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THE INFLUENCE OF ORGANIC MATTER AND pH ON TRANSFORMATIONS
OF NITRITES TO ELEMENTAL NITROGEN

by

Mohammad A. Rashid

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

of

DOCTOR OF PHILOSOPHY

in

Soil Fertility

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1966

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Mohammad A. Rashid

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INTRODUCTION

Nitrogen occupies an important and prominent place in plant and animal nutrition. Large quantities are required by plants and must be available in the soil. Recently the use of nitrogenous fertilizers has increased manifold. In view of the importance of this element in crop production, even greater use is anticipated in the coming years. In order to make full use of the applied nitrogen, continued investigations of soil-nitrogen relationship are imperative; however, this element presents many complex and challenging problems to investigators.

The most important problem in nitrogen relationships concerns its losses, inasmuch as it is depleted and lost from the soil very easily. No other plant element suffers so varied losses as does nitrogen. Losses through leaching, volatilization, biological process, and chemical reactions, under different soil conditions, have been investigated and proved beyond any doubt. The extent and magnitude of nitrogen losses are much more severe than originally suspected, and future investigators may reveal more hitherto unknown complexities of the problem.

Losses of nitrogen under poor aeration have long been known. One of the surprising revelations of recent years is that such losses do occur and assume considerable proportions even in soils kept in good aerated condition. Much attention has been focused on this aspect of the problem and several mechanisms, reactions, and pathways have been suggested by the workers to account for the loss. At present, investigations are being made into the reactions of nitrite ion. This ion has long been known as an intermediate in the process of biological

oxidation of ammonia to nitrate and also in the process of denitrification. Although it is a transitory intermediate in these processes and rather unstable under normal soil conditions, in the nitrogen economy of soils it assumes considerable importance, in view of its high degree of reactivity. It is believed to react with many soil components, resulting in the formation of diversified nitrogen compounds and losses of nitrogen in varied forms.

Some of the most recent investigations in the Nitrogen Laboratory of Utah State University suggest that nitrites may also react with soil organic matter, yielding elemental form of nitrogen. The work reported here was undertaken to determine some reactions of nitrite with organic matter, which result in losses of nitrogen in elemental form.

REVIEW OF LITERATURE

It has long been suspected that losses of N may occur in nature in appreciable quantities. Attempts to make soil nitrogen balance sheets have revealed substantial deficits in total N (Allison, 1955). These deficits were attributed to the losses of N by volatilization (Allison et al., 1952; Clark et al., 1960; Soulides and Clark, 1958; Wagner and Smith, 1958). In recent years there has been renewed interest in volatile losses of N from soils and much information has accumulated on the subject. The best studied mechanisms of volatile N losses involve loss of ammonia under certain soil conditions and microbial denitrification occurring under conditions of limited air supply (Martin and Chapman, 1951; Broadbent and Stojanovic, 1952; Allison, 1955; Marshall et al., 1953; Clark et al., 1960; Cady and Bartholomew, 1960). These have been used by many workers to explain N deficits.

Smith and Clark (1960) have reported loss in excess of 50 percent of the applied N under conditions where neither volatilization of ammonia nor enzymatic denitrification of nitrate were of any consequence. Many workers (Allison et al., 1952; Gerretsen and DeHoop, 1957; Soulides and Clark, 1958; Wagner and Smith, 1958; Clark et al., 1960) have presented evidences that such losses may occur even in well aerated arable soils. For a long time the investigators have felt that the losses of N were tied up with the nitrite ion but they were undecided on the mechanism of such losses as they lacked experimental evidences. Allison et al. (1952), Gerretsen and DeHoop (1957), Soulides and Clark (1958), Wagner and Smith (1958), Clark et al. (1960),

Wullstein and Gilmour (1964), Ruess and Smith (1965), and others are a few of the many investigators who have presented experimental evidences to show that N losses are tied up with reactivity of nitrite ions.

Conditions Favoring Nitrite Accumulation in Soils

Nitrites may accumulate in the soils for a temporary period as transitory intermediates of the process of nitrification and denitrification. The accumulation of large quantities of nitrite has been observed in both field and laboratory experiments by many workers (Kelley, 1916; Martin et al., 1942; Chapman and Liebig, 1952; Broadbent et al., 1957; Stojanovic and Alexander, 1958; Justice and Smith, 1962). The accumulated nitrites may persist in the soil from a few days to several weeks and the quantity may vary from traces to sizable amounts. In view of the important role of nitrite in the losses of N and its toxic effects on plants, it is important to see under what soil conditions the nitrite accumulation takes place.

Soil reaction

Alkaline soil conditions. Soil reaction is considered to be the most important factor governing the accumulation of nitrite. Under alkaline soil conditions greater nitrite accumulation takes place than in acid soils. Kelley (1916) found large accumulation when small amounts of sodium carbonates were incorporated with soils to which dried blood or ammonium sulfate was added. In some cases the nitrite content greatly exceeded the nitrate content. He noted distinct increase in nitrite content and suppression in nitrate formation with the addition of alkali salts.

Robinson (1923) reported rapid loss of nitrite in all acid soils without any nitrate formation. The greater the acidity, the more quick were the losses. When calcium carbonate was incorporated in soils receiving sodium nitrite treatment, the nitrites were retained for longer periods.

Midgley (1932), in his studies of nitrification in heavily limed soils, noticed nitrite accumulation sixteen times greater than those soils heavily limed than in the same soils receiving light doses of lime. His results were identical with those of Albrecht and McCalla (1937) who, while studying the nitrification of ammonia, found that soils with lime had seven times more nitrite than soils receiving no lime. Similar results have been reported by Fraps and Sterges (1939).

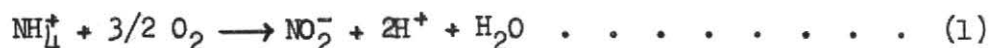
Martin et al. (1942) made a comparative study of nitrification of ammonium sulfate and urea in six typical Arizona desert soils and suggested that the complete oxidation of ammonia to nitrite does not take place in alkaline desert soils until the pH has been reduced to a threshold value of 7.7 ± 0.1 . They found accumulation of nitrites in well aerated alkaline soils and concluded that in excessively alkaline soils the nitrite oxidation phase of the process of nitrification will be delayed until other natural chemical processes have reduced the pH value to the point where nitrification can take place. If this reduction in pH is not accomplished, the formation of nitrates will be greatly reduced.

Chapman and Liebig (1952), in their field and laboratory studies on accumulation of nitrites, found substantial amount of nitrite accumulation with the application of urea, anhydrous ammonia, ammonium or ammonia forming fertilizers to neutral or alkaline soils. From this

observation they concluded that in neutral and alkaline soils of arid and semi arid regions heavy application of ammonia or ammonia forming fertilizers are likely to lead to nitrite accumulation. Such accumulation may persist for months or may disappear rather rapidly, depending on soil conditions, especially the resistance of soil to change in pH. Wahhab and Fazaluddin (1954) have reported accumulation and persistence of large quantities of nitrite in soils of Punjab with a pH of 8.0 to 8.3, fertilized with ammonium sulfate. There are many others who agree to this viewpoint. Duisberg and Buehrer (1954), Martin and Cox (1956), Broadbent et al. (1958), and Stojanovic and Alexander (1958) found high accumulation of nitrite in alkaline soils treated with large quantities of ammonium hydroxide, ammonium sulfate and ammonium nitrate.

From the foregoing discussions it is clear that alkaline soil conditions favor larger accumulation of nitrite. It will be of interest to see how the accumulation of nitrite occurs under alkaline conditions.

The process of nitrification is a two-step process and takes place in two stages, each stage being brought about by a different set of bacteria. The nitrite bacteria convert ammonia to nitrite



and in the second step nitrate bacteria convert nitrite to nitrate



Kelley (1916), Martin et al. (1942), and Chapman and Liebig (1952) have reported that alkaline conditions are not conducive to nitrite oxidizers.

Lees and Quastel (1946) have suggested that high concentrations of ammonium ion inhibit the growth and metabolism of Nitrobacter and hence the oxidation of nitrite. The degree of inhibition increases as the pH

of the medium increases. This may be because at pH greater than 7.0, the free ammonia concentration, known to be injurious to the cells in general, rises sharply. Broadbent (1957) and Stojanovic and Alexander (1958) have supported this viewpoint, suggesting that at high pH free ammonia is present in the soil in large quantities and this free ammonia is selective as an inhibitor of the Nitrobacter species. Once the pH has fallen sufficiently low to eliminate or reduce the level of free ammonia to a non-inhibitory concentration, or the ammonium added has been oxidized to a point where the remaining substrate has a non-toxic quantity of free ammonia, nitrite oxidation commences. Aleem and Alexander (1960) have also illustrated selective inhibitory effect of free ammonia on Nitrobacter agile. Under similar condition they have noticed only slight inhibition in the activity of Nitrosomonas.

Acid soil conditions do not favor the accumulation of nitrite. The inhibitory effects mentioned in the foregoing discussions do not exist under these conditions and hence the biological oxidation of nitrite to nitrate is rapid. In addition, under acid soil conditions, the accumulated nitrite may rapidly decompose resulting in the loss of N. There are, however, some instances to show that nitrite may accumulate even under acidic conditions. Wagner and Smith (1958) reported that a soil having an initial pH of 5.6 treated with 500 ppm N as urea, accumulated nitrite to the extent of 94 ppm after 2 weeks. Patil (1963) has reported large accumulation of nitrite in acid Walla Walla soil (pH 6.5) when treated with 1000 ppm N as urea. With the application of urea the soil pH shifted to alkaline condition. Within 3 days the pH rose as high as 8.8 and then declined gradually. After 6 weeks of incubation the pH came down to 6.7. The maximum accumulation of nitrite reported by him

was 187 ppm. At the end of 6 weeks of incubation the nitrite content of the soil was still high (138 ppm).

Other conditions

In addition to alkaline soil condition the next most important factors favoring nitrite accumulation are the kind and rate of application of nitrogenous fertilizers. Ammonia or ammonia yielding fertilizers give rise to larger quantities of nitrites than does the nitrate form of nitrogen fertilizer. Stojanovic and Alexander (1952) have emphasized the concentration of ammonia in relation to nitrite accumulation.

Smith and Burns (1965) have reported higher concentration of nitrite in the zone of decomposition of organic material incorporated in the soil.

Soil temperature is another factor which may influence nitrite accumulation or its persistence in the soil. Chapman and Liebig (1952) have reported that once the nitrites have formed they can persist for several months if the soil temperature is not sufficiently warm to permit rapid microbial activity. Tyler and Broadbent (1960) have shown that the nitrite oxidizers are very sensitive to low temperatures. Justice and Smith (1962) have found high accumulation of nitrite both at low and high temperatures. With the application of 450 ppm of N as ammonium sulfate, the nitrite accumulated in soils incubated for 70 days at 10 C was as high as 72 ppm.

Clark et al. (1960), working with many poorly buffered soils of pH value ranging from 6.7 to 7.2, noted mineral N deficits exceeding 25 percent of the applied N. Such soils accumulated nitrite during

incubation following the addition of urea. With the addition of urea to such soils the pH shifted towards greater alkalinity due to the release of ammonia. This shift in pH favored nitrite accumulation in the poorly buffered soils.

Chemical or photochemical processes

Rao and Dhar (1939) have suggested that under the action of sunlight and in the presence of photo-sensitizers like oxides of titanium, cadmium, zinc, aluminum, magnesium, and silicon, there is a vigorous oxidation of ammonia or ammonium salts to nitrite. They have suggested that in the presence of sunlight and photo-sensitizers the O_2 of atmosphere is converted to ozone (O_3). Ozone interacts with ammonia or ammonium salts according to the following reaction:



They have suggested that nitrification in soil is, at least in part, a photochemical reaction that takes place on the surfaces of the various photo-catalysts present in the soil under the influence of sunlight. In a separate publication Dhar and Bhattacharya (1933) have pointed out that even in the sterilized soil ammonium phosphate undergoes considerable oxidation in the presence of light. They found no difference in oxidation in sterilized and nonsterilized soils. They, therefore, suggested that nitrification in tropics is more a photochemical than a bacterial process. It was pointed out by them, however, that ammonium salts are photochemically oxidized to nitrites and not to nitrates.

From these discussions it is clear that nitrites may become an important intermediate product under field conditions and may pose problems with regard to nitrogen losses.

Reactions of Nitrites in Soils Resulting
in the Loss of Nitrogen

In the foregoing discussions ample evidences have been presented showing an accumulation of nitrite as transitory intermediate of the process of nitrification. The nitrite ions are not stable. Depending upon the soil conditions the accumulated nitrites undergo rapid transformations, resulting sometimes in the loss of N. In the recent years information has begun to accumulate on these losses of N arising due to nitrite reactivity. There is good agreement among the workers that acid soil conditions aggravate the problem but considerable disagreement exists with regard to the mechanism and end products of nitrite reactions. For ease in discussions nitrite reactions are categorized into the following broad groupings:

1. Self decomposition of nitrite under acid conditions
2. Reaction of nitrite with ammonia or urea resulting in the formation of ammonium nitrite
3. Reactions with various components of organic matter
 - (a) Reaction with amino acids
 - (b) Reactions with other fractions
4. Reactions involving hyponitrous acid.

Self decomposition of nitrite under acid conditions

The spontaneous or self decomposition of nitrite under acid soil conditions, resulting in the loss of N, is one of the well accepted mechanisms. The majority of workers (Temple, 1914; Robinson, 1923; Fraps and Sterges, 1939; Clark et al., 1960) have reported nitric oxide (NO) as the product of decomposition. However, N₂ has also been

reported in some instances (Tyler and Broadbent, 1960; Thorne, 1961).

Temple (1914) was probably the first person to suggest the possibility of self decomposition of HNO_2 according to the following reaction:



He suggested that the NO may react with O_2 resulting in NO_2 production and setting up a cycle with gaseous intermediates which could be re-absorbed to form nitrous acid or could result in gaseous losses.

In later years Robinson (1923) noticed a rapid loss of nitrite from acid soils with the addition of NaNO_2 . The greater the acidity the more rapid were the losses. He observed complete loss of added nitrite in a very short period and did not notice any accumulation of nitrate. Where the soil had received a calcium carbonate treatment, the nitrites were retained for a longer period and the losses were retarded. The loss of N in acid soil was attributed to the instability of nitrous acid formed in acid soils as:



Nitrous acid is very unstable and escapes rapidly after decomposition depending on the degree of acidity.

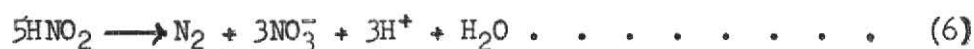
Fraps and Sterges (1939) added 100 ppm of nitrite-N to a group of soils ranging in pH from 4.4 to 6.9 and recorded losses from 10 to 95 ppm over an 8-day period. They assumed the loss to be due to the nitrous acid decomposition, according to the reaction No. 4.

Clark et al. (1960) recorded deficits greater than 50 percent of the applied nitrite-N from acid soils and suggested that even a weak acidity is important in causing the breakdown of nitrite. They also found that with the addition of KNO_2 to 41 different soils larger N deficits occur from soils in pH range of 5.5 to 6.5 than from soils

ranging in pH from 4.5 to 5.5. This finding is not in agreement with the fact that nitrous acid instability increases with increase in acidity. The explanation suggested by them is that under more acid conditions there is rapid conversion of nitrite to nitrate, thereby reducing the supply of nitrite for conversion to a volatile form.

Tyler and Broadbent (1960) reported N_2 evolution from acid soils upon the addition of nitrite. A trapping system failed to show more than traces of NO or NO_2 . Mass spectrometer data confirmed N_2 as the major component and failed to reveal the presence of NO or NO_2 . In their studies the loss from sterile and non-sterile soil was the same.

Another interesting possibility has been brought to light by Thorne (1961). While investigating the HNO_2 decomposition, he identified N_2 as one of the gases evolved from the system. This was apparently arising from a spontaneous conversion of HNO_2 to N_2 and nitrate and could be described by equation 6.



In addition to this he has proposed one more pathway of HNO_2 decomposition.



At pH 1.4, reaction 7 accounted for 90 percent of HNO_2 decomposition in Thorne's studies. He has hypothesized that hyponitric acid ($H_2N_2O_3$) is an intermediate in reactions 6 and 7.

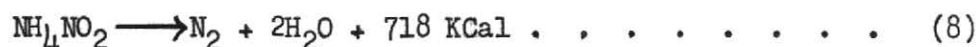
Reactions of nitrite with ammonia or urea forming ammonium nitrite

Another suggested mechanism for the loss of N is the reaction of nitrite with ammonia or urea forming ammonium nitrite which ultimately decomposes to yield N_2 . In the literature there are many proponents

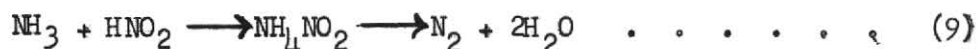
(Lohnis, 1913; Dhar, 1960; Allison, 1963) of this idea and a few opponents (Sabbe and Reed, 1964), too. The ammonium nitrite mechanism of nitrogen loss is distinctly different from that of Van Slyke mechanism in view of the fact that the former may occur even in alkaline conditions whereas the latter is strictly restricted to acid conditions. Allison (1963) has pointed out that ammonium nitrite can also form from interaction of ammonia or ammonium ions and nitrite in alkaline media where there is no nitrous acid.

In the early part of this century Lohnis (1913) postulated that nitrites accumulated in soil may react with ammonia to yield N_2 . He considered ammonium nitrite to be the intermediate in this reaction.

Dhar (1960) observed copious evolution of nitrogen gas when a concentrated solution of ammonium sulfate was mixed with a concentrated solution of nitrite, even under feebly acidic conditions. It was suggested by him that the nitrite interact with ammonia forming unstable ammonium nitrite which decomposes, yielding N_2 according to the following reaction:



Allison (1963) has also favored the idea of formation and decomposition of ammonium nitrite.

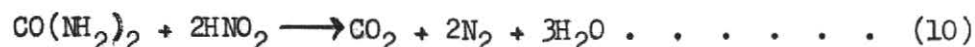


Ammonia and nitrite instantaneously unite to form ammonium nitrite. This unstable compound then decomposes to N_2 gas and water.

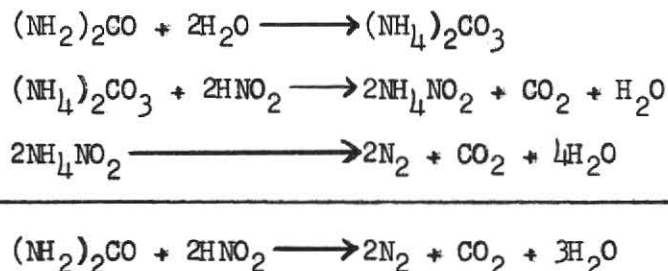
Sabbe and Reed (1964) have reported that when N is added to the soil in a band, the inorganic N ions could reach the minimum concentrations necessary for the ammonium nitrite reaction to occur. However, the conditions for an accumulation of nitrite to occur (i.e., alkaline

media and high ammonia concentrations) are contrary to those under which ammonium nitrite reactions will readily occur.

There is less certainty as to the nature of reactions between nitrous acid and urea. Krase (1932) stated that there was very little direct reaction between the two substances unless a strong acid, such as nitric or hydrochloric, is present. Sneed and Brasted (1956) have suggested the following reaction between urea and nitrite.



Allison (1963) has favored the above reaction but he thinks the reaction goes to completion with certain intermediate steps. The intermediate steps suggested by him involve the formation of $(\text{NH}_4)_2\text{CO}_3$ and NH_4NO_2 according to the following reactions:



The sum total of all the intermediates is the same as suggested by Sneed and Brasted (reaction No. 10).

Patil (1963) noted an increased N_2 evolution with the addition of alfalfa, ammonium sulfate, and urea to nitrite. The increase in N_2 evolution suggests reaction of nitrite with these substances. In his studies a higher amount of N_2 was evolved with urea than with ammonium sulfate. It is possible that the N_2 recorded by him was evolved by the mechanism suggested by Allison (1963) and others which involves the formation and decomposition of ammonium nitrite.

From these discussions it is evident that in both the reactions of ammonia and urea with nitrite, ammonium nitrite could be formed. At

present there is not much information available to show how concentrated the ammonium nitrite solution must be before decomposition becomes appreciable. Sabbe and Reed (1964) are of the opinion that the concentrations of both urea and nitrite needed to evolve N are seldom, if ever, found in the soil.

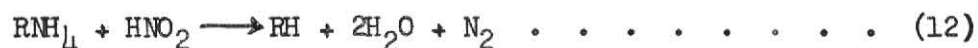
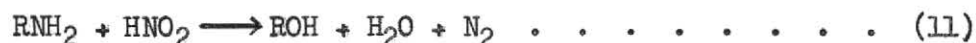
It is generally recognized that ammonium nitrite in concentrated solution is extremely unstable but in very dilute solutions is moderately stable. It decomposes intramolecularly according to reaction No. 9. Thorne and Roberts (1954) suggested that one part of the molecule is oxidized and the other reduced and, hence, the complete change appears to be one of direct decomposition. The rate of decomposition of ammonium nitrite increases with temperature and is appreciably greater if the medium is acidic instead of alkaline.

Reactions of nitrites with various components of organic matter

van Slyke type of reaction. Of all the mechanisms suggested for nitrite reactivity, the Van Slyke type of reaction involving the alpha amino acids of organic matter is probably the most controversial. There are many workers (Barritt, 1931; Wilson, 1943; Wahhab and Fazal Uddin, 1954; Gerretson and DeHoop, 1957) who strongly favor this type of reaction and there are many more (Allison and Doetsch, 1951; Jones, 1951; Clark and Beard, 1960; Smith and Clark, 1960; Allison, 1963; Sabbe and Reed, 1964) who are strongly opposed to the idea.

It has long been known that nitrite react readily with many organic nitrogenous substances producing nitrogen gas. Van Slyke studied this aspect and found wide difference in the ratio of reaction of different compounds. It was found by him that amino group in the alpha position

reacts with nitrite most readily while the N of ammonia, methylamine, and purine react at a slower rate. This reaction is represented by the following equation:



In the Van Slyke apparatus the reaction is carried out in the presence of glacial acetic acid and in an atmosphere of NO. Under such conditions it proceeds rapidly and goes to completion.

Many investigators have tried to apply this reaction to soil conditions and it has become a subject of much controversy.

Barritt (1931) found loss of amino nitrogen as a result of interaction of nitrite and glycine in solution, when maintained at pH 5.8 but not at 6.5. He concluded that direct conversion of amino nitrogen to N_2 would not occur by biological reactions but could occur through the reaction of nitrous acid with amino compounds.

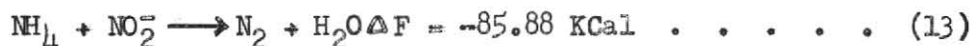
Wilson (1943) is one of the proponents of this idea who reported that Van Slyke type of reaction occurs throughout the nature. He presented data to show that N_2 would be evolved from the reaction of nitrite in the soil and such a reaction could also occur in plant exudates at pH 6.3 to 6.7.

Wahhab and Fazal Uddin (1954) investigated the possibility of interaction of NH_4^+ and nitrite in soils of Pakistan with pH 8.0 to 8.3. They found that even under alkaline conditions such a reaction takes place provided the reactants are in high concentrations. They also noticed slow evolution of elemental nitrogen when concentrated solutions of ammonium sulfate and sodium nitrite were mixed at pH 7.2.

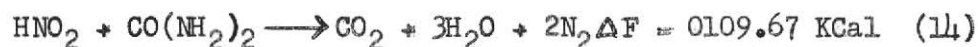
Most notable among those who favor the occurrence of Van Slyke

type of reaction in soils are Gerritsen and DeHoop (1957). They have emphasized that the nitrite can react with amino acids and ammonia in well aerated acid soils, resulting in loss of molecular N. In pot experiments with acid sandy soils, losses up to 74 percent of added ammonium sulfate were reported by them. While accounting for the losses encountered, they ruled out the possibility of volatilization of ammonia because the soils were acid and the nitrogen losses disappeared when the pH was raised above 5.5. Under the conditions of their experiment there were no chances of denitrification. These losses were, therefore, attributed by them to the reaction of ammonium sulfate with nitrite even though nitrite levels were low in all cases. They have also demonstrated that sterile buffer solutions containing KNO_2 and ammonium sulfate can lose lot of N_2 gas at pH 4.0 to 5.5 but not above. It is suggested that added nitrite gives rise to HNO_2 when the concentration of H^+ is raised. It is this compound which reacts with ammonia. They have suggested that during the process of microbial decay of organic matter a great variety of amino acids are produced in the soil which can react with HNO_2 resulting in the loss of N.

Chao and Kroontje (1963) have reported the possibility of evolution of free N from reactions between nitrite and ammonium ions



and between nitrite and urea



Patil (1963), working with acidified Fort Collins Soil (pH 4.2), noted that upon the addition of ammonium sulfate or organic matter to the soil treated with 400 ppm of nitrite-N, the losses of N_2 were high. With the addition of alfalfa he found greater loss of N_2 as compared to

the addition of ammonium sulfate. This indicates that some nitrogenous compound of organic matter is more reactive than ammonium ions. On the basis of this observation, Patil suggested that Van Slyke type of reaction may take place in the soil.

There are many investigators who do not agree to the possibilities of occurrence of this type of reaction in the soil. Allison and Doetsch (1951) did not find any reaction of amino acids or ammonia with nitrite to form nitrogen gas in dilute solutions under conditions commonly occurring in nature. In their view the N gas production is possible only when the reacting materials are present in appreciable concentrations and the reaction is sufficiently acid. In the presence of air and moisture, they suggested strong possibility of conversion of nitrite to nitrate than the reaction between nitrite and amino acids. They have totally ruled out the possibility of such a reaction occurring in normal soils on the grounds that conditions requiring for the completion of this type of reaction are almost absent, and if at all some reaction takes place, it will be of minor importance.

Jones (1951) studied N_2 production in acid soil (pH 5.8) under aerobic conditions using various nitrogen sources tagged with N^{15} . In his work he did not find Van Slyke type of reaction occurring in the soil. Clark and Beard (1960), while studying the influence of organic matter on volatile losses of nitrogen from soil, found some component of organic matter responsible for dismutation of nitrite but they totally overruled the possibility of any reaction of amino acids with nitrites.

Smith and Clark (1960) were not able to get results similar to those reported by Gerretsen and DeHoop. They compared the losses of

nitrogen from the treatment to which ammonium and nitrite were added with the treatment in which nitrite plus an appropriate buffer was added. As much nitrogen evolved from the latter treatment from which ammonium sulfate was omitted as where it had been added. They, therefore, concluded that evolution of N was not due to the reaction between ammonia and nitrite but rather the reduction of nitrite by some other soil agent. However, in the presence of heavy concentrations of both reactants, they noticed some reaction between ammonia and nitrite.

In highly acid cation exchange resin, Reuss and Smith (1962) noted NO evolution but no N₂ on treatment with NaNO₂ and ammonium sulfate. When nitrite was added to soil of the same pH, they observed N₂ besides NO. However, no additional N₂ was evolved when ammonium sulfate was added along with nitrite source. They, therefore, concluded that ammonium sulfate may not react with nitrite under many conditions.

In his most recent publication, Allison (1963) has reemphasized the exclusion of true Van Slyke type of reaction occurring in soil. Sabbe and Reed (1964) agree with Allison's view and are of the opinion that this type of reaction is of minor importance in the soils.

Reactions with other components of organic matter

In addition to amino acids, it has recently been suspected that some other component of organic matter may also react with nitrite and accentuate the losses of nitrogen.

Robinson (1923), while studying the fertilizer value of sodium nitrite, observed its rapid loss from neutral soils high in organic matter as compared to the soils low in organic matter. He suggested

that organic matter may cause rapid decomposition of nitrite.

Bremner (1957) encountered difficulties in determining nitrogen from free amino groups and suggested that lignin or lignin derived material may react with nitrite yielding nitrogen, resulting in apparent high amino-N values.

Soluides and Clark (1958) in their nitrification studies on grassland soils in comparison with intertilled soils found higher retention of ammonia and lesser production of nitrate, in all grassland soils than intertilled counterparts when they were amended with 0.1 percent urea. In almost all cases they noticed nitrite accumulation and consequently N deficits. Greater N deficits were commonly encountered in the grassland than in the intertilled soils. Smith and Clark (1960) studied N losses in Fort Collins soil containing 3.86 percent organic matter and Durham soil with 0.54 percent organic matter. They noticed greater evolution of nitrogen in Fort Collins than Durham and suggested that some component of organic matter was more reactive in reducing nitrite than was ammonium ion.

Clark and Beard (1960) have presented data strongly supporting that organic matter has strong influence on dismutation of nitrite and consequent losses of N from soils at comparable pH values. They observed that at a given pH value some component in soil served to promote the reactivity of nitrite and development of much greater N deficits than occurred in sand. This specific influence in soil was greatly reduced by pretreatment of the soil with hydrogen peroxide (H_2O_2). Organic amendments such as peptone or alfalfa meal added to quartz sand increased the mineral nitrogen deficits. They, however, have not reported the forms in which the N was lost.

In recent years Stevenson and Swaby (1963), Stevenson and Kirkman (1964), and Stevenson and Swaby (1964), in their studies, observed reaction of nitrous acid (HNO_2) with humic acid, fulvic acid, lignin and lignin building units and polyphenols resulting in evolution of N_2 , N_2O , CO_2 , and CH_3ONO (methyl nitrite).

It is evident from the foregoing discussions that many investigators have mentioned the "deficits" of nitrogen as influenced by organic matter. No attempts have been made to define the "deficits" in clear terms.

Reuss (1963) has reported N_2 production when nitrite is added to the acid soil. In his view the N_2 evolved was not the result of reaction of nitrite with ammonium ion. He thought that it might have evolved either due to the interaction of HNO_2 and organic matter or direct reduction of nitrite to N_2 , catalyzed by some fraction of organic matter.

In another recent study, Patil (1963) has observed N_2 evolution with addition of nitrite to alfalfa. He noticed greater N_2 production in nitrite-alfalfa reaction than nitrite-ammonium sulfate reaction. This lead him to conclude that labile organic nitrogen compounds are more reactive than ammonium sulfate.

Reactions of nitrite with hyponitrous acid

The reactions related to the process of nitrification have been presented in simple equations (No. 1 and 2) but they are not as simple as presented. There may be many intermediates. As early as the beginning of this century, Mumford (1914) obtained evidences about hyponitrous acid being an intermediate product between ammonia and nitrite. Corbot (1934) and Lees (1948) have also favored the idea of hyponitrous acid

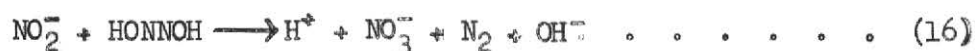
formation as an intermediate compound in the biological or photo-chemical oxidation of ammonia to nitrous acid.

Thorne (1961), while suggesting certain pathways of nitrous acid decomposition, has postulated hyponitrous and hyponitric acids as intermediates. More recently Chao and Kroontje (1963) have suggested that the formation of hyponitrous acid as an intermediate product of nitrification is thermodynamically feasible.

Hyponitrous acid undergoes a variety of reactions. By ionic decomposition it gives rise to N_2 as suggested by Buchholz (1963) and Chao and Kroontje (1963).



By free radical decomposition it yields N_2 and nitrate. Buchholz (1963) has presented evidences to show that by adding sodium nitrite to acidified solution of hyponitrous acid ($H_2N_2O_2$), N_2 , and nitrate, small amounts of nitric oxide (NO) and nitrous oxide (N_2O) evolve. He has proposed the following reaction:



Nitric oxide (NO) and nitrous oxide (N_2O) may be the product of decomposition of nitrous acid and hyponitrous acid ($H_2N_2O_2$) respectively.

From the foregoing discussions it is apparent that there is good agreement among the investigators that in acid soils significant amount of nitrogen is lost during the process of nitrification of NH_4^+ or urea. It is also well agreed that these losses occur due to the reactivity of nitrite ion. But as far as the mechanisms of losses are concerned, there is no good agreement among the investigators. Various workers have suggested various mechanisms, some of which conflict with others. Because of the high reactivity of nitrous acid and nitrite ion,

diversity in the suggested mechanisms is not altogether unexpected. In view of the diversified reactions of nitrite resulting in many intermediates and end products, reader of this subject comes across many contradictory views in the literature.

STATEMENT OF THE PROBLEM

In the foregoing discussions sufficient experimental evidences of the various workers have been reported to show that in the process of nitrification of ammonia or ammonia forming fertilizers, nitrites may accumulate in well aerated alkaline as well as acid soils. There are also evidences that in the process of denitrification, nitrates are first reduced to nitrites and then to gaseous products, N_2O and N_2 . Thus in both the processes of nitrification and of denitrification, nitrites are formed as transitory intermediates. Depending upon the soil conditions and degree of acidity, nitrites undergo rapid chemical transformations. Under alkaline soil conditions the accumulated nitrites may persist for a long period, but under acid conditions the transformations are sufficiently rapid to result in large deficits of N.

Most of the workers have assumed that the gas, NO, is the product of nitrous acid decomposition. Recent investigations carried out at Utah State University have indicated that one of the major products of the decomposition of nitrite is the elemental form of N (Reuss, 1963). This has not been identified or reported by other workers.

There are very few references concerning the possibilities of organic matter enhancing the losses of N from added or accumulated nitrite (Soulides and Clark, 1958; Clark and Beard, 1960; Stevenson and Swaby, 1963 and 1964). The exact role of organic matter in these losses is not clearly defined. The end products of these losses have been vaguely reported as "deficits of N" without attempting to identify them. No organized efforts have been made in the past to evaluate the

role of organic matter in nitrite transformations.

Organic material, both dry and green, is often turned into the soil in large quantities. The effect of added material is manifest in overall improvement in soil conditions and productivity. There are, however, some short term effects which are not always beneficial to the crop. The interaction of organic matter with the nitrites of the soil resulting in the losses of N may be one of the short term adverse effects. In view of the common practice of incorporation of organic matter in soil and in view of the fact that some level of nitrites will usually be present in soil, the postulated interaction of these two soil components may be of great significance in the N economy of soil. It was, therefore, considered pertinent to investigate and evaluate the role of organic matter, both dry and green, on the losses of elemental form of N arising from reaction with accumulated nitrite.

Incorporation of organic matter brings in certain changes in soil pH, and the soil pH is important in nitrite transformations. A simultaneous study of the effect of pH on loss of N along with the study of the role of organic matter is considered desirable in clearly defining the pattern and magnitude of losses of elemental N.

The objectives of the investigations reported here were

1. To determine and compare the effect of incorporation of dry and green organic matter, incubated for varying lengths of time, on the pattern of loss of elemental N from soils of different pH to which nitrites were added.
2. To evaluate the effect of initial soil pH and the changes arising in pH due to the addition of organic matter on the magnitude of losses of elemental N.

3. To determine the effect of pH and organic matter individually and collectively on the pattern of loss of N_2 from a simple system which is devoid of all complexities present in soil.
4. To identify or pin point the fractions of organic matter which react readily with nitrites, giving rise to N_2 .

MATERIALS AND METHODS

Different experiments were set up to evaluate the role of both the dry and green organic matter on nitrite transformations resulting in the losses of elemental form of N. The work reported here consists of three major experiments. In Experiment I the soils of different pH were incubated for varying lengths of time with and without green or dry organic matter additions at 1 percent level on the dry weight basis. Nitrites were then added to see the pattern of evolution of N_2 .

Experiment II was designed to see the effect of pH and organic matter individually and collectively and also to evaluate the role played by the soil complex in enhancing the losses of N_2 . The media of this experiment was a sand-bentonite mixture acidified to various levels of pH and incubated for varying lengths of time with and without dry organic matter additions at 1 percent level.

In Experiment III an attempt was made to determine the fractions of organic matter which may react with nitrite, resulting in the evolution of N_2 . Humic acids, fulvic acids, various phenolic compounds, organic acids, and various amino acids commonly reported to be found in plant material were included in this study.

In the studies reported here, four acid soils, Clermont silt loam (pH 5.2), Olympic silty clay loam (pH 4.6), Dayton silty clay loam (pH 4.8) and Miami silt loam (pH 5.6) were used. Some characteristics of these soils are given in table 1.

Procedural details specific to each experiment are outlined along with the results and discussions of that experiment, Methods and

Table 1. Some characteristics of the soils used in these experiments

Soils	pH	Texture	Organic matter percent	Moisture level (1/3 bar) percent
Clermont	5.2	Silt loam	1.72	25.7
Olympic	4.6	Silty clay loam	6.98	34.0
Dayton	4.8	Silty clay loam	3.76	30.2
Miami	5.6	Silt loam	2.01	24.2

procedure commonly applicable to all the experiments are discussed here.

Preparation of Soil Samples

The air dried soil, passed through a 40 mesh sieve, was weighed in 100 g lots and placed in waxed paper cartons. Alfalfa, either green or dry, was added at 1 percent level on dry weight basis to the samples intended to receive organic matter amendments. Finely ground alfalfa was used in the study related to dry organic matter addition, and finely chopped green alfalfa was used in green organic matter studies. Appropriate quantities of water were added to each soil to bring it to the field capacity (1/3 bar moisture tension). After addition of water, soil and organic material were thoroughly mixed with a spatula and transferred to 250 ml suction flasks. The samples were then incubated for the desired lengths of time in a constant temperature chamber at 30 ± 1 C.

Changing the Gaseous Atmosphere in the Flasks

The original atmosphere in the flasks of the incubated and non-incubated samples was evacuated with the aid of a vacuum pump. Pure

helium (He) was passed through the flasks several times and then evacuated. After flushing the samples with He several times, the final flask atmosphere was adjusted with a mixture of He and O₂ in 80:20 proportion. Gas samples from the flask atmosphere were analyzed to see that N₂ had been reduced to a negligible level.

Addition of Nitrite

In all the studies reported here, nitrite-N at a level of 200 ppm was added as NaNO₂. Addition of nitrite was made after evacuating the original flask atmosphere and filling the flasks with the He:O₂ mixture. To accomplish this, a small vial containing the nitrite solution was placed in the flask before exchanging the atmosphere in the flask. After adjusting the flask atmosphere with He:O₂ mixture and making sure that no N₂ existed in the flask atmosphere, the vial containing the nitrite solution was gently tilted to mix all the solution in the soil.

Gas Sampling

Special arrangements were made to draw the gas samples for analysis purposes. The flask stoppers were bored through in the center, making a hole of 12 mm in diameter. A cut portion of a serum cap was placed tightly in the bottom of the bored stopper. A tightly fitting silicon disc was placed on this portion to make the sealing arrangement more secure. This sealing was further tightened by placing a serum cap on the top portion of the boring. The entire arrangement was so efficient that there were no leakages of gases from the flask atmosphere. Gas samples were drawn from the flasks with hypodermic needle and syringe and analysis was carried through a gas chromatograph.

A Beckman GC-2 Chromatograph with a 6-foot molecular sieve and a 12-inch charcoal column, connected to a Bristol recorder, was used for N_2 and N_2O separation respectively. Helium gas was used as carrier. The machine setting for analysis was:

Carrier gas flow rate . . . 3.3 liter/min.
Filament current 260 ma
Temperature 40 C
Gas volume 1 ml for N_2 and
5 ml for N_2O

Peak heights were used for calculations, as it was found by previous investigations (Cooper and Smith, 1963) that peak heights proved to be a linear function of the amount of gas present. The volume of the flask was determined and corrected for the volume of soil, water, trap solution, and vial in the flask. Calculations for N_2 were based on the perfect gas law.

Oxygen Supply

Care was taken to see that complete aerobic conditions prevailed in the flasks in all the studies reported here. After adjusting the flask atmosphere with a mixture of helium and oxygen in proportion of 80:20, the reading of oxygen peak was normally 10 at an attenuation of 2. During the period of study of N losses, when the peak of oxygen of the flask atmosphere went below 10 at an attenuation of 1, oxygen was supplied to the flask to bring the peak back to its original level. Oxygen supply was accomplished by attaching the hypodermic needle to the oxygen supply line and feeding the individual flask through the sealing arrangement.

Soil Analysis

Soil reaction

The pH was determined with a glass electrode pH meter on a 1:1 soil water ratio using 10 g soil.

Nitrite and nitrate determinations

To determine nitrite and nitrate, 10 g sample of the soil was weighed out from each flask. The soil samples were placed in a 250 ml Erlenmeyer flask and 100 ml saturated calcium hydroxide solution was added to the flask. After shaking the flask for 10 minutes on a mechanical shaker, the solution was filtered. Aliquots of the filtrate were taken for nitrite and nitrate analysis.

Nitrites were determined by the method of Shinn (1941), employing a diazo coupling of sulfanilic acid and N-(1-Naphthyl)-ethelene diaminedihydro chloride coupling reagent. A standard phenol disulphonic acid method similar to that described by Jackson (1958) was used for determining nitrate after destroying nitrite with sulfamic acid. Complete analytical procedures are included in the appendix.

Trap Analysis

The alkaline permanganate solution (5 ml 0.20 M KMnO_4 in 2 M KOH) was used to trap NO and NO_2 gases in the flask arising during the course of reaction (Reuss, 1963). The absorbed NO and NO_2 would be oxidized to nitrate in the system. The traps were removed every 24 hours and analyzed. The solution was washed into a beaker, and KMnO_4 was destroyed by addition of 0.6 M $\text{Na}_2\text{C}_2\text{O}_4$ in 7 N H_2SO_4 . The solutions were then made alkaline with 5 N NaOH and the Mn compounds which precipitated were

filtered off. The precipitate on the filter paper was thoroughly washed several times with hot water and the volume of filtrate was made to 100 ml. Aliquots of this filtrate were analyzed for nitrate by the phenol disulphonic acid method. The method was calibrated by adding the known amounts of KNO_3 to the original solutions of KMnO_4 and preparing a calibration curve of added N versus optical density. Calibration curves using either NaNO_2 or KNO_3 as a standard were identical. The complete procedure is also included in the appendix.

EXPERIMENT I. N_2 EVOLUTION FROM REACTIONS OF NITRITE
WITH DRY AND GREEN ALFALFA ADDED TO VARIOUS SOILS
INCUBATED FOR VARYING LENGTHS OF PERIODS

This experiment was designed to determine the effect of additions of dry and green organic matter on the pattern and magnitude of N_2 evolution arising from nitrite reaction in soils of different pH, non-incubated and incubated for varying lengths of time.

Materials and Methods

The soils used in this experiment were Clermont silt loam (pH 5.2), Dayton silty clay loam (pH 4.8), Olympic silty clay loam, (pH 4.6), and Miami silt loam (pH 5.6). The soil samples were prepared as outlined earlier. Dry ground alfalfa and finely chopped fresh green alfalfa were used as the source of organic matter. Organic matter was added to the soil at the 1 percent level on a dry weight basis. $NaNO_2$ was used to provide nitrite-N at 200 ppm. In addition to the nitrite-N, those treatments receiving organic matter have received additional organic-N. It is estimated that in the experiment conducted with dry alfalfa, addition of organic-N to the samples receiving organic matter was about 250 ppm on the basis of 2.5 percent N content in dry alfalfa. The nitrogen content of green alfalfa was not determined. Since the immature plant material was used as a source of green organic matter, the N content of this material must be higher than that of dry alfalfa. On the basis of approximately 3.0 percent N content in green alfalfa, the amount of organic-N added may be about 300 ppm.

In the dry alfalfa studies the experiment was a factorial arrangement in a completely randomized design with two replications. The treatments were: three soils, Olympic silty clay loam, Dayton silty clay loam, and Clermont silt loam; two organic matter levels, no organic matter and 1 percent organic matter on the dry weight basis; and three incubation periods, 0, 3, and 14 days. The studies were extended to 35 days for the Dayton and Olympic soils. The statistical analysis presented is for the N_2 evolved at 24 hours after the nitrite-N addition.

In green alfalfa studies the experiment was factorial with treatments in a completely randomized design with two replications. The treatments were: three soils, Olympic silty clay loam, Dayton silty clay loam, and Miami silt loam; two organic matter levels, no organic matter and 1 percent organic matter on the dry weight basis; and two incubation periods, 0 and 21 days. For the Dayton and Olympic soils, studies were also made at the 3-, 6-, and 35-day incubation periods. In this study the statistical analysis presented is for the N_2 evolved at 48 hours after the addition of the nitrite-N.

Results and Discussions

Dry organic matter addition

The N_2 recovered at 12, 24, 36, and 48 hours from Clermont, Dayton, and Olympic soils, non-incubated and incubated for varying periods, is presented in tables 2, 3, and 4. The figures reported in these tables are after deducting the quantity of N_2 found in the soil used as a control to which no nitrites were added. It is evident from the data contained in these tables that a considerable portion of the added nitrite gets converted to the elemental form of nitrogen in acid soils. There was a distinct difference in the pattern and magnitude of loss of

Table 2. N_2 recovery after various time periods following the reactions of nitrite with and without 1 percent dry alfalfa in Clermont silt loam nonincubated and incubated at 30 C for 3, 6, and 14 days prior to the addition of 200 ppm N as $NaNO_2^a$

Treatment	Incubation time			
	None	3 days	6 days	14 days
	ppm	ppm	ppm	ppm
		<u>12 hours</u>		
Soil + NO_2^-	35.8	20.0	15.0	27.9
Soil + NO_2^- + alfalfa	25	18.6	9.7	10.6
		<u>24 hours</u>		
Soil + NO_2^-	42.4	27.2	18.0	36.0
Soil + NO_2^- + alfalfa	36.2	25.6	16.0	11.8
		<u>48 hours</u>		
Soil + NO_2^-	55.0	36.0	35.0	41.0
Soil + NO_2^- + alfalfa	43.0	26.0	24.0	13.0
Standard error	± 1.14			
L.S.D. at 0.05 level	3.38			

^aQuantity of N_2 is reported after deducting that found in the soil used as a control to which no nitrites were added.

Table 3. N_2 recovered after various time periods following the reactions of nitrite with and without 1 percent dry alfalfa in Dayton silty clay loam nonincubated and incubated at 30 C for 3, 14, and 35 days prior to the addition of 200 ppm N as $NaNO_2^a$

Treatment	Incubation time			
	None ppm	3 days ppm	14 days ppm	35 days ppm
			<u>12 hours</u>	
Soil + NO_2^-	55.0	51.9	50.7	35.9
Soil + NO_2^- + alfalfa	52.4	54.0	56.7	57.9
			<u>24 hours</u>	
Soil + NO_2^-	70.1	55.5	57.3	39.1
Soil + NO_2^- + alfalfa	63.7	60.0	62.2	67.8
			<u>36 hours</u>	
Soil + NO_2^-	-	57.6	59.9	46.6
Soil + NO_2^- + alfalfa	-	66.5	65.6	70.6
			<u>48 hours</u>	
Soil + NO_2^-	76.4	65.1	59.6	50.2
Soil + NO_2^- + alfalfa	71.4	74.4	79.6	75.4
Standard error	± 1.14			
L.S.D. at 0.05 level	3.38			

^aQuantity of N_2 is reported after deducting that found in the soil used as a control to which no nitrites were added.

Table 4. N_2 recovered after various time periods following the reaction of nitrite with and without 1 percent dry alfalfa in Olympic silty clay loam nonincubated and incubated at 30 C for 3, 14, and 35 days prior to the addition of 200 ppm N as $NaNO_2^a$

Treatment	Incubation time			
	None	3 days	14 days	35 days
	ppm	ppm	ppm	ppm
			<u>12 hours</u>	
Soil + NO_2^-	69.9	65.8	61.2	47.1
Soil + NO_2^- + alfalfa	56.9	71.4	80.0	64.9
			<u>24 hours</u>	
Soil + NO_2^-	82.3	80.0	76.2	73.0
Soil + NO_2^- + alfalfa	75.2	85.7	87.0	89.6
			<u>36 hours</u>	
Soil + NO_2^-	86.8	84.0	83.3	80.9
Soil + NO_2^- + alfalfa	83.7	90.1	86.4	97.6
			<u>48 hours</u>	
Soil + NO_2^-	Leak	83.3	Leak	82.3
Soil + NO_2^- + alfalfa	Leak	91.6	Leak	103.4
Standard error	± 1.14			
L.S.D. at 0.05 level	3.38			

^aQuantity of N_2 is reported after deducting that found in the soil used as a control to which no nitrites were added.

N_2 from soils receiving organic matter as compared to their counterparts to which no organic matter was added. The pattern of evolution of N_2 from nonincubated soils was clearly different from soils incubated. The initial pH of the soil or the changes in pH due to organic matter additions seemed to have a profound influence on the magnitude of losses.

Nonincubated soils

The pattern of evolution of N_2 arising from the various nonincubated soils is presented in figure 1. It can readily be seen from this figure that addition of dry organic matter to nonincubated soils had some lowering effect on N_2 evolution. This phenomenon can be explained on the basis of several postulated possibilities.

With the addition of organic matter, there may be a tremendous increase in microbial population. The rapidly multiplying organisms need some source of energy for their growth, and N for the body-building. It is possible that these organisms use up the active fraction of the added organic matter as a source of energy which would, otherwise, have reacted with nitrite and possibly resulted in N_2 evolution. It is also possible that a fraction of the added nitrite, as it is originally added or in some other transformed condition, is used up by these multiplying organisms. Thus the concentration of both the reactants might be reduced, resulting in reduced evolution of elemental N.

Nitrites or their products of decomposition may form complexes and reduce the concentration of one of the reactants. Silver (1960) has suggested that nitrites and their products of decomposition have great affinity for the hemes and for the respiratory enzymes of heterotrophs. Under acid conditions one of the products of decomposition of nitrite is NO, which is believed to have evolved by catalytic function of

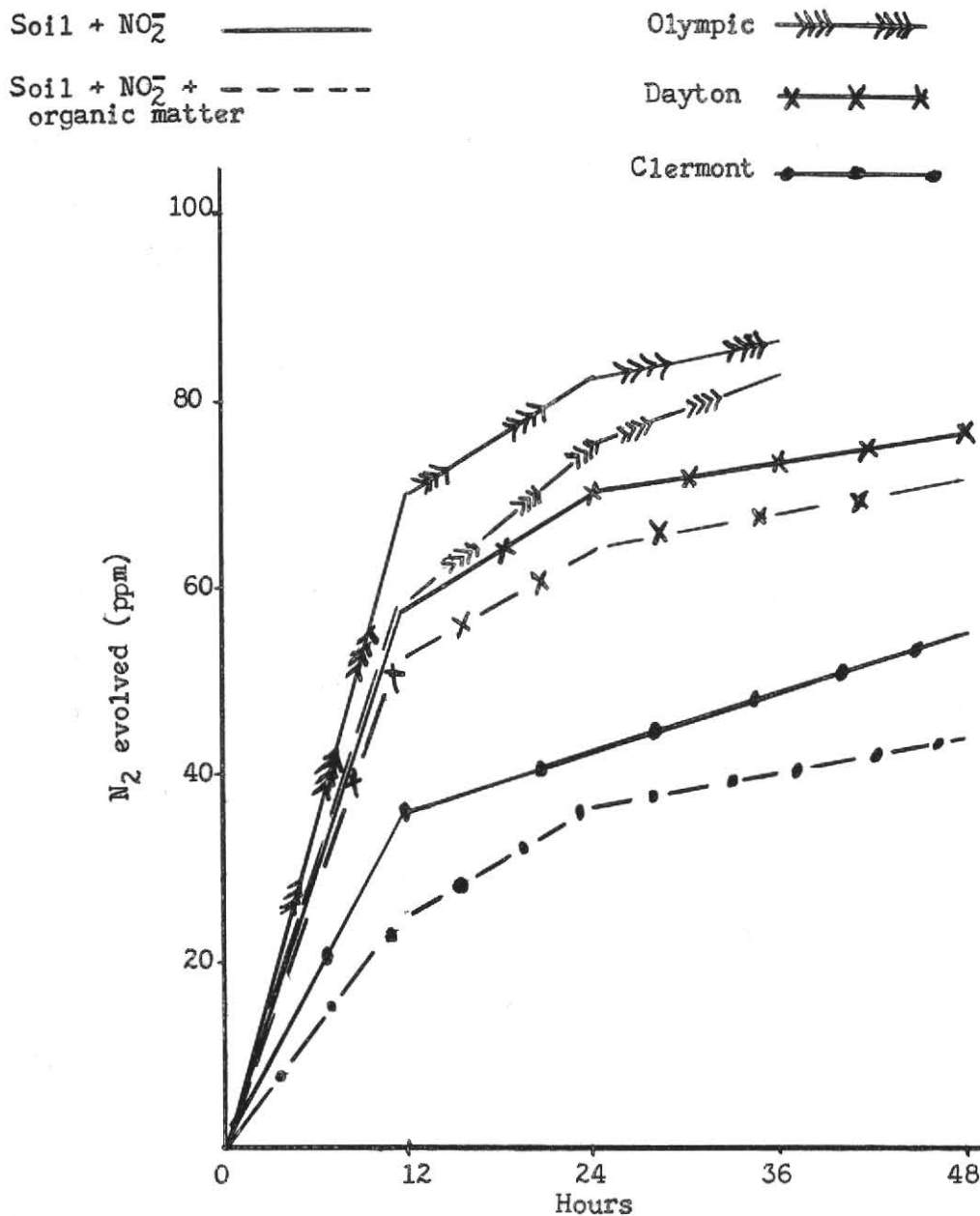
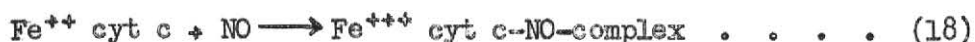
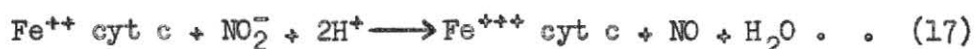


Figure 1. N_2 evolved at various time periods from nonincubated Olympic silty clay loam, Dayton silty clay loam, and Clermont silt loam soils treated with and without 1 percent dry alfalfa and 200 ppm nitrite-N added as $NaNO_2$.

ferrous iron. It is quite likely that the ferrous iron of cytochrome c performs the catalytic function and gets converted to ferri-cytochrome-NO-complex. Silver has suggested the following reactions:



In view of the initial rapid multiplication of micro-organisms with the addition of organic matter, cyt c-NO₂⁻/NO-complex formation may assume a significant proportion resulting in reduced N₂ evolution.

In the presence of ferrous iron, Chao and Bartholomew (1964) were not able to extract the NO fully. They have, therefore, suggested the possibility of an NO-ferrous iron complex formation. It is possible that such a complex formation may occur in nonincubated soils which may reduce N₂ evolution.

Führ and Bremner (1964a) have reported that the reactions between soil organic matter and nitrite lead to the fixation of nitrite-N (i.e., conversion of nitrite-N to organic forms of N) and also to gaseous losses of nitrite-N. The fixation of nitrite-N by organic matter increases with increase in nitrite concentration and with decrease in pH. In another paper (1964b) Führ and Bremner have confirmed these findings by using NaN¹⁵O₂ and have reported that air drying of acidic or neutral soils containing nitrite leads to the fixation of nitrite by soil organic matter, and this fixed nitrogen is not easily extractable.

It is possible that in the studies reported here such a fixation of nitrite on added organic matter may occur, which in turn may reduce evolution of N₂.

In recent years evidences have been presented emphasizing the role of transition metals in initiating the losses of N. Wullstein and

Gilmour (1964) have reported that the reduced state of transition metals like copper, iron, manganese, and certain salts of aluminum can be quite active in promoting nitrite losses. It is quite likely that with the addition of organic matter these transition metals are complexed and rendered temporarily inactive. Ample evidences have been presented regarding the chelation of metals by organic matter. Even fresh leaves and water extracts of fresh leaves have the ability to chelate the metals (Baughman, 1956; Bloomfield, 1952a and 1952b; Broadbent, 1957; Himes and Barber, 1957; Schnitzer and Skinner, 1963; Mortensen, 1963).

Incubated soils

Incubation seems to have a marked influence on the pattern of N_2 evolution from different soils. The quantity of N_2 evolved from various soils incubated for 3 days is depicted in figure 2. It is interesting to note that the pattern of N_2 evolution reverses with incubation in Dayton and Olympic soils but not in Clermont (figure 2). With incubation, higher amounts of N_2 were recorded from treatments receiving organic matter as compared to their counter parts to which no organic matter was added. This reversal in pattern was noticed in a period as short as 3 days, suggesting that with incubation the added organic material undergoes microbial transformations resulting in the release of some fractions which can react with nitrite giving rise to N_2 . Results from the short incubation period suggest that the fraction of organic matter reacting with nitrite has not undergone extensive microbial transformation. The reacting fractions may be the degradation products of lignin, protein, carbohydrates, or some other constituent of the plant tissue.

It is interesting to note that the pattern of N_2 evolution does

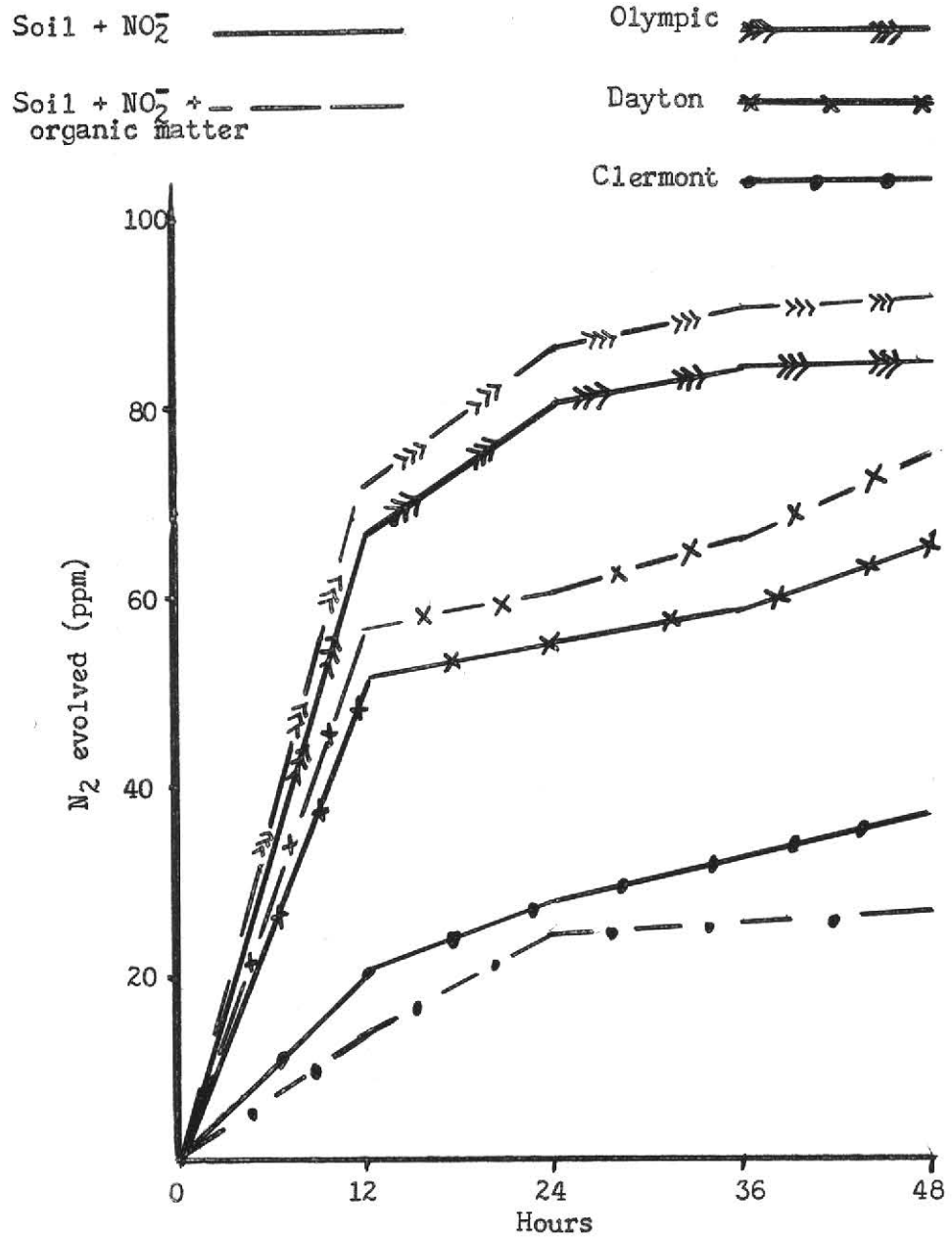


Figure 2.— N₂ evolved at various time periods from Olympic silty clay loam, Dayton silty clay loam, and Clermont silt loam soils treated with and without 1 percent dry alfalfa and incubated at 30 C for 3 days prior to the addition of 200 ppm nitrite-N added as NaNO₂.

not change with incubation in the Clermont soil. The addition of organic matter lowered the evolution of N_2 in both the incubated and nonincubated soils. This lowering effect was more pronounced with longer incubations as could be seen in table 2. Out of the 200 ppm of added nitrite-N, 43 ppm N were recorded in 48 hours from nonincubated soil. With 3 days of incubation the amount of N_2 evolved was reduced to 26 ppm and with 14 days of incubation it was further reduced to 13 ppm. This unusual behavior of the Clermont soil as compared to the Dayton or Olympic soils is rather hard to explain fully. The continued reduction in the evolution of N_2 can partly be due to the inherent properties of this soil. To some extent it can be explained on the basis of changes in pH occurring in this soil (table 5).

Table 5. The pH of different soils incubated for different periods with and without 1 percent dry alfalfa

Incubation period days	Clermont silt loam		Dayton silty clay loam		Olympic silty clay loam	
	With alfalfa	Without alfalfa	With alfalfa	Without alfalfa	With alfalfa	Without alfalfa
0	5.2	5.2	4.8	4.8	4.6	4.6
3	5.8	5.3	5.0	4.9	4.8	4.6
6	6.2	5.4	5.1	4.8	4.8	4.6
14	5.5	-	5.2	4.8	4.6	4.6
25	-	-	5.0	4.9	-	-
35	-	-	4.8	4.8	4.6	4.5

The clear cut differences in the pattern and magnitude of N_2 evolved from various acid soils (figures 1 and 2) may be attributed to the inherent properties of these soils. However, in accounting for these differences, the role of the pH of these soils cannot be overlooked. A slight change in pH causes a considerable difference in the magnitude of N_2 evolved. The greater the degree of acidity, the greater will be the evolution of N_2 . It was noticed that with 3 days of incubation of the Clermont soil with dry alfalfa additions the pH rose from 5.2 to 5.8, which further increased to 6.2 with 6 days of incubation. In no other soil was there such a rapid change in pH occurring when dry alfalfa was added and allowed to incubate.

The sharp rise in pH of the Clermont soil, with incubation of 3 or 6 days, may explain the reduction in N_2 evolved. The effect of pH probably overcame the effect of added organic matter and reduced the N_2 evolved. But this line of approach cannot be used to explain the reduction in loss with 14 days of incubation. It was noticed that in a period of 14 days the pH of this soil went down to 5.5, which is close to the original pH of 5.2. In spite of this drop in pH, the N_2 recorded was lowest of all (13 ppm). This suggests that some other inherent property of the soil may also influence the pattern or magnitude of N_2 evolved. It is rather difficult to say what that inherent property could be. The clays present in Clermont may either fix the nitrite or interact with some organic fraction making such an organo-mineral complex that the organic fraction is not able to react with the added nitrite.

The pattern and magnitude of the evolution of elemental-N recorded from the Olympic, Dayton, and Clermont soils with 14 days of

incubation are presented in figure 3. It is evident from this figure that the differences in N_2 evolved from various soils amended or non-amended with organic matter widen with 14 days of incubation. In Clermont soil, the added alfalfa depressed the N_2 evolution considerably as compared to the treatment where no organic matter was added. The reverse was true for the Dayton and Olympic soils.

As the time of incubation increased, the differences in the magnitude of N_2 evolved from organic matter treated and non-treated Dayton and Olympic soils became more prominent (tables 3 and 4). This may be attributed to the decrease in a reactive fraction of organic matter of the non-amended soil due to microbial decay and an increased proportion of such substance in treated soils due to mineralization of added organic matter.

It was noticed that in the Dayton soil the addition of organic matter enhanced the evolution of N_2 , the highest peak being reached with 14 days of incubation. Further incubation (35 days) resulted in a slight decline in the magnitude of N_2 evolved as compared to that recorded with 14 days incubation. It is interesting to note that addition of alfalfa to this soil resulted in an increase in the pH from 4.8 to 5.2 in a period of 14 days. In spite of this rise in pH the reactive fraction of organic matter released from added alfalfa enhanced N_2 production. With further incubation (35 days), the pH declined to its original level, yet there was no increase in the evolved N_2 , suggesting that with the longer incubation the reactive substances have either been exhausted from the system or were transformed into some non-reactive form.

The role of added organic matter in enhancing the evolution of N_2

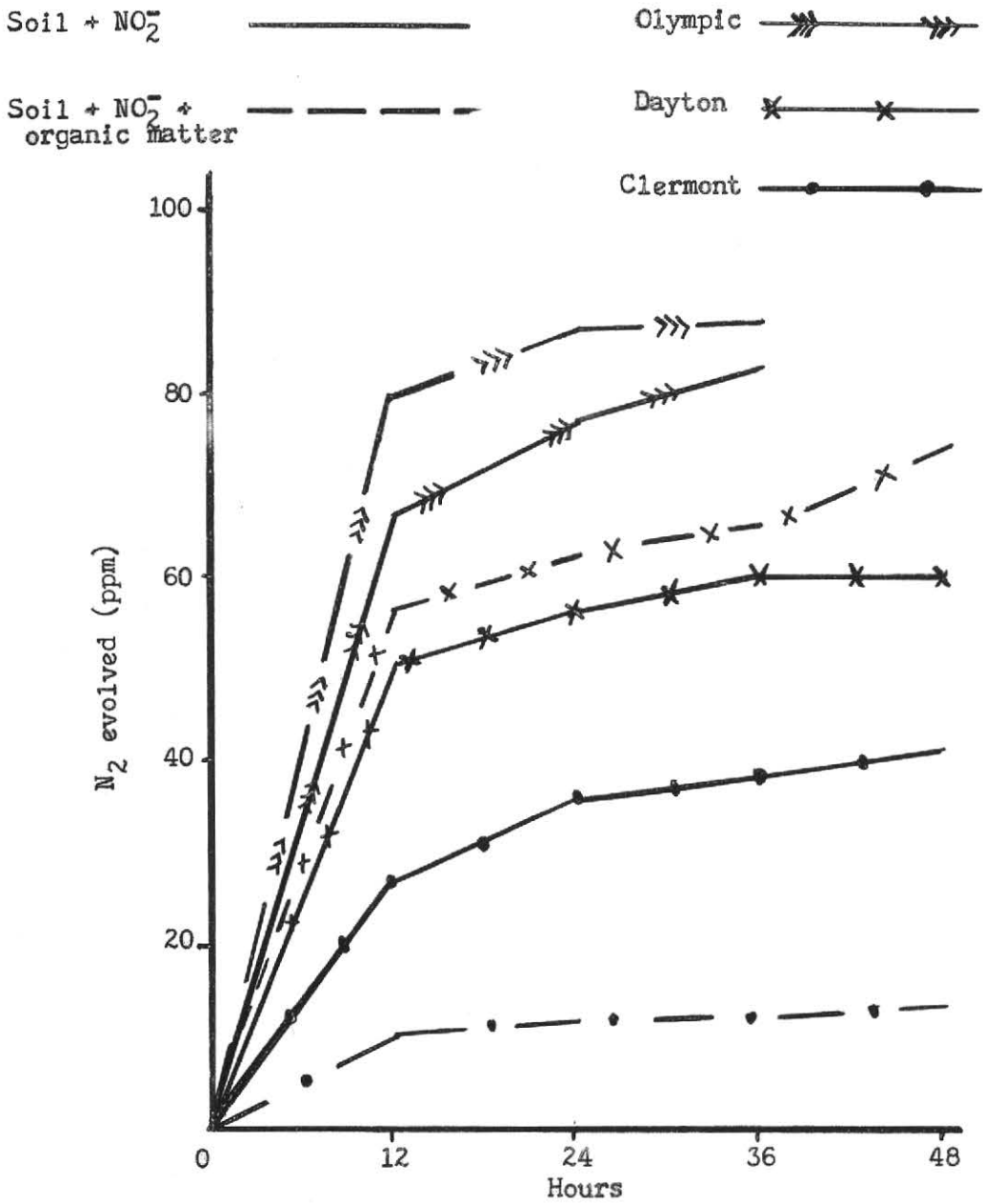


Figure 3. N₂ evolved at various time periods from Olympic silty clay loam, Dayton silty clay loam, and Clermont silt loam soils treated with 1 percent dry alfalfa and incubated for 14 days prior to the addition of 200 ppm nitrite-N added as NaNO₂.

can clearly be seen in table 3. Without any incubation the organic matter addition was responsible for a slight reduction in N_2 evolved as compared to the soil receiving no organic matter. With 3 days of incubation the pattern reversed and treatment receiving organic matter gave 9.3 ppm more N_2 than the control. In 35 days the difference increased to 25.2 ppm. This difference speaks in favor of our hypothesis that organic matter reacts with nitrite resulting in the production of N_2 .

In spite of the fact that the Dayton and Olympic soils follow pretty much the same pattern in N_2 evolution from added nitrite, yet certain distinct differences were noticed especially at longer incubation periods. In the Dayton soil a gradual decrease in N_2 evolution was noticed in treatment receiving no organic matter additions whereas such a downward trend was not noticed in the Olympic soil, where the change in magnitude of N_2 evolution was only slight. This may be due to the original high organic matter content of the Olympic soil (6.98 percent), which probably did not diminish appreciably by microbial activity. The Olympic soil treated with organic matter showed no signs of reduction in evolution of N_2 with longer incubation as was noticed in the Dayton soil. Of all the incubations studied, the longest incubation gave the highest amount of N_2 loss. The role of added organic matter in increasing the evolution of N_2 can clearly be seen in this soil also. With 35 days of incubation the N_2 evolved from the soil treated with organic matter was 21.1 ppm more than the treatment receiving no organic matter.

N_2 evolution from H_2O_2 treated Olympic soil

The role of organic matter in initiating or enhancing the production

of N_2 on reaction with nitrite is more clearly elucidated from the results of a supplementary experiment involving destruction of the original organic matter by H_2O_2 .

The destruction of inherent organic matter of the Olympic soil was accomplished by treating the soil with 30 percent H_2O_2 for a prolonged period of many days at room temperature. Addition of H_2O_2 was continued until there was no more frothing from the soil, which indicated that all the active or readily decomposed fraction of organic matter was destroyed. The soil was then rinsed thoroughly with distilled water to get rid of unused H_2O_2 . Suspecting that the H_2O_2 treatment may have been detrimental to the microorganisms, the treated soil was inoculated from extracts of incubated samples of untreated Olympic soil.

The N_2 evolved from the H_2O_2 treated soil was determined without any incubation and with 3 days of incubation, in the presence and absence of freshly added dry alfalfa at 1 percent level.

The pattern of the N_2 evolved from H_2O_2 treated soil, in comparison with untreated soil, is presented in figure 4. It is clear from this figure that destruction of organic matter resulted in significant reduction in loss of N_2 . In H_2O_2 treated soil the loss of N_2 was reduced by about 30 to 50 percent, as compared to the non-treated soil. This supplementary experiment presents a very strong evidence to the effect that nitrite can react with organic matter resulting in the evolution of N_2 .

It was pointed out in earlier discussions that the addition of organic matter reduced the loss of N_2 from all non-incubated soils. Certain postulated explanations were suggested to account for this reduction. The possibility of NO/NO_2^- -cytochrome c-complex formation or

Soil + NO₂⁻ ————— Ordinary Olympic ×——×——
Soil + NO₂⁻ + organic matter - - - - H₂O₂ treated Olympic ●——●——

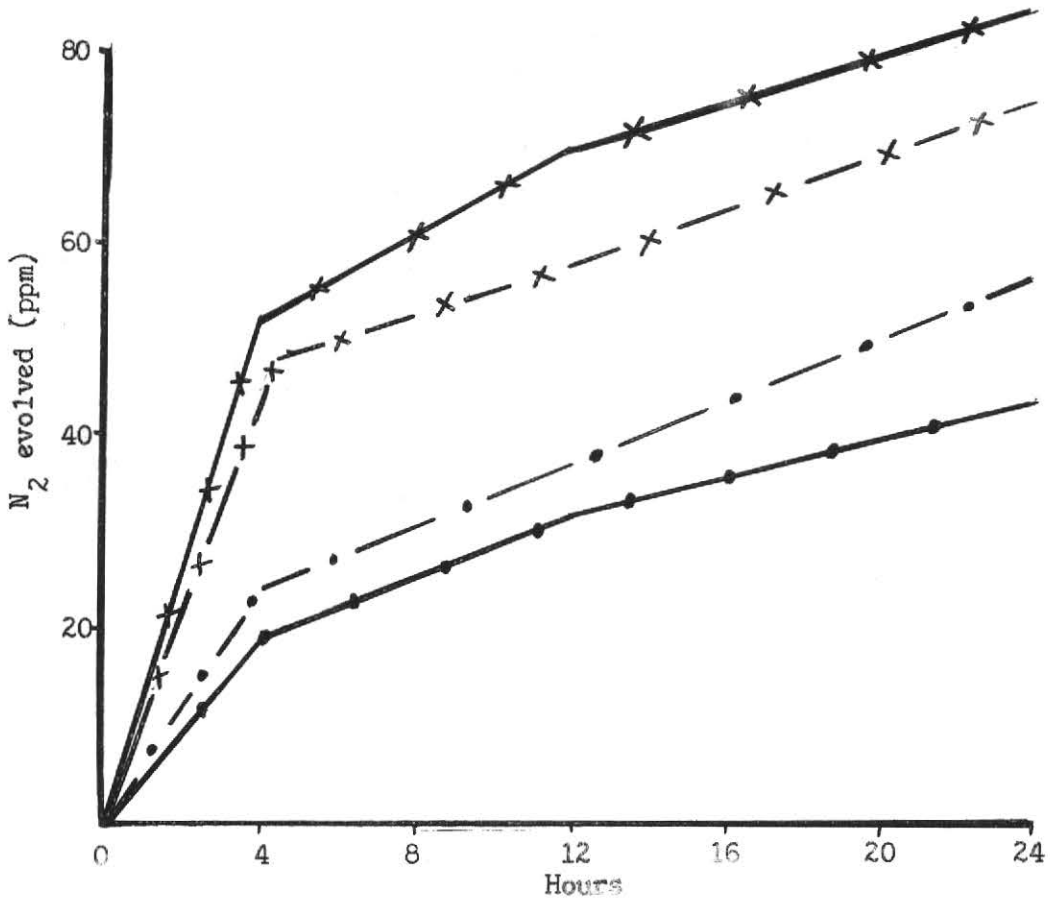


Figure 4. N₂ evolved at various time periods from nonincubated H₂O₂ treated and ordinary Olympic silty clay loam soil treated with 1 percent dry alfalfa and 200 ppm nitrite-N added as NaNO₂.

a utilization of the active fraction of added organic matter by microbes as a source of energy were among the postulated explanations. It is evident from figure 4 that the addition of organic matter to H_2O_2 treated soil yielded greater N_2 as compared to the treatment receiving no organic matter. This suggests that the postulated explanation given with regard to the use of organic fraction by microorganisms or complex formation of NO/NO_2^- with the cytochrome c of organisms is valid to some extent. With H_2O_2 treatment the microbial population or activity was probably reduced which lowered the complex formation or utilization of the active fraction of organic matter by microorganisms. The ultimate result was an increase in N_2 evolution from the treatment receiving organic matter.

In H_2O_2 treated soil the N_2 evolution was drastically reduced as compared to non-treated soil, yet the loss was not reduced to a negligible level. About 20 percent of the added N was lost from H_2O_2 treated soil receiving no organic matter. This indicates that in addition to organic matter probably some other component of the soil complex may also be responsible for the evolution of N_2 .

In figure 5 a comparison is made between the nonincubated and H_2O_2 treated soils incubated for 3 days. The higher losses with 3 days of incubation confirm the postulation that nitrite-organic matter interaction results in release of elemental form of N.

Green organic matter additions and N_2 evolution

Some experiments were conducted to study the effect of added green alfalfa addition on the pattern of evolution of N_2 arising from nitrite reactivity in various soils. Finely chopped fresh green alfalfa was

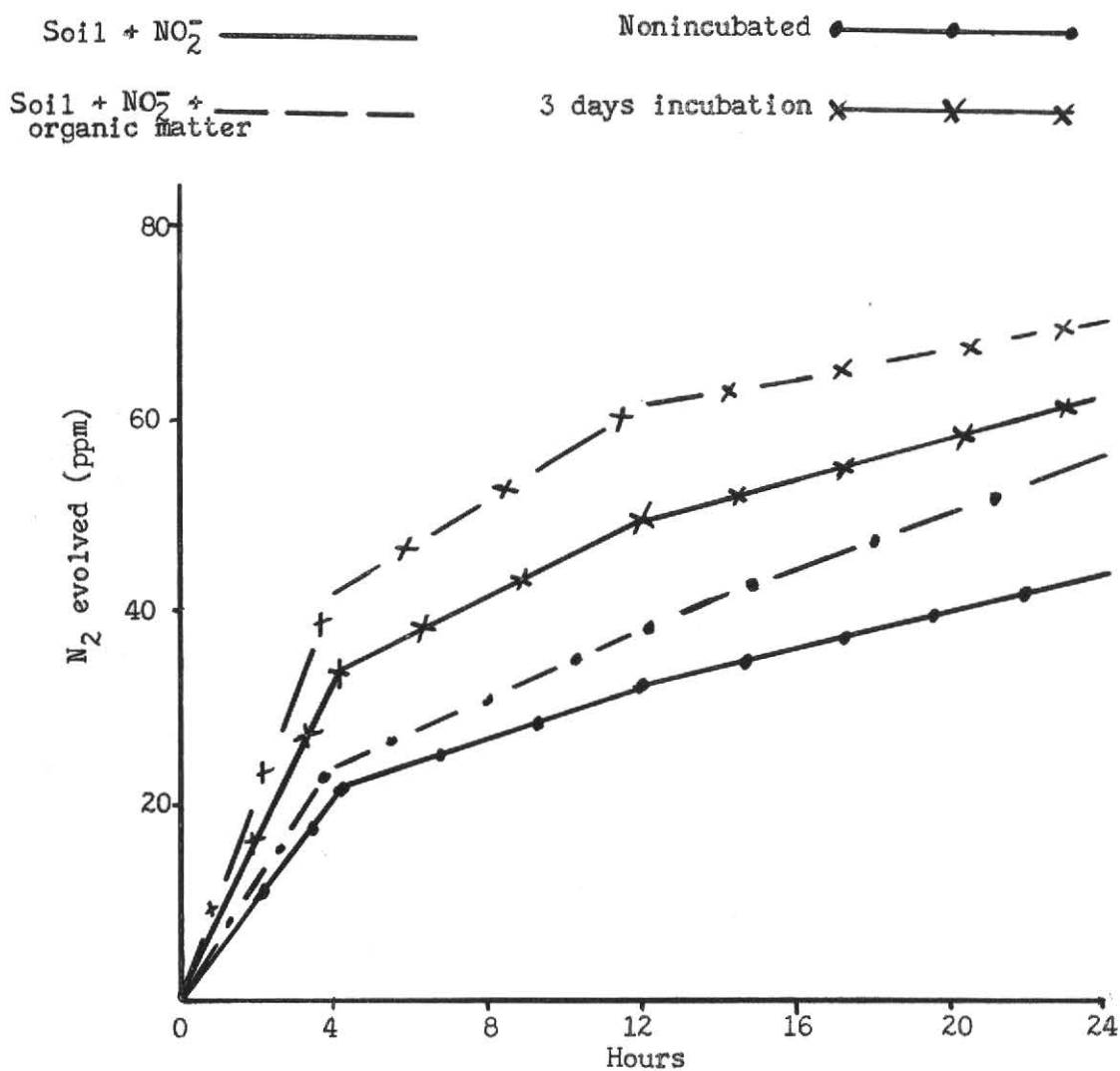


Figure 5. N_2 evolved at various time intervals from H_2O_2 treated Olympic silty clay loam nonincubated and incubated at $30^\circ C$ for 3 days with and without 1 percent dry alfalfa prior to the addition of 200 ppm nitrite-N added as $NaNO_2$.

used as organic matter additions. Dayton silty clay loam and Olympic silty clay loam were incubated for 0, 6, 21, and 35 days, and the Miami soil was incubated for 0 and 21 days only.

The results of this experiment are interesting and important from a theoretical as well as from a practical point of view. The N_2 evolved from Dayton, Olympic, and Miami soils is shown in tables 6, 7, and 8.

Nonincubated soils. It is interesting to note that the pattern of evolution of N_2 arising from these soils receiving green alfalfa additions, without any incubation, was just the reverse of that noticed with dry alfalfa additions. The results presented in tables 2 to 4 show that addition of dry organic matter reduced the evolution of N_2 in all soils as compared to the treatment to which no organic matter was added. But with the addition of green alfalfa the pattern of N_2 evolution reversed completely. Green alfalfa not only gave higher loss of N_2 as compared to the treatment receiving no organic matter, but it also enhanced the losses of N_2 considerably as compared to the dry alfalfa.

The pattern and magnitude of N_2 evolved from nonincubated Dayton and Miami soils treated with green and dry alfalfa are presented in figure 6, which shows clear cut differences in the effectiveness of dry and green organic matter in reacting with nitrite resulting in the subsequent release of N_2 .

The total production of N (as N_2) from the Dayton soil treated with dry alfalfa was 71.4 ppm. With addition of green alfalfa it increased to 105 ppm. Thus the amount of N_2 evolved due to the green alfalfa addition was 34 ppm more than the dry alfalfa. Similar increases were noticed in the Olympic and Miami soils, also. This suggests that green alfalfa has certain components which are very reactive and can react

Table 6. N_2 evolved at various time periods from Dayton silty clay loam non-treated and treated with green alfalfa (1 percent on dry weight basis) and incubated at 30 C for 0, 3, 6, 14, 21, and 35 days prior to the addition of 200 ppm nitrite-N added as $NaNO_2$

Treatment	Incubation time					
	None	3 days	6 days	14 days	21 days	35 days
	ppm ^a					
	<u>12 hours</u>					
Soil + NO_2^-	58.0	41.3	45.0	48.7	39.6	35.9
Soil + NO_2^- + alfalfa	63.2	44.8	33.6	24.3	25.9	27.3
	<u>24 hours</u>					
Soil + NO_2^-	76.0	58.3	57.2	56.7	45.2	39.1
Soil + NO_2^- + alfalfa	85.6	67.6	45.5	42.7	32.4	35.6
	<u>36 hours</u>					
Soil + NO_2^-	85.0	64.0	64.0	64.8	51.8	46.6
Soil + NO_2^- + alfalfa	94.6	77.0	50.0	44.0	36.2	42.1
	<u>48 hours</u>					
Soil + NO_2^-	84.0	66.3	64.0	65.3	56.2	50.2
Soil + NO_2^- + alfalfa	105.0	82.0	60.8	45.2	40.1	46.0
Standard error	± 1.48					
L.S.D. at 0.05 level	4.55					

^aReported after deducting that found in the soil used as a control to which no nitrites were added.

Table 7. N_2 evolved at various time periods from Olympic silty clay loam treated with and without green alfalfa (1 percent on dry weight basis) and incubated at 30 C for 0, 6, 21, and 35 days prior to the reaction with 200 ppm nitrite-N added as $NaNO_2$

Treatment	Incubation time			
	None	6 days	21 days	35 days
		ppm ^a		
		<u>12 hours</u>		
Soil + NO_2^-	63.4	58.2	52.7	47.1
Soil + NO_2^- + alfalfa	68.1	44.9	73.4	52.6
		<u>24 hours</u>		
Soil + NO_2^-	75.4	78.9	63.1	73.0
Soil + NO_2^- + alfalfa	87.8	61.2	81.7	77.0
		<u>36 hours</u>		
Soil + NO_2^-	87.0	87.0	71.9	80.9
Soil + NO_2^- + alfalfa	97.7	75.8	86.4	82.7
		<u>48 hours</u>		
Soil + NO_2^-	89.5	94.6	77.7	82.3
Soil + NO_2^- + alfalfa	97.6	84.3	90.1	92.4
Standard error	± 1.48			
L.S.D. at 0.05 level	4.55			

^aReported after deducting that found in the soil used as a control to which no nitrites were added.

Table 8. N_2 evolved from Miami silt loam treated with and without dry and green alfalfa (1 percent on dry weight basis) and incubated at 30 C for 0 and 21 days prior to the addition of 200 ppm nitrite-N as $NaNO_2$

Treatment	Incubation time	
	None	21 days
	ppm ^a	
	<u>12 hours</u>	
Soil + NO_2^-	20.6	27.3
Soil + NO_2^- + dry alfalfa	17.7	21.8
Soil + NO_2^- + green alfalfa	27.9	6.6
	<u>24 hours</u>	
Soil + NO_2^-	27.2	30.9
Soil + NO_2^- + dry alfalfa	22.1	23.8
Soil + NO_2^- + green alfalfa	39.5	9.9
	<u>48 hours</u>	
Soil + NO_2^-	36.9	44.2
Soil + NO_2^- + dry alfalfa	32.3	34.3
Soil + NO_2^- + green alfalfa	48.2	12.6
Standard error	± 1.48	
L.S.D. at 0.05 level	4.55	

^aReported after deducting that found in the soil used as a control to which no nitrite was added.

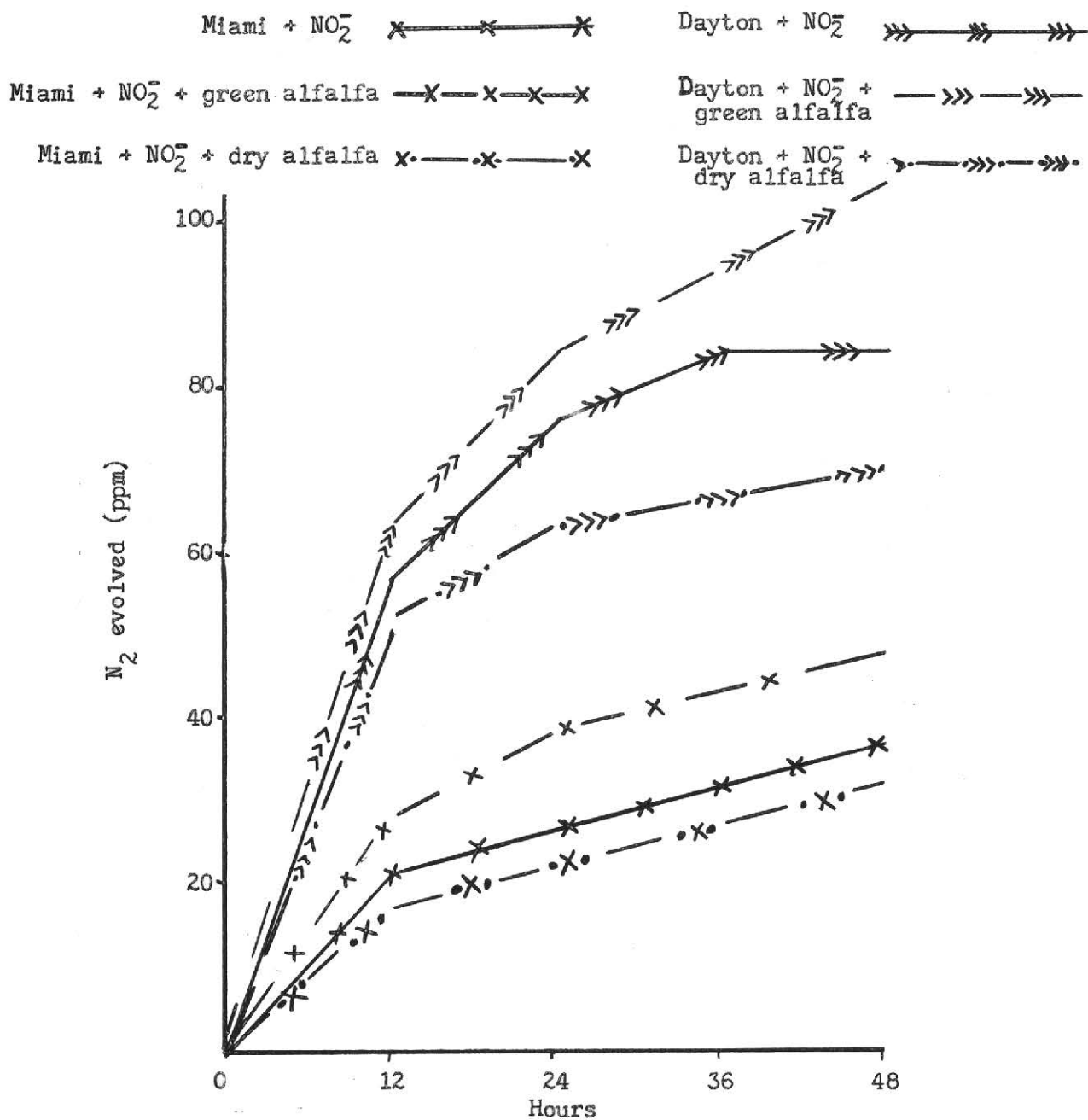


Figure 6. Comparison of elemental-N evolved in nonincubated Dayton silty clay loam and Miami silt loam soils treated with and without dry and green alfalfa at 1 percent level on dry weight basis and 200 ppm nitrite-N added as NaNO₂.

Incubated soils. It is evident from table 6 that in the Dayton soil, even with 3 days of incubation, the N_2 evolution is higher in treatments receiving green alfalfa than in the treatments receiving no organic matter. It is interesting to note here that with 3 days incubation the pH of treated soil rose from 4.8 to 5.5, yet the evolution of N_2 from the treated soil was higher than from the control. Previously it had been postulated that a slight change in pH was responsible for a considerable reduction in the evolution of N_2 . The rise in pH from 4.8 to 5.5, with 3 days of incubation, should have reduced the N_2 evolution considerably as compared to the control. The recorded high N_2 , in spite of high pH, suggests that with 3 days of incubation there were yet many more active components in the organic matter which react with nitrite. Probably, at this stage, the proportion of active fraction has increased considerably, which counter balances the effect of increased pH.

With 6 days of incubation the N_2 evolved from treatment receiving green alfalfa addition was lower than the control, suggesting that most of the active fractions were either transformed to inactive form or have been exhausted from the system due to rapid microbial activity. With 14 and 25 days of incubation, the reduction in N_2 evolution from organic matter treated soil became more pronounced. With 35 days incubation there was a slight increase in N_2 loss from green alfalfa treated soil which could be due to slight decrease in pH of this soil.

The difference in the pattern of N_2 evolved from the Dayton soil receiving green and dry alfalfa, incubated and nonincubated for a period of 35 days, can clearly be seen in figure 7. Without any incubation the magnitude of N_2 so evolved was 84 ppm without any organic

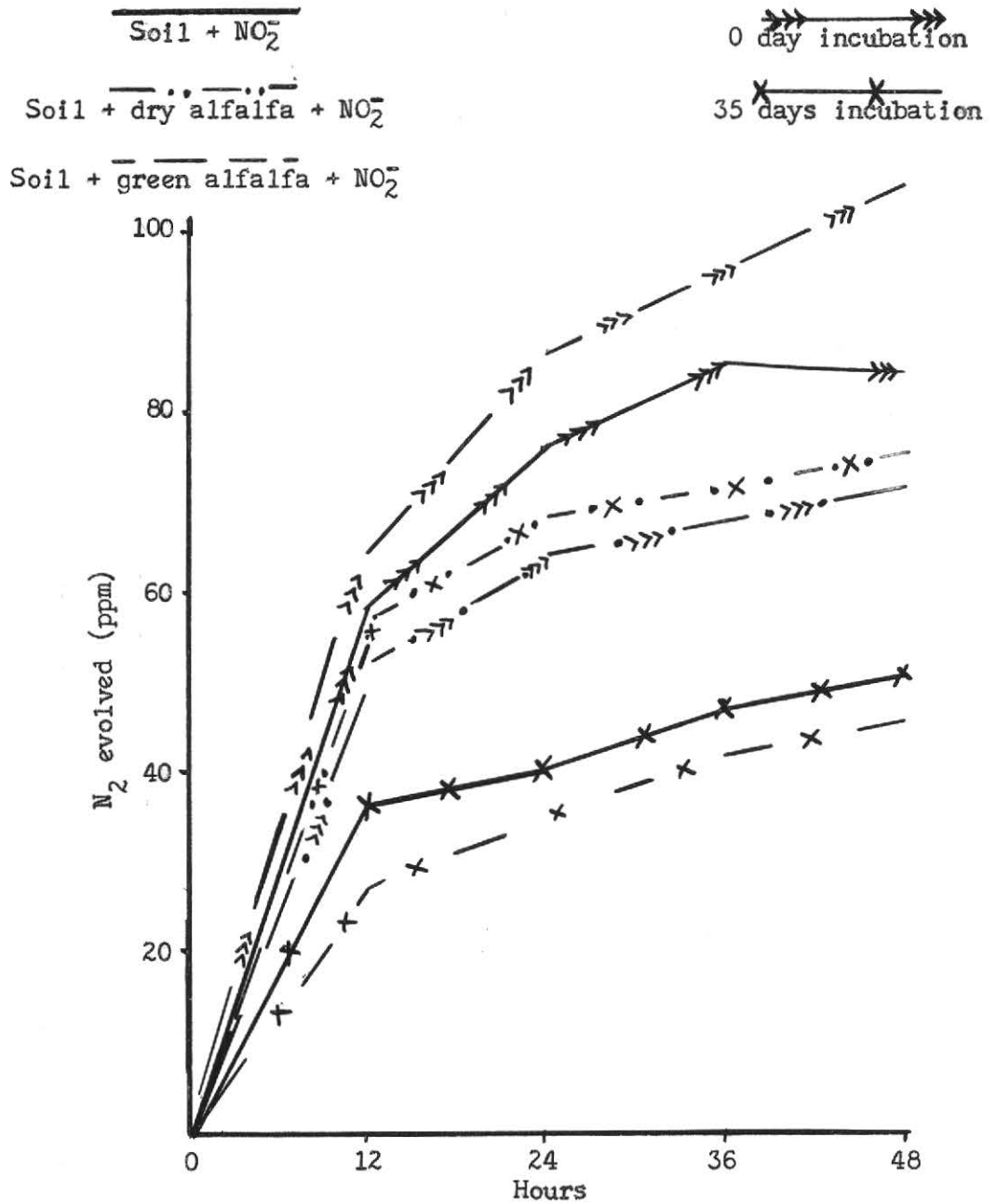


Figure 7. Comparison of elemental-N evolved from Dayton silty clay loam soil amended with dry alfalfa (1 percent on dry weight basis) and incubated for 0 and 35 days prior to the addition of 200 ppm nitrite-N added as NaNO_2 .

matter addition, 71.4 ppm with dry alfalfa addition, and 105 ppm with green alfalfa addition. A comparison of this pattern with that of 35 days of incubation shows the evolution of N_2 was 50.2 ppm without any organic matter addition, 75.4 ppm with dry alfalfa addition, and 46.0 ppm with green alfalfa addition. Thus the reaction of fresh green alfalfa with nitrite resulted in considerably enhanced evolution of elemental-N from nonincubated soils which was drastically reduced on incubation for 35 days.

Before proceeding further to the discussions of the N_2 evolution in the Olympic and Miami soils, it would probably be worthwhile to look at the changes in pH of these soils due to the application of green alfalfa, because the effect of any change in pH is reflected in the pattern or magnitude of N_2 evolved.

The data presented in figure 8 give an overall picture of the changes occurring in the Dayton, Olympic, and Miami soils with the application of green or dry alfalfa. It is evident from the figure that addition of dry alfalfa has very little effect on the pH of these soils but green alfalfa has a sharp and pronounced effect.

With application of green alfalfa, the pH of the Dayton soil rose from the original level of 4.8 to 5.5 in 3 days, 5.8 in 14 days, and dropped to 5.4 in 25 and 35 days of incubation. Even up to a period of 35 days the effect of added green material on the pH of this soil is clearly noticeable. Somehow the Olympic soil did not retain the effect of added green alfalfa for a long period. In 3 days the pH of this soil rose from 4.6 to 5.5 but then dropped down to 5.2 in 14 days, 4.8 in 21 days, and 4.7 in 35 days. The effect of added green organic material on increasing the pH was almost completely lost in 14 days.

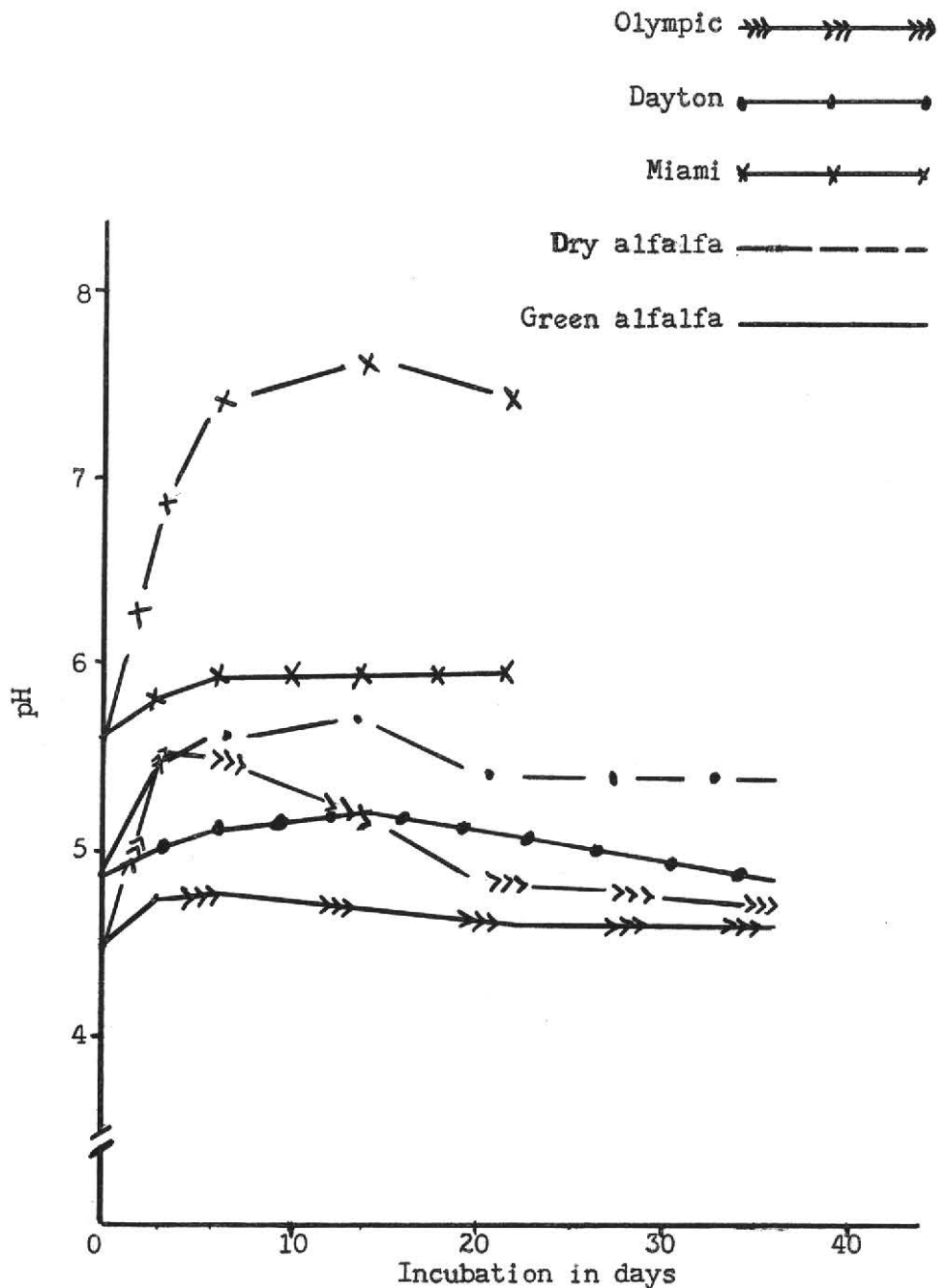


Figure 8. Changes in pH of Olympic silty clay loam, Dayton silty clay loam, and Miami silt loam soils incubated for varying time periods with the addition of dry or green alfalfa at 1 percent level on dry weight basis.

In the Miami soil the changes are quite pronounced. The pH of this soil rose from 5.6 to 6.8 in 3 days, 6.7 in 14 days, and 7.4 in 21 days.

In the light of these changes in pH, it will be easy to follow the pattern of loss of N_2 from the Olympic and Miami soils presented in figures 9 and 10.

In the Olympic soil the highest (97.6 ppm) N_2 was evolved with the reaction of nitrite and green alfalfa when the soil was not incubated (figure 9). With 6 days of incubation the evolution of N_2 was reduced to 84.3 ppm. In 6 days the pH of this soil rose from 4.6 to 5.5. Probably this increase in pH was responsible for the decrease in evolution of N_2 . In 21 days the pH dropped to 4.8. This decrease in pH was reflected in higher (90.1 ppm) evolution of N_2 as compared to 6 days (84.3 ppm N). With further incubation up to 35 days, there was no appreciable change in the pH as well as in the magnitude of N produced.

Like the Dayton and Olympic soils the nonincubated Miami soil treated with nitrite and green alfalfa evolved considerably higher (48.2 ppm) N_2 as compared to the treatments receiving dry alfalfa (32.3 ppm) or no alfalfa (36.9 ppm) (figure 10). With 21 days of incubation, the green alfalfa amended treatment yielded only 12.6 ppm N_2 as compared to 34.3 and 44.2 ppm from treatments receiving dry organic matter or no organic matter. It is interesting to note that the addition of both dry and green organic matter reduced the evolution of N_2 with 21 days incubation. This could be attributed to the high pH noticed in both the treatments after 21 days of incubation or due to the depletion of an active fraction of organic matter.

Trap and soil analysis

The detailed results of trap and soil analysis of the various

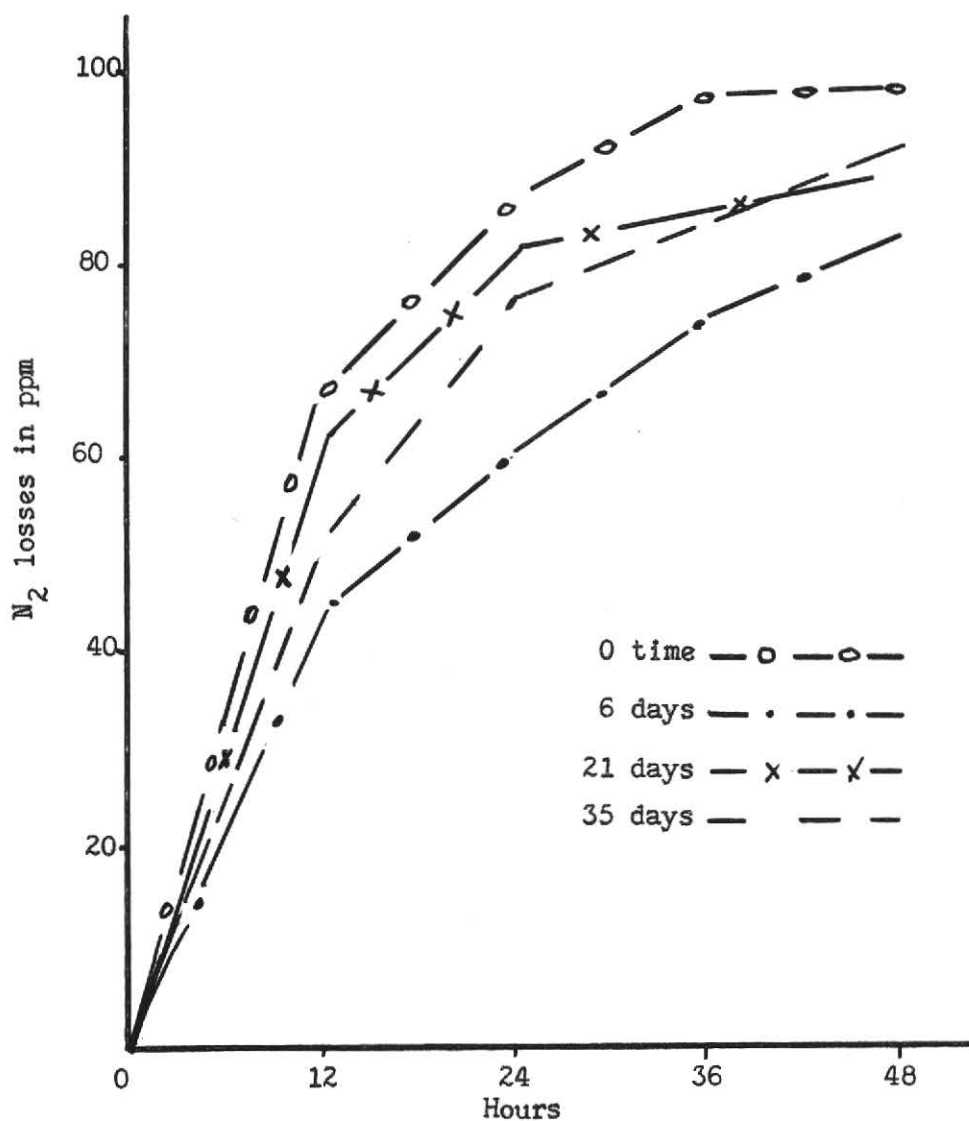


Figure 9. Comparison of the N_2 losses recorded at various time periods from Olympic silty clay loam soil incubated for 0, 6, 21, and 35 days with green alfalfa at 1 percent level on dry weight basis prior to the reaction with 200 ppm nitrite-N added as $NaNO_2$.

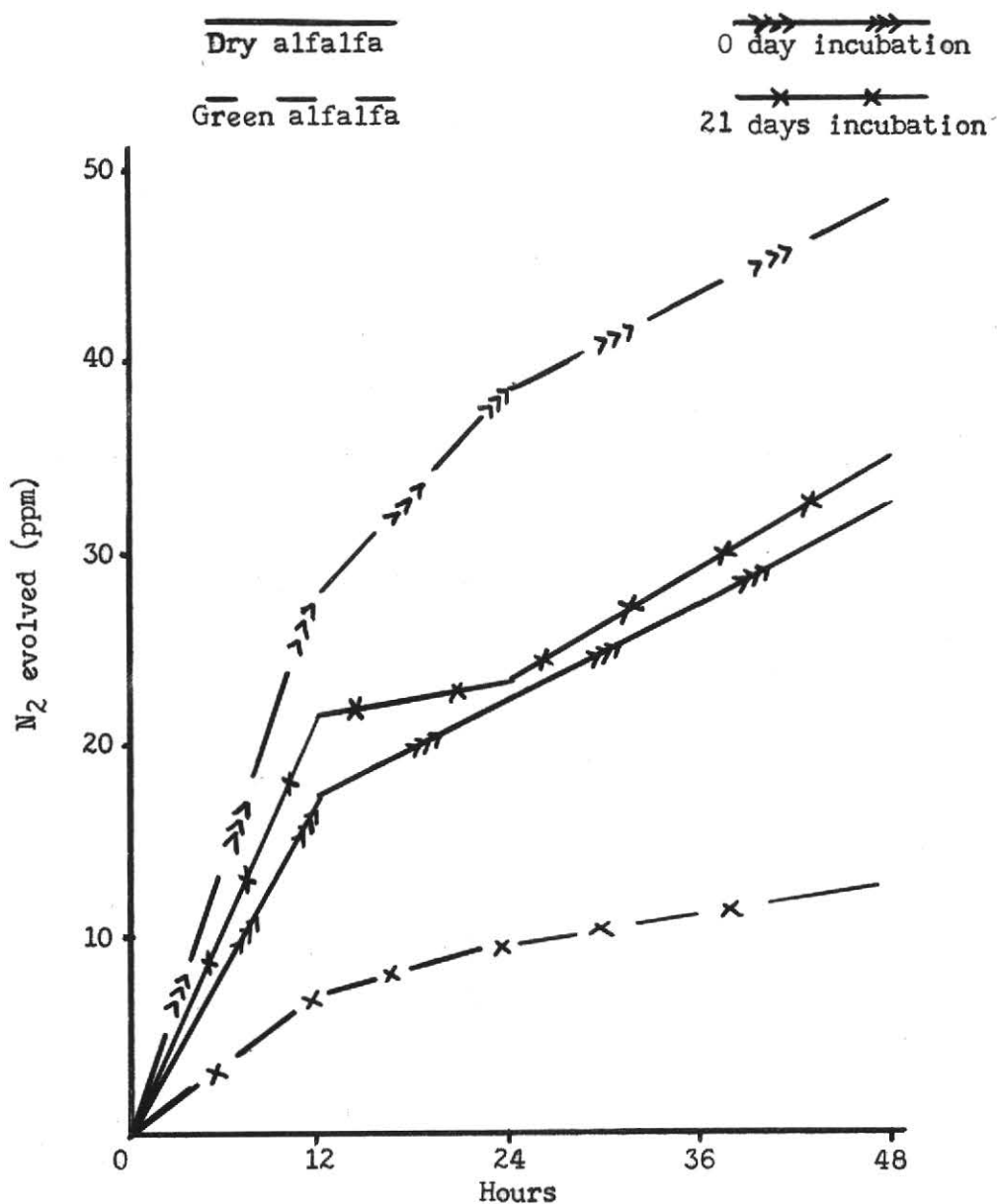


Figure 10. N_2 evolved at various time periods from Miami silt loam soil incubated for 0 and 21 days with dry or green alfalfa additions at 1 percent level on dry weight basis prior to the addition of 200 ppm nitrite-N added as $NaNO_2$.

experiments are presented in appendix tables 14 through 18. Since the conversion of nitrite to nitrate is out of the purview of these studies, a brief account of the other oxides of N recorded and total nitrogen recovered is given.

Oxides of nitrogen. From the results of trap analysis it is evident that, under aerobic conditions, the evolution of the oxides of N (NO and NO₂) is of minor importance, especially when the soil pH is near 5.0. In more acid soils, like the Olympic soil (pH 4.6), the losses were high in the initial stages of incubation but decreased considerably with longer incubation.

Temple (1914), Clark et al. (1960), Wullstein and Gilmour (1964), and a few others have suggested the gas NO as the major product of decomposition of nitrite. Wullstein and Gilmour (1964) have especially emphasized NO as the major product. The low recovery of NO in the present studies could be due to the oxidation of evolved NO to nitrite and nitrate as postulated by Temple (1914) and Cady and Bartholomew (1961):

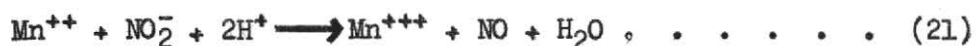


It is possible that some of the NO evolved gets absorbed on clays as suggested by Mortland (1964). When the exchange complex was saturated with transition metals, he observed chemical absorption of NO on Montmorillonite. Chao and Bartholomew (1964) have also suggested the possibility of NO absorption on Al-saturated bentonite.

As pointed out earlier, a certain amount of NO can form a complex with Fe cytochrome c of the microorganisms or with other metals in the soil. This complex formation may result in low recovery of NO.

Lu
60

In the presence of metals and H^+ ions the nitrites get converted to NO as suggested by Wullstein and Gilmour (1964):



It is quite possible that this reaction proceeds further and NO is reduced to N_2 under the catalytic influence of various transition metals present in the soil. This postulation was confirmed by Mahendrappa (1966) who has reported N_2 formation from the soil treated with only NO.

Due to these various possibilities, the evolution of NO may have been reduced in these studies. There are instances in the literature (Tyler and Broadbent, 1960) to show that no NO or NO_2 evolves in nitrite transformations.

Total nitrogen. From the analytical data presented in the appendix tables 14 through 18, it is apparent that the mineral and gaseous forms of N recovered from soil and gas analysis do not add up to the original amount of N added (200 ppm N). In very rare cases the total makes up the 200 ppm N or little over, but in most of the cases the balance sheet shows a deficiency. In some cases the deficiency is as great as 25 to 35 percent of the added N.

This shows that the determinations made in these experiments do not cover all the transformation products of N evolved during the course of the nitrite reactions. One such product is methyl nitrite (CH_3ONO) which has very recently been identified by Stevenson and Swaby (1964). They have suggested that when nitrite reacts with lignin or lignin building units, methyl nitrite evolves. There could be more N products which have not been identified so far. Gray (1958) has suggested that nitrite can get converted to N_2O_4 , and N_2O_4 then be ionized, yielding NO^+ and NO_3^- .



NO^+ once formed undergoes many reactions and nitrosation is one of them. It can react with various unsaturated hydrocarbons forming many nitrosation products.

The deficiency in total amount of N may also be due to the fixation of nitrite on organic matter or due to a complex formation of NO with heme, Fe cytochrome c, or by its adsorption on clays. Führ and Bremner (1964) have reported that the reaction between soil organic matter and nitrite results not only in conversion of N to gaseous forms but also to the fixation of nitrite-N on organic matter, i. e., the conversion of nitrite-N to organic N. Silver (1960), Chao and Bartholomew (1964), and Mortland (1964) have reported complex formation of NO with certain metals like aluminum and iron, heme, and cytochrome c and also a chemical adsorption of NO on montmorillonite or bentonite clays in the presence of certain transition metals.

EXPERIMENT II. N₂ EVOLUTION FROM REACTIONS OF NITRITE
WITH ORGANIC MATTER IN SAND-BENTONITE MIXTURE

This experiment was designed to see the effect of pH and organic matter on the pattern of N₂ evolution from organic matter-nitrite reactions in a system devoid of all complexities present in the soil. A sand-bentonite mixture acidified to pH 4.0, 4.5, and 5.0 was selected for this work.

Materials and Methods

Pure quartz sand and bentonite clay were used as the media in this experiment. The sand was washed several times with distilled water and dried. Bentonite clay, free of organic matter, was finely ground and thoroughly mixed with sand, keeping sand-bentonite in 90:10 proportion. The pH of this mixture was adjusted to 4.0, 4.5, and 5.0 with 0.3 N H₂SO₄. One hundred g of the mixture were placed in a waxed paper carton. Dry ground alfalfa was added at 1 percent level to those samples intended to receive organic matter additions. The mixture was inoculated with water extracts from the Olympic soil which in turn was incubated with organic matter additions for about 8 days. One-half of the quantity (3 ml) of water needed to bring the mixture to field capacity was from the soil extract, and the other one-half (3 ml) was distilled water. After being thoroughly mixed, the mixture was transferred to suction flasks and incubated for 0, 3, and 6 days. After incubation 200 ppm nitrite-N was added as NaNO₂. In addition to nitrite-N, the samples receiving organic matter received about 250 ppm of organic-N on the

basis of 2.5 percent N content in dry alfalfa. The N_2 evolved was determined at 12, 24, and 48 hours.

Statistical analysis of N_2 evolved at 48 hours after nitrite-N addition was made at each pH level, treating it as a factorial experiment in completely randomized design with two replications. The treatments were: two organic matter levels, no organic matter and 1 percent organic matter on the dry weight basis; and three incubation periods, 0, 3, and 6 days.

Results and Discussions

The results of this experiment are presented in tables 9, 10, and 11. A comparison of these data with that of the soils (tables 2 to 4) brings to light certain interesting differences. The most notable difference is related to the magnitude of evolution of N_2 . In the soil system the evolution of N_2 was considerably higher than in the sand-bentonite mixture of comparable pH. For instance, in the Olympic soil (pH 4.6) without any incubation, the N_2 recorded in 36 hours was 83.7 and 86.8 ppm from treatments with and without organic matter additions. In sand-bentonite mixture of comparable pH (4.5), the evolution of N_2 was only 26.0 from treatments receiving organic matter and 8.2 ppm from treatments receiving no organic matter. This abnormality may be attributed to some extent to the inherent high organic matter content of the Olympic soil, but this alone does not fully explain the difference. The results presented in figure 4 show that in H_2O_2 treated Olympic soil the evolution of N_2 was considerably higher than in the sand-bentonite mixture. This suggests that some other component or components of the soil complex serve to increase the production of N_2 .

Table 9. Elemental N recovered at various time periods from sand-bentonite mixture (acidified to pH 4.0) with and without 1 percent dry alfalfa and incubated at 30 C for 0, 3, and 6 days prior to the addition of 200 ppm nitrite-N added as NaNO_2

Treatment	Incubation time		
	None ppm	3 days ppm	6 days ppm
		<u>12 hours</u>	
Sand-bentonite mix + NO_2^-	6.4	3.3	3.5
Sand-bentonite mix + NO_2^- + dry alfalfa	20.6	13.2	8.5
		<u>24 hours</u>	
Sand-bentonite mix + NO_2^-	10.0	8.1	4.9
Sand-bentonite Mix + NO_2^- + dry alfalfa	25.4	24.6	11.4
		<u>48 hours</u>	
Sand-bentonite mix + NO_2^-	11.6	9.9	6.3
Sand-bentonite mix + NO_2^- + dry alfalfa	28.0	28.4	16.1
Standard error	± 0.8		
L.S.D. at 0.05 level	2.75		

Table 10. Elemental N recovered at various time intervals from sand-bentonite mixture (acidified to pH 4.5) with and without 1 percent dry alfalfa and incubated at 30 C for 0, 3, and 6 days prior to the reaction with 200 ppm nitrite-N added as NaNO_2

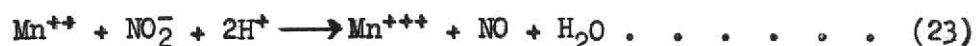
Treatment	Incubation time		
	None ppm	3 days ppm	6 days ppm
		<u>12 hours</u>	
Sand-bentonite mix + NO_2^-	5.0	3.3	3.3
Sand-bentonite mix + NO_2^- + dry alfalfa	16.6	13.2	6.2
		<u>24 hours</u>	
Sand-bentonite mix + NO_2^-	6.7	4.8	3.3
Sand-bentonite mix + NO_2^- + dry alfalfa	24.5	19.7	8.6
		<u>48 hours</u>	
Sand-bentonite mix + NO_2^-	8.29	7.0	4.9
Sand-bentonite mix + NO_2^- + dry alfalfa	26.0	27.2	16.2
Standard error	± 1.04		
L.S.D. at 0.05 level	3.19		

Table 11. N_2 recovered at various time intervals from sand-bentonite mixture (acidified to pH 5.0) with and without 1 percent dry alfalfa and incubated at 30 C for 0, 3, and 6 days prior to the reaction with 200 ppm nitrite-N added as $NaNO_2$

Treatment	Incubation time		
	None ppm	3 days ppm	6 days ppm
		<u>12 hours</u>	
Sand-bentonite mix + NO_2^-	5.0	3.3	3.2
Sand-bentonite mix + NO_2^- + dry alfalfa	11.8	9.9	4.9
		<u>24 hours</u>	
Sand-bentonite mix + NO_2^-	6.5	4.9	4.5
Sand-bentonite mix + NO_2^- + dry alfalfa	16.7	14.6	6.8
		<u>48 hours</u>	
Sand-bentonite mix + NO_2^-	9.5	8.2	8.1
Sand-bentonite mix + NO_2^- + dry alfalfa	24.3	24.0	12.9
Standard error	± 0.95		
L.S.D. at 0.05 level	2.92		

From the results of the experiment on nitrite transformations in cation exchange resins, Reuss and Smith (1965) have concluded that the reactions leading to the evolution of gaseous N require the presence of some soil complex or constituent.

In recent years evidences have been presented by some investigators emphasizing the role of transition metals in nitrite-N transformations. Wullstein and Gilmour (1964), in a series of experiments, have found that the reduced state of transition metals like copper, iron, and manganese and certain salts of aluminum is quite active in promoting nitrite loss. In some of their studies they have accredited one-half of the nitrite loss to Mn^{++} activity.



In this reaction the metal is oxidized and nitrite is reduced to NO.

According to their findings, NO is the major product of Mn^{++} activity.

As pointed out in an earlier discussion, it is likely that this reaction proceeds still further, resulting in reduction of NO to elemental-N.

The transition metals or some other constituent of the soil complex may serve as a catalyst in this reduction reaction.

Chao and Bartholomew (1964) have also emphasized the role of these metals in nitrite transformations. They have reported that the rate of nitrite-N transformations was directly proportional to the aluminum and iron content. They have not, however, reported the end products of these transformations. The role of some other soil complex in these losses cannot be ruled out.

Since the sand-bentonite mixture is devoid of all transition metals and other soil complexes, the reduction in N_2 evolution was not unexpected in this simple system.

A modification of figure 1 with inclusion of the losses of N_2 from the sand-bentonite mixture acidified to pH 4.5 is presented in figure 11. It is noted from this figure that the sand-bentonite mixture at the highest degree of acidity yielded the lowest elemental-N when compared to various soils. It was pointed out earlier that a slight change in the degree of acidity in the soils caused a considerable change in the magnitude of N_2 evolution. But the data presented in table 9 (pH 4.0) and table 11 (pH 5.0) show that a considerable change in pH did not make much difference, and the magnitude of N_2 evolved was almost the same at both the levels of pH. The evolution of N_2 from the sand-bentonite acidified to 4.0 was 28.0 and 11.6 ppm with and without organic matter additions without any incubation. When the same system was acidified to pH 5.0, the N_2 evolved was 24.3 and 9.5 ppm with and without organic matter additions. Thus, there was no significant difference in the extent of evolution of N_2 in the sand-bentonite mixture of these two different pH. This suggests that pH alone is not the sole factor affecting the evolution of N. Tyler and Broadbent (1960) have come to similar conclusions in their experiment of N losses from limed and unlimed soils.

Another distinct difference in the soil and the sand-bentonite mixture was noticed in the pattern of evolution of N_2 . In earlier discussions, it was pointed out that without any incubation, the addition of organic matter to the soil reduced the evolution of N_2 as compared to the treatment receiving no organic matter. Various explanations were suggested to account for this reduction. It is noticed in figure 11 that the addition of organic matter to the sand-bentonite mixture did not lower the production of N_2 . On the contrary, it increased N_2

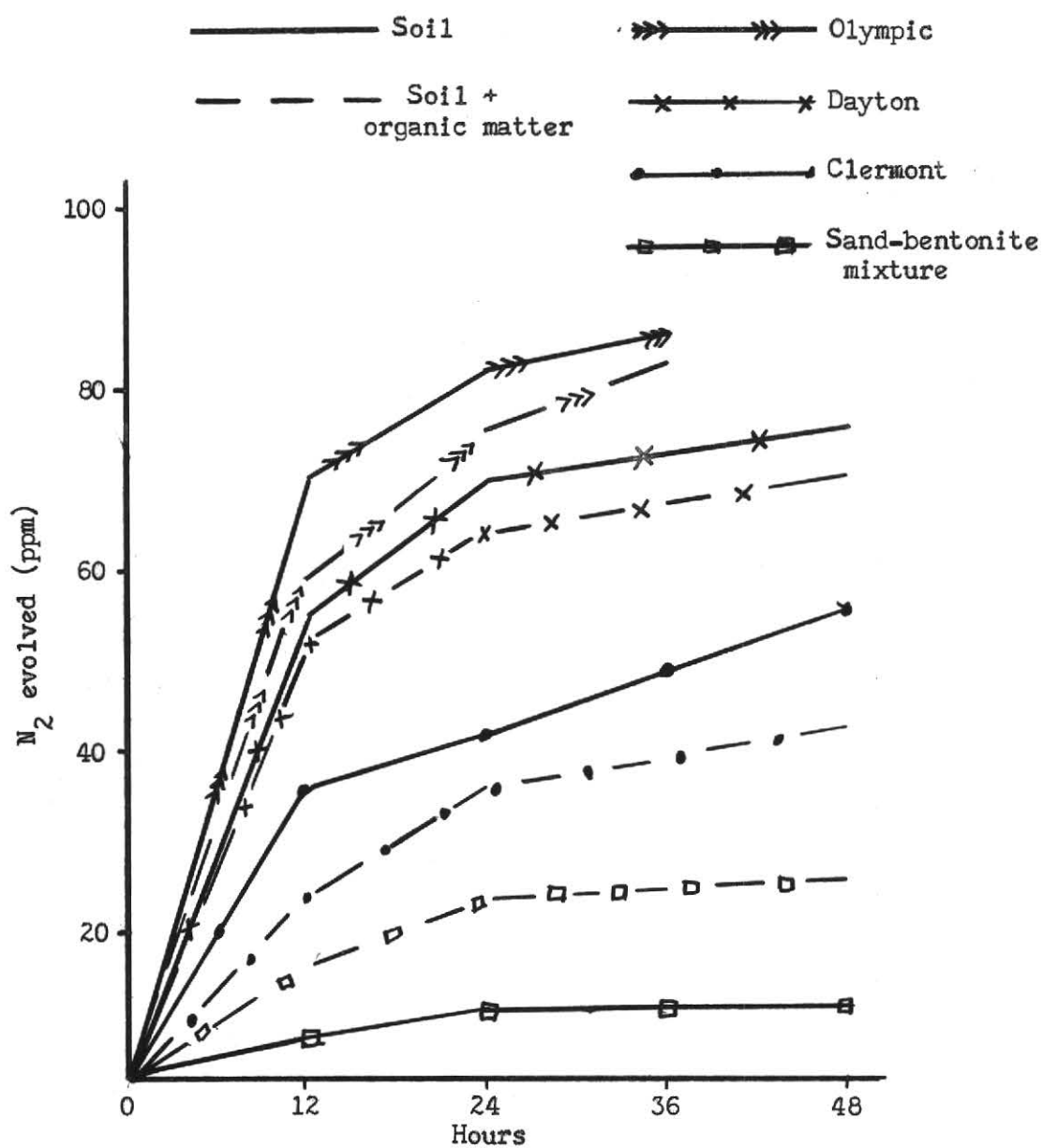


Figure 11. Comparison of elemental-N evolved at various time periods from various soils and sand-bentonite mixture (pH 4.5) treated with dry alfalfa at 1 percent level on dry weight basis and 200 ppm nitrite-N added as NaNO_2 .

evolution. The active fraction present in the added organic matter probably reacted with the nitrite resulting in the N_2 production. In this simple system, the effect of organic matter addition can be seen clearly. Without any organic matter the evolution of N_2 was 8.2 ppm which increased to 26.0 ppm with the addition of organic matter. The difference can be accredited to the reaction of organic matter with added nitrite.

Incubation of soils with dry organic matter additions had a marked effect on increasing N_2 evolution. In the sand-bentonite studies incubation had a reducing effect on N_2 evolution. With three days of incubation, there was no significant change in the extent of N_2 evolved. With six days of incubation, however, the losses were reduced considerably in the treatment receiving organic matter additions in all three levels of pH. This observation is quite contrary to the findings reported earlier in all studies conducted with dry alfalfa additions to various soils wherein greater amounts of N were recorded with longer incubations. The reduction in quantity of N in this experiment could be due either to fixation of nitrite or organic matter or the formation of an organic matter-clay complex which does not make organic fraction available to react with the added nitrite. Evans and Russell (1959) have pointed out that bentonite clay can form a complex very easily when allowed to react with organic matter.

It is also possible that with incubation there was some initial increase in microbial population in which the active fraction of the added organic matter was used as a source of energy but the microbial activity could not proceed further due to the lack of metals. Iswaran and Rao (1960), Waksman (1952), and Webley (1960) have emphasized the

need of various trace elements like copper, nickel, molybdenum, cobalt, boron, and iron for the growth and functioning of microorganisms. Chandra et al. (1962) and Singh and Pathak (1962) have reported rapid decomposition of organic matter in the presence of trace elements. With longer incubation, the active fraction of organic matter, instead of increasing, may actually decrease due to utilization of this fraction in the limited initial microbial activity and cessation of this activity at later stages and lack of mineralization of organic matter due to the restricted supply of various trace elements. Though this media was inoculated with soil extracts, the quantity added was quite small and the trace elements present in the extract may not have been sufficient to meet the requirements of microbial activity and decomposition of organic matter. Thus, the resulting decrease in active fraction of organic matter may reduce the N_2 evolved.

EXPERIMENT III. REACTIONS OF NITRITE WITH VARIOUS MODEL
PHENOLIC COMPOUNDS, HUMIC AND FULVIC ACIDS, AND
VARIOUS AMINO ACIDS IN THE SOIL COMPLEX

In the earlier discussions it was pointed out that when green alfalfa and nitrite are allowed to react in the soil, N_2 evolves copiously. Similarly, higher losses of N_2 were recorded with incubated dry alfalfa as compared to the treatment receiving no alfalfa. These suggest that green and decomposed dry alfalfa have certain components which are reactive and react with nitrite and result in the release of N_2 . Some experiments were designed to find out the fractions of organic matter which can react with nitrite. At this stage, it would be helpful to look at the nature or components of organic matter.

Plant and animal residues incorporated in the soil under natural conditions undergo intense microbial transformations resulting in the formation of a group of organic matter compounds known as "humic substances." Kononova (1961) has reported that this group includes humic acids, fulvic acids, haematomelonic acids, humins, and ulmins. The most important of these are humic acids and fulvic acids. Both are complex in nature and structure and are believed to be the products of condensation of aromatic compounds with products of protein decomposition with the possible participation of substances of carbohydrate nature. The aromatic structure of fulvic acids is weakly expressed, and the molecular weight is lower than the humic acids group.

Many workers have attempted to analyze the fresh and decomposed fraction of soil organic matter to isolate the structural units and

functional groups. Bloomfield (1957, 1958) and Coulson, et al. (1960) have extracted and identified many polyphenols from leaves, litter, and humus. Esh and Circar (1940), Morrison (1958), Hayashi and Nagai (1961), and Kononova (1961) have reported catechol, protocatechuic acid, resorcinol, vanillin, p-hydroxybenzoic acid, vanillic acid, phloroglucinol, veratric acid, p-hydroxy benzaldehyde, syringaldehyde, and dihydroxybenzoic acid as building stones of the various phenolic polymers. Dubach and Mehta (1963), reviewing the work of many authors, have reported the presence of carboxyls, phenols, enols, and alcohols, hydroxyls, quinones, hydroquinones, and other carbonyls, esters, lactones, and ethers in soil organic matter. Mortensen (1963) has reported mixtures of lignin, polysaccharides, proteins, tannins, and other polyphenols, perhaps quinoides and melonoides, as major components of organic matter.

Material and Methods

Model phenolic compounds

Some model phenolic compounds were used in this study to see the pattern of evolution of N_2 from these compounds when allowed to react with nitrite. The compounds selected were protocatechuic acid, pyrogallol, quinone, hydroquinone, vanillin, phloroglucinol, and cinnamic acid. Each of these was added to 100 g of Dayton soil at 0.3 g per treatment. After chemicals and soil had been thoroughly mixed, nitrites were added to the soil as outlined earlier. The N_2 evolved was determined at 4, 8, and 12 hours.

The experiment was in completely randomized design with eight treatments (seven model phenolic compounds and a control consisting of

Dayton soil with 200 ppm nitrite-N) with two replications. The statistical analysis presented is in respect of N_2 evolved at 12 hours.

Humic and fulvic acids

Green alfalfa was added to Dayton soil at the 5 percent level on a dry weight basis. This treated soil was incubated at 30 C for 10 days. The humic and fulvic acids were then extracted from the soil following the procedure outlined in detail by Henry (1966).

To extract the fulvic acid fraction, 20 g of incubated soil was placed in an Erlenmeyer flask to which 100 ml of 0.5 N H_2SO_4 was added. The mixture was shaken well on a mechanical shaker for about an hour and then filtered. The filtrate containing fulvic acids fraction was used to treat Dayton soil. The soil was brought to field capacity by using only the filtrate containing fulvic acid fraction. The pH of the soil was then adjusted to 4.8, the original pH of Dayton soil. The adjustment of pH was accomplished by the addition of dilute solution of sodium hydroxide (NaOH).

To obtain humic acid extract, another 20 g soil was placed in an Erlenmeyer flask to which 50 ml of 0.1 M $Na_4P_2O_4$ (sodium pyrophosphate) and 50 ml of 0.5 N NaOH were added. The mixture was shaken for one hour on a mechanical shaker. It was then filtered. This filtrate contained a mixture of humic and fulvic acids. The humic acid group was separated from the mixture by adding 1 N H_2SO_4 . With the addition of sulfuric acid, the humic acids group precipitated. The precipitate was collected on a filter paper. The entire quantity obtained from 20 g soil was used to treat 300 g of Dayton soil, which was thoroughly mixed and brought to field capacity by adding additional water. The pH was adjusted to 4.8. Nitrite-N was added at 200 ppm level as $NaNO_2$. The experiment was

started without any incubation following the same general procedure as outlined earlier.

Amino acids

Three different amino acids, tyrosine, tryptophan, and cystine, were used in this study. In view of the limited availability of amino acids, uniformity in the amount used could not be maintained. The study was conducted in the non-incubated Dayton soil to which either 0.30 g of tyrosine, 0.05 g of tryptophan, or 0.20 g of cystine per 100 g soil was added. The treatments receiving above quantities of tyrosine, tryptophan, and cystine have received 230, 66, and 230 ppm N respectively in addition to 200 ppm nitrite-N added as NaNO_2 . The N_2 evolved was determined at 12, 24, 36, and 48 hours. The design of the experiment was completely randomized with four treatments (including a control) and three replications. The statistical analysis was made in respect of N_2 evolved at 48 hours after the addition of NaNO_2 .

Results and Discussions

Model phenolic compounds

It is evident from the data (table 12) that some of the phenolic compounds have the ability to enhance the evolution of N considerably as compared to the control, and some of them have the reverse effect and reduce the evolution. The largest amount of N_2 evolved was from the treatment receiving hydroquinone (figure 12). The total quantity of N_2 evolved from the treatment (81.4 ppm) was almost double the N_2 evolved from the control (41.6 ppm). Cinnamic acid also showed fairly good reactivity. Appreciable quantities of N_2 were also evolved from the treatment receiving vanillin.

Table 12. Losses of elemental N recorded from Dayton silty clay loam treated with (0.3 g) various model phenolic compounds on reaction with 200 ppm added nitrite-N as NaNO_2

Treatment	N_2 evolved		
	4 hours ppm	8 hours ppm	12 hours ppm
Dayton + NO_2^- (control)	35.2	38.8	41.6
Dayton + NO_2^- + protocatechuic acid	5.5	10.9	14.1
Dayton + NO_2^- + pyrogallol	11.1	12.6	13.2
Dayton + NO_2^- + quinone	30.0	35.8	38.8
Dayton + NO_2^- + hydroquinone	49.3	74.5	81.4
Dayton + NO_2^- + vanillin	48.4	51.6	50.1
Dayton + NO_2^- + phloroglucinol	11.3	15.1	15.2
Dayton + NO_2^- + cinnamic acid	46.8	53.3	57.0
Standard error	± 1.49		
L.S.D. at 0.05 level	4.85		

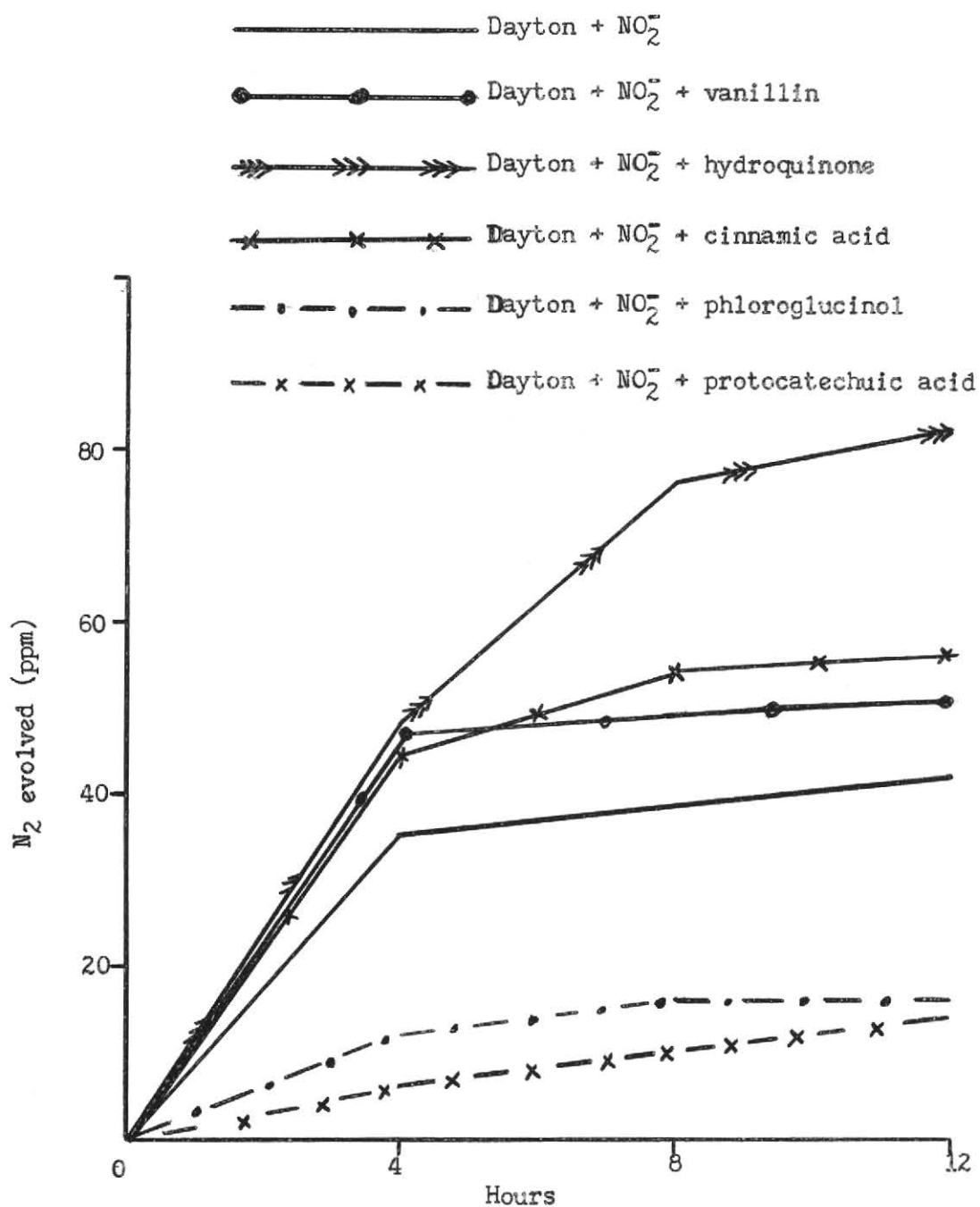
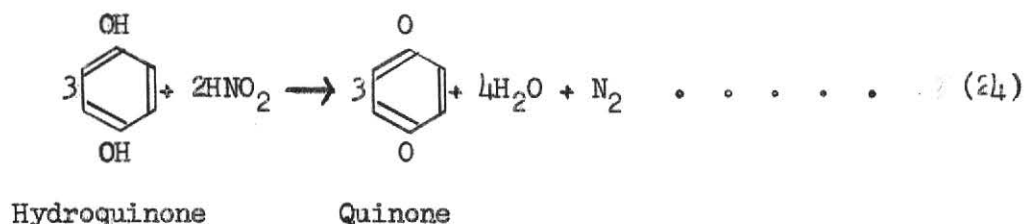


Figure 12. N_2 evolved from Dayton silty clay loam soil treated with various model phenolic substances (at 0.3 g/100 g soil) and 200 ppm nitrite-N added as NaNO_2 .

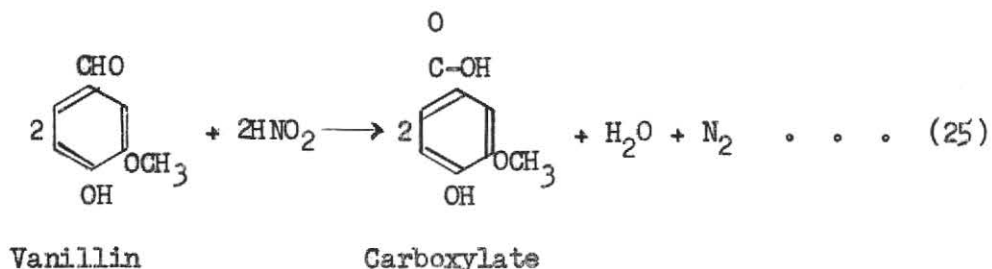
It is interesting to note that some of the phenolic substances have the astonishing ability of reducing the evolution of N_2 . Protocatechuic acid and phloroglucinol, for example, evolved 14.1 and 15.2 ppm N as compared to 41.6 ppm N evolved from the control.

The higher yields of N_2 recorded from some of the phenolic substances like hydroquinone and vanillin indicate that these substances react with nitrite. The reaction between phenolic compounds and nitrite may be represented by the following postulated model equation:



In this reaction, the hydroquinone is oxidized and nitrite is reduced, resulting in N_2 evolution.

Vanillin may react with nitrite according to the following model reaction:



The degree of reactivity of the various phenolic compounds may depend on the nature and position of substitution.

The other interesting observation with regard to the reduction in N_2 evolution in pyrogallol, phloroglucinol, and protocatechuic acid can best be explained by assuming the phenomenon of fixation of nitrite on these compounds.

Führ and Bremner (1964) have reported that the reaction between the soil organic matter and nitrite leads to the fixation of nitrite on organic matter. The fixation increases with an increase in nitrite concentration. The results of this experiment are in agreement with their findings. In addition to N_2 there could be some other end product of nitrogen which could not be determined in these studies.

The phenomenon of fixation of nitrite on some phenolic compounds may serve to explain the reduced amount of N_2 recorded in all non-incubated soils to which dry alfalfa additions were made. Probably the dry alfalfa is rich in phenolic compounds which have nitrite fixing ability. With incubation, those compounds may get transformed to some other forms which promote reaction with nitrite.

The work done in this area is very limited. The only reference available in the literature is that of Stevenson and Swaby (1964) who have reported production of elemental N, N_2O , CO_2 , and CH_3ONO (methyl nitrite) when some model phenolic compounds were allowed to react with nitrite in solution studies.

Humic and fulvic acids

It is evident from figure 13 that extracts of both the humic and fulvic acids enhanced the evolution of N_2 . In a period of 48 hours, 74.1 ppm N was recovered as N_2 from the control treatment, whereas the N_2 evolved from the humic and fulvic acids treatments was 85.2 and 93.5 ppm respectively. The question rises as to what fraction of these complex substances could react with nitrite to yield N_2 . The humic and fulvic acids are a series of acidic predominantly aromatic phenolic polymers widely varying in their molecular weight. Kononova (1961) has

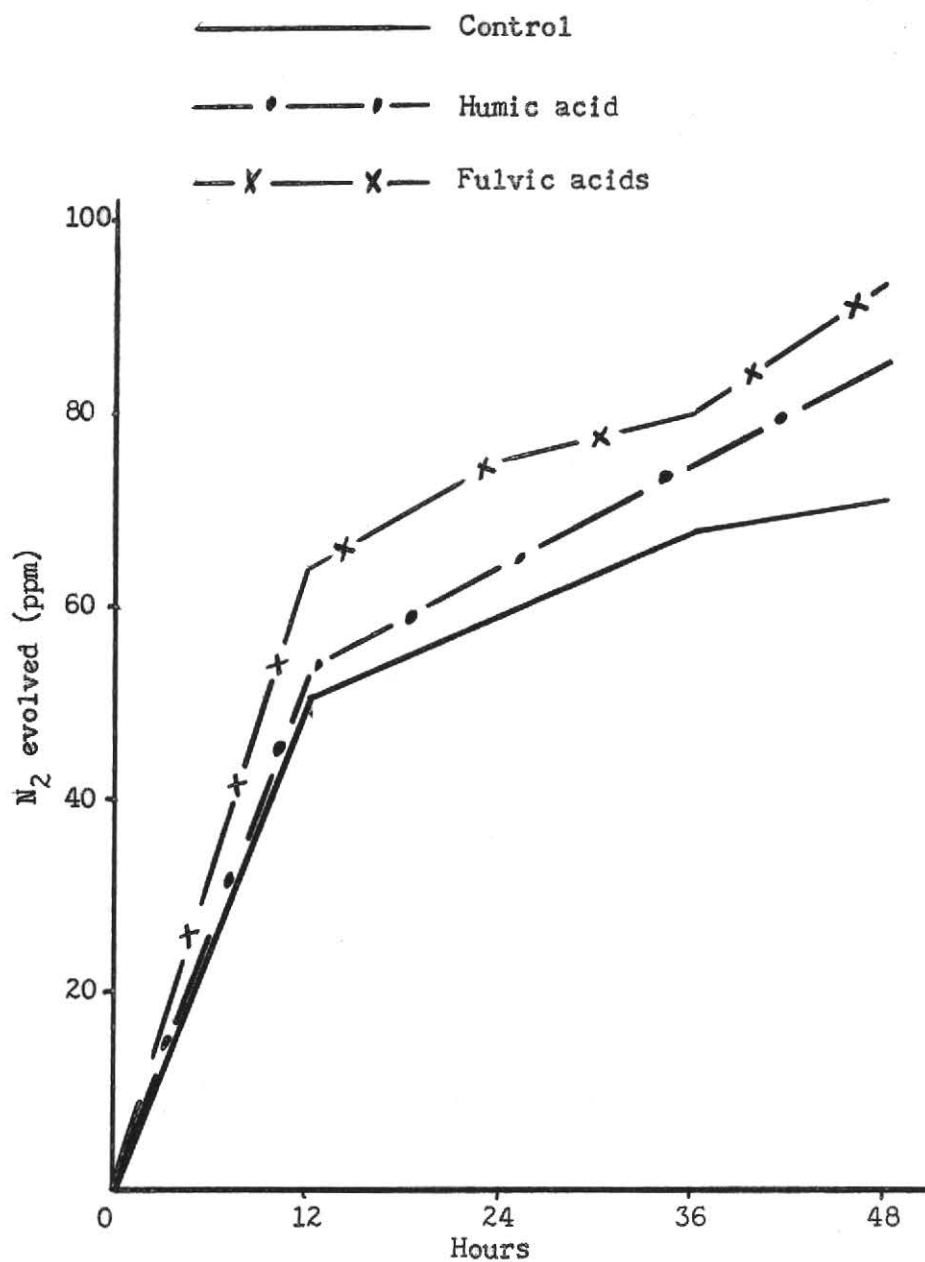


Figure 13. N_2 evolved at various time periods from Dayton silty clay loam treated with humic and fulvic acids and 200 ppm nitrite-N added as $NaNO_2$.

reported that both substances are made of the same structural units, i. e., compounds of an aromatic nature, N-containing substances, and reducing substances. She has reported that the aromatic structure in fulvic acids is weakly expressed, thereby giving predominance to the peripheral chains. Aromatic rings containing various phenolic substances are probably the portion of molecule of these substances which reacts with nitrite giving rise to N_2 .

Dubach and Mehta (1963) have reported the presence of over 30 phenolic compounds in humic and fulvic acids. Coffin and Delong (1960) have reported that 30 percent of the humic fraction from the B horizon of a podzol was phenols, and they were able to isolate 19 different phenolic substances.

Coffin and Delong (1960), Kononova (1961), and Dubach and Mehta (1963) have reported the presence of quinones, hydroquinones, vanillic acid, protocatechuic acid, m-hydroxybenzoic acid, p-hydroxy benzoic acid, 2, 4-, 3, 4-, 3, 5-dihydroxybenzoic acid, various methoxyl-containing phenols, pyrogallol, gallic acid, and various other acids in the structure of humic acids. As reported earlier, the various phenolic compounds have the ability to react with nitrite yielding elemental N.

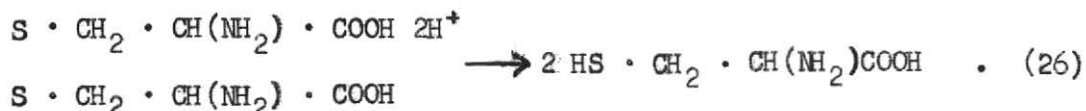
It is obvious from figure 13 that the fulvic acid fraction is more reactive than humic acids fraction. Higher reactivity of fulvic acids with nitrite could probably be attributed to the presence of reducing substances and active acids in higher amounts than in the humic acids group. Kononova (1961) has reported the work of Ponomoreva who has suggested that fulvic acids have a greater percentage of reducing substances than humic acids. In addition, the same worker has reported the presence of large amounts of active acids group in the fulvic acids

fraction. Though she has not identified the active acid groups, Gillam (1940), Broadbent and Bradford (1952), Lewis and Broadbent (1961), and Kononova (1961) have reported the presence of phenolic, enolic, alcoholic, carboxyl, and carbonyl groups in humic substances. The presence of reducing substances and the active acid groups individually or collectively may account for the higher reactivity of fulvic acids group.

Amino acids

It is important and interesting to note that greater N_2 evolved from treatments receiving amino acids as compared to the control (figure 14 and table 13). In the first 24 hours the losses of N_2 from the cystine treatment were less than from the control, but in the remaining 24 hours higher losses were recorded from this treatment.

Cystine is readily reduced to cysteine



This conversion explains the low losses in the initial period of the experiment. Once cystine is converted to cysteine, the reaction proceeds faster as the cysteine and its derivatives are extremely active in oxidation-reduction reactions.

Tyrosine and tryptophan both yielded higher N_2 as compared to the control. In the initial stages, tyrosine appeared to be more effective in N_2 evolution than tryptophan, but at later stages, in spite of the low rate of application, tryptophan evolved more N_2 than tyrosine. In 48 hours, 73.7 ppm N was recovered from the control treatment as N_2 . Recovery of N_2 from tyrosine, tryptophan, and cystine was of the order

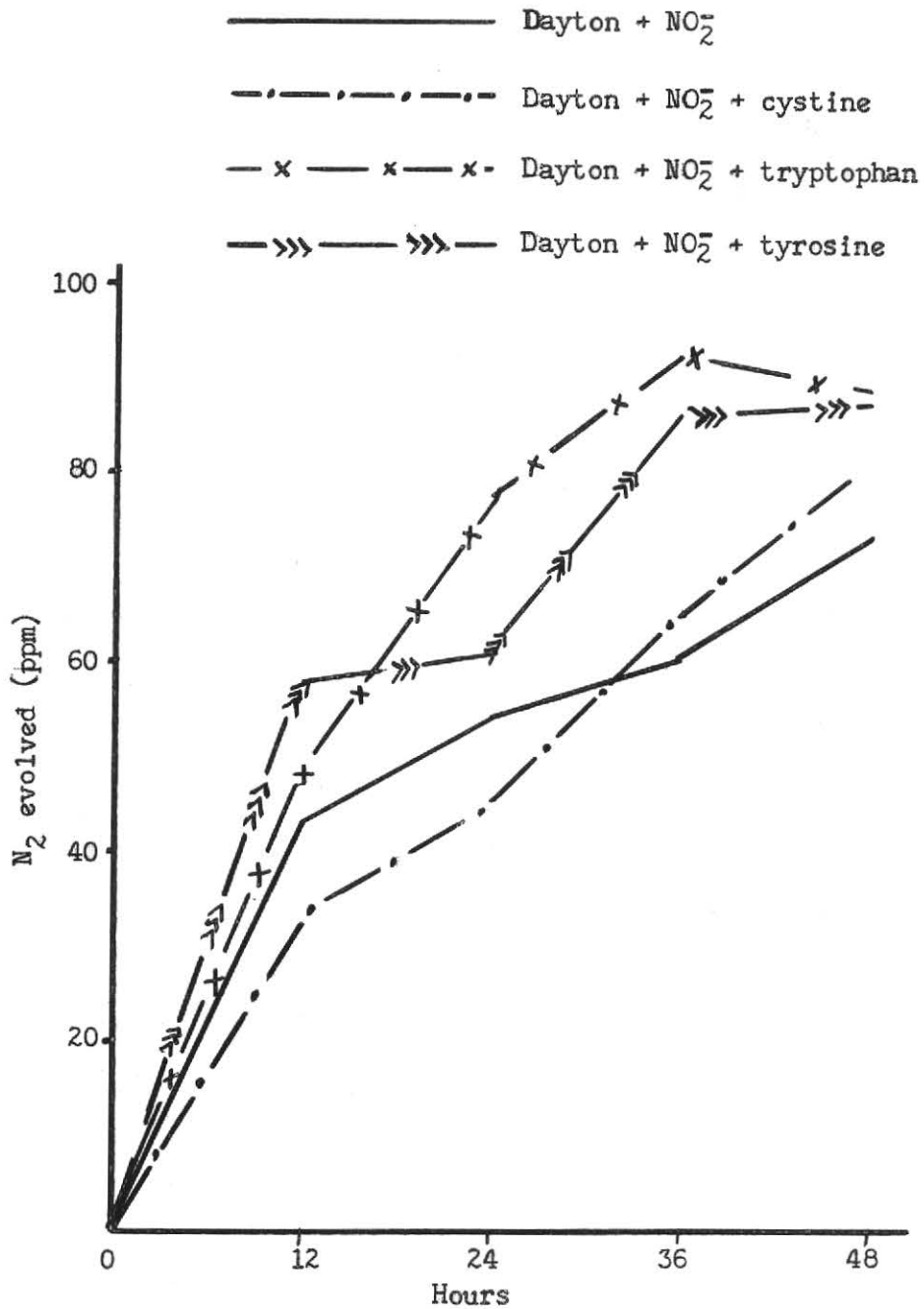


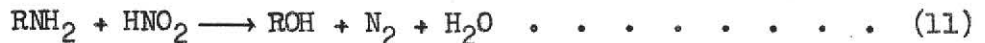
Figure 14. Elemental-N evolved at various time periods from Dayton silty clay loam soil treated with various amino acids (cystine--0.2 g/100 g soil, tyrosine--0.3 g/100 g soil, and tryptophan--0.05 g/100 g soil) and 200 ppm nitrite-N added as NaNO_2 .

Table 13. Losses of elemental-N recorded from Dayton silty clay loam soil treated with various amino acids and reaction with 200 ppm nitrite-N added as NaNO_2^a

Treatment	N_2 evolved			
	12 hours ppm	24 hours ppm	36 hours ppm	48 hours ppm
Dayton + NO_2^- (control)	43.1	53.9	60.3	73.4
Dayton + NO_2^- + tyrosine	58.0	61.8	86.0	87.2
Dayton + NO_2^- + tryptophan	48.3	77.4	92.0	88.3
Dayton + NO_2^- + cystine	33.4	45.2	65.5	82.3
Standard error	± 2.70			
L.S.D. at 0.05 level	8.59			

^aQuantity of N_2 is reported after deducting that found in the soil used as a control to which no nitrites were added.

of 87.2, 88.3, and 82.3 ppm respectively. The reaction may proceed according to the classical Van Slyke reaction:



All the treated samples evolved significantly more N_2 as compared to the control but they were not significantly different among themselves. There are some investigators who have supported the view that amino acids can react with nitrite yielding elemental-N. Barritt (1931) has reported loss of N as a result of the interaction of nitrite and glycine at pH 5.8. Wilson (1943) and Gerretson and DeHoop (1957) have strongly favored nitrite-amino acids reaction. Patil (1963) has suggested the possibility of reaction of amino acids with nitrite. The results of this study are in conflict with those reported by Allison and Sterling (1948), Allison and Doetsch (1951), Allison et al. (1952),

Jones (1952), and Smith and Clark (1960), who did not find any appreciable loss of elemental N from the reaction of amino acids and nitrites.

GENERAL DISCUSSION OF THE RESULTS

Evaluation of Elemental-N from Organic Matter--

Nitrite Interactions

The present investigations were based on the hypothesis that N_2 is one of the products of the decomposition of nitrite. This N_2 may evolve either from the direct reaction of some component of organic matter with nitrite or from the reduction of nitrite to N_2 in which the reaction is catalyzed by some fraction of organic matter. The work reported here definitely supports the view that nitrite does react with organic matter giving rise to elemental-N. The pattern and extent of the N_2 evolution depend not only on the inherent properties of soil such as pH, organic matter content, and mineralogical make-up, but also on the nature of added organic matter and the incubation treatment given to the soil. There was a considerable difference in the magnitude of N_2 evolving from nitrite reactions with dry and green organic matter and from incubated and non-incubated soils. The pattern of N_2 evolved from dry alfalfa treatment incubated for varying lengths of time was totally different from the pattern noted in green alfalfa studies.

The initial pH of the soil or the shift in pH due to the application of organic matter had profound influence on the extent or even the pattern of N_2 evolution.

Nonincubated soils

The addition of dry organic matter to soils without any incubation had a temporary lowering effect on N_2 evolution as compared to the

treatment receiving no organic matter. This might be explained on the basis of (a) an immediate utilization of an active fraction of added organic matter by soil microorganisms as a source of energy, (b) a complex formation of NO or nitrite with Fe cytochrome c or with other substances, (c) the fixation of nitrite on organic matter, or (d) a chelation or complexing of the various transition metals which are believed to be important in nitrite transformations.

In the H₂O₂ treated Olympic soil, the addition of organic matter did not exhibit any lowering effect on N₂ evolution as was noted in non-treated soil. This strongly suggests that H₂O₂ treatment had affected the factor or factors which reduced N₂ evolution. Of the four postulated explanations suggested above, the most susceptible to H₂O₂ treatment seems to be the microbial population. The drastic and prolonged H₂O₂ treatment would have destroyed most of the microorganisms initially present in the soil. Although the soil was inoculated by extracts from incubated soil samples, the inoculation treatment may not have restored the microbial population to its initial level. The decreased number of microorganisms may not use up all the reactive fraction present in added organic matter. Thus, additions of organic matter to H₂O₂ treated soil resulted in higher loss of N₂. Incidentally, this explanation illustrates the other suggested possibility in which emphasis is laid on a NO/NO₂⁻-cytochrome c-complex formation as suggested by Silver (1960). In the absence of intense microbial activity in the H₂O₂ treated soil, the complex formation may not be as important as in non-treated soil.

The results of some of the experiments reported herein favored the explanation suggesting the possibility of adsorption of nitrite on

organic matter. The experiments conducted with various phenolic substances showed that some of these compounds can enhance the evolution of N_2 when allowed to react with nitrite and some of them have the reverse effect. Protocatechuic acid, pyrogallol, and phloroglucinol are some of the substances which reduced the evolution of N_2 considerably. The low amount of N_2 recovered from these treatments can be explained on the basis of adsorption of nitrite on these compounds. The literature is devoid of information on this subject. The only available reference is that of Führ and Bremner (1964) who have reported nitrite fixation on organic matter. Their findings are in favor of these results wherein we have been able to isolate some of the compounds which have nitrite fixing ability. There could be many more compounds in the plant material capable of fixing nitrite.

Incubated soils

The lowering effect of dry organic matter on evolution of N_2 did not last long. A brief incubation, as short as three days, was enough to reverse the pattern and enhance the amount of N_2 evolved.

Incubation of soils with dry organic matter additions clearly established the role of organic matter in enhancing the N_2 evolution. With incubation the N_2 evolved from organic matter treated soils increased in all cases except for the Clermont soil. This unusual behavior of Clermont soil may partly be attributed to the rapid rise in pH of this soil with organic matter addition and partly to the inherent properties. In the Olympic and Dayton soils, the difference in N_2 evolution of treated and non-treated soils became more prominent with incubation. With 35 days of incubation, the difference in the

magnitude of N_2 evolved between the treated and non-treated Olympic soil was 20 ppm and 25 ppm in the Dayton soil. Higher N_2 evolved from organic matter treated soils. The difference reported here favors our hypothesis that organic matter reacts with nitrite to evolve N_2 .

H_2O_2 treated soil

The results of H_2O_2 treated Olympic soil further confirm our hypothesis that interaction of nitrite with organic matter results in loss of N in elemental form. As compared to the normal soil, the magnitude of N_2 evolution in H_2O_2 treated soil was drastically reduced, indicating that the higher losses in non-treated soils may be due to the inherent organic matter content of the soil.

Green alfalfa amendments

With the addition of green alfalfa to various soils about 10 to 20 ppm more N was recorded as compared to the treatments receiving no organic matter additions. The clear-cut higher N_2 recorded in these experiments strongly confirms the role of organic matter in nitrite transformations.

Sand-bentonite mixture

The experiment conducted with the sand-bentonite mixture is another evidence supporting our hypothesis. In the soil system, in addition to organic matter, many other components of the complex, like transition metals, etc., may enhance N_2 evolution or nitrite transformations, but in a simple system like the sand-bentonite mixture which is devoid of all complexities the difference can clearly be seen and definitely be attributed to organic matter. The organic matter treatment yielded 18

to 20 ppm more N_2 than its counterpart receiving no organic matter treatment.

The results of these investigations illustrate and explain the large "N deficits" noted by many investigators in their studies of nitrite in relation to organic matter. Robinson, as early as 1923, noted rapid loss of N from soils high in organic matter and suggested that organic matter may cause rapid decomposition of nitrite. Soulides and Clark (1958), in their studies of nitrification in grassland soil, encountered greater N deficits in grasslands than intertilled soils. Smith and Clark (1960) studied N losses in Fort Collins loam (3.86 percent organic matter) and Durham loam (0.54 percent organic matter). They noted greater N losses in the Fort Collins than in the Durham soil and suggested that some component of organic matter was more reactive in reducing nitrite. Clark and Beard (1960) have observed that at a given pH value, organic matter of the soil served to promote the reactivity of nitrite and the development of a much greater N deficit.

The studies reported here confirm the above findings and explain that the losses or deficits encountered by them were due to evolution of elemental N from reaction of nitrite with organic matter.

Role of pH in N_2 Losses

The degree of acidity is of over-riding importance in nitrite transformations. Kelly (1916), Robinson (1923), Fraps and Sterges (1939), Martin et al. (1942), Clark et al. (1960), and Smith and Clark (1960) have emphasized the effect of H^+ in nitrite transformations. The greater the degree of acidity, the more severe are the losses of N_2 . The results of the present investigations are in accordance with

the findings of these workers. A slight change in the degree of acidity in the soils was seen responsible for a considerable change in the magnitude of N_2 evolved. The difference in the pH of the Clermont soil (5.2) and the Olympic soil (4.6) is not much, but the extent of N_2 loss recorded in the Olympic soil is almost double that in the Clermont soil. Such a vast difference in the magnitude of evolved N_2 may be due to the inherent properties of these soils. However, the role of pH cannot be overlooked in accounting for the differences.

The changes occurring in pH of the various soils due to the application of organic matter had a considerable influence on the magnitude of N_2 evolution. In some instances, the changes in pH changed even the pattern of N_2 evolution. The best example of this is the Clermont soil wherein the N_2 loss was reduced considerably due to a rise in pH when the soil was incubated with organic matter. In this soil, the effect of pH seems to have overcome the effect of added organic matter. The changes in pH of various soils incubated with green alfalfa and consequent changes in magnitude of evolved N_2 also serve as a good illustration. Incubation of soils with green alfalfa resulted in a rise in pH of the soils so treated, which consequently reduced N_2 evolution from added nitrite. The Olympic soil treated with green alfalfa serves as good evidence of the influence of pH on N_2 evolution. As shown in figure 9, without any incubation when the pH of this soil was 4.6, the total amount of N_2 evolved was 97.6 ppm. With six days of incubation when the pH went up to 5.5, the evolution of N_2 was reduced to 84.3 ppm; with further incubation for 21 days when the effect of added green material on pH waned considerably and the pH came down to 4.8, the amount of evolved N_2 went up again to 90.1 ppm. Thus the N_2 evolved at

21 days was higher than the N_2 evolved with six days of incubation.

However, it is interesting to note that in a simple system of sand-bentonite mixture, acidified to various pH levels, the over-riding influence of pH was not seen. The quantities of N_2 recorded at pH 4.0 were in no way different in magnitude from those recorded at pH 5.0. This suggests that the effect of pH gains prominence only in the presence of the soil complex.

Characteristic of the Reactions

An important aspect of all the studies reported here is that the major fraction of the evolved N_2 is recorded in the first 12 hours. The amount of N_2 recorded in the subsequent 36 hours is not as high as recorded in the first 12 hours, as could be seen in figures 1 and 2. This strongly suggests that the evolution of N_2 is the result of chemical reaction and not the product of biological activity. There are many evidences in the literature to show that the nitrite transformations yielding N_2 are chemical in nature. Clark and Beard (1960) and Tyler and Broadbent (1960), from their studies of nitrite dismutation, have reached similar conclusions. While studying the transformations of nitrite-N in sterile and non-sterile Bath soil, Reuss (1963) found no differences in the amount of N_2 evolved. He, therefore, concluded that the microbial denitrification was not contributing to the N_2 production. Reuss and Smith (1965) have also suggested that the nitrite transformations are chemical rather than microbiological. This is further confirmed by the most recent investigations carried out by Mahendrappa (1966), who did not find any differences in the amount of N_2 evolved from nitrites added to Miami, Dayton, and Olympic acid soils.

Nature and Composition of Organic Matter and its Probable
Reactions with Nitrite Giving Rise to N₂

Nature of organic matter

The higher amounts of N₂ recorded with green alfalfa additions or with incubated dry alfalfa suggest that these substances are rich in a fraction or fractions which are reactive and can react with nitrite. It was noted that a short incubation of three days was enough to convert the inactive form of organic matter fraction in dry alfalfa to an active form. It is obvious that in three days the stage of decomposition did not proceed to an advanced stage. In this short period the carbohydrates, proteins, and lignins may have disintegrated due to microbial activity to simpler substance like mono-, di-, and tri-saccharides, amino acids, amines, and various phenolic or aromatic compounds.

Bloomfield (1957, 1958) and Coulson et al. (1960) have extracted and identified many phenolic substances from leaves, litter, and humus. Esh and Circar (1940), Morrison (1958), Hayashi and Nagai (1961), and Kononova (1961) have reported the presence of various phenolic substances like catechol, protocatechuic acid, resorcinol, p-hydroxybenzoic acid, dihydroxybenzoic acid, and various quinones and hydroquinones in decomposing organic matter. In addition to the phenolic substances, various aldehydes are also present in the plant tissues. Kononova (1961), Robinson (1963), and Dubach and Mehta (1963) have reported the presence of salicyl aldehyde, vanillin, and syringaldehyde in plant material.

Plant tissues are also rich in carbohydrates. Notable among them from the point of view of reactivity are the mono- and di-saccharides. The monosaccharide group is the most important and abundant in plants.

The presence of various amino acids and amines and proteins is well

accepted by all.

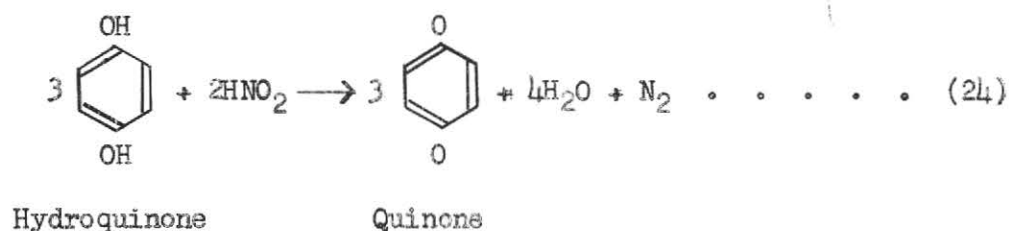
In addition, the green alfalfa is rich in various pigments which can be oxidized easily. Robinson (1963) has reported the presence of many flavonoids in the entire plant kingdom from fungi to angiosperm. Quercetines, carotinoids, and many other easily oxidizable pigments have also been identified.

From the point of view of nitrite reactions or its reduction to elemental N₂, any of the substances discussed above may be important.

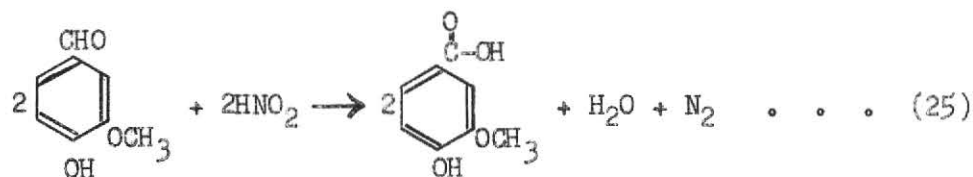
Probable reactions

Nitrite-organic matter reactions. In view of the high degree of reactivity, diversification in the nature of reactions of nitrite is not unexpected. The complexities in reaction grow in magnitude when a reactive substance like nitrite reacts with a complex substance like organic matter. Without a very detailed and thorough research, it is rather difficult to outline the reactions occurring that lead to the formation of N₂.

The experiments carried out with various phenolic compounds, humic acids, fulvic acids, and various amino acids indicate that many of these substances do react with nitrite. As pointed out earlier, various phenolic compounds and aromatic structures are present in plant tissues and decomposed organic matter. Probably one of the most important and active groups is that of hydroquinones. Its high degree of reactivity with nitrite is evident from the fact that it yielded twice as much N₂ as the control. The reaction between hydroquinone and nitrite may be represented by the following postulated model equation:

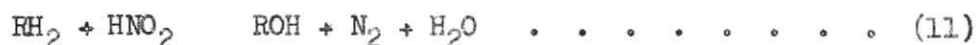


In addition, there are many aldehydes in the plant tissues or decomposing organic matter. In view of the reducing nature of aldehyde, reduction of nitrite to N_2 is not unexpected. It was observed in the present studies that when vanillin reacted with nitrite, the N_2 evolved was greater than that found in the control treatment. A model reaction of vanillin and nitrite may be as follows:



The various pigments present in the plant body are made of easily oxidizable aromatic substances. The possibility of the reaction of various pigments with nitrite cannot be ruled out.

The green plant tissues and the decomposing dry organic matter are rich in various amino acids, some of which can easily be oxidized. The higher N_2 evolution reported from tyrosine, tryptophan, and cystine shows that these amino acids can react with nitrite evolving N_2 . The pathway of N_2 evolution from these reactions is not known; it is quite likely that the reaction proceeds according to the classical Van Slyke type of reaction.

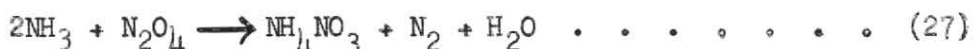


The results of this experiment are in conflict with the findings of Allison and Sterling (1948), Allison and Doetsch (1951), Jones (1951), Allison et al. (1952), and Smith and Clark (1960), who have seriously doubted about the occurrence of such a reaction in soils. In view of

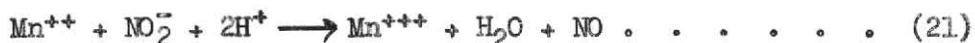
the higher N_2 recorded in this study, the possibility of occurrence of the Van Slyke type of reaction cannot be totally ruled out. In the literature there are some evidences to show that such a reaction might occur in the soil. Barritt (1931) and Gerretsen and DeHoop (1957) are strongly in favor of the occurrence of this reaction in soils.

The contribution of various reducing sugars present in green organic matter and various organic acids in evolution of N_2 needs further research.

The ammonia evolved during the process of decomposition of organic matter and N_2O_4 present in the system from conversion of NO_2^- ($2NO_2^- \rightleftharpoons N_2O_4$) may contribute to N_2 production as suggested by Gray (1958):

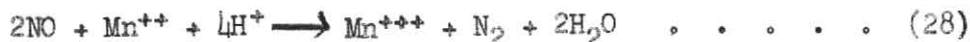


Other nitrite reactions. From the various studies reported here, it is obvious that the nitrite-organic matter reactions are not the only reactions which contribute to the N_2 evolution. In addition to organic matter, some other component of the soil complex may also contribute to N_2 production. The best example is that of the H_2O_2 treated soil wherein the losses of N_2 to the extent of 44 ppm were noted with nitrite addition. In view of the drastic H_2O_2 treatment, obviously the decomposed organic fraction was destroyed completely. N_2 evolution from such a treatment strongly suggests that some soil complex is also contributing to N_2 production. Wullstein and Gilmour (1964) have suggested that in the presence of a reduced form of transition metals, nitrite is converted to NO:

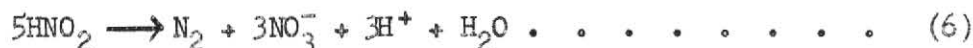


When nitrite can be reduced to NO in the presence of transition metals, the reduction can proceed still further, resulting in formation

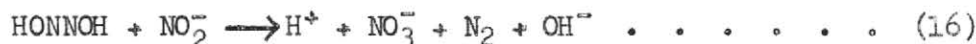
of elemental-N from NO.



The evolution of N_2 from acidified sand-bentonite mixture, which was devoid of all complexities, suggests that self decomposition of nitrite under acid conditions may evolve N_2 as suggested by Thorne (1961):



Nitrite may also react with hyponitrous or hyponitric acid evolving N_2 as suggested by Buchholz (1963):



In addition to these, there could be many more reactions of nitrite with the various components of organic matter and the soil complex resulting in N_2 production.

SUMMARY AND CONCLUSIONS

Experiments were carried out to see the effect of non-incubated and incubated dry and green alfalfa additions on transformations of nitrite to elemental N in Clermont silt loam (pH 5.2), Dayton silty clay loam (pH 4.8), Olympic silty clay loam (4.6), and Miami silt loam (pH 5.6) soils. In another study evolution of N_2 was determined from nitrite reactions with dry alfalfa in a sand-bentonite mixture acidified to various pH levels. Experiments were also conducted to see the extent of N_2 evolution from the reactions of nitrite with various model phenolic compounds, fulvic acids, humic acids, and various amino acids.

From the results of these experiments, the following conclusions are made.

1. Nitrites do react with organic matter, releasing N. The elemental form of N was the major product of nitrite reduction. Some oxides of N, however, also evolved in small quantities.

2. In non-incubated soils the addition of dry alfalfa resulted in a reduction in N_2 production as compared to the treatment receiving no organic matter additions. With incubation the pattern reversed, resulting in a higher production of N_2 with organic matter additions, the only exception being Clermont soil in which no increase in N_2 production was noted with incubation. The differences in evolved N_2 between the treated and non-treated Dayton and Olympic soils became more distinct with longer incubation. The organic matter addition resulted in 20 to 25 ppm more N_2 production than the control treatment.

3. The supplementary experiment involving H_2O_2 treatment to

destroy organic matter confirmed the hypothesis that nitrite-organic matter reactions result in N_2 production. With the destruction of organic matter, the amount of N_2 evolved was reduced to about 50 percent of the soil not treated with H_2O_2 .

4. Green alfalfa reacted more rapidly and effectively with nitrite than dry alfalfa, yielding elemental-N. The evolution of N_2 was higher in non-incubated soils than the incubated soils. Incubation of soils reduced the amount of N_2 produced in most of the cases which could be attributed to rise in pH of the soil with incubation. The pattern of evolution of N_2 with green alfalfa additions was just the reverse of that of dry alfalfa additions; i. e., without any incubation, as compared to the control, the amount of N_2 evolved reduced with dry alfalfa whereas with green alfalfa it increased considerably. With incubation dry alfalfa addition yielded higher N_2 whereas the green alfalfa treatment reduced the N_2 evolved in general.

5. The evolution of N_2 from nitrite-organic matter reaction was further confirmed in studies on a sand-bentonite mixture where the treatment receiving organic matter additions yielded 18 to 20 ppm more N_2 than the treatment receiving no organic matter.

6. In addition to organic matter, some other soil complex also seemed to mediate the reactions of nitrite resulting in evolution of N_2 .

7. The soil pH was found of over-riding importance in nitrite-organic matter reactions, the trend being in high N_2 evolution with increased acidity. The changes occurring in pH with organic matter additions governed, in many instances, the pattern of evolved N_2 . However, in the simple media of the sand-bentonite mixture, the effect of pH remained largely unexhibited, suggesting that the soil complex

may have some complementary effect.

8. Some aromatic compounds like hydroquinone, vanillin, and cinnamic acid, when allowed to react with nitrite in soil, enhanced the amount of N_2 evolved. The N_2 evolved from hydroquinone treatment was twice as much as that of control. This suggests that the various phenolic compounds present in organic matter can react with nitrite evolving N_2 .

9. It was also noticed that some other phenolic compounds like protocatechuic acid, pyrogallol, and phloroglucinol reduced N_2 production when allowed to react with nitrite. The N_2 evolved from these treatments was 13 to 15 ppm as compared to 42 ppm from the control treatment receiving only nitrite, suggesting that these compounds may fix up added nitrite.

10. The reactions of nitrite with humic and fulvic acids resulted in higher N_2 production than was found in the control treatment. Higher evolution of N_2 was noted in the fulvic acid treatment than in the case of humic acid. This suggests that organic matter, at an advanced stage of decomposition, has the ability to react with nitrite resulting in evolution of N.

11. Some amino acids like cystine, tyrosine, and tryptophan also reacted with nitrite, yielding higher amounts of elemental-N as compared to the control treatment receiving only nitrite.

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A P P E N D I X

Nitrate-Nitrogen Determination

Extraction of soil for nitrite and nitrate

1. Weigh out 10 or 20 g soil into 250 ml erlenmeyer flask.
2. Add 100 ml $\text{Ca}(\text{OH})_2$ solution.
3. Place flasks on mechanical shaker and shake for 5 minutes.
4. Let stand for 20 minutes.
5. Filter into numbered 125 ml erlenmeyer flasks, using small funnels. Mix the last portion of soil and extract by swirling the flask quickly and pour the soil and liquid onto the filter paper. Let stand until the extract has stopped dripping from the funnels.
6. Retain filtrate for NO_2 and NO_3 determination.

Reagents:

Phenoldisulfonic acid ($\text{C}_6\text{H}_3\text{OH}(\text{HSO}_3)_2$).--Dissolve 200 g pure white phenol in 1200 ml concentrated H_2SO_4 . Add 600 ml fuming sulfuric acid (15% free SO_3); stir well, heat for 2 hours on hot water bath.

Acetic acid solution.--One part glacial acetic acid and 3 parts distilled water.

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

Ammonium hydroxide.--One part concentrated NH_4OH to one part distilled water.

Standard KNO_3 solution.

Stock.--Dissolve 0.722 g anhydrous KNO_3 and dilute to 1 liter with distilled water. Contains 0.1 mg N/ml.

Standard.--Evaporate 50 ml stock KNO_3 solution to dryness; dissolve residue by rubbing with 2 ml phenoldisulfonic acid, dilute to 500 ml with distilled water. 1 ml = 0.01 mg N.

For preparation of standard curve use 0, 0.5, 1.0, 2.0, 3.5, 8.0, 10.0, 15.0, 20.0 ml of standard KNO_3 solution (1 ml = 0.01 mg N).

Treat and evaporate as outlined in "Procedure."

Procedure

1. (a) Pipette 5 ml of the filtered Ca(OH)_2 extract into 100 ml tall beakers.

(b) Pipette into extra beaker 5 ml of the Ca(OH)_2 extracting solution.
2. Add 5 drops of acetic acid; swirl to mix.
3. Add 0.5 ml of ammonium sulfamate solution; swirl to mix. (Add 1 ml to those samples having high NO_2^- content.)
4. Place on hot plate.
5. When warmed to 60 to 80 C, add 5 drops of 10% NaOH solution; swirl to mix.
6. Let contents evaporate to dryness.
7. Remove beakers from hot plate.
8. Add (from pipette) 2 ml of phenoldisulfonic acid to the beakers in such a way that the residue will be moistened quickly and thoroughly.
9. Rub sides of beakers with a stirring rod to bring the phenoldisulfonic acid in contact with any of the nitrates that may have spattered onto the wall on drying.
10. Let stand about 20 minutes (or longer) or until the residue is dissolved.
11. Add 3/4 ml of distilled water to beaker.
12. Add NH_4OH solution (1:1) to beaker until a permanent yellow color remains (1/4 ml). Perform this step under the fume hood.
13. Let cool. Make further dilution if color is too intense. Record final volume.
14. Mix contents by stirring with stirring rod or swirling carefully and read on electrophotometer after balancing the bridge and setting null adjustment with a blank Ca(OH)_2 solution).

Nitrite-Nitrogen Determination

Reagents:

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

Hydrochloric acid.--1 to 1 dilution of the concentrated acid with distilled water.

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

Sodium nitrite standard

1. Employing assayed NaNO_2 prepare an accurately known solution containing about 0.005 mg of N per ml (Solution A).
2. Dilute 50 ml of above solution to 100 ml in volumetric flask. (Solution B).
3. With a volumetric pipette measure 0.5, 1.0, 1.5, 1.8 ml of A, and 1, 2, 4 ml of B into 50 ml volumetric flasks.
4. Add 20 ml of Ca(OH)_2 extracting solution (filtered) to each.
5. Treat as outlined in steps 2 to 7 of "Procedure."
6. Prepare graph.
7. Keep standard sodium nitrite solution tightly stoppered, in refrigerator.

Procedure:

1. (a) Pipette 20 ml of the filtered Ca(OH)_2 soil extract into 50 ml volumetric flasks. Use smaller aliquot (2 to 5 ml) if NO_2^- content of sample is high. Make up to 20 ml with Ca(OH)_2 solution.
 (b) Pipette 20 ml of the Ca(OH)_2 extracting solution into an extra 50 ml volumetric flask.
 (c) Fill burettes with 50% HCl, 0.2% sulfanilamide and coupling reagent.
2. From a burette add 1 ml of 50% HCl.
3. From a burette add 5 ml of 0.2% sulfanilamide solution.
4. Wait 3 minutes.

Note: Steps 2 and 3 can be performed on 10 samples within the 3 minutes as follows: Add the HCl and the sulfanilamide to

the first sample, then set the time clock for 3 minutes. Then quickly add the HCl and sulfanilamide to the other 9 samples in succession.

5. At the end of the 3-minute interval, begin adding 1 ml of the coupling reagent to each sample, timing the additions so that each sample will have remained at 3 minutes after the addition of sulfanilamide.
6. Make up to volume with distilled water.
7. If the color is too dark, take smaller quantities of aliquot and develop color.
8. Read on electrophotometer, using the blank to set the null point. Use 525-B filter or read at 550 m μ wavelength.

Sodium Nitrite

Tests for identity:

Dry about 1 g sodium nitrite to constant weight over sulfuric acid, weigh accurately in a stoppered weighing-bottle, dissolve the salt in a volumetric flask with sufficient distilled water to make 100 ml and add 10 ml of this solution, from a pipette, to a mixture of 40 ml of tenth-normal potassium permanganate, 100 ml of distilled water, and 5 ml of sulfuric acid. When adding the sodium nitrite solution, immerse the tip of the pipette beneath the surface of the permanganate mixture. Warm the liquid to 40 C, allow it to stand for 5 minutes, and add 25 ml of tenth-normal oxalic acid or sodium oxalate. Heat the mixture to about 80 C and titrate with tenth-normal potassium permanganate. Each ml of tenth-normal potassium permanganate is equivalent to 0.003450 g NaNO₂.

Storage:

Preserve sodium nitrite in air-tight containers in refrigerator.

Analysis of Alkaline Permanganate Traps for Oxides of Nitrogen

The oxides of nitrogen that dissolve in the traps will be oxidized to nitrate by the 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 $\text{Na}_2\text{C}_2\text{O}_4$ in 7 N H_2SO_4

Place about 1200 ml distilled water in a 2 liter beaker. Add carefully 390 ml concentrated H_2SO_4 . Weigh out and add 160.6 g $\text{Na}_2\text{C}_2\text{O}_4$. 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 200 g reagent grade NaOH and dissolve in about 800 ml distilled water. Allow to cool; transfer to 1 liter volumetric flask and make to volume.

Procedure:

Wash the entire contents (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 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 spectrophotometer.

Standard curve and calculations:

KNO_3 standard - 200 $\mu\text{g N/ml}$

1.44 g KNO_3 made up to 1 liter volume.

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 $\mu\text{g N}$ per 50 ml. Read on spectrophotometer and plot curve.

Determine from the curve the ratio $\mu\text{g N/optical density}$ (inverse of slope if optical density is plotted on the y axis and μg on the x).

$$\frac{\text{Ratio x opt. den. x 20}}{1000} = \frac{\text{mg N}}{\text{trap}}$$

if 2 ml aliquot was used

$$\frac{\text{Ratio x opt. den. x 50}}{1000} = \frac{\text{mg N}}{\text{trap}}$$

Ratio determined by Reuss (1963) was 128.

Notes

Do not use excess water to wash the trap solution into the beaker or for washing the precipitate as the total volume must be less than 1000 ml. The destructure of KMnO_4 by CO_2 requires high acidity, and incomplete reaction at this point is often the result of insufficient acidity. If the traps are to be exposed to large amounts of 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 14. Mineral and gaseous nitrogen recovered from Clermont silt loam treated with 1 percent dry alfalfa and incubated for 0, 3, 6, and 14 days prior to the reaction with 200 ppm nitrite-N added as NaNO_2

Treatment	Time		Mineral-N		Oxides of N ppm	Elemen- tal N ppm	Total N ppm
	Incubation days	Reac- tion hours	NO_2^- -N	NO_3^- -N			
			ppm	ppm			
Soil + NO_2^-	0	24	80	18.6	7.7	42.4	148.7
Soil + NO_2^- + alfalfa	0	24	124	15.5	6.6	36.2	182.3
Soil + NO_2^-	0	48	62	34.2	8.8	55.0	160.0
Soil + NO_2^- + alfalfa	0	48	75.2	21.0	7.1	43.0	146.3
Soil + NO_2^-	3	24	124	9.3	7.7	27.2	143.2
Soil + NO_2^- + alfalfa	3	24	112	24.8	5.5	25.0	167.3
Soil + NO_2^-	3	48	112	21.0	8.25	36.0	177.2
Soil + NO_2^- + alfalfa	3	48	80	34.0	6.6	26.0	146.6
Soil + NO_2^-	6	24	94	31.1	6.6	18.0	149.7
Soil + NO_2^- + alfalfa	6	24	94	19.3	5.5	16.0	134.8
Soil + NO_2^-	6	48	81	40.4	7.7	35.0	164.1
Soil + NO_2^- + alfalfa	6	48	75	12.4	6.05	24.0	117.8
Soil + NO_2^-	14	24	75.2	15.5	5.5	36.0	181.7
Soil + NO_2^- + alfalfa	14	24	137.7	9.3	4.4	11.8	202.8
Soil + NO_2^-	14	48	43.8	46.6	7.15	41.0	138.5
Soil + NO_2^- + alfalfa	14	48	72.6	15.5	5.5	13.0	106.6

Table 15. Mineral and gaseous nitrogen recovered from Dayton silty clay loam treated with 1 percent dry alfalfa and incubated for 0, 3, 14, and 35 days prior to the reaction with 200 ppm nitrite-N added as NaNO_2

Treatment	Time		Mineral-N		Oxides of N ppm	Elemen- tal N ppm	Total N ppm
	Incubation	Reac-	NO_2^- -N	NO_3^- -N			
	days	tion hours	ppm	ppm			
Soil + NO_2^-	0	24	27.0	46.6	11.5	70.1	155.2
Soil + NO_2^- + alfalfa	0	24	40.0	31.1	9.6	63.7	143.8
Soil + NO_2^-	0	48	26.0	52.8	14.8	76.7	170.0
Soil + NO_2^- + alfalfa	0	48	30.0	55.9	19.2	71.4	176.5
Soil + NO_2^-	3	24	56.4	77.7	13.0	55.5	202.6
Soil + NO_2^- + alfalfa	3	24	62.6	31.1	14.3	60.0	208.0
Soil + NO_2^-	3	48	25.0	99.5	17.0	65.1	206.5
Soil + NO_2^- + alfalfa	3	48	18.8	37.3	20.3	74.0	162.4
Soil + NO_2^-	14	24	48.8	24.8	10.4	57.3	141.3
Soil + NO_2^- + alfalfa	14	24	43.8	52.8	9.0	62.2	167.8
Soil + NO_2^-	14	48	42.6	105.7	15.9	59.6	223.8
Soil + NO_2^- + alfalfa	14	48	21.2	65.3	12.6	79.6	178.7
Soil + NO_2^-	35	24	28.8	108.0	5.5	39.1	181.4
Soil + NO_2^- + alfalfa	35	24	26.8	46.6	5.5	67.8	146.7
Soil + NO_2^-	35	48	18.8	121.0	6.6	50.2	196.6
Soil + NO_2^- + alfalfa	35	48	13.4	99.5	7.7	75.4	196.0

Table 16. Mineral and gaseous nitrogen recovered from Olympic silty clay loam treated with 1 percent dry alfalfa and incubated for 0, 3, 14, and 35 days prior to the reaction with 200 ppm nitrite-N added as NaNO_2

Treatment	Time		Mineral-N		Oxides of N ppm	Elemen- tal N ppm	Total N ppm
	Incubation days	Reac- tion hours	NO_2^- -N	NO_3^- -N			
			ppm	ppm			
Soil + NO_2^-	0	24	30.0	62.2	11.5	82.3	186.0
Soil + NO_2^- + alfalfa	0	24	42.0	65.3	7.7	75.2	190.2
Soil + NO_2^-	0	48	18.8	71.5	13.2	86.8	190.3
Soil + NO_2^- + alfalfa	0	48	22.0	80.7	14.8	83.7	201.2
Soil + NO_2^-	3	24	34.0	46.6	6.0	80.0	166.6
Soil + NO_2^- + alfalfa	3	24	38.8	59.1	16.5	85.7	200.1
Soil + NO_2^-	3	48	18.8	84.0	16.3	83.3	202.6
Soil + NO_2^- + alfalfa	3	48	12.4	99.5	33.5	91.6	237.0
Soil + NO_2^-	14	24	86.0	21.8	12.1	76.2	196.7
Soil + NO_2^- + alfalfa	14	24	62.6	15.6	10.4	87.0	175.6
Soil + NO_2^-	14	48	27.4	84.0	13.2	83.3	207.9
Soil + NO_2^- + alfalfa	14	48	16.2	87.1	24.2	86.4	213.9
Soil + NO_2^-	35	24	21.2	31.1	5.5	73.0	130.8
Soil + NO_2^- + alfalfa	35	24	34.8	49.7	5.8	89.6	179.9
Soil + NO_2^-	35	48	16.0	46.6	6.6	82.3	151.5
Soil + NO_2^- + alfalfa	35	48	13.2	52.8	8.5	103.4	177.9

Table 17. Mineral and gaseous nitrogen recovered from Dayton silty clay loam treated with green alfalfa at 1 percent level on dry weight basis and incubated for 0, 3, 14, 25, and 35 days prior to the reaction with 200 ppm nitrite-N added as NaNO_2

Treatment	Time		Mineral-N		Oxides of N ppm	Elemen- tal N ppm	Total N ppm
	Incu- bation days	Reac- tion hours	NO_2^- -N	NO_3^- -N			
			ppm	ppm			
Soil + NO_2^-	0	24	81.4	31.1	12.6	76.0	132.7
Soil + NO_2^- + alfalfa	0	24	87.0	37.3	8.8	85.6	218.7
Soil + NO_2^-	0	48	44.0	62.2	14.8	84.0	205.0
Soil + NO_2^- + alfalfa	0	48	56.0	52.8	11.5	105.0	225.3
Soil + NO_2^-	3	24	56.4	55.6	4.4	58.3	174.7
Soil + NO_2^- + alfalfa	3	24	81.4	31.1	3.3	67.6	183.4
Soil + NO_2^-	3	48	50.0	77.0	8.2	66.3	201.5
Soil + NO_2^- + alfalfa	3	48	73.2	46.0	6.6	82.6	207.8
Soil + NO_2^-	14	24	56.4	77.7	7.7	56.7	198.5
Soil + NO_2^- + alfalfa	14	24	64.0	40.9	4.9	42.7	151.6
Soil + NO_2^-	14	48	28.4	83.9	13.2	65.3	190.8
Soil + NO_2^- + alfalfa	14	48	56.4	46.6	5.5	45.2	153.7
Soil + NO_2^-	25	24	42.8	83.7	8.8	45.2	180.5
Soil + NO_2^- + alfalfa	25	24	15.2	62.2	5.5	32.4	115.3
Soil + NO_2^-	25	48	16.0	93.7	8.8	56.2	174.7
Soil + NO_2^- + alfalfa	25	48	9.4	77.7	9.3	40.1	136.5

Table 17. Continued

Treatment	Time		Mineral-N		Oxides of N	Elemen- tal N	Total N
	Incubation	Reac- tion	NO ₂ ⁻ -N	NO ₃ ⁻ -N			
	days	hours	ppm	ppm			
Soil + NO ₂ ⁻	35	24	28.8	108.0	5.5	39.1	181.4
Soil + NO ₂ ⁻ + alfalfa	35	24	48.0	62.2	2.7	35.6	148.5
Soil + NO ₂ ⁻	35	48	18.8	121.2	6.6	50.2	196.8
Soil + NO ₂ ⁻ + alfalfa	35	48	13.4	93.3	8.2	46.0	160.9

Table 18. Mineral and gaseous nitrogen recovered from Miami silt loam treated with dry or green alfalfa at 1 percent level on dry weight basis and incubated for 0 and 21 days prior to the reaction with 200 ppm nitrite-N added as NaNO_2

Treatment	Time		Mineral-N		Oxides of N	Elemen- tal N	Total N
	Incubation	Reac- tion	NO_2^- -N	NO_3^- -N			
	days	hours	ppm	ppm			
Soil + NO_2^-	0	24	-	-	-	27.2	-
Soil + NO_2^- + dry alfalfa	0	24	-	-	-	22.1	-
Soil + NO_3^- + green alfalfa	0	24	-	-	-	39.5	-
Soil + NO_2^-	0	48	112.8	21.7	3.3	36.9	174.7
Soil + NO_2^- + dry alfalfa	0	48	94.7	31.1	5.0	32.3	163.1
Soil + NO_2^- + green alfalfa	0	48	81.4	18.6	6.6	48.2	157.5
Soil + NO_2^-	21	24	106	15.5	2.2	30.9	154.6
Soil + NO_2^- + dry alfalfa	21	24	103	6.2	3.8	23.8	136.8
Soil + NO_2^- + green alfalfa	21	24	158	3.1	1.6	9.9	172.6
Soil + NO_2^-	21	48	77	40.0	4.4	44.2	165.6
Soil + NO_2^- + dry alfalfa	21	48	94	15.5	4.9	34.3	148.7
Soil + NO_2^- + green alfalfa	21	48	116	6.2	3.3	12.6	138.1