BIOLOGICAL ACTIVITY
AS A MEASURE OF SOIL FERTILITY

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CONTENTS

PART I

MANNITOL DECOMPOSITION IN SOIL AS A MEASURE OF CROP RESPONSE TO FERTILIZERS AND SOIL PRODUCTIVITY

PART II

THE EFFECT OF SOIL MICROORGANISMS ON SOIL REACTION

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PART I

MANNITOL DECOMPOSITION IN SOIL AS A MEASURE OF CROP RESPONSE TO FERTILIZERS AND SOIL PRODUCTIVITY

INTRODUCTION

Microbiological methods used in soil investigations are based upon a relation in the requirement of crop plants and soil microorganisms for nutrients. For those elements which both crops and soil microorganisms require in large quantities, biological methods may indicate the quantities present or the response of the plants to the added elements.

Crop response to fertilizers and different levels of soil fertility are measured by crop yields; soil microorganic response to fertilizers and variation in soil fertility may be determined by:

1. Numbers of soil microorganisms.
2. Rate of ammonification.
3. Rate of nitrification.
4. Rapidity of organic matter disappearance
5. Carbon dioxide production.

Of these methods, the determination of carbon dioxide production is one of the easiest. Carbon dioxide is an end-product of respiration, and as such is indicative of the activity of the organisms concerned.

The object of this investigation was to determine the relation between the response of crop plants, as measured by crop yields, and the response of soil microorganisms in mannitol treated soil, as measured by carbon dioxide production, to applied nitrogen, phosphorus, potassium, calcium, magnesium, copper, zinc, manganese, and arsenic, and to soil productivity.

REVIEW OF LITERATURE

The production of carbon dioxide by soil microorganisms has been found to be closely related to the number of bacteria by Russell and Appleyard (39), Petersen (32), van Suchtelen (46), and Stoklasa (44).

The effect of various salts on the production of carbon dioxide or the decomposition of organic matter by the soil microorganisms has been investigated by Corbet (9), Fred and Hart (13), Lemmerman and coworkers (25), Lundegardh (26), Merkle (27), Potter and Synider (34), and Remy (37). Christensen and Jensen (8) made a review of literature dealing with the use of carbon dioxide production in soil fertility investigations.

Of the materials added to soils to supply microorganism energy, cellulose has probably been used more than any other. The supply of available nitrogen in the soil affects the rate of decomposition of cellulose very markedly. Carbon dioxide production is the measure generally used to indicate its decomposition. The method as it has been used requires up to twenty-four days or more and several carbon dioxide determinations are necessary.-- References: Waksman and Heukelekian (49), Starkey (41), Anderson (3), Carter (6), Holben (18), Shunk (40), and Nicklewski (31). Holben found that "plots receiving incomplete fertilizer treatments rank too high in carbon dioxide production to show any relation to crop yields. Several other high crop producing plots rank too far down the list in carbon dioxide evolution to credit this method as a reliable indication of soil productivity. . . . . When soil acidity is eliminated as a limiting factor . . . . . the carbon dioxide production and cellulose decomposing powers show very close agreement to crop yields".

The production of carbon dioxide in the soil from cellulose,dextrose, rye straw, alfalfa meal, dried blood, and mixed spores and mycelium of fungi was studied by Starkey (41). He found that the production of carbon dioxide from organic materials is affected by soil productivity, and that the differences in the production of carbon dioxide from the carbon contained in the soil are greater than the differences in the carbon dioxide produced by the same soils from added organic matter.

Neller (30) found a correlation between crop yield and the carbon dioxide produced from 200 gm. soil plus 0.75 gm. soybean hay. Merkle (27) added soybean hay to soil and found that nitrate of soda and basic slag increased carbon dioxide production. Ammonium phosphate, superphosphate, sulphate of potash, and raw bone had little effect, whereas muriate of potash and kainit reduced the production of carbon dioxide.

Dextrose and glucose have been used in soil investigations to supply the microorganisms with energy. König and Hasenbäumer (22), König, Hasenbäumer, and Glenk (23) used carbon dioxide production from glucose in soil investigations. Waksman and Starkey (51) and Starkey (41) found that carbon dioxide production from dextrose may be used in soil fertility investigations; the former authors (Waksman and Starkey) found that it may serve as a means of grading soils on a basis of their fertility.

The quantity of nitrogen fixed by soil microorganisms has been found to be indicative of the amount of available phosphorus in a soil. Waksman and Karunaker (50), Turk (48), Stoklasa (43), Given, Kuhlman, and Kern (15) determined the nitrogen fixed in standard mannitol solutions inoculated with soil and incubated for as long as twelve weeks. Waksman and Karunaker (50) concluded that information concerning the nitrogen fixing bacteria and the available phosphorus of a soil may be obtained by the mannitol disappearance method of Christensen (7), in which 2% mannitol is added to a soil and the residual mannitol determined every five days for thirty days. Water equal to 75% of the maximum water-holding capacity of the soil was used.

Andrews (4) found that the production of carbon dioxide in soils to which mannitol had been added furnished a basis for determining the nitrogen and the phosphorus requirement of soils for cotton. The time used for carbon dioxide production was twenty-four hours. The water used was one-third of the maximum water-holding capacity of the soil.

When the work reported in this paper was started, it was arbitrarily decided to determine the effect of fertilizer treatment on the production of carbon dioxide in mannitol treated soil and to correlate the data thus obtained with the effect of a similar treatment on the crop yield. During the course of the investigations, the following questions arose:

1. What is the effect of the soil water content on the production of carbon dioxide?

Gainey (14) found 12% water for one soil to be the water content for maximum carbon dioxide production, and that higher quantities had little effect, whereas smaller quantities reduced it. König, Hasenbäumer, and Glenk (23) used 50% of the maximum water holding capacity for carbon dioxide production experiments with dextrose. Christensen (7) and Waksman and Karunaker (50) used 75% of the maximum water-holding capacity in mannitol disappearance experiments. The optimum water content for carbon dioxide production from cellulose decomposition was found by Shunk (40) to be 50% saturation. Van Suchtelen (46) found 75% of the maximum water-holding capacity to be more favorable than 30% and 90% for the production of carbon dioxide and numbers of bacteria (dextrose was used, and the temperature was 10 to 12° C.).

2. Should soils be air-dried before the biological tests are made?

Darbishire and Russell (11) investigated the effect of partial sterilization of a soil by heating to 100° C. or by use of volatile antiseptics which were subsequently removed and found that partial sterilization increased the available N, P, and K, and also the absorption of oxygen by the soil micro-organisms on rewetting the soil.

Air-drying a soil and rewetting it has been found to increase the numbers of bacteria and/or carbon dioxide production.—- References: 17, 20, 21, 35, 36, 38, and 42. Achromeiko (1), Gustafson (16), and Lebedjantzev (24) found that air-drying soil increased the soluble quantities of one or more of the following: Organic matter, nitrogen, phosphorus, and other minerals. Klein (21) found no increase in soluble K, Ca, and P due to air-drying.

Waksman and Starkey (55) said that protozoa and fungi are not destroyed by air-drying soils. They (53) report the following data from Lennan (without reference):

Influence of Desiccation of Soils on
Development of Colonies of Fungi on Agar

Preparation	Av. No. of Colonies Per Plate	
	Before Desiccation	After Desiccation
Only spores present in the soil	31.0	30.0
Only mycelium present in the soil	20.0	0.8
Field Soil No. 1	10.6	0.6
" " " 2	11.6	1.0
" " " 3	8.3	1.1
" " " 4	25.2	3.0
" " " 5	199.0	13.7
" " " 6	326.3	5.6

Waksman and Starkey (54) said that a high fungus flora may limit the increase in bacterial numbers. They (56) found that partial sterilization practically eliminated fungi.

Andrews (4) found that air-drying soil increased the production of carbon dioxide by one soil and had little effect on another after they were aerated and treated with mannitol.

3. Does the length of time a soil remains air-dry affect the production of carbon dioxide?

Waksman and Starkey (55) found that increasing the length of time a soil remains in a dry state increased its production of carbon dioxide when it was rewetted. Andrews (4) found that storing one soil air-dry decreased the increase in production of carbon dioxide due to N and NP, whereas with another soil a small increase was obtained.

4. What is the effect of protozoa on the production of carbon dioxide by the soil microorganisms?

Cutler (10) said that when protozoa are inoculated as cysts they will, judging from analogy in fluid cultures, probably remain as such for relatively long periods (24 - 28 hours) before excistation occurs. Cutler (10) and Telegdy-Kovats (47) found that protozoa reduce the number of bacteria.

5. Do bacteria, fungi, and Actinomyces produce equal quantities of carbon dioxide when they have consumed equal quantities of energy material?

Waksman and Starkey (52) said that fungi assimilate 20 to 50%; bacteria, 1 to 30%; and Actinomyces, 15 to 30% of the organic compounds decomposed.

Buchanan and Fulmer reviewed the literature on the toxic and stimulative effects of arsenic (5-a), copper (5-b), zinc (5-c), and manganese (5-d) on microorganisms.

The literature reviewed shows that:

A. Soil productivity is related to:

1. Numbers of soil microorganisms
2. Carbon dioxide production by soils receiving
    a. No additional energy material
    b. Energy materials
        (1) Cellulose
        (2) Glucose

(3) Dextrose

(4) Other organic materials

3. Mannitol disappearance

4. Nitrogen fixation

B. Crop response to applied nitrogen and phosphorus is related to the response of soil microorganisms to nitrogen and phosphorus when mannitol has been added to the soil.

C. Carbon dioxide production by a soil is affected by:

1. Air-drying or partial sterilization
2. The length of time a soil remains air-dry
3. The water content of the soil

EXPERIMENTAL

METHODS

Preliminary studies made during the development of the method (4) used in this investigation brought out some observations which are worthy of comment. In taking soil samples, the immediate surface of the soil was removed and the sample taken to a depth of approximately $2\frac{1}{2}$ inches at several places in an experimental plot or field. After air-drying, the samples were screened and thoroughly mixed, preparatory to their use in carbon dioxide production determinations after addition of mannitol and other materials, as designated. The letters in the tables presented indicate that the following quantities of fertilizers were added to 100 gm. of soil:

L = 50 mgm. finely ground limestone

P = 25 mgm. 20% superphosphate

K = 10 mgm. 50% muriate of potash

N = 30 mgm. nitrate of soda

S = 55 mgm. 9% basic slag

R = 15 mgm. Rhum's rock phosphate

The quantity of water to add was determined by "the feel" of the moistened soil for the first experiments; however, a uniform quantity was used throughout an experiment. Later the water used was based upon the maximum water-holding capacity of the soil, which was taken as that quantity of water which is retained by 100 gm. of air-dry soil in a funnel after water had been added in excess and the excess allowed to drain off. The moist soils were then put into 1000 cc. Erlenmeyer flasks and incubated for twenty-four hours, or the time specified, at laboratory temperature, or at the specified temperature.

After incubation, the flasks were connected in gas trains for the collection of the carbon dioxide. The carbon dioxide was removed from the soil flasks by drawing 3600 cc. of air through the trains, and it was absorbed by ascarite. The quantity of carbon dioxide was determined by weighing the ascarite tubes before and after its absorption.

RESULTS AND DISCUSSION

Effect of Fertilizers and Lime on Carbon Dioxide Production

The soils selected for the first experiments were Norfolk and Ochlocknee fine sandy loams. The Ochlocknee soil came from the unfertilized plots of a field on which a fertilizer analysis test had been conducted for a number of years. This soil was very deficient in potash and nitrogen was also a limiting factor in crop production. Lime has never been found beneficial to crops on this Ochlocknee soil. The Norfolk soil was taken from an area adjoining the plots of a phosphorus sources test on limed and unlimed soil. This soil was deficient in nitrogen, phosphorus, potash, and lime for the production of cotton.

Lime, muriate of potash, and superphosphate when used without nitrogen had little affect on the production of carbon dioxide (Table 1) by the soil microorganisms, whereas nitrogen trebled its production. These results indicate that lime, phosphorus,and potash were present in sufficient quantity to satisfy the needs of the soil microorganisms with the nitrogen level found in the untreated soil.

Ammonium sulphate was equally as efficient as nitrate of soda for carbon dioxide production on the Norfolk soil which was deficient in lime for carbon dioxide production and crop growth. The Ochlocknee soil was not deficient in lime for biological activity and probably not for crop growth, and nitrate of soda produced 101 mgm. of carbon dioxide, whereas ammonium sulphate produced 89 mgm. On the basis of the neutralizing effect of the sodium, nitrate of soda might have been considered more efficient for carbon dioxide production on the lime deficient soil. When lime, muriate of potash, and superphosphate were applied, singly and in combinations, to the Ochlocknee soil, in addition to nitrogen, the NPL treatment was the only one that produced more carbon dioxide than nitrogen alone and the increase was only 8 mgm. Supplying lime or superphosphate in addition to nitrogen to the Norfolk soil increased the production of carbon dioxide 38 mgm. This behavior raises the question "Was the microbiological response to superphosphate at least partially due to its calcium content?"

There are no data on the effect of muriate of potash on the yield of crops on the Norfolk soil, but it was apparently deficient in potash for cotton production; muriate of potash increased the yield of seed cotton 473 pounds per acre (28) on the Ochlocknee soil. Generally muriate of potash had little effect on the production of carbon dioxide, even though these soils were deficient in potash for crop production. In order to check these results, soil was obtained

Table 1. Effect of fertilizers and lime on the production of carbon dioxide by soil microorganisms.

TREATMENT	NORFOLK F.S.L.	OCHLOCKNEE F.S.L.	TREATMENT	NORFOLK F.S.L.	OCHLOCKNEE* F.S.L.	TREATMENT	OCHLOCKNEE* F.S.L.
	Mgm. CO_2	Mgm. CO_2		Mgm. CO_2	Mgm. CO_2		Mgm. CO_2
Ck	59.6	65.0	$(NH_4)_2SO_4$	76.2	89.0	NK	100.1
L	69.6	70.4	" + L		90.9	NKL	99.3
K	64.9	56.6	N	75.8	101.6	NPL	108.6
P	57.4	63.1	NL	114.2	99.4	NPK	98.4
N	175.4	201.6	NK	75.7	99.3	NPKL	101.3
			NP	113.3	100.9	PKL	34.2
Time - hours	29	22		18	18		17
Water - c.c.	15	15		15	8		8

The maximum water-holding capacity for the Ochlocknee soil was 35 c.c. per 100 gm. soil, whereas that for the Norfolk was 30 c.c.

*50 gm. of soil and half of the usual quantities of fertilizer were used.

from the check plots of a field test on Ochlocknee soil, in which potash had increased the yield of seed cotton 343 pounds per acre (28) over a period of six years. The addition of potash with nitrogen decreased the production of carbon dioxide 10 mgm., which is in harmony with the above data.

Effect of the Duration of the Experiment and the Amount of Fertilizer on the Production of Carbon Dioxide

The data in Table 2 show that reducing the fertilizers to half reduced the differences in the production of carbon dioxide between the treatments. The quantities of carbon dioxide produced were greater on the first day than on any other day; the relative quantities of carbon dioxide produced were different on the different days. The decrease in the production of carbon dioxide from the first to the fifth day was greater where the usual quantities of fertilizers were used than where half the usual quantities were used. The total production of carbon dioxide for the full amounts of fertilizer was only slightly more than for the half amounts.

Effect of the Water Content of the Soil on the Production of Carbon Dioxide

The data on the effect of the water content of the soil on the production of carbon dioxide are reported in Table 3. The maximum water-holding capacity of this soil was 30 cc. per 100 gm. of soil. The data show that 15 cc. of water was slightly more efficient for the production of carbon dioxide than was 10 and 12 cc., whereas 8 cc. was decidedly inferior to the 12 cc. application. The 25 and 50 cc. applications of water were decidedly inferior to smaller applications. The greatest response to superphosphate and the least response to basic slag was obtained with the smallest applications of water; when the water was increased, the response to basic slag increased, whereas that to superphosphate decreased.

Table 2. Effect of the duration of the experiment and the amount of fertilizer on the production of carbon dioxide by Norfolk fine sandy loam.

TIME IN DAYS ---	FULL AMOUNTS OF FERTILIZERS						ONE -HALF OF THE USUAL AMOUNTS OF FERTILIZERS					
	1	2	3	4	5	Total	1	2	3	4	5	Total
TREATMENT	MGM. CARBON DIOXIDE						MGM. CARBON DIOXIDE					
NK	154.7	120.0	78.7	48.6	21.0	423.0	129.3	86.6	81.1	64.2	40.1	401.3
NPK	180.8	144.1	87.3	30.8	19.1	462.1	136.0	82.8	74.1	59.0	38.9	390.8
NSK	190.0	103.9	62.6	40.5	21.8	418.8	141.2	85.5	82.1	58.1	35.8	402.7
NRK	150.8	118.1	78.2	48.3	20.5	415.9	127.9	86.1	80.7	61.3	40.9	396.9
NKL	163.5	101.1	61.0	45.5	33.8	404.9	133.6	85.4	77.1	55.2	35.4	386.7
NPKL	200.7	107.1	60.4	39.6	24.4	432.2	140.1	82.2	73.4	51.1	35.2	382.0
NSKL	202.9	89.6	51.9	39.7	29.1	413.2	147.6	85.6	74.4	52.2	33.7	393.5
NRKL	167.6	102.7	63.7	47.0	34.7	415.7	131.1	79.2	69.5	51.2	35.3	366.3
NP	178.1	141.0	87.9	31.2	17.0	455.2	131.0	83.3	72.0	58.3	37.7	382.3
NPL	203.0	107.0	61.8	42.7	26.4	440.9	141.2	75.8	59.8	48.1	34.9	359.8
PK	52.7	34.1	22.6	22.7	21.6	153.7	51.0	36.6	32.2	28.8	27.5	176.1
PKL	64.8	23.3	26.6	29.6	32.7	177.0	57.6	35.2	30.0	33.9	29.6	186.3

Maximum water-holding capacity 30 c.c.; water used 15 c.c.

Table 3. Effect of the water content of the soil on the production of carbon dioxide in Norfolk fine sandy loam.

TIME - HOURS	11	17 1/3			24			48
C.C. WATER ---	25	8	12	25*	10*	12*	15	50
TREATMENT	MGM. CARBON DIOXIDE							
NK	15.2	50.5	76.9	33.5	132.8	136.2	154.7	129.2
NPK	10.5	103.8	118.3	30.1	172.4	173.4	180.8	127.6
NSK	27.1	97.5	124.2	56.4	171.6	178.7	190.0	155.4
NRK	11.8	50.1	72.1	26.1	133.6	142.9	150.8	125.4

*K omitted, because it was not beneficial in any of the other tests.

The greater response by the microorganisms on the dry soil is in harmony with the recent work of Emmert*,which showed that the phosphorus content of tomato plants was much lower when they were grown on a dry soil than when grown on a wetter soil.

Summarizing the preliminary data, they show that both cotton and soil microorganisms respond to nitrogen, lime, and superphosphate, and that the response of the latter to muriate of potash is either negative or small. The greatest differences due to the treatment were obtained during the first twenty-four hours. The largest quantities of carbon dioxide were obtained when the water content of the soil was 50% of the maximum water-holding capacity, whereas the largest quantities of carbon dioxide due to the addition of superphosphate were obtained when the water content of the soil was 27% of the maximum water-holding capacity. The water content used in the experiments which followed was one-third of the maximum water-holding capacity of the soil, except in one instance which is noted. This water content was selected on the basis of the response of the soil micro-organisms to additions of nitrogen and phosphorus and the ease with which this quantity of water can be mixed with the soil and the ease with which a soil of this water content may be handled.

Effect of Different Phosphorus Carriers on the Production of Carbon Dioxide and Seed Cotton

The Norfolk soil was obtained from a field where phosphorus carriers had been tested with and without lime. The effect of different phosphorus carriers and lime on the production of carbon dioxide and seed cotton is shown by the data in Table 4. When used without lime, basic slag produced more carbon dioxide and more seed cotton than any other phosphorus carrier; with lime, superphosphate was more effective for both carbon dioxide and crop production. Rock phosphate was decidedly

*Emmert, E. M. 1936. The Effect of Drouth on the Nutrient Levels in the Tomato Plant. Soil Sci. 41:67-70

Table 4. Effect of different phosphorus carriers and lime on the production of carbon dioxide and seed cotton in Norfolk fine sandy loam.

TREATMENT	MGM. CARBON DIOXIDE IN 17 HOURS	POUNDS PER ACRE OF SEED COTTON*
		1931-1932 Average
NP	147.2	440
NS	161.8	729
NR	108.1	378
NLP	170.8	855
NLS	166.6	766
NLR	136.8	665

The maximum water-holding capacity was 30 c.c.; the water used was 17 c.c.

*All plots received K.

inferior to the other carriers of phosphorus, both for carbon dioxide and crop production. The relative response of the soil microorganisms and cotton to the carriers of phosphorus and lime was not the same. There was probably an accumulation of lime in the field from the addition of basic slag. Three tons of lime per acre was used in the field, whereas 1000 pounds per acre equivalent was used in the laboratory.

Effect of Different Quantities of Superphosphate on the Production of Carbon Dioxide

The effect of different quantities of superphosphate on the microbiological activity of the soil without nitrogen and with one rate of application is reported in Table 5. Without added nitrogen, superphosphate had no effect on the production of carbon dioxide. The 25 mgm. application of superphosphate supplied P_2O_5 equivalent in weight to the nitrogen in the nitrogen treatment. With nitrogen present, the first increment of superphosphate increased the production of carbon dioxide 89 mgm.; the second, 18 mgm.; the third, 1 mgm; whereas the fourth reduced the production of carbon dioxide 10 mgm., and the fifth reduced it 18 mgm. These data show that the soil microorganisms used superphosphate efficiently when a sufficient quantity was used to supply P_2O_5 equal in weight to half the nitrogen added, and that additional superphosphate sufficient to supply P_2O_5 equal in weight to the nitrogen added was slightly beneficial, and that additional quantities were harmful.

Effect of Temperature on the Production of Carbon Dioxide

Raising the temperature had practically no effect on the increase in microbiological activity due to the addition of nitrogen or nitrogen plus superphosphates (Table 6). The laboratory temperature in July was about 26 to 27° C. The increase in the production of carbon dioxide due to the addition of nitrogen alone was 111, 114, and 108 mgm. and that due to the addition of

Table 5. Effect of the quantity of superphosphate on the production of carbon dioxide by a Ruston fine sandy loam with and without the addition of nitrogen.

QUANTITY OF SUPERPHOSPHATE	MGM. CARBON DIOXIDE IN 24 HOURS	
	Without Nitrogen	With Nitrogen
None	24.0	110.2
12.5 Mgm.	24.5	198.9
25.0 "	24.3	217.4
50.0 "	25.0	218.2
100.0 "	24.6	207.5
200.0 "	23.2	190.0

Table 6. Effect of temperature on the production of carbon dioxide by an Orangeburg fine sandy loam.

TREATMENT	MGM. CARBON DIOXIDE IN 24 HOURS		
	LABORATORY TEMPERATURE July 17 and 18, 1933 26-27° C.	30° C.	33° C.
None	31.5	42.0	67.4
N	143.2	155.6	175.6
P	30.3	45.3	75.9
NP	150.0	167.3	196.1

superphosphate and nitrogen together was 120, 122, and 120 mgm. for the summer laboratory temperature (27° C.), 30° C., and 33° C., respectively. The carbon dioxide produced by the check for the respective temperatures was 32, 42, and 67 mgm.

Relation Between the Response of Cotton and Soil Microorganisms to Superphosphate and Their Relation to the 0.002 N Sulphuric Acid Soluble Phosphorus of the Untreated Soil

The relation between the response of cotton and soil microorganisms to superphosphate and their relation to the phosphorus content of the untreated soil, as indicated by Truog's method, is shown in Tables 7 and 8. There were only thirteen determinations. The correlation coefficient between the increase in crop yield due to superphosphate and the increase in the carbon dioxide produced due to superphosphate is $.60 \pm .176$. The correlation coefficient between the increase in crop yield due to superphosphate and the phosphorus soluble in dilute acid is $-.59 \pm .181$. The correlation coefficient between the increase in carbon dioxide due to superphosphate and the phosphorus soluble in dilute acid is $-.49 \pm .210$.

Dividing the dilute acid soluble phosphorus by the combined silt and clay content, or the maximum water-holding capacity of the soil, increased the correlation coefficient of increases in yield of cotton and soluble phosphorus from $-.59 \pm .181$ to $-.70 \pm .143$ and $-.63 \pm .167$, respectively. These data show that the heavy soils require a greater supply of dilute acid soluble phosphorus than the light soils to supply the needs of cotton without the addition of superphosphate. The silt and clay content of the soil was determined by the Bouyoucos hydrometer method.

Effect of Air-Drying Soils on the Microbiological Activity

The literature reviewed shows that fungi and protozoa go into inactive states when soil is air-dried. When the soil is rewetted, they do not become active immediately. Increases in the number of bacteria due to air-drying and

Table 7. Relation between the increase in carbon dioxide production and crop yield due to phosphorus and soluble phosphorus, as determined by Truog's method, and the use of the maximum water-holding capacity and silt + clay content of the soil in the interpretation of the data.

SOIL TYPE	P.P.M. P	INCREASE DUE TO P		SILT + CLAY
		CARBON DIOXIDE	SEED COTTON Lbs. Per Acre	
Houston Clay	8.3	70	385	75.6
Ruston F.S.L.(?)	10.0	52	174	56.8
Orangeburg F.S.L.	35.8	7	33	27.8
Norfolk F.S.L.	17.6	49	194	23.8
Oktibbeha Clay	7.3	44	297	72.6
Sarpy F.S.L.	105.5	15	-1	61.8
Trinity Clay	94.0	7	77	63.6
Denham Silt L.	8.4	16	50	66.8
Ruston F.S.L.	13.1	7	145	34.3
Yohola V.F.S.L.	85.8	14	125	63.8
Ruston F.S.L.	9.5	32	138	19.8
Ruston F.S.L.	9.4	30	238	29.3
Memphis Silt L.	4.2	18	409	64.8

Table 8. Correlation of some of the data in Table 7.

FACTORS BEING CORRELATED	CORRELATION COEFFICIENT
Increase in crop yield due to phosphorus and increase in carbon dioxide due to phosphorus	+.602 ± .1768*
Increase in carbon dioxide due to phosphorus and available phosphorus in soil	-.492 ± .2102
Increase in crop yield due to phosphorus and available phosphorus in soil	-.591 ± .1805
Increase in crop yield due to phosphorus and available phosphorus divided by silt + clay content	-.695 ± .1434
Increase in crop yield due to phosphorus and available phosphorus divided by maximum water-holding capacity	-.632 ± .1666

*Standard error

rewetting have been reported many times. Many investigators report changes in the solubility of the soil nutrients--most of them being increases. In a later paper it will be shown that soil bacteria take up an excess of acidic substances over basic substances and that fungi take up an excess of basic substances over acidic substances. A general effect of bacterial growth in the soil is to increase the pH, whereas fungal growth decreases it. On this basis, when bacteria are decomposed the minerals incorporated in their bodies are returned to the soil and the pH is decreased. On the contrary, when fungi are decomposed the mineral constituents of their bodies increase the pH of the soil. The disappearance of the fungi on air-drying a soil is, therefore, in harmony with an increase in the pH and solubility of certain basic constituents, as was brought out in the literature reviewed. On air-drying and rewetting a soil, there exists a period of time when the competition from the fungi is reduced, due to their smaller numbers or decreased activity. It seems logical that the bacteria may be able to consume at least a part of the energy and other constituents of the fungi as desiccation proceeds. If this is true, the reported increases in numbers of bacteria due to air-drying and rewetting may be coincident with desiccation.

The decomposition of 250 pounds of fungal mycelium containing 6% nitrogen will return fifteen pounds of nitrogen to the soil, which is equal to one-sixth of the nitrogen application used in these experiments. From this standpoint, the death and decomposition of fungi on air-drying and rewetting certain soils may increase the amount of soluble nitrogen considerably.

Immediate Effect of Air-Drying on the Production of Carbon Dioxide: The carbon dioxide production data are for twenty-four hours, which is too short a period after rewetting for fungi to be revived and be very active in the production of carbon dioxide. The carbon dioxide produced during the first twenty-four hours after air-drying a soil is, therefore, considered to be due to the activity of the bacteria. The data in Table 9 show that the production of carbon dioxide by two of the check soils was almost doubled on air-drying; and that of three of the N treated soils was very much higher; whereas fourteen of the soils showed little change in carbon dioxide production; and one showed a big decrease. The increase in carbon dioxide due to the addition of phosphorus to the Olivier soil was nearly three times as great after air-drying as before.

Effect of Prolonged Air-Drying on the Production of Carbon Dioxide: The effect of air-drying soil for eight months on the biological activity is shown by the data in Table 10. The effect of fertilizers on the production of carbon dioxide in these soils was determined in November, 1933, after which the remainder of the soils were put in pots and rewetted. The rewetted soils were permitted to become air-dry during December, and they remained air-dry until the following September, when they were given similar fertilizer treatments and carbon dioxide production was determined again. The incubation temperature was 30° C. in both cases. In every case, the production of carbon dioxide by unfertilized soil was increased by prolonged air-drying. In one out of six cases, the N treated soil produced considerably more carbon dioxide after the prolonged air-drying, which indicates a favorable change in the available phosphorus and/or calcium. In two cases, the soil receiving N produced considerably less carbon dioxide after prolonged air-drying, which may have been due to a decrease in numbers of bacteria. In five cases, the increase in the production of carbon dioxide due to P was very much greater after prolonged air-drying.

Response of Soil Microorganisms to Calcium and Magnesium

The laboratory method used below was identical with that reported above, except 500 c.c. Erlenmeyer flasks were used, the air was drawn through the train by means of a suction pump which was run for five minutes, P = P_2O_5 equivalent to

Table 9. Effect of air-drying the soil on the production of carbon dioxide - Mgm. carbon dioxide.

SOIL CONDITION	MOIST	AIR-DRY	MOIST	AIR-DRY	MOIST	AIR-DRY
TREATMENT						
	Houston Clay 1		Olivier Silt Loam 1		Norfolk Fine Sandy Loam	
Ck	60.9	68.0	49.2	45.2	33.2	31.7
N	70.5	108.9	109.6	101.0	67.8	65.2
NP	163.5	177.9	114.3	113.7	124.4	124.8
	Houston Clay 2		Olivier Silt Loam 2		Lufkin Clay	
Ck	45.4	49.6	52.3	70.8	22.9	27.0
N	63.2	54.3	42.1	52.7	18.9	23.6
NP	159.8	122.3	75.0	146.6	23.5	40.8
	Ruston Fine Sandy Loam 1		Cahaba Silt Loam		Orangeburg Fine Sandy Loam 1	
Ck	28.2	60.0	32.4	42.2	31.7	40.4
N	149.9	183.4	73.8	70.8	164.0	173.6
NP	153.7	193.2	—	103.9	159.0	177.4
	Ruston Fine Sandy Loam 2		Grenada Silt Loam		Orangeburg Fine Sandy Loam 2	
Ck	32.7	63.2	32.1	31.9	40.9	62.3
N	79.8	102.6	40.2	39.0	191.0	207.7
NP	134.9	178.6	85.7	75.5	181.6	—
	Ruston Fine Sandy Loam 3		Fulton*		Lintonia Silt Loam	
Ck	22.5	35.0	73.7	52.3	68.6	65.1
N	81.1	88.3	181.2	170.1	184.3	177.8
NP	132.1	147.6	187.2	164.0	211.4	202.8
	Montrose Clay Loam		Oktibbeha Clay		Pheba Loam	
Ck	60.9	58.1	36.1	46.8	31.2	24.2
N	103.7	68.4	39.1	39.2	31.3	24.3
NP	127.7	91.2	66.4	—	56.6	41.6

*Soil type not known

Table 10. Effect of maintaining soils in an air-dry condition for eight months on the carbon dioxide production at 30° C. - Mgm. carbon dioxide.

TREATMENT	YOHOLA VERY FINE SANDY LOAM		OLIVIER SILT LOAM		LINTONIA SILT LOAM	
	Before Storage	After Storage	Before Storage	After Storage	Before Storage	After Storage
Ck	50.8	78.1	47.1	51.6	44.8	74.3
N	140.4	194.6	68.2	48.6	136.7	131.3
NP	155.1	202.8	90.8	121.1	143.1	198.0
	RUSTON FINE SANDY LOAM 1		RUSTON FINE SANDY LOAM 2		RUSTON FINE SANDY LOAM 3	
	Before Storage	After Storage	Before Storage	After Storage	Before Storage	After Storage
Ck	22.1	37.9	28.3	46.5	14.7	26.0
N	62.5	45.9	126.3	120.1	70.1	73.3
NP	91.9	107.9	133.1	164.3	101.5	133.4

12.5 mgm. 20% superphosphate, and N = 15 mgm. $NaNO_3$.

Soil was obtained from the check plots of three experimental fields on which lime had produced large increases in the yield of crops. The soils were used in the laboratory to determine the response of soil microorganisms to calcium and magnesium. The data are reported in Table 11.

The first Fox soil was so low in lime that it was impossible to establish a stand of alfalfa on it. The data show that the addition of a three-ton equivalent of calcium carbonate in the laboratory increased the production of carbon dioxide five fold. Replacing calcium carbonate with an equivalent quantity of magnesium carbonate up to 75% had a favorable effect on the production of carbon dioxide.

The production of carbon dioxide by the soil microorganisms of the Warsaw soil was reduced from 75 to 59 mgm. by the addition of calcium carbonate. Substituting 25 and 50% of the calcium carbonate with magnesium carbonate increased the production of carbon dioxide to 93 and 98 mgm., respectively.

The production of carbon dioxide by the second Fox soil was doubled by the addition of calcium carbonate. However, the substitution of a part of the calcium carbonate with magnesium carbonate increased the production of carbon dioxide only very slightly with a three-ton application and reduced it slightly with a one-ton application. This soil came from a field where dolomitic limestone was more effective for alfalfa production than was a high calcium limestone.

The beneficial effect of the substitution of a part of the calcium with magnesium, in addition to the nutritive function of magnesium, may be due to the higher solubility of magnesium phosphates below pH 7 (73).

Effect of Certain Rare Elements on the Microbiological Activity of the Soil

<u>Effect of Calcium Arsenate on Carbon Dioxide Production</u>: Soil was obtained from a Norfolk sandy loam field which had received a toxic quantity of

Table 11. Response of soil microorganisms to calcium and magnesium, as measured by production of carbon dioxide.

RATE OF APPLICATION OF LIME		3 TONS	3 TONS	3 TONS	1 TON
PERCENT		SOIL TYPE			
$CaCO_3$	$MgCO_3$	FOX SANDY LOAM	WARSAW LOAM	FOX SANDY LOAM	
		Mgm. CO_2	Mgm. CO_2	Mgm. CO_2	Mgm. CO_2
0	0	9.8	74.7	32.6	—
100	0	59.6	59.2	65.8	64.6
75	25	64.9	92.9	66.2	—
50	50	69.7	98.2	71.4	59.8
25	75	80.4	—	—	—
0	100	79.4	—	—	—

calcium arsenate through dusting cotton for boll weevils. Data in the South Carolina Annual Report, page 35, show that superphosphate decreased the yield of cowpeas on this soil and that iron sulphate overcame the superphosphate-induced arsenic toxicity. Manganese sulphate, and lime did not overcome the superphosphate-induced arsenic toxicity. Data showing the effect of superphosphate on the production of carbon dioxide by this soil are shown in Table 12. The laboratory treatment was nitrogen and water. The data show that quantities of superphosphate up to 800 pounds per acre increased the production of carbon dioxide and that 3200 pounds per acre decreased it only a little below the quantity obtained with the 800-pound treatment. Evidently, the soil microorganisms and cowpeas do not respond to superphosphate in the same manner where arsenic is present.

The data on page 34 of the 1931 S. C. Annual Report show that the addition of calcium arsenate in large quantities materially reduced the yield of cowpeas, soybeans, corn, and sorghum. When increasing quantities of calcium arsenate were applied (Table 13), the 500 to 2000 pound applications markedly reduced the production of carbon dioxide during the first twenty-four hours; but the carbon dioxide produced where 6000 pounds was added was equal to that where none was added. The carbon dioxide produced during the 24 - 48 hour period shows no harmful effects due to calcium arsenate. The data indicate that the effect of calcium arsenate toxicity to certain crop plants may be determined by the production of carbon dioxide during the first twenty-four hours where quantities of calcium arsenate are used which are likely to be applied in the field. However, additional work is needed before the test can be fully adapted for determining the toxic limits of crop plants.

<u>Effect of Iron Sulphate and Other Salts on Arsenic Toxicity to Microorganisms</u>: Iron sulphate overcame the superphosphate-induced calcium arsenate toxicity to cowpeas in the field. After finding that soil microorganisms are less active in the presence of certain quantities of calcium arsenate for a

Table 12. Effect of superphosphate on the production of carbon dioxide by an arsenic sick Norfolk fine sandy loam.

LABORATORY TREATMENT - PER ACRE*	MGM. CARBON DIOXIDE IN 24 HOURS
Check	56.9
100 Pounds Superphosphate	90.1
300 " "	126.3
400 " "	130.9
600 " "	141.2
800 " "	143.1
1600 " "	140.0
3200 " "	134.4

*600 pounds per acre of nitrate of soda was added to all treatments.

Table 13. Effect of calcium arsenate on the production of carbon dioxide by a Greenville sandy loam.

LABORATORY TREATMENT - PER ACRE*	MGM. CARBON DIOXIDE	
	0 - 24 HOURS	24 - 48 HOURS
Check	49.6	157.8
500 Pounds Calcium Arsenate	28.2	172.5
750 " " "	26.5	171.8
1000 " " "	26.8	172.0
1500 " " "	32.7	177.4
2000 " " "	32.4	171.1
3000 " " "	36.2	169.1
4000 " " "	42.5	168.4
5000 " " "	45.2	164.6
6000 " " "	49.5	167.8

*600 pounds nitrate of soda and 1000 pounds superphosphate were added.

Table 14. Effect of sodium sulphate, magnesium sulphate, aluminum sulphate, ferrous sulphate, copper sulphate, and manganese sulphate on the production of carbon dioxide by calcium arsenate treated soil.

LABORATORY TREATMENT - PER ACRE	MGM. CARBON DIOXIDE IN 24 HOURS
Check	139.2
500 Pounds Calcium Arsenate	100.9
500 Pounds Calcium Arsenate + 400 Pounds Sodium Sulphate	91.1
500 Pounds Calcium Arsenate + 400 Pounds Magnesium Sulphate	92.8
500 Pounds Calcium Arsenate + 400 pounds aluminum sulphate	64.8
500 Pounds Calcium Arsenate + 400 Pounds Iron Sulphate	92.0
500 Pounds Calcium Arsenate + 200 Pounds Copper Sulphate	61.6
500 Pounds Calcium Arsenate + 200 Pounds Manganese Sulphate	92.7

twenty-four hour period, it was attempted to overcome this reduced activity by means of iron, sodium, magnesium, aluminum, copper, and manganese sulphates. The data in Table 14 show that these additions decreased the production of carbon dioxide by the soil microorganisms. Aluminum and copper sulphates decreased the production of carbon dioxide the greatest amounts.

Effect of Zinc, Manganese, and Copper on the Production of Carbon Dioxide: Soils were obtained from the Florida Experiment Station which were known to be deficient in zinc, manganese, and copper for certain crops, and the influence of these elements on the microbiological activity of the soil was determined. The data in Table 15 show that neither copper, zinc, nor manganese increased the production of carbon dioxide by the respective soils.

Relation of Crop Yields on Fertility Plots to Carbon Dioxide Production by Soils from These Plots

The data reported above deal with the effect of fertilizers on the production of crops and carbon dioxide (on mannitol treated soil); the data which follow deal with the relation between the yield of crops and the production of carbon dioxide by soils from fertility plots, and soil of different horizons receiving fertilizer treatment in the greenhouse.

Relation of Fertilizer and Lime Treatment in the Field to Crop Yields and Carbon Dioxide Production: Soil samples were obtained from a series of soil fertility plots of a "Fox Sandy Loam Experimental Field" for carbon dioxide production in the laboratory. The carbon dioxide data are reported in Table 16, and the field data and part of the laboratory data are illustrated in Figure 1.

The carbon dioxide produced during the first twenty-four hours was very low and added fertilizers had little effect on the quantity produced. The production of carbon dioxide for this period without the addition of fertilizers, based on the literature reviewed, is considered the microbiological activity of these plots, which, as such, is indicative of the numbers of bacteria and the

Table 15. Effect of zinc sulphate, manganese sulphate, and copper sulphate on the production of carbon dioxide by soils on which crop plants respond to the respective treatment in the field.

LABORATORY TREATMENT - LBS. PER ACRE	MGM. CARBON DIOXIDE
NORFOLK FINE SAND	
Check	155.5
80 Pounds Zinc Sulphate	153.4
200 " " "	149.3
MARL	
Check	126.3
100 Pounds Manganese Sulphate	113.0
200 " " "	111.0
LEON SAND	
Check	37.3
40 Pounds Copper Sulphate	35.2
80 " " "	35.6

Table 16. Production of carbon dioxide, under different laboratory treatments, by soil taken from plots of a Fox sandy loam fertilizer experimental field - Mgm. carbon dioxide.

LABORATORY TREATMENT	FIELD PLOT NUMBERS AND PLOT TREATMENT												
	CHECK	L	LNPK	LNP	LNK	LPK	CHECK	L	LN	LK	LP	RP $CaSO_4$	RP
	301N	301S	302	303	304	305	306N	306S	307	308	309	310N	310S
	0 - 24 HOURS												
Ck	6.3	14.0	22.8	30.5	25.2	24.8	11.4	16.6	25.1	25.0	20.3	25.7	13.7
N	6.2	22.9	17.7	28.7	30.3	30.7	9.5	27.2	36.2	34.0	29.1	34.1	40.3
P	6.8	10.3	20.1	28.0	24.7	24.9	10.7	16.2	23.5	23.5	17.2	21.4	17.0
NP	6.6	23.9	18.0	30.5	36.8	33.9	9.4	27.1	41.1	39.7	26.6	34.5	42.5
	24 - 48 HOURS												
Ck	36.0	21.0	44.9	39.1	30.3	28.1	29.1	18.8	27.3	26.1	22.1	37.1	18.4
N	71.9	74.7	97.6	100.0	78.7	90.1	83.6	73.0	65.4	70.0	75.6	91.8	70.8
P	35.7	22.7	46.4	40.3	29.2	27.4	28.7	18.9	21.0	26.0	19.3	33.0	16.6
NP	81.1	79.2	108.8	109.2	94.3	92.9	87.2	76.9	74.9	78.6	79.3	92.2	71.5

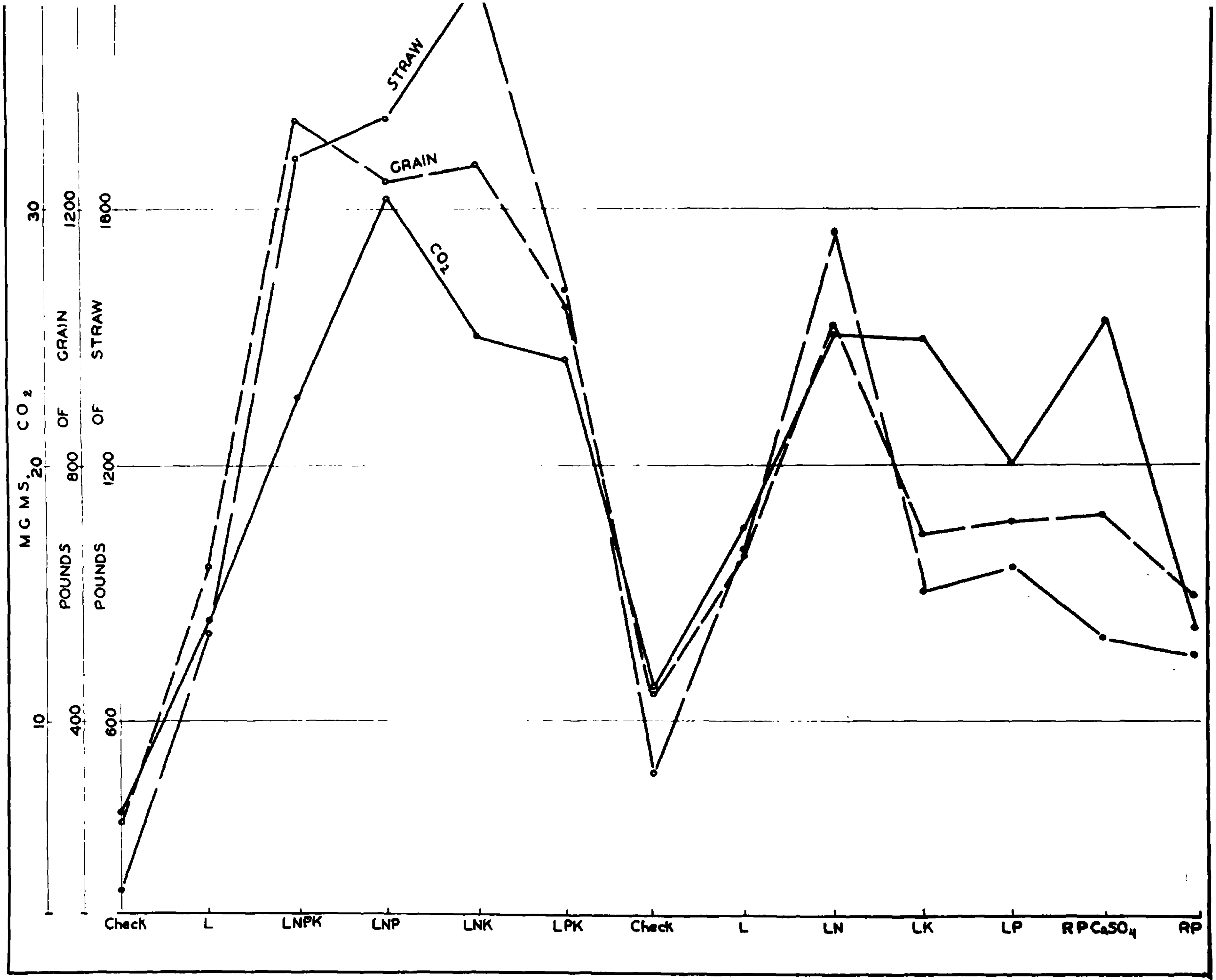

Figure 1. Relation of fertilizer and lime treatment in the field to crop yield and carbon dioxide production.

fertility of the soil. The carbon dioxide produced by the laboratory check during the first twenty-four hours was plotted in Figure 1, and average grain and straw yields at least two seasons after plowing under a legume are also plotted in Figure 1. These yields were selected because the unlimed plots did not produce legumes, and the turning under of the legume crops probably exaggerated differences between limed and unlimed plots. In general, the production of carbon dioxide follows the production of grain and straw closely, but the yields of the LNPK, LNK, and LN plots are slightly higher than the carbon dioxide production indicates, and the yields of the LK and RPCaSO$_4$ plots are lower than the carbon dioxide production indicates. These results may be attributed to one or the other of the following reasons:

1. The crop plants used up the added nitrogen, leaving the plots which received nitrogen no richer in nitrogen than the plots which received no nitrogen; and at the same time, the nitrogen may have induced a greater removal of the other elements by the plants.

2. Winogradsky (57) and Miss Ziemieka (58) found that the addition of nitrogen materially reduced the Azotobacter population of the soil.

These plots were laid out in 1917, at which time the lime was applied. Lime produced very large increases in the crop yields, and the plots were all badly in need of lime when the samples were taken (1934).

Based upon the above considerations, the crop yields obtained during the past seventeen years, which were compared with the carbon dioxide produced, do not give a true indication of the present fertility level of these plots, and it would not be good logic to attempt to correlate them directly. All that can be said is that the treatments have changed the biological activity of the soil and that, in this case, the biological activity expressed as gm. of carbon dioxide produced probably more nearly represents the present relative fertility

Table 17. Sanborn Field, Missouri, (Putnam silt loam). Average yields 1914-1928, for the last fifteen years of the forty-year period the field has been in operation, and the production of carbon dioxide in the laboratory in mgm.*

PLOT	TREATMENT	MGM. CARBON DIOXIDE 0 - 24 HOURS	24 - 48 HOURS	CORN	OATS	WHEAT	CLOVER	TIMOTHY
				Bu.	Bu.	Bu.	Lbs.	Lbs.
2	N, P, and K annually equal to that in 40 bushels of wheat	52.9	64.7			20.65		
9	No treatment	18.5	44.3			10.19		
10	Manure annually	54.1	189.1			20.71		
17	No treatment	21.8	31.9	19.03				
18	Manure annually	112.0	85.8	34.91				
21	Manure up to 1913; since, 200 pounds superphosphate	32.6	49.9			23.41		
22	Manure annually	44.4	117.0					5367
23	No treatment	19.0	32.3					1944
24	Manure to 1913; since, nothing	30.2	51.4			18.35		
29	Manure 1908-1913; since, ammonium sulphate	15.4	64.5			13.36		
30	Manure to 1913; since, $NaNO_3$	22.4	52.0			19.23		
35	Manure to 1913; since, nothing	15.3	50.8	55.79	43.47	27.55	3430	5828
36	Manure to 1913;since, 150 pounds 3-10-4 annually	19.3	54.3			20.50		
37	Manure to 1913;since 3-10-4 on corn and wheat	25.7	83.1	56.22	48.35	32.84	5194	6610
38	Limestone & Manure to 1913;since,3-10-4 on corn and wheat	62.6	68.8	59.15	48.29	31.25	5537	6080

*The field data were supplied by the Soils Department of the University of Missouri.

carbon dioxide as did the soil from Plot 2, which indicates that nutrients from the manure were made available during the second day.

Plot 9 is a no-treatment continuous wheat plot, and Plot 17 is a no-treatment continuous corn plot. The production of carbon dioxide by soil from both of these plots was very low during the first twenty-four hours. However, during the second twenty-four hours, soil from the wheat plot produced slightly more carbon dioxide. Plot 23 grew timothy continuously without treatment, and the soil produced practically the same quantity of carbon dioxide during both days as did the soil from Plot 17, which grew corn continuously without treatment.

Plots 10, 18, and 22 received manure and grew wheat, corn, and timothy, respectively. The carbon dioxide produced was 54, 112, and 44 mgm. on the first day and 189, 86, and 117 mgm. on the second day for the wheat, corn, and timothy plots, respectively. On this basis, there is less available nutrients in the timothy soil, and the wheat soil had more available nutrients than the corn soil. Part of these differences may be due to the cultural practices used in growing the different crops and the method of application of the manure.

Plot 29 received manure from 1908 - 1913, and Plot 30 received manure from 1889 - 1913. Since 1913, Plot 29 has received ammonium sulphate, whereas Plot 30 has received an equivalent quantity of sodium nitrate. The soil from the sodium nitrate plot produced nearly 50% more carbon dioxide during the first twenty-four hours, and this plot produced nearly 50% more wheat for the 1914 - 1928 period. The differences are not entirely due to the fertilizer treatment, but they should be largely due to it. Ammonium sulphate tends to reduce the supply of calcium and other bases in the soil. The production of carbon dioxide for the first day by the soil from these plots was about the same as that of the no-treatment plots growing the same crop continuously; but on the second day, the no-treatment plots produced significantly less carbon dioxide.

Plots 35, 37, and 38 received manure until 1913, after which the manure was discontinued; and since then, Plots 37 and 38 have received 3-10-4 fertilizer on corn and wheat. Plot 38 has had lime. The addition of 3-10-4 fertilizer increased the crop yields and carbon dioxide production. Lime increased the production of carbon dioxide, but it had little effect on the yield of the crops. The fertilizer had its greatest effect on clover production.

<u>Effect of Green Manures on Crop Yields and Carbon Dioxide Production</u>: An experiment testing green manures was laid out at Michigan State College in 1926. Soil was obtained from the plots where rye, and sweet clover were removed and turned under, respectively, for carbon dioxide production studies in the laboratory. The green manures were plowed under or removed in 1926 and 1931. The grain and the carbon dioxide data are reported in Table 18. There was little difference in the quantity of carbon dioxide produced by the soil from the "sweet clover under" and the "sweet clover off" plots without fertilizer treatment in the laboratory. The soil from the "sweet clover off" plot produced 131 mgm. carbon dioxide, whereas that from the "sweet clover under" plot produced 119 mgm. when these soils were treated with nitrogen in the laboratory. Likewise, the "rye off" soil produced more carbon dioxide than the "rye under" where nitrogen was added in the laboratory. The addition of phosphorus brought the production of carbon dioxide where rye and sweet clover were plowed under up to that where they were taken off. These data indicate that the additional plant material where the crops were plowed under brought about a tie-up of the phosphorus or other nutrients. It is possible that the products derived from the additional organic material when it underwent decomposition covered up part of the colloidal fraction of the soil, and thus hindered the normal exchange of calcium and phosphorus. Jenny and Shade (19) advanced the idea as an explanation of the variable results obtained by various research workers on the effect of calcium on the exchangeable potassium.

Table 18. Effect of green manures on crop yield and carbon dioxide production - Hillsdale sandy loam.

PLOT TREATMENT*	YIELD - BUSHELS OF GRAIN PER ACRE					MGM. CARBON DIOXIDE			
	1928 WHEAT	1929 WHEAT	1930 WHEAT	1932 WHEAT	1933 CORN	LABORATORY TREATMENT			
						CHECK	N	P	NP
Sweet clover - under	52.5	19.9	48.5	38.1	35.9	43.4	119.4	41.2	127.6
Sweet clover - off	43.9	19.6	47.9	33.3	33.6	41.1	131.2	43.4	130.2
Rye - under	42.7	21.4	49.3	36.9	39.5	31.9	104.2	37.2	101.6
Rye - off	36.8	19.8	53.6	30.4	25.8	42.6	117.7	37.8	125.8

*The green manures were plowed under in 1926 and 1931.

In the field there were twelve plots between the sweet clover and the rye plots, which is too far for direct comparisons. The data in Table 18 show that plowing sweet clover and rye under increased the yield slightly over that obtained where they were removed.

Effect of Superphosphate on the Production of Small Grain and Carbon Dioxide: In 1930 a series of plots was laid out to determine the effect of different quantities of phosphorus in fertilizer on the yield of grain. The treatments at the beginning of the test were 300 pounds per acre of 3-48-10 and 3-0-10, but in 1933 the plots were divided and an additional 3% nitrogen was added to half of each plot. The samples of soil for carbon dioxide production were taken in the fall of 1934. The carbon dioxide production and crop yield data are reported in Table 19.

The yields obtained during the last two years were slightly less where no phosphorus was applied. The addition of superphosphate in the field increased the production of carbon dioxide in the laboratory. The addition of phosphorus in addition to nitrogen in the laboratory increased the production of carbon dioxide where no superphosphate was added in the field; but where superphosphate was added in the field, phosphorus did not increase carbon dioxide production. Increasing the nitrogen in the field from 3 to 6% decreased the production of carbon dioxide in the laboratory where nitrogen was applied, which may be due to:

1. An increased consumption of phosphorus and lime in the field, or

2. A reduction in the nitrogen fixing bacteria, as was found by Winogradsky (57) and Ziemeika (58).

Effect of Fertilizer Treatment on the Production of Sudan Grass and Carbon Dioxide by the A_1, A_2, and B Horizons of Four Soil Types: The data on the effect of fertilizer on the microbiological activity of the A_1, A_2, and B

Table 19. Effect of fertilizers on the yield of small grain and on the production of carbon dioxide - Hillsdale sandy loam.

PLOT TREATMENT	YIELD - BU. GRAIN PER ACRE				MGM. CO_2 - 48 HOURS			
					LABORATORY TREATMENT			
	1930 OATS	1931 RYE	1932 WHEAT	1933 BARLEY	CHECK	N	P	NP
3-48-10	42.8	22.0	27.7	7.6	79.3	165.3	81.0	164.9
6-48-10	—	—	—	7.6	85.2	153.2	71.8	156.4
3-0-10	41.2	22.7	24.3	6.8	62.3	113.2	61.8	128.0
6-0-10	—	—	—	4.0	64.1	104.4	62.0	135.0

Table 20. Production of carbon dioxide in the laboratory by the A_1, A_2, and B horizons of several soil types, which had grown two crops of sudan grass in the greenhouse, and the greenhouse yields.*

GREENHOUSE TREATMENT	CROP YIELD 1st	CROP YIELD 2nd	CARBON DIOXIDE	CROP YIELD 1st	CROP YIELD 2nd	CARBON DIOXIDE	CROP YIELD 1st	CROP YIELD 2nd	CARBON DIOXIDE
	Gm.	Gm.	Mgm.	Gm.	Gm.	Mgm.	Gm.	Gm.	Mgm.
	A_1 HORIZON			A_2 HORIZON			B HORIZON		
				BELLEFONTAINE SANDY LOAM					
Ck	3.00	2.16	25.7	0.69	0.75	10.3	0.85	1.64	9.5
NK	2.55	2.51	26.5	1.19	1.45	8.0	0.88	1.20	6.7
NP	6.63	3.02	(47.1** 33.2	2.71	1.02	9.9	3.23	0.80	8.4
KP	7.34	3.13	40.3	2.72	0.79	8.4	2.50	0.83	6.3
NPK	9.05	3.46	39.4	3.75	1.02	9.3	3.74	1.66	7.8
				CONOVER LOAM					
Ck	3.29	6.15	46.0	2.35	2.11	(27.7** 19.0	2.59	1.60	11.4
NK	6.19	10.00	41.9	2.76	4.53	16.5	2.55	2.06	10.8
NP	5.31	7.57	70.0	3.16	3.16	16.9	3.66	2.48	(19.8** 11.9
KP	10.40	7.82	51.4	4.24	2.57	(12.7** 21.2	2.70	1.64	13.9
NPK	10.74	10.01	51.3	4.71	3.58	13.7	3.63	2.31	(10.4** 16.1
				HILLSDALE SANDY LOAM					
Ck	9.48	6.87	36.7	0.16	0.79	8.1	0.24	0.54	13.1
NK	12.68	8.49	33.4	0.23	0.28	8.0	0.19	0.26	9.2
NP	12.96	6.64	34.7	0.25	1.12	8.5	4.20	1.72	10.6
KP	14.28	6.86	29.5	1.44	0.66	6.9	0.75	3.63	8.8
NPK	14.61	7.10	34.1	2.61	1.30	7.7	3.94	2.52	8.4
				MIAMI LOAM					
Ck	2.58	3.12	16.5	1.84	1.25	8.3	3.62	1.53	5.2
NK	1.83	2.38	12.7	0.22	0.57	5.2	0.78	1.06	(1.1** 6.9
NP	14.29	4.53	29.3	4.74	2.51	14.5	5.96	1.80	8.0
KP	9.79	5.82	(27.3** 18.7	4.40	1.64	10.4	4.78	1.10	2.7
NPK	15.16	5.05	(27.2** 21.1	6.85	1.70	10.2	7.15	2.23	6.8

*The yields of Sudan grass were taken from Ellis' thesis (12).

**The carbon dioxide produced by soil from duplicate plots did not check closely.

horizons of four soil types on which the nutrient deficiencies had been determined in the greenhouse by Ellis (12) are reported in Table 20. He grew two crops of Sudan grass on the A_1, A_2, and B horizons of Conover loam, Hillsdale sandy loam, Bellefontaine sandy loam, and Miami loam in the greenhouse. By means of laboratory tests, he maintained the phosphorus, potassium, and nitrogen at the same levels. Six months after Ellis had harvested the second crop of Sudan grass, these soils were sampled for carbon dioxide production studies. The state of fertility of the soils as they were used in the laboratory was more nearly like their fertility during the growth of the second crop than that existing when the first crop was grown. Both the greenhouse and the field data are reported in Table 20.

"Data for the first crop show that the addition of a complete fertilizer to the A_2 and B horizons of all soil types studied, except the Hillsdale, resulted in a yield in excess of that obtained from the unfertilized A_1 horizon . . . . . . The data for the second crop show that the untreated A_1 horizon gave a greater yield in all cases than that of any treatment of the A_2 and B horizons".

The carbon dioxide produced during the twenty-four hour period is considered an index of the numbers of microorganisms involved and the microbiological activity of the soil at this time. The production of carbon dioxide by the A_1 horizon of all four soil types was usually several times as large as the production of carbon dioxide by the A_2 and B horizons. In certain cases, the NK treatment reduced the production of carbon dioxide. In a few cases, the NP treatment increased the production of carbon dioxide.

SUMMARY

The effect of fertilizer treatment on the production of carbon dioxide by mannitol treated soils was studied, and the data were correlated with crop yields. The data show that:

A. Nitrogen alone, and lime, superphosphate, and basic slag in combinations

with nitrogen increased the production of carbon dioxide by mannitol treated soils. Potash did not increase the production of carbon dioxide by the microorganisms of soils which were deficient in potash for cotton production.

B. A twenty-four hour period of incubation brought out the greatest differences in carbon dioxide production due to soil treatment. The effects of the treatment on carbon dioxide production were more evident with the larger than with the smaller amounts of fertilizers.

C. The increases in carbon dioxide production due to the addition of superphosphate were greatest with a water content of 27% of the maximum water-holding capacity, whereas more carbon dioxide was produced when water equal to 50% of the maximum water-holding capacity was used.

D. The phosphorus carriers which produced the largest increases in seed cotton on both limed and unlimed soil produced the largest increases in carbon dioxide produced by the soil microorganisms.

E. The soil microorganisms used P_2O_5 efficiently when supplied in quantities equal to one-half the quantity of nitrogen supplied.

F. Increasing the temperature from about 27° to 30° and 33° C. increased the production of carbon dioxide, but the increases due to the fertilizer treatments were practically identical at all temperatures.

G. There was a fair correlation between the increase in crop yield and carbon dioxide production due to phosphorus treatment, and a fair negative correlation between the phosphorus and the 0.002 N H_2SO_4 soluble phosphorus (Truog's method). Clay soils require a higher soluble phosphorus content than do sandy soils to supply the needs of cotton for phosphorus.

H. Air-drying increased the production of carbon dioxide on rewetting, by some soils, but it usually did not have a great effect except with prolonged air-drying. One soil showed a decrease in carbon dioxide production due to prolonged air-drying.

I. On soils which were deficient in calcium for crop growth and microbiological activity, the substitution of magnesium for part of the added calcium increased the production of carbon dioxide in two cases out of three.

J. The effect of certain elements seldom applied to soils on the production of carbon dioxide by the soil microorganisms:

1. Calcium arsenate had no effect on carbon dioxide production when used in large or small amounts, but intermediate quantities reduced it.
2. Contrary to the results obtained with crops, superphosphate did not intensify arsenic toxicity to soil microorganisms, nor did iron sulphate and other salts alleviate the harmful effects of arsenic, as measured by carbon dioxide production.
3. Zinc, manganese, and copper did not increase the production of carbon dioxide by soils known to be deficient in these elements for certain crops.

K. The relation of crop yields on fertility plots to carbon dioxide production by soils from these plots in the laboratory:

1. There was a close relation between the carbon dioxide produced by soil from the plots of a Fox sandy loam experimental field and the average yield of grain and straw two or more years after the plowing under of a legume crop. The carbon dioxide produced by the nitrogen treated plots was not as high as the yields indicate it would have been, which may be explained on

the basis that the added nitrogen was largely used up by the plants before the samples were taken.

2. There was a relation between the production of carbon dioxide and the crop yields by a Putnam silt loam from Missouri. The experimental field contained rotations, as well as different fertilizer treatments. The cultural practices with the different crops probably influenced carbon dioxide produced very materially.

3. Green manure tops plowed under increased the yield of grain insignificantly over where they were removed. The carbon dioxide produced by the soil in the laboratory was significantly less where the tops were plowed under when nitrogen alone was applied. The addition of superphosphate in addition to nitrogen brought the production of carbon dioxide to the same level.

4. The superphosphate added in 300 pounds per acre of 3-48-10 in the field was sufficient to supply the needs of the micro-organisms in the laboratory.

5. Fertilizer treatment at a rather high level did not increase the production of carbon dioxide by the A_2 and B horizons of four soil types to that of the no-treatment A_1 horizon.

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PART II

THE EFFECT OF SOIL MICROORGANISMS ON SOIL REACTION

INTRODUCTION

The "reaction of soils" has justifiably received considerable attention during recent years. Emphasis has been placed on the means of determining the concentration of hydrogen ions. Changes in reaction over short or long periods of time have been recorded as facts and, in most cases, the reasons for the changes have not concerned the investigators. The object of this paper is to present data on the effects of microorganisms on the reaction of the soil.

REVIEW OF LITERATURE

Andrews (1) reviewed several papers on the effect of air-drying soils on certain chemical changes, and determined the effect of air-drying on the microbiological activity of the soil. The conclusion was reached that the changes produced on air-drying a soil are directly due to changes in the soil flora. The fungal mycelium disappears during desiccation; and between the time of desiccation and shortly after rewetting, the bacteria increases in number in many cases. Certain investigators have reported increases in Ca, Mg, P_2O_5, etc. on air-drying, whereas others have reported no increases. Evidently, a large part of the fungus mycelium is available for bacterial consumption during slow drying or immediately after rewetting the soil.

Feher (3) and Nehring (4 and 5) have recently reported that the pH of the soil has been found to be as much as two units lower during the colder months of the year than during certain of the warmer months. Nehring (4) gave a general summary of the seasonal changes in pH which he quoted from Feher, D. in Wiss. Archiv. f. Landw., A, 9, 172 (1932), as follows:

"Auch der regelmassig bebaute Ackerboden sowie die Wiesenboden zeigen im allgemeinen das gleiche Verhalten wie der Waldboden und der unberuhrter Brache.

Da aberletztere der ausgleichenden Wirkung des schutzenden Waldsbestandes entbehrt so kommen bei ihr die Klimaschwankungen besonders deutlich zum Ausdruck und infolgedessen zeigt sie auch gewohnlich grossere Veraderungen in der Bodenaziditat wie die Waldboden. Das gleiche gilt auch fur den Ackerboden."

The data presented by Waksman (7a) concerning the numbers of bacteria and fungi at different seasons of the year should be considered in connection with the seasonal changes in pH reported above. The numbers of fungi are much higher in the winter and early spring than in the summer and fall, whereas with variations the opposite is true for bacteria.

Turk and Millar (6) found that where organic matter was used alone and combined with nitrogen, the pH decreased over a period of two years. The pH also decreased in the untreated soil. The pH at the end of twelve months was usually much higher than at the end of eight or sixteen months. A further check of the data revealed that the water content of the soil was usually lower at the end of the twelfth month than when any other determination was made. The low water content probably resulted in the removal of the fungal mycelium. The change in the fungal flora may have influenced the change in the soil reaction.

Clevenger and Willis (2) attributed increases in pH where cottonseed meal and urea were added to soil in combination with dolomitic limestone to the accumulation of ammonia; but where sulphate of ammonia was added, the increase in pH was of about the same magnitude and no explanation was offered. Without dolomitic supplements, the changes in pH were small. They also reported other unexplained changes in pH.

EXPERIMENTAL

Effect of Soil Microorganisms on the Reaction of Sand Cultures and Agar Media

The production of carbon dioxide by soil suspensions inoculated into nutrient solutions containing mannitol was investigated. The method was identical with that reported by Andrews (1), except all nutrients were added to sand. The nutrient solutions used and the data obtained are reported in Table 1. The 4:1 ratio of calcium to magnesium was much superior to the no magnesium, 3:2, 2:3, 1:4, ratios and no calcium in the production of carbon dioxide by the soil microorganisms.

The production of carbon dioxide above was rather low, and the pH of the nutrient solutions was low. The affect of increasing the quantity of calcium and magnesium on the production of carbon dioxide was next determined. The calcium to magnesium ratio was maintained at 4:1. The solutions were added as shown in Table 2 with the data obtained. The $Ca(OH)_2$ was added in solution and evaporated to dryness before the other nutrients were added. Sufficient calcium was added to produce a pH range of 3.37 to 7.68. Increasing the quantity of calcium and magnesium increased the production of carbon dioxide from 2.8 mgm. at the original pH of 3.37 to 71 mgm. where the original pH was 7.68. During this part of the experiment, it was accidentally discovered that the pH of one of the nutrient solutions increased during the period of incubation. The nutrient solutions were then set up again and inoculated, and the pH data were obtained as reported in Table 2 (The original pH values, as discussed above, were taken from this part of the experiment).

The pH of the sand cultures varied from 3.37 to 7.68 at the beginning of the experiment; after seventeen days, they varied from 6.73 to 7.78. The changes produced were gradual and reasonably uniform, which indicates that they were coincident with the growth and development of the soil flora which was inoculated into the cultures. The extreme difference in pH at the beginning of the test was 4.31 units; the extreme difference seventeen days later was 1.13 pH units.

The nitrogen used in this experiment was nitrate nitrogen, and the removal of an excess of nitrate nitrogen over the bases it is connected with would have increased the pH of the solution. In order for the soil micro-

Table 1. Effect of the Calcium : Magnesium ratio of a nutrient solution on the production of carbon dioxide by inoculated sand cultures.*

NO.	C.C. USED FOR EACH CULTURE						Ca:Mg RATIO	MGM. CO_2 72 Hours
	.1 N $Ca(NO_3)_2$	.1 N $Mg(NO_3)_2$	.1 N H_3PO_4	.1 N K_2CO_3	.1 N H_2SO_4	H_2O		
1	2.50	0.00	2.50	0.63	0.63	3.75		31.4
2	2.00	0.50	2.50	0.63	0.63	3.75	4:1	60.5
3	1.50	1.00	2.50	0.63	0.63	3.75	3:2	25.2
4	1.00	1.50	2.50	0.63	0.63	3.75	2:3	29.7
5	0.50	2.00	2.50	0.63	0.63	3.75	1:4	13.5
6	0.00	2.50	2.50	0.63	0.63	3.75		23.2

*Used 100 gm. of washed sand plus 0.5 gm. mannitol and in addition given treatment indicated in table.

Table 2. Effect of calcium and magnesium on the production of carbon dioxide by inoculated sand cultures and the effect of soil microorganisms on the pH of sand culture media.*

NO.	C.C. USED FOR EACH CULTURE							MGM. CO_2 48 Hours	TIME - DAYS							
	.024 N $Ca(OH)_2$	.1 N $Ca(NO_3)_2$	.1 N $MgNO_3$	.1 N H_3PO_4	.1 N K_2CO_3	.1 N H_2SO_4	H_2O		0	1	2	5	7	9	13	17
									pH							
1	0.0	2.00	0.50	2.50	0.63	0.63	3.75	2.8	3.37	3.50	3.55	4.41	5.80	6.17	6.50	6.73
2	8.3	1.75	0.75	2.50	0.63	0.63	3.75	10.0	6.10	6.28	6.36	5.95	6.46	7.39	7.33	7.22
3	16.7	1.50	1.00	2.50	0.63	0.63	3.75	26.4	6.78	6.96	7.02	6.40	6.89	7.55	7.63	7.51
4	33.3	1.00	1.50	2.50	0.63	0.63	3.75	55.4	7.18	7.26	7.35	6.65	7.31	7.94	7.70	7.58
5	50.0	0.50	2.00	2.50	0.63	0.63	3.75	60.0	7.68	7.77	7.60	6.79	7.50	8.00	7.68	7.86
6	66.7	0.00	2.50	2.50	0.63	0.63	3.75	71.3	7.68	7.75	7.61	6.79	7.63	8.03	7.78	7.78

*Used 100 gm. of washed sand plus 0.5 gm. mannitol and in addition given treatment indicated in table.

organisms to increase the pH of the culture from 7.37 to 6.73, they must have consumed considerably more acids than bases. The culture with an original pH of 6.10 had nearly enough bases present to neutralize the acids, yet the growth of microorganisms increased the pH. The other four cultures had sufficient bases present to more than neutralize the acids and the growth of the microorganisms increased the pH.

It follows that if the soil microorganisms involved in these cultures consumed a greater quantity of acids than of bases, the return of the assimilated acids and bases through the decomposition of the microorganisms to the culture would decrease the pH to the original value.

In a later experiment, fungi reduced the pH of a dextrose agar medium from 4.20 to 3.00, whereas bacteria increased the pH of a mannitol agar medium from 6.31 to 6.99 in eight days. The pH of the fungal mycelium was 5.39. This affect of fungi and bacteria on the pH of agar media is apparently in agreement with the base and acid content of bacteria and fungi, as reported by Waksman (7b). The combined affect of bacteria and fungi on the reaction of a soil will, therefore, depend upon the changes which occur in the equilibrium between the bacteria and the fungi. The effect of the Actinomyces on the reaction of the soil has not received any attention.

Microbiological Effects of Fertilizers on Soil Reaction

The data obtained above indicate that the "Immediate effects of fertilization on soil reaction", reported by Clevenger and Willis (2) and reviewed above, may have been due to the effect of the fertilizers on the microbiological activity of the soils treated with the respective fertilizers, and that their unexplained changes in soil reaction might be accounted for. Therefore, an experiment was set up to determine the relation between microbiological activity and soil reaction. Clevenger and Willis' experiment was duplicated in part. Fertilizer was applied at the rate of 16,000 pounds of 3-8-6 per acre to 1,000 gm. portions of Fox sandy loam soil. The sources of nitrogen were ammonium sulphate, nitrate of soda, and cottonseed meal. The fertilizers were applied in duplicate, alone, and with calcium carbonate equivalent to the nitrogen. The soil and the fertilizers were mixed and water equal to one-third of the maximum water-holding capacity of the soil was added. The soil was then put into 3000 c.c. Erlenmeyer flasks and maintained at room temperature. The carbon dioxide produced, the nitrate, and the ammonia nitrogen, and the pH were determined on the dates indicated in Table 3 and Figures 1 and 2. The pH was determined by the quinhydrone electrode method; the carbon dioxide was absorbed in ascarite; the nitrate and ammonia nitrogen were removed from the soil with dilute HCl (5 c.c. of concentrated HCl per liter) and determined by the usual distillation method. The carbon dioxide was removed from the flasks and then the soil was thoroughly mixed and samples were taken for the pH and the nitrogen determinations. Two gm. of mannitol per 100 gm. of soil was added on the thirty-first day. The experiment was set up November 18, 1935. The data are reported in Table 3 and Figures 1 and 2.

Immediate Effect of the Fertilizers on the Soil Reaction: Clevenger and Willis (2) found that "in all cases on mixing the fertilizers with the soil a drop in the pH of the soil ranging from 0.6 to 1.0 unit took place immediately", which was attributed to the superphosphate and muriate of potash.

In this experiment (Table 4) the pH of the original soil was 4.88. The addition of the 0-8-6 fertilizer reduced it to 4.28. Nitrogen in addition to the 0-8-6 increased the pH slightly. The addition of lime increased the pH of the soil from 4.88 to 6.58; the addition of the 0-8-6 to the limed soil reduced the pH from 6.58 to 5.98. Nitrogen in addition to the 0-8-6 and lime reduced the pH from 6.58 to 6.48, 6.46, and 6.31, respectively, for ammonium sulphate, nitrate

Table 3. Effect of fertilizers and lime on the production of carbon dioxide by the soil microorganisms - mgm. carbon dioxide per 100 gm. soil in twenty-four hours.

SOURCE OF NITROGEN	WITHOUT MANNITOL								WITH MANNITOL								
	TIME - DAYS																
	1	2	3	5	7	11	14	19	31	32	33	34	35	36	38	40	42
	NORMAL FERTILIZERS																
$(NH_4)_2SO_4$	2.8	3.0	5.2	4.1	3.3	2.6	2.8	1.6	1.2	3.3	4.9	128	290	271	240	156	75
$NaNO_3$	1.6	5.9	4.0	3.2	2.4	1.8	2.0	1.3	0.8	1.8	3.1	47	174	381	291	168	63
C. S. M.	2.6	37	50	36	22	13	14	6.3	3.1	9.3	126	275	157	115	97	75	72
	NORMAL FERTILIZERS PLUS LIME EQUIVALENT TO THE NITROGEN																
$(NH_4)_2SO_4$	37	13	7.1	6.1	3.2	2.6	3.4	1.8	0.8	4.3	71	281	206	221	280	177	67
$NaNO_3$	35	12	6.5	4.9	3.2	2.6	2.8	2.1	1.3	4.3	61	376	276	186	175	158	138
C.S.M.	55	43	35	44	23	12	10	5.9	4.0	13	13	249	147	129	133	124	121

Table 4. The immediate effect of fertilizers and other chemicals on soil reaction and hydrogen ion concentration.

TREATMENT	WITHOUT LIME			WITH LIME[b]	
	NH_4OH	$(NH_4)_2CO_3$			
	pH	pH	H Ion Concentration x 1,000,000	pH	H Ion Concentration x 1,000,000
None	4.88	4.88	13.10	6.58	.262
0-8-6[a]	4.28	4.28	52.60	5.98	1.050
0-8-6 + H_2SO_4[b]	3.31	3.31	492.00	4.96	10.900
0-8-6 + Na_2CO_3[c]	6.20	6.20	.63	7.10	.079
0-8-6 + 1.875 mgm. N/100 gm. soil	4.28	4.28	52.60	6.40	.398
0-8-6 + 3.75 " " " "	4.45	4.28	52.60	6.46	.347
0-8-6 + 7.50 " " " "	4.55	4.62	24.00	6.48	.331
0-8-6 + 15.00 " " " "	5.00	4.96	10.90	6.65	.224
0-8-6 + 30.00 " " " "	5.72	5.73	1.86	6.99	.102
0-8-6 + 60.00 " " " "	6.90	6.82	.15	7.41	.038

[a] All the treated soils received a general application of an 0-8-6 fertilizer (Superphosphate and KCl) at the rate of 16,000 pounds per acre.

[b] Added in quantities equivalent to the nitrogen in 16,000 pounds per acre of a 3-8-6 fertilizer.

[c] Equivalent to the H_2SO_4 treatment.

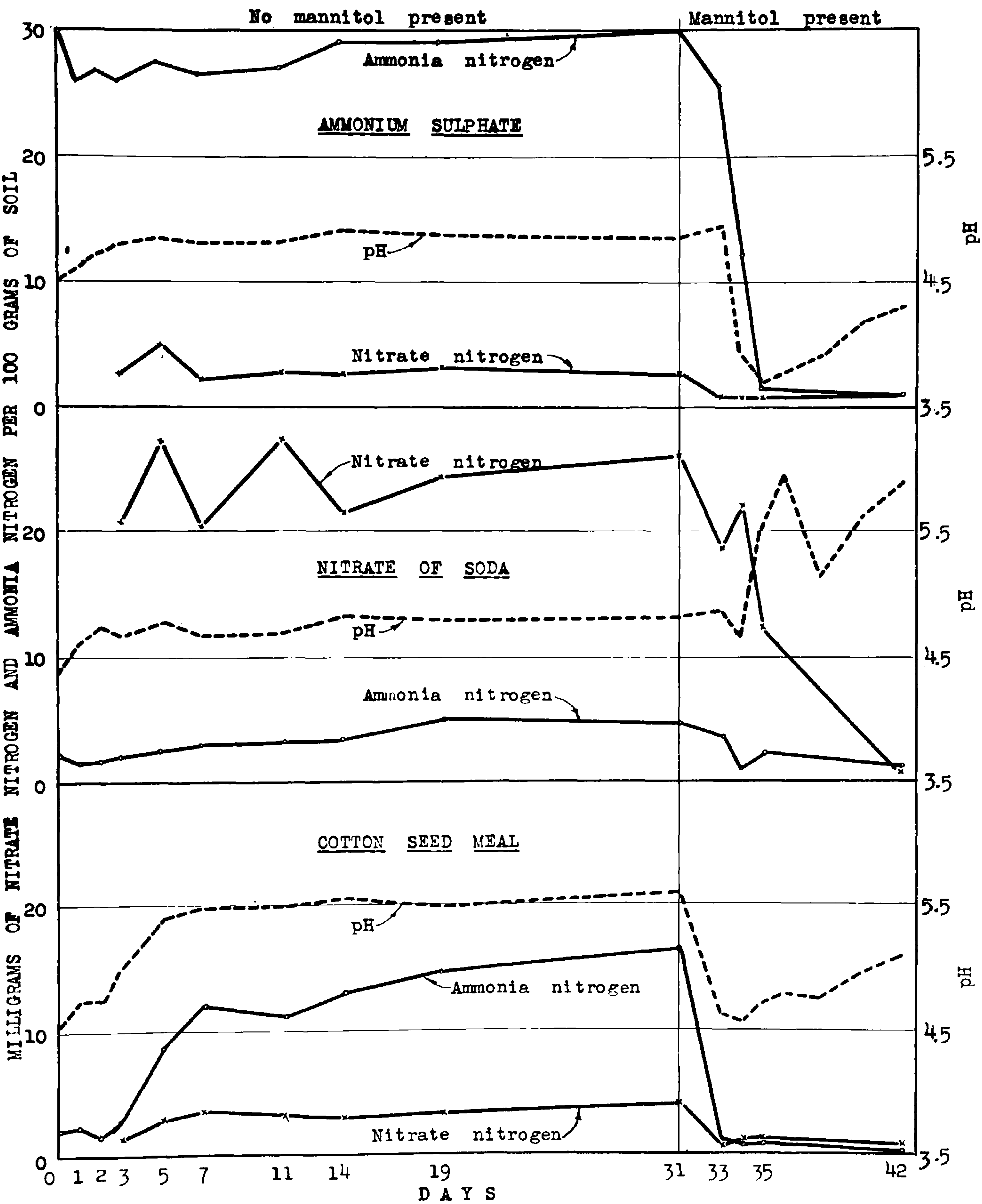

Figure 1. Changes in pH, ammonia and nitrate nitrogen of soil following the addition of ammonium sulphate, nitrate of soda, and cottonseed meal in normal

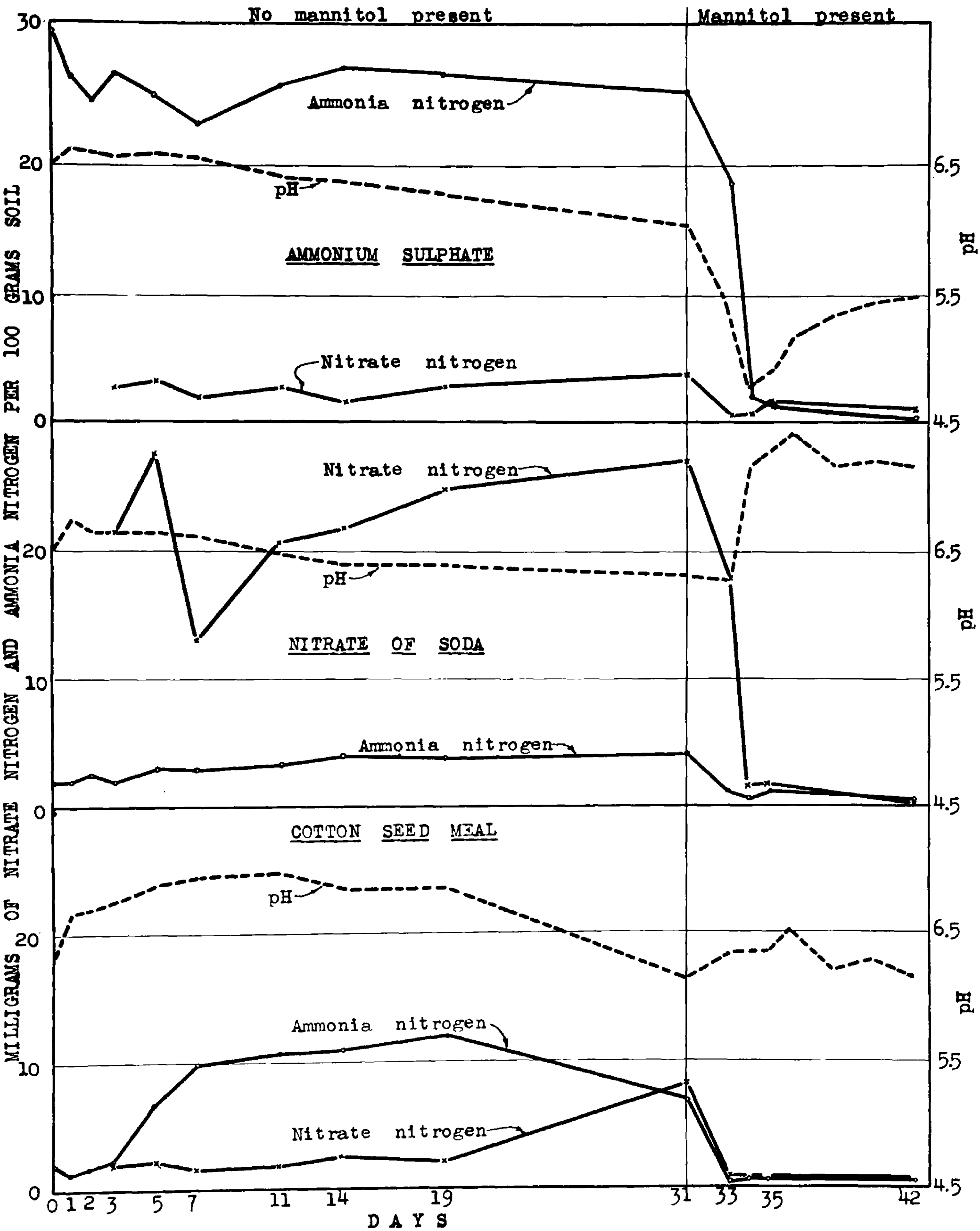

Figure [illegible]. Changes in pH, ammonia and nitrate nitrogen of soil following the addition of ammonium sulphate, nitrate of soda, and cottonseed meal to normal fertilizers plus lime of soda, and cottonseed meal.

<u>Relation of Microbiological Activity to the Reaction of the Soil Where Normal Fertilizers were Applied</u>: Before mannitol was added (Figure 1), the pH of the soil increased 0.4, 0.5, and 1.1 units where the source of nitrogen was sulphate of ammonia, nitrate of soda, and cottonseed meal, respectively. The pH values obtained on the fifth day were nearly a maximum for all three sources of nitrogen. At this time, the maximum production of carbon dioxide had also been reached. From the fifth to the thirty-first day, the rate of production of carbon dioxide was on the decline and only small increases in pH took place.

The pH increased from 4.50 to 4.85 from the first to the fifth day where sulphate of ammonia was applied. During this time, the ammonia nitrogen decreased slightly and the nitrate nitrogen increased slightly, whereas the ammonia nitrogen increased and the nitrate nitrogen decreased from the fifth to the thirty-first day and the pH increased only from 4.85 to 4.88. These changes were brought about through the soil microorganisms taking out an excess of acids over bases without consideration of the nitrogen. The decreases in the ammonia and increases in nitrate nitrogen while the pH was increasing from 4.50 to 4.85 in the absence of the soil flora would have decreased the pH.

The decreases in the nitrate nitrogen where nitrate of soda was applied were much greater than the changes in ammonia nitrogen where sulphate of ammonia was applied, and the pH increased about the same in both cases. The production of carbon dioxide was significantly less where nitrate of soda was applied than where sulphate of ammonia was applied. Since the removal of ammonia nitrogen, on a chemical basis, from the sulphate of ammonia would decrease the pH, and an increase was obtained, and the removal of nitrate nitrogen from the nitrate of soda would have increased the pH, and the pH was increased about the same in both cases by the fifth day even though there had been as much nitrate nitrogen from the nitrate of soda as ammonia from the sulphate of ammonia taken up by microorganisms, combined with the fact that less carbon dioxide was given off where nitrate of soda was applied, indicates that the microbiological flora was different in the two cases. These data indicate that bacteria predominated where sulphate of ammonia was applied and that fungi predominated where nitrate of soda was applied - on the basis that bacteria consume more acids than bases and that fungi consume more bases than acids (See data above).

Coincident with the increase of 6.6 (8.6 - 2.0) mgm. of ammonia nitrogen and at least 1.7 (3.1 - 1.4) mgm. of nitrate nitrogen per 100 gm. of soil from the first to the fifth day where cottonseed meal was applied the pH increased from 4.54 to 5.41. If it is assumed that nitrate nitrogen and ammonia nitrogen are equally effective in changing the reaction of the soil, 4.9 (6.6 - 1.7) mgm. of ammonia nitrogen was responsible for changing the pH of the soil from 4.54 to 5.41. From the fifth to the thirty-first day, the ammonia nitrogen increased 6.5 mgm. more than the nitrate nitrogen, and the pH increased from 5.41 to 5.59. The decrease in hydrogen ion concentration coincident with the formation of the excess of 4.9 mgm. of ammonia nitrogen was twenty times as great as the subsequent decrease with the appearance of the 6.5 mgm. more of ammonia nitrogen than of nitrate nitrogen.

During the time the 4.9 mgm. of ammonia was accumulating, the microorganisms were very active, as indicated by the carbon dioxide produced (Table 3); whereas during the time the 6.5 mgm. was accumulating, the carbon dioxide produced was very much less, which indicates that the activity of the soil microorganisms had a greater effect on the pH of the soil than the small amounts of ammonia.

When mannitol was added on the thirty-first day, there was approximately as much nitrogen present in the form of ammonia and nitrate nitrogen where ammonium sulphate and nitrate of soda were added to the soils, respectively, as was added by them. By the thirty-fifth day, the pH where sulphate of ammonia and cottonseed meal were applied had gone down, whereas it had gone up where nitrate of soda was applied.

The ammonia and nitrate nitrogen had practically all been removed from the ammonium sulphate treated soil by the thirty-fifth day, and the pH decreased from 4.88 on the thirty-first day to 3.69. The data in Table 4 show that the pH of the soil receiving the 0-8-6 fertilizer and the H_2SO_4 equivalent to the sulphate of ammonia had a pH of 3.31. If the pH changes were determined by the ammonia nitrogen present, as concluded by Clevenger and Willis, the pH of the ammonium sulphate treated soil would have dropped to 3.31 on removal of the ammonia and nitrate nitrogen. The concentration of hydrogen ions at pH 3.31 is two and one-half times that at pH 3.69. The soil microorganisms prevented the pH from reaching the low level it should have reached on the basis of the removal of the nitrogen. This action of microorganisms is in harmony with the effect of bacteria noted above on the pH of the media, and it indicates that the bacteria predominated over the fungi in that apparently the acids were absorbed in greater quantity than the bases, which prevented the pH from dropping to 3.31. From the thirty-fifth to the forty-second day the pH increased from 3.69 to 4.30 coincident with the removal of 1 mgm. nitrate nitrogen and 0.6 mgm. of ammonia nitrogen, which leaves a negligible removal of nitrate nitrogen to increase the pH from 3.69 to 4.30.

The increase in pH from 3.69 to 4.30 took place with a negligible change in ammonia and nitrate nitrogen, and at the same time the production of carbon dioxide was very high. The pH change may be accounted for on the basis of the bacteria increasing relative to the fungi, which, in the absence of available soil nitrogen,would necessitate a destruction of the fungi. "Are bacteria able to derive nitrogen from fungi?" Waksman (7c) said that "when the amount of available nitrogen is low - - - - - a part of the synthesized protoplasm of the microorganisms will be decomposed, liberating some of the nitrogen which is immediately again assimilated - - - -."

The pH at the beginning of the experiment was 4.50; that at the end was 4.30. At the beginning of the experiment, there were 480 pounds per acre of ammonia nitrogen present; at the end, there was a negligible amount of soluble nitrogen. The difference in pH at the end of the experiment and that (3.31) when the 0-8-6 and H_2SO_4 equivalent to the ammonium sulphate was applied may be attributed to the removal of an excess of acids over bases, disregarding the nitrogen. On this basis, the microorganisms reduced the hydrogen ion concentration more than twenty-four times as much as the removal of 480 pounds of ammonia nitrogen by the soil flora reduced it.

The affect of the organic acid decomposition products of mannitol on the pH of the soil is not to be overlooked. Decreases in pH in soil and in culture solutions have often been attributed to the increase of organic decomposition products, and subsequent increases in pH to their decomposition. If the decrease in pH from 4.88 to 3.69 was considered to be due to the effect of organic acids and the removal of the ammonia nitrogen, and the increase (with no accumulation in ammonia nitrogen) in pH from 3.69 to 4.30 was due to the decomposition of the organic acids, then it is concluded that the soil microorganisms consumed much larger quantities of mineral acids than of bases than would be necessary to produce this action with the organic acids playing only an insignificant role in the changes in pH.

When mannitol was added to the nitrate of soda soil, the pH increased from 4.81 on the thirty-first day to 5.91 on the thirty-sixth day when the nitrate and ammonia nitrogen were almost completely removed. The pH obtained when 0-8-6 and Na_2CO_3 were added to the soil was 6.80. The soil microorganisms, therefore, prevented the pH from increasing to the point calculated on the basis of complete removal of the nitrate and ammonia nitrogen. In contrast to the sulphate of ammonia data, these data indicate that the fungi predominated over the bacteria, which is brought out again in the decreases in pH which followed; but on the last date, the pH indicates that the bacteria were increasing relative to the fungi. Does the presence of sodium in a soil relatively low in bases favor fungal activity over bacterial activity?

When mannite was added to the cottonseed meal soil, the pH decreased from 5.59 on the thirty-first day to 4.55 on the thirty-fourth day, at which time the nitrate and ammonia nitrogen had been almost completely removed. The pH increased from 4.55 to 5.09 with little change in nitrate and ammonia nitrogen, and the increase was probably due to an increase in the bacteria or a decrease in the fungi or both. This indicates that the bacteria may obtain nitrogen at the expense of the fungi.

The production of carbon dioxide was much greater where cottonseed meal was applied than where ammonium sulphate and nitrate of soda were applied. More carbon dioxide was produced where ammonium sulphate was applied than where nitrate of soda was applied. The nitrate of soda soil produced much less carbon dioxide than the sulphate of ammonia soil at the beginning of the experiment and immediately after putting on the mannitol.

<u>Relation of Microbiological Activity to the Reaction of the Soil Where Normal Fertilizers Plus Lime Were Applied</u>: The quantity of lime added was equivalent to the nitrogen; the same quantity of lime was added where nitrate of soda, and cottonseed meal were applied as where sulphate of ammonia was applied.

Without mannitol, the pH of the ammonium sulphate and the nitrate of soda treated soil increased slightly at first, followed by small decreases. There were small changes in the ammonia and nitrate nitrogen, but there was no apparent relation between them and the pH changes. Where cottonseed meal was used, the pH increased from 6.31 to 6.77 on the fourth day, with no change in content of ammonia nitrogen and probably a slight increase in nitrate nitrogen content.

It took 28 mgm. ammonia nitrogen to change the pH of the soil from 6.40 to 6.99 (Table 4). The soil microorganisms reduced the hydrogen ion concentration .00000038 moles per liter, whereas the 28 mgm. (448 pounds per acre) of nitrogen reduced it only .000000296 moles per liter. These data indicate that the soil flora had consumed acids equivalent to 448 pounds per acre of ammonia nitrogen.

When mannitol was added to the ammonium sulphate soil, the pH decreased from 6.06 to 4.79 in three days, at which time practically all of the ammonia and nitrate nitrogen had been removed; but coincident with a small decrease in the ammonia nitrogen, the pH increased again to 5.51, which indicates that changes in the flora took place, thus leaving bases available to increase the pH. This would take place with the increase of the bacteria at the expense of the fungi. The pH of 4.79 is lower than that, 4.96, obtained from the addition of the 0-8-6 and H_2SO_4 equivalent to the ammonium sulphate.

When mannitol was added to the sodium nitrate soil, the pH increased from 6.35 to 7.32 when about all of the ammonia and nitrate nitrogen had been removed, and it increased to 7.45 and decreased to 7.19, which indicates that changes in the soil flora were taking place. The pH obtained when Na_2CO_3 instead of nitrate of soda was put on the soil was 7.10.

When mannitol was added to the cottonseed meal treated soil, the pH increased from 6.15 to 6.36 on removal of about all of the nitrate and ammonia nitrogen. The pH continued to increase and decrease with little change in soluble nitrogen.

The data in Table 3 show that usually sulphate of ammonia was more efficient for carbon dioxide production than was sodium nitrate; cottonseed meal was more efficient than either, except where mannitol was added.

DISCUSSION

The data show that soil microorganisms exerted a marked effect on the soil reaction. The fungi decreased the pH of a medium, whereas the bacteria increased it. The change in the pH of the medium due to the growth of the bacteria and the fungi is considered to be due to the absorption of more acids than bases by the bacteria and more bases than acids by the fungi, which is in harmony with the chemical composition of fungi and bacteria reported by Waksman in his book "Principles of Soil Microbiology". If the bacteria increase the pH by an absorption of an excess of acids over bases and the fungi decrease it by an absorption of an excess of bases over acids, a decrease in the bacterial population will have the same effect on the pH as an increase in the fungal population, and a decrease in the fungal population will have the same effect as an increase in the bacterial population, due to the reentrance of the absorbed constituents into the soil solution. The effect of the soil population on the pH of the soil depends upon the microbial equilibrium which exists at any one time. It is logical to assume that organisms other than bacteria and fungi played a part in the reaction changes which took place.

Upon complete removal of the nitrogen, in several instances, the resulting pH was very much different from the pH obtained on addition of all of the fertilizing constituents except nitrogen, which indicates that there was an excess absorption of acids or bases. Changes in the pH took place which were significant without changes in the soluble nitrogen content; these changes apparently were brought about by a shift in the equilibrium between the bacteria and fungi. These data, therefore, indicate that the bacteria are able to derive nitrogen and probably other products from fungi, and under certain conditions fungi may increase at the expense of bacteria. There is an indication that the sodium from the nitrate of soda is more favorable for the development of fungi than for bacteria.

The ammonia and nitrate nitrogen play a part in the reaction of the soil, but the part played by the soluble nitrogen is probably secondary to the removal of an excess of acids or bases by the soil microorganisms. It would be possible to imagine a condition in which the increase or decrease in soluble nitrogen would determine the pH changes. This condition could exist when the equilibrium between the fungi and bacteria was such that they would remove or add to the soil solution equivalent hydroxol and hydrogen ion producing substances. Under field conditions, decreases in pH obtained when organic matter is turned under is probably due to the predominance of fungi in the decomposition of the added material rather than to the production of organic acids. Certain data presented in this paper show that if the organic acids did play an important part, much more of the mineral acids would need to have been taken up to produce the results obtained.

SUMMARY

The following data presented in this paper show that soil microorganisms influenced the pH of the soil:

A. Soil microorganisms increased the pH of a sand culture from 3.37 to 6.73; the pH of other cultures was also increased.

B. Fungi decreased the pH of dextrose agar medium from 4.20 to 3.00; the pH of the fungal mycelium was 5.39.

C. Bacteria increased the pH of a mannitol agar medium from 6.31 to 6.39.

D. The pH of soil changed significantly with insignificant changes in ammonia and nitrate nitrogen.

E. With changes in nitrate and ammonia nitrogen greater where nitrate of soda was applied than where ammonium sulphate was applied, the pH increased in both cases about the same amount.

F. The soil microorganisms prevented the pH of the soil where ammonium sulphate was applied from dropping below 3.69, whereas the pH of the soil receiving H_2SO_4 equivalent to the ammonium sulphate was 3.31.

G. The pH (see F above) increased from 3.69 to 4.30 without a change in the ammonia and nitrate nitrogen.

H. The results under F and G were obtained immediately after applying mannitol. If organic acids should be considered as playing a significant role in reducing the pH, the data (F and G) would have to be explained on the basis of a much greater absorption of acids than if they are considered to play an insignificant part.

I. The pH where nitrate of soda was applied on the unlimed soil did not go as high as was calculated on the basis of complete removal of the nitrate nitrogen, whereas on the limed soil it went higher.

J. Where cottonseed meal was added to soil, increases in pH took place with small increases in ammonia nitrogen, which were much greater than those which took place through the addition of the nitrogen as ammonium carbonate.

K. The pH changes which took place on the unlimed soil without mannitol were much greater where cottonseed meal was applied than where either nitrate of soda or sulphate of ammonia were applied.

The literature which was reviewed in this paper shows the following, which point in the same direction as the data reported:

A. The pH of a soil may vary as much as two units from winter to summer.

B. The fungi in the soil may be higher in winter than in summer (relative to bacteria), whereas the reverse is true for bacteria.

C. Drying soils may increase their pH.

D. Drying a soil tends to decrease the quantity of fungal mycelium.

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