From the Plant Nutrition Laboratory, The University of Michigan, Ann Arbor, Michigan, U.S.A., Paper No. 32

Factors Limiting Microbial Activities in Soil

I. The Level of Substrate, Nitrogen, and Phosphorus

By

G. STOTZKY and A. G. NORMAN

With 12 Figures in the Text

(Received April 13, 1961)

Despite the numerous studies of microbiological events in soil, there have been few investigations into the relative significance of the various factors that influence the activity of the microbial population in soil. Most studies in soil microbiology have been concerned with either specific groups of the soil microflora or specific activities such as nitrification, organic matter transformations, or repression of plant pathogens. On the other hand, there is considerable information on the growth requirements of many soil organisms in pure culture. Soil as an environment for microbial growth possesses certain unique features which, in conjunction with the complications involved in dealing with mixed populations, have made it difficult to obtain equally definitive information about soil cultures. Although the soil environment does not change the biochemical potentialities of microorganisms, it may influence the expression of these potentialities.

Some of the contradictory results found in the soil microbiological literature may be the result of not knowing fully the degree of interaction between factors that affect microbial activity. As an example, the disagreement over the formulas for the kinetics of glucose decomposition in soil suggested by LEES (1949, 1950), DROBNIK (1958, 1960), and CHASE and GRAY (1957), may be due, in part, to the different abilities of the soils used in their investigations to satisfy the factors which limit microbial development.

The present communication describes the influence of several environmental factors on the microbial activity of a sandy soil. Subsequent publications will describe others. Although an attempt was made to investigate the influence of each of these factors individually, it is obvious that the expression of some is influenced by interactions with others.

In order to minimize the influence of the native soil organic matter and structural aggregation, a soil low in organic matter and clay was employed. Glucose was used as the substrate, because the great majority of soil

organisms can readily utilize this as a carbon source. Microbial activity
was followed indirectly by the determination of CO_2 evolution; this was
assumed to be the best single parameter for measuring the activity of the
soil micropopulation.

Experimental

The soil used in these investigations was classified in the field as an Ottawa
loamy fine sand (Veatch et al. 1930), but as an Ottawa sand by mechanical analysis
in the laboratory (Table 1). The top four inches of soil were sampled in the field

Table 1. *Some chemical and physical properties of Ottawa loamy fine sand*

Property	Value[1]	Method of Analysis
Organic matter	0.85%	Weight loss on dry combustion at 600° C
Total carbon	0.16%	Wet combustion
Total nitrogen	0.02%	Kjeldahl[2]
Carbon/nitrogen ratio	8.0	
Available phosphorus	0.014%	NH_4F-HCl extraction[3]
Available potassium	0.007%	NH_4 acetate extraction[3]
Available sulfur	0.00038%	Morgan solution extraction[4]
Magnesium	0.0034%	$(NH_4)_2SO_4$-HCl extraction[3]
Manganese	0.00025%	Morgan solution extraction[3]
Boron	negative test	H_2O extraction[3]
Nitrates	negative test	Morgan solution extraction[3]
Soluble salts	negative test	H_2O extraction; conductivity[3]
pH	6.2	1:2 soil: water ratio
Lime requirement	1 ton/acre	Buffer solution[3]
Base exchange capacity	0.92 meq/100 g	NH_4HCO_3 replacement
Particle density	2.68 g/cc	Pycnometer[5]
Bulk density	1.40 g/cc	(on disturbed cores)[5]
Sand	96.3%	Sieve method[6]
Silt	1.1%	Sieve method[6]
Clay	2.6%	Sieve method[6]
Air-dry moisture	0.41%	Dried under fan
Wilting coefficient moisture	1.8%	Pressure membrane (15 atmos.)[5]
1/3 atmos. pressure moisture	4.7%	Tension table[5]
Aeration porosity moisture	12.6%	Tension table (60 cm tension)[5]
Field capacity moisture	16.3%	Column method

[1] All values calculated on an oven-dry basis.

[2] Analysis courtesy of Dr. A. R. Wolcott, Michigan State University.

[3] Procedures are those employed by the Ohio State Soil Testing Service. Analysis
courtesy of Mr. Ray Linville, The Ohio State University.

[4] Analysis courtesy of Dr. H. V. Jordan, USDA, A.R.S., Eastern Section of
Soil and Water Management, State College, Mississippi.

[5] Analysis courtesy of Dr. G. S. Taylor, The Ohio State University.

[6] Analysis courtesy of Dr. M. R. Heddleson, The Ohio State University.

in December under a shallow mantle of melting snow. For two years prior to
sampling, the soil had been cropped to barley followed by alfalfa. Yields had been
low. Some physical and chemical properties of the soil are presented in Table 1.

Because a uniform supply of soil was required for studies extending over several years, a large volume of soil was air-dried, sieved through a 3 mm screen, mixed by quartering, and stored. Although it has been reported that air-drying changes the microbial activity of soils (BIRCH 1958; LEBEDJANTZEV 1924; STEVEN-SON 1957), it was concluded that relatively fewer changes would occur between experiments if the original volume of soil was allowed to dry. Subsequent experiments have confirmed this assumption, as it has been possible to obtain virtually identical results from experiments repeated after intervals as great as 15 months. All experiments, unless otherwise noted, were conducted with 200 g samples of soil in $1/2$ pint (275 cc) widemouth canning jars. The various additives were mixed with sufficient water to bring the soil moisture content to field capacity; the final free water content was 16% (w/w). After addition of the solutions, the jars were stoppered, the soils and additives thoroughly mixed, and the jars placed at 3° C for at least 24 hours. Preliminary experiments indicated that this equilibration period was required to obtain uniform distribution of the solutions throughout the soil, without loss of glucose. After equilibration, the jars were attached to an aspiration manifold in a constant temperature room maintained at 25° $\pm$ 1° C. The soils were continuously flushed with CO_2-free air at a rate of 14 l/hr, and the CO_2 evolved from the soils was collected in NaOH and determined titrimetrically (STOTZKY and MORTENSEN 1957).

Results

Effect of various levels of glucose on the rate of CO_2 evolution

In order to determine the microbial activity of this soil, various levels of glucose were added. The CO_2 evolution curves obtained (Fig. 1)
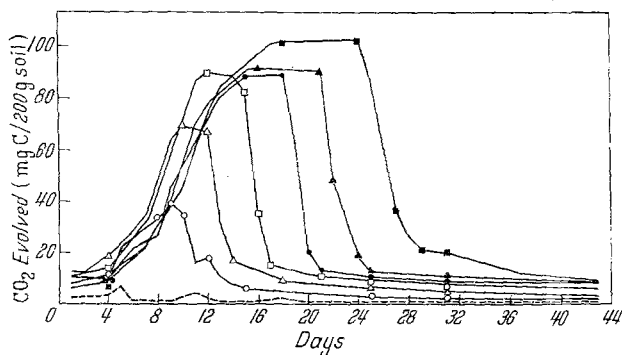

Fig. 1. Daily rate of CO_2 evolution from soil treated with various levels of glucose. % Glucose: --- None; o 0.5; △ 1.0; □ 1.5; ● 2.0; ▲ 2.5; ■ 3.75

were considerably different than the respiration patterns that are usually observed with soils amended with an available carbon source. Approximately 9—15 days were required to achieve a maximum rate of CO_2 evolution; the usual early sharp peak was not apparent; and the height of the CO_2 evolution curves did not increase significantly with additions of glucose greater than 1.5%, suggesting that some factor other than the supply of energy material was limiting. Instead of the usual respiration

23*

peaks, the CO_2 evolution curves exhibited plateaus, the duration of which appeared to be a function of the amount of glucose added. Due to the small amount of carbon added, the duration of the plateaus in the 0.5 and $1.0\,^0/_0$ glucose treatments was considerably shorter and occurred at lower levels than the plateaus with the higher additions of glucose. Once the available carbon was exhausted, the rate of CO_2 evolution sharply decreased. The rate of decrease was approximately the same, regardless of the original concentration of carbon.

The patterns of CO_2 evolution suggested that the maximum microbial potential of the soil was not realized. Inasmuch as the large additions of glucose considerably raised the C/N ratio, the amount of nitrogen available may have limited the development of the microorganisms. Accordingly, the experiment was repeated with various levels of nitrogen added.

Effect of various levels of nitrogen and glucose on the rate of CO_2 evolution

Various levels of glucose and nitrogen were added with sufficient water to bring the soil samples to field capacity. NH_4NO_3 was used as the nitrogen source in order not to affect the p_H and to provide both the nitrate and ammonium ions. The results, illustrated in Fig. 2, clearly indicate that the supply of available nitrogen did limit glucose utilization in the previous experiment. The peaks in CO_2 evolution occurred within the first 3 days as compared to 9—15 days, and the maximum rate of evolution was more than twice that obtained when nitrogen was not added. The attainment of peak CO_2 evolution after 2 to 3 days of incubation was still at variance with the CO_2 patterns obtained with some soils from which the maximum rate of respiration is usually observed within the first 24 hours (CHASE and GRAY 1957; DROBNIK 1958; KATZNELSON and STEVENSON 1956; KOEPF 1954; LEES 1949; NEWMAN and NORMAN 1941; STOTZKY et al. 1956; STOTZKY and MORTENSEN 1957, 1958). This lag in respiration was probably due to the low endogenous microbial activity of this soil, so that 2 to 3 days were required to permit the microbial population to increase to a level that enabled maximum utilization of the added glucose.

Essentially the same respiratory patterns were obtained with all three levels of nitrogen. Although the peaks in the CO_2 evolution curves were considerably higher than in the absence of nitrogen, there was no significant increase in the rate of evolution with levels of glucose greater than $1.0\,^0/_0$. Once the peak in evolution was attained, respiration decreased rapidly in the samples treated with 0.5, 1.0, and $1.5\,^0/_0$ glucose. The samples which received more than $1.5\,^0/_0$ glucose exhibited a plateau in the rate of respiration approximately half-way between the minimum and maximum rates. The duration of this plateau was again dependent

upon the amount of glucose added, and the rate of decrease in CO_2 evolution was approximately the same for all levels of glucose, again indicating that microbial activity was maintained at a high level only until the amount of available carbon was depleted.

The presence of plateaus and the similarity in the height of the evolution peaks of samples treated with levels of glucose greater than 1.0% may have been caused by the limitation of some nutrient elements other than nitrogen. When nitrogen was added, the rate of glucose decomposition increased until other elements became limiting. Only a portion of these elements was irreversibly immobilized, because the plateaus in respiration did not occur at the level of the peaks. The constant rate of respiration in the region of the plateaus indicated that the rates of mineralization and re-utilization of a portion of the limiting nutrient element or elements were rapid and essentially equal. There apparently was a sufficient quantity of these nutrients present in the soil to affect com-
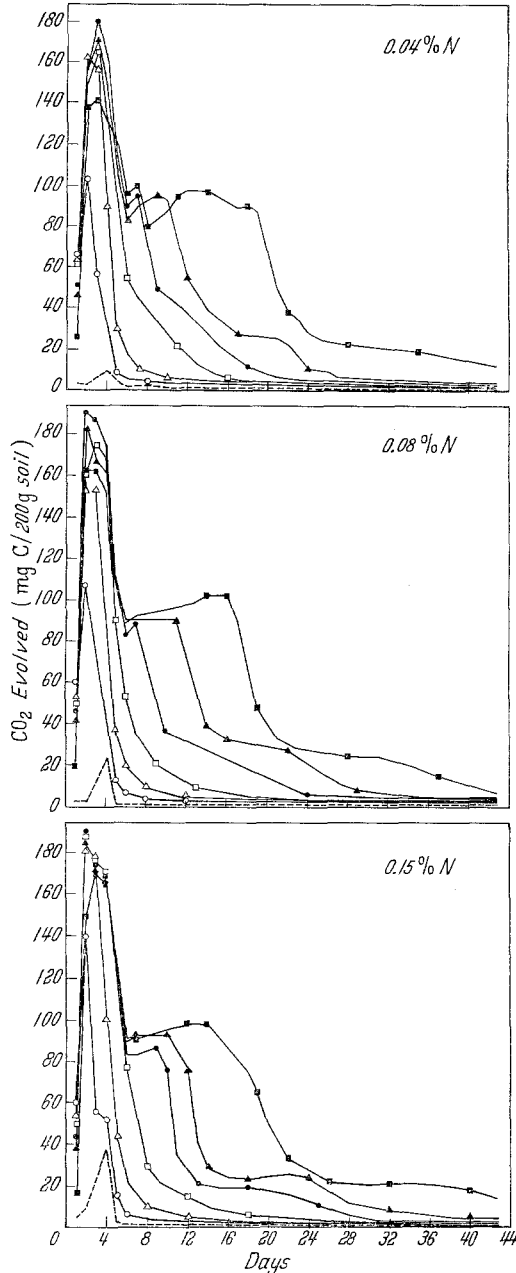

Fig. 2. Daily rate of CO_2 evolution from soil treated with various levels of glucose and nitrogen. $\%$ Glucose: --- None; ○ 0.5; △ 1.0; □ 1.5; ● 2.0; ▲ 2.5; ■ 3.75

plete decomposition of glucose added at the 0.5 and 1.0% levels, as evidenced by the absence of plateaus. The turnover rate of the limiting elements was not as rapid as that of nitrogen, inasmuch as the plateaus occurred at approximately the same level of respiration even when nitrogen was not added (Fig. 1).

The plateaus and the similarity in peak heights might also have resulted from changes in the microbial population and the lack of space for development of these populations. The sequential development of microbial populations after the addition of carbonaceous materials to soil was suggested in 1924 by WINOGRADSKY (see RUSSELL 1950) in his concept of "autochthonous" and "zymogenous" populations, and again recently by GARRETT (1956) in ecological classifications of soil-inhabiting fungi. There is ample evidence supporting the concept of sequences of organisms in the decomposition of heterogeneous substrates such as plant residues. When only a single soluble substrate such as glucose is involved, other possibilities must be considered. The addition of glucose supported rapid development of a microbial population which perhaps only incompletely decomposed the glucose. The resulting intermediary products may have inhibited further development of this "primary" population but were metabolized by "secondary" organisms. The development of the "secondary" population may have been limited by the high concentrations of glucose (ENGLESBERG 1959, FORBES and SEVAG 1951, KLIGLER et al. 1943, WESSMAN et al. 1958), a slow growth rate, or the need for growth factors synthesized by the "primary" population.

Microorganisms in soil develop primarily in water films, contact rings, and capillaries between soil particles (BARTHOLOMEW and NORMAN 1946, BHAUMIK and CLARK 1947, ELLINGER and QUASTEL 1948, QUASTEL and SCHOLEFIELD 1951); thus, the spatial distribution of organisms in these areas may also have been responsible for the CO_2 evolution patterns obtained. With the ample supply of substrate, this space may have become virtually filled with cells of the "primary" population, the senescent tissues of which were protected from decomposition by the presence of more available energy sources, i. e., glucose and its products. The activity of the "secondary" population may have been restricted by the space available for its development, despite the presence of available substrates. The dead cells ultimately decomposed, as indicated by the gradual decline in the rate of CO_2 evolution in the latter part of the incubation period, but, inasmuch as all available carbon had been utilized by this time, the space limitations no longer restricted activity. The data in Fig. 2 do not make it possible to distinguish between these and other possibilities.

At least 0.08% N was required for maximum decomposition of the added glucose (Tables 2, 3). There were no significant differences between

Table 2. *Recovery of added glucose as CO_2 from soil treated with various levels of glucose and nitrogen and incubated for 43 days*

Additions		C/N Ratio		Total CO_2 evolved (mg C/ 200 g soil)	Recovery of carbon as CO_2 (%)		
Glucose (mg C/ 200 g soil)	NH_4NO_3 (mg N/ 200 g soil)	Additions only	Additions + native soil organic matter[1]		Added glucose only		Added glucose + native soil organic matter[4]
					Including control[2]	Excluding control[3]	
0	0	—	8	52	—	—	16
0	80	—	2.7	56	—	—	18
0	160	—	1.6	73	—	—	23
0	300	—	0.9	110	—	—	34
400	0	—	18	344	86	73	47
400	80	5	6	342	86	72	47
400	160	2.5	3.6	367	92	74	50
400	300	1.3	2.1	423	106	78	58
800	0	—	28	669	84	77	60
800	80	10	9.3	637	80	73	57
800	160	5	5.6	637	80	71	57
800	300	2.6	3.2	715	89	75	64
1200	0	—	38	969	81	76	64
1200	80	15	12.6	966	81	76	64
1200	160	7.5	7.6	947	79	73	62
1200	300	4	4.4	1063	89	79	69
1600	0	—	48	1137	71	68	59
1600	80	20	16	1342	84	80	70
1600	160	10	9.6	1342	84	79	70
1600	300	5.3	5.6	1442	90	83	75
2000	0	—	58	1353	68	65	58
2000	80	25	19.3	1614	81	78	70
2000	160	12.5	11.6	1750	88	84	75
2000	300	6.6	6.8	1746	87	82	75
3000	0	—	83	1973	66	64	59
3000	80	37.5	27.5	2188	73	71	66
3000	160	18.8	16.6	2340	78	76	70
3000	300	10	9.7	2425	81	77	73
Mean ± standard error				1037 ± 137.1	83 ± 1.7	75 ± 1.0	58 ± 3.1 (63 ± 1.7)[5]
Coefficient of variation				68.8%	10.2%	6.8%	28.9% (13.3%)[5]

[1] Soil contained 320 mg C and 40 mg N/200 g soil; C/N = 8.

[2] (Total C evolved/C added as glucose) × 100.

[3] (Total C evolved − C evolved from control/C added as glucose) × 100; assume no "priming action".

[4] (Total C evolved/C added as glucose + C present in native soil organic matter) × 100.

[5] Excluding controls which received no glucose.

the 0 and 0.04% N additions in total amount of CO_2 evolved nor in per cent carbon recovered as CO_2 from the added glucose. A significant increase in per cent carbon recovery was obtained, however, with the 0.04% N addition when carbon recovery was calculated on the basis of the total carbon present in the soil (i.e., added glucose plus the native soil carbon).

Table 3. *Summary table for analysis of variance of data in Table 2* [1]

| | Total CO_2 evolved (mg C/200 g soil) | Recovery of carbon as CO_2 (%) | | | |
| | | Added glucose only | | Added glucose + native soil organic matter | |
		Including control [2]	Excluding control [3]	Including control [4]	Excluding control [5]
Glucose added (mg C/200 g soil)		Grand mean			
0	72.7	—	—	22.8	—
400	369.0	92.5	74.2	50.5	50.5
800	664.5	83.3	74.0	59.5	59.5
1200	986.2	82.5	76.0	64.8	64.8
1600	1315.7	82.3	77.5	68.5	68.5
2000	1615.7	81.0	77.2	69.5	69.5
3000	2231.5	74.5	72.0	67.0	67.0
Level of probability		LSD			
0.05	129.20	6.90	6.59	5.38	5.46
0.01	177.00	9.55	9.11	7.37	7.54
NH_4NO_3 added (mg N/200 g soil)		Grand mean			
0	928.1	76.0	70.5	51.9	57.8
80	1020.7	80.8	75.0	56.0	62.3
160	1065.1	83.5	76.1	58.1	64.0
300	1132.0	90.3	79.0	64.0	69.0
Level of probability		LSD			
0.05	97.70	5.63	5.37	4.08	4.45
0.01	133.83	7.78	8.69	5.58	6.16

[1] See footnotes in Table 2.

The addition of nitrogen increased the decomposition of the native soil organic matter, even though the C/N ratio of the soil organic matter was probably low enough for maximum decomposition (Table 1). Whether the added nitrogen was used directly in decomposition of the soil organic matter or whether the microorganisms preferentially utilized some of the native soil nitrogen (Broadbent 1948; Jansson 1955; Stojanovic and Broadbent 1956; Stotzky and Mortensen 1958), could not be determined in this study.

A significant difference in glucose recovery was apparent only between the 0.5 and 1.0% glucose levels, and then only when recovery was calculated on the basis of added glucose alone. This difference was due primarily to the amount of carbon evolved from the control soil. The per cent recoveries from any of the levels of glucose added were not significantly different when the recovery values were corrected for the CO_2 evolved from the control soils, indicating little "priming action" in this experiment (BROADBENT 1948; STOTZKY and MORTENSEN 1958). The recovery values calculated on the basis of both the added and residual soil carbon were lower than when calculated on the basis of added glucose

Table 4. *Organic matter content of soil after 43 days of incubation with various levels of glucose and nitrogen*

NH_4NO_3 (% N)	Glucose added (% C)							Nitrogen grand mean
	0	0.2	0.4	0.6	0.8	1.0	1.5	
	Organic matter (%)							
0	0.93	0.94	0.93	1.15	1.30	1.38	1.46	1.157
0.15	1.21	1.27	1.29	1.34	1.37	1.44	1.56	1.352
Carbon grand mean	1.075	1.103	1.110	1.243	1.335	1.410	1.508	

LSD	$P = 0.05$	$P = 0.01$
Carbon	0.0618	0.0857
Nitrogen	0.0331	0.0460
Carbon × nitrogen	0.0875	0.1215

alone, further showing that the residual soil organic matter was quite resistant to decomposition. Although the rate of CO_2 evolution was considerably different when nitrogen was added than when it was not, the amount of glucose utilized by the end of the incubation period was essentially the same for all nitrogen treatments, indicating again a rapid turnover in soil nitrogen.

The C/N ratio of either the additives alone or of the additives plus the soil organic matter did not seem to be significant in the decomposition of glucose. Only at C/N ratios greater than 38 was there any decrease in the amount of glucose recovered, and this difference would, undoubtedly, have been eliminated had the incubation period been extended. The C/N ratio of all systems was constantly decreasing, due to removal of carbon as CO_2, and the most important factor controlling the rate and amount of decomposition appeared to be the level of available carbon (STOTZKY and MORTENSEN 1957).

Although the per cent of glucose recovered as CO_2 was essentially the same at all levels of addition, the final organic matter content of the soil increased with an increase in level of glucose added (Table 4).

Conversely, the mineral nitrogen content at the end of the incubation period was lower with the higher levels of glucose (Table 5). There was no significant increase in organic matter content and no significant decrease in mineral nitrogen with levels of glucose less than 1.0%. Products rich in nitrogen apparently accumulated in the soils which received the higher levels of glucose. These products probably consisted primarily of cell protoplasm, indicating that the plateaus in respiration may have resulted from a limitation of space caused by accumulation of dead cells. The high recovery of added mineral nitrogen and the small

Table 5. *Recovery of mineral nitrogen from soil after 43 days of incubation with various levels of glucose and 0.15% N as NH_4NO_3*

Glucose added ($\%$ C)	0	0.2	0.4	0.6	0.8	1.0	1.5
Mineral nitrogen recovered ($\%$)	97	90	91	86	84	84	82

Mean $\pm$ standard error $= 87.7 \pm 1.99$. Coefficient of variation $= 6.0\%$.

Table 6. *pH of soil after 43 days of incubation with various levels of glucose and nitrogen* (pH determined on a 1:2 soil:water slurry)

NH_4NO_3 added ($\%$ N)	Glucose added ($\%$ C)						
	0	0.2	0.4	0.6	0.8	1.0	1.5
	pH						
0	6.9	7.1	7.3	7.4	7.5	7.2	7.2
0.15	6.6	6.6	6.6	6.7	7.0	7.0	7.3
0.04	*	*	*	*	*	*	7.3
0.08	*	*	*	*	*	*	7.4

* No measurements made.

amount of CO_2 evolved from the soil which received no glucose further showed that microbial activity was low when available carbon was not present.

The pH of the soils was higher at the end than at the beginning of the incubation period (Table 6). The higher pH of the soils treated with the larger quantities of glucose was correlated with the amount of nitrogen immobilized. Because approximately equal amounts of ammonium- and nitrate-nitrogen were recovered at the end of the incubation, the pH changes could not be attributed to the added nitrogen source.

An inverse relationship between rate of CO_2 evolution and level of glucose added was noted on the first day of incubation. This relationship was consistent with all levels of nitrogen, but was more pronounced when nitrogen was added (Fig. 1,2). This apparent restriction in development of the microbial population may have been caused by the toxicity of glucose to certain organisms (ENGLESBERG 1959; FORBES and SEVAG 1951; KLIGLER et al. 1943; WESSMAN et al. 1958) or by osmotic

pressure effects. The amount of CO_2 evolved at each level of glucose decreased as the concentration of nitrogen increased, indicating that the restriction was probably the result of hypertonic osmotic pressures. The osmotic effect was also manifest in the height of the respiration peaks which were lower with 2.5 and 3.75 than with 1.0, 1.5, or 2.0% glucose.

Definition of the CO_2 evolution curves

To define further the CO_2 evolution curves obtained in the previous experiment, a multiple sub-sample technique was employed (STOTZKY et al. 1958). This method enabled continuous collection of evolved CO_2 and concomitant sampling of soil without disturbing the environmental equilibrium of the remainder of the incubating soil. Seventy-five g of air-dried soil were placed in vials (40×90 mm) and treated with 1, 2, or 4% glucose, 0.08% N as NH_4NO_3, and sufficient water to bring the soils to 16% moisture. The soils and solutions were thoroughly mixed, and the vials were covered with aluminum foil. After 36 hours of equilibration at 3°C,

Table 7. *Recovery of glucose from soil incubated at 3° C* [1]

Glucose added (%)	Length of incubation (days)					
	0	1	2	9	15	28
	% recovery					
1	98	97	102	104	101	97
2	98	100	96	101	102	98
4	97	100	97	100	99	98

[1] One ml toluene added to soil.

the aluminium foil was removed, 17 vials identical in treatment were placed into large jars which were attached to the manifold, and evolved CO_2 was collected. Individual vials were withdrawn periodically during the incubation period. From these, subsamples of 10 g for the determination of moisture, 5 g for pH determination, and 5 g for dilution plating of microorganisms were removed. A small amount of detergent was added to the first dilution, and bacteria and fungi were plated in duplicate at two dilutions on soil extract (JAMES 1958) and rose bengal-streptomycin (MARTIN 1950) media, respectively. One ml of the highest soil dilution was also placed into test tubes containing nutrient broth and brom-cresol purple indicator[1], in order to measure the acid-producing potential of the soil population. Plates and tubes were incubated at 25° C. A further 20 g of soil was shaken with 75 ml of distilled water on a reciprocal shaker for 1 hour, the mixture filtered with the help of diatomaceous earth[2], and the filtrate diluted to 100 ml with distilled water. A portion of the filtrate was treated with $Ba(OH)_2$ and $ZnSO_4$ in order to remove all polar material (SOMOGYI 1952), and the glucose remaining in the soil was determined enzymatically[3]. The optical density of the residual filtrate was measured at 260, 280, and 400 mμ.

[1] Bacto Purple Broth Base, Difco No. B 227.

[2] Celite Analytical Filter Aid, Johns-Manville.

[3] This procedure involved oxidation of glucose to gluconic acid and H_2O_2 by glucose oxidase, and reaction of the H_2O_2 with o-dianisidine in the presence of peroxidase. The color produced was measured spectrophotometrically at 401 mμ. The enzymes and chromogenic acceptor were obtained from the Worthington Biochemical Corporation, Freehold, New Jersey, under the name of *Glucostat*.

To determine if glucose added to this soil could be recovered by this method of extraction, 1, 2, or 4% glucose was added to 50 g samples of air-dried soil. The samples were brought to 16% moisture, 1 ml of toluene was added before mixing, and the samples were placed at $3°$ C. After various periods of time, samples were removed, extracted, and analyzed for glucose by the above procedure. The results (Table 7), in agreement with those of Greenland (1956), show that glucose was not irreversibly adsorbed by the soil and could be extracted quantitatively with water.

The carbon balance throughout the incubation period (Fig. 3) was calculated only on the basis of glucose added, i. e., the native soil organic matter was not included. Although the rate of CO_2 evolution fell rapidly to that of the control soil once the glucose was completely utilized, the decrease occurred several days after glucose could no longer be recovered, indicating that during this interval intermediates between glucose and CO_2 were being decomposed. The longer time lag between the disappearance of glucose and the decrease in rate of CO_2 evolution showed that more intermediates were produced in samples which had been treated with the higher levels of glucose.

Fig. 3. Carbon balance of soil treated with various levels of glucose and 0.08% nitrogen. Calculated on basis of added glucose only. ◇ recovered as CO_2; △ recovered as glucose; ○ total recovered; □ total not recovered

The curves for glucose disappearance were similar in profile to the CO_2 evolution curves (Fig. 4a). At least four distinct slopes were apparent in both curves from samples treated with 4% glucose, indicating changes in the rate of microbial activity during the incubation period. The rate of activity was probably governed by (1) the turnover rate of limiting elements, (2) the rate at which more space became available, and/or (3) the differential rates of utilization of intermediates by various components of the microbial population present at any specific time.

The per cent recovery of added glucose as CO_2 and unchanged glucose during the first few days of incubation was greater with the higher levels of addition. If the amount of glucose that was not recovered either as unchanged glucose or as evolved CO_2 was a measure of cell mass and intermediates, then proportionately more of these were produced during

the early part of the incubation in samples treated with the lower levels of glucose. These results, in addition to the inverse relationship between addition level and initial rate of CO_2 evolution, suggested again that microbial activity was restricted when high concentrations of glucose were added.

Changes in p_H during the course of the incubation were plotted on an inverse scale (Fig. 4 b) to show the similarity between the CO_2 evolution (Fig. 4 a) and p_H curves. The reaction of the soil was acid as long as the rate of CO_2 evolution was high, presumably due to accumulation of acidic products during the period of high microbial activity. Measurement
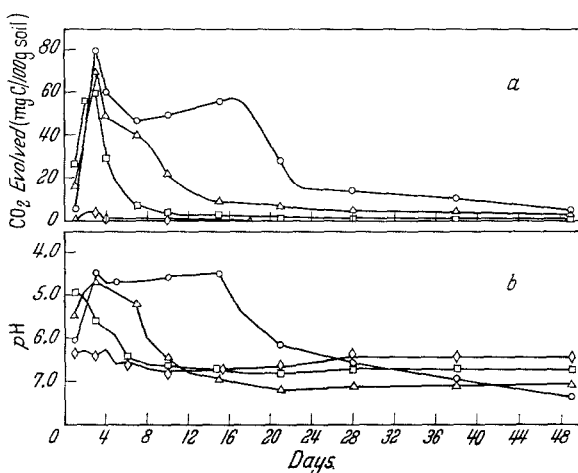
Fig. 4. a Daily rate of CO_2 evolution. b Daily changes in p_H. Soil treated with various levels of glucose and 0.08 % nitrogen. % Glucose: ◇ none; □ 1.0; △ 2.0; ○ 4.0

of the acid-producing potential of the soil population confirmed this accumulation and indicated that acids formed at any specific time were not decomposed immediately after formation, but persisted for several days. The p_H again increased several days before the rate of CO_2 evolution decreased to a constant level. Inasmuch as added glucose had completely disappeared before this time (Fig. 3), acidic intermediates were apparently decomposed preferentially, and basic products accumulated. The final p_H of the soils was directly related to the amount of glucose added, indicating a greater production and/or accumulation of basic products with the higher levels of carbon.

The p_H and carbon balance curves suggested that, although added glucose was rapidly attacked, not all was directly nor completely converted to CO_2 or cellular material; some was transformed into metabolic intermediates which accumulated. These intermediates were probably fermentative products, as suggested by the rapid decrease in p_H, the

odious smell of the soils, the low recovery of glucose as CO_2 during the first two days of incubation, and the high respiratory quotient indicative of anaerobic breakdown of glucose (STOTZKY 1960). The acidity during the first 24 hours of incubation was inversely related to the level of glucose added, supporting the observation that microbial activity was repressed by high levels of glucose. The pH data of this and the previous experiment show that pH should be determined throughout the course of an incubation period, as measurements only at the beginning and end do not reveal changes occurring during incubation and may result in erroneous interpretations.

The numbers of bacteria and fungi estimated by dilution plating (Fig. 5) were not correlated with the activity of the microorganisms as
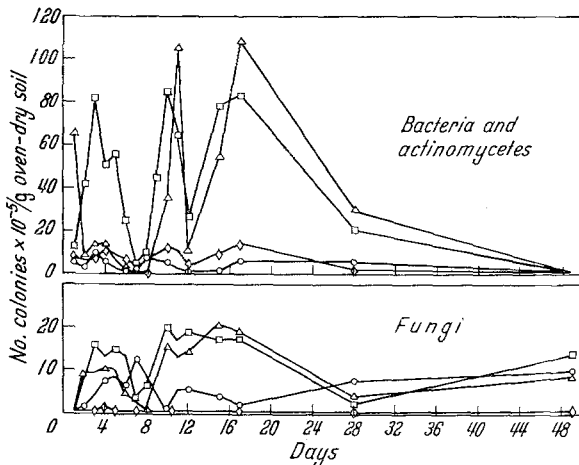

Fig. 5. Number of microorganisms enumerated in soil treated with various levels of glucose and 0.08°/₀ nitrogen. °/₀ Glucose: ◇ None; □ 1.0; △ 2.0; ○ 4.0

indicated by CO_2 evolution, glucose disappearance, or pH changes. The number of bacteria included both bacteria and actinomycetes; no attempt was made to differentiate these groups. Greater numbers of bacteria and fungi were obtained from soils treated with the two lower levels of glucose than from the soil which received 4°/₀ glucose. Considerably fewer organisms were enumerated during the period of high CO_2 evolution than after the rate of CO_2 evolution had decreased to a constant level. This lack of correlation between microbial activity and microbial numbers, as determined by dilution plating, has been observed previously by HAUSENBUILLER (1950), JENSEN (1936) and VANDECAVEYE (1939), and may have resulted from inhibition of some cells by high osmotic pressures. The high respiratory quotients (STOTZKY 1960) during the early part of the incubation and the larger numbers of bacteria and fungi

observed after the rate of CO_2 evolution had decreased, also suggested that a portion of the microbial population active during the early part of the incubation may have consisted of anaerobes. Inasmuch as the soil dilution plates were incubated under aerobic conditions, obligate anaerobes did not develop. If a limited number of microbial groups, the distribution of which did not vary, was selected by the environmental conditions under which the dilution plates were incubated and by the media used, then little change would be expected in the number of organisms developing. This was not the case, and the fluctuation in number of microorganisms over relatively short periods of time indicated that there were qualitative as well as quantitative changes in the soil population. The qualitative changes were probably associated with changes in the type of metabolic intermediates present at any specific time during the course of the incubation.

The quantitative changes in the fungal population were further complicated by the fact that fungi which develop on dilution plates arise primarily from spores (WARCUP 1951). The sporulation of some fungi is related to the amount and nature of food available (HAWKER 1957); i. e., vegetative growth predominates over spore formation when food supply is ample, and spores are produced profusely only when the supply decreases; apparently this effect is especially pronounced when glucose is the source of carbon. The number of fungi estimated by dilution plates may, therefore, be inversely related to the number and activity of fungi in the soil. This may explain the higher apparent numbers of fungi in soils treated with 1 or $2^0/_0$ glucose after the 9th day when glucose had been depleted and the rate of CO_2 evolution was decreasing, as well as the inverse relationship between the rate of CO_2 evolution and number of fungi obtained from soil which had been treated with $4^0/_0$ glucose. The soils which received glucose became covered with a dense mycelial growth of mucoraceous fungi which did not sporulate until the rate of CO_2 evolution decreased. The density of the hyphal growth appeared to be proportional to the level of glucose added. These species are rapid colonizers of soluble carbon substrates (GARRETT 1956, HAWKER 1957) and were probably part of the "primary" population. Prior to sporulation of the mucoraceous fungi, the genera present on dilution plates were primarily *Aspergillus, Trichoderma, Penicillium,* and *Cephalosporium*. Once the mucoraceous fungi had sporulated, however, they were also observed frequently.

When the soils were extracted with water for determination of residual glucose, it was noted that the intensity of the yellow color of the filtrates was related to the level of glucose added. The absorption spectra of the extracts were measured in order to determine if differences in color intensity could be related to the concentration of any particular fractions. The absorption spectra (Fig. 6) of filtrates from soils incubated for 7 days did not show any definitive peaks or shoulders,

but did indicate quantitative differences in absorption throughout the entire spectrum. Inasmuch as the amount of absorption was correlated with the level of glucose added, the color intensity of the soil extracts was presumably a measure of metabolic products or intermediates. The optical density of the soil extracts, therefore, was measured throughout the incubation period at wave lengths of 260 and 280 mμ and expressed in units calculated from a formula (Layne 1957)
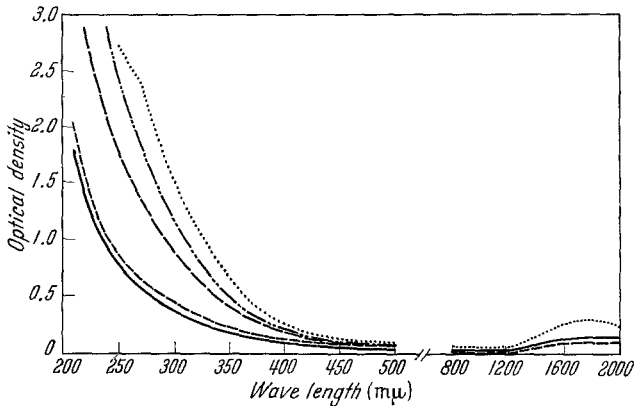
Fig. 6. Absorption spectra of water extracts of soil treated with various levels of glucose and 0.08% nitrogen and incubated for 7 days. % Glucose: —— None; — — —0.5; —— 1.5; —·—·—2.0; ······ 2.5
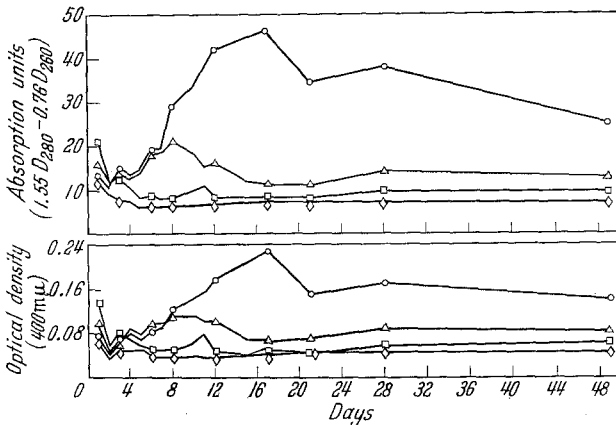
Fig. 7. Concentration of water-soluble intermediates present in soil treated with various levels of glucose and 0.08% nitrogen. % Glucose: ◇ None; □ 1.0; △ 2.0; ○ 4.0

for determining protein concentrations[1]. The use of this formula was arbitrary and does not imply that proteins contributed significantly to the color intensity. To be certain that production of proteins during the course of incubation would not unduly bias the use of the 260/280 ratio formula, absorption was also measured at 400 mμ. The similarity in the curves derived from these two measurements (Fig. 7), the absence of distinct shoulders at 260 or 280 mμ, and the essentially negative results obtained with the Folin-Ciocalteu reagent or on precipitation

[1] mg protein/ml solution = 1.55 D_{280} — 0.76 D_{260}.

with trichloroacetic acid (LAYNE 1957) indicated that the concentration of protein in the extracts was low.

The shapes of the absorption curves approximated those of the CO_2 evolution curves (Fig.4a), once the peak of microbial activity had passed. During the early part of incubation there was little accumulation of absorbing material, indicating that water-soluble intermediates were rapidly decomposed during the period of maximum microbial activity. The concentration of intermediates in samples that received $1^0/_0$ or $2^0/_0$ glucose decreased rapidly after the peak of microbial activity had been attained, but the concentration with $4^0/_0$ glucose increased during the first 17 days and was correlated with the plateau obtained in the CO_2 evolution curves. The factors that restricted decomposition of the added glucose also apparently inhibited decomposition of the intermediates. The level of intermediates increased slightly during the period when the rate of CO_2 evolution was at the plateau level, suggesting that glucose was being decomposed with the formation of intermediates. The decreases in both the rate of CO_2 evolution and the level of absorbing material were directly correlated after the 17th day, at which time the glucose had essentially disappeared, indicating that these intermediates constituted the major substrates between the 17th and 21st days.

An inverse relationship, similar to that obtained with the CO_2 evolution, glucose recovery, and p_H curves (Fig.3 and 4), was noted after the first day of incubation between the level of glucose added and the concentration of intermediates, again showing the inhibitory effects on microbial activity of the higher levels of glucose. More intermediates were formed during the 1st than during the following 6 or 7 days of incubation with all levels of glucose, in agreement with the patterns of glucose recovery (Fig.3). Glucose was apparently decomposed rapidly but not completely, resulting in the formation of intermediates, the concentrations of which were proportional to the amount of glucose added. The increase in intermediates during the first 24 hours of incubation was possibly the result of anaerobic dissimilation of the glucose, as oxygen may not have diffused to the sites of active microbial activity at a sufficiently rapid rate (STOTZKY 1960). A high concentration of metabolic intermediates, apparently resistant to decomposition, persisted in soils treated with 2 or $4^0/_0$ glucose, inasmuch as the absorption curves did not decrease to as low a level as did the CO_2 evolution curves. The intermediates that persisted were of a basic nature, as indicated by the p_H curves (Fig.4b).

Effect of phosphorus on the rate of CO_2 evolution

Because a marked response in the rate of respiration was obtained by the addition of nitrogen, the possibility that the level of available

soil phosphorus was also limiting maximum microbial activity was investigated. Two-hundred g samples of soil were incubated with 1, 2, or $4^0/_0$ glucose, $0.08^0/_0$ N as NH_4NO_3, and 0, 0.004, or $0.04^0/_0$ P as KH_2PO_4 and K_2HPO_4 combined to give a p_H of 6.95. The higher respiration peaks, the elimination of the plateau with $2^0/_0$ glucose, and the increase in the height of the first plateau with $4^0/_0$ glucose (Fig. 8), showed that the concentration of phosphorus did limit the rate of glucose decomposition in previous experiments. Although addition of phosphorus increased the rate of respiration, in agreement with the results of other investigators (CHANG 1940; DROBNIK and KRAMER 1960; HARDEN and YOUNG 1905; KAILA 1949), the increase was obtained only with $0.04^0/_0$ P, and a slight depressive effect in the rate of respiration occurred with the addition of $0.004^0/_0$ P. Not only was the rate of respiration decreased by the addition of $0.004^0/_0$ P, but also the total amount of glucose recovered as CO_2 was reduced (Tables 8 and 9). The depressive effect of $0.004^0/_0$ P was apparent even when glucose was not added. No explanation for these responses to $0.004^0/_0$ P is offered.
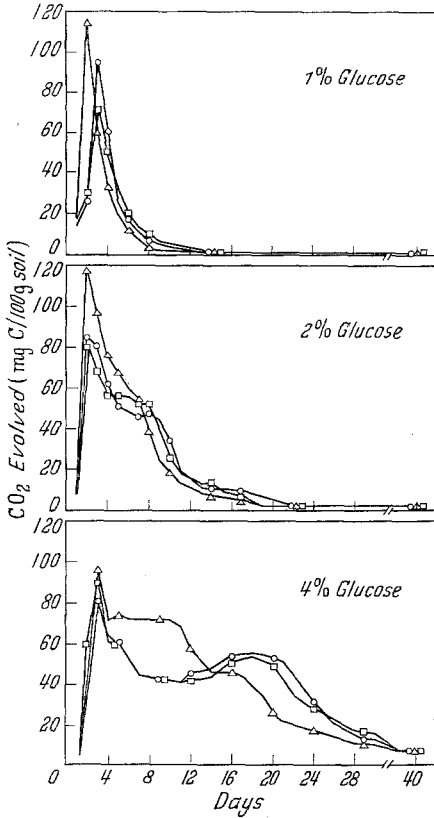

Fig. 8. Daily rate of CO_2 evolution from soil treated with $0.08^0/_0$ nitrogen and various levels of glucose and phosphorus. $^0/_0$ P: ○ None; □ 0.004; △ 0.04

Although addition of $0.04^0/_0$ P increased the rate of respiration, the plateau in the CO_2 evolution curve of the soil which received $4^0/_0$ glucose was not eliminated; instead, two plateaus were obtained. The double plateau and the increased height of the first plateau suggested that some other factor was still limiting maximum microbial activity. The increase in activity, resulting from addition of phosphorus, may have rapidly depleted the supply of some other limiting nutrient element or elements, the turnover rate of which was not rapid enough for synthesis of new cells. When the available supply of the element(s) was depleted, respiration decreased until more became available for synthesis of new cells, at which time glucose was further decomposed and a second

plateau was obtained. The decrease in respiration after attainment of the second plateau was due to depletion of the carbon substrate, as discussed previously. Experiments with higher levels of phosphorus did not result in increased respiration, and 0.04% P apparently satisfied the phosphorus requirements of the soil microorganisms, even at the highest level of glucose addition.

Table 8. *Recovery of added glucose as CO_2 from soil treated with various levels of glucose and phosphorus and incubated for 41 days*

Additions		C/P Ratio		Total CO_2 evolved (mg C/ 200 g soil)	Recovery of carbon as CO_2 (%)		
Glucose (mg C/ 200 g soil)	KH_2PO_4 and K_2HPO_4 (mg P/ 200 g soil)	Additions only	Additions + native soil organic matter[1]		Added glucose only		Added glucose + native soil organic matter[4]
					Including control[2]	Excluding control[3]	
0	0	—	11.4	31	—	—	10
0	8	—	8.9	28	—	—	9
0	80	—	3.0	38	—	—	12
800	0	—	40.0	600	75	71	54
800	8	100	31.1	585	73	70	52
800	80	10	10.4	624	78	73	57
1600	0	—	68.6	1276	80	78	67
1600	8	200	53.3	1244	78	76	65
1600	80	20	17.8	1322	82	80	69
3200	0	—	125.7	2626	82	81	75
3200	8	400	97.7	2590	80	80	74
3200	80	40	32.6	2680	84	83	76
Mean ± standard error				1013 ± 292.8	79 ± 1.2	77 ± 1.5	52 ± 7.6 (65 ± 3.0)[5]
Coefficient of variation				89.1%	4.4%	5.9%	50.8% (14.0%)[5]

[1] Soil contained 320 mg C and 28 mg P/200 g soil; C/P = 11.4.

[2] (Total C evolved/C added as glucose) × 100.

[3] (Total C evolved — C evolved from control/C added as glucose) × 100; assume no "priming action".

[4] (Total C evolved/C added as glucose + C present in native soil organic matter) × 100.

[5] Excluding controls which received no glucose.

The presence of microbial inhibitors

The accumulation of products inhibitory to certain groups of soil microorganisms could, as previously suggested, account for the anomalous CO_2 evolution curves obtained. The following experiments were conducted to determine if direct evidence for the presence of inhibitors of microbial activity could be obtained. One hundred g samples of air-dried soil were mixed with 100 g samples of soil which had previously been incubated for 69 days with various levels of glucose

Table 9. *Summary table for analysis of variance of data in Table 8*[1]

| | Total CO_2 evolved (mg C/200 g soil) | Recovery of carbon as CO_2 (%) | | | |
| | | Added glucose only | | Added glucose + native soil organic matter | |
		Including control[2]	Excluding control[3]	Including control[4]	Excluding control[5]
Glucose added (mg C/200 g soil)		Grand mean			
0	32.3	—	—	10.1	—
800	603.0	75.4	71.5	54.2	54.2
1600	1280.7	80.0	78.1	66.7	66.7
3200	2632.0	82.0	81.3	74.8	74.8
Level of probability		LSD			
0.05	36.78	0.82	1.20	1.02	1.32
0.01	55.72	1.35	1.99	1.54	2.19
KH_2PO_4 and K_2HPO_4 added (mg P/200 g soil)		Grand mean			
0	1133.2	79.0	76.9	51.1	64.9
8	1111.8	77.0	75.2	49.9	63.5
80	1166.0	81.4	78.7	53.4	67.3
Level of probability		LSD			
0.05	31.86	0.82	1.20	0.88	1.32
0.01	48.27	1.35	1.99	1.33	2.19

[1] See footnotes in Table 8.

and subsequently dried at 75° C. One per cent glucose plus sufficient water to bring the soils to field capacity were added, and the soils were thoroughly mixed. Nitrogen and phosphorus were not added, as a slow rate of microbial activity was desired to avoid masking the effects of possible inhibitors.

The CO_2 evolution curves (Fig. 9) did not indicate the presence of any inhibitory material, but rather the presence of stimulatory substances. There was a marked increase in the rate of respiration during the early part of the incubation in the samples which contained 100 g of soil previously incubated with 0.5 and 1.0% glucose. This stimulation in respiration was possibly due to the presence of nitrogen mineralized from the soil organic matter during the initial incubation. A greater concentration of readily available nitrogen was probably present in these soils than in those which received higher levels of glucose, because not as much nitrogen was immobilized in microbial cells (Table 5).

The greater height of the CO_2 evolution peak of the sample which received no pre-incubated soil was probably due to the slower utilization of glucose prior to attainment of the peak. The greater respiration during the early part of the incubation period and the higher recovery of added

glucose as CO_2 from samples which received pre-incubated soils (Table 10) indicated that some material, most probably inorganic nitrogen, was present in the pre-incubated soils which enabled more rapid utilization of glucose.

Aliquots of extracts of the pre-incubated soils were added to different soil samples on the 8th, 11th, and 21st day, to determine if possible inhibitory materials were destroyed by drying the pre-incubated soil at 75° C and if the stimulatory substances were acting on different segments of the microbial population at specific times during the course of the incubation. The extracts were obtained by shaking 100 g of pre-incubated soil with distilled water and filtering through a Seitz bacterial filter. The amount of organic matter present in the extracts was determined by wet combustion and by absorption measurements at 260 and 400 mμ. A high correlation was obtained between the two methods for determining the organic content of the extracts. The amount of or-

Fig. 9. Daily rate of CO_2 evolution from a mixture of fresh soil and soil pre-incubated with various levels of glucose. Mixture received 1% glucose. 100 g new soil + 100 g soil preincubated with glucose: ◇ None; □ 0.5%; △ 1.0%; ○ 1.5, 2.0, or 2.5% (average curve); − − − not preincubated (200 g new soil)

ganic material in each aliquot of the extracts was equivalent to that contained in 20 g of the pre-incubated soils added in the previous experiment.
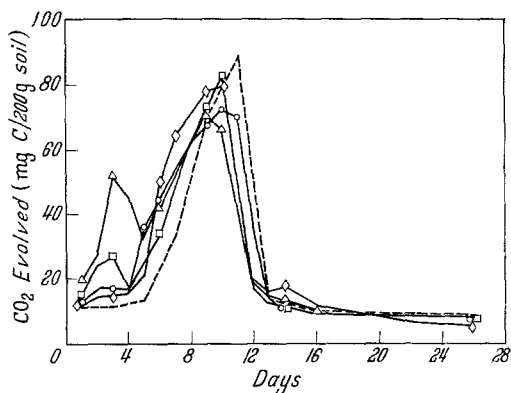
Table 10. Recovery of added glucose as CO_2 from a mixture of 100 g pre-incubated and 100 g fresh soil after 26 days of incubation

Glucose added to original soil (mg C/200 g)[1]	C added in 100 g preincubated soil[2]	Total C added (mg C/200 g)	Recovery of carbon as CO_2[3]	
			(mg C/200 g)	(%)
Control[4]	—	800	586	73
0	0	800	624	78
400	4	804	601	75
800	11	811	645	80
1200	17	817	614	75
1600	24	824	654	79
2000	31	831	624	75
Mean ± standard error			621 ± 8.94	76.4 ± 0.97
Coefficient of variation			3.8%	3.4%

[1] Original soil incubated with glucose for 69 days prior to addition to fresh soil.

[2] Carbon remaining in soil after 69 days of incubation, as determined by wet combustion and spectral analysis of water extracts of the soils.

[3] Based only on C added; does not include native soil organic matter.

[4] 200 g of fresh soil.

No reduction in respiration was noted when extracts were added at various times to soils treated with 1% glucose (Fig. 10). The slight
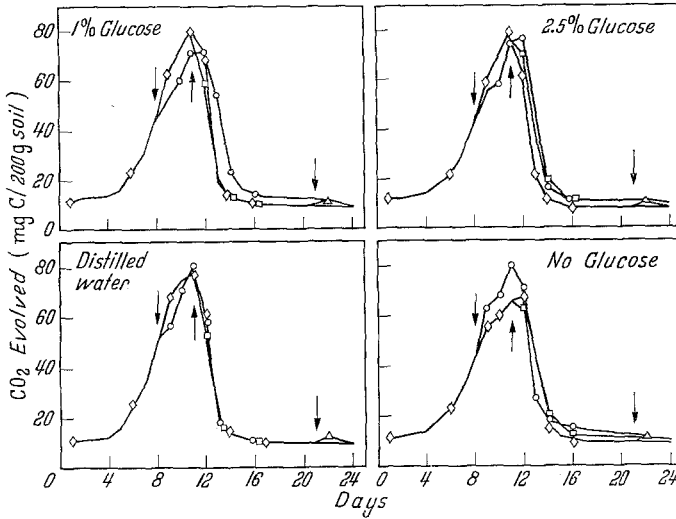

Fig. 10. Daily rate of CO_2 evolution from soil treated with 1% glucose and periodic additions of water extracts of soils pre-incubated with various levels of glucose. Arrows indicate time of additions. Extract added on: ◇ No extract added; ○ 8th day; □ 11th day; △ 21st day

Table 11. *Recovery after 24 days of incubation of added glucose as CO_2 from soil treated with 1% glucose and periodic additions of water extracts of pre-incubated soils*

Glucose added to original soil (mg C/200 g)[1]	C added in extract (mg C/200 g)	Time of addition (days after start of incubation)	Recovery of carbon as CO_2	
			(mg C/200 g)	(%)
Control[3]	0	8	583	73
	0	11	587	73
	0	21	591	74
0	0	8	602	75
	0	11	602	75
	0	21	585	73
800	2.2	8	606	76
	2.2	11	588	73
	2.2	21	595	74
2000	6.1	8	593	74
	6.1	11	601	75
	6.1	21	585	73
Mean ± standard error			593.2 ± 2.45	74 ± 0.30
Coefficient of variation			1.4%	1.4%

[1] Original soil incubated with glucose for 69 days prior to extraction.

[2] Based only on C added as glucose and in extracts; does not include native soil organic matter.

[3] Received distilled water.

decrease in rate of CO_2 evolution after addition on the 8th day of extracts from soils originally treated with 1.0 and 2.5% glucose apparently was not the result of an inhibitor, inasmuch as a similar effect was noted when distilled water alone was added. A stimulation in the rate of respiration was obtained with the addition on the 8th day of an extract from soil which originally received no glucose. This stimulation was also probably due to inorganic nitrogen derived from the prior decomposition of the soil organic matter. The total amount of carbon recovered at the end of the incubation period was the same for all treatments, further indicating the absence of inhibitory materials in the soil extracts (Table 11).
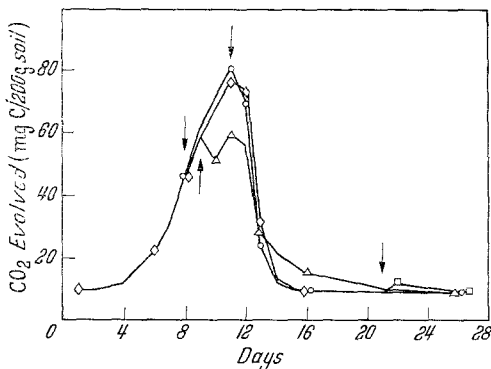

Fig. 11. Daily rate of CO_2 evolution from soil treated with 1% glucose and either periodic additions of water extract of soil previously preincubated with 2% glucose or $5 \cdot 10^{-4}$ M 2,4-dinitrophenol. Arrows indicate time of additions. ◇ No extract added; ○ extracts on 8th and 11th days; □ Extract on 21st day; △ DNP on 9th day

The concentration of organic material added in each aliquot of the extracts was equivalent only to that present in 20 g of the preincubated soils, thus, the concentration of inhibitors may have been insufficient to give a measurable response. An extract of soil which previously had been incubated with 2% glucose was concentrated in vacuo at 30° C, and aliquots were added to the same soil samples after 8 and 11 days of incubation and to other samples after 21 days of incubation. The extracts added were equivalent to the amount of organic material present in 75 or

Fig. 12. Daily rate of CO_2 evolution from soil treated initially with 1% glucose and subsequently with either 0.125 % glucose alone or with $5 \cdot 10^{-4}$ M 2,4-dinitrophenol. Arrows indicate time of additions. ◇ No subsequent additions; ○ Water on 8th and 11th days; △ 0.125 % Glucose + DNP on 12th day; □ 0.125% Glucose on 12th day

25 g of pre-incubated soil, respectively. All extracts were added to the surface of the soils with a pipette, and the soils were not mixed after addition. Because there was a possibility that the extracts did not diffuse throughout the soil, 5 ml of 2,4-dinitrophenol (DNP) was added separately by the same method to other
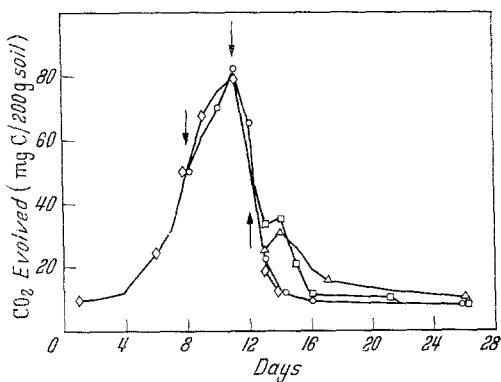
samples to give a final concentration in the soil solution of $5 \cdot 10^{-4}$ M. All samples received 1% glucose initially.

Although addition of the concentrated extracts did not significantly affect the rate of CO_2 evolution (Fig. 11), the addition of DNP on the 9th day resulted in an immediate reduction in the evolution rate. The lack of inhibition with the soil extracts was, therefore, not due to failure of inhibitory materials to become distributed through the soil mass.

To note the effect of an inhibitor on respiration once the peak in microbial activity had passed, DNP plus 0.125% glucose or 0.125%

Table 12. *The recovery after 26 days of incubation of added glucose as CO_2 from soil treated with 1% glucose and periodic additions of water extracts of pre-incubated soil; 2,4-dinitrophenol; or 0.125% glucose*

Additions	Time of addition (days after start of incubation)	Recovery of carbon as CO_2	
		mg C/200 g	$\%$ C
Control	—	585	73
H_2O	8 and 11	571	71
12 mg C[1]	8 and 11	576	72
6 mg C[1]	21	561	70
DNP[2]	9	584	73
100 mg C[3]	12	633	70
100 mg C[3] and DNP[2]	12	665	74
Mean $\pm$ standard error			71.8 ± 0.60
Coefficient of variation			2.2%

[1] Extract from soil originally incubated with 2% glucose for 69 days; 6 mg C added at each addition.

[2] Final concentration of 2,4-dinitrophenol (DNP) in soil solution was $5 \cdot 10^{-4}$M.

[3] C added as glucose.

glucose only were added on the 12th day to samples which had been initially treated with 1% glucose. The addition of DNP slightly decreased the rate of CO_2 evolution when compared to that from soil which had received glucose only (Fig. 12). The more pronounced effect of DNP added on the 9th day (Fig. 11) was probably the result of the larger microbial population and the higher concentration of glucose present in the soil on the 9th than on the 12th day. Although the addition of DNP decreased the rate of glucose decomposition, the total amount decomposed during the incubation period was the same for all treatments (Table 12). Because DNP inhibits assimilatory processes by interfering with oxidative phosphorylation and energy transferring mechanisms (LOOMIS and LIPMAN 1948) but does not interfere directly with dissimilative activity (CLIFTON 1937), the slower respiration rate of samples treated with DNP was probably due to inhibition of synthesis of new cells.

The results of these experiments indicated that the CO_2 evolution patterns obtained with this soil were not the result of inhibitory materials produced during decomposition of glucose. BIRCH (1958) also was unable to extract appreciable levels of inhibitory materials from soil. The question of whether microbial inhibitors are produced in soil can not be answered from the present study, inasmuch as extracts were obtained only from soils incubated for 69 days. Although inhibitory materials are undoubtedly produced during periods of high microbial activity, they are probably decomposed rapidly by sequentially developing populations.

Discussion

In these experiments, an ample supply of a soluble substrate, glucose, was provided in order to ascertain the nature and something of the quantitative expression of factors which may limit microbial activities in soil. No support was obtained for the recent suggestion of DROBNIK (1960) that the initial degradation and oxidation of glucose in soils is accomplished by cell-free enzymes. The conversion of glucose to CO_2 requires an enzyme complex in a spatial arrangement which enables the transfer of both metabolic intermediates and energy from one enzyme system or sequence to another. Although enzymes are released from lysed cells, they are probably rapidly diluted and dispersed in the soil solution as well as adsorbed on colloidal soil particles (ESTERMAN and McLAREN 1959). The results of the present study clearly indicate that glucose added to soils is utilized first for synthesis of new cells and then in their respiration, and that the amount of glucose assimilated is increased when nitrogen and phosphorus are added to fulfill the structural requirements of cell synthesis. The indications of repression of microbial activity by large additions of glucose and inorganic elements will be discussed in detail in a subsequent paper.

Although the initial concentration of essential inorganic nutrients determined the rate of utilization of added glucose, the degree of utilization was controlled by the turnover rate of the nutrient elements. The rate of turnover appeared to be nitrogen > phosphorus > other elements, the last of which will be discussed in a subsequent paper (STOTZKY and NORMAN 1961). Because the turnover rate of inorganic nutrients was fairly rapid, the extent of decomposition of glucose was independent of the original C/N or C/P ratios of the system. CHASE and GRAY (1957) suggested that the kinetics of organic matter decomposition in soil are composed of a series of superimposed first-order reactions. The results of the present study support this suggestion; at least four superimposed first-order reactions were apparent, presumably due to the limitation of nitrogen, phosphorus, other elements, and space, respectively. Despite the number of limiting factors, the over-all kinetics of the decomposition

of glucose was essentially a first-order reaction, inasmuch as the velocity
of a complex reaction is determined by the order of the slowest step.
The adherence of glucose decomposition in soil to first-order kinetics
indicates that the factors which influence the activity of pure cultures
and natural populations developing on single substrates are similar.

When, as in these studies, a substrate is uniformly distributed through-
out the soil water, the situation may arise in which population develop-
ment may be limited by restrictions of space. Microorganisms in soil
develop largely in the water films encompassing the soil components,
particularly in the contact rings or necks between particles (BARTHOLO-
MEW and NORMAN 1946; BHAUMIK and CLARK 1947; ELLINGER and
QUASTEL 1948; QUASTEL and SCHOLEFIELD 1951). The available space
for colonization appears to be directly related to the water content
(BHAUMIK and CLARK 1947). Although the soil-water system is, in effect,
continuous, there is probably little adjustment and movement of cells
as in liquid culture. Even in the latter, however, restriction of microbial
development resulting from limitations of space has been reported at
high population densities (BAIL 1929; CONTOIS 1959).

Limitations of space assume considerable importance in mixed culture
systems because of effects on the sequential development of specialized
populations capable of decomposing metabolic intermediates or in-
completely oxidized products. The decomposition of even a simple
substrate in soil, such as the glucose added in this study, apparently
requires a sequence of organisms. Under natural conditions the substrates
are far more complex, and numerous specialized groups of organisms
are probably required to prevent accumulation of these products. The
importance of space as a factor in limiting a specific microbial trans-
formation in soil, viz., nitrification, was also demonstrated by QUASTEL
and SCHOLEFIELD (1951), who suggested that, once the surface of "soil
crumbs" were saturated with cells, further growth of microorganisms
will not occur except to replace cells which have died and disintegrated.
The number of microorganisms in this soil was not correlated with the
level of activity, presumably due, in part, to the limitation of space;
however, the activity per cell may have increased through adaptive
mechanisms, as suggested by QUASTEL and SCHOLEFIELD (1951) and
KATZNELSON and STEVENSON (1956). Nevertheless, the final determinant
of microbial activity was the concentration of an available carbon source,
and when this was depleted, activity decreased rapidly.

The soil had a low endogenous microbial level and exhibited a lag
period before an appreciable amount of respiration occurred. It is doubt-
ful if air-drying this soil prior to use affected the respiration rates, as
has been suggested by STEVENSON (1957), BIRCH (1958), CHASE and
GRAY (1957) and LEBEDJANTZEV (1924). Air-drying probably did not

appreciably increase the amount of soluble organic matter, inasmuch as the level of native soil organic matter was not only low, but also relatively resistant to decomposition. Even if drying resulted in some solubilization of organic matter, its effect was overshadowed by the high levels of glucose added. Nor should drying have had a marked effect on the physiological state of the microorganisms, as suggested by BIRCH (1958); the soil was sampled in winter, and the number of microorganisms, as well as their endogenous activity, was low.

Although the diffusion rate of liquids was adequate in this soil, that of gases may not have been sufficiently rapid to keep pace with the oxygen demand when microbial activity was at a maximum. The anaerobic breakdown of added glucose under these experimental conditions is significant (STOTZKY 1960). Gas diffusion is, undoubtedly, impeded when there has been heavy development of microbial tissue in the water films, especially at points of contact between soil particles. The soils were superficially aerated with a constant flow of CO_2-free air at a rate (14 l/hr) which was considerably higher than the rate of gas exchange under natural conditions (RUSSELL 1950). The rate was probably adequate to remove effectively liberated CO_2 from the soil surface but may not have ensured rapid oxygen replacement in the soil mass. Transitory anaerobiosis may be of more frequent occurrence in the field when carbonaceous materials are rapidly decomposing than is ordinarily recognized.

The influence of various environmental factors on microbial activity, only a few of which have been discussed in this paper, appeared to be very similar in soil as in pure culture, indicating the value and validity of applying pure-culture techniques to soil microbiological studies. The influence of other factors will be discussed in subsequent communications.

Summary

The influence of several environmental factors on microbial activity in soil was measured by CO_2 evolution, substrate disappearance, formation of intermediates, and changes in p_H. Although good correlation was obtained between these parameters, microbial activity was not directly correlated with the numbers of bacteria and fungi estimated by dilution plating. Possible explanations for this are presented.

The results obtained indicated that the decomposition in soil of even a simple substrate, such as glucose, is complex. The kinetics of glucose decomposition appeared to be composed of a series of first-order reactions, presumably resulting from the rate-limiting influence of nitrogen, phosphorus, other elements, and space for development, respectively. Although the concentration of inorganic nutrient elements limited the rate of

glucose decomposition, it did not affect the extent of decomposition. The influence of each of the limiting factors was discussed.

No inhibitors of microbial activity were detected in the soil after 69 days of incubation with glucose. Some inhibition was noted when high levels of glucose and minerals were added, presumably the result of hypertonic osmotic pressures and/or toxicity of glucose.

The diffusion rate of liquids through the soil was rapid, but that of gases was not, and some anaerobic breakdown of glucose occurred.

A limitation of available space for development of sequential microbial populations was inferred, and the implications of this as an ecological factor were discussed.

The results of this study indicated that the techniques of the pure-culture microbiologist can successfully be applied to soils, and that factors which limit microbial activity in pure culture are also operative in soil.

References

BAIL, O.: Z. Immun.-Forsch. **60**, 1—14 (1929).
BARTHOLOMEW, W. V., and A. G. NORMAN: Soil Sci. Soc. Amer. Proc. **11**, 270—279 (1946).
BHAUMIK, H. D., and F. E. CLARK: Soil Sci. Soc. Amer. Proc. **12**, 234—238 (1947).
BIRCH, H. F.: Plant and Soil **10**, 9—31 (1958).
BROADBENT, F. E.: Soil Sci. Soc. Amer. Proc. **12**, 246—249 (1948).
CHANG, S. C.: Soil Sci. **49**, 197—202 (1940).
CHASE, F. E., and P. H. H. GRAY: Canad. J. Microbiol. **3**, 335—349 (1957).
CLIFTON, C. E.: Enzymologia 4, 246—253 (1937).
CONTOIS, D. E.: J. gen. Microbiol. **21**, 40—50 (1959).
DROBNIK, J.: Folia biol. (Prague) 4, 137—146 (1958).
DROBNIK, J.: Plant and Soil **12**, 199—211 (1960).
DROBNIK, J., and M. KRAMER: Folia microbiol. (Prague) **5**, 59—61 (1960).
ELLINGER, G., and J. H. QUASTEL: Biochem. J. **42**, 214—218 (1948).
ENGLESBERG, E.: Proc. nat. Acad. Sci. (Wash.) **45**, 1494—1507 (1959).
ESTERMAN, E. F., and A. D. MCLAREN: J. Soil Sci. **10**, 64—78 (1959).
FORBES, M., and M. G. SEVAG: Arch. Biochem. **31**, 406—415 (1951).
GARRETT, S. D.: Biology of Root-infecting Fungi. Cambridge: Cambridge Univ. Press 1956.
GREENLAND, D. J.: J. Soil Sci. 7, 319—334 (1956).
HARDEN, A., and J. W. YOUNG: Proc. Chem. Soc. **21**, 189 (1905).
HAUSENBUILLER, R. L.: Trans. Fourth. Intern. Congr. Soil Sci. **3**, 89—93 (1950).
HAWKER, L. E.: The Physiology of Reproduction in Fungi. Cambridge: Cambridge Univ. Press 1957.
JAMES, N.: Canad. J. Microbiol. 4, 363—375 (1958).
JANSSON, S. L.: Z. Pflanzenernähr., Düng., Bodenkunde **69**, 190—195 (1955).
JENSEN, H. L.: Proc. Linnean Soc. N. S. W. **61**, 27—55 (1936).
KAILA, A.: Soil Sci. **68**, 279—289 (1949).

KATZNELSON, H., and I. L. STEVENSON: Canad. J. Microbiol. **2**, 611—622 (1956).

KLIGLER, I. J., N. GROSSOWICZ and S. BERGNER: Proc. Soc. exp. Biol. (N.Y.) **52**, 332—334 (1943).

KOEPF, H.: Z. Acker- u. Pflanzenbau **98**, 289—312 (1954).

LAYNE, E.: Methods in Enzymology **3**, 447—454. New York: Academic Press Inc. 1957.

LEBEDJANTZEV, A. N.: Soil Sci. **18**, 419—447 (1924).

LEES, H.: Plant and Soil **2**, 123—128 (1949).

LEES, H., and J. W. PORTEOUS: Plant and Soil **2**, 231—241 (1950).

LOOMIS, W. F., and F. LIPMAN: J. biol. Chem. **173**, 807—808 (1948).

MARTIN, J. P.: Soil Sci. **69**, 215—227 (1950).

NEWMAN, A. S., and A. G. NORMAN: Soil Sci. Soc. Amer. Proc. **6**, 187—194 (1941).

QUASTEL, J. H., and P. G. SCHOLEFIELD: Bact. Rev. **15**, 1—53 (1951).

RUSSELL, E. J.: Soil Conditions and Plant Growth (8th Ed. E. W. RUSSELL). London: Longmans, Green & Co. 1950.

SOMOGYI, M.: J. biol. Chem. **195**, 19—23 (1952).

STEVENSON, I. L.: Plant and Soil **8**, 170—182 (1957).

STOJANOVIC, B. J., and F. E. BROADBENT: Soil Sci. Soc. Amer. Proc. **29**, 214—218 (1956).

STOTZKY, G.: Canad. J. Microbiol. **6**, 439—452 (1960).

STOTZKY, G., W. P. MARTIN and J. L. MORTENSEN: Soil Sci. Soc. Amer. Proc. **20**, 392—396 (1956).

STOTZKY, G., and J. L. MORTENSEN: Soil Sci. **83**, 165—174 (1957).

STOTZKY, G., and J. L. MORTENSEN: Soil Sci. Soc. Amer. Proc. **22**, 521—524 (1958).

STOTZKY, G., u. A. G. NORMAN: Arch. Mikrobiol. **40**, 370—382 (1961).

STOTZKY, G., T. M. RYAN, and J. L. MORTENSEN: Soil Sci. Soc. Amer. Proc. **22**, 270—271 (1958).

VANDECAVEYE, S. C.: Trans. Third Comm. Intern. Soc. Soil Sci. A, 120—130 (1939).

VEATCH, J. O., L. C. WHEETING and A. BAUER: U. S. Dept. Agric. Soil Survey Ser., No. 21 (1930).

WARCUP, J. H.: Trans. Brit. Mycol. Soc. **34**, 376—399 (1951).

WESSMAN, G. E., D. J. MILLER and M. J. SURGALLA: J. Bact. **76**, 368—375 (1958).

Dr. G. STOTZKY

Central Research Laboratories, United Fruit Company, Norwood, Mass. (USA)