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FIXATION AND UTILIZATION OF NITROGEN IN DESERT VEGETATION

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Summary

Some seasonal surveys were made to see if nitrogen fixation reactions could be detected in the root zone of desert vegetation by the acetylene reduction method. Corroborative field studies also were made to determine the response of desert vegetation to nitrogen fertilization with and without supplementary moisture.

Root-soil samples from several different plant species showed positive reactions which are presumed to primarily involve symbiotic microbial endophytes because of the sensitivity of the reaction to temperature and moisture and its dependence upon the presence of plant roots. The following species of nonleguminous plants gave positive reactions: Artemisia spinescens, Artemisia tridentata, Hymenoclea salsola, and Tetradymia canescens of the Compositae family; Coleogyne ramosissima of the Rosaceae family; Bromus rubens of the Gramineae family; and Krameria parvifolia of the Krameriaceae family. Three members of the Leguminosae family resulted in a positive test: Lupinus argenteus, Dalea fremontii, and Astragalus lentiginosus. Lichens which were not identified also resulted in a positive test.

The greatest response of desert vegetation to nitrogen fertilizer occurred in winter annual species with a two- to three-fold increase in standing crop biomass. Among the perennial shrubs studied, Eurotia lanata and Grayia spinosa showed the greatest growth response to added moisture. Plant growth on plots fertilized at levels of 100 and 200 kg of

nitrogen per hectare indicated no beneficial influence from excess nitrogen input into desert soil. Added nitrogen increased the total nitrogen content in the foliage of nearly all plants examined.

Introduction

The nitrogen deficiency symptoms commonly seen in the foliage of cultivated crop plants seldom appear in the foliage of desert vegetation. Although nitrogen deficiency would be expected to occur in the desert environment, its symptomatic appearance might be masked by other limiting factors. For example, the seasonal moisture may be insufficient to sustain an amount of vegetation that could seriously deplete the available nitrogen in desert soils. Actually, the sparse stands of vegetation typical of most desert areas should require much less nitrogen than would be required of cultivated crop plants. Furthermore, most of the nitrogen used by plants is subsequently returned to desert soils through leaf fall and decomposition of litter. Leaching and erosion from rainfall probably accounts for the greatest loss of nitrogen from a given area in the desert; yet, in some cases, that which is lost by run-off at one site might be compensated for by run-in from another site.

Another factor to consider is the degree to which certain species of desert plants might fix atmospheric nitrogen through endophyte symbiosis or free-living microbial forms. This important life process has been the subject of several recent reviews ^{6 8 14 19 20}. With this in mind, some seasonal surveys were made to see if nitrogen fixation could be detected in the root zone of desert plants by the acetylene reduction method. Corroborative field studies were also made to determine the response of desert vegetation to nitrogen fertilization with and without supplemental moisture.

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Materials and Methods

These studies were conducted at the Nevada Test Site of the U.S. Atomic Energy Commission in southern Nevada. Most of this area lies in the transition zone between the Mojave and Great Basin Deserts where a number of plant species common to each of these deserts grow together in dominant or co-dominant association. Allred, Beck, and Jorgensen² and Beatley³ have described the biotic communities of the Nevada Test Site.

Surveys were made at different seasons of the year to learn the extent to which nitrogen fixation might occur in the desert environment either from symbiotic microbial endophytes or from free-living forms. The species of plants examined are listed in Table 1; taxa synonymy follows that of Munz and Keck¹⁷. The acetylene reduction method was used to detect the presence of a nitrogen-fixation system without attempting to resolve its specific nature. This indicator method has received broad application^{10 13 15 18 21}. Briefly, 50 g samples containing roots and soil, or soil alone, were taken from the root zone of plants growing in the field and placed in serum bottles. A few drops of water were added to moisten the soil, if needed, and the samples were immediately purged with a gas mixture (80% Argon, 20% oxygen, 0.04% CO₂) and then charged with a measured volume of acetylene. After selected incubation intervals, the samples were analyzed for the presence of ethylene by gas chromatography (Wheelco Flame Detector). Control samples with Clostridium pasteurianum were run in conjunction with these field samples. Although the results of these preliminary surveys are qualitative, all verifications were made with multiple samples having from two to five replicates.

Studies on the utilization of nitrogen fertilizers involved 730 m² field

plots which received surface applications of NH_4NO_3 at levels equivalent to 100 and 200 kg of nitrogen per hectare. These treatments were added to plots in natural desert conditions and to plots periodically receiving supplementary moisture by overhead sprinkler irrigation to maintain soil moisture above 5 per cent by weight. This was equivalent to from 25 to 30 cm of additional rainfall in an area which normally receives from 10 to 15 cm of rainfall annually. Plant and soil samples from treated and control areas were analyzed for total nitrogen contents by the Coleman Model 29A Nitrogen Analyzer II. Nitrate-nitrogen in soil was determined by the method of Chapman and Pratt⁹.

Results and Discussion

Nitrogen Fixation in Desert Vegetation

Results of seasonal surveys on some prominent desert plant species appear in Table 1. At no time was the presence of a nitrogen-fixation system detected in soil samples without roots; all positive results were with root-soil samples except for the lichens. Root nodules were prevalent on two of the legumes, Astragalus letiginosus and Lupinus argenteus. We detected what were thought to be root nodules on Artemisia tridentata and Krameria parvifolia, but no intensive effort was made to examine whole-root systems for the presence of nodules or to isolate microbial endophytes; such work forms the basis for future investigations. As one might suspect, no evidence of a continuing fixation reaction was seen after the soil was depleted of its seasonal moisture with the onset of the hot, dry summer months. Negative results also were obtained in spot checks after some late summer and fall rains. Although these surveys did not identify the specific nature of a nitrogen-fixation reaction, we presume that symbiotic microbial endophytes are primarily involved because of the sensitivity of the reaction to tempera-

ture and moisture and its dependence upon the presence of plant roots. Went and Stark²² reported that "typical endotrophic mycorrhiza was observed microscopically in the roots of Franseria dumosa, Peucephyllum schottii, and Hymenoclea salsola. All other desert plants, both annuals and shrubs, had many hyphae in their rhizosphere and surrounding soil. The dark colored hyphae could often be seen penetrating through the root epidermis."

Farnsworth and Hammond¹¹ observed nodules on the roots of Artemisia ludoviciana and found that the endophyte isolated from the nodules was bacterial, and presumably functioned in the assimilation of atmospheric nitrogen. The presence of these nodules seemed to be correlated very closely with the supply of early spring moisture and cool soil temperature. Nodules could not be found after soil had dried out near the end of June.

The gas chromatogram tracings in Figure 1 show some examples of the relative activity of the acetylene reduction—ethylene production reaction by root-soil samples with common incubation time. Bromus rubens and Lupinus argenteus were the most reactive samples detected by our qualitative survey method. Bromus rubens is an introduced grass species⁴ of ecological significance because it grows primarily within the sheltered canopies of shrubs where nitrogen fixation may be of greatest advantage to native vegetation.

It is well known that leguminous plants may contribute substantially to the nitrogen status of soils; however, symbiotic nitrogen fixation is not restricted to legumes. Diverse non-leguminous angiosperms also fix atmospheric nitrogen by root-nodule symbiosis in association with actinomycetes⁶. More than 100 species of 14 genera of 7 families of the non-leguminous angiosperms are reported to have root nodulation^{1 6 7 20}. Should further investi-

gations on species suggested by our surveys show conclusive presence of root nodule symbiosis, the following families, genera and species of non-leguminous plants could be added to those already identified.

Compositae: Tetradymia canescens, Hymenoclea salsola, Artemisia spinescens, Artemisia tridentata. Farnsworth and Hammond ¹¹ have identified Artemisia ludoviciana.

Rosaceae: Coleogyne ramosissima. The genera Cercocarpus, Dryas and Purshia have been identified in this family ⁶.

Gramineae: Bromus rubens.

Krameriaceae: Krameria parvifolia.

Three of the other species which tested positive to the acetylene reduction reaction are Leguminosae: Lupinus argenteus, Dalea fremontii, and Astragalus lentiginosus.

The nitrogen fixation capabilities of lichens is of ecological importance because of their widespread occurrence in desert soils. At our study sites the lichens are most active during the cool, moist winter and early spring months. These associations are probably the most representative of the free-living forms of nitrogen-fixing systems in desert soils. Fuller et al. ¹² identified many genera of algae obtained from desert soils as blue-green algae, many of which are autotrophic both with respect to nitrogen as well as carbon. Their ability to fix atmospheric nitrogen resulted in algae and lichen crusts being four to five times as high in nitrogen as the soil below. This nitrogen was shown to be available to plants. Mayland et al. ¹⁶ found that semi-arid desert algae crusts fixed nitrogen at rates of 0.16 and 0.10 pounds of nitrogen per acre of crust surface per day under continuous wet and wet-dry cycling conditions, respectively.

Utilization of Nitrogen by Desert Vegetation

Our greatest response from nitrogen fertilization of desert soil came from winter annual plants with a two- to three-fold increase in standing crop biomass. There was, however, no significant increase in the number of plants per unit area. Some perennial shrubs showed visible growth response but the effect was less striking than with winter annuals. Growth was equally as good on plots fertilized with nitrogen at levels equivalent to 100 and 200 kg of nitrogen per hectare during the 2-year period of our observations indicating no beneficial influence from excess nitrogen input into the soil. The nitrogen contents of plant samples harvested the first season after nitrogen was applied in 1968 are shown in Table 2. In nearly all species tested the nitrogen contents were higher in leaf tissues grown on nitrogen-treated plots. Additional effects of nitrogen fertilization and supplementary moisture on desert vegetation are shown by data in Table 3. In natural dry plots no growth response to added nitrogen was measured except for Eurotia lanata. This same species along with Grayia spinosa responded significantly in plots receiving supplementary moisture, and all species listed showed growth response to added moisture with the exception of Ephedra nevadensis, Krameria parvifolia, and Larrea tridentata. Growth response from added moisture was greater than the response from added nitrogen fertilizer. For most species, however, the nitrogen treatments tended to increase the nitrogen contents in foliage. Our results indicate that very little practical advantage could be gained from applying nitrogen to desert range areas unless there is also an input of supplementary moisture.

At our study areas on the Nevada Test Site we have found the above-ground biomass of perennial shrubs to vary from 1500 to 5000 kg per hectare

and the new annual photosynthetic production on these shrubs to vary from 200 to 800 kg per hectare. Since the primary productivity markedly depends upon the seasonal supply of soil moisture, it varies widely from year to year for both annual plants and perennial shrubs. We have observed the standing crop of winter annuals to range from 50 to 300 kg per hectare at our study sites. Beatley⁵ has reported biomass values for winter annual plants ranging from 0 to 616 kg per hectare on undisturbed soils.

The total nitrogen content of desert soils generally is low. In bare areas we have found the total nitrogen level in soil to vary from less than 0.01 to 0.08 per cent. Under shrub clumps the range is from 0.01 to 0.15 per cent and in rare instances we have detected levels up to 0.25 per cent in zones of high organic matter deposition and nitrogen accumulation. The NO_3 -nitrogen levels in soil are low, ranging from 2 to 80 parts per million. Fixed forms of nitrogen on the soil colloids range within a factor of 10 higher than the levels of NO_3 -nitrogen. The highest levels of nitrogen are not always associated with the higher concentrations of organic matter usually found in the upper horizons of the soil profile. In many soil profiles we have found the nitrogen to be quite uniformly distributed, and zones of accumulation sometimes appear in the B and C horizons, especially under shrub clumps. This indicates that leaching of nitrogen into the soil profile is of ecological significance in desert soils.

From our investigations of nitrogen in desert soils and vegetation, we can make the following suppositions: (1) the standing crop contains about 2 per cent nitrogen, (2) about 2 per cent of the nitrogen in soil organic matter could be delivered to the vegetation per year, and (3) about 25 per cent of the total nitrogen in the crop is needed for annual growth. In an Artemisia community

in the Great Basin-type desert area, about 240 kg of nitrogen per hectare was in the standing crop with an estimated 60 kg of nitrogen per hectare needed annually. The soil organic matter contained approximately 5,000 kg of nitrogen per hectare, and this could deliver around 100 kg of nitrogen annually for plant growth. This represents one of the more fertile systems in the desert ecosystem and probably reflects the nitrogen fixation reactions seen in the Artemisia species. A less fertile system is represented by a Larrea-Franseria community in the Mojave Desert-type area in which the soil organic matter contained approximately 2200 kg of nitrogen per hectare. The standing crop contained about 45 kg of nitrogen per hectare and of this about 10 kg is needed annually for new plant growth. Since the soil organic matter could supply as much as 44 kg of nitrogen per hectare, nitrogen would not be deficient nor would atmospheric nitrogen need be fixed to maintain balance on a short-time basis. The input of nitrogen to soil from rainfall and from symbiotic and free-living microbial endophytes, coupled with the sparsity of natural vegetation and relatively high retention of nitrogen in the desert ecosystem, all lead to an explanation for the apparent lack of nitrogen deficiency in desert vegetation around well-established shrub clumps. The implication is that soil moisture rather than nitrogen is the major limiting factor in primary productivity in this desert ecosystem.

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Table 1. Plant species surveyed for nitrogen fixation by the acetylene reduction method. An x indicates positive detection of the reaction in root-soil samples.

Plant species	Date of sample collection			
	Dec. 9	Feb. 17	Apr. 16	June 12
<u>Acamptopappus shockleyi</u>				
<u>Artemisia arbuscula</u>				
<u>Artemisia spinescens</u>		x		
<u>Artemisia tridentata</u>		x	x	
<u>Astragalus lentiginosus</u>			x	x
<u>Atriplex confertifolia</u>				
<u>Bromus rubens</u>		x	x	
<u>Coleogyne ramosissima</u>		x		
<u>Dalea fremontii</u>		x	x	
<u>Ephedra funerea</u>				
<u>Ephedra nevadensis</u>				
<u>Eriogonum inflatum</u>				
<u>Eurotia lanata</u>				
<u>Franseria dumosa</u>				
<u>Grayia spinosa</u>				
<u>Hilaria rigida</u>				
<u>Hymenoclea salsola</u>		x		
<u>Kochi americana</u>				
<u>Krameria parvifolia</u>		x		
<u>Larrea tridentata</u>				
<u>Lepidium fremontii</u>				
Lichens (not identified)		x		
<u>Lupinus argenteus</u>			x	x
<u>Lycium andersonii</u>				
<u>Lycium pallidum</u>				
<u>Lycium shockleyi</u>				
<u>Oryzopsis hymenoides</u>				
<u>Sphaeralcea ambigua</u>				
<u>Tetradymia canescens</u>		x		
<u>Yucca schidigera</u>				

Table 2. Nitrogen contents in samples of vegetation harvested in 1969 from plots treated with 0 and 100 kg of nitrogen per hectare.

Plant species	Nitrogen content in dry leaf tissue*	
	Control plot	100 kg N/ha
	%	%
<u>Astragalus lentiginosus</u>	3.33	3.07
<u>Atriplex confertifolia</u>	1.70	2.01
<u>Chaenactis carphoclinia</u>	1.38	1.68
<u>Ephedra nevadensis</u>	1.77	2.02
<u>Eurotia lanata</u>	1.66	2.35
<u>Franseria dumosa</u>	1.67	2.30
<u>Grayia spinosa</u>	1.71	1.90
<u>Krameria parvifolia</u>	1.47	2.05
<u>Larrea tridentata</u>	1.70	2.25
<u>Lycium andersonii</u>	2.10	2.23
<u>Lycium pallidum</u>	1.89	2.69
<u>Mentzelia albicaulis</u>	1.42	1.74

*Mean of three replicates from pooled samples of leaves collected at random within plots.

Table 3. New shoot yield and nitrogen content of leaf tissues grown in plots fertilized with nitrogen with and without supplemental moisture.

Plant species	Control		100 kg N/ha		200 kg N/ha	
	Yield*	N	Yield*	N	Yield*	N
	g	%	g	%	g	%
Natural desert conditions						
<u>Acamptopappus shockleyi</u>	6.6	1.9	7.5	2.1	5.5	2.5
<u>Ephedra nevadensis</u>	4.1	2.3	4.4	2.8	4.2	2.8
<u>Eurotia lanata</u>	8.6	2.6	9.9	3.1	10.2	3.1
<u>Franseria dumosa</u>	8.0	3.6	9.3	3.9	7.4	3.9
<u>Grayia spinosa</u>	7.2	2.2	7.9	2.4	7.6	2.4
<u>Krameria parvifolia</u>	1.0	2.0	1.2	2.5	1.1	2.9
<u>Larrea tridentata</u>	2.9	2.8	2.8	4.1	2.9	4.3
<u>Lycium andersonii</u>	3.6	2.2	3.3	2.4	3.4	2.4
Supplemental moisture added						
<u>Acamptopappus shockleyi</u>	7.7	1.8	9.9	2.6	8.1	2.3
<u>Ephedra nevadensis</u>	3.0	1.8	4.1	2.7	3.6	2.6
<u>Eurotia lanata</u>	13.8	2.9	16.3	3.5	14.7	3.1
<u>Franseria dumosa</u>	9.0	3.8	8.0	3.4	8.3	4.1
<u>Grayia spinosa</u>	11.7	2.3	13.2	2.6	10.3	2.6
<u>Krameria parvifolia</u>	1.0	1.9	0.8	2.3	1.0	2.5
<u>Larrea tridentata</u>	3.2	2.7	2.6	3.3	2.9	3.1
<u>Lycium andersonii</u>	4.0	2.2	4.2	2.6	4.1	2.5

*Data are means from three pooled samples consisting of 50 new shoots picked at random from 10 shrubs of each species in each plot.

Figure 1. Gas chromatogram tracings of ethylene production in root-soil samples with common incubation time.