A CORRELATION BETWEEN BACTERIAL ACTIVITY AND LIME REQUIREMENT OF SOILS

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INTRODUCTION

Limestone regions are noted for their fertility. Alfalfa, red clover, blue-grass, and corn are among the crops which thrive best on limestone soils. Those soils which do not naturally contain carbonate of lime are usually made more productive by applications of lime or limestone. Extensive investigations carried out by the Rhode Island, Maryland, Pennsylvania, Ohio, Illinois, and other agricultural experiment stations have demonstrated the value of lime in either the oxide, hydrate or carbonate form on soils which are acid to litmus. An excellent review of the most important investigational work on the use of lime on acid soils is given by Frear (9).

The investigations of Wheeler at the Rhode Island Agricultural Experiment Station, indicate, however, that a number of plants of economic importance thrive on soils which contain no solid carbonate of lime. Some of these plants are benefited by lime, but others are injured by applications of lime. Wheeler (36) says that orchard grass (*Dactylis glomerata*, L.) and meadow fescue (*Festuca elatior*, L.) are less injured by soil acidity than Kentucky blue-grass (*Poa pratensis*, L.) and timothy (*Phleum pratense*, L.) and that awnless brome grass (*Bromus inermus*, L), red top (*Agrostis alba var. vulgaris*, Thurb.), and Rhode Island bent (*Agrostis canina*, L.) do not seem to be susceptible to injury even on decidedly acid soils. He also states (37) that Concord grapes are apparently indifferent to the lack of lime and that cranberries, raspberries, and lima beans are injured by liming, the last named growing splendidly on soils so acid as to entirely destroy lettuce, spinach, onions, beets and asparagus. In his latest publication on this subject Wheeler (38) gives a summary of his work in which he shows that plants vary in their requirements from those which are injured by applications of lime even to a very acid soil, to those which are unable to live on an acid soil and are benefited remarkably by lime.

Coville (6) states that the blueberry, cranberry, strawberry, blackberry, red respberry, potato, sweet potato, rye, oats, millet, buckwheat, red top, carrot, turnip, cowpea, hairy vetch, crimson clover, soybean, lupine, and serradella are adapted to acid soils. He concludes, "soil acidity is not always an objectionable condition which invariably requires lime" and "under certain conditions, a complete system of acid agriculture is practicable."

Harter (14) writes that liming has been shown to be beneficial to all crops on Norfolk soils with the exception of beans, peas, and tomatoes. Kossovitch and Althausen (26) report that, while the liming of acid podzol soils strikingly increases the yields, the limit of increase is at about the point of neutralization and that an excess injures the plants. No statement is made as to how the point of neutralization was determined. Heinrich (15) concludes that the determination of lime in a soil, by digesting with 10 per cent hydrochloric acid, can be used as an index in determining what crops will thrive. According to his report, the least amounts of lime which will permit of successful growth are:

Crops	*Calcium carbonate in the soil* per cent
Lupines, potatoes, and rye	0.05
Oats and barley	0.05 to 0.10
Peas and vetch	0.10
Red clover	0.10 to 0.12
Alfalfa	0.20 to 0.30

Fred and Graul (10) experimenting with alfalfa, soybeans, and red clover on acid soils of two series, conclude that half enough lime to neutralize the soil acidity as measured by the Truog (32) method is sufficient for the production of good yields of these crops on acid soils of these two series.

THE RELATION BETWEEN BACTERIAL ACTIVITY AND THE REACTION OF SOILS

One of the reasons usually given for the maintenance of a neutral or slightly alkaline reaction in soils is that the soil microörganisms, which have to do with the processes of decay and the changes by which certain organic and inorganic substances become available for higher plants are unable to work to best advantage in an acid medium. The ammonifying, nitrifying, and nitrogen-fixing bacteria are thought to prefer a neutral or slightly alkaline medium. However, it is probably true that the various groups of soil bacteria are differently affected by the soil reaction. The influence of acidity and alkalinity on the development of pathogenic bacteria has been studied by a number of investigators. The literature on this subject is reviewed quite fully by Itano (21). The degree of acidity or alkalinity which the organisms are able to withstand varies with the species. Certain forms, e.g., *Bacterium tuberculosis*, are able to live in the presence of a considerable degree of acidity. It is reasonable to believe that soil microorganisms show similar differences in this respect. The fact that many acid soils are supporting vegetation, indicates that bacterial processes are being carried on in them, although these processes might be materially hastened if lime were applied.

The number of bacterial colonies from soil aliquots which will develop on agar plates is influenced by the reaction of the medium. Hoffmann (16) finds in counting the number of bacteria in soils that a medium slightly acid to phenolphthalein is more favorable than a medium which is neutral or slightly alkaline to phenolphthalein. Fischer (8), who conducted probably the most extensive investigations on the effect of lime on the number of bacteria in soils, shows that an application of either calcium oxide or calcium carbonate has a very marked effect in increasing the total number of bacteria.

That the rate of ammonification is increased by applications of lime is shown by Voorhees and Lipman (35). Coville (6) points out that many soils acid to litmus contain large amounts of ammonia. Kopeloff (25) shows that "where the soil reaction is unfavorable for the activities of the soil bacteria concerned in ammonification, the soil fungi may prove to be an important compensating factor."

The rate of nitrification is increased by applications of lime on soils which give an acid reaction with litmus. The results obtained by Lyon and Bizzell (27) are typical. A number of other investigators report similar effects from the use of lime. Scales (29), studying the activities of nitrifying organisms, finds they are most active in the presence of 50 per cent of the calcium-carbonate requirement (Veitch) of the soil. An excess of calcium carbonate seems to be toxic to the nitrifying organisms. Temple (31) finds that if an organic source of nitrogen is used instead of ammonium sulfate, the formation of nitrates is much greater in acid soils. He explains this increased nitrification on the basis of the formation of neutral zones, caused by the production of ammonia, at which points conditions are favorable for nitrification. Temple also shows that calcium salts of organic acids can be used as effectively as calcium carbonate in overcoming the toxic effect of ammonium sulfate on an acid soil. Miller (28), working with a sandy soil acid to litmus, finds that an application of 0.1 per cent of calcium oxide caused a decrease in the ability of the soil to nitrify ammonium sulfate and that 0.5 per cent of calcium oxide stopped the process entirely. Hutchinson (19) finds that calcium oxide acts not alone as a neutralizing agent, but also as a partial sterilizing agent. Since in the experimental work following applications of neutralizing agents are confined to calcium carbonate, it does not seem necessary to include any further discussion on the effect of calcium oxide on the bacterial processes in the soil.

It should be remembered that it has been shown that nitrate nitrogen is not necessary for all plants. Hall and Miller (12) call attention to the fact that ammonium sulfate, on the Park plats of the Rothamsted Farm, produces very good crops of grass, although the soil is deficient in lime and very little nitrification takes place. Hutchinson and Miller (20) find that peas are able to utilize ammonia nitrogen as well as nitrate nitrogen, although the opposite is true with wheat. Kelley (24) shows that rice, grown in swamp land, secures its nitrogen in the form of ammonia. If ammonification processes are less affected than nitrification processes by a deficiency of lime in the soil, then plants which are able to utilize ammonia can survive where those depending on nitrate nitrogen cannot live.

Hopkins (18) notes that the application of lime increases the power of *Bacillus radicicola* in certain legumes to fix atmospheric nitrogen. Whiting (39) writes that nodules are often found in abundance on legumes on very acid soils. Japanese clover (*Lespedeza*) has often been observed by the writer growing on soils strongly acid to litmus and the roots were well supplied with nodules. These nodules were mostly near the surface of the soil. Kellerman and Robinson (22) find that crimson clover inoculation is little affected by the reaction of the soil. Fred and Graul (10) find that, if acid Colby silt loam soil is previously inoculated with *B. radicicola*, nitrogen fixation by soybeans is little influenced by applications of calcium carbonate. They also find this true on acid Colby silt loam with red clover. Both clover and alfalfa were able to fix considerable amounts of nitrogen when growing on Colby silt loam and Plainfield sand having only one-half of their acidity (Truog method) neutralized. The Colby silt loam required 10,400 and the Plainfield sand 5200 pounds of calcium carbonate to neutralize one-half of the acidity in 2,000,000 pounds of soil. Determinations of the lime requirement (Veitch) on the Colby silt loam soil, chosen from the same locality the year previous, showed a need of 3234 pounds of calcium carbonate per 2,000,000 pounds of soil. The authors state that "the Truog method shows much larger amounts of soil acidity than the Veitch."

Ashby (1) shows that the use of lime on the Rothamsted soils more than doubled the nitrogen-fixing power of the *Azotobacter*. Hoffman and Hammer (17) find that calcium carbonate is essential to non-symbiotic nitrogen fixation, but that the amount required is very minute and was present in sufficient amount in all the soils tested. These soils were chosen from various localities in Wisconsin and must have included some soils acid to litmus, since Whitson and Weir (40) estimate that two-thirds of the soils of Wisconsin are acid. Christensen and Larsen (4) find that if Ashby's solution is inoculalated with a soil in need of lime, the brownish film usually produced by *Azotobacter* does not develop. They suggest this as a method of determining the need of a soil for lime.

Gimingham (11) describes several organisms capable of bringing about the formation of carbonates from calcium salts of organic acids. Hall and Miller (13) also report that calcium salts of organic acids are transformed to the carbonate by soil organisms, the organic acids being decomposed to form carbon dioxide and water. Drew (7) shows that marine bacteria precipitate calcium carbonate from sea water. He names the organism responsible for this reaction, *Bacillus calcis*. Kellerman and Smith (23) write that it is possible in the laboratory to produce calcium carbonate by three types of biological processes; by the action of ammonium carbonate on calcium sulfate; by the action of ammonium hydroxide on calcium acid carbonate, and by the decomposition of calcium salts of organic acids. They state that Drew's organism is *Pseudomonas calcis*. This is a denitrifying organism which produces ammonia by the reduction of nitrates. Bear and Salter (2) show that the lime requirement (Veitch) of the West Virginia Agricultural Experiment Station fertility plots is less where the content of organic matter has been increased, and suggest that this decrease may have been due to the precipitation of calcium from solution by the humus in the soil, whereby it was prevented from being lost in the drainage water. This calcium might later be freed as the carbonate, as the decomposition of the organic matter was brought about by the soil organisms.

OBJECT OF THESE INVESTIGATIONS

In view of the fact that large areas of land are acid and that the distance from the supply of lime often makes the cost of applying large amounts of lime or limestone prohibitive, it was thought it might be desirable to consider more carefully the possibilities of a system of acid agriculture as suggested by Coville (6). Since the problem of the economy of nitrogen and its availability for the use of crops is largely a bacterial problem, it seemed important to study the relation of the reaction of the soil to the activities of the bacteria concerned in nitrogen accumulation and transformations. Recognizing the fact that plants do grow on soils which are acid to litmus, how are these plants supplied with nitrogen? We know that lime and limestone are valuable soil amendments, but might it not be possible that small applications of these materials would be relatively more effective in promoting the activities of the bacteria concerned in the nitrogen problem than large applications? If the *B. radicicola* of some legumes is more resistant to acidity than the *B. radicicola* growing on other legumes, might it not be possible to select legumes adapted to the reaction of the soil instead of adding lime to the soil to make the reaction suitable for the legumes we desire to grow? Even if nitrogen-fixing organisms are able to grow in acid soils, are they able to fix atmospheric nitrogen in such an environment? To answer these questions, it was proposed to measure the activities of those bacteria concerned in the nitrogen economy of plants as influenced by various amounts of calcium carbonate applied to acid soils.

DEFINITION OF "LIME REQUIREMENT"

In the preceding discussion, a rather loose construction is given to the term "soil acidity." This is simply in accordance with precedents set by the various investigators whose work is reviewed. As a rule, an "acid" soil means a soil which changes blue litmus paper red. The "degree of acidity" of soils has no such definite meaning, consequently the investigations reported are not strictly comparable. The writer sees no reason to disagree with Truog (33) as to what "soil acidity" really is. Truog writes that acid silicates are the main cause of soil acidity in upland soils. His excellent review of this subject gives a select bibliography of the investigational work along this line. Truog (32) also writes that the acidity of soils may be conveniently divided into two classes, "active" and "latent" acidity. He states that "latent" acidity is undoubtedly much less injurious to plants than "active" acidity. He also shows the desirability of knowing the "avidity" of the active soil acids. Sharp and Hoagland (30) attempt to measure the lime requirement of soils by determining the hydrogen-ion concentration of the soil suspensions. The recent review of Clark and Lubs (5) of the literature on this subject, indicates that the hydrogen-ion concentration of the medium is the important factor to consider in the relationship between acidity and biological processes. The hydrogen-ion concentration of a soil in suspension in water is, however, not a measure of the amount of lime necessary to add to an acid soil to produce a neutral reaction of the soil. This is partly because of the slow solubility of the acid-forming constituents present in soils.

At the time this investigation was begun, most of the recent work on soil acidity had not been published. The writer felt at that time that the most satisfactory measure of the "lime requirement" of a soil was that obtained by the Veitch (34) method. Accordingly, this method was used in determining the quantitative need of the soils used for lime. It is interesting to note in this connection that when the two soils which were used most largely in these investigations had been treated with the quantity of calcium carbonate necessary to satisfy their lime requirements (Veitch) and had been mixed once each week for 12 weeks, they were found to be neutral to litmus paper.

HISTORY OF THE SOILS USED IN THESE EXPERIMENTS

A large part of the work reported has been done on samples of soil from two different localities belonging to different soil series. Both of these were acid in reaction, as will be shown later.

Soil I was secured from plot 18 of the West Virginia Agricultural Experiment Station farm. The soil is classified by the United States Bureau of Soils as Dekalb silt loam. It is a residual soil which has been formed by the disintegration of sandstone and greenish gray shales overlying the Pittsburg coal. The original timber was largely oak and chestnut with an occasional locust. The analysis of this soil is as follows:

Element	*Pounds per 2,000,000 of soil*
Nitrogen	1,940
Phosphorus	600
Potassium	25,100
Carbon	23,900
Calcium	2,300
Magnesium	4,300
Calcium carbonate requirement (Veitch)	3,500

Plot 18 has not received any fertilizer, lime or manure since the beginning of the fertilizer tests in 1900. Only a partial record of the produce of this plot is available. During a part of the time since 1900 a tile drain, which passed near this plot, was not working, and, since the yields of the plot were somewhat abnormal, no permanent records of the plot were kept. Later the record of the produce of this plot was continued. This record shows that plot 18 corresponds normally in productivity to plot 21, which also received no fertilizer, lime or manure. The sample of soil was chosen from plot 18 because its record was incomplete and any change due to the removal of a large sample of soil would not interfere with the plot experiments. Since 1900 the following crops have been grown on these plots; rye, 1900 and 1907; wheat, 1901 and 1914; clover, 1902, 1909, and 1915; corn, 1903, 1905, and 1912; cowpeas, 1904; potatoes, 1906; timothy, 1909, 1910, and 1911, and oats, 1913. Table 1 gives the records of the fertilizer treatment and total produce of all the plots up to and including 1915.

TABLE 1

Total amounts of fertilizers applied and total produce per acre from 1900 to 1915 on soil I

PLOT	TREATMENT	NITRATE OF SODA	ACID PHOSPHATE	SULFATE OF POTASH	LIME (CaO)	MANURE	TOTAL PRODUCE
		pounds	*pounds*	*pounds*	*pounds*	*tons*	*pounds*
19	N, P, K, CaO	4200	4200	1625	4500		120,605
20	M, CaO				4500	210	152,400
21	Check						38,600
22	CaO				5500		36,615
23	Ash M, N	300	Ash of 40 tons of manure until 1912				39,270
24	Check						43,075
25	M					190	139,670
26	N, P, K	4200	4200	1625			117,910
27	Check						42,170
28	P, K		4200	1625			76,995
29	N	4200		1625			52,215
30	Check						39,480
31	N, P	4200	4200				95,940
32	K			1625			41,565
33	Check						36,845
34	P		4200				63,415
35	N	4200					41,195

N, indicates nitrate of soda; P, acid phosphate; K, sulfate of potash; M, manure.

Soil II was secured from the Ohio Agricultural Experiment Station farm at Wooster. This soil is classified by the Bureau of Soils as Wooster silt loam. It has been formed from the disintegration of sandstone and shales of the Mississippian period, under the influence of glacial action. The analysis of the soil used is as follows:

Element	Pounds per 2,000,000 of soil
Nitrogen	1,775
Phosphorus	664
Potassium	34,000
Carbon	22,200
Calcium	4,470
Magnesium	6,596
Calcium carbonate requirement (Veitch)	3,500

It will be observed that soil II has the same calcium-carbonate requirement as soil I.

Soil II has never received any fertilizer, lime or manure since the beginning of the fertilizer tests in 1893. Continuous records since that time have been kept on soil of the same history as this soil in a 5-year rotation experiment at the Wooster station. The rotation has been corn, oats, wheat, clover, and timothy. A summary of the effect of lime and fertilizers on this soil is given by Williams (41) in table 2. An experiment has also been in progress on this same type of soil which had been kept in a fair state of fertility by a good rotation and an occasional application of manure previous to the beginning of the experiment. The rotation since practiced has been corn, oats, and clover. The records of this experiment are shown in table 3. It will be seen by a study of tables 2 and 3, that both lime and acid phosphate are very effective in increasing the yields of the crops grown in these two rotations. While lime is very efficient, it seems remarkable that such large yields of these crops can be produced by the use of acid phosphate alone on a soil which has a calcium-carbonate requirement of 3500 pounds per 2,000,000 pounds of soil.

The other samples of soil used in these experiments were Dekalb soils chosen from various localities in West Virginia. These soils vary greatly because of differences in the systems of management they have undergone. Analyses of these soils are shown in subsequent tables.

PLAN OF THESE EXPERIMENTS

Large samples of soils, acid to litmus, were secured, sent immediately to the laboratory, made to pass a 2-mm. sieve, and stored in large galvanized iron cans. From these cans soil was removed as needed. Careful analyses of the soils were made for the total amount of nitrogen, phosphorus, potassium, calcium, magnesium, and carbon. Lime-requirement determinations were made by the Veitch method as indicated above. Amounts of C. P. calcium carbonate varying from 250 pounds to 40,000 pounds per 2,000,000 pounds of soil were added to the soils. A study was made of the effects of these applications on: (a) the number of bacteria, (b) the rate of ammonification, (c) the rate of nitrification, (d) the fixation of nitrogen by non-symbiotic organisms, and (e) the development of *B. radicicola* of the soybean. All analyses were made according to the methods given by Bear and Salter (3).

The calcium carbonate was applied and mixed thoroughly with the soil, which was then placed in 1-gallon stone jars. Enough water was added to the soil to give it an optimum moisture content. Each week the soil was removed from the jars and mixed thoroughly and the loss of moisture, due to

TABLE 2

The effect of lime on the yields of crops on soil II

PLOT	TREATMENT	YIELD PER ACRE									
		Corn 1900–1915		Oats 1901–1916		Wheat 1906–1916		Clover 1903–1916		Timothy 1909–1916	
		Unlimed	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed	Limed
		bus.	*bus.*	*bus.*	*bus.*	*bus.*	*bus.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
2	Phosphorus*...............	35.51	42.32	39.16	42.85	21.48	25.17	1848	2680	3058	3810
8	Phosphorus,* potassium.....	43.95	51.08	42.62	46.38	22.17	26.38	2144	3166	3125	3881
11	Phosphorus*, potassium, nitrogen..................	47.67	55.12	49.77	49.71	31.27	31.86	2683	3388	3445	4124
17	All three with less nitrogen but more phosphorus*....	47.23	55.67	51.84	52.38	27.32	30.85	2492	3598	3364	4543
18	Barnyard manure..........	56.31	61.68	43.62	44.93	29.51	32.49	3448	4393	4525	5531
24	Same as 17 but nitrogen in sulfate of ammonia.......	46.23	55.98	48.21	51.36	24.70	31.26	2139	3544	3111	4409
26	Same as 17 but phosphorus in bone meal............	46.01	51.17	46.37	46.81	27.78	28.65	2945	3772	3504	4585
29	Same as 17 but phosphorus in basic slag.............	46.27	51.69	47.77	47.85	29.76	28.93	2981	3371	3741	4306
Average unfertilized.............		26.48	32.32	27.19	32.08	12.74	16.09	1276	1841	2500	3069

* Phosphorus in the form of acid phosphate.

TABLE 3

The effect of lime and acid phosphate on soil II

TREATMENT	AMOUNT PER ACRE	CORN 9 YEARS		OATS 9 YEARS		CLOVER 8 YEARS
		Grain	Stover	Grain	Straw	Hay
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
No fertilizer..............		51.50	2759	44.94	1961	4074
Calcium oxide............	1000	57.33	3149	47.53	2079	4580
Ground limestone.........	1780	54.84	2820	45.35	1876	4362
Acid phosphate...........	320	60.18	3056	46.16	1912	4277

evaporation, was restored. This was continued for 12 weeks in order that the soil microörganisms should have time to adjust themselves to the changes in soil reaction. At the end of that time, the determinations of nitrifying power, ammonifying power, etc., were made. These determinations required about one-half of the soil.

Since the analyses showed that these soils were very deficient in total phosphorus, a thing which is commonly true of acid soils, it seemed advisable to apply phosphorus in a readily available form in order to remove it from being a possible limiting factor in the various bacterial activities studied. Accordingly, 0.2 per cent of mono-calcium phosphate, equivalent to 1000 pounds of phosphorus per 2,000,000 pounds of soil, was added, the moisture content was again restored, and the mixing was continued for another period of 12 weeks. At the end of this time, the above determinations were repeated. In some of the later experiments the calcium carbonate was added just previous to the time of studying the rate of nitrification, ammonification, etc.

THE EFFECT OF CALCIUM CARBONATE ON THE NUMBER OF BACTERIA

Soils I and II were used in these experiments, after they had received the various applications of calcium carbonate and had been mixed thoroughly each week for 12 weeks, as previously outlined. Plate counts of the number of microörganisms were made at the end of the 12-week period. After the 0.2 per cent of mono-calcium phosphate had been added and mixed with the remainder of the soil each week for a second 12 weeks, plate counts were again made. Aliquots of the soil suspension were plated on Heyden agar. The plates were incubated at room temperature and counts were made at the end of 6 days. Table 4 shows the results of these counts. Each figure represents the average of four plates.

As might be expected, the greatest relative change in the number of bacteria occurred after the neutral point had been passed. This was true in

TABLE 4

The effect of calcium carbonate on the number of bacteria

CALCIUM CARBONATE PER 2,000,000 POUNDS OF SOIL	BACTERIA PER GRAM OF SOIL			
	Soil I without phosphorus	Soil I with phosphorus	Soil II without phosphorus	Soil II with phosphorus
pounds				
0	3,341,000	4,150,000	3,503,000	3,438,000
250	4,127,000	4,320,000	3,418,000	3,536,000
500	3,537,000	3,540,000	4,614,000	4,421,000
1,000	3,439,000	2,750,000	3,781,000	5,207,000
2,000	3,930,000	2,520,000	4,472,000	5,781,000
3,000	4,127,000	4,090,000	4,919,000	5,683,000
Neutral point (Veitch method)				
4,000	4,422,000	5,820,000	7,348,000	10,005,000
5,000	5,306,000	7,700,000	9,741,000	17,392,000
7,500	5,601,000	7,070,000	15,827,000	14,297,000
10,000	3,341,000	6,680,000	14,973,000	7,959,000
20,000	6,682,000	10,750,000	14,892,000	3,635,000
40,000	9,335,000	13,050,000	18,199,000	9,826,000

both soils, as shown in figure 1. The 4000 and 5000-pound applications of calcium carbonate resulted in relatively large increases in numbers. Additions of calcium carbonate in excess of 7500 pounds per 2,000,000 pounds of soil gave somewhat uncertain results. There was a decrease in every case accompanying an application of 10,000 pounds as compared with 7500 pounds of calcium carbonate. The 20,000 and 40,000-pound applications brought about marked increases in numbers. These fluctuations were probably due to the adjustment of the soil reaction to the point where it was more suitable to the requirements of some forms which developed in vast numbers under this optimum soil reaction. It seems quite evident that the application of calcium carbonate caused decided changes in the number of bacteria in these soils. The maximum increases in numbers had apparently not been reached in three of the four cases by applications of 40,000 pounds of calcium carbonate per 2,000,000 pounds of soil. Similar trials with calcium oxide produced marked decreases in the number of bacteria in these soils following the larger applications. This was probably due to the partial sterilizing action of calcium oxide previously referred to.

THE EFFECT OF CALCIUM CARBONATE ON THE RATE OF AMMONIFICATION

Soils I and II, after the treatments with calcium carbonate and monocalcium phosphate previously referred to, were used in the experiments on ammonification. The source of nitrogen was Hammarsten's casein. Enough casein was added to supply 160 mgm. of nitrogen per 100 gm. of soil. The soil was then given an optimum moisture content and incubated in tumblers for 3 days at room temperature, after which analyses were made for ammonia by distillation with magnesium oxide. Each figure given in table 5 represents the average of two determinations which checked usually within less than 1 mgm. per 100 gm. of soil. Other determinations, not reported in this paper, were made in which only 40 mgm. of nitrogen were added, with very satisfactory results. The author believes that 160 mgm. of nitrogen per 100 gm. of soil are likely to produce abnormal conditions in a soil, although in most of the ammonification experiments reported in the literature even larger amounts of nitrogen were supplied.

The greatest relative increase in the rate of ammonification of casein, per unit of calcium carbonate applied, occurred with applications of 2000 pounds of calcium carbonate per 2,000,000 pounds of soil, as shown in figure 2. There was no marked increase in ammonification as the neutral point was passed. As previously shown, this was also the case in the number of bacteria. Applications of 250 pounds of calcium carbonate per 2,000,000 pounds of soil had a tendency to cause a decrease in the rate of ammonification. Applications of calcium carbonate in excess of 5000 pounds caused only a slight increase in the amount of ammonia produced. The 20,000 and 40,000-pound applications caused slight decreases in ammonia in several cases. There was apparently no definite correlation between the number of bacteria and the amount of ammonia produced, although in general, increased amounts of calcium carbonate resulted in larger numbers of bacteria and more rapid ammonification.

THE EFFECT OF CALCIUM CARBONATE ON THE RATE OF NITRIFICATION

The effect of calcium carbonate on the rate of nitrification in soils I and II is shown in table 6. All figures in this and in succeeding tables of nitrification

TABLE 5

*The effect of calcium carbonate on the rate of ammonification of casein**

CALCIUM CARBONATE PER 2,000,000 POUNDS OF SOIL	NITROGEN AS AMMONIA PER 100 GM. OF SOIL					
	Soil I without phosphorus	Soil I with phosphorus	Soil II† without phosphorus	Soil II without phosphorus	Soil II with phosphorus	Soil II with phosphorus
pounds	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0	72.40	60.70	38.85	22.89	29.51	39.20
250	71.00	61.00	40.25	22.05	25.55	37.38
500	72.80	59.60	45.36	29.05	28.00	37.80
1,000	75.40	62.40	48.02	29.40	28.63	44.31
2,000	78.50	68.00	57.54	41.65	43.40	55.44
3,000	79.00	70.00	56.00	44.59	45.36	57.75
Neutral point (Veitch method)						
4,000	78.40	70.60	60.41	45.85	56.84	63.00
5,000	76.30	70.80	67.27	57.12	64.61	71.54
7,500	85.30	74.80	71.40	57.05	52.29	67.97
10,000	83.60	74.80	74.48	62.79	63.07	71.61
20,000	85.40	77.80	77.40	60.76	55.27	68.81
40,000	87.50	77.60	77.42	61.25	51.61	59.36

* The results in each vertical column were obtained on the same day. Fluctuations in the temperature in the room are responsible for some of the differences observed in horizontal columns.

† Four-day periods of incubation.

represent averages of two determinations. As a rule, the duplicates agreed within less than 0.1 mgm. per 100 gm. of soil. Accordingly, only averages are reported.

These soils were treated with calcium carbonate in varying amounts and with mono-calcium phosphate as previously outlined. At the end of the 12-week periods, samples of these soils of 100 gm. each were placed in 1000-cc. Erlenmeyer flasks for the nitrification experiments. To each flask were added 20 mgm. of nitrogen in the form of either ammonium sulfate or ammonium carbonate. After adding water to the optimum content, the soils were incubated for 21 days at room temperature, after which the nitrate determinations were made by the phenol-disulphonic acid method.

A study of table 6 and figure 3 shows that the addition of calcium carbonate is followed by an increased nitrification which correlates almost directly with the increased application of calcium carbonate. This correlation holds fairly well in every case with applications up to 5000 pounds per 2,000,000 pounds of soil. There is no sudden break in the correlation as the neutral point is passed. Applications of calcium carbonate in excess of 5000 pounds are followed by increased nitrification, although the curve of increase begins to incline more toward the horizontal. In half of the experiments the curve was still ascending with applications of 40,000 pounds of calcium carbonate.

TABLE 6

The effect of calcium carbonate on the rate of nitrification

CALCIUM CARBONATE PER 2,000,000 POUNDS OF SOIL	NITROGEN AS NITRATE PER 100 GM. OF SOIL							
	Source of nitrogen, ammonium sulfate				Source of nitrogen, ammonium carbonate.			
	Soil I* without phosphorus	Soil I with phosphorus	Soil II without phosphorus	Soil II with phosphorus	Soil I without phosphorus	Soil I with phosphorus	Soil II without phosphorus	Soil II with phosphorus
pounds	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0	1.08	4.06	5.28	6.07	1.38	7.22	5.29	7.50
250	1.38	4.32	4.39	5.34	1.93	8.24	5.00	8.00
500	1.43	4.60	4.40	6.38	2.11	8.42	5.42	8.40
1,000	1.82	5.24	6.15	6.75	2.21	9.52	6.60	8.55
2,000	2.29	6.38	8.50	8.73	3.01	12.42	9.03	11.55
3,000	2.96	9.34	10.48	10.43	3.55	15.30	10.04	12.50
Neutral point (Veitch method)								
4,000	3.13	11.92	15.74	12.50	3.28	17.50	11.87	15.12
5,000	3.44	13.86	15.96	15.00	4.11	18.00	15.77	18.75
7,500	3.48	16.37	18.18	15.38	4.69	19.00	17.27	16.35
10,000	4.44	19.35	20.98	15.98	4.30	20.00	20.30	16.16
20,000	4.00	20.45	22.87	16.00	4.32	20.96	19.80	16.00
40,000	4.20	22.55	19.88	15.00	5.18	23.30	20.57	15.00

* An error was made in calculating the optimum moisture content and the soil in this experiment was too dry.

This is directly contrary to the work of Scales previously referred to, which indicated that 50 per cent of the amount of calcium carbonate necessary to supply the lime requirement of the soil is sufficient to attain the maximum rate of nitrification. Additional amounts are reported to have acted injuriously.

The marked increase in the rate of ammonification observed with the addition of 2000 pounds as compared to 1000 pounds of calcium carbonate per 2,000,000 pounds of soil was not followed by a coresponding increase in the nitrification. No correlation was found between the increased number of bacteria in the soil and the rate of nitrification except that in general the application of increased amounts of calcium carbonate caused an upward tendency in the number of bacteria, as well as in the rate of nitrification. Since the agar plate method is not designed to include the nitrifying bacteria, no data are available as to the actual number of nitrifying organisms which were present in the soils following the applications of varying amounts of calcium carbonate.

EFFECT OF CALCIUM CARBONATE ON THE RATE OF NITROGEN FIXATION BY NON-SYMBIOTIC SOIL ORGANISMS

Samples I and II were employed again in these experiments after they had been treated as previously described. Shallow dishes having a depth of about 3 inches and a capacity of 400 gm. of soil were used for this work. Soil from the various pots to which the calcium carbonate and mono-calcium phosphate had been applied was placed in the dishes and mixed thoroughly with 2 per cent of mannit. Optimum moisture conditions were secured and maintained as nearly as possible by adding water twice daily to restore that lost by evaporation. The soils were incubated 21 days at room temperature. After thorough drying, the entire samples were pulverized to pass a 100-mesh sieve and the total nitrogen was determined in triplicate. The triplicates agreed usually within 0.05 mgm. of nitrogen on 10-gm. samples.

TABLE 7

The effect of calcium carbonate on nitrogen fixation by non-symbiotic soil organisms

CALCIUM CARBONATE PER 2,000,000 POUNDS OF SOIL	NITROGEN FIXED PER 100 GM. OF SOIL		
	Soil I without phosphorus	Soil II without phosphorus	Soil II with phosphorus
pounds	*mgm.*	*mgm.*	*mgm.*
0	0.7	0.3	2.0
250	0.3	0.8	3.6
500	0.5	0.8	3.6
1,000	1.0	0.6	4.6
2,000	0.7	1.4	3.8
3,000	0.5	2.3	11.3
Neutral point (Veitch method)			
4,000	0.1	2.8	12.2
5,000	1.8	4.1	12.7
7,500	2.0	6.0	15.8
10,000	1.5	5.8	10.1
20,000	2.1	4.5	12.6
40,000	1.4	4.4	9.9

From a study of table 7, it appears evident that both calcium carbonate and mono-calcium phosphate were essential to the highest fixation of nitrogen. The mono-calcium phosphate had such a marked effect in increasing the nitrogen-fixing power of soil II, as shown in figure 4, that it would seem that phosphorus was equally as important as lime for the nitrogen-fixing organisms in this soil. The largest relative increase in nitrogen fixation followed an application of 3000 pounds of calcium carbonate per 2,000,000 of soil when accompanied by the use of mono-calcium phosphate. Heavier applications of calcium carbonate caused an increase in nitrogen fixation until as much as 10,000 pounds per 2,000,000 pounds of soil had been applied. This amount and heavier applications caused a decrease in nitrogen fixation. Apparently phosphorus was a limiting factor in nitrogen fixation in soil I, although time did not permit an experimental test of this point. The good effects resulting from the use of acid phosphate on these soils under field conditions may be due in part to this increased nitrogen fixation accompanying its use. This again is indicated by the analyses of the West Virginia Station fertility plots from which the author and others have shown that the plot receiving acid phosphate and sulfate of potash has accumulated 1173 pounds of nitrogen per acre during the last 15 years which could not be accounted for except by nitrogen fixation from the air. The evidence shown in table 7 indicates that calcium carbonate is necessary in addition to the phosphorus for the most effective nitrogen fixation.

Following the suggestion of Christensen and Larsen (4), soil from each of the pots to which varying amounts of calcium carbonate had been applied, was used to inoculate Ashby's solution in order to study the relation between the film development on the surface of the liquid and the lime requirement of the soil. The brownish film was very well developed in the flasks inoculated with soil which contained an amount of calcium carbonate in excess of the requirement of the soil and practically disappeared as the quantity of calcium carbonate applied was reduced below the amount necessary to satisfy the requirement of the soil. The development of brown pigment is apparently closely related to the amount of lime in the soil and may be used as an index of the need of lime by the soil. Other experiments which will be discussed later indicated, however, that nitrogen fixation in Ashby's solution may take place when the solution is inoculated with soils having calcium-carbonate requirements as high as 4600 pounds per 2,000,000 pounds of soil. Apparently, the lack of development of the brownish film is not accompanied by the loss of ability to fix nitrogen, since no soil was found which did not show nitrogen fixation when inoculated into Ashby's solution and allowed to stand for 21 days at room temperature.

THE EFFECT OF CALCIUM CARBONATE ON THE FIXATION OF NITROGEN BY B. RADICICOLA OF THE SOYBEAN (SOJA MAX PIPER)

Soils I and II were again used in these experiments. One-gallon pots were filled with these soils and the calcium carbonate was added. Each pot received an application of 0.2 per cent of mono-calcium phosphate. The pots were planted to soybeans, the beans having been previously inoculated with

B. radicicola. Six beans were planted in each pot and later thinned to three per pot. After the beans had reached the stage where pods were formed, they were harvested, and records were taken of their green and dry weight, the number of nodules, the dry weight of nodules and the milligrams of nitrogen in the roots, tops and nodules. One crop was harvested from each pot during the summer of 1915 and another crop during the summer of 1916. The records are shown in tables 8 and 9.

The number of nodules had a tendency to increase slightly with small applications of calcium carbonate. Applications of more than 3000 pounds of calcium carbonate per 2,000,000 of soil caused a decrease in the number of nodules. This decrease was proportional to the amount of calcium carbonate applied. The dry weight of nodules was also decreased with large applications of calcium carbonate. The rate of decrease in dry weight of nodules with increased amounts of calcium carbonate was more marked than the rate of decrease in the number of nodules. The amount of nitrogen in the nodules was almost directly correlated with the dry weight of nodules, and decreased with additional quantities of calcium carbonate. It will be noticed that in both cases the dry weight and total nitrogen of both stems and roots had a tendency to increase with small applications of calcium carbonate, but that applications in excess of 2000 pounds per 2,000,000 of soil had a tendency to cause a decrease in dry weight and total nitrogen of the stems and roots.

The total nitrogen fixed by soils I and II during the two years in which the two crops of soybeans were grown was determined. Analyses of the soil were made before and after the beans were grown. The difference in the nitrogen content of the soil at these two periods plus the nitrogen removed in the nodules, stems and roots, after subtracting the nitrogen content of the seed and water used in watering the plants, represents the nitrogen secured from the air.

The total nitrogen fixed in two years per 2,000,000 pounds of soil, as shown in the last columns of tables 8 and 9, indicate that soil II has had a more active nitrogen-fixing flora than soil I. In so far as the chemical composition is concerned, the two soils correspond fairly well, as will be found by referring to the analyses of these two soils previously shown. By referring again to table 7, showing the rate of nitrogen fixation by *Azotobacter*, it will be seen that soil II was much more active in this respect than soil I. It is possible that a greater part of the nitrogen accumulated in soil II during the growing of the legumes was fixed in the soil through the agency of the non-symbiotic organisms. The nitrogen fixation had a tendency to decrease with applications of calcium carbonate in excess of 2000 pounds per 2,000,000 pounds of soil, although the lime requirement of both soils indicated a need of 3500 pounds. Apparently, with increased applications of calcium carbonate the rate of nitrification was so high, as indicated in table 6, that the soybeans were able to secure a greater part of their nitrogen in the form of nitrates. Large numbers of *B. radicicola* were present in all the pots whether treated with

TABLE 8

The effect of calcium carbonate on nitrogen fixation by B. radicicola of the soybean in soil I

POT	CALCIUM CARBONATE PER 2,000,000 POUNDS OF SOIL	NUMBER	NODULES		STEMS		ROOTS		SOIL		
			Dry weight	Total nitrogen	Dry weight	Total nitrogen	Dry weight	Total nitrogen	Nitrogen in beginning per pot	Nitrogen at end per pot	Nitrogen fixed per 2,000,000 pounds of soil
	pounds		*mgm.*	*mgm.*	*grams*	*mgm.*	*grams*	*mgm.*	*grams*	*grams*	*pounds*
1	0	113	653	31	13.2	348	4.3	40	3.0009	2.8985	93
2	250	67	887	39	14.2	377	5.1	42	3.0039	2,8675	98
3	500	88	749	33	14.3	368	4.4	39	3.0039	2.8985	107
5	2,000	100	1017	47	17.3	464	3.7	35	3.0039	2.8272	130
6	3,000	72	560	27	14.9	303	3.4	38	3.0039	2.7900	+8
Neutral point (Veitch method)											
7	4,000	65	317	16	11.9	342	2.9	37	3.0039	2.7683	−6
8	5,000	79	537	26	14.1	363	3.6	39	3.0039	2.8923	94
9	7,500	84	464	22	13.9	426	3.8	46	3.0039	2.7218	23
10	10,000	66	212	11	11.2	342	3.6	52	3.0039	2.6660	−60
11	20,000	45	220	13	10.3	298	5.1	58	3.0039	2.6815	−87
12	40,000	57	221	13	12.2	383	3.4	50	3.0039	2.6505	−56

0.1727 gm. of nitrogen in the soybeans planted.
0.0043 gm. of nitrogen in the water used in watering the soybeans.

TABLE 9

The effect of calcium carbonate on nitrogen fixation by B. radicicola of the soybean in soil II

POT	CALCIUM CARBONATE PER 2,000,000 POUNDS OF SOIL	NUMBER	NODULES		STEMS		ROOTS		SOIL		
			Dry weight	Total nitrogen	Dry weight	Total nitrogen	Dry weight	Total nitrogen	Nitrogen in beginning per pot	Nitrogen at end per pot	Nitrogen fixed per 2,000,000 pounds of soil
	pounds		*mgm.*	*mgm.*	*grams*	*mgm.*	*grams*	*mgm.*	*grams*	*grams*	*pounds*
1	0	79	781	38	14.8	394	4.1	46	2.6181	3.2008	570
3	500	75	744	37	15.2	440	4.0	42	2.6181	3.1860	587
6	3,000	148	577	29	13.5	371	4.7	45	2.6181	3.1418	511
Neutral point (Veitch method)											
8	5,000	71	330	18	12.3	338	3.2	41	2.6181	3.1358	476
9	7,500	85	251	13	12.7	370	3.8	45	2.6181	3.1270	490
10	10,000	66	207	12	11.8	335	3.3	57	2.6181	3.0238	408
11	20,000	54	124	5	11.5	341	4.0	68	2.6181	2.9648	377
12	40,000	33	126	7	14.4	393	3.0	40	2.6181	2.9205	265

0.1727 gm. of nitrogen in the soybeans planted.
0.0043 gm. of nitrogen in the water used in watering the soybeans.

calcium carbonate or not, although quantitative determinations were not made of their numbers.

THE EFFECT OF CALCIUM CARBONATE ON SOYBEANS UNDER FIELD CONDITIONS

In order to determine whether soybean yields are increased by the use of calcium carbonate on soil I under field conditions, it was decided to grow soybeans on the fertility plots of the station farm during the summer of 1916. Three varieties of soybeans were sown in rows across the plots and cultivated during the growing season. One-half of each plot received an application of calcium carbonate at the rate of 2 tons per acre in the form of ground limestone. The yields of hay produced are given in table 10. The previous crop records and the analyses of the soils of these plots are given in tables 1 and 11.

TABLE 10

Effect of ground limestone on yield of soybean hay on soil I

PLOTS	TREATMENT	CALCIUM-CARBONATE REQUIREMENT PER 2,000,000 POUNDS	YIELD OF HAY PER ACRE		INCREASE WITH LIMESTONE
			No limestone	Limestone	
		pounds	*pounds*	*pounds*	*per cent*
19	N, P, K, CaO	0	5270	5400	+2
20	M, CaO	0	6390	6850	+7
21	Check	2800	1605	1400	−13
22	CaO	0	1920	2430	+26
26	M	2800	7150	7360	+3
26	N, P, K	3200	5300	5370	+1
28	P, K	3600	2820	4690	+66
29	N, K	3400	1285	2280	+77
31	N, P	3200	3375	4460	+32
32	K	3600	1495	1995	+34
34	P	3400	3285	4105	+25
35	N	3400	1220	1705	+40
Averages			3426	4004	+17

From a study of the plots and the crop records, it would seem that the use of 2 tons of limestone per acre did not give sufficient increase in yield to justify the conclusion that soybeans will not grow well except on soils which have had their lime requirement satisfied. On plots 25 and 26, the soils of both of which have rather high calcium-carbonate requirements, but which also contain a fairly high content of nitrogen, the yield of soybeans was little affected by the limestone. This might mean that more nitrogen was secured from the soil on these plots and for this reason the crop was larger. However, the nodules were plentiful on the roots of the soybeans on plots 25 and 26 and, therefore, we could assume that nitrogen fixation from the air was taking place.

EFFECT OF CALCIUM CARBONATE ON THE BACTERIAL ACTIVITIES OF DEKALB SOILS HAVING VARYING LIME REQUIREMENTS

A large number of samples of acid soils all belonging to the Dekalb series were chosen from various parts of West Virginia and sent to the laboratory. From this number 12 samples were chosen which had calcium-carbonate requirements varying from 400 to 4600 pounds per 2,000,000 pounds of soil. The analyses of these soils are shown in table 11. These soils differ mostly because of the different systems of management practiced by the men who have farmed them since the areas from which the samples were chosen were cleared from the forest. Many of these areas had been farmed for from seventy-five to one-hundred years and others had not been farmed for more than a few years.

TABLE 11

Analyses of soils of table 12

SAMPLE	POUNDS PER 2,000,000 POUNDS OF SOIL			
	Nitrogen	Phosphorus	Carbon	Calcium-carbonate requirement
	pounds	*pounds*	*pounds*	*pounds*
III	3870	1203	41,420	400
IV	1904	586	21,790	1000
V	1669	680	17,600	1200
VI	3374	697	47,230	1400
VII	3142	902	32,140	1600
VIII	2042	1216	20,280	2000
IX	2602	662	32,450	2200
X	4142	1135	48,680	2600
XI	3384	660	39,490	2800
XII	2750	706	32,140	3200
XIII	1960	608	21,900	3800
XIV	3124	753	48,280	4600

The rates of nitrification, ammonification, and nitrogen fixation were studied in an attempt to determine whether there was any relation between the activities of the soil organisms and the calcium-carbonate requirements of these soils.

In nitrification studies 100 gm. of soil to which varying amounts of calcium carbonate had been added were placed in 1000-cc. Erlenmeyer flasks and incubated with optimum moisture content at room temperature for 21 days, using ammonium sulfate as the source of nitrogen, adding a sufficient amount to supply 20 mgm. of nitrogen per 100 gm. of soil. In ammonification studies 100-gm. samples of soil were used and varying amounts of calcium carbonate were added as in the nitrification tests. Casein was used as the source of nitrogen, 160 mgm. of nitrogen being added to 100 gm. of soil. The soil was incubated in tumblers at optimum moisture content for 3 days and the ammonia determined by distillation with magnesium oxide. Nitrogen-fixation tests were carried on by placing 10 gm. of soil in 100 cc. of Ashby's solution in 800-cc. Kjeldahl flasks for a period of 21 days at room temperature. To one set of flasks enough calcium-carbonate was added to be equivalent to 10,000 pounds per 2,000,000 pounds of soil. At the end of 21 days the total nitrogen was determined. All of the determinations on nitrification, ammonification, and nitrogen fixation were performed in duplicate and these duplicates as a rule checked very closely. The results of these experiments are tabulated in table 12.

In general the highest rates of ammonification occurred with soils having the lowest calcium-carbonate requirements. The applications of 2000 pounds and 5000 pounds of calcium-carbonate brought about marked increased in the rate of ammonification. Applications of 10,000 pounds of calcium carbonate per 2,000,000 pounds of soil caused a decreased ammonification except in soils XIII and XIV, which had calcium-carbonate requirements of 3800 and 4600 pounds, respectively. Apparently the application of 10,000 pounds of calcium carbonate per 2,000,000 of soil on soils having calcium-carbonate requirements of less than 3800 pounds is injurious to ammonifying organisms.

There was no very definite correlation between the rate of nitrification of ammonium sulfate and the calcium-carbonate requirement of the soils. In general, the soils having high calcium-carbonate requirements had a very low nitrifying power. With soils having calcium-carbonate requirements in excess of 2200 pounds per 2,000,000 pounds of soil, the nitrifying organisms did not become markedly active even with large applications of calcium carbonate. Either the nitrifying organisms were almost entirely absent or had become very inactive because of the unfavorableness of the medium in which they were living.

There was no very marked correlation between the calcium-carbonate requirement of these soils and the nitrogen-fixing power of the soil organisms in Ashby's solution. Soil XIV, having a calcium-carbonate requirement of 4600 pounds, was able to fix nitrogen to the extent of 2.9 mgm. per 100 cc. of Ashby's solution in 21 days. The rate of nitrogen fixation was increased in every case by the addition of calcium carbonate, but the effect was more marked on soils having a high requirement than in soils having a low calcium-carbonate requirement. It seems remarkable, however, that nitrogen fixation took place in all cases even though some of the soils had very high lime requirements.

EFFECT OF FERTILIZERS ON THE BACTERIAL ACTIVITIES OF SOILS

These experiments were conducted in order to determine what effect differences in the fertilizer treatments of the same soil would have on the bacterial activities in the soil. Samples of soil were chosen from 12 plots of the fertilizer series of the fertility plots on the West Virginia station, some of which differ considerably because of the fertilizer applications they have received during the last 15 years. Records of the treatments of the soil on these plots have

TABLE 12

The effect of calcium carbonate on the activities of soil bacteria in Dekalb soils having varying lime requirements

SOIL	CALCIUM-CARBONATE REQUIREMENT PER 2,000,000 POUNDS OF SOIL	CALCIUM CARCONATE APPLIED PER 2,000,000 POUNDS OF SOIL	NITROGEN PER 100 GM. OF SOIL				NITROGEN FIXED IN 100 CC. OF ASHBY'S SOLUTION	
			Nitrogen as ammonia from casein		Nitrogen as nitrates from ammonium sulfate			
	pounds	*pounds*	*mgm.*	*relative*	*mgm.*	*relative*	*mgm.*	*relative*
III	400	0	68.9	100	8.0	100	7.6	100
		2,000	76.5	111	12.6	158		
		5,000	87.6	127	12.5	156		
		10,000	82.4	118	9.4	118	8.7	114
IV	1,000	0	67.1	100	4.4	100	4.2	100
		2,000	81.2	121	8.4	191		
		5,000	86.4	129	13.5	307		
		10,000	74.6	111	14.0	318	5.6	133
V	1,200	0	68.9	100	1.3	100	2.7	100
		2,000	80.5	117	7.8	600		
		5,000	78.1	114	12.0	969		
		10,000	72.6	105	16.0	1231	4.1	152
VI	1,400	0	73.5	100	5.8	100	4.4	100
		2,000	83.2	113	7.3	126		
		5,000	86.9	118	8.0	138		
		10,000	87.7	119	12.6	217	5.6	127
VII	1,600	0	56.8	100	1.5	100	4.9	100
		2,000	77.3	136	3.0	200		
		5,000	91.8	161	5.7	380		
		10,000	87.5	154	16.0	1067	5.9	120
VIII	1,800	0	67.0	100	0.4	100	5.1	100
		2,000	76.2	114	1.5	375		
		5,000	96.6	129	8.0	2000		
		10,000	87.3	130	15.7	3925	6.5	127
IX	2,200	0	69.6	100	0.8	100	3.1	100
		2,000	83.1	119	5.1	638		
		5,000	90.1	129	9.8	1225		
		10,000	82.7	119	4.6	575	4.7	151
X	2,600	0	62.9	100	2.7	100	3.4	100
		2,000	75.1	119	4.8	144		
		5,000	92.2	147	5.2	156		
		10,000	91.8	146	13.0	390	4.8	141
XI	2,800	0	51.6	100	0.8	100	2.3	100
		2,000	68.3	132	2.2	275		
		5,000	88.9	172	1.7	213		
		10,000	92.2	159	2.1	263	3.2	139

TABLE 12—(Continued)

SOIL	CALCIUM-CARBONATE REQUIREMENT PER 2,000,000 POUNDS OF SOIL	CALCIUM CARBONATE APPLIED PER 2,000,000 POUNDS OF SOIL	NITROGEN PER 100 GM. OF SOIL				NITROGEN FIXED IN 100 CC. OF ASHBY'S SOLUTION	
			Nitrogen as ammonia from casein		Nitrogen as nitrates from ammonium sulfate			
	pounds	*pounds*	*mgm.*	*relative*	*mgm.*	*relative*	*mgm.*	*relative*
XII	3,200	0	49.6	100	0.4	100	4.4	100
		2,000	70.5	142	1.4	350		
		5,000	93.0	187	2.0	500		
		10,000	89.5	180	3.2	800	5.6	127
XIII	3,800	0	46.0	100	1.1	100	5.4	100
		2,000	62.1	135	3.0	273		
		5,000	76.3	166	4.5	410		
		10,000	81.3	177	3.8	345	7.1	131
XIV	4,600	0	30.0	100	0.3	100	2.9	100
		2,000	45.5	152	0.7	233		
		5,000	72.4	241	1.1	367		
		10,000	86.3	288	0.7	233	4.4	152

been given in table 1 previously referred to. Analyses of the soil on the various plots were made and are recorded in table 13.

The studies in nitrification, ammonification, and nitrogen fixation were conducted in the same manner as previously mentioned in the discussion of the 12 soils of the Dekalb series with varying calcium-carbonate requirements. It will be remembered that the soil of the fertility plots is also Dekalb soil. The records of these experiments are shown in table 14.

TABLE 13

Analyses of soils of table 14

PLOT	TREATMENT	POUNDS PER 2,000,000 POUNDS OF SOIL			
		Nitrogen	Phosphorus	Carbon	Calcium-carbonate requirement
		pounds	*pounds*	*pounds*	*pounds*
19	N, P, K, CaO	2130	765	24,500	0
20	M, CaO	2700	1045	32,500	0
21	Check	1830	590	21,200	2800
22	CaO	1750	510	19,400	0
25	M	3240	1220	36,800	2800
26	N, P, K	2665	900	30,400	3200
28	P, K	2280	850	26,000	3600
29	N, K	2290	640	27,000	3400
31	N, P	2395	880	28,000	3200
32	K	2310	740	29,200	3600
34	P	2300	885	28,200	3400
35	N	2100	620	28,800	3400

N indicates nitrate of soda; P, acid phosphate; K, sulfate of potash; M, manure.

TABLE 14

The effect of calcium carbonate on the activities of soil bacteria in Dekalb soils which have received varying fertilizer treatments

TREATMENT	CALCIUM-CARBONATE REQUIREMENT PER 2,000,000 POUNDS OF SOIL	CALCIUM CARBONATE APPLIED PER 2,000,000 POUNDS OF SOIL	NITROGEN PER 100 GM. OF SOIL: Nitrogen as ammonia from casein		NITROGEN PER 100 GM. OF SOIL: Nitrogen as nitrates from ammonium sulfate		NITROGEN FIXED IN 100 CC. OF ASHBY'S SOLUTION	
	pounds	*pounds*	*mgm.*	*relative*	*mgm.*	*relative*	*mgm.*	*relative*
N, P, K, CaO	0	0	78.3	100	15.3	100	5.6	100
		2,000	80.1	102	18.5	121		
		5,000	78.3	100	19.5	127		
		10,000	77.4	99	20.3	133	7.5	134
N, CaO	0	0	87.8	100	17.5	100	6.2	100
		2,000	89.5	102	21.5	123		
		5,000	87.0	99	22.5	129		
		10,000	84.3	96	22.0	126	6.4	103
Check	2,800	0	60.6	100	1.2	100	3.7	100
		2,000	65.8	108	5.4	450		
		5,000	78.2	129	11.8	983		
		10,000	79.7	131	15.5	1275	4.2	113
CaO	0	0	71.8	100	8.5	100	2.6	100
		2,000	75.2	105	15.8	187		
		5,000	82.9	115	18.3	215		
		10,000	82.7	115	22.0	259	4.8	185
M	2,800	0	71.7	100	6.7	100	5.1	100
		2,000	75.3	105	12.5	186		
		5,000	90.5	126	16.3	243		
		10,000	90.0	125	21.5	321	7.2	141
N, P, K	3,200	0	70.1	100	2.9	100	4.2	100
		2,000	80.3	114	7.0	241		
		5,000	85.5	122	9.3	321		
		10,000	86.6	126	13.8	475	6.1	145
P, K	3,600	0	58.4	100	1.4	100	4.3	100
		2,000	66.2	109	4.3	301		
		5,000	76.3	131	8.5	601		
		10,000	82.4	141	13.0	928	5.5	128
N, K	3,400	0	56.6	100	1.5	100	5.1	100
		2,000	65.9	116	4.8	320		
		5,000	75.5	133	7.0	467		
		10,000	81.8	144	10.0	667	6.8	133
N, P	3,200	0	60.9	100	1.8	100	4.4	100
		2,000	68.7	113	5.8	322		
		5,000	82.9	136	9.7	504		
		10,000	85.2	140	12.8	701	5.5	125

TABLE 14—(Continued)

TREATMENT	CALCIUM-CARBONATE REQUIREMENT PER 2,000,000 POUNDS OF SOIL	CALCIUM CARBONATE APPLIED PER 2,000,000 POUNDS OF SOIL	NITROGEN PER 100 GM. OF SOIL: Nitrogen as ammonium from casein		Nitrogen as nitrates from ammonium sulfate		NITROGEN FIXED IN 100 CC. OF ASHBY'S SOLUTION	
	pounds	*pounds*	*mgm.*	*relative*	*mgm.*	*relative*	*mgm.*	*relative*
K	3,600	0	47.9	100	1.1	100	7.4	100
		2,000	57.4	120	2.5	237		
		5,000	75.7	158	5.0	454		
		10,000	84.0	174	6.6	600	7.6	103
P	3,400	0	49.5	100	1.1	100	5.3	100
		2,000	65.0	131	3.4	301		
		5,000	79.4	160	7.0	636		
		10,000	84.3	170	8.5	772	7.6	143
N	3,400	0	50.2	100	1.1	100	3.8	100
		2,000	64.9	129	2.9	272		
		5,000	78.6	156	5.5	500		
		10,000	83.1	165	7.3	663	6.9	182

Nitrification of ammonium sulfate was not very active in these soils except on the plots where lime had been applied in the field. Even the soil of plots 26, 28 and 31, which had been producing very satisfactory crops as indicated in table 1, did not contain vigorous nitrifying organisms. The rate of nitrification was materially increased by applications of calcium carbonate. The nitrifying organisms were much more active in the soil from the manure plots than in the soil from any of the other plots except where lime had been applied. There was a general tendency for the rate of ammonification of casein to decrease with an increase in the lime requirement of the soils. There were some marked exceptions to this tendency, notably plots 25 and 26. A study of the analyses of these plots shows a high total content of nitrogen and organic matter. No lime has ever been applied to plots 25 and 26. This increased nitrogen in the form of protein represents an increased amount of material available for the action of ammonifying organisms. If a large amount of nitrogen has been stored up in the soil, the amount of ammonia produced without any applications of calcium carbonate would be sufficient to produce satisfactory yields of those crops which are able to utilize ammonia, on a soil having lime requirements no higher than those of plots 25 and 26. The tendency for small applications of calcium carbonate to be relatively much more effective than larger applications was again shown in these experiments. It was evident that ammonification proceeds fairly satisfactorily without the application of calcium carbonate, especially, as suggested in the preceding discussion, if the content of organic nitrogen is high. Large applications of calcium carbonate had a tendency to reduce the rate of ammonification.

Nitrogen fixation took place very readily in Ashby's solution when inoculated with soil from any of the plots. There did not seem to be any correlation between the calcium-carbonate requirement and nitrogen fixation. The addition of calcium carbonate to Ashby's solution caused an increase in nitrogen fixation in every case, but this increase was no more marked in soil having a high lime requirement than in soil having a low lime requirement.

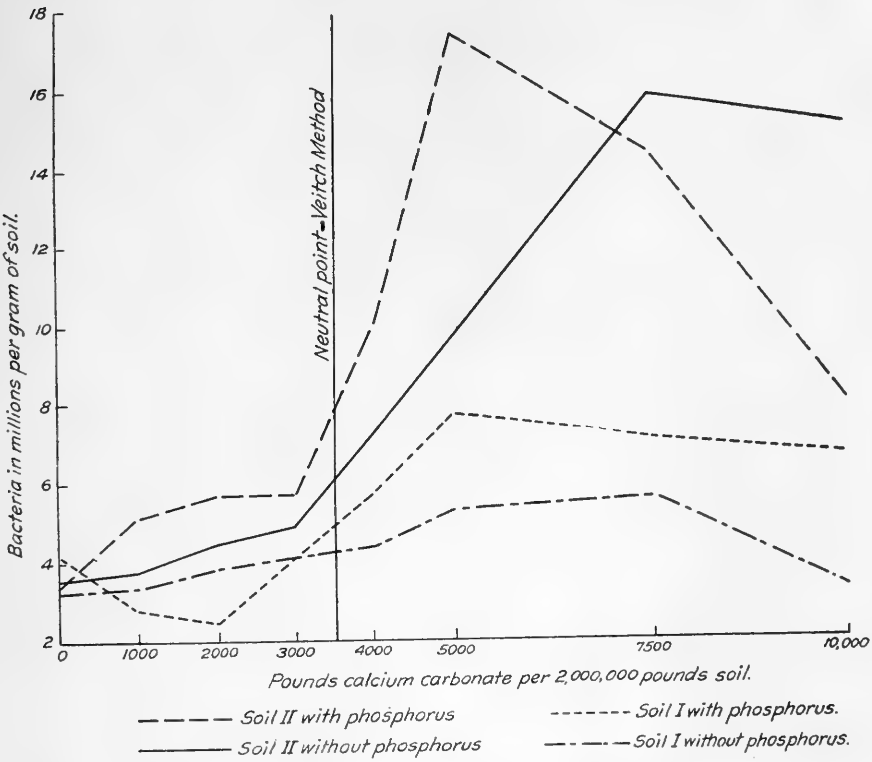

FIG. 1. THE EFFECT OF CALCIUM CARBONATE ON THE NUMBER OF BACTERIA IN SOILS I AND II

SUMMARY AND CONCLUSIONS

This investigation was undertaken as a preliminary step in the study of the possibilities of a system of acid agriculture on soils somewhat distantly removed from a source of lime. A study was made of the relation between the activities of the soil bacteria concerned in nitrogen accumulation and nitrogen transformations and the lime requirement of certain soils. The lime requirement of these soils varied from none to 4600 pounds of calcium carbonate per 2,000,000 pounds of soil. To different portions of these soils calcium carbonate was added in amounts ranging from 0.01 per cent to 2 per cent of the weight of the soil. The data accumulated show that the various groups of soil organisms vary in their response to applications of calcium carbonate.

Ammonification proceeded fairly satisfactorily in most of the soils without the application of lime. The use of moderate amounts of calcium carbonate increased the rate of ammonification in most cases. Small applications were much more effective, relatively, than large applications.

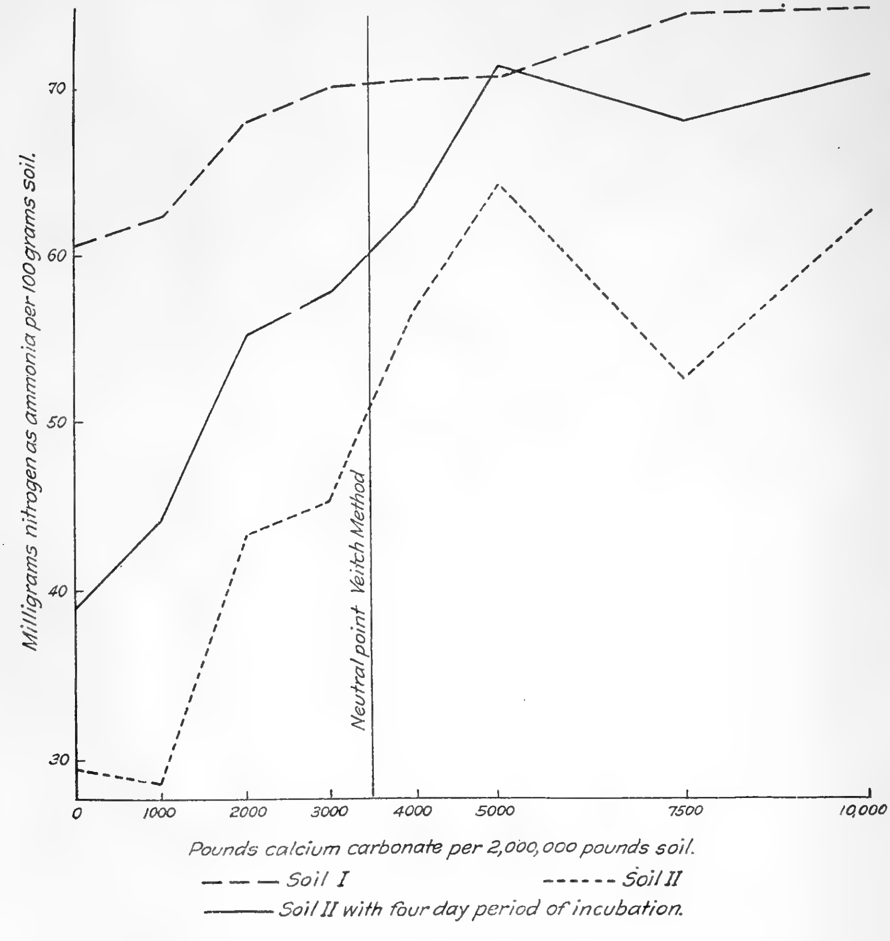

FIG. 2. THE EFFECT OF CALCIUM CARBONATE ON THE RATE OF AMMONIFICATION IN SOILS I AND II WITH PHOSPHORUS

The rate of nitrification was almost directly correlated with the amount of calcium carbonate supplied. Excessive applications were not injurious to the nitrifying organisms. Soils having high lime requirements showed practically no nitrification until calcium carbonate had been mixed with them.

Nitrogen fixation by non-symbiotic soil organisms was considerably increased by the addition of calcium carbonate. The application of mono-calcium phosphate also was necessary for maximum nitrogen fixation. All of the soils studied accumulated considerable amounts of nitrogen when incubated in Ashby's solution without the addition of calcium carbonate, although its use increased the rate of nitrogen fixation.

A lime requirement of 3000 pounds was not sufficient to prevent a good growth of soybeans on soil well fertilized with acid phosphate or manure. Nitrogen fixation accompanying the growth of soybeans took place readily

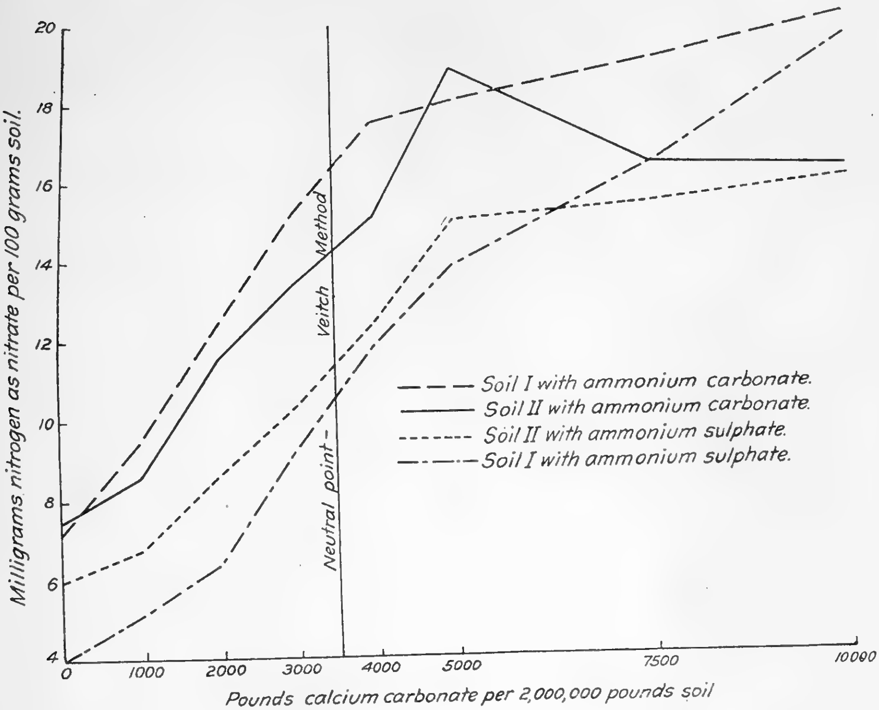

FIG. 3. THE EFFECT OF CALCIUM CARBONATE ON THE RATE OF NITRIFICATION IN SOILS I AND II WITH PHOSPHORUS

in acid soils. This fixation was increased by small applications but decreased by large applications of calcium carbonate.

From these facts the following conclusions seem justified:

1. Plants which are able to utilize ammonia nitrogen need not suffer from nitrogen hunger when grown on soils having lime requirements no higher than those studied in these investigations.

2. Plants which depend on nitrates as their source of nitrogen may suffer from the lack of available nitrogen in soils having high lime requirements, unless these requirements have been at least partially satisfied.

3. The supply of nitrogen in acid soils may be maintained by growing acid-resistant legumes, of which the soybean is one. Undoubtedly, the use of acid phosphate aids materially in the nitrogen-fixation processes in acid soils.

4. Small applications of calcium carbonate are, as a rule, relatively more effective than large applications as a means of increasing the bacterial activities in acid soils.

Acknowledgment is due Dr. E. B. Fred of the University of Wisconsin for many helpful suggestions and criticisms offered during the progress of this investigation.

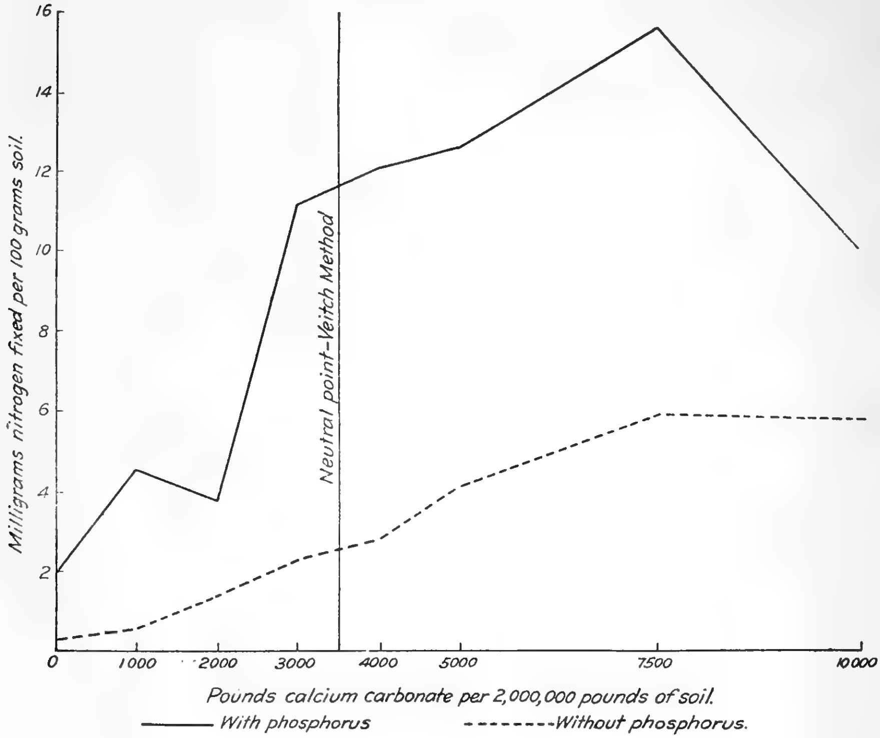

FIG. 4. THE EFFECT OF CALCIUM CARBONATE ON NON-SYMBIOTIC NITROGEN FIXATION IN SOIL II

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