The Relationship between Micro-organisms and Soil Aggregation

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SUMMARY: The physical condition of soil is improved by adding readily decomposable organic material. Microbial cells and metabolic products affect soil structure by binding loose soil particles into water-stable aggregates.

Experimentally, the relative aggregating power of pure cultures of micro-organisms was as follows: fungi > actinomycetes and a few gum-forming bacteria > many gum-producing bacteria > yeasts, proactinomycetes, and many bacteria; the last three groups did not improve aggregation. Fungal hyphae entangled soil particles into stable aggregates; weaker crumbs were formed by the frailer threads of actinomycetes. A few bacterial strains produced gums capable of glueing soil into water-stable aggregates, but the majority of bacterial slimes were almost useless because they remained water-soluble after drying. The cementing properties of these gums was not improved by treatment with H or Ca ions. Bacterial gums stabilized the aggregates produced from completely dispersed soils and kaolin, but not those formed with bentonite or ferric hydroxide. The pH value of the soil played a very minor part in influencing the aggregation produced by pure cultures of micro-organisms or even by soil inoculum.

Mixed cultures of fungi or of actinomycetes gave slightly better aggregation than pure cultures, but neither capsulated nor non-capsulated bacteria in mixtures gave better results than single strains. More complex mixtures containing fungi, actinomycetes and bacteria gave good aggregation when all micro-organisms were compatible, but poor results when antagonistic bacteria inhibited the growth of either fungi or actinomycetes. The fair aggregation obtained with soil inoculum was reproduced in the laboratory by inoculating sterilized soil with complex mixtures of micro-organisms.

A study was made of the relative merits of glucose, starch, blood, yeast, fungal mycelium, straw, clover and farmyard manure for encouraging aggregation by mixtures of fungi, actinomycetes and bacteria.

Aggregates bound by mycelia did not last long because the hyphae were decomposed by bacteria. The temporary improvement of soil structure after the addition of organic materials can be partly explained by the action of microbes, but the permanent crumb structure of many soils must be due mainly to other causes.

Many of the older countries of the world have succeeded in maintaining large populations only by conserving the physical condition of the soil with rotational cropping or alternate husbandry. It has long been known that the maintenance of soil friability depends on periodically supplying readily decomposable organic matter, and that ley farming methods are not only profitable, but improve the structure of all types of soil. Ley farming might be adopted more readily if more were known of the mechanism whereby soil crumbs are restored by grass roots and other organic materials. The task of combating erosion is closely linked with the problem of restoring the crumb structure of soil. This paper deals with some aspects of the role of micro-organisms and organic matter in improving the aggregation of soil.

The biological factors influencing soil structure have been reviewed in a recent publication of the Commonwealth Bureau of Soil Science (1948). Geltser (1936, 1937, 1943) found that readily decomposable organic materials temporarily improved the crumb structure of soils, whereas materials more resistant to decomposition were less beneficial. She considered that stable aggregates were formed only after fungal hyphae had been replaced by bacteria, which in turn were lysed and formed organic cements. Martin (1945) inoculated sterilized soil containing sucrose with pure cultures of fungi, actinomycetes and bacteria and found that the hyphae of *Cladosporium* spp. and gum from *Bacillus subtilis* greatly increased aggregation, but that actinomycetes and other bacteria were less effective. Subsequently Martin (1946) isolated polysaccharides from several bacteria, including fructosans from *Bacillus subtilis* and *Azotobacter indicum* and dextrans from *Leuconostoc dextranicus* and two unidentified strains, and found them to be better crumb cements than casein or lignin. These polysaccharides were slowly decomposed by several micro-organisms. In pure culture studies McCalla (1945, 1946) classified the aggregating power of soil micro-organisms as follows: fungi and a few gum-forming bacteria > actinomycetes > yeasts > most bacteria.

Most workers have tried to interpret field phenomena by experiments with pure cultures of micro-organisms, often specially selected because they possessed good aggregating properties. Little account was taken of the fact that the soil contains numerous organisms all competing with one another; and no attempt was made to estimate the relative proportion of micro-organisms beneficial, useless and harmful to aggregation, that are present in soil after the addition of organic materials.

EXPERIMENTAL METHODS

Micro-organisms. Throughout the text the micro-organisms are designated by a prefixed letter and a number. Thus B stands for a bacterium, A for an actinomycete, and F for a fungus. The reference number, name, a brief description and place of origin of the cultures most commonly used are as follows.

B 2 *Bacillus mycoides*; Haywards Heath.
B 8 *Micrococcus* sp., Gram-positive, white mucoid colony; Haywards Heath.
B 35 *Aerobacter* sp., slimy colony; Rothamsted.
B 45 *Achromobacter* sp., Gram-negative, gummy colony; Haywards Heath.
B 50 *Sarcina* sp., Gram-positive, yellow waxy colony; Rothamsted.
B 68 *Achromobacter* sp., Gram-negative, gummy colony; Rothamsted.
B 91 *Bacillus* sp., rubbery colony; Rothamsted.
B 163 *Pseudomonas fluorescens*, non-mucoid colony; London brick earth.
B 169 *Bacillus polymyxa*, gelatinous colony; from Prof. Kluyver, Delft.
A 3 *Actinomyces* sp., white powdery colony; Haywards Heath.
A 16 *Actinomyces coelicolor*, purple colony; Rothamsted.
A 20 *Micromonospora* sp., brown gelatinous colony; Haywards Heath.

F 7 *Absidia glauca*; Haywards Heath.

F 27 *Aspergillus nidulans*; London brick earth.

F 51 *Trichoderma lignorum*; Rothamsted.

Most of the work was carried out with three English soils: (1) Rothamsted allotment grey clay loam, of pH 6·9; (2) Haywards Heath light, grey-brown, sandy loam, podsol of pH 4·7; (3) Woburn light brown, sandy loam, podsol, limed to pH 6·7.

Production of microbial gums. Bacteria and fungi were cultivated for 20 days at 25° in shallow layers of the following medium: 20 g. glucose, 5 g. peptone, 2 g. NH_4NO_3, 0·5 g. K_2HPO_4, 0·25 g. $MgSO_47H_2O$, 0·1 g. $CaCl_2$, 10 ml. soil extract, made up to 1 l. with distilled water. The bacterial cells were removed by centrifugation and the fungal hyphae by filtration. Bacterial gums were precipitated from the resulting fluid by 3 volumes of ethanol, collected on a Buchner filter, washed with ethanol, dispersed in water and dialysed to remove salts. Proteins were removed by repeatedly shaking with chloroform and the gums reprecipitated with 3 volumes of ethanol, washed with ethanol and dried at room temperature.

Crude fungal gums were prepared by fragmenting the fungal hyphae in a Waring Blendor, treating them with sodium carbonate and trypsin overnight to digest proteins and boiling the residue left after filtration with 0·1 N-NaOH. The gums were precipitated by freezing, washed with water then ethanol and dried at room temperature.

Cultivation of micro-organisms in soil. The soils were air-dried, crushed, passed through a 2 mm. sieve and 50 g. lots weighed into large test-tubes, 7 in. long and $1\frac{1}{4}$ in. diameter. It was found that a 2 mm. fraction gave better results than a 1 mm. fraction because better aeration was obtained. After plugging, the tubes of soil were dry sterilized for at least 4 hr. at 150°. Insoluble foodstuffs, such as oat-straw and dried blood, were ground and 0·5 % mixed with the soil before heat sterilization. Soluble nutrients, such as glucose, peptone and salts, were dissolved in sufficient water to bring the 50 g. of soil to a water content just up to field capacity. These solutions were tubed and autoclaved separately. The sterile soils were inoculated by adding a loopful of micro-organisms first to the tube of sterile nutrient solution, mixing thoroughly, and pouring the contents aseptically into the tube of soil. Ultimately the soil contained 0·5 % sugar, 0·036 % peptone or 0·014 % NH_4NO_3, 0·01 % K_2HPO_4, 0·005 % $CaCl_2$ and 0·005 % $MgSO_4$. In all experiments tubes were prepared in triplicate for each treatment so that for all standard errors quoted $n = 2$. The tubes were incubated at 25° and losses due to evaporation restored by adding sterile water at intervals of approximately 20 days. At the end of incubation the plugs were removed, and the soils were dried in an oven for 48 hr. at 50°. After this they could be stored without deterioration until aggregate analyses were carried out.

Aggregate analysis of soils. At first the soils were analysed both for micro-aggregates and for macro-aggregates, but when it was found that the results were parallel, only the macro-aggregates were estimated. Usually 50 g. of soil were used for each determination. Soils that had become compacted during

incubation were gently broken by hand into lumps of about 1·5 cm. Then, instead of allowing the moisture gradually to soak through by capillarity, a more vigorous action was obtained by flooding the lumps or crumbs of soil in a Petri dish, where they were soaked for 24 hr. This relatively long period of soaking was chosen to give closer approximation to field conditions, where soils are often moist for weeks on end. It was found that many bacterial gums were not fully hydrated after only a few hours soaking, so that a false value for their cementing power was obtained unless they were soaked for 24 hr.

Micro-aggregates were determined by transferring the wet soil to a measuring cylinder, filling up to the 1 l. level, shaking end over end 20 times, floating a Bouyoucos hydrometer in the suspension, and reading after 30 sec. settling. A temperature correction was made and this gave the amount of suspended non-aggregated material $< 0{\cdot}07$ mm. in diameter, from which the weight of micro-aggregates and sand $> 0{\cdot}07$ mm. in diameter was found by difference. Allowance was made for the sand $> 0{\cdot}07$ mm. by determining the amount present after completely dispersing the soil by shaking overnight with sodium oxalate solution.

Macro-aggregates > 1 mm. in diameter were determined by transferring the soaked soil to a sieve with 1 mm. holes and hand-sieving under water until all fine particles had passed through. This usually required sixteen to twenty strokes. The aggregates and sand retained on the sieve were dried at 50° and weighed. In any one experiment the amount of sand was considered to be constant, so no deduction was made.

EXPERIMENTAL RESULTS

Myers & McCalla (1941) counted the number of micro-organisms in soil containing sucrose and found that maximum aggregation occurred after the bacterial population had reached its peak. They concluded that bacterial products, rather than cells, were responsible for aggregation.

Aggregate formation in unsterilized soil. A test for correlation between the bacterial and fungal population and the degree of aggregation was made as follows. Two 5 kg. samples of Haywards Heath soil in glass pots were treated respectively with 0·5 % dried blood and 0·5 % soluble starch, and one sample was untreated. The pots were moistened to field capacity with a mineral solution containing soil inoculum, and incubated at 25°. At intervals over a period of 86 days, micro- and macro-aggregation was estimated, and at the same time dilution plate counts of fungi were made on acid glucose peptone agar, and of bacteria and actinomycetes on peptone soil-extract agar. The pH was determined on all soil samples. On two occasions the bacterial numbers and fungal lengths were determined by the Jones & Mollison (1948) direct microscopic method.

The percentages of micro-aggregates almost paralleled the percentages of macro-aggregates throughout the whole period of incubation (Fig. 1). Both the control and the blood treatment produced an initial decrease in the amount of aggregation. After 8 days the percentage aggregation of untreated soil

remained fairly constant except for minor fluctuations. The percentage of micro-aggregates in soil containing blood improved fairly steadily throughout the whole experiment, but the percentage of macro-aggregates decreased somewhat at the 52nd day. The starch-treated soil aggregated rapidly and aggregation reached a peak between the 17th and 26th days, but then declined.

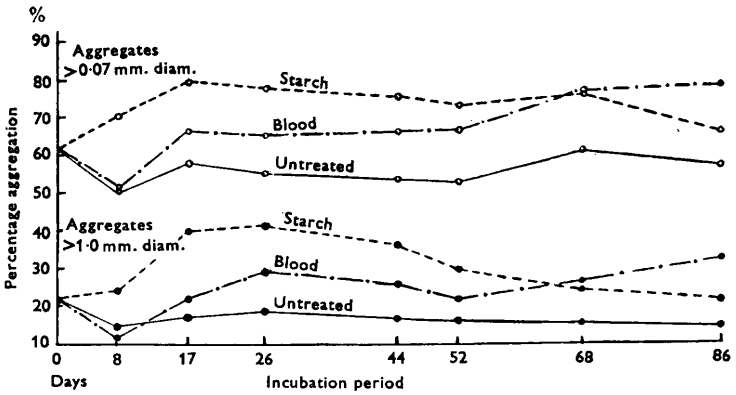

Fig. 1. Influences of starch and blood in aggregation of Haywards Heath soil.

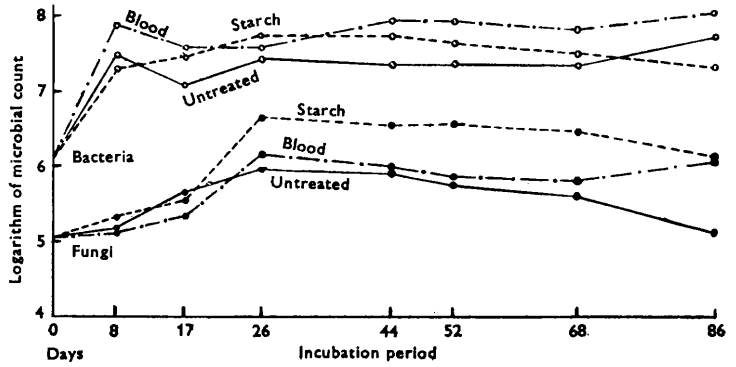

Fig. 2. Bacterial and fungal counts of Haywards Heath soil treated with starch and blood.

There was an initial sharp rise in the bacterial plate count in all three treatments (Fig. 2). In general, blood produced the highest counts and even after 86 days the peak was not reached. With starch the bacterial count reached a maximum after 26–44 days, then declined slightly. Throughout most of the incubation period the untreated soil contained fewer bacteria than the two treated soils. There were no obvious differences in the types of bacteria and actinomycetes attributable to the various treatments.

The fungal population as estimated by plate counts was always less than that of bacteria. The fungal count of all three treatments rose steadily until the 26th day, remained fairly constant until the 68th day, and then fell in the untreated and starch treated soils. During most of the experiment starch gave

higher counts than either blood or untreated. There were no obvious differences in the types of fungi isolated from starch, blood or untreated soils.

On the two occasions when direct microscopic counts were made by the method of Jones & Mollison (1948), the figures greatly exceeded the number found by dilution plating. At the start of the experiment all treatments contained 12·7 m. of fungal hyphae and 1350 million bacteria per g. soil. On the 17th day starch, blood and untreated soil contained 96·2, 49·5 and 25·8 m. of fungal hyphae and 9500, 11,330 and 3·470 million bacteria respectively per g. soil.

The pH of the untreated soil remained between 4·7 and 5·3 during the whole period. Blood-treated soil became neutral on the 26th day and remained thus until the ammonia began to nitrify after 52 days, when the pH fell to 4·7. Contrary to expectation, soil containing starch gradually became less acid with incubation and finally reached a pH of 6·1. Doubtless microbial activity was influenced by fluctuations in soil pH, but it is doubtful whether aggregation was greatly affected by pH alone, since artificial adjustment of the reaction with alkali from pH 4·7 to 7·5 had no appreciable effect on macro-aggregation.

It is difficult to draw from plate counts any definite conclusions about the relative importance of bacteria and fungi in improving the structure of the differently treated soils. It is doubtful whether either population can be even approximately estimated by dilution plating. When breaking the clods of soil by hand it was observed, however, that good aggregation was invariably associated with a visibly luxuriant growth of fungi throughout the soil.

After 17 days' incubation, the lengths of fungal mycelium were correlated with the degree of aggregation (see p. 252), but neither plate nor microscopic counts of bacteria closely paralleled the increase in aggregation during the earlier stages of incubation, perhaps because such counts included a majority of organisms having no aggregating power. Hence it was decided to isolate various microbes from Haywards Heath and Rothamsted soils and to test their aggregating power in pure culture.

Aggregation of soil by pure cultures

Seventy-five strains of bacteria, twenty-one of actinomycetes, five of pro-actinomycetes, five of yeast and fifty of fungi were cultured in tubes of sterile Rothamsted allotment soil (<2 mm.) containing 0·5 % of glucose, 0·036 % of peptone and the usual mineral salts. After 20 days at 25° the soils were subjected to macro-aggregate analysis by wet sieving. Table 1 classifies the seventy-five bacterial strains according to their ability to produce fair (13–16 g.), poor (10–13 g.) or no (7–10 g.) aggregation of 50 g. soil. Observations on the presence of mucoid colonies on glucose peptone agar, the formation of chains of cells and Gram-staining are also given. In general bacteria were not good aggregators of soil despite the fact that most of them grew well. Twenty-two of the strains gave fair aggregation, twenty-five gave poor, while twenty-eight did not aggregate the soil at all. There was no consistent correlation between

the degree of aggregation and the production of mucoid colonies on agar, cell morphology or the Gram-reaction. The best aggregator from Haywards Heath soil was B45, which produced 15·02 g. crumbs/50 g. soil, while the best from Rothamsted was B68, which produced 14·86 g. crumb/50 g. soil. Both organisms were species of *Achromobacter* that produced very gummy colonies. Many other strains also produced gums which bound the soils when dry or after a short period of soaking, but after 24 hr. in water most of these gums redispersed and the aggregates were slaked.

Table 1. *Characters of some soil bacteria as related to their ability to aggregate soil in pure culture*

			No. of strains having the characters shown					
			Consistency of colonies on agar		Cell grouping of bacteria		Gram stain	
Aggregation	Mean weight of aggregates >1 mm./50 g. soil (g.)	Total no. of strains	Mucoid	Non-mucoid	Chains	Single cells	+	−
Fair	13–16	22	10	12	2	20	8	14
Poor	10–13	25	21	4	2	23	10	15
None	7–10	28	12	16	10	18	16	12
	Totals	75	43	32	14	61	34	41

Table 2. *Influence of actinomycetes, proactinomycetes and yeasts on soil aggregation*

Type of micro-organism	Aggregation	Mean weight of aggregates >1 mm./50 g. soil	No. of strains
Actinomycetes	Good	21–29	7
Actinomycetes	Fair	13–21	12
Actinomycetes	Poor	10–13	2
Proactinomycetes	None	7–10	5
Yeasts	None	7–10	5

Subsequently many other bacterial strains from various sources were tested and only two gave better results than B45 and B68, viz. *Bacillus subtilis* (Geoghegan's strain) and *B. polymyxa* (B169). The aggregating powers of actinomycetes, proactinomycetes and yeasts are summarized in Table 2.

Usually actinomycetes were found to be better aggregators than bacteria, but proactinomycetes and yeasts did not cement the soil at all. Seven vigorously growing actinomycetes gave good aggregation (21–29 g.), twelve of them produced fairly stable crumbs (13–21 g.), while two species of *Micromonospora* formed only a few stable aggregates (10–13 g.). It was observed that strains which produced numerous waxy spores prevented the soil from being properly wetted so that amyl alcohol had to be added to the water. The best aggregator was strain A3 (28·46 g.) which also inhibited wetting of the soil with water. Many other actinomycetes from other soils were tested and the majority gave good to fair aggregation. Table 3 classifies the fungi according to their influence on soil aggregation and to the type of mycelium produced

on glucose peptone agar. In general pure cultures of fungi produced far better aggregation than all other micro-organisms tested. The best strains were vigorous growers, producing woolly mycelia on both soil and agar, e.g. species of *Absidia*, *Mucor*, *Rhizopus*, *Chaetomium*, *Fusarium* and *Aspergillus*. Slightly poorer results were obtained by slower growing strains which gave a more prostrate growth on soil or agar, e.g. some species of *Penicillium*, *Cladosporium*, *Alternaria* and *Rhizoctonia*. Fair aggregation only was obtained by a very

Table 3. *Influence of fungi on soil aggregation*

Aggregation	Mean weight of aggregates >1 mm./50 g. soil (g.)	Total no. of strains	Growth on agar (no. of strains)	
			Woolly	Prostrate
Excellent	37–45	21	18	3
Very good	29–37	15	10	5
Good	21–29	11	3	8
Fair	13–21	3	0	3
	Totals	50	31	19

slow-growing unidentified fungus and by *Oospora lactis* and a *Monilia* sp. whose hyphae tended to break up into fragments. Again it was observed that vigorous sporers like penicillia and aspergilli impeded wetting of the soil. The best aggregator isolated from Haywards Heath soil was *Absidia glauca* (F7) (44·62 g.), but similar strains were isolated subsequently from many other types of soil.

It was rather surprising that most bacteria were less effective than actinomycetes or fungi, since other workers had found that bacterial gums were very effective in glueing soils into water-stable crumbs. In most cases these workers carried out aggregate analysis after wetting the soils for a shorter time than the 24 hr. needed to dissolve water-soluble gums; and they used specially selected strains of bacteria without stating whether they were abundant in field soils. Before dismissing bacteria as unimportant aggregators of soil enriched with organic materials, some further experiments were carried out to find whether the addition of liquid cultures to soil gave better results than growing the bacteria *in situ*.

Aggregation by bacterial liquid cultures

Previously the bacterial strains had been divided according to their ability to produce fair, poor, or no aggregation, and for the present experiment eight strains were selected from each of the three groups. They were grown for 20 days at 25° in shallow liquid medium containing 2·0 % glucose, 0·5 % peptone and the usual salts, shaken and their turbidity and viscosity estimated. Samples (15 ml.) were mixed with 50 g. of Rothamsted allotment soil (< 1 mm.) and after drying at 50° macro-aggregate analyses were carried out in the usual way. During soaking it was noticed that some of the treated soils wetted slowly, while others wetted readily. Table 4 classifies the bacteria according to their aggregating power, their influence on the wettability of soil, and the turbidity and viscosity of the cultures in the liquid medium.

In most cases the aggregation produced by liquid cultures was practically the same as that produced by bacteria grown in soil, with the exception that two strains formerly classified as fair and poor aggregators respectively were reclassified as poor and non-aggregators respectively. There was no perfect correlation between cementing power of the bacteria and any of their other properties, but better aggregation tended to be associated with poor wettability of soil and with high turbidity and viscosity of liquid cultures.

Table 4. *Influence of various bacterial properties on the aggregation and wettability of soil treated with liquid cultures*

			No. of strains with characters shown					
			Waterproofing action on soil		Turbidity of liquid culture		Viscosity of liquid culture	
Aggregation	Weight of aggregates 1 mm./50 g. soil (g.)	Total no. of strains	Low	High	High	Low	High	Low
Fair	12–15	7	2	5	5	2	5	2
Poor	9–12	8	2	6	6	2	2	6
None	6–9	9	4	5	6	3	3	6
	Totals	24	8	16	17	7	10	14

Six of the same strains from each of the three aggregation classes were grown in fifteen different media, all containing 0·5 % of peptone as the source of nitrogen, the usual salts and 2·0 % of various metabolites including one pentose, three hexoses, three disaccharides, one trisaccharide, three polysaccharides, one salt of an organic acid, two hexahydric alcohols and one peptone. During incubation it was noticed that the turbidity and viscosity of the cultures varied greatly, depending on the nutrients present. In the majority of cases glucose gave the best growth and the highest viscosity, but galactose, maltose and mannitol were often almost as good. The liquid cultures were added to soil as previously and the stability of the aggregates was determined (Table 5). It is evident that the stability of the aggregates depended on the bacterial culture medium. Metabolic products from glucose produced the most stable aggregates. Strains originally classified as fair aggregators also usually produced stable crumbs when cultivated in galactose, maltose and mannitol. Some poor aggregators also gave a few fairly stable crumbs when grown in mannitol and maltose. With few exceptions the strains from the non-aggregating class produced no stable aggregates from any media, although B 35 and B 163 both from this last group yielded fairly stable aggregates with mannitol and peptone respectively.

Two factors might influence the cementing power of strains grown in different media. Some nutrients might favour the production of larger quantities of gum than others. Judging by the various amounts of gum precipitated by ethanol from some of the media this is almost certainly true. It is also possible that the chemical composition of the gums and their glueing properties might depend on the source of carbon, but this point was not investigated. However, most bacteria isolated from soil do not produce very stable aggregates.

Properties and effects of microbial gums

In view of the large amounts of uronide carbon found in soils by Fuller (1946, 1947*a*, 1947*b*) an attempt was made to correlate the physical and chemical properties of various microbial gums with their cementing power after mixing 0·5 % of gum with sifted Rothamsted allotment soil (< 1 mm.). Gums from

Table 5. *Influence of bacterial foodstuff on stability of soil aggregates*

	Effect of groups of six bacterial strains originally classified by their behaviour with glucose as					
	Fair		Poor		None	
No. of strains giving aggregates that were ...	Stable	Unstable	Stable	Unstable	Stable	Unstable
Metabolite:						
Xylose	1	5	0	6	0	6
Glucose	6	0	6	0	0	6
Laevulose	1	5	1	5	0	6
Galactose	6	0	2	4	0	6
Sucrose	3	3	1	5	0	6
Maltose	6	0	3	3	0	6
Lactose	2	4	2	4	0	6
Raffinose	0	6	0	6	0	6
Dextrin	2	4	1	5	0	6
Soluble starch	1	5	1	5	0	6
Inulin	2	4	1	5	0	6
Calcium gluconate	0	6	2	4	0	6
Mannitol	5	1	4	2	1	5
Sorbitol	0	6	1	5	0	6
Peptone	0	6	1	5	1	5

four bacteria and two fungi were prepared by the methods given previously (p. 238). In addition, fungal hyphae of strain F 27 were composted for 20 days at 25° after inoculating with soil suspension and a gum was isolated from the sludge by the technique used for preparing bacterial polysaccharides. A gum from the fulvic acid fraction of soil humus was isolated by the method of Forsyth (1947). Sodium α-humate was extracted from Rothamsted allotment soil by the method used by Waksman (1938). A few of the physical and chemical properties of these eight gums are summarized in Table 6. For comparison the properties of a typical soil humate are also given. Only two gums were insoluble in water, viz. from F 7 and F 27, and they produced of course very stable aggregates when their hot alkaline solutions were added to soil. Of the remaining six gums, all were soluble in aqueous solutions of NaOH, HCl and $CaCl_2$, except for a rather impure gum isolated from F 27 compost, which was precipitated by calcium salts. Half these polysaccharides were flocculated by $FeCl_3$; all were precipitated from aqueous solution by 75 % ethanol. The difference in behaviour of soil humate from most of the gums is very striking. Evidently the other constituents in humus either mask the properties of the polyuronides also present, or else polysaccharides different from those studied are involved.

There was no clear correlation between the cementing powers of the gums and the particular properties studied.

Most of the bacterial gums when mixed with soil gave only poor to fair aggregation because most of the gums diffused out into the water during sieving operations. Treatment with H^+ or Ca^{++} ions did not improve matters because the gum films were not precipitated by these ions. It was thought that

Table 6. *Some properties of gums from bacteria, fungi, decomposing fungi, and soil*

Test or reagent	*Achromobacter* sp. (B45)	*Achromobacter* sp. (B68)	*B. polymyxa* (B169)	*B. subtilis**	*Absidia glauca* (F7)	*Aspergillus nidulans* (F27)	Decomposing F27	Soil gum†	Na-α-humate (Rothamsted)
Cold H_2O	Sol.	Sol.	Sol.	Sol.	Insol.	Insol.	Sol.	Sol.	Sol.
Boiling H_2O	Sol.	Sol.	Sol.	Sol.	Insol.	Insol.	Sol.	Sol.	Sol.
0·1 N-NaOH	Sol.	Sol.	Sol.	Sol.	Sol.-hot, insol.-cold	Sol-hot, insol.-cold	Sol.	Sol.	Sol.
0·1 N-HCl	Sol.	Sol.	Sol.	Sol.	Insol.	Insol.	Sol.	Sol.	Insol.
0·1 N-$CaCl_2$	Sol.	Sol.	Sol.	Sol.	Insol.	Insol.	Sl. sol.	Sol.	Insol.
0·1 N-$FeCl_3$	Insol.	Sol.	Insol.	Sol.	Insol.	Insol.	Sl. sol.	Sol.	Insol.
75 % ethanol	Insol. (floc.)	Insol. (powder)	Insol. (strings)	Insol. (powder and strings)	Insol. (floc.)	Insol. (floc.)	Insol. (floc.)	Insol. (floc.)	Sol.
Sugars in hydrolysate	Glucose	Glucose	Glucose mannose	Laevulose	—	—	—	Glucose xylose arabinose	—
Uronic acid group	Present	Present	Present	—	—	—	—	Present	Present
Aggregation (%)	34·10 ±1·42	12·28 ±0·98	53·48 ±2·34	90·80 ±2·12	97·26 ±2·76	96·51 ±3·26	65·19 ±2·22	8·20 ±0·86	27·70 ±1·88

* Obtained from M. J. Geoghegan, Jealott's Hill Research Station

† Obtained from W. G. C. Forsyth, Macaulay Institute.

merely mixing them with soil paste might not give adequate contact between the colloidal micelles of gum and those of clay. Consequently an attempt was made to separate the clay particles by water molecules and to insert the polysaccharides between them.

Stability of aggregates formed by co-precipitation

Suspensions (4 %) of Na-bentonite, Na-kaolin and freshly prepared ferric hydroxide were shaken overnight to disperse them completely. Similar concentrations of Rothamsted surface soil and subsoil were dispersed with sodium oxalate. Colloidal solutions of gums from *Achromobacter* spp. (B45, B68) and *B. polymyxa* (B169) were added to these suspensions of clays and soils in a concentration of 0·02 % by weight of gum. The mixtures of ferric hydroxide and gums began to coagulate and settle almost immediately but the other suspensions remained stable. Co-precipitates were formed by adding 0·05 N-$CaCl_2$ and allowing the floccules to settle overnight. The supernatant was decanted and tested for gums by adding 3 volumes of ethanol and looking for a precipitate after 24 hr. No gums were detected from ferric hydroxide treatments, and

only traces were obtained from other treatments. The co-precipitates were dried at 50°, the flakes were resoaked for 48 hr. and wet-sieved. The weight of aggregates from 10 g. of material are given in Table 7. Ferric hydroxide gave extremely stable aggregates both in the presence and absence of bacterial gums. Bentonite flakes swelled and slaked more when gums were added than when they were absent. The reverse was the case with kaolin, Rothamsted surface soil and subsoil, with which the gums improved aggregation.

Table 7. *Stability of aggregates formed by co-precipitating clays or soils with bacterial gum*

	Bacterial gum used (Mean weight (g.) of aggregates > 1 mm./10 g. soil)			
Clay or soil	B45	B68	B169	Untreated
$Fe(OH)_3$	10·02 ± 0·26	10·00 ± 0·20	10·03 ± 0·29	9·96 ± 0·27
Na-bentonite	8·51 ± 0·19	8·30 ± 0·33	8·68 ± 0·48	9·63 ± 0·35
Na-kaolin	6·85 ± 0·25	6·96 ± 0·37	7·72 ± 0·41	4·87 ± 0·13
Rothamsted soil (0–4 in.)	8·90 ± 0·46	—	—	7·53 ± 0·50
Rothamsted soil (18 in.)	9·31 ± 0·23	—	—	6·25 ± 0·18

In natural soils it is possible that over a long period of time micelles of bacterial gum might diffuse in between platelets of clay and thereby cement them better. This would presuppose that the bacterial gums were fairly resistant to microbial attack. Martin (1945, 1946) found that bacterial polysaccharides were slowly attacked by soil organisms. Gums isolated from B45, B68, B169 and *B. subtilis* (Geoghegan) certainly lose their viscosity after inoculation with micro-organisms. Ensminger & Gieseking (1942) found that proteins were protected from enzymic digestion by clay and it is possible that adsorbed polyuronides might also survive microbial attack.

Influence of pH *on aggregation by pure cultures*

An experiment was planned to determine the effect of soil pH on the aggregation brought about by different micro-organisms.

Haywards Heath soil of pH 4·7 was chosen for this experiment, because unlike Rothamsted allotment soil it contained no free lime and its reaction could be changed readily by the addition of KOH or H_3PO_4. After adjustment to pH values of 3·5, 4·7, 6·0, 7·2 and 8·5, sterile soil samples were treated with 0·5 % of glucose and 0·036 % of peptone and minerals, then separate samples inoculated respectively with bacteria B2 and B45 from the fair-aggregation class, bacteria B35 and B91 from the poor cementing group, and bacteria B50 and B163 from the non-aggregation class. In addition an actinomycete, A3, two fungi, F7 and F27, and Rothamsted soil inoculum were also used in the experiment. After 3 weeks at 25° aggregate analyses were made. The results for the bacterial treatments may be summarized by saying that B2 gave no significant difference in aggregation at the acid pH values of 3·5, 4·7 and 6·0 and the alkaline value of 8·5, but better aggregation at pH 7·2;

B45 produced no stable aggregates at pH 3·5, but fairly stable crumbs at all other values; B35 did not grow at pH 3·5, and B35 and B91 produced little or no aggregation at any reaction; and finally B20 failed to grow at pH 3·5, and B50 and B163 formed no stable crumbs throughout the entire pH range. In contrast to the poor performance of bacteria, A3 formed fairly stable crumbs equally well at all pH values between 4·7 and 8·5, but was killed by pH 3·5. The fungi F7 and F27 were checked a little at the extreme acid values, but produced very good aggregation at most other pH values. Soil inoculum gave results intermediate between the actinomycete and the fungi. Soil pH did not affect the aggregation of sterile soil. The aggregation of uninoculated soil receiving sterile glucose, peptone and salts was no different from that receiving sterile water only.

Evidently the pH value plays a very minor part in influencing the aggregation produced by pure cultures of micro-organisms or even by soil inoculum. This is in agreement with field experience, where the addition of readily decomposable organic matter improves the structure of all soil types irrespective of pH value. It is realized, however, that some organisms may be dominant at one pH and other types at another pH; but the resultant effect on structure is much the same.

Tests with pure cultures can hardly be expected to elucidate effects obtained in the field, so experiments were made with mixed cultures of micro-organisms.

Aggregation of soil by mixed cultures

Two bacteria (B45 and B169), two actinomycetes (A3 and A16) and two fungi (F7 and F27) were added singly and in combinations of two, three and six organisms into 50 g. of sterilized Rothamsted allotment soil (<2 mm.), together with 0·5 % of glucose, 0·036 % of peptone and minerals; sterile soil and soil inoculum were also included in the treatments. After 3 weeks at 25° the soil aggregates were analysed.

As already observed, pure cultures of the fungi gave very good aggregation, the actinomycetes produced good aggregation, while single cultures of bacteria formed only a fair weight of crumbs. Pairs of fungi or actinomycetes produced significantly more aggregates than single organisms, possibly because they mutually assisted one another; but the pair of bacteria was no better than strain B169 alone. When a fungus or an actinomycete was grown in association with a bacterium, there was a small increase in aggregation which in some combinations attained significance. Possibly the bacterial gums were prevented by the network of hyphae from diffusing into the water during soaking of the soil. There was also a small but insignificant increase in effect in mixtures of a fungus with an actinomycete.

Mixed cultures of three micro-organisms consisting of a pair of bacteria, of actinomycetes or of fungi together with a third organism sometimes produced a significant improvement as compared with the same pair or the same three components growing separately. Mixtures containing fungi always produced more aggregates than mixed cultures containing only actinomycetes and bacteria.

The effects of a bacterium, an actinomycete and a fungus alone, and of mixtures of these organisms are shown in Pl. 1, fig. 1.

The best aggregation of all was obtained when all six organisms were grown together. In view of this fact it was rather surprising to find that soil inoculum, containing numerous microbial types, formed much fewer aggregates.

Influence of inhibitory bacteria on soil aggregation

The unexplained behaviour of soil inoculum stimulated further investigation of the compatibility of mixtures of other microbial strains. Bacteria capable of inhibiting the growth of fungi and actinomycetes growing on agar were easily found, and it was suspected that the anomalous behaviour of soil inoculum might be due to the presence of such bacterial antagonists. Accordingly, an experiment was conducted to find whether inhibitory bacteria could prevent the formation of aggregates by fungi and actinomycetes.

Two bacteria, B 8 and B 163, antagonistic to the growth of F 7 and A 3, were inoculated singly and in combinations into the usual amount of sterile Rothamsted soil enriched with glucose, peptone and minerals. After 20 days at 25° the soils were analysed by wet sieving; the figures are given in Table 8.

Table 8. *Influence of inhibitory bacteria on aggregation of soil by actinomycetes and fungi*

Micro-organisms used	Mean weight of aggregates > 1 mm./50 g. soil (g.)
B 8	9·58 ± 0·71
B 163	8·32 ± 1·12
A 3	19·98 ± 0·43
F 7	36·27 ± 1·16
B 8 + B 163	8·46 ± 0·27
B 8 + A 3	11·10 ± 0·20
B 8 + F 7	13·84 ± 1·79
B 163 + A 3	10·75 ± 0·49
B 163 + F 7	15·17 ± 1·39
A 3 + F 7	39·95 ± 0·91
B 8 + A 3 + F 7	24·20 ± 0·32
B 163 + A 3 + F 7	20·78 ± 1·12
B 8 + B 163 + A 3 + F 7	18·63 ± 0·74
Soil inoculum	21·54 ± 0·46
Sterile	8·77 ± 0·14

B 8 and B 163, grown singly, did not significantly improve soil structure. On the other hand, A 3 in pure cultures produced good aggregation, F 7 also gave excellent results, and the pair together gave the best effect of all. The presence, in mixtures, of the inhibiting bacteria B 8 and B 163, whether combined with one or two other organisms, always decreased the aggregation. This is seen in Pl. 1, fig. 2 which shows the effect of growing B 163, A 3 and F 7 in various combinations.

Such inhibition can hardly be explained by competition for food because free glucose was often found at the end of the experiment. It seems more likely that it was due to the production by the bacteria of antibiotic substances which hindered the growth of the fungus and the actinomycetes. This suggested imitating the effect of soil inoculum on aggregation by using very complex mixtures of bacteria, actinomycetes and fungi, some of which were perfectly compatible and others antagonistic. By using selected micro-organisms it was possible to use not only glucose as foodstuff but also more complex materials such as plant and animal products. It was hoped that this would lead to a better understanding of the improvement in soil structures obtained when crop residues, green manure crops, or ley pastures were ploughed in.

Influence of mixed cultures and complex organic materials on aggregation

Various organic materials (0·5 %) were added to soil. As examples of readily available carbohydrates, glucose and soluble starch were used in conjunction with 0·036 % of peptone and minerals. Blood was tried as a readily decomposable protein. Oat-straw, with a low nitrogen content and a moderately high percentage of lignin, was added as an example of a typical crop residue. Red clover with a fair nitrogen content was used as representing a common green manure crop. The nitrogen supply was augmented by adding 0·014 % of NH_4NO_3. Rotted farmyard manure was included as a material that had become humified through composting. It is probable that dead bacterial and fungal cells serve as nutrients for other soil micro-organisms; so killed and dried yeast cells and mycelium of *Aspergillus niger* were included in the trial. Each of these organic materials was tested in Rothamsted clay loam, and glucose, straw and clover were also tested in Woburn sandy loam. Soils with each added material were divided into sets inoculated respectively with twenty-two fungal, thirty-three actinomycete and sixty-six bacterial strains and with a mixture of the above 121 strains. A set with soil inoculum and a sterile control were also included. All inocula contained micro-organisms capable of decomposing cellulose, chitin, starch, pectin, agar, lignin, resins, fats, proteins and sugars. Soil inoculum was included for comparison. Macro-aggregate analyses were carried out after 3, 6 and 9 weeks at 25°.

In comparable treatments the heavier textured Rothamsted soil aggregated better than the light soil from Woburn, especially with oat-straw and red clover. Apart from this difference the various inocula and organic materials behaved similarly in both soil types. The mean aggregating influence of different groups of organisms utilizing the same foodstuffs ran in the following order: fungi > soil inoculum = fungi + actinomycetes + bacteria > actinomycetes > bacteria > none. This suggested that soil inoculum could be closely imitated by using a complex microbial mixture containing numerous fungi, actinomycetes and bacteria, some of which were compatible and others incompatible. In Rothamsted soil the relative effect of the organic materials varied greatly with the inoculum and length of incubation. In Woburn soil the superior effect of the readily

available glucose was striking. Pl. 2, fig. 3 shows the influence of the various inocula on Rothamsted soil supplied with glucose.

Previous workers have found that soil structure was restored more rapidly by readily decomposable materials than by resistant materials; this finding is supported by the good effects of glucose, yeast and starch. It appears that good crumb structure can be achieved only when maximum microbial activity is promoted by a plentiful supply of easily digested food, especially materials which encourage fungi, actinomycetes and gum-forming bacteria.

Disaggregation of soil stabilized by fungal hyphae

An experiment was designed to find which organisms caused disaggregation of a soil that had been mechanically stabilized by fungal hyphae.

Fifty portions of Rothamsted allotment soil (<2 mm.) were sterilized in percolation funnels plugged at the bottom with glass wool and on the top with cotton wool. A sufficient volume of medium containing spores of the fungi, F7 or F27, was added to bring the soil moisture to field capacity and to provide 0·5 % of glucose, 0·014 % of NH_4NO_3 and the usual minerals. The fungi were allowed to grow for 3 weeks at 25° until they had stabilized all the soil aggregates and the residual soluble metabolites and products were then leached out aseptically with Hoagland & Arnon's complete nutrient medium sterilized and diluted. Some of the tubes of soil were autoclaved to kill the fungi, while others were left unheated. Six sets were then inoculated respectively with mixtures containing ten different fungi, ten strains of actinomycetes, seven, fifteen and thirty of selected bacteria and soil inoculum. A sterile control was also included. The mixtures of fungi, actinomycetes and bacteria had previously been tested for ability to grow in a shallow liquid medium containing only sterilized fungal fragments and minerals. The bacterial inocula contained organisms capable of digesting a wide variety of substances including cellulose, chitin, agar, starch, sugars, resins, butter-fat and proteins. The inoculum containing seven bacteria included two *Cytophaga* spp. *Pseudomonas fluorescens*, *Bacillus mycoides*, an *Achromobacter* sp., and two unidentified strains capable of digesting agar and butter-fat. Inocula comprising fifteen and thirty strains included these seven strains together with various isolates from soil previously enriched with dead fungal fragments. Macro-aggregate analyses were carried out at intervals over a period of 8 weeks.

Under sterile conditions dead hyphae of F27 continued to bind the soil together throughout the experimental period. Growth from the inoculum containing the ten fungi caused no loss of structure during the period of the experiment, probably because dead fungal threads were replaced by living hyphae, so that the soil remained aggregated. With the ten actinomycetes, however, aggregation declined, probably because the fungal mycelia were replaced by weaker threads of actinomycetes. Treatment with seven bacterial strains and soil inoculum caused a marked deterioration of structure even after 2 weeks at 25°. This effect was accentuated when fifteen and thirty bacterial strains were used. Evidently the products of bacterial decomposition of the

fungus F27 were not as effective in aggregating the soil as the original fungal hyphae. Pl. 2, fig. 4 shows the effect of the various inocula on soil aggregates originally stabilized by F27. Similar results were obtained with living hyphae of F27, but here the treatments gave smaller losses in aggregation than with dead hyphae. In contrast to the results from fungus F27, the other soil stabilizing fungus F7 proved to be very resistant to bacterial attack. Fungi and actinomycetes certainly grew on the dead mycelium of F7, but as before, only the actinomycetes caused any disaggregation. Microscopic examination of the soil inoculated with numerous bacteria showed that they were growing but were not decomposing the cell walls of the hyphae of F7, so that soil aggregation remained practically unchanged over a period of 8 weeks. Similarly, soil inoculum caused no disaggregation until after 6 weeks' incubation.

Estimated influence of micro-organisms on aggregation of field soil

An attempt was made to calculate the amount of macro-aggregation that might be attributed to fungal hyphae and to gum-forming bacteria in Rothamsted allotment soil that had been cultivated for many years.

Pure cultures of *Absidia glauca* (F7) and *Aspergillus nidulans* (F27), growing in sterilized Rothamsted soil enriched with glucose, formed 242 and 374 m. of hyphae/g. of soil as determined by the method of Jones & Mollison (1948) and they entangled 96·5 and 80·3 % respectively of the soil into stable aggregates. The length of fungal hyphae, found in fresh Rothamsted allotment soil was 38·8 m./g. The length of hyphae should thus aggregate 15·5 % of the soil, if they behaved like species F7 and 8·4 % if like species F27. The fresh soil actually contained 38 % of aggregates, an appreciable fraction of which might hence be attributed to the effect of fungi.

A connexion between fungal mycelium and aggregation is also shown in the data previously quoted from Haywards Heath soil incubated for 17 days with the addition respectively of blood, starch and untreated. Here the percentage of macro-aggregates (a), though very different with the various treatments, yet shows a regular relationship to the lengths of mycelium (m) in which $a = 0{\cdot}3m + K$, where K is a constant of value 9·5 %. This is shown in the following figures.

		Percentage aggregation a	
Treatment	m	Calculated	Found
Untreated	25·8	17·2	17·0
Blood	49·5	24·3	22·2
Starch	96·2	38·4	39·0

The estimate of aggregation attributable to slimy bacteria was based on a test with strain B45 and thus assumes this strain to be representative. It also assumes that the proportion of slimy bacteria was the same in the natural field soil as it was amongst plate colonies derived from a suspension of that soil.

On these assumptions the glueing power of the native bacteria accounts for only about 2 % of aggregation out of a total of 38·0 % in the field soil. On these

calculations the combined effect attributable to mycelial threads and to bacterial gums can account for less than half of the macro-aggregates found in fresh Rothamsted soil. While it is true that microbial threads and gums could largely account for the increased temporary aggregation of soils supplied with readily decomposable organic matter, it is evident that existing fungal and actinomycete hyphae plus capsulated bacteria cannot account for the aggregation of natural Rothamsted soil to which fermentable material has not recently been added. One must thus conclude that the formation of more permanent crumbs found in certain soils must be due to other cementing substances, e.g. colloidal clay, humus or resistant gums produced by micro-organisms no longer visible.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Influence of pure and mixed cultures of micro-organisms on aggregation. The bacterium was B45, the actinomycete, A3, and the fungus, F7.

Fig. 2. Influence of inhibiting bacterium B163 on aggregation by actinomycete A3 and fungus F7.

Plate 2

Fig. 3. Influence on mixed inocula on aggregation of soil enriched with glucose.

Fig. 4. Microbial disaggregation of soil previously stabilized by hyphae of fungus F27. 1, sterile; 2, ten strains of fungi; 3, ten strains of actinomycetes; 4, seven strains of bacteria; 5, fifteen strains of bacteria; 6, thirty strains of bacteria.

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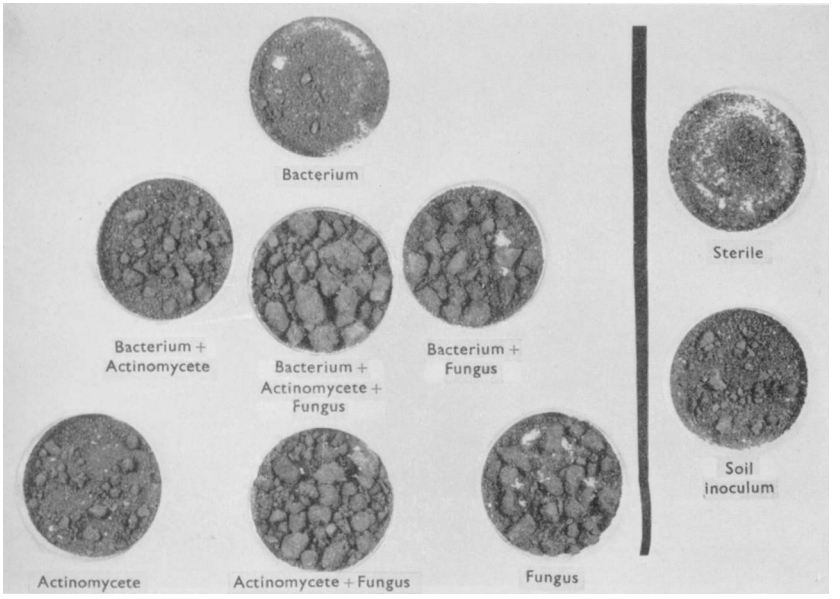

Fig. 1

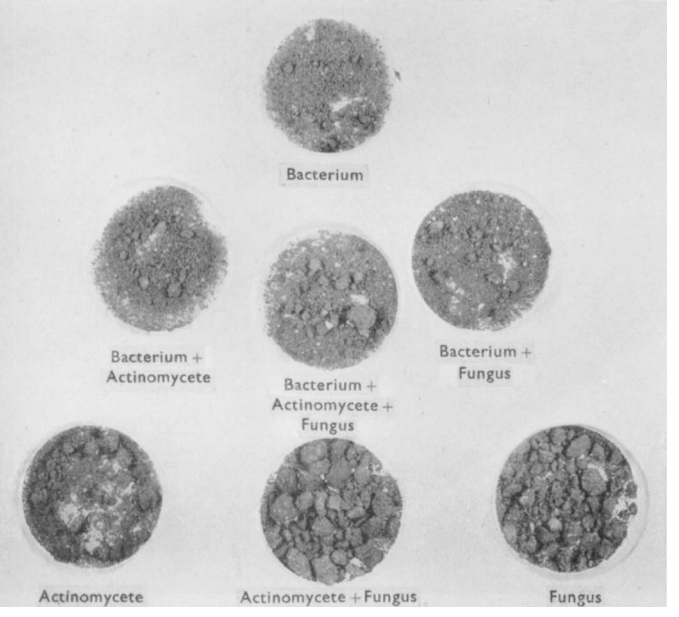

Fig. 2

R. J. Swaby—Micro-organisms and soil aggregation. Plate 1

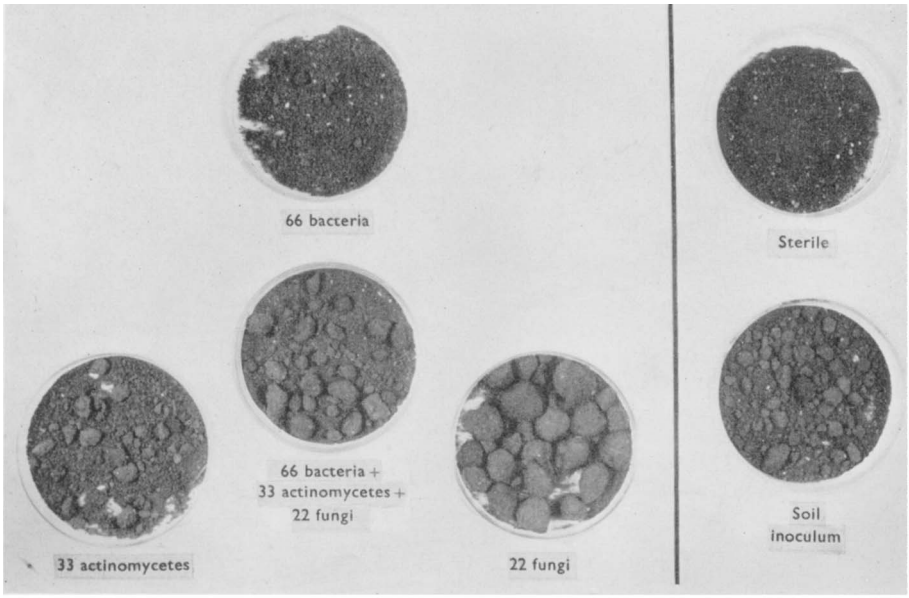

Fig. 3

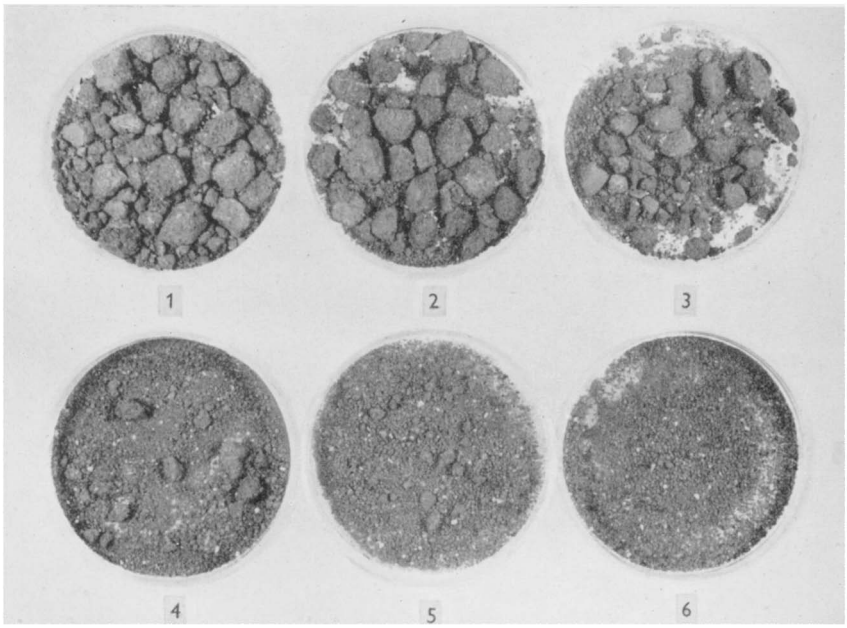

Fig. 4

R. J. SWABY—MICRO-ORGANISMS AND SOIL AGGREGATION. PLATE 2