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SOIL PROTOZOA FROM A CEDAR GLADE IN RUTHERFORD COUNTY, TENNESSEE

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ABSTRACT

Soil samples from gravel, grass, shrub, shrub-cedar, and cedar-hardwood communities in a cedar glade southwest of Murfreesboro, Tennessee were examined for protozoa. Samples were collected in June and October of 1974 and examined weekly for five months and then monthly through June of 1976. One hundred sixty-six species were found, including 43 flagellates, 28 naked amoebae, 43 testacea, 4 heliozoans, and 48 ciliates. There were more aquatic species than edaphic and ubiquitous species encountered from each community. The species observed in the spring samples and fall samples were approximate in number and ecological group ratios, but only 67 species were found common to the two samplings. *Colpoda* species, typically the most common soil ciliates, were conspicuously rare at all sites.

INTRODUCTION

Cedar glades are located in the middle portion of Tennessee within a basin surrounded by the rim of the Interior Low Plateau. Cedar glades occur on thin-bedded, horizontal, Lebanon limestone of the Stones River Series, a dolomitic rock of Ordovician age (Quarterman 1950). Rutherford County lies in the eastern part of this basin and has glade communities scattered throughout. Quarterman (1950) recognized six glade communities: rocky glade, gravel glade, grassy glade, shrub glade, shrub-cedar, and cedar-hardwood glade. The glades have a characteristic flora that includes endemic and near endemic species (Baskin 1968).

The cedar glade selected for this study contained all of the major plant communities described by Quarterman (1950) as typical of cedar glades. Located 11 miles southwest of Murfreesboro, Tennessee, at 35° 42′35″N latitude and 85° 32′40″W longitude it was approximately 800 feet above sea level and had a slope of 2-15%. No streams or bodies of water were in the area.

Five glade communities, excluding the rocky glade, were determined on the basis of depth of soil and floral cover. Depth of the soil in the gravel glade was 5 cm or less and was sparsely covered with low herbs: *Sedum pulchellum, Petalostemon gattingeri, Sporobolis vaginiflorus, Eragrostis spectabilis, Potentilla simplex, Reullia humilis, Senecio smallii,* and *Pleurochaete squarrosa. Juniperus virginiana* seedlings were scattered over the gravel glade, and the bare areas in the community were covered with *Nostoc commune.*

The soil depth of the grass glade was 5-20 cm, and was covered with tall herbs: *Andropogon virginicus, Sporobolis vaginiflorus, E. spectabilis, Aristida longespica, Croton capitatus, Senecio smallii,* and *Potentilla recta. Juniperus virginiana* seedlings were also found here.

The soil outside of crevices in the shrub glade had accumulated to 20-30 cm. Dominant shrubs included *Forestiera ligustrina, Symphocarpos orbiculatus, Hypericum frondosum, Rhus aromatica,* and *Rhamnus caroliniana.* There were also well-established shrub-sized cedars, *J. virginiana,* and winged elms, *Ulmus alata.* Dominant herbs were *Potentilla recta, Oxalis* sp. and *Sisyrinchium albidum.* The ground was covered with *Pleurochaete squarrosa* and large clumps of three lichen species: *Cladonia alpestris, C. rangiferina,* and *C. apodocarpa.*

The soil in the shrub-cedar glade outside crevices had accumulated to more than 30 cm. Ground cover and shrubs were the same as in the shrub glade, but this community supported some trees. Red cedar dominated, but also present were *U. alata* and *Fraxinus quadrangulata.*

The soil in the cedar-hardwood glade was sufficiently deep in wide crevices to support large trees. The same species as the shrub-cedar glade plus two additional species, *Celtis laevigata* and *Quercus muhlenbergii,* occurred there.

The purpose of this study was to determine the protozoan species in the soil of a cedar glade in Rutherford County, Tennessee, and to compare the species composition of the various communities in a glade.

METHODS AND MATERIALS

A composite soil sample from the cedar glade was analyzed for texture, organic matter, and chemical properties by the United States Testing Company, Memphis, Tennessee. Each glade was also tested for pH. A Delmhorst Model KS Soil Moisture Tester was used to determine the percentage of available soil moisture. Soil moisture readings were taken weekly during the months of June through October to determine which glades retained the most moisture.

A representative sampling site was selected in each of the five glade communities. On 5 June 1974 and again on 5 October 1974, the plant cover was carefully removed and two 50 cc samples of the top 5 cm of soil were collected from each community. Each sample was mixed with 50 ml of distilled water. From each of these mixtures, a 10 ml sample was inoculated into 90 ml of distilled water and stored in individual jars with screw type lids. All samples were examined weekly for five months and then monthly until 16 June 1976. Several species remained active after two years in the closed jars.

Three additional soil samples were taken from each glade on 10 July 1982. Water was immediately added to one sample, another sample was allowed to partially dry for two days, and the third sample was allowed to com-

pletely dry before water was added. These additional samples were observed daily for 9 days to make sure that *Colpoda* sp. had not been missed during the early stages of succession in previous samples.

Protozoa were identified by the use of keys and descriptions of Kudo (1966), Leidy (1879), Kahl (1932,1933,1935), Lackey (1959), Deflandre (1959), Thompson (1959), Bonnet (1958,1961a,1961b,1964,1968), Thomas (1959), Penard (1902,1905), Sandon (1927), Rosa (1957,1962), Calaway and Lackey (1962), Borror (1972), Godeanu (1972), Page (1976), Leedale (1967), Alexander (1961), Chardez (1959, 1960a,1960b, 1962a,1962b), and Varga (1935a,1935b).

Results

The soil in the glade was silt loam, rich in organic matter, and contained a high percentage of calcium (Table 1). The sampling sites differed in moisture content; more moisture was retained as the soil depth increased from the gravel through the cedar-hardwood glade (Table 2). Weekly readings showed moisture varied from 0-98% in all plant communities during the months of June through October. All sites registered no available moisture during the month of August. The pH of the glade communities ranged from 6.0 to 7.4 (Figs. 1 and 2).

TABLE 1. *Characteristics of the Cedar Glade Soil.*

Soil Texture	Silt Loam
Organic Matter	7.9%
Calcium (base saturation)	95.7%
Magnesium (base saturation)	3.1%
Potassium (base saturation	1.0%
Hydrogen (base saturation)	0.0%
Calcium	5650.0 ppm
Phosphorus (P_1)	3.0 ppm
Potassium	119.0 ppm
Magnesium	110.0 ppm
Nitrate	13.0 ppm
Cation Exchange Capacity	29.5 meg/100g
Hydrogen	0.0 meg/100g

One hundred sixty-six species representing 104 genera were found: flagellates—29 genera and 43 species, naked amoebae—13 genera and 28 species, testacea—21 genera and 43 species, heliozoans—4 genera and 4 species, and ciliates—37 genera and 48 species (Table 3). The ecological classification of each species as defined by Bamforth (1969) is included in Table 3. Group A encompasses species which are well adapted to the environmental conditions of the soil. Group B consists of species which are not as well adapted to the soil environment, and their occurrence in the soil is less frequent. Group C includes the aquatic species, which are more specialized and more exacting in their ecological requirements.

There were more aquatic than edaphic and ubiquitous species encountered in each glade community. Of the 166 different species found in the June and October samples, 39 belong to group A, 39 to group B, and 88 to group C. The total yield of the June samples was 114 species, of which 25 belong to group A, 27 to group B, and 62 to group C. The October samples contained 118 species of which 32 belong to group A, 28 to group B, and 58 to group C. Only 67 species were found common to June and October samples. These 67 species were composed of 19 group A, 16 group B, and 32 group C organisms. *Colpoda* sp., normally the most edaphic and common of the soil ciliates were few in number in all samples collected.

Figure 1 shows a composite plot of the June and October ecological group A, B, and C forms for each glade. The pH and average moisture for each glade are also shown in this figure.

Figure 2 indicates the number of ciliate, flagellate, testacean, and naked amoebae species found in each glade. Heliozoans were not included because only four species were found. Average moisture and pH are also included.

Numerous microorganisms, other than protozoa, were found in the glade soil samples. Rotifers, nematodes, and diatoms were encountered frequently; several gastrotrichs, a few tardigrades, and one *Catinula* were also observed.

Discussion

Moisture availability was most constant in the shrub-cedar and cedar-hardwood communities and fluctuated most radically in the gravel glade community. However, all glades showed no available moisture during the month of August (Table 2). When soil protozoa were plotted according to ecological groups (Fig. 1) available moisture

TABLE 2. *Average, Maximum, and Minimum Percentages of Available Soil Moisture of Each Glade Community for June through October.*

	Gravel	Grass	Shrub	Shrub-cedar	Cedar-hardwood
	% Moisture	% Moisture	% Moisture	% Moisture	% Moisture
Month	Avg. Max. Min.	Avg. Max. Min.	Avg. Max. Min.	Avg. Max. Min.	Avg. Max. Min
June	51 99— 0	68 99— 0	73 98— 0	87 98— 72	91 98—74
July	85 97—64	93 99—35	94 99—35	95 98—86	96 98—91
Aug.	41 97— 0	37 98— 0	50 98— 0	66 98— 0	65 97— 0
Sept.	80 98—45	93 99—81	93 99—81	92 98—82	91 98—78
Oct.	96 97—94	96 96—94	96 96—94	95 97—83	95 98—83
June-Oct. Average	70.6	77.4	81.2	87	87.6

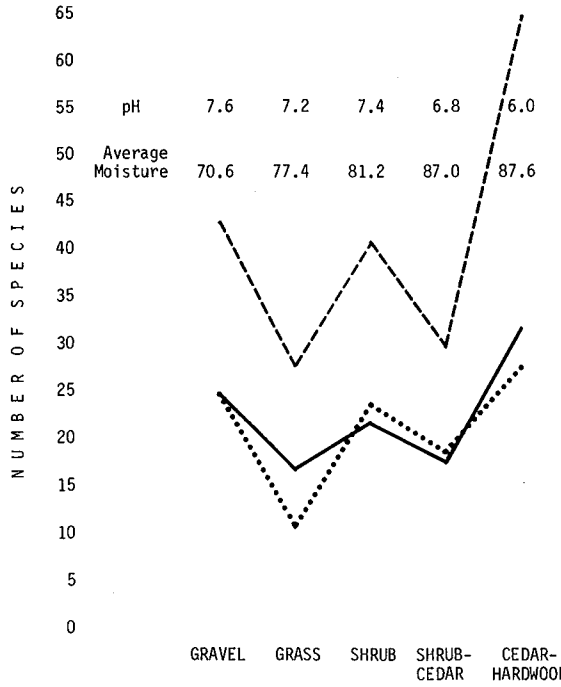

FIG. 1. *Average moisture, pH, and plot of ecological groups A, B, and C soil protozoa from June and October samples combined. Group A includes species well adapted to environmental conditions of the soil, group B species are not so well adapted, and group C species are aquatic.*
Group A ——————
Group B ·····
Group C – – – – – –

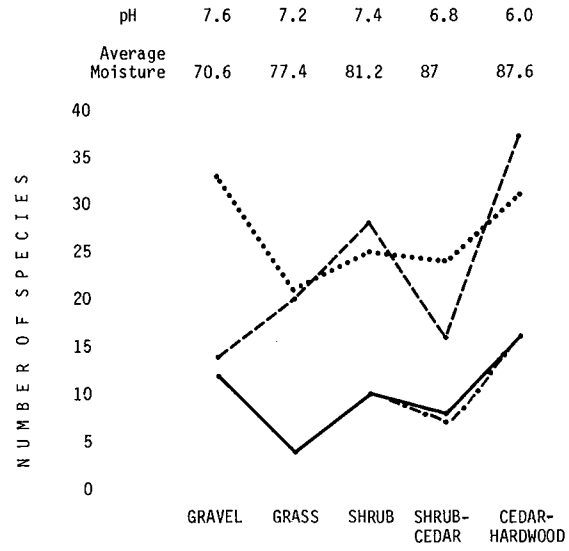

FIG. 2. *Average moisture, pH, and number of species of ciliates, flagellates, testaceans, and naked amoeba from each glade.*
Flagellates ·····
Ciliates – – – – –
Testaceans ——————
Naked Amoebae –·–·–·–

TABLE 3. *Species occurrence (X) and Ecological Classification of Protozoa in the Soil of the (1) Gravel, (2) Grass, (3) Shrub, (4) Shrub-cedar, and (5) Cedar-hardwood Glade Communities. Ecological group A species are well adapted to environmental conditions of the soil, group B species are not so well adapted, and group C are aquatic.*

Ecological Groups	Species	June Samples 1	2	3	4	5	October Samples 1	2	3	4	5
	Flagellates										
A	*Oikomonas termo* Ehrenberg	X	X	X	X	X		X		X	X
A	*Oikomonas* sp.	X				X	X				
B	*Cyathamonas truncatus* Fromental			X		X	X				X
C	*Polytomella agilis* Doflein										X
C	*Eutreptia viridis* Perty					X					
C	*Thylacomonas compressa* Schewiakoff										X
C	*Astasia klebsi* (Klebs)										X
C	*Petalomonas mediocanella* (Klebs)	X		X	X	X					
C	*P. carinata* France	X									X
C	*Rhabdomonas incurva* Fres				X					X	X
B	*Scytomonas pusilla* Stein	X	X	X			X	X	X		X
C	*Parmidium scutulum* Christen	X									
C	*Atractomonas laevis* Christen	X		X							X
C	*Anisonema ovale* Klebs	X	X				X				
C	*A. acinus* Klebs		X	X			X				
C	*A. emarginatum* Dujardin	X	X	X							X
C	*Tropidoscyphus octocostatus* Stein										X
C	*Notosolenus obicularis* Stokes		X								
C	*Entosiphon sulcatum* Dujardin			X		X	X				X
C	*Peranema trichophorum* (Ehrenberg)				X	X			X		
C	*Distigma proteus* Stein	X								X	X
C	*Phalansterium solitarium* France					X					
B	*Monas* sp.	X									X
B	*M. vulgaris* Cienk	X									
C	*Dinomonas vorax* Kent				X	X	X				
B	*Pleuromonas jaculans* Perty							X			
C	*Rhynchomonas nasuta* Klebs	X	X	X	X	X	X	X		X	
B	*Bodo mutabilis* Klebs	X	X	X	X	X	X		X	X	
C	*B. edax* Klebs	X	X	X						X	
A	*B. globosus* Stein	X	X	X	X	X	X	X	X	X	X
B	*B. minimus* Klebs								X		
B	*B. caudatum* Dujardin	X	X	X	X	X		X	X		
C	*B. repens* Klebs	X	X		X	X					
B	*Cercobodo bodo* Meyer		X	X	X						
B	*C. agilis* Moroff									X	
A	*C. crassicauda* Alexeieff			X	X	X	X	X	X	X	X
B	*C. longicauda* Stein								X		
A	*Allantion tachyploon* Sandon	X				X					
B	*Mastigamoeba longifolium* Stokes	X			X	X				X	X
B	*M. invertens* Klebs	X		X	X	X			X		
B	*M. limax* Moroff	X									
C	*Clautriava parva* Schouteden					X					
C	*Helkesimastix faeciola* Woodcock-Lapage			X							
	Naked Amoebae										
A	*Nuclearia simplex* Cash										X
C	*Amoeba guttula* Dujardin	X	X			X			X	X	X
C	*A. limicola* Rhumbler	X							X		
C	*A. brachiata* Dujardin		X			X					
C	*A. hylobates* Penard	X									
C	*A. alveoata* Mereschkovaki	X				X					
C	*A. silvatica* Varga	X									

C *A. villosa* Leidy								X	X		
C A. gorgonia Penard			X		X						
C *A. velata* Parona					X						
B *Hartmannella* sp.			X								
B *H. vermiformis* Page	X										
A *Saccamoeba limax* Page	X	X								X	
B *Glaeseria mira* Glaser	X				X						
B *Thecamoeba striata* Penard									X	X	
B *Vanella simplex* Wohlforth-Bottermann	X		X		X						
B *Platyamoeba placida* Page					X						
A *Mayorella spatula* Bovee					X						
B *M. ambulans* Bovee					X						
B *M. bicornifrons* Bovee				X							
A *Acanthamoeba* sp.			X								
A *Dinamoeba* sp.			X								
A *Vahlkampfia* sp.				X						X	X
A *V. limax* Lepsi	X	X		X							
A *V. spinifera* Lepsi			X				X				
A *V. albida* Lepsi									X		
A *Naegleria gruberi* Alexeieff							X				
A *N. bistadialis* (Kuhn) Testacea					X				X		
C *Artodiscus saltans* Penard	X										
C *Pseudodifflugia gracilus* Cash	X										
C *Arcella vulgaris* Ehrenberg							X				
C *A. arenaria* Greef									X		
C *Pyxidicula operculata* Ehrenberg									X		
C *Parmulina cyathus* Penard					X						
C *Cochliopodium* sp.	X	X	X	X	X		X	X	X	X	X
C *C. obscura* Penard							X				
C *Bullinula indica* (Wailes)							X				
A *Centropyxis plagiostoma* Bonnett and Thomas							X		X		
A *C. aerophila* Deflandre							X				X
A *C. sylvatica* var. *minor* Bonnet and Thomas							X			X	
A *C. sylvatica* Deflandre	X		X				X				
A *C. deflandriana* Bonnet											X
A *C. elongata* Penard					X		X		X		
B *C. constricta* (Ehrenberg)				X				X	X	X	
B *C. vandeli* var. *globulosa* Bonnet							X				
A *Cyclopyxis kahli* Bonnet and Thomas											X
A *Plagiopyxis minuta* Bonnet									X		
A *P. callida* Penard							X		X		
A *P. oblonga* Bonnet and Thomas							X				
C *Difflugia lucida* Penard	X	X		X	X						X
C *D. oblonga* Ehrenberg	X		X	X	X						X
C *D. fallax* Penard		X		X			X	X			X
C *D. minuta minor* Godeanu											X
C *D. bipartis* Godeanu											X
C *D. lancelata* Penard							X				
C *Pontigulasia varadi* Godeanu				X						X	X
B *Heleopera sphagni* Leidy					X					X	
B *Nebela collaris* (Ehrenberg)	X			X	X		X	X	X		X
C *N. dentistoma* Penard							X				
C *Hyalosphenia* sp.							X		X	X	
B *Phryganella* sp.											X
B *P. acropodia* Hartwig and Lesser							X				
A *Corythion dubium* Taranek										X	X
C *Euglypha laevis* Ehrenberg				X		X		X			X
A *E. compressa* Carter				X			X	X		X	
A *E. tuberculata* Dujardin	X				X				X		X
C *Assulina seminulum* Wailes											X
A *Trinema complanatum* Penard							X	X			X
B *T. enchelys* Ehrenberg				X		X		X		X	X
A *T. lineare* Penard	X	X	X	X	X		X	X	X	X	X
C *Pseudawerintzewia calicola* Bonnet											
Heliozoans											X
C *Actinophrys sol* Kudo					X	X				X	
C *Ciliophrys infusionum* Butschli									X		X†
C *Clathrella foreli* Penard				X	X	X					
A *Elaster greeffi* Penard										X	X
Ciliates											
C *Placus* sp.										X	X
B *Chaenea* sp.											X
C *Enchelymorphia vermicularis* Smith		X									
C *Dileptus* sp.	X		X								
B *Chilodonella uncinata* Ehrenberg			X		X						
A *Colpoda steini* Maupas		X	X	X	X						
A *Leptopharynx sphagnetorum* (Kahl)			X		X					X	
B *Microthorax stimulans* (Kahl)										X	
B *Drepanomonas dentata* Fresenius	X	X	X	X							X
B *D. revoluta* Penard										X	X
A *Sathrophilus muscorum* Kahl		X			X					X	
C *Cohnilembus fusiformis* Kahl								X			
C *Frontonia depressa* (Stokes)								X			
C *Platynematus sociale* (Penard)					X				X		X
C *Dexiotrichides centrale* Kahl									X		
B *Cyrtholophosis* sp.									X		
C *Cyclidium* sp.					X						
B *C. glaucoma* Muller		X	X		X				X		
C *C. versatile* Kahl					X			X		X	
C *C. brandoni* Maskell			X								
C *Pleuronema* sp.					X						
C *Ctedoctema* sp.			X								
C *Cristigera* sp.			X	X			X				X
C *Blepharisma* sp.	X	X	X	X	X						X
A *Protocrucia* sp.		X						X		X	X
C *Condylostoma* sp.			X								
C *Halteria grandinella* Muller	X	X	X	X	X				X	X	X
C *Strombidinopsis gyrans* Kent	X										
C *Oxytricha* sp.	X										
A *O. minor* Kahl					X		X				
C *O. setigera* Stokes		X	X								X
C *Tachysoma* sp.	X		X	X	X		X				
A *T. pellionella* Stokes		X			X						X
B *Gonostomum affine* Stein	X						X				
C *Psilotricha accuminata* Stein					X				X		X
C *Uroleptus* sp.		X	X	X						X	X
C *Uroleptus* sp.	X	X			X						X
C *Uroleptus* sp.			X								
C *Uroleptus* sp.		X	X		X						
B *Uroleptus muscorum* Kahl	X				X				X		
C *Histrio histrio* (Muller)								X			
C *Gastrostyla muscorum* Kahl			X					X			
A *Keronopsis muscorum* Kahl			X					X			
C *Balladyna fusiformis* Kahl											X
C *Stylonychia mytilus* Ehrenberg			X								
C *Pleurotricha* sp.											X
C *Urosoma caudata* Stokes											X
B *Vorticella microstoma* Ehrenberg	X			X	X		X			X	X

and pH did not appear to be limiting factors on the number of species found in the gravel, grass, shrub, and shrub-cedar glades, although the average available moisture increased from 70.6% in the gravel glade to 87% in the shrub-cedar glade. The number of species increased slightly in the cedar-hardwood glade, but the average available moisture was only 0.6% higher than the shrub-cedar glade. In all cases the more aquatic C group made up approximately 50% of the protozoan population and the A and B groups approximately 25% each in both June and October

samples. This was also true of the 67 species that were common to the June and October samples.

To further analyze the effect of moisture on soil protozoa, the number of ciliate, flagellate, testacean, and naked amoebae species in each glade community were compared in Fig. 2. The flagellates, testaceans, and naked amoebae appeared tolerant to moisture fluctuations. They were fairly constant in species number across all glades although average available moisture was quite variable. The ciliates, which were reported by Bamforth (1973) to be less tolerant to moisture fluctuations, generally increased in number from the gravel glade, where average available moisture was lowest (70.6%), to the cedar-hardwood glade, where average available moisture was highest (87.6%). The only exception to this trend occurred in the shrub-cedar glade where there was a decrease in the number of species despite an increase in available moisture to 87%.

The samples from the shrub-cedar glade were taken from an area covered by *Juniperus virginiana* leaves and stems. Lamer-Zarawska (1975) reported the isolation of biflavonoids from the leaves and twigs of several *Juniperus* sp. including *J. virginiana*. Rice (1974) found flavonoids toxic to seed germination and some bacteria. A possible explanation for the decrease in number of ciliate species in the shrub-cedar glade (Fig. 2) could be the decrease in bacterial populations since most ciliates are bacterial feeders. The decrease also could be caused by a toxic effect directly on the ciliates. The latter seems more logical since testaceans, naked amoebae and particularly flagellates (Fig. 2) also show a decrease in number of species in this glade.

The most frequently observed aquatic species from all sites were *Blepharisma* sp. and *Halteria grandinella*. The *Colpoda* sp. were conspicuously rare. These species are the most edaphic and the most common of the ciliates (Bamforth 1967, 1973; and Stout and Heal 1967), yet only a few *Colpoda steinii* were found in the June and October 1974 samples. Additional samples taken in July 1982 also revealed few *Colpoda* sp. The July 1982 samples were observed daily for 9 days to make sure that *Colpoda* sp. had not been missed during the early stages of succession in previous samples. During the present study a few ml of the soil samples were inoculated into boiled hay infusions and *Colpoda* sp. grew more rapidly in number. In soil samples collected from old fields in the local area *Colpoda* sp. were noted in large numbers within 48 hours after the addition of water. This was not true in glade samples and possibly indicates a deficiency in the proper bacterial or plant factor to induce excystment.

The difference was negligible between the total number of species found in the soil samples collected in June (114) and those collected in October (118), but the species composition varied greatly. Only 67 species were found common to both spring and fall samples. It appears that seasonal variations in species here are greater than community variations.

Acknowledgments

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