An Investigation of the Soil Microflora of Two Grassland Plots

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Ecological investigations of plant communities and soil conditions can hardly be complete without some analysis, both quantitative and qualitative, of the microorganisms of the soil. Vegetation and soil conditions of the University of Oklahoma Grassland Investigation Plots have been studied extensively; however, no studies of the soil microflora of these plots have previously been made. The investigations presented here represent a preliminary attempt to describe the microflora of two of the plots and to discover any differences in soil population between the two.

The two plots studied were an area of virgin prairie and a revegetating abandoned field. The plots are one-fourth mile apart and both are located eight miles southwest of Norman, Oklahoma. The five acre virgin prairie plot is a relatively undisturbed area of tall grass which includes as dominants *Andropogon scoparius*, *Andropogon furcatus*, *Aster ericoides*, *Panicum virgatum*, and *Sorghastrum nutans* (5). The revegetating abandoned field has not been cultivated for eleven years; its dominant species are *Aristida oligantha*, *Lespedeza stipulacea* (planted), and *Panicum scribnerianum* (5). Both plots were burned six months before this study was made.

The soil samples on which the following results are based were taken July 9, 1952. Samples of the top 3 inches of soil were taken with a 1½ inch soil augur. The 10 samples from each plot were located 15 feet apart

on a transect line across the plot. The samples from each plot were thoroughly mixed to give one composite sample. Each composite sample was then shaken through a seive (U. S. Standard 590 micron) in order to exclude plant roots from samples for measurements of physiological activity and to give more uniform samples for dilution plates.

Measurements of the total physiological activity of the soil microorganisms, represented by rates of respiration, were made for each plot. Numbers of bacteria, fungi, and actinomycetes per gram of soil were determined for both plots. The species of fungi and the most prevalent species of bacteria were also found for each plot. In addition, determinations of the *p*H and organic carbon of the soils were made using the technique described by Piper (6).

Total Microbial Activity

The total microbial activity of each soil was determined by measuring the rate of respiration in a Warburg constant volume respirometer. Following the technique of Umbreit, Burris, and Stauffer (7), the oxygen uptake of each soil sample was measured at a constant temperature of 33.5° C. The respiration rates of dry soil, wet soil, soil enriched with 1M glucose, and soil enriched with 5 per cent soluble starch were found for the virgin prairie plot and the revegetating field plot.

Although rates of respiration in both soils increased when water was added, the respiration rate of the revegetating field was greater than that of the virgin prairie (Fig. 1). Glucose enrichment caused an increased

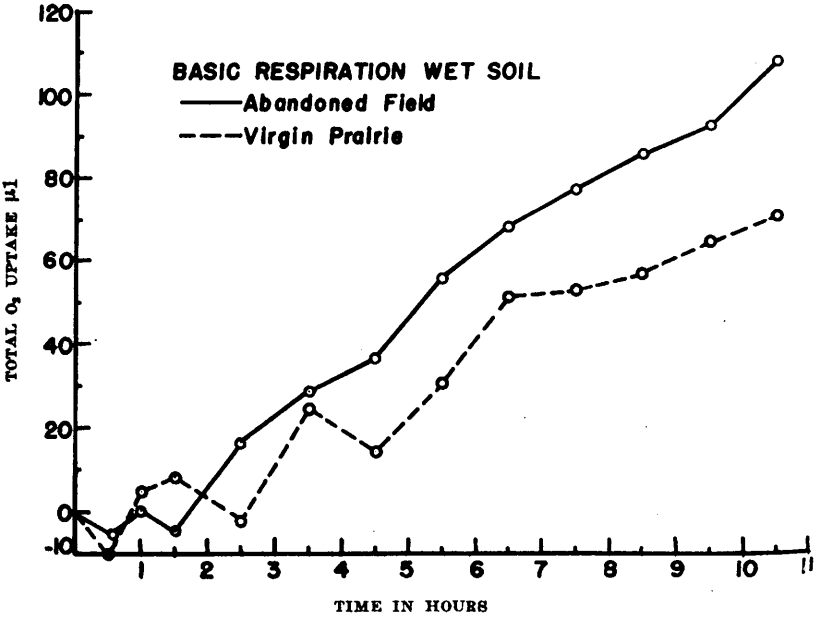

FIGURE 1. Relation of Oxygen Uptake of Wet Soils from Virgin Prairie and Abandoned Field. Data represent cumulative O_2 uptake above that of dry soil.

rate of respiration, especially in the revegetating field soil. Starch enrichment caused the greatest increase in respiration rate, but resulted in the

least difference in rate between the two plots (Fig. 2). Respiration rates of virgin prairie soil were more variable than those of the revegetating field soil of all cases.

The data indicate that addition of water and addition of certain carbohydrates greatly increase the activity of soil microorganisms. In addition, these results suggest that microbial respiration rates are higher in the revegetating field than in the virgin prairie. However, a close examination of the data from the virgin prairie shows a regularly fluctuating

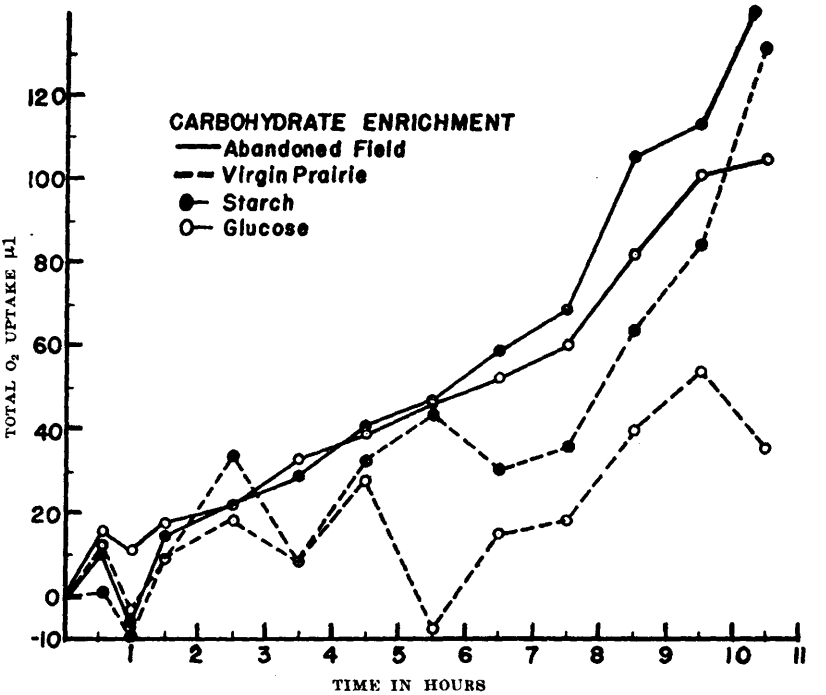

FIGURE 2. Relation of Oxygen Uptake of Soils from Virgin Prairie and Abandoned Field After Enrichment with Starch or Glucose. Data represent cumulative O_2 uptake above that of wet soil.

rate with periodic decreases. These decreases do not represent a negative oxygen uptake or experimental error. They probably result from the production of some gas which is not absorbed by the potassium hydroxide used to absorb carbon dioxide. Although this investigation did show that there are differences in the rates of respiration of the microorganisms of the plots, only further study will determine the exact nature and magnitude of these differences.

Bacteria and Actinomycetes

In order to determine the numbers and principal species of bacteria of the plots, dilution plates were made in triplicate on nutrient agar, Sabouraud's agar, nutrient gelatin, and gelatin agar. Separate samples, as well as composite samples, were used for making dilutions. Species determinations were made using the method of Conn (3). With this method, the most common soil bacteria may be classified according to Gram stain morphology, colony morphology, and gelatin liquefaction.

TABLE I

Total Bacterial Counts per Gram of Separate Samples on Nutrient and Sabourauds Agar.

Medium	Virgin Prairie	Revegetating Field
Nutrient agar	26 x 10^5 to 45 x 10^5	9.6 x 10^5 to 11.2 x 10^5
Sabourauds agar	15.6 x 10^5 to 19.5 x 10^5	10.2 x 10^5 to 11.6 x 10^5

The numbers of bacteria per gram of soil (Tables I and II) were higher on all media in the virgin prairie than in the revegetating field. *Bacillus mycoides* and *Streptomyces sp.* were found to be present in about equal numbers in both soils. Other important species determined on nutrient agar were *B. megatherium* and *Arthrobacterium sp.* in the virgin prairie, and *Agrobacterium sp.*, *Pseudomonas sp.*, and *Micrococcus sp.* in the revegetating field. Species determinations made on gelatin showed that the principal species of the virgin prairie were *B. mycoides*, *Streptococcus sp.*, *B. megatherium*, and *B. cereus*, while those of the revegetating field were *B. mycoides*, *Streptococcus sp.*, and *Agrobacterium sp.* These data indicate not only a quantitative difference between plots but also some qualitative differences.

TABLE II

Total Bacterial Counts of Composite Samples on Nutrient and Sabourauds Agar.

Medium	Virgin Prairie	Revegetating Field
Nutrient agar	42.4 x 10^5	10.9 x 10^5
Sabourauds agar	17.5 x 10^5	13.0 x 10^5

The numbers of actinomycetes in the soil of each plot were found using Jensen's medium and the method described by Allen (1). The number of actinomycetes per gram in the revegetating field (2,540,000) was found to be almost double that of the virgin prairie (1,588,000).

Fungi

The numbers and species of fungi were determined by using the method of Warcup (9). According to this method, the soil itself, rather than dilutions of soil, is used as the innoculum in acidified Czapek's agar plus yeast extract. Species numbers are recorded on the basis of per cent occurrence (per cent of plates on which a species occurs) rather than average number of colonies per plate. This method reduces the advantage given to heavily sporulating fungi and gives more meaningful numbers. Thirty plates were made from each composite sample. Species were identified following a key prepared by Gilman (4).

According to colony counts, the number of fungi per gram of soil for the virgin prairie and the number for the revegetating field were essentially the same. Thirty-two species were isolated from the virgin prairie soil; thirty-six species were isolated from revegetating field soil. Of the total species identified (47), 36.2 per cent were common to both plots. Principal species (those found on 30 per cent or more of the plates) common to both plots were *Fusarium decemcellulare*, *Fusarium* (section *Martierella*), and *Fusarium neoceras*. Species appearing on more than 30 per cent of the plates of the virgin prairie soil in addition to the above were *Mucor sphaerosporus*, *Penicillium notatum*, and *Trichoderma lignorum*, but these species also occurred in the revegetating field in lower numbers. Principal species of the abandoned field in addition to the principal ones common to both plots were *Fusarium moniliforme*, *Fusarium nivale*, *Mucor globosus*, and *Penicillium fellutanum;* these species occurred in the virgin prairie in

lower numbers. Species which were isolated from the virgin prairie soil only and which occurred on 10% or more of the plates were *Botrytis terrestris*, *Fusarium orthosceros*, *Penicillium biforme*, and *Sporotrichum pruinosum*. Species occurring in the revegetating field only and which occurred on 10 per cent or more of the plates from this plot were *Fusarium lateritum*, *Phycomyces nitens*, *Torula allii*, and *Trichosporium nigricans*. These results indicate a difference in species composition of fungi of the two plots even though the numbers of fungi were essentially the same in both plots.

Conclusions

The quantitative results of this investigation are summarized in Table III.

TABLE III

Total Numbers of Bacteria, Fungi, and Actinomycetes per Gram of Soil.

	Virgin prairie	Revegetating Field	Ratio
Bacteria (nutrient agar)	4,220,000	1,090,000	4/1
Fungi	4,450	4,250	1/1
Actinomycetes	1,588,000	2,540,000	1/2
Total	5,812,450	3,634,250	1.6/1

Although the number of organisms per gram was greater in the virgin prairie soil, the total microbial activity appeared to be greater in the revegetating field soil. This indicates that numbers alone should not be taken to represent total physiological activity of microorganisms.

Determinations of the *p*H and the organic carbon (Walkley-Black method) of the two soils in July, 1952, showed no significant difference between the organic carbon content of the two soils and very little difference in *p*H. Since organic carbon determinations were made using sieved soil, the results may not represent the true organic carbon content. The *p*H range of the virgin prairie soil was 6.7-7.3, while that of the revegetating field was 6.6-6.8.

Differences between the two soils which may affect the microflora are:

1. Topsoil. Virgin prairie topsoil is sandy loam; revegetating field topsoil is silt loam (5).
2. Compaction. Revegetating field soil is more compact (5).
3. Field capacity and soil moisture. Virgin prairie soil has a greater field capacity and more soil moisture (5).
4. Mulch. The virgin prairie has a heavy natural mulch (5).
5. Temperature. The revegetating field soil has a higher summer temperature (2).

Possibly the greater amount of soil moisture in the virgin prairie soil was responsible for the differences in numbers of microorganisms. The fact that wetting very dry soil greatly increases the activities of microorganisms may account for the unexpectedly increased rate of microbial activity, as measured by oxygen uptake, in the revegetating field soil. Waksman (8) has shown that remoistening soil after desiccation gives a pronounced increase in numbers of microorganisms and in physiological activity as measured by carbon dioxide evolution. Soil which has not been desiccated does not show this pronounced increase upon wetting.

It is evident that there are quantitative and qualitative differences between the microflora of these two plots. Whether these differences are

responsible for differences in vegetation and soil conditions or are the result of differing soil conditions and vegetation is not known. Probably differences in soil microflora appear as both causes and effects of the differences between plant communities. Future investigations correlating soil microbiology and plant ecology should prove of value in both fields.

SUMMARY

A study was made of the revegetating field and virgin prairie plots of the University of Oklahoma Grassland Investigation Plots in July, 1952, to provide some information on the microflora of these areas.

Total activity of microorganisms, as measured by rate of respiration, appeared to be greater in the revegetating field.

Bacterial numbers were significantly higher in the virgin prairie. In addition, some differences in principal species of the two plots were noted.

Numbers of actinomycetes were significantly higher in the revegetating field.

Numbers of fungi in the two soils were nearly the same; however, differences in species composition of the two plots were found.

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