# A Continuation of the Investigation of the Soil Microflora of Two Grassland Plots

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During the summer of 1953, investigations on the soil microbiology of some of the plots in the University of Oklahoma Grassland Research Project were continued. The research area is situated eight miles southwest of Norman, Oklahoma. The two plots studied, a virgin prairie and a revegetating field, differ greatly in vegetation and soil conditions. These areas are about one-quarter of a mile apart, separated by a highway. The virgin prairie consists of five acres of relatively undisturbed tall grass prairie, and has not been burned or grazed in the past 25 years. The revegetating field was farmed for a period of 20 to 25 years before 1941, but has been undisturbed since except for grazing. In 1941, it was planted with Korean lespedeza, and is now covered with low growing grasses. The revegetating field, as compared with the virgin prairie, has a greater number of species of plants and a higher percentage of living cover.

Previously, both vegetation and soil conditions were studied to help to explain the differences in the two fields (3). This problem extends the work formerly done concerning numbers and activity of bacteria, fungi, and actinomycetes (4). The problem of differences in vegetation was studied also in regard to the nitrogen content of the soil and an investigation of possible microbial antagonism was carried out.

## Materials and Methods

Soil samples were taken on June 29, 1953, by means of a 1 ½-inch soil auger. The samples were collected every 15 feet along a transect line from the top four inches of soil and were thoroughly mixed to insure a uniform sample. Samples were screened to break up clumps and remove plant roots and twigs.

Soil extract agar, Thornton's agar, and nutrient agar were used for bacterial counts. Actinomycete counts were made on Jensen's medium and fungi counts on Rose Bengal agar (1). Incubation was at 30° C. for three to eight days during which period several counts were made on the triplicate plates at each dilution.

Respiration studies of the soil microflora were made by using a Warburg constant volume respirometer for determination of carbon dioxide evolution (5). Weighed samples of each sieved soil were placed in the manometric flasks and enough water added to bring each sample's moisture content to 75 per cent of the moisture holding capacity. This was done in order that a valid comparison could be made between the two fields (2).

## Results and Discussion

The results of the counts on the soil microorganisms and a comparison with last year's results are shown in Table I. A great difference is noted in numbers of bacteria found in the virgin prairie for the two years. A great variation would be normally found from month to month or even from day to day, and makes a long, comprehensive study of plots such as these necessary before any conclusions may be drawn. Conclusions drawn from a single monthly or yearly investigation of soil microorganisms cannot be considered applicable to other periods. These fragments of knowledge, however, do fit with those previously gathered and begin to give an understanding of at least part of the difference in the two plots.

TABLE I

*Comparison of the Relative Numbers of Microorganisms per Gram of Soil for the Two Year Period.*

| 1953 | Virgin prairie | Revegetating field | Ratio |
|---|---|---|---|
| Bacteria | 2,150,000 | 1,770,000 | 1.2:1 |
| Fungi | 18,600 | 23,200 | 1:1.25 |
| Actinomycetes | 4,520,000 | 3,730,000 | 2:1 |
| Total | 6,688,600 | 4,523,000 | 1.5:1 |
| 1952 (4) | | | |
| Bacteria | 13,600,000 | 1,750,000 | 13:1 |
| Fungi | 4,450 | 4,250 | 1:1 |
| Actinomycetes | 1,588,000 | 2,540,000 | 1:2 |
| Total | 15,192,450 | 4,334,250 | 3.5:1 |

Factors which might account for differences in the microflora of the two plots are temperature, moisture content, *p*H, compaction, color, and nitrate content. Temperatures have been found to be higher in summer in the soil of the abandoned field. The moisture content of the revegetating field, 3.96 per cent, was found to be higher than that of the virgin prairie, 2.99 per cent. This is contrary to previous results and could be attributed to drouth conditions at the time of sampling. The moisture-holding capacity of sieved samples of the virgin prairie was slightly greater than that of the revegetating field with percentages of 46.2 and 42.6. The *p*H's of the two soils, as previously noted (3), were practically identical. The virgin prairie was darker and was therefore, probably a richer soil. Little variation was found in the nitrate and nitrogen content of the two soils. Total nitrogen in the virgin prairie was 1.47 per cent and that for the revegetating field was 1.28 per cent. The soil from the virgin prairie contained 0.04 per cent nitrate, while that from the revegetating field contained 0.03 per cent. Another important factor which might cause variation in microbial composition in the two fields is the effect of higher plants growing on the fields. Further work should be carried out before any conclusions are drawn as to the causes of the differences in the two fields.

Both carbon dioxide evolution and oxygen uptake were measured for samples from each field, using different percentages of moisture-holding-capacity (2). The total microbiological activity of the virgin prairie was found to be greater than that of the revegetating field, in direct contrast to the results found in the summer of 1952. Such a result is in accord with the higher number of microorganisms in the virgin prairie. This yearly variation may be due to the intensive and early drouth suffered in this section of the state in the summer of 1953. Such results point out the need for a year-round investigation of the two fields.

A new approach to the explanation of the differences in the microflora in the two fields, which may affect the differences in higher plants, was made during the summer of 1953. In a study of possible microbial antagonism, organisms found only in the revegetating field or only in the virgin prairie were isolated, with no attempt at identification. One fungus, five bacteria, and four actinomycetes were isolated from the revegetating field, while four each of bacteria, fungi, and actinomycetes were isolated from the virgin prairie. The revegetating field organisms were streaked on plates seeded with soil from the virgin prairie. An aqueous soil extract from the virgin prairie was also streaked on seeded plates made from the isolated organisms. Jensen's medium was used for the actinomycetes, Rose Bengal agar was used for the fungi, and soil extract agar was used

for the bacteria (1). Plates were incubated at 30° C. and readings were made at three, five, and eight days. The virgin prairie organisms were tested in the same manner with soil from the revegetating field. Inhibition was seen in only one case in which one of the actinomycetes found only in the revegetating field slightly affected the growth of a fungus found only in the virgin prairie. All other results were negative.

Extracts of revegetating field and virgin prairie soils were also tested against the bacteria, fungi, and actinomycetes found in the two fields, respectively. Solvents used were cold water, hot water, cold 4 per cent HCL, cold 4 per cent NaOH, absolute ether, 95 per cent ethanol, chloroform, and acetone. Thirty-five grams of soil were added to 50 cc. of solvent and the solutions were allowed to set overnight. After this period, the solvents were evaporated and water was added to the residue. Penicylinder discs were used in the sensitivity tests and Felsen quadrant plates were used in testing extracts against organisms. Readings, except in the case of the fungi, were made after 48 hours. A growing period of 96 hours was allotted to the fungi. Plates were incubated at room temperature. Again no significant results were found that would account for the differences in the two plots. There was slight inhibition from the NaOH extract, but this was undoubtedly due to the high pH of 13. In fact, in many cases, a stimulating effect was noted.

On the basis of these preliminary experiments it appears that the differences in microflora are not due to microbial antagonism. However, additional tests are necessary to substantiate this view.

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