

**THE MICROBIOLOGICAL OXIDATION OF VARIOUS
NITROGEN FERTILIZERS IN DESERT SOILS,
WITH SPECIAL REFERENCE TO THE BEHAVIOR
OF ANHYDROUS AMMONIA**

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TO THE BEHAVIOR OF ANHYDROUS AMMONIA

by

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INTRODUCTION

During the last ten years an interesting development has taken place in the application of nitrogen fertilizers to arable soils in the agricultural regions of the West, namely, the use of anhydrous ammonia, applied by way of irrigation water. Aside from providing an outlet for the synthetic and by-product ammonia of industry, this method offers several advantages to the agriculturist: Anhydrous ammonia has the highest nitrogen content of all of the common nitrogen compounds used as fertilizers; the time and labor-consuming practices of mixing, and spreading or drilling are eliminated; relatively good distribution is attained; and the fertilizer can be applied by way of the irrigation water even after the crop is too high for the usual mechanical methods of fertilizer application.

There are, however, other important advantages. Irrigation water is an ideal vehicle for applying anhydrous ammonia; and, by the same token, anhydrous ammonia is the source of nitrogen best adapted to this method of application. It is very soluble in water, and carries the highest

nitrogen content of all nitrogen fertilizers -- 176% as much per unit weight, for example, as urea, and 388% as much as ammonium sulfate. Ammonia is promptly "fixed" in the soil by base exchange, hence losses by volatilization are negligible. Moreover, it can be shipped in compressed, liquid form, thus reducing transportation costs to a minimum. Its application to the water by way of reducing valves is simple in operation and the amount of ammonia added can be readily controlled.

Because of the interest in this means of supplying nitrogen to the soil in the irrigated sections of Arizona where citrus, truck crops, small grains, and hay are extensively grown, an investigation of the behavior of anhydrous ammonia when so introduced into the soil has been undertaken. As a fundamental approach to the problem it seemed desirable to study the chemical and microbiological changes which ammonia undergoes in the soil in the hope that such information might be useful in determining how best to use this form of nitrogen in the production of different crops. This thesis sets forth the results of such a study.

REVIEW OF LITERATURE

Inasmuch as the use of free ammonia as a fertilizer is a very recent development in agricultural practice, the amount of published research on its properties and behavior is of necessity very limited. Furthermore, it is hazardous to make even provisional deductions relative to the probable behavior of ammonia from results of investigations of the various ammonium salts commonly used as fertilizers. Nevertheless, the background of existing research on the varied factors affecting the microbiological oxidation of nitrogen in both its inorganic and organic forms and the utilization of the various forms of nitrogen by plants has yielded valuable hints and suggestions as to a desirable experimental approach to the present problem. With this thought in mind, and recognizing the paucity of published literature on ammonia per se, the present literature review has been assembled.

A. Ammonia as Compared with Nitrate Nitrogen in Plant Nutrition

A thorough review of the early research on nitrification has been made by Waksman (1). Although nitrification studies have been conducted along numerous lines, many specific problems yet remain to be solved in the study of

anhydrous ammonia as a fertilizer on desert soils.

Any introductory investigation of the applicability of anhydrous ammonia as a source of nitrogen for plants must include nitrification studies because the ability of plants to utilize ammonia as such has not yet been definitely established. It is true that certain investigators, for example, Prianishnikov (2, 3), have found ammonia nitrogen preferable to nitrate nitrogen. Prianishnikov considers nitrification an energy-wasting process since more energy is required to convert nitrates than ammonia to protein. He also demonstrated that in a neutral medium ammonia gives better nutritional results than nitrates, whereas in an acid medium the reverse is true. An extension of Prianishnikov's finding to alkaline soils would, a priori, favor ammonia nitrogen. Such an extension of thought can only be tenable, of course, when it has been confirmed experimentally. It is in agreement, however, with Buehrer's (4) reasoning relative to the absorption of the negatively charged phosphate ion. In soils sufficiently alkaline, if the exudations of the roots do not lower the pH in the immediate vicinity of the roots below their isoelectric point, the roots will possess a negative charge. In this event, since the ions must be adsorbed before they can be absorbed, as indicated by Buehrer, it is reasonable to expect that positive ammonium ions would be more readily available to plants in such soils than negative nitrate ions.

Studies on ammonium ion absorption have generally been conducted using neutral salts as the source of the ammonium ion in nutrient solution, sand-and-water culture, or water-logged soil. Such studies have been made by Hutchinson and Miller (5), Pantanelli and Severini (6, 7), Prianishnikov (8), Shulov (9), Stewart et al (10), and McGeorge (11).

Kelley (12) and Janssen and Metzger (13) studied the transformations occurring in submerged soils and reported rapid disappearance of nitrates. Breazeale and McGeorge (14), using puddled soils, found nitrification completely stopped by puddling.

As Arnon (15) recently noted, the possibility of nitrification of ammonium ion in any study comparing the nutritional value of ammonia with nitrate nitrogen is a complicating factor which renders the use of soil undesirable. The writer feels, however, that it is hazardous to translate results from nutrient solution studies directly to the soil.

The fact that microbiological activities differ under aerobic and anaerobic conditions is definitely established. The evidence that higher plants can utilize ammonia nitrogen equally as readily as nitrate nitrogen, however, is not so positive. Physiological differences in plants with respect to the intake of other nutrient elements, in the development of adequate root systems, and in their fruiting abilities for example, have been noted repeatedly when plants have been

subjected to conditions in which either nitrate or ammonium ion was present as the sole source of nitrogen.

Shive and his coworkers (16, 17, 18, 19, 20, 21, 22) have made a substantial contribution to our scientific knowledge relative to the behavior of ammonia and nitrate nitrogen. They have shown (16, 17, 18, 22) that the absorption and assimilation of the ammonium ion is a function of both the age and the species of plant. Arrington and Shive (22) attribute the difference in absorption characteristics noted between oats (16, 17) on the one hand and tomato seedlings (22) on the other to the more vegetative nature of the latter.

Stahl and Shive (17, 18) noted, too, that whereas neither the absorption of ammonia nor nitrate nitrogen ceased entirely at any point in the growth cycle of the oat plant, the rate of nitrate nitrogen absorption for buckwheat was zero at the start of growth, and increased to time of blossoming. They concluded that ammonia nitrogen plays a more important role during most of the life cycle of buckwheat than does nitrate nitrogen.

Clark and Shive (19) and Davidson and Shive (20), in the study of tomato plants and young peach trees, respectively, found a definite relationship between the reaction of the external medium and the rate of absorption of ammonium and nitrate ions. The former observed the rate of ammonia nitrogen absorption from solutions of pH 7.0 to be nearly

twice that at pH 4.0, and that for nitrate nitrogen was only about half as great from a solution at pH 7.0 as at pH 4.0. Arrington and Shive (22), on the other hand, failed to find this same positive correlation using tomato seedlings. Their observations were that at pH 4.0 the nitrate rate was 4.7 times the ammonia rate, and at pH 7.0 it was still 1.2 times the ammonia rate.

Clark and Shive (21) obtained a positive correlation in the case of the tomato plant among pH, concentration of the ammonia nitrogen in the roots of the plants, and the rate of absorption from the nutrient solution; they found further that the ammonium ion was assimilated readily by the plant even at the pH favoring most rapid absorption.

The findings of Stahl and Shive (16, 17) that ammonia nitrogen is the more readily absorbed during early growth are substantiated by those of Jones and Skinner (23) for soybeans and corn, and by Naftel (24) for cotton.

The foregoing review of the literature dealing with the absorption and assimilation of ammonia versus nitrate nitrogen, and the effect of the pH of the external solution is by no means exhaustive. It illustrates, however, that our knowledge is still quite inadequate on the subject.

The preponderance of evidence indicates that nitrates are essential at some time in the growth of plants, but the fact remains that ammonia applied to soil will be nitrified

if conditions are favorable. It would be highly desirable, therefore, to study the rates of nitrification in desert soils to determine what form of nitrogen is available to growing plants at various periods after application of the fertilizer.

B. Biological Nitrification vs. Chemical Nitrification

During the sixty-five years since Schloesing and Müntz (25, 26, 27) performed their classical experiments demonstrating the biological nature of nitrification, the pertinent literature has become extensive. The work of Warington (28, 29, 30) on the effect of organic matter on nitrification, and on the oxidation of organic nitrogen, as well as Winogradsky's (31) ingenious isolation of the nitrite and nitrate organisms, constitutes basic historical knowledge familiar to all students in the field.

For many years following the work of Schloesing and Müntz, the opinion that the nitrification process is solely biological was nearly unopposed. In most quarters this opinion still predominates, but a series of recent investigations on chemical nitrification cannot be disregarded. Light is considered by adherents to this newer school of thought to be a causal factor.

Berthelot and Gaudechon (32) were probably the first exponents of the idea when they demonstrated that ammonia can

be oxidized to nitrite by ultraviolet radiation. Chemical nitrification of an aqueous ammonia solution of humus under the influence of sunlight was reported by Zolcinski (33) in 1923. Gopal Rao and Dhar (34) reported the oxidation of ammonia and its compounds in solution to nitrite in the presence of photosensitizers, and that basicity favors the oxidation. Dhar, Bhattacharya, and Biswas (35), in 1933, concluded that nitrification in soil, "especially in tropical countries, is a more photochemical than a bacterial process."

Gopal Rao (36) assembled much evidence, direct and indirect, supporting the photochemical theory of nitrification, and reported the oxidation not only of ammonium salts but also aqueous solutions of urea, acetamide, hydroxylamine hydrochloride, hydrazine hydrochloride, ethylamine, and methylamine in the presence of suitable photosensitizers.

Waksman and coworkers (37, 38) and Fraps and Sterges (39) have opposed the theory of photonitrification because they failed to obtain positive results. Corbet (40) criticized the technic of the latter, however, because they used ordinary window glass to cover the samples which they exposed to sunlight, thus eliminating the ultraviolet radiations, and he reported (41) further that he had "confirmed in a large measure" the photonitrification of ammonium salts in sterile soils previously reported by earlier works. He expressed the opinion, however, that the mechanism of nitrification may be either biological or chemical. Singh and Nair (42)

concur in this opinion with Corbet.

de Rossi (43), on the other hand, concluded that non-biological nitrification is the result not of light but of heat -- "a moderate degree of heat, such as may be experienced in any land through the agency of the sun's rays in the spring, summer, and autumn."

Although a number of references have been cited which indicate that photonitrification may be effective in areas of extended periods of sunshine, the prevailing belief is still that nitrification is a biological process. For this reason it was decided to approach the study of the nitrification of ammonia in desert soils first from the biological viewpoint, and this is the only phase of the study reported in this thesis. As stated earlier, however, the chemical point of view should not be ignored in desert regions, and future work on the subject should include studies on photonitrification.

C. Nitrogen Sources Previously Used in Nitrification Studies

Although many compounds containing nitrogen have been studied as a nitrogen source in fertilizing programs, it is interesting to note that practically no work has been published on the use of anhydrous ammonia. In addition, with nitrogen fertilizers other than ammonia the large preponderance of investigations have related to acid rather than

alkaline soils. To cite a few examples of the nitrogen fertilizers which have been investigated:

Lipman and Burgess (44) studied the availability of fourteen nitrogenous fertilizers in twenty-nine soil types of importance in California. Included in the study were twelve commonly used organic waste and by products and two inorganic compounds--calcium cyanamid and ammonium sulfate.

Batham (45, 46) compared the relative rates of nitrification of ammonium sulfate and thirteen of the amino acids, three alkaloids, and two purines. This work was done on soils in India which showed fluctuations in the nitrate content during the year similar to those found in Iowa soils.

Jones (47) performed ammonification and nitrification experiments with urea.

Crowther and Richardson (48), Mukerji (49), and Moyer (50) have studied the nitrification of calcium cyanamid.

Davis, Miller, and Scholl (51) used ammoniated peat, dextrose, lignin, and starch, and magnesium ammonium phosphate, ammonium sulfate, and guanidine carbonate in a study of nitrification characteristics.

Brown and Reid (52) have investigated the nitrifying ability of formamid and its hydrolysis product, ammonium formate.

Ayers and Jenny (53) and Waynick (54) have used anhydrous ammonia and ammonium sulfate in base exchange and leaching

studies on some California soils. They included some nitrification studies which indicate that in many respects anhydrous ammonia compares favorably with ammonium sulfate.

D. Nitrification as Affected by pH

Ammonium sulfate has been used extensively as the standard inorganic form of nitrogen in nitrification studies in which several compounds have been compared. Anhydrous ammonia has as yet received relatively little attention at the hands of investigators. The introduction of the pH factor by the dissociation of the base is of particular importance in the nitrification of ammonia -- especially in soils which have an initial pH value above 7.0. The effect on soil reaction of the added base, the buffering capacity of the soil and its consequent ability to maintain the normal reaction, the effect on the micropopulation-- especially the nitrifiers--of changes in reaction, and their ability to remain active at all pH values likely to result from fertilizing an already alkaline soil with ammonia are a few of the fundamental factors involved in the present study.

The necessity of a neutral or slightly alkaline reaction for efficient nitrification is a rather well established fact. This is one of the principal reasons for liming acid soils in humid regions. Fraps and Sterges (55) examined a

large number of soils which failed to nitrify ammonium sulfate, and found that the condition could be remedied by the addition of cultures from actively nitrifying soils, or of calcium carbonate, or both. They (56) likewise found that calcium carbonate had a more favorable effect on nitrification rate than dicalcium phosphate, rock phosphate, magnesium carbonate, or dolomite in acid soils. Tandon and Dhar (57) similarly found that nitrification was favored more by calcium carbonate than by magnesium carbonate.

Waksman (58) has stressed the necessity of neutralizing the acid formed in the oxidation of ammonium sulfate, and has pointed out the effect of low pH on nitrification. He observed that the accumulation of nitrates from the oxidation of this ammonium salt stopped when a pH value of 4.4 to 4.8 was reached. He concludes that the amount of nitrate which accumulates in the soil depends on the initial reaction, buffer capacity, and final reaction "more so than on the bacteriological activities."

The minimum pH value noted by Waksman is in essential agreement with that noted by Humfeld and Erdman (59) and Gaarder and Hagem (60). The latter, using acid solutions, placed the minimum for the nitrifiers at 3.9 to 4.5

Naftel (61) used five soils varying widely in base and calcium saturation to study the effect of reaction and base

saturation upon nitrification, and found that nitrification increased, in general, with these factors. His results indicate, however, that nitrification may vary considerably in different soils at the same reaction. The soil with highest percentage base and calcium saturation was the only one which nitrified ammonium sulfate at pH 4.1.

Olsen (62) used strongly acid humus soil in which he adjusted and maintained the pH with lime, and concluded that nitrification can proceed between pH 3.7 and 8.8, with an optimum, if ammonia is not a limiting factor, at pH 8.3. He also concluded that the organisms are capable of adjusting themselves to wide variations in soil reaction.

Gowda (63) and Meek and Lipman (64) have reported results of most definite interest in the alkaline range. The former reported the optimum reaction for nitrite formation was pH 8.0, whereas that for nitrate formation was 8.5 to 8.8. The latter found both nitrite- and nitrate-forming organisms from garden soil were able to live and function at pH 13.0; but the nitrifiers from acid peat could not produce nitrates above pH 9.5. These workers considered that their data, in general, indicate that the nitrate forming bacteria are slightly more resistant to alkalinity than the nitrite forming bacteria. Neither Gowda nor Meek and Lipman employed soils in their studies, however.

Ayers and Jenny (53) and Waynick (54) included some nitrification studies of anhydrous ammonia and ammonium

sulfate with investigations of several physical and chemical changes in the soil following application of these fertilizers. The former used one acid and one slightly alkaline soil. Waynick reported that the soils used had initial pH values of 7.1 to 8.1; but these values were taken on the soil suspension rather than the moist soil and probably indicate that the soils used appeared to be more alkaline than they actually were.

Although it has been noted many times that a neutral or alkaline reaction favors nitrification, no work has appeared on optimum or limiting reactions for nitrification in alkaline, calcareous soils.

E. Nitrification as It Is Affected by Soluble Salts

Because of the relatively high salt content of desert soils under irrigation, the effect of the various ions upon nitrification is of great importance in cases where the nitrogen is applied in forms other than nitrate -- assuming, of course, that the nitrate form is the available form to the majority of plants.

Nelson (65) studied the effect of manganese salts and found, in general, that increasing concentrations caused increasing toxic effects. Manganese toxicity was reduced, however, by additions of lime, probably due, according to this worker, to a change in reaction toward a pH value more

favorable to nitrification.

Lipman (66) used the ammonifying and nitrifying power of the soil as a criterion in studying anion antagonism and found the antagonism between anions to be strikingly shown by their effect on the nitrifying power of the soil. He concluded that antagonism between anions, if the proper concentration of the antagonistic ions are present, can cause the nitrogen-transforming power of soils to become greater than that of normal soil.

Brown and Hitchcock (67) similarly found that nitrification in normal soils is stimulated by small amounts of sodium chloride, sodium sulfate, and magnesium sulfate, and by large amounts of calcium carbonate. They concluded that "in general, it seems that nitrification and crops are very similarly affected by alkali salts."

Greaves (68) found the common soil alkalis--sodium chloride, calcium chloride, and sodium sulfate--effective if present to any great extent in greatly reducing the ammonia produced in the soil. This fact emphasizes the necessity of adding ammonia as a fertilizer to such soils because of diminution of the natural supply. Greaves (69) also found the antagonism between magnesium chloride and iron nitrate to be such that the latter increased the nitrification 420.7% over that in soil treated with magnesium chloride alone. He reported that the cations exert the greater influence, but that the anions are not without effect.

Kelley (70) found the effect produced by the addition of alkali salts to vary greatly when different concentrations of nitrogen-containing materials were present in the soil. In some cases the ratio of concentration of salt to amount of fertilizer added proved distinctly toxic to nitrifying bacteria whereas in other cases such relative concentrations were markedly stimulating.

It appears from the references cited that the effect of naturally occurring salts in arid, alkaline soils upon the nitrification rate is highly variable, depending upon the salts present, and to some extent upon the amount and possibly kind of nitrogenous material in the soil. Whether nitrification will occur, then, in any particular soil can be proved only by examining its nitrification characteristics, or possibly by analysis for the kinds and amounts of the salts present, or both.

F. Toxicity of Ammonia to the Nitrifying Bacteria

Willis and Piland (71) found that free ammonia formed by the hydrolysis of diammonium phosphate was toxic when used in pot culture experiments. Such toxicity was not observed in the case of the sulfate, chloride, or nitrate; nor did the alkalinity of the diammonium phosphate appear to contribute to the injurious effect. They found further that calcium salts were able to counteract the toxicity of

the ammonia; hence, it is reasonable to expect that such toxicity might not prevail in calcareous soils even though ammonia itself were used as the fertilizer.

Waksman (58) reported similarly that the rapid decomposition of dried blood in alkaline or poorly buffered soils evolves sufficient free ammonia to have an injurious action upon the activity of the nitrifying bacteria in soil. He did not mention the antagonistic effect of calcium salts reported by Willis and Piland, however, and since his report referred to alkaline soils, further research is needed to determine the validity of their deductions.

SCOPE OF THE PRESENT INVESTIGATION

Because of the economic importance of ammonia and its adaptability to irrigation agriculture, it seemed desirable to make a study of certain fundamental aspects of its behavior in the alkaline calcareous soils of the desert.

The assimilation of nitrogen as ammonia by crops such as citrus, small grains, grasses, cotton, and truck has not yet been definitely established, whereas the assimilation of nitrate nitrogen by these crops is accepted generally as an established fact. It therefore appeared reasonable to initiate this investigation by a study of the oxidation of ammonia to nitrates.

Since the greater amount of evidence supports the biological rather than the chemical theory of nitrification, especially under temperate and subtropical conditions, the biological aspects were investigated first.

The fact that most nitrification studies have been made on acid soils, low in water-soluble salts, emphasizes the desirability of studying both pH and salt effects on the nitrification of ammonia. The pH relationships have been accorded a prominent place in this investigation because of the generally alkaline character of desert soils.

In brief, the results to be presented are based upon

an investigation of the nitrification rates of anhydrous ammonia, ammonium sulfate, and urea in six typical desert soils of Arizona, three of which have been cultivated for a number of years and the remaining three being still in the virgin state. These findings revealed an unusual relationship between pH and nitrification which suggested the desirability of studying nitrite formation as related to the lowering of the reaction in alkaline soils. The most important outgrowth of the foregoing studies was an investigation of the prime factors which affect the speed of nitrate formation and build up of nitrites in desert soils.

EXPERIMENTAL PROCEDURE AND RESULTS

A. Description and Characteristics of the Soils Studied

Six soils were chosen for this study, each of them representing agriculturally important areas in Arizona. Three of the soils have been cultivated for a number of years, and three are still in the virgin condition but lie adjacent to areas under cultivation. All of the six soils are either irrigated or are suitably situated for irrigation. Among them are included two which comprise a pair of the same series and type, lying within fifty feet of each other, only one of which has been cultivated.

The six soils selected for study are:

- (1) Superstition sand, from near the University Farm on the Yuma mesa. This is a calcareous, uncultivated soil.
- (2) Gila sandy loam, from a field immediately adjacent to the Cortaro Farms at Marana, Arizona. This is a calcareous, virgin soil, and with that given next below constitutes the pair of soils referred to in the preceding paragraph.
- (3) Gila sandy loam, from a field in cotton on the Cortaro Farms at Marana, Arizona. This soil is calcareous.
- (4) Pima clay loam, from the river-bottom at Safford, Arizona. This is a mildly calcareous, cultivated soil.
- (5) Laveen loam, from the Experimental Farm at Mesa, Arizona. This is a calcareous, cultivated soil.

- (6) Palos Verdes sandy loam, from just north of the Rancho Palos Verdes near Tucson, Arizona. This soil is non-calcareous and uncultivated.

There are no available records to show that any of the six soils have ever been artificially fertilized.

Preparation of the Soils for Study

All of the soils used in this study were collected within a period of about one month--June 19 to July 29, 1939. Several tons were collected at the time to avoid the necessity of additional collections at a later period under different conditions.

The soils were reduced to a condition permitting uniform mixing and sampling in the following manner:

Soils too moist to reduce in particle size were spread out on tables and allowed to dry. The air-dry soil was transferred to a steel buckboard on which the large aggregates were broken down to approximately 10-mesh under a hand roller. All twigs, grass, stones, and other foreign matter were carefully picked out. They were then screened to 10-mesh.

After screening, the soil was shovel-mixed on the smooth, cement floor of the greenhouse and transferred to bins.

Mechanical Analysis of the Soils

Mechanical analyses were made on the six soils according

to the method of Bouyoucos (72), with a slight modification for the temperature correction. The time of reading the hydrometer was corrected to account for the change of viscosity of the solution with temperature. The data for the change in viscosity with temperature were taken from Wright (73).

The results of the mechanical analyses of the six soils are presented in Figure 1. Attention should be called to the fact that although soils 2 and 3, virgin and cultivated Gila series soils, are both sandy loams, the virgin soil is almost a sand, and the cultivated soil approached a loam. The heavier texture of the cultivated sample is quite marked; it is easily possible to distinguish between the two soils visually.

The Laveen loam, soil 5, was so classified because the duplicate analyses fell upon two lines of the textural triangle -- one upon the clay loam-loam boundary, and the other upon the sandy loam-loam boundary.

Physical Constants of the Soils

The so-called Keen-Raczkowski measurements (73) -- the apparent density, the moisture content of the saturated soil, pore space, specific gravity, and volume expansion -- were determined, using the modified technic suggested by Fletcher (74) which employs Gooch crucibles instead of the

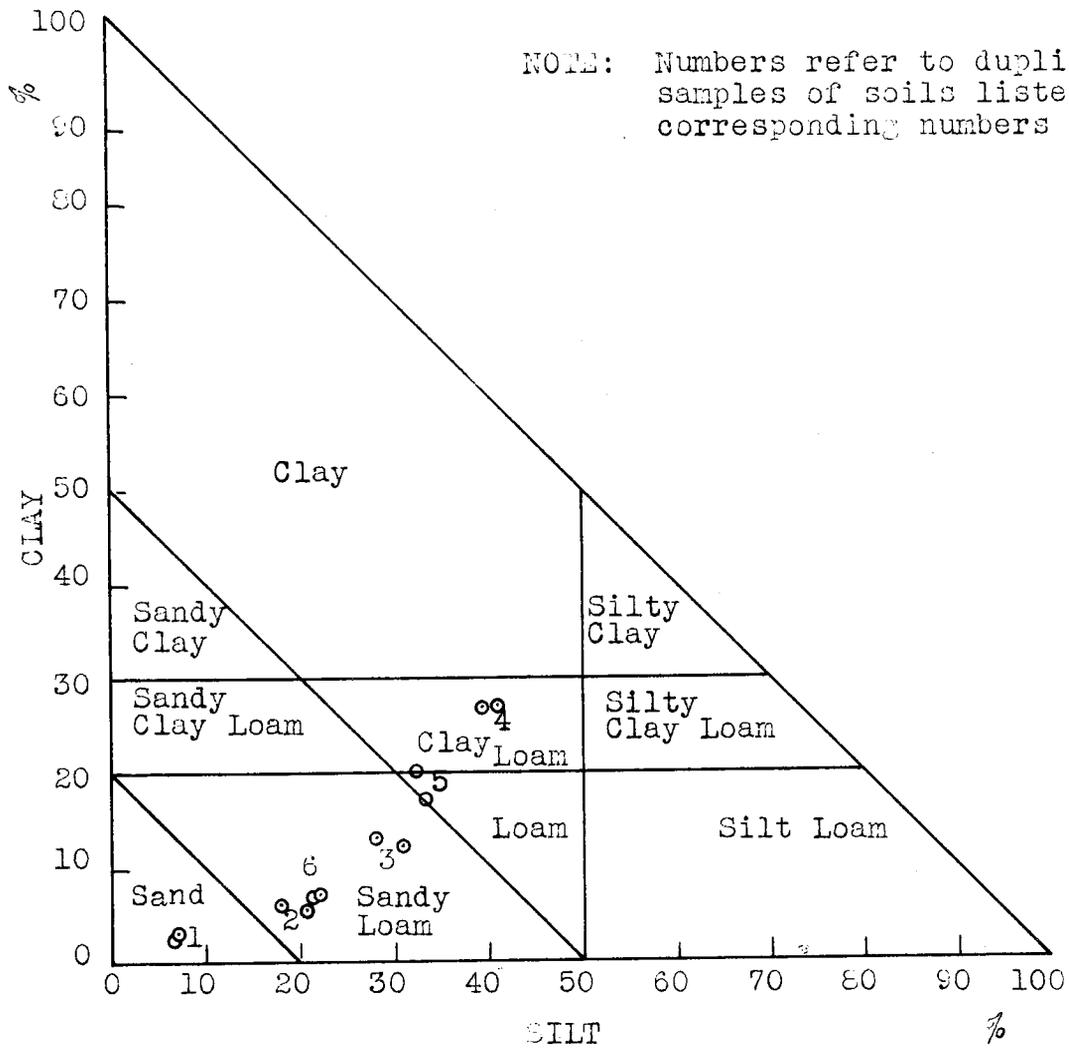


Fig. 1. Textures of the Soils Studied in Relation to the Textural Triangle.

cylindrical boxes suggested by the original authors. The volumes of the crucibles were determined by using powdered tin and measuring the volume with a burette and tapping, and the data so obtained, when statistically analyzed, gave no values outside of the standard deviation. This method was used, therefore, in measuring the volumes.

It was deemed inadvisable to determine the volumes of the crucibles by sealing the pores with paraffin and filling the crucibles with a liquid because of the error introduced by the meniscus. In many soils the volume expansion may be represented by a volume no greater than the error introduced by using the liquid method of measuring. Similarly, it was impossible to obtain the volume from the specific gravity and weight of paraffin used to fill the crucibles because, on cooling, the wax would contract; further, if the measurements were made on the wax before it had contracted by cooling, an error in volume would be introduced because of the expansion of the crucible.

In addition to the Keen-Raczkowski measurements, the water-holding capacity, as conventionally determined by allowing the saturated soil to drain for several days in a saturated atmosphere, and the moisture equivalent as measured by a slight modification of Bouyoucos' (75) suction method were determined. A Gooch crucible was substituted in the latter method for the Buchner funnel used by Bouyoucos.

The following values were used in making the above

measurements:

- a = weight of crucible + dry filter paper
- b = weight of crucible + dry filter paper + air-dry soil
- c = weight of crucible + wet filter paper + saturated soil
- d = weight of crucible + wet filter paper + saturated residual soil, the expanded portion having been removed
- e = weight of crucible + dry filter paper + oven-dry residual soil, the expanded portion having been removed before drying
- f = same as c except that saturated soil has been allowed to drain in a saturated atmosphere for about 40 hours
- g = same as c except that the soil has been drained by suction
- v = the internal volume of the crucible
- y = the moisture content of the wet filter paper

The foregoing values are used in the following formulas to arrive at the desired measurements:

$$\text{Apparent density} = \frac{(b-a)}{v}$$

$$\text{Water of saturation in per cent} = \frac{d-(e+y)}{e-a} \times 100$$

$$\text{Pore space in per cent} = \frac{d-(e+y)}{v} \times 100$$

$$\text{Specific gravity} = \frac{e-a}{v-(d-e)}$$

$$\text{Volume expansion in per cent} = \frac{c-d}{d-(a+y)} \times 100$$

$$\text{Water holding capacity in per cent} = \frac{f-(e+y)}{e-a} \times 100$$

Moisture equivalent as per cent of oven-dry weight =

$$\frac{g-(e+y)}{e-a} \times 100$$

The values obtained by these measurements are presented in Table 1.

TABLE 1

PHYSICAL CONSTANTS OF SOILS USED IN NITRIFICATION STUDIES

Soil	Appar- ent density g/ml.	Satur- ation mois- ture %	Pore space %	Speci- fic grav- ity	Volume expan- sion %	W.H.C. %	Moisture equiva- lent %
Superstition sand	1.73	19.9	31.9	2.36	8.2	20.4	8.0
	1.74	19.1	31.0	2.35	7.8	19.9	9.8
Gila sandy loam-virgin	1.70	22.8	35.9	2.47	7.3	22.7	14.9
	1.70	23.3	36.5	2.48	7.3	23.2	15.9
Gila sandy loam-cult.	1.58	28.2	40.7	2.45	8.0	28.2	21.9
	1.59	28.0	40.9	2.48	7.7	28.0	21.5
Pima clay loam	1.39	42.5	48.8	2.26	16.9	43.1	39.1
	1.40	43.4	50.2	2.33	17.1	44.0	38.7
Laveen loam	1.51	36.3	46.5	2.40	15.3	36.1	27.8
	1.55	34.3	45.6	2.46	14.0	34.2	29.3
Palos Verdes sandy loam	1.74	24.7	38.4	2.54	11.0	24.6	13.3
	1.76	23.8	37.6	2.55	10.8	23.6	13.4

The effects of cultivation are quite marked if one examines the values of the constants for the virgin and cultivated Gila sandy loam samples. (The differences between the cultivated and uncultivated soils in general may be observed

by comparing the data for the third, fourth and fifth soils --all cultivated--with the remaining three which are uncultivated. Such a comparison is suggested here only as a matter of interest, however, since the differences in texture and series are recognized.) It is evident that cultivation favors a decrease in apparent density (volume weight), making for a heavier soil, and an increase in saturation moisture, pore space, volume expansion, water-holding capacity, and moisture equivalent. These changes which the virgin soil undergoes with cultivation probably result from a decrease in particle size, and would be expected to result from tillage operations, root penetration, microbiological activity, and the comminution of soil by higher forms of animal life.

The specific gravity of the soil particles, on the other hand, does not show any change. Except for an appreciable introduction of foreign material, not arising from the parent material of the soil, an alteration of the specific gravity should not be expected.

The saturation moisture as determined by Keen and Raczkowski and the water-holding capacity as usually determined represent the same physical measurement involving two slightly differing technics. It is not to be expected, therefore, that differences in these quantities will be very great; as a matter of fact, the differences are much smaller between the average values for all samples between

the two methods (0.28%) than the average difference between duplicate samples with either the water-holding capacity (0.82%) or the saturation moisture (0.88%) determinations themselves. Since the Keen-Raczkowski technic for measuring the saturation moisture is much more rapid than the usual method of determining the water-holding capacity, it may be recommended for more general use unless other factors in certain soils make for larger differences between the values than those here reported.

Buffer Capacities and Carbonate Contents of the Soils

The buffer curves were determined by potentiometric titration. A series of 20-gram samples was made up for each soil up to 1 to 5 suspension by the addition of water plus varying amounts of 0.5 N H_2SO_4 , shaking, and the pH value recorded. All pH measurements reported in this study were made with a Beckman pH meter.

The buffer curves characteristic of Superstition sand, virgin Gila sandy loam, cultivated Gila sandy loam, Pima clay loam, Laveen loam, and Palos Verdes sandy loam are presented in Figures 2, 3, 4, 5, 6, and 7, respectively.

Since the soils were all alkaline, their buffer capacities toward acid were calculated as the number of milliliters of normal sulfuric acid required per 100 grams of soil to reduce the pH from the normal value to 4.8, the lower limit

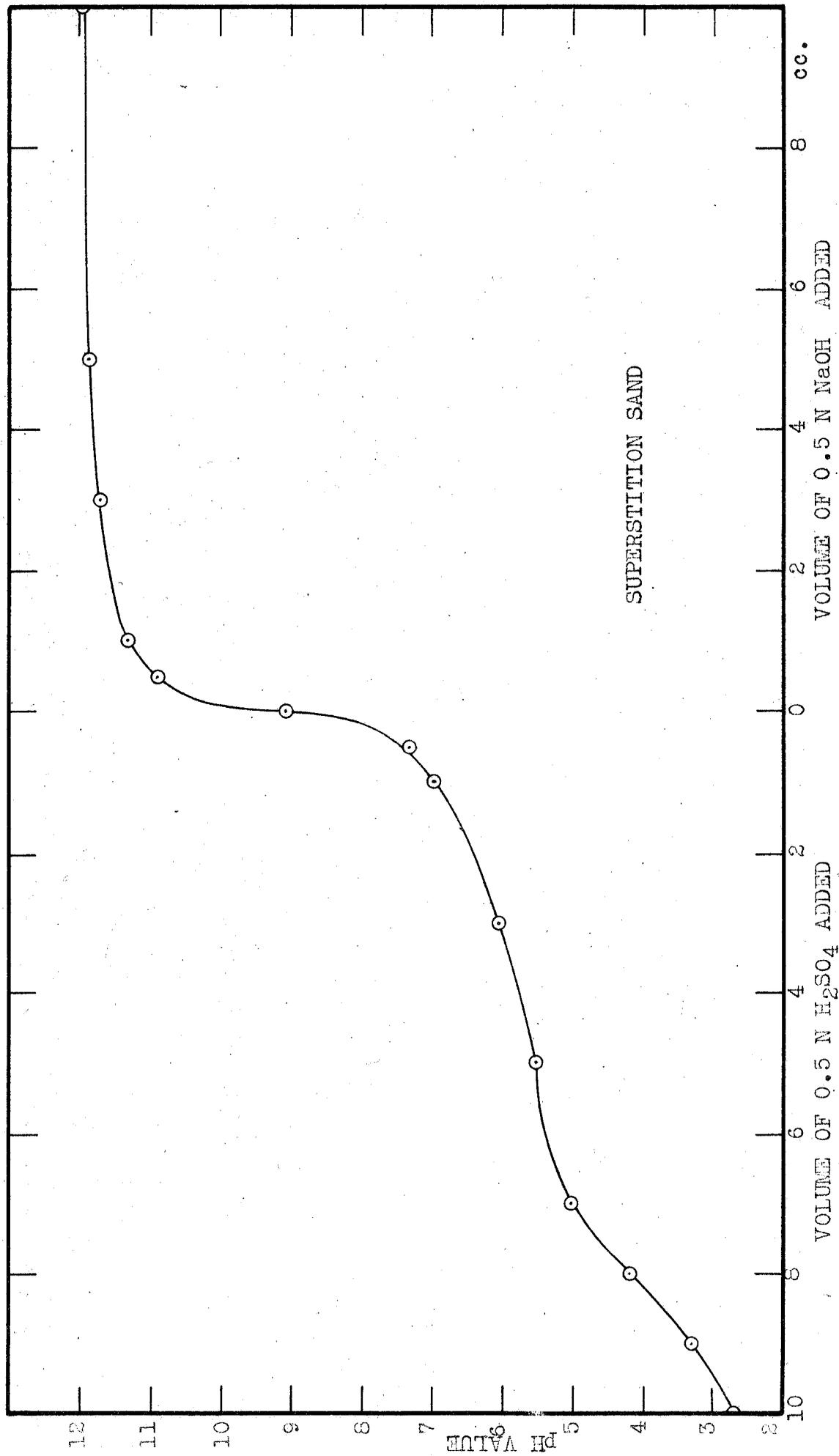


Fig. 2. Buffer Curve for Superstition Sand.

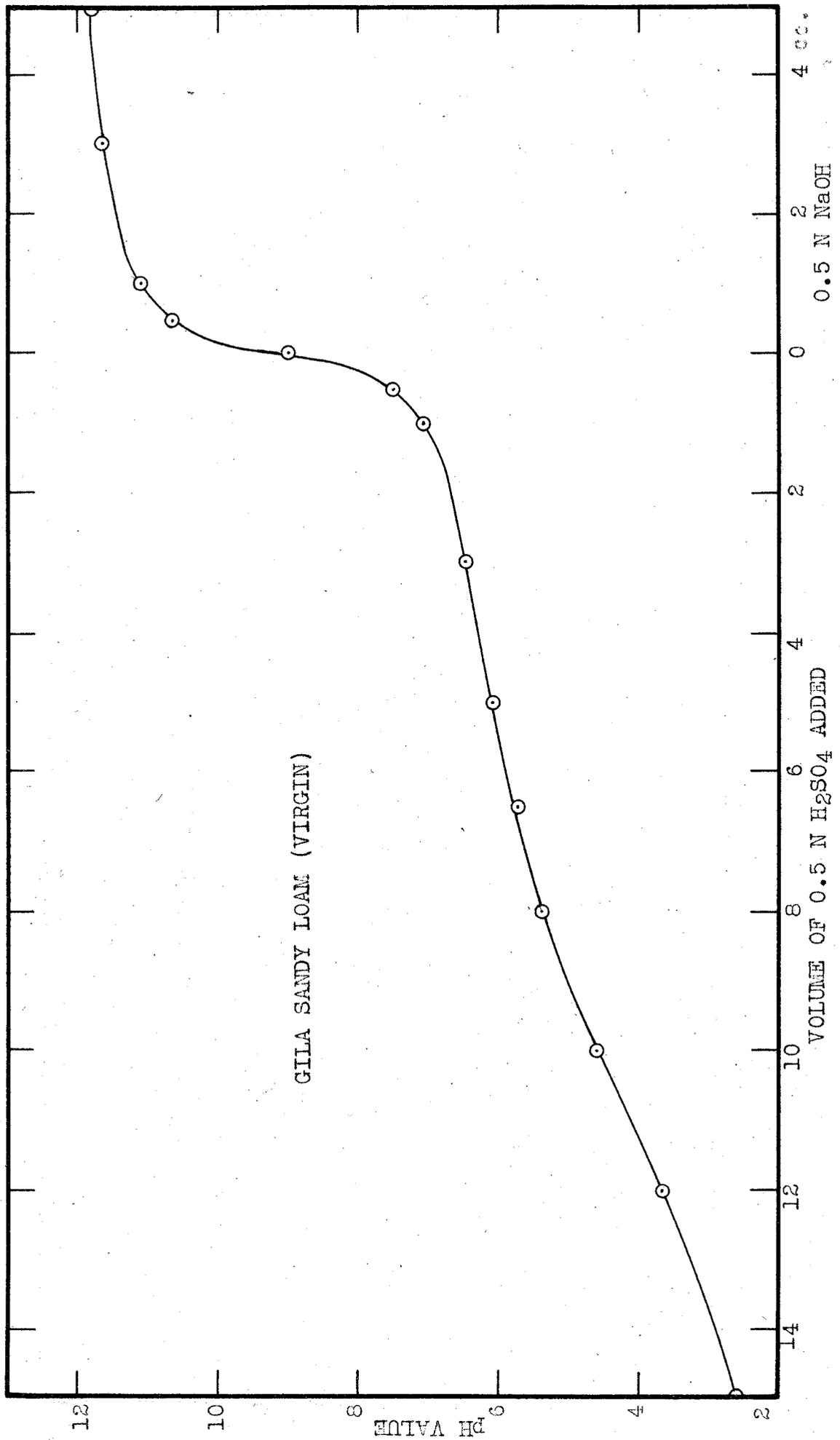


Fig. 3. Buffer Curve for Virgin Gila Sandy Loam.

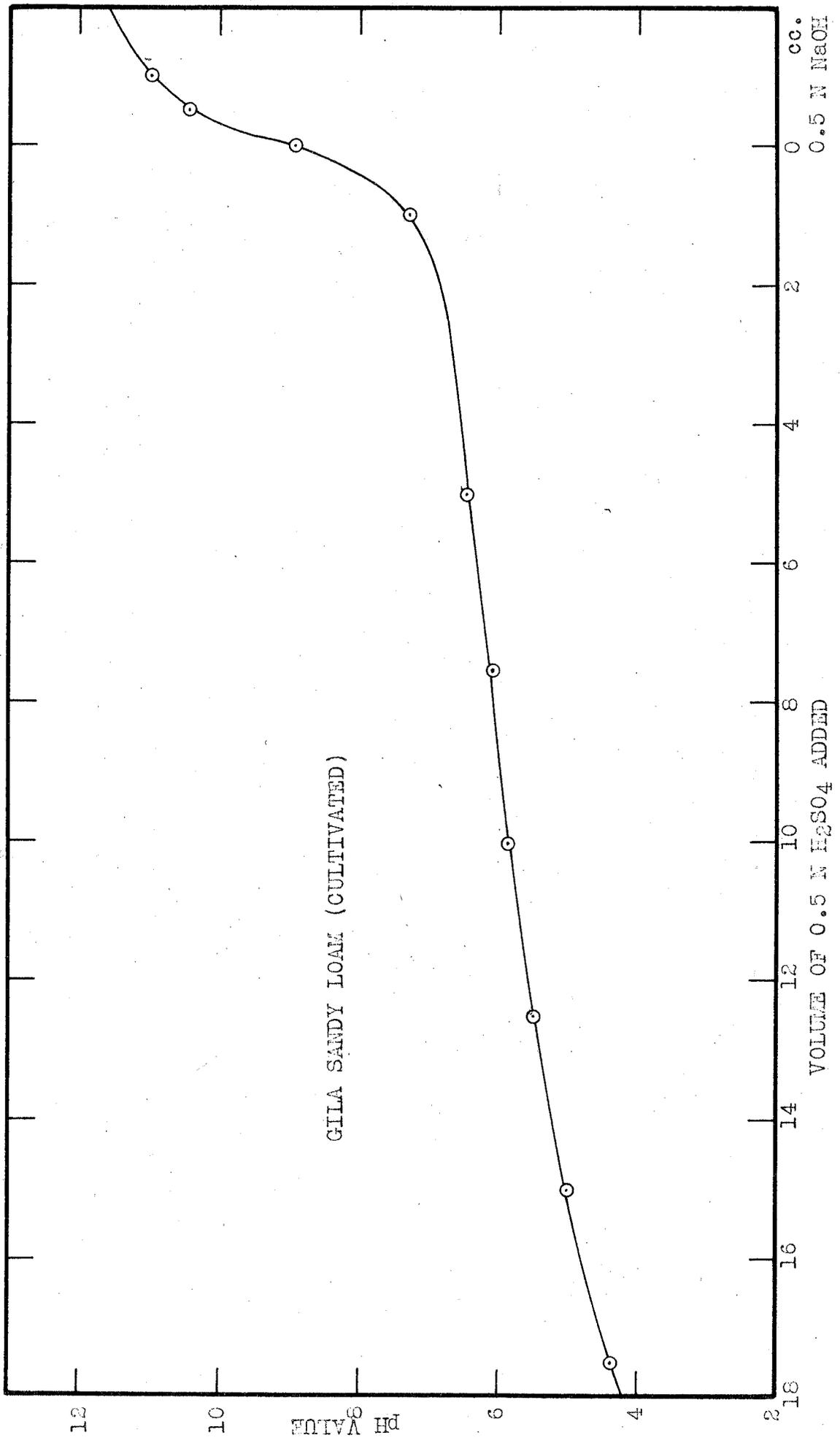


Fig. 4. Buffer Curve for Cultivated Gila Sandy Loam.

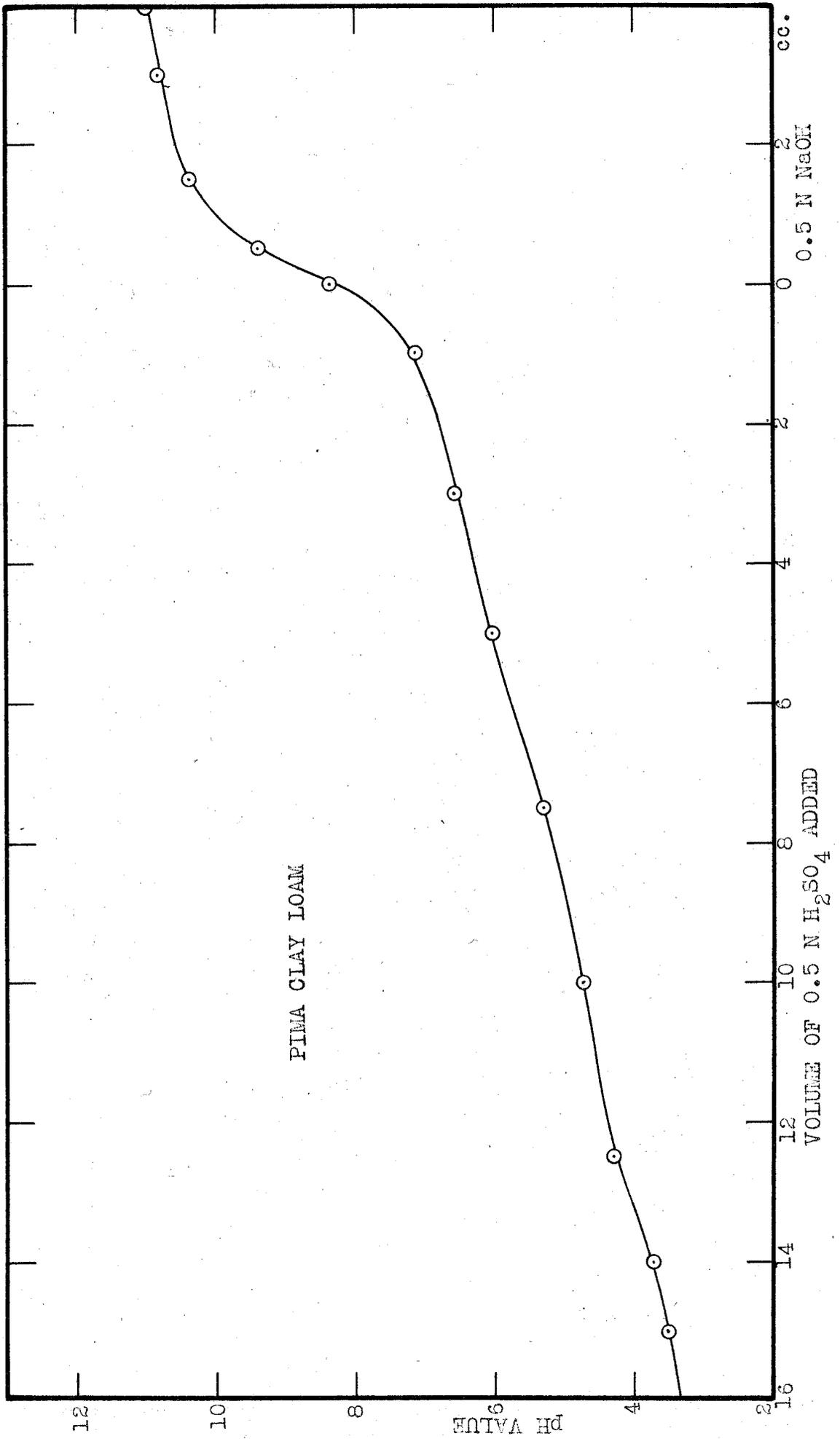


Fig. 5. Buffer Curve for Pima Clay Loam.

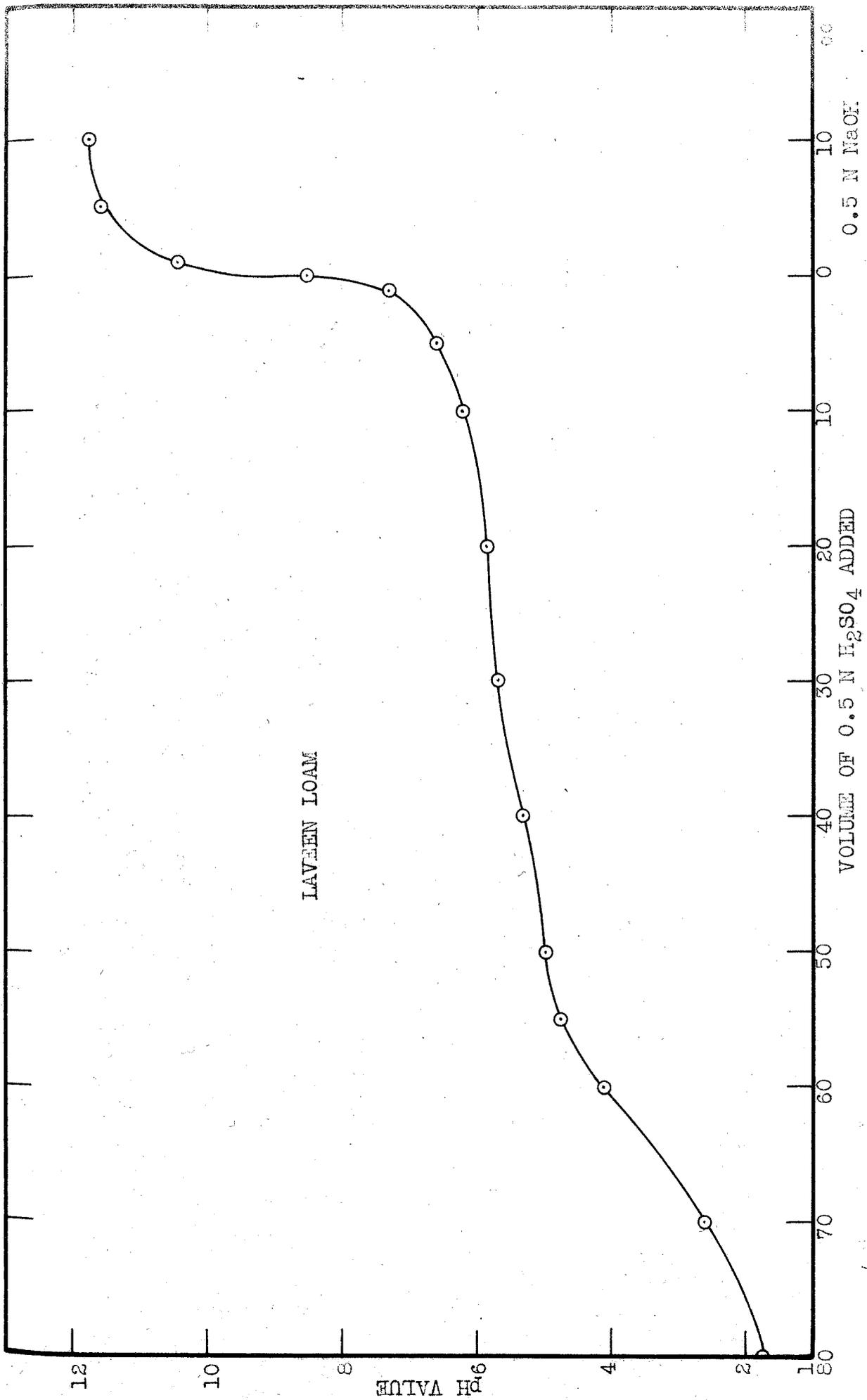


Fig. 6. Buffer Curve for Laveen Loam.

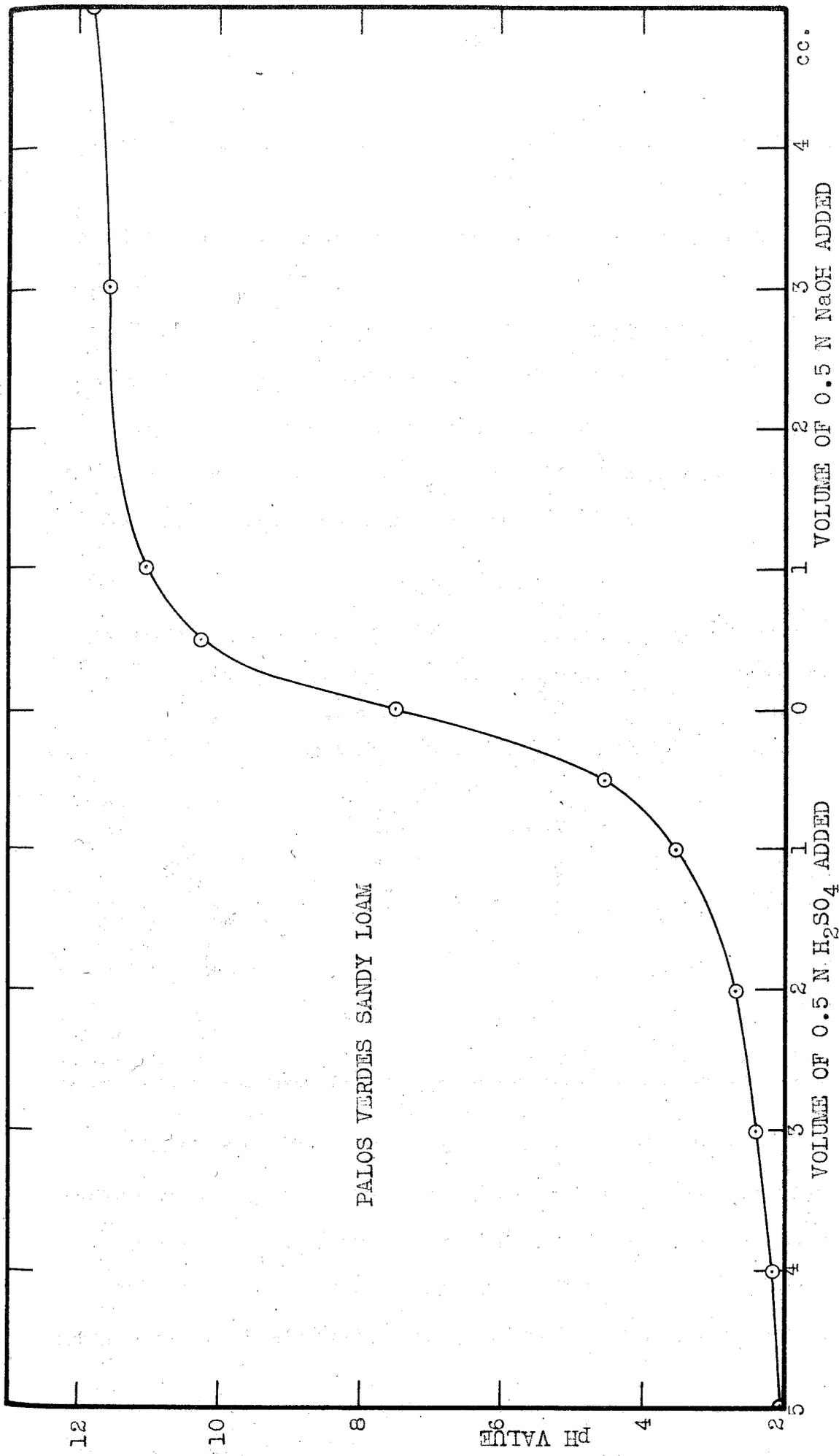


Fig. 7. Buffer Curve for Palos Verdes Sandy Loam.

set by Pierre (76) for tolerance of most agricultural plants.

The specific buffer capacities were determined by dividing the total buffer capacities by the change in pH produced.

The total carbonates were determined gasometrically by the official method of the A.O.A.C. (77).

The pH of the 1 to 5 soil suspension, the total and specific buffer capacities, and the total carbonate contents of the soils studied are presented in Table 2.

TABLE 2

THE BUFFER CAPACITY TOWARD ACID OF THE SOILS STUDIED

Soil	pH of 1 to 5 soil suspension	Total buffer capacity ml.	Specific buffer capacity ml.	Total carbonate content %
Superstition sand	9.1	18.3	4.3	1.18
Gila sandy loam (virgin)	9.0	24.0	5.7	0.77
Gila sandy loam (cult.)	8.9	39.3	9.6	1.10
Pima clay loam	8.3	24.0	7.0	0.85
Laveen loam	8.5	136.3	36.8	3.51
Palos Verdes sandy loam	7.5	1.0	0.4	Trace

The correlation between carbonate content and specific buffer capacity is very pronounced on soils having the same or similar texture, as in the case of the three sandy loams and the one loam. The relationship in the sand, however, does not correlate with the values for the other five soils.

This suggests the influence of colloid content on the buffer capacity of soils.

It should be noted that all of these soils have an alkaline reaction in 1 to 5 suspension, and that none has a pH below 8.3 with the single exception of the Palos Verdes sandy loam. That a correlation is lacking between pH and either total or specific buffer capacity, however, is very marked. Whereas the Laveen loam has a pH value only 0.2 unit higher than the Pima clay loam, its total buffer capacity exceeds that of the Pima clay loam nearly six times. On the other hand, the cultivated Gila sandy loam has a pH value only 0.2 unit lower than the Superstition sand but its total buffer capacity is over twice as great as that of the sand.

The total and specific buffer capacities of the six soils cover an extremely wide range, the Palos Verdes sandy loam having minimum values of 1.0 ml. and 0.4 ml., respectively for the two buffer values, and the Laveen loam having maximum values of 136.3 ml. and 36.8 ml. for the corresponding buffer capacities.

The differences between the virgin and cultivated Gila sandy loam, as shown by the physical constants, are again of interest. Although the two soils have pH values only 0.1 unit apart, the cultivated soil has a total buffer capacity over 1.6 times that of the virgin, a specific buffer capacity nearly 1.7 times as great, and a total carbonate

content 1.43 times that of the uncultivated soil. If it were true that the carbonates were alone effective in buffering these soils, the ratio of carbonate in the two soils would be the same as the ratios of the buffer capacities. This emphasizes the effect of the clay and organic matter content, as already noted above.

The difference in total carbonate content of the virgin and cultivated soil, lying adjacent and arising from the same parent material, emphasizes the effect of cultivation and cropping on the properties of a virgin soil.

B. Rate of Nitrification of Ammonia and Other Nitrogen Compounds in Desert Soils

Replicate 100-gram soil samples were weighed into glass tumblers with close-fitting covers. The weighed samples were divided into four equal groups and treated as follows: (1) check or untreated, (2) ammonia-treated, (3) ammonium sulfate-treated, and (4) urea-treated. The fertilizers were added in solution form in amounts calculated to put 30 mg. of nitrogen into each 100-gram soil sample, i.e., the nitrogen was added in each case in a concentration of 300 p.p.m. on the basis of the air-dry soil.

The ammonium sulfate solution was prepared by dissolving 56.6004 g. of the pure salt in 2 l. of freshly distilled water; the urea solution by dissolving 25.7208 g. of the compound in a like volume. This gave a concentration in

each solution such that 5 ml. contained the desired 30 mg. of nitrogen. The ammonia solution was standardized to 0.2141 N, a concentration sufficient to deliver 30 mg. of nitrogen in each 10 ml. of solution. The weaker solution was used in the case of ammonia because of the high vapor pressure of ammonia solutions. The solution used had a vapor pressure of 2.79 mm. of ammonia at 25° C., calculated from the equation (79)

$$PNH_3(\text{mm.}) = 12.9C(1+0.046C).$$

This relationship holds up to $C = 1.6$ mols of ammonia per liter of solution.

Since ammonia is fixed very rapidly by the soil, there is little danger of its being lost from the samples once applied, and the danger of loss from the standardized fertilizer solution was further reduced to a minimum by keeping it at all times in a closed delivery system.

The study of ammonium sulfate is of interest, first, because large amounts of this compound are being used in fertilization programs in Arizona, and, second, because it has been used by a number of previous investigators in studies of nitrification and of ammonium ion utilization by crop plants.

Urea was selected as the organic fertilizer because it is also a commonly used fertilizer and can be obtained chemically pure.

Following the application of the fertilizer solutions, sufficient freshly distilled water was added to bring the moisture content of the samples up to 70% of their water-holding capacities; the covered tumblers were weighed immediately. Moisture losses were compensated for at frequent intervals during the study, but the losses were considerably reduced by the close-fitting covers.

The samples were kept at a temperature of $30 \pm 1^{\circ}$ C. in a thermostatically controlled incubator throughout the course of the study.

Periodically, duplicate samples for each of the four treatments were removed from the incubator, and the pH value determined on the moist soil. The entire 100 grams of soil were then transferred to large, wide-mouthed bottles, and 500 ml. of freshly distilled water added to each. The 1 to 5 suspensions were shaken for 20 minutes, removed from the shaker, and the pH value determined on the suspensions.

Next, the total soluble salts were determined by the use of a conductivity bridge. The samples, still in the large, wide-mouthed bottles, were then aspirated for 15 minutes with CO_2 , according to the procedure devised by McGeorge and Breazeale (84) as a measure of the "available phosphate" in alkaline calcareous soils.

Following aspiration with CO_2 , the suspensions were filtered, and the clear filtrates were analyzed for nitrate by the official (77) phenoldisulfonic acid method. The

phosphates were likewise determined on the filtrates, using a slight modification of the method of Zinzadze (78). Experiment showed that neutralization of the extract in the presence of alpha dinitrophenol was unnecessary. Also, the gum arabic suggested by Zinzadze for use as a protective colloid with stannous chloride was omitted; fresh aqueous stannous chloride solution of the same strength recommended by Zinzadze was prepared for each set of analyses.

The nitrate and phosphate values, as well as nitrites which were determined in subsequent phases of the study using the official (77) alpha naphthylamine-sulfanilic acid method, were read with a Cenco photometer. Calibration curves were prepared with solutions of purified, recrystallized salts.

All of the analytical data in this entire study are reported as parts per million on the basis of the air-dry soil.

Throughout this study the analyses have been made on duplicate soil samples -- never on single samples or on aliquots from the same soil sample. This should be borne in mind in interpreting the results which follow; likewise, that the curves although continuous do not represent a continuous change in any one sample.

With the exception of the pH data obtained in connection with the buffer determinations presented above, the pH measurements presented in the remainder of this thesis were

made on the moist soil. This was done because the latter value is thought to be more significant than that of the 1 to 5 suspension.

The total soluble salt determinations were discontinued in later phases of this study, and are not being reported because their accurate interpretation would involve more complete chemical data than could be obtained at the time the measurements were made.

Because of the various measurements mentioned above, it was necessary to divide the rate studies into two parts. In the first, Superstition sand, virgin Gila sandy loam, and cultivated Gila sandy loam were used, and incubation was continued for 107 days; in the second, Pima clay loam, Laveen loam, and Palos Verdes sandy loam were used, and incubation was continued for 40 days.

Results of the Nitrification Rate Studies

The relative rates of nitrification of the three fertilizers and the soil nitrogen in the soils studied are given in Figures 8, 9, 10, 11, 12, and 13 respectively.

Two facts are strikingly apparent in Figure 8. First, nitrates did not begin to accumulate for nearly 30 days with any of the fertilizers, and then with only the ammonium sulfate and urea. Second, during the entire 107 days of incubation the ammonia was not oxidized to the nitrate form; in fact, the ammonia curve drops almost immediately below the

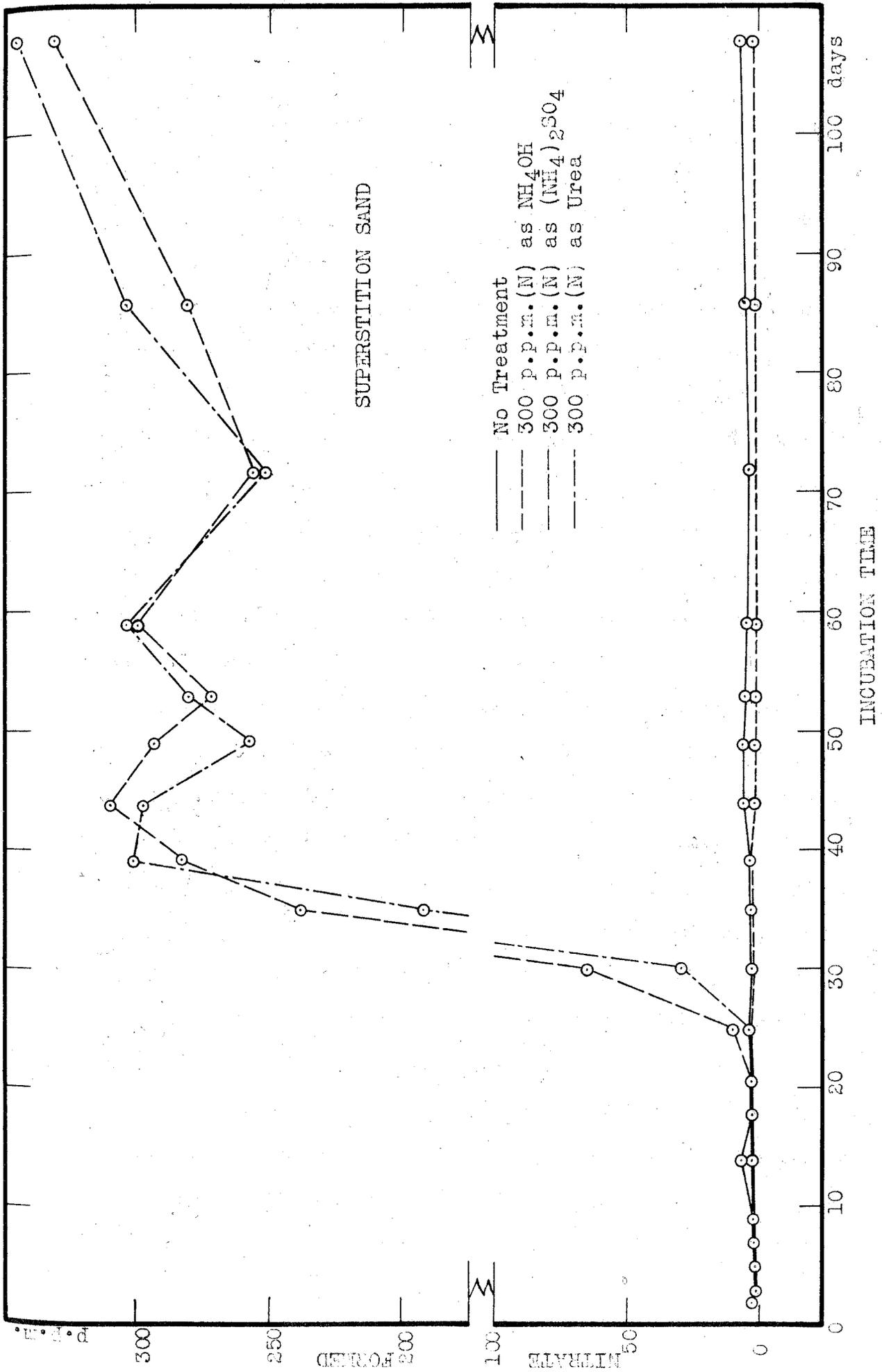


Fig. 6. Relative Rates of Nitrification in Superstition Sand Under Different Treatments.

soil nitrogen curve and remains there throughout the experiment. This indicates that the ammonia application to a sandy soil represses what little nitrification would normally have occurred. The effect of the ammonia is believed to be related to the change in pH induced in the soil, which, as indicated by Figure 2, has a very low buffering capacity toward hydroxyl ion. The addition of ammonia raised the pH of this soil to an initial value of 9.5.

That the failure of the ammonia to nitrify may be due to the large amount of nitrogen applied, as Halversen (83) has suggested for arid, sandy soils, is refuted by the complete oxidation of the ammonium sulfate and urea in about six weeks' time.

Justification for the opinion that high alkalinity retards nitrification is based largely upon the pH-nitrate relationships revealed by a study of Figures 14 to 28, inclusive. The results indicate that there is a threshold pH range above which nitrification will not occur, and to which the pH value of alkaline soils must be reduced in some manner before nitrification will occur.

In only two instances out of the 133 in which nitrification was observed did the phenomenon occur at pH values above 7.6 to 7.7. The two exceptions are found in Figures 19 and 23: in the former, one of duplicate samples of ammonia-treated virgin Gila sandy loam showed an increase in nitrates at pH 7.9; in the latter case, nitrates were first observed

in the ammonia-treated cultivated Gila sandy loam sample at pH 7.8. There is no reason to believe that these two pH values are in error except for the preponderant evidence that nitrates did not form in any of the other samples until the pH value had been decreased to 7.6 to 7.7. It should be remembered, also, that the pH determinations here discussed were made on the moist soil, and not on soil suspensions where greater reproducibility of the measurements is possible.

Attention was first directed to the possibility of a threshold pH for nitrate formation in alkaline soils by the anomalous behavior of duplicate samples in the ammonia-treated virgin Gila sandy loam, Figures 9 and 19. Although nitrates first began to accumulate in the ammonium sulfate and urea treated samples at the end of about two weeks, no nitrates were noted in the ammonia-treated samples until the 49th day, and then only in one of the duplicate samples. Since duplicates had been in good agreement in all previous instances, it was at first assumed that an error had crept into the technic employed with one of the samples. Particular care was accordingly taken with the corresponding pair of samples on the 53rd day of incubation; but again an irregularity was noted between samples. Similar irregularity was noted throughout the course of the experiment: on the 59th day nitrates were not detected in either sample;

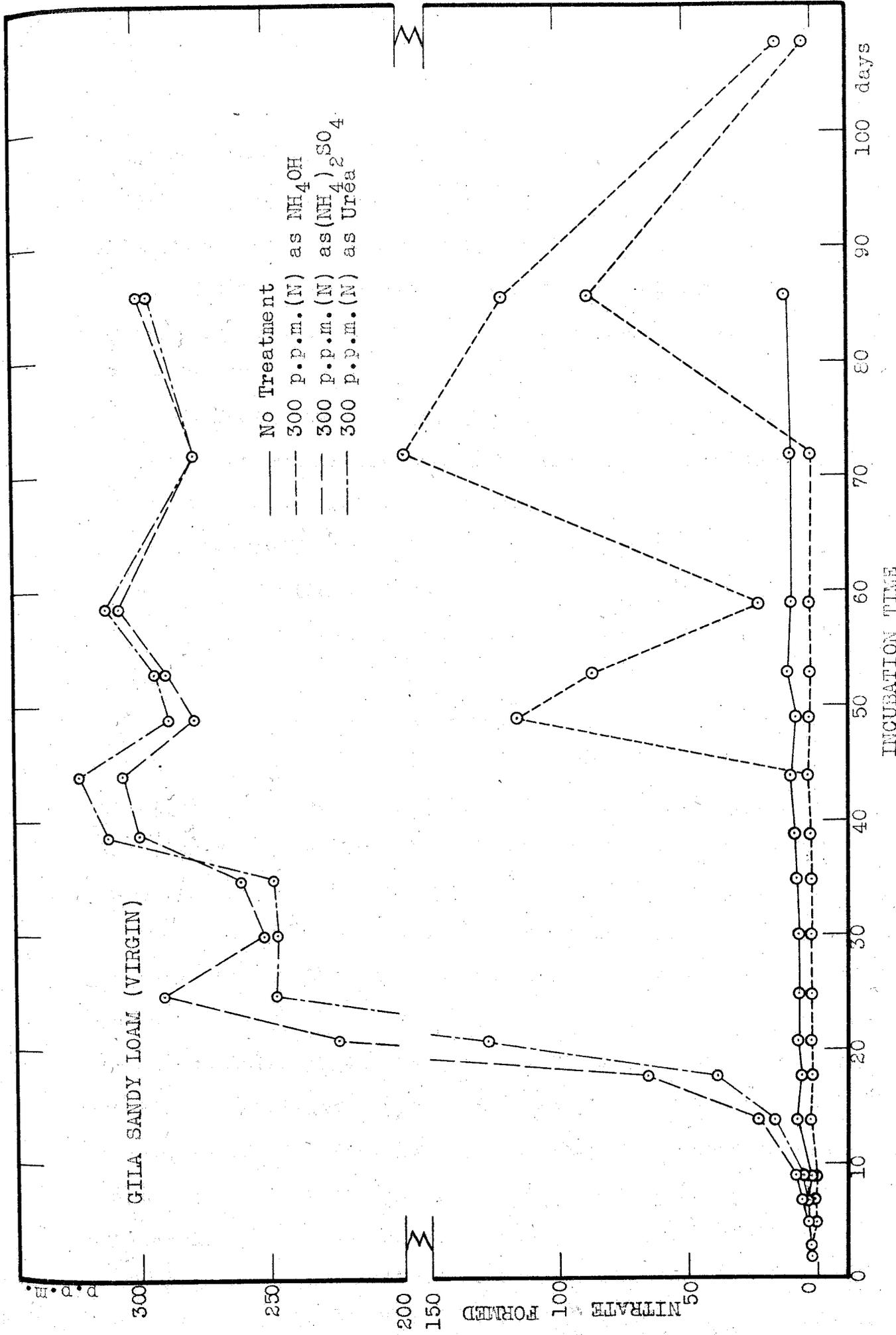


Fig. 9. Relative Rates of Nitrification in Virgin Gila Sandy Loam under Different Treatments.

on the 72nd day they were found in one sample only; on the 86th day in both samples; and on the 107th day, neither sample showed any nitrates to be present.

An examination of Figure 19, representing the relationship between changes in pH and rate of nitrification, shows that on the 49th day when the first nitrates appeared in one of the duplicate samples, the pH of this sample was 7.9. (This is one of the two exceptions to a threshold pH range of 7.6 to 7.7 noted previously.) In all other instances the failure of the ammonia to nitrify in this soil correlated with a pH above 7.6 to 7.7; similarly, all samples which contained nitrates had pH values below 7.6 to 7.7.

Figures 10, 11, and 12 reveal that in all three soils which have been under the plow for a number of years -- cultivated Gila sandy loam, Pima clay loam, and Laveen loam, respectively -- the three fertilizers were completely nitrified. Only in the Gila sandy loam was the initial rate of nitrification of the ammonia-treated samples appreciably less than that of the ammonium sulfate- and urea-treated samples.

In the Palos Verdes sandy loam, Figure 13, the ammonium sulfate nitrified less rapidly than the urea or ammonia -- the reverse of the situation in the other soils tested. This phenomenon probably results from the fact that the reaction is lowered by the ammonium sulfate to a point which hinders

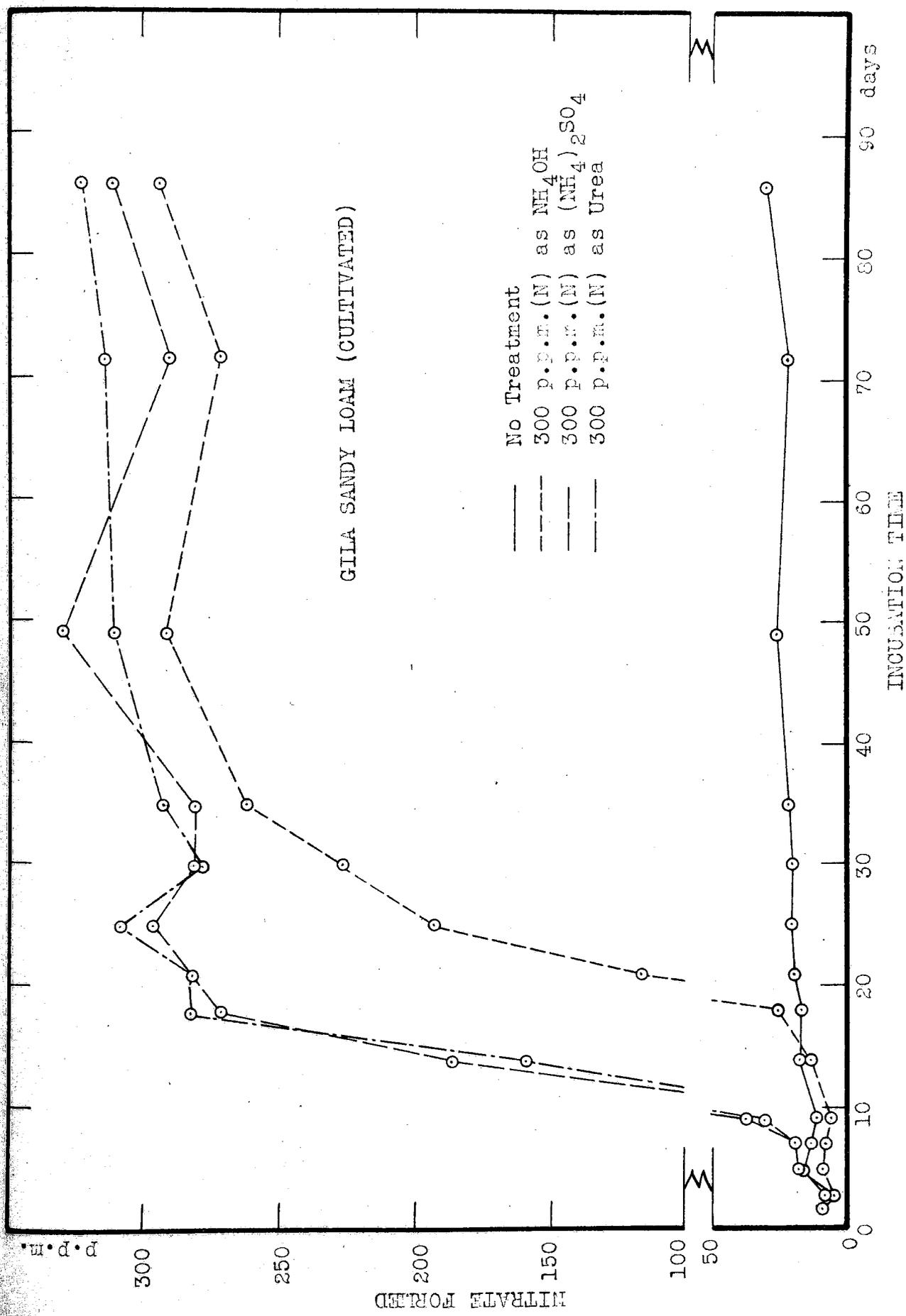


Fig. 10. Relative Rates of Nitrification in Cultivated Gila Sandy Loam Under Different Treatments.

NITRATE FORMED

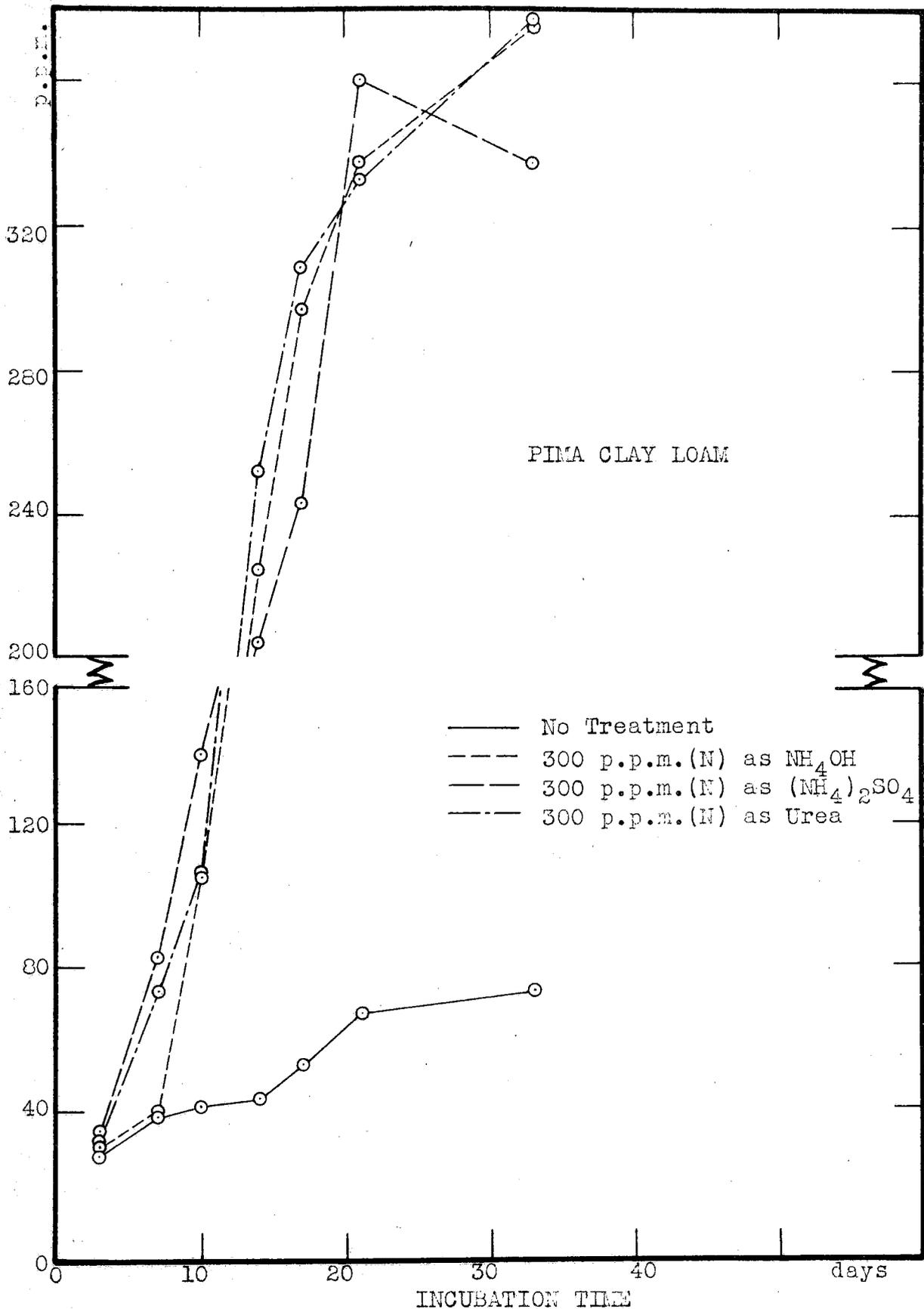


Fig. 11. Relative Rates of Nitrification in Pima Clay Loam under Different Treatments.

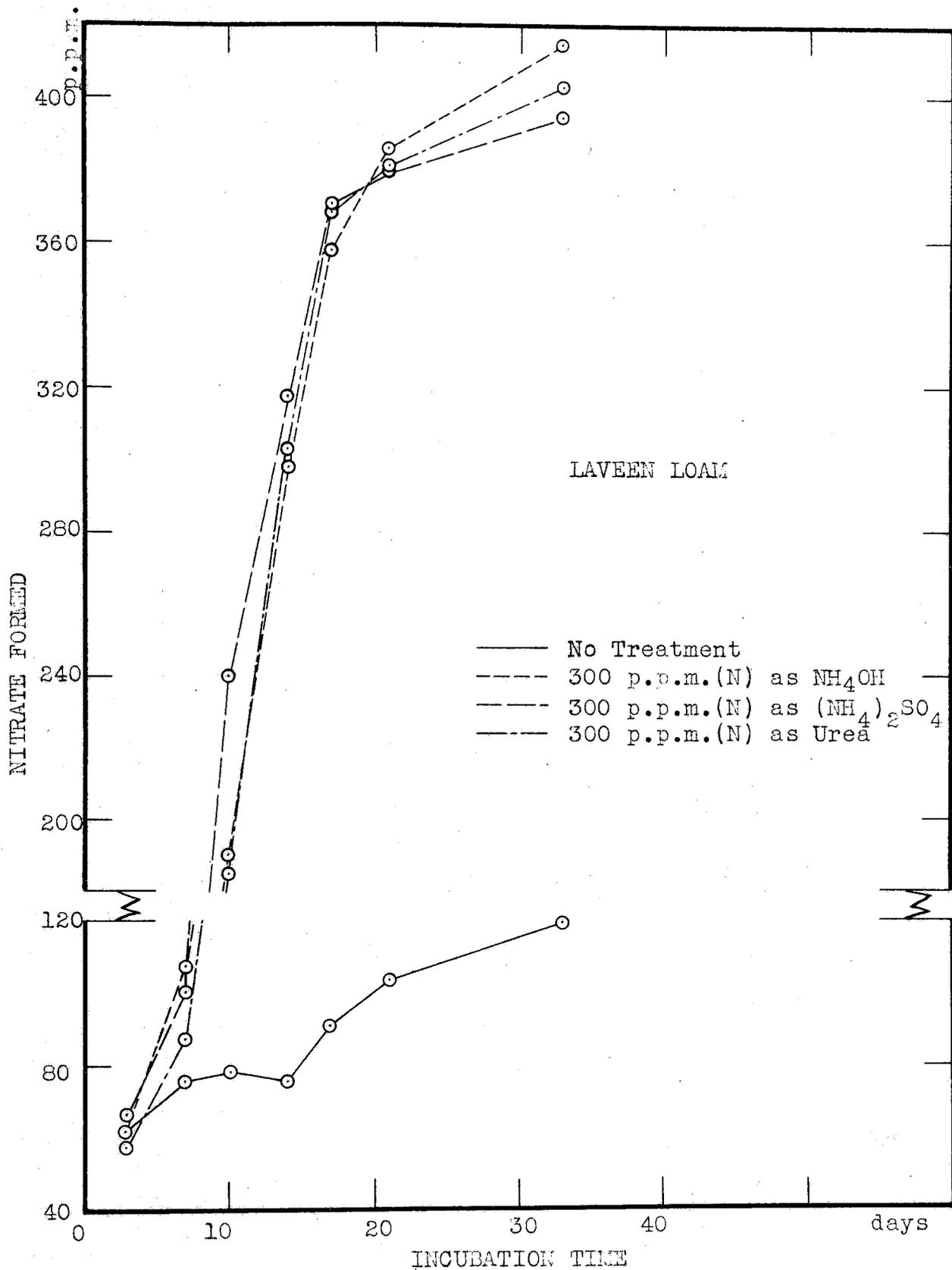


Fig. 12. Relative Rates of Nitrification in Laveen Loam under Different Treatments.

the nitrification process. At the end of 40 days of incubation and with but one-third of the ammonium nitrogen oxidized, the pH had already dropped to the low value of 4.65, as shown in Figure 28, which is very close to the value of pH 4.4 reported by Waksman (58) and Humfeld and Erdman (59) as the limiting reaction for nitrification on the acid side of neutrality.

Attention is called to the fact that the scale along the ordinate axis of Figure 13 has been expanded considerably beyond that in Figures 11 and 12 to show more clearly the relative rates of nitrification; hence, the extent of nitrification is not so great even in the case of urea as might be thought at first glance. Although the urea-treated sample of Palos Verdes sandy loam is initially only slightly above the threshold pH range of 7.6 to 7.7, at the end of forty days incubation -- when nitrification was complete in the three cultivated soils -- less than 60% of the urea had been oxidized to nitrate. This difference probably is due to a less extensive and less active micropopulation in the uncultivated soil.

Another point of interest with respect to Figure 13 is that all three fertilizers began to show an accumulation of NO_3 at approximately the same time. This is particularly significant when considered with the relative rates of nitrification of urea and ammonia. If hydrolysis of urea occurs to give carbon dioxide and ammonia before nitrification begins,

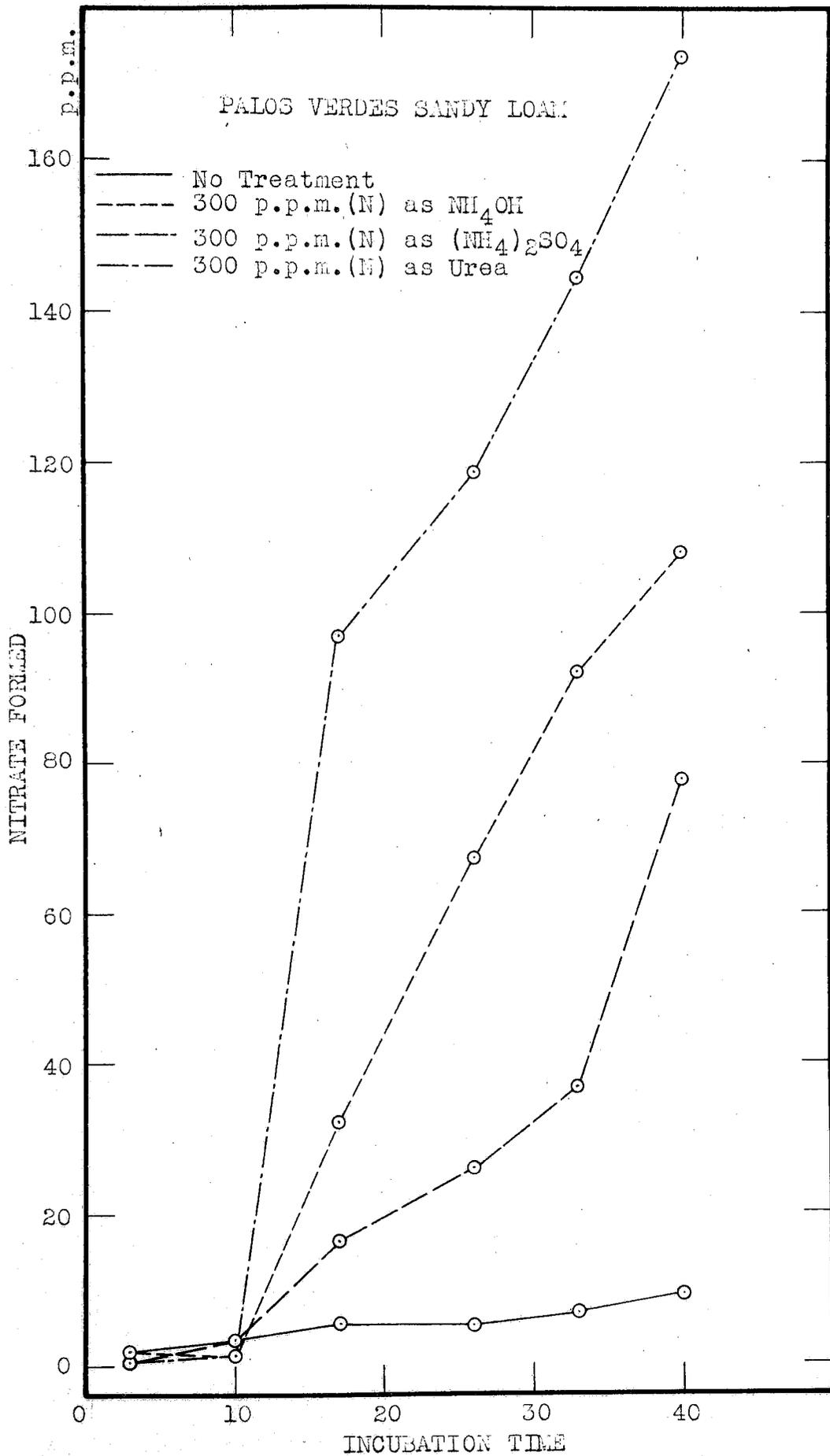


Fig. 13. Relative Rates of Nitrification in Palos Verdes Sandy Loam under Different Treatments.

as is generally assumed, the oxidation process should be limited by the equilibrium of the hydrolysis. On the other hand, if ammonia is directly added to the soil, nitrification is not limited by any previous hydrolysis equilibrium, and once the pH has been lowered to the threshold pH range of 7.6 to 7.7, the rate should be expected to be more rapid in the case of ammonia than in that of urea. This suggests that urea does not hydrolyze to produce ammonia prior to nitrification, but may form some intermediate product having a state of oxidation lying between ammonia and nitrite, hence can show a faster nitrification rate than ammonia once the threshold pH has been reached. Since no experimental evidence is at hand to support the supposition, it remains pure speculation at this time; but it still remains to be proved that urea could not, under soil conditions, hydrolyze to give formaldehyde and hydroxylamine.

The foregoing discussion has assumed that in every instance the threshold pH above described is characteristic for the nitrifying bacteria occurring in the alkaline soils of Arizona, and is the same irrespective of the fertilizer used. So far this assumption appears to be borne out, for with the three fertilizers used, the threshold pH range of 7.6 to 7.7 appears valid.

The writer is not unaware that the slower nitrification rate of ammonia in this more acid soil may be due also to the intermediate formation of ammonium nitrite, and the subsequent

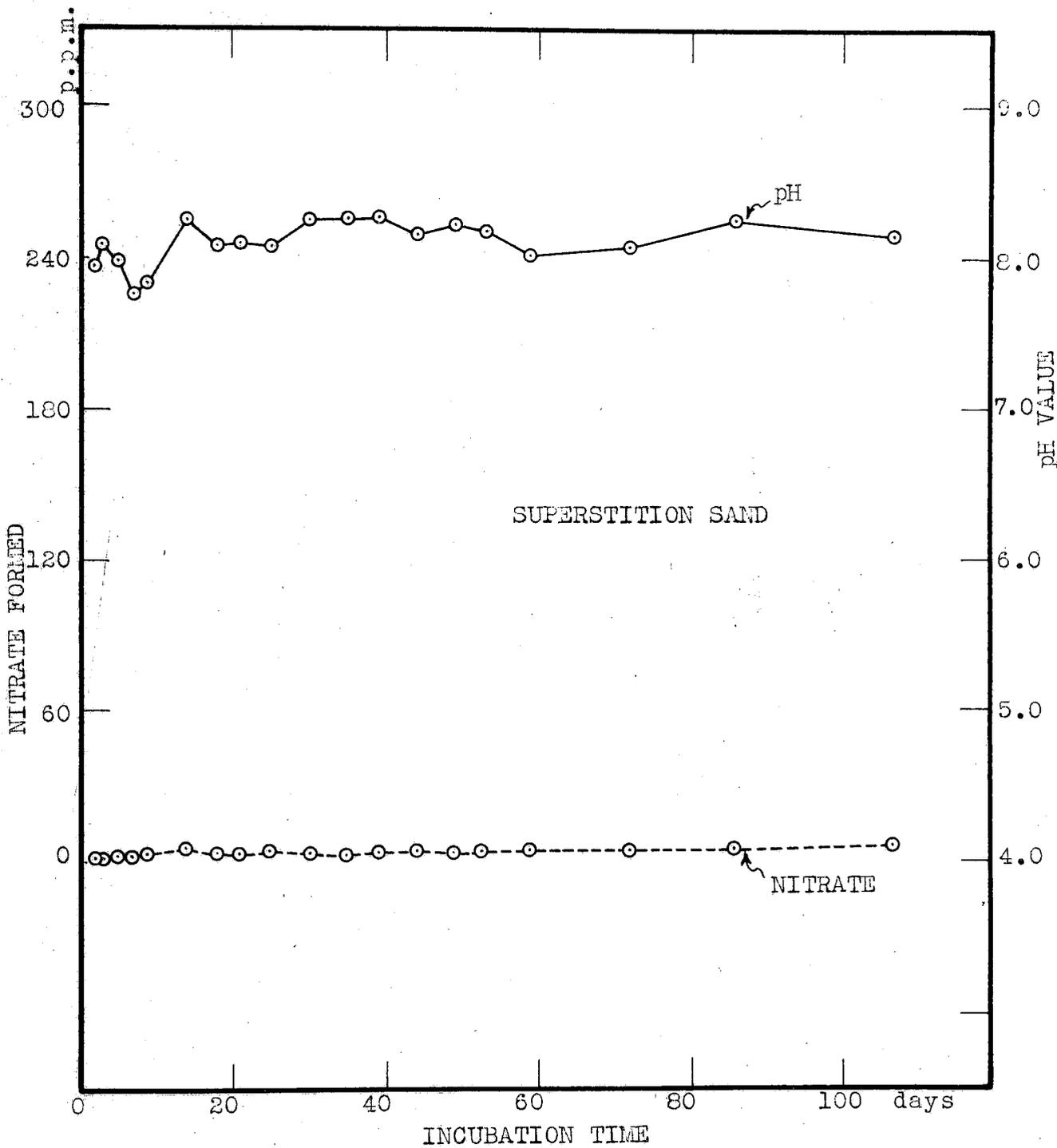


Fig. 14. Relative Changes in pH and Nitrate Content in Untreated Superstition Sand.

loss of nitrogen by its decomposition into nitrogen and water, as suggested by Dhar and Mukerji (80).

pH Expressed as "Active" Acidity or Alkalinity

In order better to appreciate the magnitude of the initial effect when Superstition sand is treated with ammonia, as shown in Figure 15, the increase in hydroxyl ions can be expressed as a multiple of the hydroxyl ion concentration at pH 7.0. Wherry (81) advocated the use of "active" acidity and alkalinity and this arithmetical means of indicating magnitudes represented by various pH values. He assigned a value of 0 to pH 7.0. Pettinger (82) presented a chart similar to that of Wherry for use in explaining pH, but used the more logical value of 1 for pH 7.0. The values of active alkalinity above pH 7.0 calculated on the basis of Pettinger's scale are assembled in Table 3.

TABLE 3

pH VALUES IN TERMS OF "ACTIVE" ALKALINITY

pH	Active alkalinity	pH	Active alkalinity
7.00	1.00	8.25	32.50
7.05	1.45	8.30	37.00
7.10	1.90	8.35	41.50
7.15	2.35	8.40	46.00
7.20	2.80	8.45	50.50
7.25	3.25	8.50	55.00
7.30	3.70	8.55	59.50
7.35	4.15	8.60	64.00
7.40	4.60	8.65	68.50
7.45	5.05	8.70	73.00
7.50	5.50	8.75	77.50
7.55	5.95	8.80	82.00
7.60	6.40	8.85	86.50
7.65	6.85	8.90	91.00
7.70	7.30	8.95	95.50
7.75	7.75	9.00	100.00
7.80	8.20	9.05	145.00
7.85	8.65	9.10	190.00
7.90	9.10	9.15	235.00
7.95	9.55	9.20	280.00
8.00	10.00	9.25	325.00
8.05	14.50	9.30	370.00
8.10	19.00	9.35	415.00
8.15	23.50	9.40	460.00
8.20	28.00	9.45	505.00
		9.50	550.00

The magnitude of the effect is exactly the same for a pH value a given number of units below pH 7.0 as for the same number of units above that value. For this reason, a separate table has not been included to show "active" acidity values.

Attention is called to several interesting values expressed in this manner. The initial pH value in the ammonia-treated Superstition sand, Figure 15, represents a concentration of hydroxyl ions 550 times that present at pH 7.0. The rapid drop in pH in this case reduced the hydroxyl ion concentration to only about 50 times the amount existing at pH 7.0; however, since the threshold range for nitrification appears to be at pH 7.6 to 7.7 -- at approximately seven times the hydroxyl ion concentration at pH 7.0 -- the concentration of hydroxyl ions was still about seven times greater than the nitrifying organisms could tolerate.

The virgin and cultivated Gila sandy loam samples are likewise of interest in this connection. In the virgin samples, the initial pH of the ammonia-treated samples, Figure 19, represents an hydroxyl-ion concentration of about 325 times that at pH 7.0, whereas the initial pH in the corresponding sample of cultivated soil, Figure 23, represents an hydroxyl-ion concentration of only about 75 times that at pH 7.0.

The effects of ammonium sulfate and urea on the pH value are not marked in any of the soils, except in the Palos Verdes sandy loam, Figure 28, in which the ammonium sulfate produced a drop from an initial pH value of 6.19 to 4.65 on the 40th day of incubation; this pH corresponds to a concentration of hydrogen ions nearly 400 times the hydrogen-ion

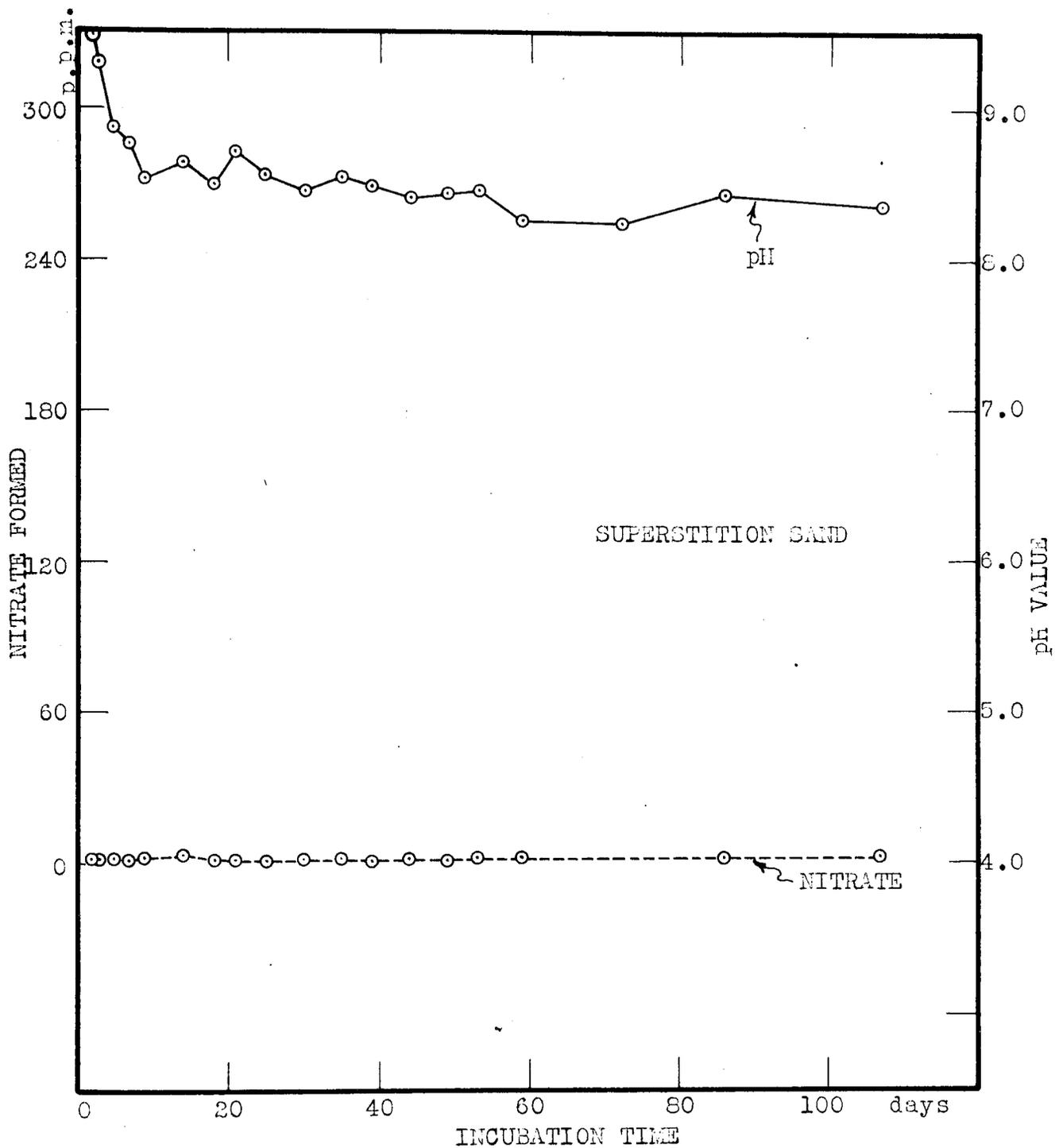


Fig. 15. Relative Changes in pH and Nitrate Content of Superstition Sand Treated with 300 p.p.m. of Nitrogen as Ammonia.

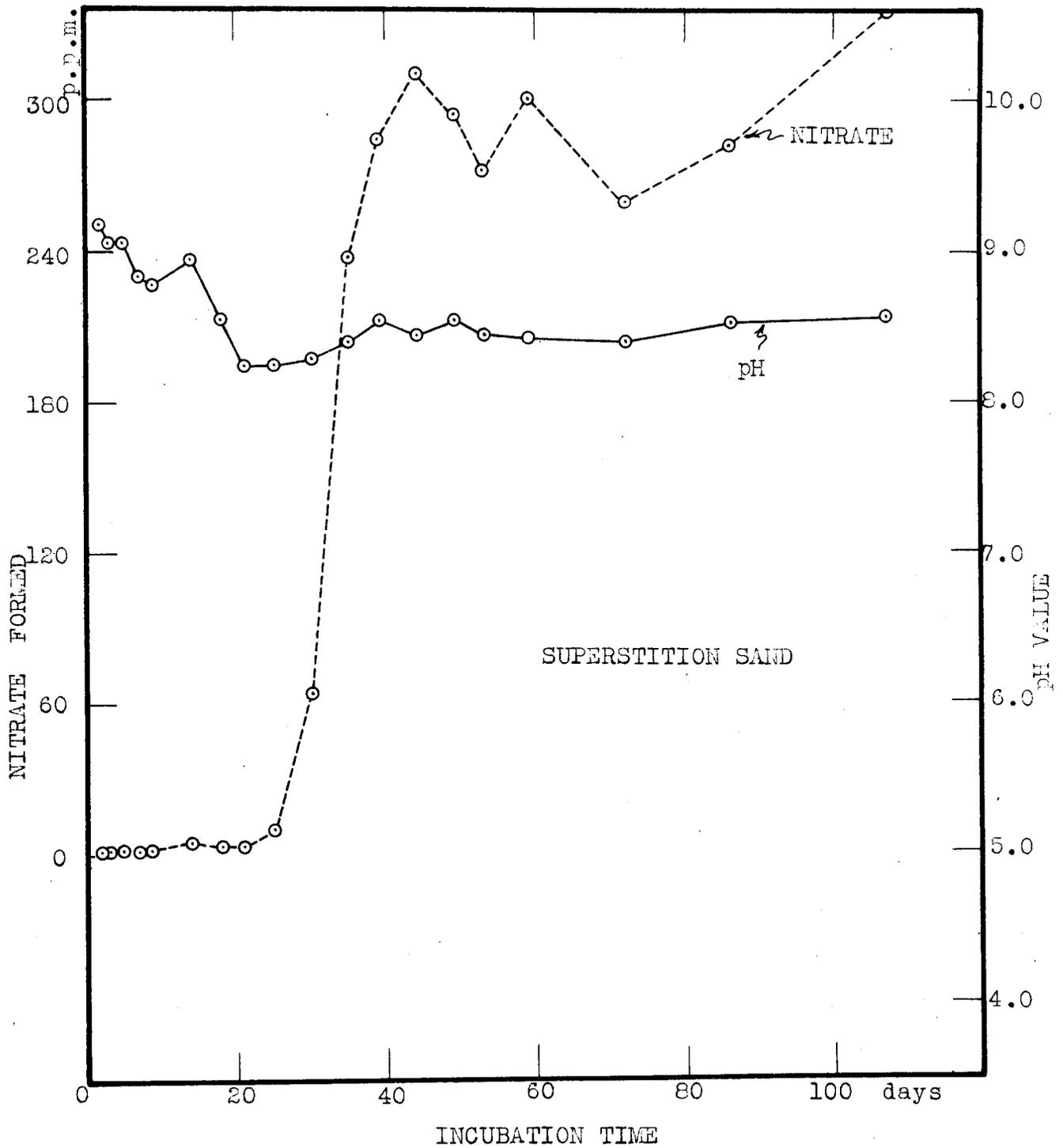


Fig. 16. Relative Changes in pH and Nitrate Content of Superstition Sand Treated with 300 p.p.m. of Nitrogen as Ammonium Sulfate.

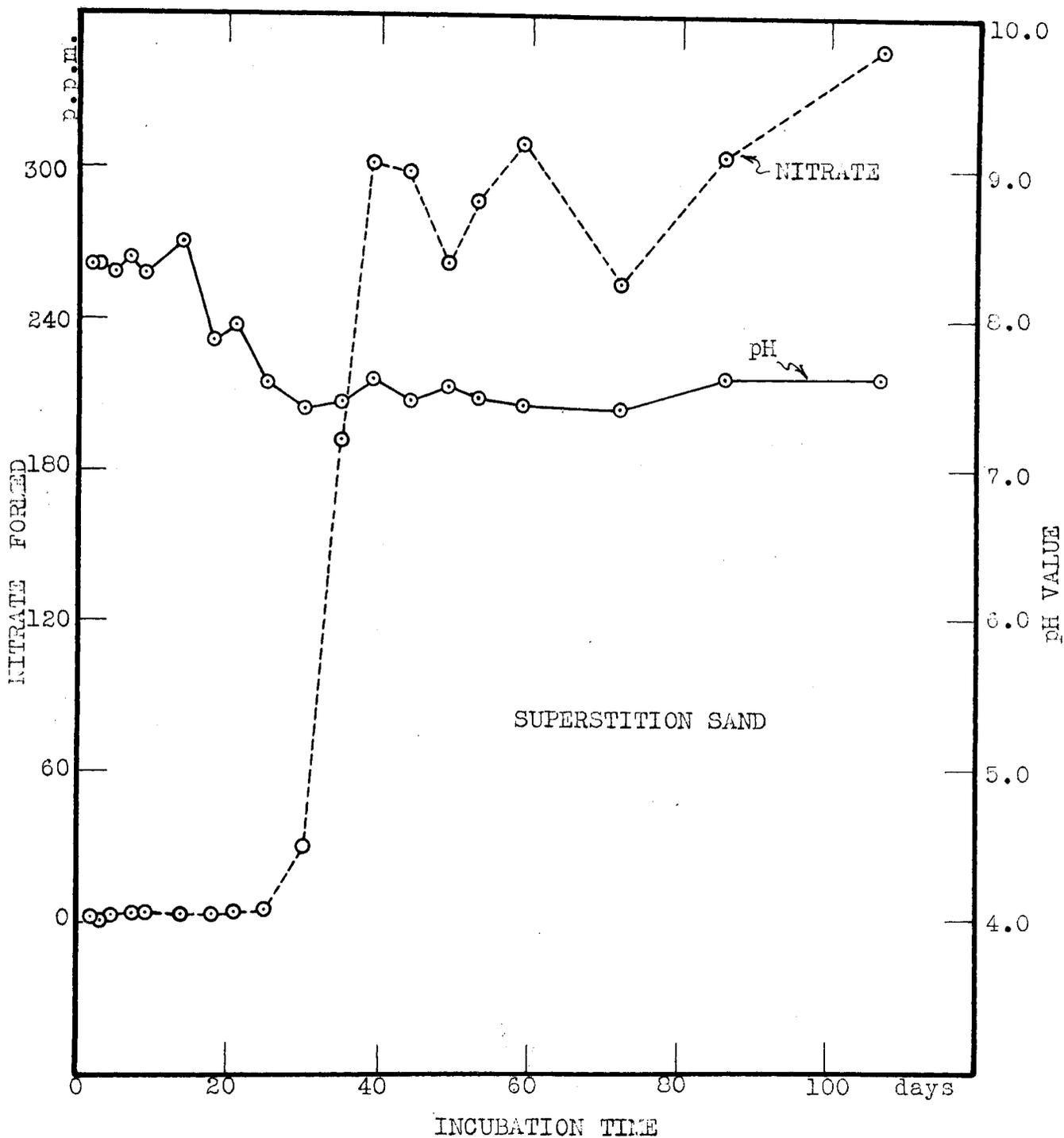


Fig. 17. Relative Changes in pH and Nitrate Content of Superstition Sand Treated with 300 p.p.m. of Nitrogen as Urea.

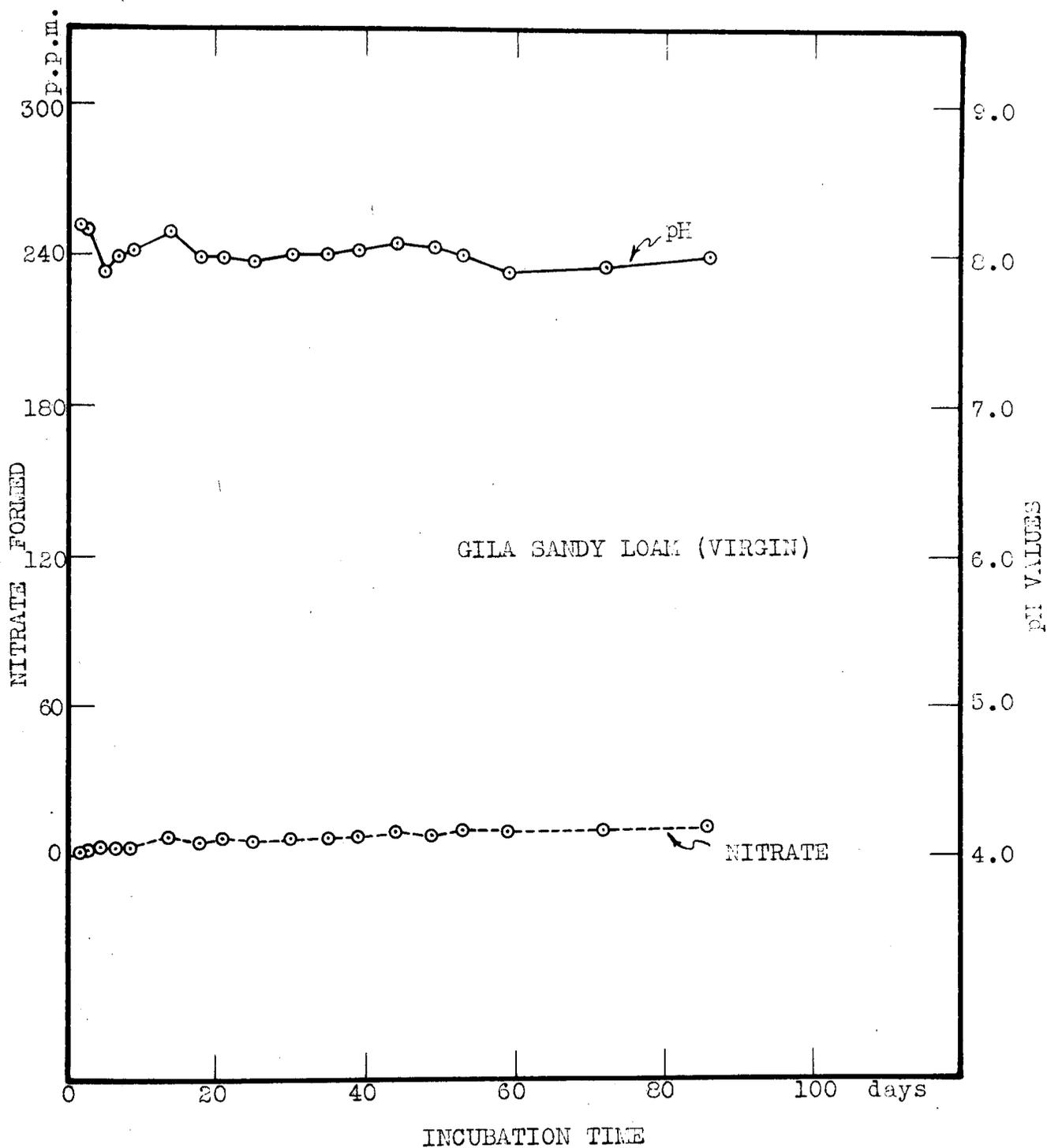


Fig. 18. Relative Changes in pH and Nitrate Content in Untreated Gila Sandy Loam (Virgin).

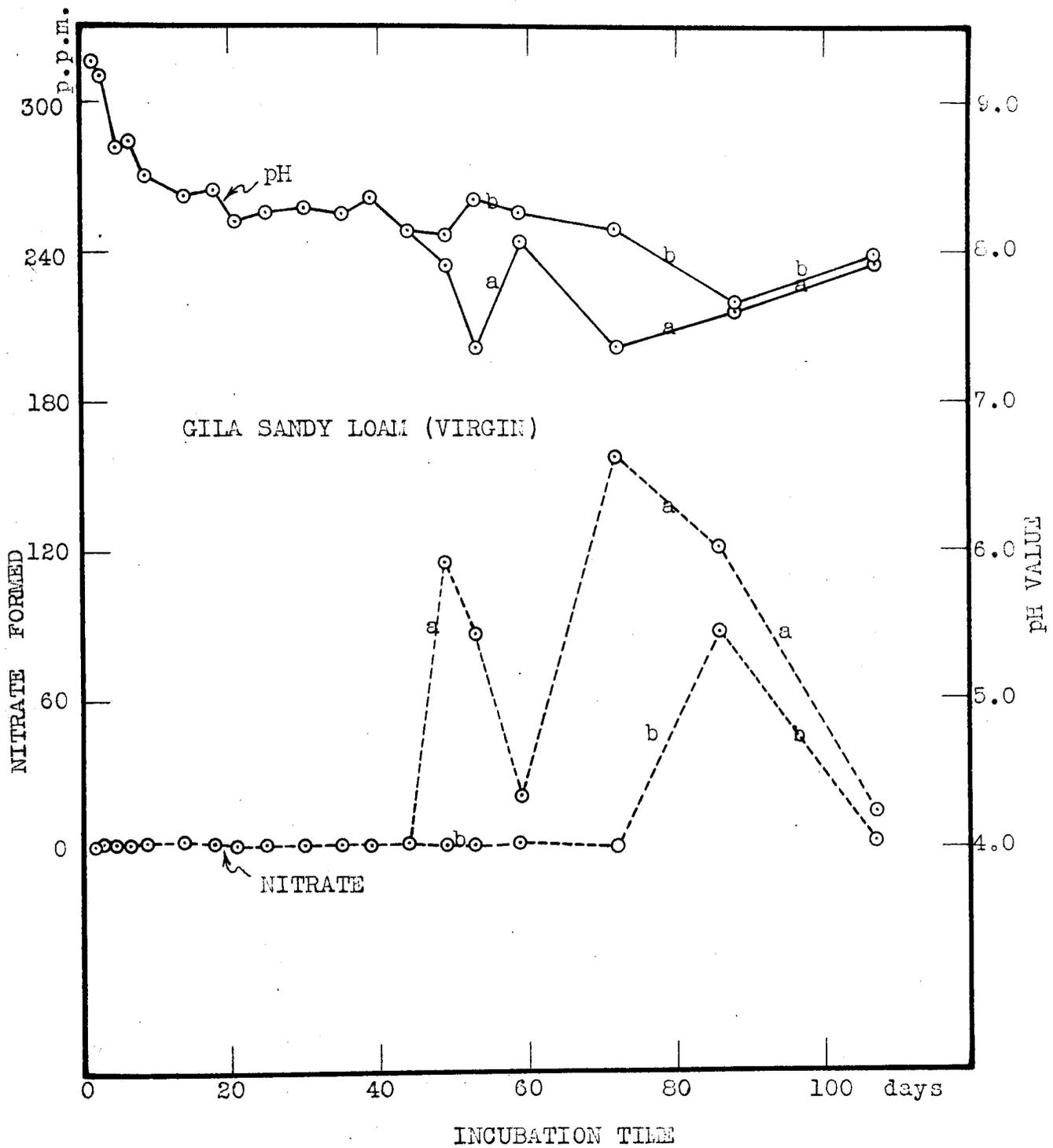


Fig. 19. Relative Changes in pH and Nitrate Content of Virgin Gila Sandy Loam Treated with 300 p.p.m. of Nitrogen as Ammonia.

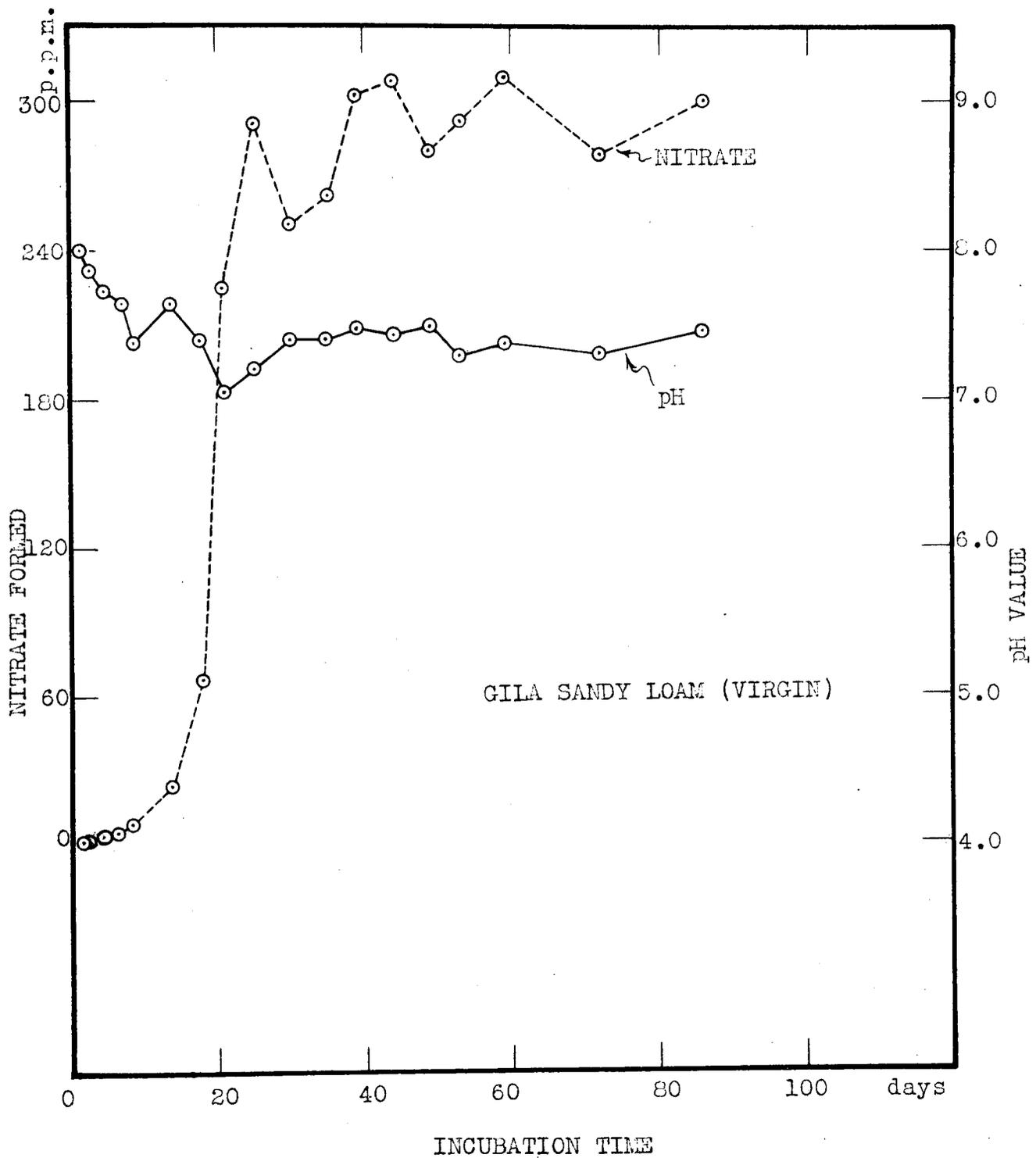


Fig. 20. Relative Changes in pH and Nitrate Content of Virgin Gila Sandy Loam Treated with 300 p.p.m. of Nitrogen as Ammonium Sulfate.

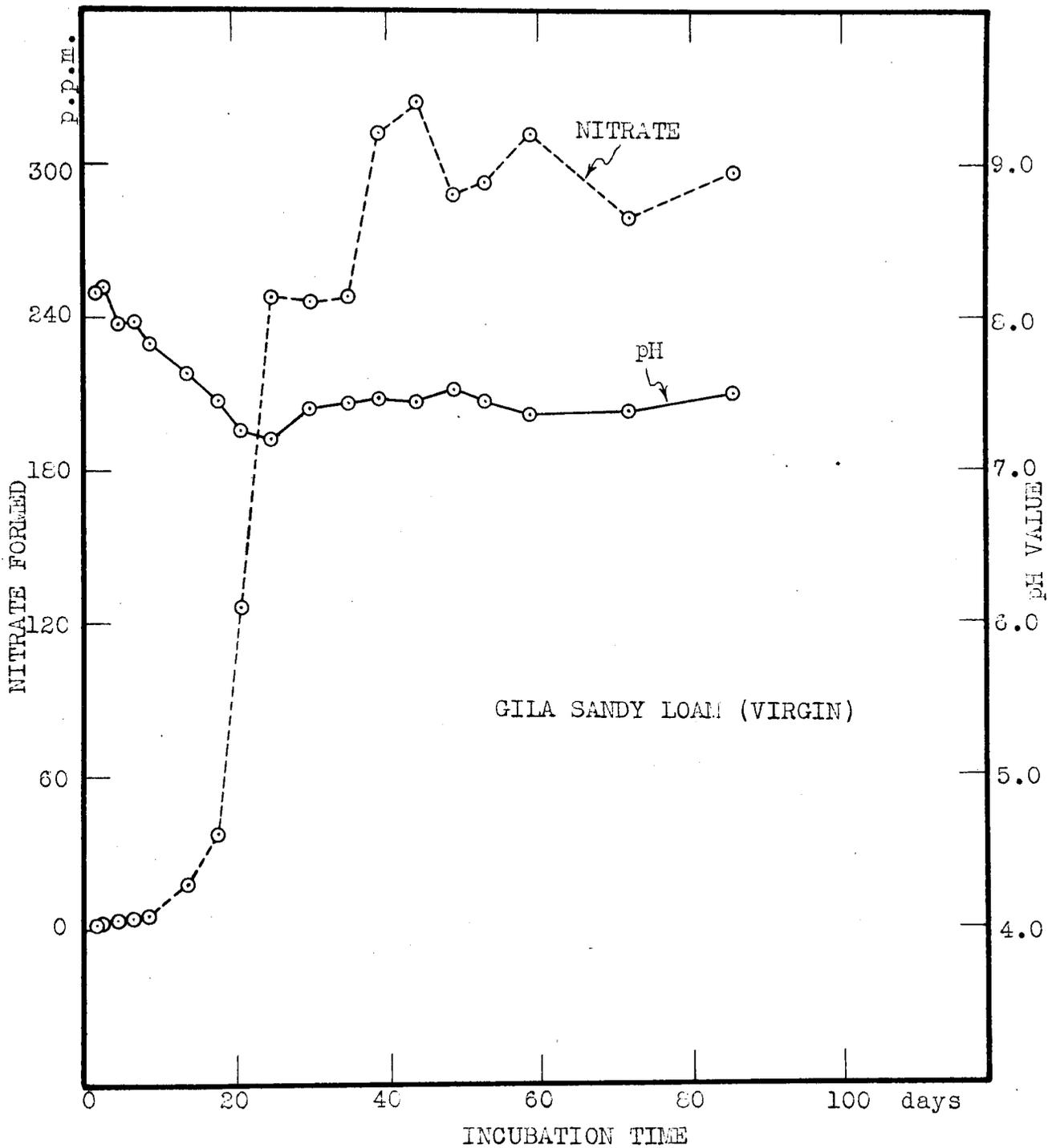


Fig. 21. Relative Changes in pH and Nitrate Content of Virgin Gila Sandy Loam Treated with 300 p.p.m. of Nitrogen as Urea.

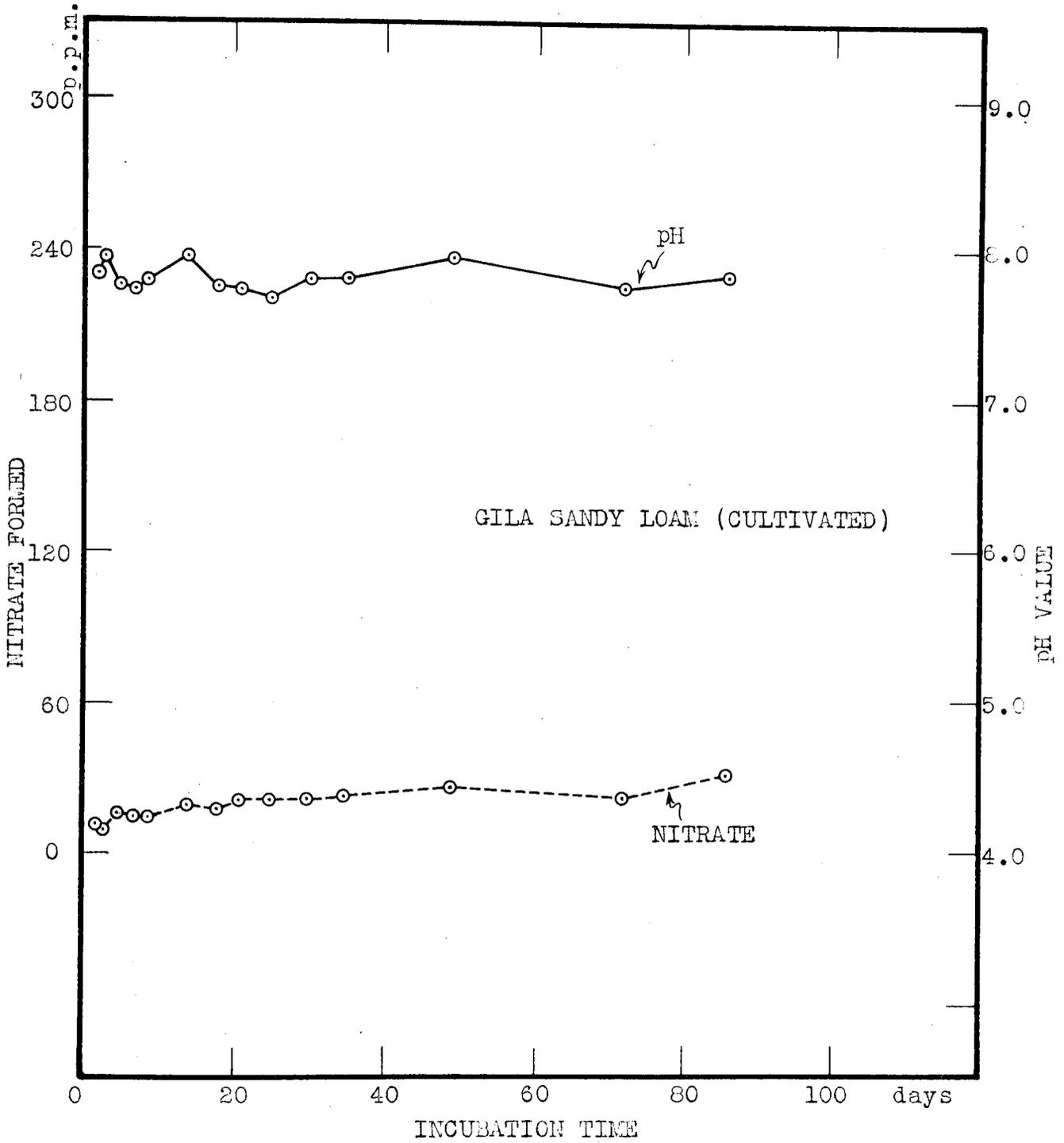


Fig. 22. Relative Changes in pH and Nitrate Content in Untreated Gila Sandy Loam (Cultivated).

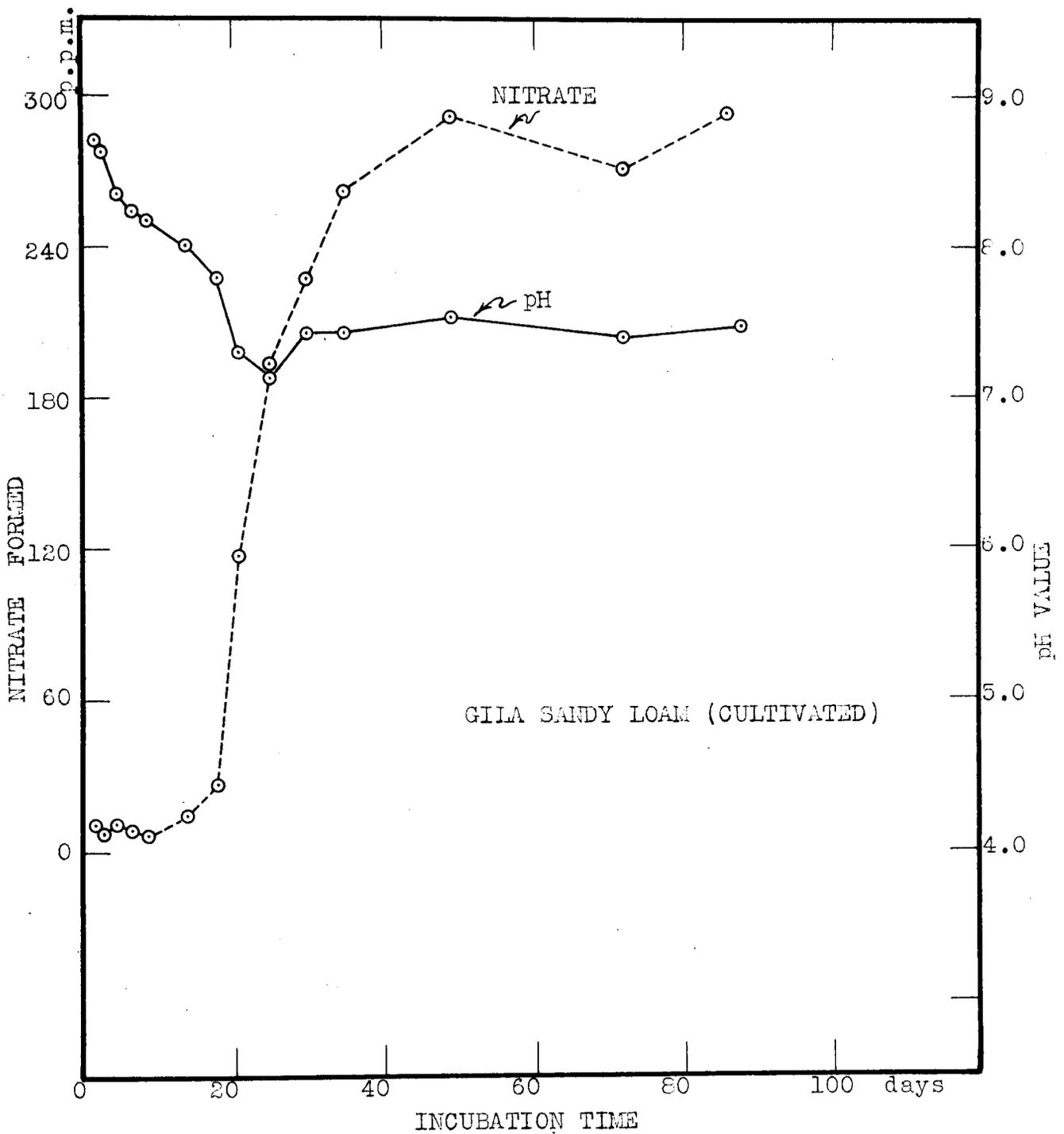


Fig. 23. Relative Changes in pH and Nitrate Content of Cultivated Gila Sandy Loam Treated with 300 p.p.m. of Nitrogen as Ammonia.

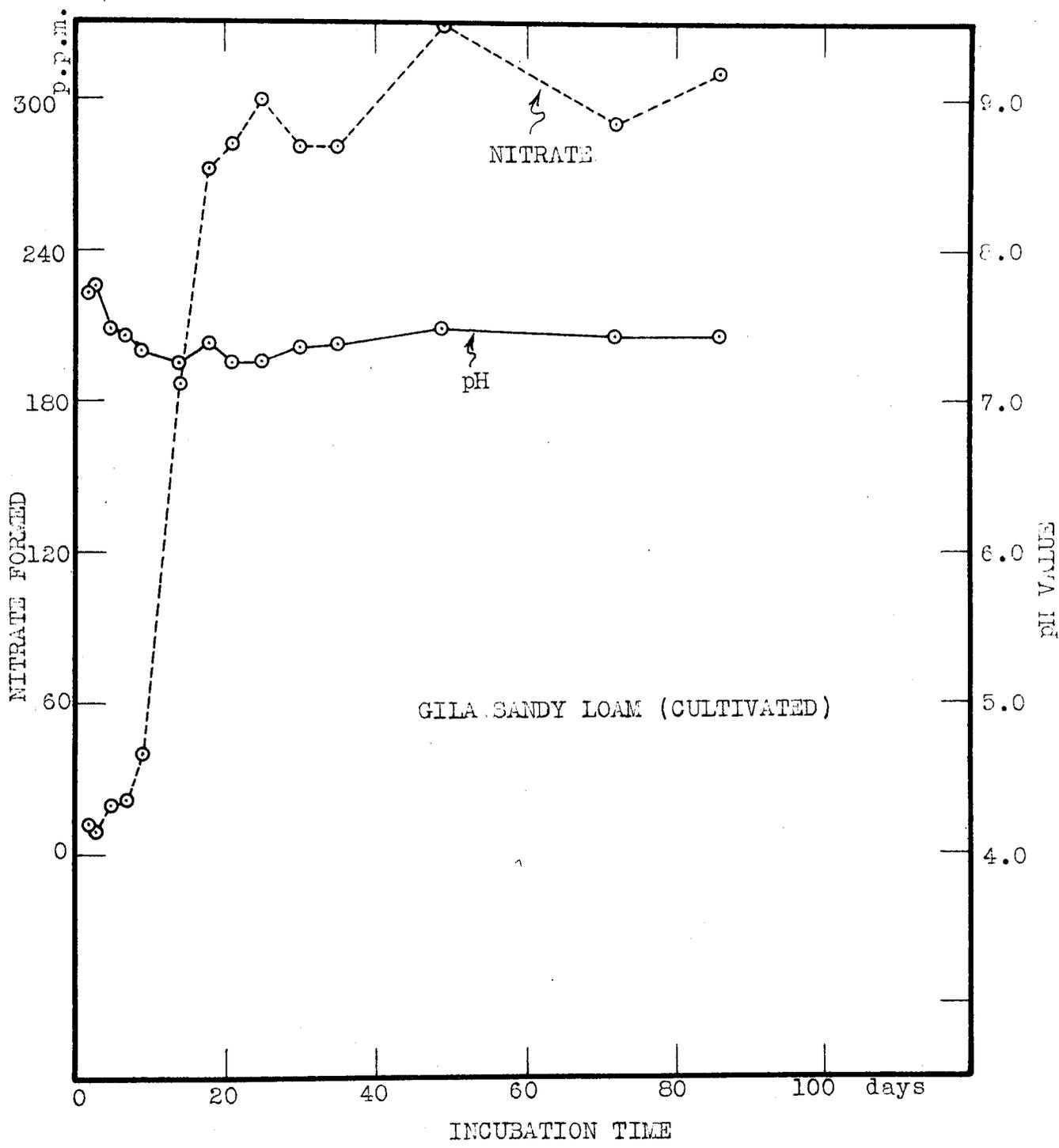


Fig. 24. Relative Changes in pH and Nitrate Content of Cultivated Gila Sandy Loam Treated with 300 p.p.m. of Nitrogen as Ammonium Sulfate.

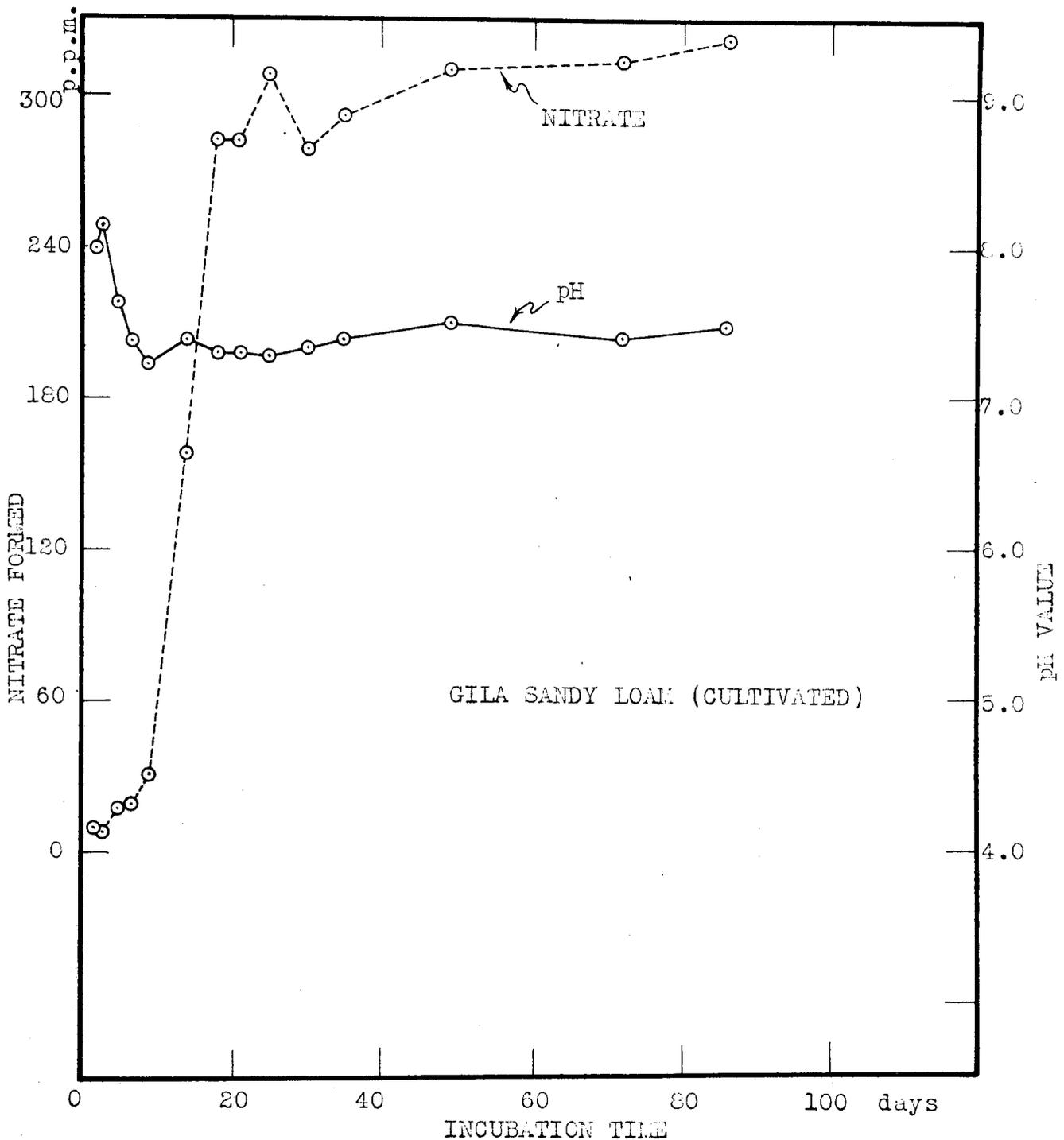


Fig. 25. Relative Changes in pH and Nitrate Content of Cultivated Gila Sandy Loam Treated with 300 p.p.m. of Nitrogen as Urea.

concentration existing at pH 7.0.

The incomplete character of the pH data in Figures 26 and 27 is due to a cracked glass electrode on the pH meter. The limited data presented are in agreement, however, with the threshold pH range of 7.6 to 7.7 before inception of nitrification, which appears to be a general characteristic of this process.

C. Further Evidence Substantiating a Threshold pH Value of 7.65 ± 0.05 for Nitrification in the Alkaline Soils of Arizona

In the preceding portions of this thesis, the process of nitrification has been spoken of in its broad meaning-- including the oxidation of nitrogen through its various intermediate steps from ammonia to nitrate. In this and subsequent sections, however, it will be desirable to distinguish between nitrite formation and nitrate formation. Hence the term nitritification will be used to refer specifically to that phase of the oxidation in which nitrites are formed from nitrogen in a lower stage of oxidation, and nitratification to signify the step in which nitrite is oxidized to nitrate.

The drop in pH prior to nitratification from such high initial values as 8.2, 8.3, 8.7, and even 9.3, as shown in Figures 16, 17, 25, and 21, respectively, is of particular interest in areas of alkaline soils, especially if the

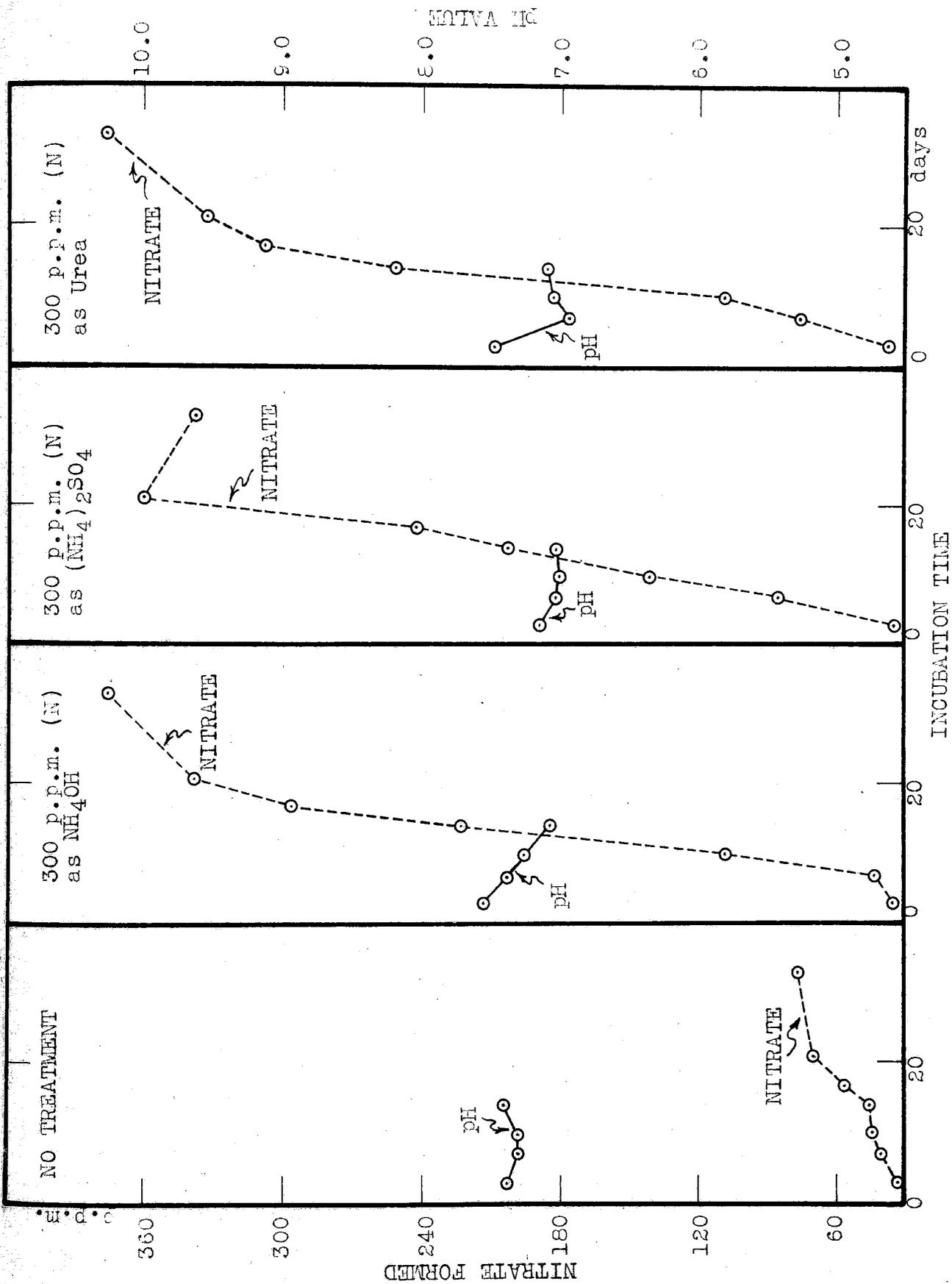


Fig. 26. Relative Changes in pH and Nitrate Content of Pima Clay Loam under Different Treatments.

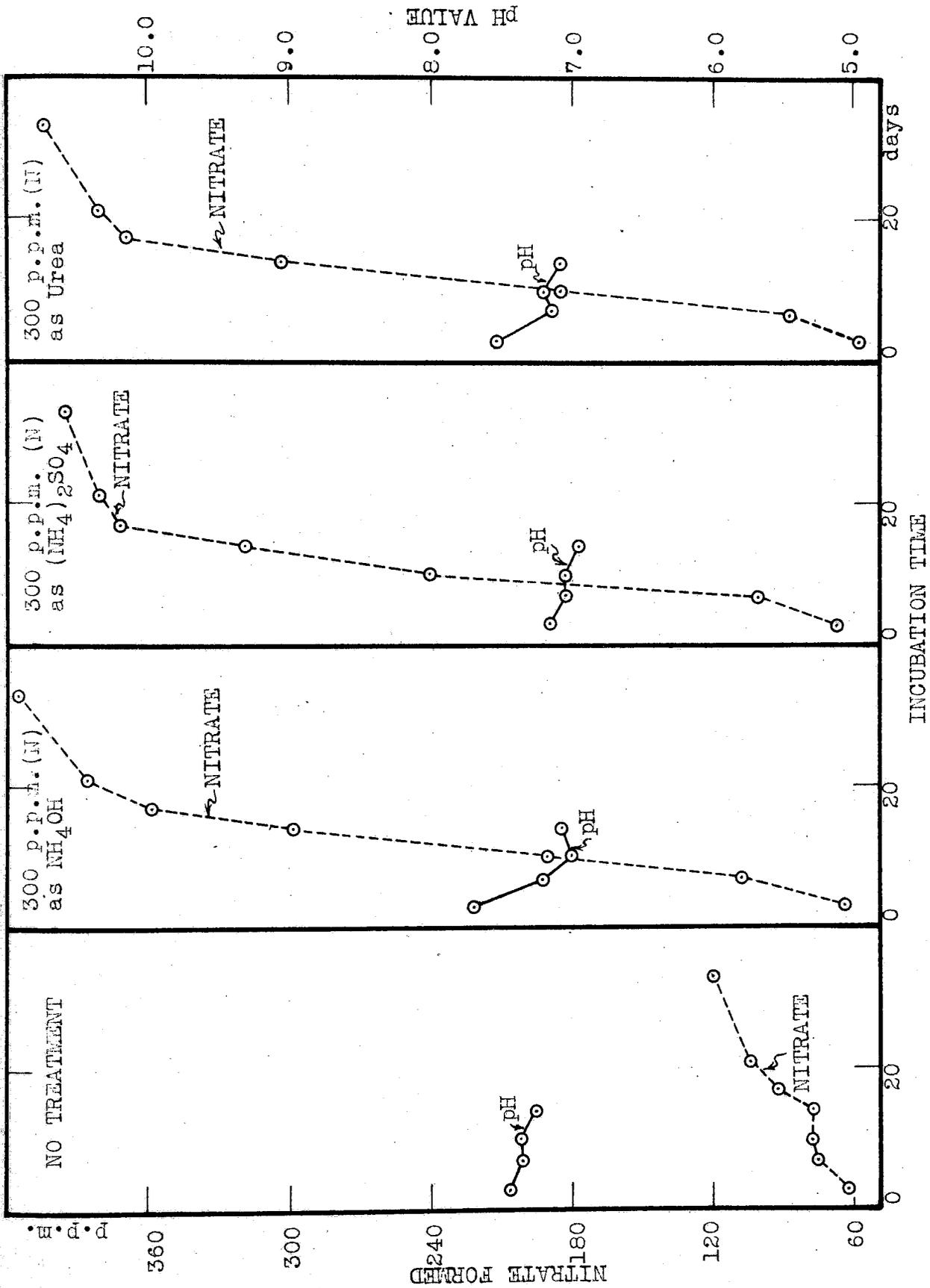


Fig. 27. Relative changes in pH and Nitrate Content of Laveen Loam under Different Treatments.

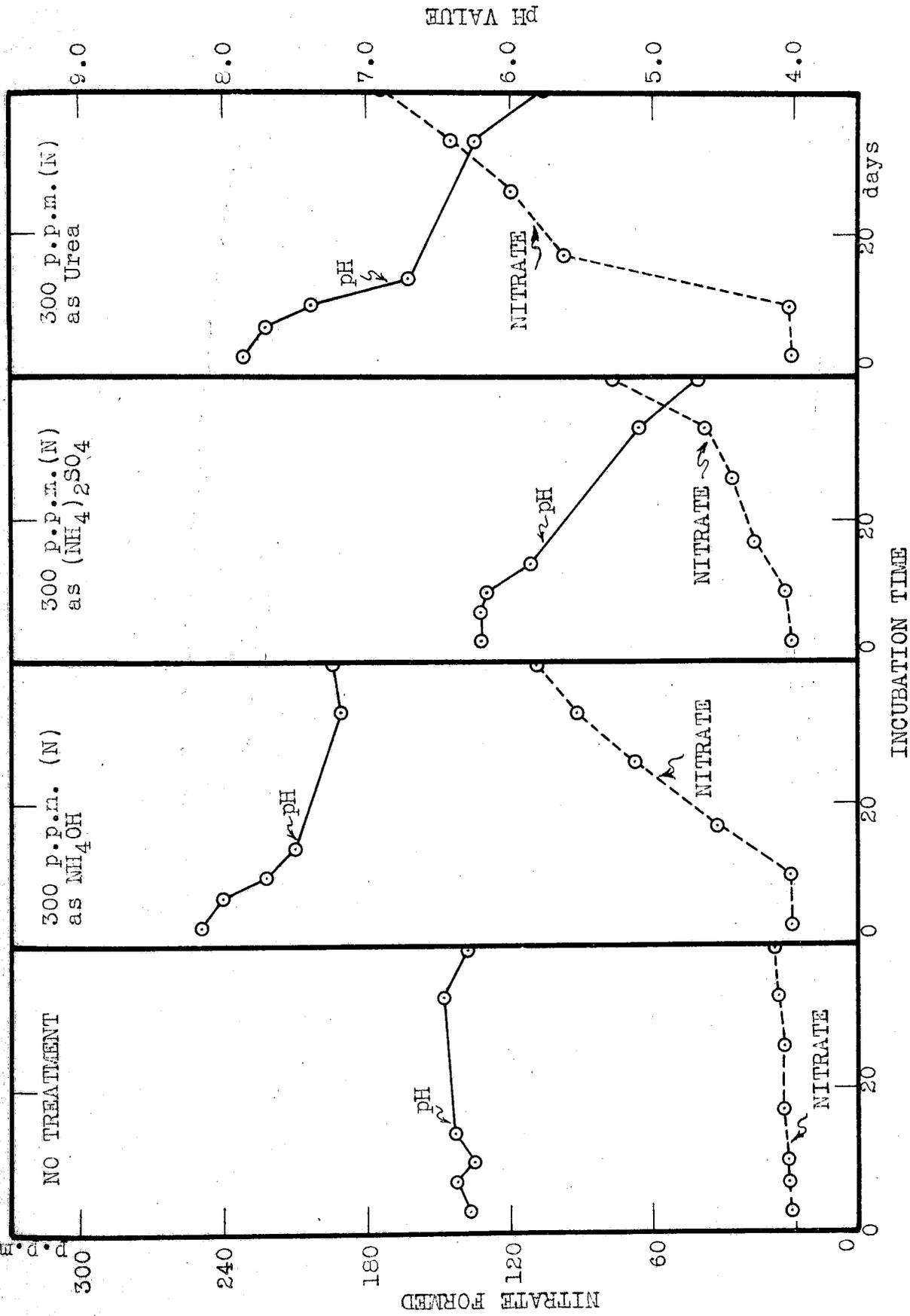


Fig. 28. Relative Changes in pH and Nitrate Content of Palos Verdes Sandy Loam under Different Treatments.

Nitrobacter native to such soils does not function effectively until a threshold range has been reached.

Further, the observation made in this study, namely that a drop in pH occurs prior to nitrification, is unique in view of the fact that it has been generally assumed that a decrease in pH occurs concurrently with nitrification as the result of the formation of nitric acid during the oxidation. For example, Waksman (58) writes:

"In the oxidation of ammonium sulfate, nitric and sulfuric acid are formed. These acids increase the hydrogen-ion concentration of the soil, till a point is reached which becomes injurious to the activity of the nitrifying bacteria."

That the formation of nitric and sulfuric acid does cause a drop in pH is indisputable; but the drop prior to the formation of nitrates, particularly in the case of ammonia and urea fertilization in which the sulfate radical is lacking, must be caused by other compounds than nitric and sulfuric acids.

Albrecht and McCalla (85) noted the effect of the decrease in pH with nitrification upon the base saturation of prepared clay suspensions. They did not indicate, however, whether the replacement of the adsorbed ions was observed prior to, concurrent with, or following the formation of nitrates.

Inasmuch as the microbiological oxidation of ammonia-nitrogen to nitrate-nitrogen is known to occur in a minimum

of two stages of oxidation, and since the drop in pH occurred prior to the detection of nitrates in the samples, it is reasonable to assume that the drop in pH resulted either from the over-all oxidation of ammonia to nitrites, thus



or from the formation of some intermediate product, the formation of which is obscured in this equation.

If the foregoing over-all reaction actually accounts for the changes in pH observed, it seemed likely that nitrites might be detectable in the soil in appreciable amounts as the oxidation proceeded, particularly if a considerable difference existed between the initial pH of the soil and the threshold range. Under normal conditions in arable soils, nitrites are considered to be too transitory, however, to occur in significant amounts.

It was considered of interest, therefore, to study this phase of the ammonia problem in an effort to determine whether the over-all reaction suggested above coincides with the decrease in pH or exceeds it.

In conjunction with this phase of the study, experiments were also carried out to determine (1) the effect of maintaining the pH at a level above the threshold pH value of 7.65 by the addition of saturated calcium hydroxide, and (2) the possibility of inducing nitrite formation in samples fertilized with ammonium sulfate and urea by starting incubation at an initially high pH value.

Procedure and Methods

In order to permit greater variation of other factors, only one soil, the cultivated Gila sandy loam, was used in this phase of the study. It seemed advisable to use a soil which nitrified ammonia rapidly and in which both an abnormally high salt content and a high or low buffering capacity were known to be absent. The three cultivated soils studied in the first series of experiments had exhibited the highest nitrification rates, as shown in Figures 10, 11, and 12; but the Laveen loam had an abnormally high specific and total buffer capacity toward acid, and the Pima clay loam contained an unusually high concentration of salts, particularly chlorides. The cultivated Gila sandy loam, on the other hand, had neither of these undesirable qualities, and was moderately well buffered; it seemed, therefore, to be a suitable choice.

The specific procedure was as follows:

(1) Influence of initial pH on the rate of nitrate formation and the build up of nitrites. Replicate 100-gram samples of the soil were weighed into glass tumblers with close-fitting covers, as in the preceding study, and were then divided into five major groups which were treated as follows: (A) an untreated sample; (B) addition of 10 ml. of ammonia solution containing 30 mg. of nitrogen plus 10 ml. of saturated calcium hydroxide solution; (C) addition of

10 ml. of ammonia solution plus 10 ml. of freshly distilled water; (D) addition of 10 ml. of ammonia solution plus 10 ml. of 0.5 N sulfuric acid; and (E) 10 ml. of ammonia solution plus 10 ml. of 1 N sulfuric acid. The purpose of these treatments was to fix the initial pH value of the soil at different levels with respect to the threshold pH value of 7.65.

Since 20 ml. of solution moistened the samples to approximately 70% of the water-holding capacity, this volume was used to expedite the addition of the solutions. Further, to facilitate the additions, the solutions which were to be added to the same type of sample were combined. In order to insure rapid and even distribution of the added liquids throughout the soil mass, each 100-gram soil sample to be moistened was emptied out of the tumbler onto a clean sheet of paper. The sample was then divided roughly into quarters with a spatula. Five ml. of the solution to be used was then put into the empty tumbler, and a one-fourth portion of the soil sample added to it; a second 5 ml. of the solution was added to the tumbler, and a second one-fourth portion of the soil, etc. In this manner the initial pH of the soil was made as uniform as possible throughout the entire sample and the danger of ammonia fixation in the upper layers of the soil largely eliminated.

As in the previous work, the weights of the moistened

samples were recorded immediately after wetting for purposes of restoring lost moisture; similarly, the samples were incubated at $30 \pm 1^{\circ}$ C.

For analysis, duplicate samples of each treatment were removed from the incubator and handled exactly as described under the procedure for the determination of nitrification rate, with the addition that nitrites were determined on an aliquot of the filtrate (77).

(2) The effect of maintaining the pH above the threshold value of 7.65. Twenty-six 100-gram samples of the cultivated Gila sandy loam were fertilized with 30 mg. of nitrogen as ammonia in 20 ml. of solution; the solution was added in 5 ml. portions as described above. The samples were weighed and were put into the incubator without the close-fitting covers. This practice was followed to allow evaporation to proceed sufficiently rapidly so that moisture could be restored with a saturated solution of calcium hydroxide, pH 12.10, before the pH had time to drop to the threshold value.

Duplicate samples were taken for analysis. The pH values were determined on the moist soil at 70% of the water-holding capacity. Nitrates, nitrites, and phosphates were analyzed for in the filtrate from the 1 to 5 suspension, prepared and treated with CO_2 gas as already described.

The measurement of pH values of the 1:5 suspensions and of total soluble salts was discontinued in this and

succeeding studies.

(3) Inducing nitrite formation in samples fertilized with ammonium sulfate and urea by starting incubation with an initially high pH. The samples were prepared by adding in 5 ml. portions, as already described, fertilizing solutions of ammonium sulfate and urea which contained 30 mg. of nitrogen and 10 ml. of saturated calcium hydroxide for each 20-ml. addition made to the samples. The weighed samples were put in the incubator, from which duplicates were periodically removed for analysis until such time as it was evident that all nitrogen applied as either ammonium sulfate or urea had been converted to nitrates. Determinations were made on these samples for pH, nitrite, nitrate, and phosphate content. Moisture losses during incubation were restored with distilled water in this experiment since it was not desired to maintain the pH above the threshold value.

Results of Study of Nitrification at Different Initial pH Values

The results obtained in the study of the threshold pH range are shown in Figures 29 to 35, inclusive. Although phosphate data appear on these and many subsequent figures, discussion of them is being deferred until a later section.

The purpose of this study was considered achieved as soon as the pH had dropped to a value sufficiently low so

that nitrification could proceed rapidly. For this reason the data shown on the graphs were made to extend only through the 25th day. A final analysis was made on the 58th day to determine the maximum amount of nitrate that had formed.

The last analysis was made primarily to check the results of the (C) treatment (see above) with the data already reported in Figures 10 and 23 for nitrification of the same nitrogen compound, in the same soil, under identical conditions of incubation. These data will be discussed later.

The analytical data obtained on the 58th day of incubation are presented in Table 4.

TABLE 4

EXTENT OF OXIDATION OF AMMONIA IN GILA SANDY LOAM AT VARIOUS INITIAL pH VALUES AFTER 58 DAYS OF INCUBATION

Treatment	Initial pH	Final pH	Nitrates p.p.m.	Nitrites p.p.m.	Phosphates p.p.m.
A	7.68	7.93	23.5	Trace	13.5
B	9.21	7.60	302.5	Trace	13.9
C	8.98	7.64	297.5	Trace	13.9
D	7.47	7.47	355.0	Trace	19.9
E	7.50	7.54	333.8	Trace	26.4

Series (A)--No Treatment -- Figure 29

The pH values, nitrate, and nitrite contents of the samples which had received no treatment remained practically

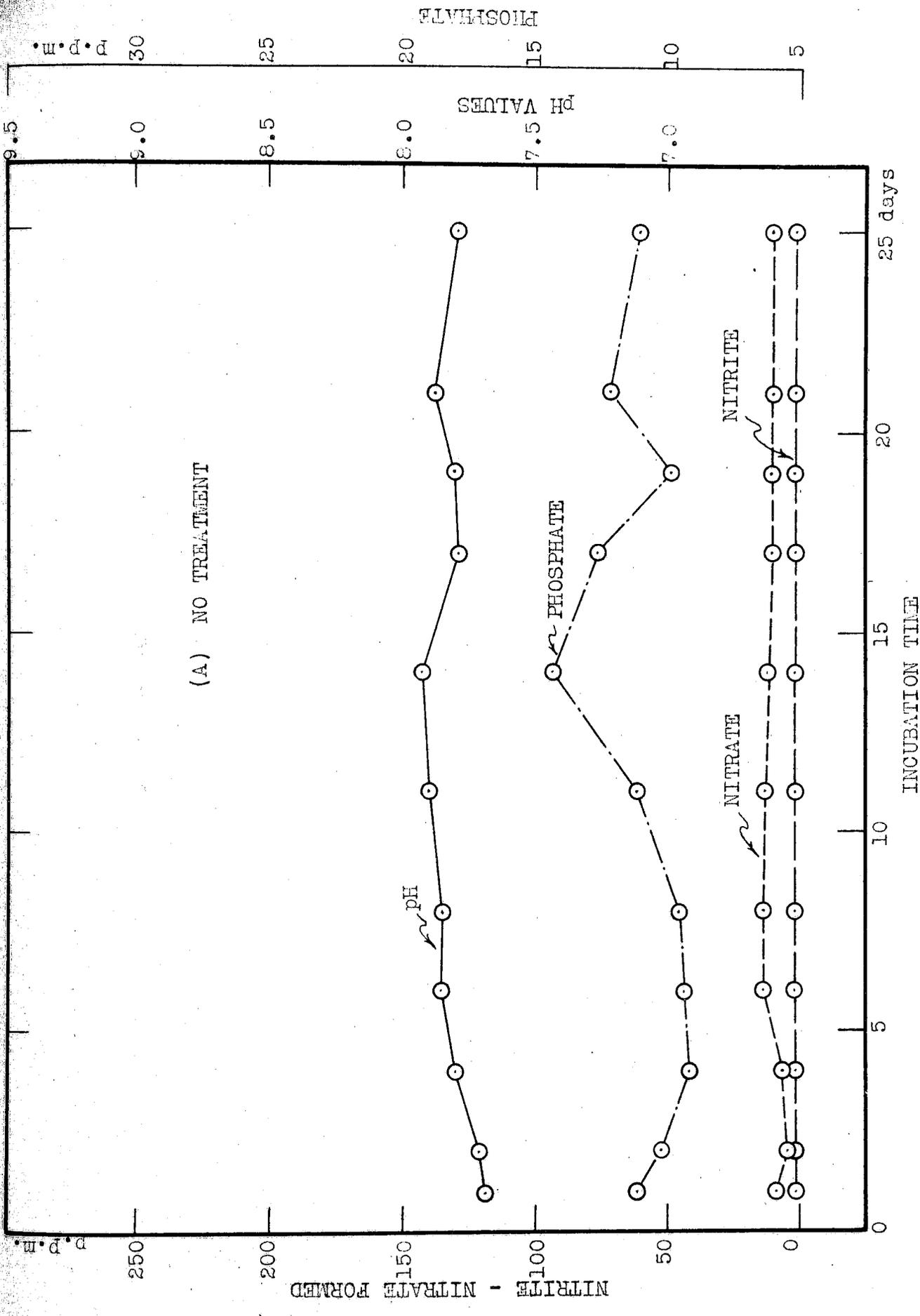


FIG. 29. Changes in pH, Phosphate, Nitrite, and Nitrate in Untreated Gila Sandy Loam.

unchanged over a period of twenty-five days. The initial pH value was at the threshold pH value and in subsequent samples varied upward as high as 7.9. The nitrates had an initial value of slightly under 10 p.p.m., and never varied more than \pm 5 p.p.m. from this value during the period of 25 days. The nitrites showed only trace quantities throughout.

Series (B)--NH₄OH + Ca(OH)₂ -- Figure 30

This series had the highest initial pH value, namely 9.21. It will be noted in Figure 30 that the pH gradually decreased during the first eight days of incubation, the total decrease being about 0.8 unit. It then remained relatively constant until the seventeenth day at which time a drop of about 0.6 of a pH unit was noted, accompanied by the appearance of nitrites. Thereafter the pH remained at a level considerably less than the original, while the nitrites increased, reaching a maximum of 71 p.p.m. on the twenty-first day. On the twenty-fifth day, as the pH value approached the threshold value of 7.65, nitrates first made their appearance and nitrites began to disappear.

Series (C)--NH₄OH alone -- Figure 31

The data from this series of samples are of special interest since similar data were obtained under identical experimental conditions on this soil and are reported in Figures 10 and 23.

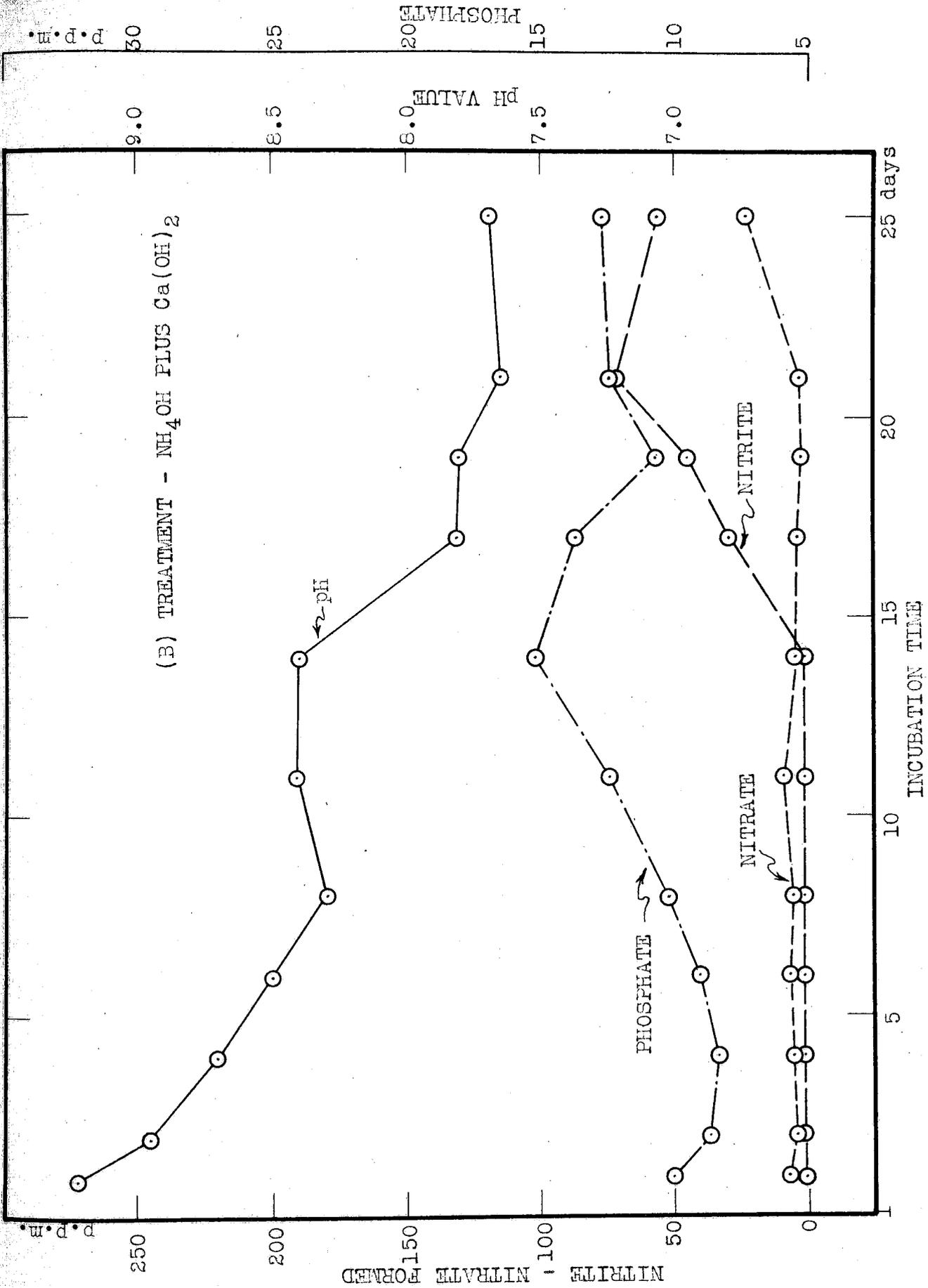


Fig. 30. Changes in pH, Phosphate, Nitrite, and Nitrate in Gila Sandy Loam Treated with 300 p.p.m. of Nitrogen as Ammonia Plus Saturated Calcium Hydroxide Solution.

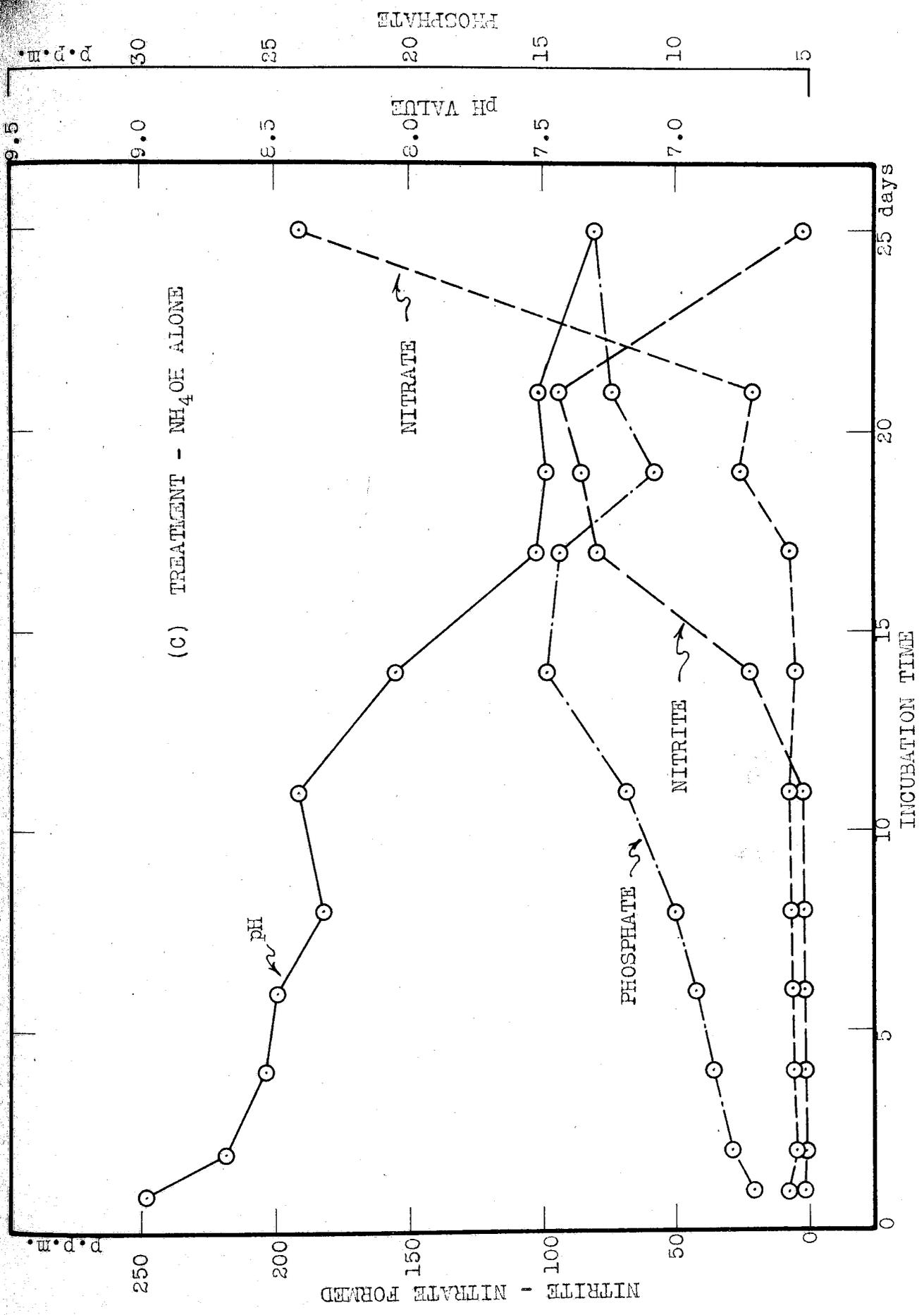


Fig. 31. Changes in pH, Phosphate, Nitrite, and Nitrate in Gila Sandy Loam Treated with 300 p.p.m. of Nitrogen as Ammonia Alone.

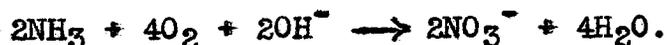
This series of samples (Figure 31) had a relatively high initial pH value -- nearly 9.0 -- from which it decreased approximately 1 pH unit during the first eleven days of incubation. Thereafter a sharp drop of approximately 0.75 unit was observed between the eleventh and fourteenth days. Up to this time no nitrate accumulation was observed, but a definite quantity, 22 p.p.m., of nitrites had made its appearance. The pH dropped slightly lower as the build-up of nitrites continued, and reached a point just below the threshold pH range at the time of the maximum formation, 94 p.p.m., of nitrites. This very high maximum was reached on the twenty-first day of incubation, and decreased sharply to trace quantities by the twenty-fifth day.

Almost immediately after the threshold pH value of 7.65 was reached on the seventeenth day, nitrates began to accumulate.

It should be noted in Figure 31 that the first and last observations of nitrites were made on the fourteenth and the twenty-first days of incubation, respectively. From this it is known that nitrites existed in appreciable amount--up to nearly one-third of the total nitrogen application--for at least one week. Since a 3-day interval preceded the analysis on the fourteenth day and a 4-day interval followed that on the twenty-first, it is possible that nitrites persisted in the soil for nearly two weeks.

This period of nitrite accumulation is approximately the same as that observed in Figure 30 for the (B) series of highly alkaline samples, and is noticeably longer than that observed in the (D) and (E) series of lower initial pH values, shown in Figures 32 and 33 which follow.

Since a general tendency is apparent in several of the figures for the pH to continue downward even after the reduction of nitrites to trace quantities, it should be pointed out that this decrease is not to be attributed to the oxidation of nitrites to nitrates and the removal of hydroxyl ions thereby, as occurs in the over-all equation for the oxidation of ammonia to nitrites. This is apparent if the formation of nitrates is represented as being formed directly from ammonia without the intermediate formation of nitrites, thus:



The balanced equation involves the same amount of hydroxyl ions as that for nitrite formation; it is obvious, therefore, that the transformation of nitrites to nitrates does not affect the pH in this manner. The difference in the degree of dissociation of nitrous and nitric acid, however, and the simultaneous oxidation of still unoxidized ammonia in the system may be responsible for this remaining drop in pH even though nitrification is occurring.

It is of interest to compare the nitrate curve in Figure 31 with that reported in Figures 10 and 23. In the

present phase of the study, the first appreciable nitrification was noted on the nineteenth day of incubation, and on the last day shown in the graphed data -- the twenty-fifth -- a maximum of approximately 190 p.p.m. of nitrogen as nitrates was found. This represents a recovery of about 63%. In the previous work, the first nitrification was noted on the eighteenth day of incubation, and on the twenty-fifth day, a maximum of approximately 195 p.p.m. of nitrogen as nitrates was observed. This represented a recovery of 65% of the total nitrogen applied as ammonium hydroxide. Inasmuch as a recovery of 97% was obtained at the end of seven weeks in the earlier study, a final set of samples was allowed to incubate approximately the same period -- 58 days -- to see how reproducible the final results would be, as already explained in connection with Table 4. It will be noted that with the (C) treatment, a recovery of 99% was obtained on the 58th day.

Series (D)--NH₄OH + 0.5 N H₂SO₄ -- Figure 32
and Series (E)--1 N H₂SO₄ -- Figure 33

The initial pH values were adjusted to 7.47 and 7.50 (appreciably below the threshold pH value of 7.65) by the addition of one-half normal and normal sulfuric acid, respectively, in the (D) and (E) series. It had been expected that such treatment, using the same volume of the two strengths of acid, would give considerably different initial

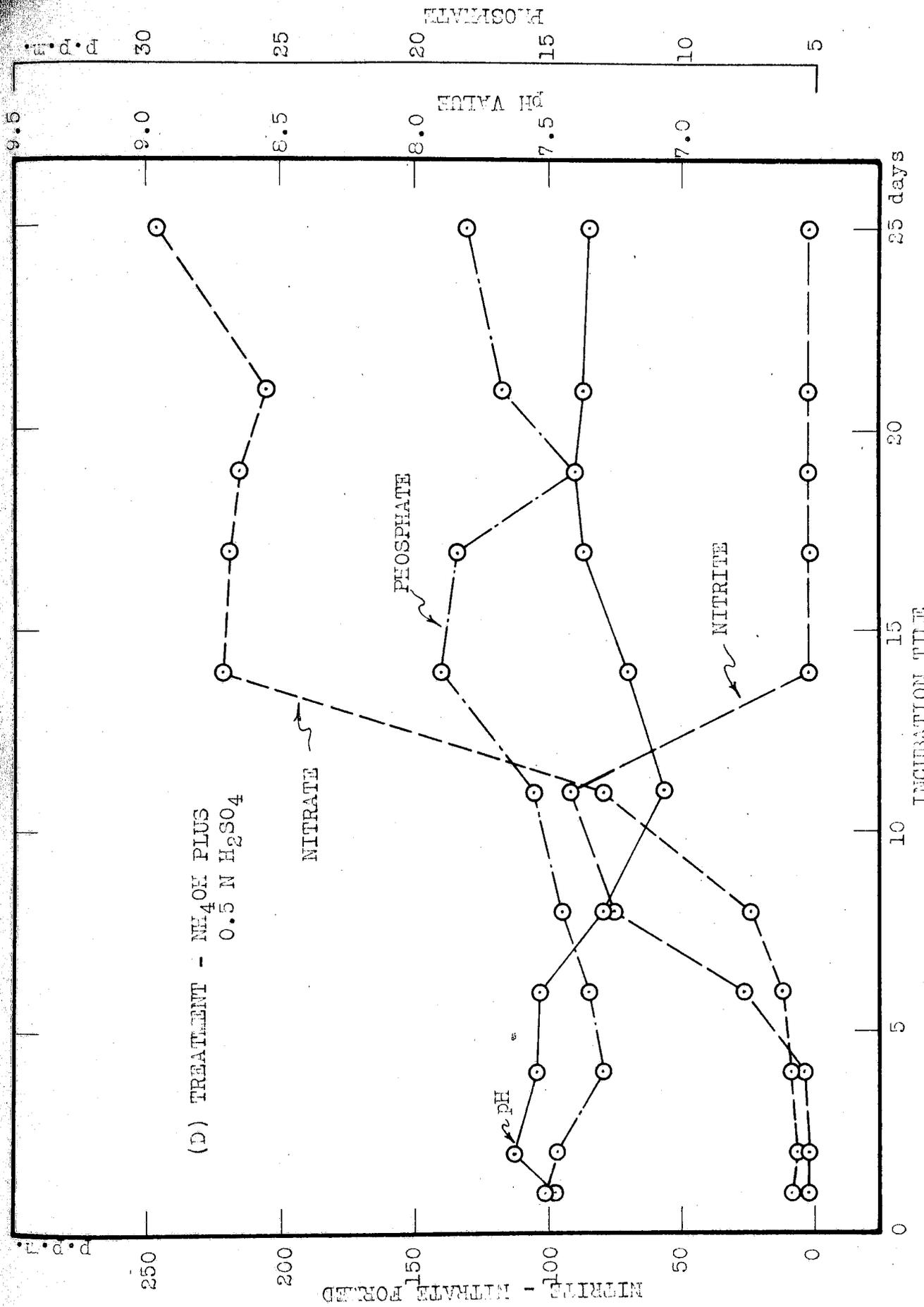


Fig. 32. Changes in pH, Phosphate, Nitrite, and Nitrate in Gila Sandy Loam Treated with 300 p.p.m. of Nitrogen as Ammonia Plus 0.5 N Sulfuric Acid.

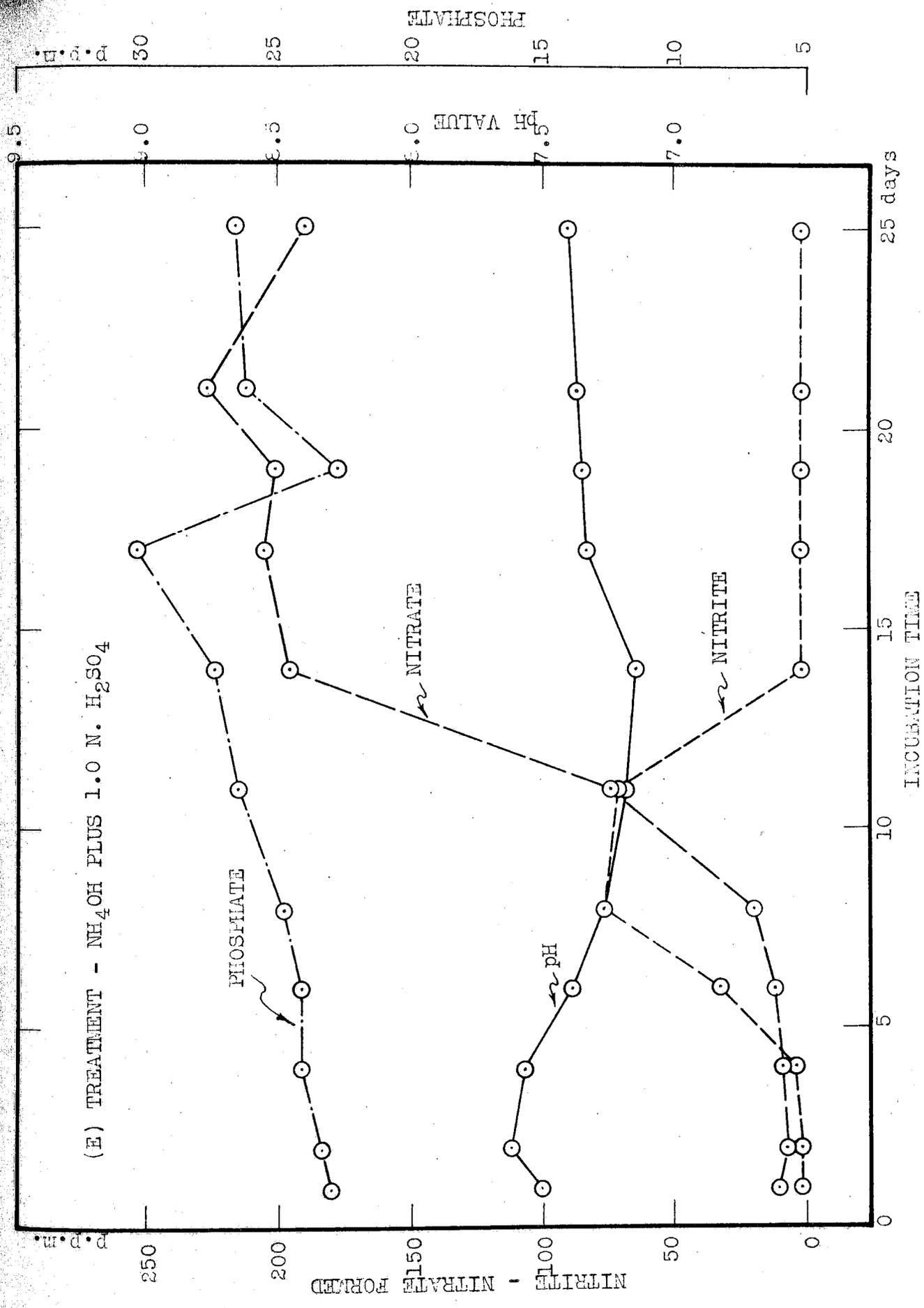


Fig. 53. Changes in pH, Phosphates, Nitrite, and Nitrate in Gila Sandy Loam Treated with 300 p.p.m. of Nitrogen as Ammonia Plus 1.0 N Sulfuric Acid.

values. The buffer capacity of the moist soil apparently was sufficiently high, however, to overcome this effect.

The curves in Figures 32 and 33 are of particular interest in that they show how reproducible the extent of the microbiological transformation becomes if the pH is constant. The respective pH, nitrite, and nitrate curves in the two figures are so nearly identical that they are practically superimposable. This similarity is easily observed in Figure 34.

Moreover, it is to be noted that the nitrite build-up comes at an earlier period during the incubation study with these two treatments -- ammonia plus half normal and normal sulfuric acid -- than with the treatments applied to the (B) and (C) series -- ammonia plus saturated calcium hydroxide, and ammonia only -- discussed in Figures 30 and 31. The period during which nitrites appear in appreciable amounts is likewise considerably shorter than in the more alkaline series. Similarly, nitrates were first observed much earlier in these acid-treated series; hence their accumulation is far greater on the twenty-fifth day of incubation than in the more alkaline series in which nitrate formation was retarded by the high pH.

Relationship between pH and Nitrate Formation -- Figure 34

In Figure 34 is shown the dependence of nitrification upon the attainment of the threshold pH value of 7.65.

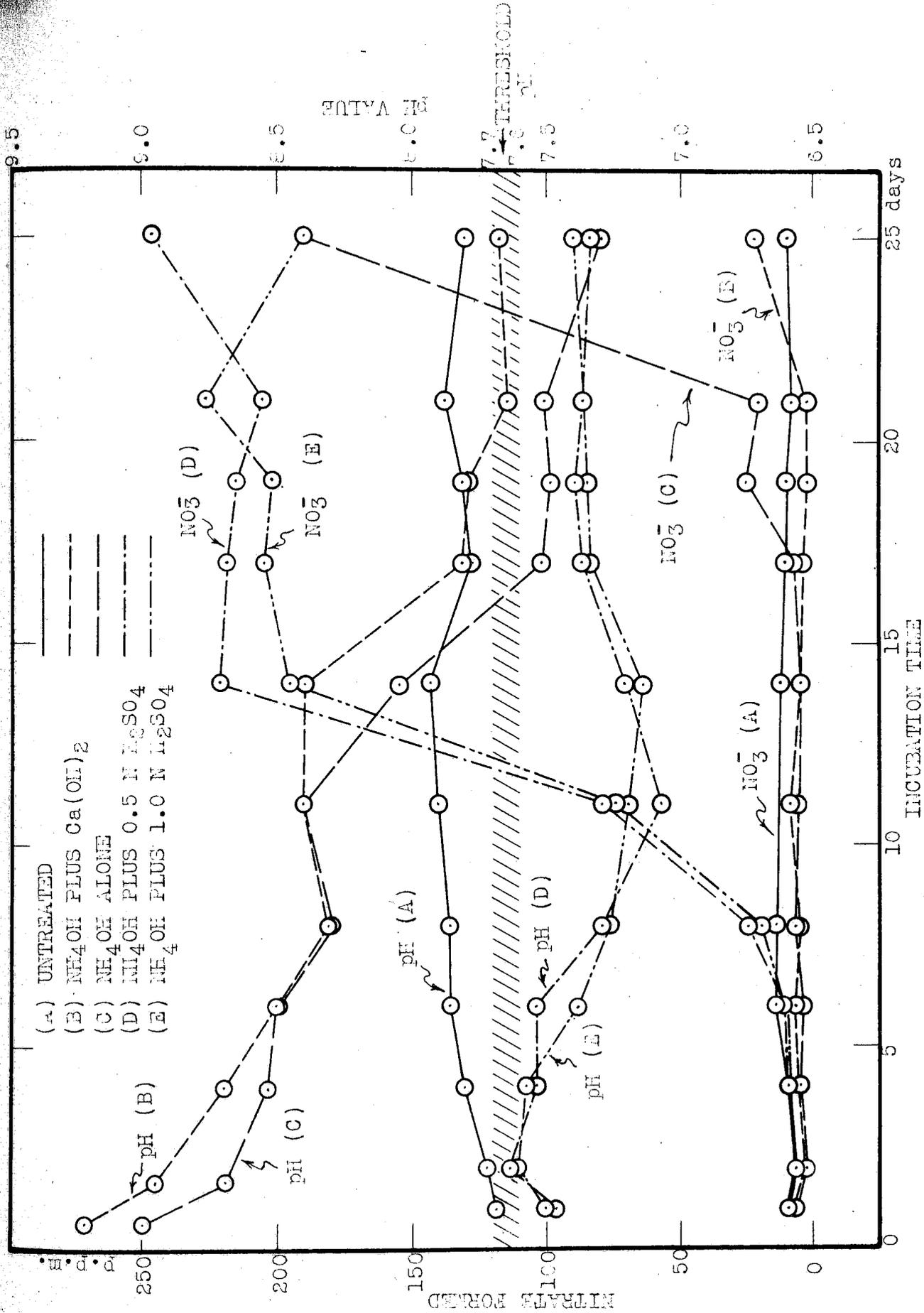


Fig. 34. Correlation between pH and Nitrate Formation in Gila Sandy Loam under Different Treatments.

It was not expected that nitrification would be appreciable in the untreated samples, (A), containing only the naturally occurring forms of nitrogen; therefore, the correlation between a lack of nitrate formation and pH values lying above the threshold value is of no real significance. On the other hand, it is quite significant that nitrates are absent in the (B) and (C) series (in which the initial pH values were far above 7.65 -- 9.21 and 8.98, respectively) until the pH curves for these series had reached the threshold pH value.

A similar indication of the importance of this range is the prompt nitrate formation -- perceptible on the sixth and appreciable on the eighth day of incubation -- in both the (D) and (E) series in which the initial pH values -- 7.47 and 7.50, respectively -- were below 7.65.

Figure 34 indicates that nitrification begins promptly after the threshold range has been reached in samples whose pH is initially above that value, and that nitrification in samples with initial pH values below 7.6 depends simply upon the beginning of an appreciable biological activity following the normal lag phase. The six- or eight-day period prior to nitrate formation in the acid series, (D) and (E), indicates a relatively prompt response by the microorganisms to a favorable environment. It must be borne in mind that the soil in storage had been

neither moistened nor brought to a favorable incubation temperature prior to this study, over a period of about eighteen months.

Correlation of pH, NO_2^- , NO_3^- , with Treatment -- Figure 35

Figure 35 is a bar graph showing the influence of the various treatments upon the pH value of the soil samples: the initial pH values of the soil samples; the values finally attained at the end of twenty-five days of incubation; the values at which the nitrites were first observed; the values at which the nitrites were observed to be at a maximum; and the values at which nitrates first began to accumulate. At the top of each bar is given the day of incubation on which the observation was made.

The drop in pH obviously is large in the alkaline series of samples when the initial and final values are compared.

That a relationship exists between the oxidation of ammonia and a decrease in pH is indicated by the fact that nitrites are observed in the alkaline series, (C) and (B), five and eight days, respectively, prior to nitrates.

In the most alkaline series, (B), the threshold pH range was not attained until the maximum build-up of nitrites was reached, as shown in Figures 30, 34, and 35; hence the maximum value for nitrites was observed prior to the time when nitrates began to appear. This observation differs

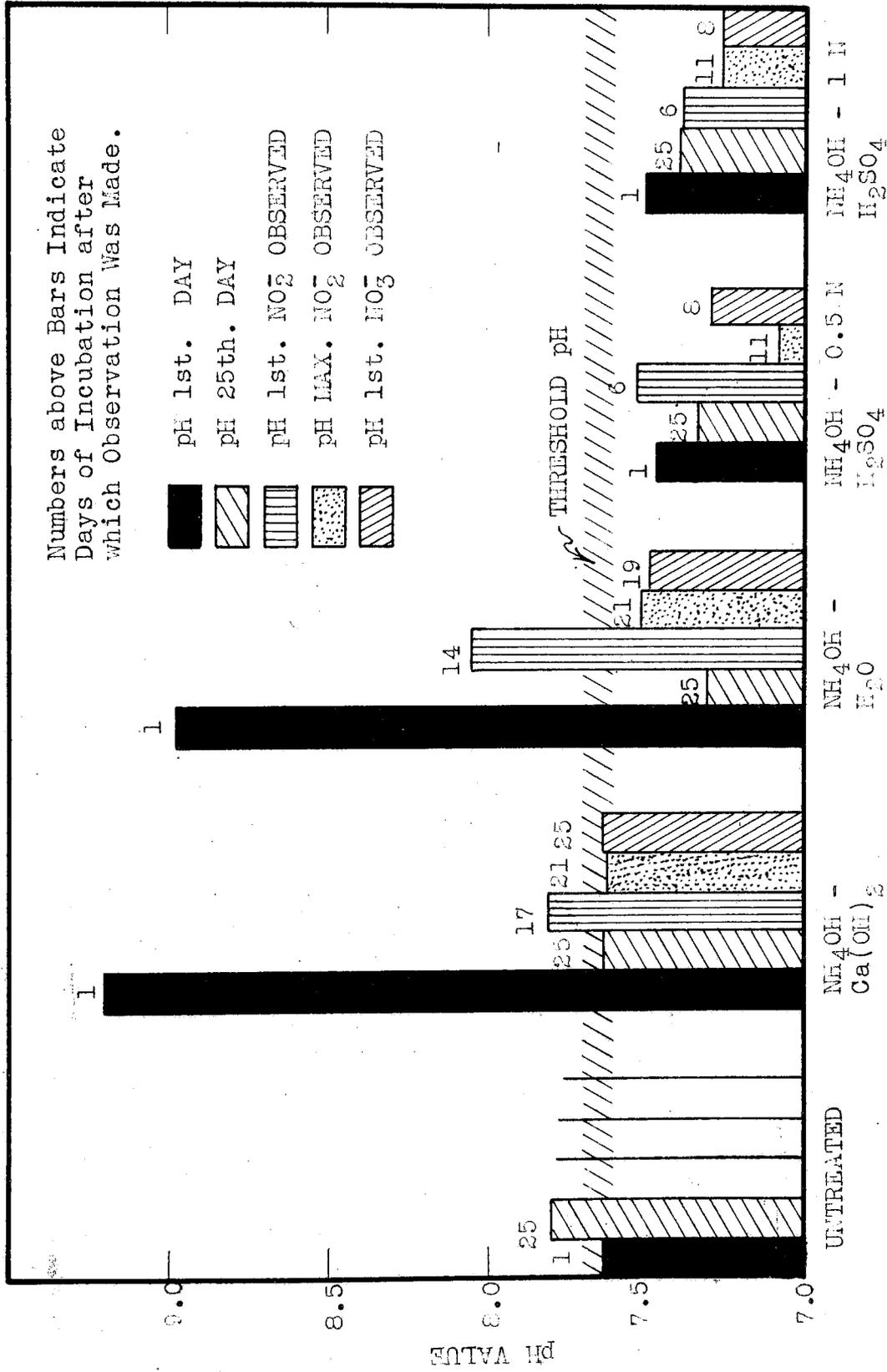


Fig. 35. Threshold pH Range in the Nitrification of Ammonia in Gila Sandy Loam.

from the results obtained in this study on series (C), (D), and (E), all of which had initial pH values lower than series (B).

In the (C) series, treated with ammonia alone, the maximum amount of nitrite was not formed until the pH value had fallen to the threshold limit and some nitrate formation was observed before the maximum value for nitrites was reached as shown in Figures 31, 34, and 35. This observation is likewise true for the two acid-treated series, (D) and (E), whose initial pH values were below the threshold value.

The correlation of nitrate formation with the threshold pH value is obvious from Figure 35. The time lag in nitrification before this value is reached is apparent from the number of days required by the treated series before nitrates were observed. In the most alkaline series, (B), twenty-five days had elapsed before nitrates were first observed, and then they were found to be only about 10% of the value for nitrates in the acid series, (D) and (E), for the same period of incubation. In the next alkaline series, (C), nineteen days had elapsed before nitrates first made their appearance, and in the two acid series, (D) and (E), the time interval was only eight days.

It thus appears quite definite that the initial pH value of the soil at least in part determines the time

interval after which initial nitrification will occur in alkaline calcareous soils.

Effect of Sustained High pH on Nitrite- and Nitrate-Formation -- Figure 36

It will be noted in Figure 36 that on the third day of incubation (i.e., the first day of sampling for analysis) the pH value of this series of samples was 8.72. Checking the weights of the samples, the loss in moisture was compensated for by adding a saturated solution of calcium hydroxide which had a pH value of 12.10. During the first twenty-two days of incubation, the pH gradually dropped to 7.9 in spite of the added base, but nitrate accumulations were very slight, amounting to only 12 to 15 p.p.m. On the twenty-second day, however, although the pH value was still 7.9, the nitrate content had increased to nearly 35 p.p.m. This was not a very high value, to be sure, but the accumulation was nevertheless significant, considering that the pH value for the soil as a whole was still 0.2 of a pH unit above the threshold value of 7.65. This was taken to indicate that the drop in pH value within soil aggregates is sufficient to enable all of the nitrifying bacteria to function feebly, or more likely a small percentage of them to function normally.

Since the nitrite content had mounted to nearly 60 p.p.m. and remained there, in contrast with over 90 p.p.m.

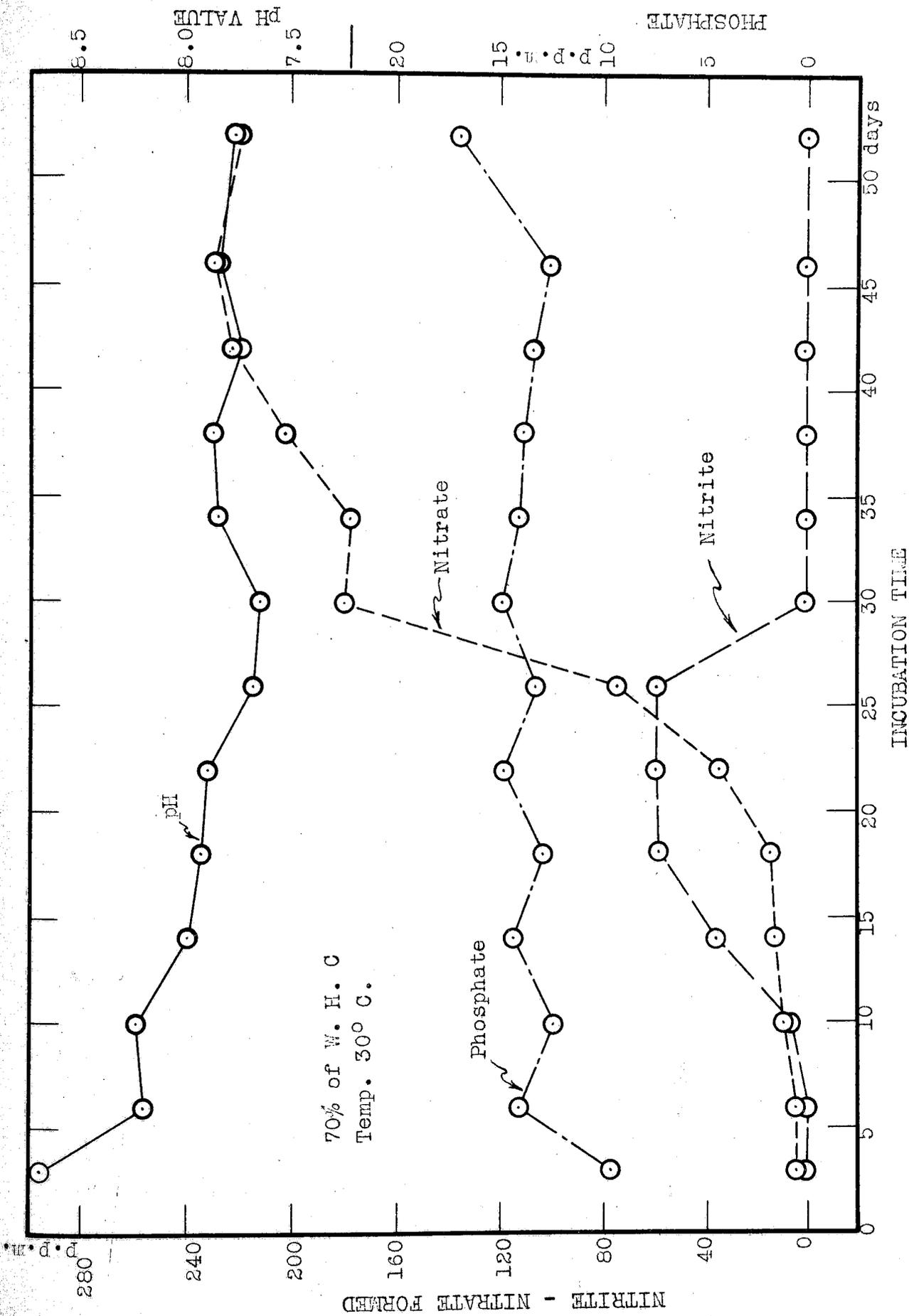


Fig. 36. Rate of Nitrification of Ammonia in Gila Sandy Loam as Influenced by Controlled pH.

at the same period of incubation in the sample treated with ammonia alone (Figure 31) and 70 p.p.m. of nitrite for the same period in this soil treated with ammonia plus saturated calcium hydroxide initially only (Figure 30), it appears likely that the sustained high pH value is detrimental to the nitrite-forming organisms as well as to the nitrate formers. This deduction may, however, be in error since, as pointed out earlier in this section, the sample may not have been analyzed at the exact time of maximum nitrite formation, so that the duplicates represented by the point on the twenty-second day of incubation may be an ascending or descending value. A similar statement may be made for the value plotted for the twenty-sixth day. In either case, the maximum value was not reached on the day of analysis.

In view of the fact that the nitrites appeared to remain at a stationary value, however, and apparently nitrates were being formed within soil aggregates, it was decided to withhold additional base until the previously noted threshold pH value of 7.65 had been reached and to observe whether such treatment favored nitrification. The results are quite conclusive: On the twenty-sixth day the pH value was 7.68 and the nitrate value had risen to 75 p.p.m., and on the thirtieth day, with a pH of 7.65, the nitrates had more than doubled, attaining a value of

180 p.p.m.

If high alkalinity were instrumental in preventing nitrification, it might be expected that the addition of saturated calcium hydroxide to the soil surface to bring up the moisture content would retard or prevent the activity of the nitrate formers with which it came in contact. It could hardly be expected, however, since the base was added directly to the surface of the sample that it would affect all the organisms throughout the sample equally. Following the thirtieth day of incubation, the pH was restored to and maintained above a value of 7.70. The rate of accumulation of nitrates decreased immediately and even after 52 days of incubation had reached a value of only 225 p.p.m. This observation is exceedingly significant in view of the fact that in this soil without the addition of calcium hydroxide (Figure 10) the nitrates reached a concentration of slightly below 300 p.p.m. on the forty-ninth day of incubation.

That the nitrite falls to a mere trace as soon as nitrification attains a rapid rate is shown in Figure 36, and was observed in all of these studies.

Nitrite Formation from Ammonium Sulfate and Urea -- Figures 37 and 38

Having observed the retarding effect of calcium hydroxide when applied with ammonia (Figure 30), it seemed desirable to determine whether nitrification would be

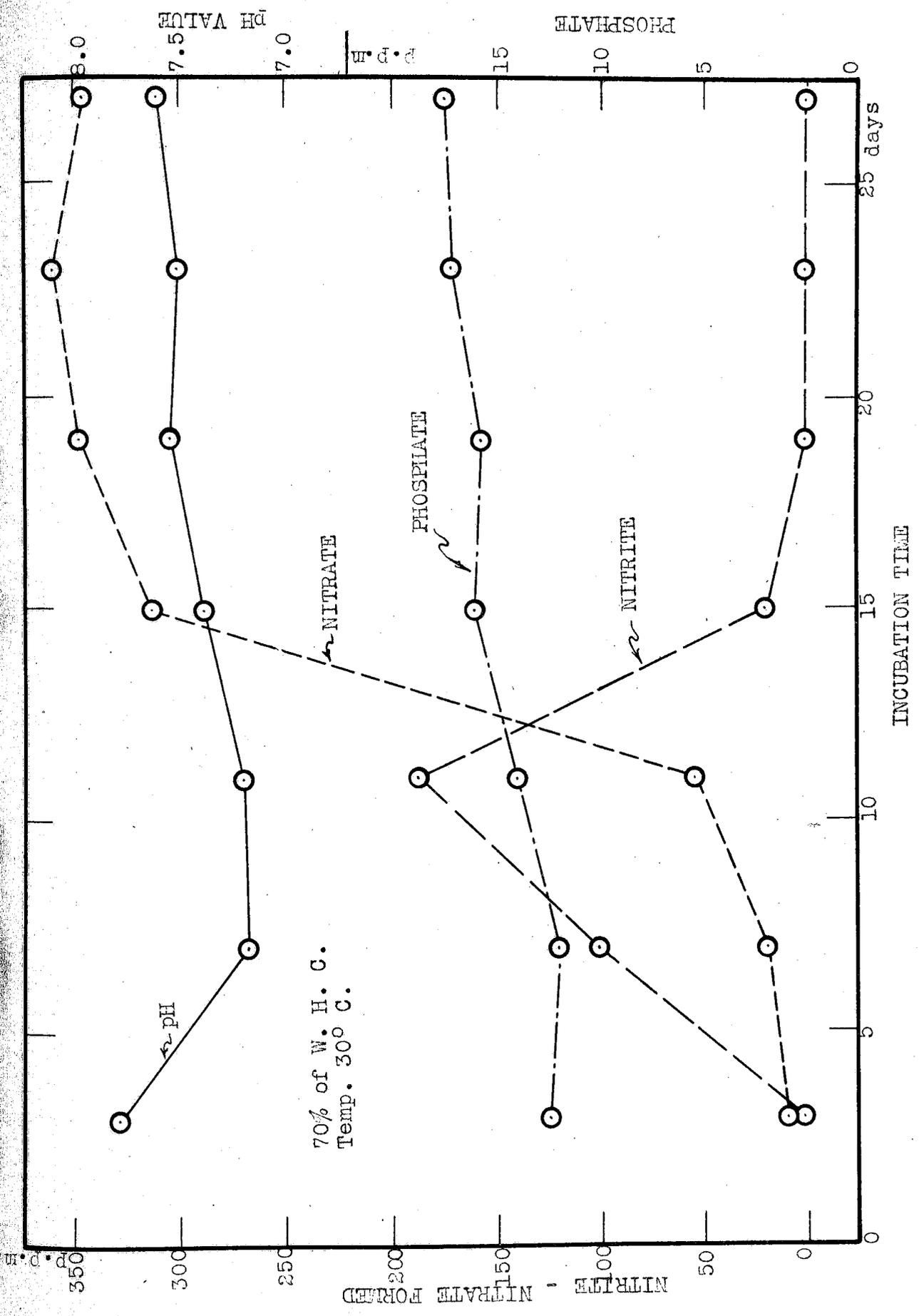


Fig. 37. Rate of Nitrite and Nitrate Formation from Ammonium Sulfate in Gila Sandy Loam at an Initially High pH.

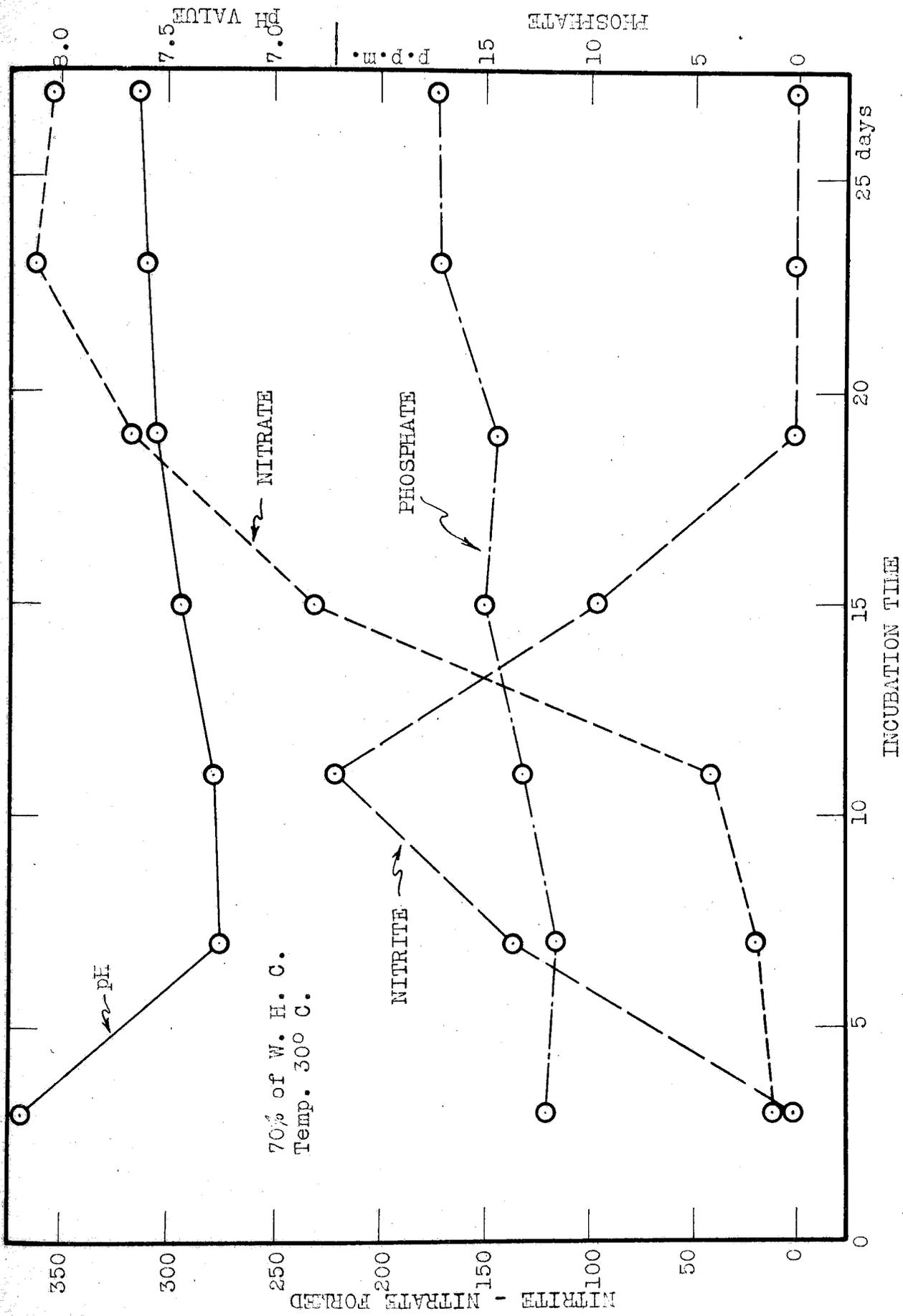


Fig. 38. Rate of Nitrite and Nitrate Formation from Urea in Gila Sandy Loam at an Initially High pH.

retarded and nitrites would accumulate if this base were added with ammonium sulfate and with urea. The data are plotted in Figures 37 and 38, respectively. The results are most striking: not only is the nitrification rate not reduced, but nitrite accumulation is the greatest of any reported throughout this study.

A comparison of the nitrification rates in Figures 37 and 38 with those in Figure 10 is of interest. In the former, calcium hydroxide was added with the fertilizing solutions of ammonium sulfate and urea; in the latter, the base was not added. Figure 10 reveals that without the base present, the ammonium sulfate had nitrified to give 186 p.p.m. of nitrates, and urea, 158 p.p.m. on the fourteenth day. Figures 37 and 38 show a respective accumulation, on the other hand, for ammonium sulfate and urea of 313 and 231 p.p.m. on the same day of incubation for the base-treated samples.

The writer does not consider, however, that the faster rate of nitrification was necessarily the result of the calcium hydroxide treatment. The results of the earlier experiment without added calcium hydroxide were recorded on October 17, 1939, whereas those with the base added were recorded on March 9, 1941. These dates are significant because they indicate that nearly a year and a half had elapsed between these experiments, during which the soil

had lain in bins, under the glass of the greenhouse, with no moisture added and with summer temperatures reaching values which undoubtedly had a partial sterilizing effect upon the soil. The effect of drying and of partial sterilization, as pointed out by Waksman (1), is always to increase the activity of the microorganisms when favorable conditions are restored.

As previously mentioned, the nitrite accumulation in the ammonium sulfate and urea samples was exceedingly high. In the former (Figure 37) the nitrites reached a maximum of 186 p.p.m. on the eleventh day of incubation. The urea samples (Figure 38) also reached their nitrite maximum at this time--221 p.p.m.; this is over 60% of the possible value of nitrite nitrogen based upon the maximum amount of nitrates eventually formed. The writer is unable to explain why nitrites accumulated so much more in the ammonium sulfate and urea treated samples than in the ammonia treated ones, except to offer the suggestion that the low maximum value for nitrites already discussed in connection with Figure 36 is correlated with a pH of 7.93, 7.90, and 7.68 on the eighteenth, twenty-second, and twenty-sixth days of incubation, respectively, whereas the peak nitrite values for ammonium sulfate (Figure 37) and urea (Figure 38) are correlated with much lower pH values, namely 7.20 and 7.28, respectively.

Attention must be called to the general shape of the pH curves in Figures 37 and 38. The tendency for the pH values to increase as the amount of nitrogen applied, either as ammonium sulfate or as urea, is exhausted suggests that the nitrogen which had been assimilated by the microorganisms is being released as ammonia at this time. This eventual upward trend of the reaction curves is especially striking in Figure 58.

The incubation studies reported in Figures 37 and 38 were extended over a period of only twenty-seven days since 100% of the nitrogen applied as ammonium sulfate and urea, respectively, (plus soil nitrogen) had been recovered as nitrates at the end of that time.

D. Factors Affecting Nitrite and Nitrate Accumulation in Arid, Alkaline Soils

The significance of the observations recorded in the foregoing sections, namely that nitrite is formed even in well-aerated desert soils under optimum moisture conditions, and that a threshold pH value exists in the nitrification process, suggested a detailed study of some of the fundamental soil factors which govern the process.

The variables studied were moisture content, incubation temperature, amount and source of nitrogen, and application of certain amendments.

The following experiments were accordingly carried out:

(1) The moisture content was varied to give 20%, 40%, 55%, 70%, and 100% of the water-holding capacity; the incubation temperature was held at $30 \pm 1^{\circ}$ C., and nitrogen applied to the soil to the extent of 300 p.p.m. as ammonia.

(2) The temperature of incubation was varied, covering the range 10° C., 20° C., 30° C., 40° C., and 60° C.; the moisture content was maintained at 70% of the water-holding capacity, and 300 p.p.m. of nitrogen as ammonia was used.

(3) The amount of nitrogen applied was varied over the range 0 p.p.m., 50 p.p.m., 100 p.p.m., 200 p.p.m., and 300 p.p.m. of nitrogen as ammonia, applied on the basis of the air-dry soil; the moisture content being maintained at 70% of the water-holding capacity, and the incubation temperature held constant at $30 \pm 1^{\circ}$ C.

(4) The source of nitrogen was varied and the effect of amendments studied by applying ammonium sulfate, mono-ammonium phosphate, ammonia plus calcium sulfate, and ammonia plus sulfur to the soils in such quantities that 300 p.p.m. of nitrogen were added in each case. The samples were incubated at 70% of the water-holding capacity and at $30 \pm 1^{\circ}$ C. The sulfur was added at the rate of 1 gm. per 100 gm. of soil; the calcium sulfate in the amount of 5.4 gm. per 100 gm. of soil. These additions were in both instances approximately equivalent with respect to sulfur

added. The sulfur and calcium sulfate were added to the air-dry soil and uniformly mixed prior to addition of the ammonia solutions. All of the nitrogen compounds were added in the form of water solutions.

Duplicate samples were analyzed periodically for pH of the moist soil, and the nitrite, nitrate, and phosphate content of the filtrate from the 1 to 5 suspension.

Effect of Moisture Content on Nitrite and Nitrate Formation

The result of maintaining the samples at different moisture levels during incubation are presented in Figures 39 to 45, inclusive.

At 20% of the water-holding capacity, the rates of nitrification and nitratification were much slower, as shown in Figure 39. Nitrites failed to accumulate in appreciable amounts throughout the fifty-two days of incubation, the highest nitrite value, 13 p.p.m., being recorded on the twenty-sixth and thirtieth days. Since no determination was made between the thirtieth and fifty-first day of incubation, it might appear that the nitrite content had reached a maximum during that period. This is improbable, however, since the nitrate value had reached 42 p.p.m. on the thirtieth day, and previous data have indicated that nitrites tend to drop almost immediately after nitrates begin to form.

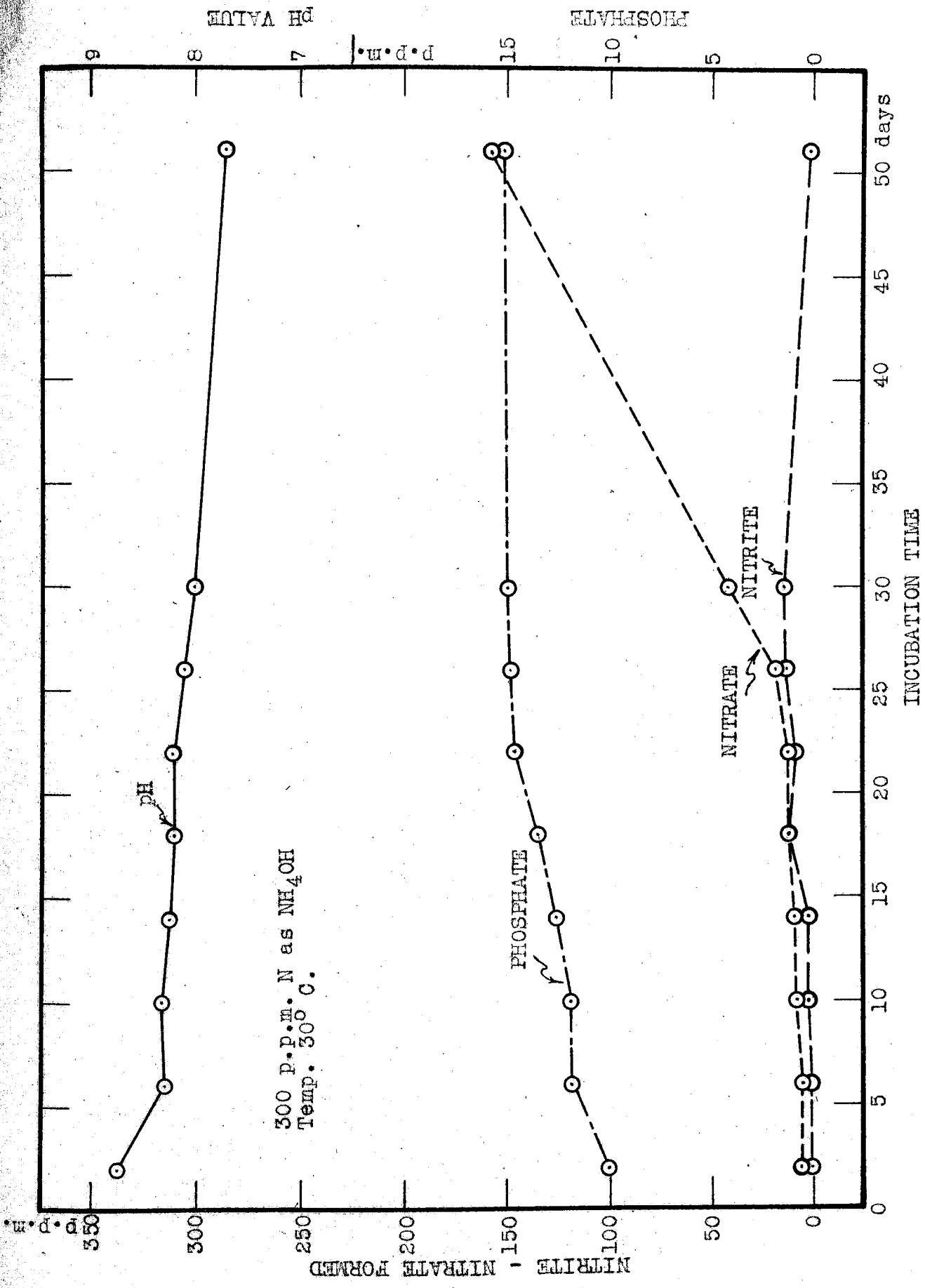


Fig. 39. Rate of Nitrite and Nitrate Formation from Ammonia in Gila Sandy Loam at 20% of the Water Holding Capacity.

The value of 42 p.p.m. of nitrates on the thirtieth day was recorded at a pH value of 8.00. The amount of nitrates is small, and the time for the initial formation of nitrate is over twice that required for nitrate formation under more favorable moisture conditions (Figures 41 and 42). This finding substantiates the opinion that if conditions are kept favorable for a sufficient length of time, the pH value within soil particles may be reduced to a value permitting the nitrate formers to function normally. On the fifty-first day of incubation, when the nitrate value had increased to nearly 160 p.p.m., the pH value was 7.7.

At 40% of the water-holding capacity (Figure 40), the first appreciable amount of nitrate formed, namely 47 p.p.m., was observed on the eighteenth day, coincident with a nitrite maximum of 51 p.p.m. The pH value on this day was 7.73, a reaction much nearer the threshold pH value of 7.65 than noted for initial nitrification in the samples moistened to only 20% of the water-holding capacity. This constitutes additional evidence for an inverse relationship between time of incubation and nitrate accumulation at pH values above 7.65. At 40% of the water-holding capacity, only 60% as much incubation time had lapsed prior to the first nitrate formation as at 20% of the water-holding capacity. If longer periods of time allow for

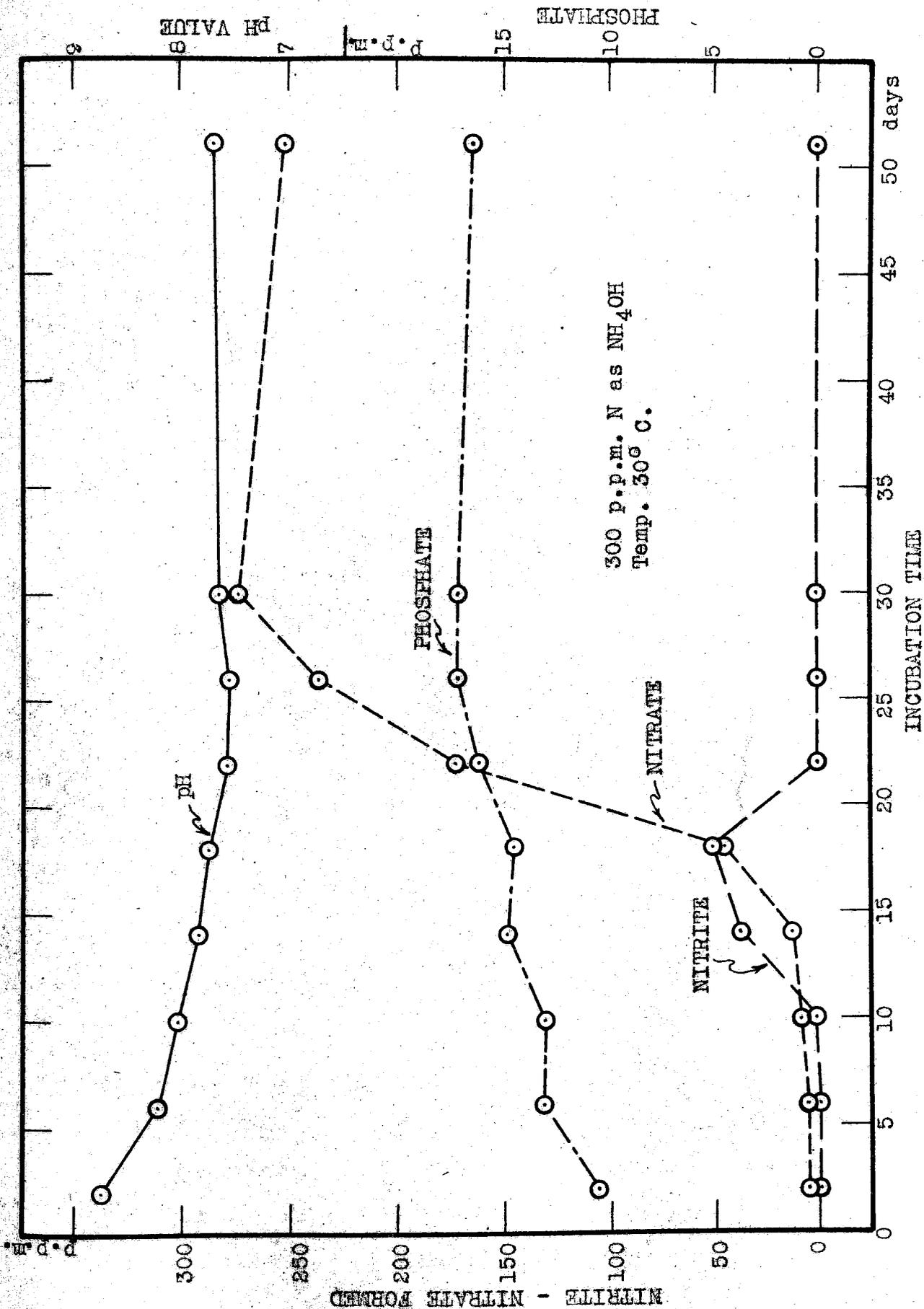


Fig. 40. Rate of Nitrite and Nitrate Formation from Ammonia in Gila Sandy Loam at 40% of the Water Holding Capacity.

adjustment of pH within the small soil aggregates, some nitrate may be observed at a pH value which, taken for the entire mass of the soil, is considerably above the threshold value; but if nitrates are observed at an earlier period of incubation the pH of the entire soil mass must have been nearer to or even below the threshold value, otherwise the incubation period would have been inadequate to overcome an unfavorable pH condition even in the small aggregates.

The nitrates reached a maximum on the thirtieth day with 274 p.p.m. formed at a pH of 7.63.

At 55% of the water-holding capacity (Figure 41) the rate of nitrification is nearly identical with the rate at 40%, but it starts about sixty hours earlier in point of incubation time. The first appreciable accumulation of nitrates was observed on the eighteenth day at which time the pH value of the soil mass was 7.60.

The maximum nitrite value of 65 p.p.m. was noted on the fourteenth day.

At 70% of the water-holding capacity (Figure 42), nitrite accumulation reached a maximum of 96 p.p.m. on the fourteenth day of incubation. On the same day, at a pH value of 7.63, the nitrate content was 14 p.p.m.; four days later, however, with the pH at 7.55, the nitrates had risen to 175 p.p.m. and the nitrites had fallen off to 13 p.p.m. The most complete nitrification in any of the moisture

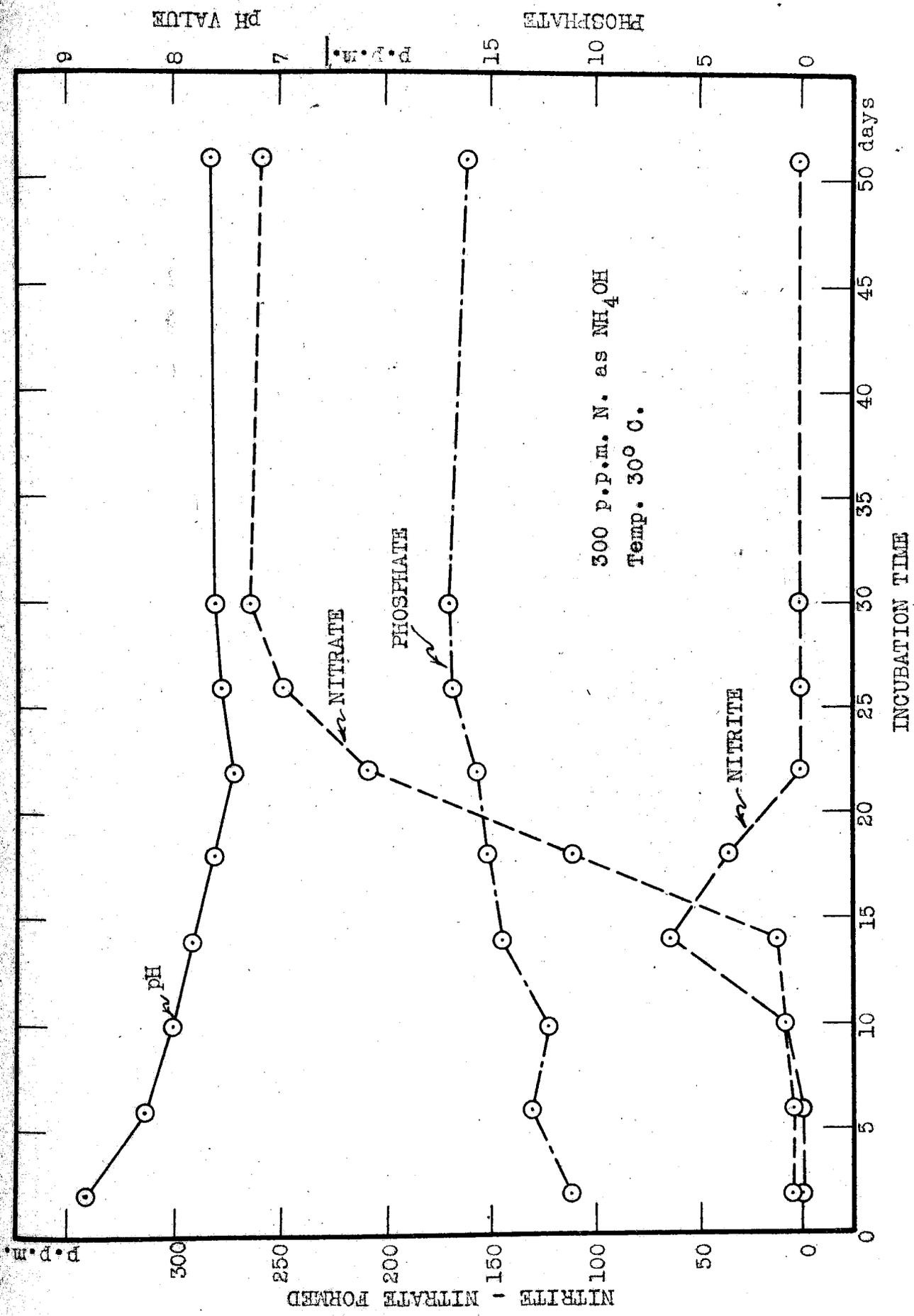


Fig. 41. Rate of Nitrite and Nitrate Formation From Ammonia in Gila Sandy Loam at 55% of the Water Holding Capacity.

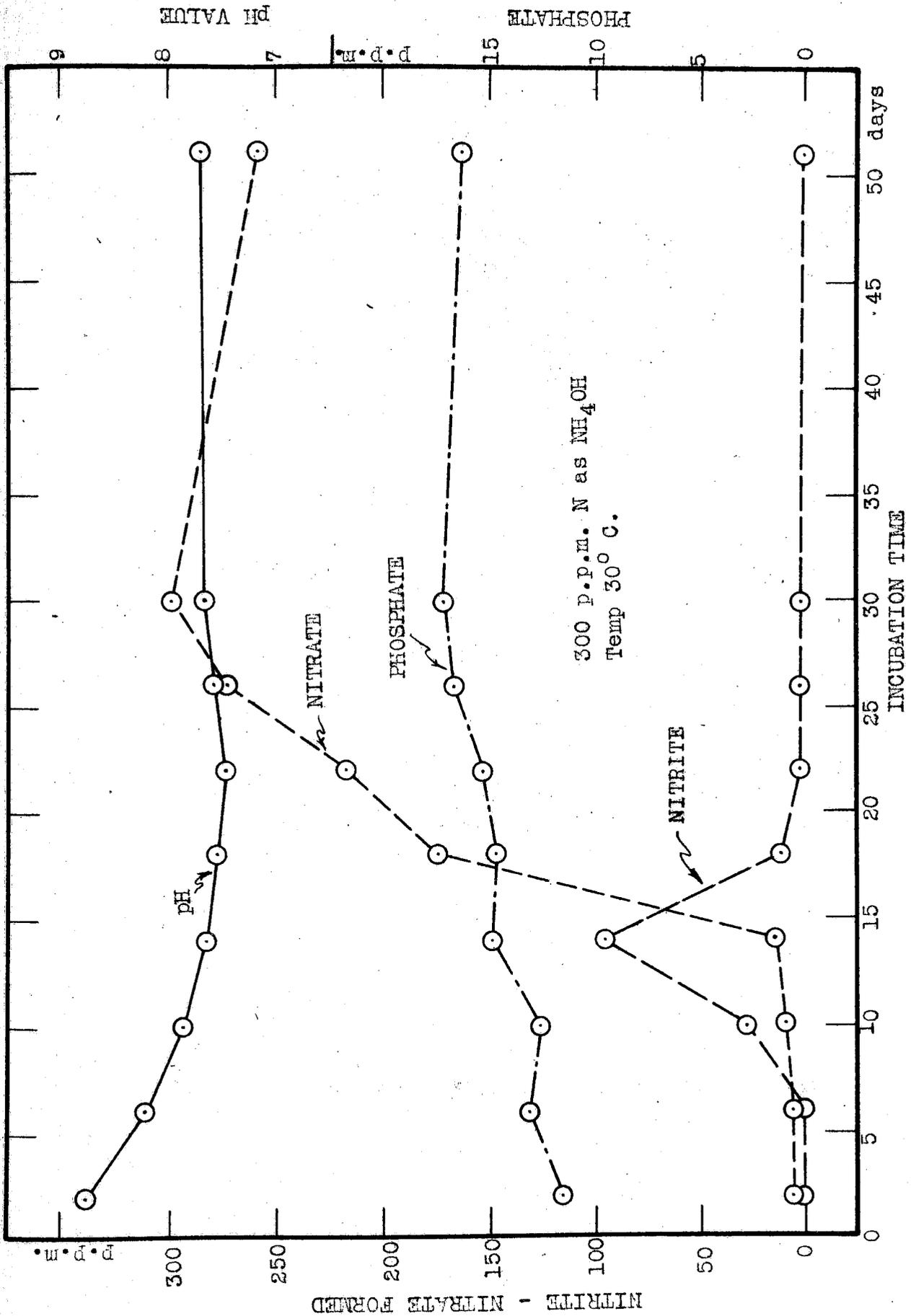


Fig. 42. Rate of Nitrite and Nitrate Formation from Ammonia in Gila Sandy Loam at 70% of the Water Holding Capacity.

studies was observed in the 70% samples on the thirtieth day when 298 p.p.m. of nitrates were found.

At 100% of the water-holding capacity (Figure 43), differences between duplicate samples were noted in several instances; but these differences serve only to emphasize the correlation between soil reaction and extent of nitrification and nitratification.

The pH and nitrification values obtained with these samples are of particular interest because as the water content increases, the continuity of the water phase should increase. This would favor more rapid establishment of equilibrium within the soil mass and more nearly complete elimination of the effect of the individual soil aggregates. The pH values of the soil mass would consequently be nearer that of each individual particle and, therefore, the effect of pH upon the nitrification process should be much more pronounced.

It will be observed in Figure 43 that duplicate samples analyzed on the tenth day of incubation gave results which did not agree with each other. One sample had a pH value of 8.15, a nitrite value of 12 p.p.m., and a nitrate value of only 3 p.p.m.; the duplicate sample had a pH of 7.60, a nitrite value of 53 p.p.m., and a nitrate value of 20 p.p.m. It becomes increasingly apparent, therefore, that the pH value must have some effect upon nitrite formation, as already suggested in a previous discussion. (Figures

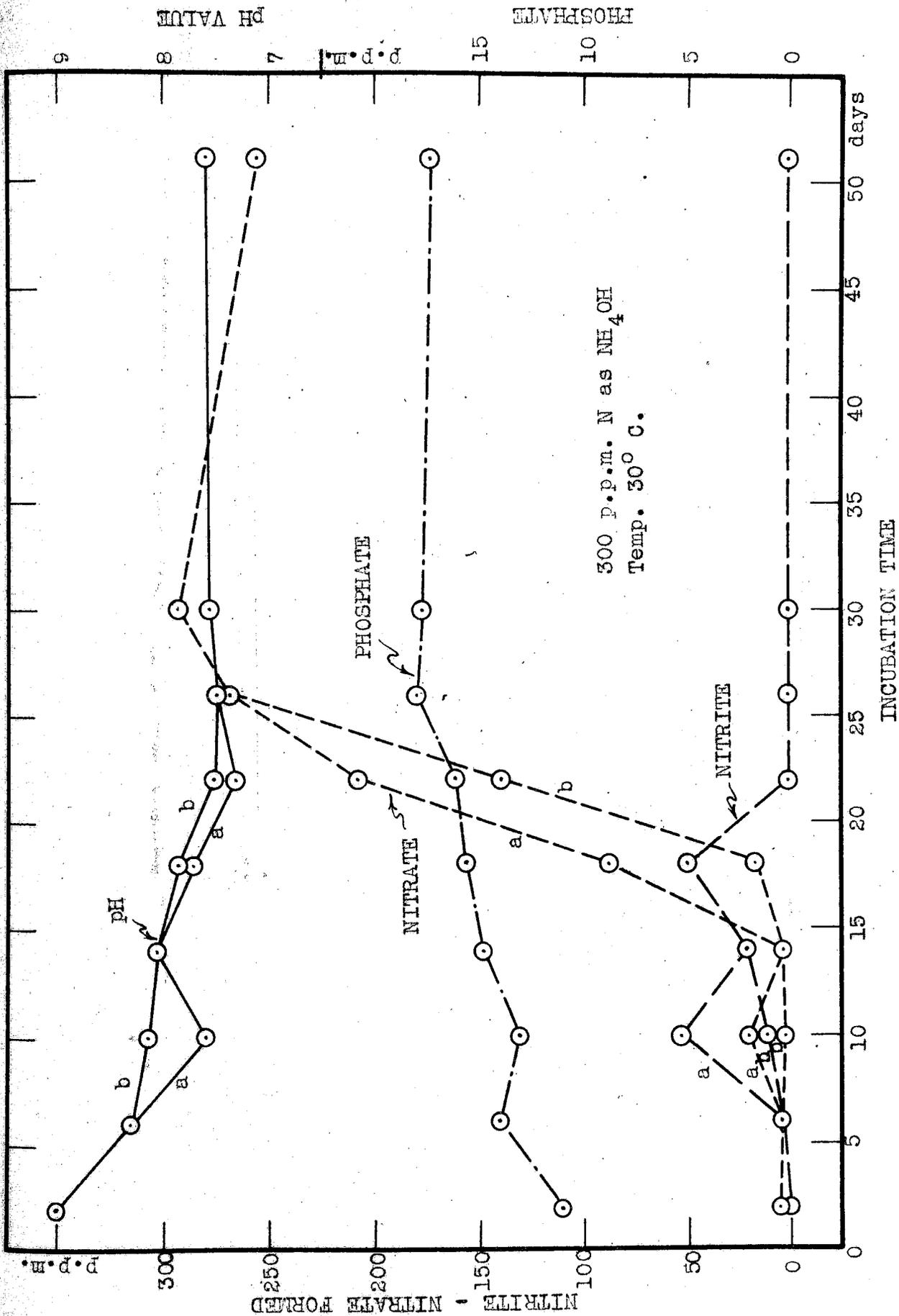


Fig. 43. Rate of Nitrite and Nitrate Formation from Ammonia in Gila Sandy Loam at 100% of the Water Holding Capacity.

36, 37, and 38.)

The correlation between reaction and the amount of both nitrites and nitrates formed is again brought out on the fourteenth day, at which time duplicate samples were in good agreement with average pH values of 8.08, nitrite values of 22 p.p.m., and nitrate values of only 3 p.p.m.

The threshold pH value for nitrites appears to lie above that for nitrates. This is shown by the data obtained on the eighteenth day of incubation when duplicate samples had identical nitrite contents of 50 p.p.m., while the pH values were 7.70 and 7.85. The lower of these pH values coincides with the threshold pH value of 7.65 ± 0.05 , which has been noted throughout this work, and the nitrate content of this sample was 89 p.p.m. compared with only 17 p.p.m. in the duplicate sample where the pH value was only 0.15 of a unit higher.

On the twenty-second day, the nitrite content of both samples had dropped to trace quantities, but a close correlation between reaction and rate of formation of nitrates was again apparent; the sample at a pH of 7.50 contained 140 p.p.m. of nitrates, whereas that with a pH of 7.30 had 208 p.p.m.

A maximum of 293 p.p.m. of nitrates was observed in samples at 100% of their water-holding capacities on the thirtieth day of incubation.

In Figure 44 is illustrated the correlation between the

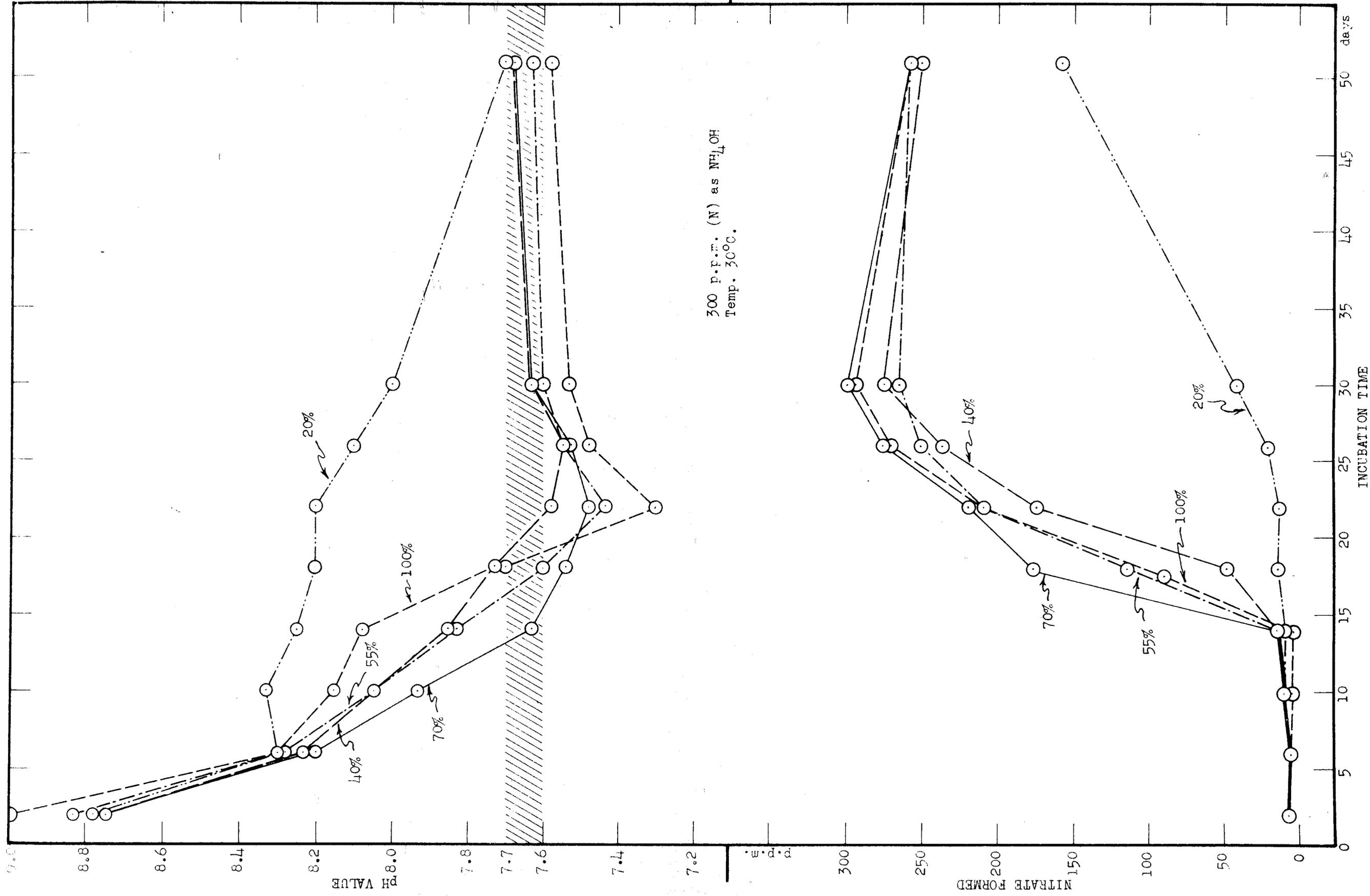


Fig. 44. Parametric Moisture-Content Curves Showing the Rate of Nitrate Formation from Ammonia in Gila Sandy Loam Relative to the Threshold pH Range.

threshold pH value of 7.65 ± 0.05 (denoted by shading) and the inception of nitrification. Moisture content is in this case the parameter.

A study of the rates of change in pH and nitrate content reveals the following significant facts:

The pH values in the several series of samples reached the threshold value in the order 70%, 55%, 100%, 40%, and 20%.

The occurrence of nitrates and the rate of nitrification were in the order 70%, 55%, 100%, 40%, and 20%.

Thus the correlation between the threshold pH and nitrate formation again is positive.

Figure 44 reveals the upward trend of pH after nitrification had been completed and the nitrates began to decrease, as pointed out in a previous section.

Nitrite formation is shown in Figure 45 with the ordinate scale considerably expanded. It will be noted that a moisture content of 70% of the water-holding capacity favored the most rapid nitrite accumulation, 55%, second greatest, 40% and 100%, about equal amounts, and 20% least of all. This correlates very closely with the order of decrease in pH as shown on Figure 44 and the rates of nitrification.

Of the moisture contents studied, 70% of the water-holding capacity is most favorable for nitrite accumulation and for rate of nitrification; 20% is least favorable.

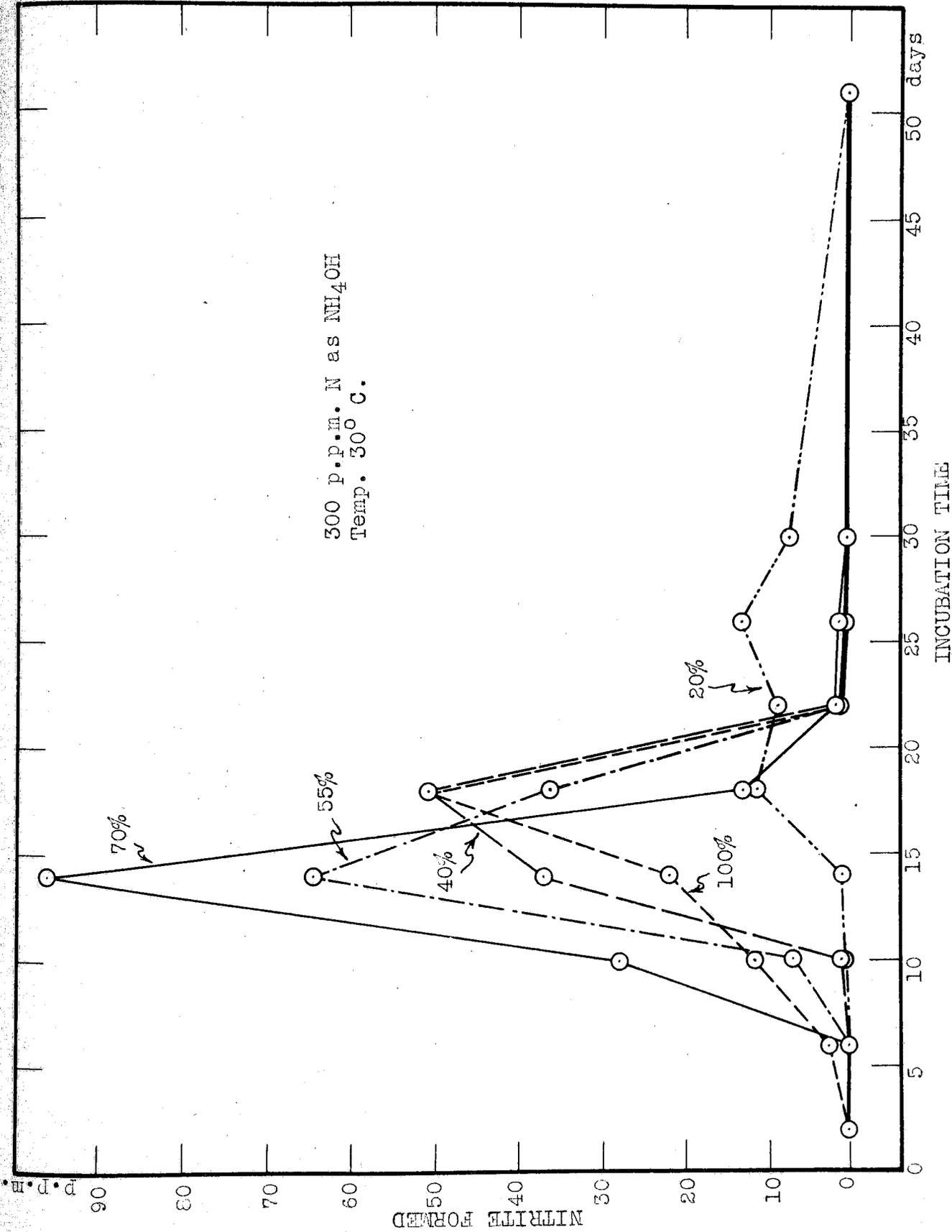


Fig. 45. Parametric Moisture-Content Curves Showing the Rate of Nitrite Formation from Ammonia in Gila Sandy Loam.

Effect of Varying Temperature upon Nitrite and Nitrate Formation

The data for the effect of temperature on the nitrification and nitrification processes are presented in Figures 46 to 52, inclusive.

At 10° C. (Figure 46) none of the ammonia had been oxidized even after forty-six days of incubation. Traces of nitrites were found throughout the period, and nitrates at concentrations of 7 ± 2 p.p.m. The reaction of the samples at the start of incubation was 9.38 and decreased to only 8.10 during the entire period.

At 20° C. (Figure 47) the initial activity of the nitrifying bacteria was slow. A peak value of 74 p.p.m. of nitrites was observed on the twenty-sixth day, however, followed by an immediate increase in nitrates, which indicates that the microorganisms are able to function at this temperature. Nevertheless, this temperature obviously is not very favorable for incubation. The pH on the second day was 9.20 but dropped successively to 7.80 on the twenty-second day when 16 p.p.m. of nitrates were present, to 7.50 on the twenty-sixth day when 25 p.p.m. of nitrates were present, and to 7.40 on the thirty-fifth day at which time the nitrate content had mounted to 200 p.p.m.

The oxidation of ammonia was most rapid at 30° C. (Figure 48). Nitrites mounted rapidly to an average value of 62 p.p.m. on the tenth day, and to 81 p.p.m. on the

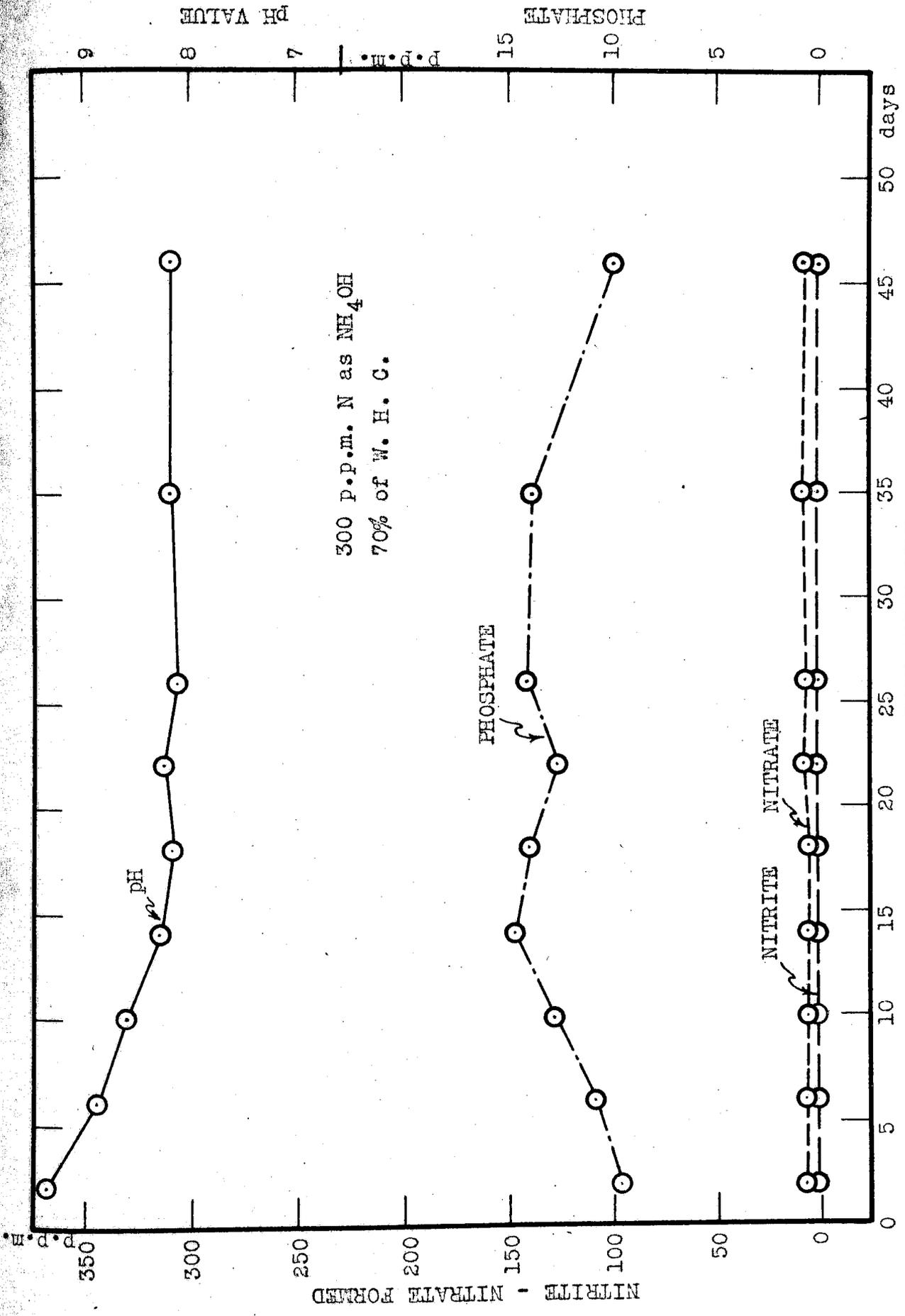


Fig. 46. Rate of Nitrite and Nitrate Formation from Ammonia in Gila Sandy Loam at 10 Degrees Centigrade.

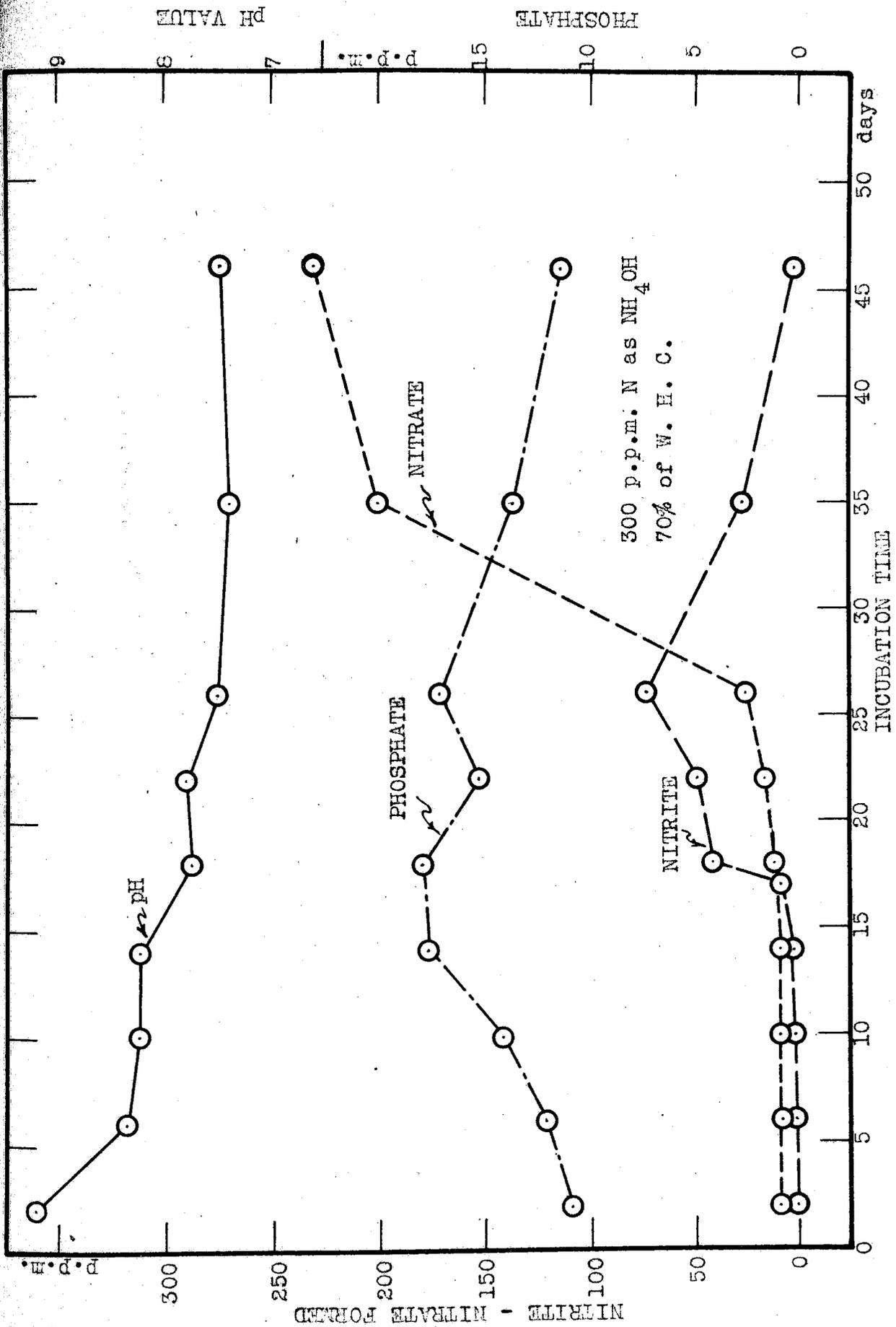


Fig. 47. Rate of Nitrite and Nitrate Formation from Ammonia in Gila Sandy Loam at 30 Degrees Centigrade.

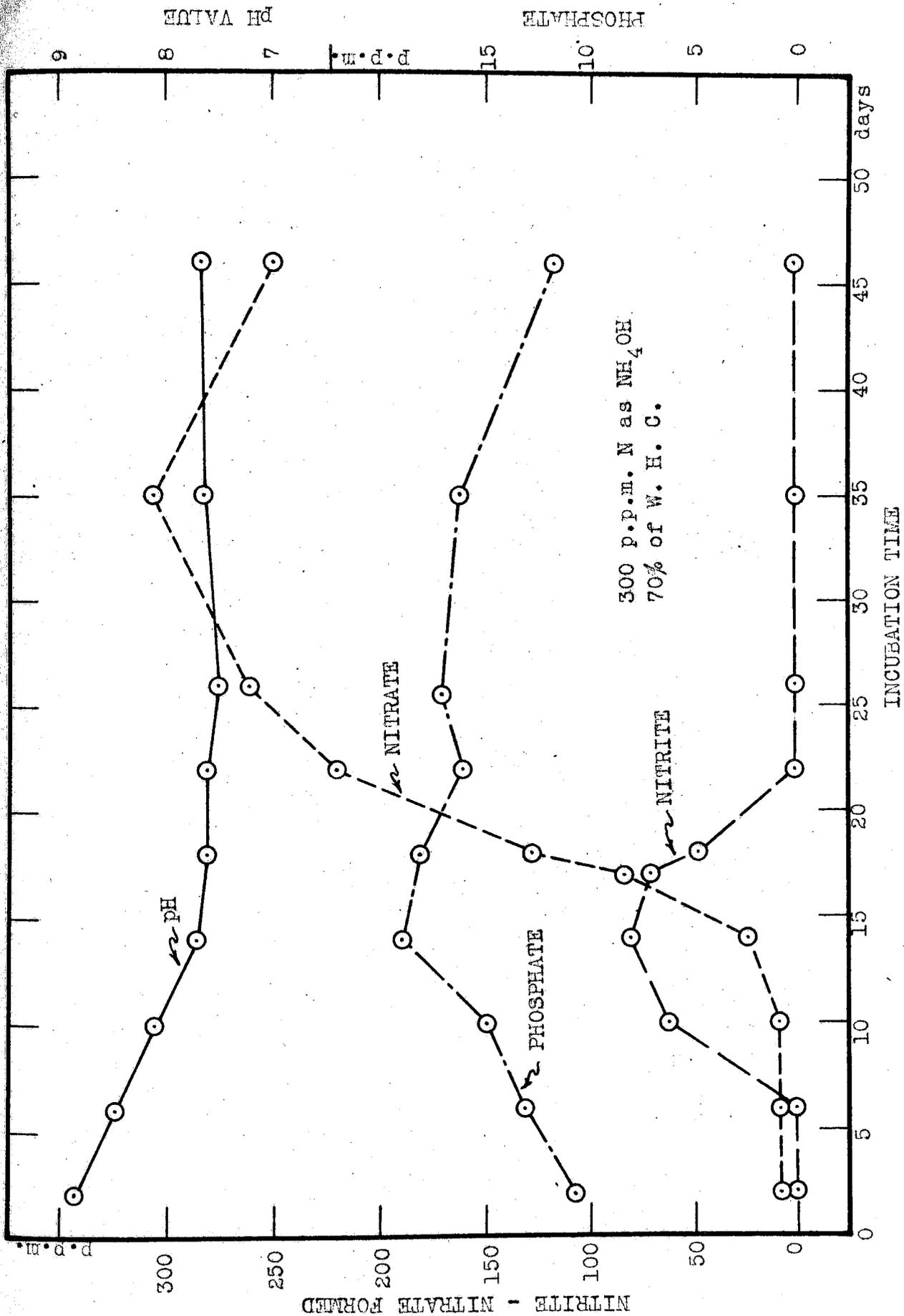


Fig. 48. Rate of Nitrite and Nitrate Formation from Ammonia in Gila Sandy Loam at 30 Degrees Centigrade.

fourteenth day, at which time the first nitrates--25 p.p.m. --appeared with the pH at 7.70. As soon as nitrification began, the nitrites dropped to trace quantities, while the nitrates mounted rapidly, attaining a concentration of over 300 p.p.m. on the thirty-fifth day.

At 40° C. (Figure 49) the activity of the nitrifiers is depressed to some extent. The maximum nitrite accumulation, noted on the seventeenth day, was only 21 p.p.m., but nitrates were not noted until the twenty-sixth day. This is the first time a decrease in nitrites had been observed for such a long period of time prior to nitrification, and may indicate a difference in the effect of this temperature on the nitrite and nitrate forming organisms. When the first significant amounts of nitrates were observed on the twenty-sixth day, duplicate samples failed to agree, but the correlation with soil reaction is such as would be expected from previous observations. One of the duplicate samples, having a pH value of 8.05, had a nitrate content of only 30 p.p.m. whereas the other, with a pH value of 7.50, contained 193 p.p.m.

At 60° C. (Figure 50) microbiological activity was inhibited as completely as at 10° C. Trace quantities of nitrites were detectable throughout the experiment; the nitrates varied from 6 to 8 p.p.m., and the pH value did not vary from 8.3 by more than 0.1 of a pH unit during the entire period of forty-six days.

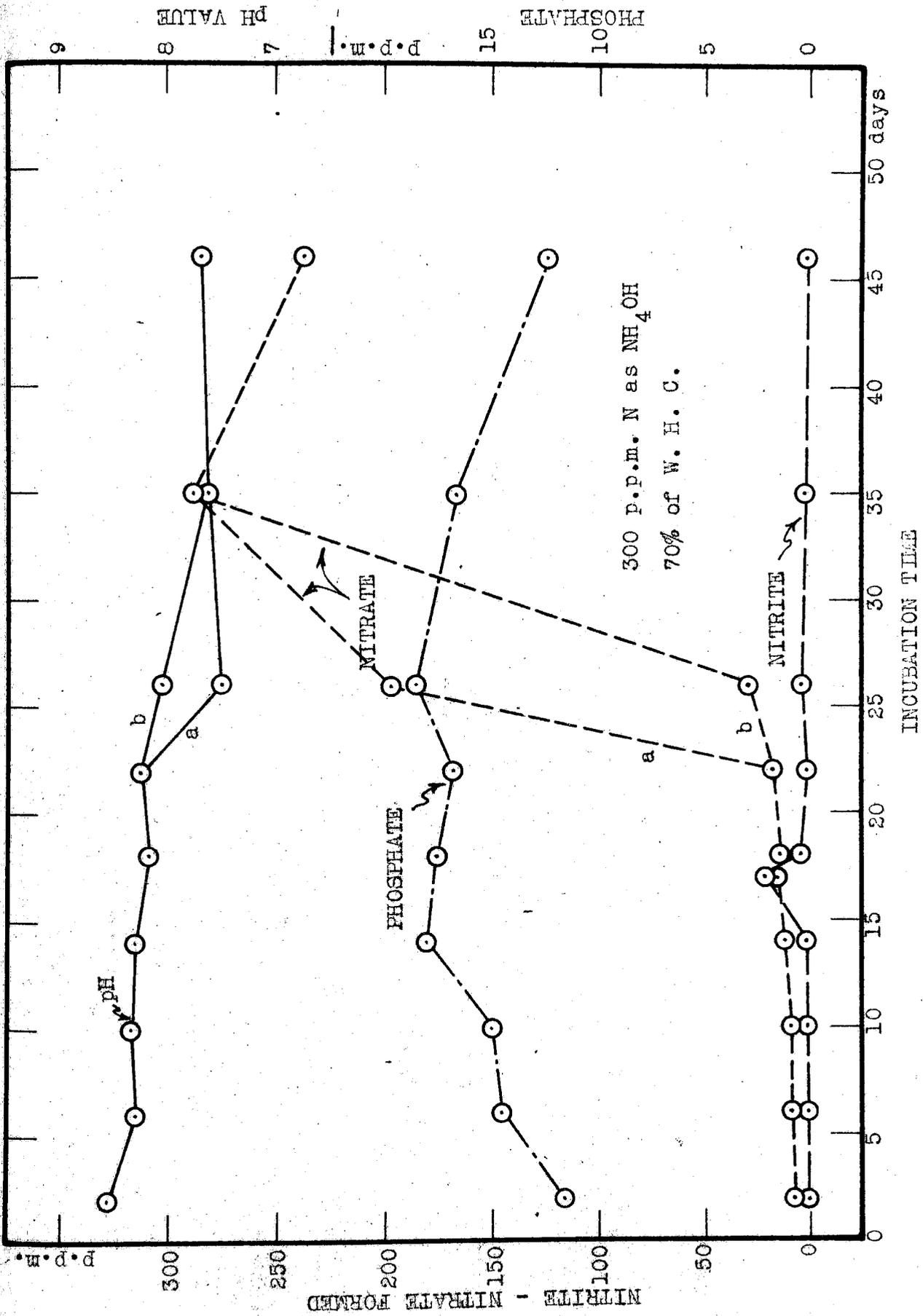


Fig. 49. Rate of Nitrite and Nitrate Formation from Ammonia in Gila Sandy Loam at 40 Degrees Centigrade.

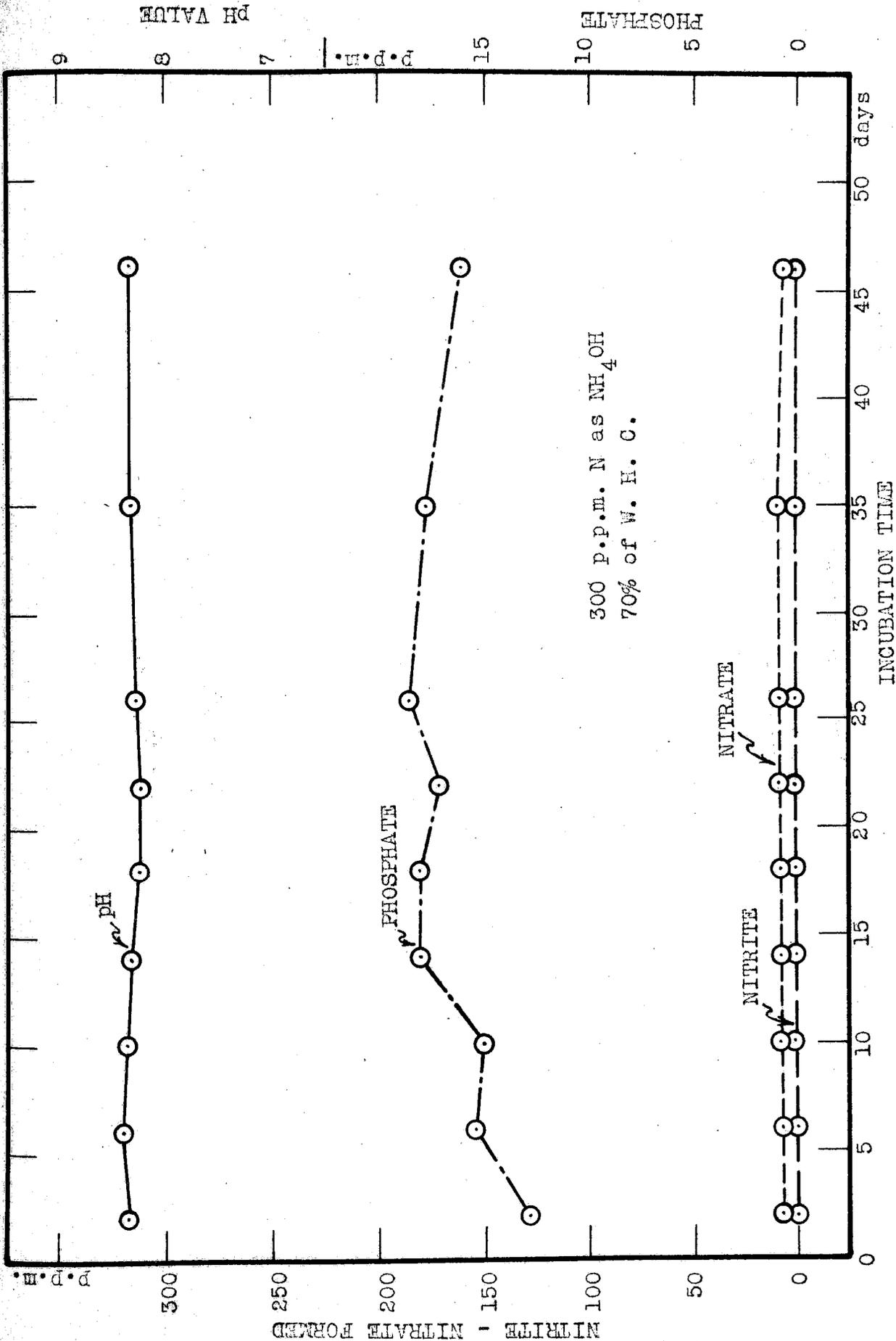


Fig. 50. Rate of Nitrite and Nitrate Formation from Ammonia in Gila Sandy Loam at 60 Degrees Centigrade.

The response of the nitrate formers to a favorable pH in the soil is, however, definitely retarded by a temperature of 20° C., as indicated in Figure 51. Whereas the 30° C. samples were first to reach the critical pH value and had the highest nitrification rate, the 20° C. samples were second to reach a favorable reaction, but had a considerably lower nitrification rate than the 40° C. samples. This appears to indicate that a temperature of 40° C. was less harmful to the nitrate formers than to the nitrite formers, or, by the same token, that 20° C. is a less harmful temperature to the nitrite formers than to the nitrate formers.

Although the accumulation of nitrites was not so great at 20° C. as at 30° C., as shown in Figure 52, the effect on the length of time during which considerable quantities of nitrites are formed in the samples at these two temperatures is very striking. Whereas it can be stated definitely that nitrites are formed at 30° C. during the interval from the tenth through the eighteenth day of incubation, they are known to have been present in the 20° C. samples to a highly significant extent from the eighteenth through the thirty-fifth day of incubation.

Of the temperatures studied, 30° C. was the most favorable for the extent of nitrite formation and rate of nitrification; 20° C. favored the accumulation of nitrites over the longest experimental period; 10° C. and 60° C. were

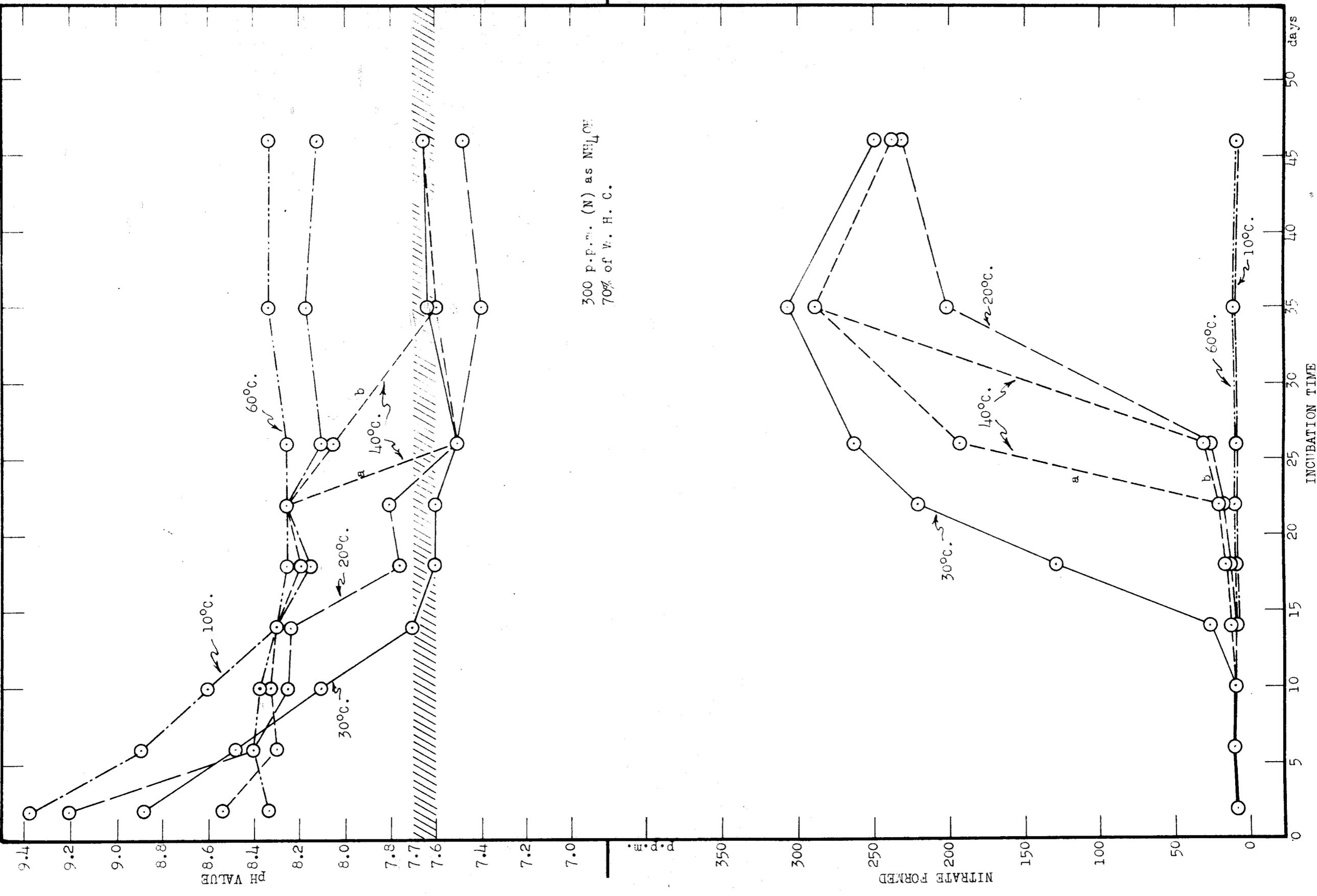


Fig. 51. Parametric Temperature Curves Showing Rate of Nitrate Formation from Ammonia in Gila Sandy Loam Relative to the Threshold pH Range.

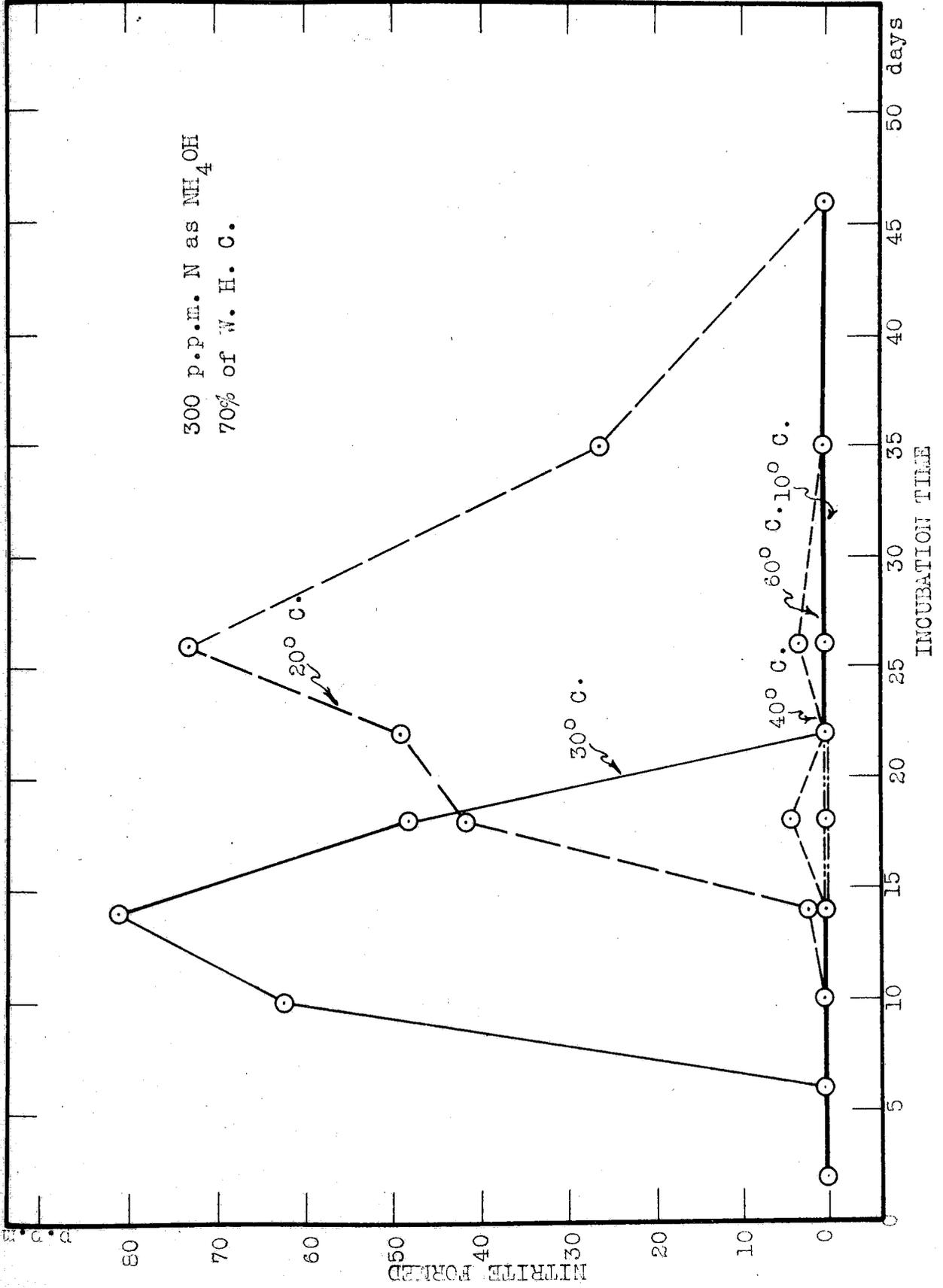


Fig. 52. Parametric Temperature Curves Showing the Rate of Nitrite Formation from Ammonia in Gila Sandy Loam.

definitely inhibitory to the nitrifiers.

Effect of Varying the Amount of Nitrogen Applied
on Nitrite and Nitrate Formation

The data showing the extent to which the amount of nitrogen applied affects the nitrification of ammonia are presented in Figures 53 to 59, inclusive.

The untreated samples (Figure 53) show an accumulation of about 30 p.p.m. of nitrates during the fifty days of incubation. During this entire period the pH values remained between 7.8 and 8.0, indicating further that if optimum conditions other than pH prevail sufficiently long, the reaction may become favorable for the activity of the organisms within the soil aggregates.

The samples treated with 50 p.p.m. of nitrogen as ammonia (Figure 54) show a maximum of 19 p.p.m. of nitrite as early as the sixth day of incubation. By the tenth day nitrites decreased and nitrates had increased to 43 p.p.m. while the soil reaction was 7.65. From this time on the nitrates increased steadily until the thirtieth day of incubation, attaining a concentration of 80 p.p.m. This value agrees well with the nitrates observed in the untreated samples. After nitrification has once begun, the pH of the samples usually remains constant at 7.75 ± 0.05 .

The samples treated with 100 p.p.m. of nitrogen as ammonia (Figure 55) show a maximum nitrite accumulation of

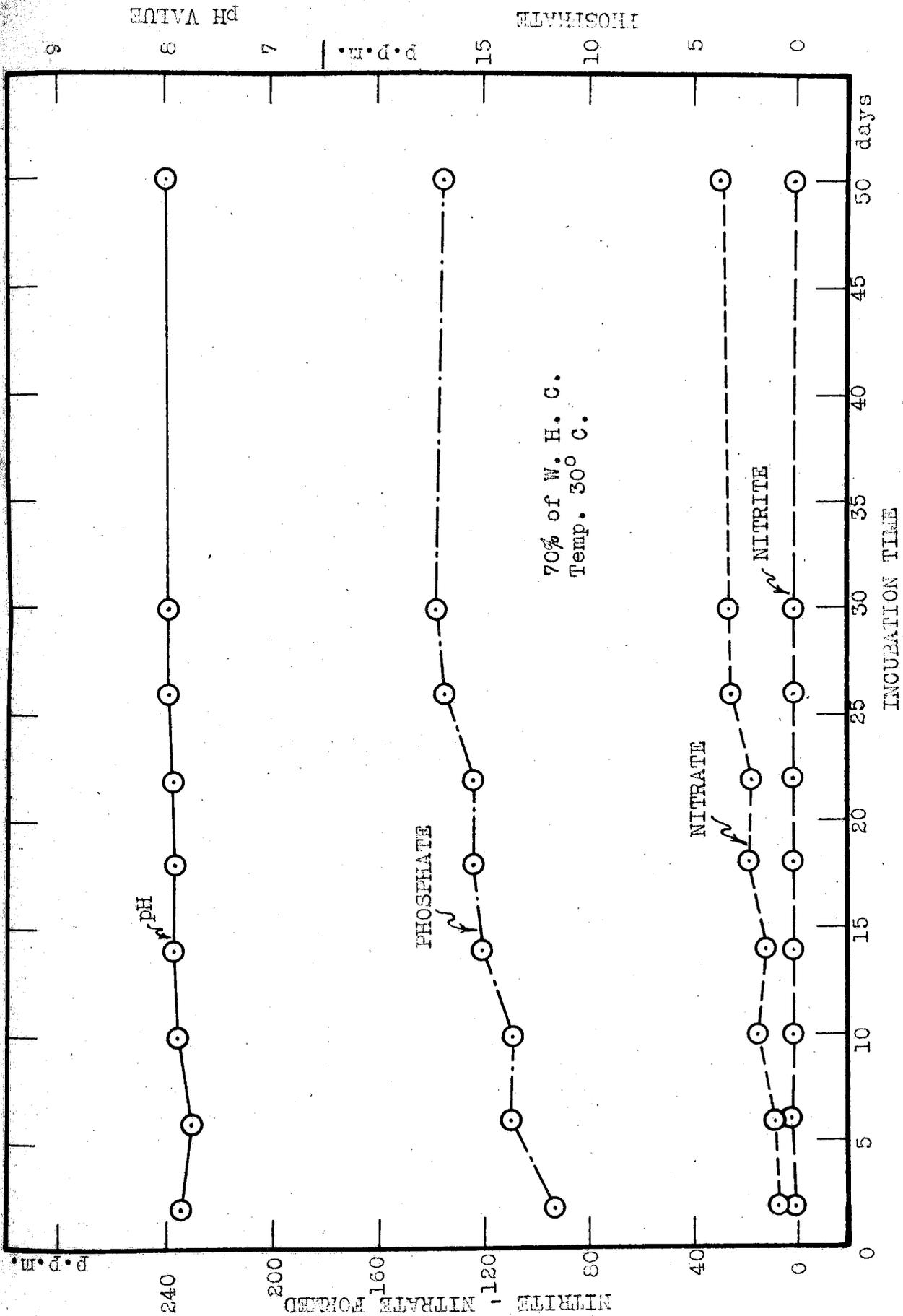


FIG. 53. Rate of Nitrite and Nitrate Formation in Gila Sandy Loam in Relation to an Initial Application of 0 p.p.m. of Nitrogen as Ammonia.

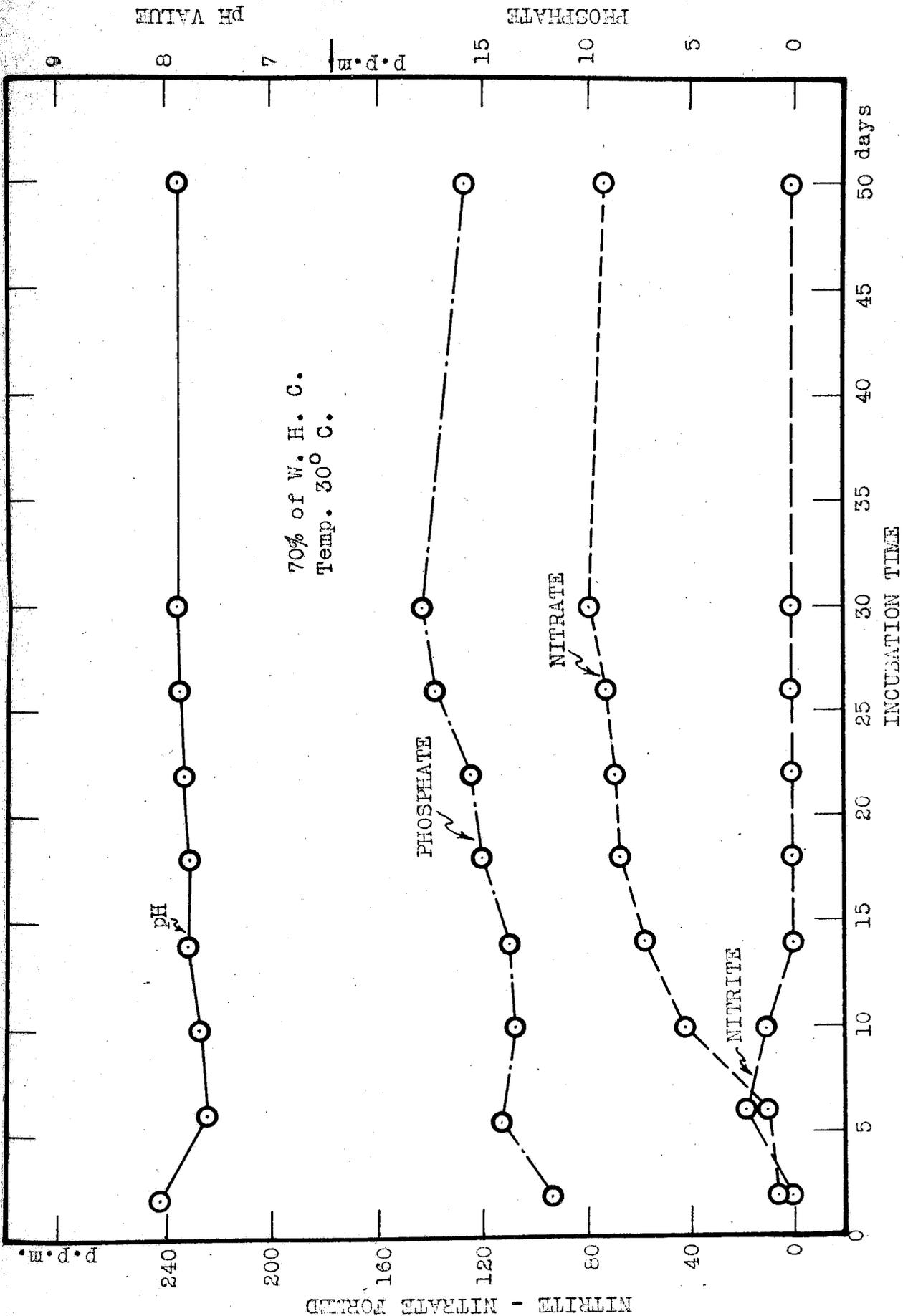


FIG. 54. Rate of Nitrite and Nitrate Formation in Gila Sandy loam in Relation to an Initial Application of 50 p.p.m. of Nitrogen as ammonia.

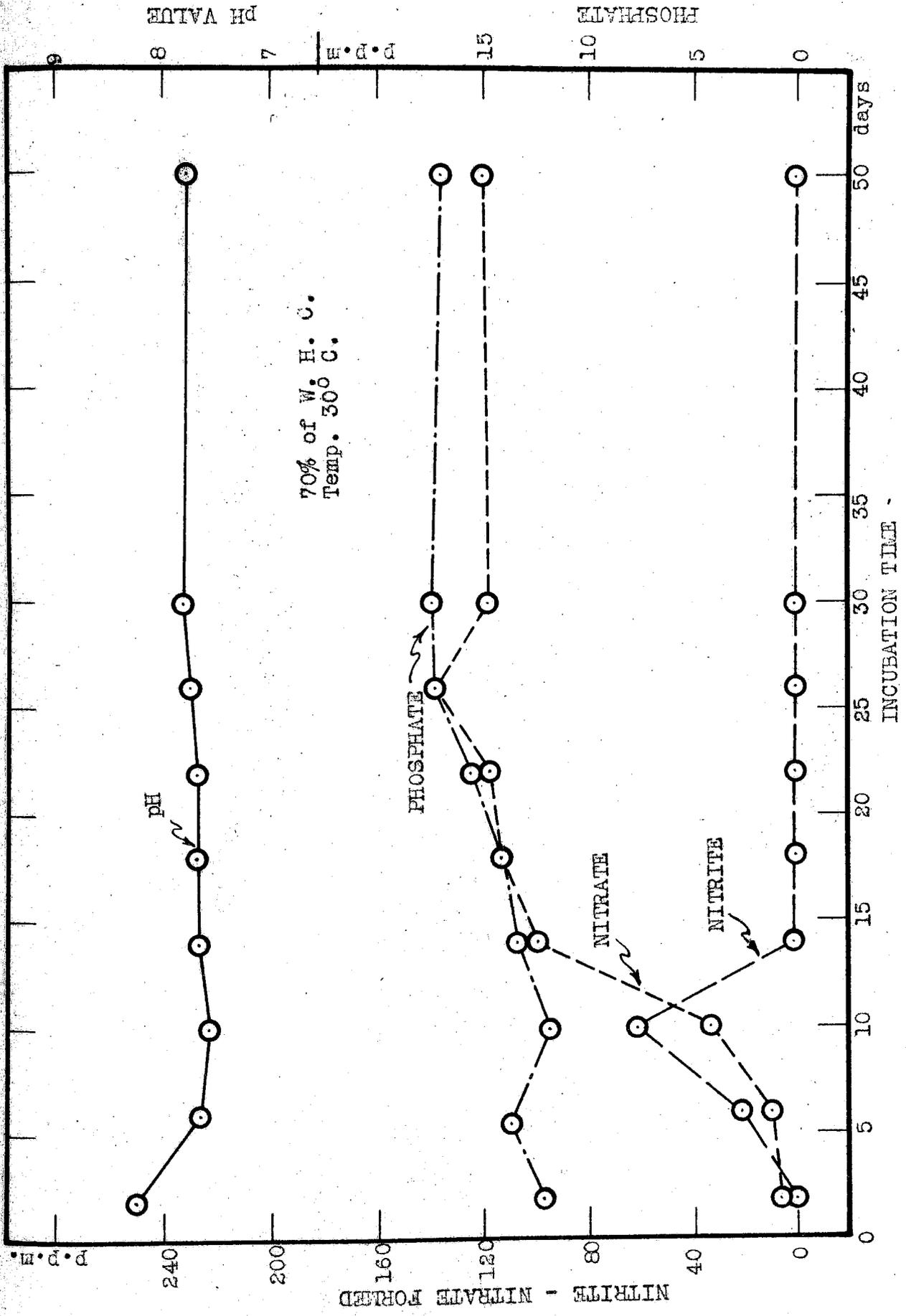


Fig. 55. Rate of Nitrite and Nitrate Formation in Gila Sandy Loam in Relation to an Initial Application of 100 p.p.m. of Nitrogen as Ammonia.

62 p.p.m. on the tenth day of incubation. This is the highest nitrite build up (expressed in terms of percentage of the nitrogen applied to the soil) to be found in any of the experiments on this phase of the problem. The accumulation equals 62% of the total nitrogen added as ammonia, or 45% of the nitrogen corresponding to the peak value for nitrates on the twenty-sixth day.

The nitrates in these samples first appeared to increase appreciably on the tenth day when 33 p.p.m. were observed at a pH value of 7.58. As noted in previous cases, the nitrites fall off to trace quantities as soon as the nitrates begin to accumulate.

The samples treated with 200 p.p.m. of nitrogen as ammonia (Figure 56) showed a behavior very similar to those treated with 100 p.p.m. except for the magnitude of values. The first appearance of nitrite, 17 p.p.m., was observed on the sixth day, increasing to 73 p.p.m. by the tenth day. Since the decrease in nitrites is not so rapid as had been observed previously, it may be assumed that the 73 p.p.m. is a false maximum and that the actual maximum probably occurred sometime between the tenth and fourteenth days.

The first nitrates, 22 p.p.m., appeared when the pH had dropped to 7.60, then climbed rapidly to a value of 224 p.p.m. on the thirtieth day. The value of 165 p.p.m. on the twenty-second day which breaks the smoothly ascending rate curve is the average of duplicate samples and is

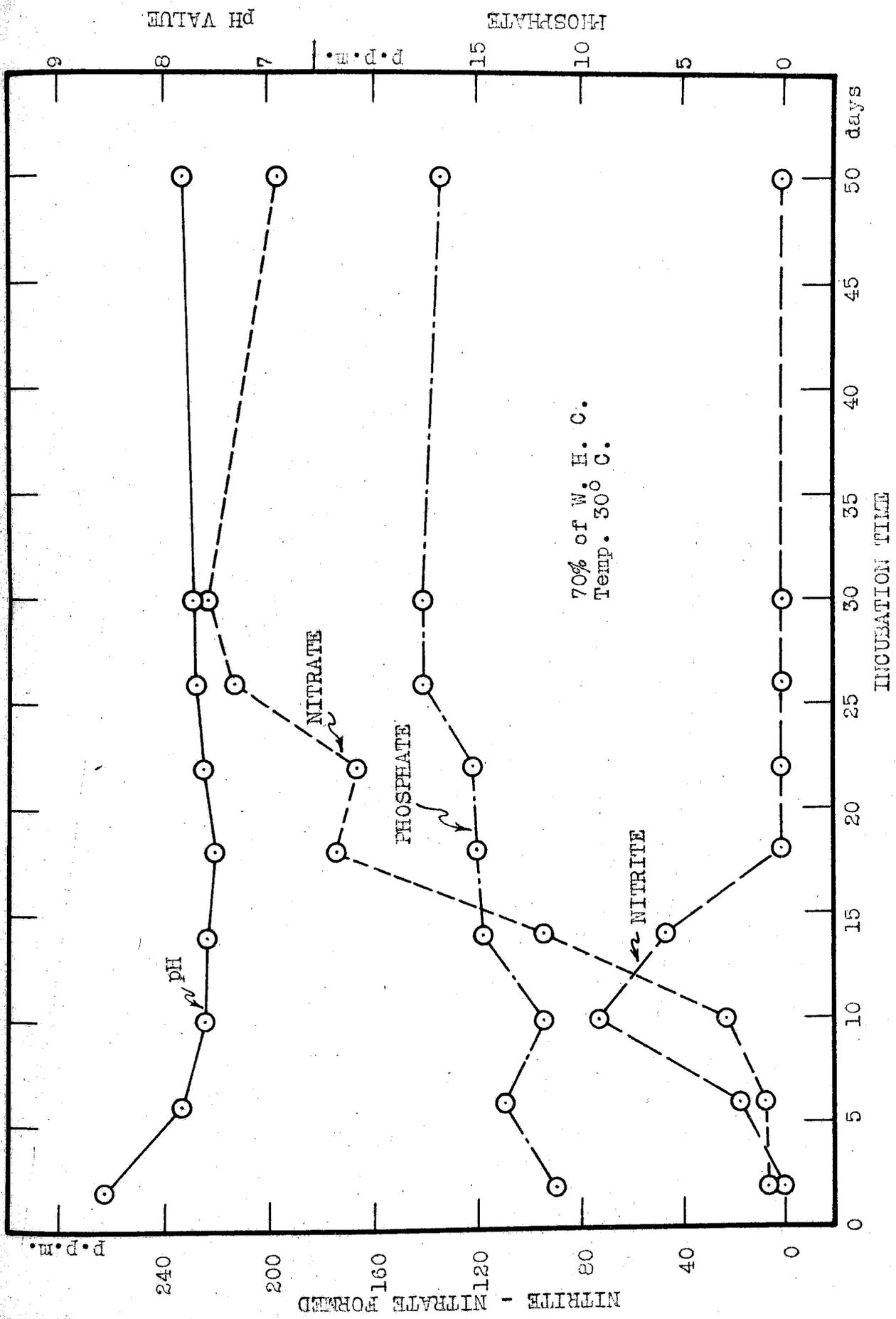


Fig. 56. Rate of Nitrite and Nitrate Formation in Gila Sandy Loam in Relation to an Initial Application of 200 p.p.m. of Nitrogen as Ammonia.

considered to be significant. One of the samples contained 140 p.p.m. of nitrates at a pH of 7.55 and the other 190 p.p.m. at a pH of 7.65. Since the pH value of each of the two samples was below the critical value of 7.65, there seems to be no justification for discarding either of the values.

Whereas the first appreciable nitrate was noted in the samples treated with 50 p.p.m., 100 p.p.m., and 200 p.p.m. on the tenth day of incubation (Figures 54, 55, and 56), it will be noted that both nitritification and nitrification occurred at an appreciably slower rate when a concentration of 300 p.p.m. of ammonia was present (Figure 57). Duplicate samples on the tenth day showed an average nitrite value of 19 p.p.m. at a pH value of 8.10. The nitrates on this date were less than 10 p.p.m. On the fourteenth day, however, with the pH value 7.68, the nitrites had jumped to 81 and the nitrates to 21 p.p.m. The nitrates mounted rapidly to a maximum of 284 p.p.m. on the thirtieth day, and the nitrites fell off to trace quantities after having attained a concentration of 82 p.p.m. on the eighteenth day. The plateau in the nitrite curve from the fourteenth to the eighteenth day probably indicates that the value taken on the fourteenth day was an ascending, and that on the eighteenth day a descending value, and that the actual maximum is not known.

Of the concentrations at which nitrogen was applied,

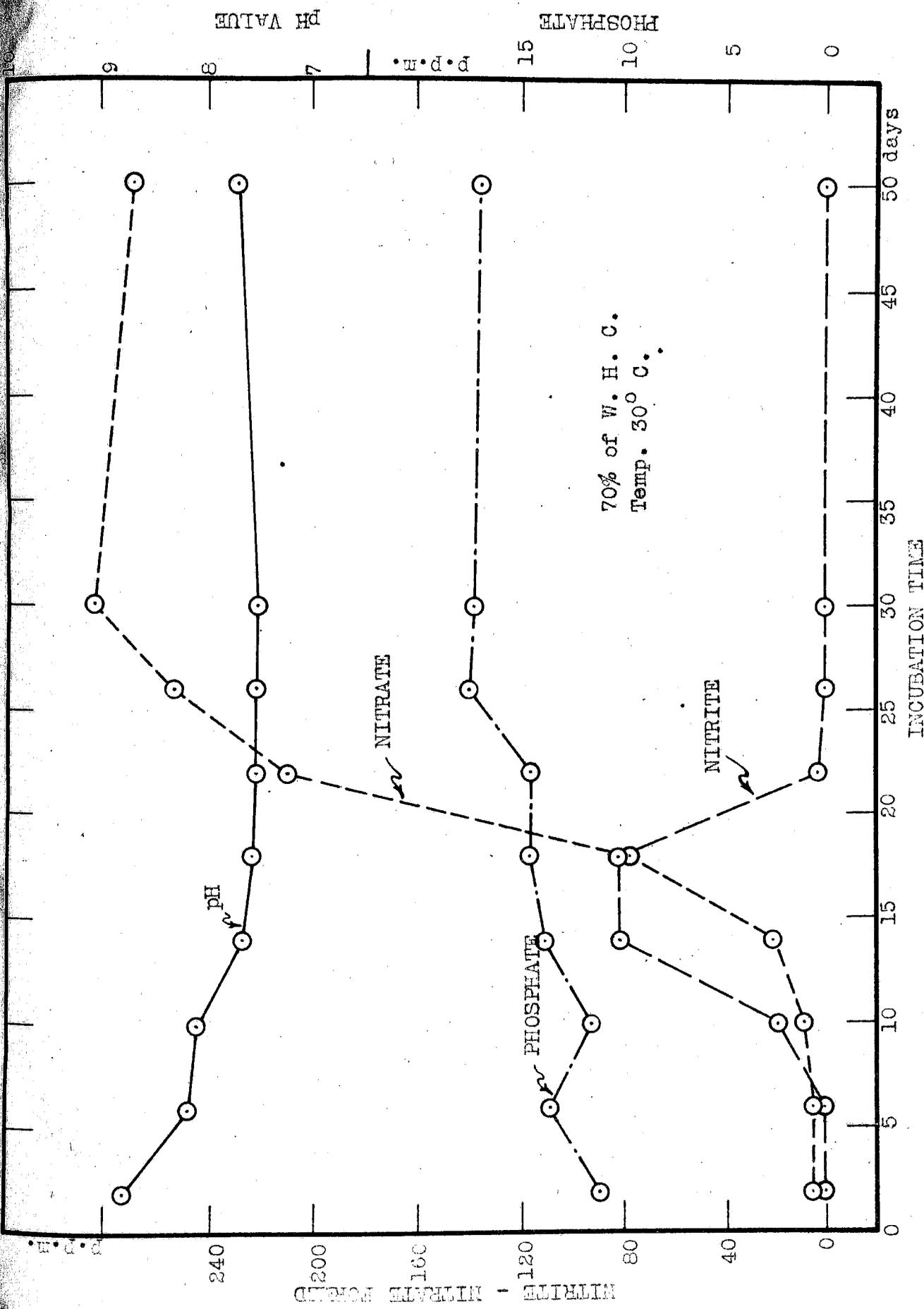


Fig. 57. Rate of Nitrite and Nitrate Formation in Gila Sandy Loam in Relation to an Initial Application of 300 p.p.m. of Nitrogen as Ammonia.

100 p.p.m. favored the largest nitrite accumulation in terms of percentage of nitrogen observed at the peak value for nitrates, but 300 p.p.m. favored the greatest nitrite accumulation on the basis of weight concentration. Rates of nitrification and nitratification were appreciably higher with applications of 50 p.p.m., 100 p.p.m., and 200 p.p.m. than they were with 300 p.p.m.

Relationship between pH Value and Nitrate Formation at Various Ammonia Concentrations

The data presented in Figure 58 give a clear picture of the relationship between the pH value and rate of nitratification of ammonia. Disregarding the untreated sample which had a pH value above the critical value of 7.65, and in which nitrification occurred only over a long period of time, it will be noted that the initial pH values of the treated samples are directly proportional to the amounts of ammonia added to them, e.g. applying 300 p.p.m. of nitrogen as ammonia gave the samples an initial pH of over 8.8; 200 p.p.m., nearly 8.6; 100 p.p.m., over 8.2; and 50 p.p.m., just above 8.0. Likewise, the time lag observed and discussed in Figure 35 is again in evidence here; i.e., the higher the initial pH value, the longer it takes for the critical pH value to be reached, hence the longer it takes for nitratification to occur. There is a close correlation between the amount of ammonia applied, the decrease in pH

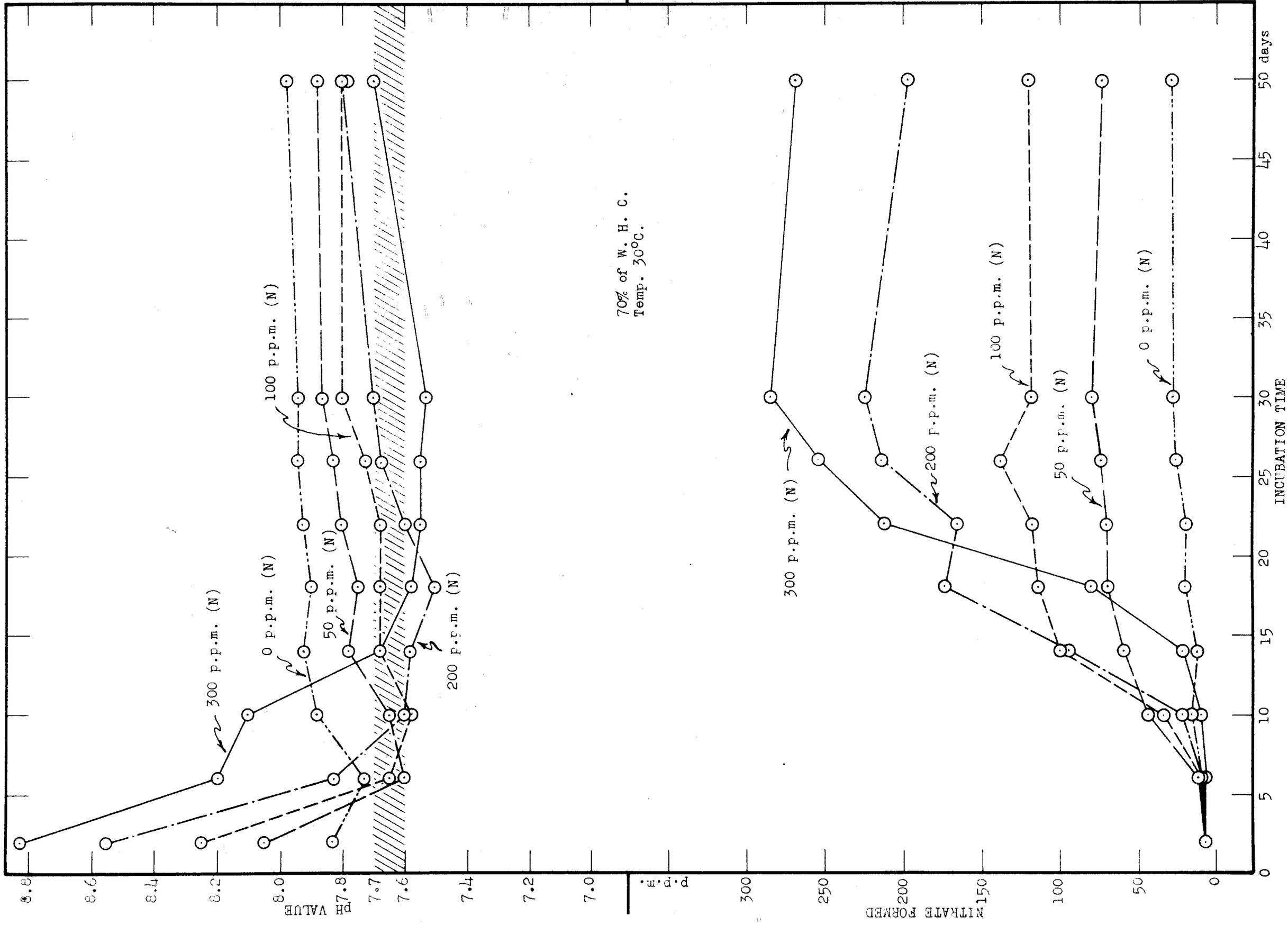


Fig. 58. Parametric Ammonia-Treatment Curves Showing the Rate of Nitrate Formation from Ammonia in Gila Sandy Loam Relative to the Threshold pH Range.

to 7.65, and the rate of nitrification.

This study would indicate that the application of ammonia at even as high a concentration as 300 p.p.m. of nitrogen is a safe practice, at least on soils of the sandy loam type as used here. A continuation of this study to determine the nitrogen concentration at which ammonia becomes toxic to the nitrifiers would be of considerable practical and theoretical interest.

As previously noted, there is a tendency for the pH curves to increase and the nitrates correspondingly to decrease in value after the maximum has been reached.

In the study of nitrite changes with time for the several levels of ammonia application, it is interesting to note the shapes of the nitrite curves in Figure 59. In the discussion of Figure 55 the fact was noted that the percentage of nitrogen observed as nitrites was greatest in the sample treated with 100 p.p.m. of nitrogen. Considering the entire family of curves, the one representing the 100 p.p.m. concentration appears most likely to represent the true maximum value. In the 50 p.p.m. samples, the maximum appears to have been missed between the tenth and the fourteenth days; and in the 300 p.p.m. samples, between the fourteenth and the eighteenth days. Since we do not know the true shape of the nitrite curve, accurate extrapolation is not possible, but the similarity between the 100 p.p.m. curve and portions of the 300 p.p.m. curve conveys the impression

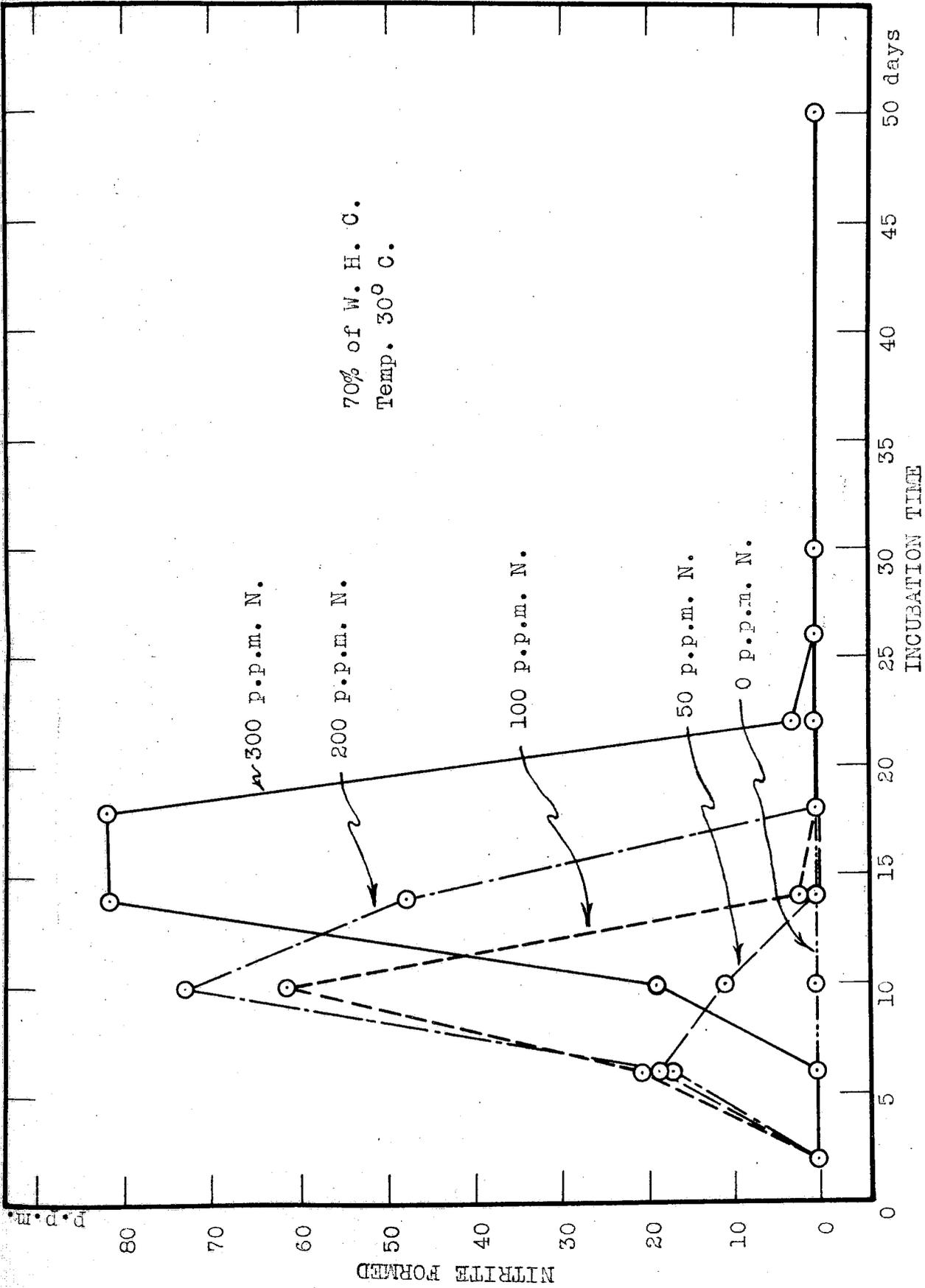


Fig. 59. Parametric Ammonia-Treatment Curves Showing the Rate of Nitrite Formation from Ammonia in Gila Sandy Loam.

that the shape of the latter may be the correct one. In view of this observation it is interesting to note that if the ascending and descending lines of the 300 p.p.m. curve are continued until they cross, the peak has a value of approximately 120 p.p.m. of nitrite, which corresponds to 40% of the total nitrogen applied.

Before more definite conclusions can be drawn concerning the formation of nitrites and their relationship to the incubation conditions, it is desirable that the shape of this curve be more accurately established.

Rate of Nitrification of Ammonia in Comparison
with that of Other Nitrogen Compounds

Figures 60 to 65, inclusive, present the results of a study showing the rates of nitrite and nitrate formation in soils treated with ammonium sulfate, monoammonium phosphate, ammonia in the presence of calcium sulfate, and ammonia in the presence of sulfur.

Although the rate of nitrification of ammonium sulfate in cultivated Gila sandy loam was reported in Figures 10 and 24, and the nitritification and nitratification rates for ammonium sulfate in conjunction with saturated calcium hydroxide were reported in Figure 37, the nitritification and nitratification characteristics have not heretofore been separately determined for ammonium sulfate when used alone. The results of such a study are presented in Figure 60, and

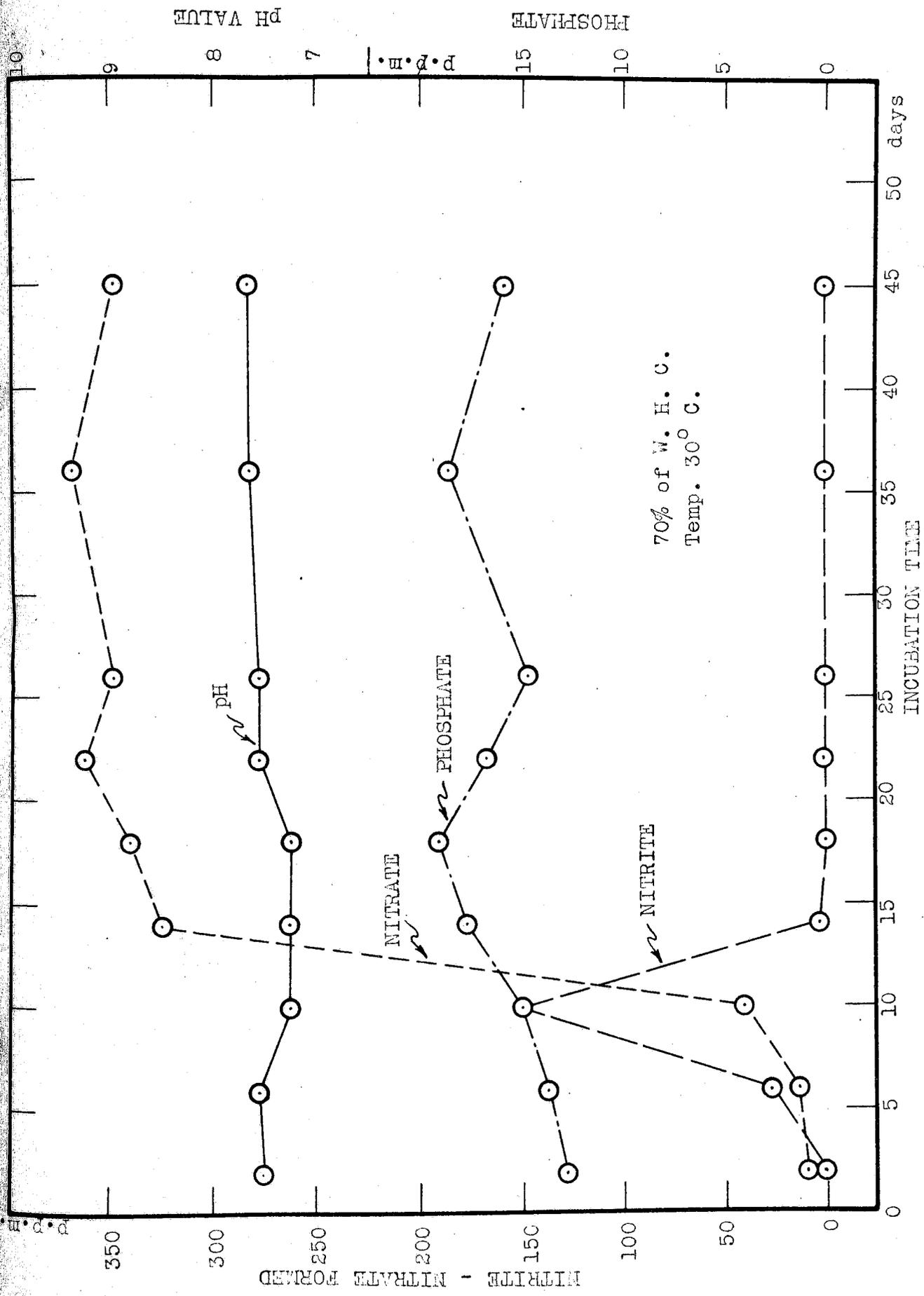


Fig. 60. Rate of Nitrite and Nitrate Formation in Gila Sandy Loam Treated with 300 p.p.m. of Nitrogen as Ammonium Sulfate.

are interesting when considered in the light of the three figures mentioned above.

On the sixth day of incubation, 27 p.p.m. of nitrite were already present, and on the tenth day a maximum value of 146 p.p.m. had been reached, in contrast with approximately 185 p.p.m. on the eleventh day of incubation in the series of samples to which calcium hydroxide was initially added with the ammonium sulfate (Figure 37). This may be in apparent contradiction to the conclusion regarding the effect of calcium hydroxide on nitrites presented in the discussion of the effect of maintaining the pH above the threshold value (Figure 36), but it must be remembered that in the latter study the initial pH was above 8.7 whereas it was only 7.75 in the study in which calcium hydroxide was added to the soil only at the time of applying the ammonium sulfate (Figure 37), and only 7.50 in the present study in which ammonium sulfate was added to the soil without calcium hydroxide (Figure 60).

The first nitrate formation was observed on the tenth day of incubation when 41 p.p.m. of nitrates were observed in samples having a pH value of 7.25. Then, in four days' time, the nitrates increased to 324 p.p.m., the pH remaining constant. A maximum nitrate value of 369 p.p.m. was recorded on the thirty-sixth day.

Since the soil nitrogen in the untreated samples (Figure 53) showed a nitrate accumulation of only about 30 p.p.m.,

this value of nearly 370 p.p.m. of nitrates would appear to be in error by about 40 p.p.m. It is not definitely known, however, whether all of the naturally occurring nitrogen in the untreated samples appeared as nitrates before the end of the incubation, since the pH remained above the critical value of 7.65 throughout the entire study. On the other hand, the maxima reached in the series of samples treated with 50 p.p.m., 100 p.p.m., and 200 p.p.m. of nitrogen (Figures 54, 55, and 56) make it appear that 30 p.p.m. closely approximates the nitrifiable nitrogen naturally present in the soil. When it is realized that a factor of 250 had to be applied to the photelometer readings at these high nitrate concentrations, an error of 0.16 p.p.m. or a variation in the soil sample of only five times this figure -- 0.80 p.p.m. -- would cause this apparently large error. An error of this magnitude is therefore not serious.

The rates of nitrification of ammonium sulfate as shown in Figures 10, 37, and 60 are almost identical, notwithstanding the addition of saturated calcium hydroxide. Likewise, there is very little, if any, difference in the time lag before nitrification begins.

In Figure 61 are presented the data for the study involving monoammonium phosphate. Like ammonium sulfate, its maximum nitrite value of 96 p.p.m. was observed on the tenth day.

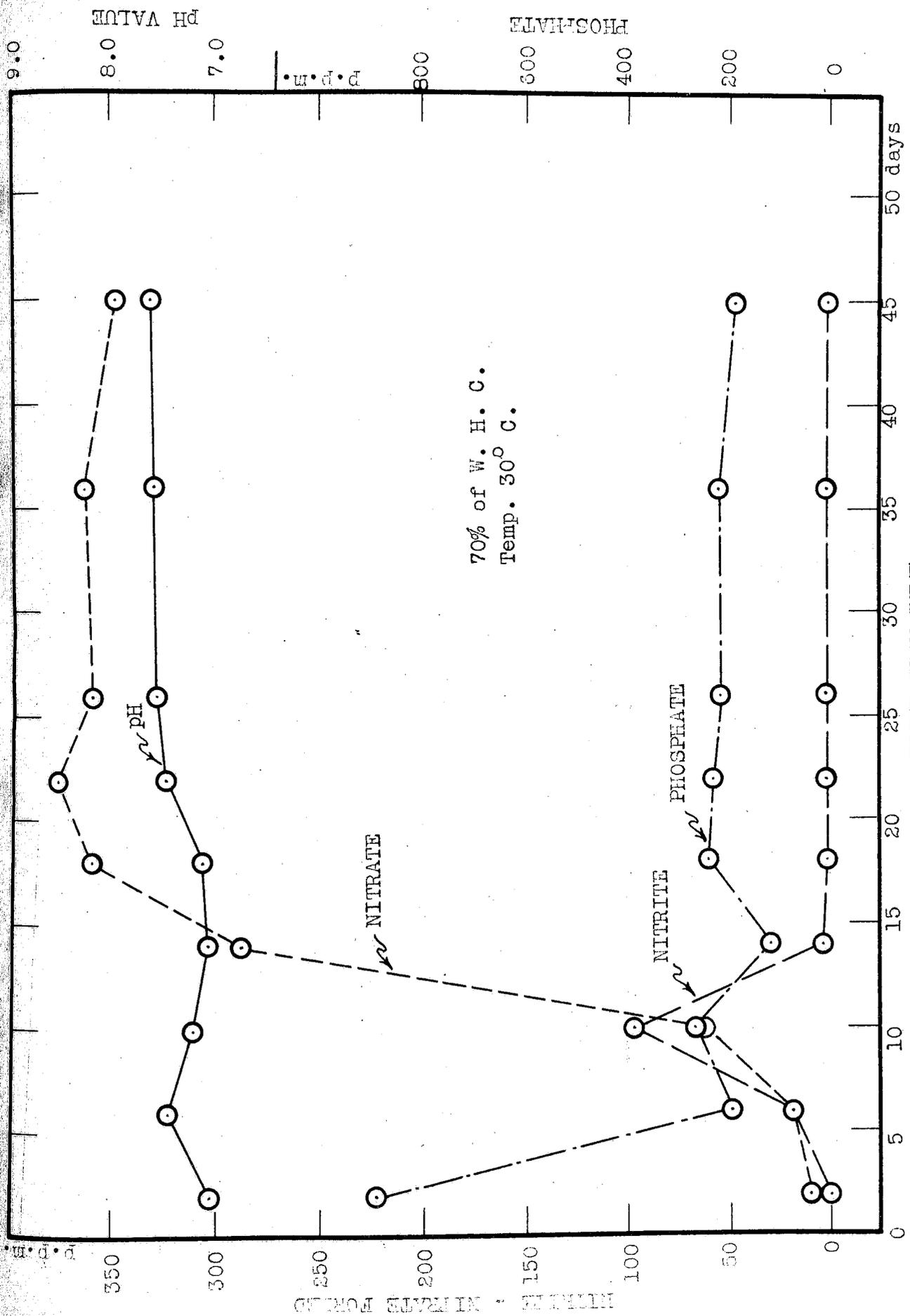


Fig. 61. Rate of Nitrite and Nitrate Formation in Gila Sandy Loam Treated with 300 p.p.m. of Nitrogen as Monoammonium Phosphate.

The nitrification rate in monoammonium phosphate was slightly slower at the beginning as compared with ammonium sulfate, but once nitrates began to form, the rates became so nearly identical that the curves are superimposable. The maximum nitrate value of 375 p.p.m. was recorded on the twenty-second day. The pH value in all cases remained below the critical value of 7.65 throughout the forty-five days of incubation.

Effect of Calcium Sulfate on the Nitrification of Ammonia

In Figure 62 are presented the results of a study on the effect of applying calcium sulfate to the soil in conjunction with ammonia. A comparison of this figure with Figure 31 in which ammonia alone was applied to the soil shows that with the exception of an approximate initial pH value of 9.0 in the latter case as compared with 8.5 when calcium sulfate was added with the ammonia, the results in the two cases are identical. In both instances the first nitrite formation was noted on the fourteenth day and the first nitrate on about the nineteenth day. The nitrite accumulation occurred over a somewhat longer period when the ammonia alone was used, and attained a greater maximum, approximately 90 p.p.m. as compared with 60 p.p.m., when calcium sulfate was added. The shape of the nitrite curve in Figure 62 suggests, however, that the maximum value was not recorded for nitrites.

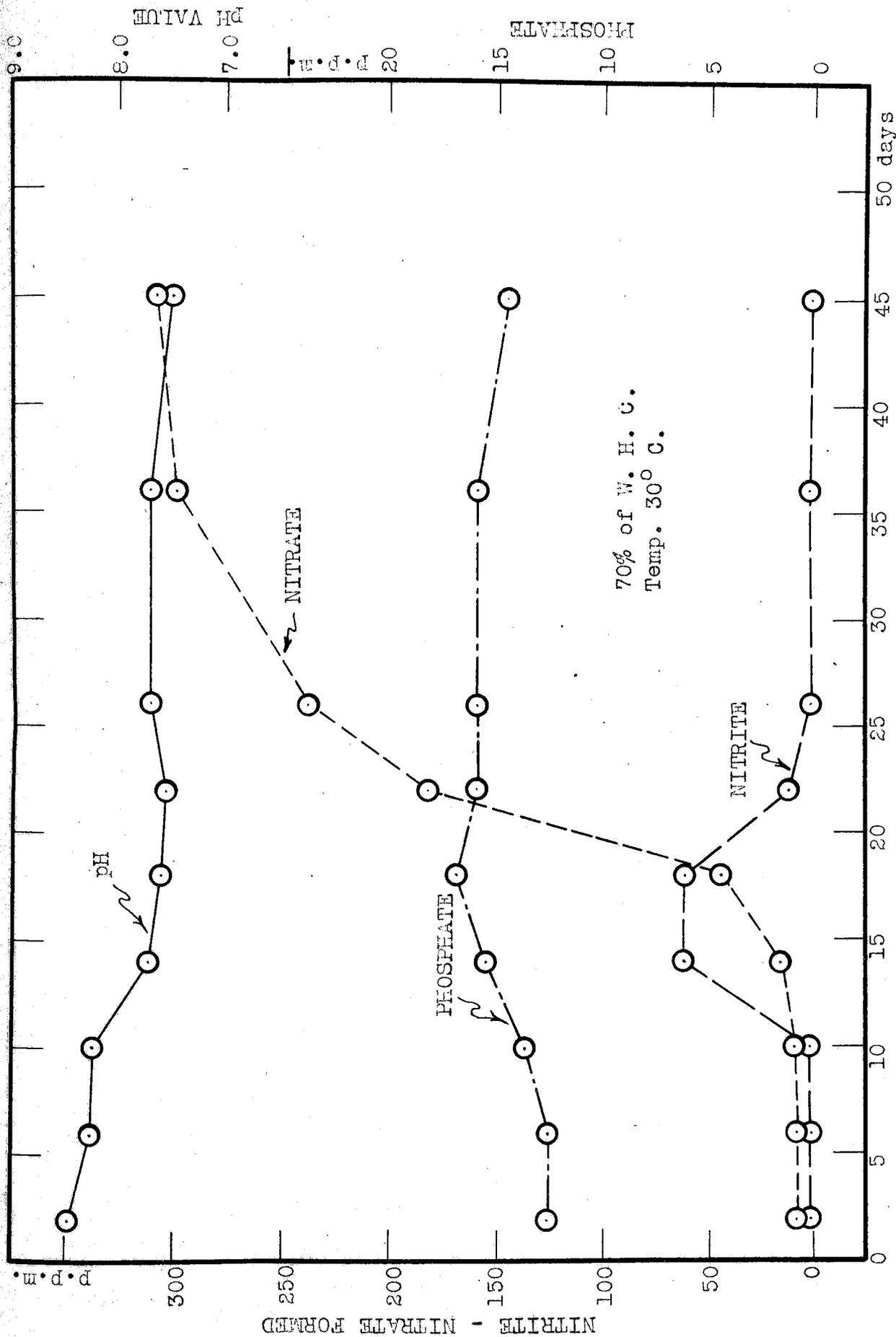


Fig. 62. Rate of Nitrite and Nitrate Formation in Gila Sandy Loam Treated with 300 p.p.m. of Nitrogen as Ammonia Plus Calcium Sulfate.

The initial rates of nitrification for the treatments ammonia plus calcium sulfate (Figure 62) and ammonia alone (Figure 31) are almost identical. In both studies the pH of the samples had decreased to a value below 7.65 before nitrification began.

Effect of Sulfur on the Rate of Nitrification
of Ammonia

The effect of added sulfur on the nitrification and nitrification rates of ammonia is shown in Figure 63. Although the pH decreased from an initial value of 7.98 to as low as 5.43 in duplicate samples by the thirty-sixth day of incubation, only trace quantities of nitrites were ever observed, and the maximum nitrate value obtained was 7 p.p.m. on the thirty-sixth day.

The pH values of the duplicate samples on the last day of incubation were for some unaccountable reason widely at variance, namely 7.20 and 5.20, but there was only a trace of nitrite and an insignificant quantity of nitrate present in either case.

The drop in pH in the samples treated with sulfur probably results from the oxidation of the sulfur to sulfuric acid by the sulfur bacteria of the soil. It is significant that no nitrites or nitrates accumulated during this oxidation process. Perhaps the oxidation of ammonia was prevented by the formation of toxic products for the nitrifi-

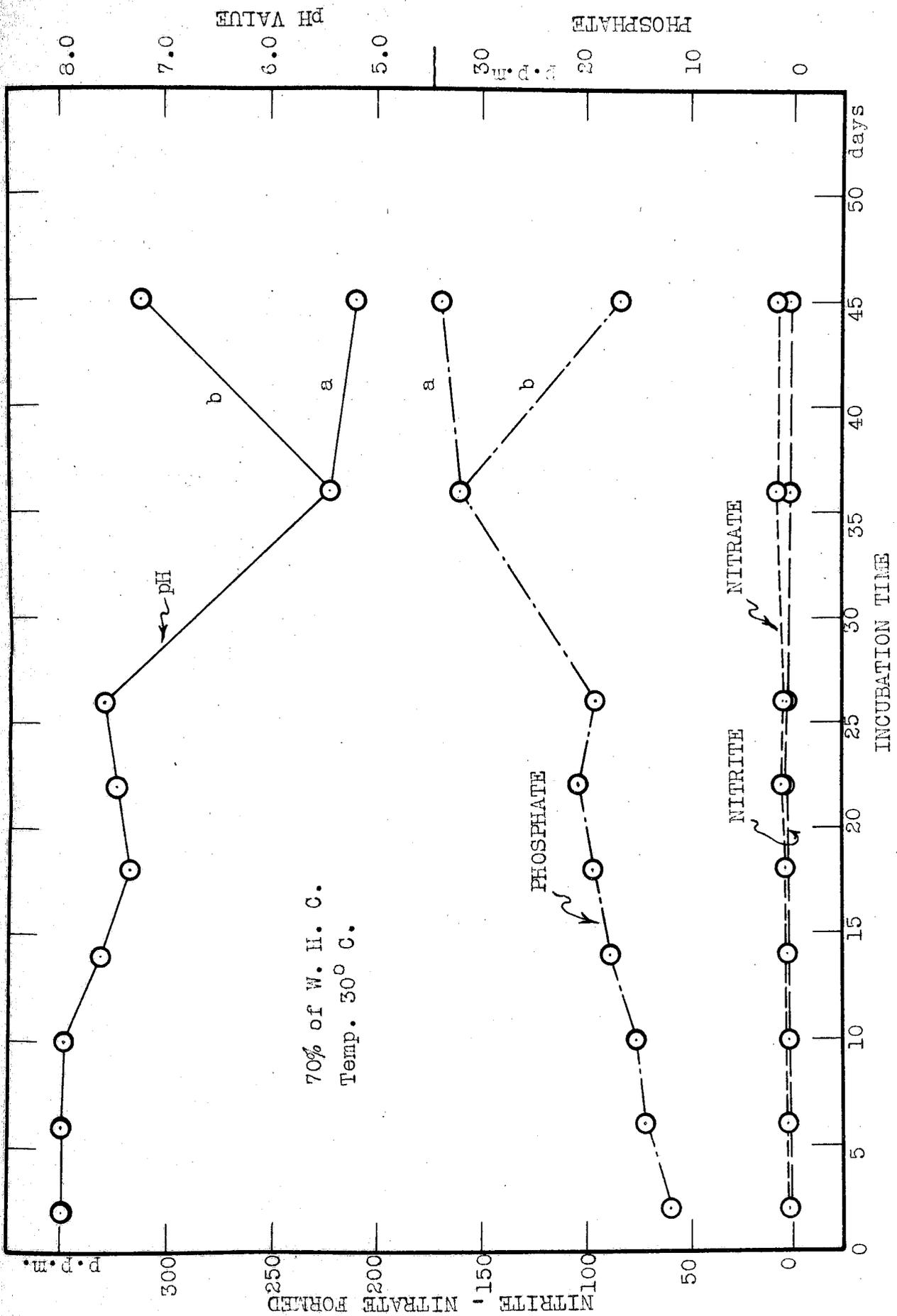


Fig. 63. Rate of Nitrite and Nitrate Formation in Gila Sandy Loam Treated with 500 p.p.m. of Nitrogen as Ammonia Plus Sulfur.

fyng bacteria. Perhaps the nitrites and nitrates were utilized by the sulfur bacteria as rapidly as formed. In any event, the significance of negative nitrification during the oxidation of sulfur in alkaline soils constitutes a problem worthy of further investigation.

Changes in pH and nitrate concentrations with time are shown in Figure 64; the nitrogen source and amendments constitute the parameters. It is apparent that the initial pH values of both series of samples fertilized with the ammonium salts were below the threshold pH value of 7.65, and those of the samples fertilized with ammonia were above the threshold value.

The nitrification rate curves for samples treated with the ammonium salts show, too, that the oxidation is subject only to the delay of a normal lag phase when the initial pH is below 7.65.

The correlation between the threshold pH value and nitrification is again evident in the samples treated with calcium sulfate, but is entirely lacking in the samples in which the presence of free sulfur forestalled any accumulation of nitrates. It must be stressed, nevertheless, that nitrates may be formed as soon as the pH has decreased to 7.65, but are being utilized in some manner which prevents their accumulation in the soil.

Ammonium sulfate and monoammonium phosphate exhibit similar behavior with respect to nitrite and nitrate forma-

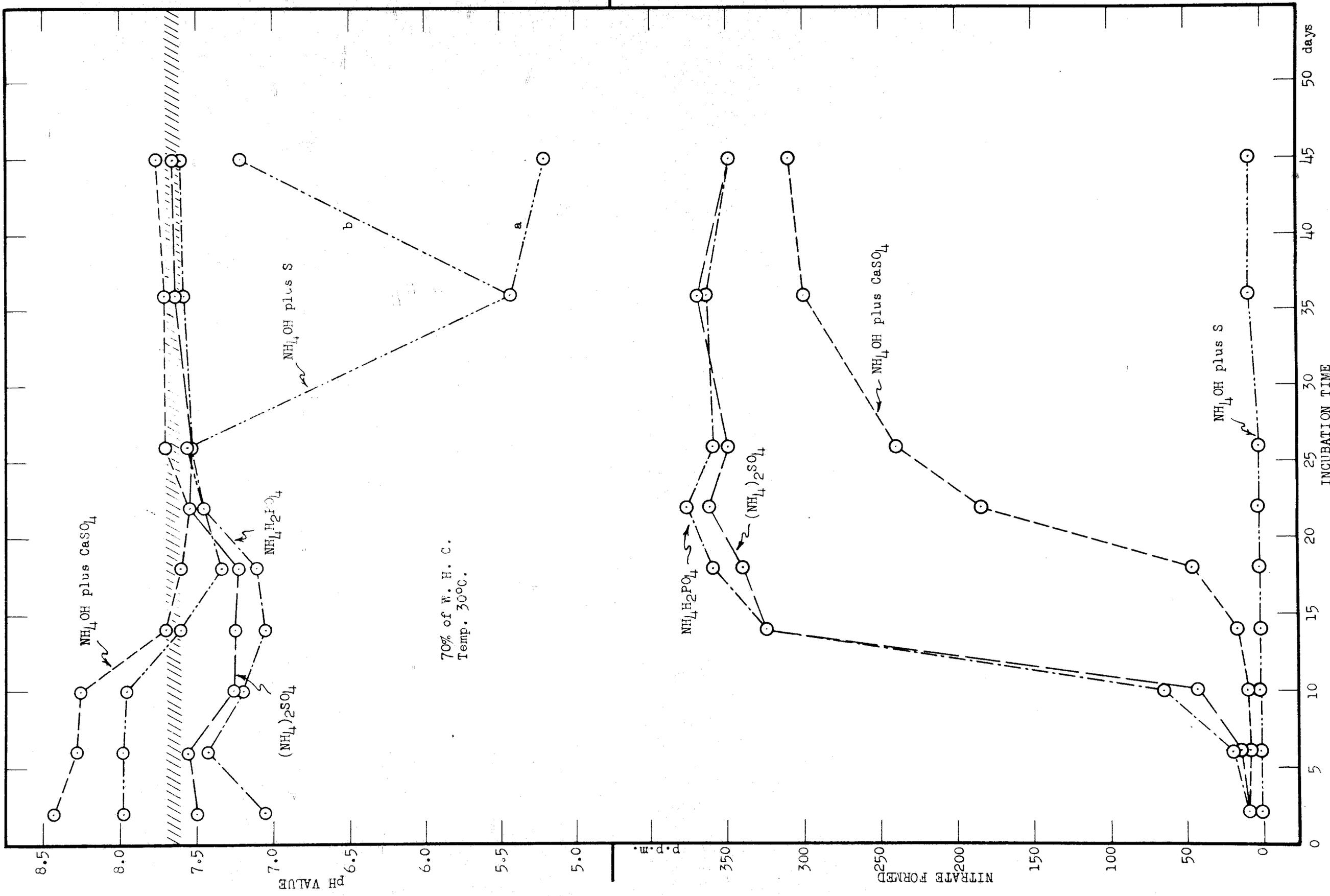


Fig. 64. Parametric Nitrogen-Source Curves Showing the Rate of Nitrate Formation in Vila Sandy Loam Relative to the Threshold pH Range.

tion; the addition of calcium sulfate with ammonia has no significant effect on the nitrification behavior of the latter; and the addition of sulfur with ammonia prevents both nitritification and nitrification.

Comparison of the Various Treatments with Respect to Nitrite Formation

In the final graph, Figure 65, are presented the nitrite curves for the various treatments in this study. The curves for the samples treated with the ammonium salts are probably very close to the true curves since the values on the fourteenth day have not yet quite dropped to the trace quantities found subsequently. It is possible, of course, that the maxima reported are too low, and that the descending curves should be even steeper. The precipitous drop in nitrites, accompanied by an almost instantaneous increase in nitrates (Figures 60 and 61) is an observation which merits future study, for the transfer of energy in this process must of necessity be extremely rapid, thus casting doubt upon the purely biological nature of the phenomenon.

The greatest nitrite accumulation was noted with ammonium sulfate, second greatest with monoammonium phosphate, third greatest with ammonia plus calcium sulfate, and only traces when the ammonia was applied with sulfur. The need for greater study of the shape of the nitrite curve is stressed by the plateau observed as a maximum in the ammonia-

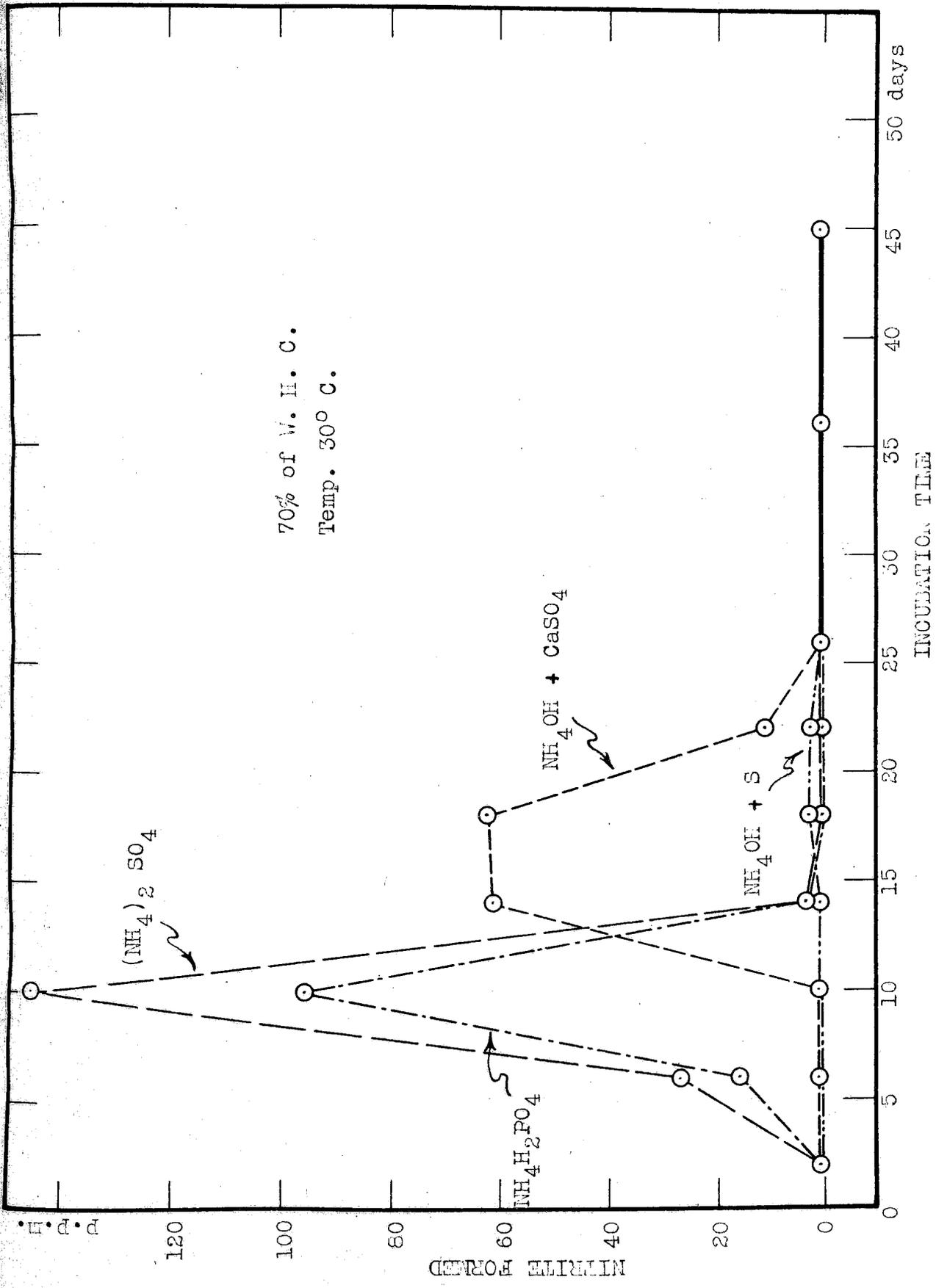


FIG. 65. Parametric Nitrogen-Source Curves Showing the Rate of Nitrite Formation in Gila Sandy Loam.

plus-calcium sulfate curve.

E. Phosphate Availability as Related to Nitrification

The close agronomic relationship between nitrogen and phosphorus in the soil made it desirable to study the solvent effect of the acids produced from the oxidation of ammonia upon the insoluble phosphates of the soil. The solubility of phosphate is a function of various factors: (a) the chemical form in which it exists in the soil, (b) the pH value of the soil, (c) the concentration of calcium and magnesium salts, both soluble and insoluble, (d) the presence of iron and aluminum, and (e) the quantity and rapidity of the decomposition of complex organic substances.

These relationships have been treated both theoretically and experimentally by Buehrer (4), McGeorge (88), and McGeorge and Breazeale (84, 87). Among the factors enumerated, the most important appear to be the pH value of the soil, and the amount of both soluble calcium and magnesium and their insoluble carbonates.

According to the calculations of Buehrer (4), the concentration of the various intermediate ions of orthophosphoric acid is a function of the pH value, such that below pH 6.8 H_2PO_4^- ion predominates and above this value, HPO_4^{--} ion is predominant.

A reduction in pH value by virtue of nitrification in a calcareous soil should result in either of the following

two reactions involving phosphates:



Although the first of these reactions removes hydrogen ion, such removal does not result in an increase in phosphate ion in solution. It must be noted, however, that one mol of calcium ion has entered the solution by reason of this reaction.

In the second reaction, both Ca^{++} ion and H_2PO_4^- ion have been rendered soluble. Both of these reactions are, therefore, directly related to the calcium ion concentration. In view of the fact that the soil was calcareous, an increase in calcium ion in the soil solution also results from the reaction:



Since, therefore, a fair concentration of calcium ion may accumulate under these conditions and, furthermore, since calcium ion tends to have an effect opposite to that of hydrogen ion, it is conceivable that there may be conditions in which one effect just offsets the other in respect to the amount of phosphate rendered soluble. In such an event, one would not expect an appreciable change in the soluble phosphate content as nitrification of the ammonia progressed,

although hydrogen ion was being continuously formed. On the other hand, it is equally probable that an actual reduction in phosphate might occur where the calcium was greatly in the predominance, thus overshadowing the pH effect.

The above effects have also been verified experimentally by McGeorge and Breazeale (84) who observed that calcium reduces the concentration of phosphate ion in solution, the effect being enhanced by high alkalinity.

In the light of the foregoing consideration, a study was made of the effect of nitrification upon the solubility of phosphates. The experimental procedure was presented in detail in an earlier section.

The nitrogen transformations were brought about by nitrifying bacteria which, in association with other soil microorganisms, were the only forms of life present to make demands upon, and utilize, the available phosphate formed. It is probable, therefore, that some of the phosphate thus rendered available may have been converted to complex organic forms by virtue of its utilization by the microorganisms. Since the analytical method yielded only the inorganic phosphorus, it is possible that the amounts of phosphate found may have been somewhat low as a result of such conversion. Such organic phosphorus is evidently not "available" in the sense in which the term is usually understood, and can only

become so after the organisms themselves, or their by-products, have been decomposed by microörganic action.

The results of the phosphate studies are presented in part in the following sets of figures all of which refer to incubation studies made on cultivated Gila sandy loam. This soil had a pH value of 7.6, a carbonate content of 1.1%, and a total buffer capacity of 39.3

A summary of available phosphate in relation to treatment is presented in Table 5.

Figures 29-33: These samples had been variously treated with acid and base solutions to adjust the initial pH value to either side of the threshold value of 7.65. Actually, the initial pH values resulting from these treatments ranged from 7.5 to 9.2.

An inspection of this group of curves shows in general that the effect of nitrification on phosphate liberation in the treated samples is very small, differing from the check sample by amounts which are well within the limits of experimental error of the analytical method. In fact, the curves are so nearly alike that they are practically superimposable.

Figures 36-38: Incubation of soil samples treated with ammonia, ammonium sulfate, and urea at relatively high initial or sustained pH values produced by the addition of calcium hydroxide is reported in these figures.

Figures 39-43: These figures report available phosphate as a function of moisture content during nitrification. The latter ranged from 20 to 100% of the water-holding capacity.

Figures 46-50: These figures report available phosphate as a function of the incubation temperature. The temperature range extended from 10° C. to 60° C.

Figures 53-57: In these experiments the amount of nitrogen applied as ammonia varied from zero to 300 p.p.m. calculated on the basis of the air-dry soil.

Figures 60-63: These figures show the effect of the nitrification of ammonia, ammonium sulfate, and ammonium phosphate, either in the presence or absence of calcium sulfate and sulfur.

The curves in this series of experiments are not genetically related, and the differences in the curves emphasize this point.

In the samples treated with over 2,000 p.p.m. of phosphate as ammonium phosphate (Figure 61), it is evident that the soluble phosphate in the presence of calcium ions reverts to an insoluble form. The curve exhibits a phenomenal decrease of about 1800 p.p.m. of soluble phosphate within the first six days of incubation. The decrease in soluble phosphate from the time of application to the first analysis, after a two-day incubation period, amounted to over 1,100 p.p.m.

The relation between hydrogen-ion concentration and the solubility of phosphate is evident from Figure 63 which represents the data for the samples fertilized with ammonia in the presence of free sulfur. A steady increase in soluble phosphate occurred during incubation, the increase being attributable to a decreasing pH value resulting from oxidation of the sulfur.

Phosphate Solubility in Relation to Treatment

The foregoing groups of figures may now be quantitatively studied to obtain an estimate of the comparative amounts of phosphate rendered soluble as a result of the various treatments. The phosphate curves in all cases exhibit fluctuations which upon critical analysis must be regarded as having resulted from errors inherent in the colorimetric method of determining phosphate. For this reason it is not considered justifiable to regard the apparent upward trend of the phosphate values as indicative of significant increases. It seemed of interest, however, to estimate, if possible, the average amount of phosphate rendered soluble by the various treatments. The most rational basis for such a calculation seemed to be the simple arithmetical mean. In view of the dominant influence of hydrogen-ion concentration on phosphate solubility it seemed of interest also to correlate the amounts of phosphate so calculated with

the minimum pH values resulting from the treatments. The phosphate and pH data together with the respective treatments are assembled in Table 5.

TABLE 5
CORRELATION OF SOLUBLE PHOSPHATE WITH pH AND TREATMENT

Fig. no.	Source of nitrogen	Treatment	Minimum pH value	Mean soluble phosphate p.p.m.
029	None	None	7.7	10.8
30	NH ₄ OH	Ca(OH) ₂ , initially	7.7	11.2
031	NH ₄ OH	None	7.3	10.9
32	NH ₄ OH	0.5 N H ₂ SO ₄ , initially	7.1	15.7
33	NH ₄ OH	1 N H ₂ SO ₄ , initially	7.1	25.6
36	NH ₄ OH	Sustained high pH	7.7	13.7
37	(NH ₄) ₂ SO ₄	Ca(OH) ₂ , initially	7.2	14.9
38	Urea	Ca(OH) ₂ , initially	7.3	14.3
039	NH ₄ OH	20% of W. H. C.	7.7	13.3
040	NH ₄ OH	40% of W. H. C.	7.6	14.7
041	NH ₄ OH	55% of W. H. C.	7.4	14.6
042	NH ₄ OH	70% of W. H. C.	7.5	14.7
043	NH ₄ OH	100% of W. H. C.	7.3	15.3
046	NH ₄ OH	10° C.	8.1	12.5
047	NH ₄ OH	20° C.	7.4	14.4
048	NH ₄ OH	30° C.	7.5	15.1
049	NH ₄ OH	40° C.	7.5	15.6
050	NH ₄ OH	60° C.	8.2	16.5
053	None	None	7.8	15.1
054	NH ₄ OH	50 p.p.m. nitrogen	7.6	14.9
055	NH ₄ OH	100 p.p.m. nitrogen	7.6	14.7
056	NH ₄ OH	200 p.p.m. nitrogen	7.5	14.8
057	NH ₄ OH	300 p.p.m. nitrogen	7.5	14.6
60	(NH ₄) ₂ SO ₄	None	7.3	15.9
61	NH ₄ H ₂ PO ₄	None	7.0	209
62	NH ₄ OH	CaSO ₄	7.5	14.6
63	NH ₄ OH	S	5.4	20.1

0 Samples receiving no treatment or NH₄OH only

The simple correlation coefficient between the minimum pH value obtained during incubation and the mean phosphate content was calculated (90) and found to be -0.483 , a statistically significant value. This indicates that the general trend was for the soluble phosphate content of the sample to increase as the pH value decreased with treatment. All treatments were considered in the calculation except that in which ammonium phosphate was added to the samples.

In order to relate the pH changes more directly to the process of nitrification, a second simple correlation coefficient was calculated between the minimum pH values of samples receiving no additions, or ammonia only, and the mean soluble phosphate content. This analysis of the data gave a coefficient of $+0.785$. This latter value indicates (1) that the foregoing significant negative correlation between pH values and soluble phosphate was the result of variable treatments to which the samples were subjected other than the addition and nitrification of ammonia, and (2) that a highly significant decrease in soluble phosphate resulted from nitrification of ammonia and soil nitrogen.

The highly significant decrease in soluble phosphate found in the nitrification of ammonia is not specific with respect to ammonia, however, but is characteristic of the nitrification process. This is shown by a statistical

analysis of the phosphate data obtained during the nitrification of ammonia, ammonium sulfate, and urea in the incubation study in which Superstition sand, and the virgin and cultivated Gila sandy loams were used. The data used in these calculations have not been presented elsewhere in this thesis. The statistical analyses are presented in Tables 6, 7, and 8.

TABLE 6

STATISTICAL ANALYSIS OF THE EFFECT OF VARIOUS FERTILIZERS
UPON THE AVAILABILITY OF PHOSPHATES IN
SUPERSTITION SAND

Incubation time days	Fertilizer added						
	None	Ammonia	Ammo.sulfate		Urea		
	p.p.m.	p.p.m.	a	b*	a	b*	
18	a	8.3	7.9	-	8.3	o	8.6
	b	9.0	8.6	+	9.0	o	10.9
21	a	9.4	10.3	+	9.4	o	9.8
	b	9.0	9.5	+	8.6	-	7.9
25	a	13.4	11.6	-	12.2	-	12.8
	b	15.3	11.9	-	12.2	-	13.1
30	a	24.5	14.5	-	13.1	-	13.6
	b	15.2	14.8	-	12.9	-	13.7
35	a	8.8	7.5	-	8.3	-	9.0
	b	7.4	10.6	+	16.5	+	9.0
39	a	11.3	11.5	+	9.6	-	11.0
	b	10.6	11.7	+	9.6	-	9.9
44	a	12.1	11.7	-	11.0	-	11.6
	b	11.9	12.5	+	11.3	-	12.3
49	a	13.4	14.1	+	11.2	-	12.3
	b	13.3	14.6	+	12.3	-	13.3
59	a	11.6	12.0	+	11.4	-	12.5
	b	12.4	12.9	+	12.3	+	12.6
72	a	-----	13.3	-	12.2	-	12.9
	b	14.1	13.9	-	12.3	-	12.4
86	a	14.2	13.7	-	13.1	-	13.4
	b	17.8	15.0	+	13.3	-	13.9
107	a	12.2	13.4	+	12.3	+	12.8
	b	12.9	14.5	+	12.9	o	13.4

* + or - refers to relative magnitude of phosphate value with respect to the check sample indicated by a and b.

TABLE 7

STATISTICAL ANALYSIS OF THE EFFECT OF VARIOUS FERTILIZERS
UPON THE AVAILABILITY OF PHOSPHATES IN
VIRGIN GILA SANDY LOAM

Incubation time	None	Fertilizer added						
		Ammonia		Ammo. sulfate		Urea		
		p.p.m.	p.p.m.	a b*	a b*	a b*	a b*	
days	p.p.m.	p.p.m.		p.p.m.		p.p.m.		
18 --	a	15.8	16.2	+	16.0	+	15.3	-
	b	-----	16.2	+	16.6	+	14.4	-
21 --	a	14.0	17.5	+ +	15.8	+ o	15.0	+ -
	b	15.8	18.0	+ +	15.8	+ o	14.0	o -
25 --	a	20.7	20.0	- -	21.4	+ -	20.5	- -
	b	22.0	20.7	o -	22.6	+ +	21.1	+ -
30 --	a	22.8	23.3	+ -	22.9	+ -	22.3	- -
	b	24.1	24.5	+ +	22.2	- -	22.2	- -
35 --	a	17.5	17.8	+ +	16.5	- -	17.5	o o
	b	17.5	18.5	+ +	16.5	- -	16.5	- -
39 --	a	19.3	20.0	+ +	18.3	- -	18.2	- -
	b	18.6	20.2	+ +	17.2	- -	17.2	- -
44 --	a	21.2	21.4	+ +	20.6	- -	19.4	- -
	b	20.8	21.2	o +	19.9	- -	20.0	- -
49 --	a	22.3	23.6	+ -	20.7	- -	22.4	+ -
	b	23.7	25.0	+ +	20.8	- -	21.8	- -
59 --	a	20.0	19.4	- -	20.5	+ -	20.8	+ -
	b	30.0	21.6	+ -	20.1	+ -	18.9	- -
72 --	a	22.2	22.8	+ -	20.7	- -	23.1	+ o
	b	23.1	20.5	- -	23.4	+ +	23.4	+ +
86 --	a	25.0	24.2	- +	24.0	- +	23.4	- +
	b	23.0	15.2	- -	24.2	- +	22.9	- -

* + or - refers to relative magnitude of phosphate value with respect to the check sample indicated by a and b.

TABLE 8

STATISTICAL ANALYSIS OF THE EFFECT OF VARIOUS FERTILIZERS
UPON THE AVAILABILITY OF PHOSPHATES IN
CULTIVATED GILA SANDY LOAM

Incubation time	Fertilizer added							
	None	Ammonia		Ammo.sulfate		Urea		
		p.p.m.	p.p.m.	a b*	p.p.m.	a b*	p.p.m.	a b*
18	a	21.6	20.5	- o	19.8	- -	17.3	- -
	b	20.5	20.5	- o	19.4	- -	18.7	- -
21	a	24.9	24.1	- -	22.8	- -	20.5	- -
	b	24.9	22.8	- -	22.0	- -	18.5	- -
25	a	31.7	29.3	- -	31.3	- -	31.1	- -
	b	32.7	32.2	+ -	34.3	+ +	31.6	- -
30	a	35.8	32.7	- +	33.3	- +	30.0	- -
	b	32.4	31.0	- -	30.6	- -	27.4	- -
35	a	26.8	24.6	- -	26.3	- o	24.9	- -
	b	26.3	24.2	- -	25.0	- -	24.6	- -
49	a	33.5	34.4	+ -	32.3	- -	31.1	- -
	b	35.6	32.3	- -	33.0	- -	31.6	- -
72	a	33.4	33.4	o -	31.4	- -	31.1	- -
	b	34.0	32.1	- -	33.4	o -	33.4	o -
86	a	37.5	31.7	- -	33.1	- -	31.2	- -
	b	34.5	33.0	- -	35.5	- +	33.4	- -

* + or - refers to relative magnitude of phosphate value with respect to the check sample indicated by a and b.

The data have been subjected to a significance test by the method of Brandt (89). As indicated in the tables, each value for every treatment was compared with both check values of the same date in order to double the number of comparisons.

In Table 9 are presented the results of the test for significance. In all three soils, the increase in phosphate availability with time in the untreated samples was highly significant. This test was made by comparing each check sample with all samples following it (excepting its own duplicate); in this manner the samples were completely randomized and a very large number of comparisons was made.

TABLE 9

SIGNIFICANCE TEST FOR DIFFERENCE IN PHOSPHATE AVAILABILITY DETERMINED BY THE STATISTICAL ANALYSES IN TABLES 5, 6, and 7

Soil	P _o for change in phosphate availability with treatments indicated			
	Check vs. time	Ammonia vs. check	Ammo.sulfate vs. check	Urea vs. check
Superstition sand	(+) 0.67 ⁰⁰	(+) 0.56	(-) 0.78 ⁰⁰	(-) 0.58
Gila sandy loam (virgin)	(+) 0.76 ⁰⁰	(+) 0.64	(-) 0.64	(-) 0.76 ⁰⁰
Gila sandy loam (cultivated)	(+) 0.84 ⁰⁰	(-) 0.86 ⁰⁰	(-) 0.84 ⁰⁰	(-) 0.98 ⁰⁰

$$P_o = \frac{\text{Total } + \text{ or } - \text{ Differences} + 1/2 \text{ Number of Zeros}}{\text{Total Number of Differences}}$$

⁰⁰ Highly significant values

Highly significant decreases in soluble phosphate were found in the following instances:

- (1) The ammonium sulfate caused a decrease which averaged 1 p.p.m. of phosphate on the basis of the air-dry weight of Superstition sand.
- (2) The urea resulted in a decrease which averaged 1.3 p.p.m. in the virgin Gila sandy loam.
- (3) In the cultivated Gila sandy loam, ammonia caused an average decrease in phosphate of 1.7 p.p.m.; ammonium sulfate, 1.4 p.p.m.; and urea, 3.1 p.p.m.

Although these values are all highly significant statistically, it is doubtful whether any of them -- unless possibly the last given -- is of practical importance.

DISCUSSION

The foregoing investigation has brought to light several new and highly significant facts: first, that ammonia exhibits a nitrification rate similar in magnitude to that of ammonium sulfate and urea; secondly, that there exists a threshold pH value for the nitrification of these compounds, namely 7.65, above which nitrification will not occur to an appreciable extent; thirdly, that nitrites form in considerable amounts, even in a well-aerated desert soil maintained at the optimum moisture content; and finally, that the relative amounts of nitrite and nitrate are a function of pH value, temperature, moisture content, and amount of nitrogen applied.

The observation that nitrate formation does not occur in alkaline calcareous soils above a pH value of 7.65 ± 0.05 is of particular interest in view of the findings of Gowda (63), Olsen (62), Gerretsen (112) and others.

Gowda (63) reported an optimum value for nitrate formation of 8.5 to 8.8, a pH unit or more above the threshold value observed in this work. He employed culture media in his study, however, and it is a dangerous practice to translate results from such studies to the soil. In culture

studies the mineral nutrients required by the organisms are supplied in an easily available form; in the soil, such is not the case. If the needed minerals are tied up in the soil in a manner which makes them either chemically or physiologically unavailable to the organisms above a specified pH value, the organisms will not exhibit activity in a soil medium until the pH has been so adjusted as to make the required nutrients available. Thus the threshold pH value is a factor which not only affects the nitrifying bacteria as such but their nutrition as well. Under these circumstances it is not necessary to assume an abnormally rapid adaptation of the microorganisms in culture media to pH values above the threshold value found in soils, for the nutrient elements are already in available form, and nitrification occurs at pH values above 7.65.

It is fairly well established that adaptation of the nitrifiers to pH probably does occur over sufficiently long periods of time in view of the observation of Meek and Lipman (64) that these organisms were able to adapt themselves to a pH value of 13.0 in solution cultures.

Olsen (62) worked with a strongly acid humus soil to which he added lime to adjust the pH to various values. He reported nitrification between 3.7 and 8.8. Although such adaptation is possible even in soils, it appears more logical to attribute nitrification to localized pH effects when it

occurs above 7.65. For example, Figure 53 shows that in the untreated cultivated Gila sandy loam a considerable amount of nitrate was formed even though the pH did not decrease to the threshold value of 7.65 throughout the entire incubation period.

The suggestion has already been advanced that a reduction of pH within the soil aggregates is responsible for this apparent deviation from the threshold value found to be characteristic of the nitrification process. This suggestion is supported by the fact that in the samples incubated at the water-holding capacity and having a pH above the threshold value, no nitrates were formed, and vice versa. Since Olsen (62) worked with distinctly acid humus soils, it is more probable that he was unable to neutralize the acid condition within soil aggregates (even though the pH of the entire soil mass may have been on the alkaline side of neutrality) than that an adaptation of the microorganisms occurred, as he suggests.

Gerretsen (112) reported that nitrate formation may be retarded in alkaline soils during nitrification in such a manner that nitrites will accumulate. Although this work suggests a threshold pH value for nitrification, no definite value is given. Nevertheless, he fixed the limits for nitrate formation between pH values of 5.2 and 10.0, with an optimum between 8.3 and 9.2, with possible adaptation of the nitrate-forming bacteria to a pH value of 11.9.

In further support of the existence of a threshold value as found in this investigation, we may cite the work of Waynick (54) who reported nitrification studies with ammonia on alkaline soils having pH values ranging from 7.1 to 8.1. According to his data there were six of twenty-four soils in which nitrification occurred at values above the 7.65 threshold value. The six samples gave pH values of 7.8, 7.9, 8.0, and 8.1. It is significant that this apparent discrepancy may be attributed to a difference in soil:water ratio, a condition which is not specified in Waynick's report. It has been shown by McGeorge and Martin (114) that the pH value of alkaline soils at a soil:water ratio of 1:2 exceeds the value at or near the moisture equivalent by 0.61 pH units.

Among the nitrification studies of ammonia, ammonium sulfate, and urea on the six desert soils used in this study, three instances are of outstanding interest: first, the failure of ammonia to nitrify in Superstition sand (Figures 8 and 15); second, the irregularity of nitrification in the virgin Gila sandy loam samples which were fertilized with ammonia (Figures 9 and 19); and third, the slower nitrification of all three fertilizers in the Palos Verdes sandy loam (Figures 13 and 28).

The application of ammonia to Superstition sand, which had a specific buffer capacity of only 4.3 toward acid and

practically none toward base (Figure 2), raised the hydroxyl-ion concentration to such an inordinately high value that nitrate formation could not occur. Failure to nitrify in this case can scarcely be attributed to toxic effects resulting from the amount of nitrogen applied, for nitrification occurred with similar applications of nitrogen in the form of ammonium sulfate and urea.

The buffer effect in the case of the Palos Verdes sandy loam is just the opposite of that observed in Superstition sand. Whereas the latter was not well buffered toward base, the former is poorly buffered toward acid (Figure 7), having a specific buffer capacity of less than 0.5 toward hydrogen ion. This buffering behavior most nearly approaches that of the acid type of soils with which Waksman (58) worked when he recommended the addition of a base to neutralize the acid formed during nitrification. This soil, with an initial reaction of about 6.5, showed much slower nitrate formation after nitrification had begun than was noted in soils with a more favorable pH value. During the nitrification of ammonium sulfate in this soil, the rate of nitrate formation decreased simultaneously with the pH. The retarded rate of nitrification at the lower pH values is in agreement with the findings of Waksman (58), Naftel (61), Gaarder and Hagem (60), and Humfeld and Erdman (59). In the present investigation, however, the incubation was not carried far enough to verify the minimum values of 3.9 to 4.4 obtained

by the foregoing investigators. It will be noted that the incubation and analytical determinations were nevertheless continued until the pH value had in the ammonium sulfate-treated samples decreased to 4.65, a value 0.15 of a pH unit below the tolerance of economic plants set by Pierre (76).

The irregularity of nitrification of ammonia observed in the virgin Gila sandy loam (Figures 9 and 19) is of particular interest since it appears to be a border-line case with respect to the threshold pH value for nitrification. It was this series of samples which first directed attention to the threshold value.

Although no definite threshold pH value for nitrite formation has been established, the data recorded in Figures 30, 31, 36, 43, 47, 48, 49, 54, 55, 56, 57, and 62 indicate that Gowda's (63) reported optimum of 8.0 for nitrite formers is an approximate threshold value for nitritification.

The Possible Role of Products Intermediate between Ammonia and Nitrite in Decreasing the pH

The second major phase of this study was undertaken to confirm the threshold pH value of 7.65 ± 0.05 , and to determine whether the drop in pH from high initial values was accompanied by or resulted from the formation of nitrites.

In Figures 30, 31, and 35 it will be noted that a greater drop in pH occurred prior to nitrite formation than

after they had begun to form. Several factors may have contributed to this decrease: for example, there was a period immediately following the application of solutions during which some diffusion must have occurred as the soil-moisture and base exchange equilibria established themselves. This period was probably of only short duration, however, and any effect upon the decline in pH should be considered as an immediate, initial drop in the pH curve. It is well known, of course, that the pH value of an alkaline soil tends to decrease for a short time after wetting, but this factor, too, is insufficient to account for even an appreciable fraction of the observed decrease in this case.

It may be mentioned also that the first observations of nitrite as reported in Figures 30 and 31 were not made promptly after the inception of nitrite formation. The first appearance of nitrites in Figure 30 was observed on the seventeenth day, when approximately 30 p.p.m. of nitrogen as nitrites had accumulated -- nearly 50% of the maximum which finally accumulated in that series. Similarly, about 22% of the maximum value for nitrites observed in Figure 31 had appeared at the time the first nitrites were detected on the fourteenth day. However, if this reasoning is correct, the remaining 50% of the total nitrite build-up in Figure 30 and the 78% in Figure 31 was much less effective in reducing the pH than the amounts corresponding to 50 and 22% of the maxima, as noted above. Since each p.p.m. of

the total nitrite formed would require the same stoichiometric removal of hydroxyl ions -- and the influence of their removal on the pH value would increase logarithmically as 7.0 was approached -- this deduction is probably incorrect.

About the only alternative to such wishful reasoning appears to be the conclusion that some intermediate reaction is responsible for the lowering of the pH prior to the formation of nitrites. This would mean that the over-all reaction for the oxidation of ammonia to nitrite,



with the removal of hydroxyl ions is too inclusive.

Other investigators have obtained data in various types of study from which they have deduced the presence of one or more intermediate compounds between ammonia and nitrite.

Beesley (91), Mumford (96), Kluyver and Donker (97), and Corbet (41, 41, 98, 99) have all produced either direct or indirect evidence of such intermediate products in oxidation studies. Beesley (91) found that as much as 44% of the applied ammonia nitrogen disappeared prior to conversion of nitrites and concluded that the nitrogen passed through some intermediate compound "which must be regarded as more or less hydroxylated." Mumford (96) advanced the theory that the oxidation of ammonia proceeds with the successive hydroxylation of the hydrogen atoms and the removal of water.

He reported finding hydroxylamine salts and salts of hyponitrous and nitrous acid.

Kluyver and Donker (97) incline to the theory that the oxidation of ammonia passes through the hydroxylamine and hyponitrous acid stages. Corbet (40, 41, 98, 99) definitely reported hydroxylamine and hyponitrite ion in nitrification processes, and wrote as follows:

"It appears that the reaction proceeds through the formation of hydroxylamine and hyponitrous acid as intermediate compounds, but while the first-named can never have more than an ephemeral existence under the experimental conditions, hyponitrous acid present in the form of the calcium salt may account for as much as 40 per cent of the total nitrogen present."

On the other hand, Sandara Rao, Krishnamurti, and Gopal Rao (100) have attempted to identify hydroxylamine and hyponitrous acid, using culture technic, but without success. They are of the opinion that Corbet's analytical method does not give a specific test for hyponitrites.

The presence of hydroxylamine has been suggested as an intermediate product not only in the oxidation of ammonia to nitrites, but in several other processes as well.

Korsakova (92, 93), and Korsakova and Lupatina (94), as reported by Russell (95) and Waksman (1), found while working with a group of microorganisms which quantitatively reduce nitrates that an intermediate product appeared during the reduction shortly after the first nitrites were formed and increased to a maximum as the nitrites fell away from

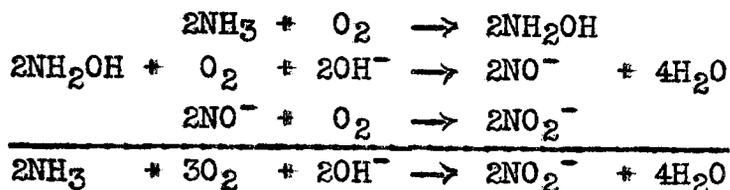
their highest value.

Also Blom (101, 102), studying nitrate-reduction, proposed a test for hydroxylamine by which he professed to have established the presence of this compound. Similarly, Franzen and Löhmann (103), Kostychev and Tsvetkova (104) and Joss (105), as noted by Waksman (1), have all advanced the theory that hydroxylamine is an intermediate product in the reduction of nitrates, and Lindsay and Rhines (106) concluded that hydroxylamine is in general a characteristic product of bacterial reduction of nitrates.

Blom (107) has also proposed the presence of hydroxylamine in a series of reactions to explain the mechanism of nitrogen fixation.

Maze (108) suggested a mechanism whereby hydroxylamine enters into the assimilation of carbon dioxide by green plants with concurrent oxidation of nitrogen compounds to give products which he identified in the leaves of several trees.

If, on the strength of the evidence presented by the foregoing investigators, it is assumed that hydroxylamine and hyponitrites do form between the stages of oxidation of nitrogen as ammonia and as nitrite, the equations which may be considered as representative of the intermediate reactions are as follows:



It is evident that even though these three reactions can be added together to give the over-all reaction for the oxidation of ammonia to nitrite, the second of the three equations is the one in which hydroxyl ions are removed, and on the basis of the evidence cited would be the reaction responsible for the reduction of the pH value prior to nitrite formation. The oxidation of hydroxylamine to hyponitrite ion would evidently have the same effect upon the pH of the medium, per unit of nitrogen oxidized, as the over-all oxidation of ammonia to nitrites.

If this hypothesis be true, it is to be expected that the pH will continue to fall until all of the added nitrogen still in a state of oxidation lower than hyponitrite is oxidized through this stage; conversely, it is not to be expected that the pH will decrease as a result of nitrite formation except as affected by the dissociation of the acid.

Conditions Favorable to Nitrification

The observation made in this study that nitrites are formed during nitrification is certainly not new; the presence of nitrites can be verified in any study of the nitrification process. However, the fact that nitrites can

accumulate to a considerable extent even in a well-aerated soil is not generally recognized. This misconception may be attributed to several reasons: first, much of the work on nitrification in the past was done in solution cultures in which soil conditions do not prevail; secondly, nitrite determinations have not been stressed (and have even been omitted entirely) in studies on nitrification since nitrite ion is generally considered to be toxic to plants and nitrate ion assimilated by them; thirdly, analyses have not been made with sufficient frequency in the past to determine significant quantities of nitrites which may accumulate; and fourth, the use of colorimeters which required the visual matching of colors has probably led to erroneous results.

In the present investigation all four of these conditions have been avoided. It was realized from the start that to know how a substance nitrifies in soils, the soil must be used as a medium in the study, notwithstanding the value of the definitive work which previous investigators have done using solution and sand-and-water cultures. This point of view is the more pertinent, because of differences in aeration, salt contents, adsorptive surface forces in the colloid fraction, competition and antagonism between various soil microorganisms, and interphase relationships which invariably exist in a system so complex as the soil.

Furthermore, nitrites have been assigned an importance on a par with nitrates since they exert an influence upon the decrease in pH prior to nitrate formation by the removal of hydroxyl ion in the conversion of ammonia to nitrite. In the course of this study, nitrites were determined at intervals of about four days, and even that time interval was not short enough to yield sufficient data to determine precisely the course of the nitrification curve.

Nitrite accumulations as reported by other workers have not, in general, been characteristic of soil conditions favorable to crop growth. For example, Fraps and Sterges (55) applied 500 p.p.m. of nitrogen as ammonium sulfate to soils of low nitrifying capacity and demonstrated a build-up of as much as 288 p.p.m. of nitrite. Nitrate-forming bacteria were absent, however, so that nitrates failed to accumulate. These workers (109) also reported appreciable accumulations of nitrite in Lake Charles series soil, but this soil is characterized by poor drainage (110) and is not generally farmed. Fraps (111) reports that no natural soil in Texas has been found in which nitrites occur to any appreciable extent. He has reported (109) the practice of analyzing at the end of 28 days of incubation, and, unless nitrate-forming bacteria were lacking or some other soil factor favored a long-time accumulation of nitrites, their presence would be entirely missed by incubating the samples for such a long period before making the analyses.

The conditions most favorable for nitrate formation were found to be a moisture content of 70% of the water-holding capacity, an incubation temperature of 30° C., and an application of not more than 200 p.p.m. of nitrogen as ammonia. These conditions may be deduced from Figures 44, 51, and 58. It is evident from Figure 58 that nitrates accumulate most rapidly in the soil treated with the smallest amount of nitrogen and vice versa. Most laboratory investigations involving nitrification have been based upon a concentration of 300 p.p.m. of nitrogen, or even higher concentrations, as used by Fraps and Sterges (55). It may therefore be concluded that smaller amounts of nitrogen would give the desired information more quickly.

The conditions most favorable for nitrite formation (Figures 45, 52, 59) are the same as those for nitrate formation, except that the optimum quantity of the nitrogen varies. A concentration of 300 p.p.m. of nitrogen applied favors the maximum amount of nitrite formed; on the other hand, the greatest percentage conversion is realized with a concentration of 100 p.p.m.

The optimum incubation temperature of 30° C. is in close agreement with the value reported by Tandon and Dhar (57) for the nitrite formers. These workers found the optimum at 35° C. in tropical soils, and 25° C. in soils from temperate countries.

The differences in influence of moisture upon nitrite

accumulation (Figure 45) are of considerable interest in view of the finding by Greaves, Stewart, and Hirst (113) that the application of irrigation water has no appreciable influence upon the nitrous-nitrogen content of irrigated soils. Since these workers were not employing the controlled conditions of the laboratory, however, they were unable to maintain the moisture constant over a sufficiently long period of time to ascertain the actual effect of moisture content upon nitrite formation.

The samples kept at the water-holding capacity, although puddled by the slightest mechanical agitation, were at no time anaerobic. Had the structure of the soil not been definitely maintained even at this maximum moisture content, anaerobic conditions with concurrent reduction of nitrate would most probably have occurred. Since the soil structure was maintained, however, the high water content served to hasten establishment of the pH equilibrium throughout the soil, and nitrate began to form promptly when the threshold value of 7.65 was reached. The slightest tendency toward anaerobic conditions would have been indicated by an increase in nitrite as a result of nitrate reduction, but it will be observed in Figure 45 that the nitrite values at 40% and 100% of the water-holding capacity are nearly identical. These observations suggest that normal nitrification processes may be expected to obtain in saturated soils even though they

cannot be worked. This statement does not apply, of course, to water-logged conditions.

The configuration of the nitrite curve is of special interest with respect to its relative position to the nitrate curve. The nitrite values increase rapidly to a maximum, and a precipitous drop occurs in these values as nitrates begin to enter the solution. This form of curve is especially manifest in Figures 45, 52, 59, and 65.

The nitrite curves here referred to have maxima which, in general, occur at the intersection of straight lines drawn as extensions of the ascending and descending portions of the curve. Since the maximum nitrite values tend to decrease almost immediately once they have been attained, a plateau in the curve is not thought to represent a true maximum value. Additional research is needed, however, to determine whether in such cases the true maximum value has been missed.

The nitrite curves obtained in this study are, however, in general agreement with the finding of Whitfield and Henry (86) who wrote:

"On treating the natural soil with the optimum amount of water the nitrite nitrogen rises immediately to a maximum and then falls rapidly to the normal low figure.....A little before the point of maximum nitrite the nitrate begins to increase while the free ammonia undergoes a corresponding decrease. The same series of changes is observed when ammonium sulfate is added to the soil."

The results of the present investigation are in accord with

those just cited. The statement that the nitrite nitrogen rises "immediately to a maximum" more accurately describes the phenomenon observed in the samples having the lowest initial pH values, the appearance of nitrites having been delayed at higher pH values.

In order to know accurately the shape of the nitrite curve and its absolute relative position with respect to the nitrate curve, determinations would have to be made at intervals of only a few hours from the beginning of nitrite formation and continued over the period of time during which nitrite occurred in appreciable quantities. The value of such a study is at least two-fold:

First, the liberation of considerable quantities of energy in the conversion of nitrite to nitrate, as indicated by the equation,



casts doubt upon the purely biological nature of the oxidation providing the descending portion of the accurately determined curve indicates that the energy release occurs during a short-time interval.

Secondly, the shape of the curve and the relative position of the maximum nitrite value with respect to the appearance of nitrate may indicate the intra- or extra-cellular nature of the oxidation as a function of biological activity. If the oxidation occurs outside of the cell, no decrease in

nitrogen, measured as the sum of nitrite and nitrate, would be found; but if the oxidation occurs within the cell, the nitrite value should be expected to have passed its maximum before nitrates are observed, and the total of nitrite and nitrate would be diminished by the amount of nitrogen involved in the intracellular oxidation.

From the foregoing studies it would appear that the nitrification behavior of ammonia is analogous to that of ammonium sulfate, urea, and ammonium phosphate in soils sufficiently well buffered toward base to prevent the occurrence of excessive changes in the hydroxyl-ion concentration. All of the fertilizers studied were observed to be subject to the same threshold pH value of 7.65 ± 0.05 before nitrates started to form. Nitrite formation and accumulation was characteristic of ammonia as well as the other forms of nitrogen. In every instance in which the initial pH value was above 7.65 a considerable decrease in the pH value was observed prior to either nitrite or nitrate formation.

SUMMARY

1. A comparative study has been made of the relative rates of nitrification of ammonia, ammonium sulfate, and urea in six typical Arizona soils.
2. Nitrification does not occur in alkaline soils with any of the three fertilizers studied until the pH has been reduced to a threshold value of 7.65 ± 0.05 .
3. Nitrification is more rapid and more nearly complete in the cultivated than in the uncultivated soils.
4. In the microbiological oxidation of ammonia there is considerable nitrite formation even in well-aerated soils under favorable conditions of temperature and moisture. It was greatest in the case of ammonium sulfate and urea, applied in the presence of calcium hydroxide.
5. A pronounced decrease in pH value occurs in the soil prior to nitrite or nitrate formation, which may be due to the formation of some intermediate nitrogen compound prior to its conversion to nitrite.
6. Nitrite accumulation is inhibited by high calcium ion concentration and/or high pH value.
7. The most favorable moisture content for nitrite accumulation and for rate of nitrification is 70% of the water-holding capacity. At a moisture content as low as

20% of the water-holding capacity, nitrite and nitrate formation is very slow. There is a close correlation between decrease of pH to 7.65 and nitrate formation in samples of various moisture contents.

8. The most favorable temperature for the accumulation of nitrite and for rapid nitrification is 30° C. The temperature at which nitrite persisted over the longest experimental period was 20° C. Temperatures as low as 10° C. and as high as 60° C. definitely inhibit nitrification.

9. A study of the effect of nitrogen concentration as ammonia covering a range between zero and 300 p.p.m. on the basis of the air-dry soil showed that at a concentration of 100 p.p.m., 62% of the applied nitrogen had been converted to nitrites, whereas at a concentration of 300 p.p.m. the conversion was only 27%.

10. A comparative study of various nitrogen compounds including ammonium sulfate, ammonium phosphate, and ammonia in the presence of calcium sulfate and sulfur, respectively, showed that nitrite accumulation occurred in the various samples in the following (decreasing) order as to treatment: ammonium sulfate, ammonium phosphate, and ammonia plus calcium sulfate. Nitrites were entirely absent in the samples treated with ammonia and sulfur. In the presence of sulfur, nitrate did not form throughout the duration of the experiment even though the pH had decreased below the threshold value of 7.65.

11. In all instances in which significant accumulations

of nitrites were noted, the nitrite values decreased almost simultaneously with the formation of nitrates. The conversion of nitrites to nitrates was, in all instances, so rapid as to cast doubt upon the purely biological nature of the process, because of the sudden release of a considerable quantity of energy.

12. Ammonia is not toxic to the nitrifying organisms even at a concentration as high as 300 p.p.m. Failure to obtain nitrification in some cases may be attributed to high alkalinity.

13. No evidence was obtained to indicate a loss of nitrogen by the volatilization of ammonia, or by the spontaneous decomposition of ammonium nitrite.

14. Under constant experimental conditions the activities of the microorganisms and the amount of nitrate formed were shown to be highly concordant in different series of experiments.

15. Statistical analysis reveals that a highly significant decrease in available phosphate occurs in Superstition sand, and the virgin and cultivated Gila sandy loams fertilized with ammonia, ammonium sulfate, and urea. An examination of the arithmetical means, however, shows that these highly significant decreases are within the range of experimental error of the analytical method, and are, in any event, not of practical significance.

16. When ammonia is used as a source of nitrogen on an alkaline calcareous soil, it nitrifies nearly as rapidly as ammonium sulfate and urea provided the soil is sufficiently well buffered toward base to withstand the initial change in pH. By virtue of microbial oxidation ammonia is capable of reducing the pH of a soil to substantially the same lower limiting value as ammonium sulfate and urea when applied in equivalent amounts with respect to nitrogen.

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