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TRANSFORMATIONS OF ^{15}N LABELED AMMONIUM SULFATE AND UREA FERTILIZERS IN SOILS (WITH SPECIAL REFERENCE TO EARLY IMMOBILIZATION AND NITRIFICATION PROCESSES)

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Gilbert Uwahamaka Okereke

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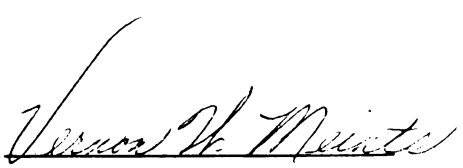

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TRANSFORMATIONS OF ^{15}N LABELED AMMONIUM SULFATE AND UREA FERTILIZERS IN SOILS (WITH SPECIAL REFERENCE TO EARLY IMMOBILIZATION AND NITRIFICATION PROCESSES)

By

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ABSTRACT

TRANSFORMATIONS OF ^{15}N LABELED AMMONIUM SULFATE AND UREA FERTILIZERS IN SOILS (WITH SPECIAL REFERENCE TO EARLY IMMOBILIZATION AND NITRIFICATION PROCESSES)

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The primary objective of the investigation was to provide evidence that nitrogen (N) immobilization occurs within a matter of hours after application of N fertilizers. The secondary objective was to study some of the factors that influence early immobilization and nitrification of applied labeled fertilizers in soils. The investigation was therefore conducted as a laboratory incubation study under controlled conditions to obtain a comparison between the rate of immobilization and nitrification of ammonium sulfate $(NH_4)_2SO_4$ and urea fertilizers applied to organic soils and arable and forest mineral soils. Fertilizers labeled with ^{15}N were used to distinguish organic-N and NO_3-N produced by immobilization and nitrification, respectively, of fertilizer N from the soil organic-N and NO_3-N already present. Nitrogen immobilization was measured by the appearance of ^{15}N in the organic fraction while nitrification was measured by the appearance of ^{15}N in the soil NO_3^- fraction.

Comparison of immobilization and nitrification processes in the soils show that marked differences may exist in the ability of the

various soils to immobilize and nitrify labeled fertilizers when evaluated under comparable conditions. For the lower pH organic soil, 10.4% of the labeled $(NH_4)_2SO_4$ fertilizer was recovered as organic-N while for the lower pH arable mineral soil and forest mineral soil, 1.4% and 5.7%, respectively,were recovered as organic -N after 12 hours of incubation. Application of labeled urea resulted in immobilization of 8.6% and 1.8% of the applied urea fertilizer in the lower pH organic soil and the lower pH arable mineral soil, respectively, after 12 hours incubation. After the same period of incubation, 12.4%, 2.3% and 1.4% of the applied labeled $(NH_4)_2SO_4$ was nitrified in the lower pH organic soil, lower pH arable mineral soil and forest mineral soil, respectively. Addition of urea caused 15.3% and 2.1% of the labeled urea fertilizer to be nitrified in the lower pH organic and lower pH arable mineral soils, respectively, These data indicate that early immobilization and nitrification of ^{15}N labeled $(NH_4)_2SO_4$ and urea fertilizers does exist.

In an effort to further study the influence of pH and readily-available carbon (C) on microbial activity and subsequent N transformation, several additional experiments were conducted. Changes in pH due to the addition of a fertilizer source exercised a considerable effect on the rate and amount of N immobilized and nitrified. In general, a higher pH favored immobilization and nitrification. Immobilization of urea fertilizer in the presence of readily available glucose-C occurred immediately and was a rapid process. In the presence of glucose, the net immobilization was 97.0% in the lower pH (pH 5.7) arable mineral soil. For the higher pH (pH 6.5) arable mineral soil, addition of glucose-C caused 88% immobilization compared to 6.0% in unamended soil while in the

higher pH (pH 6.8) organic soil, 90% was immobilized by the glucose amended soil compared to 26.0% in unamended soil. After addition of glucose-C and incubation for 96 hours, the net nitrification was 10.3% of the applied urea-N in the higher pH (pH 6.8) organic soil while in the lower and higher pH arable mineral soils, it approached zero. These data show that the state at which N is found in the soils studied is determined to a high degree by the presence or absence of a readily-available C source, i.e. energy.

TO MY WIFE AND CHILDREN

This thesis is dedicated to my beloved wife, Victoria and children for their courage, prayers and encouragement all these years.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table | Page

LIST OF FIGURES

Figure Page

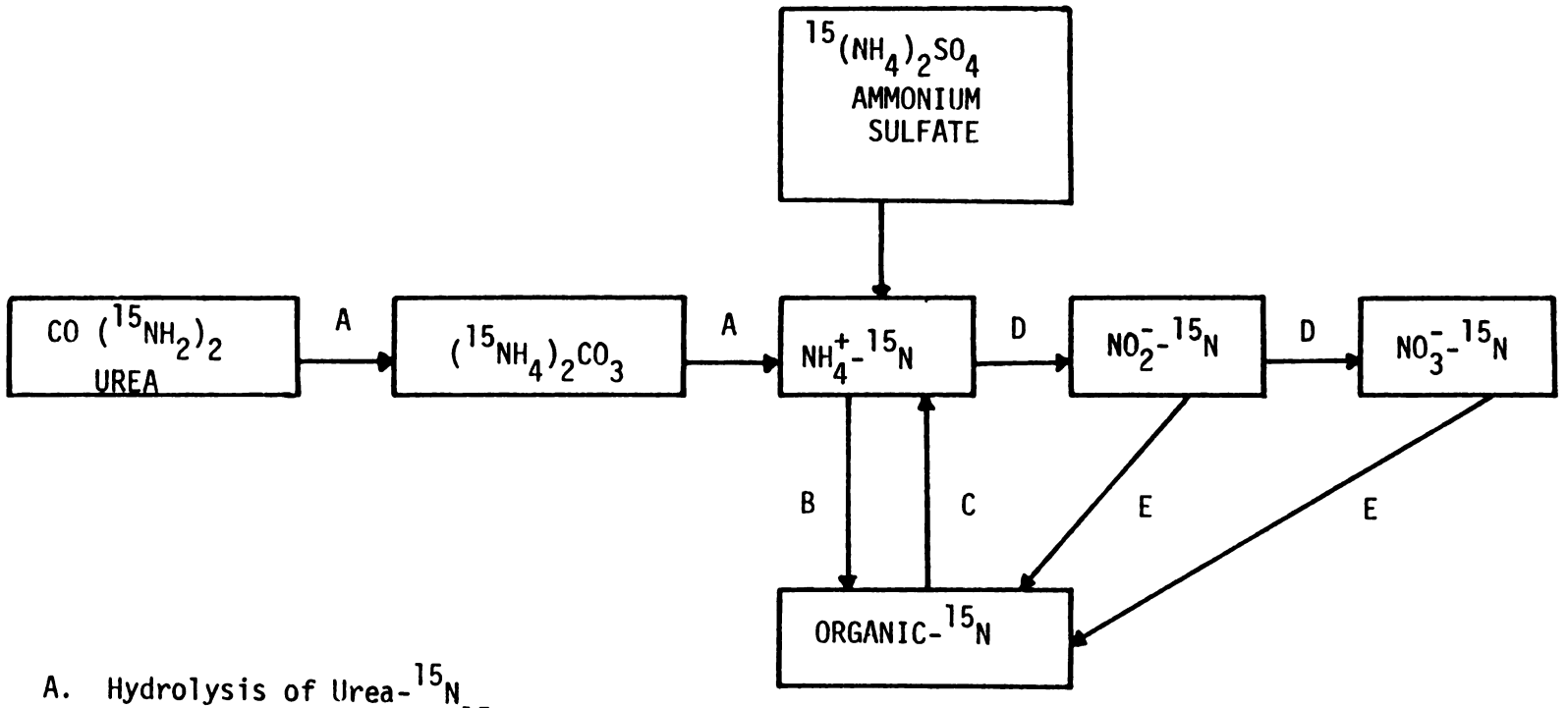
Figure Page

INTRODUCTION

It has been well established that nitrogen (N) is an essential plant nutrient; thus, the series of transformations of applied fertilizer N is very important in plant nutrition.

The application of fertilizer N in agriculture and forestry has increased recently without quantitatively assessing the efficiency, economy and environmental effects of these increasing rates of N application. A clearer understanding of fertilizer N transformations in soils, especially immobilization and nitrification, are therefore needed.

Most studies in this area have been conducted with N fertilizer added to mineral agricultural soils. To my knowledge, little such work has been done using organic soils or forest mineral soils. Organic soil constitutes an important potential soil reserve in Michigan and other parts of the world. There are 4-1/2 million acres of organic soils in Michigan of which less than 5% is farmed. (Davis and Lucas, 1959). Forest soils also represent a major soil reserve since 50.7% of Michigan is forested (Somers, 1977). Forest soils have an accumulation of organic N which results from N cycling and forest floor development. The N transformation activities are closely associated with forest soil productivity as it is influenced by forest succession, stand development, forest fertilization, harvesting, weed control and site preparation



A. Hydrolysis of Urea-^{15}N
B. Immobilization of NH_4-^{15}N
C. Mineralization
D. Nitrification
E. Assimilation of NO_2^--^{15}N and NO_3^--^{15}N

Figure 1. Transformations of ^{15}N labeled $(NH_4)_2SO_4$ and urea fertilizers in soils.

activities.

Although immobilization does not represent loss of N from the soil, it does compete with plant uptake. On the other hand, it reduces volatilization, denitrification and leaching of the applied N fertilizer.

Nitrification, the microbial oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) and nitrate (NO_3^-), is of great environmental significance for many reasons. Except for fertilizers and certain chemical reactions that form NO_3^- from oxides of N in the atmosphere, biological nitrification is the sole natural source of NO_3^- in the biosphere. The importance of the process is that it produces an oxidized form of N which may participate in denitrification reactions, resulting in the loss of readily available N from the soil environment.

Under conditions favoring a high degree of microbial activity, the addition of inorganic N to soils causes an adjustment in the equilibrium of the system resulting in an interchange between inorganic and organic forms of N (Kirkham and Bartholomew, 1955). The extent of the adjustment depends on soil microorganisms, C/N ratio, temperature, moisture, pH, etc. (Hideaki, et al., 1969).

A diagram showing some of the recognized processes involved in fertilizer N transformations under aerobic conditions is presented in Figure 1. The diagram shows that immobilization and nitrification make use of the same substrate with different end products.

The primary objective of the investigation was to provide evidence that N immobilization occurs within a matter of hours after application of N fertilizers. The study was modified to provide information on the relationship between immobilization and other competing processes which

utilize inorganic soil N. A knowledge of the magnitudes of these competing processes is essential in determining the availability of N applied as fertilizer to plants. The secondary objective was to study some of the factors that influence immobilization and nitrification under laboratory incubation conditions.

The investigation was conducted as a laboratory incubation study under controled conditions to obtain comparison between the rate of immobilization and nitrification of labeled ammonium sulfate [$(NH_4)_2SO_4$] and urea fertilizers.

Nitrogen immobilization was measured by the appearance of ^{15}N in the organic fraction. Nitrification was measured by the appearance of ^{15}N in the soil NO_3^- fraction.

LITERATURE REVIEW

Immobilization

"Immobilization" generally denotes the process of conversion of inorganic N to the organic form during decomposition (Hutchinson and Richards, 1927).

Bierema (1909, cited by Jansson, 1958) was the first to establish that a variety of micororganisms present in soils were able to immobilize NH_4- and NO_3-N. Initially, this immobilization of N was regarded as being detrimental to crops as they consumed N that could be available to plants. Further research gradually accumulated evidence that showed that the detrimental effect was temporary and changed into a favorable effect.

Tracer and non-tracer experiments reviewed by Allison (1966) indicate average recoveries of fertilizer N under field conditions in a single harvest to range between 50 and 70%. A review by Kundler (1970) of research utilizing ^{15}N- labeled fertilizers reports first year recovery in the crop of 30 to 70% with 10 to 40% retained in soil, 5 to 10% removed by leaching and 10 to 30% unaccounted for and presumed lost. Similar ranges of values were reported in a review by Hauck (1971) in which N balance data are given for different cropping systems and experimental methods. Studies with ^{15}N-labeled fertilizers demonstrate that much of the fertilizer N not recovered by the initial crop becomes immobilized and slowly available to succeeding crops (Legg, et al., 1971)

Several investigators in recent years have indicated that rapid interchange between added fertilizer N and organic N in soil occurs. According to Stewart, et al., (1963) most of the N initially immobilized appears in the amino acid fraction. Low plant recovery of tagged N fertilizer in several greenhouse and field experiments is evidence that a substantial part of a N fertilizer application becomes at least temporarily unaviailable to crops (MacVicar, et al., 1950; Bartholmew, et al., 1950; and Bartholomew, 1957). Tyler and Broadbent (1958) used successive crops of ryegrass to recover ^{15}N which had been immobilized into organic form and found that uptake of fertilizer N was substantially greater from newly synthesized organic forms than would be expected if this N had been uniformly diluted with the total N present. Jansson (1958) and Tyler and Broadbent (1958) have suggested that a small portion of the total soil organic matter may be acting as an "active" fraction. This "active" fraction is only a minor fraction of the soil N and a large portion of immobilized fertilizer N will enter this fraction.

Broadbent (1966) and Broadbent and Nakashima (1965) showed that if the level of soil inorganic N is measured during a period when conditons favor net immobilization, there is at first a rapid decrease followed by a somewhat more gradual increase in soil N. The length of the intervening period of N depression may vary from a day or two (Broadbent and Tyler, 1962) to several months (Bartholomew, 1965) depending on the nature of the organic matter undergoing decomposition in soil.

Work with ^{15}N-tagged fertilizer, however, indicates that highly stabilized forms of organic N are produced in relatively short periods (Legg and Allison, 1967). Yoneyama and Yoshida (1977) found that under

lowland conditions, the amount of N immobilized was small during the first week, but became large after two or three weeks. While under upland conditions, the immobilized N reached its maximum during the first week, but the amount was not as large as under lowland conditions. Broadbent and Tyler (1962) found that immobilization attained a maximum in 6 to 10 days after addition of tracer N to two different soils.

A number of environmental conditions have been shown to influence the rate of N immobilization and subsequent mineralization. The influence of moisture on N tie-up was studied by Jansson and Clark (1952). Their data showed that more accumulation of N in organic form occurred during a 10 day incubation period under a moisture content of 1/6 of the saturation capacity than at full saturation.

In soil, the optimum moisture content for decompositon of C was reported by Stotklassa and Ernst (1907) to be about 50 percent of the saturation capacity. Bollen (1947), on the other hand, reported the optimum to be at 75 percent of the saturation capacity. De and Digar (1954) reported that at the higher moisture contents, especially in waterlogged soil, N is generally lost in a gaseous form.

Norman (1931) studied the effect of H^+ ion concentration on the rate of immobilization of N and found that the process was more rapid under slightly alkaline conditions and ultimately more N was retained. He attributed the observed effects to differences in character of the active microflora induced by changes in pH. Nommick (1968) found that liming an originally acid raw humus substantially increased immobilization of mineral N.

Richards and Shrikhande (1935) were the first to demonstrate that there is preferential utilization of NH_4^+ over NO_3^- by heterotrophic microorganisms in the decomposition of straw.

Bremner and Shaw (1955), Broadbent and Norman (1947) showed that application of carbonaceous materials to the soil with or without N tied up the N and that its release occurred after a time.

Waksman (1924) studied the immobilization of N from decomposing plant residue and concluded that organic materials with N content of 2.0 to 2.5 percent or more tend to decompose with the immediate production of NH_3, whereas materials with less N will show a lag period before N liberation; or in the case of low N residues will fail to liberate any NH_3 because all the N will be immobilized by the microorganisms which carry out the decomposition.

Net immobilization of N during the process of plant residue decomposition has been shown to reach a maximum very quickly after addition of a C source to soil, (Allison and Klein, 1962, Windsor and Pollard, 1956). They concluded that the N requirement in the decomposition process depended on composition of the materials, on environmental conditions which affect the nature of the microflora and rate of decay, and on the time of incubation. In this regard, Hiltbold, et al., (1950) noted that two to four times more N was immobilized in cropped than in fallowed soils. In a N balance field study, it was observed that considerable applied N was immobilized by microorganisms during the growing season (Kissel, et al., 1976).

A soil perfusion technique was used by Lees (1948) to study immobilization of NO_3-N in the presence of organic compounds. With glucose and sucrose, immobilization of N reached its maximum in two to four days. The relationship between C/N ratio and N transformations in the soil is influenced by the ease of decomposition of the various constituents present (Rubins and Bear, 1942).

Allison (1927) noted that an immediate harmful effect resulted from adding materials of a wide C/N ratio to soil. However, the ultimate effect of this action was beneficial provided sufficient time was allowed for the NO_3^- supply to return to normal. He attributed these results to a temporary increase in biological activity followed by a slowing up of this activity until a point was reached where the proteins assimilated in microbial cells were made available to plants through their death and the subsequent ammonification and nitrification of the microbial remains.

Nitrification

The classic forerunner of all studies in this field was by Schloesing and Muntz (1877, cited by Jansson, 1958). After demonstrating the microbial nature of NH_4^+ oxidation, these workers went on to show that almost as much nitrification occurred in soils at an oxygen (O_2) concentration of 11 percent as at 21 percent. Work done by Gainey and Metzler (1917) explored the relationship between O_2 concentration and nitrification. Their results substantiated those of Schloesing and Muntz (1877) and showed that soil air does not vary greatly in composition with depth in the profile. They concluded that conditions are rarely met where there is not sufficient O_2 potentially available to insure maximum nitrification.

Studies by Amer and Bartholomew (1951) confirmed the observation that decreasing the O_2 concentration from 20 to 11 percent has a negligible effect on the nitrification rate. They also stated that a minimum level of O_2 concentration exists somewhere between 0.2 and 0.4 percent where nitrification does not occur. The partial pressure of O_2 in the soil air is rarely more than 1 or 2 percent lower than that in the

atmosphere, except in water-logged soil or in heavily compacted subsoils. Nitrification is evidently an aerobic process since it does not occur in the absence of O_2. The process is, however, less sensitive to conditions of a limited O_2 supply than would be expected (Woldendorp, 1975). Nitrifying bacteria can survive for long periods in anoxic environment even though they cannot grow (Painter, 1970).

Nitrosomonas has a broad pH optimum which may vary with strain, but usually lies between pH 6.0 and 9.0 (Engel and Alexander, 1958). Nitrobacter strains have optima between pH 6.3 and 9.4 (Winogradsky and Winogradsky, 1933). Chen, et al., (1972) found no appreciable nitrification in acid, soft water lake sediment.

Upon hydrolysis, urea is converted to ammonium carbonate $(NH_4)_2\ CO_3$. This leads to a high concentration of NH_4^+ and a rise in pH near the fertilizer application. These conditons adversely affect the activity of Nitrobacter spp. and an accumulation of NO_2^- may result (Aleem and Alexander, 1960; Alexander, 1965; and Pang, et al., 1973). Such accumulations of NO_2^- have been reported to result in losses of N from the soil system through chemodenitrification (Broadbent and Clark, 1965; Reuss and Smith, 1965). In acid soils, the increased alkalinity can stimulate nitrification (Stojanovic and Alexander, 1958). Turk, (1939), showed that the nitrifying capacity of two acid soils (pH 4.3 and 3.4) was increased about fourfold by liming.

Temperature has a marked effect upon nitrification. Generally higher temperatures accelerate nitrification. Nitrobacter seem to be more sensitive to cool temperatures than Nitrosomonas since NO_2^- accumulates more at 5^oC than at 25^oC (Gasser, 1964). Urea hydrolysis is also temperature sensitive. Hydrolysis increases as the temperature

increases (Broadbent, et al., 1958 and Fisher and Parks, 1958) and at $10^{o}C$ hydrolysis may be the rate limiting step in the nitrification of urea (Fisher and Parks, 1958).

Soil water content has a significant effect upon nitrification. Nitrification is reported to be an almost linear function of water content between 0.2 and 15 bars of soil moisture tension (Reichman, et al., 1960). Nitrification of 150 ppm NH_4-N in a slightly basic loam soil was completed in about 56, 28, 20 and 12 days at 15, 10, 7 and 1 bars water tension, respectively (Justice and Smith, 1962).

Nitrifiers are mostly autotrophs and it is probable that they are not sensitive to growth inhibition by small organic molecules, which is not surprising because they generally exist in rich organic environment. Characteristically, they adhere to particles, probably many of them organic, in soil (Gray, et al., 1968). It is necessry for nitrifying bacteria to be in a region where NH_4^+ is available and close to a zone of organic decomposition. It has been shown that Nitrobacter is capable of heterotrophic growth (Smith and Hoare, 1968). Nitrosomonas is also not a true autotroph, in that its growth is stimulated by pyruvate and amino acids, (Clark and Schmidt, 1967).

In most organic soils, a valuable product of organic matter decomposition by bacteria is NO_3^- (Davis and Lucas, 1959). The rate of formation depends upon soil moisture, temperature, aeration and total N content.

Roberge and Knowles (1966) found that over a 42 day period, there was no appreciable NO_3-N production in Black spruce humus. Total absence of nitrification is unusual in agricultural soils (Broadbent, et al., 1957) but has been reported by other workers, especially in forest soils (Harmsen and Van Schreven, 1955).

The pH optimum for NO_3 oxidation was shown to be over the range 6.8 to 8.2 in phosphate buffer (Silver, 1961). Similar results were also obtained by Lees and Simpson (1957).

Lack of nitrification in a forest soil may be due to the absence of nitrifying microorganisms rather than the presence of substances inhibitory to nitrification (Nakos, 1975). The population of nitrifying organisms is an important factor affecting the amount of nitrification occuring in soils (Anderson and Purvis, 1955; Frederick, 1957). Theobald and Smith, (1974) showed that soil incubation tests indicated that the microflora of two forest soils had a weak capacity to produce NO_3. Their results agree with those of Maftoun and Pritchett, (1970) and Smith, et al., (1971) who detected negligible amounts of nitrification in other forest soils of the low coastal plain.

Ureolysis

Urea is unique among commonly used N fertilizer materials in that it is an organic compound which generally requires an enzymatic hydrolysis to make its N available to plants (More, 1967). Urea as a fertilizer has been commonly considered to resemble NO_3^- in its susceptibility to leaching and to be like NH_3 in transformations following hydrolysis (Broadbent, et al. 1958). Gibson (1938) noted that although urea is not absorbed by soils, its rapid conversion to NH_3 will generally prevent losses by leaching.

The N transformations occuring following addition of urea-N and of $(NH_4)_2CO_3$-N (3,500 ppm) to the raw humus in laboratory experiments have been reported by Roberge and Knowles (1966). They found that ureolysis was complete after 2 days; that in spite of the high C/N ratio,

immobilization was small and that in most treatments, nitrification was negligible. Hydrolysis would occur within 3 to 4 days or less under favorable temperature conditions (Broadbent, et al., 1958).

Many micro-organisms possess the enzyme urease, the catalyst responsible for hydrolyzing urea.

Bacteria, fungi and actinomycetes synthesize urease and therefore can use urease as an N source for growth (Alexander, 1977).

$$CO(NH_2)_2 + H_2O \longrightarrow H_2NCOO\ NH_4 \downarrow 2NH_3 + CO_2$$

Following hydrolysis of urea biochemical transformations begin immediately. These are nitrification and immobilization of the NH_4-N produced.

MATERIALS AND METHODS

Soils

Soil samples were collected to represent agricultural mineral soils, (W1 and WZ); a forest mineral soil, (F1); and organic soils, (MB18 and MC15). The agricultural mineral soils, W1 and WZ, were collected from plots 40 and 24, respectively, of an experimental area formerly utilized by Dr. A. R. Wolcott on the Michigan State Old Soils Farm. Soil WZ received applications of dolomitic agricultural lime in the spring of 1965 and the spring of 1966. Annual applications of sodium nitrate fertilizer at the rate of 136 kg N $acre^{-1}$ yr^{-1} were made between 1959 and 1972. Soil W1 did not receive any lime and fertilizer during the same period of time. Details about the history, fertilizer treatments and crops grown in these plots are contained in an M. S. thesis by Burutolu (1977). Soil F2 was collected from Baker Woodlot, a research area under the supervision of the Department of Forestry, Michigan State University. This area is predominantly a hardwood forest that has been clearcut in the last 100 years. Tree species presently growing at the sample site include beech, maple, red oak, black cherry and basswood.

Soils MB18 and MC15 are muck soils collected from the Michigan State University Muck Farm supervised by Department of Crop and Soil Sciences, Michigan State University. Soil MB18 was planted to potatoes which received 68 kg N $acre^{-1}$ as urea fertilizer in 1978. Soil MC15 was planted to sod which received no fertilizer in 1978. In 1979, the sod was plowed.

Table 1. Chemical and physical properties of the soils used.

Properties	Soils				
	WI	WZ	F2	MC15	MB18
Texture	Sandy Loam	Sandy Loam	Sandy Loam	Organic	Organic
pH	5.1	6.5	5.7	6.8	5.7
Classification	Typic Hapludalf	Typic Hapludalf	Typic Hapludalf	Typic Mediprists	Typic Medisaprists
% Organic-N	0.05	0.07	0.13	2.15	2.12
% Organic-C	1.4	1.2	3.2	45.0	47.4
C:N	29:1	17:1	24:1	20:1	22:1
Ammonium-N(ppm)	4.9	0.04	5.1	0.04	1.0
Nitrate-N(ppm)	3.2	3.8	2.8	393.4	373.6
% moisture at 1/3 atms.	15	14.8	17.0	158.0	175.0

Soil sampling and characterization

Bulk samples of the top soil (0-15 cm) were collected from each site in the fall of 1979, sieved (2 mm screen), thoroughly mixed in a small cement mixer and stored in their moist condition in a cold room at $2^{o}C$ until used.

Some relevant physical and chemical properties of the soils are summarized in Table 1. The method used to determine these are as follows: The pH values refer to a water suspension (soil:water ratio, 1:1 for mineral soils and 1:2.5 for organic soils). The field moisture capacity of the soils was determined by use of pressure membrane plates that were allowed to equilibrate at a tension of 1/3 atmosphere for 48 hours after which the moisture content was determined gravimetrically. The method used was a modification of a method used by Richards and Weaver (1943). Organic C was determined using Leco carbon analyzer (Model 750-100, Laboratory Equipment Corporation, St. Joseph, Michigan). Ammonium-N and NO_3-N were determined by alkaline distillation of KCl extract of the soil with MgO and Devarda's alloy, respectively, (Bremner and Keeney, 1966).

Incubation and extraction

An incubation and extraction method was used that allowed determination of various forms of soil N at regular intervals over an extended period of time. Unless otherwise stated, the general procedure was to add 2 mg N as labeled $(NH_4)_2SO_4$ (90.2 atom percent ^{15}N) or urea (96 atom percent ^{15}N) in solution to soil equivalent to 20 g of oven dry soil weighed in duplicate or triplicate into 300 ml Erlenmeyer flasks. Additions corresponded to 100 ppm N on the soil basis (2 mg N/20 g oven

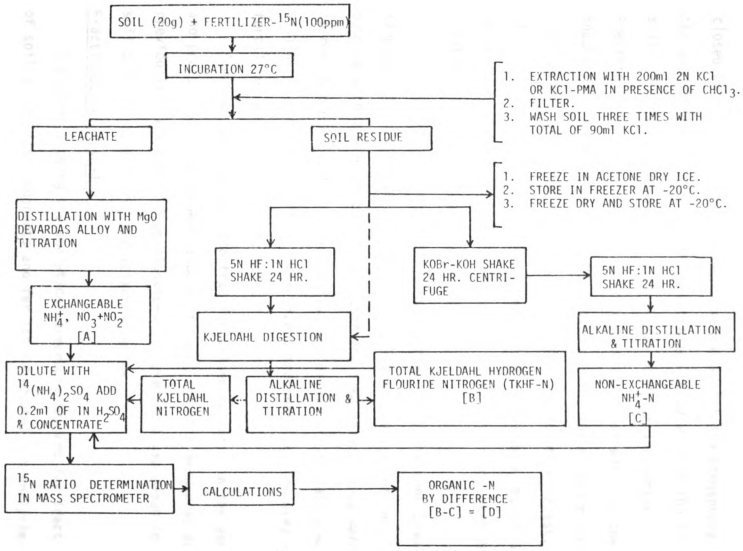


Figure 2. Fractionation of soil N for ^{15}N analysis.

dry soil). Soils were adjusted to their 1/3 atmosphere moisture percentage, thoroughly mixed with a spatula and evenly distributed over the bottom of the flask by tapping the flask gently. The flasks were closed with cotton to allow gaseous exchange with the atmosphere and at the same time control excessive evaporation of water from the incubated soil. A constant temperature cabinet was used for incubation. Preliminary study showed that by providing basins of water in the incubation chamber during the incubation period, the moisture content on a dry weight basis did not decline more than 1 percent per flask. The flasks were incubated at $27^{o}C$ for periods of either 0, 3, 6, 12, 24, 48, 96 and 120 hours or 0, 12, 24, 48, and 96 hours. Analyses were made after each time interval.

In order to provide comparisons of urea transformations among samples differing in pH but otherwise having similar properties, soil samples with varied pH collected within the same general area were used in one of the incubation experiments. The method of incubation was similar to the one described above except that only labeled urea fertilizer was applied to the soil.

Ammonium sulfate and urea transformations in soils in the absence and presence of glucose were studied in much the same manner as described previously, except that 4 mg glucose-C per gram soil was added to each soil prior to addition of fertilizer solution.

Fractionation of soil N

A diagram showing the analytical procedure adopted for fractionation of soil N into its components and for its subsequent ^{15}N analysis is presented in Figure 2. The fractionation procedure adopted was based on the fact that in soil systems, the problem of evaluating N fertilizer

transformations is complicated and better understood by analyzing all the possible soil N fractions. It is known that NH_4^+ sources of N are utilized preferentially by the heterotrophic population, (Jannson et al., 1955) and that an NH_4^+ source cannot be analyzed independently under normal conditions since it is rapidly converted to NO_3^-. Disappearance of NH_4-N cannot be used as a reliable estimate of immobilization in soils capable of nitrification and fixation of significant quantities of NH_4^+ into nonexchangeable forms. Hence, the initial experiments involved determination of the various possible N fractions.

(a) Extraction of exchangeable NH_4^+-N, NO_2^--N and NO_3^--N from incubated soils

At the end of each incubation period, the soil samples were brought out from the incubation chamber for extraction of the mineral N with 2N KCl. The extraction and distillation procedures used were similar to those described by Bremner and Keeney, (1966). Sahrawat, (1979) recently confirmed that 2N KCl is the best extractant when recovery of a known amount of NH_4-N applied to soils was used as a criterion for evaluating extracting efficiency.

The soil extracts were prepared by adding 200 ml of 2N KCl into the entire contents of the 300-ml Erlenmeyer flask without subsampling, thus avoiding possible sampling errors due to uneven distribution of inorganic N. Then 0.8 ml of chloroform was added to the flasks before being stoppered and shaken on a mechanical shaker for one hour. Addition of chloroform served to inhibit microbial transformations of inorganic N forms. After shaking, the suspension was transfered to Buchner funnels containing 11-cm Whatman No. 42 filter paper which had been moistened and sealed firmly by suction on Buchner funnels mounted on 500-ml Buchner

flasks. The soil was washed three times with a total of 90 ml of 2N KCl solution. Finally, the soil extract was transfered to 300-ml volumetric flasks and the volume made up to mark. The extracts for use in the determination of mineral N were stored in polyethylene plastic bottles. Determinations were always performed immediately after extraction. The remaining KCl extracts were stored at -20°C for possible recheck of results.

The washed soils were immediately frozen in a mixture of dry ice and acetone or liquid N. The frozen soils were stored in a freezer at -20°C until dried in a freeze dryer (Repp Industries Inc., Gardiner, New York) for 48 hours. After freeze drying, the soils were packaged and stored in a freezer at -20°C for subsequent determination of organic N and nonexchangeable NH_4-N.

Extraction of urea

In experiments involving treatment of the soil with urea, 20 ml of 2N potassium chloride - phenylmercuric acetate (2N KCl-PMA) solution was added to each incubation flask containing the 20 g soil. Data by Douglas and Bremner (1970) showed that in the 2N KCl-PMA solution adopted for extraction of urea from soils does not interfere with extraction and steam distillation of exchangeable NH_4^+, NO_2^- or NO_3^-. Their data also show that phenylmercuric acetate completely inhibits urease. A preliminary study was conducted to prove the effectiveness of the phenylmercuric acetate inhibitor before adopting it in the major experiments. The rest of the procedure was the same as that used above for extraction of mineral N in the soils treated with $(NH)_2SO_4$.

Distillation Procedure for Analysis of Various Soil N Fractions

The distillation apparatus used was designed to take a micro-Kjeldahl distillation flask (100 or 250 ml capacity). The

apparatus was a modification of the one shown by Bremner and Edwards (1965). The distillation procedure was that of Keeney and Bremner (1966) and (1967). The steam generation rate during distillation was adjusted to 7 to 8 ml distillate per minute and the water flow through the condenser was regulated to ensure that the temperature of the distillate did not exceed $22^{o}C$. Plastic tubes were connected to each steam outlet such that each distillation tube was approximately 4 mm from the bottom of the distillation flask. Preliminary experiments were carried out to determine the percentage recovery of known amounts of NH_4-N before use in the distillation of experimental samples.

Before distillation of any batch of samples, 30 ml of ethanol was first distilled through the apparatus for 10 min. to remove any traces of NH_4^+ present in the distillation apparatus. For most of the mineral N analyses 20 ml of each soil extract were pipetted using wide tip pipettes into 100-ml distillation flasks made alkaline with 0.2 g ignited MgO. The NH_3 was distilled into 10 ml of 2% boric acid solution mixed with bromocresol green-methyl red indicator contained in 125 ml Erlenmeyer flasks. The NO_3^-- plus NO_2^--N was determined by addition of 0.2 g of Devarda's alloy to the same flask after the removal of NH_4^+-N and distilling the NH_3 produced into 10 ml of 2% boric acid indicator solution. Urea-N was determined colorimetrically according to the method of Douglas and Bremner (1970).

The distillation for all forms of mineral N was stopped after collecting about 35 ml of the distillate. Cross contamination of samples which can lead to serious error in ^{15}N analyses was prevented by detaching the sample flask and immediately distilling 15 ml of ethanol into the receiver flask up to the 65 ml mark. To further reduce any

potential errors, samples expected to have low ^{15}N content were always distilled before those with expected high ^{15}N content. Titrations were carried out (to determine the NH_4-N in the distillate) with 0.005N or 0.025N H_2SO_4 from a 5 ml microburette graduated at 0.01 ml intervals. For organic N, the higher concentration of H_2SO_4 was used for titration.

Determination of organic-N and nonexchangeable NH_4-N

Total N in the soil samples from which NH_4-N, urea-N, NO_3-N and NO_2-N had previously been extracted was determined by a modification of the Kjeldahl procedure (Stewart and Porter, 1963, Meints and Peterson, 1972). The N extracted by this method is referred to in this thesis as total Kjeldahl hydrogen flouride-N (TKHF-N). Nonexchangeable NH_4^+ (fixed) was determined by the procedure of Silva and Bremner (1966). Organic N in the soil was obtained by subtracting nonexchangeable NH_4-N from the TKHFN.

Total Kjeldahl-hydrogen floride-N

This method involved pretreatment of soil samples with an HF-HCl acid mixture before the normal Kjeldahl digestion. One gram of the mineral soils or 0.2 g of the organic soils and 20 ml of 5N HF-1N HCl solution were shaken in a centrifuge tube for 24 hours on a mechanical shaker. The mixture (both soil and solution) was then transfered into a 100-ml Kjeldahl digestion flask. The catalyst mixture (100 g of K_2SO_4, 10 g of $CuSO_4 \cdot 5H_2O$, and 1 g of Se) and concentrated H_2SO_4 (4 ml) were added to the flask. A few drops of octyl alcohol were added to prevent bumping and frothing during digestion. The samples were digested for three hours (one hour after clearing). After completion of digestion 10 ml of water was added to the flask to bring any insoluble material into suspension. The contents were transfered into a 300-ml Kjeldahl flask

marked to indicate a volume of 60 ml. The digestion flasks were subsequently washed three times with about 20 ml of water to complete the transfer. Water was added to the flask to bring it up to the 60-ml mark. The digested soil was distilled with 13N NaOH (20 ml of cold 50% NaOH) into 10 ml boric acid indicator solution contained in a 125-ml Erlenmeyer flask. The amount of N in the distillate was determined by titration with H_2SO_4 as previously described.

Total Kjeldahl N (TKN)

Because the soils used had relatively low NH_4-N fixing capacities, TKHF-N and nonexchangeable NH_4-N determinations were discontinued after the first group of experiments. For the rest of the experiments organic-N to include NO_3-N and NO_2-N were determined according to the method by Bremner (1965). The procedure was a micro-Kjeldahl method that included NO_3^- and NO_2^- using one tenth of the amounts of soil and reagents recommended by Bremner in his regular macro-Kjeldahl methods. The size of soil sample was 0.2 gm for organic soil and 1 or 2 g for mineral soils. Four milliliters of concentrated H_2SO_4 and 1.5 g catalyst mixture were used with a digestion time of 6 hours. Titration of the distillate was the same as previously described.

ANALYSIS OF ^{15}N SAMPLES

Preparation of distillates for ^{15}N analysis

The distillate from each N determination was prepared for mass spectrometer analysis according to the procedure described by Porter and O'Deen (1977). To avoid loss of NH_4^+ upon evaporation, the distilled and titrated samples were slightly acidified with 2 ml of 0.1N H_2SO_4 before

drying, (Edwards, 1978). To provide sufficient N for ^{15}N analysis on a V. G. Micromass 622 isotope-ratio mass spectrometer, 1 ml of unenriched $(NH_4)_2SO_4$ solution containing 3 mg N ml^{-1} was added to distillates with low N content (Tiedje, et al., 1980). One source of reagent grade $(NH_4)_2SO_4$ contained in one bottle was used for all dilutions. For highly-enriched distillates, 1 ml of unenriched $(NH_4)_2SO_4$ solution (ranging from 3 mg N ml^{-1} to 15 mg N ml^{-1}) was added to dilute the distillates to less than 3 atom percent ^{15}N. The exact amount of unenriched $(NH_4)_2SO_4$ added to the distillates therefore depended on the concentration of N and the expected ^{15}N enrichment in each distillate as determined by preliminary experiments.

After addition of the unenriched $(NH_4)_2SO_4$, the distillates contained in the 125-ml Erlenmeyer flasks were vigorously mixed before and during drying. The contents were dried down to about 5 ml on a hot plate (80^oC) and then transfered to 10 ml disposable vials. The samples in the vials were evaporated to dryness and analyzed immediately or covered with parafilm and stored in a refrigerator for later analysis.

^{15}N analysis in the V. G. Micromass 622 Isotope Ratio Mass Spectrometer

Isotopic assay of the N gas was performed in a Micromass 622 isotope-ratio mass spectrometer. The Micromass 622 is a 90^o sector magnetic collection instrument of 6 cm radius with twin Faraday bucket collectors coupled to a ratio recording output stage for the determination of precise isotope ratios. The Micromass 622 is designed for operation in two distinct modes, namely single beam and ratio. The single beam mode of operation is used for background spectra, and checking for leaks and contamination. In the ratio mode, the ratio of the minor signal to the major signal is displayed directly. The signals

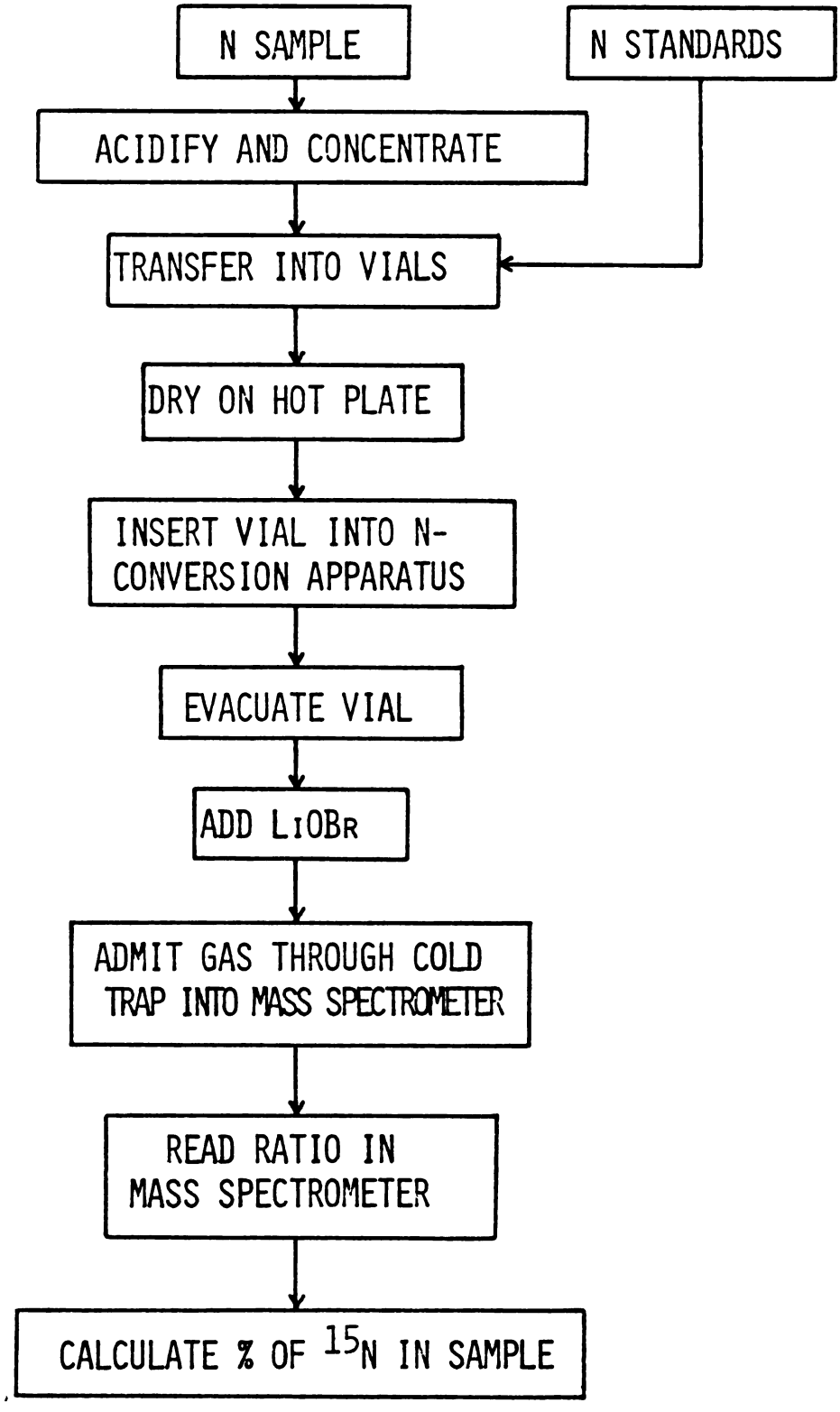


Figure 3. Sample preparation and ^{15}N analysis in isotope ratio mass spectrometer.

of the twin head amplifiers are fed to the analogue ratio unit where the major and minor signals are attenuated and the resulting signals displayed on the major and minor meters, respectively. Figure 3 shows the analytical steps involved in sample preparation and its isotope ratio analysis.

Checks on the precision of the instrument were performed by several determinations of both labeled and unlabeled standard $(NH_4)_2SO_4$ salts over a period of three months. Several reagent $(NH_4)_2SO_4$ reference samples were run each time a batch of unknown samples were analyzed. To minimize errors in ^{15}N ratio due to possible leakage of air into the mass spectrometer, the O_2 peak (m/e 32) was checked after each sample run. The effect of partial pressure of N on isotope ratios was also determined. The effect of partial pressure is important, because it is related directly to sample size at a given volume. Throughout the analysis, the N pressure of standards and samples were adjusted to a constant and similar pressure, using the variable reservoir on the mass spectrometer.

To minimize memory effects during isotope ratio analysis, samples with high expected ^{15}N content were analyzed after those with lower ^{15}N content.

CALCULATION OF RESULTS

Atom percent ^{15}N in samples

From the measured ratios of the samples, an apparent percentage of ^{15}N was calculated after correction for the major and minor gain settings used. The equation used in calculation of apparent atom % ^{15}N at low

abundance where it was difficult to measure the mass peak 30 with accuracy was as follows:

$$\text{Atom \% } {}^{15}N = \frac{100}{2R+1}$$

$$\text{Where } R = \frac{\text{Intensity of 28}}{\text{Intensity of 29}} = \frac{{}^{14}N{}^{14}N}{{}^{14}N{}^{15}N}$$

R is corrected for gain setting used

or

$$\text{Atom \% } {}^{15}N = \frac{R}{2 + R} \quad \text{Where } R = \frac{29N}{28N}$$

Correction for isotope dilution calculation

Correction for isotope dilution was made in cases where natural abundance NH_4-N was added to the distillates before drying as previously explained. From the ratio measured, the atom % ^{15}N in the equilibrated NH_4^+ fraction was calculated by means of the equation shown above. The actual atom % ^{15}N of the labeled NH_4-N before dilution was calculated using the equations below. These equations are based on isotope dilution principles as explained by Gest, et al., (1946).

$$AX + BY = (A + B)\ Z$$

$$X = (A + B)\ Z - BY$$

where:

A = milliequivalents of N in sample (^{14}N + ^{15}N) before dilution.

X = actual atom % ^{15}N in sample A before dilution

B = milliequivalents of natural abundance N added.

Y = Atom % ^{15}N in the added natural abundance N.

Z = isotope ratio of A and B after equilibration.

All calculations of N were made on equivalent mass basis.

Calculation of fertilizer N in different soil N fractions

The quantity of labeled fertilizer N found in each soil N fraction was caluclated using the equation stated below (Stojanovic and Broadbent, 1956, and Edwards, 1978). The ^{15}N content of the standard with which all samples were compared in computing milligrams fertilizer N was found to be 0.367 atom % ^{15}N.

$$Y = \frac{Z(c-a)}{(b-a)}$$

where:

a = fraction ^{15}N coming from natural compound

b = fraction ^{15}N in added N [$(NH_4)_2SO_4$ or urea].

c = fraction ^{15}N in recovered N (NH_4-N, NO_3-N or organic -N).

Y = quantity N from added source (mg. fertilizer N)

Z = quantity N recovered in given fraction

Based on the above calculations, the percentage total recovery of applied tagged fertilizer at each sampling period were computed as follows:

$$\% \text{ Total Recovery} = \frac{\text{Total Fertilizer N Recovered}}{\text{Total Fertilizer N Applied}}$$

The percentage of fertilizer-N found in each soil N fraction was determined as follows:

$$\% \text{ Recovery of Fertilizer-N in Nitrogen Fraction} = \frac{\text{mg fertilizer-N present} \times 100}{\text{mg fertilizer-N applied}}$$

Statistical analysis

A statistical analysis was conducted on data obtained from two or three replications as indicated in Appendixes A and B. Fishers least significant difference (FLSD) was employed to statistically evaluate differences between treatment means. The statistical analysis procedures were those outlined in Steel and Torrie (1960). Regression analysis and all other statistical analysis were performed using the Michigan State University's computer facilities. The objectives of these analysis were to show the relationship between the amount of fertilizer N recovered in the various fractions and the length of incubation period.

The regression models used were as follows:

$$Y = a+bT$$

$$Y = a+bT+cT^2$$

where:

T = incubation period (hours)

Y = Percent (%) of applied N fertilizer recovered as NO_3-N or organic-N

a, b and c are regression coefficients in the equation

The quadratic term (T^2) was added only in cases where additional sums of squares was significant. Values for R^2 for both linear and quadratic

equations including other statistical tests of these equations are shown in figures to be presented later. Pairwise comparison of the different curves were also made. Simple correlation coefficients (r) between the different N forms and incubation time, the dependent variable, were calculated.

Table 2. Clay fixation of labeled $(NH_4)_2SO_4$ fertilizer in soils.

Soil	Fertilizer-N Recovered as Clay-fixed NH_4-N	
	Zero Hour[a]	48 Hours[a]
WL	0.005	0.01
F2	0.01	0.04
MB18	0.02	0.07

a, Hours of incubation

Table 3. Total recovery of N Added as $(NH_4)_2SO_4$ and urea fertilizer applied to soils.

	Total Fertilizer Recovered in Soils[a]				
	MB18[b]	F2[b]	W1[b]	W1[c]	MB18[d]
Hours	%				
0	95.9±1.7*	94.1±0.23*	94.8±0.1*	99.6±2.0	100 ±1.4
3	N.D.[e]	N.D.	N.D.	96.7±1.2	98.8±1.8
6	94.9±0.32*	99.0±0.1	94.9±0.1*	100.4±1.3	99.4±1.3
12	97.2±2.8	98.7±0.4	96.7±1.4	95.8±1.3*	100.4±1.9
24	94.1±1.7*	94.9±0.5*	95.2±0.2*	91.7±0.9*	97.7±4.4
48	96.2±1.9	92.0±1.5*	95.5±0.22*	94.2±1.6*	98.3±0.9
96	93.8±1.8*	96.5±1.1	97.0±1.24	98.1±1.4	101.6±2.3
144	94.1±0.13*	93.4±1.2*	95.7±1.2*	N.D.	N.D.

a, Mean and standard deviations (of 3 replications) of percentage total fertilizer recovered at each sampling period

b, Treated with ^{15}N labeled $(NH_4)_2SO_4$ fertilizer (100 ppm NH_4-N)

c, Treated with ^{15}N labeled urea (100 ppm urea-N) and glucose (4000 ppm glucose-C)

d, Treated with ^{15}N labeled urea (100 ppm urea-N)

e, N. D., Not determined

* Statistically different from 100 percent recovery $P(\simeq 0.05)$

RESULTS AND DISCUSSION

Preliminary experiment to determine NH_4^+ fixation capacities of soils

Preliminary experiments (Table 2) indicated that in all the soils the amount of labeled NH_4^+ fertilizer fixed within 48 hours was not significant (less than 1%). Similar results about NH_4^+ fixation were observed by Broadbent (1965) whose data show that ion exchange between fixed and exchangeable NH_4^+ and between exchangeable NH_4^+ and amino N was negligible. Sowden et al., (1977) reported that in a clay fixation experiment most of the NH_4^+ fixation was complete in 2 hours with a much slower rate of fixation in the next 3 days.

In view of the results of this preliminary experiment, clay-fixed NH_4^+ was not determined as a component of the N fertilizer transformations reported in this research.

Overall recovery of tracer N

In tracer experiments dealing with interchange between inorganic and organic forms of N, it is essential to provide a complete accounting of tracer N in the system. This serves as a check on the reliability of the analytical procedures and indicates whether losses are taking place.

As shown in Table 3, the ^{15}N labeled fertilizer $(NH_4)_2SO_4$ and urea were quantitatively recovered as mineral N at zero time and subsequently at other sampling times during the period of incubation. The recovery ranges from 92 to 102%. These recoveries indicate that clay fixation of the tagged NH_4^+ fertilizer was negligible during the incubation period.

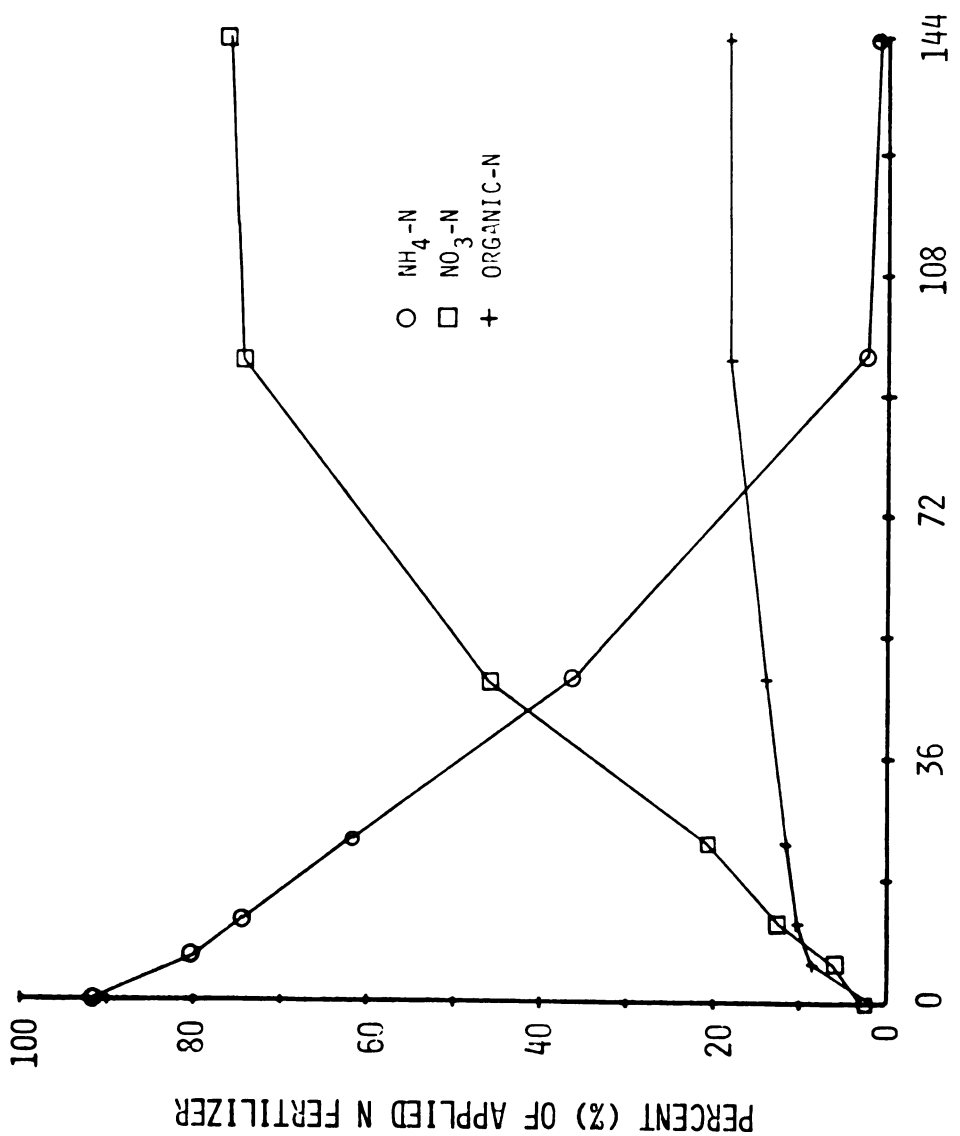


Figure 4. Changes in NH_4-, NO_3- and organic-N after addition of labeled $(NH_4)_2SO_4$ to soil MB18.

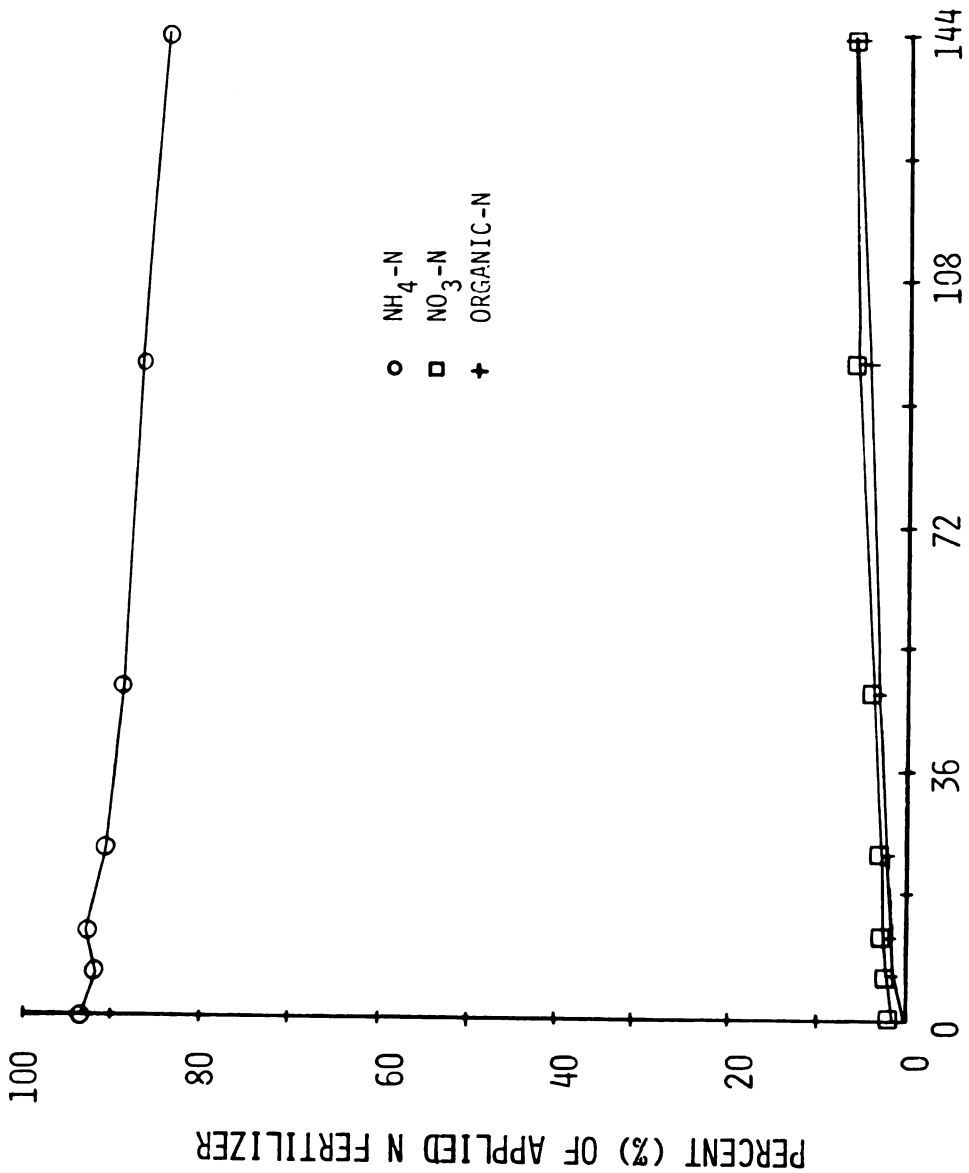


Figure 5. Changes in NH_4-, NO_3- and organic-N after addition of labeled $(NH_4)_2SO_4$ to soil W1.

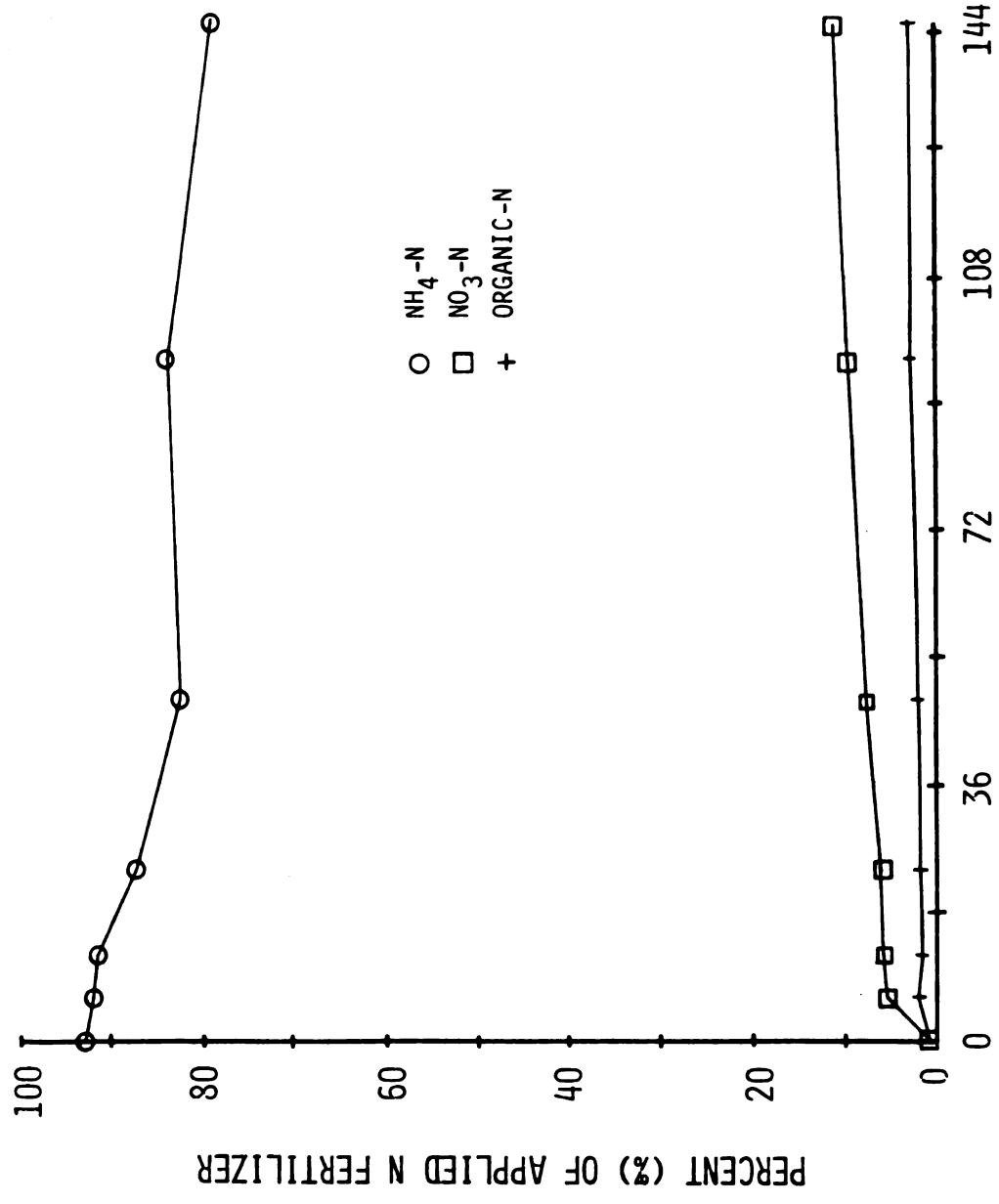


Figure 6. Changes in NH_4-, NO_3- and organic-N after addition of labeled $(NH_4)_2SO_4$ to soil F2.

Statistical analysis shown in Table 3 indicate the recovery values that were significantly different from 100% recovery. Low recoveries in some cases may be due to experimental errors involved in ^{15}N analysis. There is no evidence with the present data to attribute these low recoveries to volatilation or denitrification. The recovery data compares favorably with similar recoveries by Broadbent (1966) and Craswell and Martin, (1975).

Transformations of $(NH_4)_2SO_4$ and urea fertilizers in soils

The aim of this section is to show an overall view of the transformations of fertilizer N added as labeled $(NH_4)_2SO_4$ and urea in soils without any soil amendments. This is to present the soil as a continuously functioning biological system carrying out the series of transformations upon which the higher plants are highly dependent in obtaining the N necessary for their development. Specific details of the individual transformations are discussed later in the thesis.

Ammonium sulfate transformations in soils

Figures 4, 5 and 6 show the NH_4-, NO_3- and organic-N from labeled $(NH_4)_2SO_4$ fertilizer as a function of time in the soils used. As shown in Figures 4, 5 and 6, the rate of disappearance of fertilizers NH_4-N varied depending on the type of soil and the associated microbial processes which have taken place in the soil. In the case of soil MB18, the NH_4-N disappeared at a rapid rate (1.5% per hour) with no significant lag period, (Figure 4). At the same time, NO_3-N began to increase rapidly almost at the same rate (1.2% per hour) but the increase in NO_3-N did not equal the decrease in NH_4-N. This net decrease in

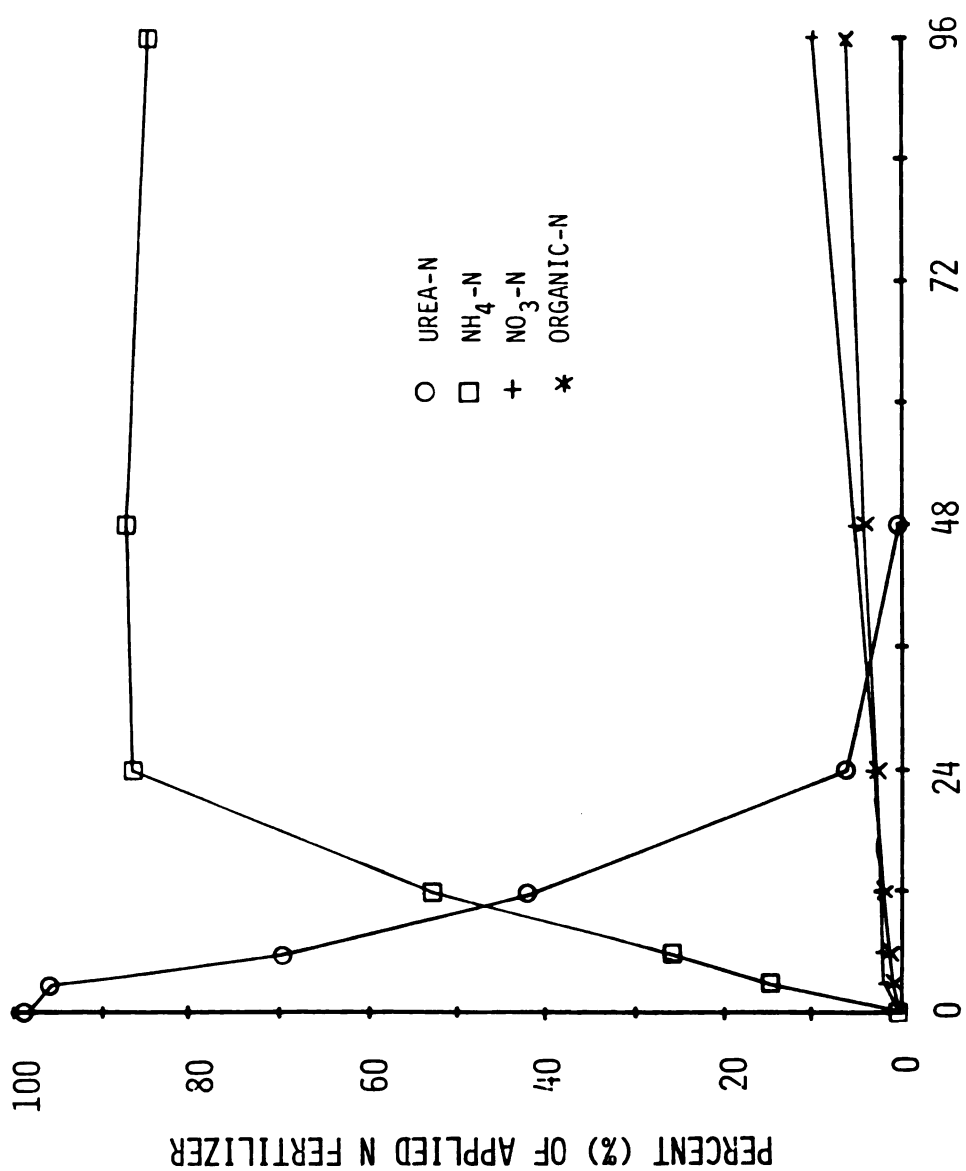


Figure 7. Changes in urea-, NH_4-, NO_3- and organic-N after addition of labeled urea fertilizer to soil W1.

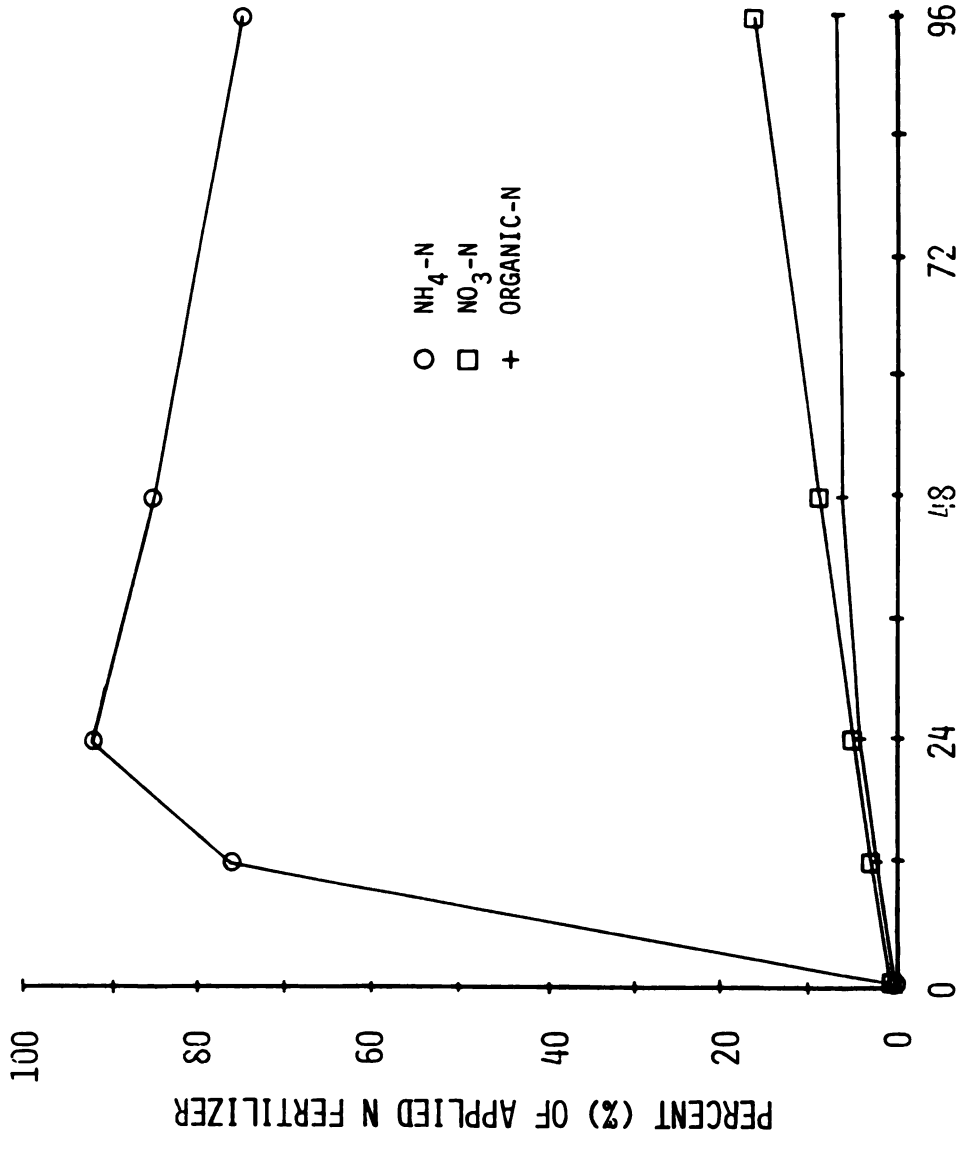


Figure 8. Changes in NH_4-, NO_3- and organic-N after addition of labeled urea fertilizer to soil WZ.

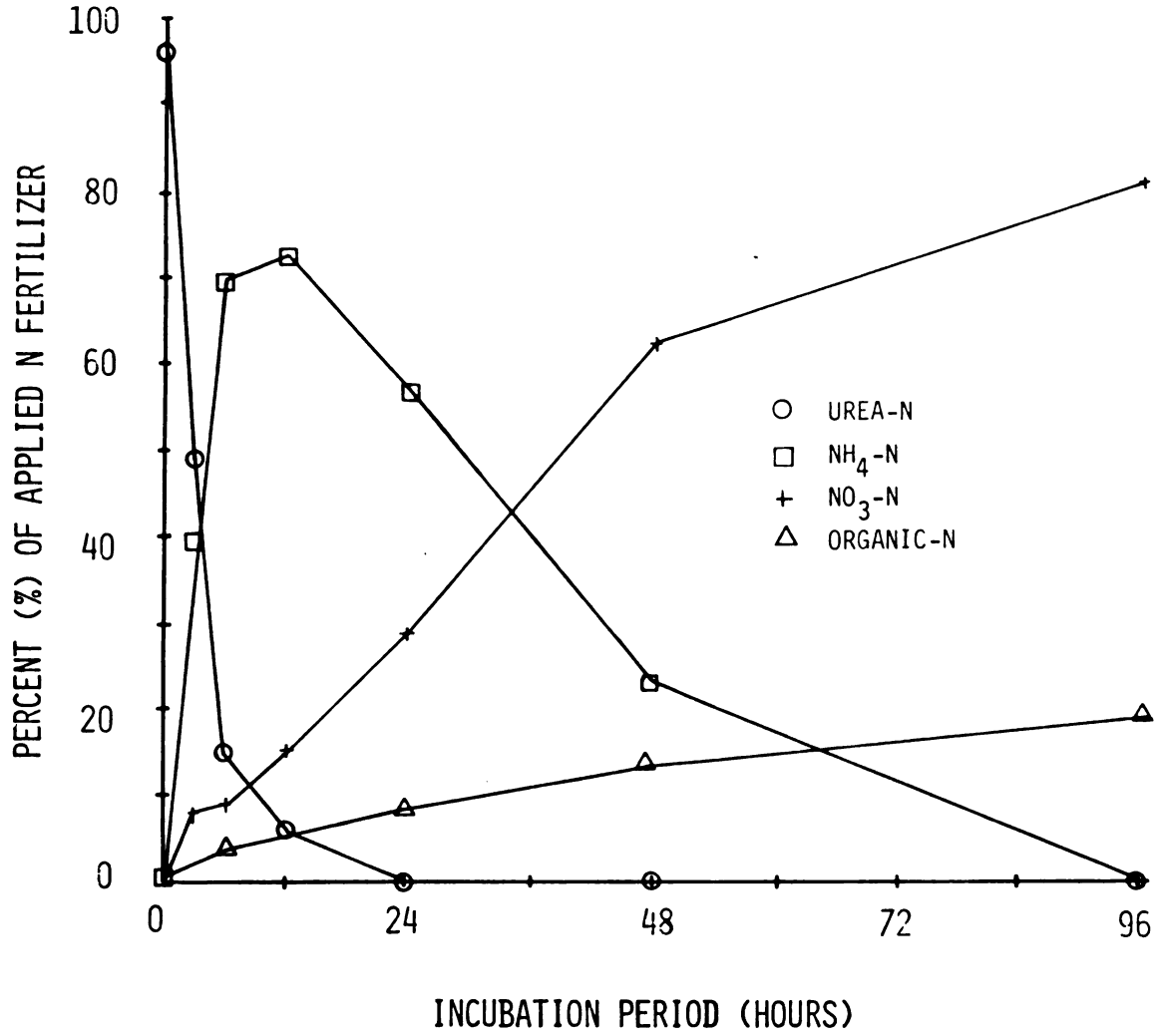


Figure 9. Changes in urea-, NH_4-, NO_3- and organic-N after addition of labeled urea fertilizer to soil MB18.

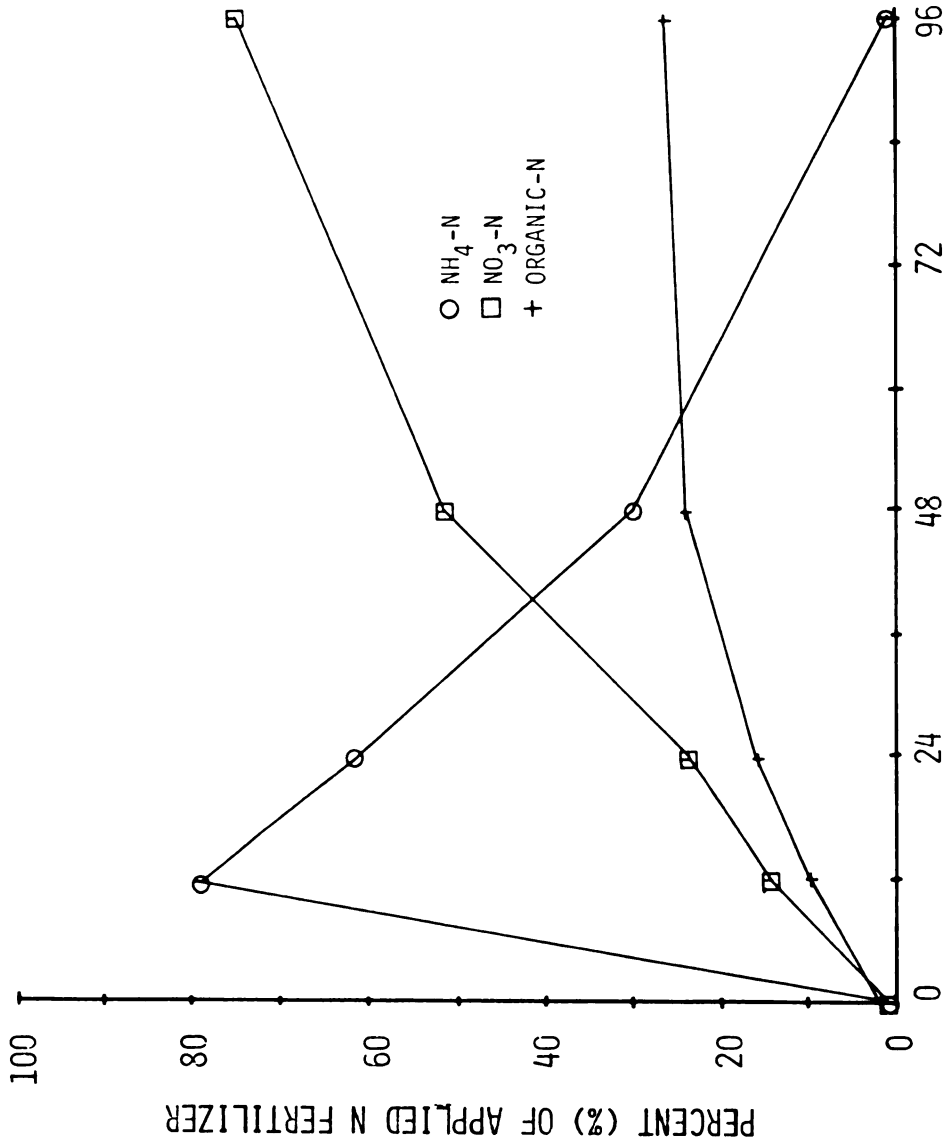


Figure 10. Changes in NH_4-, NO_3- and organic-N after addition of labeled urea fertilizer to soil MC15.

Table 4. Changes in pH due to addition to labeled urea and $(NH_4)_2SO_4$ fertilizer.

Incubation Period	Soil Reaction		
	Soil W1[b]	Soil W[c]	Soil MB18[c]
	pH		
0 (Before fertilizer addition)	5.1	5.1	5.7
0 (After fertilizer addition)	4.8	5.1	5.7
3	4.8	5.4	5.7
6	4.7	5.5	5.7
12	4.7	5.6	5.7
24	4.7	6.1	5.7
48	4.7	6.0	5.6
96	4.7	6.0	5.9

a, Mean of duplicate determinations

b, Labeled $(NH_4)_2SO_4$ added at the rate of 100 ppm fertilizer N (w/w basis).

c, Labeled urea fertilizer added at the rate of 100 ppm fertilizer-N.

mineral N was due to immobilization of the NH_4^+-N because the rest of the fertilizer was found in the organic fraction. After 96 hours of incubation, the percentage of fertilizer N in each fraction appears to remain relatively constant with NO_3-N and organic-N comprising 76% and 18%, respectively, of the N applied.

Ammonium sulfate transformations in soil W1 and F2, (Figures 5 and 6) were small even though they were statistically different from zero hour readings. In general, there was little disappearance of fertilizer NH_4-N or appearance of the fertilizer NO_3-N and fertilizer organic-N. This indicates early nitrification and immobilization, even though they are minimal when compared to soil MB18. The interesting thing is that significant changes in the NO_3-N and organic-N were observed in soil MB18 as early as 6 hours while in the case of soils W1 and F1, the significant increases were observed at 12 to 24 hours. The reasons for these differences are explained in later paragraphs.

Urea transformations in soils

In general, the urea applied was very readily hydrolyzed and much of the added urea N was transformed to NH_4-N in 2 days (Figures 7, 8, 9 and 10). The pH of soil W1 increased during this urea hydrolysis. The pH of MB18, the organic soil, did not significantly change because of its high buffering capacity (Table 4).

In soil W1 (initial pH of 5.1), net nitrification and immobilization of urea at 96 hours of incubation were 10% and 6% of the applied fertilizer, respectively. Disappearance of NH_4-N produced by hydrolysis of urea was greater than in treatments where $(NH_4)_2SO_4$ was applied.

In soil WZ (Figure 8), after 24 hours the majority of applied urea was in the form of NH_4-N. After 24 hours, there was a greater disappearance

Table 5. Immobilization of ^{15}N-labeled $(NH_4)_2SO_4$ and urea fertilizers after 12 hours of incubation.

Soil	Fertilizer and Carbon Treatment	Fertilizer N Immobilized[a]
		%
W1	$(NH_4)_2SO_4$	1.4 ± 0.03
W1	Urea	1.8 ± 0.06
W1	Urea + Glucose-C[b]	17.8 ± 1.3
F2	$(NH_4)_2SO_4$	5.7 ± 0.04
MB18	$(NH_4)_2SO_4$	10.4 ± 0.25
MB18	Urea	8.6 ± 0.8
WZ	Urea	2.3 ± 0.03
WZ	Urea + Glucose-C[b]	21.7 ± 0.01
MC15	Urea	9.7 ± 0.4
MC15	Urea + Glucose-C[b]	24.8 ± 0.01

a, Mean and standard deviations of percent labeled fertilizer recovered as organic-^{15}N

b, Applied at the rate of 4000 ppm glucose-C.

of NH_4-N because of rapid transformations of NH_4-N to oxidized forms of N and the immobilization of some of the NH_4-N into the organic-N

In soil MC15 and MB18, the NH_4^+ form produced after hydrolysis decreased very rapidly and totally disappeared by the end of the incubation period and NO_3-N became the major form of N from urea (Figures 9 and 10). These two soils appear to have identical trends in their transformations of applied urea.

Results of urea hydrolysis show that the reaction is rapid and varies in rate from one soil to another. Urea added to soils is apparently hydrolyzed in a matter of a few days (Overrein, 1967). The ammonical-N resulting from this enzymatic process becomes at least partly equilibrated with the mineral-N pool of the soil. After the rapid hydrolysis of urea, a sizeable pool of tracer NH_4-$^+$N developed in the soil and its subsequent behavior depended on the biological properties of the soil. Similar results were obtained by Broadbent, et al., (1958) who stated that urea as a fertilizer has been considered to resemble NH_3 in transformations following hydrolysis.

Comparison of immobilized ^{15}N labeled $(NH_4)_2SO_4$ and urea fertilizers in soils

The percent $(NH_4)_2SO_4$ and urea fertilizer immobilized in various soils after 12 hours of incubation with and without glucose amendments is shown in Table 5. Mean and standard deviations of labeled fertilizer immobilized during laboratory incubation of different soils throughout the incubation period are shown in Appendix A. These data were obtained by adding an enriched $(NH_4)_2SO_4$ or urea fertilizer and following the distribution of the isotope in the organic form at definite intervals as discussed previously.

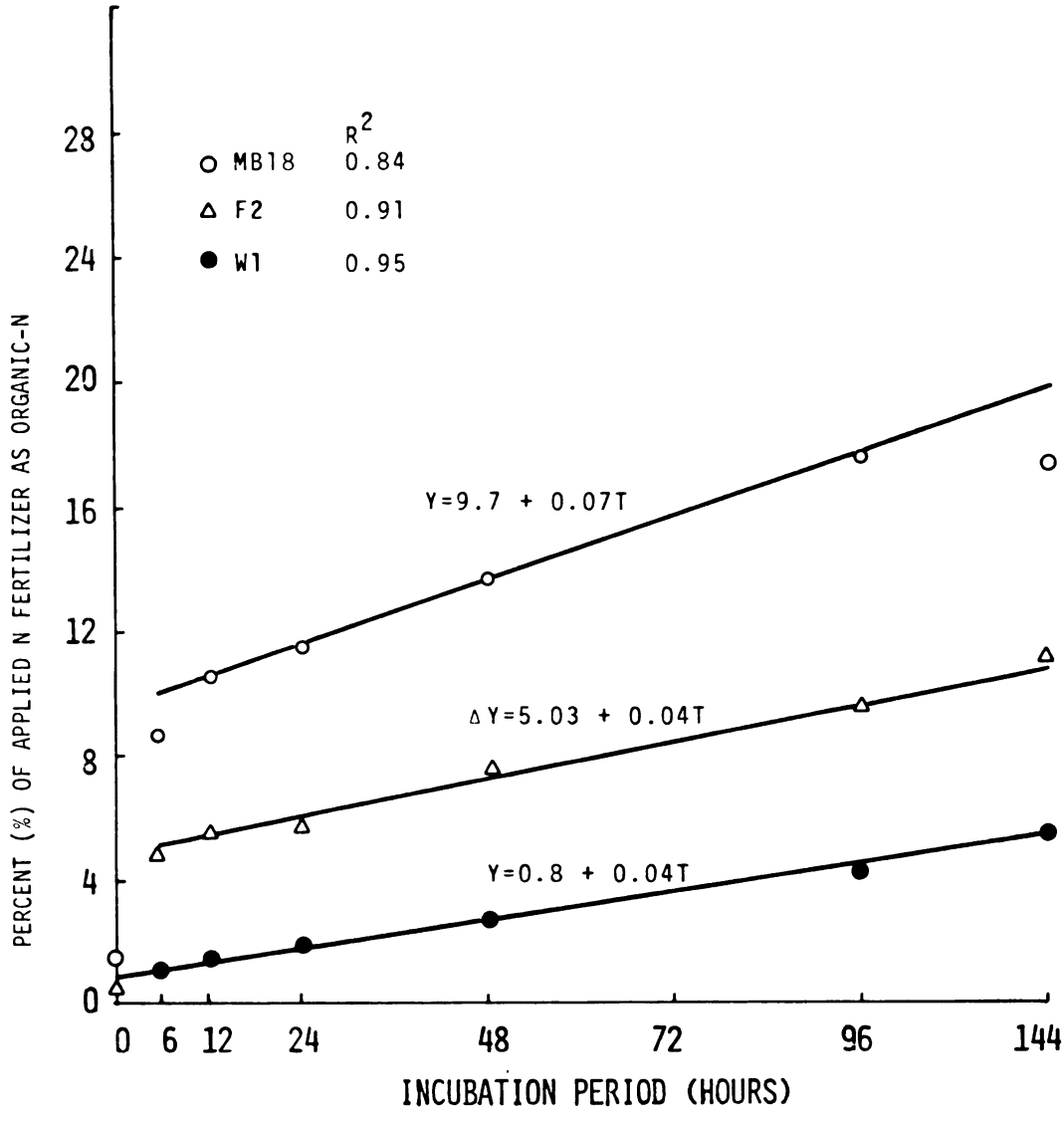


Figure 11. Comparison of immobilized labeled $(NH_4)_2SO_4$ fertilizer in various soils.

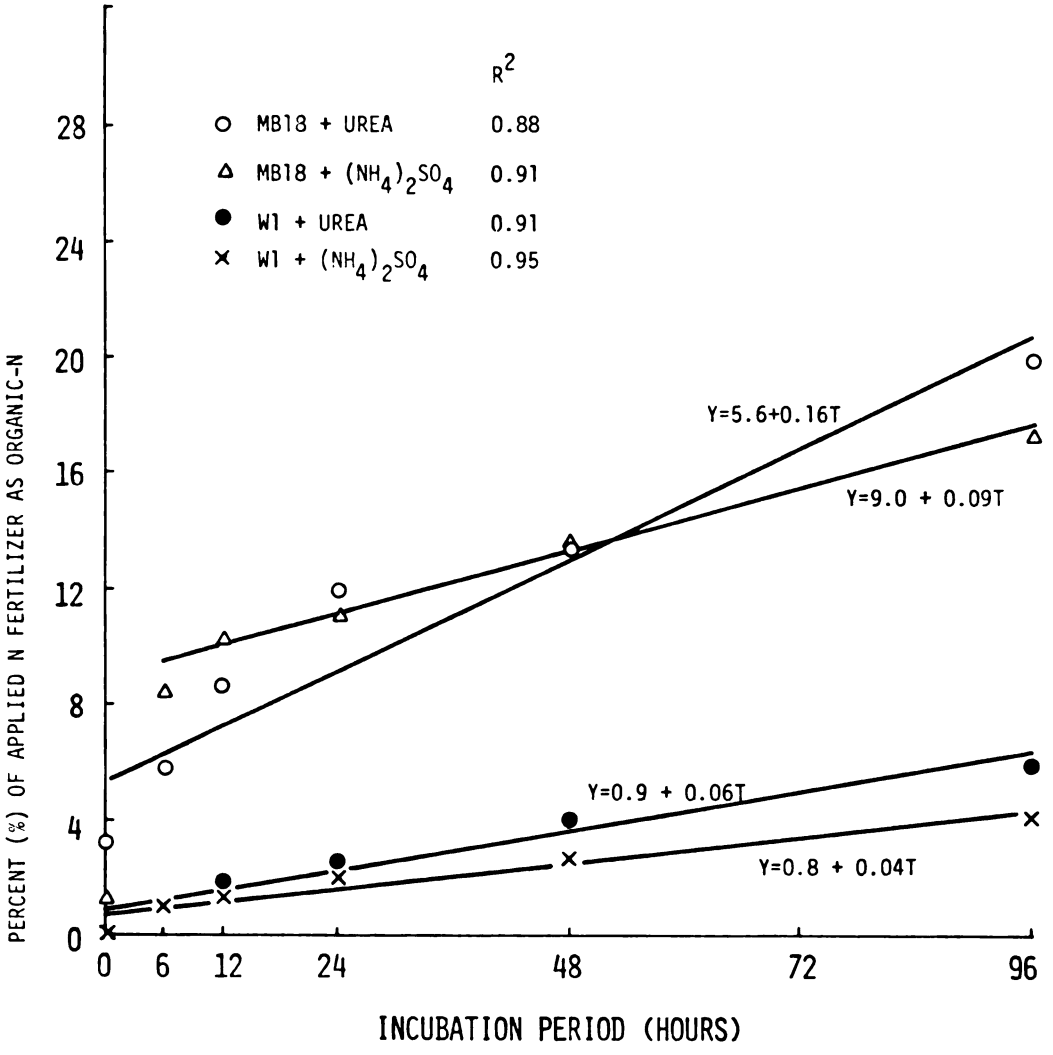


Figure 12. Effect of fertilizer source on immobilization of labeled fertilizer N in soils W1 and MB18.

In Figures 11 and 12, incubation time has been plotted against the percent fertilizer N immobilized. The results show a linear relationship. Inclusion of the zero hour reading for soils MB18 and F2 treated with labeled $(NH_4)_2SO_4$ (Figures 11 and 12) did not permit the prediction of N for the first 6 hours after the addition of the labeled fertilizer. Regression equations with the zero hour reading for such soils tend to underpredict the values at 0 to 6 hours. Because the provision for separate reactions appeared to be anomolous and a departure from the trend of the remainder of the data, it was necessary to delete the zero point in the regression analysis. Based on the data of this experiment, this anomoly may be attributed to the various experimental errors associated with the different steps in sample preparation and analysis.

Appendices C and D show that better prediction was achieved when the zero hour was deleted. The most practical use of this information would be for times between 6 to 96 hours. A significant increase in immobilization ($P \leq 0.05$) occurred in all the soils during the incubation period.

The high correlation coefficients show a high association between the immobilized N and time of incubation. For soil MB18, 10.4% of the applied labeled $(NH_4)_2SO_4$ fertilizer was recovered as organic-N while for soils W1 and F2, 1.4% and 5.7%, respectively, were recovered as organic-N 12 hours of incubation.

The rate of immobilization of $(NH_4)_2SO_4$ fertilizer indicated by the linear regression coefficients are as follows, MB18, 0.07% N $hour^{-1}$; F2, 0.04% N $hour^{-1}$ and W1 0.04% N $hour^{-1}$. Soil MB18 was found to be significantly different from soils W1 and F2 using a pairwise comparison procedure.

Application of urea resulted in immobilization of 8.6% and 1.8% of the applied urea fertilizer in soils MB18 and W1, respectively, after 12 hours of incubation (Table 5). The rates of immobilization of urea fertilizer were as follows, MB18 0.2% N $hour^{-1}$ and W1 0.06% N $hour^{-1}$ (Figure 12). Tests for statistical significance made at the 5 percent probability level showed that all the regression curves in Figures 11 and 12 were statistically significant.

The range in the amount of $(NH_4)_2SO_4$ and urea fertilizers immobilized during the incubation period suggests very different levels of microbial activity in the various soils. The higher rate of immobilization of $(NH_4)_2SO_4$ and urea fertilizers seen in soils MB18 and MC15 (organic soils) could be attributed to a higher population of heterotrophic organisms and the presence of a readily-available C source for microbial activity.

In an experiment to be reported later, all the soils effectively immobilized the urea fertilizer almost at the same rate when treated with glucose-C. This shows that the soils had the potential to immobilize N, but differences seen may have been due to differences in the immediate availability of a C source.

In an effort to further study the influence of pH and readily-available C on microbial activity and subsequent immobilization, several additional experiments were conducted.

Influence of changes in soil pH on immobilization of labeled $(NH_4)_2SO_4$ and urea fertilizers

The pH effect is an important consideration as protons are liberated in the oxidation of NH_4^+ to NO_3^-. When acid conditions are produced in poorly buffered environments, it may cause inhibition of nitrification.

The changes in pH during the incubation period as affected by the type

of fertilizer, were shown previously in Table 4 for soils W1 and MB18. In soil W1, application of urea fertilizer resulted in an initial marked increase in pH, while $(NH_4)_2SO_4$ application resulted in a slight decrease in pH. After the initial decrease in pH on the $(NH_4)_2SO_4$ treated soil, and after 12 hours on the urea treated soil, the pH remained relatively constant throughout the rest of the incubation period. There were relatively minor changes in the pH of the organic soil (MB18) with the addition of urea which is probably due to the higher buffering capacity of this soil. In soil W1, after 24 hours of incubation, the pH had increased from initial value of 5.1 to 6.1 due to hydrolysis of the urea.

The net effect of these increases in soil pH are evidenced in the increased rate of immobilization (Figure 12). There was a significant increase ($P \leq 0.05$) in N immobilization for soil W1 when urea was applied. After 96 hours of incubation, application of urea and $(NH_4)_2SO_4$ resulted in immobilization of 6.1% and 4.2%, of the applied N, respectively.

Net immobilization of fertilizer N was greater in the soil MB18 that received urea fertilizer. Initially, in the early hours of incubation, the amount of fertilizer N immobilized in the $(NH_4)_2SO_4$ treated soil seemed to be greater though this was not statistically significant ($P \leq 0.05$). This may have been due to the time factor involved in the hydrolysis of urea to NH_4-N. As soon as this process was underway, the immobilization became higher in urea amended soils and continued to be higher until the end of incubation.

From the results presented, it seems that changes in H^+ ion concentration exercise a considerable effect on the rate and amount of N immobilization. The wide range of organisms present in the soil and the cooperative nature of their activities make possible a large number of

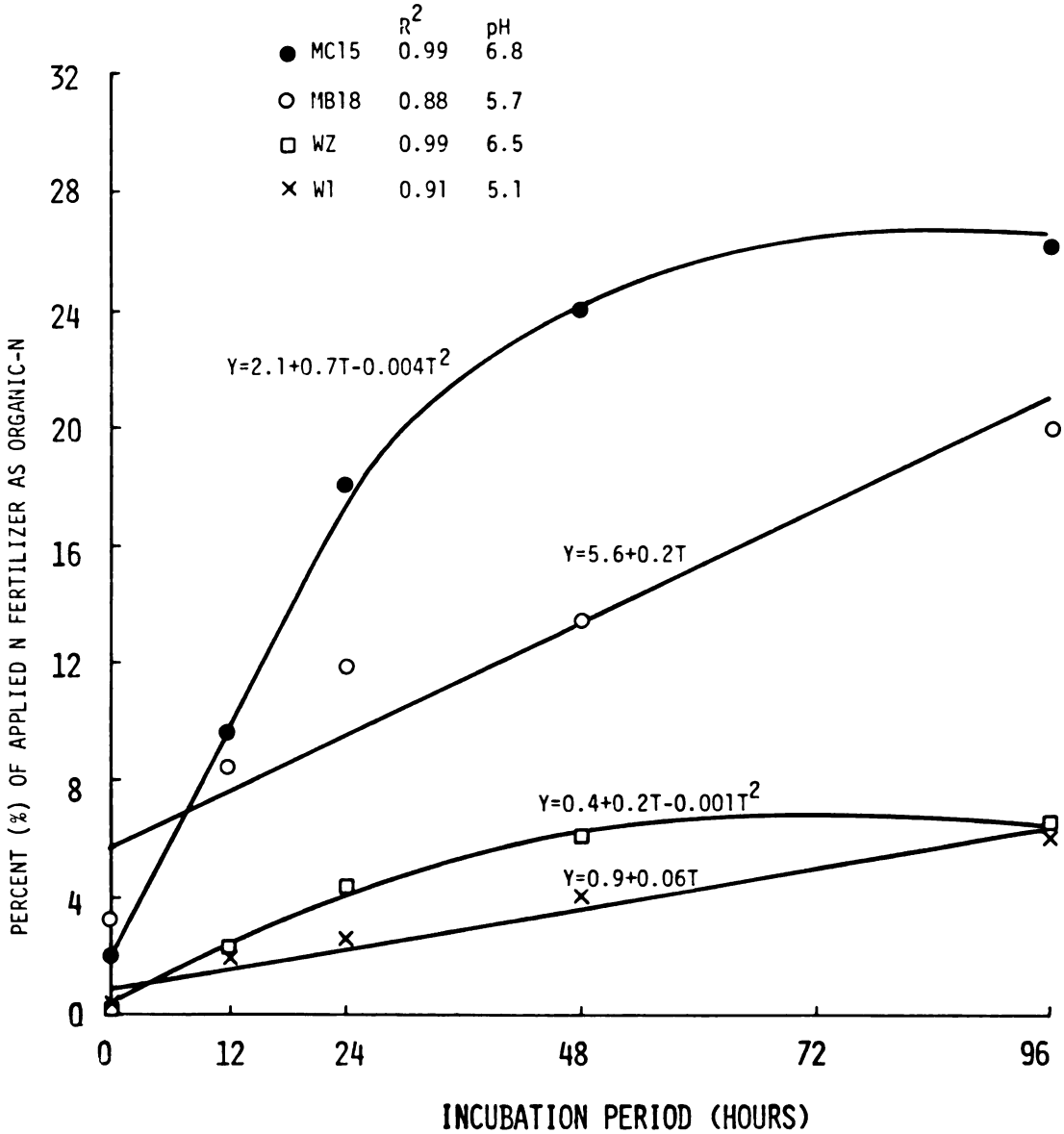


Figure 13. Influence of initial soil pH on immobilization of labeled urea fertilizer in various soils.

balance reactions, each capable of bringing about the same ultimate result, though the route and rate of achieving them may differ. The effects and differences observed are undoubtedly due to a modification in nature of the active microflora. Similar results were obtained by Norman (1931) who found that under slightly alkaline conditions, immobilization was more rapid than in either neutral or slightly acid conditions. He attributed the observed effects to differences in the character of the active microflora influenced by changes in pH.

Influence of initial soil pH on immobilization of labeled urea fertilizer in soils

Soils with similar physical and chemical properties collected within the same general area were used for this comparison. It was assumed that the only variable factor in these soils was the difference in their pH caused by prior agricultural practices, even though this assumption may not have been completely correct.

The second assumption was that because the soils were formed from the same parent material, they had comparatively similar microbial transformations under identical conditions. The soils were selected to include a low and a high pH. For the mineral soils, W1 had a pH of 5.1 and WZ a pH of 6.5. For the organic soils, the pH of soil MB18 was 5.7 while that of MC15 was 6.8. All the soils received the same quantity of fertilizer N (100 ppm urea-N) and were incubated under the same conditions.

Results as shown in Figure 13 indicate significant differences ($P \leq 0.05$) in the rate of immobilization of fertilizer N in identical soils with differing pH. In general, immobilization of N was greater in the organic soil series (MC15 and MB18) than in the mineral soil series (WZ and W1).

These differences were all statistically significant ($P \leq 0.05$). In the absence of direct microbiological data of the soils used, it was not possible to assess the extent to which the relationship between the pH of the soils and immobilization of N was due to differences among the microorganisms present. However, there is abundant evidence that the pH of the soil does influence the numbers of and types of organisms present in soils and it is generally considered that acid conditions favor fungi rather than bacteria. In the case of the acid soil, the immobilization may have been due mainly to the work of fungi, the growth of which will be favored by such conditions. In the more alkaline soil, MC15, the condition is more suitable for bacterial development and less favorable for fungi. It is not surprising that the immobilization rate was consistently higher than under the conditions of higher pH since in general, bacteria have a higher N content than fungi. Waksman (1922) showed that addition of lime stimulated the development of bacteria and actinomyces, but not fungi. By analogy, it is possible that the relationship between pH and immobilization of N in the present investigation was in part due to differences among the soil organisms present.

Effect of addition of glucose-C on immobilization of labeled urea fertilizer in soils

In view of the low rate of immobilization in some of the soils reported previously, an explanation to this was sought. At least the previous experiments mentioned in this thesis show that low pH was partly responsible for the low rate of immobilization. In a subsequent experiment, glucose-C applied at the rate of 4 mg glucose-C gm^{-1} soil (4000 ppm glucose-C) was added to soils W1, WZ and MC15 after the addition of 100 ppm of urea-N. This resulted to a C/N ratio of 40/1

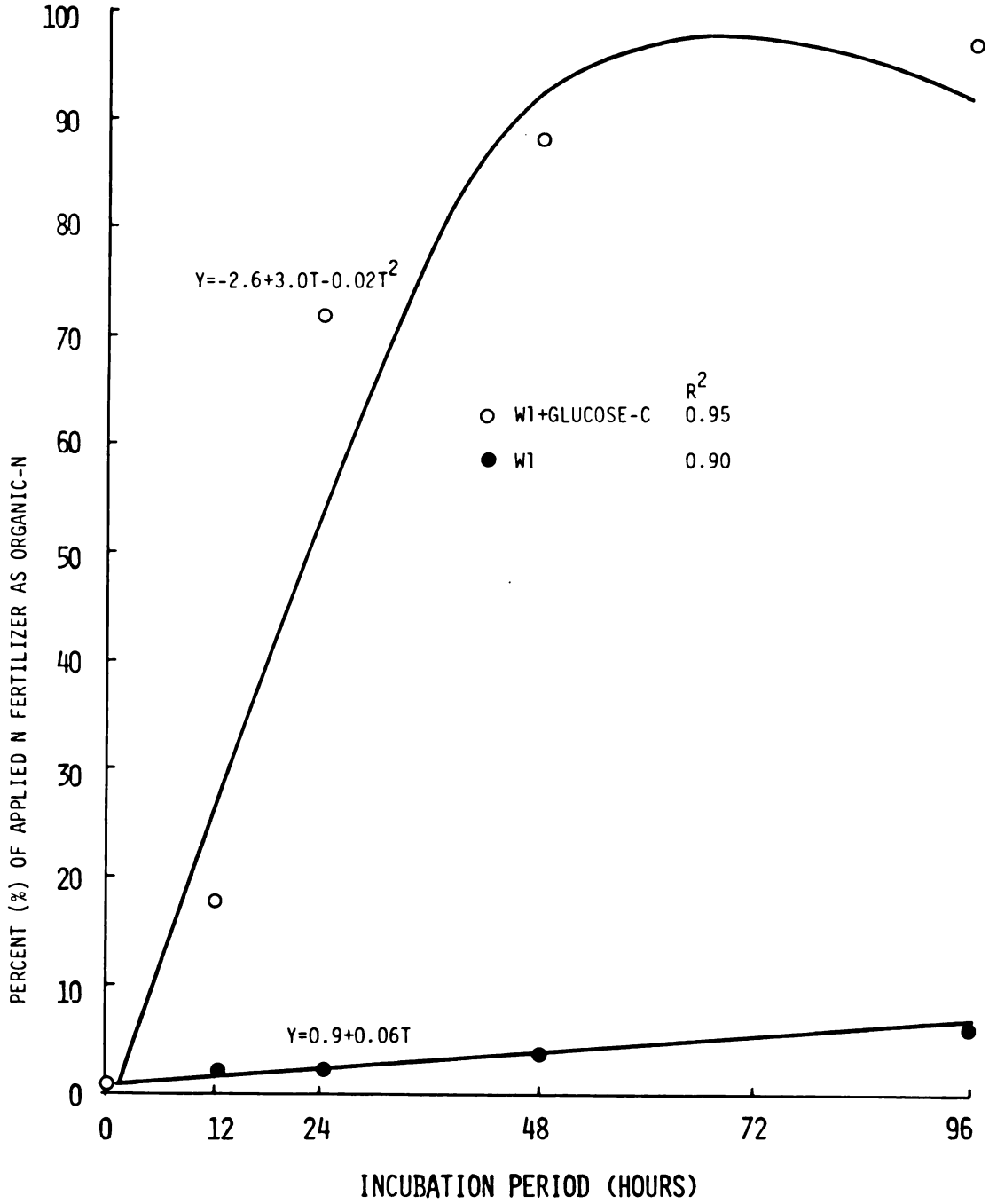


Figure 14. Effect of addition of glucose-C on immobilization of labeled urea fertilizer in soil W1.

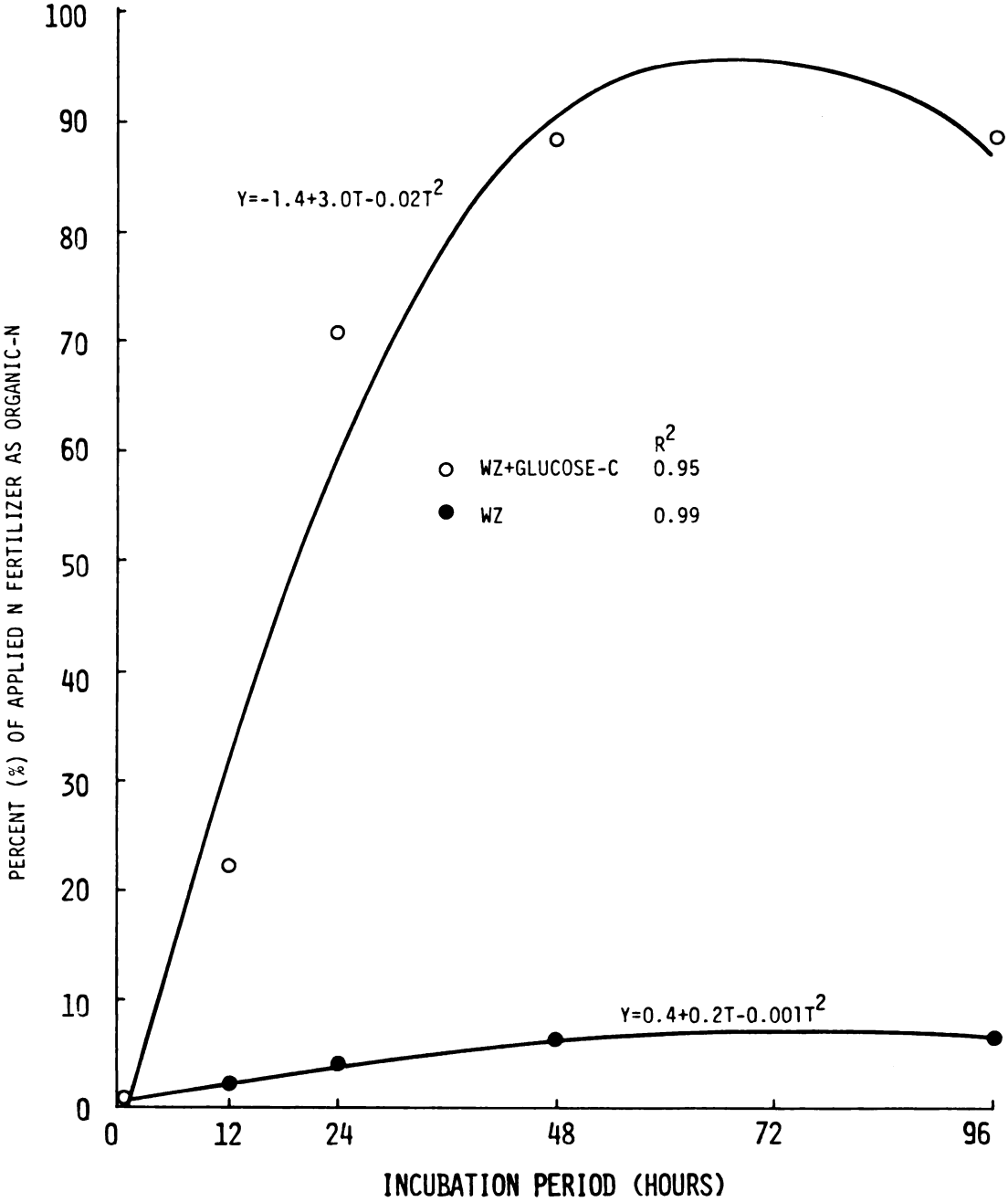


Figure 15. Effect of addition of glucose-C on immobilization of labeled urea fertilizer in soil WZ.

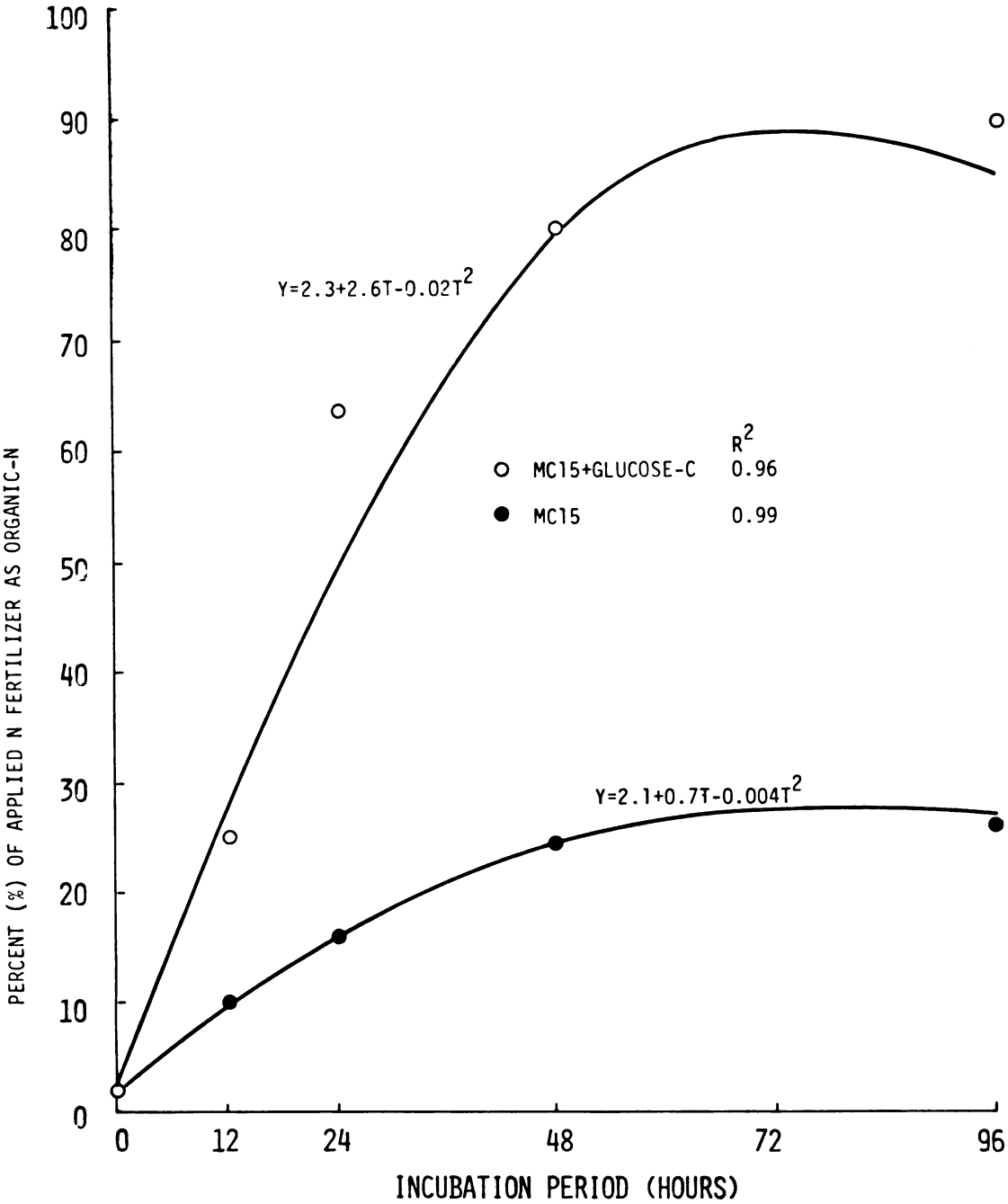


Figure 16. Effect of addition of glucose-C on immobilization of labeled urea fertilizer in soil MC15.

Table 6. Comparison of rate of immobilization of fertilizer-N in the presence and absence of Glucose-C[a]

Soil	Fertilizer[b] Source	Rate of Immobilization[c] Percent of Applied N hour^{-1}	
		No Carbon	With Carbon
		%	
W1	Urea	0.6	2.3
WZ	Urea	0.2	2.3
MC15	Urea	0.7	2.0

a, Applied at the rate of 4,000 ppm glucose

b, Added as 100 ppm urea-N

c, Represents the rate between 12 and 24 hours incubation period

(glucose-C/urea-N). Determinations of NH_4-^{15}N, NO_3-^{15}N and organic-^{15}N were made at intervals on duplicate flasks of soil in the manner previously described.

The results, expressed in percentage recovery of fertilizer in organic-N fractions in the soil, have been plotted against the period of incubation up to 96 hours as shown in Figures 14, 15 and 16. These figures show comparisons with and without addition of glucose-C. As shown in these figures, the addition of a readily-available C source caused very rapid rates of immobilization which, in all cases, were found to be significantly different ($P \leq 0.001$) when compared to their unamended counterparts. Table 6 shows that when the three different soils are compared with respect to the rate of immobilization (between 12 and 24 hours) after glucose-C addition, they all show a similar rate of immobilization: soil W1, 2.3% N $hour^{-1}$; soil WZ, 2.3% N $hour^{-1}$; and soil MC15, 2.0% N $hour^{-1}$. In the presence of glucose, the net immobilization was 97.0% in soil W1 after the 96 hour incubation period, compared to 6.0% in the absence of glucose amendment. For soil WZ, addition of glucose-C (after a 96 hour incubation period) caused a net immobilization of 88.0% compared to 6.0% in unamended soil, while in soil MC15 90.0% was immobilized by the glucose amended soil as compared to 26.0% in unamended soil.

In the three soils studied, immobilization of urea fertilizer in the presence of readily-available glucose-C occurred immediately, and was a rapid process, reaching its maximum in these soils within 48 hours. Although the results indicate that unamended soils have lower rates of immobilization, the immobilization potential under optimum conditions (presence of readily-available C) was high and similar for all soils (Table 6).

Immediate and rapid immobilization of N in soils W1, WZ and MC15 amended with glucose-C was due to the ready availability of this C source to microorganisms for performance of a complex series of reactions. When the glucose-C was added, heterotrophic organisms of the soil likely used the readily-available source of energy and increased rapidly in numbers. Part of the added C was evolved as CO_2 while part was used by the organisms in the synthesis of their own cell substance. This synthesis of microbial tissue was responsible for the immobilization of fertilizer N.

In a similar experiment but using a soil perfusion technique, Lees (1948) showed that with addition of glucose and sucrose immobilization of N reached its maximum in two to four days. Rubins and Bear (1942) also indicated that the relationship between C/N ratio and N transformations in the soil is influenced by the ease of decomposition of the various constituents present.

Agronomic implication of immobilization in the presence of available carbonaceous material

Under agricultural situations where plant materials which contain a wide C to N ratio are added to soils, the size of the decay population and the quantity of N immobilized in microbial cell materials may increase temporarily to levels which seriously deplete the soil of the mineral N (principally NO_3^-) for plant growth. Nitrogen immobilization has a significant bearing on the interpretation of any chemical or biological tests used to make recommendations on crop management practices and N fertilizer recommendations. Because of immobilization, the results of chemical or biological tests for N will vary, not only with the time of year when soil samples are taken, but also with the point in the rotation

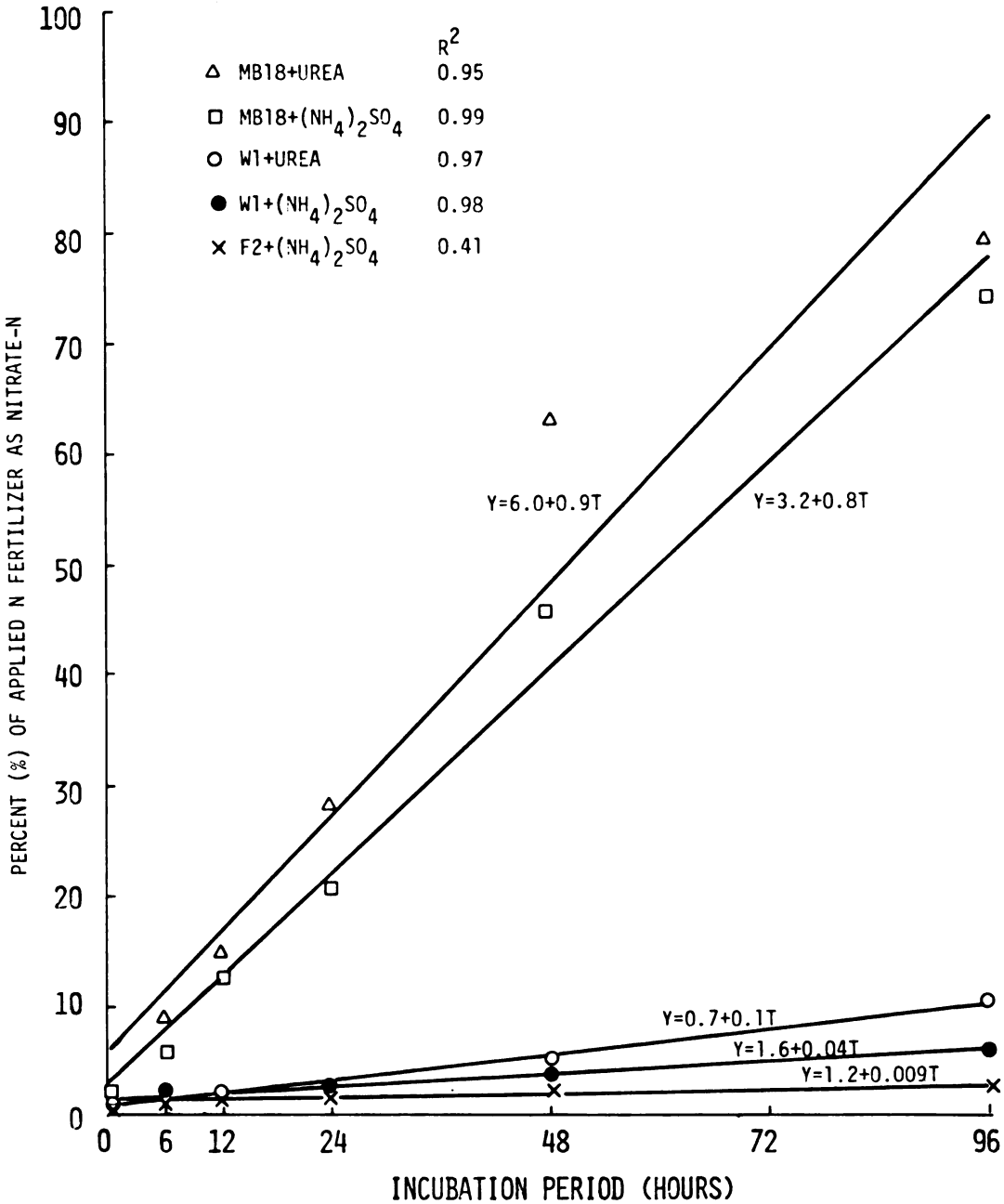


Figure 17. Effect of fertilizer source on nitrification of labeled $(NH_4)_2SO_4$ and urea fertilizers in various soils.

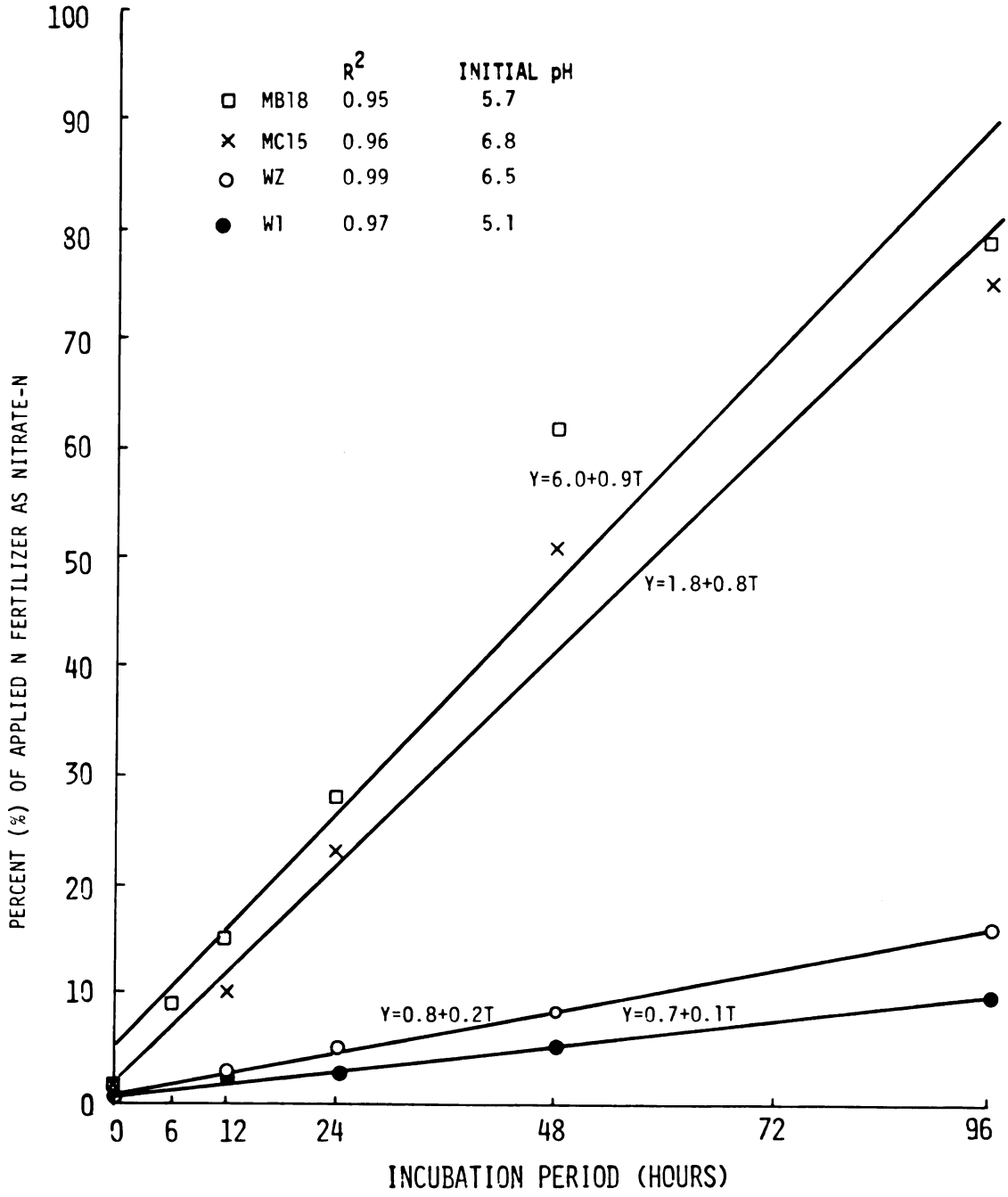


Figure 18. Effect of initial soil pH on nitrification of labeled urea fertilizer in various soils.

Table 7. Nitrification of ^{15}N labeled $(NH_4)_2SO_4$ and urea fertilizers after 12 hours incubation.

Soil	Fertilizer and Carbon Treatment	Fertilizer N Nitrified[a]
		%
W1	$(NH_4)_2SO_4$	2.3 ± 0.15
W1	Urea	2.1 ± 0.28
F2	$(NH_4)_2SO_4$	1.4 ± 0.12
MB18	$(NH_4)_2SO_4$	12.4 ± 1.10
MB18	Urea	15.3 ± 1.0
WZ	Urea	3.1 ± 0.68
WZ	Urea + Glucose-C[b]	2.8 ± 0.21
MC15	Urea	5.7 ± 0.0
MC15	Urea + Glucose-C[b]	9.0 ± 0.47

a, Mean and standard deviations of percentage labeled fertilizer recovered as NO_3-^{15}N after 12 hours of incubation

b, Applied at the rate of 4000 ppm glucose-C

when they were taken. It appears, therefore, that any direct correlation between chemical and biological soil tests and crop response to N will be difficult to achieve unless ways are found to evaluate the immobilizing potential of carbonacious residues in the soil.

Effect of fertilizer source and initial soil pH on nitrification on ^{15}N labeled $(NH_4)_2SO_4$ and urea fertilizers in soils

Figure 17 shows the effect of different fertilizer sources (urea and $(NH_4)_2SO_4$) on nitrification of ^{15}N-labeled fertilizer N in different soils, while Figure 18 shows the effect of initial soil pH on the nitrification of ^{15}N-labeled urea fertilizer in soils MB18, MC15, WZ and W1.

Nitrification of $(NH_4)_2SO_4$ and urea fertilizers was significantly greater in soils MB18 and MC15 than in soils W1, F2 and WZ ($P \leq 0.05$). At an application rate of 100 ppm NH_4-N as $(NH_4)_2SO_4$, soil MB18 had converted 74% of the NH_4-N to NO_3-N by the end of the 96 hours of incubation. At the end of the same time period (96 hours), 2.6% and 5.5% of the N applied was recovered as NO_3-N in soils F2 and W1, respectively. At the same application rate of 100 ppm N as urea 79.6%, 75.3%, 16.2% and 10% of the applied urea-N was converted to NO_3-N at the end of the 96 hours of incubation in soils MB18, MC15, WZ, and W1, respectively.

The percentages of applied $(NH_4)_2SO_4$ and urea fertilizers nitrified within 12 hours of incubation are shown in Table 7. It is apparent that the different soils did not nitrify the $(NH_4)_2SO_4$ and urea fertilizers at the same rate.

Ammonium sulfate and urea, in general, exhibited similar nitrification behavior in all soils; however, specific differences in the rates relate directly to the influence of acidity. Urea as a fertilizer has been considered to be like NH_3 in transformations following hydrolysis (Broadbent, et al., 1958).

In the case of soil W1 treated with $(NH_4)_2SO_4$, the nitrification rate was 0.04% N $hour^{-1}$ while the nitrification rate for the same soil treated with urea was 0.1% N $hour^{-1}$ (Figure 17). Data on pH changes (already reported) due to addition of fertilizer show that the $(NH_4)_2SO_4$ decreased the pH of this soil, while urea treatment increased the pH of the same soil upon hydrolysis. There was also a higher rate of nitrification in the urea treatment of soil MB18 (0.9% N $hour^{-1}$) compared to the same soil treated with $(NH_4)_2SO_4$ (0.8% N $hour^{-1}$). This observation indicates that soil acidity may have limited, or even prevented the nitrifying bacteria from oxidizing the NH_4^+ to NO_3^- in the acidic soils. Since soil W1 was initially acid, the rise in pH accompanying the addition of urea and its hydrolysis apparently provided a somewhat more favorable environment for nitrifying microorganisms. This contributed to the earlier and higher rate of nitrification of labeled NH_4^+-N from the urea application compared to the $(NH_4)_2SO_4$ application.

Roberge and Knowles (1966) found that over a 42 day period, there was no appreciable NO_3-N production in black spruce humus. Total absence of nitrification is unusual in agricultural soils (Broadbent et al., 1957) but has been reported by other workers especially in forest soils (Harmsen and Van Schreven, 1955).

In the experiment reported in this study, there was no evidence to prove that the slow rate of nitrification in these soils (W1 and F2) was due to lack of nitrifying bacteria or due to the existence of inhibitory substances. The only evidence to prove that it was a pH effect was the noticeable but not statistically significant increase in nitrifying capacity of soil W1 when urea was applied compared to an application of $(NH_4)_2SO_4$.

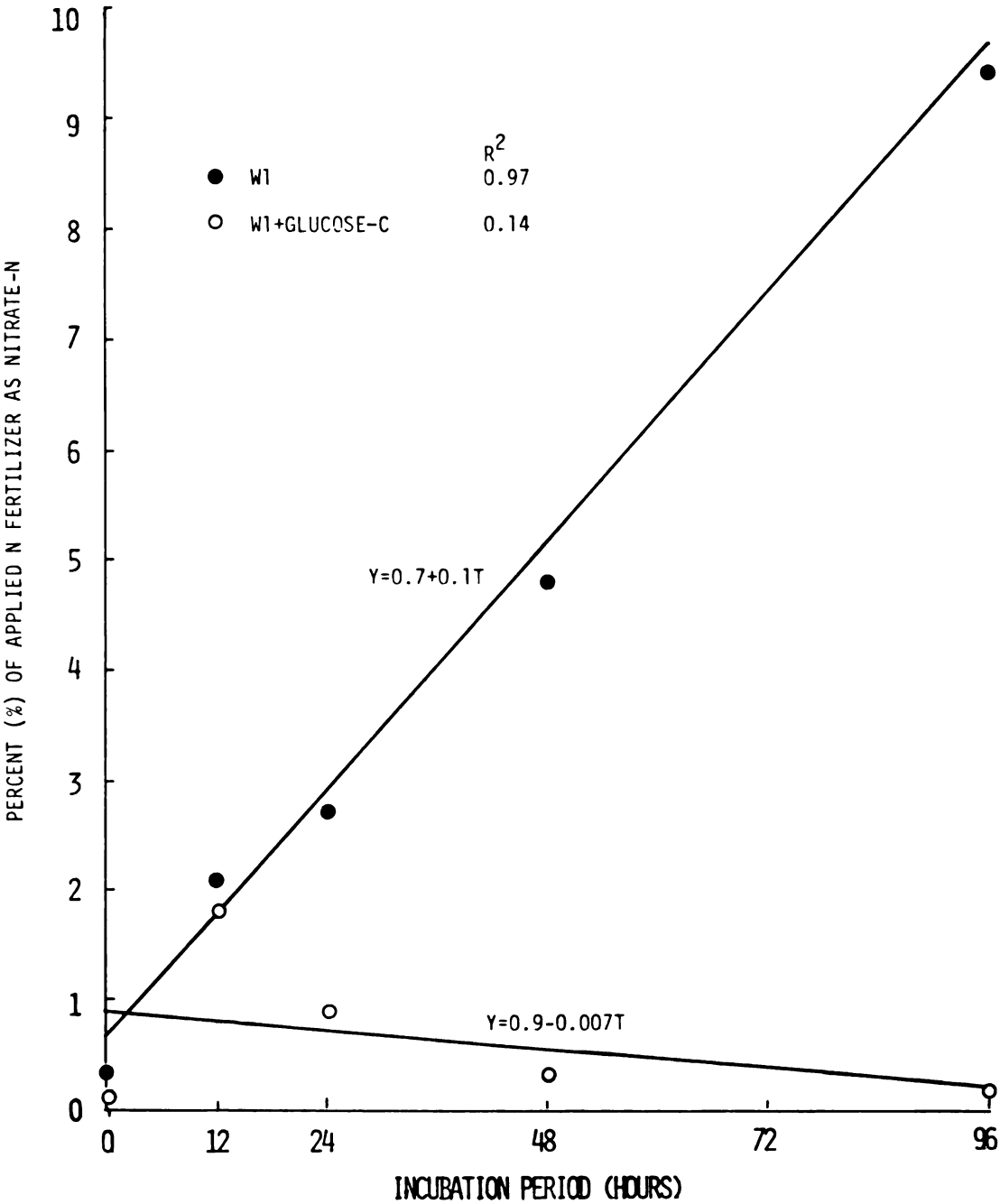


Figure 19. Effect of addition of glucose-C on nitrification of labeled urea fertilizer in soil W1.

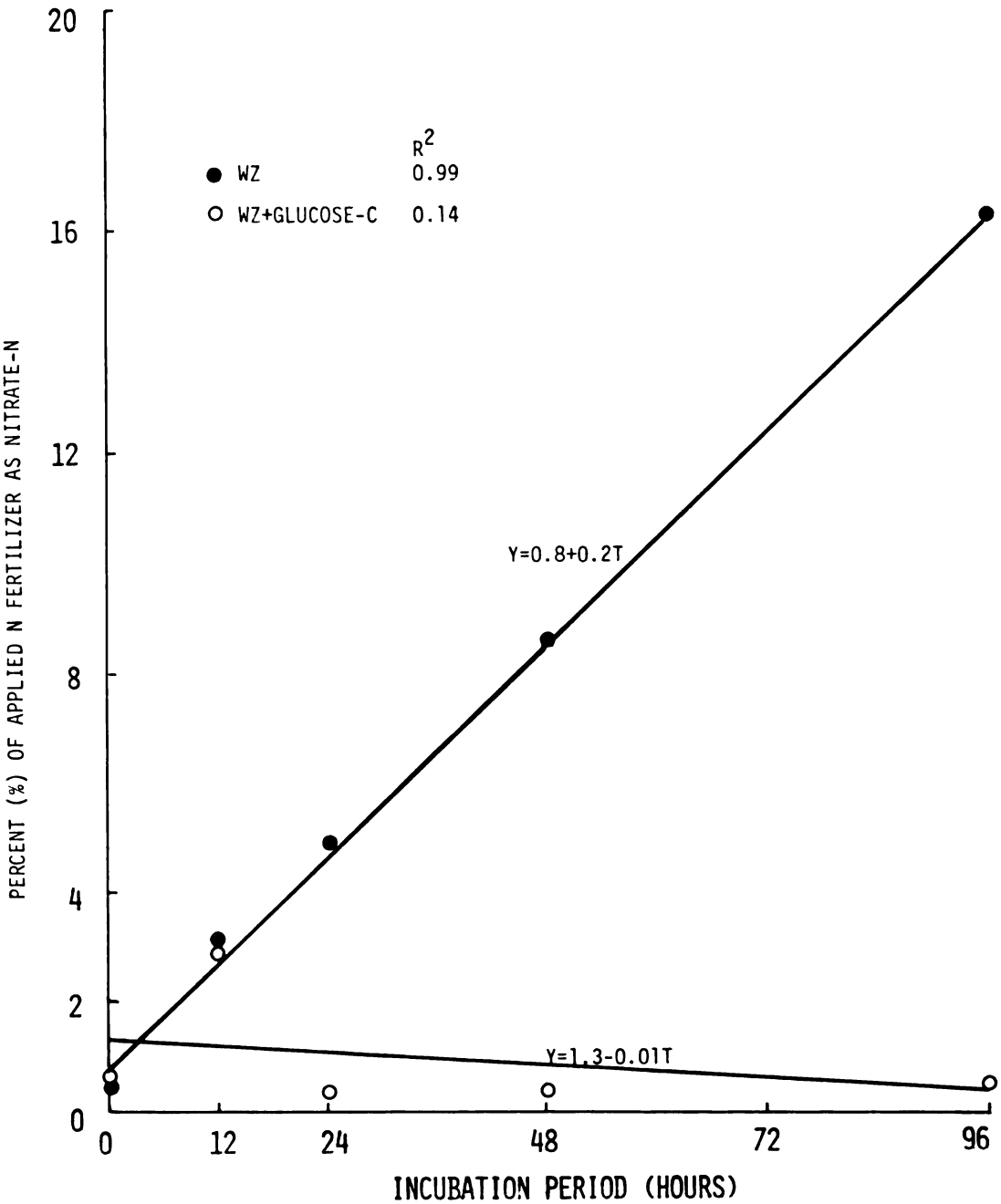


Figure 20. Effect of addition of gluclose-C on nitrification of labeled urea fertilizer in soil WZ.

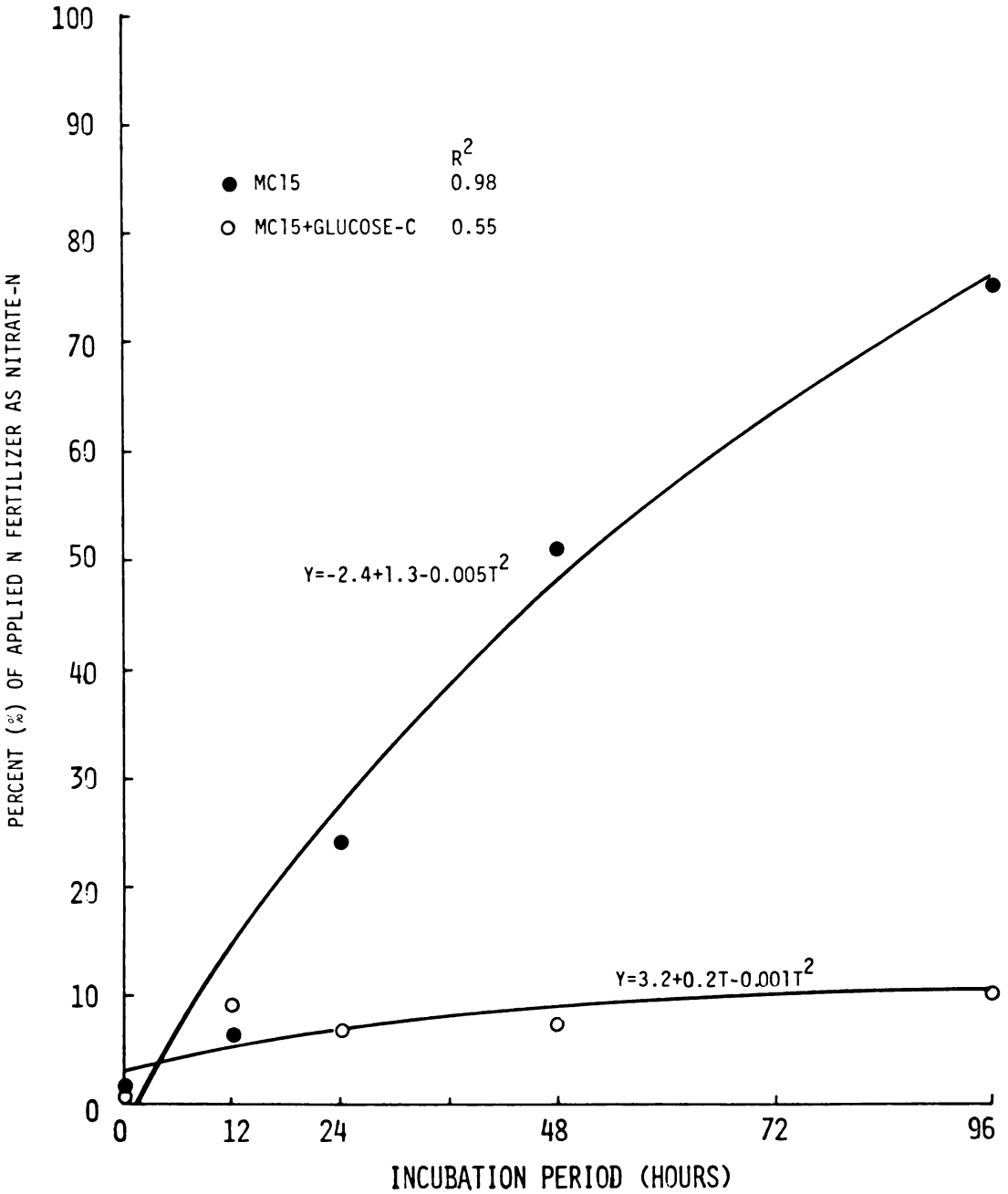


Figure 21. Effect of addition of glucose-C on nitrification of labeled urea fertilizer in soil MC15.

Table 8. Comparison of rate of nitrification of fertilizers-N in the presence and absence of Glucose-C[a]

Soil	Fertilizer[b] Source	Rate of Nitrification	
		No Carbon	With Carbon
		%	
W1	Urea	0.1[c]	-0.7×10^{-2}[c]
WZ	Urea	0.2[c]	-0.01[c]
MC15	Urea	1.12[d]	-0.17[d]

a, Applied at the rate of 4000 ppm glucose-C

b, Added as 100 ppm urea-N

c, Represents the rate between 0 and 96 hours incubation period

d, Represents the rate between 0 and 24 hours incubation period

Table 9. Net nitrification and immobilization of urea fertilizer in the presence and absence of glucose-C[a].

SOIL	Fertilizer N			
	Immobilized[b] no Carbon	Nitrified[c] no Carbon	Immobilized[b] with Carbon	Nitrified[c] with Carbon
	%			
W1	6.1± 0.13	10 ± 1.1	97 ± 2.0	0.19±0.08
WZ	6.5± 0.34	16.2 ± 2.0	87.8 ± 0.5	0.49±0.00
MC15	26.2± 0.20	75.3 ± 3.2	89.8 ± 4.0	10.30±0.26

a, Applied at the rate of 4000 ppm glucose-C
b, Mean and standard deviations of percent labeled fertilizer recovered as organic-N at 96 hours
c, Mean and standard deviations of percent labeled fertilizer recovered as NO_3-N at 96 hours

For the comparison of nitrification of urea in an initially acid soil with a counterpart that was initially less acid, soils W1 and WZ were used as well as soils MB18 and MC15. The nitrification rate for soil WZ (pH 6.5) was greater than that for soil W1 (pH 5.1) (Figure 18). On the other hand, the rate of nitrification in soil MB18 (pH 5.7) was greater than that in soil MC15 (pH 6.8). The result for soils W1 and WZ demonstrate the pH effect discussed earlier. The only explanation for the opposite effect shown by soils MB18 and MC15 is that in these two soils, the differences in rate of nitrification may not necessarily have been controlled by pH alone, but by other soil factors as well. One of these factors may have been an initially greater microbial population in soil MB18 than in soil MC15.

Effect of addition of glucose-C on the rate of nitrification of urea fertilizer

The rate of nitrification of urea in the presence of glucose-C was determined on soils W1, WZ, and MC15. These results are presented in Figures 19, 20, and 21 which show that in absence of C, the urea-N nitrified very readily. When glucose-C was added, the nitrification rate was slowed considerably in soil MC15 and completely reduced in soils W1 and WZ (Table 8). After 96 hours, net nitrification was 10.3% of applied urea-N in soil MC15 while in soils W1 and WZ, it approached zero (Table 9).

It is impossible to know for sure from these data, but it seems that some nitrification of the labeled urea fertilizer occurred initially in soils W1 and WZ treated with glucose-C but that the NO_3-N produced was later immobilized during the incubation. After 12 hours of incubation, 2% of the fertilizer N applied was recovered as NO_3-N in soil W1 while for

the subsequent incubation period, the amount decreased until it was about 0.1% after 96 hours of incubation. A similar observation was noticed in soil WZ where 3% fertilizer N was recovered as NO_3-N after 12 hours incubation and only 0.5% after 96 hours. In soil MC15, no further NO_3^- seems to have been produced after 12 hours. The net NO_3-N produced during 12 hours was 9% and this remained almost the same throughout the incubation period.

These results show that when glucose-C was added to the soils after addition of labeled urea fertilizer, the nitrifying bacteria were unable to compete effectively with heterotrophic microorganisms immobilizing the added N. In soil MC15 in the absence of glucose-C, the nitrifying bacteria were able to compete effectively for NH_4-N since a substantial part of the tracer N added as urea-N was recovered in the NO_3-N fraction. The increase reached a maximum shortly after the NH_4^+ had disappeared, which required only 96 hours.

The interesting thing in this experiment was that when sufficient energy was available in the form of a readily-available C source, the energy demanding steps of the N cycle predominate, i.e. immobilization of N (Table 9). In the absence of energy, nitrification is the predominant process leading to the most oxidized state of N, viz. NO_3-N (Woldendorp, 1975). From the foregoing, it is clear that the state in which N is found in the soil is determined to a high degree by the presence or absence of a readily available C source, i.e. energy. Nitrifiers are autotrophic bacteria and are very inefficient, relative to heterotrophs, in converting the energy available from their respective oxidations into cellular materials (Fenchel and Blackburn, 1979). This suggests that the quantity of N nitrified in a given situation will depend to a considerable extent

upon the activity of the heterotrophic flora in relationship to that of the nitrifiers.

A point of difference with Jansson's (1958) findings is in relation to his conclusion that in competition between heterotrophic flora and the nitrifiers for NH_4-N, the nitrifiers are left with the N not needed by the heterotrophs. The experimental data on soil MB18 and MC15 previously discussed showed that nitrifiers competed effectively for NH_4-N in the absence of added C, but in the presence of an available C source, the heterotrophic organisms outcompeted the nitrifiers. Broadbent and Tyler, (1962) in a similar experiment with Sacramento clay that received $(NH_4)_2SO_4$ plus straw, found that nitrifying bacteria were able to compete effectively with the immobilizing flora for N whereas in the Moreno soil, this was not the case.

SUMMARY AND CONCLUSION

These data show significant immobilization and nitrification of ^{15}N labeled $(NH_4)_2SO_4$ and urea fertilizers applied to soils after 12 hours of incubation. Urea applied to soils was very rapidly hydrolyzed and much of the added urea N was transformed to NH_4-N in two days. Following hydrolysis, urea transformations resembled that of NH_3. Marked differences existed in the ability of the various soils to immobilize and nitrify labeled $(NH_4)_2SO_4$ and urea fertilizers in soils in the absence of a glucose-C amendment when evaluated under comparable conditions.

Changes in the pH of the soil which resulted from fertilizer application exercised a considerable effect on the rate and amount of N immobilized and nitrified. Alkaline reaction caused greater immobilization and nitrification while an acidic reaction slowed them down. Soils with initial high pH had greater immobilization and nitrification than those with initial low pH. In one case, nitrification in soil MB18 (pH 5.7) was greater than that in soil MC15 (pH 6.8). This shows that nitrification may not necessarily have been controlled by pH alone, but by other soil factors as well.

In conclusion, the quantity of ammonical fertilizer nitrified in a given situation will depend in part on the rate at which it is immobilized into organic-N. In these experiments with organic soil (MB18 and MC15), the nitrifying bacteria were able to compete effectively for NH_4^+ with the heterotrophic flora, but the opposite was the case in the presence of an

available C source. In addition, the quantity and rate of fertilizer N transformation are dependent not only on pH and available C source, but also on other soil factors.

RECOMMENDATIONS FOR FUTURE RESEARCH

Laboratory studies do not adequately reflect the importance of some major soil factors under natural field conditions. For more reliable estimates of N transformations, field experiments should be developed to obtain practically useful prediction of the pattern of N transformations after fertilizer application. The time taken for immobilized fertilizer N to be re-mineralized is something about which very little is known. This requires incubation experiments at extended periods of time.

Long soil columns can be used to simulate field conditions in N transformations experiments that involve amendment of soils with natural organic materials after application of fertilizer N.

It may also be interesting to study the effect of other nutrients on N transformations. For example, addition of readily available organic matter stimulates microbial activity. This is accompanied by the immobilization of nitrogen, which requires phosphorous (P) and other elements essential to growth. Such a data can explain the relationship between the different N transformations and the levels of P and other elements in the soils.

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APPENDICES

Appendix A. Mean and Standard Deviations of Labeled Fertilizer Immobilized During Laboratory Incubation Period of Different Soils.

Incubation Period	Labeled Fertilizer as Organic-N									
	Soil + Urea				Soil + Urea + Glucose-C			Soil + $(NH_4)_2SO_4$		
	WZ[a]	MB18[b]	W1[b]	Mc15[a]	WZ[a]	W1[b]	MC15[a]	F2[b]	MB18[b]	W1[b]
(Hours)	%									
0	0.37±0.07	3.3±0.83	0.16±0.04	1.9±0.02	0.37±0.07	0.2±0.03	1.9±0.0	0.8±0.22	1.6±0.13	0.21±0.02
6	N.D.[c]	5.8±0.2	N.D.	N.D.	N.D.	N.D.	N.D.	4.9±0.23	8.7±0.31	1.13±0.1
12	2.3 ±0.03	8.6±0.77	1.8 ±0.06	9.7±0.14	21.7 ±0.01	17.8±1.3	24.8±0.01	5.7±0.04	10.4±0.25	1.4 ±0.03
24	4.3 ±0.25	12.0±2.0	2.6 ±0.1	15.9±2.54	70.4±7.7	72.2±0.79	64.1±0.64	5.7±0.15	11.6±0.24	1.9 ±0.08
48	6.1 ±0.4	13.5±0.92	4.1 ±0.43	23.9±1.7	87.6±0.18	88.72±1.8	79.4±7.0	7.5±1.1	13.8±1.84	2.8 ±0.1
96	6.4 ±0.34	20.0±2.3	6.1 ±1.3	26.2±0.19	87.8±0.5	97.2 ±0.76	89.8±4.4	9.6±1.2	17.7±1.25	4.2± 0.09
144	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	11.2±0.76	17.6±0.3	5.7± 0.87
L.S.D.(0.05)	0.7	2.5	1.1	3.6	8.8	2.0	9.5	1.2	1.5	0.6

a, Mean and standard deviations (2 replications) of percentage labeled fertilizer recovered as organic-N.

b, Mean and standard deviations (3 replications) of percentage labeled fertilizer recovered as organic-N.

c, N.D. No determination

Appendix B. Mean and Standard Deviations of Labeled Fertilizer Nitrified During Laboratory Incubation Period of Different Soils.

Incubation Period	Labeled Fertilizer as NO_3-N									
	Soil + Urea				Soil + Urea + Glucose-C			Soil + $(NH_4)_2SO_4$		
	WZ[a]	MB18[b]	W1[b]	MC15[a]	WZ[a]	W1[b]	MC15[a]	F2[b]	MB18[b]	W1[b]
(Hours)	%									
0	0.49±0.3	0.17±0.12	0.32±0.16	0.78±0.2	0.65±0.071	0.077±0.082	0.65±0.11	0.5±0.01	2.6±0.06	1.3±0.63
6	N.D.[c]	9.0 ±0.36	N.D.	N.D.	N.D.	N.D.	N.D.	1.7±0.11	6.1±0.34	1.9±0.12
12	3.1 ±0.7	15.3±0.45	2.1 ±0.28	5.7 ±0.0	2.82±0.21	1.75 ±.17	9.3 ±0.47	1.4±0.12	12.4±1.1	2.3±0.15
24	4.9 ±0.2	28.6±0.45	2.7 ±0.43	23.5±0.06	0.35± .064	0.89±0.053	6.76±0.45	1.6±0.49	20.6±1.1	2.7±0.02
48	8.6 ±0.1	62.45±0.53	4.8 ±0.09	51.4±1.2	0.38±0.11	0.28±0.046	7.36±0.56	1.7±0.46	46.0±0.23	3.6±0.04
96	16.2 ±2.0	79.6± 4.6	9.9 ±1.05	75.3±3.4	0.49±0.0	0.19±0.08	10.3 ±0.26	2.6±0.06	74.4±0.63	5.5±0.2
144	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2.1±0.79	76.1±0.32	5.8±0.04
L.S.D.(0.05)	1.6	2.0	1.1	2.5	0.30	0.24	1.04	0.7	1.2	0.2

a, Mean and standard deviations (2 replications) of percentage labeled fertilizer recovered as NO_3-N
b, Mean and standard deviations (3 replications) of percentage labeled fertilizer recovered as NO_3-N
c, N.D. No Determination

Appendix C. Linear regression coefficients relating percent $(NH_4)_2SO_4$ fertilizer immobilized during different time intervals.

Soils		Time Interval (Hours)		
		0-144	6-144	12-144
MB18[a]	R2	0.69	0.84	0.82
	a	7.55	9.74	10.50
	b	0.09	0.07	0.06
F2	R2	0.78	0.91	0.90
	a	3.89	5.03	5.09
	b	0.06	0.04	0.04
W1	R^2	0.95	0.96	0.96
	a	0.82	1.05	1.11
	b	0.04	0.03	0.03

a, Intercept

b, Linear regression coefficient.

Appendix D. Linear regression coefficients relating percent $(NH_4)SO_2$ and urea fertilizer immobilized during different time intervals.

Soils		Time Interval (Hours)		
		0-96	6-96	12-96
MB18 + Urea	R^2	0.88	0.89	0.88
	a	5.58	6.66	7.83
	b	0.16	0.14	0.13
MB18 + $(NH_4)_2SO_4$	R^2	0.72	0.91	0.90
	a	6.64	8.95	9.53
	b	0.13	0.06	0.09
W1 + Urea	R^2	0.91	0.91	0.89
	a	9.90	1.25	1.40
	b	0.06	0.05	0.05
W1 + $(NH_4)_2SO_4$	R^2	0.95	0.99	0.99
	a	0.82	1.01	1.06
	b	0.04	0.03	0.03

a, Intercept

b, Linear regression coefficient