THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

RELATION BETWEEN SOIL FUNGI AND SEED PLANTS IN THREE SUCCESSIONAL FOREST COMMUNITIES IN OKLAHOMA

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

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Norman, Oklahoma

1964

RELATION BETWEEN SOIL FUNGI AND SEED PLANTS IN THREE SUCCESSIONAL FOREST COMMUNITIES IN OKLAHOMA

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ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to professor Elroy L. Rice for his guidance and assistance on this problem. He is grateful to Dr. H. W. Larsh, Dr. D. E. Anderegg, Dr. Wm. T. Penfound, Dr. G. J. Goodman, and Dr. J. B. Clark for their valuable suggestions and encouragements during the investigations. Thanks are due to Mr. R. L. Parenti and to many others who helped the author in a number of ways.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

RELATION BETWEEN SOIL FUNGI AND SEED PLANTS IN THREE SUCCESSIONAL FOREST COMMUNITIES IN OKLAHOMA

INTRODUCTION

During the last three decades, the study of soil fungi has aroused great interest throughout the globe. Most literature on the subject is concerned with the fungi of agricultural soils. Ling-Young suggested in 1930 that one should study the soil from such localities as forests and mountains undisturbed by man to obtain an idea of endemic microflora. In spite of his suggestion, little attention has been paid to the study of microfloras from such localities. Where such studies have been made, sampling sites have not been described adequately to obtain meaningful relationships from an ecological viewpoint. Warcup (1951) made a detailed investigation of microfungi from natural grassland and attempted to interpret his results ecologically. In the analysis of their data, Tresner, Backus, and Curtis (1954) used techniques analogous to those used in the study of above ground vegetation. Their results indicated that there existed a complex interrelationship between the cover vegetation and the soil microfungi. Although many species were ubiquitous in nature, occurring in nearly all the stands, some species showed an optimum in a definite portion of the stand gradient thus simulating the behavior of the higher plants. Orpurt and Curtis (1957) reported that the total population of fungi and bacteria and

the total number of species of soil fungi reached a maximum in mesic prairie stands, whereas the actinomycetes were more numerous in dry prairies. England and Rice (1957) found both qualitative and quantitative differences in the soil populations of a tall grass prairie and a revegetating old field. Christensen, Whittingham, and Novak (1962) showed that the soil microfungal population of a wet mesic forest was different from that of upland forest and prairie.

Brown (1958) found a succession of fungal species correlated with the succession of higher plants occurring across a British dune system. Similar results were obtained by Wohlrab, Tuveson, and Olmsted (1963) in Indiana sand dunes. Odum (1963) mentioned that "the number of species of heterotrophs increases until relatively late in the sere".

Knowledge of total metabolic activity of soil microorganisms is useful in understanding soil microbial ecology. Gray and Taylor (1935) could not find any correlation between the bacterial number and the amount of carbon dioxide evolved from the A1 horizon of a podzol soil. Swartz et al. (1953) indicated that the total metabolic activity and the number of microorganisms per gram of soil were higher in virgin prairie than in a revegetating field. This was contradictory to the findings of McElroy, Jones, and Rinehart (1954). Stevenson (1956) found a higher respiratory rate in air dried soil than in fresh soil despite a lower bacterial population in air dried soil. Gray and Wallace (1957) showed a direct correlation between the bacterial number and amount of carbon dioxide evolved from agricultural soil as determined by the barium-hydroxide absorption technique.

The present investigation was concerned with soil microorganisms of three well defined natural areas representing successional stages in forest vegetation. The soil fungi from these areas were compared throughout the year. An attempt was made to determine the interrelationships existing between the soil fungi, the above ground vegetation, the moisture and organic carbon content of the soil, and the reaction of the soil. The metabolic activity of the soil microorganisms was compared. No effort was made to classify the bacteria and actinomycetes; however, their numbers per gram of soil were determined.

DESCRIPTION OF STUDY AREAS

The three natural areas, selected for this study represent stages of primary succession in forest vegetation along the South Canadian River near Norman in Cleveland County, Oklahoma. These consisted of the following: a) a pioneer area, b) a transitional area, and c) a climax area.

The area chosen as representative of the pioneer stage is located about 2 miles southwest of the University Campus (Fig. 1). Ware and Penfound (1949) designated this area as "the first level of the river". The soil is composed of fine sand and silt. Evaporation and surface temperatures during summer months are high. The vegetation consists of seedlings of Salix interior*, Tamarix gallica, and Populus deltoides as the predominant woody species. Cyperus esculentus, Panicum dichotomiflorum, Desmanthus illinoensis, and Xanthium strumarium are the important herbaceous species.

The cottonwood savanna, chosen as a transitional stage, is located about 5 miles southwest of Norman (Fig. 2). A rivulet runs by one side of the plot. The open stand consists almost entirely of Populus deltoides. The important ground cover includes Muhlenbergia asperifolia, Panicum virgatum and Symphoricarpos orbiculatus. Most of

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*Nomenclature of grasses follows Hitchcock and Chase (1950) and of other vascular plants, Waterfall (1962).

FIG. 1. The pioneer area. Tree seedlings of Populus deltoides, Salix interior, and Tamarix gallica with many herbaceous species.

FIG. 2. The transitional area. Scattered trees of Populus deltoides interspersed with Salix nigra; fairly good ground cover.

FIG. 3. The climax area. Large trees of Fraxinus pennsylvanica, Ulmus americana, and Populus deltoides; thick ground cover.

the trees were 15 years old suggesting that the river was diverted away from this area about 15 years ago. The soil is sandy loam and reddish in color. The forest was burned very lightly in the recent past, as evidenced from fire scars. The area was not grazed for several years prior to the summer of 1962 (when this work was started) but has been heavily grazed since early spring of 1963.

The mature bottomland forest representing the climax is located in the University's Oliver Wild Life Preserve about 1 mile south of the main campus of the University (Fig. 3). In a complete census of the area, Penfound and Rice (1956) found Fraxinus pennsylvanica var. subintegerrima to be the dominant species with Ulmus americana, Diospyros virginiana and Populus sargentii (designated as P. deltoides in present study) as important secondary species. The floor of the forest contains thick herbaceous vegetation which includes Bromus japonicus, Cyperus esculentus, Iva ciliata, and Leersia virginica. Smilax bona-nox and Symphoricarpos orbiculatus are common ground cover also. The soil is dark grey, ramified heavily by plant roots, and contains a high amount of humus. The area has remained fairly undisturbed for a considerable period except for grazing prior to 1961.

MATERIALS AND METHODS

Vegetational Analyses

Arboreal Vegetation. - Quantitative analyses of the vegetation were done during August, September, and October of 1963. The arboreal vegetation was analysed by the modified variable-radius method described by Rice and Penfound (1955) along four compass lines in the transitional area. Basal area was determined by using an angle gauge at intervals of 30 paces. The arm-length rectangle method was employed to determine density and frequency of trees and shrubs. Any woody plant with a diameter breast high (DBH) of 1-2.9 in. was classified as a sapling if it belonged to a species which eventually reaches a DBH of at least 3 in. in the study areas. From these data, the number of saplings and trees per acre, relative density, relative frequency and relative basal area were calculated. The importance percentage was determined by averaging the relative density, relative frequency and relative basal area. The data on arboreal vegetation of the climax bottomland forest were obtained from Rice and Penfound (1956).

Herbaceous Vegetation. - Basal cover and relative composition of the herbaceous vegetation and shrubs in the transitional area were obtained by the point frame method (basal contacts). The total number of points sampled was 1000. A list was made of the more common herbaceous

species not contacted by the point frame. The herbaceous vegetation in the pioneer area and in the climax was analysed by 75 quarter square-meter quadrats in each area. Density and frequency data were taken and relative density and relative frequency were calculated. The importance percentage was determined by averaging the relative density and relative frequency.

Isolation of Soil Microorganisms

Soil samples were collected at intervals of two months for one full year starting in July, 1962. Samples were collected at intervals of 50 paces along each of two compass lines oriented lengthwise in each of the plots. Eight samples were collected from each of the three study areas at each sampling period. Prior to the collection of samples, the surface soil from the upper half inch was scraped off with a spatula. A small trench was dug to a depth of 6 in.; the soil was scraped in from each wall of the trench and thoroughly mixed before removing a portion of the soil to a sterilized bottle. The spatula was sterilized with an alcohol swab each time before use. Duplicate samples were taken in each case for the determination of soil moisture content. Composite samples were also collected for the determination of pH, organic carbon content, and metabolic activity. All samples used for microfloral analyses were plated within 12 hours of their collection. James's (1959) soil extract medium for fungi with streptomycin added was employed for the isolation and determination of the numbers and kinds of fungi. A soil extract medium for bacteria recommended by James (1959) was

used for the determination of the numbers and kinds of bacteria and actinomycetes. Soil extracts were made following the recommendation of James (1958), but were centrifuged to separate the colloidal particles rather than filtered. Soil extract obtained from a particular soil was used in the preparation of media for the isolation of microflora present in that soil. The pH of the media was adjusted to 6.0 for fungi and 7.0 for bacteria and actinomycetes. About 20 ml of medium were poured into sterilized petri plates one day before soil sampling.

The dilution-plate technique was employed for microfloral analyses. Eight to 30 colonies of fungi and 30 to 300 colonies of bacteria and actinomycetes were desired per plate. Dilutions of 1:1,000; 1:2,000; and 1:4,000 for the isolation of fungi and 1:100,000; 1:2000,000; and 1:1000,000 for bacteria and actinomycetes were found to be appropriate for the pioneer, the transitional, and the climax areas respectively. A 0.5 ml aliquot from the appropriate dilution was pipetted onto the top of each of the prepared plates. By gentle vertical and horizontal rotation the soil suspension was spread evenly over the plate. Three replicates for fungi and three for the bacteria and actinomycetes were employed for each soil sample from each plot. During the entire period of preparation of soil suspension and inoculation, three prepared plates were kept exposed in the laboratory so that any laboratory aerial contaminants could be noted. Such contaminants which occurred frequently on the check plates were not included in the list of species from the soil plates. Czapek's agar medium (Thom and Raper 1945) and Sabouraud's medium (Difco) were enployed for identification.

Procedure of Fungal Analyses

After incubation for an appropriate period (four days for bacteria and seven to ten days for fungi), the number of colonies of microorganisms were counted and the average numbers of bacteria, actinomycetes, and fungi per gram of soil (on the dry weight basis) were calculated from the plate counts. No effort was made to identify the bacteria and actinomycetes. The fungal colonies were sorted and identified. The organisms which could not be identified from the original plates were transferred to Czapek's agar slants and identified later. Frequency and relative density were calculated for each species in each sample by the formulae suggested by Tresner et al. (1954).

$$\text{Frequency percentage} = \frac{\text{Number of plates in which a particular species occurred}}{\text{Total number of plates}} \times 100$$

$$\text{Relative density} = \frac{\text{Total number of colonies of a species}}{\text{Total number of colonies of all species}} \times 100$$

Soil Analyses

The soil moisture content and pH of the soil were determined by standard techniques (Piper 1944). The total metabolic activity of each of the soil types was determined by measuring the oxygen uptake of the soil microorganisms. The water holding capacities of the soil types were found to be 24.1, 30.9, and 35.2 per cent for the pioneer, the transitional, and the climax areas respectively. Sufficient distilled

water was added to 2 gm of air dried and sieved soil in the Warburg respirometer to obtain a concentration of 75% of water holding capacity. A period of 30 minutes was allowed for the flasks to reach equilibrium before the first reading was taken. The readings were taken over a period of five hours. The temperature of the water bath was maintained at 29°C. The conventional method was used for calculation of the rate of respiration (Umbreit, Burris, and Stauffer 1959).

For the determination of the organic carbon content of the soil, the modified Walkely and Black method suggested by Piper (1944) was employed.

RESULTS

Vegetational Analyses

Of the three arborescent species in the pioneer area, Tamarix gallica had the highest density and importance percentage (Table 1). This species had four times as many seedlings as Salix interior and over 35 times as many Populus deltoides. The dominant in the transitional area was Populus deltoides. This area could be designated as a cottonwood savanna because of the widely scattered trees and the heavy ground cover of grasses. In this area Tamarix gallica, which rarely attains tree size, had the highest number of saplings and Populus deltoides the lowest of the species present. Apparently Populus deltoides was not reproducing well, but the data indicated that Tamarix gallica and Salix nigra were not reproducing well either. The low density of Salix nigra could be due to the high susceptibility to fire damage. Fraxinus pennsylvanica was just starting to invade the transitional area, whereas it was dominant in the climax. Species of secondary importance in the climax were Ulmus americana, Diospyros virginiana and Populus deltoides. Minor species in order of descending importance were Celtis laevigata, Quercus macrocarpa, Carya illinoensis, Salix nigra, Acer negundo, Catalpa speciosa, Crataegus viridis, Bumelia lanuginosa, and Gymnocladus dioica. "The minor status of this group is indicated by the fact that

TABLE I. Results of quantitative analysis of arborescent species[1]

SPECIES	DENSITY PER ACRE					IMPORTANCE PERCENTAGE		
	Seedlings	Saplings		Trees				
	Pioneer	Transi-tional	Climax	Transi-tional	Climax	Pioneer[2]	Transi-tional[3]	Climax[3]
Fraxinus pennsylvanica	-	0.5	0.9	-	79.7	-	-	40.5
Ulmus americana	-	-	0.1	-	19.5	-	-	15.7
Diospyros virginiana	-	-	9.6	-	27.7	-	-	14.3
Populus deltoides	680	0.3	-	45.0	3.3	24.0	94.4	11.9
Salix nigra	-	1.0	-	2.5	-	-	5.6	-
Salix interior	6,194	-	-	-	-	20.0	-	-
Tamarix gallica	25,589	1.7	-	-	-	56.0	-	-
Minor species (9)	-	-	0.8	-	14.5	-	-	17.6
Total	38,546	3.5	11.4	47.5	144.7	100.0	100.0	100.0

1 Data on the climax area obtained from Rice and Penfound (1956).
2 Average of relative density and relative frequency.
3 Average of relative density, relative frequency, and relative basal area.

TABLE II. Results of quantitative analysis of shrubs and vines

SPECIES	IMPORTANCE PERCENTAGE*	
	Transitional	Climax
Smilax bona-nox	-	37.3
Symphoricarpos orbiculatus	92.3	35.9
Vitis palmata	-	12.4
Rhus radicans	-	5.3
Cornus drummondii	-	3.0
Rosa setigera	-	3.0
Smilax tamnoides	-	3.0
Ampelopsis cordata	7.7	-
Totals	100.0	99.9

* Consists of relative composition in the transitional area; and average of relative density and relative frequency in the climax.

the total importance percentage of these nine species was about the same as that of any one of the major secondary species" (Rice and Penfound 1956). Diospyros virginiana was reproducing well in this area as was evidenced by the number of saplings.

The predominant shrub in the transitional area and in the climax was Symphoricarpos orbiculatus (Table II). Of the total of eight species of shrubs and vines in these two areas, only Symphoricarpos orbiculatus occurred in both. The only woody vine in the transitional area was Ampelopsis cordata, and the most important woody vine in the climax area was Smilax bona-nox. Other shrubs and vines not listed in Table II included Amorpha fruticosa and Sambucus canadensis in the transitional area, and Smilax rotundifolia in the climax. No shrubs or woody vines occurred in the pioneer area.

Cyperus esculentus was the predominant herb in the pioneer area and in the climax with importance percentages of 36.4 and 19.8 respectively (Table III). This species did not occur in the transitional area. Other important species in the pioneer area in order of descending importance percentage were Desmanthus illinoensis, Panicum dichotomiflorum, Xanthium strumarium, and Panicum virgatum. None of these occurred in the other two areas except Panicum virgatum which was the predominant herb in the transitional area. Muhlenbergia asperifolia was an important species in the transitional area but it had a low importance percentage in the pioneer area. Of the 12 herbaceous species encountered in the transitional area in the analyses, only three were common to both pioneer and transitional areas and two to both the transitional and climax areas. In the climax area, 19 species were

TABLE III. Results of quantitative analysis of herbaceous species

SPECIES	IMPORTANCE PERCENTAGE*		
	Pioneer	Transitional	Climax
Cyperus esculentus	36.3	-	19.8
Bromus japonicus	-	-	13.6
Iva ciliata	-	-	12.8
Leersia virginica	-	-	12.0
Muhlenbergia sylvatica	-	-	5.7
Cynodon dactylon	-	-	5.2
Sporobolus neglectus	-	-	4.9
Ambrosia trifida	-	-	4.1
Ruellia strepens	-	-	4.0
Tridens flavus	-	-	3.3
Artemisia sp.	-	-	2.9
Elymus virginicus	-	-	2.8
Paspalum ciliatifolium	-	-	2.2
Galium circaezans	-	-	1.9
Solidago missouriensis	-	4.8	1.6
Aster eriocoides	-	-	1.2
Panicum malacophyllum	-	-	0.8
Solanum sp.	-	-	0.6
Verbena urticifolia	-	1.4	0.6
Panicum virgatum	6.0	44.5	-

*Consists of average of relative density and relative frequency in the pioneer and the climax; and relative composition in the transitional areas.

TABLE III. Continued -

SPECIES	IMPORTANCE PERCENTAGE		
	Pioneer	Transitional	Climax
Muhlenbergia asperifolia	3.3	35.6	-
Lespedeza sp.	1.8	3.4	-
Elymus canadensis	-	2.7	-
Viola missouriensis	-	2.0	-
Cyperus brevifolius	-	1.4	-
Euphorbia marginata	-	1.4	-
Sonchus asper	-	1.4	-
Oxalis stricta	-	0.7	-
Strophostyles leiosperma	-	0.7	-
Desmanthus illinoensis	8.1	-	-
Panicum dichotomiflorum	7.1	-	-
Xanthium strumarium	6.0	-	-
Panicum capillare	5.4	-	-
Aster sericeus	5.0	-	-
Amaranthus hybridus	4.6	-	-
Verbesina encelioides	2.6	-	-
Vernonia baldwini	2.4	-	-
Ambrosia artemisiifolia	2.1	-	-
Melilotus alba	1.8	-	-
Erigeron canadensis	1.7	-	-
Setaria geniculata	1.7	-	-

TABLE III. Continued -

SPECIES	IMPORTANCE PERCENTAGE		
	Pioneer	Transitional	Climax
Oenothera biennis	1.1	-	-
Andropogon gerardi	0.6	-	-
Digitaria sanguinalis	0.6	-	-
Echinochloa colonum	0.6	-	-
Helianthus annuus	0.6	-	-
Setaria lutescens	0.6	-	-
Totals	100.0	100.0	100.0

encountered. In addition to the predominant species, Cyperus esculentus, the other important species included Bromus japonicus, Iva ciliata, Leersia virginica, Muhlenbergia sylvatica, and Cynodon dactylon with importance percentages ranging from 13.6 to 5.2. The following species were present but not encountered in the analyses: Andropogon glomeratus in the pioneer area; Aster patens, Ambrosia psilostachya, Eupatorium coelestinum, Gutierrezia dracunculoides, Parthenium hysterophorus, Polygonum punctatum, Setaria lutescens, Solidago gymnospermoides, Cassia nictitans, Apocynum cannabinum, and Lobelia cardinalis in the transitional area; and Polygonum aviculare in the climax area.

Analyses of Microorganisms

The number of microorganisms in the climax area was nearly seven times as high as in the pioneer area and two times as high as in the transitional area (Table IV). The fungal counts per gram of dry soil were generally higher than those reported by England and Rice (1957) who used a soil plate technique. The higher count in this study might have been due to the different technique used. Conversely, Tresner et al. (1954) and Christensen et al. (1962) reported higher fungal and lower bacterial counts than in the present study. The high pH of the soil in all plots provided favorable conditions for bacterial proliferation whereas it affected the fungi adversely. The total oxygen uptake was lowest in the pioneer and highest in the climax areas (Fig.4). The organic carbon content of the climax area was about three times higher than that of the transitional area and ten times higher than that of the pioneer area (Table IV). The soil moisture of both the climax

TABLE IV. Results of soil analyses and number of microorganisms per gram dry soil in the three areas throughout the year.

MONTHS	PIONEER			TRANSITIONAL			CLIMAX		
	Numbers of Microorganisms*								
	Bacteria	Actino-mycetes	Fungi	Bacteria	Actino-mycetes	Fungi	Bacteria	Actino-mycetes	Fung
July	8,310	600	8.78	69,830	1,098	30.50	63,740	2,099	67.69
September	9,890	640	14.63	44,550	1,430	38.08	75,750	2,060	71.86
November	6,650	179	13.41	29,530	634	31.25	70,670	1,900	73.57
January	13,320	44	13.30	34,020	1,460	34.10	72,910	1,320	70.96
March	18,430	87	12.74	51,950	1,588	35.17	158,620	5,140	97.40
May	29,770	1,480	12.06	84,590	5,590	46.34	163,510	4,770	120.47
Average	14,395	505	12.49	52,411	1,633	35.90	100,866	2,881	83.65

Soil Analyses

	% Org. Carbon	% Soil Moisture	pH	% Org. Carbon	% Soil Moisture	pH	% Org. Carbon	% Soil Moisture	pH
July	0.154	15.49	9.0	0.460	22.82	8.5	1.520	20.69	8.6
September	0.093	21.21	8.8	0.233	25.86	8.0	1.596	28.46	8.1
November	0.154	20.64	9.0	0.513	25.85	8.5	1.260	26.12	8.4
January	0.154	21.32	8.6	0.491	28.75	8.2	1.897	29.53	8.2
March	0.168	21.23	8.7	0.577	30.53	8.3	1.786	31.35	8.1
May	0.106	16.89	8.8	0.570	26.89	8.3	1.503	31.25	8.4
Average	0.138	19.46	8.8	0.474	26.78	8.3	1.594	27.90	8.3

* in thousands

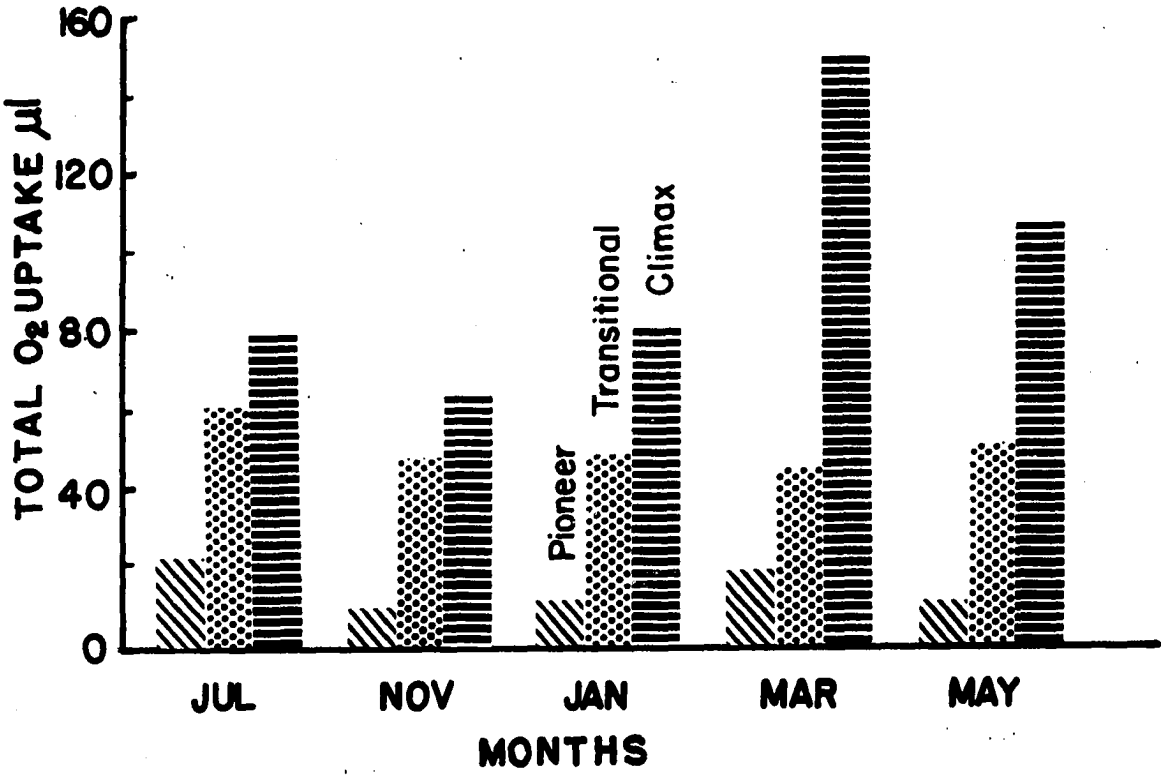

FIG. 4. Comparison of amounts of oxygen uptake in the three areas.

and the transitional areas was about 8% higher than that of the pioneer area. The pH of the soil in all three areas was alkaline, the highest average, 8.8, being in the pioneer area.

Although the numbers of microorganisms fluctuated in different months, a trend was apparent. The fungal counts were lowest in July with secondary lows in November or January. The bacterial counts were lowest in November except in the climax area. The counts of actinomycetes were generally lowest in late fall or winter. These counts increased appreciably in all three areas in spring. The percentage increase in the number of microorganisms was higher in the climax than in the other areas. The fungal counts in the pioneer area, however, showed no increase from September through May.

The total oxygen uptake was lowest in November in all three areas (Fig. 4). A higher oxygen uptake was apparent in March and May along with the increase in the number of microorganisms. The very high oxygen uptake in March in the climax area, however, could not be explained. The correlation between oxygen uptake and the number of microorganisms was poor.

The organic carbon content of the soil did not change appreciably in different months except in September in both the pioneer and the transitional areas. A slight increase in winter and in spring was probably due to the leaf fall during the previous autumn. The soil moisture generally increased from September through March reaching its peak in March.

Species Distribution

The pioneer area yielded lowest number (average 28.8) and the

TABLE V. Number of fungal species isolated in different months in the three areas.

MONTHS	PIONEER	TRANSITIONAL	CLIMAX
July	12	40	36
September	24	42	40
November	35	49	50
January	28	38	39
March	43	51	49
May	31	64	59
Average	28.8	47.3	46.5

transitional area highest number (average 47.3) of fungal species throughout the year (Table V). The difference between the number of fungal species in the transitional and the climax areas was very small. The number of fungal species was low in July and January, and increased in March and May in general conformity with the total counts.

Of the total of 157 species distributed in 64 genera, 73 occurred in the pioneer area, 125 in the transitional, and 115 in the climax (Table VI). Thirty four per cent of the species were common to all three areas. Only 7% of the species were common to the pioneer and the transitional areas, and 4% were common to the pioneer and the climax areas. Eighteen per cent of the species, however, were common to the transitional and the climax areas. The number of species restricted to a particular area was very few, 1.3% in the pioneer area, 17.8% in the transitional, and 14.6% in the climax.

The majority of the fungi belonged to the Deuteromycetes. Phycomycetes and Ascomycetes were very poorly represented, none occurring in the pioneer area. The important genera with respect to the number of species isolated were Penicillium, Fusarium, Aspergillus, Cephalosporium, and Gliocladium (in order of descending importance). Aspergillus was the most common of these in the pioneer area. Fusarium was prominent in both the transitional and climax areas. Aspergillus and Cephalosporium were more numerous in the transitional area than in the climax, whereas Penicillium and Gliocladium were more prominent in the climax area than in any other. Trichoderma was common in all three areas.

The species which occurred in at least four of six sampling periods

TABLE VI. Average frequency percentage of fungi in the three areas.

SPECIES*	PIONEER	TRANSITIONAL	CLIMAX
Absidia spinosa	-	-	1.5
Acremonium sp.	7.9	11.7	15.8
Alternaria grophila	20.2	-	2.4
Alternaria humicola	5.5	1.0	1.2
A. tenuis	13.5	6.0	6.9
Alternaria sp.	7.7	-	1.5
Aspergillus candidus	6.4	10.0	45.0
A. flavipes	-	1.0	-
A. flavus	-	-	1.2
A. fumigatus	3.7	8.5	7.8
A. glaucus	1.6	1.6	-
A. luchuensis	1.3	3.2	-
A. nidulans	1.4	1.6	2.3
A. niger	17.7	35.9	31.9
A. niveus	-	1.0	-
A. orchraceous	-	3.9	-
A. sydowi	-	1.7	-
A. terreus	1.7	1.7	0.8
A. ustus	-	5.3	1.2
A. versicolor	6.3	6.0	6.4
A. wentii	1.8	-	2.9

*Nonemclature of Aspergillus follows Thom and Raper (1945); of Penicillium Raper and Thom (1949); and of the rest, Gilman (1959), Barnet (1960), and Clements and Shear (1954)

TABLE VI. Continued -

SPECIES	PIONEER	TRANSITIONAL	CLIMAX
Aspergillus sp.	4.1	3.8	8.0
Botrytis sp.	5.9	9.0	14.7
Brachysporium sp.	-	-	1.2
Candida sp.	-	0.8	-
Cephalosporium acremonium	3.3	10.3	1.5
C. asperum	7.2	16.1	3.3
C. curtipes	33.4	45.2	14.6
C. humicola	-	1.7	6.2
C. roseo-griseum	-	20.7	-
Cephalosporium sp. #1	-	30.3	1.2
Cephalosporium sp. #2	-	10.0	1.2
Cephalosporium sp. #3	1.3	5.5	2.8
Cephalothecium sp. ?	-	5.8	21.5
Chaetomella sp.	2.1	1.0	-
Chaetomium magnum	-	-	0.8
Chloridium sp.	-	-	1.6
Cladosporium epiphyllum	31.8	11.2	10.2
C. herbarum	19.6	-	7.3
C. lignicolum	3.7	4.6	3.0
Cladosporium sp.	0.8	3.1	1.2
Coniothyrium fuckelii	-	3.9	4.5
Corethropsis sp.	-	-	1.2
Cunninghamella verticillata	-	-	4.6

TABLE VI. Continued -

SPECIES	PIONEER	TRANSITIONAL	CLIMAX
Curvularia geniculata	5.0	5.2	0.8
C. tetramera	-	1.0	3.2
Curvularia sp.	5.0	4.0	0.8
Cylindrocarpon heteronemum	-	2.7	-
C. radicicola	-	-	3.3
Cylindrocephalum sp.	-	2.6	1.5
Dematiaceae sp.	4.3	-	1.7
Dicoccum sp.	-	1.0	0.8
Fusarium avenaceum	-	2.6	-
F. coeruleum	-	-	1.5
F. conglutinans	-	1.7	-
F. decemcellulare	-	1.3	4.5
F. dimerum	-	9.8	4.5
F. equiseti	-	-	5.3
F. graminearum	1.3	1.7	1.7
F. merismoides	3.7	10.7	7.7
F. moniliforme	-	-	7.8
F. neoceras	1.3	1.9	8.7
F. nivale	-	6.8	14.3
F. oxysporum	4.2	1.7	23.9
F. poae	-	4.3	-
F. solani gr.	6.8	19.2	31.3

TABLE VI. Continued -

SPECIES	PIONEER	TRANSITIONAL	CLIMAX
F. trichothecioides	-	1.7	-
Fusarium sp. #1 (Discolor gr.)	6.4	12.5	16.4
Fusarium sp. #2 (Spicari-oides)	-	4.3	9.1
Fusarium sp. #3 (Martiel-la)	4.2	3.6	10.7
Fusicladium	-	1.7	-
Fusidium viride	8.3	2.6	3.2
Geotrichum sp.	-	1.7	1.7
Gliobotrys alboviridis	3.3	-	-
Gliocladium catenulatum	-	6.0	18.6
G. fimbriatum	-	-	20.0
G. penicilloides	4.7	24.0	59.7
G. salmonicolor	-	3.9	-
Gliocladium sp.	-	2.8	9.3
Haplosporangium sp.	-	0.8	-
Helminthosporium anomalum	5.1	6.9	10.2
H. microsorum	-	-	1.2
H. sativum	-	1.9	1.2
Helminthosporium sp.	1.7	-	1.2
Heterosporium terrestre	-	2.7	-
Hormiscium sp.	-	5.0	-
Hormodendrum pallidum	15.9	2.2	-
H. viride	4.2	1.0	-

TABLE VI. Continued -

SPECIES	PIONEER	TRANSITIONAL	CLIMAX
Hyalopus sp.	-	1.0	-
Masoniella sp.	-	1.0	-
Mortierella sp.	-	1.7	10.8
Mucor corticolus	-	1.0	-
Mucor sp.	-	3.9	0.8
Mycelia sterilia[1] #1	5.3	7.8	9.0
Mycelia sterilia[2] #2	8.9	26.8	26.6
Mycelia sterilia[3] #3	4.3	15.2	16.5
Mycelia sterilia[4] #4	0.7	16.4	9.1
Nigrospora sp.	-	1.9	-
Oospora sp.	-	1.7	2.6
Paecilomyces sp.	-	-	1.0
Papularia sp.	9.3	5.0	3.2
Penicillium brevi-compactum	-	1.0	7.5
P. chrysogenum	-	-	1.2
P. citrinum	-	1.2	6.2
P. commune	-	-	5.7
P. digitatum	-	-	2.4
P. frequentans	2.1	3.3	13.6
P. funiculosum	1.0	5.0	1.7
P. herquei	2.1	11.4	4.4

[1]with white sclerotia like. [2]white cottony growth. [3]with terminal and intercalary chlamydospores. [4]dark grey.

TABLE VI. Continued -

SPECIES	PIONEER	TRANSITIONAL	CLIMAX
P. janthinellum	1.7	4.0	8.0
P. lilacinum	-	3.4	8.2
P. nigricans	-	-	1.3
P. purpurogenum	-	1.7	3.3
P. raistrickii	-	1.7	-
P. rugulosum	-	1.7	10.6
P. thomii series	-	3.3	-
Penicillium Sp. #1	0.8	10.6	7.1
Penicillium Sp. #2	0.8	2.5	1.7
Penicillium Sp. #3	-	-	7.1
Periconia Sp.	1.7	1.7	-
Phoma glomerata	6.3	4.6	2.5
P. hibernica	2.8	8.3	-
P. humicola	-	4.1	-
Phoma sp.	3.8	8.0	2.4
Phycomyces sp.	-	1.7	-
Pullularia sp.	-	3.8	-
Pyrenochaeta decipiens	-	3.2	-
Rhizoctonia sp.	5.9	40.0	3.6
Rhizopus nigricans	-	4.8	1.2
Sclerotium sp.	5.7	41.0	9.8
Scopulariopsis sp.	-	1.0	3.3
Sphaeronaema spinella	3.3	2.6	1.5

TABLE VI. Continued -

SPECIES	PIONEER	TRANSITIONAL	CLIMAX
Spicaria divaricata	2.9	1.0	1.9
S. violancea	4.2	3.4	15.0
Spicaria sp.	-	1.0	10.4
Spondylocladium sp.	-	-	3.9
Sporotrichum epigaeum	-	2.6	-
Stachybotrys atra	5.0	7.8	-
S. cylindrospora	0.8	4.9	-
S. lobulata	-	2.1	1.9
Stemphylium sp.	-	-	0.8
Stilbella sp.	2.9	2.6	-
Thielavia terricola ?	4.2	-	-
Tilachlidium sp.	-	-	1.2
Torula allii	2.9	8.6	1.7
Trichoderma album	2.4	1.0	3.2
T. glaucum	3.7	3.2	0.8
T. koningi	18.6	23.4	17.5
T. lignorum	19.8	40.1	31.2
Tubercularia sp.	-	-	2.3
Verticillium candelabrum	-	1.9	1.7
V. effusum	-	2.9	3.9
V. terrestre	4.4	8.2	35.8
Volutella ciliata	-	5.3	6.2
V. piriformis	1.2	5.1	-
V. roseola	-	2.2	-

were considered as the "normal inhabitants" of the particular area (Waksman 1944). On this basis, there were nine normal inhabitants in the pioneer area, 26 in the transitional, and 21 in the climax (Table VII). Seven species were common in all three areas, and nine species were normal inhabitants of both the transitional and climax areas. The other species were considered "invaders" brought in by the wind. In such a classification system, a normal inhabitant of one area might be included among the invaders of another area. The species considered normal inhabitants were of greater significance and importance since these were present during a greater period of the year. Importance percentages of these species were computed by averaging relative density and relative frequency. Plates having more than seven colonies of the same species were excluded from the calculation. Objections may be raised about the validity of using relative density when considering fungi because heavily sporulating species are likely to give higher densities. It was found, however, that the species with supposedly equal sporulating capacity did not always give similar densities. Moreover, the species with high frequencies generally had high densities. Therefore, the importance percentage rather than the frequency percentage alone was employed to determine the "principal species" (England and Rice 1957). The species with importance percentages of at least three were arbitrarily termed as principal species. The principal species in the pioneer area, in order of decreasing importance percentage, included Cladosporium epiphyllum, Cephalosporium curtipes, Aspergillus niger, Trichoderma lignorum, Alternaria geophila, and Trichoderma koningi; in the transitional area, Cephalosporium curtipes, Trichoderma

TABLE VII. Average importance percentages[1] of normal inhabitants[2] in the three areas.

SPECIES	PIONEER	TRANSITIONAL	CLIMAX
Acremonium sp.	-	1.5	1.5
Alternaria geophila	3.5	-	-
Aspergillus candidus	-	1.1	5.4
A. fumigatus	-	0.8	0.8
A. niger	4.3	5.0	4.5
A. versicolor	-	0.6	-
Botrytis sp.	-	0.9	-
Cephalosporium curtipes	6.2	6.4	1.4
Cephalosporium sp. #1	-	2.8	-
Cephalothecium sp.	-	-	3.6
Cladosporium epiphyllum	7.4	1.2	1.0
Fusarium dimerum	-	1.0	-
F. merismoides	-	1.2	0.6
F. oxysporum	-	-	2.4
F. solani gr.	1.2	2.8	3.7
Fusarium sp#1 (discolor gr.)	-	1.1	1.7
Gliocladium penicilloides	-	3.8	9.6
G. fimbriatum	-	-	2.9
Mycelia sterilia #1	-	0.8	0.8
Mycelia sterilia #2	1.6	3.8	4.0
Mycelia sterilia #3	-	2.9	2.7

[1]Average of relative density and relative frequency. [2] Species present during at least four of six sampling periods in a given area.

TABLE VII. Continued -

SPECIES	PIONEER	TRANSITIONAL	CLIMAX
Mycelia sterilia #4	-	1.6	-
Papularia sp.	1.6	-	-
Penicillium frequentans	-	-	1.2
P. herquei	-	1.4	-
Phoma sp.	-	1.3	-
Rhizoctonia sp.	-	4.7	-
Sclerotium sp.	-	4.8	-
Spicaria violacea	-	-	1.7
Stachybotrys atra	-	1.0	-
Trichoderma konnigi	3.4	3.0	2.8
T. lignorum	4.3	5.6	5.8
Verticillium terrestre	-	0.9	3.7

lignorum, Aspergillus niger, Scherotium sp., Rhizoctonia sp., Mycelia sterilia #2, Gliocladium penicilloides, and Trichoderma koningi; and in the climax area, Gliocladium penicilloides, Trichoderma lignorum, Aspergillus candidus, Aspergillus niger, Mycelia sterilia #2, Verticillium terrestre, Fusarium solani, and Cephalothecium sp. Gliocladium fimbriatum with an importance percentage of 2.9 was restricted to the climax area.

DISCUSSION

Pronounced changes in the selected soil factors and in the microflora of the soil were found to be correlated with the changes in the vegetation during forest succession. The pioneer area had the lowest organic carbon content, water holding capacity, and soil moisture content. These soil factors increased with the progress of succession all the way to the climax. The total counts of microorganisms followed the same pattern. The number of fungal species also increased with the progress of succession up to the transitional stage, but it decreased slightly in the climax area. The slight decrease may not have been significant, but it does appear significant that no further increase occurred after the transitional cottonwood savanna stage. Tresner et al. (1954) also found a decrease in the number of fungal species in a climax stand of successional sequence. These findings conform in general with the principle stated by Odum (1963) that the number of species of heterotrophs increases with succession until relatively late in the sere.

The low counts of microorganisms in the pioneer area were probably due to low soil moisture, water holding capacity, and organic carbon. The increase in these soil factors in the transitional area enabled a larger number of microorganisms to survive there. The high organic carbon content in the climax area undoubtedly provided better

food sources resulting in maximum counts of microorganisms. A greater percentage of fungal species was common to the transitional area and the climax than to any other combinations of plots. This probably was due to a closer similarity between the transitional and the climax areas in regard to soil factors and vegetation. The slightly higher number of fungal species in the transitional area may have been due to the fact that species from both the pioneer and climax stages found at least sufficient minimal conditions for growth in that area. The higher plants also exhibit overlapping of species in many different communities including those under investigation.

The total count of all microorganisms reached its maximum in spring which agrees with the findings of Witkamp (1963), Wright and Bollen (1961), England and Rice (1957), and Tresner et al. (1954). These correlated well with the increase in soil moisture and organic carbon contents. The total oxygen uptake also increased with the increase of microorganisms in spring, although the correlation in other seasons was not well established. This is in contrast with the result of Witkamp (1963) who found no correlation between the respiratory rate and the number of microorganisms. The fungal count was lowest in summer, and similar summer minima in the fungal counts were noted by England and Rice (1957) and others (Tresner et al., 1954, Wright and Bollen 1961). The minimal counts of bacteria and actinomycetes were not reached until late fall and winter which agrees with the results of Wright and Bollen (1961) but contrasts with the results of Tresner et al. (1954). The summer minima in fungal counts may be due to unfavorable soil moisture.

Many of the fungal species were common to all three areas, indicating their wide tolerance of environmental conditions. Many of them were among common soil fungi isolated by Christensen et al. (1962), Goos (1960), Orpurt and Curtis (1957) McElroy (1953), and Warcup (1951). Since the colonies which developed on the plates could come either from the spores brought to a given area by wind or from the mycelia or spores developed in the soil in the area, it is difficult to distinguish the normal inhabitants of the soil from the invaders or ephemeral species. The number of species of normal inhabitants in each area was low and so was the number of restricted species. The pattern of the change in the number of normal inhabitant species from the pioneer to the climax areas was similar to that of the total number of fungal species. Here too a greater number of species of normal inhabitants was common to the transitional and the climax areas than to any other combination of plots.

When the principal species in each area were determined it was found that each area had a different species composition. Even in those instances where the same species was a principal species in more than one area, it had different importance percentages in the areas indicating closer relationships to a specific successional stage than to others. As an example, Gliocladium penicilloides was better adapted to the climax area than to the transitional area even though it was fairly prominent in both.

The preference of Aspergillus over Penicillium in the pioneer area and conversely, Penicillium over Aspergillus in the climax is comparable with the results of Christensen et al. (1962), and Orpurt and

Curtis (1957) who found more Penicillium than Aspergillus in forest soil. In contrast with Christensen et al. (1962), two species of Aspergillus were prominent in the climax area. The average frequency percentage of Mortierella sp. and the other members of Phycomycetes was highest in the climax area, but this class was not represented at all in the pioneer area. The results of Orpurt and Curtis (1957) who concluded that the Phycomycetes are a mesic group and of Wohlrab et al. (1963) who did not find Phycomycetes in the early stages of succession are in agreement with the results of this study. Apparently Gliocladium and Penicillium are favored in bottomland forests. Christensen et al. (1962) also reported Penicillium and Gliocladium roseum as prominent in the maple-elm-ash forest in southern Wisconsin.

The results of the present study and the studies of England and Rice (1957), Tresner et al. (1954), and Warcup (1951) indicate that the species composition of fungi does not change much in different seasons, but differs appreciably from one ecological area to the other, being influenced by plant cover and soil factors. Each area harbors a different species composition. The species of broader amplitude occur with different frequencies in different areas. Similar situations are also observed among green plants. Overlapping in the occurrence of fungal species is not unusual since the leaf litter plays an important role in determining the fungal population (Witkamp 1963). The correlations found between the higher plants and fungi in this investigation are in agreement with those of Christensen et al. (1962), England and Rice (1957), Orpurt and Curtis (1957), and Tresner et al. (1954).

SUMMARY

1. The soil fungi from three areas representing successional stages in bottomland forest vegetation in central Oklahoma were compared throughout the year at two months intervals. The fungi were isolated on James's soil extract medium by a dilution plate technique. The numbers of accompanying bacteria and actinomycetes were also determined. Importance percentages based on relative density and relative frequency were calculated for both green plants and fungi.

2. The pioneer area had a high density of arboreal seedlings and no trees, the transitional area had a low density of trees, and the climax area had a high density of trees. Each area had a different type of herbaceous vegetation. There was some overlapping in the occurrence of both arboreal and herbaceous species. The soil moisture, organic carbon, water holding capacity, and total number of microorganisms were low in the pioneer stage of succession, intermediate in the transitional stage, and high in the climax stage. The number of fungal species was low in the pioneer area, and slightly higher in the transitional area than in the climax. Apparently the maximum number of fungal species attained during succession is reached long before the climax stage.

3. The total count of microorganisms was high in spring. The minima for fungi occurred in summer, and for bacteria and actinomycetes

in late fall or winter. The correlation between the number of microorganisms and the oxygen uptake in different seasons was poor except for spring.

4. Many of the fungal species were sporadic in occurrence. The fungal species occurring in at least two-thirds of the sampling periods in a given area were termed normal inhabitants. The number of such inhabitants in each area was low, but there were characteristic populations. The number of species restricted to any given area was low also.

5. Although many of the fungal species were ubiquitous in occurrence, they appeared in considerably different frequencies in different areas indicating definite community relationships. This overlapping in the occurrence of fungal species in different plant communities simulates that of the green plants. In other words, a continuum exists rather than completely discrete communities.

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