

THE UNIVERSITY OF MANITOBA

INTERACTION BETWEEN AMMONIUM ION AND SOIL

BY

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OLALEKAN OLUWOLE AKINREMI

A thesis submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
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ABSTRACT

Fixation of ammonium by four Manitoba soils: peat; clay; loam; and sand was monitored under various soil treatments. The extent of organic matter involvement in fixation reaction was also investigated using NH_4^+ from urea, ammonium carbonate and ammonium sulphate. These nitrogen sources were applied to give concentration of 1000 ug of nitrogen per gram of soil. Fixation was determined by both the "difference" and the direct isotopic methods.

The level of ammonium fixation observed from both urea and ammonium carbonate (as calculated by the difference method) was high in all the four soils. One week after incubation, the percent fixation of NH_4^+ from applied urea nitrogen, was 56%, 41%, 32%, and 33% for peat, clay, loam and sand, respectively. These levels of fixation appeared quite high and were not in agreement with those obtained using ^{15}N -labelled nitrogen sources.

A substantial portion of the mineral fraction of the soil was removed by HF acid treatment leaving residue highly enriched in organic matter. Ammonium fixation from ammonium carbonate applied to acid-treated soils followed the same pattern as observed for the intact soils. However, urea was not hydrolysed and there was no observable urea fixation in acid-treated soil.

With the aid of ^{15}N -labelled nitrogen sources, it was found that ammonium fixation in all the soils was a function of incubation time and concentration of applied ammonium. The absolute amount of fixed NH_4^+ increased with increase in the level of applied ammonium, but the

percent fixation decreased as the concentration of applied ammonium increased. A relationship between ammonium fixation the soil organic matter content was observed. Peat had the highest level of NH_4^+ fixation from urea, with 16% of applied nitrogen fixed by peat one week after incubation. This was followed by clay and loam, which fixed 7%, and sand had the lowest level of NH_4^+ fixation of 5%. Organic matter accounted for over 80% of the nitrogen retained in the soil.

Soil sterilization by heat and gamma radiation was employed to investigate the possibility of a direct urea involvement in fixation reaction. There was no urea hydrolysis in heat sterilized soil, and no apparent fixation of urea was observed in all the soils. With two levels of gamma radiation, 6 and 12×10^4 Gy, complete soil sterilization was accomplished, but urea hydrolysis still occurred in these sterilized soils. Ammonium fixation in sterilized soils followed the same pattern as that in unsterilized soils, indicating a minimal role of microorganisms in ammonium fixation.

Isotopic exchange of fixed ammonium was carried out by incubating soil containing fixed $^{15}\text{NH}_4^+$ with a nitrogen source containing $^{14}\text{NH}_4^+$. On the average, 30% of the fixed nitrogen was isotopically exchangeable. The amount of fixed ammonium exchanged bore a direct relationship with the amount of nitrogen fixed in the soil. Isotopic exchange was rapid, reaching equilibrium in most cases 1 hr after the initiation of incubation.

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INTRODUCTION

That nitrogen is the most important and the most limiting nutrient element in crop production is not an overstatement. Application of nitrogen fertilizer has increased crop production over the years more than, perhaps, any other single farm operation. It has been estimated, that, about 30% of all the total inputs (in energy units of Kcal per year) to crop production is in the form of nitrogen fertilizer (Pimental et al 1975). From this viewpoint, the attention paid to nitrogen seems justified.

Emphasis has been placed on the efficiency of applied fertilizer, because of the high production cost of nitrogen fertilizers, coupled with the ever increasing need for agricultural products. Nitrogen applied to the soil must be available as and when needed by the plant. However, the efficiency of plant recovery of fertilizer nitrogen tends to be quite low, due to the various transformations that nitrogen is capable of undergoing in the soil. Values of percent recovery ranging from 0-60% have been obtained by various workers. Some of the factors limiting the efficiency of applied nitrogen fertilizers are: chemical and biological denitrification; gaseous loss or volatilization; leaching; ammonium fixation and biological immobilization. Since ammonium fixation constitutes one of the pathways through which the efficiency of nitrogen may be lowered, it is an area worth exploring.

It has been observed by several workers that plant recovery of nitrogen from NH_4^+ -yielding fertilizers (such as urea and ammonium sulphate), is usually inferior to that of nitrate-yielding fertilizers.

To the contrary, the amount of nitrogen retained in the soil, is higher for NH_4^+ -yielding fertilizers. Usually NH_4^+ fixation has been used to explain such observations (Obi 1982, Rennie and Rennie 1973).

The ammonium ion, because of its positive charge is capable of interacting with the negative charges on the soil colloids either at their surfaces or in the interlattice spaces. It is believed that fixation results from the entrapment of the ammonium ion within the clay lattice and reaction with the organic fraction of the soil. Several studies have implicated soil organic matter in ammonium retention by the soil (Sohn and Peech 1958, Burge and Broadbent 1961, Nyborg 1968). In some of these studies organic matter was shown to play a dominant role in NH_4^+ fixation.

It is essential to know the extent and mechanism of ammonium retention by the soil and the role of organic matter in NH_4^+ retention. It was, thus, the aim of this study to investigate the role, if any, that organic matter played in NH_4^+ fixation by some Manitoba soils. Particular emphasis was placed on the effect of ammonium concentration and time of incubation on NH_4^+ fixation by soil organic matter. Attempts were made to see if such reaction or interaction can be characterized by physical parameters.

LITERATURE REVIEW

A. Definition and methods of studying fixation.

(1) Definition.

The term fixation is an overused one in soil science, in the sense that it is often used to describe several processes going on in the soil some of which are unrelated and sometimes even antagonistic from the viewpoint of plant nutrition. For example, the entrapment of NH_4^+ in the clay lattice is called fixation, reaction between NH_3 and organic matter is fixation, biological immobilization of nitrogen is sometimes termed fixation and incorporation of N_2 into the soil by free living or symbiotic bacteria is termed fixation. Indeed, the term fixation is easily confusing to the uninitiated.

The classical definition of NH_4^+ fixation was given by the Soil Science Society of America as - "The absorption or adsorption of ammonium ion by the mineral or organic fraction of the soil in a manner that they are relatively insoluble in water and relatively unexchangeable by the usual method of cation exchange." Also, fixed ammonium has been defined as the NH_4^+ which results when interlayer cations such as Ca^{++} or Mg^{++} in the expanded lattice of certain clay minerals (vermiculite, montmorillonite and illite) are replaced by NH_4^+ ion causing the collapse of the lattice. Barshard (1951), proposed that the fixed ammonium be defined as that which is not replaceable by prolonged extraction and leaching of soil with a potassium salt solution and conversely that fixed K^+ should be defined as that which is not replaceable by NH_4^+ .

One fact that comes out clearly from these definitions is that clay fixation of NH_4^+ is indeed an arbitrary concept. It is defined relative to the extracting cation, thus, NH_4^+ is considered fixed if it cannot be extracted by a lattice contracting cation such as K^+ , but extraction might still be possible using a lattice expanding cation such as Na^+ or Ca^{++} . This arbitrary nature of fixation makes comparison of results from various studies extremely difficult if not impossible. Each study adopts its own unique definition or datum for fixation. Thus, results, vary depending on the type of extracting cation, concentration of extracting solution, intensity and method of extraction etc. According to Young and McNeal (1964), factors that contribute to the diverse finding in fixation studies include "necessarily arbitrary experimental approach, different analytical methods and unrecognized properties of mineral components of the reacting system".

(2) Methodologies

The method of studying clay fixation of ammonium is relatively straightforward and is implied in the definition of fixation given earlier. Since clay fixation is quite rapid, reaching equilibrium usually in 24 hours (Nommik and Vahtras 1982), an NH_4^+ -salt can directly be applied to the soil, equilibrated and extracted with K^+ -salt solution. This, in fact, is the method employed by most workers studying fixation of ammonium by the mineral fraction. However, the extracting salt, i.e. KCl , is perhaps the only item standardized, other factors such as concentration of ammonium used for equilibration,

concentration of the extracting K^+ salt, soil to solution ratio, length of time of incubation and shaking, are generally left to the individual researcher to define.

Jenny et al (1943), working on the comparative behaviour of ammonia and ammonium salts in soil employed the following procedure. An electrolyte solution containing 2-500 mmol of NH_4OH or $(NH_4)_2SO_4$ were added to a 50g portion of air dry soil. All samples were brought to volume of one liter. The suspensions were kept at room temperature for 24 hours with occasional shaking. Samples of clear supernatant liquid were obtained by centrifugation or by means of a Pasteur-Chamberland filter". Nommik (1957), in his work "fixation and defixation of ammonium in soils", used 10g of air dry soil in a 200 mL extraction flask, with 10 mL of standard $(NH_4)_2SO_4$ solution and incubated at room temperature for 48 hours. The samples were then shaken with 100 mL of 1 mol L^{-1} KCl solution for two hours and the filtrate washed with a further 100-150 mL KCl solution in small portions. In a more recent study, Okereke and Meints (1985), used ^{15}N -labelled urea and ammonium sulphate. They added 2 mg of nitrogen in solution to 20 g of oven dry soil in a 300 mL Erlenmeyer flask. Soils were adjusted to their -0.03 Mpa moisture percentage. The flasks were closed with cotton and incubated in a constant temperature cabinet at 27 °C for various periods of time ranging from 0-144 hours. The extraction procedure was as described by Bremner and Keeney (1966) for ammonium sulphate-treated soil. While for urea, the method of Douglas and Bremner (1970) was used.

Methods of studying fixation of NH_4^+ by organic matter, are not

standardized. Methodologies are as numerous as the number of studies conducted. What probably complicates the situation is that both the mineral and the organic fractions are capable of fixation reactions. Yet, there is no defined procedure for discerning the actual fraction involved in NH_4^+ fixation. In general, two methods can be identified, the indirect method and the direct method. In the direct method, effort is made to eliminate the mineral fraction (clay) by the use of HF acid, leaving soil residue highly enriched in organic matter.

An example of the indirect method was that adopted by Nommik and Nilson (1963), where the soil was pretreated with dilute K^+ salt solution. This was to saturate the expanding lattice of clay minerals present in the soil with potassium, thus, reducing their capacity to fix added ammonia. Two weaknesses in this procedure concern the possible interference of K^+ with ammonia fixation, and the effectiveness of K^+ , in actually preventing the fixation of ammonia by the mineral fraction.

According to Bremner and Harada (1958), one of the main difficulties encountered in work on the nature and properties of soil organic matter is that of separating the organic matter from the mineral constituents of the soil. The problem is further complicated, perhaps, as a result of the formation of clay-organic matter complexes. The classical method of separation, using alkali, permits isolation of a considerable fraction of organic matter. But the main disadvantage is that materials extracted by alkali are undoubtedly modified in the process. This is where hydrofluoric acid shows promise. It has the ability to decompose the clay fraction and as a weak acid it has been

shown not to cause serious modification to the organic matter (Rather 1917, Alexander and Byers 1932, Burge and Broadbent 1961). This is, thus, the basis of the HF acid method for removing the clay fraction of the soil. The method was originally used by Rather in 1917, it was modified by Alexander and Byers (1932), and has been successfully used by several workers since then.

Broadbent and Bradford (1952), used this method to obtain soils free of inorganic exchange materials for investigating the cation exchange properties of soil organic matter. Gottlieb and Hendricks (1946), adapted Rathers method to prepare a mineral soil fraction containing a high percentage of organic matter. The main problem with this method involves organic matter dissolution as a result of HF acid treatment. Studies, however, have shown that this effect seems to be very slight and within the acceptable range. Using data from Rather (1917), Bremner and Harada (1958), estimated that HF-HCl treatment dissolved 4% of carbon in the soil. A stronger treatment with the reagents of Alexander and Byers (1932), dissolved 16-23% of soil organic matter.

Direct use of the HF treatment in studying fixation of ammonia by soil organic matter was employed by Burge and Broadbent (1961), where it was used to dissolve the clay fraction leaving residue high in organic carbon. The effectiveness of this method is indicated by the significant increase in the carbon content of the soil residue. That it is a mild and reliable treatment is portrayed by the fact that data for acid-treated soil seem to fall on the same line as that of the untreated soil. According to these authors, this is an indication that

the contribution of the mineral fraction to ammonium fixation in these particular soils is slight. It is doubtful, however (unless neutralization is carried out), that the pH regime of treated soil will be identical to the untreated ones.

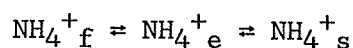
B Fixation of NH_4^+ by clay.

(1) Mechanism of NH_4^+ fixation by clay.

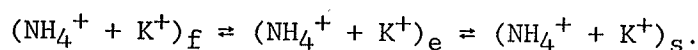
Extensive work has been done on the ability of the clay fraction of the soil to fix NH_4^+ . This is in such a manner as to be relatively unexchangeable by the normal exchange process. Such fixation is usually ascribed to the 2:1 layered clay minerals such as montmorillonite, vermiculite, illite etc. The commonly accepted theory of NH_4^+ fixation is that proposed by Page and Baver (1940). The exposed surface and surfaces between the sheet of 3-layered minerals consist of oxygen atoms arranged hexagonally. The opening within the hexagon is equal to the diameter of the oxygen ion, and this is approximately 0.28 nm. Ions having this diameter (e.g. K^+ and NH_4^+) can fit into this hole barely. Strong electrostatic attraction will cause the two layers to come together, thus, preventing expansion and rehydration. Cations with diameter larger than 0.28 nm cannot enter this hole. Hence, they leave the lattice open, subjecting it to rehydration and the cations themselves to exchange by other cations. A strong support of this theory is the usual reduction in cation exchange capacity following fixation of NH_4^+ (Page et al 1967). However, fixation has been observed in nonexpanding layer silicates such as kaolinite and feldspar, and in the nonclay fraction of the soil (Nash and Marshall

1956, Nommik 1957). This shows that this theory, plausible as it is, may not give a complete explanation of the mechanism of fixation by all the components of the mineral fraction.

It is assumed that there exists an equilibrium between fixed ammonium ($\text{NH}_4^+_f$), exchangeable ammonium ($\text{NH}_4^+_e$) and ammonium in solution ($\text{NH}_4^+_s$). This can be expressed as follows:



Nommik (1957), used this assumption to explain the reduction of NH_4^+ fixation brought about by the addition of cations which do not contract the lattice e.g. Ba^{++} and Ca^{++} . This reduction, he attributed to competition with NH_4^+ for exchange sites thus keeping $\text{NH}_4^+_e$ low, hence, low fixation. However, another observation of the influence of K^+ on NH_4^+ fixation with simultaneous addition of K^+ and NH_4^+ , brought about the modification of the above relationship. It was observed that NH_4^+ fixation increased with simultaneous addition of small amount of K^+ to soil that fixes NH_4^+ in preference to K^+ . Nommik (1957), then postulated that the sum of NH_4^+ and K^+ should be inserted in the above equation to give



It is generally believed that NH_4^+ and K^+ are fixed by the same mechanism in the soil, hence, fixed ammonium has been defined relative to K^+ extraction and vice-versa. Osborne (1976a), proposed that the

term fixed ammonium should be discarded and instead a new term "intercalary" ammonium be used. He defined intercalary ammonium as the NH_4^+ released by HF-HCl treatment of soil subsequent to KBr/KOH treatment and leaching with 0.5 mol L^{-1} KCl to remove organic nitrogen.

The concept of clay fixation of ammonium was challenged by Freney (1964). He suggested that native fixed ammonium was a laboratory artifact caused by (1), the use of K^+ containing salts as extractant. This leads to the collapse of the clay lattice and entrapment of otherwise exchangeable NH_4^+ . (2) The use of reagents such as HF. This causes considerable hydrolysis of soil organic matter releasing ammonium which is then determined as fixed NH_4^+ . This comment has not been supported by the preponderant evidence on NH_4^+ fixation.

Several investigations have been carried out on the subject of ammonium fixation by clay. It has generally been observed that fixation in most soils increased with depth. The actual reason for this is not known. This is probably due to increase in clay content with depth, decline in organic matter with depth, and saturation of the topsoil lattices by native K^+ and NH_4^+ . This saturation of the topsoil will serve to reduce it's fixing capacity (Allison et al 1953).

Osborne (1976b), working with six different soils in Southern New South Wales, found a heavy-gray-textured soil that fixed significant amount of NH_4^+ on addition of $(\text{NH}_4)_2\text{SO}_4$. The level of fixed ammonium increased by 55% in the surface soil and 100% in the subsoil. Fixation was high enough to warrant recommendation against the use of NH_4^+ -fertilizers on this soil. Kowalenco and Cameron (1976), reported that approximately one-half of $0\text{-}1000 \text{ ug g}^{-1}$ of ammonium sulphate was fixed

two days after application to the soil. The same authors in 1978, found that their experimental soil fixed 34-60% of 150 Kg ha^{-1} of NH_4^+ -fertilizer immediately after application. Sowden (1976), observed that about 40% of NH_4^+ from manure and fertilizer was fixed in his laboratory incubation.

In all these fixation studies, clay minerals have been held responsible for the NH_4^+ fixation. The authors either did not consider the mineralogy of the clay fraction or give much thought to the role of organic matter as a means of establishing the actual soil component involved in the large fixation observed.

(2) Factors affecting clay fixation of NH_4^+ .

(a) Clay mineralogy and particle size.

The mineralogy of the clay fraction is an important factor in the fixation of NH_4^+ by the soil. This may even have a modifying effect on the role of particle size. Studies have shown that vermiculite has the largest capacity to fix ammonium. In fact it is the only mineral that has been shown to fix more ammonium than potassium (Nommik 1957). Montmorillonite does not generally fix appreciable amounts of NH_4^+ under moist conditions. Oven-drying, however, has been found to increase fixation by a factor as high as 10 (Young and McNeal 1964). Illite, on the other hand, is intermediate between vermiculite and montmorillonite with respect to NH_4^+ fixation.

Aoram and Evan (1983), working on six soil profiles of varying mineral composition, showed that regression analysis indicated that vermiculite was more important in ammonium fixation than illite.

Similarly, Young and McNeal (1964), found that vermiculite mineral under wet and air-dry conditions fixed quantities of ammonium in excess of amounts fixed by other minerals. Stevenson and Dhariwal (1953), investigated the distribution of fixed NH_4^+ in soils. They found that the amount of fixed NH_4^+ in the soil depends on the type and amount of clay minerals present. With respect to clay mineral type, the order found was illite > montmorillonite > kaolinite. Thus, a consideration of the mineralogy of the soil could give an indication of the ability of the various soils to fix added ammonium. It should be noted, however, that there is a need to exercise restraint in extrapolating data from a few mineral types to a complex mixture as generally encountered in the soil.

The effect of particle size on NH_4^+ fixation is closely related to that of the mineralogy. In general, NH_4^+ fixation decreases with increase in particle size, however, results from the literature has not been consistent, perhaps, because of the difference in the mineralogy of the various size fractions. Nommik (1957), separated the soil into five size fractions (<1, 1-2, 2-5, 5-50 and >50 μm). He found the highest fixation in the 1-2 μm size fraction and attributed the lower fixation in the finest fraction (< 1 μm) to its high montmorillonite content. There was also relatively high fixation in the coarse fraction. Similarly, Mclean and Brydon (1961), worked on fixation of potassium in different size fractions. They reported that fixation of K^+ against extraction by ammonium acetate, decreased with increase in the particle size. But there was considerable fixation in the fine and medium silt fraction.

(b) Influence of potassium ion.

It is generally believed that NH_4^+ and K^+ are fixed by the same mechanism in the soil. The similarity in their size and electronic configuration is, perhaps, a possible reason for this. Much of the knowledge gained on NH_4^+ fixation was derived from the study of K^+ fixation. Because of this similarity, there is a competitive and depressive effect of one ion on the fixation of the other. The influence of K^+ on NH_4^+ fixation varies depending on the history of the soil how and when K^+ is added to the soil, and also the soil mineralogy.

The addition of K^+ prior to NH_4^+ will depress fixation of NH_4^+ by the soil. Stanford and Pierre (1947), reported that the magnitude of this depression is proportional to the amount of K^+ initially added. Simultaneous addition of K^+ and NH_4^+ gives variable results depending on the type of fixing mineral, the concentration level and the ratio of NH_4^+ to K^+ . For example, Joffe and Levine (1946), reported that when equivalent amounts of potassium and ammonium were simultaneously added to a H-saturated montmorillonite, more K^+ than NH_4^+ was fixed at lower concentration. The situation was reversed at higher concentration. Nommik (1957), found that a clay soil containing vermiculite, fixed 10-15% more ammonium than potassium. This was valid for a wide range of concentrations. The fixation of ammonium was increased by a small but significant amount, with simultaneous addition of K^+ and NH_4^+ . Nielsen (1972), noted that NH_4^+ and K^+ were fixed in similar proportion when added separately to the soil but potassium was fixed preferentially with simultaneous addition.

Addition of K^+ after NH_4^+ has no appreciable effect on the amount

of fixation. Perhaps, more important is the effect of K^+ added after ammonium on the release of fixed NH_4^+ . Hanway and Scott (1956), concluded from their study that the presence of a very small amount of K^+ interfered with the replacement of fixed NH_4^+ by NaOH distillation. This has been termed the blocking effect and may have a profound effect on the use of the Barshard double distillation technique of determining fixed ammonium. Legget and Moodie (1963) confirmed the above finding. They showed that 86-98% of fixed NH_4^+ was released during alkaline aeration or distillation. But with a K^+/Na^+ ratio of 0.005, only 14-35% was released. This blocking effect may also be important in the availability of fixed ammonium to plants and nitrifying microorganisms. As Axley and Legg (1960), pointed out, plant uptake of nitrogen from urea or ammonium sulphate is not greatly affected by the capacity of the soil to fix NH_4^+ unless sufficient K^+ was present to block its release. These authors suggested that a nitrate source should be used when nitrogen and potassium are to be applied simultaneously to soils capable of fixing ammonium.

(c) Soil pH and base saturation

The pH of the soil and the degree of base saturation affect the magnitude of fixation by the soil. Nommik (1957), reported that saturation of the soil with cations like Na^+ , Mg^{++} and Ca^{++} which leaves the lattice expanded did not influence fixation of NH_4^+ to any great extent. On the other hand, K^+ , Rb^+ , Ba^{++} and La^{+++} reduced NH_4^+ fixation considerably. Raju and Mukhopadhyay (1975), concluded that the influence of the initially adsorbed cations on NH_4^+ fixation under

the condition of ammonium preceding potassium and when added simultaneously was in the order of $Ba^{++} > Na^+ > Ca^{++}$. But when K^+ was added before NH_4^+ the order was $Ca^{++} > Na^+ > Ba^{++}$. These authors were not explicit, however, about the direction of the influence of cation on ammonium fixation.

Similarly, an increase in the H^+ ion concentration resulted in decrease in NH_4^+ fixation. The high relative replacing power of H^+ is presumably the important factor influencing the equilibrium between exchangeable and fixed ammonium. It has been shown that lowering the pH of the soil reduces fixation of K^+ and NH_4^+ and that fixation is insignificant or negligible in hydrogen saturated soils (Harada and Katsuna 1954). Barshad (1954b), suggested that the decrease in ammonium fixation in the presence of exchangeable H^+ may be due to the expanded state which the adsorbed H^+ leaves the crystal lattice, rendering interlattice NH_4^+ more accessible to a replacing cation. In acid soils, NH_4^+ fixation generally shows low values and this may possibly be explained by the presence of Al, Fe, and H ions in the interlayer positions. These ions are only difficultly replaceable by K^+ and NH_4^+ , (Stanford 1948). It is thus, probable that, liming acid soils could increase their ammonium fixing capacity.

(d) Concentration of ammonium

The amount of ammonium added to the soil affects the magnitude of fixation. In general, fixation increases with increase in concentration of applied ammonium. Percent fixation, however, decreases with increase in initial ammonium concentration. Doram and Evans (1983),

reported that the amount of NH_4^+ fixed increased with increasing concentration up to the highest rate of application (2000 $\mu\text{g g}^{-1}$ of soil). A result similar to this was obtained by Nommik (1957). A possible explanation of the concentration effect on fixation can be seen in the equilibrium relationship alluded to earlier. An increase in solution NH_4^+ will disturb the equilibrium already established. This causes a change in exchangeable ammonium concentration and subsequent change in the amount of ammonium at the fixation sites.

(e) Effect of temperature on ammonium fixation by clay

Rate of ammonium fixation is governed by the diffusion of ammonium ion from the site of application to the clay surface. Rate of NH_4^+ fixation is thus at maximum at the period immediately after ammonium addition and then slows down with time. Using data generated by Harada and Katsuna (1954), Nommik and Vahtras (1982), stated that from 60-90% of the total fixation may occur within the first few hours following ammonium application to the soil. Since diffusion is a temperature dependent phenomenon, an increase in the rate of fixation has been obtained within the temperature interval of 0-60 $^{\circ}\text{C}$ (Nommik 1957). Higher temperature has also been found to increase the magnitude of fixation considerably in minerals such as montmorillonite. Young and McNeal (1964), reported a 20-fold increase in the ammonium fixation by montmorillonite upon drying at 105 $^{\circ}\text{C}$. Two different reactions may be responsible for this (Nommik and Vahtras 1982). Drying removes water and causes an increase in NH_4^+ concentration which may then affect diffusion to clay surfaces. Removal of water also results in dehydra-

tion of the interlayer surfaces causing contraction of the clay surface and entrapment of ammonium ion.

(f) Effect of soil moisture content

The influence of soil moisture content on fixation depends on the nature of the fixing material. For example, there is no appreciable difference in ammonium fixation by vermiculite under wet and dry conditions. Montmorillonite on the other hand, does not fix appreciable amounts under wet conditions, this is because of the expanded state of the lattice when occupied by water. Walsh and Murdock (1959), investigated the effect of freezing soil on NH_4^+ fixation. They reported that on the average, freezing the soil after addition of ammonium resulted in twice as much fixation as that occurring under moist condition.

(3) Availability of clay-fixed ammonium to plants and microorganisms

That the soil is capable of tying up ammonium is generally accepted as a fact. Some controversies, however, still surround the agronomic significance of this phenomenon. Availability of fixed ammonium to plants and microorganisms is of agronomic importance. As such, several studies have been conducted on this subject, but their results are quite inconclusive. A survey of literature reveals a general consensus amongst earlier studies using nonlabelled fertilizer nitrogen - that fixed ammonium is only slightly available to plants and microorganisms (Bower 1950, Allison et al 1952, 1953). In general, considerably less than 20% of fixed NH_4^+ was oxidized and made available to plants in most of these studies. These results tend to over-

emphasize the importance of fixation in the nitrogen economy of the soil and they are almost diametrically opposed to those results obtained recently from greenhouse and field studies which employed ^{15}N -labelled nitrogen sources and reported figures between 30-70% for availability of fixed NH_4^+ (Kowalenco and Cameron 1976, 1978).

At this juncture, possible explanation for some of these discrepancies will not be out of place. Firstly, most of the earlier studies were conducted in the laboratory or greenhouse and direct extrapolation to field situations is often not possible. As Legg and Allison (1958) rightly pointed out, "their experimental procedure was best served by saturating soil samples with an ammonium salt solution to fill their fixing capacity, and then removing the excess ammonium with strong salt solutions which may markedly affect the release of the trapped ions. Such procedures permit the study of ammonium availability under maximum fixation conditions in the absence of readily exchangeable ammonium, but the results do not necessarily represent the availability of ordinary field applied NH_4^+ ". Secondly, availability of fixed ammonium is related to the release of NH_4^+ from the fixing sites and this does vary depending on several factors. Some of these are, type of fixing mineral, absence or presence of K ion, method of application of NH_4^+ and K^+ etc. The release of ammonium from fixing sites on vermiculite is not identical with that from montmorillonite. Since fixing minerals do vary from soil to soil, availability of fixed ammonium should also be expected to vary.

Allison et al (1953), found that only 7% of the applied NH_4^+ fixed was available to millet when fixation was by air drying, and 12% when

fixation was brought about by oven drying. Some years later, Legg and Allison (1958), reported that uptake of nitrogen applied to two NH_4^+ fixing subsoils by Sudan grass was similar to the uptake from nonfixing surface soils. The availability of applied ammonium fixed by semi-arid soil was determined by Bower (1951). He reported that when cultures were not inoculated by nitrifying bacteria, he obtained only 10% recovery by barley compared to 13-28% with an inoculated culture. This indicated that plants were unable to utilize fixed NH_4^+ in excess of that made available through nitrification. Considerably higher recovery of fixed NH_4^+ by plants was recorded in other greenhouse trials. Using a heavy soil containing vermiculite, Nommik (1957), found that the growth response of oats to $\text{NH}_4^+\text{-N}$ was only slightly lower than that of $\text{NO}_3^-\text{-N}$.

In a more recent study, Kowalenco and Cameron (1978), showed that about 34-60% of NH_4^+ -fertilizer applied at the rate of 150 Kg ha^{-1} as ammonium sulphate was fixed immediately upon application. About 71-96% of the fixed ammonium was available to barley over the growth period. Similarly, Kowalenco (1977), reported that clay fixation of $\text{NH}_4^+\text{-N}$ was significant in an Ottawa area clay-loam soil. Fifty nine percent of the fertilizer applied at the rate of 150 Kg ha^{-1} was immediately fixed. Over one-half (66%) of this fixed $\text{NH}_4^+\text{-N}$ was released within the first 86 days. It seems that with the use of ^{15}N -labelled nitrogen sources the precise fate of fixed ammonium can be followed and plant recovery of fixed NH_4^+ from these studies looks impressive.

Theoretical consideration of the equilibrium relationship proposed by Nommik (1957), suggests that fixed ammonium should ultimately be

available to plants and microorganisms. Plant uptake of ammonium should result in the depletion of both solution and exchangeable NH_4^+ and a disruption of the equilibrium already established. But as Nommik pointed out in 1965, not all fixed NH_4^+ appeared to participate in this equilibrium. With clay fixation, availability is generally high except in instances where K^+ interference is involved. As Walsh and Murdock (1963) pointed out, most of the differences in the availability of applied NH_4^+ fixed by clay minerals or soil can probably be traced to the varying levels of exchangeable K^+ . These workers reported that the highly significant correlation between exchangeable K^+ in most soils and the amount of NH_4^+ fixed (as measured by differential nitrogen uptake), showed that the level of exchangeable K^+ in the soil controlled the release of fixed ammonium to the crop. This is in harmony with the results of Axley and Legg (1960), who reported that plant uptake of nitrogen from ammonium sulphate or urea was not greatly affected by the capacity of the soil to fix NH_4^+ , unless sufficient K^+ was present to block its release.

C Fixation of NH_4^+ by soil organic matter

1. Mechanism of NH_4^+ fixation by organic matter

It is generally believed that fixation of NH_4^+ by soil organic matter is via the reaction of NH_3 with the organic fraction. Hence discussion is often limited to ammonia with the NH_4^+ ion being excluded. It is doubtful, however, if such dichotomy exists in the soil, where NH_4^+ fixation is via the mineral fraction and NH_3 through the organic fraction. It should be noted that a dynamic equilibrium exists

between NH_4^+ and NH_3 in the soil which is a function of concentration and pH. Both species are always present simultaneously in the soil system. Application of an NH_4^+ salt to the soil is bound to generate NH_3 , particularly from alkaline sources such as urea and anhydrous ammonia. In the following discussion of organic matter fixation, the term ammonia and ammonium will be used interchangeably.

The organic fraction of the soil is capable of fixing NH_3 which is released from NH_4^+ -compounds, added to the soil or used as a fertilizer, making it unexchangeable by the normal exchange process. The earlier literature on the subject of ammonia fixation by soil organic matter has been reviewed by Mortland (1958). Fixed ammonia is defined as that fraction of added NH_4^+ which is retained by the soil organic fraction after intensive extraction and leaching with acid solution (Broadbent et al, 1961; Burge and Broadbent 1964), or neutral salt solution (Nommik and Nilsson, 1963a). It has been observed that NH_3 fixed by organic matter is very stable, only released in mineral forms with difficulty, hence, slowly available to plants and microflora.

Though the actual mechanism is unknown, fixation is thought to be as a result of the reaction between NH_3 and organic matter. This leads to the incorporation of the NH_3 into the structure of the organic matter. Mattson and Anderson (1943), considered that a chemical reaction involving simultaneous oxidation and ammoniation was responsible for the increase in the stable organic nitrogen they found in peat and humus materials after aerating them in the presence of NH_4OH . Based on their work on humus materials and polyphenolic compounds, they theorized that fixation took place in the lignin fraction of soil

organic matter and requires the presence of aromatic rings possessing more than one hydroxyl group.

In support of this theory, Bennets (1949), reported that aerobic ammoniation of commercial lignin reduces its capacity to form methoxyl groups upon treatment with methyl sulphate. Conversely methylation reduced the amount of NH_3 that could be fixed. Burge and Broadbent (1961), found that progressive blocking of the hydroxyl groups with dimethyl-sulphate decreased the capacity of organic matter to fix ammonia, indicating the involvement of this group in fixation reaction. They contended that the presence of hydroquinone and quinone polymers in soil organic matter could account for the anaerobic fixation of ammonia and the increase in fixation in the presence of oxygen.

Flaig (1950), suggested a type of reaction which might account for ammonia fixation in soil organic matter. He considered that alkaline oxidation of hydroquinone or catechol produces a polymer composed of ether-linked monomers capable of reacting with NH_3 after further oxidation. The possible mechanism for oxidation and ammoniation of a hydroquinone polymer suggested by Flaig (1950) is shown below. According to this author, fixation of ammonia by aromatic compounds is dependent on extensive polymerization. On polymerization, the nitrogen is combined into bridging structures, analogous to those in phenoxazine dyes.

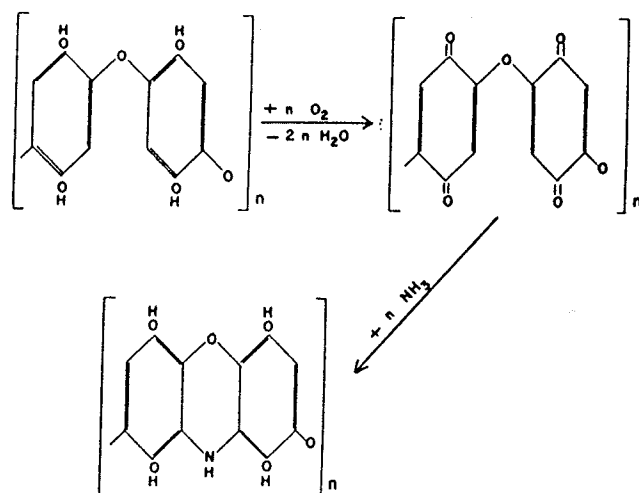


Figure 5—Possible mechanism for oxidation and ammoniation of a hydroquinone polymer as suggested by Flaig (8).

Mattson and Koutler-Anderson (1943), considered that ammonia fixation may occur under natural soil conditions and that most of the nitrogen in humus originates from the fixation reaction. The same opinion is maintained by Broadbent et al. (1961). They suggested that a substantial part of the non-protein organic nitrogen in soil is formed by the reaction between ammonia and lignin derivatives.

Sohn and Peech (1958), measured the ammonia fixing capacity of a number of soils when treated aerobically with anhydrous NH_3 . They concluded that at least half of the ammonia fixed must be attributed to fixation by the organic matter and that the fixation reaction was probably that referred to as oxidation and ammoniation by Mattson and Anderson (1943). They found that the highest amount of ammonia was fixed by acid soils containing high level of organic matter. Perhaps, the most notable result was that of Burge and Broadbent (1961). They

showed that ammonia fixation in organic soils of varying carbon content was linearly correlated with percent carbon. They pointed out that this is evidence of a fixation mechanism completely unrelated to that involving the entrapment of NH_4^+ within the crystal lattice. Ammonium fixation amounting to 161 mmol per 100g of organic matter was obtained in the presence of oxygen, and 106 mmol per 100g, where oxygen was excluded. Approximately 1 molecule of NH_3 was fixed for every 29 atoms of carbon under aerobic condition and 1 molecule of NH_3 for every 45 atoms of carbon under the anaerobic state. Similarly, Tomar (1982), working on immobilization of nitrogen in soil, obtained a linear relationship between the amount of nitrogen fixed and the humic acid content of the soil. A highly significant (at 1% probability level) correlation coefficient of 0.75 was obtained.

Nyborg (1968), working on fixation of gaseous NH_3 by soil, reported that the mineral soil fixed 2.5-8.5 mmol per 100g of soil compared to organic soil that fixed 39-190 mmol. He concluded that organic matter accounted for most of the fixation in mineral soil because little of the fixed NH_3 was extractable with Na^+ . His conclusion is in agreement with the result obtained by Obi (1982). He found that most of the nitrogen fixed after addition of urea to the soil was organically bound and the soil organic matter fixed about 5-10 times as much nitrogen per unit weight as clay.

Direct comparison of these studies, however, is quite difficult and may even be erroneous to do since different criteria were used to define fixation. Also, the method of distinguishing between minerally fixed and organically bound nitrogen is not uniform and sometimes even

questionable. Sounding a note of warning, Young (1964), said "it should be remembered that the level of NH_3 retention and fixation can be varied substantially by experimental conditions. Moreover, there were methodical weaknesses in this, (referring to his own study) and studies by Sohn and Peech (1958), for arriving at the precise values of organically fixed ammonia." Nevertheless, substantial evidence of organic matter involvement abounds in the literature and a considerable role can be played by the organic fraction in nitrogen retention.

2. Factors influencing ammonia-organic matter reaction

(a) Concentration effect

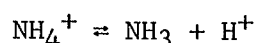
Factors that influence the fixation of ammonia by soil organic matter include, concentration of ammonia, pH of the reacting medium, presence or absence of oxygen and temperature (Broadbent et al 1960). Just as in the case of clay fixation, the amount of NH_4^+ fixed by organic matter increases with the concentration but the proportion of added ammonium fixed decreases as concentration increases (Broadbent et al 1960). A similar result was reported by Nommik (1970), using ^{15}N -labelled NH_4Cl adjusted to pH 11.3 by the addition of CaO .

(b) pH effect

Perhaps the most important factor affecting the reaction between organic matter and ammonia is the pH of the reacting medium. Reaction between NH_3 and organic matter increases with increase in soil pH. Using $\text{Ca}(\text{OH})_2$ to adjust the soil pH, Broadbent et al (1960), obtained an almost linear relationship between pH and the amount of NH_4^+ fixed.

It is probable that the profound pH effect upon ammonium fixation might have generated the consensus that ammonia rather than the ammonium ion is involved in the reaction with soil organic matter. Since the former is the dominant specie at high pH.

The effect of increasing pH on fixation is often attributed to the increase in the polarized and reactive -COOH and -C=O groups. Also, pH affects the ratio $\text{NH}_4^+/\text{NH}_3$ in equilibrium. At higher pH most of the nitrogen is present as ammonia. This is portrayed by the equilibrium relationship shown below.



$$[\text{NH}_3] [\text{H}^+]/[\text{NH}_4^+] = 10^{-9.5}$$

Thus, at pH 9.5 the ratio of $\text{NH}_4^+/\text{NH}_3$ is unity and NH_3 concentration increases rapidly above this pH. Optimum pH for fixation has been shown to occur at pH 10-11 (Nommik 1970). He found that fixation of ammonium and glycine was exceedingly low in H^+ -saturated raw humus (pH 3.3-3.4), but the magnitude of fixation was rapidly increased by increasing the pH during aerobic incubation. Considerable deamination of nitrogen compounds at pH 12 and above was reported to occur (Nommik and Nilson 1963), causing the net fixation as measured by the difference method to be lower at these pHs.

Some other results, however, do not lend full support to this profound pH effect. Nommik and Nilson (1963), using a high concentration of NH_4^+ , 14 and 140 mmol/100g and pH variation from 6-12, reported that fixation showed a weak tendency to increase with

increasing NaOH addition.

In fact, some observations and results contradict the earlier position. Sohn and Peech (1958), reported that the highest amount of ammonia fixation was obtained in acid soils containing large amounts of organic matter. Nyborg (1968), observed that fixation of ammonia tended to increase with decreasing soil pH and increasing clay content. Obi (1982), asserted that NH_4^+ fixation, following the application of urea, increases with decreasing pH and that organic matter was responsible for most of the observed fixation in the soil used. Even in the results of Broadbent et al (1960), where a profound pH effect was demonstrated, it was noticed that a small but significant fixation occurred at pH 6 and below.

A safe conclusion from all these varied observations is that perhaps it is not the absolute soil pH that is a factor of import, but the direction and magnitude of the pH change of the medium, resulting from the imposed treatment. Another important factor that can probably have a modifying effect on pH is the oxidation state of the soil organic matter and how this is affected by the pH change.

(c) Effect of oxygen

Mattson and Anderson (1942), suggested that ammonia fixation by organic matter involves a simultaneous oxidation reaction indicated by oxygen uptake. The functional groups formed during auto-oxidation are responsible for the capacity to fix ammonia. Thus, a positive correlation may be expected to occur between the magnitude of oxygen consumed and the extent of ammonia fixation. These authors observed that a

large quantity of oxygen was absorbed when samples were treated with NH_4OH and that preoxidation of the organic matter preparations reduced their capacity to bind NH_3 . This view is consistent with the observation that ammonia fixation increased with increasing pH, since spontaneous oxidation of organic matter in contact with the air occurs under alkaline conditions.

Broadbent et al (1960), found no effect of varying partial pressure of oxygen on NH_3 fixation by three organic matter preparations. These authors concluded that the reactive groupings capable of ammonia fixation, possibly quinone produced by oxidation of polyphenols, apparently, are present in soils in sufficient quantities to permit the reaction to occur. On the other hand, Nommik and Nilson (1963), demonstrated a definite relationship between the volume of oxygen consumed and the amount of NH_3 fixed, in which the higher the oxygen consumed, the more ammonia was generally fixed. A similar result was obtained by Nommik (1970), where, in the nitrogen gas atmosphere, the fixation figures were 40-50% lower than for corresponding treatments in an air atmosphere.

That the presence of oxygen was not always essential to fixation was demonstrated by the data of Burge and Broadbent (1961). They reported that the ammonia fixing capacity of the organic fraction as 161 mmol/100g in the presence of oxygen and 106 mmol/100g when oxygen was excluded. The influence of oxygen on fixation depends, however, on the oxidation status of the organic matter. A varied results can thus be obtained depending on the soil type.

(d) Temperature effect

Feustel and Byers (1933), demonstrated that heating peat with NH_4OH increased the nitrogen content of the peat very substantially. In their work, they treated the peat with ammonium hydroxide solution in a sealed container at elevated temperature and pressure. Nommik (1970) found that at room temperature, 1.7 percent of the added glycine nitrogen was fixed by raw humus during a reaction period of 24 hours. The corresponding figure at 100°C was 14.1 percent.

Work done by Broadbent et al (1960) at atmospheric pressure but varying temperature also indicated that ammonia fixation was increased by increasing the temperature. These authors also investigated the thermal stability of ammoniated organic matter samples. This is rather interesting in the light of the relatively unstable nature of ammonium compounds at elevated temperatures. Surprisingly, at all temperatures up to 400°C , the ammoniated samples retained more organic nitrogen than those not ammoniated. It is also instructive to note that very little of the exchangeable ammonium survived heating at temperature above 200°C . This thermal stability may have implication on the availability fixed ammonium to plants.

3. Availability of organically fixed nitrogen to plants and micro-organisms

With respect to organic matter, considerable evidence has accumulated showing its ability to react with NH_3 and bind it in a form resistant to chemical hydrolysis or microbial attack. However, it is not clearly understood if these reactions occur in the field, and their

extent is still a subject of speculation. Theoretically, the reaction is most likely to occur on calcareous soils having a high organic matter content. The reaction will be influenced by the nature of the organic fraction, the soil moisture content, and the nitrification capacity of the soil. Also, with the increased use of aqueous ammonia and banded urea, local pH may be high enough in the field to have substantial amounts of organically bound NH_3 .

An insight into the availability of fixed ammonium can be gained by considering the nature of the reaction product. According to a great majority of observations, NH_3 fixed by soil organic matter is exceedingly resistant to both acid and alkaline treatments. Thermal stability has also, been previously mentioned.

Broadbent and Thenabadu (1967), found that some of the NH_3 fixed against extraction with $0.1 \text{ mol L}^{-1} \text{ HCl}$ or acidified NaCl was resistant to refluxing for 16 hours with 6N HCl . A similar result was reported by Nommik (1970). The ammonia-organic matter complexes synthesized in the laboratory have been found to be rather stable compounds, being only slowly mineralized by soil microflora (Feustel and Byers 1933, Bremner and Shaw 1957). Thus, it seems likely that the capacity of organic matter to fix ammonia may be a factor of agronomic significance, as it probably influences the efficiency of the $\text{NH}_3/\text{NH}_4^+$ fertilizers. The resistant nature so reported, if it has any effect on plant availability of organically bound ammonium, may also partly explain the low residual effect of nitrogen fertilizer often encountered in the field.

The chemical resistance of NH_3 -organic matter complexes indicates

a low availability of fixed ammonium to soil heterotrophic microflora and to higher plants. Unfortunately, only limited research has been carried out on this subject. Burge and Broadbent (1961), in a pot experiment showed that only 4.3% of the fixed tagged NH_4^+ was recovered in the first cutting of Sudan grass. In the second cutting, only 1.3% of the remaining tagged N was taken up by the plant, suggesting, perhaps, that a conversion to a more resistant compound gradually occurred. It should be noted that NH_4^+ fixation cannot be considered an entirely unfavourable reaction from an agronomic point of view. Under certain soil and climatic conditions, fixation may be a positive factor in preventing losses through leaching, denitrification, volatilization etc. and ensure a more even nitrogen supply throughout the growing season. Furthermore, fixation may even be the mechanism through which the residual nitrogen effect can be exploited.

D Interaction between organic matter and clay in NH_4^+ fixation

Both the mineral and the organic fractions of the soil have the capacity to retain the NH_4^+ ion in a manner not exchangeable by the usual methods of cation exchange (Sohn and Peech 1958, Nyborg 1968, Nommik and Nilsson 1963). But there is no consensus of opinion in the literature with regards to the actual role played by these fractions and the interaction between them.

Hinman (1974) reported that the destruction of organic matter prior to ammonium fixation increased the amount of NH_4^+ fixed. This increase was greater as the organic matter content was higher. He attributed this to the role of organic matter in preventing ammonium

fixation by clay through (a) blocking the entry of NH_4^+ into the fixation sites or, (b) by preventing the collapse of the basal spacing. In agreement with this, was the observation of Porter and Stewart (1962), that the pretreatment of subsoil or Vermiculite with organic compounds interfered with fixation when NH_4Cl was added. On the other hand, Sohn and Peech (1958), showed that treatment of soil with hydrogen peroxide to remove organic matter, markedly decreased the capacity of the soil to fix NH_3 into nonexchangeable form. While Young (1964), working on ammonia and ammonium fixation in some Pacific North Western soils, expressed the amount of NH_4^+ retained as a function of %clay and %carbon in a quadratic equation as follows:

$$\begin{aligned} \text{NH}_3 \text{ retention (ug g}^{-1}\text{)} = & 102 \times \%C + 139 \times \%Cl - 2.53 \times \%C \times \%Cl + \\ & 50.94 \times \%C^2 - 1.31 \times \%Cl^2 - 327. \end{aligned}$$

where Cl and C represent clay and carbon, respectively.

He stated that the organic and the mineral fraction apparently have little influence on each other with respect to NH_3 retention as their interaction term was not significant.

More than anything else, the discrepancies reported above confirmed the ability of the various fractions to fix NH_4^+ . And that the mechanism of fixation, perhaps, differs between the various soil fractions.

MATERIALS AND METHODS

Soil samples

The four surface soils used in this experiment were collected in the Spring of 1984 from 0-150mm depth of cultivated fields. The soils were air dried immediately after collection, ground to pass through 2 mm sieve and stored at room temperature. The soils were chosen to give some variations in the organic matter content. The soils used were Almasippi sand, Red River clay, Elm Creek loam and an organic soil and are designated as sand, clay, loam and peat, respectively. Some of the physical and chemical properties of these soils are shown in Table 1.

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

<u>Properties</u>	<u>Experimental Soils</u>			
Designation	Peat	Clay	Loam	Sand
Name	Organic	Red river	Elm creek	Almasippi
pH	6.6	6.8	6.8	6.9
O.Matter(%)	72.79	7.33	5.77	5.0
NH ₄ ⁺ -N ug g ⁻¹	40.81	30.58	22.72	23.61
NO ₃ ⁻ -N ug g ⁻¹	49.7	10.3	7.1	1.0
Total N(%)	2.01	0.34	0.26	0.16
Clay (%)		73	33	5
Silt (%)		24	23	6
Sand (%)		3	44	89

Experiment 1 - Ammonium fixation from urea and ammonium carbonate

A laboratory incubation study was carried out to determine the extent of ammonium fixation in soils with urea and ammonium carbonate. Five grams of soil (on air dry basis) was placed into a plastic container with lid (diameter of 85 mm). Nitrogen carrier was applied dropwise, in solution form, using an automatic pipet to give the desired NH_4^+ concentration (1000 ug of nitrogen per g of soil). This method of application was such that the solution was uniformly distributed throughout the soil making mixing unnecessary for the three mineral soils. For peat, however, the hydrophobic nature of this soil made thorough mixing of the soil and the applied solution necessary. The moisture content of the soils during incubation were maintained at field capacity, and temperature of 20°C. After a predetermined time interval (which varied from 1 hour to 1 week) the soil samples were extracted for exchangeable ammonium. Extraction involved shaking the sample with 50 mL of 1 mol L^{-1} KCl for 1 hour, and then filtering through Whatman No 42 filter paper. The filtrate was either analyzed immediately or stored in a freezer for subsequent determination of ammonium and urea.

Since nonlabelled nitrogen sources were used, the "difference method" was used to calculate the fixation values. This essentially, entailed determining the exchangeable ammonium of both the control and treated soils. Fixation was calculated as follows:

$$\text{Fixed } \text{NH}_4^+ = \text{Applied } \text{NH}_4^+ - \text{Exch. } \text{NH}_4^+ \text{ of treated soil} + \\ \text{Exch. } \text{NH}_4^+ \text{ of control soil.}$$

The same method was used for soils treated with urea except that in addition to the exchangeable ammonium in the filtrate, urea concentration was also determined. Fixed NH_4^+ was calculated as follows:

$$\text{Fixed } \text{NH}_4^+ = \text{Conc. of applied urea} - \text{Urea-N in filtrate} - \text{Exch. } \text{NH}_4^+ \text{ of treated soil} + \text{Exch. } \text{NH}_4^+ \text{ of control soil}$$

There are, however, inherent limitations with this method. Firstly, there is the possibility of increased error as a result of the numerous measurements used in the calculation. Also, if fixation is small, the various measurements might not be sensitive enough to pick it up. Limitation of this method becomes apparent when a comparison is made between the results of this experiment and those obtained with the use of ^{15}N isotope.

Experiment 2 - Fixation of NH_4^+ in HF-treated soils

To eliminate the interference of clay minerals in NH_4^+ fixation by the organic fraction, an attempt was made to destroy the clay fraction through the use of hydrofluoric acid. Several workers have successfully used HF acid treatment to remove the clay fraction while causing minimal destruction to the organic matter (Rather 1914, Alexander and Bayer 1934, Burge and Broadbent 1961). Treatment involved the addition of a solution containing $5 \text{ mol L}^{-1} \text{ HF} : 1 \text{ mol L}^{-1} \text{ HCl}$ to the bulk soil, (e.g. 300 g), with a soil to solution ratio of 1:10 in a 4 L polyethylene container. The soil was allowed to stand for 24 hours with occasional stirring. After decanting the supernatant solution, the

remaining soil was washed several times with distilled water and oven dried at low heat.

Both urea and ammonium carbonate were used with five grams of acid-treated soil in a plastic container for the fixation studies. Other procedures were as enumerated for untreated soil. Table 2 shows some of the properties of acid-treated soils.

Table 2. Chemical and physical properties of HF-HCl treated soils.

Properties	Experimental Soils			
Designation	Peat	Clay	Loam	Sand
Name	Organic	Red River	Elm Creek	Almasippi
pH	3.56	3.56	3.56	3.56
O.Matter (%)	72.07	29.74	9.35	5.18
NH ₄ ⁺ -N μgg^{-1}	174.60	32.80	26.70	16.90
Clay (%)		35.30	4.70	3.20
Silt (%)		37.70	16.20	4.30
Sand (%)		27.00	79.10	92.50

Experiment 3 - Ammonium fixation with ¹⁵N-labelled nitrogen sources

This experiment was similar to the initial experiment described above, except that the urea and ammonium sulphate used were labelled with 98.6% and 95.5% ¹⁵N isotope, respectively. The use of ¹⁵N allowed a direct and precise measurement of applied NH₄⁺ remaining in the soil by mass spectrometry, after prolonged extraction of exchangeable NH₄⁺

with 1 mol L⁻¹ KCl. Four replicates of each soil sample were used for this experiment, with 2 replicates for total nitrogen and the remaining 2 for the organic nitrogen determination.

Extraction of the exchangeable NH₄⁺ was carried out by shaking the soil with 50mL of 1 mol L⁻¹ KCl for 1 hour. Filtration was carried out under suction and the soil on the filter paper was leached with 75mL KCl in three portions of 25mL each. The extractant used for soils treated with urea contained phenyl mercuric acetate (pma) at a concentration of 50 ug g⁻¹ to inhibit urease activity in the filtrate. Urea and ammonium concentrations in the filtrate were also determined and a comparison between the direct ¹⁵N and the "difference" methods of fixed ammonium measurement made. The soil in the filter was quantitatively transferred into plastic container for both total and organic nitrogen determination.

Experiment 4 - Fixation of ammonium in sterilized soils

It was noted from the results of experiment 3 that more nitrogen was retained in the soil from urea than from ammonium sulphate. This suggested that urea per se might be directly involved in fixation reactions. Experiment was thus carried out to investigate the possibility of a direct urea involvement in the interaction with soil organic matter. Soils were sterilized to arrest microbial and urease activities, leaving the urea unhydrolyzed in the soil.

Two sterilization techniques were employed, the second, only when the first gave an inconclusive result. The first technique was heat sterilization of soils in an autoclave for 24 hours at a temperature of

121°C and a pressure of 10.35×10^4 Pa. Nonlabelled urea was used and other procedures were similar to those previously described. The second sterilization technique involved gamma radiation at a dose of 2×10^4 Gy. With gamma radiation, two levels of sterilization were used corresponding to 6 and 12×10^4 Gy. A sterility test was performed on the irradiated soil by streaking on a nutrient agar broth and incubating at room temperature for 1 week. Labelled urea was used, and all sample preparation took place in a sterile laminar flow chamber, model V6MW97T, to prevent contamination of the sterilized sample.

Experiment 5 - Isotopic exchange of fixed ammonium

The results of previous studies showed a substantial level of ammonium fixation. Thus, it became desirable to determine what fraction of this so called fixed ammonium was labile or isotopically exchangeable. Unsterilized soils were used with ^{15}N labelled urea-N in concentration of 1000 ug g^{-1} .

This experiment had a factorial design with three factors, each at four different levels (ie. a 4^3 factorial). The three factors were: soil; amount of fixed ammonium; and periods of secondary incubation. The four soils constituted the levels of soil. Four different levels of fixed ammonium were obtained by varying the length of time that ^{15}N -urea was incubated with the soils (ie. 1 day, 2 days, 4 days and 7 days), these were termed primary incubation periods. Soils containing varying levels of fixed ^{15}N were in turn incubated with ^{14}N ammonium sulphate in concentration of 1000 ug g^{-1} for 1 hr, 6 hr, 12 hr and 24 hr, and these were termed the secondary incubation periods.

Extraction of these samples were carried out as previously described. After extraction, the soil residues containing fixed labelled NH_4^+ were quantitatively transferred into plastic containers and 25 mL of unlabelled ammonium sulphate solution was added to give a nitrogen concentration of 1000 ug g^{-1} which was equal to the concentration of the labelled urea-N used for the primary incubation. These newly treated soils were allowed to equilibrate for 1, 6, 12 and 24 hr. The samples were then shaken for 1 hr (the 1 hr shaking period formed part of the secondary incubation time). Just before filtration, 25mL of 1 mol L^{-1} KCl was added. This was to precipitate dissolved organic matter and allow filtration to proceed normally. The soil in the filter paper was then washed with an additional 25mL of KCl to ensure complete extraction of added $^{14}\text{N-NH}_4^+$. The ammonium content of the filtrate was determined by distillation while its ^{15}N enrichment was measured with the aid of a mass spectrometer. These values were then used to calculate isotopically exchangeable ammonium.

ANALYTICAL PROCEDURES.

(1) Soil pH : pH was measured with a digital pH meter equipped with glass and calomel electrode (Fisher Accumet pH-meter model 620). A soil to water ratio of 1:2 was used for the mineral soils while that of the organic soil was 1:7. The soil suspensions were allowed to equilibrate for thirty minutes before measurements were taken.

(2) Electrical conductivity : The electrical conductivity was measured on the samples prepared for the measurement of pH using a conductivity

meter (Basch-Simpson Ltd. type CDM 2e No. 223159pTd).

(3) Moisture content at field capacity : Soil, ground to pass through a 2mm sieve was placed in a one-liter graduated polyethylene cylinder. Sufficient water was added to wet the upper half of the soil column. The column was covered with a plastic film, fastened with a rubber band, and allowed to equilibrate for 48 hours. The wet soil was then subsampled and the moisture content determined by oven drying for 24 hours at a temperature of 105°C.

(4) Texture : Particle size analysis was performed on all samples by the standard pipet method after the required pretreatment of the soil with H_2O_2 to remove soil organic matter.

(5) Soil organic matter : Soil organic matter was determined by the Walkley-Black method (1934). Potassium dichromate was used for the oxidation of carbon and the excess potassium dichromate was back-titrated with 0.5 mol L^{-1} ferrous sulphate using an automatic titrator.

(6) Ammonium nitrogen : Ammonium nitrogen was determined on the soil extract using a Fisher model 801A digital pH/mV meter equipped with a specific ammonium ion electrode. Because of the high ionic concentration of the extract relative to the reference solution in the electrode, the soil extract was diluted 10 times (to 0.1 mol L^{-1} KCl), to improve the accuracy of the measurements. One-half ml of 10 mol L^{-1} NaOH was added to 50 ml of diluted solution and thoroughly mixed with a

magnetic stirrer. The electrode was inserted into the solution, allowed to equilibrate for 30 or more seconds and a reading taken. Readings for the unknown solution were compared to those of known concentrations and ammonium concentration of the solution calculated.

(7) Nitrate nitrogen : The phenol disulfonic acid method was used for determining the concentration of nitrate in the soil. The soil was extracted with copper sulphate, and the concentration of the nitrate measured colorimetrically with a spectrophotometer set at a wavelength of 415nm.

(8) Urea nitrogen : Urea remaining in the soil was extracted with 1 mol L⁻¹ KCl solution containing phenyl mercuric acetate (pma). The pma was added to arrest urea hydrolysis pending the analysis of the filtrate. Urea in the extracted solution was determined colorimetrically, employing the method of Douglas and Bremner (1970).

(9) Total nitrogen : Total nitrogen of the soil was determined by Kjeldahl digestion, modified to include clay fixed NH_4^+ according to the method of Stewart and Porter (1962). The soil residue was quantitatively transferred into plastic container, 4mL of 50% HF and 1mL of concentrated sulphuric acid was added. This was left overnight to destroy the clay fraction.

This soil was then transferred into a Kjeldahl flask and digested at full heat for 1 hour after the addition of two Kelpak (K_2SO_4) tablets and 30mL of concentrated sulphuric acid. The digested soil was

distilled with 50mL of 50% NaOH and the distillate collected into 50mL of 0.05 mol L⁻¹ H₂SO₄ (100mL for peat soil), containing mixed indicator. The excess acid was back-titrated with 0.1 mol L⁻¹ of NaOH and the nitrogen content of the soil was then calculated.

(10) Organic nitrogen : Two replicates of the soil residue obtained after the extraction of exchangeable NH₄⁺ with KCl, were quantitatively transferred into Kjeldahl flasks. Fifty mL of 1 mol L⁻¹ H₂SO₄ was added, and the soil was digested at low heat with 30% H₂O₂ in small portions of 5mL for 24 hours according to the method of McKenzie and Wallace (1953). Here the H₂O₂ digestion was intended to oxidize the organic matter only without affecting the release of minerally fixed NH₄⁺. Subsequent procedures for nitrogen determination were similar to those described earlier for total nitrogen.

(11) ¹⁵N Analysis.

Titrated solution was acidified with one drop of concentrated H₂SO₄ and then oven-dried to 5mL. Ammonium in this concentrated solution was converted to nitrogen gas by treatment with sodium hypobromite (NaOBr) in a vacuum system. The nitrogen gas was analyzed for mass to charge ratio (m/e) of 28 and 29 and 30 using a micromass 602 mass spectrometer (V.G.Micromass Ltd. Winsford, England) as described by Cho and Sakdinan (1978). Determination of ¹⁵N abundance was carried out on the concentrated distillate obtained from both the total nitrogen and the organic nitrogen measurements. ¹⁵N abundance was expressed as atom percent ¹⁵N excess, calculated by using the following

equation.

$$\text{Atom } ^{15}\text{N Excess} = 100/(2R+1) - B.$$

where R is the ratio of the peak heights of m/e 28 and 29. The value B represents atom percent ^{15}N in the atmosphere i.e. the background which was determined for each set of measurements.

Simple proportion was used to obtain fixed ammonium, after determining total nitrogen of the soil residue, and its percent ^{15}N excess. For example, if the applied source of nitrogen contained 95.5% ^{15}N , and A (mg) was the amount of total nitrogen in the experimental soil after extraction, which had B% ^{15}N excess, then the amount of ammonium fixed, NH_{4f} (mg) by the soil is given by:

$$\text{NH}_{4f} = (A \times B) / 95.6 .$$

RESULTS AND DISCUSSION.

Experiment 1 : Ammonium Fixation From Urea And Ammonium Carbonate

The first experiment was a preliminary experiment, carried out to investigate the extent of ammonium fixation in the set of soil samples collected. It was important to first of all determine the magnitude of NH_4^+ fixation in the available soils before further work could be done. The information collected, formed the basis for further investigations.

Two ammonium sources were used: urea; and ammonium carbonate. These were applied to the soil in solution at a concentration equivalent to 1000 ug of nitrogen per g of soil. A very high concentration was used to simulate nitrogen concentrations which occurred in or near fertilizer bands and to allow for a measurable level of fixation to occur. An important consideration for the use of these nitrogen carriers in this initial experiment was their alkaline reaction in the soil. Previous research indicated that fixation of ammonium by organic matter occurred to a reasonable extent in the alkaline range (Nommik 1970, Broadbent et al 1960). Using urea and ammonium carbonate at high concentrations provided an optimum condition for the fixation reaction to occur.

Samples treated with nitrogen were incubated for periods of time ranging from 1 hour to 1 week to provide information on the kinetics of the reaction. Since steps to inhibit microbial activity in the soil samples were not taken, relatively short incubation period was adopted to eliminate as much as possible the influence of microbial immobilization. The amounts of nitrogen fixed were calculated by the "difference

method" and are shown in Table 5, and Figure 1.

Urea hydrolysis

The concentration of urea remaining in the soil was determined on the extract using the method of Douglas and Bremner (1970). This is a very sensitive analytical procedure capable of determining urea concentration in the range of 0-2 $\mu\text{g g}^{-1}$. However, because of the suitability of this method in the low concentration range, soil extracts containing high levels of urea were diluted to give a concentration level falling within this range. The process of dilution was a potential source of error. The error becomes magnified the higher the dilution factor. This may explain the variability observed in some of the data on urea. With some care, however, this method gave accurate and quite satisfactory results.

The hydrolysis of urea, as measured by the concentration of urea remaining in the soil, was quite rapid in all soils. Rate of urea hydrolysis generally increased with the amount of organic matter in the soil. This was expected since urea hydrolysis involves an enzymatic reaction. The level of urease enzyme in the soil should be related to microbial population and activities both of which are a function of the soil organic matter content.

Peat, containing 72% organic matter, had the most rapid rate of urea hydrolysis. Over 50% of applied urea was converted into the ammonium ion form during 6 hr of incubation and the hydrolysis of the 1000 $\mu\text{g g}^{-1}$ of urea-nitrogen was almost complete after 24 hr of incubation.

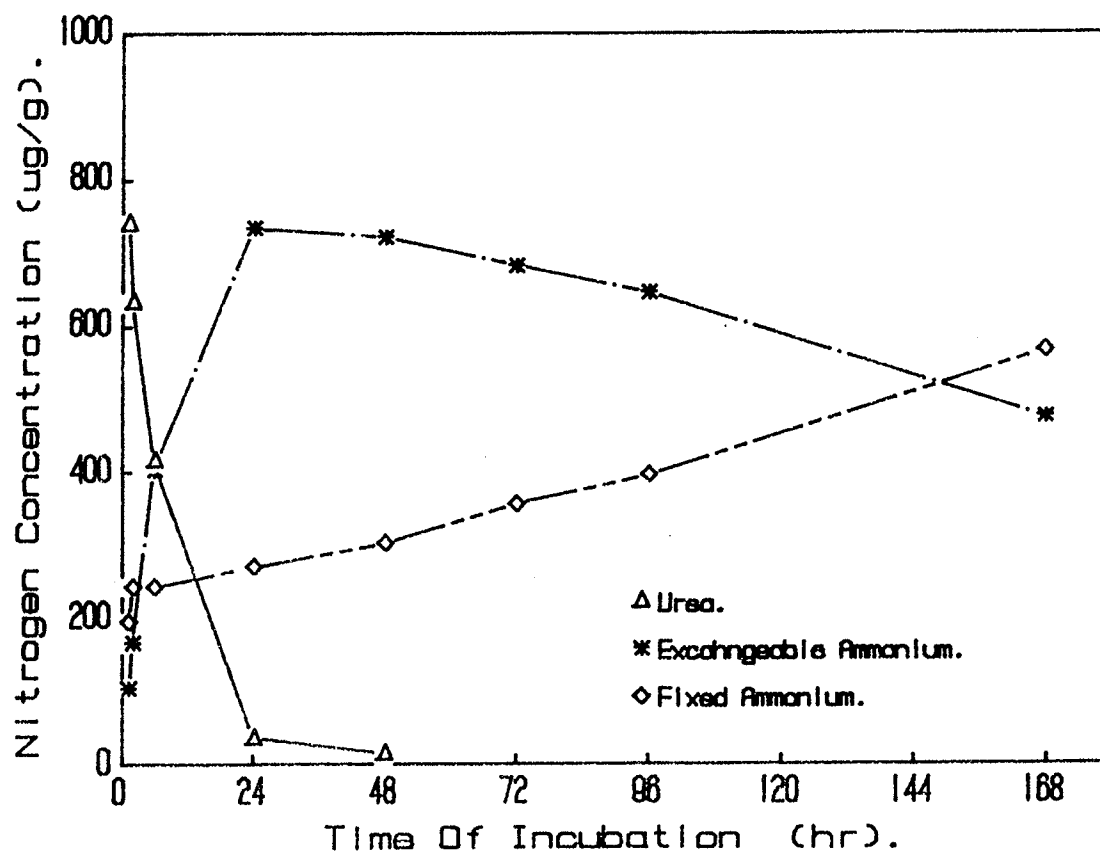


Fig. 1. Levels of Urea, Ammonium and Fixed Ammonium in Peat Soil.

Table 3 - Concentration of urea-N ($\mu\text{g g}^{-1}$) in the soils as a function of time.

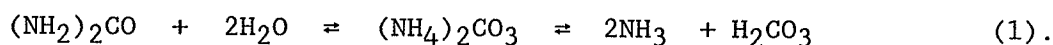
Sample	Incubation Time (hr)							
	1	2	6	24	48	72	96	168
Peat	740	632	414	37	16	0	0	0
Clay	918	821	744	413	28	0	0	0
Loam	967	941	802	661	342	60	0	0
Sand	95	926	903	688	335	37	0	0

This rapid rate of urea hydrolysis in peat may have significant implication on the timing of fertilizer application to peat soil, especially when urea is the nitrogen carrier. Application should be made to coincide with the period of maximum demand and uptake by the crop. Considerable loss could occur if there was a long time span between the time of urea application and that of plant uptake. This will be via the interaction between the product of urea hydrolysis and the soil in the form of ammonium fixation.

Clay also showed a high rate of urea hydrolysis. The concentration of urea-N declined from $1000 \mu\text{g g}^{-1}$ (the amount applied) to $413 \mu\text{g g}^{-1}$, 24 hr after the initiation of incubation. Virtually all the urea applied to the clay soil was hydrolysed to ammonium 48 hr after application. In the loam and sand soils, measurable levels of urea (60 and $37 \mu\text{g g}^{-1}$, respectively), were still present in the soil 3 days after the initiation of incubation. Complete urea hydrolysis was achieved in these soils only when incubation was extended to the fourth day.

In sand, there was a considerable time lag before an appreciable level of urea hydrolysis occurred. Only about 100 ug g^{-1} of urea-N was hydrolyzed by sand, compared to over 500 ug g^{-1} hydrolyzed in peat after 6 hr of incubation. This initial lag, could be due to a low level of urease enzyme in this soil. Thus, some time was needed (more than 6 hr in this case), for the build up of this enzyme to occur. After an initial lag period, however, the rate of hydrolysis in sand and loam became about equal.

Using the data presented in Table 3, an attempt was made to describe the kinetics of urea hydrolysis in the four soils. The data show that hydrolysis of urea could be described by first order reaction kinetics, with the rate of hydrolysis being a linear function of the concentration of urea remaining in the soil. The above conclusion was arrived at as follows: The overall reaction for urea hydrolysis can be written as



The rate of disappearance of urea can be described by

$$\text{Rate} \propto [(\text{NH}_2)_2\text{CO}][\text{H}_2\text{O}] \quad (2).$$

Equation 2 can be rewritten as

$$\text{Rate} = K [(\text{NH}_2)_2\text{CO}][\text{H}_2\text{O}] \quad (3).$$

where K is the rate constant of the reaction. The concentration of water in equations (2) and (3) can be assumed to be constant during entire period of the hydrolysis. Substituting K' for $K[\text{H}_2\text{O}]$, equation (3) becomes

$$\text{Rate} = K' [(\text{NH}_2)_2\text{CO}] \quad (4).$$

This relationship can be described mathematically as follows.

$$dc/dt = -K'[c] \quad (5).$$

Here c is the concentration of urea extracted from the soil at time t .
Solution of equation (5) gives

$$\ln c = -K't + \ln c_0 \quad (6).$$

where c_0 is the initial urea concentration at time $t=0$.

A plot of $\ln c$ against time (t), should give a straight line with K' being the slope and $\ln c_0$ the intercept. This plot is shown for the four soils in Figure 2. Shown, also, are the regression equations in which the values of K' represent the rate of urea hydrolysis. The values of K' obtained for peat, clay, loam and sand were 0.52, 0.15, 0.04 and 0.04 hr^{-1} , respectively.

Strictly speaking, however, the hydrolysis of urea is considered to be a pseudo-first order reaction. This is because the first order kinetics was obtained by assuming that the concentration of water does not change appreciably during the course of hydrolysis.

Similar reaction kinetics have been proposed by other workers. For example, Chin and Kroontje (1963) showed that the chemical hydrolysis of urea was in accordance with the first order rate law. But the reaction rate of chemical hydrolysis of urea was low compared to biochemical hydrolysis. It should be noted however, that the order of reaction may vary simply by varying the experimental method employed. Thus, Vlek and Carter (1983), reported that when urea was uniformly distributed throughout the soil, hydrolysis was adequately described by zero-order equations but application of prilled urea creates a heterogeneous system in which urea hydrolysis was described by a first order kinetics.

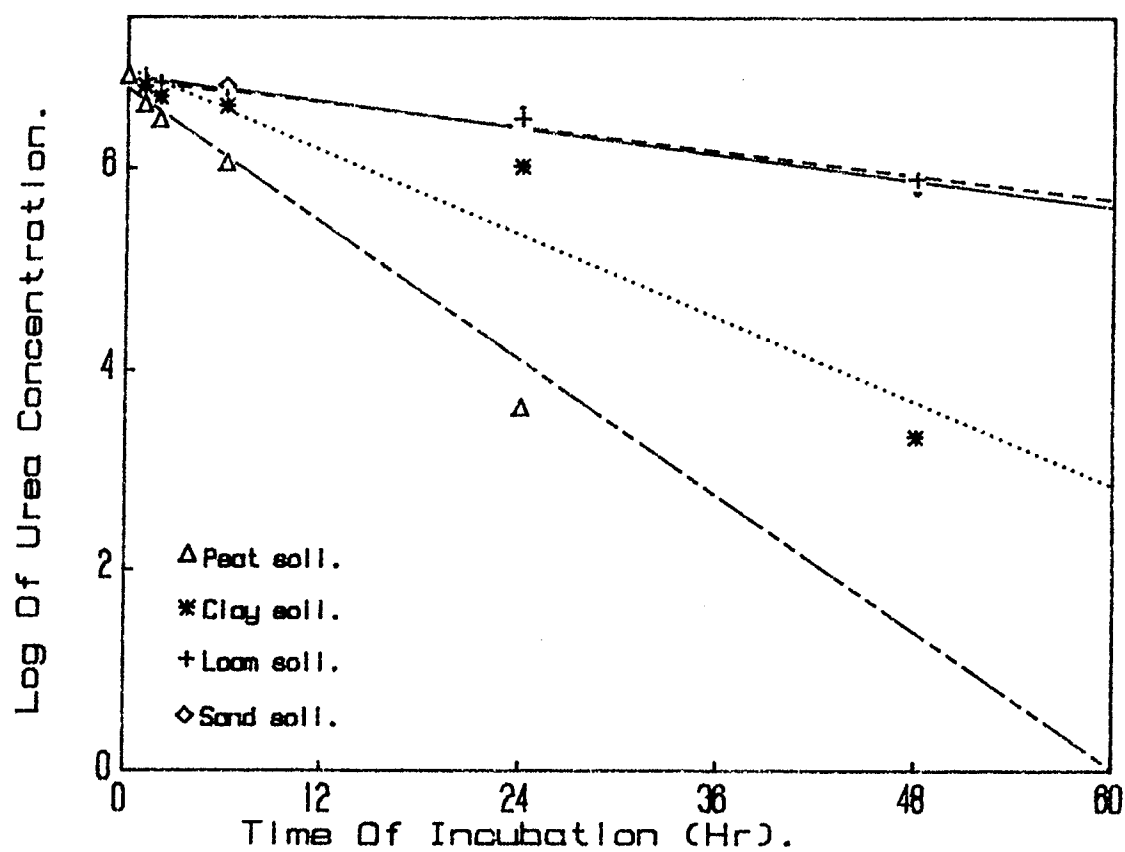


Fig. 2. The Kinetic Model of Urea Hydrolysis in Four Manitoba Soils.

Also, the hydrolysis of urea was profoundly affected by soil and environmental conditions. Temperature, moisture and pH were shown by the above named authors to control the rate of hydrolysis considerably. This may limit the extent to which results on urea hydrolysis can be compared, and the extrapolation of laboratory incubation results to field situations.

Ammonium concentration

Since soil extraction was carried out with 1 mol L⁻¹ KCl solution, the ionic concentration of this solution is higher than that of the standard ammonium solutions. For the standard solutions to be of any use in the preparation of a calibration curve they must be of the same ionic strength as the sample ie. 0.1 mol with respect to each ionic specie. To overcome the problem of high ionic strength of the sample, the extracted solution was diluted 10 times. This greatly improved the accuracy of the NH₄⁺ measurement by the electrode method. The solution on which NH₄⁺ determination was to be made also contained unhydrolyzed urea. It was suspected that addition of 10 mol L⁻¹ NaOH necessary to convert NH₄⁺ to NH₃ (in order to measure ammonium by electrode method), may also hydrolyze some urea in solution, thereby increasing the concentration of ammonium above that originally extracted. A test was therefore performed to see if a measurable level of ammonium could be detected from pure urea solution by the ammonium electrode. The ammonium concentration determined from urea solution was not different from that from pure water indicating that the hydrolysis of urea did not occur during ammonium determination.

In all four soils, the level of NH_4^+ in solution increased concomitant with the hydrolysis of urea (Tables 3 and 4). Ammonium concentration increased from levels slightly above that of the native exchangeable ammonium, just immediately after incubation to a concentration commensurate to that of the applied urea during one week of incubation. In peat, for example, ammonium concentration after 1 hr of incubation was 105.4 ug g^{-1} , slightly higher than the exchangeable ammonium content in the native peat. This increased to 732.8 ug g^{-1} after 24 hr of incubation, and after 1 wk the concentration declined to 474.0 ug g^{-1} . This pattern of ammonium buildup and decline with time, was evident in all soils. Rate of buildup of ammonium approximated the rate of disappearance of urea from the soil. Ammonium concentrations attained its maximum value at about the time that all the urea was hydrolyzed. After the maximum, NH_4^+ concentration started to decrease in all the soils. This decline was partly due to further ammonium fixation after the disappearance of urea or to nitrification of NH_4^+ . The time required for the ammonium concentration to reach its maximum level, varied amongst the four soils. The soil with the highest rate of urea hydrolysis reached the maximum point earliest.

Not only did the duration of time required to attain maximum ammonium concentration vary from soil to soil, the absolute value of this maximum was not the same for all the soils. The values of maximum ammonium concentration were inversely related to the organic matter content of the soils or their potential to fix NH_4^+ . For peat, the maximum value of 732.85 ug g^{-1} was attained after 24 hr of incubation.

Table 4 - Ammonium concentration ($\mu\text{g g}^{-1}$) in the experimental soils incubated with urea ($1000 \mu\text{g g}^{-1}$) as a function of time.

Sample	Incubation Time (hr)							
	1	2	6	24	48	72	96	168
Peat	105.4	166.7	402.7	732.8	722.2	685.2	646.4	474.0
Clay	31.0	46.2	129.8	448.4	641.2	799.4	750.9	617.7
Loam	20.2	28.1	81.1	306.2	542.8	776.9	786.3	698.0
Sand	20.2	32.2	88.1	282.2	533.4	752.6	811.5	684.0

In clay maximum value of $799.4 \mu\text{g g}^{-1}$ was attained after 3 days of incubation while loam and sand had their maximum ammonium concentration 4 days after the application of urea, and the values were 786.2 and $811.5 \mu\text{g g}^{-1}$, respectively.

Ammonium fixation in soil from urea

Using the difference method, the levels of ammonium fixation in the four soils were calculated. This is given as the difference between the concentration of urea applied and the sum of urea and ammonium concentration extracted from the soil after correcting for the exchangeable ammonium in the control soil. The values of ammonium fixation obtained are shown in Table 5. A plot of fixation level as a function of time in peat is shown in Figure 1.

For all soils, the amount of ammonium fixed increased with time during the incubation period (1 wk). The increase in the magnitude of

ammonium fixation as a function of time throughout the incubation period was not in agreement with results of previous studies (Nommik 1957, Black and Waring 1972). Published results show fixation as a rapid process attaining a maximum or equilibrium value after 24 hr (Harada and Katsuna 1954). It is therefore surprising that the fixation, as measured in this experiment, increased continuously with time for a period of 1 wk without showing any sign of saturation. This may be explained by the possible sources of error mentioned later under the discussion of the limitations of the "difference method" or it may indicate a fixation mechanism quite unrelated to physical sorption by the clay lattices.

Table 5 - Fixed $\text{NH}_4\text{-N}$ ($\mu\text{g g}^{-1}$) in the experimental soils calculated by the "difference method".

Sample	Incubation Time (hr)							
	1	2	6	24	48	72	96	168
Peat	195.4	242.1	224.1	271.0	302.6	355.6	394.4	566.8
Clay	81.6	163.4	156.8	169.2	361.4	231.2	279.7	412.8
Loam	35.5	53.6	139.6	55.5	137.9	185.8	236.4	324.7
Sand	8.4	65.4	32.5	53.4	155.2	234.0	212.1	339.6

The level of ammonium fixation in peat was higher than those of the three mineral soils. There appeared to be a relationship between organic matter content and the amount of ammonium fixed by the mineral soils. During the first hour of incubation, for example, peat with 72%

organic matter content, fixed 195 ug g^{-1} corresponding to 19% of the added nitrogen. Clay fixed 81 ug g^{-1} or about 8% of the added ammonium nitrogen, whereas loam and sand, fixed 35 and 8 ug g^{-1} , respectively. The amount of ammonium fixed after 1 wk of incubation, increased considerably in all the soils. Peat fixed about 57% of the added urea, clay 41%, whereas loam and sand fixed 32 and 34% of the applied urea, respectively. This trend was rather interesting in the light of the fact that other workers have observed a relationship between the levels of ammonium fixed and the organic matter content of the soil. Burge and Broadbent (1961), obtained a linear relationship between soil organic carbon and ammonia fixation in their study. While Sohn and Peech (1958), reported that the highest level of NH_3 fixation was obtained in acid soils having high organic matter contents. The possibility of establishing a quantitative relationship between NH_4^+ fixation and organic matter content will be explored when a more precise ^{15}N method is used to determine fixation.

Ammonium fixation from ammonium carbonate

The end product of urea hydrolysis is ammonium carbonate. It should then be possible to deduce the behaviour of NH_4^+ from urea in soil by direct application of ammonium carbonate. This incubation study was carried out with analytical reagent grade $(\text{NH}_4)_2\text{CO}_3$ applied to the soil in solution.

Results obtained for this incubation study are summarized in Tables 6 and 7. Ammonium fixation from $(\text{NH}_4)_2\text{CO}_3$ was quite similar to

that from urea (Tables 5 and 7) except that ammonium fixation during the first 48 hours of incubation was higher with ammonium carbonate than with urea. The rate limiting factor of urea hydrolysis was perhaps responsible for this. Since fixation possibly involves the NH_4^+ ion, urea has to be converted to ammonium before fixation can occur. Since the hydrolysis of urea is not instantaneous but a function of time, some time is required for ammonium to be rendered available in appreciable quantity. For example, an ammonium fixation value of 8 ug g^{-1} was obtained for urea after 1 hour of incubation in sand. The corresponding value for ammonium carbonate was 235 ug g^{-1} after 1 hour. But the concentration of ammonium in soil after 1 hour in sand treated with urea was 20 ug g^{-1} , whereas with ammonium carbonate, the ammonium concentration in the soil after 1 hour was 789 ug g^{-1} . Hence, the initially high magnitude of ammonium fixation observed in all soils with ammonium carbonate, was an indirect manifestation of the effect of ammonium concentration on ammonium fixation in soils.

In sand, the values of ammonium fixation (calculated using the difference method) with ammonium carbonate, was consistently higher than with urea throughout the incubation period. This could be due to a much higher loss of NH_3 from ammonium carbonate in the sandy soil. The low cation exchange capacity, and high pH of the sandy soil could be responsible for this higher loss and an over-estimation of fixed NH_4^+ . Apart from some observed differences, it can safely be concluded that there is some similarity between urea and ammonium carbonate with respect to their behaviour in soil.

Table 6 - Ammonium concentration ($\mu\text{g g}^{-1}$) in the experimental soils incubated with $1000 \mu\text{g g}^{-1}$ of ammonium carbonate.

	Incubation Time (hr)						
Sample	1	2	24	48	72	96	168
Peat	797	768	766	633	630	574	484
Clay	892	903	828	755	702	682	687
Loam	802	762	738	689	733	673	673
Sand	789	759	764	722	659	560	595

Table 7 - Fixed ammonium ($\mu\text{g g}^{-1}$) in experimental soils calculated by the "difference method".

	Incubation Time (hr)						
Sample	1	2	24	48	72	96	168
Peat	244	273	275	408	411	467	557
Clay	139	128	203	276	329	349	344
Loam	221	261	285	334	290	350	350
Sand	235	265	260	302	365	464	429

Fixation of ammonium, as measured by the difference method was considered high. No definite relationship was observed between fixation and time of incubation. Nevertheless, there was a definite trend of higher ammonium fixation by soil with higher organic matter

contents. Possible sources of error and limitations of this technique are given after the discussion of Experiment 3.

Experiment 2 : Fixation Of Ammonium In HF-Treated Soils

The main objective of this experiment was to measure NH_4^+ fixation in soil after the clay fraction has been eliminated. Fixation measured on the soil residue, after the destruction of the clay fraction, can be exclusively ascribed to organic matter. To achieve this, treatment with HF:HCl was employed for the destruction of the clay fraction.

The physical properties of the acid-treated soils (Table 2), reflect the effectiveness of the acid treatment. As a result of acid treatment the organic matter content of clay soil increased from 7% to 30%, while the clay content declined by about 50% from 73 to 35%. The treated soil was thus considerably enriched in organic matter, but complete removal of either the clay or the mineral fraction was not achieved. A similar effect of HF-HCl treatment on soil was observed by Burge and Broadbent (1961). They reported that the organic carbon of acid-treated soil increased considerably.

Another measure of the destruction of the mineral fraction not shown in Table 2, is the percent recovery of the soil residue following acid treatment. Clay which experienced the highest level of organic matter enrichment due to HF:HCl treatment, also had the lowest percent recovery. Only 30% of the 300g of bulk clay soil was recovered after acid treatment. Recovery figures of 45% and 67% were obtained for loam and sand, respectively. The proportion of soil organic matter

that was dissolved as a result of acid treatment was not estimated. The higher level of exchangeable NH_4^+ in acid-treated soils compared to untreated soils, indicated either the hydrolysis of organic matter or the release of native clay-fixed NH_4^+ or both. It was not possible to identify the source of this NH_4^+ in this experiment.

Similar to Experiment 1, urea and ammonium carbonate were applied in concentration of 1000 ug g^{-1} to acid-treated soils. After the desired period of incubation, the soils were extracted. Both urea and NH_4^+ concentrations were determined as previously described. The concentrations of urea and ammonium nitrogen measured after various periods of incubation are shown in Tables 8 and 9. Throughout the entire incubation, the concentrations of NH_4^+ in the acid-treated soils, were much lower than those observed with intact soils indicating that urea hydrolysis was inhibited by the acid treatment. This could be the result of a direct acid effect on soil microorganisms and/or the urease enzyme. Though the soils were thoroughly-rinsed with distilled water following acid treatment, the pH of the acid-treated soils were much lower than untreated soils (Table 2). The number of times the soils could be rinsed was limited because repeated leaching resulted in the dissolution and loss of organic matter as the soil suspension became less and less acidic. Direct neutralization of the soil was attempted using NaOH, but since this caused extensive dissolution of organic matter, it was discontinued.

Even though hydrolysis of urea did not appear to have taken place in these soils, the urea concentration in extracted solution from the peat soil was much less than that applied (Table 8). While a concen-

tration of 1000 ug g^{-1} of urea as nitrogen was applied to peat, only an average of 650 ug g^{-1} of urea was extracted from the soil following incubation. This observed shortfall in urea concentration in peat soil was without a concomitant increase in NH_4^+ concentration. It is not known if this shortfall could be attributed solely to experimental error. Ammonia volatilization is likely to be minimal since the soil is highly acidic. Because of the high organic matter content of the peat soil, it was suspected that there might be a direct interaction between the urea molecule and the soil organic matter.

Table 8 - Concentration of urea-N (ug g^{-1}) in acid-treated soil.

Sample	Incubation Time (hr)					
	1	2	4	6	24	48
Peat	636.6	643.5	657.9	775.9	687.6	612.3
Clay	852.0	866.2	870.8	862.6	795.8	804.6
Loam	930.5	-	-	981.0	913.0	-
Sand	956.7	895.4	-	-	940.0	915.8

Table 9 - Ammonium concentration (ug g^{-1}) in soil treated with HF-HCl and incubated with urea-N.

Sample	Incubation Time (hr)					
	1	2	4	6	24	48
Peat	172.3	168.4	170.4	147.6	160.6	155.2
Clay	36.7	40.3	34.4	38.1	41.5	37.0
Loam	16.1	16.4	18.8	19.8	17.5	18.1
Sand	14.5	16.8	15.7	19.3	17.7	18.5

Ammonium carbonate in HF-treated soil

Since there was no urea hydrolysis in the HF-treated soils a direct comparison could not be made between treated and untreated soils incubated with urea. With ammonium carbonate, however, the levels of ammonium fixation in HF-treated soils (Table 11), bear some resemblance to that of the native (untreated) soils (Table 7). The amount of nitrogen fixed by native peat, clay, loam and sand after one week of incubation with ammonium carbonate were 557, 344, 350, and 429 $\mu\text{g g}^{-1}$, respectively. After acid treatment, the corresponding values were 455, 278, 278, and 461 $\mu\text{g g}^{-1}$ after two days of incubation.

Table 10 - Concentration of $\text{NH}_4^+\text{-N}$ ($\mu\text{g g}^{-1}$) in HF-treated soils incubated with 1000 $\mu\text{g/g}$ of nitrogen as ammonium carbonate.

Sample	Incubation Time (hr)						
	1	2	4	6	12	24	48
Peat	695.9	697.1	683.3	686.3	647.1	647.3	648.9
Clay	763.9	760.9	805.3	668.9	736.1	701.6	751.9
Loam	783.9	792.9	782.2	752.4	711.5	723.0	739.6
Sand	706.8	742.1	766.3	694.4	660.3	639.4	553.2

Some pertinent differences in the pattern of NH_4^+ fixation could be noted between HF-treated and untreated soils. In the native soils ammonium fixation following application of ammonium carbonate was time dependent, with fixation increasing as incubation time increased.

Table 11 - Ammonium fixation ($\mu\text{g g}^{-1}$) in acid-treated soils incubated with $1000 \mu\text{g g}^{-1}$ of ammonium carbonate.

Sample	Incubation Time (hr)						
	1	2	4	6	12	24	48
Peat	408.1	424.8	420.7	417.7	456.9	456.7	455.1
Clay	266.1	269.1	224.7	361.1	293.9	328.4	278.1
Loam	224.1	226.1	235.8	265.6	306.5	295.0	278.3
Sand	307.2	271.9	247.7	319.6	353.7	374.6	460.8

But in the acid-treated soils, the dependency of fixation on time was quite weak particularly for the peat and sand soils. In peat, for example, ammonium fixation of $408 \mu\text{g g}^{-1}$ was obtained 1 hr after the initiation of the experiment. Extending incubation time to 48 hr increased fixation to only $455 \mu\text{g g}^{-1}$. Fixation of NH_4^+ increased from 243 after 1 hr to $408 \mu\text{g g}^{-1}$ 48 hr of incubation for the peat soil not treated with acid (Table 7).

Not only was the effect of time on NH_4^+ fixation removed with acid treatment, but the values of fixation at the initial stages of the incubation were much higher for acid-treated soils. This initially higher fixation levels in acid-treated soils was very pronounced for the peat and sand. The actual reason for this observation is unknown. Perhaps, after acid treatment, fixation sites were more accessible as a result of breakdown of organic-clay complexes. This will also result in earlier saturation of the fixing sites removing the effect of time on fixation.

The clay and loam soils, after acid treatment, exhibited similar behaviour with respect to their ammonium fixation levels, with the levels of fixation being generally close in magnitude to that obtained in Experiment 1.

It was found from Experiment 2 that HF:HCl treatment was very effective in destroying the clay fraction of the soil, however, complete elimination of the mineral fraction was not achieved. Acid treatment resulted in increased soil organic matter content, reduced clay content, reduced soil pH, and increase in exchangeable ammonium. Acid-treated soil incubated with ammonium carbonate showed a weak tendency of higher ammonium fixation than their untreated counterpart. Hydrolysis of urea was inhibited in acid-treated soil, but in the peat soil, a decline in urea concentration was observed without a corresponding increase in NH_4^+ concentration. On application of ammonium carbonate to acid treated soil, the fixation values obtained at the initial stage of incubation was much higher than for the untreated soil.

Experiment 3 : Ammonium Fixation With ^{15}N -Labelled Nitrogen Sources

The results obtained using the "difference" method in the two previous experiments, were inconclusive. Thus, it was decided that a direct method be employed to measure ammonium fixation. This experiment was initiated, utilizing ^{15}N labelled nitrogen sources so that the amount of NH_4^+ -N held in soil against KCl extraction could be determined using mass spectrometry.

An alternative method would be to determine the change in total nitrogen content of the soil devoid of free ammonium following the fixation reaction. But because ammonium fixation may not be high enough to produce a measurable change in nitrogen content of the soil, this method was not attempted.

(3a) Fixation from urea and ammonium sulphate

Soil samples were incubated with either ammonium sulphate or urea containing 95.5 and 98.6% ^{15}N , respectively. High precision in the measurement of fixed ammonium, requires a high percent ^{15}N enrichment. Incubation was carried out for various time intervals (ranging from 1 hour to 1 week), after which the soil was then extracted with 1 mol L^{-1} KCl solution. The applied nitrogen remaining in the soil against KCl extraction was termed fixed ammonium. This was then determined through Kjeldahl's digestion, titration, and mass spectrometric analysis. As earlier mentioned, not only was the total fertilizer nitrogen remaining in the soil residue determined, an attempt was also made to determine what fraction of the fixed NH_4^+ was in the organic fraction.

Unlike the result obtained in the two previous experiments, NH_4^+ fixation in all the soils was a function of incubation time (Fig. 3). The fixation rate was initially rapid and gradually declined with time. In all the soils, the period of rapid rate of fixation extended to 12 hours after the initiation of incubation. After 12 hr of incubation, the rate of fixation became quite slow, with magnitude of fixation levelling off in all the three mineral soils. An almost constant level of fixation was maintained during the 12 hr to 1 wk incubation period

(Figure 3).

The peat soil behaved somewhat differently from the three mineral soils. It showed an initially rapid fixation rate between 1-12 hr, with a slope change at about the 12 hr incubation period. There was no levelling off or saturation point throughout the incubation period. Time dependent NH_4^+ fixation was observed throughout the incubation period suggesting a higher capacity of peat to fix ammonium than the mineral soils. This observation is quite important and could have significant implications on nitrogen application to peat. Where $\text{NH}_4^+\text{-N}$ is involved, a considerable proportion of applied nitrogen could be tied up by the soil and rendered unavailable to the plant (in the short run at least). Though this experiment was extended to 1 wk, there is no doubt that fixation will continue in peat beyond this period. Hence, the magnitude of ammonium fixation after 1 wk by no means represents the equilibrium value.

The fixation pattern shown in Figure 3 is in agreement with previously published work on ammonium fixation, with about 60-90% of the total fixation occurring in clay, loam and sand in the first 12 hr of incubation and then levelling off. The levelling off of fixation in the three mineral soils could be due to the saturation of the available fixing sites. The leveling off indicates the attainment of equilibrium under which the rate of adsorption of NH_4^+ ion from soil solution is equal to the rate of desorption of the ion from the fixing site. Nommik and Vahtras (1982), used the data obtained by Harada and Katsuna (1954), to show that 60-90% of total ammonium fixation may occur within the first few hours of incubation. The result obtained with the peat

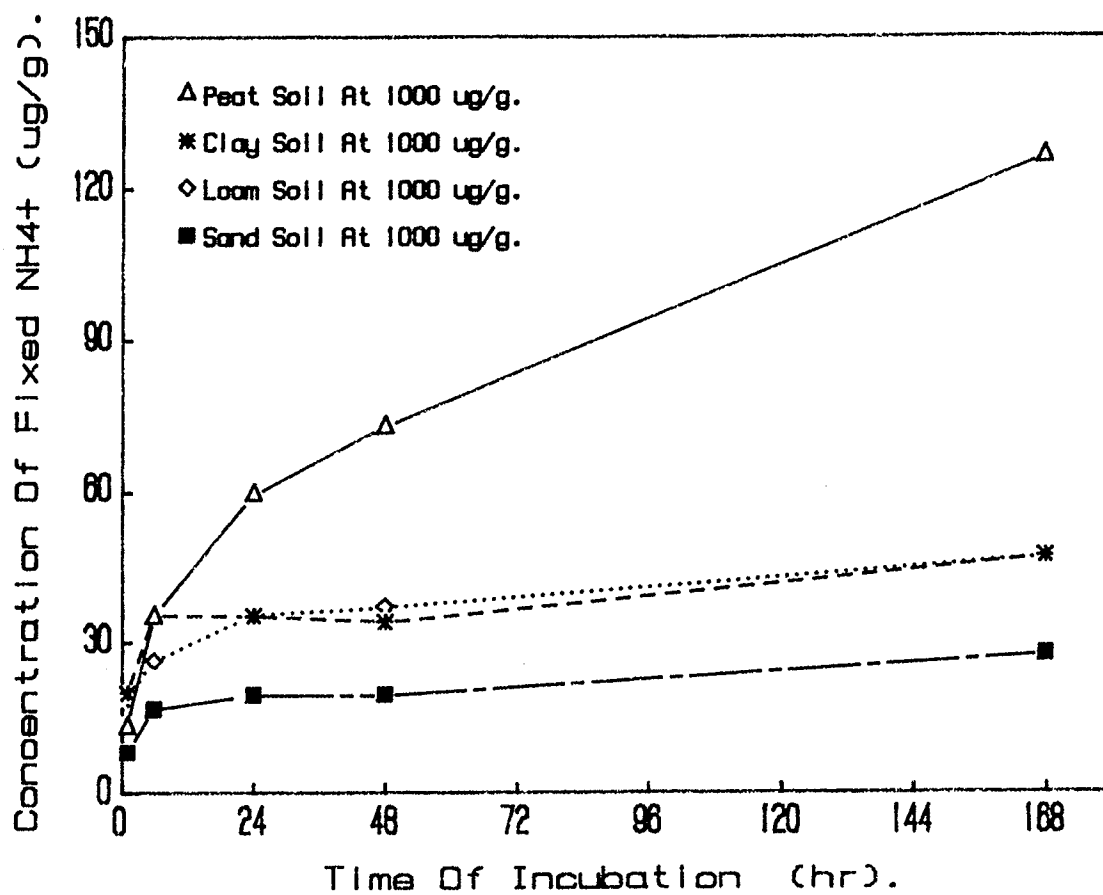


Fig. 3. Ammonium Fixation from Labelled Ammonium Sulphate in Four Manitoba Soils.

soil, however, was an exception to this rule, suggesting that, perhaps, the fixation mechanism here was different.

The curves shown in Figure 3 could be divided into two phases. Phase 1, being the phase of rapid fixation, where fixation might be predominantly due to the physical sorption of the NH_4^+ ion into the inter-lattice space of the clay mineral of the soils. In phase 1, ammonium fixation rate, being controlled mainly by ion diffusion, was highest in the period immediately after ammonium addition. It then slowed down as the equilibrium point was approached, which for this experiment was very near the 12 hr period. In phase 1, a linear relationship between ammonium fixation and time of incubation could be observed. During phase 2, equilibrium seemed to have been attained, indicating saturation of the fixing sites and further increases in the magnitude of NH_4^+ fixation during this phase was rather small. The analysis given here may not be applicable to the peat soil, considering it's small proportion of the mineral fraction. Also, the peat soil did not show any sign of saturation throughout the entire incubation period.

The magnitude of NH_4^+ fixation in the clay soil during phase 1 was higher than that of the loam soil. This is understandable since the former has a higher content of both the clay and organic matter. But in phase 2, fixation levels were virtually identical in the two soils, and their two curves actually converged near the 1-week period. The reason for this similarity in behaviour is not known. It should be remembered that despite all the limitations of Experiments 1 and 2, similarities between the clay and loam soils with respect to ammonium

fixation were consistently observed. Similarities in the NH_4^+ fixing capacities of these soils could not be related to the physical and chemical properties of the two soils shown in Table 1. Perhaps, the similarity exists in the mineralogy of the clay fraction or other mineral size fractions of these soils.

Sand with the lowest clay and organic matter content, also had the lowest level of NH_4^+ fixation at any point in time. It also appears, from Figure 3, that sand approached the saturation point much earlier than the other mineral soils. This should be the case if sand has the lowest number of fixation sites which is exposed to an equal NH_4^+ concentration as the other soils.

An obvious relationship between the level of ammonium fixation and the soil organic matter content could be seen (Figure 3 and Table 12). Peat, with the highest organic matter content also had the highest level of fixation during the entire incubation period. Being obvious, is perhaps, the only thing that can be said about this relationship.

An attempt was made to plot fixation level against organic matter content of the four soils. This plot, shown in Figure 4, represents fixation after 1 week of incubation with 1000 ug g^{-1} of nitrogen from urea and ammonium sulphate. A linear relationship between organic carbon and ammonium fixation was obtained by Burge and Broadbent (1961). However, probably because of the limited number of soil samples, and the wide range of their organic matter content (most especially between peat and the mineral soils), not much could be made out of the relationship shown in Figure 4. In retrospect, perhaps, if more soil samples had been utilized, including ones with organic matter

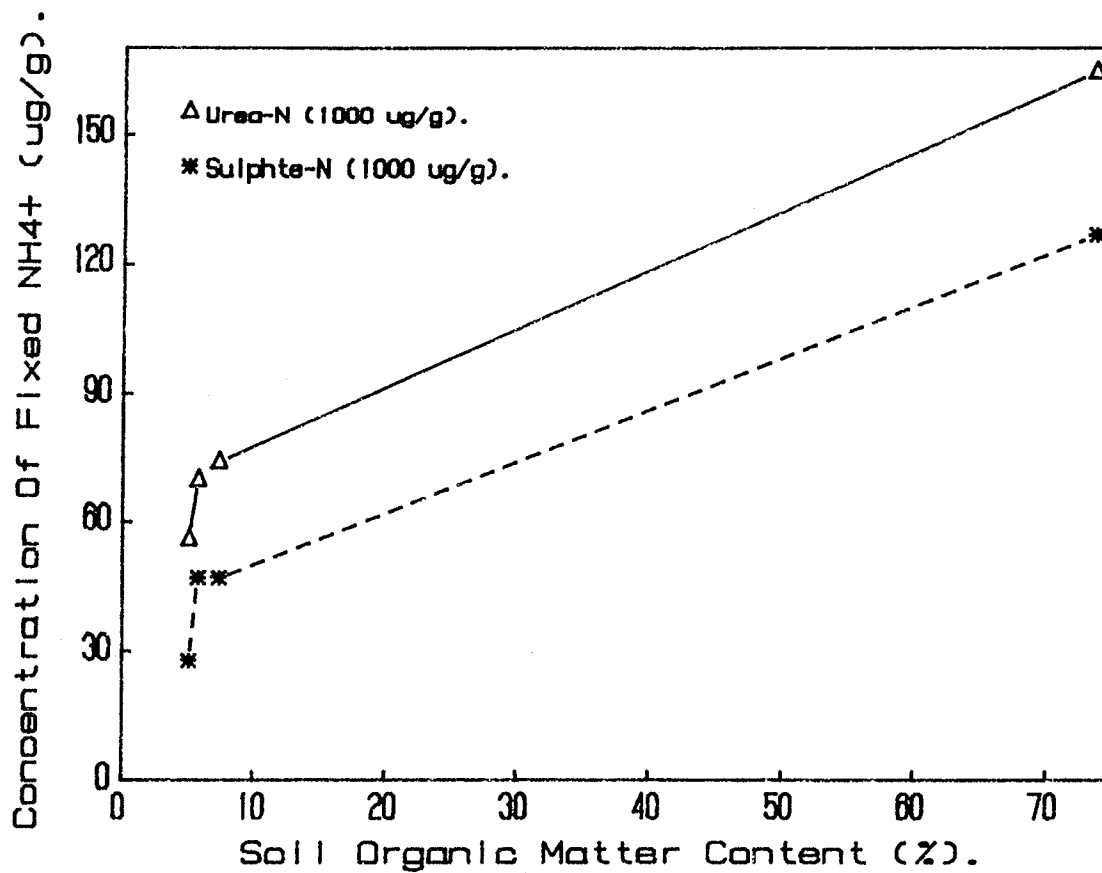


Fig. 4. Fixed Ammonium as a Function of Soil Organic Matter Content.

in the range of 10-50%, the data obtained could be subjected to regression analysis. The limitation of time and space, with the unavailability of soils with organic matter content in between 10 to 50%, precluded the realization of the above suggestion.

As previously mentioned, this experiment was carried out over a relatively short period of time, terminating after 1 wk. A short incubation time was used to eliminate as much as possible the effects of microbial immobilization, which could further complicate the interpretation of the results. As will be later demonstrated, from direct evidence, microbial contribution was negligible or nil during this period.

(3b) Effect of concentration of applied ammonium on fixation

The main objectives of this study were: to characterize the nature of the reaction between organic matter and ammonium; to determine the effect of initial concentration of NH_4^+ on the rate of fixation; and to describe the kinetics of the reaction.

To achieve these objectives, increasing levels of $^{15}\text{N}-(\text{NH}_4)_2\text{SO}_4$ was used for the incubation experiment. Concentrations of 250, 500, 750, 1000 and 1500 $\mu\text{g g}^{-1}$ of $^{15}\text{NH}_4^+-\text{N}$ were added to each of the four soils. These concentration levels were chosen to make the effect of increasing ammonium concentration on ammonium fixation, if any, easily discernible. The level of fixation was monitored at various times up to 1 wk. The results obtained from this experiment after 1 wk of incubation are summarized in Table 12. A plot of fixation as a function of

time and concentration in the peat soil is shown in Figure 5.

Lower percent ^{15}N enrichment was used for the 750 and 1500 ug g^{-1} , treatments to minimize costs. Since the concentrations of NH_4^+ for these treatments were high, reasonable level of ammonium fixation should be obtained from their application, hence, lowering the percent ^{15}N by dilution, should be possible without jeopardizing the accuracy of ^{15}N measurement on the soil residue by mass spectrometry. Two parts of the stock ammonium sulphate containing ^{15}N was added to one part of ^{14}N -ammonium sulphate. This resulted in a sample containing 63.67% ^{15}N . Due to the above dilution, the percent ^{15}N of soil residues incubated with 750 and 1500 ug g^{-1} (shown in Table 7) is lower than those treated with 500 and 1000 ug g^{-1} . This dilution, however, did not affect the overall result since 63.67% was the factor used to convert the ^{15}N measured in the soil residue to total nitrogen fixed by the soil where the diluted N-source was applied.

Table 12 shows the total nitrogen content of the soil residues after extraction with KCl. The variation in this value for each soil at various concentrations of applied NH_4^+ , is a reflection of the error in the analytical method. The magnitude of total nitrogen should actually increase with an increase in the amount of applied ammonium to reflect the increased level of fixed ammonium in the soil. That this was not the case, (see Table 12), confirmed the earlier suspicion, that, measurement of the change in total nitrogen of the soil, following fixation, is not a suitable method to employ in the determination of fixed ammonium in the soil.

In peat soil, because of the very high level of total nitrogen in

the soil residue, the percent ^{15}N in the soil was relatively low. This did not imply low NH_4^+ fixation, but, that the ^{15}N retained by this soil was diluted by the high proportion of native ^{14}N .

In all soils, the amount of $^{15}\text{NH}_4^+$ fixed increased with the amount of ammonium applied. The effect of increasing concentration on NH_4^+ fixation was more pronounced at the lower than at the higher concentration ranges and more so in peat than in the mineral soils. Increasing ammonium concentration from 500 to 1000 ug g^{-1} , almost doubled the magnitude of fixed ammonium-N in peat from 66 to 126 ug g^{-1} . At the 1500 ug g^{-1} level, however, ammonium fixation only increased to 146 ug g^{-1} . The reduced effect of increasing NH_4^+ concentration on fixation at the high concentration range may indicate that the capacity of these soils to fix ammonium is finite. The lower the capacity of the soil to fix ammonium, the less dramatic was the influence of increasing concentration on the magnitude of fixed NH_4^+ . Thus, in both the clay and loam soils there was virtually no difference in the amount of ammonium held in the soil after 1 week, at the concentrations of 750, 1000 and 1500 ug g^{-1} . But there was a marked difference in the amount of fixation at these high concentrations (750, 1000 and 1500 ug g^{-1}) and that fixed when 500 ug g^{-1} was applied.

The effect of increasing concentration of applied ammonium on the magnitude of fixation in sand was erratic. No definite pattern could be observed at either the high or the low concentration ranges. The sand soil fixed 24 ug g^{-1} when 500 ug g^{-1} of nitrogen was added, but doubling the applied ammonium to 1000 ug g^{-1} resulted in fixation increasing to only 28 ug g^{-1} . At the highest concentration level of

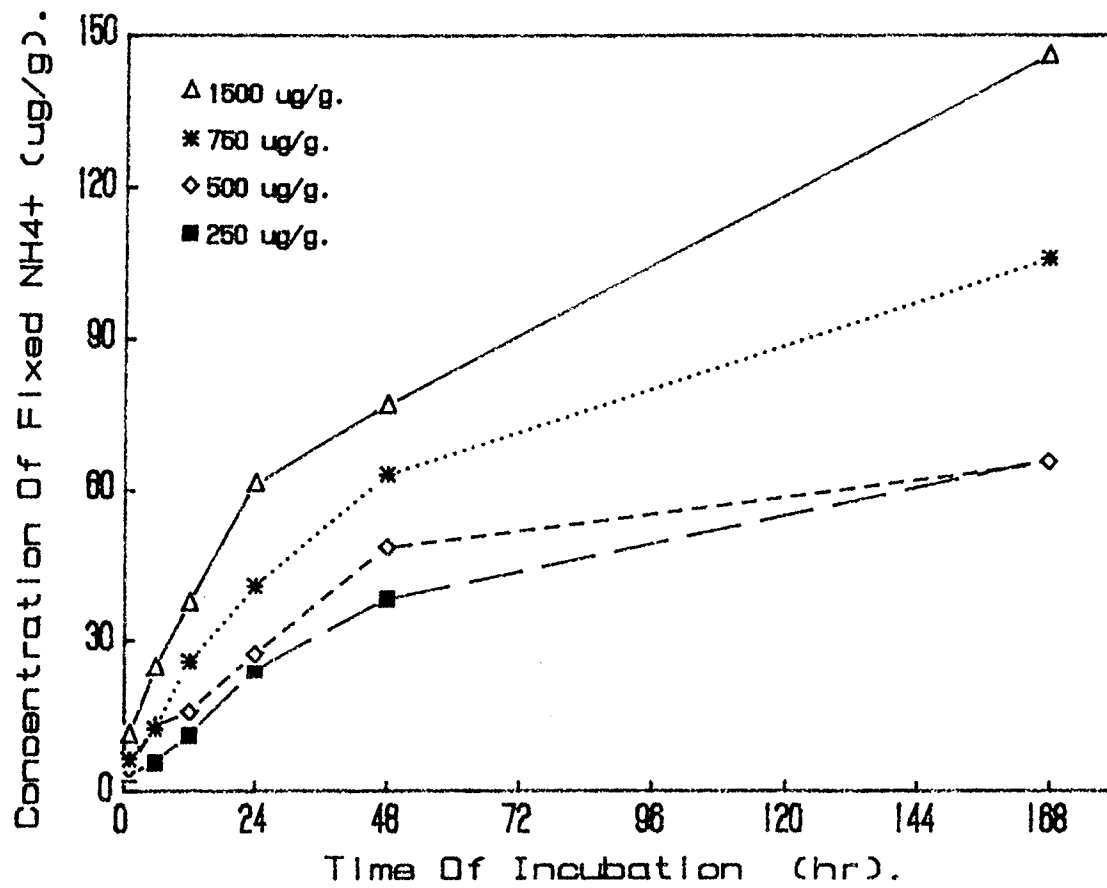


Fig. 5. Fixation as a Function of Applied Ammonium-N Concentration in Peat.

1500 ug g^{-1} , ammonium fixation increased to 40 ug g^{-1} .

A general pattern of NH_4^+ fixation as influenced by the concentration of applied NH_4^+ could be observed from Table 12 for all the soils. Though the absolute amount of ammonium fixed increased as the concentration of applied ammonium was increased, the proportion of the applied nitrogen that was retained in the soil actually declined with increasing concentration. In loam, for example, as concentration of applied NH_4^+ increased from 500 to 1500 ug g^{-1} , the amount of fixed NH_4^+ increased from 33.8 to 55.4 ug g^{-1} , but the proportion of applied ammonium fixed, expressed as a percentage decreased from 6.7 to 3.7. This result is in agreement with previously reported results by Nommik and Vahtras 1982.

The pattern of NH_4^+ fixation, as related to the concentration of applied NH_4^+ , shown in Figure 5 for peat, is representative of all the other soils. Ammonium fixation was a function of the initial concentration of ammonium applied to the soil. The absolute value of fixed NH_4^+ increased with an increase in the initial concentration of $(\text{NH}_4)_2\text{SO}_4$. But, the percent fixation decreased with increasing ammonium concentration (Table 12).

This concentration effect on the level of NH_4^+ fixation may have an implication on nitrogen retention by the soil in field situations, especially in cases where banding of $\text{NH}_4^+\text{-N}$ fertilizer is employed. Though the overall concentrations of nitrogen in the field may not be high, banding of NH_4^+ -fertilizer may produce microsite concentration far above the field average. Considerable nitrogen fixation may occur in these microsites.

Table 12 - Effect of concentration of applied ammonium, on ammonium fixation in experimental soils incubated for 1 wk.

	Applied NH_4^+ ($\mu\text{g g}^{-1}$)	Total N (mg)	% ^{15}N	Fixed N ($\mu\text{g g}^{-1}$)	% Fixation
Peat	500	100.0	0.312	65.6	13
	*750	101.0	0.334	105.7	14
	1000	100.0	0.607	126.5	13
	1500	100.0	0.465	145.8	10
Clay	500	16.8	0.806	28.2	5.7
	750	17.5	0.771	42.3	5.6
	1000	17.3	1.306	47.4	4.7
	1500	17.3	0.890	48.4	3.2
Loam	500	13.2	1.227	33.8	6.8
	750	12.9	1.084	44.0	5.9
	1000	13.0	1.721	47.0	4.7
	1500	12.9	1.367	55.4	3.7
Sand	500	8.1	1.410	24.0	4.8
	750	7.4	1.144	26.5	3.5
	1000	8.2	1.626	27.8	2.8
	1500	9.2	1.375	39.7	2.6

* For the concentrations of 750 and 1500 $\mu\text{g g}^{-1}$, ^{15}N enrichment was 63.7% in the applied stock, while for other concentrations (500 and 1000 $\mu\text{g g}^{-1}$) the ^{15}N enrichment was 95.6%.

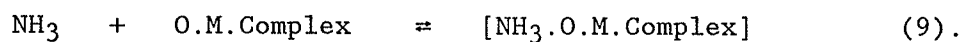
(3c) Reaction Kinetics

Using the results plotted in Figure 5, an attempt was made to formulate a reaction mechanism between organic matter and the ammonium ion. A kinetic model will also be proposed, from which the rate constant of the reaction can be calculated.

The reaction between organic matter and NH_4^+ can be summarized as follows :



note that
$$\frac{[\text{NH}_3][\text{H}^+]}{[\text{NH}_4^+]} = 10^{-9} \quad (8).$$



Employing a method similar to that used for urea in Experiment 1, the rate equation can be written as :

$$\text{Rate of NH}_3 \text{ fixation} \propto [\text{NH}_3][\text{O.M.Complex}] \quad (10).$$

which becomes

$$\text{Rate of NH}_3 \text{ fixation} = K [\text{NH}_3][\text{O.M.Complex}] \quad (11).$$

where K is the rate constant of the reaction.

Since the level of organic matter is appreciable relative to applied ammonium, it is reasonable to assume that the level of the reactive organic fraction does not change to any significant extent during the course of the reaction. An indication that organic matter has a large fixing capacity, is the lack of saturation of ammonium fixation in peat throughout the 1 week incubation period. Equation (11) can then be re-written as:

$$\text{Rate} = K' [\text{NH}_3] \quad (12).$$

here K' is the new rate constant incorporating the former K and

[O.M.Complex].

It should be noted that it is not the absolute concentration of ammonia that should be inserted into equation (12). The capacity of the soil to fix ammonium is not infinite. At a particular time (time t equals infinity) equilibrium has to be reached. Also, the concentration of ammonium in soil will never equal zero. Hence, the rate of disappearance of ammonia is not a function of the NH_4^+ concentration in soil solution per se, but rather, a function of the effective ammonium concentration. The effective ammonium concentration is defined as the difference between the ammonium concentration at any point in time and the NH_4^+ concentration when equilibrium is reached.

The actual concentration of ammonium in the soil at various points in time up to 1 week is known. The problem then arises, of finding the concentration of NH_4^+ when equilibrium is reached. Since the experiment was terminated after 1 wk of incubation the equilibrium ammonium concentration (termed C_{inf}) was obtained by approximation. Through the aid of a computer, estimated values that will give the best correlation coefficient was obtained.

This was through a computer program labelled "search". This program starts with the "equilibrium" ammonium concentration obtained after 1 wk of incubation and by adding small increments (eg. 0.25), it looks for the value of C_{inf} such that a plot of $\ln (C_t - C_{\text{inf}})$ against time (t) will give the maximum correlation coefficient. The output of this program was various values of C_{inf} and their corresponding correlation coefficients. It was from this array that a particular C_{inf} having the maximum correlation coefficient was chosen. This value was

then substituted into equation (12) to give:

$$\text{Rate} = K'(C_t - C_{\text{inf}}) \quad (13).$$

where C_t is ammonium concentration at any time t , C_{inf} is the estimated ammonium concentration at equilibrium i.e., when time t equals infinity. $(C_t - C_{\text{inf}})$ is the effective concentration ($C_{\text{effective}}$).

A plot of $\ln(C_{\text{effective}})$ against t should produce a straight line with K' as the slope. This plot for the peat soil is shown in Figure 6, together with the regression equation for the line. The rate constant obtained from this plot was 0.017 hr^{-1} and the correlation coefficient r for this plot was 0.99. This analysis is only applicable to peat soil and is another example of a pseudo-first order reaction. The data obtained for the mineral soils did not lend itself to such analysis. At a very early stage of the incubation, for example, ammonium fixation in the mineral soils levelled off, and was no longer a function of time. Whereas in peat no such levelling off was observed.

(3d) Ammonium fixation from N^{15} -labelled urea

The results obtained when soil was incubated with 1000 ug g^{-1} of ^{15}N -urea nitrogen is shown in Table 13. Figure 7 is a graph comparing fixation from urea with that from $(\text{NH}_4)_2\text{SO}_4$ in the loam soil. The pattern of NH_4^+ fixation obtained from urea was similar to that obtained from $(\text{NH}_4)_2\text{SO}_4$ in all the soils. As shown in Figure 7, the magnitude of fixation from urea was lower at the initial stages of incubation than that from $(\text{NH}_4)_2\text{SO}_4$. However, as incubation time

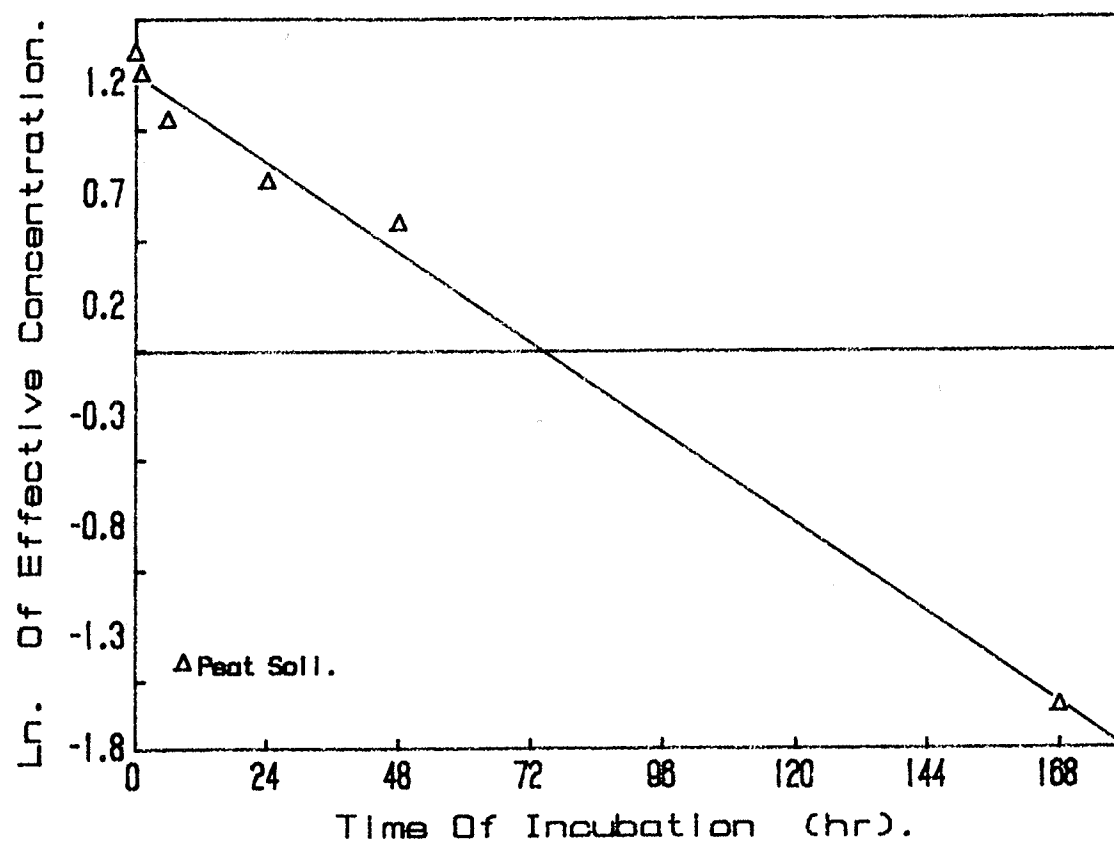


Fig. 6. Kinetics of Fixation Reaction in Peat Soil.

progressed, the magnitude of fixation from urea became greater than that from ammonium sulphate. This pattern was then maintained throughout 1 wk of incubation. This may be an indication that urea hydrolysis was the rate limiting process here. As urea hydrolysis progressed with time, and more NH_4^+ was rendered available, fixation from urea increased and was above that from $(\text{NH}_4)_2\text{SO}_4$.

Within the first four days, ammonium fixation from urea was a linear function of incubation time in all soils. The linear relationship became distorted, however, between the fourth and the seventh day of incubation, perhaps, as a result of saturation of the system with NH_4^+ . When incubation time progressed from 1 day to 2 days, ammonium fixation was doubled in all the soils. Fixation increased in peat from 49 to 90, in clay from 22 to 45, in loam from 17 to 32 and in sand from 14 to 29 $\mu\text{g g}^{-1}$. Similar increases were obtained in the fixation values between 2nd and the 4th days. An initially rapid rate of fixation was also noticed with ammonium sulphate, but the duration of the period was only shorter (12 hr).

The reason for the higher level of ammonium fixation with urea than with $(\text{NH}_4)_2\text{SO}_4$ may be related to the nature of fertilizer elements and their local pH effects. A profound effect of pH on NH_3 fixation has been previously reported (Nommik 1970). The pH of the soil following urea application, may be high enough to provide a favourable regime for NH_3 fixation. The often reported low plant uptake of urea fertilizer compared to other N-carriers, may be partially explained by this higher level of fixation.

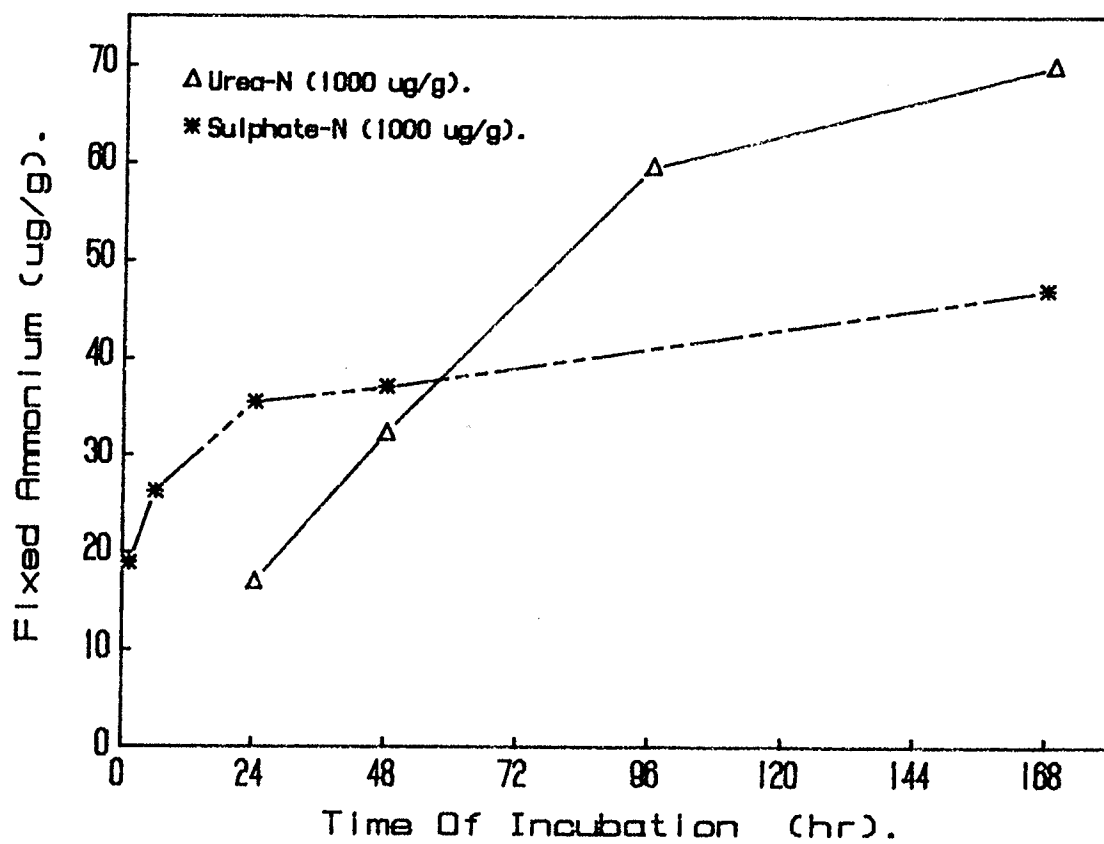


Fig. 7. Effect of Nitrogen Carrier on Ammonium Fixation in Loam Soil

Table 13 - Ammonium-N fixation ($\mu\text{g g}^{-1}$), from ^{15}N -labelled urea-N applied at the rate of $1000 \mu\text{g g}^{-1}$.

	Incubation time (days)			
	1	2	4	7
Peat	49.4	89.6	127.5	164.7
Clay	21.6	44.8	64.2	74.4
Loam	16.9	32.3	59.5	69.8
Sand	13.6	29.4	49.2	55.9

(3e) Organically Bound NH_4^+

The organic nitrogen content of soil residue containing fixed $^{15}\text{NH}_4^+$ was determined using the method described by Mike and Wallace (1953). Obi (1982), employed this method to arrive at the proportion of fixed NH_4^+ that is organically bound. The method involves digesting soil (to which dilute H_2SO_4 has been added), with H_2O_2 . In this experiment, the hydrogen peroxide was added in small portions until there was no more CO_2 effervescence with further addition. Also, the digestion was carried out under low heat, so as to ensure minimal disturbance to the mineral fraction. The use of this method to determine organic nitrogen assumes that both the dilute acid treatment, and peroxide oxidation of soil organic matter do not result in liberation of clay-fixed ammonium. This assumption, in a sense, is a limitation of the H_2O_2 oxidation method.

The percent ^{15}N excess in the organic fraction of peat was lower

than those of other soils because of the dilution by the large amount of soil nitrogen. The organic nitrogen in the four soils, based on five grams of soil, ranged from 90.3 mg in peat to 7.4 mg in sand (Table 14).

Table 14 - Fixed $\text{NH}_4^+\text{-N}$ (ug g^{-1}) in the organic Nitrogen fraction after 1 wk of incubation with $(\text{NH}_4)_2\text{SO}_4$ (1000 ug g^{-1}).

	Total N (mg)	Organic N (mg)	% ^{15}N	Fixed N (ug g^{-1})	% Of Total Fixation
Peat	99.0	90.3	0.572	108.2	85.5
Clay	17.2	14.4	1.248	37.5	79.17
Loam	13.0	11.2	1.609	37.7	80.34
Sand	8.2	7.4	1.764	27.4	98.78

Organic nitrogen constituted the largest proportion of the total nitrogen in the soil (Table 14). In five grams of peat, soil organic nitrogen measured by H_2O_2 oxidation was 90.3 mg while total soil nitrogen by Kjeldahl's method was 99 mg. Hence, organic nitrogen constituted more than 90% of the total soil nitrogen. In clay, the proportion was 83%, while loam and sand had 86% and 90.5% of their total nitrogen in the organic fraction, respectively.

In the peat soil, which fixed 126.5 ug g^{-1} of applied $\text{NH}_4^+\text{-N}$ (Table 12), 108.2 ug g^{-1} or 85.5% of the total nitrogen fixed by the soil was found in the organic nitrogen fraction. For the clay soil, the amount of nitrogen fixed by the soil was 47.7 ug g^{-1} . The organic fraction contained 37.5 ug g^{-1} or about 79% of the total fixation. The

similarity between clay and loam was also reflected by both the absolute amount of fixed ammonium contained in their organic fraction and the proportion of total fixation that could be ascribed to organic matter.

In the sand soil, the situation was somewhat different. The figure obtained showed that the organic fraction was responsible for nearly all the ammonium fixed by this soil. The reason for this high contribution to total fixation by the organic matter in sand is unknown. It is perhaps, an indication that the contribution of the mineral fraction to NH_4^+ fixation in this soil was negligible.

It is not quite surprising that on the average, organically bound nitrogen constituted about 80% of the total ammonium retained by the soils. This, conversely, showed that the contribution of the mineral fraction was low. Though the mineralogy of these soils was not determined, Manitoba soils contain predominantly montmorillonite in their clay fraction. This mineral is known to fix little or no ammonium under wet conditions. Air drying, however, can increase fixation quite dramatically (Young and McNeal 1964). This experiment was performed under wet conditions without air drying. The low contribution of the mineral fraction to overall ammonium fixation was not unexpected. The results reported here concur with those obtained by Obi (1982), who found that over 80% of ammonium fixed in his experimental soils was organically bound.

It was not possible to recommend the H_2O_2 oxidation method as a standard method for determining organically bound nitrogen. This was because of the limitations earlier mentioned.

With the aid of ^{15}N labelled nitrogen sources, it was possible to

determine precisely the amount of added NH_4^+ retained in the soil against KCl extraction. This $\text{NH}_4^+\text{-N}$ was then termed fixed ammonium. Ammonium fixation in all the soils was a function of time, increasing rapidly at the initial stage of the incubation and later attaining an equilibrium level in the mineral soils. Peat soil did not show any sign of this saturation throughout the incubation period.

Ammonium fixation was not only a function of time, but was also concentration dependent. With the absolute value of fixation increasing with an increase in the level of applied $\text{NH}_4^+\text{-N}$. The proportion of fixation, however, decreased, with increasing NH_4^+ concentration. Fixation of ammonium in all the soils was related to the level of organic matter.

First order reaction kinetics was proposed for the interaction between NH_4^+ ion and soil organic matter content. The data collected on the mineral soils did not lend itself to such an analysis.

(3f) Limitation of the "difference method"

It appeared at this point that the "difference method" used to measure fixation in Experiment 2 grossly overestimated the level of NH_4^+ fixation. This became obvious when the result obtained from Experiment 2 was compared to that obtained using ^{15}N -labelled carriers (see Tables 5 and 12). A comparison between the two methods of determining fixed ammonium revealed a lack of agreement. Such a lack of agreement has been previously observed by workers employing the two methods. Msumali (1977), reported that immobilization, as measured by

the recovery of nitrogen, was not consistent with the result obtained for immobilization of added nitrogen using ^{15}N . Also, Nommik and Nilsson (1963b), observed discrepancies between the difference method and the direct ^{15}N method of estimating fixed ammonium. While Stojanovic and Broadbent (1959), working on recovery of nitrogen from soils, reported that the tracer nitrogen recovery was invariably less than that of total NH_4^+ .

The exact cause of the discrepancy observed between the "difference method" and the ^{15}N technique of measuring ammonium fixation is unknown. Possible reasons for the over-estimation of fixed ammonium by the difference method will be presented. One fundamental assumption made in this discussion is that the ^{15}N method (see Expt. 3, Table 12), gives results nearer to the true fixation values than the difference method. On the basis of this, the possible sources of error in the difference method will be enumerated.

Firstly, the difference method by definition is a combination of several independent measurements. The errors in these individual measurements when combined into a single value, could result in an increase in the magnitude of the error.

Also the amount of nitrate and nitrite in the soil solution were not determined. It was assumed that the nitrification of ammonium during the short duration of this experiment, was negligible. If this assumption did not hold, and appreciable amounts of NH_4^+ were converted to nitrate and nitrite (most especially at the latter part of the incubation), then the value of ammonium from urea would be underestimated thereby over-estimating ammonium fixation.

Finally, any loss of applied fertilizer nitrogen in the form of ammonia volatilization or denitrification, would result in the over-estimation of fixation value. The likelihood of denitrification and its extent under the experimental condition of this study is unknown. Ammonia volatilization on the other hand could be an important source of error in the difference method of calculating NH_4^+ fixation. Overrein and Moe (1961), reported that the rate of ammonia volatilization from soil during laboratory incubation increased exponentially with an increase in the rate of urea application. Considering the relatively high level of urea applied in these experiments, significant amounts of nitrogen could be lost through ammonia volatilization. This loss will then be reflected in a higher level of fixation calculated by the difference method.

Any or a combination of these sources of error could be responsible for the lack of any general or specific trend in the data obtained from Experiments 1 and 2. Hence, the data obtained in the first two experiments could not be subjected to further analysis.

Experiment 4 : Fixation Of Ammonium In Sterilized Soils

This experiment derives from the observation made in Experiments 2 and 3. In Experiment 2, a considerable amount of urea appeared to be adsorbed by the acid-treated peat soil against extraction with 1 mol L^{-1} KCl solution, while in Experiment 3, higher levels of NH_4^+ fixation were obtained from urea than from ammonium sulphate. From these observations, a direct interaction between organic matter and the urea

molecule was suspected. The aim of this experiment was to investigate the possibility of a direct involvement of urea with organic matter in fixation reactions.

One problem worthy of consideration, however, is the ease with which urea is converted to ammonia in the soil system. To separate the reaction of ammonia from that of the parent urea, a means had to be devised which ensured that urea applied to the soil remains intact. The use of a urease inhibitor would have been plausible. However, highly effective urease inhibitors are not available. Sterilization of the soil was the most practical option.

(4a) Heat sterilization

Bulk soil in Erlenmeyer flask was subjected to heat sterilization in an autoclave. To ensure complete soil sterilization and inactivation of the urease enzyme, sterilization was conducted over a 24 hr period. Studies of NH_4^+ fixation in sterilized soils were carried out employing the methods previously described.

Table 15 shows the results obtained from this experiment. Data in Table 15 are the average of two separate incubation studies with each incubation carried out in duplicate. The results obtained were almost identical to that obtained in Experiment 2 where urea was applied to HF-treated soils (Table 9). The similarity was perhaps, due to the sterile condition of the soil samples used in both experiments, with urea hydrolysis being effectively inhibited.

The concentrations of NH_4^+ extracted from each soil increased slightly with an increase in the incubation time, but ammonium concen-

tration can generally be considered nearly constant with time, when compared to unsterilized soil. This should not be the case if urea hydrolysis took place in the soil. It was an indication of the sterility of the soil, and the inactivation of the urease enzyme.

Table 15 - Ammonium-N concentration ($\mu\text{g g}^{-1}$) in heat-sterilized soil incubated with $1000 \mu\text{g g}^{-1}$ of urea-N.

Sample	Incubation Time (hr)				
	1	2	4	6	24
Peat	139.2	144.7	147.7	144.5	146.7
Clay	54.3	55.7	61.1	61.4	77.4
Loam	42.3	41.5	48.3	46.6	52.2
Sand	33.2	33.5	41.2	38.8	44.3

Table 16 - Urea-N concentration ($\mu\text{g g}^{-1}$) in heat-sterilized soil incubated with $1000 \mu\text{g g}^{-1}$ of urea-N.

Sample	Incubation Time (hr)				
	1	2	4	6	24
Peat	840	894	809	924	920
Clay	923	902	911	889	950
Loam	931	894	856	931	983
Sand	886	899	882	865	951

In a 24 hr incubation period, concentration of ammonium extracted from peat soil ranged from 139 to 146.7 $\mu\text{g g}^{-1}$. This was not the case when urea was applied to unsterilized peat (Tables 3 and 4). The level of ammonium extracted from the clay soil ranged from 54.3 to 77.4 $\mu\text{g g}^{-1}$ in a 24 hr incubation period. NH_4^+ concentration increased from 42.3 to 52.2 and 33.2 to 44.3 $\mu\text{g g}^{-1}$, in the loam and sand soils, respectively. The small increase in NH_4^+ concentration with incubation time (Table 15), may be due to the contamination of the soil sample by atmospheric urease. Though, for all practical purposes NH_4^+ concentration could be considered to be constant with time, however, the concentration of ammonium extracted from heat-sterilized soil incubated with urea was higher than the exchangeable ammonium in the control soil (Table 1). If biochemical hydrolysis did not presumably take place in these soils, the question can be raised as to the source of the higher levels of exchangeable ammonium in heat-sterilized soils. Chemical hydrolysis of urea could be partly responsible for this. This is not likely to be a significant factor, however, Chin and Kroontje (1963), reported that the reaction rate of chemical hydrolysis of urea is very slow and insignificant in comparison with biochemical hydrolysis. The increase in the level of exchangeable ammonium following soil sterilization, could be attributed to the release of native clay-fixed NH_4^+ ion from the clay lattice as a result of thermal treatment. Porter and Stewart (1966), reported loss of fixed NH_4^+ from ammonium-treated and untreated soil samples, when subjected to temperatures above 400°C . The temperature used in this experiment was 121°C , but the duration of the heat treatment was long enough, that it might have

caused some release of native clay-fixed NH_4^+ from the soil.

Corroborating evidence that the soil was sterile, was based on the data on urea concentration as a function of time as shown in Table 16. Since urea hydrolysis normally occurred quite rapidly in these soils (Table 4), maintenance of a high and almost constant levels of urea with time, gave further evidence of inactivity of the urease enzyme. In peat soil sterilized with heat, for example, the concentration of urea in a 24 hr period ranged from 809 to 920 ug g^{-1} (Table 16). When unsterilized peat was incubated with urea, the concentration of urea declined from 1000 to 37 ug g^{-1} after 24 hr of incubation (Table 4).

It may not be possible to make any strong conclusion regarding the direct involvement of the urea molecule in the fixation reaction with soil organic matter. The concentration of extracted urea in all the soil samples was below the amount of urea-N applied to the soil. Urea was applied to all the soils to give a nitrogen level of 1000 ug g^{-1} , but the level extracted in peat ranged from a low of 809 to a high of 924 ug g^{-1} in a 24 hr incubation period. The variation was much less in the clay soil where the extracted urea concentrations ranged from 899 to 950 ug g^{-1} . A similar pattern was obtained for the loam and sand soils. This incomplete recovery of urea in all the soils would suggest probable adsorption of urea by the soil. But there is enough variation in the values of urea, measured as a function of time, that the shortfall in urea concentration might be safely attributed to experimental error. Considering the high level of urea and organic matter (especially in peat), any appreciable fixation of urea should be detectable by the method employed. On the basis of this, it is either

that urea fixation was small, or that there was no direct interaction between urea and soil organic matter. It was because of the inconclusive nature of this experiment, that another sterilization technique was employed.

(4b) Sterilization using gamma radiation

A Co-60 source was used to irradiate the soil at a dose of 2×10^4 Gy per hr for 3 and 6 hr periods to give two total radiation levels of 6 and 12×10^4 Gy, respectively. It should be noted that both of these doses are far above the levels required for either soil sterilization or complete inactivation of the enzyme systems. Quoting data from Roberge and Knowles (1968), Forster et al (1985) reported that soil sterilization was achieved at 1.1×10^4 Gy, while microbial and enzyme activities were completely inhibited by gamma radiation of 4.55×10^4 Gy. After gamma radiation, a sterility test was performed on the sterilized soil samples. Nutrient agar broth was used for this test under sterile conditions. The test media were sealed and examined for microbial growth for 7 days. No microbial growth was observed at both levels of sterilization throughout the test period, indicating that the soils were sterile.

The data generated on NH_4^+ fixation from urea in gamma-irradiated soils is shown in Tables 17-19. Figure 8 compares fixation of ammonium from urea under sterilized and non-sterilized conditions in sand. This plot was representative of the results obtained with other soils. The concentration of extractable NH_4^+ in the soil increased rapidly with time of incubation. The pattern of NH_4^+ buildup shown in Table 17 is

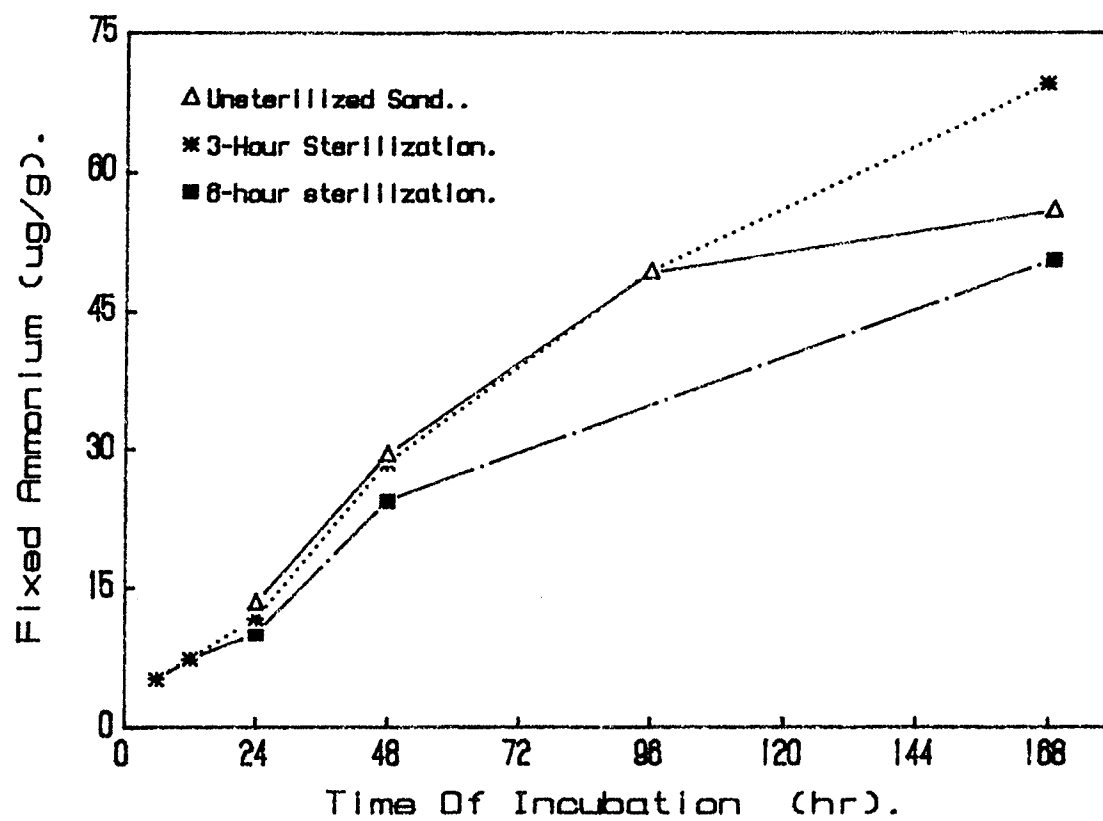


Fig. 8. Ammonium Fixation from Labelled Urea N in Sterilized and Unsterilized Sand.

similar to that obtained for unsterilized soil as shown in Table 3. In peat, the concentration of ammonium increased from 263 $\mu\text{g g}^{-1}$ after 6 hr of incubation to a maximum of 923 $\mu\text{g g}^{-1}$ by 4 days. The concentration of ammonium subsequently declined slightly to 902 $\mu\text{g g}^{-1}$ 1 wk after the initiation of the experiment. Compared to unsterilized soil, the rate of buildup of ammonium in soils at the two levels of sterilization were quite low. This was an indication of a lower rate of urea hydrolysis following sterilization.

Table 17 - Exchangeable ammonium-N ($\mu\text{g g}^{-1}$) in gamma-radiated soils incubated with urea-N (1000 $\mu\text{g g}^{-1}$).

	Incubation Time (hr)					
	6	12	24	48	96	168
Peat A	263	491	749	735	923	902
Peat B	169	265	475	671	-	906
Clay A	128	265	492	697	738	762
Clay B	114	168	309	452	-	726
Loam A	96	177	330	590	707	702
Loam B	64	101	150	264	-	656
Sand A	55	77	142	258	455	641
Sand B	38	38	51	81	-	291

Note A = Soil sterilized for 3 hours (6×10^4 GY).

B = Soil sterilized for 6 hours (12×10^4 GY).

Table 18 - Extractable urea-N ($\mu\text{g g}^{-1}$) in sterilized soil.

	Incubation Time (hr)					
	6	12	24	48	96	168
Peat A	549	362	44	24	-	-
Peat B	787	627	420	73	-	-
Clay A	720	502	269	13	-	-
Clay B	794	690	530	245	-	-
Loam A	799	686	417	114	14	-
Loam B	888	821	763	464	234	-
Sand A	899	797	697	551	305	67
Sand B	913	908	900	795	-	533

Table 19 - Ammonium-N fixation ($\mu\text{g g}^{-1}$) in sterilized soil incubated with ^{15}N -urea.

	Incubation Time (hr)					
	6	12	24	48	96	168
Peat A	12.2	18.5	30.3	73.4	119.0	159.2
Peat B	13.0	18.3	28.5	80.6	-	153.2
Clay A	6.9	13.2	21.8	42.0	78.8	89.9
Clay B	6.9	9.6	16.8	42.4	-	82.9
Loam A	8.6	12.8	23.6	45.1	68.2	82.6
Loam B	6.4	9.7	16.7	42.9	-	76.0
Sand A	5.2	7.4	11.9	28.6	49.4	69.6
Sand B	5.0	7.5	10.1	24.6	-	50.7

Note A = Soil sterilized for 3 hours (6×10^4 GY).

B = Soil sterilized for 6 hours (12×10^4 GY).

Because of this apparent reduction in the rate of urea hydrolysis in sterilized soil, it took a longer time before maximum NH_4^+ concentration could be achieved in these soils compared to their unsterilized counterparts. Even after the attainment of the maximum in sterilized peat, the subsequent rapid decline in NH_4^+ concentration (shown in Figure 1 for unsterilized peat) did not occur. The reduced rate of urea hydrolysis in sterilized soils could be attributed to the decline in the level of urease following gamma radiation. The high ammonium concentration maintained in peat was likely due to the absence of microbial activity to convert NH_4^+ to NO_3^- .

In peat sterilized for 6 hr the rate of urea hydrolysis was even lower than for samples sterilized for 3 hr (see Table 18). For example, after 6 hr of incubation, exchangeable ammonium was 402.7, 263 and 169 ug g^{-1} in unsterilized peat, peat sterilized for 3 and 6 hr, respectively. Thus, gamma radiation did have a profound effect on the rate of urea hydrolysis. This was indicated by the progressive decline in the rate of ammonium build up in the soil with increasing levels of radiation.

In the mineral soils, the rate of urea hydrolysis appeared to be more affected by gamma radiation than in peat. The rate of urea hydrolysis was low enough in these soils that maximum ammonium concentration was not attained during the 1 week incubation period. In clay, NH_4^+ concentration increased from 128 ug g^{-1} after 6 hr of incubation to 762 ug g^{-1} after 1 wk, when sterilized for 3 hr. With soil sterilized for 6 hr, corresponding figures of 114 to 728 ug g^{-1} were obtained. Perhaps, it may be necessary to incubate these soils for more than 1

week for their ammonium concentration to be comparable to that obtained in unsterilized peat.

The probable reason why gamma radiation caused a more pronounced decline in the rate of urea hydrolysis in mineral soils than in peat, is their lower level of organic matter. The organic matter content affects the amount of urease enzyme remaining in the soil before and after gamma radiation. Peat, was obviously richer in urease than the mineral soils. Exposure of these soils to the same dosage of radiation will cause a higher proportionate decline in the urease level of the mineral soils compared to peat. This was then reflected in the lower rate of urea hydrolysis by these mineral soils following gamma radiation.

Unlike the situation with heat sterilization, urea hydrolysis occurred in soils sterilized with gamma radiation (Table 18). The hydrolysis of urea was quite surprising, considering that, very high levels of gamma radiation were employed in this experiment, and that the sterility test performed on these soils produced a negative result. It is difficult to believe that urease could withstand a dose of 12×10^4 Gy. At first, chemical hydrolysis of urea catalyzed by soil organic matter was thought to be operative in the sterilized soils. This reaction, however, was most unlikely because, there was virtually no urea hydrolysis even in peat soil which has high level of organic matter, with heat sterilization (Tables 15 and 16). If urea hydrolysis was chemical in nature, it should have occurred in all sterilized soils regardless of the method of sterilization. It was thus concluded, that, even though soil sterilization was achieved, the doses of gamma

radiation employed here were unable to completely inactivate the urease enzyme in the soil.

It is not clear, why, with such high levels of gamma radiation, was complete inactivation of urease enzyme not achieved. The high radiation flux of the gamma source (2×10^4 Gy hr⁻¹) used for the irradiation, necessitated a short exposure time to obtain the required doses. Roberge and Knowles (1967), reported that, urease was almost completely inactivated by a $4-5 \times 10^4$ Gy radiation. But for a given dose, the measured urease activity was somewhat lower, when a lower radiation flux was used with a longer exposure time. A similar finding was reported by Forster et al (1984), who claimed to have achieved complete soil sterilization and enzyme inactivation at a dose of 4.55×10^4 Gy (much lower than any of the doses used in this experiment). To obtain this dose, however, their soil was exposed for 17.5 hr to a source having a radiation flux of 2.6×10^3 Gy hr⁻¹ (10 times lower than the flux employed here). With a longer exposure time, the gamma ray has more time to interact with the soil and a better chance of inactivating the enzyme system. In using gamma ray as a source of sterilization, the radiation flux as well as the exposure time should be considered *pari passu*.

Because of the incomplete inactivation of urease enzyme, and subsequent urea hydrolysis, it was not possible to determine in this experiment, whether or not urea interacts directly with soil organic matter. A very significant discovery, perhaps, as important as the objective earlier set out, was the observation that NH_4^+ fixation in all the soils was not affected by sterilization. The soils used in

this experiment were sterile. Also, the rate of urea hydrolysis was affected by sterilization. But from Figure 8 (a plot of fixed ammonium as a function of incubation time in sterilized and unsterilized sand), the pattern of ammonium fixation was virtually identical in sterilized and unsterilized sand. Data in Table 19 shows that similar results were obtained for all the other soils. This observation is important, for it completely eliminates biological activities as the pathway for the ammonium fixation measured. It can, thus, be concluded that, biological immobilization within the time period investigated, if it existed at all, was negligible. Ammonium fixation can then be said to occur via a physico-chemical process.

From published works on nitrogen immobilization, it seems as if much emphasis has been placed on the role of microorganisms in immobilization of nitrogen in the soil. A probable explanation for this is, that the bulk of immobilized nitrogen is usually found in the soil organic nitrogen fraction. If this is the case, it may lead to the conclusion that fixed nitrogen is microbial in origin. In most of the studies, no consideration was given to the ability of the soil organic fraction to undergo reaction with NH_3 . But as can be seen from the results of the analysis of organically-bound nitrogen in Experiment 3 (Table 14), about 80% of the ^{15}N fixed was found in the organic fraction. This will be the case if organic matter was responsible for sorbing NH_4^+ . Okereke and Meints (1984), reported that approximately 10% of the applied $(^{15}\text{NH}_4)_2\text{SO}_4$, was immobilized in organic (muck) soil after 12 hr of incubation. These authors believed that the observed retention of nitrogen was due to microbial immobilization. A very

strong point against such a conclusion is the unlikelihood of a microbial effect being exhibited within such a short period of incubation (12 hr). The direct evidence obtained in this experiment clearly points to the contrary. Microorganisms should ultimately contribute to nitrogen immobilization in the long run. It is perhaps, an exaggeration to attribute nitrogen retention, in a 12 hr period, entirely to microorganisms.

It was found in this experiment that heat sterilization resulted in the liberation of native fixed NH_4^+ . Complete sterilization was achieved as signified by lack of urea hydrolysis. From the data generated, it was not possible to state unequivocally that urea interacted directly with soil organic matter. If such interaction existed at all, the magnitude was probably small.

With gamma radiation, soil sterilization was achieved but the urease enzyme was not completely inactivated. The high flux of the radiation source, resulting in short exposure time, may be partially responsible for the incomplete urease inactivation. Rate of urea hydrolysis was significantly reduced by gamma radiation. Ammonium fixation was not affected by soil sterilization, confirming that the mechanism of NH_4^+ fixation was nonbiological.

Experiment 5 : Isotopic Exchange Of Fixed Ammonium

Substantial amounts of $^{15}\text{N-NH}_4^+$ were fixed during 1 wk of incubation. In the field the NH_4^+ ion would definitely interact with the soil for a longer period of time than was the case in the laboratory

studies conducted. It is then reasonable to believe that ammonium fixation, in field situations, may be more than that measured in the laboratory. The fixed NH_4^+ , could be a significant proportion of the applied nitrogen, particularly in soils of high organic matter and/or clay content. It becomes of importance, (from the viewpoint of soil nitrogen economy), to determine directly or indirectly, the fraction of fixed NH_4^+ that is labile and may be available to plants.

The extent to which the fixed $^{15}\text{N-NH}_4^+$ can be replaced (ie. isotopically exchanged), by $^{14}\text{N-NH}_4^+$, may be a good measure of the labile nature of fixed nitrogen. This, essentially, was the objective this experiment set out to achieve. It is known from Experiment 3 that fixed ammonium increased with time of incubation. It is then possible to vary the quantity of fixed ammonium in the soil by simply varying the length of the incubation time. The relationship between the amount of NH_4^+ initially fixed and the level of $^{15}\text{NH}_4^+$ isotopically exchangeable, and the effect of time of contact between $^{14}\text{NH}_4^+$ and the soil containing fixed $^{15}\text{NH}_4^+$ was investigated. A factorial design was employed in this experiment, where the various time of $^{15}\text{NH}_4^+$ fixation (primary incubation time: 1 day; 2 days; 4 days and 1 week), were factorially combined with time of ^{14}N equilibration with soil containing fixed ammonium (secondary incubation time: 1 hour; 6 hours; 12 hours and 24 hours), for each of the four soils. The primary incubation time was the length of time that applied urea was allowed to interact with the soil. Variation of this time resulted in soil samples containing varying levels of fixed NH_4^+ . The secondary incubation time on the other hand was the length of time $^{14}\text{NH}_4^+$ was

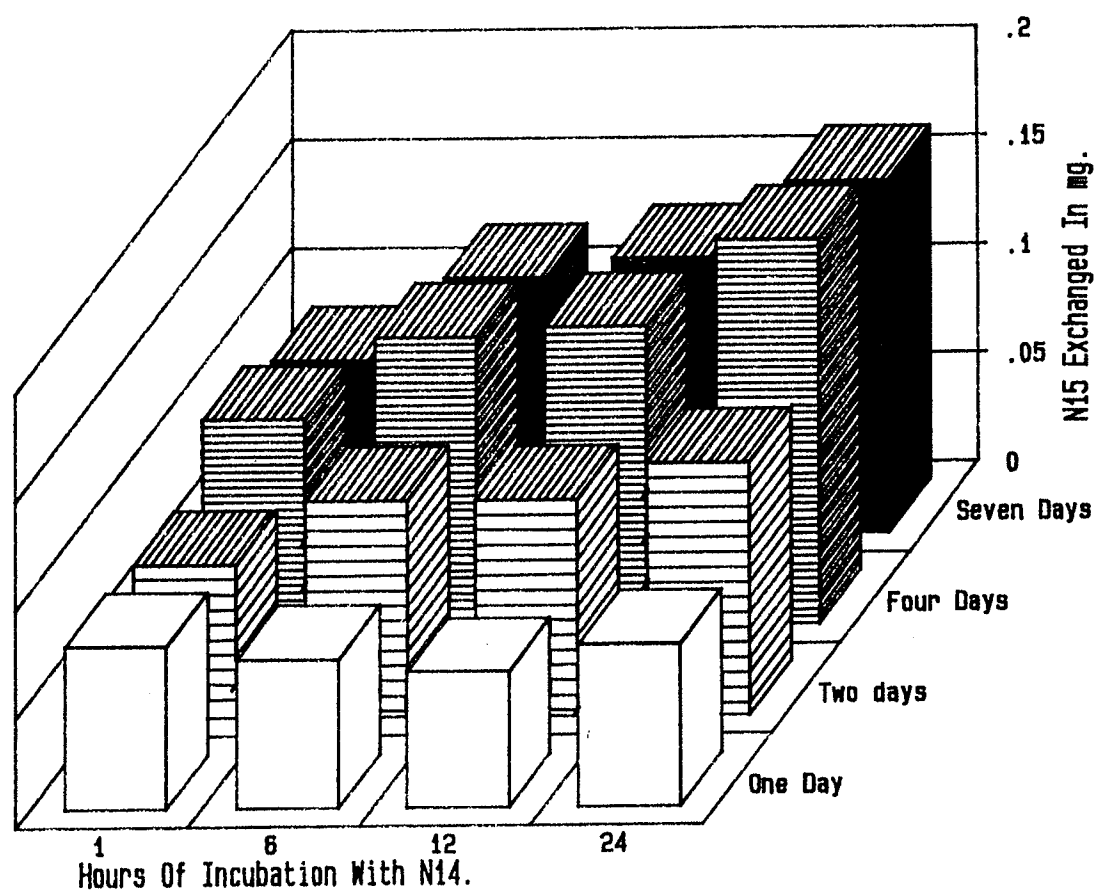


Fig. 9. Isotopic Exchange of Fixed Ammonium in Peat Soil.

equilibrated with soil containing fixed $^{15}\text{NH}_4^+$.

The results obtained in this experiment are shown as a 3-dimensional bar graph for each soil in Figures 9-12. After incubation of $^{14}\text{NH}_4^+$ with the soil containing fixed ^{15}N , a reasonable level of ^{15}N was obtained from the extracted solution. The ^{15}N level was high enough to be accurately measured by the mass spectrometer. The relatively high ^{15}N in this extract was not expected. Some previously published studies have indicated that fixed ammonium was isotopically unexchangeable. Newman and Oliver (1966), concluded from their study on isotopic exchange of fixed ammonium that, fixed ammonium was not isotopically exchangeable. Broadbent (1965), working on the effect of fertilizer nitrogen on the release of soil nitrogen, asserted that little if any exchange occurred between fixed ^{15}N and applied $^{14}\text{N-NH}_4^+$. On the contrary, Nommik (1957), found, in his study on fixation and defixation of NH_4^+ in soils, that a part of the fixed $^{14}\text{N-NH}_4^+$ that could not be replaced by K^+ was in equilibrium with $^{15}\text{NH}_4^+$ in solution. At higher NH_4^+ concentrations, this part was rather high, making up nearly 13% of the NH_4^+ determined to be fixed by the KCl leaching method.

The data generated from this experiment, (part of which was plotted in Figures 9-12), are shown in the Appendix. For individual soils, the amount of $^{15}\text{NH}_4^+$ replaced by $^{14}\text{NH}_4^+$ was greater as the amount of ammonium fixed by the soil was higher. In five grams of peat, for example, with a primary incubation time of 1 day, the amount of ^{15}N isotopically exchanged was 0.07 mg. This value increased to 0.12 mg when the primary incubation time was 2 days, then to 0.18 mg

with the 4-day sample, when ^{14}N was allowed to equilibrate with the soil for 24 hr.

In the peat soil, with the primary incubation time of 1 day, there was no effect of increasing secondary incubation time on the magnitude of ^{15}N exchanged. Thus, the amount of nitrogen isotopically exchanged 1 hr after the initiation of incubation with ^{14}N was of the same order of magnitude as that exchanged after 24 hr of incubation (see the Appendix). But when the primary incubation time was increased to 2 days (with the soil containing more fixed nitrogen than the 1 day sample), the effect of the length of secondary incubation on the magnitude of fixed ^{15}N exchanged was manifested. About 0.07 mg of ^{15}N was exchanged after 1 hr of secondary incubation in five grams of soil, this increased to 0.12 mg when the secondary incubation time was extended to 24 hr. A similar effect of increasing time of secondary incubation on the magnitude of isotopically exchanged nitrogen was observed for 4 days and 1 week with the peat samples.

In clay and the rest of the mineral soils, the pattern of isotopic exchange of fixed NH_4^+ was quite similar to that described for the peat soil above. Here, too, the magnitude of fixed ^{15}N that exchanged with $^{14}\text{NH}_4^+$ increased with increasing level of fixed ammonium. In these soils also, increasing the secondary incubation time, had no effect on the amount of ^{15}N that exchanged with ^{14}N in the soil pre-incubated for 1 day. Increasing the primary incubation time beyond 1 day, however, produced a response of the amount of ^{15}N exchanged to increasing secondary incubation time.

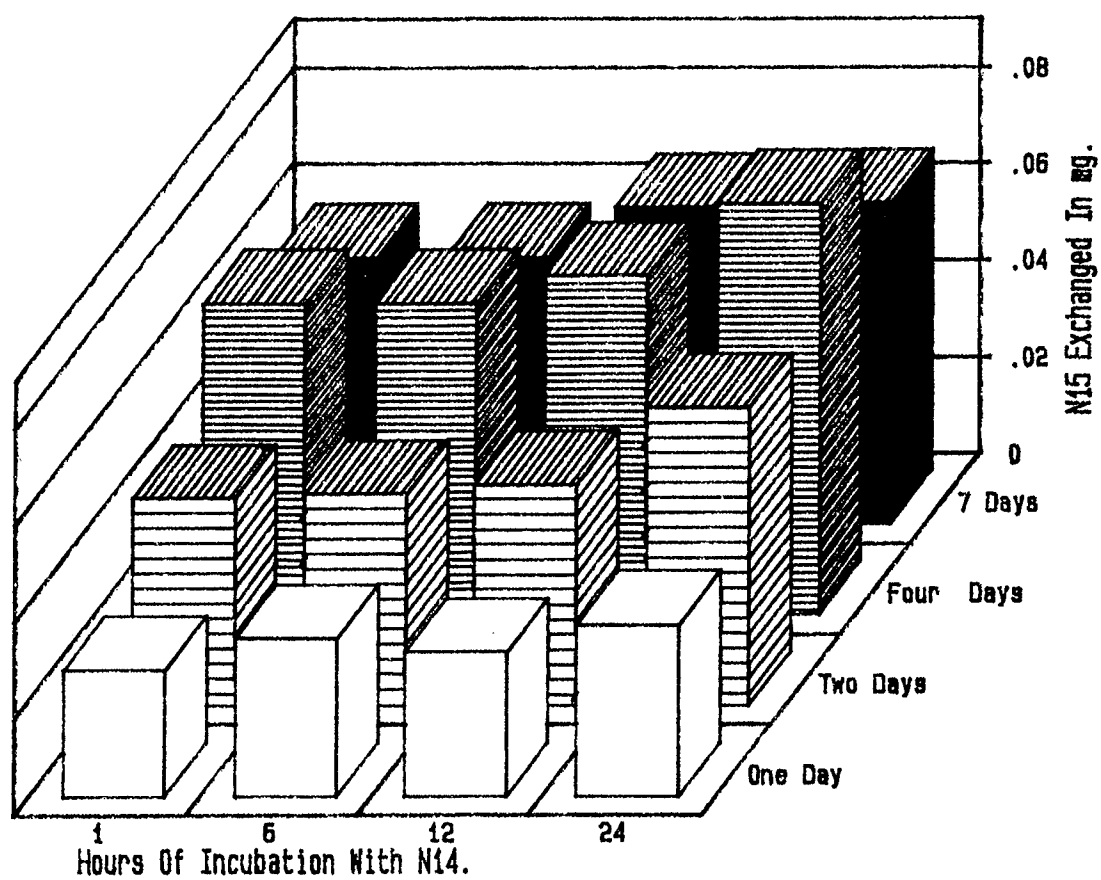


Fig. 10. Isotopic Exchange of Fixed Ammonium in Clay Soil.

Within each soil, the observation was made that the amount of nitrogen exchanged increased as the amount of ^{15}N originally fixed increased. This also holds for comparison between the four soils. Peat, which at any point in time contained the highest amount of fixed NH_4^+ , also had the highest magnitude of isotopically exchangeable NH_4^+ .

As portrayed by the four plots (Figures. 9-12), there were some differences in the behaviour of individual soils, either with respect to the effect of the levels of initially fixed ammonium or the length of time of the secondary incubation. Nevertheless, some general trends could still be observed.

On the average, about 30% of fixed $^{15}\text{NH}_4^+$ was isotopically exchangeable during the period of secondary incubation with ^{14}N . This percentage, was reasonably constant for all levels of fixed ammonium, indicating a linear relationship between the level of ammonium fixed and the amount of ^{15}N coming into solution. Also, an interaction between the length of time the ^{15}N has had to react with the soil and the rate of isotopic exchange could be observed. For example, when the primary incubation time between ^{15}N and the soil was 1 day, isotopic exchange was completed within 1 hr of ^{14}N addition in all soils. Increasing this secondary incubation time to 24 hr had no effect on the amount of ^{15}N that exchanged (see Appendix). The implications of this observation are two-fold. Firstly, that isotopic exchange is a rather fast process, sometimes reaching equilibrium in less than 1 hour. This concurs with the result of Newman and Oliver (1966), who reported that most of the isotopic dilution (in their study of isotopic exchange of fixed ammonium), occurred within 10 minutes of adding solution to the

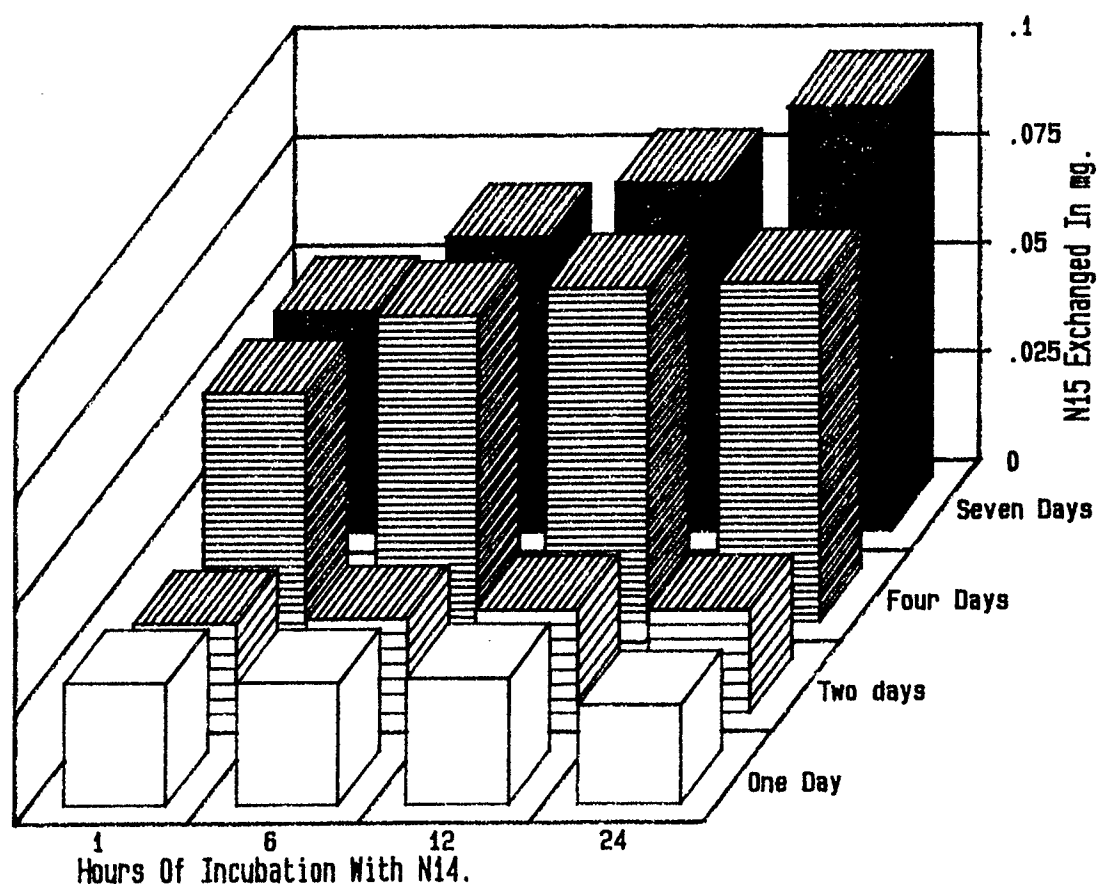


Fig. 11. Isotopic Exchange of Fixed Ammonium in Loam Soil.

solid. Secondly, it implies that the effect of period of secondary incubation on the amount of isotopically exchangeable NH_4^+ , depends on the length of time of primary incubation of ^{15}N with soil (ie. how rapidly the fixed NH_4^+ came out into solution, depended on the length of time it had to react with the soil). Thus, while length of time of secondary incubation had no effect on the level of ^{15}N exchanged for samples initially incubated for 1 day, a response was observed on samples initially incubated for 2 to 7 days. For samples incubated initially for 4 days, for example, not only was the amount of fixed nitrogen isotopically exchangeable at any point in time higher than with 1 or 2-day samples, but the level of ^{15}N exchanged also increased as the contact time increased from 1 to 24 hours.

The result for sample initially incubated for 1 wk did not follow the general pattern observed with the other samples. The amount of ^{15}N exchanged was actually lower for samples incubated initially for 1 week than was for samples incubated for 4 days. This observed deviation may be entirely due to experimental error or it may have resulted from a change of the NH_4^+ -organic matter complex into a more stable form with time. The actual cause is unknown.

The relatively high level of isotopic exchange obtained in this experiment would suggest that a portion of the fixed ammonium could ultimately be available to plants. This could be through their participation in exchange reactions. There is a possibility though, that the NH_4^+ -organic matter complex could undergo a condensation reaction resulting in fixed ammonium being rendered less available with time. The result obtained with the samples incubated for 1 week seemed

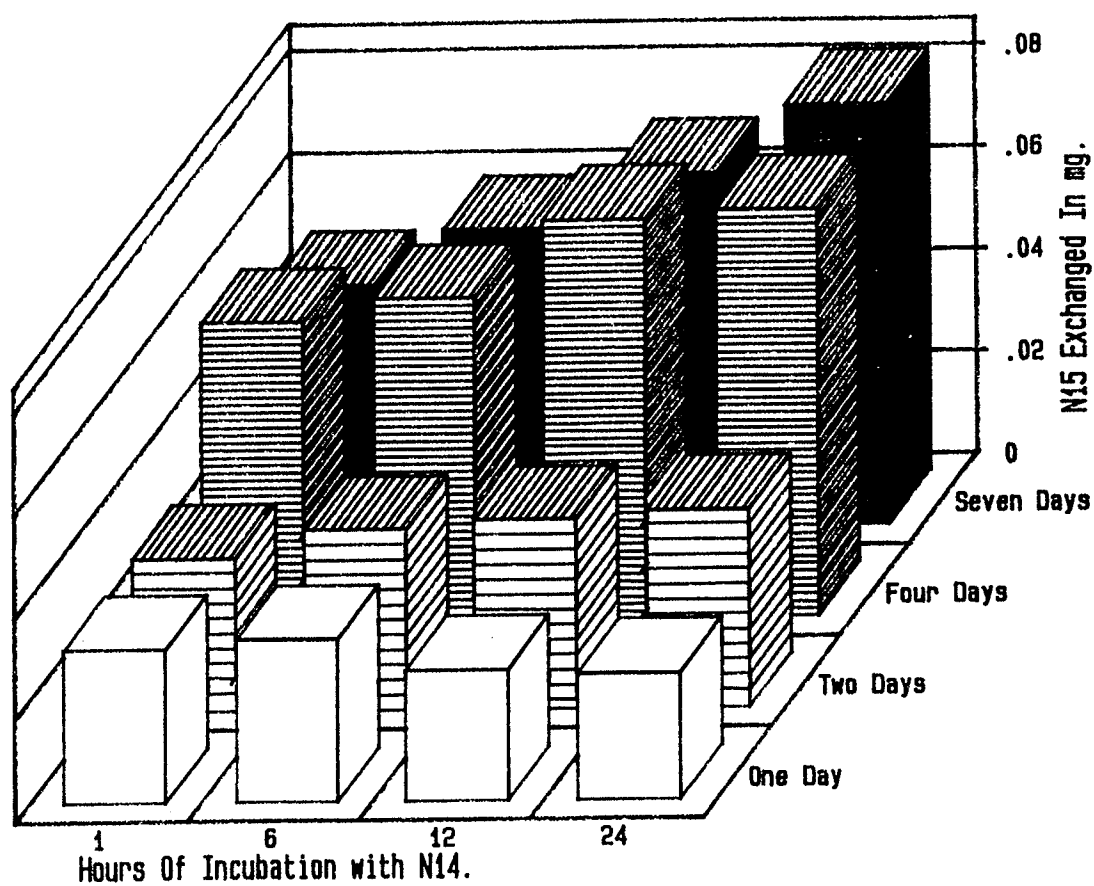


Fig. 12. Isotopic Exchange of Fixed Ammonium in Sand Soil.

to show that some fixed $^{15}\text{NH}_4^+$ may not be as readily exchangeable as those contained in samples which had been subjected to a shorter period of incubation. Burge and Broadbent (1961), utilized an organic soil containing fixed NH_4^+ to grow Sudan grass. They observed that progressively less and less of fixed ammonium was picked up by successive cuttings. They attributed this observation to possible stabilization of the fixed ammonia.

By incubating soil containing fixed $^{15}\text{N-NH}_4^+$ with an ^{14}N -source, significant amounts of isotopic exchange was obtained. The level of ^{15}N exchanged was a linear function of the amount of NH_4^+ initially fixed. The rate of exchange was found to be quite rapid in soils containing low levels of fixed ammonium. As the level of fixed NH_4^+ increased, the amount of ^{15}N exchanged with time also increased. The result of the samples that were initially incubated for 1 wk - which had less isotopically exchanged ^{15}N than samples initially incubated for 4 days - may suggest a change to a more stable form with time.

SUMMARY AND CONCLUSIONS

Using unlabelled ammonium carbonate and urea, the extent of NH_4^+ fixation was studied in four Manitoba soils. Nitrogen was applied at a concentration of 1000 ug per gram of soil as a solution and incubated for varying periods of time ranging from 1 hr to 1 wk. Fixation was determined using the difference method (ie. the amount of NH_4^+ -N added - the amount of NH_4^+ -N extracted by 1 mol L^{-1} KCl).

Enzymatic hydrolysis of urea was found to follow first order reaction kinetics. Fixation of NH_4^+ increased continuously up to 1 week of incubation (maximum length of time studied), without showing any sign of saturation. The magnitude of ammonium fixation was positively correlated with the organic matter content of the soil.

Treatment of the soil with HF-HCl was employed to destroy the clay fraction thereby isolating the organic matter which was then used directly for fixation studies. The acid treatment was very effective in destroying the clay fraction, however, complete elimination of the mineral fraction was not achieved. Acid treatment resulted in increased soil organic matter, reduced clay content, reduced pH and an increase in the level of exchangeable ammonium in the soil.

Hydrolysis of urea was inhibited in acid-treated soil. Peat treated with HF, showed a decline in the level of extractable urea without a corresponding increase in ammonium concentration. Acid-treated soils incubated with ammonium carbonate showed a weak tendency of higher ammonium fixation than their untreated counterpart.

With the aid of ^{15}N -labelled nitrogen sources, it was possible to

determine precisely the amount of added nitrogen retained in the soil against KCl extraction. Ammonium fixation in all the soils was found to be a function of incubation time. Fixation increased rapidly at the initial stage of the incubation and later attained an equilibrium level in the mineral soils. Peat did not show any sign of this saturation throughout the incubation period.

Ammonium fixation was not only a function of time, but was also concentration dependent. The absolute amount of fixed NH_4^+ increased with an increase in the level of applied ammonium nitrogen. The proportion of applied ammonium fixed, however, decreased as the amount of ammonium applied was increased. Fixation of ammonium in all the soils was positively correlated with the organic matter content. Soil organic matter accounted for over 80% of fixed ammonium in the soil. The interaction between soil organic matter and ammonium could best be described by first order reaction kinetics.

To investigate the possibility of direct urea involvement in fixation reaction, soil sterilization was carried out. Soils were sterilized using heat and gamma radiation. This was to ensure that the urea remained intact in the soil by eliminating microbial and enzymatic activities. It was found that heat sterilization resulted in the liberation of clay fixed ammonium. Complete sterilization was achieved as signified by the absence of urea hydrolysis in heat-sterilized soil. From the data generated, it was not possible to state unequivocally that urea interacted directly with soil organic matter. If such an interaction existed at all, the magnitude was probably small.

With gamma radiation, soil sterilization was achieved, but the

urease enzyme was not completely inactivated. The high radiation flux of the source, necessitating a short exposure time, may be partly responsible for the incomplete inactivation of urease. Rate of urea hydrolysis was significantly reduced by gamma radiation. Ammonium fixation was not affected by soil sterilization confirming that the mechanism of NH_4^+ fixation was nonbiological.

By incubating soil containing fixed $^{15}\text{NH}_4^+$ with an ^{14}N -source, a significant amount of isotopic exchange was obtained. The level of ^{15}N exchanged was a linear function of the amount of ammonium initially fixed. The rate of isotopic exchange was found to be quite rapid in soil containing a low level of fixed ammonium. As the level of fixed ammonium increased, the level of fixed ^{15}N exchanged also increased with time. The results from samples initially incubated for 1 week, which had less isotopically exchanged ^{15}N than the samples incubated initially for 4 days, might suggest reversion to a more stable form with time.

From the level of ammonium fixation obtained in the four soils it could be concluded that ammonium fixation is unlikely to be a factor of importance in the nitrogen economy of sandy soils such as the Almasippi. In soils of high clay and/or organic matter content, however, significant amount of applied $\text{NH}_4^+\text{-N}$ may be tied up by the soil and rendered unavailable to plants.

PROPOSAL FOR FURTHER STUDIES

From the insight gained on ammonium fixation in this study, the following areas are suggested as worthy of further investigation.

1. The effect of temperature on ammonium fixation (-5 to 100°C).
2. The effect of varying soil pH on ammonium fixation.
3. Fixation of ammonium in HF-treated soil using ^{15}N -labelled urea and ammonium sulphate.
4. Fractionation of the soil organic matter containing fixed ammonium to determine the amount of fixed ammonium in the different fractions.
5. Determination of the portion of fixed ammonium that can be taken up by plants, correlating this with the amount that is isotopically exchangeable.
6. Long term fixation study. To determine, for example, the equilibrium point for peat soil.
7. To determine whether or not fixed ammonium does revert to a less labile form with time. ie. what is the effect of the length of time after fixation on isotopic exchange of fixed ammonium or plant availability of fixed ammonium ?

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APPENDIX

Data Generated On the Isotopic Exchange Of Fixed NH_4^+ in 5g of soil

Isotopic Exchange After 1 Day Of Primary Incubation					
	Time (hrs)	Total N2 (mg)	%N15 (2 ^{0d} incu.)	%N15 (1 ⁰ incu.)	Isoexch. (mg)
Peat	1	4.37	1.75	81.58	0.075
	6	4.14	1.68	81.58	0.068
	12	4.28	1.54	81.58	0.063
	24	4.23	1.77	81.58	0.074
Clay	1	4.48	0.88	88.94	0.026
	6	4.57	1.01	88.94	0.033
	12	4.82	0.92	88.94	0.030
	24	4.82	1.03	88.94	0.036
Loam	1	4.75	0.89	88.15	0.028
	6	4.71	0.89	88.15	0.028
	12	4.83	0.89	88.15	0.029
	24	4.91	0.78	88.15	0.023
Sand	1	4.58	0.94	87.75	0.030
	6	4.67	0.97	87.75	0.032
	12	4.78	0.84	87.75	0.026
	24	4.72	0.83	87.75	0.025

Isotopic Exchange After 2 Days Of Primary Incubation

	1	4.64	1.49	75.71	0.070
Peat	6	4.63	1.96	75.71	0.100
	12	4.80	1.89	75.71	0.099
	24	4.61	2.24	75.71	0.117
	1	4.80	1.15	89.01	0.043
Clay	6	4.91	1.17	89.01	0.044
	12	4.89	1.19	89.01	0.046
	24	4.81	1.49	89.01	0.062
	1	4.79	0.76	88.67	0.021
Loam	6	4.80	0.78	88.67	0.022
	12	4.86	0.81	88.67	0.024
	24	4.75	0.82	88.67	0.024
	1	4.81	0.92	88.98	0.030
Sand	6	4.96	0.99	88.98	0.035
	12	5.00	1.02	88.98	0.037
	24	4.89	1.08	88.98	0.039

Isotopic Exchange After 4 Days Of Primary Incubation

Peat	1	4.54	1.84	71.23	0.096
	6	4.61	2.36	71.23	0.133
	12	4.30	2.57	71.23	0.138
	24	4.26	3.20	71.23	0.177
Clay	1	4.65	1.56	86.94	0.065
	6	4.53	1.59	86.94	0.065
	12	4.49	1.71	86.94	0.071
	24	4.54	1.96	86.94	0.085
Loam	1	4.68	1.38	90.25	0.053
	6	4.69	1.71	90.25	0.071
	12	4.61	1.84	90.25	0.077
	24	4.54	1.89	90.25	0.078
Sand	1	4.58	1.49	90.68	0.058
	6	4.63	1.58	90.68	0.063
	12	4.50	1.91	90.68	0.078
	24	4.58	1.92	90.68	0.080

Isotopic Exchange After 7 Days Of Primary Incubation

	1	4.33	1.60	67.54	0.081
Peat	6	4.58	2.08	67.54	0.119
	12	4.55	2.20	67.54	0.127
	24	4.72	2.60	67.54	0.162
	1	4.59	1.16	85.74	0.043
Clay	6	4.45	1.22	85.74	0.045
	12	4.73	1.55	85.74	0.066
	24	4.73	1.56	85.74	0.067
	1	4.56	1.35	89.76	0.051
Loam	6	4.45	1.72	89.76	0.068
	12	4.56	1.92	89.76	0.080
	24	4.73	2.19	89.76	0.098
	1	4.59	1.28	88.83	0.048
Sand	6	4.53	1.50	88.83	0.058
	12	4.63	1.68	88.83	0.069
	24	4.61	1.92	88.83	0.082