CHANGES IN SOIL NITROGEN AS AFFECTED BY CHEMICAL

TREATMENT AND RESPONSE OF CROPS TO NITROGEN FERTILIZATION

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Introduction

Most of the nitrogen in the soil is present in organic form. It is insoluble in water and cannot be assimilated by higher plants until it is changed to simple compounds. This process is carried out in the soil by the action of microorganisms in which protein material is hydrolyzed to amino acids and the nitrogen changed to ammonia or nitrate as a result of biological activity. If some proteins are more easily hydrolyzed than others differences in availability should be secured by methods which would measure any one or all of the products of decomposition under different conditions. This is very important from the point of view of the availability of nitrogen for plant growth. As the nitrogen content of a productive soil gradually declines, a point will eventually be reached where soil fertility will become the limiting factor in plant development rather than climatic conditions. This value will vary depending upon the nitrogen requirement of the crop, the season of year it matures, the profile characteristics as whether it is a droughty or shallow soil and the availability of the nitrogen that is present.

Various chemical methods have been developed to determine the availability of the different inorganic elements in the soil such as phosphorus, potash and calcium since total analyses have very little value in determining nutrient availability for plant growth. Total nitrogen and organic matter in soil may or may not be an index of nitrogen availability. This problem is more important in a region where soil still contains a high percentage of the nitrogen which was present under virgin conditions than in areas where the nitrogen content is low and practically all crops will respond to nitrogen fertilization.

Review of Literature

Biological methods have been used more extensively than chemical analysis to study the availability of nitrogen in soil. Nitrate accumulation is affected by a number of different factors and requires a considerable period of time before an analysis can be completed. While nitrogen is considered to be one of the most useful constituents of soil organic matter, or humus, it is the available nitrogen which is of special value. Numerous investigators have shown that the amount of ammonia in soils is quite small and there is a tendency of nitrates to accumulate in the soil under certain conditions. Even under conditions favorable for high accumulation, the nitrate nitrogen accounts for a very small fraction of the total nitrogen.

Waksman (19) and Lipman (9, 10) maintained that the nitrifying power of the soil indicates that nitrification studies can yield information for the differentiation of soil fertility although in some instances they did not always find a correlation with field experiments.

Waksman (21) presented results to show that determination of the power of a soil to decompose cellulose can yield information not only on the microbiological conditions of the soil but also on its available nitrogen. For each milligram of nitrogen that is available or can become available in the soil, in the particular period of time, there will be decomposed approximately 40 to 50 milligrams of cellulose in the given amount of soil.

Schreiner and Skinner (15) showed that a nitrate determination in the soil does not give a complete valuation of its readily assimilable nitrogen, for nitrification may be low and yet appreciable quantities of such beneficial nitrogenous compounds as histidine, hypoxanthine, creatine, etc., be present. They have shown that these compounds can be used by the plant without further decomposition. Although nitrate formation cannot occur without protein decomposition much stress on the nitrate content of soils is unwarranted in the light of their results.

Jodidi (8) found all other things being equal, the rate of transformation of the amino acids and acid amide nitrogen into ammonia is greatly influenced by their chemical constitution so that amino acids and acid amides of similar structure yield about the same portion of ammonia.

Schreiner and Shorey (16) in their studies of the chemical nature of soil organic matter showed that in the decomposition of protein there were two groups of nitrogenous compounds. The monamino and diamino acids contain a carboxyl group, and these compounds are mainly acid in character. In addition to protein which breaks up into amino acids there are nucleoprotieds which form pyrimidine derivatives and purine bases as products of decomposition. These compounds contain no carboxyl group, are of ring structure and the nitrogen may be either combined with carbon or with hydrogen as NH or NH_2.

Jodidi (7, 8) in his studies on the chemical nature of the organic nitrogen in the soil found that hydrochloric acid digestion would change 75 to 80 percent of the total nitrogen to a soluble form. The different forms of nitrogen in the soluble fraction were as follows: 25 to 33 percent nitrogen as acid amides, 11 to 13 percent as diamino acids, and 22 to 50 percent was present as monamino acids.

The Jones alkaline permanganate method and the Street neutral permanganate method were proposed to differentiate between organic nitrogenous materials of good quality and high availability and those of poor quality and low availability in commercial fertilizers (1). These methods have been accepted by the Association of Official Agricultural Chemists and have been used for many years to determine the relative value of different forms of organic nitrogenous fertilizers. Although experience in the past has shown that it is somewhat difficult to obtain concordant results from several different laboratories by either method Moore and White (11) and others (13, 18) have pointed out some of the factors which may have caused these discrepancies and made recommendations which they thought would give more uniform results. Haskins (3) set up some vegetative pot experiments to determine nitrogen availability of low grade fertilizing materials valued chiefly for their organic nitrogen as determined by the alkaline and neutral permanganate methods. He found a fairly close agreement between crop response and the fertilizers tested except Peruvian guano which seemed to have a low activity as determined by treatment with alkaline permanganate.

Fraps (2) in his studies on the organic constituents of the soil studied the nitrogen insoluble in neutral permanganate but found no relationship between the soluble and insoluble nitrogen and the results of pot experiments with nitrogen on soils.

Waksman reported that Istcherekoff (4, 5) has done considerable work on the determination of humus by treatment with potassium permanganate but information concerning the method of analysis or results obtained were not given.

In a region where many cultivated soils still contain enough nitrogen to produce maximum yields when climatic conditions are favorable general recommendations for soil improvement by the use of legume crops cannot be made for all soils. It is the purpose of this study to investigate the possibility of using different chemical methods which will change the nitrogen in soil organic matter to a soluble form and correlate these results with response of various crops to nitrogen fertilization.

Methods of Analysis

1. Acid hydrolysis--Twenty grams of 100 mesh soil was placed in a 500 ml. Erlenmeyer flask and 200 ml. of sulfuric acid of various strengths was added. A reflux condenser was connected to the flasks and the soil suspension boiled for various periods of time. The hot soil suspension was filtered on a Buchner funnel and the filtrate evaporated to about 50 ml. This solution was poured into a 500 ml. Kjeldahl flask and several glass beads were added to prevent bumping during the sulfuric acid digestion, and nitrogen was determined by the regular Kjeldahl method.

2. Alkaline permanganate method--The alkaline permanganate solution is prepared by dissolving 25 grams of potassium permanganate in 400 ml. of distilled water which should be added to a cold solution containing 80 grams of sodium hydroxide dissolved in 200 ml. of distilled water. This mixture should be diluted to one liter. If hot sodium hydroxide is added to a potassium permanganate solution some decomposition of the permanganate will occur.

Ten grams of finely ground soil is placed in a 500 ml. Kjeldahl flask (in soils containing 3000 pounds or more of total nitrogen use a five gram sample) and 200 ml. of alkaline permanganate solution added. Connect the flask with an upright condenser to the lower end of which has been attached an Erlenmeyer flask containing 10 ml. of 1/10 normal acid. Digest slowly until danger from frothing has passed. Then gradually raise the temperature and distil over 100 ml. of distillate. If the soils are sandy bumping will probably occur and they will have to be distilled rather carefully. If they are low in organic matter a small amount of zinc may be added to prevent bumping without affecting the final results. Titrate the excess acid with standard alkali using a mixed indicator containing methyl red and methylene blue or any other indicator which has a color change between pH 4 and 6.

3. Neutral permanganate method--Ten grams of soil is placed in a 500 ml. Kjeldahl flask. Five grams of potassium permanganate, 1 gram of sodium carbonate and 200 ml. of water are added. The flask is connected to an upright condenser and 100 ml. of distillate collected in a 500 ml. Erlenmeyer flask containing 10 ml. of 1/10 normal acid. Titrate excess acid with standard alkali using the same indicator as recommended in the preceding paragraph.

4. Method for nitrates--The residue after distillation with permanganate was filtered and washed to remove the nitrates. The filtrate was collected in a 500 ml. flask and a saturated solution of sodium sulfite was added until all of the potassium permanganate was reduced to MnO_2. The manganese dioxide was removed by filtering and was washed free of nitrates with distilled water. The remaining clear solution was neutralized with 20% sulfuric acid using phenolphthalin as an indicator and transferred to a 500 ml. Kjeldahl flask. Five ml. of 50% sodium hydroxide, three grams of Devarda's alloy and a small piece of paraffin were added. The flask was immediately connected to an upright condenser and the nitrogen was collected as ammonia in 10 ml. of 1/10 normal sulfuric acid. The excess acid was titrated with standard alkali and the nitrogen calculated as nitrate nitrogen.

5. Total nitrogen was determined by the Kjeldahl method using selenium as a catalyist.

6. Organic matter was determined by the method as recommended by Schollenberger (14).

Experimental

As no chemical methods have been recommended to indicate whether any relationship existed between nitrogen availability, several series of experiments have been set up in an attempt to find a method that might give some correlation with crop response as related to chemical treatment.

Nitrogen Extraction by Acid Hydrolysis

In the first series of experiments a study was made to determine the rate at which nitrogen compounds in the soil were hydrolized or made soluble by treatment with various strengths of sulfuric acid. For this study three soils were selected. Two samples were taken from a fertility study of continuous wheat on Kirkland very fine sandy loam at Stillwater, Oklahoma. One plot has received applications of manure at varying intervals and has an organic matter content of 2.76 percent and total nitrogen content of 2212 pounds per acre. The other plot has not been treated and has an organic matter content of 2.10 and 1848 pounds per acre of nitrogen. The other sample of soil was a Lake Charles clay obtained from Angleton, Texas. It contained 3.43 percent of organic matter, and 3024 pounds of nitrogen to the acre. The data obtained by boiling 20 grams of finely ground soil in 200 ml. of various strengths of sulfuric acid in a 500 ml. Erlenmeyer flask connected with a reflux condenser are given in the following table.

Table 1 The Amount of Nitrogen Made Soluble by Boiling with Various Strengths of Sulfuric Acid for Two Hours.

Strength of Acid Used and Pounds of Nitrogen per Acre and Percent of Total Nitrogen Obtained

	.02N		.1N		.2N		.5N		1N	
Soil Type	%T.N	Lbs/A N	%T.N	Lbs/A N	%T.N	Lbs/A N	%T.N	Lbs/A N	%T.N	Lbs/A N
Lake Charles Clay	5.9	180	12.9	390	15.5	470	22.8	690	30.4	920
Kirkland V.F.S.L. Untreated	4.3	80	14.0	260	17.3	320	22.7	420	28.4	525
Kirkland V.F.S.L. Manured	5.4	180	14.9	330	16.5	387	25.3	560	32.5	720

These data show that there were considerable variations in the amount of nitrogen made soluble in the different soils, but are closely related to the quantity of total nitrogen in the soils. A higher percentage of the total nitrogen was hydrolyzed when the manured soil was treated with strong solutions of sulfuric acid as compared with similar treatments of soil from the untreated plot. The greatest differences were obtained by treatment with .5 normal and 1 normal acid, in which there was approximately 2.6 and 4.1 percent more nitrogen hydrolized. Although more nitrogen was obtained by acid treatment of the Lake Charles clay, the percentages of the total nitrogen extracted were similar to that obtained from the unmanured Kirkland very fine sandy loam. As the strength of the acid was increased the amount of nitrogen made soluble was also increased and there was a tendency for the curve of the amount extracted to fall in a straight line as is indicated in Figure 1. This would indicate that either the character of the organic nitrogen in these three soils was probably similar in nature or that the different protein materials which may be present appear to hydrolyze at the same rate.

Further studies were made on the manured and untreated plot using .1 normal and 1 normal sulfuric acid extractions with a ratio of soil to extracting solution of 1-10 and 1-20. The periods of digestion were 1,2, and 4 hours. The results of these studies are give in Tables 2 and 3.

Table 2 Nitrogen Made Soluble by Boiling Soil Obtained from a Manured and Untreated Plot at Stillwater, Oklahoma, with 1 Normal Sulfuric Acid for 1, 2, and 4 Hours.

Treatment and Soil Acid Ratio

Time in Hours	Manured				Untreated			
	1 to 10 Ratio		1 to 20 Ratio		1 to 10 Ratio		1 to 20 Ratio	
	Percent Total N	Lbs. N. per Acre	Percent Total N	Lbs. N. per Acre	Percent Total N	Lbs. N. per Acre	Percent Total N	Lbs. N. per Acre
1	27.6	610	30.7	680	25.4	470	30.8	570
2	32.9	728	43.0	952	28.5	526	42.4	784
4	39.9	882	50.6	1120	36.4	672	48.5	900

Figure 1.
Nitrogen Extracted from Soil by Boiling with Sulfuric Acid for Two Hours

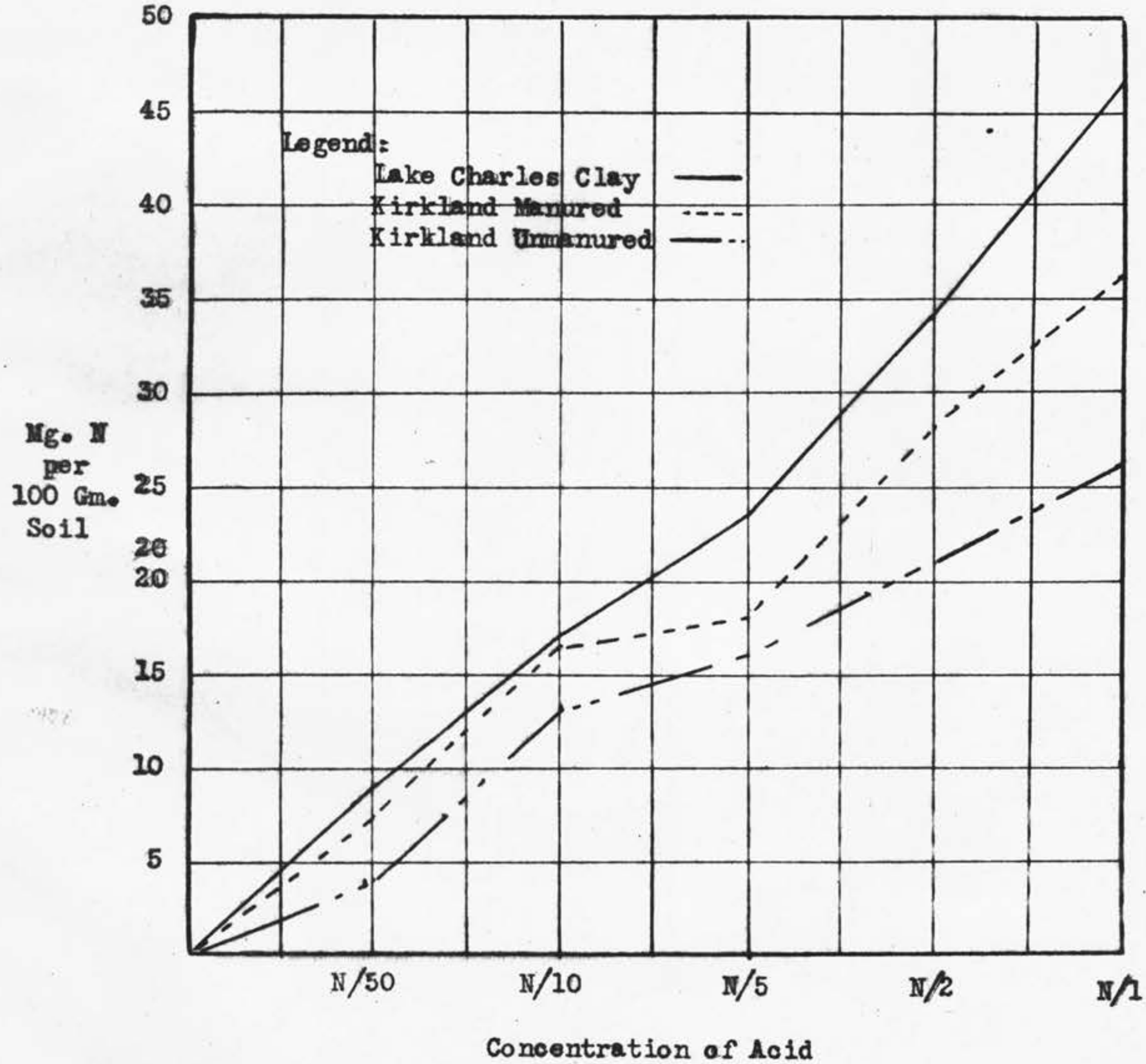


Table 3 Nitrogen Made Soluble by Boiling Soil Obtained from a Manured and Untreated Plot at Stillwater, Oklahoma, with .1 Normal Sulfuric Acid for 1, 2, and 4 Hours.

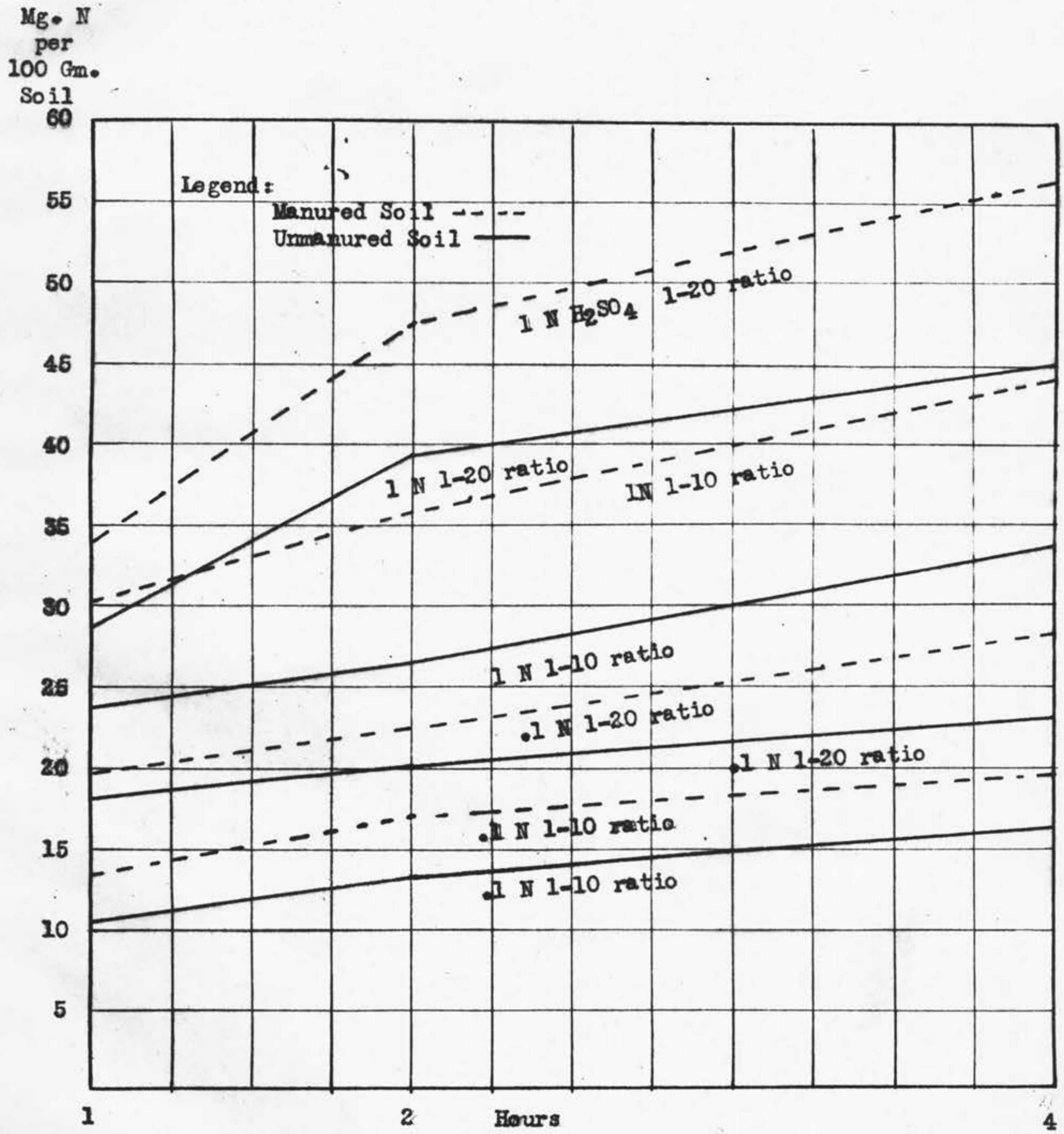
Time in Hours	Manured: 1 to 10 Ratio, Percent Total N	Manured: 1 to 10 Ratio, Lbs. N. per Acre	Manured: 1 to 20 Ratio, Percent Total N	Manured: 1 to 20 Ratio, Lbs. N per Acre	Untreated: 1 to 10 Ratio, Percent Total N	Untreated: 1 to 10 Ratio, Lbs. N. per Acre	Untreated: 1 to 20 Ratio, Percent Total N	Untreated: 1 to 20 Ratio, Lbs. N. per Acre
1	12.2	270	17.6	390	11.4	210	19.5	360
2	15.2	336	20.3	448	14.4	266	22.0	406
4	17.5	386	25.3	560	17.9	330	25.0	462

As the strength of the acid was increased to 1 normal, larger quantities of nitrogen were extracted but the amount in relation to time of extraction tended to run almost parallel where different strengths of acid were used. When the period of extraction was increased to four hours there was a wider variation in the amount of nitrogen extracted from the fertilized and unfertilized soil by 1 normal acid than .1 normal acid. The maximum difference in nitrogen extraction was 320 pounds per acre for the 1 normal acid and 100 pounds per acre for .1 normal when a 1-20 ratio was used. Although more nitrogen was secured from the manured soil the percentage of the nitrogen removed as compared to the total nitrogen in the soil were very similar.

The amount of nitrogen extracted in relation to the time of extraction as shown in Figure 2 conforms to the suggestion previously made that the nitrogen compounds extracted from the soil studied are closely related. As the strength of the acid was increased the percentage of the nitrogen made soluble in relation to the total nitrogen was similar for the two soils. As the time of extraction is prolonged there is a proportional increase of the nitrogen made soluble in each of the two soils.

Figure 2.

Nitrogen in Kirkland Very Fine Sandy Loam Made Soluble by Boiling with .1 and 1. N Sulfuric Acid



Since a short period of extraction is important in routine analysis samples of soil from the manured and unfertilized plot were boiled for ten minutes with .1 normal sulfuric acid. The suspension was immediately filtered and an aliquot taken for analysis. An attempt was made to oxidize the amino and acid amide nitrogen to ammonia with bromine water and potassium iodate. Nessler's solution was added and the solution was made up to a 100 ml. volume and compared with standards. Differences of several pounds of nitrogen to the acre were obtained from the treated and untreated plot, and in the light of the results thus far obtained the method should warrant further study.

Nitrate Accumulation in Soil in Relation to Total Nitrogen, Organic Matter and Ammonia Liberated by Treatment with Neutral and Alkaline Permanganate

In this study samples of soil were obtained from various agricultural experiment stations and fertility plots in Kansas, Oklahoma, Texas, Louisiana, Mississippi, Arkansas, and Ohio. This was done in order to obtain a group of soils that varied widely in their mode of formation and prevailing climatic conditions. The twenty-eight soil samples obtained varied greatly in physical and chemical characteristics. The maximum nitrogen content was 3024 pounds per acre and the minimum 700 pounds per acre. The organic matter content ranged from $3\frac{1}{2}$ percent to .5 percent. The soil reactions of the different samples were basic to strongly acid. The organic matter-nitrogen ratio ranged from 14 to 1 to 29 to 1. The texture varied from fine sandy loam to clay.

Nitrification studies were conducted on all the soils by weighing out 50 gram samples and incubating at 30 degrees Centigrade at an optimum water content for a period of four weeks at which time nitrates were determined by the phenoldisulfonic method. The optimum moisture content was maintained during inculbation by weighing twice a week and adding water lost by evaporation. Organic matter determinations were made by the chromic acid method. Nitrogen liberated as ammonia by the alkaline and neutral permanganate was obtained by the method that has been previously described. Total nitrogen was determined by the regular Kjeldahl method. Data on nitrate nitrogen and the results of chemical analyses are given in Table 4.

No correlation could be made between total nitrogen and the results of the nitrification studies. The acidity, physical condition of the soil, different carbon-nitrogen ratios and perhaps the presence of nitrogenous compounds that do not decompose at the same rate are factors that could be responsible for these variations.

Table 4 A Study on the Nitrate Accumulation in Soil in Relation to Total Nitrogen, Organic Matter and Ammonia Liberated by Treatment with Neutral and Alkaline Permanganate.

Location	Soil Type	Total Nitrogen	Alkaline Permanganate Nitrogen	Percent of Total Nitrogen	Neutral Permanganate Nitrogen	Percent of Total Nitrogen	pH	Nitrification Studies P.P.M.-NO_3	Organic Matter	Nitrogen Organic Matter Ratio
St. Joseph, La.	Sarpy v.f.s.l.	.0658	.0378	57.4	.0091	13.2	7.5	8.2	1.71	25.9
St. Joseph, La.	Sarpy v.f.s.l.	.0658	.0336	51.0	.0077	11.7	7.3	8.4	1.58	24.0
St. Joseph, La.	Sarpy v.f.s.l.	.0644	.0328	50.9	.0084	13.0	7.7	8.2	1.53	23.7
St. Joseph, La.	Sarpy v.f.s.l.	.0812	.0455	56.0	.0126	15.5	6.2	10.1	2.10	25.8
College Station, Tex.	Lufkin f.s.l.	.0336	.0224	67.2	.0077	22.9	5.7	8.8	.90	26.7
Angleton, Tex.	Lake Charles Clay	.1512	.0882	58.3	.0217	14.3	5.4	21.5	3.43	22.6
Manhattan, Kansas		.1344	.0826	61.4	.0182	13.5	5.1	11.2	3.62	26.9
Manhattan, Kansas		.1246	.0791	63.5	.0175	14.0	5.0	10.0	3.38	27.1
Crowley, La.		.0784	.0462	58.9	.0126	16.0	4.4	13.8	1.75	22.3
Crowley, La.		.0852	.0448	52.6	.0133	15.6	4.5	12.2	1.90	22.3
State College, Mississippi	Lufkin Silt loam	.0532	.0252	47.4	.0098	18.4	4.7	5.5	1.50	28.1
State College, Mississippi	Ochlocknee f.s.l.	.0420	.0224	53.3	.0070	16.6	6.1	5.1	1.12	26.6
State College, Mississippi	Sharkey Clay loam	.0868	.0434	50.0	.0112	12.9	6.2	8.6	2.52	29.0
Wooster, Ohio	Wooster Silt loam	.0672	.0357	53.1	.0140	20.8	6.3	9.4	1.56	23.2
Stillwater, Oklahoma	Kirkland v.f.s.l.	.1106	.0644	58.2	.0210	18.2	5.1	9.7	2.76	24.9

Table 4 Continued

Location	Soil Type	Total Nitrogen	Alkaline Permanganate Nitrogen	Percent of Total Nitrogen	Neutral Permanganate Nitrogen	Percent of Total Nitrogen	pH	Nitrification Studies P.P.M.-NO_3	Organic Matter	Nitrogen Organic Matter Ratio
Stillwater Oklahoma	Kirkland v.f.s.l.	.0924	.0518	56.0	.0154	16.6	4.9	10.4	2.10	22.7
Marianna, Arkansas,	Surface	.0476	.0154	33.3	.0056	11.7	6.5	5.7	.68	14.2
Marianna, Arkansas,	Subsoil	.0420	.0147	35.0	.0056	13.3	5.5	2.6	.59	14.0
Marianna, Arkansas,	Surface	.0658	.0210	31.9	.0070	10.6	5.5	6.0	1.24	18.8
Marianna Arkansas,	Subsoil	.0658	.0189	28.7	.0070	10.6	5.0	2.4	.98	14.8
Marianna, Arkansas,	Surface	.0364	.0168	46.1	.0063	17.3	5.4	4.9	.78	21.4
Marianna, Arkansas,	Subsoil	.0392	.0168	42.8	.0056	14.3	5.1	2.1	.78	19.8
Marianna, Arkansas,	Surface	.0392	.0126	30.0	.0042	10.7	5.8	4.3	.66	16.8
Marianna, Arkansas,	Subsoil	.0350	.0126	36.0	.0035	10.0	5.2	1.6	.53	14.8
Marianna, Arkansas,	Surface	.0448	.0231	51.5	.0077	17.1	6.3	7.9	1.01	22.5
Marianna, Arkansas,	Subsoil	.0504	.0252	50.0	.0077	15.2	5.7	6.1	1.12	22.2
Marianna, Arkansas,	Surface	.0420	.0231	55.0	.0070	16.6	5.9	6.7	.88	20.9
Marianna Arkansas,	Subsoil	.0532	.0259	48.6	.0070	13.1	5.5	5.5	.85	15.9

It was found that alkaline permanganate solution liberated on the average approximately 58 percent and the neutral permanganate approximately 14.8 percent of the total soil nitrogen as ammonia. Soil samples obtained from Marianna, Arkansas, yielded low results as compared with other samples. From 30 to 55 percent of the total nitrogen in these soils was liberated by the alkaline permanganate treatment. Excluding the soils from Arkansas there is a very close correlation between the nitrogen extracted in the form of ammonia by boiling a soil with an aqueous solution of alkaline permanganate and the total nitrogen content of the soil. Assuming that the alkaline permanganate extracted 58 percent of the total nitrogen and calculating the total nitrogen from this basis, it was found that the widest variations that occurred in all of the samples were equivalent to an error of only .4 of a ml. in the titration of total nitrogen by the Kjeldahl method. This quantity would be 224 pounds of total nitrogen per acre.

Apparently the chemical character of the nitrogen in the Arkansas soils is somewhat different than in the other samples. The organic matter content of these soils is exceptionally low, varying from .5 percent to slightly above 1 percent. The organic matter-nitrogen ratio ranged as low as 14 to 1 and there is a possibility that the nitrogen compounds in these soils are more resistant to decomposition than in the other soils studied. If this assumption is true there are some organic nitrogenous compounds in soils which are more resistant to decomposition than others and alkaline permanganate does not convert them into ammonia as rapidly as the more easily oxidized forms under similar conditions. The quantity of nitrate formed as a result of chemical treatment was not determined on these samples.

There is a parallel agreement between the amount of nitrogen liberated as ammonia by the alkaline and neutral permanganate solutions as shown in Table 4. Approximately four times as much nitrogen was secured from the alkaline permanganate treatment as compared with neutral permanganate oxidation.

In some samples data obtained from nitrification experiments and the percent of ammonia liberated by treatment with neutral permanganate compare favorably, in others there is no correlation whatsoever. For example, the unfertilized plot at Stillwater had a slightly higher accumulation of nitrates than did the manured plot, while neutral permanganate liberated 2 percent more nitrogen on the manured than on the untreated plot calculated on the basis of total nitrogen in the soil. On the other hand, although sample number 5 had a low quantity of total nitrogen a large percentage was available as determined by neutral permanganate and nitrification studies. The soils from Arkansas that showed such low availability as determined by the permanganate methods were also low in nitrate accumulation.

Comparison of the Ammonia Liberated by Alkaline Permanganate with Total Nitrogen in Cropped and Virgin Samples of Surface and Subsurface Soils

In this study forty soils were selected from official soil samples that represent soils in eleven widely distributed counties in Oklahoma. They contain a maximum content of 6.36 percent of organic matter and a minimum content of .7 percent. Total nitrogen ranged from 5280 pounds per acre to 728 pounds per acre. The relationship between total nitrogen and the quantity of nitrogen obtained as ammonia by alkaline permanganate distillation is given in Table 5. It was found that on the average the alkaline permanganate converted 58 percent of the total nitrogen over to ammonia. No significant differences could be observed in the amount of nitrogen obtained from virgin and cropped soils or between surface and subsurface samples.

In Figure 3 these soils studied were arranged in the order of their total nitrogen content and compared with the quantity of nitrogen obtained as ammonia by alkaline permanganate distillation. As the total nitrogen in the soil declines the amount of nitrogen liberated as ammonia by alkaline permanganate is also decreased giving the plotted curves a wedge-shaped appearance.

When a calculation of total nitrogen was made by multiplying the amount of nitrogen obtained as ammonia in the alkaline permanganate distillation by 1.7 the greatest differences were not more than .01 percent of the total nitrogen as determined by the regular Kjeldahl method. This would be equivalent to .4 of a ml. variation in the titration of regular Kjeldahl nitrogen or 224 pounds of nitrogen per acre.

The possible advantages of using this method are that it requires only one-half as much time as the regular Kjeldahl method since no digestion is necessary before distillation, no irritating fumes are given off, it is a relatively inexpensive method as commercial grades of sodium hydroxide and permanganate may be used. Very little nitrogen was present in the reagents used in this study. Blank determinations varied from .05 to .1 ml. of .1 normal acid. In Kjeldahl determinations commercial sulfuric acid may contain considerable amounts of nitrogen and blank determinations equivalent to .6 ml. of .1 normal acid are not unusual. Also better agreement between duplicate analyses were obtained by the alkaline permanganate method as compared with Kjeldahl determinations which are made with different catalysts. Incomplete oxidation of some nitrogen compounds to ammonia may result although digestion of organic matter appears to be complete.

Fig. 3. Comparison of Total Nitrogen on Surface and Subsurface Soil and Nitrogen Liberated as Ammonia by Distillation with Alkaline Permanganate

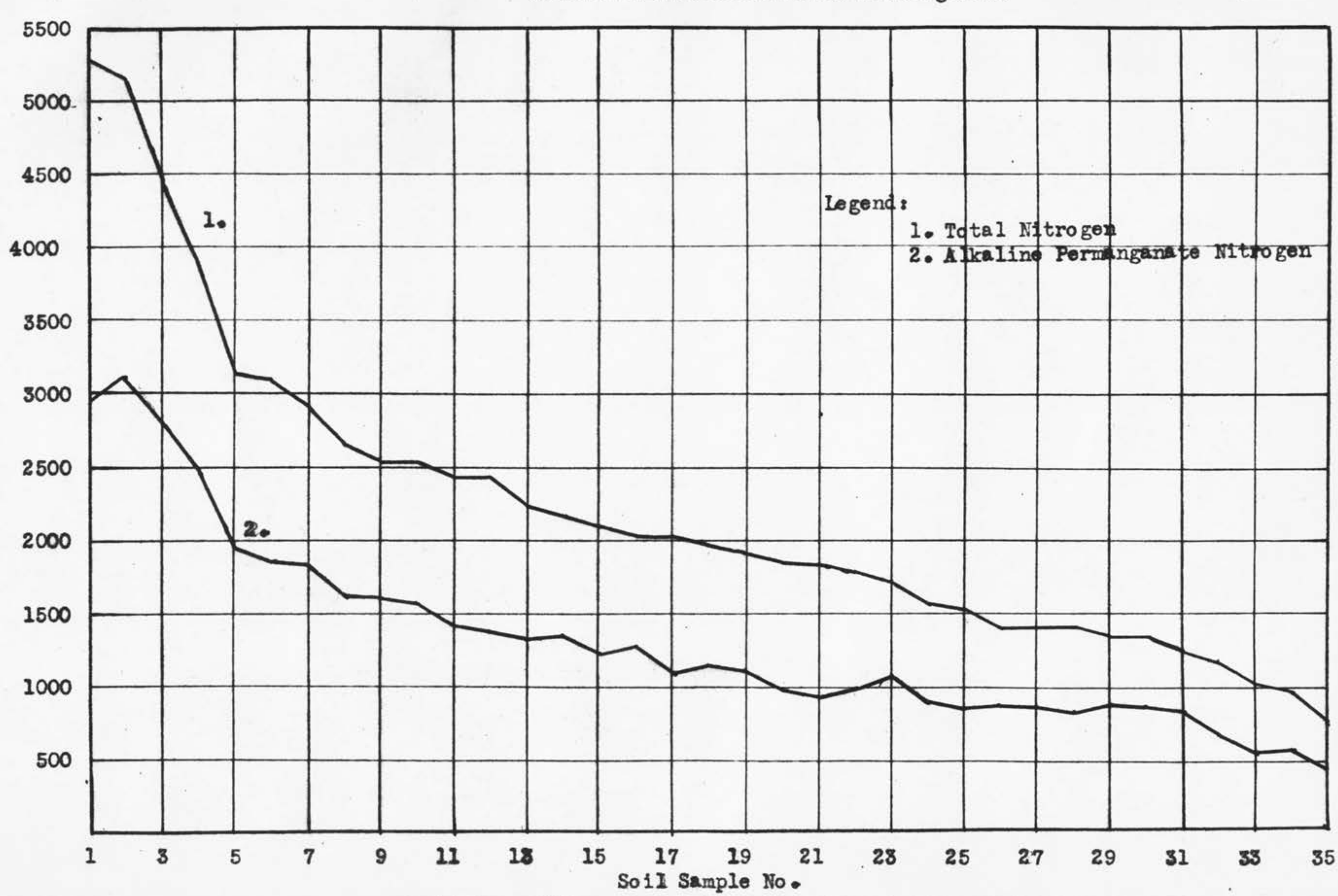


Table 5. Comparison of the nitrogen liberated as ammonia by alkaline permanganate with total nitrogen in cropped and virgin sample in surface and subsurface layers.

County	Official Sample Number	Description of Sample	Percent of Organic Matter	N by Alkaline Permanganate Distillation	Total Nitrogen	Percent of Total Nitrogen
				Lbs./A	Lbs./A	
Pittsburg	4136	Sur. cropped	1.39	1148	1904	60.1
	4137	Sub. cropped	1.26	1260	2016	62.5
	4138	Sur. virgin	2.93	2772	4480	61.9
	4139	Sub. virgin	.72	686	1176	58.3
Woods	4160	Sur. cropped	1.81	1624	2632	61.7
	4161	Sub. cropped	1.33	1218	2072	58.7
	4162	Sur. virgin	3.31	1820	2906	62.6
	4163	Sub. virgin	.98	868	1344	64.2
Alfalfa	4196	Sur. cropped	1.29	980	1848	53.0
	4197	Sub. cropped	1.24	896	1560	57.4
	4198	Sur. virgin	6.36	3170	5152	61.5
	4199	Sub. virgin	1.39	980	1760	55.6
Greer	4220	Sur. cropped	1.96	1330	2240	59.3
	4221	Sub. cropped	1.49	924	1792	55.3
	4222	Sur. virgin	5.30	2968	5280	56.2
	4223	Sub. virgin	2.47	1568	2520	62.2
Muskogee	4168	Sur. cropped	1.44	880	1512	55.5
	4169	Sub. cropped	.82	576	1008	54.1
	4170	Sur. virgin	1.75	1078	1736	62.0
	4171	Sub. virgin	1.18	658	1008	65.2
Jackson	4212	Sur. cropped	2.32	1358	2464	55.1
	4213	Sub. cropped	1.65	1092	1960	55.7
	4214	Sur. virgin	3.81	2464	3920	62.8
	4215	Sub. virgin	1.90	1392	2352	59.1
Mayes	4244	Sur. cropped	2.06	1344	2156	62.3
	4245	Sub. cropped	3.80	1946	3136	62.0
	4246	Sur. virgin	2.68	1848	3080	60.0
	4247	Sub. virgin	.77	560	952	58.8
Custer	4269	Sur. cropped	1.06	812	1400	58.0
	4270	Sub. cropped	1.29	868	1400	62.0
	4271	Sur. virgin	2.47	1664	3136	56.2
	4272	Sub. virgin	1.93	1400	2464	56.8
Mc Lain	4097	Sur. cropped	3.19	1596	2520	63.3
	4096	Sub. cropped	1.45	868	1344	64.5
	4098	Sur. virgin	.75	434	828	59.6
	4099	Sub. virgin	1.33	812	1232	60.4
Grady	4104	Sur. cropped	1.45	880	1400	00.0
	4105	Sub. cropped	1.24	728	1288	56.5
	4106	Sur. virgin	2.61	1358	2128	63.7
	4107	Sub. virgin	1.58	1106	1904	58.0

The Effect of Various Basic Compounds with and without Permanganate on the Liberation of Ammonia from Soils

Treatment of a soil with alkali will cause a hydrolytic cleavage of the complex organic nitrogenous compounds ultimately producing amino acids and acid amides. Decomposition of some of these compounds will occur especially when the solution is heated and ammonia will be liberated. The presence of permanganate hastens the decomposition of the organic nitrogen in the soil. Data in Table 6 show the quantity of ammonia liberated from soils obtained from manured and untreated plots when solutions containing various basic compounds with and without potassium permanganate were used.

Table 6 Ammonia Liberated by Digestion of the Soil with Magnesium Hydroxide, Sodium Carbonate and Sodium Hydroxide with and without Potassium Permanganate

Treatment	pH	Manured Mg.N/10 Gm. Soil	Untreated Mg.N/10 Gm. Soil	Treatment	Manured Mg.N/10 Gm. Soil	Untreated Mg.N/10 Gm. Soil
Saturated $Mg(OH)_2$	9.65	.21	.07	$Mg(OH)_2$ $KMnO_4$	2.74	1.68
$Ca(OH)_2$	10.0	.42	.25	$Ca(OH)_2$ $KMnO_4$	2.73	1.75
1 Gm.200 ml. Na_2CO_3	10.65	.56	.42	Na_2CO_3 $KMnO_4$	2.10	1.75
.1N NaOH	14.0	1.19	1.12	NaOH $KMnO_4$	5.60	4.41

These data show that as the hydroxyl ions increase there is an increase in the quantity of ammonia liberated, but that the amount liberated due to the action of the alkali alone is small as compared with the solutions to which potassium permanganate was added. More nitrogen was obtained as ammonia by all treatments on the fertilized plot than on the untreated plot. Differences in ammonia liberated were not as great when the strength of the alkaline solution was increased.

Waksman (20) assumes that a bacteriological breakdown of amino acids in the soil is caused by enzymatic activity and may occur according to the following reactions.

1. Hydrolytic deaminization.

$$R\text{-}CH.NH_2.COOH + HOH \rightarrow NH_3 \quad R\text{-}CH.OH.COOH \quad R\text{-}CH_2OH \quad CO_2$$

Amino Acid   Hydroxy-fatty Acid   Alcohol

This process is carried out by various aerobic organisms and is of common occurence among bacteria, fungi, and yeasts.

2. Decarboxylation with amine formation.

$$R\text{-}CH.NH_2.COOH \rightarrow R.CH_2.NH_2 + CO_2$$

$$R\text{-}CH_2.NH_2 + HOH \rightarrow R.CH_2.OH + NH_3$$

This process of amino acid decomposition through the amine stage, with the formation of alcohol and ammonia, has been described for yeasts and fungi.

3. Oxidative deaminization.

$$R.CH.NH_2COOH + O_2 \rightarrow R.COOH + CO_2$$

This process is thought to be carried out by aerobic organisms, especially fungi.

As Waksman has shown that by biological activity ammonia formation from amino acids may involve processes of hydrolysis, oxidation or a combination of the two resulting in the splitting of the amino or carboxyl groups or both, it may be that similar reactions take place with the alkaline and neutral permanganate solution.

A Study of the Quantity of Ammonia and Nitrates Produced by Digestion of Soils with Neutral and Alkaline Permanganate

In previous studies all of the total nitrogen in soil could not be recovered when the alkaline and neutral permanganate methods were used and a Kjeldahl determination was made on the residue. Approximately 58 percent of the total nitrogen was distilled over as ammonia in the alkaline solution and 15 percent with the neutral permanganate. When a Kjeldahl determination was made on the remaining solution and residue after distilling over the ammonia it was found that a large portion of the nitrogen was still undetermined. Data in Table 8 show that there was .0275 percent nitrogen changed to some form other than ammonia when soil was treated with the alkaline permanganate and .0635 was not recovered when neutral permanganate was used. Studies on the nitrate content of the digested solutions indicate that some of the organic nitrogen compounds were oxidized directly to nitrates.

The formation of nitrate by the action of neutral and alkaline permanganate solutions on the soil is not clearly understood. When ammonium carbonate was treated with alkaline permanganate to determine the amount of ammonia liberated all of the ammonia was recovered by distillation. When ammonium carbonate was added to a neutral permanganate solution 92.8 percent of the nitrogen was liberated as ammonia. This would indicate that if any ammonium carbonate was formed in the decomposition of the organic matter that it could not account for any of the nitrogen that is being oxidized to nitrate by the alkaline permanganate treatment but that some of the ammonia may be oxidized to nitrates by a neutral permanganate solution.

Studies on the liberation of ammonia from urea treated with alkaline permanganate indicated that a very high percentage of the urea was changed to nitrate and very little ammonia was liberated. The neutral permanganate distillation liberated a smaller amount of ammonia and had a higher percentage of nitrates formed.

If time of contact between the soluble nitrogen material and the permanganate solution is a factor in nitrate accumulation, a difference in time of distillation of ammonia could result in a greater formation of nitrate from longer exposure to an oxidizing medium. Soil suspensions were heated just below the distillation point for forty-five minutes before distilling over any ammonia. This treatment did not vary the results obtained from previous determinations. Heating the permanganate solution to boiling before it was added to the soil sample also did not increase the amount of ammonia liberated.

It has been found that approximately 90 percent of the ammonia distilled over by the alkaline permanganate was distilled over in the first 20 ml., 4 to 7 percent in the second 20 ml., and no ammonia was obtained after distilling over 80 ml. of distillate. The neutral permanganate distilled over only 73 percent during the first 20 ml. of distillate, but the quantity received thereafter was about the same as that obtained by the alkaline permanganate although the percentages were lower. The results of these determinations are given in Table 7 and indicate that the liberation of ammonia by boiling soil with the permanganate solution occurs rapidly.

Table 7 Rate at Which Ammonia Is Liberated from Soils by Distillation with Alkaline and Neutral Permanganate Solutions.

Treatment and Plot	1st. 20 ml.		2nd 20 ml.		3rd 20 ml.		4th 20ml.		5th 20ml.	
Alkaline permanganate	Mg.N	%	Mg.N	%	Mg.N	%	Mg.N	%	Mg.N	%
Manured	5.88	89.6	.47	7.2	.14	2.1	.07	1.0	--	--
Untreated	4.34	91.2	.21	4.4	.14	2.9	.07	1.5	--	--
Neutral permanganate										
Manured	1.54	73.3	.28	13.3	.14	6.7	.07	3.3	.07	3.3
Untreated	1.26	72.0	.28	16.0	.14	8.0	.07	4.0	--	--

It is not known how much of the nitrogen compounds in soil are oxidized directly to the nitrate form, but it is known that some of the ammonia is being oxidized to nitrates in the case of neutral permanganate for 57 percent of the total nitrogen in one sample was oxidized to nitrates as shown in the following table.

Table 8 The Quantity of Ammonia and Nitrate Produced by Alkaline and Neutral Permanganate Digestion of Soils

	Method of Treatment and Nitrogen Obtained			
	Alkaline Permanganate		Neutral Permanganate	
	Lbs/A	% Total N	Lbs/A	% Total N
Nitrogen as Ammonia	1246	56.3	374	16.9
Nitrogen as Nitrate	550	24.8	1270	57.4
Insoluble Nitrogen	308	13.9	406	18.3
	2104	95.1	2050	90.6

These data are the average of triplicate analysis. The sample of soil was taken from a manured plot on Kirkland very fine sandy loam. It had a total nitrogen content of 2212 pounds per acre. Although there was an error of 5 to 9 percent in the recovery of the nitrogen in this soil it is believed that this could be due to incomplete extraction of the nitrate from the reduced permanganate solution. In the alkaline permanganate solution 25 percent of the total nitrogen was oxidized to nitrates while in the neutral permanganate solution 57 percent was oxidized to nitrates. It is not clearly understood why so-called neutral permanganate should oxidize more nitrogen to nitrates than the alkaline solution. The solution of the neutral permanganate is not neutral. It has one gram of sodium carbonate in 200 ml. of water which has a pH value of 10.6. Under these conditions it is doubtful if organic acids would be formed that would combine with ammonia and hold it in solution until it was oxidized to nitrates.

A Comparison of the Nitrogen Content of Soil in Relation to Response of Various Crops to Nitrogen Fertilization

Soils which contain a sufficient quantity of nitrogen to produce as large a yield as climatic conditions will permit will not respond to applications of manure, the use of green manures, or nitrogenous fertilizers under average conditions. At times too much available nitrogen will cause a harmful effect on plant development as a result of increased vegetative growth. During periods of limited rainfall large quantities of available nitrogen will increase the forage growth of the plant, and as a result of the increased growth a greater demand is made on the moisture present in the soil. If there is not enough moisture present to take care of this increased demand a decline in the yield of grain will result.

It has been observed that small grains such as oats, wheat, and barley grow during the cool portion of the year when climatic conditions are unfavorable for organic matter decomposition and mature at the time when rainfall is usually favorable for plant development. These crops require a larger quantity of soil nitrogen for the production of maximum yields than crops which grow and mature during midsummer or early fall. The physical condition of the soil, moisture holding capacity and depth of the profile are factors that will also affect the yields of small grains. The results of some analyses of soils from fertility plots where oats, wheat, corn, and cotton have been grown are given in Table 9.

A study of this table will indicate that cotton does not respond to the use of legumes when the total nitrogen content of the soil is above 1176 pounds per acre or alkaline permanganate nitrogen 728 pounds per acre. Profitable responses were obtained when green manures were plowed under for wheat on soil containing less than 2000 pounds of nitrogen per acre of total nitrogen and 1162 pounds per acre of alkaline permanganate nitrogen. No response was obtained when wheat was grown on a soil that contained 2295 pounds per acre of total nitrogen. Oats gave increased response to plowing under legumes in northeastern Oklahoma when the total nitrogen content was better than 2200 pounds per acre and 1288 pounds of nitrogen was liberated by the alkaline permanganate method. The effect of fertilization on corn showed no increased response in central Oklahoma when the total nitrogen content was 1792 pounds per acre or the alkaline permanganate nitrogen was 938 pounds per acre.

These data tend to show that beneficial effects of legume crops will not appear until after the original nitrogen content in the virgin soil is reduced to a point where nitrogen is the first limiting factor in plant development. This point will vary for different crops. As the data in Table 9 indicates, especially those crops that mature in different seasons of the year will vary.

Table 9. A study of the nitrogen content of soils collected from land where experimental plots have been conducted in different parts of Oklahoma in relation to the increase in yield of grain or cotton from the use of legumes or nitrogenous fertilizers.

Soil No.	Location	Total Nitrogen Lbs./A	Alkaline Permanganate N Lbs./A	Percent of Total N	Crop	Response
100	Watova	2240	1288	57.1	Oats	+
671	Watova	2265	-----	-----	Oats	+
1374	Stillwater	1764	938	53.1	Oats	+
2783	Pryor	1848	1008	54.5	Oats	+
117	Stillwater	1932	1162	60.0	Wheat	+
119	Stillwater	1950	980	50.2	Wheat	+
137	Stillwater	1905	-----	-----	Wheat	+
344	Carrier	952	520	54.5	Wheat	+
722	Granite	2295	-----	-----	Wheat	-
776	Stillwater	1960	1148	58.5	Wheat	+
4439	Inola	2408	1344	55.8	Wheat	-
4481	Thomas	840	476	56.6	Wheat	+
1044	Coweta	2260	1442	54.2	Corn	-
95	Chandler	408	224	54.9	Corn	+
339	Chandler	560	280	50.0	Corn	+
1379	Okemah	2288	1568	58.3	Corn	-
2167	Chandler	1792	938	53.1	Corn	-
2196	Pleasant Valley	1848	980	53.0	Corn	-
483	Fayetteville, Arkansas.	1232	700	56.8	Corn	+
470	Shawnee	940	574	61.0	Cotton	+
513	Stigler	1848	1092	59.0	Cotton	-
813	Stigler	1400	728	52.0	Cotton	-
879	Scott, Ark.	952	434	46.6	Cotton	+
915	Stillwater	1970	1204	61.1	Cotton	-
922	Stillwater	1176	728	61.9	Cotton	-
1370	Lone Grove	1288	644	50.0	Cotton	-
1371	Heavenor	810	490	60.4	Cotton	+
209	Tifton	728	420	57.6	Cotton	+

Summary

A series of experiments were made to determine the rate at which nitrogen compounds in soil were hydrolized or made soluble with various strengths of sulfuric acid. There was a considerable variation in the amount of nitrogen made soluble in the different soils, but the percentage of nitrogen was closely related to the quantity of total nitrogen in the soil. As the strength of the acid was increased, the amount of nitrogen extracted was also increased. As the time of extraction was prolonged there was reduction in the amount of nitrogen occurring in the soil filtrate indicating that some forms of soil nitrogen are more easily hydrolyzed by acid treatment.

Nitrate accumulation in the soil in relation to total nitrogen, organic matter and ammonia liberated by alkaline and neutral permangarate was studied on 28 samples of soils obtained from various states. No correlation could be made between total nitrogen and nitrate accumulation. There is a parallel agreement between the amount of nitrogen liberated as ammonia by treatment with alkaline or neutral permanganate in which approximately four times as much nitrogen was secured from the alkaline solution as compared with the neutral permanganate.

A close correlation was found between the amount of ammonia liberated by alkaline permanganate and total nitrogen. An average of 58 percent of the total nitrogen in the soil samples analyzed was collected as ammonia by distillation.

In the alkaline and neutral permanganate digestion a portion of the nitrogen was found to be oxidized to nitrate. The percentage of ammonia liberated by neutral permanganate is small as compared with the nitrate content of the solution whereas the alkaline permanganate solution liberates a much higher percentage of ammonia as compared with

nitrates produced.

A study of nitrogen content of surface soils from fertility plots showed that wheat did not respond to nitrogen fertilization when a soil contained 2295 pounds of nitrogen per 2,000,000 pounds of surface soil. Oats gave increased response to the plowing under of legumes in eastern Oklahoma when the soil contained as much as 2200 pounds of nitrogen per acre. No effect of nitrogen fertilization on corn was obtained in central Oklahoma when the total nitrogen content of the surface soil was 1792 pounds per acre. Cotton did not respond to green manure crops or legume residues when the total nitrogen content of the soil was above 1176 pounds per acre.

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