# EFFECT OF ADDED ORGANIC MATTER ON NITROGEN CHANGES IN CHICKASHA SILT LOAM

# EFFECT OF ADDED ORGANIC MATTER ON NITROGEN CHANGES IN CHICKASHA SILT LOAM

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# PREFACE

One of the critical agronomic problems in the wheat producing area of Oklahoma and the Southwest is to find a satisfactory method for the disposal of wheat straw that will, at the same time, reduce the destructive effect of wind and water erosion. The carbon-nitrogen ratio in the straw must be reduced to less than 20 to 1 before the nitrogen will be released for crops growing on the land. It has been considered quite possible that during the decomposition of straw, some of the nitrogen may be lost by conversion to gaseous nitrogen by soil microorganisms.

With the general availability of the stable isotope of nitrogen, $N^{15}$, a new and potentially useful method of following nitrogen changes in the soil has been introduced. As an application of this technique, the following experiments have been conducted in an effort to trace nitrogen changes in soils under normal aerobic conditions, particularly in the presence of large amounts of added organic matter.

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# INTRODUCTION

Much time and energy have been devoted to studies of denitrification in soils, that is, the reduction of nitrates with the setting free of gaseous nitrogen. That reduction of combined nitrogen takes place in anaerobic fermentation due to the activity of various microorganisms has been known for years. Waksman (21) has summarized the available microbiological information concerning these organisms. A critical survey of bacterial denitrification to 1931 has been presented by Lloyd (7), and the practical aspects of denitrification in soil and sewage to 1927 have been summarized by Buswell and Neave (3).

Despite these extensive investigations, however, little information is available regarding the magnitude of this process under normal conditions of soil aeration.

Wagner (20) declared that applications of manure and nitrate to soil are often not only unprofitable, but harmful, that, when applied together, microorganisms bring about the destruction of nitrate. Though several investigators later proved that the results were grossly exaggerated, Wagner's statement served to give the question practical as well as scientific interest. Voorhees (19), in experiments with soil and manure, came to the conclusion that the phenomenon of denitrification is of no economic significance in well-aerated, not too moist soils, in presence of moderate amounts of organic matter and nitrate. In contrast, Murray (10) reported significant nitrogen losses in soil to which 10 ccs. of 2% $KNO_3$ had been added occurred only under anaerobic conditions. Arnd (2) observed that denitrification in limed bog soils is dependent upon the amount of aeration, loosening of the soil being accompanied by a decrease in the loss of nitrogen. In peat soils nitrogen is lost from nitrates even in layers

one cm. thick, with neither aeration nor the removal of $H_2O$ being sufficient to inhibit denitrification to a satisfactory degree.

Shutt (16) observed large losses in nitrogen following cultivation of Canadian prairie soils. In certain pot experiments, Wilshaw (22) noted that it was possible to recover by leaching with water less than 13% of added nitrogen fertilizer. He believed that large losses of nitrogen as elemental nitrogen must have occurred. Meggitt (9) and Annette et al. (1) reported copious nitrogen losses from tropical soils.

In extensive in vitro studies of the nitrogen cycle in biological systems, Corbet and Wooldridge (4) found that rapid denitrification occurred. No appreciable losses could be attributed to such physical factors as repeated leaching with distilled water, rapid upward aeration for several weeks, exposure to relatively high temperatures in the presence of a saturated atmosphere, or exposure to ultra-violet light. Likewise, no measurable losses in sterile soils could be attributed to the reaction between ammonium and nitrite ions as had been postulated by several workers. Extensive losses, however, occurred during the aeration of unsterilized mixtures of soil, ammonium sulfate, and potassium nitrite. In sewage sludge digestion, in the absence of available ammonium nitrogen, the conversion of organic matter into more complex forms containing nitrogen can be effected at the expense of any nitrate or nitrite present. It was shown that during such digestion conversion of nitrate occurs concurrently with losses of nitrogen, which may be considerable. The stabilized organic matter in soil consists essentially of ligno-protein (11) so that there is little or no organic nitrogen available for the synthesis of bacterial cells. It would appear, therefore, that addition of nitrite or nitrate under these conditions would not result in nitrogen losses. When

large amounts of available carbonaceous materials are present, however, Corbet and Wooldridge found no loss of nitrogen when ammonium ion was supplied, but significant losses with addition of nitrite or nitrate. Losses as high as 70% of added nitrate were observed under in vitro conditions. It seems quite possible that losses may occur in cultivated soils during the decomposition of organic matter, particularly when the C/N ratio is very wide.

In recent experiments on the influence of oxygen on nitrate and nitrite reduction, Sacks and Barker (13) observed a suppression of the formation of nitrate- and nitrite-reducing enzyme systems in bacteria when grown in the presence of oxygen. When these reducing systems are present, oxygen decreased the rate of the reduction processes. The conclusions were that nitrification and denitrification can occur together at oxygen tensions of 5 percent or less, but at higher values, the simultaneous occurrence of both processes is unlikely unless the bacteria have been able to move from an adjacent region of lower oxygen tension within a short period of time. However, they point out that in a heterogeneous system such as soil the oxygen tension may change rapidly over very short distances. Under such circumstances the diffusion of nitrite and nitrate as well as the movement of bacteria may be a factor in determining the rate of denitrification.

The use of the stable isotope of nitrogen to label physiologically important compounds in metabolic studies is well known (14). Extension of this technique to studies of the biochemical changes occurring in plants has been limited. Studies by Hevesy and co-workers (6) and Vickery and Schoenheimer (18) were the only extensive investigations using $N^{15}$ prior to those undertaken by MacVicar and Burris (8). These investigations clearly demonstrated the usefulness of $N^{15}$ in such studies.

So far as is known only one group of workers has undertaken investigations to determine nitrogen losses occurring in soils using such tracer techniques. Several years ago, workers in the U.S.D.A. Experiment Station at Beltsville made a study of the possible loss of nitrogen from soils due to the Van Slyke reaction (the reaction of nitrous acid and primary amines to yield gaseous nitrogen). Under the conditions employed it was found that this reaction did not occur to a detectable extent (5).

## EXPERIMENTAL

### Materials

The soil used in these experiments was Chickasha loam derived from Permanin sandstone collected 10 miles north of Stillwater, Oklahoma. It is a well-aerated soil, the profile being 3 to 4 feet deep. Chemical analysis showed the following composition for the virgin and cultivated soils, respectively: total nitrogen—0.136%, 0.072%; readily available phosphorus—0.0 ppm., 3.0 ppm.; exchangeable potassium—50.6 ppm., 26.0 ppm.; organic matter—2.86%, 2.01%. The pH of the virgin soil was found to be 5.7 and that of the cropped soil 5.6.

Experiment I: The soil was collected, air dried, and ground to 20 mesh. Six hundred grams of well-mixed virgin and cultivated soil were placed in 1000 ml. beakers and 169.8 mg. of $(N^{15}H_4)SO_4$ containing 32 atom percent $N^{15}$ excess added. This treatment provided the equivalent of 120 lbs. of nitrogen per acre. In addition, 11.0 grams of cellulose (filter paper pulp) or sucrose, equivalent to 40,000 lbs. of carbonaceous material per acre, were added. Table I shows the treatment of each sample.

Table I

Treatment of Soils Used in Experiment I

| Sample No. | Soil | Treatment |
|---|---|---|
| 1 | Virgin | Plus 120 lbs. N |
| 2 | Virgin | Plus 120 lbs. N<br>Plus 40,000 lbs. cellulose |
| 3 | Virgin | Plus 120 lbs. N<br>Plus 40,000 lbs. sucrose |
| 4 | Cropped | Plus 120 lbs. N |
| 5 | Cropped | Plus 120 lbs. N<br>Plus 40,000 lbs. cellulose |
| 6 | Cropped | Plus 120 lbs. N<br>Plus 40,000 lbs. sucrose |

The water content was maintained at 16% of saturation value by semi-weekly additions of water.

Auxiliary Experiments II and III were performed to determine the presence of denitrifying bacteria in the soil. In Experiment II 5 grams of untreated soil were incubated with 500 ccs. of Klaeser's solution (21) at 37° for a period of 288 hours with no artificial aeration.

In Experiment III the above procedure was repeated using the same solution with one modification. Nitrogen was supplied as the ammonium ion in the form of $(NH_4)_2HPO_4$, $NH_4H_2PO_4$, $(NH_4)_2SO_4$, and $NH_4Cl$, the total nitrogen added being equivalent to nitrogen supplied as nitrate in Experiment II.

## Methods

Experiment I: Samples were taken at intervals of 15, 30, 60, 90, and 120 days. The soil was well-mixed and aliquot portions representing 100 grams of the original air dry soil were taken from each treatment. A sample, representing 10 grams of air dry soil, was immediately analyzed for ammonium and nitrate nitrogen by the aeration procedure of Umbreit and Bond (17), using Devarda's alloy to reduce the nitrates after ammonium analysis. Aeration was continued for four hours. The remainder of the 100 gram sample was dried rapidly at 65° for further analyses.

Total nitrogen was determined by a semi-micro modification of the Kjeldahl procedure. Two gram samples of the dried virgin soil were used and 3 gram samples of the dried cropped soils were used for total nitrogen analyses.

Experiment II and III: At time of sampling the culture medium was agitated thoroughly to disperse bacterial cells throughout the solution.

Fifty cc. samples were removed and immediately diluted to 100 cc. with 1:1 sulfuric acid. An aliquot portion of 20 cc. of the acid-sample mixture, representing 10 cc. of the original sample, was analyzed for total nitrogen by the micro-procedure above. Any nitrates present were reduced before digestion by the iron procedure as developed by Pucher, Vickery, et al. (12). This reduction was deemed necessary in Experiment III because of the possibility of nitrification of the ammonium ion.

$N^{15}$ analyses were made on a Westinghouse mass spectrometer under the supervision of Dr. R. F. Wall of the Department of Electrical Engineering, A. and M. College of Texas. The conversion apparatus used was similar to that shown by Schoenheimer (15), and the Kjeldahl titration mixtures from all phases of Experiment I were prepared for conversion as suggested by him.

## RESULTS AND DISCUSSION

The total nitrogen content of the variously treated soils sampled during a 120 day incubation period is presented in Table 2. In every case

Table 2

Total Nitrogen Content in Milligrams N per Gram of Soil

| | No added Organic Matter | | Cellulose added | | Sucrose added | |
|---|---|---|---|---|---|---|
| Days | Virgin | Cultivated | Virgin | Cultivated | Virgin | Cultivated |
| 15 | 1.32 | 0.78 | 1.34 | 0.78 | 1.31 | 0.81 |
| 30 | 1.34 | 0.78 | 1.30 | 0.79 | 1.31 | 0.84 |
| 60 | 1.32 | 0.74 | 1.27 | 0.77 | 1.25 | 0.82 |
| 90 | 1.31 | 0.73 | 1.25 | 0.78 | 1.31 | 0.84 |
| 120 | 1.26 | 0.70 | 1.27 | 0.76 | 1.27 | 0.76 |

the total nitrogen content was found to be slightly less at the end of the experiment than at the beginning. In all but one case, however, the range of variation between samples was less than the analytical variation within samples. In our hands, the semi-micro Kjeldahl procedure for total nitrogen has not been applied to soil samples with an accuracy greater than about five percent. In one treatment, however, the decrease appeared to be significant. The nitrogen content of the cultivated soil without added organic matter showed a consistent decline from 0.78 mg. of nitrogen per gram of dry soil initially to 0.70 mg. at 120 days. This is an apparent loss of 10.3 percent and is, therefore, appreciably in excess of the error of the determination. If extensive nitrification had occurred, incomplete recovery of nitrate nitrogen might have accounted for a portion of these small decreases. Reduction of nitrate prior to digestion, however, failed to give any appreciable increase in total nitrogen, thus eliminating this explanation for the apparent loss.

Data on the $N^{15}$ content of these soils are shown in Table 3. No

Table 3

$N^{15}$ Content Expressed as Atom Percent Excess $N^{15}$

| | No added Organic Matter | | Cellulose added | | Sucrose added | |
|---|---|---|---|---|---|---|
| Days | Virgin | Cultivated | Virgin | Cultivated | Virgin | Cultivated |
| 15 | 1.28 | 2.01 | 1.22 | 1.92 | 1.28 | 2.00 |
| 30 | 1.26 | 1.84 | 1.24 | 2.05 | 1.34 | 2.02 |
| 60 | 1.22 | 1.86 | 1.22 | 2.08 | 1.27 | 1.98 |
| 90 | 1.22 | 1.55 | 1.25 | 2.05 | 1.26 | 1.95 |
| 120 | 1.04 | 1.47 | 1.27 | 1.91 | 1.27 | 1.96 |

apparent decline in isotope content was observed except in the soils without added organic matter, particularly the cultivated sample, the same one that had shown an apparent loss in total nitrogen. There are two possible explanations for such a finding, selective loss of the added isotope or contamination of the soil with added nitrogen with a resultant dilution in the isotope concentration. The latter was found not to be a probable explanation, since the nitrogen content fell rather than increased. Nearly identical isotope concentration values were obtained before and after reduction of nitrate, thus eliminating selective nitrification and incomplete recovery on Kjeldahl digestion as an explanation. Evidence to be presented later on the $N^{15}$ content of the nitrate fraction would cause a selective loss of $N^{15}$ if, as seems likely, nitrate is the source of the nitrogen converted to $N_2$ by denitrifying bacteria. That strictly anaerobic conditions are not required for such conversion in solution culture is shown by the data presented in Table 4. It will be seen that extensive loss of total nitrogen occurred as long as nitrate was present (during the first 24 hours) and accounted for a total loss of some 33.9 percent

Table 4

Total Nitrogen Content in Milligrams N per 10 cc. Sample

| Days | Nitrate culture | Ammonia culture |
|---|---|---|
| 0 | 2.36 | 2.42 |
| 1 | 1.57 | 2.38 |
| 3 | 1.45 | 2.39 |
| 6 | 1.45 | 2.38 |
| 12 | 1.40 | 2.40 |

of the total nitrogen. This finding was in accordance with the observations of Murray (10) who showed that extensive denitrification occurred in solution cultures with mixed soil bacteria even during continuous and rapid aeration.

These two different types of approach both indicate that in soils relatively low in organic matter available for bacterial decomposition appreciable losses of nitrogen may occur under what approximates normal soil conditions. In the case of the cultivated soil, the quantity lost was approximately equal to the quantity added in the form of ammonium sulfate. The addition of organic matter to these soils, either in the form of sucrose or as highly purified cellulose, provided a carbon source for the soil bacteria. Under these conditions no apparent loss of nitrogen was found by quantitative determinations of the total nitrogen content; isotope data likewise indicated that any denitrification of the added $N^{15}$ was of a low order of magnitude. In virgin soils, which contained 2.86 percent organic matter as compared with 2.01 percent in the cultivated sample, the nitrogen loss as determined by Kjeldahl nitrogen analyses was not significant. Isotope data, however, indicates that some denitrification has occurred nonetheless. These findings strongly suggest that

under normal conditions of aeration the addition of organic matter of widely varying C/N ratio does not result in the loss of nitrogen during a 120 day incubation period. On the contrary, losses of added nitrogen which occurred in soils low in organic matter were prevented by the addition of readily available carbohydrate in quantities to provide a C/N ratio equivalent to wheat straw.

Changes in ammonia and nitrate concentration of variously treated soils are indicative of the microbiological activity at the time of sampling. Ammonia and nitrate levels at varying times of incubation are shown in Figures 1-6.

The ammonia content of these soils was invariably of a low order of magnitude at all sampling dates, accounting for from 1.3 to 10.7 percent of the total nitrogen in the cultivated and from 0.8 to 5.0 percent in the virgin soil for minimum and maximum values, respectively. Although the relative change in ammonia concentration was relatively large, it will be seen that with respect to the total nitrogen present or the nitrogen added as $(N^{15}H_4)_2SO_4$, the changes were less marked. The quantity present in soils containing large amounts of available organic matter was not consistently either more or less than the soils to which no such additions were made. The variations in the ammonia nitrogen content of soils supplied with organic matter were more pronounced and irregular, probably a result of prolific microbiological activity. The increased microbiological activity in the presence of readily available carbonaceous matter is also shown by the $N^{15}$ content of the ammonia fraction at various dates of sampling (Figures 7-12). The $N^{15}$ content in the ammonia fraction of all treatments would be expected to be initially high since the heavy nitrogen isotope was added as $(N^{15}H_4)_2SO_4$. This was found to be true with but one exception,

FIGURE 1

AMMONIA AND NITRATE CONTENT IN MILLIGRAMS NITROGEN PER GRAM OF SOIL
VIRGIN SOIL - NO ADDED ORGANIC MATTER

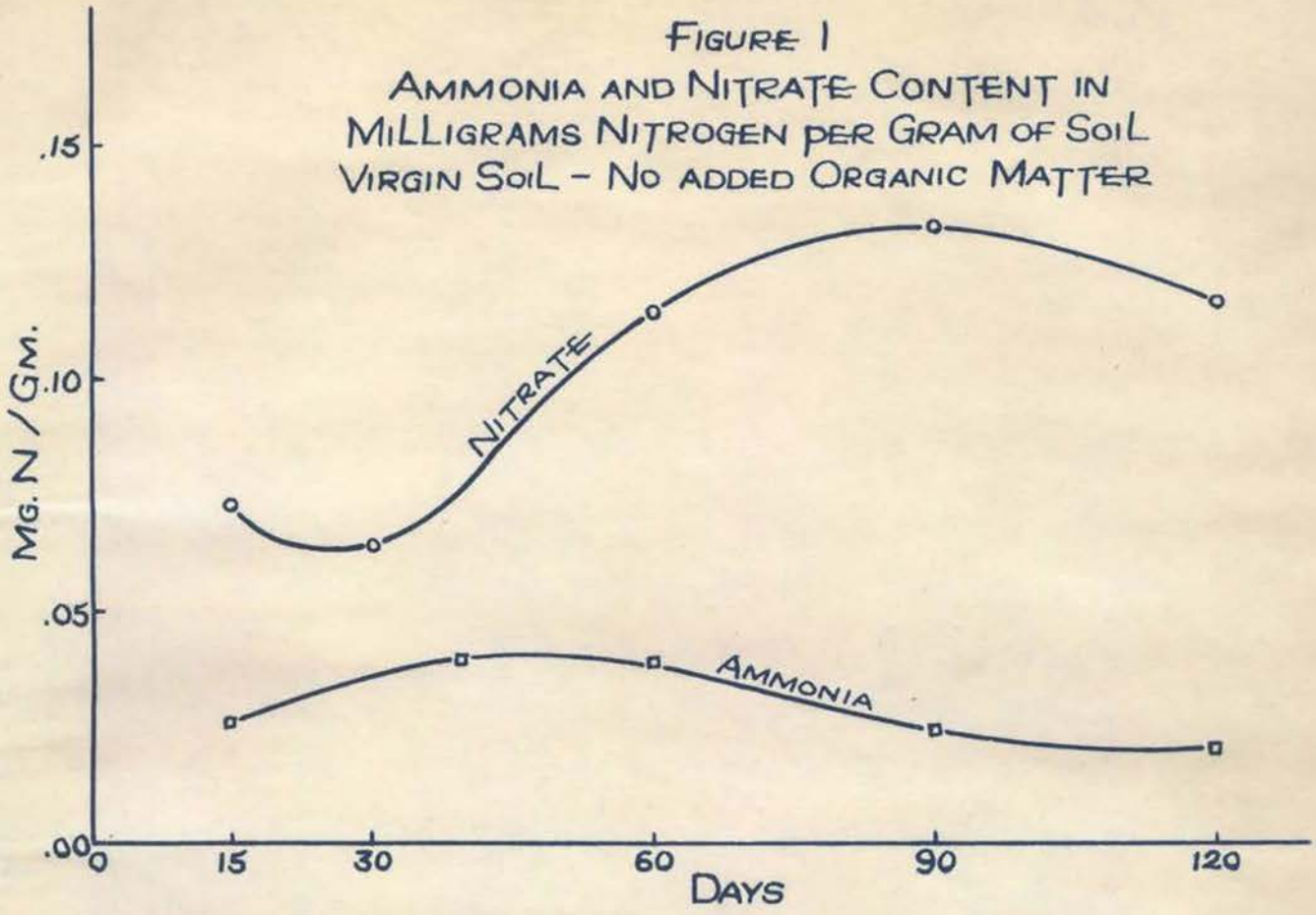



FIGURE 2

AMMONIA AND NITRATE CONTENT IN MILLIGRAMS NITROGEN PER GRAM OF SOIL
CULTIVATED SOIL - NO ADDED ORGANIC MATTER

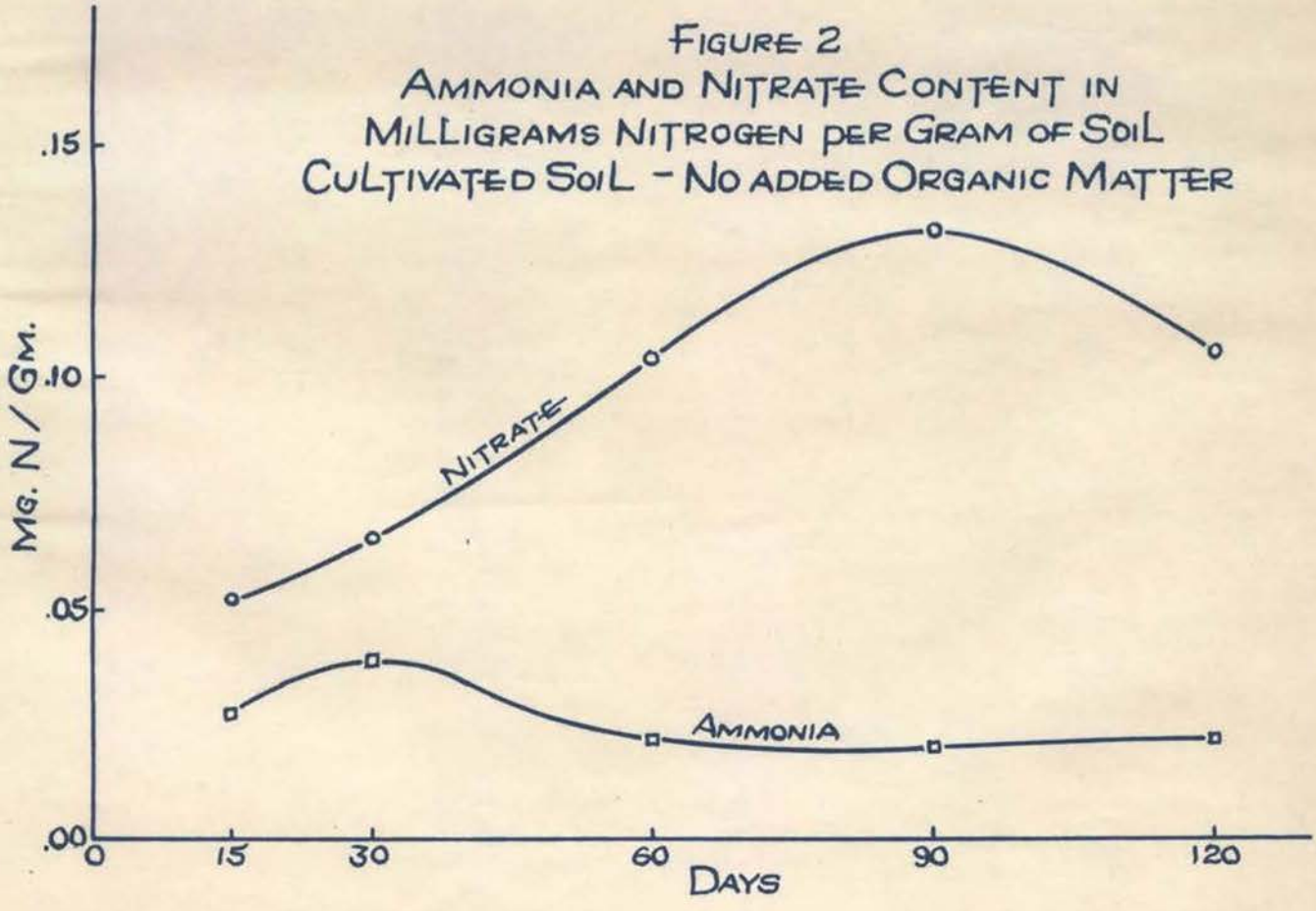


FIGURE 3

AMMONIA AND NITRATE CONTENT IN MILLIGRAMS NITROGEN PER GRAM OF SOIL
VIRGIN SOIL - CELLULOSE ADDED

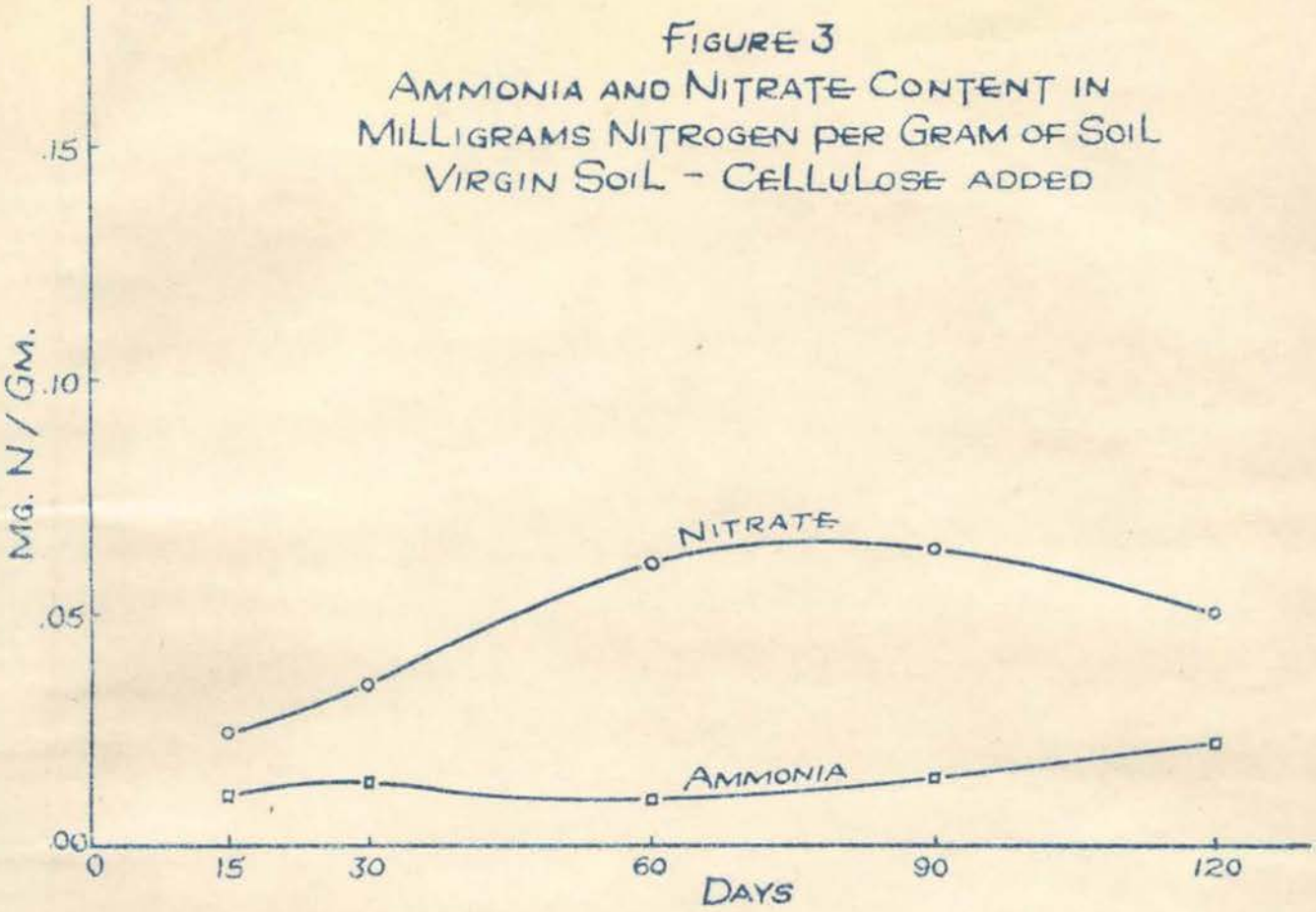



FIGURE 4

AMMONIA AND NITRATE CONTENT IN MILLIGRAMS NITROGEN PER GRAM OF SOIL
CULTIVATED SOIL - CELLULOSE ADDED

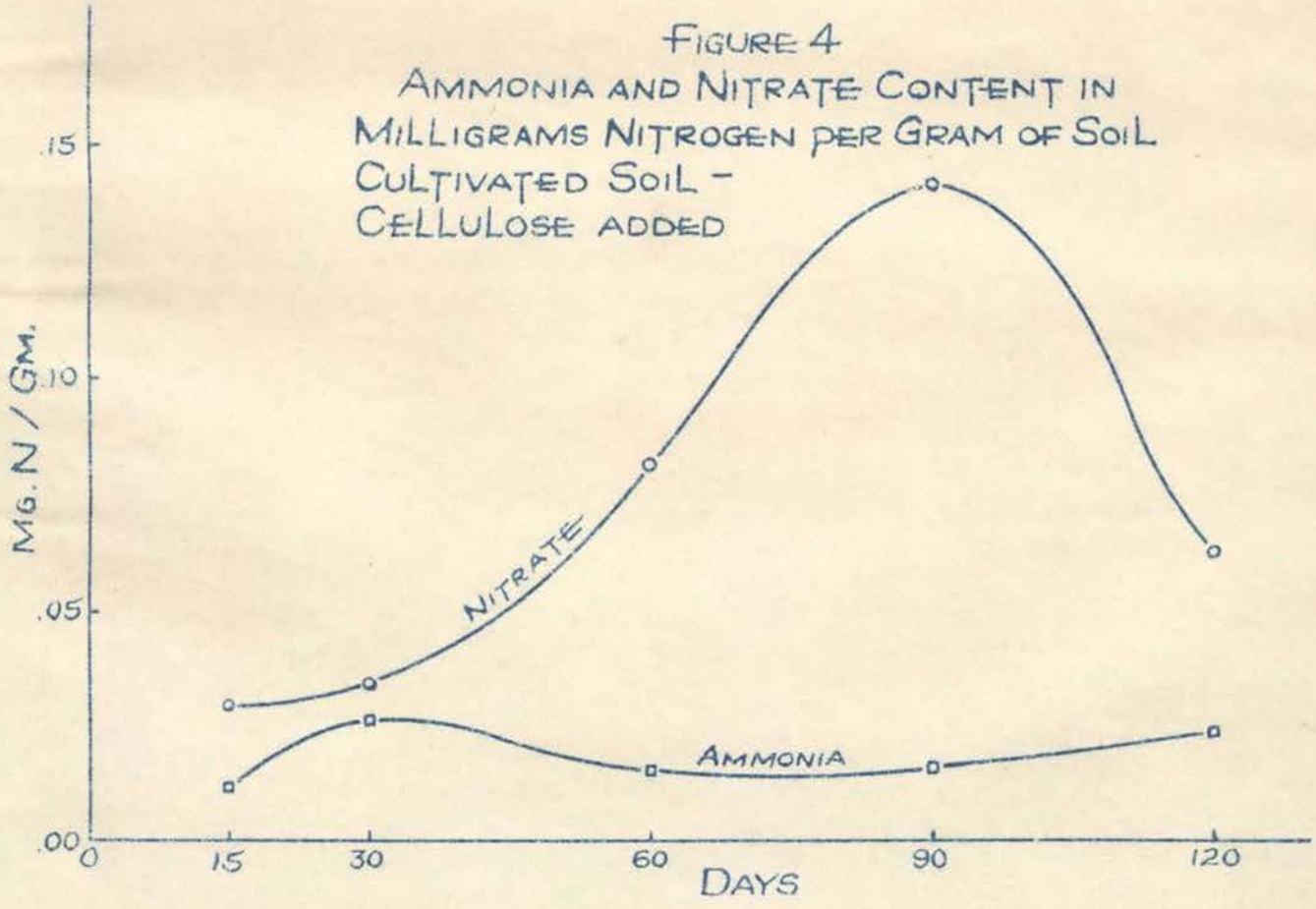


FIGURE 5

AMMONIA AND NITRATE CONTENT IN MILLIGRAMS NITROGEN PER GRAM OF SOIL
VIRGIN SOIL – SUCROSE ADDED

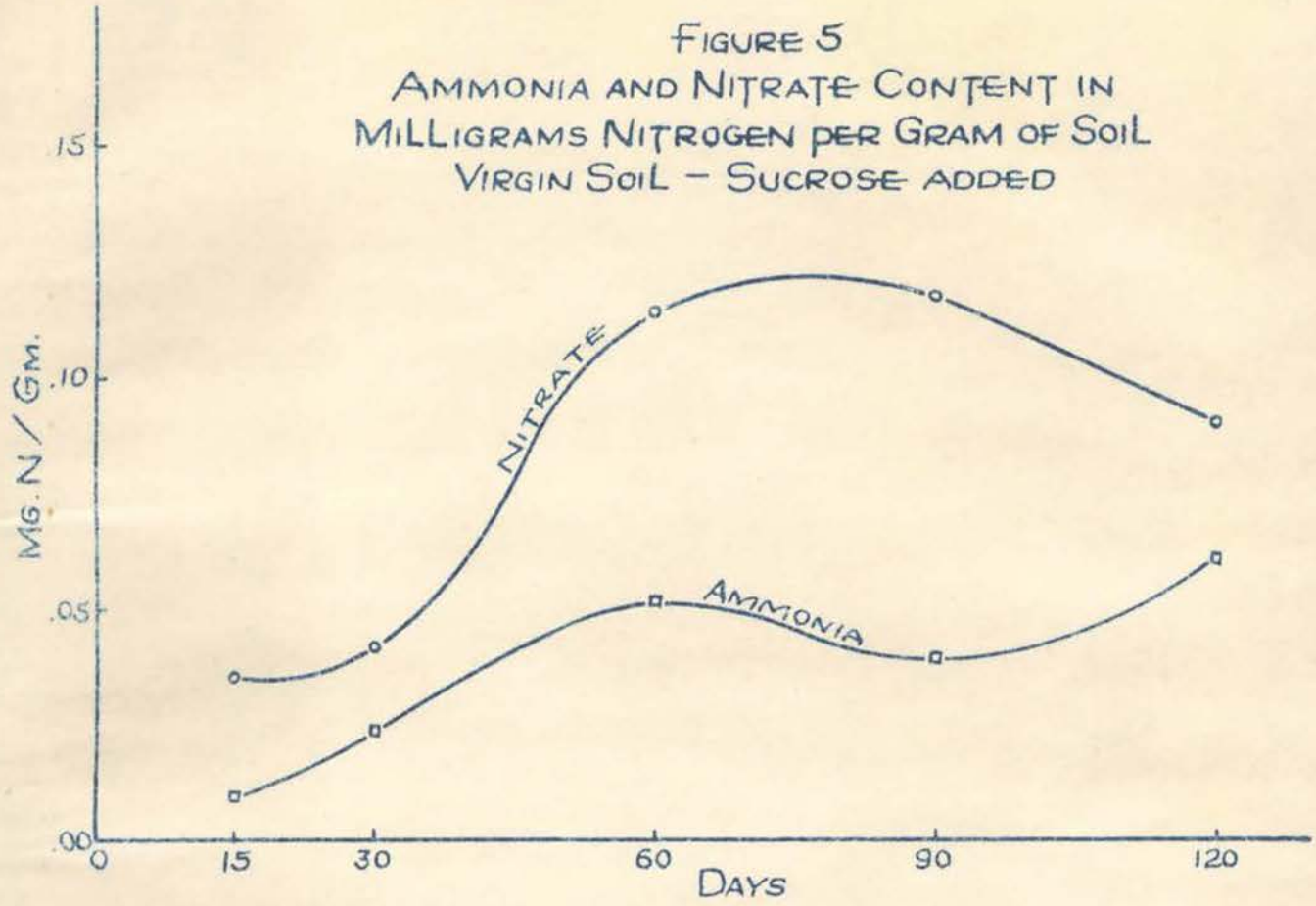



FIGURE 6

AMMONIA AND NITRATE CONTENT IN MILLIGRAMS NITROGEN PER GRAM OF SOIL
CULTIVATED SOIL –
SUCROSE ADDED

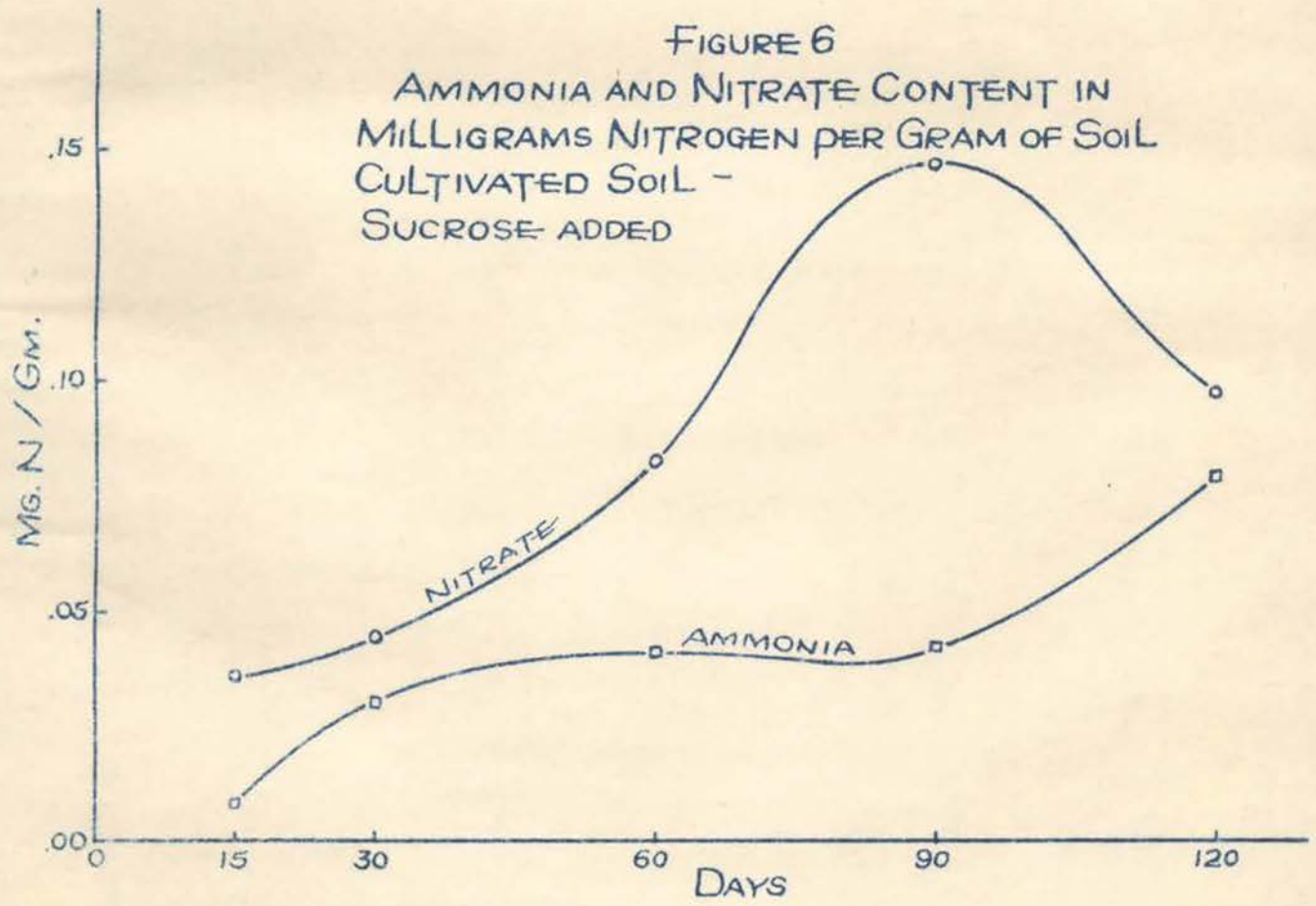


FIGURE 7

$N^{15}$ CONTENT OF AMMONIA AND NITRATE FRACTIONS
VIRGIN SOIL - NO ADDED ORGANIC MATTER

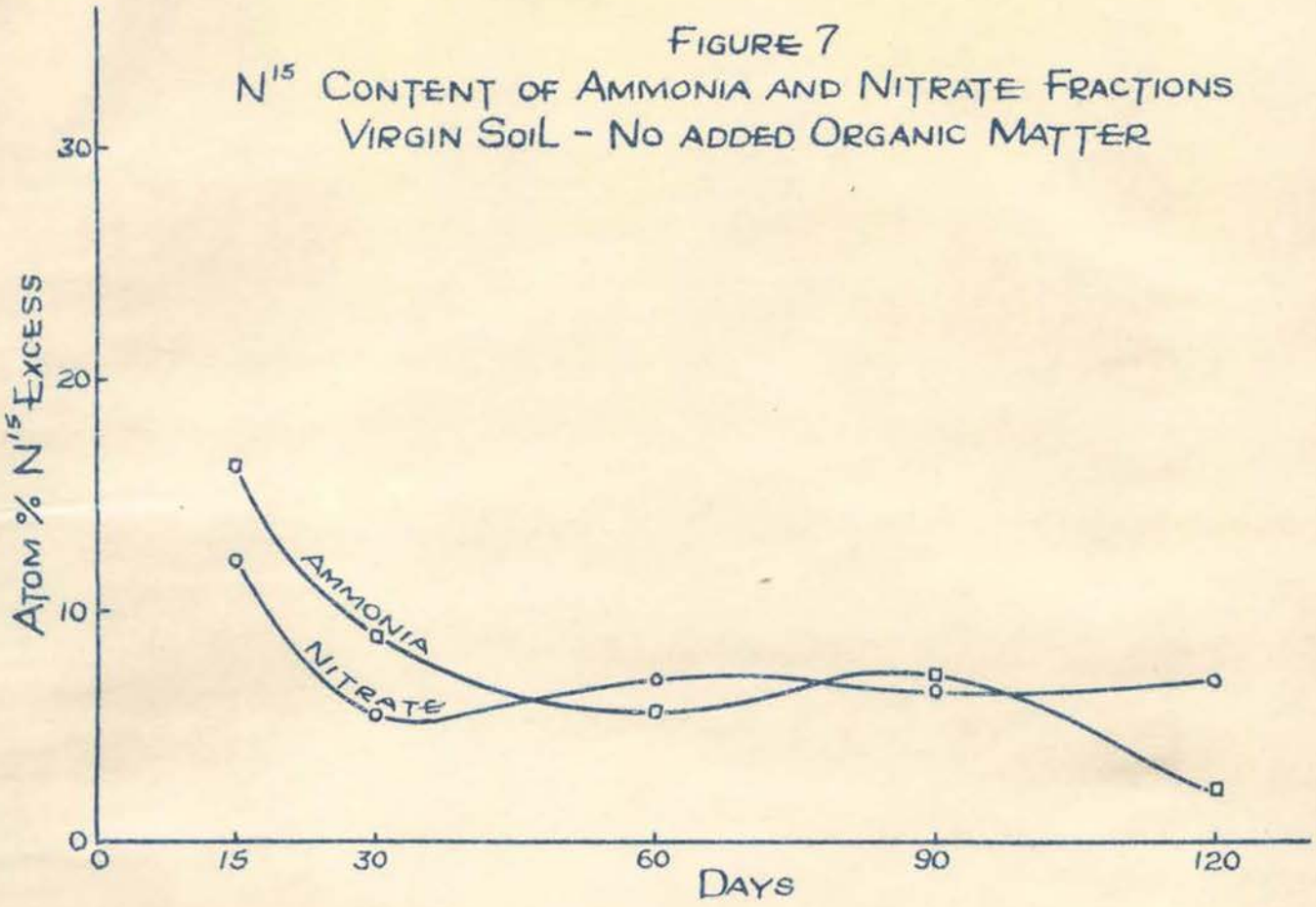



FIGURE 8

$N^{15}$ CONTENT OF AMMONIA AND NITRATE FRACTIONS
CULTIVATED SOIL - NO ADDED ORGANIC MATTER

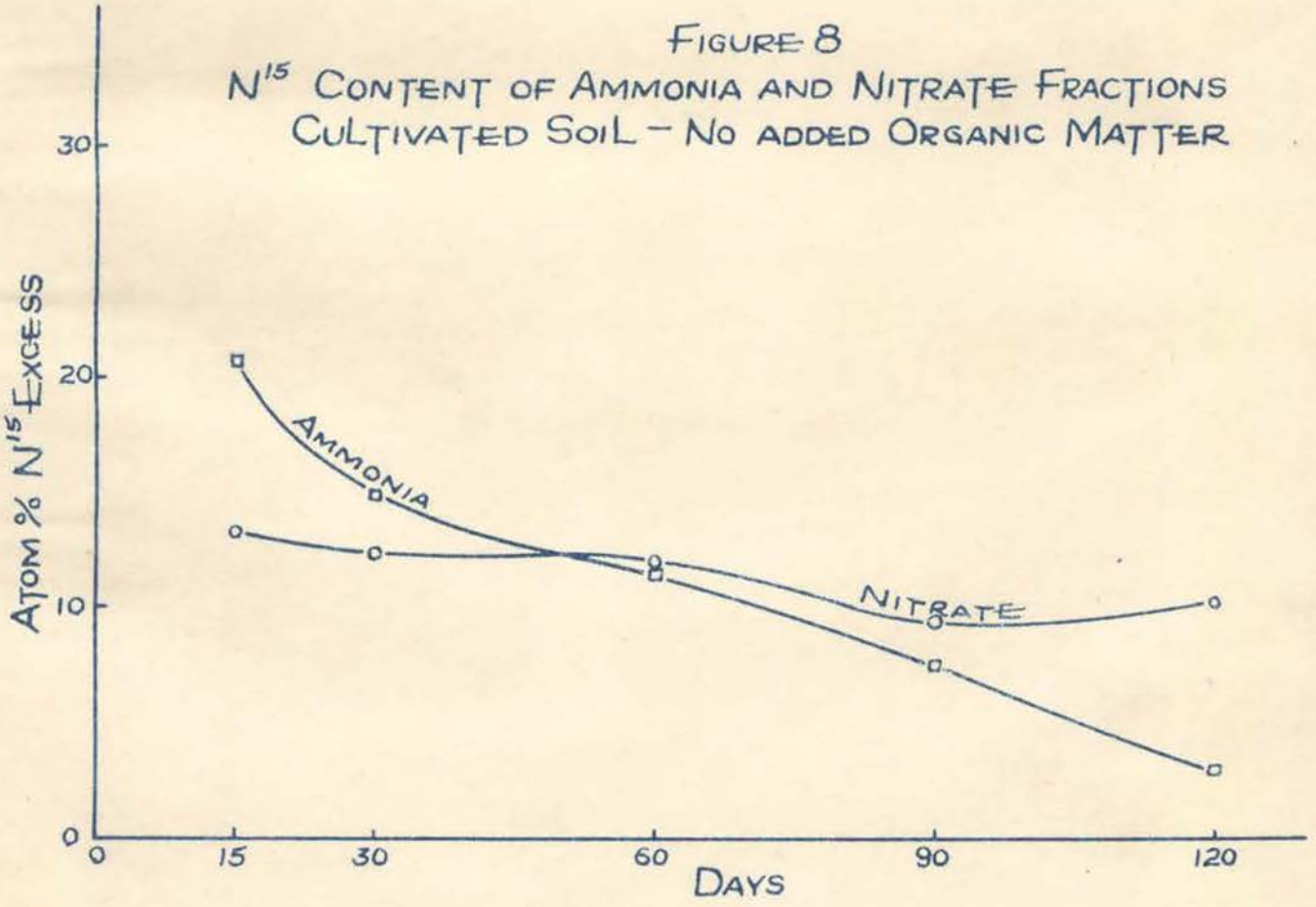


FIGURE 9

$N^{15}$ CONTENT OF AMMONIA AND NITRATE FRACTIONS

VIRGIN SOIL - CELLULOSE ADDED

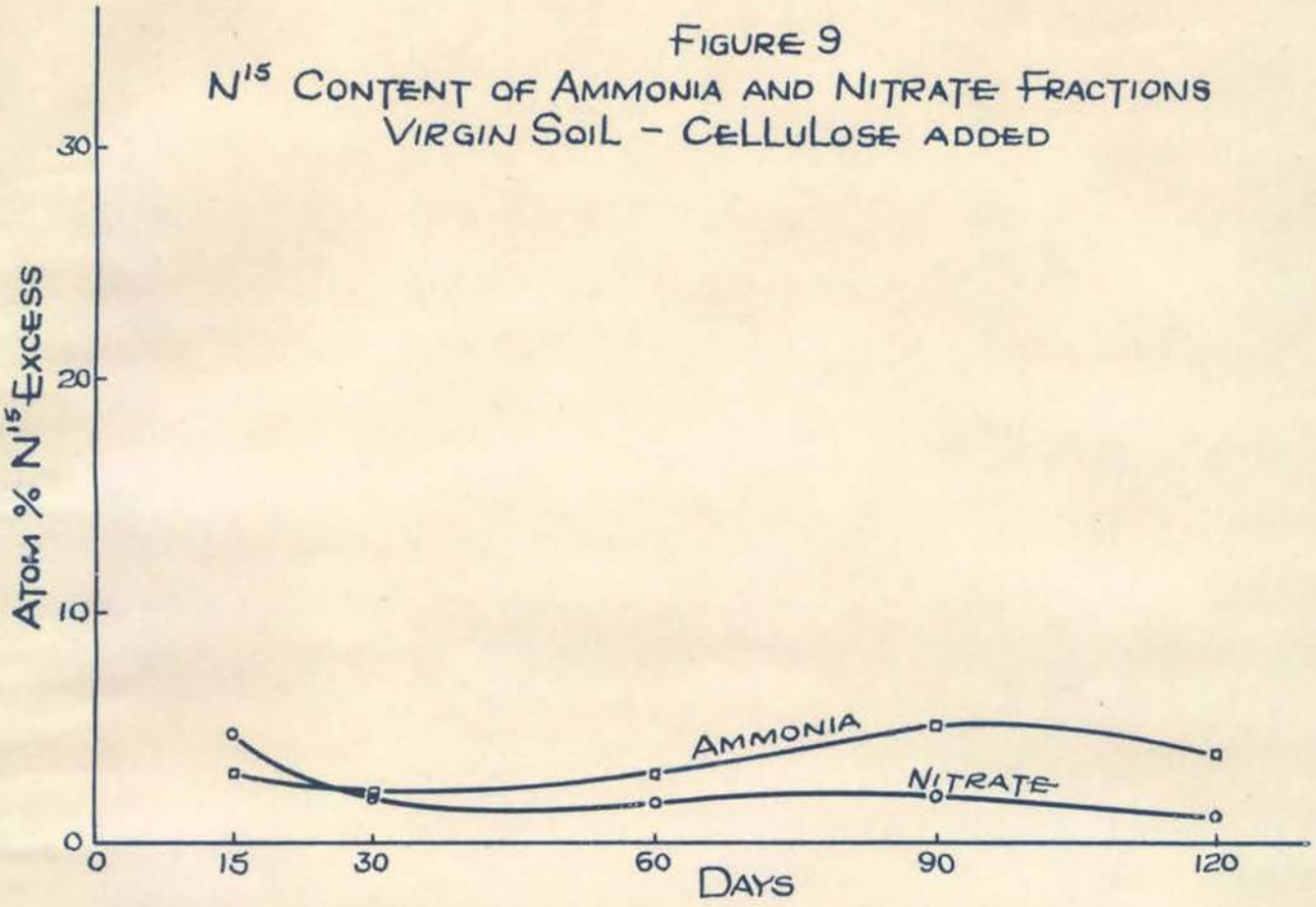



FIGURE 10

$N^{15}$ CONTENT OF AMMONIA AND NITRATE FRACTIONS

CULTIVATED SOIL - CELLULOSE ADDED

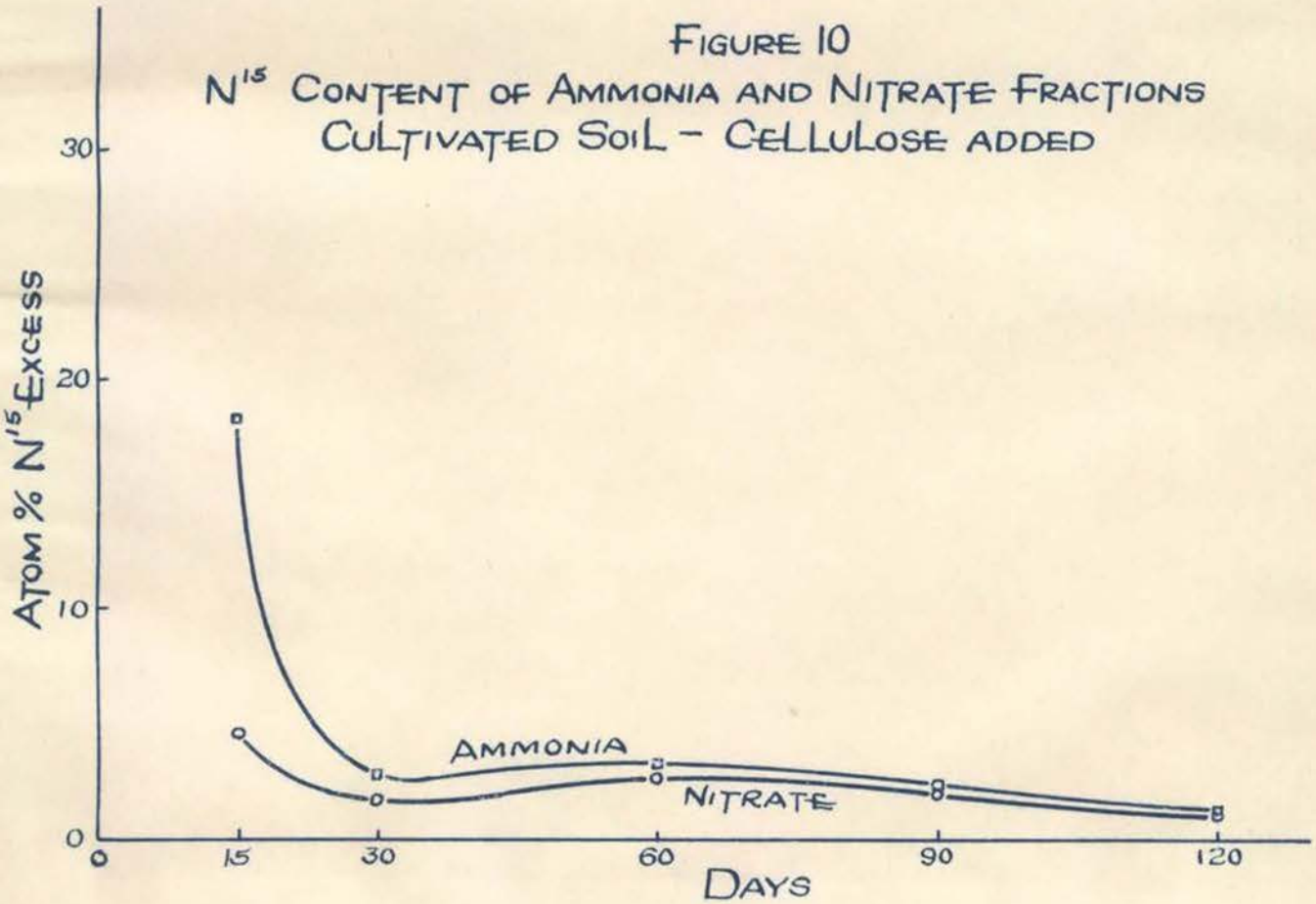


FIGURE 11

$N^{15}$ CONTENT OF AMMONIA AND NITRATE FRACTIONS

VIRGIN SOIL - SUCROSE ADDED

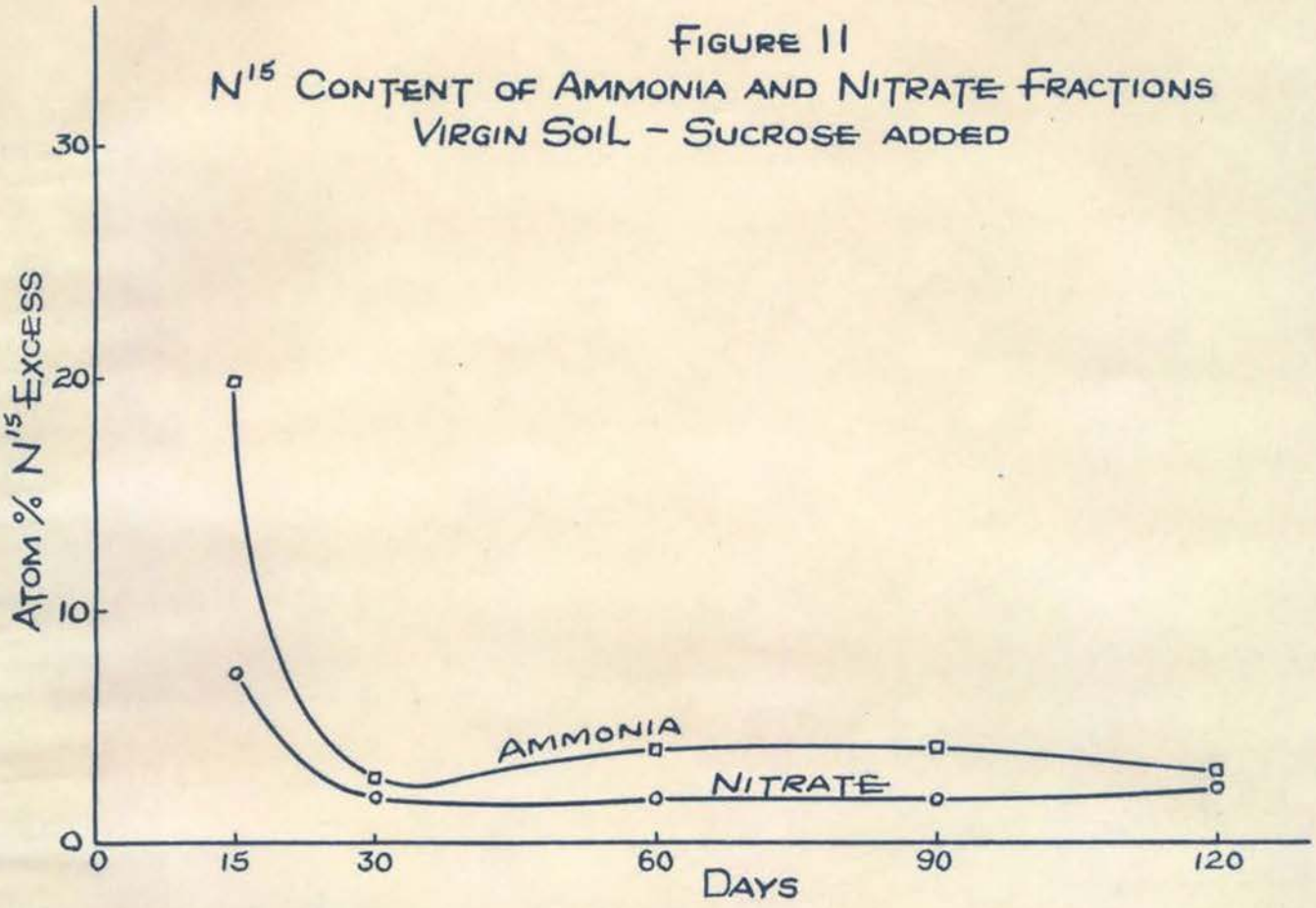
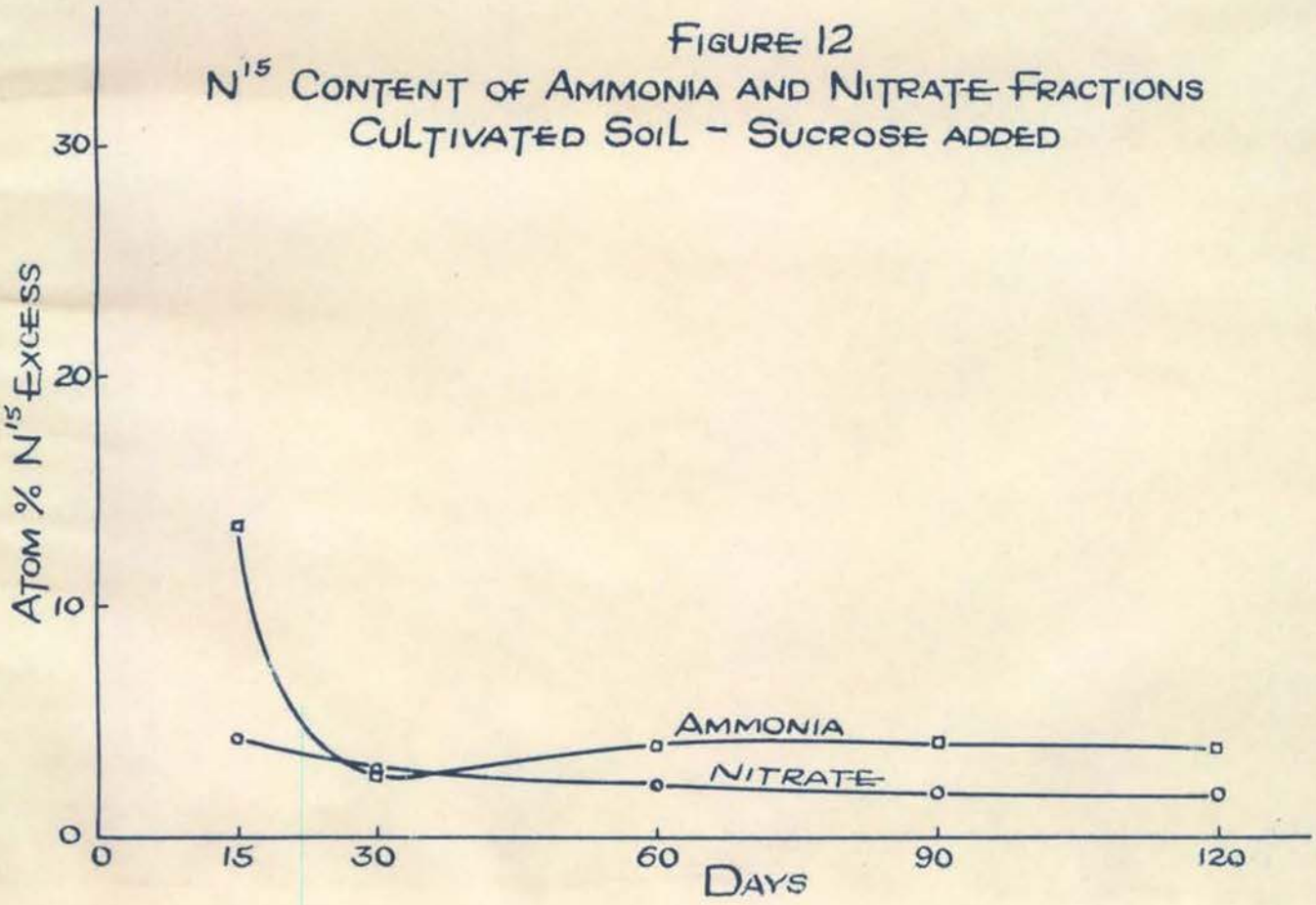



FIGURE 12

$N^{15}$ CONTENT OF AMMONIA AND NITRATE FRACTIONS

CULTIVATED SOIL - SUCROSE ADDED



the virgin soil treated with sucrose. In presence of excess carbonaceous matter, however, the rate of turnover of the added ammonia was much greater, as shown by the more rapid decline in the $N^{15}$ content when compared with the low organic matter soils. The virgin soil sample containing sucrose showed a low level of $N^{15}$ even at the first sampling date. This can be explained by a very rapid turnover of the added nitrogen within that sample. There was also a noticeable difference in the rate of turnover of the added $N^{15}$ between the virgin and cultivated soils untreated with excess organic matter. The organic matter in virgin soil which represented this difference was partly in the form of relatively unstable organic matter; that in the cultivated soil consisted of highly stable organic materials which were not readily available for use by bacteria.

The nitrate content of the various samples is likewise given in Figures 1-6. It will be seen that generally speaking the nitrate content was somewhat greater than the ammonia. It rose gradually during the first two months, indicating that nitrifying bacteria were present in the soil. At the final sampling (120 days of incubation) all soils showed a decline in the nitrate content, suggesting that utilization of nitrate as a nitrogen source was occurring. As shown in Figures 7-12, the $N^{15}$ content of the nitrates at the fifteenth day of incubation in the cultivated soils without organic matter was 13.4 atom percent $N^{15}$ excess. Since the original $N^{15}$ contained 32.0 percent excess $N^{15}$, roughly two-fifths of the nitrate present on this date had been derived by the nitrification of added ammonium ion. The $N^{15}$ content of the virgin soil with added sucrose was approximately one-half this value (7.2); all other treatments were nearly equal at 4.5 atom percent $N^{15}$ excess. At thirty days, all treatment containing added organic matter had attained isotope concentrations of from two to

three atom percent $N^{15}$ excess; changes during the ensuing 90 days of incubation were very slight. In the soils to which no organic matter had been added, however, the concentration of isotope in the nitrate fraction was appreciably higher and declined only slightly during the last three months of incubation. Final values for cultivated and virgin soils were 10.2 and 7.0 atom percent $N^{15}$ excess, respectively. The greater quantity of $N^{15}$ incorporated into the nitrate fraction of those soils containing no excess organic matter and the higher level of $N^{15}$ in this fraction throughout the experiment substantiated the evidence of less microbiological activity in those soils of low organic matter content as found in the data from the ammonia fraction.

## SUMMARY

Nitrogen changes in soils of varying organic matter content (virgin and cultivated) have been traced by quantitative procedures and by utilization of the stable, heavy isotope of nitrogen, $N^{15}$. Total nitrogen, ammonia, and nitrate contents were determined at periodic intervals during a 120 day incubation period in soils to which had been added $(N^{15}H_4)_2 SO_4$. Excess organic matter (sucrose or highly purified cellulose) was added to some samples.

Analyses for total nitrogen showed a significant decrease only in the cultivated soil to which no organic matter had been added. Isotopic analysis confirmed this observation, and further indicated that losses not detectable by chemical procedures had occurred in the corresponding treatment with virgin soil. These findings suggest that under normal conditions of soil aeration the addition of organic matter of widely varying C/N ratios such as wheat straw is probably not conductive to losses of nitrogen by denitrification. On the contrary, additions of organic matter appeared to stabilize the soil nitrogen.

Ammonia content was found to be of a low order of magnitude at all sampling dates. More pronounced and irregular changes in ammonia content in those soils containing excess organic matter and the rapid decrease of $N^{15}$ content in the ammonia fraction of those same soils signify a prolific microbiological activity in the presence of excess organic matter.

The nitrate content, generally higher than that of ammonia, showed a general increase in all samples, signifying the presence of nitrifying bacteria. The greater quantity of $N^{15}$ incorporated into the nitrate fraction of those soils containing no excess organic matter and the higher level of $N^{15}$ in this fraction throughout the experiment substantiated the

evidence of less microbiological activity in those soils of low organic matter content as found in the data from the ammonia fraction.

In experiments on denitrification in solutions containing added nitrate a loss of approximately 34 percent of the total nitrogen added as nitrate was found to occur during the first 24 hours under normal aerobic conditions of incubation.

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