CHANGES IN THE ORGANO-MINERAL COMPLEX OF SOILS DUE TO MANURE APPLICATIONS

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#### ABSTRACT

# CHANGES IN THE ORGANO-MINERAL COMPLEX OF SOILS DUE TO MANURE APPLICATIONS

By

### Kenneth Wayne Linville

Profile distributions of N and P were examined in soils which had received nine annual applications of beef cattle manure at moderate rates or single applications at massive rates. Surface and subsoil samples from selected treatments were subjected to two different extraction sequences involving 1.7% KCl, 0.1N H<sub>2</sub>SO<sub>4</sub>, and 0.1N NaOH or 0.1M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>. The extracts were analyzed for total N and, after perchloric acid digestion, for Ca, Mg, Fe and Al.

After nine annual applications at 10, 20 and 30 T/a, more organic N was retained in the plow layer where continuous corn was harvested for grain and stover was returned than where silage was removed. Downward movement of organic N was detected to depths of 4 to 6 ft and was greater where silage was removed. Ammonium in subsoils was greater with silage harvest, whereas nitrate in the plow layer was greater where only grain was removed. Extractable P in the plow layer was less with manure treatments than where high rates of fertilizer P had been used, but downward movement was greater with manure and was detectable to 15 inches. Eight months after single applications of 100, 200 and 300 T/a, essentially all of the input N could be accounted for in the plow layer, although some downward movement to depths up to 18 inches was detected. About 16% of input P could be accounted for as extractable in the plow layer, with no evidence for downward movement.

Fifteen percent or less of input Ca and Mg could be accounted for in exchangeable forms in the plow layer; whereas nonexchangeable but extractable forms were equivalent to 90% of input or more. Most of the nonexchangeable Ca and Mg appeared to be present in mineral salts or complexes and was associated with Fe and Al in  $H_2SO_{\mu}$  extracts. Nevertheless, all cations were present in organo-mineral complexes which contained 27 to 58% of total soil N and were extractable with NaOH or pyrophosphate. The quantities present in complexes with N were, in general, related directly to valence and inversely to ionic radius of the metal cation: Ca < Mg < Fe < Al. Wide fulvic/humic recovery ratios for Ca in NaOH extracts of the plow layer indicate that Ca was the cation mainly responsible for precipitating fulvic acids and stabilizing N in the fulvic acid of the upper profile. Total nonexchangeable Ca and N in the plow layer were highly correlated (r = .84, P = .01). In the subsoil, Al appeared to be the principal stabilizing cation. Iron in NaOH extracts was associated mainly with the humic acid fraction. То a lesser extent, this was true also for Mg.

## CHANGES IN THE ORGANO-MINERAL COMPLEX OF SOILS DUE TO MANURE APPLICATIONS

By

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#### CHAPTER I

#### INTRODUCTION

Soil organic-N and N fixed by legumes or recycled through crop residues and barnyard manures were the major sources of nitrogen for crop production until the end of World War II. After the war, industrially fixed N became available in large quantities at a relatively cheap price.

At present, higher levels of crop production are being maintained by liberal use of N from industrial sources, balanced with other fertilizer nutrients. Numerous agronomists have estimated that commercial fertilizers now account for one third of the total food production in the United States and predict that the quantity of fertilizer used will increase as population grows.

Increased human populations have also affected the livestock industry. The demand for more meat has resulted in the development of large feedlots. With this increase in animal populations, concentrated in small areas, have come the problems of disposing of large quantities of animal waste and increased potential for pollution of streams and ground water.

Metropolitan areas are also faced with problems of waste disposal. Under recent Federal legislation, state and local agencies are imposing increasingly rigorous requirements

for renovation of water in waste effluents before discharge into surface waterways. They too are now looking to the soil as a possible medium for treating waste water and for disposing of solid wastes.

In the context of water treatment or waste disposal. the tendency is to go to very high application rates because of high costs for land and distribution facilities. It is recognized that excessive rates can exceed the capacity of soils and crops to remove N and that the excess N will appear very quickly as nitrate in drainage or ground water. Most soils have a large capacity for removing soluble P from percolating water, but this capacity can be saturated over a period of years at high rates of input, thus reducing the useful life of a waste disposal site. Potentially toxic industrial metals are retained effectively in many soils. Some of these metals are essential nutrients but, at high rates of input, available concentrations can build up over time to the point where plants are injured or the concentrations taken up into vegetation become toxic to livestock.

Some sort of compromise must be made between excessively high rates of waste application, with emphasis on disposal, and lower rates which will permit much of the nitrogen to be removed into standing or harvested vegetation or by denitrification. With many wastes, even if application rates are regulated to control N, the inputs of P and other nutrients will still exceed, sometimes greatly, the quantities that can be removed by vegetation. As yet, there is

insufficient data from long-term experiments to predict reliably how long soils can remove P or metals before becoming saturated.

Mechanisms whereby P and metals are retained in soils (exchange, adsorption, precipitation, complexation) have been studied extensively, but seldom under conditions where their effectiveness could be related to long-term inputs in the field.

These considerations have prompted this investigation to study (1) the profile distributions of N and P and (2) the fractional distribution of N and cations in soils from field experiments involving long-term differential applications of manure and commercial fertilizers or recent heavy applications of manure.

#### CHAPTER II

#### LITERATURE REVIEW

Traditionally, industry has looked to agriculture as a potential market for waste products, many of which are worthless or harmful to plants. Fortunately, the waste from animal feeding can be utilized in crop production.

As large amounts of feedlot manures or fluid materials are applied to valuable crop land, two important considerations must be taken into account: (1) the impact of these additions on surface and ground waters and (2) the composition of waste materials and their soil reactions, since yields and crop composition may be adversely affected by improper use.

#### Surface and Ground Water

High concentrations of  $NO_3$  in water consumed by infants or livestock is of direct concern because of methemoglobinemia. Nitrate may be harmful to ruminants. When consumed, this ion may be reduced to nitrite, enter the blood stream (Garmer 1973, Wright 1964), and tie up the oxidative mechanism of the blood (Winter 1962). Drinking water standards of the U. S. Public Health Service (1962) list ten ppm  $NO_3$ -N (45 ppm  $NO_3$ ) as the level that should not be exceeded for infants.

Investigation by Smith (1965) of 6,000 rural water supplies in Missouri showed that animal wastes, improperly

constructed shallow wells and septic tank drainage are the main sources of contaminating  $NO_3$ . His investigation revealed that livestock was a more important source of contamination in improperly constructed wells than N fertilizers. These findings are substantiated by Stewart <u>et al</u>. (1967) by work done in Colorado.

To determine the effect of fertilizer N on contamination of ground water Linville and Smith (1971) studied five soil types in Missouri treated with various rates of nitrogenous fertilizers and under continuous culture of corn. Their results indicated that N additions which exceeded N removal increased the potential for downward movement of NO3. These results are substantiated by Adriano et al. (1972) in a recent study of nine row-crop sites in California. In another study in California, Lund et al. (1974) found significant correlation between soil profile characteristics and average NO3 concentration below the root zone. He recommends that soil profile characteristics be considered in selecting land for high N input through fertilization or waste disposal. A detailed discussion of the fate of N in soils, water and plants has been given by Viets and Hageman (1971).

Eutrophication is a term applied to the situation where an increase in nutrients, notably P or  $NO_3$ , in surface waters stimulates the growth of aquatic plants. This leads to excessive production followed by depletion of  $O_2$  due to decomposition of large masses of dead plant materials. In

lakes, ponds or slow moving streams, a bog or swamp will develop eventually. Engineers responsible for producing potable water for domestic use are concerned about N and P concentrations in water that stimulate this aquatic growth. The most likely processes that can lead to P contamination from the soil-plant system are surface runoff and erosion. Any P-containing particles of soil, organic matter or manure present on the surface are subject to being washed away unless adequate erosion control practices are followed. Organic or mineral forms of N are also subject to runoff and erosion. Nitrate can move into surface waters by seepage or through agricultural drainage systems.

Concentrations of P in the water are usually more critical than  $NO_3$  in eutrophication. Phosphate content in fresh water that will limit the growth of aquatic plants is about 0.02 ppm and, for  $NO_3$ , it is from 0.05 to 1.0 ppm (Taylor 1967, Smith 1968). Therefore, the levels of nutrients that will restrict eutrophication present more critical limits for agricultural and waste disposal operations than do the higher levels of  $NO_3$  that can be tolerated in water for animal or infant consumption.

### Manure Additions to Soils

Numerous investigations have been conducted on the effects of manure additions on soils. Massachusetts researchers report no economic advantage from manure applications

in excess of 20 tons per acre (Weeks et al. 1972). A Michigan study indicates ten tons per acre was the most favorable rate for corn grain on a sandy loam soil (Vitosh et al. 1973). High salt content of the soil solution or toxic concentrations of  $NH_3-N$  in the soil atmosphere, or both, affected germination on barley, sudangrass, spinach and radish in a greenhouse study (Adriano et al. 1973). Murphy reports depressed yields due to salt injury to corn grown on soils receiving large additions of solid beef feedlot manure. He found significant accumulations of  $NO_3-N$ , total N, and available P in the soil. The high soil concentrations of P were reflected in high P values in corn silage (Murphy et al. 1972). An extensive review of the literature and analysis of research needs has been conducted (Powers et al. 1974). They concluded that there is a large volume of research data available on shortterm effects of applying animal waste to land, but there is little information available on long-term effects of animal wastes on physical, chemical and biological properties of There are few guidelines for calculating animal soils. waste loading rates. Results from a recently completed study suggest that housing type in feedlots can modify climatic influence and conserve nutrients from loss by evaporation, runoff and leaching (Adriano et al. 1974), thereby increasing the quantities which must be managed in ways that will give more positive control over their movement through the environment.

It is known that N can be removed by denitrification

in the presence of plant residues and root exudates under conditions of low oxygen tension which occur during waterlogging or even in microhabitats in well-drained soils (Allison 1973, Broadbent 1973). N can also be immobilized in organic forms if carbonaceous residues are returned in quantities sufficient to give actual increases in soil organic matter content (Bartholomew and Clark 1965, Broadbent and Stevenson 1966). How soils can be managed to maximize such removals over indefinite periods of time is not known (Jacobs 1974).

From 15 to 80% of the P in soils has accumulated in organic forms over geological periods of time (Allison 1973). Most soils have large capacities to adsorb or precipitate  $PO_4$  in mineral fractions, but this capacity can be saturated over a period of years where inputs are high (Ellis <u>et al</u>. 1972, Ellis 1973).

Metal cations are retained in soils by exchange reactions, precipitation or complexation with mineral and organic colloids (Ellis <u>et al</u>. 1972, Ellis and Knezek 1972). On the other hand, most metals can form soluble salts or complexes with mineral acids, such as  $NO_3$ , Cl,  $SO_4$ , or with organic acids and chelating compounds which are formed during decomposition of wastes in soils. How these competing processes will balance out over long periods of time at high levels of input is not known.

#### Organo-Mineral Complexes

Numerous studies and extensive reviews have been published on the nature of complexes formed between metals and organic compounds found in soils (Mortenson 1963, Ellis and Knezek 1972, Schnitzer and Khan 1972, Khan 1969, Stevenson 1960, Stevenson and Gascho 1968, Stevenson and Ardakani 1972).

There is general agreement that the interacting organic species include simple monomeric organic acids, phenols, etc., microbial polysaccharides and polyuronides, and a more or less continuous series of complex humus substances related to each other but increasing in molecular weight from less than 2,000 to more than 300,000, in the order: fulvic acid < humic acid < humin.

Most surface functional groups which can interact with metals contain oxygen: carboxyl (-COOH), acidic hydroxyl ( $\downarrow$ COH), carbonyl ( $\downarrow$ C=O). The ratio of active functional groups attains a maximum in the more highly oxidized and lower molecular weight fulvic acids, some of which are soluble. In larger fulvic acid and humic acid molecules, an increasing proportion of potentially active sites is bound up by various bridging structures which hold together the basic skeleton of aromatic rings derived mainly from lignin or plant and microbial pigments (Stevenson 1969).

Important bridging structures include: -O-, -CH<sub>2</sub>-, -NH-, =N-, -S-. Bridges involving N arise by condensation

reactions between NH<sub>3</sub> or amino acids and intermediates which arise during the oxidation of lignin or aromatic-based pigments. Nitrogen bridges apparently involve heterocyclic rings which are very resistant to decomposition.

The acidic functional groups in microbial polyuronides and humus substances give to these materials a cation exchange capacity which greatly exceeds that of clay minerals like vermiculite or montmorillonite (Ahlrichs 1972). Because of the diversity of oxygen-containing groups, they also enter into coordinate-covalent complexes with polyvalent cations. Some of these complexes are extremely stable, as with Cu and Zn (Allison 1973). Low molecular weight chelates are soluble and may be responsible for the downward displacement of Fe and Al during podzolization. On the other hand, the formation of complexes with Ca, Fe, Mn and Al is an important factor in stabilizing and retaining fulvic acids, humic acids and humin in soils (Kononova 1966). The reverse, of course, is also true: metals are stabilized in these complexes with humus substances; their mobility in soils and their availability to plants is regulated to an important degree in this way (Allison 1973).

The importance of organo-metal interactions in soils used for waste treatment has been pointed out by numerous workers. However, few systematic attempts have been made to assess their significance for soil solution-solid phase equilibria among competing cations (Lindsay 1973). No reports were encountered where the distribution of cations associated

with different soil organic fractions has been studied in relation to applications of manure or other wastes.

Methods for Extracting Soil Organic Matter

A wide variety of methods has been used to extract and fractionate organic matter from soils (Ahrlichs 1972, Felbeck 1971, Stevenson 1965, 1969, Mortenson and Himes 1964, Kononova 1966, Kononova and Dokuchayev 1967, and Aleksandrova 1967).

The quantity of water soluble organic compounds in soils is very low, except during the early stages of decomposition of added organic materials (plant trash, animal remains). A "bitumen" fraction, including fats, waxes and resins, is soluble in organic solvents but is normally a small proportion (one to six percent) of the total soil organic matter. About ten to 20% of soil organic matter can be shown to be carbohydrate in nature because constituted sugars and sugar acids are released by acid hydrolysis. Similarly, 30 to 50% of the organic N in soils can be hydrolyzed to amino acids. Much of the carbohydrate is present in the form of polysaccharides and polyuronides. Only a very small proportion of the amino nitrogen is present as protein, the remainder being intimately associated with or incorporated in humus substances (fulvic acids, humic acids, and humin).

Methods for extracting humus substances are all based on the principle of breaking complexing bonds with minerals through use of alkali, chelating agents or reducing agents

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(to reduce ferric iron).

The most frequently used procedure involves extraction with dilute NaOH (0.1 to 0.5  $\underline{N}$ ). The largest yields of organic matter are obtained with 0.1 $\underline{N}$  NaOH. This concentration also coextracts the largest quantities of complexing minerals (Al, Fe, Si, and P). For studies of organic matter composition and properties, Levesque and Schnitzer (1966) found this high ash content to be undesirable. In the present study, however, the principal objective was to identify metals associated with organic fractions, so  $0.1\underline{N}$  NaOH was used. This is also the concentration recommended by Russian workers (Kononova 1966).

Workers outside of Russia have been concerned with the possibility that oxidation in alkaline extracts would alter the properties of organic matter extracted. Milder agents include sodium pyrophosphate adjusted into the lower alkaline range (pH 7.0 to 8.0). The Russian workers have examined this possibility and conclude that negligible alteration occurs during alkaline extraction. But they have found that sodium pyrophosphate (0.1M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> in 0.1N NaOH) gives efficient extraction without the need for prior decalcification with acid.

Alkaline extracts are further fractionated into fulvic acid and humic acid by acidification to pH 2 or 3, at which point the humic acids are precipitated. The ratio of fulvic acid to humic acid has a diagnostic value in the Russian scheme for soil organic matter studies (Kononova 1966).

Additional quantities of organic matter can be extracted by repeated sequences of acid and alkaline extraction (Kononova 1966). The above extractants and fractionations were used in sequential extraction schemes which are described in the next chapters.

### CHAPTER III

#### EXPERIMENTAL PROCEDURES AND METHODS

### Selection and Description of Research Sites

The proposed research undertook to utilize existing research plots of Michigan State University, Department of Crop and Soil Sciences. The plots selected were established in 1963 at the soils farm at East Lansing, and were designed to study the long-term effects of fertilizer and manure on continuous corn. In addition, a new study was established in 1972 adjacent to the existing plots where very high rates of manure were applied and corn was grown. Harvesting procedures were the same for both studies.

These two research sites were worthy of study in regard to the influence of long-term lower rates versus a single large application of manure on the chemical properties of the soil. The research plots selected for study are described in detail below.

#### Experiment I

The research plots selected were part of a long-term study to determine the optimum rate of manure to be applied annually to a sandy loam soil which was intensively cropped with continuous corn and to compare the efficiency of cattle manure <u>vs</u> commercial fertilizer with respect to corn

production for grain and silage (Vitosh <u>et al</u>. 1973). The treatments were replicated three times.

The field experiment was a split-block design with each replication divided into grain and silage areas. The size of the whole plots was  $42 \times 128$  feet. The sub-plot size was  $42 \times 64$  feet.

The study consisted of five treatments: (1) 10 T/a manure, (2) 20 T/a manure, (3) 30 T/a manure, (4) 160-40-40( $1b/a N-P_2O_5-K_2O$ ), and (5) 160-190-190. In the spring of 1971, two treatments were added to the experiment by the application of three tons of lime per acre to one-half of the plots receiving 160-40-40 and 160-190-190 1b/a (treatments 6 and 7). The N for treatments 4, 5, 6 and 7 was supplied as ammonium sulfate and the P and K as 0-20-20, plowed down prior to planting.

The manure used came from the loose-housing beef barns at Michigan State University and was applied annually in the fall. This material contained considerable amounts of straw bedding. Manure obtained in 1971 showed the following chemical analysis: 73% water, .55% N, .15% P, .46% K, .18% Ca, .12% Mg, .06% Na, 179 ppm Fe, 70 ppm Al, 34 ppm Mn and 24 ppm Zn (wet basis) (Vitosh <u>et al</u>. 1973). Totals applied for elements and treatments of special interest to this report are given in Table 1.

All treatments received 200 lb/a 5-20-20 starter fertilizer each year, banded two inches to the side and two inches below the seed at planting. Minimum tillage practices

			Total a	applied
Element	Analysis	Quantity/10T application	30T (9years)	300T (lyear)
		lbs	<b></b> lb	/a
N	0.55%	110	2970	3300
Р	0.15%	30	810	900
Ca	0.18%	36	972	1080
Mg	0.12%	24	648	720
Fe	179 ppm	3.58	97	107
Al	70 ppm	1.40	38	42

Table 1. Manure analysis and approximate loading<sup>†</sup>.

<sup>†</sup>Chemical analysis on wet basis (73% water) 1971.

were employed. Weeds were controlled with herbicides. Plant populations varied from 13,600 plants per acre in 1963 to 28,000 in 1970. Row spacings were 42 inches, except in 1967-70 when two row spacings (28 and 42 inches) were used and in 1971, when only the 28-inch spacing was used. Corn hybrids were different each year.

#### Experiment II

A new study was started in the spring of 1972 across the alley from Experiment I. The purpose of the new study was to determine the effect on the soil and crop of a single application of manure at very high rates on a sandy loam soil.

The field experiment was a randomized block design with four replications. The plot size was 14 x 115 feet.

The study consisted of four manure treatments: (1) no manure, (2) 100 T/a, (3) 200 T/a, (4) 300 T/a.

The manure used came from the same source as in Experiment I. It was applied in February of 1972 and plowed under.

All treatments received 200 lb/a 5-20-20 starter fertilizer, applied as in Experiment I. The no manure treatment received 150 lb/a N as  $NH_4SO_4$  just prior to planting.

### Description of soils

Replication 1 of Experiment I and the four replications of Experiment II were classified as a Hodunk sandy loam (Ochreptic Fragiudalfs) with 0-2 percent slope. This soil series consists of moderately well drained gray-brown podzolic soils with fragipans, developed on calcareous sandy loam glacial till. The typical profile consists of an Ap 0-7 in, sandy loam;  $A_2$  7-16 in, sandy loam;  $B_{1x}$  16-25 in, sandy loam to light sandy clay loam;  $B_{2t}$  25-46 in, sandy clay loam; and C 46 in+, sandy loam. The profile observed on the site is described in Table 2. The predominent soil type in replications 2 and 3 of Experiment I was classified as a Metea loamy sand (Arenic Hapludalfs) with 0-2 percent slope. This soil series consists of well drained to moderately well drained soils with 20 to 40 in of sands or loamy sands over loam to clay loam till materials. The typical profile consists of an Ap 0-8 in, loamy sand;  $A_{21}$  8-15 in, loamy sand or sand;  $A_{22}$ - $B_1$  15-28 in, loamy sand; II  $B_{2t}$ 28-40 in, clay loam; II C 40 in+, loam or clay loam till.

#### Sampling Procedures

The soil samples were taken with hydraulic probe mounted on a truck. Samples were taken in the fall of 1971 to a depth of 10 feet from three replications of each treatment in Experiment I. Experiment II was sampled in the fall of 1972; samples were taken to a depth of five feet from three replications of each treatment.

The surface cores were taken with a 1 1/2 inch by 48 inch probe. The cutting point was 1/8 inch smaller than the inside diameter of the tube. This reduced friction, and prevented compaction of the cores. Compaction of samples was slight, seldom being more than one inch for a 48-inch

Horizo	Depth (in)	
Ap	0-9	
A <sub>21</sub>	9-12	
A <sub>22</sub>	12-15	
A <sub>3x</sub>	15-27	
<sup>B</sup> 2t	27-39	
BC	39-42	
С	42-120	)

Table 2.	Hodunk sandy	loam	profile	exposed	at	the	site	of
	Experiments 1	[ (Rep	) 1) and	II.				

depth core. For the lower depths a smaller one-inch tube was substituted. This reduced side friction, and made possible deeper sampling. After the probe was inserted to the desired depth, it was brought to the surface and disengaged from the hydraulic equipment. A wooden rod was used to push the core of soil from the probe.

The core was then cut into the following increments: Ap horizon 0-9 inches, three-inch increments from nine inches to five feet, and six-inch increments from five to ten feet. Two cores were taken from each plot. One core was taken to a depth of ten feet and a second adjacent core was taken to five feet. Sampling increments from the two cores to five feet were combined. Each soil sample was placed in a paper bag and stored in an ice-cooled, insulated chest while being transported to the laboratory. The results of three replications were averaged for the values reported.

### Laboratory Methods of Analysis

Soil samples were refrigerated in the field and were held below 0 C until dried for analysis. Soil samples to be tested were dried in a forced air oven at a temperature not exceeding 55 C. These samples were then ground with a mortar and pestle to pass through a two mm sieve and stored in tightly stoppered glass bottles.

#### Sequential extractions

Two sequential extracting procedures were employed (Figure 1) to study the distribution of N, C, and metals
(Ca, Mg, Fe, and Al) in soluble and exchangeable forms (KCl extraction) and in various nonexchangeable fractions. Sequences of acid and alkaline extraction were used to effect differential recoveries from mineral and organic complexes in the soil solid phase (Kononova 1966).

Forty grams of selected soil samples from Experiments I and II were reground in a Spex Mixer/Miller (This grinding rendered the soil to the texture of face powder.) The pulverized soil was thoroughly mixed and stored in plastic airtight bags. Three 1.00 g samples were removed and total N was determined. Two 0.100 g samples were used for determination of total C. Two 12.5 g samples were then weighed into two 200 ml Nalge centrifuge bottles, one for each of the two extraction sequences in Figure 1.

# 1. KCl (Seq. I-a and II-a)

Thirty ml of 1.7% KCl was added to each of the two 12.5 g samples of soil in the Nalge centrifuge bottles. The bottles were covered and shaken on a rotary shaker for 15 minutes. The bottles were then removed and centrifuged in a Sorvall Refrigerated Centrifuge at -7 C and at 10,000 RPM for 20 minutes. The supernatants were decanted and the soil freed of Cl by resuspending and centrifuging in four successive 50 ml aliquots of cold deionized water. Extract II-a and washings were discarded. Extract I-a and washings were combined, allowed to come to room temperature, and then made up to 250 ml. Three 25 ml aliquots were analyzed for Kjeldahl N immediately. The remaining solution was stored





in a plastic bottle for metal analysis.

# 2. H<sub>2</sub>SO<sub>4</sub> (Seq. I-b)

To the Nalge centrifuge bottle containing the soil from extract I-a, 200 ml of  $0.1\underline{N} H_2SO_4$  was added. The bottle was capped and shaken by hand for a few minutes to insure mixing and left to stand for 24 hr.

At the end of the 24-hr period, the bottle was centrifuged and the liquid decanted into a 500 ml volumetric flask. The remaining soil was then washed twice with 100 ml aliquots of cold 0.1<u>M</u>  $H_2$ SO<sub>4</sub> acid. The washings were recovered by decanting after centrifugation and combined with the 24 hr extract. The combined solution was allowed to come to room temperature before making up to 500 ml with deionized water. Three 25 ml aliquots were then analyzed for Kjeldahl N and the remaining solution was stored in a plastic bottle for analysis of metals.

# 3. Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> (Seq. I-c)

To the soil residue from extract I-b, 200 ml of 0.1MNa<sub>4</sub>P<sub>2</sub>O<sub>7</sub> in 0.1N NaOH was added in the evening. The bottle was capped and mixed thoroughly by hand.

The next morning the bottle was shaken and centrifuged. The liquid was decanted into a 250 ml volumetric flask and allowed to come to room temperature before making up to volume. Three 25 ml aliquots of this extract were analyzed for Kjeldahl N. A portion was stored in plastic for metal analysis, and the remainder was separated into fulvic and humic acid

fractions (extracts I-d and I-e).

### 4. NaOH (Seq. II-b)

To the soil residue from extract II-a, 200 ml of 0.1NNaOH was added. After capping and mixing thoroughly by hand, the bottle was allowed to stand for 15-16 hours before resuspending and centrifuging.

The extract was decanted into a 250 ml volumetric flask and allowed to come to room temperature before making up to volume. Three 25 ml aliquots were analyzed for Kjeldahl N. Part of the remaining solution was separated into fulvic and humic acid fractions (extracts II-c and II-d). The remainder was stored in a plastic bottle for analysis of metals.

### 5. Fulvic acid (Seq. I-d and II-c)

Fulvic acid/humic acid separations were performed on 100 ml aliquots of the pyrophosphate extract (I-c) and of the NaOH extract (II-b). After acidifying from pH 13 to 2.5 with concentrated sulfuric acid, the solutions (in covered 200 ml beakers) were heated for 30 minutes on a boiling water bath and allowed to stand overnight at room temperature. The next day, the precipitate (humic acid) was removed on Whatman No. 42 filter paper. Cold  $0.05\underline{N}$  H<sub>2</sub>SO<sub>4</sub> was used to effect complete transfer and separation of acid-soluble fulvic acids from the precipitate. The fulvic acid fractions and washings were collected in 200 ml volumetric flasks and allowed to come to room temperature before making up to volume. Aliquots were analyzed for Kjeldahl N and the remaining solution stored in plastic bottles for metal analysis.

# 6. Humic acid (Seq. I-e and II-d)

The precipitated humic acid fractions were taken up again in warm 0.05<u>N</u> NaOH. The filters were washed several times to assure complete recovery. The recovered solutions were made up to 200 ml at room temperature. Aliquots were analyzed for Kjeldahl N and the balance stored in plastic for metal analysis.

# 7. Extended series, H<sub>2</sub>SO<sub>4</sub> extract (Seq. I-f and II-e)

Organic matter remaining in soil residues from  $Na_4P_2O_7$ extraction (I-c) and from NaOH extraction (II-b) were subjected to a further succession of extractions in acid and alkali to get some indication of the extent to which metals may have been stabilized in complexes associated with more resistant organic or mineral fractions in soils.

The residues from extracts I-c and II-b were extracted for 24 hr in 0.1<u>N</u> H<sub>2</sub>SO<sub>4</sub>, as has been described for extract I-b. Aliquots of the combined extract and washings were analyzed for Kjeldahl N and the remainder stored in plastic for metal analysis. The soil after this acid extraction was dried in a forced air oven at 20 C and pulverized again in the Spex Mixer/Miller. A 10.0 g aliquot was returned to the Nalge centrifuge bottles for alkali extraction (I-g and II-f) and the remainder set aside for organic C analysis. 8. Extended series, NaOH extract (Seq. I-g and II-f)

The 10.0 g aliquots of dried soil residue from extracts I-f and II-e were extracted for 15-16 hr in 190 ml of  $0.1\underline{N}$ NaOH as has been described for extract II-b. Aliquots were analyzed for Kjeldahl N and the remainder stored in plastic for metal analysis. The soil residue after this extraction was dried and pulverized again, then transferred into  $0.1\underline{N}$ HCl for about 24 hr to destroy carbonates. After thorough washing with deionized water, they were dried at room temperature, ground with mortar and pestle and set aside for determination of Kjeldahl N and organic C.

### Perchloric acid digestions

Perchloric acid digestion was used to destroy complexing organic matter in all extracts after KCl so that a quantitative measurement of the metals could be made (Levesque and Schnitzer 1966).

The basic procedure followed was developed by Johnson and Ulrich (1959). A 50 ml aliquot of the extraction liquid was pipetted into a 100 ml Kjeldahl flask. To this flask five ml of concentrated nitric acid was added and digestion was carried out on an electric digesting unit until about ten ml of solution remained. At that point the digestion was stopped; the flask was removed and allowed to cool. Then ten ml of concentrated nitric acid and two ml of 70% perchloric acid were added. The digestion was resumed until about two ml of solution was left and the dense white perchloric acid fumes had subsided. (Sometimes this last

digestion with nitric and perchloric had to be repeated due to high organic matter content of the liquid or because of high concentrations of phosphate.) The flask was removed from the digesting unit and allowed to cool.

At this point two different procedures were developed to promote higher quantitative measurement of the metals to be analyzed. The need for two procedures arose when Al could not be determined in the presence of diethylenetriaminepentaacetic acid (DTPA<sup>1</sup>).

### 1. Procedure for Ca, Mg and Fe

After the digestion mixture had cooled, a few ml of deionized water was used to wash down the side of the long neck flask. Then ten ml of  $0.5\underline{M}$  DTPA was added to complex all metal cations and to keep them in solution. After heating to the boiling point, the contents were swirled to insure that nothing adhered to the walls of the flask. After cooling overnight, the contents of the flask were transferred quantitatively to a 50 ml volumetric flask and brought up to a concentration of  $0.1\underline{M}$  DTPA. The solution was then analyzed for Ca, Mg and Fe.

#### 2. Procedure for Al

After the flask had cooled, a few ml of deionized water was added to wash down the neck and sides. Then five ml of 9N HCl was added. After heating to the boiling point,

<sup>&</sup>lt;sup>1</sup>Compliments of Ciba-Geigy Chemical Company

the contents were swirled to insure that nothing adhered to the walls of the flask. After cooling, four drops of 2, 4-dinitrophenol indicator and about three ml of 10N NaOH were added to bring the solution to a light yellow (pH 3.5). The contents were then transferred quantitatively to a 50 ml volumetric flask, made to volume, and analyzed for Al.

Chemical analysis

# 1. Nitrogen

Ammonium and  $NO_3$  in the KCl soil extracts were recovered by steam distillation in the presence of MgO, using Devarda's alloy to reduce  $NO_3$  to  $NH_3$  (Bremner 1965a). Kjeldahl N (excluding  $NO_3$ ) was determined in soils, extracts and extracted soil residues by a semimicro-Kjeldahl procedure, using  $CuSO_4$ ,  $K_2SO_4$  and Se as catalysts (Bremner 1965b). Ammonia in distillates was collected in two percent  $H_3BO_4$ containing two drops of methyl purple mixed indicator (Fleisher Chemical Co.) and titrated against standard acid.

### 2. Carbon

A 0.100 g sample was used to determine organic C in selected soils and extracted residues, using a Leco Carbon Analyzer. Where necessary, the sample was pretreated with 0.1N HCl to remove carbonate.

# 3. Phosphorus

Available P in soil was recovered in Bray's P-1 extracting solution (0.025<u>N</u> HCl and 0.03<u>N</u> NH<sub>U</sub>F). P was

determined by Jackson's Method I (Jackson 1958), except 1amino-2-naphthol-4-sulfonic acid (Eastman 360) was used as the reducing reagent instead of chlorostannous acid (Fiske and Subbarow 1925).

### 4. Calcium and magnesium

Ca and Mg were determined directly in KCl extracts or, in the case of other extracts, in the presence of  $0.1\underline{M}$ DTPA after perchloric acid digestion. A Perkin-Elmer 303 Atomic Absorption Spectrophotometer was used. Solutions for analysis contained five ml of sample and five ml of a 16,000 ppm solution of LaCl<sub>3</sub>, made up to 25 ml with deionized water. Standard solutions of Ca and Mg were made to be .1<u>M</u> DTPA plus 20% of the LaCl<sub>3</sub> stock solution in a perchloric acid matrix for the perchloric acid extracts or 1.7% KCl plus 20% LaCl<sub>3</sub> solution for the KCl extracts.

# 5. Iron

Fe was determined directly in the  $0.1\underline{M}$  DTPA perchloric acid digests and the 1.7% KCl extracts using the Atomic Absorption Spectrophotometer. Some of the samples required dilution. The standards were made to be  $0.1\underline{M}$  DTPA with a perchloric acid matrix the same as the samples. Lanthanum chloride was not used.

# 6. Aluminum

The procedure developed by McLean (1965) was used, with the following alterations. (1) A 50 ml volumetric

flask was used instead of a graduated test tube. (2) Due to extreme difficulty in adjusting pH in the perchloric acid digest, 15 ml of pH 4.0 ammonium acetate buffer was added to perchloric acid samples and standards before making to final volume (50 ml). Additions of buffer kept the pH at 3.8. (3) A stock solution of perchloric acid was made. This solution served as a blank and was added to the standards to provide the same matrix as in the unknown samples. (4) The 1.7% KCl extracts were analyzed as stated by McLean, except 1.7% KCl was used in the standards and blank instead of 1<u>N</u> KCl.

## 7. pH and buffer pH

Soil water and buffer pH was determined, on selected soils samples, according to Schoemaker et al. (1961).

# Statistical Methods

Data for Experiment I were analyzed as described by Cochran and Cox (1957) for a split plot, randomized block in three replications, with systematic main plots (corn or silage harvest) and randomized sub-plots (nitrogen treatments). Experiment II was treated as a randomized block with three replications.

The data for sequential extractions were analyzed as for a split-split plot in three replications, with manure treatments as randomized main plots, depths as sub-units and extracts or soil and soil residues as sub-sub-units. Simple

and multiple correlations among N and metals within depths and extracts were calculated.

Facilities and programs of the Michigan State University Computer Laboratory were used.

#### CHAPTER IV

### RESULTS AND DISCUSSION

# Influence of Manure and Fertilizers on the Soil Environment

Experiment I

Experiment I was designed to study the long-term effects of fertilizers and manure on continuous corn. After nine years of annual treatment, soil samples were obtained for analysis. To compare soil horizons to sampling depths, see Table 2.

### Distribution of Soil N

The results of the 1971 sampling are summarized in Table 3. Tables 23 to 30 in the Appendix give the N content of the soil by incremental depths.

Soil variation was great and only a few differences associated with treatment were statistically significant. The significant differences which were found, however, serve to identify trends which appear to be important and which were expressed more generally through the data.

Organic N in the plow layer was higher at the 30T level of manure application than for other treatments (Table 3). It tended to be higher where more organic residues for immobilizing N were returned as stover after grain harvest than where all above ground production was removed as

Depth	Corn				Trea	tments		
(in)	harvested as		Manure		160 Jb N	160 15 N	160 16 1	N NPK
		10 <b>T</b>	20T	30 <b>T</b>	100 Ib N	P & K	lime	lime
				*******	ppm			
		a) Org	anic-N					
0-9‡	Grain	8331	7659	10745	7572	7816	6098	7341
	Silage	7812	3810	9792	7217	6231	6569	5772
9-120	Grain	4607	4312	4829	3960	5152	4870	5438
	Silage	4600	4902	5478	4781	5000	4666	4147
		ь)NH <sub>4</sub> -	N					
0-9	Grain	38	40	54	55	41	22	41
	Silage	22	30	61	17	37	25	23
9-120 <sup>\$</sup>	Grain	17	46	55	24	26	50	70
	Silage	82	81	82	62	45	28	56
		c) NO <sub>3</sub>	-N					
0-9 <sup>¶</sup>	Grain	60	56	95	88	34	32	45
	Silage	41	27	37	26	18	18	35
9-120	Grain	116	126	180	119	188	161	235
	Silageq	103	153	213	250	220	290	159
		d) Tot	al miner	al-N				
0-9#	Grain	98	96	149	143	75	54	86
	Silage	63	57	98	43	55	43	58
9-120	Grain	133	172	235	143	214	211	305
	Silage	185	234	299	312	265	318	215

Table 3. Summary of	nitrogen forms recovered	in the plow layer and in subsoils t	o 10
feet after corn. <sup>†</sup>	nine annual applications	of manure or commercial fertilizers	for

<sup>+</sup>Mean of three replications.

# Organic-N (0-9 in. only): Treatments within crop LSD (.05) = 2481.
% NH<sub>4</sub>-N (9-120 in. only): Treatments within crop LSD (.05) = 35
LSD (.05) = 51
% NO<sub>3</sub>-N (0-9 in. only): Treatments within crop LSD (.05) = 57
# Total mineral-N (0-9 in. only): Crop within treatments LSD (.05) = 72.

silage.

There is evidence in Tables 23 and 24 that mobile forms of organic N have been displaced to considerable depths into parent materials below 42 inches. Thus, the average for the bottom five increments (90 to 120 in) at the 10T rate is about 80 ppm organic N, whereas the values for increments between 42 and 60 inches are significantly greater for most treatments. The indicated depth of penetration is greater for the 20 and 30T rates than for 10T, particularly where silage was removed. The addition of lime to plots harvested for silage tended to reduce the depth to which organic N was displaced, but the reverse was true for plots where only grain was harvested. These differences are reflected in organic N totals for 9-120 inches in Table 3, although not with statistical significance.

Ammonium is the first mineral form of N released when plant materials decompose. Since it is a cation, it is not very mobile in soil. It is also quickly nitrified. Twenty to 70% of the total  $NH_4-N$  encountered was found in the plow layer where most of the decomposition of manure and corn residues would have taken place (Table 3). Concentrations found at greater depths were very much lower (Tables 25 and 26). Nevertheless, when subsoil quantities were totaled to 120 inches, significant relationships to treatment and cropping system were expressed. Since much of this  $NH_4$  was probably produced <u>in situ</u> or released from organic matter during air drying of samples, these data provide indirect evidence

to support the data for organic N which indicate that the nature and properties of soil organic matter were influenced to considerable depths in the soil by treatment and the quantity of corn residues returned.

Nitrate and total mineral N in the plow layer tended to be lower where silage was harvested than where only grain was removed (Table 3). This reflects the more efficient harvest removal of N and other nutrients. Over the nineyear period, silage yields have responded to increasing manure rate and additional P and K, whereas grain yields have not responded to more than ten tons of manure and were frequently depressed by the addition of P and K with fertilizer N (Vitosh <u>et al. 1973</u>).

On the other hand,  $NO_3$  and total mineral N in subsoils tended to be lower where grain was harvested (Table 3). Of the total  $NO_3$ -N recovered from plots under grain harvest, 57 to 85% was found below the plow layer, whereas 72 to 94% was recovered from subsoils under silage harvest. Thus, it seems that, over the years, the additional organic matter in returned stover has contributed to closer cycling of N by immobilization and/or greater losses by denitrification.

Nitrate which passes beyond the influence of plant roots and associated microflora can be expected to remain stable in the percolation stream at greater depths (Viets and Hageman 1971). Concentrations below 48 inches ranged from two to 12 ppm, on a dry soil basis (Tables 27 and 28). Concentrations in percolating solution above field capacity

would be several-fold greater.

### Distribution of Soil P

The soil analyses for extractable P (Bray  $P_1$ ) for all N treatments (see Table 4) showed no significant differences between corn harvested as grain or silage. The Ap horizon shows higher accumulations of P where 30T of manure, 160 lb N + P & K, and NPK + lime were applied. The values were 121, 143 and 153 lb/a respectively. Downward movement of P was greater for the manure treatments, even though the Ap horizon had less extractable P than for mineral treatments.

### Experiment II

Experiment II was designed to study the effects of manure at high rates on soils and continuous corn. The soils were sampled eight months after the first applications of 100, 200 and 300 T/a of beef cattle manure.

#### Distribution of Soil N

The 1972 sampling results are summarized in Table 5. Tables 31 to 34 in Appendix give the N concentrations by increments of depth.

Organic N in the Ap horizon (0-9 in) increased with rate of manure application (Table 5). The increase for 300T (3,500 lb/a) would account totally for the estimated 3,300 lb N applied (cf. Table 1). The quantities found, however, were substantially less than in Experiment I where essentially similar total applications of manure had been made over a

Table 4.	Distribu profile for corn	tion of after n	extract ine annu	able phosph al applicat	iorus (Bra tons of m	y P-1) wit anure or c	h depth commercia	in the al fertilizers
Depth				Treatmen	lts			Treatment within depth
(in)		Manure		160 1b N	160 Jb N +	160 Jb N +	NPK +	LSD (.05)
	loT	20T	30Т		P&K	lime	lime	
					wdd			
6-0	86	96	121	52	143	60	153	26.6
9-12	42	0 †	59	19	26	21	35	su
12-15	20	23	29	12	16	10	15	su
15-18	16	18	17	10	16	7	10	ns
Depth wit LSD (.0	hin treat 5)	ment						
	7.2	7.2	7.2	7.2	7.2	7.2	7.2	
tMean of	three rep	licatio	ns.					

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 ${}^{\sharp}{}_{
m No}$  significant difference between corn harvested as grain or silage.

Depti	า		r	Tons of man	ure per a	acre
(in)			None	100T	200T	300T
	- )	Organization N		]	b/a	
0_0 <b>‡</b>	a)	Urganic-N	μ <b>7</b> 10	5074	7110	8220
0-91			-110	7777	7110	0239
9-60			2639	2259	2887	2787
	b)	NH <sub>4</sub> -N				
0-9			41	37	36	58
9-60			54	50	30	20
	c)	<sup>NO</sup> 3-N				
0-9			235	151	580	406
9-60			176	222	367	241
	d)	Total mineral-N				
0-9			276	188	616	464
9 <b>-</b> 60			230	272	39 <b>7</b>	261

Table 5. Summary of nitrogen forms recovered in the plow layer and in subsoils to five feet eight months after a single application of manure at high rates for corn harvested as grain.<sup>†</sup>

<sup>†</sup>Mean of three replications.

**‡Organic-N (0-9 in. only):** Treatment LSD (.05) = 2280.

nine-year period at one-tenth these annual rates. Since there were no control plots in Experiment I, the increase due to manure cannot be estimated to resolve this apparent discrepancy.

Organic N in the subsoil was increased, but only to depths of 15 to 18 inches (Table 31). The subsoil concentrations were less than for the same depth increments under manure treatments in Experiment I (cf. Tables 23 and 24).

Ammonium in the plow layer was not related to treatment (Table 5). However, subsoil levels were significantly influenced over most depth increments to 60 inches (Table 32). The extractability of subsoil  $NH_4$  tended to decrease with increasing manure application. This is the reverse of that observed in Experiment I (cf. Tables 3 and 25).

It was difficult to incorporate these very heavy applications of manure. The surface soil lost its infiltration capacity for a time, becoming very wet, with ponded water observed on several occasions. The potential low oxygen tensions are the most likely influence which could have been transmitted through 60 inches of profile over the short period of eight months to reduce the extractability of  $NH_4$ . The mechanism of the effect is not clear, however.

Variable oxygen status may have contributed also to great variability in the  $NO_3$  analyses. Nitrate increased generally with increasing manure rate, both in surface and subsoils (Table 5). Variations in total mineral N were due mainly to variations in  $NO_3$ .

The concentrations of  $NO_3$  in the plow layer were several-fold greater than in Experiment I (cf. Table 3). Significant differences for manure rate were encountered in subsoils to 27 inches (Table 33). Below 27 inches,  $NO_3$ concentrations were the same as for comparable depths in Experiment I (Tables 27, 28).

### Distribution of Soil P

The results of soil analysis for extractable P is tabulated in Table 6. Phosphate content increased in the Ap horizon with additions of manure. The ranges were 57 ppm for no manure to 105 ppm for the 300T. There was no evidence for any downward movement.

### Sequential Extraction of Organic Matter and Associated Metals

Sequential extractions were used to study exchangeable and nonexchangeable N and metals, and their interactions. For this study, the no manure, 300T (1 year) and 30T (9 years) manure treatments (stover returned to the soil plots) were used. The essential treatment variable was time, since the total manure applications were essentially the same (Table 1). A further source of variation must be recognized and that is that the 30T/year manure treatment was in a separate experiment (Experiment I) which was, however, immediately adjacent to Experiment II from which samples were taken for no manure and 300T/a in one application eight months before sampling.

Depth	Tons	of manu	re per	acre	Treatment
(in)	None	100T	200T	300T	within depth LSD (.05)
				ppm	
0-9	58	69	97	105	18.0
9-12	19	12	21	20	ns
12-15	10	7	12	8	ns
15-18	8	7	13	4	ns
Depth within tre LSD (.05)	eatment				
	11.5	11.5	11.5	11.5	

Table 6. Distribution of extractable phosphorus (Bray P-1) with depth in the profile eight months after a single application of manure at high rates for corn.

<sup>†</sup>Mean of three replications.

•

Surface (0-9 in) and subsoil (18-21 in) samples from each of the three field replications of each treatment were carried through the extraction schemes in Figure 1. (The 18-21 in depth was used because it was a sufficient distance from the plow layer and preliminary C/N ratios increased with manure applications.) All chemical analyses were in duplicate or triplicate and the average value reported. Statistical analyses were based on the three field replications.

# Exchangeable Forms of N and Metals

It appeared that the level of exchangeable N in the plow layer increased with time and with increasing total organic N rather than with the annual rate of manure application (cf. Tables 7, 23 and 31).

Calcium was the dominant exchangeable cation (Table 7). In the plow layer, it tended to increase with manure application over time, and exchangeable Ca and N were positively correlated (r = .89, P = 0.01). Exchangeable Ca was significantly higher in the subsoil than in the plow layer in no manure plots. There was a marked tendency for it to decline in the subsoil with manure application over time.

Magnesium showed a similar trend in the subsoil, and Ca and Mg were positively correlated in the subsoil (r = .85, P = 0.01).

Exchangeable Fe and Al were very low, the values for Al being at the lower limit for detection.

Flements		Treatment	ts	Treatment
PTCHCI109	No manure	300T (1 year)	30T (9 years)	LSD (.05)
		ppm		
Plow layer (0-9 in.)				
Ν	24	46	59	28.9
Ca	321	376	389	ns
Mg	116	132	107	ns
Fe	4	10	40	ns
Al	20	20	20	ns
Subsoil layer (18-21 in.)				
Ν	8	9	5	ns
Ca	728	622	387	ns
Mg	130	115	110	ns
Fe	8	4	18	ns
Al	20	20	20	ns
Depth within treatment LSD (.05)				
N	ns	23	23	
Ca	340.9	ns	ns	
Mg	ns	ns	ns	
Fe	ns	ns	ns	
Al	ns	ns	ns	

Table	7.	Recoveries	of	exchangeable	N	and	metals	in
		1.7% KC1'.						

<sup>†</sup>Mean of three replications.

Nonexchangeable Forms of N and Metals

# 1. Overall recoveries

Vitosh <u>et al</u>. (1973), in studies which included Experiment I of this study, observed that a major portion of nutrients applied in manure could not be accounted for by crop removals or by standard soil tests for available P or exchangeable cations. It was, therefore, of interest to compare the capacity of soils to retain cations by exchange mechanisms with their capacity for retaining them by precipitation or complexation in soil fractions extractable with relatively mild reagents which have been used for isolating important organic constituents of soils.

Total recoveries of N and metals in nonexchangeable fractions, averaged over both extraction sequences, are presented in Table 8 for comparison with exchangeable quantities in Table 7.

The N in Table 8 is organic and provides an estimate of extracted organic matter. The increase in extractable organic N in the plow layer (0-9 in) for the 300T manure treatment over no manure was 171 ppm or about 500 lb/a. This is equivalent to about 15% of input N (cf. Table 1). The indicated increase for 30T annually over nine years would be 700 lb/a, or 24% of input; however, this estimate must be questioned, since the 30T plots were in a separate, though immediately adjacent experiment (Experiment I).

Extractable Ca in the plow layer (Table 8) increased

		Treatment	ts	Treatment		
Elements	No Manure	300T (l year)	30T (9 years)	within depth LSD (.05)		
		ppm				
Plow layer (0-9	in.)					
Ν	388	559	625	140.2		
Ca	489	993	1470	900.9		
Mg	436	657	461	ns		
Fe	3555	3234	2142	1074.8		
Al	2202	2526	1863	ns		
Subsoil layer (l	8-21 in.)					
N	81	76	89	ns		
Ca	228	343	1301	900.9		
Mg	914	879	735	ns		
Fe	6879	4635	3028	1074.8		
Al	3806	3443	2260	969.8		
Depth within tre LSD (.05)	atment					
N	106.2	106.2	106.2			
Ca	ns	ns	ns			
Mg	347.0	ns	ns			
Fe	1274.9	1274.9	ns			
Al	941.4	ns	ns			

Table 8.	Total recoveries of nonexchangeable N and metals
	by sequential extraction. <sup>†</sup>

Mean for two extraction sequences and three replications.

as organic N increased. The extractable concentrations were much greater than the exchangeable concentrations in Table 7. The increase in exchangeable Ca in the plow layer for the 300T treatment over no manure was 165 lb/a, or 15% of input, whereas the increase in extractable but nonexchangeable Ca was 1,500 lb/a, or 139% of input. The indicated increase for the 30T (9 years) treatment would have been 2,900 lb/a, or 300% of input. This is clearly not a treatment effect and is due to unsuspected differences in soils between Experiments I and II. Extractable Ca was related directly to pH; 6.0, 6.1, 6.9 in the plow layer for no manure, 300T and 30T, respectively, and 5.6, 5.6, 6.9 in the subsoil.

In the subsoil layer (18-21 in), exchangeable Ca (Table 7) was greater than nonexchangeable (Table 8) except for the 30T (9 years) treatment. There was no relation between extractable N and Ca in the subsoil. Nevertheless, the very high level of extractable Ca in the subsoil under the 30T treatment (Table 8) was associated with much higher levels of organic N at this depth in Experiment I (Table 23) than in Experiment II (Table 31).

Larger quantities of Mg were recovered by sequential extraction (Table 8) than were present in exchangeable form (Table 7). The indicated increases over no manure in the plow layer for the 300T manure treatment were 48 lb/a exchangeable Mg (seven percent of input) and 660 lb/a extractable (92% of input).

Very much larger quantities of Fe and Al were extracted

than were present exchangeably. In both surface and subsoil layers, extractable Fe was related inversely to extractable Ca. This is the most striking relationship to be seen in Figure 2. In the subsoil this was true also for Al.

# 2. Fractional recoveries

The summed recoveries of nonexchangeable N and metals for Extraction Sequences I and II are presented separately for comparison in Table 46.

Both sequences removed very similar quantities of organic N. In general larger quantities of metals were removed by Sequence I.

By reference to Figures 3 and 4 and to Appendix Tables 35 through 41, it can be seen that most of the organic N came out in the  $Na_4P_2O_7$  extract (I-c) in Sequence I and in the first NaOH extract (II-b) in Sequence II. In the case of plow layer samples, additional quantities of organic N were removed in the final NaOH extraction I-g and II-f (Figure 3); only small amounts appeared in this final extract from subsoils (Figure 4).

Total extractable Ca was correlated positively with total extractable N (Table 9). Nevertheless, relatively little Ca was in fact associated with organic N in the same extracts (Figures 3 and 4). Most of the non-exchangeable Ca came out in the initial  $0.1\underline{N} + \underline{N}_2SO_4$  extract (I-b) in Sequence I and in the  $0.1\underline{N} + \underline{N}_2SO_4$  extract (II-e) after NaOH in Sequence II.



Figure 2. Distributions of the total recoveries of non-exchangeable N and metals by sequential extraction.



Figure 3. Distributions of the recoveries of N and metals in sequential extractions of the plow layer (0-9 in.).



Figure 4. Distributions of the recoveries of N and metals in sequential extractions of the subsoil layer (18-21 in.).

	N	Ca	Mg	Fe	N	Ca	Mg	Fe
Sequence	e I				Sequer	nce II		
Plow lay	ver (0-	9 in.)						
Ca	.84 <b>**</b>	ł			•85 <b>*</b> 1	ł		
Mg	.16	.12			•57	.58		
Fe	<b></b> 65	<b></b> 66	•47		<b>-</b> .60	<b></b> 72 <b>*</b>	<b></b> 07	
Al	.02	11	.69 <b>*</b>	.63	12	38	• 38	•79 <b>**</b>
Subsoil	layer	(18 <b>-</b> 21	in.)					
Ca	.87 <b>**</b>	F			<b>-</b> .07			
Mg	• 36	.45			.03	.80 <b>*</b> 1	ł	
Fe	29	47	• 34		• 39	<b>-</b> •57	07	
Al	20	38	•57	•71 <sup>*</sup>	.09	42	.13	•74 <sup>*</sup>
*Signii	ficant	at P(.	05); 1	c > .666	5.			

Table 9. Simple correlations (r) between totals for nonexchangeable N and metals recovered in extraction sequences I and II.

**\*\***Significant at P(.01); r > .798.

In the Russian scheme, an initial acid extraction is employed before alkali extraction to "decalcify" the soil and permit larger quantities of organic matter to be removed (Kononova 1966). Some fulvic acid materials will be removed also in this initial acid extraction. In the soils examined here, Fe and Al were removed in quantities equal to or greater than Ca, and substantial quantities of Mg were removed also.

Due to prior removal in acid, and probably also to chelation and precipitation by pyrophosphate, cations were very low in extract I-c as compared with the parallel NaOH extract II-b. The quantities of organic N recovered by I-c and II-b were very similar (cf. Tables 36 and 37).

The dominant cation removed in the first NaOH extract in Sequence II was Al (Figures 3 and 4). The effect of this alkali extraction on the subsequent acid extraction (II-e) was to increase the quantities of Fe removed as compared with the first acid extraction (I-b) in Sequence I which was not preceded by an alkaline extraction (cf. Tables 35 and 39). The Al extracted in II-e was much less than in I-b, but the total for the alkali followed by acid extractions (II-b plus II-e in Tables 37 and 39) was greater than for the single acid extraction I-b in Table 35. This data is in line with the principle that organo-mineral complexes in soils are mutually protective and that fractional removal of one component can increase the extractability of another (Kononova 1966, Schnitzer and Khan 1972).

The second  $H_2SO_4$  extraction (I-f) in Sequence I removed additional quantities of Mg, Fe and Al, together with much smaller quantities of Ca (Figures 3 and 4, Table 38). This second acid extraction is largely responsible for the larger total recoveries of cations in Sequence I than in Sequence II (Table 46).

The two acid extractions in Sequence I may have increased the extractability in the final NaOH extract of Al in the plow layer (Figure 3) and of Fe in the subsoil (Figure 4), but they had little effect on the residual extractability of organic N, Ca or Mg (cf. Tables 40 and 41).

# 3. Interactions among N and metals

The positive correlations between Ca and N in total recoveries from the plow layer (Table 9) were reflected in similar positive correlations between Ca and N exchangeable to KCl and between Ca and N in the first NaOH extract of Sequence II (Table 10). There was no strong relationship between Ca and N in any other fractional extract from the plow layer (Tables 10 and 11) or in any of the extracts from the subsoil (Tables 12 and 13). Overall recoveries of Ca and N from the subsoil were positively correlated in Sequence I but not in Sequence II (Table 9).

Thus, there is little evidence that Ca was extensively complexed with extractable organic compounds containing N. The same conclusion can be drawn for Mg. The divalent cations were recovered mainly in acid extracts (Figures 3

	N	Ca	Mg	Fe	N	Ca	Mg	Fe
		<u>H<sub>2</sub>SO<sub>4</sub> (</u>	<u>I-b)</u>		KCl	(I-a a	nd II-	<u>a)</u>
Ca	30				•89 <sup>**</sup>			
Mg	08	.66			.12	.43		
Fe	.09	58	.09		.02	.10	.27	
Al	.21	29	•25	.65	.00	.00	.00	.00
		<u>Na4P207-</u>	(I-c)			<u>NaOH (</u>	II-b)	
Ca	<b></b> 33				•77 <b>*</b>			
Mg	• 32	.42			• 39	•43		
Fe	•49	57	48		<b>-</b> .79 <b>*</b>	81**	39	
Al	29	26	.04	07	28	47	.19	.14
		Ful <b>vic (</b>	I-d)			<u>Fulvic</u>	(II-c)	
Ca	.31				•54			
Mg	• 34	•90 <b>**</b>			<b></b> 34	• 55		
Fe	.07	78*	80*	*	29	71#	40	
Al	•44	36	<b>-</b> .20	.48	30	11	.46	.18
		<u>Humic (</u>	I-e)			Humic (	(II-d)	
Ca	.61				.11			
Mg	.49	•95 <b>**</b>			24	•75 <b>*</b>		
Fe	65	<b></b> 83 <b>**</b>	79	ł	27	•44	<b>.</b> 84*	×
Al	.21	22	31	11	53	.27	•04	04

Table 10. Simple correlations (r) between N and metals in sequential extracts (through humic acid) in the plow layer (0-9 in.)

**\*\***Significant at P(.01); r > .798.

	N	Ca	Mg F	e N	Ca	Mg	Fe
		<u>H<sub>2</sub>SO4</u>	(I-f)		<u>H<sub>2</sub>SO<sub>4</sub> (</u>	II-e)	
Ca	.66			<b></b> 25			
Mg	.29	•71 <b>*</b>		04	<b>.</b> 69 <b>*</b>		
Fe	09	.11	.71*	•33	70*	18	
Al	•04	.27	•75* •	93** .49	• 34	.49	.24
		NaOH	(I-g)		<u>NaOH (</u>	II-f)	
Ca	14			04			
Mg	76*	.42		60	•76 <b>*</b>		
Fe	72*	24	•75*	11	.48	.45	
Al	•77*	06	50	3743	•77 <b>*</b>	<b>.</b> 85 <b>**</b>	.83**

Table 11. Simple correlations (r) between N and metals in extended sequential extracts of plow layer (0-9 in.)

\*Significant at P(.05); r > .666.

**\*\***Significant at P(.01); r > .798.

	N	Ca	Mg	Fe	N	Ca	Mg	Fe	
*****************	<u>н<sub>2</sub>so<sub>4</sub> (I-ъ)</u>				KC	KCl (I-a and II-a)			
Ca	37				.40				
Mg	.09	<b>.</b> 87 <b>**</b>			.03	<b>.</b> 85 <b>*</b> *	F		
Fe	.68*	-/45	09		.09	50	36		
Al	•87 <b>**</b>	<b></b> 32	.05	•59	.00	.00	.00	.00	
	<u>Na4P207 (I-c)</u>					NaOH (II-b)			
Ca	<del>-</del> •59				.13				
Mg	30	•74*			.03	.42			
Fe	.45	97**	67*		02	43	.60		
Al	06	.41	•35	49	02	.09	•57	•58	
	Fulvic (I-d)				١	Fulvic (II-c)			
Ca	26				33				
Mg	15	.49			.00	.00			
Fe	• 30	<b>-</b> .76 <b>*</b>	<b></b> 39		16	•75*	.00		
Al	.60	14	<b>-</b> .28	<b>-</b> .21	16	.13	.00	41	
	Humic (I-e)					Humic (II-d)			
Ca	<b>-</b> •59				.18				
Mg	.00	.00			.22	•95 <b>*</b> 1	ŧ		
Fe	<b>.</b> 82**	34	.00		55	.12	.26		
A1	64	•91**	.00	27	44	10	.08	.64	

Table 12. Simple correlations (r) between N and metals in sequential extracts (through humic acid) in the subsoil layer (18-21 in.).

\*Significant at P(.05); r > .666.
\*\*Significant at P(.01); r > .798.
	N	Ca	Mg Fe	N	Ca	Mg	Fe
		<u>H<sub>2</sub>SO<sub>4</sub> (</u>	<u>I-f)</u>		H	( <u>2804 (</u>	II-e)
Ca	30			12			
Mg	29	.81**		09	•89 <b>**</b>	ł	
Fe	24	• 39	•71 <b>*</b>	.64	58	<b>-</b> .29	
Al	46	01	.47 .47	•27	37	.06	.65
		<u>NaOH (</u>	<b>I-g</b> )		NaOH (	II-f)	
Ca	30			12			
Mg	<b></b> 39	.50		14	.13		
Fe	37	.08	.89**	.01	26	•90 <del>*</del>	¥
Al	45	<b>.</b> 80 <b>**</b>	•72 <b>*</b> •48	.48	.18	.06	.14

Table 13. Simple correlations (r) between N and metals in extended extracts of the subsoil layer (18-21 in.).

\*Significant at P(.05); r > .666.
\*\*Significant at P(.01); r > .798.

and 4), in which they were positively correlated with each other (Tables 10 through 13).

However, the small amounts of Ca and Mg which did appear in pyrophosphate extracts (Table 36, Figures 3 and 4) were negatively correlated with Fe (Tables 10 and 12), either in the extract itself (I-c) or in its fulvic (I-d) or humic (I-e) sub-fractions. Calcium was negatively correlated with Fe in the first NaOH extract (II-b) of the plow layer and its fulvic sub-fraction (II-c). In the NaOH extract, Fe was also correlated negatively with N.

Iron and aluminum are characteristically complexed with organic matter extracted from soils, and the complexes are referred to as Fe- and Al-humates (Schnitzer and Khan 1972). The negative correlations noted for Fe in the preceding paragraph would be expected, since the cations would be competing for complexing sites in organic matter.

Incorporation of N can also be competitive for the same sites (Stevenson and Butler, 1969). However, where incorporation or release of N and metals occurs in association with substantial increases or decreases in quantity of humus, positive correlations can be expected since N becomes a measure of the quantity of complexing organic matter. Thus, in Sequence I extracts of subsoils (Table 12), Fe and Al are correlated positively with N in the first  $H_2SO_4$  extract (I-b), and Fe and N are positively correlated in the humic subfraction (I-e).

Iron and aluminum were the dominant cations extracted

(Table 46). Overall, they tended to be correlated with each other (Table 9). However, this inter-correlation did not carry over into many of the fractions in Tables 10 through 13, and many of their interactions with Ca and Mg were distinctly different. The pattern of interaction varied with extraction sequence and with position in the profile.

Thus, in Table 11, Fe and Al were both positively correlated with Mg and with each other in the second  $H_2SO_4$ extract (I-f) of Sequence I. In the following NaOH extract (I-g), the positive Fe <u>vs</u> Mg relationship remained, but the positive relationships between Al and Fe, Al and Mg were lost; Al became positively related to N, and Mg and Fe became negatively related to N. In the parallel NaOH extract of Sequence II, Al is positively correlated with all three cations but not with N.

Significant correlations between Fe and Mg in extracts I-f and I-g in Table 11 appear again in the same extracts from subsoils in Table 13, as well as in II-f. Relationships to Al in extracts I-g and II-f are very different in subsoils than in the plow layer.

It is to be expected that interactions of metals with soil organic matter will vary with specific properties of individual cations and of organic species varying in their content of oxygen, nitrogen and aliphatic side chains (Kononova 1966, Stevenson and Butler 1969). Environmental conditions at the interface with the soil solution will also have a profound effect.

Schnitzer and Khan (1972) note that humic substances can be considered as true solutions of macro-ions or negatively charged hydrophilic colloids which vary in their susceptibility to coagulation by different cations. Coagulation depends also on pH and the chemical potential of the solution. In general, cations of the same valence with the largest ionic radius are the most effective coagulants. This rule does not apply to trivalent cations which, because of higher charge density, do not occur as simple cation species in solution and do form stable complexes with humic substances. They note also that complexes of  $Fe^{3+}$  and  $Al^{3+}$ with fulvic acids and humic acids are more susceptible to coagulation by  $Ca^{2+}$  as .99,  $Mg^{2+}$  as .66,  $Fe^{2+}$  as .74,  $Fe^{3+}$ as .64 and  $A1^{3+}$  as .51 Å. It is of interest to note that Ca, Mg, Fe and Al were associated with N in NaOH extracts in quantities related directly to valence and inversely to ionic radius (Tables 37, 40, 41). There were deviations for Fe in the no manure treatment (Tables 40, 41) and for Ca in the plow layer (Table 37). The relationship was less clearly expressed in pyrophosphate extracts (I-c, Table 36) but appeared again in the following  $H_2SO_4$  extract (I-f) OTable 38), except for Al which appeared in smaller quantities than Fe.

It may be postulated from these observations that cations may have been complexed with humic substances in quantities related directly to charge density and inversely to ionic radius. Extracted complexes were stable in NaOH extracts

but were subject to breakdown through chelation of the metals by pyrophosphate. The chelated metals may have been precipitated and contributed to metal recoveries in the succeeding  $H_2SO_{\mu}$  extract (cf. Figures 3, 4).

Aluminum appears to have been the dominant complexed cation in the initial NaOH extract (II-b). There was an increase in the proportion of Fe in the final NaOH extracts (I-g, II-f), specifically in the more acid soils of Experiment II (no manure and 300T).

Since most of the extracted Ca and Mg appeared in  $H_2SO_4$ extracts, it seems likely that these cations were present mainly in precipitated salts or colloidal mineral complexes. The highly significant positive overall correlations between total extracted Ca and organic N (Table 9) indicate that the acid-extracted Ca (Ca salts) served as the major coagulant for precipitating and stabilizing the portion of soil organic N recovered in these two extraction sequences. Thus, some of the extractable Ca may have been present as humate salts from which, however, it was more readily displaced by H<sup>+</sup> than were Fe or Al.

### 4. Regression of N on metals

The overall extractability of N was influenced mainly by Ca. However, it appeared reasonable to expect that other cations would be influential at different stages of extraction.

Accordingly, Tables 14 and 15 give the results of stepwise least squares regression which was used to estimate

Extmante	Depth	Reg	ressions (b	) of N on		Coefficients
Extracts	(11)	Ca	Mg	Fe	Al	determination
. 1.7% ксі	0-9	0.48012**	-0.31906	Delete	Delete	R <sup>2</sup> = .88**
	18-21	0.02335*	-0.08892	Delete	Delete	$R^2 = .51$
. 0.1 <u>м</u> н <sub>2</sub> so,	0-9	Delete	Delete	Delete	Delete	
	18-21	-0.00952#	-0.03037*	Delete	-0.00595	$R^2 = .92^{**}$
. 0.1 <u>M</u> Na <sub>4</sub> P <sub>2</sub> + 0.1N NaC	2 <sup>0</sup> 7 Он 0-9	Delete	15.20115*	1.37745*	Delete	R <sup>2</sup> = .65 <sup>≢</sup>
-	18-21	-0.85518	Deiete	Delete	Delete	$r^2 = .35$
. Fulvic Acid	0-9	Delete	Delete	Delete	Delete	
	18-21	Delet⊬	Delete	Delete	0.26320	$r^2 = .36$
. Humic Acid	0-9	0.78170	Delete	Delete	Delete	$r^2 = .37$
	18-21	Delete	Delete	1.02561	-0.21906	$R^2 = .86**$
. 0.1 <u>м</u> н <sub>2</sub> so,	0-9	1.15496**	-0.53961*	0.07969*	Delete	$R^2 = .89*$
	18-21	Delete	Delete	Delete	Delete	
. 0.1 <u>N</u> NaOH	0-9	Delete	Delete	0.12349#	0.19213#	R <sup>2</sup> = .81**
	18-21	Delete	Delete	Delete	Delete	

Table 14. Optimal solution's for linear regressions of N on metals in extracts of Sequence I.

\*Significant at P(.05).

**\*\***Significant at P(.01).

Delete = deleted from optional solution of P(.10)

1440040	Depth	Reg	ressions (b)	) of N on		Coefficient
EA UT GUUS	(117)	Ca	Mg	Pe	A1	or determination
. 1.7% KC1	6-0	0.48012**	-0.31906	Delete	Delete	R <sup>2</sup> = .88**
	18-21	0.02335*	-0.08892	Delete	Delete	R <sup>2</sup> = .51
. 0.1 <u>N</u> NaOH	6-0	Delete	Delete	-1.39905#	Delete	r <sup>2</sup> = .61*
	18-21	Delete	Delete	Delete	Delete	2
. Fulvic Acid	6-0	0.95610**	-16.28878**	Delete	0.10225##	R <sup>2</sup> = .97**
	18-21	Delete	Delete	Delete	Delete	1
. Humic Acid	6-0	1.91870*	-7.54290	Delete	-0.52755*	R <sup>2</sup> = .77*
	18-21	Delete	Delete	-0.07772	Delete	R <sup>2</sup> = .30
. 0.1N H2SO4	6-0	Delete	Delete	Delete	Delete	1
	18-21	Delete	Delete	0.00744	Delete	r <sup>2</sup> = .41
. 0.1N NaOH	6-0	4*61703.4	Delete	0.21212##	-0.51081**	R <sup>2</sup> = ,92##
	18-21	Delete	Delete	Delete	Delete	8

Optimal solution's for linear regressions of N on metals in extracts of Sequence II. Table 15.

#Significant at P(.05).

##Significant at P(.01)

Delete - Deleted from optional solution at P(.10)

the best relationship between N, the dependent variable, and metals, the independent variables. In this analysis, all of the metals were in the initial regression equation. They then were deleted, one by one, if the significance probability of the F statistic for the least squares coefficient for the metal was higher than the significance level of P(.10). The above process was repeated at each step. The stepwise procedure was terminated when only metals meeting the deletion criterion were left.

The cause and effect relationships implied in these regressions are subject to question. Nevertheless, the approach was used as a tool to see which metals strongly interacted with N.

The results of multiple regression analyses within extracts are presented in Tables 14 and 15. Several significant to highly significant regressions were found, accounting for 65 to 95% of the variation in fractional recoveries of N. The partial regression coefficients in these two tables may be compared as to sign and level of significance with the simple correlation coefficients in Tables 10 to 13.

In the case of the KCl extractions for exchangeable N and metals, only Ca and Mg were retained in the multiple regressions for both sampling depths. The relationship of N to Ca remained positive, as it had been in the simple correlations (Tables 10 and 12), and there was an increase in significance for the partial over the simple correlation

in the subsoil.

There were several surface soil fractions where significant positive regressions of organic N on Ca were expressed in the multiple functions but not in the simple correlations (extracts I-f, II-c, d and f). In each case, there was also an increase in significance for the coefficients of two other cations, usually accompanied by a change in sign for one of them.

Thus, the overall positive correlation between organic N and Ca in surface soils (Table 9) was borne out in important soil fractions when the more variable interactions with Mg, Fe and Al were also taken into account. Again this supports the inference made earlier that Ca was the dominant cation involved in precipitating and stabilizing organic N in these soils.

Partial regression coefficients for organic N in subsoil fractions were rarely significant at P(.05). Negative regressions on Ca and Mg were indicated for extract I-b. Regression coefficients significant at P(.10) involved Ca in extract I-c and Fe and/or Al in a few other fractions.

# 5. Fulvic acid/humic acid separations

The quantities of N and metals found in fulvic and humic sub-fractions are given in Appendix Tables 42 and 43 for the pyrophosphate extracts and in Tables 44 and 45 for the NaOH extracts.

In Table 16, the sums for the fulvic plus humic subfractions are compared with the parent extracts. Summed

Element	Depth			Fulv: for	ic plus hum r extracts	ic acid and trea	recoverie atments	8
	(in)		No mai	nure	30	OT	30T	•
			Na4P207	NaOH	Na4P207	NaOH	Na4P207	NaOH
					pp	m		
N	0-9	Extract	282	293	387	429	412	473
		<b>F' +</b> H	289	323	394	393	361	459
		Recovery	102\$	110\$	101\$	92\$	88\$	97\$
	18-21	Extract	36	56	28	60	44	54
		F + H	59	69	37	66	70	78
		Recovery	163 <b>%</b>	123\$	132%	110%	159\$	144%
Ca	0-9	Extract	28	5	28	78	5	133
		F + H	20	63	167	203	26	128
		Recovery	71%	126%	596\$	260\$	520 <b>\$</b>	96 <b>%</b>
	18-21	Extract	5	5	19	27	5	9
		F + H	20	20	96	133	20	23
		Recovery	400\$	400\$	505 <b>%</b>	492\$	400\$	255 <b>%</b>
Mg	0-9	Extract	5	23	12	35	5	29
-		F + H	20	27	45	38	20	20
		Recovery	400\$	117\$	375\$	108%	400\$	69\$
	18-21	Extract	6	32	11	34	5	14
		F + H	20	27	27	54	20	22
		Recovery	33\$	84\$	245\$	159%	400\$	157
Fe	0-9	Extract	47	287	41	190	122	191
		F + H	110	263	50	248	138	185
		Recovery	234\$	92\$	121\$	130\$	113\$	97
	18-21	Extract	27	314	18	158	26	128
		F + H	- 38	250	31	223	64	1 39
		Recovery	140\$	- 80≸	172\$	141%	246\$	108%
Al	0-9	Extract	71	880	59	1028	56	655
	-	F + H	87	781	96	811	146	581
		Recovery	122\$	89\$	162\$	79\$	260\$	89\$
	18-21	Extract	<u>44</u>	1643	69	1223	61	775
		F + H	102	1346	101	1010	53	608
		Recoverv	231\$	_J.0 82≰	146 <b>4</b>	825	90 <b>%</b>	784
			<b>*</b> **C <b>-</b>					

Table 16.	Comparisons of summed recoveries in fulvic and humic fractions with analysis
	made directly on pyrophosphate or sodium hydroxide extracts.

recoveries of N in the plow layer were in fairly good quantitative agreement with the analyses made on the parent extracts. The sums for the subsoil were greater than in the parent solutions. The lack of agreement for metals was great. In particular, the sums for Ca and Mg were frequently four or five times greater than in the parent extract.

The source of these analytical difficulties was not resolved. The discrepancies for pyrophosphate were generally greater than for NaOH. About 80% of the time, the percentage recovery in pyrophosphate sub-fractions was greater than for NaOH; only about 13% of the time was the reverse true.

There is the possibility that cations complexed with pyrophosphate or with soil P were less completely solubilized by DTPA after perchloric acid digestion of the parent extracts than in the case of the sub-fractions. DTPA was not used with Al, and excess recoveries for sub-fractions were much less than for Ca or Mg. On the other hand, DTPA was used with Fe, but recoveries were generally similar to Al.

If the analytical procedures were, in fact, more sensitive to metals in sub-fractions than in parent extracts, it would have served to accentuate rather than detract from interactions already described between cations and organic N. At least, this inference is supported by the increased information and increased significance of regression coefficients in multiple regressions for fulvic and humic fractions as compared with the function for the parent NaOH

extract of surface soil (Table 15).

# 6. Fulvic/humic ratios

In recent reviews, Stevenson and Butler (1969) and Schnitzer and Khan (1972) note that fulvic acids are more highly oxidized than humic acids, contain more oxygen, and much of the oxygen is present in surface functional groups of an acidic nature (-COOH, COH). Humic acids have a higher proportion of their oxygen content in interiorized carbonyl groups (C=O) and structural linkages (C-O-C).

It appears that substantial quantities of polyvalent cations are trapped or bonded in the interior structure of humic acid molecules, since ash constituents, such as Al, Fe, Si, are extremely difficult to eliminate in preparing highly purified humic acids (Stevenson and Gascho 1968). This is true also of fulvic acids although to a lesser extent because of their smaller molecular size (Levesque and Schnitzer 1966).

Due to their lower molecular weight and greater surface concentration of acidic groups, fulvic acids are more soluble and tend to be more mobile in soils than humic acids. Nevertheless, both form insoluble salts and complexes with polyvalent cations through electrovalent and chelate bonding to surface functional groups. Their salts with monovalent cations, such as Na and  $NH_4$ , are soluble, and this is in part the basis for their extraction from soils using alkaline reagents. Elimination of precipitating polyvalent cations through replacement with H<sup>+</sup> in acid solutions and by removal

as insoluble hydroxides, salts or chelates at alkaline pH is also involved in extraction procedures such as those used here.

The ratio of fulvic acid to humic acid provides an estimate of the degree of oxidation of soil organic matter (Kononova 1966), Schnitzer and Khan 1972). The fulvic/humic recovery ratios in Tables 17 and 18 show the distribution of N and cations between these two fractions. This sort of comparison for cations does not appear to have been reported in the literature.

The actual quantities of cations in the parent pyrophosphate extracts were very low because of the preceding extraction with  $H_2SO_4$  and additional removals by precipitation during the pyrophosphate extraction (cf. Figures 3, 4). Thus, the fulvic/humic ratios in Table 17 are for the more tightly complexed or interiorly trapped cations. The range of ratios is smaller than for the NaOH sub-fractions in Table 18. In both cases, more of the extracted N was present in potentially mobile fulvic acid fractions in the subsoil than in the plow layer.

A significantly larger proportion of tightly bound Ca in Table 17 was associated with fulvic acids in the plow layer than in the subsoil, whereas the reverse was true for Al. These relationships are even more striking in Table 18, where 84 to 92% of the extracted Ca in the plow layer and similar proportions of Al in the subsoil appeared in the fulvic fraction for no manure and the 30T treatment. The

Elements	<u></u>	Treatme	ents	Treatment
	No Manure	300T (1 year)	30T (9 years)	within depth LSD (.05)
Plow layer (0-9	in.)			
N	1.3	1.0	1.1	ns
Ca	1.0	1.3	1.6	ns
Mg	1.0	1.0	1.0	ns
Fe	1.5	1.7	2.9	ns
Al	2.4	1.3	1.0	ns
Subsoil layer (1	18-21 in	.)		
Ν	2.2	2.2	1.4	ns
Ca	1.0	0.9	1.0	ns
Mg	1.0	1.7	1.0	ns
Fe	1.7	0.9	1.4	ns
Al	4.1	0.6	1.7	1.5
Depth within tro LSD (.05)	eatment			
N	ns	1.1	ns	
Ca	ns	0.3	0.3	
Mg	ns	ns	ns	
Fe	ns	0.7	0.7	
Al	0.9	ns	ns	

Table 17. Fulvic/humic recovery ratios for extract c  $(0.1M \text{ Na}_4P_2O_7 + 0.1M \text{ NaOH})$ , Sequence I.<sup>+</sup>

 $^{\dagger}$ Mean of three replications.

		Treatme	nts	
Elements	No Manure	300T (1 year)	30T (9 years)	Treatment within depth LSD (.05)
Plow layer (0-	9 in.)			
N	0.7	0.6	0.6	ns
Ca	5.3	1.9	11.8	2.4
Mg	0.7	0.5	1.0	0.3
Fe	0.5	0.3	0.5	ns
Al	2.7	2.4	2.8	ns
Subsoil layer	(18-21 in.	)		
N	1.2	1.0	1.0	ns
Ca	1.0	1.5	1.3	ns
Mg	0.6	0.2	0.7	0.3
Fe	0.2	0.3	0.4	0.2
Al	5.2	3.8	12.2	ns
Depth within t LSD (.05)	reatment			
Ν	ns	ns	ns	
Ca	2.0	ns	2.0	
Mg	ns	ns	ns	
Fe	0.1	ns	ns	
Al	ns	ns	ns	

Table 18. Fulvic/humic recovery ratios for extract b  $(0.1\underline{N} \text{ NaOH})$ , Sequence II.<sup>+</sup>

<sup>†</sup>Mean of three replications.

proportion was very much lower for the 300T treatment, reflecting the much lower oxidation status of organic matter due to the massive manure addition and periodic waterlogging associated with it. This effect appears also for the more tightly bound pyrophosphate-extracted Al in the subsoil (Table 17) and for NaOH-extracted Mg in the plow layer and in the subsoil (Table 18).

More of the tightly bound Fe in Table 17 was associated with fulvic than humic acids, whereas much of the additional Fe extracted with NaOH was apparently associated with humic acids (Table 18). The same trend was shown for Mg.

It may be visualized that the tightly bound cations in pyrophosphate extracts are not associated with surface functional groups and, therefore, have little influence on surface properties of the fulvic or humic acid molecule. Those complexed in this way with fulvic acids will move with the fulvic acid if surface acidic groups are occupied by monovalent cations or if conditions of pH and chemical potential in soil solution are favorable for mobilization (Schnitzer and Khan 1972).

On the other hand, the additional polyvalent cations found here in NaOH extracts are more likely to have been associated with surface functional groups and would act as coagulants to precipitate and stabilize fulvic and humic acids at soil pH's commonly associated with crop production. The data in Table 18 indicate that Ca was the dominant precipitating cation responsible for stabilizing otherwise

mobile fulvic acids in the plow layer. In the subsoil, it was Al.

### 7. Carbon-nitrogen relationships

Nitrogen was used as the measure of organic matter in all extracts. Due to time restrictions and lack of suitable procedures and equipment, carbon was determined only in the original soil and in residual soil before and after the final NaOH extraction (Residues a and b in Table 20). Nitrogen was determined in the original soil and in Residue (b) (Table 19).

In the surface soil, both N and C were related significantly to treatment, both in the original soil and in residues from both extraction sequences. The lowest values were for no manure and the highest for 30T annually over nine years. Simple correlations between N and C were significant at P(.01): r = 0.947 for the original soil, r = 0.928 for Residue (a) and r = 0.746 for Residue (b).

Values in the subsoil were significantly lower than in the plow layer, but there were no significant differences for treatment and simple correlations between N and C were not significant at P(.05).

Percent removals in Table 21 were calculated from the differences between original soil and Residue (b) in Tables 19 and 20. Percentage removals of N and C from the plow layer were similar and ranged from 55 to 64%, with no relation to treatment. These are consistent with commonly

		Nitr	ogen in s	toil or res	ldue		Treatment	
Soll			)				within depth	
or residue		manure t Sec II	<u>300T (1</u>	. year) Sec II	30T (9 Y	ears)	within sequence LSD (.05)	<b>υ   ⊢</b>
	ד סעל מ	TT ·bac	T ·hac	TT •hac	- hac	nt .peq.	• hac I • hac	-
	           	* * * * * * * * * * * * * * * * * * *	 	1d	, 			
Plow layer (0-9 in.	<b>•</b>							
Soll	Ŷ	II	10	200	1226		291.9	
Residue (b)‡	235	239	412	352	478	447	128.7 131.8	
Subsoil layer (18-2	21 <b>1</b> n.)							
Soll	-	-92	г	-59	196		ns	
Residue (b)‡	136	128	106	96	97	81	su su	
Depth within treatm sequence LSD (.05	nent withi 5)	ц						
Soll	lu	7.11	()	7.11	112	.7		
Residue (b)	88.2	รน	88.2	118.6	88.2	118.6		

Nitrogen recoveries in original soil and in residual soil after final NaOH extraction Table 19.

Tho significant differences between Seq. I and II within treatments.

 $^{\dagger}$ Residue (b) taken after final NaOH extraction.

Soll		Ca	rbon in s	oil or resi	due		Tre: with1	atment n depth
or residue	NON	manure	300T (	l year)	30T (9	) years)	within LSD	sequence (.05)
	Seq. I	' Seq. II	Seq. I	Seq. II	Seq. I	Seq. II	Seq. I	Seq. II
	8 8 9 9							
Plow layer (0-9 in								
Soll	0	.88	ŗ.	37	Γ.	.77	0	• 35
Residue (a) ‡	0.30	0.29	0.69	0.91	1.22	1,6.0	0.22	0.25
Residue (b) <sup>§</sup>	0.34	0.30	0.52	0.59	0.78	0.75	0.17	0.12
Subsoll layer (18-	(.ni 15.							
Soll	0	.17	0	21	0	.45	1	IS
Residue (a)	0.07	0.03	0.16	0.22	0.19	0.17	su	su
Residue (b)	0.08	0.11	0.14	0.05	0.16	0.16	รน	มร
<pre>Depth within treat sequence LSD (.0</pre>	ment with 15)	ln						
Soil	0	.37	0	37	0	.37		
Residue (a)	su	0.19	0.24	0.19	0.24	0.19		
Residue (b)	0.19	0.12	0.19	0.12	0.19	0.12		

•

<sup>†</sup>Residue (a) taken before final NaOH extraction.

<sup>§</sup>Residue (b) taken after final NaOH extraction.

		111 1100 10110				Totonon hor		• 6110
ст 10 10 10 10 10 10 10 10 10 10 10 10 10			Treat	ments			Treat within	cment depth
shilamata		No manure	300T (1	r year)	30T (9 3	rears)	within : LSD (.(	sequence 05)
	Seq.	I <sup>†</sup> Seq. II	Seq. I <sup>†</sup>	Seq. II	Seq. I <sup>†</sup>	Seq. II	Seq. I	Seq. II
Plow layer (0.	-9 in.)							
N	64	63	58	64	61	63	su	su
U	61	64	61	56	55	56	มร	ns
Subsoll layer	(18-21 in.)							
N	28	33	34	39	51	58	11	11
U	48	38	35	75	64	64	su	su
Depth within sequence LSI	treatment w1 0 (.05)	thin						
N	14	14	14	14	su	su		
U	su	bsu	ns	su	su	su		
† <sub>No</sub> significa	nt differenc	e between Seq.	. I and II	( within t	reatments.			

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reported values (Kononova 1966).

In the subsoil, a significantly lower proportion of soil organic N was removed by both extraction sequences for the no manure and 300T treatments than for 30T. A similar (although not significant) result was obtained for carbon, except for what may be an erroneously high value for 300T in Sequence II.

In Table 22, C/N ratios have been calculated for original soil, extracted fractions and final residue. The unique feature in these data is the very wide C/N ratios for soil, extracted fractions and residues in subsoil under the 30T manure treatment. The soil and extracted ratios are significantly higher than in the plow layer.

Soil samples from the 30T treatment were taken from plots harvested for grain in Experiment I. It is possible that the wide C/N ratios in subsoil reflect effects of carbonaceous organic matter returned in the form of stover. This inference is consistent with the larger retention of organic N in the surface and lesser movement into subsoils indicated in Table 3 for grain <u>vs</u> silage. If these subsoil C/N ratios were in fact greater than under silage harvest, they would also explain the significantly lower levels of NH<sub>4</sub> recovered from subsoils under grain harvest (cf. Table 3 and text on page 34).

A similar wide C/N ratio was calculated for fractions extracted in Sequence II from subsoils under the 300T treatment. This value derives from the unusually low C analysis

Soll, extracted fraction or		C/N F	atios in	soll or re	stdue		Treat within	:ment depth	
residue	No ma	nure	300T (1	year)	30T (9	years)	within s LSD (	(.05)	
	Seq. I <sup>†</sup>	Seq. II	Seq. I <sup>†</sup>	Seq. II	Seq. I <sup>†</sup>	Seq. II	Seq. I	Seq. II	
Plow layer (0-9 in.)									
Soll	13.	36	13.	83	14.	33	su		
Extracted fractions	12.79	13.50	14.88	12.32	13.05	12.86	รน	ns	
Residue (b)‡	14.40	13.07	12.67	16.76	16.49	17.47	su	รน	
Subsoll layer (18-21	l <b>in.</b> )	-							
Soll	8	95	13.	50	23.	72	6.8	32	
Extracted fractions	16.59	10.46	13.70	26.10	28.73	28.26	su	ns	
Residue (b)‡	6.24	8.27	13.13	5.48	20.79	20.78	8.57	8.57	
Depth within treatme sequence LSD (.05)	ent within	_							
Soil	r	ß	su		7.	05			
Extracted fractions	su	su	su	12.51	12.51	12.51			
Residue (b)	su	su	su	6.78	su	su			
t No significant diff	erence be	tween Seq.	I and II	within tr	eatments				

\*Residue (b) taken after final NaOH extraction.

Table 22. C/N Ratios in original soil, extracted fractions, and in residual soil.

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for Residue (b) in Table 20 and is to be questioned.

There is little basis for speculating what the interactions of C with cations may have been. The wide C/N ratios in subsoil for the 30T treatment were associated with substantially higher levels of extractable Ca and lower levels of Mg, Fe and Al than in subsoils from the other two treatments (cf. Figure 2, Table 46). The functional groups which can interact with cations are associated with C rather than N in fulvic acid and humic acid molecules. It is, therefore, essential that accurate C determinations be used as the measure of organic matter in future studies of this sort.

#### CHAPTER V

## SUMMARY AND CONCLUSIONS

Profile distributions of N and P were examined in sandy loam soils to a depth of 10 feet after nine annual applications of 10, 20 and 30 T/a beef cattle manure or variable inputs of N and P in commercial fertilizer for continuous corn (Experiment I). In a second closely adjacent experiment, profile distributions were examined, to a depth of 5 feet, eight months after additions of 100, 200 and 300 T/amanure for corn. Fractional distributions of N, Ca, Mg, Fe and Al were examined in the plow layer and in the 18-21 in subsoil increment from plots which received no manure and 300T in a single application in Experiment II and 30T annually over nine years in Experiment I. Two fractionation sequences were used: Sequence I (1.7% KCl + 0.1<u>N</u> H<sub>2</sub>SO<sub>4</sub> + 0.1<u>M</u> Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> in  $0.1\underline{N}$  NaOH  $\rightarrow 0.1\underline{N}$  H<sub>2</sub>SO<sub>4</sub>  $\rightarrow 0.1\underline{N}$  NaOH); Sequence II (1.7% KCl + 0.1<u>N</u> NaOH + 0.1<u>N</u> H<sub>2</sub>SO<sub>4</sub> + 0.1<u>N</u> NaOH). The alkaline pyrophosphate extract of Sequence I and the first NaOH extract of Sequence II were separated into fulvic acid and humic acid sub-fractions. Except for the exchangeable fraction (1.7% KCl), fractional extracts were subjected to perchloric acid digestion to remove organic matter before analyzing for the metal cations.

In the long-term Experiment I, more organic N was retained in the plow layer (0-9 in.) where corn was

harvested for grain and stover was returned than where silage was removed. Downward movement of organic N (detectable to depths of 4 to 6 feet) was greater under silage harvest and increased with increased additions of manure; it was also greater where fertilizer N, P and K were used without lime. Nitrate and total mineral N in the plow layer were higher with grain harvest, whereas  $NH_{4}$  and total mineral N were lower in the subsoil than with silage harvest. This suggests closer cycling of N due to carbonaceous stover residues and perhaps greater losses of N by denitrification over the years. Extractable P (Bray P-1) in the plow layer increased with rate of manure addition, but not to levels as great as where commercial fertilizer supplying 190 lb/a  $P_2O_5$  annually was used. However, downward movement of P, detectable to 15 in, was greater with manure than with commercial fertilizer.

There were no control plots in Experiment I, so recoveries from soil of N and P could not be compared with inputs. In Experiment II, however, the increase in organic N in the plow layer (3,500 lb/a) for 300T/a manure applied eight months earlier would account totally for the estimated 3,300 lb N applied. There was some evidence for downward movement of organic N to depths up to 15 or 18 in. Ammonium in the plow layer was similar in the two experiments, with no relation to treatment; nitrate was much higher in Experiment II, ranging from 50 to 194 ppm NO<sub>3</sub>-N for manure treatments, as compared with 9 to 32 ppm for applications of 10 to 30T in Experiment I. Nitrate concentrations below about

27 inches were again similar at comparable depths to 5 feet in both experiments (7 to 17 ppm NO<sub>3</sub>-N for 100 to 300T manure in Experiment II <u>vs</u> 7 to 11 ppm for 30T with silage harvest in Experiment I). In the subsoil,  $NH_4$  concentrations in 3inch increments to 5 feet were significantly lower for 200 and 300T applications than for no manure; this appeared to be due to a lower oxidation status due to temporary loss of infiltration capacity in the plow layer and periodic ponding associated with these massive manure additions. Extractable P in the plow layer increased with manure application in Experiment IF, but there was no evidence for downward movement out of the plow layer; the increase for 300T was 140 lb/a P, or about 16% of estimated input.

Only about 15% of the Ca and seven percent of the Mg applied in 300T of manure could be accounted for in exchangeable forms in the plow layer. Nonexchangeable but extractable forms were equivalent to 139% of input Ca and 92% of input Mg. Most of the nonexchangeable Ca and Mg appeared to be present in minearal salts or complexes and was found in  $H_2SO_4$  extracts. Nevertheless, Ca and Mg were present, together with larger quantities of Fe and much larger quantities of Al, in organo-mineral complexes which contained 27 to 58% of the total soil organic N and were removed in pyrophosphate or NaOH extracts. There was a strong tendency for the quantities present in these complexes to be related directly to valence and inversely to ionic radius of the metal cation in the order: Ca < Mg < Fe < Al.

Significant interactions among the cations themselves and with N appeared in simple and multiple correlation analyses of data for individual fractions and for their sums in the two extraction sequences. These correlations indicated that Ca was the dominant cation responsible for precipitating and stabilizing organic N in the plow layer. This inference was supported by fulvic/humic recovery ratios which showed that 84 to 92% of the Ca in NaOH extracts was associated with the fulvic fraction. In the subsoil similar percentages of the NaOH-extracted Al appeared in the fulvic fraction, indicating that Al was principally responsible for precipitating and stabilizing fulvic acids in the subsoil.

Fulvic/humic recovery ratios for all cations were sharply reduced at the 300T level of manure addition as compared with no manure or 30T. This effect was expressed either in surface or subsoil and, in the case of Mg, in both. As with  $NH_4$  levels in subsoils of Experiment II, this reflects the lower oxidation status of organic matter due to reduced infiltration and periodic waterlogging which occurred with this massive manure application.

Although only a small proportion of the total Ca extracted appeared in the same fractions with organic N, total extracted N and Ca in both sequences were positively correlated at P(.01) in the surface soil. This indicates that Ca in mineral fractions extractable with  $H_2SO_4$  were also influential in stabilizing N.

Calcium, overall and in extracted organo-mineral

fractions, was negatively correlated with Fe, both in surface and subsoils, and tended to be negatively correlated with Al, particularly in subsoils. Interactions involving Mg were more variable but were also consistent with the inference that cations compete for similar sites in organic matter. Nitrogen undoubtedly competes for some of the same sites but no strong evidence for this was found.

Much smaller quantities of cations remained in association with N in pyrophosphate extracts than in NaOH. It was inferred that these are tightly complexed or trapped inside organic molecules and would be subject to movement with fulvic acids under appropriate conditions. The additional quantities of cations in NaOH extracts are more likely to be associated with surface functional groups and to act as coagulants for precipitating organic matter. This postulation appears to be promising to pursue in further research. For this purpose, the four extraction schemes in Figure 5 are proposed.

To avoid problems of interpretation encountered in this study, soil samples should be taken from a single experiment and from the same soil type. Analytical difficulties in quantitating cations in the presence of phosphate in extracts will need to be resolved. Both C and N should be determined in all fractions and in original and residual soil, using procedures tested for reliability on similar preparations.





BIBLIOGRAPHY

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#### BIBLIOGRAPHY

- Adriano, D. C. 1974 Effects of housing type on nutrient composition of beef cattle manure. <u>In</u> "Proceedings Agriculture Waste Conference Emphasis-Animal Waste", Michigan State University, East Lansing, Michigan.
- Adriano, D. C., A. C. Chang, P. F. Pratt, and R. Sharpless. 1973. Effect of soil application of dairy manure on germination and emergence of some selected crops. J. Environ. Quality 2: 397-399.
- Adriano, D. C., F. H. Takatori, P. F. Pratt, and O. A. Lorenz 1972. Soil nitrogen balance in selected rowcrop sites in southern California. J. Environ. Quality 1: 279-283.
- Ahlrichs, J. L. 1972. The soil environment. <u>In</u> "Organic Chemicals in the Soil Environment", C. A. I. Goring and J. W. Hamaker, eds. Marcel Dekker, Inc., New York.
- Aleksandrova, L. N. 1967. Organomineral humic acid derivatives and methods of studying them. Soviet Soil Sci. 909-913.
- Allison, F. E. 1973. Soil organic matter and its role in crop production. Elsevier Scientific Publishing Company, New York.
- Bartholomew, W. V., and F. E. Clark 1965. Soil nitrogen. Agronomy Monograph No. 10, American Society of Agronomy, Madison, Wisconsin.
- Broadbent, F. E. 1973. Factors affecting nitrification-denitrification in soils. <u>In</u> "Recycling Treated Municipal Wastewater and Sludge through Forest and Cropland", W. E. Sopper and L. T. Kardos, eds. The Pennsylvania State University Press, University Park, Pennsylvania.
- Broadbent, F. E., and F. J. Stevenson 1966. Organic matter interactions. <u>In</u> "Agricultural Anhydrous Ammonia Technology and Use", M. H. McVickar, et al., eds. American Society of Agronomy, Madison, Wisconsin.
- Bremner, J. M. 1965a. Inorganic forms of nitrogen. <u>In</u> "Methods of Soil Analysis", C. A. Black, et al., eds. Agronomy Monograph No. 9, Part 2. American Society of Agronomy, Madison, Wisconsin.

- Bremner, J. M. 1965b. Total nitrogen. <u>In</u> "Methods of Soil Analysis", C. A. Black, <u>et al</u>., eds. Agronomy Monograph No. 9, Part 2. American Society of Agronomy, Madison, Wisconsin.
- Cochran, W. G., and G. M. Cox 1957. Experimental Designs. 2nd. ed. John Wiley & Sons, Inc., New York.
- Ellis, B. G. 1973. The soil as a chemical filter. <u>In</u> "Recycling Treated Municipal Wastewater and Sludge through Forest and Cropland". W. E. Sopper and L. T. Kardos eds. The Pennsylvania State University Press, University Park, Pennsylvania.
- Ellis, B. G., A. E. Erickson, B. D. Knezek, and A. R. Wolcott 1972. Impact of wastewater on soils. Technical Report No. 30, Institute of Water Research, Michigan State University, East Lansing, Michigan.
- Ellis, B. G., and B. D. Knezek 1972. Absorption reactions of micronutrients in soils. <u>In</u> "Micronutrients in Agriculture", J. J. Mortvedt, <u>et al</u>. eds. Soil Science Society of America, Inc., Madison, Wisconsin.
- Felbeck, Jr., G. T. 1971. Chemical and biological characterization of humic matter. <u>In</u> "Soil Biochemistry", A. D. McLaren and J. Skujins, eds. Marcel Dekker, Inc., New York, Vol. II.
- Fiske, C. H., and V. Subbarow 1925. Colorimetric determination of phosphorus. J. Biol. Chem. 66: 37.
- Garner, G. B. 1963. Nitrate: A factor in animal health. Proc. of The New Zealand Soc. of Animal Prod. 23: 28-38.
- Jackson, M. L. 1958. Soil Chemical Analysis. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.
- Jacobs, L. W. 1974. Utilizing the nutrients in animal manures. In "Proceedings Agriculture Waste Conference Emphasis — Animal Waste", Michigan State University, East Lansing, Michigan.
- Johnson, C. M., and A. Ulrich 1959. II. Analytical methods for use in plant analysis. Calif. Agr. Exp. Bull. 766.
- Khan, S. U. 1969. Interactions between the humic acid fraction of soils and certain metallic cations. Soil Sci. Soc. Am. Proc. 33: 851-854.
- Kononova, M. M. 1966. Soil organic matter: Its nature, its role in soil formation and in soil fertility. 2nd. ed. Pergamon Press, New York.

- Kononova, M. M., and V. V. Dokuchayev 1967. Methods of determining humus composition and their rationalization. Soviet Soil Sci. 894-903.
- Levesque, M., and M. Schnitzer 1966. Effects of NaOH concentrations of the extraction of organic matter and of major inorganic constituents from a soil. Can. J. Soil Sci. 46: 7-12.
- Lindsay, W. L. 1973. Inorganic reactions of sewage waste with soils. In "Proceedings of the Joint Conference on Recycling Municipal Sludges and Effluents on Land", National Association of State Universities and Land-Grant Colleges, Washington, D.C.
- Linville, K. W., and G. E. Smith 1971. Nitrate content of soil cores from corn plots after repeated nitrogen fertilization. Soil Sci. 112: No. 4. 249-255.
- Lund, L. J., D. C. Adriano, and P. F. Pratt 1974. Nitrate concentrations in deep soil cores as related to soil profile characteristics. J. Environ. Quality 3: 78-82.
- McLean, E. O. 1965. Aluminum. <u>In</u> "Methods of Soil Analysis", C. A. Black, <u>et al.</u>, eds. Agronomy Monograph No. 9, Part 2. American Society of Agronomy, Madison, Wisconsin.
- Mortensen, J. L. 1963. Complexing of metals by soil organic matter. Soil Sci. Soc. Amer. Proc. 27: 179-186.
- Mortensen, J. L., and F. L. Himes 1964. Soil organic matter. <u>In</u> "Chemistry of the Soil", F. W. Bear ed. American Chemical Society, Monograph Series No. 160, Reinhold Publishing Corporation, New York.
- Murphy, L. S., G. W. Wallingford, W. L. Powers, and H. L. Manges 1972. Effects of solid beef feedlot wastes on soil conditions and plant growth. Proc. Cornell Univ. Agr. Waste Management Conf., Syracuse, New York.
- Powers, W. L., G. W. Wallingford, and L. S. Murphy 1974. Status of research on the effects of land disposal of animal wastes. EPA Project #803021. US-EPA, Ada, Oklahoma.
- Public Health Service Drinking Water Standards 1962. Public Health Service, Washington, D.C.
- Schnitzer, M., and S. Khan 1972. Humic substances in the environment. Marcel Dekker, Inc., New York.

- Schoemaker, H. E., E. O. McLean, and P. F. Pratt 1961. Buffer methods for determining lime requirement of soils with appreciable amounts of extractable aluminum. Soil Sci. Soc. Am. Proc. 25: 274-277.
- Smith, G. E. 1968. Contribution of fertilizers to agriculture. Joint Seminar, Water pollution as related to agriculture. University of Missouri and Missouri Water Pollution Board.
- Smith, G. E. 1965. Water form, Nitrate problems in water as related to soils, plants and water. Mo. Agr. Exp. Sta. Spec. Rpt. 55: 42-52.
- Stevenson, F. J. 1960. Chemical nature of the nitrogen in the fulvic fraction of soil organic matter. Soil Sci. Soc. Proc. 24: 472-477.
- Stevenson, F. J. 1965. Gross chemical fractionations of organic matter. <u>In</u> "Methods of Soil Analysis", C. A. Black, <u>et al.</u>, eds. Agronomy Monograph No. 9, Part 2. American Society of Agronomy, Madison, Wisconsin.
- Stevenson, F. J., and M. S. Ardakani 1972. Organic matter reactions involving micronutrients in soils. In "Micronutrients in Agriculture", J. J. Mortvedt, et al., eds. Soil Science Society of America, Inc., Madison, Wisconsin.
- Stevenson, F. J., and J. H. A. Butler 1969. Chemistry of humic acids and related pigments. <u>In</u> "Organic Geochemistry Methods and Results", G. Eglinton and M.T. J. Murphy eds. Springer-Verlag, New York.
- Stevenson, F. J., and G. J. Gascho 1968. An improved method for extracting organic matter from soil. Soil Sci. Soc. Am. Proc. 32: 117-119.
- Stewart, B. A., <u>et al</u>. 1967. Distribution of nitrates and other water pollutions under field and corrals in the middle south Platte Valley of Colorado. USDA ARS 41-134.
- Taylor, A. W. 1967. Phosphorus and water pollution. J. Soil and Water Conserv. 22: 228-231.
- Viets, F. G., and R. H. Hageman 1971. Factors affecting the accumulation of nitrate in soils, water, and plants. Agr. Handbook No. 413. ARS-USDA, Washington, D.C.
- Vitosh, M. L., J. F. Davis, and B. D. Knezek 1973. Longterm effects of manure, fertilizer and plow depth on chemical properties of soils and nutrient movement in a monoculture corn system. J. Environ. Quality 2: 296-299.

- Weast, R. C. 1973. Handbook of chemistry and physics. 54th ed. CRC Press, Cleveland, Ohio.
- Weeks, M. E., M. E. Hill, S. Karczmarczyk, and A. Blackmer 1972. Heavy manure applications: benefit or waste? Proc. Cornell Univ. Agr. Waste Management Conf., Syracuse, New York.
- Winter, A. J. 1962. Studies on nitrate metabolism in cattle. Amer. Jour. of Vet. Res. 23: 500.
- Wright, M. J., and K. L. Davidson 1964. Nitrate accumulations in crops and nitrate poisioning in animals. Adv. Agron. 16: 197-247.

APPENDIX
Depth				Treatme	nts			Treatment
(in)		Manure			160 lb N	160 1b N	NPK	within depth LSD (.05)
	10T	20T	30T	160 16 N	+ P & K	+ lime	+ lime	
					ppm			
0-9	2777	2553	3582	2524	2605	2033	2447	650
9-12	302	251	388	203	325	339	392	ns
12-15	219	175	289	171	239	207	315	ns
15-18	198	203	217	148	253	217	237	ns
18-21	177	183	220	157	247	251	187	ns
21-24	213	193	183	163	238	213	186	ns
24-27	225	178	192	218	247	170	281	ns
27-30	232	199	184	208	219	154	226	ns
30-33	218	224	185	169	206	175	209	ns
33-36	301	197	196	175	197	221	310	ns
36-39	176	214	181	212	197	217	211	ns
39-42	189	230	175	<b>2</b> 20	192	231	270	ns
42-45	205	211	182	166	175	207	195	ns
45-48	212	216	180	174	215	219	193	ns
48-51	212	184	182	173	199	223	187	ns
51-54	194	180	173	144	188	215	201	ns
54-57	177	164	180	120	188	213	176	ns
57-60	157	164	180	115	190	205	202	ns
60-66	137	178	182	113	189	184	188	ns
66-72	130	142	174	120	210	121	183	ns
72-78	110	126	162	128	160	126	179	ns
78-84	97	94	143	117	1 39	134	181	ns
84-90	95	84	130	132	124	133	141	ns
90-96	74	77	147	97	145	111	105	ns
96-102	91	74	128	63	133	93	211	ns
102-108	89	89	85	90	128	101	113	ns
108-114	86	44	98	85	103	102	84	ns
114-120	89	39	92	79	103	86	75	ns
Depth within LSD (.05)	treatme	ent						
	96	96	96	96	96	96	96	

Table 23. Distribution of organic-N with depth in the profile nine years after annual applications of manure or commercial fertilizers for corn harvested as grain.<sup>†</sup>

Depth				Treatme	nts			Treatment
(in)	<u>-</u>	Manure		160 lb N	160 15 N	160 16 N	NPK	within depth LSD (.05)
	10 <b>T</b>	20 <b>T</b>	30T	100 10 %	P&K	lime	lime	
					ppm			
0-9	2604	2770	3265	2406	207 <b>7</b>	2190	1924	650
9-12	254	317	449	258	335	312	311	ns
12-15	209	20 <b>9</b>	275	175	209	252	165	ns
15-18	200	232	210	197	224	248	158	ns
18-21	198	191	177	217	197	253	179	ns
21-24	187	239	200	238	185	282	153	ns
24-27	147	211	218	226	228	244	173	ns
27-30	195	190	213	214	269	225	192	ns
30-33	205	158	216	224	246	211	226	ns
33-36	186	176	257	210	232	220	182	ns
36-39	187	179	211	176	212	181	182	ns
39-42	181	190	176	207	223	186	158	ns
42-45	239	206	243	218	217	157	195	ns
45-48	255	166	207	203	199	177	177	ns
48-51	246	178	231	146	224	148	182	ns
51-54	224	202	226	191	220	139	179	ns
54-57	195	165	214	182	189	158	174	ns
57-60	188	187	196	197	191	195	162	ns
60-66	174	213	202	162	154	105	124	ns
66-72	146	204	156	135	142	109	112	ns
72-78	141	200	106	126	114	112	114	ns
78-84	129	145	135	135	120	124	103	ns
84-90	142	145	153	171	125	122	93	ns
90-96	84	143	139	153	101	111	101	ns
96-102	78	130	164	127	109	102	95	ns
102-108	96	128	172	104	105	104	90	ns
108-114	49	98	157	98	121	99	85	ns
114-120	63	103	172	91	107	89	81	ns
Depth within LSD (.05)	n treatmo	ent		·				
	96	96	96	96	96	96	96	

Table 24. Distribution of organic-N with depth in the profile nine years after annual applications of manure or commercial fertilizer for corn harvested as silage.<sup>†</sup>

<sup>†</sup>Mean of three replications.

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Depth		Treatment						
(in)		Manure		160 lb N	160 15 N +	160 15 N	NPK +	LSD (.05)
	10T	20T	30T		P&K	lime	lime	
					ppm			
0-9	12.6	13.3	17.9	18.2	13.8	7.3	13.8	ns
9-12	2.2	3.0	3.3	1.3	1.3	1.8	3.1	ns
12-15	1.1	2.3	3.5	2.1	2.1	3.4	2.6	ns
15-18	1.4	2.2	2.9	1.2	1.3	1.3	2.2	ns
18-21	1.3	1.9	2.9	1.3	1.2	1.2	11.4	ns
21-24	0.8	2.7	2.9	1.5	2.3	2.1	2.4	ns
24-27	1.3	1.3	2.2	0.9	1.3	1.3	1.8	ns
27-30	0.7	1.7	2.5	1.5	1.2	2.2	2.1	ns
30-33	0.6	2.4	2.3	0.9	0.7	2.2	2.3	ns
33-36	0.7	1.1	1.9	0.6	0.9	1.3	1.6	ns
36-39	0.1	1.7	1.8	0.4	1.1	1.5	2.4	ns
39-42	0.5	1.8	2.2	0.4	0.8	1.9	2.3	ns
42-45	0.5	1.5	2.9	0.2	0.9	1.5	2.7	ns
45-48	0.3	1.6	1.7	1.1	1.1	1.1	2.7	ns
48-51	0.3	1.8	1.6	0.7	0.7	1.6	1.9	ns
51-54	0.5	1.4	1.8	1.0	0.7	1.8	2.6	ns
54-57	0.2	1.9	1.2	0.6	0.9	1.6	2.4	ns
57-60	0.4	1.6	1.1	0.6	0.3	1.8	2.3	ns
60-66	0.3	1.7	1.8	0.6	0.6	1.7	2.5	ns
66-72	0.4	1.5	1.8	1.2	0.2	2.4	2.5	ns
72-78	0.5	1.5	2.0	1.5	0.5	1.7	2.7	ns
78-84	0.1	1.3	1.6	0.4	0.7	1.2	2.4	ns
84-90	0.4	1.1	1.1	0.5	0.9	1.5	2.3	ns
90-96	0.4	1.4	1.6	1.0	0.6	2.1	2.0	ns
96-102	0.2	1.3	1.5	0.4	0.7	1.9	1.8	ns
102-108	0.4	1.4	1.0	0.1	0.7	1.7	1.8	ns
108-114	1.0	1.1	1.6	0.8	0.7	2.4	1.5	ns
114-120	0.5	1.1	1.7	0.6	0.8	4.3	1.7	ns
Depth withi LSD (.05)	n treatm	ent						
	ns	ns	ns	ns	ns	2.7	2.7	

Table 25. Distribution of ammonium-N with depth in the profile nine years after annual applications of manure or commercial fertilizers for corn harvested as grain.<sup>†</sup>

				Treatme	nts			
Depth (in)	Manure				160 1b N	160 1b N	NPK	Treatment within depth
	10 <b>T</b>	20T	30 <b>T</b>	160 lb N	+ P&K	+ lime	+ lime	LSD (.05)
					ppm			
0-9	7.4	9.9	20.5	5.2	12.5	8.4	10.9	ns
9-12	2.8	4.1	5.4	2.9	7.1	1.8	2.6	ns
12-15	2.7	3.8	5.5	3.4	2.8	3.4	3.4	ns
15-18	2.4	4.0	5.3	2.2	2.8	1.8	2.3	ns
18-21	3.9	1.4	4.3	3.1	1.8	2.1	2.3	ns
21-24	3.1	3.3	3.9	2.5	2.0	2.4	2.3	ns
24-27	3.4	3.7	3.5	2.5	1.3	1.4	2.5	ns
27-30	3.5	3.3	4.2	2.9	0.9	1.0	2.6	ns
30-33	4.1	3.4	5.2	2.8	1.2	1.9	2.5	ns
33-36	2.8	2.5	3.4	2.4	1.5	0.9	2.6	ns
36-39	3.9	4.1	3.2	2.7	1.9	0.4	2.1	ns
39-42	2.8	2.2	2.9	2.8	1.3	0.7	2.4	ns
42-45	3.4	2.2	4.5	1.9	1.2	0.6	1.9	n <b>s</b>
45-48	2.9	3.4	2.5	1.7	1.4	0.8	2.5	ns
48-51	3.3	3.3	6.2	1.7	1.7	1.0	2.1	ns
51-54	3.1	3.3	2.7	2.1	1.0	0.8	1.9	ns
54-57	3.2	2.8	2.9	2.1	1.1	0.5	2.1	ns
57 <b>-</b> 60	2.9	2.9	2.3	2.2	1.1	0.7	1.2	ns
60-66	2.8	2.7	3.2	2.3	1.7	0.9	1.7	ns
66-72	4.4	3.9	2.5	2.3	0.9	0.6	2.2	ns
72-78	3.1	2.6	1.0	2.0	1.0	0.8	1.8	ns
78-84	3.2	3.3	1.2	1.8	1.0	0.2	1.6	ns
84-90	2.9	2.3	2.2	2.4	0.5	0.3	1.5	ns
90-96	4.1	2.9	0.9	2.0	1.2	0.4	1.3	ns
96-102	1.8	1.9	2.2	2.1	0.9	0.6	1.4	ns
102-108	2.3	2.6	1.1	1.8	1.8	0.5	1.6	ns
108-114	1.9	3.5	2.4	1.7	1.7	0.4	1.8	ns
114-120	1.5	0.9	1.3	1.8	1.3	0.8	2.1	ns
Depth within LSD (.05)	n treatme	ent						
	2.7	2.7	2.7	ns	2.7	2.7	ns	

Table 26. Distribution of ammonium-N with depth in the profile nine years after annual applications of manure or commercial fertilizer for corn harvested as silage.<sup>†</sup>

				Treatme	nts			Treatment
Depth (1n)		Manure		160 lb N	160 15 N	160 15 N	NPK +	within depth LSD (.05)
	10T	20 <b>T</b>	30T		P& K	11me	11me	
					ppm			****
0-9	20.1	18.8	31.7	29.3	11.4	10.6	15.0	19.0
9-12	28.2	5.1	6.9	4.2	0.9	1.5	10.2	10.3
12-15	25.8	4.3	5.9	1.3	1.9	2.2	12.2	10.3
15-18	1.9	3.2	6.3	1.3	3.6	2.0	9.9	ns
18-21	1.7	2.7	5.7	0.2	3.1	3.2	8.8	ns
21-24	1.9	2.2	5.5	1.3	3.1	3.0	8.4	ns
24-27	1.8	3.6	4.3	1.7	4.1	3.9	12.5	10.3
27-30	1.9	4.2	5.5	2.2	5.3	5.2	14.0	10.3
30-33	2.2	5.7	5.6	2.2	7.1	7.6	11.3	ns
33-36	3.1	5.1	5.3	4.1	9.9	8.4	9.7	ns
36-39	2.7	4.9	5.4	4.6	10.4	6.4	9.2	ns
39-42	2.7	5.9	6.9	6.0	11.9	7.8	9.7	ns
42-45	3.3	6.3	7.2	6.0	9.9	8.7	8.9	ns
45-48	2.9	5.6	7.5	6.4	12.4	8.6	8.4	ns
48-51	3.6	6.6	7.7	7.4	10.9	8.2	8.8	ns
51-54	3.4	5.5	8.1	7.1	10.4	8.8	8.8	ns
54-57	3.3	6.2	9.2	6.8	10.5	9.8	8.8	ns
57-60	3.8	5.9	9.2	7.0	10.0	8.6	9.1	ns
60-66	2.7	5.9	9.0	5.9	8.7	8.1	7.8	ns
66-72	3.1	5.8	7.7	7.2	8.6	7.0	8.6	ns
72-78	2.4	6.1	8.7	7.5	8.4	6.4	8.9	ns
78-84	2.5	4.4	6.9	6.1	6.5	5.8	7.2	ns
84-90	1.6	4.5	7.8	5.7	6.5	5.6	6.7	ns
90-96	2.3	4.3	7.8	3.6	4.8	6.5	5.3	ns
96-102	1.5	3.4	7.3	3.3	5.1	4.8	5.7	ns
102-108	2.0	3.0	3.8	3.3	6.7	4.0	5.2	ns
L08-114	1.6	3.1	4.4	3.6	4.2	4.2	5.4	ns
114-120	2.2	2.3	4.1	2.3	3.2	4.5	5.4	ns
epth with LSD (.05	in treatm	ent						
	7.2	ns	ns	7.2	7.2	7.2	7.2	

Table 27. Distribution of nitrate-N with depth in the profile nine years after annual applications of manure or commercial fertilizers for corn harvested as grain.<sup>†</sup>

				Treatme	nts			Treatment
Depth (in)		Manure		160 lb N	160 15 N +	160 15 N +	NPK +	within depth LSD (.05)
	10T	20 <b>T</b>	30T		P & K	lime	lime	
					ppm			
0-9	13.8	8.9	12.5	8.6	5.9	6.1	11.6	ns
9-12	2.8	4.2	11.6	2.9	7.1	10.2	3.9	ns
12-15	2.8	3.5	6.9	4.2	4.0	19.9	3.2	10.3
15-18	2.6	3.9	5.6	5.2	2.5	26.1	2.1	10.3
18-21	2.7	4.1	5.9	10.3	2.3	20.0	3.1	10.3
21-24	4.2	4.3	8.2	12.3	3.3	21.1	4.3	10.3
24-27	3.9	4.7	10.4	15.8	5.6	14.9	4.1	10.3
27-30	3.3	4.7	11.1	15.8	8.5	15.3	6.8	10.3
30-33	3.5	6.8	8.2	13.0	11.5	15.3	6.9	10.3
33-36	2.1	4.0	6.6	13.6	13.5	14.6	6.3	10.3
36-39	3.1	7.4	. 7.2	10.9	17.2	11.7	7.6	10.3
39-42	3.6	5.5	9.5	12.6	13.6	12.4	7.0	ns
42-45	4.2	6.6	10.9	12.2	14.7	9.6	8.0	10.3
45-48	4.5	7.0	9.5	10.9	11.4	10.3	7.5	ns
48-51	4.4	7.1	10.2	10.4	13.4	9.9	7.3	ns
51-54	4.8	7.9	8.9	10.5	11.8	8.5	7.6	ns
54=57	4.6	6.8	9.9	10.0	12.1	9.3	7.8	ns
57-60	4.6	6.7	8.7	12.6	9.7	11.8	8.3	ns
60-66	5.0	7.5	8.5	8.7	6.3	4.6	6.9	ns
66-72	4.1	8.1	7.2	7.3	6.3	5.8	6.3	ns
72-78	4.3	6.8	6.5	8.4	6.5	6.9	6.0	ns
78-84	4.5	7.7	5.9	7.4	6.2	6.0	6.2	ns
84-90	4.7	5.7	6.7	7.7	6.1	5.6	5.3	ns
90-96	4.6	6.0	6.2	6.4	6.2	5.2	5.3	ns
96-102	4.5	5.4	6.4	5.5	6.0	4.2	5.1	ns
.02-108	4.4	4.6	4.8	5.5	5.4	3.4	5.6	ns
.08-114	2.7	4.1	5.7	4.6	3.7	4.2	5.3	ns
14-120	2.9	2.1	5.8	5.3	5.1	2.8	4.8	ns
)epth withi	n treatm	ent						
	ns	ns	7.2	7.2	7.2	7.2	ns	

Table 28. Distribution of nitrate-N with depth in the profile nine years after annual applications of manure or commercial fertilizers for corn harvested as silage.<sup>+</sup>

D				Treatme	nts			Treatment
Uepth (in)		Manure			160 1b N	160 16 N	NPK	within depth LSD (.05)
	10 <b>T</b>	20 <b>T</b>	30T	160 16 N	+ ₽ & K	+ lime	+ lime	
					ppm			
0-9	32.7	32.1	49.6	47.5	25.2	17.9	28.8	ns
9-12	30.4	8.1	10.3	5.5	2.3	3.3	13.4	11.0
12-15	26.9	6.6	9.4	3.4	3.9	5.7	14.8	11.0
15-18	3.4	5.5	9.3	2.6	4.9	3.4	12.1	ns
18-21	3.0	4.6	8.5	1.6	4.4	4.5	20.2	ns
21-24	2.7	4.9	8.4	2.8	5.4	5.1	10.8	ns
24-27	3.2	5.0	6.6	2.6	5.4	5.2	14.3	ns
27-30	2.6	5.9	8.1	3.6	6.6	7.5	16.1	ns
30-33	2.8	8.1	7.9	3.1	7.8	9.9	13.5	ns
33-36	3.8	6.2	7.2	4.7	10.8	9.7	11.3	ns
36-39	2.9	6.6	7.2	5.0	11.5	7.9	11.6	ns
39-42	3.2	7.8	9.1	6.4	12.7	9.8	12.0	ns
42-45	3.8	7.8	10.1	6.3	10.9	10.1	11.6	ns
45-48	3.3	7.2	9.2	7.6	13.6	9.7	11.1	ns
48-51	3.9	8.4	9.3	8.1	11.7	9.8	10.8	ns
51-54	4.0	6.9	10.0	8.1	11.1	10.5	11.4	ns
54-57	3.5	8.0	10.4	7.4	11.5	11.4	11.2	ns
57-60	4.2	7.5	10.3	7.6	10.3	10.3	11.4	ns
60-66	3.0	7.7	10.8	6.6	9.3	9.8	10.3	ns
66-72	3.5	7.3	9.6	8.5	8.8	9.4	11.1	ns
72-78	2.9	7.6	10.7	9.0	8.9	8.0	11.7	ns
78-84	2.6	5.8	8.5	6.5	7.3	7.1	9.6	ns
84-90	1.9	5.6	8.9	6,2	7.4	7.1	9.0	ns
90-96	2.7	5.7	9.5	4.7	5.4	8.6	7.3	ns
96-102	1.7	4.7	8.8	3.8	5.8	6.7	7.5	ns
102-180	2.4	4.4	4.7	3.4	7.4	5.6	7.0	ns
108-114	2.6	4.3	6.0	4.5	4.9	6.6	7.0	ns
114-120	2.7	3.4	5.8	2.9	4.1	8.8	7.1	ns
Depth with: LSD (.05	in treatm )	ent						
	7.8	ns	ns	ns	7.8	7.8	7.8	

Table 29. Distribution of total mineral-N ( $NH_4-N+NO_3-N$ ) with depth in the profile nine years after annual applications of manure or commercial fertilizers for corn harvested as grain.<sup>†</sup>

				Treatme	nts			
Depth (in)		Manure		160 15 N	160 15 N +	160 1b N	NPK +	Treatment within depth LSD ( 05)
	10 <b>T</b>	20T	30T		P & K	lime	lime	
				****	ppm			
0-9	21.2	18.8	33.0	13.8	18.4	14.5	22.5	ns
9-12	5.5	8.3	17.0	5.8	14.2	11.9	6.5	11.0
12-15	5.4	7.3	12.4	7.6	6.8	23.3	6.7	11.0
15-18	5.1	8.0	10.8	7.5	5.3	27.8	4.4	11.0
18-21	6.6	5.5	10.2	13.4	4.1	22.1	5.4	11.0
21-24	7.3	7.6	12.1	14.8	5.3	23.5	6.6	11.0
24-27	7.3	8.5	13.9	18.3	7.1	16.4	6.6	11.0
27-30	6.9	7.9	15.4	18.6	9.5	16.2	9.4	ns
30-33	7.7	10.2	13.4	15.8	12.8	17.2	9.5	ns
33-36	4.9	6.5	10.0	16.0	15.0	15.6	8.9	ns
36-39	7.0	11.5	10.4	13.6	19.2	12.2	9.7	ns
39-42	6.4	7.7	12.4	15.4	14.9	13.2	9.4	ns
42-45	7.6	8.8	15.5	14.2	15.9	10.1	9.9	ns
45-48	7.4	10.4	12.0	12.6	12.9	11.2	10.0	ns
48-51	7.7	10.4	16.4	12.1	15.1	11.0	9.4	ns
51-54	7.9	11.1	11.6	12.6	12.9	9.3	9.6	ns
54-57	7.8	9.6	12.9	12.2	13.2	9.8	9.9	ns
57-60	7.6	9.6	11.0	14.8	10.9	12.5	9.6	ns
60-66	7.8	10.3	11.8	11.0	8.0	5.6	8.6	ns
66-72	8.5	12.0	9.7	9.6	7.3	6.5	8.5	ns
72-78	7.4	9.4	7.4	10.4	7.5	7.7	7.7	ns
78-84	7.7	11.0	7.0	9.2	7.1	6.3	7.7	ns
84-90	7.6	8.0	8.9	10.1	6.6	6.0	6.8	ns
90-96	8.7	8.9	7.0	8.5	7.4	5.6	6.7	n <b>s</b>
96-102	6.4	7.3	8.6	7.6	6.9	4.8	6.5	ns
102-108	6.8	7.2	5.9	7.3	7.2	3.9	7.3	ns
108-114	4.6	7.7	8.2	6.3	5.4	4.6	7.1	ns
114-120	4.4	3.0	7.1	7.1	6.4	3.6	6.9	ns
Depth withi LSD (.05)	n treatm	ent						
	ns	7.8	7.8	7.8	7.8	7.8	ns	

Table 30. Distribution of total mineral-N ( $NH_4$ -N+NO<sub>3</sub>-N) with depth in the profile nine years after annual applications of manure or commercial fertilizers for corn harvested as silage. <sup>†</sup>

 $\ensuremath{^{+}\text{Mean}}$  of three replications.

Depth	То	Tons of manure per acre					
(in)	None	100 <b>T</b>	200T	300T	LSD (.05)		
			p	pm	، بې بې نو		
0-9	1570	1991	2370	2746	761		
9-12	260	297	433	341	ns		
12-15	144	230	304	193	ns		
15-18	125	145	186	163	ns		
18-21	126	107	163	123	ns		
21-24	191	162	183	175	ns		
24-27	174	131	177	145	ns		
27-30	139	118	174	140	ns		
30-33	141	108	158	155	ns		
<b>33-</b> 36	155	114	122	140	ns		
36 <b>-</b> 39	153	125	124	166	ns		
39-42	156	86	121	172	ns		
42-45	165	90	134	177	ns		
45-48	160	136	126	157	ns		
48-51	123	76	119	139	ns		
51-54	135	96	126	127	ns		
54-57	119	127	106	140	ns		
57-60	109	109	129	134	ns		
oth within tr LSD (.05)	eatment						
	87	87	87	87			

Table 31.	Distribution	of organic-N with depth	in the	profile	eight
	months after for corn.	a single application of	manure	at high	rates

†Mean of three replications.

Depth	То	ns of manu	re	Treatment	
(in)	None	100 <b>T</b>	200T	300T	within depth LSD (.05)
			p	pm	
0-9	13.8	12.5	11.9	19.4 .	ns
9-12	3.8	3.9	2.7	2.6	ns
12-15	3.4	3.2	2.4	1.9	ns
15-18	3.1	3.0	2.4	1.2	1.5
18-21	3.5	3.1	2.5	2.3	ns
21-24	3.9	3.0	2.3	1.6	1.5
24-27	2.9	2.8	1.6	1.2	1.5
27-30	2.7	2.7	1.9	1.1	1.5
30-33	2.8	2.6	1.5	0.8	1.5
33 <b>-</b> 36	2.5	2.5	1.1	0.7	1.5
36-39	3.3	2.3	1.7	1.0	1.5
39-42	3.3	2.8	1.2	1.0	1.5
42-45	3.4	2.7	1.5	1.1	1.5
45-48	3.4	3.1	1.1	0.8	1.5
48-51	3.0	3.1	1.6	0.6	1.5
51-54	2.6	2.6	1.7	0.4	1.5
54-57	3.1	3.6	1.6	0.7	1.5
57-60	3.1	3.0	1.5	1.1	1.5
Depth within tr LSD (.05)	eatment				
	0.9	0.9	0.9	0.9	

Table 32.	Distribution of ammonium-N with depth in the profile eight
_	months after a single application of manure at high rates for corn. <sup>+</sup>

 $\dagger_{Mean}$  of three replications.

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Depth	Т	ons of mar	Treatment		
(in)	<sup>-</sup> None	100T	20 OT	300T	within depth LSD (.05)
			pi	)m	
0-9	78.4	50.2	193.5	135.5	ns
9-12	25.3	26.8	63.4	32.1	18.6
12-15	19.6	28.7	53.7	31.8	18.6
15-18	8.2	23.6	41.7	25.4	18.6
18-21	4.1	13.6	37.7	19.6	18.6
21-24	4.6	10.5	30.3	21.3	18.6
24-27	4.9	10.3	24.3	12.7	18.6
27-30	5.2	10.4	16.8	10.6	ns
30-33	6.7	10.0	15.2	9.1	ns
33-36	7.2	10.8	11.7	8.4	ns
36-39	8.5	8.9	11.9	9.5	ns
39-42	9.4	10.9	10.9	9.5	ns
42-45	14.9	11.3	10.5	9.8	ns
45-48	12.0	11.9	9.3	9.4	ns
48-51	10.9	9.4	8.0	8.0	ns
51-54	11.4	8.5	7.1	8.4	ns
54-57	11.9	7.9	6.8	7.6	ns
57-60	11.0	8.8	7.8	7.9	ns
Depth within th LSD (.05)	reatment				
	14.2	14.2	14.2	14.2	

Table 33.	Distribution	of nitra	te-N with dep	oth in the	profile	eight
	months after for corn. <sup>†</sup>	a single	application	of manure	at high	rates

Depth	То	ns of manu	Treatment		
(in)	None	100T	200T	300T	within depth LSD (.05)
<u></u>			pi	om	
0-9	92 <b>.2</b>	63.0	205.4	154.9	ns
9-12	29.0	30.7	66.2	34.7	17.4
12-15	23.1	32.0	56.1	33.6	17.4
15-18	11.4	26.6	44.1	26.6	17.4
18-21	7.6	16.7	40.2	21.9	17.4
21-24	8.6	13.6	32.7	22.9	17.4
24-27	7.8	13.1	25.9	14.0	17.4
27-30	7.9	13.2	18.7	11.8	ns
30-33	9.5	12.6	16.7	9.9	ns
33-36	9.7	13.3	12.8	9.1	ns
36 <b>-</b> 39	11.8	11.2	13.6	10.5	ns
39-42	12.7	13.7	12.2	10.5	ns
42-45	18.4	14.0	12.0	11.0	ns
45-48	15.5	15.0	10.4	10.2	ns
48-51	14.0	12.5	9.6	8.6	ns
51-54	14.0	11.1	8.8	8.9	ns
54-57	15.0	11.6	8.3	8.3	ns
57-60	14.2	11.8	9.3	9.1	ns
Depth within to LSD (.05)	reatment				
	14.2	14.2	14.2	14.2	

Table 34. Distribution of total mineral-N ( $NH_4-N + NO_3-N$ ) with depth in the profile eight months after a single application of manure at high rates for corn.<sup>†</sup>

Elements		Treatment within depth		
	No Manure	300T (1 year)	30T (9 years)	LSD (.05)
		***	ppm	
Plow layer (0-9 in.)				
Ν	46	48	40	ns
Ca	481	893	1485	ns
Mg	261	459	399	ns
Fe	2152	2313	1552	ns
Al	1311	1560	1150	ns
Subsoil layer (18-21 in.)				
Ν	26	16	11	ns
Ca	212	248	1386	ns
Mg	559	382	580	ns
Fe	5109	2849	2350	865.4
Al	2201	1812	1574	ns
Depth within t LSD (.05)	reatment			
Ν	17.8	17.8	17.8	
Ca	ns	ns	ns	
Mg	ns	ns	ns	
Fe	892.4	ns	ns	
Al	ns	ns	ns	

Table	35.	Recoveries of N and metals in extract b ((	).1N
		H <sub>2</sub> SO <sub>4</sub> ), Sequence I. <sup>T</sup>	

Elements		Treatment within depth		
	No Manure	300T (1 year)	30T (9 years)	LSD (.05)
			ppm	
Plow layer (0-9 in.)				
N	282	387	412	76.3
Ca	28	62	5	ns
Mg	6	12	6	ns
Fe	47	41	122	37.3
Al	71	65	56	ns
Subsoil layer (18-21 in.)				
N	36	28	44	ns
Ca	5	19	5	ns
Mg	7	12	5	ns
Fe	27	17	26	ns
Al	44	69	61	ns
Depth within tr LSD (.05)	eatment			
Ν	63.3	63.3	63.3	
Ca	ns	ns	ns	
Mg	ns	ns	ns	
Fe	ns	ns	31	
Al	ns	ns	ns	

Table 36.	Recoveries of N and metals in extract c (	0.1M
	$Na_4P_2O_7 + 0.1N$ NaOH), Sequence I. <sup>+</sup>	_

Elements		Treatments						
Liementos	No Manure	300T (1 year)	30T (9 years)	LSD (.05)				
			ppm					
Plow layer (0-9 in.)								
N	293	429	473	115.8				
Ca	8	78	133	19.4				
Mg	23	35	29	ns				
Fe	287	190	191	ns				
Al	880	1028	655	ns				
Subsoil layer (18-21 in.)								
N	56	60	54	ns				
Ca	5	27	9	19.4				
Mg	32	34	14	ns				
Fe	314	158	128	ns				
Al	1643	1223	775	ns				
Depth within the LSD (.05)	reatment							
N	45.4	45.4	45.4					
Ca	ns	16.3	16.3					
Mg	ns	ns	ns					
Fe	ns	ns	ns					
Al	ns	ns	ns					

Table 37	. I	Recoveries	of	N	and	metals	in	extract	b	(0.1N
	1	NaOH) Seque	ence	e ]	[1.+					-

†Mean of three replications.

		······································						
Elements		Treatments						
	No Manure	300T (1 year)	30T (9 years)	LSD (.05)				
		* * * * * * * * * * * * * *	ppm					
Plow layer (0-9 in.)								
N	4	26	7	ns				
Ca	10	91	98	ns				
Mg	211	281	119	130.1				
Fe	851	643	588	252.2				
Al	561	502	470	ns				
Subsoil layer (18-21 in.)								
N	2	9	16	ns				
Ca	10	96	12	ns				
Mg	434	642	194	130.1				
Fe	1524	1473	634	252.2				
Al	1048	770	535	ns				
Depth within t LSD (.05)	creatment							
Ν	ns	ns	ns					
Ca	ns	ns	ns					
Mg	97.7	97.7	ns					
Fe	200.2	200.2	ns					
Al	342.2	ns	ns					

Table 38.	Recoveries of N and metals in extract f (	(0.1N
	H <sub>2</sub> SO <sub>4</sub> ), Sequence I. <sup>+</sup>	_

Elements		Treatment within depth		
	No Manure	300T (1 year)	30T (9 years)	LSD (.05)
			ppm	
Plow layer (0-9 in.)				
Ν	31	34	17	ns
Ca	441	802	1291	ns
Mg	286	343	385	ns
Fe	2980	2586	1779	ns
Al	749	781	748	ns
Subsoil layer (18-21 in.)				
Ν	18	14	3	ns
Ca	214	242	1180	ns
Mg	549	466	657	ns
Fe	4397	3633	2792	ns
Al	1635	1317	799	ns
Depth within t LSD (.05)	reatment			
N	ns	14.6	ns	
Ca	ns	ns	ns	
Mg	ns	ns	ns	
Fe	1038.5	ns	ns	
Al	713	ns	ns	

Table	39.	Recoveries	of	Ν	and	metals	in	extract	е	(0.1N
		H <sub>2</sub> SO <sub>4</sub> ), Sec	quer	nce	e II.	•				

Elements		Treatments	3	Treatment within depth
	No Manure	300T (1 year)	30T (9 years)	LSD (.05)
			ppm	
Plow layer (0-9 in.)				
Ν	55	91	152	27.0
Ca	5	35	5	1.3
Mg	54	52	12	ns
Fe	493	222	124	ns
Al	441	533	660	ns
Subsoil layer (18-21 in.)				
N	13	15	34	ns
Ca	5	26	5	1.3
Mg	125	146	12	ns
Fe	1359	824	69	ns
Al	522	975	269	391.2
Depth within tr LSD (.05)	reatment			
N	28.8	28.8	28.8	
Ca	ns	1.2	ns	
Mg	ns	ns	ns	
Fe	854.9	ns	ns	
Al	ns	362.9	362.9	

Table 40.	Recoveries	of N	and	metals	in	extract	g	(0.1N
	NaOH), Sequ	lence	I. <sup>T</sup>				-	

Elements		Treatment	5	Treatment within depth
	No Manure	300T (1 year)	30T (9 years)	LSD (.05)
			ppm	
Plow layer (0-9 in.)				
N	64	103	149	24.3
Ca	5	27	5	ns
Mg	30	41	12	ns
Fe	300	472	116	ns
Al	390	583	153	ns
Subsoil layer (18-21 in.)				
N	12	11	15	ns
Ca	5	28	5	ns
Mg	122	75	8	35.6
Fe	990	315	58	530.0
Al	519	720	506	ns
Depth within t LSD (.05)	reatment			
N	20.6	20.6	20.6	
Ca	ns	ns	ns	
Mg	30.2	30.2	ns	
Fe	500.6	ns	ns	
Al	ns	ns	ns	

Table 41.	Recoveries of N	and metals	in	extract	f	(0.1N
	NaOH), Sequence	II.†				_

 $^{\dagger}Mean$  of three replications.

Elements		Treatments	3	Treatment within depth
	No Manure	300T (1 year)	30T (9 years)	LSD (.05)
			ppm	
Plow layer (0-9 in.)				
N	161	196	190	ns
Ca	10	96	16	22.9
Mg	10	23	10	ns
Fe	67	36	100	13.8
Al	58	55	72	ns
Subsoil layer (18-21 in.)				
N	41	26	39	ns
Ca	10	46	10	22.9
Mg	10	17	10	ns
Fe	24	15	37	13.8
Al	82	37	33	42.1
Depth within tr LSD (.05)	eatment			
Ν	33.4	33.4	33.4	
Ca	ns	14.2	ns	
Mg	ns	ns	ns	
Fe	12.5	12.5	12.5	
Al	22.3	ns	22.3	

Table 42. Recoveries of N and metals in extract d (Fulvic Acid), Sequence  $I.^{\dagger}$ 

\* Mean of three replications.

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Elements		Treatments	3	Treatment within depth
	No Manure	300T (1 year)	30T (9 years)	LSD (.05)
			ppm	
Plow layer (0-9 in.)				
N	128	198	171	48.9
Ca	10	71	10	11.3
Mg	10	22	10	4.3
Fe	43	21	37	12.4
Al	29	41	74	28.3
Subsoil layer (18-21 in.)				
N	18	11	30	ns
Ca	10	50	10	11.3
Mg	10	10	10	ns
Fe	14	16	26	12.4
Al	20	64	20	28.3
Depth within t LSD (.05)	reatment			
Ν	39.4	39.4	39.4	
Ca	ns	11.3	ns	
Mg	ns	3.8	ns	
Fe	5.5	ns	5.5	
Al	ns	ns	23.3	

Table 43.	Recoveries of N	and	metals	in	extract	е	(Humic
	Acid), Sequence	· I.T					

Clements		Treatment	3	Treatment within depth
	No Manure	300T (1 year)	30T (9 years)	LSD (.05)
			ppm	
low layer (0-9 in.)				
N	129.8	147	180	30.8
Ca	53	132	118	26.5
Mg	10	14	10	2.2
Fe	84	63	60	11.3
Al	538	571	428	ns
ubsoil layer (18-21 in.)				
N	38	31	39	ns
Ca	10	78	13	26.5
Mg	10	10	10	ns
Fe	35	46	36	ns
Al	<b>7</b> 95	739	536	ns
epth within th LSD (.05)	reatment			
N	26.6	26.6	26.6	
Ca	19.4	19.4	19.4	
Mg	ns	1.9	ns	
Fe	7.0	7.0	7.0	
Al	ns	ns	ns	

Table 44. Recoveries of N and metals in extract c (Fulvic Acid), Sequence II.<sup>†</sup>

Elements		Treatments	5	Treatment within depth
	No Manure	300T (1 year)	30T (9 years)	LSD (.05)
			ppm	
Plow layer (0-9 in.)				
N	194	246	279	ns
Ca	10	71	10	ns
Mg	17	24	10	ns
Fe	179	185	124	ns
Al	243	240	153	ns
Subsoil layer (18-21 in.)				
Ν	31	34	39	ns
Ca	10	55	10	ns
Mg	17	44	12	ns
Fe	215	177	103	ns
Al	552	271	72	ns
Depth within t LSD (.05)	reatment			
Ν	69.8	69.8	69.8	
Ca	ns	ns	ns	
Mg	ns	ns	ns	
Fe	ns	ns	ns	
Al	ns	ns	ns	

Table 45. Recoveries of N and metals in extract d (Humic Acid), Sequence II.<sup>†</sup>

lement	S	sequence I		Ň	equence II		Treatment	s within	Sequence
	No manure	300T (1 year)	30T (9 years)	No manure	300T (1 year) (	30T 9 years)	sequen depth LS Seq. I	ce and ED (.05) Seq. II	within treatment and depth LSD (.05)
'low laye	r (0-9 1n	)							
N	387	552	612	388	556	639	198.2	198.2	ព
Ca	524	1080	1511	454	906	1429	ns	ns	ពន
Mg	532	850	196	339	510	426	n8	<b>n</b> 8	ns
Ре	3543	3219	2198	3567	3249	2086	ns	su	su
IN	2384	2660	2171	2020	2392	1556	ns	suu	ព
ubsoll le	ayer (18-	.21 1n.)							
N	76	67	105	87	85	73	n8	ns	มร
C &	232	389	1408	224	297	1194	ns	SU	su
Mg	1125	1183	161	203	576	619	80	SU	445.5
Ре	8056	5163	3079	5702	4107	2978	1520.0	1520.0	1470.5
IN	3814	3627	5440	3797	3260	2080	1371.5	1371.5	ពន
epth with	h <b>in trea</b> t	ment and a	iequence LSI	(30.) (					
Z	150.1	150.1	150.1	150.1	150.1	150.1			
Ca	su	ns	su	ns	ns	ns			
Mg	490.8	su	8u	su	ns	ns			
Рe	1803.1	1803.1	ns	1803.1	ns	ns			
Al	1331.4	ns	su	1331.4	<b>n</b> 8	នព			

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