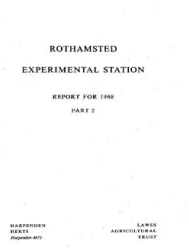
ROTHAMSTED RESEARCH

Rothamsted Experimental Station Report for 1968 - Part 2



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9. MICROBIOLOGY OF BROADBALK SOILS

JANE MEIKLEJOHN

Microbiological work began on Broadbalk after interest had been aroused in the practical benefits of partial soil sterilisation in increasing the growth of plants, and reasons for its effects were being sought. The number of bacteria in soil was known to increase greatly after partial sterilisation and this was suspected to be the reason for the increased fertility. Russell and Hutchinson (1913) suggested that partial sterilisation kills some organisms, and they favoured protozoa, that usually restricted bacterial populations and so fertility. Hence, other evidence for the beneficial effects of bacteria and possible harmful ones of protozoa was sought. The extremes of fertility represented by the Broadbalk plots 2, FYM, and 3, unmanured, provided good potential sites and were the first to be examined.

The comparisons of bacterial counts gave some support to the idea that bacteria might be beneficial, because plot 2 contained two or three times as many per gram of soil as plot 3, and the amounts of nitrate in these and several other soils also fluctuated with the numbers of bacteria. That soils did have a specific protozoal fauna was also first shown by work on these plots by Martin and Lewin (1915), but produced little evidence that they were harmful because plot 2 contained more individuals than plot 3, though fewer species. In both plots, numbers were fewer when the soil contained only 10% water than when it contained 20%.

Crump (1920) found that flagellates were the most abundant group of protozoa in plot 2, and averaged 28400 cells per gram in the top inch of soil. Amoeba were also numerous, averaging 7700, whereas ciliates were few, averaging only 56 per gram. However, counts made by Cutler and Crump (1920) at short intervals showed that the populations of three different species of flagellates fluctuated greatly between counts and that the active forms exhibit a daily periodicity, a day with many was followed by a day with few. Their finding that the number of bacteria and active amoeba in the soil were correlated, varying inversely over a period of 16 days, again seemed to suggest that protozoa might be limiting soil fertility, but much work since has shown that although bacterial predators, including protozoa, may limit numbers, they do not restrict bacterial activity (Thornton & Crump, 1953).

The algae have been rather neglected, and studied only by Bristol Roach (1927). Plots 2 and 3 had similar numbers of species, but plot 2 more than twice as many individuals (38000/g of top soil) as plot 3 (15000). These numbers omit blue-green algae, which could not be counted by the method Roach used, but these occurred and were sometimes plentiful enough to form a crust on the soil surface. It was of interest that she found algae living 12 in. below the soil surface, for then it was not known that many algae can live heterotrophically.

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Work since 1930

Since 1930 methods of estimating the numbers of organisms have greatly improved, and although Skinner, Jones and Mollison (1952) confirmed that plot 2 contained more than plot 3, the populations they reported enormously exceeded those reported by Russell and Appleyard (1917). The direct-count method they used gives the best measure of total populations, and showed an average in plot 2 of 2500 and a maximum of 4000 million/g of soil, and in plot 3 an average of 1500 and a maximum of 2500 million. They also examined plot 7 (N_2PKNaMg), and this had similar populations to plot 3, although its wheat yields resemble those of plot 2, so bacterial populations did not reflect the fertility as indicated by crop yields.

Singh (1937) was the first to examine fungi and actinomycetes, not only on plots 2B and 3, but also on plot 10 (N_2) plot 11 (N_2P) and plot 13 (N_2PK). He found the same species in all plots, but differences between the numbers in different plots, with plot 2B having most and plot 3 fewest (Table 9·1).

TABLE 9·1

Plate counts of fungi and actinomycetes

Plot	Average numbers in top 6 in.: thousands per gram soil	
	Fungi	Actinomycetes
2B	490	2350
13	320	1500
11	250	1100
10	225	1000
3	175	700

Skinner (1951) also found many actinomycetes in plots 2, 3 and 7, though most were present as spores and not as growing mycelium. A recent series of counts of actinomycetes and fungi (see Etheridge, p. 181) was made to seek a biological explanation for take-all being less prevalent on Broadbalk than on nearby fields and to study the differences between the populations in the soil surrounding the roots (rhizosphere) and those on the roots (rhizoplane).

Protozoa. Singh (1946, 1949) having improved the method for counting soil micro-predators, estimated the population of amoebae in the soils of plots 2, 3 and 7. Plot 3 had fewest, averaging 17000/g, of which 10000/g were active (not encysted) cells and plot 2 most, averaging 72000/g (52000 active), and plot 7, which gives comparable yields to plot 2 average 48000/g (40000 active). Singh (1947a) also isolated some peculiar and little-known soil micro-organisms, including several of the higher Myxobacteria, from plot 3. Myxobacteria, which differ from true bacteria in lacking a rigid cell wall, include the widely-distributed cellulose-decomposer *Sporocytophaga myxococcoides*, discovered at Rothamsted by Hutchinson & Clayton (1919), and several species that break down chitin. However, the species Singh found were all predatory on other bacteria, which they killed by toxins and then digested by extra-cellular enzymes, and they formed characteristic fruiting bodies.

Singh (1947b) also found several members of the Acrasiae in Broadbalk

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soils. In their active form these unusual organisms are small amoebae, which feed and reproduce as do other soil amoebae, but sometimes they collect into a pseudo-plasmodium which produces a sporangium and the spores hatch into new small amoebae. Species of *Dictyostelium*, a typical member of the Acrasiae, were obtained from the soil of Broadbalk plots 3 and 7.

Another peculiar soil organism isolated was *Leptomyxa reticulata* (Singh, 1948), which is a very large amoeboid creature consisting of a thin multi-nucleate sheet of protoplasm that can attain a width of 3 mm, and feeds on bacteria. As these three organisms occurred in the unmanured plot, Singh concluded that they were indigenous to soil and not introduced in dung, but this conclusion may not be fully justified because Broadbalk was for long cultivated by horse-drawn implements.

Nitrifiers and nitrogen fixers

Nitrifiers. As part of a study on Winogradsky's Azotobacter test for soil fertility, Ziemiecka (1932) counted ammonia-oxidising bacteria on several of the Rothamsted Classical field experiments, using the silica-gel plate method. She found most in plots with bulky organic manures, and fewest in plots with no added nitrogen. The counts for Broadbalk were: plot 2, (FYM) 2243 cells/g; plot 19 (rape cake) 1336/g; plot 8 (N_3PKNaMg) 1949/g; plot 5 (PKNaMg) 406/g; and plot 3 (unmanured) 252/g.

TABLE 9·2

Numbers of nitrifying bacteria per gram dry soil, 1962

Date	Plot	Ammonia oxidisers	Nitrite oxidisers
1958	2A	360	45
1961 July	3 Fallow	6480	100
	3 Cropped	6300	390
1961 Oct.	5 Fallow	26300	412
1961 Aug.	7 Fallow	>11500	>11500
	7 Cropped	>11500	>11500
1961 Oct.	7 Fallow	26500	830
1958	8	720	720
1961 Aug.	10 Fallow	3350	210
	10 Cropped	6670	210
1961 Jan.	Wilderness Wood	4070	128
June	Wilderness Wood	3450	108
1961 Aug.	Wilderness Grass (grazed by sheep)	2870	45
Oct.	Wilderness Grass	2010	15
1962 Jan.	Wilderness Grass	8580	33
May	Wilderness Grass	>16000	65
June	Wilderness Grass	>13000	1700

The ammonia-oxidisers operate the first stage in the process of nitrification that makes combined nitrogen available to crop plants. The nitrite they make by the oxidation of ammonia is further oxidised to nitrate by other nitrifying bacteria, the nitrite-oxidisers. Meiklejohn (1962) found adequate populations of both kinds of nitrifier in six of the Broadbalk plots, 2, 3, 5, 7, 8 and 10, with no evident differences between plots. Soil from the Wilderness at the west end of Broadbalk also contained nitrifiers of both kinds, both in the wooded end and in the end under grass. This is of interest

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because many woodland soils in Britain do not contain nitrifiers (Table 9·2)

An ammonia-oxidising bacterium, *Nitrosomonas europaea*, was isolated from the soil of plot 2 (Meiklejohn, 1949); it, and the nitrite-oxidiser *Nitrobacter winogradskyi*, seem to be the only two abundant nitrifiers in Rothamsted soils.

Nitrogen fixers. The free-living aerobic bacterium *Azotobacter chroococcum* adds nitrogen to the soil by taking it from the air and building it into its own cell substances. Ashby (1907) discovered it in soil from Broadbalk Wilderness and in the Broadbalk drains. Ziemiecka (1932) used Winogradsky's plate method to estimate populations in various fields at Rothamsted in September, October and November 1931. Her counts on Broadbalk were largest on plots 5 (PKNaMg but no nitrogen) 8400/g in September and 2055/g in October, and on plot 9 (N_1*PKMaMg) 1593/g in October. In November she found 338 Azobacter per g. in the Wilderness grass, and 120/g in the Wilderness wood soil. In 1960 Burlingham counted Azotobacter in rhizopheres and in soil between the rows of plants in plots 2, 5 and 8 (Table 9·3). Counts in January and March 1960 showed no 'rhizophere effect', i.e. populations were no larger in the soil immediately around the roots than in the inter-row soil. Plot 2 has many more Azotobacter than plots 5 or 8, and contrary to the results of other workers she found no Azotobacter in soil under the Wilderness grass.

TABLE 9·3

Numbers of Azotobacter in rhizosphere and in other soil, 1960

	Number of Azotobacter (cells/g dry soil)			
	Plot 2		Plot 5	Plot 8
	Rhizosphere	Soil	Soil	Soil
Jan. 1960	4500	2060	115	14
	4600	3080	74	64
	13800	11000	108	38
	1800	3500	35	69

	Plot 2		Plot 5	
	Rhizosphere	Soil	Rhizosphere	Soil
Mar. 1960	1200	238	0	124
	700	6000	234	126
	12000	56800	160	256
	2300	3500	110	260
	3500	1400	0	87
	1100	5300	0	125

Wilderness grass: none found in any sample.

After the system of a fallow every fifth year was found to act like a dressing of quick-acting nitrogen, it was thought some of the effect of fallow might reflect nitrogen fixation in the fallow soil. Therefore the populations of nitrogen fixers were estimated before, during and after the fallow year in several of the plots. Most counts were of *Azotobacter chroococcum*, an easily recognisable species, but a few counts were also made of the anaerobe, *Clostridium pasteurianum*. Counts made during the 1955–58 seasons seemed

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to support the idea that fallowing stimulated nitrogen fixation for the first crop after fallow. Azotobacter were few in sections under the 4th successive wheat crop and in the soil being fallowed, but increased greatly in the first wheat crop after the fallow. The increase in Azotobacter was greatest in the plots where the increased yield from fallow was greatest (Table 9·4). Plots given ammonium sulphate every year had fewer Azotobacter than the other plots (Meiklejohn, 1965).

However, Azotobacter in the Broadbalk soils seemed too few to fix enough nitrogen to affect the crop growth. Although an improved technique (Brown, Burlingham & Jackson, 1962) increased the count, the largest population found was only 9400 cells/g dry soil, which again is too few for Azotobacter to affect crop growth through nitrogen fixation. Nevertheless the Azotobacter numbers indicate that conditions are suitable for nitrogen fixation by other species, such as the anaerobe *Clostridium pasteurianum*, which occurs all over Broadbalk in much larger numbers than Azotobacter, ranging up to 100 000 cells per gram dry soil (Meiklejohn, 1956). The possibility that an anaerobe is the active fixing agent in Broadbalk soil is supported by laboratory observations of nitrogen fixation by soil from Broadbalk plot 3 incubated anaerobically with straw (Barrow & Jenkinson, 1962).

TABLE 9·4

Effect of fallow on Azotobacter numbers in 1962

		Largest number (cells/g soil)	Mean
Plots without added nitrogen			
3	Fallow	137	53 ± 16
	1st crop after fallow	1194	430 ± 135
5	Fallow	282	166 ± 30
	1st crop after fallow	894	400 ± 121
Plots with annual ammonium sulphate			
7	Fallow	410	134 ± 49
	1st crop after fallow	1429	631 ± 190
10	Fallow	555	129 ± 76
	1st crop after fallow	260	146 ± 27
Other nitrogen treated plots			
16	Fallow	4524	1003 ± 594
	1st crop after fallow	3782	1803 ± 435
17	Fallow	2620	580 ± 343
	1st crop after fallow	9406	2442 ± 1276

Symbiotic Nitrogen Fixation: Legume Nodule Bacteria

By P. S. NUTMAN

Four species of the genus *Rhizobium*, which form nodules on the roots of leguminous plants occur in Broadbalk soils, but in populations that are much smaller than in neighbouring fields.

Nodule bacteria were counted in samples of soil taken in March 1968

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and was especially rich in reticulate spores. Yellow vacuolate spores were most abundant in the FYM plot. Of the four types, laminate spores were fewest, whereas in the nearby field, Little Knott, they were the only ones found and were numerous in some plots, especially those not given nitrogen.

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