was followed by Dayton silty clay loam and Miami silt loam. Olympic soil has the lowest and Miami soil the highest pH among these soils (Table 1). Thus, pH effect can partly account for these variations in the ability of the soils to transform nitrite. This is in agreement with earlier reports of Olendskii (1931), Robinson (1923), and Corbet (1934, 1935), who found the rate of nitrite decomposition reaction to increase with acidity. In acid media nitrite exists in the form of nitrous acid (HNO_2) which is more reactive than nitrite ion (Sneed and Brasted, 1958; Reuss and Smith, 1965). Thus, in Olympic soil with the highest acidity, greater quantities of nitrite were transformed than in others with higher pH. The decrease in hourly increments of nitrite decomposition was greater in Miami and Dayton soils than in Olympic soil. Thus, pH also was found to affect the completeness of the reaction. At higher acidity the reaction proceeded to more completion than at higher pH. Thus, apart from the concentration effects of nitrite, pH also plays an important role in nitrite decomposition.

Cation resin with pH 4.0 transformed lesser quantities of nitrite when treated with 300 ppm nitrite N than the Olympic silty clay loam with pH 4.6 (figure 3). In this case the nitrite concentration was the same in both media; thus the reasoning on the basis of pH effect does not account for all the differences observed. This indicates the importance of the role played by both the mineral and the organic components of the soil on the process of nitrite decomposition. Yost and Russell (1944), Wulstein and Gilmour (1964), and Chao and Bartholomew

(1964) showed that various transition metals present in the soil catalyze nitrite decomposition. In addition, Austin (1961), Stevenson and Swaby (1964), and Reuss and Smith (1965) found that organic matter also enhances nitrite decomposition. Thus resin without organic matter and mineral elements showed less nitrite disappearance than Olympic soil. Nitrite could also be fixed by organic matter (Turtschin, 1936; Führ and Bremner, 1964a, 1964b). Such fixation also is reported to increase with acidity (Führ and Bremner, 1964a). However, fixation is probably not completely responsible for lesser recovery of nitrite in this investigation since most of the nitrite disappearing was recovered in other forms of mineral N.

Both the concentration of added nitrite and the reaction of the media probably influence the self degradation of nitrite. Such an effect is clearly shown in figure 4, which represents the various quantities of nitrite transformed at the end of 12 hours reaction time in Miami silt loam acidified to various pH values and treated with three levels of nitrite. Percentages of the added nitrite that decomposed were found to increase significantly with decrease in both pH values and the quantities of added nitrite.

Concentration of the reactant added (nitrite N) was found to influence the quantities of nitrite decomposed in all the soil, including the acidified Miami soil. Thus, nitrite decomposition in soils is not a simple equilibrium as postulated by earlier workers (Bray, 1932). Therefore, some factors present in the soils limit either the forward or reverse reactions involved in nitrite decomposition. Again, the fixation of nitrite by organic matter (Führ and Bremner, 1964a) and/or the adsorption of oxides of N by mineral components of the soil

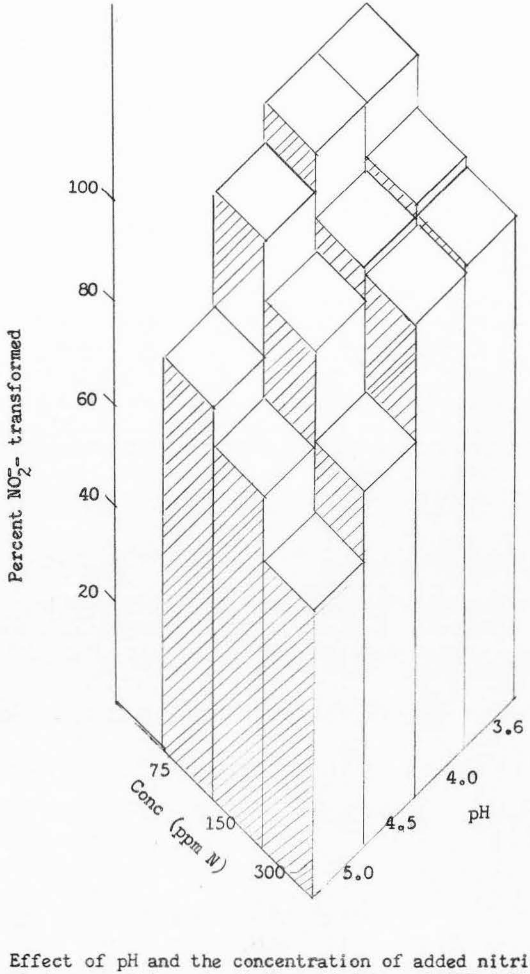


Figure 4. Effect of pH and the concentration of added nitrite on the percent autodecomposition of nitrite added to acidified Miami silt loam, and incubated for 12 hours.

(Mortland, 1965) may in part explain the results. However, these processes are not the probable causes (see the section on Theoretical Considerations).

Keeping in mind that nitrosation of organic matter (Austin, 1961) and nitrite fixation by organic matter (Führ and Bremner, 1964a) can cause the disappearance of nitrite, it can be concluded that factors such as the reaction of the media, the quantity of the reactant added, and the soil component that catalyzes the reaction, influence the process of non-biological nitrite decomposition. Overall evaluation of the results indicates the complex interaction between the above-mentioned factors. For instance, Olympic silty clay loam transformed more nitrite than the resin system with a lower pH value than the former. Thus factors in the soil dominated the pH effects. At the same time, Dayton silty clay loam with twice the amount of EDTA extractable iron (Table 1) as compared to Olympic silty clay loam transformed comparatively lesser quantities of nitrite N than the latter (figures 1, 2, and 3). Though iron is shown to catalyze nitrite decomposition (Wulstein and Gilmour, 1964; Chao and Bartholomew, 1964), Olympic soil with lower pH and higher organic matter content promoted nitrite decomposition to an extent greater than that by Dayton silty clay loam with higher pH and smaller quantities of organic matter than the former.

Formation of the oxides of nitrogen

Nitric oxide (NO) and nitrogen dioxide (NO_2) were the two oxides of N determined quantitatively. These two oxides were absorbed in alkaline permanganate traps placed within the incubation flasks containing samples. Analysis of the gases in the flasks for nitrous oxide (N_2O) was done with gas chromatograph fitted with a 14-inch column filled with active carbon. Nitrous oxide was not detected until the oxygen level reduced to about one-half the initial level. Thus, it was not possible to consider it as one of the products of nitrite decomposition under aerobic conditions.

Various quantities of NO and NO_2 recovered at different reaction times in Olympic and Dayton silty clay loams and in resins system adjusted to pH 4.0 are presented in figure 5. There was considerable doubt with regard to the ability of the trap solution to absorb all the oxides of N produced in the system. Unlike the trend of nitrite decomposition, there was a linear relationship between the quantities of the oxides of N absorbed and the reaction time. Thus, although NO and NO_2 are the products formed immediately following nitrite decomposition (Sneed and Brasted, 1958), there is no quantitative relationship between the reactants transformed and the accumulation of products. Throughout the reaction time and in all the media studied, the proportions of nitrite N recovered as NO and NO_2 were found to increase with decrease in the concentration of added nitrite.

In Olympic silty clay loam maximum quantities of NO and NO_2 were recovered. The quantity recovered in Olympic soil at all the nitrite levels was greater than that recovered in Dayton soil and in resin systems. Here again the pH effect was clear only when two soils were compared. On the other hand, both soils with pH values higher than that of the resin system yielded more nitrogen oxides than the latter. This was probably the effect of catalysts present in the soil systems and not in resin media.

Oxides of N were obtained in all the samples of Miami soils. Figure 6 represents various proportions of NO and NO_2 recovered in

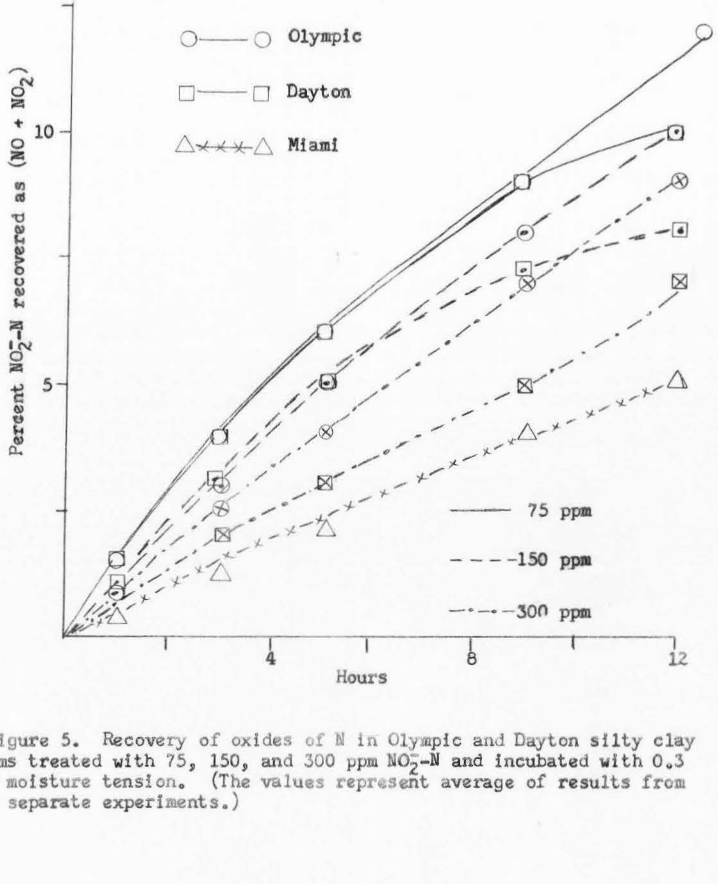


Figure 5. Recovery of oxides of N in Olympic and Dayton silty clay loams treated with 75, 150, and 300 ppm NO_2^--N and incubated with 0.3 bar moisture tension. (The values represent average of results from two separate experiments.)

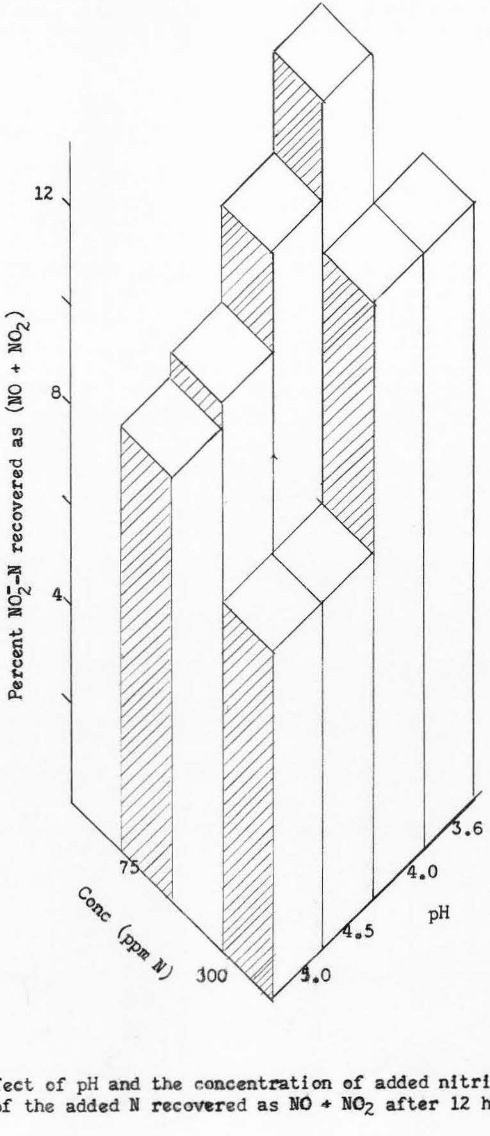


Figure 6. Effect of pH and the concentration of added nitrite on the percentage of the added N recovered as NO + NO_2 after 12 hours of reaction time.

Miami silt loam adjusted to different pH values and treated with 75, 150, and 300 ppm nitrite N (NO_2^--N).

The proportions of nitrite N converted to oxides of N were found to vary inversely with the reaction of the medium (pH) and the concentration of reactants. Formation of NO and NO_2 in Miami silt loam with pH 5.8 is contradicing the earlier postulations (Corbet, 1934; Fraps and Sterges, 1939; Gerretson and DeHoop, 1957) that above pH 5.5 nitrite decomposition may cease. Yet it is in agreement with some of the recent reports (Allison, 1963; Cady and Bartholomew, 1963; Clark and Smith, 1960; Reuss and Smith, 1965), according to which nitrite could decompose to NO even at pH values around 6 and 6.5.

In this investigation, relatively small proportions of added nitrite were recovered as NO and NO_2. This is in agreement with earlier reports of Smith and Clark (1960) and Tyler and Broadbent (1960), who found only traces of oxides of N. But Gerretson and DeHoop (1957) and Reuss and Smith (1965) obtained much larger quantities of N as NO and NO_2. Reuss and Smith (1965) attributed this contradiction to differences in the techniques used to absorb the nitrogen oxides. However, in this investigation the trapping system used was identical to that adapted by Reuss and Smith (1965). Therefore, it is suggested here that the temperature conditions under which the experiments are carried out limit the extent of nitrogen oxides produced from nitrite. Gray (1958) reported nitrogen dioxide as the common reactant at higher temperatures, and at temperatures above 27 C it is the major gaseous N compound that would remain in the system. Although this may account for the differences between results obtained by Reuss and Smith and those by Tyler and Broadbent, it evidentally is not the sole reason. In spite of the fact that the temperature of the incubation chamber used in this study was the same as that used by Reuss and Smith, less oxides of N were recovered in this experiment. Alternatively it can be suggested that the quantity of NO and NO_2 recovered from decomposing nitrite depends on temperature (Gray, 1958), the rate of the removal of oxides from the system (Gerretsen and DeHoop, 1957), the ability of trap solution to absorb the oxides produced (Reuss and Smith, 1965), and the kind and amount of clay colloids and cations present on clay particles (Mortland, 1965). All these factors exhibit some complex interaction, and the net effect is determined by the interactions instead of by individual factors. Therefore it is suggested that investigations in simple systems must be carried out.

Nitrate (NO_3^-) production (nitrification)

Biochemical conversion of ammonia and its decomposition products to nitrate is called nitrification. Therefore, it would be appropriate to term the process of nitrate formation through chemical decomposition of nitrite as "non-biological nitrification." Throughout this paper the terms nitrate production from nitrite and/or the conversion of nitrite to nitrate will be used to refer to the process of non-biological nitrification and will be used interchangeably with one another.

The patterns of non-biological nitrification observed in different acid media treated with 75, 150, and 300 ppm nitrite N are presented respectively in figures 7, 8, and 9. Regardless of the nature of the media and nitrite concentration added, the rate of nitrate formation was the fastest during the first 3 hours after the addition of nitrite to the samples. After the period of fastest nitrification, there was

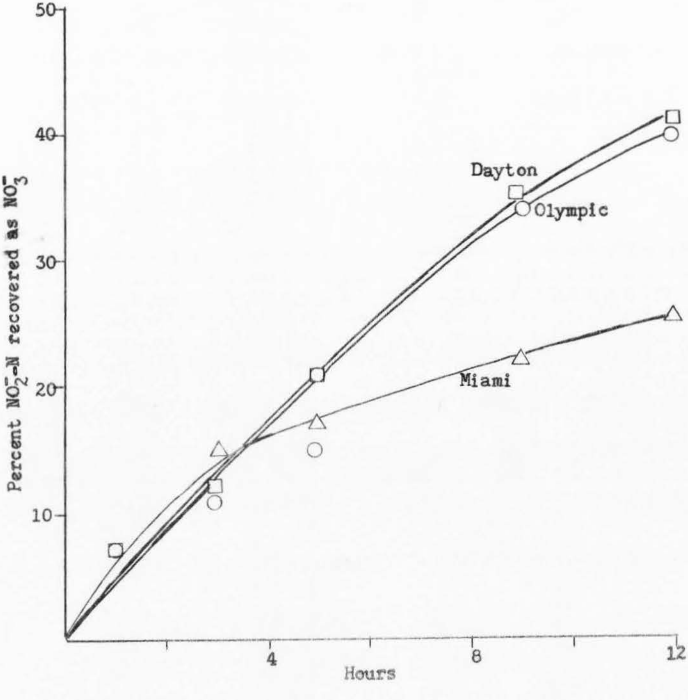


Figure 7. Nitrate N recovery in different soils treated with 75 ppm nitrite N and incubated for various periods of time.

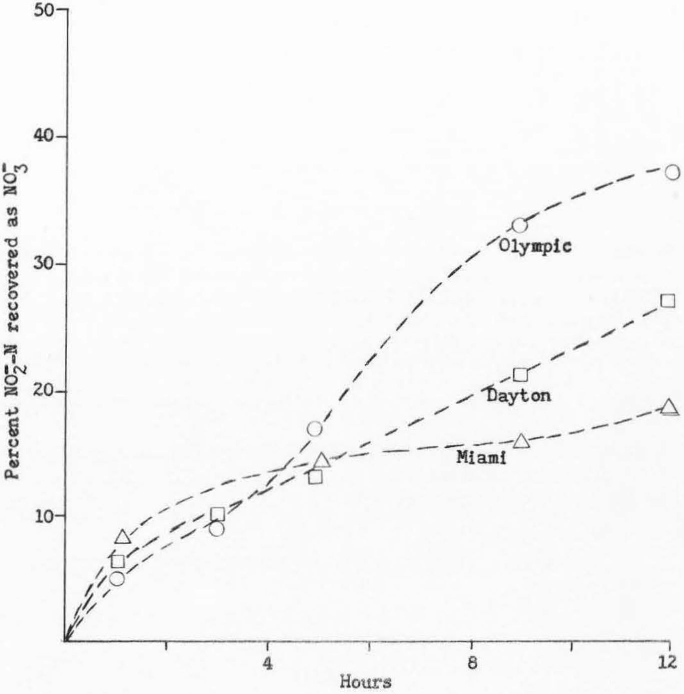


Figure 8. Nitrate N recovery in different soils treated with 150 ppm nitrite N and incubated for various periods of time.

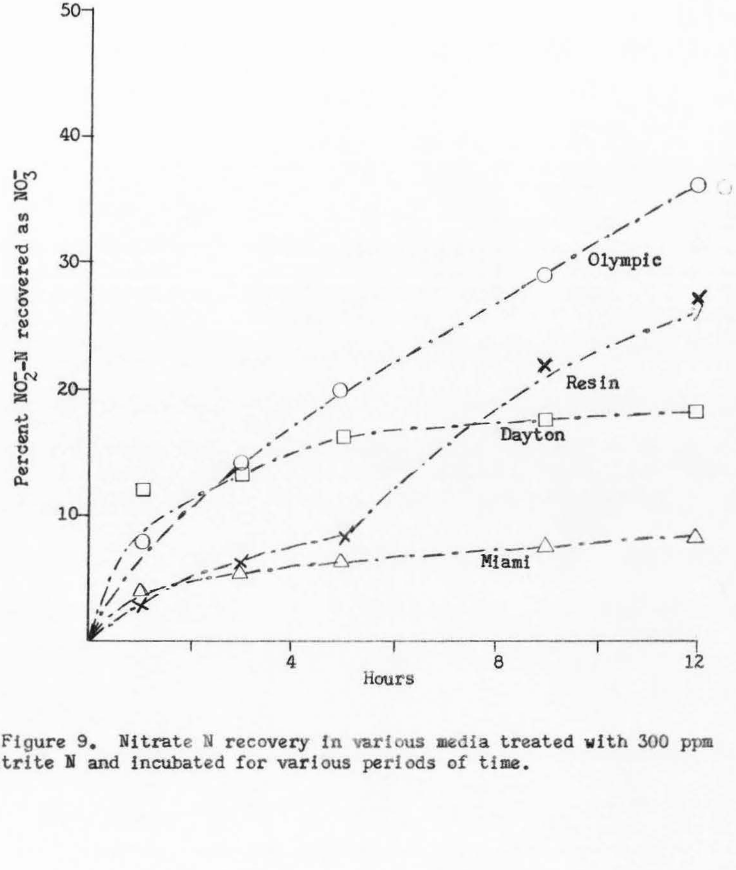


Figure 9. Nitrate N recovery in various media treated with 300 ppm nitrite N and incubated for various periods of time.

a steady decrease in the rate. Such a decrease was smaller in samples to which smaller quantities of nitrite N were added. Ultimately, at the end of 12 hours reaction time, higher percentages of the added nitrite were recovered from the media incubated with smaller amounts of nitrite N (figure 10). This is contrary to earlier reports (Corbet, 1934; Fraps and Sterges, 1939) who found complete nitrification of all the nitrite only in samples that contained larger N content. But those data do not sound logical for one main reason, that if the process was more nearly completed in samples with greater quantities of nitrite N, higher concentrations of products must promote the reaction. If that is true, the nitrification pattern must resemble the curves of autocatalytic reactions. Instead, there is neither any resemblance between the nitrification pattern and autocatalytic reactions, nor any support for the view that the products favor forward reaction. Bray (1932) found the reaction (1) to be inhibited by higher NO pressures. Similarly, Yost and Russell (1944) and Thorne (1961) have postulated the probable inhibitory effects of nitrate N on the process of nitrification.

Thus in samples containing larger quantities of nitrite N and exhibiting faster nitrification in the initial stages of reaction time, the probable accumulation of inhibitory products in greater quantities caused a greater reduction in the rate of the nitrification process. On the other hand, in samples treated with smaller quantities of nitrite N, the probable inhibitors may not accumulate in toxic quantities. Thus, the reaction proceeded to a greater extent and higher percentages of the added nitrite were converted to NO_3^--N. Until 5 hours of reaction time, non-biological nitrification patterns were identical in all the three soils treated with 75 and 150 ppm nitrite N. At the lowest level

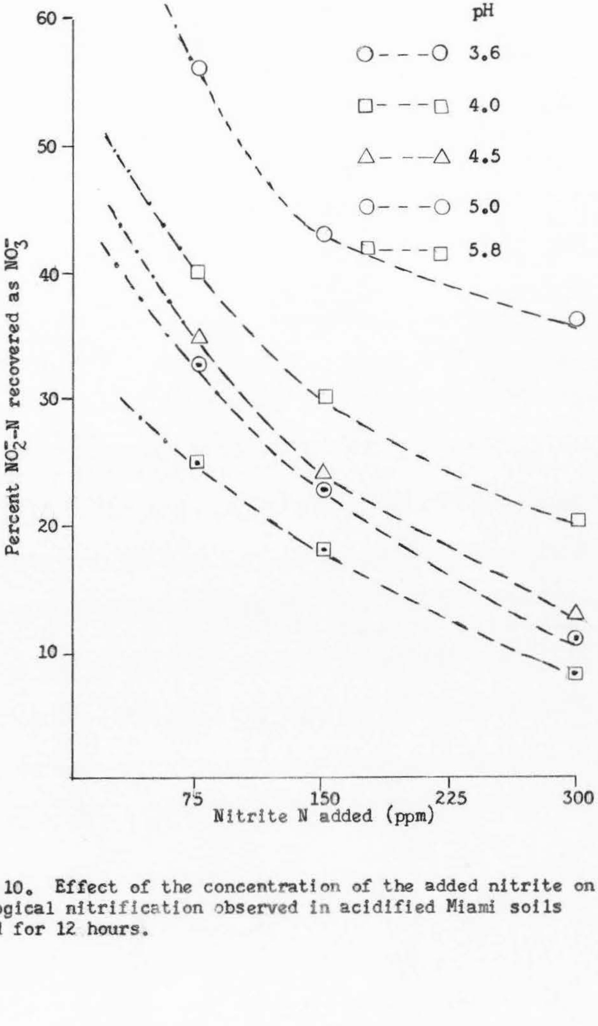


Figure 10. Effect of the concentration of the added nitrite on the non-biological nitrification observed in acidified Miami soils incubated for 12 hours.

of nitrite treatment, nitrification observed in Olympic and Dayton silty clay loams was identical throughout the incubation period, while in samples treated with 300 ppm nitrite N, they were similar to each other only up to 3 hours of reaction time. In most cases the differences were observed after approximately 4 to 5 hours.

In all the media the decrease in the rate of non-biological nitrification was found to be greater in samples treated with higher concentrations of nitrite. The smallest decrease in the rate was observed in Olympic silty clay loam, and Miami silt loam showed the greatest reduction in the rate of nitrification with time. Dayton silty clay loam was in between these two limits. Probably this was the effect of pH. Higher acidity, as was the case with Olympic soil, favors the reaction.

These results support the concept that nitrous acid is the reactive species of nitrite which undergoes decomposition to yield nitrate and various other products (Reuss and Smith, 1965). The quantity of nitrite that exists in the reactive form of nitrous acid is shown to be a function of the reaction of the media (Yost and Russell, 1944; Gray and Yoffe, 1955; Sneed and Brasted, 1958; Reuss and Smith, 1965). Thus, Olympic silty clay loam with highest acidity showed maximum nitrification as compared to Dayton and Miami soils which have higher pH values than the former. However, any other possible effects of higher acidities on nitrite decomposition cannot be overruled. Catalytic effects of H^+ (Olendskii, 1931) may be a factor, but it is not possible to differentiate these effects. It may also be concluded with caution that inhibitors are probably competitive in the nature of their action and such a competition is related to quantity of reactive species of nitrite N (HNO_2) present in the medium.

The reaction of the medium cannot acount for all the variations observed. Rates of non-biological nitrification in resin system with pH 4.0 and in Olympic silty clay loam, both treated with 300 ppm nitrite N, indicate pH effects contrary to those discussed earlier. Here some mineral and/or organic components of Olympic silty clay loam probably hastened the process of non-biological nitrification and thus dominate the effects of the lower pH of the resin system. Reuss and Smith (1965) postulated the possibility of either mineral or organic soil components being responsible for catalyzing the chemical conversion of nitrite to nitrate. Evidences have been presented (Wulstein and Gilmour, 1964; Chao and Bartholomew, 1964) to show that mineral components of the soil, such as various transition metal ions, and organic matter in the soil (Stevenson and Swabey, 1964) interact with nitrite and transform it into other compounds. On the basis of the above results, it is suggested that the substances catalyzing nitrite decomposition are also involved in the reactions that lead to the formation of nitrate N.

Much has been predicted and shown about the pH effects on non-biological nitrification. On the other hand, the influence of the concentration of nitrite N on such a process has not been worked out. Quantities of nitrate N produced in Miami soil samples acidified to different pH values and incubated for 12 hours are shown in figure 10 as the functions of nitrite N added to the soils. This figure clearly indicates the effects of reactant concentration on the formation of nitrate. At all the pH values, higher percentages of the added nitrite were transformed to nitrate N in the samples receiving smaller doses of nitrite N. The influence of nitrite concentration on non-biological nitrification was more distinct at lower pH (higher acidity) values. Thus, if nitrite exists in small concentrations in acid soils, the chances of its being chemically nitrified are greater than if it were found in large quantities. The dotted lines in figure 10 represent the data extrapolated to smaller nitrite levels than those studied. If the extrapolation represents the real situation, it would clearly explain why under normal conditions nitrite accumulates only in alkaline soils and not in acid soils (Tyler and Broadbent, 1960). The smaller quantities of nitrite produced are probably either lost in some form or immediately nitrified to nitrate.

The data so far presented were collected by incubating the samples with alkaline permanganate trap solution placed within the incubating flasks to absorb oxides of N produced. These data were useful to verify the validity of reaction (1) which was proposed in the beginning of the 20th century on the basis of the results obtained by conducting experiments on non-biological nitrification that were not highly technical.

According to reaction (1), only one-third the number of moles of nitrous acid transformed must be recovered as nitrate N. On the other hand, if the oxides of N are not absorbed by the traps but allowed to re-enter the system to undergo further transformation, they must be converted to nitric and nitrous acid according to reactions (6), (7), and (8). Under these conditions, all of the nitrous acid must be transformed to nitric acid as shown in reaction (10). In the systems without the trap solutions, therefore, more nitrate must be recovered than that in the systems from which the oxides of N are removed by the trap solution.

In support of the above hypothesis, data were obtained. Table 2 represents the additional quantities of nitrate N recovered in different media. These values were obtained by subtracting the percent of nitrite N recovered as nitrate N in the samples with the traps, from those obtained in the samples where no trap solutions were placed. These results indicate positively that oxides of N are the intermediate compounds produced during the process of non-biological nitrification. Though the pressure of NO is said to reduce the rate of nitrite decomposition (Bray, 1932), nitrate formation was probably not influenced by NO pressure. The difference in the quantities of nitrite that decomposed in the samples with and without the trap solution were negligible. Thus, the reactions observed in solutions also occur in soil and resin systems without being greatly modified by the mineral and organic components of soil.

These values presented in Table 2 are supposed to represent the quantities of oxides of N recovered. But as against the effects of pH and nitrite concentration observed on the recovery of the oxides of N, their influences on the formation of additional quantities of nitrate N were not recognizable. Thus, the data presented here do not agree completely with the theory. In addition, the systems without the trap solutions did not show one to one relation between the quantities of nitrite N transformed to nitrate N recovered. In the light of the present knowledge, of course, such a ratio was not expected because nitrite can also be decomposed to N_2 (Tyler and Broadbent, 1960; Smith and Clark, 1960; Thorne, 1961; Reuss and Smith, 1965). Oxides of N are also shown to enter equilibrium reaction with non-gaseous nitrogenous compounds in the soil (Cady and Bartholomew, 1963). Thus, all

Table 2. Additional quantities of NO_3^--N recovered in the systems where NO and NO_2 were not trapped in alkaline permanganate solution

Media	pH	NO_2^--N added (ppm)		
		75	150	300
		percent	percent	percent
Cation resin	4.0	--	--	5
Miami silt loam	3.6	20	7	15
Miami silt loam	4.0	6	8	13
Miami silt loam	4.5	7	9	12
Miami silt loam	5.0	10	13	10
Miami silt loam	5.8	8	9	9
Olympic silty clay loam	4.6	5	3	4
Dayton silty clay loam	4.9	3	3	10

the oxides of N may not be oxidized to nitrate. Mortland (1965) reported that nitric oxides can be adsorbed on clay colloids and either be retained or be transformed to other N compounds. He has also shown that nitric oxide can form certain complexes by reacting with the cation in the soil. Therefore, part of the oxides of N that re-enter the system probably do not get further transformed to nitrate N. In addition to the above mentioned causes, the quantitative disagreement between the oxides of N recovered and the additional nitrate formed could as well arise due to the inability of the trap solution to absorb all the oxides of nitrogen produced (Reuss and Smith, 1965; Gerretsen and DeHoop, 1957).

Gray and Yoffe (1955), Gray (1958), and Sneed and brasted (1958) supported the concept of Bray (1932) that reaction (8) is the rate limiting step in both forward and reverse reactions of nitrite decomposition. Gray (1958) has shown the reaction (9) to be faster than reaction (8). Thus, under conditions where either reaction (8) is rather slow or the system is at high temperature (Gray, 1958), reaction (9) will be the additional major pathway of nitrite transformation. Then the sequence of reactions involved in non-biological nitrification will be different from that presented earlier. A new sequence of reactions is suggested here as

$$9HNO_2 \rightarrow 6NO + 3H^+ + 3NO_3^- + 3H_2O \quad . \quad . \quad . \quad . \quad . \quad (20)$$

$$6NO \xrightarrow{O_2} 6NO_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (21)$$

$$6NO_2 + 3H_2O \rightarrow 2NO + 4H^+ + 4NO_3^- + H_2O \quad . \quad . \quad . \quad (22)$$

The sum of the above reactions will yield

$$9HNO_2 + 3O_2 \rightarrow 2NO + 7H^+ + 7NO_3^- + H_2O \quad . \quad . \quad . \quad (23)$$

According to reaction (23), the proportion of nitrous acid transformed to nitric acid would be higher than that expected according to reaction (1). It is possible that the process of nitrification under field conditions is better represented by reaction (23) than (1) because the oxides of N produced in moist soil may further be transformed before they completely escape into the atmosphere (Allison and Doetsch, 1951).

The results indicate that NO and NO_2 produced as a result of nitrite decomposition are further converted to nitrate. Various oxides of N such as NO_2^-, N_2O_3, NO, NO_2, N_2O_4, and NO_3^- are said (Latimer, 1952) to have valences of +3, +3, +2, +4, +4, and +5, respectively. It is interesting to note that nitrite with +3 valency undergoes reduction to NO (+2) before being oxidized to nitrate with +5 valency. Of course, it is known that nitric oxide can exist in three forms (Gray, 1932; Sneed and Brasted, 1958) such as NO^- (+1), NO (+2), and NO^+ (+3). However, the scheme of reactions proposed by Sneed and Brasted (1958) and Gray and Yoffe (1955) includes NO (+2) and not the other chemical forms. Definite conclusions about the validity of the reactions cannot be drawn on the basis of the results presented here. Infra-red spectrophotometric studies may reveal in great detail the mechanism of the non-biological nitrification process.

N_2 production during nitrite decomposition

One of the major objectives of this investigation was to study the nature of reactions involved in the conversion of nitrite to N_2. In the latter sections of this manuscript, mechanism of N_2 production will be dealt with in detail. At this point, various quantities of N_2 recovered only in acidified Miami silt loam are presented here. This is done to prepare balance sheets of nitrogen and to verify the validity of certain pathways of nitrite decomposition.

Percentages of three levels of nitrite N recovered as N_2 in various samples of acidified Miami silt loam incubated for 12 hours are presented in figure 11. At the end of 12 hours reaction time, higher proportions of added nitrite were transformed to N_2 at lower pH values and in samples receiving smaller nitrite concentrations.

Production of N_2 from nitrite is postulated (Thorne, 1961) to be represented by the reaction

$$5HNO_2 \rightarrow N_2 + 3HNO_3 + H_2O \quad . \; . \; . \; . \; . \; . \; . \quad (14)$$

Here also nitrous acid is shown to be involved in the reaction rather than nitrite ion. Therefore, it is logical to expect higher quantities of N_2 at lower pH values.

So far it was seen that the quantities of nitrite transformed and the accumulation of various products was promoted by higher acidities. Thus, influence of pH on nitrite decomposition was found to affect the formation of various products in a similar manner.

Total nitrogen recovered

The sum of nitrite, nitrate, and oxides of N and N_2 recovered in different media after 12 hours of reaction time is presented in Table 3. In all the media the total recovery was smaller in samples with higher concentrations of added nitrite. Similarly, the sum of all the N forms determined decreased with the increase in pH up to 4.5, and an increase in the total N recovery was observed at pH above 4.5. This is contrary to the findings of Clark et al. (1960) who found the greatest deficit in samples with final pH of 5.5 to 6.5.

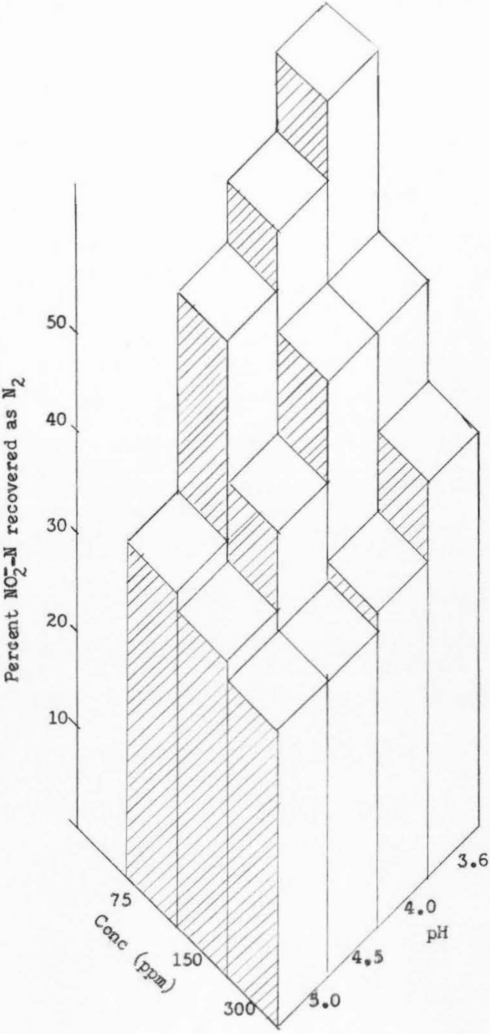


Figure 11. Effect of pH and the concentration of added nitrite on the quantity of N_2 recovered in acidified Miami silt loam.

Table 3. Sum of all the forms of nitrogen recovered after various concentrations of nitrite nitrogen were incubated in different media for 12 hours

Media	pH	NO_2-N concentration (ppm)		
		75	150	300
		percent	percent	percent
Cation resin	4.0	--	--	86
Miami silt loam	3.6	88	74	71
Miami silt loam	4.0	73	64	66
Miami silt loam	4.5	71	56	63
Miami silt loam	5.0	81	78	73

Deficits as encountered here can be caused either by the loss of the oxides of N (Reuss and Smith, 1965; Gerretsen and DeHoop, 1957) or due to the fixation of the oxides of N by mineral (Mortland, 1965; Cady and Bartholomew, 1963) and/or organic (Austin, 1961; Führ and Bremner, 1964a) components of the soil. Hence, the factor that influences the process of fixing various forms of N also affects the degree of deficits that can be encountered. Therefore, due to the differences in the physical and chemical make-up of the soil, discrepancies in the total N recovered in different soils can be expected. Smith and Clark (1960) suggested that the loss of N occurs as the oxides of N. This probably cannot be the cause of deficits observed in this investigation since the oxides of N were absorbed by the trap solution. If the deficiencies were caused by the fixation of oxides of N by organic soil components, the deficiencies are expected to increase with decrease in pH (Führ and Bremner, 1964a). On the contrary, the reverse effect was observed. Also, if the mineral components of the soil were the responsible factors, greater deficits are expected in systems treated with lower nitrite concentrations, because the quantity of mineral component is constant and fixing power also must be constant. Therefore, higher proportions of nitrite added in smaller concentrations must be fixed. Again, the results obtained were contrary to these expected values.

Alternatively, it can be proposed that deficits of N observed were probably caused due to the formation of intermediate compounds other than those determined. Stevenson and Swaby (1964) have found the formation of methylnitrite (CH_3NO_2), which is a gas that was not determined in this study. Hydroxylamine and hyponitrous acid could probably be produced. Although both hydroxylamine and hyponitrous acid are very unstable in acid media, their mineral salts are stable (Corbet, 1935), and concentrations as high as nearly 40 percent of the added N were reported to have been recovered in the form of the salts of these compounds. Beesley (1914) predicted the occurrence of such intermediate compounds.

The above results indicate clearly the inadequacy of the reactions proposed earlier to describe nitrite decomposition. Although the reaction sequences discussed earlier seem to be logical, they are unable to show the occurrence of other intermediate compounds. Above all, the conversion of the part of the added nitrite to N_2 alone is a positive indication of the inadequacy of these reactions. However, evidences show that most of the reactions proposed earlier do occur in acid media.

Theoretical considerations

On the basis of the observed results, it could only be shown that the reactions proposed earlier are inadequate to describe completely the process of nitrite decomposition. The occurrence of these reactions had been shown (Reuss and Smith, 1965) and was confirmed in this investigation. Now an attempt is made to determine conditions which may favor each of the reactions proposed. For the convenience of understanding the discussions, the reactions are listed here. They are

$$3HNO_2 \rightarrow 2NO + H^+ + NO_3^- + H_2O \quad . \; . \; . \; . \; . \; . \quad (1)$$

$$9HNO_2 \rightarrow 2NO + 7H^+ + 7NO_3^- + H_2O \quad . \; . \; . \; . \; . \quad (23)$$

$$5HNO_2 \rightarrow N_2 + 3H^+ + 3NO_3^- + H_2O \quad . \; . \; . \; . \; . \quad (14)$$

The occurrence and validity of reaction (14) are discussed later. Here attention is paid mainly to reactions (1) and (23).

The following theoretical considerations are based on certain assumptions as mentioned here. First, only the above mentioned reactions are considered as the pathways of nitrite decomposition. This also implies that nitrate is assumed to be the only N compound left in solution. Although other intermediate compounds are postulated to be formed, this assumption seems quite valid since the main purpose is to show the inadequacy of these reactions and to determine the conditions that favor the reactions. In addition, all the nitrogen recovered is assumed to have originated only from the added nitrite N and not from the native N.

On the basis of reactions (1), (14), and (23) it is possible to calculate the moles of nitrous acid that decompose to yield given quantities of N_2 and nitrate N. If expected and observed quantities of nitrous acid transformed coincide, these reactions would completely describe nitrite decomposition. The quantities of nitrous acid that could be transformed according to individual reactions can be calculated if the moles of nitrate and N_2 formed are known.

Given x and y as the moles of respectively nitrate and N_2 recovered in the system, the total moles of nitrous acid transformed through reactions (1) and (14) can be calculated as follows.

1. Moles of HNO_2 decomposed through the reaction (14) will be 5y.

2. Moles of HNO_3 formed = 3y.

3. Moles of HNO_3 formed according to reaction (1) = x - 3y.

4. Moles of HNO_2 transformed according to reaction (1) = 3(x - 3y).

5. Total number of moles of nitrous acid decomposed is given by the sum of it used up in two reactions

$$= 5y + 3(x - 3y)$$

$$= 5y + 3x - 9y$$

$$= (3x - 4y) \text{ moles.}$$

Similarly, the total quantity of HNO_2 decomposed through reactions (14) and (23) is equal to

$$1.14\ x + 1.3\ y = \text{moles of } HNO_2 \text{ decomposed.}$$

Some of the values were calculated from the results of acidified Miami silt loam incubated for 12 hours. Tables 4, 5, and 6, respectively, present the data of the samples treated with 75, 150, and 300 ppm nitrite N. Reaction (14) could not account for all the nitrous acid transformed in any of the samples. Combination of reactions (1) and (14) seemed to represent most of the reactions that probably occurred in resin systems with pH 4.0 and treated with 300 ppm nitrite N (Table 6). This clearly indicates that reaction (1) which was originally considered to describe completely the nitrite decomposition is incomplete. In addition, reaction (14) seems to represent very

Table 4. Comparison between the quantities of NO_2^--N transformed in Miami soil treated with 75 ppm NO_2^--N and those expected on the basis of theory

		Nitrite transformed (millimoles x 10^3)		
			Expected according to reactions	
Media	pH	Observed	1 and 14	14 and 23
Miami silt loam	3.6	536	346	394
Miami silt loam	4.0	536	341	338
Miami silt loam	4.5	493	338	310
Miami silt loam	5.0	422	342	286
Miami silt loam	5.8	268	269	255

Table 5. Comparison between the quantities of NO_2^--N transformed in Miami soil treated with 150 ppm NO_2^--N and those expected on the basis of theory

		Nitrite transformed (millimoles x 10^3)		
			Expected according to reactions	
Media	pH	Observed	1 and 14	14 and 23
Miami silt loam	3.6	1061	630	655
Miami silt loam	4.0	1036	402	524
Miami silt loam	4.5	922	343	452
Miami silt loam	5.0	761	366	430
Miami silt loam	5.8	472	151	368

Table 6. Comparison between the quantities of NO_2^--N transformed in various media treated with 300 ppm nitrite N and the expected values calculated on the basis of proposed reactions

		Nitrite transformed (millimoles x 10^3)			
			Expected according to reactions		
Media	pH	Observed	14	1 and 14	13 and 14
Cation resin	4.0	1469	716	1286	841
Miami silt loam	3.6	1450	895	1600	1113
Miami silt loam	4.0	2060	855	2735	1679
Miami silt loam	4.5	1540	780	234	543
Miami silt loam	5.0	1250	780	60	468

very closely the stoichiometry of N_2 production. Slight differences between the observed and expected values of N_2 produced were probably caused by the occurrence of some other intermediate compounds. The combination of reactions (14) and (23) seemed to underestimate the quantity of nitrous acid decomposed.

The effects of pH on each of these reactions can be seen when the results of Miami silt loam are compared. The quantities of HNO_2 decomposed in samples with pH 3.6 and 4.0 are better approximated by the combination of reactions (1) and (14) and/or (14) and (23), than by reaction (14) alone. On the other hand, the samples with pH 4.5 and 5.0 yielded less nitrate and thus the amount of nitrous acid decomposed is drastically underestimated. This may suggest that reactions (1) and (23) are more sensitive to pH than reaction (14). This is in close agreement with Corbet's (1935a) report that nitrate production may cease in media with pH above 5.0. In parallel with the underestimation of nitrous acid transformed, these samples also showed greater deficiencies

(Table 3) than the others. This may partly support the suggestion that some intermediate compounds other than those known also occur.

So far it was seen that nitrite undergoes non-biological self degradation in acid media. The degree of decomposition depended on pH of the medium and the concentration of added nitrite. At lower pH and lower nitrite concentration, the tendency for the compound to decompose was greater. Nitrate, oxides of N, and N_2 were definitely produced during the chemical transformation reactions. Higher quantities of all the products were recovered at lower pH values. The reactions proposed earlier are inadequate to describe the pathways of nitrite decomposition. The reactions proposed to explain non-biological nitrification were found to be more sensitive to pH than the N_2 producing reaction. Inability of all the forms of N determined to add up to the quantity initially present in the sample suggests that possibly other intermediate compounds occur.

Loss of Elemental Nitrogen

In the previous section evidences were presented to show the formation of N_2 through nitrite decomposing reactions. Here the factors affecting the conversion of nitrite to N_2 are considered in detail. In addition, the nature of N_2 producing reactions are discussed, giving due consideration to the possible intermediate compounds involved in this process.

Effects of pH and nitrite concentration on the loss of N_2

Six acid soils varying in pH from 4.6 to 6.1 were treated with 75, 150, and 300 ppm nitrite N and incubated at 30 C for 12 hours. Figures

12, 13, and 14, respectively, present the quantities of N_2 produced in various soils treated with 75, 150, and 300 ppm nitrite N. Regardless of the concentration of nitrite added, N_2 production was most rapid in all the soils during the first 3 hours of reaction time. Following this, the rate of N_2 production reduced until it ceased. It was interesting to note that N_2 production ceased although considerable proportions of the substrate remained in the system. At all the nitrite levels and during each sampling period larger quantities of N_2 were recovered in soils with higher acidities. Differences among the soils increased as the reaction time advanced. Thus, in soils with higher pH values the rate of N_2 production declined more rapidly than in those with higher acidities. In addition, the decrease in the rate of N_2 production was more drastic in all the soils that received higher doses of nitrite N.

The most interesting point in the results presented in figures 12, 13, and 14 is the fact that N_2 production stopped when appreciable quantities of reactant were present in the medium. The cause of such a process is unknown. Active transition metals that catalyze nitrite decomposition (Wulstein and Gilmour, 1964) also may be responsible for N_2 production. Exhaustion of such catalysts could result in the cessation of the reaction. But cessation of N_2 production even in the samples treated with the smallest concentration of nitrite N (75 ppm) overrules such a possibility. Instead, the results indicate that one or more of the intermediate compounds of nitrite decomposition probably act as inhibitors to the reactions producing N_2. Inhibition may be caused either by poisoning the catalysts or by preventing some of the reactions. In such a case the inhibition will be competitive.

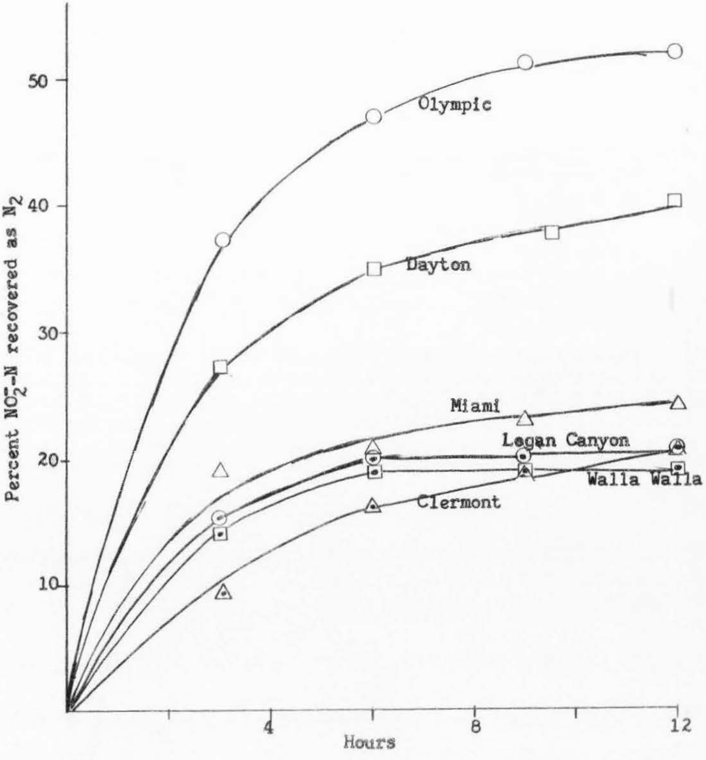


Figure 12. Gaseous nitrogen recovery in various acid soils treated with 75 ppm NO_2^--N and incubated for various periods of time.

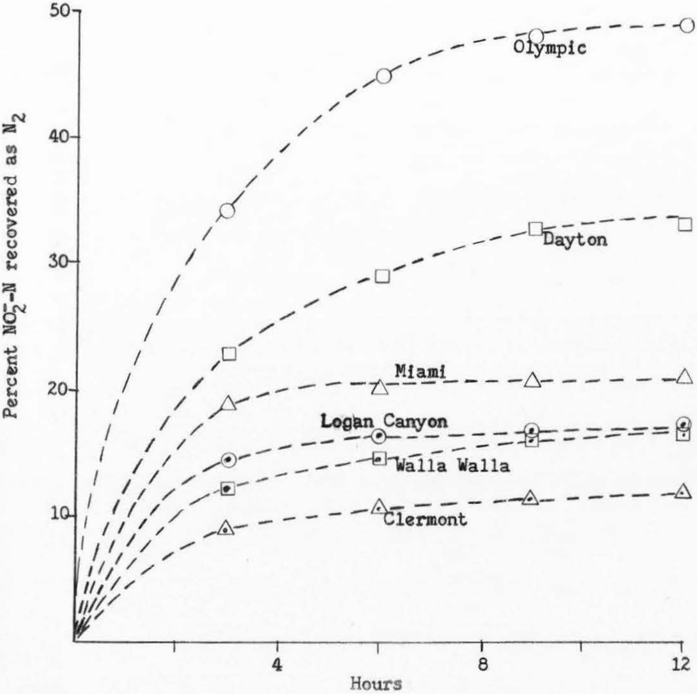


Figure 13. Gaseous nitrogen recovery in various acid soils treated with 150 ppm NO_2^--N and incubated for various periods of time.

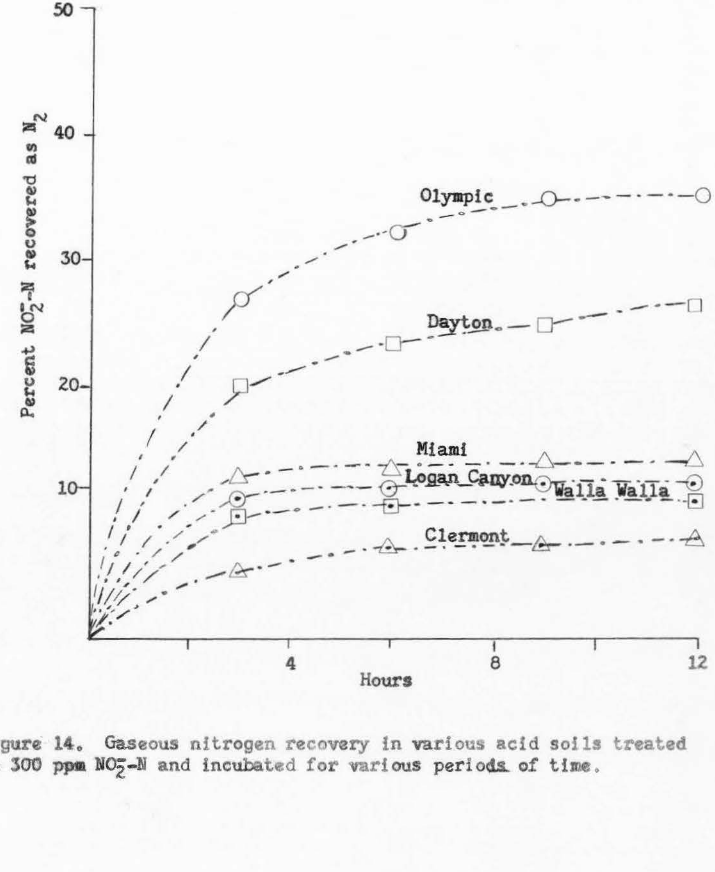


Figure 14. Gaseous nitrogen recovery in various acid soils treated with 300 ppm NO_2^--N and incubated for various periods of time.

The effect of the concentration of added nitrite on the quantity of N_2 produced is presented in figure 15. The values represent the quantity of N_2 recovered in various media treated with 75, 150, and 300 ppm nitrite N and incubated for 12 hours. Larger percentages of nitrite N were converted to N_2 when the substrate was present in smaller concentrations. In acid soils, therefore, under field conditions, if nitrite is produced in small quantities and the rapid loss of it as N_2 continuously occurs in large proportions, very large quantities of the added fertilizers can be lost into the air. Such a mechanism of loss of N can also answer some of the questions on the enigma of soil nitrogen.

Precursors of N_2 formation

In the previous section, indirect evidences were presented to show the occurrence of some unknown intermediate compounds of nitrite decomposition. Hydroxylamine (Corbet, 1934 and 1935; Martin et al., 1942), hyponitric acid (Emelius and Anderson, 1938; Thorne, 1961), and hyponitrous acid (Corbet, 1935; Mumford, 1914) have been postulated to occur during the transformation of either ammonia to nitrite or nitrite to various products. It is interesting to note that all the predicted compounds are free radicals and could be destroyed or be prevented from being active in the reaction sequence.

Here attention was focused on the occurrence of hyponitrous acid as an intermediate compound. Breakdown of nitrous acid in which N has a valence of +3 to nitric oxide (+2) was postulated earlier and was shown to be true. Formation of N_2 with zero valence was also observed. Thus, it is logical to choose a compound wherein nitrogen has a valence between +2 (NO) and 0 (N_2) to be the most plausible intermediate

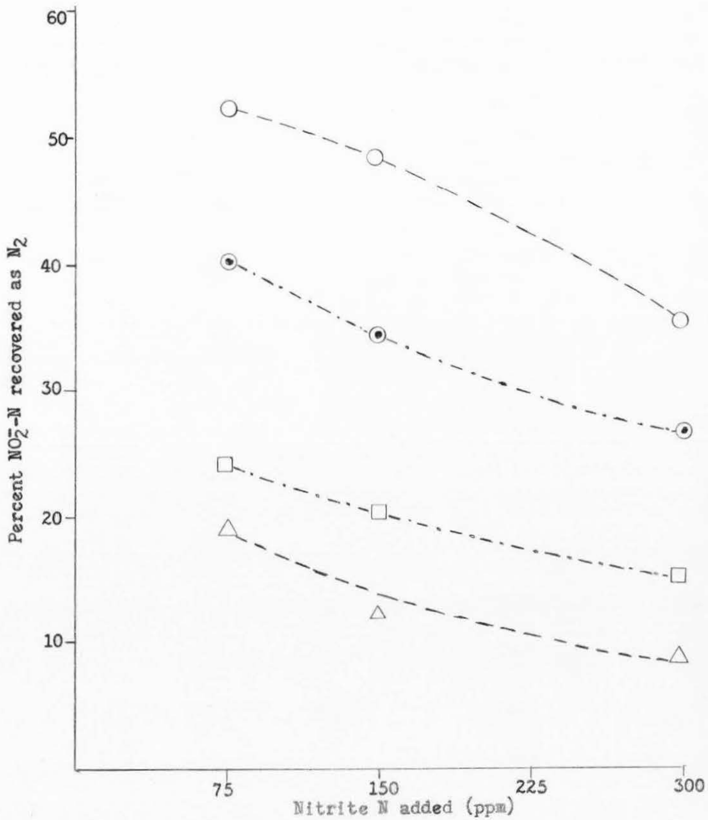


Figure 15. Effect of nitrite concentration on the quantity of N_2 produced in different soils.

compound. In hyponitrous acid nitrogen has a valence of +1, and it is a free radical. Thus, hyponitrous acid could possibly be an intermediate. Hyponitric acid ($H_2N_2O_3$) which is said to be very unstable (Emelius and Anderson, 1938) instantly breaks down to hyponitrous acid and nitrous acid. But the formation of $H_2N_2O_3$ is very slow (Emelius and Anderson, 1938) as compared to the rate of its breakdown.

The occurrence of any free radical as a precursor of N_2 formation was investigated by treating the sampes with 5 ml of 95 percent ethyl alcohol. Ethyl alcohol is said to act as a scavanger of free radicals in the medium and thus disrupt the reaction sequence. Thus, if free radicals are involved in N_2 producing reactions, less N_2 must be recovered in alcohol treated samples. Accordingly, in the samples treated with alcohol, smaller quantities of N_2 were produced as compared to the samples that were free of alcohol. Table 7 presents the percent inhibition of N_2 production that was observed in samples with alcohol. These values represent the quantities of N_2 recovered in alcohol treated samples expressed as the percent fraction of those quantities recovered in samples free of alcohol. Dayton silty clay loam showed maximum reduction in the quantity of N_2 formation. There was no regular variation in the reduction that was brought about by alcohol, although reduction was observed in all the soils.

Nitric oxide is definitely known to be produced by the decomposition of nitrous acid. Similarly, the data show the formation of N_2 during autodecomposition of nitrous acid. Therefore, the possibility of nitric oxide being one of the precursors of N_2 was tested by adding approximately 10 mg NO-N to the soil system. Nitric oxide was introduced in gaseous form into the flasks containing soil samples after the

Table 7. Ratios between the quantities of N_2 produced in alcohol treated and untreated samples of various soils that received three levels of nitrite and incubated for 12 hours

Soil	pH	NO_2^--N level (ppm) 75	150	300
		percent inhibition		
Olympic silty clay loam	4.6	34	42	42
Dayton silty clay loam	4.9	50	50	33
Clermont silt loam	5.2	28	24	23
Logan Canyon silt loam	5.8	27	37	36
Miami silt loam	5.8	43	35	44
Walla Walla (Washington) silt loam	6.1	18	22	30

atmosphere within the flasks was made free of N_2. In both Olympic and Dayton silty clay loams treated with NO, the elemental form of nitrogen was observed (figure 16). Olympic soil produced slightly more N_2 than Dayton silty clay loam, and it may probably be due to the variations between them in pH and organic matter contents. Thus, nitric oxide is one of the precursors of N_2.

On the basis of the results obtained in samples treated with alcohol and nitric oxide, the following scheme of reactions can be proposed as a probable pathway of converting nitrite to elemental nitrogen.

$$6HNO_2 \rightarrow 4NO + 2H^+ + 2NO_3^- + 2H_2O \quad \ldots\ldots \quad (24)$$

$$4NO + 2H_2O \rightarrow 2H_2N_2O_3 \quad \ldots\ldots \quad (25)$$

$$2H_2N_2O_3 \rightarrow 2HNO_2 + H_2N_2O_2 \quad \ldots\ldots \quad (26)$$

$$H_2N_2O_2 + HNO_2 \rightarrow N_2 + H^+ + NO_3^- + H_2O \quad \ldots\ldots \quad (27)$$

The sum of the above reactions yields reaction (14) that was said to represent the stoichiometry of N_2 production. Considering the above

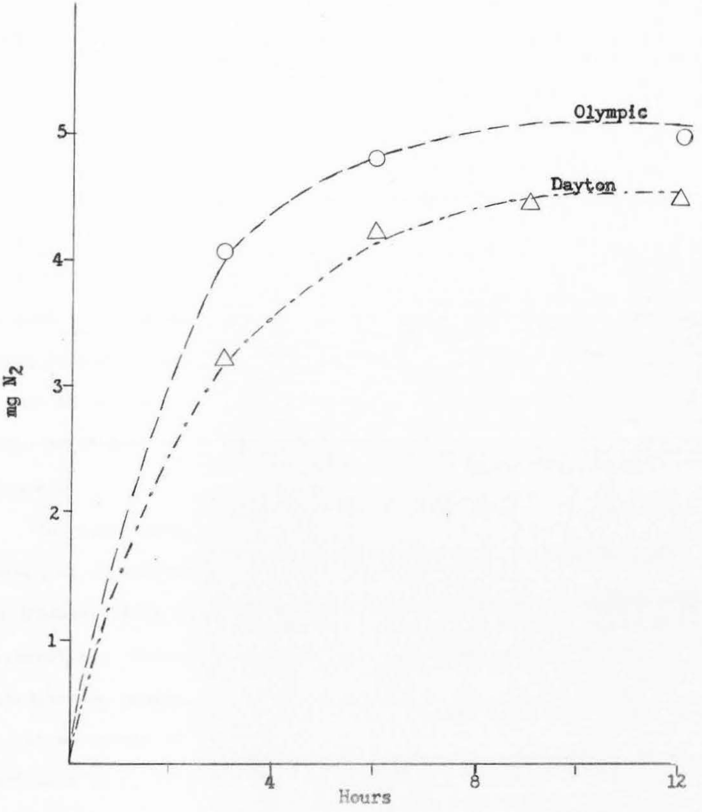


Figure 16. Recovery pattern of N_2 from Olympic and Dayton silty clay loam treated with NO.

results, it is possible to write the sequence of products formed in the conversion of nitrite to N_2 as $NO_2^- \rightarrow NO \rightarrow H_2N_2O_3 \rightarrow H_2N_2O_2 \rightarrow N_2$.

Inhibitors of N_2 producing reactions

It was seen that N_2 formation stopped in all the soil even when considerable proportions of the added nitrite remained in the system. It was postulated (Thorne, 1961) that such an effect probably resulted from the inhibitory effects of one or more of the intermediate compounds of nitrite decomposition. Although the possibility of the catalyst being exhausted was ruled out, it is probable that the catalysts may either be poisoned by the inhibitors or the inhibitors may compete with other precursors of N_2 for the catalysts. Thus, N_2 formation may be stopped.

In either case, the addition of excess catalyst must result in the formation of additional quantities of N_2. Iron has been found (Wulstein and Gilmour, 1964; Chao and Bartholomew, 1964) to catalyze nitrite decomposition. Therefore, it was decided to find out whether or not iron catalyzes N_2 producing reactions also. Soil samples were treated with different levels of iron before adding nitrite N. Figure 17 shows the influence of 0, 250, 500, and 1000 ppm iron, added as ferrous sulfate to Miami silt loam with pH 4.6. The quantities of N_2 produced in Miami soil were unaffected by the freshly added iron. However, the possibility of the inactivation or poisoning of some other metallic or nonmetallic catalysts that may be involved in the process of producing N_2 is not precluded.

Thorne (1961) postulated that the accumulation of nitrate probably stops N_2 formation, but N_2 was formed in samples that received 15 mg

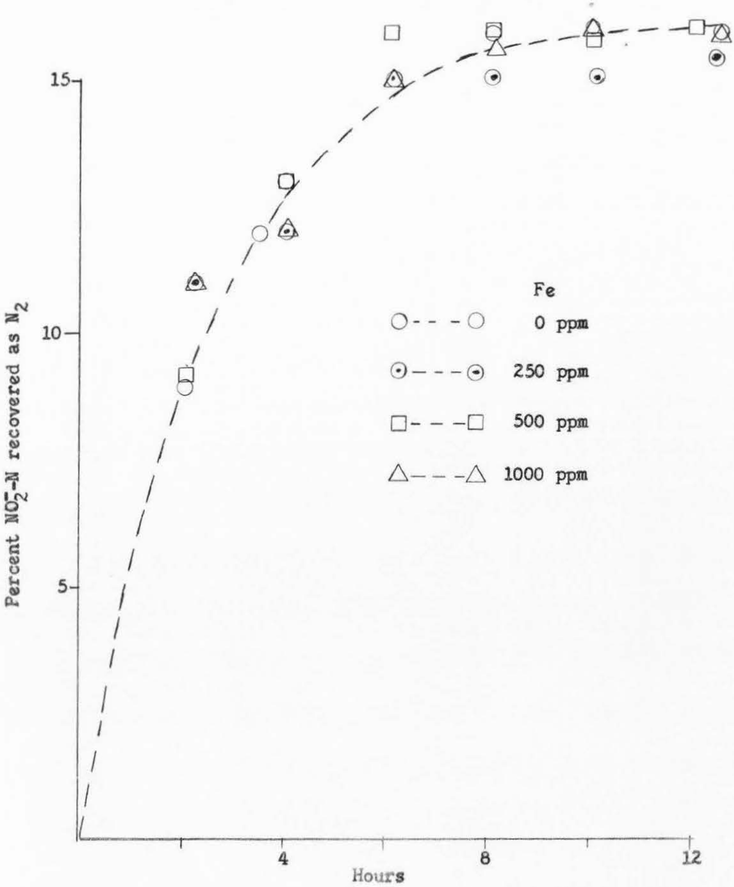


Figure 17. Effect of Fe added as $FeSO_4 \cdot 7H_2O$ to Miami silt loam (pH 4.6) on N_2 production from 30 mg NO_2^--N.

nitrate N prior to the adding of nitrite N (figure 18). Incubation of nitrate containing samples with NO alone or in combination with nitrate did not influence N_2 production except that NO was converted to N_2 (figure 18). Thus, it was clear that neither nitrate nor NO alone or in combination slows down or prevents N_2 production.

So far two schemes of nitrite decomposition pathways have been presented. During non-biological nitrification, the sequential products expected are $NO_2^- \rightarrow N_2O_3 \rightarrow NO + NO_2 \rightarrow NO_2 \rightarrow N_2O_4 \rightarrow NO_3^-$. It was postulated that the sequence $NO_2^- \rightarrow NO \rightarrow H_2N_2O_3 \rightarrow H_2N_2O_2 \rightarrow N_2 + HNO_3$ can be expected in the process of N_2 formation. In the systems most of the sequential products are in equilibrium with each other (Bray, 1932; Gray and Yoffe, 1955; Sneed and Brasted, 1958). Thus, it is not an easy task to point out the particular compounds responsible for the slowing down of N_2 production. However, separate addition of each of the suspected compounds may give some idea about the inhibitory effects.

Nitric oxide (NO) was used in an experiment to find some clue to the nature of inhibition. Samples were incubated with NO for various lengths of time prior to adding nitrite and the patterns of N_2 formation were determined. Figures 19 and 20, respectively, present the patterns of N_2 formation observed in the samples of Dayton and Olympic silty clay loams incubated with NO for various lengths of time. Incubation of the samples with NO for 3 hours did not have any appreciable effect on N_2 production in either soil. During the early stages of reaction time, however, more N_2 was produced in Olympic soil incubated 3 hours than in unincubated samples. When the samples with NO were pre-incubated for 6 hours or more, the formation of N_2 was in smaller quantities and N_2 production ceased sooner than that observed in the

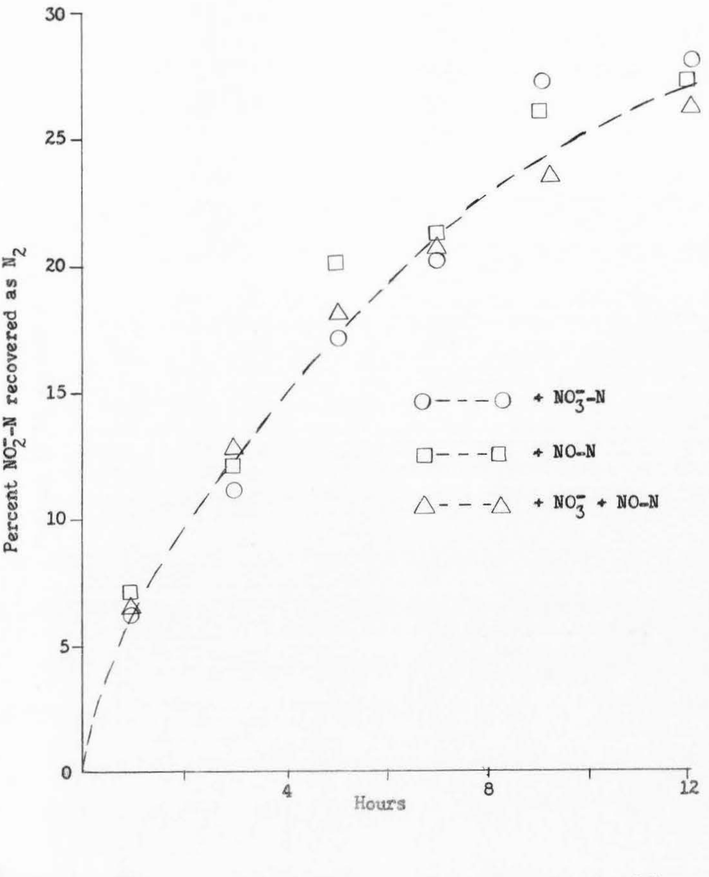


Figure 18. Effect of excess nitrate and NO on the pattern of N_2 formation in Dayton soil.

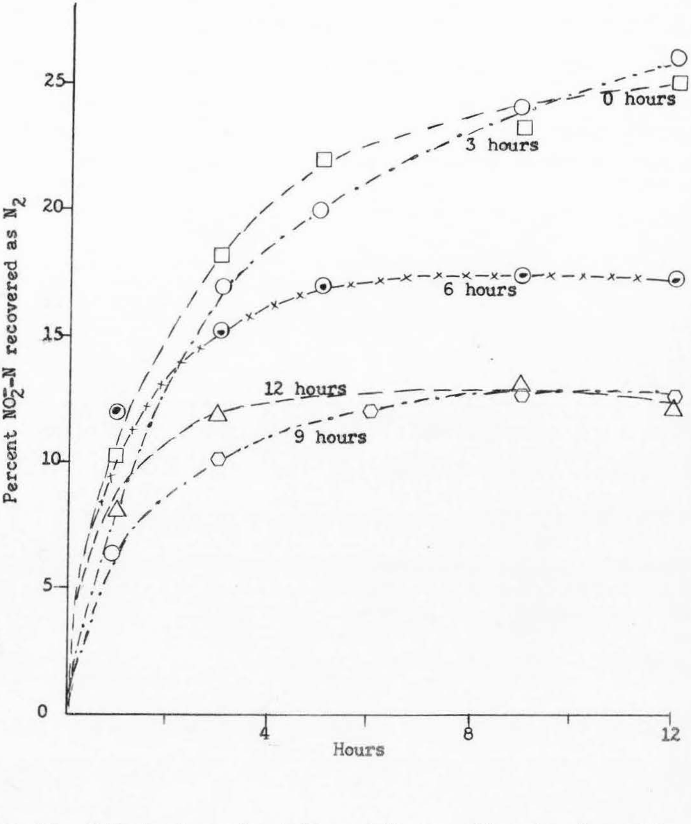


Figure 19. Effect of pre-incubation of Dayton silty clay loam with NO on the N_2 recovery pattern from 300 ppm NO_2^--N added.

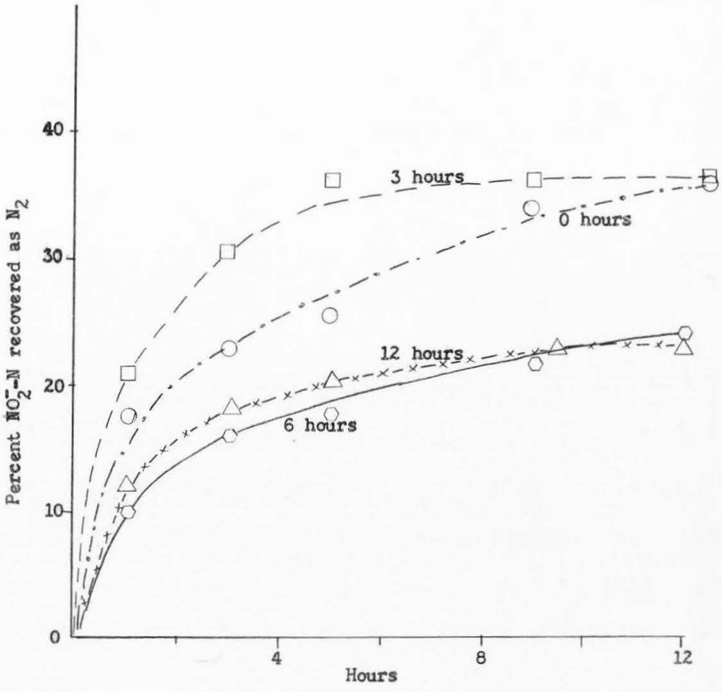


Figure 20. Effect of pre-incubation of Olympic silty clay loam with NO on the N_2 recovery pattern from 300 ppm NO_2^- added.

unincubated samples. Nine and 12 hour pre-incubation of Dayton soil samples resulted in further decrease in the quantity of N_2 produced as compared to 6 hours pre-treatment. In Olympic soil there was no difference between 6 and 12 hours of pre-incubation time.

It was seen that the added nitrite and nitric oxide can be converted to N_2 within an hour after they are added to the medium. Also nitrate was recovered an hour after the addition of nitrite to the sample. But nitrate and/or nitric oxide were not responsible for the slowing down and eventual stopping of N_2 formation. Thus, it can be safely concluded that the compounds formed during the conversion of nitric oxide to nitrate probably do not influence N_2 formation. Pre-incubation of the samples with NO for 3 hours did not alter the pattern of N_2 formation. Therefore, the compounds responsible for inhibiting the N_2 yielding reaction required longer than 3 hours to build up effective concentrations. In a way, similar to the formation of nitrate, nitrite was produced according to reactions (6), (7), and (8). If the decomposition of nitrite is restricted to the pathways discussed earlier, it is certain that there are some compounds produced prior to the formation of NO and that particular compound probably is responsible for stopping N_2 production. According to reaction (2), N_2O_3 would be formed prior to NO, but it could also arise when NO was added to the medium because they attain equilibrium instantaneously (Gray, 1958). After 3 hours of pre-incubation time, most of the nitric oxide probably was converted to nitrate and nitrous acid. Thus, nitrite probably decomposed through different pathways producing some unknown unidentified compounds. Such compounds may be involved in modifying N_2 production. The buildup of that compound responsible to inhibit N_2 production may have required more than 3 hours. Alternatively, there are other pathways of nitrite decomposition in which the compounds capable of inhibiting N_2 producing reactions occur.

Thus, two main points are clearly seen in the results presented in this section of the manuscript. First of all, the proposed pathways of nitrite decomposition are incomplete and are not able to describe all the results observed. Secondly, there are positive indications that some intermediate compounds other than those included in the pathways of reactions proposed also occur.

SUMMARY AND CONCLUSIONS

Non-biological decomposition of 75, 150, and 300 ppm nitrite N added to various acid soils varying in pH values from 4.6 to 6.1 was studied. In some cases similar studies were carried out in cation resin adjusted to pH 4.0. In one instance, one of the soils was studied after adjusting to different pH values by adding the required quantities of sulfuric acid. All the samples, except resin systems, were incubated after adjusting the moisture content to 0.3 bar tension. Nitrite was added to the samples in the form of sodium nitrite solution, after the system was made free of N_2. Samples were incubated in constant temperature cabinets maintained at 30 C. On the basis of the results obtained, the following conclusions were drawn.

1. It is positively proven that autodecomposition or self-degradation of nitrite occurs in sterile soils when the pH is in acid range. Nitrate, elemental nitrogen, and various other oxides of N such as nitric oxide and nitrogen dioxide are produced during non-biological nitrite decomposition.

2. Regardless of the pH of the medium and the concentration of N added, nitrite decomposition was very rapid during the first 4 to 5 hours of reaction time. Following this the rate of nitrite disappearance decreased gradually until the process ceased, although large proportions of the added nitrite remained in the system.

3. The degree of nitrite decomposition was found to depend on both nitrite concentration and the reaction of the medium. Greater proportions of the added nitrite decomposed in a medium with lower pH

and smaller nitrite level.

4. The quantities of nitric oxide and nitrogen dioxide recovered in the traps also were found to depend on pH and concentration of the reactants, but unlike the nitrite disappearance, the recovery of the oxides of N was found to have linear relation to the reaction time.

5. The term non-biological nitrification is used herein to refer to the process of chemical oxidation of nitrate. Non-biological nitrification occurred in all the media including resin systems. Higher percentages of the nitrite were recovered as nitrate in the samples with lower pH and smaller nitrite concentrations. Thus under field conditions, in acid soils, if nitrite occurs in smaller concentrations, nitrate could be formed in the absence of nitrifying organisms.

6. Both the disappearance of nitrite and the nitrate formation were greater in soil than resin systems. Thus either organic or mineral soil components alone or in combination hasten the non-biological nitrification process.

7. The results support the earlier concepts that nitric oxide is eventually oxidized to nitrate. Thus when no traps were placed in the flask, allowing these oxides to re-enter the medium, more nitrate was recovered.

8. The data do not show the relationship between the expected quantities of nitrite decomposed and the nitrate recovered. Therefore it is concluded that the reactions proposed earlier to describe nitrite decomposition are inadequate.

9. Under normal field conditions gas phase reaction may occur between nitric oxide and water vapor. Assuming this reaction to be one

of the major pathway of nitrite decomposition, a new reaction is proposed to show non-biological nitrification as

$$9HNO_2 + 3O_2 \rightarrow 2NO + 7H^+ + 7NO_3^- + H_2O \quad . \quad . \quad . \quad (23)$$

10. At no sampling time did the total quantities of N recovered equal that added. Calculated quantities of nitrite disappearance were always lower than the observed amounts. Thus the nitrite is used up in other reactions that yield different compounds, such as elemental nitrogen.

11. Elemental nitrogen was produced from the added nitrite. Higher percentages of the nitrite were recovered as N_2 in the medium treated with lower nitrite concentrations. Formation of N_2 was also greater in systems with smaller pH values.

12. The effect of pH and reactant concentration was identical on both the decomposition of nitrite and the formation of various products. Evolution of elemental nitrogen from the nitrite in acid media suggests that a large fraction of the added fertilizers can be lost through this mechanism. Thus, it also reduces the enigmatic nature of soil nitrogen.

13. The results from the samples treated with alcohol indicate the possibility that some free radicals are involved in N_2 producing reactions. Reduction up to as high as 50 percent was observed in the quantity of N_2 produced.

14. Nitric oxide added in gaseous form to soil systems was recovered as N_2. Thus it is concluded that nitric oxide is an intermediate compound involved in the conversion of nitrite to elemental nitrogen.

15. If nitric oxide is one of the precursors of N_2, it forms a

sequence of compounds wherein nitrogen has valencies of +3 (NO_2^-), +2 (NO), and zero (N_2). Thus it is logical to consider a compound like hyponitrous acid ($H_2N_2O_2$), in which N has a +1 valency, as the immediate precursor of N_2. On the basis of the above assumption and the results from the samples treated with alcohol and nitric oxide, the following scheme of reactions is proposed as a probable pathway of N_2 production during non-biological nitrite decomposition.

$$6HNO_2 \rightarrow 4NO + 2H^+ + 2NO_3^- + 2H_2O \quad . \; . \; . \; . \; . \; . \tag{24}$$

$$4NO + 2H_2O \rightarrow 2H_2N_2O_3 \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \tag{25}$$

$$2H_2N_2O_3 \rightarrow H_2N_2O_2 + 2HNO_2 \quad . \; . \; . \; . \; . \; . \; . \; . \tag{26}$$

$$H_2N_2O_2 + HNO_2 \rightarrow N_2 + H^+ + NO_3^- + H_2O \; . \; . \; . \; . \tag{27}$$

$$5HNO_2 \quad N_2 + 3H^+ + 3NO_3^- + H_2O \; . \; . \; . \; . \; . \; . \; . \tag{14}$$

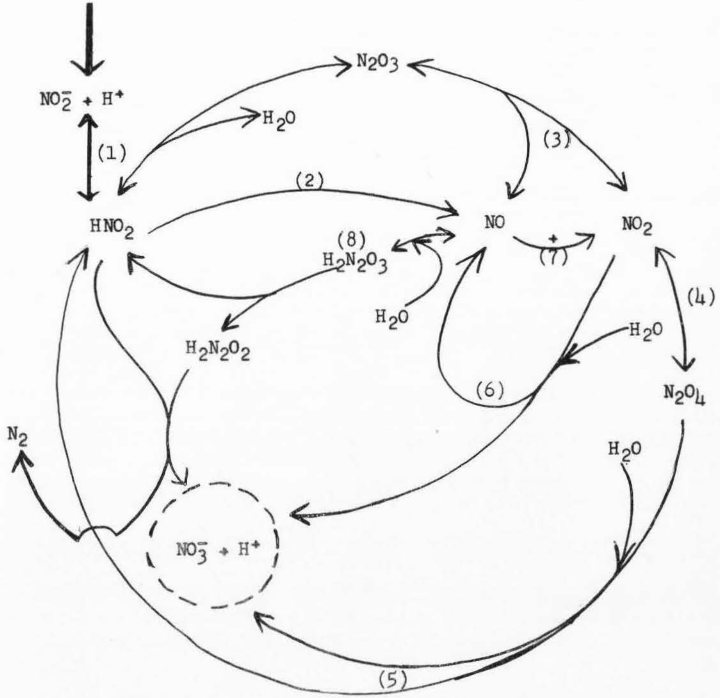
16. Accumulation of neither nitrate nor nitric oxide alone or together was found to alter the N_2 recovery pattern in acidified Miami silt loam. Addition of iron in the form of iron sulfate to the soil system did not influence the quantity of N_2 evolved.

17. Cessation of N_2 production occurs in the presence of large proportions of the reactant in the system. Such an effect is thought to be due to the occurrence of some unknown products of nitrite decomposition, which probably inhibits the process. This view was supported by the reduced quantity of N_2 recovered in samples that were pre-incubated with NO for various lengths of time prior to adding nitrite. During the pre-incubation of the samples with NO, part of it was oxidized to nitrous acid, which in turn probably decomposed to other compounds that inhibit N_2 production.

18. Both the inability of all the forms of nitrogen recovered to equal the quantity added and the disagreement between the expected and

observed quantities of nitrite N transformed strongly suggest the occurrence of some intermediate compounds other than those known to be produced during nitrite decomposition in acid media. It is, therefore, concluded that the known reactions of nitrous acid decomposition are inadequate to explain the process.

19. All the above results are summarized schematically in figure 21. The scheme shows three sequences of reactions that form nitrate N. In all cases both oxidative and reductive processes are involved. Elemental nitrogen also is suggested to be formed simultaneously with nitrate production.



1. pH sensitive
2. Hastened by both organic and inorganic soil components
3. Equimoles of NO and NO_2 are produced.
4. Instantaneous process
5. Equimoles of products are formed.
6. Temperature sensitive gas phase reaction
7. Instantaneous aerobic reaction
8. Extremely unstable in acid media

Figure 21. Schematic summarization of proposed reactions of HNO_2 in acid media wherein both nitrate and elemental N are produced.

PRESENT STATUS AND FUTURE OUTLOOK

Although this work is not a pioneering research in a new field, it has to be admitted that neither is it the concluding experiment on nitrite decomposition in acid media. However, the data presented so far are preliminary in nature and give clues to many lines of possible investigation in the future. As mentioned in the introduction to this thesis, more questions are left unanswered than those solved.

The author has gained some insight on various reactions that take place during non-biological nitrite decomposition as well as on some of the practical applications of the results. The following few words are written with two main objectives in mind. The first is to pass on the experience and insight gained by the author during the present investigation to future workers who may find this information useful in writing up research projects. The second objective is to keep these data in a form that could be readily used for the purpose of refreshing the minds of author and readers. At any time proper blending of some of the insights presented here and a person's forethought may result in good research work.

Some of the results obtained are more conclusive than others. On the basis of the conclusive results, two kinds of research work are suggested here. Some work could be carried out in the field similar to the one reported here. In addition these data are useful in conducting a research program in other fields of science, such as plant physiology and plant nutrition. In the case of ideas that are not very obvious and conclusive from the results, some of the logical viewpoints of the author are presented to substantiate and vindicate the concepts.

Many reactions have been proposed to represent the overall stoichiometry of nitrite decomposition process. The inadequacy of these reactions is clear from the fact that elemental nitrogen, which is not included as an end product, is given off during nitrite degradation. Nitrate and elemental nitrogen are shown to be the end products. It would be interesting to know if both end products are formed simultaneously or through two separate equations. If both are produced through a reaction, a single reaction sequence could be formulated to describe the process of nitrite decomposition. On the other hand, if they are formed as a result of separate reactions, one should be able to investigate the importance of each of the reactions under different field conditions and use the information to increase the efficiency of nitrogen fertilizers. At the same time it should be remembered that these compounds may be formed through both separate and common reactions.

Evidences have been presented to show that in none of the cases was all the added nitrogen recovered. In spite of the fact that various processes responsible for causing the deficiencies are proposed, there are indications to show the occurrence of certain unknown intermediate compounds. One would find it an interesting challenge to isolate such compounds. In the light of the author's experience and personal contact with others, it is suggested that such an intermediate may either be in inorganic or organic form or both. Turtstein (1936) and Stevenson and Swaby (1964) have postulated the possibilities of certain organic compounds being involved in such a process.

More information is needed on the nature of N_2 producing reactions and various intermediate compounds involved. Hyponitrous and hyponitric acids are postulated here as the probable intermediate compounds that precede N_2 formation from nitrite. It should be relatively easy either to prove or disprove the occurrence of these compounds. Use of isotopic nitrogen ought to render such a study much easier.

It is positively shown that the formation of nitrate and elemental nitrogen from nitrite stops even when considerable quantities of added nitrite remain unaltered in the system. Isolation of such an inhibitor would be of great value both in reducing the loss of nitrogen and in the more efficient use of controlled availability fertilizers. Both the inhibitors and unknown intermediate compounds could be isolated by solvent extraction procedure.

In addition to the above mentioned results, there are some indications that some of the reactions are more likely to be the major pathway than others. Nitrate formation results from the dissolving of dinitrogen tetroxide (N_2O_4) in water and by the gas phase reaction between nitrogen peroxide (NO_2) and water. Thus both the moisture content of the system and temperature of the surroundings may influence the quantities of the nitrate produced. The effects of temperature and moisture content of the system on non-biological nitrification needs to be investigated. These data may be quite useful in explaining the differences in quantities of oxides of N recovered by various workers. It will be interesting to know the temperature and moisture effects on the quantities of various oxides of nitrogen produced.

Elemental nitrogen formation from nitrite is inevitable. There are some indications that nitrate production is more pH dependent than the release of elemental nitrogen. In the recent years many workers have reported (Gerretsen and DeHoop, 1957; Clark et al., 1960) that chemical conversion of nitrite to nitrate stops in media with pH above 4.5 to 5.0. However no such effect has been reported on N_2 formation. If N_2 formation is less pH dependent, more serious attention should be paid to it. More nitrogen fertilizers may be lost through this pathway.

Organic matter seems to have tremendous influence on the evolution of N_2. Reuss and Smith (1965) have shown that organic matter plays some role in N_2 formation. Rashid (1966) has found distinct differences between the effects of fresh and dry plant material added to the soil. Their influence is shown to be different following the incubation of organic matter in soil. Stevenson and Swaby (1964) have gone a few steps ahead in isolating some of the compounds produced. In all the above cases acid soils were used. Recently Meek and McKenzie (1965) have reported the loss of N_2 even in calcareous soils. Thus, on account of the organic matter effects, evolution of N_2 may occur in both acid and neutral soils. In organic matter certain functional groups with high pK values may be involved in N_2 production, and this may make it less pH sensitive. Many aspects of organic matter effects on N_2 production from nitrite need to be investigated in order to understand the chemistry of nitrite in soils.

Oxides of nitrogen are the immediate products of nitrite decomposition. Apart from the reactions that convert these to other nitrogen compounds, numerous transformation processes may take place. Nitric oxide can enter equilibrium reaction with non-nitrogenous compounds (Cady and Bartholomew, 1963) and can be transformed to other compounds (Mortland, 1965). Similarly it is suggested that these oxides may react with both organic and inorganic nitrogenous compounds.

Interaction of oxides of nitrogen and other compounds is also important. These compounds, being reactive, can react with other nitrogenous compounds leading to the formation of different products. Waksman (1952) has postulated the reaction between ammoniacal nitrogen and various oxides of nitrogen which probably result in the evolution of elemental nitrogen. Gray (1958) has presented a possible reaction involving ammonia and dimer form of nitrogen peroxide as

$$N_2O_4 + NH_3 \rightarrow N_2 + H^+ + NO_3^- + H_2O \quad . \quad . \quad . \quad . \quad . \quad (28)$$

This mechanism of N_2 production may be responsible for the loss of N reported to occur during nitrification of ammoniacal fertilizers (Clark et al., 1960). If this is true, accumulation of nitrite alone may not be sufficient to cause the loss of N. This field of research can be developed to a great extent and valuable information also can be obtained.

Although phyto-toxicity of nitrite has been reported, not much work has been done on this. Toxicity is said to be more severe in acid media than in alkaline conditions. The decomposition products of nitrite produced in acid conditions may be responsible for the phyto-toxicity since in alkaline conditions nitrite is stable and not acid media. Thus the toxicities of various intermediate products (oxides of N) on plants ought to be investigated. Excess nitrite is shown to affect adversely the respiration of plant roots. Thus it would be interesting to know the effects of nitrite and its decomposition products on respiratory enzymes in plants.

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APPENDIX

Analysis of Alkaline Permanganate Traps for Oxides of Nitrogen

The oxides of nitrogen that are absorbed in trap solutions were oxidized to nitrate by the $KMnO_4$. Thus this procedure is designed to destroy the color of the $KMnO_4$ and permit colorimetric nitrate determination.

Reagents

1. Trapping solution of alkaline permanganate; 0.2 M $KMnO_4$ in 2 N KOH

   Dissolve 112.2 g reagent grade KOH in about 800 ml of distilled water. Add 31.7 g $KMnO_4$, warm and stir till 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 M $Na_2C_2O_4$ in 7 N H_2SO_4

   Place about 1200 ml distilled water in a 2 liter beaker. Add carefully 390 ml Conc. H_2SO_4. Weigh out and add 160.6 g $Na_2C_2O_4$. Stir until oxalate is completely dissolved, transfer to 2 liter volumetric flask and make up to volume. Store in brown plastic bottle; the oxalate will attack glass.

3. 5 N NaOH

   Weigh out 400 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 (5 ml) of the trap into a 100 ml beaker. Avoid using excess water. A blank of unused trap solution should be included. From a burette add 5 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 8 ml of reagent 3 (5 N NaOH) to each beaker. Precipitation of Mn as MnO_2 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 MnO_2 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 100 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 100 ml and an aliquot (2 ml) taken for the standard phenoldisulphonic acid nitrate determination. The addition of ammonium sulfamate to destroy nitrites is unnecessary.

The blank carried through the entire procedure is used to set the colorimeter.

Standard curve and calculations

Weigh accurately 1.44 g KNO_3 into a clean beaker; dissolve in distilled water and transfer to a liter flask. Then make up to volume. This solution has 200 ppm N.

Add 0, 1, 2, 3, ... 8 ml of KNO_3 standard to a series of beakers containing 5 ml $KMnO_4$ 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 μ g N per 50 ml. Read on spectrophotometer and plot curve.

Determine from the curve the ration μ g N/Opt density (inverse of slope if Opt density is plotted on the y axis and μ g on the x axis).

$$\frac{\text{Ratio x Opt den x 20}}{1000} = \frac{\text{mgm N}}{\text{Trap}}$$

if 2 ml aliquot was used

$$\frac{\text{Ratio x Opt den x 50}}{1000} = \frac{\text{mgm N}}{\text{Trap}}$$

Ratio obtained during the preparation of standard curve was 110.

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 $KMnO_4$ by $C_2O_4^=$ 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 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.

Table 8. Quantities of different nitrogen compounds recovered at various intervals in sterile and non-sterile Olympic silty clay loam incubated with 300 ppm nitrite N

Reaction time	Nitrogen recovered			
	NO_2^-	NO_3^-	N_2	$NO + NO_2$
hours	mg[a]			
	Sterile			
1.0	22.0	2.2	5.2	0.3
3.0	17.9	4.2	8.0	0.8
5.0	14.2	6.0	9.2	1.2
9.0	9.5	8.9	10.8	2.1
12.0	6.2	10.8	11.0	2.8
	Non-sterile			
1.0	22.1	2.3	5.1	0.2
3.0	18.3	4.5	8.1	1.0
5.0	14.0	6.5	9.0	1.3
9.0	10.0	9.1	11.0	2.3
12.0	6.0	12.0	11.0	3.0

[a] Average of 2 replicate samples.

Table 9. Quantities of different nitrogen compounds recovered at various intervals in Miami silt loam adjusted to pH 3.6 and incubated at 30 C with 75 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
			With trap		
0.5			13.0		
1	13.0	10.0	23.0	3.7	51.0
2			21.3		
3	4.0	16.0	32.0	5.6	67.0
4			31.0		
5	3.0	29.0	31.0	8.0	69.0
7			31.0		
9	0.5	39.0	31.0	9.6	80.0
12	0.0	43.0	31.0	12.3	86.2
			Without trap		
1	17.0	18.6	19.0		55.0
2			23.0		
3	13.0	28.0	31.0		72.0
4			29.0		
5	8.0	51.0	27.0		85.0
7			29.0		
9	4.0	63.0	29.0		96.0
12	3.0	64.0	28.0		95.0

[a] Average of 3 separate experiments with 2 replicates in each.

Table 10. Quantities of different nitrogen compounds recovered at various intervals in Miami silt loam adjusted to pH 3.6 and incubated at 30 C with 150 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
			With trap		
0.5			3.4		
1.0	20.0	34.2	15.2	2.9	72.0
2.0			20.0		
3.0	6.6	27.3	21.7	5.3	60.9
5.0	3.3	48.7	26.7	6.9	86.0
8.0	2.0	49.3	24.7	7.2	83.0
12.0	0.6	56.0	25.3	8.0	90.0
			Without trap		
0.5			5.3		
1.0	22.7	21.3	8.0		52.0
2.0			18.6		
3.0	9.3	30.0	21.6		61.0
5.0	7.0	33.3	32.0		88.0
8.0	6.0	60.6	32.0		100.0
12.0	3.0	61.0	32.0		96.0

[a] Average of 2 separate experiments with 2 replicates in each.

Table 11. Quantities of different nitrogen compounds recovered at various intervals in Miami silt loam adjusted to pH 3.6 and incubated at 30 C with 300 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
			With trap		
0.5			7.0		
1.0	67.0	19.3	10.4	3.3	100.0
2.0			16.4		
3.0	53.0	21.0	17.0	7.0	98.0
4.0			15.0		
5.0	46.0	25.7	14.0	8.7	94.3
7.0			16.0		
9.0	38.7	26.7	17.8	11.0	94.2
12.0	32.3	36.0	16.7	11.7	96.7
			Without trap		
0.5			7.7		
1.0	61.0	27.3	10.3		98.7
2.0			14.7		
3.0	50.3	31.6	15.1		97.0
4.0			15.0		
5.0	36.0	39.6	15.0		90.6
7.0			15.1		
9.0	23.0	46.0	16.6		85.7
12.0	10.0	51.3	16.0		83.3

[a] Average of 3 separate experiments with 2 replicates in each.

Table 12. Quantities of different nitrogen compounds recovered at various intervals in Miami silt loam adjusted to pH 4.0 and incubated at 30 C with 75 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
			With trap		
0.5			13.3		
1.0	5.3	14.7	20.0	3.3	43.3
2.0			22.7		
3.0	2.6	21.3	24.0	5.3	53.2
5.0	1.3	32.0	25.3	8.0	66.6
9.0	0.0	34.7	26.7	8.0	69.4
12.0	0.0	35.0	26.7	10.0	71.7
			Without trap		
0.5			14.7		
1.0	8.0	20.0	24.0		52.0
2.0			25.3		
3.0	4.0	26.7	26.7		53.4
5.0	2.7	37.4	24.0		63.1
9.0	1.3	41.4	25.3		68.0
12.0	0.5	42.0	26.0		68.5

[a] Average of 3 separate experiments with 2 replicates in each.

Table 13. Quantities of different nitrogen compounds recovered at various intervals in Miami silt loam adjusted to pH 4.0 and incubated at 30 C with 150 ppm nitrite N

Reaction	Percent[a] of added nitrite recovered as				
time	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
			With trap		
0.5			9.3		
1	38.0	8.6	14.7	3.5	64.8
2			14.7		
3	18.0	12.7	22.0	6.3	59.0
5	12.0	14.0	28.7	7.7	62.4
7			--		
9	5.0	14.7	25.4	8.7	53.8
12	3.3	17.3	25.4	10.3	53.3
			Without trap		
0.5			11.0		
1	38.0	13.3	13.3		64.6
2			18.7		
3	11.3	17.3	17.3		45.9
5	11.0	23.0	22.7		56.7
7			--		
9	8.0	23.0	25.3		56.3
12	5.3	25.0	24.7		55.0

[a] Average of 4 separate experiments with 2 replicates in each.

Table 14. Quantities of different nitrogen compounds recovered at various intervals in Miami silt loam adjusted to pH 4.0 and incubated at 30 C with 300 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
			With trap		
0.5			6.0		
1.0			9.3		
2.0	33.0	38.0	12.7	2.8	87.0
3.0			14.2		
5.0	21.0	44.3	17.0	8.0	91.0
7.0			22.0		
9.0	7.0	55.0	26.0	9.7	97.7
12.0	4.0	60.0	26.0	11.7	100.7
			Without trap		
0.5			3.0		
1.0			7.3		
2.0	24.3	40.0	7.7		72.0
3.0			15.0		
5.0	20.7	60.7	19.0		100.4
7.0			22.0		
9.0	9.7	66.7	27.0		103.0
12.0	3.3	73.3	26.0		103.0

[a] Average of 3 separate experiments with 2 replicates in each.

Table 15. Quantities of different nitrogen compounds recovered at various intervals in Miami silt loam adjusted to pH 4.5 and incubated at 30 C with 75 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
		With trap			
0.5			2.8		
1.0	40.0	18.0	9.0	2.0	69.0
3.0	25.0	25.0	13.0	4.3	67.3
5.0	19.0	28.0	18.0	5.0	70.0
9.0	16.0	30.0	20.0	8.0	74.0
12.0	14.0	35.0	22.0	9.0	80.0
		Without trap			
0.5			5.0		
1.0	50.0	18.0	9.0		77.0
3.0	30.0	22.0	23.0		75.0
5.0	25.0	25.0	22.0		72.0
9.0	23.0	30.0	22.0		75.0
12.0	20.0	38.0	22.0		80.0

[a] Average of 2 replicates.

Table 16. Quantities of different nitrogen compounds recovered at various intervals in Miami silt loam adjusted to pH 4.5 and incubated at 30 C with 150 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
			With trap		
0.5			1.6		
1.0	46.7	16.0	5.9	1.4	70.0
2.0			11.3		
3.0	31.3	20.7	17.3	4.0	73.3
5.0	22.0	21.3	17.4	4.4	65.1
9.0	21.0	22.0	18.7	6.1	68.8
12.0	18.0	24.0	20.0	7.7	69.7
			Without trap		
0.5			1.7		
1.0	66.0	17.3	4.0		87.3
2.0			7.3		
3.0	34.7	20.0	16.7		71.4
5.0	26.7	24.0	18.7		69.4
9.0	24.0	26.7	19.3		70.0
12.0	22.7	33.0	19.0		74.7

[a] Average of 4 replicates.

Table 17. Quantities of different nitrogen compounds recovered at various intervals in Miami silt loam adjusted to pH 4.5 and incubated at 30 C with 300 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
			With trap		
0.5			2.7		
1.0	65.0	6.0	9.3	1.3	81.6
2.0			10.7		
3.0	51.0	7.3	13.3	3.3	74.9
5.0	35.3	7.7	14.3	4.7	62.0
9.0	27.7	8.7	14.3	5.3	56.0
12.0	28.0	13.0	15.0	7.0	63.0
			Without trap		
0.5			5.3		
1.0	60.7	10.0	8.7		79.4
2.0			11.0		
3.0	41.0	13.0	13.3		67.3
5.0	35.0	19.0	14.0		68.0
9.0	32.7	19.3	14.3		66.3
12.0	25.0	25.0	14.0		64.0

[a] Average of 4 replicates.

Table 18. Quantities of different nitrogen compounds recovered at various intervals in Miami silt loam adjusted to pH 5.0 and incubated at 30 C with 75 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
			With trap		
0.5			1.3		
1.0	70.7	9.3	11.2	2.3	93.5
2.0			13.2		
3.0	46.7	12.0	15.3	4.0	78.0
5.0	42.7	16.0	17.7	5.3	81.7
9.0	22.7	29.4	17.9	7.3	79.3
12.0	21.3	33.3	18.0	8.5	81.1
			Without trap		
0.5			0		
1.0	80.4	10.0	10.1		100.5
2.0			12.5		
3.0	60.7	16.0	16.0		92.7
5.0	48.0	26.7	17.7		92.4
9.0	40.0	42.7	17.8		100.5
12.0	38.7	42.7	18.1		99.5

[a] Average of 2 replicates.

Table 19. Quantities of different nitrogen compounds recovered at various intervals in Miami silt loam adjusted to pH 5.0 and incubated at 30 C with 150 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
			With trap		
0.5			0.0		
1.0	72.0	11.3	4.0	1.1	88.4
2.0			9.3		
3.0	52.0	16.7	16.0	3.7	88.4
5.0	48.7	18.7	16.7	5.7	89.8
9.0	33.3	20.7	17.3	6.7	78.0
12.0	29.0	23.0	18.0	8.3	78.3
			Without trap		
0.5			2.0		
1.0	74.7	14.7	4.7		94.1
2.0			8.7		
3.0	56.7	28.0	14.3		99.0
5.0	50.0	33.3	15.7		89.0
9.0	36.0	34.7	16.7		87.4
12.0	36.0	34.0	17.0		87.0

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[a] Average of 4 replicates.

Table 20. Quantities of different nitrogen compounds recovered at various intervals in Miami silt loam adjusted to pH 5.0 and incubated at 30 C with 300 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
			With trap		
0.5					
1.0	69.4	3.3	3.3	1.9	77.9
2.0			10.0		
3.0	54.3	8.3	11.7	2.5	76.8
5.0	45.0	11.3	13.7	3.7	73.7
9.0	43.3	13.3	14.0	6.8	77.4
12.0	40.0	15.0	14.3	7.3	76.6
			Without trap		
0.5			0.5		
1.0	70.7	2.7	5.3		78.7
2.0			10.0		
3.0	69.4	6.0	11.8		87.2
5.0	61.0	8.0	13.7		82.7
9.0	49.3	9.3	14.0		71.6
12.0	41.7	10.7	15.0		67.4

[a] Average of 2 replicates.

Table 21. Quantities of different nitrogen compounds recovered at various intervals in Olympic silty clay loam incubated at 30 C with 75 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
		With trap			
1	73.0	7.0	24.0	1.3	102.3
2					
3	53.0	11.0	29.0	4.0	97.0
4					
5	38.0	21.0	33.0	6.0	98.0
9	13.0	34.0	40.0	9.0	96.0
12.0	5.0	40.0	43.0	12.0	100.0
		Without trap			
1	80.0	11.0	17.0		108.0
2					
3	60.0	20.0	32.0		~~102.0~~ 112.0
4					
5	40.0	27.0	37.0		104.0
9	20.0	37.0	44.0		101.0
12	10.0	45.0	47.0		102.0

[a] Average of 4 replicates.

Table 22. Quantities of different nitrogen compounds recovered at various intervals in Olympic silty clay loam incubated at 30 C with 150 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
		With trap			
1	63.3	5.3	11.0	1.3	80.9
2			21.0		
3	52.0	9.0	28.0	3.0	92.0
4					
5	41.0	17.0	32.0	5.0	95.0
9	21.0	33.0	35.0	8.0	97.0
12	18.0	37.0	37.0	10.0	102.0
		Without trap			
1	67.0	8.0	15.0		90.0
2			20.0		
3	49.0	22.0	29.0		100.0
4					
5	44.0	25.0	33.0		102.0
9	28.0	33.0	41.0		102.0
12	21.0	40.0	42.0		103.0

[a] Average of 4 replicates.

Table 23. Quantities of different nitrogen compounds recovered at various intervals in Olympic silty clay loam incubated at 30 C with 300 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
			With trap		
1	73.3	7.3	17.3	1.0	98.9
2			19.6		
3	59.6	14.0	26.6	2.6	102.8
4					
5	47.3	20.0	30.6	4.0	101.9
9	30.6	29.6	36.3	7.0	103.5
12	20.6	36.0	36.6	9.3	102.5
			Without trap		
1	71.6	6.6	19.3		97.5
2			22.0		
3	60.6	17.3	25.0		102.9
4					
5	54.0	21.6	27.0		102.6
9	33.0	33.3	36.0		102.3
12	20.0	40.0	37.6		97.6

[a] Average of 4 replicates.

Table 24. Quantities of different nitrogen compounds recovered at various intervals in Dayton silty clay loam incubated at 30 C with 75 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
			With trap		
0.5			11.0		
1.0	64.0	7.0	22.0	1.0	94.0
3.0	44.0	12.0	27.0	4.0	87.0
5.0	41.0	15.0	36.0	6.9	98.0
9.0	24.0	35.0	38.0	9.0	106.0
12.0	21.0	40.0	39.0	10.0	104.0
			Without trap		
0.5			9.0		
1.0	62.0	9.0	25.0		96.0
3.0	50.0	16.0	27.0		93.0
5.0	40.0	19.0	37.0		96.0
9.0	36.0	30.0	33.0		99.0
12.0	31.0	43.0	36.0		110.0

[a] Average of 2 replicates.

Table 25. Quantities of different nitrogen compounds recovered at various intervals in Dayton silty clay loam incubated at 30 C with 150 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
		With trap			
0.5					
1.0	67.0	6.0	17.0	1.0	91.0
3.0	60.0	10.0	28.0	3.0	101.0
5.0	45.0	13.0	31.0	5.0	94.0
9.0	37.0	21.0	33.0	7.0	98.0
12.0	30.0	27.0	33.0	8.0	98.0
		Without trap			
0.5					
1.0	74.0	7.0	17.0		98.0
3.0	66.0	14.0	20.0		100.0
5.0	60.0	17.0	28.0		105.0
9.0	48.0	28.0	29.0		105.0
12.0	45.0	30.0	30.0		105.0

[a] Average of 2 replicates.

Table 26. Quantities of different nitrogen compounds recovered at various intervals in Dayton silty clay loam incubated at 30 C with 300 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
			With trap		
0.5					
1.0	69.0	12.0	10.0	1.0	102.0
3.0	56.0	13.0	19.0	2.0	90.0
5.0	43.0	16.0	23.0	3.0	85.0
9.0	39.0	17.0	25.0	5.0	86.0
12.0	33.0	18.0	27.0	7.0	85.0
			Without trap		
0.5					
1.0	68.0	8.0	8.0		84.0
3.0	63.0	18.0	22.0		93.0
5.0	43.0	23.0	24.0		90.0
9.0	37.0	27.0	26.0		80.0
12.0	27.0	28.0	27.0		82.0

[a] Average of 4 replicates.

Table 27. Quantities of different nitrogen compounds recovered at various intervals in cation exchange resin systems adjusted to pH 4.0 and incubated at 30 C with 300 ppm nitrite N

Reaction time	Percent[a] of added nitrite recovered as				
	NO_2^-	NO_3^-	N_2	$NO + NO_2$	Total
hours					
		With trap			
0.5			1.0		
1.0	91.0	2.0	6.0	0.4	99.4
3.0	70.0	6.3	8.7	1.5	85.5
5.0	65.0	7.7	8.0	2.0	82.7
7.0	62.0	15.7	8.0	2.9	88.6
9.0	47.0	21.6	8.3	3.3	80.2
12.0	40.0	27.0	8.0	4.0	79.0
		Without trap			
0.5			4.6		
1.0			5.9		
3.0	74.0	2.0	10.7		86.7
5.0	56.0	9.0	11.0		76.0
7.0	52.0	13.0	11.0		76.0
9.0	40.0	28.0	11.0		79.0
12.0	33.3	33.3	11.0		77.6

[a] Average of 2 replicates.