ROTHAMSTED RESEARCH

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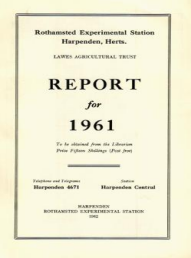

Microbiological Observations of the Classical Fields at Rothamsted

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MICROBIOLOGICAL OBSERVATIONS ON THE CLASSICAL FIELDS AT ROTHAMSTED

JANE MEIKLEJOHN

The Rothamsted field experiments offer great opportunities for the study of Soil Microbiology. Here, as nowhere else, the effects of long-continued treatments of the same soil on its population of micro-organisms can be observed. Perhaps the opportunities have not been fully exploited, but several fundamental discoveries of the true nature of the microscopic population of soils have been made, notably that Protozoa are true soil inhabitants, and that the total population must be reckoned in thousands of millions per gram of soil. (Much work has also been done on soil-borne plant pathogens in the Rothamsted fields, but this has been most ably reviewed by others (see, for instance, Glynne & Salt, 1957), and little would be gained by repeating what has been better done already.)

The earliest microbiological observations on Rothamsted soils were made by Robert Warington in 1883. In that year he took soil samples at different depths on Agdell field, which at that time carried a long-term experiment on two four-course rotations (turnips, barley, bare fallow, wheat; turnips, barley, clover, wheat) and found that surface soil, and soil down to but not below 18 inches, contained nitrifying bacteria. He repeated the work the next year, when he failed to find nitrifiers at a depth greater than 9 inches (Warington, 1884).

There was then a gap of more than twenty years before Ashby (1907) examined a series of soils collected at Rothamsted for the then recently discovered bacterium, *Azotobacter chroococcum*, which could fix nitrogen from the air. He found that it was abundant in the soil of Broadbalk Wilderness, and fairly abundant in the Drain Gauges. In Agdell it was present in the limed but not the unlimed plots, and it could not be found in three Park Grass plots (1, 4 and 9) or in Geescroft Wilderness (which has a more acid soil than the Broadbalk Wilderness).

Two years later, in 1909, appeared the first of a series of papers by Russell and Hutchinson on the effects of partial sterilisation of soil on crop growth and on the numbers of bacteria. Their first experiments were made with unmanured soil taken from the headland of Barnfield. They treated this soil with steam, and with toluene, and counted the bacteria by the plate method on nutrient gelatine. The count in the untreated soil was 5–9 million bacteria/g.; treatment with steam or toluene diminished the count to about half this value at first, but 10 days later the count had risen to 40 million bacteria/g. The same gelatine plate method showed bacterial numbers of 14–77 millions/g. in the dunged plot of Barnfield, but only 12 millions/g.

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in Hoos Field unmanured plot (Russell and Hutchinson, 1909, 1913; Hutchinson, 1913). Partial sterilisation thus increased crop yields and bacterial numbers after a temporary fall; from these results Russell and Hutchinson concluded that there was some factor in soil which limited both bacterial growth and soil fertility, and which was removed by partial sterilisation. As they succeeded in growing the ciliate Protozoon *Colpoda* in hay infusion inoculated with soil, they thought that it must be the soil Protozoa that were the harmful factor. Goodey (1911) found several species of Protozoa, mostly ciliates but some amoebae, in fresh and in old stored soil samples taken from Barnfield and Hoosfield unmanured plots. Critics of his work suggested that the Protozoa were not true soil inhabitants, but were accidentally blown into the soil in their encysted stage, and remained encysted. Martin and Lewin (1915), however, discovered that Protozoa lived in the soil as active forms, mobile and able to feed, and not only as cysts. They examined samples taken from Broadbalk plots 2 and 3 (FYM and unmanured), and from Agdell fallow.

In 1917 Russell and Appleyard published counts made on gelatine plates of bacteria from soil samples collected during a whole year from Broadbalk plots 2 and 3 (and from Great Harpenden field). Numbers fluctuated on all the plots and their attempt to relate the fluctuations to the nitrate content of the samples (not surprisingly) failed. The fluctuations were not related to soil temperature or soil moisture, and Russell and Appleyard said that they might have been caused by the predatory activities of soil Protozoa. The carbon dioxide output of incubated soil samples was shown to be related to the numbers of bacteria in them, and the output of carbon dioxide has been used since by many workers as a convenient index of microbial activity in soils. This paper also includes one of the earliest estimations of the amount of nitrate lost from the soil in autumn by leaching and otherwise.

In 1920 Cutler published a method for counting total numbers of Protozoa, and also the numbers of active (i.e., not encysted) Protozoa in soil samples. This method, which depended on the development of Protozoa on peptone–meat-extract–agar plates inoculated with dilutions of a soil suspension, was used by Crump (1920) to study the soil Protozoa in plots 2 and 3 of Broadbalk, the dunged plot (1–0) of Barnfield and an unmanured plot on Great Harpenden. She found that the most numerous Protozoa were small flagellates, which varied in numbers from 1,000 to 100,000/g. of soil. Amoebae were fewer, from 100 to 50,000/g., and ciliates were not always present, and never more than 1,000/g. Very few Protozoa were found deeper than 6 inches below the soil surface. In surface samples there were more Protozoa in dunged plots than in unmanured plots. In all the plots the numbers fluctuated similarly, with peaks at the same time of year; the fluctuations could not be correlated with changes in soil temperature or moisture, or with rainfall, but there was some indication that they went in the opposite direction to changes in the plate count of bacteria.

This point was investigated further by Cutler and Crump (1920), who found that the numbers of active forms of three common soil flagellates (*Oicomonas*, *Cercomonas* and *Bodo* spp.) in the soil of

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Broadbalk plot 2 fluctuated from day to day. Once again, these changes could not be related to soil moisture or temperature. In the same soil the numbers of active amoebae varied inversely with the plate counts of bacteria. A year later a really heroic experiment was undertaken by Cutler, Crump and Sandon (1922). They took a soil sample from the dunged plot (1–0) of Barnfield on every one of the 365 days from 5 July 1920 to 4 July 1921, and from these samples they obtained plate counts of bacteria on Thornton's agar (Thornton, 1922), and counted total and active Protozoa by Cutler's method. The results were examined in great detail, and showed that, on many occasions, the bacterial numbers were low when the Protozoa numbers were high, and vice versa. A common picture was to see a sudden rise in the bacterial count followed two or three days later by a rise in the numbers of one or other of the Protozoa. These results seemed at the time to indicate that the predatory action of the Protozoa was limiting the numbers of bacteria in soils; but subsequent work showed that knowledge of both the predators and the prey was far from complete. (See Thornton and Crump, 1952.)

It had become apparent for some time that the counts of bacteria obtained by plate methods were gross underestimates of the real soil population. No agar medium, however " unselective ", permits every bacterial species to develop, and it was obvious that anaerobic bacteria, and autotrophs, were not being included in the counts. Just how gross the underestimate was, however, came as a surprise to most bacteriologists. The plate counts, even on a better medium than the nutrient gelatine used by Russell and Hutchinson, estimated the bacterial population of a fertile soil at some tens of millions per gram. The first method of total counts, the ratio method of Thornton and Gray (1934), showed that the real bacterial population might be more than a hundred times as great. In this method a known small quantity of soil was mixed with a suspension of indigo containing a known number of indigo particles per ml., and drops of the mixture spread on microscope slides and stained with a red dye. The bacteria appeared as red dots and the indigo particles as blue dots, and comparing the numbers of each in random microscope fields gave an estimate of the soil population, of the order of thousands of millions per gram of soil. For instance, counts on Barnfield soil ranged from 1,900 to 2,900 millions/g. on different plots, and counts on Hoosfield from 1,700 millions on plot 1–0 (unmanured) up to 3,700 millions on plot 4-AA (nitrate of soda, phosphate and potash).

Another total counting method, the agar-film method of Jones and Mollison (1948), gives estimates of the same order of numbers. Counts done on Broadbalk by this method showed that Plot 2 (FYM) had the most bacteria and actinomycetes, and Plots 3 (unmanured) and 7 (ammonium sulphate and minerals) had similar numbers. The average count by the direct method was 2,500 million cells/g. of soil, and the average plate count 50 millions/g., $\frac{1}{50}$ of the direct count (Skinner, Jones and Mollison, 1952).

As well as the total numbers, several particular groups of micro-organisms have been counted at Rothamsted. Algae were counted in samples from Broadbalk plots 2 and 3 by Bristol Roach (1927), who found the same species on both plots, but more numerous on the

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dunged plot 2. J. Singh (1937) made plate counts of fungi and actinomycetes, on an agar medium, pH 5·2, that was not really favourable to either group. On both Broadbalk and Barnfield he found most in the plots with organic manures, and fewest in the unmanured plots. Skinner (1951) found that the high numbers of actinomycetes in Broadbalk plots 2, 3 and 7 were nearly all in the spore stage at the time of counting, with very little mycelium present.

Jensen (1931) used soil from two plots on Park Grass to study the decomposition of farmyard manure. In the limed soil from plot 1, pH 7, he found that the numbers of bacteria and actinomycetes were increased when the soil was incubated with farmyard manure and straw; but in the acid unlimed soil, pH 3·8, from plot 14, the same treatment increased only the numbers of fungi. From the soil of plot 1 he isolated a cellulose-decomposing bacterium, a species of Vibrio, which appeared to be the most numerous cellulose decomposer at pH 7. From the acid plot 14 he isolated cellulose decomposing fungi; and from Hoosfield soil, of intermediate acidity (pH 6·3), he obtained the Vibrio, and also the Myxobacterium *Sporocytophaga*, which had been discovered at Rothamsted by Hutchinson and Clayton in 1919 (Jensen, 1931b). From the Hoosfield soil Jensen also isolated two actinomycetes active in breaking down keratin (Jensen, 1930) and two species of fungi able to decompose chitin (Jensen, 1932).

In 1946 B. N. Singh described an improved method of counting Protozoa in soils. This was based on his discovery that the Protozoa were selective in their choice of food, eating some species of bacteria readily and others not at all. Small circles of an edible bacterium were spread inside glass rings embedded in plain agar, and inoculated with serial dilutions of the soil sample. As the Protozoa developed they ate the bacterial circle, and the different kinds were easy to detect in the small space. Singh (1949), by this method, counted amoebae in plots on Barnfield and Broadbalk. On both fields numbers were highest in the dunged plot and lowest in the unmanured, with a plot with sulphate of ammonia and minerals giving a count between the two (see Table 1).

TABLE 1

Numbers of amoebae per gram dry soil (Singh, 1949)

Barnfield (mean of 9 observations)			
Unmanured (8–0)	FYM (1–0)	Ammonium sulphate and minerals	
8,000	34,000	26,000	(4 A)
Broadbalk (mean of 6 observations)			
Unmanured (3)	FYM (2)	Ammonium sulphate and minerals	
17,000	72,000	48,000	(7)

Singh also discovered, by this same method, that several micropredators, once thought to be rare, were in fact widely distributed and numerous in soils. Some of these, the Acrasieae, for instance, were thought to live in dung only, and not to be true soil inhabitants. But Singh found that the Acrasian *Dictyostelium*, the gigantic amoeboid predator *Leptomyxa reticulata*, and the higher Myxobacteria *Myxococcus*, *Chondrococcus* and *Archangium*, were all pre-

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sent and quite numerous in the soil of Broadbalk Plot 3, which has received no manure of any kind for more than a hundred years (Singh, 1947a, 1947b, 1948).

Some specialised groups of bacteria have also been counted in the Rothamsted classical fields. In the autumn of 1930 and 1931 Ziemięcka (1932) took a series of soil samples, and used Winogradsky's method of crumbs of soil on silica gel to count the nitrogen fixer *Azotobacter chroococcum*, and also the ammonia-oxidising bacteria in them. Her counts of Azotobacter, given in Table 2, show more in plots without added nitrogen.

TABLE 2

Azotobacter: cells per gram dry soil (Ziemięcka, 1932)

	Mean Nos. of Azotobacter
Unmanured (6 observations)	
Broadbalk 3, Hoosfield 1–0 and 7–1, Agdell 5 and 6 ...	852
P, K, *no* N (6 observations)	
Broadbalk 5, Hoosfield 4–0, Agdell 3 and 4, Barnfield 4–0	2,382
N: *Ammonium sulphate* (10 observations)	
Broadbalk 6, 7, 8, 10, 11, 12, 13, 14, Hoosfield 4–1 ...	231
N: *Sodium nitrate* (7 observations)	
Broadbalk 9 and 16, Hoosfield 3–AA and 4–AA, Barnfield 4–N	568
N: *Organic* (7 observations)	
Broadbalk 2 and 19, Hoosfield 3–0, 4–0 and 7–2	550
All plots without nitrogen (12 observations)	1,617
All plots with nitrogen (24 observations)	423

The ammonia oxidisers, by contrast, seemed to be most numerous in the plots with added organic nitrogen (farmyard manure or rape cake) (Table 3). Ammonia-oxidising bacteria have now been isolated from Broadbalk Plot 2, and identified as *Nitrosomonas europaea* Winogradsky (Meiklejohn, 1949).

TABLE 3

Ammonia oxidisers: cells per gram dry soil (Ziemięcka, 1932)

	Broadbalk	Barnfield
Unmanured	252	384
P, K, no N	406	334
Ammonium sulphate	1,949	252
Sodium nitrate	—	987
Rape cake	1,336	3,687
Farmyard manure	2,243	3,198

The results in these two tables are based on single observations. Recently I have counted *Azotobacter chroococcum* at intervals over a period of 3½ years in samples taken from eight plots on Broadbalk (Meiklejohn, 1962). The number of cells always fluctuated from sample to sample, but in spite of this there were real differences between plots. In general, the plots which showed the greatest increase in the yield of wheat after fallowing had the most Azotobacter. Plot 10, for instance, which receives sulphate of ammonia only (no P or K), and has an average increase in yield of 3 cwt. of grain/acre, has consistently fewer Azotobacter than Plot 5, receiving P and K but no N, which has an average yield increase of 9 cwt./acre

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after fallow. Table 4 shows the average counts of Azotobacter for eight Broadbalk plots.

TABLE 4

Azotobacter: cells per gram dry soil (Meiklejohn, 1962)
(Broadbalk)

Plot	Treatment	Number of observations	Mean No. of Azotobacter
3	Unmanured	56	167
5	Minerals (P, K, no N)	49	178
7	Ammonium sulphate, P, K	50	107
10	Ammonium sulphate only	52	77
11	Ammonium sulphate, P	35	140
13	Ammonium sulphate, P, K	35	139
17 & 18	Alternate years: P, K, no N	50	220
17 & 18	Alternate years: Ammonium sulphate	50	199

Few Azotobacter were found, probably because of the medium used, which has since been found to underestimate the numbers of Azotobacter. The error, however, appears to affect all counts in the same proportion, so that, though the real numbers are all higher than those shown, plots 17 and 18, for instance, really have more Azotobacter than plot 10. And in any case, Azotobacter is not the only nitrogen fixer in the Broadbalk soil. It is not even the most numerous. A few counts made of the anaerobic nitrogen-fixing bacterium *Clostridium pasteurianum* show consistently higher numbers than those of Azotobacter. In all the eight plots sampled, nitrogen-fixing Clostridia were found to amount to 100,000/g. or more on at least one occasion, and very few counts numbered fewer than 1,000 cells/g. of soil. There were not enough data for any differences between plots to be detected (Meiklejohn, 1956).

It is obvious, I think, that much remains to be done before the Rothamsted classical fields can be said to be adequately studied. The work already done has disclosed that the population of micro-organisms in cultivated soils must be numbered in thousands of millions per gram, and has given some indication of the complexity of this population. Future advances in useful knowledge are, however, more likely to be made not by attempting to study the " whole " soil population, but by more detailed study of the groups of micro-organisms responsible for particular processes important to soil fertility. The best line of approach is indicated by the work of Jensen. In his study of the decomposition of manure he was able to use two soils, from Park Grass plots 1 and 14, that had been changed by fertiliser treatment so that one was neutral and the other very acid, and so to find out that soils of different acidity contain different groups of cellulose decomposers. Further studies of this kind on the classical fields, where the soil properties are so well known, and all the yields are recorded, would take some useful advantage of the unique opportunities offered by the Rothamsted classical fields.

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