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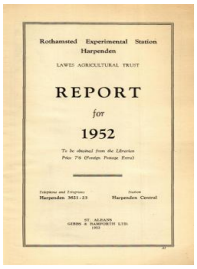

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H. G. Thornton and L. M. Crump

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MICROPREDATORS IN SOIL

By

H. G. THORNTON AND L. M. CRUMP

Introduction

The growth and health of crops is greatly affected by the activities of the soil micropopulation so that the factors that influence the size and composition of this population are a fundamental interest in soil science. The quantity of living matter comprising the soil population is, of course, determined and limited by environmental factors such as the quantity and availability of food material and such variables as pH, moisture, aeration and temperature. But the dominance of certain groups of organisms in the soil population and the prevalence of organisms having specific effects, whether beneficial or harmful, are of far greater importance than is the total size of that population. Here again factors external to the population itself must ultimately determine its biological composition. In this case, however, their action may be indirect, for the varied organisms comprising the micropopulation interact in a very complex manner, so that the effect of an external agent on any one of them may be influenced by its effect on the other organisms. Any attempt therefore to produce a beneficial change in the soil population, whether it be the encouragement of organisms producing desirable biochemical changes or the discouragement of harmful organisms such as pathogens, must involve knowledge of the interaction of such organisms with their associates and competitors in the soil. Competition between organisms in the soil takes place in three principal ways. Firstly, there is a keen competition for the limited supply of available nutrients. Secondly, there are the toxic effects of the products of growth of one organism on others, of which the action of specific antibiotic secretions is an example. This may be quite incidental in that the organism producing them may gain no benefit other than some possibly increased freedom from competition. Thirdly, there are organisms that feed directly upon others as their only or main method of nutrition. These organisms may conveniently be called "micropredators." There are a few organisms remarkable for their modes of nutrition such as fungi that attack nematodes or amœbæ, and the recently described rhizopod protozoa that consume nematodes, but the great majority of micropredators in the soil feed on bacteria. They include protozoa, the active stages of certain myxomycetes and also some Myxobacteria that feed by dissolving bacterial cells and absorbing the products of lysis. There are also some small metazoa such as nematodes that eat bacteria but the work summarized in this report deals only with micropredators having an active unicellular stage.

Interest in soil protozoa has long been maintained at Rothamsted and dates from the work of Russell and Hutchinson on partial sterilization of soil (1909), and their suggestion that, in untreated soil, the numbers of bacteria might be limited by the feeding activities of soil protozoa and that the destruction of the latter might

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account for the observed increase in the number and activity of bacteria in soil after partial sterilization. This theory instigated work at Rothamsted on soil protozoa by T. Goodey from 1910 to 1913, the late Martin and Lewin from 1913 to 1915, Crump who came here in 1915, Cutler who came in 1919 and others who came later. The Protozoology Department was set up in 1919 under the leadership of Ward Cutler originally to study the protozoan fauna of soil, although its scope was later widened. Investigation at Rothamsted and elsewhere showed that soil contained a large and varied protozoan population, amongst which amœbæ and flagellates were predominant, and also that the protozoa existed in an active condition in field soil. This discovery, coupled with the theory of Russell and Hutchinson, made it important to find out whether the numbers of bacteria in soil were controlled by the feeding activities of protozoa. It was therefore necessary to determine what relationship the numbers of bacteria in field soil have to those of the active protozoa.

A technique was devised for the estimation of numbers of protozoa that fed on bacteria, based on a series of soil dilutions (Cutler, 1920), and the plating method for counting soil bacteria was improved. Preliminary counts showed that the numbers both of bacteria and of protozoa changed at short intervals in field soil. In 1920-21 therefore, a series of soil samples was taken from the Barnfield dunged plot at daily intervals for a year and the numbers of bacteria and of the active and encysted individuals of two species of amœbæ and four of flagellates were estimated. (Cutler, Crump and Sandon, 1922.) Marked fluctuations in the numbers of bacteria and protozoa are found ; these were not clearly related to weather conditions but there was a general rise in all groups during the spring and autumn. Changes in bacterial numbers were not related to those of the flagellates. Of the two amœbæ, one, then identified as Dimastigomœba, was much the more abundant in most samples. The frequency of occurrence of high numbers of the active form of this amœba (above 100,000 per gram of soil) was significantly related to that of low bacterial numbers (below 30 millions per gram). This indicated that the amœbae when sufficiently numerous exercised a controlling effect on the changing numbers of bacteria found in the plot by the plating method used. Experiments also showed that amœbæ did keep down the numbers of bacteria when both were inoculated into sterilized soil (Cutler, 1923).

In 1941, on the death of Mr. Ward Cutler, the department was merged with that of Bacteriology to form the present Soil Microbiology Department and it is with the work carried out since then that this summary is mainly concerned.

The selective feeding of soil protozoa

At the time when the surveys of bacterial and protozoan numbers in Rothamsted field soil were made, the quality of the bacterial food supply was not considered, but somewhat later work, both at Rothamsted (Cutler and Crump 1927 and 1935) and elsewhere, showed that soil bacteria differ in their edibility by protozoa. But it was important to discover whether amœbæ, supplied with a mixture of bacterial species, as happens in fresh soil, will feed selec-

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tively and whether they can in consequence change the relative numbers of different bacteria in the soil. B. N. Singh investigated the feeding of amœbæ and of a flagellate on different species of bacteria using an ingenious method in which an inoculum of amœbæ placed at the centre of an agar surface in a petri dish, was presented with a series of radially disposed streaks of different bacterial species whose subsequent rate of consumption could thus be compared. In his first series of experiments (1941a) he tested two species of soil amœbæ on five strains of Aerobacter, and twelve species of soil bacteria; in his second series (1942) two amœbæ and the flagellate Cercomonas were tested on sixteen strains of the nodule organism Rhizobium, twenty other assorted species of soil bacteria and twelve species of plant pathogenic bacteria; in his third series (1945) two amœbæ were tested on sixty-three species of soil and thirty-nine of other bacteria. The species of soil bacteria tested in these experiments differed widely in their characters. About half of them were eaten by the protozoa and showed a range of edibility from some that were readily and completely consumed to others that were but slightly attacked. There were small differences between the two species of amœbæ and rather larger differences between the amœbæ and the flagellate in respect of the particular bacterial species that were eaten. Amœbæ when placed in contact with closely adjacent parallel streaks of readily and less readily eaten species of bacteria usually consumed the whole of the former before making a noticeable inroad on the less edible species. They could, however, be adapted by previous feeding on the less edible species and would then eat both species together. Of eight species of bacteria producing red or pink pigment, seven were not eaten, nor were strains of *Chromobacterium violaceum* or *Pseudomonas æruginosa*. Apart from this relationship to pigment, no clear relationship emerged between the edibility of a bacterial species and any other of its characters, such as gram staining, or slime production.

Species that are eaten differ greatly in their nutritive value as measured by the multiplication rate and by the mean cell size of protozoa fed on them. This was shown, for example, with amœbæ in early work of Cutler and Crump (1927) as more recently for a ciliate by Singh (1941b). Some bacterial species produce secretions highly toxic to amœbæ (Singh 1945). A number of these produce pigments and, in several cases studied, the pigment itself was found to be toxic. This was the case with pyocyanin, prodigiosin and the violet-blue pigment of *Chromobacterium violaceum*.

Not only is the total number of amœbæ affected by the quality of the bacterial food supply but also the percentage of them that is in the active condition. For not only do they tend to form cysts in the presence of unfavourable bacterial food, but Crump has shown that with some species of soil amœbæ, hatching of the cysts is stimulated by the presence of bacteria of which the correct species must be present to ensure maximum excystment (1950).

A highly specific relationship between soil amœbæ and bacteria can thus be demonstrated on laboratory media, and Singh showed that amœbæ are also specific in their consumption of bacteria in soil (1941a). He inoculated samples of sterilized soil with two species of bacteria each in pure culture and with both species together,

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with and without the further addition of soil amœbæ. In the absence of amœbæ, the bacteria fluctuated greatly in numbers in a manner previously observed under similar conditions by Taylor (1936). In the presence of amœbæ, however, one species of bacterium was greatly reduced in numbers and eventually almost extinguished. The other less edible bacterium was little affected and after a month had regained the numbers found without the amœbæ. Thus the amœbæ were able in soil to alter the relative numbers of the two bacterial species.

It seems likely therefore that quality of bacterial flora in differently treated field soils may both influence the numbers of amœbæ and be itself influenced by their selective feeding. That the nutritive quality of the bacterial flora to amœbæ does in fact differ with soil treatment is suggested by counts from field plot samples described below.

Improved method for estimating the numbers of amœbæ in a soil sample

Our knowledge of the soil population is limited by the adequacy of our methods for estimating the numbers of organisms belonging to each of the different groups of which it is composed. Methods for doing this involve making a suspension of the soil sample and, in most cases, diluting this suspension to known degrees. Direct microscope counts are only possible for groups, such as bacteria, present in very large numbers. Otherwise a less direct method must be used. If the organisms to be counted will grow as colonies on a jelly medium, the numbers present at certain known dilutions can then be counted by plating methods.

But important groups such as the protozoa will not do this satisfactorily and here we can only base our estimate on the presence or absence of the organisms in samples from a series of dilutions. Such samples are incubated under conditions ensuring growth of the organisms by which growth their presence is detected. With amœbæ these conditions include the supply of bacteria edible by them. Selective feeding tests showed that strains of Aerobacter were readily eaten by a range of species of amœbæ and other soil protozoa and this knowledge enabled Singh (1946 a and b) greatly to improve the counting method by using as food supply a pure culture of Aerobacter placed in a petri dish on the surface of non-nutrient agar or silica jelly, on which possibly harmful or inedible bacteria from the soil dilution would make little or no growth. The accuracy of estimates by the dilution method is dependent on the number of replicate samples at each dilution that are examined. This was increased by using, for each dilution to be examined, a petri dish in which eight small glass rings were imbedded in the agar or silica jelly, in each of which a sample of the dilution was tested for the growth of amœbæ on Aerobacter.

This improved technique gave consistent results between duplicate samples from field soils, but " recovery " tests from sterilized soil to which known numbers of amœbæ were added showed a consistent loss of about 30 per cent, most of which could be accounted for by non-viability of individual amœbæ in laboratory culture. Thus counts from soil probably represent a systematic underestimate of this order, inherent in any cultural method of counting.

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The numbers of amœbæ and bacteria in differently treated plots

The above technique has been used by Singh to survey the content of active and encysted amæbœ (1949) in plots on Barnfield and Broadbalk and in partially sterilized field plots at Ampthill, Bedfordshire. The samples examined from Barnfield and Broadbalk were taken at nine and six approximately monthly intervals respectively from the plots with no manure (8·0 and 3) farmyard manure (1·0 and 2) and complete artificials (4A and 7) in each field. Over the periods of sampling marked fluctuations in numbers of amœbæ took place. In both fields the numbers of amæbœ, both total and active, were much the lowest in the untreated plots but did not differ appreciably as between the plots treated with farmyard manure or artificials. On the other hand bacterial numbers, determined by both microscope and plate counts, from the same Broadbalk samples by Skinner, Jones and Mollison (1952) were much higher in the farmyard manure plot (2) than in the other two plots (3 and 7), whose bacterial numbers were similar to each other. In other words the ratio of the number of amœbæ to those of bacteria was much higher in plot 7 than in plot 2. This suggests a qualitative difference in food value to amœbæ of the bacterial populations in the two plots.

The setting up by the Chemistry Department of a plot experiment at Ampthill, Bedfordshire, to test the effects of partial sterilization on Sitka spruce nursery beds gave an opportunity to study its action on soil protozoa in the field. An untreated plot and plots whose soil had been partially sterilized with steam and with formalin were sampled at intervals after the treatment and the numbers of bacteria and of amœbæ were estimated by Crump and Singh (1953). Both treatments caused an immediate fall in the numbers of amœbæ and bacteria, the latter estimated by plate counts. After this, in the steamed plot the numbers both of amœbæ and of bacteria rose far above those in the untreated plot. But after formalin treatment the number of bacteria rose well above those in the untreated plot but numbers of amœbæ remained persistently depressed. This result shows that the effects of soil partial sterilization on the micropopulation differ according to the type of treatment used. This conclusion is supported by the different effects produced by steam and formalin on the fungal population of the plots (Mollison 1953).

Classification of soil amœbæ

A difficulty constantly met with in studying the soil protozoa is that of identifying them. Correct identification is of added importance because of the specific reactions which different amœbæ show towards soil bacteria. The taxonomy of small amœbæ was hitherto based to a large extent on characters too uncertain to be of practical value, such as the occasional production of flagella. The type of nuclear division is a more stable character and differs strikingly between different groups of amœbæ, but the difficulty in finding specimens of the different stages of nuclear division has until now limited the usefulness of this character. The discovery of a satisfactory bacterial food supply for cultures of soil amœbæ enabled Singh to devise a beautiful and simple technique in which

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thick cultures of these amœbæ including all stages of nuclear division can be grown on cover slips coated with films of agar supplied with suitable bacterial food (1950). A fortunate habit of the amœbæ to wander through the agar on to the glass surface enables the agar to be removed and the amœbæ to be left adhering to the cover slip, where they can be fixed and stained. With this method he has studied the nuclear division of a number of soil amœbæ and has proposed a classification of amœbæ based on this character (1952).

Giant Rhizopods from soil

The use of a generally edible bacterial food supply for counting amœbæ and for isolating them from soil, resulted in several other types of bacterial predators appearing in cultures from field soil. One of these was a giant multinucleate Rhizopod of the genus Leptomyxa which may attain a diameter of nearly 3 mm. The history of work on this organism is interesting. In 1913 T. Goodey, who was then studying soil protozoa at Rothamsted, found and described three Rhizopods of a type new to the soil fauna and related to the Proteomyxæ. On these he founded the two genera Leptomyxa and Gephyramœbæ (Goodey 1915). Sandon in 1927 found Gephyramœbæ in several soil samples in the course of a survey of protozoa from a range of soils. He however failed to find Leptomyxa although this organism was again found in Australian soil by McLennan in 1930. After this it was not recorded again until Singh (1948a), using Aerobacter as food supply, found that it could be isolated regularly from field soil and obtained it from thirty-eight out of fifty-nine soil samples derived from localities widely scattered over Great Britain and from nine of the plots on Barnfield and Broadbalk. He studied its life-cycle and nuclear division (1948b) and showed that like true amœbæ it was selective in its bacterial food requirements but differed from the amœbæ with which he compared it, in the species of bacteria that it would eat (1948a). A few estimates made by the dilution method from the soil of Barnfield plot 1·0 revealed its presence in dilutions up to 1/1,000.

Soil Acrasieæ

The improved methods of culture used for soil Rhizopods also revealed the abundance and widespread occurrence in soil of a second group of amœboid Protista, the Acrasieæ, particularly the genus Dictyostelium, which was first described by Brefeld in 1869. Singh obtained this organism from soil samples collected from widely scattered localities in Great Britain. He found it in 33 out of 38 arable soils examined but only in 3 out of 29 grassland soils (1947a). He also found it in all the plots from Barnfield and Broadbalk. The Acrasieæ pass through a remarkable life cycle, in one stage existing as amœba-like forms, " myxoamœbæ," which later, under suitable conditions, collect together and form fruiting bodies superficially resembling those of certain fungi. Inside these, spores are formed which are released and from which the amœboid forms are hatched. In the amœboid stage they feed on bacteria and in this stage Dictyostelium, like other predators, was found to be specific in the species of bacteria that it would attack (1947a and b). It

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will also develop and form fruiting bodies when grown in sterilized soil supplied with suitable bacterial food and was then found greatly to reduce the numbers of bacteria in the soil (1947b). The growth of the organism in sterilized soil as judged by the development of fruiting bodies on the soil surface, was found to be dependent on the species of bacteria supplied. The spread of the organism through the soil was dependent on its moisture content. There was little evidence of spread at moisture contents below 25 per cent, and below 15 per cent moisture no fruiting bodies were found even at the point of inoculation, perhaps because the amœboid forms could not assemble in such dry soil. The organism will also pass through its life cycle in fresh unsterilized soil.

Soil Myxobacteria

The Myxobacteria were recognized as a group by Thaxter in 1892 but the group has been comparatively little studied till recently and even now many forms are known only by their fruiting bodies. The more highly developed types of Myxobacteria pass through a life-cycle. In the active stage they consist of thin rods, capable of a sliding motion the mechanism of which is not understood. After a while these rods collect to form swarms each of which may become covered with a coating to form a fruiting body. Inside this the rods turn into the so-called " microcysts " which are usually round or oval bodies but which in some species have the form of short rods. They are eventually released and develop into the active rod stage. Some of the Myxobacteria found in soil do not swarm to produce fruiting bodies. Important amongst them is a group attacking cellulose and placed in the genus Sporocytophaga (Stanier 1942), which was originally found and studied at Rothamsted in 1912 by Hutchinson and Clayton, who mistakenly considered them to be Spirochætes. Another genus, Cytophaga (Winogradsky), even lacks the microcyst stage. Some species in the genus also attack cellulose while others have a more generalized nutrition. One of these that can attack chitin, was isolated by Stanier (1947) during a short visit to Rothamsted. The " higher " Myxobacteria from soil, that have been studied by Singh, belong to the genera Myxococcus, Chondrococcus and Archangium. These organisms are micropredators since they feed readily on true bacteria previously killed and dissolved by their secretions. In a joint investigation Oxford and Singh (1946) found that Myxococcus produced two types of secretion one of which had a toxic effect on a considerable range of bacterial species while the other was a powerful bacteriolytic and proteolytic enzyme that would lyse dead bacteria, though not attacking live ones. Myxobacteria of this predator type are again selective in the bacterial species which they will attack (Singh 1947c). At one time they were regarded as dung inhabiting organisms but they have been found to be widely distributed in British soil and to occur in all the various plots of Barnfield and Broadbalk most of which do not receive dung, so that their status as soil inhabitants is no longer in doubt. Dilution counts from the soil of Barnfield plot 1 ·0 gave numbers of predaceous Myxobacteria ranging from 2,000 to 76,000 per gram.

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Conclusion

There is no means of estimating the effect on the bacterial flora of soil of the micropredator population as distinct from other competitive and antagonistic factors. The daily counts of amœbæ and bacteria from Barnfield made by Cutler, Crump and Sandon (1922) showed evidence of a limitation of bacterial numbers when the number of amœbæ in an active state exceeded 100,000 per gram of soil. In view of the variety of other micropredators now known to inhabit the soil it is not surprising that the effects of any one gronp such as the amœbæ should be distinguishable only when present in exceptionally high numbers. Any assessment of the quantitative effect of the micropredators as a whole would require that the numbers of each type should be estimated from a range of soil samples and compared with bacterial counts. Such a task is at present beyond the capabilities of our counting technique.

But the selective attack on different bacterial species, evidence for which has been found with all groups of micropredators, adds greatly to their interest from the point of view of soil ecology. Singh tested eighty-seven very varied strains of soil bacteria against eight micropredators, comprising a large and a small soil amœba, the giant Rhizopod *Leptomyxa reticulata* Goodey, the myxamœbæ of two species of Acrasieæ (Dictyostelium) and three species of predaceous Myxobacteria (Anscombe and Singh 1948). Any one of these predators was found to attack about half of the bacterial species tested, but owing to the dissimilarity in feeding habits of the various predators there were only seven of the bacterial strains that were not attacked by any of the predators and only twelve were attacted by all of them. Certain groups of bacteria such as the nodule bacteria seem to be generally resistant to attack by micropredators while others such as strains of Aerobacter are attacked by all of them. If it is desired to establish any kinds of bacteria in soil, their resistance to predators should be considered. The presence of micropredators also complicates the unravelling of the effects of soil treatments and especially those like partial sterilization that are liable to check the predators. This was appreciated by Russell and Hutchinson in their original hypothesis although this now appears to us as an over-simplification of the complex perturbations that must occur when the balance of micro-organic life is radically upset by soil treatment.

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