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THE EFFECT OF TEMPERATURE AND MOISTURE ON NITRIFICATION OF APPLIED
AMMONIACAL FERTILIZER IN A NONCALCAREOUS SOIL

by

Merwin Allen Stevens

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

of

MASTER OF SCIENCE

in

Soil Fertility

UTAH STATE UNIVERSITY
Logan, Utah

1961

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This work is dedicated to my wife, Hermese, who has spent many hours typing and proof reading.

Merwin Allen Stevens

TABLE OF CONTENTS

	Page
Introduction	1
Review of literature	3
The nitrifying bacteria	3
Moisture effects on nitrogen transformation	5
Temperature effects on nitrogen transformation	7
Nitrogen loss	9
Experiment I. Incubation with decreasing moisture	13
Methods and procedure	13
Results and discussion	20
Summary	24
Experiment II. Incubation with constant moisture	26
Methods and procedure	26
Results and discussion	27
15 bars moisture tension	27
0.3 bars moisture tension	31
Conclusions	35
Summary	38
Experiment III. Incubation with decreased temperature	39
Methods and procedure	39
Results and discussion	40
Summary	43
General summary and conclusions	44
Literature cited	46

LIST OF TABLES

Table	Page
1. Chemical and physical characteristics of Wysaro clay . .	14
2. Changes in ammonium nitrogen, nitrite nitrogen, and nitrate nitrogen with incubation at 25° C and 35° C with decreasing moisture percentage	23
3. Changes in ammonium nitrogen, nitrite nitrogen, and nitrate nitrogen with incubation at 25° C and 35° C with 15 bars moisture tension	28
4. Changes in ammonium nitrogen, nitrite nitrogen, and nitrate nitrogen with incubation at 25° C and 35° C with 0.3 bars moisture tension	32
5. Changes in ammonium nitrogen, nitrite nitrogen, and nitrate nitrogen with incubation at 2° C and 0.3 bars moisture tension	42

LIST OF FIGURES

Figure	Page
1. Bottles containing the humidifying solutions through which the air passes before reaching the plastic tubes inside the bath	17
2. Partially filled bath showing the capillaries connected to the air manifolds and to the samples	18
3. A capillary with its protective shield	19
4. Changes in ammonium nitrogen, nitrate nitrogen, and percent water with incubation at 25° C	21
5. Changes in ammonium nitrogen, nitrate nitrogen, and percent water with incubation at 35° C	22
6. Changes in ammonium nitrogen, nitrate nitrogen, and their sum with incubation at 25° C and 15 bars moisture tension	29
7. Changes in ammonium nitrogen, nitrate nitrogen, and their sum with incubation at 35° C and 15 bars moisture tension	30
8. Changes in ammonium nitrogen, nitrate nitrogen, and their sum with incubation at 25° C and 0.3 bars moisture tension	33
9. Changes in ammonium nitrogen, nitrate nitrogen, and their sum with incubation at 35° C and 0.3 bars moisture tension	34
10. Changes in nitrate nitrogen with incubation at 25° C and 35° C with 0.3 and 15 bars moisture tension	36
11. Changes in the sum of ammonium nitrogen plus nitrate nitrogen with incubation at 25° C and 35° C with 0.3 and 15 bars moisture tension	37
12. Changes in ammonium nitrogen, nitrate nitrogen, nitrite nitrogen, and their sum with incubation at 2° C and 0.3 bars moisture tension	41

INTRODUCTION

The importance of nitrogen in world agriculture has been known for many years. But in the past few decades the enormity of the problem of nitrogen economy has been recognized. Along with the recognition of this problem there has developed a great increase in the use of nitrogen fertilizers. Coupled with the increased use of nitrogen there has come about an increasing awareness of the problem involved in the use of nitrogen fertilizers.

In recent years the use of ammonium fertilizers to improve soil fertility has increased a great deal. The use of these fertilizers has resulted in the possibility of lengthening the period from the date of the fertilizer application to the time of utilization by the crop.

This advanced application has many advantages, but it also presents many problems. Is this advanced application economically feasible or is the applied nitrogen lost through leaching and volatilization in the ensuing period between application and utilization? If this applied ammonia is needed for plant growth does it become available at the rate at which it is needed? How do temperature and moisture affect the loss of nitrogen and the rate at which it becomes available?

This study is an attempt to answer some of the questions that heretofore have not been answered completely. A previous study of this type, at this institution, was conducted on a calcareous soil. It is hoped that this study on a noncalcareous will supplement the answers found in the previous study.

The problems coupled with nitrogen economy are varied and

numerous. This study should aid in answering some of these questions.

REVIEW OF LITERATURE

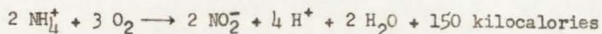
The Nitrifying Bacteria

Despite its great importance in the field of agriculture and despite the intriguing question of the intermediary metabolism involved, the process of nitrification has received relatively little attention and that only spasmodically. When Pasteur demonstrated in 1862 the microbiological nature of the oxidation of alcohol to acetic acid, he suggested that the oxidation of ammonia might have a similar origin (Frobisher, 1957). The next advance did not come until 1916 when Meyerhof undertook an extensive investigation of the respiratory activity of these organisms and a study of the effects of inhibitors (Waksman, 1957). A variety of reports on the subject of nitrification made their appearance in the next thirty years but no serious attempt to study the metabolic activities of these organisms was made until the advent of the soil perfusing apparatus in 1944. Since then several reports of results obtained using this technique have been published and they obviously indicate a renewed interest in the subject of soil nitrification and in particular in the biochemical mechanisms involved. The first studies on the process of nitrification using the soil perfusing apparatus confirmed it as a comparatively slow process accomplished entirely by microorganisms (Quastel and Scholefield, 1951).

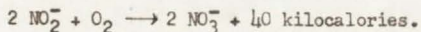
In a study on nitrate accumulation Calder (1957) concluded that only when microbiological processes are permitted does nitrate status improve. Waksman and Madhok (1937) concluded that the formation of nitrate in soil is the biological oxidation of ammonia to nitrite and of nitrite to nitrate and must still be considered as the all-important

process in the formation of nitrate in soil.

The nitrifying bacteria were first isolated in pure culture by Winogradsky in 1891. The nitrifying bacteria are autotrophic, that is to say, they are entirely dependent for energy on the oxidation reaction they carry out. They cannot use the breakdown of organic materials as a source of energy, as the ordinary heterotrophic bacteria do. For the ammonium-oxidizers the source of energy is the reaction:



and for the nitrite oxidizers:



In 1895 Godlewski found that the only source of carbon which the nitrifiers can use for the building up of their cell substances is carbon dioxide (Meiklejohn, 1953).

The ammonia oxidizers are of the genus Nitrosomonas. The nitrite oxidizers are of the genus Nitrobacter, of which there appears to be two species. There also appears to be two species of Nitrosomonas.

Heterotrophic bacteria which produce small quantities of nitrite from ammonia have been described by several workers. The amounts are very small. An actinomycete producing very small quantities of nitrite from ammonia has recently been described (Isenberg, Schatz, and Hunter, 1952). It has been claimed that the united activities of such organisms account for the nitrification observed in soil, but Nitrosomonas, or a related autotrophic organism, has been found in every soil where it has been seriously looked for, and in which nitrification takes place. Since Nitrosomonas produces much greater amounts of nitrite than any of the heterotrophs, the hypothesis that the latter are important nitrifiers seems unlikely. The only bacteria which have been adequately described, and are certainly known to produce nitrite in quantity from

ammonium ion, or to produce nitrate by oxidation are the autotrophic species described by Warington and the Frandlands, and isolated by Winogradsky (Meiklejohn, 1953).

Lees and Quastel (1946) studied the biochemistry of nitrification using a soil perfusing apparatus. They concluded that when nitrification takes place in a soil perfused with ammonium sulfate, little or no nitrification takes place in the perfusate or soil solution. Nitrification in soil takes place wholly at the soil surfaces. It occurs at those areas of the soil surface where ammonium ion is combined or adsorbed. The rate of nitrification in soil is (a) proportional to the fraction of the total ammonium ion which is adsorbed or combined in the base-exchange complexes of the soil, and (b) apparently independent of the concentration of ammonium ion in the soil solution. The rate of nitrification in a soil is increased by the addition of sterile soil, the amount of increase being roughly proportional to the base exchange capacity of the added sterile soil. Addition of sand leads to no increase in the rate of nitrification. It was concluded that the rate of nitrification in soil is a function of the base-exchange capacity of a soil.

Moisture Effects on Nitrogen Transformation

Although a considerable volume of work has been published during the past thirty or forty years on the subject of ammonia and nitrate nitrogen formation and accumulation in soils under controlled laboratory conditions, surprisingly little clear information of this type is available for close soil moisture intervals in the wilting-point moisture range and below. Results such as those of Greaves and Carter (1920) and Russel, Jones and Bahrt (1925) have shown that no nitrate nitrogen formation occurs in soils at the air dry soil moisture content,

but that at soil moisture values which are apparently slightly above the permanent wilting percentage, active nitrification takes place. Greaves and Carter (1920) provide data which show that ammonia nitrogen formation takes place in soils at moisture levels below the permanent wilting percentage, i.e., heavy textured soils held at 10 percent of the water-holding capacity. The writer was able to find very little published work concerning the study of ammonia and nitrate nitrogen formation simultaneously under conditions of soil moisture near the permanent wilting percentage. Justice and Smith (1961) found in their study of nitrification under reduced moisture conditions that ammonia oxidation proceeded slowly at moisture as low as 70 bars tension.

Practically speaking, when one is concerned with the inorganic forms of soil nitrogen in the topsoil, the soil moisture range from the field capacity percentage down to values well below the permanent wilting percentage is of the greatest importance. It is within this moisture range that the nitrate nitrogen which is produced will remain in the top two or three feet of the soil profile where the roots of a crop are most active, i.e., there is little or no leaching loss (Robinson, 1957).

In a recent study Calder (1957) reported that between the roughly defined limits of soil air dryness (10 to 15 percent moisture) and waterlogging (45 percent moisture) there was a broad range of soil-moisture conditions under which, in his experiments, large quantities of nitrate appeared in the soil. The failure of any well-defined optimum moisture range to emerge may be interpreted in two ways: either it is so limited as not to have been achieved by the techniques used, or it does not exist. Evidence has been given showing variability in conditions in soil columns, and if the hypothetical moisture optimum

represents a sensitive and highly unstable condition it may not have been produced, except transitorily, in small zones within the soil mass. If, on the other hand, no optimum exists, the only other possibility is that nitrate production occurs in response to change in moisture status.

The nitrate productivity of the soil, stored dry in the laboratory for three years, was almost unimpaired, and the organisms involved in nitrate production must be robust forms capable of withstanding long drought. This is known to be true of the classical nitrifiers, Nitrosomonas and Nitrobacter (Calder, 1957).

Robinson (1957) reported that laboratory incubation studies with topsoil samples of the Kikuyu red loam coffee soil have shown that active nitrification of the natural soil nitrogen stops at soil moisture level just below the permanent wilting percentage. At moisture levels between the permanent wilting and field capacity percentages ammonification and nitrification took place in a recognized manner.

Temperature Effects on Nitrogen Transformation

The literature is considerably more voluminous with respect to temperature effects in nitrification than it is in relation to moisture effects. Stojanovic and Broadbent (1956) studied nitrogen transformation, under laboratory conditions, at 5 and 10° C. Ammonification occurred at both temperatures. The rate of formation was approximately twice as high at 10° at it was at 5° C.

There has been considerable work reported in relation to the optimum temperature for nitrification. Sabey et al. (1956) studied the general relationships between temperature and nitrification rates in given soils. Nitrification rate decreased with diminution in soil temperature; however, the relationship was not linear over the entire temperature range. Complete inhibition was not attained until soil

temperatures approached the freezing point. Only slight oxidation of ammonium occurred, however, under field conditions in soils that were fertilized after soil temperatures had decreased below 50° F. Their field data showed that nitrification proceeded rather rapidly in early fall and that complete thermal inhibition did not occur until soil temperatures approached the freezing point. However, when the soil temperature decreased below 50° F the rate of nitrification was slow and the possibility of oxidation of large amounts of ammonium seem remote, especially if temperatures continue to decline with the advancing fall season.

Frederick (1956) found that the increase in the rate of nitrification with an increase in temperature was quite rapid, with the greatest change occurring between 7 and 15° C. The nitrification rate at temperatures near freezing was still appreciable in soils which had favorable conditions for nitrification. When some unfavorable condition such as pH or moisture existed, as indicated by a considerable lag period, appreciable nitrification was not found below 7° C.

Meiklejohn (1953) reported that nitrification did not proceed at 40° C in a tropical Uganda soil, and Warrington found a similar limit with English garden soil. It is usually found that the lower temperature limit at which nitrification proceeds is influenced by the soil used (Frederick, 1956).

Soils rarely exceed 35° C under natural conditions when sufficient moisture is present to allow biological activity. Higher temperatures are not generally studied.

Tyler et al. (1959) concluded that lowering the temperature from 75 to 45° F materially decreased the rate of the nitrification process. At 45° F nitrification still proceeded at a moderate rate. No minimum

temperature for nitrification was suggested, since this apparently depended on the nature of the soil and ammonium ion concentration. A greatly reduced though still measurable rate of nitrification was observed in California soils as low as 37° F.

Nitrogen Loss

The factors affecting the loss of nitrogen through volatilization and leaching have been studied in some detail. Bremner and Shaw (1958) studied the factors affecting denitrification by determining the loss of nitrogen from soil under various conditions by total nitrogen analysis. It was found that the rate of denitrification of nitrate in soil was dependent upon various factors such as the pH, temperature and water content of the soil, and that, under conditions conducive to denitrification, 80 to 86 percent of nitrate nitrogen added to Rothamstead soils was lost by denitrification in five days. The rate of denitrification was greatly affected by the pH of the soil. The rate of denitrification increased rapidly with rise in temperature from 2 to 25° C. The optimum temperature for denitrification was about 60° C. The degree of water saturation of the soil had a profound influence on the rate of denitrification. Below a certain moisture level practically no denitrification occurred; above this level denitrification increased rapidly with increase in moisture content. The critical moisture level was about 60 percent of the water-holding capacity of the soil. No loss of nitrogen by denitrification could be detected when moist soils were incubated with or without nitrate and glucose and were aerated continuously during incubation. It was shown that the rate of denitrification in soil depends upon the amount and type of organic material present. The results obtained support the view that denitrification occurs only when the supply of oxygen required by the soil micro-organism is restricted.

Martin and Chapman (1951) studied the loss of nitrogen through volatilization as ammonia from surface-fertilized soils. It was found that 9 to 51 percent of the nitrogen added in the form of ammonium hydroxide to soils ranging from pH 4.5 to 8.0 was lost; 1 to 27 percent was lost from ammonium sulfate applied to the same soils. The pH of the soil was important in determining losses. The moisture content of the soil had little effect except that evaporation of water was necessary for appreciable volatilization of ammonia from the soil. Losses increased with temperature increase.

Wahhab et al. (1957) found that twice as much ammonia volatilized from a sandy soil than from a sandy loam soil. They found that negligible losses of ammonia occurred from air dried soil. Maximum loss of ammonia took place at 25 percent moisture saturation. The losses subsequently decreased with increase in moisture percentage. They concluded that a relationship exists between loss in moisture and loss in ammonium nitrogen. Jewitt (1942) found that gaseous ammonia was lost in considerable quantities when ammonium sulfate was applied to certain alkaline Sudan soils. He concluded that the loss takes place over long periods from the moist soil at a rate greatly influenced by the rate of application of the fertilizer and little influenced by the moisture content except when this approaches air-dry levels.

Loewenstein et al. (1957) concluded that denitrification and nitrification in the soil proceeded simultaneously. Either nitrates produced in the aerobic soil area moved to oxygen-poor regions and became subject to denitrification or the aerobic soil area becomes anaerobic. It is also possible that an aerobic area may have become anaerobic as a result of rapid oxygen consumption or because of concurrent carbon dioxide evolution by the soil microflora. In either of

these two instances, nitrification would likely be followed by denitrification. Broadbent et al. (1952) found that denitrification of added nitrate was inversely related to partial pressure of oxygen, but was of appreciable magnitude even under seemingly fully aerobic conditions.

Not only are nitrates more susceptible to leaching, but they are also subject to loss through denitrification under a wide variety of soil conditions. The findings of Bizzell (Broadbent et al., 1952) are particularly relevant to this point. In lysimeter experiments where sodium nitrate was used as a fertilizer, losses due to unknown causes, presumably volatilization, were found to be substantially larger than those due to leaching.

In a study on the losses of nitrous oxide from the soil, Arnold (1954) found that an almost immeasurably slow release of nitrous oxide can take place at moderate moisture contents (0.1 bars moisture tension), while under extremely wet conditions large releases of the gas are likely. It is to be expected that nitrous oxide evolution would be a seasonal phenomenon related to the available-nitrogen content of the soil, the degree of aeration, and probably the supply of easily decomposable organic matter, the soil pH, and the temperature, to mention the most obvious factors. From the soil-fertility side of the question it does not matter whether the gaseous escape is as nitrous oxide or molecular nitrogen. Insufficient aeration, caused by enough excess moisture to render the environment reducing, can lead to a gaseous escape of nitrous oxide and presumable molecular nitrogen. It is probably enough that the conditions tend towards the anaerobic. A state may be visualized in which the rate of oxygen consumption exceeds replacement by diffusion to the most inaccessible centers. Such statements as Meiklejohn's (1953), "it is very difficult to eliminate

denitrification by means of increased aeration", provide further evidence of the difficulty of avoiding conditions favoring denitrification. Winogradsky (Arnold, 1954) states, even when a soil is far from saturated with moisture, anaerobic organisms find conditions favorable for their development at the very surface of the soil. When an environment is truly anaerobic, molecular nitrogen may be the sole product of denitrification (Jones, 1951).

Data (Arnold, 1954) obtained from infra-red spectra of soil airs collected both in laboratory experiments and in the field indicate that nitrous-oxide evolution is a factor of considerable significance to the nitrogen economy of soils. Soils which are approaching saturation with moisture rapidly release large amounts of their available nitrogen as nitrous oxide; at lower moisture contents very slow evolution of the gas can take place.

EXPERIMENT I. INCUBATION WITH DECREASING MOISTURE

Methods and Procedure

This study was undertaken to determine the minimum moisture at which nitrification of applied ammonium fertilizer would take place in a noncalcareous soil.

The soil used throughout this study was Wysaro clay. The physical and chemical characteristics of this soil are outlined in table 1. The sample used in this study was taken from Sheridan, Wyoming.

The moisture percentage corresponding to 15 bars moisture tension (approximately permanent wilting percentage) was used as a basis for the moisture additions used in this study. The moisture level in the soil at the beginning of incubation was 18 percent, 2.6 percent above the 15 bars tension level. This moisture percentage was determined by the use of a pressure membrane apparatus.

The soil, treated with 150 ppm of nitrogen as ammonium sulfate, was incubated under varying temperature and moisture conditions and sampled at various intervals to test for different forms of inorganic nitrogen and moisture.

Ammonium sulfate was dissolved in water and added to 33.3 grams of soil in 250 milliliter Erlenmeyer flasks with sufficient water to bring the moisture to 18.0 percent. Care was taken to make sure that the samples were uniformly mixed. After the ammonium sulfate and water had been added, the soil was thoroughly mixed with a spatula. The samples were mixed and put into the constant temperature baths as soon as possible.

Table 1. Chemical and physical characteristics of Wysaro clay

pH (paste)	6.5
pH ₅	6.6
Total soluble salts ^a (percent)	0.05
Electrical conductivity of saturation extract (mmhos)	0.64
Cation exchange capacity (me./100 gms.)	29.5
Potassium ^b (ppm)	450
Organic carbon (percent)	1.54
Organic matter (percent)	2.65
Nitrogen ^c (percent)	0.151
Carbon-nitrogen ratio	11.0
P ₂ O ₅ ^d (lbs. per acre)	56
Air dry moisture (percent)	2.7
Saturation percentage	51
Moisture tensions	
0.3 bars (percent)	25.0
15 bars (percent)	15.4
Particle size distribution ^e (percent)	
Sand	18.5
very coarse sand (2-1 mm.)	0
coarse sand (1-0.5 mm.)	0.3
medium sand (0.5-0.25 mm.)	1.4
fine sand (0.25-0.10 mm.)	5.0
very fine sand (0.10-0.05 mm.)	11.8
Silt (0.05-0.002 mm.)	37.9
Clay (0.002 mm.)	43.6
Texture	Clay

^aPaste^bAmmonium acetate soluble^cKeldahl^dSodium bicarbonate extraction^ePipette method

The apparatus described by Justice and Smith (1961) was used to control temperature and moisture in this study. The moisture was not actually controlled but was allowed to drop from the 18.0 percent which was in the soil when it was introduced into the tanks. This was done by producing a pressure differential in the system which would cause a moisture deficit in the air passing over the soil samples. The incoming air was saturated under a known pressure and then released to atmospheric pressure when passing over the samples. The formula:

$$R. H. = P_f/P_i \quad (1)$$

where R. H. = relative humidity, P_f = the final total pressure (atmospheric) and P_i = the pressure under which the air was humidified, was used as a basis for the moisture control. This formula, making use of a fundamental principle described by the gas laws, is given by Bartholomew and Broadbent (1949).

The air was humidified under a pressure of 6 centimeters of mercury. Using 910 centimeters of water as the barometric pressure for this location, the calculated relative humidity to the nearest percent was 92. Using the formula:

$$\Psi = RT \ln R. H. \quad (2)$$

where Ψ = moisture tension, $R = 8.314 \times 10^7$ ergs/deg/mole, T = absolute temperature and R. H. = relative humidity, the calculated moisture tension would be 115 bars. Using this moisture tension would allow for a moisture drop and tension increase in the soil in an attempt to determine the minimum moisture percentage at which nitrification would occur.

Two metal soil storage tanks (24 by 36 inches in size) were insulated, lined with galvanized iron and painted black. Water pumps

were mounted on the tanks to circulate the water. A 500 watt heating element was mounted in the center of each tank. To control the temperature a bimetallic thermoregulator was connected to a supersensitive relay.

The moisture was controlled by passing the air through distilled water in 2-liter bottles and then through three large plastic tubes (4 inches in diameter) which were mounted in the bottom of the tanks (figure 1). These tubes were filled to one-half capacity with distilled water. As the air passed slowly over the water in these tubes it became saturated. From these tubes the air was directed by rubber tubing into manifolds which in turn were connected by a capillary tube to the 250 milliliter Erlenmeyer flasks (figure 2).

The capillary tubes were made by drawing a 1.0 millimeter capillary tubing to a very small opening. These were calibrated so that at 6 centimeters of mercury pressure the air flow into the flasks would be approximately 5 milliliters per minute. After calibration the capillary tube was inserted into a protective shield (figure 3). The large plastic tubes, used to insure moisture equilibrium of the air, the manifolds, and the 250 milliliter Erlenmeyers were all submerged in the water of the baths.

Each tank had a capacity of 54 samples. The samples were tied in the tank by means of rubber bands and paper clips. The rubber band was placed over the glass tubing in the rubber stopper in the Erlenmeyer and held to a wire grating in the bottom of the tank by means of a paper clip (figure 2).

Two tanks were operated simultaneously, one at 25° C and one at 35° C. The pressure in the system was controlled by means of manometers



Figure 1. Bottles containing the humidifying solutions through which the air passes before reaching the plastic tubes inside the bath

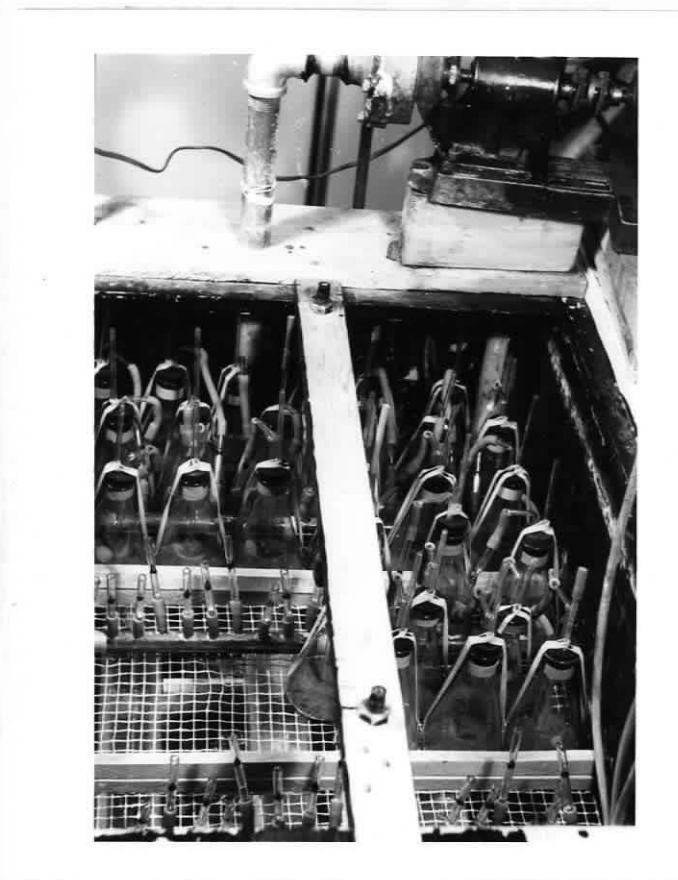


Figure 2. Partially filled bath showing the capillaries connected to the air manifolds and to the samples



Figure 3. A capillary with its protective shield

and a screw type pressure regulator.

The soil was sampled, in triplicate, from each tank at various intervals. Immediately after removal from the tank a moisture sample was taken from each Erlenmeyer flask and the samples were treated with 0.5 milliliters of toluene. The samples were then placed in a refrigerator at 0° C until the analyses could be made. In most instances the samples were analyzed within three days of sampling.

In addition to the moisture determination made on the samples, they were all analyzed for ammonium nitrogen, nitrate nitrogen and nitrite nitrogen. Ammonium nitrogen determination was made on a potassium chloride extract of the soil by the Nessler method; alkaline tartrate and gum acacia were used in a modification of the method given by Jackson (1958). Nitrate nitrogen and nitrite nitrogen were determined on a saturated calcium hydroxide extract. Nitrate nitrogen was determined using the phenodisulfonic acid method in which nitrites were destroyed by use of ammonium sulfamate (Justice and Smith, 1961). Nitrite nitrogen was determined by the method given by Shinn (1941) which employs sulfanilamide and coupling reagent.

Results and Discussion

At the first sampling, there had been nitrification at both 25 and 35° C as shown in figures 4 and 5. The complete nitrification data are shown in table 2.

The moisture sprayed around the mouth of the Erlenmeyer flask and on the rubber stopper was apparently enough to keep the moisture in the soil constant for the first week. There was no moisture loss at 25° C and the moisture loss in the soil incubating at 35° C was less than 1 percent. Starting with the second week of incubation there was a rapid drop in the moisture content of the soil at both temperatures. There

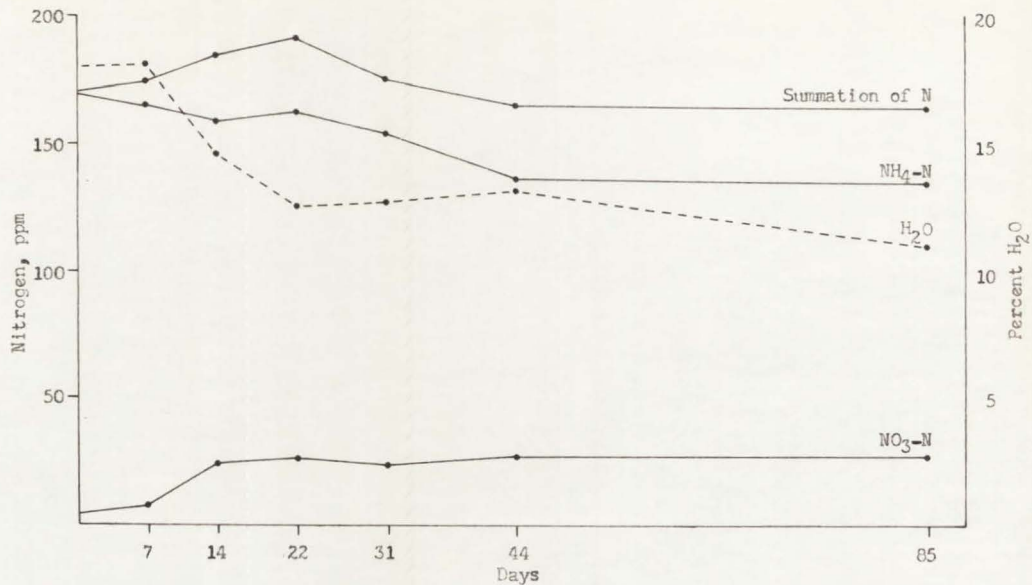


Figure 4. Changes in ammonium nitrogen, nitrate nitrogen and percent water with incubation at 25°C.

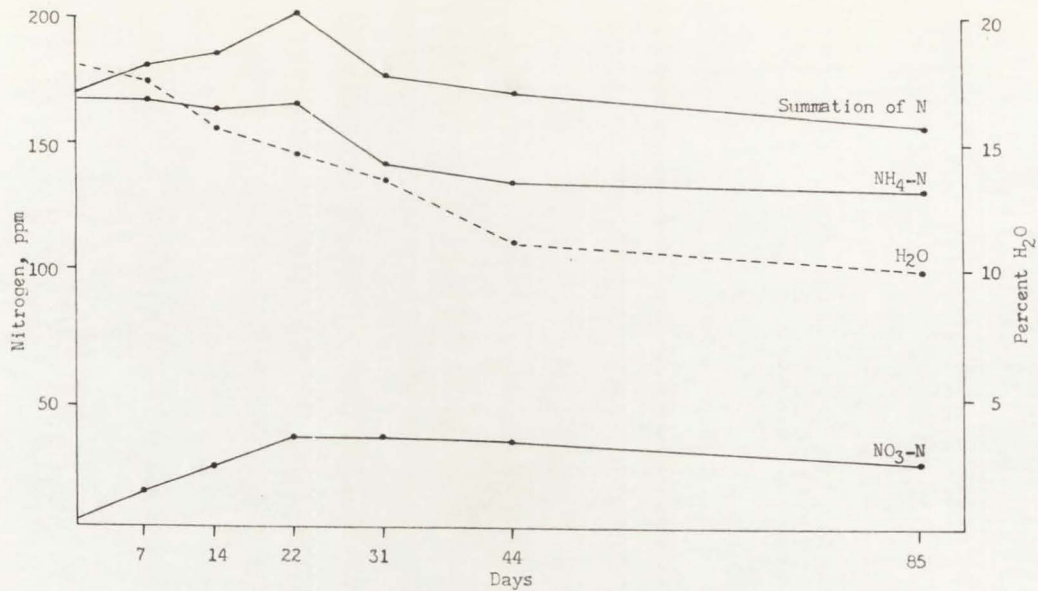


Figure 5. Changes in ammonium nitrogen, nitrate nitrogen and percent water with incubation at 35°C.

Table 2. Changes in ammonium nitrogen, nitrite nitrogen, and nitrate nitrogen with incubation at 25° C and 35° C with decreasing moisture percentage^a

Temperature	Days incubated	Percent water	NH ₄ -N	NO ₂ -N	NO ₃ -N	Sum
				ppm		
25° C	7	18.0	165	1	8	174
	14	14.6	159	0	24	183
	22	12.4	162	0	28	190
	31	12.7	152	0	23	175
	44	13.2	137	0	28	165
	85	10.9	134	0	28	162
35° C	7	17.4	168	0	12	180
	14	13.6	162	0	23	185
	22	14.6	165	0	36	201
	31	13.8	141	0	37	178
	44	11.1	136	0	35	170
	85	10.1	132	0	28	160

^aValues are means of three replications.

was a drop of 6 percent moisture in the soil at 25° C within the next two weeks of incubation. At 35° C there was a considerable drop in moisture for the next four weeks, about 7 percent total. After the soil moisture had apparently reached equilibrium with the moisture in the air passing over the samples, the drop in moisture was very slow.

The production of nitrate nitrogen continued until the soil moisture reached the minimum level at which nitrification could proceed. At 25° C there was considerable nitrate production for two weeks, and then nitrification stopped almost completely. There was nitrification at 35° C for three weeks before stoppage occurred. The moisture in the soil at 25° C was 14.6 percent at the second sampling while at 35° C the soil moisture did not reach 14.6 percent until the third sampling. This 14.6 percent water corresponds, therefore, quite closely to the point at which nitrification has essentially stopped.

Since nitrification stopped at the same moisture for both temperatures, the conclusion can be reached that for this soil the minimum moisture at which nitrification can occur is independent of the temperatures studied.

It is interesting to note that nitrification stopped at a moisture percentage just slightly below the 15 bars moisture tension (approximately permanent wilting percentage) level (table 1). Robinson (1957) also showed that active nitrification stopped at a soil moisture level just below the permanent wilting percentage.

The significance of the ammonium nitrogen trends observed in this study are discussed in a later section.

Summary

The purpose of this study was to determine the minimum moisture

at which nitrification would occur. This was found to be about 14.6 percent. This moisture percentage corresponds to a point just slightly below the permanent wilting percentage for this soil.

EXPERIMENT II. INCUBATION WITH CONSTANT MOISTURE

Methods and Procedure

Since the first study showed that the moisture percentage which corresponds to 15 bars tension was the minimum at which any appreciable nitrification would occur, it was decided to study the soil under the same conditions of temperature and aeration but with the moisture held constant. Two moisture levels were used in this study, 15 bars tension (approximately permanent wilting percentage) and 0.3 bars tension (approximately field capacity). This was done to give a comparison between nitrification at rather restrictive moisture conditions and optimum moisture conditions. Calder (1957) found that nitrification proceeded at a near optimum rate at field capacity, which corresponds closely to 0.3 bars moisture tension.

The treatment of the soils in this study was the same as in Experiment I, except the moisture percentage was adjusted to different levels. For the 15 bars moisture tension study the percent water in the soil was 15.4. The percent water in the soil to be incubated at 0.3 bars moisture tension was adjusted to 25.

The apparatus used in Experiment I was modified slightly for this study. The capillary tubes were all removed and replaced with tubes which contained a larger orifice. This was done to permit aeration of samples at the rate of 5 milliliters of air per minute with less pressure in the system (14 centimeters of water). Using formula 1 with the same assumptions proposed in the previous experiment, the calculated relative humidity was 98.5. It was found that both the 15.4 and the 25.0 moisture percentage held constant under this physical setup.

The soil was sampled, in triplicate, weekly from each tank. All other methods and procedures were the same as those used in Experiment I.

Results and Discussion

15 bars moisture tension

The nitrate nitrogen was produced and increased steadily throughout the 77 days of the study (table 3). The rate was slightly faster at 35° C (figures 6 and 7). It is interesting to note that there was no lag period at the beginning of the incubation (figures 6 and 7). Nitrification started the first week and continued at very nearly the same rate throughout the study. Although the previous study showed that nitrification stopped at a point very near 15 bars moisture tension (14.6 percent), there was considerable nitrification at this point (15.4 percent). The average rate of nitrification at 25° C was about 10 ppm of nitrate nitrogen production per week. At 35° C this figure was slightly higher (11 ppm). This nitrification rate would amount to about 20 lbs. per week of nitrate nitrogen being produced per acre furrow slice (2×10^6 lbs.).

The trend of the changes in ammonium nitrogen is harder to interpret. There was a period of buildup for about three weeks with a subsequent drop for most of the remainder of the incubation period. The initial buildup may be explained on the theory that the organic forms of soil nitrogen are being converted to an inorganic form. The loss of ammonium nitrogen from the soil would, of course, be explained by a conversion to nitrate nitrogen, loss by volatilization and conversion to organic forms by soil microorganisms. There was no accumulation of nitrite nitrogen found in this study.

Table 3. Changes in ammonium nitrogen, nitrite nitrogen, and nitrate nitrogen with incubation at 25° C and 35° C with 15 bars moisture tension^a

Temperature	Days incubated	Percent water	NH ₄ -N	NO ₂ -N	NO ₃ -N	Sum
				ppm		
25° C	7	15.4	226	0	19	245
	14	15.1	215	0	29	244
	21	15.3	227	0	32	259
	28	15.6	206	0	48	254
	35	15.4	183	0	55	238
	42	15.4	162	0	61	223
	49	15.1	143	0	72	216
	56	14.7	131	0	81	212
	63	15.0	98	0	93	191
	70	14.7	95	0	104	199
	77	15.1	93	0	110	203
35° C	7	15.2	233	1	21	255
	14	14.8	227	0	34	261
	21	15.0	237	0	47	284
	28	15.1	227	0	62	289
	35	15.0	189	0	72	261
	42	15.6	171	0	77	249
	49	15.3	155	0	78	233
	56	14.9	166	0	90	257
	63	14.5	150	0	96	247
	70	14.5	150	0	110	260
	77	15.1	116	0	115	231

^aValues are means of three replications.

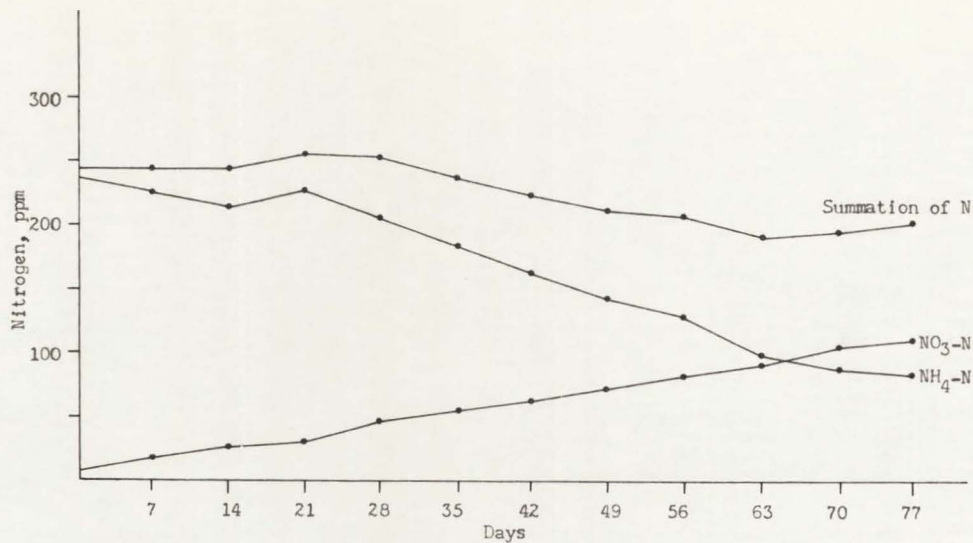


Figure 6. Changes in ammonium nitrogen, nitrate nitrogen and their sum with incubation at 25°C and 15 bars moisture tension.

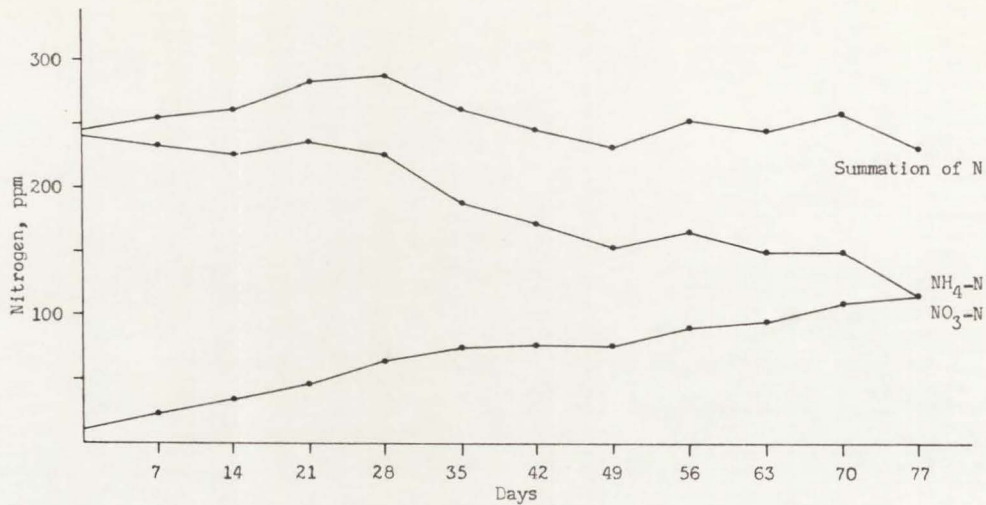


Figure 7. Changes in ammonium nitrogen, nitrate nitrogen and their sum with incubation at 35°C and 15 bars moisture tension.

The loss in the ammonium plus nitrate nitrogen (summation of nitrogen) is shown in figures 6 and 7. At 25° C there was a total loss of 42 ppm nitrogen. At 35° C there was a total loss of 24 ppm nitrogen. If the increase due to the release of soil organic nitrogen is considered, the loss of inorganic nitrogen would be greater.

0.3 bars moisture tension

The nitrate nitrogen production started with the first week of incubation and continued at a fairly constant rate for the duration of the study (table 4). Here again the rate was slightly faster at 35° C (figures 8 and 9).

It is interesting to note that at 35° C there was a very steady decrease in the ammonium nitrogen and very little buildup or decrease in summation of nitrogen. At 25° C, however, a considerable increase in summation of nitrogen was noted, followed by a much greater decrease. There was a small increase in ammonium nitrogen during the second week of incubation.

The increase in nitrate nitrogen was not as steady at 25° C as at the other temperature. The rate was faster the third week than at any other time in the study. The differences in the trends at the two temperatures can best be explained by the differences in nitrification rate. At 35° C the rate was rapid from the beginning of the incubation. The rate was such that there was no ammonium nitrogen buildup. At 25° C there was a slight buildup of ammonium nitrogen because it took three weeks for nitrification to reach the rate needed to prevent a buildup. Apparently the 25° C temperature was more conducive to the release of soil organic nitrogen at this moisture tension.

Table 4. Changes in ammonium nitrogen, nitrite nitrogen, and nitrate nitrogen with incubation at 25° C and 35° C with 0.3 bars moisture tension^a

Temperature	Days incubated	Percent water	NH ₄ -N	NO ₂ -N	NO ₃ -N	Sum
					ppm	
25° C	7	24.7	225	0	24	249
	14	22.9	232	0	42	274
	21	23.0	224	0	58	281
	28	24.5	153	0	91	245
	35	22.9	86	0	113	199
	42	28.0	73	0	126	199
35° C	7	24.0	219	0	32	251
	14	23.0	199	0	60	259
	21	23.3	171	0	81	252
	28	23.6	151	0	113	264
	35	24.0	115	0	133	248
	42	23.7	95	0	145	240

^aValues are means of three replications.

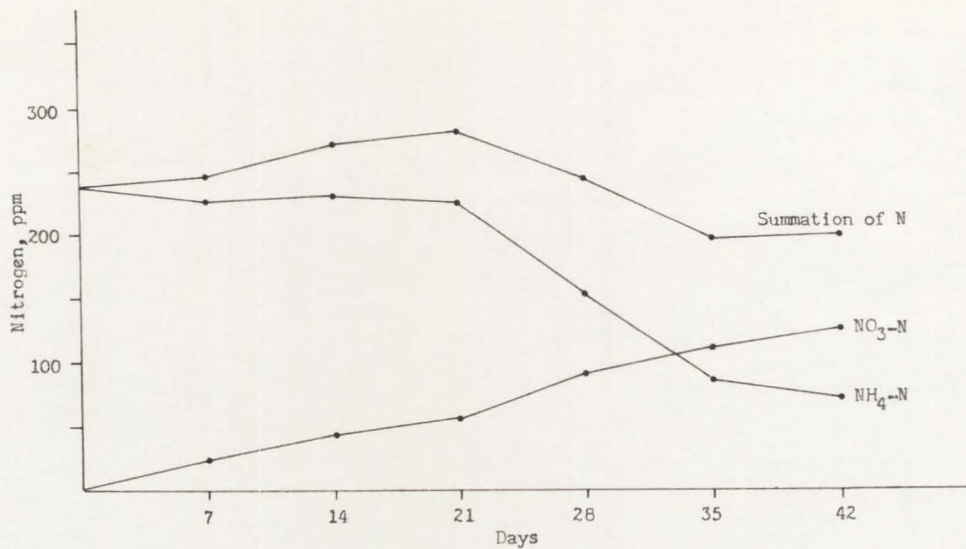


Figure 8. Changes in ammonium nitrogen, nitrate nitrogen and their sum with incubation at 25°C and 0.3 bars moisture tension.

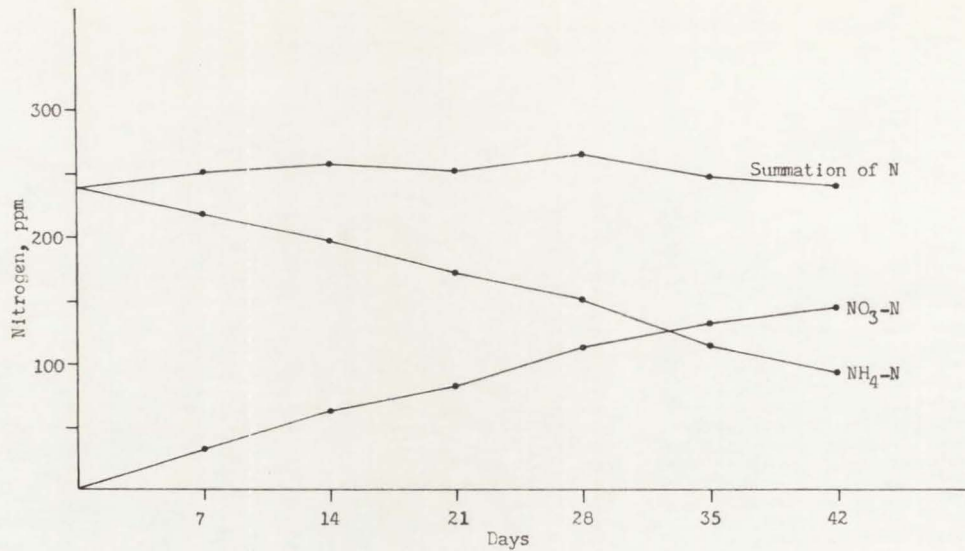


Figure 9. Changes in ammonium nitrogen, nitrate nitrogen and their sum with incubation at 35°C and 0.3 bars moisture tension.

Conclusions

A comparison of the nitrate production at the various temperatures and moistures studied in this experiment are shown in figure 10. The rate of nitrification was twice as fast at 0.3 bars moisture tension as at 15 bars. At 0.3 bars the average rate of nitrification was about 2.4 ppm of nitrate nitrogen per day. The corresponding value for 15 bars was about 1.2 ppm per day. This observation confirms the theory that decreasing moisture has a restricting effect on nitrification rate (Sabey et al., 1956).

Figure 11 compares the ammonium plus nitrate nitrogen sum for the various temperature and moisture levels used in this study. Perhaps the most interesting comparison here is the effect of temperature. There was a considerably greater loss of nitrogen at 25° C than at 35° C. At 0.3 bars moisture tension and 25° C the total loss of inorganic nitrogen was 50 ppm. If the buildup is considered the loss was considerably greater (82 ppm). At 35° C and 0.3 bars, nitrification from the beginning to the end of incubation was 10 ppm of nitrogen. At 15 bars moisture tension, the loss at 25° C was 42 ppm and at 35° C the loss was 24 ppm nitrogen. This would indicate that there was either a greater tieup of nitrogen by soil microorganisms at 25° C or that the loss by denitrification and/or volatilization was greater.

Lowenstein et al. (1957) observed that denitrification and nitrification in the soil proceed simultaneously. The trends of the summation of nitrogen in this study would tend to confirm this observation. At all of the temperature-moisture levels there was, near the beginning of the incubation period, a buildup of the summation of nitrogen followed by a decrease. The buildup would indicate a release

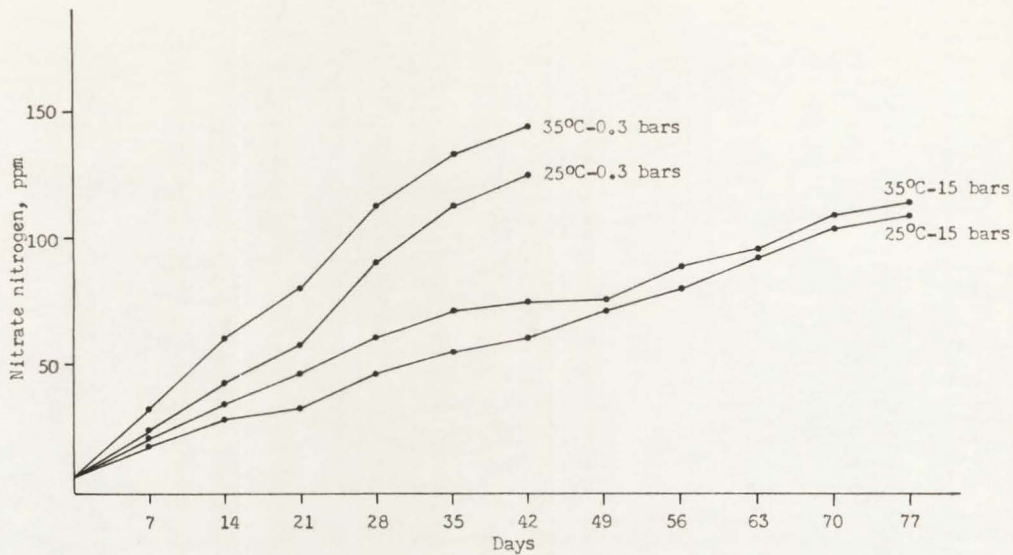


Figure 10. Changes in nitrate nitrogen with incubation at 25 and 35°C with 0.3 and 15 bars moisture tension.

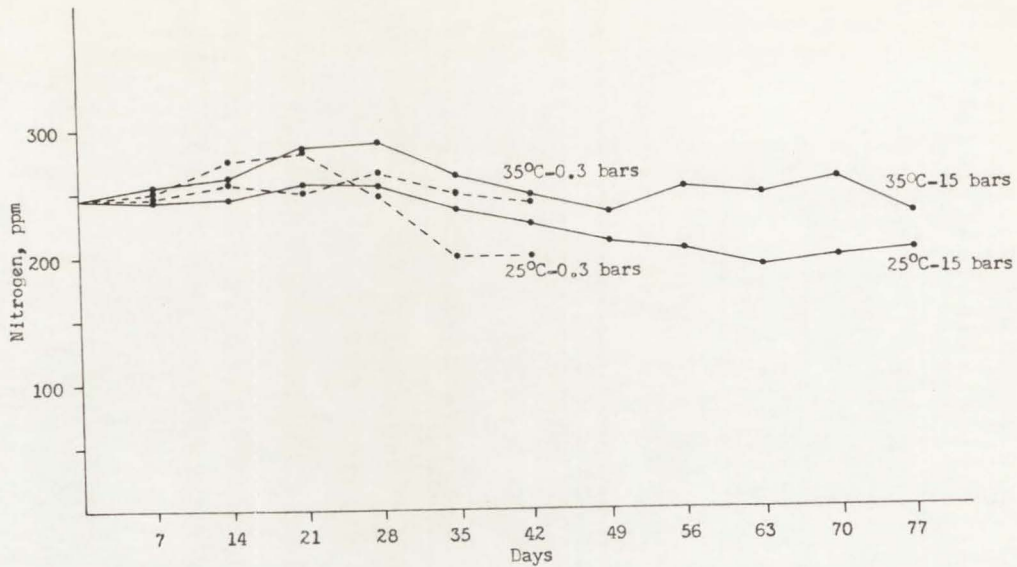


Figure 11. Changes in the sum of ammonium nitrogen plus nitrate nitrogen with incubation at 25 and 35°C with 0.3 and 15 bars moisture tension.

of soil organic nitrogen as nitrification proceeds. Concurrent with this buildup there might be a large expansion of the microbiological populace which are concerned with nitrification. The easily oxidizable materials could soon be diminished to the point that there was not enough left to support the organisms present, consequently the only possible avenue left was the tieup of some of the inorganic nitrogen in the soil. This would account for the decrease in summation of nitrogen. There was probably some loss of nitrogen by volatilization of ammonia and denitrification, with the subsequent loss of nitrogen gases. This, however, was not proven.

Summary

This was a comparative study of nitrification rate at 15 and 0.3 bars moisture tension. It was found that nitrification proceeded at a moderate rate, 8.5 ppm nitrate nitrogen per week, at 15 bars tension. At 0.3 bars the nitrification rate was about twice as fast as it was at 15 bars.

There was considerable loss of total inorganic nitrogen at 25° C with less loss at 35° C.

EXPERIMENT III. INCUBATION WITH DECREASED TEMPERATURE

Methods and Procedure

This study was undertaken to study the nitrification rate of added ammonium fertilizer in a noncalcareous soil at a temperature of 2° C and 0.3 bars moisture tension. It was felt that in light of the previous study it might be fruitful to study this soil under conditions of reduced temperature.

The Wysaro clay was also used for this study. The soil, treated with 150 ppm of nitrogen, added as ammonium sulfate, was incubated at 2° C for 140 days. The soil was sampled at a biweekly interval, in triplicate, and tested for the different forms of inorganic nitrogen and moisture.

Ammonium sulfate was dissolved in water and added to the 100 grams of the soil in pint jars with sufficient water to bring the moisture content to 25.0 percent which corresponds to 0.3 bars moisture tension. After adding the desired water and ammonium sulfate, the samples were thoroughly mixed with a spatula and put in the constant temperature room as soon as possible. The lid and mouth of the bottle were sprayed with a fine mist of water before the lid was put on to assure a high humidity in the jar. In addition to the treated samples put in the constant temperature rooms, there were enough blank samples (no ammonium sulfate added) put in to allow that one could be sampled each time the treated soils were sampled.

The pint jars were opened twice weekly and the air in the flask exchanged using a squeeze bulb. Justice and Smith (1961) reported that

this aeration twice weekly was sufficient for the highest rate of microbial activity in a soil at this low temperature. The rate of course depends on the soil, but it was felt that if nitrification proceeded at a rapid rate the interval between aerations could be decreased. After each aeration, a fine mist of moisture was sprayed inside each jar and onto the lid to compensate for the moisture lost during aeration.

At each biweekly interval three treated jars and a jar containing a blank sample were removed from the constant temperature room. A moisture determination sample was taken and the remaining sample was treated with toluene and placed in the refrigerator. The samples were analyzed for inorganic nitrogen components as described in Experiment I within a week of sampling.

Results and Discussion

There was no nitrification of applied ammoniacal fertilizer at 2° C and 0.3 bars moisture tension. The nitrification trends are shown in figure 12 and the results of this study are tabulated in table 5. There was a slight accumulation of nitrite nitrogen during the last four weeks of incubation. This would indicate that there was some factor, presumably temperature, affecting the ability of the nitrite oxidizers (Nitrobacter) to do their work. The oxidation to nitrite occurred, but the process of nitrification stopped at this point.

The changes encountered in the ammonium nitrogen during this study are considerably harder to interpret and, to say the least, confusing (figure 12). During the course of the incubation there was a loss of about 20 ppm ammonium nitrogen. This erratic nature can best be explained by the increase and decrease of soil organic nitrogen with

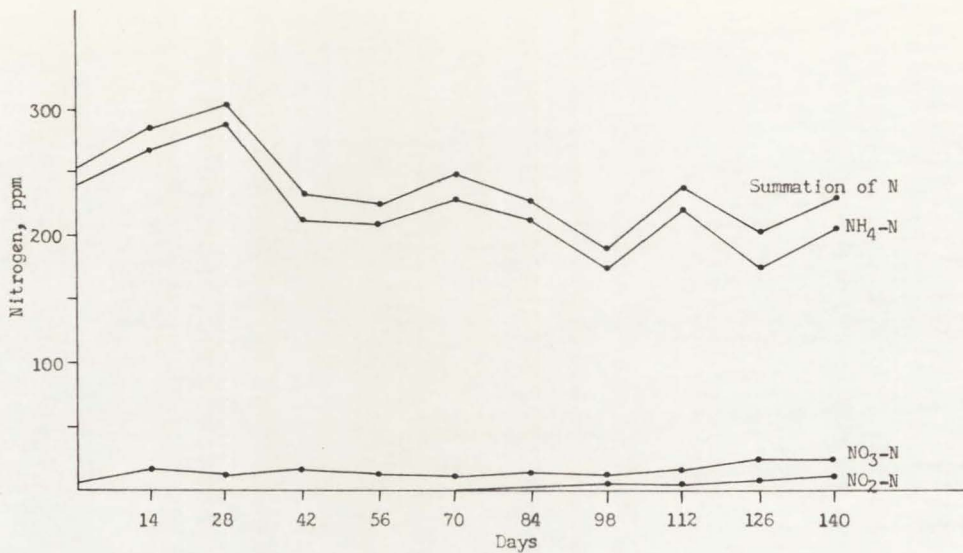


Figure 12. Changes in ammonium nitrogen, nitrate nitrogen, nitrite nitrogen and their sum with incubation at 2°C and 0.3 bars moisture tension.

Table 5. Changes in ammonium nitrogen, nitrite nitrogen, and nitrate nitrogen with incubation at 2° C and 0.3 bars moisture tension^a

Temperature	Days incubated	Percent water	NH ₄ -N	NO ₂ -N	NO ₃ -N	Sum
					ppm	
2° C	14	23.8	267	0	19	286
	28	24.6	293	0	11	304
	42	23.7	214	1	18	233
	56	23.5	204	0	17	221
	70	23.6	236	0	14	250
	84	24.6	218	0	18	236
	98	24.5	176	2	14	192
	112	23.7	221	1	19	241
	126	23.7	179	3	24	206
	140	24.4	210	5	23	238

^aValues are means of three replications.

the resulting and inversely proportionate changes in ammonium nitrogen. This undoubtedly does not explain the mechanism completely and there are almost certainly other causes of the effects observed. A more complete explanation would require further study.

Summary

This study was designed to determine whether or not nitrification would occur at 2° C. The answer according to the data obtained would be a decisive "no." An interesting sidelight to this study were the fluctuations in ammonium nitrogen. These can best be explained by the release and accumulation of soil organic nitrogen.

GENERAL SUMMARY AND CONCLUSIONS

This was a series of incubation studies designed to determine the effect of temperature and moisture on the nitrogen transformation of applied ammonium sulfate. The variables investigated in this study included temperatures ranging from 2 to 35° C and moisture levels ranging from 0.3 to approximately 115 bars tension.

The study consisted of three experiments: The first at 25 and 35° C was designed to permit determination of the minimum moisture level at which nitrification would occur. The second experiment was designed to study nitrification at 15 bars moisture tension and, as a comparative study, nitrification rate at 0.3 bars moisture tension. The third study was at 2° C and 0.3 bars moisture tension.

The following conclusions can be reached from these studies:

1. Nitrification stopped at a point just below the moisture percentage corresponding to 15 bars moisture tension.
2. At 15 bars moisture tension there was considerable nitrification. The rate was slightly faster at 35 than at 25° C.
3. The nitrification rate at 0.3 bars was approximately twice as fast as it was at 15 bars moisture tension. Here again nitrification was slightly faster at 35° C.
4. Nitrate nitrogen production proceeded at a fairly constant rate for the duration of the incubation period at 0.3 and 15 bars moisture tension. There was no lag period at the beginning of incubation.

5. There was no nitrification at 2° C. There was a slight nitrite nitrogen accumulation at this temperature, presumably because of a restriction placed on the bacteria oxidizing the nitrite to nitrate.

6. There are fluctuations in the soil inorganic nitrogen that can best be explained by the release and accumulation of soil organic nitrogen.

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