

AMMONIA AND NITRATE NITROGEN TRANSFORMATIONS
IN SEMI-ARID SOILS OF NORTHEASTERN OREGON

by

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AMMONIA AND NITRATE NITROGEN TRANSFORMATIONS
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INTRODUCTION

The conservation and wise use of nitrogen in soil presents a variety of problems. This important plant element may be immobilized in the form of bacterial protoplasm or be tied up in a relatively unavailable state in the colloidal complex of the soil. This process may be offset by active soil mineralization which releases nitrogen in a form available to plants. However, coincident with the above nitrogen transformations, direct soil nitrogen losses may result from water leaching and through the action of the denitrifying bacteria of the soil. All these aspects of the problem make the nitrogen balance of soil a challenging subject of both fundamental and applied importance.

In the Umatilla area of northeastern Oregon, nitrogen fertilizers are added to the soil along with waste wheat straw. This practice is followed so as to guard against depletion of the soil's nitrogen reserves during the decomposition of added wheat straw. Invariably, however, the nitrogen added quickly disappears and rarely can be accounted for in terms of crop uptake and leaching losses. For this reason, it was felt that these soils may be undergoing serious economic losses of nitrogen.

The present study was planned to investigate this aspect of the soil nitrogen problem with two primary objectives in mind: 1) to trace the overall fate of added nitrate in soil and 2) to investigate possible soil nitrogen losses via gaseous oxides of nitrogen.

HISTORICAL

For many years soil scientists have recognized problems relating to the N balance in soil. Processes involving N immobilization, mineralization and factors associated with the movement or translocation of N in soil have received considerable attention.

In soil the native N or added N of fertilizers does not remain stationary, but undergoes a variety of transformations. Waksman (49, pp.166-189) devoted an informative chapter to these changes. Presented below in diagrammatic form are the transformations of N which are known to occur in soil.

Decomposing proteins

Ammonification (microbial)

Salts ----- NH_3 ----- Base exchange

Nitrification (microbial)

NO_2

Plant use ----- NO_3 + Carbon ----- NO_2 + H_2O + energy

Reduction (microbial)

NH_3 ---- NH_2OH

$\text{H}_2\text{N}_2\text{O}_2$ --- $\text{N}_2, \text{N}_2\text{O}$

In the panhandle area of Oklahoma, Colorado, Kansas, and Texas a decline in wheatland production of 7 bushels per acre in 30 years was observed (21, pp.1-6). Of this loss, 4.2 bushels/acre were attributed to erosion losses and 2.8 bushels to crop removals. Wheat yields were maintained by offsetting soil decline with improved varieties and more efficient farm practices. Yet the need for fertility maintenance was strongly indicated.

Carpenter, Haas, and Miles (11, p.122), in studying the N content of wheat, found that crop uptake of N fell off rapidly on low N soils, but continued on high N soils. The N in the wheat at all stages of growth was closely correlated with grain yields. In this respect Coie (13) showed that early fertilization of wheat with N increased the total yield and thereby created an additional need for N. He suggested that three fertilizations be applied in soils of low fertility. Williams and Smith (52, p. 58), by using N fertilizers also showed net increases in the yield of wheat. Ammonium nitrate produced greater yields than calcium cyanamide. The type of fertilizer carrier had little effect on the protein percentage contained in wheat. In testing commercial fertilizer on winter wheat, Olson and Rhoades (40, p.30), found that the wheat responded consistently to N fertilization in all but three soil series. Among the fertilizers used

NH_4NO_3 and urea proved superior in the spring, with anhydrous ammonia equally effective in the fall. Spring applied $(\text{NH}_4)_2\text{SO}_4$ was efficient and calcium cyanamide was consistently inferior (40, pp.32-33). Significant relationships were found between nitrification rate of soil and check yield of wheat and between nitrification rate and yield response to N fertilizer. Nitrification rate proved to be more reliable than total organic matter for predicting the N status of Nebraska soils (40, p.37). In this respect Fitts, Bartholomew and Heidel (22, p.121) proposed nitrification rate as a procedure for evaluating N requirements of soil. Using corn, these workers found a significant correlation between nitrification rate and crop response.

Huppert and Buchner (28) reported the results of some 4971 experiments performed during the last 25 years using different forms of N fertilizer singly and in various combinations. In most instances the N was carried in the form of the NH_4 or NO_3 radical. They found that the observed superiority of $\text{NO}_3\text{-N}$ over $\text{NH}_4\text{-N}$ became less apparent with increasing soil moisture. With the exception of potatoes, $\text{NO}_3\text{-N}$ performed better on light soils than on soils of heavy texture. At higher applications of N the differences between the various forms of fertilizer were not as obvious.

Dueso Tello (20) concluded that activated lignite is favorable for the conservation of N in the soil. The use of this product improved soil texture and humidity, corrected alkalinity, and regulated the C/N ratio in compact soils. The use of lignite also increased the N (gluten) content and production of varieties of wheat used in Spain. It was suggested that the lignite sets free organic substances of an acid character which form NH_4 salts, and that these salts are more easily transformed into nitrates by the soil bacteria than are salts of the inorganic type.

In a field experiment Jacob and Wiegard (29) applied 200 kg. per hectare to a loamy sand soil. The $\text{NH}_4\text{-N}$ was converted completely to $\text{NO}_3\text{-N}$ within six months by soil nitrifiers. The rather high microbiological activity in the soil indicated that the heavy application of mineral fertilizer had no deleterious effects on the soil. However, Fox, Olson, and Mazurak (23, pp.504-513), after broadcasting $\text{NH}_4\text{-N}$ on the surface of an eroded clay soil were able, five months later, to recover the $\text{NH}_4\text{-N}$ almost completely in the surface 1.5 inch. Appreciable amounts of the NH_4 remained in the surface 1.5 inch after two additional months. The long persistence of the NH_4 ion at the surface of the soil caused a deflocculated condition resulting in crusting of surface soil, even at low

(20 lbs/acre) application rate of $\text{NH}_4\text{-N}$. It was advised that broadcasting of $\text{NH}_4\text{-N}$ fertilizer salts on the surface of poorly aggregated, fine textured soils low in organic matter should not be done when climatic conditions are unfavorable for rapid nitrification. Drouineau (19) reported that $\text{NH}_4\text{-N}$ may be quantitatively higher than $\text{NO}_3\text{-N}$ independent of the nature of N fertilization when the rate of nitrification is low such as in winter, in acid soils, or in soils with a high organic matter content. Furthermore, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ content varied according to the season and past history of a given soil. Mineral N content was very low during the winter and early spring, while it increased during the late spring and summer to reach a maximum value through mineralization; hence the necessity of spring N fertilization.

Allison, Kefauver, and Roller (2, pp.107-110) found that subsoils fix more $\text{NH}_4\text{-N}$ than do surface soils. They reported that considerable moisture fixation may occur in soil if the predominant clay mineral is illite or vermiculite, and these values are increased by drying or heating (2, p.109). Low nitrification values for added NH_4 salts were shown to be characteristic of soils that fix NH_4 under moist conditions. The percent nitrification in such soils was markedly increased when fixation was prevented (2, p.110).

Bower (7, p.119), because of the possible influence of NH_4 fixation upon the N economy of soils, studied the nitrification rate and availability to plants of difficultly exchangeable $\text{NH}_4\text{-N}$ as compared to readily exchangeable $\text{NH}_4\text{-N}$. In all the nitrification tests the production of $\text{NO}_3\text{-N}$ practically ceased after 2 weeks incubation, at which time 78-87% of the readily exchangeable $\text{NH}_4\text{-N}$ was nitrified, but only 13-28% of the difficultly exchangeable $\text{NH}_4\text{-N}$ supplied was nitrified (7, p.120). When cultures were not artificially inoculated with nitrifying bacteria it was found that 75% of the readily exchangeable $\text{NH}_4\text{-N}$ supplied was recovered by barley after 17 days of growth as compared to a recovery of only 10% of the difficultly exchangeable $\text{NH}_4\text{-N}$ supplied. In the case of artificially inoculated cultures, the percentages of fixed $\text{NH}_4\text{-N}$ obtained through nitrification agreed closely with the percentages of fixed $\text{NH}_4\text{-N}$ recovered by barley plants (7, p.121). This indicated that the plants were not able to utilize any fixed $\text{NH}_4\text{-N}$ in excess of that made available through nitrification.

The movement of $\text{NH}_3\text{-N}$ in soil has also been studied by Dow, Moodie, and Stanberry (18, pp.353-356) and by Nelson (37, pp.154-157) who found that the method of application of N fertilizers had a direct bearing upon the distribution of N in the soil. In addition, Lorez and

Johnson (34, p.127) showed that the form of N fertilizer applied influences crop yields on certain light, slightly alkaline soils; NH_4 fertilizers providing greatly increased yields over NO_3 fertilizers. These workers attributed the difference to the effect the added fertilizer has on the native soil phosphate: the physiological acid salt $(\text{NH}_4)_2\text{SO}_4$ effectively released this soil phosphate, whereas N from NO_3 did not.

Broadbent and Stojanovic (9, p.359) studied the effect of oxygen on soil N transformations. Ammonium and NO_3 salts were added to soil and the distribution of inorganic N determined at the end of each experiment. In two experiments the stable isotope of N (N^{15}) was used to determine accurately the fate of added NO_3 -N. Immobilization of added inorganic N occurred whether or not ample N was available to the soil population, probably due to preferential assimilation (9, p.362). Reduction of NO_3 ion to NH_3 was almost negligible at all oxygen concentrations. Denitrification of added NO_3 -N was inversely related to the partial pressure of oxygen, but was of appreciable magnitude even under fully aerobic conditions. No evidence of N losses from the soil was obtained when NH_4 salts were added. In a related study Amer and Bartholomew (3, p.217) investigated the effect oxygen exerts on soil N transformations. They found

that the minimum oxygen concentration for nitrification was 0.2 to 0.4%, and that nitrification was reduced to approximately half the rate when the oxygen concentration was maintained at 2.1% as at 20%. Reducing the oxygen concentration from 20 to 11% had only a negligible influence upon the rate of nitrification. Their data indicated that the optimum concentration of oxygen for nitrification in soil is comparable to that contained in the air.

The gaseous phase of soil was reviewed by Russell and Appleyard (43, pp.1-48). They pointed out that at a depth of 6 inches the free air in the pores of soil is similar in composition to ordinary air in all but two respects: 1) soil air contains more CO_2 and correspondingly less oxygen, averaging 0.25% CO_2 and 20.6% oxygen as compared to 0.03% CO_2 and 20.96% oxygen in the atmosphere. 2) It shows greater fluctuations in composition. Usually the sum of CO_2 and oxygen is only slightly less than in normal air, but at periods when nitrates rapidly increase there is a perceptible falling off of oxygen, and a still greater one in waterlogged soils. Apparently the fluctuations in composition of the free soil air are mainly due to changes in the rate of biochemical reactions in the soil. The rate of biochemical activity attains a maximum value in the late spring and again in

autumn, with minimum values in summer and winter. In autumn the bacteria increase first, then CO_2 rises, and finally the $\text{NO}_3\text{-N}$ increases. From November to May the activity curves closely follow those for the soil temperature which thus appears to be one dominating factor. Observed differences between rainfall and soil moisture indicate that rainfall does something more than add water to the soil. It was shown that the dissolved oxygen brought in is probably a factor of considerable importance in renewing the dissolved soil atmosphere and facilitating biochemical changes.

Quastel and Webley (41, pp.257-266) showed that the admixture of straw to soil greatly improved its aeration, resulting in higher respiratory rates. Sylvestre (46) felt, however, that if straw is to be turned under, N should also be added to the soil in order to prevent depletion of soil N reserves.

A study was conducted for 89 weeks by Barnes (5, pp.365-368) to determine the fate of soluble N in moist aerated soil to which straw was added. The soluble fraction of N changed little during the study. Ammonia N was reduced to a trace in all cases. Little change took place after 11 weeks apart from a very slow and slight increase of $\text{NO}_3\text{-N}$. With straw there was less accumulation of NO_3 during the duration of the study than with

no additive. Only in the case in which $(\text{NH}_4)_2\text{SO}_4$ was added was there an appreciable loss in N. In the latter case the loss was 30%.

Nitrification was studied by Wilson (53, pp.3-36) and by Chapman and Liebig (12, pp.276-282). The latter workers found that under field conditions, especially where the soils were not warm enough for rapid biological activity, the NO_2 produced persisted for several months (12, p.277). In none of their field studies was there any evidence of NO_2 accumulation from denitrification, even though occasionally heavy fertilization with $\text{NO}_3\text{-N}$ fertilizer supplemented with straw was immediately followed by heavy rains or irrigation. They concluded that wherever high NH_4 concentrations appear in neutral or alkaline soils, more or less NO_2 accumulation may be expected (12, p.281).

Soil N losses will occur regardless of the form in which N is added. Martin and Chapman (35, p.10) tested the rate of volatilization of N fertilizers from the soil. They found that when the pH of the surface soils exceeds 7.0 and the $\text{NH}_4\text{-N}$ exists at or near the surface, some NH_3 (14-26%) will be lost by volatilization. Appreciable amounts were lost only when there was a simultaneous loss of water, and the rate was accelerated by rise in temperature. This observation was true whether the

source of NH_3 was $(\text{NH}_4)_2\text{SO}_4$, NH_4NO_3 , urea, or anhydrous NH_3 . Losses of NH_3 occurred in the acid soils only when urea and anhydrous NH_3 were added and were negligible when the NH_3 was in the form of neutral or acid salts. Jewitt (31, p.404) found that gaseous NH_3 was lost in considerable quantities when $(\text{NH}_4)_2\text{SO}_4$ was applied to certain alkaline Sudan soils, the loss taking place over long periods from moist soil, at a rate influenced mostly by the rate of application of the fertilizer. He suggested a mechanism for the loss, according to which the base exchange equilibrium in the soil tends to maintain the concentration of NH_3 in the soil solution at a constant level, whereas the normal buffered state of the soil solution maintains constant the hydroxyl ion concentration. Under these circumstances the NH_3 is lost as from a dilute solution, at a constant rate proportional to the NH_3 concentration in the soil solution (31, p.405). Jewitt felt that commercially important losses of NH_3 occur in the field when $(\text{NH}_4)_2\text{SO}_4$ is broadcast on alkaline soil. In this he is in agreement with Martin and Chapman (35, pp.9-10) and with VanSchreven (47, p.260) who mentioned that appreciable amounts of $\text{NH}_3\text{-N}$ may be lost by evaporation when NH_4 -containing fertilizers are added to such soils.

It is now a well established fact that N losses may occur anaerobically via the oxides of N (49, p.184). Jones (32, p.194) reported that under anaerobic conditions about 80% of the N in added NO_3 is lost from a soil as N gas within 3 days after the air dry soil is moistened. Interestingly enough, the rate and extent of the loss was almost as great where no source of energy was supplied as where 0.5% sucrose was added. By the use of the N^{15} tracer method Jones determined that over the pH range of 4.0 to 6.0 only a negligible trace of the evolved N came from sources other than nitrates and nitrites formed in or added to the soil. The evolution of N gas was probably the result of the activities of denitrifying bacteria that utilize oxidized forms of N as hydrogen acceptors (32, p.195). The results of De and Digar (15, pp.129-132) agree, showing that low oxygen levels favor N losses. Broadbent (8, p.131) found, however, that when NO_3 was added to soil samples (in the presence of ground clover), losses of N occurred at oxygen levels ranging from 0.14 to 47% oxygen. Below 4.5% oxygen, all NO_3 -N disappeared from untreated soils. Some evidence was obtained that oxygen in the NO_3 radical is used for the oxidation of organic matter. His results suggest that aerobic denitrification may account for part of the large NO_3 losses

known to occur in many California soils.

Sacks and Barker (44, p.19) undertook to investigate the direct effects oxygen has upon reduction of nitrates and nitrites. He found that oxygen has a twofold action on denitrification: it suppresses the formation of the nitrate and nitrite reducing enzyme systems in bacteria and when these systems are present it decreases their activities. Quantitative data were presented illustrating both these effects of oxygen. He also showed that inhibition of denitrification by air was largely reversible over short periods of time. The ability of bacterial cells to denitrify did not decline appreciably as a result of continuous growth under aerobic conditions (44, p.20).

Nitrogen losses may occur at the NO_2 stage also. Experiments were conducted by Fraps and Sterges (24, p. 177) and by Jansson (30, pp.330-334) and both research groups concluded that nitrites are decomposed by acid soils. Fraps indicated that in rare cases nitrites may be formed during the nitrification of $(\text{NH}_4)_2\text{SO}_4$ and decomposed chemically in acid soils with loss of N. Wahhab and Uddin (48, p.120) studied loss of N due to interaction of NH_4 and NO_2 ions in aqueous media and alkaline soil. They observed loss at high concentrations only after dessication, which caused further concentration of

the reacting ions. Interaction of ions was independent of soil as a substrate and of temperature of desiccation. At slightly alkaline pH, losses due to volatilization of $\text{NH}_4\text{-N}$ and spontaneous decomposition of NaNO_2 were much greater than those due to $\text{NH}_4\text{-NO}_2$ ions interaction. In highly alkaline soil, loss in $\text{NO}_2\text{-N}$ ions due to interaction was very high. Allison, Doetsch, and Sterling (1, p.312) reported N gas losses due to interactions of nitrites with amino acids, but felt that the required environmental conditions for this reaction to proceed makes improbable any appreciable loss of gaseous N from soils by this mechanism.

Wijler and Delwiche (51, pp.155-169) followed denitrification quantitatively with KN^{15}O_3 as a tracer, and identified three products: N_2O , free N, and NO. Nitrous oxide was found to be the major product of denitrification under most soil conditions. The moisture content of the soil indirectly influenced denitrification by inhibiting the diffusion of oxygen into the soil, resulting in more denitrification. Below the permanent wilting point limiting moisture depressed denitrification directly. Total denitrification rates were quite constant above pH 6.0, but the proportion of N_2O and N_2 were pH dependent. Above pH 7.0 N_2O could be readily reduced to N; below pH 7.0 its reduction was strongly inhibited. Below pH 6.0

the formation of NO became more prominent and the rate of denitrification was decreased. The suppressing effect of oxygen on soil denitrification was found to be very pronounced. Oxygen tensions as low as 5 mm mercury decreased denitrification to rates almost 1/10 of the anaerobic denitrification rate. The initial NO₃-N concentration did not influence the rate of denitrification. Isotope content of all denitrification products showed them to be wholly derived from NO₃, indicating that an enzymatic denitrification was taking place. Reduction of NO₃-N to NH₃ was found to be very small, even when all the NO₃-N was lost during denitrification. Nitrate-N was reduced to NH₃ in larger amounts only when excessive amounts of organic matter were added under anaerobic conditions. Incorporation of labelled N into the organic fraction was always very small. Wijler concluded that rapid oxidation of NH₃-N to NO₃-N may result in higher N losses under conditions of fluctuating oxygen tensions than under strict anaerobiosis.

Najjar and Allan (36, pp.209-214) performed some experiments on the formation of N₂, N₂O, and NO by extracts of denitrifying bacteria which paralleled nicely the work of Wijler's group. From Pseudomonas stutzeri and a denitrifying strain of Bacillus subtilis, they obtained cell-free extracts which reduce NO₂ to a mixture of gaseous

products. It was found that the gas formed by Ps. stutzeri extracts was principally N, while B. subtilis preparations produced an appreciable quantity of N_2O . Both formed nitric oxide from NO_2 .

Further studies were made by Arnold (4, pp.116-128) on the loss of N from soils. He found that soils approaching saturation with moisture rapidly release large amounts of their available N as N_2O ; at lower moisture contents very slow evolution of gas can take place (4, p.118). Nitrous oxide evolution is a factor of considerable significance to the N economy of soils, not only from the standpoint of actual N losses, but because a N_2O atmosphere may greatly inhibit N fixation. In respect to this, Wilson, Gilchrist, and Roberts (54, p.87) found N_2O to be a specific competitive inhibitor of N fixation by Azotobacter vinlandii.

EXPERIMENTAL METHODS

Two separate lines of investigation were followed in studying the transformation of nitrogen in soil. These two approaches are referred to as chemical and manometric studies. Each is treated separately in the following sections on experimental methods and results.

Chemical Studies

Soil

A Palouse silt loam was used throughout the present study. The parent material of this soil was derived from loess. The reaction of this soil is usually on the acid side to a depth of approximately 4 feet. In general, this soil has proven to be highly productive, primarily because of the relatively high and deep organic matter accumulation.

Soil treatment

The soil was collected from the Agriculture Experiment Station at Pendleton, Oregon. It was passed through a 10 mesh screen and stored in large metal cans in a dry cool storage bin.

Fifty gram samples on a water-free basis were weighed out and placed in 125 ml. Erlenmeyer flasks. Those soils treated with wheat straw (at rates of 1% and 2% equivalent

to 10 tons and 20 tons per acre respectively) were intimately mixed with the wheat straw prior to being weighed and added to the flasks.

The wheat straw was ground to pass 60 mesh. Other soil additives such as glucose and nitrate salts were dissolved in water to a concentration which would contain the desired weight of additive in the volume of water added to each flask. Sets were run to determine the influence of glucose on nitrate reduction anaerobically at carbon-nitrogen ratios of 6:1 and 2:1. Sets were also run using weights of wheat straw and of sodium nitrate yielding carbon-nitrogen ratios of 25:1 and 13:1. These latter sets were incubated under both aerobic and anaerobic conditions and the influence of two levels of wheat straw upon the added nitrogen under these conditions determined. Further sets of flasks were prepared which were similarly treated to those mentioned above except that ammonium nitrate was used as the nitrogen source. In the ammonium nitrate sets the soils contained carbon-nitrogen ratios of 7:1 and 3.5:1. Weights of sodium nitrate and ammonium nitrate were adjusted to yield the same weight of nitrate nitrogen to all sets. Control flasks of soils receiving no treatment and of soils treated with nitrogen alone accompanied all sets. Also, a control flask containing glucose alone was run in sets where the

soil received the glucose treatment. In all cases, moisture was adjusted to 60% of the saturation capacity of the soil.

In the aerobic sets cotton plugs were loosely placed over the flasks to allow maximum aeration. The weight of the final flask in the aerobic sets was checked daily. On the assumption that rate of evaporation was uniform in all flasks, the moisture lost from the check flask was replaced in the aerobic set when necessary.

The anaerobic series required additional care to ensure a closed system. 1.5 ml. of 30% KOH was placed in small culture tubes (50 X 0.9 mm). A folded piece of filter paper (55 X 25 mm) was then inserted into each tube and the tube carefully pressed through the moistened soil to the bottom of the flask with a pair of forceps. The flasks were then tightly closed with rubber stoppers and a vaspar seal was made as an added measure to insure a closed system. The center wells containing KOH served to capture evolved CO_2 and convert it to the soluble carbonate form. The inserted filter paper afforded a larger surface area in contact with the CO_2 within the closed atmosphere. Thus the center wells served a double function. First they allowed for collection of evolved CO_2 in a form which could later be measured by titration. This gave a direct index of microbiological activity in

the flasks. Secondly, the absorption of CO₂ prevented the production of gas pressures within the flasks great enough to break the seals.

All flasks were incubated at 30°C and chemical determinations carried out at designated time intervals.

Analytical procedures

Chemical determinations were made to trace with time the transformations of nitrogen and the evolution of gases resulting from microbiological activity within the soil.

Total nitrogen

Total nitrogen was determined according to the standard Kjeldahl method described in the A.O.A.C. handbook (33, p.32). The procedure was modified by having a saturated solution of boric acid in the receiving flask to capture the released ammonia. The boric acid was then titrated with N/14 sulfuric acid using bromocresol green-methyl red indicator.

Ammonia nitrogen

Ammonia nitrogen was determined by placing a known weight of soil into a Kjeldahl flask, adding the buffer described by Nichols and Foote (39, p.312), 300 ml. distilled water, and steam distilling the contents into

30 ml. of saturated solution of boric acid. The distillate was then titrated with N/14 sulfuric acid, using bromocresol green-methyl red indicator.

Nitrite nitrogen

The nitrite nitrogen was determined by the procedure outlined by Nelson, Kurtz, and Bray (38, p.1081). The standard curve was prepared and samples of soil extract read in a Beckman Model B spectrophotometer, set at 520 millimicrons and sensitivity 3.

The procedure of Nelson, Kurtz, and Bray included modifications for determination of nitrate nitrogen as well, but this test for nitrates was discarded after repeated attempts over several months failed to yield reliable values. Their method for nitrates cannot, apparently, be used for testing soil extracts.

Nitrate nitrogen

The procedure followed for determination of nitrates was that of Harper (27, p.182). It was modified, except where the nitrate level was at only a few ppm, in that only a 1.0 ml. aliquot was evaporated to dryness. The standard curve and extracts of treated soils were also determined by the Beckman Model B spectrophotometer. Settings were 430 millimicrons at sensitivity 4.

H-ion concentration

The H-ion concentration was determined by use of the Fisher automatic titrimeter which, after allowing ten minutes to warm up, was adjusted with standard buffer, pH 7.0.

Carbon dioxide

Carbon dioxide determinations were made using the Fisher automatic titrimeter. The instrument was standardized as above. Base from the center well (and the filter paper as well) was transferred to a 250 ml. beaker, and about 50 ml. distilled water added. The beaker was placed on the titrimeter stand, the mechanical stirrer turned on, and 1 N sulfuric acid added cautiously to remove excess base. At about pH 9.0 to 9.5 N/12 sulfuric acid was substituted for the 1 N and the pH was lowered to 8.5. The N/12 acid was then added until the pH was brought to 5.0. If more than 10 ml. of the acid were required then pH 4.6 was used as the end point. The volume in ml. of N/12 acid required to lower the pH from 8.5 to 5.0 or to 4.6 gave the mg. of carbon as CO₂ in the sample (14, p.468).

Glucose

Glucose content was determined chemically by the oxidation-reduction and titration method of Shaffer and

Somogyi (45, pp.695-713).

Manometric Studies

Chemical tests gave evidence that when nitrate is added to a soil containing a readily oxidizable carbon source, the nitrate will diminish in quantity or disappear altogether. Further, these chemical data showed that when the nitrate disappeared the nitrogen itself could not be picked up appreciably, if at all, in other forms, namely nitrite, ammonia, or total nitrogen. The only other form in which this nitrogen could disappear would be as a gas. This implied the possibility of free nitrogen gas or gaseous oxides of nitrogen being evolved from soils where loss of nitrate occurred. A manometric procedure, therefore, seemed logically suited to check for this possible gas evolution.

125 ml. Warburg flasks with center wells and single vented side arms were used. By means of forceps the center wells were capped with small rubber stoppers to prevent soil entering the flask from spilling into them. After 50 grams water-free soil had been added to each flask, the flask was tamped a few times to allow the soil to settle. The water and other components were added, and the small stopper was then removed and 2 ml. KOH and filter paper added to the center well. As before, the

wheat straw was intimately mixed with the soil before placing it in the flask, and the soluble components added with the water. Moisture was adjusted to 60% of saturation in all cases. The ground glass stoppers attached to the manometers were coated with vaseline, and by rotating the mouth of the flasks onto the stoppers a closed system connecting the flasks to the manometers was obtained. The temperature of the water bath was maintained at 30°C.

The soils tested received various treatments and gas exchange was measured under these conditions. Studies were made of soils receiving no treatment, soils receiving nitrate alone, and soils receiving wheat straw alone. The latter two served as controls. In addition, soils received treatment of nitrogen in several forms along with wheat straw. Two levels of nitrate were run to determine what effect the nitrate level of a soil may have on gas losses.

EXPERIMENTAL RESULTS

All chemical data, with the exception of hydrogen ion concentration, have been computed on the basis of 100 grams of water-free soil. The manometric determinations are based on 50 grams of water-free soil.

Chemical Studies

Influence of glucose

Reference to Table 1 shows that where sodium nitrate alone is added to soil and the soil is incubated under semi-anaerobic conditions, no reduction of nitrate occurs, even after 14 days incubation. A very different picture occurs, however, when glucose is added to soil which contains nitrate. Table 2 shows clearly the complete disappearance of 7.5 mg. native nitrate in a soil to which glucose has been added.

The data from Tables 3 and 4 are graphically demonstrated in Figures 1 and 2. Here it is again evident that when glucose is added to a soil, under anaerobic conditions, nitrates rapidly disappear. Figure 1 also shows that a longer period of time (8 days) is required for the complete removal of the higher level (600 mg.) of nitrate. Figure 2 shows that, coincident with nitrate removal, detectable quantities of nitrite begin to appear

in about 16 to 20 hours. The nitrite at its heaviest concentration may account for as much as 33% of the added nitrogen, but the nitrite production falls off rapidly after reaching its peak and then it too disappears.

Influence of wheat straw

The data listed in Tables 5 and 6 are depicted graphically in Figure 3, and contents of Tables 7 and 8 are shown in Figure 4. Tables 6 and 8 compare the results obtained from flasks treated and held under similar conditions to those with glucose, but substituting wheat straw for the readily oxidizable glucose. The marked effect of 1% wheat straw upon added sodium nitrate, shown in Figure 3, gives essentially the same picture of nitrate disappearance as in the presence of glucose. A study of Figure 4 reveals this effect to be even more pronounced when the level of wheat straw in soil is doubled. Significantly, there is some loss of nitrate even under fully aerobic conditions, and this is also accelerated by the higher level of wheat straw. Aerobically, 20% of the nitrate disappeared at the 1% wheat straw level at the end of 12 days, whereas, in the same time period 47% of the nitrate disappeared at the 2% level. There was no detectable appearance of nitrite or ammonia in the aerobic sets and the increase in total

nitrogen was negligible.

Under anaerobic conditions 75% of the nitrate had disappeared in 12 days at the 1% level of wheat straw and the nitrate had disappeared entirely after 5 days at the 2% level. As can be seen in Figure 5, nitrites appeared anaerobically at both levels of wheat straw, reaching higher values with the more rapid disappearance of nitrate. In general, nitrite seemed to disappear at about the same time as the nitrate. Anaerobically there is no appearance of ammonia. The loss in total nitrogen was 5% at the lower, 10% at the higher level of wheat straw. The pH remained constant under aerobic conditions, but under anaerobic conditions it shifted markedly to the alkaline side coincident with the disappearance of nitrate.

From Tables 9 and 11, which are graphically shown in Figures 6 and 7, the effect of wheat straw upon nitrogen transformations is only slightly different. There is no loss of nitrate aerobically but an actual increase of about 3% at both levels of wheat straw. This is accompanied, however, by a loss of ammonia nitrogen of 22% at the lower and 54% at the higher level of wheat straw. Again, totaling the ammonia and the nitrate nitrogen values shows a loss of ammonia nitrogen of 5% and 26% respectively. Nitrites did not make their appearance

Table 1. Check determinations on soil treated with
200 mg. NaNO_3 /100 gm soil

Time	milligrams*			pH
	NO_3	NO_2	CO_2	
3 hrs.	--	0	2.6	--
8 hrs.	--	0	3.4	--
11 hrs.	38.8	0	3.8	--
17 hrs.	37.5	0	3.8	--
21 hrs.	37.5	0	5.7	--
33 hrs.	37.5	0	3.2	--
5 days	42.5	0	5.6	--
7 days	42.4	0	7.3	--
8 days	43.2	0	9.1	5.7
9 days	44.8	0	9.0	5.6
11 days	44.0	0	10.4	5.7
14 days	42.4	0	11.1	5.4

* Soil incubated under semi-anaerobic conditions

Table 2. Check determinations on soil treated with
500 mg. glucose/100 gm. soil

Time	milligrams*			
	NO ₃	NO ₂	CO ₂	glucose
3 hrs.	7.5	0	8.0	--
8 hrs.	4.8	0	16.5	262
11 hrs.	2.5	0	27.4	238
14 hrs.	0	0	32.6	98
16 hrs.	0	0.1	36.6	105
18 hrs.	0	0.2	39.8	94
26 hrs.	0	trace	42.1	86
33 hrs.	0	0	50.2	50
2 days	0	0	60.2	0
5 days	0	0	63.6	0
7 days	0	0	97.6	0
8 days	0	0	--	0

* Soil incubated under semi-anaerobic conditions

Table 3. Influence of 500 mg. glucose upon fate of NaNO_3 added at the rate of 200 mg./100 gm. soil

Time	milligrams*			
	NO_3	NO_2	CO_2	glucose
3 hrs.	--	0	2.6	--
8 hrs.	--	0	6.5	312
11 hrs.	38.8	0	12.2	306
17 hrs.	27.5	1.1	31.6	--
21 hrs.	22.8	4.8	37.3	--
27 hrs.	20.0	8.3	43.0	26.4
34 hrs.	16.0	10.4	48.2	--
41 hrs.	15.6	12.0	47.6	28.4
45 hrs.	13.7	12.2	52.4	--
50 hrs.	13.7	13.0	48.0	--
57 hrs.	11.7	12.0	48.0	--
82 hrs.	10.4	7.6	51.0	--
5 days	5.0	1.0	63.2	--
7 days	0	0	74.2	0

* Soil incubated under semi-anaerobic conditions

Table 4. Influence of 500 mg. glucose upon fate of NaNO_3 added at the rate of 600 mg./100 gm. soil

Time	milligrams*				pH
	NO_3	NO_2	CO_2	glucose	
3 hrs.	112.0	0	2.6	444	--
14 hrs.	110.0	0	8.2	372	--
16 hrs.	114.0	0	18.2	324	--
20 hrs.	110.0	trace	21.8	252	5.8
22 hrs.	95.0	0.2	27.6	210	5.8
24 hrs.	102.5	0.5	30.8	194	5.9
26 hrs.	94.5	1.2	34.8	--	5.9
28 hrs.	96.0	3.0	46.2	--	6.1
40 hrs.	82.5	10.2	50.7	--	6.0
3 days	60.5	11.7	57.1	--	6.9
4 days	66.3	7.3	68.3	--	7.3
5 days	42.7	9.8	74.6	--	7.7
8 days	0.3	0.8	90.1	0	8.6
11 days	0	0	106.3	--	7.4

* Soil incubated under semi-anaerobic conditions

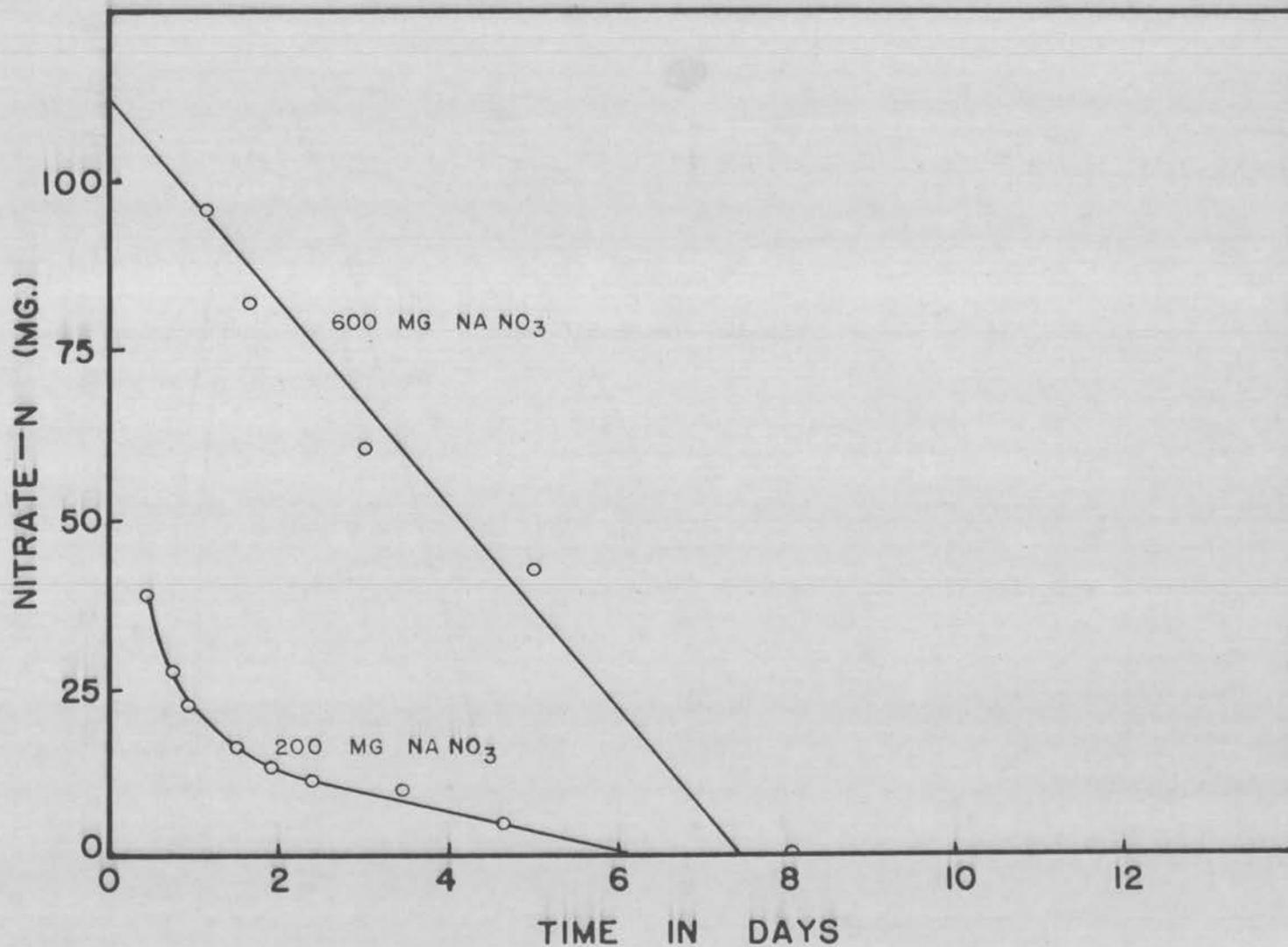


FIG. 1. OBSERVED DISAPPEARANCE OF NITRATE (NaNO_3) IN SOIL TREATED WITH 500 MG GLUCOSE

Table 6. Influence of 1% wheat straw on NaNO_3 added at the rate of 200 mg./100 gm. soil and incubated under anaerobic conditions

Time (days)	milligrams											
	NO_3		NO_2		NH_3		Total N		CO_2		pH	
0.1	46.7	(55.0)*	0	(0)	0	(0)	39.9	(41.1)	5.1	(2.7)	5.9	(5.7)
1	41.5		0		0		--		15.3		6.1	
2	41.0		0.1		0		--		28.9		6.3	
3	40.5	(56.0)	1.4	(0)	0	(0)	40.4	(42.0)	31.7	(4.5)	6.3	(6.0)
4	35.3		1.6		0		--		41.0		6.3	
5	29.4		1.1		0		--		36.3		6.6	
6	23.7	(56.6)	1.4	(0)	0	(0)	38.6	(44.0)	42.2	(7.3)	7.1	(5.8)
7	--		0.6		0		--		43.9		--	
8	19.8		0.6		0		--		47.1		7.1	
9	18.0	(56.6)	0.6	(0)	0	(0)	38.8	(43.5)	49.6	(7.7)	7.2	(5.7)
10	13.8		0.7		0		--		48.2		7.4	
11	10.6		0.6		0		--		54.3		7.9	
12	11.9	(56.6)	0.8	(0)	0	(0)	37.7	(44.7)	50.5	(8.8)	7.7	(5.7)

* Figures in parenthesis refer to control determinations carried out in absence of wheat straw.

Table 7. Influence of 2% wheat straw on NaNO_3 added at the rate of 200 mg./100 gm. soil and incubated under aerobic conditions

Time (days)	milligrams								
	NO_3		NO_2		NH_3		Total N		pH
0.1	52.8	(53.6)*	0	(0)	0	(0)	41.9	(40.5)	5.7 (5.4)
1	46.0		0		0		--		6.2
2	39.4		0		0		--		6.3
3	38.2	(45.6)	0	(0)	0	(0)	42.2	(40.0)	6.4 (5.5)
4	34.7		0		0		--		6.5
5	32.8		0		0		--		6.5
6	31.7	(51.6)	0	(0)	0	(0)	43.1	(41.7)	6.3 (5.5)
7	29.9		0		0		--		6.6
8	28.1		0		0		---		6.7
9	29.4		0		0		--		6.2
10	29.2		0		0		--		6.5
11	28.9		0		0		--		6.4
12	29.9	(52.8)	0	(0)	0	(0)	43.1	(41.1)	6.3 (5.6)

* Figures in parenthesis refer to control determinations carried out in absence of wheat straw.

Table 8. Influence of 2% wheat straw on NaNO_3 added at the rate of 200 mg./100 gm. soil and incubated under anaerobic conditions

Time (days)	milligrams											
	NO_3		NO_2		NH_3		Total N		CO_2		pH	
0.1	51.3	(55.0)*	0	(0)	0	(0)	42.3	(41.1)	14.4	(2.7)	6.1	(5.7)
1	45.5		0.2		0		--		31.2		6.3	
2	36.0		5.0		0		--		38.3		6.5	
3	27.0	(56.0)	6.8	(0)	0	(0)	40.7	(42.0)	43.7	(4.5)	7.0	(6.0)
4	13.2		6.3		0		--		52.1		7.2	
5	3.9		5.5		0		--		54.6		7.7	
6	0		0.8	(0)	0	(0)	37.5	(44.0)	65.5	(7.3)	8.2	(5.8)
7	0		0		0		--		71.0		--	
8	0		0		0		--		73.0		8.0	
9	0	(56.6)	0	(0)	0	(0)	39.4	(43.5)	75.4	(7.7)	8.0	(5.7)
10	0		0		0		--		75.6		8.0	
11	0		0		0		--		80.4		8.0	
12	0	(56.6)	0	(0)	0	(0)	38.1	(44.7)	84.8	(8.8)	8.0	(5.7)

* Figures in parenthesis refer to control determinations carried out in absence of wheat straw.

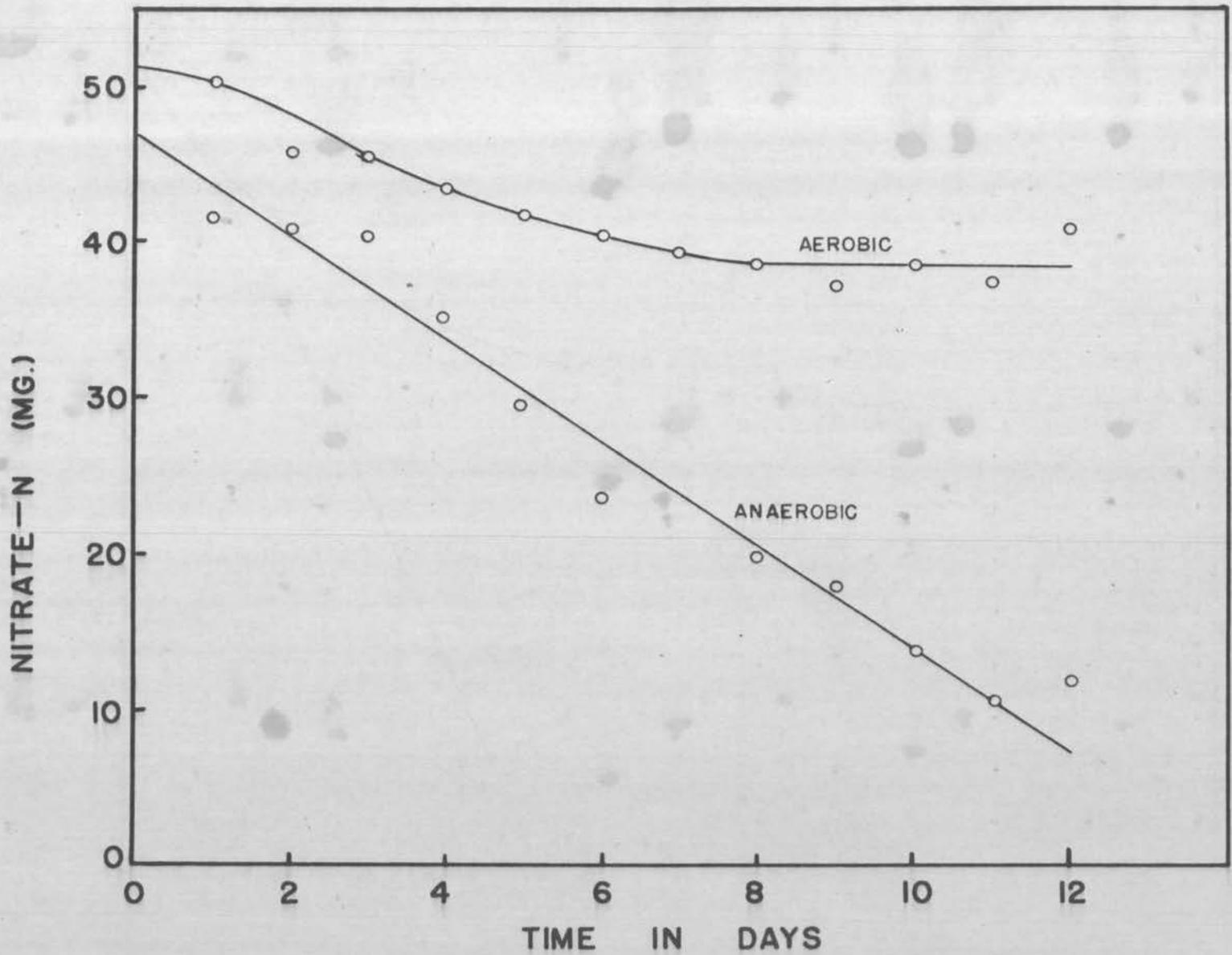


FIG. 3. OBSERVED DISAPPEARANCE OF NITRATE (NaNO_3) IN SOIL TREATED WITH 1% WHEAT STRAW

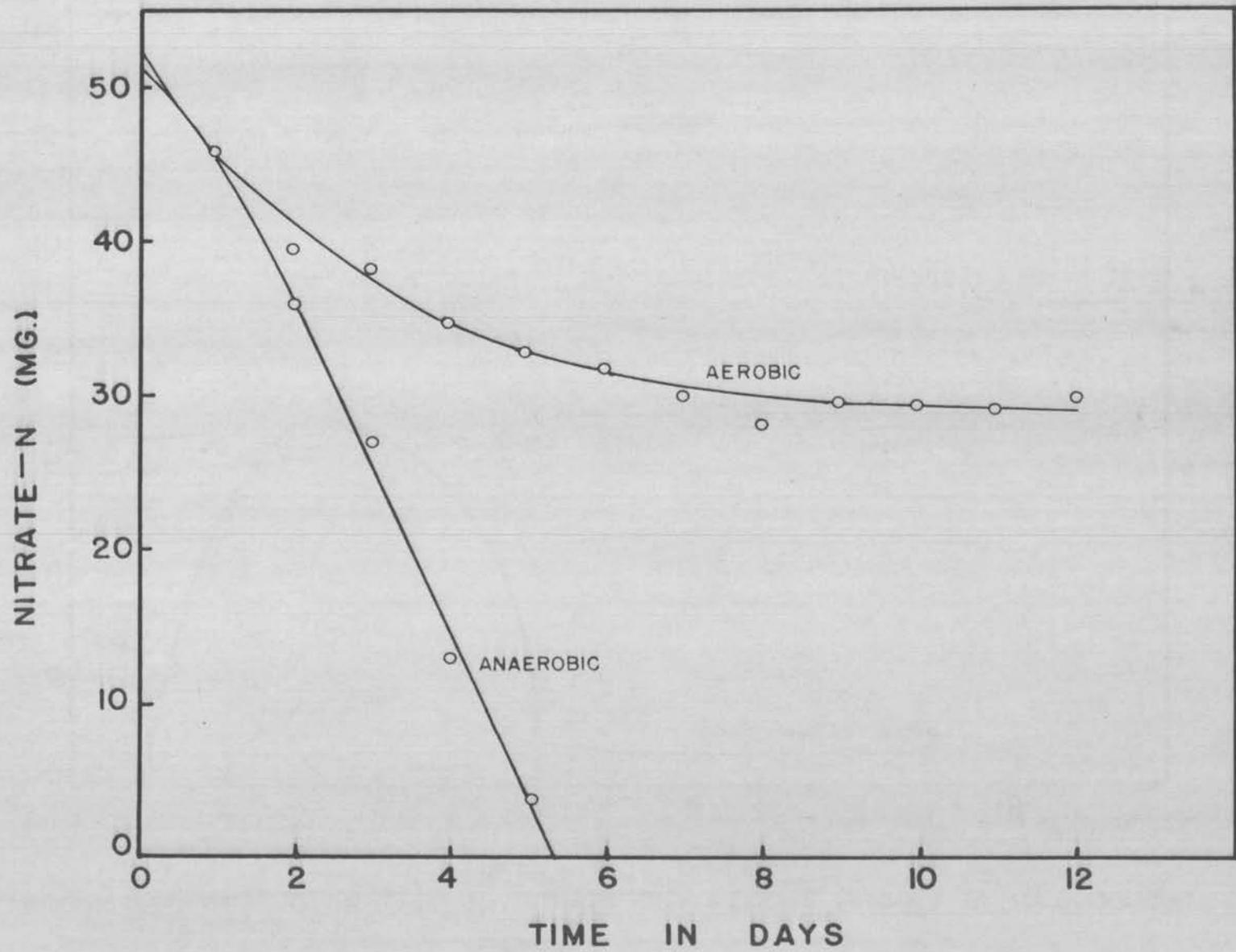


FIG. 4. OBSERVED DISAPPEARANCE OF NITRATE (NaNO_3) IN SOIL TREATED WITH 2% WHEAT STRAW

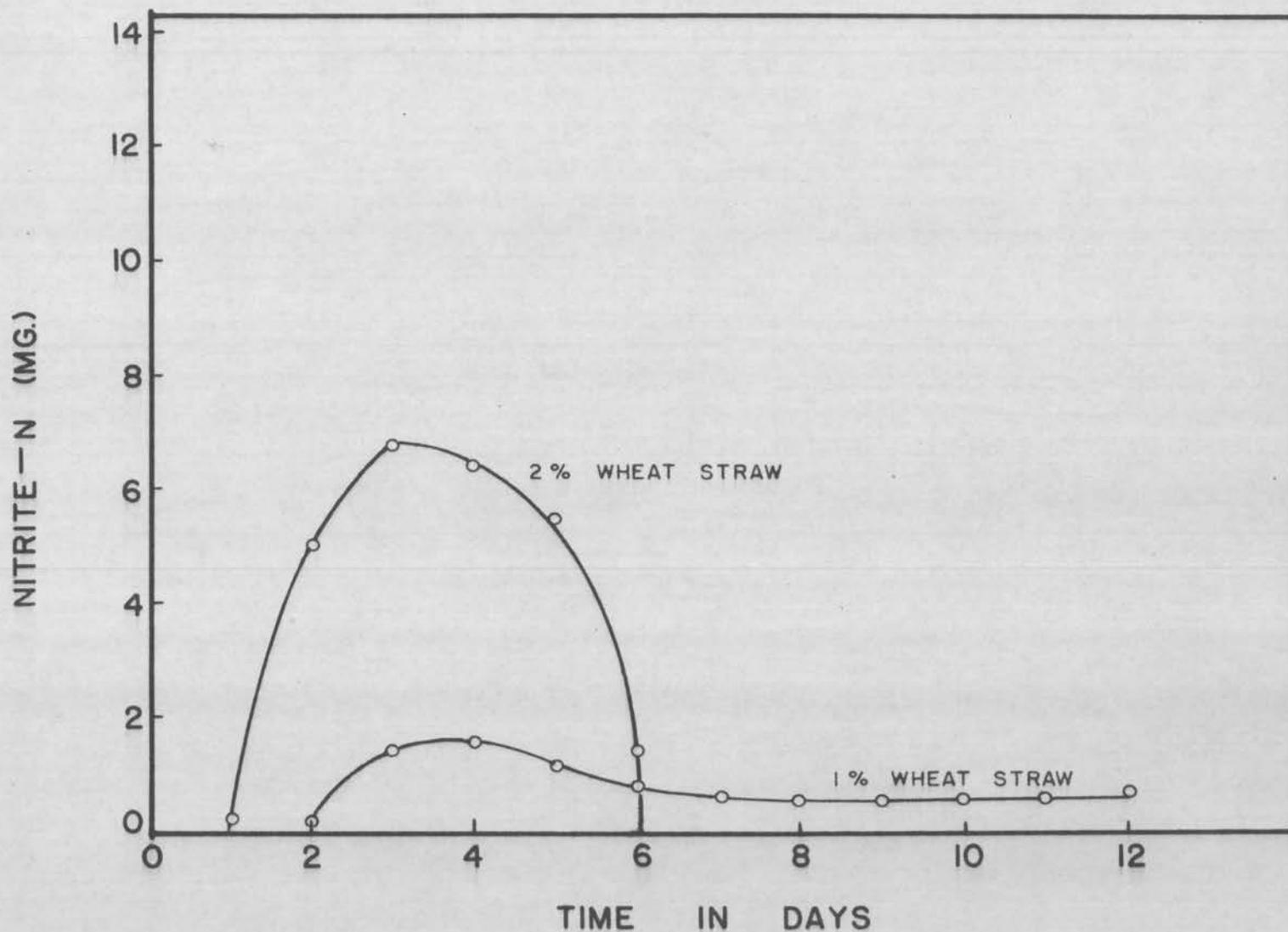


FIG. 5. ANAEROBIC PRODUCTION OF NITRITE FROM NITRATE (NaNO_3) IN SOIL TREATED WITH WHEAT STRAW

aerobically in these sets.

Under anaerobic conditions, however, nitrates disappeared completely after 12 days at the 1% wheat straw level and after 7 days at the 2% level. Nitrites made their appearance more slowly in the ammonium nitrate sets and the rate of production of nitrites was significantly lower than in the sodium nitrate sets. In addition to disappearance of nitrate, 24% of the ammoniacal nitrogen disappeared at the 1% level of wheat straw and 38% at the 2% level.

As in the sodium nitrate sets, the pH remained constant under aerobic conditions of incubation, but under anaerobic conditions the soils moved over to the basic side.

Manometric Studies

Soil differentiation

In early experiments various soil types were tested in the absence of added fertilizers or organic crop residues. Presumably the obtained data would result from the natural soil condition and might be used as a means for comparison of the test soils. Reference to Figure 8 discloses that a wide variation in oxygen uptake values were observed in the three test soils. Soil 1, Chehalis silt loam, consumed over 1500 microliters of oxygen after 10

Table 9. Influence of 1% wheat straw on NH_4NO_3 added at the rate of 188.4 mg./100 gm. soil and incubated under aerobic conditions

Time (days)	milligrams						pH	
	NO_3		NO_2		NH_3			
0.1	46.9	(46.0)*	0	(0)	34.8	(43.5)	--	(--)
1	49.3		0		41.5		5.8	
2	48.6		0		33.6		5.8	
3	49.5	(53.6)	0	(0)	30.4	(41.1)	5.7	(5.9)
4	49.2		0		30.2		5.4	
6	52.4		0		27.1		5.8	
10	51.0	(52.4)	0	(0)	27.7	(34.4)	5.7	(5.7)
12	51.0		0		32.5		5.6	
15	51.0		0		27.7		5.7	
18	51.0	(52.4)	0	(0)	27.0	(30.4)	5.6	(5.7)

* Figures in parenthesis refer to control determinations carried out in absence of wheat straw.

Table 10. Influence of 1% wheat straw on NH_4NO_3 added at the rate of 188.4 mg./100 gm. soil and incubated under anaerobic conditions

Time (days)	milligrams									
	NO_3		NO_2		NH_3		CO_2		pH	
0.1	46.9	(46.0)*	0	(0)	34.8	(43.5)	--	(--)	--	(--)
1	49.0		0		44.8		22.4		5.7	
2	47.3		0.9		42.2		27.3		5.7	
3	42.4	(49.6)	0.8	(0)	39.7	(34.3)	31.7	(6.6)	6.0	(5.6)
4	36.3		0.9		33.5		39.5		6.3	
6	28.9		0.5		38.1		50.6		6.6	
10	14.9	(51.0)	0.9	(0)	34.9	(34.7)	58.0	(10.0)	7.2	(5.9)
12	5.7		1.1		35.7		59.4		7.2	
15	0		0		35.6		64.3		8.4	
18	0	(50.0)	0	(0)	34.1	(31.5)	67.6	(16.0)	8.4	(6.7)

* Figures in parenthesis refer to control determinations carried out in absence of wheat straw.

Table 11. Influence of 2% wheat straw on NH_4NO_3 added at the rate of 188.4 mg./100 gm. soil and incubated under aerobic conditions

Time (days)	milligrams						pH
	NO_3		NO_2		NH_3		
0.1	46.8	(46.0)*	0	(0)	46.5	(43.5)	-- (--)
1	47.0		0		31.4		5.9
2	48.5		0		28.9		5.8
3	48.6	(53.6)	0	(0)	27.8	(41.1)	5.8 (5.9)
4	50.0		0		24.2		5.3
6	48.4		0		19.2		5.7
10	46.4	(52.4)	0	(0)	17.0	(34.4)	5.6 (5.7)
12	48.0		0		22.4		5.3
15	48.0		0		20.2		5.6
18	48.0	(52.4)	0	(0)	21.2	(30.4)	5.6 (5.7)

* Figures in parenthesis refer to control determinations carried out in absence of wheat straw.

Table 12. Influence of 2% wheat straw on NH_4NO_3 added at the rate of 188.4 mg./100 gm. soil and incubated under anaerobic conditions

Time (days)	milligrams									
	NO_3		NO_2		NH_3		CO_2		pH	
0.1	46.8	(46.0)*	0	(0)	46.5	(43.5)	--	(--)	--	(--)
1	47.5		0.7		34.7		25.8		5.7	
2	44.0		1.5		30.6		29.0		5.7	
3	35.7	(49.6)	1.0	(0)	30.8	(34.3)	34.0	(6.6)	6.2	(5.6)
4	32.3		1.1		28.2		38.0		6.3	
6	16.0		1.9		29.9		48.2		7.0	
10	0	(51.0)	0	(0)	27.5	(34.7)	67.8	(10.0)	7.2	(5.9)
12	0		0		31.4		61.2		7.5	
15	0		0		29.9		71.5		7.4	
18	0	(50.0)	0	(0)	29.0	(31.5)	75.1	(16.0)	7.3	(6.7)

* Figures in parenthesis refer to control determinations carried out in absence of wheat straw.

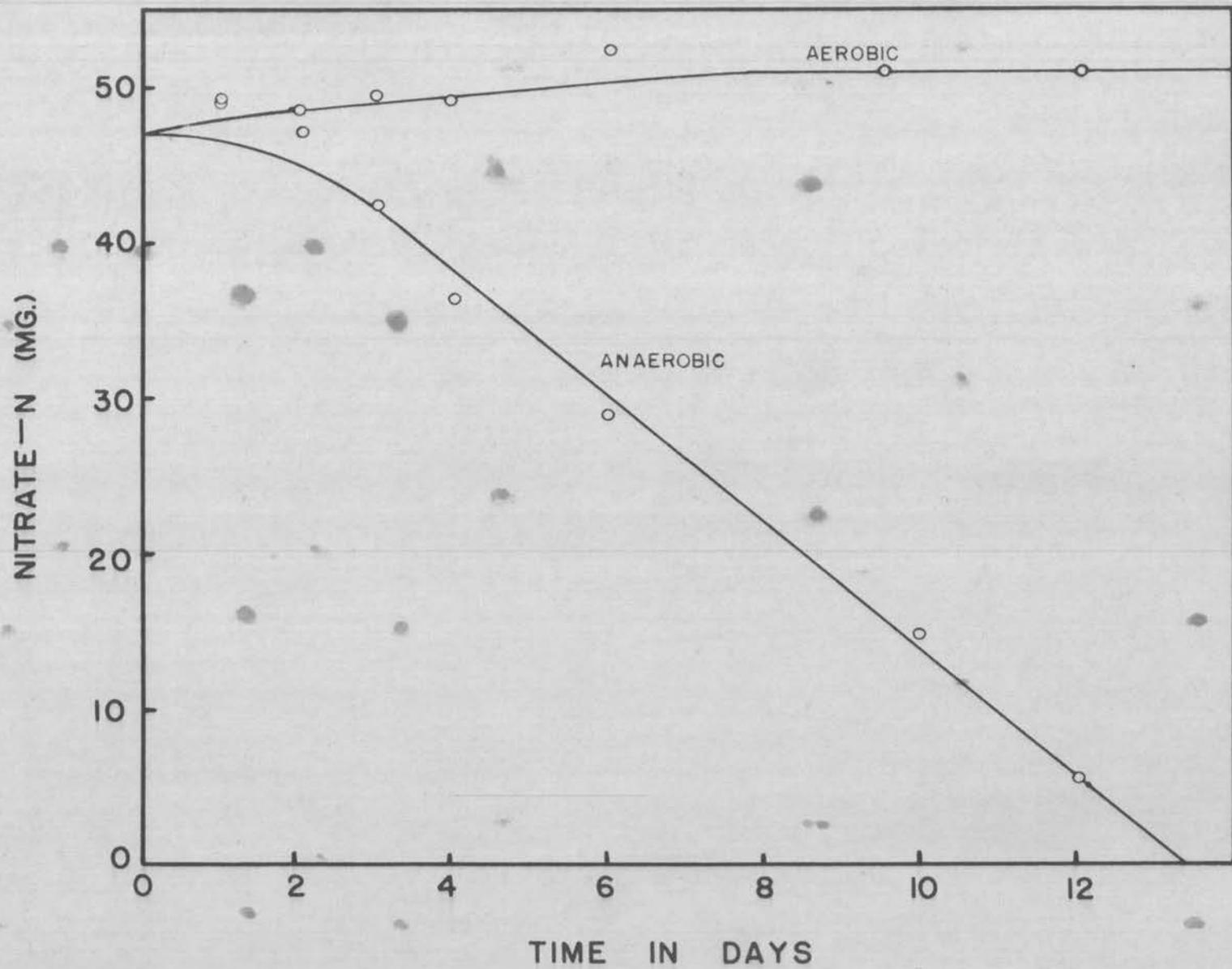


FIG. 6. OBSERVED DISAPPEARANCE OF NITRATE (NH_4NO_3) IN SOIL TREATED WITH 1% WHEAT STRAW

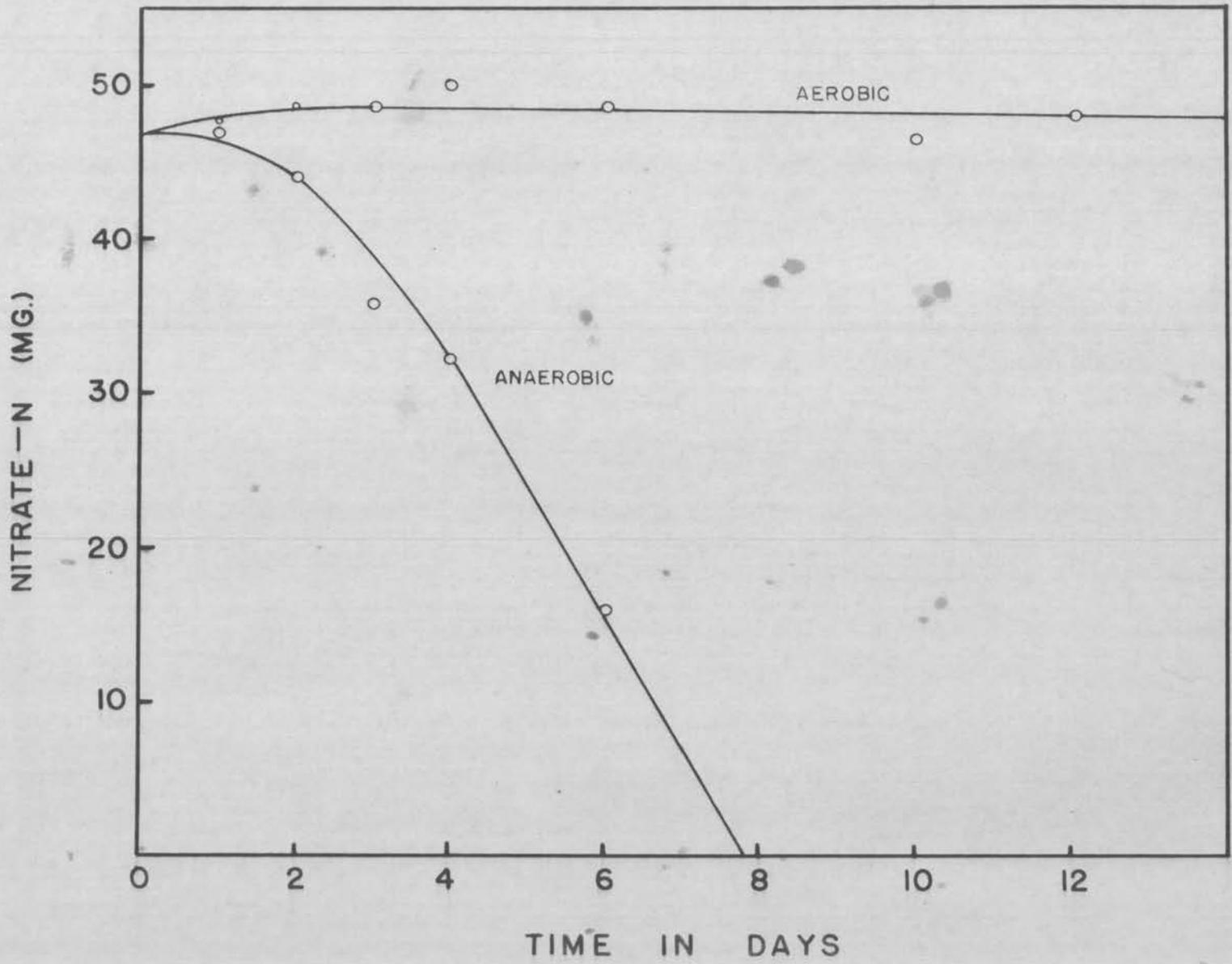


FIG. 7. OBSERVED DISAPPEARANCE OF NITRATE (NH_4NO_3) IN SOIL TREATED WITH 2% WHEAT STRAW

hours incubation; soil 2, Palouse silt loam, approximately 1000 microliters; and soil 3, Brookston silt loam, 400 microliters of oxygen.

Influence of glucose on soil respiration

The fate of organic substances added to soil may be studied by measurement of the total oxygen consumed by the soil flora. In a typical experiment 0.25 grams of glucose was added to a 50 gram sample of Palouse silt loam and in a second test 0.25 grams of glucose plus 0.30 grams of sodium nitrate were used. The results are depicted in Figure 9. As might be expected, the inclusion of glucose stimulated soil respiration 30 to 40 times over that observed in the untreated soil. However, the inclusion of sodium nitrate with glucose caused a decrease in oxygen uptake when compared to the oxygen consumption observed with glucose alone. The plateau shown in Figure 9 begins at 18-20 hours and results from depletion of oxygen in the flask. At the point indicated by the jagged line, oxygen was re-introduced and normal uptake began as before.

Influence of wheat straw on soil respiration

It may be observed in Figure 10 that wheat straw added at the rate of 10 tons/acre greatly stimulated soil respiration over the indicated 6 hour test period.

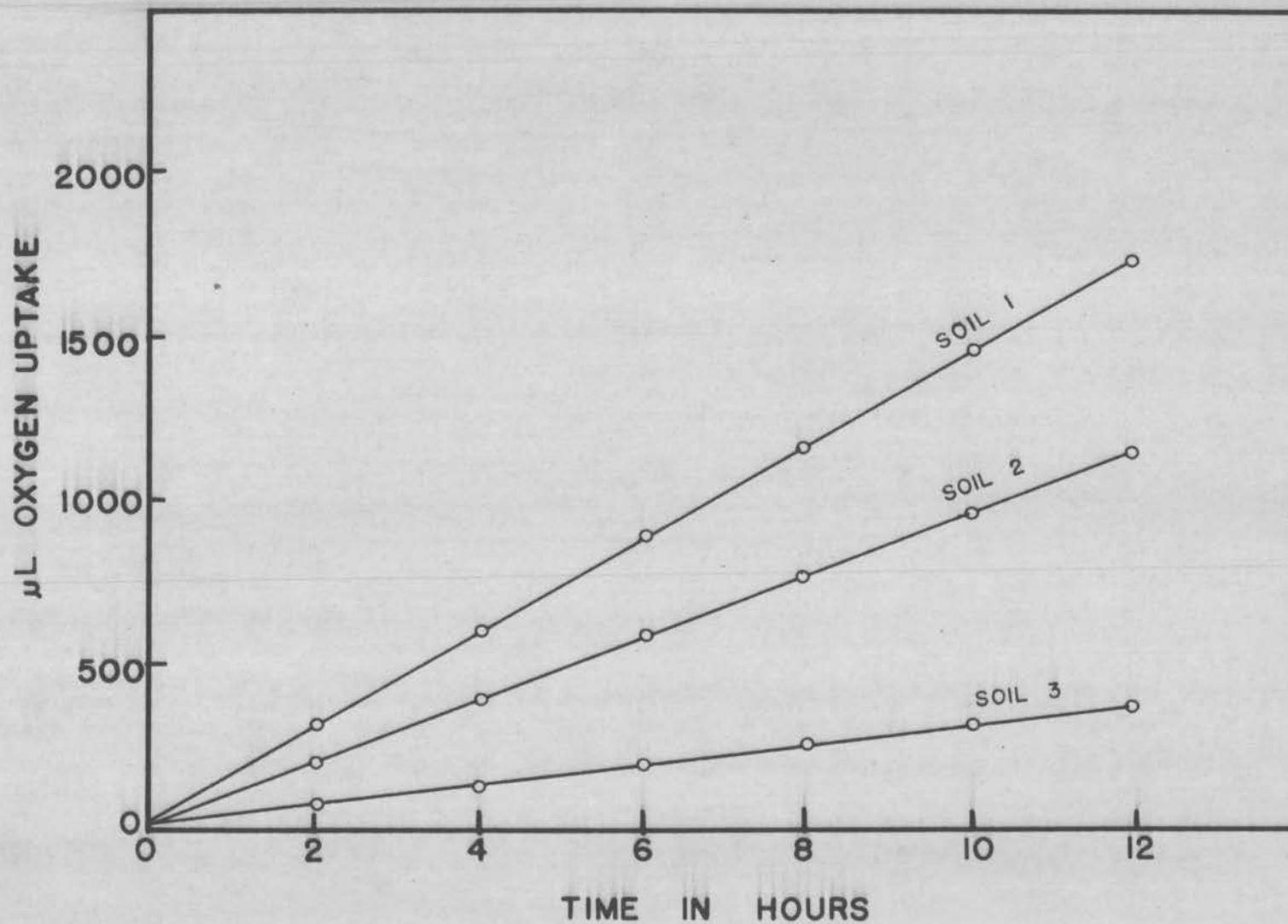


FIG. 8. RESPIRATION OBTAINED WITH 3 SOIL TYPES

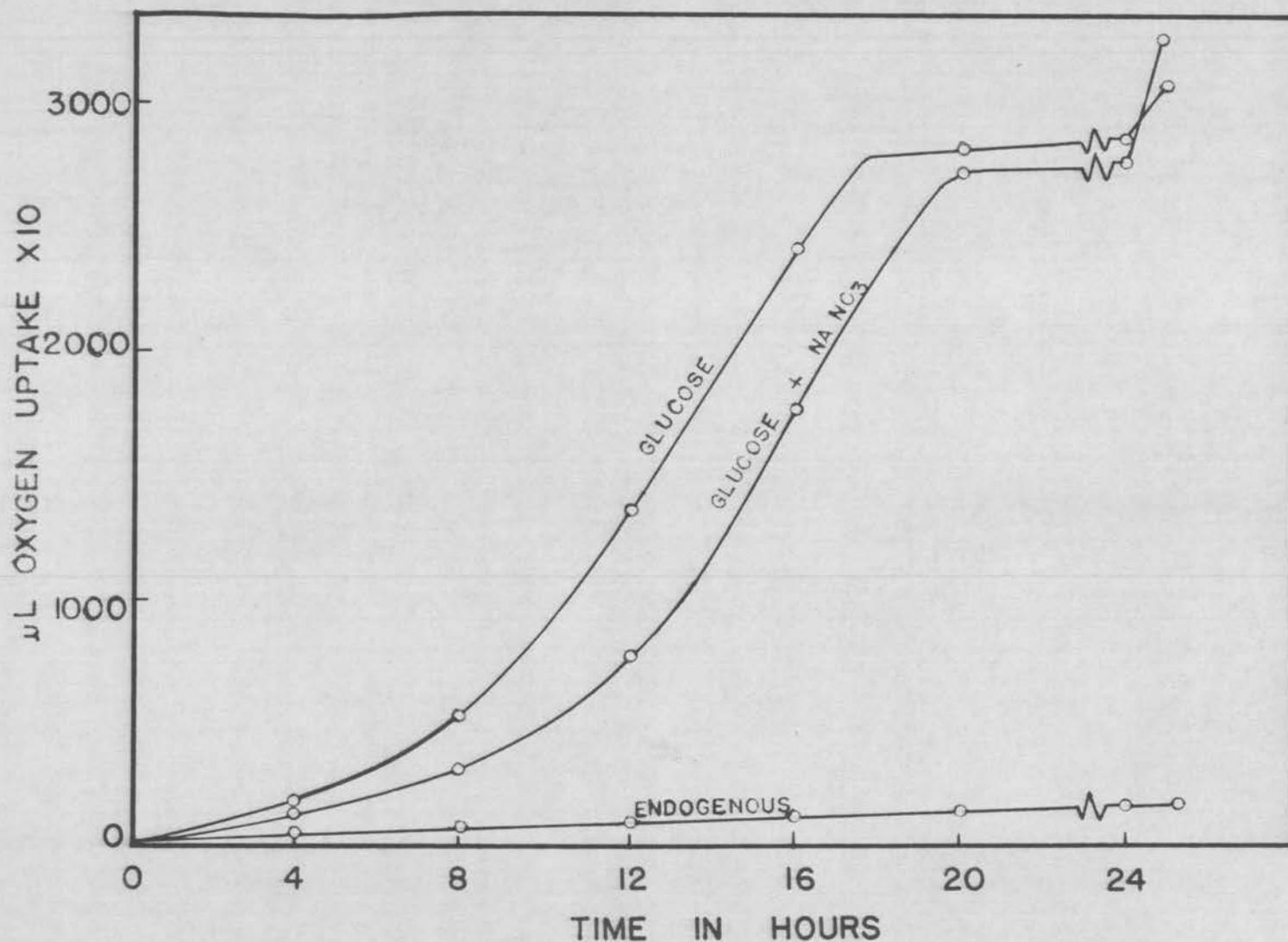


FIG. 9. THE INFLUENCE OF GLUCOSE AND ADDED NITROGEN ON LEVEL OF SOIL RESPIRATION

Presumably this increase was due to the cold water soluble fraction or that fraction immediately available for utilization by the soil flora. As is indicated in Figure 11, the soil became oxygen depleted at 20 hours after which a plateau exists. However, as mentioned previously, if the oxygen supply is readmitted to the flask then oxygen consumption starts again. As with glucose, the inclusion of nitrate with the wheat straw reduced the apparent uptake of oxygen. On the other hand, the ammonium salt (ammonium sulfate) stimulated the overall respiration values.

At this point in the study a need was felt to examine the soil respiration picture after depletion of the free oxygen. An examination of Figure 12 provides information relative to this point. As noted in prior data, oxygen becomes limiting after 20 hours continuous incubation.

No subsequent change in manometer levels were observed until a short lag period had passed after which positive evidence of gas evolution from the soil treated with wheat straw and nitrate became evident. It is evident that ammonium nitrate and sodium nitrate effected the greatest increase in gas evolution whereas only a slight rise was observed with wheat straw and wheat straw plus ammonium sulfate. Since CO_2 was observed in each instance and only traces of ammonia gas existed, it is

considered probable that nitrogen and nitrous oxide were the principle gases involved.

Figures 13 and 14 illustrate the effect of the nitrogen level on the evolution of gas. It is evident that higher levels of nitrogen sources resulted in greater gas evolution.

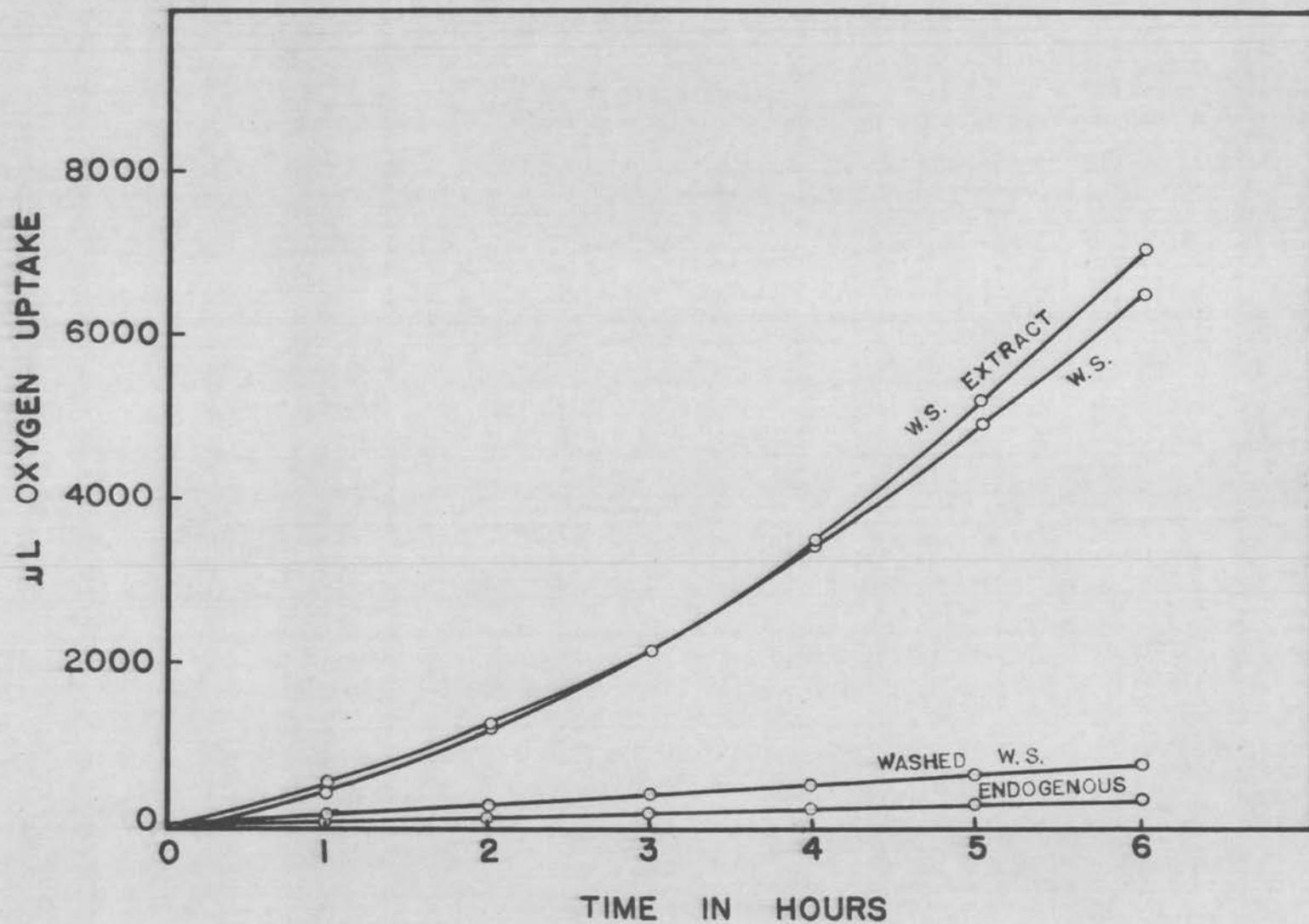


FIG. 10. INFLUENCE OF VARIOUS ORGANIC RESIDUES ON SOIL RESPIRATION

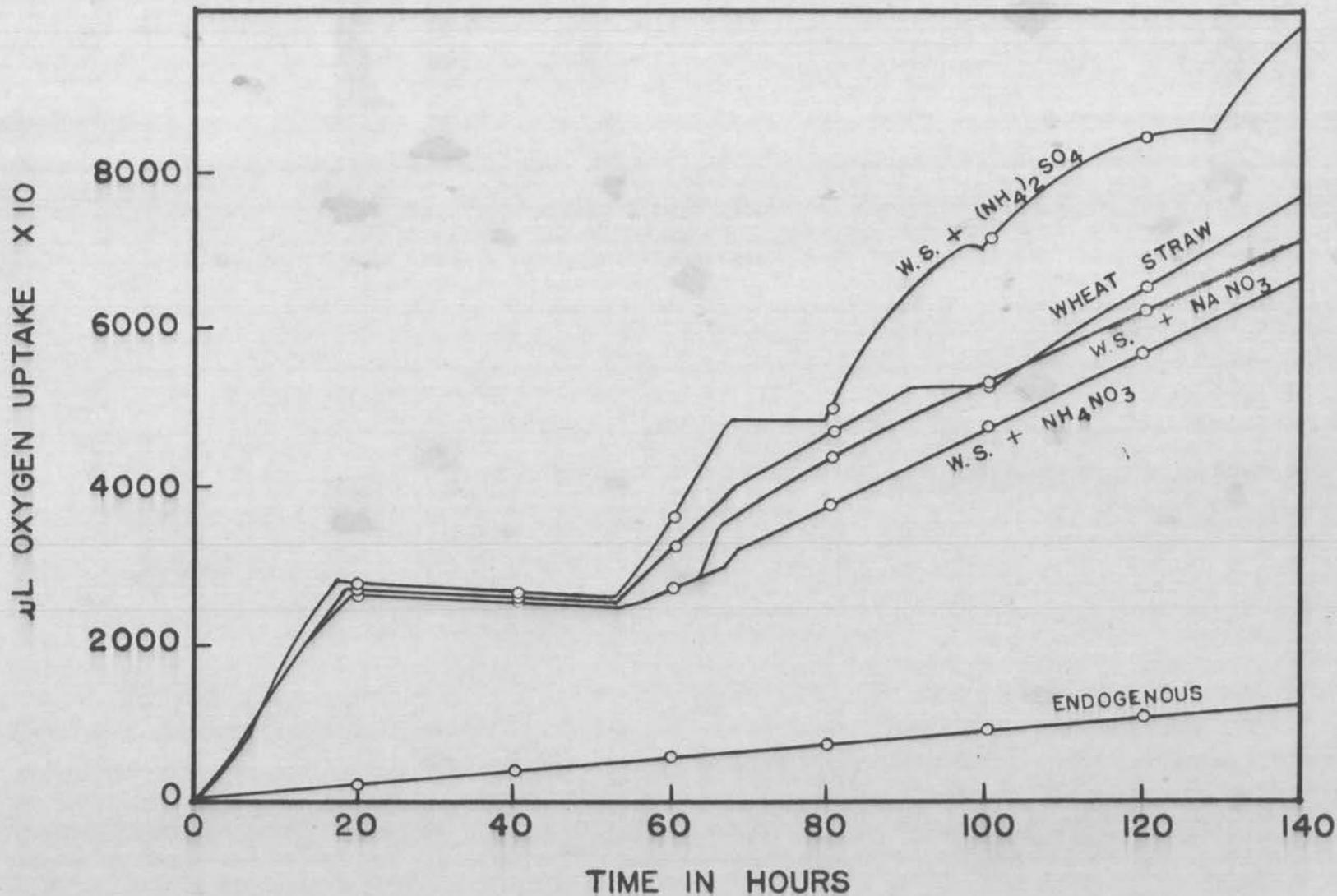


FIG. 11. ACCUMULATIVE OXYGEN CONSUMPTION VALUES UNDER SEMI-AEROBIC CONDITIONS

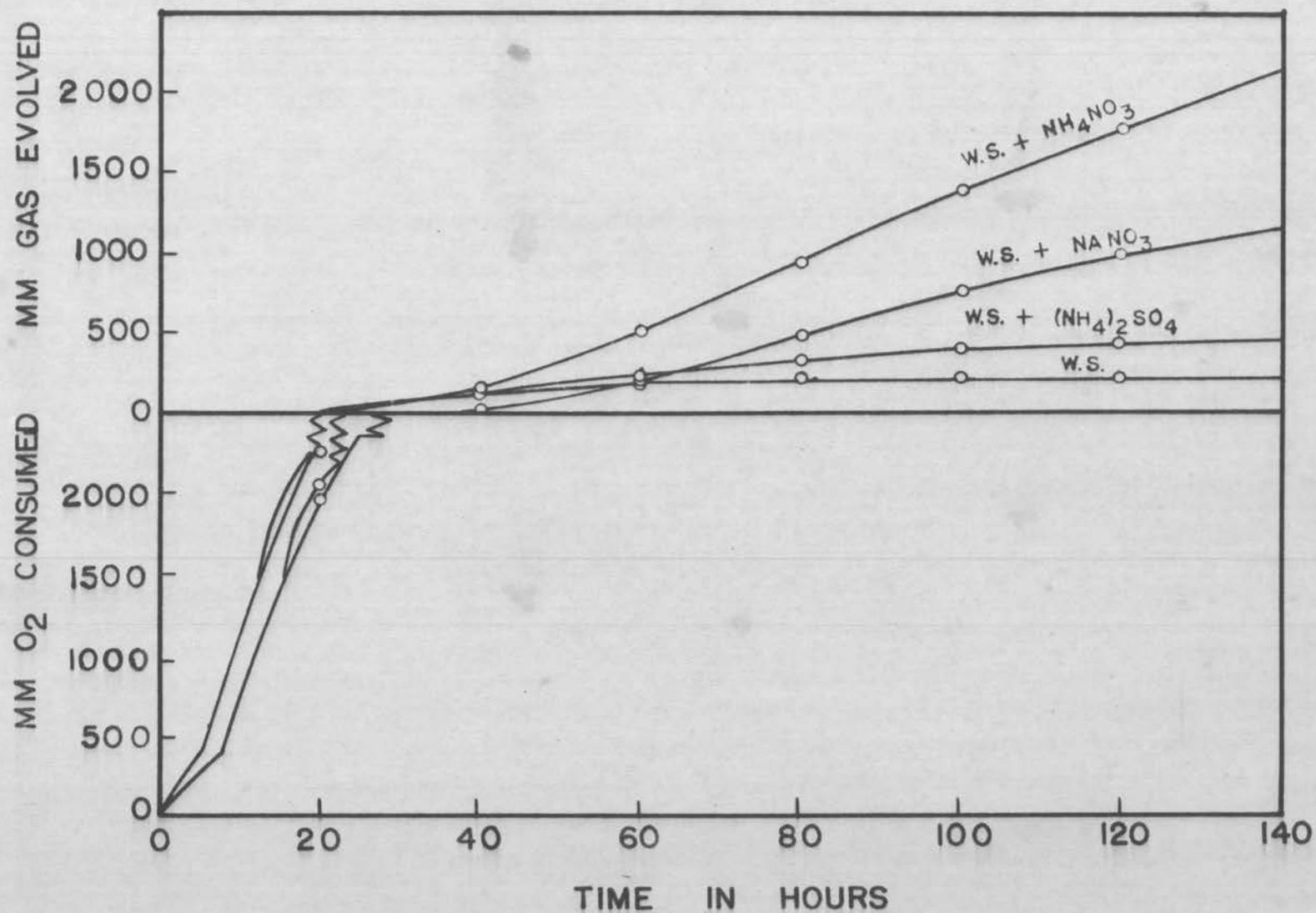


FIG. 12. EVOLUTION OF GAS OBSERVED UNDER ANAEROBIC CONDITIONS

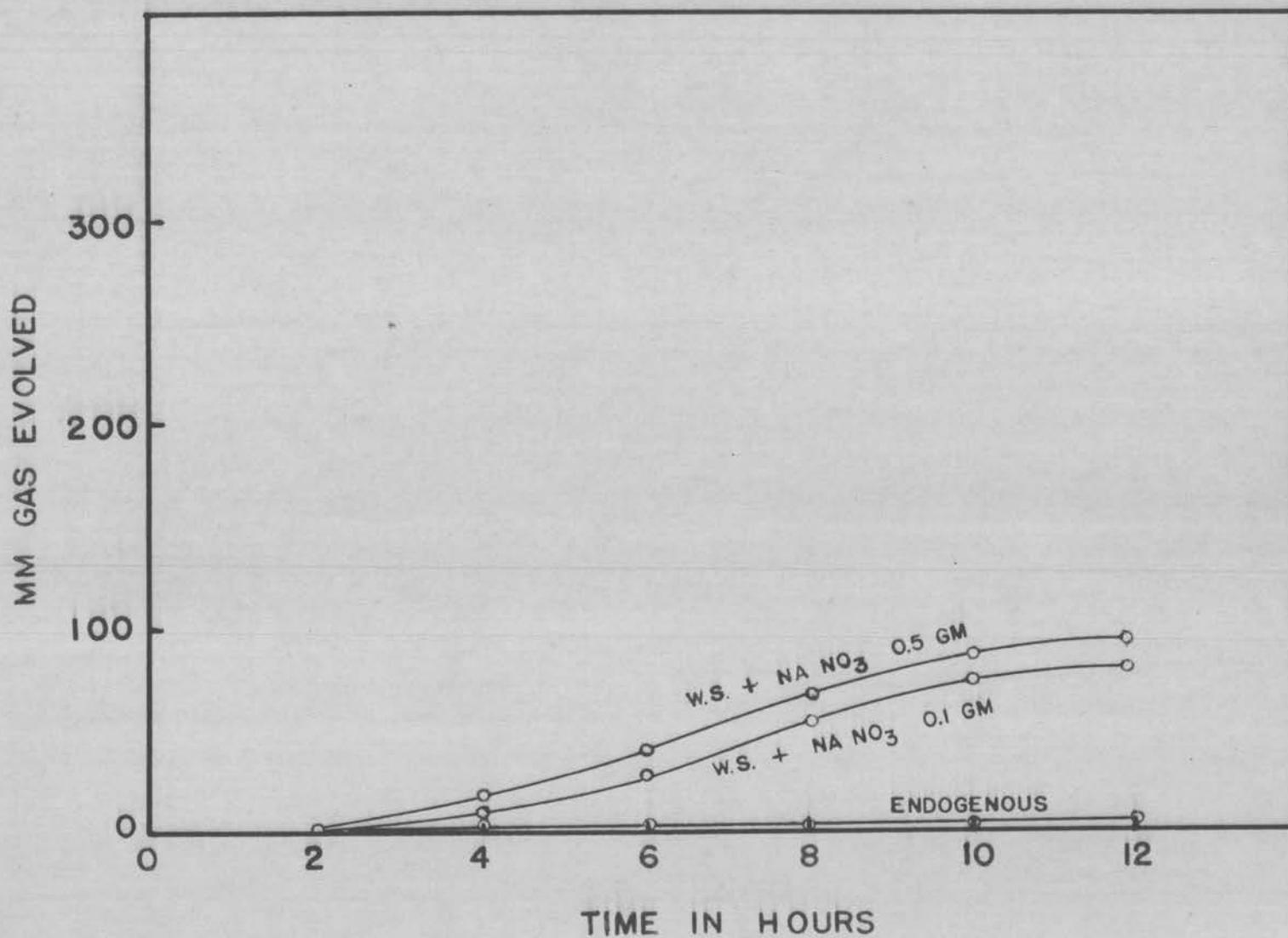


FIG. 13. EVOLUTION OF GAS AS INFLUENCED BY NITRATE LEVEL IN SOIL

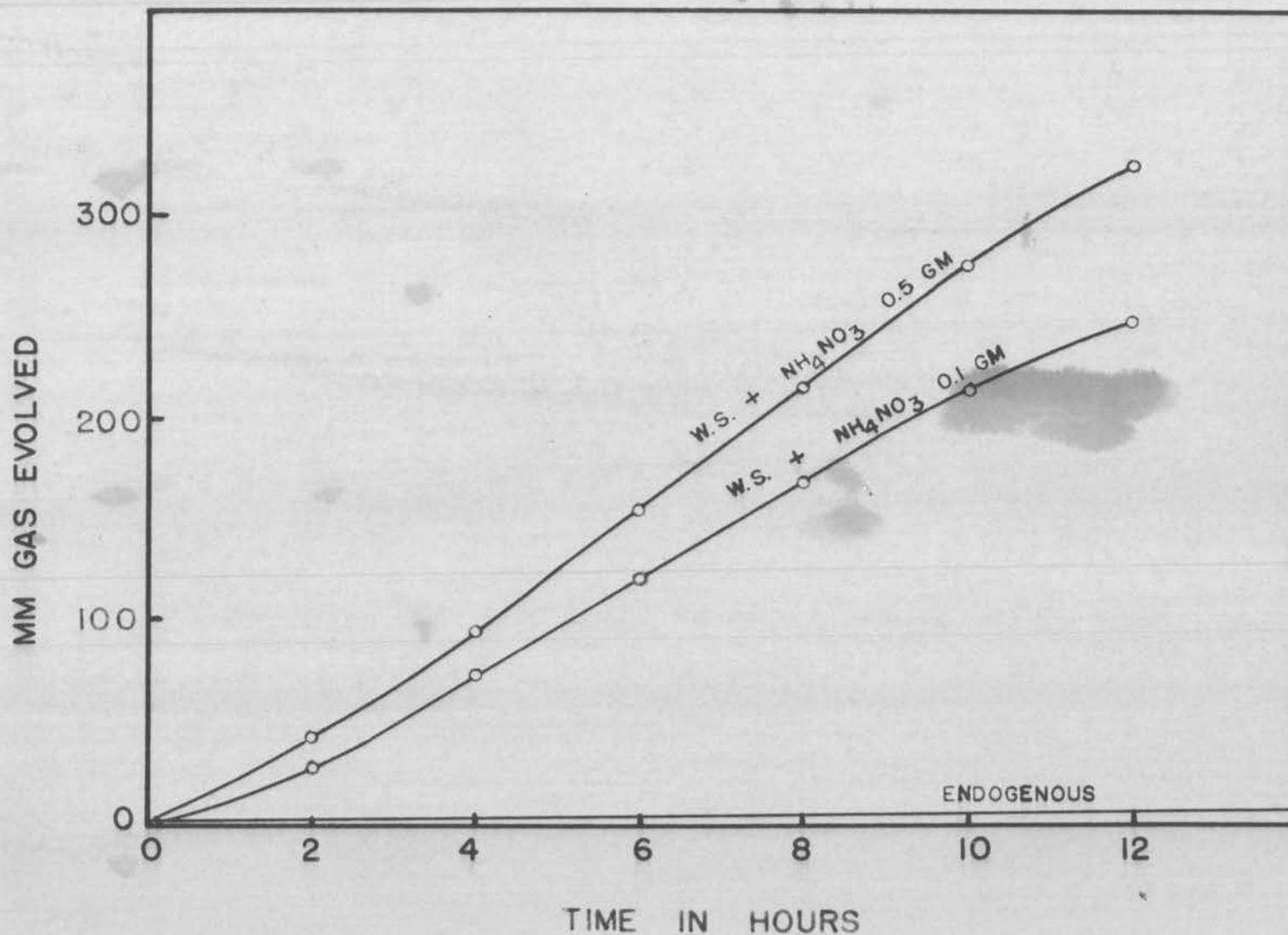


FIG. 14. EVOLUTION OF GAS AS INFLUENCED BY NITRATE LEVEL IN SOIL

DISCUSSION

The problem of tracing the transformations of nitrogen in soil may be resolved by the use of several methods. In the present study the chemical and manometric approaches were used, and correlated data from these were analyzed. The results clearly show that in the presence of wheat straw a rapid loss of native nitrate or any added nitrate fertilizer will occur anaerobically. Heavier applications of nitrate will not prevent this process; the greater amounts merely require some added time to disappear. Conversely, heavier applications of the wheat straw reduce the time period for nitrate losses.

Two considerations are shown to be significant, bearing directly upon this disappearance of nitrate. One is the levels of oxygen tension in the soil atmosphere, the other is the presence of a readily oxidizable carbon source. These are interrelated, since economic losses occur in soil only as a result of low oxygen levels in the presence of high carbon content.

That oxygen tensions bear a decided effect on nitrate utilization was demonstrated throughout this work, in both chemical and manometric studies. The aerobic incubation of soils with added sodium nitrate and wheat straw results in significant disappearance of nitrate, whereas incubation of soils with ammonium nitrate as the

nitrogen carrier does not. The disappearance of nitrate in this aerobically treated soil may be accounted for by its CO_2 evolution. Carbon dioxide, being heavier than air, may form a blanket over the soil. As more CO_2 accumulates, this overlaying blanket becomes thicker and probably interferes with the diffusion of free oxygen into the soil. Since there is no disturbance of the atmosphere immediately above the soil in the flask (analogous to winds, rainfall, etc. under natural field conditions) an effective degree of anaerobiosis could result over extended time periods in "aerobically" treated soils. Soil treated aerobically with added ammonium nitrate and wheat straw did not exhibit nitrate disappearance. There was instead a marked disappearance of ammonium nitrogen which, under anaerobic conditions, showed a similar rapid rate of disappearance up to the end of the first day, about the time when strict anaerobic conditions prevailed. This implies that ammonium nitrogen was incorporated by the aerobic flora which would continue to thrive so long as free oxygen was available. Thereafter, as oxygen tensions in the soil atmosphere decreased, their activity correspondingly disappeared and the denitrifiers gained the advantage.

Manometric data show that the inclusion of sodium nitrate brought about an apparent decrease in oxygen

consumption from that observed with glucose alone. Other tests, using a variety of inorganic fertilizers, lead one to attribute this effect to the nitrate radical. Chemical data showed that considerable reduction of nitrate to nitrite occurs during the indicated time period. Thus it is highly probable that nitrate is to some degree replacing oxygen as the final acceptor of hydrogen in the terminal respiration of the soil flora. This would give an apparent decrease in oxygen consumption and, should the terminal oxidative cycle be affected, might cause a decrease in CO_2 output.

The elevation of pH in the anaerobic soils and the stable pH in the aerobic sets afford a more complete picture. Obviously nitrate disappearance results in accumulation of sodium ions which will, in water, attach themselves to hydroxyl ions. Coincident with this formation of sodium hydroxide, the hydrogen ions of the water combine with the evolved CO_2 to form carbonic acid. The overall reactions (production of a strong base and a weak acid) manifest themselves by elevating the pH. The residual ion effect is absent in the ammonium nitrate series, where losses were not encountered. The easily accessible nitrate ion can be used as a hydrogen acceptor by the denitrifying bacteria in those portions of a well aerated soil where essentially anaerobic conditions

nevertheless do exist. Conceivably, greater evolution of gaseous nitrogen or oxides of nitrogen would result from use of ammonium nitrate as against sodium nitrate. Data depicted in Figures 11, 12, 13, and 14 bear out this supposition. Having a greater affinity for the nitrate than for water, the ammonium ion would remain stable.

Under anaerobic conditions the nitrate ion disappears completely, and at a much more rapid rate, regardless of which carrier of nitrate is used. The residual sodium and ammonium effects are reflected in the constantly rising pH. The increase of CO₂ evolution clearly demonstrates that these chemical transformations occurring in the soil result from microbiological activity. The observation that nitrites will always appear anaerobically but never aerobically in nitrate reduction implies the possibility that the metabolic pathway chosen by these denitrifiers is dependent upon oxygen tensions in the soil atmosphere. Here an interesting question arises as to why nitrites do not appear during aerobic loss of nitrate as they do under anaerobic loss. One possibility is that oxygen tensions may inhibit the nitrate reduction pathways and under such conditions the nitrate may bypass the nitrite stage in any new reduction scheme. The validity of such an explanation, however, seems questionable. A second hypothesis offered for the lack of

nitrite appearance in aerobic nitrate reduction may be the more reliable one. It may be recalled that less nitrate is reduced aerobically than anaerobically, since the denitrifiers will use free oxygen preferentially to the nitrate as a hydrogen acceptor. Since the aerobic rate of reduction is slower, sufficient enzyme is present to reduce the nitrite the instant it appears. Anaerobic nitrate reduction is so rapid, however, that the pileup of nitrite in the soil (before its inevitable disappearance) would imply an enzyme saturation effect upon the nitritase.

A re-examination of the chemical data with a view to the effects produced by added glucose reveals that the presence of a readily oxidizable carbon source is necessary for increased activity of the denitrifiers present among the soil flora. Supporting manometric data show an increased oxygen consumption or gas evolution only in those flasks containing such an energy source. Other manometric data, of wheat straw extract and of washed wheat straw additions to the soil, reveal that such a readily oxidizable carbon source is present in wheat straw in the water soluble fraction. Under actual field conditions this soluble fraction will enter the soil as soon as the straw is moistened and encourage the growth of denitrifiers as well as other forms. Rainfall

would help to create such anaerobic conditions by both the physical barrier it would set up against oxygen diffusion, and by washing this soluble fraction from the straw into areas of the soil favorable for the rapid development of denitrifiers. The increased activity of these denitrifiers would create even greater anaerobic conditions in the field conducive to soil nitrate losses.

Three different soils received treatment of added moisture only, yet they showed different rates of oxygen consumption. Other soils, treated with various organic residues in the presence and absence of nitrogen fertilizer salts, showed consistent trends in increased rate of oxygen uptake or of gas evolution as a result of such treatments. Results of these manometric studies were correlated with chemical data for the indicated time periods. They show that highly accurate data could be collected by using this technique with soils, and the trends of soils undergoing treatment could be established in a matter of hours. These advantages open the possibility of future use of manometric studies as a valuable tool, used either alone or in conjunction with other procedures in soil microbiology studies.

SUMMARY AND CONCLUSIONS

The fate of nitrate fertilizers added to soil in the presence and absence of oxidizable carbon has been studied.

Under aerobic conditions, a significant amount of nitrate-nitrogen disappeared from the soil when incubated with wheat straw. However, no nitrite appeared, possibly because of enzyme inhibition by free oxygen, but more probably because rate of nitrite production was lower than under anaerobic conditions and hence the nitritase enzyme system was not saturated.

The nitrate nitrogen in wheat straw treated soils disappeared completely when incubated under anaerobic conditions. Nitrite appeared, but eventually was lost. Calculations based on total nitrate-nitrogen added and an approximate estimation of the fraction used by the soil flora indicated that a large proportion of the added nitrate-nitrogen remained unaccounted for. Gas evolution as observed using the manometric procedure supported the conclusion that a part of the aforementioned loss occurred via gaseous forms of nitrogen. Thus the use of nitrate-nitrogen fertilizer in combination with wheat straw requires exhaustive re-examination in light of the present findings.

The manometric procedure, modified in the present

study for use in soils research, proved of genuine value in respect to soil population studies and as a means of detecting gas evolution. It is believed that this method will prove to be a useful tool in other soil microbiological investigations.

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