

Technical Report No. 102  
MICROBIOLOGICAL STUDIES AT THE  
OSAGE SITE, 1970

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## ABSTRACT

A review is given of the 1970 microbiological investigations at the Osage Site. Data is presented covering microbial biomass measurements by the plate count and direct microscopic measurement of bacteria, actinomycetes, and fungi decomposition rates of buried filter paper and standard litter samples, and respiratory activity by carbon dioxide release and oxygen uptake measurements.



Osage Site. Early counts done with both poured and spread samples showed the latter yielded useful data in less than  $\frac{1}{4}$  the plates prepared. Secondly, plates were counted after 5 days incubation at 30°C, since two-week incubation gave no higher colony counts.

The results of the plate count data for the 0-5 cm depth in the ungrazed area are shown in Fig. 1, while comparable data from samples at the 10-20 cm zone are shown in Fig. 2. The mean values are plotted with maximum and minimum counts indicated for each date. As with all plate count data, considerable variation can be seen in the determinations. Population fluctuations throughout the season are more apparent at the surface of the soil than at the 10 cm level. This would be expected since moisture, temperature, and food supply would fluctuate more at the surface. Comparisons with the grazed areas show that populations of the soil, as determined by plate counts, appear to be slightly higher than in the ungrazed section. This has not yet been subjected to statistical analysis.

The vertical distribution of the microbial population is indicated in Table 1, showing mean plate count values on September 30 for a sample from the grazed and ungrazed areas. Although the soil at the Osage Site is rather heavy with considerable clay, it is apparent that the bacteria are growing in considerable numbers even at the lower depths of sampling. It will be most interesting to compare the root biomass data with microbial populations at various depths, since the most obvious food source for these organisms would be slough-off material from living roots and the decomposition of dead roots.

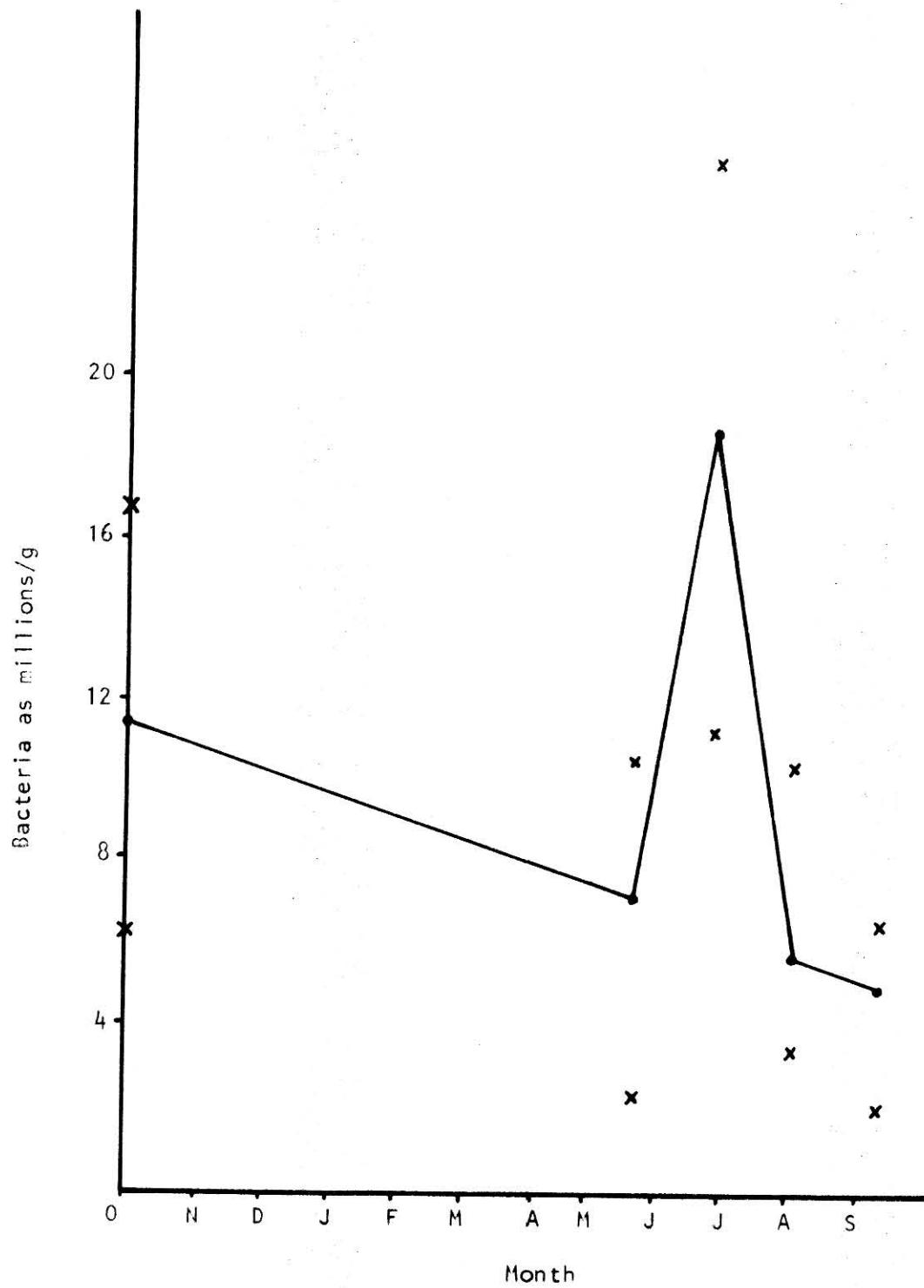


Fig. 1. Plate counts of the 0 to 5 cm depth ungrazed soil.

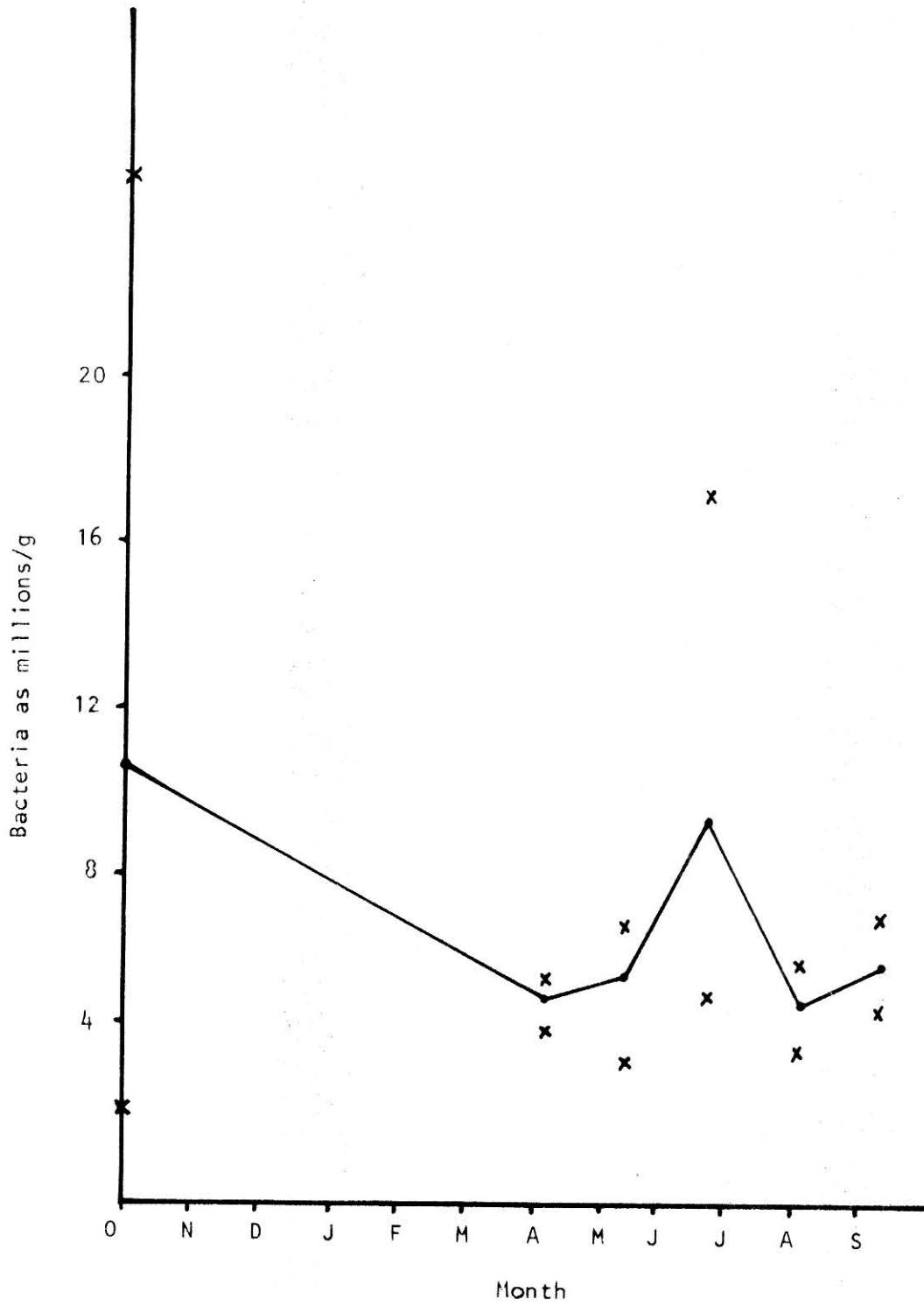


Fig. 2. Plate counts of 10 to 20 cm depth ungrazed soil.

Table 1. Total bacterial numbers (plate count) at different depths in ungrazed and grazed sections, September 30, 1970.

	0-5 cm	5-10 cm	10-20 cm	20-30 cm	30-50 cm
Ungrazed					
1	13.77	10.97	7.00	5.80	4.40
2	10.97	4.80	3.80	15.35	8.82
Grazed					
1	5.65	3.97	5.57	2.77	.925
2	5.12	6.97	3.87	14.00	3.95

The bacterial populations at the Osage Site are somewhat lower than soils of comparable cultivated areas in the Oklahoma-Kansas region. The low pH of the soil may be one major reason for this, but the vertical distribution of these organisms suggests a major difference in microbial activities in the cultivated zone of farmland and the root area of grasslands. Further investigation of this is desirable.

*Bacillus* spores. Comparisons of the usual plate count data and spore counts obtained by heating the soil-dilution fluid suspension for 10 minutes at 80°C show that most of the *Bacillus* species were present as endospores in the soil. Table 2 shows results for two samples from the ungrazed area samples at three depths. The total numbers of *Bacillus* endospores exceeds the so-called "total" count which would include the spores as well as the vegetative cells of all types. The explanation for this anomaly is simply that many spores do not germinate in ordinary plating procedures but do

germinate following the heat-shock treatment. Limited study of the species present in germinating *Bacillus* spores showed about half or more to be *Bacillus cereus*, while the remainder were *B. subtilis*, *B. megaterium*, and other species. Since these bacteria are predominant among the starch, hemicellulose, and protein decomposers, it is apparent that a high zymogenous population of *Bacillus* species exists at the Osage Site.

Table 2. Plate count data comparing numbers of bacteria and spore counts from heat shocked soil-water dilutions. Ungrazed area, April 13, 1970.

Sample	Depth in cm	Counts in millions/g	
		Total	Spores
3	0- 5	7.37	8.20
	5-10	7.20	7.18
	10-20	7.20	11.00
4	0- 5	8.97	8.92
	5-10	4.10	5.58
	10-20	4.36	9.10

*Actinomycetes* from plate count data. Colonies of typical members of the actinomycete group were counted on plates used for estimating bacterial populations. The criteria for this group were conidia formation, colonial morphology, and pigmentation. The raw data show much variation in individual plates, though the numbers are in the range of hundreds of thousands per

gram or about 10% of "total" counts. The method of despersing the soil by using a Waring type blender effectively destroys all non-sporulating propagules of this group of microorganisms. Thus, the counts reported in all the plate counts should be considered as inactive conidia. On the microscopic examinations for mold mycelia (discussed later), there was scant evidence of vegetative hyphal masses of these organisms. The effects of the blending treatment are shown in Table 3 from a study of a pure culture of *Streptomyces* sp. isolated from these soils. Clearly, blending over three minutes greatly reduces viable propagules in non-sporulating cultures.

Table 3. Plate count data showing the destructive effect of blender treatment on vegetative propagules of *Streptomyces* sp.

Time in Blender	Plate count of:	
	Vegetative Cells	Conidia
0 sec	9.46 million	7.23 million
15 sec	11.81 million	7.21 million
60 sec	8.33 million	7.18 million
120 sec	5.20 million	7.28 million
180 sec	1.76 million	7.19 million
240 sec	0.80 million	7.20 million

Just as the bacterial numbers are lower than those in cultivated soils, the counts of the actinomycetes show only about one-fourth to one-tenth the levels seen in cultivated soils. Factors causing this are likely those mentioned for bacteria. No unique features were apparent as far as the distribution of the common genera of this group encountered at the Osage

Site. *Streptomyces* was the most common, *Nocardia* was often seen, while *Micromonospora* was seldom encountered.

*Random isolation, digestive capability, and antibiotic sensitivity.* In order to secure some sampling of the types of bacteria present without making a detailed taxonomic study, several hundred bacterial colonies were picked from the plates used for the population estimates. Streaked plate isolation gave pure cultures. Gram's stain, microscopic morphology, and digestive action on starch and protein (casein) were determined plus sensitivity to various antibiotics. The antibiotic tests were done using Bacto-Sensidisco and results of inhibition recorded in mm of the diameter of growth inhibition zone. Results are given in Appendix A. While this information, per se, gives little information regarding those isolates, comparisons with similar studies in future years or other sites will furnish a tool for indicating population fluctuations both from a quantitative and qualitative point of view.

#### Fungal Hyphal Measurements

Since the blender treatment was shown to be disruptive of actinomycete vegetative cells, all data reported on the fungal hyphae were from soil samples dispersed by hand shaking. These were measured into 0.5% agar, and 1 x 1 cm smears were prepared. Following heat fixing, these were stained 15 seconds with 0.2% methylene blue, washed, and air dried. Hyphal strands were measured using a camera lucida with oil immersion objectives and a map-measuring device for length. Statistical analysis has not been carried out on the raw data, but it appears that this measurement of the fungal biomass may be the least variable of the methods used the past year. In Table 4, mean values are shown for fungal hyphal length for two samples from grazed and ungrazed areas

of the Osage Site. There are no marked differences between the grazed and ungrazed but, as stated above, statistical studies have not been made. The depth distribution of hyphae follows the same general pattern as the plate counts for bacteria and the actinomycetes with numerous fungi, even at the 30-50 cm level. These indications of bacterial, actinomycete, and fungal populations throughout the profile suggest aerobic conditions at considerable depths even in the heavy clay soil of the Osage Site. Since organic matter of these soils is relatively high for this region, one might conclude that soil acidity may be the factor limiting humus decomposition. Furthermore, these data emphasize the importance of attempting to learn the pathways of food penetration into the upper and lower levels of the soil.

Table 4. Total length of fungal hyphae (m/g soil) at different depths in ungrazed and grazed sections, June 19, 1970.

	0-5 cm	5-10 cm	10-20 cm	20-30 cm	30-50 cm
Ungrazed					
1	1000	1132	678	1560	1211
2	996	1372	2650	1229	993
3	1181	1300	1618	2030	1010
Grazed					
1	1022	925	1754	1041	1811
2	3003	2150	1635	1251	1383
3	1378	814	1300	2530	1843

#### Bacteria by Direct Microscopic Counts

Smeared slides prepared from various dilutions were made at the time of plate counts. Raw data from these are incomplete and are not being submitted at this time. Although an estimated 800 hours have been spent on these counts, we have only a small part of the samples counted. The apparent variations in counting, between fields, between smears, and between samples, lead us to conclude that this method will be unsatisfactory. The slides are being retained and the data held, should we wish to make future use of these.

#### DECOMPOSITION OF BURIED MATERIAL

Three types of organic matter were buried during the past year, removed, and decomposition rates estimated. Most data were obtained using filter paper (Whatman No. 1) in 14 × 14 cm squares. These were sandwiched between nylon mesh of approximately 2 mm mesh and buried at 5 cm below the surface, with as little disturbance as possible. Raw data are presented on the standard report forms. A summary of means of three replicates showing rates of decomposition of both filter paper and the standard hay sample are shown in Table 5. During the winter, as would be expected, cellulose decomposition was slow. Only about 1/3 decomposed during the 141 days prior to April 13. Samples removed May 5 showed about  $\frac{1}{2}$  decomposition in slightly over a month's burial. Variability in these tests was great, due to several factors. For instance, seven of the samples were completely removed by termite and ant action before the May 5 sampling date. Field mice or other small animals caused the loss of a number of other samples simply by digging them out of the soil. We could not determine what attracted the animals to the buried material.

Table 5. Rate of decomposition of buried cellulose and standard prairie hay.

Date Removed	Days Buried	Percent Decomposed	
		Ungrazed	Grazed
<i>Cellulose</i>			
April 13	141	39	
May 5	176	79	
May 5	35	55	
June 19	79	97	96
July 16	60	94	91
Sept. 30	77	100	100
<i>Standard Prairie Hay</i>			
June 19	33	29	37
July 16	60	72	49
Sept. 30	137	82	84

Data from the buried hay (both the standard prairie hay sample and material clipped on the site) were fewer in number and subject to greater variation. It was most difficult to retain the chopped dried grass within the mesh bags, and variable losses occurred in this way before burial. The dimensions of the mesh bag and the compactness of the buried hay gave further variations. Finally, recovery of the buried material was only partially successful. However, this type of investigation has considerable merit and, if continued, should allow comparisons of decomposition rates at various

grassland sites. In our limited studies, we could see the cellulose degraded more rapidly than the native plant material. There are a number of these tests continuing on both grazed and ungrazed sections of the Osage Site.

#### RESPIRATION STUDIES

##### Carbon Dioxide Measurements

Attempts were made to measure  $\text{CO}_2$  released as the result of decomposition and respiration in the soil. We attempted to simplify the method using readily available materials so, if desired, similar studies could be done at other sites. For the measurements in the field, one pound coffee cans were driven into the ground after cutting off the bottom. Only about 5 cm of the cans projected above the soil surface. Care was taken in placement of cans to include only about one crown of a little blue stem plant and to disturb the plants as little as possible. These cores were in place throughout the growing season. To measure  $\text{CO}_2$  evolved, 100 ml beakers of standard KOH (10 ml of 1.0 M) were placed in the can. Heavy polyethylene bags were then placed over all plants and fastened tightly about the lip of the can. As plants grew, it was necessary to bend the grasses to accommodate them within the enclosure. After 24 hours, the alkali was removed, carbonate precipitated with barium chloride, and the remaining alkali titrated with standard acid using phenolphthalein as indicator. The results include carbon dioxide measurements for four replicates in grazed and ungrazed areas on three days during the 1970 season. These values of  $\text{CO}_2$  represent the total gaseous exchange during the 24 hour period. Except for the June date, shading was not necessary to prevent solar heating, as the surrounding vegetation was high enough to shield the experimental plastic enclosed

can from the sun. We have done a few measurements on areas with top growth, with and without surface litter removed. Differences seemed to be within experimental error. Likewise, photosynthetic removal of carbon dioxide would decrease  $\text{CO}_2$  values. Future studies should include enough determinations so that a statistical evaluation could be used.

We have been following carbon dioxide release in the laboratory under controlled moisture and temperature conditions. Fig. 3 shows  $\text{CO}_2$  evolved during the week, following removal from the site by comparable weights of soil, but after different physical treatment. Sample A was a coffee can core, disturbed only at the bottom and sides, but with plants clipped and litter removed. Sample B was screened through a 10 mm mesh screen and piled loosely in a closed container with an alkali absorbing vessel. In both instances, the atmosphere was allowed to equilibrate with the room atmosphere for 30 minutes daily. Moisture in the two samples was the same. As seen in Fig. 3, both showed an initial burst of  $\text{CO}_2$  release. The core sample gave more variation throughout the following week than did the screened soil. These and similar data from other tests indicate that it may be useful to use "disturbed" soils for critical evaluation of such things as root and plant respiration, and to perhaps gain some measurement of the soil microfaunal respiration.

#### Oxygen Uptake by Warburg Method

Encouraged by the carbon dioxide experiments cited above, a series of respirometer tests were started using screened soil samples from the Osage Site. Because moisture relations represent a major controlling environmental factor not only on decomposition, but also on all biological activity,  $\text{O}_2$

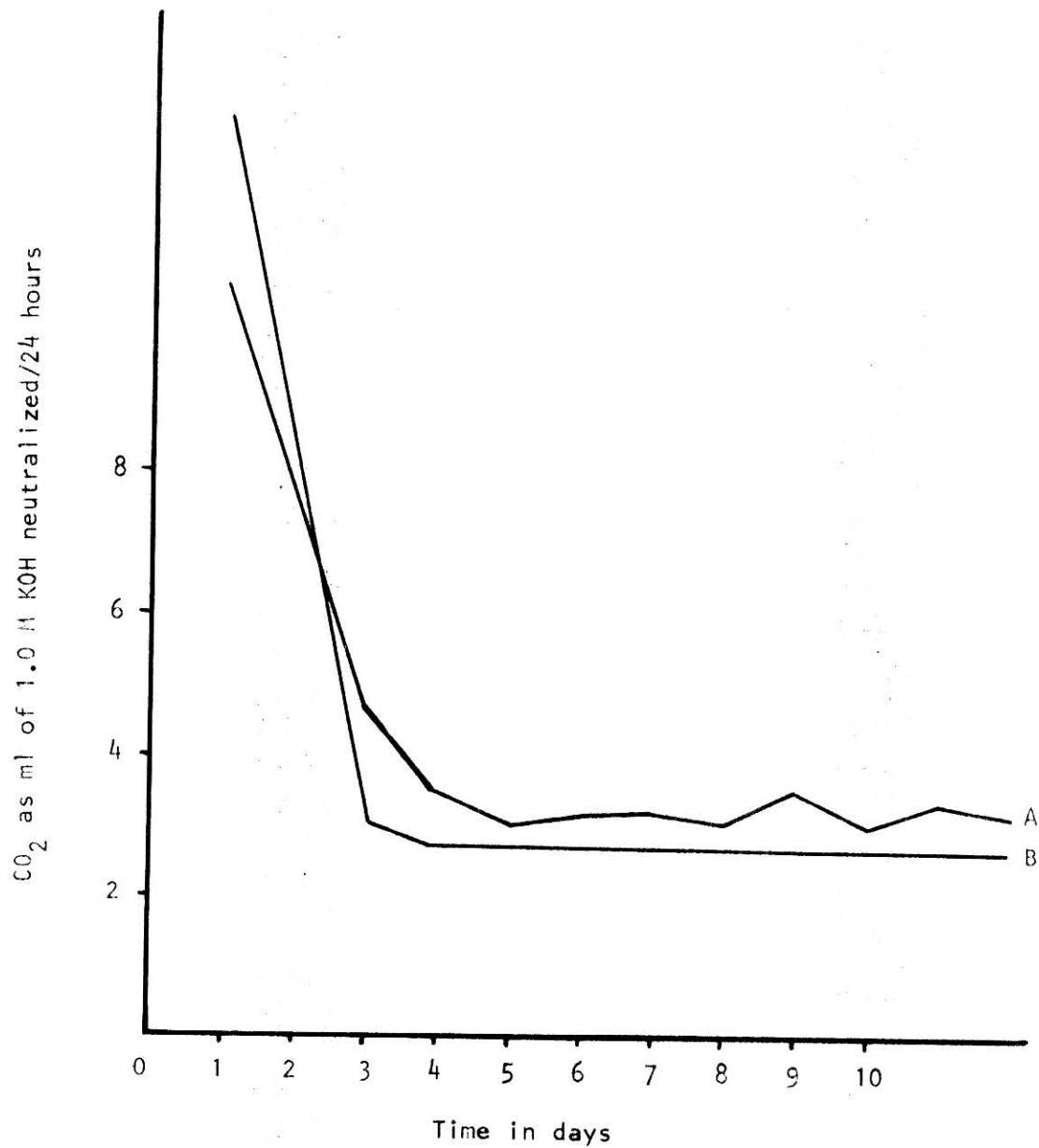


Fig. 3. Carbon dioxide released by 2000 g soil core (A) and same soil sieved loose (B).

uptake at different water contents of soil were determined. Values for soil from ungrazed sections are shown in Fig. 4, while similar data for grazed soil is shown in Fig. 5. As would be expected, low water content shows decreased respiratory activity, and too much water does not allow adequate gas exchange so that oxygen uptake is again depressed. In both grazed and ungrazed samples, the optimum water content is about 45% of the water holding capacity of the soil. This is considerably lower than the often quoted 55% to 60% optimum for microbial activity. This probably reflects the fine texture of these soils.

Respiration studies have shown good reproducibility, and other investigations have shown that soils may be stored under refrigeration for over six months and still show unimpaired respiratory capacity (Funke and Harris 1968). Studies are currently in progress investigating further aspects of this technique which can be used in future research.

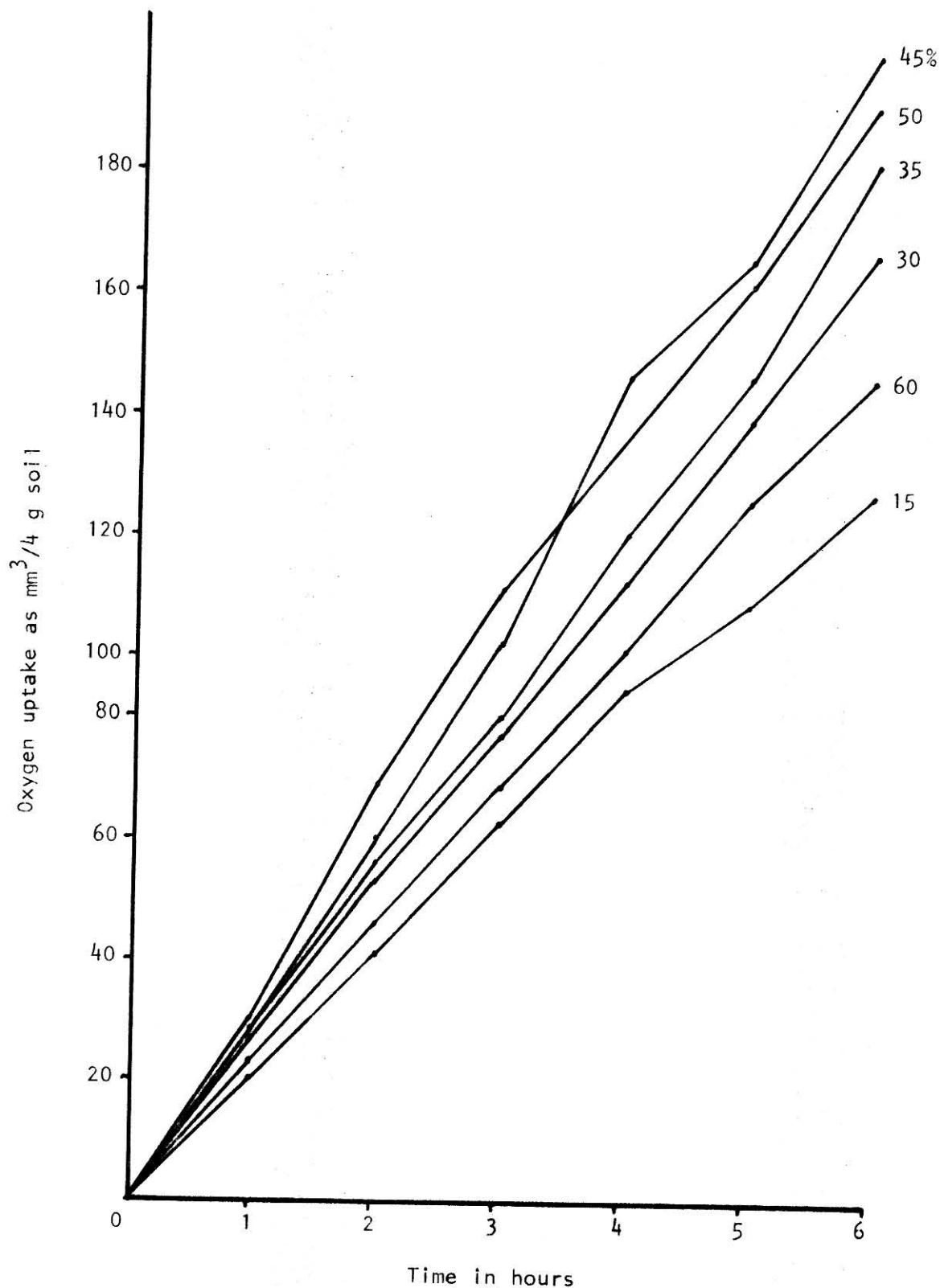


Fig. 4. Respiratory activity of ungrazed soil at different moisture levels.

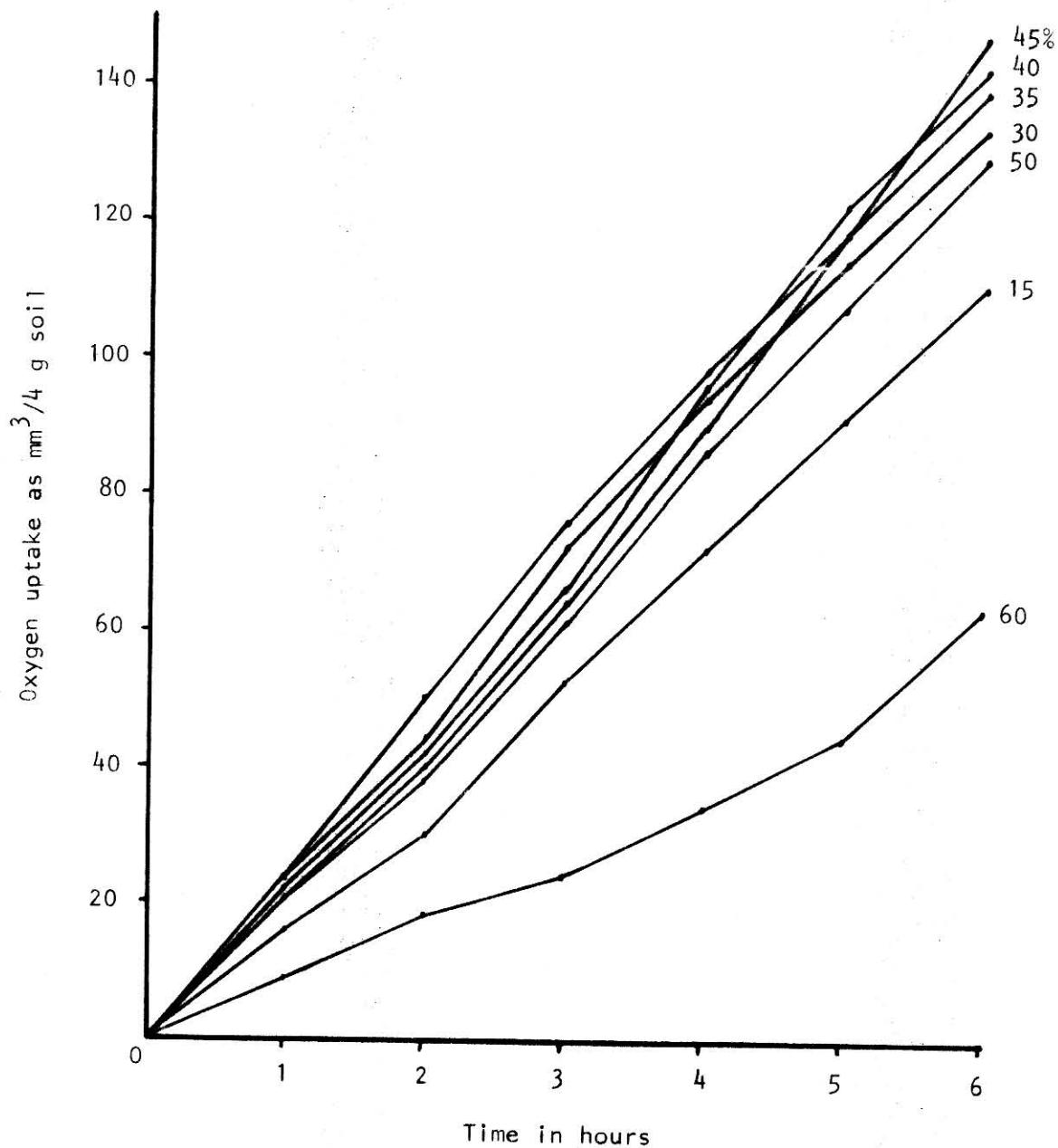


Fig. 5. Respiratory activity of grazed soil at different moisture levels.

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APPENDIX A

CHARACTERISTICS OF INDIVIDUAL RANDOM ISOLATES OF BACTERIA

Code Index

Gram Reaction	+	Positive
Morphology	-	Negative
Starch and Protein Hydrolysis	+	Hydrolysis
Antibiotic Sensitivity (measured in mm)	-	Nonhydrolysis
0 Completely resistant	P	Penicillin
1 1-5 mm	B	Bacitracin
2 5-10 mm	E	Erythromycin
3 10-15 mm	N	Neomycin
4 15-20 mm	Ch	Chloromyctin
5 20-25 mm	Au	Auromycin
	M	Magnamycin
	T	Terramycin
	Tt	Tetracycline
	OI	Oleandomycin
	Nb	Novobiocin
	K	Kanamycin
	St	Streptomycin

Antibiotic Sensitivity of the General Bacterial Population

Culture Number	Gram Reaction	Morphology	Starch Hydrology	Protein Hydrology	Antibiotic Sensitivity						
<i>Group I--Freshly Isolated Cultures</i>											
1	+-	Rod C	-	-	P1	B1	E1	N2	Ch2	Au1	
2	-	Rod C	-	+	P1	B0	E3	N1	Ch2	Au1	
3											
4	-	Rod S	-	-	-----No Growth-----						
5	-	Rod S	-	-	P2	B0	E0	N2	Ch3	Au3	
6	-	Rod S	+	-	-----No Growth-----						
7	-	Rod S	-	-	-----No Growth-----						
8	-	Rod C	+	+	P1	B2	E2	N1	Ch2	Au2	
9	+-	Rod CS	-	-	P3	B2	E3	N1	Ch2	Au3	
10	+-	Rod G	+	-	P5	B5	E4	N2	Ch3	Au4	
11	-	Rod SP	+	-	P4	B3	E4	N1	Ch2	Au3	
12	+-	Rod C	+	-	P4	B2	E4	N2	Ch3	Au3	
13	+-	Rod SP	-	-	-----No Growth-----						
14	-	Rod GP	+	+	P0	B0	E3	N2	Ch1	Au1	
15	-	Rod C	-	+	P2	B2	E3	N1	Ch1	Au1	
16	-	Rod G	-	+	P2	B2	E3	N1	Ch2	Au2	
17	-	Rod S	-	-	P4	B4	E4	N2	Ch3	Au3	
18	-	Rod S	-	-	-----No Growth-----						
19	-	Rod G	+		P3	B2	E3	N1	Ch2	Au3	
20	-	Rod C	-	-	P1	B0	E2	N2	Ch3	Au3	
21	-	Rod C	-	-	P5	B5	E4	N2	Ch3	Au4	
22	-	Rod S	-	-	--Complete Inhibition--						
23	-	Rod S	+	-	P5	B3	E4	N2	Ch3	Au3	
24	+	Rod G	+	+	P0	B1	E3	N1	Ch2	Au1	
25	-	Rod C	-	+	P3	B1	E2	N1	Ch2	Au2	
26	-	Rod S	+	+	P2	B2	E3	N1	Ch2	Au2	
27	-	Rod C	+	+	P4	B2	E4	N2	Ch3	Au3	
28					P2	B2	E3	N1	Ch2	Au2	
29	-	Rod C	-	+	P2	B2	E3	N1	Ch2	Au2	

Culture Number	Gram Reaction	Morphology	Starch Hydrology	Protein Hydrology	Antibiotic Sensitivity							
30	+	Rod G	+	+	P0	B1	E3	N1	Ch2	Au1		
31	+	Rod G	+	+	P3	B2	E3	N1	Ch2	Au3		
32	+-	Rod C	-								No Growth	
33	-	Rod SP	-	-							No Growth	
34	-	Rod P	-	+	P3	B2	E3	N1	Ch3	Au2		
35	-	Rod G	+	+	P3	B2	E3	N1	Ch3	Au3		
36	-	Rod G	+	+	P2	B2	E3	N1	Ch2	Au2		
37	+-	Rod G	-	+	P1	B2	E2	N2	Ch1	Au2		
38	-	Rod G	+	+	P2	B2	E2	N1	Ch2	Au2		
39	-	Rod S	-	-							Complete Inhibition	
40	-	Rod S	+	+	P4	B3	E3	M2	Ch3	Au4		
41	-	Rod S	-	+	P2	B3	E3	M2	Ch2	Au2		
42	-	Rod C	+	+	P3	B2	E3	M2	Ch3	Au3		
43	-	Rod G	-	-	P4	B2	E4	M4	Ch2	Au2		
44	-	Rod G	+	-	P5	B5	E5	M3	Ch1	Au5		
45	-	Rod SG	-	+	P2	B2	E2	M2	Ch2	Au2		
46	+	Rod S	-	-								
47	-	Rod SG	+	+	P1	B2	E2	M2	St2	Au2		
48	-	Rod G	+	-	P4	B3	E4	M3	St2	Au4		
49	-	Rod S	-	+	P0	B0	E1	M0	St2	Au2		
50	-	Rod C	-	-							Complete Inhibition	
51	+-	Rod SG	+	+	P4	B2	E3	M3	St2	Au3		
52	+	Rod G	+	+	P0	B1	Tt1	M2	St2	Au1		
53	-	Rod C	-	+	P0	B2	M1	N1	Ch2	Au2		
54	-	Rod C	+	+	P1	B2	M2	N1	Ch2	Au1		
55	-	Rod S	-	-	P5	B2	E3	M3	St2	Au3		
56	-	Rod S	-	-	P0	B0	E0	M0	St0	Au0		
57	-	Rod G	+	+	P0	B2	M1	N1	Ch2	Au2		
58	+	Rod C	-	+	P1	B1	M1	N1	Ch2	Au2		
59	+	Rod SP	+	+	P0	B1	E2	M2	St2	Au2		

Culture Number	Gram Reaction	Morphology	Starch Hydrology	Protein Hydrology	Antibiotic Sensitivity						
60	-	Rod S	-								
61	-	Rod C	-	+	P0	B1	M2	N1	Ch1	Au1	
62	+	Rod C	+	+	P0	B1	E3	M3	St2	Au2	
63	-	Rod S	-	-	P0	B1	M3	N1	Ch2	Au2	
64	-	Cocci	-	-	P0	B2	E2	M2	St1	Au2	
65	+	Rod C	+	+	P3	B1	Tt3	M3	St2	Au3	
66	-	Rod C	+								
67	-	Rod S	-	-	P0	B1	M2	N1	Ch1	Au1	
68	+	Rod C	-	+	P3	B2	Tt2	M2	St2	Au2	
69	-	Rod C	+	+	P1	B2	M1	N1	Ch2	Au1	
70	-	Rod C	+	+	P1	B2	M2	N1	Ch2	Au2	
71	+	Rod C	+	+	P0	B1	M2	N1	Ch1	Au1	
72	+-	Rod C	+	+	P0	B2	M2	N1	Ch2	Au2	
73	-	Rod G	-	+	P2	B2	Tt2	M2	St1	Au2	
74	-	Rod C	+	+	P0	B1	Tt1	M2	St2	Au1	
75	-	Rod C	-								
76	-	Rod C	+	+	P3	B1	Tt2	M3	St1	Au2	
77	-	Rod S	-	-	P0	B0	E1	M0	St0	Au2	
78	+	Rod SC	+	+	P0	B1	M2	N1	Ch1	Au1	
79	+-	Rod C	+	-	P2	B1	M2	N1	Ch2	Au2	
80	-	Rod S	-	-	P0	B0	M0	N1	Ch1	Au1	
81	-	Rod SP									
82	+	Rod C	-	+	P2	B1	M2	N1	Ch2	Au2	
83	+	Rod C	+	+	P0	B1	M3	N1	Ch1	Au1	
84	+	Rod G	+	+	P0	B1	M2	N1	Ch2	Au0	
85	-	Rod C	+	+	P1	B1	M2	N1	Ch2	Au2	
86	+	Rod C	+	+	P0	B1	M2	N1	Ch1	Au1	
87	-	Rod C	+	+	P1	B2	M1	N1	Ch2	Au2	
88	-	Rod C	+	-	P1	B2	M2	N1	Ch2	Au2	
89	+-	Rod C	+	+	P0	B2	M2	N1	Ch2	Au2	

Culture Number	Gram Reaction	Morphology	Starch Hydrology	Protein Hydrology	Antibiotic Sensitivity					
90	-	Rod C	+	+	P0	B2	M2	N1	Ch2	Au2
91	-	Rod PS	+		P0	B2	M2	N2	Ch3	Au2
92	-	Rod C	-	-	P0	B0	M2	N1	Ch1	Au1
93	+-	Rod C	-	+	P0	B1	M2	N1	Ch2	Au2
94	+	Rod C	-	+	P1	B1	M2	N1	Ch2	Au2
95	+	Rod C	+	+	P1	B2	M3	N2	Ch3	Au3
96	-	Rod S	-	-	P0	B0	M0	N1	Ch1	Au1
97	-	Rod S	-	-	P0	B1	M0	N1	Ch0	Au1
98	-	Rod	+	+	P0	B1	M2	N1	Ch2	Au2
99	-	Rod C	+	-	P2	B2	M3	N2	Ch3	Au3
100	-	Rod S	+	-	St2	B2	M2	N1	Ch2	Tt2
101	-	Rod C	-	+	-----No Growth-----					
102	-	Rod C	-	+	St2	B2	M3	N1	Ch2	Tt3
103	+	Rod S	+	+	St1	B0	M1	N1	Ch2	Tt1
104	-	Rod SP	+	-	St2	B3	M3	N2	Ch3	Tt3
105	+-	Rod C	-	+	St1	B1	M2	N1	Ch2	Tt2
106	+-	Rod PS	+	+	St1	B1	M1	N1	Ch1	Tt1
107	+-	Rod C			P0	B2	M2	N1	Ch2	Au1
108	-	Rod C			P0	B2	M2	N1	Ch2	Au2
109	-	Rod C			P0	B2	M2	N2	Ch2	Au2
110	+	Rod C	+	+	P0	B0	M2	N1	Ch1	Au3
111	-	Rod S	+	+	P0	B2	M2	N1	Ch2	Au2
112	-	Rod C	-	+	St1	B1	M1	N1	Ch2	Tt2
113	-	Rod SP	+	+	St2	B1	M1	N1	Ch1	Tt1
114	-	Rod P	-		-----No Growth-----					
115	+	Rod C	-	-	St1	B1	M2	N1	Ch2	Tt2
116	-	Rod S			P0	B0	M0	N0	Ch1	Au1
117	+	Rod			P1	B2	M3	N2	Ch3	Au3
118	-	Rod SP	+	-	St0	B2	M2	N1	Ch2	Tt2
119	-	Rod SP	-	-	B0	Ch1	M2	T3	Tt3	o12

Culture Number	Gram Reaction	Morphology	Starch Hydrology	Protein Hydrology	Antibiotic Sensitivity					
120	-	Rod C	-	+	B2	Ch2	M2	T1	Tt2	o12
121	+-	Rod PS	+	+	B1	Ch2	M2	T1	Tt2	o12
122	-	Rod C	-	-	-----No Growth-----					
123	-	Rod PS	-	-	-----No Growth-----					
124	+-	Rod SP	-	-	B1	Ch3	M2	T3	Tt3	o12
125	-	Rod C	-	-	-----No Growth-----					
126	-	Rod C	+	+	B1	Ch2	M1	T2	Tt2	o11
127	-	Rod CP	-	+	B1	Ch2	M2	T2	Tt2	o12
128	-	Rod C	+	+	B2	Ch2	M1	T2	Tt2	o13
129	-	Rod C	-	+	B2	Ch2	M3	T3	Tt3	o13
130	-	Rod S	-	+	B1	Ch2	M2	T2	Tt2	o13
131	+-	Rod G	+	+	B2	Ch2	M2	T2	Tt2	o12
132	+-	Rod C	-	+	B3	Ch2	M2	T2	Tt2	o12
133	-	Rod C	-	+	B2	Ch2	M2	T2	Tt2	o12
134	-	Rod C	+	-	B3	Ch3	M3	T4	Tt4	o13
135	+-	Rod C	-	+	B1	Ch2	M2	T2	Tt2	o12
136	+-	Rod C	-	+	B2	Ch2	M2	T2	Tt2	o12
137	-	Rod C	-	-	-----No Growth-----					
138	+	Rod G	+	+	B2	Ch2	M2	T2	Tt3	o12
139	-	Rod C	+	+	B2	Ch2	M2	T2	Tt2	o12
140	+-	Rod C	+	+	B2	Ch2	M2	T2	Tt2	o12
141	-	Rod C	+	+	B2	Ch3	M2	T2	Tt2	o12
142	+	Rod C	+	+	St1	B0	M2	T1	Tt2	o11
143	-	Rod C	-	+	St1	B0	M2	N1	Ch2	Tt1
144	-	Rod C	-	-	St1	B1	M2	N1	Ch2	Tt2
145	+-	Rod G	+	+	St1	B1	M2	N1	Ch2	Tt2
146	+-	Rod G	+	+	St2	B1	M2	N1	Ch2	Tt3
147	+-	Rod C	+	+	St2	B2	M2	N2	Ch2	Tt2
148	-	Rod C	-	-	St1	B1	M0	N1	Ch0	Tt1
149	+	Rod P	+	+	St1	B1	M2	N1	Ch1	Tt2

Culture Number	Gram Reaction	Morphology	Starch Hydrology	Protein Hydrology	Antibiotic Sensitivity						
150											
151	+-	Rod C	-	-	St2	B2	M2	N1	Ch1	Tt2	
152	+-	Rod C		-	St2	B2	M2	N2	Ch2	Tt3	
153	+-	Rod PS	+	-	B1	Ch1	M2	T3	Tt3	o12	
154	+	Rod C		+	B1	Ch1	M2	T1	Tt1	o12	
155	+	Rod C	-	-	St2	B2	M2	N2	Ch2	Tt3	
156	-	Rod C	+	+	St1	B2	M2	N1	Ch2	Tt2	
157	+	Rod C	-	-	St2	B3	M3	N2	Ch3	Tt3	
158	-	Rod C	+	-	St1	B2	M2	N1	Ch2	Tt2	
159	+	Rod G	+	-							
160	-	Rod GS	+	-	B3	Ch2	M2	T3	Tt3	o13	
161	-	Rod C	-	-	B1	Ch2	M2	T2	Tt2	o12	
162	-	Rod C	-	-	St1	B1	M0	N1	Ch0	Tt1	
163	-	Rod G	-	+							
164	+	Rod C	+	-	St2	B2	M2	N2	Ch2	Tt3	
165	+-	Rod G	-	+	St2	B1	M2	N1	Ch2	Tt2	
166	-	Rod C	-	-	-----No Growth-----						
167	+-	Rod P	-		St2	B4	M3	N2	Ch3	Tt3	
168	+	Rod C	+	+	St1	B0	M2	N2	Ch1	Tt1	
169											
170	+-	Rod GP	-	-	St1	B1	M2	N1	Ch2	Tt2	
171	+-	Rod C	-	-	St2	B3	M2	N2	Ch2	Tt3	
172	+	Rod C	+	+	St1	B2	M2	N2	Ch2	Tt2	

*Group I - Old Cultures*

004	-	Rod Ss	-	+	B1	Ch2	M2	T2	Tt2	o11
012	-	Rod	-	-	-----No Growth-----					
014	-	Rod G	-		-----No Growth-----					
018	-	Rod S	+	+	B3	Ch2	M2	T3	Tt3	o13
020	-	Rod C	+		B2	Ch2	M2	T2	Tt3	o12

Culture Number	Gram Reaction	Morphology	Starch Hydrology	Protein Hydrology	Antibiotic Sensitivity						
032	-	Rod C	-	+	B2	Ch2	M1	T2	Tt2	o12	
028	-	Rod Ss	-	-	-----No Growth-----						
027	+-	Rod CS	-	-	-----No Growth-----						
036	+-	Rod CG	+		B2	Ch2	M2	T2	Tt2	o12	
037	-	Rod Ss		+							
056	-	Rod G	+	+	B2	Ch2	M2	T3	Tt2	o12	
057	-	Rod S	+	+	B2	Ch2	M2	T2	Tt2	o12	
064	-	Rod S	-	+	B2	Ch2	M2	T2	Tt2	o12	
084	-	Rod S									

*Group II*

220	-	Rod C	+	+	P2	T2	M2	o12	B2	K1	
221	+-	Rod C	+	+	P0	T0	M0	o10	B0	K0	
224	-	Rod G	+	+							
227	+-	Rod C	+	+	P1	T2	M2	o11	B2	K2	
229	+-	Rod Ps	+	+							
230	+-	Rod C	+	+	P4	T4	M3	o13	B3	K3	
231	+-	Rod C	+	+	P2	T2	M2	o11	B1	K2	
232	+	Rod C	+	-	P4	T4	M3	o12	P3	K3	
234	+-	Rod C	-	+	P2	T2	M1	o11	B2	K2	
236	+-	Rod C	-	+	P2	T2	M2	o12	B2	K2	
237			-	-							
238	+-	Rod C	+	+	P2	T3	M2	o12	B2	K2	
239	+	Rod S	+	+	P2	T3	M2	o12	B2	K2	
240	-	Rod S	+	-	P2	T2	M2	o12	B2	K2	
242	-	Rod S	+	+	P1	T2	M1	o11	B1	K1	
243	+-	Rod C	+	+	P0	T0	M0	o10	B0	K0	
244	-	Rod S	-	+	P2	T2	M2	o11	B2	K2	
246	-	Rod Ss	-	-	-----No Growth-----						
248	+-	Rod C	+	+	P1	T2	M2	o12	B2	K2	
249	+	Rod C	+	+							

Culture Number	Gram Reaction	Morphology	Starch Hydrology	Protein Hydrology	Antibiotic Sensitivity					
252	-	Rod C	+	+	P2	T2	M2	o12	B2	K2
256	-	Rod C	+	+	P2	T2	M2	o11	B2	K2
257	+-	Rod C	+	+	P0	T1	M2	o12	B0	K2
258	+	Rod C	-	+	P1	T2	M2	o12	B2	K2
259	-	Rod C	+	+	P1	T2	M2	o12	B2	K2
261	-	Rod S	-	+	P0	T0	M0	o10	B0	K2
262	+-	Rod SP	+	-	P2	T1	M2	o11	B1	K2
263	+-	Rod CS	-	+	P4	T4	M3	o12	B1	K2
264	-	Rod C	+	+	P2	T2	M2	o11	B2	K1
266	-	Rod Ss	-	+	P1	T2	M1	o11	B1	K1
268	+	Cocci G	-	-	P1	T1	M1	o10	B1	K1
269	+	Rod C	+	+	P0	T1	M1	o11	B0	K1
270	+	Rod C	+	+	P2	T2	M2	o12	B2	K2
271	+	Rod C	+	-	P2	T3	M2	o12	B1	K2
272	+-	Rod C	+	-	P3	T4	M3	o13	B1	K0
273	-	Rod SG	-	+	P0	T0	M0	o10	B0	K0
275	+	Rod C	-	-	P2	T3	M2	o12	B1	K2
276	+	Rod C	+	+	-----No Growth-----					
277	+-	Rod C	+	+	P2	T2	M2	o11	B?	K2
279	-	Rod Ss	-	-	-----No Growth-----					
280	+	Rod C	+	+	P0	T2	M1	o11	B0	K1
281	+	Rod	+	-	P2	T3	M2	o12	B1	K2
283	-	Rod P	+	+						
285	+-	Rod C	+	+	--Complete Inhibition---					
286	-	Rod G	-	+	P0	T0	M0	o10	B0	K0
287	-	Rod G	+	-	P0	T2	B0	o10	St1	M2
289	-	Rod C	-	+	P0	T2	M2	o11	B2	K2
290	+-	Rod C	+	+	P3	T3	B2	o12	St2	M2
292	+	Rod P	+	+	P0	T2	B0	o12	St2	M2
293	+-	Rod C	+	+	P2	T2	B2	o12	St1	M2

Culture Number	Gram Reaction	Morphology	Starch Hydrology	Protein Hydrology	Antibiotic Sensitivity					
296	+	Rod C	+	+	P0	T2	B1	o11	St2	M2
297	+-	Rod C	+	+	P2	T2	M2	o11	B2	K2
298	+-	Rod G	+	-	P4	T3	B1	o11	St2	M2
299	-	Rod S	+	+	P3	T3	B2	o12	St2	M2
301	-	Rod P	+	+	P2	T2	M2	o11	B2	K2
302	+	Rod C	+	-	P3	T1	B1	o10	St2	M2
303	-	Rod P	-		P2	T3	B2	o12	St2	K2
304	-	Rod C	+	+	-----No Growth-----					
305	+	Rod P	+	+	P3	T3	B2	o12	St2	M2
306	-	Rod C	-	-	-----No Growth-----					
307	-	Rod C	+	+	P2	T3	B2	o12	St2	M2
308	-	Rod C	-	-	P3	T3	B1	o12	St0	M3
309	+-	Rod C	+	+	P1	T3	B2	o12	St2	M2
310	-	Rod P	-	-	P3	T3	B1	o13	St3	M3
311	+-	Rod G	+	+	P3	T3	B2	o12	St2	M2
312	+	Rod C	-	-	-----No Growth-----					
314	-	Rod S	-	-	P2	T1	B0	o12	Nb3	Tt2
315	-	Rod S	-	-	P1	T3	B0	o10	St0	M0
318	-	Rod C	+	+	P4	T3	B2	o12	St2	M2
322	-	Rod C	+	+						
323	-	Rod S	+	-	P4	T4	B2	o13	St3	M3
324	-	Rod P	+	-	P5	T4	B3	o13	St3	M4
326	-	Rod S	-	-	P1	T2	B0	o10	Nb1	Tt2
327	-	Cocci G	-	-	P0	T1	B1	o10	St1	M0
329	-	Cocci G	-	-	P0	T2	B0	o11	St0	M0
331	+	Rod C	-	-	-----No Growth-----					
332	-	Rod Ss	-	-	-----No Growth-----					
333	-	Rod Ss	+	-	P3	T3	B2	o12	Mb3	Tt3
334	+	Rod C	-	-	-----No Growth-----					
335	--	Rods & Cocci	+	+						
336	+-	Rods SP	-	-	P4	T3	B1	o12	St0	M3

Culture Number	Gram Reaction	Morphology	Starch Hydrology	Protein Hydrology	Antibiotic Sensitivity					
337	-	Rod Ss	-	-	-----No Growth-----					
338	+	Rod C	+	+	P0	T1	B1	o12	St2	M2
339	-	Rod P	+	+	P3	T3	B2	o12	St2	M2
340	+-	Rod C	+	+	P3	T3	B2	o12	St2	M3
341	-	Rod C	+	+	P3	T3	B2	o12	St2	M2
342	-	Rod C	+	+	P3	T3	B2	o12	St2	M2
343	-	Rod C	+	-	P3	T4	B1	o12	St3	M3
344	-	Rod	-	-	-----No Growth-----					
345	+-	Rod C	+	+	P3	T3	B3	o12	Nb3	Tt3
346	-	Rod C	+		P2	T3	B2	o12	Nb2	Tt2
347	-	Rod	-	-	P2	T3	B1	o12	Nb3	Tt3
349	+	Rod C	+	+	P0	T2	B1	o11	Nb2	Tt2
350	+	Rod C	+	+	P3	T2	B2	o12	Nb2	Tt2
351	-	Rod C	+	+	P3	T3	B2	o12	Nb1	Tt2
352	+	Rod C	+	-	P5	T4	B1	o12	Nb3	Tt4
353	-	Rod S	-	-	P2	T4	B0	o10	Nb1	Tt4
354	-	Rod S	+	-	P2	T3	B3	o12	Nb2	Tt3
356	-	Rod C	+	+	P3	T3	B3	o12	Nb2	Tt3
357	-	Rod C	+	+	P3	T3	B3	o12	Nb2	Tt3
360	-	Rod C	+	+	P0	T3	B2	o11	Nb2	Tt2
361	-	Rod S	-	-	P0	T2	B0	o12	Nb2	Tt2
362	+	Cocci	-	-	P2	T0	B2	o12	Nb3	Tt1
363	+-	Rod C	+	+	P4	T3	B3	o12	Nb3	Tt3
364	+-	Rod C	+							
366	-	Rod P	+	+	P3	T2	St1	o12	N1	M2
367	-	Rod P	+		P2	T3	B2	o12	Nb2	Tt3
368	-	Rod C	+	+	P3	T3	B2	o12	Nb2	Tt3
369	-	Rod C	+	+	P3	T3	St2	o12	N2	Tt2
370	-	Rod C	+	+	P3	T3	B3	o12	Nb2	Tt3
372	-	Rod C	+	+	P3	T2	B3	o12	Nb2	Tt3
373	+-	Rod C	+	+	P3	T3	B3	o12	Nb2	Tt3
374	-	Rod C	+	+	P4	T4	B3	o13	Nb3	Tt4

Culture Number	Gram Reaction	Morphology	Starch Hydrology	Protein Hydrology	Antibiotic Sensitivity					
375	+	Rod Ss	+	+	P0	T1	B1	o12	Nb1	Tt0
376	-	Rod C	+	+	P5	T5	St5	o13	N2	Tt3
379	+-	Rod C	+	-	P4	T2	B2	o12	Nb2	Tt3
380	+-	Rod C	+	-	-----No Growth-----					
381	-	Rod C	-							
387	-	Rod C	+	+	P4	T4	B3	o13	Nb2	Tt4
388	-	Rod Ss	+	+	P0	T1	B0	o10	Nb0	Tt0
389	-	Rod Ss	+	+	P2	T2	B1	o12	Nb1	Tt2
392	+	Rod S	+	+	P0	T1	B0	o12	Nb2	Tt1
393	-	Rod Ss	-	-	P0	T0	B0	o10	Nb1	Tt0
394	+	Rod PC	+	+	P0	T2	B1	o12	Nb2	Tt2
396	-	Rod Ss G	-	-	P0	T1	St1	o10	NO	M0
397	-	Rod C	-	-	--Complete Inhibition---					
400	+	Rod C	-	-	-----No Growth-----					
401	+	Rod G	-	-	-----No Growth-----					
402	-	Rod C	-	-	-----No Growth-----					
406	+-	Rod G	+	+	-----No Growth-----					
407	-	Rod C	+	+	P4	T3	B3	o12	Nb2	Tt3
408	-	Rod C	+	+	P3	T2	St1	o12	N1	M2
409	+	Rod P	+	+	P0	T0	B1	o11	Nb2	Tt1
412	-	Rod Ss	-	-	-----No Growth-----					
413	-	Rod Ss	+	+	P0	T3	B2	o12	Nb2	Tt3
414	-	Rod Ss	-	-	P0	T0	B0	o10	Nb0	Tt0
416	-	Rod G	+	-	-----No Growth-----					
419	-	Rod C	+	-	P1	T3	B2	o12	Nb1	Tt2
420	+-	Rod SP	+	+	P2	T2	B2	o12	Nb2	Tt2
429	+-	Rod Ps	+	+	P5	T4	B2	o13	Nb2	Tt5
430	+	Rod C	-	+	-----No Growth-----					
431	-	Rod C	+	-	--Complete Inhibition---					
432	+	Rod C	-	-	-----No Growth-----					
433	+-	Rod Ps	+	+	P2	T2	B1	o13	Nb2	Tt3

Culture Number	Gram Reaction	Morphology	Starch Hydrology	Protein Hydrology	Antibiotic Sensitivity					
434	+	Rod SP	+	+	P0	T2	B1	o12	Nb2	Tt1
435	-	Rod C	+	-	P2	T2	B1	o11	Nb2	Tt2
437	-	Rod SP	-	-						
440	+	Rod PS	+	-	P2	T2	B2	o12	Nb2	Tt2
442	+-	Rod S	+	-	-----No Growth-----					
447	-	Rod Ss P	-	-	-----No Growth-----					
448	-	Rod	+	+	P2	T3	B2	o12	Nb2	Tt2
449	-	Rod S	+	+	P3	T3	B2	o12	Nb2	Tt2
454	-	Rod S	-	-	P3	T2	B0	o10	Nb3	Tt2
458	+-	Rod SP	+	+	P0	T2	B0	o11	Nb2	Tt1
463	-	Rod C	-	-	P2	T2	B1	o12	Nb2	Tt2

APPENDIX B

FIELD DATA

Microbiology--Biomass

Microbiology--biomass data collected in 1970 at the Osage Site is Grassland Biome data set A2U4029. Data were collected on form NREL-42. A sample data form and a sample of the data follow.

GRASSLAND BIOME  
U.S. INTERNATIONAL BIOLOGICAL PROGRAM

## FIELD DATA SHEET - MICROBIOLOGY - BIOMASS

TYPE	DATE	INITIALS	DATE			PLOT SIZE	DEPTH, TOP	DEPTH, BOTTOM	CORE WT.	SAMPLE WT.	COUNT- LENGTH	
			Day	Mo	Yr							
I-2	3-4	5-7	8-9	10-11	12-13	14	15	16-19	21	23-25	27-29	31-35
<b>DATA TYPE</b>												
01	Aboveground Biomass											
02	Litter											
03	Belowground Biomass											
10	Vertebrate - Live Trapping											
11	Vertebrate - Snap Trapping											
12	Vertebrate - Collection											
20	Avian Flush Census											
21	Avian Road Count											
22	Avian Road Count Summary											
23	Avian Collection - Internal											
24	Avian Collection - External											
25	Avian Collection - Plumage											
30	Invertebrate											
40	Microbiology - Decomposition											
41	Microbiology - Nitrogen											
42	Microbiology - Biomass											
43	Microbiology - Root Decomposition											
	Microbiology - Respiration											
<b>SITE</b>												
01	Ale											
02	Bison											
03	Bridger											
04	Cottonwood											
05	Dickinson											
06	Hays											
07	Hopland											
08	Jornada											
09	Osage											
10	Pantex											
11	Pawnee											
<b>TREATMENT</b>												
1	Ungrazed											
2	Lightly grazed											
3	Moderately grazed											
4	Heavily grazed											
5	Grazed 1969, ungrazed 1970											
6												
7												
8												
9												
<b>TYPE</b>												
1	Bacteria											
2	Bacterial spore											
3												
4												
5	Fungus											
<b>HORIZON</b>												
1	AO											
2	A											
3	B											
4	C											

+++ EXAMPLE OF DATA +++

	1	2	3	4	5	6	7	8
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890
4209JOH03067011		00 05	0004 5 81					
		00 05	0004 5 163					
		00 05	0004 5 215					
		00 05	0004 5 208					
		00 05	0004 5 348					
		05 10	0004 5 273					
		05 10	0004 5 271					
		05 10	0004 5 204					
		05 10	0004 5 349					
		05 10	0004 5 443					
		10 20	0004 5 83					
		10 20	0004 5 83					
		10 20	0004 5 26					
		10 20	0004 5 129					
		10 20	0004 5 93					
		10 20	0004 5 25					
4209JOH03067012		10 20	0075 5 357					
		10 20	0075 5 361					
		10 20	0075 5 376					
		10 20	0075 5 136					
		10 20	0075 5 287					
4209JOH03067013		00 05	0004 5 205					
		00 05	0004 5 299					
		00 05	0004 5 292					
		00 05	0004 5 257					
		00 05	0004 5 360					
		05 10	0004 5 240					
		05 10	0004 5 270					
		05 10	0004 5 299					
		05 10	0004 5 204					
		05 10	0004 5 242					
		10 20	0080 5 449					
		10 20	0080 5 303					
		10 20	0080 5 253					
		10 20	0080 5 537					
		10 20	0080 5 190					
4209JOH03067031		00 05	0004 5 302					
		00 05	0004 5 511					
		00 05	0004 5 411					
		00 05	0004 5 317					
		00 05	0004 5 361					
		05 10	0004 5 132					
		05 10	0004 5 254					
		05 10	0004 5 86					
		05 10	0004 5 113					
		05 10	0004 5 121					
		10 20	0086 5 227					
		10 20	0086 5 341					
		10 20	0086 5 398					
		10 20	0086 5 348					
		10 20	0086 5 326					

4209JOH03067032

00	05	0004 5 141
00	05	0004 5 88
00	05	0004 5 220
00	05	0004 5 205
00	05	0004 5 210
05	10	0004 5 201
05	10	0004 5 232
05	10	0004 5 299
05	10	0004 5 229
05	10	0004 5 299
10	20	0086 5 335
10	20	0086 5 470
10	20	0086 5 353
10	20	0086 5 357
10	20	0086 5 412

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00	05	0004 5 133
00	05	0004 5 107
00	05	0004 5 230
00	05	0004 5 192
00	05	0004 5 300
05	10	0004 5 141
05	10	0004 5 103
05	10	0004 5 163
05	10	0004 5 224
05	10	0004 5 393
10	20	0076 5 459
10	20	0076 5 511
10	20	0076 5 450
10	20	0076 5 512
10	20	0076 5 190

**Microbiology--Decomposition**

Microbiology--decomposition data collected in 1970 at the Osage Site is Grassland Biome data set A2U4009. Data were collected on form NREL-40. A sample data form and a listing of the data follow.



## GRASSLAND BIOME

## U.S. INTERNATIONAL BIOLOGICAL PROGRAM

## FIELD DATA SHEET - MICROBIOLOGY - DECOMPOSITION

**DATA TYPE**

- 01 Aboveground Biomass
  - 02 Litter
  - 03 Belowground Biomass
  - 10 Vertebrate - Live Trapping
  - 11 Vertebrate - Snap Trapping
  - 12 Vertebrate - Collection
  - 20 Avian Flush Census
  - 21 Avian Road Count
  - 22 Avian Road Count Summary
  - 23 Avian Collection - Internal
  - 24 Avian Collection - External
  - 25 Avian Collection - Plumage
  - 30 Invertebrate
  - 40 Microbiology - Decomposition
  - 41 Microbiology - Nitrogen
  - 42 Microbiology - Biomass
  - 43 Microbiology - Root Decomposition
  - Microbiology - Respiration

SITI

- 01 Ale  
 02 Bison  
 03 Bridger  
 04 Cottonwood  
 05 Dickinson  
 06 Hays  
 07 Hopland  
 08 Jornada  
 09 Osage  
 10 Pantex  
 11 Pawnee

## TREATMENT

- 1 Ungrazed
  - 2 Lightly grazed
  - 3 Moderately grazed
  - 4 Heavily grazed
  - 5 Grazed 1969, ungrazed 1970

## SAMPLE MATERIAL

- 1 Cellulose
  - 2 Litter
  - 3 Standing dead
  - 4
  - 5

\* \* \* FIELD DATA \* \* \*

4009.JOH30097011	2 05 180570 137 10.00 04.14 02.16 05.00 04.75
	2 05 180570 137 10.00 06.32 04.33 05.00 04.75
	2 05 180570 137 10.00 04.58 03.14 05.00 04.75
4009.JOH30097021	2 05 180570 137 10.00 03.37 01.17 05.00 04.75
	2 05 180570 137 10.00 05.51 04.40 05.00 04.75
	2 05 180570 137 10.00 05.73 05.18 05.00 04.75
4009.JOH30097011	4 05 180570 137 10.00 04.72 01.86 05.00 04.75
	4 05 180570 137 10.00 03.80 02.26 05.00 04.75
	4 05 180570 137 10.00 05.15 04.32 05.00 04.75
4009.JOH30097021	4 05 180570 137 10.00 01.69 00.83 05.00 04.75
	4 05 180570 137 10.00 04.92 01.71 05.00 04.75
	4 05 180570 137 10.00 03.77 02.85 05.00 04.75