

SOIL ALGAE IN FIELD AND FOREST ENVIRONMENTS¹

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Abstract. The prokaryotic and eukaryotic algal components of soil were quantified and identified generically over a 17-mo period in 2 successional fields and a climax forest. A correlation of the quantitative and qualitative changes was sought with other biological components (bacteria, actinomycetes and fungi) and major chemical and physical parameters. The highest counts obtained for the eukaryotic algae were 3.3×10^7 , 2.2×10^7 , and 1.2×10^8 cells/g soil for a 1-yr old field, an 11-yr old field and the forest, respectively. The highest counts obtained for the prokaryotic (bluegreen) algae were 8.2×10^5 , 2.3×10^5 and 1.6×10^4 cells/g soil for the same sites. The total number of algae in all 3 sites declined during the summer. There was no further decline in winter. The greatest number of algal genera was found in the 1-yr old field, 28 of a total 35 isolated or 80%; fewer were observed in the 11-yr old field and the forest, 57% and 63%, respectively.

A predictive model for numbers of soil algae was formed by multiple regression analysis. The significant factors that showed a relationship with the bluegreen algae were pH, time, Mg, precipitation and the eukaryotic algae, $R^2 = 45\%$; significant factors with the eukaryotic algae were time, Mg and the bluegreen algae, $R^2 = 37\%$. Prediction equations were tested against additional data with success.

Key words: algae; multiple regression analysis; New Jersey; predictive model; soil; soil ecosystem.

INTRODUCTION

Algae have been isolated from soils worldwide, including the Antarctic (Cameron and Devaney 1970, Curl and Becker 1970), tropics (Durrell 1964) and desert regions (Forest and Weston 1966, Friedman et al. 1967). Their importance in soil has been discussed earlier (Lund 1962, Shields and Durrell 1964, Dommergues and Mangenot 1970, Round 1973). As photoautotrophs, algae are thought to be important contributors to the organic carbon content of soil. Bluegreen algal nitrogen fixation can increase soil organic nitrogen content (Mayland and McIntosh 1966, Mayland et al. 1966). When growth is extensive, production of mucilage is thought to stabilize the soil against erosion (Fritsch 1907, Booth 1941, Durrell and Shields 1961, Bailey et al. 1973).

Many previous studies have resulted in taxonomic lists (e.g., Forest 1962, Fairchild and Willson 1967, Jurgenson and Davey 1968, MacEntee et al. 1972, Dystra et al. 1975) and these studies have relied on relatively small numbers of soil samples (one in many cases). Round (1973) has broadly classified the soil alga microflora into 2 groups: the ephemerals which appear at random and multiply rapidly under suitable

conditions and the perennials which are constantly subjected to the extremes of the soil habitat. A single soil isolation may give an incomplete picture of the algae present, both taxonomically and numerically. Recently, investigators have broadened the scope of soil algal studies to include changes in populations in response to land use and soil treatment (Drew and Anderson 1977, King and Ward 1977). The purpose of the present study was to continue to expand the nature of soil algal studies by determining the qualitative and quantitative changes in the algal microflora over an extensive period and to relate these changes to the biological, chemical and physical environment. Using multiple regression equations we offer a statistical model of the microflora. Different isolation procedures were used to quantify each component of the microflora. Macro and microclimatic conditions were considered as well as concentrations of the major elements. Three sites with differing vegetation were selected for this study: a newly-plowed field left abandoned, an 11-yr-old successional field, and a climax forest.

MATERIALS AND METHODS

Research area

Hutcheson Memorial Forest, 1.6 km east of East Millstone, Somerset County, New Jersey, was selected for this research because of the availability of

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fields in various successional stages. The red shale soil of the area is part of the Upper Triassic Brunswick formation on the Piedmont Plateau (Ugolini 1964).

Sampling

Three sites separated by 50 to 100 m were investigated from April 1974 to August 1975: a field last plowed in late April 1974 and abandoned after plowing, (1-yr-old field), a field abandoned after hay harvest in the fall 1963 (11-yr-old field), and a forest, > 250 yr old. The predominant macro-vegetation in the 1-yr-old field after plowing was wild radish, *Raphanus raphanistrum* L. During the summer the predominant vegetation was common ragweed, *Ambrosia artemisiifolia* L. and giant foxtail, *Setaria faberii* Herrm. Lesser dominants were hedge bindweed, *Convolvulus sepium* L., woodsorrel, *Oxalis stricta* L. and common crabgrass, *Digitaria sanguinalis* (L.) Scop. Dominants in the 11-yr-old field were aster, *Aster pilosus* Willd., and the goldenrods, *Solidago rugosa* Mill., *S. milifora* and *S. juncea* Ait. The forest site, located 30 m in from the periphery of the forest, had an overstory of white oak, *Quercus alba* L., an understory of dogwood, *Cornus florida* L., a shrub layer of mapleleaf viburnum, *Viburnum acerifolium* L. and an herb layer of mayapple, *Podophyllum peltatum* L. Complete descriptions of these sites are in Bard (1952), Small et al. (1971) and Sulser (1971). Map reference points are D5 for the 1-yr field, C5 for the 11-yr field and B5 for the forest (Small et al. 1971).

Quadrats (10 × 10 m) were randomly selected at each site and were equally subdivided into 12 sections. Three randomly selected sections of each site were sampled monthly by collecting 1–2 cm thickness of soil over an area of approximately 30 cm², after first removing the litter and 4–7 mm of surface soil to avoid recent contamination from airborne cells. The same section was not disturbed for a minimum of 4 mo. The sampling instrument, a scoop spatula, was wiped clean after each use. Samples were held in commercial plastic bags and the bags were discarded after use.

Chemical and physical analyses

All sites were assumed to have identical climatological conditions. Minimum and maximum air temperature and precipitation were measured daily at the forest weather station located within 200 m of each site. Snowfall was converted to rain equivalents. In March 1975, light intensity readings were taken at the soil surface in all 3 sites using a Weston Illumination Meter.

For pH readings, a 10 g portion of each sample was aseptically mixed 1:1 with water. After 1 h the pH was determined using a Sargent-Welch Model LS pH meter with a glass, combination electrode. Additional soil was sieved through a United States Standard Sieve #10 (pore size 1.0 mm). Freshly sieved samples were

weighed onto dried aluminum trays, heated at 105–110°C for 24 h, and reweighed to determine moisture loss. These readings were used to normalize all microbial counts from numbers per g wet weight to numbers per g dry weight of soil. All samples were held in commercial paper bags at 3–5°C, for no longer than 48 h until microbial isolations were completed. Soils were then air dried for further analyses.

Analyses for Mg, Ca, P and K were run on leachates of dried samples as described by Black et al. (1965) and Chapman and Pratt (1961). Total nitrogen was determined by Kjeldahl extraction and subsequent colorimetric analysis. Organic carbon was calculated by loss on ignition with oven dried samples.

Organism isolations and identifications

One g soil samples were used to initiate a 10-fold dilution series, each dilution being vortexed to ensure uniformity of suspension.

Soil algae were isolated using the 5 tube Most Probable Number technique (MPN) (Meynell and Meynell 1965). One ml of suspended inoculum was aseptically transferred to each of 5 plastic capped test tubes (18 × 150 mm) for each 10-fold dilution step. Four to 5 serial dilution levels were used.

BG-11 medium (Allen and Stanier 1968), incubated at 35 ± 1°C, was used for preferential isolation of blue-green algae. The eukaryotic algae were isolated in Bristol's medium (Nichols 1973) at 20 ± 1°C. Both were incubated on a 16/8 h light-dark cycle with a light intensity of 3200 to 4300 lx. For one 4-mo period, the medium (TBIM) of Smith and Wiedeman (1964) also was used to isolate eukaryotic algae.

Following incubation of 4 to 7 weeks, all tubes were examined and numbers of viable algae estimated (Meynell and Meynell 1965). Generic identifications of algae in the highest dilution tubes were made using numerous resources (Smith 1950, Chapman 1962, Brown and Bold 1964, Smith and Bold 1966, Groover and Bold 1969, Kantz and Bold 1969, Archibold and Bold 1970, Baker and Bold 1970, Prescott 1970, Whitford and Schumacher 1973, Lee and Bold 1974).

For plating and isolation of bacteria, actinomycetes, and fungi, 1 g of soil was placed in 10 ml of sterile physiological saline (0.9% NaCl) and serially diluted in 10⁻¹ steps. Three dilution levels were plated (0.1 ml) in triplicate on media preferential for the growth of each group (see below). Plates for bacteria and actinomycetes were incubated at 37°C for 1 to 14 days and fungi at 25–28°C for 5 to 14 days.

Bacterial population estimates were made after plating on nutrient agar (Difco) supplemented with antifungal antibiotics (Pimaricin 100 g/ml and Actidione 10 g/ml). The actinomycete microflora was estimated after growth on a starch-nitrate casein medium modified from that of Kuster and Williams (1964) by the substitution of soluble starch (4 g/l) for glycerol and supplemented with the antifungal antibiotics. Fungi were

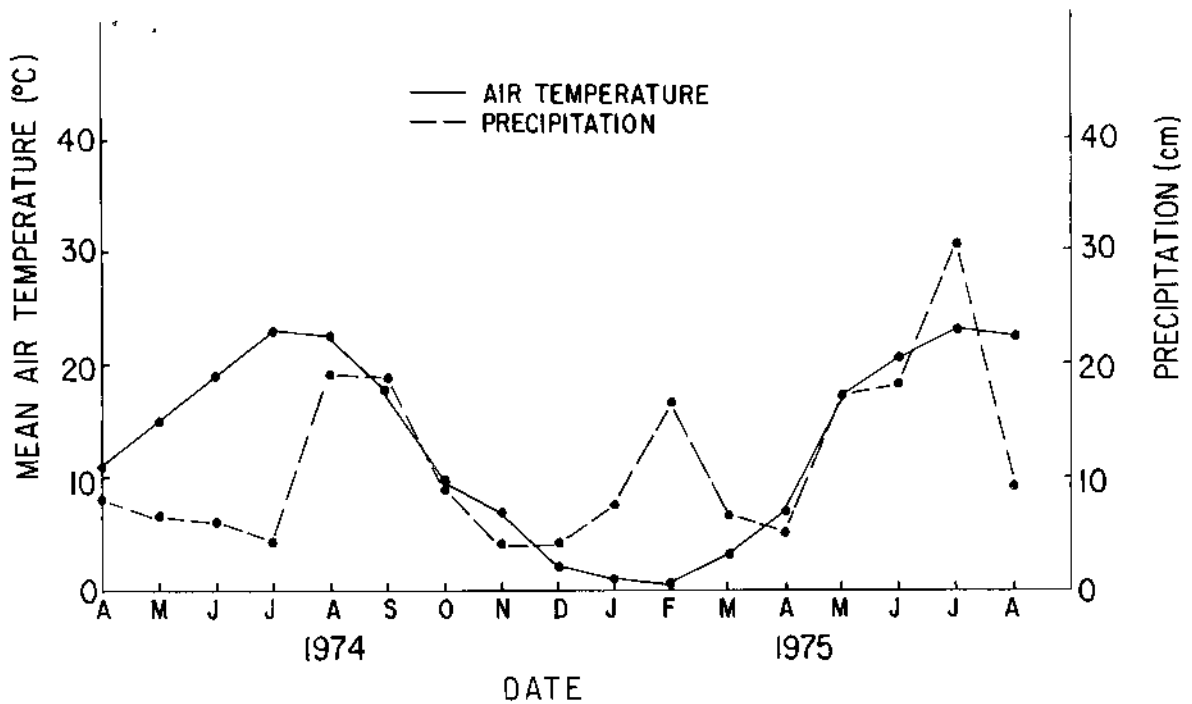


FIG. 1. Monthly precipitation and monthly means of air temperature readings.

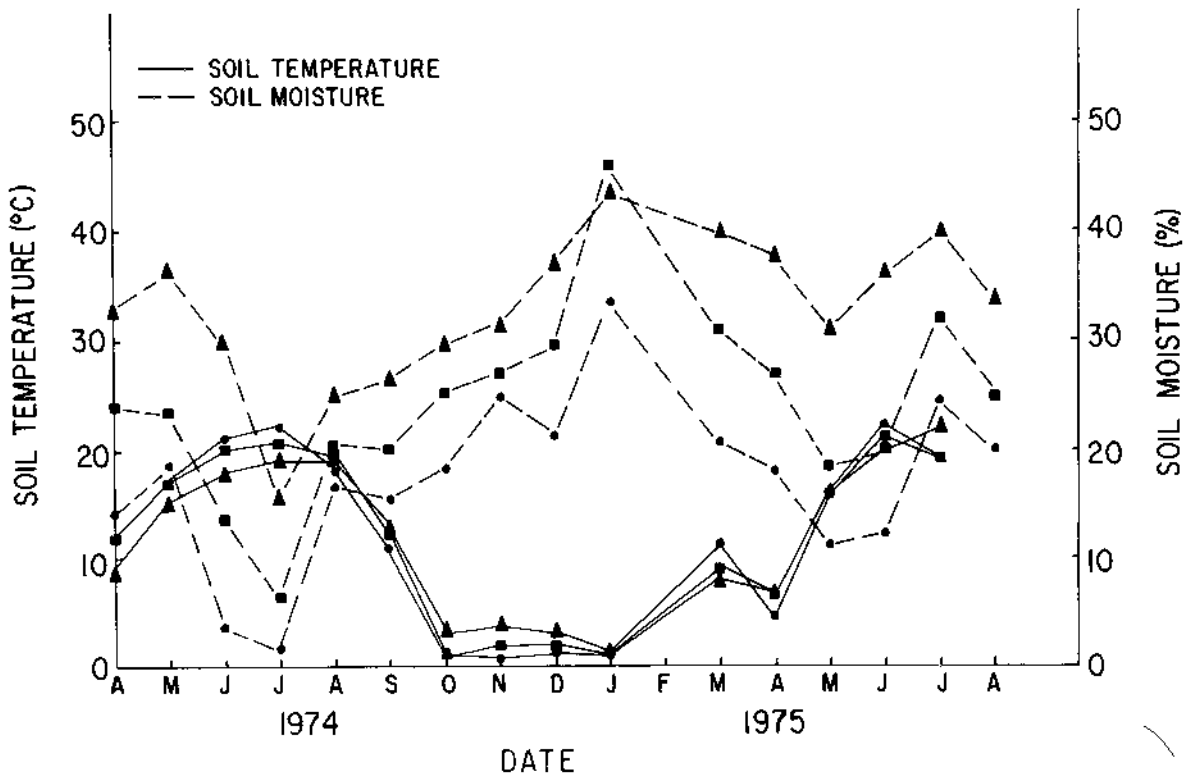


FIG. 2. Soil moisture and temperature at time of collection. Each point is the mean of triplicate samples. ● = 1-yr field, ■ = 11-yr field, ▲ = forest.

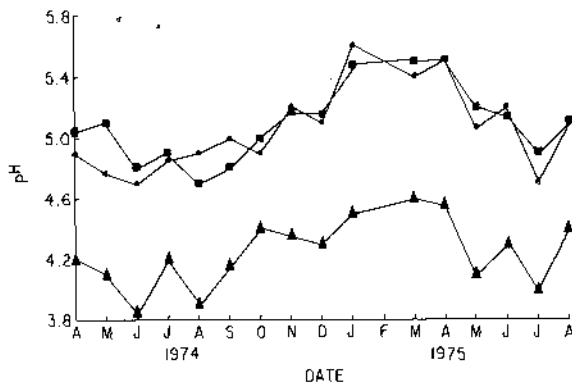


FIG. 3. Soil pH at time of collection. Each point is the mean of triplicate samples. ● = 1-yr field, ■ = 11-yr field, ▲ = forest.

plated onto dextrose-peptone yeast extract medium modified from that of Papavizas and Davey (1959) by the substitution of sodium deoxycholate (0.3 g/l) for oxgall and added antibacterial antibiotics (oxytetracycline 5 mg/l and polymyxin 5 mg/l). Previous experiments (*personal observations*) had shown this medium to be selective for fungi and spreading was not a problem.

Ten representative bacterial colonies at the highest dilution levels were selected for identification. They were gram stained and gram morphology noted.

Isolated actinomycete colonies were held at 4°C on slants of the isolation medium (minus the antibiotics) and then plated onto Peptone Iron Agar (Difco), Synthetic Starch Medium (Merck soluble starch 10 g, NaNO₃ 1 g, K₂HPO₄ 0.3 g, NaCl 0.5 g, CaCO₃ 3g, MgSO₄·7H₂O 1 g, Bacto Agar 15 g, distilled water 1 l) and Oatmeal Agar (Gerber's Baby Oatmeal 20 g, trace salts 1 ml, Bacto Agar 18 g, distilled water 1 l). The trace salts solution consisted of FeSO₄·7H₂O 0.1 g, MnCl₂·4H₂O 0.1 g, ZnSO₄·7H₂O 0.1 g, distilled

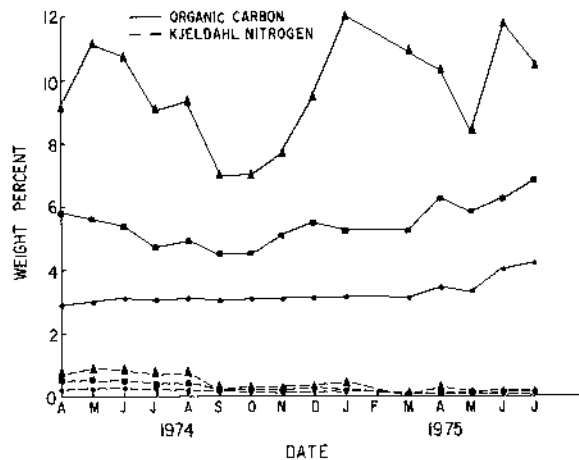


FIG. 4. Organic carbon and Kjeldahl nitrogen content of soil. Each point is the mean of triplicate samples. ● = 1-yr field, ■ = 11-yr field, ▲ = forest.

TABLE I. Summary of most frequently occurring soil algae over a 15-mo period, April 1974 through July 1975, sampled once monthly (except February 1975)

Phylum	Genera	Months observed (N)		
		1-yr Field	11-yr Field	Forest
Cyanophyta	<i>Anabaena</i>	13	15	4
	<i>Gloeocapsa</i>	0	1	0
	<i>Lyngbya</i>	3	1	1
	<i>Nostoc</i>	15	13	2
	<i>Oscillatoria</i>	11	5	1
	<i>Phormidium</i>	0	0	1
	<i>Plectonema</i>	1	0	0
	<i>Synechococcus</i>	0	1	0
	<i>Synechocystis</i>	6	7	9
	Chlorophyta	<i>Bracteacoccus</i>	10	6
<i>Characium</i>		1	0	1
<i>Characiopsis</i>		1	0	3
<i>Chlamydomonas</i>		1	2	7
<i>Chlorella</i>		14	12	10
<i>Chlorococcum</i>		14	15	13
<i>Chlorosarcina</i>		0	1	2
<i>Chlorosarcinopsis</i>		2	0	3
<i>Gleocystis</i>		0	1	2
<i>Klebsormidium</i>		2	0	0
<i>Mesotaenium</i>		1	0	0
<i>Myrmeclia</i>		1	0	0
<i>Nannochloris</i>		9	7	8
<i>Neochloris</i>		2	1	1
<i>Oocystis</i>		2	1	2
<i>Palmellococcus</i>		4	2	2
<i>Pleurastrum</i>	2	0	0	
<i>Stichococcus</i>	9	11	13	
<i>Tetracystis</i>	0	5	1	
Unidentified	1	0	0	
Chrysochyta	<i>Bumilleria</i>	4	0	0
	<i>Gomphonema</i>	1	0	0
	<i>Monocilia</i>	0	0	2
	<i>Navicula</i>	6	12	3
Euglenophyta	<i>Euglena</i>	1	0	0

water 100 ml. Identifications were based on Pridham et al. (1958) and Waksman (1967).

Fungal colonies were refrigerated at 4°C on potato dextrose agar slants (Difco) until time permitted identification. Isolates were then streaked onto 2 different media: Czapek Dox (Difco) and Egg Albumin (egg albumin 0.15 g, glucose 10 g, K₂HPO₄ 0.5 g, MgSO₄·7H₂O 0.2 g, Fe(SO₄)₃ trace, Bacto Agar 20 g, distilled water 1 l). Identifications were based on Barnett and Hunter (1972) and Gilman (1957).

Data analyses

Analysis of variance and multiple stepwise regression techniques were used for data analysis. Library programs of the Bio-Med series at the Rutgers University Computer Center were employed.

In the analysis of variance the response variable was the population estimates of each of the biological groups obtained from the triplicate samples taken each month. Log transformations were used to give homogeneous variances.

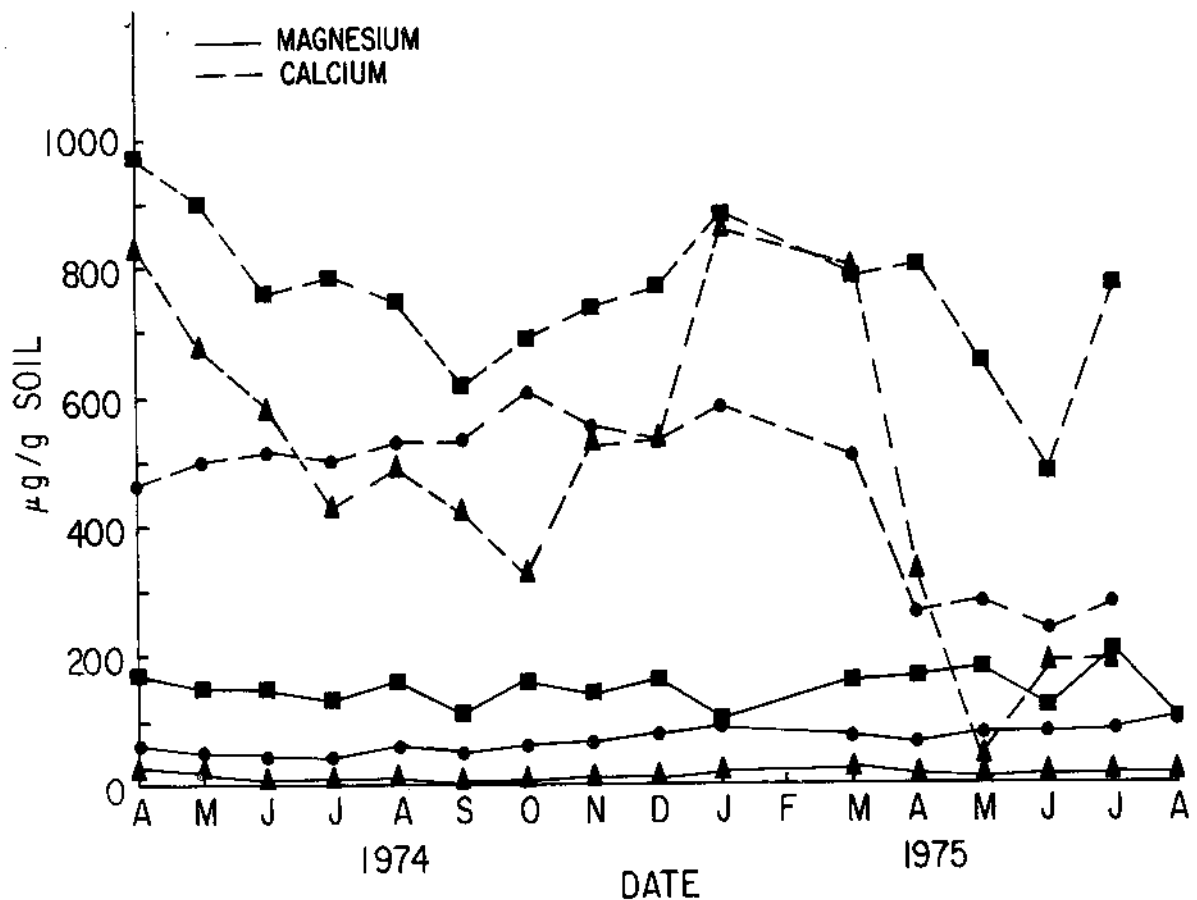


FIG. 5. Magnesium and calcium content of soil. Each point is the mean of triplicate samples. ● = 1-yr field, ■ = 11-yr field, ▲ = forest.

In the regression analysis, 18 variables were considered: microorganisms (5 variables)—bluegreen algae, eukaryotic algae, bacteria, actinomycetes and fungi; macroclimatic conditions (2 variables)—air temperature and precipitation; microclimatic conditions (2 variables)—soil moisture and soil temperature; soil microenvironment (7 variables)—pH, C, N, Mg, Ca, P and K; Time, with September as 1 and August as 11, there being no February sample; and Time Squared, i.e., this numerical designation being squared. Months were coded so that the functions were either linear or quadratic; if January had been coded 1, the functions would have been more complex. August 1975 samples were not included in the analysis. Quadratic and interactive effects of significant independent variables were also considered. Regression equations were accepted only if F , R^2 values and all coefficients were significant at the 0.05 level or lower. The presence of outliers in the data set was tested using the methods outlined by Ott (1975). A regression equation was calculated for each of the groups of microorganisms. For each group, the remaining 4 groups were considered as predictor vari-

ables. The appropriate equations were then used to predict the numbers of algae for August 1975 and compared to actual values obtained as a test of the regression equations.

RESULTS

Macroclimatic conditions

Precipitation and air temperature readings at Hutcheson Memorial Forest from April 1974 through August 1975 are shown in Fig. 1. The lowest mean monthly temperature was 0.4°C in February 1975 and the highest was $22\text{--}23^{\circ}\text{C}$ in July and August 1974 and 1975. The first summer was dry until late August with June and July having 6.4 and 4.6 cm of rain, respectively, while the second summer was relatively wet with the same months having 18.2 and 30.2 cm of rain.

Light intensity readings were recorded in March 1975 for each of the 3 sites. The 1-yr field had the highest readings at the soil surface ($\bar{x} = 85\,000\text{ lx}$) and the 11-yr field the next highest ($\bar{x} = 61\,000\text{ lx}$). The readings for the forest floor were approximately that

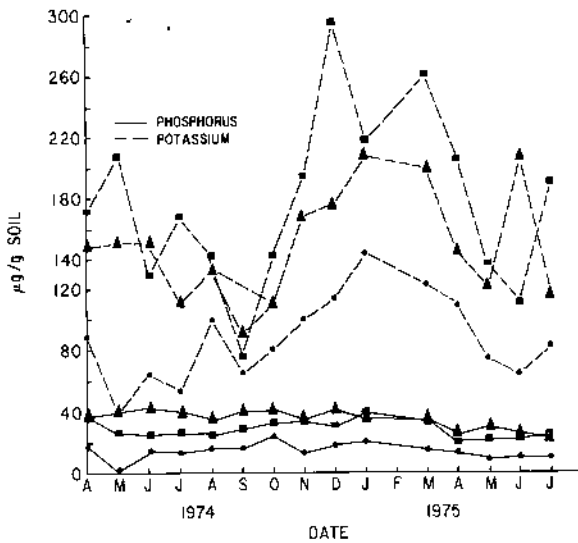


FIG. 6. Phosphorus and potassium content of soil. Each point is the mean of triplicate samples. ● = 1-yr field, ■ = 11-yr field, ▲ = forest.

seen in the 11-yr field ($\bar{x} = 54\ 500\ \text{lx}$), and as expected, readings taken beneath the leaf litter were considerably less ($\bar{x} = 11\ 200\ \text{lx}$).

Microenvironmental conditions

Soil temperature and moisture content at sampling times are shown in Fig. 2. January samples were chiseled from the frozen ground resulting in the high moisture readings and March samples were taken

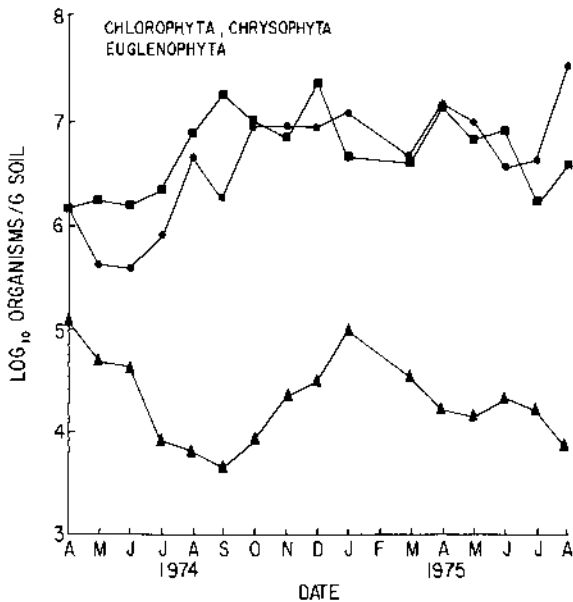


FIG. 7. Log₁₀ changes in numbers of soil eukaryotic algae (Chlorophyta, Euglenophyta, Chrysophyta). Each point is the mean of triplicate samples. ● = 1-yr field, ■ = 11-yr field, ▲ = forest.

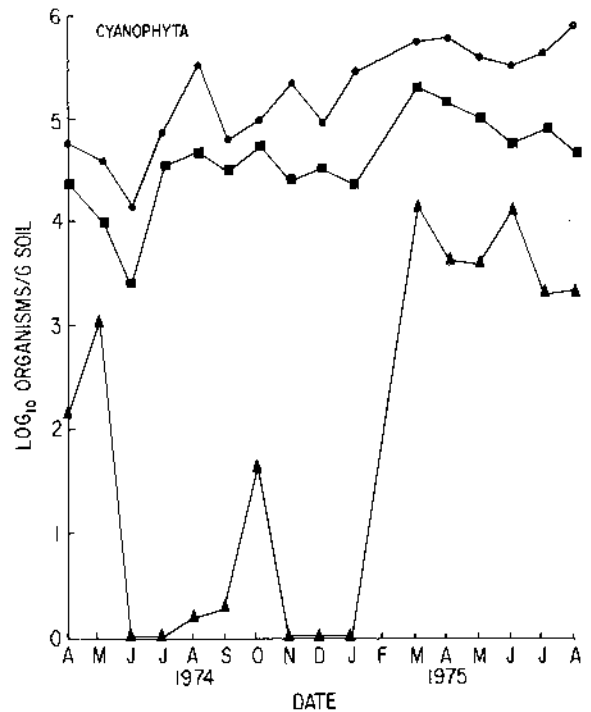


FIG. 8. Log₁₀ changes in numbers of soil bluegreen algae (Cyanophyta). Each point is the mean of triplicate samples. ● = 1-yr field, ■ = 11-yr field, ▲ = forest.

4–5 h later than usual. Seasonal soil temperature response patterns were similar, but the forest exhibited the slowest rate of change and the 1-yr field the fastest. Except for the January samples, the forest had the highest soil moisture content and the 1-yr field the lowest. The lowest soil moisture for all 3 fields was in July 1974: 2% for the 1-yr field, 6.5% for the 11-yr field and 15.5% for the forest.

Soil pH readings for the 3 sites are shown in Fig. 3. The pH values for the 2 fields are similar with a low of 4.7 and a high of 5.7. The forest pH was considerably lower, 3.9 to 4.6.

Figures 4–6 show site concentrations of organic carbon, Kjeldahl N, Mg, Ca, P and K. The newly plowed field had no measurable change in organic carbon content until the second summer. The forest had the highest organic carbon content, 15%, compared to 6.8% in the 11-yr field and 4.2% in the 1-yr field. The forest also had a higher concentration of Kjeldahl N and P than either of the other sites. In contrast, concentrations of Mg and Ca were lowest in the forest, highest in the 11-yr field, and intermediate in the 1-yr field. The 11-yr field concentrations of K were only slightly > that of the forest, while the 1-yr field again was lowest.

Algae

In all 3 sites, eukaryotic algae (Fig. 7) were present in greater numbers than prokaryotic algae (Fig.

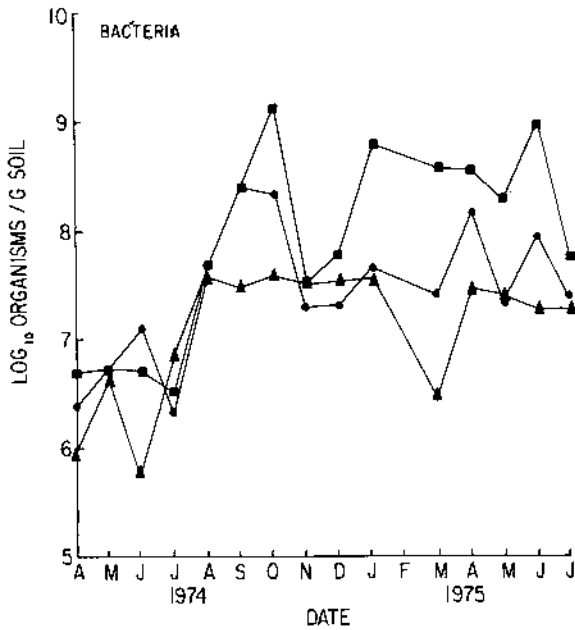


FIG. 9. Log_{10} changes in numbers of soil bacteria. Each point is the mean of triplicate samples. ● = 1-yr field, ■ = 11-yr field, ▲ = forest.

8). Numbers of eukaryotic algae were very similar in the 1-yr and 11-yr fields; forest numbers were considerably lower. Bluegreen numbers were consistently higher in the 1-yr field than either the 11-yr field or forest. The differences from site to site, month to month and interaction of fields with months is highly significant for both algal groups ($P < .001$).

The 1-yr and 11-yr fields for both groups of algae show similar patterns of slight declines in numbers in late spring and early summer (Figs. 7, 8). The forest, however, was remarkably dissimilar. There was a decided decrease in eukaryotic algal numbers starting in spring, reaching a low (4.6×10^3 cells/g soil) in early fall, and an increase through the fall reaching a high in late winter-early spring (1.2×10^5 cells/g soil). Bluegreen algal numbers in the forest also showed a similar decrease in late spring-early summer of 1974 but not in 1975. The highest bluegreen algal numbers in the forest were 1.6×10^4 /g soil compared to a low

TABLE 2. Bacteria: predominant morphological types isolated over the 10-mo period, April 1974 through January 1975

Types	Months observed (N)		
	1-yr Field	11-yr Field	Forest
Gram positive rod, spore-forming	9	10	8
Gram positive rod, no spores	6	6	6
Gram positive cocci	1	0	0
Gram negative rod	5	7	5
Gram variable rod	4	3	5
<i>Nocardia</i>	0	1	1

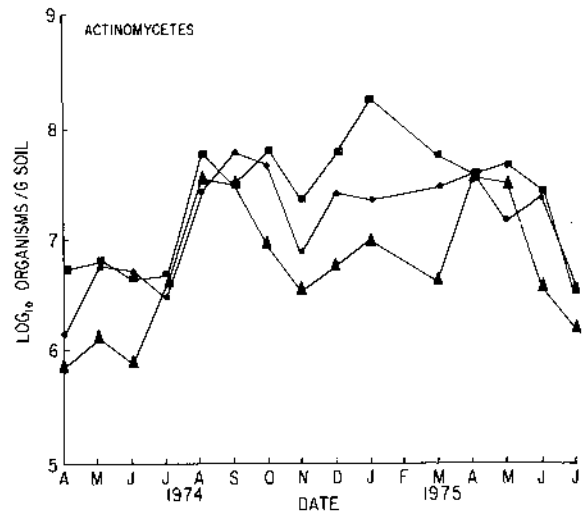


FIG. 10. Log_{10} changes in numbers of soil actinomycetes. Each point is the mean of triplicate samples. ● = 1-yr field, ■ = 11-yr field, ▲ = forest.

of zero (June, July and December 1974). In additional forest samples taken from a second area in July 1974, no bluegreen algae were observed.

The algal genera, the fields in which they were found and the frequency of occurrence are shown in Table 1. Of 9 different bluegreen genera isolated over the entire study, 6 were observed in the 1-yr field, 7 in the 11-yr field, and 6 in the forest. *Anabaena*, *Nostoc*, *Oscillatoria* and *Synechocystis* were the most frequently observed. *Synechocystis* was most common in the forest while *Anabaena* and *Nostoc* predominated in the open fields. Of those genera observed only once *Plectonema* was isolated from the 1-yr field, *Gloeocapsa* and *Synechococcus* from the 11-yr field and *Phormidium* from the forest.

Of 19 different Chlorophyta identified, 16 were from the 1-yr field, 12 from the 11-yr field and 14 from the forest. The most frequently observed eukaryotic algae were *Chlorococcum*, *Chlorella* and *Stichococcus*. *Bracteacoccus*, *Nannochloris* and *Navicula* were observed approximately 50% of the time. *Klebsormidium*, *Mesotaenium*, *Myrmecia*, *Pleurastrum*, *Bumilleria*, *Gomphonema* and *Euglena* were only observed in 1-yr field samples. *Tetracystis* and *Chlorosarcina* were never found in the 1-yr field but were present in the other 2 sites. The unidentified genus was a sphere, 18–22 μ in diameter, with a cup-shaped chloroplast and no pyrenoid. *Monocilia* was the only eukaryotic alga unique to the forest.

Of 4 different Chrysophyta isolated, 3 were from the 1-yr field and 2 from the forest. One genus of the Euglenophyta was found in the 1-yr field.

Comparison of algal numbers in Bristol and TBIM media showed no significant difference in counts and no new algal genera were found. Visual observation

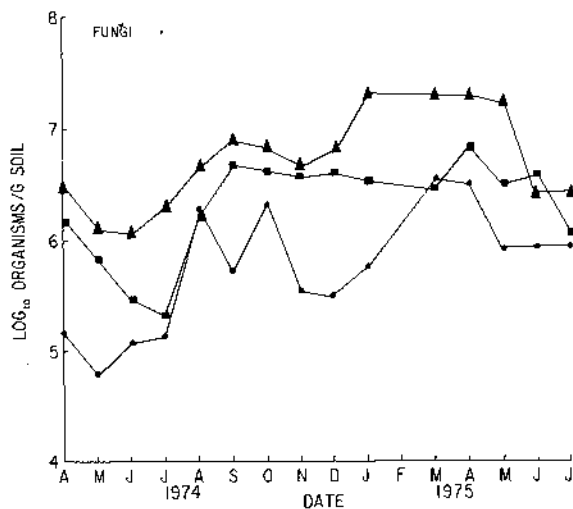


FIG. 11. Log_{10} changes in numbers of soil fungi. Each point is the mean of triplicate samples. ● = 1-yr field, ■ = 11-yr field, ▲ = forest.

of tubes, however, indicated less dense and slower growth in TBIM.

Other soil microorganisms

A decrease in bacteria (Fig. 9) occurred during the winter months in all 3 sites. Increases in numbers occurred in fall and early spring. Field to field and month to month differences and the interaction of fields with months are highly significant at the $P < .001$ levels. Table 2 shows the frequency of occurrence of the bacterial morphological types over the first 10 mo of this study. Gram positive spore forming rods predominated.

In all fields, lowest counts of actinomycetes were in July with an increase in early fall and a slight decrease in winter (Fig. 10). Differences between fields, between months and the interaction of fields with months are all highly significant at the $P < .001$ levels. Actinomycete genera isolated during the first 4 months of this study are listed in Table 3. The majority were *Streptomyces* sp.

Fungi were found in consistently higher numbers in

TABLE 3. Actinomycetes: predominant genera isolated over the 4-mo period, April 1974 through July 1974

Taxa	Months observed (N)		
	1-yr Field	11-yr Field	Forest
<i>Micromonospora</i>	1	2	0
<i>Micropolyspora</i>	1	0	0
<i>Streptomyces</i>			
<i>monoverticillus</i>	1	0	0
<i>rectus-flexibilis</i>	2	2	1
<i>retinaculum-apertum</i>	1	2	2
<i>spira</i>	4	4	4
Unidentified	4	2	1

TABLE 4. Fungi: predominant genera isolated over the 9-mo period, April 1974 through December 1974

Genera	Months observed (N)		
	1-yr Field	11-yr Field	Forest
<i>Apergillus</i>	2	2	0
<i>Botryotrichium</i>	1	0	0
<i>Botrytis</i>	0	1	1
<i>Cephalosporium</i>	4	1	1
<i>Fusarium</i>	1	3	0
<i>Fusidium</i>	0	1	0
<i>Gliocladium</i>	1	0	0
<i>Gliomastix</i>	0	0	1
<i>Haplosporangium</i>	1	2	1
<i>Hormodendrom (Cladosporium)</i>	1	2	2
<i>Menispora</i>	1	0	0
<i>Monilia</i>	1	0	0
<i>Monocillium</i>	0	1	0
<i>Mucor</i>	3	3	4
<i>Penicillium</i>	6	5	7
<i>Phycomyces</i>	0	1	0
<i>Scytalidium</i>	1	0	0
<i>Spicaria (Paecilomyces)</i>	6	4	6
<i>Sporotrichium</i>	0	0	1
<i>Staphylotrichium</i>	0	1	0
<i>Trichoderma</i>	1	6	4
<i>Verticillium</i>	0	0	1
Unidentified—Sterile Hyphae	5	4	6

the forest (Fig. 11) with lowest numbers in summer and winter. Field to field and month to month differences and the interaction of field to months were all highly significant at the $P < .001$ levels. During the first 9 months *Penicillium*, *Mucor* and *Spicaria* were most frequently isolated (Table 4).

Multiple regression analysis

The eukaryotic and prokaryotic algae were reciprocally correlated, with the numbers of 1 group showing up in the prediction equation of the other. Bacteria and actinomycetes were similarly correlated. The fungi equation contained only environmental variables (Table 5). In each multiple regression equation the dependent variable was the log of the number of organisms. The variation accounted for, R^2 , varied from 35 to 65%.

The regressions all meet the significance criteria previously listed; 3 data points out of a total of 810 were rejected as outliers (Ott 1975).

An additional test of the algal equations also indicated the validity of the multiple correlations. The August 1975 data were not used to calculate the equations. Rather the equations, based on the earlier data, were used to estimate the August numbers. A comparison of predicted versus actual numbers shows that the actual values fall within the predicted range (Table 6).

DISCUSSION

Physical parameters

An area frequently plowed in the past and plowed just after April 1974 sampling, the 1-yr field repre-

TABLE 5. Significant independent variables used in regression equations for determination of various soil microbial groups (all coefficients significant at $P \leq .05$ level), $N = 45$

Microbial Group	Significant independent variables						Total R^2
	B_0	B_1	B_2	B_3	B_4	B_5	
Bluegreen algae	-14681.51	+3146.31 (pH)	+139.73 (time)	-16.58 (Mg)	+129.28 (precip.)	+0.74 (eukary. algae)	45%
Eukaryotic algae	+452.61	-65.72 (time)	+4.07 (Mg)	+0.10 (prokary. algae)			37%
Bacteria	-26.64	+3.54 (Actinomycetes)	+0.44 (Mg)				58%
Actinomycetes	-8.65	+0.005 (Bacteria- Soil Moisture)	-0.00005 (Bacteria ²)	+0.92 (pH ²)			65%
Fungi	-118.24	+42.10 (C)	-0.51 (Air Temp., C)	-0.47 (N, P)	+1.74 (Air Temp.)		54%

sented a disturbed area open for colonization. The low concentrations of mineral elements were expected as significant litter accumulation had not been encouraged and water percolation was rapid. As a result of the more open vegetation and open space, higher light intensities reached the soil surface and this soil responded very rapidly and sharply to both temperature and moisture changes. This response pattern probably represents an environmental stress for those organisms living within the first 2 centimetres of soil.

The 11-yr-old field had an accumulated litter layer and greater foliage cover than the 1-yr field. Approximately 30% light reduction occurred in March. As the soil was undisturbed for a longer period of time, higher concentrations of mineral elements were seen and greater microfloral growth was expected. The slightly higher pH readings and considerably higher magnesium readings in the 11-yr field were most likely due to the past history of the field, i.e., it was limed and fertilized and then undisturbed for 10 years, whereas the 1-yr field, which had a similar history, was repeatedly plowed during the last 10 years.

The climax forest represents a mature system. The highest values of organic carbon, Kjeldahl nitrogen and phosphorus were reached in the forest. The low values of magnesium in the forest may relate to the acidity of the soil which results in increased mobility of this cation (Millar 1955). The values for potassium were generally higher in the forest and the 11-yr field, probably due to the high water solubility of potassium.

Insulation by litter and fermentation layers in the forest was responsible for the soil retaining higher and more constant moisture levels and for slowing the response to temperature changes.

All 3 sites had the same pattern of pH changes over the seasons, a situation similar to that described by Bayer (1927), i.e., a continual increase in hydrogen ion concentration from May through September. The magnitude of change observed is also similar to that seen by Bayer, approximately 0.9 pH units. The causes of the pH changes with time may relate to the accumulation of inorganic salts during the summer and to the dehydration of colloidal silicates with subsequent decreased buffering capacity. Decreased production of carbon dioxide during the winter decline in microbial activity probably also affected the pH values.

Light measurements on the forest floor show that the forest canopy can significantly decrease light penetration (Wales 1967). In our 1 spring reading, incident light penetrating to beneath the leaf litter was considerably < the amount of light reaching the soil surface of the open areas. During the winter the differences between the areas would certainly be much smaller.

Soil algae—Quantitative changes

Other researchers have found algae to be relatively numerous in new fields (Flint 1958, Fairchild and Willson 1967, Hofstetter 1968, MacEntee 1970, MacEntee et al. 1972). Linear regression analysis showed a significant relationship of the bluegreen algae with pH, time,

TABLE 6. Observed and predicted numbers of soil algae in 2 successional fields and a climax forest for August 1975. Predictions made from multiple linear regression equations (\pm SD)

Sites	Bluegreen algae (cells/g soil)		Eukaryotic algae (cells/g soil)	
	Predicted	Observed	Predicted	Observed
1-yr field	$4.4 \times 10^5 \pm 0.8$	$8.2 \times 10^5 \pm 2.5$	$9.6 \times 10^6 \pm 0.1$	$3.3 \times 10^7 \pm 3.1$
11-yr field	$2.1 \times 10^6 \pm 0.08$	$4.7 \times 10^6 \pm 1.3$	$6.6 \times 10^6 \pm 7.5$	$3.7 \times 10^6 \pm 0.9$
Forest	$9.8 \times 10^4 \pm 6.0$	$2.2 \times 10^3 \pm 1.9$	$2.2 \times 10^3 \pm 2700$	$6.8 \times 10^3 \pm 5.1$

magnesium, precipitation and the eukaryotic algae, $R^2 = 45\%$ (Table 5). Significant independent variables for the eukaryotic algae were time, magnesium and the bluegreen algae, $R^2 = 37\%$. The presence of the corresponding algal group in each regression equation was thought to follow from their similar responses to these variables.

The bluegreen algae are known to be intolerant of low pH conditions (Lund 1962, Jurgensen and Davey 1968, Bold 1970, Brock 1973, Dooley and Houghton 1973) and lower cyanophyte numbers in the forest may reflect this characteristic. The summer decline in pH could be responsible for the summer decrease in bluegreen numbers.

The response to precipitation by the cyanophytes may be an indication of germination of akinetes or increased fragmentation of filaments resulting in an increased number of potential colony-forming units. As certain of the bluegreen algae (*Nostoc* and *Anabaena*) maintain their integrity by mucilage, they probably would not fragment in response to precipitation. However, whether mucilage is produced to any extent beneath the soil surface has not been shown.

Gerloff and Fishbeck (1969) reported that requirements for critical cell concentrations of magnesium are higher than for Ca or K in 3 bluegreens and 2 of 3 green algae studied, while requirements for calcium were very low and requirements for potassium were variable. This may explain why Mg was the only soil element found to be significant for the algae (Table 5). Mg, essential for metabolism and the synthesis of chlorophyll, was found in lowest concentrations in the forest, possibly accounting for the negative expression of Mg in the regression analysis for the bluegreens (see Table 5).

Both algal regression equations contain time as a significant variable, and as can be seen for all 3 fields, a decline in algal numbers occurred in late spring and early summer with the pattern for the forest being most exaggerated and extended over the longest period. This pattern was not seen for the heterotrophic organisms. Wales (1967) in his study on climatic dissimilarities between the southern and northern boundaries of Hutcheson Memorial Forest found that light penetration 1 metre from the forest floor started to decline at the end of April, with mayapple (*Podophyllum peltatum* L.) emergence the first week of April and dogwood (*Cornus florida* L.) bud break in the middle of April. By mid-May light penetration ranged from 0 to 5% at the northern boundary near the forest site for this study. Penetration during the summer was found to vary 50-fold within inches, but generally ranged from 1.5 to 1.9%. Sulser (1971) also found the lowest light penetration in the forest during the summer, approximately 1.0%, and the highest values were in November and April.

Although some algae are capable of heterotrophic growth (Parker 1961, Carr 1973, Smith 1973, Droop

1974,) and nutritive interrelationships are known to occur with other organisms (Parker and Bold 1961, Whitton 1973), most are photoautotrophic. The decline was probably due to obligate autotrophic metabolism of the algae. A possible secondary explanation was the lack of adequate soil moisture in the more open sites; however, while the late July rains resulted in a slight increase in the 2 fields, no response was found in the forest. The October 1974 peak in cyanophyte numbers in the forest was preceded and followed by lows for the bluegreens. The low pH values during the summer and the slight decline in pH after the October sampling period may have been responsible.

The R^2 values for both the bluegreen ($R^2 = 45\%$) and the eukaryotic algae ($R^2 = 37\%$) are extremely good considering the complexity of the soil environment. The regression equations successfully predicted the algal numbers for August 1975 (Table 6). Both predicted and observed numbers for August 1975 were substantially higher than August 1974. The major difference between these sampling periods was the greater amount of precipitation for June and July of 1975 resulting in a larger standing crop for August. In addition, forest pH values showed an approximate 0.4 pH unit increase from July to August. The regression equations demonstrated the influence of an environmental factor on the soil algae.

Allen and Stanier (1968) showed that at 35°C, Bristol's medium supported less bluegreen algal growth than BG-11 both in terms of total numbers and number of different genera observed. In the present study, bluegreen growth in Bristol's medium at 20°C was also low.

Comparison of eukaryotic algal numbers isolated in Bristol's medium with TBIM showed no significant differences between the 2, however, reliance on the latter medium might prove unfeasible. Slower growth rates in TBIM require longer incubation periods and result in concomitant sparse growth.

The use of selective isolation media and temperature is important in determining actual numbers of algae in soil. Tchan and Whitehouse (1953) reported some daily fluctuation in numbers from sandy soils and as seen in this study, dependence upon summer sampling alone also could result in an inaccurate analysis of the soil algal population.

Soil algae—Qualitative changes

The greatest number of algal genera was found in the newly plowed field, i.e., 28 of a total of 35 found in all sites, or 80%, while the forest and the 11-yr field had 63% and 57%, respectively. Of the genera observed once, 56% were in the 1-yr field, 33% in the 11-yr field, and 11% in the forest. Light penetration was probably the most important factor for these results.

Chlorella, *Chlorococcum*, and *Stichococcus* appeared as the dominant algae in all 3 fields while *Brac-*

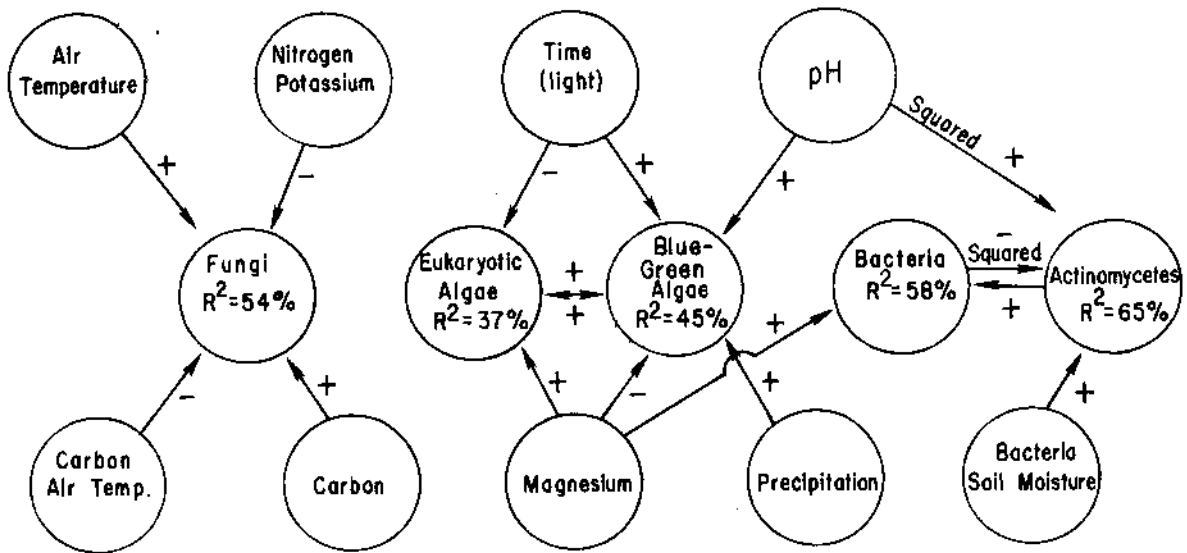


FIG. 12. A conceptual model of the microbial components in a soil ecosystem based on multiple regression analyses.

teacoccus and *Nannochloris* were less frequent. We have no explanation for the sporadic occurrence of certain genera. Three of the 4 filamentous or semi-filamentous algae were predominant in, but not restricted to, the 1-yr field: *Klebsormidium*, *Pleurastrum* and *Bumilleria*. Increased light penetration and a more open soil structure would aid development of these filamentous forms. The absence or scarcity of a species from the 1-yr field may relate to its sensitivity to certain microenvironmental conditions. For example, *Chlamydomonas* was found in lowest numbers in the 1-yr field. As a motile alga, moisture would be a requirement for growth, a condition least favorable in the 1-yr field. Likewise, intolerance to low light and pH conditions might also hinder growth of other genera (e.g., *Tetracystis* and *Navicula*) in the forest. Seasonal changes in the predominant algal genera were not apparent, possibly because the mild winter had a moderating influence.

Bacteria, actinomycetes, and fungi

The abundance of the fungi and the reduction in actinomycetes in the forest is thought to be related to their respective tolerance and intolerance to acidic conditions. Also, air temperature as a significant variable may be an indication of the influence of increased litter fall or nutrient source.

The presence of magnesium in the bacterial regression equation may be related to the requirement of Mg for normal metabolic growth, to the bias inherent in the lower concentrations present in the forest or to the reduced numbers of bacteria there. The numbers of actinomycetes paralleled the bacteria.

The actinomycete microflora correlated with the quadratic effects of bacteria and pH and to the interrelationships of bacteria with soil moisture. With both

quadratic relationships, increasing values of pH and bacteria correlate with increasing numbers of actinomycetes until a point is reached where increasing values of the independent variables correlate with decreasing numbers of actinomycetes. This suggests that there is an optimum level of pH and bacteria numbers for actinomycete growth.

Conceptual model

The multiple correlations were used to construct a conceptual model of the microbial component of the soils at the study sites (Fig. 12). Based on the regression equations, it is suggested that the 2 algal groups are independent of the other 3 groups, and the algal groups (because of the positive coefficients, Table 5) have a positive interaction, i.e., an increase in the numbers of one group correlates with an increase in numbers of the other. This may be due to similarities in the algal groups and to dissimilarities with the other groups. Competition might not be biologically significant between the photoautotrophs and the heterotrophs. Another possibility is that none of the microbial groups are living under optimum conditions in the study sites.

The influence of one algal group on another can be predicted by application of their respective equations (Table 7). In the 1-yr-old successional field, a 3 log change in either bluegreen or eukaryotic algal numbers results in a slight increase in the contrasting algal group. Seasonal (time-light) changes show a different pattern with a one and a half log decrease in eukaryotic algae and a slight increase in prokaryotic algal numbers when comparing the months of October to July. Theoretical increases in precipitation result in calculation of greater numbers of cyanophytes. For example, the month of July would have 4.2×10^3 algae/

TABLE 7. Predicted changes in numbers of eukaryotic algae and bluegreen algae with time and with changes in corresponding algal group in a 1-yr-old successional field. Values calculated were for magnesium = 53 $\mu\text{g/g}$ soil, pH = 5.4, and precipitation = 13 cm of rain

Predicted numbers (cells/g soil)									
Eukaryotic algae					Bluegreen algae				
Selected levels of bluegreen algae	Months				Selected levels of eukaryotic algae	Months			
	Oct	Jan	Apr	Jul		Oct	Jan	Apr	Jul
1.0×10^2	5.4×10^6	3.4×10^6	2.1×10^6	1.0×10^5	1.0×10^4	2.4×10^5	2.8×10^5	3.1×10^5	3.5×10^5
1.0×10^5	6.4×10^6	4.4×10^6	3.1×10^6	1.1×10^6	1.0×10^7	3.1×10^5	3.5×10^5	3.8×10^5	4.2×10^5

g soil with 15 cm of rain. Increases in pH also show increases in numbers of cyanophytes. For example, an increase of pH to 5.8 combined with an increase in precipitation to 38 cm for the month would result in 6.8×10^5 algae/g soil.

The soil fauna was not examined in this study. The mites, however, are the most abundant arthropod group to be found in soil (Wallwork 1970). Radioisotope experiments by Coleman and McGinnis (1970) have shown that the 3 major orders of mites consume approximately 0.2% of fungal inoculum in soil. They conclude that these microarthropods have little direct effect in soil. However, selective quantitative and qualitative removal of any biological component and dissemination of propagules could affect the microflora (Milliger et al. 1971, Mitchell and Parkinson 1976).

This is the first study of this type to continue over a prolonged period of time. The data illustrate the importance of taking multiple samples over several seasons and the use of selective isolation media and temperatures to characterize the algal microflora. Algal numbers in soil are very high, equivalent to that of the fungi and only 1 or 2 orders of magnitude lower than the actinomycetes and bacteria. The proposed model can serve as an analog of the real system and as such it can be used to suggest hypotheses. The predictions of algal numbers of Hutcheson Memorial Forest corresponded well with the actual numbers obtained. The next tests would appear to be the comparison to other terrestrial situations.

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