Technical Report No. 22

BACTERIAL ECOLOGY OF GRASSLAND SOILS

PAWNEE SITE

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U. S. International Biological Program

November 15, 1969

INTRODUCTION

This report describes the work that has been conducted in the study of the bacterial population in the soil of the Pawnee National Grassland IBP research station. The primary objectives were to obtain estimates of the bacterial population with depth in the soil profile and to determine if there was any indication of variation in the population of the different treatments the grazing plots had been subjected to in the past years. These treatments were ungrazed exclosures, lightly grazed areas, heavily grazed areas and moderately grazed areas. The preliminary work involved the use of soil samples to determine which methods would be best suited for determining the viable bacterial biomass of the soils. A comparison was made of bacterial culture media in order to determine which medium would be best suited for determining the bacterial population of the soils. A comparison was made of bacterial culture media in order to determine which medium would be best suited for the bacterial enumerations. Treatment of the soil by different methods, that is, sonication, blending, or other means of mechanical agitation, were examined in order to determine which procedure would yield the best and most consistent results.

METHODS

One of the first objectives of this research program was to determine which medium would be best suited for enumeration of the bacterial populations in the Pawnee Grassland soils. Three commonly used laboratory media were compared with the same soil to determine the ability of each medium to support the growth of the maximum numbers of the soil population. These three media were (1) plate count agar, (2) soil extract agar, and (3) sodium caseinate agar. Comparative dilution plate counts on these three selective media show that plate

count agar generally gave the highest total count of the three media. Sodium caseinate, which is generally used to enumerate the actinomycetes, gave the lowest count of the three media. As a result of repeated comparative studies, plate count agar was selected as the medium of choice for future studies involving the enumeration of the general bacterial population. Soil extract agar, however, also was used for comparative counts on occasion, particularly when major changes in the bacterial population were suspected, such as may occur immediately after a rainfall.

After the medium of choice had been selected, studies to determine the best method of dispersing the bacterial soil suspension were initiated. Normal agitation of soil suspensions by hand (25 strokes in a one-foot radius) were compared with the use of sonic energy and with the use of a Waring blender. The use of sonic energy for the dispersion of the bacterial cells gave erratic results. In other words, some samples gave counts much lower than the control, while other samples gave counts approximately equal to the hand-shaken controls. We concluded that the use of sonic energy for the dispersion of bacteria from soil suspensions was not suitable to reproducible results.

The second treatment that was attempted was a comparison of normal hand agitation vs. agitation in a Waring blender. Blending for as short a time as one minute gave counts approximately four times greater than agitation by hand. It was decided that the Waring blender be the instrument of choice for the dispersion of the soil bacterial suspensions.

Comparisons of two quantities of soil suspensions were used in the blending study. The first consisted of the addition of 10 g of soil to 95 ml of sterile buffered water in the blender; the second quantity was 20 g of soil in 190 ml sterile buffered water. The soil suspensions consisting of 95 ml of

water and 10 g of soil provided more consistent results and less variation, as well as higher total counts, than did the suspensions of 20 g of soil in 190 ml of water.

An additional study with the blender involved exposure of the soil bacterial suspensions to different blending times to determine whether there was a maximum count which could be reached by blending for a time period which would not harm the organisms as a result of heating or mechanical agitation. The results showed a relatively uniform count obtained during periods of blending ranging from three to 10 minutes.

One particular problem encountered during the blending studies was the significant increase in temperature in relation to the blending time. In a study to determine the temperature ranges as related to the blending times, temperatures were recorded at the end of each blending time. Two different blenders were used for two different studies, using only room temperature water blanks on one blender and using both room temperature water blanks and refrigerated blanks on the second blender. Using the first blender, a 10-degree increase in temperature was registered over the initial blending periods of up to 10 minutes. However, when multiple samples were being analyzed, a greater increase in temperature was noted. A significant increase in temperature is noted with the increase in blending times where the second blender was used, regardless of whether the water blanks were refrigerated or not. Although a significant increase in counts is observed with increase in blending time through 15 minutes, it is impractical to use such long blending times because of the increase in temperature that is observed. Other workers have generally settled on a blending time of two minutes in order to avoid problems due to heating, and we have decided upon the use of

refrigerated water blanks to further offset any additional heating problems encountered.

A study was conducted to determine whether or not the addition of a surface active agent would facilitate the dispersion of the bacterial particles. Several test runs, with counts made at both 7 and 14 days did not indicate any advantage to adding the surface active agent to the soil bacterial suspensions. The agent we employed was Tween-80, and due to the results obtained from the study, no such agent was added to any of the subsequent test suspensions.

Treatment of Samples

Field samples were initially taken to a depth of 90 cm by use of a hand corer and later to the same depth with an hydraulic corer. The cores were removed from the corer and cut into sections representing the appropriate depth increments. Each section of the core was placed in a polyethylene bag which was then tied shut and placed in a paper specimen carton for returning to the laboratory. Once removed to the laboratory, the samples were pulverized and mixed. A 10 g sample was weighed for each depth interval and then added to 95 ml sterile buffered distilled water in a sterile Waring blender jar. The samples were blended for 10 minutes and then 10-fold serial dilutions were prepared through a 10^{-6} dilution. One-tenth ml aliquots were added to triplicate plate count agar plates and spread over the surface with sterile glass rods. For depths of soil samples from 0 to 45 cm, aliquots on the agar plates were made to give 10^{-5}, 10^{-6}, and 10^{-7} dilutions. For depths from 45 to 90 cm, aliquots were made to give 10^{-4}, 10^{-5}, and 10^{-6} dilutions. After inoculation using the spread-plate technique, the plates were incubated in an inverted position at 30°C in a humidified incubator for 7 to 14

days. The plates were then counted with a New Brunswick Colony Counter. In addition to the total bacterial count, an estimate was made of the actinomycete count. Those dilutions containing between 20 and 200 colonies were used to estimate the total population.

Field Studies

After the development of the techniques we felt would provide us with most reliable counts, samples were taken in the field to a depth of 90 cm for the purpose of determining bacterial counts. Most of the early sampling through winter and early spring was done on one site. Initial studies were made only on cores taken from the heavily-grazed area of the Pawnee Site. Composite samples were made of the material for each sample depth. Sample depths were 0 to 7.5 cm, 7.5 to 15 cm, 15 to 30 cm, 30 to 45 cm, 45 to 60 cm, 60 to 75 cm, and 75 to 90 cm.

In May, we began sampling all four grazing treatment areas independently of other investigators. However, later in the summer, our sampling was coordinated with that of the investigators studying the root biomass distribution in the soil profile. As a result, our sampling interval changed, and the total depth of sampling was decreased to 80 cm. Sampling intervals were changed to 0 to 10 cm, 10 to 20 cm, 20 to 40 cm, 40 to 60 cm, and 60 to 80 cm.

RESULTS

General distribution of the bacterial population for the sampling times is illustrated graphically in Fig. 1-6. One can see that the general distribution of microbial population with depth decreases and that the major change in numbers occurs below 45 cm. In those samples which were

correlated with the root biomass studies, Fig. 10-15, the breaking point in the population numbers occurs in the 40 cm range. Those samples taken below 40-45 cm generally had a bacterial population of less than 10×10^6 per g of soil. The bacterial population in the upper layers of soil generally ran from 10×10^6 to 75×10^6 per g of soil.

Data obtained from the early sampling studies are not included here, since contamination and spreading colonies on the plates made it quite difficult to interpret or evaluate the counts obtained. In some instances, the actual count should have been higher than was observed and in others the count represents an over-estimation of the population. After adequate measures were taken to avoid or eliminate the sources of contamination, reproducibility of results was much improved.

Fig. 1-4 show counts obtained from the four grazing treatment areas at seven depth levels down to 90 cm. Fig. 4 summarizes data from one sampling site (heavily grazed), obtained from the three separate experiments shown in Fig. 1, 2, and 3.

Fig. 5 and 6 show the counts obtained from sampling the four grazing treatments in coordination with the root biomass determinations. Note the difference in sampling depths in these and subsequent figures. Due to the apparent variability of counts between the sites and within the same treatment site, we decided to begin taking triplicate samples at each grazing treatment area. Replicate samples showed that, with the exception of the heavily grazed area samples, the variability within a given site may not be significant. However, statistical verification of non-significance has not been completed.

CONCLUSIONS

The preliminary results of population estimation on the four major grazing treatments included in the overall experimental design indicate that there may be differences in the bacterial population detected by the plate count technique. However, the sampling system does not allow adequate statistical analysis between treatments, since only one core sample was taken from each treatment. The latter experiments in which core samples were taken in triplicate showed that it is possible to get as much difference in counts within a treatment as among treatments. Statistical analysis of these data has not been completed. The results of these analyses and the evaluation of our data as compared to that of other groups, especially the root biomass studies and total biomass studies, should dictate changes in sampling as well as call for additional information to provide a more complete study. This information and suggested changes should be available before field sampling begins in the spring.

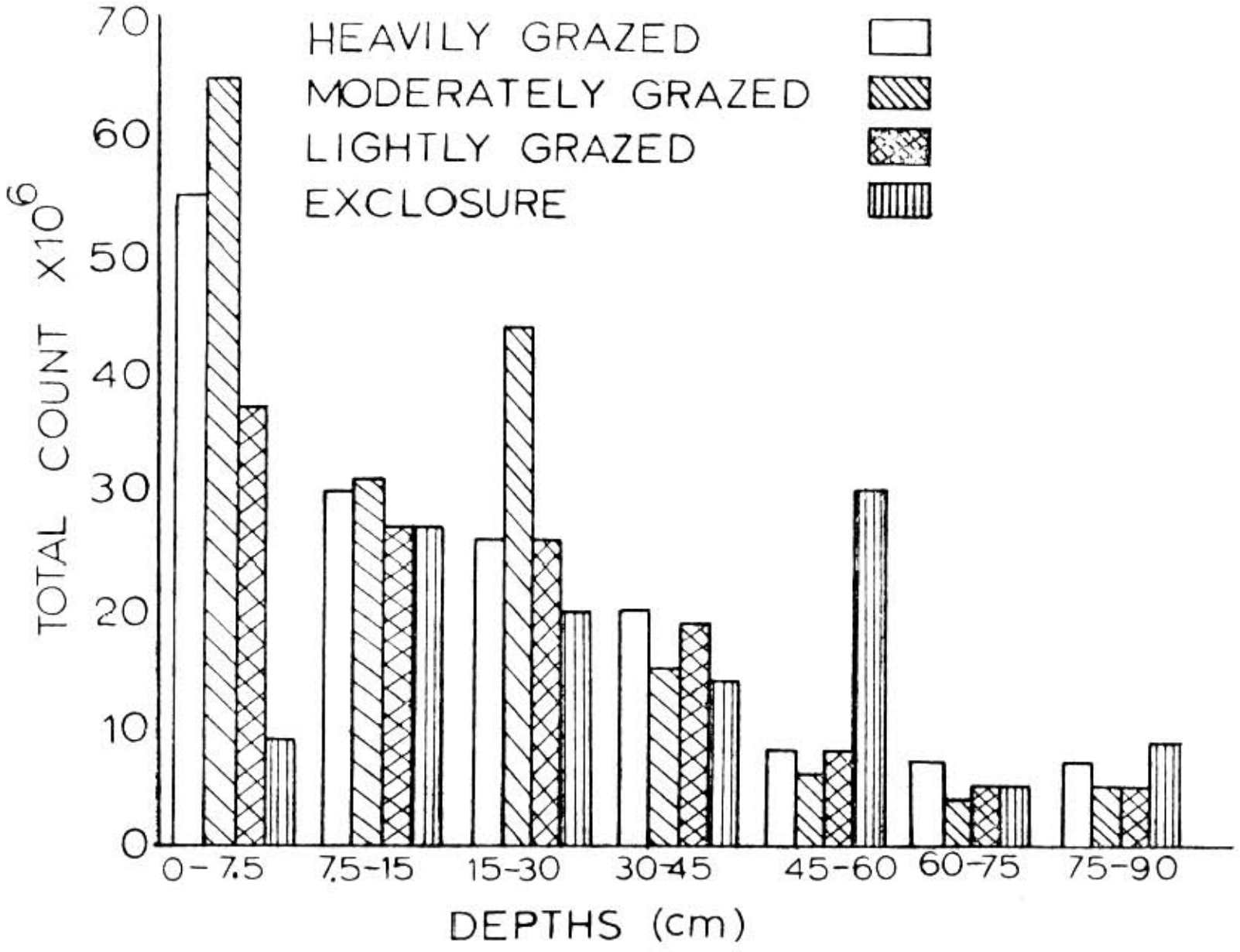

Fig. 1. Comparison of total plate counts of bacteria from soil taken from the heavily grazed, moderately grazed, lightly grazed and ungrazed exclosure areas. Sampling date: May 8, 1969.

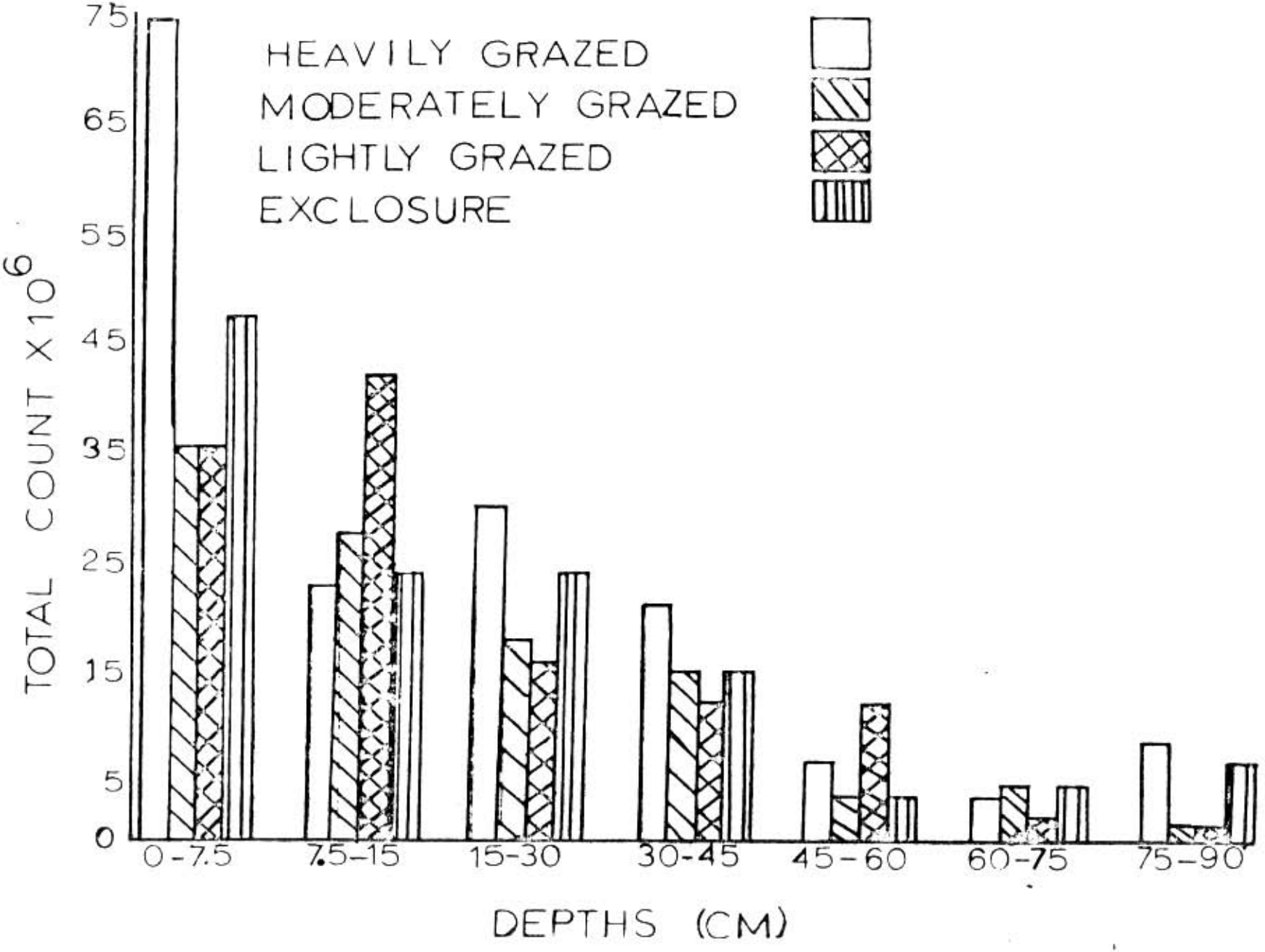

Fig. 2. Comparison of total plate counts of bacteria from soil taken from the heavily grazed, moderately grazed, lightly grazed and ungrazed exclosure areas. Sampling date: May 21, 1969.

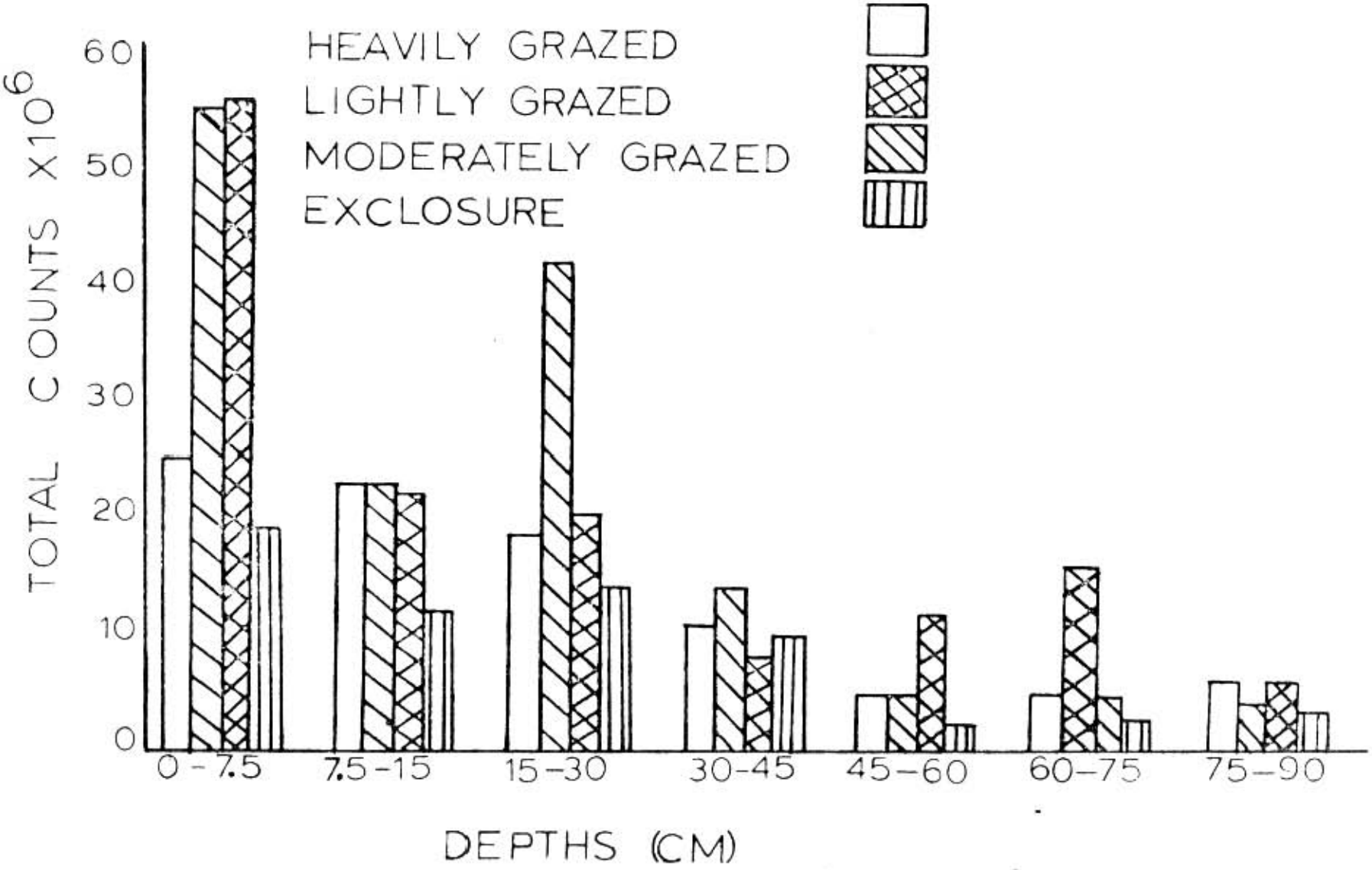

Fig. 3. Comparison of total plate counts of bacteria from soil taken from the heavily grazed, moderately grazed, lightly grazed and ungrazed exclosure areas. Sampling date: May 28, 1969.

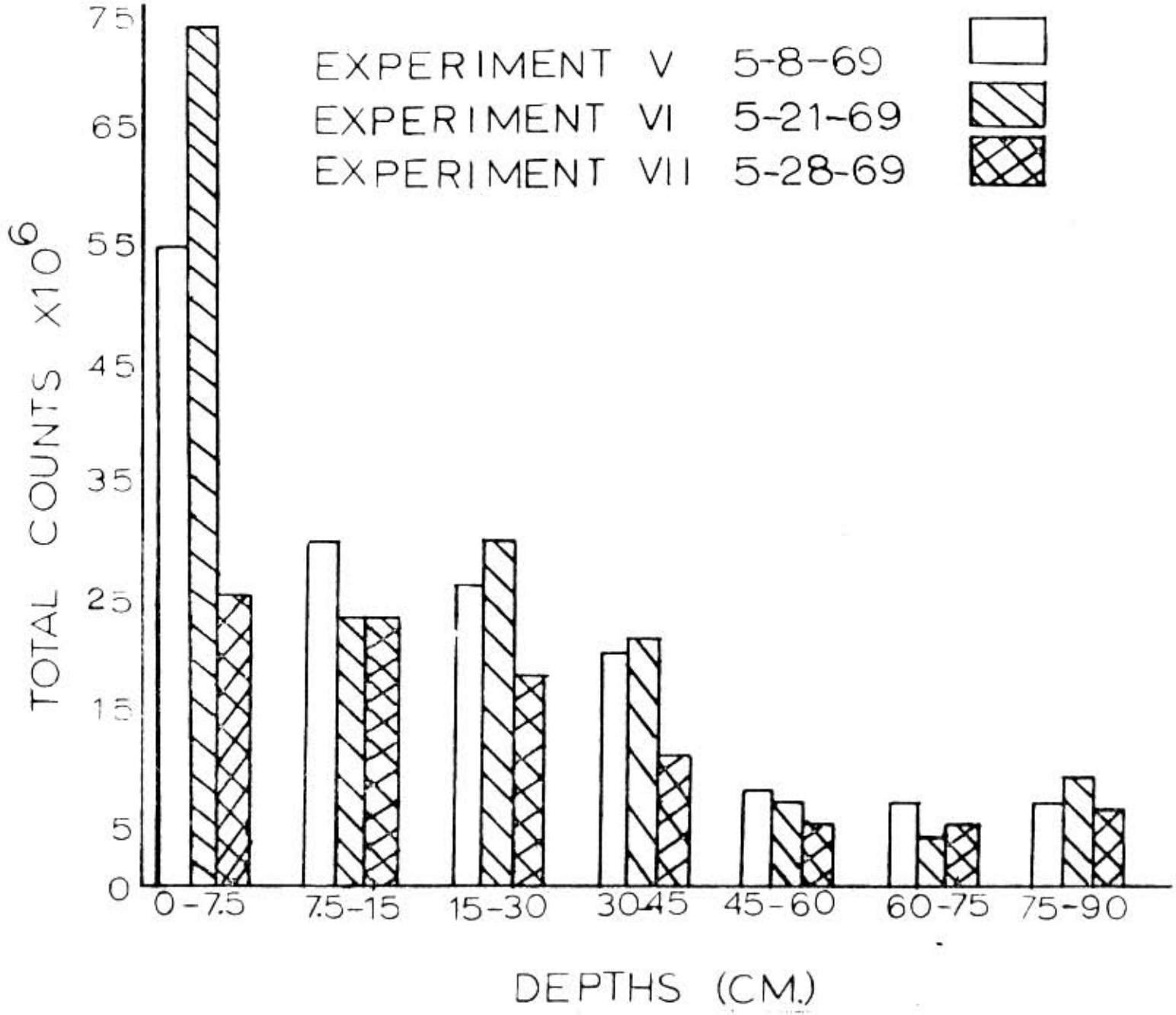

Fig. 4. Comparison of bacterial plate counts from the heavily grazed area on three sampling dates.

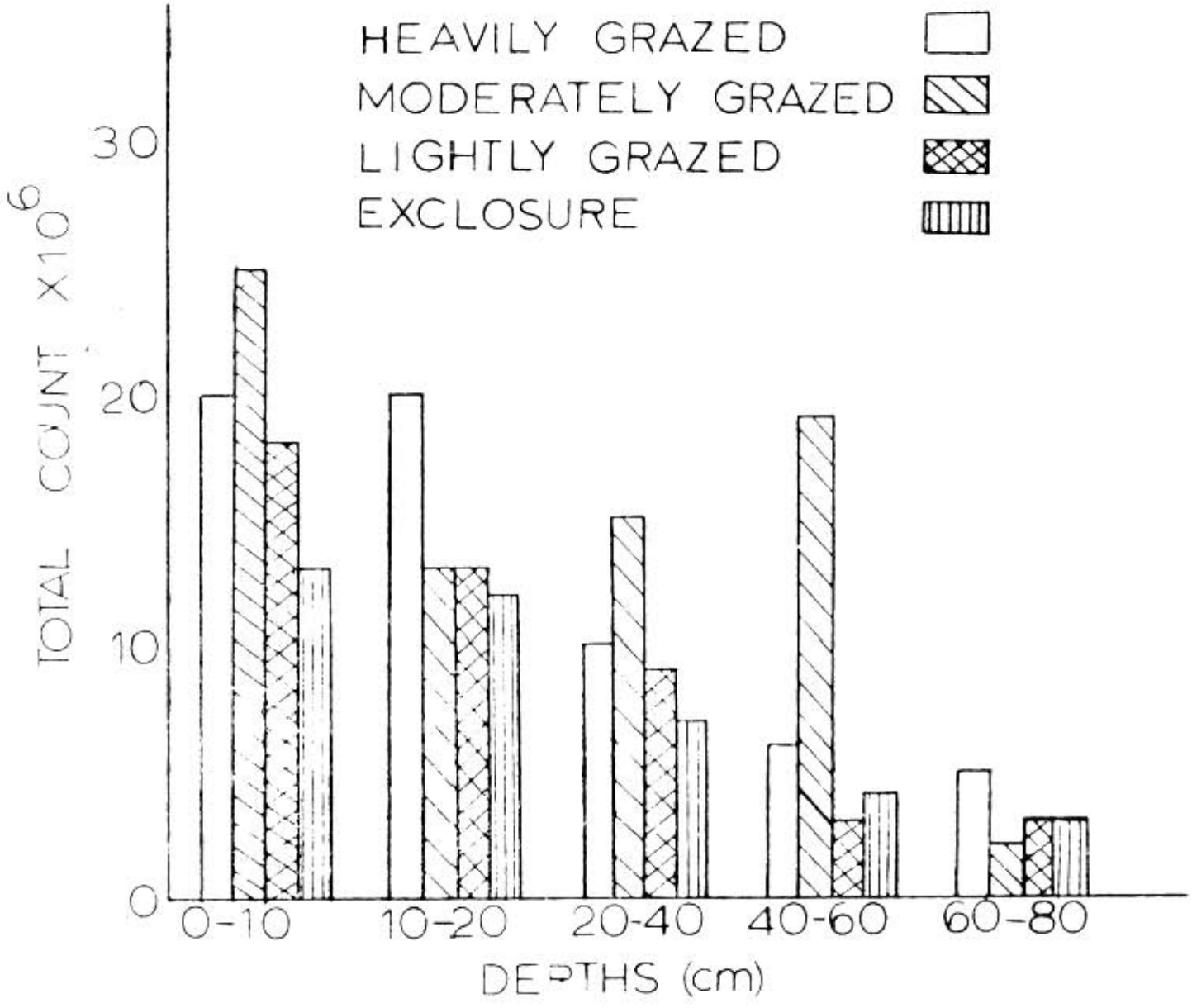

Fig. 5. Comparison of bacterial plate counts from the heavily grazed, moderately grazed, lightly grazed and ungrazed exclosure areas, using soil sample depths in coordination with root biomass determinations.

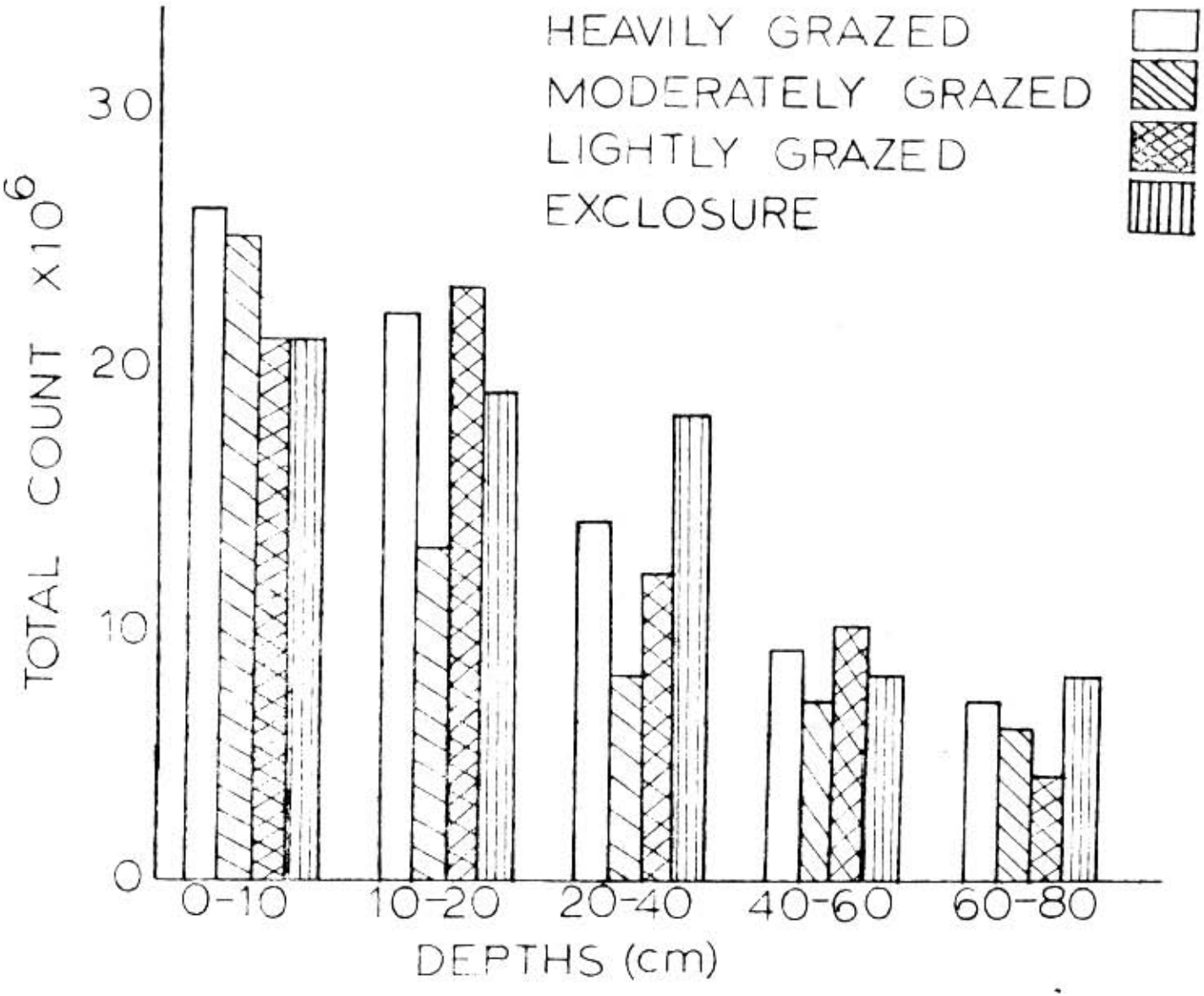

Fig. 6. Comparison of bacterial plate counts from the heavily grazed, moderately grazed, lightly grazed, and ungrazed exclosure areas, using soil sample depths in coordination with root biomass determinations.