Technical Report No. 126

DECOMPOSER STUDIES AT THE COTTONWOOD SITE

Jack Turner and Robert M. Pengra

Bacteriology Department

South Dakota State University

Brookings, South Dakota

GRASSLAND BIOME

U. S. International Biological Program

August 1971

TABLE OF CONTENTS

	Page
Title Page	i
Table of Contents	ii
Abstract	iii
Carbon Dioxide Measurement	1
Introduction and Literature Review	1
Materials and Methods	3
Procedure for trapping CO_2	3
Analysis of CO_2 samples using the gas analyzer	5
Cellulose and Litter Decomposition	7
Introduction and Literature Review	7
Materials and Methods	8
Results and Conclusions	10
Literature Cited	15

ABSTRACT

Carbon dioxide collection and measurement for a known surface area and time period was made in an attempt to assess decomposer activity under good and poor range conditions. Data collected and the method employed have aided in devising methods that will be more quantitative for use in subsequent studies.

Through litter and filter paper studies we have also developed and adapted methods to be used in further studies of decomposer activity at the Cottonwood Site.

CARBON DIOXIDE MEASUREMENT

Introduction and Literature Review

If we assume that an energy value can be set for soil organic matter and that we can measure organic matter decomposition in soil by measuring CO_2 liberated through microbial decomposition of that organic matter, then we can measure the energy trapped by primary producers that is lost from the ecosystem by "soil respiration." Included in soil respiration are plant-root and soil-animal respiration as well as heterotrophic breakdown of organic matter by microbes. We will eventually need to separate the role of each of these components, but it is sufficient for this study to lump these as energy loss through "soil respiration."

There are several techniques that can be used to determine the amount of CO_2 evolved from the soil. The object of this paper is to describe the methods which we have tried. We have evaluated our methods by their ability to provide data needed to determine the energy released from decomposition of soil organic matter in our grasslands ecosystem.

The most common method of CO_2 collection is absorption of CO_2 in an excess of known strength dilute alkali solution followed by back titration with a standard acid. These titration data can be converted into the weight of CO_2 trapped. This method was used by Wallis and Wilde (1957). Utilizing a small vacuum pump attached to a canopy, they circulated the air from the canopy through a series of vessels containing dilute alkali. They showed that when samples were taken hourly there was a rapid evolution of CO_2 followed by a leveling off in about two to three hours. This burst of CO_2 in the first few hours probably reflected the reservoir of CO_2 stored within

the soil. The pump created a partial vacuum on the canopy unless the rate of pumping was very slow. This method of measurement is useful if one is measuring the relatively high rates of evolution of CO_2. Wallis and Wilde (1957) measured amounts of 2.2 g to 7.0 g of CO_2 liberated per square meter per hour from forest soils.

Carbon dioxide concentration can be determined by measuring its partial pressure with a special electrode attached to a pH meter. The measure of CO_2 is made under a canopy placed over the soil. This type of detector is manufactured by Chemtronics, Inc. of San Antonio, Texas and will measure from 2 to 100 mm Hg CO_2 partial pressure. The manufacturer states that these data correlate well with measures made using a gas chromatographic method. It has possibilities for field analysis.

Several other methods are available. These methods include gas-liquid chromatography, equilibrium pH alkali solutions, electrical conductivity of deionized water, and thermal conductivity. All of these methods have several things in common: they require a considerable amount of manipulation; they require some type of collection device; and some require expensive equipment such as a gas-liquid chromatograph or a mass spectrometer. Gravimetric and titrimetric methods require considerable manipulation and are subject to errors.

The use of a pump to circulate the atmosphere from under a canopy through a dilute alkali solution appears to bias the samples. It is not only measuring CO_2 from the area under the canopy, but is also drawing the air out of the adjacent soil by creating a partial vacuum.

The best available method of monitoring CO_2 content of a gaseous sample is the use of an infrared spectrophotometer such as the Beckman IR 215 gas

analyzer. This instrument can be adapted to measure large amounts of CO_2 from 0 to 20%, or very small amounts from 0 to 600 ppm. This is the method used by Johnson and Kelly (1970) in an Arctic study and by Kucera and Kirkham (1970) at the University of Missouri in a study of CO_2 evolved from soil in the tallgrass region of Missouri. Kucera and Kirkham determined CO_2 evolved from the soil and also the biomass of roots in an attempt to calculate the CO_2 from root respiration. They found that the roots contribute as much as 40% of the CO_2 evolved. Other authors think this may be even higher (Wiant 1967). Kucera and Kirkham indicated in their study that they obtained 450 mg CO_2/m^2/hour for an annual total of 1675 g CO_2/m^2. We assume that the root respiration was included in this total as they did not indicate otherwise.

Witkamp (1969) was able to show that CO_2 evolution was cyclic during a 24-hour period. He observed a midday maximum and a minimum during late evening and early morning.

Materials and Methods

The remainder of this section describes a simple technique that was developed employing both absorption of the CO_2 into an alkali solution and the use of the Beckman IR 215 gas analyzer.

Procedure for trapping CO_2. The procedure first involved trapping the CO_2 in a "CO_2-free" solution of 1N NaOH. This solution was returned to the laboratory where the CO_2 was released by acidification and measured using the gas analyzer.

The CO_2-free solution of 1N NaOH was prepared from a commercially prepared NaOH solution which was guaranteed to be CO_2-free. Twenty-five ml of this solution was dispensed into each of 25 25-ml serum bottles. To

insure that a minimum of CO_2 would be absorbed during transfer, the solution was made up in a separatory funnel. To facilitate this a piece of rubber tubing was attached to the separatory funnel, and a hypodermic needle was attached to the other end of the rubber tube. The transfer was done in this manner: enough NaOH was bled out to fill the tubing; the needle was pushed into the serum stopper; and another needle was placed beside the first needle. The second needle allowed gas to escape as the desired amount of NaOH was let into the serum bottle.

Carbon dioxide evolution was measured immediately after vegetation, litter, insects, and a core soil sample had been removed from the test plot. The CO_2-collecting vessel was a 25-ml plastic funnel with the stem sealed. The canopy employed was a metal cylinder, open at one end and covering an area of 0.1 m^2. Two holes in the top permitted insertion of a thermometer and a rubber serum stopper through which NaOH could be "injected" into the funnel. The edges of the canopy were sharpened to facilitate cutting of roots when it was pushed down into the soil.

Following cleaning of the soil surface and extraction of the soil sample, the stem end of the funnel was pushed into the soil to a depth sufficient to maintain it in a vertical position, and the canopy was placed over the top and pushed into the soil to a depth of 2 cm. A 10-ml plastic syringe was used to inject the 1N NaOH through the stopper into the collecting funnel. The NaOH was allowed to remain under the canopy for 10 minutes to remove the initial CO_2. This NaOH was removed, again using the syringe, and 10 ml of distilled H_2O was injected into the vessel and removed to clean out the remaining alkali. Then 7 ml of 1N NaOH was injected into the funnel and allowed to remain for 30 minutes, after which time 3 ml was removed and

placed in an Evaco tube (Beckman-Dickers). The remaining alkali was removed at the end of 60 minutes and again was placed in an Evaco tube. These tubes had been flushed with nitrogen three times to remove all CO_2 and then evacuated down to 0.95 mm of Hg. The partial vacuum made injection of the NaOH easier. Three ml of CO_2-free NaOH was placed in a tube for a zero time sample. The temperature of the soil surface inside the canopy and 5 cm below the surface was recorded at 0, 30, and 60 minutes.

In order to maintain a constant temperature inside the canopy, it was necessary to provide shade. When the shade was not used, the canopy temperature increased from 30°C to as high as 50°C. The samples from 20 plots were brought back to the laboratory at Brookings to finish the analysis.

Analysis of CO_2 samples using the gas analyzer. The Beckman IR 215 gas analyzer belongs to the USDA Northern Grain Insects Laboratory which is located at Brookings.

In order to measure the CO_2 in the samples, a 250-ml Ehrlenmeyer flask was connected to the machine. The flow of air was from a pump which pumped 1.8 liters per minute through the flask into the machine and back through the flask forming an airtight continuous flow system. The whole system was flushed with nitrogen gas. When the meter on the gas analyzer read zero, indicating that no CO_2 was present, the system was closed to the atmosphere. One ml of the CO_2-containing NaOH sample was injected into the 250-ml flask through a rubber tube attached at the top of the flask. This was followed by the addition of 3 ml of 5N H_2SO_4 to release the CO_2. The concentration of CO_2 was then registered as milliamperes on a read-out chart. The milliamperes were then converted to ppm using a graph provided by Beckman Instrument Company. A different 250-ml Ehrlenmeyer flask was used for each sample. In

order to test this system we attached a 250 ml Ehrlenmeyer flask, as described above, and filled the system with a gas of known CO_2 concentration. Then, with the pump running, we added 1 ml of 1N NaOH which immediately removed all CO_2. After the meter reading returned to zero we acidified the CO_2-containing NaOH solution by adding 3 ml of 5N H_2SO_4. The reading immediately returned to its original position reflecting the known CO_2 concentration.

The results show that there is a difference between good and poor range condition plots in the amount of CO_2 evolved during the 60-minute sample collection period. The poor range areas evolved an average of 11.4 mg of CO_2/hour/m^2 during the sample period of September 6, 1970. In comparison, the good range areas for the same date evolved an average of 21.8 mg of CO_2/hour/m^2. This same trend was observed in the October collection during which the good range areas evolved 15.2 mg CO_2/hour/m^2 and the poor range areas produced 18.5 mg CO_2/hour/m^2.

The calculated figure of about 100 g CO_2/m^2 evolved annually from the good range areas is only a relative value. This total is computed from 10 one-hour samples. The data are averaged and projected to a daily rate and then to an annual rate. The rate does not take into account the cyclic variations in CO_2 concentration during the day, nor does it take into account the fact that for about five months of the year there is little CO_2 being evolved because of frozen soil.

As part of the total data collection at the Cottonwood Field Station, the surface litter was removed and placed with the herbage material before CO_2 measurement. Kucera and Kirkham (1970) indicated that CO_2 from litter accounts for 15 to 20% of the CO_2 evolved from undisturbed soil. Therefore,

this is lost to the overall measurement when our method of collection is used.

If an exact figure for the amount of CO_2 evolved is needed, it will be necessary to monitor CO_2 production continually. But, if the only data needed are the amount of CO_2 given off during each sample period, then it appears necessary to continually monitor CO_2 production for at least 24 hours. We feel a good way to do this is through use of the gas analyzer.

If the only information desired is the quantity of CO_2 produced by microbial respiration, then it will be necessary to substract the amount of CO_2 produced through plant respiration. There are other biological systems at work in the soil besides microbes and plant roots. These systems include insects, earthworms, and other soil animals. In many areas in the two treatments there are large populations of algae and lichens which will also affect CO_2 evolution. If all we need to know to determine the energy flow is how much carbon is lost through evolution to CO_2, then any system for monitoring CO_2 would provide this information. But if CO_2 evolution is going to be used as a measure of microbial activity, then the amount that root and soil-animal respiration contribute must be taken into account.

From some exploratory studies done recently, it appears that a direct titration method may be more satisfactory and will be attempted in 1971-72.

CELLULOSE AND LITTER DECOMPOSITION

Introduction and Literature Review

Dr. Francis Clark proposed burying filter paper as a means of determining cellulose decomposer activity in the soil. This material was readily

available and provided a means of comparing decomposer activity in different soils and different sites. In addition to filter paper for decomposer activity studies, it was proposed that each site use some type of native litter. It was felt that the material would be much more standard if it came from a single source. The material of choice this time was big bluestem (*Andropogon gerardii* Vitm.) provided by Dr. John Harris at Kansas State University. The use of a standard litter again provided a means of comparing decomposer activity at the different sites.

Witkamp (1966), in a study conducted in a forest environment at Oak Ridge, Tennessee, reported 12-month weight losses from decomposition of native leaf litter, including red mulberry (*Morus rubra* L), redbud (*Cercis canadensis* L), white oak (*Quercus alba* L), and American beech (*Fagus grandifolia* Ehrh.) ranging from 63 to 90%. Temperature, bacterial density, moisture, and length of time since leaf drop were the most important factors controlling decomposition of the leaf material.

Materials and Methods

A piece of filter paper 7 cm^2 was cut, weighed, and placed in a nylon bag. This bag was made from "Bridal Veil" nylon net, having a mesh size of 20 squares/cm^2. This bag was sewn together using nylon thread. Each bag was numbered using a brass sheep ear-tag.

The procedure for handling the big bluestem litter was much the same as that for cellulose, although it presented many more problems. The material sent to us from Kansas State University was so finely chopped that much of it would not stay in the nylon bag which was used for the filter paper. It was deemed necessary to obtain nylon net of smaller mesh. A fine mesh nylon

having 30 squares/cm^2 was used, greatly reducing the problem but not eliminating it.

The field procedure was the same for both types of organic material. The plots in each treatment were selected at random. Each treatment had six plots in each of two replicates in each of two treatments.

A sharpened tilling spade was used to cut the area where the bag was to be buried. A piece of sod larger than the bag, cut on three sides, was raised by cutting down 5 to 7 cm and slicing horizontally under the sod. A bag was placed under each sod and the slice was replaced and tamped down. The sites within each replication were marked by placing a stake at each end of the row of bags with a nylon rope connecting them. A piece of tape marked the position of each bag. The litter bags were placed adjacent to the row of filter paper bags.

Three bags per replicate were carefully removed each month; a total of 12 samples were removed during each sample period. The samples were transferred to 9 × 4.5 inch plastic bags and stored in a 10°C cooler to slow down microbial activity. A soil sample from each bag site was analyzed for organic matter content. The filter paper bags were removed the first sample period of each month, and the litter bags were removed during the second sample period.

The samples were brought back to the laboratory where they were dried to a constant weight at 60°C for 24 hours. Each sample was weighed and placed in a porcelain crucible to assay for the amount of clinging soil by ashing the samples at 600°C for four hours. This will oxidize the organic matter. Filter paper has less than 0.1 mg of ash per gram of paper; therefore any measurable amount of ash remaining should be soil.

The soil samples were treated in the same manner as the filter paper bags to determine how much of the weight lost in ashing was from soil organic matter.

Results and Conclusions

Fig. 1 shows the rate of loss of material on a gram per month loss basis; each month was arbitrarily calculated as 30 days. The graph indicates that the rate of loss under the two treatments was not the same. One factor biasing the decomposition rate in May, and more obviously in June, is that the bags within good range condition plots were placed immediately prior to a 30-day cold period during which snow fell. The other set on the poor range condition plots was placed 27 days later, followed by more cold weather. It was not until the soil started to warm that both sets of material began to decompose. When the rate of loss was calculated it was seen that the loss was greater on the grazed pastures.

Cutting the roots of the plants to insert the bags resulted in the death of many of the plants. The dead plants in the bag sites created an unnatural habitat. The killed area affects the micro-environment under it in two ways: (i) the soil is bared, increasing the temperature, and thus increasing the decomposition rate; (ii) this increased rate is offset by the increase in loss of moisture which tends to retard decomposition. Killing of the vegetation will also increase the available organic matter for decomposition, thus increasing the bacterial and fungal mass which should lead to increased activity. This will be true only if there is adequate moisture and a temperature suitable for microbial activity.

The dead vegetation on top of each plot is quite noticeable. There is one outstanding difference between the ungrazed and grazed areas. The

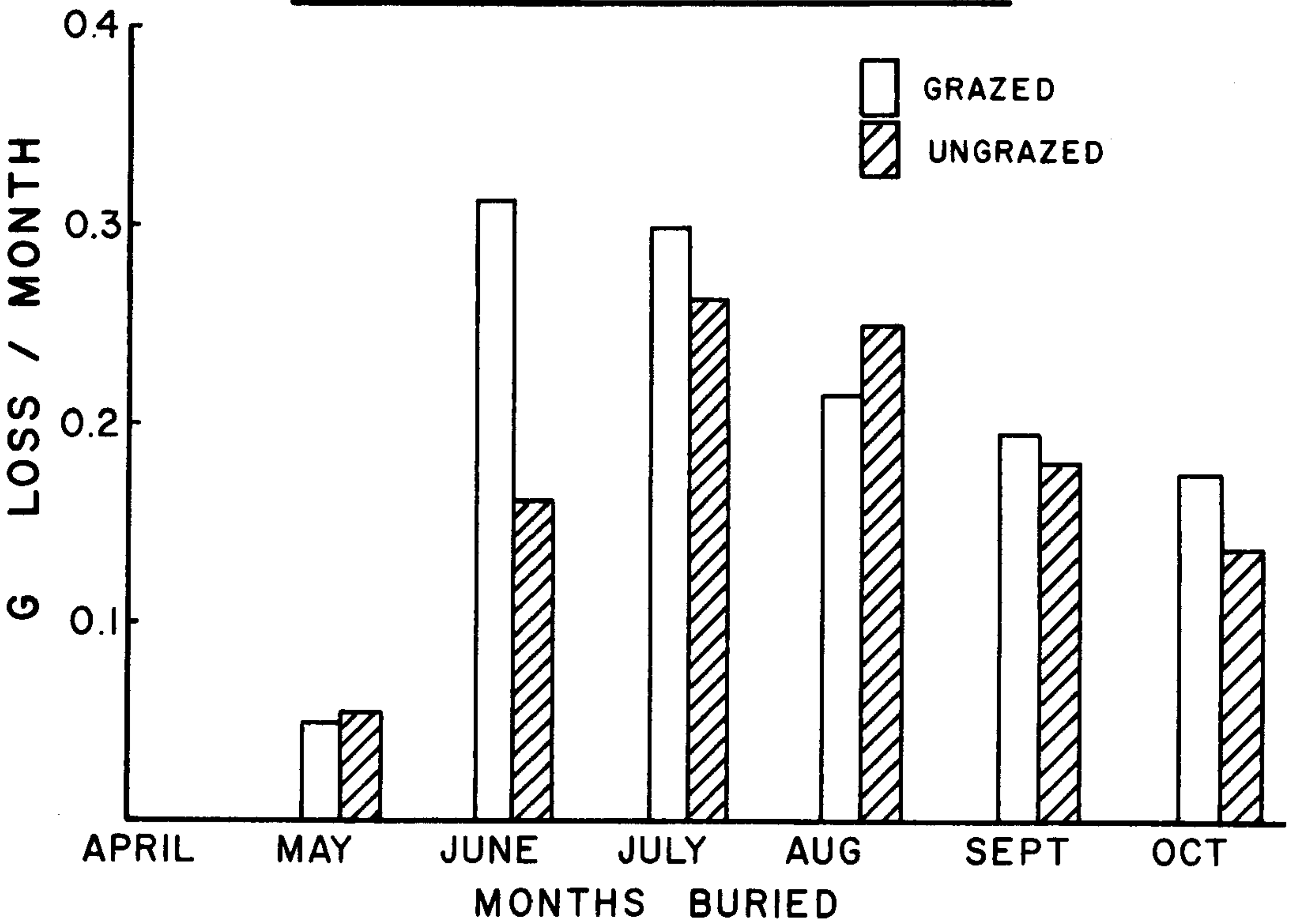

Fig. 1. Decomposition of cellulose as filter paper expressed as loss in grams per month.

soil in the ungrazed area is loose and friable in comparison to the grazed area which tends to be more compact. Difficulty was experienced in removing the sod over the bags on the ungrazed area since the sod fell apart, whereas the sod on the grazed plot was compacted and could be raised easily. Soil under grazed sod tended to be more moist than the ungrazed plots.

Fig. 1 shows that there is a rapid decomposition rate when adequate moisture is available followed by a decline in the rate of decomposition about the time that soil moisture declines--in this case, early August.

It is hoped that by placing the cellulose bags in the soil in the fall of 1970, dying out of the plants can be eliminated.

Judging from past experience it will be necessary to place two sets of cellulose bags in the soil, one set in the fall of 1970 and one set in July 1971. The material placed in April of 1970 was completely decomposed by August. Therefore, the material placed in the fall of 1970 should be decomposed in April or June, thus necessitating the placement of more filter paper bags.

.The litter decomposition date (Fig. 2) should be disregarded for this season for several reasons. Leaves, the most readily decomposed material, are lost during handling while the slower-decomposing stems are retained by the mesh bags. Weight of leaf matter lost appears in the data to be the result of decomposition.

It would seem that in order to study loss by decomposition of native litter one must use native litter. Therefore, it is proposed that each site provide its own litter and that filter paper be used as a means of comparison of decomposer activity between sites. We will, however, also bury bags containing a standard litter used throughout the biome.

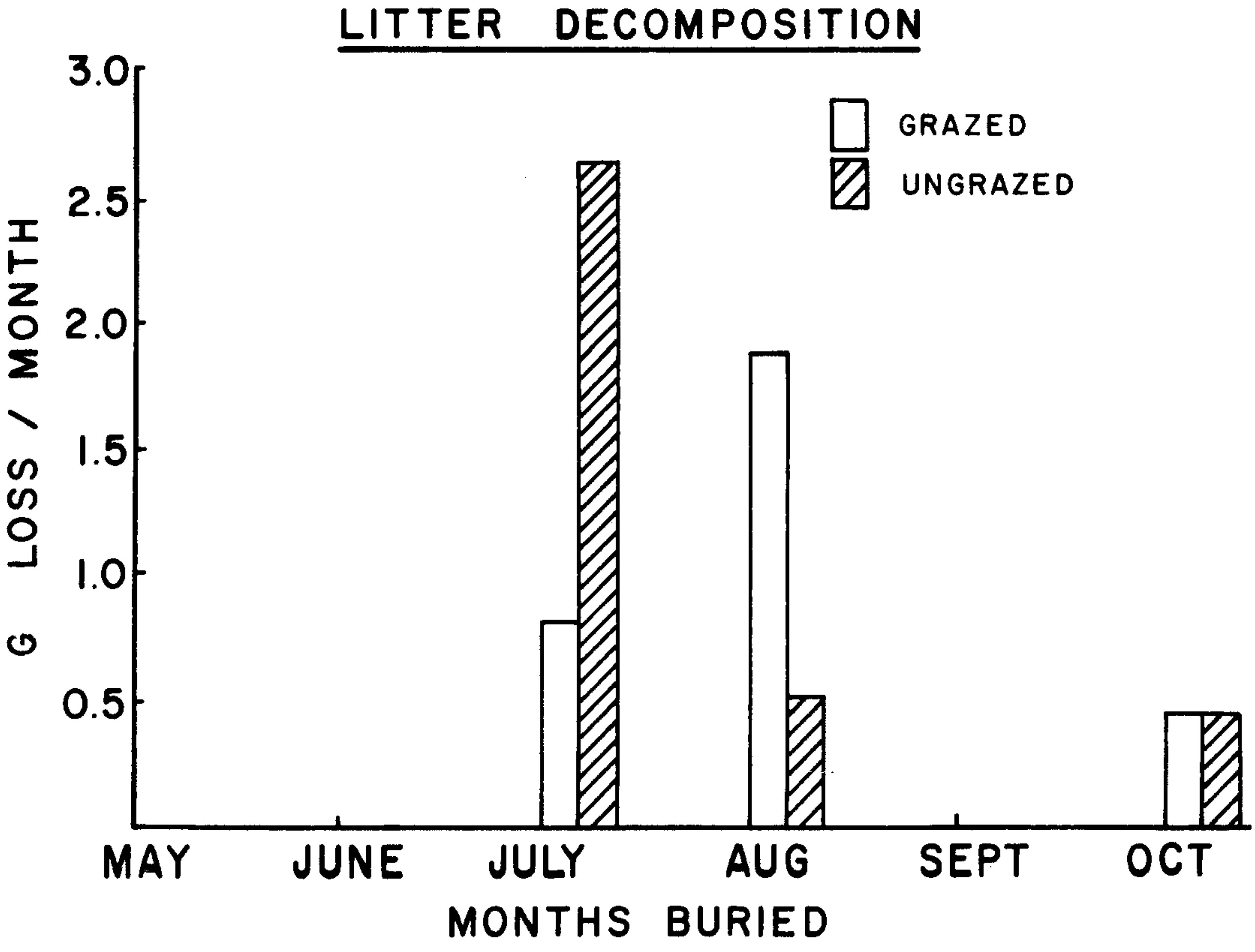

Fig. 2. Decomposition of big bluestem (*Andropogon gerardii* L.) expressed as rate of loss in grams lost per month. These data were very inadequate as explained in the text.

Killing of the vegetative mass over each bag might be avoided by placing the material to be decomposed in the soil vertically. This will also conserve space, which at Cottonwood is of some importance. This technique will be attempted this next season in addition to the regular method of placing the bags.

LITERATURE CITED

Johnson, P. L. and J. J. Kelley, Jr. 1970. Dynamics of carbon dioxide productivity in an arctic biosphere. Ecology 51:73-80.

Kucera, C. L. and D. R. Kirkham. 1970. Soil respiration studies in tallgrass prairie in Missouri. (Manuscript submitted to Ecology).

Wallis, G. W. and S. A. Wilde. 1957. Rapid method for the determination of carbon dioxide evolved from forest soils. Ecology 38:359-361.

Wiant, H. V. 1967. Contribution of roots to forest "soil respiration." Advancing Frontiers of Plant Sci. Ecology 18:136-138.

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

Witkamp, M. 1969. Cycles of temperature and carbon dioxide evolution from litter and soil. Ecology 50:922-924.