THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

CONDENSED TANNINS IN SOIL:
INPUTS AND EFFECTS ON MICROBIAL POPULATIONS

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

1976

CONDENSED TANNINS IN SOIL:

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-- Dziękuję --

ACKNOWLEDGMENT

I wish to extend my sincere appreciation to my major professor, Dr. Elroy L. Rice, for the stimulation, advice, and encouragement necessary to complete this research. I would like to thank the other members of my advisory committee, Drs. Norman H. Boke, James R. Estes, John S. Fletcher, and Simon H. Wender for their comments during the research and their critical reading of the manuscript. I am grateful to Drs. Paul G. Risser and Eddie C. Smith for their discussions and technical assistance.

I wish to acknowledge the financial assistance of the National Science Foundation, which made this research possible.

I would like to thank my families for their encouragement, especially my parents and my aunts Louise and Phyllis, whose financial aid allowed a semblance of normalcy in our daily existence.

To all those individuals who contributed to the success of this project, may their satisfaction come from knowing that without their assistance the project would not have been completed.

To my wife, Cheryl, who silently maintained a family unit against tremendous pressures and never succumbed, I acknowledge her sacrifices and offer my love in return.

Finally, I would like to dedicate this research to my daughters, Kristen Marie and Michele Ann, in recognition of their able assistance in the field and in the laboratory. They share with their father, a love of plants and a concern for the environment.

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ABSTRACT

The monthly condensed tannin content of the soil, the decomposing leaves, and the roots was determined in a post oak-blackjack oak forest in Oklahoma. The tannin concentration in the 0-15 cm level of the soil increased in the early spring, decreased in May and June, and increased through the early fall. The tannin content of green leaves of Quercus stellata and Q. marilandica was high, increasing slightly during senescence. Decomposition rates were highest during the late spring, with no significant difference among species. The amount of original condensed tannin remaining after one year was approximately 15%. Even though condensed tannins are water soluble, none were present in the throughfall or the stemflow. The tannin content of the roots increased in May and June but decreased through October. The below-ground plant material was apparently the major source of condensed tannins in the soil, based on relative biomass and was responsible for the agreement between loss of tannins from plant materials and increases in the soil content.

The condensed tannin content of the soil was negatively correlated with numbers of bacteria and actinomycetes in

both the undisturbed forest and an adjacent clear-cut plot but was not correlated with the fungal population. The annual amount of carbon dioxide evolved was 1953 g/m^2 in the cleared area and the number of microbes was significantly ($P < .01$) correlated with the evolved CO_2. The forest had a higher annual amount evolved because of root respiration. Nitrate-nitrogen accumulated in the cleared plot but was not detected in the forest. The numbers of Nitrosomonas and Nitrobacter were higher in the clear-cut area and were positively correlated ($P < .01$), but the numbers of these nitrifiers were not correlated in the forest. The condensed tannin content was significantly higher in the soil of the cleared area than in the forest in early spring and in August. Correlation coefficients between abiotic factors and numbers of nitrifiers indicated that these factors were more conducive to nitrification in the forest than in the clear-cut area. Thus, the evidence supports the hypothesis that the lower amount of nitrate in the forest was due to an inhibition of nitrification, probably by condensed tannins, the uptake of nitrate by the vegetation, or a combination, and not due to a change in microclimate.

CONDENSED TANNINS IN SOIL:
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CHAPTER I

INTRODUCTION

Tannins have been shown to inhibit many enzymes such as the pectolytic enzymes, cellulase, hemicellulase, catalase, peroxidase, amylase, myrosinase, pepsin, proteinase, dehydrogenases, invertase, phosphatases, B-glucosidase, aldolase, polyphenoloxidase, lipases, urease, trypsin, and chymotrypsin (Williams, 1963; Benoit and Starkey, 1968a, b). Thus, it is not surprising that these compounds have been found to inhibit the growth of many organisms from the bacteria to higher plants (Rice, 1965a, b, 1969; Blum and Rice, 1969; Parks and Rice, 1969; Olmsted and Rice, 1970) and to inhibit decomposition of organic matter (Benoit and Starkey, 1961, 1968a, b).

Rice and Pancholy (1972) reported an inverse relationship between ammonium-nitrogen and nitrate-nitrogen during the successional process in three different Oklahoma vegetation types. Nitrate-nitrogen was highest in the pioneer

stage, intermediate in the second stage, and lowest in the climax stands. The numbers of nitrifiers decreased as succession progressed. These results indicate that nitrification was inhibited during succession. Inhibition of nitrification is probably important in preventing loss of nitrogen from the ecosystem by leaching and surface drainage and also in conserving energy (Rice and Pancholy, 1972; Woodwell, 1974).

Berlier et al. (1956), Dommergues (1956), and Jacquemin and Berlier (1956) noted that nitrification increased greatly upon disturbance of climax African forest communities by either clearing or cultivation. At the Hubbard Brook Experimental Forest in New Hampshire, a small watershed was clear-cut and sprayed with the herbicide Bromacil (Bormann et al., 1968). An accelerated loss of nitrate ions from the soil was observed in the associated stream water. Smith et al. (1968) measured the numbers of nitrifying bacteria and recorded an 18-fold increase in Nitrosomonas and a 34-fold increase in Nitrobacter after clear-cutting. Three hypotheses might be advanced to explain the pronounced increase in nitrifiers: (1) clear-cut areas have a more favorable physical environment, (2) the trees may have produced an inhibitory substance, or (3) the prior vegetation had eliminated the nitrate by uptake. Rice and Pancholy (1973) showed that with the successional decrease in nitrate-nitrogen and in the numbers

of nitrifiers, there was a concomitant increase in the amounts of soil condensed tannins. These results suggested that condensed tannins are inhibitory to the autotrophic nitrifying population.

The primary purpose of this research was to quantify the soil condensed tannins and their rate of decomposition in the soil; to measure the major inputs into the soil and the effect of condensed tannins on microbial populations and their activity (Fig. 1). Secondly, the relationship between clear-cutting and nitrification was examined.

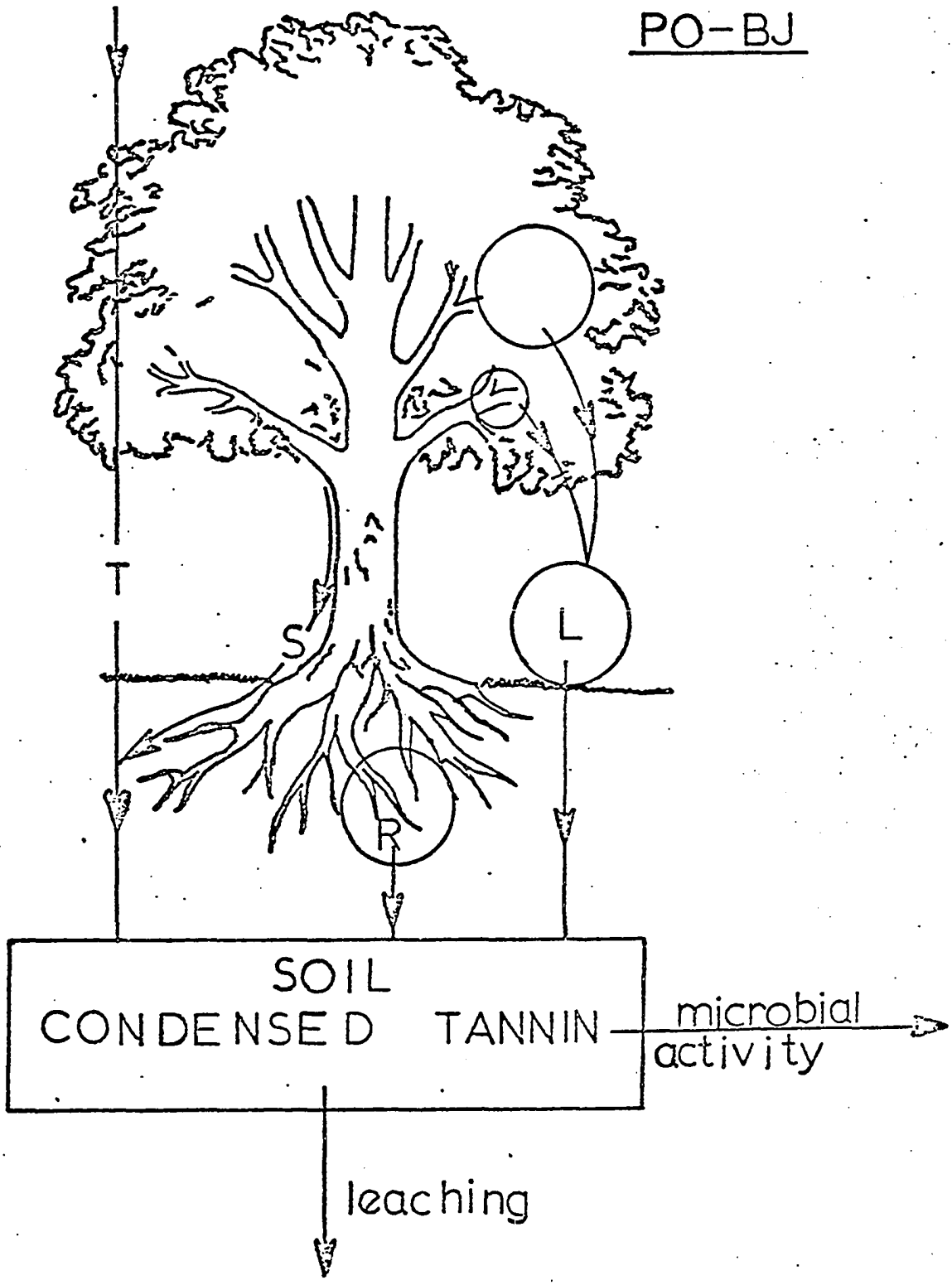


Figure 1. Flow diagram for soil condensed tannins: Inputs and Outputs. (T = throughfall, S = stemflow, R = root, L = litter)

CHAPTER II

STUDY AREA

The field study was conducted in the 25-hectare University of Oklahoma Lake Thunderbird Research Area located in the NE¼ of Section 25, T9N, R1W in Cleveland County. An earlier study (Johnson and Risser, 1974) on mineral cycling was performed at the site. Thus, any new data will add to our understanding of this forested area. The area has a gently rolling topography with a two-five degree slope and is located 17 km east of Norman. Dense vegetation is confined to the lower slopes and ravines, and an open savanna occupies the upper slopes and ridgetops (Johnson and Risser, 1975). The forest vegetation is almost exclusively *Quercus stellata* Wang. and *Q. marilandica* Muench. (Johnson and Risser, 1974), characteristic of the Western Cross Timbers as described by Dyksterhuis (1948). The climate is classified as subtropical humid (Trewartha, 1968). Mean annual precipitation for the area is 88.09 cm, with a January normal temperature of 2.2 C and a normal July temperature of 26.1 C (Curry, 1970). The soil is a shallow sandy, red-yellow podzolic, covering sandstone parent material at depths ranging from 98-66 cm along

the ESE slope. Textural classes are as follows: 0-15 cm, sandy loam and 45-60 cm, sandy clay loam.

CHAPTER III

METHODS

Site Selection, Design, and Sampling Procedure

The selected site was representative of the forest vegetation and occupied the lower slope approximately 75 m from the lake shore. This area was divided into two sections (Fig. 2). The larger tract contained 0.2 hectare. The undisturbed area was marked off into twenty permanent 10x10 m quadrats. This plot corresponded to the same 0.2 hectare study area used by Johnson and Risser (1974). The quadrats were further subdivided, with one square meter being the basic sampling unit. The location of various sampling devices is shown in Figure 3.

A clear-cut zone 20x20 m was located to the north of the undisturbed area. The inner 64 m^2 of this plot were subdivided for soil sampling, and the outer margins served as a buffer zone. In the fall of 1973 all the woody vegetation was inventoried, cut, and removed. In addition, all herbaceous material and litter were removed. The corresponding biomass values were calculated from regression equations (Johnson, personal communication).

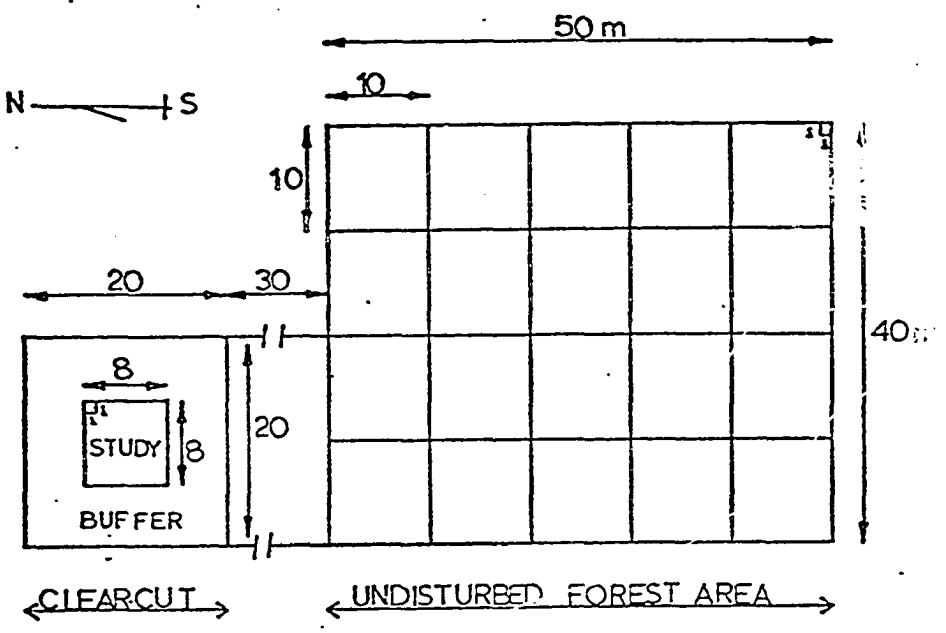


Figure 2. Location and dimensions of the study area.

C 1	C 2 C	C 3 S	C 4 C	L 5 C
L S 6	L 7 S	8 L	S 9 C	10 L
L 11	12	13 C	S L 14	C 15
C 16	C L 17 C	S 18 L C	C 19 L	20 C

Figure 3. Undisturbed forest area showing location of sampling devices. Symbols are as follows: C = CO_2 cylinder; S = stemflow and throughfall collectors, L - litterfall traps.

Runoff into the area was prevented by a surrounding mechanical barrier. A trench was dug to the depth of the parent material around the inner zone and was lined with 12 mil polyethylene sheets. McPherson and Thompson (1972) reported that this thickness prevents penetration by roots. Thus, no input of condensed tannin could be expected from the adjacent below-ground biomass. The remaining stumps were painted with 2,4-D, which has been reported (Young et al., 1969) to prevent root suckers. As a consequence, this area would not have an increase in the amount of photosynthate, and my assumption is that metabolic activities would be directed toward the production of energy rather than the synthesis of secondary metabolites, such as condensed tannins. The inner portion of the control was covered with a cheese-cloth canopy to simulate the microclimate of the undisturbed test plot.

The sampling schedule is presented in Table 1. With the exception of the CO_2-cylinders, all sample points were selected by a stratified random design.

Microclimate

Measurements were made of those climatic variables considered to be driving forces of the ecosystem (Table 1). In an attempt to determine the effect of abiotic parameters, the appropriate recording instruments were installed at three locations. One set occupied the open savanna adjacent to the

Table 1. Sampling Schedule

Weekly:

air temperature	relative humidity
soil temp - surface and 7.5 cm depth	
soil moisture	insolation
wind	precipitation
stemflow	throughfall

Mid-Month:

decomposition bags	decomposition line
litter quadrats	leaf & branches

End of Month:

- soil for tannins analysis
  - organic carbon microbes
  - nitrogen-ammonia + nitrate
- litter traps
- CO_2 - evolution

sampling area on the ridgetop. The others were associated with each plot.

Air temperature, relative humidity, air movement, and evaporation from white and black Livingston spherical atmometer cups were monitored 1.5 m above ground. Precipitation was measured by a standard Weather Bureau rainguage located in the savanna portion minimizing the influence of the forest canopy. Soil temperatures at the surface and at 7.5 cm depth were recorded for each plot. All temperature and humidity measurements were made by recording instruments, and air movement was measured by totalizing cup anemometers. The hygrothermograph for measuring air temperature and humidity was enclosed in a standard Weather Bureau shelter and was located in the forested zone separating the forest plot from the clear-cut plot. The soil thermographs were also sheltered.

Measurements were made throughout the year, except for evaporation, which was restricted to June through November. Calculations were based on average daily values for weekly and monthly periods. Average daily values of temperature were based on averages of measurements recorded every 2 hr. After the practice of the U.S. Weather Bureau, days were from midnight to midnight.

Soils

Monthly soil cores were taken to a depth of 60 cm at five random locations within each treatment and trans-

ported under sterile conditions. A soil corer with a 4.72 cm cutting diameter was used. Soil cores were removed from the 0-15, 15-30, and 30-60 cm levels for chemical, physical, and biological analyses. This maximum depth was selected because the soils are high in clay at the lower levels and compaction occurs during sampling. Each sample in the top 15 cm was individually processed whereas soils from other levels were composited. The samples were stored at -5 C if immediate determinations were not possible. Nelson and Bremner (1972) reported this procedure satisfactory for preserving inorganic nitrogen.

All analyses were made with air-dry soil passed through a 2 mm sieve and ground in a soil mill to pass a 0.5 mm sieve. Results were converted to an oven-dry basis.

Each month the organic carbon content was determined by the Walkley and Black method (Piper, 1942). Soil moisture was obtained gravimetrically (Gardner, 1965). Exchangeable-ammonium nitrogen was analyzed by steam distillation with MgO (Bremner, 1965). Nitrate nitrogen was determined by a specific ion electrode after the soil was extracted with distilled water (1:2 soil-water suspension) and treated with sodium citrate (Raveh, 1973) to remove interfering ions.

The soil reaction, pH, and textural analysis were performed at the onset of the field sampling. The soil pH (1:5 soil-water suspension) was determined by the glass electrode method described by Rice (1968) with soil that was

sieved but not ground. Mechanical analyses were performed with a modified Bouyoucos hydrometer method (Bouyoucos, 1936; Piper, 1952; Rice, 1968).

The field capacity of the soil was determined in the field (Gardner, 1965) for the 0-15 cm level. The characteristic permanent wilting percent was also obtained by the method of Briggs and Schantz (1912). The test species used was Helianthus annuus L. cultivar. Russian Mammoth at the three-leaf stage. The bulk density (Rice, 1968) was determined for each plot.

Plant Material

The plant material collected consisted of leaf and root samples. The leaf sample consisted of composited material from five randomly selected post oak and blackjack oak trees. Equal portions of upper sun leaves and lower shade leaves were obtained from each tree, and the criteria of Feeny (1970) for harvesting and transportation were followed. Root material was collected and composited according to soil level from each monthly soil sample from the forested plot. Adhering soil was removed by gentle shaking and rinsing with benzene (Stecher, 1968). The plant material was oven dried (60C) to constant weight, weighed, and ground in a Wiley Mill to pass a 1 mm sieve before subsequent analysis.

Throughfall, Stemflow, and Interception

Rainfall was intercepted by the tree canopy and redistributed as throughfall and stemflow, with some being evaporated from the vegetation. Several different schemes for the associated terminology have been proposed (Voight, 1960; Helvey and Patric, 1965a; Helvey and Patric, 1965b; Leyton et al., 1968). For this study the following terms were used: gross rainfall is the total rainfall incident on the canopy; throughfall is the amount reaching the ground via gaps in the canopy or from leaf and branch drip; stemflow is that portion of the rainfall which runs down the stem; interception loss is the amount of rainfall that is held by the vegetation or evaporated.

Gross rainfall was measured in the open savanna with a Standard Weather Bureau raingauge that employed the location criteria of Middleton and Spilhaus (1953). Five trees of each species were fitted with polyurethane stemflow collectors (Likens and Eaton, 1970), and throughfall collectors were placed under each of these trees. The latter were supported at a level one meter above the ground to prevent contamination by soil splash. Each collector consisted of a 5 liter polyethylene bottle fitted with a 10 cm funnel equiped with a wire screen and a glass-wool plug. The number per tree followed the design recommendations of Eaton et al. (1973). At weekly intervals the total volume in each stemflow and throughfall collector was

recorded and a 250 ml aliquot removed. The samples from each species were combined.

Gross rainfall was also determined on a volume basis by a throughfall collector located adjacent to the standard raingauge. Percent throughfall per tree was determined by dividing the measured volume by the volume of gross precipitation. Percent stemflow was obtained by calculating the total volume of precipitation falling on the individual tree crowns and dividing this amount into the volume of stemflow. In order to determine the incoming volume, the canopy area was calculated. Homogenous portions of each crown were measured as to radius and the area of each portion was computed. The total area was the summation of these individual measurements. Assuming 100% closure, the total volume of precipitation per crown is the product of the incoming precipitation, ml per cm^2, and the crown area in cm^2. Percent interception loss was calculated by subtracting the stemflow and throughfall percents from 100.

The monthly condensed tannin input values were determined by analyzing composite samples. Because of rainfall variation per storm, the individual weekly samples were combined porportionately according to the weekly amounts of precipitation. This procedure was followed for both stemflow and throughfall for each oak species. The presence of condensed tannin was determined by the ferric chloride reagent (Smith, 1960). If tannins were present, quantification followed.

Litterfall

Litter inputs were determined with 1x0.5 m traps. Collected material was separated into leaves, budscales, catkins, acorns, and branch components for the overstory species only. The monthly amounts were expressed on an oven-dry basis (60 C) by species.

Each month the amount of litter on the ground was quantified by collections from five 25x25 cm random quadrats. The material was separated into leaf and non leaf catagories, ovendried (60 C), and weighed.

Decomposition

Litter dynamics were analyzed by the litter-bag technique of Bocock and Gilbert (1957). Oak leaves of each species were removed from trees in the fall of 1973 after the first killing frost and oven dried. Bags 10x10 cm were filled with approximately seven grams, with the exact weight being determined and recorded. The amount chosen was determined by actual measurement of existing litter in early November. The mesh diameters employed were 1 mm and 3 mm. Before placing the bags in the field, they were wetted and allowed to equilibrate with the air. This wetting has a negligible effect on decomposition (Suffling and Smith, 1974). Thus loss was compared between the decomposition action of microorganisms and decomposition by invertebrates (Curry, 1969a). Bags were placed randomly in the field, with

sufficient numbers to accomodate the following retrieval schedule. Once a month, five bags of each mesh size were collected, oven-dried (60 C), and assayed. One treatment, consisting of ten bags (5 of each mesh size), was used for determining organic matter loss; another treatment focused on the loss of condensed tannins per gram leaf material. The latter represented a composited determination. Because of soil contamination, a correction factor was required to determine rate of decomposition. Percent decomposition was calculated from the Garland equation (May, 1974) as follows:

$$\text{Fraction of material lost} = \frac{0_o - 0_r}{0_o}$$

Where: (1) 0_o = original dry wt., i.e. original wt. minus original ash wt.

(2) 0_r = ash free wt. of retrieved substrate

$$= B - \left[\left(\frac{B_r - A \cdot C}{S}\right) + A \cdot C\right]$$

where:

A = original plant wt.

B = wt. of retrieved plant material + soil

B_r = ash wt. of retrieved plant material + soil

C = fraction ash in original plant material

S = fraction of soil remaining after ashing, (based on a soil

sample taken in same area),

i.e. $\frac{\text{soil weight after ignition}}{\text{original soil weight}}$

In addition, the rate of decomposition of organic matter was evaluated by the nonconfinement method of Witkamp and Olson (1963).

Microbial Populations

Five random soil cores (0-15 cm) were taken from each treatment each month. The soil samples were placed in sterile plastic bags for transport to the laboratory. The samples were allowed to air-dry overnight, and the major groups of heterotrophic microbes were quantified by the dilution-plate count method (Clark, 1965) and the appropriate medium for each group. Difco Plate Count agar was employed for bacterial counts, Martin's media with streptomycin and Rose Bengal for total fungi (Martin, 1950), and one of Aaronson's select media for soil actinomycetes (Aaronson, 1970, p. 187). For each plot the relative numbers of microorganisms were reported. Subsamples were used for moisture determination, and these conversion factors were used in correcting to soil dry weight.

In addition, each soil sample was analyzed for numbers of Nitrosomonas (oxidizer of NH_4 to NO_2) and Nitrobacter (oxidizer of NO_2 to NO_3) by a modification of the most probable number (MPN) method of Alexander and Clark (1965). The modification involved porcelain spot

plates and dimethyl-alpha-naphthylamine and sulfanilic acid reagents instead of the Griess-Ilosvay reagent (Society of American Bacteriologists, 1957).

Microbial Activity

Carbon dioxide evolution as an indicator of microbial activity was determined by field cylinders and titration of the absorbing alkali (Coleman, 1973). Plastic cylinders 30 cm long with a 10 cm diameter were selectively set into the soil to a depth of 20 cm in both treatments. The slope position and degree of insolation as influenced by the amount of canopy closure were of primary concern. Widemouth jars containing 10 ml of 1M NaOH were placed in the cylinders, which were then covered with air tight plastic caps (Sinclair and Rush, St. Louis). The protruding portion of the cylinder was covered with aluminium foil to shield against heat buildup. After 24 hr, the jars were collected. In the laboratory 10 ml of 1M $BaCl_2$ were added before titrating with the appropriate concentration of HCl. Blank cylinders 10 cm long and 10 cm in diameter were set on the ground and were sealed at both ends with plastic caps. They were used to measure the ambient CO_2 concentration within the cylinder. The amount of CO_2 evolved was calculated, based on the area of the cylinder (78.5 cm^2) and the CO_2 equivalence of the acid, after correcting for the ambient CO_2. Sixteen permanent cylinders wereused in the forested area and ten in the clear-

cut plot. At each sampling, seven blanks were placed throughout the two treatments. In the event of a forecast for a drastic weather change samples were not taken because the technique is susceptible to changes in barometric pressure. On such occasions the tests were conducted as soon as weather conditions permitted.

Tannin Analysis

Several methods for the quantification of tannins (Hillis, 1962; Burns, 1963; Haslam, 1966; Ribéreau - Gayon, 1972) are available, but they are inappropriate because they fail to separate the hydrolyzable tannins from the condensed tannins. One of the earliest attempts to quantify condensed tannins involved the Stiansy Reagent (Hathway, 1962). This method is based on the formation of an insoluble complex between the acidified formaldehyde and the condensed tannin. McConnell and Longo (1971) found this technique discriminated between the two kinds of tannins.

Commercial Mimosa tannin (Tannins and Chemicals, Inc., Jersey City, N.J.), a condensed tannin, was analyzed to determine percent recovery. Determinations were run on Mimosa tannin alone and on extract amended with gallic acid, a monomer of hydrolyzable tannin. Reactions were performed under reflux conditions for 3 hr, and by autoclaving at 15 psi for 15 min. The results (Table 2) indicated a low recovery and a poor separation. This discrepancy could

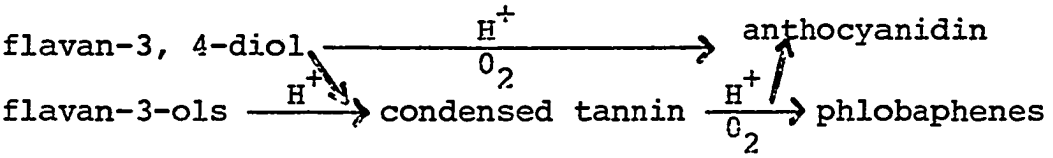
possibly be accounted for by the wide variability in condensed tannins.

The Feeny and Bostock (1968) method involves the precipitation of a tannin complex which is ultimately separated on a Sephadex column into hydrolyzable and condensed tannins. The disadvantages of the technique are: (1) loss of condensed tannin in the column (Feeny, 1969), and (2) incomplete separation of hydrolyzable from condensed tannins by the column. The latter was substantiated by qualitative tests. There were several fractions which gave a positive cyanidin chloride test (Bate-Smith and Swain, 1953) for condensed tannins and a positive nitrous acid test (Bate-Smith 1972) for hydrolyzable tannins. A spectrophotometric procedure (Feeny, 1969) was attempted but was unsuccessful because a reproducible standard curve was not obtained.

Since groups of tannins are classified according to their response to dilute acid hydrolysis (Swain, 1965), a technique was developed based on that property. Rice and Pancholy (1973) subjected soil to a mild alkali hydrolysis and recovered the condensed tannin following the Feeny-Bostock (1968) procedure. This procedures was specific for the condensed tannins because the hydrolyzable tannins were removed during dialysis. Separation of the final tannin preparation on a Sephadex column showed only the presence of condensed tannins. Similar treatment of plant material

yields favorable results (Table 2). However, the procedure requires large volumes of acetone in making the original 70:30 acetone solution after the NaOH hydrolysis. Consequently, an alternate method was developed. It represents a combination of the Feeny-Bostock (1968) method and the property of resistance to hydrolysis (Bate-Smith, 1953).

The condensed tannins are primarily composed of flavan-3-ols (catechin type) and flavan-3, 4-diols (leucoanthocyanins) according to Brown (1964). Their response to acid hydrolysis (Robinson, 1975) is as follows:



Based on the results of the tests described above I adopted the procedure described below. Five grams of milled plant material were mixed with 100 ml acetone-water solution (70:30 v/v) and homogenized in a Waring Blendor. Plant debris was removed by filtration through miracloth. The acetone layer was separated by adding an excess of NaCl. The tannin content was determined on a 25 ml acetone subsample. Twice its volume (50 ml) of 95% ethanol was added, followed by 5-volumes of anhydrous diethyl ether, precipitating a mixture of tannins and sodium chloride. The precipitate was collected (centrifugation 10 min at 2000 g) and dissolved in 5% HCl. The solution was subjected to acid hydrolysis. Two approaches were employed, acid hydrolysis in a water bath (95C) for 3 hr, and hydrolysis under 15 psi pressure for 10 min in an auto-

Table 2. Comparative efficiency of extraction methods.

Technique	Mimosa Tannin Extract % Recovery ± SE	Mimosa Tannin Extract + Gallic Acid[a] % Recovery ± SE
Feeny Spectrophotometric	No Results[b]	No Results[b]
Feeny-Bostock Method	47 ± 4	57 ± 3
NaOH Hydrolysis	96 ± 0.5	120 ± 9[c]
Stiansy Reagent (Reflux 3 hr)	40 ± 25	37 ± 19
Stiansy Reagent (Autoclave)	24 ± 12	34 ± 9
HCl Hydrolysis (3 hr 95°C)	61 ± 8	81 ± 14
HCl Hydrolysis (Autoclave)	98 ± 8	113 ± 2

a/ Expressed as % of condensed tannin

b/ Unable to obtain meaningful standard curve

c/ Difference between tannin extract and extract plus gallic acid significant at .1 level.

clave. Any precipitate was collected, dried, and weighed. In both treatments, the hydrolysate was dialysed against running water until a negative silver nitrate test was obtained. The volume of the dialysate was recorded and a 10 ml subsample was lyophilized enabling gravimetric quantification. Total condensed tannin was calculated from the appropriate dilution factors and was expressed as mg condensed tannins per gram dry weight. The procedure is summarized in Figure 4.

Autoclaving gave better yields than the slower hydrolysis (95 C for 3 hr) (Table 2). The mixture of condensed tannin and gallic acid gave a higher percent recovery than condensed tannin alone, indicating some contamination by gallic acid. However, the two results were not significantly different by the student t-test (Woolf, 1968). Furthermore, when plant material was assayed, the final product gave negative tests for hydrolyzable tannin, indicating minimal contamination.

Statistical Treatment

Appropriate statistical tests were based on procedures of Snedecor and Cochran (1967), Woolf (1968) and Sokal and Rohlf (1969). Linear and curvilinear regressions were calculated with computer programs from the University of Oklahoma Ecology Laboratory. Regression equations were derived for the analyzed variables (Table 17, 18) on a

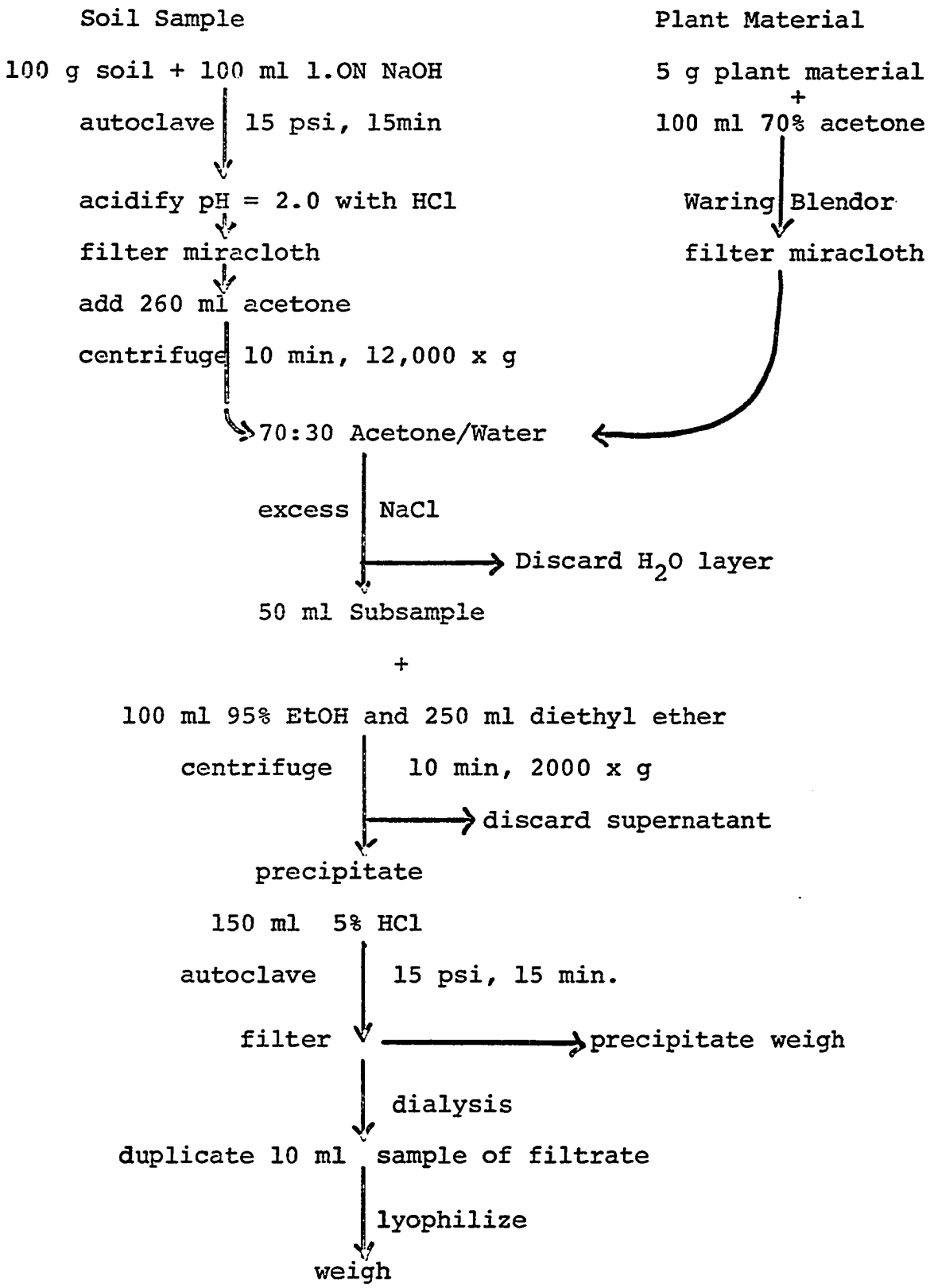


Figure 4. Flow chart for condensed tannin analysis.

yearly basis and by growing season and non-growing season. The growing season was May through November. The non-growing season consisted of the remaining months.

CHAPTER IV

RESULTS AND DISCUSSION

Study Area

The vegetation of the undisturbed forest plot was earlier described (Johnson and Risser, 1974). The composition of the woody vegetation removed during clear-cutting is summarized in Table 3. The basal area was almost identical between plots, 18.75 m^2/ha in the cleared zone vs. 18.28 m^2/ha in the forest. There was a slight variation in density, with more stems in the clear-cut area. In addition, the proportion of Q. stellata to Q. marilandica was different. Blackjack oak, Q. marilandica was the major dominant in the cleared area, and Q. stellata in the undisturbed plot. Johnson and Risser (1975) proposed that blackjack oak is more successful in disturbed areas. The number of multistem trunks observed in the clear-cut zone indicated a past disturbance. Nevertheless, the initial values of the sampled parameters were not statistically different between the two areas.

From the measured DBH, estimated biomass values for the clear-cut area were calculated (Table 4). Since no

Table 3. Analysis of all woody vegetation larger than 2.5 cm DBH in the clear-cut plot.

Species	Inner Study Area (64 m^2)		Buffer Zone (336 m^2)	
	Number of Trees	Basal Area, cm^2	Number of Trees	Basal Area, cm^2
Q. stellata	6	93	32	2052
Q. marilandica	23	1410	63	3943

Table 4. Estimated Biomass (kg/400 m^2) of the clear-cut plot calculated from regression equations (Johnson, personal communication).

	Q. stellata	Q. marilandica
Leaf	42.60	122.06
Branch	457.43	462.10
Trunk	583.88	1422.95
Root[a]	162.58--270.98	301.96--501.77

[a/] Based on data of Rodin and Bazilevich (1967). Calculated from total above-ground biomass: lower limit = 15%, upper limit = 25%.

regression equation existed for below ground biomass, the mass of roots was determined between upper and lower limits. Rodin and Bazilevich (1967) state that root biomass ranges from 15% - 25% of the total above-ground biomass.

Microclimate

Lake Thunderbird had a pronounced moderating influence on the adjacent land mass (Fig. 5), with characteristic spring and winter lags in air temperature. The mean annual temperature was 15.7 C. The daily average extremes were -9.3 C and 31.2 C. The winter months (Dec.-Feb.) averaged 4.5 C, and the summer (June-Aug.) value was 25.2 C. The growing season consisted of 224 days with the last killing frost occurring April 15 and first zero degree reading on November 25. The only extended period of daily mean temperatures below freezing was in early January and lasted 8 days. Fifty calendar days had daily averages exceeding 26 C.

The total 1974 precipitation measured 84.81 cm at the study site. Even though this amount approximated the annual normal, the distribution pattern was atypical (Fig. 6). Months receiving below average amounts were January, May, June, and July. Above normal rainfall was recorded in September and October.

The effectiveness of the artificial cheese-cloth canopy was evaluated by comparing amounts of evaporation and wind movement (Tables 5, 6), soil temperatures (Fig. 7,8),

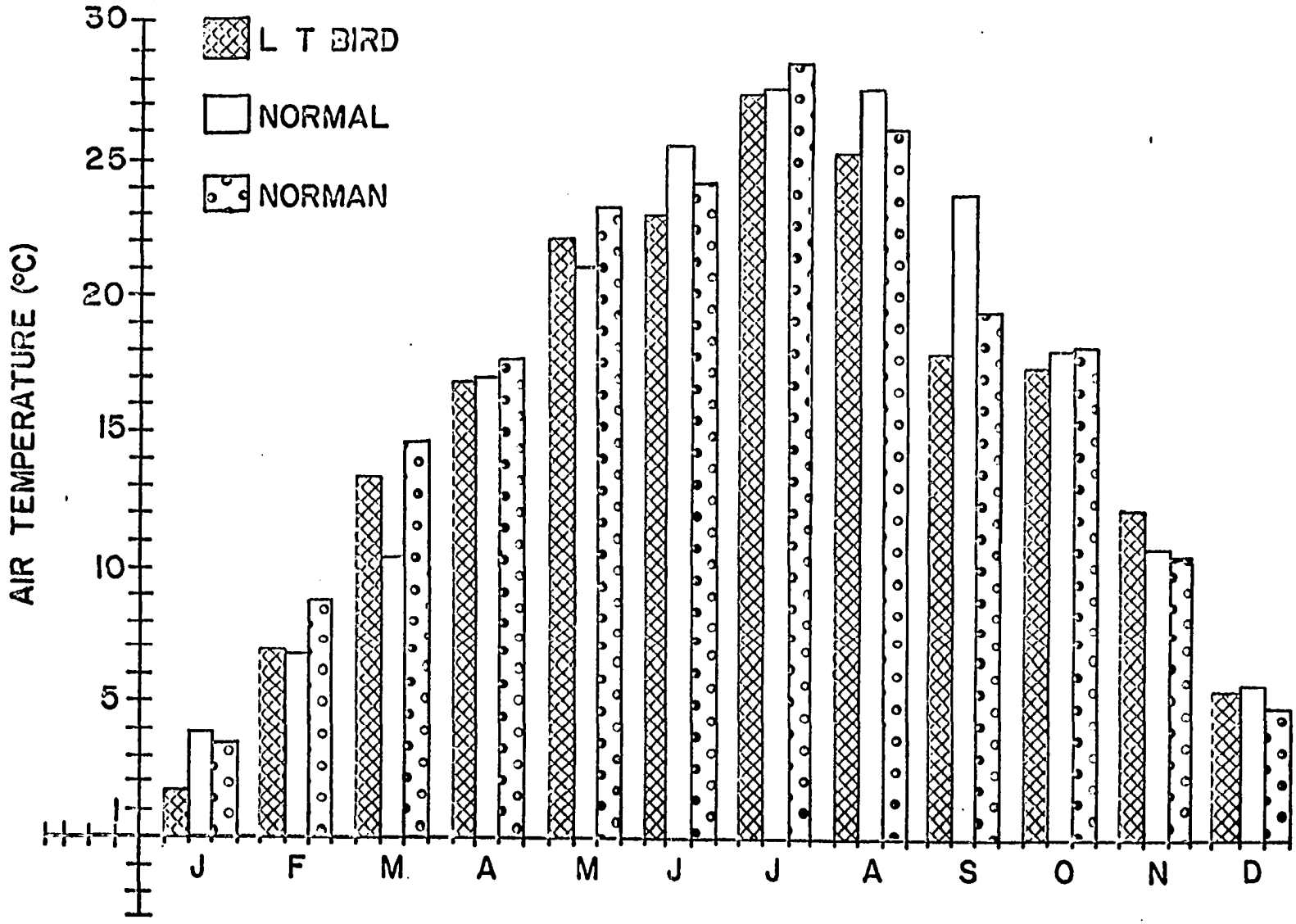


Figure 5. 1974 average monthly temperature for Lake Thunderbird Research Site, and Norman, Oklahoma plus the 30 year normal for Norman (U.S. Dept. of Commerce, 1975).

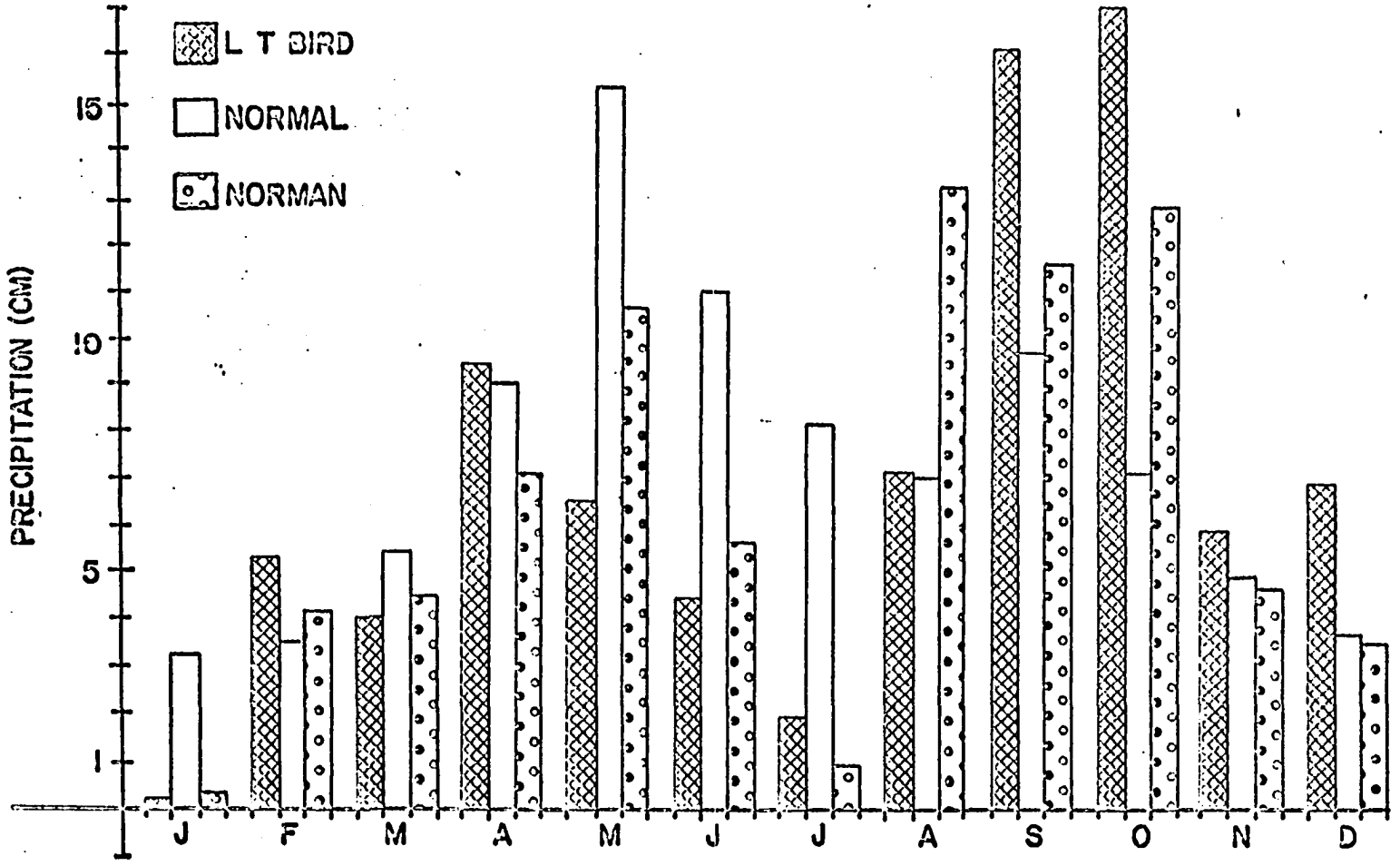


Figure 6. 1974 monthly precipitation totals for Lake Thunderbird and Norman, Oklahoma the 30 year normal for Norman, Oklahoma (U.S. Dept. of Commerce, 1975).

Table 5. Average daily evaporation (ml/day) for each month determined by the use of Livingston white, spherical, porous-cup atmometers.

Months	Savanna	Clear-cut	Forest
June	28.86	19.73	16.93
July	50.41	33.90	30.03
August	36.49	24.70	18.98
September	20.59	13.31	9.46
October	22.93	14.52	15.03
November	22.32	14.63	13.75

Table 6. Monthly wind movement (km/day).

Months	Savanna	Clear-cut	Forest
January	56.1	26.1	18.1
February	127.0	34.1	22.1
March	126.8	32.4	22.8
April	171.5	49.1	35.2
May	72.4	11.1	4.6
June	50.1	5.1	2.0
July	45.1	2.7	1.0
August	45.0	3.2	2.1
September	41.5	9.4	5.1
October	50.9	6.6	4.1
November	187.0	25.2	19.9
December	113.6	35.2	21.2

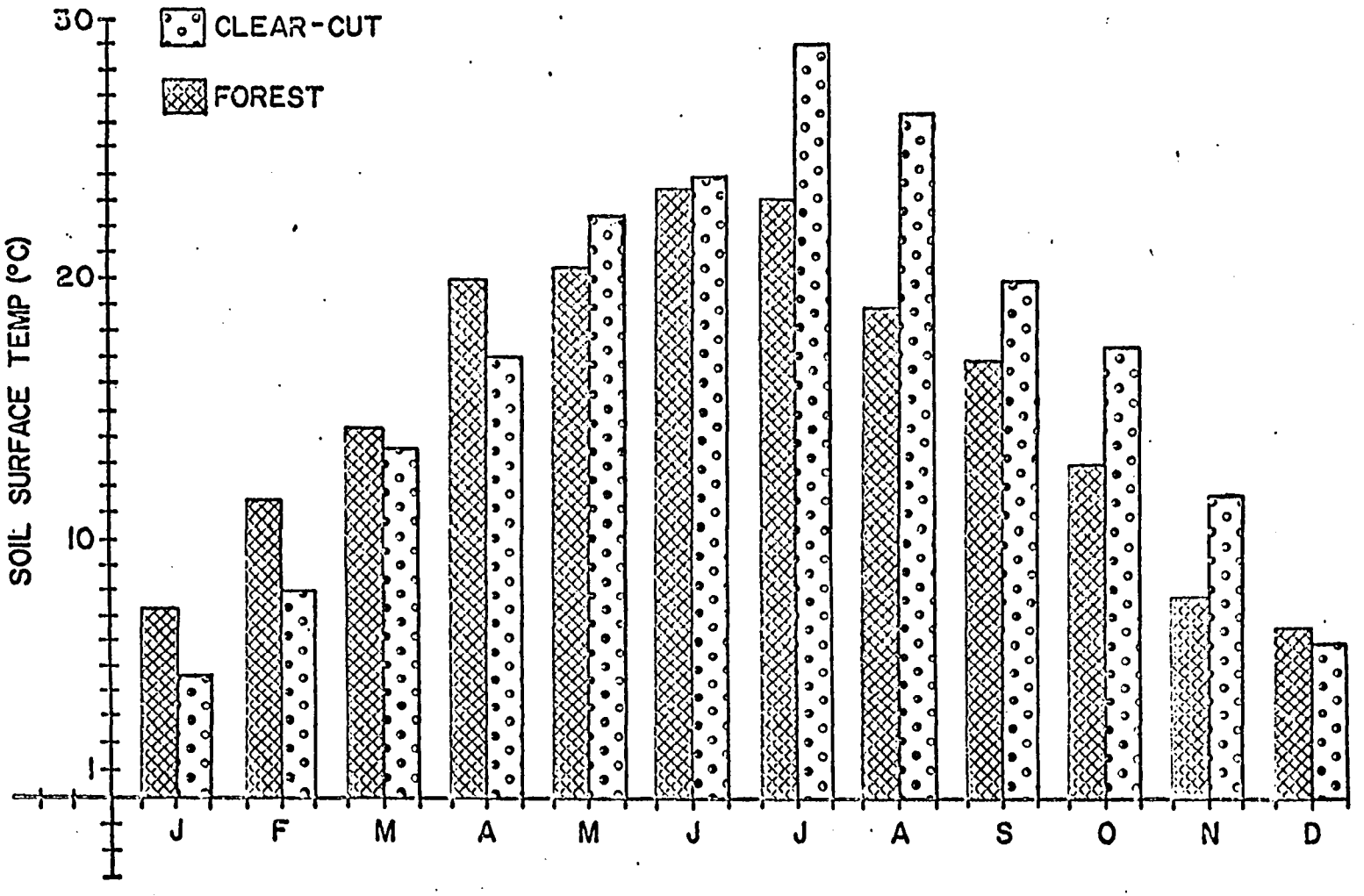


Figure 7. Monthly average of daily soil surface temperatures. Differences for April, May, June, July and August were significant at the .05 level or better.

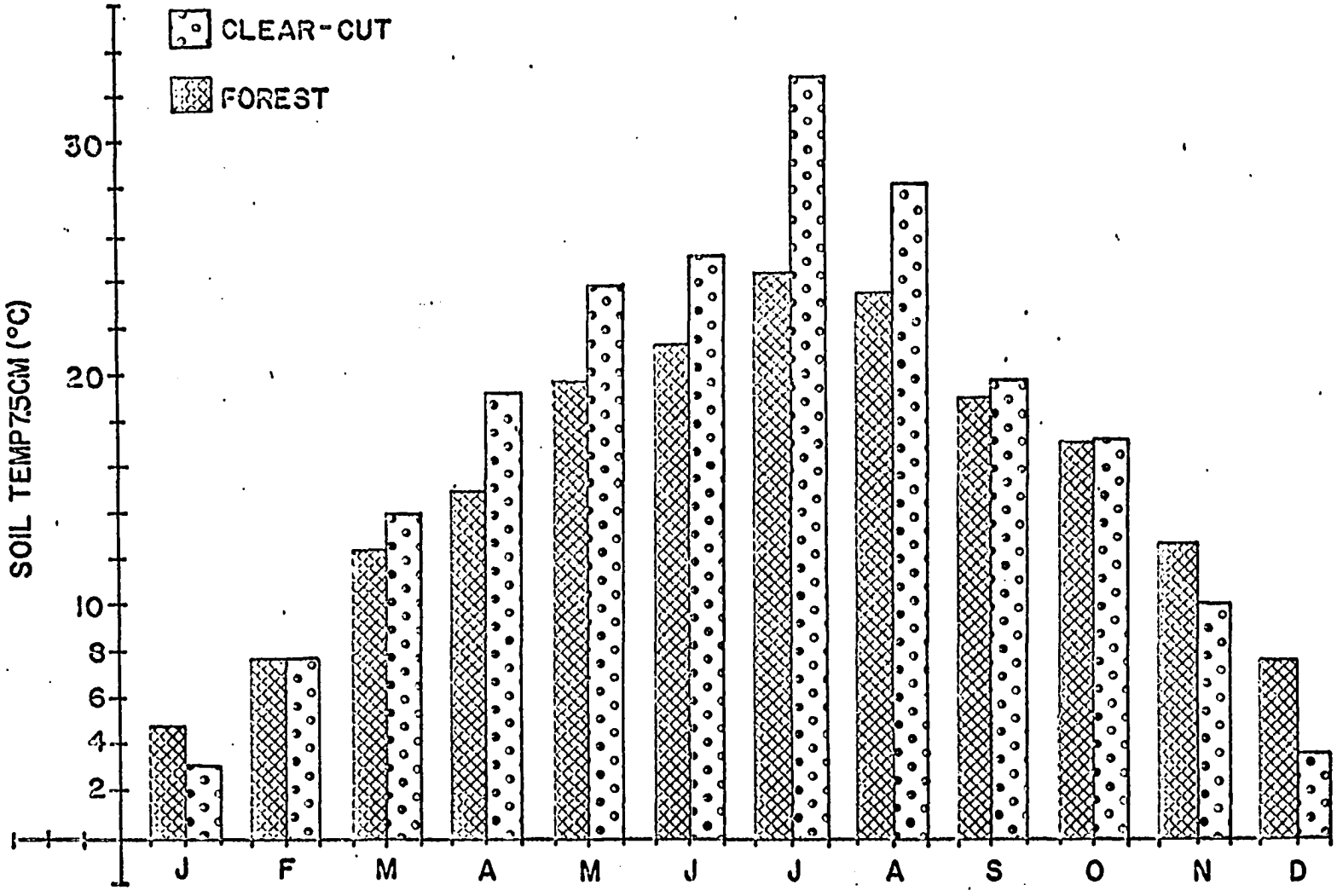


Figure 8. Monthly average of daily soil temperatures at a 7.5 cm depth. All differences were significant at the .05 level or better except for February, September, and October).

and insolation (Table 7). The values for evaporation and wind movement were intemediate in the cleared area but were more similar to the forest. The highest values were recorded in the open savanna. The amount of solar energy absorbed by the soil can be influenced by soil color, the slope, and the vegetation (Buckman and Brady, 1960; Johnson, Bell & Sipp, 1975). Differences in evaporation between black and white Livingston atmometers give a measure of absorbed radiation. Insolation in the clear-cut area was similar to that in the savanna (Table 7). This relationship was also evident in the soil temperatures (Fig. 7,8). The surface temperature of the cleared area was lower in the winter and higher in the summer. The daily average ranged from -4.8 to 36.4 C. Daily temperatures were characterized by large fluctuations. Temperature changed as much as 22 degrees on a given date in January. Thirty degree fluctuations were daily occurrences during the summer. This phenomenon was due to the lack of a litter layer. Due to the insulating effect of the litter, the forest soil approached but never reached a freezing daily average temperature during the winter. Daily fluctuations were less than five degrees, even in the summer. Soil temperatures at the 7.5 cm depth behaved similarly in pattern and fluctuation (Fig. 8). I concluded that the two sampling sites exhibited different microclimates. Therefore, these differences had to be considered in explaining changes in microbial population.

Table 7. Insolation measured by differences between evaporation rates from black and white Livingston atmometers.

Months	Savanna	Clear-Cut	Forest
June	8.11	7.42	0.23
July	10.75	6.65	3.99
August	11.90	3.90	0.98
September	8.85	1.25	2.00
October	7.82	4.16	5.84
November	4.97	3.13	1.33

Soil

The soil was characterized by a particle distribution of 81% sand, 11% clay, and 8% silt at the 0-15 cm depth. The bulk density was 1.06 g per cm^3. Percent soil moisture was 16.2 at field capacity and 3.1 at the permanent wilting point. The initial soil reaction was 5.9. At the conclusion of the field sampling the bulk density was 0.93 g/cm^3 in the clear-cut area, possibly due to erosion, and the soil pH was 4.9. The undisturbed forest did not show any significant change.

Soil moisture values in the forest were high until a steady decline was initiated in mid-May (Fig. 9). The soil moisture remained low throughout July and fluctuated in August, September, and October before regaining high values for the remainder of the year. The same pattern occurred in the clear-cut area. Soil water content is biologically more meaningful when expressed in terms of field capacity and the permanent wilting point. Soil moisture was at 100% of field capacity for the major portion of the year (Fig. 10). Three divergent periods were recorded in June and July, September, and October, but the soil moisture percentage never reached the permanent wilting percent in either plot.

Differences in amounts of soil organic matter between the two treatments were statistically significant only in April and May (Table 8). However, the cleared area had

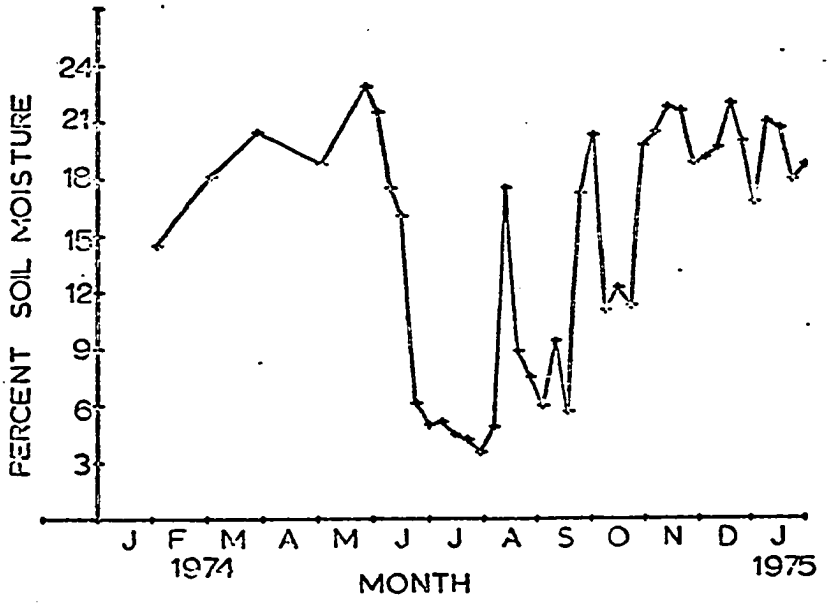


Figure 9. Soil water determinations for the undisturbed forest.

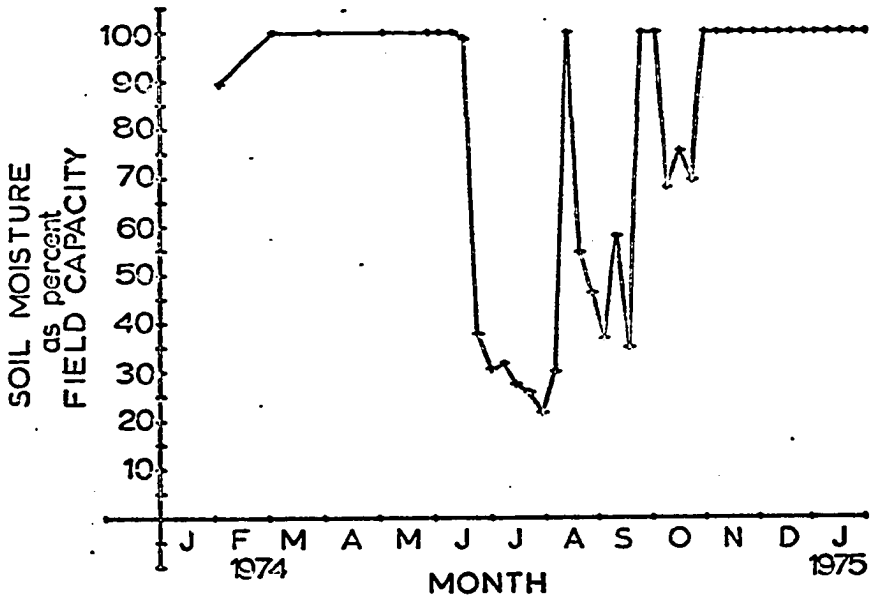


Figure 10. Soil moisture expressed as percent of field capacity for the undisturbed forest

Table 8. Monthly soil organic matter reported as Walkley and Black values (mean ± SE).

Date	Clear-cut	Forest
1/31/74	0.52 ± .05	0.44 ± .06
3/03/74	0.59 ± .04	0.53 ± .06
3/28/74	0.55 ± .10	0.41 ± .07
4/30/74	0.68 ± .05	0.39 ± .07[a]
6/01/74	0.44 ± .04	0.61 ± .05[a]
6/29/74	0.52 ± .08	0.38 ± .04
7/27/74	0.50 ± .03	0.49 ± .06
8/31/74	0.56 ± .05	0.48 ± .07
9/28/74	0.49 ± .04	0.37 ± .06
10/05/74	0.48 ± .06	0.36 ± .05
11/30/74	0.60 ± .11	0.43 ± .11
12/28/74	0.47 ± .05	0.38 ± .04

[a/] Significantly different at the .05 level or better

consistently higher monthly values. The differences between monthly values for the year were statistically significant using the paired-t test.

The amount of ammonium-nitrogen for the two treatments was variable but generally high (Table 9) (Russell and Russell, 1961; Rice and Pancholy, 1972). Both plots, however, had very little ammonium-nitrogen in April and December. Ammonification is responsible for most of the ammonium-nitrogen, and the ammonifying microbes are a complex population of bacteria and fungi (Harmsen and Kolenbrander, 1965).

Nitrate level in the forest soil were below the reproducible, detectable amount of 2.5 ppm with the nitrate electrode, except for September and November. The clear-cut area had values ranging from 2.7-30 ppm. There was a steady increase in nitrate-nitrogen from May through August followed by a decrease in September and October. This loss in nitrate-nitrogen was probably the result of considerable leaching accompanying above normal precipitation. No statistical treatment was performed because of the undetectable amounts in one plot or the other during most months. It is obvious, of course, that concentrations in the clear-cut area were significantly higher from May through October.

Quantities of condensed tannins were calculated on the basis of mg/g of oven-dry soil and in kg/ha in the different soil levels (Table 10). The tannin concentration

Table 9. Amount of soil inorganic nitrogen in parts per million (ppm).

Month	Forest Site		Clear-cut Site	
	Ammonium-Nitrogen	Nitrate-Nitrogen[a]	Ammonium-Nitrogen	Nitrate-Nitrogen
Jan 15	3.9	NR[b]	4.9	2.5
Jan 30	3.5	NR	4.9	NR
Feb	2.5	NR	1.8	NR
Mar	7.0	NR	5.3	NR
Apr	1.8	NR	0.0	NR
May	6.9	NR	6.1	5.0
June	6.9	NR	7.5	4.9
July	3.5	NR	4.2	8.0
Aug	2.1	NR	3.9	30.0
Sept	3.1	4.5	8.4	15.0
Oct	4.6	NR	5.3	4.8
Nov	6.9	7.5	6.9	NR
Dec	0.7	NR	0	NR

a/ Lower limit of reproducibility was 2.5 ppm

b/ NR = Sample below the 2.5 ppm limit

Table 10. Amounts of condensed tannins in the forest soil.

Level in Soil (cm)	January	February	March	April	May	June
			mg/gm			
0-15	2.02	2.01	4.08	4.37	2.47	1.45
15-30	0.14	0.07	0.02	1.72	2.77	0.61
30-60	0.05	0.06	1.48	0.02	0.02	1.56
			kg/ha			
0-15	2834	2822	5717	6129	3471	2175

	July	August	Sept	October	Nov	Dec
			mg/gm			
0-15	4.31	4.00	4.19	4.32	3.14	3.32
15-30	0.29	0.15	3.08	1.06	1.53	0.55
30-60	0.61	0.06	0.03	1.06	0.04	0.13
			kg/ha			
0-15	6049	4205	5883	6060	4411	4662

in the 0-15 cm level of the forest increased in early spring, decreased in May and June, and increased through the early fall. The condensed tannin concentration decreased with depth, but the design of the experiment did not enable me to determine if movement of condensed tannins to the lower levels occurred. The tannin contents of the different levels were generally positively correlated, but most differences were not statistically significant (Table 11). A radioisotope procedure would be required to elucidate the movement of condensed tannins from one level to the next. Rice and Pancholy (1973) stated that the tannins are probably held by chemical union with peptide linkages in the proteins of the humus and other organic matter in a manner analogous to that in the tanning of leather. Therefore, the tannins would not be expected to move downward until all the possible linkage sites were saturated in a given layer of the soil.

The sampling of the cleared area was restricted to the 0-15 cm level because the microbes are concentrated there (Eicker, 1970; Ardakani et al., 1974). The pattern was essentially identical to the forest zone except that higher values occurred in the early months and again in late fall (Table 12). Tannin concentrations were significantly higher statistically in the clear cut area in January, February, April, and July. This was probably due to a release of condensed tannins from the roots after the clear-

Table 11. Correlation coefficients for linear regressions between the condensed tannins at different soil levels in the undisturbed forest.

	0-15 cm vs. 15-30 cm	15-30 cm vs. 30-60 cm
Whole Year Data Set	0.020	0.002
Growing Season	0.342	-0.779[a]
Non-Growing Season	0.364	0.407
Whole Year (Lag[b])	0.387	0.472
Growing Season (Lag)	0.000	0.814[a]
Non-Growing Season (Lag)	0.973	0.263

[a/] Significant correlation at 0.05 level or better

[b/] Lag means the lower soil level was 1 month out-of phase.

Table 12. Amounts of condensed tannins in the clear-cut soil (0-15 cm level).

Months	mg/gm	kg/ha
January	5.31	8444
February	3.80	6050
March	3.76	5977
April	5.99	9521
May	1.54	2448
June	1.29	2050
July	4.39	6977
August	4.60	7308
September	5.24	8330
October	4.08	6481
November	4.59	7307
December	3.68	5858

cutting, because the design of the area excluded all other forms of tannin input.

One of the objectives of this research was to determine the rate of decomposition of condensed tannin in the soil. The clear-cut area was to offer such a natural condition. From the data (Table 10, 12) it becomes evident that all inputs were not successfully excluded. The pre-existing roots and their high tannin content were presumed responsible for the measured increases. These increases made it impossible to determine the amount of soil tannin decomposed.

Plant Material

The condensed tannin in leaves was determined from green and senescent material. The June (6/14) samples of Q. stellata contained 71.03 ± 6.64 mg/g and Q. marilandica contained 43.33 ± 2.02 mg/g. The condensed tannin content in early November (11/02) for the respective species was 73.62 ± 9.88 mg/g and 48.48 ± 5.32 mg/g. These data are inconsistent with those obtained by Feeny (1970) on Q. robur where the condensed tannin concentration increased throughout the growing season, more than doubling in amount. However, Dement and Mooney (1974) found relatively uniform concentrations of condensed tannins throughout the year in the leaves of the shrub Heteromeles arbutifolia.

Catkins and budscales were analyzed for condensed tannin and both had appreciable amounts. The respective quantities were 77.08 ± 6.02 mg/g and 77.20 ± 2.72 mg/g.

Root biomass was determined for the different soil levels. Since the root material from each soil core was composited and quantified, the averages for the monthly values were calculated. The results were: 0-15 cm, 16,864 ± 2,276 kg/ha; 15-30 cm, 6,503 ± 1,358 kg/ha; 30-60 cm, 16,488 ± 3,968 kg/ha. The total biomass 39,855 ± 7,602 kg/ha compares favorably with the 39,000 kg/ha estimate of Johnson and Risser (1974). The distribution of roots in the soil is of interest with 42% in the top 15 cm, and only 16% in the 15-30 cm level. This distribution is significant in relation to soil respiration.

The condensed tannin content was relatively constant in the combined root material in the early months of the year, but it increased in May and June (Fig. 11). A decrease was noted after June and continued through October. This reduction in condensed tannin could be the result of root exudation. An increase was again detected in November and December. Roots of post oak and blackjack oak were collected separately in October, 1973 and analyzed for condensed tannin content. The roots of blackjack oak had a high concentration (64.58 ± 2.54 mg/g dry wt) than post oak (49.80 ± 12.29 mg/g dry wt), but these concentrations were not significantly different by the Student's-t test.

Throughfall, Stemflow, and Interception

This portion of the study suffered from lack of proper instrumentation. In analyzing the data, it was

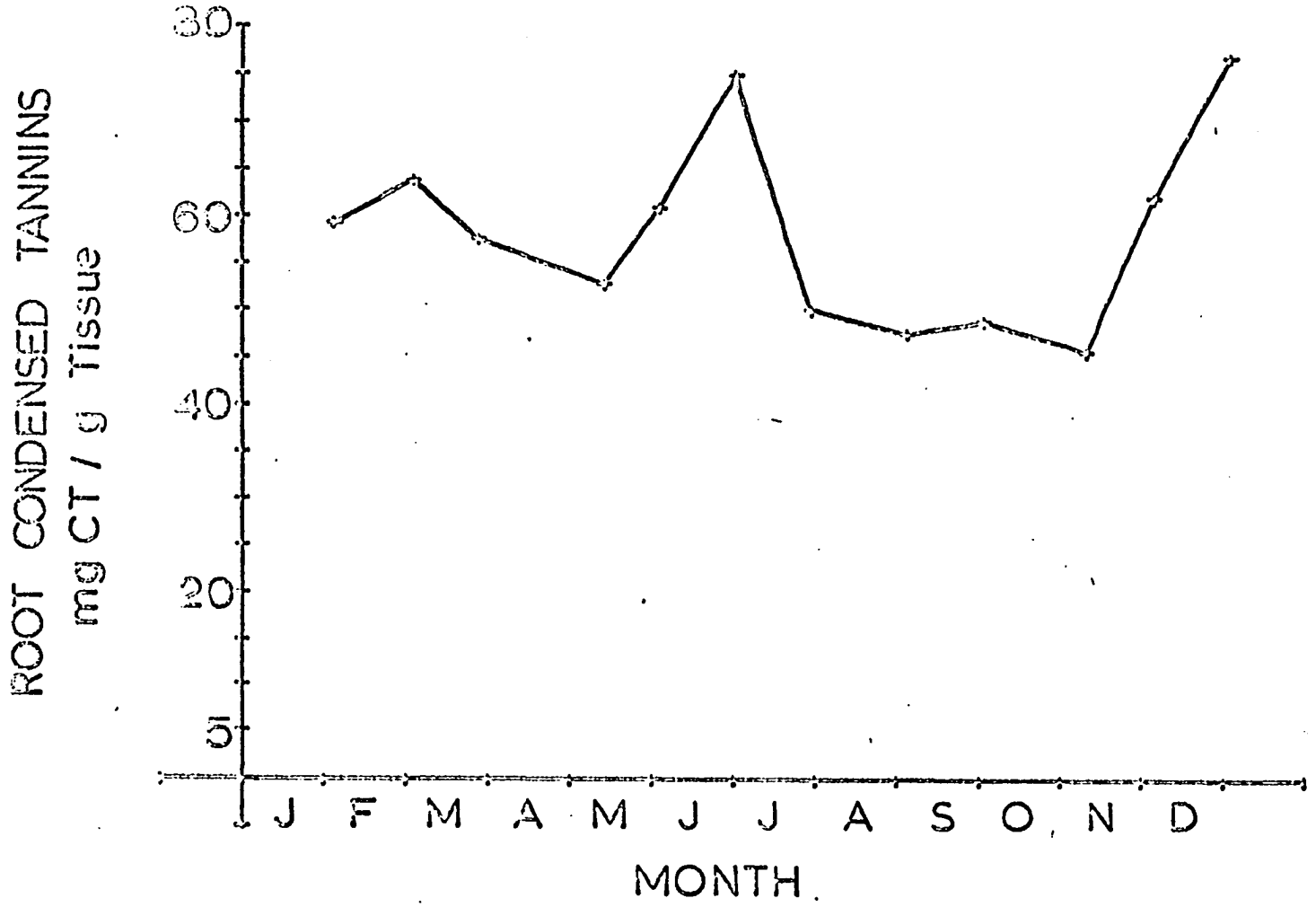


Figure 11. Concentration of condensed tannin in root material (mg condensed tannin per gram dry weight of tissue).

apparent that individual storm intensities were required. There was no significant correlation between weekly precipitation and the amount of stemflow, throughfall, and interception for the individual species. However, the monthly percentages by species (Tables 13,14) provided some meaningful data (Table 15). Loss of intercepted rainfall in forest ranges from 10-35% of the annual precipitation (Swank et al., 1972). White and Carlisle (1968) reported that stemflow was not very great if compared with total rainfall. Their annual allocation to stemflow was only 2.1% for an oak forest. The effect of defoliation on the amount of throughfall is surprisingly minimal (Mitchell, 1930; Rutter et al., 1975), ranging from 3.6-8.8% increase. The data collected for Q. stellata and Q. marilandica were consistent with the cited observation.

The ferric-chloride tests for tannins on collected samples of throughfall and stemflow were all negative. As a precaution, nevertheless, the monthly samples were analyzed for condensed tannins and none was found. It was concluded that even though condensed tannins are water soluble the large polymer is retained by the plant material and not leached by precipitation.

Litterfall

The annual composition of litterfall for the forest stand was 52.6% leaves, 18.2% woody material, 23.7% acorns,

Table 13. Distribution of gross precipitation for blackjack oak (mean % ± SE).

Months	Stemflow	Throughfall	Interception
January	6.14 ± 2.1	85.69 ± 4.9	8.19 ± 7.0
February	8.73 ± 4.0	87.55 ± 4.7	3.72 ± 8.7
March	10.46 ± 5.1	78.33 ± 5.5	11.21 ± 10.6
April	17.01 ± 9.2	82.46 ± 6.1	0.53 ± 15.3
May	1.68 ± 0.9	86.98 ± 6.0	11.34 ± 6.9
June	0.56 ± 0.5	83.53 ± 9.7	15.91 ± 10.2
July	0.0	76.43 ± 10.4	23.57 ± 10.4
August	1.62 ± 0.9	77.13 ± 9.5	21.25 ± 10.1
September	1.24 ± 0.7	74.85 ± 11.8	23.91 ± 12.5
October	1.14 ± 0.7	95.67 ± 8.6	3.19 ± 9.5
November	0.5 ± 0.3	93.43 ± 0.9	6.07 ± 1.2
December	2.44 ± 1.4	82.05 ± 4.0	15.5 ± 5.4

Table 14. Distribution of gross precipitation for post oak (mean % ± SE).

Months	Stemflow	Throughfall	Interception
January	3.20 ± 1.8	83.42 ± 5.4	13.38 ± 7.2
February	4.31 ± 2.0	82.95 ± 5.8	12.74 ± 6.0
March	4.62 ± 1.5	80.90 ± 1.4	14.58 ± 3.0
April	4.33 ± 0.3	89.60 ± 2.3	6.07 ± 2.6
May	0.54 ± 0.2	76.60 ± 6.1	22.86 ± 6.3
June	0.41 ± 0.2	80.38 ± 4.6	19.21 ± 4.8
July	0.0	79.24 ± 4.6	20.76 ± 7.2
August	0.65 ± 0.2	73.45 ± 6.9	25.90 ± 7.1
September	0.48 ± 0.2	66.15 ± 7.2	33.37 ± 7.4
October	0.47 ± 0.2	93.40 ± 10.6	6.13 ± 10.8
November	1.77 ± 1.4	82.21 ± 7.3	16.02 ± 8.7
December	2.55 ± 1.3	82.33 ± 6.1	15.12 ± 7.4

Table 15. Correlation coefficients for the linear regressions between the monthly distribution of gross rainfall for each oak species (df = 10, critical value = .576 at .05 level and .708 at .01).

	Postoak Throughfall	Postoak Stemflow	Postoak Interception	Blackjack Throughfall	Blackjack Stemflow	Blackjack Interception
Precipitation	.234	-.501	-.192	.337	-.428	-.232
POT		.435	-.998	.900	.354	-.990
POS			-.491	.315	.865	-.468
POI				-.967	-.403	.990
BJT					.201	-.981
BJS						-.389

3.9% catkins, and 1.7% budscales (Table 16). Many leaves of both oaks remain attached and continue to fall throughout the winter. Since the tree species surrounding each trap influence the species composition in that trap, the monthly leaf fall data were expressed as a percent of the yearly amount by species on a per trap basis. These monthly percents were statistically tested according to oak species. There was no significant difference between oaks regarding leaf fall and retention. Forty-eight percent of the leaf fall occurred in October. Johnson and Risser (1974) reported a secondary peak in April, but my data did not show any substantial increase in the spring (Fig. 12). The maximum input was 44 kg/ha/day and less than 1 kg/ha/day through the early summer.

Decomposition

The decomposition process can be approached either as the rate of loss according to environmental influences, or as the successional sequence of specific microbes and their biological action. This study was concerned with the rate of decomposition regardless of which organisms were responsible. The losses of organic matter, and of condensed tannins from leaves were analyzed.

The rate of weight loss of leaves was not significantly different in the two oak species in either the confined technique or the tethered procedure (Fig. 13). The relative

Table 16. Litterfall (kg/ha).

Month	Leaves	Branches & Bark	Acorns	Catkins	Bud Scales
January	206.4	92.0	7.2		
February	258.0	56.0	5.5		
March	176.6	16.9	0.2		
April	81.4	81.6	0.0	231.2	98.6
May	11.0	532.0	0.0		
June	15.6	16.0	3.6		
July	15.4	8.20	29.0		
August	76.0	23.9	108.4		
September	100.0	48.6	364.4		
October	1491.4	112.9	746.1		
November	406.6	55.6	128.0		
December	279.4	37.2	12.0		
TOTAL	3117.8	1080.9	1404.4	231.2	98.6

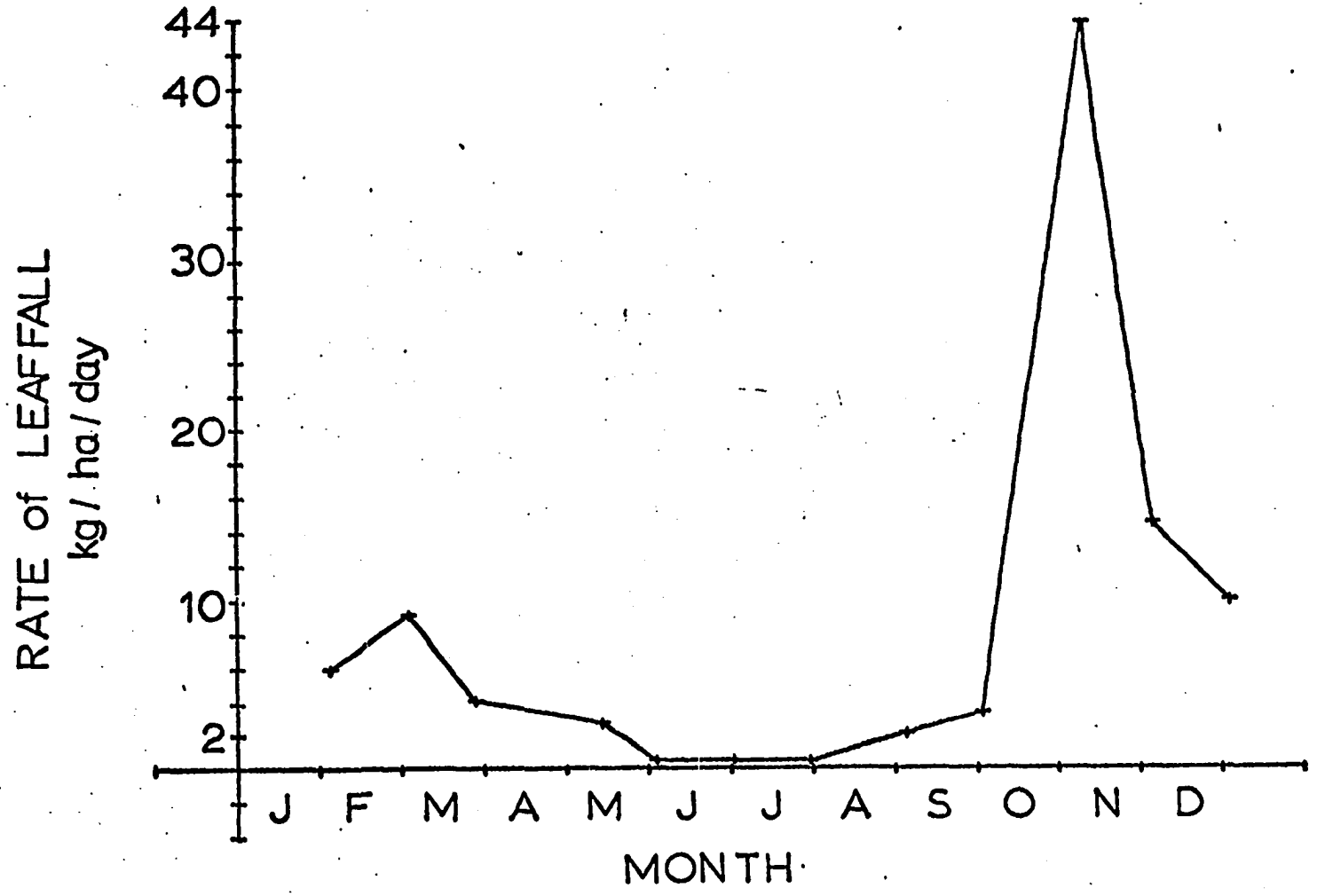


Figure 12. Leaffall rates for combined Q. stellata and Q. marilandica.

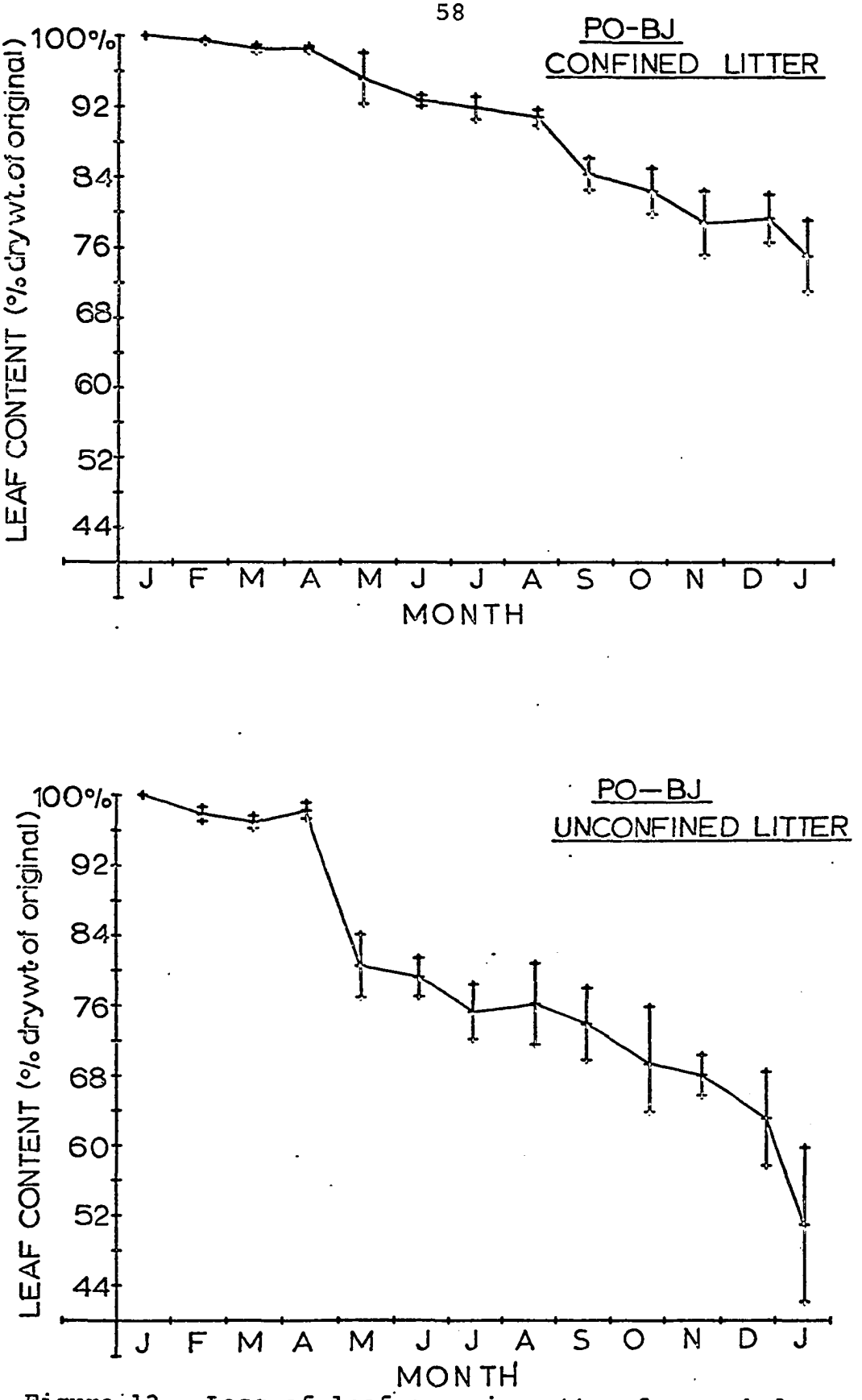


Figure 13. Loss of leaf organic matter from oak leaves using two different techniques. Vertical lines represent 1 SE.

merits of these techniques were reviewed by Witkamp and Olson (1963). The mesh size of the decomposition bags had no significant effect, and the reported results are a composite. However, the 3 mm mesh bags had slightly higher decomposition rates in the last two months. Curry (1969a, 1969b) had reported substantial differences for grassland herbage according to mesh size. The larger mesh allowed arthropods and other small invertebrates access to the leaf litter, increasing the rate of disappearance. The discrepancy could be due in part to the different tannin contents of the herbaceous plant material and the oak leaves. Rice and Pancholy (1972) reported higher tannin values for tree leaves than for the shoots of the herbaceous plants. After one year in the field, the average loss for the unconfined leaves was 49%, whereas it was 24% for the bags. Since the mesh-bag technique is an underestimation and the unconfined method overestimates because of excessive fragmentation, the true decomposition rate lies between these two limits. The highest decomposition rates occurred during April, August, October, and December. The minimal loss from May through July could be attributed to a decreasing soil moisture. Excessive soil moisture could account for the low November value.

The annual losses of condensed tannins from leaves were different for the oak species because of the difference in the original amounts present, but the general pattern was

similar (Fig. 14,15). Q. stellata, with a higher initial content, lost 67% of the original condensed tannins and Q. marilandica lost 78%. There were no significant differences in monthly rates between species. The pattern was characterized by a rapid initial loss, March through June, followed by a steady decline. The data were based on a combination of the two mesh sizes because there was no significant difference in rates of loss. The amount of the original condensed tannin remaining after one year ranged between 11 and 17%, based on the amount of organic matter lost and the decomposition rate of the tannins.

Heterotrophic Microbes

The techniques employed for the quantification of microbial populations have been re-evaluated in recent years. The use of dilution-plate counts has been critized because organisms respond differently to a medium so that counts vary depending on the medium used, and the kinds of organisms which grow vary with the medium used (Parkinson, 1973). Even though these conventional techniques may not be considered absolute, the general trends may be objectively compared (Mishustin, 1975). Furthermore, Sparrow and Doxtader (1973) did numerous laboratory studies which indicated high correlation coefficients between numbers of viable bacteria, actinomycetes, and fungi, as indicated by dilution plate counts and ATP levels.

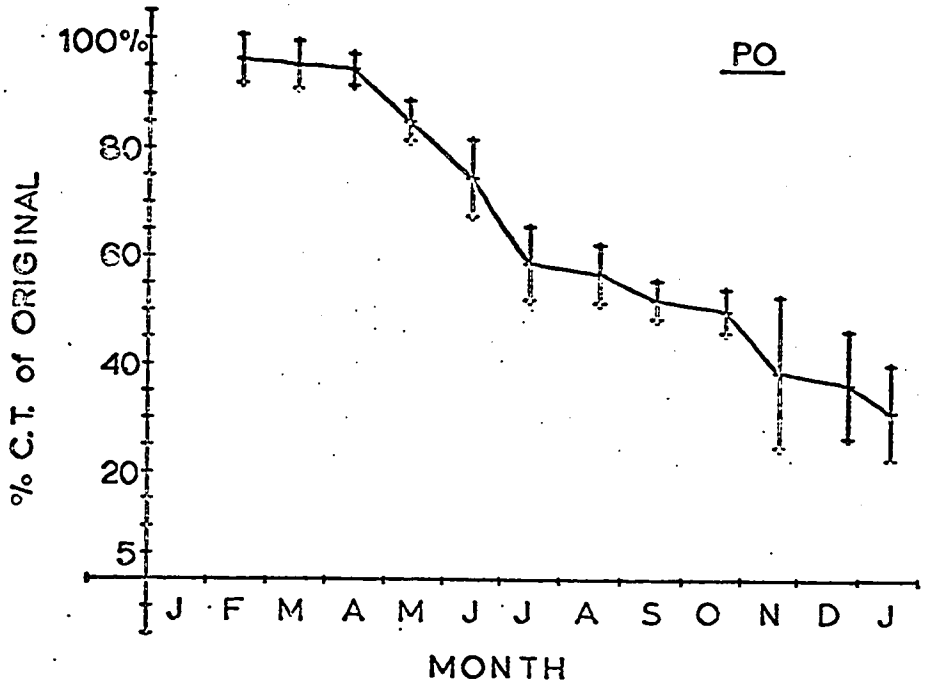


Figure 14. Loss of condensed tannin from post-oak leaves during decomposition. Vertical lines represent 1 SE.

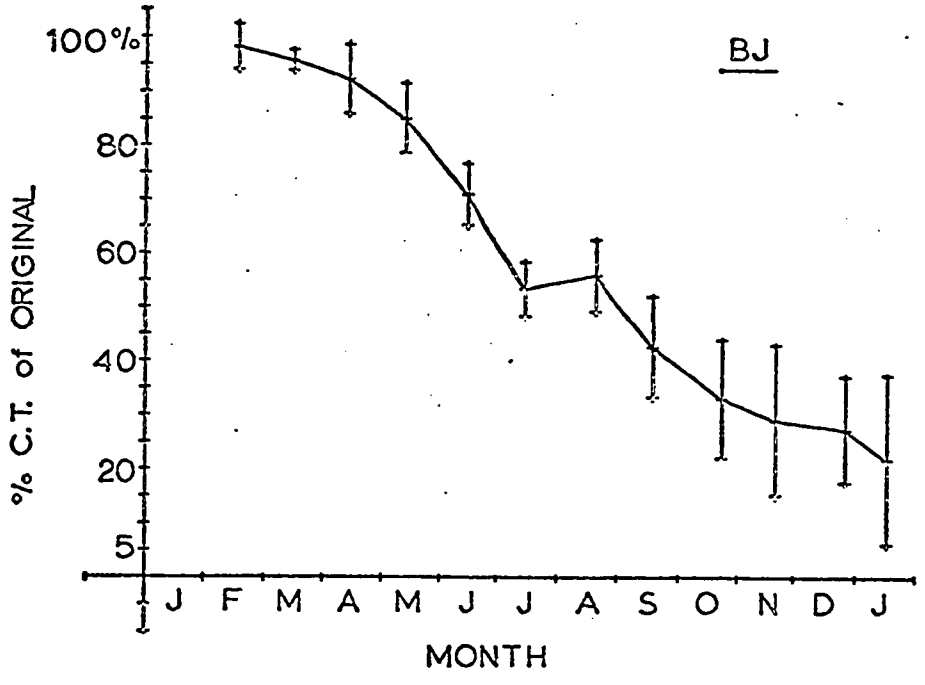


Figure 15. Loss of condensed tannin from blackjack oak leaves during decomposition. Vertical lines represent 1 SE.

The heterotrophic microbes of each treatment were quantified (Fig. 16, 17, 18). The bacterial counts were not significantly different between the two plots, and after March, were almost identical. The initial difference may have been due to the high tannin content of the clear-cut area, since tannins are inhibitory to bacteria (Kramer and Doetsch, 1950; Lewis and Starkey, 1969) (Table 17,18). The fungal population fluctuated during the year and was not correlated with the condensed tannins in the soil. Soil moisture and soil temperature were thus apparently more important in regulating the population than the amount of condensed tannin. This is not surprising, since several workers (Knudson, 1913; Lewis and Starkey, 1969) showed that the deuteromycetes are the primary tannin decomposers which indicates that these organisms are not inhibited by condensed tannins. The responses of the actinomycetes to both treatments were similar (Fig. 18). Peak population densities occurred in the summer. The low numbers corresponded to times of high soil tannin content, and there was a negative correlation (Table 17,18). However, other factors were apparently of equal importance. Goodfellow and Cross (1974) stressed the limited knowledge available regarding this group.

Microbial Activity

Carbon dioxide evolution can be used as a measure of microbial activity (Witkamp, 1963, 1966; Witkamp and

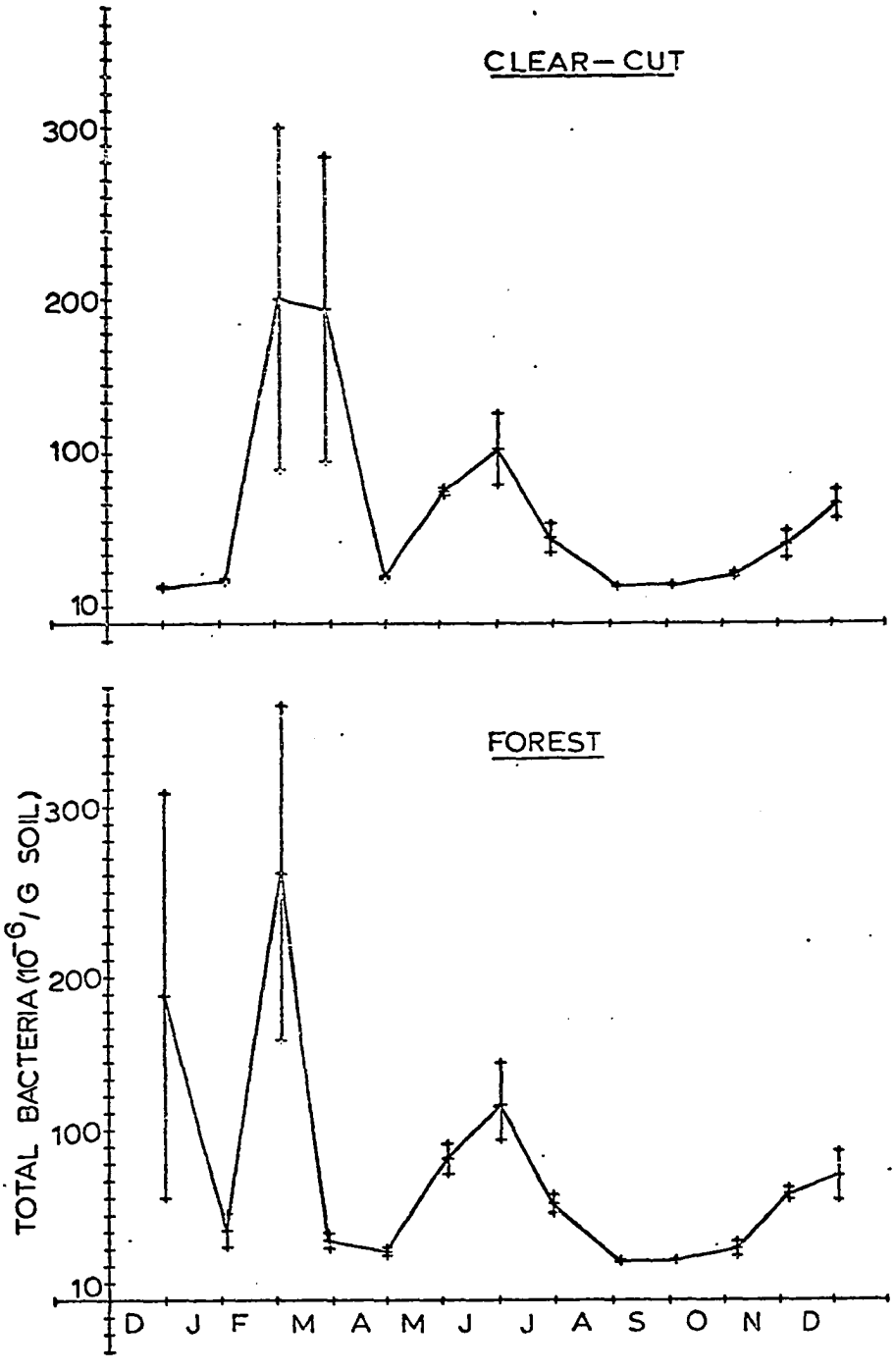


Figure 16. Numbers of total bacteria ($\bar{X} \pm SE$) for each treatment.

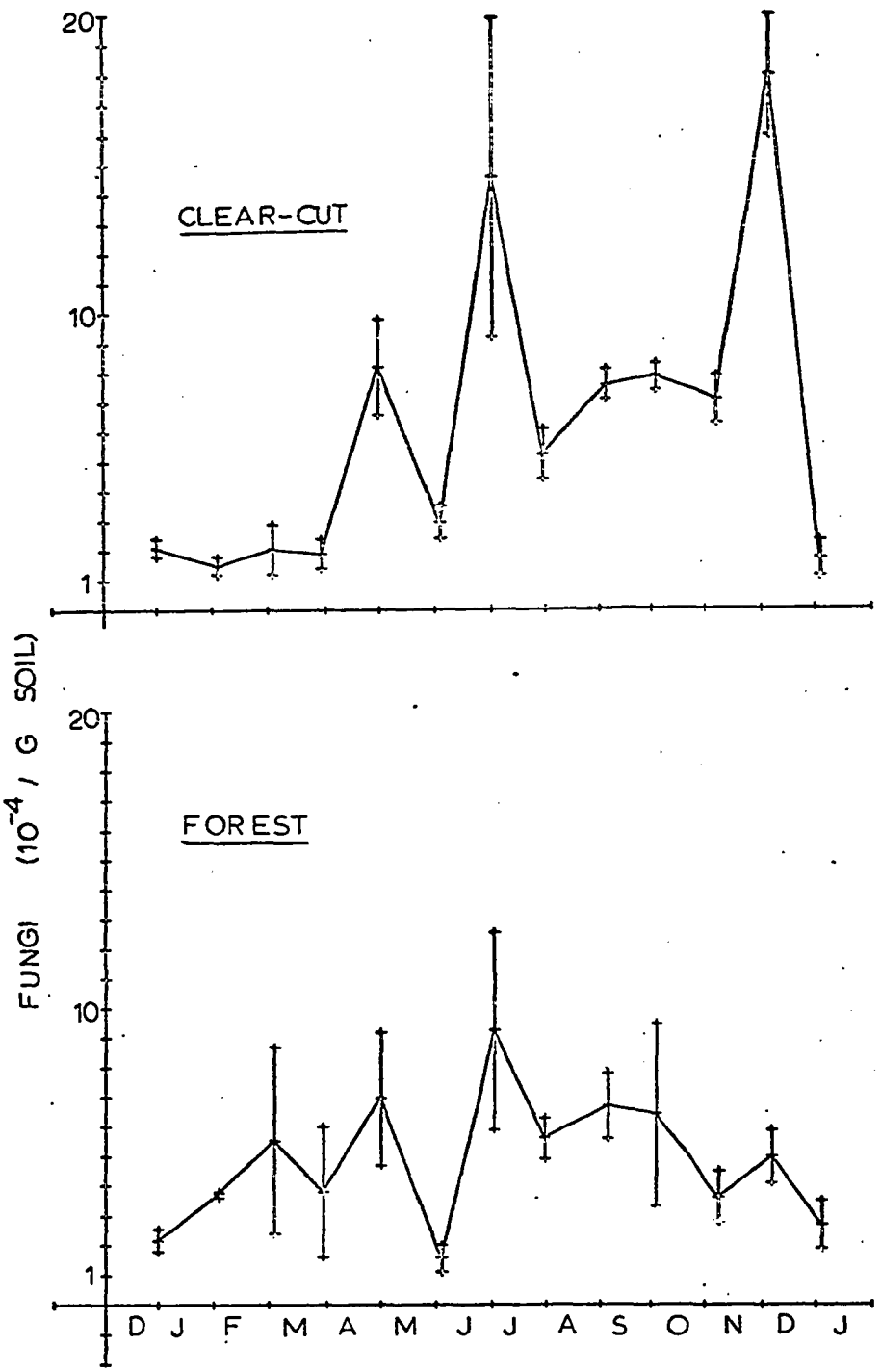


Figure 17. Numbers of fungi ($\bar{X} \pm$ SE) for each treatment.

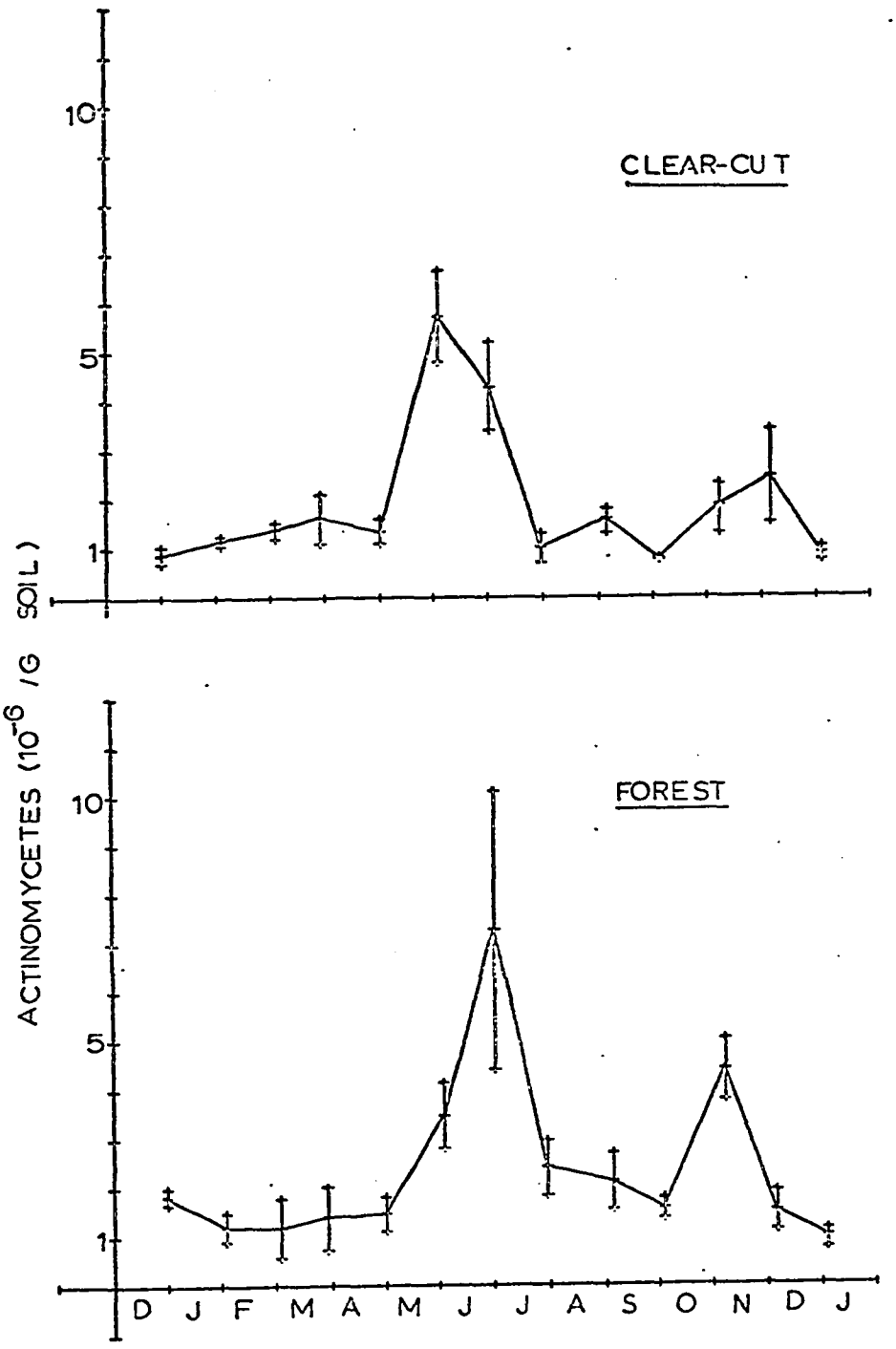


Figure 18. Numbers of actinomycetes ($\bar{X} \pm SE$) for each treatment.

Table 17. Correlation coefficients for linear regressions between microbes and selected environmental parameters of the forest. Critical values at the .05 level are .576 for the whole year, .754 for the growing season, and .878 for the non-growing season. Symbols are : $\bar{X}$ = mean, SM = % soil moisture, PWP = permanent wilting percent.

		Condensed Tannin 0-15 cm	% Soil Moisture	% Field Capacity	Ratio SM/PWP	Organic Matter	$\bar{X}$ Date Soil Temp Surface	High Surface Temp for Date	Low Surface Temp for Date	$\bar{X}$ Date Soil Temp 7.5 cm	7.5 cm High Temp	7.5 cm Low Temp	$\bar{X}$ 3.75 cm Depth Temp	Surface Temp 3 wk $\bar{X}$	Surface Temp 2 wk $\bar{X}$	Surface Temp 1 wk $\bar{X}$	7.5 cm Temp 3 wk $\bar{X}$	7.5 cm Temp 2 wk $\bar{X}$	7.5 cm Temp 1 wk $\bar{X}$
Nitrosomonas	Year	-.268	-.444	-.445	-.447	.157	.417	.359	.453	.428	.395	.461	.428	.399	.403	.395	.423	.416	.405
	Grow	-.357	-.402	-.383	-.401	.220	.430	.378	.493	.474	.417	.533	.463	.476	.475	.402	.525	.515	.368
	Non-Grow	.260	-.617	-.190	-.680	-.652	-.790	-.826	-.459	-.852	-.855	-.868	-.819	-.574	-.573	-.548	-.730	-.715	-.668
Nitrobacter	Year	-.482	-.503	-.540	-.509	-.353	.406	.401	.343	.381	.374	.356	.386	.425	.432	.466	.416	.422	.447
	Grow	-.521	-.483	-.507	-.483	-.365	.461	.546	.294	.412	.426	.331	.422	.545	.555	.626	.508	.533	.450
	Non-Grow	.132	-.264	-.304	.240	-.838	-.447	-.557	-.184	-.369	-.449	-.183	-.419	.499	.609	.627	.353	.345	.272
Bacteria	Year	-.599[a]	.006	.042	.000	.391	-.026	.126	-.165	-.153	-.115	-.149	-.090	.289	-.262	-.234	-.135	-.130	-.057
	Grow	-.950[a]	.053	.319	-.008	.439	-.536	.598	-.106	-.406	-.401	-.505	.482	.162	-.024	-.236	-.497	-.591	-.722
	Non-Grow	-.568	-.313	-.370	-.313	.462	.374	.352	.460	.309	.399	.291	.246	.277	.293	.208	.236	.259	.324
Fungi	Year	-.016	-.560	-.576[a]	-.577[a]	.543	.513	.502	.478	.515	.500	.511	.515	.442	.451	.433	.467	.469	.509
	Grow	.001	.571	-.522	-.571	.781[a]	.373	.386	.355	.391	.365	.352	.382	.196	.201	.154	.157	.156	.281
	Non-Grow	.559	.221	.366	.380	.498	.420	.419	-.027	.382	.343	.563	.374	.416	.597	.734	.837	.913[a]	.920[a]
Actinomycetes	Year	-.258	-.412	-.478	-.421	.463	.386	.547	.481	.500	.481	.476	.516	.513	.523	.506	.499	.510	.394
	Grow	-.473	-.303	-.306	-.303	.537	.051	.023	.182	-.619	.052	-.017	.023	-.017	.005	-.033	-.012	.005	-.124
	Non-Grow	.071	.602	.110	.679	.429	.429	.274	.110	.281	.236	.382	.185	.844	.881	.766	.646	.609	.468

a/ Significant at the .05 level or better.

Table 18. Correlation coefficients for linear regressions between microbes and selected environmental parameters of the cleared area. Critical values at the .05 level are .576 for the whole year, .576 for the whole year, .754 for the growing season, and .878 for the non-growing season. Symbols are: $\bar{X}$ = mean, SM = % soil moisture, PWP = permanent wilting percent.

		Condensed Tannin 0-15 cm	% Soil Moisture	% Field Capacity	Ratio SM/PWP	Organic Matter	$\bar{X}$ Date Soil Temp Surface	High Surface Temp for Date	Low Surface Temp for Date	$\bar{X}$ Date Soil Temp 7.5 cm	7.5 cm High Temp	7.5 cm Low Temp	$\bar{X}$ 3.75 cm Depth Temp	Surface Temp 3 wk $\bar{X}$	Surface Temp 2 wk $\bar{X}$	Surface Temp 1 wk $\bar{X}$	7.5 cm Temp 3 wk $\bar{X}$	7.5 cm Temp 2 wk $\bar{X}$	7.5 cm Temp 1 wk $\bar{X}$
Nitrosomonas	Year	-.331	-.331	-.239	.248	-.088	-.012	.013	.074	.300	-.190	-.082	.440	-.199	-.223	-.154	.409	-.333	-.066
	Grow	-.474	-.709	-.315	.366	.143	.412	.349	.383	.536	-.024	-.095	.435	-.181	-.985[a]	-.460	.455	-.083	.135
	Non-Grow	-.368	-.264	-.368	.013	.560	-.303	-.613	-.269	-.081	.527	.307	.180	.586	-.316	-.513	.539	-.116	-.310
Nitrobacter	Year	-.333	-.349	-.190	.411	-.086	.097	-.061	-.322	.175	-.260	-.166	.454	-.310	-.401	-.129	.436	-.305	-.007
	Grow	-.589	-.713	-.368	.714	-.578	.519	-.101	-.222	-.475	-.097	-.131	.436	-.330	-.831[a]	.093	.458	-.611	-.369
	Non-Grow	-.513	-.208	-.476	.207	.552	.132	.565	-.456	-.364	.433	.153	-.081	.484	-.640	-.133	.518	-.465	-.015
Bacteria	Year	-.112	-.125	-.191	-.252	.910	.548	-.094	-.116	-.082	.912	-.146	-.103	-.162	-.323	-.029	-.082	-.196	-.028
	Grow	-.189	-.381	-.369	-.515	-.381	.388	-.172	-.308	-.277	.730	-.369	-.203	-.147	-.345	-.081	-.174	-.236	.264
	Non-Grow	-.252	-.546	-.323	-.571	.995[a]	.070	-.232	-.154	.335	.980[a]	.771	-.526	-.267	-.563	.002	.969[a]	.295	-.401
Fungi	Year	-.093	-.094	-.196	-.318	-.161	-.221	-.091	-.081	-.088	-.020	-.272	-.091	-.006	-.007	-.109	-.092	-.031	-.142
	Grow	-.169	-.372	-.350	-.507	-.116	-.371	-.164	.393	-.474	-.087	-.372	-.155	-.116	-.445	-.159	-.169	-.342	.183
	Non-Grow	-.251	-.555	-.323	-.579	-.223	-.348	-.249	.987	.771	-.119	-.314	.441	-.104	.789	-.033	-.073	-.597	-.050
Actinomycetes	Year	-.104	-.109	-.206	-.304	-.233	-.153	-.091	-.090	-.079	-.224	-.251	.004	-.053	-.330	-.159	-.073	-.103	-.339
	Grow	-.322	.963[a]	-.019	-.773[a]	.533	.332	-.281	-.281	-.446	-.580	-.005	.449	-.513[a]	-.734	-.383	.454	-.341	-.066
	Non-Grow	-.212	.355	-.110	.780	-.390	-.192	.338	.338	-.666	-.464	-.511	.613	.944[a]	-.001	-.435	-.456	-.680	-.653

[a] Significant at the .05 level or better

Frank, 1969). Initial determinations utilized the alkali absorption method, whereas infrared gas analysis is currently gaining in popularity. Reiners (1973) reviewed the basic assumptions of the alkali method. Permanently installed chambers have been criticized (Edwards, 1974) because they restrict the movement of materials in and out of the chambers creating a nonrepresentative sample of the area.

Carbon dioxide evolution from the forest floor ranged from 1.76 g/m^2/day to 12.24 g/m^2/day (Fig. 19). The annual output was 2,473 g/m^2. Cumulative CO_2 evolution for the year in the clear-cut area was 1,953 g/m^2 with daily values ranging from 1.97-9.08 g/m^2/day. Significantly different amounts between the two plots occurred in the late fall of 1974. There was an inverse correlation between condensed tannin and CO_2 evolution, but it was generally not statistically significant. Effects of temperature and moisture on CO_2 evolution were apparently similar to that of tannin.

The relationship between numbers of microbes and evolved CO_2 was striking in the cleared area (Table 19). This reflected the approximate rate of microbial respiration because of the elimination of root respiration. The pretreatment of the clear-cut area to kill the roots was evidently successful. Furthermore, the length of the cylinder was great enough that it excluded root material in the forest to a depth of 20 cm. Most of the root material was concentrated in the 0-15 cm level. This is further substantiated

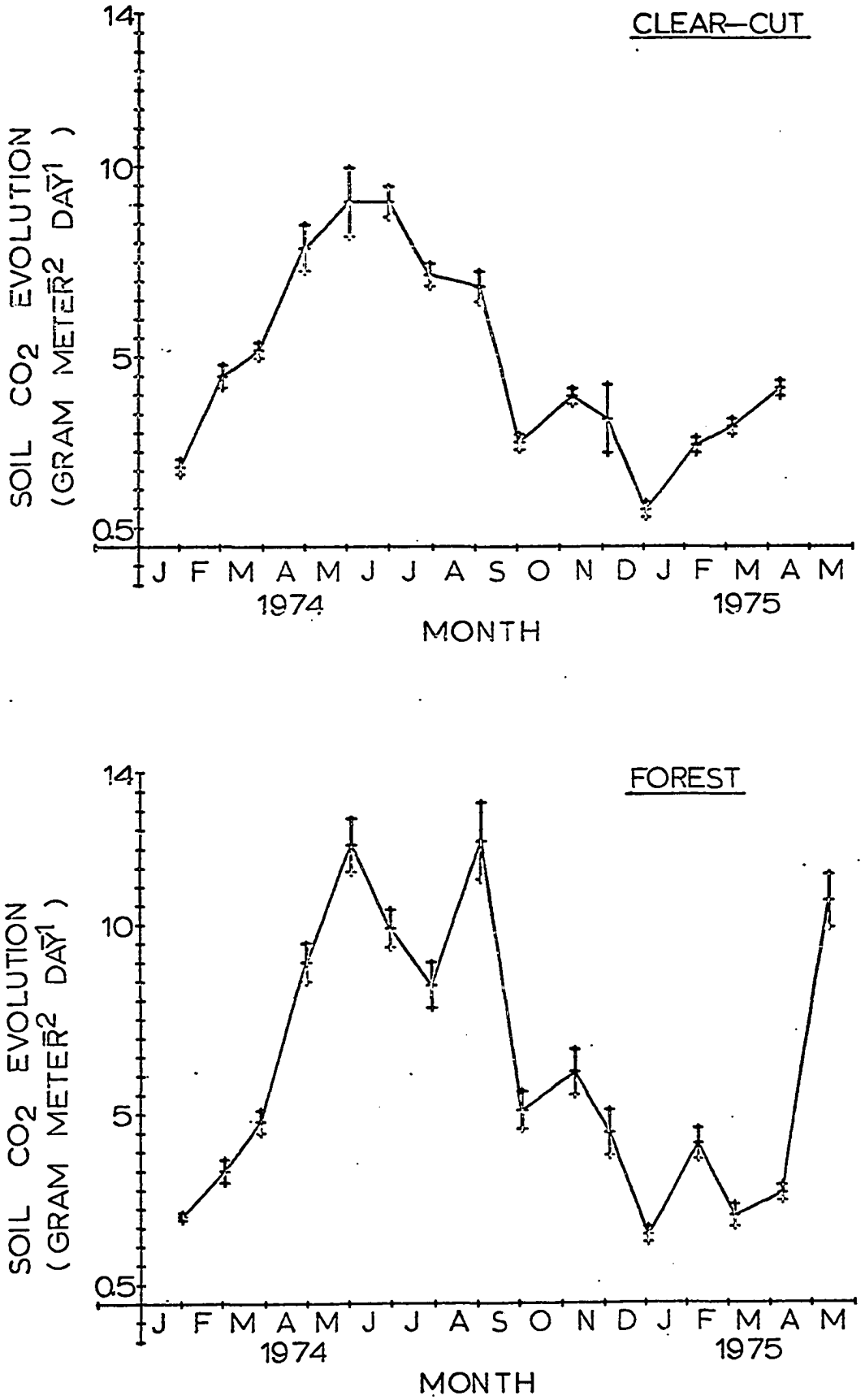


Figure 19. Soil respiration for the two plots (mean ± SE).

Table 19. Correlation coefficients for linear regressions between the amount of evolved CO_2 and selected biotic and abiotic parameters. Critical values at the .05 level are: .576 for the whole year; .754 for the growing season, and .878 for the non-growing season.

	Bacteria	Fungi	Actinomycetes	% Field Capacity	Surface Temp	7.5 Temp & cm
FOREST						
Year	-.180	.378	.479	-.484	.867[a]	.911[a]
Grow	-.360	-.023	-.036	-.256	.601	.633
Non-Grow	.234	.727	.812	-.501	.645	.743
CLEAR-CUT						
Year	.995[a]	-.093	-.108	-.318	.535	-.089
Grow	.997[a]	-.172	-.355	-.350	.387	-.266
Non-Grow	1.000[a]	.251	.349	-.323	.072	.351

[a/] Significantly correlated at the .05 level or better

by comparing CO_2 rates from other forested areas. Reiners (1968) found an annual rate of 2,900 g CO_2/m^2 from an oak forest floor in Minnesota. Woodwell and Dykeman (1966) reported 3,400 g $CO_2/m^2/yr$ evolved from the Brookhaven National Forest. An eastern Tennessee mesophytic forest produced 3,800 g $CO_2/m^2/yr$ (Edwards and Sollins, 1973) and Garrett and Cox (1973) measured 3,700 g $CO_2/m^2/yr$ in Missouri. Annual litter fall at Lake Thunderbird Research Site was 593 g/m^2. If the carbon content of the litter is estimated to be 50%, annual litter fall would provide 296 g/m^2 of carbon for eventual conversion into CO_2. At a steady state, the calculated CO_2 output would be 1,087 $g/m^2/yr$. The measured rate for the forest floor was over two times the predicted value. This discrepancy can not be explained totally by the fact that the forest is still in an active growth phase (Johnson and Risser, 1975). Consequently, it was concluded that there was some contamination by root respiration in the forest but it was reduced by the design of the cylinder.

Nitrification

The temperature effects on ammonification and nitrification are well known (Alexander, 1961; Myers, 1975). From 10-35 C, nitrification proceeds faster than ammonification, thus accounting for nitrate accumulation. Based on the amounts of ammonium-nitrogen in the soils (Table 9), nitrate should be present. In the clear-cut area, a steady

decrease in ammonium-nitrogen was detected through the summer, with a concomitant increase in nitrate-nitrogen (Table 9). Numbers of nitrifiers in the cleared area followed a similar pattern (Fig. 20). The numbers of Nitrosomonas and Nitrobacter were positively correlated at the 0.01 significance level.

As in the cleared area, the undisturbed forest soil possessed adequate ammonium-nitrogen (Table 9). However, there was practically no accumulation of nitrate-nitrogen. One possible explanation is interference with nitrification. This would be consistent with the conclusions of Rice and Pancholy (1972) for a revegetating post oak-blackjack oak forest. They found that numbers of Nitrosomonas were high in the first successional stage and decreased to a very low level in the climax. This hypothesis is supported in my project by the reduced number of nitrifiers in the forest soil (Fig. 21). The numbers of Nitrosomcnas were significantly different between the two treatments during the growing season except in July when numbers in both plots were low, probably because of low soil moisture.

The interference may have been due to either an unfavorable microclimate or the presence of an inhibitory substance. The activity of the nitrifying bacteria is markedly influenced by certain environmental conditions, chief among which is soil pH (Richards, 1974). Nitrate production rapidly decreases below pH 6.0 and is generally

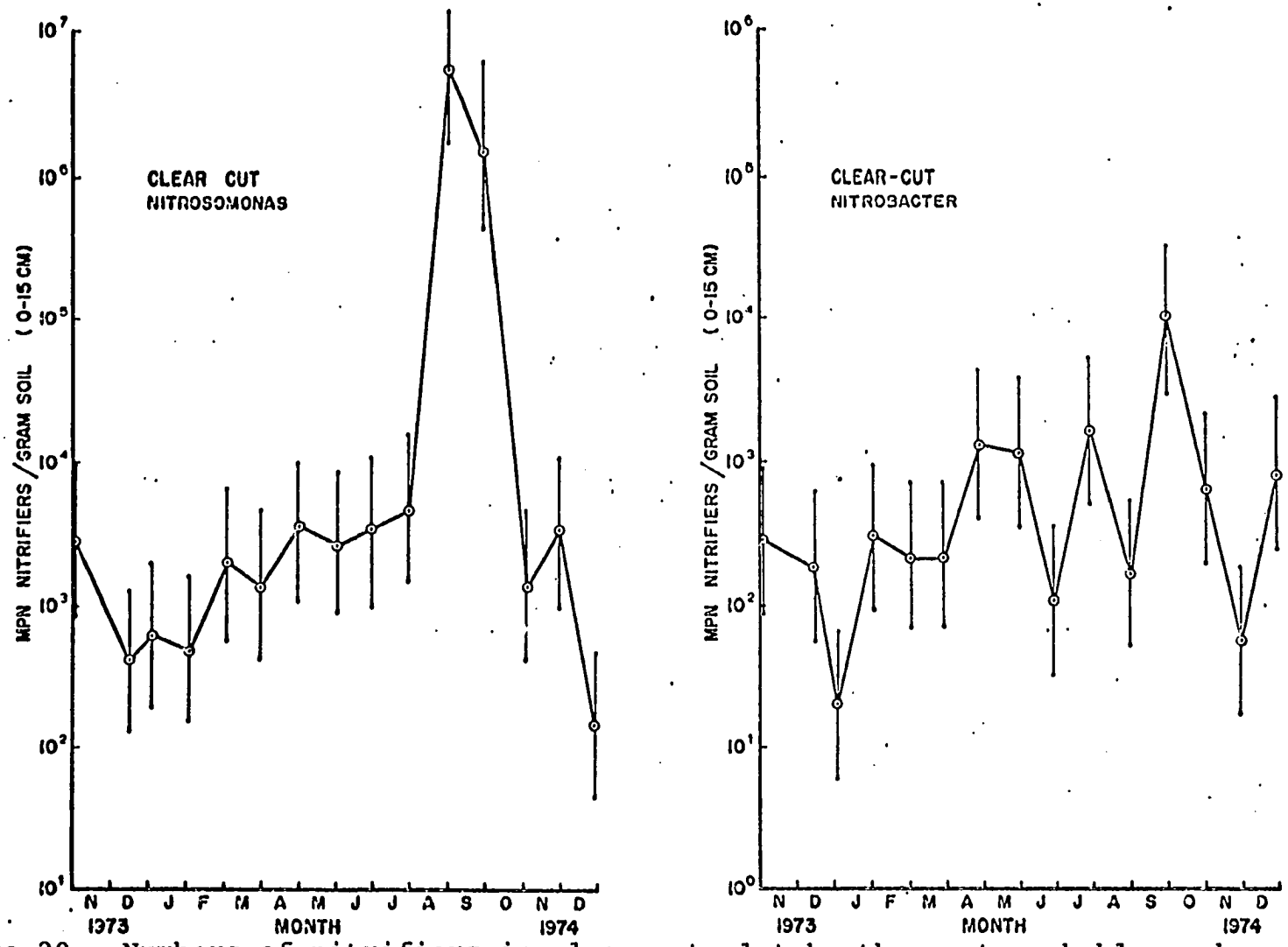


Figure 20. Numbers of nitrifiers in clear-cut plot by the most probable number method (Average of 5 samples with vertical lines indicating the 95% confidence interval).

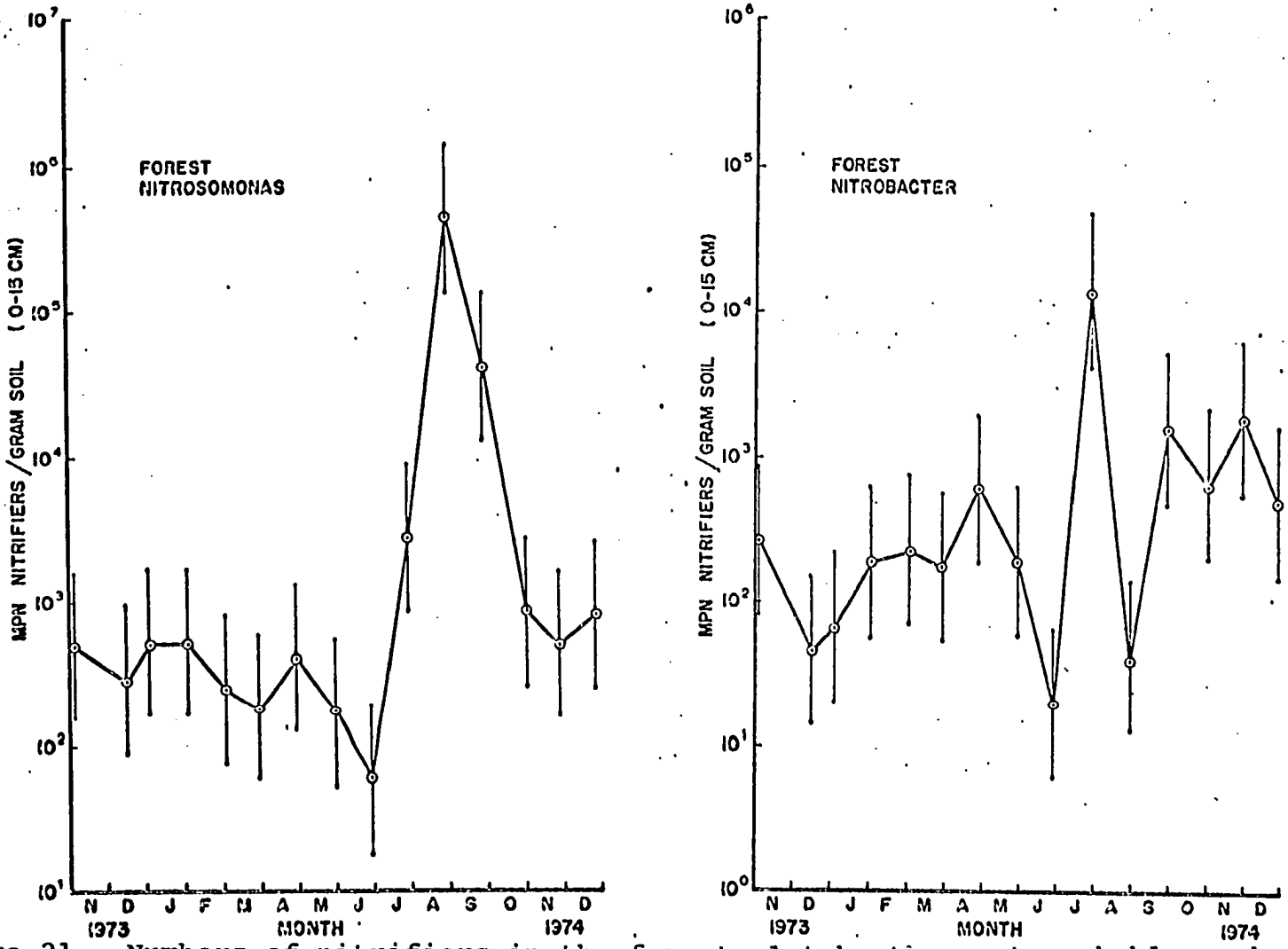


Figure 21. Numbers of nitrifiers in the forest plot by the most probable number method (Average of 5 samples with vertical lines indicating the 95% confidence interval).

negligible below pH 5.0. However, nitrification can occur at a soil pH of 4.0 (Richards, 1974). The clear-cut area had the lower soil pH, and this would eliminate pH as a major factor. In both treatments an inverse relationship existed between the numbers of nitrifiers and percent field capacity, whereas soil temperature was positively correlated at the surface, 3.75 cm depth, and at the 7.5 cm level (Table 17, 18). Therefore, the environmental conditions during the growing season would favor nitrification, but the observed supression in the forest soil is contradictory. Soil condensed tannins were negatively correlated with the nitrifiers (Tables 17, 18). This response was similar to the results of Basaraba (1964) who used soil samples amended with condensed tannin. He found that the addition of tannins lowered the rate of nitrification. Rice and Pancholy (1973) found that concentrations of condensed tannins as low as 2 ppm in soil suspensions completely inhibited oxidation of NH_4 to NO_2 by Nitrosomonas. In the clear-cut area the soil tannins decreased during the early summer months, with a corresponding increase in Nitrosomonas. However, the forest soil retained higher amounts of tannins during the same period and this could be responsible for the reduction of populations of nitrifiers and in nitrification.

The reduced amounts of nitrate could be due to two other phenomena. The nitrate-nitrogen may be immobilized by microbial assimilation. This does not seem to be the case,

however, since the soil in the clear-cut area had higher microbial populations (Figs. 16, 17, 18). The other possible explanation is that the vegetation removes the inorganic nitrogen as fast as it is mineralized. This hypothesis can not be eliminated by the current experimentation.

CHAPTER V

CONCLUSIONS

The condensed tannin content in leaves of Q. stellata and Q. marilandica was high throughout the year, and the annual rate of disappearance from leaves was about 75%. However, all of this condensed tannin was not incorporated into the soil. Approximately 40% of the leaf tannin was probably assimilated directly by the mycoflora and thus not added to the soil. This amount corresponded to the period January through July, when the microbial populations increased and the soil tannins decreased. The remaining loss could have been added to the soil. Throughfall and stemflow did not contribute tannins. The roots, with their high condensed tannin content, were probably the major contributor to the soil ecosystem. There was a positive correlation between percent increase in soil tannin and percent decrease in root tannin during the growing season. The 40% reduction in root tannin corresponded to the period of increasing soil tannin. Furthermore, the root biomass was three to five times higher than the leaf biomass (Table 4). I concluded that the major input is from the below-ground biomass.

The influence of condensed tannin on microbial populations was prounounced on the bacteria, moderate on the actinomycetes and neutral on the fungi. The increased numbers of nitrifiers and amounts of nitrate in the soil of the clear-cut area indicated that the rate of nitrification was higher there than in the forest. Evidence based on most abiotic factors suggested that nitrification should have been more rapid in the forest. The condensed tannin content of the soil was the one factor which seemed to be most closely correlated with the overall results. The concentration was significantly lower in the clear-cut area during the time nitrification appeared to be higher. My data support the conclustion, therefore, that nitrification was inhibited by the condensed tannin, but the immobilization of inorganic nitrogen by the surrounding vegetation could not be excluded.

LITERATURE CITED

Aaronson, S. 1970. Experimental microbial ecology. Academic Press. New York.

Alexander, M. 1961. Introduction to soil microbiology. John Wiley and Sons, Inc., New York.

_____ and F.E. Clark. 1965. Nitrifying bacteria, p. 1477-1483. In C.A. Black et al., (ed.), Methods of soil analysis, Vol. 2, Amer. Soc. Agron., Madison, Wisconsin.

Ardakani, M.S., R.K. Schulz, and A.D. McLaren. 1974. A kinetic study of ammonium and nitrite oxidate in a soil field plot. Soil Sci. Soc. Amer. Proc. 38:273-277.

Basaraba, J. 1964. Influence of vegetable tannins on nitrification in soil. Plant and Soil 21:8-16.

Bate-Smith, E.C. 1953. Color reactions of flowers attributed to (a) flavanols and (b) carotenoid oxides. J. Exp. Bot. 4:1-9.

_____. 1972. Detection and determination of ellagitannins. Phytochemistry 11:1153-1156.

_____ and T. Swain. 1953. Identification of leucoanthocyanins as "tannins" in foods. Chem. and Ind. 1953: 377-378.

Benoit, R.E. and R.L. Starkey. 1961. Influence of vegetable tannin on decomposition of some organic compounds and plant materials. Bacteriol. Proc. 1961:54.

_____. 1968a. Enzyme inactivation as a factor in the inhibition of decomposition of organic matter by tannins. Soil Science 105:203-208.

_____. 1968b. Inhibition of decomposition of cellulose and some other carbohydrates by tannin. Soil Science 105:291-296.

Berlier, Y., B. Dabin, and N. Leneuf. 1956. Comparison physique, chimique et microbiologique entre les sols de foret et de savane sur les sables tertieries de la Basse Côte d'Ivoire. Trans. Int. Congr. Soil Sci., 6th, 1956E, pp. 499-502.

Blum, U. and E.L. Rice. 1969. Inhibition of symbiotic nitrogen-fixation by gallic and tannic acid, and possible roles in old-field succession. Bull. Torrey Bot. Club 96:531-544.

Bocock, K.L., and O.G.W. Gilbert. 1957. The disappearance of leaf litter under different woodland conditions. Plant and Soil 9:179-185.

Bormann, F.H., G.E. Likens, D.W. Fisher, and R.S. Pierce. 1968. Nutrient loss accelerated by clear-cutting of a forest ecosystem. Science 159:882-884.

Bouyoucos, G.J. 1936. Directions for making mechanical analysis of soils by the hydrometer method. Soil Sci. 42:225-229.

Bremner, J.M. 1965. Inorganic forms in nitrogen, p. 1179-1237. In C.A. Black et al. (ed.), Methods of soil analysis, Vol. 2. Amer. Soc. Agron., Madison, Wisconsin.

Briggs, L.G. and H.L. Schantz. 1913. The water requirements of plants. Bulletin 285, Bureau of Plant Industry, U.S. Dept. of Agriculture.

Brown, S.A. 1964. Lignin and tannin biosynthesis, p. 361-398. In J.B. Harborne (ed.), Biochemistry of phenolic compounds. Academic Press, New York.

Buckman, H.O., and N.C. Brady. 1960. The nature and properties of soils. 6th ed. Macmillan Co., New York, N.Y.

Burns, R.E. 1963. Methods of tannin analysis for forage crop evaluation. Georgia Agricultural Experiment Station Technical Bull. NS. 32. Athens, Georgia.

Clark, F.E. 1965. Agar-plate method for total microbial count, p. 1460-1466. In C.A. Black et al. (ed.), Methods of soil analysis, Vol. 2. Amer. Soc. Agron., Madison, Wisonsin.

Coleman, D.C. 1973. Soil carbon balance in successional grassland. Oikos 24:195-199.

Curry, J.P. 1969a. The decomposition of organic matter in soil. Part I. The role of the fauna in decaying grassland herbage. Soil Biol. Biochem. 1:253-258.

_____. 1969b. The decomposition of organic matter in soil. II. The fauna of decaying grassland herbage. Soil Biol. Biochem. 1:259-266.

Curry, B. 1970. Climates of the states: Climate of Oklahoma. U.S. Dept. Commerce No. 60-34.

Dement, W.A., and H.A. Mooney. 1974. Seasonal variation in the production of tannins and cyanogenic glucosides in the chaparral shrub, Heteromeles arbutifolia. Oecologia (Berl.) 15:65-76.

Dommergues, Y. 1956. Study of the biology of soils of dry tropical forest and their evolution after clearing. Trans. Int. Congr. Soil Sci, 6th, 1956 E, pp. 605-610.

Dyksterhuis, E.J. 1948. The vegetation of the western cross timbers. Ecol. Monog. 18:325-376.

Eaton, J.S., G.E. Likens, and F.H. Bormann. 1973. Throughfall and stemflow chemistry in a northern hardwood forest. J. Ecol. 61:495-508.

Edwards, N.T. 1974. A moving chamber design for measuring soil respiration rates. Oikos 25:97-101.

_____, and P. Sollins. 1973. Continuous measurement of carbon dioxide evolution from partitioned forest floor components. Ecology 54:406-412.

Eicker, A. 1970. Vertical distribution of fungi in Zululand soils. Trans. Br. Mycol. Soc. 55:45-59.

Feeny, P. 1969. Spectrophometric method for rapid determination of oak leaf condensed tannin. Anal. Chem. 41:1347-1348.

_____. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology 51:565-581.

_____, and H. Bostock. 1968. Seasonal changes in the tannin content of oak leaves. Phytochemistry 7: 871-880.

Gardner, W.H. 1965. Water content, p. 82-127. In C.A. Black, et al. (ed.), Methods of soil analysis, Vol. I. Amer. Soc. Agron., Madison, Wisconsin.

Garrett, H.E. and G.S. Cox. 1973. Carbon dioxide evolution from the floor of an oak-hickory forest. Soil Sci. Soc. Amer. Proc. 37:641-644.

Goodfellow, M., and T. Cross. 1974. Actinomycetes, p. 269-302. In C.H. Dickinson and G.J.F. Pugh (ed.), Biology of litter decomposition, Vol. 2. Academic Press, New York.

Harmsen, G.W. and G.J. Kolenbrander. 1965. Soil inorganic nitrogen, p. 43-92. In W.V. Bartholomew and F.E. Clark (ed.), Soil nitrogen. Amer. Soc. Agron., Madison, Wisconsin.

Haslam, E. 1966. Chemistry of vegetable tannins, Academic Press, New York.

Hathaway, D.E. 1962. The condensed tannins, p. 191-228. In W.E. Hillis (ed), Wood extractives and their significance to the pulp and paper industries. Academic Press, New York.

Helvey, J.D., and J.H. Patric. 1965a. Canopy and litter interception of rainfall by hardwoods of eastern United States. Water Resources Res. 1:193-206.

_____ and _____. 1965b. Design criteria for interception studies, p. 131-137. In Design of hydrological networks, Int. Ass. Hydrol. Publ. 67.

Hillis, W.E. (ed.). 1962. Wood extractives and their significance to the pulp and paper industries. Academic Press, New York.

Jacquemin, H., and Y. Berlier. 1956. Evolution du pouvoir nitrifiant d'un sol de bosse Côte d'Ivoire sous l' action du climax et de la végétation. Trans. Int. Contr. Soil Sci. 6th, 1956 C, pp. 343-347.

Johnson, F.L., D.T. Bell, and S.K. Sipp. 1975. A comparison of urban and forest microclimates in the midwestern United States. Agric. Meteorol. 14:335-345.

_____, and P.G. Risser. 1974. Biomass, annual net primary production, and dynamics of six mineral elements in a post oak-blackjack oak forest. Ecology 55:1246-1258.

Johnson, F.L. and P.G. Risser. 1975. A quantitative comparison between an oak forest and an oak savannah in central Oklahoma. Southwest. Natural.20:75-84.

Knudson, L. 1913. Tannic acid fermentation. I.J. Biol. Chem. 14:159-184.

Kramer, N., and R.N. Doetsch. 1950. The growth of phenol-utilizing bacteria on aromatic carbon sources. Arch. Biochem. 26:401-405.

Lewis, J.A., and R.L. Starkey. 1969. Decomposition of plant tannins by some soil microorganisms. Soil Sci. 107:235-241.

Leyton, L., E.R.C. Reynolds, and F.B. Thompson. 1968. Interception of rainfall by trees and moorland vegetation, p. 97-108. In R.M. Wadsworth (ed.), The measurement of environmental factors in terrestrial ecology. Blackwell Scientific Publications, Oxford, England.

Likens, G.E. and J.S. Eaton. 1970. A polyurethane stemflow collector for trees and shrubs. Ecology 51:938-939.

Martin, J.P. 1950. Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. Soil Sci. 69:215-232.

May, S.W. 1974. Microbial decomposition of cellulose and native plant litter in a true prairie. Ph.D. dissertation, University of Oklahoma, Norman, Oklahoma.

McConnell, W.G. and A. Longo. 1971. Litter phenolics of possible limnological importance in southeastern Arizona. J. Ariz. Acad. Sci. 6:235-237.

McPherson, J.K. and G.L. Thompson. 1972. Competitive and allelopathic suppression of understory by Oklahoma oak forests. Bull. Torrey Bot. Club. 99:293-300.

Middleton, W.E.K., and A.F. Spilhaus. 1953. Meterological instruments. University of Torronto Press, Toronto, Canada.

Mishustin, E.N. 1975. Microbial associations of soil types. Microb. Ecol. 2:97-118.

Mitchell, J.A. 1930. Interception of rainfall by the forest. J. For. 28:101-102.

Myers, R.J.K. 1975. Temperature effects on ammonification and nitrification in a tropical soil. Soil Biol. Biochem. 7:83-86.

Nelson, D.W. and J.M. Bremner. 1972. Preservation of soil samples for inorganic nitrogen analysis. Agron. J. 64:196-199.

Olmsted, C.E., III and E.L. Rice. 1970. Relative effects of known plant inhibitors on species from first two stages of old field succession. Southwestern Naturalist 15:165-173.

Parkinson, D. 1973. Techniques for the study of soil fungi. Bull. Ecol. Res. Comm. (Stockholm) 17:29-36.

Parks, J.M. and E.L. Rice. 1969. Effects of certain plants of old-field succession on the growth of blue-green algae. Bull. Torrey Bot. Club 96:345-360.

Piper, C.S. 1942. Soil and plant analysis. The University of Adelaide, Adelaide, Australia.

Raveh, A. 1973. The adaptation of the nitrate-specific electrode for soil and plant analysis. Soil Sci. 116:388-389.

Reiners, W.A. 1968. Carbon dioxide evolution from the floor of three Minnesota forests. Ecology 49:471-483.

_____. 1973. Terrestrial detritus and the carbon cycle, p. 303-327. In G.M. Woodwell and E.V. Pecan (ed.), Carbon and the biosphere. National Technical Information Service, Springfield, Virginia.

Ribéreau-Gayon, P. 1972. Plant phenolics. Hafner Publ. Co., New York.

Rice, E.L. 1965a. Inhibition of nitrogen-fixing and nitrifying bacteria by seed plants. II. Characterization and identification of inhibitors. Physiol. Plant. 18:255-268.

_____. 1965b. Inhibition of nitrogen-fixing and nitrifying bacteria by seed plants. III. Comparison of three species of Euphorbia. Proc. Okla. Acad. Sci. 45:43-44.

_____. 1968. Physiological ecology laboratory procedures. University of Oklahoma Bookstore, Norman, Oklahoma.

Rice, E.L. 1969. Inhibition of nitrogen-fixing and nitrifying bacteria by seed plants VI. Inhibitors from Euphorbia supina Raf. Physiol. Plant. 22:1175-1183.

_____, and S.K. Pancholy. 1972. Inhibition of nitrification by climax ecosystems. Amer. J. Bot. 59: 1033-1040.

_____. 1973. Inhibition of nitrification by climax ecosystems II. Additional evidence and possible role of tannins. Amer. J. Bot. 60:691-702.

Richards, B.N. 1974. Introduction to the soil ecosytem. Longman Inc., New York.

Robinson, T. 1975. The organic constituents of higher plants. Third ed. Cordus Press, North Amherst, Massachusetts.

Rodin, L.E. and N.I. Bazilevich. 1967. Production and mineral cycling in terrestrial vegetation. Oliver and Boyd, London, England.

Russell, E.J., and E.W. Russell. 1961. Soil conditions and plant growth. 9th ed. John Wiley and Sons, New York.

Rutler, A.J., A.J. Mortor, and P.C. Robins. 1975. A predictive model of rainfall interception in forests. II. Generalization of the model and comparison with observations in some coniferous and hardwood stands. J. Appl. Ecol. 12:367-380.

Smith, I. (ed.). 1960. Chromatographic and electrophoretic techniques. Vol. 1: Chromatography, Interscience, New York.

Smith, W.H., F.H. Bormann, and G.E. Likens. 1968. Response of chemoautotrophic nitrifiers to forest cutting. Soil Sci. 106:471-473.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical methods. Sixth ed. Iowa State University Press, Ames, Iowa.

Society of American Bacteriologists. 1957. Manual of microbiological methods. McGraw-Hill Book Co., New York.

Sokal, R.R. and F.J. Rohlf. 1969. Biometry. W.H. Freeman and Co., San Francisco, California.

Sparrow, E.B. and K.G. Doxtader. 1973. Adenosine triphosphate (ATP) in grassland soil: its relationship to microbial biomass and activity. U.S. IBP Grassland Biome Tech. Rep. No. 224, Colo. State Univ., Fort Collins.

Stecher, P.G. (ed.). 1968. The Merck index. Eighth ed. Merck and Co., Rahway, New Jersey.

Suffling, R. and D.W. Smith. 1974. Litter decomposition studies using mesh bags: spillage inaccuracies and the effects of repeated artificial drying. Can. J. Bot. 52:2157-2163.

Swain, T. 1965. The tannins, p. 552-580. In J. Bonner and J.E. Varner (ed.), Plant Biochemistry. Academic Press, New York.

Swank, W.T., N.B. Goebel, and J.D. Helvey. 1972. Interception loss in loblolly pine stands of the South Carolina piedmont. J. Soil and Water Conserv. 27:160-164.

Trewartha, G.T. 1968. An introduction to climate. 4th ed. McGraw-Hill, New York.

U.S. Department of Commerce. 1975. Climatological Data, Annual Summary for Oklahoma for 1974. 83(13):1-15. Weather Bureau, Asheville, North Carolins.

Voight, G.K. 1960. Distribution of rainfall under forest stands. For. Sci. 6:2-10.

White, E.J. and A. Carlisle. 1968. The interception of rainfall by mixed deciduous woodland. Quart. J. For. 62:310-320.

Williams, A.H. 1963. Enzyme inhibition by phenolic compounds, p. 87-96. In J.B. Pridham (ed.), Enzyme chemistry of phenolic compounds. MacMillan Co., New York.

Witkamp, M. 1963. Microbial population of leaf litter in relation to environmental conditions and decomposition. Ecology 44:370-377.

_____. 1966. Decomposition of leaf litter in relation to environment, microflora, and microbial respiration. Ecology 47:194-201.

Witkamp, M., and M.L. Frank. 1969. Evolution of CO_2 from litter, humus, and subsoil of a pine stand. Pedobiologia 9:358-365.

_____, and J.S. Olson. 1963. Breakdown of confined and nonconfined oak litter. Oikos 14:138-147.

Woodwell, G.M. 1974. Success, succession, and Adam Smith. Bio-Science 24:81-87.

_____, and W.R. Dykeman. 1966. Respiration of a forest measured by CO_2 accumulation during temperature inversions. Science 154:1031-1034.

Woolf, C.M. 1968. Principles of biometry. Van Nostrand Co., Princeton, New Yersey.

Young, L.J., B. Wilson, J. Tabler, and R. Ellis. 1969. A study of the ecology and control of the Texas persimmon, Diospyros texana, p. 90-91. Noxious Brush and Weed Control Research Reports #33. Texas Tech., Lubbock, Texas.