APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1993, p. 3021–3026
0099-2240/93/093021-06$02.00/0

Vol. 59, No. 9

Effect of Water and Nitrogen Additions on Free-Living Nitrogen Fixer Populations in Desert Grass Root Zones

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

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

Received 29 April 1993/Accepted 23 June 1993

In this study we measured changes in population levels of free-living N_2-fixing bacteria in the root zones of potted *Bouteloua eriopoda* and *Sporobolus flexuosus* plants as well as the photosynthetic indices of the plants in response to added nitrogen, added water, and added water plus nitrogen treatments. In addition, N_2 fixer population changes in response to added carbon source and nitrogen were measured in plant-free soil columns. There were significant increases in the numbers of N_2 fixers associated with both plant species in the water and the water plus nitrogen treatments. Both treatments increased the photosynthetic index, suggesting that plant exudates were driving N_2 fixer population changes. Population increases were greatest in the water plus nitrogen treatments, indicating that added nitrogen was synergistic with added water and suggesting that nitrogen addition spared bacteria the metabolic cost of N_2 fixation, allowing greater reproduction. Plant-free column studies demonstrated a synergistic carbon-nitrogen effect when carbon levels were limiting (low malate addition) but not when carbon was abundant (high malate), further supporting this hypothesis. The results of this study indicate the presence of N_2 fixer populations which interact with plants and which may play a role in the nitrogen balance of desert grasslands.

Nitrogen and water deficiencies are usually described as the major limits to growth in desert and desert/grassland ecosystems (24). Recent studies have emphasized the heterogeneity of desert ecosystems and the importance of "islands" of biological activity. These sites, usually centered on shrubs, represent fertile islands where on-site primary productivity is recycled through mineralization, organic matter from upslope or upwind is trapped and recycled, and the biological community modifies the water status of the island soils (1, 2, 5, 11, 12, 22).

Upland (bajada) grassland sites in the Chihuahuan Desert do not have the advantage of much upslope or upwind vegetation as the source of nutrient inputs and therefore must rely on activities within site for nutrient uptake. Ammonia-N levels in the grassland zone are 53% of those in the shrubland and 11% of those in the downslope playa. Nitrate-N levels are similar between the grassland and shrubland but are 8.4% of those in the downslope playa (17). Nevertheless, the bajada zone supports healthy stands of perennial grasses, especially *Bouteloua eriopoda* (black grama) and *Sporobolus flexuosus* (mesa dropseed).

Nitrogen inputs to the grassland zone can come from recycling of above- and below-ground biomass, atmospheric deposition, N_2 fixation by cryptogamic crusts, or N_2 fixation by heterotrophic organisms in the root zone of the grasses (23). The potential importance of root zone N_2 fixation is highlighted by the finding that the addition of water plus nitrogen was no more effective in stimulating biomass production by *B. eriopoda* than was the addition of water alone (19), suggesting that nitrogen may be available as the result of microbial activity in the root zone.

The goal of the present study was to assess the population size of organisms with the potential to contribute nitrogen in the root zone of upland desert grasses and to examine the influence of water, nitrogen, and carbon energy on those population levels.

* Corresponding author. Electronic mail address: rpeter@nmsu.edu

MATERIALS AND METHODS

Site characteristics. Field studies and soil and plant collections were carried out on the Jornada Long Term Ecosystem Research area 40 km NNE of Las Cruces, N.M. Specifically, the site was located in bajada grassland at the base of Summerford Mountain (R1E T21S, Sec. 35 NE 1/4, Doña Ana County, N.M.). Soil from this area is a Ustollic Haplargid (6) composed of 76% sand, 7% clay, 0.68% $CaCO_3$, and 0.48% organic matter (25). Single-year chemical characteristics include a pH of 7.02, 1.13 mg of ammonia-N kg^{-1}, and 0.57 mg of nitrate-N kg^{-1} (17). The average total nitrogen for the bajada grassland is 46 mg kg^{-1} (26). Vegetation is dominated by *B. eriopoda* (black grama) and *S. flexuosus* (mesa dropseed).

Media. All isolations were carried out on an almost nitrogen-free malate-mannitol medium (NF/MM), a modification of *Azospirillum* semisolid nitrogen-free malate medium (18). Composition of solid NF/MM was as follows (in grams per liter): KH_2PO_4, 0.4; K_2HPO_4, 0.1; $MgSO_4$, 0.097; NaCl, 0.1; $CaCl_2$, 0.0196; $FeCl_3 \cdot 6H_2O$, 0.17; $NaMoO_4 \cdot 2H_2O$, 0.002; yeast extract, 0.001; L-malic acid, 3.58; mannitol, 5.0; agar, 15. Medium pH was adjusted to 7.0 with KOH prior to the addition of the agar. Liquid NF/MM for nitrogen-free growth confirmation was made as above but without the agar and yeast extract, to remove the major sources of trace nitrogen.

Greenhouse studies. For greenhouse pot studies, 28 plants each of *B. eriopoda* and *S. flexuosus* were transferred individually into 1-gallon (3.8-liter) plastic pots in the field and returned to the greenhouse. Plants were held in the greenhouse for a period of 3 weeks prior to the start of experimental studies. Pots with healthy plants (24 from each species) were numbered, set out in a four-by-six grid on the greenhouse bench, and randomly assigned to one of the four treatment groups. The control group received 60 ml of water

three times weekly; the nitrogen-amended group received 60 ml of water containing 0.1 g of NH_4NO_3 liter^{-1}; the water-amended group received 180 ml of water three times weekly; and the water plus nitrogen group received 180 ml of water containing 0.033 g of NH_4NO_3 liter^{-1}. The watering rate for the control and nitrogen-amended groups was equivalent to a rainfall of 10 mm week^{-1}, whereas the water-amended and water plus nitrogen groups received the equivalent of 30 mm week^{-1}. Application rates of NH_4NO_3 were the equivalent of 2 g m^{-2}. All water additions were made by pouring the measured volume evenly over the soil surface. Soil cores (1 cm by 10 cm, at the plant crown edge) for measurements of free-living N_2 fixer populations were taken just prior to treatment (day zero) and after 8 and 14 days of treatment. Soil without plants (eight pots) was obtained from the same general location as the plants, returned to the greenhouse, and assigned to treatment groups as above. Soil cores for measurement of free-living N_2 fixer populations were taken from these pots immediately (day zero) and after 14 days of treatment.

Column studies. A series of 24 polyvinyl chloride columns (7.5 by 30 cm) were filled with bajada zone soil, watered periodically for 2 weeks to allow the soil to settle to a consistent level about 6 cm below the top of the column, numbered, sampled for N_2 fixer numbers, and randomly assigned to one of six groups; control, nitrogen only, low malate, high malate, low malate plus nitrogen, and high malate plus nitrogen. Control columns received 60 ml of water three times weekly. Treated columns received 60 ml of water three times weekly with additions at the following rates: nitrogen at 0.1 g of NH_4NO_3 liter^{-1}, and DL-malic acid at 0.015 g liter^{-1} for low malate and 0.15 g liter^{-1} for high malate. Soil cores were removed at intervals over a period of 3 months.

Isolations. Pot and column soil cores were removed by using an alcohol flame-disinfected cork borer (1 by 10 cm). The entire core was aseptically transferred to a plastic bag and returned to the laboratory for processing within 30 min.

Cores were manually broken and mixed in their plastic bags. Two 1-g samples per pot core and one per column core were aseptically weighed and transferred to dilution bottles containing 100 ml of sterile deionized water. Bottles were allowed to stand for 15 min and then shaken manually for 15 s. Two dilution series were made per bottle onto solid NF/MM. Final dilutions ranged from 10^4-fold to 10^7-fold. Soil samples were weighed, dried for 24 h at 105°C, and reweighed to calculate percent moisture. Plates were incubated in snap-lid plastic tubs for 5 days at room temperature (~22°C). Colonies were counted after the incubation. Obviously filamentous colonies were ignored, and plates obscured by fungal growth or with large zones of inhibition surrounding fungal colonies were discarded. In no case were enough plates discarded to have an effect on the statistical analysis. Data were expressed as CFU per gram of dry soil.

pO_2 values in the snap-lid containers were periodically monitored with an Applied Electronics S3A Oxygen analyzer. The pO_2 dropped from ambient levels to about 95 mmHg during the incubation, levels comparable to those measured in freshly made candle jars.

Photosynthesis measurements. Photosynthetic activity of healthy leaf tissue from greenhouse-grown plants was estimated by measuring CO_2 flux with a LiCor LI6200 portable photosynthesis system fitted with the 0.25-liter cuvette. Leaf areas were measured by using a video imaging digitizer, and photosynthesis was calculated as micromoles of CO_2 depleted per second per square meter. The proportion of leaf tissue which was photosynthetically active was estimated to the nearest 5% at each measurement period. Photosynthesis data were collected within 1 day of each microbiological sampling. At the end of the 2-week experiment, plants were harvested and divided into green and nongreen tissue. Total plant dry weights were measured. A photosynthetic index for each pot was calculated as the product of proportion of photosynthetically active tissue × total plant weight × photosynthetic rate.

ARA assay. Two separate measures, using acetylene reduction activity (ARA), were carried out to confirm that colonies growing on the isolation plates on NF/MM in closed boxes did represent N_2 fixers. In the first, 20 isolation plates with 80 to 150 colonies per plate were selected at random from the plates enumerated during the column study. All colonies were counted and separated into groups on the basis of colony morphology. Colonies were picked proportional to the relative abundance of their morphology (more than 50%, had 50 colonies screened from the 20 plates; more than 3% but fewer than 50% had 10 colonies; and fewer than 3% had 5 colonies). Picked colonies were cut from the plate, placed in 1 ml of liquid NF/MM in 10-ml serum bottle under an atmosphere of 10% (vol/vol) acetylene, and incubated for 60 h at room temperature. The headspace was sampled with a gastight syringe, and acetylene reduction was measured by gas chromatography. The 60-h incubation was chosen because, although it is not optimal for all isolates, it was the incubation time which provided the best comparative data.

In the second test, 12 soil samples were split. Half the sample was processed for counting as described above. A 3-g (wet weight) sample of the other half was placed in a 10-ml serum vile, flooded with 2 ml of NF/MM liquid medium, gassed with 100 nmol of acetylene, and incubated at room temperature for 60 h. The headspace was sampled and analyzed for acetylene reduction by gas chromatography. Acetylene reduction activity was plotted against nitrogen fixer number as calculated by dilution plating.

Gas chromatography was carried out with a Hewlett Packard 5710A gas chromatograph with a Porapak N column (2 m by 1/8 in. [0.32 cm]). The N_2 carrier gas flow rate was 40 ml min^{-1}. Isothermal runs were carried out at 80°C. The injector port temperature was 80°C, and the flame ionization detector temperature was maintained at 250°C. Data were collected on a Hewlett Packard 3390A integrating recorder.

Statistics. All data were analyzed by analysis of variance (ANOVA) and multivariate ANOVA (MANOVA) using SAS General Liner Models Procedures (16). Data on figures are displayed as means ± standard errors of the mean (SEM). Groups were considered significantly different when $P < 0.05$.

RESULTS

Colonies isolated after plating on NF/MM routinely fell into one of 10 colony types. In validation studies, 4 of the 10 colony types tested positive for acetylene reduction activity (ARA). In three colony types all members were positive, and in the fourth 66% tested positive. Positive colonies were calculated to represent 76.8% of all colonies counted. The results of these control studies are summarized in Table 1.

To determine whether there was a significant relationship between N_2-fixing ability and numbers as determined by plate counts on NF/MM, we plotted N_2-fixing ability (measured as ARA) against cell counts. The positive relationship is described by the equation $y = 3.28 \times 10^{-7}x + 1.29 \times 10^{-2}$ with $r^2 = 0.81$.

TABLE 1. Properties of colonies selected from 20 nitrogen-free dilution plates

Colony type[a]	No.	Frequency[b]	% Relative abundance[c]	No. of colonies screened	No. of N_2-fixing colonies	% of N_2-fixing colonies	Total no. of N_2-fixing colonies[d]	Cell description[e]
sw	975	0.95	50.8	50	33	66	33.5	g^- rods ~1.5 by 0.3 μm
mw	271	0.70	14.1	10	10	100	14.1	g^- rods ~1.8 by 0.3 μm
lw	305	0.60	15.9	10	10	100	15.9	g^- rods ~2.5 by 0.5 μm
tf	256	0.25	13.3	10	10	100	13.3	g^- rods ~4.0 by 1.5 μm
ma	76	0.35	4.0	10	0	0	0	g^- rods ~2.0 by 0.3 μm
lm	11	0.25	0.6	5	0	0	0	g^- rods ~2.0 by 0.4 μm
sm	3	0.10	0.2	5	0	0	0	g^- rods ~1.0 by 0.3 μm
am	7	0.20	0.4	5	0	0	0	g^- rods ~1.5 by 0.3 μm
ye	11	0.30	0.6	5	0	0	0	g^- rods ~2.0 by 0.5 μm
dk	6	0.25	0.3	5	0	0	0	g^- rods ~2.0 by 0.3 μm
Total	1,921		100.2	115	63	54.7	76.8	

[a] Colony types: 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; dk, dark brownish black.
[b] Number of plates on which colony type appeared/20.
[c] (Number of colonies of type/1,921) × 100.
[d] Unrounded relative abundance × percentage screened positive for colony type.
[e] g^-, gram negative.

Plant-photosynthetic status and nitrogen addition influenced the population of free-living N_2 fixers in pots containing either *B. eriopoda* or *S. flexuosus*. Figure 1 shows the effect of added nitrogen, added water, and added water plus nitrogen on the photosynthetic index (Fig. 1B) and free-living N_2 fixer populations (Fig. 1A) associated with *B. eriopoda*. There were no initial differences in photosynthetic index among the treatment groups. By day 8, the photosynthetic indices for the water-amended (W) and the water- plus nitrogen-amended (B) treatments were significantly higher than for either the control (C) or nitrogen-amended (N) group. This difference persisted, although at a much lower level, at day 14. At this time point, plants were showing signs of postflowering senescence. As with the photosynthetic index, there were no initial differences in numbers of free-living nitrogen fixers. By day 8, numbers in the water- plus

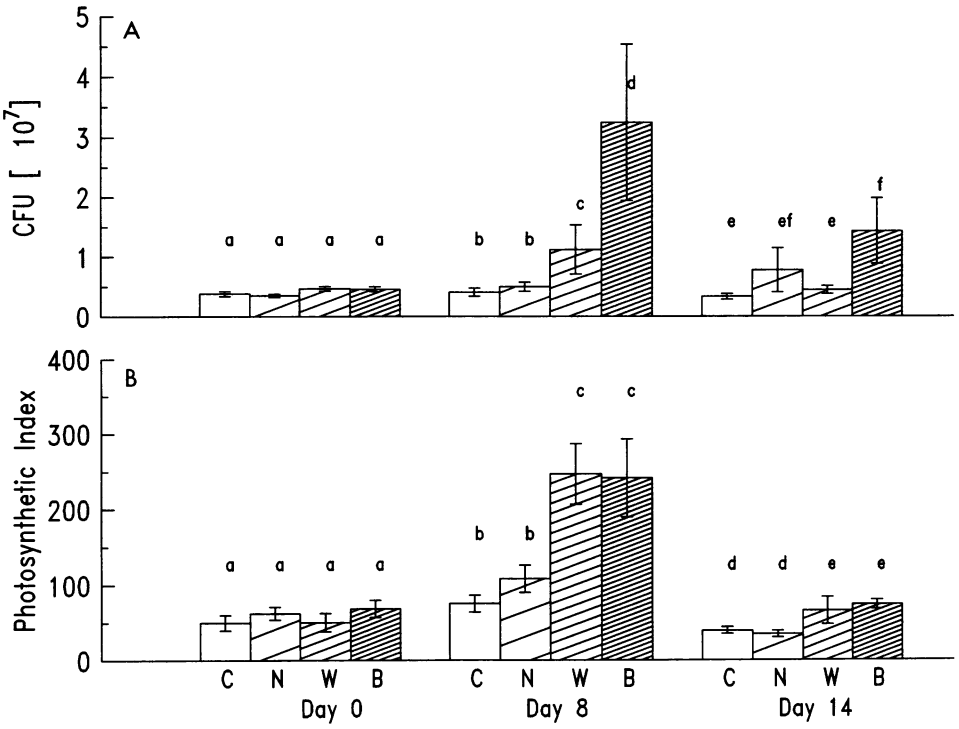

FIG. 1. Effects of no addition (C), added nitrogen (N), added water (W), and added water plus nitrogen (B) on population levels of organisms (CFU per gram of dry soil) capable of growing on nitrogen-free medium from the root zone of pots containing *B. eriopoda* (panel A) and on the photosynthetic index of *B. eriopoda* plants (panel B) (n = 6 for each treatment). Population values are means of 20 to 24 determinations ± SEM. Bars within a sample period marked by the same letter are not significantly different ($P < 0.05$).

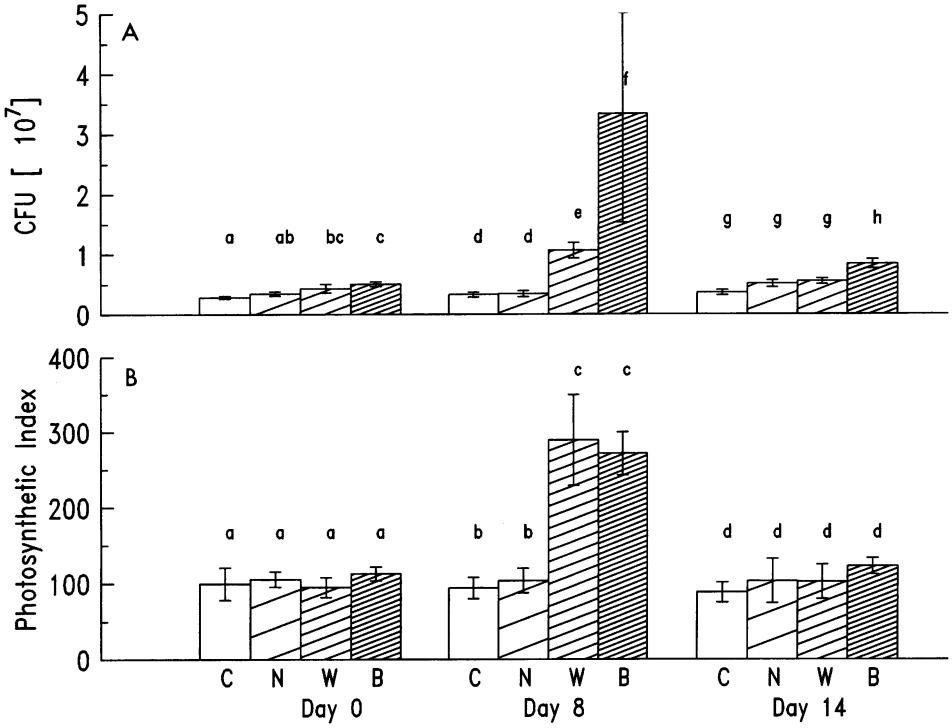

FIG. 2. Effects of no addition (C), added nitrogen (N), added water (W), and added water plus nitrogen (B) on population levels of organisms (CFU per gram of dry soil) capable of growing on nitrogen-free medium from the root zone of pots containing *S. flexuosus* (panel A) and on the photosynthetic index of *S. flexuosus* plants (panel B) (n = 6 for each treatment). Population values are means of 20 to 24 determinations ± SEM. Bars within a sample period marked by the same letter are not significantly different ($P < 0.05$).

nitrogen-amended group (B) were larger than for all other treatments and the numbers of free-living N_2 fixers in the water-amended group (W) were significantly larger than the control (C) and nitrogen-amended (N) groups. The clear pattern disappeared by day 14, at which time the values for the control, nitrogen-amended, and water-amended groups were indistinguishable. The water plus nitrogen treatment led to population numbers larger than those for the control and the water treatments but was indistinguishable from the nitrogen-only treatment.

Figure 2 shows the results of the same treatments on the photosynthetic index and nitrogen fixer population levels associated with the pots containing *S. flexuosus*. As with *B. eriopoda*, the only differences in photosynthetic index were in the day 8 W and B groups, which were associated with significantly higher indices than were all other samples. There were small but significant differences in population levels among the treatment groups in the initial sample. The same pattern as seen with *B. eriopoda* emerged on day 8 for *S. flexuosus*. The population levels in the C and N groups were the same and were lower than in the W group. Populations in the B group were higher than in the three other groups. As before, the strong pattern disappeared in the day 14 samples, with the populations remaining slightly higher in the B group than in the C, N, and W groups.

To determine whether the treatments alone, in the absence of plants, would influence N_2 fixer population levels, we treated a series of plantless pots as described above. There were no differences in the initial populations prior to any water or nitrogen additions. After 2 weeks of watering with the same treatments as above, the cell numbers in all pots increased to an equal extent, ~2.3-fold. The numbers in the watered, plant-free soil were smaller than the initial values in the *B. eriopoda* controls (1.1×10^6 and 2.8×10^6, respectively).

The results of the plant experiments suggested that carbon energy sources leaked from roots stimulates the number of free-living N_2 fixers and that the addition of N potentiated this effect. Column experiments carried out in the absence of plant roots examined the effect of high and low carbon source additions (as malate) on free-living N_2-fixing organism numbers in the presence and absence of added N. The numbers of free-living N_2 fixers increased to significantly elevated levels in the high-malate treatment groups. The increase was the same in the presence or absence of added N (Fig. 3A). In the low carbon source addition treatments, populations were significantly elevated over controls. Populations were increased to a significantly greater degree when both low levels of malate and N were added to the soils (Fig. 3B). The addition of N in the absence of an exogenous carbon source resulted in no significant difference from control populations (Fig. 3C).

DISCUSSION

The effect of added nitrogen on overall bacterial numbers in the rhizosphere or root surface zone has yielded conflicting results, with some authors finding increases (4, 10, 14, 21) and others finding no change or decreases (13, 20). Still other studies have shown a strong dependence of the response on plant age (7). The results for free-living N_2 fixers are similarly contradictory (4, 9, 27).

In our studies, the addition of nitrogen without extra water had little effect on the root-associated population of microorganisms able to grow on nitrogen-free medium. Similarly,

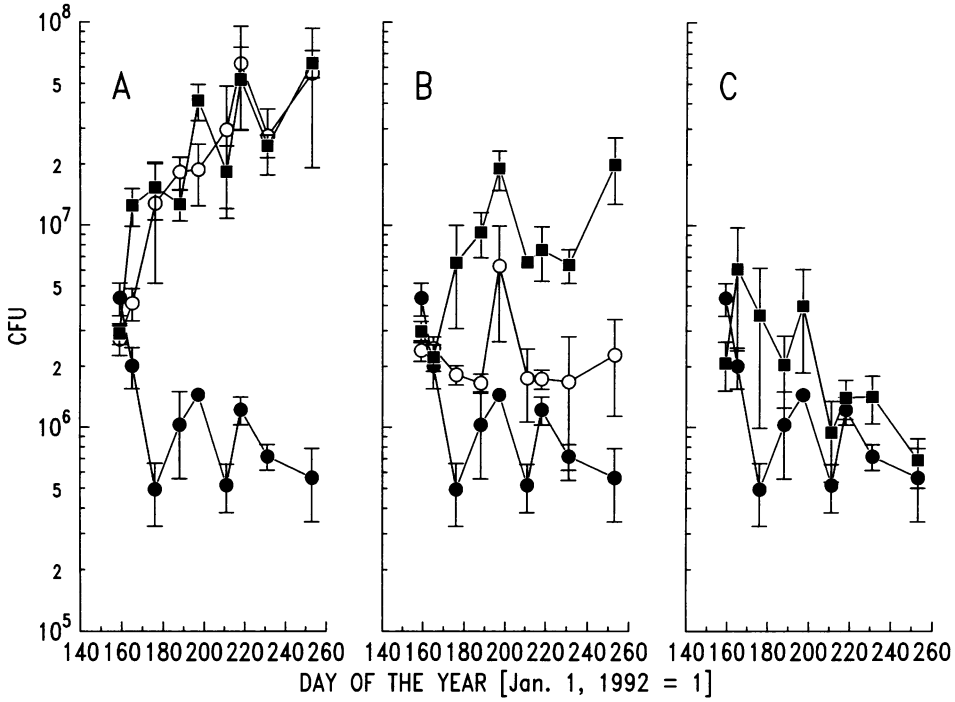

FIG. 3. Effect of carbon source and nitrogen addition on population levels of organisms (CFU per gram of dry soil) capable of growing on nitrogen-free medium isolated from soil columns. In all cases, each point represents the mean ± SEM of duplicate determinations for four columns. (A) Symbols: ●, no addition; ○, addition of a high concentration of carbon source (0.15 g of malate $liter^{-1}$); ■, addition of a high concentration of carbon source plus nitrogen. (B) Symbols: ●, no addition; ○, addition of a low concentration of carbon source (0.015 g of malate $liter^{-1}$); ■, addition of a high concentration of carbon source plus nitrogen. (C) Symbols: ●, no addition; ■, addition of nitrogen alone.

Kolb and Martin reported that nitrogen additions did not stimulate, and frequently decreased, the absolute or relative abundance of root-associated free-living N_2 fixers from temperate grassland soils (4). They suggest that N_2 fixers may be poor competitors with general heterotrophs for added resources.

Root exudation of photosynthate (15) may be responsible for the observed increases in microbial populations associated with roots. Although the proportion of photosynthate actually leaked is highly variable (11) and depends on the growth stage and nutritional state of the plant, exudates can represent as much as 66% of the dry weight increase of roots (7). In our study, the addition of water (treatment W) resulted in significant population increases and the addition of water plus nitrogen (treatment B) caused greater increases than did the addition of water alone. Photosynthesis-driven root exudation could explain these increases in N_2 fixer population. This hypothesis is supported by the change in N_2 fixer populations and photosynthetic indices at day 14 in both grass species. When the photosynthetic index decreased at day 14 of the W and B treatments, the N_2 fixer population levels associated with these plants decreased as well. The observed decrease of photosynthesis is associated with the onset of postflowering senescence in the experimental plants. The observation that numbers of N_2 fixers decrease with plant senescence is consistent with data which show a decrease in acetylene reduction with the onset of flowering in soil from potted *B. eriopoda* plants collected from the same field site (3).

The difference in the increases in N_2 fixer populations between the water and the water plus N treatments has at least two possible explanations. The first is that the removal of the need to fix nitrogen allows the N_2 fixer cells to devote a larger portion of the increased carbon source availability to cell reproduction. Even if competition of generalist heterotrophs suppresses the proportion of N_2 fixers in the total population (4), the added N may allow an absolute increase in N_2 fixer numbers. The second major possibility is that the plants themselves may be allocating more carbon to the roots in treatment B than in treatment C. Wheat plants grown at high nitrogen levels release more ^{14}C-labeled exudates from their roots than do plants grown at low nitrogen levels (8).

Although additional allocation of carbon to roots in the presence of nitrogen may play a role in the plants treated with water plus nitrogen, it appears that data from the column studies support the hypothesis that "metabolic cost savings" resulting from added nitrogen is largely responsible for increases in numbers of free-living N_2 fixers. Population response patterns in the low-carbon groups, in which carbon is potentially limiting, mimic the pattern found in the pots that contain plants. There are significantly greater numbers of free-living N_2 fixers in the low-carbon-source group over controls, and this response is significantly increased with the addition of nitrogen (Fig. 3B). Any cost saving would be most strongly reflected by population increases when carbon energy is limited. This hypothesis is further supported by the lack of difference in numbers during treatments when carbon might not be limiting growth (Fig. 3A). That carbon is not limiting in the high-carbohydrate treatment is suggested, although not proved, by the relatively small difference (twofold) in numbers between the high-carbon plus nitrogen group and the low-carbon plus nitrogen group resulting from a 10-fold difference in carbon addition levels.

Significant time × treatment interactions in the MANOVA of the column populations indicated that populations were

changing from their initial levels in response to treatment for the first four to six sampling periods, depending on the treatment. The lack of significant time × treatment interactions after this point suggested that the populations had equilibrated in response to treatment.

Not all putative nitrogen fixers enumerated in this study were, in fact, nitrogen fixers. Some organisms were capable of scavenging the limited quantities of nitrogen in the agar and yeast extract. The four colony types which represented 94.1% of the colonies counted contained isolates with demonstrated ARA as a measure of nitrogen fixing ability. Three of the four colony types were made up entirely of nitrogen fixers, whereas 66% of the isolates in the fourth colony type demonstrated ARA. The remaining six colony types clearly contained organisms with extremely efficient nitrogen uptake. Our calculation that 76.8% of the screened isolates represent true fixers suggests that the numbers of free-living N_2 fixers presented in this study are probably overestimated by 25 to 30% (Table 1). The relatively good yield of small gram-negative enteric-appearing rods which were capable of fixing nitrogen was probably due to the reduced O_2 tension in the snap-lid tubs in which they were incubated. It is also clear that the counts obtained are positively correlated with the N_2-fixing potential of field-collected soils.

The results of this study demonstrate that organisms capable of utilizing atmospheric N_2 as their sole nitrogen source exist in soils associated with *B. eriopoda* and *S. flexuosus* in desert bajada grasslands. They also show that N_2 fixer populations fluctuate in response to the photosynthetic status of their associated plants and the nitrogen and carbon levels in the soil. This association suggests the possibility that nitrogen fixation by free-living N_2 fixers and/or the mineralization of dead N_2 fixers makes a significant contribution to the nitrogen balance of desert grassland communities.

ACKNOWLEDGMENTS

This work was supported in part by National Science Foundation Research Experience for Undergraduate stipend supplement to the JORNADA LTER BSR-8811160 (to G.M.S. and K.R.P.), National Institutes of Health Minority High School Student Research Apprenticeship Program Stipend SO3RR03100-08 (to K.R.P.), and Minority Access to Research Careers Fellowship GMO7667-16 (to R.J.T.).

We thank Dennis Clasen for assistance with the statistical analyses and Walter G. Whitford for helpful discussions about the manuscript.

REFERENCES

1. **Caldwell, M. M., and J. H. Richards.** 1989. Hydraulic lift: water efflux from upper roots improves effectiveness of water uptake by deep roots. Oecologia **79:**1–5.
2. **Elkins, N. G., G. V. Sabol, T. V. Ward, and W. G. Whitford.** 1896. The influence of subterranean termites on the hydrological characteristics of a Chihuahuan Desert ecosystem. Oecologia **68:**521–528.
3. **El Shahaby, A. F.** 1988. Associative nitrogen fixation with C_4 grasses of the northern Chihuahuan Desert. Ph.D. dissertation. New Mexico State University, Las Cruces.
4. **Kolb, W., and P. Martin.** 1988. Influence of nitrogen on the number of N_2-fixing and total bacteria in the rhizosphere. Soil Biol. Biochem. **20:**221–225.
5. **Lajtha, K., and W. H. Schlesinger.** 1986. Plant response to variations in nitrogen availability in a desert shrubland community. Biogeochemistry **2:**29–37.
6. **Lajtha, K., and W. H. Schlesinger.** 1988. The biogeochemistry of phosphorus and phosphorus availability along a desert soil chronosequence. Ecology **69:**24–39.
7. **Liljeroth, E., E. Bååth, I. Mathiasson, and T. Lundborg.** 1990. Root exudation and rhizoplane bacterial abundance of barley, *Hordeum vulgare* L., in relation to nitrogen fertilization and root growth. Plant Soil **127:**81–89.
8. **Lijleroth, E., J. A. Van Veen, and H. J. Miller.** 1990. Assimilative translocation to the rhizosphere of two wheat lines and subsequent utilization by rhizosphere microorganisms at two soil nitrogen concentrations. Soil Biol. Biochem. **22:**1015–1021.
9. **Martin, P., A. Glatzle, W. Kolb, H. Omay, and W. Schmidt.** 1989. N_2-fixing bacteria in the rhizosphere: quantification and hormonal effects on root development. Z. Planzenernahr. Bodenkd. **152:**237–245.
10. **Merckx, R., A. Dijkstra, A. DenHartog, and J. A. VanVeen.** 1987. Production of root-derived material and associated microbial growth in soil at different nutrient levels. Biol. Fertil. Soils **5:**126–132.
11. **Newman, E. I.** 1985. The rhizosphere: carbon sources and microbial populations, p. 107–122. *In* A. H. Fitter (ed.), Ecological interactions in soil—plants, microbes and animals. Blackwell Scientific Publications, Oxford.
12. **Nulsen, R. A., K. J. Bligh, I. N. Baxter, E. J. Solin, and D. H. Imrie.** 1986. The fate of rainfall in a mallee and heath vegetated catchment in southern Western Australia. Aust. J. Ecol. **11:**361–371.
13. **Okano, S., K. Sato, and K. Inoue.** 1991. Negative relationship between microbial biomass and root amount in topsoil of a renovated grassland. Soil Sci. Plant Nutr. **37:**47–53.
14. **Roswell, T., and K. Paustian.** 1984. Cycling of nitrogen in modern agricultural systems. Plant Soil. **76:**3–21.
15. **Rovira, A. D.** 1969. Plant root exudates. Bot. Rev. **35:**35–57.
16. **SAS Institute.** 1982. SAS users guide. SAS Institute, Cary, N.C.
17. **Schlesinger, W. H., and W. T. Peterjohn.** 1991. Processes for controlling ammonia volatilization from Chihuahuan Desert soils. Soil Biol. Biochem. **23:**637–642.
18. **Smibert, R. M., and N. R. Krieg.** 1981. Systematics: general characterization, p. 409–443. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
19. **Stephens, G. A., and W. G. Whitford.** Response of black grama grass, *Bouteloua eriopoda*, to water and nitrogen. J. Arid Environ., in press.
20. **Turner, S. M., and E. I. Newman.** 1984. Growth of bacteria on the roots of grasses: influence of mineral nutrient supply and interactions between species. J. Gen. Microbiol. **130:**505–512.
21. **VanVuurde, J. W. L.** 1978. The rhizosphere microflora of wheat grown under controlled conditions. I. The effect of soil fertility and urea leaf treatment on rhizoplane microorganisms. Plant Soil **50:**447–460.
22. **Virginia, R. A., and W. M. Jerrell.** 1983. Soil properties in a mesquite-dominated Sonoran Desert ecosystem. Soil Soc. Am. J. **47:**138–144.
23. **West, N. E., and J. O. Klemmedson.** 1978. Structural distribution of nitrogen in desert ecosystems, p. 1–19. *In* N. E. West and J. J. Skujins (ed.), Nitrogen in desert ecosystems. Dowden, Hutchinson and Ross, Stroudsburg, Pa.
24. **Whitford, W. G.** 1986. Decomposition and nutrient cycling in deserts, p. 93–118. *In* W. G. Whitford (ed.), Pattern and process in desert ecosystems. University of New Mexico Press, Albuquerque.
25. **Whitford, W. G., J. F. Reynolds, and G. L. Cunningham.** 1987. How desertifuication affects nitrogen limitation of primary production of Chihuahuan Desert watersheds, p. 143–153. *In* C. E. Gonzales-Vicente and C. E. Moir (ed.), Strategies for classification and management of native vegetation for food production in arid zones. General Technical Report RM-150. U.S. Department of Agriculture Forest Service, Rocky Mountain Forest and Range Experiment Station, Ft. Collins, Colo.
26. **Wierenga, P. J., J. M. H. Hendricx, M. H. Nash, J. Ludwig, and L. A. Dougherty.** 1987. Variation of soil and vegetation with distance along a transect in the Chihuahuan Desert. J. Arid Environ. **13:**53–63.
27. **Wright, S. F., and R. W. Weaver.** 1981. Enumeration and identification of nitrogen-fixing bacteria from forage grass roots. Appl. Environ. Microbiol. **42:**97–101.