Seasonal and Spatial Population Dynamics of the Nitrogen-Efficient Guild in a Desert Bajada Grassland

R. P. HERMAN,* KERRI PROVENCIO, RUBEN TORREZ, AND GWEN M. SEAGER

Department of Biology, New Mexico State University, Las Cruces, New Mexico 88003

Received 19 July 1993/Accepted 13 January 1994

This study examined the temporal and spatial variation in the populations of bacteria from a Chihuahuan desert bajada grassland that could grow on nearly nitrogen-free medium, a nitrogen-efficient guild (NEG). A Curtis similarity index of 0.876 and nearly identical diversity and equability indexes (H' = 1.22, J = 0.46 and H' = 1.27, J = 0.48 for the crown edge and interplant samples, respectively) indicated that there was no qualitative difference between the NEG assemblages isolated from samples taken at Bouteloua eriopoda plant crowns and in nonvegetated areas 45 cm from crowns. The difference in NEG populations between the crown and interplant samples was only 2-fold, despite a 9.5-fold difference in root biomass between the sites. These differences, while consistent, were statistically significant at only 50% of the sampling times. There was over an order of magnitude difference in NEG numbers in root-associated soil and in bulk soil from the crown or intercrown sites. The typical trend for temporal variation in NEG numbers was that they increased in the spring, fluctuated dramatically over the summer, and declined at the summer’s end. The pattern of soil moisture change was the only abiotic variable which showed the same fluctuation pattern as NEG numbers.

The quantity of microorganisms in soil varies both temporally and spatially (15, 32). Temporal and spatial variability in the quantity and quality of available resources is generally thought to be responsible for this pattern of variability in microorganism numbers (15, 32). Resources to which microbial populations have been shown to respond include organic matter (1, 7, 8, 12, 13, 18, 32), nitrogen (1, 8, 31, 32), and soil moisture or water potential (2, 8, 23, 30, 32).

Soil resources, for example, nitrogen, often have extremely variable distribution on a fine spatial (20) to a very large landscape (21, 24) scale. The soil resource variability influences and is, in turn, influenced by the covering vegetation. Soil resource variability also influences rates of microorganism-mediated processes, microbial biomass, and, by extension, microbial populations (15, 32).

Another major influence on microbial populations is proximity to roots. Numerous studies have shown increased bacterial numbers in soils intimately associated with roots, the rhizosphere or rhizospheres (9, 12–16, 31). There is a diminishing or less consistent pattern of influence as samples are collected greater distances from this immediate contact zone. Some studies show increased numbers (31), whereas others demonstrate little effect of proximity to roots (17).

In arid regions, water and nitrogen are generally the limiting resources for plants (33). Their levels are low, and their distribution is often patchy and dependent on the type of vegetation present. In shrub-dominated areas, soil resources are centered on individual shrubs, which serve as “islands” of biological activity where primary production is recycled and where the biological community influences the water status of the soil (3, 5, 10, 16). In grasslands, the distribution of resources is usually much more homogeneous in space and time (27). The stress-induced invasion of shrubs into grasslands during desertification results in a shift from even to patchy resource distribution. This change in resource distribution appears to prevent the reestablishment of grasses and is an underlying mechanism for the maintenance of the resulting shrubby vegetation (27).

Work from this laboratory involving potted desert grasses and grassland soils in columns showed that plants and simulated plant exudates strongly influence the numbers of free-living nitrogen fixers and other organisms capable of growing on nearly N-free medium (6). The present study is an extension of these controlled studies and examines field population dynamics of these N2 fixers and efficient N scavengers, a nitrogen-efficient guild (NEG). These laboratory data suggested that the NEG might provide a useful tool to investigate factors controlling microbial population fluctuations in response to temporal and spatial soil resource patterns. Because of the importance of the soil resource pattern in the maintenance of desert grasslands, microbial population changes may provide a useful indicator of soil resource status. To this end, we ask whether the relatively homogeneous resource distribution, proximity to grass plants, abiotic factors, or some combination of these factors most strongly influences the spatial and temporal distribution of NEG members.

MATERIALS AND METHODS

Site characteristics. Field studies were carried out on the New Mexico State University College Ranch Long Term Ecological Research area 40 km NNE of Las Cruces, N.M. Soils were collected from a bajada grassland at the base of Summerford Mountain (R1E T21S, Sec. 35 NE 1/4, Doña Ana County, N.M.) dominated by Bouteloua eriopoda (black grama) with significant amounts of Sporobolus flexuosus (mesa dropseed). Soil from this area is a Ustolic Haplargid (11) composed of 76% sand, 7% clay, 0.68% CaCO3, and 0.48% organic matter (34). Single-year chemical characteristics include a pH of 7.02, 1.13 mg of ammonium nitrogen kg⁻¹, and 0.57 mg of nitrate nitrogen kg⁻¹ (26). The average total nitrogen for the

* Corresponding author. Mailing address: Department of Biology, New Mexico State University, Box 30001, Dept. 3AF, Las Cruces, NM 88003. Phone: (505) 646-4532. Fax: (505) 646-5665. Electronic mail address: rpeter@nmsu.edu.
bajada grassland is 46 mg kg⁻¹ (35). Meteorological data (air temperature; relative humidity; soil temperature at depths of 0, 5, 10 and 20 cm; and rainfall) were monitored with a Campbell CR10 data logger at a site approximately 0.75 km SE of the study area.

**Media.** All isolations were carried out on an almost nitrogen-free malate-mannitol medium (NF-MM), a modification of \textit{Azospirillum} semisolid nitrogen-free malate medium (28). The composition of solid NF-MM (in grams per liter) was as follows: KH₂PO₄ 0.4; K₂HPO₄ 0.1; MgSO₄·7H₂O 0.097; NaCl 0.1; CaCl₂·2H₂O 0.0196; FeCl₃·6H₂O 0.017; NaMoO₄·2H₂O 0.002; yeast extract 0.001; l-malic acid, 3.58; mannitol, 5.0; and agar 15. The medium pH was adjusted to 7.0 with KOH prior to the addition of the agar. Known N addition from yeast extract represents a concentration of <1 μM prior to the agar impurities providing an additional source of N in unknown quantities.

**Sample collection.** We selected six \textit{B. eriopoda} clumps which were isolated from other plants by 90 to 100 cm. Soil cores were collected at the edge of the plant crown and at a point 45 cm from the crown by using a 19-mm (inner diameter) Oakfield soil probe. To eliminate variability associated with surface disturbance, the top 5 cm of each core was discarded and the soil between 5 and 10 cm deep was stored in plastic bags and brought back to the laboratory for analysis. The probe was flamed between collections. To determine root biomass associated with each plant, 115-mm-diameter cores from depths of 5 to 10 cm were collected at the crown and at the interplant collection site 45 cm from the plant crown. Roots were extracted from the soil by dry sieving and hand picking. To determine populations associated with root-adhering soil, we took samples at the crown and at 45 cm from the crown of plants as described above. The plants were then dug up and returned to the laboratory in pots.

Cores were manually broken and mixed in their plastic bags. A 1-g sample from each core was aseptically weighed and transferred to dilution bottles containing 100 ml of sterile deionized water. For rhizoplane samples, roots at a depth of 5 to 10 cm were clipped, gently shaken to remove soil that was not closely associated with the root surface, weighed, and transferred to dilution bottles.

Bottles were allowed to stand for 15 min and then shaken manually for 15 s. Three dilution series were made per bottle onto solid NF-MM. Final dilutions ranged from 1:10,000 to 1:10,000,000. Soil samples were weighed, dried for 24 h at 105°C, and reweighed to calculate the percent moisture. Root samples were removed from the dilution bottles, dried, and reweighed. Comparable roots were brushed clean of soil, weighed, dried, and reweighed to calculate a wet weight-to-dry weight ratio for the plant roots. The wet weight of adhering soil was determined by subtraction of calculated root wet weights. Soil dry weight was calculated by using the wet weight-to-dry weight ratio from the crown sample.

Plates were incubated in snap-lid plastic tubes for 5 days at room temperature (−22°C) and counted. Obviously filamentous colonies were ignored, and plates obscured by fungal growth, with large zones of inhibition surrounding fungal colonies, or overrun with swarming bacteria were discarded. Data were expressed as CFU per gram of dry soil. Previous experiments indicated that repeated 1-g within-core samples showed less variability than between-core or between-plant samples.

**Colony characterization.** Isolation plates from crown and interplant samples \((n = 20\) for each distance) with between 75 and 150 colonies per plate were selected at random from the plates enumerated during the second summer of the study. All colonies were counted and separated into groups based on colony morphology. Representative colonies of each type were Gram stained. Acetylene reduction activity assays were carried out to determine the proportion of colonies growing on the isolation plates which were capable of N₂ fixation. Colonies were picked haphazardly proportional to the relative abundance of their morphology (>30%, 30 colonies on 20 plates; >1% but <30%, 10 colonies; <1%, 5 colonies). Picked colonies were cut from the plate, placed in 1 ml of liquid NF-MM in 10-ml serum bottles under an atmosphere of 10% (vol/vol) acetylene, and incubated for 60 h at room temperature. The headspace was sampled with a gas-tight syringe, and acetylene reduction was measured by gas chromatography.

Gas chromatography was carried out in a Hewlett-Packard 5710A gas chromatograph with a Poropac N column (2 m by 1/8 in. [0.32 cm]). N₂ carrier gas flow was 40 ml min⁻¹. The isothermal oven temperature and injector port temperature were 80°C, while the flame ionization detector was maintained at 250°C. Data were collected on a Hewlett-Packard 3390A Integrating Recorder.

**Similarity and diversity indices.** To assess the similarity of community composition between crown and interplant communities, we calculated the Curtis similarity index \(2w/(a + b)\), where \(a\) and \(b\) are the sums of the frequencies of the taxa in communities A and B, respectively, and \(w\) is the sum of the cover frequency for each taxon as described by Curtis (4). The diversity of the two communities was calculated by using the Shannon-Weaver index and equitability index as described by Pielou (22).

**Statistics.** All data were analyzed by analysis of variance (ANOVA) and multivariate ANOVA (MANOVA) by using SAS general linear models procedures (25) on log-transformed data. Data are expressed as means ± standard errors of the means. Groups were considered significantly different when \(P < 0.05\).

**RESULTS**

NEG member numbers fluctuated over the sampling period in both the crown samples (from \(1.8 \times 10^5\) to \(3.8 \times 10^6\)) and the interplant samples (from \(9.4 \times 10^4\) to \(2.5 \times 10^5\)). This fluctuation was similar irrespective of the distance from the plant (Fig. 1). Although there was considerable variation during the three springs in the sampling period, populations typically increased in the spring, fluctuated dramatically over the summer, and declined at the summer’s end (Fig. 1). In general, NEG numbers were greater in samples taken at the plant crown than in interplant samples collected 45 cm from the plant crown (15 of 16 sample periods). These differences were not uniform and were only significant at about 50% of the sampling times. On one occasion, NEG numbers in the interplant samples exceeded those in the crown samples (day 533; 16 June 1992). The largest difference between the crown and interplant samples occurred on day 269 (26 September 1991), with the crown population 3.8-fold higher than the population at 45 cm from the crown. On average, the crown populations were double the interplant populations (1.95-fold). In contrast, the quantity of roots in the crown samples was 9.5-fold greater than in the interplant samples (307.6 ± 70.6 versus 32.4 ± 6.4 g m⁻² in the top 10 cm).

Although there is no convincing evidence of a rhizosphere effect when comparing the NEG populations from the crown and interplant sampling sites, it is clear that the rhizosphere and rhizoplane contained significantly more NEG members than did either the crown or interplant sample (Fig. 2). At each
sampling period, the number of NEG organisms in soil associated with the root surface was at least 10-fold greater than in soil from the more distant samples.

To determine whether an abiotic variable was associated with NEG numbers, we plotted graphs of daily maximum, minimum, and mean air temperatures; daily maximum, minimum, and mean relative humidities; daily maximum, minimum, and mean soil temperatures at depths of 0, 5, 10, and 20 cm; daily rainfall; and soil moisture associated with the microbiological samples. The only meteorological variable which was associated with NEG populations was soil moisture (Fig. 3).

There was little difference in the NEG assemblage between the crown samples and the interplant samples. On the basis of relative abundance of colony morphology (Table 1), both had similar Shannon-Weaver diversity indices and equitability indices (1.22, 0.48 and 1.27, 0.46 for crown samples and interplant samples, respectively) (22). The Curtis similarity index (4) between the two sites was 0.876. On the basis of estimates from the colonies screened, only 27.7% of the colonies isolated were able to fix nitrogen, the balance being efficient scavengers of fixed nitrogen. The frequency, relative abundance, and nitrogen-fixing ability of the colony types from the crown and interplant samples are also listed in Table 1.

**DISCUSSION**

The bacteria isolated and enumerated in this study were capable of growing on a nearly N-free medium. However, when screened for the ability to fix N\(_2\), only 27% of the isolates were positive (Table 1). This indicates that the majority of the isolated organisms were efficient scavengers of fixed N rather than N\(_2\) fixers. These two groups, free-living N\(_2\) fixers and efficient N scavengers, represent a NEG. It is not surprising that NEG members should be found in large numbers in the Jornada bajada grassland, given the low total N of 46 mg kg\(^{-1}\) (35) and the even lower availability of rapidly assimilated forms such as nitrate N (0.57 mg kg\(^{-1}\)) and ammonium N (1.12 mg kg\(^{-1}\)) (26). Because the NEG has two different components, it is difficult to predict its overall effects on the grasses. On one hand, the N-scavenging ability of some NEG members may contribute to low N availability for grasses. On the other hand, the N\(_2\) fixers, particularly those in the rhizoplane, may contribute either directly or indirectly, upon death and mineralization, to plant N supplies.

The proportion of the NEG represented by N fixers is lower
TABLE 1. Community characteristics for interplant and crown samples

<table>
<thead>
<tr>
<th>Colony type</th>
<th>Interplant sample</th>
<th>Crown sample</th>
<th>Colony type nitrogen fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequencyd</td>
<td>Total no. of colonies</td>
<td>% Relative abundancee</td>
</tr>
<tr>
<td>vsw</td>
<td>1.0</td>
<td>919</td>
<td>52.96</td>
</tr>
<tr>
<td>sw</td>
<td>1.0</td>
<td>544</td>
<td>31.35</td>
</tr>
<tr>
<td>mw</td>
<td>0.6</td>
<td>79</td>
<td>4.55</td>
</tr>
<tr>
<td>lw</td>
<td>0.4</td>
<td>31</td>
<td>1.79</td>
</tr>
<tr>
<td>tf</td>
<td>0.3</td>
<td>18</td>
<td>1.04</td>
</tr>
<tr>
<td>ma</td>
<td>0.3</td>
<td>93</td>
<td>5.36</td>
</tr>
<tr>
<td>lm</td>
<td>0.25</td>
<td>11</td>
<td>0.63</td>
</tr>
<tr>
<td>sm</td>
<td>0.25</td>
<td>16</td>
<td>0.92</td>
</tr>
<tr>
<td>am</td>
<td>0.2</td>
<td>5</td>
<td>0.29</td>
</tr>
<tr>
<td>ye</td>
<td>0.2</td>
<td>7</td>
<td>0.40</td>
</tr>
<tr>
<td>or</td>
<td>0.25</td>
<td>6</td>
<td>0.35</td>
</tr>
<tr>
<td>dk</td>
<td>0.2</td>
<td>5</td>
<td>0.29</td>
</tr>
<tr>
<td>pk</td>
<td>0.0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>ow</td>
<td>0.05</td>
<td>1</td>
<td>0.06</td>
</tr>
<tr>
<td>Total</td>
<td>1,735</td>
<td></td>
<td>640</td>
</tr>
</tbody>
</table>

---

a Colony types: vsw, very small white; sw, small white; mw, medium white; lw, large white; tf, translucent/opalescent flat and darkening with age; ma, irregular flat white; lm, large mucoid; sm, small mucoid; am, amber; ye, yellow; or, peach/orange; dk, dark brownish black; ow, highly opaque white.

b Shannon-Weaver diversity indexes ($H' = -\sum p_i \ln p_i$, where $p_i$ is the relative abundance for each species) were 1.27 for interplant samples and 1.22 for crown samples. Equitability indexes ($J = H'/H_{max}$, where $H_{max} = \text{the maximum Shannon-Weaver diversity index for a community of the same number of taxa}$) were 0.48 for interplant samples and 0.46 for crown samples.

c g−, gram negative; g+, gram positive.

d Number of plates on which colony type appeared/20 colonies.

e Number of colonies of type/total × 100.

Unrounded relative abundance in both areas combined multiplied by percentage screened positive for colony type.

---

in this study (27.7 versus 76.8%) than previously found in greenhouse studies with soils from this site (6). The isolates in the previous study came either from within the crowns of potted plants or from columns in which a carbon source was evenly applied throughout each column. It is possible that this increased availability of energy allowed for a greater population of organisms capable of energetically costly N₂ fixation.

There was no qualitative difference in the NEG assemblages between the crown and interplant sampling sites. Both sites displayed similar diversities (Shannon-Weaver indices $H' = 1.22$ and $H' = 1.27$ for crown and interplant sites, respectively). The equitability measures and taxon richness ($J = 0.46, 13 taxa$, and $J = 0.48, 14 taxa$, for crown and interplant sites, respectively) indicate that the components of diversity were similar as well.

The Curtis similarity index provides a more direct measure of the similarity of community makeup. The Curtis index of 0.876 between the sites falls within the range of indices obtained when samples 1 to 10 are compared with samples 11 to 20 or odd samples are compared with even samples within a site. This overlap of between-sample to within-sample indices indicates that the community composition is not different.

The diversity indices observed in this study are lower than those usually found in unstressed microbial communities (values from 2.5 to 5 are common). This might be expected for a number of reasons. The first is that this study reports diversity of a heterotrophic guild rather than a whole heterotrophic community. The latter strict isolation conditions would be expected to select against some proportion of the total community. The second is that taxa were defined morphologically, which would underestimate the number of taxa present, decreasing diversity measures and increasing similarity indices.

There are consistent but not always statistically significant differences in NEG numbers between the crown edge and interplant samples (Fig. 1 and 2). The 2-fold increase in numbers near the crown does not reflect the nearly 10-fold difference in root biomass associated with the two sites. However, the positive influence of organic matter, as would arise from root turnover, has been frequently demonstrated (1, 7, 8, 12, 13, 18, 32). Only rarely has root biomass not been correlated with increases in microbial populations (17). Although a direct relationship in the response of NEG numbers to root biomass would not necessarily be expected, in soil column studies a 10-fold increase in added carbon, as malate, resulted in a 10-fold increase in NEG numbers (6).

We found dramatic increases in NEG numbers in soils intimately associated with roots (Fig. 2). The populations at the root surface were 27-fold larger than those in bulk soil at the crown edge and 44-fold larger than those in the sample taken at a distance of 45 cm from the crown. These findings are consistent with large bacterial populations reported for root-associated soils (9, 12, 14, 15, 29, 31). The relatively small influence of increased root biomass on populations in the crown edge sample compared with the interplant sample, despite the obvious positive influence of roots on NEG numbers, may result from a combination of factors. First, the majority of soil in even the crown edge samples was not intimately associated with roots, and second, the movement of materials lost from roots by leakage or sloughing has only limited movement in soil (12, 31).

NEG populations fluctuated dramatically, approximately twofold, over an annual cycle (Fig. 1). This variability is greater than reported for other guilds such as nitrate reducers (19). The only environmental variable measured which appeared to track NEG populations was soil moisture. When soil moisture was plotted against NEG numbers for all samples, there was no correlation ($r^2 = 0.005$ for the regression). On the other hand, the pattern of change in soil moisture closely tracks the changes in NEG numbers (Fig. 3). For the few points at which
NEG numbers are greater than would be predicted from the change in soil moisture, there was significant rainfall 1 to 2 weeks before the samples were taken. This suggests that although the soil moisture had dropped by sampling time, the NEG numbers had not yet fully declined.

The positive correlation between soil moisture effects and soil microorganism numbers is well documented (2, 8, 23, 30, 32). Microbial populations may respond quite quickly to the type of wet-dry cycles experienced in arid environments (23). It is not clear that soil moisture alone will have as strong an influence on NEG numbers in other environments. Jenkins et al. found that although a single environmental variable could account for much of the variation seen in the numbers of rhizobia capable of nodulating mesquite (Prosopis glandulosa) at a particular site, no single factor could explain the variation across the desert sites studied (8).

Our finding that there is little qualitative or quantitative difference in NEG populations near to and far from grass plants supports the hypothesis that grasslands have a relatively equitable distribution of resources (27). These data also suggest that the plant roots exert a minimal influence on NEG numbers in soil that is not in intimate contact with the roots. Finally, this study supports the proposition that the pattern of water availability is correlated with biological activity in desert systems (33).

ACKNOWLEDGMENTS

This work was supported in part by a National Science Foundation Research Experience for Undergraduate stipend supplement to Jornada LTER-BSR-8811160 (G.M.S., K.R.F.), National Institutes of Health Minority High School Student Research Apprenticeship Program stipend S03RR03100-08 (K.R.F.), and Minority Access to Research Careers fellowship GM07667-16 (R.J.T.).

REFERENCES

32. Wardle, D. A. 1992. A comparative assessment of factors which...

