FIXATION OF ADDED AMMONIUM BY THE CLAY

AND ORGANIC FRACTIONS OF SOIL

by

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INTRODUCTION

Ammonia fixation has been defined as adsorption of ammonium ions by soils or minerals in such a form that they are neither water soluble nor readily exchangeable. Forty-five years ago McBeth (1917) reported that ammonium added in the salt form to certain soils could not be quantitatively recovered as soluble or exchangeable ammonium. Since then, many investigations have been made to determine the factors responsible for ammonium fixation in soil.

Vermiculite and degraded illite type clay minerals have been found to react with ammonium or potassium ions to form a clay-ammonium or clay-potassium complex under moist conditions at room temperatures. Studies of ion-fixation by clay minerals have been considered important since the location of the fixed-ion between the layers of micaceous minerals such as illite, vermiculite and montmorillonite was the same site as that formerly occupied by potassium in the primary mineral structure. Information about the nature and weathering of micaceous minerals has been obtained from studies of ammonium and potassium ion fixation. The fixed ion has been considered to be less available to biological systems than readily exchangeable or water-soluble forms of the same ion.

Mattson and Koutler-Andersson (1943) reported that ammonium hydroxide would react with organic soils and plant materials, particularly lignin, to form complex organic

nitrogen compounds. The ammonium which had reacted was considered fixed since it was no longer water-soluble nor readily exchangeable ammonium. While a small amount of ammonia was found to be fixed by organic materials under acid conditions, a much larger amount of ammonia was found to be fixed under alkaline conditions. Mattson and Koutler-Andersson were primarily interested in fixation of ammonia by organic materials as a means of determining the type of organic structure(s) which might account for the accumulation of organic nitrogen in soil. If the chemical structure of soil organic nitrogen compounds could be established, it might help explain why only about one to three percent of the organic nitrogen in soil becomes available to plants during one year.

Investigations of the ammonium fixation by soil clay minerals have been restricted largely to subsoils low in organic matter and clay minerals from natural deposits that are relatively pure. The ammoniation of clays and surface soils has been carried out primarily with ammonium salts under neutral or acidic conditions to avoid interference by organic materials. Studies on the fixation of ammonia by organic materials have been restricted mainly to organic soils or organic materials free of clay minerals capable of fixing added ammonium.

The increased use of alkaline forms of ammonium fertilizers, such as anhydrous ammonia, aqua ammonia, and nitrogen

solutions would seem to increase the possibility for reaction
of the ammonium with the organic matter normally found in sur-
face soils. Few data have been available to indicate whether
added ammonium would react with the organic fraction of min-
eral soils.

Studies on ammonium fixation with mineral surface soils
have been handicapped by the lack of a method which could
quantitatively separate the soil organic and mineral frac-
tions without drastic chemical alteration of both fractions.
Determination of ammonium fixation by organic and mineral
fractions without separation was handicapped by the follow-
ing problems: (1) part of the soil organic fraction is
solubilized in the presence of alkaline solutions; (2) ex-
traction of a surface soil by neutral salt solutions removes
some soil organic matter; (3) some ammonium may be released
during alkaline distillation of soil extracts as a result of
partial decomposition of soluble organic matter; (4) release
of ammonium from soil organic nitrogen has interfered with
certain methods used to estimate ammonium fixation by the
clay fraction.

Recent developments in methods appeared to overcome
problems 3 and 4 above. The proper use of the new methods
gave promise of allowing investigation of the following
hypothesis: that ammonium added to surface mineral soils,
i.e. those containing less than 20 percent organic matter,

would react with both the organic and the clay fraction of the soil to become "fixed" ammonium.

The objectives of the studies in this dissertation were as follows:

1. Develop a reliable procedure for estimating the total amount of added ammonium which was fixed by a mineral surface soil. The procedure used to estimate ammonium fixation must be independent of the conditions employed for ammoniating the sample.

2. Fractionate the total ammonium fixed by a mineral soil into the amount fixed by the mineral fraction and the amount fixed by the organic fraction.

3. Investigate some factors which may influence the fixation of added ammonium by the organic and mineral fractions of mineral soils.

4. Determine the amount of added ammonium fixed by some Iowa soils.

LITERATURE REVIEW

Ammonium added to soil may be retained as exchangeable, soluble, or fixed ammonium. Physically sorbed ammonia can react with a proton to become ammonium and then be retained in one of the forms described above. The chemical, physical and biological reactions of ammonium in soils have been reviewed by Mortland (1958) and McIntosh (1958).

Fixation of added ammonium was first reported by McBeth in 1917 after he found that extensive leaching with 10 percent HCl would not remove more than 81 percent of the ammonium that had been added to a soil. The ammonium which was not removed was termed "fixed" by McBeth. The definition of fixed ammonia as approved by the Soil Science Society of America (1956) is "adsorption of ammonium ions by soils or minerals in such a form that they are neither water soluble nor readily exchangeable." Factors affecting ammonium fixation would be expected to include: (1) the conditions employed for the determination of the amount of ammonium fixed; (2) the kind of fixing material under study; and (3) the reaction conditions employed during the fixing process.

Methods of Determining Ammonium Fixation

A variety of methods have been used to estimate ammonium fixation. Procedures employing extraction as a means of recovering added ammonium have been used most frequently and,

historically, were the first methods used. As evidence accu-
mulated on the structures and chemical reactions of ammonium
fixing clay minerals, attempts were made to devise methods
which would recover specifically the fixed ammonium retained
in the clay minerals. Pure clay mineral systems or subsoils
devoid of appreciable organic matter were analyzed for fixed
ammonium (following removal of soluble and exchangeable
ammonium) by either Kjeldahl or HF digestion and subsequent
alkaline distillation. Only recently have procedures been
developed (Dhariwal and Stevenson, 1958; Bremner, 1959) where
the ammonium fixed in soil clay minerals could be isolated
quantitatively from samples containing soil organic matter.
A summary of the several methods which have been employed for
ammonium fixation studies are presented below. It should be
kept in mind that, except where noted, these investigations
assumed that all of the ammonium fixation in mineral soils
was due to clay minerals. Usually the ammoniation reaction
was carried out with NH_4Cl, $(NH_4)_2SO_4$, or ammonium acetate
as the ammonium source.

Methods employed with clay minerals

Extraction methods

Extract analysis   In the extract analysis pro-
cedure, a known amount of ammonium salt was added to the
sample. Following the reaction period the sample was leached

with a solution containing a cation other than ammonium, thus removing the water soluble and exchangeable ammonium. The extract was then analyzed for ammonium content. Fixation of added ammonium was calculated by the difference between the amount of ammonium added and the amount of ammonium recovered in the extract less the ammonium content found in the extract from a control sample.

Residue analysis   Following the ammoniation reaction with an ammonium salt, the sample was extracted as described above. The residue left after extraction was then analyzed for total nitrogen content by Kjeldahl analysis. Ammonium fixation was calculated by difference in nitrogen content between the ammoniated sample and control sample.

Aeration method   Leggett (1958) proposed the addition of K_2CO_3 solution to the ammoniated surface soil and that the system then be aerated as a means of distilling off ammonium without concurrent release of soil organic nitrogen as ammonium. With this method, solubilization of organic matter by addition of alkaline ammonium sources would not cause interference in the determination. The K_2CO_3 should serve to replace exchangeable ammonium while simultaneously blocking the release of clay fixed ammonium. The method was relatively fast and simple. However, after trial of Leggett's method, the following limitations were observed: (1) the length of aeration and the aeration rate were critical and difficult

to control; (2) it was difficult to obtain good replication
of results; (3) the effect of pH on ammonium fixation could
not be studied; and (4) native clay-fixed ammonium was not
determined.

Alkali distillation   Barshad (1951) proposed that
clay-fixed ammonium in soils could be determined by an alka-
line distillation procedure. One member of a sample pair was
distilled with NaOH while the other sample was distilled with
KOH. The procedure was based on the assumption that sodium
ions would replace all of the clay-fixed ammonium while
potassium ions would not replace the clay-fixed ammonium.
Clay-fixed ammonium was based on the ammonium recovered by
NaOH distillation minus the ammonium recovered by KOH dis-
tillation.

Hanway and Scott (1956) obtained comparable results for
the extraction methods employed by Allison et al. (1951) and
the alkali distillation method of Barshad (1951) for deter-
mination of ammonium fixation. However, Hanway et al. (1957)
found that the presence of potassium ions in the NaOH solution
during distillation may prevent the complete release of clay-
fixed ammonium.

Hydrofluoric acid   Rodriguez (1954) was the first to
estimate clay-fixed ammonium directly by digesting the clay
mineral fraction with HF and distilling the released fixed
ammonium. However, the method did not preclude the inter-

ference of organic nitrogen nor did he present evidence on the recovery of fixed ammonium from clay.

Dhariwal and Stevenson (1958) and Bremner (1959) developed reliable methods of determining clay-fixed ammonium directly in surface soils; i.e., in the presence of organic matter. The Dhariwal-Stevenson method was based on (1) destruction of alkali-labile organic nitrogen by autoclaving the sample in the presence of KOH; (2) separation of the residue containing the clay-fixed ammonium from the alkali-soluble organic matter; (3) destruction of the silicate clay mineral fraction of the residue with HF, thereby releasing the clay-fixed ammonium into solution; (4) recovery of the clay-fixed ammonium by distillation.

The Bremner method was based on (1) extraction of soluble and exchangeable ammonium by KCl extraction; (2) digestion of the silicate clay mineral fraction by HF; (3) separation of the residue from the HF solution by filtration; (4) distillation of the HF filtrate with MgO at 25° C which does not cause release of nitrogen as ammonium from soil organic matter.

Both methods base the amount of clay-fixed ammonium on determination of the fixed ammonium and not on differences which include nitrogen released from other sources.

Methods employed with organic materials

Few reports were found in the literature describing methods for measuring ammonium fixation by the organic fraction of soil. Studies of ammonium fixation in mineral soils have been carried out using neutral or acidic ammonium sources, and hence, the investigators assumed that the ammonium did not react with soil organic matter to form organic nitrogen compounds. Most alkaline ammoniations of organic materials, peats, coal, sawdust, and lignin, have been carried out with the objective of getting maximum retention of ammonium in soluble, exchangeable and organic forms so that the material could be used as nitrogen fertilizer.

The three investigations cited below represent the only significant contributions found.

Mattson and Koutler-Andersson (1941, 1942, and 1943) electrodialyzed or leached with HCl and electrodialyzed the samples following the removal of volatile ammonia. The ammonium fixation was calculated by difference in the total (Kjeldahl) nitrogen content of ammoniated and control samples.

Sohn and Peech (1958) employed paired samples in their analysis scheme. One member of the sample pair was analyzed for total nitrogen by Kjeldahl immediately following removal of volatile ammonia. The remaining sample was extracted with NaCl and the extract analyzed for ammonium by MgO macrodistillation or Nesslerization. Total ammonium fixation was

calculated by difference in the total nitrogen content of
ammoniated sample and control sample less the difference in
the ammonium content of the NaCl extracts of the ammoniated
sample and control sample. Fixation by the organic fraction
was estimated by subtraction of the amount of potassium fixed
by the mineral fraction from the total ammonium fixation of
the soil. This procedure of estimating organic ammonium fixa-
tion was subject to the following errors: (1) the method
employed for estimating the fixing capacity of clay was inade-
quate and (2) the conditions of ammoniation were not constant
between samples; thus, there may have been variation in the
amount of total ammonium fixed.

Burge and Broadbent (1961), working with organic soils,
digested and removed the clay mineral fraction with a HF-HCl
mixture prior to ammoniation with gaseous ammonia. Following
ammoniation they removed the soluble and exchangeable ammonia
by HCl extraction and determined the nitrogen content of the
residue by Kjeldahl analysis. Ammonium fixation was calcu-
lated by difference in residual nitrogen content of ammoniated
and control samples.

The three investigations cited above have not properly
accounted for organic nitrogen solubilized and removed by
extraction and have not properly accounted for clay-fixed
ammonium. The samples were ammoniated by massive amounts
of ammonium hydroxide or gaseous ammonia under conditions

that would be hard to reproduce. Following the ammoniation
reaction, the water soluble and physically sorbed ammonia
have been removed by acid extraction or volatilization of
the ammonia by drying the sample with heat, aeration or vac-
uum. These conditions are difficult to reproduce and may
affect the amount of added ammonium fixed. Thus, valid
comparisons of ammonium fixation values between different
samples and different investigations would be difficult.

Ammonium Fixation by Clay Minerals

Clay minerals have been studied very extensively. As a
result the theories for both the crystal structure and the
chemical behavior of the clay-ammonium fixation reaction have
been supported by many researchers. Gieseking (1949),
Reitemeier (1951), Kardos (1955), and Rich and Thomas (1960)
have written excellent reviews which cover the extensive
literature in this subject matter area. A summary of points
important for the development and understanding of this dis-
sertation will be presented below.

Ammonium fixing clay minerals

Ammonium fixation by clays has been shown to occur with
silicate clay minerals of the 2:1 (silica to alumina) layer
lattice type. Silicate clay minerals of the 1:1 layer lat-
tice type have not shown any appreciable capacity to fix

ammonium. Of the 2:1 type clay minerals, only the vermicu-
lite type have the capacity to fix added ammonium under moist
conditions at room temperature. The illite type clays exhibit
ammonium fixation under the same reaction conditions as ver-
miculite only after the illite has been partially degraded by
the removal of some interlayer potassium before the addition
of ammonium. Montmorillonite type clays exhibit ammonium
fixation only when the system has been dried by either heat-
ing or freezing in the presence of ammonium.

Structure of 2:1 type clay minerals

The structure of 2:1 type clay minerals has been postu-
lated as follows. A layer of aluminum hydroxide (gibbsite)
in the form of octahedrons has been sandwiched between two
layers of silicon oxide tetrahedrons. The apex of the silica
tetrahedron is pointed inward toward the aluminum octahedron.
The apical oxygen of the silica tetrahedron is shared with
one of the oxygens of the alumina octahedron. The silica
tetrahedral layer has a hexagonal arrangement so that voids
surrounded by six oxygens occur in the outer oxygen (base
of the tetrahedron) layer. The diameter of the void has
been estimated to be 2.5 $\overset{o}{A}$ which is only a few tenths of
an angstrom smaller in diameter than the diameter of a
dehydrated potassium-ion, 2.66 $\overset{o}{A}$, or dehydrated ammonium,
2.86 $\overset{o}{A}$. Up to 15 percent isomorphous substitution of aluminum

for silicon has been found in the silica tetrahedran and some
isomorphous substitution of divalent ions has been found for
aluminum in the aluminum octahedral layer; these substitutions
result in a net negative charge in the clay crystal which must
be balanced by a cation exterior to the crystal lattice.
Vermiculite and illite have been found to have a larger amount
of isomorphous substitution of aluminum for silicon than
found in montmorillonite. Isomorphous substitution of
divalent ions for aluminum in the aluminum octahedral layer
has been found to account for most of the negative charge
found in montmorillonite. Thus, the distance from the charge
site to the clay mineral crystal surface is greater in mont-
morillonite than it is in vermiculite and illite.

Mechanism of fixation

If the force of attraction between the positive hydrated
potassium or ammonium ion and the negatively charged clay
mineral is of sufficient strength to strip the water shell
from the positive ion, the clay plates and the positive ion
are drawn together. The positive ion may then be positioned
within the voids of the silica tetrahedral layer (Page and
Baver, 1939). The unit lattice spacing decreases from
approximately 14 Å before fixation to approximately 10 Å
after fixation has occurred. After fixation, the space
between the unit lattices of the clay mineral will be smaller

than the diameter of the fixed ammonium or potassium ion or
of other hydrated cations; thus, exchange of the fixed ion
with another ion in the external solution can not take place
unless the clay plates can be forced apart.

Some factors affecting ammonium fixation by clay

Cation exchange   The fixation value obtained for clay
fixed ammonium depends upon the type of cation employed for
replacement of the "readily exchangeable ammonium".

Barshad (1951) found that ammonium fixed in vermiculite
could be replaced by Li, Na, Mg, Ca, and Ba ions, but could
not be replaced by K, Rb, and Cs ions. Allison and Roller
(1955a) compared extracting solutions composed of chlorides
of K, Na, Ca, Mg, and H with respect to the ability of these
cations to exchange with ammonium fixed by illite and vermicu-
lite clay minerals. NaCl was found to extract the greatest
amount of ammonium and KCl extracted the smallest amount of
ammonium. They suggested that fixed ammonium be designated
as that ammonium not removed by KCl extraction.

Potassium   Stanford and Pierre (1946) found that
ammonium and potassium could be fixed by the clay fraction of
soil either singly or simultaneously, but that the total
amount of potassium and/or ammonium fixed was constant.

Hanway et al. (1957) found that the presence of small
amounts of potassium ions or ammonium in the solution external

to the clay mineral containing "fixed" potassium or ammonium
ions would block the release of the fixed ion from the clay
mineral. Fixed ammonium in vermiculite or bentonite (mont-
morillonite) could be completely released by 0.2 normal NaOH
distillation provided less than two potassium ions were
present per 100 sodium ions. The release of fixed ammonium
from clays by NaCl extraction could be blocked by the presence
of either ammonium or potassium ions in the NaCl extracting
solution. These investigators suggested that the presence of
exchangeable potassium in soils might be of importance in
restricting the release of clay-fixed ammonium to nitrifiers.
This hypothesis was later supported by the work of Welch
(1958).

<u>Aluminum, hydrogen and iron</u>   McBeth (1917) reported
that Al and Fe salts added to soils prior to ammoniation re-
duced the magnitude of ammonium fixation. Stanford (1948)
observed that Al, H, and Fe ions, which he classed as diffi-
cultly exchangeable, reduced potassium fixation by illite and
bentonite clay minerals. Edwards (1960) found that extraction
of Fe and Al oxides and interlayer Al tended to increase the
exchange capacity and the ammonium fixing capacity of benton-
ite. It has been suggested that the reduction in ion fixation
caused by Al, H, and Fe may be due to one of the following:
(1) blockage of the collapse of adjacent crystal units;
(2) inability of potassium or ammonium ions to effect complete

exchange with the Al, Fe, or H ions or (3) formation of insoluble precipitates with phosphate anions present in the system as described below.

<u>Anions</u>   Soluble phosphate has been shown to react with soluble iron and aluminum to form insoluble complexes. If the cation associated with the phosphate is ammonium, then an ammonium-aluminum-phosphate or ammonium-iron-phosphate precipitate may be formed (Haseman <u>et al.</u>, 1950a and 1950b). The pH, time, phosphate, and iron or aluminum concentration required for formation of the precipitate would indicate that the amount of precipitate formed during ammoniation of most soils would be negligible. Bremner[1] found only 25-50 parts per million of ammonium fixed as an iron phosphate in a soil known to be high in soluble iron under ideal reaction conditions, i.e., high levels of soluble ammonium and phosphate added.

Leggett (1958) found that changing the anion accompanying the ammonium had little effect on ammonium fixation by exfoliated vermiculite. The vermiculite was found to fix 4.1, 4.0, 4.7, 3.8, 4.0 and 4.2 m.e. NH$_4$-N/100g following ammoniation with the ammonium salts of chloride, sulfate, phosphate (dibasic), citrate, tartrate and acetate respec-

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[1]J. M. Bremner, Agronomy Department, Iowa State University, Ames, Iowa. Formation of taranakites in soils. Personal communication. 1961.

tively.

Time   Leggett (1958) reported that ammonium fixation
by soil clay minerals and vermiculite was rapid. The fixation
of ammonium was 92 and 98 percent complete after ammoniation
of the sample for 1 and 24 hours respectively when based on
the total ammonium fixation which occurred after ammoniation
for 7 days.

Nõmmik (1957) reported ammonium fixation by soil clay
minerals was 77 and 90 percent complete after 1 and 24 hours
of ammoniation when total fixation was based on an ammonia-
tion period of 6 days.

Concentration of fixable ion   Levine and Joffe (1947)
found a linear relationship between the amount of potassium
added and the amount fixed until the amount of potassium added
was equal to the cation exchange capacity of the fixing min-
eral. Fixation of added potassium increased slowly at a
decreasing rate when the amount of potassium added exceeded
the CEC of the fixing mineral. Nõmmik (1957) reported that
the amount of added ammonium fixed by the mineral fraction of
soil was 0.83, 3.18, 4.48 and 6 me NH_4-N/100g when ammonium
was added at concentrations of 1, 10, 40, and 500 me
NH_4-N/100g.

pH of fixing system   Potassium and ammonium fixation
by clay minerals tends to increase with increasing calcium
saturation (Reitemeier, 1951). It is difficult to separate

the effect of associated ions such as Ca, Mg, and Na from the effect of H and OH ions. Studies of the effect of pH on fixation have been performed by (1) saturating the exchange complex of the fixing mineral with Ca and/or Na and/or H ions and then adding ammonium or potassium, or by (2) adjusting the pH of the ammoniating solution by additions of $Ca(OH)_2$ or NaOH.

Ammonium Fixation by the Soil Organic Fraction

In contrast to the knowledge of clay structures and their chemical reactions, relatively little information has been gained thus far about nature and properties of soil organic matter. Several reviews summarize the research in the field of soil organic matter. Waksman (1938) in his book, Humus, has given detailed coverage to literature related to soil organic matter dating back into the 18th century. Progress since 1936, suggestions for needed study and critical evaluations of some areas of organic matter research have been presented by A. G. Norman (1942), J. M. Bremner (1951), F. E. Broadbent (1953), and G. K. Frazer (1955). Therefore, only a summary of the points considered of direct importance to this dissertation will be presented below.

Composition of organic matter

The definition of soil organic matter, as approved by
the Soil Science Society of America (1956), is:

> The organic fraction of the soil. Includes plant
> and animal residues at various stages of decompo-
> sition, cells and tissues of soil organisms, and
> substances synthesized by the soil population.
> Commonly determined as those organic materials
> which accompany the soil when put through a
> 2-mm sieve.

Humus, a term frequently found in the literature, has been
considered synonymous with soil organic matter by the Society
(ibid.). Substances synthesized by the soil micro-organisms
and residues of plant, animal and microbial tissues, portions
of which are decomposed very slowly, have been considered to
constitute the bulk of the organic fraction of soil. Attempts
to isolate inidividual organic compounds from the soil have
met with only limited success. Classical organic analysis
methods, and more modern methods, such as infrared and ultra-
violet, spectroscopy and electrophoresis, have yielded scant
useful information when applied to these complex organic
compounds and their reaction products. The organic fraction
of soil by gross analysis, consists of 50-52 percent carbon,
5 percent nitrogen and less than 1 percent sulfur and phos-
phorus.

Approximately 30 to 35 percent of the organic nitrogen
in soil has been isolated as amino acid-nitrogen following
acid hydrolysis. Only about 3.2 mg/100g soil of free amino

acids have been isolated from soil (Paul and Schmidt, 1960 and 1961). Bremner (1958) isolated from 3 to 10 percent of the organic nitrogen in the form of amino sugars. Another 13 to 30 percent of the organic nitrogen has been found to remain as insoluble organic nitrogen following acid or alkaline hydrolysis (Bremner, 1949). The remaining 10 to 20 percent of the organic nitrogen in soil has been recovered as soluble organic nitrogen and ammonium nitrogen following acid or alkaline hydrolysis.

A lignin-like fraction constitutes approximately 40 to 45 percent (Waksman, 1952) of the organic fraction of prairie derived mineral soils. The question of whether the lignin-like substances found in soils have been obtained directly from plant residues or indirectly through microbial synthesis has not been resolved. Both plant and soil lignin have an aromatic nucleus. But, soil lignin contains fewer methoxyl, phenolic hydroxyl, and aliphatic groups and more carboxyl groups than does plant lignin. Only a trace of nitrogen has been found in plant lignin, whereas soil lignin apparently contains appreciable amounts of nitrogen, part of which appears to be incorporated into heterocylic rings.

According to the proximate analysis data of Waksman (1952), approximately 12 percent of the organic fraction of mineral soils should consist of carbohydrates, such as hemicelluloses and celluloses.

Fixation reaction

Mattson and Koutler-Andersson (1941, 1942, and 1943) have made an extensive study of ammonia fixation by natural organic materials. They suggested that ammonium fixation was an oxidative process involving aromatic compounds containing two or more hydroxyl groups. The phenolic components contained in lignin-like materials were first oxidized to quinoid type structures followed by introduction of ammonia to form quinone imide type structures.

Evidence has not been reported to support the presence of imide groups in the reaction products of ammoniated organic materials.

Mattson and Koutler-Andersson (1943) found that hydrolysis with strong alkali released about half of the ammonium fixed by an organic soil and lignin while hydrolysis with strong concentrated acid released only about 10 percent of the fixed ammonium. They suggested that more than one hydroxyl group must be present in a molecule before ammonium fixation could occur from evidence obtained by ammoniating simple organic compounds. Resorcin, hydroquinone, pyrogallol, gallic acid, tannin and glucose fixed appreciable amounts of added NH_4OH-N whereas phenol, cinnamic acid, salicylic acid, and sucrose did not. Oxygen was consumed during ammoniation only by those compounds which fixed ammonium. The amount of ammonium fixed could be reduced by auto-oxidation of the

materials with NaOH prior to ammoniation or by ammoniating under anaerobic conditions.

The author has ammoniated several aromatic compounds containing hydroxyl groups. Phenol and a-naphthol did not fix ammonium, whereas catechol, pyrogallic acid, hydroquinone and quinhydrone fixed 15, 44, 75, and 132 me NH_4-N/100g of material respectively. The reaction was carried out in a 100 ml stoppered flask using 2 g of material incubated at room temperature for 24 hours with 1 ml of 10 normal NH_4OH.

Bennett (1949), using a commercial lignin, found that methylation prior to ammoniation greatly reduced the amount of ammonium fixed by Meadol, while ammoniation prior to methylation reduced the number of methoxyl groups that could be introduced. Similar results have been obtained by Shoji (1958) and Burge and Broadbent (1961). These results would tend to support the hypothesis that phenolic hydroxyl groups were involved in the ammonium fixation reactions.

Mattson and Koutler-Andersson (1943) found greater amounts of ammonium were fixed by peats, plant materials, lignins, and organic soils when aerobic rather than anaerobic conditions accompanied alkaline ammoniation of the sample. The oxygen uptake curve was found to parallel the ammonium fixation curve when organic matter was ammoniated with 12 percent NH_4OH. Burge and Broadbent (1961) found 2.9 me

NH_4-N fixed per gram of carbon with oxygen present versus
2.1 me NH_4-N fixed per gram of carbon under anaerobic condi-
tions when organic soils were ammoniated with gaseous ammonia.

Chang et al. (1961) investigating the ammoniation of
sugar cane bagasse found that the pentosans rather than the
lignins were the ammonium fixing reactants. They presented
evidence to show that most of the added ammonium was fixed
as 4 (or 5) methylimidazole and 4 (or 5) hydroxymethyl-
imidazole. According to Hoffmann (pp. 33-41, 1953) D-glucose,
D-fructose, D-mannose, L-sorbose, D-xylose and L-arabinose can
react with zinc hydroxide and ammonia to form imidazoles but
that maltose, lactose and D-galactose yield only trace amounts
of product under similar conditions. The reaction involves
oxidative cleavage of the sugar to methylglyoxal. The reac-
tion with free sugar could not be expected to account for much
fixation of added ammonium by soil organic matter since the
amount of free sugar in soil is normally very low.

The question of how ammonium is fixed by soil organic
matter or lignin remains unanswered. The fixation reactions
appear to involve hydroxyl groups and in some cases are asso-
ciated with oxidation.

Some factors affecting ammonium
fixation by organic materials

Solubilization of organic matter   Solution extraction
of soil samples containing organic matter will result in

solubilization and removal of some organic material in the extract. Thus, a Kjeldahl analysis for residue-nitrogen only could yield a low organic nitrogen value because part of the organic nitrogen had been removed during extraction. Bremner and Lees (1949) found sodium halide salt solutions extracted from 1 to 2.5 percent of the soil organic nitrogen. Alkaline conditions have been found to result in increased solubilization of the organic fraction with approximately 20 to 25 percent of the organic nitrogen removed by 0.5 molar NaOH extraction (ibid.). Alkaline conditions will exist following the ammoniation of a sample with aqua or gaseous ammonia. Thus, neutral normal KCl extraction following alkaline ammoniation of samples containing organic nitrogen will result in the removal of sizable quantities of organic nitrogen in the extracting solution. Alkaline macro-distillation of soil extracts even with MgO may result in the decomposition and release of some organic nitrogen as ammonium (Bremner and Shaw, 1955). Sohn and Peech (1958) claimed that MgO distillation of KCl extracts, containing organic nitrogen from gaseous ammoniated surface soils did not result in appreciable decomposition and release of the organic nitrogen as ammonium.

pH and oxygen uptake   Mattson and Koutler-Andersson (1943) and Burge and Broadbent (1961) used massive amounts of NH_4OH or gaseous ammonia for ammoniation of sample materials. Under aerobic alkaline ammoniation conditions the

fixation of ammonium was accompanied by simultaneous oxygen uptake as previously discussed.

Bremner (1950) reported that auto-oxidation of soil organic matter increased as the pH of the system was increased by the addition of alkaline and alkali solutions. The oxygen uptake (mm^3) during a 5 hour period with neutral sodium pyrophosphate soil extracts was 0, 18, 21, 57, and 230 at pH 7, 10, 11, 12, and 13 respectively when the pH was adjusted by NaOH additions. The uptake of oxygen was 205, 907, and 896 mm^3 when the soil was extracted with 0.5 molar solutions of NH_4OH, KOH, and NaOH, respectively. Leaching with acid prior to the addition of alkali increased the amount of oxygen consumed. Therefore it is imperative that known oxygen levels be maintained during alkaline ammoniation in order to make valid comparisons between experiments.

Time   Mattson and Koutler-Andersson (1943) ammoniated beech leaf lignin with 12 percent NH_4OH. Of the total ammonium fixed within a 7 day period, 70 percent had been fixed within 24 hours under aerobic conditions and 80 percent had been fixed within 24 hours under anaerobic conditions.

Ammonium concentration   Quantitative information has not been reported as to the effects of ammonium concentration on the fixation of ammonium by soil organic matter.

Importance of Fixed Ammonium

Availability of ammonium to biological systems

Soluble and exchangeable forms of ammonium have been shown to be readily available or nearly so to biological systems (Allison et al., 1951, 1953b; Bower, 1951). The level of soluble and exchangeable ammonium in a field soil approachs zero if there is an active population of nitrifiers present and there has not been a recent addition of ammonium contain- ing or forming material.

Conflicting results have been obtained as to the avail- ability of clay-fixed ammonium to biological systems. It appears that part of the inconsistent results obtained for biological availability of clay-fixed ammonium have been due to faulty techniques employed in these studies (Edwards, 1960). Generally, it is now recognized that not all ammonium fixed by clays against extraction with potassium ions is held by the same amount of energy (ibid.). Thus, some clay-fixed ammonium may be as available to biological systems as ex- changeable ammonium (Allison et al., 1953b; Legg and Allison, 1959; Nõmmik, 1957) while some clay-fixed ammonium may be completely unavailable to biological systems (Allison et al., 1951, 1953a; Bower, 1951; Nõmmik, 1957). In most investi- gations, clay-fixed ammonium has been shown to be less avail- able to biological systems than an equivalent amount of

soluble or exchangeable ammonium.

The reduced availability of clay-fixed ammonium to biological systems is further supported by data indicating that many soils contain several hundred pounds of clay-fixed ammonium-nitrogen/acre (Walsh and Murdock, 1960; Allison et al., 1953b, 1953a) under conditions where an active biological population has reduced the level of exchangeable and soluble ammonium to near zero pounds per acre. Most soils which contain clay-fixed ammonium have been found to have the capacity to fix additional ammonium in the clay fraction; the amounts sometimes range up to 1000 pounds of nitrogen per acre (Sohn and Peech, 1958; Walsh and Murdock, 1960; Allison and Roller, 1955b).

The availability to biological systems of added ammonium fixed by the soil organic fraction has not been investigated since little was known about the amounts of ammonium that may be fixed. Bremner and Shaw (1957) found that the added ammonium fixed by ammoniated lignins was less than 15 percent available to soil micro-organisms.

In a pot experiment, the first and second cutting of sudan grass recovered 4.3 and 1.3 percent respectively of the tagged ammonium fixed by an organic soil (Burge and Broadbent, 1961).

Economic considerations

The amount of added ammonium fixed by soils and the
subsequent availability of fixed ammonium to biological
systems may be important economically since greater than 40
percent of the nitrogen fertilizer applied to soils in the
United States in 1959 was in the ammonium form (Scholl et al.,
1960). The amount of nitrogen fertilizer applied as anhydrous
ammonia and aqua ammonia has increased from less than 1 per-
cent in 1948 to greater than 25 percent of the total nitrogen
applied to soils in the United States in 1959. Thus, the
amount of ammonium fixed by soils when ammonium is added
either in the neutral salt or alkaline form has become of
considerable potential economic importance.

MATERIALS AND METHODS

Materials

Chemicals

Chemicals employed in this study were of reagent grade unless otherwise specified.

Isotopic nitrogen

$(N^{15}H_4)_2SO_4$ containing 98 atom percent $N^{15}H_4$-N was converted by distillation with NaOH to $N^{15}H_4OH$, and the distillate was diluted with nonenriched NH_4OH until the desired NH_4-N concentration and isotopic enrichment were obtained.

Indicator-boric acid mixtures

Boric acid solution containing a mixed indicator devised by Moraghan (1961) was prepared as follows: Solution A, 0.0066 g methyl red plus 0.0132 g brom cresol green was dissolved in 225 ml of 95 percent ethanol; Solution B, 20 g of H_3BO_3 was dissolved in 700 ml of H_2O. Solutions A and B were mixed and diluted to 999 ml with H_2O. One ml of 0.1 normal NaOH was added.

Dilution of 1 ml of indicator with 1 ml of H_2O yielded a faint green color.

Boric acid solution containing a mixed indicator devised

by Conway and O'Malley (1942) was prepared as follows: 20 g
of H_3BO_3 was dissolved in 700 ml of H_2O to which 200 ml of
95 percent ethanol and 2 ml of indicator solution B were
added. The resulting mixture was neutralized (so that 1 ml
of solution plus 1 ml H_2O yields a faint green color) with
approximately 50 ml of 0.1 normal NaOH and diluted with water
to one liter. Indicator B was prepared as follows: 0.165 g
of brom cresol green and 0.330 g of methyl red were dissolved
in 500 ml of 95 percent ethanol.

Nessler's reagent

Nessler's reagent was made by dissolving 50 g KI in 50
ml of H_2O. $HgCl_2$ was added until a slight excess was indi-
cated by the formation of a precipitate; 400 ml of a 50 per-
cent (w/v) solution of KOH was added and the solution mixed.
The precipitate was allowed to settle and the clear liquid
decanted.

Soils

Table 1 lists the chemical and physical characterizations
made on the soils used for the ammonium fixation investiga-
tions.

Glencoe silty clay loam, Webster clay loam, Nicollet
clay loam and Clarion loam were obtained from the Iowa State
University Agronomy Farm. The vegetation was permanent grass.

Table 1. Characterization of the soils used in ammonium fixation investigations

Properties	Soil							
	Marion	Edina	Marshall	Clarion	Grundy	Nicollet	Webster	Glencoe
pH	6.4	5.8	6.4	7.2	6.7	7.1	6.4	6.8
CEC (me/100 g)	12.5	18.1	20.5	16.9	18.6	33.4	36.7	59.8
Sand (%)[a]	1.2	0.6	2.2	42.3	2.0	28.6	22.0	8.3
Silt (%)	79.1	73.9	61.9	33.3	68.4	40.6	45.8	51.0
Clay (%)	19.7	25.5	36.0	24.4	29.7	31.3	32.2	40.8
Carbon (%)[b]	1.36	1.66	1.99	2.14	2.27	3.97	4.44	10.2
Nitrogen (%)[c]	0.137	0.162	0.201	0.205	0.207	0.339	0.354	0 928
(me/100 g)	9.79	11.5	14.4	14.6	14.8	24.2	25.3	67.2
Clay fixed NH_4-N (me/100 g)	1.03	0.91	1.60	0.61	1.35	0.77	0.88	1.01

[a] Percentage based on oven dry weight of the mineral material recovered from the soil suspension employed for particle size determination.

[b] Percentage based on oven dry weight of whole soil.

[c] Includes organic-N and native clay fixed NH_4-N but not NO_3-N.

Marshall silty clay loam was obtained from plot 14-VIII of
the rotation-fertility experiments on the Soil Conservation
Experimental Farm, Shenandoah, Iowa. The rotation was CCOMM
and the plot was sampled during the first year corn. Grundy
silty clay loam was obtained from plot 216 of the rotation-
fertility experiments on the Grundy-Shelby Experimental Farm,
Beaconsfield, Iowa. The rotation was CCOMM and the sample
was obtained during first year corn. Edina silt loam was
obtained from plot 618 of the rotation-fertility experiments
on the Southern Iowa Experimental Farm, Bloomfield, Iowa.
The rotation was CCOM with the sample obtained under first
year corn. Marion silt loam was obtained from plot 205 of
the rotation-fertility experiments on the Pasture Experimental
Farm, Albia, Iowa. The rotation was CCOM and the plot was
sampled during first year corn.

Clay minerals

The following clay minerals were obtained from Dr. A.
D. Scott:[1] Volclay-bentonite, Goose Lake Grundite (Illite)
and Montana Vermiculite (High Exchange).

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[1]A. D. Scott, Agronomy Department, Iowa State University of Science and Technology, Ames, Iowa.

Lignin

Indulin AT was obtained from the West Virginia Pulp and Paper Company.[1] Indulin AT has properties similar to Indulin A.[2] Indulin A has been described (West Virginia Pulp and Paper Co., 1957) as

> ... an alkali lignin derived from southern wood. Indulin is a naturally occurring polymer, characterized by a series of closely linked benzene, pyrane and furane rings carrying methoxyl, hydroxyl and other substituent groups.

The following properties were listed for Indulin A: 99 percent organic material, 3.49 m^2/g surface area, approximately 13.9 percent methoxyl groups (four methoxyl groups per lignin unit of molecular weight 840), and insoluble in water but soluble in alkali. The Indulin AT (hereafter referred to as lignin) was found to have a pH of 2.5 and a nitrogen content of 0.074 percent (5.3 me N/100 g).

Water

Deionized H_2O was obtained by passing distilled H_2O (condensed steam) through a column of Amberlite MB-3 ion exchange resin. Deionized water was used to make up all reagents and as final wash for cleaning glassware.

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[1]West Virginia Pulp and Paper Company, Polychemicals Division, Charleston A, South Carolina.

[2]B. Harril, West Virginia Pulp and Paper Company, Charleston A, South Carolina. 1959. Properties of Indulin. Private communication.

Equipment

Shaker   An International Bottle Shaker, size 2, was
used for shaking of samples unless another form of shaking
is specified.

Centrifuge   An International Centrifuge, size 2, was
used for all steps where centrifugation is specified. An
eight place head which accomodated 100 ml centrifuge tubes
was used. The maximum speed obtainable was 1800 rpm. The
maximum distance from the axis of rotation to the end of the
centrifuge tube was approximately 10 in.

Mass spectrometer   A Consolidated Engineering Cor-
poration model 21-620 mass spectrometer fitted with a direct
current isotope ratio attachment was used for isotopic
analysis of nitrogen gas samples. The N29/28 ratio was
obtained by direct reading from the spectrometer.

Analytical Methods

Reaction

The determination of pH on a 1:2.5 (w/v) soil to water
suspension was made by a line-operated, glass-electrode pH
meter.

Cation exchange capacity

Cation exchange capacity was determined by the neutral normal ammonium acetate method described by Black (1957) as modified for micro technique by Keeney and Bremner.[1] The modification consisted of reducing the sample size so that a range of 1 mg to 2 mg of NH_4-N was obtained in the KCl extract. The ammonium obtained in the KCl leachate was determined by the micro steam distillation procedure described below.

Carbon

Carbon analysis was made by the wet oxidation method of Shaw (1959).

Preparation of sample materials

All experiments were performed using air dried material. The sample material was weighed on the basis of the desired equivalent of oven dry weight.

The soils employed in this study were air dried and ground to pass a 60 mesh sieve.

The bentonite and illite clay minerals were already finely divided as supplied. Vermiculite was ground to pass

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[1]D. Keeney and J. M. Bremner, Iowa State University, Ames, Iowa. 1961. Micro methods for determination of cation exchange capacity by the ammonium acetate method. Personal communication.

a 20 mesh sieve.

The lignin was finely divided as supplied. Octyl alcohol was used as a wetting agent for the lignin. After adding 5 drops of octyl alcohol per 2 g lignin, the sample was shaken vigorously until the lignin and alcohol were well mixed.

Particle size analysis

The pipette method (Kilmer and Alexander, 1949) as modified by Edwards and Bremner[1] was used for particle size analysis. The modification consisted of substituting 1.5 g (dry weight) of a sodium saturated Amberlite IRC-50 ion exchange resin as a dispersing agent in place of sodium hexametaphosphate. Determinations of the $<50\mu$ and $<2\mu$ size fractions were made by pipette. The $>50\mu$ fraction was determined by wet sieving of the sample following the pipette analysis.

Nitrogen determinations

Pretreatment of extracts for NH_4-N distillation

Extractions were made in this study with H_2O and/or normal KCl and/or normal acetic acid and/or alcohol. The extracts, with the exception of those containing alcohol, were analyzed

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[1]A. P. Edwards and J. M. Bremner, Iowa State University, Ames, Iowa. 1961. The use of ion exchange resin as dispersing agent for particle size determination. Personal communication.

for NH_4-N content by the micro steam distillation procedure described below. Extracts which contained alcohol were acidified with normal H_2SO_4 and the alcohol removed by steam distillation. The residue in the distillation flask was then analyzed for NH_4-N by the micro steam distillation procedure described below.

Ammonium by micro steam distillation   The solution containing NH_4-N was placed in a 50 or 100 ml micro distillation flask.[1] Sufficient (heavy) MgO was added to make the solution alkaline to phenolphthalein indicator (pH 9). The distillation flask was then connected to a Bremner[2] micro steam distillation apparatus and distilled by passing steam into the solution in the flask. The distillation was carried out at a rate sufficient to collect 25 ml of distillate within four minutes. For distillations of 6 mg of NH_4-N or less, only the first 25 ml of distillate was retained. For distillations of 6 to 10 mg of NH_4-N, the second 25 ml of distillate was retained in a second receiving flask. The NH_4-N contained in the distillate was absorbed in 5 ml of Moraghan's boric acid-indicator solution, if titration was to be made by

[1]Flask number MC 22/50 or MC 22/100 obtained from Scientific Suppliers Co. Ltd., Scientific House, Vine Hill, Clerkenwell Road, London E.C. 1, London, England.

[2]J. M. Bremner, Iowa State University, Ames, Iowa. 1959. Micro steam distillation. Personal communication.

0.005 normal H_2SO_4. Conway and O'Malley's boric acid-
indicator solution (5 ml) was used to absorb NH_4-N if titra-
tion of the distillate was to be made with 0.01 or 0.1 normal
H_2SO_4. A micro-burette was used to deliver the acid used for
titration.

Total-N   The Kjeldahl procedure recommended by Bremner
(1960) was followed. The digestion mixture consisted of a
100:10:1 ratio by weight of K_2SO_4:$CuSO_4$:Se that was added in
a 1:3 (w/v) ratio with concentrated H_2SO_4 to the sample prior
to heating. Allowance was made for 1) the salt content of the
sample and 2) the H_2SO_4 consumed by the carbon in the sample
during digestion, before adding the calculated additional
H_2SO_4 required prior to heating. Digestion was continued
for 5 hours with soil samples or 2 hours with other materials
after the digest was clear. No provision was made to include
nitrate or nitrites. Following digestion and cooling, H_2O
was added to dilute the mixture. The NH_4-N contained in the
digest was determined by the micro steam distillation method
described above except that 10 normal NaOH was used in place
of MgO.

Clay-fixed NH_4-N   The method of Dhariwal and Stevenson
(1958) was used under the conditions described for determina-
tion of clay-fixed NH_4-N. Approximately 20 ml of 2 normal
KOH was added to 2 g of sample. The mixture was then auto-
claved at 15 pounds pressure for 4 to 6 hours. The KOH

solution was separated from the residue by centrifugation and the residue was washed three times with normal KCl. Approximately 15 ml of a solution with final concentrations of 5 normal HF, 0.76 normal HCl and 0.6 normal H_2SO_4 was added per 2 g of sample. The mixture was digested in a 100 ml polypropylene test tube at room temperature with occasional stirring for at least 16 hours. The sample-solution mixture was then transferred to a 100 ml micro distillation flask and neutralized with 10 normal NaOH to approximately pH 5-6 by using methyl red indicator. The flask was then stoppered and shaken thoroughly by hand. After cooling, additional 10 normal NaOH was added, until the mixture was alkaline to phenolphthalein (pH 9). The flask was immediately connected to a steam distillation unit and distilled as described above for micro steam distillation.

Isotopic nitrogen analysis

The distillates to be analyzed for N29/28 ratios were titrated in the usual manner and then either subdivided or the replicates bulked so that a sample containing between 0.5 and 1.5 mg of NH_4-N was obtained. The sample was then steam distilled with heavy MgO. The distillate was caught in a vial containing 2 ml of 0.1 normal H_2SO_4. The distillate totalling 25-30 ml was then evaporated to dryness and stored in this condition. Immediately before determination of the

N29/28 ratio the sample was diluted to a volume of 2-3 ml.
The techniques used for the conversion of $(NH_4)_2SO_4$ to N_2
were essentially those of Rittenberg (1946). However, the
NaOBr was diluted prior to use with an equal volume of 0.1
percent KI. The use of a KI-NaOBr solution was suggested by
Sims and Cocking (1958) to eliminate the catalytic breakdown
of NaOBr to NaBr and O_2 by Cu ions. Approximately 1 ml of
the NaOBr-KI solution was pipetted into one arm of the
Rittenberg tube and 2 ml of the sample solution was pipetted
into the second arm of the Rittenberg tube. The Rittenberg
tube was evacuated in an apparatus similar to that described
by Sprinson and Rittenberg (1949). After evacuation, the
solutions in the tube arms were mixed and the nitrogen gas
introduced into the mass spectrometer.

Calculations   The ratio of N29/28 was converted to
atom percent N^{15} by the following formula: atom $\% = \dfrac{100 \times R}{2 + R}$
where R was equal to the ratio of N29/28. The following cal-
culations were based on the assumption that there was no dif-
ference in the chemical rate of reaction between compounds
containing N^{14} and those containing N^{15}. The nitrogen (X)
found in any fraction that came from the NH_4-N initially added
to the sample was calculated as follows:

$$\frac{(me\ T)(A\%_t) - (me\ C)(A\%_c)}{(A\%_i)} = X$$

where me T = me NH_4-N/100 g found in the treated fraction

me C = me NH_4-N/100 g found in the comparable control
sample fraction

$A\%_t$ = the atom percent of N^{15} found in the treated
fraction

$A\%_c$ = the atom percent of N^{15} found in the control
fraction

$A\%_i$ = the atom percent of N^{15} contained in the ammonium
initially added to the sample

X = me NH_4-N/100 g that was derived from the ammonium-
nitrogen initially added to the sample.

When the sample of ammonium in a control fraction was too
small for analysis of N29/28, the atom percent N^{15} found in
the untreated soil was substituted in the equation for $(A\%_c)$.
Bremner[1] has observed that the atom percent N^{15} found in soils
may differ from the atom percent N^{15} normally found in the
atmosphere (0.366 atom percent); therefore, it is imperative
that the N^{15} content of the control sample be measured.

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[1]J. M. Bremner, Iowa State University, Ames, Iowa. 1961.
Isotopic nitrogen content of soil. Personal communication.

DEVELOPMENT OF A PROCEDURE FOR ESTIMATING
AMMONIUM FIXATION BY MINERAL SOILS

The primary objective of this investigation has been to determine whether the organic fraction of mineral soils under normal field conditions would fix significant quantities of added ammonium.

The following approach appeared to solve most satisfactorily the problem of estimating the amount of added ammonium which had reacted with and was held by the soil organic matter in such a manner that the added ammonium was no longer water soluble nor readily exchangeable. 1) Develop a method for estimating total ammonium fixation by the soil system. 2) Determine the amount of clay-fixed ammonium by one of the HF methods. 3) Estimate the amount of added ammonium fixed by the organic fraction by calculating the difference between the total ammonium fixation of the soil and ammonium fixation by the clay fraction.

It seemed desirable that the procedure employed for estimating fixation of added ammonium by mineral soils would satisfy the following requirements: 1) the method must quantitatively remove and/or measure water-soluble ammonium; 2) the method must quantitatively remove and/or measure "readily exchangeable ammonium"; 3) the method must include and measure organic nitrogen in both the soluble and insoluble forms; 4) the estimation of ammonium fixation should be based

on the amount of added ammonium recovered as fixed ammonium
rather than that amount of added ammonium not recovered as
water soluble and exchangeable ammonium; 5) the procedure used
to estimate ammonium fixation should be independent of the
reaction conditions employed during the ammoniation of the
sample; 6) the method should be adaptable with little or no
modification for estimating ammonium fixation by surface
soils, subsoils, pure clay minerals and/or complex organic
materials; 7) the method should differentiate the added
ammonium fixed by the organic fraction from that fixed by
the clay fraction.

First Approach - The Leaching Method

The ammonium acetate method employed for the determina-
tion of cation exchange capacity of soils as described by
Black (1957) was considered to provide the means necessary
for measuring total ammonium fixation by a soil. The follow-
ing modifications were made: 1) instead of leaching with
ammonium acetate, a small measured amount of standardized
ammonium solution was added to the soil; 2) the system was
closed so that volatile ammonium could not escape during the
ammoniation reaction.

Soluble nitrogen

Following the ammoniation reaction the ammoniated sample was leached with 150 ml of methanol or ethanol. Alcohol was used to displace soluble NH_4-N so that hydrolysis of exchangeable NH_4-N would be held to a minimum. The alcohol leachate was diluted with water to a volume of 500 ml. One aliquot was removed for the determination of soluble NH_4-N by macro distillation with MgO. A second aliquot was taken for the determination of total-N by the Kjeldahl method.

KCl extractable-N

Following the alcohol extraction the sample was leached with 500 ml of neutral normal KCl. Potassium ions were used to replace "readily exchangeable ammonium" without the concurrent release of clay-fixed ammonium. Chloride was selected as the anion since chloride salts are water soluble and chloride has not been reported to form complexes with the alumino silicates of iron and aluminum hydrous oxides in soil (Rich and Thomas, 1960). The KCl leachate was diluted with water to a volume of 1000 ml. One aliquot was removed for the determination of exchangeable NH_4-N by macro distillation with MgO. A second aliquot was taken for the determination of total-N by the Kjeldahl method.

Residual-N

The extracted residue was then analyzed for nitrogen content by the Kjeldahl method.

Calculations

The alcohol-soluble organic-nitrogen was calculated as the total nitrogen content less the soluble NH_4-N content of the alcohol extract. The KCl-soluble organic-N values were determined from the total-N content less the exchangeable NH_4-N content of the KCl extract. Total ammonium fixation was calculated by summation of the alcohol-soluble organic-N plus the KCl-soluble organic-N plus the residual-N less the nitrogen content of the respective fractions from a control sample.

Discussion

After a few trials with the above method the following favorable observations were made. 1) Recovery of the total nitrogen in the system ranged from 97 to 101 percent. 2) Simulation of field conditions during the ammoniation reaction would be possible; a small amount of ammonium solution could be introduced into a large volume of soil. Modification of the sample holder would permit the use of undisturbed soil cores taken directly form the field.

The following limitations in the method were observed.

1) Large volumes of solution were required for leaching the sample and consequently a considerable length of time was required to leach the sample and large volumes of extracts were obtained. 2) The alcohol leachate contained a large amount of ammonium and a small amount of organic nitrogen. Therefore, an experimental error of only 1 percent in the determination of NH_4-N or total-N could mask the true magnitude of the alcohol-soluble organic-N quantity when the organic-N was calculated by subtraction of the NH_4-N content from the total-N content of the alcohol extract. 3) Bremner and Shaw (1955) reported that some organic nitrogen compounds decomposed with the liberation of ammonia during macro distillation with MgO. An attempt was made to use the Bremner and Shaw micro diffusion method of distilling ammonium at room temperature with MgO whereby labile organic nitrogen compounds are not decomposed. However, the range of ammonium concentrations and solution volumes which may be distilled by micro diffusion were too restricted for use with the extracts obtained by the leaching method. 4) The amount of ammonium solution that was added to the sample for the ammoniation reaction caused some difficulty. If enough solution were added to completely wet the sample, some leaching occurred. The packing material, sand and glass wool, employed above and below the sample retained some of the added ammonium solution so that it would not be available for reaction with

the soil. 5) A provision for determination of clay-fixed NH_4-N was not included in the method.

Second Approach – Single Sample Method

The single sample method was developed from the basic principles of the leaching method combined with the Bremner micro distillation technique and the Dhariwal and Stevenson method for determination of clay-fixed ammonium.

Bremner[1] reported that a modified steam distillation procedure he had devised was capable of quantitatively separating soluble-ammonium from labile-amines contained in the same solution. The procedure consisted of steam distillation for four minutes in the presence of MgO. Bremner reported that at least 6 mg of NH_4-N could be quantitatively distilled within the four minute distillation period. After becoming familiar with the equipment and distillation procedure, the following observations were made. 1) By lengthening the distillation time to seven minutes at least 10 mg of NH_4-N could be quantitatively recovered. 2) The residue left in the micro distillation flask following steam distillation could be analyzed for organic-N by the Kjeldahl procedure. 3) The NH_4-N found in the extracts from HF digestion of soil could

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[1] J. M. Bremner, Iowa State University, Ames, Iowa. 1959. Determination of ammonium by steam distillation. Private communication.

be determined by steam distillation providing the HF was neutralized prior to distillation.

Preliminary investigations of HF procedures for determination of clay-fixed ammonium (Dhariwal and Stevenson, 1958; Bremner, 1959) were made. Satisfactory recoveries of NH_4-N were obtained by both methods after a known amount of NH_4-N had been added to samples of vermiculite or soil. The Dhariwal and Stevenson HF method was considered best suited for use with the Bremner steam distillation.

The Dhariwal and Stevenson HF method employed centrifugation as a means of separating a soil residue from a treatment solution. Shaking and centrifugation of the sample with extracting solution was substituted for leaching as a means of reducing the time required for extraction of the sample and reducing the volume of extractant required.

The five limitations cited for the leaching method were overcome by the inclusion of the techniques described above. The procedures employed in the single sample method for determination of ammonium fixation have been described below. A flow diagram of the single sample method is shown in Figure 1.

Ammoniation

Sample materials were prepared as previously described in the Material and Methods chapter. A 2 g sample was placed

Figure 1. Flow diagram of the single sample procedure for estimating ammonium fixation by the clay and organic fraction of mineral soils

```
                                        ─── KOH

AUTOCLAVE

STEAM DISTILL ─────────────────────────────→  ┌─────────────────────────────┐
                                               │  ALKALI − LABILE  NH₄ − N   │
                                               └─────────────────────────────┘
                                               ┌─────────────────────────────┐
                   ALKALI                       │     ALKALI − SOLUBLE        │
CENTRIFUGE ──→    EXTRACT    ──→  KJELDAHL ──→  │      ORGANIC − N            │
                                               └─────────────────────────────┘


          ──── HF  +  H₂SO₄  +  HCl


          ──── NaOH

STEAM  DISTILL ────────────────────────────→  ┌─────────────────────────────┐
                                               │   CLAY − FIXED  NH₄ − N     │
                                               └─────────────────────────────┘

          ──── H₂SO₄

          ─→ HF

KJELDAHL ──────────────────────────────────→  ┌─────────────────────────────┐
                                               │   RESIDUAL  INSOLUBLE − N   │
                                               └─────────────────────────────┘
```

AUTOCLAVE

STEAM DISTILL → ALKALI − LABILE $NH_4 − N$

CENTRIFUGE → ALKALI EXTRACT → KJELDAHL → ALKALI − SOLUBLE ORGANIC − N

HF + H_2SO_4 + HCl

NaOH

STEAM DISTILL → CLAY − FIXED $NH_4 − N$

H_2SO_4

HF

KJELDAHL → RESIDUAL INSOLUBLE − N
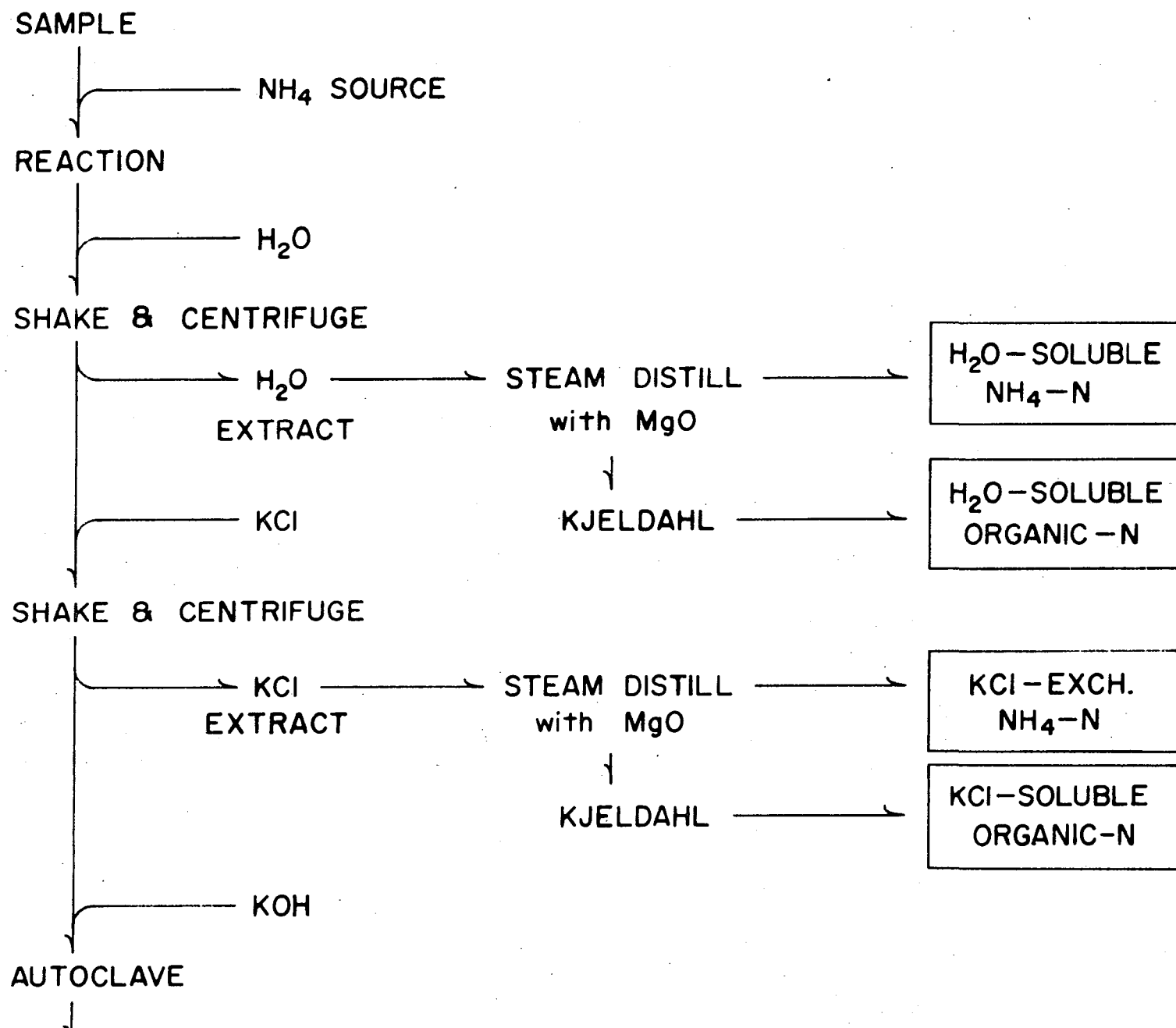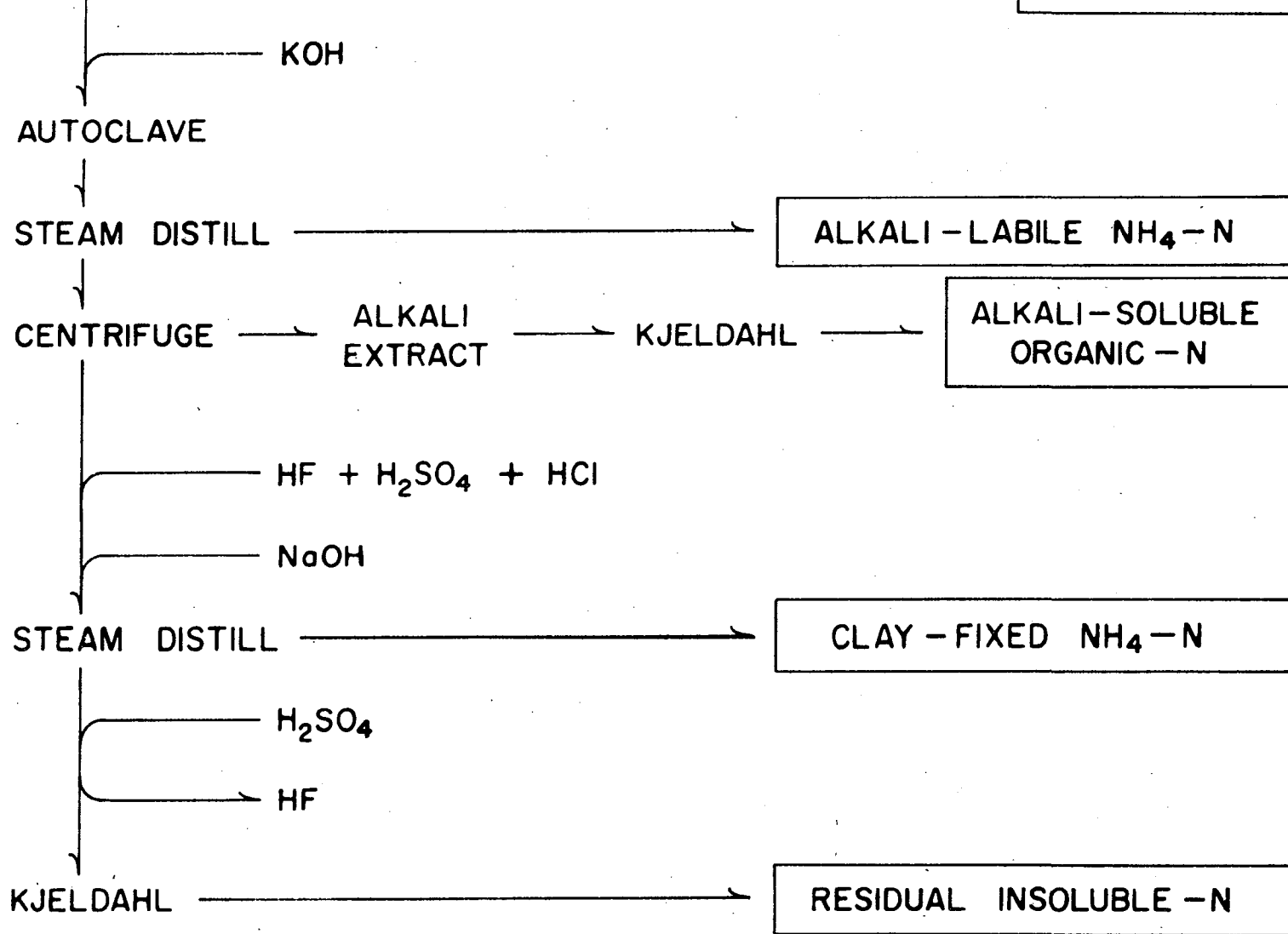
in a 100 ml glass centrifuge tube fitted with a female, ground glass, standard taper joint so that the tube could be attached to the micro steam distillation unit.

The volume of solution required to completely wet a sample was found to vary with the type of sample material used. Soils and vermiculite were completely wetted with a 1:1 ratio (w/v) of sample material to solution. Bentonite, illite and lignin were not wetted untilthe ratio of solution to sample approached a 2.5:1 ratio and then wetted only to the stage of a thick paste. The 2.5:1 solution to sample ratio was used with all sample materials during the development of the centrifuge method. Also, the pH of the reaction mixture could be determined by standard methods using the 2.5:1 ratio of solution to sample material.

Aeration

Following the ammoniation reaction, the sample was aerated by a forced air system. The volatile ammonium was caught in boric acid and determined by titration with standard acid. The aeration step was later abandoned since the information obtained was not significant enough to warrant the time and effort involved.

Water-extractable-N

Initially, alcohol was added to the samples for removal of soluble ammonium by shaking and separation of the liquid

and solid by centrifugation. However, alcohol was found to be unsuitable for removal of ammonium added in the form of $(NH_4)_2SO_4$ or NH_4Cl because of the low solubility of these salts in alcohol. Lignin was partially dissolved by repeated alcohol extraction. However, bentonite and illite would not precipitate during centrifugation unless the liquid phase was 95 percent alcohol or the aqueous phase contained a high concentration of ionic salts. In view of the above conditions, the following procedures were adapted. Soils, vermiculite and lignin, following the ammoniation reaction, were extracted with water as a means of removing soluble NH_4-N. Soluble NH_4-N was removed from bentonite or illite by one of the following methods. 1) If NH_4OH was used in the ammoniation reaction, then 95 percent alcohol was used for extraction since NH_4OH is soluble in alcohol. 2) If ammonium salts were used in the ammoniation reaction, extraction was made with alcohol diluted with sufficient water to dissolve the ammonium salts. The alcohol concentration was increased in each of the subsequent extractions up to a concentration of 95 percent, in order to precipitate the clays during centrifugation.

The extraction procedure and the analysis of the extract for H_2O-extractable NH_4-N and H_2O-extractable organic-N has been completely described in the next chapter.

KCl-extractable-N

A neutral normal solution of KCl was used for the re-
placement of exchangeable NH_4-N. The extraction with KCl
did not present any difficulties. The extraction procedure
and the analysis of the extract for exchangeable NH_4-N and
KCl-extractable organic-N has been completely described in
the next chapter.

Fractionation of residual-N

Alkali-labile   Following the KCl extraction, 20 ml of
2 normal KOH was added to the residue in the centrifuge tube.
A small glass test tube containing 2 ml of normal H_2SO_4 was
suspended from the glass stopper used to plug the centrifuge
tube. The centrifuge tube was then placed in an autoclave
for 4 to 6 hours under 15 pounds of steam pressure. After
cooling, the small glass test tube was removed and the centri-
fuge tube was fitted immediately to the steam distillation
apparatus and steam distilled. The distillate was caught in
5 ml of normal H_2SO_4. The distillate was diluted to a known
volume and an aliquot redistilled for the determination of
the NH_4-N content. The H_2SO_4 in the small test tube was
analyzed for NH_4-N content by the micro steam-distillation
method. Alkali-labile NH_4-N was calculated as the sum of the
NH_4-N recovered from the small test tube and the centrifuge
tube.

Alkali-soluble organic-N

The centrifuge tube was removed from the steam-distillation unit and the alkali-soluble organic-N was separated from the residue by centrifugation, which included three washings of the residue with 15 ml of normal KCl. The insoluble residue was transferred to a polypropylene 100 ml test tube during the KCl washing and centrifugation. The pooled extracts were diluted to a known volume and an aliquot was analyzed for alkali-soluble organic-N by the Kjeldahl method.

Clay-fixed NH_4-N

The residue was then analyzed for clay-fixed NH_4-N by the Dhariwal and Stevenson method as described previously, beginning at the HF addition step.

Insoluble residual-N

Following the steam distillation of the alkaline HF solution and residue mixture for clay-fixed NH_4-N, the mixture in the distillation flask was transferred to a 250 ml beaker. The mixture was acidified with H_2SO_4 and placed on a steam plate overnight in a force ventilated hood. Most of the HF was removed from the mixture by volatilization. The mixture in the 250 ml beaker was then analyzed for insoluble residual-N by the Kjeldahl method.

Calculations

Fixation of added NH_4-N by the organic fraction of soil was calculated as the sum of the, H_2O-extractable organic-N plus KCl extractable organic-N plus alkali-labile organic-N plus alkali-soluble organic-N plus insoluble residual-N, fractions obtained from the ammoniated sample less the amounts of organic-N contained in the respective fractions separated from a control sample.

Added NH_4-N fixed by the clay mineral fraction was obtained by the difference between the NH_4-N extracted by HF from the ammoniated sample less the NH_4-N extracted by the HF from a control sample.

Total fixation of added NH_4-N was expressed as the sum of the added NH_4-N fixed by the organic fraction plus the added NH_4-N fixed by the clay mineral fraction.

Discussion

Satisfactory recovery was made of the total-N contained in the system. However, the method was not satisfactory for routine work for the following reason. 1) Many of the samples were lost during the fractionation sequence either by breakage of the glass centrifuge tubes during the centrifugation and autoclaving processes or by popping of stoppers during the autoclaving process. 2) The single sample method was very time consuming because of the large number of transfers and

distillations necessary.

Third Approach - The Paired Sample Method

The following modifications were made in the single sample method in order to reduce 1) the number of samples lost during the fraction procedure and 2) the time required for completion of the determination.

Polypropylene tubes were substituted for the glass centrifuge tubes.

An identical pair of soil samples was substituted for each single sample formerly employed in the procedure.

A pair of soil samples was ammoniated under identical conditions. Following the ammoniation reaction the sample pairs were fractionated for water-soluble nitrogen and KCl-soluble nitrogen as described previously for the single sample method. One member of the sample pair was then analyzed for residual-N by the Kjeldahl method. The second member of the sample pair was then analyzed for clay-fixed ammonium by the Dhariwal and Stevenson method. The test tubes were not stoppered during the autoclaving step of the HF determination. The alkali-labile NH_4-N, alkali-soluble organic-N and insoluble residual-N were not isolated or determined in the paired sample method. The procedure for the paired sample method has been completely described in the next chapter.

THE PAIRED SAMPLE METHOD FOR DETERMINATION
OF AMMONIUM FIXATION

Sample Preparation

Sample weight

Sample materials were collected and prepared as described
previously. A sample of the air dry material, the equivalent
of 2 g oven dry weight, was placed in a glass or polypropylene
100 ml test tube (approximately 32 mm x 200 mm).

Experimental design

The fractionation procedures and calculation methods
that were employed required that one replication consist of
two control samples and two samples for each ammoniation
treatment (see Figure 2).

Ammoniation Conditions

A measured amount of ammonium solution was added to the
samples to be ammoniated and the test tubes immediately stop-
pered with either a glass or rubber stopper. Deionized H_2O
was added to the control samples in a volume equal to that
volume of ammonium solution added to the treated samples.
The ammoniation conditions were varied according to the
objective of the experiment.

Figure 2. Flow diagram of the paired sample procedure for
determination of ammonium fixation in mineral
soil – Step 1 – ammoniation of sample material

PAIRED SAMPLES
(for both control & treatment)
of
SOIL, CLAY or ORGANIC MATERIAL

— NH_4^+ SOURCE

(measured amount)

REACTION
(closed system)

REACTED SAMPLE PAIRS
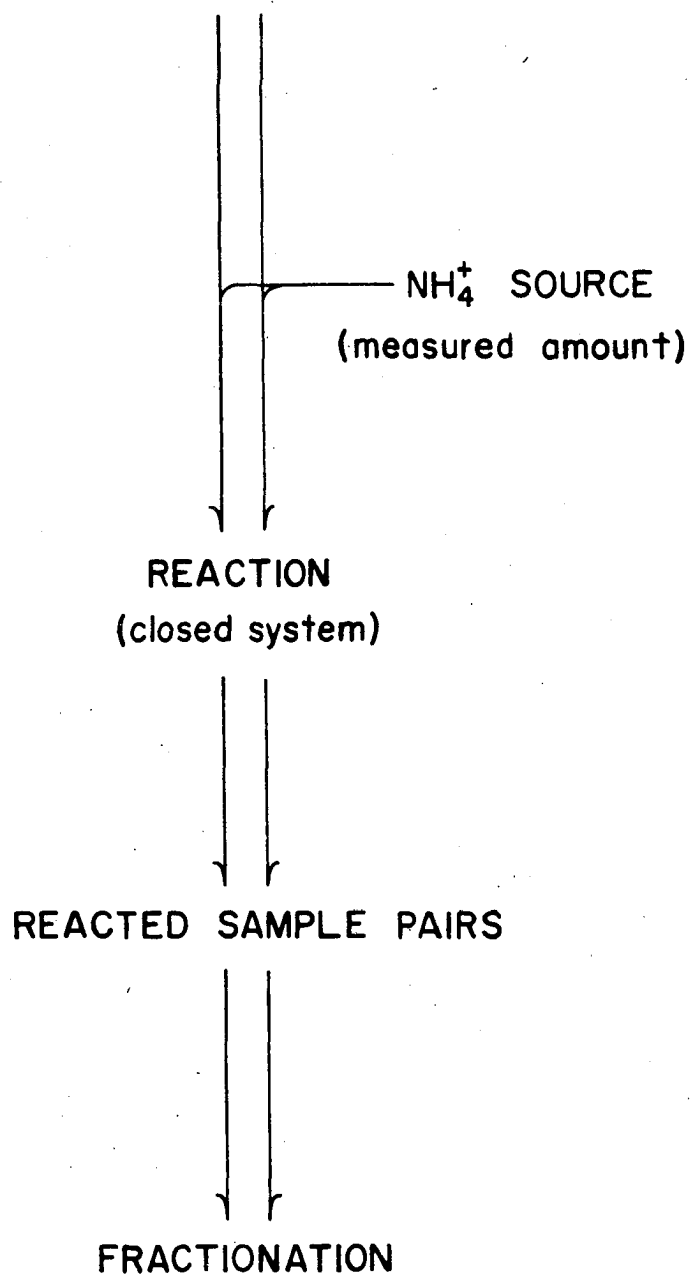
FRACTIONATION

Fractionation

The several steps in the nitrogen fractionation procedure have been outlined in the flow diagram shown in Figure 3. Table 2 illustrates typical results obtained by the paired sample method when 2 g of Glencoe soil was ammoniated for 24 hours at room temperature with 5 ml of normal NH_4OH. The sizes specified below for volumetric flasks and aliquots have been based on the addition of 5 me of NH_4-N to 2 g of Glencoe soil.

H_2O extractable-N

Approximately 50 ml of H_2O was added[1] to each sample and the test tube immediately restoppered. The tube was shaken by hand until the sample material and H_2O were mixed. The stopper was removed and the sample material washed from the stopper and the sides of the tube into the tube. The tube was covered with aluminum foil and centrifuged at 1800 rpm for 5 minutes. Following centrifugation the supernatant liquid was decanted into a 250 ml volumetric flask. The extraction was repeated with additions of 40 ml of H_2O until a negative Nessler's test was obtained on the centrifuged supernatant. Usually at least three extractions but not more

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[1]The alcohol-H_2O modification described previously for the single sample procedure must be employed with samples of bentonite or illite.

Figure 3. Flow diagram of the paired sample procedure for
determination of ammonium fixation in mineral
soil - Step 2 - fractionation of the ammoniated
sample and analyses required

PAIRED SAMPLES

├──────── NH₄ SOURCE

REACTION

├──────── H₂O

SHAKE & CENTRIFUGE

├──── H₂O ──→ STEAM DISTILL ──→ | H₂O-SOLUBLE NH₄-N |
     EXTRACT   with MgO

              KJELDAHL ──── | H₂O-SOLUBLE ORGANIC-N |

├──── KCl

SHAKE & CENTRIFUGE

├──── KCl ──→ STEAM DISTILL ── | KCl-EXCH. NH₄-N |
     EXTRACT   with MgO

              KJELDAHL ──── | KCl-SOLUBLE ORGANIC-N |

├──── KOH   KJELDAHL

├──── ALKALI-LABILE-N | RESIDUAL-N |

├──── HF

├──── NaOH

STEAM DISTILL ──────→ | CLAY-FIXED NH₄-N |
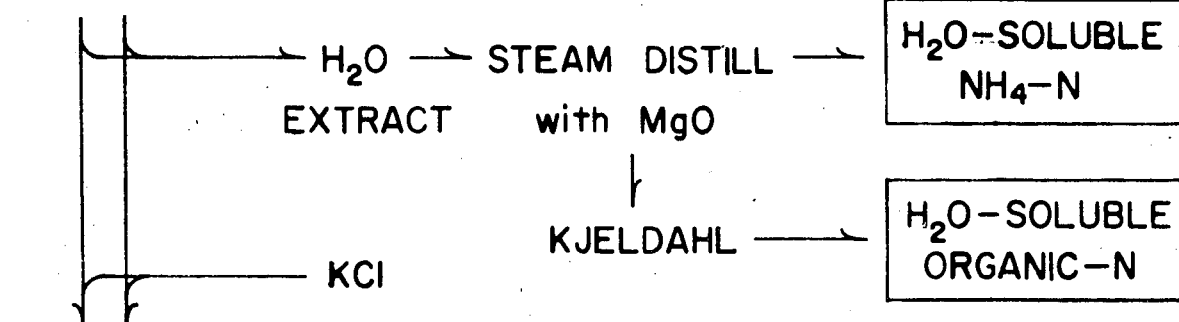
RESIDUE
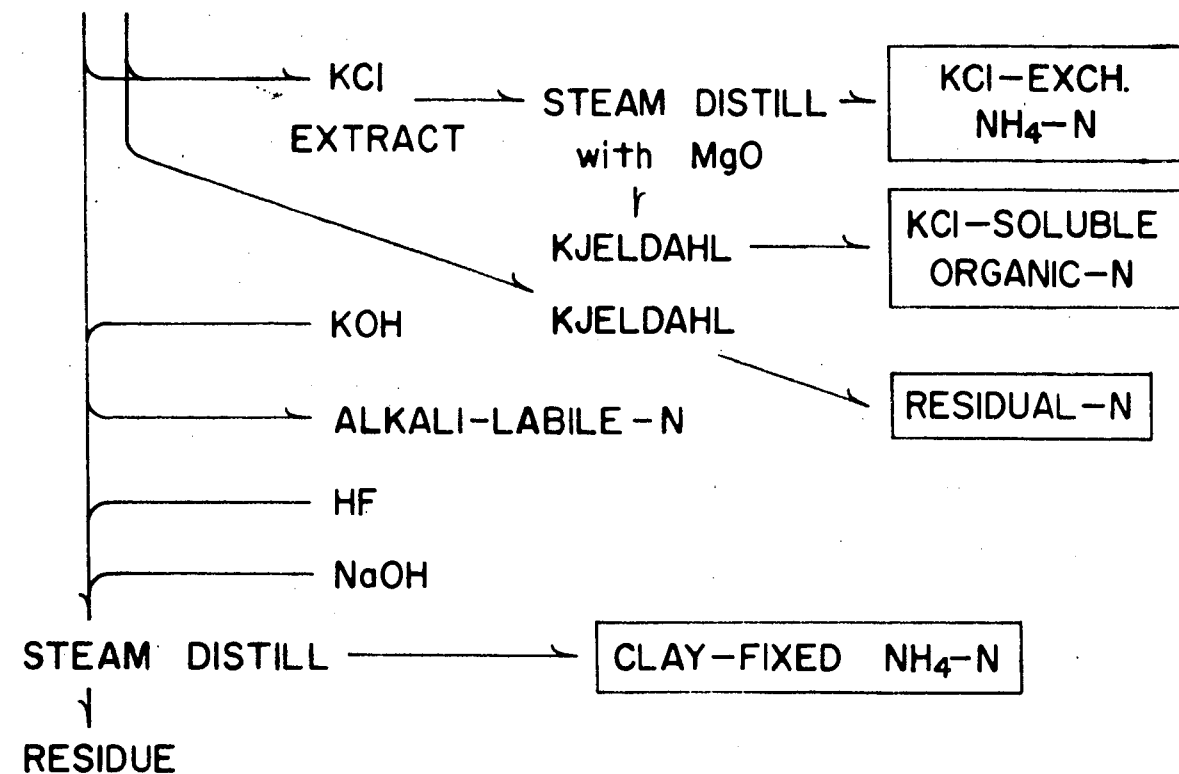
Table 2. Fixation of NH_4OH by Glencoe soil

Line	Treatment[a] column	Control (u)	Ammoniated (t)
A	me NH_4-N added/100 g	0	267.2
	Fractionation	me N/100 g	me N/100 g
B	H_2O-soluble NH_4-N	0.31	245.6
C	H_2O-soluble organic-N	1.30	6.87
D	KCl-exchangeable NH_4-N	0.36	15.11
E	KCl-soluble organic-N	trace	trace
F	Residual-N	65.33	65.45
G	HF extracted NH_4-N	1.01	1.75

Calculation of added NH_4-N fixed and nitrogen recovered

Line	Treatment	Control (u)	mid	Ammoniated (t)
H	Total Kjeldahl-N	66.63		72.32
I	Total fixed NH_4-N		5.69	
J	Clay-fixed NH_4-N line G(t-u)		0.74	
K	Organic fixed NH_4-N line I-J		4.95	
L	Total N recovered line B + C + D + E + F	67.3		333.0
M	Native soil-N	67.2		67.2
P	Total N in system line A + M	67.2		334.4
R	Percent N recovered line (L/P)(100)	100		99.6

[a]Reaction conditions: 5 ml of solution was added to 2 g soil. The mixture was allowed to react for 24 hours at room temperature in a stoppered tube.

than five extractions were required, depending upon the sample material, ammonium source and concentration. The control samples were extracted in the same manner and the same number of times as were the ammoniated samples. The H_2O extracts were combined in a 250 ml volumetric flask, made to volume with H_2O and thoroughly mixed.

Thus, paired extracts were obtained for each treatment. The two members of the extract pair could be pooled and analyzed or the extracts could be analyzed individually and the results averaged for each pair. The results presented in this dissertation for extract analysis employed the latter option – extracts were analyzed individually and the results averaged for each pair.

$\underline{H_2O\text{-soluble } NH_4\text{-N}}$ H_2O- soluble NH_4-N (line B, Table 2) was determined on a 25 ml aliquot of the H_2O extract by steam distillation with MgO as described previously.

$\underline{H_2O\text{-soluble organic-N}}$ H_2O-soluble organic-N (line C, Table 2) was determined by Kjeldahl analysis of the residue remaining in the distillation flask following the distillation of the H_2O extract for H_2O-soluble NH_4-N. Two grams of Kjeldahl catalyst and 6 to 7 ml of concentrated H_2SO_4 were added to the flask and the determination was carried out as described previously for total-N. It was assumed that all of the nitrogen in the H_2O extract was either NH_4-N or organic-N, therefore, the Kjeldahl determination determined soluble organic-N.

KCl extractable-N

Approximately 50 ml of normal KCl was added to the tube containing the H_2O extracted residue and the tube was placed on a shaker for one hour. The sample was centrifuged and decanted as previously described for the H_2O extraction. The extraction was repeated with 40 ml additions of KCl and 10 minute shaking periods until a negative Nessler's test was obtained on the centrifuged supernatant. Usually not more than three extractions were required. The KCl extracts were combined in a 250 ml volumetric flask, made to volume with H_2O and thoroughly mixed.

KCl exchangeable NH_4-N   KCl exchangeable NH_4-N (line D, Table 2) was determined on a 25 ml aliquot of the KCl extract by steam distillation with MgO as described previously.

KCl-soluble organic-N   KCl-soluble organic-N (line E, Table 2) was determined by Kjeldahl analysis of the residue remaining in the distillation flask following the distillation of the KCl extract for KCl exchangeable NH_4-N. One gram of Kjeldahl catalyst and 6 to 7 ml of concentrated H_2SO_4 were added. The determination was then carried out as described previously for total-N by Kjeldahl.

Residual-N

One member of the extracted sample pair was transferred to a 500 ml Kjeldahl flask. The residual-N determination (line F, Table 2) was then carried out using 7 g of catalyst and 22 ml of H_2SO_4 as previously described for total-N.

Clay-fixed NH_4-N

The remaining member of the extracted sample pair was transferred to a 150 ml tall-form glass beaker. Clay-fixed NH_4-N was then determined as described previously (line G, Table 2).

Calculations

From the above procedure, analysis for the following fractions had been obtained for both control and ammoniated samples as shown on lines B through G in Table 2.

B = H_2O-soluble NH_4-N

C = H_2O-soluble organic-N

D = KCl-exchangeable NH_4-N

E = KCl-soluble organic-N

F = residual-N (residual-N includes clay-fixed NH_4-N)

G = HF extracted NH_4-N (includes native + added NH_4-N fixed by clay)

In addition the following data must be available before the amount of added nitrogen which was recovered can be calculated.

A = NH_4-N added to sample for ammoniation

M = native sample-N

The following calculations were made where subscript t refers to ammoniated sample and u refers to control sample as shown on lines H through R in Table 2:

I = total added NH_4-N fixed = $(C_t + E_t + F_t)$ - $(C_u + E_u + F_u)$

J = added NH_4-N fixed by the clay fraction = $G_t - G_u$

K = added NH_4-N fixed by the organic fraction = total added NH_4-N fixed (I) - NH_4-N fixed by the clay fraction (J)

L = total nitrogen recovered = B + C + D + E + F

P = total nitrogen in system = A + M

R = percent N recovered = $\dfrac{\text{total-N recovered (L)}}{\text{total-N in system (P)}}$ (100)

FACTORS AFFECTING AMMONIUM FIXATION

Knowledge of 1) conditions necessary for non-biological fixation of ammonium, 2) the reactive group(s) and 3) the chemical nature of the product(s) formed, would be the ultimate goal for studies on ammonium fixation. Investigation in any one of the three areas will eventually lead to knowledge in the other areas. Owing to the complexity of the mineral and organic fractions of soil, study of reaction conditions appeared most likely to provide useful information. From an agronomic standpoint it would be desirable to be able to predict how much ammonium added to given soil under known conditions might become fixed.

The experiments described in the following sections were carried out to ascertain the effect of certain conditions on the reactions of ammonium with organic matter and clay minerals in order that "standard ammoniation conditions" could be established for use in determining the importance of ammonium fixation by the organic fraction of mineral soils.

Selection of Model Compounds

Mineral soil is a heterogeneous mixture of complex inorganic and organic material. Either or both components may be capable of fixing added ammonium. The possibility also exists that either component might influence the fixation of ammonium by the other component. Therefore, comparative experiments

with soil and model compounds were carried out, in order to determine whether different responses to various conditions, or whether any interaction between soil components might occur during ammonium fixation.

Based on the information obtained from the literature and the preliminary experiments cited below, vermiculite was selected as a model compound to represent the clay fraction of soil, and lignin was chosen as a model compound for the organic fraction of soil.

Preliminary experiments

Bentonite, illite and vermiculite, which represented each of the three major categories of 2:1 type clay minerals found in soils, were ammoniated 24 hours with 5 ml of the ammonium solutions shown in Table 3. The samples were not dried prior to analysis. Vermiculite fixed a considerable amount of ammonium (from about 20 to 40 me/100 g). The results, which indicated that illite and bentonite did not exhibit a similar tendency to fix ammonium under these conditions, were in agreement with previous reports of similar studies. From these results, vermiculite was selected as a model compound to represent the clay fraction of soils.

It has been shown that both lignin and soil organic matter have aromatic structures and contain similar functional groups. Fixation of added ammonium by lignin was 18.8 me

Table 3. Preliminary experiments investigating the fixation of added NH_4-N by clay minerals and lignin

Treatments Ammonium source Normality of added solution[a]	none	NH_4OH		$(NH_4)_2SO_4$	
		1	5	1	5
me NH_4-N/100 g found as fixed-N					
Bentonite	0.13	0.16[b]	0.14[b]		0.15
Illite	3.29	3.04[b]	2.94[b]		3.28
Vermiculite	0.7	21.1[b]	30.9[c]		38.7[c]
Lignin		18.8[b]		0.2[b]	

[a]5 ml of solution was added per 2 g material.

[b]Average of duplicates.

[c]Average of triplicates.

NH_4-N/100 g or 0.2 me NH_4-N/100 g respectively when the lignin was ammoniated 24 hours with 5 ml of normal NH_4OH or 5 ml of normal $(NH_4)_2SO_4$. The organic fraction of Glencoe soil was found to fix about 5 me NH_4-N/100 g soil (Table 2) when ammoniated with NH_4OH under similar conditions. Preliminary experiments indicated that the organic fraction of Glencoe soil did not fix appreciable amounts of ammonium when the soil was ammoniated with $(NH_4)_2SO_4$ solutions. Lignin was chosen as a model compound to represent the organic fraction of soil based on the results obtained above and those of Mattson and Koutler-Andersson (1943).

Experimental Conditions

Ammoniation conditions

The ammoniation reaction was carried out in a stoppered 100 ml test tube at room temperature. With the exception of the experiment which involved the effect of pH on fixation by vermiculite, the samples were not agitated in any manner during the ammoniation reaction. The analysis values represent one replication unless stated otherwise.

Analysis procedures

The ammoniated samples were analyzed by the paired sample method for determination of ammonium fixation. No significant differences were found in the amounts of added ammonium fixed under similar ammoniation conditions when the two modifications of the analysis procedures, described below, were used.

In order to stop or greatly reduce the reaction of added ammonium with organic materials at the end of the desired ammoniation time, extraction with normal acetic acid was substituted for water as the first extraction. The acetic acid reduced the pH of the ammoniation system below pH 6. All subsequent steps in the paired sample procedure were carried out as described previously or in combination with the modification described below.

To reduce the time required for analysis by the paired sample procedure the H_2O and KCl extractions were combined

as follows. The sample was extracted twice with H_2O and then extracted by the KCl extraction procedure. Nitrogen analyses were performed on the pooled H_2O and KCl extract.

In the Appendix tables, extractions made with acetic acid have been listed as HAc + H_2O and HAc + KCl, or when combined with the second modification as HAc + H_2O + KCl. The combined H_2O and KCl extraction procedure has been listed as H_2O + KCl.

The graphs and tables used in the following sections were compiled from the more complete information obtained in each experiment which can be found in the Appendix tables.

Influence of Ammoniation Time

Before comparative studies of other factors could be meaningful it was necessary to establish the time required for near completion of the ammonium fixing reactions by clay and organic materials. Previous studies had indicated that the reactions were fairly rapid.

Fixation by lignin and soil organic matter

The reaction of ammonium with lignin or the organic fraction of Glencoe soil was rapid (Figures 4 and 5). The fixation reaction was found to be 75 to 85 per cent complete within 24 hours when based on the total ammonium fixed after 96 hours of ammoniation.

Figure 4. Influence of ammoniation time on the fixation of
NH_4OH by the organic fraction of Glencoe soil

me NH₄−N FIXED BY ORGANIC
FRACTION/100g GLENCOE SOIL

AMMONIATION TIME IN HOURS

● 1ml OF 9.7N NH₄OH/2g SOIL

○ 5ml OF 0.8N NH₄OH/2g SOIL

74
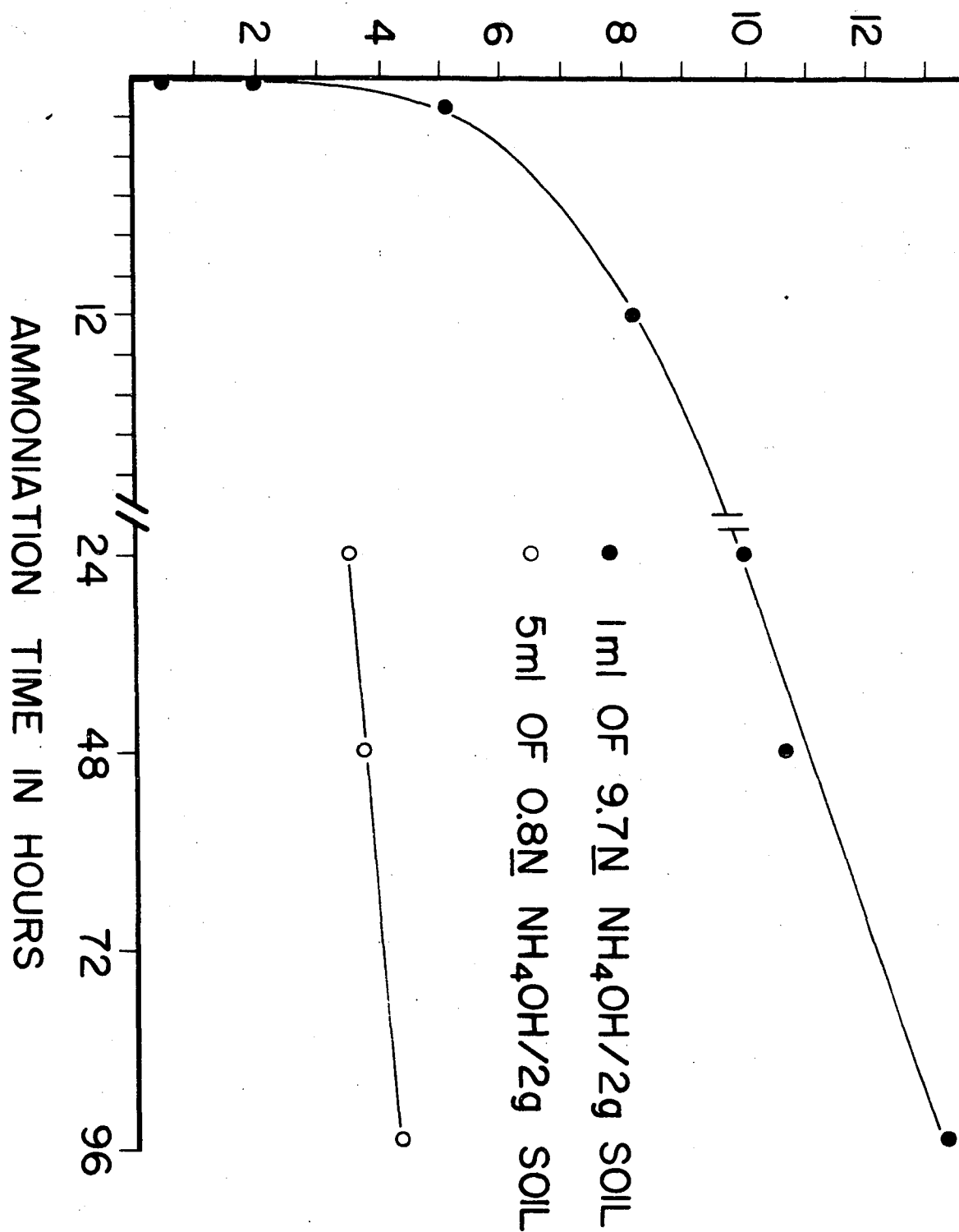
Figure 5. Influence of ammoniation time on the fixation of NH_4OH by lignin
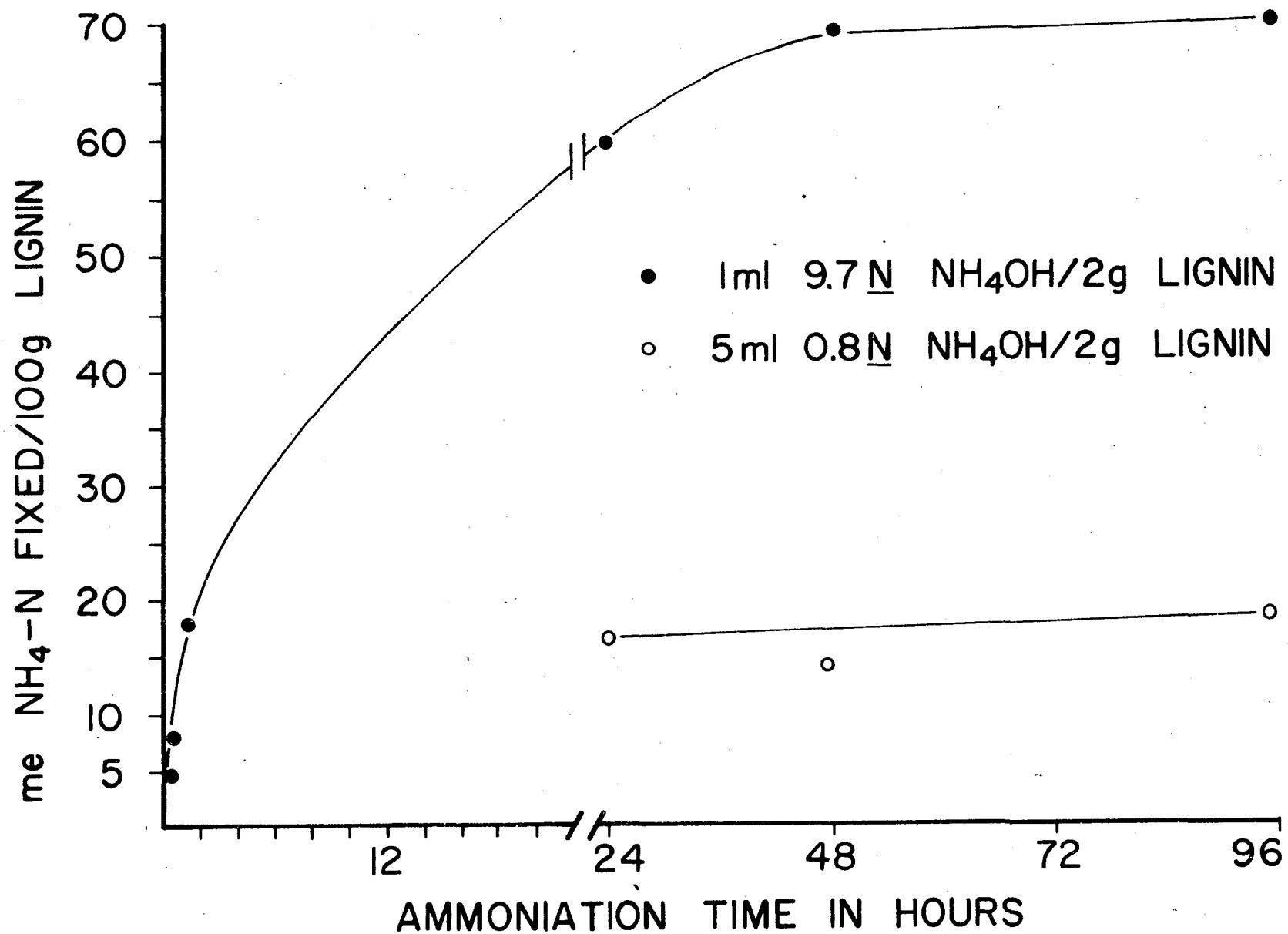
The soil organic matter (Figure 4) fixed approximately 0.6, 2, 5, 8, 10 and 13 me $NH_4-N/100$ g soil after ammoniation for 1, 10, 100 minutes, 12, 24, and 96 hours when 487 me NH_4-N was added. Lignin (Figure 5) fixed 5, 8, 18, 58, and 70 me $NH_4-N/100$ g after ammoniation with 487 me NH_4-N for 1, 10, 100 minutes, 24, and 96 hours respectively. Changing the concentration of added ammonium affected the amount of ammonium fixed but had little effect on the time required for completion of the reaction. When 209 me $NH_4-N/100$ g was added the soil organic matter fixed 3.6, 3.9, and 4.0 me $NH_4-N/100$ g of soil and lignin fixed 15, 14, and 18 me $NH_4-N/100$ g after respective ammoniation times of 24, 48, and 96 hours.

When the amount of ammonium added was increased 2.5 fold (209 me $NH_4-N/100$ g sample to 487 me $NH_4-N/100$ g sample) the amount of NH_4-N fixed/100 g increased 3.1 and 3.8 fold for the soil organic matter and lignin respectively. Further investigations regarding the influence of ammonium concentration on ammonium fixation have been presented later in a separate section.

The Glencoe soil contains approximately 20 per cent organic matter. Thus, multiplication of the ammonium fixation values for the organic fraction of Glencoe soil by five would base the fixation values for soil and lignin on approximately the same weight of organic material. The Y-axis scale for Figure 4 (soil) has been expanded five times when compared to the Y-axis scale employed for Figure 5 (lignin). The

datum points, after transformation, have nearly the same mag-
nitude at any ammoniation time. The difference between the
lignin and soil curves at the high level of ammonium addition
probably was not significant since the datum points represent
only one replication.

Fixation by vermiculite and soil clay

Fixation of added ammonium by vermiculite and Glencoe
soil clay was rapid, at least 70 per cent complete after 24
hours of ammoniation (Table 4 and Figure 6). The fixation
rate for clays was equal to or greater than the rate for
organic materials. The data indicated that an ammoniation

Table 4. Influence of ammoniation time on the fixation of
NH_4OH by the clay fraction of Glencoe soil[a]

| Ammoniation time | NH_4-N added/100 g soil[b] | |
	209 me	487 me
	NH_4-N fixed, me N/100 g soil	
1 minute	--	0.54
10 minutes	--	0.61
100 minutes	--	0.64
12 hours	--	0.70
24 hours	0.55	0.73
48 hours	0.69	0.75
96 hours	0.77	0.80

[a]Native clay fixed NH_4-N (1 me/100 g) not included.

[b]NH_4OH added in 5 ml/2 g and 1 ml/2 g respectively.

Figure 6. Influence of ammoniation time and pH on the
fixation of ammonium by vermiculite
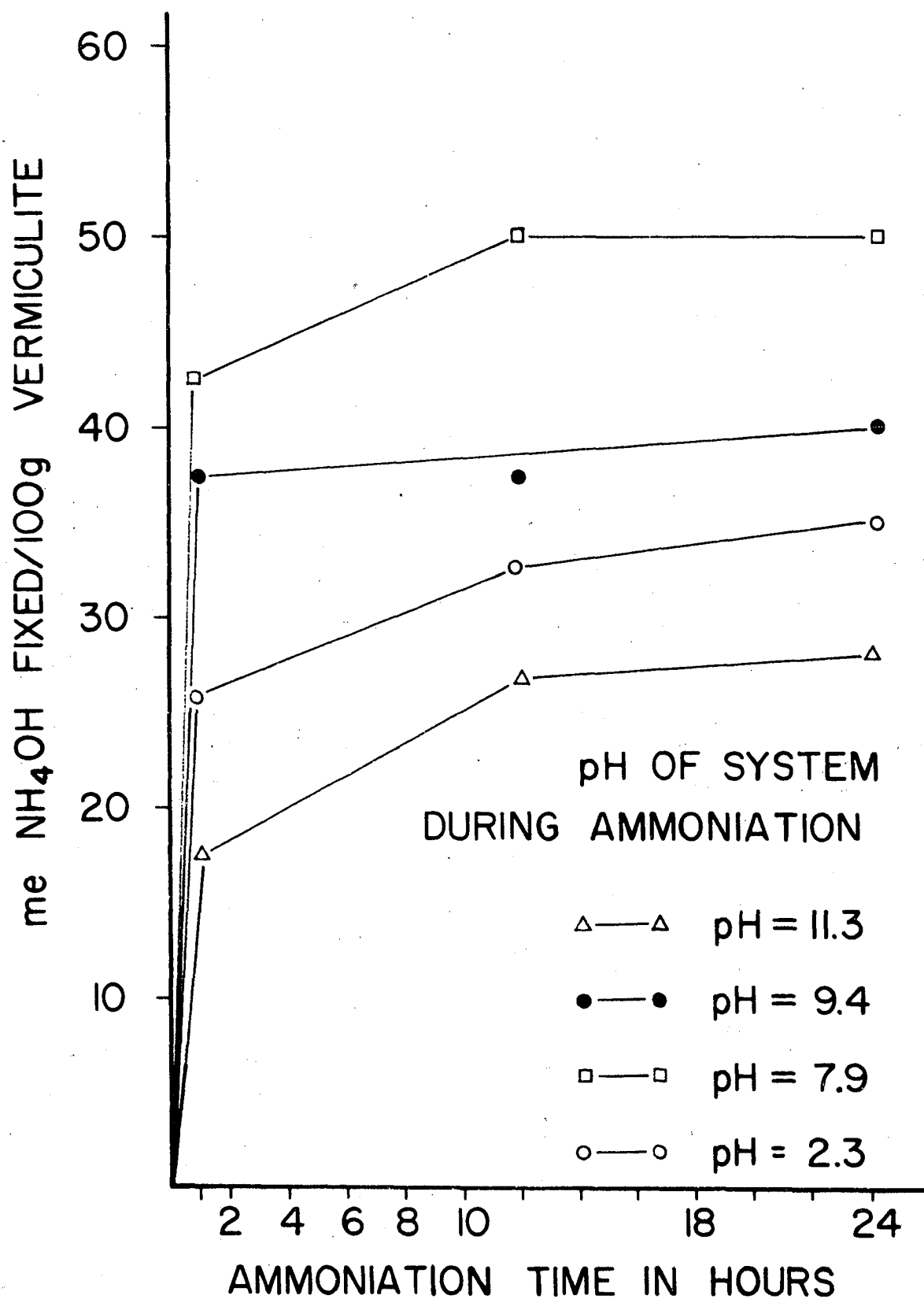
time of 24 hours should be sufficient for most investigations
on ammonium fixation by soil, vermiculite and lignin. All
further studies in this dissertation have employed an ammoni-
ation time of 24 hours.

The ammoniation conditions employed for the investigation
of soil clay was the same as those described above for soil
organic matter. Ammonium fixation by Glencoe clay was 71
percent complete within 24 hours when based on the total
fixation after 96 hours of ammoniation with 209 me NH_4-N/
100 g soil as shown in Table 4. When Glencoe soil was
ammoniated with 487 me NH_4-N/100 g soil for 96 hours, fixa-
tion by soil clay was 68, 80, and 88 percent complete after
1 minute, 12 hours, and 24 hours of ammoniation respectively.

Vermiculite was ammoniated for 1, 12, and 24 hours
with 5 ml of 1 normal NH_4-N at several different ammoniation
pH conditions as shown in Figure 6. Fixation of ammonium
by vermiculite was 65 percent complete after 1 hour and 92
percent complete after 12 hours of ammoniation when based on
24 hour fixation values. Investigations of the influence of
pH during ammoniation have been presented in the next section.

Influence of pH During the Ammoniation Reaction

Composition of the ammonium solution

The pH of the ammoniation system was controlled by adjust-
ing the pH of the added ammonium solution. Solutions normal

in NH_4-N but differing in pH were formulated by combining normal HCl, 2 normal NH_4Cl and normal NH_4OH as shown in Table 5. The ammoniation system consisted of 5 ml of solution added to 2 g of sample. The resultant pH of the reaction

Table 5. Composition of solutions used to vary ammoniation pH with vermiculite, lignin and Glencoe soil

Ratio of reagents used for 1 N NH_4-N solution					pH of 5 ml solution plus 2 g of		
HCl	NH_4Cl	NH_4OH	H_2O		Glencoe soil	Vermic-ulite	Lignin
1N	2N	1N		pH	pH	pH	pH
0	0	0	1	--	6.8	8.0	2.5
3	5	0	2	1.7	4.9	2.3	1.6
0	1	0	1	5.1	6.0	4.0	2.3
0	17.1	1	17.6	8.0	7.1	7.4	3.9
0	9	1	9.2	8.4	7.7	7.9	4.7
0	2	1	2	9.1	8.6	8.7	6.6
0	1	2	1	9.6	9.4	9.4	8.5
0	0	1	0	11.5	10.9	11.3	9.8

system was determined by the composition of the ammonium solution and the kind of material used as shown in Table 5. The pH of the system did not vary appreciably during the 24 hour ammoniation period.

Fixation by vermiculite and soil clay minerals

Ammonium fixation by vermiculite increased from 36 me NH_4-N/100 g at pH 2.3 to 47 me NH_4-N/100 g at pH 7.9 and then

decreased to 27 me NH_4-N/100 g at pH 11.3 as shown in Figure 7.

Fixation of added ammonium by the clay fraction of Glencoe soil increased from 0.4 me NH_4-N/100 g soil at pH 4.9 to 0.7 me NH_4-N/100 g soil at pH 10.9 (Table 6). In contrast to the results with vermiculite, fixation of ammonium by the soil clay did not show any tendency to decrease at pH 9.4 and 10.9 under similar ammoniation conditions.

By increasing the concentration of added ammonium, the amount of fixation by vermiculite at high pH could be increased. Addition of 1 ml of 1, 5, and 13 normal NH_4OH solutions (54, 277 and 678 me NH_4-N/100 g respectively) to 2 g of vermiculite resulted in fixation of 28, 34, and 45 me NH_4-N/100 g vermiculite. The soil clay may not have exhibited pH effects similar to vermiculite because of the much higher concentration of NH_4-N present per fixing site with soil. Based on the maximum ammonium fixation obtained by vermiculite (47 me NH_4-N/100 g) and Glencoe soil (5 me NH_4-N/100 g) at the same level of ammonium addition, there would be approximately 10 times as many sites available for fixation of ammonium in the vermiculite as there were in Glencoe soil. However, concentration of added ammonium may not have been the sole factor influencing the decreased fixation at high pH by vermiculite. As shown in Figure 7, the amount of NH_4-N retained as exchangeable NH_4-N by

Figure 7. Influence of pH during ammoniation on the
            fixation of ammonium by vermiculite
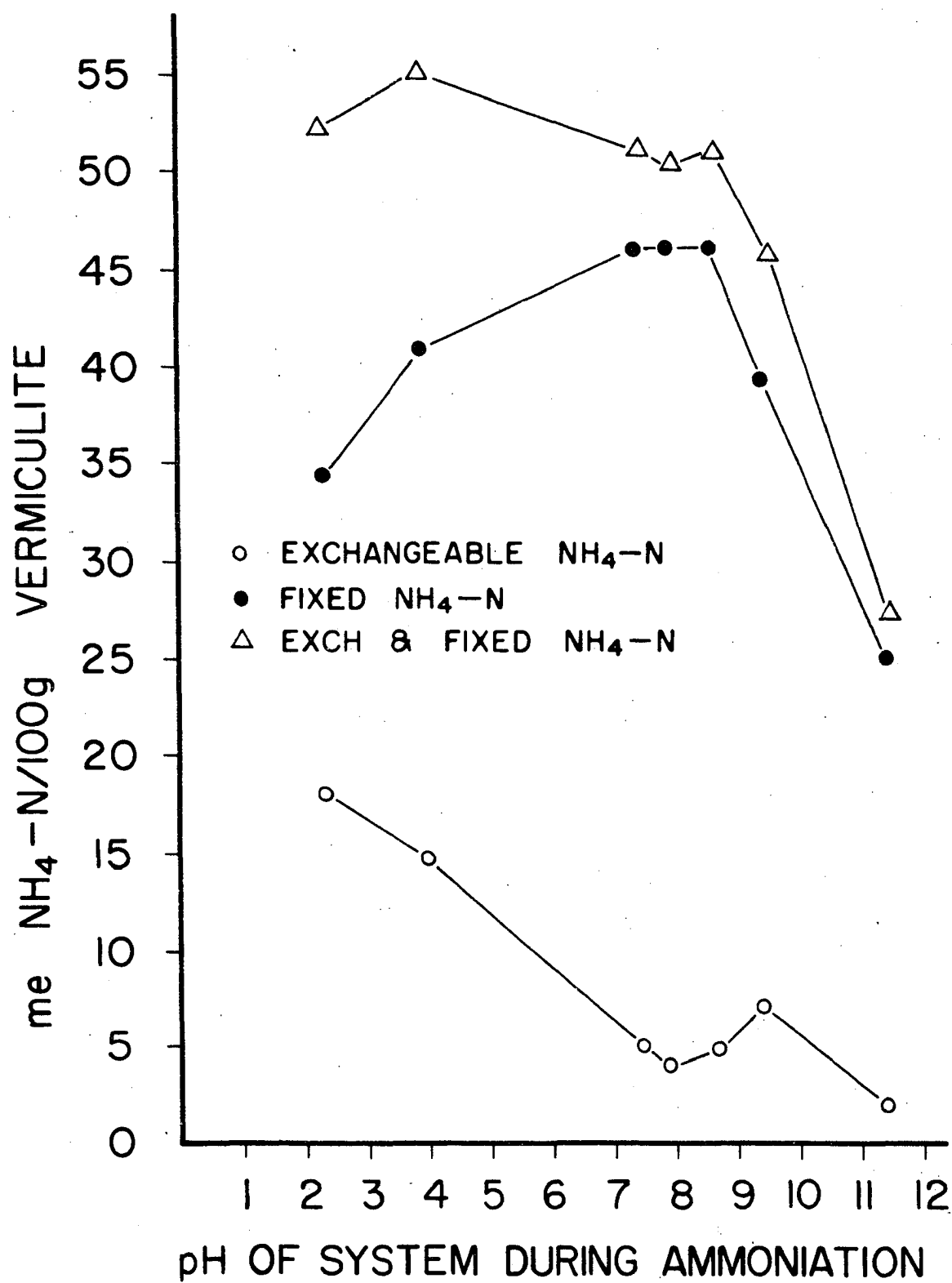
Table 6. Influence of ammoniation system pH on the fixation
of ammonium by the clay fraction of Glencoe soil

Ammoniation system[a]	NH$_4$-N fixed
pH	me/100 g soil
4.9	0.43
6.0	0.37
7.1	0.51
7.7	0.51
8.6	0.63
9.4	0.62
10.9	0.72

[a]5 ml. of 1 $\underline{N}$ NH$_4$OH (260 me N) added/2 g soil. Data
represent average of three replications.

vermiculite decreased from 18 to 4 me NH$_4$-N/100 g as the pH
increased from 2.3 to 7.9. Then the exchangeable NH$_4$-N in-
creased to 7 me NH$_4$-N/100 g at pH 9.4 followed by a decrease
to 2 me NH$_4$-N at pH 11.3. The sum of the fixed plus exchange-
able NH$_4$-N remained nearly constant from pH 2.3 to 8.6 and
then decreased as ammonium fixation decreased. The small
peak in the curve for exchangeable NH$_4$-N at pH 9.4 was inter-
esting since it corresponded with the beginning of the rapid
drop in the ammonium fixation curve. Perhaps there has been
some structural change in the clay mineral or precipitation
of a compound which restricted the collapse of the crystal
plates thus reducing ammonium fixation. The cause of the
reduced fixation of ammonium by vermiculite at high pH was

not investigated further since the ammonium fixation by soil
clay did not exhibit similar reductions in fixation. The
amount of ammonium fixed by the soil clay even if reduced by
half (0.5 me NH_4-N/100 g soil) would only result in a 10
percent reduction in the total ammonium fixed by whole Glencoe
soil (5.1 reduced to 4.6 me NH_4-N/100 g soil) under the
ammoniation conditions specified above.

Fixation by lignin and soil organic matter

Ammonium fixation by lignin and Glencoe soil organic
matter appeared to be of nearly equal magnitude at the same
pH when the fixation values of lignin and soil organic matter
were compared on an equal weight of organic material as shown
in Figure 8. It should be noted that the use of straight
lines instead of curves in Figure 8 was done only as a means
of emphasizing the effect of pH on ammonium fixation by
organic materials. As the pH during ammoniation was increased
from pH 2 to 7, fixation of added ammonium increased slightly
from approximately 1 to 3.2 me NH_4-N/100 g of organic mate-
rial. As the pH was increased above pH 7, fixation of
ammonium by lignin and organic matter increased sharply.
Fixation values of approximately 3.2, 11.5, 15, and 26 me
NH_4-N/100 g organic material were found at pH 7, 8.6, 9.4,
and 10.9 respectively.

Previous studies of ammonium fixation by mineral soils

Figure 8. Influence of pH during ammoniation on the
fixation of ammonium by lignin and the
organic fraction of Glencoe soil
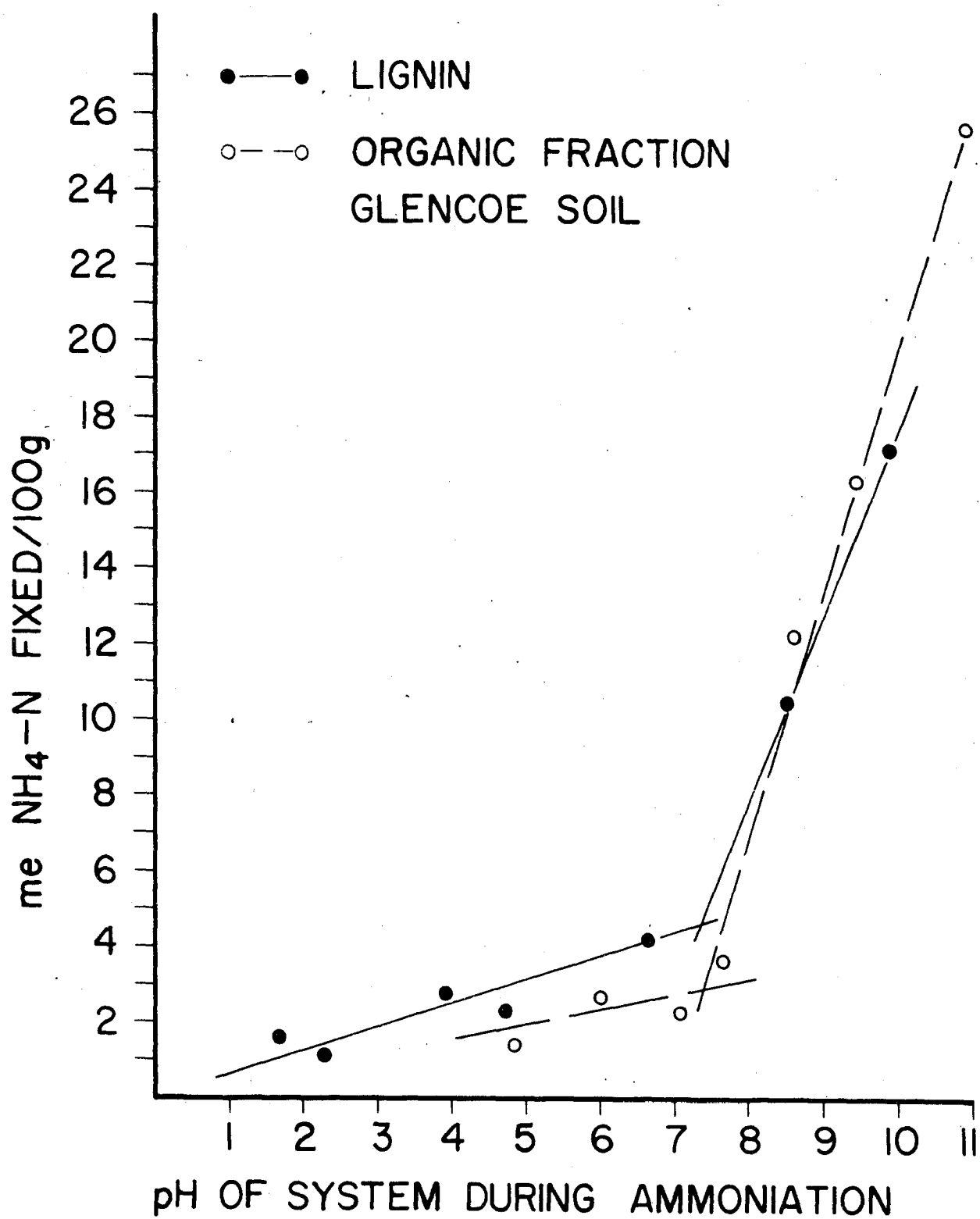
where neutral or acidic ammoniation conditions were employed, have not indicated that ammonium was fixed by the soil organic fraction. Investigators have not observed fixation of ammonium by the organic fraction due to the small amount fixed or not accounting fully for all of the organic nitrogen contained in the system under study.

In a mineral soil containing 5 percent organic matter ammoniated at pH 7 or less, fixation of added ammonium by the organic fraction would not be expected to exceed 0.15 me $NH_4-N/100$ g soil. Extraction of the soil with neutral salt solutions may remove 1 percent of the total organic nitrogen present in the soil. Thus an amount of organic nitrogen equal to or greater than the amount of NH_4-N fixed may be removed by extraction. No previous ammonium fixation studies are known to have included analysis of both the extract and the residue for organic nitrogen in the procedures used for estimating ammonium fixation.

Ammonium concentration, at a constant solution to soil ratio, appeared to be a critical factor at high ammoniation pH. The pH during ammoniation of Glencoe soil and lignin was 10.9 and 9.8 respectively when NH_4OH was added at two slightly different concentrations, 209 and 270 me $NH_4-N/100$ g. The fixation of ammonium varied with the added ammonium concentration. Glencoe organic matter fixed 3.6 and 5.1 me $NH_4-N/$ 100 g soil and lignin fixed 15.1 and 17.3 me NH_4-N at the

respective ammonium addition levels of 209 and 270 me NH_4-N/ 100 g.

Influence of Ammonium Concentration

The concentration of ammonium added for the ammoniation reaction was varied in two ways: 1) at constant volume of added solution the normality of NH_4OH was adjusted to approximately 0.1, 1, 5, and 13 normal; 2) at constant normality of NH_4OH the volume of solution added was adjusted to 0.2, 1, 2, and 5 ml. Thus, 16 combinations of ammonium concentration were obtained ranging from 1 to 3300 me NH_4-N added/100 g material.

Fixation by lignin and soil organic matter

Lignin and Glencoe soil organic matter fixed similar amounts of ammonium under comparable ammoniation conditions when the amount fixed was based on the same weight of organic material as shown in Figures 9 and 10. The scale of the y-axis of Figure 9 has been expanded by a factor of 5 when compared to the y-axis scale of Figure 10. The amount of added NH_4-N fixed increased at a decreasing rate as the amount of NH_4-N added/100 g was increased with a constant level of solution addition. Of particular interest was the observation that at the same ammonium concentration, as the solution to soil ratio decreased, the fixation increased.

Figure 9. Influence of volume and concentration of the
ammoniating solution on ammonium fixation by
the organic fraction of Glencoe soil
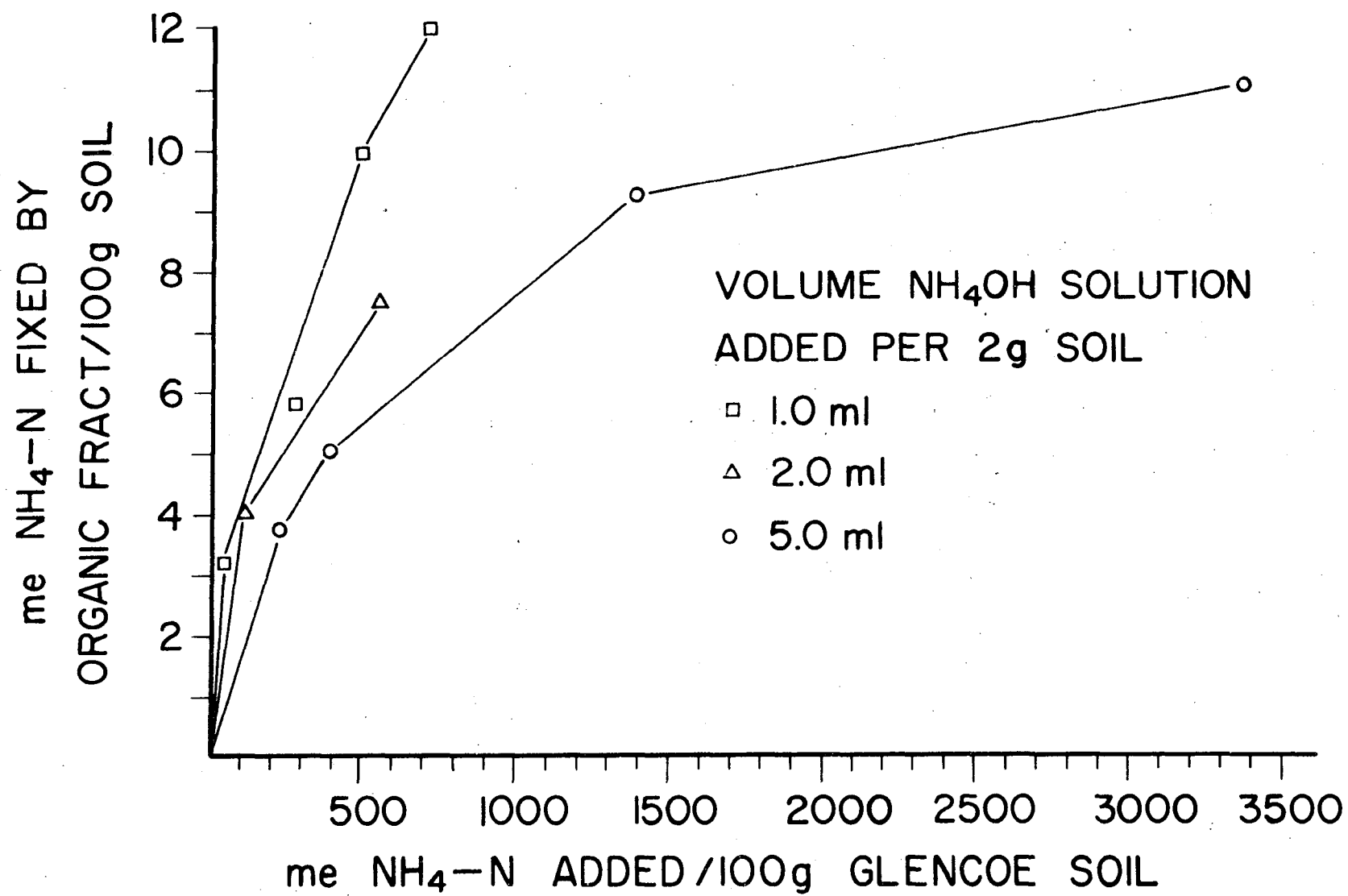
Figure 10.   Influence of volume and concentration of the
ammoniating solution on the fixation of ammonium
by lignin
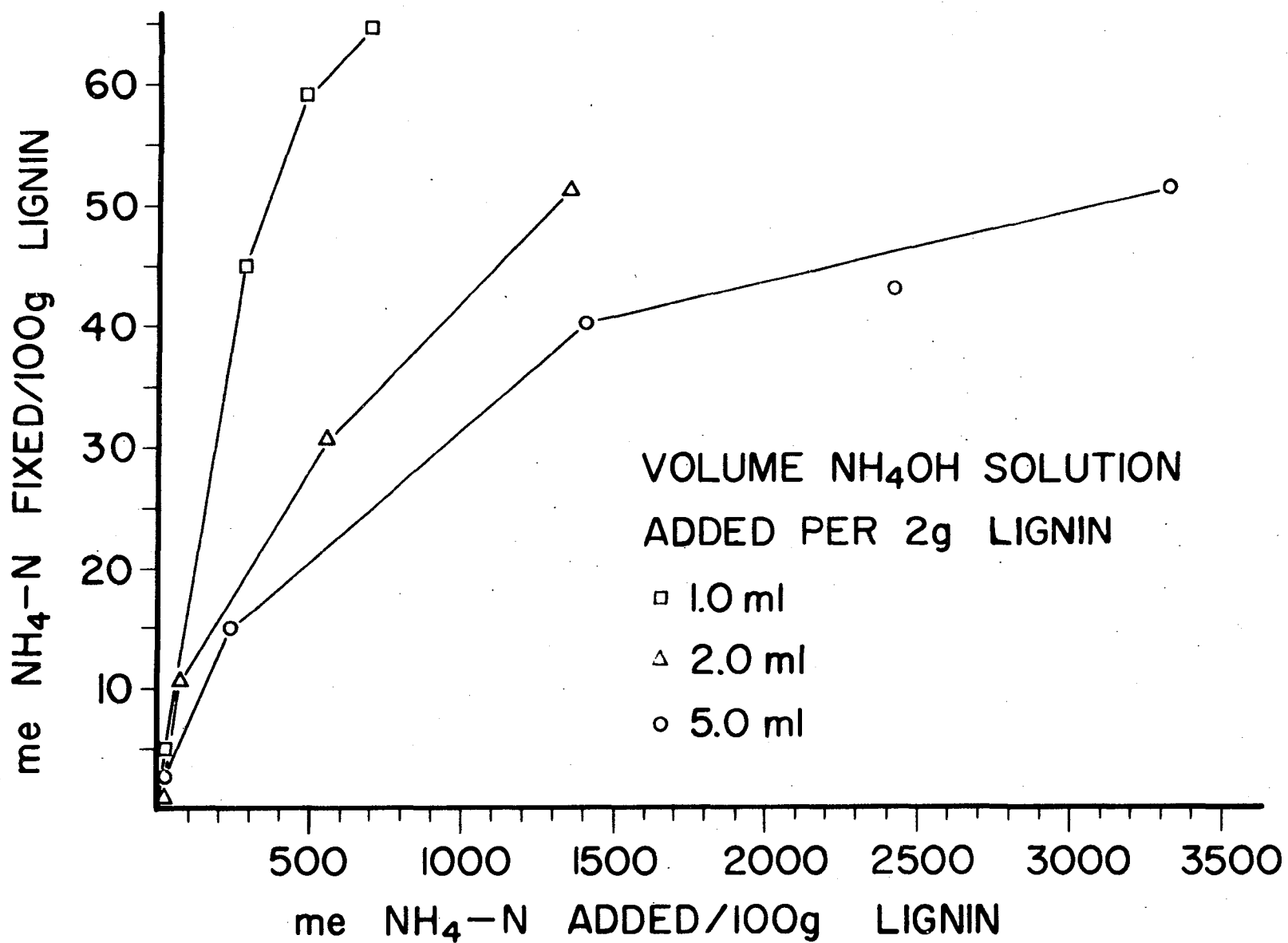
When 500 me NH$_4$-N/100 g was added, lignin fixed approximately 23, 31, and 59 me NH$_4$-N/100 g when the ammonium added was carried in 5, 2, and 1 ml of solution respectively. Under comparable conditions the organic fraction of Glencoe soil fixed 5.5, 7.5, and 10 me NH$_4$-N/100 g. soil. Addition of 1 ml of 13 normal (650 me/100 g) to Glencoe soil resulted in fixation by the organic fraction of 12 me NH4-N/100 g while only 11 me NH$_4$-N was fixed with the addition of 5 ml of 13 normal NH$_4$OH (3300 me NH$_4$-N/100 g). Under comparable conditions lignin fixed 53 and 65 me NH$_4$-N/100 g when 13 normal NH$_4$OH was added in 5 and 1 ml of solution volume respectively. Thus, the presence of H$_2$O may be inhibiting the reaction of ammonia with organic materials.

Fixation by soil clay minerals

The fixation of added NH$_4$-N by the clay fraction of Glencoe soil appeared to depend upon the total amount of ammonium added and the fixing capacity of the clay but not on the volume or concentration of the added NH$_4$-N solution. Fixation of NH$_4$-N by the soil clay increased from 0 to 0.7 me NH$_4$-N/100 g soil as the amount of ammonium added was increased from 0 to 10 me NH$_4$-N/100 g. With the addition of 3300 me NH$_4$-N/100 g soil the clay fraction fixed 0.8 me NH$_4$-N/100 g which was not significantly different from the 0.7 me NH$_4$-N fixed when only 10 me of NH$_4$-N was added per

100 g of soil.

Fixation of NH₄OH by Mineral Soils

Eight Iowa soils, ranging in carbon content from 1.4 to 10 percent, were ammoniated under two conditions. 1) High level ammoniation was carried out by addition of 1 ml of approximately 10 normal NH_4OH to 2 g of soil (460 me NH_4-N/ 100 g) followed by incubation for 24 hours at room temperature in stoppered 100 ml test tubes. The high level ammoniation provided conditions, similar to those found in the investigations previously described, which resulted in a high NH_4-N fixation by the soil clay and organic matter. 2) Low level ammoniation was obtained by addition of 5 ml of approximately 1 normal NH_4OH to 2 g of soil (250 me NH_4-N/100 g) followed by incubation as described above. Isotopic enrichment of the added NH_4OH was possible with low level ammoniation. Four of the soils were ammoniated with enriched $N^{15}H_4OH$ as a means of tracing the distribution of added ammonium within the several fractions obtained by the paired sample procedure. The low level ammoniation corresponded to the conditions employed for development of the paired sample procedure and for several investigations previously described. Following ammoniation, the samples were analyzed by the paired sample procedure for determination of ammonium fixation.

Total fixed NH$_4$-N

The eight soils ammoniated at the low level (250 me NH$_4$N/100 g) fixed from 1.3 to 5.7 me NH$_4$-N/100 g of soil while ammoniation at the high level (500 me NH$_4$-N/100 g) resulted in the fixation of 1.9 to 10.8 me NH$_4$-N/100 g as shown in Table 7. Fixation of added NH$_4$OH resulted in a significant increase in the total nitrogen content of the eight mineral soils investigated. At the low level of ammoniation, the native total soil nitrogen content was increased by 8.5 to 14.7 percent; at the high level of ammoniation the soil nitrogen content was increased by 16 to 26 percent.

Fixation of NH$_4$OH by the soil organic matter accounted for more than half of the total ammonium fixed. As the level of ammonium addition was increased, fixation by organic matter accounted for a higher percentage of the total ammonium fixed by the soil. In the Glencoe soil, the organic matter accounted for greater than 85 percent of the total ammonium fixed by the soil. These results are in agreement with the suggestion of Sohn and Peech (1958) that at least 50 percent of the amount of ammonia fixed by mineral surface soils when ammoniated with gaseous ammonia was due to fixation of ammonia by the soil organic matter.

Table 7. Fixation of NH_4OH by Iowa soils

Soil	Native soil		Added NH_4-N fixed added at							
			250 me/100 g				500 me/100 g			
	Carbon	Nitrogen	Total fixed	Clay fixed	Organic fixed	Increase in total soil-N	Total fixed	Clay fixed	Organic fixed	Increase in total soil-N
	%	me/100 g	me/100 g	%	%	%	me/100 g	%	%	%
Marion	1.36	9.79	1.44	43.7	56.3	14.7	2.61	21.8	78.2	26.7
Edina	1.66	11.52	1.39	12.2	87.8	12.1	1.94	25.3	74.7	16.8
Marshall	1.99	14.37	1.31	34.4	65.6	9.1	2.93	21.8	78.2	20.4
Clarion	2.14	14.59	1.83	28.4	71.6	12.5	3.17	30.3	69.7	21.7
Grundy	2.27	14.81	1.85	34.6	65.4	12.5	2.74	19.3	80.7	18.5
Nicollet	3.97	24.22	2.42	31.4	68.6	10.0	4.99	20.2	79.8	20.6
Webster	4.44	25.28	2.70	31.9	68.1	10.7	4.94	22.3	77.7	19.5
Glencoe	10.2	67.2	5.69	13.0	87.0	8.5	10.8	6.5	93.5	16.1

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Clay-fixed NH_4-N

The amount of added NH_4-N fixed by the clay fraction appeared to be independent of the two levels of ammonium addition as shown in Table 8. The soil clay fractions fixed an average of 0.7 me NH_4-N/100 g of soil within a range of 0.33 to 0.98 me NH_4-N/100 g. The clay fraction initially contained

Table 8. Fixation of NH_4OH by the clay fraction of eight Iowa soils

| Soil | Clay content | Native clay fixed NH_4-N | | Added NH_4-N fixed by clay | | |
		Direct[a]	P.S.M.[b]	250	500	Av.
	%	me/ 100 g	me/ 100 g	me/ 100 g	me/ 100 g	me/ 100 g
Marion	20	1.03	0.89	0.63	0.57	0.60
Edina	26	0.91	0.80	0.17	0.49	0.33
Marshall	36	1.60	1.44	0.45	0.64	0.55
Clarion	24	0.61	0.58	0.52	0.68	0.60
Grundy	30	1.35	1.17	0.64	0.53	0.59
Nicollet	31	0.77	0.78	0.76	1.01	0.89
Webster	32	0.88	0.90	0.86	1.10	0.98
Glencoe	41	1.00	1.01	0.74	0.73	0.73

[a]Direct determination of native clay fixed NH_4-N was made by the Dhariwal and Stevenson (1958) procedure.

[b]P.S.M. determination of native clay fixed NH_4-N was obtained from control sample when analyzed by the paired sample method.

from 0.6 to 1.6 me NH_4-N/100 g soil prior to ammoniation.
Fixation of ammonium by the clay fractions accounted for 6.5
to 43 percent of the total ammonium fixed by the soil.

Organic fixed NH_4-N

The amount of NH_4-N fixed by the soil organic fraction
was found to increase linearly with the increase in soil
carbon content as shown in Figure 11 and Table 9. At the
high level of ammoniation, approximately 0.95 me NH_4-N was
fixed per gram of soil carbon. Burge and Broadbent (1961)
reported that 2.9 me NH_4-N was fixed per gram of carbon in
organic soils (carbon contents ranged from 15 to 60 percent).
However, the regression equation, $Y = 3.17X - 21.6$ (ibid.)
predicted 10.7 me NH_4-N/100 g would be fixed when the soil
contained 10.2 percent carbon. The regression equation in
Figure 11, $Y = 0.947X + 0.21$, predicts 9.87 me NH_4-N would be
fixed when the soil contains 10.2 percent carbon. The
organic fraction of Glencoe soil, which contains 10.2 percent
carbon fixed 10 me NH_4-N/100 g soil when ammoniated at the
high level. The two regression equations, cited above, have
different slopes which might be due to one or more of the
following. 1) Ammoniation conditions: Burge and Broadbent
ammoniated with an excess of gaseous ammonia in the presence
of oxygen which should result in maximum fixation. 2) Method
of analysis: Burge and Broadbent estimated ammonium fixation

Figure 11.   Relationship of soil carbon content to the
              fixation of NH_4OH by soil organic matter

me NH₄–N FIXED BY ORGANIC FRACTION / 100g SOIL vs PERCENT SOIL CARBON

NH₄OH ADDED AT

○ 500 me NH₄–N/100g SOIL
● 250 me NH₄–N/100g SOIL

$\hat{Y} = 0.947X + 0.21$
r = 0.99

$\hat{Y} = 0.456X + 0.14$
r = 0.98
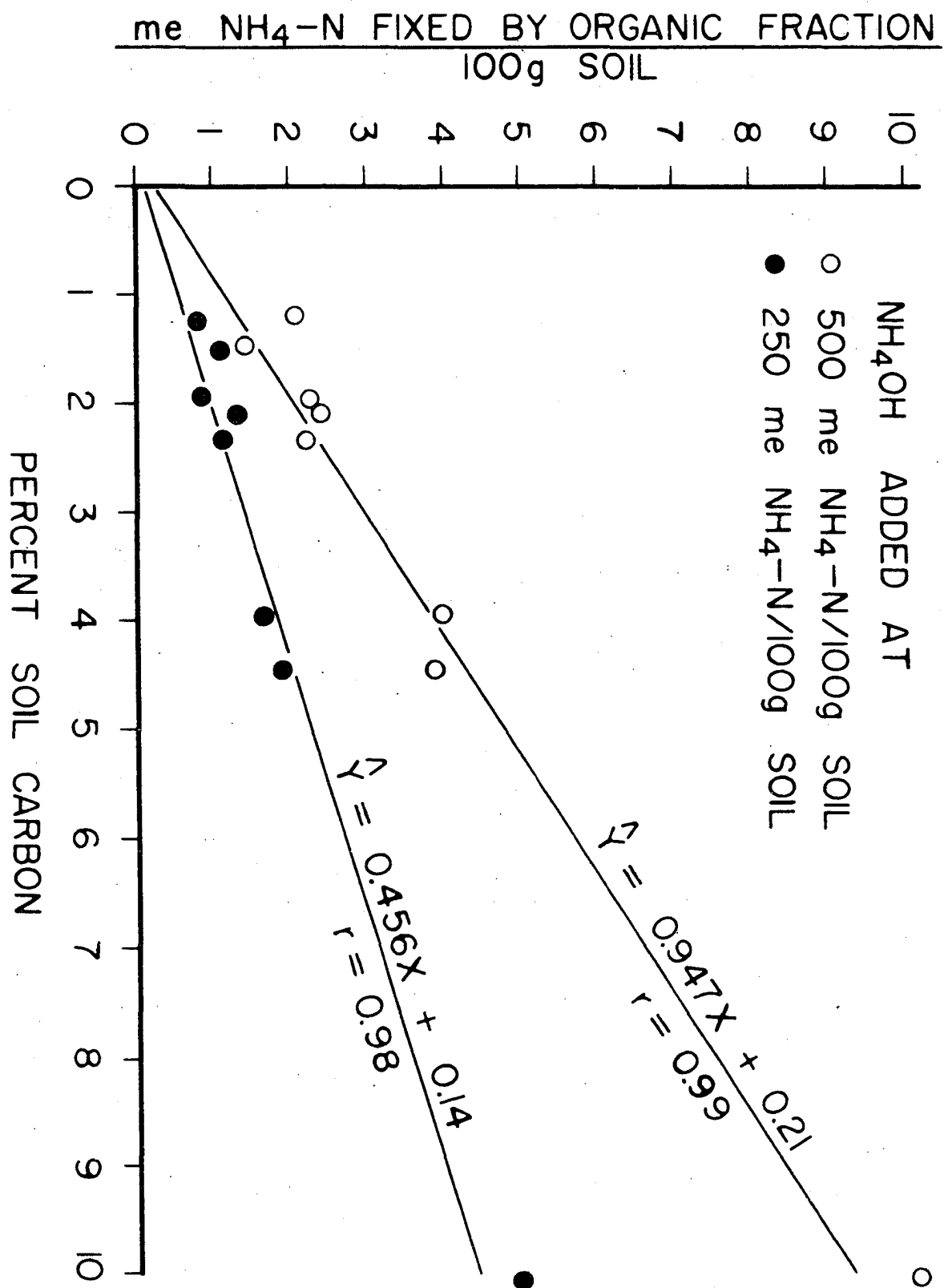
Table 9. Fixation of NH_4OH by the inorganic fraction of eight Iowa soils

Soil	Carbon content	Native org.-N content	NH4OH fixed by organic fraction			
			250 me NH4-N/100 g added		500 me NH4-N/100 g added	
			NH4-N fixed	Increase in org.-N	NH4-N fixed	Increase in org.-N
	%	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	%	$\frac{\text{me N}}{100 \text{ g}}$	%
Marion	1.36	8.76	0.81	9.2	2.04	23.3
Edina	1.66	10.61	1.22	11.5	1.45	13.7
Marshall	1.99	12.77	0.86	6.7	2.29	17.9
Clarion	2.14	13.98	1.31	9.4	2.49	17.8
Grundy	2.27	13.46	1.21	9.0	2.21	16.4
Nicollet	3.97	23.45	1.66	7.1	3.98	17.0
Webster	4.44	24.40	1.84	7.5	3.84	15.7
Glencoe	10.2	66.2	4.95	7.5	10.1	15.3

by difference in the residual-N content of ammoniated and control samples which had been extracted with acid in order to remove soluble and exchangeable NH_4-N. Therefore, any organic N contained in the acid extract would lead to low fixation values. 3) Soil properties: The nature and properties of the soil organic fraction may change as the organic content of the soil increases above 10 percent carbon. Thus, the relationship between soil carbon content and ammonium fixation may be curvilinear rather than linear over a range of 1 to 60 percent soil carbon.

At the low level of ammoniation, approximately 0.45 me NH_4-N was fixed per gram of carbon (Figure 11). Thus, increasing the ammonium addition from approximately 250 me NH_4-N/100 g to 500 me NH_4-N/100 g resulted in a two fold increase in the amount of ammonium fixed by soil organic matter. As shown in Figure 9, the relationship between ammonium solution concentration and volume was unique; further increases in the total ammonium concentration at the solution soil ratios employed above would not result in the same proportional difference in the amount of ammonium fixed.

A considerable increase in the organic nitrogen content of the soil organic matter was attained as a result of ammonium fixation. As shown in Table 9, the high level of ammoniation resulted in an average 17 percent increase in the content of soil organic nitrogen. An average increase

in the soil organic nitrogen content of 8.5 percent occurred with the low level of ammoniation.

Distribution of Fixed $N^{15}H_4$-N in the Fractions Obtained by the Paired Sample Procedure

Total, clay, and organic-fixed NH_4-N

The several fractions obtained from soil fractionated by the paired sample procedure were analyzed for NH_4-N content. The distillates containing ammonium were then analyzed for $N29/28$ ratio. The total amount of NH_4-N derived from added $N^{15}H_4OH$ was calculated as described in the chapter on methods.

Comparison of the total, clay, and organic ammonium fixation values obtained by claculations based on added $N^{15}H_4OH$ and by the calculations normally employed with the paired sample method have been shown in Table 10. The amount of total, organic and clay-fixed NH_4-N was substantially the same when calculated by both methods. The variation between the values could have been due to the extra distillation required for each fraction analyzed for isotopic nitrogen ratio, insufficient replication, and possible errors encountered with the isotope analysis procedure. The complete data obtained for the four soils ammoniated by $N^{15}H_4OH$ can be found in the Appendix tables 27, 28, 29, and 30.

Table 10. Fixation of ammonium by soils as determined with
and without isotopic nitrogen[a]

Soil	Calculation[b]	Total fixed NH_4-N	Clay fixed NH_4-N	Organic fixed NH_4-N
		me/100 g	me/100 g	me/100 g
Edina	P.S.M.	1.39	0.17	1.22
	P.S.M.+N^{15}	1.01	0.29	0.72
Marshall	P.S.M.	1.31	0.45	0.86
	P.S.M.+N^{15}	1.29	0.49	0.80
Webster	P.S.M.	2.70	0.86	1.84
	P.S.M.+N^{15}	Lost	1.13	Lost
Glencoe	P.S.M.	5.69	0.74	4.95
	P.S.M.+N^{15}	5.34	0.72	4.62

[a]5 ml of 1 $\underline{N}$ NH_4OH added/2 g.

[b]P.S.M.: difference between appropriate fractions
obtained for treated and control sample by paired sample
procedure.
P.S.M.+N^{15}: from added $N^{14+15}H_4OH$ as calculated from
isotopic analysis of fractions obtained by paired sample
method.

Distribution of enriched NH_4-N

The distribution of fixed NH_4-N, based on N^{15} content,

within the several fractions obtained by the paired sample

procedure, HF extract (total clay-fixed NH_4-N), H_2O-soluble

organic-N, and residual-N have been shown in Table 11. The

H_2O-soluble organic-N fraction was found to contain from 20

to 37 percent of the total NH_4-N fixed by the soil organic

Table 11. Distribution of fixed NH_4-N within the fractions obtained by the paired sample method using N^{15}

Soil	Fraction	Total-N in fraction	Fixed NH_4-N	Fixed NH_4-N as percentage of		
				Total-N in fraction	Total fixed NH_4-N	Organic fixed NH_4-N
		me/100 g	me/100 g	%	%	%
Edina	Total clay-fixed NH_4-N	0.96	0.29	30.2	28.7	
	Residual-N	8.61	0.81	9.4	80.2	
	Residual organic-N	7.65	0.52	6.8	51.5	72.2
	H_2O-soluble organic-N	3.64	0.2	5.5	19.8	27.8
	Total organic-N	11.29	0.72	6.4	71.3	
	Total fixed NH_4-N		1.01			
Marshall	Total clay-fixed NH_4-N	1.85	0.49	26.5	38.0	
	Residual-N	12.77	1.13	8.9	87.6	
	Residual organic-N	14.62	0.64	4.4	49.6	80.0
	H_2O-soluble organic-N	2.90	0.16	5.5	12.4	20.0
	Total organic-N	17.52	0.80	4.6	62.0	
	Total fixed NH_4-N		1.29			
Webster	Total clay-fixed NH_4-N	1.77	1.13	63.8		
	H_2O-soluble organic-N	3.68	1.10	29.9		
Glencoe	Total clay-fixed NH_4-N	1.75	0.72	41.1	13.5	
	Residual-N	65.45	3.62	5.5	67.8	
	Residual organic-N	63.70	2.90	4.6	54.3	62.8
	H_2O-soluble organic-N	6.87	1.72	25.0	32.2	37.2
	Total organic-N	70.57	4.62	6.5	86.5	
	Total fixed NH_4-N		5.34			

fraction. The remaining 63 to 80 percent of the total NH_4-N fixed by the organic fraction was contained in the residual-N fraction. Water extraction following ammoniation with NH_4OH was in fact a dilute, alkaline extraction which may have removed a portion of the soil organic fraction that would normally have been water soluble. However, as shown in the Appendix tables, extraction by H_2O, KCl, or acetic acid removed some organic-N from both ammoniated and control samples. It is imperative that extracts obtained from soils be analyzed for organic-N content in any investigation involving ammonium fixation.

The fixed NH_4-N contained within the H_2O-extractable organic-N fraction may be more available to biological systems than the fixed NH_4-N contained in the residual organic-N fraction. It would appear that the H_2O-soluble organic-N fraction from ammoniated soil should be investigated separately from the residual-N fraction as to the availability of fixed NH_4-N to biological systems.

The organic-fixed NH_4-N constitutes only a small percentage (from 4 to 7 percent) of the total-N contained within the residual-N fraction. Thus, attempts to identify the nature of the compounds containing fixed NH_4-N would be extremely difficult due to the interference of the large amount of native organic-N present. Approximately 25 percent of the total-N in the H_2O-soluble organic-N fraction extracted

from Webster and Glencoe soils was fixed NH_4-N. Investigation of the organic-N compounds in the H_2O extract from soils high in organic matter might offer some clues as to the nature and properties of the compounds containing fixed NH_4-N.

Nitrogen fertilization and ammonium fixation

Ammonium fixation could be an important factor in plant nutrition particularly when soils high in organic matter are fertilized with alkaline ammonium-nitrogen. McIntosh (1958) found that anhydrous ammonia, applied in bands 40 inches apart in a clay loam soil, was retained in a zone 1.5 to 2.5 inches in radius. The ammonia would thus contact from 54,000 to 150,000 pounds of soil per acre. If ammonia were applied to a soil having the capacity to fix 1 me NH_4-N/100 g, then it would be possible in theory to fix a maximum of 7.5 to 20 pounds of NH_4-N per acre. However, concentrations of ammonia high enough to allow maximum fixation occur in only a small portion of the total retention zone, perhaps 1 to 10 percent of the total area. Thus in a soil containing 10 percent carbon, application of 100 pounds of NH_3-N per acre might result in fixation by soil organic matter of 2 to 20 pounds of the applied nitrogen. Further investigation will be necessary to find 1) how much of the ammonium applied under field conditions may be fixed by various soils and 2) how readily the fixed ammonium may become available to biological systems.

SUMMARY AND CONCLUSIONS

Determination of Ammonium Fixation

A method was developed for quantitatively estimating the non-biological fixation of ammonium added to mineral surface soils. Paired samples were ammoniated with a measured amount of ammonium in a closed system. Following ammoniation the samples were extracted successively with H_2O and 1 normal KCl by shaking and centrifugation. The extracts were analyzed for ammonium-N by steam distillation with MgO and for organic-N by Kjeldahl analysis of the residue from this distillation. One member of the extracted sample pair was then analyzed for residual total-N by Kjeldahl analysis. The remaining member of the sample pair was analyzed for clay-fixed ammonium-N by determination of the ammonium released by HF after treatment with hot KOH. Total ammonium-N fixed was determined from (H_2O-extractable organic-N plus KCl-extractable organic-N plus residual-N in the ammonitated sample) minus (the sum of the corresponding values obtained from a control sample). Ammonium-N fixed by the clay fraction was calculated as the difference in the amount of ammonium-N extracted by HF from ammoniated and control samples. Ammonium-N fixed by the organic fraction was calculated from the total ammonium-N fixed by subtraction of the ammonium-N fixed by the clay fraction.

The procedures employed in estimating ammonium fixation were independent of the conditions employed for ammoniation of the sample. In addition to soils, the method could be used without modification for estimating ammonium fixation by vermiculite and lignin. Substitution of an alcohol-water combination in place of water for extraction of soluble ammonium would allow use of the method for estimating ammonium fixation by illite and montmorillonite type clay minerals.

Distribution of organic fixed ammonium

Using $N^{15}H_4OH$ as a tracer, it was found that 20 to 37 percent of the total NH_4-N fixed by the soil organic matter was contained in a fraction extractable by H_2O following ammoniation. The remaining 63 to 80 percent of the NH_4-N fixed by the soil organic matter was retained as residual-N following H_2O and KCl extraction. Therefore, if information on total ammonium fixation is desired, the methods employed must provide for the measurement of the ammonium that has been fixed in both soluble and insoluble forms.

Factors Affecting Ammonium Fixation

A study was made of various conditions which may affect fixation of ammonium by the organic and mineral fractions of soil. Comparative experiments were conducted with model compounds, vermiculite and lignin.

Ammoniation time

The fixation of ammonium by lignin and soil organic matter was found to be 75 to 85 percent complete within 24 hours when based on the total ammonium fixed after 96 hours of ammoniation.

Ammonium fixation by vermiculite was 92 percent complete within 12 hours when based on the total ammonium fixed after 24 hours of ammoniation. When Glencoe soil was ammoniated with 487 me NH_4-N/100 g for 96 hours, fixation by the soil clay was 68 and 88 percent complete after 1 minute and 24 hours, respectively.

Ammoniation pH

Ammonium fixation by organic materials occurred at all pH values between 2.3 and 10.9. At pH 10.9 approximately 26 me NH_4-N was fixed per 100 g of organic material. The amounts fixed decreased as the pH decreased, so that below pH 7 the amounts fixed were less than 3.2 me NH_4-N per 100 g of organic material. Fixation of ammonium by the soil organic fraction and lignin appeared to be of similar magnitude when compared on the basis of an equal weight of organic material.

Vermiculite and soil clay minerals fixed ammonium at all pH values studied. When 260 me NH_4-N was added per 100 g vermiculite, the fixation increased from 36 me NH_4-N/100 g at

pH 2.3 to 47 me NH_4-N/100 g at pH 7.9, then decreased to 27 me NH_4-N/100 g at pH 11.3. Fixation of ammonium by Glencoe soil clay was found to increase from 0.43 to 0.72 me NH_4-N/ 100 g soil as the pH was increased from pH 4.9 to pH 10.9.

Ammonium concentration

With a constant solution to soil ratio, ammonium fixation by soil organic matter and lignin was found to increase as the total amount of ammonium added as NH_4OH was increased. However, at the same ammonium concentration, as the solution to soil ratio decreased, the fixation increased. When 500 me NH_4-N/100 g sample was added, approximately 25, 34, and 54 me NH_4-N/100 g of organic material was fixed when the ammonium added was carried in 5, 2, and 1 ml of solution respectively.

Ammonium fixation by Glencoe clay increased from 0 to 0.7 me NH_4-N/100 g soil as the amount of ammonium added was increased from 0 to 10 me NH_4-N/100 g soil but further increases in the amount of ammonium added did not result in a significant increase in the amount of ammonium fixed by the soil clay. The amount of ammonium fixed by soil clay was not affected by changing the solution to soil ratio used for ammoniation.

Fixation of Ammonium by Mineral Surface Soils

Eight Iowa mineral surface soils were ammoniated with NH_4OH added at the approximate rate of either 250 me NH_4-N/ 100 g soil employing a 2.5:1 solution to soil ratio or 500 me NH_4-N/100 g soil using a 1:1 solution to soil ratio. The carbon content of the eight soils ranged from 1.4 to 10.2 percent.

Total fixation

The total soil nitrogen content was increased from 9 to 26 percent by the fixation of the added ammonium. Addition of 250 me NH_4-N/100 g in the different soils resulted in the fixation of from 1.3 to 5.7 me NH_4-N/100 g. From 1.9 to 10.8 me NH_4-N/100 g was fixed in the eight soils when 500 me NH_4-N/100 g was added.

Clay fixation

The amount of ammonium fixed by any one soil clay fraction was the same at both levels of ammonium addition. The amounts fixed in the eight soils ranged from 0.3 to 1.0 me NH_4-N/100 g soil when either 250 or 500 me NH_4-N/100 g was added.

Organic fixation

From 50 to 90 percent of the increase in the total soil nitrogen content was due to ammonium fixation by the soil organic fraction. A linear relationship was found between the soil carbon content and the amount of added ammonium fixed by the soil organic matter. Approximately 0.45 me NH_4-N was fixed per gram of carbon when 250 me NH_4-N was added per 100 g of soil while addition of 500 me NH_4-N/100 g soil resulted in about 0.95 me NH_4-N fixed per gram of soil carbon.

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APPENDIX

Table 12. Influence of ammoniation time on the fixation of NH_4OH by Glencoe soil

Treatment[a]								
1. Time of contact	1 min	10 min	100 min	12 hrs	48 hrs	48 hrs	96 hrs	96 hrs
2. Milliliters of solution	1	1	1	1	1	5	1	5
3. Approximate normality	10	10	10	10	10	1	10	1
4. me NH_4-N added/100 g	487.2	487.2	487.2	487.2	487.2	209.5	487.2	209.5

	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$
Fractionation[b]								
5. HAc + H_2O + KCl - sol. org.-N	1.22	1.45	3.1	3.75	5.34	3.49	7.03	4.40
6. Residual-N	64.68	65.98	67.51	69.83	70.77	65.75	71.99	65.47
7. Total Kjeldahl-N (line 5 + 6)	65.90	67.43	70.61	73.58	76.11	69.24	79.02	69.87
8. Total fixed NH_4-N (line 7 - control)[c]	1.13	2.66	5.84	8.81	11.34	4.47	14.25	5.10
9. HF extract NH_4-N	1.54	1.61	1.64	1.70	1.75	1.69	1.80	1.77
10. Clay-fixed NH_4-N (line 9 - control)[c]	0.54	0.61	0.64	0.70	0.75	0.69	0.80	0.77
11. Organic fixed NH_4-N (line 8 - 10)	0.59	2.05	5.20	8.11	10.59	3.78	13.45	4.33
12. HAc + H_2O + KCl - sol. + exch. NH_4-N	470.6	467.5	457.8	448.2	438.2	202.6	406.1	195.7
13. Total N recovered	536.5	534.9	528.4	521.8	504.3	271.8	485.1	265.6

[a]For 24 hour ammoniation time, see Table 25 for 458 me NH_4-N/100 g and Table 14 and Table 23 for 209 me NH_4-N/100 g.

[b]Data represent one replication.

[c]Control values: total Kjeldahl - N = 64.77 me N/100 g; clay-fixed NH_4-N = 1.0 me N/100 g.

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Table 13. Influence of ammoniation time on the fixation of NH_4OH by lignin

Treatment[a]							
1. Time of contact	1 min	10 min	100 min[b]	2 days	2 days	4 days	4 days
2. Milliliters of solution	1	1	1	1	5	1	5
3. Approximate normality	10	10	10	10	1	10	1
4. me NH_4-N added/100 g	487	487	487	487	209.5	487.0	209.5
	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$
Fractionation[c]							
5. HAc + H_2O + KCl - sol. org.-N	1.08	1.31	2.25	8.06	2.37	8.07	2.91
6. Residual-N	9.49	12.38	22.93	67.32	18.95	68.00	21.25
7. Total Kjeldahl-N (line 5 + 6)	10.57	13.69	24.18	75.38	20.32	76.07	24.16
8. Total fixed NH_4-N (line 7 - control)[d]	4.78	7.90	18.39	69.59	14.53	70.28	18.37
9. HAc + H_2O + KCl - sol. + exch. NH_4-N	462.9	450.4	447.0	383.4	192.1	412.3	187.4
10. Total N recovered	467.7	458.3	465.4	453.0	206.6	482.6	205.8

[a]For 24 hour ammoniation time see Table 24 for 487 me N/100 g and Tables 17 and 19 for 209 me NH_4-N/100 g.

[b]One replication.

[c]Data represent average of two replications.

[d]Control value: total Kjeldahl - N = 5.79 me N/100 g.

Table 14. Influence of pH on the fixation of added NH_4-N by Glencoe soil

Treatments[a]

1. pH of system	6.8	4.9	6.0	7.1	7.7	8.6	9.4	--
2. me NH_4-N added/100 g	0.0	258.9	256.4	252.9	253.0	258.9	265.1	209.5

Fractionation[b]	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$
3. H_2O + KCl - sol. + org.-N	1.17	1.12	0.84	1.14	1.22	1.56	1.99	3.16
4. Residual-N	63.03	64.07	64.16	63.97	64.16	65.10	65.66	64.72
5. Total Kjeldahl-N (line 3 + 4)	64.20	65.19	65.00	65.11	65.38	66.66	67.65	67.88
6. Total fixed NH_4-N (line 5 - control)	--	0.99	0.80	0.91	1.18	2.46	3.45	3.68
7. HF extract NH_4-N	1.02	1.37	1.36	1.41	1.43	1.49	1.50	1.52
8. Clay-fixed NH_4-N (line 7 - control)	--	0.35	0.34	0.39	0.41	0.47	0.48	0.50
9. Organic fixed NH_4-N (line 6 - 8)	--	0.64	0.46	0.52	0.77	1.99	2.97	3.18
10. H_2O + KCl - sol. + exch. NH_4-N	1.81	256.1	254.6	250.7	251.4	251.3	254.3	204.4
11. Total N recovered	66.01	321.2	309.6	315.8	316.8	318.0	322.0	272.3

[a]See Table 5 for solution compositions.

[b]Data represent one replication.

Table 15.   Influence of pH on the fixation of added NH_4-N by Glencoe soil when acetic acid was used for the first extraction

Treatment[a]								
1. pH of system	6.8	4.9	6.0	7.1	7.7	8.6	9.4	10.9
2. me NH_4-N added/100 g	0.0	258.9	256.4	252.9	253.0	258.9	265.1	270.5

	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$
Fractionation[b]								
3. HAc + H_2O - sol. org.-N	1.39	2.28	2.08	1.98	2.08	2.97	3.39	5.11
4. KCl sol. org.-N	0.28	0.29	0.26	0.27	0.30	0.25	0.26	0.35
5. Residual-N	64.72	63.46	64.44	64.37	64.62	65.57	66.29	66.06
6. Total Kjeldahl-N (line 3 + 4 + 5)	65.69	66.03	66.78	66.62	67.00	68.79	69.94	71.52
7. Total fixed NH_4-N (line 6 - control)	--	0.34	1.09	0.93	1.31	3.10	4.25	5.83
8. HF extract NH_4-N	1.02	1.44	1.46	1.53	1.58	1.69	1.67	1.70
9. Clay-fixed NH_4-N (line 8 - control)	--	0.42	0.44	0.51	0.56	0.67	0.65	0.68
10. Organic fixed NH_4-N (line 7 - 9)	--	0.08	0.65	0.42	0.75	2.43	3.60	5.15
11. HAc + H_2O - sol. NH_4-N	0.58	243.7	242.5	238.9	238.5	241.0	243.9	247.3
12. KCl exch. NH_4-N	0.12	10 6	10.9	10.9	11.0	11.8	10.5	12.3
13. Total N recovered (line 6 + 11 + 12)	66.39	320.3	320.2	316.4	316.5	321.6	324.3	331.1

[a]See Table 5 for composition of solution.

[b]Data represent one replication.

Table 16. Influence of pH on the fixation of added NH_4-N by lignin

Treatments[a]								
1. pH of system	2.5	1.6	2.3	3.9	4.7	6.6	8.5	9.8
2. me NH_4-N added/100 g	0.0	258.9	256.4	252.9	253.0	258.9	265.1	270.5

	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$
Fractionation[b]								
3. H_2O org.-N	0.50	0.86	0.87	0.35	1.1	1.56	2.94	14.25
4. KCl org.-N	0.31	0.30	0.30	0.31	0.31	0.56	1.55	0.78
5. Total sol. org.-N (line 3 + 4)	0.81	1.16	1.17	0.66	1.41	2.12	4.49	15.03
6. Residual org.-N	4.81	6.28	6.05	6.82	6.80	8.02	13.34	10.32
7. Total org.-N (line 5 + 6)	5.62	7.44	7.22	7.48	8.21	10.14	17.83	25.35
8. NH_4-N fixed (line 7 – control)	--	1.82	1.60	1.86	2.59	4.52	12.21	19.73
9. H_2O sol. NH_4-N	0.00	251	251	241.6	239.9	217.7	197.4	211.6
10. KCl exch. NH_4-N	0.05	1.3	1.9	4.9	9.5	35.6	49.5	35.2
11. Total inorg. NH_4-N (line 9 + 10)	0.05	252.3	252.9	246.5	249.4	253.3	246.9	246.8
12. Total N recovered (line 7 + 11)	5.67	259.7	260.1	254.0	257.6	260.4	264.7	272.2
15. pH H_2O extract	3.8	3.0	3.8	4.9	7.0	7.3	8.5	9.2

[a]See Table 5 for composition of solution.

[b]Data represent one replication.

Table 17. Influence of pH on the fixation of added NH_4-N by lignin

Treatments[a]									
1. pH of system	2.5	1.6	2.3	3.9	4.7	6.6	8.5	--	9.8
2. me NH_4-N added /100 g	0.0	258.9	256.4	252.9	253.0	258.9	265.1	209.5	270.5

	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$
Fractionation[b]									
3. H_2O + KCl sol. org.-N	0.03	0.3	0.43	0.44	0.53	0.98	2.23	8.23	8.46
4. Residual org.-N	6.00	7.36	6.94	8.21	7.97	9.52	14.59	13.79	14.35
5. Total org.-N (line 3 + 4)	6.03	7.66	7.37	8.65	8.50	10.50	16.82	22.02	22.81
6. NH_4-N fixed (line 5 - control)	--	1.63	0.74	2.62	2.47	4.47	10.79	15.99	16.85
7. H_2O + KCl sol. + exch. NH_4-N	0.85	254.5	252.6	250.5	250.8	255.9	251.0	196.9	241.1
8. Total N recovered (line 6 + 7)	6.88	262.2	260.0	259.2	259.3	266.4	267.8	218.9	263.9
11. pH of H_2O + KCl extract	3.7	3.2	3.5	7.2	7.7	7.3	8.6	9.2	9.3

[a]See Table 5 for composition of solution.

[b]Data represent one replication.

Table 18. Influence of pH on the fixation of added NH_4-N by lignin when acetic acid was used for the first extraction

Treatments[a]								
1. pH of system	2.5	1.6	2.3	3.9	4.7	6.6	8.5	9.8
2. me NH_4-N added/100 g	0.0	258.9	256.4	252.9	253.0	258.9	265.1	270.5

	$\frac{\text{me N}}{100\ g}$	$\frac{\text{me N}}{100\ g}$	$\frac{\text{me N}}{100\ g}$	$\frac{\text{me N}}{100\ g}$	$\frac{\text{me N}}{100\ g}$	$\frac{\text{me N}}{100\ g}$	$\frac{\text{me N}}{100\ g}$	$\frac{\text{me N}}{100\ g}$
Fractionation[b]								
3. HAc + H_2O - sol. org.-N	0.0	0.0	0.0	2.1	0.9	1.3	1.93	3.43
4. KCl - sol. org.-N	0.08	0.04	0.05	0.07	0.07	0.1	0.14	0.27
5. Total sol. org.-N (line 3 + 4)	0.08	0.04	0.05	2.17	0.97	1.4	2.07	3.70
6. Residual org.-N	5.35	6.33	5.72	6.99	Lost	7.55	Lost	17.31
7. Total org.-N (line 5 + 6)	5.43	6.37	5.77	9.16	--	8.95	--	21.01
8. NH_4-N fixed (line 7 - control)	--	0.92	0.34	3.73	--	3.52	--	15.58
9. HAc + H_2O - sol. NH_4-N	1.6	253.4	249.1	242.5	243.4	251.5	254.2	246.9
10. KCl - exch. NH_4-N	0	0.9	1.1	1.8	2.1	1.9	1.1	4.7
11. Total inorg. NH_4-N (line 9 + 10)	1.6	254.3	250.2	244.3	245.5	253.4	255.3	251.6
12. Total N recovered (line 7 + 11)	7.03	260.7	256.0	253.5	--	262.4	--	272.6
15. pH of HAc + H_2O extract	2.9	2.7	2.8	3.0	3.0	3.0	3.4	3.7

[a]See Table 5 for solution composition.

[b]Data represent one replication.

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Table 19. Influence of pH on the fixation of added NH_4-N by lignin when acetic acid was used for the first extraction

Treatments[a]									
1. pH of system	2.5	1.6	2.3	3.9	4.7	6.6	8.5		9.8
2. me NH_4-N added /100 g	0.0	258.9	256.4	252.9	253.0	258.9	265.1	209.5	270.5

Fractionation[b]	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$
3. HAc + H_2O + KCl - sol. org.-N	0.07	0.86	1.13	0.97	1.05	1.42	2.1	2.49	2.98
4. Residual org.-N	6.03	6.63	6.45	7.57	7.15	9.08	13.20	17.94	19.51
5. Total org-N (line 3 + 4)	6.1	7.49	7.58	8.54	8.20	10.50	15.30	20.43	22.49
6. NH_4-N fixed (line 5 - control)	--	1.39	1.48	2.44	2.10	4.40	9.20	14.33	16.96
7. HAc + H_2O + KCl - sol. + exch. NH_4N	0.14	250.7	251.0	248.9	248.2	252.9	248.5	197.9	245.6
8. Total N recovered (line 5 + 7)	6.24	258.2	258.6	257.4	256.4	263.4	263.9	218.3	262.6

[a]See Table 5 for composition of solution.

[b]Data represent one replication.

Table 20. Influence of pH, time and shaking on the fixation of added NH_4-N by Montana vermiculite

Treatment[a]								
1. pH of system	8.0	2.3	4.0	7.4	7.9	8.7	9.4	11.3
2. me NH_4-N added/100 g	0.0	240.6	240.3	239.3	241.0	247.1	251.5	261.1

	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$
Fractionation[b]		Samples incubated one hour without shaking						
3. HF extract (fixed NH_4-N)	--	28.1	32.0	43.2	42.5	40.3	37.9	17.6
4. KCl - exch. NH_4-N	0.0	17.6	13.8	5.2	3.3	3.7	4.7	3.2
5. Fixed + exch. NH_4-N (line 3 + 4)	--	45.7	45.8	48.4	45.8	44.0	42.6	20.8
6. H_2O - sol. NH_4-N	0.0	191.4	188.8	185.9	183.5	194.7	201.4	236.0
7. Total NH_4-N recovered (line 5 + 6)	--	237.1	234.6	234.3	229.3	238.7	244.0	256.8
8. Percent recovered (line 7 ÷ 2 x 100)	--	98.5	97.6	97.9	95.1	96.6	97.0	98.4
		Samples incubated one hour with shaking						
9. HF extract (fixed NH_4-N)	0.05	34.8	40.4	46.2	47.8	48.7	40.9	20.3
10. KCl - exch. NH_4-N	0.0	16.8	13.2	4.3	3.1	3.1	4.3	1.7
11. Fixed + exch. NH_4-N (line 9 + 10)	0.05	51.6	53.6	50.5	50.9	51.8	45.2	22.0
12. H_2O - sol. NH_4-N	0.0	185.7	186.6	183.4	187.5	190.4	197.8	230.3
13. Total NH_4-N recovered (line 11 + 12)	0.05	237.3	240.2	233.9	238.4	242.2	243.0	252.3
14. Percent recovered (line 13 ÷ 2 x 100)	--	98.6	100.0	97.4	98.9	98.0	96.6	96.6

[a]See Table 5 for solution composition.

[b]Data represent one replication.

Table 20. (Continued)

Treatment[a]								
1. pH of system	8.0	2.3	4.0	7.4	7.9	8.7	9.4	11.3
2. me NH_4-N added/100 g	0.0	240.6	240.3	239.3	241.0	247.1	251.5	261.1

	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$
Fractionation[b]		Samples incubated 12 hours without shaking						
3. HF extract (fixed NH_4-N)	0.01	32.8	35.9	48.2	50.1	46.8	37.8	26.9
4. KCl - exch. NH_4-N	--	19.5	14.7	5.4	5.0	6.3	8.6	2.7
5. Fixed + exch. NH_4-N (line 3 + 4)	--	52.3	50.6	53.6	55.1	53.1	46.4	29.6
6. H_2O - sol. NH_4-N	--	185.6	177.1	176.2	179.4	183.5	194.4	221.3
7. Total NH_4-N recovered (line 5 + 6)	--	237.9	227.7	229.8	234.5	236.6	240.8	250.9
8. Percent recovered (line 7 ÷ 2 x 100)	--	98.9	94.8	96.0	97.3	95.8	95.7	96.1
		Samples incubated 12 hours with shaking						
9. HF extract (fixed NH_4-N)	--	32.6	38.0	Lost	42.9	43.2	38.1	25.3
10. KCl - exch. NH_4-N	--	17.5	12.5	Lost	4.1	5.0	6.7	1.3
11. Fixed + exch. NH_4-N (line 9 + 10)	--	50.1	50.5	--	47.0	48.2	44.8	26.6
12. H_2O - sol. NH_4-N	--	182.0	181.5	178.8	175.9	182.9	193.2	224.0
13. Total NH_4-N recovered (line 11 + 12)	--	232.1	232.0	--	222.9	231.1	238.0	250.6
14. Percent recovered (line 13 ÷ 2 x 100)	--	96.5	96.5	--	92.5	93.5	94.6	96.0

Table 20. (Continued)

Treatment[a]	2.3	4.0	7.4	7.9	8.7	9.4	11.3
1. pH of system	2.3	4.0	7.4	7.9	8.7	9.4	11.3
2. me NH_4-N added/ 100 g	240.6	240.3	239.3	241.0	247.1	251.5	261.1

	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$
Fractionation[b]	Sample incubated 24 hours without shaking						
3. HF extract (fixed NH_4-N)	35.5	43.6	43.7	50.0	48.2	40.2	27.2
4. KCl - exch. NH_4-N	18.4	13.6	5.0	4.9	5.4	7.1	1.6
5. Fixed + exch. NH_4-N (line 3 + 4)	53.9	57.2	48.7	54.9	53.6	47.3	28.8
6. H_2O - sol. NH_4-N	182.6	180.9	180.9	180.0	180.2	192.6	216.9
7. Total NH_4-N recovered (line 5 + 6)	236.5	238.1	229.6	234.9	233.8	239.9	245.7
8. Percent recovered (line 7 ÷ 2 x 100)	98.3	99.1	95.9	97.5	94.6	95.4	94.1
	Sample incubated 24 hours with shaking						
9. HF extract (fixed NH_4-N)	33.4	38.4	45.8	40.5	44.1	41.9	27.0
10. KCl - exch. NH_4-N	17.2	12.6	4.6	4.5	6.0	9.0	2.2
11. Fixed + exch. NH_4-N (line 9 + 10)	50.6	50.0	50.4	45.0	50.1	50.9	29.2
12. H_2O - sol. NH_4-N	182.6	171.8	183.5	177.4	175.6	191.4	221.3
13. Total NH_4-N recovered (line 11 + 12)	233.2	221.8	233.9	222.4	225.7	242.3	250.5
14. Percent recovered (line 13 ÷ 2 x 100)	96.9	92.3	97.7	92.3	91.3	96.3	95.9

Table 21. Influence of pH on the fixation of NH_4-N by Montana vermiculite when acetic acid was used for first extraction

Treatment[a]							
1. pH system[b]	8.0	2.3	4.0	7.4	7.9	8.7	9.4
2. me NH_4-N added/100 g	0.0	258.9	256.4	252.9	253.0	258.9	265.1
	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$
Fractionation[c]							
3. HF extract (fixed NH_4-N)	--	36.2	46.2	45.2	42.6	46.6	36.7
4. KCl exch. NH_4-N	--	16.1	7.0	3.0	2.4	3.8	6.4
5. Total fixed + exch. NH_4-N (line 3 + 4)	--	42.3	53.2	48.2	45.0	50.4	43.1
6. HAc + H_2O - sol. NH_4-N	--	193.1	191.1	184.6	182.3	188.8	199.3
7. Total NH_4-N recovered (line 5 + 6)	--	235.4	244.3	222.8	227.3	239.2	242.4
8. Percent recovery (line 7/2 x 100)	--	90.9	95.3	88.1	89.8	92.4	91.4
9. pH of HAc + H_2O extract	2.7	2.7	2.7	2.8	2.9	3.0	3.4

[a]See Table 5 for composition of solution.

[b]pH 11.3 sample lost.

[c]Data represent one replication.

Table 22. Influence of NH_4OH concentration on the fixation of NH_4-N by Montana vermiculite

Treatments[a]				
1. Approximate normality	0	1	5	13
2. me NH_4-N added/100 g	0	54.0	276.9	677.5

Fractionation[b]	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$
3. HF extract (fixed NH_4-N)	0.05	27.6	34.4	45.0
4. KCl - exch. NH_4-N	--	0.9	1.9	3.2
5. Total fixed + exch. NH_4-N (line 3 + 4)	--	28.5	36.3	48.2
6. H_2O - sol. NH_4-N	--	17.8	196.0[c]	538.7
7. Total NH_4-N recovered (line 5 + 6)	--	46.3	232.3	586.9
8. Percent recovered (line 7/2 x 100)	--	85.7	83.9	86.6
9. pH of H_2O extract	8.7	9.9	10.5	10.7

[a]One milliliter of NH_4OH of the normality indicated was added to 2 g of clay and allowed to react at room temperature for 24 hours in a stoppered test tube.

[b]Results represent one replication.

[c]Result may be low due to broken test tube.

Table 23. Influence of normality and volume of NH_4OH solution added on the fixation of NH_4-N by Glencoe soil

Treatments

1. Milliliters of solution	0.2	0.2	0.2	0.2	1.0	1.0	1.0	1.0
2. Approximate normality	0.1	1.0	5.0	13.0	0.1	1.0	5.0	13.0
3. me NH_4-N added/100 g	0.95	7.57	41.69	122	4.62	38.85	278.3	665.5

Fractionation[a]	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$
4. H_2O + KCl - sol. org.-N	0.93	1.35	2.75	3.43	0.98	3.21	3.43	7.94
5. Residual-N	63.97	64.25	67.35	67.72	62.85	65.57	67.91	69.69
6. Total Kjeldahl-N (line 4 + 5)	64.90	65.60	70.10	71.15	63.83	68.78	71.34	77.63
7. Total fixed NH_4-N (line 6 - control)[b]	0.13	0.83	5.33	6.38	-0.94	4.01	6.57	12.86
8. HF extract NH_4-N	1.08	1.75	1.73	1.63	1.31	1.64	1.74	1.75
9. Clay fixed NH_4-N (line 8 - control)[b]	0.08	0.75	0.73	0.63	0.31	0.64	0.74	0.75
10. Organic fixed NH_4-N (line 7 - 9)	0.05	0.08	4.60	5.75	--	3.37	5.83	12.11
11. H_2O + KCl - sol. + exch. NH_4-N	1.4	7.86	41.09	105.2	5.0	36.3	244.0	574.2
12. Total N recovered (line 6 + 11)	66.3	73.46	111.2	176.4	68.83	105.1	315.3	651.8

[a]Data represent one replication.

[b]Control values: total Kjeldahl - N = 64.77 me/100 g; clay fixed NH_4-N = 1.0 me/100 g.

Table 23. (Continued)

Treatments							
1. Milliliters of solution	2.0	2.0	2.0	5.0	5.0	5.0	5.0
2. Approximate normality	0.1	1.0	5.0	0.1	1.0	5.6	13.3
3. me NH_4-N added/100 g	9.15	81.39	554.8	22.93	215.9	1404	3337

Fractionation[a]	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$
4. H_2O + KCl – sol. org.-N	1.45	4.84	7.40	2.66	5.33	9.71	8.1
5. Residual-N	63.78	64.72	65.66	63.59	63.97	65.19	68.57
6. Total Kjeldahl-N (line 4 + 5)	65.22	69.58	73.06	66.25	69.30	74.90	76.67
7. Total fixed NH_4-N (line 6 – control)[b]	0.45	4.81	8.29	1.48	4.53	10.13	11.90
8. HF extract NH_4-N	1.43	1.69	1.74	1.73	1.55	1.77	1.81
9. Clay fixed NH_4-N (line 8 – control)[b]	0.43	0.69	0.74	0.73	0.55	0.77	0.81
10. Organic fixed NH_4-N (line 7 – 9)	0.02	4.12	7.55	0.75	3.98	9.36	11.09
11. H_2O + KCl – sol. + exch. NH_4-N	10.03	73.66	504.7	22.42	202.0	1300	3098
12. Total N recovered (line 6 + 11)	70.25	143.2	577.8	88.67	271.3	1375	3175

Table 24. Influence of normality and volume of NH_4OH solution added on the fixation of NH_4-N by lignin

Treatments

1. Milliliters of solution	0.2	0.2	0.2	0.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2. Approximate normality	0.1	1.0	5.0	13.0	0.1	1.0	1.0	5.0	5.0	10	13
3. me NH_4-N added/100 g	0.95	7.57	41.69	122	4.62	38.85	38.85	278.3	278.3	487.5	665.5

Fractionation[a]	me N / 100 g	me N / 100 g	me N / 100 g	me N / 100 g	me N / 100 g	me N / 100 g	me N / 100 g	me N / 100 g	me N / 100 g	me N / 100 g	me N / 100 g
4. H_2O + KCl - sol. org.-N	0.41	0.49	1.44	4.15	0.46	1.81	--	10.11	--	--	18.6
5. HAc + H_2O + KCl - sol. org.-N	--	--	--	--	--	--	0.0	--	4.12	5.76	--
6. Residual org.-N	5.9	6.5	9.9	18.2	6.0	9.1	9.68	40.9	45.31	59.09	52.1
7. Total Kjeldahl-N (line 4 + 5 + 6)	6.3	7.0	11.3	12.4	6.5	10.9	9.68	51.0	49.43	64.85	70.7
8. Fixed NH_4-N (line 7 - control)[b]	0.51	1.21	5.51	6.61	0.71	5.11	3.89	45.21	43.64	59.06	64.91

[a]Data represent one replication.

[b]Control value: total Kjeldahl-N = 5.79 me NH_4-N/100 g.

Table 24. (Continued)

Treatments											
1. Milliliters of solution	0.2	0.2	0.2	0.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2. Approximate normality	0.1	1.0	5.0	13.0	0.1	1.0	1.0	5.0	5.0	10	13
3. me NH_4-N added/100 g	0.95	7.57	41.69	122	4.62	38.85	38.85	278.3	278.3	487.5	665.5

Fractionation[a]	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$
9. H_2O + KCl sol. + exch. NH_4-N	0.97	7.69	47.21	100.6	6.57	39.39	--	223.3	--	--	544.7
10. HAc + H_2O + KCl sol. + exch. NH_4-N	--	--	--	--	--	--	40.39	--	220.6	410.1	--
11. Total N recovered (line 7 + 9 + 10)	7.3	14.5	58.5	113.0	13.1	50.3	50.07	274.3	270.0	474.9	615.4
12. Total N in system (line 3 + 5.8 me NH_4-N	6.8	13.4	47.5	127.8	10.4	44.7	44.7	284.1	284.1	493.3	671.3

Table 24. (Continued)

Treatments

1. Milliliters of solution	2.0	2.0	2.0	2.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
2. Approximate normality	0.1	1.0	5.6	13.3	0.1	1.0	1.0	5.6	5.6	9.7	13.3
3. me NH_4-N added/100 g	9.2	81.4	554.8	1357	22.9	215.9	215.9	1404	1404	2435	3337

Fractionation[a]	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$
4. H_2O + KCl - sol. org.-N	0.64	4.01	16.6	27.3	0.97	8.49	--	33.9	--	--	39.6
5. HAc + H_2O + KCl - sol. org.-N	--	--	--	--	--	--	1.85	--	16.2	15.8	--
6. Residual org.-N	7.4	13.6	20.5	30.9	7.9	13.3	17.99	13.3	30.79	33.21	18.9
7. Total Kjeldahl-N (line 4 + 5 + 6)	8.0	17.61	37.1	58.2	8.87	21.79	19.84	47.2	46.99	49.01	58.5
8. Fixed NH_4-N (line 7 - control)[b]	2.3	11.82	31.31	52.41	3.08	16.00	14.05	41.43	41.20	43.22	52.71

Table 24. (Continued)

Treatments

1. Milliliters of solution	2.0	2.0	2.0	2.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
2. Approximate normality	0.1	1.0	5.6	13.3	0.1	1.0	1.0	5.6	5.6	9.7	13.3
3. me NH_4-N added/100 g	9.2	81.4	554.8	1357	22.9	215.9	215.9	1404	1404	2435	3337

Fractionation[a]	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$	$\frac{\text{me N}}{100 \text{ g}}$
9. H_2O + KCl sol. + exch. NH_4-N	9.5	74.8	479.7	1183	24.7	191.8	--	1252	--	--	3075
10. HAc + H_2O + KCl sol. + exch. NH_4-N	--	--	--	--	--	--	195.9	--	1285	2353	--
11. Total N recovered (line 7 + 9 + 10)	17.5	92.4	516.8	1241	33.5	213.6	215.7	1299	1332	2402	3133
12. Total N in system (line 3 + 5.8 me NH_4-N)	15.0	87.2	560.6	1363	28.7	221.7	221.7	1410	1410	2441	3343

Table 25. Fixation of ammonium added at high rate by eight Iowa soils

Treatment[a]	Marion Check 0	Marion Reacted 458.5	Edina Check 0	Edina Reacted 458.5	Marshall Check 0	Marshall Reacted 458.5	Clarion Check 0	Clarion Reacted 458.5
1. me NH_4-N added/100 g	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$
Fractionation								
2. H_2O + KCl - sol.org.-N	0.57	2.43	0.77	3.12	0.43	2.67	0.60	3.23
3. Residual-N	9.03	9.78	10.49	10.08	13.69	14.38	14.47	15.01
4. Total Kjeldahl-N	9.60	12.21	11.26	13.20	14.12	17.05	15.07	18.24
5. Total fixed NH_4-N line 4 (Reacted-Check)		2.61		1.94		2.93		3.17
6. HF extract NH_4-N	0.83	1.40	0.81	1.30	1.47	2.11	0.56	1.24
7. Clay-fixed NH_4-N line 6 (Reacted-Check)		0.57		0.49		0.64		0.68
8. Organic fixed NH_4-N (line 5 - 7)		2.04		1.45		2.29		2.49
9. H_2O + KCl - sol. + NH_4-N	0.46	403.8	0.41	408.2	0.61	410.0	0.18	401.8
10. Total N recovered (line 4 + 9)	10.06	416.0	11.67	421.4	14.73	427.1	15.25	420.0
11. Total N in system (line 1 + native-N)	9.79	468.3	11.52	470.0	14.37	472.9	14.59	473.1
12. Percent recovery[b] (line 10 ÷ 11 x 100)	103	88.8	101	89.7	103	90.3	105	88.8

[a]Reaction conditions: 1 milliliter of 9.17 $\underline{N}$ NH_4OH was added to 2 g of soil and allowed to react for 24 hours at room temperature in a stoppered test tube. Data represent average of two replications.

[b]Recovery values for reacted samples may be low due to loss of volatile NH_3 between time of opening reaction tube and addition of water.

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Table 25. (Continued)

Treatment	Grundy		Nicollet		Webster		Glencoe	
	Check	Reacted	Check	Reacted	Check	Reacted	Check	Reacted
1. me NH_4-N added/100 g	0	458.5	0	458.5	0	458.5	0	458.5
	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$
Fractionation[a]								
2. H_2O + KCl-sol.org.-N	0.65	2.61	0.60	3.38	0.66	3.30	0.83	6.01
3. Residual-N	13.65	14.43	23.55	25.75	25.63	27.93	61.96	67.58
4. Total Kjeldahl-N	14.30	17.04	24.15	29.13	26.29	31.23	62.79	73.59
5. Total fixed NH_4-N line 4 (Reacted-Check)		2.74		4.99		4.94		10.80
6. HF extract NH_4-N	1.15	1.68	0.73	1.74	0.89	1.99	1.01	1.74
7. Clay-fixed NH_4-N line 6 (Reacted-Check)		0.53		1.01		1.10		0.73
8. Organic fixed NH_4-N (line 5 - 7)		2.21		3.98		3.84		10.07
9. H_2O + KCl-sol. + NH_4-N	Lost	408.3	0.07	401.0	0.27	399.6	0.59	408.1
10. Total N recovered (line 4 + 9)	--	425.3	24.22	430.1	26.56	430.8	64.39	482.7
11. Total N in system (line 1 + native-N)	14.81	473.3	24.22	482.7	25.28	483.8	64.13	522.6
12. Percent recovery[b] (line 10 ÷ 11 x 100)		89.9	100	89.1	105	89.0		

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Table 26. Fixation of ammonium added at low level by four Iowa soils

Treatment[a]	Marion Check	Marion Reacted	Clarion Check	Clarion Reacted	Grundy Check	Grundy Reacted	Nicollet Check	Nicollet Reacted
1. me NH_4-N added/100 g	0	209.5	0	209.5	0	209.5	0	209.5
	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$	$\frac{me\ N}{100\ g}$
Fractionation								
2. H_2O + KCl-sol.org.-N	0.17	1.75	0.34	2.14	0.50	2.13	0.33	2.24
3. Residual-N	9.24	9.10	14.23	14.26	13.62	13.84	23.52	24.03
4. Total Kjeldahl-N	9.41	10.85	14.57	16.40	14.12	15.97	23.85	26.27
5. Total fixed NH_4-N line 4 (Reacted-Check)		1.44		1.83		1.85		2.42
6. HF extract NH_4-N	0.96	1.59	0.61	1.13	1.19	1.83	0.84	1.60
7. Clay-fixed NH_4-N line 6 (Reacted-Check)		0.63		0.52		0.64		0.76
8. Organic fixed NH_4-N (line 5 - 7)		0.81		1.31		1.21		1.66
9. H_2O + KCl-sol. + NH_4-N	0.44	195.6	0.67	198.0	0.62	197.8	0.65	199.3
10. Total N recovered (line 4 + 9)	9.85	206.5	15.24	214.4	14.74	213.8	24.50	226.0
11. Total N in system (line 1 + native-N)	9.79	219.3	14.59	224.1	15.81	224.3	24.22	233.7
12. Percent recovery (line 10 ÷ 11 x 100)	101	95.5	104	95.7	99.5	93.4	101	96.7

[a]Reaction conditions: 5 ml of 0.835 $\underline{N}$ NH_4OH was added to 2 g of soil and allowed to react for 24 hours at room temperature in a stoppered test tube. Data represent average of two replications.

Table 27. Fixation of $N^{15}H_4OH$ added at low level by Edina silt loam

Treatment[a]	Check			Treated			N derived from added NH4-N	
	NH4-N	%N15	N15H4-N	NH4-N	%N15	N15H4-N	N15	N15+14
1. me NH4-N added/ 100 g	0	0	0	237.8	18.24	43.38		
	$\dfrac{\text{me N}}{100\ g}$	atom %	$\dfrac{\text{meN}^{15}}{100g}$	$\dfrac{\text{meN}}{100\ g}$	atom %	$\dfrac{\text{meN}^{15}}{100\ g}$	$\dfrac{\text{me N}}{100\ g}$	$\dfrac{\text{meN}}{100\ g}$
Fractionation								
2. H₂O-sol. org.-N	0.63	0.366	0.002	3.64	1.074	0.309	0.037	0.20
3. KCl-sol. org.-N	0.29	--[b]		0.27	--[b]			
4. Residual-N	10.21	0.423	0.043	8.61	2.214	0.191	0.148	0.81
5. Total Kjeldahl-N (line 2 + 3 + 4)	11.13		0.045	12.52		0.230	0.185	1.01
6. Total fixed NH4-N line 5(Treated-Check)				1.39				
7. HF extracted NH4-N	0.79	0.372	0.003	0.96	5.869	0.056	0.053	0.29
8. Clay-fixed NH4-N line 7(Treated-Check)				0.17				
9. Organic fixed NH4-N (line 6 - 8)				1.22				0.72
10. H₂O-sol. NH4-N	0.29	0.423[c]	0.001	216.4	17.85	38.63	38.63	211.8
11. KCl-exch. NH4-N	0.18	0.423	0.001	6.83	15.60	1.065	1.06	5.81
12. Total N recovered (line 5 + 10 + 11)	11.60			235.7			39.88	

[a]Ammoniation conditions: 5 ml of solution added to 2 g of soil and incubated 24 hours at room temperature. Data represent average of two replications.

[b]Insufficient N for isotope analysis.

[c]Insufficient N for isotope analysis - atom % for residual-N assumed.

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Table 28. Fixation of $N^{15}H_4OH$ added at low level by Marshall silty clay loam

Treatment[a]	Check			Treated			N derived from added NH_4-N	
	NH_4-N	%N^{15}	$N^{15}H_4$-N	NH_4-N	%N^{15}	$N^{15}H_4$-N	N^{15}	N^{15+14}
1. me NH_4-N added/ 100 g	0	0	0	237.8	18.24	43.38		
	$\dfrac{\text{me N}}{100\text{ g}}$	atom %	$\dfrac{\text{meN}^{15}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	atom %	$\dfrac{\text{meN}^{15}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$	$\dfrac{\text{me N}}{100\text{ g}}$
Fractionation								
2. H_2O-sol. org.-N	0.86	0.362	0.003	2.90	1.13	0.033	0.030	0.16
3. KCl-sol. org.-N	0.34	--b		0.36	--b			
4. Residual-N	13.52	0.365	0.049	12.77	2.006	0.256	0.207	1.13
5. Total Kjeldahl-N (line 2 + 3 + 4)	14.72		0.052	16.03		0.289	0.237	1.29
6. Total fixed NH_4-N line 5(Treated-Check)				1.31				
7. HF extracted NH_4-N	1.40	0.385	0.005	1.85	5.113	0.095	0.090	0.49
8. Clay-fixed NH_4-N line 7(Treated-Check)				0.45				
9. Organic fixed NH_4-N (line 6 - 8)				0.86				0.80
10. H_2O-sol. NH_4-N	0.28	0.366c	0.001	219.3	18.16	39.82	39.82	218.3
11. KCl-exch. NH_4-N	0.18	0.366c	0.001	7.09	17.79	1.261	1.26	6.91
12. Total N recovered (line 5 + 10 + 11)	14.90			242.4			41.32	

[a]Ammoniation conditions: 5 ml of solution added to 2 g of soil and incubated 24 hours at room temperature. Data represent average of two replications.

bInsufficient N for isotope analysis.

cInsufficient N for isotope analysis - atom % for residual-N assumed.

Table 29. Fixation of $N^{15}H_4OH$ added at low level by Webster clay loam

Treatment[a]	Check			Treated			N derived from added NH4-N	
	NH_4-N	%N15	$N^{15}H_4-N$	NH_4-N	%N15	$N^{15}H_4-N$	N15	N15+14
1. me NH_4-N added/ 100 g	0	0	0	279.1	2.739	7.643		
	$\dfrac{me\ N}{100\ g}$	$\dfrac{atom}{\%}$	$\dfrac{meN^{15}}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{atom}{\%}$	$\dfrac{meN^{15}}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$
Fractionation								
2. H_2O-sol. org.-N	0.79	0.366	0.003	3.68	0.529	0.033	0.030	1.10
3. KCl-sol. org.-N	0.19	--b		0.07	--b			
4. Residual-N	24.88	Lost		24.81	Lost			
5. Total Kjeldahl-N (line 2 + 3 + 4)	25.86			28.56				
6. Total fixed NH_4-N line 5(Treated-Check)				2.70				
7. HF extracted NH_4-N	0.91	0.366	0.003	1.77	1.933	0.034	0.031	1.13
8. Clay-fixed NH_4-N line 7(Treated-Check)				0.86				
9. Organic fixed NH_4-N (line 6 - 8)				1.84				
10. H_2O-sol. NH_4-N	0.61	0.366c	0.001	250.3	2.724	6.821	6.820	249.0
11. KCl-exch. NH_4-N	0.61	0.366c	0.002	9.02	2.919	0.262	0.260	9.16
12. Total N recovered (line 5 + 10 + 11)	26.87		0.097	287.8				

[a]Ammoniation conditions: 5 ml of solution added to 2 g of soil and incubated 24 hours at room temperature. Data represent average of two replications.

[b]Insufficient N for isotope analysis.

[c]Insufficient N for isotope analysis - atom % for residual-N assumed.

Table 30. Fixation of $N^{15}H_4OH$ added at low level by Glencoe silty clay loam

Treatment[a]	Check			Treated			N derived from added NH_4-N	
	NH_4-N	$\%N^{15}$	$N^{15}H_4-N$	NH_4-N	$\%N^{15}$	$N^{15}H_4-N$	N^{15}	N^{15+14}
1. me NH_4-N added/ 100 g	0	0	0	267.2	2.79	7.455		
	$\dfrac{me\ N}{100\ g}$	$\dfrac{atom}{\%}$	$\dfrac{meN^{15}}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{atom}{\%}$	$\dfrac{meN^{15}}{100\ g}$	$\dfrac{me\ N}{100\ g}$	$\dfrac{me\ N}{100\ g}$
Fractionation								
2. H_2O-sol. org.-N	1.30	0.381	0.005	6.87	0.769	0.053	0.048	1.72
3. KCl-sol. org.-N	trace	--[b]		trace	--[b]			
4. Residual-N	65.33	0.381	0.249	65.45	0.535	0.350	0.101	3.62
5. Total Kjeldahl-N (line 2 + 3 + 4)	66.63		0.254	72.32		0.403	0.149	5.34
6. Total fixed NH_4-N line 5(Treated-Check)				5.69				5.34
7. HF extracted NH_4-N	1.01	0.381	0.004	1.75	0.395	0.024	0.020	0.72
8. Clay-fixed NH_4-N line 7(Treated-Check)				0.75				0.72
9. Organic fixed NH_4-N (line 6 - 8)				4.95				4.62
10. H_2O-sol. NH_4-N	0.31	0.381[c]	0.001	245.6	2.767	6.796	6.795	243.5
11. KCl-exch. NH_4-N	0.36	0.381[c]	0.001	15.11	2.561	0.387	0.386	13.84
12. Total N recovered (line 5 + 10 + 11)	67.30			333.0			7.320	262.6

[a]Ammoniation conditions: 5 ml of solution added to 2 g of soil and incubated 24 hours at room temperature. Data represent average of two replications.

[b]Insufficient N for isotope analysis.

[c]Insufficient N for isotope analysis - atom % for residual-N assumed.