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# MICROBIOLOGICAL STUDIES AT THE PANTEX SITE, 1971

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## ABSTRACT

This report covers the microbiology studies and decomposition studies at the Pantex Site during 1971. Bacteria and fungi were estimated by plate count procedures. The rates of grass and paper decomposition were determined. Several physical parameters such as soil water and density were determined. Significant differences were detected in the microbial populations between grazed and ungrazed treatments, between grass and prickly pear ground cover, between shaded and unshaded plots, and between different depths in the soil. Significant populations of anaerobic as well as aerobic bacteria were found, and relatively large numbers of algae were detected in the top 5 cm of the soil. A method for determining the effect of fertilizer treatment (or other treatment) on cellulose decomposition was evaluated.

## INTRODUCTION

The purpose of this study was to provide a profile of the microbial population of the soil and its activity during a growing season of the mixed blue grama and prickly pear grassland at the Pantex Site. The climate of this area is such that large variations in temperature and moisture occurred during the year. The Pullman silty loam of this site varied from powder dry to wet clay, depending on the amount of rain received by the site. The site changed drastically in appearance during the growing season. It was very dry during the spring and much of the summer, and this was reflected in the relative abundance of prickly pear ground cover. Later in the summer and fall rather substantial amounts of rain were received which resulted in an abundant growth of the grass.

## MATERIALS AND METHODS

### Testing Sites

Two areas approximately 1 mile apart were used in this study. The ungrazed study area was a 35-acre pasture located on land belonging to the Atomic Energy Commission. This site has not been grazed for 5 years. The grazed site has been continuously grazed during the previous years. Each of the sites was subdivided into two replicates. Each replicate was divided into 200 2.74 m × 2.74 m quadrats arranged in two parallel rectangles 5.49 × 137.2 m with an access alley between the two rectangles.

### Bacterial Population Studies

Samples were obtained by coring at monthly intervals with some variation caused primarily by weather. A total of eight samples was taken covering the period from mid-May to mid-November.

Except for the first sample period, sample cores were taken to a depth of 50 cm with a 2.5-cm diameter hydraulic corer. Twelve cores were taken from six plots (two per plot) chosen at random from each replicate. During some sampling periods a further stratification was made, and cores were taken from both areas which had predominantly grass or predominantly prickly pear ground cover for a total of 24 cores per replicate. The cores were removed from the corer and divided into five different horizons:

Depth 1 0-5 cm

Depth 2 5-10 cm

Depth 3 10-20 cm

Depth 4 20-30 cm

Depth 5 30-50 cm

Each of these horizons, from each core, was placed separately in a labeled, sterile, Whirl-Pak plastic bag, sealed, and placed in an ice chest at the time of collection. The first sample period differed in that the last two horizons were from 20-50 cm and 50-90 cm, respectively.

All samples from each horizon were pooled, pulverized, and mixed; and a 10-g sample was weighed and placed in 90 ml of sterile physiological saline. The samples were blended for a period of 3 min. Serial dilutions of the sample were prepared. Pour plates were prepared with five replicate plates at each appropriate dilution. The temperature and time of incubation depended on the culture medium as follows. The number of aerobic, mesophillic, and heterotrophs were assayed with "Plate Count Agar" or "Standard Methods Agar" and incubated for 3 days at 30°C before the colonies were counted. The number of bacteria and actinomycetes were determined

from these same plates by colonial morphology. Mesophillic anaerobes and facultative anaerobes were assayed using "Anaerobic Agar" (BBL) with incubation for 5 days under nitrogen at 30°C. Aerobic and anaerobic spore formers were assayed by heat shocking the appropriate dilution at 85°C for 20 min and plating on the appropriate medium.

Fungal Population Studies

Two methods were used for preparation of serial dilutions for estimation of fungi. The first was identical to that used for bacteria including the blending step. In the second technique the blending step was eliminated, and hand shaking was substituted. The first and usual procedure probably resulted in disruption of all but the spores. Fungi were cultivated by pour plate techniques in Rose-bengal Streptomycin Agar (Johnson, 1957) at 30°C for 3 days. As for bacteria, five replicate plates per dilution were poured and the colonies on all five plates were counted at the appropriate dilution.

Algal Population Studies

Serial dilutions for the estimation of the number of algae were prepared by hand shaking of the dilution bottles. The population was estimated by the terminal dilution technique. Serial dilutions were prepared from the soil suspensions, and a 1-ml aliquot from each appropriate dilution was transferred to each of 10 replicate culture tubes. Each tube contained 50 g of washed sand and 20 ml of Bristol's sodium nitrate solution (Allen, 1957). The tubes were incubated for several weeks in the greenhouse. The number of algae was calculated by the dilution frequency method.

Nutritional Groupings of Soil Microbial Populations

The nutritional groupings of the soil microbes were studied both as a group and as separate isolates of the typical aerobic heterotrophs. In all cases the terminal dilution technique was used with duplicate tubes of medium at each dilution. The media used were: phenol red broth, phenol red broth with cellulose powder, chitin medium (Skerman, 1967), basal salts medium (Johnson et al., 1959), basal salts medium plus cellulose, basal salts medium plus amino acids, basal salts medium plus yeast extract, basal salts medium plus growth factors, basal medium plus amino acids and growth factors, soil extract medium, and soil extract plus yeast extract. The cultures were incubated for 5 days at 30°C.

Decomposition

Rates of decomposition were evaluated by putting sample material in nylon net bags with 1-2 mm mesh and placing these containers in or on the soil for later recovery. Standard techniques were used as described in Technical Report No. 85 (French, 1971). Approximately 8 g of grass furnished from the Osage Site as "standard litter" was sewn into each bag using polyester thread. The grass was laid lengthwise in the bag so that there was maximum surface exposure and was stitched into place. Filter paper squares 8 cm × 8 cm were cut from Whatman No. 1 chromatography filter paper.

Only surface grass samples were used. Filter paper samples were placed both on the surface and at a depth of 5 cm. Five samples were used per replicate of each type sample. The samples were exposed for periods of approximately 1 month before recovery.

Carbon Dioxide Evolution

Carbon dioxide evolution was estimated by covering soil from which live plant cover had been removed with a 3-lb. coffee can by inserting the can 1-2 cm into the soil with 10 ml of 0.1 M KOH under the can for a period averaging 16 hr. The cans were covered with plastic to shade them from the sun. Trapped $CO_2$ was estimated by titration. A total of five samples per replicate was taken at each sampling period.

Physical Measurements

The pooled cores from each horizon were weighed to 0.1 g. Soil water content was determined gravimetrically on 10 g samples by drying at 105°C for 24 hr. Soil water in this report is reported as g $H_2O$/100 g dry soil. The dry sample was ashed at 600°C for 6 hr. The ash content is reported as percent dry soil weight.

## RESULTS

The results of the experiments are presented in Tables 1 through 11 and Fig. 1 through 4. A small difference between the average bacterial populations at the two sites was found. The total population of both aerobic and anaerobic heterotrophs was higher at the grazed site (Fig. 1). (Note: These data have not been analyzed statistically.) The populations of both bacteria and fungi were inversely related to depth (Fig. 1 and 2). This decrease in microbial population with increasing depth may also apply to both aerobic and anaerobic spore forming bacteria (Fig. 3). The average soil water content of each horizon increases with depth to 10 cm (Fig. 4).

An analysis of the actual microbial biomass for the grazed site at an average depth of 2.5 cm is as follows: aerobic heterotrophs (approximated individual wet weight = $10^{-12}$ g) $2.72 \times 10^{-5}$ g/g wet soil; and since

Table 1. Microbial population of soil from the grazed and ungrazed site under grass during 1971 (no./g we soil). (R = replicate.)

| Microbial Population | Grazed R | Grazed Sample Date 5/11 | 6/8 | 6/29 | 8/4 | 8/23 | 9/2 | 9/30 | 11/13 | Ungrazed R | Ungrazed Sample Date 6/8 | 6/29 | 8/4 | 9/ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *0-5 cm depth* | | | | | | | | | | | | | | |
| Aerobic heterotrophs No. × $10^7$ | 1 | 2.24 | 1.00 | 1.93 | 2.36 | 5.92 | 5.62 | .44 | 14.4 | 1 | 1.41 | 2.09 | 0.995 | 0.8 |
| | 2 | 1.93 | 1.20 | 1.95 | 2.04 | | 3.84 | 9.18 | 2.15 | 2 | 1.13 | 1.70 | 1.86 | 1.3 |
| | 3 | | | | | | 1.32 | | | | | | | |
| Bacteria No. × $10^7$ | 1 | 1.98 | 0.89 | 1.80 | 2.31 | 5.48 | 5.42 | 0.40 | 14.4 | 1 | 1.25 | 1.98 | 0.96 | 0.7 |
| | 2 | 1.76 | 1.10 | 1.75 | 1.98 | | 3.58 | 9.12 | 1.93 | 2 | 1.00 | 1.55 | 1.80 | 1.2 |
| | 3 | | | | | | 1.30 | | | | | | | |
| Actinomycete type colonies No. × $10^5$ | 1 | 27 | 9.6 | 9 | 3.6 | 5.6 | 0.4 | 4.0 | | 1 | 9.4 | 5.0 | 4.6 | 8.8 |
| | 2 | 13 | 7.4 | 5.2 | 5.6 | | 24 | 0.6 | | 2 | 8.4 | 6.2 | | 5.6 |
| | 3 | | | | | | 2.6 | | | | | | | |
| Anaerobes No. × $10^4$ | 1 | | | 8.4 | | | 259 | 104 | 88 | 1 | | 32.9 | | 11.8 |
| | 2 | | | 14.5 | | | 15.3 | 115 | 172 | 2 | | 25.1 | | 21.2 |
| | 3 | | | | | | 87.6 | | | | | | | |
| Fungi No. × $10^4$ | 1 | | 10.2 | 12.2 | 2.28 | 3.12 | 9.7 | 17.48 | | 1 | 5.2 | 12.9 | | 10.9 |
| | 2 | | 5.9 | 14.7 | 11.9 | 0.43 | 6.3 | 16.98 | | 2 | 6.3 | 12.2 | | .5 |
| | 3 | | | | | | 3.3 | | | | | | | |
| Algae No. × $10^3$ | 1 | | | 9 | | | 0.5 | 40 | | | | | | |
| | 2 | | | 9 | | | 0.1 | 60 | | | | | | |
| *5-10 cm depth* | | | | | | | | | | | | | | |
| Aerobic heterotrophs No. × $10^7$ | 1 | 1.29 | 1.18 | 1.26 | 1.83 | 1.29 | 2.65 | 7.36 | 2.24 | 1 | 0.97 | 0.92 | 0.72 | 1.82 |
| | 2 | 1.08 | 1.05 | 1.12 | 2.13 | | | 1.64 | 1.38 | 2 | 1.08 | 1.01 | 1.25 | 0.99 |
| | 3 | | | | | | 2.10 | | | | | | | |
| Bacteria No. × $10^7$ | 1 | 1.21 | 1.11 | 1.16 | 1.80 | 1.26 | 2.65 | 7.22 | 2.20 | 1 | 0.92 | 0.84 | 0.68 | 1.77 |
| | 2 | .98 | .93 | 1.00 | 2.28 | | | 1.60 | 1.23 | 2 | 0.96 | 0.93 | 1.21 | 0.94 |
| | 3 | | | | | | 2.02 | | | | | | | |
| Actinomycete type colonies No. × $10^5$ | 1 | 9.0 | 6.2 | 5.0 | 3.2 | 2.8 | 0 | 12 | | 1 | 4.0 | 4.0 | 4.0 | 4.6 |
| | 2 | 11.6 | 9.6 | 8.4 | 4.0 | | 7.0 | 2.8 | | 2 | 10.4 | 2.6 | 3.6 | |
| Anaerobes No. × $10^4$ | 1 | | | 26.6 | | | 8.1 | | 73 | 1 | | 12.3 | | 16.5 |
| | 2 | | | 23.9 | | | 115.2 | 14.9 | 147 | 2 | | 35.8 | | 13.8 |
| | 3 | | | | | | 51.2 | | | | | | | |
| Fungi No. × $10^4$ | 1 | | 5.24 | 5.7 | 6.86 | 3.0 | 3.6 | 8.62 | | 1 | 3.5 | 5.4 | 7.08 | 4.96 |
| | 2 | | 7.96 | 5.7 | 11.0 | 5.7 | 5.9 | 19.9 | | 2 | 3.0 | 6.9 | 9.9 | 1.884 |
| *10-20 cm depth* | | | | | | | | | | | | | | |
| Aerobic heterotrophs No. × $10^7$ | 1 | 1.70 | 0.96 | .68 | 1.12 | $>10^5$ | $>10^6$ | 1.28 | 2.2 | 1 | 0.62 | 0.68 | | .91 |
| | 2 | 1.08 | .82 | .67 | 1.80 | $>10^5$ | 1.18 | 1.43 | .73 | 2 | 0.58 | 0.52 | 0.83 | 1.06 |
| | 3 | | | | | | $>10^6$ | | | | | | | |
| Bacteria No. × $10^7$ | 1 | 1.61 | 0.89 | 0.59 | 1.04 | $>10^5$ | $>10^6$ | 1.21 | 2.1 | 1 | 0.57 | .63 | | 0.876 |
| | 2 | 1.01 | 0.72 | 0.62 | 1.76 | $>10^5$ | 1.12 | 1.38 | 0.71 | 2 | 0.52 | .48 | 7.8 | 0.98 |
| | 3 | | | | | | $>10^6$ | | | | | | | |
| Actinomycete type colonies No. × $10^5$ | 1 | 7 | 6.3 | 5.4 | 7.4 | | | 6.6 | | 1 | 5.4 | 3.0 | 4.0 | 3.4 |
| | 2 | 7 | 7.4 | 3.0 | 4.2 | | 6.0 | 4.4 | | 2 | 3.6 | 1.6 | 6.0 | |
| Anaerobes No. × $10^4$ | 1 | | | 20.0 | | | 75.8 | 28.7 | 73 | 1 | | 14.6 | | 13.9 |
| | 2 | | | 24.2 | | | 8.48 | 6.12 | 9.0 | 2 | | 19.4 | | |
| | 3 | | | | | | 24.0 | | | | | | | |
| Fungi No. × $10^4$ | 1 | | 2.22 | 1.85 | 3.9 | 3.44 | 1.53 | 3.4 | | 1 | 1.6 | 1.67 | 1.778 | 6.92 |
| | 2 | | 2.1 | 1.35 | 5.0 | 1.52 | 2.30 | 2.8 | | 2 | 1.5 | 0.72 | 4.6 | 1.61 |
| | 3 | | | | | | 0.96 | | | | | | | |

Table 1. (Cont.)

| Microbial Population | R | Grazed | | | | | | | | R | Ungrazed | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sample Date | | | | | | | | | Sample Date | | | |
| | | 5/11 | 6/8 | 6/29 | 8/4 | 8/23 | 9/2 | 9/30 | 11/13 | | 6/8 | 6/29 | 8/4 | 9/2 |
| | | | | | | | *20-30 cm depth* | | | | | | | |
| Aerobic heterotrophs No. × $10^7$ | 1 | | 0.40 | 0.37 | 0.554 | 0.3 | 0.50 | 0.81 | 1.59 | 1 | 0.60 | 0.33 | | 0.56 |
| | 2 | | 0.38 | 0.25 | 0.78 | 0.58 | 0.38 | 1.19 | 0.52 | 2 | 0.36 | 0.38 | | |
| Bacteria No. × $10^7$ | 1 | | 0.39 | 0.32 | 0.522 | 0.3 | 0.47 | 0.76 | 1.59 | 1 | 0.57 | 0.31 | | 0.51 |
| | 2 | | 0.36 | 0.23 | 0.73 | 0.51 | 0.36 | 1.12 | 0.49 | 2 | 0.36 | 0.36 | | |
| Actinomycete type colonies No. × $10^5$ | 1 | | 1.2 | 2 | 2.8 | 0.3 | 2.4 | 4 | | 1 | 2.6 | 0.3 | 0.8 | |
| | 2 | | 1.4 | 0.8 | 4.8 | 6.8 | 2.4 | 6.4 | | 2 | 0.4 | 1.6 | 3.5 | |
| Anaerobes No. × $10^4$ | 1 | | | 22.5 | | | 58.2 | | 29.4 | 1 | | 14.5 | | |
| | 2 | | | 13.2 | | | 13.1 | 30 | 37.2 | 2 | | 16.5 | | 34.0 |
| Fungi No. × $10^4$ | 1 | | 0.62 | 0.52 | 1.09 | 0.64 | 0.66 | | | 1 | 0.22 | 0.51 | 0.614 | 0.678 |
| | 2 | | 1.0 | 0.43 | 1.42 | 1.05 | 0.62 | | | 2 | 0.4 | 0.56 | 0.654 | 0.804 |
| | 3 | | | | | | 0.97 | | | | | | | |
| | | | | | | | *30-50 cm depth* | | | | | | | |
| Aerobic heterotrophs No. × $10^7$ | 1 | | 0.23 | 0.24 | 0.34 | | | 0.38 | | 1 | 0.47 | 0.18 | | 0.36 |
| | 2 | | 0.24 | 0.16 | 0.49 | | 0.30 | 0.46 | | 2 | 0.20 | 0.26 | | 0.37 |
| | 3 | | | | | | 0.47 | | | | | | | |
| Bacteria No. × $10^7$ | 1 | | 0.21 | 0.20 | 0.32 | | | 0.36 | 8.9 | 1 | 0.46 | 0.18 | | 0.32 |
| | 2 | | 0.24 | 0.14 | 0.45 | | 0.31 | 0.45 | | 2 | 0.20 | 0.25 | | 0.406 |
| | 3 | | | | | | 0.45 | | | | | | | |
| Actinomycete type colonies No. × $10^5$ | 1 | | 1.8 | 2.2 | 2.2 | | 0 | 2.6 | | 1 | 0.6 | 0 | 2.8 | |
| | 2 | | 0.4 | 0.6 | 3.8 | | 0 | 1.6 | | 2 | 0 | 0.3 | 2.6 | 3.8 |
| | 3 | | | | | | 2 | | | | | | | |
| Anaerobes No. × $10^4$ | 1 | | | 20.4 | | | | | 22.4 | 1 | | 14.9 | | |
| | 2 | | | 15.5 | | | 14.2 | 6.3 | | 2 | | 14.9 | | 31.0 |
| | 3 | | | | | | 11.3 | | | | | | | |
| Fungi No. × $10^4$ | 1 | | 0.4 | 0.36 | 0.63 | 0.41 | 0.45 | 0.42 | | 1 | 0.14 | 0.16 | 0.388 | 0.70 |
| | 2 | | 0.9 | 0.13 | 0.46 | | 0.44 | 0.79 | | 2 | 0.2 | 0.20 | 0.338 | 0.36 |
| | 3 | | | | | | 0.67 | | | | | | | |

Table 2. Microbial population of the soil from the grazed and ungrazed site under prickly pear during 1971 (no./g wet soil). (R = replicate.)

| Microbial Population | Grazed | | | | Ungrazed | | |
|---|---|---|---|---|---|---|---|
| | R | Sample Date | | | R | Sample Date | |
| | | 5/11 | 6/8 | 8/4 | | 6/8 | 8/4 |
| | | | *0-5 cm depth* | | | | |
| Aerobic heterotrophs No. × $10^7$ | 1 | 1.72 | 1.46 | 1.722 | 1 | 1.18 | |
| | 2 | 1.96 | 1.94 | 6.84 | 2 | 0.69 | 7.5 |
| Bacteria No. × $10^7$ | 1 | 1.54 | 1.33 | 1.662 | 1 | 1.06 | |
| | 2 | 1.85 | 1.86 | 6.44 | 2 | 0.63 | 6.88 |
| Actinomycete type colonies No. × $10^5$ | 1 | 10.0 | 9.6 | 15.28 | 1 | 9.3 | 7.2 |
| | 2 | 10.0 | 6.4 | 38.0 | 2 | 2.8 | 4.8 |
| Fungi No. × $10^4$ | 1 | 80.0 | 36.0 | 14.48 | 1 | 25.0 | 9.46 |
| | 2 | 30.0 | 18.0 | 10.92 | 2 | 32.0 | 26.68 |
| | | | *5-10 cm depth* | | | | |
| Aerobic heterotrophs No. × $10^7$ | 1 | 1.30 | 0.75 | 2.05 | 1 | 0.81 | |
| | 2 | 1.20 | 1.12 | 1.878 | 2 | 0.96 | 1.838 |
| Bacteria No. × $10^7$ | 1 | 1.20 | 0.65 | 2.008 | 1 | 0.74 | |
| | 2 | 0.91 | 1.05 | 1.828 | 2 | 0.90 | 1.838 |
| Actinomycete type colonies No. × $10^5$ | 1 | 7.0 | 7.6 | 3.8 | 1 | 5.2 | 4.4 |
| | 2 | 21.0 | 5.8 | 4.4 | 2 | 5.3 | 2.4 |
| Fungi No. × $10^4$ | 1 | 26.0 | 28.0 | 13.72 | 1 | 16.0 | 5.6 |
| | 2 | 80.0 | 8.0 | 10.72 | 2 | 7.5 | 7.1 |
| | | | *10-20 cm depth* | | | | |
| Aerobic heterotrophs No. × $10^7$ | 1 | 0.88 | 0.60 | 0.886 | 1 | 0.63 | |
| | 2 | 0.79 | 0.77 | 1.342 | 2 | 0.44 | 3.86 |
| Bacteria No. × $10^7$ | 1 | 0.82 | 0.54 | 0.81 | 1 | 0.58 | |
| | 2 | 0.75 | 0.73 | 1.342 | 2 | 0.41 | 3.42 |
| Actinomycete type colonies No. × $10^5$ | 1 | 5.4 | 5.4 | 5.2 | 1 | 3.8 | 5.4 |
| | 2 | 4.0 | 3.8 | 4.4 | 2 | 2.3 | 4.4 |
| Fungi No. × $10^4$ | 1 | 10.0 | 0 | 3.16 | 1 | 5.0 | 1.388 |
| | 2 | 2.0 | 0 | 1.936 | 2 | 10.0 | 4.92 |
| | | | *20-30 cm depth* | | | | |
| Aerobic heterotrophs No. × $10^7$ | 1 | 0.33 | 0.23 | 0.63 | 1 | 0.26 | |
| | 2 | 0.14 | 0.38 | 0.776 | 2 | 0.36 | 0.428 |
| Bacteria No. × $10^7$ | 1 | 0.32 | 0.19 | 0.596 | 1 | 0.24 | |
| | 2 | 0.13 | 0.37 | 0.744 | 2 | 0.34 | 0.390 |
| Actinomycete type colonies No. × $10^5$ | 1 | 1.8 | 2.2 | 2.8 | 1 | 1.0 | |
| | 2 | 0.8 | 1.0 | 3.0 | 2 | 2.0 | 4.68 |
| Fungi No. × $10^4$ | 1 | 0 | 1.6 | 3.36 | 1 | 12.0 | 0.332 |
| | 2 | 2.0 | 0 | 1.266 | 2 | 4.0 | 0.522 |

Table 2. (Cont.)

| Microbial Population | R | Grazed | | | R | Ungrazed | |
|---|---|---|---|---|---|---|---|
| | | Sample Date | | | | Sample Date | |
| | | 5/11 | 6/8 | 8/4 | | 6/8 | 8/4 |
| | | | *30-50 cm depth* | | | | |
| Aerobic heterotrophs No. × $10^7$ | 1 | 0.17 | 0.29 | 0.484 | 1 | 0.26 | |
| | 2 | 0.094 | 0.17 | 0.752 | 2 | 0.26 | 0.366 |
| Bacteria No. × $10^7$ | 1 | 0.16 | 28.0 | 0.468 | 1 | 0.25 | |
| | 2 | 0.086 | 0.16 | 0.734 | 2 | 0.26 | 0.346 |
| Actinomycete type colonies No. × $10^5$ | 1 | 1.0 | 0.8 | 1.6 | 1 | 0.6 | 3.2 |
| | 2 | 0.8 | 1.4 | 1.8 | 2 | 0.2 | 2.0 |
| Fungi No. × $10^4$ | 1 | 0 | 0 | 3.78 | 1 | 0 | 0.542 |
| | 2 | 0 | 0 | | 2 | 0 | 0.226 |

Table 3. Microbial population of the soil with white and black frames during 1971 (no./g wet soil). (R = replicate.)

| Microbial Population | White | | | | Black | | | |
|---|---|---|---|---|---|---|---|---|
| | R | Sample Date | | | R | Sample Date | | |
| | | 9/2 | 9/30 | 11/13 | | 9/2 | 9/30 | 11/13 |
| | | | | *0-5 cm depth* | | | | |
| Aerobic heterotrophs No. × $10^7$ | 1 | 5.3 | 6.2 | 2.27 | 1 | 6.1 | 9.4 | 2.53 |
| | 2 | 4.1 | | | 2 | 2.26 | 9.2 | |
| | 3 | 11.66 | 10.1 | | 3 | 0.81 | 10.1 | |
| Bacteria No. × $10^7$ | 1 | 5.0 | 6.1 | | 1 | 5.9 | 9.2 | |
| | 2 | 3.7 | | | 2 | 2.36 | 9.0 | |
| | 3 | 1.16 | 9.9 | | 3 | 0.74 | 9.7 | |
| Actinomycete type colonies No. × $10^5$ | 1 | 32.0 | 12.0 | | 1 | 18.0 | 10.0 | 2 |
| | 2 | 34.0 | | | 2 | 5.6 | 24.0 | |
| | 3 | 1.2 | 14.0 | | 3 | | 32.0 | |
| Anaerobes No. × $10^4$ | 1 | | | 40.4 | 1 | | 79.4 | 270 |
| | 2 | | 11.98 | | 2 | | 170.4 | |
| | 3 | 178.0 | 165.0 | | 3 | | 167.6 | |
| Fungi No. × $10^4$ | 1 | 1.756 | 4.76 | | 1 | 4.1 | 13.1 | |
| | 2 | 10.32 | 11.24 | | 2 | | 16.74 | |
| | 3 | 12.1 | 11.42 | | 3 | | 14.66 | |
| | | | | *5-10 cm depth* | | | | |
| Aerobic heterotrophs No. × $10^7$ | 1 | | 1.40 | 1.75 | 1 | | 2.05 | 0.97 |
| | 2 | 1.696 | | | 2 | 3.8 | 1.91 | |
| | 3 | 1.96 | 5.4 | | 3 | 1.20 | 5.2 | |
| Bacteria No. × $10^7$ | 1 | | 1.34 | | 1 | | 1.96 | |
| | 2 | 1.614 | | | 2 | 3.4 | 1.81 | |
| | 3 | 1.942 | 5.2 | | 3 | 1.13 | 4.9 | |
| Actinomycete type colonies No. × $10^5$ | 1 | 7.0 | 5.0 | | 1 | | 8.6 | |
| | 2 | 7.6 | 9.0 | | 2 | 9.0 | 9.2 | |
| | 3 | 1.2 | 20.0 | | 3 | 7.0 | 28.0 | |
| Anaerobes No. × $10^4$ | 1 | 75.6 | 7.02 | 31.8 | 1 | | | 138 |
| | 2 | | 13.9 | | 2 | | | |
| | 3 | 74.2 | | | 3 | | | |
| Fungi No. × $10^4$ | 1 | 7.76 | 11.38 | | 1 | | 10.64 | |
| | 2 | 8.06 | 4.74 | | 2 | 5.24 | 19.02 | |
| | 3 | 8.70 | 7.0 | | 3 | 2.184 | 14.56 | |
| | | | | *10-20 cm depth* | | | | |
| Aerobic heterotrophs No. × $10^7$ | 1 | | | | 1 | 1.0 | 1.0 | 0.77 |
| | 2 | 0.798 | 1.02 | 0.88 | 2 | 1.28 | 1.33 | |
| | 3 | 1.972 | | | 3 | 0.53 | 1.10 | |
| Bacteria No. × $10^7$ | 1 | | | | 1 | 0.92 | 0.93 | |
| | 2 | 0.75 | 0.98 | | 2 | 1.19 | 1.28 | |
| | 3 | 1.944 | | | 3 | 0.48 | 1.05 | |
| Actinomycete type colonies No. × $10^5$ | 1 | 5.0 | | | 1 | 5.4 | 6.2 | |
| | 2 | 4.6 | 5.1 | | 2 | 8.6 | 5.2 | |
| | 3 | 2.4 | | | 3 | 4.4 | 4.6 | |
| Anaerobes No. × $10^4$ | 1 | | | 24.4 | 1 | | | 43.1 |
| | 2 | | 14.52 | | 2 | | 27.78 | |
| | 3 | 80.6 | | | 3 | | 4.16 | |
| Fungi No. × $10^4$ | 1 | 1.03 | 2.108 | | 1 | 1.92 | 1.996 | |
| | 2 | 1.362 | 6.52 | | 2 | | 5.72 | |
| | 3 | 4.64 | 7.68 | | 3 | 1.554 | 6.0 | |

Table 3. (Cont.)

| Microbial Population | R | White Sample Date 9/2 | 9/30 | 11/13 | R | Black Sample Date 9/2 | 9/30 | 11/13 |
|---|---|---|---|---|---|---|---|---|
| | | | *20-30 cm depth* | | | | | |
| Aerobic heterotrophs No. × $10^7$ | 1 | 0.744 | 0.60 | 0.63 | 1 | 0.68 | 0.66 | 0.48 |
| | 2 | 0.98 | | | 2 | 0.71 | 0.43 | |
| | 3 | 1.478 | 8.3 | | 3 | | 0.69 | |
| Bacteria No. × $10^7$ | 1 | 0.718 | 0.56 | | 1 | 0.64 | 0.63 | |
| | 2 | 1.97 | | | 2 | 0.66 | 0.42 | |
| | 3 | 1.47 | 7.8 | | 3 | | 0.64 | |
| Actinomycete type colonies No. × $10^5$ | 1 | 2.8 | 3.6 | | 1 | 4.6 | 3.8 | |
| | 2 | 5.0 | 0.4 | | 2 | 4.8 | 1.2 | |
| | 3 | 0.8 | 3.8 | | 3 | | 4.2 | |
| Anaerobes No. × $10^4$ | 1 | | | 24.9 | 1 | | 16.12 | 53 |
| | 2 | 32.2 | | | 2 | | | |
| | 3 | 52.6 | 22.64 | | 3 | | | |
| Fungi No. × $10^4$ | 1 | 0.814 | 1.308 | | 1 | 0.92 | 1.27 | |
| | 2 | 0.824 | 0.586 | | 2 | 0.916 | 1.238 | |
| | 3 | 1.236 | 1.332 | | 3 | 0.336 | 1.26 | |
| | | | *30-50 cm depth* | | | | | |
| Aerobic heterotrophs No. × $10^7$ | 1 | | | | 1 | 0.45 | 1.08 | |
| | 2 | | | | 2 | | 0.43 | |
| | 3 | 0.534 | 1.75 | | 3 | | 0.49 | |
| Bacteria No. × $10^7$ | 1 | | | | 1 | 0.41 | 1.09 | |
| | 2 | | | | 2 | | 0.40 | |
| | 3 | 0.522 | 1.75 | | 3 | | 0.38 | |
| Actinomycete type colonies No. × $10^5$ | 1 | 3.6 | | | 1 | 4.2 | 0.2 | |
| | 2 | | | | 2 | | 3.0 | |
| | 3 | 1.2 | 0 | | 3 | | 2.2 | |
| Anaerobes No. × $10^4$ | 1 | | | | 1 | | | |
| | 2 | 7.66 | | | 2 | | | |
| | 3 | | | | 3 | | | |
| Fungi No. × $10^4$ | 1 | 0.57 | 1.672 | | 1 | 0.57 | 1.02 | |
| | 2 | 0.53 | 0.792 | | 2 | 0.398 | 1.21 | |
| | 3 | 0.986 | 1.126 | | 3 | | 0.994 | |

Table 4. Physical properties of soil from the grazed and ungrazed grass during 1971. (R = replicate.)

| Physical Properties | Grazed R | Grazed Sample Date 5/11 | 6/8 | 6/29 | 8/4 | 8/23 | 9/2 | 9/30 | 11/13 | Ungrazed R | Ungrazed Sample Date 6/8 | 6/29 | 8/4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *0-5 cm depth* | | | | | | | | | | | | | |
| Core weight (g) | 1 | 141.1 | 288.2 | 259.0 | 273.0 | 312.2 | | 51.8 | 325.7 | 1 | 235.9 | 226.9 | 323.9 |
| | 2 | 147.1 | 247.9 | 255.5 | 253.0 | 322.0 | | 319.7 | 380.4 | 2 | 256.1 | 226.2 | 319.6 |
| g HOH/100 g dry soil | 1 | 3.70 | 6.04 | 3.1 | 4.22 | 14.2 | 5.18 | 22.5 | | 1 | 6.2 | 5.5 | 10.3 |
| | 2 | 4.10 | 6.57 | 4.7 | 4.90 | 14.0 | 4.22 | 25.6 | | 2 | 5.7 | 5.3 | 9.4 |
| | 3 | | $\bar{x}$=6.30 | | | | 4.93 | $\bar{x}$=24.0 | | | 6.0 | 5.4 | 9.9 |
| | | | | | | | $\bar{x}$=4.78 | | | | | | |
| g ash/100 g dry soil | 1 | 94.3 | 94.4 | 94.8 | 94.0 | 93.9 | 95.0 | 94.7 | | 1 | 93.3 | 93.5 | 93.15 |
| | 2 | 95.0 | 94.4 | 93.7 | 93.6 | 92.9 | 89.3 | 94.7 | | 2 | 94.2 | 94.1 | 93.43 |
| | 3 | | | | | | 95.0 | | | | | | |
| *5-10 cm depth* | | | | | | | | | | | | | |
| Core weight (g) | 1 | 175.9 | 326.7 | 304.6 | 308.7 | 392.1 | 412.9 | 412.9 | 317.5 | 1 | 353.5 | 344.5 | 370.2 |
| | 2 | 130.2 | 349.4 | 343.6 | 309.5 | 383.3 | 444.3 | | 401.4 | 2 | 322.3 | 290.8 | 465.2 |
| g HOH/100 g dry soil | 1 | 5.37 | 7.06 | 3.63 | 5.4 | 17.2 | 7.8 | 21.9 | | 1 | 7.8 | 7.0 | 11.3 |
| | 2 | 4.82 | 6.8 | 5.4 | 5.69 | 15.9 | 7.24 | 22.2 | | 2 | 6.7 | 6.3 | 12.4 |
| | 3 | | | | | | 8.23 | | | | | | |
| g ash/100 g dry soil | 1 | 95.0 | 95.7 | 95.9 | 94.8 | 95.2 | 95.4 | 95.7 | | 1 | 95.4 | 95.4 | 94.49 |
| | 2 | 94.4 | 95.6 | 95.7 | 94.9 | 95.3 | 95.5 | 95.7 | | 2 | 95.6 | 95.8 | 94.57 |
| | 3 | | | | | | 95.5 | | | | | | |
| *10-20 cm depth* | | | | | | | | | | | | | |
| Core weight (g) | 1 | 326.2 | 661.7 | 605.1 | 601.5 | 793.5 | | | 938.0 | 1 | 633.5 | 718.2 | 747.5 |
| | 2 | 272.6 | 583.0 | 561.8 | 672.5 | 762.2 | | 1933.4 | | 2 | 795.8 | 623.2 | 839.6 |
| g HOH/100 g dry soil | 1 | 8.34 | 9.9 | 5.48 | 6.59 | 20.2 | 11.2 | 23.3 | | 1 | 9.2 | 9.9 | 13.9 |
| | 2 | 8.6 | 9.9 | 8.1 | 10.53 | 13.3 | 10.99 | 24.2 | | 2 | 9.4 | 7.0 | 13.1 |
| | 3 | | | | | | 11.8 | | | | | | |
| g ash/100 g dry soil | 1 | 95.4 | 96.0 | 96.0 | 95.1 | 95.0 | 95.7 | 96.9 | | 1 | 95.2 | 96.0 | 94.62 |
| | 2 | 95.7 | 95.6 | 95.7 | 95.1 | 95.3 | 95.5 | 96.0 | | 2 | 95.7 | 96.0 | 94.57 |
| | 3 | | | | | | 95.3 | | | | | | |
| *20-30 cm depth* | | | | | | | | | | | | | |
| Core weight (g) | 1 | | 379.2 | 728.8 | 779.0 | 884.0 | | | 1050.4 | 1 | 750.4 | 735.6 | 885.5 |
| | 2 | | 657.4 | 756.9 | 716.7 | 779.7 | | 1064.5 | 875.3 | 2 | 866.3 | 728.2 | 844.5 |
| g HOH/100 g dry soil | 1 | | 11.4 | 7.1 | 9.65 | 20.7 | 12.0 | 24.6 | | 1 | 10.1 | 10.7 | 12.1 |
| | 2 | | 11.1 | 9.8 | 10.29 | 24.7 | 13.4 | 24.7 | | 2 | 10.9 | 10.4 | 12.1 |
| | 3 | | | | | | 14.8 | | | | | | |
| g ash/100 g dry soil | 1 | | 95.7 | 96.2 | 95.2 | 95.5 | 95.9 | 95.7 | | 1 | 95.8 | 97.1 | 94.2 |
| | 2 | | 95.9 | 95.9 | 95.1 | 95.0 | 95.9 | 95.7 | | 2 | 97.1 | 96.3 | 94.84 |
| | 3 | | | | | | 95.6 | | | | | | |
| *30-50 cm depth* | | | | | | | | | | | | | |
| Core weight (g) | 1 | | 1551.4 | 1441.7 | 1731.2 | 1554.9 | 2107.6 | 2107.6 | 2043.1 | 1 | 1585.2 | 1695.6 | 1734.3 |
| | 2 | | 1590.5 | 1529.3 | 1557.9 | 1558.1 | 1933.4 | | | 2 | 1673.3 | 1653.2 | 1630.4 |
| g HOH/100 g dry soil | 1 | | 11.2 | 7.9 | 9.87 | 13.6 | 10.6 | 16.3 | | 1 | 11.0 | 10.6 | 11.6 |
| | 2 | | 11.1 | 9.5 | 19.25 | 15.1 | 11.5 | 15.4 | | 2 | 10.5 | 10.9 | 11.1 |
| g ash/100 g dry soil | 1 | | 96.0 | 96.3 | 94.7 | 95.9 | 95.4 | 95.6 | | 1 | 96.4 | 97.2 | 95.22 |
| | 2 | | 96.2 | 95.9 | 95.2 | 95.3 | 95.0 | 95.8 | | 2 | 96.0 | 96.4 | 97.22 |

Table 5. Physical properties of soil for the grazed and ungrazed prickly pear during 1971. (R = replicate.)

| Physical Properties | Grazed | | | | Ungrazed | | |
|---|---|---|---|---|---|---|---|
| | R | Sample Date | | | R | Sample Date | |
| | | 5/11 | 6/8 | 8/4 | | 6/8 | 8/4 |
| | | | | *0-5 cm depth* | | | |
| Core weight (g) | 1 | 134.1 | 318.2 | 307.3 | 1 | 355.3 | 289.5 |
| | 2 | 131.7 | 299.2 | 272.5 | 2 | 299.5 | 297.2 |
| g HOH/100 g dry soil | 1 | 4.0 | 6.3 | 4.5 | 1 | 7.5 | 5.7 |
| | 2 | 4.8 | 6.9 | 4.7 | 2 | 7.0 | 8.8 |
| g ash/100 g dry soil | 1 | 93.6 | 95.0 | 94.72 | 1 | 95.2 | 93.5 |
| | 2 | 94.5 | 94.6 | 94.15 | 2 | 94.5 | 93.92 |
| | | | | *5-10 cm depth* | | | |
| Core weight (g) | 1 | 166.1 | 372.9 | 320.2 | 1 | 354.0 | 397.6 |
| | 2 | 124.1 | 357.3 | 299.4 | 2 | 322.5 | 446.3 |
| g HOH/100 g dry soil | 1 | 4.9 | 7.1 | 5.0 | 1 | 8.6 | 7.8 |
| | 2 | 4.5 | 7.3 | 5.7 | 2 | 6.8 | 11.9 |
| g ash/100 g dry soil | 1 | 95.5 | 95.8 | 95.17 | 1 | 95.9 | 93.45 |
| | 2 | 94.9 | 92.7 | 95.17 | 2 | 95.4 | 95.3 |
| | | | | *10-20 cm depth* | | | |
| Core weight (g) | 1 | 332.1 | 695.0 | 557.2 | 1 | 743.0 | 744.8 |
| | 2 | 267.3 | 723.6 | 647.6 | 2 | 599.9 | 839.6 |
| g HOH/100 g dry soil | 1 | 7.6 | 9.2 | 6.4 | 1 | 11.0 | 10.5 |
| | 2 | 8.8 | 9.5 | 8.1 | 2 | 10.4 | 18.7 |
| g ash/100 g dry soil | 1 | 95.2 | 95.3 | 95.07 | 1 | 95.7 | 95.2 |
| | 2 | 95.4 | 96.0 | 94.60 | 2 | 95.8 | 95.65 |
| | | | | *20-30 cm depth* | | | |
| Core weight (g) | 1 | 1186.6 | 862.8 | 655.9 | 1 | 715.3 | 850.5 |
| | 2 | 1148.6 | 748.2 | 706.7 | 2 | 718.2 | 844.5 |
| g HOH/100 g dry soil | 1 | 10.4 | 10.3 | 9.2 | 1 | 11.5 | 11.4 |
| | 2 | 10.9 | 6.5 | 10.6 | 2 | 10.9 | 11.5 |
| g ash/100 g dry soil | 1 | 95.7 | 96.1 | 95.20 | 1 | 95.8 | 95.45 |
| | 2 | 95.1 | 95.7 | 95.47 | 2 | 95.9 | 95.25 |
| | | | | *30-50 cm depth* | | | |
| Core weight (g) | 1 | 1611.6 | 1004.6 | 1626.0 | 1 | 1576.2 | 1681.9 |
| | 2 | 1596.7 | 1652.1 | 1451.3 | 2 | 1612.6 | 1630.4 |
| g HOH/100 g dry soil | 1 | 9.9 | 10.5 | 9.3 | 1 | 11.9 | 10.9 |
| | 2 | 10.1 | 10.3 | 7.9 | 2 | 10.7 | 11.0 |
| g ash/100 g dry soil | 1 | 95.7 | 96.1 | 95.32 | 1 | 96.3 | 95.87 |
| | 2 | 95.9 | 96.0 | 95.65 | 2 | 95.9 | 95.77 |

Table 6. Physical properties of soil with white and black frames during 1971. (R = replicate.)

| Physical Properties | White | | | Black | | |
|---|---|---|---|---|---|---|
| | R | Sample Date | | R | Sample Date | |
| | | 9/2 | 9/30 | | 9/2 | 9/30 |
| | | | *0-5 cm depth* | | | |
| Core weight (g) | 1 | | 77.55 | 1 | | 45.6 |
| | 2 | | 65.0 | 2 | | 48.7 |
| | 3 | | 42.0 | 3 | | 47.7 |
| g HOH/100 g dry soil | 1 | 13.9 | 22.7 | 1 | 21.1 | 22.9 |
| | 2 | 13.3 | 23.7 | 2 | 17.8 | 28.9 |
| | 3 | 11.8 | 27.0 | 3 | 14.8 | 27.0 |
| g ash/100 g dry soil | 1 | 94.21 | 95.77 | 1 | 95.9 | 95.16 |
| | 2 | 94.95 | 95.09 | 2 | 95.67 | 93.82 |
| | 3 | 94.97 | 93.70 | 3 | 95.45 | 95.01 |
| | | | *5-10 cm depth* | | | |
| Core weight (g) | 1 | | 57.2 | 1 | | 58.9 |
| | 2 | | 70.2 | 2 | | 72.9 |
| | 3 | | 79.3 | 3 | | 72.3 |
| g HOH/100 g dry soil | 1 | 14.5 | 21.5 | 1 | 16.8 | 24.0 |
| | 2 | 16.1 | 22.2 | 2 | 21.2 | 24.4 |
| | 3 | 13.4 | 23.2 | 3 | 14.7 | 23.6 |
| g ash/100 g dry soil | 1 | 95.82 | 96.45 | 1 | 95.37 | 95.37 |
| | 2 | 95.45 | 95.47 | 2 | 95.42 | 95.45 |
| | 3 | 95.35 | 95.48 | 3 | 95.72 | 95.92 |
| | | | *10-20 cm depth* | | | |
| Core weight (g) | 1 | | 151.2 | 1 | | 153.6 |
| | 2 | | 142.5 | 2 | | 160.5 |
| | 3 | | 145.0 | 3 | | 149.4 |
| g HOH/100 g dry soil | 1 | 19.2 | 24.2 | 1 | 22.3 | 23.9 |
| | 2 | 15.3 | 22.9 | 2 | 19.2 | 26.5 |
| | 3 | 15.7 | 24.0 | 3 | 16.7 | 25.2 |
| g ash/100 g dry soil | 1 | 95.97 | 96.00 | 1 | 95.82 | 96.08 |
| | 2 | 95.80 | 96.00 | 2 | 95.80 | 95.85 |
| | 3 | 95.65 | 95.71 | 3 | 95.35 | 95.50 |
| | | | *20-30 cm depth* | | | |
| Core weight (g) | 1 | | 178.9 | 1 | | 178.8 |
| | 2 | | 200.5 | 2 | | 197.9 |
| | 3 | | 202.1 | 3 | | 199.4 |
| g HOH/100 g dry soil | 1 | 22.5 | 20.7 | 1 | 23.0 | 24.2 |
| | 2 | 17.1 | 23.0 | 2 | 23.7 | 22.4 |
| | 3 | 19.7 | 23.9 | 3 | 16.8 | 23.9 |
| g ash/100 g dry soil | 1 | 95.70 | 95.78 | 1 | 95.82 | 95.85 |
| | 2 | 96.10 | 95.62 | 2 | 94.87 | 96.08 |
| | 3 | 95.70 | 95.84 | 3 | 95.47 | 95.95 |
| | | | *30-50 cm depth* | | | |
| Core weight (g) | 1 | | 355.2 | 1 | | 402.5 |
| | 2 | | 333.25 | 2 | | 300.2 |
| | 3 | | 402.5 | 3 | | 298.2 |
| g HOH/100 g dry soil | 1 | 17.6 | 11.6 | 1 | 19.7 | 15.8 |
| | 2 | 17.4 | 12.2 | 2 | 25.5 | 14.6 |
| | 3 | 16.1 | 14.4 | 3 | 12.3 | 16.8 |
| g ash/100 g dry soil | 1 | 96.10 | 95.70 | 1 | 95.75 | 94.89 |
| | 2 | 95.77 | 95.35 | 2 | 93.35 | 95.27 |
| | 3 | 95.82 | 95.26 | 3 | 98.82 | 96.03 |

Table 7. Carbon dioxide evolution from the surface of the soil ($g/m^2/24$ hr). (R = replicate.)

| Site | R | Date and Collection Period | | | |
|---|---|---|---|---|---|
| | | 6/8 (23 hr) | 8/4 (21 hr) | 9/2 (10 hr) | 9/30 (9 hr 45 min) |
| Grazed | 1 | | 6.11 | 14.3 | 10.6 |
| | 2 | 4.41 | 5.97 | 14.4 | 13.0 |
| Ungrazed | 1 | 5.25 | 6.09 | 14.5 | |
| | 2 | 4.40 | 5.99 | 14.6 | |

Table 8. Filter paper decomposition during 1971.

| Replicate | Placement | Treatment | | |
|---|---|---|---|---|
| | | Grazed (% loss) | Ungrazed (% loss) | Unknown (% loss) |
| | | *5/25 to 6/29* | | |
| 1 | Surface | 0.02 | 0.88 | |
| | Subsurface | 29.0 | 13.6 | |
| 2 | Surface | 0.89 | 3.01 | |
| | Subsurface | +9.79 | 8.39 | |
| | | *6/29 to 7/27* | | |
| Unknown | Surface | | | 8.02 |
| | Subsurface | | | 32.9 |
| | | *7/27 to 8/23* | | |
| 1 | Surface | 2.83 | 3.25 | |
| | Subsurface | 29.4 | 25.7 | |
| 2 | Surface | | 1.0 | |
| | Subsurface | | 16.7 | |
| | | *8/4 to 9/2* | | |
| 1 | Subsurface | 35.4 | | |
| | | *9/2 to 9/30* | | |
| 1 | Surface | 7.10 | | |
| | Subsurface | 11.6 | | |

Table 9. Grass decomposition during 1971.

| Replicate | Treatment | | |
|---|---|---|---|
| | Grazed (% loss) | Ungrazed (% loss) | Unknown (% loss) |
| | *5/25 to 6/29* | | |
| 1 | 13.99 | 17.6 | |
| 2 | 18.4 | 18.0 | |
| | *6/29 to 7/27* | | |
| Unknown | | | 24.0 |
| | *7/27 to 8/23* | | |
| 1 | 23.6 | 23.6 | |
| 2 | | 22.1 | |

Table 10. Effect of fertilizer treatment on decomposition of filter paper from August 4, 1971 to September 2, 1971.

| Weight Loss | R | Control | $(NH_4)SO_4$ | | |
|---|---|---|---|---|---|
| | | | 0.1 M | 0.06 M | 0.02 M |
| Wt loss (g) | 1 | 0.990 | | 0.590 | 0.285 |
| | 2 | 0.955 | 0.422 | 0.740 | 0.780 |
| | 3 | 0.610 | 0.825 | 0.721 | 0.360 |
| | 4 | 0.330 | 0.206 | 0.210 | 0.600 |
| | 5 | 0.195 | | 0.940 | |
| Total wt loss (g) | | 3.080 | 1.453 | 3.201 | 2.025 |
| Total original wt | | 8.700 | 5.680 | 9.370 | 7.390 |
| Avg % loss | | 35.4 | 25.6 | 34.2 | 27.4 |

| Weight Loss | R | $(NH_4)_2HPO_4$ | | | $KH_2PO_4$ | | |
|---|---|---|---|---|---|---|---|
| | | 0.1 M | 0.06 M | 0.02 M | 0.1 M | 0.06 M | 0.02 M |
| Wt loss (g) | 1 | 0.465 | 0.285 | 0.566 | 0.160 | 0.380 | 0.485 |
| | 2 | 0.540 | 0.530 | 0.265 | 0.305 | 0.600 | 0.915 |
| | 3 | 0.220 | 0.395 | 0.285 | 0.200 | 0.340 | 0.375 |
| | 4 | 1.005 | 1.095 | 0.290 | 0.295 | 1.185 | 0.835 |
| | 5 | 0.555 | | | | | |
| Total wt loss (g) | | 2.785 | 2.305 | 1.406 | 0.960 | 2.505 | 2.610 |
| Total original wt | | 8.990 | 7.240 | 7.130 | 7.430 | 7.130 | 7.470 |
| Avg % loss | | 30.97 | 31.8 | 19.7 | 12.9 | 35.1 | 34.9 |

| Weight Loss | R | $KNO_3$ | | | $NH_4NO_3$ | | |
|---|---|---|---|---|---|---|---|
| | | 0.1 M | 0.06 M | 0.02 M | 0.1 M | 0.06 M | 0.02 M |
| Wt loss (g) | 1 | 0.325 | 1.365 | 0.431 | 0.325 | 0.280 | 1.055 |
| | 2 | 0.180 | 1.181 | 0.440 | 0.620 | 1.275 | 0.505 |
| | 3 | 0.325 | 0.456 | 0.285 | 0.485 | 0.160 | 0.660 |
| | 4 | 0.275 | 0.330 | 0.174 | 0.450 | 0.845 | 0.810 |
| | 5 | | | 0.360 | 0.260 | | 0.210 |
| Total wt loss (g) | | 1.105 | 3.332 | 1.690 | 2.140 | 2.560 | 3.240 |
| Total original wt | | 7.300 | 7.60 | 8.860 | 9.34 | 7.13 | 9.30 |
| Avg % loss | 15.1 | | 43.8 | 19.1 | 22.9 | 35.9 | 34.8 |

Table 11. Filter paper decomposition under covered frames during 1971.

| Placement | Control (% loss) | Black (% loss) | White (% loss) |
|---|---|---|---|
| | *8/4 to 9/2* | | |
| Surface | | 7.52 | 4.11 |
| Subsurface | 35.4 | 17.2 | 27.6 |
| | *9/2 to 9/30* | | |
| Surface | 7.10 | 6.07 | 6.19 |
| Subsurface | 11.6 | 29.1 | 29.2 |

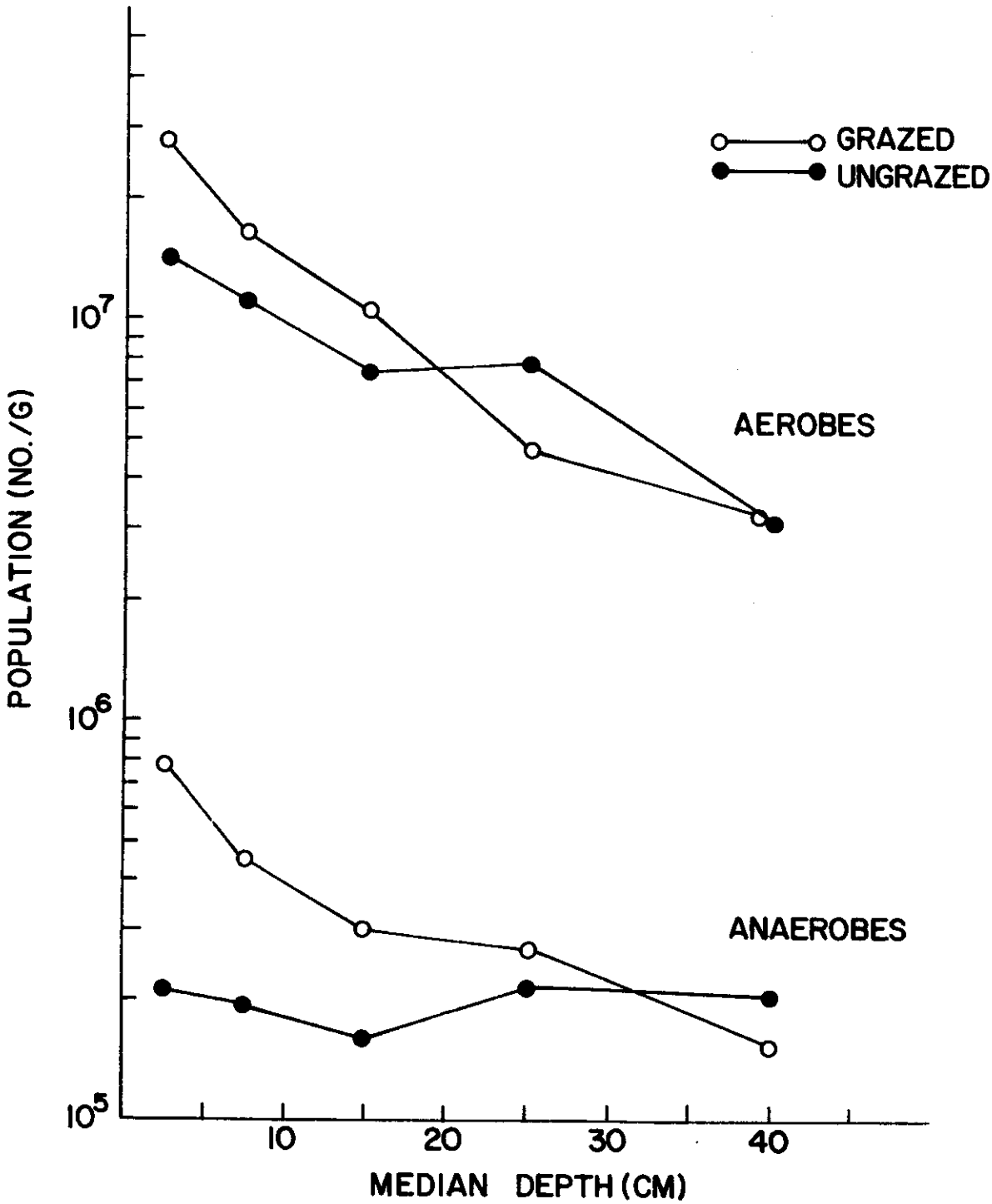


Fig. 1. Effects of depth on the average total population of heterotrophic bacteria in the soil between June 8, 1971, and September 2, 1971.

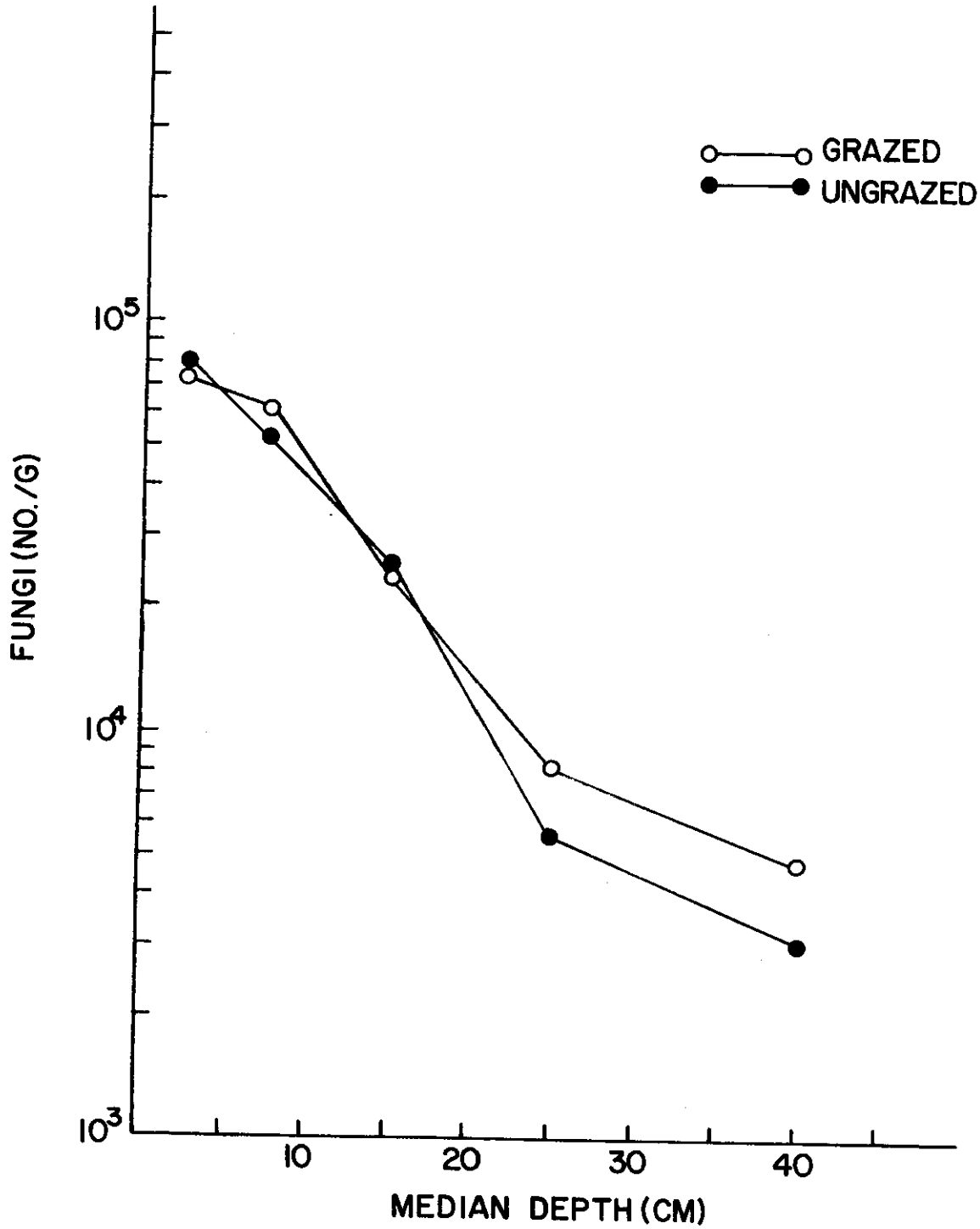


Fig. 2. Effects of depth on the average total population of aerobic fungi in the soil between June 8, 1971, and September 2, 1971.

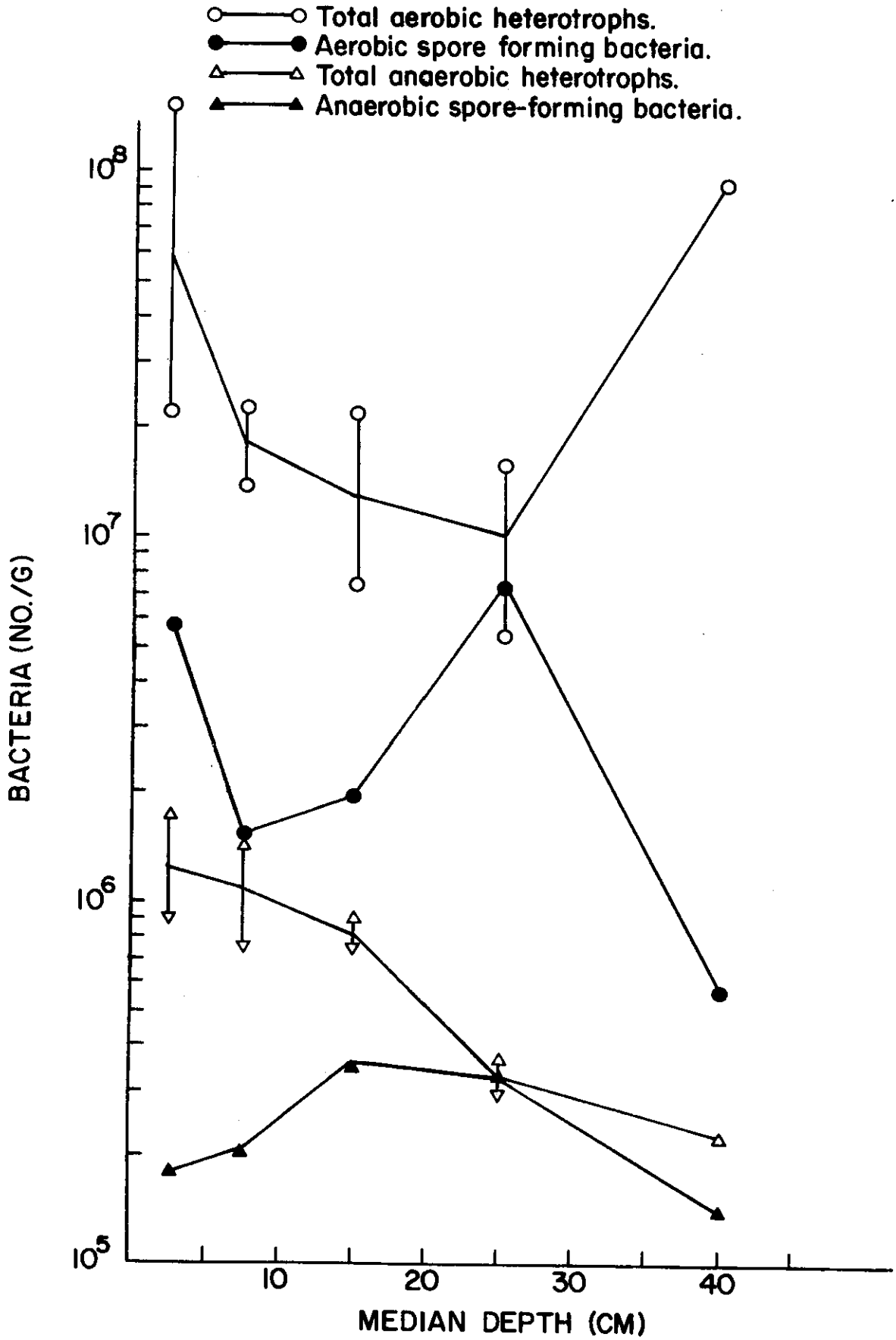


Fig. 3. Comparison of the effects of depth on the number of spore-forming bacteria and the total number of bacteria on the grazed site on November 13, 1971.

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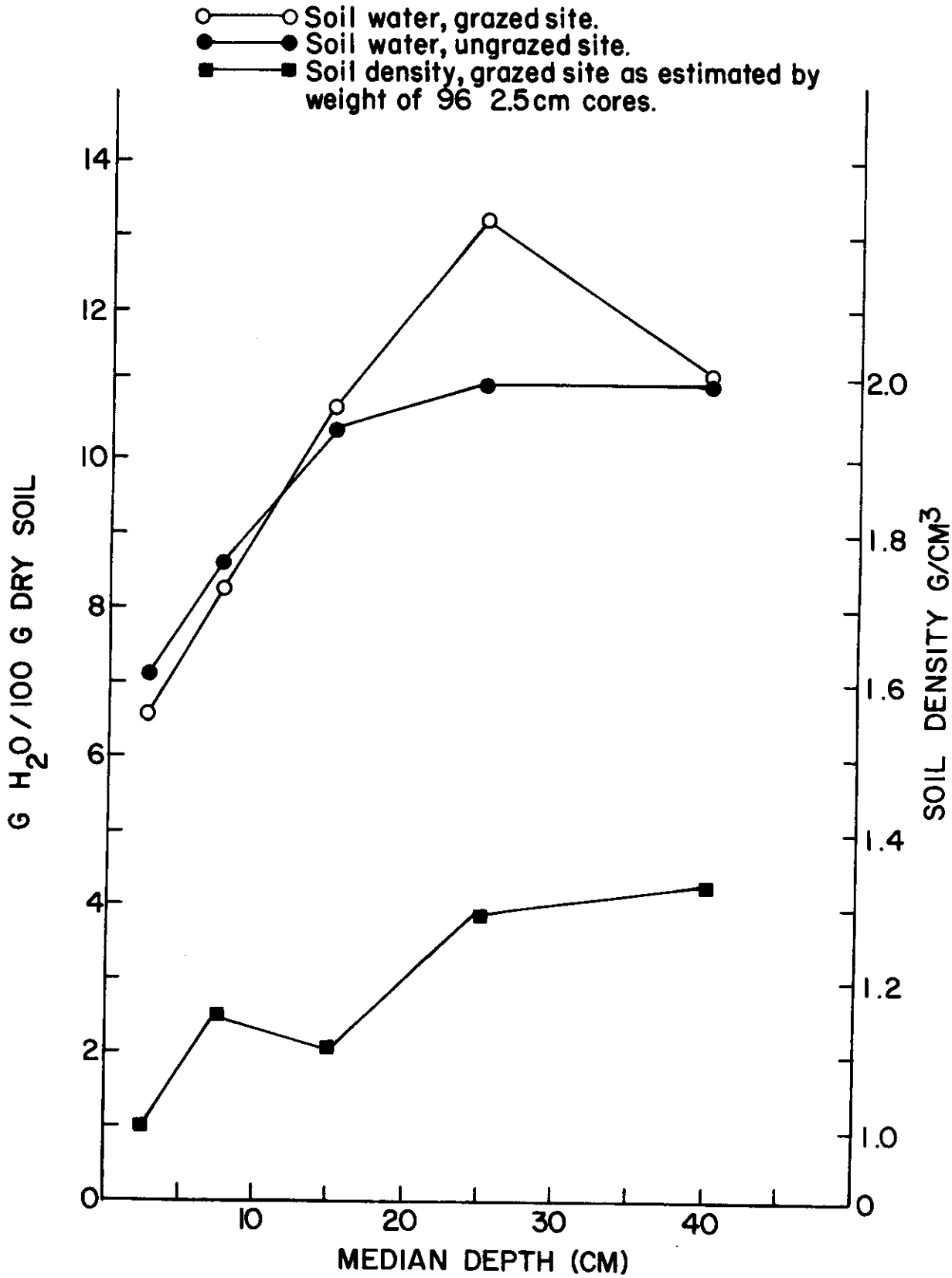


Fig. 4. Effects of depth on the average soil water and soil density during the period June 8, 1971, to September 2, 1971.

on this site the average density of the soil in this 5-cm layer was very close to 1.0, it can be calculated that there were approximately 1.36 g of aerobic heterotrophs per square meter. Further, since the number of anaerobes in this soil was very nearly as great, the actual weight could be expected to be at least twice the above weight. This does not consider the autotrophs which were not estimated, nor does it provide an estimate of the dormant or non-viable cells.

Comparison of the microbial populations under grass with those under prickly pear indicate a very pronounced increase in the number of fungi under the pear. This effect was found only in the first two horizons.

Examination of the microbial populations under the white and black frames and comparison with the grazed site on the same dates indicate some effect on the populations. Covering the ground with white cloth apparently resulted in a decrease in the fungal population. The population of the aerobic heterotrophs was significantly greater under both black and white frames in the first horizon.

In an attempt to determine the nutritional capabilities of mixed soil cultures, various media were inoculated from dilutions of soil samples obtained on June 8, 1971. Under aerobic and anaerobic growth conditions, growth of bacteria and acid production were obtained at a terminal dilution of $10^{-7}$ in phenol red broth of samples from 0-5 cm and 5-10 cm on both replicates of both grazed and ungrazed sites. The addition of cellulose did not increase the acid production by the cultures. Growth was obtained in the chitin medium only at dilutions of $10^{-4}$ or less.

The experiment described above was repeated on June 30, 1971, and was expanded to include several other media. Growth and acid production by

cultures from the first two horizons were as reported above. Below 10 cm of depth growth was not obtained at dilutions greater than $10^{-5}$. Culture dilutions from the first two horizons, from either site or replicate, in the basal medium did not result in growth at dilutions greater than $1 \times 10^{67}$. Addition of either amino acids or yeast extract increased the maximum dilution by at least a factor of 10. The addition of cellulose did not result in a greater maximum dilution than that obtained with the basal medium. Growth in the chitin medium did not occur at dilutions beyond $1 \times 10^{-4}$.

Sixteen culture isolates were obtained from the most common colony types on the aerobic standard methods agar. Three of these cultures grew in all eight media tested including the basal salts medium. One culture failed to grow only in the basal medium. Three cultures did not grow only in the basal medium plus growth factors. One culture failed to grow only in the basal medium containing both amino acids and growth factors. Three cultures did not grow in the basal medium, the basal medium plus cellulose, and the basal medium plus growth factors. One culture grew only in the basal medium containing soil extract or soil extract plus yeast extract. Two cultures did not grow in the basal medium, the basal medium plus cellulose, the basal medium plus growth factors, or the basal medium plus both growth factors and amino acids. One culture failed to grow in the basal medium, the basal medium plus cellulose, and the basal medium plus growth factors. One culture failed to grow only in the basal medium and the basal medium plus growth factors. One culture grew in every medium but the basal medium containing both amino acids and growth factors.

## DISCUSSION

The results of these experiments indicate that there is a large active microbial biomass in the soil of the shortgrass prairie. There are apparently some small differences in the number of bacteria which may be associated with grazing by cattle. There are relatively large populations of both aerobic and anaerobic bacteria and smaller numbers of fungi and algae. The numbers of algae were much higher in the fall which was probably associated with the available soil water.

The decomposition studies added very little information to the study except for the treated filter paper study. There may have been slightly more activity in the fall than in the spring, but the variability and considerable loss of samples makes this conclusion questionable. Loss of samples occurred from the surface due to cattle and apparently also jackrabbits. The cattle also pulled up the metal flags marking buried samples, apparently attracted by the plastic flags. The only conclusions that I am willing to draw from the standard decomposition study is that the decomposition rate is greater on buried samples. The number of samples which would be required to provide good statistics would be at least twice the number actually used.

An experiment was conducted using filter paper and treating each sample with various levels of phosphate or nitrogen. These results (Table 10) also were not conclusive, but did indicate that $KNO_3$ greatly enhanced filter paper decay and that very high concentrations lowered the decomposition rates. This technique with greater replication might prove valuable for evaluating the effect of various fertilizers on decomposition rates. A clear trend was seen, even in this experiment with only five replications

per treatment, between the medium and the high and low concentration treatments.

The effects of covering the ground with either a dark or white cloth were not pronounced, although differences were observed in the bacterial populations. There may also have been a considerable difference in the decomposition rates of cellulose during the second month. This experiment would probably be more valid if conducted for a much longer period.

Considerable information was obtained as to both the number and the nutritional types of bacteria in the soil of the shortgrass prairie. Of 16 isolates three were able to grow in a very simple medium. One culture apparently required only additional amino acids. Four cultures apparently were inhibited by added growth factors or growth factors plus amino acids. None of the cultures seemed to have a requirement for cellulose. One culture required unidentified substances in soil extract. Two cultures required unidentified substances in yeast extract or soil extract. Since very little chitin utilization was found either in mixed or pure cultures, it would be of considerable interest to know the turnover time for chitin in this soil.

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## LITERATURE CITED

Allen, O. N. 1957. Experiments in soil bacteriology. 3rd ed. Burgess Publ. Co., Minneapolis.

French, N. R. [Coordinator]. 1971. Basic field data collection procedures for the Grassland Biome 1971 season. U.S. IBP Grassland Biome Tech. Rep. No. 85. Colorado State Univ., Fort Collins. 87 p.

Johnson, L. F. 1957. Effect of antibiotics on the numbers of bacteria and fungi isolated from the soil by the dilution plate method. Phytopathology 47:630-631.

Johnson, L. F., E. A. Curl, J. H. Bond, and H. A. Fribourg. 1959. Methods for studying soil microflora-plant disease relationships. Burgess Publ. Co., Minneapolis.

Skerman, V. D. B. 1967. A guide to the identification of the genera of bacteria. 2nd ed. Williams & Wilkins Co., Baltimore.