Technical Report No. 175

SOIL MICROBIOLOGICAL STUDIES

ON THE ALE RESERVE, 1971  $\frac{1}{2}$ 

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## GRASSLAND BIOME

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#### **ABSTRACT**

In order to accurately predict carbon and energy flow through the terrestrial ecosystem, it is necessary to understand the influence of environmental factors on the rate of  ${\tt CO}_2$  evolution from soil. Studies were therefore initiated to determine the influence of soil temperature, soil water, and grazing on the rate of  ${\tt CO}_2$  evolution from soil.

Sampling locations were selected from both grazed and ungrazed grassland sites on the Arid Land Ecology Reserve operated by the U.S. Atomic Energy Commission in south central Washington. The soil  ${\rm CO}_2$  evolution rate was measured at periodic intervals from April to September 1971. Concurrently, surface soil water and temperature were also monitored.

To provide an estimate of the rate of plant tissue decomposition in soil, shoot and root tissues (Agropyron spicatum) in nylon-mesh bags were buried in the soil at sites employed for soil respiration measurements, and the bags were retrieved at periodic intervals during the growing season. Tissue weight losses and net decreases in organic carbon were taken as indices of decomposition.

Seasonal changes in soil temperature (ranging 6.4 to 31.0°C) were not significantly correlated with changes in soil  $CO_2$  evolution rate. Soil water content (ranging 1.2 to 19.7%) was, however, correlated (r = 0.77, p < 0.001) with  $CO_2$  evolution rate suggesting that carbon flow through unperturbed arid ecosystems during the spring and summer is limited largely by adequate soil water.

The  ${\rm CO}_2$  evolution rates on grazed and ungrazed plots were not significantly different. Grazing effects may be expected to become more pronounced as grazing (initiated in 1971) is continued in subsequent years.

Total decomposition of shoot tissues measured gravimetrically over the growing season amounted to approximately 21% of the tissues originally buried. Root tissues decomposed less rapidly exhibiting less than a 10% weight loss over the same period. Grazing did not influence the decomposition rates of shoot or root tissues.

Plant tissue organic carbon content increased, and the C/H ratio decreased with time as readily decomposable plant components were degraded and resistant lignaceous components of the tissue increased in proportion. Net carbon losses over the growing season approximated 10% for shoot tissues, whereas there was no net loss of carbon from root tissues.

## INTRODUCTION

The soil is the principal medium for return of photosynthetic energy to the atmosphere. Soil microorganisms may be represented as receiving reduced forms of carbon in plant and animal residues and ultimately oxidizing the carbonaceous materials to the lowest energy state of carbon, i.e.,  $CO_2$ . The rate of  $CO_2$  evolution from soil is largely a function of the activity of soil microorganisms and plant root respiration, but also includes respiration by soil invertebrates (Kononova, 1966). Prediction of carbon and energy flow through an ecosystem must be predicated upon an understanding of the environmental factors influencing soil respiration rate. Field measurements of soil respiration rate are perhaps the best means of evaluating the integrated influence of these effects.

As a first phase in an IBP Grassland Biome effort to determine the influence of grazing on ecological processes, soil respiration rate was measured in grazed and ungrazed sections of a field plot located on the Arid Land Ecology Reserve, Hanford, Washington.

Previous laboratory and field studies (Katznelson and Stevenson, 1956; Kononova, 1966; Wildung, Hajek, and Price, 1971) have indicated the importance of soil temperature and soil water in influencing the rate of organic matter degradation in soil. Therefore, to provide information useful for predictive purposes, soil temperature and soil water were also monitored during the growing season.

## MATERIALS AND METHODS

The grazing studies were conducted on a field plot located at an elevation of approximately 270 m on the northeast-facing slopes of the

Rattlesnake Mountains in south central Washington. The understory at this elevation is dominated by bluebunch wheatgrass (Agropyron spicatum), whereas the principal overstory shrub species is big sagebrush (Artemisia tridentata).

The soil at the experimental site, a Ritzville silt loam, developed primarily on aeolian silt parent material and contained 0.66% organic carbon (Table 1).

The field plot design is illustrated in Fig. 1. The field plot  $(600 \times 600)$  consisted of grazed and ungrazed treatments containing two replicates  $(300 \times 300)$ . Replicates were subdivided into three strata. To locate sites for analyses two blocks  $(15 \times 30)$  were randomly selected from each stratum, and two plots  $(1 \times 1)$  were randomly selected from each block (12 sample sites/treatment). Grazing (15 head) was alternated at 7-day intervals on replicates 1 and 2 from April 14 to June 10, 1971.

The soil  ${\rm CO}_2$  evolution rate was measured for 24-hr periods (8 AM to 8 AM) at regular intervals from April 1 to September 1, 1971. The measurements will be continued at less frequent intervals throughout the year.

To collect CO<sub>2</sub> evolved from the soil surface, a polypropylene bottle with the bottom removed was inserted into the soil to a depth of 20 cm (Fig. 2). A vessel containing 1.0 N NaOH (10 ml) was utilized as a CO<sub>2</sub> trap. The bottle containing the CO<sub>2</sub> trap was sealed, using a threaded lid containing a moistened "O" ring. To reduce the possibility of increased temperature in the closed vessel the unit was shaded using an angled section of galvanized sheeting. Controls were placed in the field adjacent to the

Table 1. Description of the soil (Ritzville silt loam) at the experimental site.

					Contents	Contents $(%) = \frac{a}{}$		CEC
Classification	Vegetative Understory	Elevation (m)	Hď	Organic Carbon	Sand (×50 µ)	Silt (50 to 2 μ)	Clay (<2 μ)	pH 7.0 (meq/100 g)
Andic Aridic Haplustoll	Agropyron	544	6.2	6.2 0.66	23.3	64.1	12.6	22.5

a/ Percent organic carbon based on total soil; other values based on total minerals.

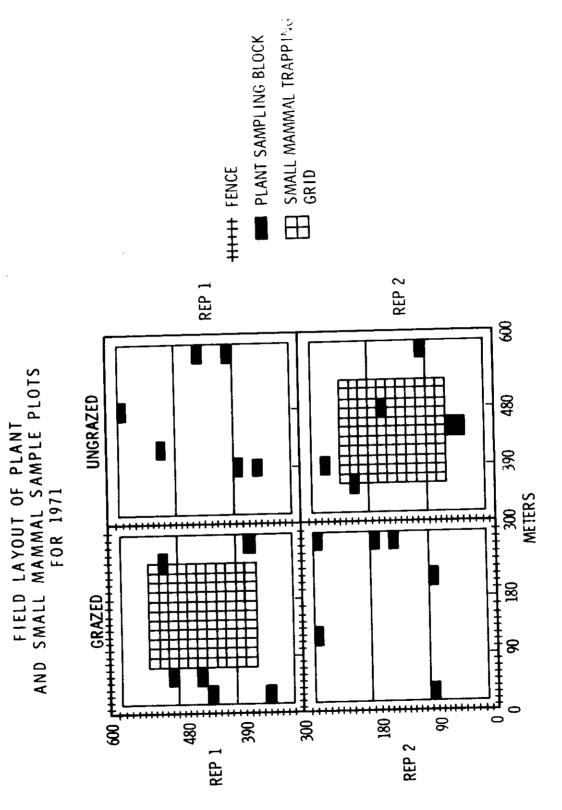


Fig. 1. Field plot design.

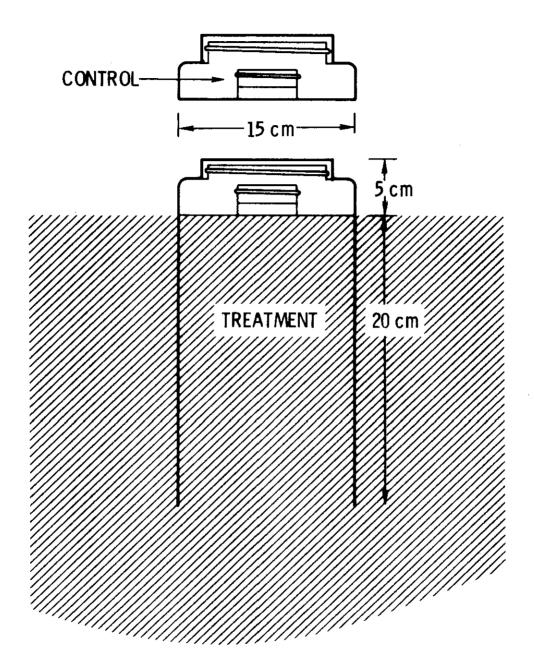


Fig. 2. Apparatus for collection of  ${\rm CO_2}$  evolved from soil.

treatment. The control systems were identical to the units utilized for collection of  ${\rm CO}_2$  evolved from soil except the volume of the controls was reduced to the equivalent volume above the soil in the closed treatment bottles (Fig. 2). The  ${\rm CO}_2$  in the soil effluent gases, absorbed as  ${\rm CO}_3^{2-}$  in the trap, was analyzed (Stotzky, 1965) by titration (1.0 N HCl) of the unneutralized NaOH after precipitation of the  ${\rm CO}_3^{2-}$  as the Ba<sup>2+</sup> salt.

Soil temperature (20-cm soil depth) was estimated by using a dial-reading, spike stem thermometer inserted into the soil. The thermometer operated on the dual metallic principle. Thermometers were placed (i) inside the  $CO_2$  collection device, (ii) inside the shade canopy, and (iii) outside the shade canopy.

Surface soil (0 to 8 cm) water content was determined gravimetrically after the drying (110°C) of samples taken concurrently with measurement of soil respiration rate.

To provide an estimate of the rate of plant tissue decomposition in soil, shoot and root tissues (Agropyron spicatum) were chopped (2 to 7 cm in length), and subsamples (1.0 to 3.0 g) were placed in nylon-mesh bags (15 x 15 cm). The bags (15 bags of each tissue) were buried in the soil (6 to 8 cm) at two locations on the grazed and at two locations on the ungrazed sections of the field plot. Thus, a total of 120 nylon bags (15 bags x 4 locations x 2 tissues) were buried at each time interval. The bags containing the tissue were buried on March 18, April 21, and June 4, 1971. Tissues buried in March were retrieved on April 21, May 26, and August 17, whereas tissues buried in April and June were retrieved on August 17.

On retrieval, the tissues were removed from the nylon bags, and subsamples were taken for gravimetric measurement of weight loss (based on ovendry 60°C weight) and for determination of ash content (600°C, 24 hr) and organic carbon content. Tissue weight loss was calculated as follows:

Organic carbon measurements were made on the original tissues and on retrieved tissues exhibiting maximum weight losses, i.e., tissues buried for 5 months. A Perkin-Elmer Model 240 Elemental Analyzer was used for carbon and hydrogen determinations. Samples (3 to 5 mg) of known moisture and ash contents were combusted at 960°C for 3 min in pure oxygen under static conditions. The combustion products ( $\rm CO_2$  and  $\rm H_2O$ ) after suitable cleanup, were analyzed automatically in a self-integrating steady-state analyzer employing thermal conductivity detectors. The method was sensitive to 2.5  $\mu \rm g$  C and 0.7  $\mu \rm g$  H. Analytical variation amounted to ±2% and ±1% for carbon and hydrogen, respectively.

Loss of tissue organic carbon was calculated as follows:

## RESULTS AND DISCUSSION

## Evaluation of Methods

The polypropylene bottles performed well in providing a closed system for collection of soil-evolved  ${\rm CO}_2$ . In moist soils the bottles

were inserted by hand pressure. However, on dry soils a preliminary shovel cut was necessary in order to place the bottle.

The  $\mathrm{CO}_2$  detected in the control bottles approximated that which would normally be present in the atmosphere within the bottle, and it is, therefore, unlikely that leakage occurred. The  $\mathrm{CO}_2$  levels in the controls did not exceed the variation between replicate treatments, and therefore, the values for soil  $\mathrm{CO}_2$  evolution rate presented herein are uncorrected for  $\mathrm{CO}_2$  present in the atmosphere within the bottles. The detection of  $\mathrm{CO}_2$  by back titration of unneutralized NaOH was accomplished using either colorimetric (phenolphathalein) or automatic potentiometric titration. The titration methods were sensitive to  $\pm 0.2$  mg of evolved  $\mathrm{CO}_2$ . The  $\mathrm{CO}_2$  evolved per collection unit ranged from approximately 25 to 100 mg in 24 hr.

Subsurface (10-cm soil depth) soil temperatures under the shaded collection vessel and outside the shade canopy were approximately equivalent throughout the monitoring period. Surface soil (2 cm) temperatures outside the shade canopy seldom exceeded surface soil temperature under the canopy by 5°C. Measurements of soil in the morning (8 AM), before maximum ambient temperatures were reached, likely served to minimize differences between shaded and unshaded surface temperatures. Subsurface soil temperatures were employed for all evaluations. However, most of the soil microbial activity likely occurs in the surface (0 to 3 cm) of the soil. If precise estimates of the influence of temperature on the microbial contributions to soil respiration are required, measurements of soil surface temperature should be employed.

Estimation of plant tissue decomposition by gravimetric determination of weight loss after field burial and retrieval was satisfactory, provided degradation exceeded approximately 10% of the original tissue weight. If tissue degradation amounted to less than 10%, interpretations were tenuous due to replicate variation. Variation may have arisen from subsampling errors (although six replicates were employed), from the presence of fresh plant roots, or from soil water in the interlayers of soil clay minerals retained by the roots after retrieval. Interlayer soil water may be considered stable at 60°C, the oven temperature at which the tissue was dried. In the present studies, the decomposition of shoot tissues over the growing season was sufficient to allow valid comparisons between treatments; however, root tissues decomposed more slowly, and evaluation of treatment effects on root decomposition over the same time interval was not feasible. Root sampling intervals will, therefore, be extended.

Seasonal Changes in Soil Temperature and Soil Water

Soil temperature (Fig. 3) generally exhibited a gradual increase from 6.6°C in April to 31.0°C near the end of July. This temperature was maintained evidently through most of August. On September 1 the soil temperature had decreased to 19°C.

Soil water content (Fig. 3) decreased from a high of 10.9% on April 1 to 2.5% at the end of May. In the interim a light rainfall on May 13 increased the soil water from 2.3 to 2.9%. A rain storm added approximately 2.5 cm of new water to the soil on June 3, raising the soil water content to approximately field water capacity (20.0%). Following the storm, surface

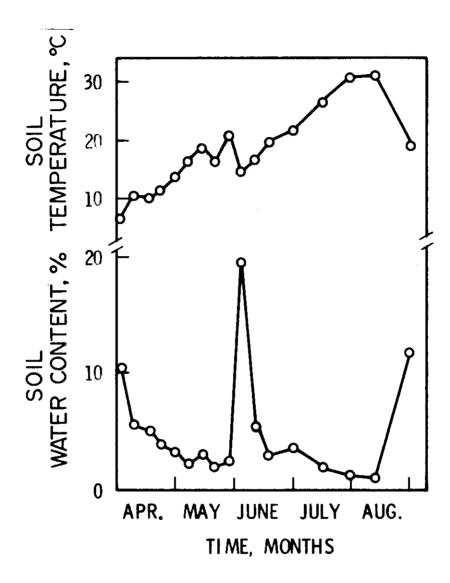


Fig. 3. Seasonal changes in soil temperature and soil water on combined grazed and ungrazed treatments.

soil water content decreased to 1.2% on August 12 due apparently to downward water movement, evaporation from the surface, and consumptive use by vegetation. Deviating from the usual climatic pattern in which July, August, and September are dry months, a storm on September 1 which deposited approximately 1.3 cm of rainfall raised the soil water level to 13.4%. Increases in soil water after rainfall were generally accompanied by reductions in soil temperature.

Seasonal Changes in Soil Respiration Rate

Seasonal changes in soil respiration rate occurring at all sample locations on grazed and ungrazed treatments are illustrated in Fig. 4. The rate of  $\rm CO_2$  evolution ranged from 68 to 205 mg/hr/m² over the sampling period. Seasonally the  $\rm CO_2$  evolution rate decreased gradually from a high of 100 mg/hr/m² on April 1 to a low of 68 mg/hr/m² on May 22. Marked increases in respiration rate to 141, 176, and 205 mg/hr/m² occurred on May 13, June 3, and September 1, respectively.

Changes in soil temperature and Soil Water on Soil Respiration Rate

Changes in soil temperature during the sampling period were not
significantly correlated with changes in soil respiration rate. In
contrast, soil respiration rate appeared to be closely related to changes
in soil water. Increases in soil water content (Fig. 3), due to rainfall
on May 13, June 3, and September 1, were accompanied by marked increases
in the rate of CO<sub>2</sub> evolution from soil (Fig. 4). Similarly, decreases in
soil water content were accompanied by decreases in soil respiration rate.

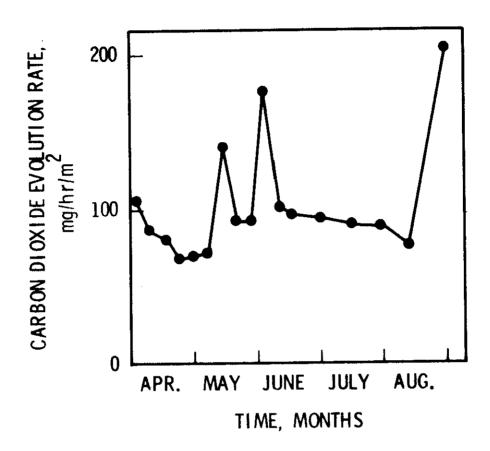


Fig. 4. Seasonal changes in soil  ${\rm CO}_2$  evolution rate on combined grazed and ungrazed treatments.

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Soil water content was significantly correlated (r = 0.77, p < 0.01) with respiration rate (Fig. 5). Although each correlative point represents the mean of 12 determinations, soil water was above 10% only at three sampling intervals, and additional soil water values above 10% should be incorporated before the correlations may be considered assured. It is likely that soil water effects are most pronounced at higher temperatures; thus, predictions of soil water influence on respiration rate based on data obtained during the summer may prove invalid when extrapolated to conditions of high soil water but lower temperatures in the fall and winter. Measurements of soil respiration rate will, therefore, be continued throughout the year.

Increases in soil respiration were apparently due to increases in microbial and plant root respiration, resulting from increased microbial proliferation and plant growth with the improved soil water regime. The results emphasize the strong dependence of higher plants and the soil microflora on soil water in arid regions and suggest that carbon flow through unperturbed arid ecosystems during the spring and summer is limited largely by adequate soil water. It is likely that temperature influences on soil respiration rate will become more pronounced in the fall, winter, and early spring as soil water increases to optimum levels.

Influence of Grazing on Soil Respiration Rate

Soil respiration rates on grazed and ungrazed plots (Fig. 6) were not significantly different. Seasonal changes on the grazed and ungrazed treatments were due largely to changes in soil water as previously described.

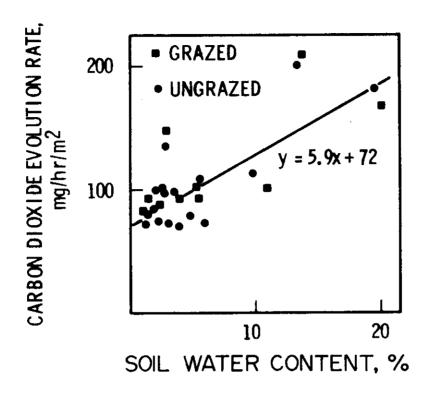


Fig. 5. Relationship between soil  ${\rm CO}_2$  evolution rate and soil water content under grazed and ungrazed conditions (r = 0.77; p < 0.01).

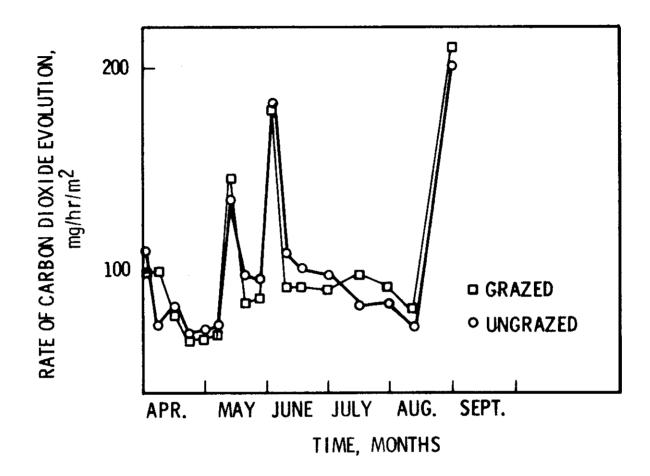


Fig. 6. Seasonal changes in soil  ${\rm CO}_2$  evolution rate under grazed and ungrazed conditions.

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Grazing may be expected to influence soil respiration rate by altering conditions which influence microbial activity and plant root respiration, e.g., alteration of soil fertility levels, increased concentrations of readily available sources of carbon, alteration of surface soil physical structure, and plant harvest. These effects may be expected to become more pronounced as grazing is continued in subsequent years.

# Decomposition of Plant Tissues in Soil

Gravimetric measurements. Decomposition of Agropyron spicatum shoot tissues, measured gravimetrically, after 5 months in the ungrazed soil, amounted to 21, 14, and 14% of tissues originally buried in March, April, and June, respectively (Table 2). Decomposition was approximately equivalent (standard deviations overlap) in August regardless of burial time. It is likely that in the spring soil temperature rather than soil water limited tissue decomposition.

Root tissues decomposed more slowly than shoot tissues (Table 2), with more than 90% of the tissues originally buried remaining on retrieval (Table 2) regardless of incubation time.

Grazing did not have a significant effect on plant tissue degradation rate (Table 2).

carbon measurements. Total organic carbon was determined on shoot and root tissues prior to burial and on retrieval after a 5-month soil incubation period (March 18 to August 17, 1971). The initial ash-free carbon contents of shoots and roots were  $46.9 \pm 0.8\%$  and  $48.8 \pm 1.5\%$ , respectively (Table 3). The carbon contents of retrieved shoot and root tissues was considerably higher, amounting to absolute increases of approximately 5% and 7%, respectively (Table 3).

Table 2. Decomposition (% weight loss) of shoot and root tissues (Agropyron spicatum) in soil. Percentage ( $\bar{x} \pm SD$ , n = 6) lost based on ovendry (60°C) ash-free weight.

	Decomposition										
Retrieval Time	Sh	oots	Ro	ots							
1 (IIIC	Grazed	Ungrazed	Grazed	Ungrazed							
		Buried March	18, 1971								
April 21	12 ± 4	6 ± 2	9 ± 2	1 ± 2							
May 26	12 ± 4	12 ± 4	3 ± 5	5 ± 3							
August 17	21 ± 5	21 ± 2	7 ± 8	8 ± 9							
		Buried April	21, 1971								
August 17	23 ± 6	14 ± 8	0 ± 0	8 ± 7							
		Buried June	4, 1971								
August 17	15 ± 5	14 ± 5	2 ± 6	5 ± 8							

Table 3. Comparison of carbon and hydrogen content (2) of shoot and root tissue of *Agropyron spicatum* before and after decomposition in the soil. Percentage (½ ± SD, n = 6) based on ovendry (60°C), ash-free weight.

		Shoots	ıts					Roots	s		
	Grazed			Ungrazed			Grazed		:	Ungrazed	
Carbon	Hydrogen	H/3	Carbon	Hydrogen	E/H	Carbon	Hydrogen	н/о	Carbon	Hydrogen	₹3
					Prior to burial	burial 2					
8.0 ± 6.94	46.9 ± 0.8 6.6 ± 0.2 7.1 ± 0.2	7.1 ± 0.2		6.6 ± 0.2	7.1 ± 0.2	46.9 ± 0.8 6.6 ± 0.2 7.1 ± 0.2 48.8 ± 1.5 6.7 ± 0.2 7.2 ± 0.1 48.8 ± 1.5 6.7 ± 0.2 7.2 ± 0.1	6.7 ± 0.2	7.2 ± 0.1	48.8 ± 1.5	6.7 ± 0.2	7.2 ± 0.1
					After re	After retrieval <sup>2</sup>					
52.1 ± 3.7	52.1 ± 3.7 7.1 ± 0.6 7.4 ± 0.2	7.4 ± 0.2		7.2 ± 0.3	7.3 ± 0.1	52.5 ± 1.9 7.2 ± 0.3 7.3 ± 0.1 56.9 ± 4.9 7.4 ± 0.7 7.7 ± 0.1 56.1 ± 3.5 7.5 ± 0.5 7.5 ± 0.1	7.4 ± 0.7	7.7 ± 0.1	56.1 ± 3.5	7.5 ± 0.5	7.5 ± 0.1

a/ Buried March 18, 1971.

b/ Retrieved August 17, 1971.

Increases in the C/H ratio apparently resulted from the more rapid degradation of plant components of lower C/H ratio, e.g., waxes and simple sugars, than the more resistant, lignaceous components of the tissue. As degradation proceeds, the C/H ratios of the plant tissues may be expected to approach the C/H ratio of the soil humic acid fraction, i.e., 9.4 (Wildung et al., 1971). As might be expected, there did not appear to be significant effects on plant tissue carbon or hydrogen contents as a result of grazing.

Although an overall increase in tissue ash-free carbon content occurred during burial, there was a net loss of carbon from shoot tissues amounting to approximately 10% (Table 4) for materials buried in soils in both grazed and ungrazed treatments. Roots did not exhibit significant net losses in carbon.

Table 4. Decomposition (organic carbon loss) of shoot and root tissues (Agropyron spicatum) in soil. Percentage ( $\bar{x} \pm SD$ , n = 6) lost based on ovendry (60°C) ash-free weight. These were buried on March 18, 1972 and retrieved August 17, 1971 with the principal variation due to measurements of weight loss on which calculation of organic carbon losses are based.

	Decomposi	tion	
Sho	oots	Roo	ts
Grazed	Ungrazed	Grazed	Ungrazed
10.4 ± 7.5	10.8 ± 4.4	0.0	0.0

# ACKNOWLEDGEMENTS

Appreciation is extended to K. R. Price who provided assistance in the litter decomposition studies.

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## APPENDIX I

# FIELD DATA

This is the soil microbiological decomposition data collected at the ALE Site for 1971. Plot size (columns 16-19) has been changed to percent organic matter.

Data were collected on form NREL-40. Data form and data follow.

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