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Nitrification Capacities of Texas Soil Types and Factors which Affect Nitrification

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Preface

Nitrification is the process which changes organic matter and ammonia in the soil to nitrates. Nitrates are readily used as plant food, are also easily soluble in water and can be leached from the soil. The nitrifying capacities of soils for ammonium sulphate may range from 0 to 100 percent but additions of bacteria and calcium carbonate will increase the nitrifying capacities of most soils and subsoils to a high extent.

Soils with low nitrification capacities have low nitrogen content, low basicity and are slightly acid or neutral. Soils with high nitrifying capacities usually contain more than .06 percent nitrogen, have basicities greater than 0.6 percent and pH values higher than 7. Upland surface soil types of East Texas and other non-calcareous soils have low nitrifying capacities. Upland calcareous soil types have high nitrifying capacities. Nitrification of 7 to 13 percent of the soil nitrogen in 28 days of incubation may be considered as normal for soils containing .06 percent or more of nitrogen.

The number of nitrate and nitrite-forming organisms are in general related to nitrification but the relations are not consistent. Nitrites produced during nitrification are not always completely oxidized in nitrates, especially if insufficient numbers of nitrate-forming organisms are present at the beginning of the nitrification. The conversion of nitrites to nitrates in soil is almost completely a biological process.

Nitrification is decreased by puddling of soils, and does not occur to an appreciable extent in water-logged soils. In soils of high nitrifying capacity, the nitrogen of cottonseed meal is nitrified to the average extent of 50 percent of that of ammonium sulphate, but in soils of low nitrifying capacity, nitrogen of cottonseed meal may be nitrified more readily than that of ammonium sulphate. When the fertilizing values of organic nitrogenous fertilizers are to be compared by means of nitrification experiments, soils of high nitrifying capacity should be used.

Depressing effects of some added organic matter upon nitrification may persist for 20 weeks. Cyanamid is not readily nitrified and depresses nitrification when applied at the rate of more than 100 parts of nitrogen per million of soil. The depressing effects of cyanamid persisted 6 to 10 months, and the substances which interfered diffused when placed in one spot in the culture. Ground sulphur interferes with nitrification, but probably not sufficiently to be of significance in cultivated soils. The maximum amounts of nitric nitrogen in 2 field soils were found in July, October and April.

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The nitrogen in soils occurs chiefly in the form of organic compounds. A small fraction may be present as ammonia or as nitrates, and more rarely as nitrites. The organic compounds are only slightly soluble in water, and retain the nitrogen. The nitrogen is released for the use of plants by the gradual decomposition of the organic compounds. The organic compounds, among other uses, serve to store nitrogen so as to avoid rapid depletion of the nitrogen of the soil.

Many changes take place in the nitrogen of the soil. These are brought about by bacteria and other living organisms. There is, first, the transformation of organic nitrogen into ammonia, termed ammonification. The ammonia is changed to nitrites. Nitrites are then changed to nitrates. This process is called nitrification. Nitrites or nitrates may be decomposed to produce free nitrogen, a change called denitrification, or they may be used by microscopic organisms to form protein and, thereby, again become a part of the organic matter of the soil. Nitrogen of the air may be fixed as protein, either by organisms in the soil or, to a greater extent, by organisms which live in nodules on the roots of leguminous plants.

Nitrates and ammonia are taken up by plants and are the chief sources of the nitrogen in plants, with the exception of the nitrogen of the air taken up by legumes. Since nitrates are the chief form in which nitrogenous plant food is provided by soils, or provided by organic fertilizers, or by manures added to soils, the changes of the forms of nitrogen in the soil have been the subject of a large number of investigations. No attempt will be made here to review all the literature. A review has been made by Waksman (74).

Nitrification in Texas soils, as the subject of a project, was studied for several years, and a number of publications were made giving

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the results of the work (17-36 inclusive). This publication includes additional work which was not published at the time the project was discontinued a few years ago.

In order to conserve space, detailed tables for some of the work are not given.

Methods

The samples used were taken for chemical analysis or for pot experiments from representative Texas soil types or areas. The samples were air dried and passed through a 6-mesh sieve after pounding up with a wooden pestle in a wooden box. Smaller samples were passed through a 1 millimeter (m.m.) sieve.

The average chemical composition of soil types of Texas is discussed in Texas Station Bulletin No. 549 (26).

In the nitrification tests (28), 100 grams of dry soil was mixed in a porcelain dish with the necessary additions. Water was next added to bring the total water content to 50 percent of the water-capacity of the soil. The soil was mixed with the water and with any other additions by cutting in with a spatula. The mixtures were transferred to 150 cubic centimeter (c.c.) beakers and kept at 35° C. in an incubator, water being added twice a week to restore the loss in weight. After 28 days, the nitrates were estimated by the phenol-disulphonic acid method and the nitrites by the alpha-naphthylamine method (29). The results are expressed in parts per million (p.p.m.) of the dry soil. In many experiments the amount of the nitrous nitrogen was small and, when not otherwise stated, it is included with the amount of nitric nitrogen.

When added, the amount of calcium carbonate was usually 1 gram, equal to 1 percent of the amount of soil taken. Bacteria were sometimes added by means of an inoculating liquid (10 c.c.). Ammonium sulphate, when added, was usually in 5 c.c. of a solution containing 0.05 grams nitrogen, equal to 500 p.p.m. of the soil used. When other nitrogenous compounds were used, the same amount of nitrogen was usually added.

To prepare the inoculating liquid, portions of actively nitrifying soil equivalent to 10 grams of dry soil were ground in a mortar. Water was added gradually to form a thin paste, the suspension was transferred to a 500 c.c. flask, and made up to volume. Just before withdrawing the inoculating liquid, the contents of the flask were thoroughly shaken. The inoculating liquid in much of the work was prepared from special cultures. To 100 grams of soil of high nitrifying capacity, ammonium sulphate, inoculating liquid and water were added as described above. After incubating 3½ to

4½ weeks, these cultures were used to prepare the inoculating liquid. With such inoculating liquid, the nitrogen was usually oxidized to nitrates; nitrites were either absent or present in very small amounts at the end of the experiment.

Basicity was determined after adding 100 c.c. of 0.2N nitric acid to 10 grams of soil, allowing the mixture to stand 30 minutes or stirring it 15 minutes, filtering, diluting 10 c.c. with water, heating to remove carbon dioxide, and then titrating with 0.1N sodium hydroxide, using phenolphthalein as an indicator. If more than 80 percent of the acid was consumed, the test was repeated with the use of normal nitric acid. Basicity is expressed in terms of percentage of calcium carbonate. The acid was neutralized chiefly by calcium derived from calcium carbonate, if present, from calcium in the base exchange complex and from silicates decomposed by the acid (25).

To determine the water capacity, 50 grams of dry soil, pounded up to pass a 10-mesh sieve, was placed on a porcelain filter plate in a carbon filter tube 1¼ inches in diameter. Water was added gradually until the soil was saturated and a little had run through. The tube was then covered and allowed to drain 30 minutes. The stem of the tube was dried with filter paper and the tube weighed. Water capacity is expressed in percentage of the dry soil.

The numbers of the organisms which convert ammonia to nitrite, and those which convert nitrites to nitrates were determined by the dilution method. Vessels plugged with cotton wool and utensils were sterilized at 140° C. for 2 hours. Distilled water was boiled 1 hour. Suspensions of different strength of the soil were inoculated into suitable sterilized culture media in 125 c.c. Erlenmeyer flasks, incubated 28 days at 35° C. and then tested for nitrates or nitrites.

The medium for determining the number of the nitrite-forming organisms consisted of 50 c.c. of distilled water in a 125 c.c. Erlenmeyer flask, .5 grams of calcium carbonate, 1 c.c. of ammonium sulphate containing .01 gram nitrogen and 5 c.c. of mineral solution. The mineral solution contained 1 gram dipotassium phosphate, .5 gram magnesium sulphate and .4 gram ferrous sulphate in 500 c.c. of water. Five cultures were inoculated with 5 c.c. and 5 with 10 c.c. of each soil suspension used. After inoculation and incubation for 28 days, nitrites were determined by the alpha-naphthylamine method (29).

From the number of cultures which when inoculated with the more dilute soil suspension gave positive tests for nitrites, the number of nitrifying organisms was estimated.

For nitrate-forming bacteria, the medium consisted of 50 c.c. distilled water, 5 c.c. lime water, 5 c.c. of the mineral nutrient used for the nitrate forming organisms, and 5 c.c. sodium nitrite solution containing .01 gram nitrite nitrogen made from silver nitrite (29). After incubation for 28 days, nitrates were determined quantitatively with the phenol-disulphonic acid method (29). The quantitative determination was necessary because chemical oxidation of nitrites produced small amounts of nitrates in some of the cultures.

To prepare soil suspensions, soil equal to 10 grams of air-dry soil was ground in a mortar with small quantities of sterilized water, and sterilized water was added gradually until a thin paste was formed. The mixture was transferred to a 500 c.c. flask, and made up to volume (suspension A). Suspension B consisted of 10 c.c. of A diluted to 250 c.c.; Suspension C, of 10 c.c. of B diluted to 200 c.c.; Suspension D, of 10 c.c. of C diluted to 200 c.c.; Suspension E, of 10 c.c. of D diluted to 200 c.c. In preliminary work, 30 flasks were used for each sample of soil, and 5 of each were inoculated with 1 c.c. or 5 c.c. of suspensions A, B and C respectively. That the liquid media were satisfactory was shown by complete oxidation of ammonia or nitrites when sufficient numbers of bacteria were added.

When 5 flasks are inoculated with 5 c.c. of dilution A, they receive a total of .5 gram soil. If one of the 5 flasks gives a positive test for nitrate or nitrite-forming bacteria, there is 1 bacteria or clump of bacteria in .5 gram soil, which is 2 bacteria per 1 gram of soil. If all 5 flasks give positive tests, there are 10 or more bacteria per 1 gram of soil. With 1 c.c. of dilution A per flask, 1 flask of the 5 with a positive test means 10 bacteria per 1 gram of soil; 5 positive tests means 50 per 1 gram. With 5 c.c. of dilution B, 1 of 5 flasks with a positive test means 50 bacteria per 1 gram; 5 flasks with a positive test means 250 per 1 gram. With 1 c.c. of dilution B, 1 of the 5 flasks with a positive test means 250 bacteria per gram; 5 flasks means 1,250. With 5 c.c. of dilution C, 1 positive flask means 1,000 bacteria per gram; with 5 flasks, 5,000 per gram. With 1 c.c. of dilution C, 1 positive flask means 5,000 bacteria or clumps of bacteria per 1 gram of soil; 5 positive flasks mean 25,000 bacteria or clumps of bacteria per gram. And so on to higher dilutions, if necessary.

To count the numbers of soil bacteria suitable suspensions of the soil were mixed with albumin agar plated in Petri dishes, and the number of colonies were counted which appeared after incubation (74, page 13).

The numbers of the autotrophic and the heterotrophic organisms were estimated by plating, using silica gel with inorganic or organic media (64).

Effect of Additions of Bacteria and Calcium Carbonate on Texas Soils

Soils vary widely in their capacity to nitrify ammonium sulphate. With some soils, additions of ammonium sulphate followed by incubation results in the production of less nitrates than are produced in the original soil. With other soils, the nitrification ranges from nearly zero to almost complete conversion of the ammonia to nitrates in the period of 28 days. Fraps and Sterges (30) found that additions of nitrifying soil, or of calcium carbonate, or of both calcium carbonate and nitrifying soil to soils of low nitrifying capacity would result in high nitrification of ammonium sulphate in many soils. In other words, the low nitrification of ammonium sulphate was due to deficiencies of nitrifying organisms, or of basic compounds needed to neutralize the nitric and sulphuric acids produced, or of both. A few soils needed additions of available phosphates for high nitrification (35) and there was a small percentage left which still did not nitrify the ammonium sulphate completely.

The object of the work here presented was to ascertain the nitrifying capacity of Texas soil types and the effect of addition of bacteria and calcium carbonate on them. For this purpose, after preliminary work, 8 cultures were usually prepared for each sample of soil, as follows: (1) no addition, (2) addition of calcium carbonate, (3) inoculating liquid, (4) calcium carbonate and inoculating liquid, (5) ammonium sulphate, (6) ammonium sulphate and calcium carbonate, (7) ammonium sulphate and inoculating liquid, and (8) ammonium sulphate, calcium carbonate and inoculating liquid. The nitrification of the ammonium sulphate was ascertained by substracting the quantity of nitric nitrogen in the culture which did not receive ammonium sulphate from the quantity in corresponding culture which received ammonium sulphate.

A summary of the effects of the inoculating liquid, the calcium carbonate, and of the two combined is given in Table 1. The soils were divided into groups according to the quantities of nitric nitrogen produced in the original soil which had received ammonium sulphate, namely, 0-25, 26-99, 101-199, 201-299, 300-399, and over 400 parts per million. The table shows the percentages of the numbers of soils in each group in which the nitrification was such as to move the soil to a higher or lower group, or leave it in the same group. For example, in the group originally nitrifying 0-25 p.p.m.,

Table 1. Effect of inoculation, addition of calcium carbonate, and both inoculation and calcium carbonate upon nitrification of ammonium sulphate. Soils are grouped according to original nitrification, and the figures are the percentages of samples nitrified in each group

Group	Surface soils			Subsoils		
	Inoculated	Calcium carbonate added	Inoculated and calcium carbonate	Inoculated	Calcium carbonate added	Inoculated and calcium carbonate
	%	%	%	%	%	%
Original nitrification 0-25 p.p.m. in 25 surface, 65 subsoils						
After treatment 0–25 p.p.m.	52	0	0	59	36	3
26–99	48	0	0	20	22	3
100–199	0	31	4	14	21	18
200–299	0	36	36	12	15	28
300–399	0	12	36	3	6	28
400 or more	0	8	24	1	0	20
Original nitrification 26-99 p.p.m. on 13 surface, 19 subsoils						
After treatment 26–99 p.p.m.	61	8	0	36	25	5
100–199	31	0	8	37	15	5
200–299	8	16	8	10	20	10
300–399	0	54	69	10	30	22
400 or more	0	22	15	5	10	58
Original nitrification 100-199 p.p.m. in 11 surface, 15 subsoils						
After treatment 100–199 p.p.m.	64	27	0	26	86	7
200–299	27	9	0	7	0	13
300–399	0	18	27	20	7	20
400 or more	9	46	73	47	7	60
Original nitrification 200-299 p.p.m. in 15 surface, 12 subsoils						
After treatment 100–199 p.p.m.	0	14	0	0	24	8
200–299	20	40	7	8	60	8
300–399	20	14	20	25	10	24
400 or more	60	32	73	67	0	60
Original nitrification 300–399 p.p.m. in 9 surface, 13 subsoils						
After treatment 200–299 p.p.m.	10	0	0	0	23	0
300–399	45	22	22	15	62	38
400 or more	45	78	78	85	15	62
Original nitrification over 400 in 50 surface, 27 subsoils	....	....	....	....	....	....

inoculation changed 48 percent of the soils to a higher group and left 52 percent of the soils in the same group.

Inoculation alone (Table 1) increased nitrification in 40 to 50 percent of the various groups of surface soils and 40 to 85 percent of the groups of subsoils sufficiently to move them to higher groups. Inoculation was more effective with the subsoils than with the surface soils, probably because the subsoils had been less subjected to nitrification in their original situation.

Calcium carbonate had more effect upon soils of low nitrifying power than those of high nitrifying power, and on surface soils than on subsoils. Calcium carbonate increased nitrification of all surface soils originally nitrifying less than 25 p.p.m., 92 percent of those nitrifying 26-99 p.p.m., 73 percent of those nitrifying 100-199 p.p.m., and 46 percent of those nitrifying 200-399 p.p.m. With the subsoils, the percentages increased were 64, 75, 14, and 10 respectively. The subsoils were more basic than the surface soils. Calcium carbonate decreased nitrification in a few of the soils originally with high nitrifying power.

Inoculation and calcium carbonate combined increased nitrification in 92 to 100 percent in the various groups of surface and subsoils. More than 60 percent of the nitrogen of the ammonium sulphate was nitrified in 60 percent of the surface and subsoils originally nitrifying 0-25 p.p.m.; approximately 80 percent of those in the group 26-99 and subsoils in groups 100-199; 100 percent in surface soils in groups 100-199; 93 percent in the surface and 84 percent in the subsoils of group 200-299.

A few of the soils still had a low nitrifying power after additions of calcium carbonate and inoculant. Applications of certain phosphates increased nitrification in some of these soils (35).

Relation of Nitrifying Capacity of Inoculated Soils to Amount of Nitrogen, Basicity and pH of the Soils

The presence of insufficient numbers of nitrifying bacteria in the soil samples was partly due to natural factors such as the depth from which the sample was taken, and partly to the extent of the suitability of the soil for nitrification of ammonium sulphate. Inoculation with inoculating liquid tends to eliminate natural deficiencies in numbers of bacteria. The results secured from inoculated soils were, therefore, used to ascertain the relation between the nitrifying capacities of the soils, and their pH values, the percentages of nitrogen, and of basicity.

These data are presented in Table 2 in percentages of the numbers of soils in each group. For example, in the group of inoculated

Table 2. Relation of nitrification of ammonium sulphate in inoculated soils to chemical composition. Numbers of soil samples expressed as percentages of the total for each group

	Nitrification 0-25 p.p.m.		Nitrification 26–100 p.p.m.		Nitrification 101–200 p.p.m.		Nitrification 201–300 p.p.m.		Nitrification 301–400 p.p.m.		Nitrification 401 p.p.m.	
	Top	Subsoil	Top	Subsoil	Top	Subsoil	Top	Subsoil	Top	Subsoil	Top	Subsoil
	%	%	%	%	%	%	%	%	%	%	%	%
Nitrogen content, percent												
0–.03	45	27	5	15	0	0	0	0	0	8	0	2
.031–.06	31	58	50	45	12	64	9	58	0	31	5	11
.061–.12	16	15	45	40	88	24	82	28	86	45	41	74
.121–.18	8	0	0	0	0	12	9	14	14	16	39	11
.181 +	0	0	0	0	0	0	0	0	0	0	15	2
Basicity, percent												
0–0.3	80	49	68	28	28	0	0	0	0	8	0	0
.31– .6	7	11	16	24	27	34	20	11	0	0	0	0
.61–2.0	13	34	11	43	27	43	50	11	50	8	13	2
2.1 –5.0	0	6	5	0	0	6	30	22	0	25	21	11
5.1 +	0	0	0	5	14	17	10	56	50	59	66	87
pH												
0–5.0	0	20	0	0	0	5	0	0	0	0	0	0
5.1–5.5	31	9	5	10	0	0	0	0	0	0	0	0
5.6–6.0	0	6	15	0	0	0	0	0	0	0	0	0
6.1–6.9	16	26	35	16	15	28	0	0	0	9	0	0
7.0–7.5	45	13	35	37	15	22	36	40	0	0	4	0
7.6 +	8	26	10	37	70	47	64	60	100	91	96	100

soils which produced 0-25 p.pm., of nitrate nitrogen from the ammonium sulphate equal to 500 p.p.m. of nitrogen, 45 percent of the top soils contained 0-.03 percent of nitrogen, 31 percent contained .03-.06 percent, 16 percent contained .061-0.12 percent, and 8 percent contained .121-.18 percent nitrogen.

Most of the soils which contained .03 percent nitrogen or less nitrified less than 25 p.p.m. of nitrogen. Nearly all the soils which contained over 0.12 percent nitrogen nitrified more than 200 p.p.m. Most of the soils with basicity 0.3 or less nitrified less than 100 p.p.m. Most of them with basicity more than 2 percent, nitrified over 200 p.p.m. Soils with pH less than 5.5 nitrified less than 100 p.p.m. Practically all soils which nitrified over 300 p.p.m. of nitrogen had pH values of 7.6 or over. Therefore, they were slightly alkaline. About one-half of the soils which nitrified over 300 p.p.m. and two-thirds of those nitrifying over 400 p.p.m. had basicities higher than the equivalent of 5 percent calcium carbonate.

In general, soils with low nitrifying capacities had low nitrogen content, low basicity, and were slightly acid. Soils with high nitrifying capacity contained more than 0.06 percent nitrogen, had basicities greater than 0.6 percent and pH values higher than 7.0. There were, however, some exceptions.

Nitrifying Capacities of Texas Soil Types

Nitrification capacities of a number of Texas soil types were studied by the method outlined above. Many tests were made before this method was fully developed in which the effect of calcium carbonate was ascertained. It was considered desirable to include the results of these tests also.

Table 3 contains the nitrification capacities of a number of surface soils of Texas soil types. The soils are arranged in the same geographical divisions as the soils whose analyses are presented in Texas Station Bulletin No. 549 (26). Many of the figures in Table 3 are averages, but some are for only one sample. When tests on different samples of the same type gave very different results, these differing tests are also included in Table 3.

The upland surface soils of the Gulf Coast Prairie, the East Texas Timber Country, the West Cross Timbers, and other noncalcareous soils have low nitrifying capacities for ammonium sulphate. These are increased little by inoculation, but may be greatly increased by additions of calcium carbonate, or both inoculation and calcium carbonate. Soils of the Lake Charles, Amarillo, Bowie, Caddo, Kirvin, Norfolk, Lufkin and Susquehanna series are some which have low nitrifying capacities.

Table 3. Nitrification of Texas soil types (surface soils only). Nitric nitrogen in parts per million

	Soil alone p.p.m.	Inoculated p.p.m.	Calcium carbonate p.p.m.	Calcium carbonate and inoculated p.p.m.
Gulf Coast Prairie				
Upland soils				
Lake Charles clay loam	34	....	397	....
Lake Charles fine sandy loam	28	....	396	....
Lake Charles clay	4	....	200	....
Edna fine sandy loam	0	....	350	....
Hockley fine sandy loam	0	....	160	....
Alluvial soils				
Miller fine sandy loam	149	....	306	....
Miller clay	237	....	170	....
Ochlockonee silt loam	28	....	316	....
Ochlockonee fine sandy loam	0	....	120	....
Trinity clay	300	....	322	....
Yahala clay	340	....	....	....
East Texas Timber Country				
Upland soils				
Bowie fine sandy loam	0	0	193	316
Caddo fine sandy loam	134	....	314	....
Caddo fine sandy loam	0	....	208	....
Kirvin fine sandy loam	0	....	175	....
Kirvin clay loam	0	....	36	....
Nacogdoches fine sandy loam	0	0	177	368
Orangeburg fine sandy loam	*0*	....	*200*	....
Norfolk fine sand	0	0	217	218
Ruston fine sandy loam	0	....	59	....
Ruston fine sandy loam	0	0	33	162
Lufkin fine sandy loam	113	217	175	395
Lufkin fine sandy loam	0	....	279	....
Susquehanna fine sandy loam	0	0	172	282
Susquehanna fine sandy loam	0	....	196	....
Susquehanna stony loam	0	....	173	....
Susquehanna very fine sandy loam	0	....	50	....
Tabor fine sandy loam	0	67	321	360
Terrace soils				
Amite fine sandy loam	0	....	225	....
Cahaba fine sandy loam	0	....	132	....
Leaf fine sandy loam	0	37	184	273
Leaf very fine sandy loam	16	59	360	330
Blackland Prairies				
Calcareous upland soils				
Houston black clay	141	280	125	320
Houston black clay	243	521	202	509
Houston black clay	447	455	417	454
Houston clay	288	420	160	397
Houston clay	53	....	430	....
Houston loam	58	....	308	....
Houston clay loam	0	....	220	....
Sumter clay	289	465	231	403
Sumter clay	484	485	460	458
Bell clay (terrace)	228	328	160	342
Bell clay	403	480	200	495
Lewisville clay	266	430	255	457
Calcareous stream bottom				
Catalpa clay	399	449	467	491
Non-calcareous upland soils				
Crockett fine sandy loam	0	23	299	407
Crockett very fine sandy loam	0	49	317	409
Crockett clay loam	0	....	374	....
Wilson very fine sandy loam	20	50	406	470
Wilson fine sandy loam	0	....	....	....
Wilson clay loam	0	0	153	416
Wilson clay loam	60	80	402	447
Wilson clay loam	225	282	470	483
Wilson clay	264	440	209	481
Wilson clay	0	....	31	....
Irving clay	36	....	199	....
Rolling Plains				
Upland soils				
Abilene clay loam	245	404	420	503
Abilene fine sandy loam	6	18	283	361
Abilene very fine sandy loam	200	228	485	502
Abilene very fine sandy loam	434	486	452	478
Foard clay loam	58	....	183	....

Table 3. Nitrification of Texas soil types (surface soils only). Nitric nitrogen in parts per million.—Continued

	Soil alone p.p.m.	Inoculated p.p.m.	Calcium carbonate p.p.m.	Calcium carbonate and inoculated p.p.m.
Rolling Plains—Continued				
Upland soils—Continued				
Miles fine sand	10	228	6	232
Miles fine sandy loam	41	78	328	356
Miles clay loam	499	491	503	495
Vernon clay	291	486	232	483
Vernon fine sandy loam	395	388	384	430
Terrace soils				
Calumet very fine sandy loam	65	....	380	....
High Plains				
Upland soils				
Amarillo fine sandy loam	0	0	244	285
Amarillo fine sand	0	....	172	....
Amarillo silty clay loam	60	....	467	....
Amarillo clay loam	10	....	0	....
Richfield fine sandy loam	210	....	161	....
Alluvial soils				
Randall clay	120	201	452	402
Grand Prairie				
Upland prairie soils				
Crawford clay loam	92	....	370	....
San Saba clay	380	....	309	....
West Coast Timbers				
Upland soils				
Bastrop sand	31	....	293	....
Denton clay	403	510	483	488
Milam fine sandy loam	0	....	0	....
Windthorst fine sandy loam	0	....	324	....
Edwards Plateau				
Upland soils				
Reagan loam	432	465	468	474
Reagan silty clay loam	502	516	505	458
Reagan fine sandy loam	383	....	393	....
Rio Grande Plain				
Upland soils				
Brennan fine sandy loam	36	....	390	....
Clareville fine sandy loam	355	512	424	456
Clareville clay loam	453	445	446	458
Duval fine sandy loam	0	27	265	355
Frio clay	96	....	368	....
Frio silt loam	435	460	482	464
Goliad fine sandy loam	198	223	453	469
Hidalgo fine sandy clay loam	87	....	110	....
Miguel fine sandy loam	326	365	435	447
Maverick fine sandy loam	384	370	315	381
Maverick clay loam	435	460	423	487
Maverick clay	518	518	520	529
Crystal fine sandy loam	0	3	62	156
Crystal fine sand	30	72	264	207
Crystal loam, fine sand	0	48	242	390
Montiola clay	395	513	413	511
Nueces loamy fine sand	152	....	353	....
Orelia clay loam	60	126	443	398
Orelia clay	307	420	482	507
Tiocana clay	289	....	391	....
Uvalde clay	499	480	498	493
Uvalde clay loam	455	492	477	499
Uvalde silty clay loam	436	469	447	481
Uvalde silty clay	466	529	447	458
Victoria clay	61	50	318	368
Victoria fine sandy loam	93	80	415	....
Webb fine sandy loam	38	200	343	410
Webb fine sandy loam	431	468	435	438
Willacy fine sandy loam	156	....	410	....
Alluvial soils				
Laredo very fine sandy loam	479	519	473	481
Rio Grande very fine sandy loam	74	....	436	....

Upland calcareous soils of the Blackland Prairies, the Rolling Plains, the Gulf Coast Plains and elsewhere have medium to high nitrifying capacities. When medium, nitrification is increased by inoculation but not by additions of calcium carbonate. This includes such soil series as Houston, Sumter, Abilene, Reagan, Clareville and Maverick. Alluvial soils, when non-calcareous, have low to medium nitrifying capacities; when calcareous, the nitrifying capacity is usually medium to high. There are exceptions to the above general statements. The agronomic significance of differences in nitrifying capacities remains to be ascertained. Ammonia nitrogen was equally as valuable as nitrate nitrogen in pot experiments in a number of soils with low nitrifying capacity (31).

Effect of Bacteria and Calcium Carbonate on Nitrification of the Soil Nitrogen

The nitrification of the organic nitrogen already present in the soil was not necessarily comparatively low when the nitrification of ammonium sulphate was low. The soil nitrogen was nitrified to a fair extent in many soils in which ammonium sulphate was not nitrified at all or in which it even depressed nitrification. Additions of bacteria, calcium carbonate or both, increased nitrification of the soil nitrogen of some soils, but did not have the same effect with some other soils. When increases occurred, they were usually relatively small, not nearly so great as occurred in many soils for nitrification of ammonium sulphate.

Data representing nitrification of soil nitrogen as compared with nitrogen of ammonium sulphate is given in Table 4 for a few of

Table 4. Representative data on nitrification of soil nitrogen compared with nitrification of ammonium sulphate. Nitric nitrogen in parts per million

Type and source of nitrogen	Soil alone	Inoculated	Calcium carbonate added	Calcium carbonate and inoculated
Webb fine sandy loam, soil nitrogen	53	51	42	53
Ammonium sulphate nitrogen	2	72	138	327
Webb fine sandy loam, soil nitrogen	84	82	74	78
Ammonium sulphate nitrogen	36	53	328	342
Maverick fine sandy loam, soil nitrogen	76	70	65	69
Ammonium sulphate nitrogen	344	370	315	381
Randall clay subsoil, soil nitrogen	64	70	71	94
Ammonium sulphate nitrogen	68	160	372	419
Crystal fine sand, subsoil, soil nitrogen	23	41	38	39
Ammonium sulphate nitrogen	0	3	62	156
Crystal fine sandy loam, subsoil, soil nitrogen	16	49	36	48
Ammonium sulphate nitrogen	0	49	147	332
Orelia clay loam, subsoil, soil nitrogen	37	64	54	80
Ammonium sulphate nitrogen	0	156	319	420
Leaf very fine sandy loam, soil nitrogen	1	4	1	10
Ammonium sulphate nitrogen	0	4	0	137

the many soils tested. With the first 3 soils, the additions had little effect on nitrification of the soil nitrogen, but had appreciable effects upon nitrification of the ammonium sulphate nitrogen. With the last 5 soils, the additions stimulated nitrification of the soil nitrogen, but the increases in the nitrification of the ammonium sulphate were relatively much greater. Calcium carbonate increased the production of nitric nitrogen from soil nitrogen in some soils, but in many other soils it had no effect.

Previous work has shown (20, 23) that, in general, the nitric nitrogen produced in nitrification tests from the nitrogen of the soil is related to the percentage of nitrogen naturally in the soil. It has also been shown that, on an average, the amount of nitrogen taken up by corn and sorghum in pot experiments is related to the amounts of nitric nitrogen produced from the soil nitrogen in nitrification experiments, the correlation coefficient with the first crop being + .708 ± .023 and with 4 crops, + .653 ± .029 (23). Nitrification tests made on samples taken from soils before and after cropping showed that the amounts of nitrates produced were reduced by cropping and related to the amounts of nitrogen withdrawn by the crops (23), the correlation coefficient being + .680 ± .029.

Differences in the ability of soils of the same nitrogen content to furnish nitrogen to crops may be related, in part, to differences in the percentages of the soil nitrogen which can be converted into nitrates by nitrification. A summary of the percentages of soil nitrogen nitrified in 115 surface soils and 115 subsoils is given in Table 5. The nitric nitrogen produced in 28 days in soils and subsoils containing .03 percent or less of nitrogen, ranged from 7 to over 27 percent of the soil nitrogen. About 50 percent of all the topsoils and 60 percent of all the subsoils nitrified 7 to 13 percent

Table 5. Number of soils which nitrified the percentages of the soil nitrogen in the groups given. Arranged according to percentages of soil nitrogen

Nitrogen content	Percentages of nitrogen nitrified				
	0–6% No.	7–13% No.	14–20% No.	21–27% No.	27% or more No.
0–.03% nitrogen, top soil	0	2	4	4	3
subsoil	0	4	3	1	1
.031–.06% nitrogen, top soil	1	10	10	3	0
subsoil	9	30	4	0	1
.061–.12% nitrogen, top soil	14	29	7	1	0
subsoil	22	25	3	0	0
.121–.18% nitrogen, top soil	6	12	3	0	0
subsoil	3	5	1	1	0
.181% or more nitrogen, top soil	2	3	1	0	0
subsoil	0	2	0	0	0
All soils, top soil (115)	23	56	25	8	3
subsoil (115)	34	66	11	2	2

of the soil nitrogen. Nitrification of 7 to 13 percent of the soil nitrogen may be considered normal when the total soil nitrogen is .06 percent or more. However, nitrification of 14 to 20 percent of the soil nitrogen might be considered as normal for surface soils containing less than .06 percent of soil nitrogen. About 20 percent of the surface soils and 30 percent of the subsoils nitrified less than 7 percent of the soil nitrogen, and about 20 percent of the surface soils and 10 percent of the subsoils nitrified from 14 to 20 percent of the soil nitrogen. These latter 2 groups of soils could be considered as poorer or better respectively, than the average in the extent to which the soil nitrogen is nitrified. A few of the soils nitrified more than 20 percent of the soil nitrogen, and in these soils the soil nitrogen could be considered as unusually easily nitrified.

Other experiments, not here presented, showed that additions of calcium carbonate increased nitrification with 20 of 56 surface soils and subsoils lower than normal in nitrification, with 18 of 53 soils higher than normal, and with 30 of 123 soils normal in nitrification of the soil nitrogen. With the last 2 groups, the effect of the calcium carbonate, if any, was usually slight, and in some cases calcium carbonate decreased nitrification of the organic matter of the soil.

Numbers of Organisms as Related to Nitrification

Comparatively little work has been done on the relation of the number of organisms in the soil to nitrification. Wilson (77) estimated the number of nitrate bacteria in soils by a dilution method. Thorne and Brown (69), and Walker, Thorne and Brown (75) used the Wilson method with some modifications.

In order to ascertain the changes in the numbers of nitrite and nitrate forming organisms during the period of incubation in nitrification studies, tests were made with surface soils of Houston black clay and Abilene fine sandy loam, both of high nitrifying power. Five cultures of each soil were prepared and incubated, and one of each culture was examined at the end of 7, 14, 21, 28 and 45 days. Nitrates, nitrites and the numbers of nitrate and nitrite-forming organisms were estimated with the results given in Table 6. The numbers of nitrite-forming organisms increased from 10 to 20,000 per gram in 21 days and then decreased. The number of nitrate-forming organisms reached a maximum of 2,000 per gram at the end of 28 days. The maximum number of nitrite-forming organisms occurred at the end of the week in which the maximum oxidation occurred; that of the nitrate-forming organisms was at the end of the 7 days succeeding the maximum oxidation. In the first 14 days

Table 6. Effect of days of incubation on the nitrification of ammonium sulphate and on the number of nitrifying organisms

Incubation period of cultures used as inoculants Days	Nitrogen found after incubation		Amount of nitrogen per period p.p.m.	Number of bacteria per gram of soil	
	Nitric N p.p.m.	Nitrous N p.p.m.		Nitrate Number	Nitrite Number
Houston black clay					
0	....	....	....	10	150
7	64	42	106	0	10
14	275	12	181	40	750
21	538	1	352	500	20,000
28	625	0	86	2,000	10,000
45	625	0	0	30	3,000
Abilene fine sandy loam					
0	....	....	....	100	750
7	84	66	150	50	2,000
14	281	49	180	150	4,000
21	538	3	241	1,000	20,000
28	588	2	49	2,000	15,000
45	650	1	61	250	10,000

the production of nitrite was faster than the production of nitrate. The numbers of nitrite-forming organisms were usually much larger than those of the nitrate organisms.

In order to study further the relation between the numbers of organisms and nitrification, cultures containing ammonium sulphate were made with 4 soils of high nitrifying capacities. At the end of each week, for 5 weeks, determinations were made of nitric nitrogen, nitrous nitrogen, and the numbers of nitrate and nitrite-forming organisms per gram of soil. Duplicate portions of 100 grams each of sterilized Houston black clay containing ammonium sulphate received at the end of each week inoculating liquid equivalent to 0.4 grams of each of the above cultures, were incubated 28 days, and analyses then made.

Table 7 shows that there was an increase of the nitrate produced each week for the 35 days. The maximum production was during the period 14-21 days for 3 of the soils, the period 28-35 days for the fourth. The numbers of organisms did not increase regularly during the period of experiment, and did not have a constant relation to the production of nitrates. With the exception of the Clareville fine sandy loam, the maximum number of organisms coincided with the end of the period of maximum production of nitrates.

The production of nitrate nitrogen in the sterilized soil cultures was related only slightly to the numbers of organisms with which they were inoculated. The maximum production of nitrates was accomplished, apparently, by inoculation with 1, 200, 750 and 2,000 nitrate-forming organisms per gram of inoculant. Inoculation with the maximum number of nitrate-forming organisms for each set produced the maximum amount of nitrates in 2 of the 4 sets. The

Table 7. Effect of the numbers of nitrifying organisms on the oxidation of ammonia in a sterilized soil

Incubation period of soil cultures used as inoculants	Inoculating cultures					Nitrogen produced in inoculated sterilized Houston black clay	
	Nitrogen oxidized p.p.m.		Oxidation per week p.p.m.	Numbers of bacteria per gram			
	Nitric N p.p.m.	Nitrous N p.p.m.		Nitrate	Nitrite	Nitric N p.p.m.	Nitrous N p.p.m.
Surface soil							
0	18	0	0	0	10	34	13
7 days	40	12	34	10	150	114	84
14 days	168	19	132	1	40	350	3
21 days	350	1	164	40	2,000	238	4
28 days	500	0	149	30	2,000	152	29
35 days	563	0	63	0	150	168	14
Clareville clay loam 7–21							
0	2	0	....	0	10	36	6
7 days	8	47	53	150	2,000	75	164
14 days	184	32	161	8	150	300	11
21 days	380	0	164	200	4,000	328	6
28 days	513	0	133	100	4,000	180	23
35 days	538	0	25	4	200	168	21
Surface soil							
0	8	0	....	2	40	28	9
7 days	14	14	20	4	1,000	32	30
14 days	19	36	27	2	200	33	30
21 days	64	22	31	10	500	116	75
28 days	231	11	156	200	25,000	253	5
35 days	420	4	183	750	20,000	376	9
Clareville fine sandy loam							
0	3	0	....	0	20	25	4
7 days	20	19	36	30	150	23	26
14 days	19	0	0	10	750	82	16
21 days	231	0	112	10	100	69	22
28 days	280	0	49	250	20,000	291	54
35 days	370	0	90	200	20,000	375	19

Table 8. Effect of different quantities of the same inoculants on nitrification

Soil inoculated	Date of collecting soil sample used as inoculant	Nitrate organisms in 20 cc. of inoculant	Nitrate N in parts per million of soil					Nitrate N p.p.m., per one cc. of inoculant				
			Inoculant added					Inoculant added				
			20 cc.	10 cc.	5 cc.	2 cc.	1 cc.	20 cc.	10 cc.	5 cc.	2 cc.	1 cc.
			p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
36498	May 9, 1938	2400	318	246	213	149	....	16	25	43	75	....
44357	May 9, 1938	....	442	466	441	334	....	22	47	88	165	....
36498	June 13, 1938	1200	....	94	73	34	9	....	9	15	17	9
44369	June 13, 1938	....	....	282	139	64	12	....	28	28	32	12
36498	September 26, 1938	1610	344	237	171	151	....	17	24	34	75	....
44369	September 26, 1938	....	531	437	354	251	....	27	44	70	125	....
36498	October 3, 1938	800	125	63	36	9	....	6	6	7	5	....
44369	October 3, 1938	....	217	121	64	47	....	11	12	13	23	....
51313	September 26, 1940	200	255	339	289	219	152	12	34	58	108	152
50302	October 14, 1940	200	291	302	265	227	130	15	30	53	115	130
36498	Culture incubated 28 days	14000	....	662	600	494	152	....	66	120	247	....
44357	Culture incubated 28 days	....	....	589	548	523	130	....	59	110	262	....

relation is not high between the number of nitrate-forming organisms used for inoculation and the quantities of nitrates formed. In each soil, however, there is a slight relation. Additional data will be given elsewhere.

In another experiment to ascertain the effect of approximately the same numbers of bacteria upon nitrification, samples of a cultivated soil were collected at several seasons of the year. The numbers of nitrate bacteria in the samples were determined. Suspensions of 10 grams of the samples in 500 cubic centimeters water were made, and 20, 10, 5, 2 and 1 c.c. were inoculated into 2 sterilized soils containing ammonium sulphate. After 28 days incubation, nitrates and nitrites were determined.

The results in Table 8 show that 2 different soils inoculated by about the same number of organisms produce different amounts of nitrates, but not in direct proportion to the quantities of inoculant used. The total production of nitrates decreases, in each set of cultures, as a rule, with the decrease in the volume of inoculant, that is, with the decrease in number of organisms introduced. The quantity of nitrates produced is to a certain degree related to the number of organisms introduced at different times, but the relationship is not high. The quantities of nitrates produced, per one c.c. of inoculant, usually increase as the volume of the same inoculant decreases. That is to say, in each series, the production of nitrates is related to the quantity of inoculant but the relation is not high.

Occurrence of Nitrites in Experimental Cultures

The nitrites which are produced during nitrification are not always completely oxidized to nitrates, but sometimes large amounts of nitrites have been found at the end of the 28-day period of incubation. This subject has been discussed in previous publications (28, 30, 33), but additional data is presented here. Nitrites have also been found to occur in appreciable amounts in field soils, and have been claimed to cause injury to citrus trees (65). In small amounts, nitrites are not injurious, but have a low availability as compared with nitrates (33).

The relative amounts of nitric and nitrous nitrogen in a soil culture at the end of the period of incubation depends upon the complex of organisms present at the beginning of the experiment. Three sterilized soils containing ammonium sulphate were inoculated with different quantities of nitrifying soil. The amounts of nitric and nitrous nitrogen found after 4 weeks incubation are given in Table 9. The quantity of nitric nitrogen increases as the quan-

Table 9. Effect of the quantities of inoculating soil on the amounts of nitric and nitrous nitrogen produced—N in p.p.m.

Grams of inoculant	Gaines clay		Falls fine sandy loam		Houston black clay	
	Nitric N p.p.m.	Nitrous N p.p.m.	Nitric N p.p.m.	Nitrous N p.p.m.	Nitric N p.p.m.	Nitrous N p.p.m.
0	0	0	14	112	8	0
0.1	21	105	26	180	218	74
0.2	24	50	76	164	243	60
0.5	84	25	120	160	225	106
1.0	117	40	240	80	360	0
2.0	220	0	337	11	400	0
5.0	287	0	329	0	440	0
10.0	293	0	430	0	487	0

tity of inoculant increases, while at the same time the quantity of nitrite nitrogen decreases. The number of nitrate-forming organisms in the small amounts of inoculant are apparently not sufficient to oxidize the nitrites as fast as they are formed, and the presence of the nitrites apparently prevents sufficient increase in numbers of the nitrate-forming organisms to oxidize all the nitrites. Inoculation with sufficient numbers of nitrate organisms usually results in complete oxidation of nitrites.

Additions of calcium carbonate to soils containing ammonium sulphate has in some cases stimulated the nitrite-forming organisms without, at the same time, stimulating the nitrate-forming organisms sufficiently to oxidize all the nitrites. As a result, appreciable amounts of nitrites may be found in the cultures at the end of the experiment. Additions of magnesium carbonate may result in greater amounts of nitrous nitrogen than additions of calcium carbonate. In one experiment, the nitrogen of ammonium sulphate was completely nitrified (575-600 p.p.m.) in 4 soils to which calcium carbonate was added, but when the same amount (1 percent) of magnesium carbonate was used from 344 to 440 p.p.m. of nitrous nitrogen were present at the end of the test, with 58 to 128 p.p.m. of nitric nitrogen.

The temperature of incubation may affect the occurrence of nitrites. Some cultures incubated at 24°C contained much nitrites and little nitrates, while portions of the same cultures incubated at 35°C contained nitrates but no nitrites. Cultures from subsoils are more likely to contain nitrites than cultures from the surface soils.

Some soils heated for an hour at 140°C were not completely sterilized and produced nitrites, but not nitrates after incubation for 4 weeks. The nitrite-forming organisms appear to be more resistant to heat than the nitrate organisms.

The relative amounts of nitrate and nitrous nitrogen found in some typical soils to which ammonium sulphate and calcium car-

Table 10. Decrease of nitric and nitrous nitrogen in cultures of some typical surface soils receiving ammonium sulphate and calcium carbonate—N in p.p.m.

	After incubation 28 days	
	Nitric N p.p.m.	Nitrous N p.p.m.
Gulf Coast Prairie—Upland soils		
Lake Charles clay	57	212
Hockley fine sandy loam	256	104
Lake Charles clay loam	116	270
Ochlockonee fine sandy loam	287	26
East Texas Timber Country		
Bowie fine sandy loam	144	168
Kirvin fine sandy loam	64	150
Amite fine sandy loam	275	52
Norfolk fine sand	144	83
Norfolk fine sand	33	245
Lufkin fine sandy loam	408	16
Orangeburg fine sandy loam	210	80
Ruston fine sandy loam	10	80
Susquehanna sandy loam	230	46
Susquehanna very fine sandy loam	30	105
Susquehanna fine sandy loam	172	368
Susquehanna fine sandy loam	37	172
Blackland Prairies		
Houston black clay	100	98
Irving clay	220	32
Wilson clay loam	460	21
Wilson clay	52	23
Crockett fine sandy loam	400	40
Rolling Plains		
Miles fine sandy loam	36	250
Miles fine sandy loam	430	7
Miles fine sandy loam	39	225
Vernon clay	250	19
High Plains		
Amarillo fine sand	85	188
Amarillo clay loam	84	235
Rio Grande Plain		
Rio Grande very fine sandy loam	352	180
Victoria clay loam	86	336

bonate had been added are given in Table 10. The relative quantity of nitrous nitrogen is high in some of these cultures. For example, the Lake Charles clay contain 57 p.p.m. of nitric nitrogen, and 212 p.p.m. nitrous nitrogen at the end of incubation for 4 weeks; Norfolk fine sand, 33 p.p.m. nitric nitrogen and 245 of nitrous nitrogen; the Susquehanna fine sandy loam, 172 of nitric, 368 of nitrous nitrogen; and the Amarillo clay loam, 84 and 235 p.p.m., respectively.

Biological and Chemical Conversion of Nitrites to Nitrates

Temple in 1914 (67) reported that nitrites are decomposed by an acid soil, with formation of nitrous oxides which could be detected by their odor. Turtschin (71) more recently reported that acid soils react with nitrites to produce gaseous nitrogenous compounds, with consequent losses of nitrogen. Fraps and Sterges (36) observed losses of nitrous nitrogen from acid soils. They found that when sodium nitrite was mixed with acid soils and water, and incubated at 35°C, the average loss of nitrogen from 36 soils was 47 percent of the nitrite nitrogen in the first 2 days, 55 percent in 4 days and 63 percent in 8 days. When 1 percent of calcium carbonate was also added, the average loss of nitrogen was 5 percent in 2 days, but 6 of the soil samples lost over 20 percent of the nitrite nitrogen in spite of the addition of calcium carbonate. Additional work showed that there was no loss of nitrogen which could be ascribed to formation and decomposition of nitrites during the nitrification of ammonium sulphate in 23 of 24 soils requiring additions of calcium carbonate for good nitrification. There was about 20 percent loss with one subsoil, however.

Puri, Rai and Kapur (55) oxidized nitrites to nitrates by shaking the solutions with acid soils, or with additions of dilute acetic acid or hydrochloric acid. The high ratio of water to soil and the closed bottle prevented loss of oxides of nitrogen and the shaking brought them into contact with air. They conclude that the oxidation of nitrites to nitrates by soils takes place, due to the acidity of the soil, by a purely physio-chemical process and quite independently of microbiological and photochemical agencies. Nitrification experiments are made in open vessels, not closed vessels, and as shown above, there are losses of nitrogen when nitrites are mixed with acid soils in open vessels. While nitrification occurs in acid soils, it takes place much more readily in soils which contain calcium carbonate, or other bases which neutralize the acid formed (Tables 1, 2). With insufficient numbers of nitrate-forming organisms, the nitrites formed are not completely oxidized to nitrates (Table 9). In sterilized culture media, a slight oxidation of nitrites to nitrates may occur, but additions of organisms are required for appreciable amounts of nitrates to be produced.

Considering these and other data, the inevitable conclusion is that the conversion of nitrites to nitrates in a nitrifying soil is due almost completely to biological processes, and only very small amounts to purely chemical reactions.

Effect of Sunlight on Nitrification

In a previous paper (32) evidence was given that, under Texas conditions, sunlight has little or no effect on the nitrification of ammonium salts in soils. Corbert (7) objected that the cultures were in pyrex beakers covered with glass which excludes ultra-violet rays, which usually bring about the photo-chemical reactions. In the work here reported, the soils were exposed to the direct rays of the sun.

Twelve surface soils previously found to be of high nitrifying capacity from different parts of Texas were used. Portions of 200 grams were placed in 5½-inch porcelain evaporating dishes with ammonium sulphate solution equal to 500 p.p.m. nitrogen in the dry soil, and enough water to equal 60 percent of the water capacity of the soil. They were exposed to sunlight, uncovered, for several days. Soil sterilized by heating for 3 hours at 150-160°C was used in some of the tests.

Because evaporation was high, the loss of moisture was restored by adding water twice a day. During rainy or very cloudy days and at night, the cultures were kept covered indoors. The experiment was carried out during 3 different seasons of the year, using different soils for each season, in order to find whether the intensity of light might affect the production of nitrates. In summer at College Station, according to Dunlap, sunlight may have the intensity of 10,000 candle power (15).

The incubation periods of the fall of 1935, of the spring of 1936, and of the summer of 1936 were, 39, 40 and 35 days respectively; and the time of exposure during these periods was 212½, 257 and 250½ hours, during which the sunlight was 153, 160½, and 198½ hours respectively, and the sky was cloudy for 591, 96 and 52 hours, respectively.

The results in Table 11, averages of 2 cultures each, show that the amount of nitric nitrogen in the sterilized soils exposed to light ranged from 0 to 8 p.p.m., which is in the limits of experimental

Table 11. Total ammonia-nitrogen nitrified in soils exposed to sunlight (nitrogen p.p.m.)

Exposure period and type name	Nitric nitrogen in original soil not exposed p.p.m.	Sterilized soil, Nitrogen nitrified p.p.m.	Sterilized soil plus inoculating liquid, Nitrogen nitrified p.p.m.	Unsterilized soil, Nitrogen nitrified p.p.m.
Sept. 3—Oct. 11				
Houston black clay	33	3	198	342
Sumter clay	4	8	215	386
Bell clay	8	0	198	327
Denton clay	12	0	240	368
April 27—June 5				
Quanah clay loam	6	0	202	252
Frio silt loam	6	3	192	199
Frio silt loam, subsoil	4	2	144	96
Uvalde silty clay loam	5	0	167	248
June 10—July 14				
Pawnee clay	2	0	11	104
Unknown	2	0	38	16
Prior clay loam	4	0	0	62
Uvalde silty clay	7	2	18	79

error. The sterilized soils, when inoculated, produced 0 to 240 p.p.m. of nitric nitrogen, while the unsterilized soils produced from 16 to 386 p.p.m. Sunlight did not produce nitrification.

Contrary to the opinion of Rao (57), the intensity of light or solar activity did not have any appreciable effect on the oxidation of ammonia.

The amount of nitrification was small in the summer series, probably due to a rapid loss of moisture, which resulted in the drying of the cultures between additions of water, and to the high temperatures, sometimes 48°C, occurring during this period.

These results show that nitrification of nitrogen in soils is due to bacterial action and not to sunlight. Light can penetrate only a short distance into soil. Nitrification usually takes place in the moist soil below the exposed surface where solar radiation does not penetrate.

According to Dhar and others (1, 2, 8, 9), light oxidizes ammonia in the presence of a catalyst. Some tests were made by exposing to sunlight for 3 hours, 50-c.c. portions of .2N ammonium hydroxide containing 0, .05, .12 and .50 grams of zinc oxide as a catalytic agent. Some of the solutions were aerated by means of an aspirator bottle. Small amounts of nitrous acid were produced in all 10 solutions, but no nitrate was produced. Oxidation of ammonia to nitrite takes place due to the action of the sunlight. These results confirm the work of other investigators (1, 2, 8, 9, 13, 56, 57).

Effect of Water Content, Puddling and Water-logging on Nitrification

Several investigators have studied the effect of the water content in the soil upon nitrification. In an experiment with 8 soils of high nitrifying capacities, the average nitrification of the soil nitrogen with water equal to 20 percent of the water capacity was 41 p.p.m. of nitric nitrogen; with 35 percent, 69 p.p.m.; with 50 percent, 82 p.p.m.; with 65 percent, 97 p.p.m.; with 80 percent, 95 p.p.m.; and with 100 percent, 89 p.p.m. When ammonium sulphate was present, the nitric nitrogen produced with 20, 35, 50, 65, 80 and 100 percent of the water capacity, was 206, 524, 483, 605, 579 and 578 p.p.m. of nitric nitrogen, respectively. The water content could vary between wide limits without appreciably affecting nitrification.

According to Waksman (74), Schloesing and Muntz reported that nitrate formation in soils is at a maximum with the highest moisture content that will not saturate the soil; when the soil approaches the saturation point, the process of nitrate formation is greatly reduced and may disappear completely. McGeorge and Breazeale (18) have discussed the effects of puddling and saturation of the soil with water upon plant growth. Although soil puddling is generally understood to interfere with nitrification, little experimental data on this subject has been found in the literature.

When the effect of puddling was studied, the soil and water were mixed by means of a spatula until in a pasty condition. The mixtures were then placed in beakers, compacted by striking against a cushion, then incubated.

When the quantity of water to be used exceeded 50 percent of the water capacity of the soil, and the soil was not to be puddled, the mixture was first made with water equal to 50 percent of the water capacity of the soil; after transfer to a beaker and compaction, the remainder of the water was distributed over the surface by means of a pipette.

When the effect of water-logging was studied, 40 grams of soil were used with 2 c.c. of nitrogenous solution containing 0.02 grams nitrogen, 5 c.c. of inoculating liquid, and water equal to 50 percent of the water-capacity of the soil. After mixing and transferring to a beaker, water was added so as to cover the surface to a depth of about one-fourth inch. The entire culture was used for the determination of nitrates and nitrites.

Table 12 shows the amount of nitric nitrogen produced in puddled and unpuddled cultures with or without ammonium sulphate. The soils used were neutral or slightly alkaline and of high nitrifying capacity. Practically all of them are clay soils which puddle easily. Puddling of the cultures containing water equal to 65 percent of the water-holding capacity, alone or with ammonium sulphate, for the average of 16 soils, produced only slightly less nitrification than the unpuddled soils. However, a marked decrease in nitrification of the soil nitrogen occurred in 3 of the 16 soils, and slight decreases in nitrification of ammonium sulphate in 5 soils. With a water content equal to 75 percent of the water capacity, nitrification was less in 12 of 16 puddled soils, with or without ammonium sulphate, with an average of about 25 percent less nitrification than in the unpuddled soils. When water equal to 85 percent of the water capacity was used, puddling decreased nitrification in practically all the soils. The average amount of nitrates in the puddled soils produced from the soil nitrogen was about 6 percent of those in the unpuddled soils, and that produced from the ammonium sulphate was about 40 percent of that in the unpuddled soils. Puddling was more detrimental to the nitrification of the soil nitrogen than

Table 12. Effect of puddling on nitrification of ammonium sulfate. (Nitric N. p.p.m.)

Laboratory number	Type of soil	Depth, inches	65% water capacity				75% water capacity				85% water capacity			
			Unpuddled		Puddled		Unpuddled		Puddled		Unpuddled		Puddled	
			O	N*	O	N	O	N	O	N	O	N	O	N
50302	Catalpa clay...........................	0–7	138	613	150	613	155	675	94	613	158	663	2	394
50303	Catalpa clay...........................	7–19	145	663	133	700	148	650	108	600	123	613	8	430
51293	Maverick clay loam	0–7	108	575	12	488	103	600	2	381	100	588	1	176
51298	Uvalde silty clay loam.................	0–7	90	563	2	450	72	563	4	132	66	588	0	34
51302	Uvalde silty clay......................	0–7	74	525	75	563	78	588	0	300	70	550	0	558
51307	Monteola clay..........................	0–7	30	488	32	450	31	488	3	400	32	500	0	161
51309	Frio silt loam.........................	0–7	60	525	44	430	55	563	2	163	59	488	0	70
51310	Frio silt loam.........................	7–17	46	538	44	513	49	525	4	332	46	500	3	197
51312	Maverick clay..........................	7–17	39	440	20	420	44	500	5	360	44	500	4	282
51313	Maverick clay loam.....................	0–7	84	588	38	475	103	550	2	329	96	550	0	21
51314	Maverick clay loam.....................	7–11	84	563	68	563	80	550	12	380	78	538	4	193
51315	Maverick clay loam.....................	12–17	135	650	123	600	144	625	80	550	136	625	53	450
53800	Abilene clay...........................	0–7	82	550	82	525	100	513	0	390	78	550	0	140
53803	Frio clay loam.........................	7–19	73	538	64	538	75	513	3	450	75	538	2	245
53807	Denton clay loam.......................	0–7	93	550	88	550	108	538	2	380	80	563	1	1
53810	Blanket clay loam......................	0–7	125	625	120	600	123	650	50	475	100	625	0	60
	Average.........................	....	88	562	68	530	92	568	23	390	84	554	5	213

*Ammonium sulphate added.

Table 13. Effect of puddling on nitrification of sodium nitrite. (Nitrogen p.p.m.)

Laboratory number	65% water capacity		75% water capacity			85% water capacity		
				Puddled			Puddled	
	Unpuddled Nitric N	Puddled Nitric N	Unpuddled Nitric N	Nitrous N	Nitric N	Unpuddled Nitric N	Nitrous N	Nitric N
50302*	613	613	688	0	363	663	0	105
50303	625	625	700	0	538	688	0	190
51293	625	575	650	0	250	613	0	513
51298	613	160	600	0	148	550	0	54
51302	613	575	625	0	220	638	0	92
51307	600	575	613	0	500	638	0	275
51309	613	563	613	7	281	588	0	215
51310	575	613	575	29	550	625	296	200
51312	500	380	513	0	331	525	58	250
51313	638	350	600	0	175	625	7	48
51314	575	588	588	0	370	588	0	230
51315	613	600	600	160	370	613	220	263
53800	600	575	588	0	210	588	0	123
53803	575	538	638	0	625	600	0	220
53807	613	638	638	0	300	575	0	125
53810	600	563	625	0	450	588	0	113
Average of nitrate and nitrite	599	533	616	....	367	607	....	225

*For type names see Table 12.

Table 14. Effect of water-logging on nitrification

Laboratory No.	Water added	No nitrogen added	Ammonium sulfate		Sodium nitrate	Sodium nitrite	
		Nitric N p.p.m.	Nitric N p.p.m.	Nitrous N p.p.m.	Nitric N p.p.m.	Nitric N p.p.m.	Nitrous N p.p.m.
50302*	50% water capacity	135	669	0	738	600	9
	water-logged	0	0	0	278	0	0
50303	50% water capacity	145	600	0	713	600	17
	water-logged	0	0	3	195	0	0
51293	50% water capacity	79	500	0	563	525	0
	water-logged	0	3	24	294	6	0
51298	50% water capacity	56	450	0	550	486	2
	water-logged	1	3	57	390	158	0
51302	50% water capacity	76	531	0	563	519	0
	water-logged	0	30	3	375	47	0
51307	50% water capacity	34	494	0	563	155	398
	water-logged	0	0	0	488	259	0
51309	50% water capacity	56	500	0	569	538	0
	water-logged	0	16	8	450	238	0
51310	50% water capacity	43	435	18	531	137	500
	water-logged	6	71	0	506	390	4
51312	50% water capacity	31	47	0	556	488	26
	water-logged	0	0	0	405	288	0
51313	50% water capacity	74	494	0	650	538	13
	water-logged	0	0	0	444	51	0
51314	50% water capacity	70	400	0	594	488	114
	water-logged	0	0	0	440	228	0
51315	50% water capacity	67	95	0	625	113	506
	water-logged	0	0	0	525	325	30
53800	50% water capacity	85	569	0	638	525	0
	water-logged	0	0	2	319	0	0
53803	50% water capacity	80	544	0	600	78	490
	water-logged	0	10	24	445	134	0
53807	50% water capacity	90	569	0	665	613	0
	water-logged	0	0	4	330	0	0
53810	50% water capacity	115	656	0	713	550	7
	water-logged	0	0	8	170	0	0

*For type names see Table 12.

to the nitrification of the ammonium sulphate. When the soils were not puddled, nitrification was practically the same in soils containing 65, 75 or 85 percent of their water capacity.

In another series of experiments, the effect of puddling upon nitrates was studied. The quantity of sodium nitrate containing 0.05 grams nitrogen (500 p.p.m.) was added to the cultures and incubated for 28 days. The average nitric nitrogen content of the unpuddled soils when the water content was 65 percent of the water capacity was 626 p.p.m.; with 75 percent, 626 p.p.m., and with 85 percent, 631 p.p.m. For the puddled soils, the averages were 609 for 65 percent, 534 for 75 percent and 461 for 85 percent. Puddling, therefore, decreased nitric nitrogen approximately 3 percent, 15 percent and 25 percent respectively.

The effect of puddling upon sodium nitrite equal to 500 p.p.m. nitrogen is shown in Table 13. The nitrite was converted completely to nitrate in all the unpuddled soils, and almost completely in the puddled soils containing water equal to 65 percent of the water capacity. The conversion of nitrite to nitrate was not complete in 3 each of the 16 soils containing water equal to 75 percent and 85 percent of the water capacity. In addition, the sum of the nitrous and nitric nitrogen was less than the amount originally present. There was a loss of nitrogen in practically all of the puddled soils. The loss averaged about 40 percent when 75 percent of the water capacity was present, and about 63 percent when the water content was 85 percent of the water capacity.

The effect of water-logging is shown in Table 14. Very little nitrification of ammonium sulphate occurred in the 16 water-logged soils, although there were slight amounts of nitrates and nitrites formed in 4 of the soils. When nitrates had been added, losses of nitrates occurred with the water-logged soils. The nitrites added were not completely oxidized in some of the soils. Nitrites persisted in only 2 of the water-logged soils, and then only in very small quantity. Appreciable proportions of the nitrite nitrogen was converted to nitrate in 10 of the 16 water-logged soils, but there were high losses of nitrous and nitric nitrogen in all the water-logged soils. The experiment does not show whether the nitrogen so lost was converted to other forms, or entirely lost.

Nitrification of Ammonium Salts of Organic Acids

Nitrification tests have been used to compare the value of nitrogen in different organic fertilizers, but the results varied in different soils (18, 78). Temple (68) reported that ammonium citrate, ammonium oxalate and ammonium tartrate nitrified faster than ammonium sulphate or chloride, and Waksman (74) states that ammonium salts of organic acids are oxidized rapidly, but little data are available on the nitrification of ammonium salts of organic acids.

Nitrification was conducted by the methods already described, with nitrogen added equal to 500 p.p.m. of the soil, in the form of ammonium sulphate, ammonium oxalate, ammonium acetate, ammonium tartrate and ammonium citrate. The nitrogen in the 4 organic ammonium salts were, in general, oxidized to a greater extent than that of the ammonium sulphate. The differences in the nitrification between the 4 organic salts were small. The average percentage of nitrogen oxidized in the 23 soils was 43 for ammonium sulphate, 59 for ammonium oxalate, 53 for ammonium acetate, 57 for tartrate and 56 for citrate. With Houston clay, Reinach silt loam and Nimrod fine sand, the nitrification of the organic compounds was less than that of the ammonium sulphate, being with the Houston clay subsoil, 32 percent for the ammonium sulphate, 29 percent for the ammonium oxalate, 0 percent for the ammonium acetate, 11 percent for the ammonium tartrate and 32 percent for the ammonium citrate. The nitrification of the organic salts in the other 2 soils were only slightly less than for the ammonium sulphate.

The greater nitrification of the organic ammonium salts might be partly due to the lower degree of acidity of the organic acids than that of the sulphuric acid liberated in the process of nitrification of ammonium sulphate. In order to ascertain if such was the case, the effect of the addition of 1 gram (1 percent) of calcium carbonate to 12 cultures was tested. With all except 3 of the soils, when calcium carbonate was added, nitrification of the ammonium sulphate was practically the same as that of the ammonium oxalate or ammonium tartrate. With Miguel fine sandy loam, Duval fine sandy loam and Nimrod fine sand, the nitrification of the organic compounds was less than that of the ammonium sulphate. The average nitrification without calcium carbonate was 40 percent for ammonium sulphate, 50 percent for ammonium oxalate and 40 percent for ammonium tartrate. With calcium carbonate, the average nitrification was 73 percent for ammonium sulphate, 66 percent for ammonium oxalate and 65 percent for ammonium tartrate.

Carbonic acid is a very weak acid. The nitrification of ammonium carbonate, alone and with 1 percent calcium carbonate, was compared in 12 soils. The soils used were neutral or slightly acid but of low basicity, except one soil. All had low nitrification capacities. The nitrogen was added at the rate of 500 p.p.m. of soil, and the cultures were incubated 28 days. The average nitrification was:

soil alone, 40 p.p.m.; ammonium sulphate, 102 p.p.m.; ammonium carbonate, 180 p.p.m.; ammonium sulphate and calcium carbonate, 336 p.p.m., and ammonium carbonate and calcium carbonate, 320 p.p.m. Ammonium carbonate alone was nitrified to a greater extent than ammonium sulphate alone. When, however, calcium carbonate was added, the nitrification in 10 of the 12 soils averaged almost the same for both salts. With Lake Charles fine sandy loam, the ammonium sulphate was nitrified appreciably less than the ammonium carbonate, but with Bowie fine sandy loam, the nitrification of the ammonium carbonate was appreciably less than that of the ammonium sulphate. On the whole, the difference between the nitrification of the ammonium carbonate alone and the ammonium sulphate alone was apparently due to the greater amount of acid produced in nitrification of ammonium sulphate.

Nitrification of Cottonseed Meal

Cottonseed meal is representative of organic compounds with which the nitrogen of the organic matter is believed to be first changed to ammonia before it is converted to nitrites and nitrates. Cottonseed meal has been reported to be nitrified to a greater extent than ammonium sulphate in some soils, but usually its nitrification is less. According to Temple (67), when tankage and other organic nitrogenous compounds are acted on by the soil organisms, ammonia is formed in excess of the acid products, neutralizes them, and allows the nitrification to proceed. The object of the work here reported is to ascertain why cottonseed meal is nitrified to a greater extent than ammonium sulphate in some soils, and the extent of nitrification of this substance in soils of different nitrifying powers. Nitrification of cottonseed meal has been studied, among others, by Lipman (43), Lipman and Burgess (44), Coleman (8), Allison (1), Carter (7) and Withers and Fraps (78).

Three groups of 4 cultures each were prepared from each soil, namely, one with no other addition, one with inoculating liquid, one with 1 gram calcium carbonate, and one with both inoculating liquid and calcium carbonate. Four similar cultures were prepared but with ammonium sulphate equivalent to 500 p.p.m. of nitrogen, and 4 similar ones with cottonseed meal containing an equal quantity of nitrogen. The amounts of nitric nitrogen found in the cultures which did not receive any nitrogen were subtracted from the amount in the corresponding cultures which received ammonium sulphate or cottonseed meal so as to ascertain the net quantities produced from these nitrogenous compounds.

Table 15. Nitrification of ammonium sulphate and of cottonseed meal. (Nitric nitrogen in p.p.m.)

	Original soil		Inoculating liquid		Calcium carbonate		Inoculating liquid plus calcium carbonate	
	Amm. sulfate p.p.m.	Cottonseed meal p.p.m.	Amm. sulfate p.p.m.	Cottonseed meal p.p.m.	Amm. sulfate p.p.m.	Cottonseed meal p.p.m.	Amm. sulfate p.p.m.	Cottonseed meal p.p.m.
Surface soils								
Duval fine sandy loam	8	23	27	75	265	208	355	203
Pryor clay	42	35	215	37	46	8	195	47
Wilson clay	60	108	30	157	432	231	447	236
Average (3)	37	55	107	90	248	149	332	162
Lazaro clay loam	110	227	127	265	477	301	476	303
Houston black clay	141	186	280	199	125	197	320	279
Lewisville clay	266	221	439	274	255	215	457	312
Webb fine sandy loam	348	232	133	226	425	226	412	233
Average (4)	216	217	245	241	321	235	416	282
Frio silt loam	435	218	460	238	482	218	464	239
Pryor clay loam	477	216	459	232	485	224	492	228
Uvalde clay	499	131	480	146	498	135	493	155
Uvalde clay loam	455	238	492	215	477	238	499	254
Uvalde silty clay loam	538	244	510	251	514	257	539	269
Uvalde silty clay	467	240	516	241	480	222	499	195
Sumter clay	484	237	485	237	460	261	458	264
Bell clay	403	241	480	289	200	287	495	283
Denton clay	403	300	510	282	483	272	488	306
Average (9)	462	229	488	237	453	235	492	244
Subsoils								
Duval fine sandy loam	0	46	0	147	181	166	279	258
Pryor clay	35	22	77	21	29	60	62	3
Uvalde silty clay	55	36	395	78	180	13	329	75
Webb fine sandy loam	0	0	0	50	108	110	129	215
Houston black clay	45	17	178	177	22	38	162	198
Wilson clay	20	58	7	152	38	73	347	266
Bell clay	92	85	305	20	31	49	272	234
Denton clay	7	33	190	149	199	97	478	300
Average (8)	32	37	144	99	99	76	257	194
Pease clay	102	91	375	163	99	57	350	166
Pryor clay loam	116	43	240	90	59	50	192	33
Uvalde silty clay loam	194	179	414	296	122	140	328	276
Lewisville clay	100	110	449	253	106	110	447	279
Uvalde clay	241	111	470	116	286	67	413	73
Average (5)	130	107	390	184	134	85	346	165
Frio silty loam	427	191	480	238	377	201	450	252

The results secured are given in Table 15. The soils are arranged in groups according to the amount of nitric nitrogen produced from the ammonium sulphate. Most of the surface soils used had high capacities to nitrify ammonium sulphate, while most of the subsoils have low nitrifying capacities. With the surface soils of high nitrifying capacity, the average production of nitric nitrogen in p.p.m. is 229 for cottonseed meal, compared with 462 for ammonium sulphate. That is, the nitrification of the nitrogen of cottonseed meal in such soils was practically 50 percent of that of the ammonium sulphate.

The additions of inoculating liquid or calcium carbonate stimulated nitrification of both cottonseed meal and ammonium sulphate in some of the soils and subsoils which otherwise nitrified below the maximum. In most of the samples with low nitrifying capacity, especially the subsoils, calcium carbonate and inoculating liquid combined gave the greatest increases in nitrification. A few soils failed to nitrify high amounts of ammonium sulphate after receiving both calcium carbonate and bacteria; this may be due to need for phosphates (35), or to other factors not yet ascertained.

The nitrification of cottonseed meal in soils of low nitrifying capacity, though low, was greater than that of the ammonium sulphate in 4 of the original soils and 3 of the original subsoils. When both calcium carbonate and bacteria were added, the ammonium sulphate was nitrified to a greater extent than cottonseed meal in all the soils.

With surface soils and subsoils of Pryor clay, of Uvalde silty clay, Pryor clay loam and Uvalde clay, the nitrification of cottonseed meal was much below normal compared with ammonium sulphate. This deficiency may have been due to insufficient conversion of organic nitrogen to ammonia during the experiment, but to ascertain the exact cause needs further investigation.

Soils of low nitrifying capacity may convert greater percentages of the nitrogen of cottonseed meal to nitrates than that of ammonium sulphate. This may be due to the production of ammonia which reduces the acidity, as suggested by Temple (67). In soils of high nitrification capacity, ammonium sulphate is nitrified to a greater extent than cottonseed meal.

Persistence of Effects of Added Organic Matter on Oxidation of Nitrogen

It is well known that microorganisms which increase during the rapid decomposition of organic materials will assimilate nitrates already present in soils and will interfere with nitrification. After the rapid decomposition which occurs when the organic matter is first added is finished, nitrates may again begin to be produced. Data as to the duration of the depressing effect of the organic matter in nitrification is inadequate, and some information on this point is herewith presented.

The depressing effect on nitrification by glucose, sucrose and starch have been reported by Lipman *et al.* (45), that of sawdust and other tree products by Gibbs and Werkman (38). Spalding and Eisenmerger (5) noticed that nitrification was depressed when the carbon-nitrogen ratio of the plant materials was greater than 1:30. Fraps (21) reported that organic materials disappear rapidly during the first 3 weeks after application, then much more slowly.

The nitrification cultures were prepared by the method already described. Different amounts of representative organic substances were first mixed with soil, then with inoculating liquid, ammonium sulphate and with water equal to 50 percent of the water capacity of the soil. The cultures were incubated at 35°C for 28 days or longer, after which nitrates and nitrites were determined.

The effect of certain organic additions on nitrification of the soil nitrogen for 28 days is indicated by O and of ammonium sulphate is indicated by N in Table 16. The relative depressing effects were approximately in the order: cottonseed oil (greatest), then starch, cane suger, grapefruit peelings and pecan shells, (least). The cocoa shells did not decrease nitrification of the soil nitrogen in any of the soils, and of ammonium sulphate in 3 of the 4 soils.

The effects on nitrification of incubation for from 4 to 20 weeks are given in Table 17. Cocoa shells and pecan hulls had no depressing effect on nitrification. The depressing effect of grapefruit peelings lasted 8 weeks. With starch, cane sugar and cottonseed oil, the amounts of nitrates produced at the end of 20 weeks was lower than those produced with the check culture, the decrease being greater with the 2 percent application than with the 1 percent. The effects of application of these substances persisted for a long time, although one would expect both the starch and sugar to be oxidized rapidly.

Tests with 16 soils were made to ascertain the effects of the various organic additions upon sodium nitrate added equal to 500 p.p.m. of nitrogen. The average p.p.m. of nitric nitrogen in the 16 soils after incubating 28 days were: no addition, 70; nitrate of soda, 643; nitrate of soda with 1 percent starch, 505; with 2 percent starch, 393; with 1 percent cane sugar, 480; with 2 percent cane sugar, 366; with 1 percent grapefruit peelings, 568; with 2 percent

Table 16. Effect of organic substances on nitrification during incubation for 28 days. (Nitrogen p.p.m.)

Organic substance in 100 gm. soil	Wilson clay		Uvalde clay loam		Reagan silty clay loam, subsoil		Uvalde silty clay loam, subsoil	
	O* p.p.m.	N** p.p.m.	O p.p.m.	N p.p.m.	O p.p.m.	N p.p.m.	O p.p.m.	N p.p.m.
None	100	550	76	588	43	563	53	588
1% starch	1	263	2	360	0	90	0	370
2% starch	1	71	2	190	0	5	0	170
1% cane sugar	16	288	4	350	0	154	4	430
2% cane sugar	1	123	2	174	0	0	0	81
1% cocoa shells	157	390	164	588	148	600	120	613
2% cocoa shells	195	430	260	613	235	588	215	650
1% pecan hulls	15	488	4	513	0	460	0	488
2% pecan hulls	2	388	2	475	0	463	0	450
1% grapefruit peelings	51	418	27	500	7	430	11	500
2% grapefruit peelings	19	299	5	360	0	325	4	369
1% cottonseed oil	3	93	2	232	0	351	0	230
2% cottonseed oil	1	12	1	2	0	160	0	59

*Organic matter added. **Ammonium sulphate added.

Table 17. Effect of organic substances on nitrification with various periods of incubation. (Nitric N. p.p.m.)

Organic substance per 100 gms. soil	Incubation period									
	4 weeks		8 weeks		12 weeks		16 weeks		20 weeks	
	O* p.p.m.	N** p.p.m.	O p.p.m.	N p.p.m.	O p.p.m.	N p.p.m.	O p.p.m.	N p.p.m.	O p.p.m.	N p.p.m.
No addition	43	563	71	475	98	588	153	563	190	663
1% starch	0	90	1	300	7	363	36	390	66	475
2% starch	0	5	2	243	0	288	0	331	14	363
1% cane sugar	0	154	13	313	43	410	56	400	113	460
2% cane sugar	0	0	3	284	11	300	8	294	55	288
1% cocoa shells	148	600	190	588	230	638	....	....	....	....
2% cocoa shells	235	588	300	625	331	688	....	....	....	....
1% pecan hulls	0	460	12	460	17	513	105	563	128	675
2% pecan hulls	0	463	3	438	1	400	28	440	55	575
1% grapefruit peelings	7	430	76	500	110	513	....	....	....	....
2% grapefruit peelings	0	325	69	388	125	463	....	....	....	....
1% Wesson oil	0	351	1	288	1	313	6	350	35	430
2% Wesson oil	0	160	3	42	2	331	0	180	0	269

*Organic matter added. **Ammonium sulphate added.

grapefruit peeling, 503; with 1 c.c. cottonseed oil, 464; with 2 percent cottonseed oil, 339. All the organic additions decreased the nitric nitrogen content of the soil, the quantity of decrease differing with different soils, and the decrease being greater with 2 percent organic material than with 1 percent. The average decrease was in the order: Wesson oil (greatest), then cane sugar, starch and grapefruit peel (least).

Nitrification of Cyanamid

Cyanamid is recognized as an excellent nitrogenous fertilizer. It is used alone and in mixed fertilizers. It is also used for the purpose of killing certain undesirable plants. Although under some circumstances it may prove injurious to plants, methods of applying it safely are well known. Previous investigations have shown that the nitrogen of cyanamid is not readily nitrified in some soils. Why this is the case, and how long cyanamid persists without nitrification, has not been clearly demonstrated.

Cowie (10) reports that cyanamid when applied alone changed to nitrate almost quantitatively in 80 days. De Grazia (12) concluded that, until nitrification begins, cyanamid has a harmful effect on the microorganisms of the soil. Crowther and Richardson (11) found that the nitrification of calcium cyanamid is slow. Wagner, as quoted by Pranke (54), states that nitrification of calcium cyanamid was normal with small applications, but with larger applications nitrification was low. Hall (39) reports that nitrification of calcium cyanamid did not occur in 2 soils. McGuinn (49), Cowie (10) and Mukerji (50) concluded that dicyandiamid hinders nitrification, but does not hinder ammonification. Murata (51) found that ammonification of dicyandiamid was very slow. Kuhn and Drecksel (42) state that calcium cyanamid increases the number of soil bacteria in neutral or alkaline soils, but has less effect on acid soils.

The procedure used is similar to that described on a preceding page. The desired amount of cyanamid or other substance was added to 100 grams of soil together with inoculating liquid and water. After incubation, nitrate and nitrite nitrogen were determined.

In preliminary work nitrification did not occur in 28 days when cyanamid equal to 500 p.p.m. of soil nitrogen was added. Soils of high nitrifying capacity were then tested, using cyanamid equivalent to 50, 100, 250 and 500 p.p.m. of nitrogen. In some of the cultures ammonium sulphate providing 500 p.p.m. of nitrogen and sodium nitrite providing 500 parts p.p.m. of nitrogen were used in addition to the cyanamid. With cyanamid added equivalent to 0, 50, 100, 250, 500 nitrogen in p.p.m. of the soil, the nitrate nitrogen found at the end of 28 days was 102, 101, 110, 33 and 12 p.p.m. (averages of 9 soils). The nitric nitrogen produced when cyanamid was added averaged less than that in the soil alone, except when 100 p.p.m. was used. When the 9 soils received 500 p.p.m. nitrogen in ammonium sulphate in addition to cyanamid nitrogen, the nitrate nitrogen produced at the end of 28 days averaged 521, 407, 146, 30 and 15 p.p.m. with 0, 50, 100, 250 and 500 p.p.m. of cyanamid nitrogen, respectively. The cyanamid was not nitrified, but decreased the nitrification of the ammonium sulphate. It did not entirely stop nitrification until more than 100 p.p.m. cyanamid nitrogen was added. When the soils received sodium nitrite equivalent to 500 p.p.m. of nitrogen in the soil, the average nitric nitrogen at the end of the incubation period was 447, 460, 351, 98 and 37 p.p.m., corresponding to additions of 0, 50, 100, 250 and 500 p.p.m. of cyanamid nitrogen. The cyanamid interfered with the oxidation of nitrite to nitrate, but did not entirely prevent it when less than 250 p.p.m. of cyanamid nitrogen was added.

Previous work has shown that additions of calcium carbonate bring about nitrification in soils which would not otherwise nitrify. Tests were made to see if 1 percent of calcium carbonate affected the nitrification of cyanamid in soils which had low nitrifying powers for ammonium sulphate when calcium carbonate was not added. Some of the cultures were incubated for 28 days only. Others, after incubation for 28 days, were reinoculated by mixing each culture thoroughly with 10 c.c. of inoculating liquid and incubated for 28 days longer. The ammonium sulphate was nitrified very little in the soils when calcium carbonate was not added, but was nitrified to the average extent of about 75 percent when calcium carbonate was added. When cyanamid was added in addition to the ammonium sulphate at the rate of 50, 100 or 250 parts per million, no nitrification occurred whether calcium carbonate was added or not, either in the first 28 days or when reinoculated and incubated 28 days longer. The calcium cyanamid decreased production of nitrates from the soil nitrogen. Calcium carbonate could not be expected to help the nitrification of cyanamid, since cyanamid itself may produce basic calcium salts.

The results of the previous experiments indicated that cyanamid either destroyed the nitrifying organisms or temporarily hindered their activity. To secure information as to how long the injurious action persists, cultures from 12 soils with high nitrifying power for ammonium sulphate received cyanamid equivalent to 500 p.p.m.

of nitrogen and were reinoculated with active nitrifying organisms after 0, 1, 2, 3, 4, 5, 6, 7 and 8 weeks of incubation. After the desired incubation period, each culture was mixed thoroughly with 10 c.c. of inoculating liquid, returned to the beaker, and incubated for a further period of 28 days.

The results with 12 soils show that the addition of cyanamid prevented nitrification although reinoculated with nitrifying organisms after 8 weeks.

With 2 soils, the experiment was conducted for periods up to 38 weeks with use of cyanamid equal to 500, 250 and 100 p.p.m. of nitrogen in the soil. The results are given in Table 18. With 500 p.p.m. of cyanamid nitrogen, nitrification did not occur with Reagan silty clay loam after incubation for 38 weeks followed by reinoculation, but some nitrification occurred in Uvalde silty clay when inoculated after 30 weeks. With 250 p.p.m. of cyanamid nitrogen, nitrification occurred in Uvalde silty clay when inoculated after 4 weeks, and after 30 weeks with Reagan silty clay loam. With 100 p.p.m. of cyanamid nitrogen, nitrification began immediately with Uvalde silty clay, and after 4 weeks with Reagan silty clay loam.

Table 18. Nitrification of different amounts of cyanamid after reinoculations. (Nitric N p.p.m.)

Addition	Reinoculation after					
	0 weeks	4 weeks	16 weeks	24 weeks	30 weeks	38 weeks
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Reagan silty clay loam 4-7						
No addition	94	208	313	438	494	525
500 p.p.m. cyanamid N	31	38	37	38	38	37
250 p.p.m. cyanamid N	31	38	41	44	51	272
100 p.p.m. cyanamid N	32	53	208	281	370	488
Uvalde silty clay 4-7						
No addition	88	215	331	415	538	569
500 p.p.m. cyanamid N	8	44	56	76	88	245
250 p.p.m. cyanamid N	41	227	385	488	588	713
100 p.p.m. cyanamid N	125	250	385	460	556	663

Cyanamid, or products of its decomposition, may interfere seriously with nitrification for long periods of time, and do not themselves nitrify. This depressing effect may persist for 38 weeks or longer, and depends upon the amount of cyanamid added and the nature of the soil to which it is applied.

Diffusion of the Toxic Substances of Cyanamid

In the preceding experiments, the cyanamid was mixed thoroughly with the soil. In order to see if the toxic substances would diffuse, experiments were made in which the cyanamid was all placed in a hole punched in the center of each culture. The cultures were incubated for 2, 4 and 6 months. The cultures received inoculating liquid at the beginning of the experiment and had no further inoculation.

Lumps of cyanamid, or its residue, were found in all the cultures at the end of each period. The results are given in Table 19. The amount of nitrate nitrogen in Reagan clay loam was less in the cultures to which cyanamid was added than in those of the soil alone, even after 6 months. The amounts of nitrate nitrogen in Uvalde silty clay which received 250 or 500 p.p.m. of cyanamid nitrogen, was less than that in the soil alone, even after 6 months, but there was some nitrification in the culture which received 100 p.p.m. of cyanamid nitrogen. The results show that the substances which interfere with nitrification diffuse to some extent from cyanamid placed in one spot in the soil.

Table 19. Diffusion of toxic substances of cynamid in soil (Nitric N p.p.m.)

Treatment	Nitrogen added p.p.m.	Incubation period			Nitrification (percent)		
		Months			Months		
		2	4	6	2	4	6
		p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Reagan silty clay loam, 7-24							
No addition	....	112	200	313	......	......	......
Ammonium sulphate	500	588	631	750	95	86	87
Cyanamid	500	32	30	32	0	0	0
Cyanamid	250	32	31	33	0	0	0
Cyanamid	100	36	72	146	0	0	0
Uvalde silty clay, 4-7							
No addition	....	119	253	347	......	......	......
Ammonium sulphate	500	638	744	813	104	98	93
Cyanamid	500	30	44	42	0	0	0
Cyanamid	250	95	108	120	0	0	0
Cyanamid	100	170	306	400	51	53	53

According to McCool (47), soil conditions are usually favorable to the rapid transformation of cyanamid to urea. Free cyanamid may also be formed, which polymerizes to dicyandiamide. To ascertain which of some products from cyanamid are readily nitrifiable, or are toxic to the nitrifying organisms, dicyandiamide, guanidine carbonate, guanylurea sulphate and urea providing 500, 250 and 100 p.p.m. nitrogen for each culture were tested in three soils.

According to the results in Table 20, urea nitrifies readily. Guanidine carbonate nitrified fairly well with Maverick loam and Uvalde silty clay loam, especially when added at the rate of 100 p.p.m. of nitrogen. The nitrification of guanidine carbonate was very low in Houston black clay. Guanylurea sulphate underwent only slight nitrification even when at the rate of 100 p.p.m. nitrogen. Dicyandiamide showed no nitrification whatever, and interfered with

Table 20. Nitrification of cynamid products (Nitrate nitrogen in parts per million)

Treatment	Nitrogen added p.p.m.	Houston black clay	Maverick loam	Uvalde silty clay loam
None		56	100	74
Cyanamid	500	6	12	12
Cyanamid	250	8	56	43
Cyanamid	100	7	176	140
Dicyandiamid	500	6	13	13
Dicyandiamid	250	7	13	16
Dicyandiamid	100	9	31	24
Guanidine carbonate	500	14	244	120
Guanidine carbonate	250	31	325	153
Guanidine carbonate	100	64	210	153
Guanylurea sulphate	500	24	73	48
Guanylurea sulphate	250	36	113	64
Guanylurea sulphate	100	60	120	84
Urea	500	588	638	625
Urea	250	313	360	380
Urea	100	168	215	185

nitrification of the nitrogen of the soil. It may be concluded that dicyandiamide and, to a lesser extent, guanylurea, hinder nitrification and would have this effect if they are formed from cyanamid applied to soils.

The effect of dicyandiamide and guanylurea on the nitrification of ammonium sulphate was also tested in 8 soils. The dicyandiamid decreased nitrification of ammonium sulphate, even when quantities containing nitrogen equal to only 100 or 50 p.p.m. of soil was added. A similar experiment was made to test the effect of dicyandiamide upon the oxidation of nitrite to nitrate on 9 soils. The dicyandiamide decreased the oxidation of nitrites, but not to such a great extent as it did the nitrification of ammonia. In quantities equal to 100 or 50 p.p.m. of nitrogen, it had only a slight depressing effect. Guanylurea did not prevent nitrification of ammonium sulphate when an amount was used containing 100 p.p.m. of nitrogen, but was not itself nitrified even when only 50 p.p.m. was used.

According to Fink (16), adsorptive substances such as activated charcoal and iron hydroxide, have the tendency to eliminate the toxic affect of cyanamid on plants. Portions of cyanamid furnishing 500, 100 and 50 p.p.m. nitrogen were mixed with fuller's earth, iron hydroxide, oat hulls, superphosphate, monopotassium and monocalcium phosphates, and elementary sulphur, and used in experiments similar to those already described. The additions did not decrease the depressing effect of cyanamid on nitrification.

Because cyanamid is mixed with superphosphate in the manufacture of mixed fertilizers, nitrification experiments were made to test the effect of superphosphate. For mixtures A, B and C, 2 grams of superphosphate were mixed with the equivalent of 100, 250 and 500 p.p.m. of nitrogen in 100 grams of soil in a porcelain dish, moistened with water and allowed to stand one week. For 3 other cultures, the 2 grams of superphosphate were mixed with 100 grams of soil, the 3 quantities of cyanamid next mixed in, and cultures then prepared. Nitrification cultures were prepared as usual and incubated for 28 days. The experiment was repeated, with practically the same results. A little nitrification occurred with 3 of the 6 soils when the amount of cyanamid nitrogen was 100 p.p.m. Practically no nitrification occurred when the cyanamid nitrogen added was equal to 250 or 500 p.p.m. The results were practically the same whether the superphosphate was mixed separately with the soil, or mixed first with the cyanamid, moistened and allowed to react for a week. That is to say, the wet superphosphate did not react with the cyanamid to produce compounds more readily nitrified than cyanamid itself.

The results offer an explanation why cyanamid occasionally does not give satisfactory crop yields. Cyanamid, on the one hand, is not readily nitrified and, on the other hand, it may hinder the activities of the nitrifying organisms. Consequently, if too much cyanamid is applied, nitrogen starvation of crops may follow. Sufficient time for the chemical decomposition of cyanamid before planting may avoid this difficulty. Small amounts of cyanamid may give more satisfactory crop yields than large amounts, because the small amounts nitrify sooner and, at the same time, do not depress so greatly the nitrification of the soil organic matter.

Effect of Sulphur on Nitrification

Elemental sulphur is applied to certain soils to promote the growth of crops in Texas and other states on the Mexican border, and along the Pacific coast. In a few of the Western States, sulphur may act as a plant food on soils that contain sufficient amounts of nitrogen and phosphorus. Where nitrogen and phosphorus are deficient, and commercial fertilizers are applied, sufficient sulphur for plant food purposes are furnished by ammonium sulphate or superphosphate (24). In Texas, especially in the Lower Rio Grande Valley, elemental sulphur is applied to calcareous soils on which certain plants, chiefly citrus trees, suffer from chlorosis. When placed in holes or furrows, the sulphur oxidizes to sulphuric acid which produces acid spots or streaks from which the plant can secure iron, manganese or other elements otherwise not available to plants in some calcareous soils. Sulphur has been used experimentally where reasonable amounts would acidify soils of low basicity, in the study of cotton root rot (66), and in control of other plant diseases.

Sulphur might interfere with nitrification by production of sulphuric acid, or possibly in other ways. Lipman, Prince and Blair (46), St. John (63), and Shedd (61) reported that sulphur had little unfavorable effect on nitrification, while Brown (5), and Ames and Richmond (2) reported depressing effects. Waksman (74) notes that certain sulphur-oxidizing bacteria may reduce nitrates to elemental nitrogen.

Soils of high nitrifying capacity were selected. Portions of 100 grams of soil received additions of 0.5, 1.0, 1.5, 2.0 and 5.0 grams of finely ground sulphur, plus inoculating liquid and water. Some of the cultures received additions of 5 c.c. ammonium sulphate solution containing .05 grams of nitrogen, equivalent to 500 p.p.m. of the soil. Next, pH values were determined with the potentiometer using quinhydrone. In one experiment, sodium nitrate equal to 500 p.p.m. of nitrogen of the soil was added with sulphur to see if the elemental sulphur caused any loss of nitrates.

All the additions of sulphur depressed nitrification of the soil nitrogen (Table 21) and of the ammonium sulphate (Table 22). The average nitric nitrogen produced from the soil nitrogen (11 soils) was 83 p.p.m. without sulphur, 31 with 0.5 percent sulphur, and 5 parts per million with 5 percent sulphur. The average nitric nitrogen production from soil plus ammonium sulphate was 553 p.p.m. without sulphur, 421 with 0.5 percent sulphur, 344 with 1 percent, 314 with 1.5 percent, 306 with 2 percent and 236 p.p.m. with 5 percent sulphur.

As shown in Table 23, the oxidation of the sulphur during the incubation period of 28 days was not sufficient to bring the pH more than slightly below the neutral point of pH 7, even with 5 percent sulphur, except with samples 47662, 51293 and 51313 with which

Table 21. Effect of quantities of sulphur on nitrification of soil nitrogen. (Nitric nitrogen parts per million)

Number	Type	Sulphur					
		0 p.p.m.	.5% p.p.m.	1.0% p.p.m.	1.5% p.p.m.	2.0% p.p.m.	5.0% p.p.m.
36482	Pawnee clay	63	40	14	4	2	0
39688	Pryor clay loam	50	4	1	0	0	0
44357	Reagan silty clay loam	64	15	3	0	0	0
44369	Uvalde silty clay	60	4	9	2	3	5
49662	Houston black clay	61	17	8	5	4	3
50302	Catalpa clay	142	109	91	64	13	17
51293	Maverick clay loam	78	34	17	18	14	8
51298	Uvalde silty clay loam	64	16	3	0	0	0
51302	Uvalde silty clay	120	28	12	0	2	0
51309	Frio silt loam	90	14	3	0	0	0
51313	Maverick clay loam	118	65	38	29	19	26
	Average (11)	83	31	18	11	5	5

Table 22. Effect of amount of sulphur on nitrification of ammonium sulphate. (Nitric nitrogen parts per million)

Number	0 p.p.m.	Sulphur .5% p.p.m.	Sulphur 1.0% p.p.m.	Sulphur 1.5% p.p.m.	Sulphur 2.0% p.p.m.	Sulphur 5.0% p.p.m.
36482*..............	588	433	321	259	299	48
39688..............	513	430	350	331	350	330
44357..............	488	440	380	356	344	263
44369..............	575	395	384	252	326	245
49662..............	500	266	14	6	8	7
50302..............	619	547	516	491	457	451
51293..............	538	156	63	75	55	25
51298..............	538	475	463	438	390	363
51302..............	600	500	430	420	380	327
51309..............	538	430	387	325	269	175
51313..............	588	563	475	500	463	360
Average (11).....	553	421	344	314	306	236

*For name of soil type see Table 21.

the pH values were 4.5, 5.0 and 5.2, respectively. The oxidation of nitrogen was lowest in soils 49662 and 51293, which were characterized by the lowest basicity (Table 23).

Complete oxidization of the 0.5 percent sulphur would produce sulphuric acid sufficient to neutralize nearly 1.5 percent calcium carbonate, which was less than the basicity of any of the soils used. Part of the basicity (Table 23) is due to replacement of bases by hydrogen in the exchange complex. It, therefore, does not require neutralization of all the basicity shown in Table 23 to produce an acid soil condition. This is shown with samples 49662 and 51293 where the pH was reduced with 0.5 percent sulphur.

The amounts of sulphur used in this study may appear excessive, since an addition of 0.5 percent is equal to 10,000 pounds on the basis of 2 million pounds of soil per acre. Sulphur is not soluble in water and cannot be distributed by soil moisture. It could not practically be intimately mixed with the entire surface layer of 7 inches.

Table 23. Effect of amount of sulphur and nitrification of ammonium sulphate upon pH of soils

Number	Basicity of original soil, CaCO	Sulphur 0	Sulphur 0.5%	Sulphur 1.0%	Sulphur 1.5%	Sulphur 2.0%	Sulphur 5.0%
	%	pH	pH	pH	pH	pH	pH
36482*.............	4.8	7.7	7.6	7.7	7.4	7.3	7.0
39688..............	6.4	7.5	7.3	7.2	7.0	6.5	6.8
44357..............	21.2	7.6	7.3	7.5	7.4	7.4	7.0
44369..............	13.6	7.4	7.5	7.6	7.5	7.2	7.5
49662..............	3.4	7.4	6.6	5.8	5.2	5.3	4.5
50302..............	21.7	7.9	7.2	7.3	7.1	7.3	7.1
51293..............	1.8	7.3	4.8	4.4	4.3	4.1	4.0
51298..............	12.5	7.3	7.3	7.2	7.3	7.0	6.6
51302..............	17.9	7.4	7.4	7.5	7.5	7.5	7.1
51309..............	51.1	7.2	7.5	7.4	7.6	7.4	7.4
51313..............	5.2	7.5	7.3	6.9	5.8	5.4	5.2

*For name of soil types see Table 21.

Consequently, when small amounts of sulphur are applied in holes or furrows, the quantity in immediate contact with the soil may be as much as or more than the proportions used in this experimental work. Although sulphur particles may depress nitrification in the soil near them, such depression in calcareous soils is limited in area and, therefore, must be regarded as not of practical significance. In soils of low basicity, a temporary or even prolonged depression of nitrification may occur when sulphur is intimately mixed in to produce an acid soil.

To ascertain whether sulphur decreases nitrates already in the soil, nitrate of soda equivalent to 500 p.p.m. of nitrogen was added with sulphur to 16 soils and nitrates and nitrites determined after 28 days incubation. The average p.p.m. of nitric nitrogen were, with soil alone, 70, sodium nitrate alone, 643, sodium nitrate with 1 gram sulphur, 587, and sodium nitrate with 2 grams sulphur, 564 p.p.m. Sulphur decreased slightly the amounts of nitrates present. This depression may have been due partly to decrease in nitrification of the soil nitrogen, or partly to reduction of the nitrates by the sulphur or sulphur-oxidizing bacteria. Apparently any such reduction is comparatively small, less than 10 percent.

Effect of Phosphorus, Magnesium and Iron on Nitrification

Additions of calcium carbonate and inoculating liquid did not produce maximum nitrification of ammonium sulphate with all the soils tested. With some of these soils, additions of available phosphates increased nitrification (35). Additional tests were made with some other samples having low nitrifying capacities, and on which the inoculation liquid, calcium carbonate and phosphates did not greatly increase nitrification.

Of 6 surface soils, nitrification was increased in Miles fine sand and Elwood fine sandy loam by dicalcium phosphate, in Refugio loamy fine sand and Crystal loamy fine sand by dicalcium phosphate, magnesium sulphate and ferrous sulphate, and in Duval fine sandy loam by dicalcium phosphate and magnesium sulphate. These additions did not increase nitrification in Pryor clay. Dicalcium phosphate increased nitrification in samples of subsoils of Pryor clay, Webb fine sandy loam, Miles fine sand, Crystal fine sand, Maverick fine sandy loam, Crystal fine sandy loam, Crystal loamy fine sand, Orelia fine sandy loam, Elwood fine sandy loam, Fannin clay loam, Houston clay and Leaf fine sandy loam. Dicalcium phosphate and ferrous sulphate increased nitrification in subsoils of Miles fine sand. Magnesium sulphate increased nitrification in subsoil of Refugio loamy fine sand, and dicalcium phosphate and ferrous sulphate increased nitrification in subsoils of Norfolk fine sand, Amarillo fine sand, Leaf sandy loam and Duval fine sandy loam. Dicalcium phosphate, ferrous sulphate and magnesium sulphate increased nitrification in subsoils of Victoria clay and Refugio loamy fine sand. Phosphorus in dicalcium phosphate increased nitrification in 4 surface and 19 subsoils, magnesium in magnesium sulphate in 3 surface and 8 subsoils, and ferrous sulphate in 2 surface and 3 subsoils. These observations apply only to the samples studied and not to the type in general.

Nitrification and Nitrifying Organisms in Two Field Soils During Various Seasons

The greatest production of nitrates in field soils usually takes place during the warm seasons when temperature and moisture conditions are favorable for bacterial action. During the winter months, in countries in temperate climates, production of nitrates is likely to be small because the nitrifying organisms are inactive at low temperatures.

Under Texas climatic conditions, however, where the temperatures are more or less moderate during the winter months, bacteria in the soil are likely to be active and thus to bring about production of nitrates. Nitrates may be moved toward the surface of the soil when evaporation is active, washed down when water penetrates, and be taken up by plants and by soil organisms. The quantity of nitrates in a cultivated soil at any given time, therefore, depends on these several factors and not on any single one.

Jensen (41) in South Dakota found that the maximum amounts of nitrates in surface soils occurred in the early part of the spring; Russell (60) in England, early spring or early fall; Whiting and Schoonover (76) in Illinois, in the early spring or early summer; Gowda (37) in Iowa, during June and September; and Dorsey and Brown in Connecticut (15), during June and July in tilled plats, but not in pasture soils. Reynolds (58) in Texas, found the maximum in July under cotton and in August under corn. Wilson (77) found the ammonia-oxidizing organisms ranged, in January, in an orchard grass plat from 4,000 to 12,000 per gram and in an orchard grass and alfalfa plat from 6,000 to 13,000. At pH 6.2 the numbers were sometimes less than 1,000, and at pH 7.0 more than 35,000 per gram of soil. Walker *et al.* (75) found that the number of the nitrite-forming organisms ranged from 0 to 10,000,000 per gram, or more, according to the conditions. Thorne and Brown (69) found them to vary from 100 to 52,000 per gram, and that the maximum number was reached in the spring or early summer.

The numbers of bacteria, fungi, actinomyces and cellulose-decomposing bacteria at different horizons of different genetic soil types were studied by Vandecaveye and Katynetson (73), and Timonin (70).

The work here reported was conducted between March 1938 and December 1940. The soil samples were collected monthly, as nearly as possible, from 2 cultivated plats at the Main Station Farm at College Station. No applications were made to one plat, while the other received annually 4,000 pounds of superphosphate and 12 tons of manure per acre beginning with 1927. The crops grown were the same on both plats, and changed annually in rotation.

The samples were collected in separate jars from 10 different spots around each plot from the surface (0-4 inches) with sterilized spatulas. In the laboratory the jars were emptied into sterilized porcelain dishes and the soil was mixed thoroughly with sterilized spatulas. Five grams were used for the estimation of moisture, the quantity equivalent to 40 grams of oven-dry soil for the determination of nitrates, and the quantity equivalent to 10 grams of oven-dry soil for the preparation of soil suspensions to be used for estimating the numbers of organisms.

Table 24. Nitric nitrogen and moisture content of untreated and treated plots

Date of collecting samples	No fertilizer		Superphosphate and manure	
	Nitric N p.p.m.	Moisture %	Nitric N p.p.m.	Moisture %
March 14, 1938—corn	3.0	10.46	8.3	11.75
April 13, 1938	2.1	8.12	9.0	10.08
May 19, 1938	1.0	11.10	10.5	12.36
June 20, 1938	1.1	7.96	6.3	9.00
July 14, 1938	3.1	3.45	12.5	3.24
August 8, 1938	1.6	2.22	7.0	2.88
September 12, 1938	.5	8.34	3.0	9.86
October 13, 1938	1.1	1.06	2.5	.94
November 14, 1938	1.1	8.08	2.4	7.64
December 15, 1938—oats	2.3	5.85	8.5	6.66
January 16, 1939	.8	18.96	1.0	19.02
February 16, 1939	.4	10.96	.8	13.18
March 20, 1939	.5	5.72	.8	7.16
April 27, 1939	.8	2.85	.8	2.50
May 29, 1939	1.6	2.16	2.8	5.22
June 29, 1939	1.3	2.10	3.9	2.50
July 31, 1939	2.0	9.26	7.5	9.34
September 4, 1939	2.1	1.92	9.0	1.70
October 12, 1939	4.5	2.42	15.3	2.04
November 13, 1939	.6	13.82	1.5	12.74
December 14, 1939	1.0	8.08	1.9	8.32
January 18, 1940	1.5	12.48	3.0	12.88
February 19, 1940	.9	15.74	.8	16.68
March 21, 1940	2.9	4.62	5.6	7.50
April 22, 1940—cotton	8.5	5.85	22.0	5.00
May 30, 1940	2.5	12.60	7.3	12.50
July 9, 1940	.5	9.02	2.4	13.80
August 5, 1940	.5	2.15	1.3	3.36
September 19, 1940	0.9	0.80	3.6	0.86
October 21, 1940	0.3	4.68	2.0	5.08
November 21, 1940	0.8	9.14	2.4	9.58
December 23, 1940	0	17.16	.4	17.98

The amounts of nitric nitrogen and moisture are found in Table 24. The maximum amounts of nitric nitrogen occur in July 1938, October 1939 and April 1940, the minimum amounts in September 1938, February 1939, November 1939, February 1940, and August and December 1940. The amounts of nitrates in the plat which did not receive fertilizer are, as a rule, lower than in the one which received manure.

From these results it may be concluded that under College Station climatic conditions, nitrates occur at almost any time during the year, much depending on weather conditions. In general, however, amounts appear to be greater in the late summer and in autumn than in the other periods of the year.

Table 25 shows the numbers of the nitrate and nitrite-forming organisms found at various dates. Organisms are present in much greater numbers in the fertilized than in the unfertilized plot, which is to be expected. The same thing applies to the numbers of the autotrophic and the heterotrophic organisms. The maximum numbers of nitrate-forming bacteria occur in June 1938, June 1939, and January and April 1940. The maximum numbers of nitrite-forming bacteria occur in July 1938, June 1939, and February and

Table 25. Numbers of nitrate and nitrite-forming bacteria in untreated and treated plots. (Number of bacteria per 1 gm. of soil)

Date of collecting samples	No fertilizer		Superphosphate and manure	
	Nitrate bacteria	Nitrite bacteria	Nitrate bacteria	Nitrite bacteria
March 14, 1938—corn	50	50	100	50
April 13, 1938	0	0	50	20
May 19, 1938	6	6	600	350
June 20, 1938	85	50	5,000	500
July 14, 1938	8	350	4,000	1,500
August 8, 1938	70	50	1,250	875
September 12, 1938	4	0	150	40
October 13, 1938	15	0	150	95
November 14, 1938	10	2	200	200
December 15, 1938—oats	6	0	50	30
January 16, 1939	4	0	150	150
February 16, 1939	8	8	500	500
March 20, 1939	30	8	750	500
April 27, 1939	20	30	750	750
May 29, 1939	20	50	500	750
June 29, 1939	225	150	1,250	750
July 31, 1939	50	60	750	200
September 4, 1939	6	2	50	150
October 12, 1939	4	2	50	150
November 13, 1939	8	0	150	40
December 14, 1939	2	0	50	95
January 18, 1940	70	0	4,000	875
February 19, 1940	35	6	1,250	3,000
March 21, 1940	8	2	150	150
April 22, 1940—cotton	20	30	1,500	500
May 30, 1940	10	4	500	875
July 9, 1940	30	40	100	1,000
August 5, 1940	6	10	50	250
September 19, 1940	8	0	100	150
October 21, 1940	6	0	250	750
November 21, 1940	10	200	500	4,000
December 23, 1940	8	150	225	4,000

Table 26. Numbers of nitrate and nitrite-forming bacteria at different depths. (Number of bacteria per 1 gm of soil)

Date of collecting samples	Surface soil		6 inches		12 inches		18 inches	
	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite
June 27, 1938	50	40	30	6	6	2	2	0
August 4, 1938	1,000	1,000	500	100	150	20	50	8
September 19, 1938	30	4	10	10	10	4	0	0
November 3, 1938	250	35	200	4	10	8	6	8
December 8, 1938	50	0	10	6	8	10	10	20
February 9, 1939	300	7	30	10	8	10	6	20
March 14, 1939	500	6	150	4	20	8	30	13
April 20, 1939	40	10	40	20	30	20	50	10
May 22, 1939	500	100	450	65	45	45	20	14
June 22, 1939	200	750	45	500	30	40	10	19
July 26, 1939	325	1,500	50	85	200	6	10	4
August 28, 1939	500	750	100	100	30	30	20	6
October 5, 1939	100	0	20	6	10	10	4	13
November 6, 1939	50	0	50	2	30	2	10	8
December 7, 1939	40	2	65	4	40	2	30	6
January 8, 1940	50	0	50	50	30	30	20	30
February 8, 1940	10	0	20	13	50	14	9	19
March 11, 1940	250	0	150	30	100	20	20	20
April 11, 1940	475	0	3,000	1,000	250	150	50 +	50 +
May 16, 1940	150	10	135	20	10	20	10	10
June 17, 1940	300	150	40	100	10	10	6	20
July 17, 1940	200	150	30	50	40	7	30	2
August 12, 1940	175	200	30	20	10	10	2	0

November 1940. There was no definite ratio between the numbers of the nitrate and nitrite-forming organisms. The ratio varies widely, but the tendency is for it to be close to 1:1 when the organisms are inactive. The seasonal variations of the numbers of the autotrophic and the heterotrophic organisms were erratic. The bacterial count is not believed to be accurate.

In Table 26 are shown the numbers of the nitrifying organisms found at 1, 6, 12, and 18 inches depth in the plot which received superphosphate and manure annually. As to be expected, the greater numbers of the organisms are near the surface soil and as a rule they diminish in number with the increase of the depth. There are a few cases in which the numbers were somewhat greater in the subsoil than in the surface soil, or between the different subsoil depths, but these are exceptions. Some variations in the numbers of all these groups of organisms occur at different depths, but it is not clear from the data whether they are due to season or some other factor.

Summary

Methods for conducting nitrification experiments, determining nitrifying capacity, and estimating numbers of nitrifying bacteria are outlined briefly.

Inoculation with nitrifying bacteria alone increased nitrification of ammonium sulphate slightly in about 50 percent of soils of originally low nitrifying capacity. Calcium carbonate increased nitrification of ammonium sulphate in soils of low nitrifying power, and both calcium carbonate and bacteria increased nitrification of ammonium sulphate in over 90 percent of the soils and subsoils.

Soils with low nitrifying capacity had low nitrogen content, low basicity, and were slightly acid or neutral. Soils with high nitrifying capacities usually contained more than 0.06 percent nitrogen, had basicities greater than 0.6 percent and pH values were higher than 7.

Upland surface soils of the Gulf Coast Prairie, the East Texas Timber Country, the West Cross Timbers and other non-calcareous soils have low nitrifying capacities. The nitrifying capacities are increased little by inoculation alone but are greatly increased by addition of calcium carbonate, or both calcium carbonate and bacteria.

Upland calcareous soils including the Blackland Prairies, the Rolling Plains, and the Gulf Coast Plains have medium to high nitrifying capacities. When the nitrifying capacity is medium, nitrification is increased by inoculation, but is not usually increased by additions of calcium carbonate.

The agronomic significance of the differences in nitrifying capacities of various soils remains to be ascertained more fully. Ammonia nitrogen, in pot experiments, was equally as valuable as nitrate nitrogen in a number of soils with low nitrifying capacities.

Natural organic nitrogen of soils and subsoils was nitrified from 7 to over 27 percent in 28 days. In about 50 percent of all surface soils and 60 percent of all subsoils, 7 to 13 percent of the soil nitrogen was nitrified. Nitrification of 7 to 13 percent of the soil nitrogen may be considered as normal for soils naturally containing 0.06 percent or more of nitrogen.

Nitrification of 14 to 20 percent of the soil nitrogen may be considered as normal for surface soils containing less than 0.03 percent of soil nitrogen. There are, of course, exceptions to these limits.

Additions of calcium carbonate or bacteria, or both, increased nitrification of soil nitrogen in many soils, but did not increase such nitrification in all of them. The increases, if any, were usually relatively small.

During the 28-day incubation period of nitrifying cultures, the number of nitrate and nitrite-forming organisms increased to a maximum and then decreased. The numbers of such organisms did not always increase regularly. When inoculated into sterilized soil, the production of nitrates was related to the numbers of organisms introduced only in a general way.

When sterilized soils were inoculated with different quantities of cultivated soils, the nitrates and nitrites were related to the quantity of inoculant, but only in a general way. The quantities of nitrates produced per unit of inoculant generally increased as the quantity of inoculant decreased.

Nitrites produced during nitrification are not always completely oxidized to nitrates. Appreciable quantities may remain in the cultures at the end of the incubation period. Insufficient numbers of organisms which convert nitrites to nitrates at the beginning of the incubation may result in incomplete oxidation of nitrites. Additions of calcium carbonate may aid the persistence of nitrites, and addition of magnesium carbonate may aid still more. Additions of small amounts of a nitrifying soil to a sterilized soil may result in persistence of nitrites, while inoculation with larger amounts of the same soil may result in complete oxidation of the nitrogen to nitrates.

The conversion of nitrites to nitrates in a nitrifying soil is due almost completely to biological processes, and only in very small amounts to chemical reactions.

Oxidation of nitrogen in soils is not appreciably due to sunlight.

The water content of soils may range between 35 to 100 percent of the water capacity without appreciably affecting nitrification.

On an average, 16 puddled soils containing water equal to 65 percent of the water capacity, nitrified the nitrogen of the soil or of ammonium sulphate only slightly less than corresponding unpuddled soils. With water equal to 75 percent of the water capacity, puddled soils nitrified about 25 percent as much of the soil nitrogen as unpuddled soils, and 60 percent of that of ammonium sulphate. With 85 percent of the water capacity, 16 puddled soils averaged 6 percent of the nitrification of the soil nitrogen of unpuddled soils and 40 percent of the nitrification of ammonium sulphate.

With 16 soils incubated 28 days, there was little average loss of added nitric nitrogen when the water content of the puddled soils was 65 percent, but the loss averaged 15 percent when the water content was 75 percent, and 27 percent when the water content was 85 percent of the water capacity.

Sodium nitrite was converted to nitrate in both the unpuddled soils and the puddled soil with the water content 65 percent of the water capacity. With water content 75 percent or 85 percent of the capacity, some nitrite remained in 3 of the 16 soils. There was an average deficiency of 40 percent of the total nitrate and nitrite nitrogen with the water content 75 percent of capacity, and 63 percent deficient when water was 85 percent of capacity.

✓ Nitrification did not occur to an appreciable extent in water-logged soils. Nitrate nitrogen added at the beginning of the incubation was partly lost. Added sodium nitrite disappeared completely from nearly all of the water-logged soils. Nitrite was converted partially to nitrates in many of the water-logged soils, but the sum of the nitrous and nitric nitrogen was less than the quantity originally introduced.

In 23 soils differing widely in nitrifying capacity for ammonium sulphate, the nitrogen of ammonium sulphate was nitrified to the extent of 43 percent in 23 days, compared with 59 percent of the nitrogen in ammonium oxalate, 53 percent for ammonium acetate, 57 percent for ammonium tartrate and 56 percent ammonium citrate, respectively. When 1 percent calcium carbonate was added to 12 soils, the average nitrification of ammonium sulphate was greater than that of ammonium oxalate and ammonium tartrate. Ammonium carbonate was nitrified much more than ammonium sulphate in 12 soils, but when 1 percent calcium carbonate was added, the ammonium sulphate was nitrified only slightly less than ammonium carbonate. The lower nitrification of ammonium sulphate as compared with ammonium salts of organic acids appears to be due chiefly to the high acidity of the sulphate ion released during the nitrification.

In soils of high nitrifying capacity, the nitrogen of cottonseed meal is nitrified in 28 days to an average of 50 percent of that of ammonium sulphate. In soils or subsoils of low nitrifying capacity, the nitrification of cottonseed meal is likewise low, and, like ammonium sulphate, is improved by additions of calcium carbonate or inoculating liquid, but in the majority of cases, both additions are required. Some soils and subsoils of low nitrifying capacity nitrified cottonseed meal more than ammonium sulphate, but when both calcium carbonate and inoculating liquid were added, the ammonium sulphate was nitrified to the greatest extent. With 1 soil and 4 subsoils the nitrification of cottonseed meal was much below normal compared with that of ammonium sulphate, evidently due to conditions which interfered with the normal production of ammonia from the cottonseed meal.

When the fertilizing value of organic nitrogen in fertilizers is to be compared by means of nitrification tests, soils of high nitrifying capacity should be used. In soils of low nitrifying capacity, the amounts of nitrates formed will depend upon the soil as well as upon the nature of the fertilizer.

In incubation for 28 days at 35°, ammonium sulphate, with 1 or 2 percent cottonseed oil, produced the least nitrates, followed by starch, cane sugar, grapefruit peel, with pecan and cocoa shells having little effect on nitrification. After 20 weeks incubation of the organic matter, the depressing effects of the additions were in the same order as given above, except that grapefruit peel and pecan shells had no depressing effect. In soils to which nitrates had been added, the quantity of nitrate nitrogen was decreased on incubation on an average in the same order as given above.

Cyanamid is not readily nitrifiable, and seriously depresses the nitrification of soil organic matter, sodium nitrite and ammonium sulphate when it is applied in the soil cultures at larger rates than 100 p.p.m. nitrogen. In soils which required additions of calcium carbonate for high nitrification, cyanamid depressed nitrification in the presence of calcium carbonate. The depressing effect of cyanamid persisted for 6 to 10 months, even though the soil was inoculated with active organisms. The substances which interfere with nitrification diffuse from cyanamid placed in one spot in the culture. Of compounds derived from cyanamid, urea nitrifies readily, guanindine carbonate was nitrified to some extent, guanylurea underwent slight nitrification, while dicyandiamide depressed nitrification of the soil nitrogen.

When applied at the rate of 0.5, 1.0, 1.5, 2.0 and 5.0 percent, ground sulphur interfered with the production of nitrates from the nitrogen of the soil and from the nitrogen of ammonium sulphate. This occurred even though the pH of the soil was over 7 at the end of the incubation period. The depressing effect on nitrification increases with the percentage of sulphur added. Where sulphur is added in holes or furrows, as is done on some calcareous soils, it will probably not affect production of nitrates to a detrimental extent. A temporary or prolonged hindrance to nitrification may, however, take place in soils of low basicity. Additions of sulphur may also reduce the nitrate nitrogen in soils to which nitrates have been added, either by depression of nitrification of the soil, or by reduction of nitrates, but the decrease was small in the work here reported.

In soils of low nitrifying capacity, in which the nitrification of ammonium sulphate was not complete after additions of inoculating liquid and calcium carbonate, dicalcium phosphate increased nitrification in 4 surface and 19 subsoils, magnesium sulphate in 3 surface and 8 subsoils, and ferrous sulphate in 2 surface and 3 subsoils.

In the 2 cultivated field soils, the maximum amount of nitric nitrogen occurred in July, October and April, the minimum in September, February, November and December. The maximum number of nitrate forming bacteria were found in June, January and April. The number of nitrate and nitrite-forming bacteria at depths of 1, 6, 12, and 18 inches are given, and generally the numbers diminish with depths. The bacterial count is not believed to be very accurate.

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