Microbial Response to Drought in a Texas Highplains Shortgrass Prairie

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Received for publication 28 February 1974

The population of the microbial flora of a mixed blue gramma grass (Bouteloua gracilis H. B. K.) and prickly pear (Opuntia polyacantha Haw.) prairie near Amarillo, Texas, was studied during 1971 after a severe drought. Bacteria, fungi, and algae were estimated by plate count and terminal dilution procedures. Rates of grass and paper decomposition were determined. The microbial flora of soil associated with bovine-grazed grass did not differ significantly from the flora associated with ungrazed grass, either qualitatively or quantitatively. During drought, a greater number of fungi were found in soil associated with prickly pear than in that associated with blue gramma grass. The microbial biomass decreased one full log between the surface and a depth of 50 cm, and the percentage of anaerobes increased with depth. The maximum numbers of fungi and algae detected were $8 \times 10^{4}$ and $6 \times 10^{5}/g$ respectively. A linear relationship existed between the microbial biomass and soil moisture. The maximum number of aerobic, heterotrophic bacteria detected was $1.5 \times 10^{8}$ viable cells per g of soil.

Clark and Paul (4) have reviewed the sparse literature on the microbial ecology of native grasslands. The biomass in the top 30 cm of a Canadian grassland is estimated by Babiuk and Paul (2), by direct microscope counts with fluorescein isothiocyanate, to be 30 to 76 g/m² on a dry weight basis. Aerobic soil extract dilution plates showed $4 \times 10^{7}$ bacteria/g in the 0- to 10-cm layer (during April) with a linear decrease of total number with depth.

This study provided a taxonomic and physiological activity profile of the microbial population of the soil during the 1971 growing season after a year of severe drought in a mixed blue gramma grass (Bouteloua gracilis H. B. K.) and prickly pear (Opuntia polyacantha Haw.) grassland. The contribution of prickly pear to the total ground cover varied from 3 to 11%. The significance of the cactus is due to its tendency to grow in thick clones. During the dry season, it may at times appear to be the predominant ground cover, because of greatly reduced amounts of grass. Minor amounts of buffalograss (Buchloe dactyloides Nutt.), sand dropseed (Sporobolus cryptandrus Torr.), and purple three-awn (Aristida purpurea Nutt.) prevail throughout the undisturbed grassland. There were specific questions of interest in this study. (i) Are there qualitative or quantitative differences between the microbial flora of soil associated with either bovine-grazed grass or ungrazed grass? (ii) Do the microbial flora associated with grass ground cover differ from that of prickly pear? (iii) Is there any change in total population or type with increasing depth? (iv) Is the microbial biomass linked with available soil moisture?

MATERIALS AND METHODS

Site description. At Texas Tech University Research Farm (Amarillo, Texas), a moderately grazed site of 32 hectares and a 14-hectare site which had been ungrazed for 5 years were selected, approximately 6.8 km apart at an elevation of 1,180 m. The weather is extremely variable, characterized by strong prevailing winds, dry winters, and sharp temperature changes (9, 12). Precipitation of only 14 cm was received at the site during 1970; the average is 56 cm. During 1971, the following amounts of precipitation were received: in April, 1.5 cm; May, 1.3 cm; July, 8.8 cm; August, 9.8 cm; September, 6.3 cm; and October, 3.5 cm. The soil at the sites is classified as Pullman silty loam (6). Each of the sites was subdivided into two replicate sites. Each replicate was further subdivided into 200 quadrats (2.74 by 2.74 m), which were arranged in two parallel rectangles (5.49 by 137.2 m) with an access alley in between.

Bacterial population studies. Two cores (2.5 by 50 cm) were taken from each of six plots, chosen at random from each replicate of both the grazed and ungrazed sites at each sampling period, for a total of 48 cores. The cores were obtained with a hollow soil sample tube hydraulically pressed into place. Two complete additional sets of cores were obtained when the sites were subdivided for comparison of prickly pear and grass ground cover. Each core was divided...
into five zones, or depths, from 0 to 5 cm, 5 to 10 cm, 10 to 20 cm, 20 to 30 cm, and 30 to 50 cm. The sections from each core were placed separately in sterile plastic bags, sealed, and immediately stored in an ice chest.

All 12 samples of each zone from each replicate were blended together in 90 ml of sterile, physiological saline for 3 min, and appropriate serial dilutions were prepared. Four plates were prepared with five replicates at each of at least three dilutions. Plate counts were made from the maximal colony development after incubation at 30 C. Aerobic heterotrophs were assayed (15) with standard methods agar (SMA; tryptone glucose yeast agar from BBL). Anaerobes were assayed with anaerobic agar (BBL) and with incubation under prepurified nitrogen; the methylene blue confirmed anaerobiosis. Aerobic and anaerobic sporeformers were assayed by heat-shocking the appropriate dilutions at 85 C for 20 min before plating. Fungi were cultivated in rose bengal streptomycin agar (7). The population of algae was estimated by the dilution-frequency method (1). The total populations of different nutritional types were also determined by the dilution-frequency method, with the basal salts media of Lockhead and Chase (8). Three additions were made to this set of media: (i) Phenol red broth base (BBL) was chosen to approximate the SMA; (ii) a 1.0 g amount of microcrystalline cellulose (Calbiochem, Los Angeles, Calif.) per 100 ml was added to one set of the basal medium; (iii) the chitin basal salts medium of Campbell and Williams (3) was used to determine chitinase activity. The most common colony types from each horizon were selected from the SMA and classified according to nutritional type as described previously (8).

Decomposition. Rates of litter decomposition were evaluated with squares (8 by 8 cm) of Whatman no. 1 filter paper or 8-g samples of blue-stem hay (Andropogon gerardi Vitman) sewn lengthwise in 1-mm mesh nylon net bags. These bags were placed horizontally on the soil surface. Blue-stem hay was used throughout the U.S. grassland study as a standard litter material. Filter paper samples were placed both on the surface and at a depth of 5 cm in the soil. Five samples were used per replicate per treatment of each sample. The samples were incubated at 50 C, and dry weights were obtained. The total sample was ashed, and the excess ash weight was subtracted from the sample weight to correct for soil contamination of the sample.

Respiration. The open end of round metal cans (12.6 cm in diameter by 17.0 cm high) were inserted 2 cm into the soil, and carbon dioxide was absorbed in 10.0 ml of 0.1 M potassium hydroxide, which was placed in open vials in the cans for an average period of 16 h. The cans were shaded with plastic covers, and five samples per replicate were taken at each period and compared to unexposed 10.0-ml samples of base. Trapped carbon dioxide was estimated by automatic titration with 0.05 M potassium biphthalate.

Physical measurements. Pooled cores from each horizon were weighed to 0.1 g. Moisture content was determined gravimetrically on 10.0-g samples by drying at 105 C for 24 h. Ash content was determined from samples ashed at 600 C for 6 h.

Statistical analysis. The total plate count data were analyzed for covariance with a prewritten computer program. Significant differences were identified by contrasts. The grazed-grass plate count data means were analyzed separately for covariance, and mean differences were then compared by the method of Tukey (14).

RESULTS

The results of viable cell counts of bacteria, fungi, and algae are presented in Fig. 1 through 4. Both the viable cell and/or spore counts in the 0- to 5-, 10- to 20, and 20- to 30-cm zones roughly followed the 5- to 10- or 30- to 50-cm zones, with appropriate corrections for the effect of depth. The computer-based analysis of all data sets for bacteria identified highly significant (1%) effects due to date, treatment, and zone depth. A marked difference (99% confidence) was found between the number of fungi associated with grass versus that associated with prickly pear ground cover. The effect of depth on the number of observed fungal colony-forming units was significant at the 99% confidence limit.

To clarify the above results, the data obtained from the grazed grass site were analyzed separately. Highly significant effects were identified for the numbers of aerobic heterotrophs due to depth but not to the date. The means from both the 0- to 5- and 5- to 10-cm zones differed significantly (95% confidence) from the means of the 10- to 20-, 20- to 30-, or 30- to 50-cm zones. The difference between the 0- to 5- and 5- to 10-cm zones was not significant. Analysis for covariance of the total population of aerobic heterotrophs to soil depth of 50 cm identified a highly significant effect due to the date. Comparison of means showed that the total population of aerobic heterotrophs on 30 September differed significantly from the populations on 4 August, 29 June, and 8 June. The total population on 2 September differed significantly from those on 29 June and 8 June 1971.

In the top two horizons, the 0- to 5- and 5- to 10-cm zones, the number of fungal colony-forming units associated with prickly pear cacti decreased nearly one-half log during the period from May until August. The number of fungi associated with grass ground cover was consistently smaller than the number associated with the prickly pear cacti (during the spring and early summer). During this same period, many of the cacti were heavily infested with Coccidae scale insects. The computer analysis of data from both sites placed a 99% confidence limit on this effect. There was a significant effect due to
the date of sampling on the number of fungal colony-forming units at the grazed site. There was a highly significant fungal-colony population change associated with increasing depth. The number of fungal colony-forming units in the 0- to 5- and 5- to 10-cm soil depths differed significantly from the numbers associated with any other depth. It was not determined whether the fungal colony-forming units were due primarily to spores or to mycelial fragments.

Limited data did not permit evaluation of algal population changes as the season progressed. The number of algae in this arid soil was greater than expected. The average populations per gram of wet soil in the 0- to 5-cm horizon on the following dates were: on 29 June 1971, $9 \times 10^3$; 2 September 1971, $0.3 \times 10^3$; and 30 September 1971, $50 \times 10^3$.

Highly significant decreases in the average populations of aerobic bacteria, actinomycetes, anaerobic bacteria, and the aerobic fungi (Fig. 3) were observed with increasing depth. Because the latter data might be misleading because of the presence of many facultatively anaerobic organisms, dilutions were heated to kill the vegetative cells and plated in the appropriate medium. The number of aerobic sporeformers tended to decrease with depth, but the number of anaerobic or facultative sporeformers did not. Additional data will be required to firmly establish this relationship. Actinomycete-type colonies were counted on the same plates as the bacteria, but represented only 2% of the colonies in the top horizon and 4.5% in the bottom horizon. The medium was not ideal for growth of the actinomycetes, and
these data provide only preliminary estimates.

In some ways SMA was an unfortunate choice for the plating medium, since it is too rich to allow the growth of many of the soil bacteria. However, its choice by U. S. International Biological Program permits the comparison of results at several sites; the usual soil extract agar would vary with the soil used for its preparation. There are, in addition, several different formulas for soil extract agar. To evaluate partially the influence of SMA on the results, terminal dilution studies were conducted with several media (8). The dilutions were prepared from the same soil suspensions used for the plate count studies. Only slightly higher counts were obtained by using a basal salts medium supplemented with amino acids. Cellulose in the basal salts medium resulted in slightly lower counts. Apparently, the SMA allowed adequate growth of the bacteria from this soil.

The most common colony types on SMA were selected and cloned from samples of each depth collected on 30 June 1971. These were compared by using basal salts medium plus various supplements, including soil extract (8). All but two of the bacteria were gram-positive rods and 12 of these were Bacillus species. Large variations existed in the nutritional requirements of these organisms; some required soil extract, amino acids or yeast extract for growth. Several cultures were inhibited by the addition of growth factors, yet all 18 isolates grew well in the presence of both soil extract and/or yeast extract. No clearly identifiable changes in nutritional requirements were associated with soil depth.

The moisture content and bulk density of the
soil increased with depth (Fig. 5). The ash content varied only slightly in the hundreds of samples examined and averaged (95.2 ± 4.0 g on a dry weight basis). The ash content increased slightly with depth. During May and June, soil samples collected at 0 to 5 cm below prickly pear had 0.5% more moisture than samples collected beneath grass.

The biomass of platable, aerobic heterotrophs on the grazed site to a depth of 50 cm on 8 June 1971 is estimated at $3 \times 10^{12}$ bacteria per square meter, or approximately 3.3 g/m² (1.1 × $10^{-12}$ g/cell; 10).

Analysis of covariance indicated that significant ($\alpha = 0.01$) effects were associated with date, treatment, and depth, but not moisture. The difference between the number of bacteria associated with the grazed and ungrazed site was not significant at 1%, but it was significant at the 5% level for the 0- to 20-cm zone. There was a marked difference ($\alpha = 0.10$) between the number of fungi associated with grass versus fungi associated with prickly pear ground cover. The negative relationship between soil moisture of individual horizons and their bacterial populations seemed questionable as populations increased after rains during July and August. A linear correlation was found to exist between the aerobic bacterial populations and total moisture per 50 cm of core (Fig. 4). This effect was identical to that for date of sampling.

Decomposition rates were significantly different for surface versus subsurface placement and may have depended on moisture. During June, grass litter samples lost 17.0% in weight. Filter paper samples which were placed on the surface lost an average of 1.2% by weight, and subsurface samples lost an average of 15.2%. During August, the grass lost 23.1% of its weight, the surface filter paper lost 2.4%, and the subsurface filter paper 23.9%.

Soil carbon dioxide evolution estimates (Table 1) indicate no significant differences between the two sites, but soil respiration increased as the season progressed.
DISCUSSION

There were minor but consistent differences in microbial populations on the two sites, resulting in the significant covariant. These differences never exceeded one-half log and were of only minor importance. Sampling difficulties made it inadvisable to continue at the ungrazed site after September.

Statistical analysis indicated a highly significant difference between the microbial flora associated with grass ground cover and that associated with prickly pear. Data in Fig. 1 showed that more fungi were associated with the prickly pear ground cover during the early summer (in the first two horizons), when the moisture content of the soil was lowest and cacti were diseased. Shade provided by cacti may have allowed the soil to retain a greater moisture content during early summer than soil under the sparse grass. Comparison of soil moisture values for grass and prickly pear indicates that the soil in the first horizon had 0.5% higher moisture content under the prickly pear. This differential was true during June, but decreased during the remainder of the season. The greater fungal population during the dry weather may have resulted both from stored moisture in the cacti and/or higher soil moisture under the cacti.

The populations of both bacteria and fungi decreased significantly in number with depth (Fig. 3). This was verified by statistical analysis. Though the total number of aerobes and anaerobes decreased with depth, analysis of the data in Fig. 3 showed that the percentage of anaerobes doubled from the top to the bottom horizon. This is in agreement with the theory that oxygen must diffuse from the soil surface downward, and that the rate of diffusion might be slow in this very hard, unworked soil. The decrease in numbers of aerobic bacterial and fungal colony-forming units with depth would be expected. There is no direct evidence that oxygen is limiting at greater depths in this soil. Such effects are usually associated with water-saturated soils. The primary producers (grass, algae, and prickly pear cacti) were at the surface. Thus, nutrients produced by photosynthesis diffused downward and decreased with depth. The ash content of the soil increased slightly with depth, which may indicate a lower percentage of organic matter at greater depths, as was found at the Amarillo Experiment Station (5). The experimental plate count data provide no information about the autotrophic bacteria. Terminal dilution studies indicated no shift with depth in nutritional types. The same results were obtained from the nutritional groupings of organisms selected from SMA. The latter data is biased, because SMA favors heterotrophic metabolism. All of the pure cultures grew in basal salts medium, supplemented both with soil and/or yeast extracts. A large percentage of the aerobic heterotrophic bacteria were Bacillus species. Values of 20% have been

| Date and period of collection | Co₂⁺ |   |   |
|-------------------------------|------|---|---|
|                               | Grazed | Ungrazed |   |
|                               | 1 | 2 | 1 | 2 |
| 8 June (23 h)                 | 0.256 | 0.261 | 0.262 | 0.219 |
| 4 August (21 h)               | 0.304 | 0.297 | 0.303 | 0.298 |
| 2 September (10 h)            | 0.720 | 0.727 | 0.712 | 0.716 |
| 30 September (9 h 45 min)     | 0.523 | 0.646 |   |   |

* Carbon dioxide was measured in grams per square meter per 24 h.

* Each site was divided into two replicates.

Fig. 5. Effect of depth on the average soil moisture and bulk density during the period from 8 June to 2 September 1971. Symbols: O, soil moisture at grazed site; ●, soil moisture at ungrazed site; □, soil density at grazed site.
given for other grasslands (4). Twelve out of 18 clones were Bacillus species, and in plate counts a preponderance of the colonies were Bacillus (Fig. 3). This may be due to lack of moisture or simply to spore survival despite insufficient water activities for vegetative cell growth.

The estimate of biomass (aerobic, heterotrophic bacteria) is a very low 3.3 g/m² in June and 6.7 g/m² in November. Babiuk and Paul (2) calculated that the active biomass based on plate counts was only ¼, that of direct counts. However, they concluded that the plate count procedure may be a better estimate of metabolizing cells in the soil. Our results were in accord with a very dry soil and a maximum carbon dioxide evolution of 0.7 g per m² per 24 h (Table 1). Several workers obtained similar plate counts with a peak of 20 to 75 × 10⁶/g of soil at the 0- to 5-cm depth at this and associated sites in 1970 (12). The maximum plate count in this study was 150 × 10⁶/g of soil at the 0- to 5-cm depth. Reuss (11) reported that another worker, Doxtader, estimated the total dry weight biomass of bacteria plus fungi to a depth of 30 cm at a grassland site in Colorado to vary from 51 to 82 g/m². Babiuk and Paul obtained counts of 4 × 10⁷ to 27 × 10⁷ bacteria per gram in the 0- to 10-cm layer on a Canadian grassland soil, by using soil extract agar. These counts compare very favorably with the results of this study. Since our figures did not include the true anaerobes, actinomycetes, autotrophs, and the fungi, and also were based on the wet soil weight, the estimate of biomass was very low. If Babiuk and Paul’s estimate (2) of the relationship between viable cell and direct count is applicable, the maximum biomass in our experiments may have been as much as 100 g/m².

On the basis of individual samples from each horizon, no correlation between moisture and the number of viable bacteria was demonstrated by statistical analysis. Yet, one would expect such a correlation if for no other reason than the dependency of grass on available moisture. Since the herbage is the primary producer in the system, the number of active bacteria should be linked to the growth of the grass or other plants. The data were reexamined. The sum of the total viable cell counts of aerobic heterotrophs for 0 to 50 cm was directly proportional to the sum of the available moisture (Fig. 4).

Lack of moisture altered the grassland drastically. The soil at the Texas Tech University Research Farm had not been studied extensively. Results obtained at the Amarillo Experiment Station, which also has Pullman soil, indicated that the moisture tensions during this study were always in excess of 15 bar. The wilting point for grass on the Pullman soil is approximately 14 bar (5). The soil moisture was always lowest in the first 20 cm (Fig. 5), exactly where one would expect the greatest microbiological and plant activity. The top 5 cm was often dust, with much of the remaining soil brittle and dry to touch. The water activities may have been below the critical points for growth of some bacterial species. The parched, sparse spring and summer vegetation changed to a luxuriant grass cover in the fall, accompanied by increased microbial biomass.

The decomposition studies provide an indication of the soil activity. Though cattle-grazing influence upon the microbial flora of this grassland was negligible, it must exist directly below their excrement. The influence of the ruminants on the decomposition of litter is not known, beyond their eating the decomposition samples. Jack rabbits ate tongue depressors marking the locations of buried samples during the spring. During June, surface grass samples lost an average of 17% in weight. The average litter biomass of the blue-gramma grass during June of 1970 (13) was estimated at 106 to 181 g/m²; thus, if the 17% decomposition is representative, then 18 to 31 g of litter decomposed per m² during the month, or 0.6 to 1.0 g per m² per day. The carbon dioxide evolution measured during this period was 0.26 g per m² per 24 h. The two figures are not greatly disproportionate. Decomposition, respiration, and microbial biomass changes were all apparent responses to rainfall and available moisture. The effects of extreme drought were the controlling factors in this grassland ecosystem.

ACKNOWLEDGMENTS

Technical assistance was provided by C. R. Allen, R. C. Cody, R. K. Ando, and C. E. Mathis. The statistical analysis was made by R. K. Steinhurst and M. Campion. The description of herbage was supplied by R. D. Pettit.

This work was supported by National Science Foundation grants GB13096 and GB-31862X to the Grassland Biome program of the U.S. International Biological Program for the Analysis of Structure, Function and Utilization of Grassland Ecosystems.

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