Nitrification in Three Different Soils in Polyethylene Bags in the Field Overwinter

William R. Olmstead
Utah State University

NITRIFICATION IN THREE DIFFERENT SOILS IN POLYETHYLENE BAGS IN THE FIELD OVERWINTER

by

William R. Olmstead

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

of

MASTER OF SCIENCE

in

Soil Fertility

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1966

ACKNOWLEDGMENTS

I am grateful to Dr. R. L. Smith for the opportunity to do graduate work and for the advice and encouragement that he gave me. I am also grateful to the other committee members, Dr. G. W. Miller, Dr. D. R. Walker, and Dr. D. McAllister, for their support and advice.

The author is also thankful to M. K. Mahendrappa, A. S. Mostafa, and V. Hunsaker for the help and advice that they extended to me.

Further appreciation is expressed for funds supplied under Regional Research Funds, W-el, which partially supported the research herein reported.

William R. Olmstead

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure | Page

INTRODUCTION

Nitrification, the process whereby ammonical-nitrogen (NH_4^+-N) is changed into nitrate-nitrogen (NO_3^--N), is one of the more important biochemical processes associated with agriculture. Nitrate appears to be the form of N that most crops can most readily utilize.

The practice of applying fertilizers to supplement the soil's reservoir of nutrients has increased tremendously in recent years. This is particularly true in the case of N, the mineral nutrient probably used in greatest quantities by plants.

For several reasons the form of N applied to arid region soils is the ammonical form. In soils the ammonia (NH_3) or ammonium ion (NH_4^+) must be converted into nitrate before much of the N becomes available to the crop. This results in a delay period between the time of fertilizer application and the time of nitrate availability. Under normal growing conditions, this delay may be as short as a day or two. Under unfavorable conditions of low temperatures or saturated soils the conversion may take months.

When applying N fertilizers, one is faced with the question of applying ammonical fertilizers in the fall with the hope that the nitrification will be delayed until spring so that nitrate will be available for early crop use or waiting until spring and hoping that the nitrification will be rapid enough for plant use. There are several reasons why the practice of fall application of ammonical fertilizers seems desirable. In the fall the farmer usually has more time than in the spring. The land is not likely to be as wet. He can get equipment on his land and can do a good job of applying a fertilizer.

However, the merit of fall-applied fertilizers, even ammonical N, is under question. It is recognized that NH_4^+ is positively charged and is held tightly by the negatively charged soil particles. For this reason, the winter moisture would be expected to remove little of the N if it remained as NH_4^+. On the other hand, the nitrate ion is negatively charged and not attracted to the negatively charged soil particles. If there are possibilities that NH_4^+-N might be converted to NO_3^--N during the fall and winter months, then the value of fall application of ammonical fertilizers might be questioned.

The purpose of this study was to check the extent of nitrification of $(NH_4)_2SO_4$ in three soils that were kept in polyethylene bags at a 4-inch depth overwinter. By placing the soils into the field at weekly intervals during the fall and measuring the nitrification periodically, it should be possible to determine the effect of an early warm temperature on prolonged nitrification when the temperature drops.

REVIEW OF LITERATURE

Many factors are known to influence the nitrification rate. The effect of moisture content, soil reaction, temperature range, and oxygen level will be discussed.

Moisture Content

It has long been known that nitrification is sensitive to soil moisture. Russel, Jones, and Bahrt (1925) suggested that low moisture interfered with nitrate production. Waksman (1952), in reviewing early work on this problem, concluded that the range most favorable for nitrification was between 0.15 and 0.5 bars moisture tension. Justice and Smith (1962) showed that considerable nitrification may proceed at 15 bars and the process is still detectable at moisture levels far below this. Although they reported the optimum moisture to be at one-third bar, they concluded that the different moistures between 0.3 and 7 bars had less effect on nitrification than did changes in temperature.

While studying the influence of moisture on nitrogen interchanges in the soil, Miller and Johnson (1964) found that carbon dioxide evolution, a measure of microbial activity, was at a minimum when the soil was air dry, and at a maximum when the soil was at 0.15 to 0.5 bar. Carbon dioxide evolution decreased as tension dropped below 0.15 bar. They also found that nitrification was greatest at 0.15 to 0.5 bars, and the nitrification slowed but was still appreciable when tensions reached 15 bars. Ammonification proceeded faster than nitrification when the soil was either air dry or saturated than in the moisture range between. Thus, an ammonium accumulation could occur at either moisture extreme.

Soil Reaction

In general, it is accepted that nitrification proceeds more rapidly in alkaline than in acid soils. Waksman (1952) suggested the pH most favorable for nitrification to be greater than 4.6. Anderson and Purvis (1955) found that as pH decreases, nitrification decreases. They limed a Nixon soil with a pH of 4.9 until its pH was 6.8. When given the same treatment under the same conditions, it was found that the acid Nixon (pH 4.9) lagged behind the limed Nixon (pH 6.8) by about 2 weeks in nitrate production.

Millbank (1959), studying Kenya highland soils that were humic red latosols, found that as the pH reached 8.5 and above, Nitrosomonas, organisms that oxidize NH_4^+-N to NO_2^-, are favored over the Nitrobacter, organisms that oxidize NO_2^--N to NO_3^--N, and a nitrite accumulation occurred. When ammonium sulfate was added to a fresh soil, the buildup of ammonium oxidizers was normal over a pH range of 6.0 to 8.8, showing no pH effects on Nitrosomonas whether lime was present or not. The Nitrobacter, however, was affected by pH values over 7.7, giving rise to a slower proliferation rate and a net nitrite buildup. Morrill and Dawson (1961) found that when substrate is not limiting, Nitrobacter grows faster than Nitrosomonas. The generation time for Nitrobacter was always less than one-half of the time required for Nitrosomonas up to a pH of 7.2. Growth rates appear to be about equal at pH 7.6 to 7.8. They found that the most favorable pH range for Nitrobacter is

6.2 to 7.0, and the most favorable range for Nitrosomonas was above pH 7.4. Silver (1961) found that Nitrobacter worked better at pH 6.8 to 7.0 than from 7.0 to 8.2.

Some workers have suggested a pH threshold for nitrification (Stojanovic and Alexander, 1958). Millbank (1959) refutes the idea of a pH threshold value as a function of pH itself. He found that when the Nitrobacter was allowed to build up at a pH of 8.5, the oxidation of nitrite would occur rapidly. Tyler, Broadbent, and Hill (1959) suggest that the apparent threshold may be related to the sluggish growth of Nitrobacter in alkaline pH's. They further indicate that the effect of high pH on the Nitrobacter may be due to the high level of NH_3 that can be present when large quantities of ammonical fertilizers are added.

Temperature Range

Panganiban (1925) found that slight daily variations in temperature depressed nitrification, but greater daily variations seemed to counteract this effect. He found nitrification to be active between 15 and 40° C. Waksman (1952) listed the most favorable temperature for nitrification as 27.5° C.

Anderson and Purvis (1955) observed nitrification in Annandale loam at 3° C. Optimum temperatures for nitrification occurred from 6 to 9° C in Washington loam, Nixon sandy loam, and Freehold loamy sand. At 11° C the nitrification rate slowed down in these soils. Frederick (1956), studying solution cultures, found nitrification to proceed between 3 and 40° C. In calcareous soils he found some nitrification at 0° C. Frederick lists nitrification rates for the soils he studied as follows: slow, 0 to 20° C; faster, 20 to 27° C; optimum, 27 to 35° C; slower, 35 to 40° C; stops above 40° C. Sabey, Frederick, and Bartholomew (1959) found the optimum temperature for nitrification to be 25° C.

Tyler, Broadbent, and Hill (1959), in studying low temperature effects on nitrification in California soils, found that Nitrobacter was more sensitive to colder temperatures than Nitrosomonas. They also found that some nitrification occurred at 0° C. Justice and Smith (1962) also found Nitrobacter to be sensitive to low temperatures and found a nitrite accumulation at 0 or 10° C.

Chandra (1962) found the optimum temperatures for nitrification in some Canadian soils to be between 27 and 30° C. He found a greater effect on nitrification when going from suboptimal to optimal temperatures than when going from optimal to suboptimal temperatures.

Anderson (1960), studying Cecil sandy loam (red-yellow-podzolic) with a pH of 6.7, found that added ammonium salts enhanced the low temperature effect on Nitrobacter. He found the nitrification was more active at 6° C and 50 ppm added NH_4^+-N, than at 6° C and 100 ppm NH_4^+-N.

Oxygen Level

It is usually accepted that the oxygen content should be about the same as that in the atmosphere in order to insure an adequate level of oxygen (Amer and Bartholomew, 1951). If the overall oxygen level of the soil drops, there are possibilities that micro-habitats will occur where denitrification can take place (Broadbent and Stojanovic, 1952). Brandt, Wolcott, and Erickson (1964) found that if the oxygen supply dropped below 20×10^{-18} $grams^{1}$ cm^{-2} min^{-}, the nitrate would be reduced (denitrification).

METHODS AND PROCEDURES

Soils

Description

The soils selected for this study were chosen from a group of 20 that had been collected from the Western United States (Mahendrappa, 1963; Mahendrappa et al., 1966). These particular three were from about the same latitude but were expected to show differing responses to temperature. Some physical and chemical characteristics of these soils are given in table 1.

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

Soil	pH	Organic matter	N	Moisture content	
				0.3 bar	Air dry
	paste	percent	percent	percent	percent
Kidman loam	7.5	2.2	0.13	14.8	1.6
Yolo loam	6.8	1.4	0.08	20.7	2.1
Millville loam	7.9	2.0	0.09	16.2	1.1

Soil preparation

The soils were obtained from the surface 6 inches, air dried, screened through a 2-mm sieve, and stored air dry until used. In all cases 50 g oven-dry soil were used. All soils were brought to about field capacity (0.3 bar) by adding distilled water. To those soils receiving N, ammonium sulfate solution was added as part of the required moisture to give 150 ppm N. Controls, soils receiving no added N, were also included. The soils were thoroughly mixed in wax-coated paper cups and then transferred to pint-size polyethylene bags and sealed with an elastic band. It had previously been shown that nitrification was not impeded in the polyethylene bags (Smith, 1963). The bags maintained the moisture but allowed the oxygen and carbon dioxide to move in or out as the need be. Sufficient sets were set up so that samples could be taken periodically to determine the extent of nitrification. Samples were taken as indicated by previous experience and by the previous analysis. Thus, samples in the cold, either in the laboratory or in the field, would have considerable time intervals between them. In all instances a control sample was taken along with the treated soils, and the amount of N found in the analysis of the soil from the control sample was subtracted from that found in the treated soil.

Soil analyses

The soils were analyzed periodically for NH_4^+-N, NO_3^--N, and NO_2^--N. Ammonium was determined by Nesslerization of a potassium chloride extract. The procedure used was the same as the one given by Jackson (1958) with a few modifications. Nitrite and nitrate were both determined from a calcium hydroxide extract. The nitrite was determined by the use of sulfonilimide and a coupling reagent (Shin, 1941). The nitrate was determined by the phenoldisulfonic acid method described by Jackson (1958).

Design of Experiments

Laboratory experiment

The laboratory experiment was set up to be used as a means for evaluating the results of the field experiment. For this experiment, different rates of nitrification were initiated at 25° C and then subjected to 2° C temperatures for sustained periods. Four rates of nitrification were studied. These were 3, 6, 9, and 14 days at 25° C. At the end of the 25° C incubation period, the samples were placed in a controlled temperature room at 2° C. A set of nine samples were removed from the cold room at different periods of time for analysis. Each set of nine samples contained two treated samples and one blank sample for each of the three soils, Millville loam, Yolo loam, and Kidman loam.

Field experiment

This study was designed to find out what effect the fall, winter, and spring temperatures have on nitrification of applied ammonical fertilizers. Six series of soil samples in polyethylene bags were buried in the field at a depth of 4 inches throughout the fall and one in the early spring. The design was to show the effect of early fall temperatures on nitrification as well as the effect that the moderate temperatures of late fall might have on the continuation of nitrification when the temperatures had dropped below the level at which nitrification was supposed to stop.

The times at which the various series were set up were:

(1) September 16, 1964

(2) September 30, 1964

(3) October 10, 1964

(4) October 16, 1964

(5) November 4, 1964

(6) November 24, 1964

(7) March 7, 1965.

The soils were buried at the 4-inch depth in such a way that they could be sampled without disturbing the remainder of the group. Thus, each unit consisted of 9 polyethylene bags, two treated and one check for each of the three soils.

A sensor probe of a maximum-minimum thermometer was also buried at the 4-inch depth. Daily records of the maximum and minimum temperatures at this depth were obtained.

RESULTS AND DISCUSSION

Laboratory Experiment

Millville loam

The results of the laboratory experiment on Millville loam are graphed in Figure 1. The upper portion of the graph plots NH_4^+-N in parts per million on the vertical axis against time in days along the horizontal axis. The lower portion shows the changes in NO_3^--N for the same time intervals. The vertical dotted line is when the samples were placed into the cold temperature room and is designated as time zero. Proceeding from time zero to the left of the graph gives days of incubation at 25° C. Proceeding right from time zero gives the length of time in cold temperature or 2° C room.

Three-day incubation at 25° C. The nitrifiers were allowed a short time to produce their numbers and probably only a small population was present when the 2° C treatment was started. As would be expected, the NH_4^+-N level dropped during the 3-day incubation period. The cold temperature treatment seems to have checked further the NH_4^+-N decrease. The slight increase in NH_4^+-N at the end of the second week is probably not significant. During the third week, the decline of NH_4^+-N may be due to nitrification as indicated by the rise in NO_3^--N. In the fourth week the NH_4^+-N showed an increase. This can only be explained as analytical variability since each week new samples are harvested. It is possible that the organisms in some sets did get started during the 3-day incubation at 25° C. During the following 10 days, the NH_4^+-N decrease indicates increased nitrifier activity. During

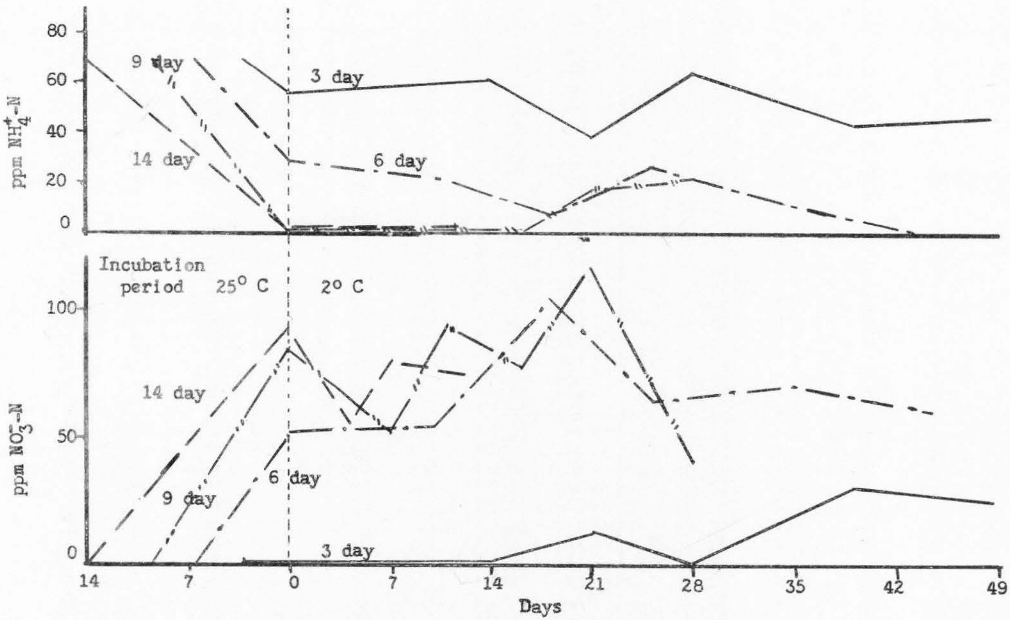

Figure 1. The nitrification patterns observed when 150 ppm N, added as $(NH_4)_2SO_4$, was incubated in Millville loam in polyethylene bags at 2° C following four different time periods, 3, 6, 9, and 14 days, at 25° C

the 7-week experiment, the NH_4^+-N level had a net drop of 10 ppm. The accumulation of nitrates was slow, and none was detected until the third week. The fourth week showed no nitrate accumulation and is balanced by the NH_4^+-N in the sample. The net NO_3^--N accumulation was about 25 ppm. Nitrites were never present in detectable amounts which would indicate that at 2° C the Nitrobacter is as active as the Nitrosomonas.

Six-day incubation at 25° C. This longer period of incubation probably allowed a larger population of nitrifiers to develop before the cold temperature treatment was begun. Apparently the organisms remained active enough to nitrify all of the added NH_4^+-N by the end of the 7-week period. The NH_4^+-N level declined rapidly during the incubation period; but when the samples were placed in the cold temperature roon, the rate of NH_4^+-N disappearance was sharply reduced. The NH_4^+-N level dropped about 40 ppm during the 6-day incubation period and only 22 ppm in the first 18 days of 2° C treatment. The nitrate accumulation for the 6-day incubation samples was greatly influenced by the 2° C treatment. In the 6 days of incubation, there was accumulated over 50 ppm NO_3^--N. The NO_3^--N line in Figure 1 would be steeper if you consider the possibility that this NO_3^--N level was reached in the last 3 days of incubation. Upon being placed in the new environment and analyzed 10 days later, the NO_3^--N level was only slightly changed. During the 10- to 18-day period, it would seem that the organisms were adapted to the new environment as the NO_3^--N level increased by more than 50 ppm. The reasons for the decline in NO_3^--N during the latter stages of the experiment are not understood. It is possible there was some incorporation in the bodies of the organisms, but it seems unlikely the pattern would be as shown. It is also possible there was some denitrification, but there was no evidence of lowered O_2 levels. It had been shown previously that the atmosphere in polyethylene bags remained sufficiently high in O_2 to carry out rapid nitrification (Smith, 1963).

Nine-day incubation at 25° C. All of the added NH_4^+-N was nitrified before the 2° C treatment was begun. However, the samples were still put into the cold temperature room for 4 weeks. The interesting thing was the NH_4^+-N buildup during the third and fourth weeks. This buildup is similar to that expressed in the 3- and 6-day incubation experiments at this period of time. The NO_3^--N accumulation seemed to be governed by an internal cycle when there was no more NH_4^+-N substrate. Each of the three peaks is higher than the one before it. It may be that the added NH_4^+-N that was never recovered and thought to be utilized for cellular growth of the organisms is now being released, giving rise to still another population buildup that soon exceeds its substrate limits and then dies off. Should this be the case, it seems that after the third week, most of the tied-up NH_4^+-N has been released and nitrified. Again, no nitrites were detected in the samples.

Fourteen days at 25° C. As would be expected, all of the added NH_4^+-N was nitrified before the 2° C treatment was begun. The NH_4^+-N level remained at zero for the following 12 days and analysis was therefore discontinued. The NO_3^--N level after 14 days of 25° C temperature was less than 10 ppm above the zero time level of the 9-day incubation samples. Although the analysis stopped after 12 days of 2° C treatment, it was apparent that the NO_3^--N line would follow the same pattern as it did in the 9-day incubation samples, and probably for the same reasons. No nitrites were detected.

Yolo loam

The graph in Figure 2 is patterned after the graph in Figure 1, only it is for the Yolo loam. The Yolo loam soil is from the warmest climate of the three soils examined and was expected to show the greatest effect of cold temperature.

Three-day incubation at 25° C. This group of samples nitrified about 10 ppm more of the added NH_4^+-N than did the Millville soil at the outset of the incubation period. Otherwise, the NH_4^+-N line patterns for the two soils are nearly identical. The rate of NH_4^+-N disappearance was checked by the 2° C temperature. After some erratic values at the 3-week sampling, the remaining weeks showed a continual decrease in NH_4^+-N. Even though there was a decline in the NH_4^+-N level, there was never any evidence of nitrite or nitrate production.

Six-day incubation at 25° C. These samples reached about the same degree of NH_4^+-N loss as did the corresponding Millville samples at the zero time analysis. However, the 2° C temperature checked the Yolo nitrification rate in the Yolo soil more than in the Millville soil. After the seventh week at 2° C, the NH_4^+-N level dropped markedly. There was a high level of NO_3^--N for the 6-day samples at zero time. The next sampling showed that there was no NO_3^--N in the soil. This could have been used up by the organisms that had been stimulated during the 6 days at 25° C. The 10- to 25-day period showed a slight buildup of nitrates, but after this period, the nitrate curve leveled off. In this respect the nitrification patterns in the Yolo soil differ markedly from that found in the Millville soil. No nitrites were found during the 7-week period.

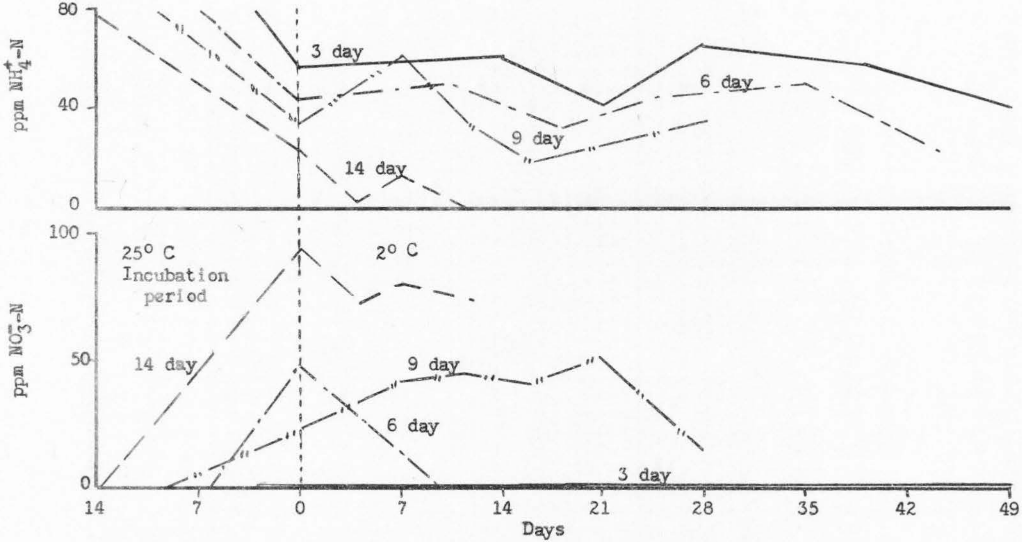

Figure 2. The nitrification patterns observed when 150 ppm N, added as $(NH_4)_2SO_4$, was incubated in Yolo loam in polyethylene bags at 2° C following four different time periods, 3, 6, 9, and 14 days, at 25° C

Nine-day incubation at 25° C. During the incubation period the nitrifiers dropped the NH_4^+-N level from 78 ppm to 43 ppm. The 2° C temperature treatment slowed the rate of NH_4^+-N loss or disappearance. Although there was an apparent increase in NH_4^+-N at the end of the first week at 2° C, the overall effect was one of slow nitrification. The NO_3^--N level at the end of the 9 days at 25° C was more in keeping with what was expected for this soil than was that found for the 6-day incubation. This rate of NO_3^--N accumulation held steady during the first week of 2° C treatment while the same samples showed an NH_4^+-N accumulation. This suggests that there may have been a stimulation of ammonification of indigenous organic matter during the period at 25° C that carried on for a few days when the soil was placed at 2° C. The overall nitrification for Yolo soil for this incubation period was less than found for the Millville soil. There was less stimulation at 25° C and also less continuation of the nitrification at 2° C. The cold temperature seemed to stop the process in case of the Yolo soil but only slow it down in the case of the Millville soil. No nitrites were detected during the 4-week period.

Fourteen days at 25° C. As would be expected, these samples reached the greatest rate of NH_4^+-N disappearance. Even when put into the cold temperature room, the NH_4^+-N was continued to be used. The NO_3^--N accumulation rate was rapid until the cold temperature treatment started. There was the drop in NO_3^--N level that had been noted in other samples. No nitrites were detected during the 12-day period at 2° C.

Kidman loam

The graph in Figure 3 is plotted for the Kidman loam used in the

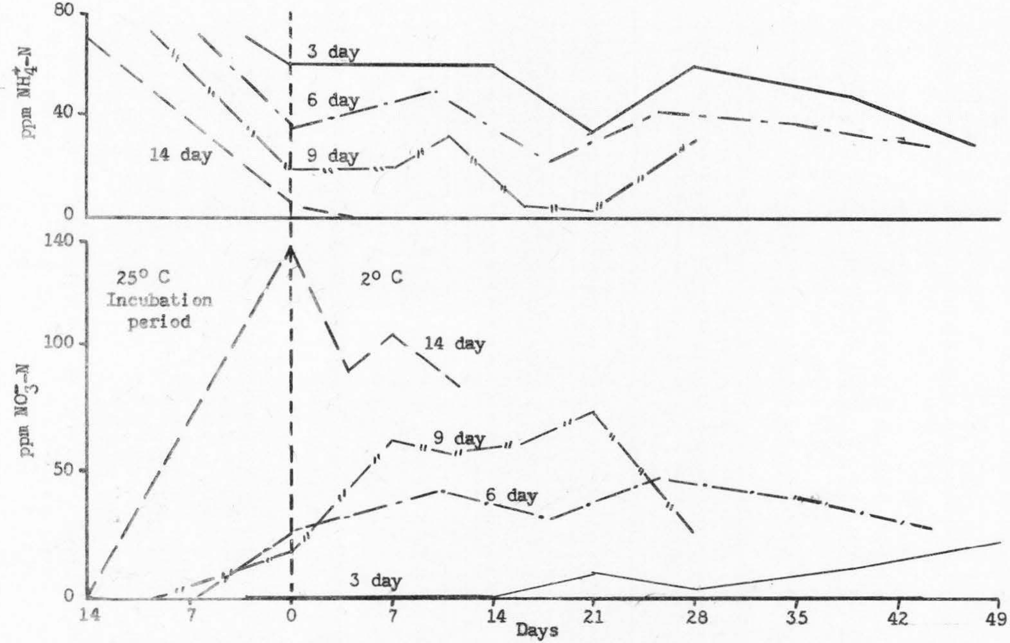

Figure 3. The nitrification patterns observed when 150 ppm N, added as $(NH_4)_2SO_4$, was incubated in Kidman loam in polyethylene bags at 2° C following four different time periods, 3, 6, 9, and 14 days, at 25° C

laboratory experiment. The pattern of the graph is the same as the graphs in Figures 1 and 2. In general, the results for the Kidman soil are intermediate between the results for the other two soils. The NO_3^--N accumulation rate was slowed by the 2° C temperature treatment in the first 10 days. The nitrification that was started in the 25° C incubation was not continued at 2° C in the same way as had been observed with the Millville loam.

Three-day incubation at 25° C. The incubation period decreased the NH_4^+-N level by about 10 ppm. When the soils were placed in the 2° C treatment, the NH_4^+-N disappearance was checked and probably didn't change much until the fourth week when there was a steady decrease until the end of the experiment. The reason for the reduction at the 21-day sampling is not obvious. No NO_3^--N was accumulated until the end of the third week of 2° C treatment; this corresponds to the NH_4^+-N decrease during the same period of time. Starting in the fourth week, the NO_3^--N started to increase gradually and by the end of the sixth week, the level had reached 22 ppm. This is about what was found in the case of the Millville soil.

Six-day incubation at 25° C. This treatment initiated a fairly active population and NH_4^+-N disappearance was rapid. The 2° C temperature treatment checked the rate of NH_4^+-N loss as effectively as it did that of the Yolo soil. In the Millville soil the NH_4^+-N loss was only slowed down. The NO_3^--N accumulation rate got off to a slow start but continued to increase in spite of the 2° C treatment.

Nine-day incubation at 25° C. This incubation reduced the NH_4^+-N level from 72 ppm to 19 ppm. This would indicate that a large population had developed. The 2° C treatment checked the NH_4^+-N loss and the level was unchanged in the first 7 days. It may be that the nitrifying population was already being slowed down due to a limited substrate supply, before the 2° C treatment was begun.

Fourteen days at 25° C. This incubation initiated a large active nitrifier population that had nearly exhausted the NH_4^+-N substrate at the time of 2° C treatment. When analyzed next after 4 days, the NH_4^+-N had totally disappeared. The NO_3^--N accumulation reached its peak during the incubation period. There was the typical drop in NO_3^--N when placed in the 2° C temperature room.

Comparison of the three soils

The graph in Figure 4 compares the three soils in the 6-day incubation study. The Millville soil, apparently being adapted to the coldest climate, utilized the NH_4^+-N substrate the fastest and also was able to produce a significant amount of NO_3^--N at 2° C. The Yolo soil came from the warmest climate and, as was expected, the nitrification was inhibited the most by the cold temperature. There was, however, a decrease in NH_4^+-N and some increase in NO_3^--N. This indicates that the organisms in this soil were more sensitive to the cold temperature but they were able to carry on some nitrification. The Kidman soil, coming from only a slightly warmer climate than the Millville soil, showed a NH_4^+-N graph very similar to that of the Yolo soil. The NO_3^--N curve for the Kidman soil, while intermediate between Millville and Yolo soils, was more like the Millville soil.

There were fluctuations observed in the NH_4^+-N and NO_3^--N in all soils. Some of these may be real, while some are the variations that will show up with these types of experiments. It appears that there

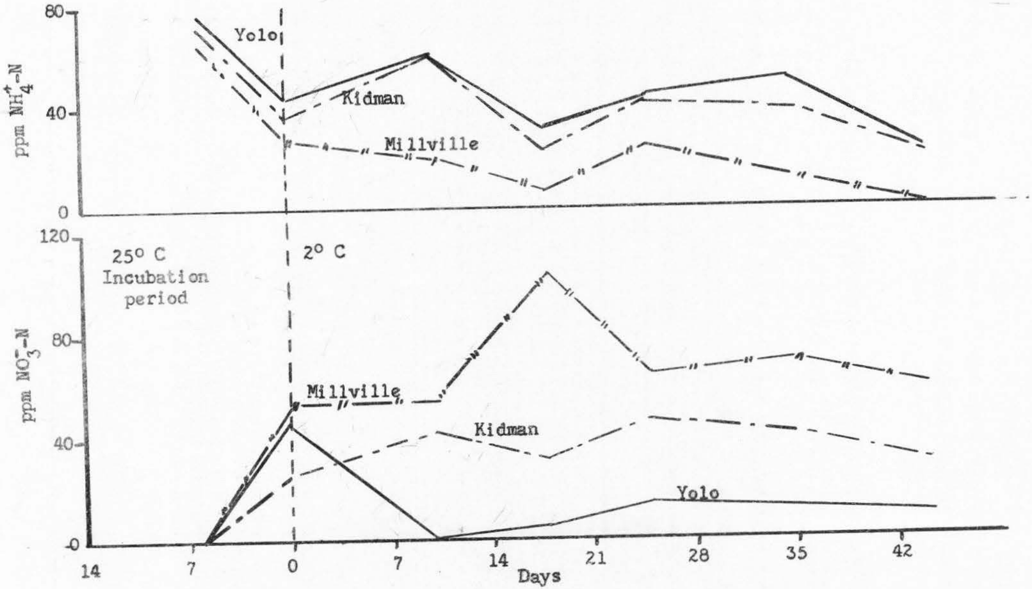

Figure 4. Comparison of the nitrification patterns observed when N, added as $(NH_4)_2SO_4$, was incubated in the three soils, Millville loam, Yolo loam, and Kidman loam, in polyethylene bags at 2° C following 6 days at 25° C

might be an accumulation of NH_4^+-N that occurs soon after the soils are taken from the 25° C room and placed at 2° C. In some cases there was also a net reduction of NO_3^--N between the time the soils were taken from 25° C and placed at 2° C.

Field Experiment

Millville loam

The results of the first field experiment with the Millville soil are graphed in Figure 5. Time is shown as the number of days buried on the horizontal axis. The vertical axis to the left indicates parts per million nitrate or ammonium nitrogen. The vertical axis to the right indicates the average temperature in degrees centigrade. There are four lines on the face of the graph, one each for NO_2^--N, NO_3^--N, and NH_4^+-N that are referenced to the ppm axis, and one for the average daily temperature which is referenced to the temperature axis. Zero time is when the samples were buried. No pre-incubation period was used.

Series one (Figure 5). These samples were buried on September 16, 1964, and by the end of 2 weeks all of the NH_4^+-N had nitrified. The average daily temperatures never dropped below 14° C. This apparently allowed the nitrifiers to expand their population and produce NO_3^--N at a rapid rate. The *Nitrobacter* was able to keep pace with the *Nitrosomonas*, and no nitrites were accumulated. There was a slight delay at the onset of nitrification that might be due to the slight drop in average temperature, but is likely just a lag period (Justice and Smith, 1962).

Series two (Figure 6). These samples were buried on September 30, 1964, and were analyzed for 3 weeks, or a week after the NH_4^+-N had

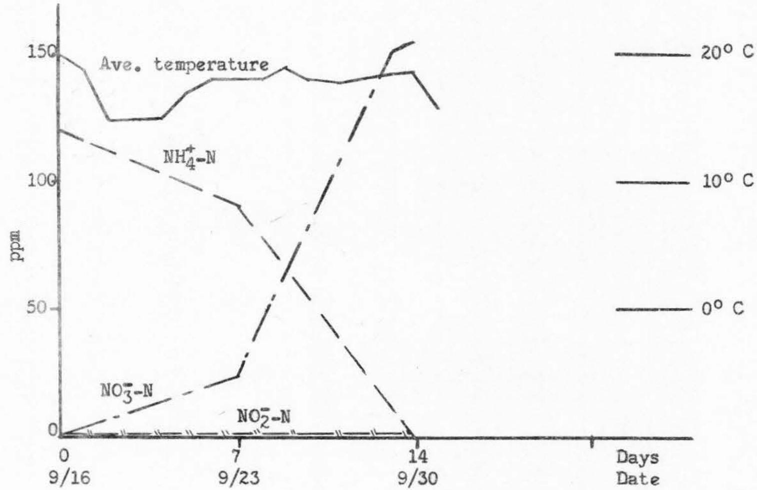

Figure 5. Millville field series one: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 9/16/64

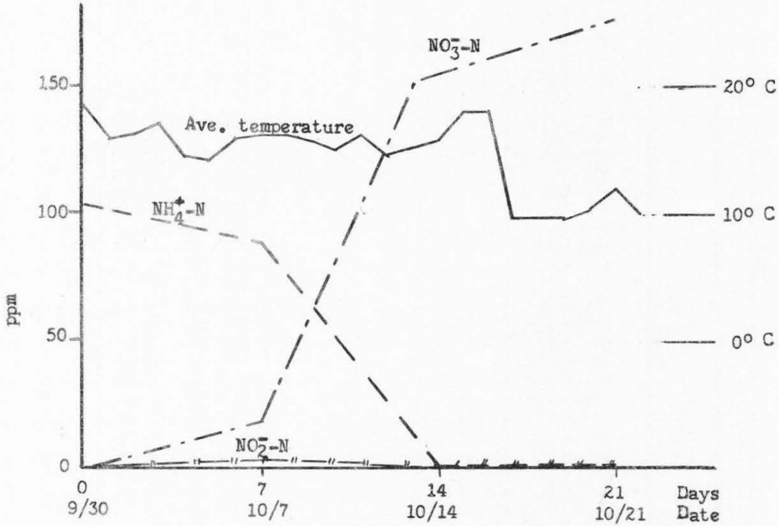

Figure 6. Millville field series two: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 9/30/64

completely disappeared. This series ended on October 21, 1964. During this 3-week period, the temperature gradually dropped until in the last week it reached 10° C. Even though the temperatures were dropping, the nitrifiers seemed to function very well once the population built up. There was the lag or delay noted, but the exact amount of lag time was not found as the first sample was not taken until a week had past. The rounding off of the NO_3^--N line in the third week is due to substrate limitations. An indication of the Nitrobacter's sensitivity to temperature is the slight NO_2^--N accumulation in the first week. Apparently the Nitrobacter did not expand as fast as the Nitrosomonas at the start, but were able to catch up.

Series three (Figure 7). These samples were buried on October 8, 1964, and were analyzed the following 5 weeks. The tests ended on November 12, 1964, after no NH_4^+-N had been recorded for 2 weeks. The temperature was still lowering gradually until on November 8, 1964, it reached 4° C. The nitrifiers were slowed by the cooler temperatures and by comparison the NO_3^--N accumulation line is not as steep as in series one and two. Again the Nitrobacter lagged a little behind the Nitrosomonas as some NO_2^--N was detected in the first week.

Series four (Figure 8). These samples were buried on October 16, 1964, which would be 1 week after series three was started, and the tests were finished on February 5, 1965. The temperatures reached 1.5° C for a low and were under 3° C for most of the time after the first 4 weeks. For the first time we observe a lag period of a week's duration in NO_3^--N accumulation. This is due to the Nitrobacter being more temperature sensitive than the Nitrosomonas, and a resultant accumulation of NO_2^--N. The NO_3^--N was gradually accumulated until the thirty-fifth

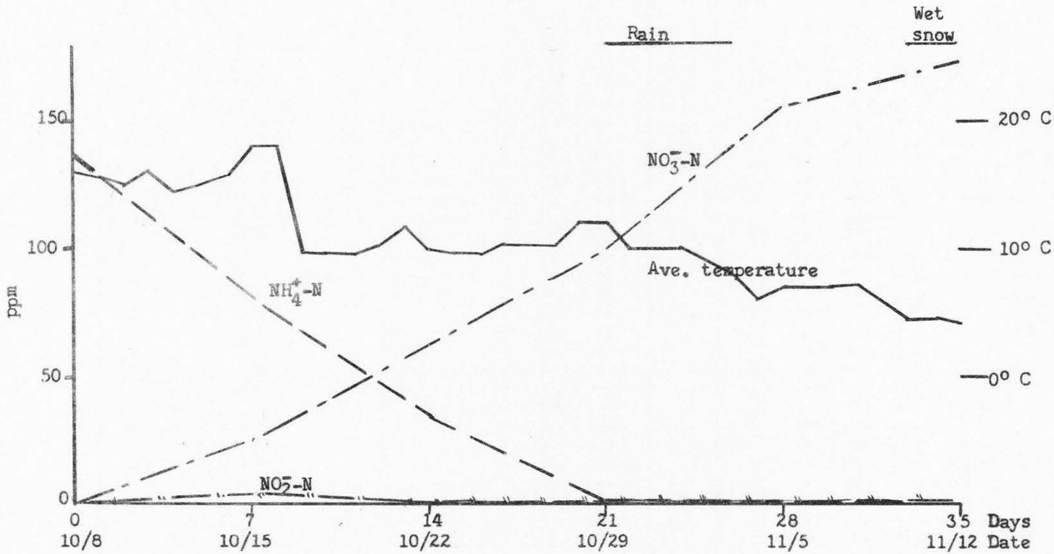

Figure 7. Millville field series three: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 10/8/64

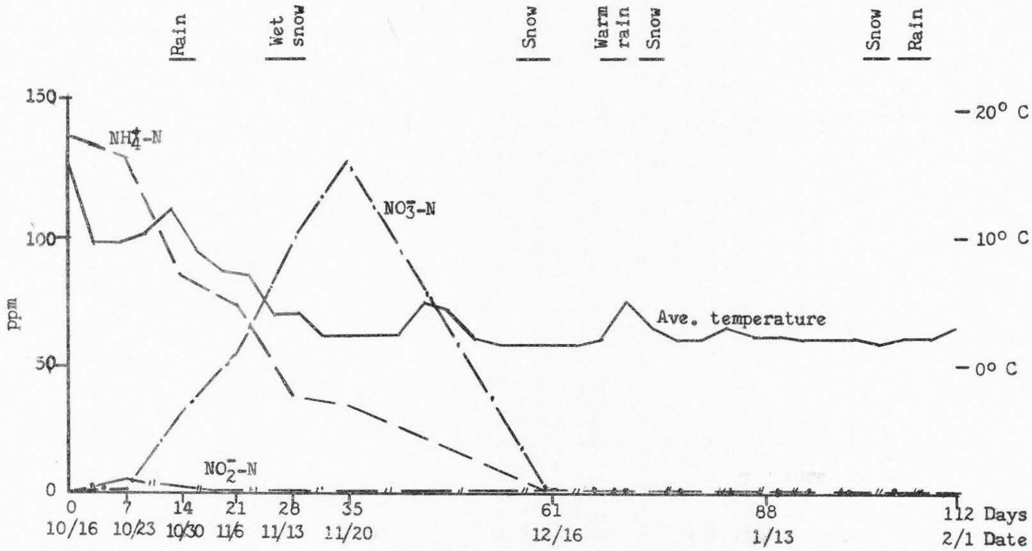

Figure 8. Millville field series four: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 10/16/64

day when a 5-day period of rain and wet snow thoroughly saturated the soil down past the 4-inch level. This apparently prevented O_2 diffusion and probably resulted in anaerobic conditions that encouraged denitrification. The overall nitrification continued, as can be seen by the continued decrease of NH_4^+-N which eventually disappeared after 61 days. The experiment was allowed to continue in hopes of observing some of the recurrent cycles of nitrate appearance which characterized the laboratory experiment.

Series five (Figure 9). This series of samples was buried on November 4, 1964, which was about 3 weeks after series four was buried, and was concluded on April 16, 1965. The average daily temperatures started at 6° C during late fall, fell to a low of 1° C just before the spring warming trend which had a high temperature of 11° C. During the major time portion of this experimental series, the temperature was near 2° C. The NO_3^--N accumulation underwent a 2-week lag period with no NO_2^--N being accumulated. This means that the Nitrosomonas were affected as much as the Nitrobacter. There was, however, a striking reduction in the NH_4^+-N level during this 2-week period. This trend continued up to about December 22, 1964, when a 3-day period of heavy rain and wet snow brought about anaerobic conditions in the soil. The result is a leveling off of the NO_3^--N line after December 16, 1964. That nitrification continued for a period of time after December 16 is verified by the greatly lowered NH_4^+-N level found on January 12, 1965. Following the initial increase in NO_3^--N between the second week (14 days) and 43 days, there was little additional NO_3^--N detected. This is true even though the NH_4^+-N continued to decrease. At the time, the NO_3^--N had levelled off at 75 ppm (43 days) the NH_4^+-N had dropped to

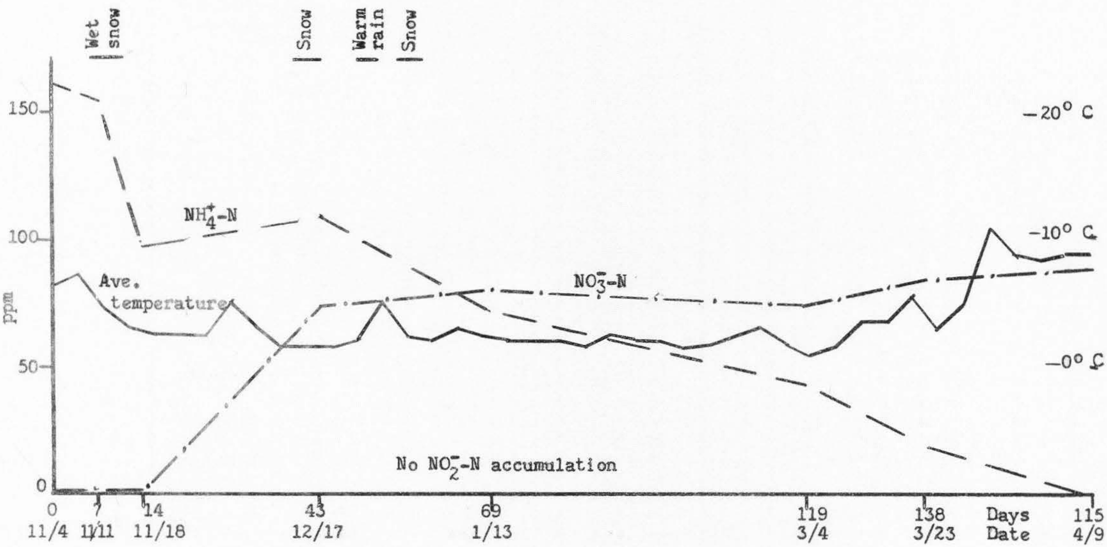

Figure 9. Millville field series five: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 11/4/64

only 100 ppm. During the next 112 days, the NH_4^+-N was gradually reduced to zero while the NO_3^--N was maintained. There is a possibility that the NO_3^--N was beginning to accumulate at 119 days (second week in March) when the weather moderated. Intermittent rain and snow in late November and early December may have made a denitrifying condition by slowing O_2 diffusion through the soil, and hence contributed to the lack of NO_3^--N accumulation found during this 100+ days when the NH_4^+-N was disappearing. It is clear that the Millville nitrifiers can function during the middle of winter.

Series six (Figure 10). This series was buried on November 24, 1964, and was terminated May 6, 1965. The nitrifiers had difficulty getting established in this case, and not much NO_3^--N was accumulated until March, although nitrification obviously started in late January (60 days) even though the average temperatures were less than 5° C. The spring warming trend started nitrification at a rapid rate. Some NO_2^--N was detected at the first analysis which indicates that the *Nitrobacter* population lagged behind the *Nitrosomonas* population at the start. This series indicates that the nitrifiers have trouble establishing a significant population under mid-winter temperatures and conditions.

Series seven (Figure 11). This series was buried on March 7, 1965, and was concluded on May 6, 1965. The difficulty in establishing a population in this series is surprising considering the warmer temperatures. It seems that temperature upward of 10° C was instrumental in the rapid NO_3^--N accumulation rate. The *Nitrobacter* population lagged behind the *Nitrosomonas* population for most of the experiment.

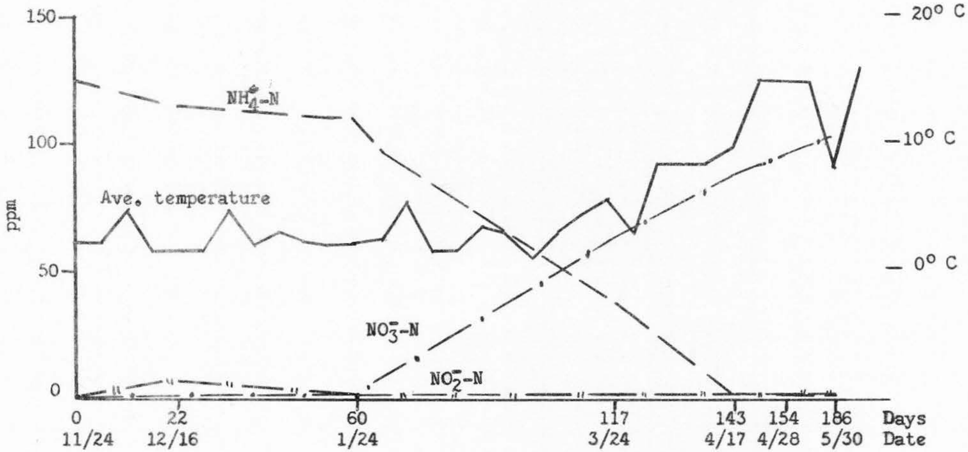

Figure 10. Millville field series six: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 11/24/64

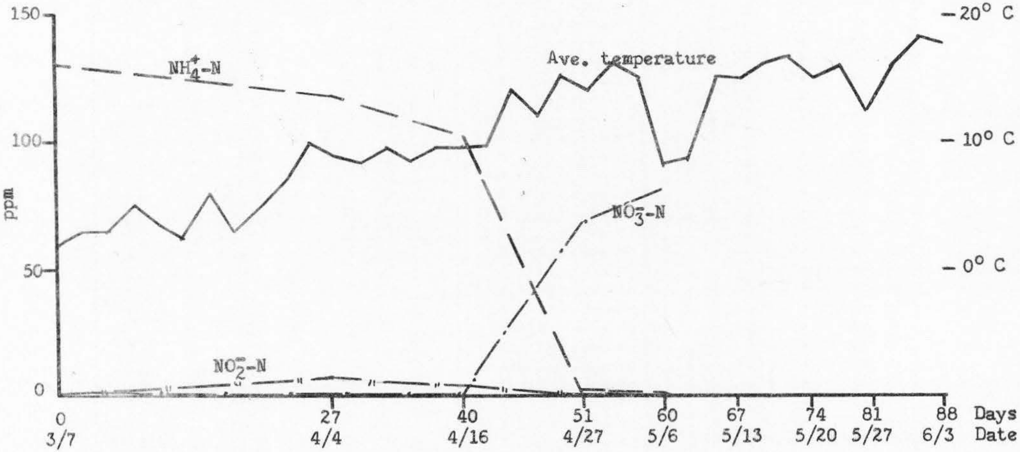

Figure 11. Millville field series seven: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 3/7/65

Millville field experiment summary (Figures 12 and 13). Figure 12 shows the individual line graph for each series from September 16, 1964, to February 1, 1965, and Figure 13 carries on from February 1, 1965, to May 6, 1965. The effect of cold temperatures on nitrification is easy to see as the NH_4^+-N lines show a more gradual decrease toward mid-winter and then a sharper slope in the spring. The same holds true for NO_3^--N accumulation. It is less in mid-winter than in either fall or spring. There is, however, evidence that nitrification, as measured by either NH_4^+-N disappearance or NO_3^--N production, can be initiated at average temperatures near 0° C. Furthermore, if the nitrification has been initiated at warmer temperatures, it proceeds quite rapidly even though the average temperature drops to 0° C.

Yolo loam

The results of the overwintering experiment for the Yolo soil are graphed similiar to the results for the Millville soil.

Series one (Figure 14). The samples for this series were buried on September 16, 1964, and the experiment was concluded on September 30, 1964. As the soil came from the warmest climate, the nitrification was somewhat inhibited by the 15° C to 20° C temperature of the first week. The *Nitrosomonas* population produced some NO_2^--N that the *Nitrobacter* were unable to oxidize. That the nitrifying populations were active is indicated by the decrease in NH_4^+-N level. In the second week, however, both groups of nitrifiers were active and complete utilization of the NH_4^+-N occurred.

Series two (Figure 15). The samples for this series were buried on September 30, 1964, and the experiment was terminated on October 21, 1964. The lag period was again present and is found in all of the Yolo

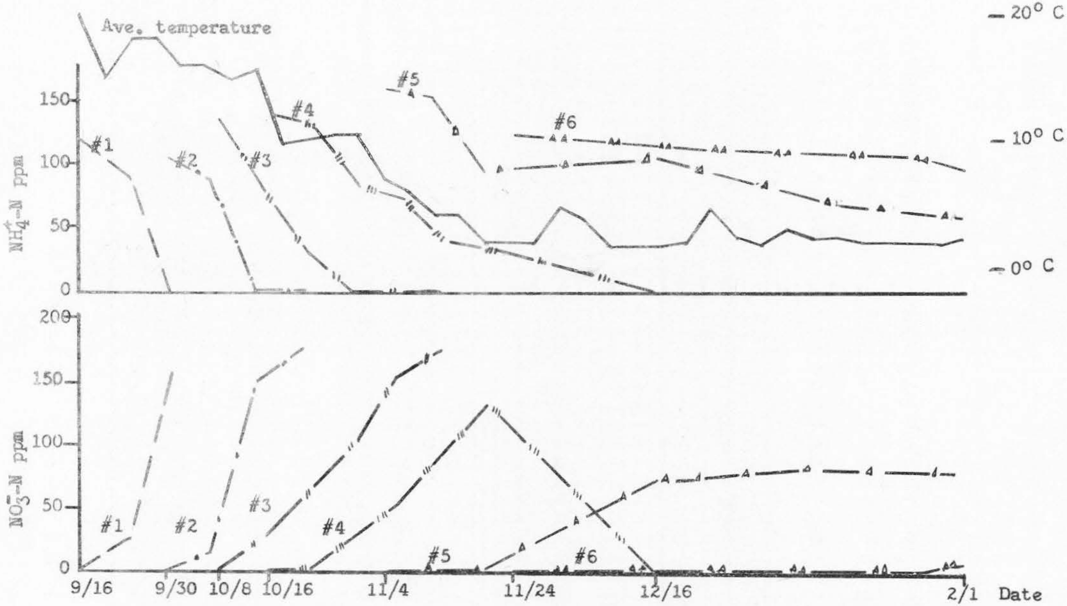

Figure 12. Summary chart for Millville series part one, 9/16/64 to 2/1/65. Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep

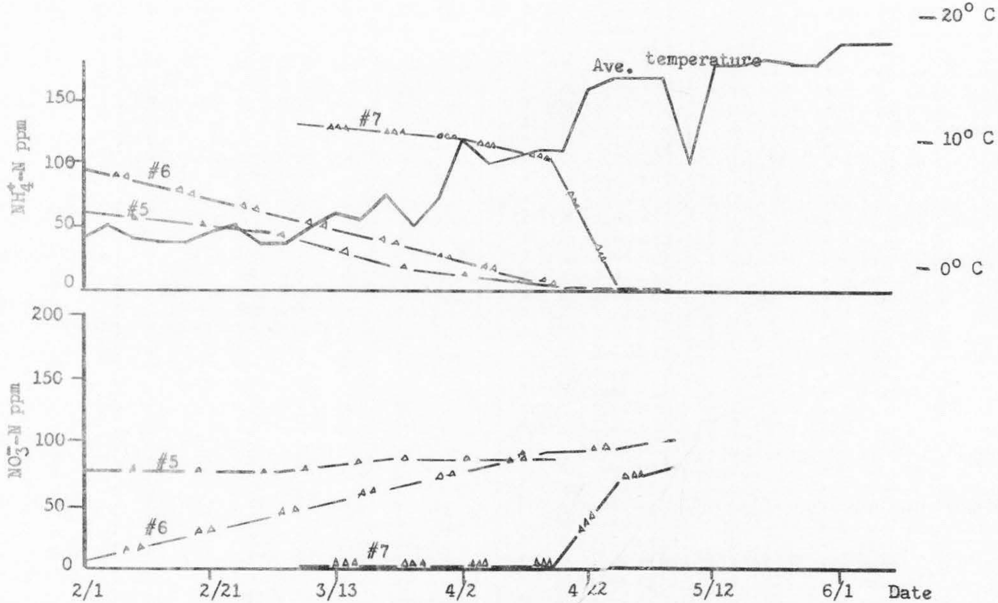

Figure 13. Summary chart for Millville series part two, 2/1/65 to 5/6/65. Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep

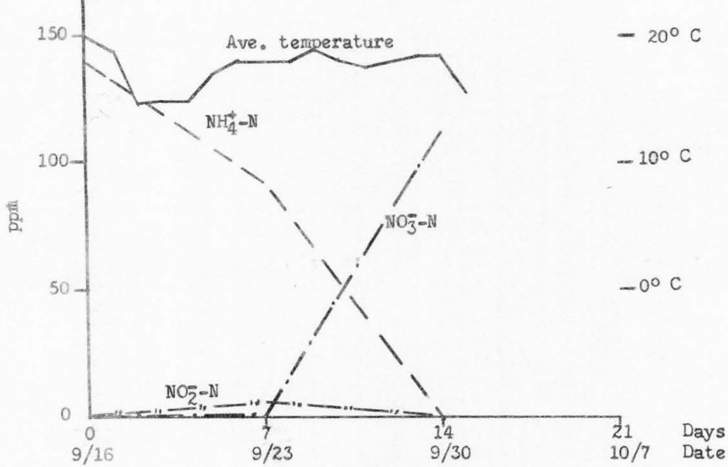

Figure 14. Yolo field series one: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 9/16/64

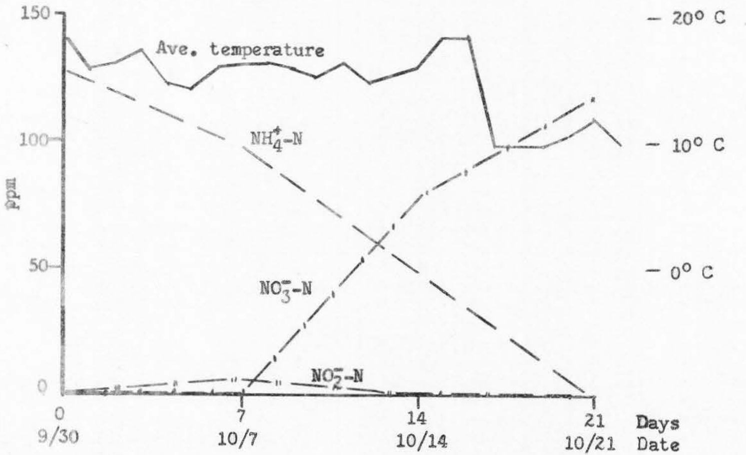

Figure 15. Yolo field series two: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 9/30/64

field series. The lag in NO_3^--N accumulation was again due to the slower expansion of the Nitrobacter population as NO_2^--N was accumulated during the lag period. The temperatures of the first 2 weeks were slightly lower than in series one, and as a result, the NH_4^+-N decrease line and the NO_3^--N increase line are not quite as steep as in series one. The effect of the sharp temperature drop during the third week is not reflected in this series.

Series three (Figure 16). The samples for this series were buried on October 8, 1964, and the experiment was concluded on November 12, 1964. Ammonium-N was constantly being used by the nitrifiers. The NO_2^--N line shows that the Nitrobacter was always behind the Nitrosomonas. The sharp temperature drop just after the seventh day slowed the Nitrobacter even more and allowed more NO_2^--N to build up during the second week. The large amount of NH_4^+-N lost during the second week is assumed to be tied up in population growth, and during the third week it was passed through the nitrifiers and deposited as NO_3^--N. Also, during the third week Nitrobacter started to reduce the level of accumulated NO_2^--N which also added to the high level of NO_3^--N accumulated during the third week. A 5-day period of rain and wet weather occurred from the twenty-first to the twenty-fifth day of this series, which probably decreased aeration enough to allow denitrification. As a result, the NH_4^+-N level decreased, but the NO_3^--N level remained about the same. As the soil dried out, the NO_3^--N level again started to rise.

Series four (Figure 17). The samples for this series were buried on October 16, 1964, and the experiment was concluded on February 5, 1965. This is the first time that the Nitrosomonas in this soil

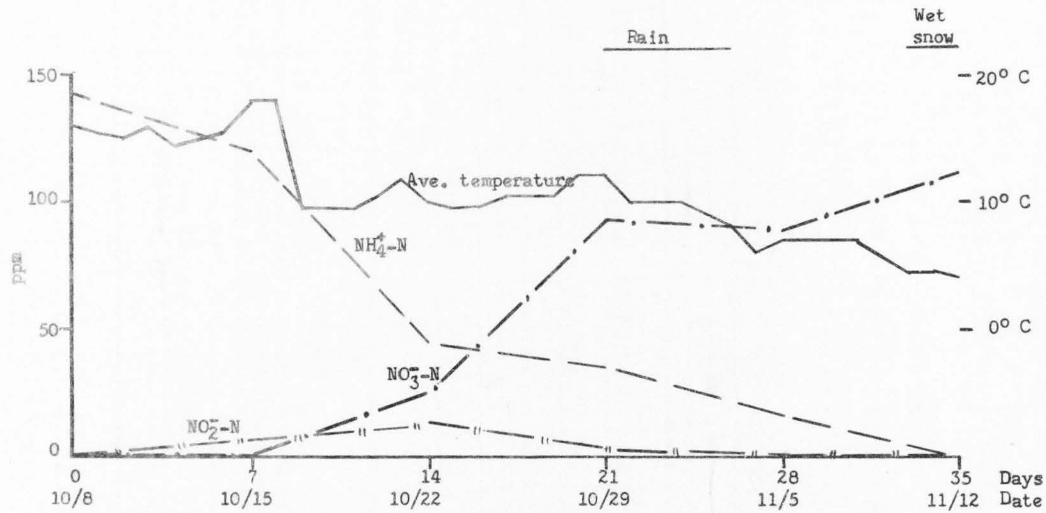

Figure 16. Yolo field series three: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 10/8/64

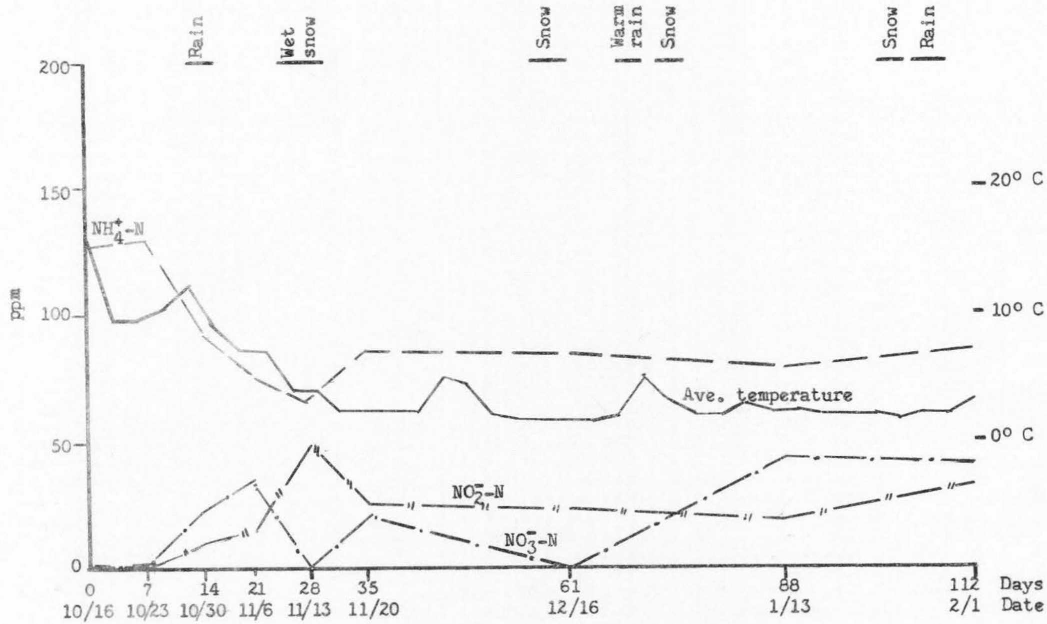

Figure 17. Yolo field series four: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 10/16/64

underwent a lag period. At the end of the first week, the NH_4^+-N level remained about the same and no NO_2^--N accumulated. In the second week both the Nitrosomonas and Nitrobacter became active. The Nitrosomonas were the most active. During the second, third, and fourth weeks, the NH_4^+-N consumption was fairly rapid. In the fifth week when the soil was saturated with rain and wet snow, a net NH_4^+-N increase occurred. This NH_4^+-N level was maintained for the remainder of this experiment. The NO_3^--N level dropped during the fourth week to zero, probably due to the denitrification process. One of the products of denitrification is NO_2^--N, and this gives a rise in the NO_2^--N curve. During the fifth week the aeration was apparently restored and part of the NO_2^--N was oxidized into NO_3^--N. The period of warm rains at the 10-week stage warmed the soil and started the nitrifiers going again. The source of the nitrifier substrate must be from one of the denitrifier populations, as the NO_2^--N level and the NH_4^+-N level change very little.

Series five (Figure 18). The samples for this series were buried on November 4, 1964, and the experiment was terminated on April 16, 1965. The first week showed a lag period with no apparent nitrogen activity. In the second week, the Nitrosomonas population expanded and utilized a lot of NH_4^+-N. Saturated soil conditions during the third week reduced aeration and thereby reduced the Nitrosomonas population, releasing much of the utilized NH_4^+-N. By the forty-third day, the Nitrosomonas were regrouping and producing more NO_2^--N. In the following 4 weeks, the Nitrobacter became active and so NO_3^--N was accumulated. However, for the remaining part of series five, the Nitrobacter never caught up to the Nitrosomonas. Between the 119th and 138th day, a 4-day period of rain and wet snow caused denitrification to occur which showed

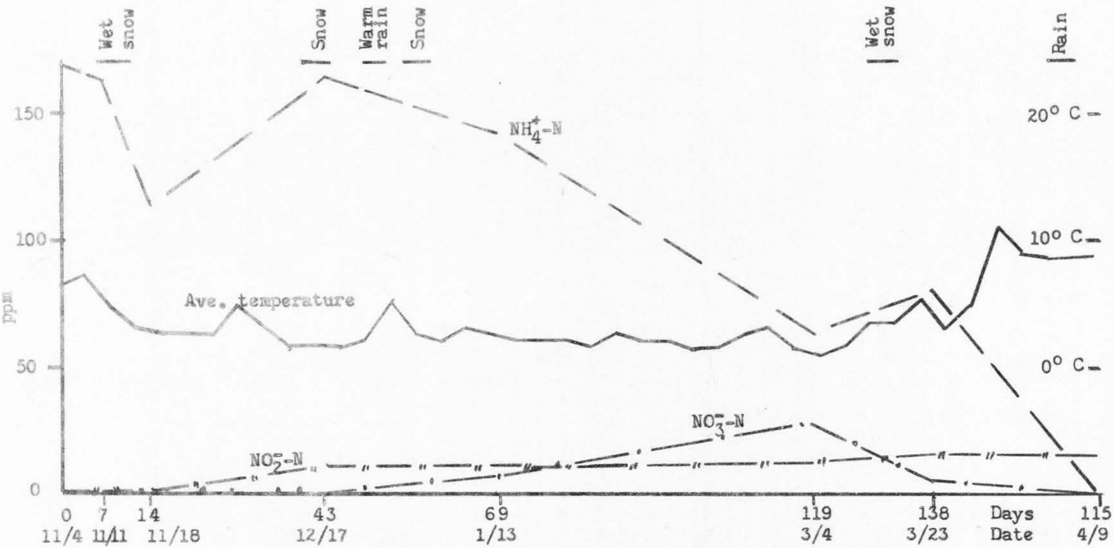

Figure 18. Yolo field series five: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 11/4/64

up as a loss in NO_3^--N. The NO_2^--N level remained steady, probably because of some donations of NO_2^--N from reduced NO_3^--N. The warm temperatures following this period started rapid utilization of NH_4^+-N, but a 4-day period of rain reduced soil aeration and denitrification again occurred. Thus, at the end of the series five experiment, both NH_4^+-N and NO_3^--N were zero.

Series six (Figure 19). The samples for this series were buried on November 24, 1964, and the experiment concluded on May 13, 1965. The nitrifiers were inactive for a long period of time. Some NO_2^--N was found at the 60-day analysis, and some NO_3^--N was found at the 117-day analysis. A 4-day period of rain between the 117-day test and the 143-day test caused net denitrification and a period of NH_4^+-N accumulation. Increasingly warm temperatures and drying soil conditions were responsible for the rapid rate of NH_4^+-N utilization, although the NO_3^--N level never did increase very much. The accumulation seemed to be mostly in the NO_2^--N form.

Series seven (Figure 20). The samples for this series were buried on March 7, 1965, and the experiment was terminated on June 3, 1965. It appears that the Nitrobacter is more temperature sensitive than the Nitrosomonas all through this experiment. It required 40 days to accumulate any NO_3^--N. The 4-day rain between the 51-day and the 60-day analysis reduced what NO_3^--N had accumulated. The following warmer, drier period started nitrification off at a good rate as evidenced by NH_4^+-N disappearance. The increase in NO_3^--N lagged behind the reduction of NH_4^+-N.

Yolo field experiment summary (Figures 21 and 22). Figure 21 shows the individual series line graphs from September 16, 1964, to

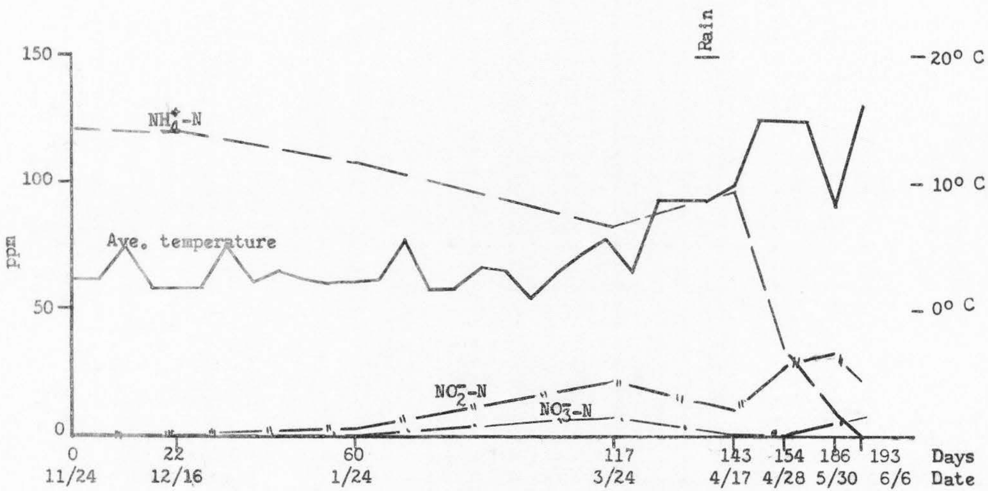

Figure 19. Yolo field series six: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 11/24/64

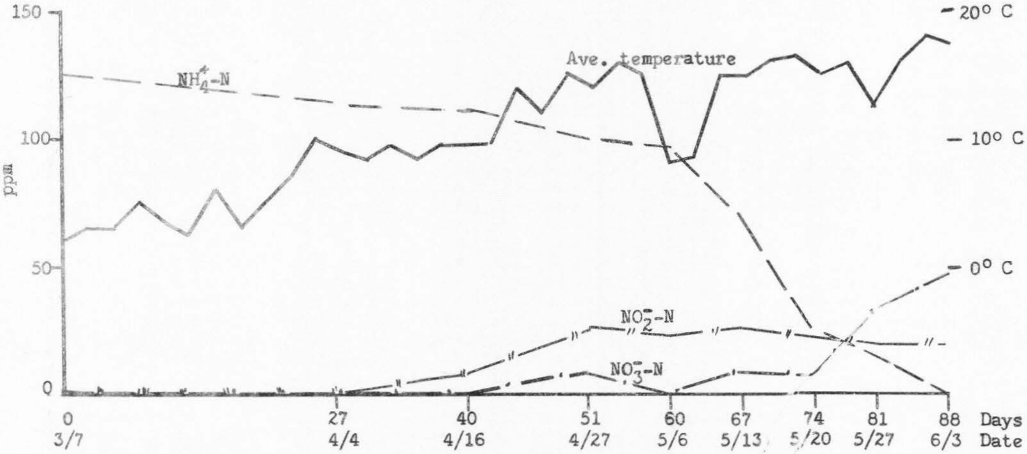

Figure 20. Yolo field series seven: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 3/7/65

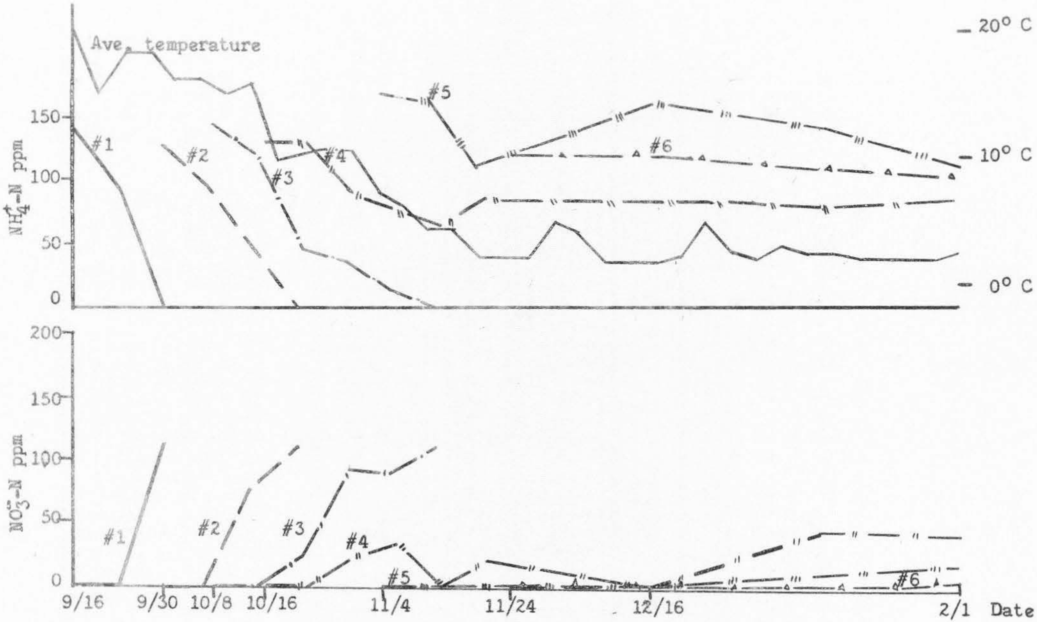

Figure 21. Summary chart for Yolo series part one, 9/16/64 to 2/1/65. Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep

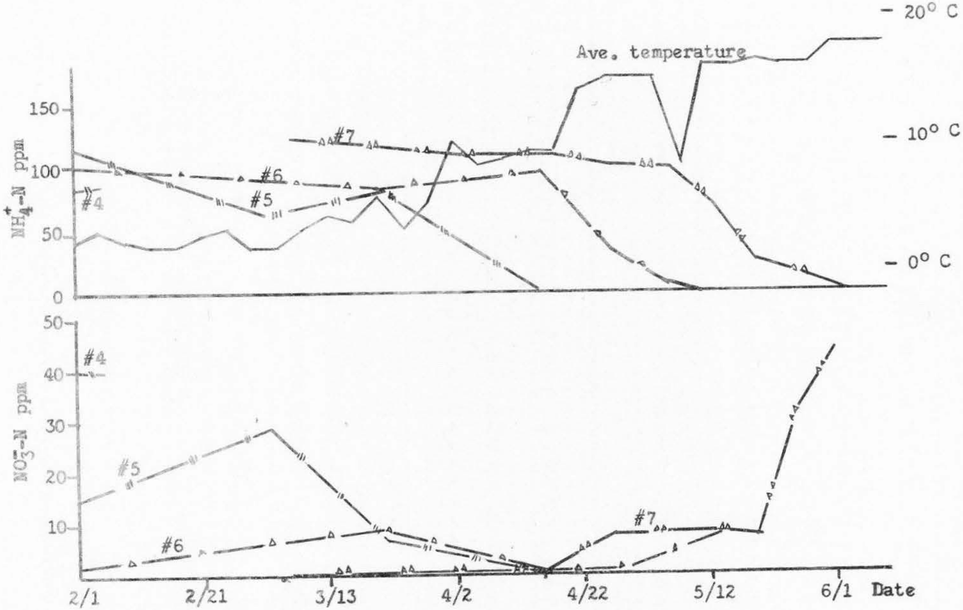

Figure 22. Summary chart for Yolo series part two, 2/1/65 to 5/6/65. Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep

February 1, 1965. Figure 22 shows the line graph from February 1, 1965, to June 3, 1965. The effect of cold temperatures is clearly shown. Low NH_4^+-N utilization and low NO_3^--N accumulation are evident during the mid-winter months.

Kidman loam

Series one (Figure 23). The samples for this series were buried on September 16, 1964, and the experiment was concluded on September 30, 1964. The Nitrosomonas were active in the first week, but the Nitrobacter was not. Thus, an accumulation of NO_2^--N was found. Both populations of nitrifiers were active in the second week and complete nitrification was found.

Series two (Figure 24). The samples for this series were buried on September 30, 1964, and the experiment was concluded on October 21, 1964. Again the Nitrosomonas were active before the Nitrobacter and a slight NO_2^--N level was found after the first week. Rapid nitrification is seen during the following 2 weeks causing the substrate to disappear on October 21, 1964.

Series three (Figure 25). The samples for this series were buried on October 8, 1964, and the experiment was terminated on November 12, 1964. The rate of NH_4^+-N utilization seemed constant throughout this series. The Nitrobacter lagged behind the Nitrosomonas during the major part of the experiment, resulting in some NO_2^--N being accumulated. The rate of NO_3^--N accumulation increased with the population expansion until the rains in the fourth week caused some denitrification.

Series four (Figure 26). The samples for this series were buried on October 16, 1964, and the experiment was concluded on February 5,

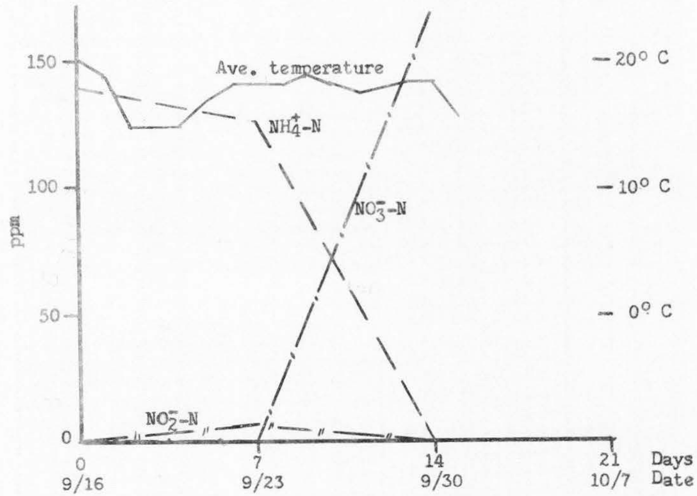

Figure 23. Kidman field series one: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ in polyethylene bags buried 4 inches deep on 9/16/64

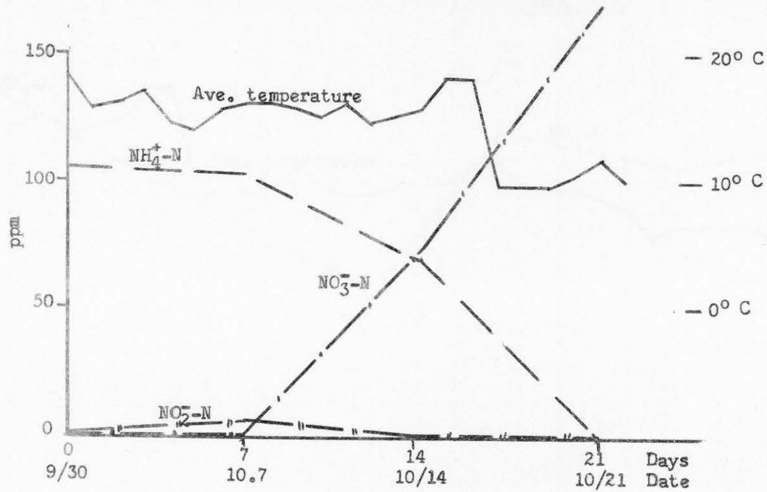

Figure 24. Kidman field series two: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 9/30/64

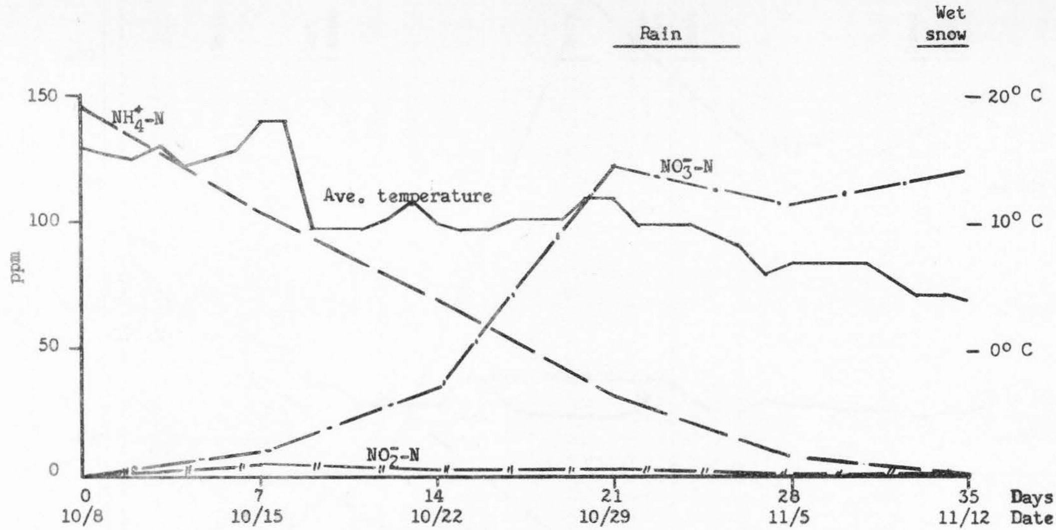

Figure 25. Kidman field series three: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 10/8/64

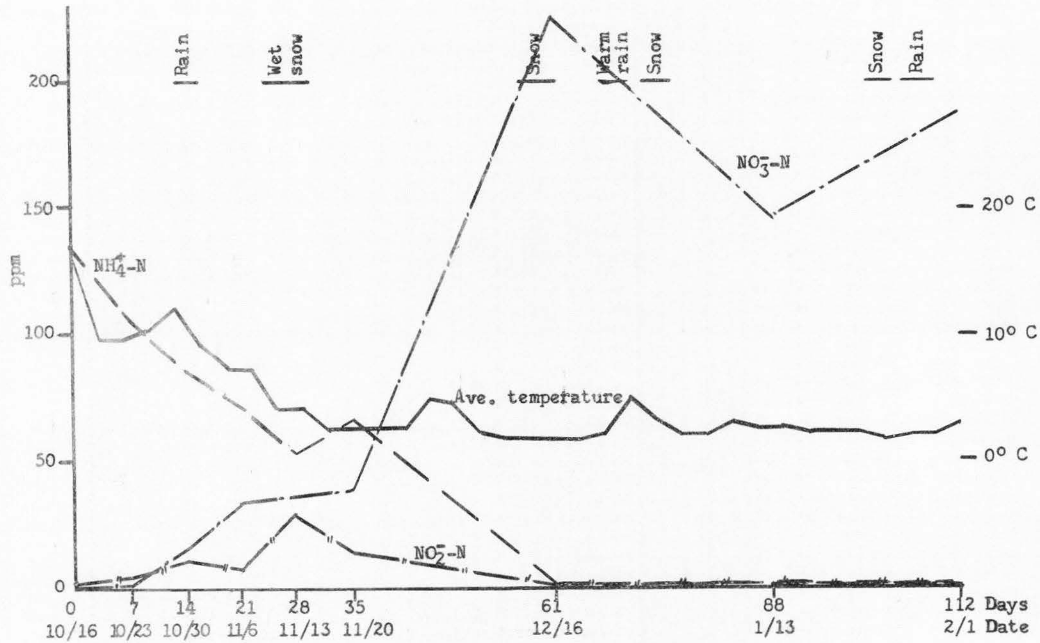

Figure 26. Kidman field series four: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 10/16/64

1965. Again the Nitrosomonas were active before the Nitrobacter and remained more active. This caused a level of NO_2^--N to be present throughout this series. Precipitation caused periods of denitrification to occur. There is no explanation for the phenomenally high level of NO_3^--N reached at the 61-day mark.

Series five (Figure 27). The samples for this series were buried on November 4, 1964, and the experiment was terminated on April 16, 1965. Both groups of nitrifiers underwent a week-long lag period before any NO_2^--N or NO_3^--N was accumulated. The temperature affected the NO_2^--N line nearly the same as the NO_3^--N line, only to a lesser degree. The recovery of significant amounts of NH_4^+-N on the forty-third day, over the amount recovered on the fourteenth day is probably due to the release of NH_4^+-N absorbed by one or both of the nitrifier populations.

Series six (Figure 28). The samples for this series were buried on November 24, 1964, and the experiment was concluded on May 6, 1965. Although the Nitrosomonas were active during the first 3 weeks, the Nitrobacter showed no activity until the analysis at 117 days. From here on the Nitrobacter became very active, as can be seen by the NO_2^--N and NO_3^--N lines.

Series seven (Figure 29). The samples for this series were buried on March 7, 1965, and the experiment was terminated on June 3, 1965. Some NO_2^--N was found on the 40-day sampling. At 51 days some NO_3^--N also was found, and that was just the start of a very rapid nitrification rate. The Nitrobacter became very active with the warmer temperature and reduced the NO_2^--N level to zero before the end of the series was reached.

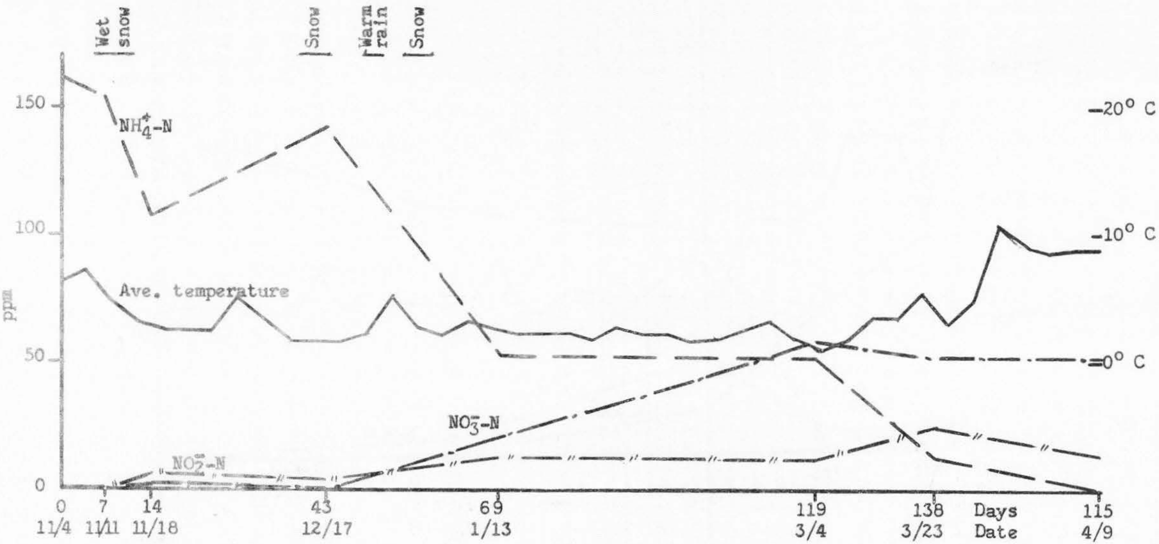

Figure 27. Kidman field series five: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 11/4/64

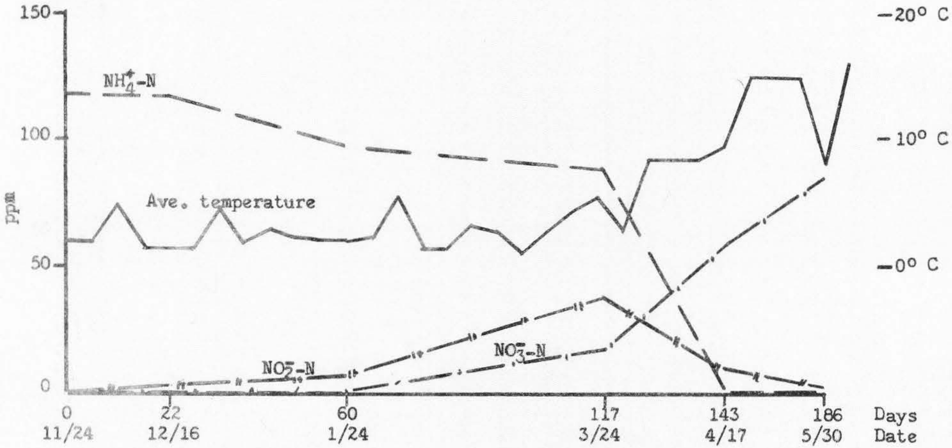

Figure 28. Kidman field series six: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 11/24/64

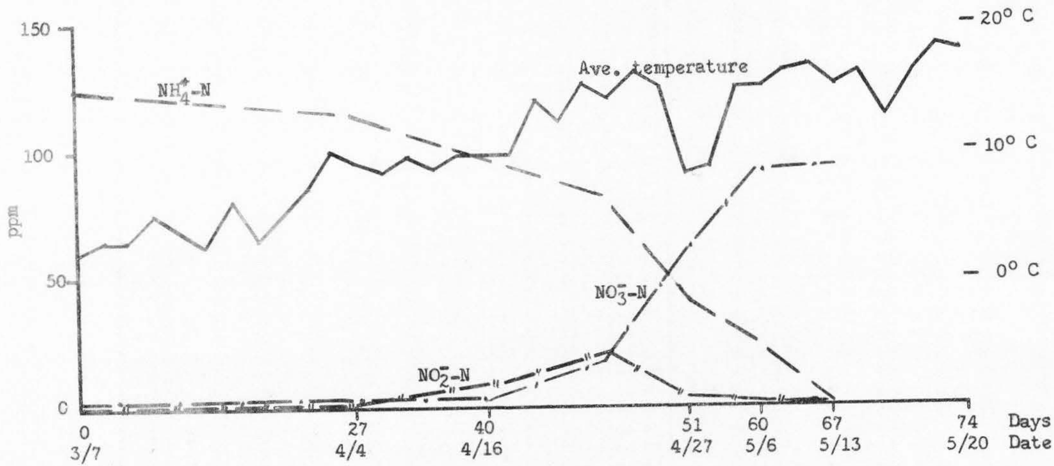

Figure 29. Kidman field series seven: Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep on 3/7/65

Kidman field experiment summary (Figures 30 and 31). As in the other two soils, the lines have more gentle slopes as the mid-winter season was encountered. This was reflected in a reduced or restricted activity of the organisms and less NO_2^--N or NO_3^--N.

Comparison of the three soils buried 10/8/64

Of the three soils involved, nitrification started earliest in the Millville soil as evidenced by the decline in the NH_4^+-N and by the increase in NO_3^--N (Figure 32). There was a pronounced lag in the nitrification for both the Kidman and Yolo soils. Once nitrification had started, the process continued even though the average temperature dropped below 10° C.

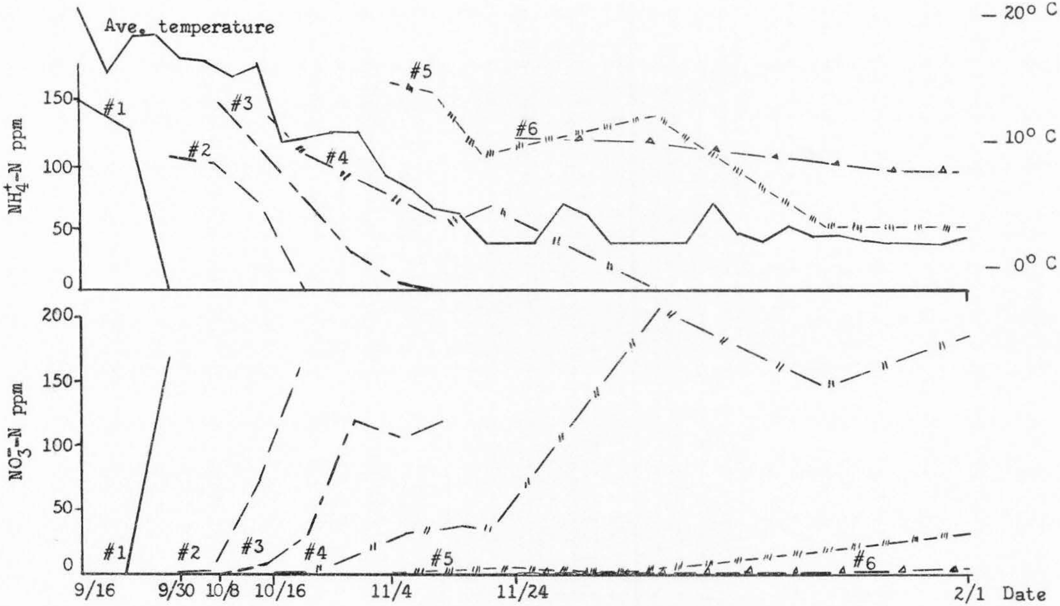

Figure 30. Summary chart for Kidman series part one, 9/16/64 to 2/1/65. Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep

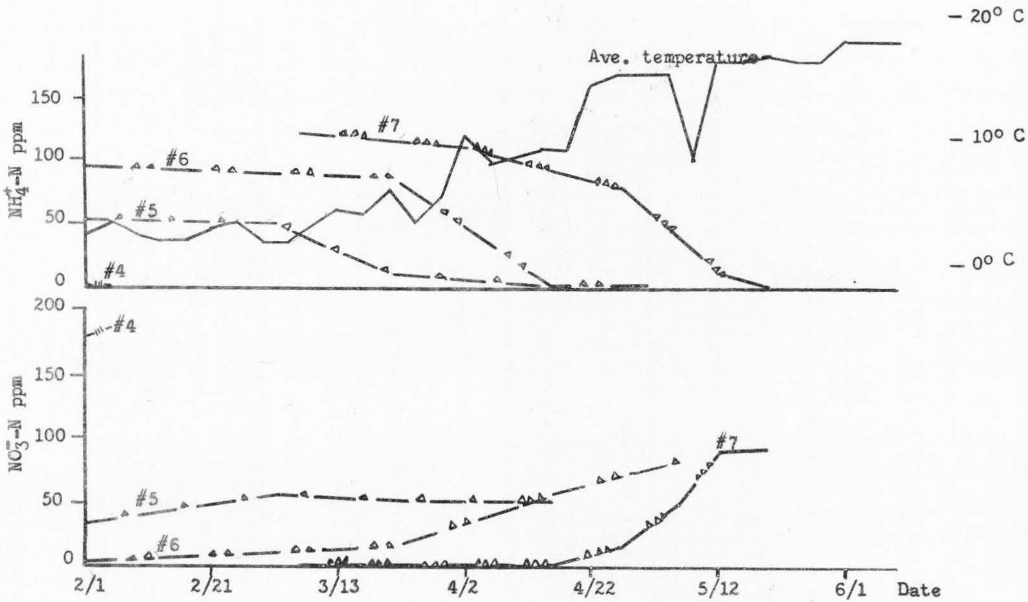

Figure 31. Summary chart for Kidman series part two, 2/1/65 to 5/20/65. Nitrification patterns of 150 ppm N added as $(NH_4)_2SO_4$ to soil in polyethylene bags buried 4 inches deep

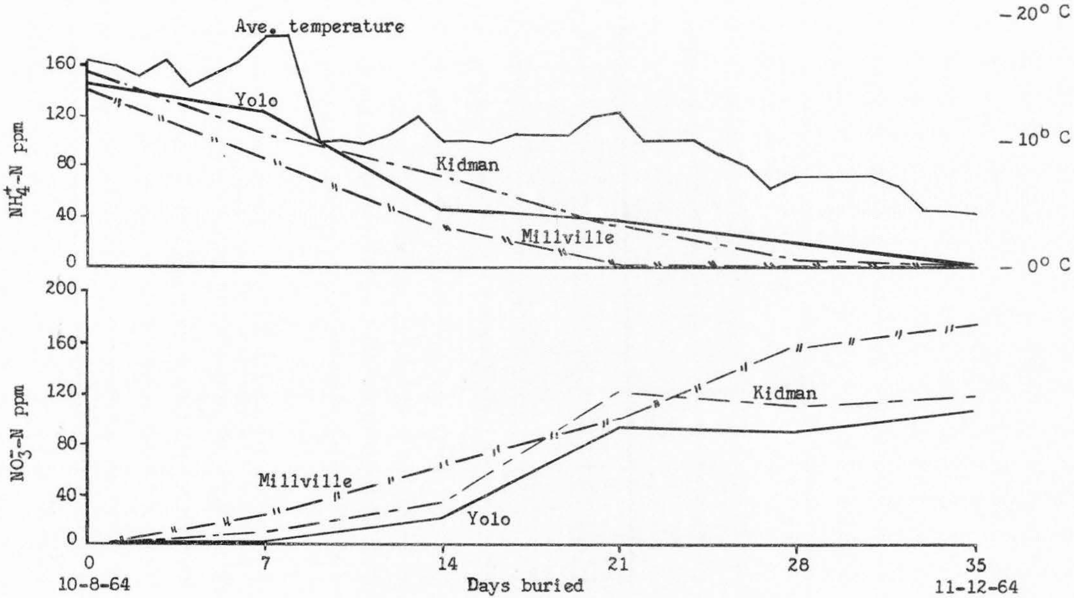

Figure 32. Comparison of the nitrification patterns for field series three for the three soils buried on 10/8/64 and sampled periodically until 11/12

SUMMARY AND CONCLUSIONS

The purpose of the study was to observe the effect that over-wintering has on the nitrification process in three soils. In the field experiment the only condition that was restricted by polyethylene bags was that of leaching. Thus it is felt that the results obtained in this study are valid, except for a portion of the NO_3^--N that may have leached away if the bags had not been present.

It was observed that for all three soils the _Nitrobacter_ organism was more sensitive to cold temperatures than is the _Nitrosomonas_ organism. This resulted in NO_2^--N accumulating under some conditions.

Nitrification was slowed but not stopped in all three soils under conditions of 2° C temperatures. The effect was most pronounced in the Yolo loam and least for the Millville soil. The effect in the Kidman soil was intermediate.

The rate of nitrification under the 2° C temperature conditions was dependent on the amount of nitrification obtained before the extreme cold, and, apparently, on the ability of the organisms to exist and multiply during the cold conditions. There is evidence that in some instances denitrification could occur during the winter months following periods of rain.

LITERATURE CITED

Amer, F. M., and W. V. Bartholomew. 1951. Influence of oxygen concentration in soil air on nitrification. Soil Sci. 71:215-220.

Anderson, O. E. 1960. The effect of low temperatures on nitrification of ammonia in Cecil sandy loam. Soil Sci. Soc. Am. Proc. 24:286-289.

Anderson, O. E., and E. R. Purvis. 1955. Effects of low temperatures on nitrification in soils. Soil Sci. Soc. Am. Proc. 20:357-360.

Brandt, G. H., A. R. Wolcott, and A. E. Erickson. 1964. Nitrogen transformations in soil as related to structure, moisture, and oxygen diffusion rate. Soil Sci. Soc. Am. Proc. 28:71-75.

Broadbent, F. E., and B. J. Stojanovic. 1952. The effect of partial pressure of oxygen on some soil nitrogen transformations. Soil Sci. Soc. Am. Proc. 16:359-363.

Chandra, P. 1962. Effect of shifting temperature on nitrification in loam soil. Can. J. Soil Sci. 42:314-315.

Eno, C. F. 1960. Nitrate production in the field by incubating the soil in polyethylene bags. Soil Sci. Soc. Am. Proc. 24:277-279.

Frederick, L. R. 1956. The formation of nitrate from ammonia nitrogen in soils: I. Effect of temperatures. Soil Sci. Soc. Am. Proc. 20:496-500.

Jackson, M. L. 1958. Soil chemical analysis. Prentice-Hall, Inc., Englewood Cliffs, N. J. 498 p.

Justice, K. K., and R. L. Smith. 1962. Nitrification of ammonium sulfate as influenced by combinations of moisture, temperature, and levels of added nitrogen. Soil Sci. Soc. Am. Proc. 26:246-250.

Mahendrappa, M. K. 1963. Preliminary studies on some of the factors affecting nitrification in soils of the western United States. Unpublished MS thesis. Utah State University Library, Logan.

Mahendrappa, M. K., R. L. Smith, and A. T. Christiansen. 1966. Nitrifying organisms affected by climatic region in western United States. Soil Sci. Soc. Am. Proc. 30:60-62.

Millbank, J. W. 1959. The physiology of nitrification in Kenya highland soils. Plant and Soil 11:293-311.

Miller, R. D., and D. D. Johnson. 1964. The effect of soil moisture tension on carbon dioxide evolution, nitrification, and nitrogen mineralization. Soil Sci. Soc. Am. Proc. 28:644-646.

Morrill, L. G., and J. E. Dawson. 1962. Growth rates of nitrifying chemo-autotrophs in soil. J. Bact. 83:205-206.

Panganiban, E. H. 1925. Temperature as a factor in nitrogen changes in the soil. J. Am. Soc. Agron. 17:1-31.

Russel, J. C., E. G. Jones, and G. M. Bahrt. 1925. The temperature and moisture factors in nitrate production. Soil Sci. 19:381-398.

Sabey, B. R., W. V. Bartholomew, R. Shaw, and J. Pesek. 1956. Influence of temperature on nitrification in soils. Soil Sci. Soc. Am. Proc. 20:357-360.

Sabey, B. R., L. R. Frederick, and W. V. Bartholomew. 1959. The formation of nitrate from ammonium nitrogen in soils: III. Influence of temperature and initial population of nitrifying organisms on the maximum rate and delay period. Soil Sci. Soc. Am. Proc. 23:462-465.

Shinn, M. B. 1941. Colorimetric method for determination of nitrate. Indus. and Engin. Chem., Analyt. Ed. 13:33-35.

Silver, W. S. 1961. Studies on nitrite oxidizing micro-organisms: Nitrobacter. Soil Sci. Soc. Am. Proc. 25:197-199.

Smith, C. D. 1963. The nitrification of ammonium sulfate in polyethylene bags in the field and laboratory. Unpublished MS thesis. Utah State University Library, Logan.

Stojanovic, B. J., and M. Alexander. 1958. Effect of inorganic nitrogen on nitrification. Soil Sci. 86:208-215.

Stojanovic, B. J., and F. E. Broadbent. 1957. Influence of low temperatures on nitrogen transformations in Honeoye silt loam. Soil Sci. 84:243-248.

Tyler, K. B., F. E. Broadbent, and G. N. Hill. 1959. Low temperature effects on nitrification in four California soils. Soil Sci. 87: 123-129.

Waksman, S. A. 1952. Soil microbiology. John Wiley and Sons, Inc., New York. 356 p.