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Moisture and Temperature Effects on the Transformations of Nitrogen from Applied Ammonium Sulfate in a Calcareous Soil

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MOISTURE AND TEMPERATURE EFFECTS ON THE TRANSFORMATIONS OF NITROGEN

FROM APPLIED AMMONIUM SULFATE IN A CALCAREOUS SOIL

by

John Keith Justice

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I dedicate this work to my wife, Vera, who did the statistical analysis of the data as well as many hours of typing and proof reading.

John Keith Justice

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INTRODUCTION

Nitrogen has commonly been a deficient element in the cultivated soils of the world since the beginning of agriculture. The general acceptance of the practice of using manures as a means of increasing plant growth, as shown by the records of ancient civilizations, attest to this fact. Since the time of von Liebig there has been an increasing awareness of the importance of this deficiency in soils. As a result of a better understanding of the problem and the increasing availability of commercial forms of nitrogen, a rapid increase in the use of nitrogen fertilizers has taken place in the last few decades.

This increasing use of commercial forms of nitrogen is accompanied by the need for more information concerning the proper use of these materials in order to accomplish the greatest benefit. For example, with the advent of increasing use of ammonium fertilizer to improve soil productivity, there has arisen a possibility of lengthening the period from the date of the fertilizer application to the time of utilization by the crop. This advanced application, especially in the fall of the year, has many advantages and is widely advocated. The following questions need to be answered in connection with the efficiency of such a practice: Will the ammonium form of nitrogen remain unoxidized in the soil over the winter months? Will it be fully oxidized to nitrates even at low temperatures and subjected to losses by leaching or denitrification in case of the high moisture commonly occurring during the winter and spring months? Could it be only partially oxidized and result in an accumulation of nitrites that may cause toxicity or be lost from the soil in a gaseous form such as nitrous oxide? What are the chances for significant losses by volatilization before it is oxidized? How does the amount of moisture and the prevailing temperature affect these transformations?

The study reported here is an endeavor to contribute to more complete answers to some of these questions. Although much research has been conducted relating to the effects of moisture or temperature on nitrogen transformations in the soil, more information is needed covering greater variations in the moisture and temperature levels along with the interactions of these. Accordingly, experiments were conducted under carefully controlled conditions to measure the changes occurring in the inorganic soil nitrogen from an applied ammonium source, at various moisture and temperature levels.

REVIEW OF LITERATURE

The Nitrifying Bacteria

Since the nitrogen transformations reported in this study are performed by the nitrifying bacteria in the soil, it is well to mention a few basic facts about these organisms as reviewed by Meikljohn (1953). They cannot use the decomposition products of organic material for their energy but are entirely dependent on the oxidation reaction that they carry out for their energy. Accordingly, they are autotrophic. One familiar group of the ammonia oxidizers is called Nitrosomonas. An important group of the nitrite oxidizers is called Nitrobacter. Carbon dioxide is the source of carbon for the cell substance of these organisms.

Other commonly accepted facts about the nitrifiers are: (1) they grow faster in darkness; (2) they are aerobic; (3) they tolerate a fairly wide range of acidity; (4) they require certain inorganic nutrients such as calcium, phosphorus and iron; and (5) they adhere to and proliferate at the surface of solid particles.

The biochemistry of nitrification in the soil has been studied by several workers (Hofman and Lees, 1953; Lees and Quastel, 1946a, 1946b) by use of the soil perfusion apparatus. One of the interesting conclusions from these experiments is that the rate of nitrification of a given quantity of ammonium sulfate is a function of the degree to which the ammonium ions are adsorbed on the particle surfaces in the soil cation exchange complex. The greater the amount of adsorption, the faster is the nitrification.

Moisture Effects on Nitrogen Transformations

Surprisingly little detailed study of the effect of moisture on nitrogen transformations was found in the literature. An early and quite extensive work concerning the influence of moisture on the bacterial activities in the soil that should be mentioned is that of Greaves and Carter (1920). Twenty-two soils of typical farm land of Cache Valley were studied. Their moisture holding capacity ranged from 31 to 78 percent of the oven dry weight. Nitrification was at its maximum at 50 to 60 percent of its water holding capacity and varied with specific soils.

Russel et al. (1925) made a study of moisture and temperature factors in nitrate production on some Nebraska soils. They found no nitrate production at the hygroscopic coefficient but observed that nitrate production increased with an increase in moisture up to 1 1/4 times the moisture equivalent. They noted some nitrification at as low as 5° C and found that it stopped at 55° C with a maximum of nitrates produced at 35° C.

The only work found dealing specifically with the low moisture range (the wilting point and below) was a recent paper from Africa by Robinson (1957). He found that active nitrification of the natural soil nitrogen stopped at a soil moisture level just below the permanent wilting percentage. Ammonification of natural nitrogen in this soil, however, did not cease at this moisture level and ammonium nitrogen accumulated substantially. Limited application could be made of these results since he was using a tropical soil (pH 4.8 to 5.6), and the method he used for controlling the moisture is questionable. The samples were kept in flasks plugged with cotton wool, and moisture was added daily with a fine spray accompanied by shaking. At the low moisture levels it seems that adequate control would be very difficult by this method.

Another recent paper from Africa by Calder (1957) used more accurate moisture control methods by means of a specially designed apparatus for humidifying the air going over the samples. However, no attempt was made to go as low as the permanent wilting point. He pointed out that the accumulation of nitrate in unenriched tropical soil was not specifically favored by any stable moisture content between the limits of 15 and 45 percent water on a dry soil basis (wilting point equal to 12.5 percent and field capacity equal to 25 percent). Change of moisture status during drying was accompanied by the appearance of much more nitrate than under steady moisture conditions. Fluctuation of moisture status about an optimum average of 22 to 23 percent seemed likely to provide the most favorable conditions according to his investigations.

An interesting observation concerning the relationship of soil moisture to the nitrification process has been pointed out in a recent paper by Birch (1960). Investigating air dry storage periods of 3 to 15 weeks, he found that the longer a soil was kept in the air dry state in storage prior to moistening, the higher the amount of nitrification after moistening. With high humic soils drying produced extra nitrogen on moistening, sometimes equivalent to over one ton of sulfate of ammonia per acre. Even with low humic soils, values of about 300 pounds were common. The mechanism possibly involved is suggested by Birch as being the result of drying increasing the surface area of the humic gels or nitrogeneous colloids. Although not suggested by him, it seems possible that this increase in nitrification could be associated with the ammonium nitrogen accumulation in dry soils mentioned above by Robinson (1957). A similar increasing effect of previous air drying on nitrification was noted recently in some South Dakota soils by Harpstead and Brage (1958).

Temperature Effects on Nitrogen Transformations

The literature is considerably more voluminous with respect to temperature effects on nitrification than it is in the case of moisture effects. Until recently, however, there have been very few studies reported on the effects of low temperatures (around the freezing point). Early studies such as that of Waksman and Madhok (1937) were primarily aimed at determining an optimum temperature. Their paper suggested 27 to 37° C as the most favorable. There is, however, much disagreement in the literature about the optimum range for nitrification.

One of the earlier and most inclusive studies of the effect of temperature as a factor in nitrogen changes was done by Panganiban (1925). He included the effect of constant and alternating temperatures in connection with ammonification, nitrification, denitrification and nitrogen fixation under both aerobic and anaerobic conditions. He found that ammonification took place at constant temperatures between 15 and 60° C and that at higher temperatures the rate is faster. He showed that nitrification took place between 15 and 40° C and that the optimum temperature in soil cultures was about 35° C.

As late as 1956 Sabey et al. (1956) made the statement that although it had been long known that temperature affects the rate of biological oxidation of ammonia, specific data showing the magnitude of this in the critical low temperature ranges was not found in the literature. They reported an optimum nitrification temperature of 25° C with no nitrification at 0° C. In general, rates were about 50, 25 and 6 percent, respectively, as rapid at 20, 15 and 8° C as at 25° C.

Later in the same year, Frederick (1956) reported on the effect of temperature on the formation of nitrate from ammonium nitrogen. Four soils were studied under laboratory conditions. The formation of nitrates took place at all temperatures studied between 2 and 35° C when other factors were favorable. The rate of nitrification increased with increase in temperature, with the greatest increase between 7 and 15° C. The optimum lay between 27 and 35° C, and no sharp break was found until the temperature went below 20° C. In Genesee silt loam, a well drained alluvial and calcareous soil of moderate organic matter and pH 7.7, nitrate nitrogen was formed at rates of 2, 10, 45, 60, 90 and 120 ppm per week at 2, 7, 15.5, 21, 27 and 35° C, respectively, until the 200 ppm of added ammonium nitrogen was oxidized. The rates in the other soils were progressively less, with the slowest rate in Clermont silt loam which is a poorly drained soil, low in organic matter and with a pH 5.0. Temperature fluctuating in a 24-hour cycle (as under field conditions) generally resulted in an increased rate of nitrification at temperatures below 15.5° C and a decreased rate above 15.5° C. An application of 50 pounds per acre of ammonium nitrogen in soils like the Genesee silt loam could be nitrified in about two months even when the average temperature is near freezing (0 to 2° C).

In a later study Frederick (1957) considered the effect of the population of the nitrifiers on the formation of nitrates and related this to the temperature over which nitrate formation from ammonium salts occurs. He concluded that differences between soils in regard to the temperature range over which nitrate formation from ammonium salts occurs appear to be due to differences in the initial population of nitrifiers. Sabey, Frederick and Bartholomew (1959) investigated the influence of temperature and initial population of nitrifying organisms on the maximum rate and delay period of nitrate formation from ammonium nitrogen. This study showed that the influence of temperature and nitrifying population on delay periods and maximum rates varied greatly among soils, indicating that other factors inherent in the soils also affect nitrification.

Anderson and Purvis (1955) and Anderson (1960) gave particular attention to the effect of low temperatures. Some nitrification at 37° F occurred in six weeks in all soils tested. They also found much variation in both delay periods and maximum rates between soils.

Stojanovic and Broadbent (1957) studied the influence of low temperatures on nitrogen transformations in Honeoye silt loam. They used 5 and 10° C with an incubation period of six weeks. One of the inorganic sources of nitrogen used was ammonium sulfate. A substantial amount of nitrate nitrogen was formed at 5° C during the zero- to two-week interval.

Another recent study of low temperature effects on nitrification was made by Tyler et al. (1959). Using California soils and temperatures of 37, 45 and 75° F, they observed that nitrification proceeded at a moderate rate at 45° F even though this was somewhat slower than at 75° F. A greatly reduced, though still measurable, rate of nitrification was observed in these soils as low as 37° F.

Fertilizer Effects on Nitrogen Transformations

Several workers have investigated the possible inhibitory effect of high amounts of ammonium fertilizers on the nitrification process. The accumulation of nitrites has been associated with this problem. Chapman and Leibig (1952) suggested that wherever fairly high ammonium concentrations and neutral to alkaline soil conditions occur, more or less nitrite accumulation may be expected. Broadbent et al. (1957) stated that the formation of considerable quantities of nitrite in the alkaline soils that they studied may be explained on the basis of the inhibitory effect of free ammonia on the activities of the Nitrobacter. In their study of six California soils they used six levels of added ammonium nitrogen ranging from 0 to 800 ppm, their purpose being to represent a typical cross section area of the fertilizer band as applied in the field. They found that nitrification was inhibited at the higher levels of applied ammonia and, in the case of one soil, this inhibition was evident as low as the 200 ppm nitrogen addition. They suggested possible causes for this inhibition other than the presence of free ammonia, especially in alkaline soils, such as pH or salt effects.

McIntosh and Frederick (1958) studied the distribution and nitrification of anhydrous ammonia applied with a field applicator by sampling an 8 x 8 x 2 inch cross section perpendicular to the applicator row. Ammonium nitrogen and nitrate nitrogen were determined on each one inch square subsample. Ammonium nitrogen decreased from a maximum concentration of 1,300 to 2,000 ppm at the center to less than 200 ppm in the area about 1 1/2 inches away from the center of the retention zone. They showed that nitrification initially proceeded more rapidly in the outside area of the retention zone where the concentration of ammonium nitrogen was less than 400 ppm. Similarly, Eno et al. (1955) found that the application of anhydrous ammonia to soil resulted in a high local concentration of ammoniacal nitrogen levels which showed a detrimental influence on the formation of nitrates.

Tyler et al. (1959) stated that low temperature and alkaline soil reaction appeared to favor nitrite accumulation from ammoniacal fertilizers even at low levels of addition. According to them, this finding suggested that the Nitrobacter group, which oxidizes nitrite to nitrate, is more sensitive to low temperature than the Nitrosomonas group responsible for the first step in nitrification; or that there is an interaction between temperature and free ammonia inhibition of Nitrobacter.

A basic study of the effect of inorganic nitrogen on nitrification was made recently by Stojanovac and Alexander (1958) using the soil perfusion technique described by Audus (1946) with the Honeoye silt loam, pH 7.7. They showed that the addition of ammonium nitrogen in quantities of 250 μg/ml perfusate, or greater, lead to a depression in the rate of nitrate formation. Analysis of the kinetics of the oxidation showed that there was no effect of ammonium nitrogen concentration on the rate of ammonium oxidation. However there did occur an accumulation of nitrite which at constant pH was proportional to the amount of ammonium sulfate initially added. Nitrites apparently accumulated only when there was some ammonium nitrogen remaining in the metabolite solution but then rapidly disappeared once the ammonium nitrogen had been oxidized. They concluded that ammonium nitrogen applied in high concentrations can cause the accumulation of nitrites in soils of high pH by virtue of the specific effects of the original substrate on the Nitrobacter-catalyzed oxidation of nitrite. These results are in agreement with cultural studies of the responsible microorganisms. Finally, they suggested that the build-up and persistance of this substance is favored by alkaline reaction and high levels of applied ammonium-forming fertilizers, possibly by means of an inhibition of Nitrobacter by the free ammonia.

EXPERIMENT I. INCUBATION WITH PERIODIC AERATION

Methods and Procedure

This study was undertaken to examine the effects of moisture, temperature, fertilizer and their interactions on nitrification under carefully controlled conditions. In order to better isolate some of these effects, the same soil was used throughout this study. The soil, Millville loam, is highly calcareous (47 percent calcium carbonate equivalent) with a pH of 7.8 and total nitrogen (Kjeldahl) of 0.13 percent. It is a highly productive soil of Cache Valley. The samples used in this study were taken from the Greenville Experiment Farm of the Utah State Agricultural Experiment Station.

The percentage moisture for this soil at 0.3 bars tension (approximately field capacity) was found to be 18.0. This figure was used in the moisture additions in this study, although Taylor and Cavazza (1954) have found the percentage for the same soil to be 21.5 at one-third atmosphere tension. The percentage moisture of the soil used in this experiment was 6.9 at 15 bars tension (approximately permanent wilting percentage). The permanent wilting percentage determined by the sunflower method was 7.9. In the air dry state this soil contained 1.6 percent moisture.

The soil, treated with different levels of ammonium sulfate, was incubated under varying temperature and moisture conditions and sampled at weekly intervals to test for different forms of inorganic nitrogen and pH.

Ammonium sulfate was dissolved in water and added to 100 grams soil in pint jars with sufficient water to bring the moisture contents to 18.0, 15.9 and 7.8 percent which correspond to 0.3, 1 and 10 bars moisture tensions. The nitrogen levels were 0, 150, and 450 ppm. Care was taken to make sure the samples were uniformly moistened. It was important to place the moistened samples in the constant temperature rooms as quickly as possible after adding the moisture and fertilizer in order to avoid the influence of uncontrolled temperature on nitrification. Since no time could be allowed for the soil to reach equilibrium with the added water, each sample was mixed thoroughly by hand with a spatula right after the predetermined amount of moisture containing the ammonium sulfate was added to the sample in the jar. The jar was then sealed immediately. Before placing the lids on the mixed sample, a fine mist of moisture was sprayed on the lids and around the mouth of the jars to cause a high humidity in the jar above the sample. The samples were then placed in the constant temperature rooms. Duplicate samples were prepared, sealed, and placed in the constant temperature rooms at 2, 10 and 22° C. Sufficient samples were made to allow for sampling at weekly intervals for four weeks. However, after the analysis was completed on the samples taken the third week, it was decided to leave the remaining samples in for a total of 10 weeks.

The jars were opened twice weekly and the air in each flask completely exchanged by using a squeeze bulb. Preliminary experiments had shown that this aeration twice weekly was sufficient for the highest rate of microbial activity in this soil. It was necessary to moisten the inside of each jar and lid with a fine mist at the time of each aeration to compensate for vapor lost when the air was exchanged. After a slight initial drop during the first week, the moisture content of all samples remained nearly constant.

The entire sample in each jar was taken for the weekly analysis. The jars were treated with toluene, resealed, and placed in a refrigerator until the analyses were made. All analyses were made during the week in which the samples were taken. The samples were analyzed for ammonium nitrogen, nitrate nitrogen, nitrite nitrogen, soil reaction and moisture. Ammonium nitrogen determination was made on a potassium chloride extract of the soils by the Nessler method, using alkaline tartrate and gum acacia in a modification of the method given by Jackson (1958). Nitrate nitrogen was determined from a saturated calcium hydroxide extract by the phenoldisulfonic acid method, after first destroying nitrites by use of ammonium sulfamate. Nitrite nitrogen was determined from the same alkaline extract using the method given by Shinn (1941) which employs sulfanilamide and a coupling reagent. The pH was determined on a saturated soil paste.

Results and Discussion

Soil reaction

With nitrification there was a corresponding reduction in pH as is normally expected. The pH ranged from 7.8 for the untreated soil to 7.2 for the treated soil where maximum nitrification occurred. This is shown in figure 1 for the three nitrogen levels at 22° C and 0.3 bar moisture tension. The complete soil reaction data are shown in table 1. After nitrification was complete, there was relatively little change in the respective pH levels with time during the 70-day period. No significant effect on soil reaction in relation to moisture or temperature was indicated within the ranges used in this study.

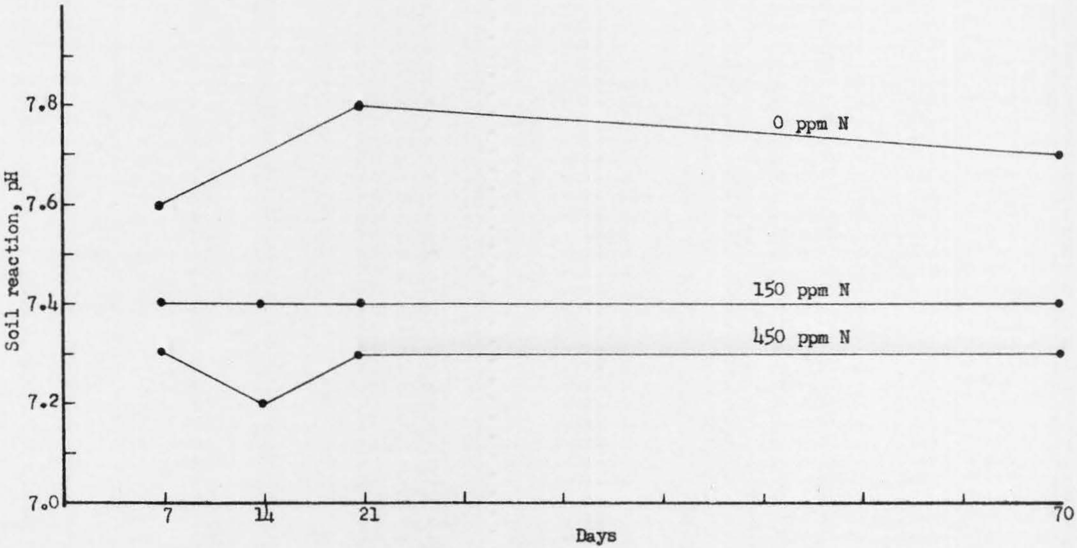

Figure 1. The effect of added nitrogen as ammonium sulfate on pH at 0.3 bar moisture tension and 22° C

in Millville loam incubated

Table 1. Changes in soil reaction at various moistures, temperatures, and levels of nitrogen (N) added as ammonium sulfate. $(NH_4)_2SO_4$.

Treatment			Days incubated			
Temperature	Nitrogen added	Moisture tension	7	14	21	70
°C	ppm	bars	saturated paste pH			
	0	10	7.8	7.8	7.8	7.9
	150	10	7.8	7.8	7.6	7.8
	450	10	7.8	7.7	7.8	7.8
	0	1	7.8	7.8	7.8	7.8
2	150	1	7.8	7.6	7.6	7.6
	450	1	7.7	7.6	7.5	7.4
	0	0.3	8.0	7.8	7.8	7.8
	150	0.3	7.8	7.6	7.6	7.5
	450	0.3	7.6	7.6	7.6	7.5
	0	10	7.7	7.8	7.8	7.8
	150	10	7.6	7.8	7.7	7.6
	450	10	7.7	7.8	7.8	7.6
	0	1	7.7	7.8	7.8	7.8
10	150	1	7.6	7.5	7.6	7.4
	450	1	7.5	7.5	7.7	7.2
	0	0.3	7.6	7.8	7.8	7.7
	150	0.3	7.5	7.4	7.5	7.4
	450	0.3	7.4	7.6	7.6	7.2
	0	10	7.6	7.8	7.8	7.8
	150	10	7.5	7.6	7.4	7.6
	450	10	7.6	7.6	7.6	7.2
	0	1	7.6	7.8	7.7	7.7
22	150	1	7.4	7.4	7.6	7.4
	450	1	7.4	7.2	7.4	7.4
	0	0.3	7.6	7.7	7.8	7.7
	150	0.3	7.4	7.4	7.4	7.4
	450	0.3	7.3	7.2	7.3	7.3

Nitrate nitrogen

One of the most interesting observations of this experiment was the relatively little effect shown by the different moisture levels on the nitrification of ammonium sulfate when the temperature was favorable. This can be seen from the data in table 2 and is pointed out more specifically by the graph in figure 2. There was no difference in either amount or rate of nitrification between the 0.3 and 1 bar moisture tensions at 22° C for the 150 ppm level. Under the same conditions at 10 bars moisture tension, there was a delay of about two weeks, in comparison to the other moisture levels, before nitrification began. After the second week the rate of nitrification at this low moisture level compared favorably with that of the higher moistures. Nitrification of the added 150 ppm nitrogen as ammonium sulfate was nearly complete at this low moisture in 70 days.

The relatively little influence of lowered moisture is amplified from the 10° C data (figure 3). Even at this low temperature there was a highly significant increase in nitrate production between the 21- and 70-day period. Thus in 70 days at 10° C and 10 bars moisture tension, about 50 percent of the added ammonium sulfate was nitrified. This would be equivalent to more than a pound of nitrogen per acre-furrow slice per day. The higher moisture levels for this same temperature gave rates of about 10 to 12 pounds nitrogen per acre per day.

Temperature exerted a greater influence on nitrification than did moisture under the conditions of this experiment. Even at the lowest moisture (10 bars tension) there was a highly significant difference between temperature levels (figure 4). The difference in nitrate production between the 2 and 10° C temperatures occurred only at the 70-day

Table 2. Changes in nitrate nitrogen in a calcareous soil incubated at various moistures, temperatures, and levels of nitrogen added as ammonium sulfate

Treatment			Days incubated			
Temperature	Nitrogen added	Moisture tension	7	14	21	70
°C	ppm	bars	ppm NO_3-N			
	0	10	55	42	45	46
	150	10	38	42	52	46
	450	10	37	42	48	44
	0	1	50	44	46	57
2	150	1	34	44	46	56
	450	1	38	41	48	50
	0	0.3	52	46	45	55
	150	0.3	40	44	50	55
	450	0.3	37	42	50	54
	0	10	46	47	48	50
	150	10	44	50	54	92
	450	10	42	46	54	70
	0	1	48	46	54	45
10	150	1	46	62	86	204
	450	1	45	54	73	462
	0	0.3	48	54	55	59
	150	0.3	50	59	92	188
	450	0.3	45	55	79	235
	0	10	47	50	58	58
	150	10	55	66	130	168
	450	10	48	69	137	440
	0	1	55	56	56	72
22	150	1	84	188	197	222
	450	1	56	288	452	482
	0	0.3	52	58	56	76
	150	0.3	88	202	190	228
	450	0.3	70	290	434	494

	LSD	
For comparison between:	.05 level	.01 level
Two treatments for same date	16	21
Two dates for same treatment	12	16

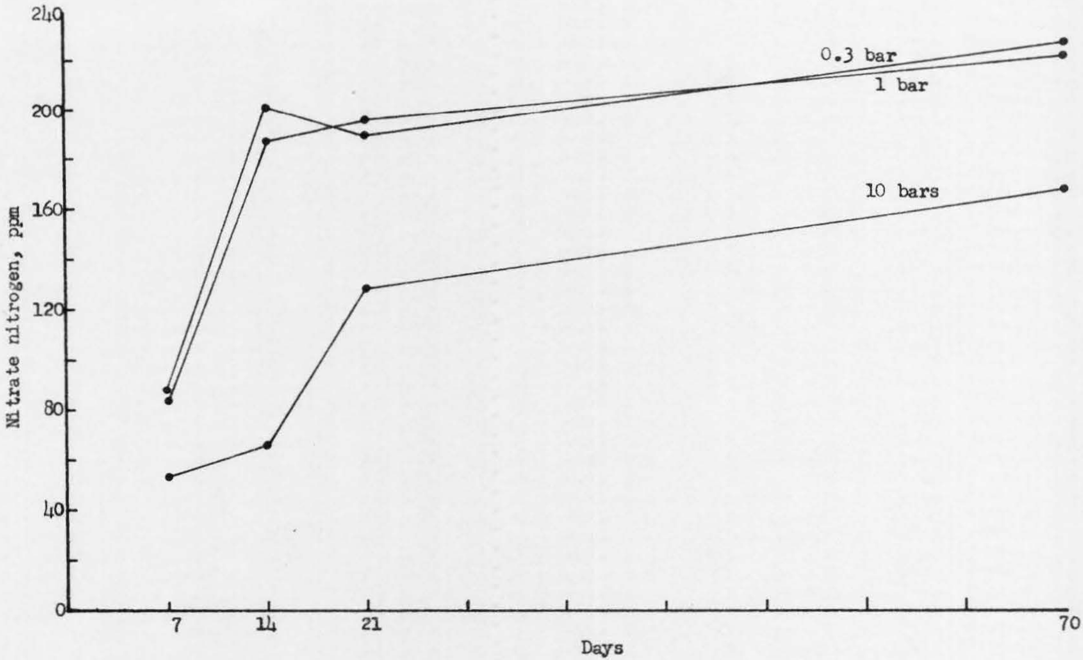

Figure 2. The effect of moisture on nitrate formation at 22° C and 150 ppm nitrogen added as ammonium sulfate

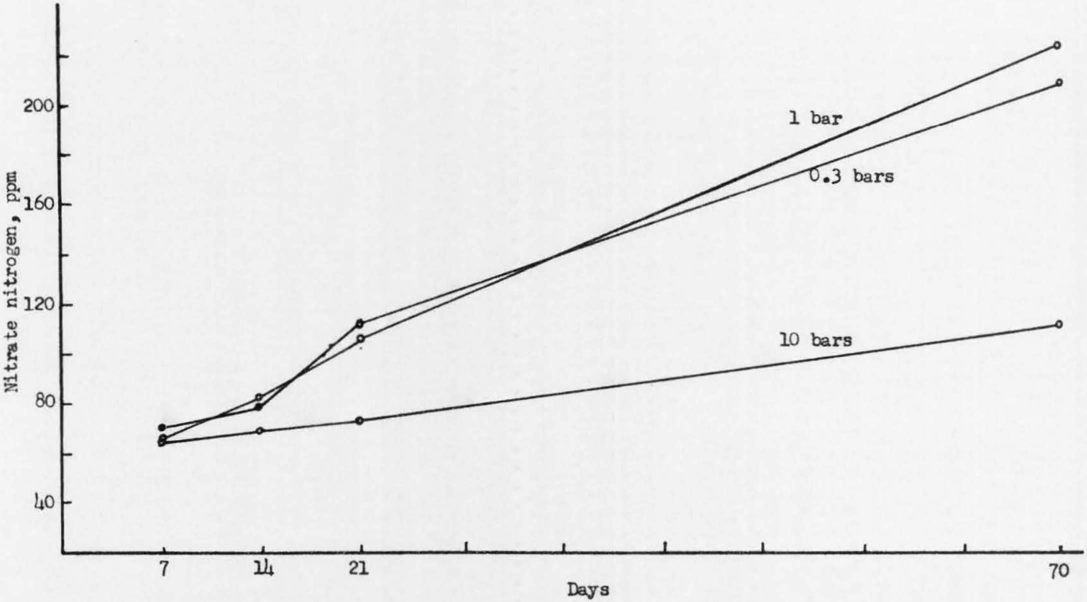

Figure 3. The effect of moisture on nitrate formation at 10° C and 150 ppm nitrogen added as ammonium sulfate

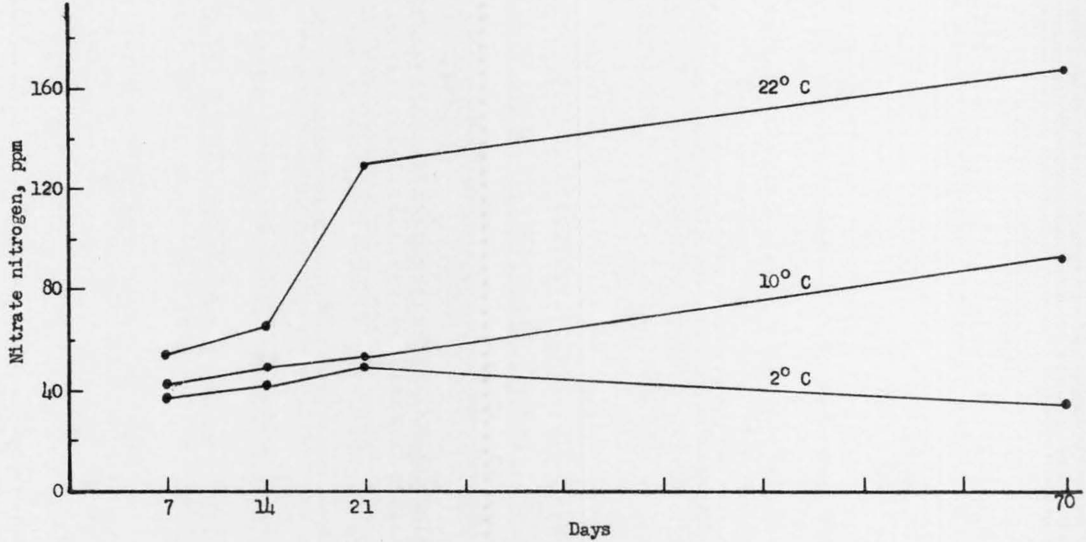

Figure 4. The effect of temperature on nitrate formation at 10 bars tension and 150 ppm nitrogen added as ammonium sulfate

test, while the difference between 10 and 22° C temperature occurred as early as 21 days. The rate of nitrification at the highest temperature (22° C) and lowest moisture (10 bars tension) was very high between 14 and 21 days. With this high temperature, at 21 days nitrification of the 150 ppm nitrogen was over half complete even at this moisture (10 bars tension) which is not far above the wilting point for higher plants.

The effect of added ammonium sulfate at high moisture (0.3 bar tension) and high temperature (22° C) is shown in figure 5. By referring to table 2, it can be noted that a significant amount of nitrification had taken place under these favorable moisture and temperature conditions even in one week. A point of interest is the fact that a delay was shown with the 450 ppm nitrogen treatment in comparison to the 150 ppm nitrogen treatment. At the 7-day period the amount of nitrification in the case of the higher nitrogen treatment was significant only at the 0.05 level, whereas the 150 ppm nitrogen treatment showed significant nitrification at the 0.01 level. At 14 days the 150 ppm nitrogen application had been completely oxidized, while the 450 ppm nitrogen treatment was hardly complete at 21 days. There was enough nitrification under these conditions (0.3 bar moisture and 22° C) even in the case of no added ammonium sulfate to be significant at the 0.05 level. This was evidently due to mineralization of the soil organic matter.

This delay effect associated with high concentrations of ammonium nitrogen has been noted by other workers. Broadbent *et al.* (1957) concluded that where ammonium concentrations were low, nitrification occurred rapidly and completely. Higher ammonium concentrations showed

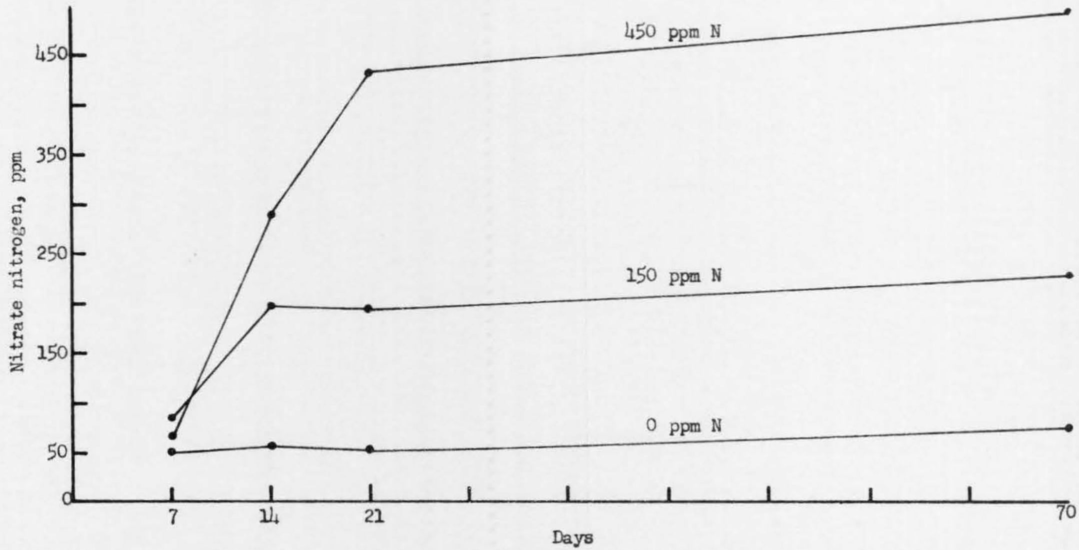

Figure 5. The effect of nitrogen added as ammonium sulfate on nitrate formation at 0.3 bar moisture tension and 22° C

an inhibiting effect on nitrification, and in the case of poorly buffered soils the nitrification was completely inhibited. The levels used in their study were 0, 50, 100, 200, 400 and 800 ppm of ammonium nitrogen. In studying the distribution and nitrification of applied anhydrous ammonia, McIntosh and Frederick (1958) showed that nitrification initially proceeded more rapidly in the outside area of the retention zone where the concentration of ammonium nitrogen was less than 400 ppm.

At 10 bars moisture tension and 22° C there were almost identical amounts of ammonium sulfate oxidized to nitrates for the 150 and 450 ppm nitrogen treatments for the first three weeks (figure 6). This also indicates a delay in the case of the 450 ppm relative to the 150 ppm nitrogen treatment. Both of these amounts of applied ammonium sulfate were nearly completely nitrified by the end of 70 days even at this low moisture (10 bars).

The effects of moisture, temperature, and fertilizer over all time periods are of interest. Since the analysis of variance (appendix, table 15) shows the interactions of the three factors to be significant, the experimental results were combined into two-way tables over all time periods.

In table 3 the interaction of moisture and temperature can be observed. At the low temperature there was no significant difference in the mean moisture effect over all fertilizer levels, while at the higher temperatures there was a significant difference between moistures. This interaction can be seen at the 150 ppm nitrogen level in figure 7. At the highest temperature there was no significant difference between the 0.3 and 1 bar moisture tensions, and even at the low moisture there was an appreciable amount of nitrate nitrogen at this high temperature.

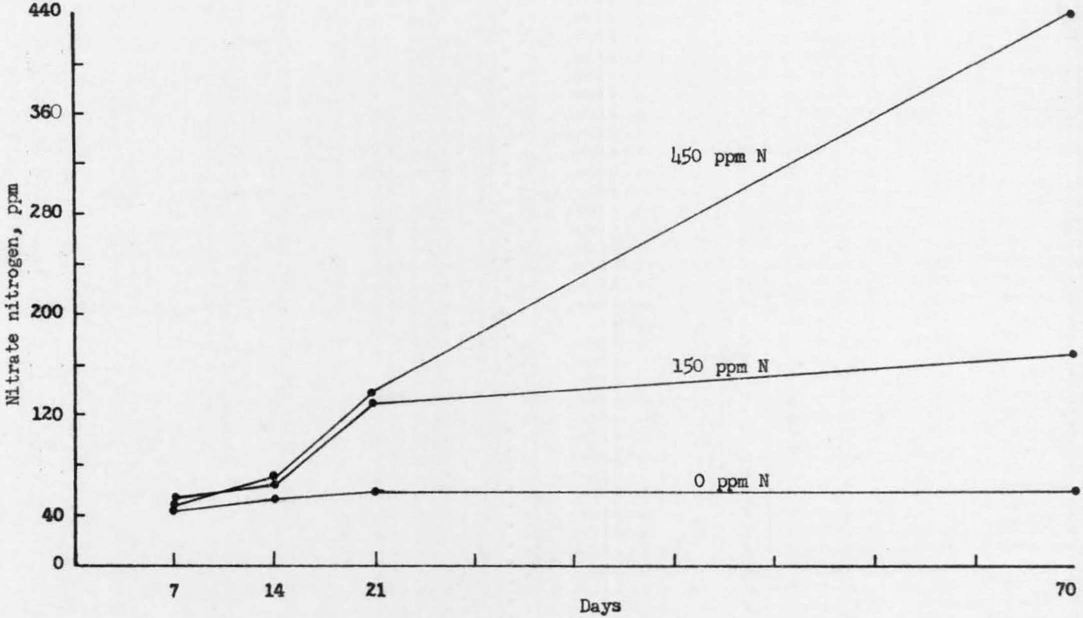

Figure 6. The effect of added nitrogen in the form of ammonium sulfate on nitrate formation at 10 bars moisture tension and 22° C

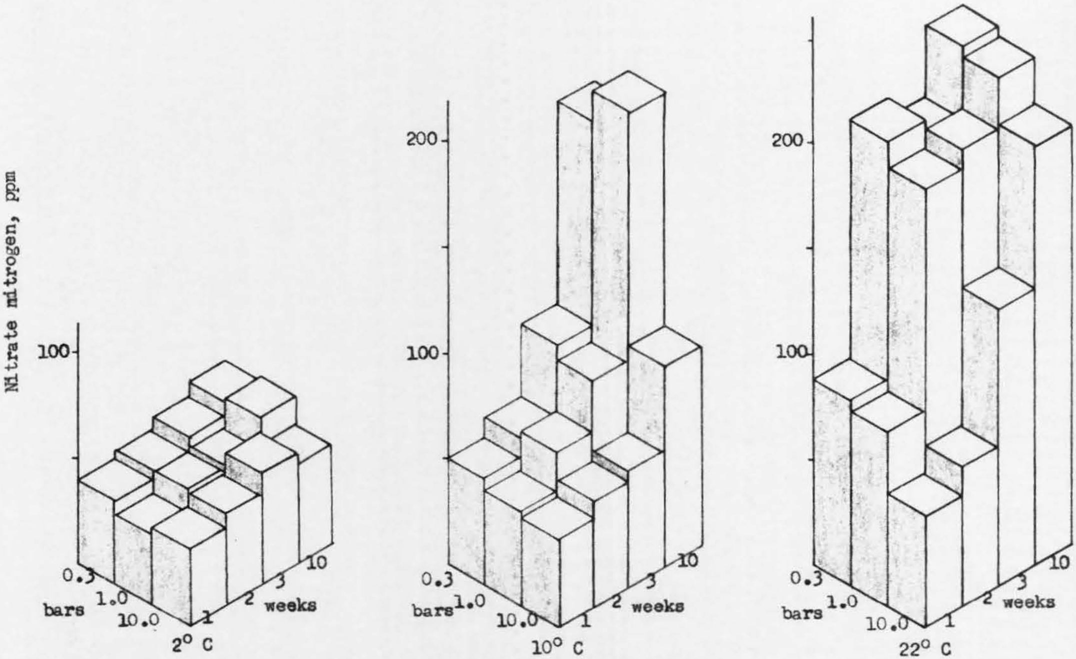

Figure 7. Rate of nitrate nitrogen production at three temperatures and three moisture levels, emphasizing the moisture influence at each temperature, from 150 ppm nitrogen added as ammonium sulfate

Table 3. Mean nitrate nitrogen content for all moistures at all temperatures

Temperature	Moisture tension, bars			Means
	10	1	0.3	
°C		ppm		
2	45	46	47	46
10	54	102	85	80
22	110	184	186	160
Means	70	111	106	95

	LSD	
	0.05	0.01
Means within table	5	6
Table means	3	5

Within the moisture range used in this experiment, there was little effect by moisture on nitrification when the temperatures were favorable. This can be seen graphically in figure 8. In contrast to this there was a strong effect of temperature as shown by the temperature means in table 3.

The interaction of moisture and fertilizer is presented in table 4. There was significant nitrification at all moisture levels with added ammonium sulfate. The mean effects of fertilizer over all moistures are in a ratio of approximately 14:9 for 450 ppm and 150 ppm added nitrogen, respectively. However, at the lowest moisture (10 bars tension) the ratio of nitrates at these fertilizer levels is only 9:7. This indicates the effectiveness of the added nitrogen depends on the amount of moisture in the soil.

There was a significantly larger amount of nitrification at the 1 bar moisture tension than at the 0.3 bar with 450 ppm added nitrogen. This is contrary to what is generally accepted about the effects of moisture on nitrification. Nitrification under these conditions should

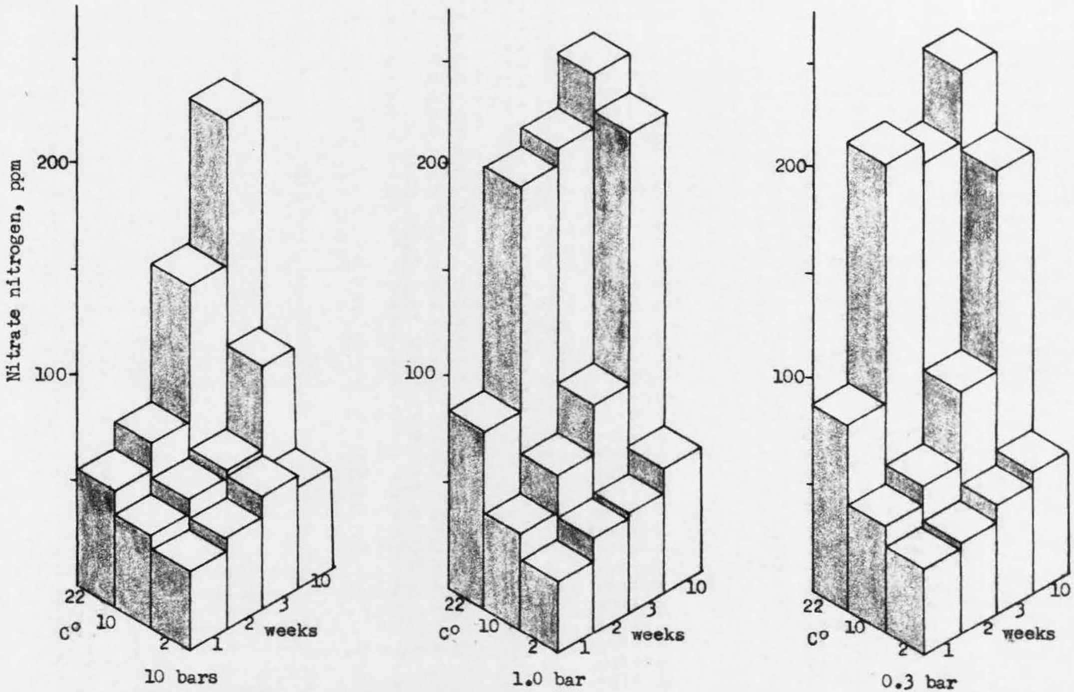

Figure 8. Rate of nitrate nitrogen production from 150 ppm nitrogen added as ammonium sulfate at three temperatures and three moisture levels, emphasizing the temperature influence at each moisture level

Table 4. Mean nitrate nitrogen content for all moistures at all fertilizer levels

Fertilizer	Moisture tension, bars			Means
	10	1	0.3	
ppm		ppm		
0	49	52	55	52
150	70	106	107	94
450	90	174	157	140
Means	70	111	106	95

	LSD	
	0.05	0.01
Means within table	5	6
Table means	3	5

be examined in greater detail by going back to table 2. It is seen here that greater nitrification occurred at the 1 bar moisture tension at 70 days with 10° C temperature. At this observation there was a significant amount (100 ppm) of ammonium nitrogen remaining, an accumulation of nitrites (72.5 ppm) and a loss (approximately 100 ppm) of total nitrogen. The moisture content was checked and found to be in line with the other experimental units. One possible explanation of these phenomena is that the oxygen supply is very near the critical level under these conditions of high moisture and high ammonium application, resulting in sufficiently adverse conditions in some samples to cause this irregularity. The 450 ppm nitrogen level requires a greater amount of oxygen, and the higher moisture level reduces the available amount of oxygen in the soil. Insufficient aeration would slow down the transformation of ammonium nitrogen to nitrite nitrogen as well as favor the accumulation of the nitrite nitrogen that is formed. A loss of nitrogen is expected under partial anaerobic conditions, as shown by other workers. Bremner and Shaw (1958) suggested that denitrification occurs when the supply of oxygen required by the microorganisms is restricted. Jones (1951), working with completely anaerobic conditions, showed that denitrification is a very rapid process. Arnold (1956) discussed another possible source of loss by pointing out that where the oxygen supply is limited the oxidation of ammonium ions may yield hydroxylamine, which, in the absence of sufficient oxygen, does not all become further oxidized but interacts with accumulated nitrite to yield nitrous oxide which escapes into the air. Broadbent and Stojanovic (1952) found that denitrification of added nitrate was inversely related to partial pressure of oxygen. It is concluded, therefore, that insufficient aeration may well account for the significantly greater amount of nitrification occurring at the 1 bar than at the 0.3 bar moisture tension at 70 days and 10° C (figure 3 and table 3).

Table 5 presents the data showing the interaction of fertilizer with temperature. Even at the zero level of fertilizer a significant amount of nitrification occurred at the highest temperature (22° C), while at the low temperature no significant amount of nitrification occurred even at the highest fertilizer level. The dependency of nitrification of the added nitrogen on temperature is further emphasized by the fact that at 10° C the ratio of nitrates at the 450 ppm nitrogen level to nitrates at the 150 ppm nitrogen level is approximately 7 to 6, while at 22° C the ratio is approximately 9 to 5. In other words, the 300 ppm increment was of little value except at the more favorable temperature.

Ammonium nitrogen

The changes in ammonium nitrogen at various moistures, temperatures and levels of added ammonium sulfate are given in table 6. Although the

Table 5. Mean nitrate nitrogen content for all temperatures at all fertilizer levels

Fertilizer	Temperature, °C 2	10	22	Means
ppm		ppm		
0	49	50	58	52
150	46	85	151	94
450	44	105	271	140
Means	46	80	160	95

	LSD 0.05	LSD 0.01
Means within table	5	6
Table means	3	5

data for ammonium nitrogen changes are slightly more erratic than that of the nitrate nitrogen changes, the two tables (tables 2 and 6) tell essentially the same nitrification story. For this reason the ammonium nitrogen changes are not discussed in detail as were the changes in nitrate nitrogen. The comparison of the changes in ammonium nitrogen and nitrate nitrogen at 0.3 bar moisture and 150 ppm added nitrogen are shown in figure 9.

In addition to what can be observed in figure 9, it can be seen from table 6 that the ammonium nitrogen disappearance proceeded at a slightly faster rate than did the formation of nitrate nitrogen at all temperature and moisture levels except at 22° C, 0.3 bar tension and 150 ppm added nitrogen. In this case they were both complete in 14 days, the changes being so rapid that weekly sampling did not provide a satisfactory rate comparison.

From these comparison curves at the 2° C temperature (figure 9) it can be seen that there is an indication of some oxidation of ammonium

Table 6. Changes in ammonium nitrogen in a calcareous soil incubated at various moistures, temperatures, and levels of nitrogen added as ammonium sulfate

Treatment			Days incubated			
Temperature	Nitrogen added[a]	Moisture tension	7	14	21	70
°C	ppm	bars	ppm NH_4-N			
	0	10	20	2	24	13
	150	10	218	148	160	136
	450	10	370	469	539	406
	0	1	20	2	16	12
2	150	1	178	146	148	130
	450	1	411	456	598	418
	0	0.3	22	2	15	14
	150	0.3	169	150	152	124
	450	0.3	438	415	610	431
	0	10	20	0	16	14
	150	10	158	142	148	105
	450	10	432	452	592	397
	0	1	18	0	18	10
10	150	1	168	112	113	10
	450	1	446	387	467	22
	0	0.3	22	0	16	14
	150	0.3	184	114	113	12
	450	0.3	453	389	541	100
	0	10	18	0	15	12
	150	10	161	112	91	38
	450	10	424	374	405	26
	0	1	24	0	24	17
22	150	1	146	14	20	12
	450	1	439	198	38	15
	0	0.3	12	0	22	12
	150	0.3	123	0	30	10
	450	0.3	412	161	26	12

a. Soil originally contained 20 ppm, NH_4-N.

For comparison between:	LSD .05 level	LSD .01 level
Two treatments for same date	49	66
Two dates for same treatment	52	69

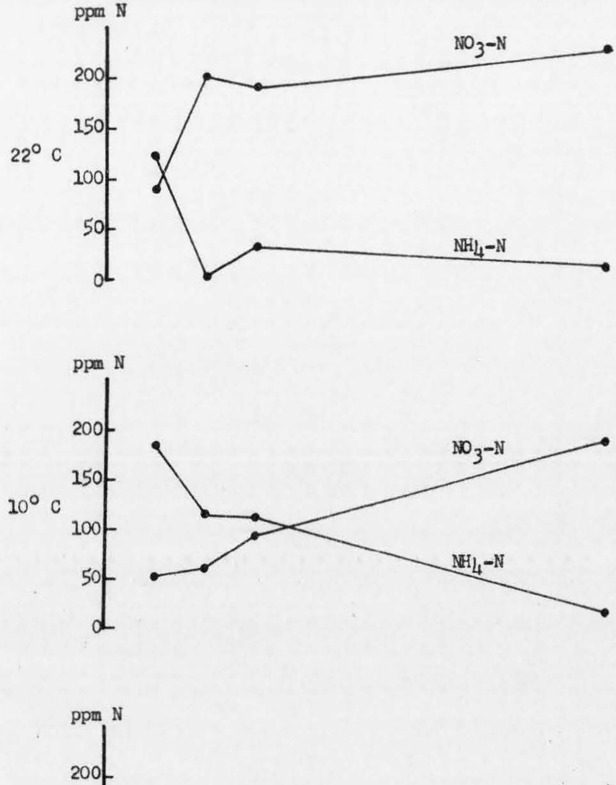

Figure 9. A comparison of changes in ammonium nitrogen and nitrate nitrogen with 150 ppm nitrogen added as ammonium sulfate, 0.3 bar moisture tension, and varying temperatures

nitrogen by the end of 70 days. This observation contributed to the decision to test these changes for a longer period of time. This is discussed later as a separate experiment.

Nitrite nitrogen

Nitrite nitrogen (table 7) did not persist long in the soils that were held at conditions felt to be optimum for nitrification in this experiment (0.3 bar, 22° C and 150 ppm added nitrogen). Rather high levels were found at the first sampling date (7 days) at the highest level of added nitrogen (450 ppm). Under the less favorable nitrifying condition of lower temperature (10° C) nitrite nitrogen persisted at moderate levels much longer for both levels of added nitrogen, and it was found to be quite high at 70 days in the case of the 450 ppm level. Tyler and Broadbent (1960) have shown that the nitrite oxidizers are very sensitive to low temperature. Under their conditions considerable nitrite persisted beyond four weeks in soils at 45° F. At the lowest temperature (2° C) in this experiment, no significant nitrite accumulation was found during the first three weeks of incubation but nitrite accumulation was very high at the 70-day test. This contrast in nitrite accumulation at 2° and 22° C is presented in figure 10 for the highest level of added nitrogen.

Nitrite formation followed closely ammonium nitrogen disappearance and preceded the formation of nitrates in some instances by as much as a week. This was observed more readily at the lower temperatures and lower moistures. There was a greater accumulation of nitrites at the 450 than at the 150 ppm level of added nitrogen except in the case of the 2° C temperature. At this low temperature the oxidation process was evidently just beginning at 70 days and had proceeded further in

Table 7. Changes in nitrite nitrogen in a calcareous soil incubated at various moistures, temperatures, and levels of nitrogen added as ammonium sulfate

Treatment			Days incubated			
Temperature	Nitrogen added	Moisture tension	7	14	21	70
°C	ppm	bars	ppm NO_2-N			
	0	10	1.1	0.8	0.6	0.1
	150	10	1.2	0.8	1.9	4.6
	450	10	0.8	1.0	0.9	2.5
	0	1	1.6	1.6	2.2	0.2
2	150	1	2.5	3.0	3.8	54.3
	450	1	2.1	3.5	4.2	32.6
	0	0.3	1.6	1.9	3.1	0.2
	150	0.3	2.6	3.2	5.8	61.1
	450	0.3	2.6	4.0	5.2	42.8
	0	10	0.6	0.1	0.5	0.1
	150	10	3.8	5.6	7.8	0.9
	450	10	3.4	4.5	4.0	52.0
	0	1	0.2	0.2	0.5	0.2
10	150	1	11.0	28.1	26.5	0.1
	450	1	9.7	28.9	47.0	0.5
	0	0.3	0.5	0.2	0.5	0.2
	150	0.3	10.6	28.5	41.5	0.4
	450	0.3	8.2	33.5	63.7	72.5
	0	10	0.1	0.1	0.4	0.4
	150	10	7.0	5.4	1.4	0.4
	450	10	7.6	8.0	2.5	0.8
	0	1	0.2	0.2	0.4	0.4
22	150	1	5.4	0.8	0.5	0.4
	450	1	27.5	2.1	0.8	0.8
	0	0.3	0.2	0.2	0.2	0.9
	150	0.3	8.2	0.8	0.8	0.9
	450	0.3	52.6	1.9	0.9	1.0

For comparison between:	LSD .05 level	LSD .01 level
Two treatments for same date	11.1	15.0
Two dates for same treatment	11.0	14.5

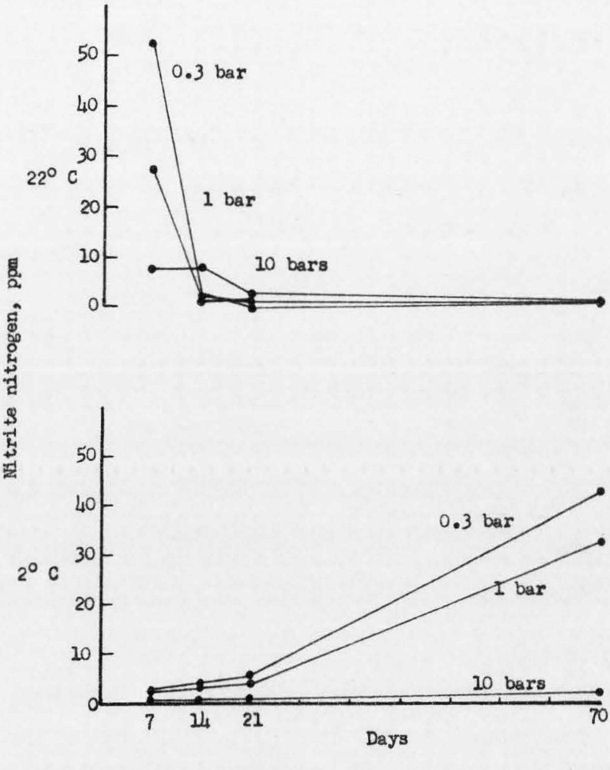

Figure 10. The effect of moisture on nitrite nitrogen at 2 and 22° C with 450 ppm nitrogen added as ammonium sulfate

the case of the 150 ppm level than at the 450 ppm treatment. Figure 11 shows this difference, which was significant at the 0.01 level at both the 0.3 and 1 bar moisture tensions. In the case of more favorable temperatures nitrate formation had already begun at the 150 ppm level, while the 450 ppm treatment remained in the nitrite accumulation stage preceding nitrate formation. All of this harmonizes with the previously mentioned delay in nitrate nitrogen production in the case of the 450 ppm treatment. This delay at the 450 ppm level is likely due to the inhibiting effect of high amounts of ammonia on the Nitrobacter which carry out the second step in the nitrification process. Stojanovac and Alexander (1958), using kinetics of the oxidation process, showed that there was no effect of ammonium nitrogen concentration on the rate of ammonium oxidation to nitrites but that high ammonium nitrogen concentrations did cause the accumulation of nitrites in soils of high pH by virtue of the specific effects on the Nitrobacter-catalyzed oxidation of nitrite.

Additional Low Temperature Experiment of Longer Duration

Because nitrite appeared at significant levels for the 70-day sampling at 2^{o} C and there was an indication of oxidation of the applied ammonium nitrogen at the 150 ppm level, it was decided to incubate the samples under these conditions for longer than 70 days.

The methods and procedures for this experiment were the same as those previously described except for the variables used. Only the 0.3 bar moisture tension was used for all fertilizer treatments, and the 10 bars tension was used with the 150 ppm treatment only. The incubation time was extended to 91 days and samples were tested for ammonium nitrogen, nitrite nitrogen and nitrate nitrogen at 70, 77, 84 and 91 days. The results are shown in table 8.

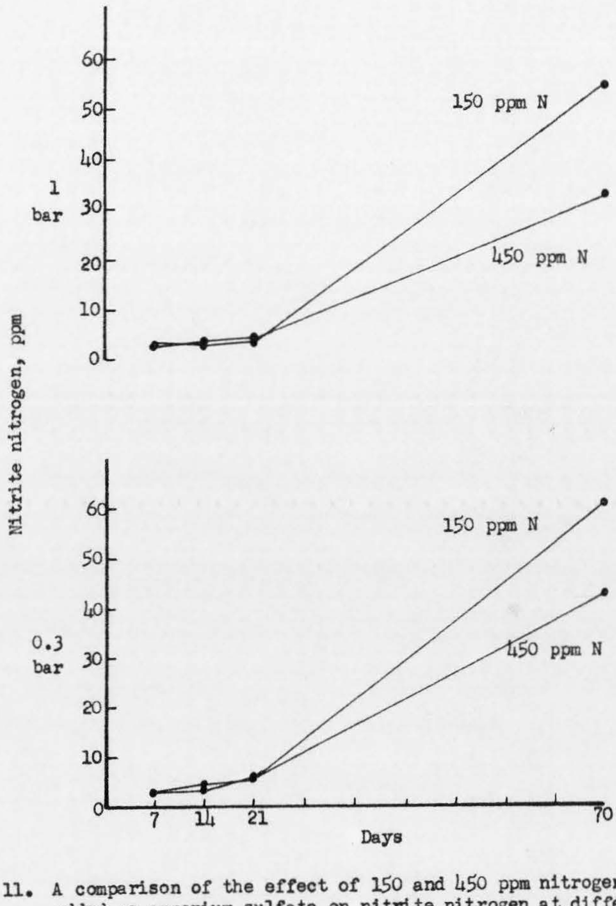

Figure 11. A comparison of the effect of 150 and 450 ppm nitrogen added as ammonium sulfate on nitrite nitrogen at different moisture levels and 2^{o} C

Table 8. Changes in ammonium nitrogen, nitrite nitrogen, and nitrate nitrogen from the incubation of ammonium sulfate in a calcareous soil at 2° C

Treatment		Nitrogen	Days incubated				Means	LSD
Nitrogen added	Moisture tension	analyzed	70	77	84	91		.05
ppm	bars		ppm					
0	0.3	NH_4-N	16	10	21	17	16	29
		NO_2-N	0	0	0	0	0	7
		NO_3-N	28	19	20	24	23	17
		Sum	44	29	41	41		
150	0.3	NH_4-N	94	70	68	74	77	29
		NO_2-N	82	72	50	82	72	7
		NO_3-N	65	78	102	81	82	17
		Sum	241	220	220	237		
450	0.3	NH_4-N	397	373	355	388	378	29
		NO_2-N	78	90	65	102	84	7
		NO_3-N	44	52	42	55	48	17
		Sum	519	515	462	545		
150	10	NH_4-N	138	135	144	133	138	29
		NO_2-N	20	10	22	26	20	7
		NO_3-N	28	23	25	27	26	17
		Sum	186	168	191	186		

	LSD 0.05 Treatment	Time
NO_3-N	35	17
NH_4-N	58	28
NO_2-N	13	7

It can be seen that the low moisture (10 bars) with this low temperature completely inhibited nitrate formation for the 13-week incubation period. There was, however, significant nitrite formation and an indication of ammonia loss even at this low moisture and temperature for these longer periods of time. Perhaps this indicates that at least the first step of the nitrification process was occurring.

In comparing the 70-day data of this experiment with that of the previous study reported, it will be noted that there was less nitrite accumulation at the 10 bars moisture level in the previous study than in this case. This difference, along with the other slight differences in actual values between the two experiments at this same incubation time, was probably due to the use of a different soil sample. These samples were obtained from the same area but, being sampled at different times, had different nitrogen levels initially. This is evident by comparing the figures for the zero treatments.

At the high moisture (0.3 bar) and 150 ppm added nitrogen there was significant nitrate formation at these longer periods of incubation. Comparing the zero ppm treatment with the 150 ppm level in table 8, there was significant nitrification even at 70 days in this experiment. Although slow, there was also significant nitrification with time for the 150 ppm treatment. In the case of the 450 ppm level no significant nitrate formation was observed under these conditions. This harmonizes with the typical delay observed with this high treatment as discussed previously.

Rather large quantities of nitrites were produced, both at the 150 and 450 ppm levels of added ammonium sulfate. This fact alone can have a significant influence on the concept of applying ammonium-containing fertilizers in the fall with the hope that this will be unchanged and, hence, free from leaching losses until spring. The nitrites produced, even though they are not oxidized to nitrates, are subject to the same leaching losses as are nitrates. The quantities produced were large, over 50 percent of the 150 ppm treatment in some cases. By the 10-week sampling about 60 percent of the added ammonium was converted to nitrates plus nitrites in the case of the 150 ppm treatment.

The results found in this experiment would be conservative since the ammonium was applied to the soil and immediately cooled and maintained at 2^{o} C throughout the period. In field soils the temperatures would come down to this low level much slower; during this period nitrification could be occurring.

Summary of Experiment I

A calcareous soil (Millville loam) was incubated in jars provided with periodic aeration to determine the transformation of nitrogen from added ammonium sulfate under controlled moisture and temperature conditions. Samples were taken at weekly intervals for a period of 21 days and again at 70 days and analyzed for soil reaction and different forms of nitrogen.

Under the conditions of this study there were definite inhibitory influences of low moisture, low temperature and high ammonium concentration on nitrification along with significant interactions of these factors. These conclusions are not altogether new, but some aspects of the study are enlightening.

At 2^{o} C there was evidence of ammonium oxidation and a distinct accumulation of nitrites at 70 days which led to a test of longer duration. In this second test nitrate production was obtained at this low temperature during the period of testing (70 through 91 days). At both 10 and 22° C nitrification was found at all moisture levels (0.3, 1 and 10 bars). Even at 10 bars moisture tension nitrification of 150 ppm added nitrogen was about 80 percent complete at 22° C in three weeks. Thus, even though the rate of nitrate production was temperature and moisture dependent, the dependency on moisture was surprisingly small.

Even under conditions felt to be relatively most optimum for nitrification (0.3 bar and 22° C) rather high levels of nitrite nitrogen were found at seven days at the highest nitrogen level (450 ppm). A delay in nitrate formation was found at this higher ammonium concentration in comparison to the 150 ppm treatment. This was evidently due to the inhibiting effect of the ammonium. Under the less favorable nitrifying conditions of lower temperature (10° C) nitrites persisted much longer and were found at 70 days to be quite high.

There was a reduction in pH with nitrification of ammonium sulfate but relatively little change in the respective pH levels with time during the 70-day period.

EXPERIMENT II. INCUBATION WITH CONSTANT AERATION

Methods and Procedures

Relationship to first experiment

Since the first experiment primarily included relatively high moisture levels in combination with comparatively low temperatures, the second study was designed to investigate the low moisture range (wilting point and below) together with relatively high temperatures.

Another major reason for undertaking the second study was to eliminate the possibility of inadequate aeration. As discussed in Experiment I, there was evidence of inadequate oxygen supply, especially in the case of the high ammonium treatment in combination with relatively high moisture and temperature.

In addition to the above reasons, which primarily arose from the first experiment, there were at least two special reasons for investigating the nitrification effects of low moisture levels. First, there were no such investigations found in the literature. Secondly, it has been postulated that the process of nitrification occurs at or on colloidal surfaces. One could theorize, then, that the small film of water surrounding the soil particles at moisture levels less than permanent wilting percentage might be sufficient to allow the process to proceed.

The soil used in this experiment (Millville loam, described in Experiment I) showed an air dry moisture percentage of 1.6 and a wilting point percentage of about 7. A series of samples were incubated at moisture levels to include this wilting percentage, two moisture levels below the permanent wilting percentage (3 and 5 percent) and one slightly above this percentage (9 percent). Sufficient water was added, along with the 150 ppm nitrogen in the form of ammonium sulfate, to bring the incubating samples to 3, 5, 7 and 9 percent moisture.

Establishing the moisture levels

Theory.—In order to be able to determine the relative humidity desirable to maintain in the air stream flowing over the soil samples to keep the moisture percentages constant, the vapor pressure data for the Millville soil determined by Thomas (1921, 1924) were used. From his data a curve was plotted of the relative humidity (P/P_0) against the moisture percentage for this soil. From this curve the 3, 5, 7 and 9 percent moisture levels corresponded to 74, 92, 99 and 99.5 percent relative humidity.

To be consistent with Experiment I in reporting all the data in terms of moisture tension so that more universal application to other soils may be made, the relative humidity percentages were converted to bars moisture tension by the formula:

$$\psi = -RT \ln R.H. \quad . \ . \ . \ . \ . \ . \ . \ . \ . \ . \quad (1)$$

where ψ = moisture tension, $R = 8.314 \times 10^7$ ergs/deg/mole, T = absolute temperature and R. H. = relative humidity. The moisture levels in terms of moisture tension from these calculations were 415, 115, 15 and 7 bars, corresponding to the 3, 5, 7 and 9 percent moistures, respectively.

Continuous aeration and relative humidity control.—An apparatus was constructed to provide continuous aeration to the samples and at the same time maintain constant moisture levels in the soil. To accomplish this, air was humidified to a definite level and passed continuously over a thin (one-fourth inch) layer of soil (33.3 grams) which was contained in a 250 milliliter Erlemeyer flask. Two methods were used to humidify the air—salt solutions and differential pressure. At the two moisture levels below the permanent wilting point the air was kept at 74 and 92 percent relative humidity, respectively, by use of salt solutions, allowing also for the differential pressure effect associated with the salt solutions. A saturated solution of sodium chloride was used for 74 percent and a saturated solution of ammonium dihydrogen phosphate for 92 percent relative humidity. In the case of the two moisture levels at and above the permanent wilting percentage, water was used to give the desired relative humidity by means of differential pressure. The incoming air was saturated under a known pressure and then released to atmospheric pressure when passing over the samples. The relative humidity needed to maintain a given moisture level was calculated from the formula:

$$R.\ H. = P_f/P_i \quad . \ . \ . \ . \ . \ . \ . \ . \ . \ . \quad (2)$$

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

The air for all moisture levels was humidified under a pressure of 10 centimeters of water and released to atmospheric pressure as it passed over the samples. A manometer was used on each air line so that the correct pressure could be maintained constantly. For the air humidified over the salt solutions, the relative humidity was that given by the saturated salt solution corrected for the 10 centimeters of water differential pressure. These corrected figures as previously mentioned were 74 and 92 percent relative humidity. The relative humidity of the air as it passed over the samples at atmospheric pressure after having been humidified with distilled water under 10 centimeters of pressure, was calculated by formula (2). Using 910 centimeters of water as the barometric pressure for this location, the calculated relative humidity to the nearest percent was 99. If this figure is carried to the same number of decimal places and expressed in terms of the activity of water as given by Taylor (1958) it is almost identical to the one given for 15.00 bars gauge pressure.

Since the relative humidity of the air is affected by temperature variation, a water bath was used to control the temperature to $\pm$ 0.01^{o} C. Temperature control was obtained by use of a bimetallic thermoregulator and supersensitive relay. The temperature levels used in this study were 25 and 35^{o} C.

Description of apparatus for moisture, temperature and aeration control

Two metal soil storage cabinets were insulated, lined with galvanized iron and painted black. A water pump was mounted on one end to circulate the water for uniform temperature throughout the bath. A 500 watt heating element was mounted in the center of each bath. A bimetallic thermoregulator was mounted and connected to a supersensitive relay for accurate temperature control. An over-all view is shown in figure 12. Three large plastic tubes (four inches in diameter) were mounted horizontally in the bottom of the bath (figure 13). These tubes were filled to about one-half capacity, two with the saturated salt solutions and the other with water. The incoming air was passed over the liquid in these tubes to insure the proper relative humidity upon entering the samples. Rubber tubing connected these large tubes to plastic manifolds which in turn were connected to 250 milliliter Erlemeyer flasks

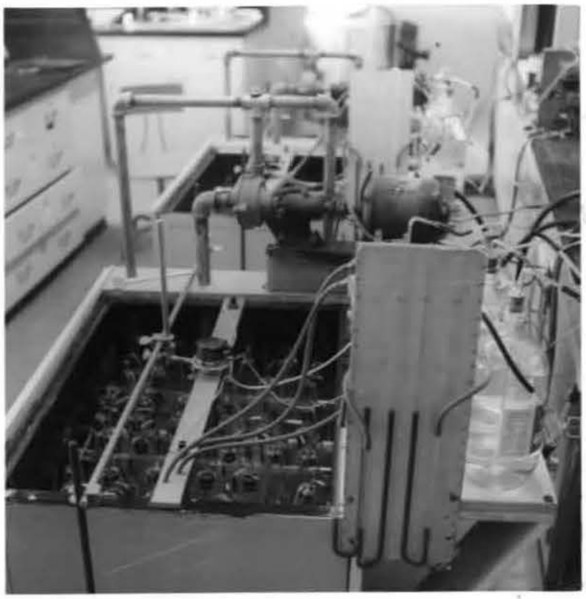

Figure 12. Over-all views of apparatus for moisture, temperature and aeration control, with (upper) and without (lower) samples

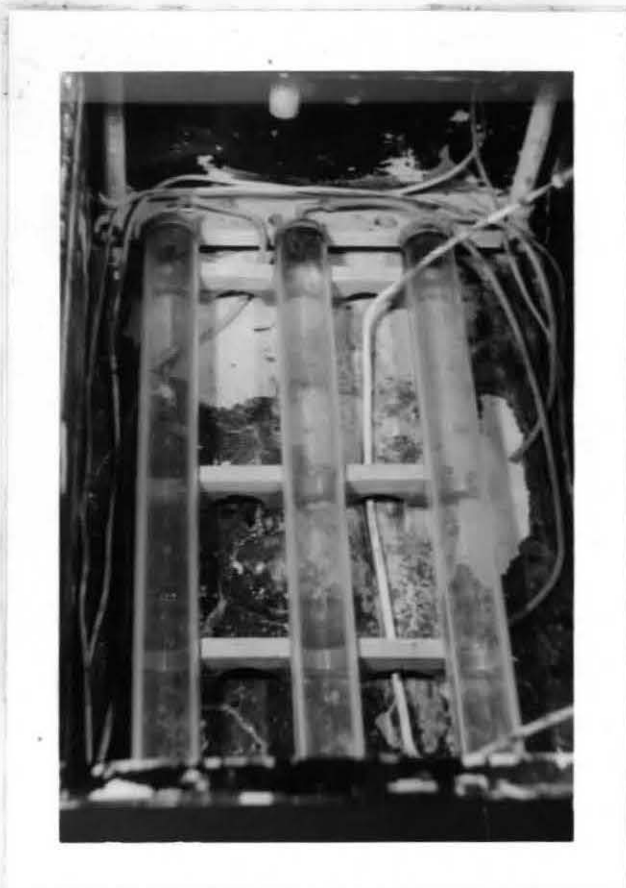

Figure 13. Plastic tubes containing the humidifying solutions over which the air is passed before reaching the samples

containing the samples. In order to secure uniform distribution of air to each sample connected to the manifold, a fine capillary was inserted in the line near the entrance into each flask. Figure 14 shows the capillaries connected to the air manifold and to the flasks. The capillaries were made by drawing down 1 millimeter capillary tubing to a very small opening, much as described by Bartholomew and Broadbent (1949). These were enclosed in a protective covering and calibrated for uniformity of air flow. Figure 15 shows the capillary and its protective shield separately.

Prior to passing the air over the solutions in the large tubes, it was passed through bottles containing the solutions and located outside the bath (figure 16). This partially humidified the air and insured more complete saturation when the air was passed over the solutions in the tubes in the bottom of the tanks to insure complete equilibrium. All of the samples, along with the tubing supplying the humidified air, were completely submerged under the water in the bath. Only the outlet air tube extended above the surface of the water (figure 17). Each week, when a group of flasks were sampled, the water level in the bath was lowered only slightly in order to disconnect the tubing. The remaining flasks were completely submerged during sampling. The flasks were anchored in the water bath, using rubber bands attached to wire hooks, to a wire mesh which was wedged in over the large air tubes (figure 14). When samples were removed from the bath without being replaced by another group of samples, a short piece of glass tubing was inserted in the air line with a cork on the other end to hold it above the surface of the water to prevent water from entering the air line and plugging the capillaries. These can be seen in figure 18 where a unit in

Figure 14. Partially filled bath showing the capillaries connected to the air manifolds and to the samples

Figure 15. A capillary with its protective shield

Figure 16. Bottles containing the humidifying solutions through which the air passes before reaching the plastic tubes inside the bath

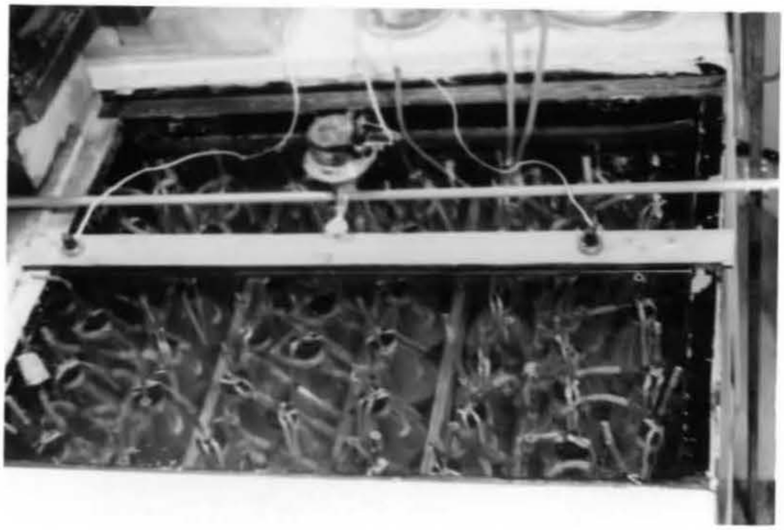

Figure 17. Apparatus for moisture, temperature and aeration control during incubation of samples

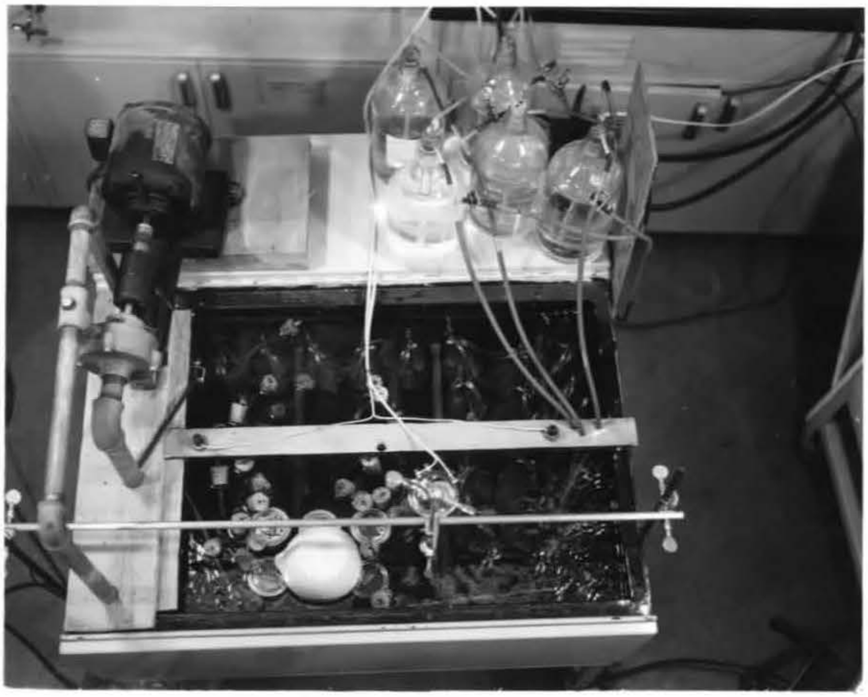

Figure 18. Apparatus for moisture, temperature and aeration control during incubation after some of the samples have been removed

operation has had part of the samples removed.

Each bath contained 54 samples. Two baths were in operation simultaneously, one for each temperature level (25 and 35° C). Three moisture levels, replicated three times, were maintained in each bath. Each sample was in a separate flask. Samples were taken at weekly intervals and replaced by others as needed to cover the various moistures, temperatures and periods of incubation. The samples were analyzed for soil inorganic nitrogen components as described in Experiment I.

Since the physical set-up limited the number of samples, fertilizer treatment was eliminated as a variable. All samples received 150 ppm nitrogen in the form of ammonium sulfate, applied with the water for proper moisture, immediately prior to being placed in the baths. The 150 ppm level was selected because the results obtained in Experiment I indicated it to be a desirable level for this type of study, since no appreciable inhibiting effects were apparent at this level.

Because only three moisture levels could be maintained at one time, incubation was begun at the 7, 115 and 415 bars moisture tension, and the 15 bars moisture tension was added later in the experiment. Time permitted only four weekly sampling periods at this moisture level, while samples were incubated at the other three moistures for eight weeks.

Results and Discussion

Moisture effects

The first samples were taken at the end of three weeks. At that time no nitrification was indicated at either the 415 or 115 bars moisture levels at either temperature; therefore, no 7- or 14-day tests were set up for these moisture levels. Actually no nitrification occurred at these moisture levels during the entire eight-week incubation period at either temperature (table 9).

Since nitrification occurred only at the 7 and 15 bars moisture levels, these data were tabulated and analyzed together for their common period of incubation (four weeks). The results, showing the nitrate nitrogen produced by incubation at these two moisture levels, are presented in table 10. At the 7 bars moisture tension and 25° C, nitrification had begun by the end of the first week and was complete at the end of three weeks. At this moisture, less nitrification occurred at 35° C. There was some indication of nitrification at the end of the first week even at the 15 bars moisture tension with 25° C temperature. At the end of four weeks over one-half of the amount of nitrogen applied in the ammonium form had been transformed to nitrates at 25° C. Only about one-half as much had been completely oxidized to nitrates in the 35° C bath as in the 25° C bath at both the 7 and 15 bars moisture tension. Figure 19 shows the comparison of the amount of nitrification at these two moisture levels nearest the wilting point.

The fact that no nitrification occurred at the 115 bars moisture level but did occur at 15 bars moisture tension indicates that the critical moisture point for nitrification is at least slightly below the wilting point for higher plants. Although the critical moisture level was not specifically determined in this experiment, it must be between 7 and 5 percent moisture for this soil, or it must lie between 15 and 115 bars moisture tension if it is expressed on a basis for comparison with other soils.

In order to investigate the idea of the critical moisture level more closely, samples were prepared at 6 percent moisture (70 bars

Table 9. Changes in ammonium nitrogen, nitrite nitrogen and nitrate nitrogen during incubation of 150 ppm nitrogen added as ammonium sulfate in a calcareous soil at various moistures and temperatures

Treatment		Nitrogen	Days incubated					
Moisture tension	Temperature	analyzed	21	28	35	42	49	56
bars	°C							
415	25	NH_4-N	159	152	147	141	129	134
		NO_2-N	0	0	0	0	0	0
		NO_3-N	17	12	15	12	13	13
		Sum	176	164	162	153	142	147
	35	NH_4-N	152	144	136	134	123	121
		NO_2-N	0	0	0	0	0	0
		NO_3-N	17	14	16	12	11	13
		Sum	169	158	152	146	134	134
115	25	NH_4-N	142	151	147	134	120	131
		NO_2-N	0	0	0	0	0	0
		NO_3-N	20	18	18	16	14	19
		Sum	162	169	165	150	134	150
	35	NH_4-N	118	129	121	105	98	94
		NO_2-N	0	0	0	0	0	0
		NO_3-N	23	17	19	19	12	13
		Sum	141	146	140	124	110	107
7	25	NH_4-N	29	31	27	13	9	11
		NO_2-N	0	0	0	0	0	0
		NO_3-N	153	151	160	161	166	164
		Sum	182	182	187	174	175	175
	35	NH_4-N	93	86	81	61	57	59
		NO_2-N	76	63	38	34	32	9
		NO_3-N	51	62	72	86	74	82
		Sum	220	211	191	181	163	150

Table 10. Changes in ammonium nitrogen, nitrite nitrogen and nitrate nitrogen during incubation of added ammonium sulfate in a calcareous soil at various moistures and temperatures

Treatment		Nitrogen analyzed	Days incubated			
Moisture tension	Temperature		7	14	21	28
bars	°C					
15	25	NH_4-N	137	128	92	80
		NO_2-N	7	1	1	0
		NO_3-N	31	42	83	97
		Sum	175	171	176	177
	35	NH_4-N	143	129	117	97
		NO_2-N	16	16	24	25
		NO_3-N	26	26	43	53
		Sum	185	171	184	175
7	25	NH_4-N	124	76	29	31
		NO_2-N	15	1	0	0
		NO_3-N	44	112	153	151
		Sum	183	189	182	182
	35	NH_4-N	129	121	93	86
		NO_2-N	30	40	76	63
		NO_3-N	28	49	51	62
		Sum	187	210	220	211

For comparison between:		LSD 0.05	LSD 0.01
Two treatments for same date	NH_4-H	8	12
	NO_2-N	19	28
	NO_3-N	19	28
Two dates for same treatment	NH_4-N	13	18
	NO_2-N	15	20
	NO_3-N	13	17

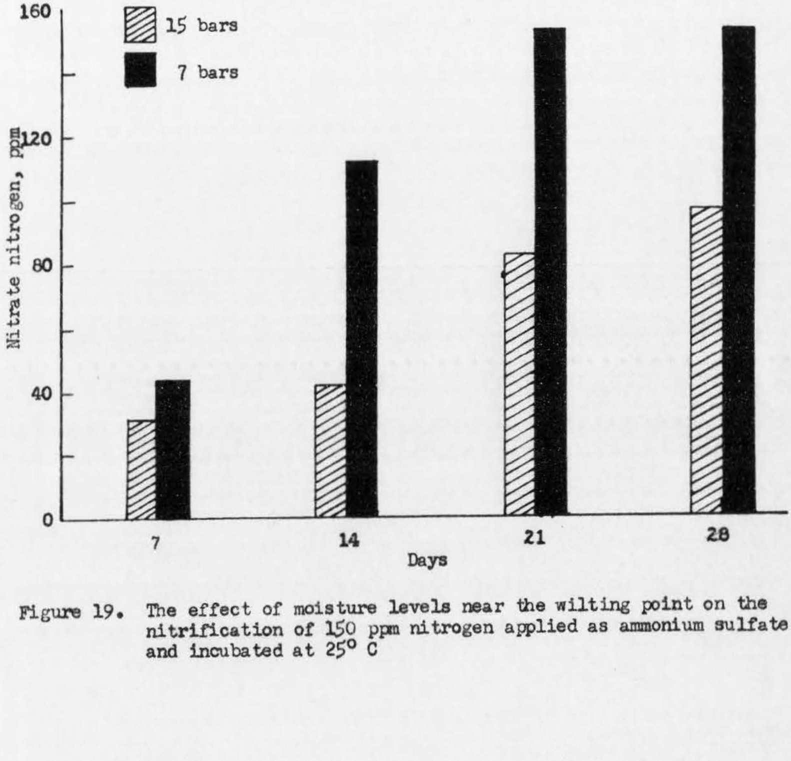

Figure 19. The effect of moisture levels near the wilting point on the nitrification of 150 ppm nitrogen applied as ammonium sulfate and incubated at 25° C

tension) and added to the 25° C bath as time and space would allow during this experiment. These were sampled at 21 and 28 days. The comparison of these two sampling dates with those of the 15 and 7 bars tension are shown in table 11. Comparing these two dates at 70 bars moisture tension, there was a significant reduction in ammonium nitrogen. The difference in nitrate formation is almost significant at the 0.05 level. There was no nitrite accumulation at any observation period shown in table 11. It should be pointed out that in the case of the 70 bars tension and 21 days incubation the total nitrogen is somewhat high in comparison with the other totals. This, of course, would indicate the possibility of an error in the ammonium nitrogen analysis which would contribute to the amount of ammonium reduction between these two dates. However, all three replications were consistent and even when an adjustment is made for this possible error, there was still a significant reduction in ammonium nitrogen.

Another possibility that might cause one to doubt the reality of this ammonium reduction being due to its oxidation is the fact that it might have been lost by volatilization from the system. It is true that there was a loss of total nitrogen from the system with time as can be seen in table 9. This loss was evidently due largely to volatilization of ammonium nitrogen as will be discussed later. It will be noted, however, that the loss was much greater at 35° C than at 25° C where the 7 bars moisture tension was used. It is concluded, therefore, that there is evidence of a slow rate of nitrification at this moisture tension which is considerably below the wilting point for higher plants. Further investigation in this moisture range is needed in order to draw any positive conclusions.

Table 11. Changes in ammonium nitrogen and nitrate nitrogen from the incubation of 150 ppm nitrogen as ammonium sulfate in a calcareous soil at 25° C

Moisture tension	Nitrogen analyzed	Days incubated	
		21	28
bars		ppm	
70	NH_4-N	172	139
	NO_3-N	29	42
15	NH_4-N	92	80
	NO_3-N	83	97
7	NH_4-N	29	31
	NO_3-N	153	151

For comparison between:	LSD NH_4-N	LSD NO_3-N
Two treatments for same date	20	27
Two periods for same treatment	15	18

Temperature effects

The comparison of nitrification at the two temperatures (25 and 35° C) is one of the most interesting observations of this experiment. As shown in figure 20, 25° C was the more favorable temperature for nitrification throughout the eight-week period at 7 bars moisture tension. At this moisture complete nitrification of the applied ammonium sulfate occurred in three weeks at 25° C. There was a slow rate of nitrification at 35° C for six weeks and then nitrification apparently stopped at approximately one-half the value for complete nitrification.

At 35° C and 7 bars moisture tension the nitrites remained relatively high throughout the first seven weeks (figure 21). At this moisture there was a significant drop in nitrites between the seventh and eighth week, reaching a level of insignificance. Since nitrate

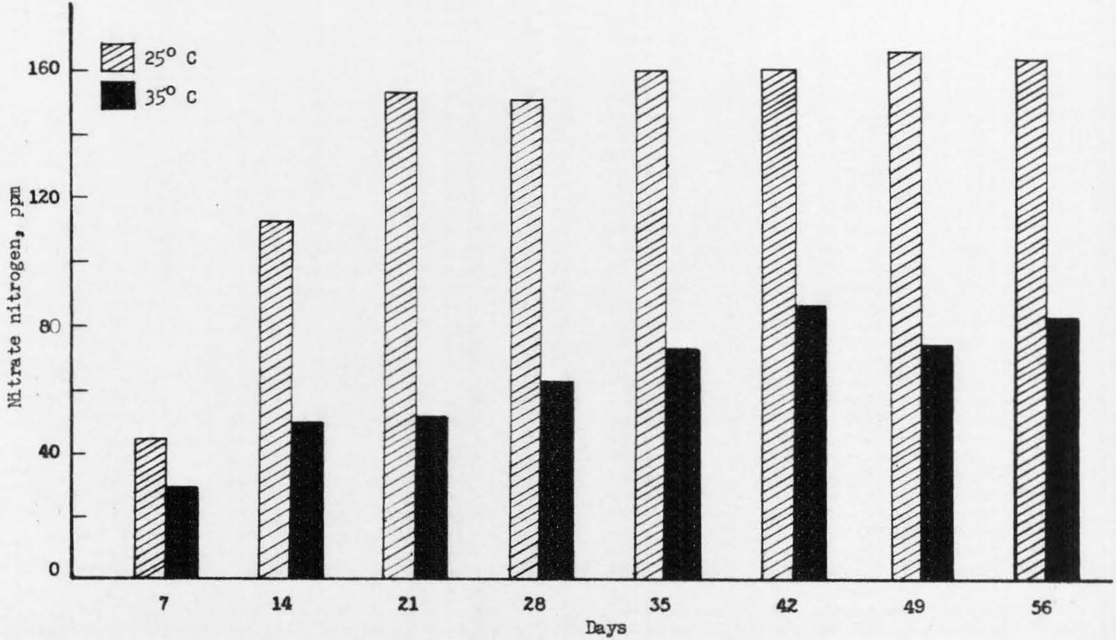

Figure 20. The effect of temperature on nitrification of 150 ppm nitrogen applied as ammonium sulfate with 7 bars moisture tension

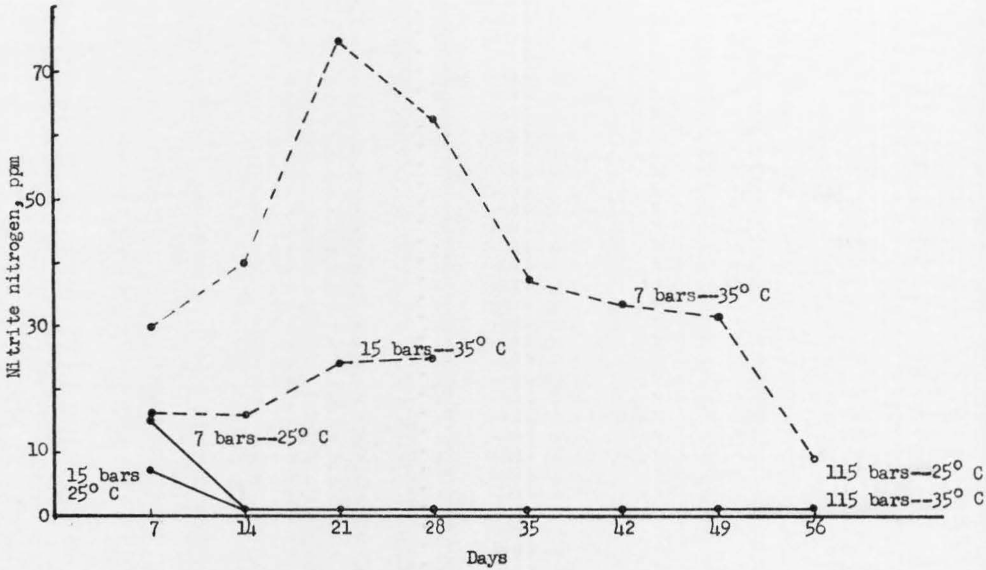

Figure 21. Changes in nitrite nitrogen during eight weeks incubation of ammonium sulfate added to Millville loam at the rate of 150 ppm nitrogen, as influenced by moisture level (7, 15, 115 bars) and temperature (25 and 35° C)

formation has tended to follow nitrite disappearance throughout this study, there is a possibility that the remaining unoxidized ammonium nitrogen may be oxidized to nitrates after the eight weeks. Figure 21 also shows that there was no nitrite accumulation with 25° C temperature and 7 bars moisture tension except at the initial (7-day) test. This is the same period at which nitrites were found in Experiment I under the more favorable conditions for nitrification. Also at 15 bars moisture tension and 25° C, nitrites were found in a measurable amount only at the 7-day period. At 35° C and 15 bars moisture tension, nitrites persisted throughout the four weeks these conditions were tested (figure 21). There was no nitrite accumulation at either 25 or 35° C with the other moisture levels.

At 35° C there was a consistently greater loss of total nitrogen from the system than from those maintained at 25° C. By the end of the incubation period (56 days) this loss was quite obvious as seen from the summation figures shown in table 9. These losses will be discussed later.

Interaction of moisture and temperature

Since the analysis of variance (appendix, table 22) shows a significant interaction between moisture and temperature, the two-way table of means (table 12) was prepared in order to examine this interaction more closely. The temperature means over all moisture levels show that 25° C was much more favorable for nitrification than 35° C. By examining the means within the table, it can be seen that the difference between temperature levels is much greater at the higher moisture, although the difference in temperature effects is significant at the 0.01 level for both moistures. The depressing effect on nitrification

Table 12. The mean nitrate nitrogen content for all moistures at all temperatures

Temperature	Moisture tension, bars		Means
	15	7	
°C	ppm		
25	63	115	89
35	37	48	43
Means	50	82	66

	LSD	
	0.05	0.01
Means within table	9	14
Table means	7	10

of the higher temperature can be seen by the fact that the difference between moisture effects is significant only at the 0.05 level, while at the lower temperature the difference in moisture effects is significant at the 0.01 level. Figures 22 and 23 show this interaction between moisture and temperature. In comparing the means of temperature and moisture, it can be seen that the differences in temperature had a greater effect on nitrification than did the differences in moisture though both differences resulted in significant effects.

Figure 24 shows the influence of moisture and temperature on the amount of nitrate nitrogen produced during the entire period of incubation. The 115 bars moisture level had shown no evidence of nitrification by the end of 56 days at either the 25 or 35° C temperature. The 7 bars moisture level at 35° C was only about 50 percent complete at the end of the 56 days and had shown no increase after the 42-day sampling period. On the other hand, nitrification of the 150 ppm added nitrogen

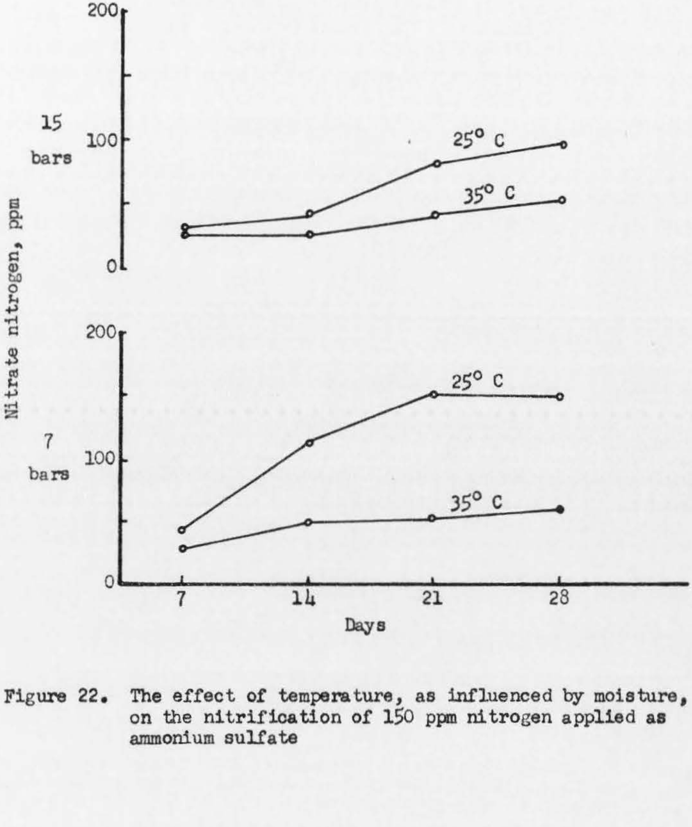

Figure 22. The effect of temperature, as influenced by moisture, on the nitrification of 150 ppm nitrogen applied as ammonium sulfate

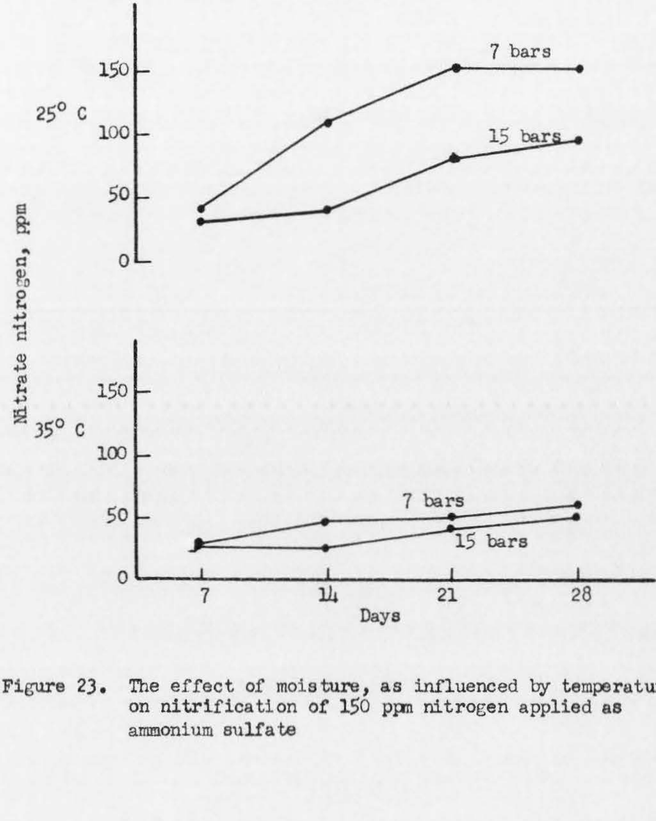

Figure 23. The effect of moisture, as influenced by temperature, on nitrification of 150 ppm nitrogen applied as ammonium sulfate

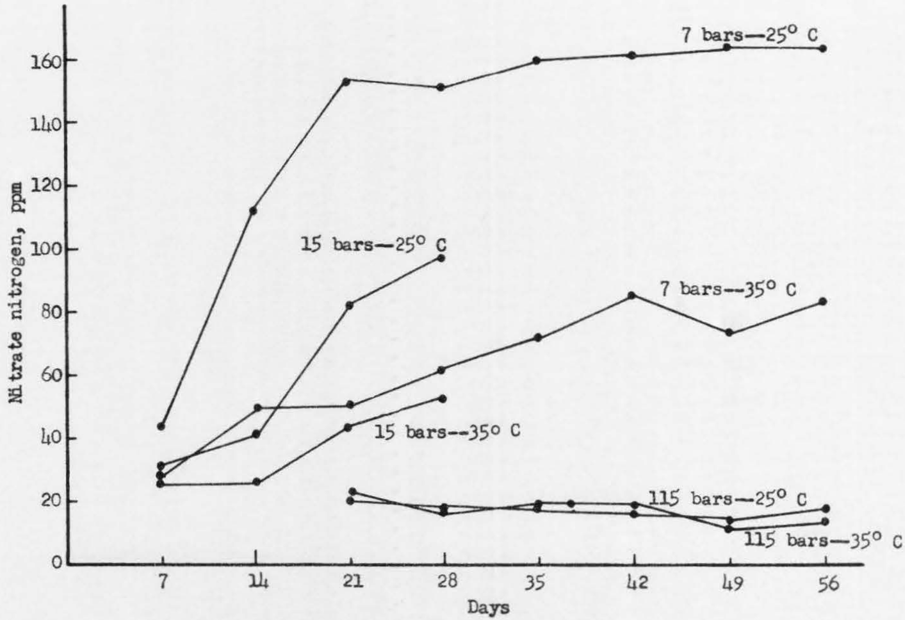

Figure 24. Changes in nitrate nitrogen during eight weeks incubation of ammonium sulfate added to Millville loam at the rate of 150 ppm nitrogen as influenced by moisture level (7, 15 and 115 bars) and temperature (25 and 35° C)

was complete by 21 days at 25° C and 7 bars moisture tension. In order to show further the magnitude of the effect of temperature, it can be seen from figure 24 that at 25° C the amount of nitrification at 15 bars moisture tension was greater than it was at 35° C even at a higher moisture (7 bars) and after a longer period of time (56 days).

Moisture control

Maintaining moisture at a constant level is one of the main problems in continuously aerated samples. In this experiment the desired amount of moisture calculated on the oven dry weight basis was mixed with the sample originally. Each week, when samples were taken for analysis, the moisture content was determined for each sample. Approximately 5 grams of moist soil was weighed, dried in the oven overnight at 110° C and the moisture expressed as a percentage of the oven dry weight. Table 13 shows the mean moisture levels for each weekly sampling period. The efficiency of moisture control during incubation is shown in table 14. More precise moisture control was obtained at the 25° C temperature than at 35° C. Better control was also generally obtained at the lower moisture levels. The best control, however, was at the 7 percent added moisture (15 bars) where the mean was 7.01, standard deviation, 0.25 and coefficient of variation, 3.57.

It should be pointed out that there was a fairly consistent loss of moisture with time in the case of the 9 percent original moisture addition (table 13). The highest variation was also found at this moisture (table 14). A plausible explanation for this is the fact that the relative humidity of the air passing over the samples at this soil moisture level was too low. The same relative humidity was used for this moisture level as was used for the samples originally receiving 7 percent

Table 13. Mean moisture levels by weekly sampling periods of Millville loam incubated at 25° and 35° C

Tempera-ture	Original moisture	Days incubated							
		7	14	21	28	35	42	49	56
°C	percent	percent							
25	3			3.08	2.89	2.79	2.86	2.84	2.93
	5			4.69	4.60	5.19	4.08	4.14	4.40
	7	7.05	6.67	7.25	7.06				
	9	8.41	8.61	8.87	8.41	8.33	7.89	8.09	7.69
35	3			2.95	2.78	3.30	2.69	2.76	2.62
	5			4.10	4.23	4.03	4.41	4.02	4.47
	7	7.04	6.36	7.17	6.88				
	9	8.45	8.23	9.40	8.65	7.74	8.74	8.28	7.02

Table 14. A comparison of various mean moistures at 25° and 35° C

Tempera-ture	Moisture		Standard deviation	Coefficient of variation
	Original added	Mean obtained		
°C	percent			percent
25	3	2.90	0.11	3.79
	5	4.59	0.42	9.15
	7	7.01	0.25	3.57
	9	8.25	0.56	6.79
35	3	2.79	0.23	8.24
	5	4.23	0.38	8.98
	7	6.85	0.45	6.57
	9	8.25	0.91	11.03

moisture. Due to the mechanical setup for this study, the relative humidity of the air stream could not be maintained above 99 percent. As previously shown, the calculated value for the 9 percent moisture level was 99.5 percent. With the size capillary being used, in order to get sufficient air to the samples, it was necessary to maintain at least 10 centimeters pressure on the line while the air was being humidified. This pressure being released as the air passed through the capillaries before going over the sample resulted in a decrease in relative humidity to 99 percent as calculated by the formula previously stated.

Nitrogen recovery

Complete recovery of the total inorganic nitrogen was not possible in either the first or second experiment of this study. This observation has been pointed out by many workers as discussed by Allison (1955) in his review. In this study, however, it was observed that nearly complete recovery was found under favorable conditions for rapid nitrification. On the other hand when nitrification proceeded very slowly, there was considerable loss of total inorganic nitrogen.

The most rapid nitrification condition of this experiment was at 7 bars moisture tension and 25° C. This is shown in figure 25 in relation to the total nitrogen recovered. It can be seen here that very little total nitrogen was unaccounted for.

At 35° C with the same moisture (7 bars), nitrification proceeded much slower (figure 26). The decrease in total recovered nitrogen with time in this case is very obvious. At the 56-day test the total recovered nitrogen was only 150 ppm in comparison to 187 ppm at the seven-day test. It is likely that some might have been lost during the first

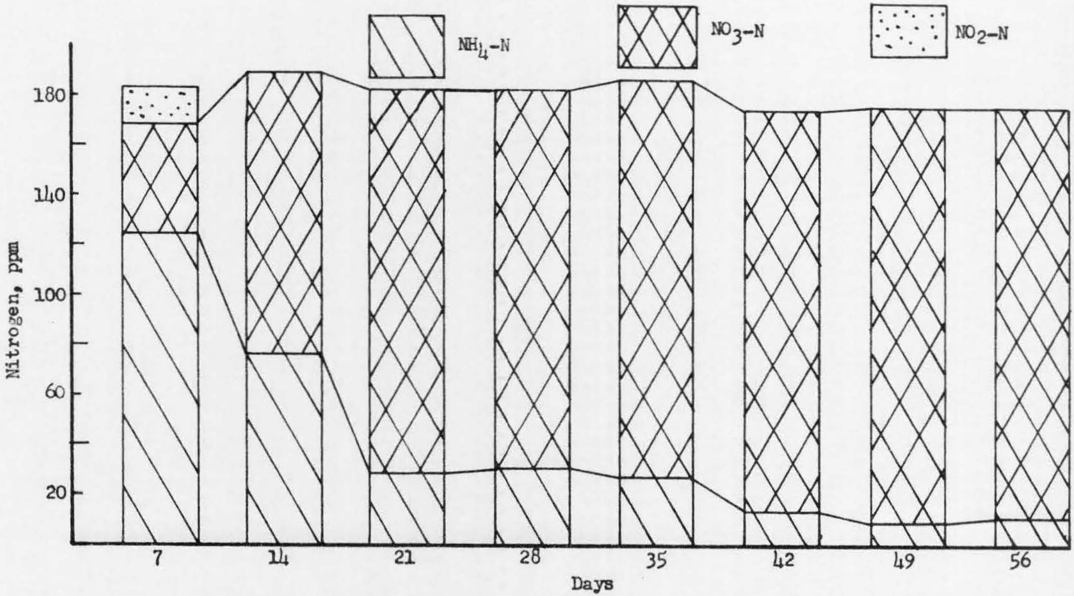

Figure 25. The total inorganic nitrogen (nitrate nitrogen, nitrite nitrogen and ammonium nitrogen) recovered at weekly intervals during the incubation of ammonium sulfate, added at the rate of 150 ppm nitrogen, at 7 bars moisture tension and 25° C

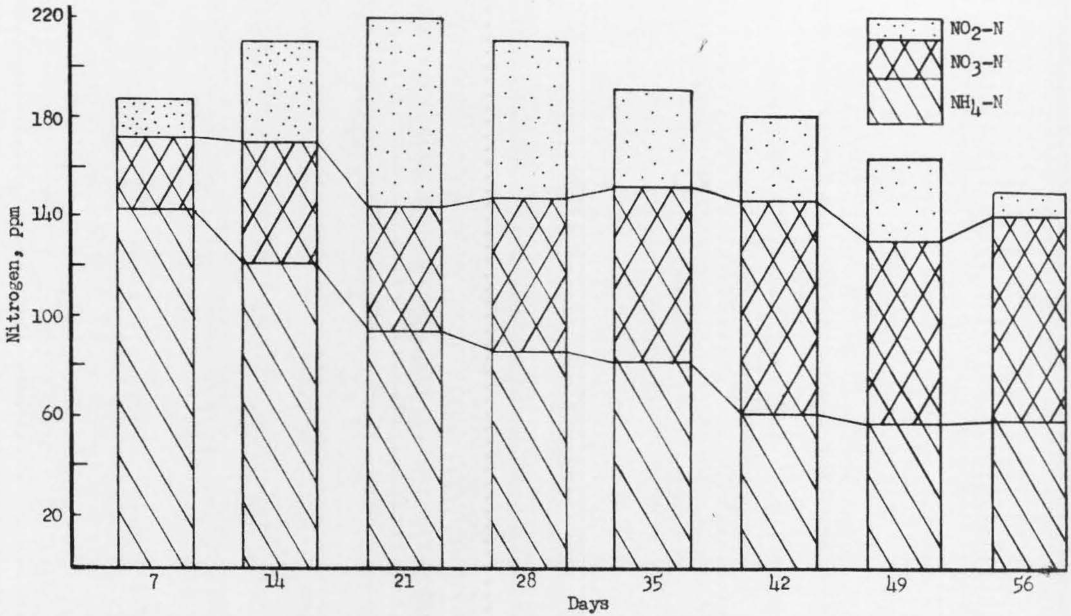

Figure 26. The total inorganic nitrogen (nitrate nitrogen, nitrite nitrogen, and ammonium nitrogen) recovered at weekly intervals during the incubation of ammonium sulfate, added at the rate of 150 ppm nitrogen, at 7 bars moisture tension and 35° C

week also. It should be noted here that there was a noticeable increase in total nitrogen up to 21 days, which is appreciably more than initially found and also more than that found for the same date for soils kept at 25° C. A probable explanation for this could be that ammonification is possibly accelerated at this high temperature while nitrification is hindered. Panganiban (1925) found that ammonification took place up to 60° C and that at higher temperatures the rate was faster. In the present study some ammonium nitrogen could have been formed from the soil organic matter at a faster rate than it was being lost for the first 21 days. Robinson (1957) has shown that ammonium nitrogen did accumulate at high temperatures where nitrification was hindered because of lowered moisture levels.

Under the conditions of this experiment the unrecovered nitrogen was evidently lost by volatilization of the ammonium nitrogen. Evidence for this is shown in figure 27. Here it will be noted that at the 415 bars moisture tension level, where no nitrification occurred, there was a loss in nitrogen with time. In fact, the greatest loss in total nitrogen occurred at the two moistures (415 and 115 bars) where there was no nitrification (table 9). Figure 27 also shows that there was greater loss of ammonium nitrogen at the higher temperature (35° C). This agrees with the conclusion made by Allison (1955) in his review on ammonia losses when he said that one of the main facts established regarding ammonia volatilization is that the losses increase with temperature.

To further establish the point that the unrecovered nitrogen was lost by volatilization of ammonium nitrogen, the 415 bars moisture tension was compared with the 7 bars moisture tension at 25° C, beginning with the 21-day incubation period (figure 28). It can be seen

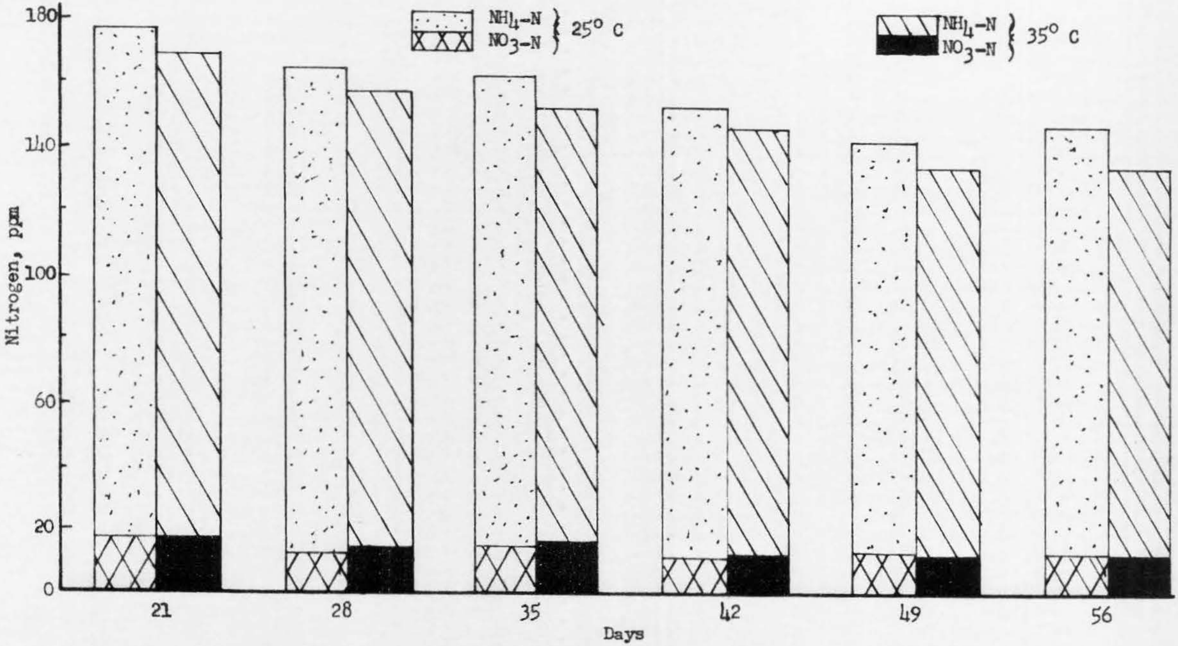

Figure 27. A comparison of total inorganic nitrogen recovered from incubation of 150 ppm nitrogen added as ammonium sulfate in a calcareous soil at 415 bars moisture tension and 25 and 35° C temperature

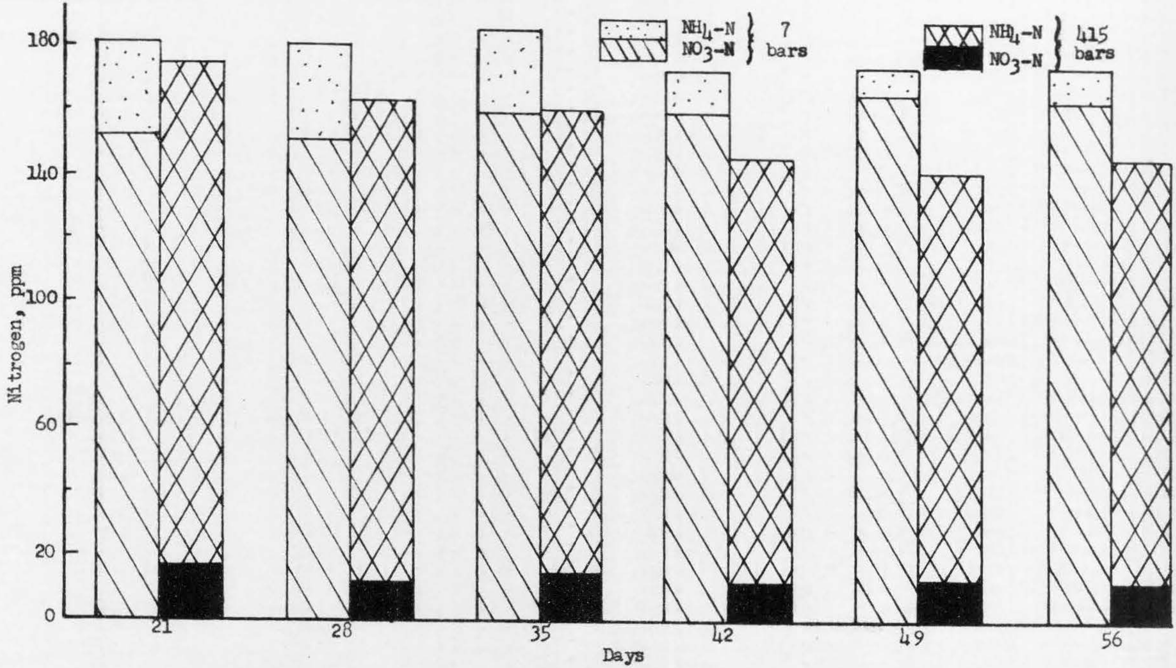

Figure 28. A comparison of total inorganic nitrogen recovered after incubation of 150 ppm nitrogen added as ammonium sulfate in a calcareous soil at 7 and 415 bars moisture tension and 25° C temperature

here that where there was little unoxidized ammonium nitrogen remaining, as was the case with the 7 bars moisture level after 21 days, there was very little reduction in total nitrogen recovered. Although the nitrates remained constant and no nitrites occurred at the 415 bars moisture, there was a consistent loss in total nitrogen which could only be accounted for as volatilization of ammonium nitrogen. Appreciable amounts of volatilization of ammonium nitrogen has been shown by other workers including Wahhab et al. (1957), Jewitt (1942), and Martin and Chapman (1951). These workers have especially associated the loss of ammonia with the loss of moisture. They agree that no loss of ammonia was observed when the loss in moisture ceased and that the ratio of ammonia loss to water loss should remain substantially constant. In the present study it was observed that the greatest loss of ammonia occurred at the highest temperature (35° C) and, as pointed out previously from table 14, the poorest moisture control was also found to be at this temperature. Although there was no consistent loss of moisture with time except in the case of the highest moisture (7 bars) discussed previously, there was a more nearly consistent moisture loss with time in the samples incubated at 35° C, where the ammonia loss was greatest, than at 25° C. It was also true, as seen by comparing tables 9 and 13, that the combination of the highest moisture (7 bars) with the highest temperature (35° C) showed the greatest loss of ammonia as well as the greatest loss of moisture with time. In other cases, however, there was a consistent loss of ammonia while under the same conditions there was no consistent loss in moisture. Under the conditions of this study, therefore, it was possible to show only a very general relationship between moisture loss and the loss of ammonia.

Summary of Experiment II

In order to expand the investigation of moisture effects on nitrogen transformations to include levels near and below the permanent wilting point for higher plants, a special apparatus was constructed to maintain these low moisture levels along with continuous aeration for long incubation periods. Constant moisture and aeration were maintained by passing a humidified air stream constantly over the samples. The moisture levels used in the study, reported in bars moisture tension for more adaptability in comparisons with other soils, were 7, 15, 70, 115 and 415 bars. Constant temperature was maintained in two water baths, one at 25 and one at 35° C. All samples were treated with 150 ppm nitrogen added as ammonium sulfate. The same soil (Millville loam) was used in this experiment as in Experiment I.

Nitrification was completely inhibited at 115 and 415 bars moisture tension. At these low moistures there was considerable loss of inorganic nitrogen with time. At 7 bars moisture tension and 25° C, nitrification was quite rapid--the 150 ppm applied nitrogen being completely oxidized in three weeks. At the wilting point for higher plants (15 bars tension) and 25° C, nitrification proceeded fairly rapidly with over half of the 150 ppm nitrogen added as ammonium sulfate being oxidized to nitrates in 28 days. Even at 70 bars moisture tension there was an indication of nitrification at 28 days.

The higher temperature (35° C) was not conducive to rapid nitrification in this soil. At 35° C nitrite accumulation persisted throughout the incubation period at both 7 and 15 bars moisture tension. Nitrate formation at 35° C was quite slow and had apparently ceased with only 50 percent nitrification after six weeks at the 7 bars moisture level. There was more effect exerted by temperature on nitrification than by moisture in this experiment as was also found in Experiment I.

There was a significant interaction between moisture and temperature. The difference between temperature effects was greater at 7 bars moisture tension than at 15 bars. The difference in moisture effects was much greater at 25° C than at 35° C.

Since one of the main problems in continuously aerated samples is that of maintaining a specific moisture level, the effectiveness of moisture control in this experiment was investigated. Moisture determinations by oven drying were made at each sampling date. In general the efficiency of moisture control was considered adequate. More precise control was obtained at 25° C than at 35° C. Better control was usually obtained at the lower moisture levels. The best control, however, was at 7 percent added moisture (15 bars). The better control on moisture at the drier levels was probably due to the fact that the air was more nearly humidified to the proper level.

Since there was a decrease in the total inorganic nitrogen recovered with time at the lowest moisture levels (115 and 415 bars) where no nitrification occurred and a negligible decrease at the highest moisture level (7 bars) where nitrification proceeded fairly rapidly, it was concluded that the loss in nitrogen from the system was due to volatilization of ammonium nitrogen.

GENERAL SUMMARY AND CONCLUSIONS

Incubation studies to determine the effects of moisture, temperature, applied ammonium nitrogen and their interactions on the transformations of nitrogen were conducted using a calcareous soil, Millville loam, under conditions of both constant and periodic aeration. The variables investigated in these studies included moisture levels ranging from 415 bars to 0.3 bar tension, temperatures from 2 to 35° C, and added nitrogen levels from 0 to 450 ppm nitrogen added as ammonium sulfate.

The studies consisted of two major experiments: one with 100 gram soil samples contained in sealed pint jars and aerated periodically, the other with 33.3 grams of soil in 250 milliliter flasks with a humidified air stream providing constant aeration. The first experiment was designed primarily to investigate the combination of relatively high moisture levels with low temperatures. In the second experiment the design was primarily for the combination of low moisture levels with relatively high temperatures.

The following observations and conclusions were noted:

1. Nitrification of applied ammonium nitrogen proceeded at a moderate rate at a soil moisture level equal to the wilting point for higher plants. There was evidence that nitrification was proceeding at a slow rate at a moisture as low as 70 bars tension. The rate of nitrification increased with an increase in soil moisture up to 1 bar moisture tension. No increase in nitrification was found between 1 and 0.3

bar moisture tension when other conditions were favorable. It is concluded, therefore, from these studies that the amount of moisture between the permanent wilting point and field capacity has less effect on the nitrification process than is indicated in the literature.

2. Temperature, within the range of 2 to 35° C, exerted considerably greater effect on nitrification than did moisture levels between the wilting point and field capacity. Some nitrification of applied ammonium sulfate was found as low as 2° C after a period of 70 days, following an extended period of nitrite accumulation. At both 10 and 35° C there was a prolonged period of nitrite accumulation along with a very slow rate of nitrate formation, indicating a fairly strong inhibition to nitrate formation by these conditions. No inhibitions resulting in a delay of more than 7 days were noted at 22 or 25° C; therefore, the optimum temperature for nitrification of ammonia in this soil must lie between 10 and 35° C and probably near 25° C.

3. In both experiments there was significant interaction between moisture and temperature. Where different levels of applied ammonium sulfate were used, there was also significant interaction between the amount of fertilizer applied and moisture, as well as between fertilizer and temperature.

4. Under the conditions of high temperature (35° C) and/or low moisture (115 and 415 bars tension) there was a considerable deficit in the amount of inorganic nitrogen recovered. Since at these low moisture levels no nitrification was shown, it is concluded that this loss was due to the volatilization of ammonium nitrogen.

5. High levels of applied ammonium nitrogen (450 ppm nitrogen) resulted in inhibition of nitrate formation along with nitrite

accumulation. This inhibition resulted in a time delay in the nitrification process which varied in length, depending upon the other conditions for nitrification.

6. Significant nitrite accumulation persisting for long periods (up to 56 days) were found at both low (2 and 10° C) and high (35° C) temperatures, especially in the case of high (450 ppm nitrogen) ammonium nitrogen application. Because of this nitrite accumulation, it is concluded that the nitrite oxidizing organisms are more sensitive to such adverse conditions than are the ammonium oxidizers.

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A P P E N D I X

Table 15. Analysis of variance for changes in nitrate nitrogen in a calcareous soil incubated at various moistures, temperatures, and levels of nitrogen added as ammonium sulfate (table 2)

Source of variation	Degrees of freedom		Sum of squares	Mean square
Treatments	26			
Moisture		2	72,206	36,603
Temperature		2	493,617	246,809
Fertilizer		2	280,478	140,239
Moisture x temperature		4	45,824	11,456
Moisture x fertilizer		4	43,728	10,932
Temperature x fertilizer		4	309,606	77,402
Temperature x fertilizer x moisture		8	33,223	4,028
Experimental error (a)	27		1,612	60
Incubation periods	3		302,477	100,826
Treatments x incubation periods	78		755,672	9,688
Experimental error (b)	81		2,777	36
Total	215		2,341,220	

Table 16. Analysis of variance for changes in nitrite nitrogen in a calcareous soil incubated at various moistures, temperatures, and levels of nitrogen added as ammonium sulfate (table 7)

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments	26	21,711	835
Experimental error (a)	27	789	29
Time	3	1,177	392
Time x treatments	78	30,140	386
Experimental error (b)	81	2,278	30
Total	215	56,095	

Table 17. Analysis of variance for changes in ammonium nitrogen in a calcareous soil incubated at various moistures, temperatures, and levels of nitrogen added as ammonium sulfate (table 6)

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments	26	5,594,743	215,182
Experimental error (a)	27	15,339	568
Time	3	375,248	125,083
Time x treatments	78	1,064,163	13,643
Experimental error (b)	81	53,275	692
Total	215	7,102,768	

Table 18. Analysis of variance for changes in nitrate nitrogen from the incubation of ammonium sulfate in a calcareous soil at 2° C (table 8)

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments	3	17,772	5,924
Experimental error (a)	4	636	159
Time	3	208	69
Time x treatments	9	1,538	171
Experimental error (b)	12	729	61
Total	31	20,883	

Table 19. Analysis of variance for changes in nitrite nitrogen from the incubation of ammonium sulfate in a calcareous soil at 2^{o} C (table 8)

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments	3	39,041	13,014
Experimental error (a)	4	89	22
Incubation periods	3	1,345	448
Treatments x incubation periods	9	1,870	623
Experimental error (b)	12	1,410	118
Total	31	43,755	

Table 20. Analysis of variance for changes in ammonium nitrogen from the incubation of ammonium sulfate in a calcareous soil at 2^{o} C (table 8)

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments	3	604,765	201,588
Experimental error (a)	4	1,700	425
Incubation periods	3	1,093	364
Treatments x incubation periods	9	2,052	228
Experimental error (b)	12	2,026	169
Total	31	611,636	

Table 21. Analysis of variance for changes in ammonium nitrogen from the incubation of 150 ppm ammonium sulfate in a calcareous soil at various moistures and temperatures (table 10)

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments	3	21,849	7,283
Experimental error (a)	8	162	20
Incubation periods	3	27,505	9,168
Treatments x incubation periods	9	4,850	539
Experimental error (b)	24	1,505	63
Total	47	55,871	

Table 22. Analysis of variance for changes in nitrate nitrogen from the incubation of 150 ppm ammonium sulfate in a calcareous soil at various moistures and temperatures (table 10)

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments			
Moisture	1	11,657	11,657
Temperature	1	26,508	26,508
Moisture x temperature	1	5,125	5,125
Experimental error (a)	8	809	101
Incubation periods	3	25,151	8,384
Treatments x incubation periods	9	10,567	1,174
Experimental error (b)	24	1,416	59
Total	47	81,233	

Table 23. Analysis of variance for changes in nitrite nitrogen from the incubation of 150 ppm ammonium sulfate in a calcareous soil at various moistures and temperatures (table 10)

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments	3	19,422	6,441
Experimental error (a)	8	840	105
Incubation periods	3	825	275
Treatments x incubation periods	9	3,959	440
Experimental error (b)	24	1,801	75
Total	47	26,847	

Table 24. Analysis of variance for changes in nitrate nitrogen from the incubation of 150 ppm ammonium sulfate in a calcareous soil at 25° C and various moistures (table 11)

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments	2	40,773	20,387
Experimental error (a)	6	1,064	177
Incubation periods	1	346	346
Incubation periods x treatments	2	232	116
Experimental error (b)	6	507	85
Total	17	42,922	

Table 25. Analysis of variance for changes in ammonium nitrogen from the incubation of 150 ppm ammonium sulfate in a calcareous soil at 25° C and various moistures (table 11)

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments	2	47,554	23,777
Experimental error (a)	6	409	68
Incubation periods	1	924	924
Incubation periods x treatments	2	883	442
Experimental error (b)	6	168	35
Total	17	49,938	