CONTRIBUTIONS TO THE MICROBIOLOGY OF AUSTRALIAN SOILS. IV.

THE ACTIVITY OF MICROORGANISMS IN THE DECOMPOSITION OF ORGANIC MATTER.

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(Eleven Text-figures.)

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Introduction.

The decomposition of the organic compounds which are continually added to soils under natural conditions, especially in the form of plant residues, is known to be due chiefly to the action of microorganisms, among which bacteria, actinomycetes and lower types of fungi are the most important. This process of decomposition results in the release of a part of the elements of the decomposed material as compounds suitable for plant nutrition (water, carbon dioxide, ammonia, nitrate, etc.) and in the accumulation of another part of the material as a slowly decomposing residue of various constituents collectively known as "humus". This "humus" is recognized as a natural constituent of most soils that carry a vegetation, and is of importance by affecting the physical properties of the soil as well as by acting as a carrier of plant food, especially nitrogen, which is gradually made available to the vegetation through the slow decomposition of the humus. The extent to which humus accumulates is known to depend largely upon the temperature relationships of the soil in question. Tropical soils, although carrying a luxuriant vegetation, are usually poor in humus, and in temperate climates Jenny (1928-31) has demonstrated an inverse relationship between the average annual temperature and the average content of organic matter and nitrogen in soils, which latter was found to increase 2 to 3 times for each 10° C. decrease in the mean annual temperature. Jenny explained these phenomena through the accelerating influence of increasing temperature on the decomposition processes in the soil according to the rule of van't Hoff. Following up this idea, Waksman and Gerretsen (1931) showed that although the rate of decomposition of straw as a whole increased markedly with the temperature, the rate of decomposition of the various constituents of the straw was not affected in the same manner by changes in temperature from about 7° C. to about 37° C. For instance, the resistant lignin was decomposed comparatively quickly at 37° C., but hardly at all at 7° C., and at this temperature (the lowest tested) there was a tendency to synthesis rather than to decomposition of proteid materials. In connection with this important contribution we shall make a brief survey of the present status of the problem of decomposition of organic matter in soil. Most of the work in this direction falls into two categories:

A. Decomposition of the soil's own organic matter ("humus").—In experiments on this subject, the evolution of carbon dioxide from the soil, either in laboratory or field experiments, has usually been used as an index of the

decomposition.* In all experiments where the influence of temperature has been studied, the rate of carbon dioxide formation has been found to increase with the temperature, at least within the limits obtaining under natural soil conditions; important contributions in this respect are due to Russell and Appleyard (1917), Fehér (1929–35), and Leroux (1934). Counts of microorganisms (especially bacteria) have frequently been combined with the CO_2-determinations, and a parallelism has sometimes been observed. The bacteria have always been counted by the plate method or other cultural methods, and determinations of the total numbers of bacteria have not been made in such experiments, although it is known that the plate method reveals only a (mostly) small fraction of the total bacterial flora of soils (Conn, 1918; Winogradsky, 1925; Thornton and Gray, 1934). The importance of fungi under these conditions is not precisely known.

B. Decomposition of organic materials added to the soil.—Laboratory experiments in this direction are almost innumerable (for references, see Waksman, 1932) and have been undertaken from widely different points of view. A very large proportion of them are simple "ammonification"—or "nitrification"—experiments with nitrogenous materials used as fertilizers, in which only the accumulation of ammonia and/or nitrate is determined. In many other experiments, with definite chemical compounds as well as with complex materials, the rate of CO_2-formation or of disappearance of added compounds has been found, sometimes together with determinations of ammonia or nitrate and chemical analysis of the complex material undergoing decomposition. Such experiments have been carried out, not only with the complex soil microflora, but also with pure cultures of soil microorganisms. In many instances, counts of bacteria and fungi have been carried out by cultural methods, but determinations of the total numbers of bacteria in quantitative decomposition experiments are lacking, with the notable exception of a few recent contributions by Jacobs (1931) and Vandecaveye and Villanueva (1934*a–b*). Finally, a large number of experiments deal merely with the influence of addition of organic materials on the number and kinds of microorganisms in soil, without quantitative estimation of the rate of decomposition. On this field microscopic methods have been widely used in recent time, particularly through the studies of Conn (1917), Winogradsky (1925) and Cholodny (1930).

Among all these investigations we find comparatively few attempts to correlate the abundance of microorganisms with the intensity of destruction of organic matter, at least on a quantitative basis. Important steps in this direction are represented by studies on pure or mixed cultures of soil microorganisms (bacteria and protozoa) by Cutler and Crump (1929), de Telegdy-Kovats (1932), and Meiklejohn (1932). As to the fungi, they have been shown again and again to develop abundantly in soil to which decomposable organic matter has been added, and to be able to carry out many processes of decomposition as actively as the bacteria or the mixed soil microflora (see Waksman, 1932), but their quantitative importance under natural conditions in comparison with the bacteria still remains unknown. The influence of temperature on separate microbial processes (nitrification, nitrogen fixation, cellulose decomposition, etc.) has been studied extensively, but its influence on the decomposition of complex organic materials as a whole has received remarkably little attention, apart from the

* Good and complete reviews of the literature on carbon dioxide production in soil are due to Waksman and Starkey (1924) and Leroux (1934).

work referred to above under (*A*) and the mainly biochemical contributions by Waksman and Gerretsen (1931), Norman (1931) and Shrikande (1934), and we have hardly any information at all concerning the influence of temperature on the number and kinds of microorganisms that arise when organic materials are added to the soil, or on the relation between the character of this microflora and the nature and intensity of the decomposition processes which it brings about.

In some preliminary experiments (Jensen, 1935) it was observed that, in soils with addition of organic materials, the bacteria and fungal mycelia generally developed most richly at lower temperatures, whereas the rate of CO_2-production increased with the temperature; correlations also appeared to exist between bacterial numbers, density of mycelium, and production of carbon dioxide. Since these results suggested a way of finding quantitative expressions for the activity of each group of microorganisms at different levels of temperature, the experiments were extended and placed on a broader basis. Two series of experiments were carried out. In the first, the rate of decomposition of the soil's own organic matter was estimated by determinations of carbon dioxide production at different levels of temperature from soils of different character, but without addition of any organic matter. In the second, similar determinations were made from soils with additions of three different organic materials. In both series, the rate of CO_2-evolution was compared with the results of periodical estimations of the density of mycelium by the Rossi-Cholodny method (Jensen, 1935) and the numbers of bacteria (including actinomycetes), which were determined by direct microscopic counting as well as on agar plates. The objection has often been raised against the method of direct counting, that these figures are of little value, since they include dead as well as living bacteria. This cannot be denied, but on the other hand Thornton and Gray (1934) pointed out that non-viable bacteria may produce chemical changes in their environment, although they are unable to multiply. This contention is undoubtedly well founded; there is ample evidence that bacterial cells incapable of reproduction may still show such fundamental biochemical activities as respiration (Cook and Stephenson, 1928; Ehrismann, 1933*), and proteolysis (Berghaus, 1908; Janke and Holzer, 1929–30; Moycho, 1933).

Methods.

Carbon dioxide production was measured by the method of Petersen (1926): a suitable quantity of moist soil, usually 50–60 gm., is placed in a cylindrical bag of copper wire gauze, approximately 3 × 12 cm., which by means of a nail and a piece of string is suspended under a rubber stopper that fits tightly into the neck of a flask of convenient size and shape (square, wide-necked reagent bottles of 600 c.c. capacity were used), containing a measured quantity of an approximately 0·05N solution of barium hydroxide. At intervals (usually 24 hours, or 2–3 days if the evolution of CO_2 is very slow) the amount of carbon dioxide liberated from the soil and absorbed by the baryta-water is determined by titration with standard oxalic acid and phenol-phthalein, similar flasks without soil serving as blanks. The results from parallel flasks usually agree within ± 4·0 per cent. In the Tables 1–4, the production of CO_2 is expressed as mgm. produced by 100 gm. of dry soil in 24 hours, unless otherwise stated. The flasks were placed

* This author sums up the matter in the paradoxical sentence: "Die Atmung als solche ist kein Beweis für das Leben."

in incubators regulated to the desired temperatures and only removed for the brief periods (not more than 15–20 min., usually less) necessary to carry out the titrations and the manipulations involved in the determinations of microorganisms. The higher temperatures, 28 and 37° C., were obtained in ordinary incubators. For the lowest range of temperature an electric refrigerator was used; the temperature of this varied somewhat (from about 4° to about 8° C.) during the whole experimental period, but not more than 1–2° C. during each single experiment. A temperature of 14–16° C., with an average of 15° C., was obtained in an ice- and water-cooled incubator; occasional fluctuations to about 13° and about 17° C. took place, but only rarely and not for more than a day at a time. A few experiments were also carried out in a water-cooled incubator at 18–21° C.; during the colder part of the year, this could also be run at a temperature of 14–15° C.

To observe the development of fungal mycelium, a clean microscopic slide was inserted vertically in the soil in each bag (or in two of them, if more than two parallels were run) with its upper edge on a level with the surface of the soil, and renewed at intervals of 3–8 days. At the same time a weighed quantity of soil was removed from each bag for the bacterial counts. After drying and heat-fixation, the slides were stained after Gram, decolorized with alcohol or acetone (preferable for heavy clay soil), and counterstained with phenolic erythrosine. The density of mycelium was then estimated as previously described (Jensen, 1935): 400–500 microscopic squares of 65μ side-length, distributed as evenly as possible over the whole area of the slide, were examined, and the percentage of fields showing presence of fungal hyphae was calculated. The figures from parallel slides usually agreed within 10 per cent., except in the case of slides very poor in mycelium (less than 2 per cent.).

Direct counts of bacteria were made by the method of Thornton and Gray (1934): shaking of the soil with a suspension containing a known number of indigo particles, and determination of the ratio bacteria/indigo. Since only a small quantity of soil was available in most cases, the same soil suspension was used for both direct and plate counts: the soil was shaken for 4 minutes with sterile agar solution, making an initial dilution of 1:3 to 1:15, according to the number of bacteria to be expected in the soil. 1 c.c. of the suspension was removed and diluted further for preparation of the agar plates, whereupon 4 or 5 c.c. of the initial suspension was shaken for 1 minute with an equal volume of the indigo suspension. Rose bengale was found to give a better staining than erythrosine.

Plate counts were made on dextrose-casein-agar (dextrose, 2·0 gm.; casein, dissolved in dilute NaOH, 0·2 gm.; KH_2PO_4, 0·5 gm.; $MgSO_4$, 0·2 gm.; $FeCl_3$, trace; agar, 20·0 gm.; H_2O, 1,000 c.c. pH 6·5–6·7). The plates were incubated for 8 days at 27–28° C., except those from experiments at 4–7° C., which were incubated for 12 days at 16–18° C. All numbers of microorganisms, direct as well as plate counts, are expressed as millions per gm. of dry soil.

Nitrate was determined by the Devarda method, after boiling of the soil extract with sodium hydroxide and potassium permanganate, and ammonia was determined by the method of Bengtsson: repeated extraction of the soil with 0·5N potassium chloride solution, and distillation of the extract with magnesium oxide.

"*Organic matter*" (Table 1) is expressed as loss on ignition.

TABLE 1.

Chemical and microbiological characters and carbon dioxide production in soils without addition of organic matter.

Soil No.		Organic Matter %.	pH.	H_2O. %.	At start of Experiment.			Mgm. CO_2 produced* in the first 24 hours at:			Total production of CO_2 in 10 days, in mgm.			Mgm. CO_2 produced per gm. of Organic Matter in 10 days at:		
					Direct Count.*	Plate Count.*										
						Bact.	Act.	15° C.	28° C.	37° C.	15° C.	28° C.	37° C.	15° C.	28° C.	37° C.
Group I: Sand soils	1	1·5	6·5	9·2	250	0·8	0·5	1·4	2·6	3·9	8·3	17·6	25·8	5·5	11·7	17·2
	2	2·1	5·7	9·0	500	4	3	2·0	—	7·7	32·8	—	76·9	10·6	—	36·6
	3	3·4	5·8	14·0	300	1	0·1	2·6	9·5	11·0	31·8	63·1	104·8	9·3	17·8	30·8
	4	5·8	6·7	17·0	2,560	134	5	6·3	16·1	21·9	50·1	119·2	148·9	8·6	20·6	25·7
Group II: Red to brown loam soils	5	3·4	6·0	17·0	1,360	22	5	3·7	7·9	12·2	60·2	98·0	136·3	17·7	28·8	40·1
	6	4·7	6·2	16·4	1,330	20	8	2·6	6·2	12·6	45·2	89·0	113·8	9·6	18·9	23·8
	7	22·4	6·1	34·6	2,180	237	3	9·5	19·6	32·6	52·8	111·3	208·6	2·4	5·0	9·4
	8	24·5	5·8	39·2	3,410	119	3	9·0	18·1	33·7	49·2	104·1	193·8	2·0	4·2	7·9
Group III: Grey to black loam soils	9	5·5	6·5	17·7	1,700	23	7	5·9	12·8	22·5	88·1	133·5	191·4	16·0	24·3	34·8
	10	5·7	6·8	15·3	1,940	84	9	4·1	6·7	10·6	23·2	38·4	62·8	4·1	6·8	11·1
	11	12·4	7·7	19·7	3,290	95	4	11·6	22·5	29·4	43·6	100·0	151·9	3·5	8·1	12·3
	12	14·5	6·9	24·6	2,360	31	2	4·9	8·5	13·2	30·9	63·7	114·1	2·1	4·4	7·9
	13	17·6	6·5	31·6	(*a*)											
					3,970	—	—	14·3	38·2	—	82·8	291·9	—	4·7	16·6	—
					(*b*)											
					3,115	161	13	—	—	46·8	—	—	388·0	—	—	22·0

* In this and the following tables, numbers of organisms are expressed as millions per gram of dry soil, and production of CO_2 as mgm. CO_2 per 100 gm. dry soil in 24 hours, unless otherwise stated.

Part I.—Production of Carbon Dioxide and Numbers of Microorganisms in Soils without Addition of Organic Matter.

The following soils were used in this series of experiments:

I. *Sand soils*: 1.—Very light sand, poor in humus, under grass, Cooper Park, Sydney. 2.—Light sand soil, poor in humus, under bushes, same locality. 3.—Coarse, dark sand soil, poor in humus, under bushes, Rose Bay Heights, Sydney. 4.—Dark sand soil, fairly rich in humus, from garden, Richmond, N.S.W.

II. *Red to brown loam soils*: 5.—Heavy, red loam, rather poor in humus, from wheat field (good crop), Wagga, N.S.W. 6.—Red-brown loam, rather poor in humus, from pasture, Burragorang Valley, N.S.W. 7.—Heavy, red-brown loam, rich in humus, from lucerne field (good crop), Northern Tablelands, N.S.W. 8.—Heavy, red loam, rich in humus, from lucerne field (crop failing), same locality.

III. *Grey to black loams*: 9.—Heavy, grey loam, fairly rich in humus, from wheat field (poor crop), Wagga, N.S.W. 10.—Heavy, grey loam, fairly rich in humus, from experimental plots, School of Agriculture, Sydney University. 11.—Heavy, dark loam, rich in lime and humus, from flower bed, Sydney University. 12.—Heavy, dark loam, rich in humus, under grass, Sydney University. 13.—Heavy, dark loam, rich in humus, under trees, covered with heavy grass, Sydney University.

After air-drying and sieving, the soils were moistened to approximately two-thirds of their water-holding capacity, and kept for about one week at room temperature before starting the experiments. Germinating seeds that appeared during this period were carefully removed. The experiments were run in duplicate over a period of 10 days, with microbiological analyses after 4 days and at the end of the experiment. For the bacterial counts, 2 or 2·5 gm. soil was removed from each parallel flask and used as a composite sample. Slides for the determination of mycelium were placed in the soil on the first and renewed on the fourth day. Three temperatures were studied: 15° C., 28° C., and 37° C. The experimental results are reproduced in Tables 1–3. Table 1 shows, besides the reaction and the humus and moisture content of the soils, their initial numbers of bacteria, and the amounts of carbon dioxide produced during the first 24 hours and during the whole 10-day period.

The direct counts of bacteria, at start as well as after 4 and 10 days (Table 2), are of the same order as those found by Thornton and Gray (1934), whose work included only clay soils with 1,000 to 4,000 mill. bacteria per gm. The figures show no correlation with the soil reaction, but show a general, although by no means proportional, increase with increasing content of organic matter. The same is true of the plate counts, which, however, vary within much wider limits; their proportion (also in Table 2) is from about 0·3 to about 13 per cent. of the direct counts. Although several important groups of soil organisms (obligate anaerobes, nitrifying bacteria, certain types of cellulose-decomposing bacteria, etc.) cannot be counted by the ordinary plate method, it does not seem necessary to assume that the bulk of the bacterial population of the soil is represented by species which, as such, are unable to develop upon agar media. It has repeatedly been shown that even in what are generally considered "young" cultures the proportion of cells capable of developing into colonies may be as low as the proportion of plate to direct counts observed here (Beijerinck, 1909; Dorner, 1924; Wohlfeil, 1932; Ehrismann, 1933), and this proportion of "viable" cells may depend not only on the age of the culture and the kind of the organism,

but also on the composition of the medium used for plate counting (Beijerinck, 1909), the nature of the diluent (Wohlfeil, 1932), and other factors.

The yields of carbon dioxide, in the first 24 hours as well as during the whole period, increase regularly with the temperature, being generally about twice as high at 28° C. as at 15° C., with a somewhat smaller increase when the temperature is raised to 37° C. There is at each temperature a marked parallelism between the direct counts of bacteria and the yields of CO_2 in the first 24 hours (see also Text-fig. 1). The total amounts of carbon dioxide increase somewhat, but very irregularly, with the content of organic matter, and the three last columns of the table show that the production of carbon dioxide per unit of organic matter generally decreases with increasing content of organic matter, i.e., the more humus there is present, the more slowly is it decomposed, as pointed out by Engel (1934); the very active soil No. 13, however, forms an exception to this rule.

Table 2 shows the counts of microorganisms and the densities of mycelium on the 4th and 10th days, and the corresponding yields of carbon dioxide in the previous 24 hours; the most important results are summarized in Table 3. The direct counts are very little influenced by temperature. In some cases, especially No. 4, there is a marked drop in the numbers after 4 and 10 days in comparison with the initial counts, but in general the changes are not of an order of magnitude different from the spontaneous fluctuations shown by Thornton and Taylor (1935) to occur in soil kept under constant conditions of moisture and temperature.* The plate counts follow the same general rule; a marked increase in bacteria, most rapidly at 37° C., is seen in the sand soils No. 1–3 and the loam soil No. 5. The actinomycetes, which never account for any very large proportion of the plate counts, do not seem to be affected by either the temperature or the time. Their mycelia were hardly ever seen in the drop-films for direct counting and were mostly not very conspicuous, although present, on the Rossi-Cholodny slides. Upon the whole, one does not get the impression that this group of organisms is of considerable importance in soil to which no decomposable material has recently been added (cf. Winogradsky, 1925). The development of fungal mycelium is strongest at 15° C. (in the sand soils equally so at 28° C.), and stronger in the sand soils than in the loams, particularly the red loams, which have also by plate counting been found very poor in fungi (Jensen, 1934). At 37° C. the growth of fungi is rather insignificant, except perhaps in the first 3 sand soils.

As on the first day, the yields of carbon dioxide increase markedly with the temperature, especially in the interval from 15° C. to 28° C., where they are approximately doubled. At each temperature, but particularly at 28 and 37° C., there is a significant correlation (Fisher, 1930) between direct counts and yields of carbon dioxide, as shown in Table 3 and Text-figure 1. On the other hand, the figures for density of mycelium show no correlation whatever with the CO_2-yields. Even when fungi are present in notable quantities, as in No. 2, they seem rather inactive, confirming the view of Winogradsky (1925), that these organisms do not partake in the breakdown of soil "humus". If we assume that the whole production of carbon dioxide is the work of the bacteria found by

* In soils incubated for longer periods (up to 3 months) a marked drop in bacterial numbers, both by direct and by plate counts, was observed at 25 to 37° C., but not at 4 to 15° C.

TABLE 2.

Carbon dioxide formation and composition of microflora in soils without addition of organic matter.

Soil No.	Time. (Day.)	Temperature. (° C.)	Direct Count of Micro-organisms.	Plate Count.		Mycelium. %.	CO_2.	Efficiency.
				Bact.	Act.			
Group I.—Sand Soils.								
1	4th	15°	230	2	0·5	4·9	0·7	0·030
		28°	230	5	0·5	6·8	1·7	0·074
		37°	200	6	0·4	2·9	2·6	0·130
	10th	15°	290	4	0·4	9·8	0·5	0·017
		28°	290	7	0·4	3·7	1·3	0·045
		37°	240	3	0·4	4·6	2·3	0·096
2	4th	15°	680	4	0·1	20·9	2·6	0·040
		37°	630	19	(0)	4·4	7·0	0·111
	10th	15°	610	21	(0)	5·5	4·0	0·066
		37°	560	15	(0)	3·2	7·2	0·129
3	4th	15°	530	4	0·1	3·9	3·1	0·058
		28°	620	5	0·1	8·5	6·3	0·102
		37°	560	19	(0)	3·4	12·3	0·220
	10th	15°	710	21	(0)	4·7	3·6	0·051
		28°	680	8	0·1	5·8	4·7	0·070
		37°	550	15	(0)	0·6	8·7	0·158
4	4th	15°	1,400	83	5	2·9	5·5	0·039
		28°	1,360	70	6	8·0	14·3	0·107
		37°	1,170	59	6	0·3	15·8	0·136
	10th	15°	1,380	71	4	4·4	3·9	0·028
		28°	850	50	6	5·5	8·1	0·099
		37°	910	37	5	0·8	8·9	0·098
Group II.—Red to Brown Loam Soils.								
5	4th	15°	1,140	21	5	3·5	6·0	0·053
		28°	1,630	35	6	0·6	12·5	0·077
		37°	1,170	83	6	0·2	13·8	0·118
	10th	15°	1,740	56	6	0·8	7·8	0·045
		28°	1,590	75	6	(0)	12·1	0·076
		37°	1,600	88	6	(0)	11·1	0·069
6	4th	15°	1,560	25	5	0·8	5·9	0·038
		28°	1,370	31	6	(0)	10·4	0·076
		37°	1,320	22	7	0·3	15·1	0·114
	10th	15°	1,570	16	4	1·5	5·3	0·034
		28°	1,310	22	5	0·6	11·8	0·090
		37°	1,480	14	6	(0)	10·7	0·072
7	4th	15°	1,810	232	3	2·5	4·7	0·026
		28°	1,800	212	4	1·6	10·3	0·057
		37°	1,560	173	3	(0)	20·0	0·128
	10th	15°	1,790	184	5	10·3	4·4	0·025
		28°	1,910	197	4	0·6	9·3	0·049
		37°	1,810	127	4	(0)	17·4	0·096

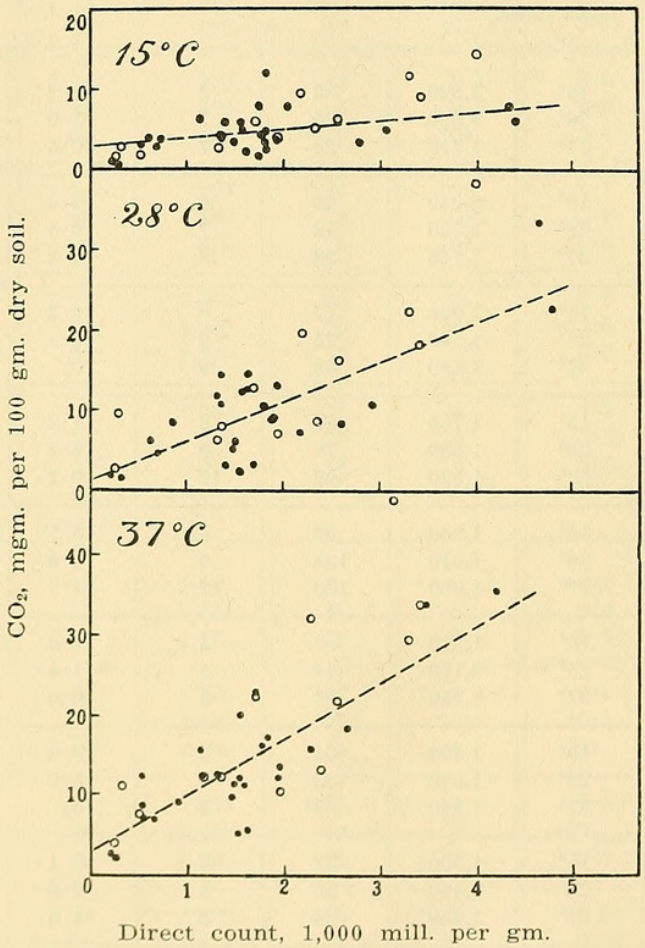
TABLE 2.—*Continued.*

Carbon dioxide formation and composition of microflora in soils without addition of organic matter.—Continued.

Soil No.	Time. (Day.)	Temperature. (° C.)	Direct Count of Micro-organisms.	Plate Count.		Mycelium. %.	CO_2.	Efficiency.
				Bact.	Act.			
Group II.—Red to Brown Loam Soils.—Continued.								
8	4th	15°	3,050	98	4	0·2	4·9	0·016
		28°	2,940	107	4	(0)	10·5	0·036
		37°	2,640	72	4	0·3	18·4	0·070
	10th	15°	2,770	108	4	(0)	3·2	0·012
		28°	2,580	91	3	0·9	8·1	0·031
		37°	2,290	65	5	(0)	15·8	0·069
Group III.—Grey to Black Loam Soils.								
9	4th	15°	1,820	30	4	5·8	12·2	0·067
		28°	1,620	25	7	1·9	14·5	0·090
		37°	1,710	28	7	0·5	22·7	0·133
	10th	15°	2,040	44	6	3·3	9·9	0·048
		28°	1,930	42	7	0·5	13·0	0·067
		37°	1,740	98	8	0·3	16·9	0·097
10	4th	15°	1,620	77	8	0·3	2·2	0·014
		28°	1,380	75	9	2·8	3·1	0·022
		37°	1,620	68	9	(0)	5·5	0·034
	10th	15°	1,750	87	9	1·2	1·6	0·009
		28°	1,550	78	9	4·2	2·4	0·015
		37°	1,530	96	12	0·7	5·2	0·034
11	4th	15°	1,930	98	5	3·1	3·7	0·019
		28°	1,910	105	4	0·6	9·1	0·048
		37°	1,960	100	7	1·3	13·6	0·069
	10th	15°	1,810	86	5	1·0	3·2	0·018
		28°	2,170	84	5	1·4	7·1	0·033
		37°	1,950	85	5	0·6	12·1	0·062
12	4th	15°	1,490	34	3	3·6	3·3	0·022
		28°	1,500	35	3	1·1	5·9	0·039
		37°	1,540	33	2	(0)	12·0	0·078
	10th	15°	1,500	37	3	2·4	2·4	0·016
		28°	1,490	36	3	2·6	5·1	0·034
		37°	1,430	34	3	1·0	9·8	0·069
13	4th	15°	4,310	285	14	11·9	7·9	0·018
		28°	4,660	272	15	4·5	33·5	0·072
		37°	4,190	165	16	1·1	35·4	0·084
	10th	15°	4,380	275	18	7·3	6·2	0·014
		28°	4,770	250	21	2·5	22·5	0·047
		37°	3,470	191	19	1·4	33·9	0·098

TABLE 3.
Summary of results in Table 2.

	15° C.	28° C.	37° C.
Number of pairs of observations	26	24	26
Mean and standard deviation of direct counts..	1,690±1,030	1,760±1,114	1,530±916
Mean and standard deviation of CO_2 production	4·56±2·699	9·8±6·96	13·6±8·08
Correlation coefficient between direct count and CO_2	0·429	0·775	0·809
Regression coefficient ($\times 10^2$) of CO_2 on direct count	0·112	0·484	0·714
Mean and standard deviation of efficiency of microorganisms in :			
Group I	0·041±0·0164	0·083±0·0244	0·135±0·0403
Groups II+III	0·027±0·0165	0·053±0·0232	0·083±0·0280
Total	0·032±0·0173	0·061±0·0264	0·099±0·0400



Text-figure 1.—Black dots: correlation between direct counts of bacteria on 4th and 10th day and yields of carbon dioxide in previous 24 hours. Regression lines corresponding to the equations:

15° C.: $y = 4{\cdot}56 + 0{\cdot}00112\ (x - 1690)$.
28° C.: $y = 9{\cdot}8 + 0{\cdot}00484\ (x - 1760)$.
37° C.: $y = 13{\cdot}6 + 0{\cdot}00714\ (x - 1530)$.

Circles: correlation between initial direct counts and yields of CO_2 in the first 24 hours.

direct counting, we may calculate the "efficiency" of the bacteria at the different levels of temperature as mgm. of carbon dioxide produced by 1,000 millions of bacteria in 24 hours. This "efficiency", shown in the last column of Table 2, varies from 0·009 to 0·067 at 15° C., from 0·015 to 0·107 at 28° C., and from 0·034 to 0·220 at 37° C. These figures, the means of which are given in Table 3, are of an order comparable with that of corresponding values found by Cutler and Crump (1929) and de Telegdy-Kovats (1932) in work with pure and mixed cultures of soil bacteria. The maximal efficiency observed here—0·220—corresponds to a daily CO_2-production about equal to the dry weight of the organisms concerned, since the dry matter of 1,000 mill. bacteria can approximately be estimated at 0·2 mgm. Table 3 also shows that the efficiency of the bacteria appears to be higher in the sand soils than in the loams, and the *t* test of Fisher (1930, p. 107) shows this difference to be significant at 28 and 37° C. This is probably due to the fact that the sand soils have lower bacterial numbers than the rest, and the efficiencies of bacterial populations will as a rule decrease with increasing density of the population (Cutler and Crump, 1929; de Telegdy-Kovats, 1932; Meiklejohn, 1932). The total plate counts (bacteria plus actinomycetes) do not show any correlation with the yields of carbon dioxide at 15° C.; although positive correlations exist at the higher temperatures, it does not seem that this fraction of the total microflora is the most important in biochemical respect. If the plate-counted organisms were alone responsible for all the carbon dioxide produced, their efficiency would at 15° C. vary from 0·17 (No. 10, 10 days) to 7·2 (No. 3, 4 days), at 28° C. from 0·27 to 12·1 (same soils), and at 37° C. from 0·48 (No. 10, 10 days) to 7·7 (No. 1, 10 days). These figures are not only rather erratic and without any clear relation to the temperature, but they also appear unreasonably high; for instance, an efficiency of 12·1 corresponds to a CO_2-production approximately 60 times the dry weight of the organisms, which exceeds the *maximal* rate of carbon dioxide production in a very young and actively multiplying culture of *Bact. coli* in peptone-water at 37° C., according to Mooney and Winslow (1935).* In the present case we are dealing with populations in comparative equilibrium and consisting of cells of all ages, where such enormous efficiencies would appear inconceivable.

The general results of this series of experiments may be summarized thus:

The rate of destruction of the soil's own organic matter ("humus") increases rapidly with increases in the temperature from 15° C. to 37° C. This is not due to any increase in the number of microorganisms, which are not significantly affected by the temperature, but to an increased metabolic activity of the organisms, among which the bacteria (and not merely the individuals capable of producing colonies on agar plates) are the most important. The total bacterial flora, as determined by microscopic counting, shows an average carbon dioxide production corresponding to approximately 16, 30 and 50 per cent. of the dry weight of the organisms in 24 hours at 15° C., 28° C., and 37° C. respectively (according to the total mean efficiencies in Table 3, and assuming that 1,000 mill. bacteria contain approximately 0·2 mgm. dry matter).

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* Mooney and Winslow estimated the maximal rate of CO_2-production by *Bact. coli* in peptone-water (after 1-2 hours at 37°) at 330 grams per hour per kilogram of bacterial substance; if it be assumed that the bacterial bodies contain 20 per cent. dry matter, this would correspond to a CO_2-production about 40 times the weight of dry matter in 24 hours.

Part II.—Decomposition of Organic Materials added to the Soil.

In these experiments, three kinds of organic materials were used: oats straw, as an example of a natural organic material poor in nitrogen; hay (mixture of young leaves and stems of grasses and white clover, from a grass lawn), as an example of a similar material richer in nitrogen; and fungal mycelium, as an example of a microbial substance synthesized and re-decomposed in the soil. The mycelium was produced by growing a common green soil *Penicillium* on a modified Czapek's solution, containing 5% saccharose, 0·5% $NaNO_3$, and 0·2% KH_2PO_4. After 6–8 days' growth at 28° C. the mats of mycelium were removed, washed several times with distilled water, dried and ground. The materials had the following elementary composition:

	In per cent. of air-dry material		
	Carbon*	Nitrogen	Ratio C/N
Straw	42·15	0·20	211 : 1
Hay	35·35	2·73	13·0 : 1
Penicillium-mycelium	51·8	3·52	14·7 : 1

The materials were used in a finely ground, air-dry condition, and were added to the following soils in quantities of 1 per cent., on the basis of air-dry soil:

1.—A "synthetic soil" made up from 80% pure sand, 18·5% pure kaolin, 1% calcium carbonate, and 0·5% ferric oxide. Small quantities of a suspension of garden soil were added, as an inoculum, to the water with which the soil was moistened. 2.—Garden soil, rich in lime and humus (same as No. 11 in the previous set of experiments), mixed with equal parts of sand. 3.—Acid sand soil (No. 2 in the previous experiments). 4.—Red loam (No. 5 in the previous experiments).

The following sets of experiments were run:

I. "Synthetic soil" + 1·0% straw + 0·1% $NaNO_3$ + 0·025% K_2HPO_4. 12·0% H_2O. Temp.: 6–8° C.; 18–21° C.; 28° C.; 37° C. Duration of experiment: 30 days.

II. Sand-mixed garden soil + 1·0% straw + 0·1% $NaNO_3$. 16·7% H_2O. Temp.: 14–16° C.; 28° C.; 37° C. Duration of experiment: 29 days.

III. Acid sand soil + 1·0% straw + 0·1% $(NH_4)_2SO_4$ + 0·03% KH_2PO_4. 9·5% H_2O. Temp.: 14–16° C.; 28° C. Duration of experiment: 19 days.

IV. "Synthetic soil" + 1·0% hay. 11·0% H_2O. Temp.: 5–6° C.; 14–16° C.; 28° C.; 37° C. Duration of experiment: 28 days.

V. Sand-mixed garden soil + 1·0% hay. 14·8% H_2O. Temp.: 14–16° C.; 28° C.; 37° C. Duration of experiment: 29 days.

VI. Acid sand soil + 1·0% hay. (*a*) 5·3%, and (*b*) 10·4% H_2O. Temp.: 19–21° C. Duration of experiment: 10 days.

VII. Red loam + 1·0% hay. 16·7% H_2O. Temp.: 14–16° C.; 37° C. Duration of experiment: 17 days.

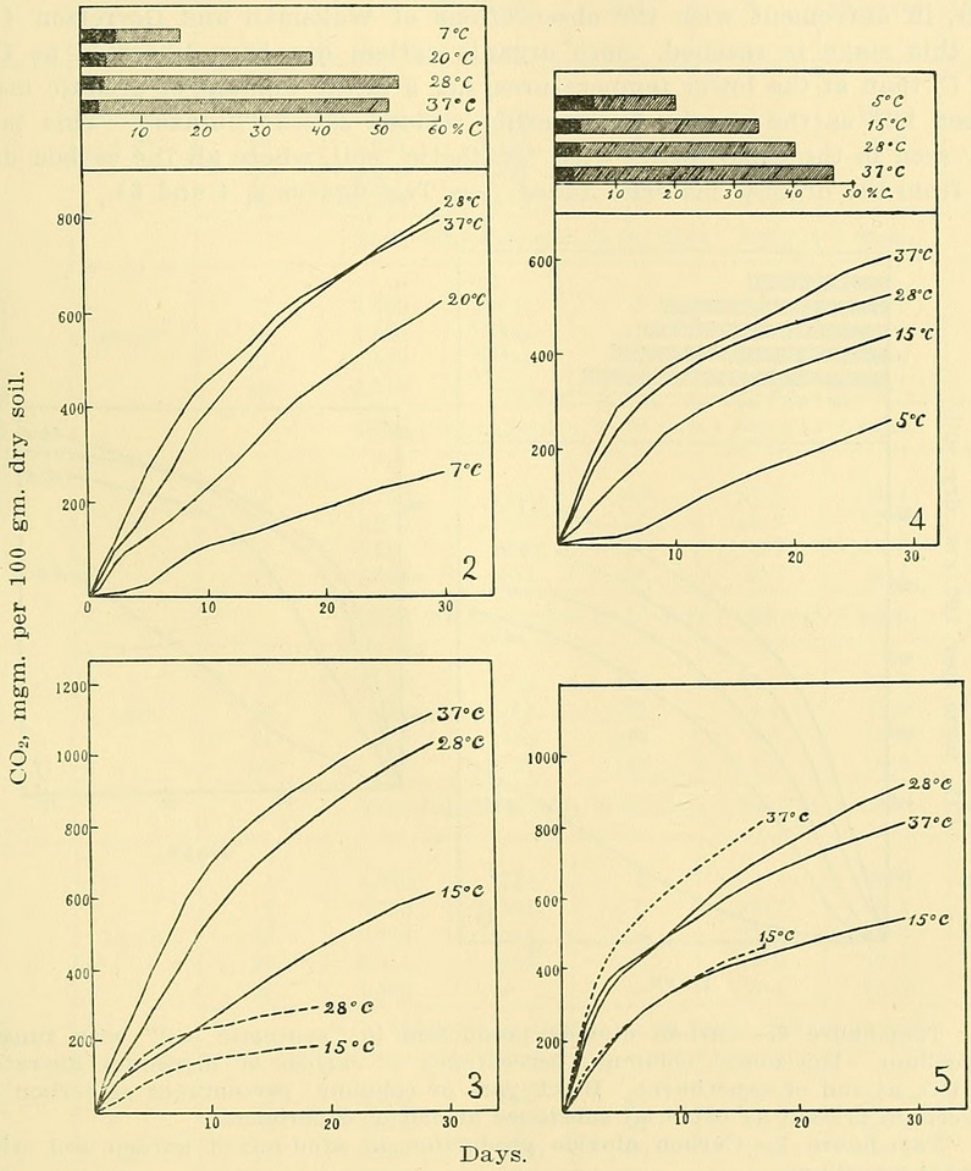
VIII. "Synthetic soil" + 1·0% fungal mycelium. 11·0% H_2O. Temp.: 4–5° C.; 13–15° C.; 18–21° C.; 28° C.; 37° C. Duration of experiment: 28–40 days.

IX. Sand-mixed garden soil + 1·0% fungal mycelium. 14·4% H_2O. Temp.: 5–6° C.; 14–16° C.; 28° C.; 37° C. Duration of experiment: 28 days.

All experiments were run in triplicate, except Nos. III and VI (duplicate).

* Carbon determinations carried out by Miss M. Cogle, B.Sc., Department of Medicine (Medical Organic Chemistry), Sydney University.

The course of carbon dioxide evolution in the various experiments is shown in Text-figures 2–7. The curves are generally of the same type as in the experiments of Starkey (1924), Petersen (1926) and Waksman and Gerretsen (1931); at the lower temperatures they are mostly of the S-shaped type suggestive of an autocatalytic reaction (accumulation of respiring organisms). The stimulating influence of increased temperature is most pronounced in the interval from about



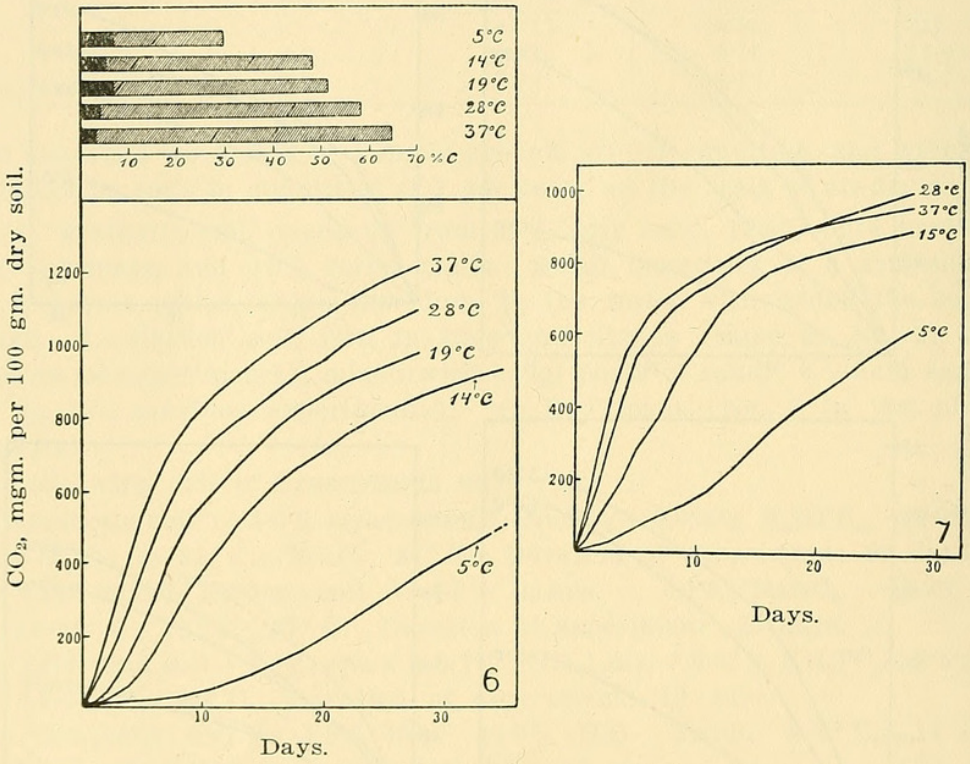
Text-figure 2.—Carbon dioxide production in "synthetic soil" with straw. Horizontal columns: percentage of carbon of added straw liberated as CO_2 at end of experiment. Black part of columns: percentage of carbon of straw present as bacterial substance at end of experiment.

Text-figure 3.—Carbon dioxide production in sand-mixed garden soil with straw (continuous lines), and acid sand soil with straw (broken lines).

Text-figure 4.—Carbon dioxide production in "synthetic soil" with hay. Horizontal columns: percentages of carbon of hay liberated as CO_2 at end of experiment. Black part of columns: percentages of carbon of hay present as bacterial substance at end of experiment.

Text-figure 5.—Carbon dioxide production in sand mixed garden soil with hay (continuous lines), and red loam with hay (broken lines).

5° to about 15° C. (cf. Petersen, 1926, and Waksman and Gerretsen, 1931), whereas the increase from 28° to 37° C. has mostly little influence except in the early stages of the process. After 2–3 weeks the rate of CO_2-production is slowing down, rather suddenly at the higher and more gradually at the lower temperatures, and after 4 weeks the curves tend to run parallel (a marked exception to this rule is only seen in Experiment No. IX), i.e., the stimulating influence of temperature on the decomposition process is most pronounced in the early stages of the process, in agreement with the observations of Waksman and Gerretsen (1931). When this stage is reached, more organic carbon has been liberated as CO_2 at 28–37° C. than at the lower temperatures, i.e., a larger amount of organic material has been left as the slowly decomposing residue called "humus". This is most clearly seen in the experiments with "synthetic" soil, where all the carbon dioxide comes from the organic material added (see Text-figures 2, 4 and 6).



Text-figure 6.—Carbon dioxide production in "synthetic soil" with fungal mycelium. Horizontal columns: percentages of carbon of mycelium liberated as CO_2 at end of experiment. Black part of columns: percentages of carbon of mycelium present as bacterial substance at end of experiment.

Text-figure 7.—Carbon dioxide production in sand-mixed garden soil with fungal mycelium.

The results of the periodical microbiological analyses, together with the corresponding yields of carbon dioxide in the previous 24 hours, are found in Table 4, which also shows the total yields of carbon dioxide, with standard deviations.

The bacteria (as estimated by direct counting, therefore including spores and mycelial fragments of actinomycetes) are seen to multiply in all cases except in the acid soil in Exp. No. 3 (pH 5·4 at the start of the experiment), where the numbers are very low and hardly change significantly. At 4–7° C. the multiplication is very slow, but eventually the numbers exceed those at the higher temperatures

TABLE 4.

Composition of microflora and production of carbon dioxide in soils with additions of organic matter.

Experiment No.	Temperature. (° C.)	Time. (days).	Direct Count.	Plate Count.		Density of Mycelium. %	Production of Carbon Dioxide.	
				Bact.	Act.		Observed.	Calculated.
I. "Synthetic soil"+straw.	6–7°	7	1,740	713	3	0·7	20·6	—
		14	2,010	1,496	3	1·2	6·9	—
		21	2,270	1,994	(0)	0·3	7·8	—
		30	2,510	1,823	(0)	0·8	6·3	—
		Total CO_2-production in 30 days: 262±14·2 mgm.						
	18–21°	7	1,880	1,341	7	1·8	17·7	—
		14	1,830	994	17	21·0	25·9	—
		21	1,530	1,034	34	14·1	18·9	—
		30	1,710	852	28	3·9	17·7	—
		Total CO_2-production in 30 days: 625±15·6 mgm.						
	28°	7	1,460	642	40	21·5	35·4	33·4
		14	1,270	591	63	12·3	27·6	21·3
		21	1,100	591	97	6·7	18·0	13·6
		30	1,640	605	63	2·4	18·1	12·9
		Total CO_2-production in 30 days: 823±48·3 mgm.						
	37°	7	1,140	619	139	38·2	38·7	63·1
		14	1,040	552	242	4·3	21·2	7·5
		21	1,310	492	193	4·5	13·8	13·9
		30	1,210	520	128	1·0	14·0	6·3
		Total CO_2-production in 30 days: 796±55·3 mgm.						
II. Sand-mixed garden soil+straw.	14–16°	4	1,750	754	5	17·8	23·5	15·0
		9	2,970	591	5	10·7	26·1	20·6
		15	2,310	544	4	27·8	22·7	23·3
		21	2,010	456	2	19·0	18·2	17·5
		29	1,650	—	—	8·4	12·3	10·4
		Total CO_2-production in 29 days: 617±8·7 mgm.						
	28°	4	2,780	951	41	25·3	57·6	48·4
		9	3,010	706	22	8·7	58·4	31·6
		15	2,800	688	20	7·8	30·2	28·3
		21	2,720	525	17	1·0	24·8	22·2
		29	2,640	432	22	0·3	18·7	18·4
		Total CO_2-production in 29 days: 1,041±3·0 mgm.						
	37°	4	2,060	564	66	17·4	75·6	52·2
		9	2,550	549	44	2·8	55·3	39·0
		15	2,390	343	31	1·7	26·8	33·5
		21	2,190	275	46	0·5	17·4	26·2
		29	1,760	180	23	0·2	15·8	13·3
		Total CO_2-production in 29 days: 1,125±13·1 mgm.						

TABLE 4.—*Continued.*

Composition of microflora and production of carbon dioxide in soils with additions of organic matter.

Experiment No.	Temperature. (° C.)	Time. (days).	Direct Count.	Plate Count.		Density of Mycelium. %	Production of Carbon Dioxide.	
				Bact.	Act.		Observed.	Calculated.
III. Acid sand soil + straw.	14–16°	6	520	—*	—	43·1	15·7	17·0
		12	550	—	—	19·4	4·6	7·3
		19	540	—	—	17·9	3·4	6·5
		Total CO_2-production in 19 days: 186±0·7 mgm.						
	28°	6	550	—	—	25·8	20·9	31·1
		12	730	—	—	7·1	6·2	11·0
		19	600	—	—	4·8	5·2	7·4
		Total CO_2-production in 19 days: 294±15·6 mgm.						
IV. "Synthetic soil" + hay.	5–6°	7	650	60	(0)	0·9	7·6	—
		12	2,620	747	(0)	7·0	14·2	—
		17	2,660	888	(0)	20·0	10·7	—
		22	1,590	547	3	21·0	9·2	—
		28	2,260	888	(0)	36·8	8·9	—
		Total CO_2-production in 28 days: 262±18·2 mgm.						
	14–16°	3	2,530	840	(0)	1·8	39·7	13·9
		7	2,430	1,708	(0)	16·1	24·3	20·4
		12	2,700	1,434	3	15·8	16·3	20·9
		17	2,010	712	9	16·6	11·0	16·5
		22	1,310	428	8	3·9	8·8	6·2
		28	1,450	275	4	4·4	8·0	7·0
		Total CO_2-production in 28 days: 444±22·3 mgm.						
	28°	3	2,790	858	1	17·3	57·0	37·9
		7	1,370	376	25	12·3	31·4	22·1
		12	1,180	638	28	2·6	16·2	9·4
		17	1,060	448	31	0·5	9·8	6·0
		22	1,220	410	28	0·2	7·8	7·0
		28	1,410	396	25	0·2	6·7	8·5
		Total CO_2-production in 28 days: 522±3·6 mgm.						
	37°	3	2,490	582	58	21·3	60·8	59·3
		7	1,360	456	94	2·8	21·2	8·6
		12	1,160	327	102	0·0	13·3	3·6
		17	860	198	102	0·0	10·8	(0)
		22	1,260	269	105	0·0	15·8	5·8
		28	1,070	205	90	0·2	7·7	1·8
		Total CO_2-production in 28 days: 608±10·1 mgm.						

* Plate counting was impossible in this experiment because of abundant growth of fungi on the agar plates.

TABLE 4.—*Continued.*

Composition of microflora and production of carbon dioxide in soils with additions of organic matter.

Experiment No.	Temperature. (° C.)	Time.	Direct Count.	Plate Count.		Density of Mycelium. %	Production of Carbon Dioxide.	
				Bact.	Act.		Observed.	Calculated.
V. Sand-mixed garden soil+hay.	14–16°	3	3,800	1,124	1	39·8	42·0	38·9
		7	2,590	932	38	34·9	30·2	28·2
		11	3,850	732	29	15·0	16·2	28·8
		16	3,190	613	20	21·5	10·9	26·8
		22	2,730	531	35	10·5	8·8	18·2
		29	2,210	435	29	2·4	6·8	11·9
		Total CO_2-production in 29 days: 536±6·0 mgm.						
	28°	3	3,420	850	53	43·5	84·1	74·4
		7	2,000	702	47	16·8	26·3	32·4
		11	3,740	631	59	25·6	30·5	56·3
		16	3,120	503	58	14·9	22·6	39·2
		22	2,230	533	45	20·7	17·8	38·6
		29	2,230	479	56	0·5	14·5	15·4
		Total CO_2-production in 29 days: 912±49·6 mgm.						
	37°	3	3,190	766	115	8·1	80·6	61·3
		7	1,730	556	171	1·8	22·4	19·0
		11	3,160	472	177	1·9	20·4	50·9
		16	2,550	547	185	2·2	21·4	37·9
		22	1,780	383	202	0·7	11·3	18·4
		29	1,990	298	164	0·4	10·2	22·5
		Total CO_2-production in 29 days: 803±17·0 mgm.						
VI. Acid sand+hay.	*a* 19–21°	4	2,420	527	2	58·8	44·2	—
		10	1,720	478	58	26·7	15·9	—
	b	4	3,030	558	(0)	32·5	40·3	—
		10	1,630	338	2	18·1	20·7	—
		Total CO_2-production in 10 days: *a*: 308 mgm.; *b*: 320 mgm.						
VII. Red loam+hay	14–16°	3	4,220	455	18	32·2	48·4	38·6
		7	2,730	337	6	43·3	25·5	32·7
		11	2,920	345	8	8·0	18·3	19·3
		17	2,530	352	11	22·6	12·2	22·6
		Total CO_2-production in 17 days: 460±6·7 mgm.						
	37°	3	3,260	377	36	27·4	112·1	92·9
		7	2,520	217	48	9·3	38·2	48·2
		11	2,300	208	47	4·4	25·8	35·8
		17	2,070	181	38	3·8	25·7	29·7
		Total CO_2-production in 17 days: 806±50·7 mgm.						

TABLE 4.—*Continued.*

Composition of microflora and production of carbon dioxide in soils with additions of organic matter.

Experiment No.	Temperature. (° C.)	Time.	Direct Count.	Plate Count.		Density of Mycelium. %	Production of Carbon Dioxide.	
				Bact.	Act.		Observed.	Calculated.
VIII. "Synthetic soil"+fungal mycelium.	4–5°	8	510	89	1	2·0	3·8	—
		14	1,270	618	1	27·6	10·7	—
		20	1,850	1,455	1	15·6	17·5	—
		28	4,610	2,865	(0)	33·2	23·4	—
		40	3,540	3,781	3	27·2	12·8	—
		Total CO_2-production in 40 days: 561±34·2 mgm.						
	13–15°	4	1,070	301	1	13·3	22·3	5·4
		8	2,580	899	(0)	54·2	55·6	36·5
		14	3,290	2,410	14	52·9	39·4	40·7
		20	3,710	1,951	29	37·6	22·1	37·3
		28	2,170	957	23	10·2	15·0	15·3
		35	2,600	739	21	8·9	9·8	16·3
		Total CO_2-production in 35 days: 915±23·5 mgm.						
	18–21°	4	2,170	1,070	3	29·6	61·5	—
		8	2,070	1,730	14	67·0	67·1	—
		14	3,240	2,697	118	34·0	35·4	—
		20	2,000	2,354	124	24·6	18·0	—
		28	3,440	1,933	124	18·0	14·2	—
		Total CO_2-production in 28 days: 978±48·9 mgm.						
	28°	4	2,890	787	62	52·0	79·8	79·5
		8	2,520	2,052	199	27·8	61·5	49·1
		14	2,160	2,132	236	8·0	25·9	23·5
		20	1,590	1,815	152	8·0	24·3	19·0
		28	2,270	1,101	258	2·9	14·7	17·7
		Total CO_2-production in 28 days: 1,097±31·6 mgm.						
	37°	4	3,090	292	292	42·5	104·3	113·7
		8	2,600	2,011	275	15·8	59·0	60·2
		14	2,190	1,281	340	3·0	30·7	30·1
		20	1,520	730	343	1·5	20·4	13·7
		28	1,690	472	157	0·3	15·4	15·8
		Total CO_2-production in 28 days: 1,218±41·8 mgm.						
IX. Sand-mixed garden soil+fungal mycelium-	5–6°	3	1,320	52	0·3	0·3	11·4	—
		7	3,420	1,426	(0)	3·7	15·6	—
		11	4,680	1,811	(0)	13·9	20·3	—
		16	7,830	2,024	(0)	55·9	35·8	—
		22	6,050	2,446	14	76·6	25·2	—
		28	5,730	2,658	3	49·8	21·5	—
		Total CO_2-production in 28 days: 623±34·8 mgm.						

TABLE 4.—*Continued.*

Composition of microflora and production of carbon dioxide in soils with additions of organic matter.

Experiment No	Temperature. (° C.)	Time.	Direct Count.	Plate Count.		Density of Mycelium. %.	Production of Carbon Dioxide.	
				Bact.	Act.		Observed.	Calculated
IX. Sand-mixed garden soil+fungal mycelium—*Continued.*	14–16°	3	4,210	1,070	2	19·5	55·4	33·3
		7	3,770	1,180	51	64·0	51·2	48·8
		11	3,780	575	131	42·2	47·8	39·7
		16	3,160	493	141	26·3	21·5	28·6
		22	2,470	497	143	18·7	9·6	19·7
		28	2,160	—	—	13·2	8·0	16·2
		Total CO_2-production in 28 days: 890±15·4 mgm.						
	28°	3	4,340	947	199	41·8	128·0	79·4
		7	3,880	1,332	145	41·0	46·0	75·1
		11	4,070	1,199	88	13·5	21·5	45·1
		16	2,570	632	94	8·5	19·7	27·3
		22	2,050	599	96	13·9	14·4	29·3
		28	2,260	547	98	2·7	11·7	13·4
		Total CO_2-production in 28 days: 998±8·5 mgm.						
	37°	3	3,990	500	201	21·3	149·1	99·6
		7	2,250	400	359	13·3	36·4	48·5
		11	2,560	278	322	3·0	21·7	39·3
		16	1,830	224	308	0·8	12·0	19·8
		22	1,860	189	355	0·2	7·8	19·5
		28	1,920	187	356	0·7	6·4	21·5
		Total CO_2-production in 28 days: 959±9·2 mgm.						

Mean and standard deviation of direct counts at 4–7° C.	2,960± 2,039
,, ,, ,, ,, ,, ,, ,, ,, 13–16° C.	2,510±992·2
,, ,, ,, ,, ,, ,, ,, ,, 28° C.	2,220± 1,001
,, ,, ,, ,, ,, ,, ,, ,, 37° C.	2,050±730·9
Mean and standard deviation of density of mycelium at 4–7° C.	19·7±21·77
,, ,, ,, ,, ,, ,, ,, ,, ,, 13–16° C.	22·6±15·39
,, ,, ,, ,, ,, ,, ,, ,, ,, 28° C.	14·3±13·83
,, ,, ,, ,, ,, ,, ,, ,, ,, 37° C.	7·1±10·81
Mean and standard deviation of CO_2-production at 4–7° C.	14·5± 7·95
,, ,, ,, ,, ,, ,, ,, ,, 13–16° C.	22·5±14·77
,, ,, ,, ,, ,, ,, ,, ,, 28° C.	31·2±26·12
,, ,, ,, ,, ,, ,, ,, ,, 37° C.	34·4±33·01

in the same experiment; this is especially the case in the experiments with mycelium. As the temperature increases, the multiplication becomes more rapid, but the numbers do not reach a higher level. The figures at 15° and 28° C. are not, on the whole, very different, and the same applies to the few results from 18–21° C. At 37° C. the numbers are, in the later stages of all the experiments, definitely less than those at the lower temperatures. What has been said of the direct counts generally applies to the plate counts of bacteria as well; here the

reduction in numbers at the higher temperatures is very pronounced. This general rule: with decreasing temperature a slower multiplication, but eventually higher maximal numbers than at temperatures favouring rapid multiplication, agrees perfectly with observations on pure cultures by Gotschlich and Weigang (1895), Graham-Smith (1920) and Hess (1934). Vanderleck (1918) has shown in an interesting (but apparently often overlooked) contribution, that surprising numbers of bacteria may accumulate during a mild frost in soil containing undecomposed plant residues. The explanation for these phenomena would seem to be that the low temperature delays the death-rate of the organisms (and possibly also the rate of disintegration of the dead cells) more than the rate of reproduction (Loeb, 1908; Cohen, 1922).

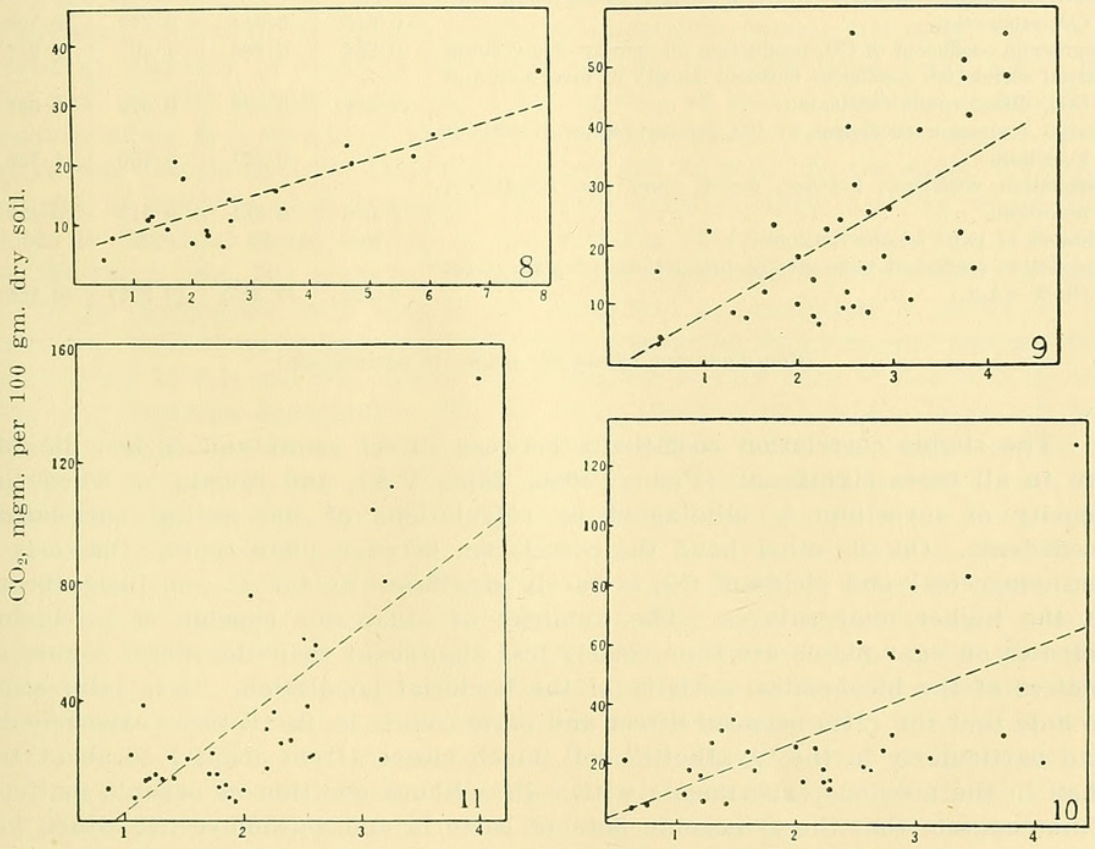
As to the kinds of bacteria observed, a large proportion of white and yellow colonies of corynebacteria (*Cor. helvolum* or related forms) was seen on the plates from experiments with hay and fungal mycelium at 5° to 15° C.; at the higher temperatures they were less conspicuous. Spore-forming bacilli, often in fine long chains, were frequently seen on the Rossi-Cholodny slides at 15° C. in the early stages of the same experiments at 15° C. They showed the presence of endospores after 3 days (cf. Winogradsky, 1925, and Ziemiecka, 1935); after this time, free spores were often seen in the films for direct counting. The slides and films from experiments with straw showed the presence of slender, curved filaments of the *Cytophaga*-group and small, curved, gram-negative rods which might be representatives of the "*Cellvibrio*"-group, but these organisms did not seem to make a large proportion of the total microflora.

The actinomycetes are shown by the plate counts to be almost absent at 4–7° C. and to be very numerous at 28° C. and 37° C., especially in the experiments with fungal mycelium. Their numbers nearly always reach their maximum after the peak period of CO_2-production, and remain high when the carbon dioxide production has become very slow. Microscopically this group of organisms showed some interesting features. On the Rossi-Cholodny slides their vegetative mycelia appeared in great abundance after 3 to 7 days at 28° and 37° C. in soils with hay and fungal mycelium, but at later periods little more was seen of them. The films for direct counting showed surprisingly few actinomyces-hyphae, even in cases where they were richly represented on the Rossi-Cholodny slides, but in such cases the films often contained many long, irregular rods which might be fragments of actinomyces-mycelia; it seems that the filaments are easily broken up by preparation of the soil suspension, and thus become included in the direct counts. These phenomena indicate that the actinomycetes are of most importance in the earlier stages of the decomposition process, where they accompany and succeed the vigorous development of fungal mycelium (cf. Ziemiecka, 1935), and that their high numbers in later stages are mainly derived from spores, which may be without biochemical significance.

The fungi, like the bacteria, developed very slowly at the lowest range of temperature, but in some cases (experiment No. IX) they gradually became very abundant at this temperature. At higher temperatures the mycelia appeared earlier on the slides. As a whole they seemed to develop most richly at 15° and 20° C., reaching a maximum after 3 to 11 days and then receding. At 28° C., and particularly at 37° C., they tend to disappear quite rapidly after a brief period of vigorous growth in the first 3–7 days. At the latter temperature their maximal density is with few exceptions considerably less than at the lower temperatures in the same experiment. The periods of strongest mycelial growth

generally coincide with the peak periods of carbon dioxide formation. This shows that the fungi are of chief importance in the early stages of intense decomposition of organic matter (cf. Waksman and Gerretsen, 1931, and Waksman, 1932), and that their period of activity is markedly shortened with increasing temperature.

The mere inspection of the figures gives only an imperfect idea of the relationships between yields of carbon dioxide, bacterial numbers, and densities of mycelium; a better picture may be obtained by calculation of the correlations between these three sets of values, as shown in Table 5. Text-figures 8–11 show definite linear correlations between the direct counts of bacteria and the yields of CO_2 at the different temperatures,* and similar types of scatter-diagrams are shown by the densities of mycelium and the yields of CO_2.



Text-figure 8.—Correlation between direct counts of bacteria and yields of carbon dioxide at 4-7° C. Regression line corresponding to the equation: $y = 14{\cdot}5 + 0{\cdot}003091\ (x - 2960)$.

Text-figure 9.—Correlation between direct counts of bacteria and yields of carbon dioxide at 13-16° C. Regression line corresponding to the equation: $y = 22{\cdot}5 + 0{\cdot}00941\ (x - 2510)$.

Text-figure 10.—Correlation between direct counts of bacteria and yields of carbon dioxide at 28° C. Regression line corresponding to the equation: $y = 31{\cdot}2 + 0{\cdot}0162\ (x - 2220)$.

Text-figure 11.—Correlation between direct counts of bacteria and yields of carbon dioxide at 37° C. Regression line corresponding to the equation: $y = 34{\cdot}4 + 0{\cdot}03088\ (x - 2050)$.

* The few determinations at 18-21° C. were insufficient to show any significant correlations; these values have been omitted from the calculations.

TABLE 5.

Correlations between CO_2 production, numbers of microorganisms, and density of fungal mycelium.

	4–7° C.	14–16° C.	28° C.	37° C.
Total correlation coefficient between direct count and CO_2-production	0·774	0·636	0·621	0·716
Regression coefficient of CO_2-production on direct count, $\times 10^2$	0·3091	0·941	1·620	3·088
Partial correlation coefficient between direct count and CO_2, density of mycelium eliminated	0·600	0·565	0·413	0·643
Partial regression coefficient of CO_2-production on direct count, $\times 10^2$	—	0·709	0·797	2·22
Total correlation coefficient between density of mycelium and CO_2-production	0·640	0·614	0·773	0·726
Regression coefficient of CO_2-production on density of mycelium	0·234	0·586	1·46	2·217
Partial correlation coefficient between density of mycelium and CO_2, direct count eliminated	(0·238)	0·536	0·675	0·657
Partial regression coefficient of CO_2-production on density of mycelium	—	0·421	1·150	1·158
Correlation coefficient between direct count and density of mycelium	0·685	0·361	0·513	0·437
Number of pairs of observations	20	36	35	36
Correlation coefficient between CO_2-production and plate count (Bact.+Act.)	0·452	(0·325)	(0·273)	(0·180)

(Non-significant values are placed in parentheses.)

The simple correlation coefficients between direct count and carbon dioxide are in all cases significant (Fisher, 1930, Table V.A), and remain so when the density of mycelium is eliminated by calculations of the partial correlation coefficients. On the other hand the correlation between plate counts (bacteria + actinomycetes) and yields of CO_2 is barely significant at 4–7° C. and insignificant at the higher temperatures. The numbers of organisms capable of producing colonies on agar plates are thus clearly less significant than the direct counts as indices of the biochemical activity of the bacterial population. It is interesting to note that the ratio between direct and plate counts is, in all these experiments, and particularly in the "synthetic" soil, much closer (from about 1 to about 10) than in the previous experiments with soils without addition of organic matter.† This suggests that the zymogenic flora of bacteria and actinomycetes, which has arisen in these experiments, consists mainly of species capable of growth on agar plates, but that the fraction of viable individuals is not necessarily the most important in biochemical respect. This is in perfect agreement with the results obtained in the previous series of experiments.

Like the direct counts, the figures for density of mycelium show significant correlations with the CO_2-yields, and at all temperatures except 4–7° C. this correlation continues to exist when calculated as a partial correlation coefficient with elimination of direct counts.

† Some experiments recorded by Jacobs (1931) seem to represent the only previous case where bacterial numbers have been determined both by direct and plate counting in soil with addition of a rapidly decomposable material (naphthalene). Here, too, the direct counts were only 2 to 3 times as high as the plate counts, but unfortunately the figures cannot be directly compared, since they were obtained from separate experiments.

The partial regression coefficients in Table 5 (calculated by means of the formulae given by Dawson, 1933) enable us in some measure to compare the effect of the bacteria and the fungal mycelia in the production of carbon dioxide. At 4–7° C., where the partial correlation between density of mycelium and yield of CO_2 is reduced below significance, the effects of the two groups of organisms cannot be separated simply, although it is obvious that their efficiency is much lower than at the higher temperatures. At 14–16° C. the partial regression coefficient $\times 10^2$ of CO_2 on direct count is 0·709; this means, that with constant density of mycelium the average daily yield of CO_2 per 100 gm. of dry soil increases with 0·709 mgm. when the number of bacteria increases with 100 mill. per gm.; expressed on the basis of 1 gm. of soil, this increase corresponds to an average production of 0·0709 mgm. CO_2 per 1,000 mill. bacteria in 24 hours, which we may regard as the average efficiency of the bacteria at this temperature. The corresponding partial regression coefficient of CO_2 on density of mycelium shows, similarly, that with constant numbers of bacteria the yield of CO_2 increases with 0·421 mgm. per 100 gm., or with 0·00421 mgm. per 1 gm. of soil, so that a quantity of mycelium corresponding to a density of about 17% produces on an average the same amount of carbon dioxide as 1,000 mill. bacteria per gm. of soil. In the cases where the growth of fungi is most abundant (50–64% density) their activity would thus seem to be about equal to that of the bacteria (3–4,000 mill. per gm.) present at the same time, but towards the end of the experiments their activity appears small in comparison with that of the bacteria. An exception is shown by the acid soil with straw (experiment No. III), where the fungi appear almost alone active, but in this soil the rate of CO_2-formation is very slow in comparison with the corresponding experiment (No. II) with alkaline soil, where large numbers of bacteria are present. In the same way we find from the partial regression coefficients at 28° C., that the average efficiency of the bacteria is 0·0797 mgm. CO_2 per 1,000 mill. bacteria, and that with constant bacterial numbers the yield of CO_2 increases with 0·0115 mgm. CO_2 per gm. soil per 1% increase in density of mycelium, i.e., 7% density of mycelium is approximately equal to 1,000 mill. bacteria per gm. soil. At this temperature the fungi seem to display their greatest activity, although their growth is usually less abundant than at 15° C. After 3 to 8 days their activity appears to exceed that of the bacteria very considerably; but after 3–4 weeks they have largely ceased to be of importance. Finally, at 37° C. the average efficiency of the bacteria is considerably increased (0·222 mgm. CO_2 per 1,000 mill. bacteria), and a density of 14% mycelium is approximately equal to 1,000 mill. bacteria per gm. This and higher densities are not very common, and only in a few cases (experiment No. I after 7 days, No. VII after 3 days, No. VIII after 4 days) does the activity of the fungi appear to exceed that of the bacteria (including actinomycetes, which are richly represented at this temperature).

It should here be pointed out that these calculations represent no more than a first attempt to estimate the relative importance of bacteria and fungi in the decomposition processes. The direct counts of bacteria include varying proportions of active and inactive cells (bacterial endospores, and large numbers of aerial spores of actinomycetes at the higher temperatures), and the method of estimating the density of mycelium is admittedly not ideal and leaves considerable room for improvement. Firstly, the number of fields showing presence of mycelium does not precisely indicate the amount of fungal protoplasm represented by these mycelia, owing to the very variable thickness (sometimes also number in each field) of the hyphae observed and, secondly, the slides were in contact with the

soils for longer periods at the end than at the beginning of the experiments. But, even with these limitations, the method is felt to be a distinct improvement in comparison with the plate method. Improvements of the method (actual measurements of the hyphae, standardization of the time of incubation, etc.) may lead to more precise estimates.

In the last column of Table 4 are shown the amounts of CO_2 that would be expected to have been produced by the amounts of microorganisms observed. These values are calculated from the partial regression formula (Dawson, 1933):

$$X_1 - \bar{X}_1 = b_{13 \cdot 3}(X_2 - \bar{X}_2) + b_{13 \cdot 2}(X_3 - \bar{X}_3),$$

where X_1, X_2 and X_3 represent yields of CO_2, direct counts of bacteria, and densities of mycelium, respectively, $\bar{X}_1$, $\bar{X}_2$ and $\bar{X}_3$ the corresponding mean values (Table 4, bottom), and $b_{12 \cdot 3}$ and $b_{13 \cdot 2}$ the partial regression coefficients of CO_2 on direct counts and density of mycelium, respectively. In some experiments the agreement between expected and observed values is very close, but in most cases the observed values are in the beginning higher and at later stages lower than expected. This is obviously due to the fact that the bacterial counts, as seen in Table 4, tend to decrease much less rapidly than the rate of carbon dioxide production, i.e., the efficiency of the bacteria decreases with advancing time (cf. Meiklejohn, 1932, and Mooney and Winslow, 1935). In the later stages of the experiments at 37° C., where the mycelium has practically disappeared, we may calculate the efficiency of the bacteria as mgm. of CO_2 per 1,000 mill. bacteria in 24 hours in the same way as in Table 2; this calculation shows efficiencies of quite the same order as in the experiments with soils without addition of organic matter (for instance, 0·079–0·081 in experiment No. II after 21–29 days, 0·072–0·125 in experiment No. IV after 11–28 days) and much lower than the average efficiency indicated by the regression coefficients. A similar decreasing efficiency has been observed in cultures of fungi, where the rate of CO_2-production per unit of weight of mycelium is high in young stages where mycelium is being synthesized, but may become very low when the already-formed mycelium is merely being maintained (Noack, 1920; cf. also Mazé, 1902; Peterson, Fred and Schmidt, 1922; and Heukelekian and Waksman, 1925). This time-factor cannot in the present experiments be eliminated simply by treating the time as a fourth variant, because the regression of carbon dioxide formation on time is far from linear, particularly at 28° C. and 37° C.

The nitrogen transformation in some of the experiments is shown in Table 6. In the soils with hay and mycelium the production of nitrate, like that of carbon dioxide, increases with the temperature and is practically the same at 28° and at 37° C. The hay, with its narrower C:N ratio, has produced more inorganic nitrogen than the mycelium, the nitrogen-compounds of which do not appear to have been attacked at all after 4 weeks at 5° C., in spite of the abundant growth of micro-organisms that took place in this experiment. The soils with straw show an analogous phenomenon: the actual amount of inorganic nitrogen consumed is rather constant at the different temperatures, but the amount of organic matter decomposed to carbon dioxide per unit of nitrogen consumed increases markedly with the temperature. This is in full agreement with the results of Norman (1931) who found the "nitrogen-equivalent" (parts of inorganic nitrogen immobilized per 100 parts of organic matter decomposed) higher at 20° C. than at 30–50° C. in decomposition experiments with straw. From a practical aspect this might indicate that the danger of exhaustion of available nitrogen by addition of straw or similar materials to the soil is graver in the colder than in the warmer seasons of the year.

TABLE 6.

Nitrogen transformations in soils with addition of organic matter.

Experiment.	NH_4–N.*	NO_3–N.*	Production (+) or Consumption (−) of Mineral N ($NH_4 + NO_3$).	mgm. C liberated as CO_2 per mgm. Mineral N consumed.
No. 2 (sand-mixed garden soil + straw and $NaNO_3$):				
At start of experiment	1·3	19·3	—	—
After 29 days at 14–16° C.	0·0	9·3	−11·3	14·9
,, ,, ,, ,, 28° C.	0·0	8·7	−11·9	23·9
,, ,, ,, ,, 37° C.	0·0	11·5	−9·1	33·9
No. 3 (acid sand soil + straw and $(NH_4)_2SO_4$):				
At start of experiment	16·8	4·0	—	—
After 19 days at 14–16° C.	11·3	3·8	−5·7	8·9
,, ,, ,, ,, 28° C.	11·7	3·7	−5·4	14·8
No. 6 (sand-mixed garden soil + hay):				
At start of experiment	1·3	5·4	—	—
After 28 days at 14–16° C.	0·8	9·3	+2·6	—
,, ,, ,, ,, 28° C.	0·0	11·6	+4·9	—
,, ,, ,, ,, 37° C.	0·8	13·2	+6·3	—
No. 8 (red loam + hay):				
At start of experiment	0·8	2·7	—	—
After 17 days at 14–16° C.	6·3	3·2	+6·0	—
,, ,, ,, ,, 37° C.	6·2	5·9	+8·6	—
No. 10 (sand-mixed garden soil + mycelium):				
At start of experiment	1·4	6·7	—	—
After 28 days at 5–6° C.	2·5	5·0	(−0·6)	—
,, ,, ,, ,, 14–16° C.	4·2	8·1	+4·2	—
,, ,, ,, ,, 28° C.	0·0	14·0	+5·9	—
,, ,, ,, ,, 37° C.	1·1	12·1	+5·1	—

* Mgm. per 100 gm. of dry soil.

CONCLUSIONS.

A general principle extends through all these experiments: the higher the temperature, the more organic matter is decomposed to carbon dioxide (and inorganic nitrogen-compounds) in proportion to the quantity of microorganisms acting; one might say that the metabolism of the total microflora becomes less economical as the temperature increases.* This explains partly the rapid destruction of soil organic matter in hot climates. At low temperatures (4–7° C.) the microorganisms develop slowly when organic matter is added to the soil, but owing to the preserving influence of the low temperature, their cell-material gradually accumulates to a greater extent than at higher temperatures, where the

* The widening influence of increasing temperature upon the ratio of carbon liberated as CO_2 to carbon converted into bacterial substance is clearly seen in Text-figures 2, 4 and 6. The quantities of carbon in bacteria have been calculated on the assumption that 1,000 mill. bacteria on an average represent 0·2 mgm. dry matter with 50% carbon. To this must be added an unspecified amount of carbon present as fungal mycelium at 4-5° C., and as residues of previous generations of bacteria.

development of the organisms is more rapid, but not more extensive, and where the re-decomposition of the synthesized microbial matter becomes increasingly rapid. These facts have another important bearing on the problem of humus-accumulation. The proteid compounds synthesized at low temperature, according to Waksman and Gerretsen (1931), are obviously identical with the protoplasm of microorganisms that accumulate under these conditions. The lignins, which also accumulate at low temperatures (Waksman and Gerretsen, 1931), may combine with the proteins of the dead organisms (or with protein-like substances actually secreted by bacteria, according to Simola, 1931), thereby giving rise to those highly resistant ligno-protein compounds (Waksman and Iyer, 1933) that account for a very considerable part of the soil humus. According to what has just been said, these two sources of humus (lignin and microbial protein) will increase in quantity as the temperature decreases; and when they have been formed, their rate of decomposition will naturally increase with the temperature.

In cases where organic matter is being decomposed chiefly by fungi, it might be imagined that more protoplasm would be synthesized than where bacteria predominate, since the fungi are usually credited with a more economic type of metabolism than the bacteria (for references, see Kruse, 1910; Stephenson, 1930; and Waksman, 1932). The generality of this principle may, however, be doubted. A favourite test object in nutritional studies with fungi has been *Aspergillus niger* and related forms, which may convert one-half or even more of the consumed food-material into cell substance. Similar values were found by Heukelekian and Waksman (1925) in *Trichoderma* and *Penicillium*, but several other fungi seem to use their nutrients far less economically (Harter and Weimer, 1921; Peterson, Fred and Schmidt, 1922 (*Pen. glaucum* less economic than *Asp. niger*); Hochapfel, 1925). As to the bacteria, Rubner (1906) found *Proteus vulgaris* able to utilize up to 25% of the energy of the medium, whereas pathogenic bacteria were far less economical; this, however, could not be confirmed by Abt (1925), who found *Cor. diphtheriae* capable of utilizing some 30 per cent. of the energy of its nutrients. A utilization of sugar nearly as economical as in *Aspergillus* has been observed in *Myc. phlei* by Stephenson and Whetham (1923) and in other bacteria by Conn and Darrow (1935), whose experiments are particularly significant in regard to our problem, since they deal with a type of bacteria that represent a large proportion of the soil microflora. Even if the fungi as a whole synthesize somewhat more cell substance from a given quantity of nutrients than the bacteria, they do not actually seem to build up more protein, since they are generally much poorer in nitrogen. For instance, the data of Waksman and Diehm (1931), expressing the ratio of decomposed hemicellulose to assimilated ammonia-N in solution and sand cultures, do not show significant differences between fungi and aerobic bacteria (both studied in large numbers), although the ratio varied strongly within each group of organisms. Simola (1931) found cellulose-decomposing bacteria consuming nearly as much nitrogen in proportion to the amount of cellulose decomposed as *Trichoderma* and *Penicillium* according to Heukelekian and Waksman (1925), and Shrikande (1934) even found the nitrogen-equivalent (parts of inorganic nitrogen immobilized per 100 parts of organic matter decomposed) much higher with bacteria than with fungi in decomposition experiments with straw. There is thus no certain evidence that a decomposition of a given amount of organic materials by fungi would lead to the formation of more protein (and hence ligno-protein compounds) than a similar process brought about by bacteria under equal conditions.

SUMMARY.

A study was made of the rate of decomposition of organic matter in soil at different ranges of temperature in relation to the abundance of microorganisms present. The rate of decomposition was measured by the production of carbon dioxide. Bacteria (including actinomycetes) were counted both microscopically and on agar plates. The density of fungal mycelium was determined microscopically by means of the Rossi-Cholodny method.

In soils without extra addition of organic matter the carbon dioxide production was generally about 100 per cent. stronger at 28° C. than at 15° C., and about 50 per cent. stronger at 37° C. than at 28° C. The numbers of bacteria were not appreciably influenced by the temperature within a period of 10 days. Direct counts showed numbers from about 8 to about 300 times as high as plate counts. At each range of temperature there was a significant correlation between the daily yields of carbon dioxide and the directly counted numbers of bacteria, which appeared to be a better index of the activity of the bacterial flora than the plate counts. The growth of fungi was more extensive in sand soils than in loam soils, and was greatly restricted at 37° C. No correlation was found between the density of mycelium and the yields of carbon dioxide. The decomposition of the soil's own organic matter ("humus") seems to be carried out almost entirely by bacteria, and the accelerating influence of increasing temperature on this process is due to stimulation of the metabolic activity of the bacteria, but not to increased numbers of these organisms.

In soils with addition of undecomposed plant materials (straw, hay, fungal mycelium) the rate of carbon dioxide formation increased steeply with increases in temperature from about 5° C. to 37° C. This effect was most pronounced in the early stages of the decomposition process and at the lower ranges of temperature. In general the microorganisms tended to develop more slowly, but eventually to a greater extent, at lower than at higher temperatures, especially 37° C., where the vegetative growth of the fungi was generally very restricted and always confined to a brief initial period. Direct counts of bacteria (including actinomycetes) showed figures only 1–10 times as high as the plate counts. The numbers of actinomycetes increased strongly with the temperature. Significant correlations existed between the daily yields of carbon dioxide and the densities of mycelium, and between yields of carbon dioxide and directly counted numbers of bacteria, but not between yields of carbon dioxide and plate counts. The efficiency of the organisms decreased rapidly with advancing time. The fungi appeared to be important agents of decomposition during the earlier stages of the process, when the most intensive destruction of organic matter takes place. The figures suggested that, especially at 28° C., their carbon dioxide production may, even in alkaline soil, considerably exceed that of the bacteria; with advancing degree of decomposition they cease to be of importance. The decreasing rate of decomposition of added organic materials, together with the increasing accumulation of microbial substance, seems to offer a natural explanation for the increasing accumulation of "humus" with decreasing soil temperature.

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