

STUDIES ON THE CORRELATION BETWEEN THE PRODUCTION OF CARBON DIOXIDE AND THE ACCUMULATION OF AMMONIA BY SOIL ORGANISMS

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Received for Publication October 15, 1917

INTRODUCTION

The decomposition of organic matter in the soil being generally a biological process, various indexes of biological activity have been used to measure it, the more common of which have been plate counting, ammonification and nitrification. Wollny (14), Lemmerman and his associates (4), Stoklasa and Earnest (10), van Suchtelen (12), and others have shown that the evolution of carbon dioxide is an excellent index of the decomposition of soil organic matter. Later work has been done by Fred and Hart (2) and by Potter and Snyder (9) concerning the influence of sulfates, phosphates and lime on carbon-dioxide production. Ammonification studies have been numerous but a survey of the literature yields little on the relationship between ammonia accumulation and carbon-dioxide production.

Discrepancies have been observed between an apparent increased biological activity in soils and an increased ammonia accumulation or vice-versa. Lipman and his associates (6) found that the addition of soluble carbohydrates decreased the accumulation of ammonia, and, in later work (7), of nitrates also, the production of carbon dioxide, however, being increased. It is evident that ammonia accumulation in itself can seldom be taken as a criterion of the intensity of action taking place. While ammonia is readily assimilated by most microorganisms and may therefore disappear as soon as liberated, the carbon dioxide produced is not used again by the same or by other species. Since carbon dioxide is not assimilated it becomes very nearly an absolute index of biological activity, with due regard to possible chemical formation under certain conditions.

In view of the fact that ammonia determinations are much more easily made and because much valuable ammonification data have been obtained, an attempt is being made to learn more of the relationship between the assimilation of ammonia and the actual activity as shown by the production of carbon dioxide. There must be an optimum ratio between these two factors necessary for the most favorable soil conditions.

Considerable attention is given here to the activity of pure cultures of some of the more common species of soil organism, both bacteria and fungi. Work with a fresh soil or soil infusions involves so many factors and species that it is difficult to interpret the results obtained, or at least to know the real effect or effects resulting from changing some of the conditions of the experiment. Results obtained with pure cultures and with mixtures of the same in the natural soil medium, except as it is changed by sterilization, ought to be of value in interpreting the action of the natural flora and to aid, ultimately, in the management and improvement of soils so that they will yield the greatest possible returns without deterioration.

Without at this time giving a more complete historical review there is here given a description of methods and of the apparatus, together with some of the data so far obtained.

THE APPARATUS AND ITS MANIPULATION

Some of the chief difficulties in obtaining a satisfactory apparatus for the determination of carbon dioxide evolved biologically, have been the lack of a source of slow, though continuous aspiration, too high a vacuum within certain of the parts, incomplete removal of the carbon dioxide from the air entering, incomplete absorption of the same gas produced, leakage, clogging of tubes, back suction and inconvenience in manipulation. It is believed that the apparatus used in this work does away with many of these defects although it may yet be much improved. Figure 1 gives a diagram of the different parts, and a photograph of a few of the units is given in plate 1. Air enters the bottle *A* containing strong sodium or potassium hydroxide and passes through the tower of glass beads inserted into the bottle. Most of the carbon dioxide and much of the moisture is removed in this tower which lies at an angle which may be varied to suit conditions. The air then passes through the soda-lime tube *B* for the more complete removal of carbon dioxide. From thence it enters the distributing bottle *C*, bubbling through a dilute acid, in this case 10 per cent sulfuric acid, before entering the bell-jar *D*. The bell-jar stands on a pine or cypress board which is prepared by painting on both sides with hot paraffin wax. A hole large enough for the insertion of a no. 5 2-holed rubber stopper is bored beneath each bell-jar which is made air-tight against the paraffined board by means of a brush and molten paraffin. The paraffin thus used was softened somewhat by melting it up with one-third its weight of paraffin oil. The air, drawn from the bottom of the bell-jar, passes in through the side neck of the suction flask and up through the barium hydroxide bead tower *E*, which removes the carbon dioxide produced by the organisms in flask *F*. From thence the line leads to the suction pump *G* which is fed by a siphon from the constant-level water tank *H*.

In the apparatus as used, a bell-jar, tower and distributing bottle are connected up in sets of six, each leading back to one soda-lime tube and ahead

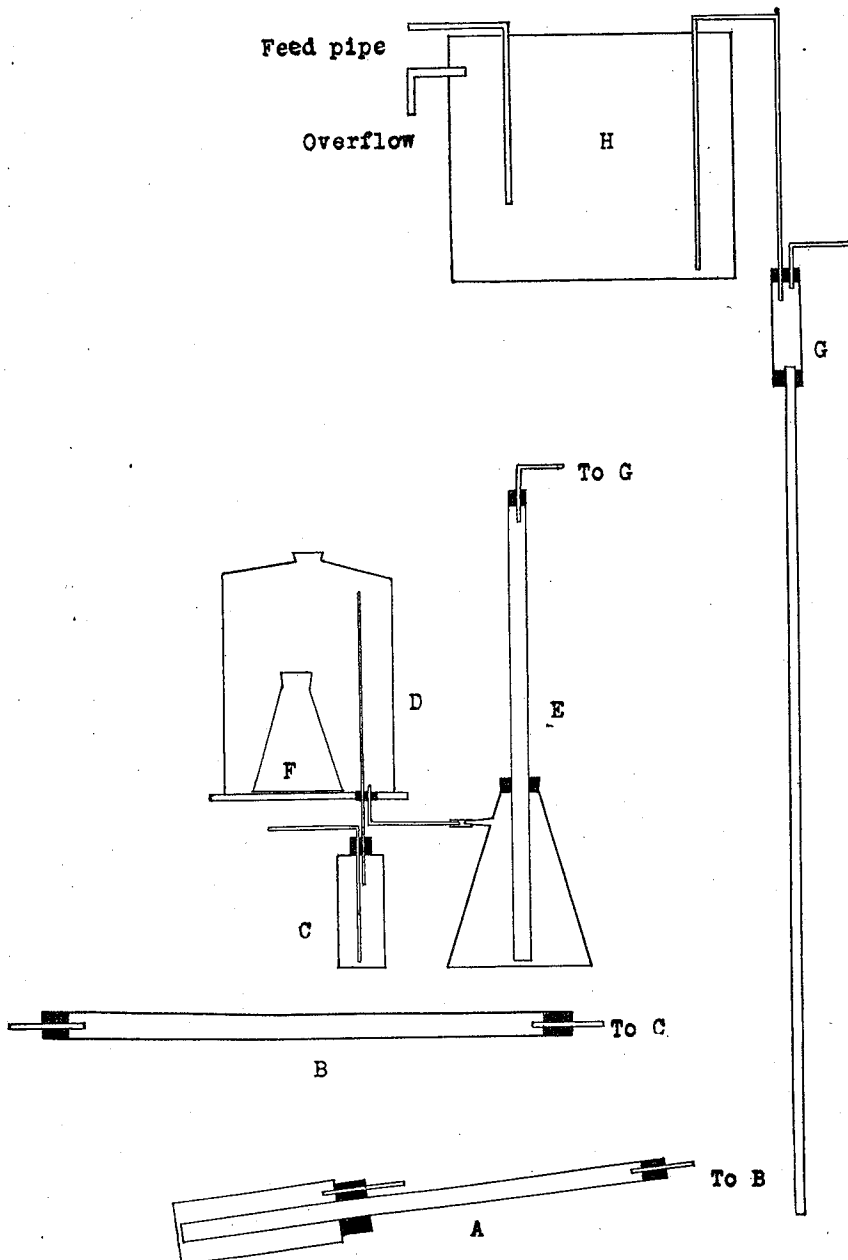


FIG. 1. A DIAGRAM OF THE APPARATUS USED TO DETERMINE CARBON DIOXIDE EVOLVED FROM SOIL¹

¹ The principle involved in the pump was obtained from apparatus used by Dr. T. J. Headlee, entomologist of the New Jersey Agricultural Experiment Station.

to one suction pump. Each bell-jar and tower is in a separate line, however, diffusion of carbon dioxide being prevented by the acid solutions of the distributing bottles *C*. The suction pump *G* as finally constructed produced a very constant, continuous suction and required but little water for its operation. The tube leading from it being larger than the intake tube, bubbles of air are drawn down by the water in its descent. The pump itself consists of an ordinary thick-walled test-tube, fitted with stoppers at top and bottom, the closed end of the tube being broken off. The length of the drain tube largely determines the suction power and it was found that a tube 4 to 5 feet long was sufficient.

Clogging of the air inlet by the formation of crystals of sodium carbonate in the strong sodium hydroxide solution was prevented by using a bead tower *A* as shown, the opening at the bottom of the tower being 24 mm. in diameter. This tower was put in a slanting position to permit a slow, constant intake of air and to prevent as much of a vacuum as possible within the bell-jars.

The barium hydroxide tower which is 24 inches high is a modification of one described by Truog (11). The side-necked suction flask is of the type ordinarily used for Gooch crucible work. The barium hydroxide solution, used to absorb the carbon dioxide liberated, was made by adding 40 gm. of $\text{Ba(OH)}_2 \cdot 8\text{H}_2\text{O}$ per liter of distilled water. The solution was siphoned off after standing over night and kept in a bottle protected from the carbon dioxide of the air, delivery being made through an automatic burette. The oxalic acid solution, used to titrate the excess of barium hydroxide, contained 8.6 gm. of the acid per liter. Both solutions were standardized in terms of milligrams of carbon dioxide per cubic centimeter. The procedure consisted of first adding the desired amount of barium hydroxide solution to the side-necked flask together with a few drops of phenolphthalein as an indicator and enough carbon-dioxide-free water to cause the liquid to rise about half way up in the bead tower when aspirating through the apparatus. When ready to make a determination the tower was partly withdrawn and washed free from barium hydroxide with carbon-dioxide-free water, the glass beads remaining in place because of the cloth gauze stretched over the bottom of the tube. The flasks were kept stoppered until titrated with oxalic acid, the residual barium hydroxide being thus determined. This method, although expeditious, results in some carbon-dioxide absorption from the air. Tests were conducted by aspirating for the customary periods with sterile flasks in the bell-jars, and the average blank thus obtained was subtracted from each determination.

The construction of the apparatus is such as absolutely to prevent any back suction of solution through the various units leading to the bell-jars. The amount of air aspirated was controlled with screw pinch cocks and was slow but continuous. Previous to a determination the air was allowed to circulate more rapidly for an hour. In view of the unquestioned value of carbon-dioxide determinations, it is hoped that a set of apparatus may finally

be perfected that will become as permanent a piece of laboratory equipment as an ammonia or a nitrogen still. Instead of bell-jars, for instance, metal jars, closed at the bottom and fitted with air-tight, removable covers, might be used.

METHODS

The bacteria used were grown on bouillon agar slants made in square bottles about 4 cm. wide and 10 cm. high. These were incubated at 28°C. for from 4 to 6 days. When ready to use, 100 cc. of a sterile 0.6 per cent salt solution was added to each bottle. A sterile rubber stopper was then inserted, and a suspension obtained by shaking gently. Erlenmeyer flasks of 250 cc. capacity, containing 70 gm. of soil were sterilized at 15 pounds steam pressure for 20 minutes and inoculated with 3 cc. of the suspensions. When mixtures of pure cultures were used for inoculating, these were obtained by pipetting equal amounts of the desired suspensions into a sterile flask from which 3-cc portions were drawn after gently shaking the mixture. This method of mixing reduces the total number of a given species but the inoculation was so heavy, at any rate, that this decrease was considered negligible.

When fungus cultures were employed the procedure was similar, except that a longer incubation of the inoculating material was necessary to secure abundant sporulation. The medium used consisted of 0.25 gm. of magnesium sulfate, 0.25 gm. of di-potassium phosphate, 10 gm. of peptone, 20 gm. of glucose, 15 gm. of agar and 1000 cc. of water. The soil infusions used were obtained from a neutral loam well supplied with organic matter.

The pure cultures employed were carefully tested as to purity and characteristics and were obtained from the stock cultures of the soil department of the New Jersey Agricultural Experiment Station. Several tests made upon flasks of soil which had been inoculated in the above manner showed no contamination with foreign organisms.

The soil was mixed with the organic material and other ingredients added by shaking for 5 minutes in a large bottle. The 70-gm. portions were then weighed out with a balance sensitive to 0.1 gm. The water content was 50 per cent of the maximum as determined by the Hilgard method. Seventy-gram instead of 100-gm. portions were used because a thinner layer with the same surface area was obtained, thus permitting better aeration. In the work done so far a soil classified as Norfolk sandy loam has been used. This contains sand of fine texture and has a maximum water-holding capacity of 32 per cent. It is low in organic matter, containing only 0.02 per cent of nitrogen, and has a lime requirement of 550 pounds of calcium oxide per acre as determined by the Veitch method.

Preliminary tests showed that the ammonia accumulation was about the same whether flasks were incubated without bell-jars or within, through which air was slowly but continuously circulated. The loss of moisture for a 12-day period was 1.2 gm. per flask within the bell-jars and 2 gm. without. Ac-

cordingly, some of the flasks for ammonia determinations were incubated apart from the apparatus during the same period that carbon-dioxide determinations were being made. The ammonia was determined by adding an excess of magnesium oxide and distilling in the usual way.

In all cases checks have been subtracted from the reported amounts of carbon dioxide and of ammonia, which are the averages of duplicate determinations. The inoculated flasks were incubated in a room kept at a temperature of from 21° to 23°C. for a period of 12 days.

TABLE 1
A comparison of duplicate determinations of carbon dioxide production and of ammonia accumulation

ORGANISMS	CO ₂ PRODUCTION				NH ₃ ACCUMULATION			
	1	2	Average	Per cent of error from mean	1	2	Average	Per cent of error from mean
Norfolk sandy loam + 1 per cent of cottonseed meal								
B. subtilis.....	134.1	138.8	136.5	3.4	10.38	11.40	10.59	9.5
B. vulgatus.....	169.1	167.0	168.1	1.3	7.85	8.61	8.23	9.2
B. subtilis + B. vulgatus.....	215.3	201.2	208.3	6.7	10.38	11.00	10.69	3.0
B. mycoides.....	60.4	Lost			2.00	2.27	2.19	12.3
B. megatherium.....	45.3	37.7	41.5	18.3	1.17	1.67	1.42	35.0
B. mycoides.....	40.7	43.5	41.6	4.1	1.42	1.42	1.42	00.0
B. mycoides + B. megatherium....	38.4	39.6	39.0	3.0	2.82	2.32	2.57	2.0
B. mycoides + B. vulgatus.....	194.2	202.3	198.3	4.0	8.00	7.40	7.70	7.7
Norfolk sandy loam + 1 per cent of alfalfa meal								
B. subtilis.....	89.5	88.4	89.0	1.2	2.38	2.26	2.32	5.2
B. subtilis (no. P ₂ O ₅ added).....	81.7	79.9	80.8	2.2	2.51	2.51	2.51	0.0
B. megatherium.....	73.9	71.9	72.9	2.7	1.12	1.37	1.25	20.0
B. megatherium (no. P ₂ O ₅ added)...	63.4	60.1	61.8	5.3	1.37	1.24	1.31	10.0
Trichoderma sp.....	160.1	146.1	153.1	9.1	0.69	0.57	0.63	*
Trichoderma sp. + Aspergillus niger.....	167.1	170.2	168.7	1.8	-0.32	-0.07	-0.20	
Aspergillus niger.....	145.2	138.9	142.1	4.4	-0.32	-0.32	-0.32	
Zygorhynchus Vuil + Trichoderma.....	133.2	133.1	133.2	0.0	0.44	0.57	0.52	
Zygorhynchus Vuil.....	104.6	90.5	97.6	14.4	0.95	0.95	0.95	
Soil infusion.....	253.8	272.4	263.1	7.1	-0.45	-0.45	-0.45	
Average.....				5.2				9.5

* These ammonia determinations from the fungus cultures were not included because of the small amounts obtained.

A COMPARISON OF THE DUPLICATE DETERMINATIONS OF CARBON-DIOXIDE PRODUCTION AND OF AMMONIA ACCUMULATION

It was observed that the variations between duplicate determinations of carbon dioxide were greater during the first few days. But at the end of 12 days the differences became less. Table 1 gives these duplicate determinations for the organisms discussed in the following pages. Averaging the percentages of error for each set of duplicates it was found that the average error for the carbon dioxide determinations was 5.2 per cent, while that for the ammonia determinations was 9.5 per cent. This indicates that carbon-dioxide production may be fairly accurately measured and that it was more uniform than the ammonia accumulation.

TABLE 2

Carbon dioxide and ammonia produced from 1 per cent of cottonseed meal in Norfolk sandy loam

TIME	B. SUBTILIS		B. VULGATUS		B. MYCOIDES		B. SUBTILIS + B. VULGATUS	
	CO ₂	NH ₃	CO ₂	NH ₃	CO ₂	NH ₃	CO ₂	NH ₃
days	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1 and 2	17.7		27.3		13.4		26.9	
3 and 4	37.1	5.30	49.1	none	9.3	0.73	64.8	3.97
5	13.2		25.7		6.2		17.6	
6	13.4	7.53	18.0	2.69	5.6	1.18	32.4	6.88
7 and 8	18.1	8.73	15.9	3.49	5.8	1.88	27.0	9.37
9 and 10	22.8	10.58	23.0	4.86	8.1	1.56	24.6	10.00
11 and 12	14.2	10.89	9.1	8.23	12.0	2.19	15.1	10.69
Totals.....	136.5		168.1		60.4		208.4	
Per cent of total nitrogen and of total carbon as found.....	17.8	26.6	21.8	20.1	7.9	5.4	27.1	26.1

THE RELATIVE OXIDIZING AND AMMONIFYING POWER OF SEVERAL PURE CULTURES AND OF MIXTURES OF THE SAME

One per cent of cottonseed meal was the source of organic matter for the series given in table 2. It contained 5.84 per cent of nitrogen and 40.3 per cent of carbon. This was mixed with Norfolk sandy loam to which was added 0.066 per cent of acid phosphate, containing about 16 per cent of water-soluble phosphorus calculated as phosphoric pentoxide. Three-tenths of 1 per cent of precipitated calcium carbonate also was added. These percentages are equivalent to about 2000 pounds and 9000 pounds per acre 9 inches. Table 3 gives data obtained under similar conditions for another series.

Considering first the relative activities of these common soil types, it may be seen that *Bacillus vulgatus* leads in carbon-dioxide production (table 2), oxidizing, in a 12-day period, 21.8 per cent of the carbon added in the cotton-

seed meal. *Bacillus subtilis*, giving considerably less carbon dioxide, caused the accumulation of more ammonia, amounting to 26.6 per cent of the total nitrogen added. A mixture of these two organisms gave a higher carbon-dioxide production than either alone, and an ammonia accumulation approximating that of *B. subtilis*. *Bacillus mycoides*, tested twice (tables 2 and 3), gave very much lower amounts both of carbon dioxide and of ammonia. *Bacillus megatherium* (table 3) was about as active as *B. mycoides* and a mixture of the two gave no increase in carbon dioxide. But mixing *B. vulgatus* with *B. mycoides* caused a very marked increase both in carbon-dioxide production and in ammonia accumulation. This increase in carbon dioxide is easily observed in figure 3. Both figure 2 and figure 3 show that the maximum rate of carbon-dioxide production always occurred before the fifth day of incubation.

TABLE 3

Carbon dioxide and ammonia produced from 1 per cent of cottonseed meal in Norfolk sandy loam

TIME	B. MYCOIDES		B. MEGATHERIUM		B. MEGATHERIUM + B. MYCOIDES		B. VULGATUS + B. MYCOIDES	
	CO ₂	NH ₃	CO ₂	NH ₃	CO ₂	NH ₃	CO ₂	NH ₃
days	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1 and 2	12.9		13.8		11.6		36.1	
3	3.0	0.50	3.9	1.19	2.0	0.75	20.6	0.63
4 and 5	5.6	1.45	8.6	1.32	4.8	1.51	43.1	4.81
6, 7, 8 and 9	11.5		9.8		14.8		75.1	
10	2.6		1.7		1.8		8.8	
11 and 12	5.8	1.42	3.7	1.42	4.1	2.57	14.7	7.70
Totals.....	41.6		41.5		39.1		198.4	
Per cent of carbon and of nitrogen as found.....	5.4	3.5	5.2	3.5	5.0	6.3	25.8	18.8

A series in which ground alfalfa hay was used instead of cottonseed meal was inoculated with cultures of *B. subtilis* and *B. megatherium* (table 4). As shown in the table, the addition of acid phosphate caused an increase in carbon-dioxide but none in ammonia accumulation. Until more data are secured no conclusions can be drawn in the comparison of carbon-dioxide production in its relation to ammonia accumulation as influenced by phosphates and by other salts.

In a second series, with alfalfa as the source of organic matter, the activities of some fungi were measured and compared with those of a soil infusion (table 5). The average oxidizing power for the bacteria (table 4) was 8.0 per cent, while that for the individual species of fungi was 16.5 per cent of the carbon added. The soil infusions greatly exceeded both, being 33.0 per cent. The bacteria are not able to utilize the carbon of cured alfalfa as readily as fungi,

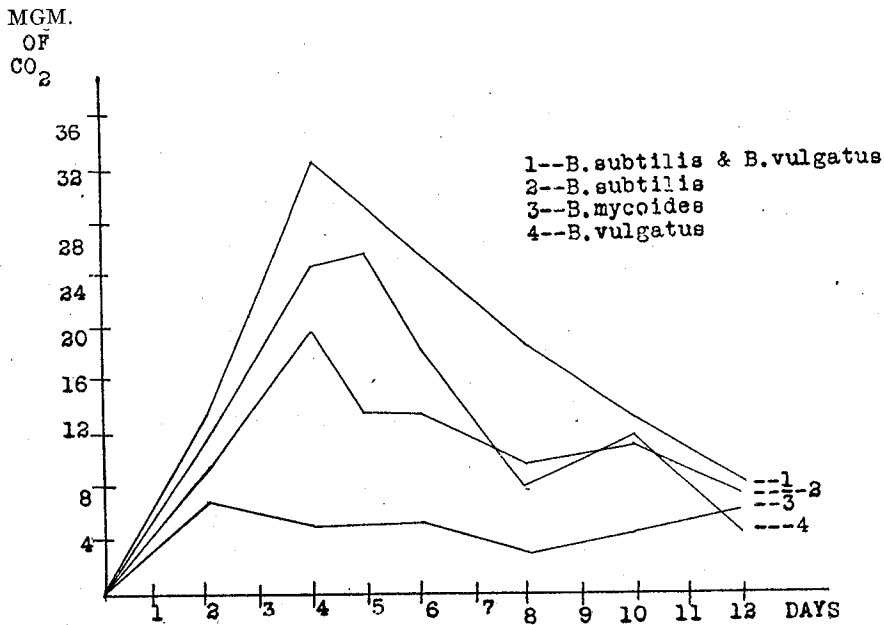


FIG. 2. THE DAILY PRODUCTION OF CARBON DIOXIDE FROM NORFOLK SANDY LOAM, PLUS 1 PER CENT OF COTTONSEED MEAL, BY PURE CULTURES OF BACTERIA (TABLE 2)

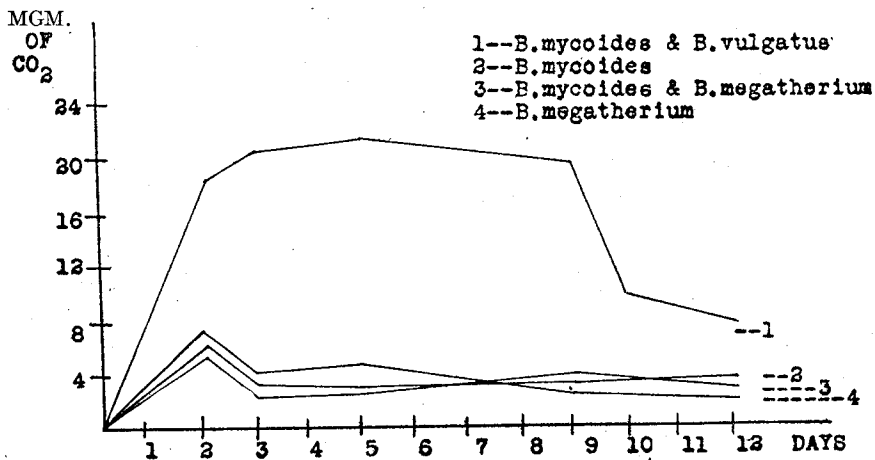


FIG. 3. THE DAILY PRODUCTION OF CARBON DIOXIDE FROM NORFOLK SANDY LOAM, PLUS 1 PER CENT OF COTTONSEED MEAL, BY PURE CULTURES OF BACTERIA (TABLE 3)

and this would be even more evident for a longer period, since the fungus cultures start more slowly but are more active at the end of 12 days.

Glancing again at table 5 it may be seen that when a mixed culture of *Trichoderma* sp. and *Aspergillus niger* is used an activity greater than that of either alone is obtained. *Trichoderma* sp. and *Zygorhynchus Vuilleminii* acting together showed an activity less than the one and greater than the other. It would be interesting to know more of the associative action of fungi and bacteria in pure cultures. The combinations of bacterial cultures so far studied, appear to be symbiotic in their behavior. Using ammonia accumulation as an index, Coleman (1) found, for low moisture contents, an antagonistic action between *B. subtilis* and *Zygorhynchus Vuilleminii*. Soil

TABLE 4

Carbon dioxide and ammonia produced from 1 per cent of alfalfa in Norfolk sandy loam with and without the addition of acid phosphate

TIME	B. SUBTILIS				B. MEGATHERIUM			
	Acid phosphate added		No acid phosphate added		Acid phosphate added		No acid phosphate added	
	CO ₂	NH ₃	CO ₂	NH ₃	CO ₂	NH ₃	CO ₂	NH ₃
days	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	7.1		5.7		7.8		7.4	
2 and 3	41.6		38.6		30.0		26.6	
4	13.0		8.3		7.4		5.0	
5 and 6	12.6	1.80	11.2	1.63	10.2	1.11	9.8	0.73
7 and 8	6.1		6.6		6.8		4.7	
9, 10, 11 and 12	8.7	2.32	10.1	2.51	10.9	1.25	8.3	1.31
Totals.....	89.0		80.8		72.9		61.8	
Per cent of carbon and of nitrogen as found.....	8.4	14.9	6.8	16.1	7.6	8.0	5.8	8.4
Per cent increase due to acid phosphate.....	10.1				17.9			

infusions are known to be more active than individual organisms. Hence it may be inferred either that the associative action of the many species in an infusion is beneficial or that the most active species have not been isolated, and if they have, that they have been attenuated by artificial culture.

Finally, it may be observed (table 5) that those fungi which oxidized the most gave the lowest accumulations of ammonia. In both cases where *Aspergillus niger* was added, as well as where a soil infusion was employed, the organisms even used up some of the ammonia shown to be present in the sterilized checks (fig. 5).

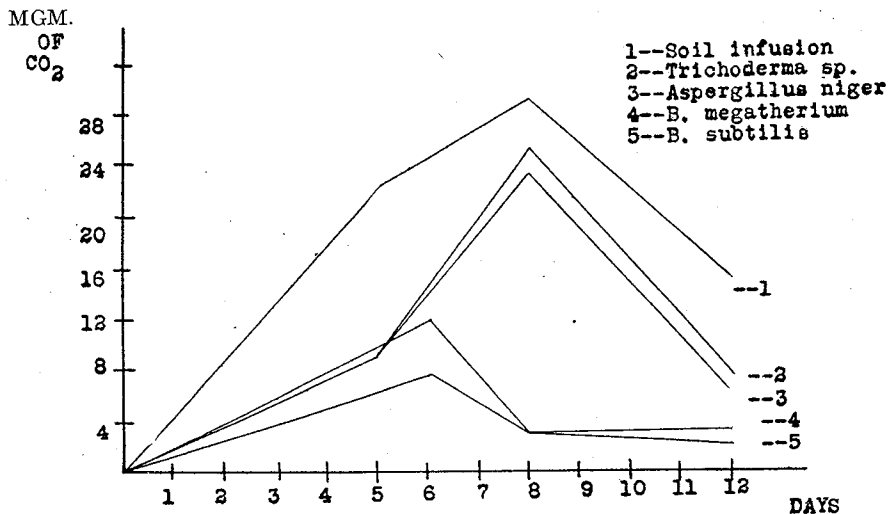


FIG. 4. THE PRODUCTION OF CARBON DIOXIDE BY CULTURES OF BACTERIA AND OF FUNGI AND BY A SOIL INFUSION, WITH 1 PER CENT OF CURED ALFALFA IN NORFOLK SANDY LOAM (TABLES 4 AND 5)

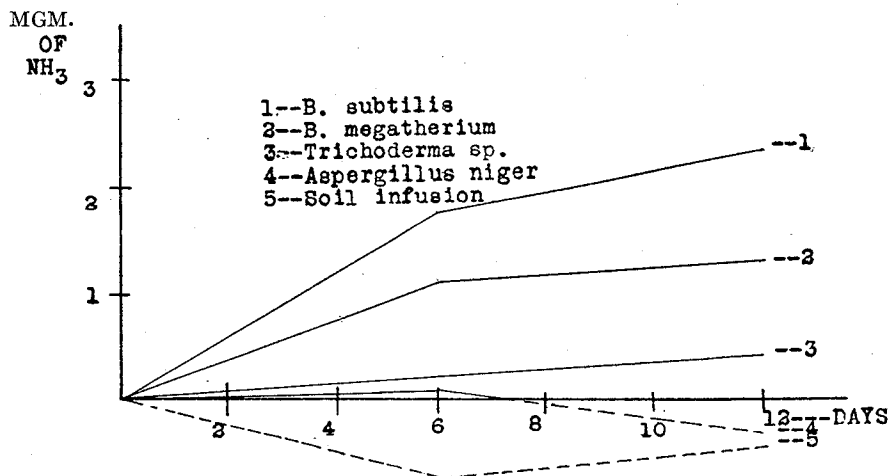


FIG. 5. THE PARALLEL ACCUMULATION OF AMMONIA BY THE ORGANISMS FOR WHICH THE CARBON-DIOXIDE PRODUCTION IS GIVEN IN FIGURE 4

THE CORRELATION BETWEEN CARBON-DIOXIDE PRODUCTION AND AMMONIA
ACCUMULATION BY PURE CULTURES OF BACTERIA AND FUNGI AND
BY SOIL INFUSIONS

In considering the relation of ammonia accumulation to the rate of organic decay it is of interest to inspect the data so far obtained from parallel determinations of ammonia and carbon-dioxide. Table 6 shows that a soil infusion decomposed nearly three times as much of the alfalfa as *B. subtilis* for a period of 8 days, the percentages of total carbon oxidized being 22.0 and 8.9, respectively. During this period *B. subtilis* caused the accumulation of 10.4 per

TABLE 5
Carbon dioxide and ammonia produced from 1 per cent of alfalfa in Norfolk sandy loam by fungi and by a soil infusion

TIME	TRICHODERMA SP.		ASPERGILLUS NIGER		ZYGORHYNCHUS VUILLEMINII		SOIL INFUSION		TRICHODERMA SP. + ASPERGILLUS NIGER		TRICHODERMA SP. + ZYGORHYNCHUS VUILLEMINII	
	CO ₂	NH ₃	CO ₂	NH ₃	CO ₂	NH ₃	CO ₂	NH ₃	CO ₂	NH ₃	CO ₂	NH ₃
days	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1, 2, 3, 4 and 5	46.0	0.04	47.5	0.13	38.0	0.63	112.8	-0.72	45.4	0.45	38.6	0.31
6, 7 and 8	77.6	0.25	70.1	0.00	44.6	0.44	88.3	-0.22	91.0	-0.14	59.8	0.70
9, 10, 11 and 12	29.6	0.63	24.5	-0.32	15.1	0.95	62.1	-0.45	32.3	-0.20	34.9	0.52
Totals.....	153.2		142.1		97.7		263.1		168.7		133.3	
Per cent of carbon and of nitrogen as found.	19.4	4.4	17.7		12.3	6.1	33.0		21.1		16.7	3.4

TABLE 6
The production of carbon dioxide and the accumulation of ammonia from 2 per cent of alfalfa in Norfolk sandy loam by B. subtilis and a soil infusion

ORGANISMS	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH AND 6TH DAYS	7TH AND 8TH DAYS	TOTALS	PER CENT OF CARBON OX- IDIZED	PER CENT OF N AMMONI- FIED
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.		
<i>B. subtilis</i>									
CO ₂	47.3	39.6	16.7	9.8	15.6	11.2	140.2	8.9	
NH ₃		1.70		2.14	3.13	3.49			10.4
Soil infusion									
CO ₂	47.0	42.0	68.2	54.6	91.8	41.8	345.4	22.0	
NH ₃		-0.60		-0.51	-1.20	-1.16			none

cent of the total nitrogen as ammonia, whereas the soil infusion not only caused no accumulation but even used up most of the small amount appearing in the checks after sterilizing.

With alfalfa as the source of energy pure cultures of fungi give results similar to those obtained with a soil infusion (table 5). The less active of the fungi tested gave a small accumulation of ammonia while the more active species, like the soil infusions, used up some of the ammonia originally present in the checks as well as all that may have been liberated.

Looking now at the action of bacteria upon alfalfa it may be noted that they (table 4) cannot utilize it as readily as fungi, the average amount oxidized by the two groups being 8.0 and 17.5 per cent, respectively. But the bacteria, although they oxidized less than half as much carbon, caused the accumulation of 14.2 per cent of the total nitrogen as ammonia, or over three times as much as was accumulated by the fungi, for which the percentage was 4.6, not including the more active fungus cultures which produced no ammonia at all (fig. 4 and 5).

Under the conditions of the experiment, an average of 12.2 per cent of the total carbon of cottonseed meal was oxidized by the bacterial cultures (tables 2 and 3), while the average amount of nitrogen found as ammonia was 13.8 per cent. The action of fungi upon cottonseed meal has not been studied with reference to the simultaneous production of carbon dioxide and accumulation of ammonia. Working with pure cultures, McLean and Wilson (8), and later Coleman (1), Kopeloff (3), and Waksman and Cook (13), have shown that fungi cause considerable accumulation of ammonia from cottonseed meal, the amounts depending upon various factors such as phosphates, length of incubation, moisture, temperature and type of soil. Lipman and Burgess (5), studying several bacteria in pure cultures, found with a given type of soil, that the species giving the highest ammonia accumulation with one kind of organic matter was not always the highest accumulator with another kind.

Referring again to the carbon-dioxide production and the ammonia accumulation from alfalfa, the data so far secured show that a low accumulation of ammonia is an indication of high rather than of low activity. With this type of organic matter the behavior of fungi was more like the action of soil infusions than the behavior of bacteria, indicating that the most active components of a soil flora, as obtained from an infusion, are fungi.

It seems evident that other kinds of organic matter and of soils, as well as the effect of various fertilizing elements and soil amendments, should be studied with reference to the optimum ratio between carbon-dioxide production and ammonia accumulation. The action of pure cultures of bacteria, actinomyces and fungi, when mixed together in different combinations, deserves further consideration and investigation. If these three groups make up the natural soil flora, studies in such combinations or floras made up synthetically might throw more light on the interactions and needs of soil organ-

isms so that they could be better controlled and aided in their function of preparing food for plants.

SUMMARY

The results obtained so far with the use of carbon dioxide as an index of biological activity and the correlation of this activity with ammonia accumulation may be summarized as follows.

1. An apparatus is described for the determination of carbon dioxide evolved biologically from soil.

2. Methods are given which were used for the study of organic decomposition by pure cultures and by mixtures of pure cultures of microorganisms.

3. Duplicate determinations indicate that the production of carbon dioxide is more uniform than the accumulation of ammonia for a 12-day period.

4. In general a high carbon-dioxide production by pure cultures of bacteria was accompanied by a high ammonia accumulation, with 1 per cent of cotton-seed meal or of alfalfa in Norfolk sandy loam.

5. Pure cultures of the fungi tested, oxidized more of the carbon of alfalfa than pure cultures of bacteria, but the bacteria caused the accumulation of much more ammonia. The more active species of fungi not only caused no accumulation of ammonia but even used up some of the small amounts appearing in the checks. Soil infusions resembled the fungus cultures with respect to ammonia accumulation but were more active in the production of carbon dioxide.

6. With alfalfa as the source of organic matter a low accumulation of ammonia is an indication of a high rather than of a low activity. Furthermore, since the behavior of the soil infusions was more like that of fungi than of bacteria, it would seem that fungi were the more active components of the natural soil flora.

7. The mixtures of pure cultures of bacteria tested showed no antagonism and in some cases a symbiotic relation seemed to exist. Mixtures of pure cultures of fungi or of fungi and bacteria have not been studied sufficiently to permit of any conclusion therefrom.

In conclusion it is a pleasure to thank Dr. J. G. Lipman for his many helpful suggestions given during the course of this work.

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