# A COMPARISON OF SOIL MICROFUNGI OF TWO GRASSLAND AREAS IN CENTRAL OKLAHOMA

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**The soil microfungi of two plots of a tall-grass prairie were surveyed using the soil dilution-plate technique. Chemical and physical analyses of the two plots revealed few differences between them although they were dominated by different grasses. The plot dominated by the taller grass species did have a slightly coarser soil texture, lesser compaction, and thicker A-horizon. Twenty-two genera of fungi were isolated, the most prevalent being *Cunninghamella*, *Penicillium*, *Paecilomyces*, and *Trichoderma*. The mycoflora of the two plots was essentially the same.**

In past years a number of workers have shown correlations between soil mycoflora and their environment (1-7). Workers in Oklahoma have studied relationships of fungi in both prairie soil (3,5) and forested areas (4).

The purpose of this study was to attempt to correlate the mycoflora found in two grassland plots located in the University of Oklahoma Grasslands Research Area with the soil types and dominant plants present. The two plots were within 25 feet of each other. The areas studied are located 8 miles southwest of Norman in McLain County, Oklahoma. The soil composition of the two plots differed only slightly. The mid-grass plot was composed of sandy loam and was dominated by *Andropogon scoparius* (little bluestem), while the tall-grass plot was composed of sand and was dominated by *Andropogon scoparius, A. gerardi* (big bluestem), and *Panicum virgatum* (switchgrass). Because of the differences in vegetation, it was decided to determine whether a similar marked difference in mycoflora existed.

## METHODS

The plots were sampled on three occasions, in February, March and April, 1970, for soil microfungi. The methods reported by Chesters and Thornton (8) were used with slight modifications. Three samples were collected along a diagonal transect across each plot at each sampling time. The litter and top half-inch of soil were removed and then approximately 10 g of soil from the top 3 inches were removed to sterile vials using a separate sterile spatula for each sample. Nine samples were taken from each plot and samples were stored at 4 C until plating was possible. The medium used for plating (ME agar) consisted of the following:

| | |
|---|---|
| malt extract | 3 g |
| peptone | 5 g |
| glucose | 10 g |
| agar | 20 g |
| penicillin G | 250,000 units |
| streptomycin sulfate | 250 mg |
| distilled water | 1,000 ml |

In preliminary experiments, we compared this medium with rose bengal streptomycin (RBS) agar (9). We chose the former medium (ME) because it effectively controlled bacterial growth and, at the same time, permitted better growth of a wider variety of fungi than did RBS agar. Dilutions of 1:1,000, 1:10,000, and 1:100,000 were plated for colony counts and isolation. Colonies were transferred to malt extract agar slants as soon as growth appeared. Identification of Zygomycetes was based on Zycha (10), whereas Barron (11) was used in the identification of Deuteromycetes. Total colony counts for each sample were also made.

Field sampling of the plants occurring in the two plots was carried out by students of a graduate physiological ecology class, as were the chemical and physical analyses of the soil. The plots were systematically sampled by the use of 10 clip quadrats (0.25m$^2$), and the dominant species of each plot were determined by the methods of Curtis and McIntosh (12) and Rice and Penfound (13).

Soil samples (minus litter) were collected during February, 1970 from each plot at two levels (0-6 inches and 12-18 inches). All soil tests were made on air-dry soil, but all calculations, with the exception of texture and pH, were based on the oven-dry weight of the soil. Soil pH was determined by the glass electrode method of

Piper (14). Mechanical analysis was performed by the method of Bouyoucos (15) as modified by Piper (14). The chromic acid digestion method (14) was employed for the determination of organic carbon content.

Total phosphorus was measured by the method of Shelton and Harper (16); exchangeable potassium and magnesium were extracted by a method modified from Pratt (17, p. 1026) and quantified on an atomic absorption spectrophotometer, Perkin Elmer Model 303. Total base exchange capacity was analyzed by a method modified from Noggle and Wynd (18) and total nitrogen by the macro-Kjeldahl method of Bremner (17, pp. 1149-1178). To determine bulk-density or compaction the following method, modified from Blake (17, pp. 377-381), was used. Holes two inches in diameter and three inches deep were made and the volume (ml) of quartz sand required to fill the holes was recorded. Oven-dry weight of the excavated soil was determined by gravimetric analysis. The volume weight (g/ml) was determined by dividing the dry weight of the soil by the volume of sand required to fill the hole.

## RESULTS

There was very little difference in chemical composition between the two plots (Table 1). There were some differences in physical factors, the most noticeable difference being in soil compaction. This indicated a greater porosity in the tall-grass prairie. The mid-grass plot had a finer textured soil and a considerably thinner A-horizon.

TABLE 1. *Results of physical and mineral analyses of the top six inches of soil in the mid-grass and tall-grass plots.*

| | Mid-grass | Tall-grass |
|---|---|---|
| pH | 6.9 | 6.5 |
| % organic carbon | 0.92 | 0.83 |
| % nitrogen | 0.086 | 0.068 |
| % phosphorus | 0.014 | 0.010 |
| % potassium | 0.018 | 0.010 |
| % magnesium | 0.035 | 0.023 |
| base exchange capacity | 9.86 | 8.10 |
| % sand | 75.7 | 83.6 |
| % clay | 17.4 | 12.4 |
| % silt | 6.8 | 4.0 |
| depth A horizon (inches) | 7.5 ± 0.4 | 13.75 ± 1.6[a] |
| % soil moisture | 20.4 ± 0.59 | 19.1 ± 0.59 |
| Compaction (bulk density) | 1.071 ± 0.060 | 0.892 ± 0.059[a] |

Estimates of fungus propagules in these plots yielded approximately 120,000 per gram of soil in the mid-grass plot and about 146,000 in the tall-grass plot. Twenty-two different genera were isolated from the plots (Table 2). The most frequently isolated genera were *Cunninghamella, Penicillium, Paecilomyces,* and *Trichoderma.* Little difference was noted between the two sampling areas; the number of species isolated from each was similar.

TABLE 2. *Genera and total number of fungal colonies obtained per gram of soil of the two grassland plots.*

| Class genus | Mid-grass | Tall-grass |
|---|---|---|
| Oomycetes | | |
| *Aphanomyces* | | X |
| Zygomycetes | | |
| *Absidia* sp. | X | X |
| *Cunninghamella*[a] *echinulata* | X | X |
| *C. elegans* | X | X |
| *Mortierella* sp. | X | X |
| *Mucor* sp. | X | X |
| *Rhizopus* sp. | X | X |
| *Zygorrhynchus* sp. | X | X |
| Deuteromycetes | | |
| *Alternaria* sp. | X | |
| *Aspergillus aculeatus* | X | |
| *A. flavus* | | X |
| *A. niger* | X | X |
| *A. nivens* | X | |
| *A. terreus* | X | |
| *Cephalosporium* sp. | | X |
| *Chloridium* sp. | X | X |
| *Curvularia* sp. | | X |
| *Cylindrocarpon* sp. | | X |
| *Dactylidium* sp. | | X |
| *Doratomyces* sp. | X | |
| *Epicoccum* sp. | | X |
| *Fusarium* sp. | X | X |
| *Gliocladium* sp. | X | X |
| *Penicillium*[a] sp. | X | X |
| *Paecilomyces*[a] sp. | X | X |
| *Trichoderma*[a] *album* | X | |
| *T. lignorum*[a] | X | X |
| *Verticillium* sp. | | X |
| Total no. per g/of soil | 120,000 | 146,000 |

[a] Predominant genera

## DISCUSSION

The major edaphic differences in the two grassland plots were the more shallow A-horizon and the greater compaction of the mid-grass plot. Not only was the depth to

the parent material, Garber sandstone, less in the mid-grass plot, but in several places there were rocky outcrops. In addition, there was a definite dark red, clay hard-pan in the mid-grass plot. Weaver and Crist (19) reported that a clay hard-pan prevents percolation of water to lower depths in the soil and that available water occurs normally only above the hard-pan. Thus, the level of the hard-pan determines the depth of penetration of roots since roots normally do not penetrate dry soil.

Weaver and Fitzpatrick (20) reported that big bluestem is more mesic than little bluestem and that it commonly provides a cover of 80-90% in well-watered areas. It has an advantage in such areas in competition for sunlight because of its greater height. *Andropogon scoparius*, however, is better adapted than big bluestem to adsorb moisture in relatively dry soil because of its very fine and extensively branched root system (20).

In a study in northern Indiana, Bliss and Cox (21) were able to identify five types of prairie communities. The big bluestem communities were found in areas with a relatively deep A-horizon, high availability of soil moisture, high organic matter, and good drainage. Little bluestem communities, on the other hand, were associated with a thinner A-horizon, lower availability of soil moisture and less organic matter. In addition, they reported a high frequency of *A. scoparius* and a greater diversity of species in the big bluestem communities and a low frequency of *A. gerardi* in little bluestem communities. Our results closely parallel those of Bliss and Cox (21) and therefore it appears that the mosaic pattern of vegetation observed is a result of differential availability of water resulting from the differences in compaction, the depth of the A-horizon, and the presence of the clay hard-pan. It is expected that these factors would be unlikely to affect the distribution of soil microfungi in the top three inches of soil, since little difference was noted in the soil moisture content of the top six inches in the two plots (Table 1).

The two plots were much alike in terms of the total number of fungal colonies obtained per gram of soil and in the genera found. Most of the genera found are of ecological significance. Several of them have species reported to be parasitic on higher plants (*e.g., Alternaria, Aphanomyces, Cercularia, Fusarium* and *Verticillium*). *Trichoderma* can exhibit antimycotic activity (22). Many of the Deuteromycete genera found in this study can be classified, according to Garrett (23), as cellulose decomposers. The presence of *Dorotomyces* in this soil agrees with the findings of the soil analyses, as it is commonly found in soils high in organic content (11).

Most of the genera isolated in this study have been previously reported by England and Rice (3), who conducted a similar study in central Oklahoma. However, *Cunninghamella*, which was frequently isolated from both plots of this grassland, was not reported in their study. Miller, Giddens, and Foster (24), in an analysis of fungi in Georgia soils, reported *Cunninghamella* as a dominant genus. Recently, Blunt and Baker (22) reported *Cunninghamella echinulata* as the most promising of a series of 175 fungi exhibiting antimycotic activity isolated from Hawaiian soils. This species was capable of antimycosis *in vitro* against all 5 species of human pathogens tested, all of which are known to occur in soil. This organism has been used in biological assays for the phosphorus content of soils (25); its presence or absence may be dependent on the quantity of this element in the soil.

*Aspergillus* was not found to be prominent, in contrast to previous reports (3, 7). Orpurt (7), by comparing his work with that of Tresner (26), indicated that *Aspergillus* is quite commonly found in undisturbed prairie regions, but only rarely in forested areas. Mallik and Rice (4) correlated the presence of *Aspergillus* with the successional stage of the area studied. They found *Aspergillus* to be less important in the climax stage, whereas it was frequently collected from both pioneer and transitional areas.

Since most of the genera found in this study have also been found in areas ranging from Costa Rica to Wisconsin, the only difference between areas seems to be in the relative frequencies, which can vary greatly with both technique and media used.

A number of workers have begun to correlate the presence of certain fungi with their environment, and two areas of

thought have arisen from this type of work: one is that fungi are cosmopolitan; the other is that the presence of fungi is directly related to soil conditions, higher plants, etc. To date, published work seems to support both concepts (6, 26). However, in future studies, it may be more important to correlate the presence of fungi that are actively growing in the soil with their environment, rather than to include all forms, some of which may be in a dormant or resting state. A study of this type might possibly produce more ecologically significant results.

## REFERENCES


1. M. CHRISTENSEN, W. B. WHITTINGHAM, and R. O. NOVAK, Mycologia 54: 374-388 (1962).
2. M. CHRISTENSEN and R. O. NOVAK, Mycologia 57: 882-896 (1965).
3. C. E. ENGLAND and E. L. RICE, Bot. Gaz. 118: 186-190 (1957).
4. M. A. B. MALLIK and E. L. RICE, Bot. Gaz. 127: 120-127 (1966).
5. C. MCELROY, W. H. JONES, and F. A. RINEHART, Proc. Okla. Acad. Sci. 33: 163-168 (1954).
6. M. TURNER and G. J. F. BAUGH, Trans. Brit. Mycol. Soc. 44: 243-252 (1961).
7. P. A. ORPURT and J. T. CURTIS, Ecology 38: 629-637 (1957).
8. C. G. C. CHESTERS and R. H. THORNTON, Trans. Brit. Mycol Soc. 39: 301-313 (1956).
9. J. P. MARTIN, Soil Sci. 69: 215-232 (1950).
10. H. ZYCHA, *Mucorineae. Kryptogamenflora der Mark Brandenburg*, Gebruder Borntraeger, Leipzig, 1935, 6a, 1-264.
11. G. L. BARRON, *The Genera of Hyphomycetes from Soil*, Williams and Wilkins Co., Baltimore, 1968.
12. J. T. CURTIS and R. P. MCINTOSH, Ecology 32: 476-496 (1951).
13. E. L. RICE and W. T. PENFOUND, Ecology 40: 593-608 (1959).
14. C. S. PIPER, *Soil and Plant Analysis*, Univ. of Adelaide, Adelaide, Australia, 1942.
15. G. J. BOUYOUCOS, Soil Sci. 42: 225-230 (1936).
16. W. B. SHELTON and H. J. HARPER, Iowa State Coll. J. Sci. 15: 408-413 (1941).
17. C. A. BLACK *et al.* (eds.), *Methods of Soil Analysis*, parts 1 and 2, Amer. Soc. Agron. Inc., Madison, Wisconsin, 1965.
18. G. R. NOGGLE and F. L. WYND, Plant Physiol. 16: 39-60 (1941).
19. J. E. WEAVER and J. W. CRIST, Ecology 3: 237-249 (1922).
20. J. E. WEAVER and T. J. FITZPATRICK, Bot. Gaz. 93: 113-150 (1932).
21. L. C. BLISS and G. W. COX, Amer. Midl. Nat. 72: 115-128 (1964).
22. F. L. BLUNT and E. BAKER, Mycologia 60: 559-570 (1968).
23. S. D. GARRETT, New Phytol. 50: 149-166 (1951).
24. J. H. MILLER, J. E. GIDDENS, and A. A. FOSTER, Mycologia 49: 779-808 (1957).
25. W. D. GRAY, *The Relation of Fungi to Human Affairs*, Holt and Co., New York, 1959.
26. H. D. TRESNER, M. P. BACKUS, and J. T. CURTIS, Mycologia 46: 314-333 (1954).