THESIS

CULTIVATION EFFECTS ON NITRIFICATION IN POTATO SOILS

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY AL-SAMARRAIE ABDUL-HAMIED ENTITLED CULTIVATION EFFECTS ON NITRIFICATION IN POTATO SOILS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

CULTIVATION EFFECTS ON NITRIFICATION
IN POTATO SOILS

Nitrification rates were found to be less rapid in newly tilled potato soils than in aged cultivated soils. Studies were undertaken to determine what factor or factors were responsible for the slower nitrification rates. Aged cultivated and virgin soils were obtained from various locations and compared in residual mineral nitrogen content, nitrification rates and in bacterial populations.

Total residual mineral nitrogen content (ammonium and nitrate) in aged cultivated soils was usually higher than that in virgin soils.

To compare nitrification rates, aged cultivated and virgin soils were enriched with 0, 50 and 100 ppm ammonium nitrogen and incubated at 24° C for 0, 10 and 21 days.

Nitrification rates were consistently lower in virgin soils than in aged cultivated soils. Also, nitrification was higher in soil samples collected in June than in those collected in December.

Determination of soil bacteria (Nitrosomonas and Nitrobacter) was done by the most probable number method. Population of Nitrosomonas in virgin soils ranged from 260 to 460 cells per gram in virgin soils and from 7, 200 to 35, 000 in cultivated soils.

Correspondingly, Nitrobacter in virgin soils ranged from 45 to 78 and in cultivated soils from 4,100 to 35,000.

The lower rate of nitrification in virgin soils was attributed to the low bacterial population.

The presence of a nitrification inhibitor in virgin soils produced by native vegetation was not considered probable. In one experiment where various amounts of aged cultivated soil were mixed with virgin soil the nitrification rates in the latter increased in proportion to aged cultivated soil added. This may not have occurred if nitrification inhibitors were present.

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INTRODUCTION

Nitrogen is often the single most limiting factor in vegetable production. Most of the shallow rooted and fast growing vegetables require continuous and adequate supply of nitrogen throughout their growing season. About 90 percent of soil nitrogen is in organic form which as such is not available to vegetables. The rate of mineralization of organic nitrogen in soil is not sufficiently fast to meet the nitrogen requirement of vegetable crops. Therefore, for higher yields, vegetable crops are generally fertilized with nitrogenous fertilizers such as ammonium sulfate, and other forms.

Nitrogen has generated considerable interest since this element is most often deficient for crop production throughout the world. Environmentalists are concerned because some nitrite moves from soils into lake and water storage reservoirs contributing to eutrophication. Nitrates are also subject to denitrification to N_2O (nitrous oxide). The latter may also be a serious atmospheric pollutant.

Nitrogen compounds in soils are exposed to a wide variety of environmental conditions. When nitrogen fertilizers are applied to a soil, reactions such as nitrification, denitrification or fixation may occur. Ammonium forms may be transformed to nitrate forms and vice versa. Microorganisms play an important role in these transformations. Nitrate is more mobile in soils than ammonium which is

adsorbed on exchange sites of soil colloids. Nitrate leached beyond the root zone is not available to plants and becomes an important environmental concern.

The rate at which applied ammonium is converted to nitrate is very important. High transformation rates may result in leaching of nitrate, accumulation of toxic species such as nitrite and low fertilizer efficiencies resulting in low yields. In potato nitrogen fertilization studies on newly cultivated sandy loam soils, the applied ammonium nitrogen was not nitrified as rapidly as expected. The results with one field are shown in Figure 1. Residual nitrogen levels were measured on April 15. One hundred eighty pounds of ammonium nitrogen was top dressed and incorporated into the soil on June 16 when the plants were four inches tall. Soil ammonium and nitrate nitrogen were measured on July 18, August 1, August 16 and September 3. The percentage of the nitrogen remaining in the ammonium form on each sampling was as follows: 4 weeks (7/18) 100 percent, 6 weeks (8/1) 72 percent, 8 weeks (8/16) 49 percent and 10 weeks (9/3) 63 percent. Nitrate nitrogen remained relatively low and constant during the season.

The conversion of ammonium to nitrate is influenced by many biological, physical and chemical characteristics of soils. Among them the microbial activities are very important. The population of nitrifying bacteria depends on the type of soils. Virgin soils may have

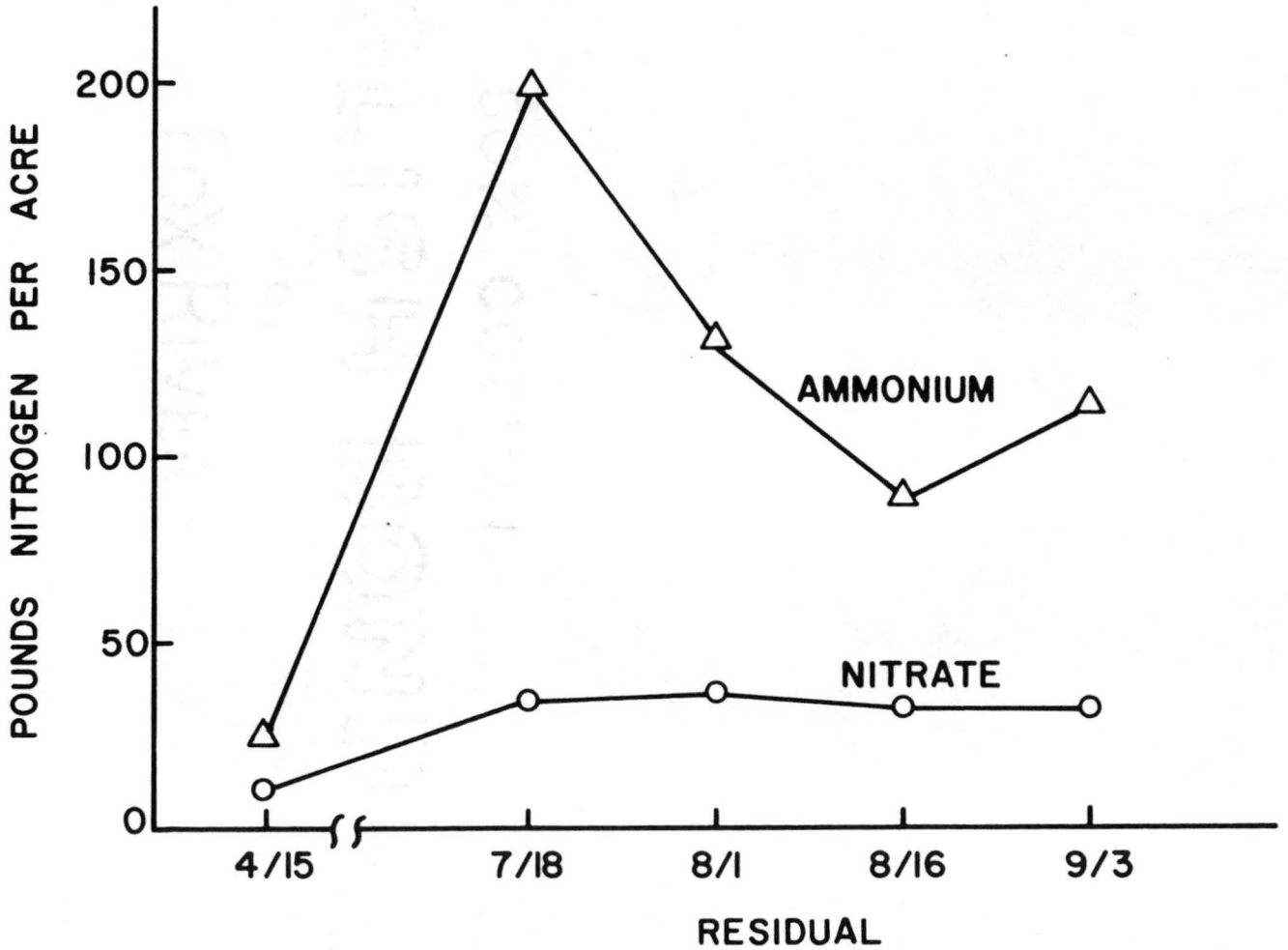


Figure 1. Seasonal changes during the first year of cultivation in ammonium and nitrate nitrogen in sandy loam soil planted to potatoes. 180 lbs. of ammonium nitrogen was applied June 16. Data taken from C.S.U. General Series Bul. No. 959.

lower microbial populations than older cultivated soils. Also, virgin soils may contain substances that inhibit nitrification. Hence, the rate of nitrification could be low in virgin soils or in recently cultivated soils. The present study was undertaken to determine to what extent nitrification rates varied between virgin and cultivated soils, and to compare bacterial populations.

REVIEW OF LITERATURE

Ammonium and Nitrate Nutrition

Barket et al. (1966) reported that beans exhibit ammonium toxicity symptoms of curling and burning of the leaf margins and necrosis of the laminae when nitrogen was supplied as $(NH_4)_2SO_4$ in sand culture. However, the symptoms were delayed by the addition of carbonates which maintained the pH of the nutrient solution near neutrality. Maynard and Barker (1969) found that beans, cucumbers and peas exhibited reduced growth rates, marginal necrosis and chlorosis of terminal leaves with ammonium salts as the N source. However, when the pH of the nutrient solution was maintained near neutrality by the addition of $CaCO_3$ plant growth increased.

Bennet et al. (1964) reported that corn plants grown in sand culture in 56 and 112 ppm nitrogen as ammonium exhibited severely wilted leaves, damaged roots, and a significant decrease in yield. Phosphorus seemed to accumulate in the roots of plants in the ammonium cultures. Tuil (1970) and Harada and Yamada (1968) reported poorer sugarbeet growth and lower organic acid content with ammonium nutrition than with nitrate nutrition. Also, in an extensive study, Dewit et al. (1963) indicated that the poor plant growth with ammonium was associated with a lower organic content. Kirkby (1968) found that leaf and stem tissue of mustard plants grown with

ammonium nitrogen had lower concentrations of inorganic cations and higher inorganic anions than plants grown with nitrate nitrogen. Also, organic acid concentrations required to balance ionic differences within the plant were much less. The accumulation of organic anions was accompanied by mineral cations to maintain ionic equilibrium.

Weir et al. (1972) reported that radish growth was increased with ammonium nitrogen concentration up to 17 ppm but then decreased at higher concentrations. Lettuce growth increased with ammonium nitrogen solution concentration up to 36 ppm but was also decreased at higher levels.

Tomato seedlings grew well in concentrated ammonium nitrogen solution with the addition of lime to maintain a solution pH which was slightly alkaline (Woolhouse et al., 1966). Sheat et al. (1958) reported tomato roots grew best with ammonium nitrogen at pH 7 or greater and with nitrate nitrogen at pH 4.6 to 5.0.

Nitrate concentration was high (767 ppm) in certain vegetable products in response to nitrate fertilization (Brown et al., 1966; Jackson et al., 1967; Maynard et al., 1972; Peck et al., 1971). However, the nitrate content of individual species appeared to differ in their response. Beets (Beta vulgaris L.), spinach (Spinacia oleraceae L.), and lettuce (Lactica sativa L.) had higher concentrations of nitrate than carrots (Daucus carota).

Influence of Soil Characteristics on Nitrification

(a) pH effects:

Dancer et al. (1973) studied the effect of pH ranging from 4.7 to 6.6 on rates of nitrification and ammonification in the soil. pH did not markedly affect the rates of ammonification. However, pH significantly affected nitrification. The rate of nitrate accumulation decreased with decreasing soil pH.

Cornfield (1952) and Cornfield (1959) reported nitrate accumulation occurred more rapidly in soils having a pH greater than 6.5. Many acid soils accumulated ammonium rather than nitrate suggesting these soils contained fewer nitrifying bacteria than neutral and alkaline soils.

Using percolation techniques to study nitrification in 16 U.S. soils ranging in pH from 4.4 to 8.8, Morrill and Dawson (1967) found four different patterns of nitrification:

(1) At pH 6.93 to 7.85, ammonium oxidized rapidly to nitrite which accumulated for extended periods of time before being oxidized to nitrate.

(2) In soils, with pH ranging from 5.01 to 6.38, ammonium and nitrite are oxidized rapidly to nitrate.

(3) Accumulation of ammonium with very little oxidation to nitrite or nitrate occurred in soils having a pH of 4.94 to 5.39.

(4) Ammonium oxidation was not detectable by either nitrite or nitrate formation at pH 4.84 to 5.12.

Gasser (1970) reported Nitrobacter activity to be favored in neutral or slightly acid media while Nitrosomonas was less sensitive to pH. Thus, nitrite is more likely to accumulate in acid or alkaline than in nearly neutral soils. Also, Gasser (1970) reported that nitrification can be inhibited partially or wholly in acid or alkaline soils, by root secretions of some plants, especially grasses, and by the extract of leaves of some plants. Low nitrification in Bladen grassland soils was found by Brar and Giddens (1968). He concluded that low pH resulted in a low population of nitrifiers.

(b) Organic carbon effect:

Sabey et al. (1956) studied nitrification in three Iowa soils under laboratory conditions. Organic carbon contents were 1.45, 3.02 and 3.31 percent with each compared at pH values of 5.6, 7.0 and 6.5. Nitrification occurred most rapidly in soil with a pH of 6.5 and organic carbon content of 3.31 percent. Sabey concluded that the rapidity of nitrification was more closely related to organic carbon content than pH.

(c) Soil aeration:

Amer and Kolenbrander (1951) reported poorly aerated soils to inhibit ammonium to nitrate conversion. However, Greenwood (1962) found that the rate of nitrification was not seriously reduced until the

oxygen concentration in the water at the bacterial surface fell to 4×10^{-6} M which is equal to air containing only 0.3 percent oxygen.

(d) Soil temperature:

Sabey et al. (1956) reported nitrification occurred slowly at 8° C, more rapidly at 15° C, and reached a maximum at about 25° C.

Harmsen and Kolenbrander (1965) reported that below the optimum temperature range, 25° C to 35° C, nitrification decreases gradually, following an asymptotic curve and practically ceases near the freezing point.

(f) Soluble salts:

Johnson and Guenzi (1963) reported little effect of sodium sulfate on nitrification until the osmotic pressure reached about 5 bars. However, sodium chloride depressed the rate of nitrification at lower osmotic pressures.

Influence of Other Soil Factors on Nitrification

Wilson (1977a) stated that nitrification in soil was totally inhibited by 1000 ppm Zn. Wilson (1977b) also found industrial sludge to contain high levels of several metals, particularly Zn, Cd and Pb. These reduced nitrification when applied at high rates in domestic sludge.

According to Frank (1943) weeds reduced nitrate accumulation in fallow soils and cutting down weeds increased the nitrate nitrogen in the top 30 cm soil from 2 to 10 ppm. Also, Frank found pastures in

the tropics to be very low in ammonium and nitrate. This could be due to a low rate of mineralization or to high rates of nitrification and subsequent leaching.

Robinson (1963) reported some New Zealand soils to be almost incapable of nitrification. However, liming and adding urea to the soils raised the pH, and provided substrate for the development of nitrifying bacteria. Also, Robinson reported that the level of ammonium nitrogen in grassland soils was higher than that in arable soil (cropped or fallow soils), but nitrate nitrogen was higher in the cropped or fallow soils.

Theron (1951) reported repression of nitrification under grass the second season after establishment. Nitrification did not take place even when grass was dormant in winter. This was a direct influence of the living root, since in fallow soil, nitrification took place throughout the winter. Theron also obtained evidence that inhibitory substances were excreted by the roots. Also, Thompson and Coup (1943) considered the lack of nitrifying bacteria in grassland soils to result from the excretion of toxic substance by grass roots.

Microorganisms Effect

Verstraete and Voets (1977) studied the soil microbial and biochemical characteristics in relation to soil management practice and crop yields. Nitrogen mineralization rate and soil respiration were found valuable to characterize the soils. Both mineralization and

respiration increased with increasing soil organic matter, clay and $CaCO_3$ content.

According to Jane (1968) ammonium oxidizers varied from 360 to over 26,000 organisms per gram of soil. Several thousand ammonium oxidizers per gram were found in well-managed pasture soils, but decreased to 100 organisms per gram in poor soils. Robinson (1963) reported that grassland soils lack nitrifying bacteria because of a substrate deficiency rather than because of the toxicity of grass roots to these bacteria.

Nitrification in Virgin and Cultivated Soils

Anderson et al. (1970) studied nitrification in fine-textured sod and in cultivated soils contiguous to each other at 6° C and 32° C. Sod soils did not greatly inhibit nitrification of ammonium at 32° C, but nitrification was strongly inhibited by the fine textured sod at 6° C, especially those high in silt content. Thus the effect was strongly temperature dependent. Also, Anderson reported that nitrification was not strongly related to cropping practices. Hinman (1964) observed total nitrogen content to decrease from an average of 0.23 percent in virgin soils to 0.15 in cultivated soils. Gomah et al. (1974) found slow nitrification with few ammonium and nitrite oxidizers in saline virgin soils. Nitrification greatly increased in leached and cultivated soils. Only 17-19.5 percent of the added $(NH_4)_2SO_4$ was nitrified in virgin soils after 28 days compared to 70-80 percent in

leached and cultivated soils. Dubey (1961) found in his studies of nitrification and denitrification in Georgia soils that significant reduction of nitrification occurred in only the grassland Bladen clay loam soils.

Soulides and Clark (1958) studied nitrification in grassland and intertilled soils contiguous to each other on seven paired soil samples. Soulides found that irrespective of pH, all grassland soils, amended with 0.1 percent Urea showed higher retention of ammonia and less nitrate production than did the intertilled counterparts.

Clark (1949) comparing cropped and fallow soils found more mineralization in cropped soils than in fallowed soils. This was attributed to the increase in the number of microorganisms around roots under cultivated conditions.

Caster et al. (1942) studied nitrification rates in virgin and cultivated sandy loam soils in relation to pH. Both virgin and cultivated soils received 300 ppm nitrogen as ammonium hydroxide. The virgin soil had an initial pH value of 9.26. After 49 days, the pH dropped to 8.0 while nitrate nitrogen accumulated to 58 ppm. Cultivated soil, receiving the same concentration of ammonium hydroxide, had an initial pH of 8.7 and rapidly oxidized the ammonium hydroxide. Two hundred ninety-one ppm nitrate nitrogen accumulated in 49 days while pH dropped to 7.53. Caster also conducted a similar experiment with the same soil types using ammonium sulfate as a nitrogen

source. Similar results were obtained after only 18 days of incubation. The cultivated soil accumulated 272 ppm nitrate nitrogen while the virgin soil accumulated only 65 ppm. The pH of both soils after incubation was 7.38, thus pH was not an important factor to explain differences between cultivated and virgin soils following addition of ammonium sulfate.

Harmsen et al. (1965) reported that net mineralization rates were lower under grass than under arable cropping.

MATERIALS AND METHODS

Cultivated and virgin soils were selected in close proximity to each other. The cultivated soils were irrigated with a pivot sprinkler thus leaving the corners uncultivated (virgin) and available for sampling. The sites were randomly sampled with a 2.5 cm diameter soil sampling tube from a depth of 0 to 30 cm. Approximately 50 cores were taken from each site. These cores were composited to represent a bulk sample for each site. The sampling tube was washed with 75 percent ethyl alcohol between sites. Twelve soil samples were collected to represent six virgin soils and six cultivated soils. The history of the soils is given in Table 1. The soil samples were air-dried, screened (2 mm sieve) and stored in plastic containers at room temperature until analyzed.

The physical and chemical characteristics of soils are given in Table 2. The texture of the soils ranged from sandy to sandy loam. The pH of the soils ranged from 6.85 to 7.56. No apparent differences in pH existed between cultivated and virgin soils. Water holding capacity and soluble salt content of the virgin and cultivated soils did not appear to differ. Organic matter of virgin soils ranged between 0.3 and 1.0 percent while cultivated soils ranged between 0.2 and 0.4 percent. Nitrate and ammonium contents of cultivated soils were higher than virgin soils.

Table 1. History of experimental soils.

Location	Field	Type	Soils History
1	Kula - Orchard I	Cultivated	Plowed, 1966; alfalfa 3 years; corn 1 year; and potatoes
1	Kula - Orchard I	Virgin	Weeds and short grass
2	Kula - Orchard II	Cultivated	Plowed, 1975; potatoes and corn, 1976/1977
2	Kula - Orchard II	Virgin	Weeds and short grass
3	Kula - Orchard III	Cultivated	Plowed, 1971; potatoes and corn, 1977
3	Kula - Orchard III	Virgin	Weeds and short grass
4	Miller - Rothe	Cultivated	Plowed, 1977; potatoes, 1977
4	Miller - Rothe	Virgin	Short grass
5	Miller	Cultivated	Aged soil (mostly potatoes)
5	Miller	Virgin	Short grass
6	McElrath	Cultivated	Plowed, 1975; potatoes, 1976/1977
6	McElrath	Virgin	Weed mixture

Table 2. Physical and chemical characteristics of experimental soils.

	Location 1		Location 2		Location 3	
	Cultivated	Virgin	Cultivated	Virgin	Cultivated	Virgin
Particle size analysis	93-1-6*	92-1-7	93-2-5	94-2-4	82-4-14	87-3-10
pH (saturated paste)	7.56	7.25	6.85	6.90	7.24	7.56
Soil field capacity (%)	10.40	10.40	10.20	9.70	12.00	10.70
Total soluble salts (mmhos/cm)	0.26	0.22	0.67	0.21	0.90	0.44
Organic matter (%)	0.40	0.30	0.30	0.40	0.40	1.00
Ammonium nitrogen (ppm-N)	6.57	7.06	24.07	3.91	23.86	2.98
Nitrate nitrogen (ppm-N)	2.28	1.73	17.58	2.13	19.16	5.07

*93-1-6 indicates 93% sand, 1% silt, 6% clay.

Table 2. Continued.

	Location 4		Location 5		Location 6	
	Cultivated	Virgin	Cultivated	Virgin	Cultivated	Virgin
Particle size analysis	87-5-8	91-1-8	92-0-8	88-3-9	96-1-3	94-2-4
pH (saturated paste)	6.95	7.05	7.32	7.05	7.50	7.05
Soil field capacity (%)	10.30	10.30	10.00	10.50	8.30	8.50
Total soluble salts (mmhos/cm)	0.88	0.22	1.02	0.20	0.27	0.18
Organic matter (%)	0.40	0.40	0.20	0.50	0.20	0.20
Ammonium nitrogen (ppm-N)	25.15	2.78	16.59	4.96	3.60	23.00
Nitrate nitrogen (ppm-N)	7.61	3.96	15.49	3.88	2.97	4.87

To determine rates of nitrification, ammonium sulfate was added to each soil at the rate of 0, 50 and 100 ppm of nitrogen and was mechanically mixed in a blender for 10 minutes. Two hundred grams of the mixed soil and ammonium sulfate were placed in 1 mil polyethylene bags. The soils were moistened to 60 percent of field capacity then incubated at 24° C for 0, 10 and 21 days. Moisture was maintained at this level during incubation. The polyethylene bags were weighed every 5 to 6 days and water loss due to evaporation was replaced. All treatments were replicated twice. During incubation, CO_2 increased to 0.14 percent while O_2 decreased to 20.4 percent within the polyethylene bags.

Each treatment was analyzed at the appropriate time for ammonium and nitrate nitrogen. Soil samples were air-dried and were ground with a mortar and pestle.

For ammonium nitrogen analyses, ten grams of air-dried soil was placed in 125 ml erlenmeyer flasks containing 50 ml 2 M KCl. The flasks were shaken on an orbital shaker, speed 3-4 (low), for 45 minutes. The soil suspensions were filtered through Whatman #42 filter paper. Ammonium nitrogen in the filtrate was determined using an Orion ammonium electrode (model 95-10). Standards were prepared with 0.1 M NH_4Cl in 2 M KCl. Concentrations of 0.5, 1, 5, 10, 50 and 100 ppm of nitrogen were prepared. Concentrations in the unknown were determined using a regression equation prepared from the standard solutions.

For nitrate nitrogen analyses, ten gram samples of air-dried soil were extracted in 125 ml erlenmeyer flasks containing 50 ml of distilled water. The flasks were shaken on an orbital shaker, speed 4 (low) for 30 minutes and speed 5 (high) for 5 minutes. The soil suspensions were centrifuged and nitrate nitrogen in the extract was determined with an Orion nitrate electrode (model 92-07). Standards were prepared with $NaNO_3$ to give 0.5, 1, 5, 10, 50 and 100 ppm of nitrogen. Nitrate nitrogen concentrations in the unknowns was determined with a regression equation prepared from the standard solutions.

The t-test was used to compare the mean differences in ammonium and nitrate content between the virgin and cultivated soils. Significant results at $P = 0.05$ level would mean the rejection of the Null Hypothesis (Leclerg et al., 1962).

Determination of soil bacteria that oxidize ammonium to nitrite and nitrite to nitrate was done by the most probable number method (Alexander, 1965). Six soil samples were used to represent three virgin soils and three cultivated soils. Based upon the incubation studies Location Numbers 3, 4 and 5 were selected for bacterial assays. The samples were ground and serially diluted with sterile distilled water from 10^{-2} to 10^{-5}. Five replicates of each soil dilution were inoculated into either Nitrosomonas or Nitrobacter liquid medium (Alexander and Clark, 1965).

Nitrosomonas medium contained 0.5 g of $(NH_4)_2 SO_4$, 1.0 g of K_2HPO_4, 0.03 g of $FeSO_4 \cdot 7H_2O$, 0.3 g of $MgSO_4 \cdot 7H_2O$, 7.5 g of $CaCO_3$ and water to make 1 liter of solution. Inoculated tubes were incubated at 25° C for 21 days and the activity of the bacteria (Nitrosomonas) was determined using the following test:

Griess-Ilosvay reagent (sulfanilic acid, N-(1-Naphytl)-ethylene diamine Dihydrochloride and acetate) was used to detect the presence of nitrite (Figure 2). Three drops were added to the culture solutions after incubation. If Nitrosomonas was present the solution immediately became purplish-red due to the presence of nitrite. If tubes yielded a negative test for nitrite, a mixture of zinc and manganese dioxide containing a trace of copper metal was added to reduce any nitrate back to nitrite. If a reddish color developed the tube was also positive for Nitrosomonas.

Nitrobacter medium contained 0.0006 g of KNO_2, 1.0 g of K_2HPO_4, 0.3 g of NaCl, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.03 g of $FeSO_4 \cdot 7H_2O$, 1.0 g $CaCO_3$, 0.3 g $CaCl_2$ and water to make 1 liter of solution. Inoculated tubes were incubated at 25° C for 21 days and the activity of the bacteria (Nitrobacter) was determined using the following test:

Griess-Ilosvay reagent was used to detect the presence of nitrate (Figure 3). Three drops were added to the culture solutions after incubation. If the medium failed to show the reddish color, the

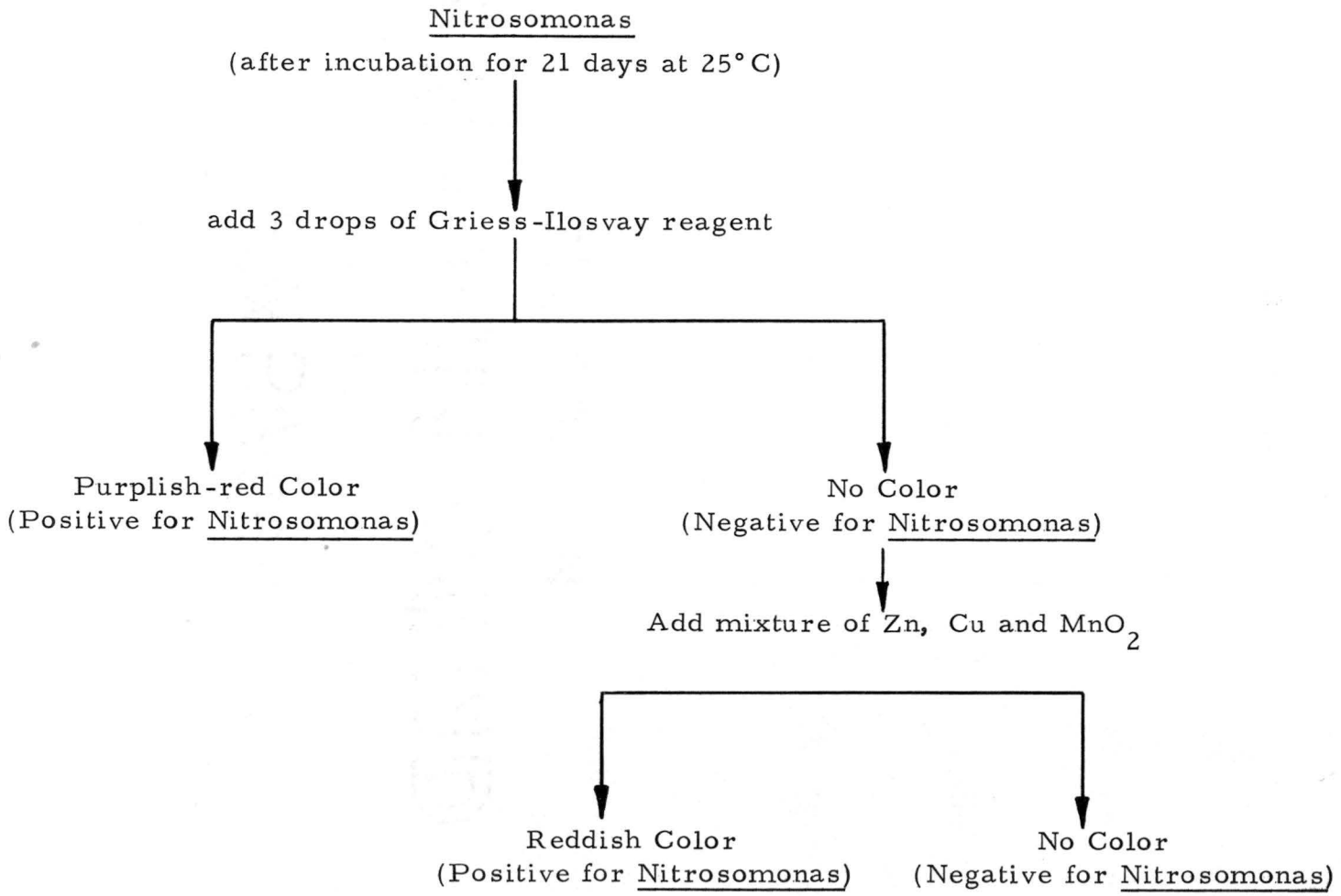


Figure 2. A schematic illustration of the determination of Nitrosomonas in selected soils (Alexander, 1965).

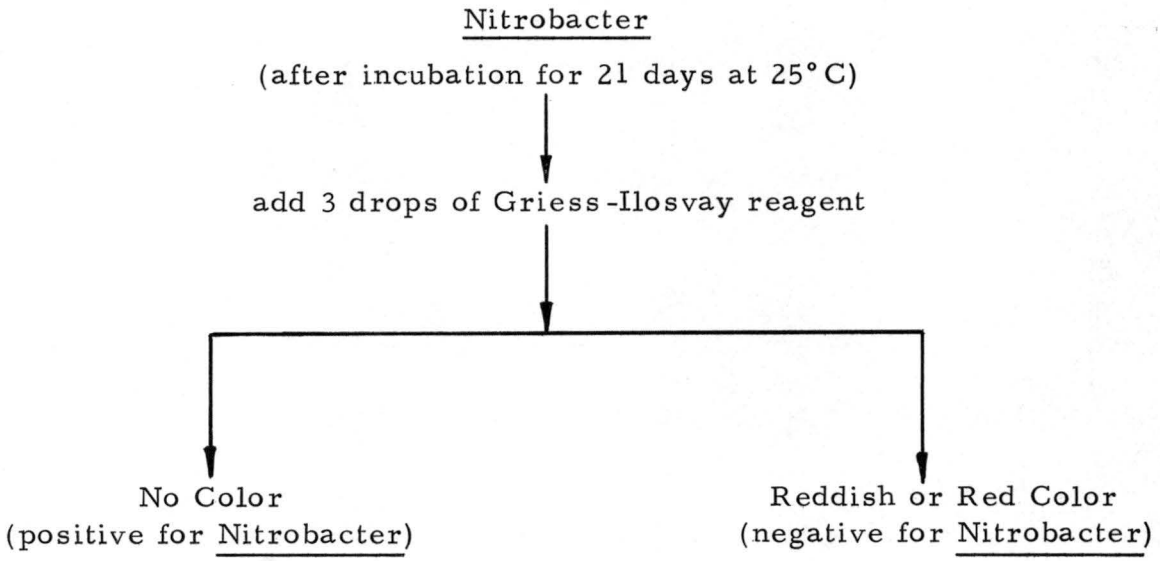


Figure 3. A schematic illustration of the determination of Nitrobacter in selected soils (Alexander, 1965).

tube was recorded as positive for Nitrobacter, or negative if red coloration developed (Alexander and Clark, 1965). The most probable number technique developed by Alexander (1965) was used to estimate the bacterial number present in the test soils.

The influences of diluting virgin soil with cultivated soils on nitrification rates was determined as follows:

The cultivated soil at rates of 10, 15, 20, 25, 30, 35, 45 and 50 percent with the virgin soil. Ammonium sulfate was added to each mixed sample at the rate of 50 ppm nitrogen. The samples were incubated for 10 days at 24°C. After incubation, the nitrate nitrogen and ammonium nitrogen were determined by the same procedure described earlier.

RESULTS

Incubation Studies

The soil samples were collected on June 10, 1977. The residual ammonium and nitrate nitrogen present in the soils collected for the incubation studies are shown in Table 3. Residual nitrate nitrogen in cultivated soils ranged from 3 to 19 ppm and in virgin soils from 2 to 5 ppm. Residual ammonium nitrogen in cultivated soils ranged from 4 to 25 ppm and in virgin soils from 2 to 7 ppm. In the data presentation the residual nitrate nitrogen was always substracted from that found after incubation. Residual ammonium nitrogen was not substracted when test soils were incubated without added ammonium nitrogen. When ammonium nitrogen was added to the test soils prior to incubation the residual ammonium was substracted. This occasionally resulted in a negative value for ammonium due to the nitrification of added ammonium and some residual ammonium.

The change in ammonium and nitrate nitrogen during incubation when no ammonium nitrogen was added to the test soils is shown in Table 4. The significance of differences between means of six cultivated and six virgin soils are indicated by the t-test. Significantly more nitrate was found in the cultivated soils perhaps as a result of their larger residual ammonium content. Nearly all the ammonium present in both cultivated and virgin soils was converted to nitrate

Table 3. Residual levels of ammonium and nitrate nitrogen present in the cultivated and adjacent virgin soils at the time of sampling.

Location	Soil Condition	Nitrate Nitrogen (ppm)	Ammonium Nitrogen (ppm)
1	Cultivated	3	7
2	Cultivated	18	24
3	Cultivated	19	24
4	Cultivated	8	25
5	Cultivated	15	17
6	Cultivated	3	4
Mean		11	17
1	Virgin	2	7
2	Virgin	2	4
3	Virgin	5	3
4	Virgin	4	3
5	Virgin	4	5
6	Virgin	5	2
Mean		4	4

Table 4. Nitrate and ammonium nitrogen present in cultivated and virgin soils receiving 0 ppm ammonium nitrogen following incubation at 24° C for 0, 10 and 21 days. Nitrate nitrogen values corrected for residuals shown in Table 3.

		Incubation Time					
		0 Days		10 Days		21 Days	
Location	Soil Condition	Nitrate Nitrogen (ppm)	Ammonium Nitrogen (ppm)	Nitrate Nitrogen (ppm)	Ammonium Nitrogen (ppm)	Nitrate Nitrogen (ppm)	Ammonium Nitrogen (ppm)
1	Cultivated	2	4	5	3	9	3
2	Cultivated	0	17	10	4	29	2
3	Cultivated	0	13	19	2	29	2
4	Cultivated	8	17	19	2	19	1
5	Cultivated	6	4	27	2	25	1
6	Cultivated	5	2	7	2	7	2
MEAN		4	10	15	3	20	2
1	Virgin	2	2	5	1	7	1
2	Virgin	3	1	4	1	4	1
3	Virgin	1	1	0	1	0	1
4	Virgin	0	1	1	2	4	1
5	Virgin	2	1	4	1	7	1
6	Virgin	1	2	0	1	1	1
MEAN		2	1	2	1	4	1
t value for MEANS		1.4	2.9	3.4	3.5	3.8	2.7
Significance Level		NS	0.05	0.01	0.01	0.01	0.05

after 21 days of incubation. The mean rate of nitrate accumulation and ammonium disappearance in the six cultivated and six virgin soils during incubation is shown graphically in Figures 4 and 5, respectively.

Table 5 shows nitrate nitrogen accumulation and ammonium disappearance in test soils during incubation after addition of 50 ppm ammonium nitrogen. Nitrification was significantly more rapid in cultivated soils. After 10 days incubation, the ammonium nitrogen concentrations of the six cultivated soils ranged from -18 to 19 ppm compared to a range of 21 to 41 ppm in virgin soils; nitrate nitrogen content in the cultivated soils ranged from 33 to 69 ppm compared to a range of 4 to 34 ppm in virgin soils. After 21 days, ammonium nitrogen ranged from -19 to 10 ppm for cultivated soils and 18 to 32 ppm for virgin soils; in cultivated soils, except in one case, all the added ammonium was nitrified. Nitrate nitrogen content in the cultivated soils ranged from 42 to 69 ppm compared to a range of 9 to 30 ppm in virgin soils after 21 days.

The mean rate of nitrate accumulation and ammonium disappearance in the six cultivated and six virgin soils during incubation is shown graphically in Figures 6 and 7, respectively.

The total of nitrate and ammonium nitrogen present in cultivated soils after 21 days of incubation ranged from 41 to 59 ppm (Table 5). Since 50 ppm ammonium nitrogen was added, this indicates losses of

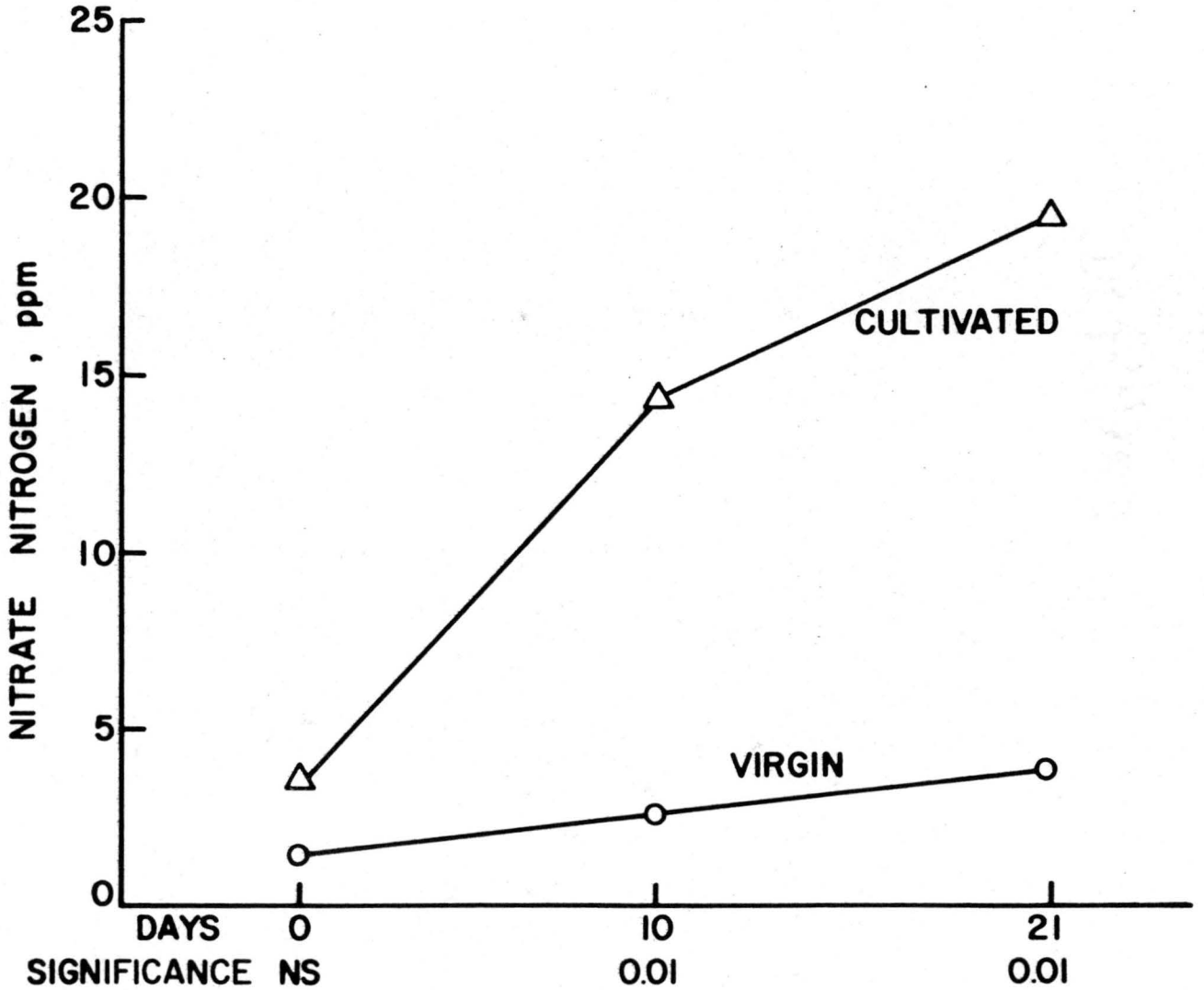


Figure 4. Mean nitrate nitrogen accumulation in six cultivated and six virgin soils during incubation at 24° C for 0, 10 and 21 days. No ammonium nitrogen was added prior to incubation.

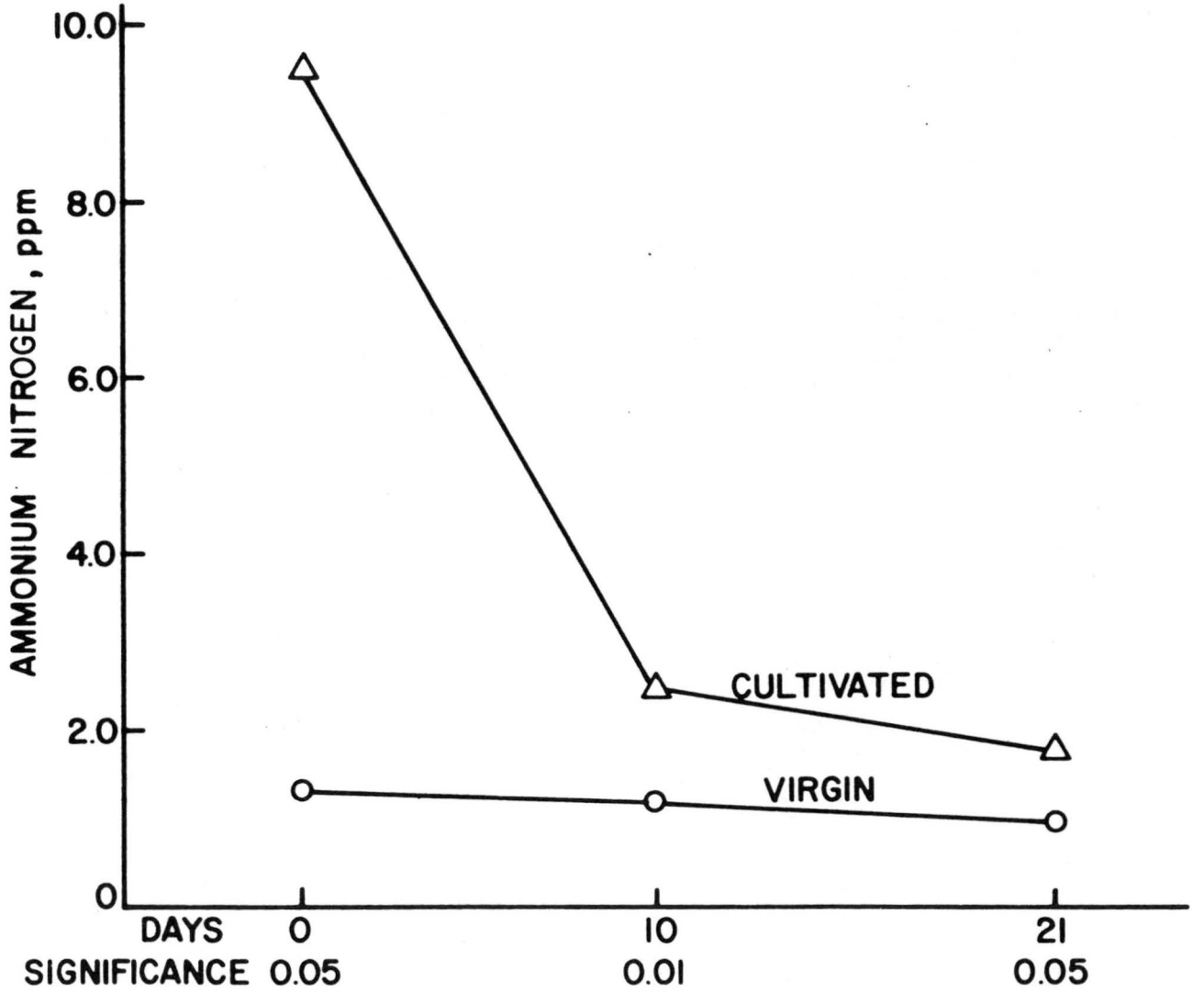


Figure 5. Mean ammonium nitrogen depletion in six cultivated and six virgin soils during incubation at 24° C for 0, 10 and 21 days. No ammonium nitrogen was added prior to incubation.

Table 5. Nitrate and ammonium nitrogen present in cultivated and virgin soils after addition of 50 ppm ammonium nitrogen following incubation at 24° C for 0, 10 and 21 days. Nitrate nitrogen and ammonium nitrogen values corrected for residuals shown in Table 3.

		Incubation Time					
		0 Days		10 Days		21 Days	
Location	Soil Condition	Nitrate Nitrogen (ppm)	Ammonium Nitrogen (ppm)	Nitrate Nitrogen (ppm)	Ammonium Nitrogen (ppm)	Nitrate Nitrogen (ppm)	Ammonium Nitrogen (ppm)
1	Cultivated	5	40	33	19	49	10
2	Cultivated	9	29	35	5	42	-1[a]
3	Cultivated	15	25	69	-18	72	-19
4	Cultivated	10	34	34	9	47	-6
5	Cultivated	28	17	58	-10	69	-13
6	Cultivated	10	31	37	8	45	-1
MEAN		13	29	44	2	54	-5
1	Virgin	3	46	22	21	27	20
2	Virgin	4	45	11	31	18	26
3	Virgin	1	42	34	23	26	25
4	Virgin	0	47	10	31	19	24
5	Virgin	3	41	21	32	30	18
6	Virgin	0	51	4	41	9	32
MEAN		2	45	17	30	22	24
t value for MEANS		3.3	4.5	3.8	4.4	5.3	6.3
Significance Level		0.01	0.01	0.01	0.01	0.01	0.01

[a]Negative values indicate nitrification of some residual ammonium.

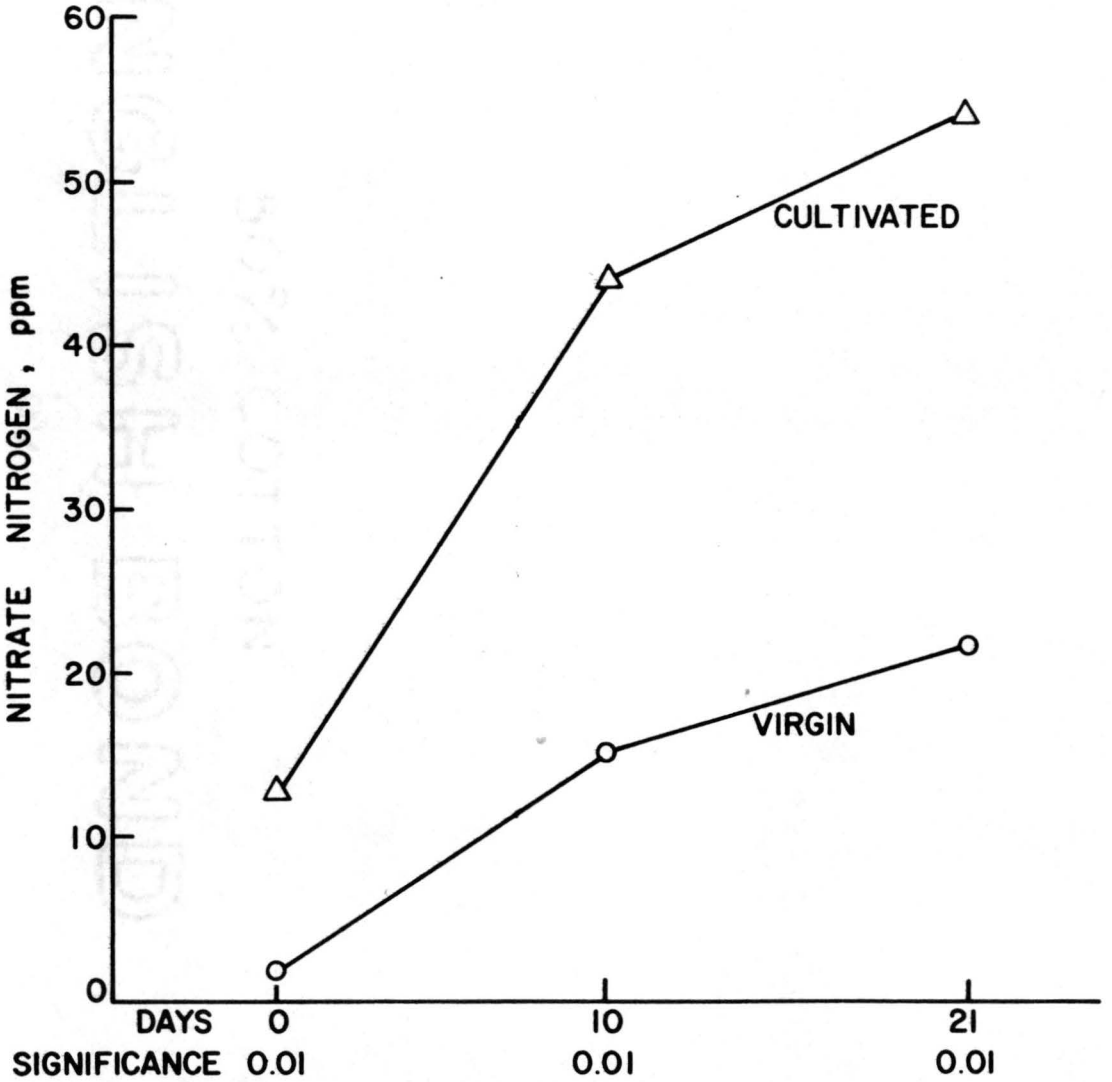


Figure 6. Mean nitrate nitrogen accumulation in six cultivated and six virgin soils during incubation at 24°C for 0, 10 and 21 days. 50 ppm ammonium nitrogen was added prior to incubation.

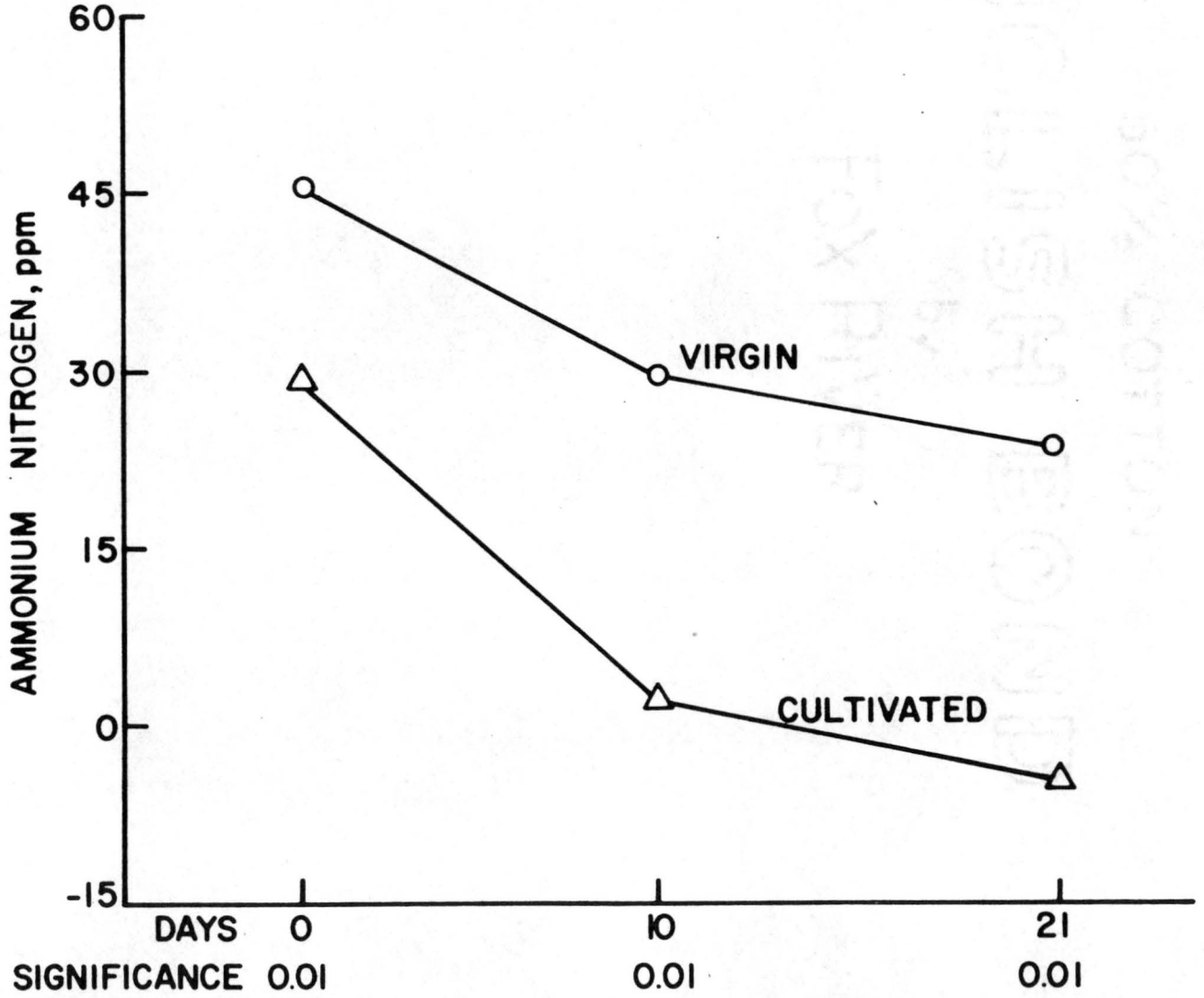


Figure 7. Mean ammonium nitrogen depletion in six cultivated and six virgin soils during incubation at 24° C for 0, 10 and 21 days. 50 ppm ammonium nitrogen was added prior to incubation.

nitrogen in some soils and gains in others. In virgin soils total nitrogen after 21 days incubation ranged from 41 to 51 ppm.

The nitrate and ammonium content of soils receiving 100 ppm of ammonium nitrogen and incubated for 0, 10 and 21 days is shown in Table 6. The rate of nitrification in soils receiving 100 ppm of ammonium nitrogen was similar to that in soils receiving 50 ppm of ammonium nitrogen. From 0 to 64 percent of the applied ammonium nitrogen was still present in cultivated soils after 21 days of incubation. Comparatively, from 36 to 98 percent remained in virgin soils.

The mean rate of nitrate nitrogen accumulation and ammonium disappearance is shown in Figures 8 and 9, respectively. Nitrate increased nearly linearly with incubation time in both cultivated and virgin soils but more rapidly in cultivated soils. Ammonium did not decrease linearly in virgin soils but did in cultivated soils.

Bacterial Assays

The soil samples for bacterial assays were taken on December 22, 1977. Locations 3, 4 and 5 were selected for assay. These three locations showed large differences in nitrification rates between the cultivated and virgin soils when evaluated in June. The residual nitrate nitrogen and ammonium nitrogen present in the three Locations on December 22 are shown in Table 7. Higher levels of residual nitrate were present in cultivated soils. Nitrate ranged between 7 to

Table 6. Nitrate and ammonium nitrogen present in cultivated and virgin soils after addition of 100 ppm ammonium nitrogen following incubation at 24°C for 0, 10 and 21 days. Nitrate nitrogen and ammonium nitrogen values corrected for residuals shown in Table 3.

		Incubation Time					
		0 Days		10 Days		21 Days	
Location	Soil Condition	Nitrate Nitrogen (ppm)	Ammonium Nitrogen (ppm)	Nitrate Nitrogen (ppm)	Ammonium Nitrogen (ppm)	Nitrate Nitrogen (ppm)	Ammonium Nitrogen (ppm)
1	Cultivated	2	86	10	92	34	64
2	Cultivated	8	81	20	88	52	60
3	Cultivated	20	76	85	32	135	-10[a]
4	Cultivated	3	89	25	82	47	61
5	Cultivated	12	87	72	34	89	20
6	Cultivated	2	99	46	60	67	41
MEAN		8	86	43	65	71	39
1	Virgin	3	89	18	88	34	68
2	Virgin	6	94	13	99	22	76
3	Virgin	0	90	42	59	57	36
4	Virgin	0	94	8	99	12	84
5	Virgin	3	88	18	90	37	67
6	Virgin	0	94	3	106	10	98
MEAN		2	92	17	90	29	72
t value for MEANS		2.03	1.5	1.9	2.0	2.5	2.2
Significance Level		NS	NS	NS	NS	0.05	NS

[a]Negative values indicate nitrification of some residual ammonium.

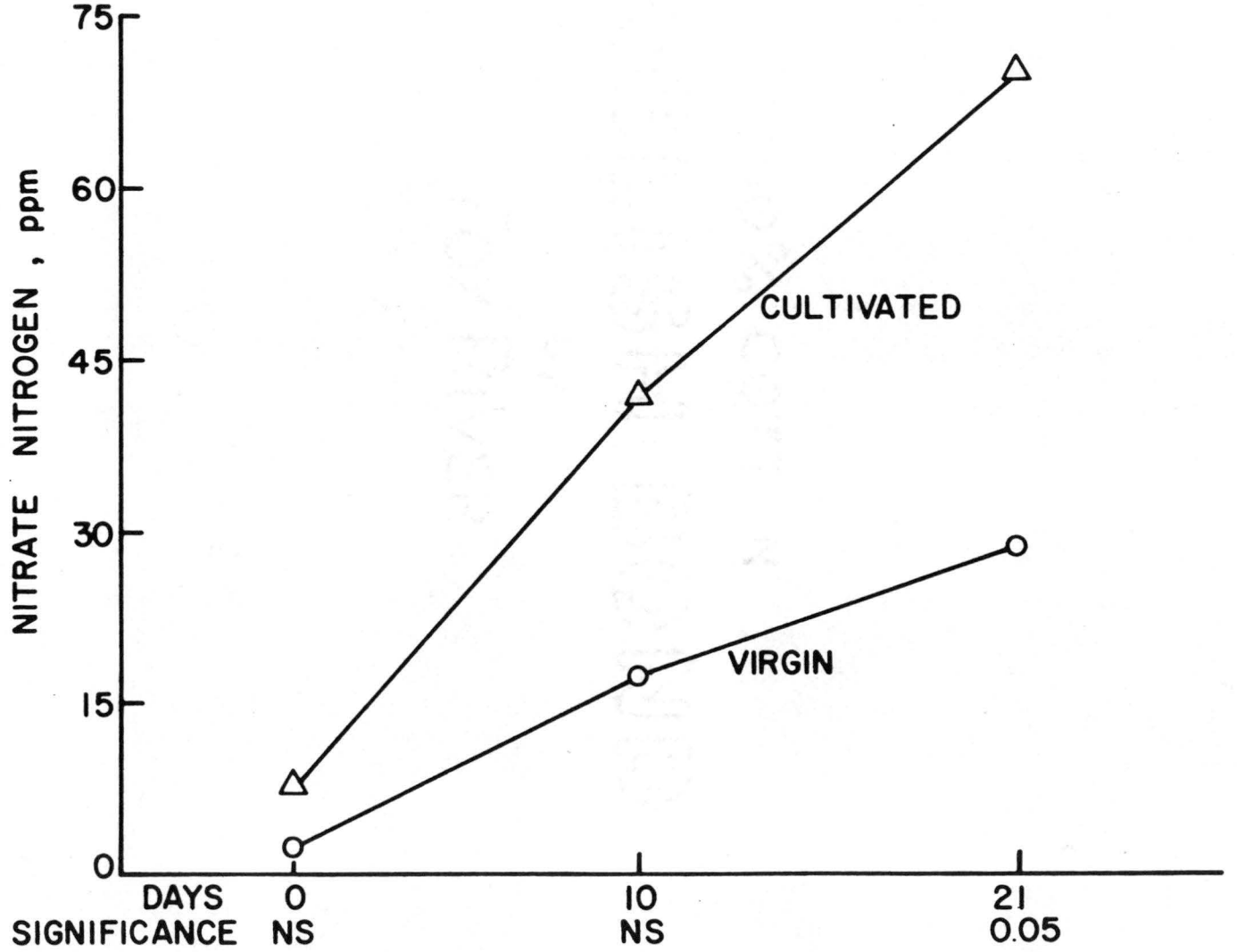


Figure 8. Mean nitrate nitrogen accumulation in six cultivated and six virgin soils during incubation at 24° C for 0, 10 and 21 days. 100 ppm ammonium nitrogen was added prior to incubation.

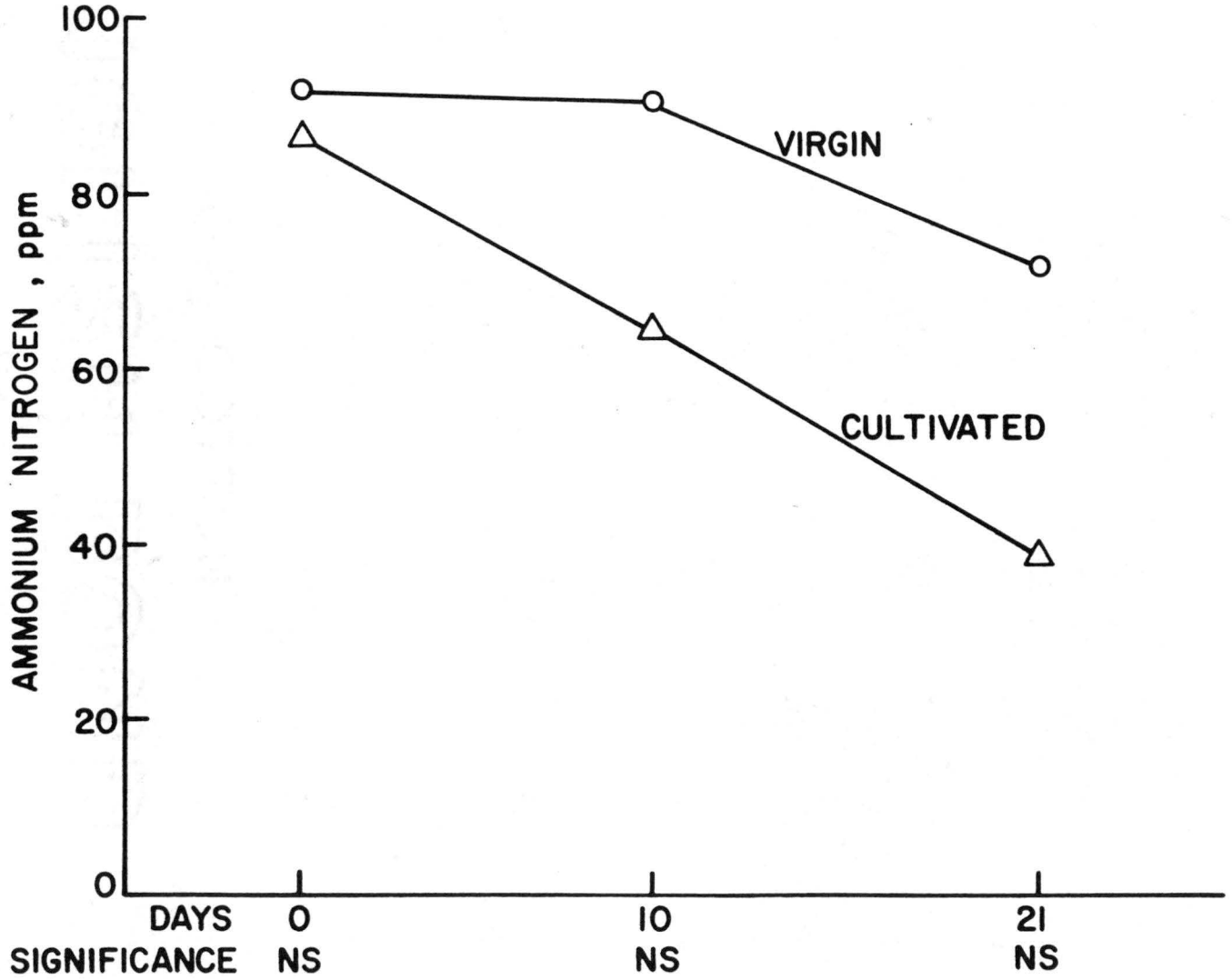


Figure 9. Mean ammonium depletion in six cultivated and six virgin soils during incubation at 24°C for 0, 10 and 21 days. 100 ppm ammonium nitrogen was added prior to incubation.

Table 7. Residual nitrate and ammonium level in test soils used in bacterial assays and their comparative nitrification ability after addition of 50 ppm of ammonium nitrogen and incubation 10 days at 24° C.

Location	Soil Condition	Residual Level		Addition of 50 ppm Ammonium-N, and 10 Days Incubation at 24° C[a]	
		Nitrate Nitrogen (ppm)	Ammonium Nitrogen (ppm)	Nitrate Nitrogen (ppm)	Ammonium Nitrogen (ppm)
3	Cultivated	9	1	33	22
4	Cultivated	13	2	30	17
5	Cultivated	7	1	50	0
MEAN		10	1	38	13
3	Virgin	5	1	24	23
4	Virgin	3	1	12	32
5	Virgin	5	1	19	24
MEAN		4	1	18	26
t value for MEANS		2.8	1	2.7	1.8
Significance Level		0.05	NS	0.05	NS

[a]Residual nitrate nitrogen and ammonium nitrogen were subtracted from that found after incubation.

13 ppm in cultivated soils compared to 3 to 5 ppm in virgin soils. Residual ammonium were similar and very low in both cultivated and virgin soils.

To obtain information on the nitrification potential of the soils, an incubation test was run for 10 days at 24° C after the addition of 50 ppm ammonium nitrogen. Similar to the June incubation studies, higher nitrification rates occurred in the cultivated soils than in virgin soils (Table 7). From 60 to 100 percent of the added ammonium nitrogen was converted to nitrate in cultivated soils compared to 24 to 48 percent in virgin soils. Ammonium nitrogen remaining after incubation ranged from 0 to 22 ppm in cultivated soils compared to 23 to 32 ppm in virgin soils.

The number of nitrifying bacteria in cultivated and virgin soil (Locations #3, #4 and #5) is shown in Table 8. Both Nitrosomonas and Nitrobacter in cultivated soils were significantly higher than in virgin soils. Nitrosomonas numbers ranged between 7,200 to 35,000 cells per gram in cultivated soils compared to a range of 260 to 460 cells per gram in virgin soils. Nitrobacter numbers ranged from 4,100 to 35,000 cells per gram in cultivated soils compared to a range of 45 to 78 cells per gram in virgin soils.

Figure 10 illustrates the positive and the negative tubes in 10^{-2} and 10^{-5} soil dilutions to test the number of Nitrosomonas in cultivated and virgin soil. Clearly at 10^{-2} dilution in cultivated and virgin soil,

Table 8. Numbers of nitrifying bacteria in cultivated and virgin soils (Location #3, #4 and #5).

Location	Soil Condition	Numbers per g soil	
		Nitrosomonas	Nitrobacter
3	Cultivated	21,000	28,000
4	Cultivated	7,200	35,000
5	Cultivated	35,000	4,100
3	Virgin	460	78
4	Virgin	260	45
5	Virgin	260	45

Figure 10. Influence of dilution on color development in Nitrosomonas tests.

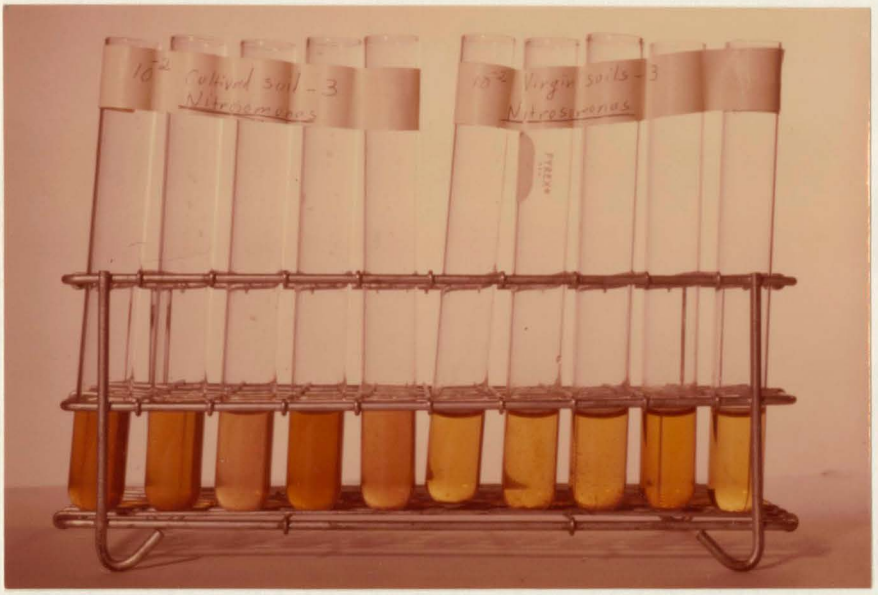
A. 5 tubes on left, 10^{-2} dilution in cultivated soil, all tubes are positive.

5 tubes on right, 10^{-2} dilution in virgin soil, all tubes are positive.

B. 5 tubes on left, 10^{-5} dilution in cultivated soil, 4 tubes are positive.

5 tubes on right, 10^{-5} dilution in virgin soil, all tubes are negative (no color) in virgin soil.

A


Cultived Soil - 3
Nitrosomonas
Virgin soils - 3
Nitrosomonas

B


Cultivated soil-3
Nitrosomonas
Virgin Soil - 3
Nitrosomonas

all the 10 tubes were positive (reddish color). However, at 10^{-5} dilution, 3 tubes were positive in cultivated soil while all 5 tubes were negative (no color) in virgin soils.

Figure 11 illustrates the positive and the negative tubes in a 10^{-2} and 10^{-5} soil dilution to test the number of Nitrobacter in cultivated and virgin soils. At 10^{-2} dilution, all 5 tubes were positive (no color) for cultivated soil while only 3 tubes were positive for virgin soil. At 10^{-5} dilution, 3 tubes were positive for cultivated soil while all the tubes were negative (red color) in virgin soil.

The effect of mixing cultivated and virgin soils in various proportions on nitrification rate is shown in Table 9. The rate of nitrate formation increased with increasing proportions of cultivated soil up to 35 percent. When the proportion of cultivated soil was increased above 35 percent, no further significant increase in nitrification rate occurred. The influence of mixing cultivated and virgin soils is shown graphically for nitrate formation in Figure 12 and for ammonium disappearance in Figure 13.

Figure 11. Influence of dilution on color development in Nitrobacter test.

A. 5 tubes on left, 10^{-2} dilution in cultivated soil, all tubes are positive (no color).

5 tubes on right, 10^{-2} dilution in cultivated soil, 3 tubes are positive (no color).

B. 5 tubes on left, 10^{-5} dilution in cultivated soil, 3 tubes are positive (no color).

5 tubes on right, 10^{-5} dilution in cultivated soil, all tubes are negative (red color).

A

B

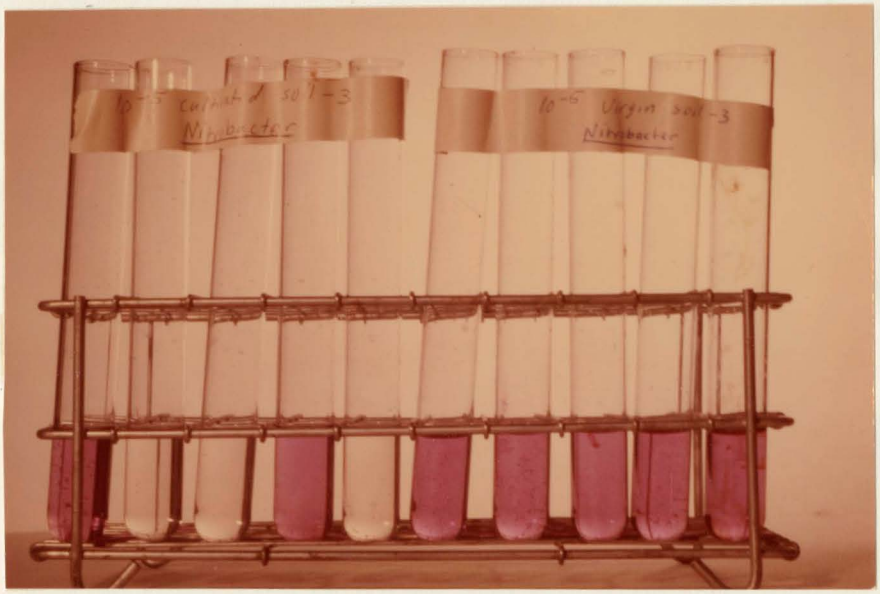


Table 9. Parts per million nitrate and ammonium nitrogen found after mixing cultivated and virgin soil after addition of 50 ppm ammonium nitrogen and incubation at 24° C for 10 days. Values corrected for residual nitrogen.

Cultivated Soil Percent	Virgin Soil Percent	Nitrate Nitrogen (ppm)	Ammonium Nitrogen (ppm)
10	90	12	52
15	85	21	45
20	80	26	41
25	75	27	37
30	70	29	33
35	65	32	33
45	55	30	30
50	50	31	31

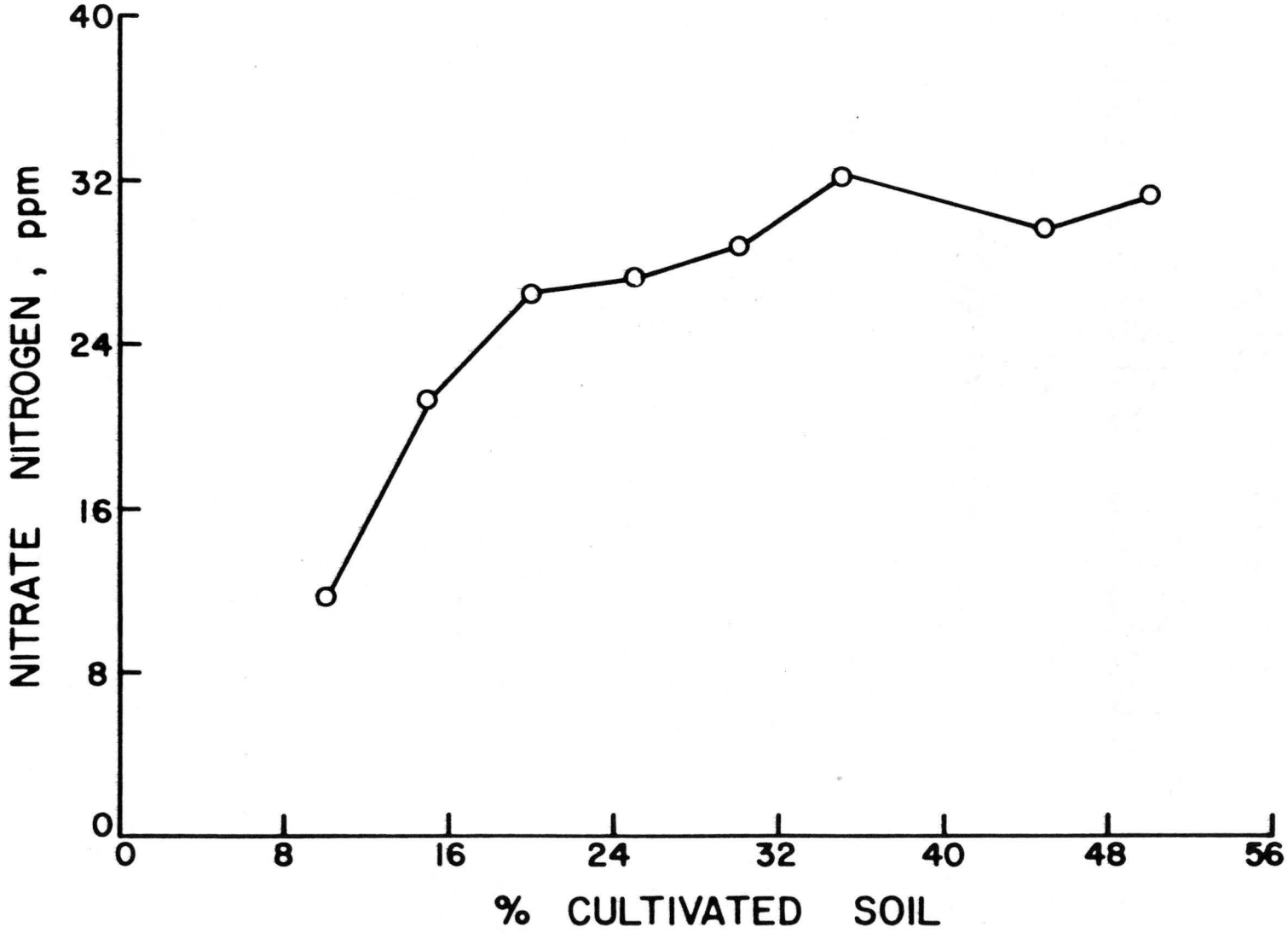


Figure 12. Influence of adding cultivated soil to virgin soil on nitrate nitrogen accumulation during incubation 10 days at 24° C. 50 ppm ammonium nitrogen added before incubation.

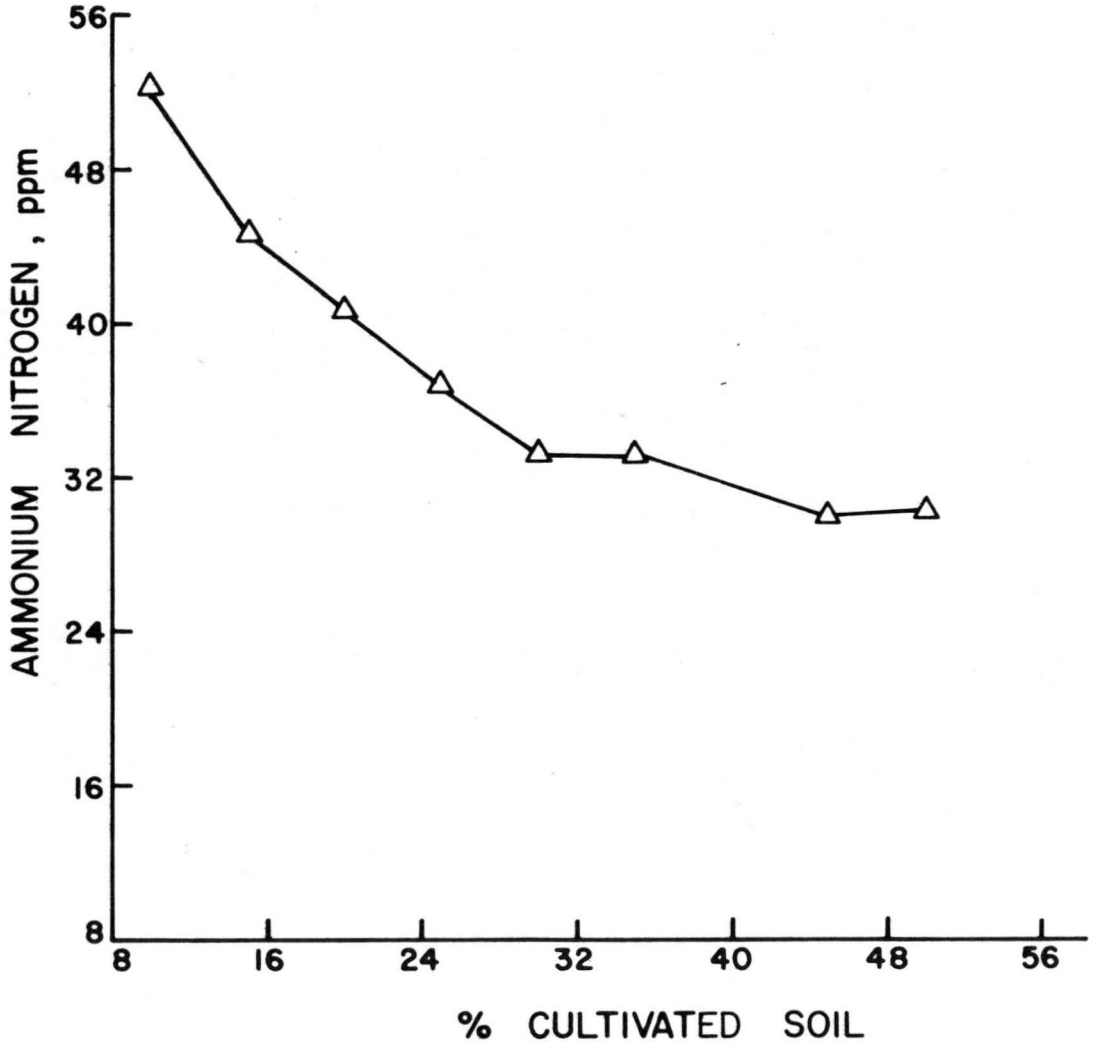


Figure 13. Influence of adding cultivated soil to virgin soil on ammonium nitrogen depletion during incubation 10 days at 24° C. 50 ppm ammonium nitrogen added before incubation.

DISCUSSION

Incubation Studies

The higher residual levels of nitrate nitrogen and ammonium nitrogen (Table 3) in most of the cultivated soils was most likely due to prior fertilization, organic matter decomposition in the spring prior to the June sampling. Also, for similar reasons, higher levels of nitrate nitrogen were present in most of the cultivated soils (Table 4) after 21 days of incubation when no ammonium nitrogen was added.

The higher nitrification rates of added ammonium nitrogen (50 and 100 ppm) in cultivated compared to virgin soils (Table 5 and 6) may be due to higher population of nitrifying bacteria present in cultivated soils, which resulted in higher levels of nitrate nitrogen. The results agreed with those reported by Brar and Giddens (1968) who observed better nitrification in a limed grassland soils with the addition of nitrifiers. Also, Watson (1976) has postulated that the slow ammonium to nitrate conversion in virgin soils is due to the low level of nitrifying bacteria.

There was some loss of total nitrogen in some cultivated and virgin soils during incubation following the addition of 50 ppm ammonium nitrogen. Broadbent and Clark (1965) reported that much of the nitrogen loss often attributed to aerobic denitrification was actually the result of formation and decomposition of ammonium nitrite

($NH_3^+ + HNO_2^- \longrightarrow NH_4NO_2 \longrightarrow N_2 + H_2O$). Clark et al. (1960) also presented evidence that nitrite instability is a major source of nitrogen loss in some alkaline soils receiving ammonium nitrogen. Alkaline soils may favor accumulation of nitrite during the process of nitrification.

Morrill et al. (1967) found ammonium oxidized rapidly to nitrite at pH 6.93 to 7.85 which then accumulated for extended periods of time before being oxidized to nitrate. Accumulated nitrite in some of these soils may explain this loss of nitrogen. There is also the possibility that some losses of nitrogen may be due to the incorporation into organic nitrogen by soil microorganisms. Bremner (1965) reported that nitrogen mineralization and immobilization cycles in soils were controlled by the availability of energy materials for microbial processes.

Meiklejohn (1940) found two species of Psuedomonas bacteria that reduced nitrate to nitrite and nitrogen gas even when well supplied with oxygen.

Accumulation of more inorganic nitrogen than expected from the added ammonium nitrogen in most of the cultivated soils and in some virgin soils (Table 6) may have resulted from organic matter decomposition during the 21 days of incubation. The finding of Harmson and Kolenbrander (1965) could explain this gain in total inorganic nitrogen. They reported that addition of nitrogenous fertilizer occasionally

stimulated the mineralization of organic matter and resulted in increased accumulation of inorganic nitrogen.

Bacterial Assays

Soil for the bacterial assays was collected in December. Lower mineral nitrogen content (Table 7) in samples of cultivated soil collected in December compared to samples from the same location collected in June could have resulted from crop removal, and slower organic matter decomposition during the fall.

Also, a difference in nitrification rate occurred in the same soils collected in June as compared with December following the addition of 50 ppm ammonium nitrogen and incubation for 10 days (Tables 5 and 7). The rate of nitrification was slower in soils collected in December. The finding by Sabey et al. (1956) could explain such findings. Sabey reported that nitrification was inhibited under field conditions when soil became frozen during December.

The population of Nitrosomonas and Nitrobacter in cultivated soils much higher than in virgin soils (Table 9). Manuring, fertilization, cropping, irrigation and tillage most likely changed the physical and chemical properties of cultivated soils and encouraged mineralization of organic matter.

The increased mineralization of organic material then stimulated the multiplication of Nitrosomonas and Nitrobacter in the

cultivated soils. The data obtained by Jane (1968) and Gomah et al. (1974) support this hypothesis.

The difference between cultivated and virgin soils in the population of Nitrosomonas and Nitrobacter was very large. Nitrification rates during incubation were higher in cultivated soils, but not proportional to the difference in bacterial populations. In fact, no correlation was apparent between nitrification rates and bacterial populations. This could be due to rapid multiplication of Nitrosomonas and Nitrobacter bacteria in virgin soils during the 10 days of incubation. Sabey et al. (1959) also reported a lack of correlation between nitrification rates and bacterial populations. Sabey found that the maximum nitrification rate was not greatly influenced by increasing the initial population of nitrifiers at temperatures above 10° C.

When cultivated and virgin soils were mixed, nitrification rates increased with increasing proportions of cultivated soils up to 35 percent (Table 9). At this proportion the bacterial population must have been optimum. This indicates that the slower nitrification rates in virgin soil was due to lack of nitrifiers.

In addition to the bacterial population, there is also possibility that nitrification inhibitors are present in some soils. Alexander (1965) reported that the concentration of the chemical Nitrapyrin (N-serve) required to cause a marked inhibition of ammonium oxidation varies from 0.05 to 20 ppm, depending upon the soil. Odu and

Akerele (1973) found that one out of four grass soil extracts and all legume soil extracts had no toxic effect on bacteria. However, root extracts did show some toxic effects. Odu and Akerele concluded that the toxic substances extracted from the roots was either not exuded into the soil or was exuded and was inactivated in the soil. Also, Rice and Pancholy (1974) identified numerous phenolic acid and phenolic glycosides produced by plant species from the intermediate and climax stage of old field successions. All of these inhibited nitrification in concentrations from 10^{-6} to 10^{-8} M. Rice and Pancholy also reported that caffeic acid was found in the year-old dead top of grasses. Further studies are needed to explore this aspect.

SUMMARY AND CONCLUSIONS

1. Total residual nitrogen content (ammonium and nitrate nitrogen) was higher in most of the cultivated soils compared to that found in virgin soils.

2. Nitrification rates were consistently lower in virgin soils than in cultivated soils following additions of 50 and 100 ppm ammonium nitrogen and incubated for 0, 10 and 21 days.

3. Nitrification rates were higher in soil samples collected in June than in those collected in December.

4. Nitrosomonas and Nitrobacter bacterial populations ranged from 4,100 to 35,000 cells per gram in cultivated soils compared to a range of 45 to 460 cells per gram in virgin soils.

5. Maximum nitrification rates occurred in virgin soil when diluted with 35 percent cultivated soil.

6. Based on the bacterial assays and the dilution study it was concluded that the low nitrification rates in virgin soils was due to low bacterial populations and most likely not due to nitrification inhibitors.

7. This study did not conclusively prove that nitrification inhibitors do not play some part in the slower nitrification rates observed under field conditions. Further work is needed in this area.

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