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MAY, Silas William, 1937-
MICROBIAL DECOMPOSITION OF CELLULOSE AND NATIVE PLANT LITTER IN A TRUE PRAIRIE.

The University of Oklahoma, Ph.D., 1974
Microbiology

Xerox University Microfilms, Ann Arbor, Michigan 48106

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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

MICROBIAL DECOMPOSITION OF CELLULOSE AND NATIVE PLANT LITTER IN A TRUE PRAIRIE

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

By

SILAS WILLIAM MAY

Norman, Oklahoma

1974

MICROBIAL DECOMPOSITION OF CELLULOSE AND NATIVE

PLANT LITTER IN A TRUE PRAIRIE

A DISSERTATION

APPROVED FOR THE DEPARTMENT OF BOTANY AND MICROBIOLOGY

By

DISSERTATION COMMITTEE

ACKNOWLEDGMENTS

I would like to thank Dr. J. Bennett Clark for his suggestions during the course of this study and in the preparation of the manuscript and Dr. Paul Risser for introducing me to the IBP and assisting in the financial support for this study. I would also like to thank the other members of my dissertation committee, Drs. William Sorenson, John Lancaster and Eddie Smith for their critical reading of the manuscript.

The helpful suggestions offered by Dr. Dave Coleman, NREL in Fort Collins, and John Harris, Kansas State, were very much appreciated.

A special thanks goes to my wife Ruby for her encouragement and understanding throughout this study.

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ABSTRACT

Microbial decomposition was studied in a true prairie and in a laboratory simulated ecosystem. Decomposition rates were determined for cellulose and native litter. Similar attempts to measure decomposition rates of roots were not satisfactory. Carbon dioxide evolution from the soil was used as an indication of microbial activity. The decomposition of ^{14}C-cellulose and native litter was determined in the laboratory to verify hypotheses based on field data. All evidence from both laboratory and field studies support the conclusion that soil temperature is a more important variable than soil moisture in determining the rate of microbial decomposition of prairie litter.

Quantitation of chemical changes in decomposing litter showed a reduction of 90% in soluble carbohydrates, 95% amino acids, 16% starch and 68% protein in 10 days under optimal conditions. A Q_{10} of 6 for cellulose and 3.18 for litter were determined for short term decomposition.

An estimate of the annual decomposition rate based on this study suggests that no more than 60% of the annual litter production in a tall grass prairie is recycled by microbial activity.

MICROBIAL DECOMPOSITION OF CELLULOSE AND NATIVE PLANT LITTER IN A TRUE PRAIRIE

CHAPTER I

INTRODUCTION

This paper is the result of a series of investigations initiated by the International Biological Program Grassland Biome. The purpose of these investigations was to quantitate the rate of microbial decomposition of grass litter and to determine the effects of soil temperature and moisture on microbial growth in a true, or tall-grass, prairie.

There are essentially two approaches to the microbiology of decomposition of organic material in the soil (Tribe 1961). Decomposition can be regarded as a process of breaking down material by microorganisms, responding to environmental parameters as moisture and temperature, without regard for the taxonomy of the microbes. The second approach, the biological analysis of decomposition, regards decomposition as a result of a succession of microbes growing on an organic substrate. In an ecosystem approach, it is the relationship between the total rate of production and the rate of decomposition, regardless of those organisms

responsible, that is of overall importance (Odum 1971). Since this research has been part of an interdisciplinary effort in ecosystem analysis of a tall-grass prairie, it has emphasized the process study approach to decomposition rather than the biological succession of specific microorganisms.

While field methodology in microbial ecology is currently undergoing rapid innovation, two techniques are still used by most ecologists studying decomposition. These are the measurement of weight lost by buried substrate and the measurement of carbon dioxide evolution from the soil. The development and use of the first method is documented by Dickinson and Pugh (1974). The carbon dioxide content of the soil was first measured by Boussingault and Lewy in 1853 (Smith and Brown 1931). Since then Lundegardh (1928), Humfeld (1930), Witkamp (1966b and 1969), Wiant (1967a), Schulze (1967), Reiners (1968), Wanner (1970), Kucera and Kirkham (1971), and Edwards and Sollins (1973) have used some modification of carbon dioxide evolution to study soil respiration and microbial decomposition. Because these carbon dioxide techniques do not distinguish between root and microbial respiration, they are subject to criticism. Nevertheless, data from thorough field studies utilizing this method yield useful information and correlations have been found in the field between temperature, moisture, bacterial density and age of the litter (Reiners 1973).

Because of the inherent problems with field methodology, a concurrent study in a simulated laboratory eco-

system was conducted to verify hypotheses based on field observations.

Recent investigations sponsored by the International Biological Program have resulted in a new awareness of the importance of decomposition in the ecosystem, especially in relation to primary production (Dickinson and Pugh 1974). This dissertation should add to our knowledge of decomposition and the role of microorganisms in the tall-grass prairie ecosystem.

CHAPTER TWO

DECOMPOSITION IN THE FIELD

Study Area

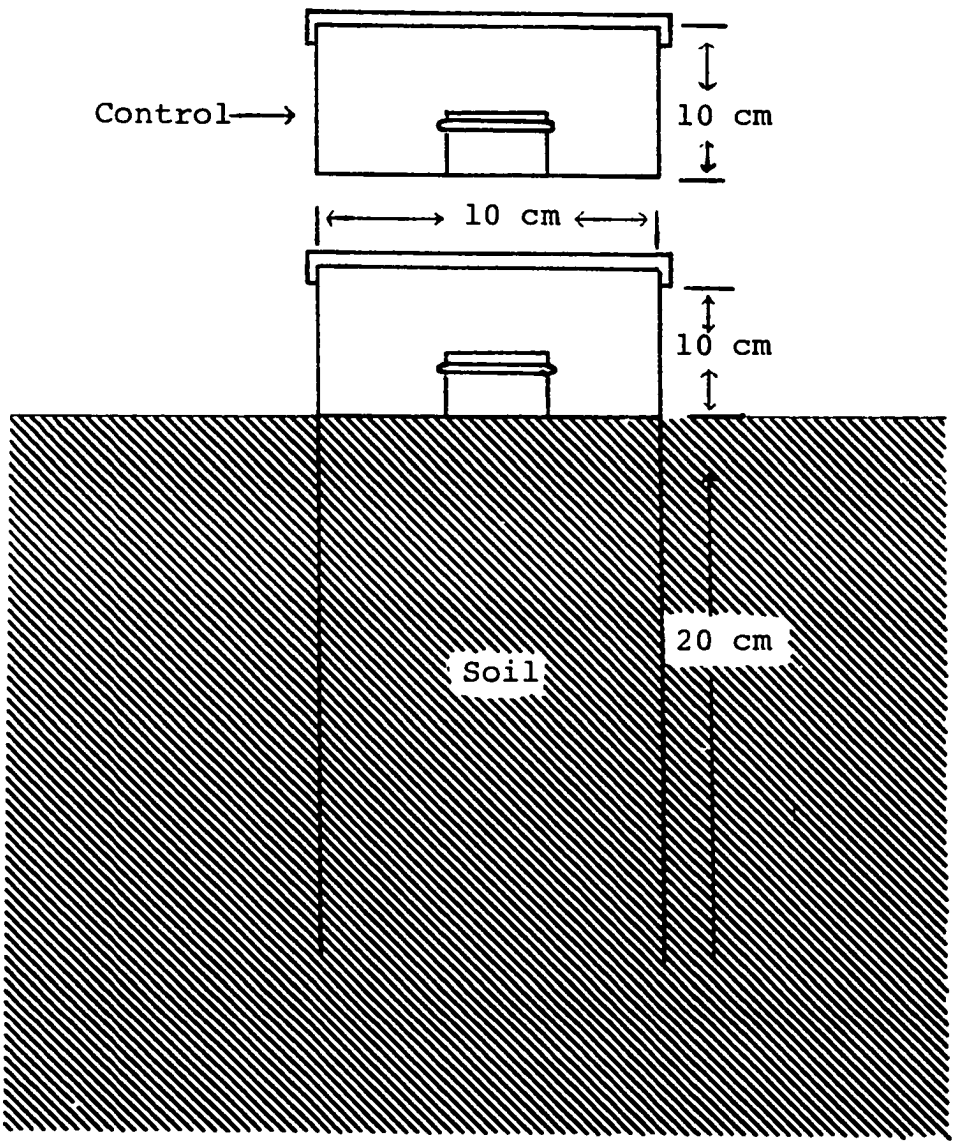
The study area, known as the OSAGE SITE in the IBP Grassland Biome from 1970 through 1973, is a true prairie located on the Adams ranch 19 km north and 5 km east of Shidler, Oklahoma, in Osage County in northeastern Oklahoma. The soils (see Table 1 in the appendix for chemical analysis) are Mollisols of the Labette-Summit-Sogan Association in a region of rolling topography (Gray 1969, Donahue 1971). Mean annual temperature is 13.2°C and the mean annual precipitation is 100 cm with 60 cm occurring from July to September (Risser and Kennedy 1972). The area includes an ungrazed and grazed treatment. The ungrazed treatment, dominated by Andropogon scoparius and Panicum scribnerianum, has existed in an ungrazed condition for at least 20 years except for occasional mowing. The grazed treatment supported light to moderate grazing during the fall and winter but was fenced during the growing season and time of the sampling from April to November. The grazed treatment is characterized mainly by the grasses Sporobolus asper, Andropogon scoparius and Panicum scribnerianum.

Materials and Methods

To collect carbon dioxide from the soil, plastic sewer pipe (30 cm long, 10 cm in diameter and 0.63 cm thick) was placed in the ground to a depth of 20 cm. Widemouth screw cap jars containing 10 ml of 1M KOH were placed in the cylinders which were then covered with air tight plastic caps (Fig. 1). The protruding cylinder was covered with aluminum foil to shield against heat buildup inside the cylinder. After 24 hr in the field, the jars were brought into the laboratory where 10 ml of 1M $BaCl_2$ was added before titrating with 1M HCl. Black cylinders were set on top of the ground to measure the carbon dioxide content in the air space inside the cylinder. Grams of CO_2/m^2/day were calculated based on the area of the cylinder (78.5 cm^2) and the milligram carbon dioxide equivalent of the acid, i.e. 1 ml of 1.0M HCl = 22 mg CO_2 (Swift and French 1972). Each of the treatments, the ungrazed and the grazed areas, were divided into two replicates. Ten randomly placed cylinders were driven in the ground in each replicate.

During the interval that carbon dioxide evolution was measured, soil temperature was monitored by a maximum-minimum soil thermometer. Soil water was measured at the time the KOH was set out by taking two 10 cm deep soil cores per replicate. These cores were dried to constant weight, approximately 48 hr, at 105 C. Soil water percentage was calculated based on the dry soil weight.

Figure 1. Apparatus for measuring carbon dioxide evolution from the soil. Control shown above.


Control
10 cm
10 cm
10 cm
20 cm
Soil

Rates of decomposition for cellulose, native litter and native roots were determined by placing these materials in net bats and placing these in the field. These bags were positioned on the ground surface or buried at a depth of 5 cm. One mm mesh nylon bags, 11cm x 11cm, were used for the below ground samples. Two mm mesh fiber glass bags of the same dimensions were used for samples placed on the surface. (Nylon tends to photooxidize if used above ground). Whatman No. 1 filter paper was used for cellulose. Native litter was obtained from recent standing dead plant material and native roots were obtained from washed soil cores. All substrates except roots were placed on the surface and at a depth of 5 cm. Native roots were placed only below the ground. With the exception of cellulose, the mass of substrate placed in each bag was calculated to simulate the actual biomass of litter and roots found in the same area at each depth for each treatment. Thus, in the ungrazed treatment 3 g of litter were placed on the surface and 5 g of litter and 6 g of roots were buried at 5 cm. In the grazed, 5 g of litter were placed on the surface and 5 g of litter and 5 g of roots were buried. Three g of cellulose were placed in each bag both on the surface and at 5 cm. Substrate bags were put out in two replicates per treatment at the beginning of the growing season on April 22. Subsets were retrieved on June 5, July 4, and August 8.

The percent organic matter for all materials was determined by ashing at 600 C for 4 hr. On retrieval the

materials were force-air-oven-dried at 60 C for 24 hr. They were then weighed, ashed and the amount of sand and mineral material included with the retrieval weight determined. At the same time a sample of soil was dried, ashed at 600 C for 4 hr and weighed to determine the loss of organic material and carbohydrate from the soil upon ignition. The percent decomposition was then calculated by the following formula:

$$\text{Fraction of material lost} = \frac{O_o - O_r}{O_o}$$

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

(2) O_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 wt.

B = wt. of retrieved material + soil

B_r = ash wt. of retrieved material + soil

C = fraction ash in original material

S = fraction of soil remaining after ashing,

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

This formula was suggested by Dr. Tom Garland of Washington State University since it incorporates organic matter loss from the soil contained in the bags. The addition of weight from the soil onto the substrate often gave the impression that no decomposition had occurred. Of the several methods tried, this one gave the best reproducibility.

Results and Discussion

Carbon Dioxide Evolution

The results of carbon dioxide evolution, together with soil temperature and moisture, are shown for both treatments in Table 1. The average rate of evolution for the ungrazed treatment was 9.15 g/m^2/day and 12.86 g/m^2/day for the grazed. The highest rates were measured in August and September when soil temperature was highest. The average for these two months in the ungrazed was 14.51 g/m^2/day and 21.79 g/m^2/day for the grazed. Using infrared gas analysis, Kucera and Kirkham (1971) found an average production of 10.8 g/m^2/day in the tall grass prairie in Missouri during the same period. The total carbon dioxide produced in the ungrazed treatment at the Osage was 2,173 g/m^2 compared to 1,676 g/m^2 at the Tucker prairie in Missouri. The grazed site at the Osage showed an even higher annual production of 2,684 g/m^2. Since the alkali method has been shown to be less efficient in measuring carbon dioxide evolving from the soil than the infrared method (Edwards and Sollins 1973, Kucera and Kirkham 1971), the Osage values are considerably higher relative to the Missouri tall grass prairie rates.

Table 2 summarizes some of the rates for carbon dioxide evolution from various ecosystems throughout the world. The Osage shows the highest rate of carbon dioxide evolution from the soil for any of these temperate ecosystems.

Table 1. Summary of carbon dioxide evolution data.

Date	Treatment	g CO_2/24 hr/m^2	Soil Temperature (°C)	Soil Water (% dry wt)
22 April	Ungrazed	5.76	14.0	37.50
	Grazed	11.09	16.0	31.50
16 May	Ungrazed	4.44	15.0	30.10
	Grazed	10.01	17.5	26.08
6 June	Ungrazed	11.85	17.5	19.05
	Grazed	11.37	20.5	12.07
7 July	Ungrazed	9.23	18.5	24.90
	Grazed	9.75 a/	19.7	24.35
8 August	Ungrazed	13.81	19.8	19.40
	Grazed	19.00	22.3	17.00
2 September	Ungrazed	15.21	19.3	30.05
	Grazed	24.58 a/	22.5	26.50
4 November	Ungrazed	3.73	9.0	24.94
	Grazed	4.20	9.0	24.66

a/ Data available for Replicate 1 only.

Table 2. Comparison of carbon dioxide evolution for several ecosystems.

Ecosystem	Method	Highest daily production g/m^2	Annual production g/m^2	Reference
Osage	alkali	13.81	2,173	May & Risser, 1972
Tall grass Missouri	infrared	10.80	1,675	Kucera and Kirkham, 1971
Decidious forest Tennessee	infrared	5.89	1,518	Witkamp, 1966
Tropical savanna	alkali	7.90	2,884	Schulze, 1967
Tropical decidious forest	alkali	8.95	3,266	Schulze, 1967
Oak forest Minnesota	infrared	--	2,912	Reiners, 1968
Swamp Minnesota	infrared	--	2,710	Reiners, 1968
Fenn Minnesota	infrared	--	2,592	Reiners, 1968

Carbon dioxide evolution at the Osage increase exponentially with rising soil temperature (Fig. 2). Although carbon dioxide evolution decreased at very high and very low soil moisture levels, no definite relationship between carbon dioxide evolution and soil moisture is observable from the data in the middle range of soil moisture, 20 to 40%. The correlations between soil temperature, carbon dioxide evolution and soil moisture are summarized for both treatments in Table 3. The high positive values for carbon dioxide and temperature are significant at the 0.1 level.

The influence of temperature on carbon dioxide evolution is well documented. Laboratory studies by Lundegardh (1921) showed that soil respiration rose rapidly up to 60 C. He attributed this to a response in biological activity. Drobnik (1962) reported that, between 8 and 28 C, increased temperature increased carbon dioxide evolution with a Q_{10} from 1.6 to 2.0. At 38 C and higher temperature, he found irregularities in carbon dioxide evolution which he attributed to inhibition of respiration. Wiant (1967a) showed that, between 20 and 40 C, carbon dioxide production from the soil increase logarithmically.

Field investigations using both the alkali absorption and infrared gas analyzers have shown that carbon dioxide evolution is significantly correlated to temperature. Witkamp (1966a) noted that in the decidious forest, tempera-

Figure 2. Exponential increase in carbon dioxide evolution with increasing soil temperatures for the ungrazed and grazed treatments. Equations for the graph are shown at the lower left.

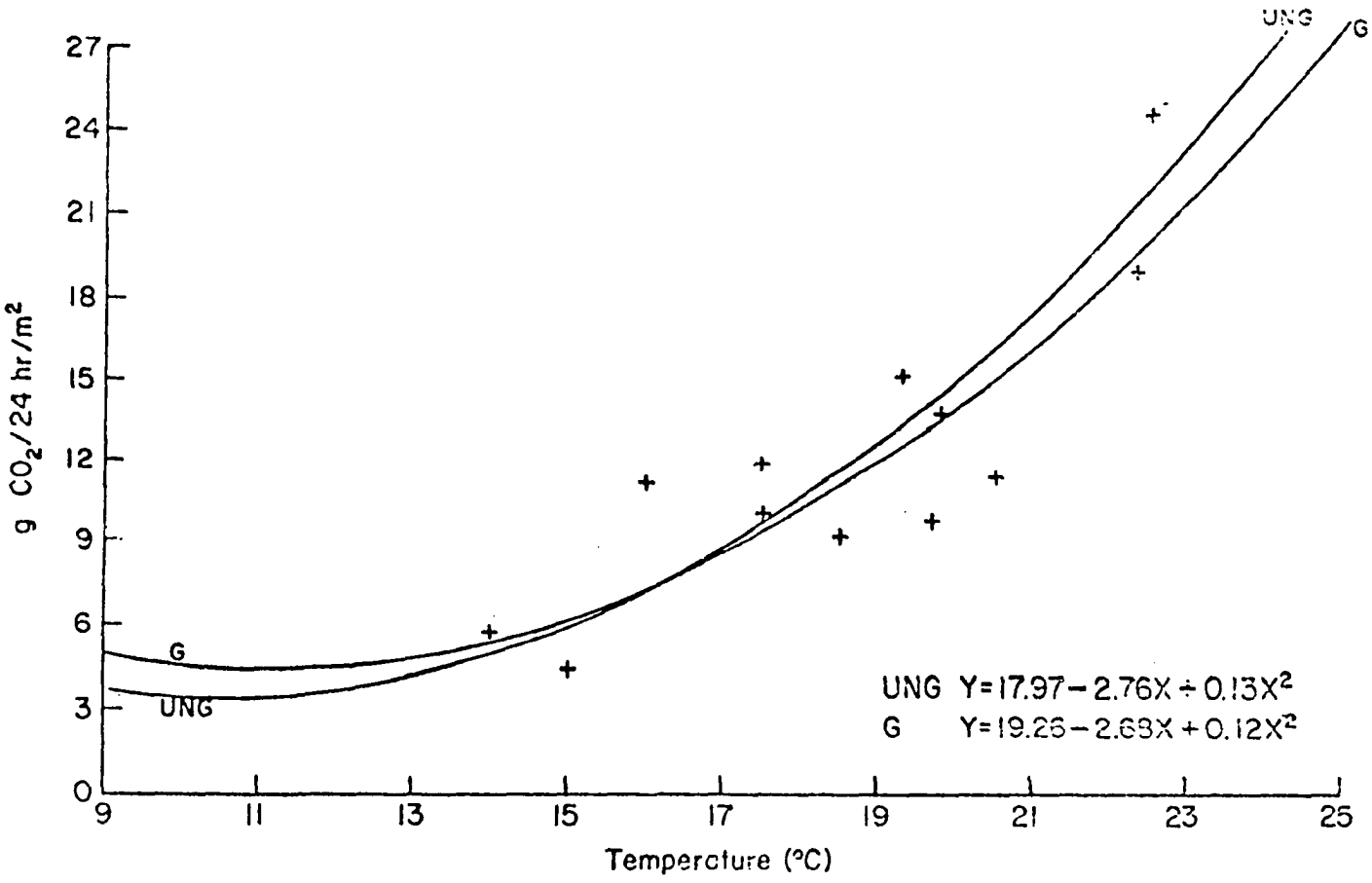

UNG
G
27
24
21
18
15
12
9
6
3
0
g CO_2/24 hr/m^2
G
UNG
UNG Y=17.97−2.76X+0.13X²
G Y=19.25−2.68X+0.12X²
9
11
13
15
17
19
21
23
25
Temperature (°C)

Table 3. Simple correlation for data from 1972 growing season. Upper right shows grazed, lower left ungrazed.

	Temp	H_2O	CO_2
Temp		−0.37	0.80
H_2O	−0.30		−0.08
CO_2	0.86	−0.43	

Grazed

Ungrazed

ture was more significantly correlated to carbon dioxide evolution than was moisture or age of the litter. Kucera (1971) reported that carbon dioxide evolution from the soil of a tall-grass prairie responded exponentially to rises in temperature within the range of field conditions.

The decrease of carbon dioxide evolution with increased soil moisture is more difficult to explain. At moisture levels near saturated or water-logged conditions, carbon dioxide evolution is reduced because of anaerobic conditions in the soil and a corresponding reduction in metabolic activity of most of the microorganisms (Cozad, et al. 1953). Since attempts were made to not measure carbon dioxide evolution at this level of soil mositure or immediately after a heavy rain, this cannot account for the observed results between carbon dioxide evolution and soil moisture at the Osage. At moisture levels near or below wilting point (15%), soil moisture is no longer available to support microbial decomposition (Waint 1967b). As long as the level of soil moisture remains between these two extremes, microbial decomposition can occur although optimal levels have been suggested to be at 60 to 70% field capacity by Koepf (1951) and 45% by Harris (1971). Since precipitation brought about a decrease in soil temperature, it may be that the decrease in carbon dioxide evolution at higher soil moistures was due to the response of microbial growth to decrease in temperature rather than an increase

in soil moisture. This response of soil temperature to soil moisture has been demonstrated also in the decidious forest (Reiners 1968).

The measurement of carbon dioxide in the soil lost due to percolation of water and by absorption of carbon dioxide in the soil water is a difficult task and was not included in this study. No references in the literature to this problem were found. However, using data adjusted for a theoretical loss of carbon dioxide to soil water, correlations between carbon dioxide, temperature and soil water were re-calculated. If it were assumed that the maximum amount of carbon dioxide possible was dissolved in the soil water available under static conditions, the correlation values were less significant statistically. However, carbon dioxide evolution still correlated better to soil temperature than to soil moisture (Table 4).

Carbon dioxide evolution was usually higher in the grazed treatment than in the ungrazed (Fig. 3). This can be explained by the higher soil temperatures in the grazed treatment (Fig. 4), although there may be other factors responsible for higher microbial activity in the grazed treatment.

The variability in the data from the grazed area is shown by the coefficient of variation (Table 5). This variability in the grazed area is reflected also in the pattern of litter fall and vegetation cover (Risser and

Table 4. Correlation of carbon dioxide to soil temperature and soil moisture after adjustment for carbon dioxide loss to soil moisture (based on theoretical calculations).

Parameters	Ungrazed	Grazed
CO_2 : T	0.60	0.58
CO_2 : H_2O	0.31	0.30

Figure 3. Graph depicting the distribution of carbon dioxide evolution between the ungrazed treatment, solid line, and grazed treatment, dotted line.

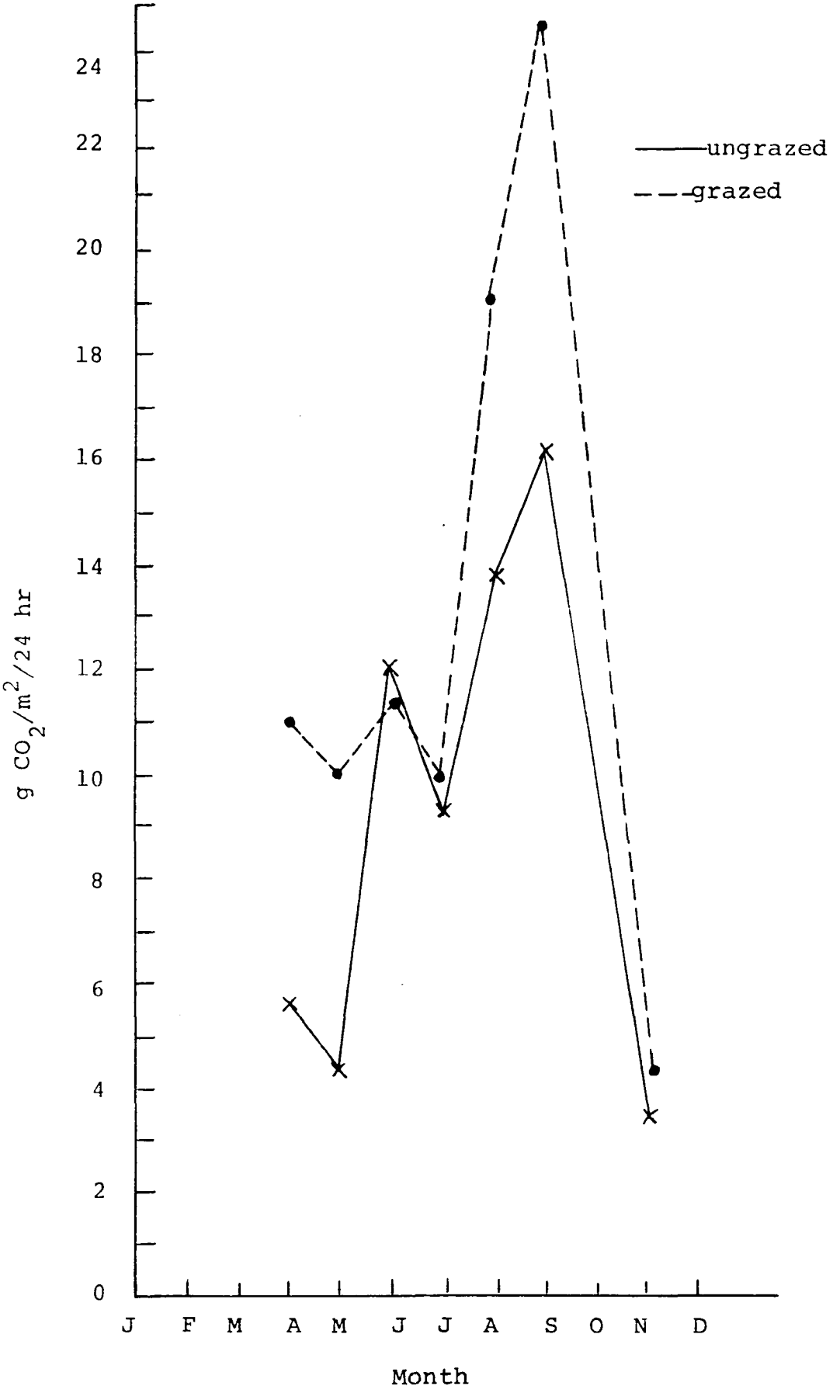

ungrazed
grazed
g $CO_2/m^2/24$ hr
0
2
4
6
8
10
12
14
16
18
20
22
24
J F M A M J J A S O N D
Month

Figure 4. Distribution of soil temperature for the ungrazed and grazed treatments.

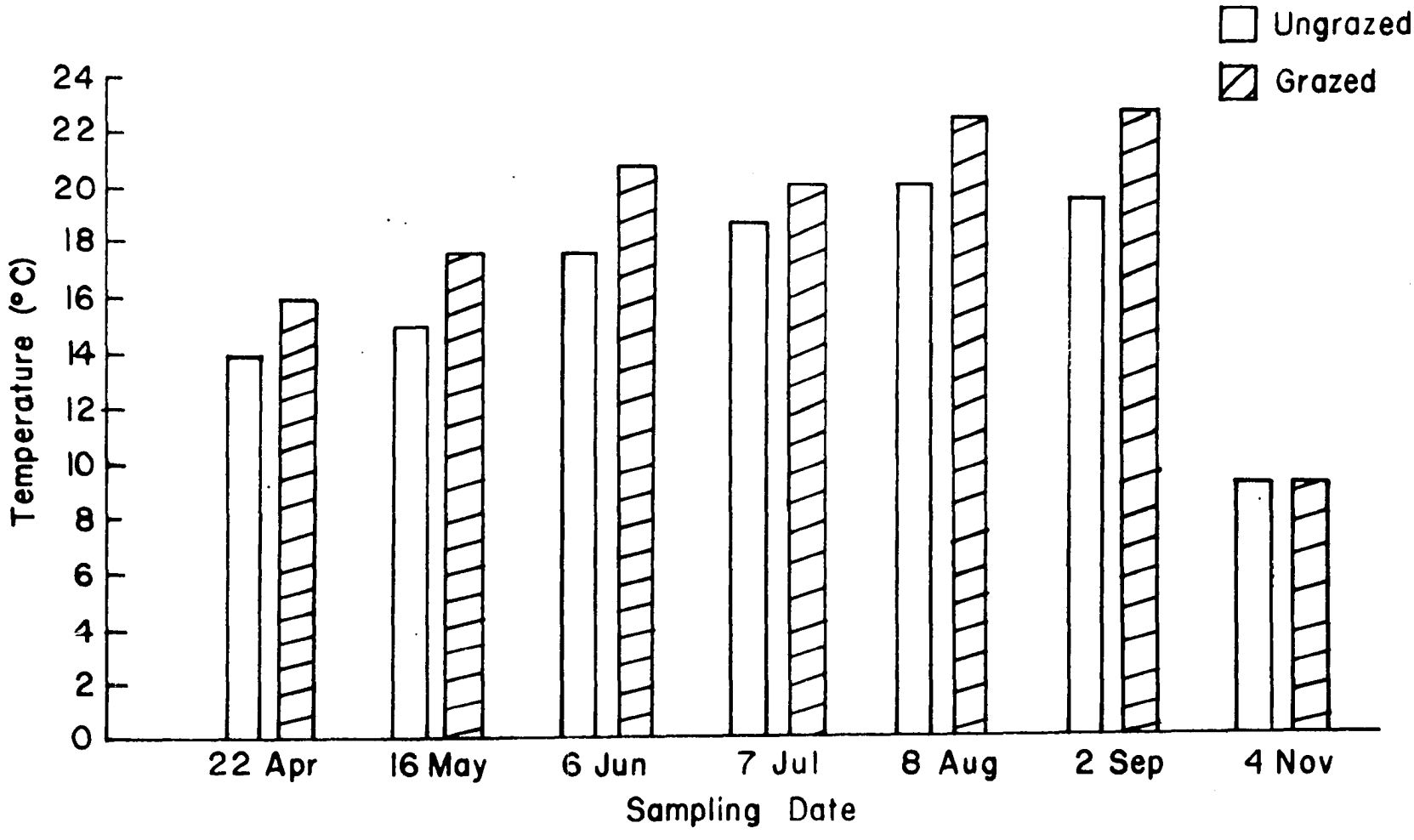

Ungrazed
Grazed
Temperature (°C)
0
2
4
6
8
10
12
14
16
18
20
22
24
22 Apr
16 May
6 Jun
7 Jul
8 Aug
2 Sep
4 Nov
Sampling Date

Table 5. Coefficient of variation for carbon dioxide evolution in the ungrazed and grazed treatments, Osage Site.

Date	Ungrazed	Grazed
April 4	21.53	24.98
May 16	59.91	44.66
June 6	14.38	32.89
July 4	17.34	27.08
August 8	11.51	28.37
September 2	28.34	5.80

Kennedy 1972). It appears that this variability may be the result of disturbance to the natural ecosystem caused by cattle grazing. Further documentation of this phenonemon is needed, however.

Decomposition of Buried Substrate

Decomposition rates for all substrates used are shown in Table 6. Detailed rates are included in Table 2 in the appendix. Cellulose buried at a depth of 5 cm had the fastest decomposition rate, followed by native litter at 5 cm, cellulose on the surface, and native litter on the surface. The rapid decomposition of cellulose is due in part to its occurrence as a homogenous compound (Gray and Williams 1971) and its physical form (Griffin 1972).

Decomposition of samples placed on the ground surface showed lower and more variable decomposition rates than those buried at 5 cm. This is due to variable soil temperature and soil water at the surface as well as the raising of some litter bags off the ground surface by sprouting grass.

Both cellulose and plant litter showed a higher decomposition rate for the first 45 days than for the remaining 63 days. Since soil water was above or at optimal levels for the entire period and soil temperature was gradually increasing throughout the period, the reduction in rate may be due to a decrease in available nitrogen in the microenvironment adjacent to the samples (Russel 1961). Despite the leveling in the decomposition rate, the buried cellulose was 96% decomposed after 3 months. Since cellulose is not a native substrate, its value has been to compare the microbial activity between the two treatments and to indicate changes in the overall decomposition pattern due to environmental parameters.

Table 6. Mean percent lost for each substrate, Osage, 1972.

Date	Treatment	Surface		Buried		
		Cellulose	Litter	Cellulose	Litter	Roots
6 June	Ungrazed	7.71	-7.15	53.88	9.93	-30.45
	Grazed	12.69	10.67	40.82	16.50	-25.95
7 July	Ungrazed	7.67	-0.48	76.00	21.75	-19.83
	Grazed	9.85	4.10	76.00	32.52	-26.38
8 August	Ungrazed	8.24	8.47	96.15	30.80	-18.31
	Grazed	10.58	11.95	96.82	33.30	-10.10

Attempts to measure the decomposition rate of native roots were not successful and thus provided no clear picture of the microbial activity of root decomposition. Samples brought into the laboratory showed no loss of weight, and in some cases, showed a gain in weight. This phenomenon has been described by other workers at several of the sites in the Grassland Biome (Pengra 1972, Coleman personal communication). Reasons for this trend are not clear. Apparently, organic carbon is added to the root material in excess of that lost by decomposition and is not corrected for in the ashing procedure. Pengra (1972) suggested that the preparation of root samples by washing soil cores may have removed nutrients needed for rapid colonization. Roots used at the Osage site had only 0.57% nitrogen compared to 0.76% for native plant litter. In this case, nitrogen may have been a factor in retarding colonization and decomposition of the roots.

The overall rates for decomposition were greater in the grazed treatment than in the native prairie (Fig. 5). Although other factors may be responsible for this, the fact that the soil temperatures are consistently higher in the grazed treatment again suggest that temperature may be a more important abiotic factor in the decomposition process than soil water. Soil water has been shown to be consistently lower in the grazed treatment than in the ungrazed treatment (May and Risser 1972).

A comparison of the decomposition rates for the years 1970, 1971, and 1972 supports this hypothesis. Fig. 6 and

Figure 5. Comparison of decomposition rates of several substrates in both the ungrazed and grazed treatments.

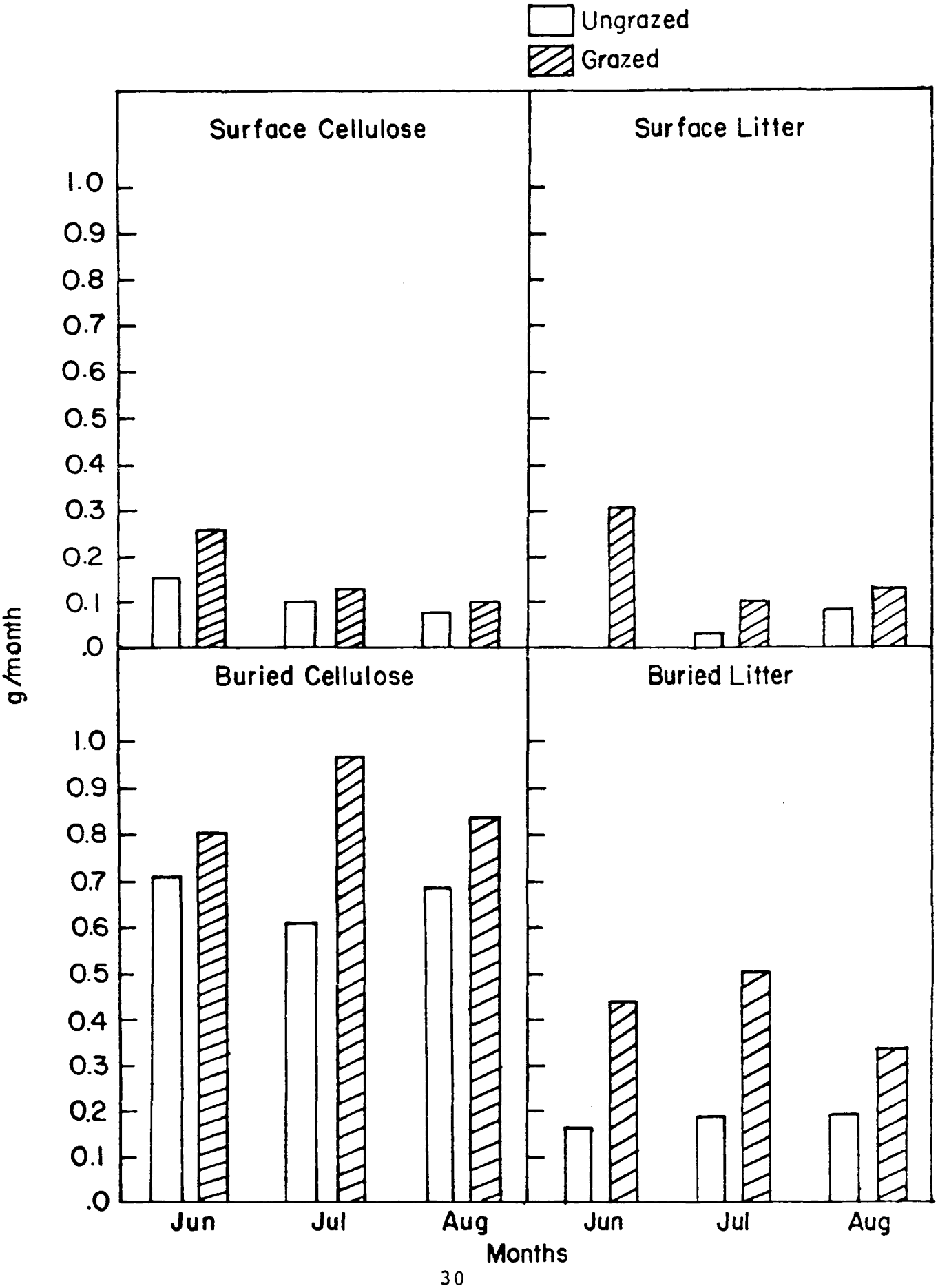

Ungrazed
Grazed
Surface Cellulose
Surface Litter
Buried Cellulose
Buried Litter
g/month
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
.0
Jun
Jul
Aug
Months

Fig. 7 show the distribution of soil temperature and soil water respectively for these three years. Fig. 8 and Fig. 9 show that the highest rates of decomposition for both cellulose and litter occurred in 1970 when the temperature was highest. The lowest rates occurred in 1972 when temperature was lowest.

Figure 6. Distribution of soil temperature at the Osage site for the growing season for 1970, 1971 and 1972.

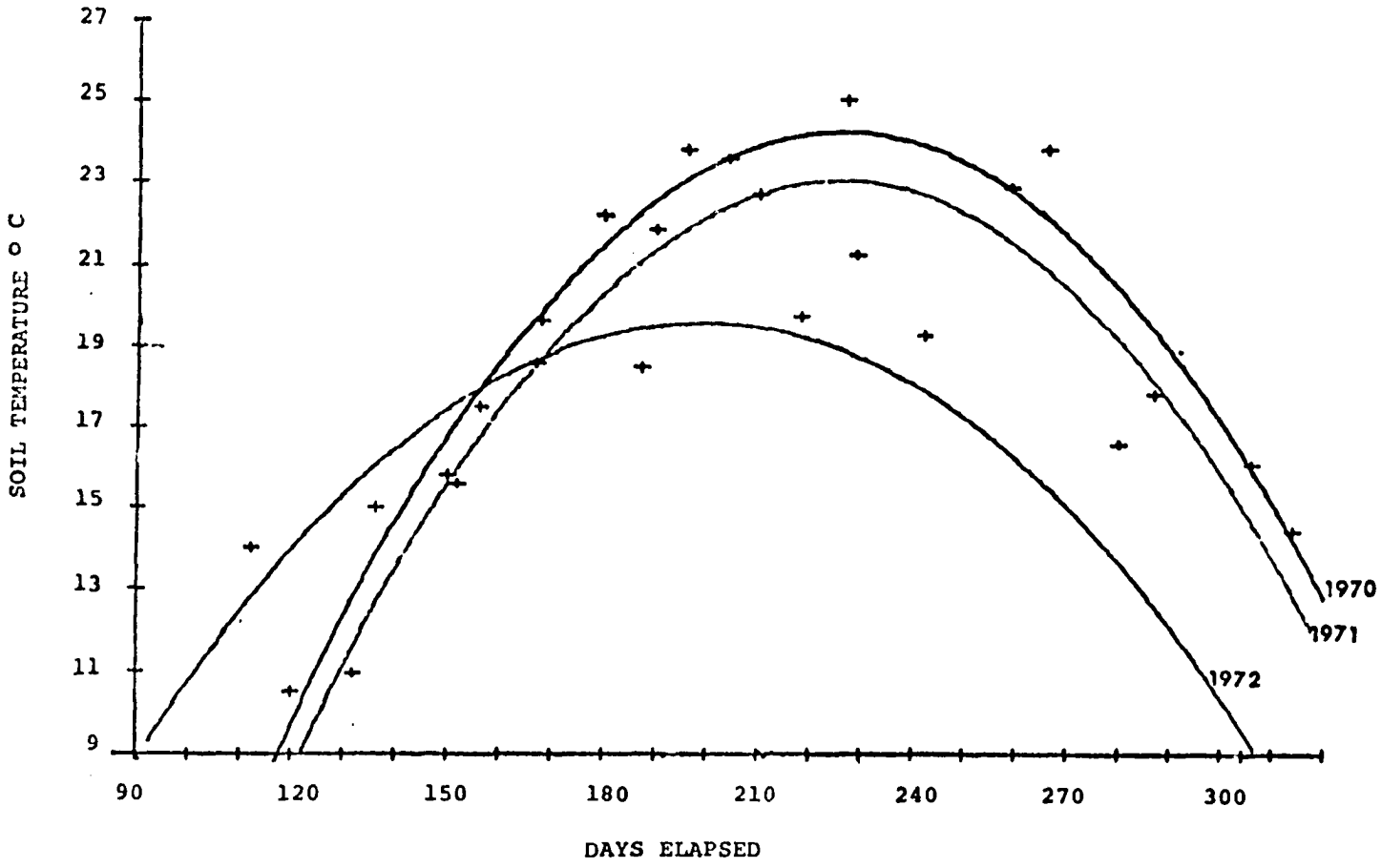
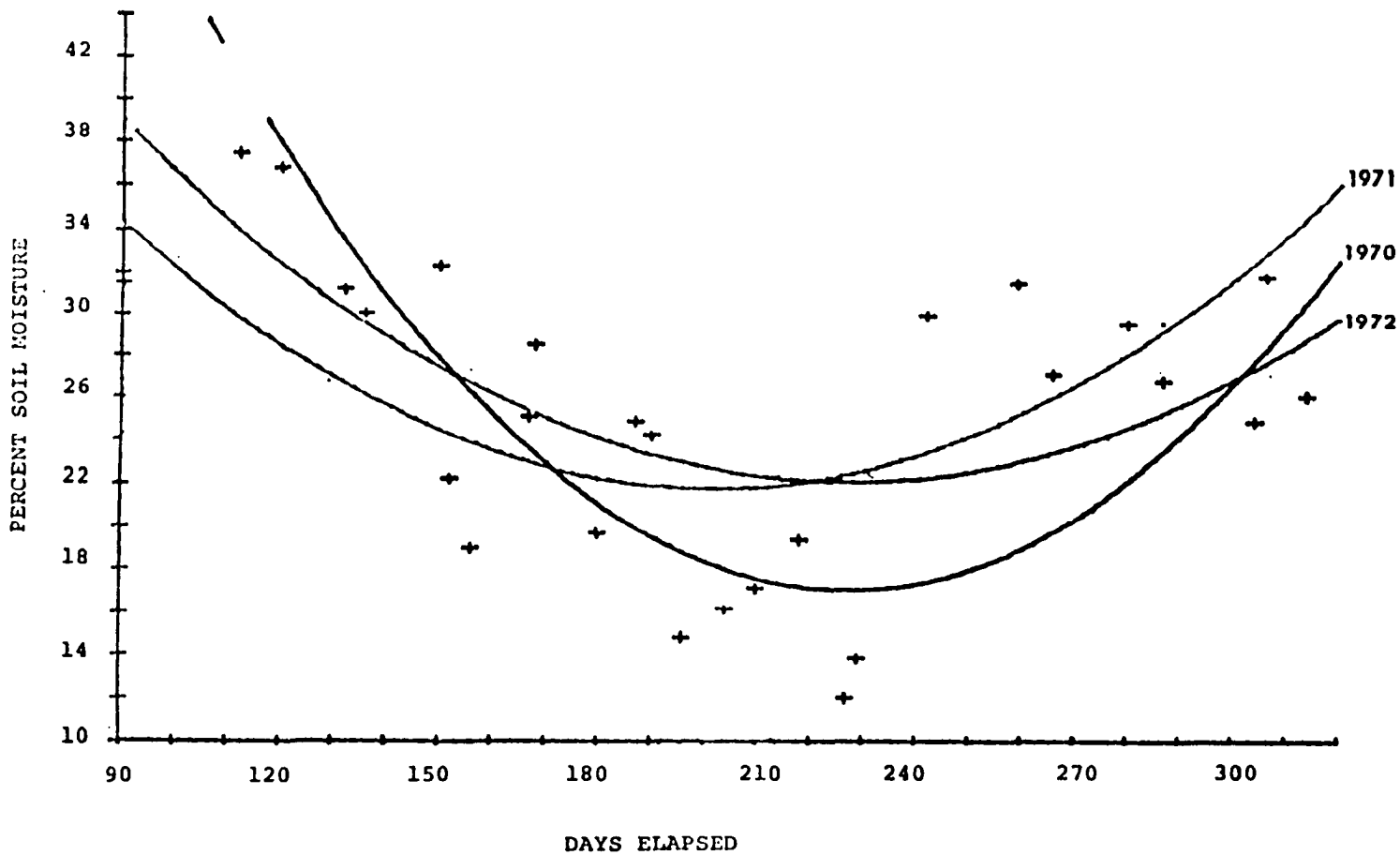

27
25
23
21
19
17
15
13
11
9
SOIL TEMPERATURE ° C
90
120
150
180
210
240
270
300
DAYS ELAPSED
1970
1971
1972

Figure 7. Distribution of percent soil water (gravimetric) for the growing season for 1970, 1971 and 1972.


42
38
34
30
26
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14
10
PERCENT SOIL MOISTURE
90
120
150
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240
270
300
DAYS ELAPSED
1971
1970
1972

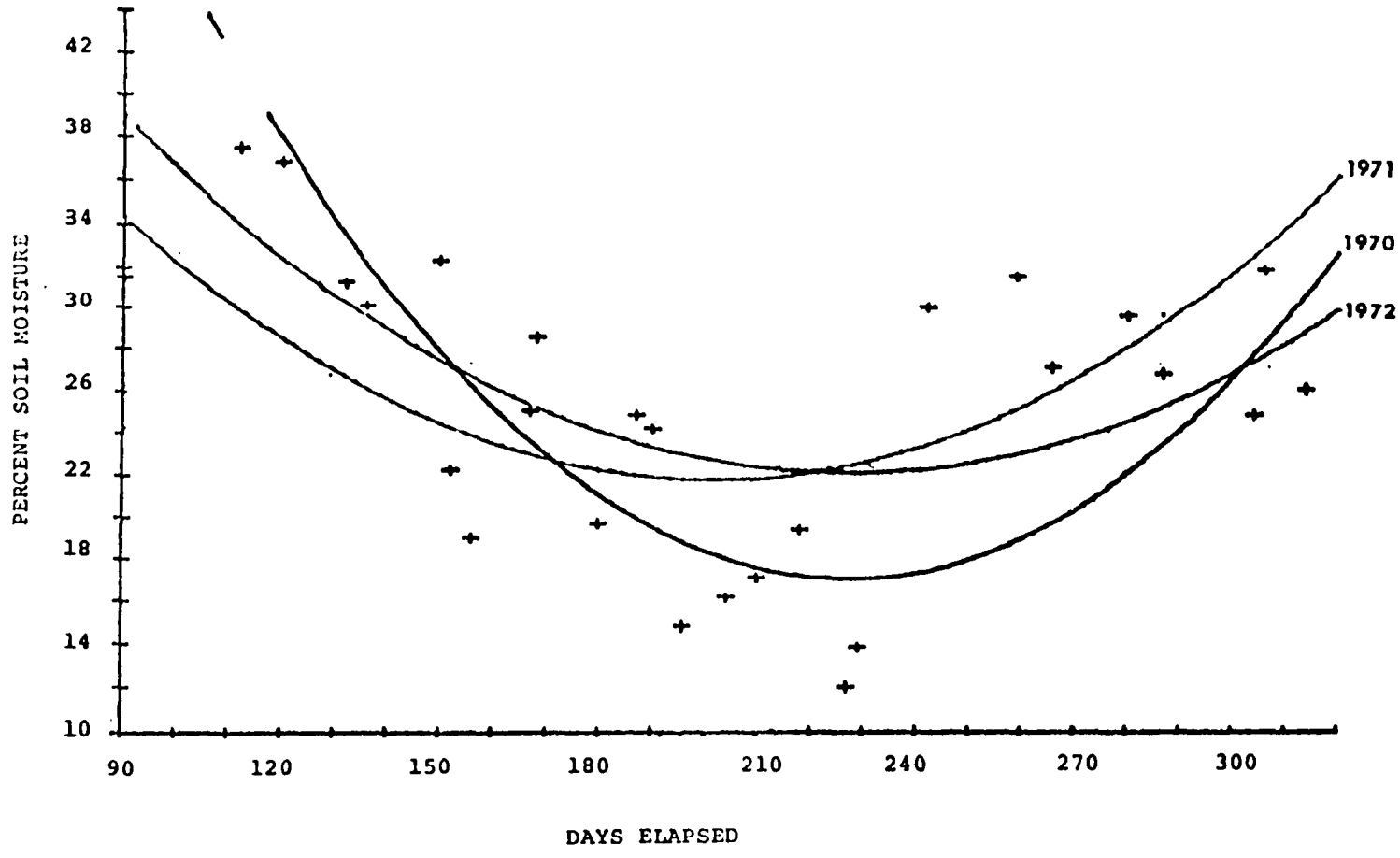

PERCENT SOIL MOISTURE
42
38
34
30
26
22
18
14
10
90
120
150
180
210
240
270
300
DAYS ELAPSED
1971
1970
1972

Figure 8. Cellulose decomposition in g/m^2/month at the Osage site for 1970, 1971 and 1972.

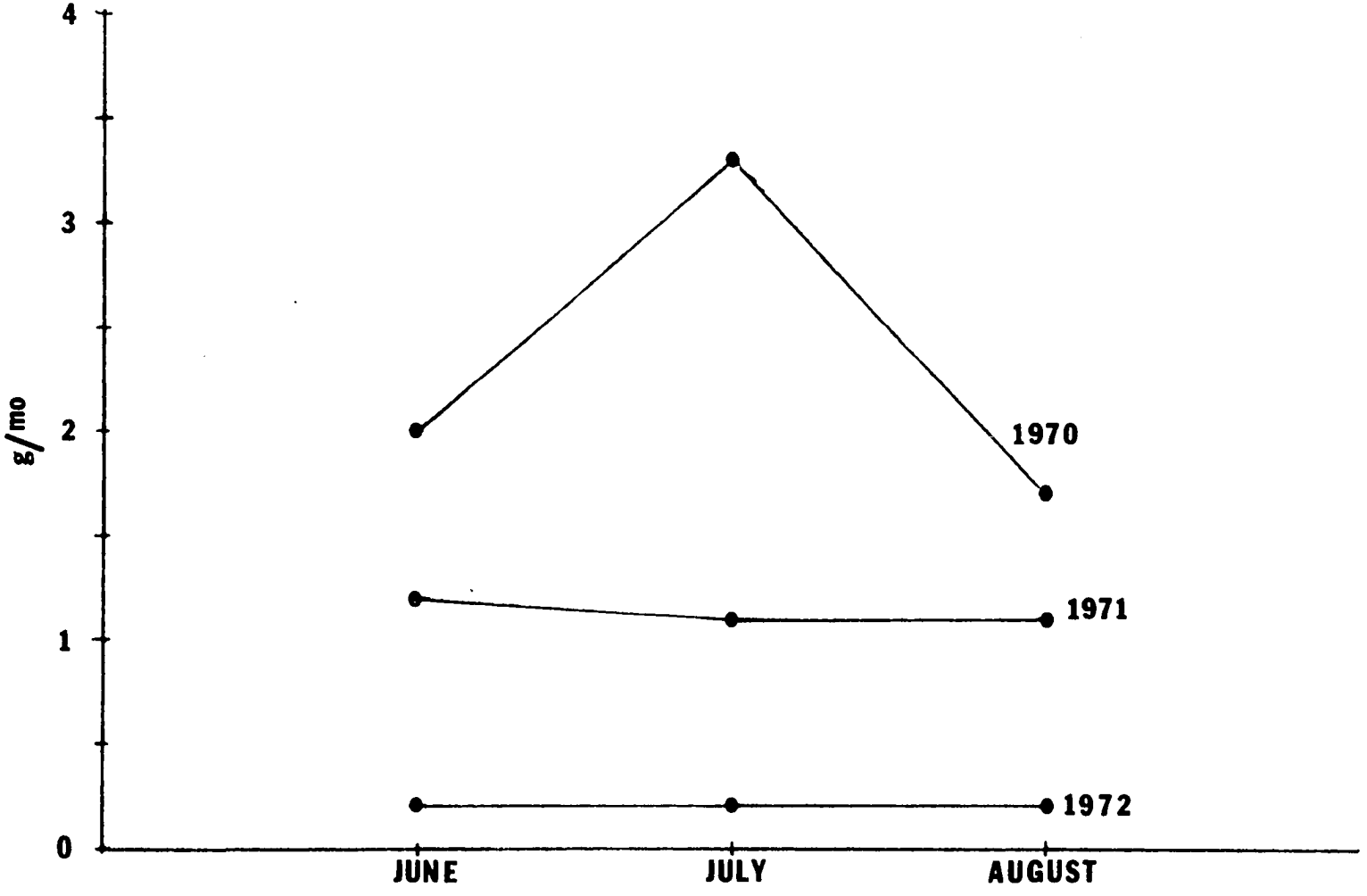
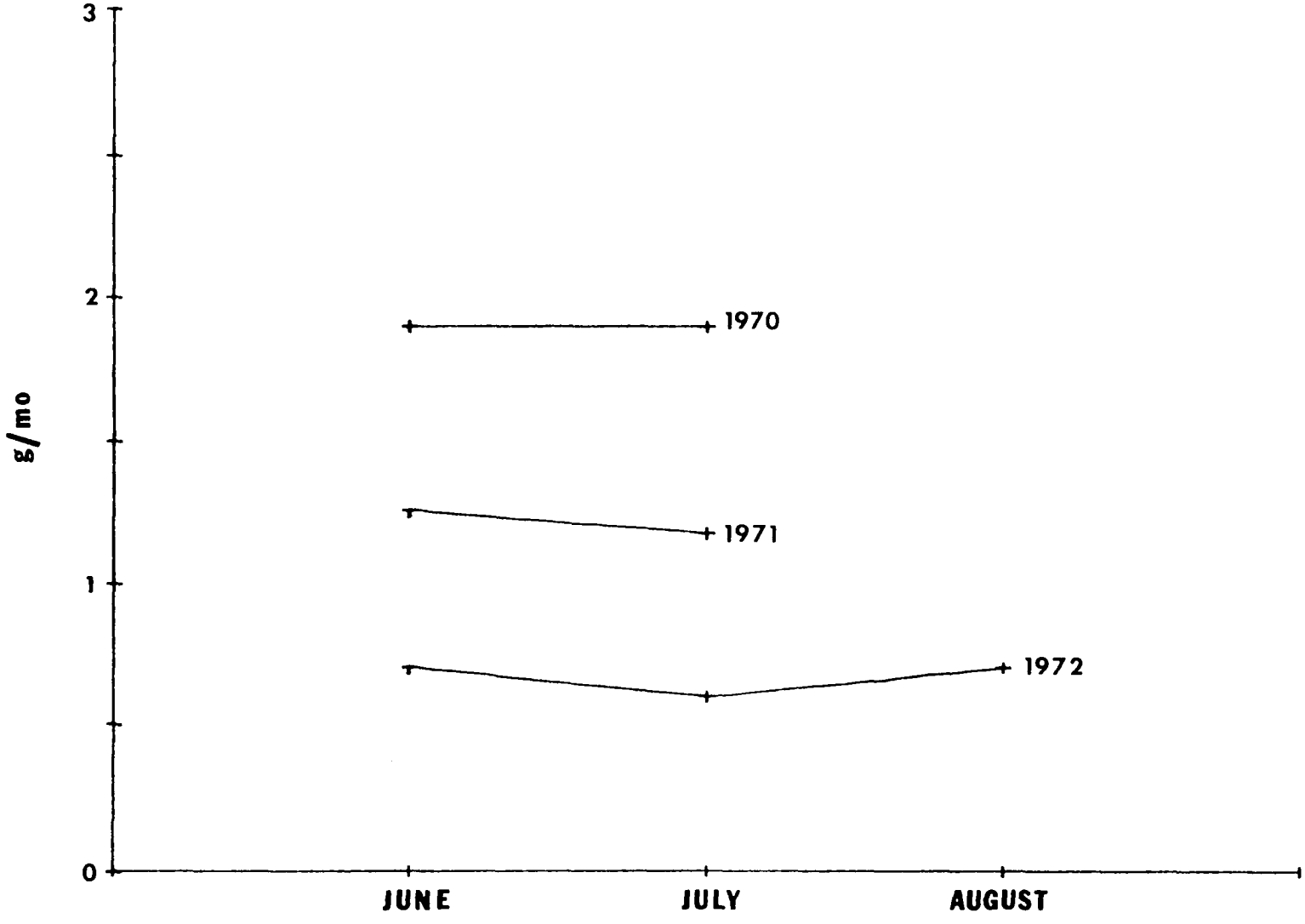

4
3
2
1
0
g/mo
1970
1971
1972
JUNE
JULY
AUGUST

Figure 9. Native litter decomposition in g/m^2/month at the Osage site for 1970, 1971 and 1972.


3
2
1
0
g/mo
1970
1971
1972
JUNE
JULY
AUGUST

CHAPTER THREE

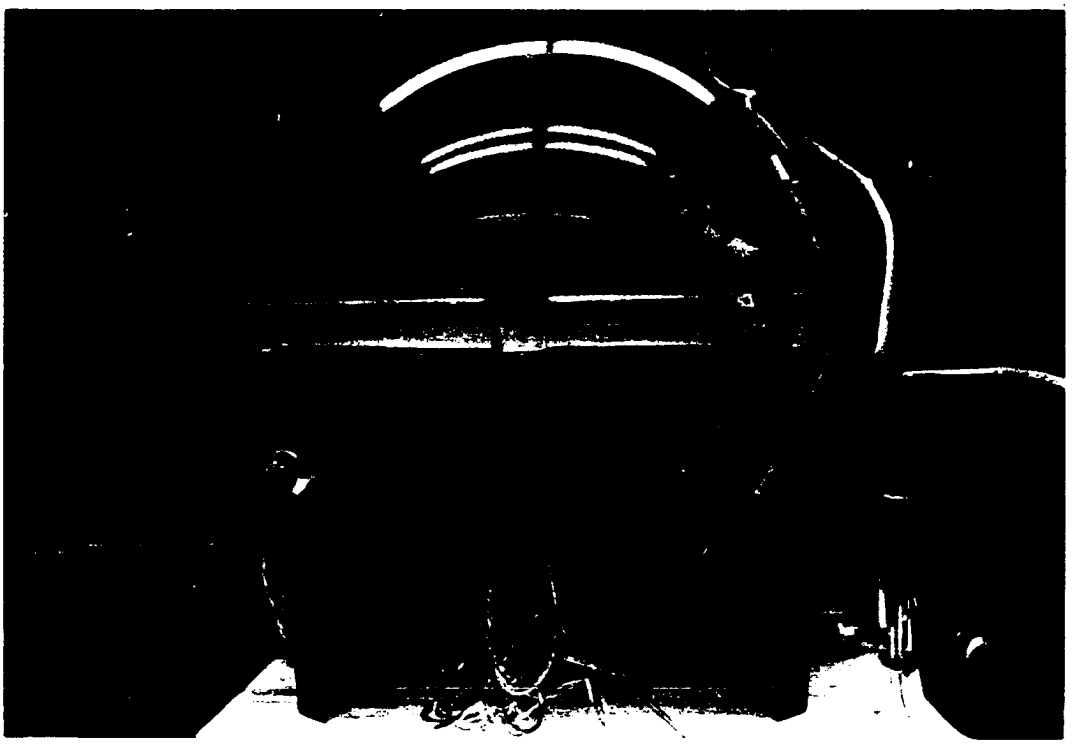
THE SIMULATED ECOSYSTEM

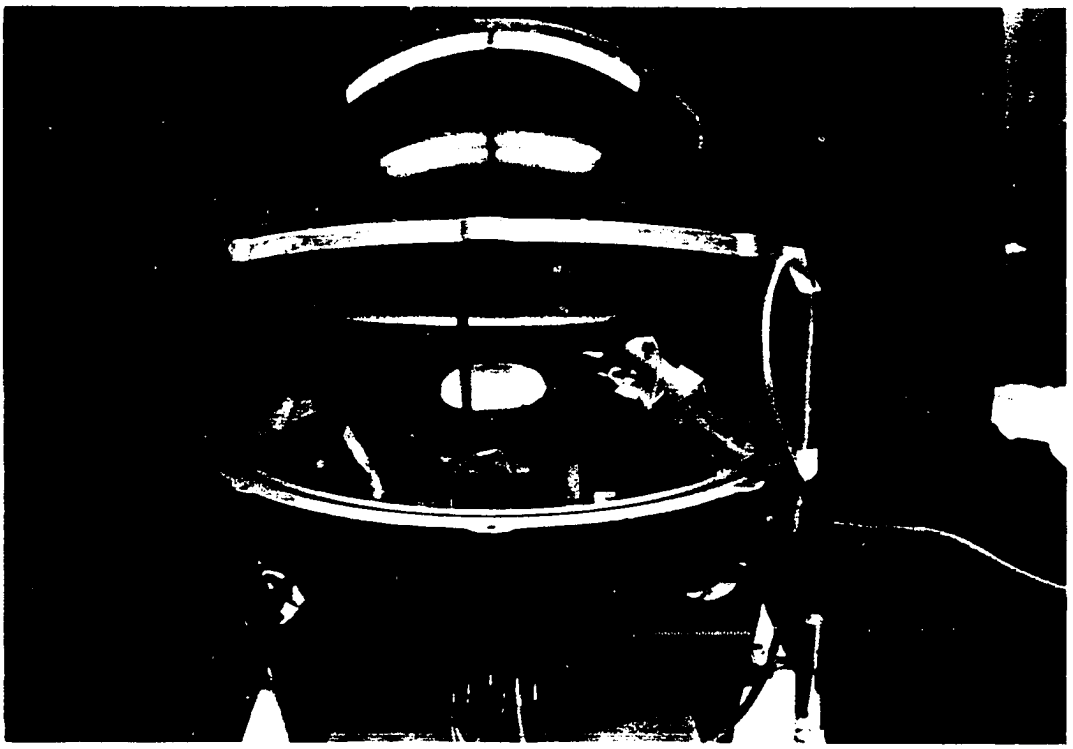
Materials and Methods

The simulated ecosystem used in the laboratory study of decomposition is shown in Fig. 10. The microcosm consisted of a soil core 122.6 cm^2 in surface area and 17.5 cm deep in an air tight sphere 2 ft in diameter. The soil core was obtained from the ungrazed treatment at Osage and placed in a Corning animal jar. Two soil psychrometers (Zollinger, Cambell and Taylor 1966) were placed in the core at depth of 1 and 5 cm to monitor soil water and soil temperature during an experiment. Water was added to the core by an irrigation tube connected to the outside of the sphere, and by a sprinkler system composed of an aspirator attached to a lead to the outside. Temperature was controlled by a high intensity heat lamp connected to a thermostat. For low temperatures, 0 to 10 C, the room temperature was adjusted. Decomposition rates were monitored by the use of ^{14}C-cellulose mixed with native litter. For each experiment 3 g of litter, oven dried to constant weight at 60 C, was mixed with 1 mg, 6.66 μCi, of ^{14}C-cellulose (International Chemical and Nuclear Laboratory). Air flow through the system was main-

Figure 10a. Photograph of the simulated laboratory ecosystem. The ethanolamine trap for $^{14}CO_2$ is seen in the lower right.

Figure 10b. Photograph of the inside of the simulated laboratory ecosystem.



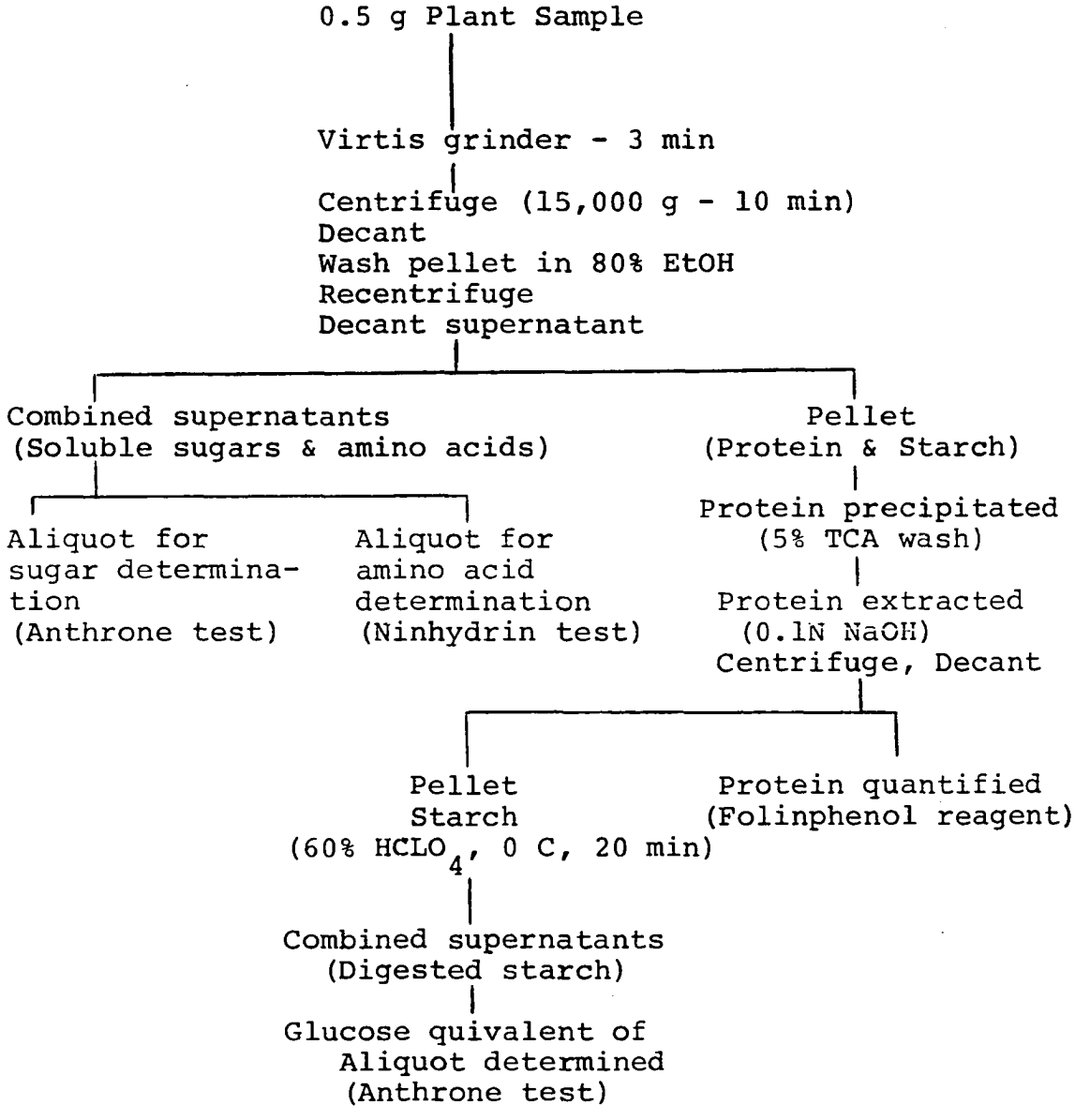


tained by connecting the system between the air input and vacuum valves in the laboratory. This was necessary to assure a steady flow of 20 to 30 cm^3/min through the system. The air outflow passed first through 50 ml of ethanolamine for trapping $^{14}CO_2$ and then a safety trap of KOH.

Decomposition rates were measured at regular intervals by taking 1 ml samples from the ethanolamine trap. CPM were determined in a Beckman LS-100 liquid scintillation counter. Samples were placed into 10 ml of scintillation fluid which consisted of 16 g 2,5 diphenyloxazole, 1 g p-bis-(o-Methylstyryl)-benzene in 1 liter of scintillation grade tolulene. One liter of this solution was mixed with 500 ml of ethanolamine and 500 ml of methanol. Soil temperature and water were monitored regularly through each decomposition experiment.

At the end of each experiment, the litter was carefully removed, dried 24 hr at 60 C, and separated manually, as completely as possible, from the fungal mycelium. The litter was dried at 60 C to constant weight, and the weight loss determined. Chemical analysis of the litter followed a method modified from Dvorak (1973)(Fig. 11). The litter was ground in a Virtis grinder and extracted with 80% ethanol. Trichloroacetic acid (5%) was added to the pellet for 24 hr, followed by protein extraction using 0.1N NaOH. Starch was extracted using 60% perchloric acid. Quantitation was made on soluble carbohydrates and starch by anthrone (Yemm and Willis 1954), on soluble amino acids by ninhydrin

Figure 11. Flow diagram for the chemical analysis of plant litter.


0.5 g Plant Sample
Virtis grinder - 3 min
Centrifuge (15,000 g - 10 min)
Decant
Wash pellet in 80% EtOH
Recentrifuge
Decant supernatant
Combined supernatants
(Soluble sugars & amino acids)
Pellet
(Protein & Starch)
Aliquot for sugar determination (Anthrone test)
Aliquot for amino acid determination (Ninhydrin test)
Protein precipitated
(5% TCA wash)
Protein extracted
(0.1N NaOH)
Centrifuge, Decant
Pellet
Starch
(60% HCLO4, 0 C, 20 min)
Protein quantified
(Folinphenol reagent)
Combined supernatants
(Digested starch)
Glucose quivalent of
Aliquot determined
(Anthrone test)

(Yemm and Cocking 1955) and on protein by Folin phenol (Lowry, Roseborough, Farr and Randall 1951).

The fungi growing on the litter were identified when possible by direct microscopic examination of the decomposing litter. Mycelium and spores were transferred to malt agar for subsequent growth and further identification.

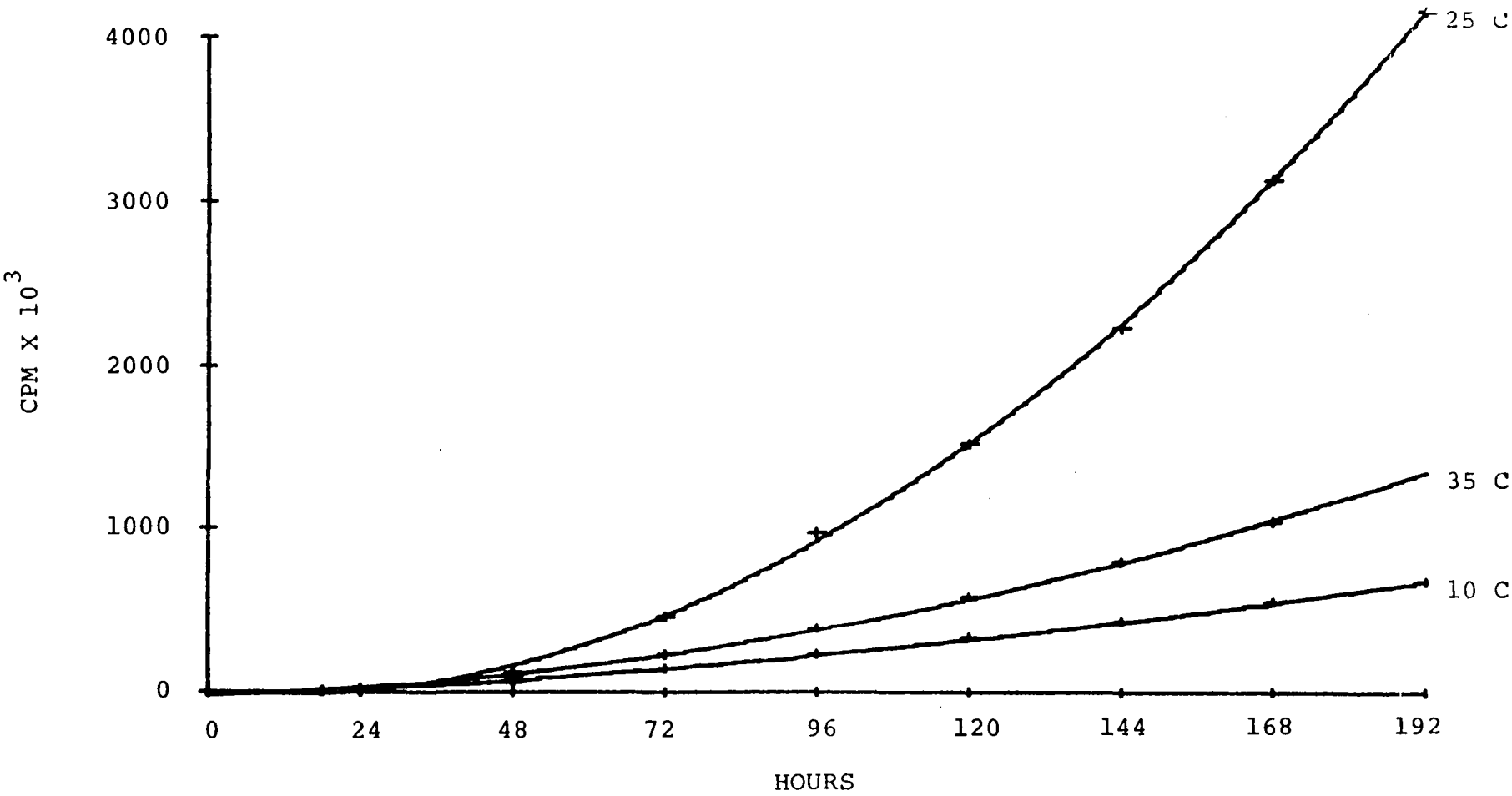
Results and Discussion

Decomposition rates for ^{14}C-cellulose and native plant litter on the soil surface were measured in the laboratory over a range of soil temperature and soil water levels as found in three years of field measurements at the Osage site. The range of soil temperatures in the laboratory were from 6 C to 35 C and the range of soil water from 0 to -40 bars.

Fig. 12 shows a comparison of decomposition rates in CMP for ^{14}C-cellulose and optimum soil moisture (0 to -.1 bars). Decomposition at 25 C was significantly higher than at 10 or 35 C. Although it is not obvious from the graph, the optimal temperature for surface decomposition of cellulose ranged from 23 to 28 C. Decomposition at 35 C was slightly greater for the same time period than at 10 C.

A lag period of 18 hr was detectable for the decomposition curves at all temperatures. This metabolic lag was measured from the time the isotope was added until detectable CPM were measured.

Figure 12. Decomposition in CPM of ^{14}C-cellulose at 10 C, 23 C and 35 C.


4000
3000
2000
1000
0
CPM X 10^3
0
24
48
72
96
120
144
168
192
HOURS
25 C
35 C
10 C

Decomposition rates for plant litter are summarized in Table 7. The rates demonstrate the same pattern in response to temperature found for the decomposition of cellulose. Optimal decomposition occurred at 25 C, followed by 35 C and 10 C.

Fig. 13 illustrates the decomposition pattern for ^{14}C-cellulose at 25 C over a range of soil water values. The curve shows an increase in the decomposition rate as moisture decreases from saturation to -1 bar. The decomposition rate is relatively consistent between 0 and -4 bars. This is the level of soil moisture tension most frequently measured in the tall-grass prairie. This suggests that within this range, soil water does not significantly alter the decomposition rate. At lower soil water levels (-10 to -40 bars) the decomposition rate decreases rapidly.

The chemical composition of native litter used during the short term decomposition experiments is shown in Table 8. Table 9 summarizes the loss for each component at selected temperatures. The pattern shown by the chemical losses reflected the same trend found in the decomposition rates of both cellulose and native litter. Losses for all components were higher at 23-25 C. Except for starch, losses for all other components were higher at 35 C than at 10 C.

The primary pioneer colonizers of plant litter and cellulose in microbial decomposition are the fungi (Gray and Williams 1971). For this reason, only the fungi were

Table 7. Short term decomposition rates of plant litter at 10 C, 25 C and 35 C.

Temperature	Percent lost
10 C	4.22
25 C	16.82
35 C	10.50

Figure 13. Decomposition of ^{14}C-cellulose at 25 C and variable soil moisture in bars.

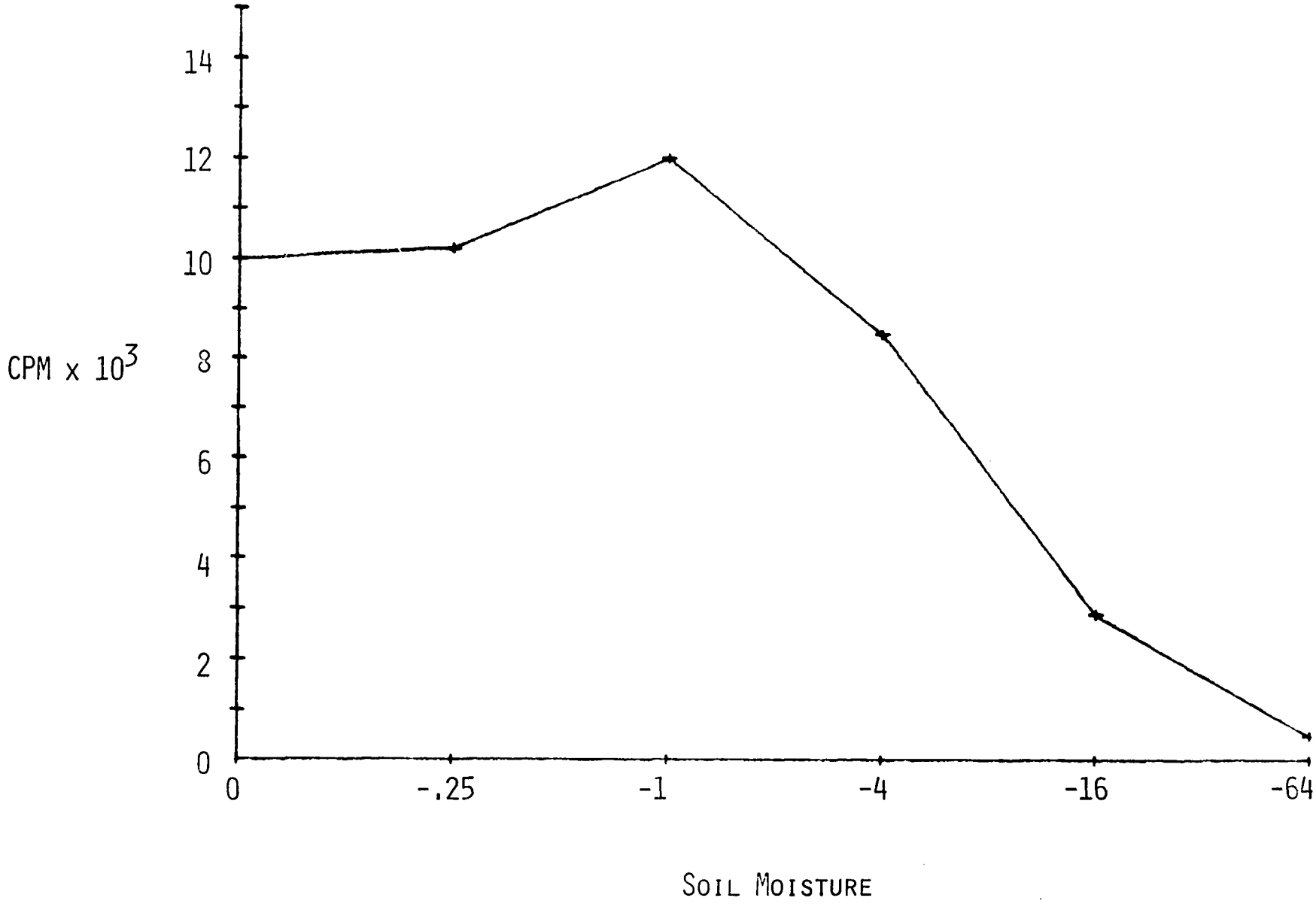

14
12
10
8
6
4
2
0
CPM x 10^3
0
-.25
-1
-4
-16
-64
Soil Moisture

Table 8. Partial chemical composition of native litter used in simulated ecosystem.

Component	Percent
Soluble carbohydrates	3.28
Soluble amino acids	0.18
Starch	4.24
Protein	0.65
Carbon	92.40
Nitrogen	0.76
$\frac{C}{n}$	121.58

Table 9. Chemical changes in native litter after short term (10 days) decomposition.

Measurement	Percent Lost		
	10°	25°	35°
Soluble CHO	76.73	90.13	88.92
Amino Acids	20.33	95.51	19.50
Starch	<1.00	16.25	11.14
Protein	3.16	68.03	44.20

identified in the short term decomposition experiments. While it is apparent that certain genera may have been overlooked by the method used in isolation and identification, it is preferable to dilution plate counts which show all fungi present with no assessment of their activity at the time of plating. Table 10 summarized the genera of fungi identified in this manner.

Table 10. Frequency distribution of fungi identified.

Genus	Frequency
Paecilomyces	100.0
Fusarium	66.6
Tricothecium	33.3
Alternaria	33.3
Cladosporium	16.6
Penicillium	16.6
Circinella	16.6

CHAPTER FOUR

DISCUSSION

Evidence obtained from both field and laboratory studies over a two year period supports the conclusions that microbial decomposition is more significantly related to soil temperature than to soil water between 0 and -4 bars in this grassland. The fact that soil temperature was a more important variable in the decomposition process was first suggested from carbon dioxide evolution experiments in the field. The correlations between carbon dioxide evolution and soil temperature were 0.86 for the native prairie and 0.80 for the grazed prairie. These values were significant to the 0.1 level. Carbon dioxide evolution has been shown to correlate to soil temperature by several workers and was documented in Chapter 2. Although these results raise the possibility that the relationship between carbon dioxide evolution and soil temperature may be in part a physical rather than a purely biological phenomenon, Reiners (1973) maintained that direct measurement of carbon dioxide evolution is of definite value in evaluating responses to environmental and biotic factors. Additional field and laboratory evidence supports this view.

A second line of field evidence supporting the conclusion was obtained from decomposition rates of cellulose and native litter from 1970 to 1972. The highest rates of decomposition occurred during 1970 when the range of soil temperature at the Osage was higher than other years of the study. The second highest decomposition rate occurred in 1971 when the soil temperature showed the second highest and the lowest soil temperature were measured. From the distribution of soil water for these years, it is obvious that the decomposition rates were lower when the soil water was higher.

Another aspect of the field data which tends to support the conclusion is the comparison of decomposition rates and carbon dioxide evolution between the ungrazed and grazed areas. The results of the investigations showed that higher rates for both decomposition and carbon dioxide evolution were measured in the grazed treatment. The grazed treatment was shown to have higher soil temperature and lower soil water than the ungrazed treatment. There are other differences between the two treatments but these do clearly relate to decomposition. The plant cover is more variable and lighter in the grazed treatment (Risser and Kennedy 1972; Conant and Risser 1974). The lighter plant cover exposes more of the ground to direct sun rays which may account for the higher soil temperature and lower soil water. Examination of soil chemical data (Table 1, Appendix) indicates that

both treatments are comparable in available nutrients and pH. The fact that the grazed area is exposed to cattle during part of the year presents another variable. The trampling and grazing of cattle on the prairie may enhance the decomposition of litter but this has not been investigated.

Data obtained in the laboratory on short term decomposition rates in the simulated ecosystem indicate further that soil temperature is a more important driving variable in the decomposition process than soil water. The decomposition rate varied only slightly when measured over the range of soil water (0 to -4 bars) characteristic of the Osage site. However, the decomposition rates varied greatly as a function of temperature. Optimal decomposition rates were obtained between 23 and 28 C, while decomposition of cellulose at 33 to 35 C fell off significantly. This suggests that the correlation between soil temperature and microbial decomposition becomes less significant and even limiting at higher temperatures. Drobnik (1962) showed that up to 28 C, soil respiration proceeded normally, but at 38 C partial inhibition of respiration occurred. Decomposition at 10 C showed the lowest rate. Generally, between 5 and 30 C, the lower the temperature, the lower the rate of decomposition (Russel 1961). Although bacteria are important in the decomposition process, it is the fungi that are the initial colonizers of substrate (Gray and Williams 1971). The fact that most fungi grow better between 20 and 30 C

(Alexopoulos 1962) would partly explain the optimal decomposition of cellulose between 23 and 28 C.

Decomposition rates for plant litter measured by weight loss followed the same trend in decomposition shown by labelled cellulose. The greatest loss was between 23 and 28 C, followed by 35 C and 10 C respectively. However, the Q_{10} for litter decomposition over the range from 10 to 25 C is 3.18 while the Q_{10} for cellulose is 6. This lower Q_{10} for plant litter is due in part to the protection of some components in the litter by their association with other substances, e.g. lignin (Gray and Williams 1971).

Chemical analysis of soluble amino acids, soluble carbohydrates, starch, and protein following short term decomposition of 10 days showed similar results for the same temperature ranges as that indicated by cellulose and litter decomposition. All components measured showed optimal loss at 23 to 28 C, a smaller loss from 33 to 35 C and the smallest loss at 10 C.

Evidence from long term field investigations on decomposition and carbon dioxide evolution and from short term decomposition of labelled cellulose and plant litter in a simulated ecosystem indicate that soil temperature is a more important variable than soil water in microbial decomposition in a tall-grass prairie. This is important in understanding the response of microorganisms to the environmental parameters in a native true prairie ecosystem and to the accurate modeling of this ecosystem.

The decomposition rate for buried plant litter has provided the most useful data for carbon modeling. For the 108 days the samples were buried, the litter in the native prairie showed a decomposition rate of approximately 9% per month and a total loss of 31%. Assuming that this monthly decomposition rate was maintained until November (soil water and soil temperature are still near the optimum for this period) no more than 57% of the litter would be decomposed. Decomposition rates for cellulose during the winter have been shown to be only 9% of the summer rate (Harris, unpublished). If this were true for litter only 2.7% of the biomass would be decomposed over the winter. Thus, only about 60% of the litter biomass is turned over by microbial activity. Jenkinson (1971) reported that about one third of the added plant carbon remains after one year. The results of our study in the true prairie tend to support this view.

CHAPTER FIVE

SUMMARY

Evidence from long term field investigations of microbial decomposition and carbon dioxide evolution and from short term laboratory studies on microbial decomposition of ^{14}C-cellulose and native grass litter in a simulated ecosystem indicate that soil temperature is a more important abiotic variable than soil water in the microbial decomposition of a true, tall-grass, prairie. This is important in understanding the response of microorganisms to the environmental parameters in a native ecosystem and to the accurate modeling of this ecosystem.

Within the range of soil water between 0 and -4 bars and a range of soil temperature from 10 to 25 C, the Q_{10} for the decomposition rate is 3.2 and 6.0 for native litter and cellulose, respectively. Under optimal conditions, prairie litter lost 90% of the soluble carbohydrates, 95% of the amino acids, 16% of the starch and 68% of the protein in 10 days.

An estimate of the annual decomposition rate based on this study suggests that no more than 60% of the annual

litter production in the tall-grass prairie, Osage site, is recycled by microbial activity.

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APPENDIX

Table 1. Soil Chemical Analysis

A. TOTAL N (WEIGHT PERCENT)

Depth	Ungrazed 1	Ungrazed 2	Grazed 1	Grazed 2
0-5	0.217	0.267	0.293	0.242
5-10	0.196	0.237	0.220	0.196
10-20	0.174	0.205	0.198	0.171
20-30	0.160	0.175	0.170	0.154
30-40	0.137	0.147	0.157	0.135
40-50	0.117	0.120	0.142	0.110
50-60	0.095	0.093	0.123	0.086

B. TOTAL P (PARTS PER MILLION)

0-5	338	378	338	338
5-10	313	363	300	313
10-20	288	338	288	320
20-30	263	313	263	313
30-40	313	363	270	338
40-50	288	363	270	300
50-60	345	378	270	188

C. pH

0-5	5.9	5.9	5.9	6.0

D. NO_3 (PARTS PER MILLION)

0-5	0.300	0.900	0.500	0.400
5-10	0.400	0.400	0.800	0.800
10-20	0.100	0.300	0.300	0.400
20-50	0.400	0.300	0.100	0.300

E. NH_4 (PARTS PER MILLION)

0-5	7.00	8.80	7.80	7.30
5-10	6.40	5.50	8.70	6.10
10-20	5.70	5.80	7.60	5.40
20-50	7.40	7.30	5.80	5.30

Table 1. (Continued)

F. EXCHANGEABLE Ca (MEQ/100g)

0-5	9.48	12.35	11.98	10.73
5-10	8.23	11.35	10.73	10.03
10-20	8.24	11.73	10.60	11.03
20-30	8.53	11.98	10.85	11.35
30-40	9.11	12.79	10.79	11.60
40-50	9.86	14.35	11.60	13.60
50-60	11.35	15.59	12.48	14.97

G. EXCHANGEABLE Mg (MEQ/100g)

0-5	1.69	1.82	2.26	2.08
5-10	1.54	1.63	2.06	2.09
10-20	1.46	1.54	1.95	2.07
20-30	1.51	1.41	1.95	2.06
30-40	1.48	1.16	1.79	1.95
40-50	1.42	1.00	1.73	2.16
50-60	1.40	0.93	1.60	2.22

H. EXCHANGEABLE Na (MEQ/100g)

0-5	0.08	0.14	0.11	0.12
5-10	0.08	0.08	0.10	0.10
10-20	0.10	0.12	0.10	0.12
20-30	0.08	0.14	0.12	0.14
30-40	0.12	0.16	0.12	0.16
40-50	0.12	0.18	0.14	0.18
50-60	0.14	0.20	0.10	0.22

I. EXCHANGEABLE K (MEQ/100g)

0-5	0.78	0.66	0.42	0.74
5-10	0.50	0.38	0.46	0.32
10-20	0.32	0.32	0.34	0.30
20-30	0.26	0.36	0.32	0.32
30-40	0.34	0.37	0.34	0.34
40-50	0.34	0.44	0.32	0.34
50-60	0.40	0.44	0.34	0.44

Table 1. (Continued)

J. CATION EXCHANGE CAPACITY

0-5	18.8	23.0	21.6	20.7
5-10	19.2	21.0	20.6	20.3
10-20	18.6	21.2	20.3	21.3
20-30	18.2	23.0	19.6	20.5
30-40	18.2	22.0	19.3	20.6
40-50	19.6	27.6	20.3	23.0
50-60	22.0	25.0	21.6	24.0

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Table 2. (Continued)

Part B. Cellulose at 5 cm.

	Sample Date	Time (days)	Decomposition rates g/month	g/period	Total % lost %/month	%/period
UNGRAZED	June 6	45	0.7285 ± 0.0309	1.0770 ± 0.2829	36.45	53.88
	July 5	74	0.6248 ± 0.0152	1.5191 ± 0.2706	31.26	76.00
	August 8	108	0.6745 ± 0.0032	2.3823 ± 0.4632	27.10	96.15
GRAZED	June 6	45	0.8278 ± 0.0352	1.2238 ± 0.3131	27.61	40.82
	July 5	74	0.6298 ± 0.0152	1.5191 ± 0.2206	31.26	76.00
	August 8	108	0.8181 ± 0.0050	2.9027 ± 0.1070	27.29	96.82

Table 2. (Continued)

Part C. Native Litter at 0 cm.

	Sample Date	Time (days)	Decomposition rates g/month	Decomposition rates g/period	Total % lost %/month	Total % lost %/period
UNGRAZED	June 6	45	-0.1121± 0.0132	-0.1658± 0.0597	-4.84	-7.15
	July 5	74	-0.0046± 0.0085	-0.0112± 0.0623	-0.20	-0.48
	August 8	108	0.0554± 0.0113	0.1965± 0.1199	2.39	8.47
GRAZED	June 6	45	0.2656± 0.1408	0.3926± 0.8898	6.87	10.67
	July 5	74	0.0652± 0.0006	0.1585± 0.0337	1.69	4.10
	August 8	108	0.1301± 0.0172	0.4678± 0.1561	3.37	11.95

Table 2. (Continued)

Part D. Native Litter at 5 cm.

	Sample Date	Time (days)	Decomposition rates g/month	Decomposition rates g/period	Total % lost %/month	Total % lost %/peiod
UNGRAZED	June 6	45	0.1557± 0.0284	0.2302± 0.1764	6.71	9.93
	July 5	74	0.2075± 0.0203	0.5044± 0.1708	8.95	21.75
	August 8	108	0.2013± 0.0123	0.7143± 0.1311	8.98	30.80
GRAZED	June 6	45	0.4313± 0.0296	0.6377± 0.1754	11.16	16.50
	July 5	74	0.5170± 0.0653	1.2569± 0.4807	13.38	32.52
	August 8	108	0.3627± 0.0201	1.2870± 0.2797	9.38	33.30

Table 2. (Continued)

Part E. Native Roots at 5 cm.

	Sample Date	Time (days)	Decomposition rates g/month	Decomposition rates g/period	Total % lost %/month	Total % lost %/period
UNGRAZED	June 6	45	-0.7922± 0.0163	-1.1713± 0.0871	-20.60	-30.45
	July 5	74	-0.3137± 0.0221	-0.7628± 0.1642	-8.16	-19.83
	August 8	108	-0.1984± 0.0157	-0.7041± 0.1666	-5.16	-18.31
GRAZED	June 6	45	-0.5625± 0.0361	-0.8813± 0.1776	-17.55	-25.95
	July 5	74	-0.3477± 0.0390	-0.8454± 0.2915	-10.85	-26.38
	August 8	108	-0.0912± 0.0246	-0.3237± 0.2634	-2.85	-10.10