Microbial diversity and activity of disturbed soil in the northern Chihuahuan Desert

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Abstract The effects of intense grazing, seasonal drought, and fire on soil microbial diversity (substrate utilization) and activity in a northern Chihuahuan Desert grassland were measured in summer 1997, winter 1998, and spring 1998. Intense livestock grazing was initiated in winter 1995, burning occurred in August 1994, and drought stresses were imposed from October 1994 to June 1997. Microbial diversity was inferred from the carbon substrate utilization patterns in both gram (+) and gram (−) Biolog plates. Microbial activity was estimated by the activity of selected enzymes. Neither microbial diversity nor activity was affected by grazing. The interaction of intense grazing and stress sub-treatments only occurred in spring for one set of diversity measurements. The maximum microbial diversity and activity occurred in the winter-drought-stress sub-plots in summer and spring. Burning reduced microbial diversity and most enzyme activities as compared to the control in summer and spring. Microbial diversity was also lower in summer-drought-stress sub-plots than in the control in summer and spring. Microbial diversity was highest in summer, intermediate in winter, and lowest in spring. Microbial activity was generally higher in summer and lower in winter. It was concluded that substrate availability was the most important factor affecting the diversity and activity of soil microorganisms within a season. Soil moisture was not the factor causing differences in microbial diversity and activity among the stress treatments, but it was a predictor for some microbial responses under a particular stress.

Key words Microorganisms · Diversity · Activity · Grazing · Stress

Introduction

Few investigations have been done on functional diversity (Zak et al. 1994). Functional diversity can be a useful tool with which to describe organism trophic levels in ecosystems and to assess both the effects of environmental stress on organisms and the effects of organisms on their environment.

Stress, both chemical and physical, can reduce microbial biomass and diversity (Atlas 1984). Physical stresses may be more common than chemical stress in most ecosystems. In rangelands, physical stresses include disturbances due to natural perturbations, such as short-term drought, and anthropogenic activities causing, e.g., overgrazing, soil erosion, compaction, and loss of organic matter. Drought may be the major stress affecting microbial population diversity and activity in semi-arid and arid areas. The stress of drought results in reduced substrate diffusion in dry soils and increased microbial demands for C and N (cited in Schimel 1995). The limited number of surviving populations under drought have specific properties, so that they can persist within their perturbed communities (Atlas et al. 1991).

Microbial activity is an important key to understanding biological processes in a soil. Changes in soil microbial activity in response to stress eventually may affect plant productivity and ecosystem functioning. Lynch and Whipps (1990) stated that the major substrate sources for microbial activity are rhizodeposition products. Those environmental factors that have effects on plant growth may influence the activity of soil microorganisms indirectly by their effects on the rhizosphere. Garcia et al. (1994) indicated that microbial activity is
influenced by soil degradation, the transformation of organic matter, and soil structure.

The relationships between microbial diversity, microbial activity, plant quality, and ecosystem sustainability of disturbed arid rangelands are still poorly understood. Ecosystem functioning before and after disturbance can be governed by soil microbial population dynamics (Kennedy and Smith 1995). Therefore, research on microbial diversity and activity may provide some advance evidence of ecosystem degradation. In managed ecosystems, the early detection of soil biological activity may allow for changes in the management system before the functioning of the entire ecosystem is impaired.

The overall objective of this study was to determine the effects of stresses on soil microbial diversity and activity in a desert grassland. The stresses included intense summer grazing, intense winter grazing, burning, summer drought, and winter drought. We hypothesized that environmental stresses would reduce the functional diversity and activity of the soil microbial communities.

**Materials and methods**

**Study site**

The study was conducted from August 1997 through June 1998 in conjunction with an experiment of the Jornada Experimental Range, United States Department of Agriculture. The experiment was established in a Chihuahuan Desert grassland located approximately 58 km north of Las Cruces, New Mexico. The long-term average annual precipitation is 230 mm, with nearly 52% of the annual rainfall occurring between July and September. The maximum summer temperature regularly reaches 40°C and the winter minimum of <0°C occurs between November and March. The vegetation is semidesert grassland dominated by black grama (Bouteloua eriopoda). Red threeawn (Aristida purpurea var. longiseta, and Aristida ternipes), and mesa mesquite (Sporobolus flexuosus) are sub-dominant grasses. Honey mesquite (Prosopis glandulosa var. glandulosa) is one of the major shrub species that has invaded or replaced the former grassland in the Jornada basin. The soil is a coarse-loamy, mixed, thermic Typic Petrocalcic, with pH 7.4–8.4.

**Experimental design**

The experiment was performed using a split-plot design that was established in 1994. The stresses included intense pulse grazing, shrub removal, short-term drought, and fire. The experiment consisted of three blocks with six completely randomized main plots per block. Each of the main plots (71 × 71 m²) was a factorial combination of intense pulse-grazing treatments (summer grazed, winter grazed, and non-grazed) and shrub treatments (shrub removed and shrub intact). Between 20 to 45 head of cattle were held in the grazed plots for 24–48 h. Stocking was adjusted to remove approximately 65–80% of available forage. This level of grazing intensity was intended to result in over-grazed plots, i.e., an intensive grazing stress. Summer grazed plots were stocked annually in August, beginning in 1995. Winter grazed plots were stocked in January or February, annually, beginning in 1995. Mesquite was completely removed from the shrub-removed plots in February and March 1994.

Three sub-treatments were applied to small plots (30 m²) which were located at random within each main plot. These sub-treatments included one time fire, summer rainout, and winter rainout. The control sub-treatment was considered to be the area within the main plot but outside the treated small plots. A fire was burned in August 1994. Transparent roof shelters were used to prevent seasonal rainfall in drought sub-treatment plots from 1994 to 1997. The drought sub-treatment plots were trenched to a depth of 1 m and lined with landscape plastic to avoid the entry of drips under the rainout shelter from the surrounding soils. Summer rainout sub-treatment plots were covered by shelters from June to September in 1994, 1995, and 1996, while winter rainout plots were covered from October to June in 1995, 1996, and 1997. Thus, the original design was 18 main plots (6 main plots × 3 blocks) with 72 sub-treatments (4 sub-treatments × 18 main plots).

**Sample collection**

Soil samples were collected in three different seasons during the study: summer (6, 11 and 18 August 1997), winter (7 and 9 January 1998), and spring (26 May, 1 and 3 June 1998). In the summer and winter, only the shrub-intact main plots were sampled (nine plots). To increase the power of statistical analysis for the following spring’s data, all 18 plots were sampled. Soil samples (0–15 cm) were collected from the rhizosphere of black grama in the sub-treatments with a 2-cm-diameter soil probe. Each sample was a composite of 10–12 soil cores taken at the base of randomly selected plants. The samples were placed into individual plastic bags and transported to the soil microbiology laboratory in a cooler. In the laboratory, each sample was completely mixed, sieved through a 2-mm sieve, and stored at room temperature. Sieving removed most of plant litter including grass roots. Soil moisture was determined on the sieved samples by drying at 105°C. In summer and winter, 36 pooled samples were collected, while 72 pooled samples were collected in the spring.

**Microbial functional diversity**

Biolig plates (Biolog, Hayward, Calif.) were used to assess microbial functional diversity. Functional diversity was based on the carbon utilization patterns of microorganisms from soil dilutions inoculated into Biolog plate wells (Garland and Mill 1991; Zak et al. 1994; Garland 1996). All dried substrates were profiled in 95 wells of the Biolog plate (Zak et al. 1994), with the first well acting as the control. Tetrazolium violet was used as a redox dye in the wells to colorimetrically indicate the utilization of the substrate by microorganisms in the soil dilutions. The wells were initially colorless when inoculated with a sample dilution. If the substrate was oxidized by microorganisms, the tetrazolium dye was reduced and a purple color was observed. If the substrate was not utilized, the well remained the same color as the reference well.

Soil samples were dispersed in 0.2% water agar with 2-min blending at high speed to ensure the homogeneous dispersion of the soil particles in the initial dilution (Zak et al. 1994). Subsequent dilutions were made in water. Aliquots (100 μl) of the final dilution (10⁻¹) were added to each of 96 wells on both graph (+) and graph (–) Biolog plates by a multiple channel pipette. The Biolog plates were incubated at room temperature and examined every 12 h for 120 h (5 days) (the number of wells changing from colorless to purple decreased after the fifth day and fungi began to grow in some wells in the preliminary studies). The number of positive wells and the density of color were recorded during each examination.

**Soil enzymes**

Soil enzyme activity was used as an indicator of microbial activity. Acid phosphatase, alkaline phosphatase, and sulfatase were determined on the fresh soil samples by the procedure of Tabatabai and Bremner (1969, 1970). Dehydrogenase activity was determined by the procedure outlined by Casida et al. (1964).
Statistical analyses

ANOVA in SAS software (SAS Institute, Cary, N.C.) was performed using a split-plot design to detect significant differences (at $P=0.05$) among both main plots and sub-treatments for gram (+) and gram (−) Biolog positive well numbers at each observation time and enzyme activities. No significant effects were attributed to shrub treatments in our previous studies (X. Liu et al., unpublished data). Therefore, the shrub treatments were treated as the replicates of intense-grazing treatments. Fisher's least significant difference was used for the multiple comparisons when the overall $P$ value in ANOVA showed differences among the main plots or sub-treatments. PROC CORR in SAS was used to correlate soil moisture with microbial diversity, and enzyme activities, both among the treatments and within individual treatments.

Results

Biolog substrate positive reactions

The number of positive reactions (number of wells changing color) increased linearly from observation hour 12 to hour 120. The intense-grazing treatments had no significant effect on soil microbial diversity as determined by substrate utilization. Significant differences among the sub-treatments in microbial diversity were observed in both gram (+) and gram (−) Biolog plates during the summer and/or spring seasons (Figs. 1, 2). The significant interaction of intense grazing and stress sub-treatments only occurred in spring 1998 in gram (+) Biolog plates at observation hour 120 (Fig. 3), with different sub-treatment distribution patterns among the intense-grazing treatments.

Significant differences of substrate utilization among the sub-treatments in gram (+) Biolog plates occurred in summer. The substrate-utilization diversity was higher in winter rainout and lower in burn and summer rainout from observation hour 72 on (Fig. 1). A similar trend was observed in spring and winter, but the difference was not statistically significant.

Substrate-utilization differences in gram (−) Biolog plates occurred at observation hour 120 in summer and observation hour 96–120 in spring (Fig. 2). Like the distribution pattern in gram (+) Biolog plates, substrate diversity was generally higher in the winter rainout and lower in the burn and summer rainout plots.

Substrate positive reactions were significantly different from one season to another in both gram (+) and gram (−) Biolog plates as determined by 1-df contrasts ($P<0.001$). The substrate diversity was highest in summer, lowest in spring, and intermediate in winter (Figs. 1, 2).

Enzyme activities

Enzymatic activities were not influenced by intense-grazing treatments, while they were affected by sub-treatments on most sampling dates (Figs. 4, 5). No interaction was found between intense-grazing treatments and stress sub-treatments for any of the enzyme activities.

Soil dehydrogenase activity was affected by sub-treatments in all seasons. Burned plots had markedly lower dehydrogenase activity than any of other sub-treatments (Fig. 4). Dehydrogenase activity was higher

![Fig. 1](image1.png) Positive reactions in gram (+) Biolog plates for sub-treatments during three sampling seasons. C Control, B burn, S summer rainout, W winter rainout. Sub-treatment bars with the same letter are not significantly different within an observation time ($P>0.05$)
highest in the winter rainout plots and lowest in the burn and control plots during the summer and spring (Fig. 5).

Enzyme activities were generally affected by seasons, as determined by 1-df contrasts ($P < 0.05$). Dehydrogenase activity and alkaline phosphatase activity had similar seasonal patterns, with highest values in summer, lowest values in winter, and intermediate values in spring ($P < 0.0001$ for dehydrogenase; $P < 0.01$ for alkaline phosphatase). Acid phosphatase activity was higher in summer, and lower in winter and spring ($P < 0.0001$). Sulfatase activity was higher in both summer and spring, and lower in winter ($P < 0.05$).

Soil moisture and microbial responses

The relationship of microbial diversity and activity with soil moisture was mostly positive but not statistically significant, based on the mean values of sub-treatments in all three seasons ($P > 0.05$). Thus, soil moisture was not the factor that caused the differences among the sub-treatments. However, soil moisture was a significant predictor for some microbial responses within a particular sub-treatment ($P < 0.05$) (Table 1).

Discussion

A number of studies have addressed soil microbial growth, population density, diversity, and/or activities in disturbed ecosystems (Atlas 1984; Atlas et al. 1991; Joshi et al. 1991) and semi-arid or arid ecosystems (Noy-Meir 1974; Parker et al. 1984; Steinberger et al. 1984; Mackay et al. 1986; Cepeda and Whitford 1989; Whitford 1989). Soil moisture, soil temperature, and/or substrate availability have been given as the most important factors that influence soil microbial growth and
population density. Soil microbial diversity (as measured by substrate utilization) and activity were generally reported to decrease with disturbance. Data from this experiment indicated that stress might have both positive and negative effects on the diversity and/or activity of microorganisms in the short term. Fire and summer drought generally reduced soil microbial substrate utilization and enzyme activities. However, winter drought, to an extent, increased soil microbial diversity and activity, contrary to expectation.

Water has been considered as the most important limiting factor for the production and activity of organisms, and biological processes, in desert ecosystems (Cutler and Dixon 1927; Noy-Meir 1973, 1974). Positive relationships between soil moisture content or water potential and microbial activities or microbial biomass have been shown (Orchard and Cook 1983; Kieft et al. 1987; Skopp et al. 1990). However, other studies indicated that water availability may not be as important as once thought as a regulator of soil biota activity (Mackay et al. 1986). Steinberger et al. (1984) found that the total numbers of nematodes in the Chihuahuan Desert were not correlated with rainfall, but were correlated with litter quantity. Parker et al. (1984) indicated that pulses in microbial biomass and activity in response to water occurred only when large accumulations of litter were produced. Therefore, the quantity of organic matter may be an important factor limiting desert soil biota (Steinberger et al. 1984).

The soil samples in our study were collected after the drought treatments were removed in June 1997. The disturbed ecosystem was in the process of recovering from the stresses. Water content was similar for all treatments during the same sampling period. Thus, water content could not be the direct cause of treatment differences. No significant correlation was found between soil moisture and microbial diversity or between soil moisture and microbial activity.

A possible reason for the higher microbial diversity and activity in winter-drought-stress sub-plots is related to the availability of substrate for soil microorganisms. Winter drought may have delayed or slowed the decomposition rate of plant litter and roots. Therefore, when favorable temperature and moisture occurred in
summer, a flush of plant residue decomposition occurred that resulted in more available substrate for soil microorganisms and caused higher microbial responses. Whitford et al. (1998) found that irrigation had no effect on rates of decomposition, but that exposure to drought reduced the rates of decomposition to half those of the controls.

The flush of organic matter decomposition also resulted in more nutrients for annual plants. In summer 1997, the annual herbaceous plant density, cover, and biomass in winter-drought-stress sub-plots were considerably higher than in any other stress sub-plots, including the control (W. G. Whitford, unpublished data). The annuals germinated in August after significant rainfall in July (135 mm), and reached their biomass production peak in early September. The roots of the dead annuals provided a substrate pulse for microorganisms during the winter and spring of 1997–1998. The differences in dead root biomass in the sub-plots accounted for the differences in microbial diversity and activity.

The interpretation that substrate availability was the key factor for soil microorganisms was also consistent with the lower microbial diversity and enzyme activity in the burn sub-plots. The perennial grasses were totally eliminated by burning in August 1994 and did not recover after the fire. Burning has been shown to reduce carbon inputs into soil systems resulting in decreased microbial metabolic diversity (Bossio and Scow 1995) and microbial biomass carbon and nitrogen (Powolson et al. 1987; Collins et al. 1992).

Substrate availability may also have caused the lower microbial diversity under summer drought stress. In the northern Chihuahuan Desert, nearly half of the annual precipitation occurs in summer from July to September. The rainout shelters reduced summer rainfall by 506 mm (55.9% of the sum of the 3-year average annual rainfall) during the experiment. Most perennial grasses were killed and the growth of annual herbaceous plants was greatly inhibited in the summer drought sub-plots.

Annual plants may be considered as an important source of organic matter variation in desert ecosystems. Their density and biomass change from season to season and year to year, mainly due to variations in timing and amount of precipitation. In addition, the roots of annual plants affect the availability of soil nitrogen by providing a pulse of energy for soil microflora (cited in Mun and Whitford 1998). The low lignin content of annual plants encourages rapid growth of the microflora, rapid immobilization of nitrogen, and results in faster decomposition rates than those of woody shrubs (Whitford et al. 1988; Mun and Whitford 1998). Therefore, in the short term, differences in annual plant density and biomass may greatly affect the substrate availability of soil microorganisms. Whitford and Herrick (1995) stated that pulses of organic matter inputs in soil systems occur with the death of annual forbs, grasses, and leaf drop in seasonal plants. These pulses would stimulate increases in the microbial biomass. In agricultural ecosystems, seasonal inputs of crop roots, rhizosphere products, and crop residues significantly alter the soil microbial biomass and mineralizable carbon and nitrogen levels of the soil (Franzluebbers et al. 1994).

Another possible reason for the higher soil microbial diversity and activity under winter drought stress may be related to the population density of bacterial predators. Abiotic factors, especially temperature and soil water content, control the functional structure of soil food webs (Whitford 1989). Winter drought may have greatly inhibited the growth of bacterial grazers, thus increasing bacterial development. The nematode fauna in a desert is dominated by bacterial feeders (Steinberger et al. 1988). A few studies indicated that nematode adult populations and larval emergence were higher during later autumn and early winter from September to January (Wallace 1973). Elliott and Coleman (1977) found that soil water had a definite effect on protozoan numbers and regulated protozoan activity under water-limited conditions. If the nematode and protozoan population in the Chihuahuan Desert followed the seasonal pattern of change mentioned above, winter drought may have severely restricted the development of the predator populations. Since soil samples were not collected in winter 1997, and little data exist concerning seasonal rainfall effects on the population density of bacterial predators, further investigation would be necessary to substantiate this explanation.

The results of the simple correlation analysis indicated that microbial diversity and activity were relatively independent of soil moisture. Moisture was not the factor that caused the differences in soil microbial responses among the stress treatments. However, the distinct seasonal changes of microbial diversity and activity indicated that moisture and temperature were two important factors for soil microorganisms. The seasonal change patterns of soil microbial diversity and activity were consistent with expectation. Soil samples in summer were collected in the rainy season – the most favorable season for biological activity in the Chihuahuan

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Season</th>
<th>Sub-treatment</th>
<th>P-value</th>
<th>$r$</th>
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<tbody>
<tr>
<td>Biolog gram (+)</td>
<td>Summer</td>
<td>Control</td>
<td>0.006</td>
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<td></td>
<td>Summer</td>
<td>Burn</td>
<td>0.021</td>
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<td></td>
<td>Summer</td>
<td>Summer rainout</td>
<td>0.008</td>
<td>0.81</td>
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<tr>
<td></td>
<td>Summer</td>
<td>Winter rainout</td>
<td>0.022</td>
<td>0.74</td>
</tr>
<tr>
<td>Biolog gram (-)</td>
<td>Summer</td>
<td>Burn</td>
<td>0.015</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>Summer rainout</td>
<td>0.043</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>Winter rainout</td>
<td>0.018</td>
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</tr>
<tr>
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<td>Spring</td>
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<tr>
<td></td>
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<td>Summer</td>
<td>Summer rainout</td>
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</tr>
<tr>
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<td>Summer</td>
<td>Burn</td>
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<tr>
<td>phosphatase</td>
<td></td>
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<td>0.84</td>
</tr>
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</table>
Desert. Before samples were collected in August 1997, 135 mm precipitation was received in July. Most of the precipitation occurred 1 or 2 weeks before sampling. The warm soil temperature and preferred soil moisture content stimulated soil microorganisms. Therefore, soil microbial diversity and activity had their highest values in this season. Others (Parker et al. 1984) observed a burst of activity of soil microorganisms after rain from day 6 to day 24 in the Chihuahuan Desert. In winter, although the soil moisture was similar to that in summer (about 4–6%), the low soil temperature was probably the overriding factor that resulted in lower microbial responses. The spring soil samples were collected in late May and early June, before the rainy season. Only 2.0 mm of precipitation occurred in April, and no rain in May. The extremely dry condition (soil moisture was <1%) plus low night temperatures probably inhibited the growth of soil microorganisms. The Biolog plate substrate positive reactions during this period were less than half of those in summer at the end of a 5-day incubation, and the enzyme activities (except for sulfatase) were 20–40% lower than those in the summer.

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References