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**FINAL REPORT**

**NITROGEN CYCLING IN GREAT BASIN DESERT SOILS**

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**US/IBP DESERT BIOME  
RESEARCH MEMORANDUM 77-21**

in

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### ABSTRACT

The inputs and losses of nitrogen from Great Basin arid soils were studied using the acetylene reduction and  $^{15}\text{N}$  isotope techniques. Filamentous blue-green algae were observed to be the predominant group in the soil crusts. The bacterial association with this group of algae suggests a phycosphere-like effect, thus allowing heterotrophic nitrogen fixation and denitrification to occur. Up to 10 mg N/100 g soil were found to have been fixed in a 3-wk incubation period; 38.4 mg N/100 g soil were fixed in a 5-wk incubation period. Ammonium sulfate and ammonium sulfate plus plant material amendments reduced the gain in nitrogen by 41-100%. Fifty to sixty percent of the applied  $^{15}\text{NH}_4^+$ -N and  $^{15}\text{NO}_3^-$ -N was denitrified during the first week of incubation while 70-80% of the applied  $^{15}\text{NH}_4^+$ -N was lost in a 3-5 wk incubation period. These data suggest that a potential for heterotrophic nitrogen fixation exists, and under optimal conditions significant gains in soil nitrogen may be achieved. However, in the presence of an allelochemic agent the potential gain in soil nitrogen may be reduced or inhibited. In addition, the denitrification potentials of these soils may also limit the realized input of nitrogen.

The application of protein (casein) to these soils resulted in an ammonification rate of 50-60%. Fixed  $^{15}\text{N}_2$  indicated a 21-48.8% ammonification rate, thus suggesting that the mineralization of  $\text{NH}_4^+$  was the rate-limiting step for nitrogen loss.

Ammonia volatilization accounted for less than a 5% nitrogen loss, regardless of experimental conditions.

The inhibitory effects of plant material, litter extracts and N-Serve on nitrogen fixation have been assessed. The results suggest that the physiological state of the cryptogamic crust organisms may play a role in the effectiveness of the allelopathic inhibitor(s).

### INTRODUCTION

This report describes continuation and conclusion of the efforts under the US/IBP Desert Biome program to describe the characteristics of nitrogen cycling in Great Basin Desert soils. The work on denitrification described here is a direct continuation of that described in the previous Research Memorandum (Skujins 1976). Previous work on nitrogen cycling in these soils has been described in the US/IBP Desert Biome Research Memoranda by Skujins (1972, 1975) and Skujins and West (1973, 1974).

### METHODS

Most of the methods have been described before (Skujins and West 1974; Skujins 1975). Additional methods or modifications of methods are given below.

#### SAMPLING SITES AND SOIL DESIGNATIONS

The Curlew Valley *Artemisia tridentata* (Curlew 5), *Ceratoides lanata* (Curlew 6) and *Atriplex confertifolia* (Curlew 7) sampling sites have been described before (Skujins and West 1973) and the Pine Valley site has been described by Skujins (1976).

#### REDUCTION OF EXOGENOUSLY SUPPLIED $\text{K}^{15}\text{NO}_3$

$\text{K}^{15}\text{NO}_3$  was the substrate used to determine the denitrification potential of the Curlew Valley and Pine Valley soils. The procedure by Tucker and Westerman (1974) was used with a minor modification. Two experimental systems were used in which the soils were amended with nonlabeled  $\text{K}^{15}\text{NO}_3$  and glucose, while in a duplicate set the soils were treated with labeled  $\text{K}^{15}\text{NO}_3$ .

To a 125-ml Erlenmeyer flask, 25 g of surface soil (0-3 cm) and labeled  $^{15}\text{N}$  (Prochem, 97% atom percent excess) were added, and moistened to 25% (vol/wt) with distilled water. In the glucose potentiated soils, a 1.5% glucose solution (0.375 g glucose/25 g soil) was added to each soil. The flasks were covered with cotton plugs and incubated in the dark for 7 days at  $22 \pm 2$  C. Following the incubation period, the soils were immersed in a dry ice-acetone bath and lyophilized, and total N,  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N were determined.

#### $^{15}\text{N}_2$ FIXATION AND CRUST DECOMPOSITION

Intact algal crusts from each of the Curlew Valley and Pine Valley sampling stations were collected from the inter-space soil, 0-3 cm deep. Approximately 10 g of crust were added to a 50-ml Erlenmeyer flask. As a definite soil crust was absent at the Pine Valley site, 1.5 g of crust, scraped from the surface of rocks, was added to 8.5 g of surface (0-3 cm depth) soil. The flasks were capped with injectable serum stoppers. To each flask was inserted a 23-gauge hypodermic needle attached to a 6-inch piece of surgical tubing and attached to a hypodermic needle inserted into a serum-capped 25-ml Erlenmeyer flask containing 5 mg ( $^{15}\text{NH}_4$ )<sub>2</sub>SO<sub>4</sub> (Prochem, 30.9% atom percent excess).

The soils were then moistened to 25% with distilled water and the air was removed by a vacuum pump.  $^{15}\text{N}_2$  was released by injecting 1 ml NaOBr into the flask containing the ( $^{15}\text{NH}_4$ )<sub>2</sub>SO<sub>4</sub>. The amount of injected NaOBr was sufficient to produce 0.35 mg  $^{15}\text{N}_2$  (Bremner 1965). The remaining vacuum was filled by injecting a CO<sub>2</sub>, O<sub>2</sub> and Ar gas mixture (Linde). The flasks were illuminated at 10,000

lux by a fluorescent source. The incubation period was 24 hr at  $22 \pm 2$  C (Skujins and West 1974).

Following the 24-hr fixation period, 1-g soil samples were then retrieved, digested and total N determined. To the remaining flasks, glass tubes (1.0 x 4.5 cm) were placed on the soil surface, and 5 ml of a 0.02 N H<sub>2</sub>SO<sub>4</sub> solution was pipetted into each tube. The flasks were again capped and incubated in the dark at  $22 \pm 2$  C for 1 wk. After incubation, the sulfuric acid was assayed for volatilized NH<sub>3</sub> by Nesslerization. The soils were frozen and lyophilized, and total N, fixed NH<sub>4</sub><sup>+</sup>-N, exchangeable NH<sub>4</sub><sup>+</sup>-N and NO<sub>2</sub><sup>-</sup>- and NO<sub>3</sub><sup>-</sup>-N were determined.

In all of the above <sup>15</sup>N experiments, the samples were analyzed in duplicate, while the non-<sup>15</sup>N controls were run in triplicate.

#### AMMONIFICATION POTENTIAL

The ammonification potential was determined by the method described by Patel (1972). Surface soil (0-3 cm) was added to a 250-ml screw-cap flask containing a center well. Each soil was amended with 125 mg casein (Nutritional Biochemical Co.) and 175 μg N-Serve (2-chloro-6 [trichloromethyl] pyridine, Dow Chemical Co.). The soils were moistened to 25% (vol/wt) and 5 ml of 0.02 N H<sub>2</sub>SO<sub>4</sub> was added to the center well. The flasks were sealed and incubated in the dark at  $22 \pm 2$  C for 1 wk. Following the incubation period, the sulfuric acid solution was analyzed for volatilized NH<sub>3</sub> by Nesslerization. The soils were frozen, lyophilized and assayed for organic N, fixed NH<sub>4</sub><sup>+</sup>-N, exchangeable NH<sub>4</sub><sup>+</sup>-N and NO<sub>2</sub><sup>-</sup>- and NO<sub>3</sub><sup>-</sup>-N. These experiments were all run in triplicate.

#### ACETYLENE REDUCTION, IN SITU

Diurnal, in situ nitrogen fixation was measured at the three Curlew Valley stations and at the Pine Valley sampling station. Within each sampling station, a 144-m<sup>2</sup> grid of 30 samples was established (Skujins 1976). A formaldehyde-treated and a nonmoistened soil core control were included in the field measurements. In all cases, five time periods were used to estimate the rates of fixation (Skujins 1976). In each study, the 24-hr period was characterized by a sunny, clear day. For each time period, the 30 soil cores (2 g soil crust in a 13 x 60 mm glass tube) were moistened with 0.5 ml of distilled water, capped and injected with 0.6 ml acetylene. The formaldehyde control was treated with 0.5 ml of 37% formaldehyde prior to acetylene injection, while the nonmoistened control was capped and injected without any further additions. After the reaction period, a 1-ml gas sample was withdrawn and injected into a sealed 6.5-ml serum bottle and returned to the laboratory for ethylene analysis. For each succeeding reaction period, the serum caps were removed from the top of the soil cores to allow ventilation of the crust. The cores were then recapped and injected with acetylene.

Ethylene assays were determined by a Varian series 1720 gas chromatograph as previously described (Skujins and West 1974).

#### ACETYLENE REDUCTION, LABORATORY MEASUREMENTS

For laboratory measurements of acetylene reduction, 2 g of soil crust were weighed out and placed in a 13 x 60 mm glass tube sealed at one end with a rubber stopper. The cores were moistened with 1 ml of distilled water, capped and injected with 0.6 ml of acetylene. The cores were then illuminated at 10,000 lux for 48 hr at  $22 \pm 2$  C. After the incubation period, a 0.2-ml gas sample was used for ethylene analysis. An ethylene release control soil core was prepared in the same fashion less the injection of acetylene. All assays were run in triplicate.

As a companion experiment to the study of <sup>15</sup>N<sub>2</sub> fixation by algal crust, a similar experimental method was used with acetylene reduction. Algal crust (10 g) was added to a 50-ml Erlenmeyer flask and moistened to 25% soil moisture. Each flask was capped and 3 ml of air were removed followed by a 3-ml injection of acetylene. The crusts were incubated at  $22 \pm 2$  C for 24 hr at 10,000 lux. After incubation, a 0.2-ml sample was removed from each flask and injected into the gas chromatograph for ethylene analysis. The flasks were then transferred to the dark and incubated for 1 wk at  $22 \pm 2$  C. Following the dark incubation, a 0.2-ml gas sample was again taken and used for ethylene analysis.

Two variations of the above procedure were employed. In one experiment following the 24-hr fixation period and assay, the serum stoppers were removed and replaced with cotton and incubated in the dark for 6 days at 22 C. After the sixth day, the flasks were again capped, with the air removed and replaced with acetylene as previously described. The flasks were incubated in the dark at the same temperature for 1 day prior to ethylene assay.

In another experiment following the 24-hr fixation and assay, the serum stoppers were removed and then reinserted for the injection of acetylene. This procedure was followed once each week for a total of 3 wk. The flasks were incubated in the dark at  $22 \pm 2$  C. In addition, 1-g samples of crust were retrieved and plated on mannitol agar plates (Aaronson 1970) prior to the moistening of the algal crusts, and after the 24-hr and 3-wk incubation periods. The dilutions of 10<sup>-4</sup> and 10<sup>-5</sup> (0.05 M phosphate dilution blanks) were plated using the spread-plate technique. All plates were incubated in the dark at room temperature for 1 wk. Following incubation, the colonies were counted and taxonomically examined.

#### NITROGEN FIXATION CALCULATIONS

The surface area of the soil core tubes is estimated to be 1.324 x 10<sup>-8</sup> ha. It is also assumed that nitrogen and acetylene are reduced by nitrogenase in a 3:1 ratio. Thus, 1 nanomole of C<sub>2</sub>H<sub>4</sub> produced equals 28 x 10<sup>-9</sup> g, equivalent to 9.33 x 10<sup>-9</sup> g of fixed nitrogen. The total nanomoles produced from a 0.2-ml gas sample (retrieved from a serum bottle) is the peak height times 30. Therefore:

$$\text{mm (peak height)} \times (1.5 \text{ nm C}_2\text{H}_4 / 100 \text{ mm}) \times 30 \times 9.33 \times 10^{-9} = \text{g of fixed N}$$

Dividing this value by  $1.324 \times 10^{-8}$  ha yields the grams of fixed nitrogen per hectare.

#### EFFECT OF TEMPERATURE ON NITROGEN FIXATION

To determine the effect of temperature on nitrogen fixation, soil crusts from the Curlew Valley and Pine Valley sites were prepared as described above. Following the injection of acetylene, one set of cores (12 total) was incubated at 22 C while a second set was placed in a 37 C incubator. Each sample was run in triplicate and illuminated at 10,000 lux for 48 hr.

#### HETEROTROPHIC NITROGEN FIXATION

To assess the rate of nitrogen fixation by the heterotrophic population, 2-g samples of soil crust were crushed and mixed with a mortar and pestle. The soil was transferred to glass tubes and the following treatments were then performed:

To two sets of soil cores, 0.5 ml of distilled water was added, capped and injected with acetylene. One set was then incubated in the dark at  $22 \pm 2$  C, the other at 37 C, both for 24 hr.

Two additional sets of soil cores were treated with a 2% glucose solution (0.01 g glucose/0.5 ml distilled water), capped and injected with acetylene. The sets were incubated at  $22 \pm 2$  C and at 37 C for 24 hr in the dark.

The third set of cores were moistened with 0.5 ml of distilled water, capped, injected with acetylene and incubated in the dark at  $22 \pm 2$  C for 3 wk.

#### INHIBITION OF NITROGEN FIXATION

To determine the effects of plant material, plant litter and N-Serve on nitrogen fixation, a number of treatments were used.

For plant material, extracts were prepared by grinding 1 g of plant leaves and stems with 20 ml of distilled water for 5 min with a mortar and pestle. The slurry was filtered through a Whatman #5 filter, followed by an additional 30 ml of distilled water.

Plant litter extracts were prepared the same way by grinding 1 g of litter (collected from beneath the canopies) with 20 ml of distilled water for 5 min and filtered through a Whatman #5 filter.

For N-Serve experiments, a stock solution of 14  $\mu$ g N-Serve per 1 ml distilled water was prepared.

The effect of plant material extracts on nitrogen fixation was assessed by the following experimental treatment schemes: To 2 g of algal crust, 0.5 ml of each extract was added to their respective cores, followed by the addition of 0.5 ml distilled water. The cores were then capped, injected and incubated at  $22 \pm 2$  C for 48 hr at a light intensity of 10,000 lux.

To 2 g of ground crust, 0.25 ml of glucose (0.05 g glucose/0.25 ml distilled water) was added, followed by a 0.25-ml

addition of plant material extract. The cores were capped, injected and incubated in the dark at  $22 \pm 2$  C and 37 C for 24 hr.

The effect of plant litter extract or N-Serve on heterotrophic fixation was assessed by the addition of 0.25 ml of extract or N-Serve (14  $\mu$ g/0.25 ml distilled water) to glucose potentiated soil cores. Following the injection of acetylene, the cores were incubated in the dark at  $22 \pm 2$  C for 24 hr.

For photosynthetic fixation, 0.5 ml of plant litter or 0.5 ml of N-Serve was added to the soil cores with crust, followed by an additional 0.5 ml distilled water. The cores were incubated at  $22 \pm 2$  C for 48 hr at a light intensity of 10,000 lux.

The combined effect of plant material extract and N-Serve was assessed by treating the algal crust with 0.5 ml of extract plus 0.5 ml N-Serve prior to acetylene injection. The cores were incubated at  $22 \pm 2$  C at 10,000 lux.

#### ALGAL IDENTIFICATION

For gross observation and identification of algae in the crusts, 15 g of soil crust samples were placed in sterile petri dishes, moistened with distilled water and illuminated at 10,000 lux for 48 hr at room temperature. Wet mounts were observed at a magnification of 400 and 1,000 times. The identification of the algae was based on Prescott's (1970) identification key.

#### C:N RATIO DETERMINATION OF CRYPTOGAMIC CRUSTS

Cryptogamic crusts were retrieved from each of the sampling stations at Curlew Valley during April, July, August and September 1976. The crusts (0-2 cm) were analyzed for organic C and total N by the Soil Test Laboratory, Utah State University, and the C:N ratios were determined.

## RESULTS

#### AMMONIFICATION POTENTIAL

Table 1 summarizes the ammonification potential data. The initial and final values for organic nitrogen,  $\text{NH}_4^+$  and  $\text{NO}_2^-$  and  $\text{NO}_3^-$  are given. It is observed that approximately 50-60% of the applied casein has been ammonified.

#### REDUCTION OF $^{15}\text{NO}_3^-$ ADDED TO SOILS

The data for the nitrate reduction potentials for Curlew Valley and Pine Valley are summarized in Table 2. Approximately 50-67.5% of the applied  $^{15}\text{N}$ -labeled nitrate is denitrified. Most of the remaining  $^{15}\text{N}$  resides in the nitrate form, while 3.3-6.6  $\mu$ g of  $^{15}\text{NO}_3^-$ -N has been reduced to nitrite, and 5-17  $\mu$ g of  $^{15}\text{N}$ -labeled nitrate has been reduced to either  $\text{NH}_4^+$ -N or organic nitrogen.

Table 3 compares the results on the difference between glucose potentiated and nonamended nitrate reduction. When glucose is supplied to the soil, more than 80% of the applied nitrate is denitrified. In nonamended soils, the

**Table 1.** Ammonification potential of Great Basin arid soils (values expressed as  $\mu\text{g N/g soil}$ )

Fraction	Site			
	Curlew Valley			Pine Valley
	Artemisia	Ceratoides	Atriplex	
Initial organic N	1814.4	1856.4	1914.8	1426.0
Final organic N	735.2	885.6	1006.4	538.4
% Ammonification	59.5	52.3	47.4	62.2
Initial $\text{NO}_2^- + \text{NO}_3^- - \text{N}$	2.8	1.6	3.6	0
Final $\text{NO}_2^- + \text{NO}_3^- - \text{N}$	3.6	3.2	4.4	2.4

**Table 2.**  $^{15}\text{NO}_3^-$  reduction at three Curlew Valley sites and at Pine Valley

N fraction	$\mu\text{g } ^{15}\text{N/g soil}$	% Loss $^{15}\text{N}$
<u>Site: Curlew Valley - Artemisia</u>		
Total $^{15}\text{N}$	9.10	
$^{15}\text{NO}_2^-$	3.34	
$^{15}\text{NO}_3^-$	29.97	
Total	42.41	
Initial $^{15}\text{NO}_3^-$	96.8	56.2
<u>Site: Curlew Valley - Ceratoides</u>		
Total $^{15}\text{N}$	5.22	
$^{15}\text{NO}_2^-$	5.08	
$^{15}\text{NO}_3^-$	21.20	
Total	31.5	
Initial $^{15}\text{NO}_3^-$	96.8	67.5
<u>Site: Curlew Valley - Atriplex</u>		
Total $^{15}\text{N}$	4.98	
$^{15}\text{NO}_2^-$	6.19	
$^{15}\text{NO}_3^-$	26.03	
Total	37.2	
Initial $^{15}\text{N}$	96.8	61.6
<u>Site: Pine Valley</u>		
Total $^{15}\text{N}$	17.11	
$^{15}\text{NO}_2^-$	6.52	
$^{15}\text{NO}_3^-$	25.07	
Total	48.7	
Initial $^{15}\text{N}$	96.8	49.7

denitrification loss also is comparatively high and it varies from 50-85%.

#### DENITRIFICATION AND VOLATILIZATION FROM $^{15}\text{NH}_4^+$ AMENDED SOILS

Table 4 gives the  $^{15}\text{NH}_3$  volatilization data for the  $^{15}\text{N}$ -labeled ammonium sulfate treated soils. Essentially all the loss of  $^{15}\text{N}$  due to volatilization occurred during the seventh day of incubation. The percent loss varied from 1.4-4.56% of the applied  $^{15}\text{N}$ .

Tables 5, 6 and 7 list the soil  $^{15}\text{N}$  analysis of the  $(^{15}\text{NH}_4)_2\text{SO}_4$  treated soils. Except for the *Artemisia* soil,

there is first an increase in  $^{15}\text{NO}_3^-$  followed by a rapid decline. Although the *Ceratoides* soil shows little change in the organic  $^{15}\text{N}$  content, the *Atriplex* soil demonstrates a substantial decline. In the *Artemisia* soil,  $^{15}\text{NO}_3^-$  declines during the entire incubation period, while exchangeable  $^{15}\text{NH}_4^+$  increases and then declines. This increase in exchangeable  $^{15}\text{NH}_4^+$  is complemented by the organic  $^{15}\text{N}$  fraction which first declines and then increases. In all three treated soils,  $^{15}\text{NO}_2^-$  does not appear in detectable amounts until the 21st day. The weekly  $^{15}\text{N}$  denitrification loss for the *Artemisia* soil was 60.9, 67.0 and 69.3%; the *Ceratoides* soil, 50.8, 61.4 and 76.0%; the *Atriplex* soil, 64.3, 67.2 and 83.8%. In all cases, the  $^{15}\text{N}$  loss due to denitrification increases with time.

#### DENITRIFICATION AND VOLATILIZATION OF $^{15}\text{NH}_4^+$ FROM AMMONIUM, PLANT MATERIAL AND N-SERVE AMENDED SOILS

Table 8 gives the ammonia volatilization data from soils amended with  $(^{15}\text{NH}_4)_2\text{SO}_4$  plus plant material and N-Serve, and with  $(^{15}\text{NH}_4)_2\text{SO}_4$  plus plant litter. Volatilization loss in the plant material plus N-Serve treatment ranged from 0.13-1.54%, while the plant litter treated soils had a volatilization loss of 0.53% (*Atriplex*) or less. Maximum volatilization losses occurred during the seventh day.

Tables 9 and 10 show analyses of the plant material plus N-Serve and plant litter treated soils. Except for the *Ceratoides* soil (both treatments) there is, in general, a net loss of nitrogen. However, only in the *Atriplex* treated soils is a reduction in the organic nitrogen fraction observed.  $\text{NO}_2^-$  is also detectable, and in the plant material plus N-Serve treated *Artemisia* soil,  $\text{NO}_2^-$  is greater than  $\text{NO}_3^-$ . The soil pH in the *Artemisia* and *Atriplex* soils is observed to have increased, while the *Ceratoides* soils experienced a decline.

Tables 11 and 12 list the volatile ammonia  $^{15}\text{N}$  losses from the plant material plus N-Serve and plant litter treatments. Except for the plant litter amended *Artemisia* soil, the greatest amount of volatilized ammonia occurred during the seventh day of incubation. The plant material plus N-Serve treated soils demonstrated a 2.0-5.67% volatilization loss of the applied  $^{15}\text{N}$  ammonium sulfate, while the plant litter amended soils were lower, with a 0.22-3.38% loss.

Table 3. Denitrification potentials from KNO<sub>3</sub> amended soils

Treatment	Curlew Valley <i>Artemisia</i>	Curlew Valley <i>Ceratoides</i>	Curlew Valley <i>Atriplex</i>	Pine Valley
Glucose (+)	86.0%	83.7%	87.2%	90.2%
Glucose (-)	49.9%	62.9%	67.8%	85.3%

Table 4. <sup>15</sup>N lost as volatilized NH<sub>3</sub> from (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> amended, air dried soils (approximately -15 bars): 21 days (all values expressed as μg <sup>15</sup>NH<sub>3</sub>-N volatilized from 100 g soil)

Time	Site		
	<i>Artemisia</i>	<i>Ceratoides</i>	<i>Atriplex</i>
7 days	11.55	19.45	39.96
14 days	1.06	0.46	0.45
21 days	<u>0.19</u>	<u>0.12</u>	<u>1.15</u>
Total Volatilized	12.80	20.03	41.56
% Volatilized	1.40	2.20	4.56

Table 5. Soil <sup>15</sup>N fractions from (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> amended, *Artemisia* air dried soil (approximately -20 bars): 21 days

N fraction	μg <sup>15</sup> N/100 g soil	Atom % excess <sup>15</sup> N	% Loss <sup>15</sup> N
<u>Time: 1 week</u>			
Exchangeable <sup>15</sup> NH <sub>4</sub> <sup>+</sup> -N	8.8	0.966	
Fixed <sup>15</sup> NH <sub>4</sub> <sup>+</sup> -N	15.9	0.265	
Organic <sup>15</sup> N	71.02	0.882	
<sup>15</sup> NO <sub>2</sub> <sup>-</sup> -N	0.0	0.0	
<sup>15</sup> NO <sub>3</sub> <sup>-</sup> -N	249.1	15.47	
<sup>15</sup> NH <sub>3</sub> -N	<u>11.55</u>	--	
Total	356.4	--	60.9
<u>Time: 2 weeks</u>			
Exchangeable <sup>15</sup> NH <sub>4</sub> <sup>+</sup> -N	20.03	3.18	
Fixed <sup>15</sup> NH <sub>4</sub> <sup>+</sup> -N	11.39	0.178	
Organic <sup>15</sup> N	51.49	0.0765	
<sup>15</sup> NO <sub>2</sub> <sup>-</sup> -N	0.0	0.0	
<sup>15</sup> NO <sub>3</sub> <sup>-</sup> -N	205.61	10.69	
<sup>15</sup> NH <sub>3</sub> -N	<u>12.61</u>	--	
Total	301.3	--	67.0
<u>Time: 3 weeks</u>			
Exchangeable <sup>15</sup> NH <sub>4</sub> <sup>+</sup> -N	5.18	0.673	
Fixed <sup>15</sup> NH <sub>4</sub> <sup>+</sup> -N	20.16	0.318	
Organic <sup>15</sup> N	80.79	0.0935	
<sup>15</sup> NO <sub>2</sub> <sup>-</sup> -N	22.72	1.73	
<sup>15</sup> NO <sub>3</sub> <sup>-</sup> -N	138.43	6.18	
<sup>15</sup> NH <sub>3</sub> -N	<u>12.8</u>	--	
Total	280.1	--	69.3

Table 6. Soil <sup>15</sup>N fractions from (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> amended, *Ceratoides* air dried soil (approximately -10 bars): 21 days

N fraction	μg <sup>15</sup> N/100 g soil	Atom % excess <sup>15</sup> N	% Loss <sup>15</sup> N
<u>Time: 1 week</u>			
Exchangeable <sup>15</sup> NH <sub>4</sub> <sup>+</sup> -N	116.42	27.72	
Fixed <sup>15</sup> NH <sub>4</sub> <sup>+</sup> -N	23.28	0.308	
Organic <sup>15</sup> N	76.43	0.089	
<sup>15</sup> NO <sub>2</sub> <sup>-</sup> -N	0.0	0.0	
<sup>15</sup> NO <sub>3</sub> <sup>-</sup> -N	213.27	10.69	
<sup>15</sup> NH <sub>3</sub> -N	<u>19.45</u>	--	
Total	448.9	--	50.8
<u>Time: 2 weeks</u>			
Exchangeable <sup>15</sup> NH <sub>4</sub> <sup>+</sup> -N	6.80	1.62	
Fixed <sup>15</sup> NH <sub>4</sub> <sup>+</sup> -N	10.93	0.135	
Organic <sup>15</sup> N	77.3	0.0923	
<sup>15</sup> NO <sub>2</sub> <sup>-</sup> -N	0.0	0.0	
<sup>15</sup> NO <sub>3</sub> <sup>-</sup> -N	237.51	7.80	
<sup>15</sup> NH <sub>3</sub> -N	<u>19.91</u>	--	
Total	352.50	--	61.4
<u>Time: 3 weeks</u>			
Exchangeable <sup>15</sup> NH <sub>4</sub> <sup>+</sup> -N	5.23	0.636	
Fixed <sup>15</sup> NH <sub>4</sub> <sup>+</sup> -N	11.52	0.179	
Organic <sup>15</sup> N	73.5	0.0862	
<sup>15</sup> NO <sub>2</sub> <sup>-</sup> -N	9.16	2.18	
<sup>15</sup> NO <sub>3</sub> <sup>-</sup> -N	99.07	2.12	
<sup>15</sup> NH <sub>3</sub> -N	<u>20.03</u>	--	
Total	218.5	--	76.0

**Table 7.** Soil  $^{15}\text{N}$  fractions from  $(^{15}\text{NH}_4)_2\text{SO}_4$  amended, *Atriplex* air dry soils (approximately  $-7$  bars): 21 days

N fraction	$\mu\text{g } ^{15}\text{N}/100 \text{ g soil}$	Atom % excess $^{15}\text{N}$	% Loss $^{15}\text{N}$
<u>Time: 1 week</u>			
Exchangeable $^{15}\text{NH}_4^+-\text{N}$	97.56	10.72	
Fixed $^{15}\text{NH}_4^+-\text{N}$	34.35	0.624	
Organic $^{15}\text{N}$	103.95	0.1335	
$^{15}\text{NO}_2^--\text{N}$	0.0	0.0	
$^{15}\text{NO}_3^--\text{N}$	49.62	--	
$^{15}\text{NH}_3-\text{N}$	39.96	--	
Total	325.4	--	64.3
<u>Time: 2 weeks</u>			
Exchangeable $^{15}\text{NH}_4^+-\text{N}$	8.87	1.49	
Fixed $^{15}\text{NH}_4^+-\text{N}$	19.14	0.35	
Organic $^{15}\text{N}$	78.49	0.1011	
$^{15}\text{NO}_2^--\text{N}$	0.0	0.0	
$^{15}\text{NO}_3^--\text{N}$	152.19	7.37	
$^{15}\text{NH}_3-\text{N}$	40.41	--	
Total	299.1	--	67.2
<u>Time: 3 weeks</u>			
Exchangeable $^{15}\text{NH}_4^+-\text{N}$	5.5	1.31	
Fixed $^{15}\text{NH}_4^+-\text{N}$	22.92	0.383	
Organic $^{15}\text{N}$	21.26	0.0301	
$^{15}\text{NO}_2^--\text{N}$	3.28	1.44	
$^{15}\text{NO}_3^--\text{N}$	53.09	1.97	
$^{15}\text{NH}_3-\text{N}$	41.56	--	
Total	147.6	--	83.8

**Table 8.** Ammonia volatilization from  $(^{15}\text{NH}_4)_2\text{SO}_4$  amended, air dried soils ( $-7$  to  $-35$  bars) treated with either fresh plant material plus N-Serve or plant litter: 21 days (all values expressed as  $\mu\text{g NH}_3-\text{N}$  volatilized from 100 g soil)

Time	Site		
	Artemisia	Ceratoides	Atriplex
<u><math>(^{15}\text{NH}_4)_2\text{SO}_4</math> plus plant material plus N-Serve</u>			
7 days	74.2	1003.1	1280.3
14 days	23.1	287.7	303.1
21 days	14.7	35.7	47.6
Total Volatilized	112.0	1326.5	1631.0
% Loss	0.13	1.43	1.54
<u><math>(^{15}\text{NH}_4)_2\text{SO}_4</math> plus plant litter</u>			
7 days	62.3	231.0	295.4
14 days	23.1	137.2	137.2
21 days	14.0	42.0	43.4
Total Volatilized	99.4	410.2	476.0
% Loss	0.13	0.41	0.53

**Table 9.** Soil analysis of organic N, total  $\text{NH}_4^+-\text{N}$ , and  $\text{NO}_2^-$ - and  $\text{NO}_3^-$ -N for  $(^{15}\text{NH}_4)_2\text{SO}_4$  plus plant material plus N-Serve amended, air dry soils ( $-7$  to  $-35$  bars): 21 days (values expressed as  $\mu\text{g N/g soil}$ )

Fraction	Site		
	Artemisia	Ceratoides	Atriplex
<u>Initial content</u>			
Exchangeable $\text{NH}_4^+-\text{N}$	6.3	0.0	2.8
Fixed $\text{NH}_4^+-\text{N}$	67.9	77.35	67.32
Organic N	643.07	683.32	820.28
$\text{NO}_2^- + \text{NO}_3^-$ -N	8.87	8.17	6.07
*Total N	894.0	929.0	1057.0
pH	8.10	8.40	8.81
<u>Soil sample time: 21 days</u>			
Exchangeable $\text{NH}_4^+-\text{N}$	3.5	3.85	3.15
Fixed $\text{NH}_4^+-\text{N}$	64.83	74.67	74.78
Organic N	689.07	808.03	753.67
$\text{NO}_2^-$ -N	4.2	13.3	0.0
$\text{NO}_3^-$ -N	0.0	83.65	53.67
$\text{NH}_3$ -N	112.0	1326.5	1631.0
Total N	763.0	997.0	902.0
pH	8.27	8.20	8.80

\*Total N includes 3.0 mg of added  $(^{15}\text{NH}_4)_2\text{SO}_4$  + plant nitrogen (1% amendment).

**Table 10.** Soil analysis of organic N, total  $\text{NH}_4^+-\text{N}$ , and  $\text{NO}_2^-$ - and  $\text{NO}_3^-$ -N for  $(^{15}\text{NH}_4)_2\text{SO}_4$  plus plant litter amended, air dry soils ( $-7$  to  $-10$  bars): 21 days (values expressed as  $\mu\text{g N/g soil}$ )

Fraction	Site		
	Artemisia	Ceratoides	Atriplex
<u>Initial content</u>			
Exchangeable $\text{NH}_4^+-\text{N}$	8.05	1.05	6.07
Fixed $\text{NH}_4^+-\text{N}$	73.33	76.54	65.98
Organic N	678.36	755.89	830.49
$\text{NO}_2^- + \text{NO}_3^-$ -N	4.67	7.94	3.04
*Total N	899.0	1007.0	1142.0
pH	8.19	8.31	8.74
<u>Soil sample time: 21 days</u>			
Exchangeable $\text{NH}_4^+-\text{N}$	3.5	6.48	7.18
Fixed $\text{NH}_4^+-\text{N}$	111.65	74.03	72.65
Organic N	737.8	855.58	746.0
$\text{NO}_2^-$ -N	11.38	33.95	7.88
$\text{NO}_3^-$ -N	32.55	61.08	19.43
$\text{NH}_3$ -N	99.4	410.2	476.0
Total N	898.0	1035.0	858.0
pH	8.23	8.25	8.85

\*Total N includes 3.0 mg of added  $(^{15}\text{NH}_4)_2\text{SO}_4$  + plant nitrogen (1% amendment).



**Table 11.**  $^{15}\text{N}$  lost as volatilized  $\text{NH}_3$  from  $(^{15}\text{NH}_4)_2\text{SO}_4$  plus plant material plus N-Serve amended, air dried soils ( $-7$  to  $-35$  bars): 21 days (all values expressed as  $\mu\text{g } ^{15}\text{NH}_3\text{-N}$  volatilized from 100 g soil)

Time	Site		
	<i>Artemisia</i>	<i>Ceratoides</i>	<i>Atriplex</i>
7 days	13.52	38.98	48.88
14 days	2.30	2.47	
21 days	0.95	0.65	0.38
Total Volatilized	18.2	41.9	51.7
% Volatilized	2.0	4.59	5.67

**Table 12.**  $^{15}\text{N}$  lost as volatilized  $\text{NH}_3$  from  $(^{15}\text{NH}_4)_2\text{SO}_4$  plus plant litter amended, air dried soils ( $-7$  to  $-10$  bars): 21 days (all values expressed as  $\mu\text{g } ^{15}\text{NH}_3\text{-N}$  volatilized from 100 g soil)

Time	Site		
	<i>Artemisia</i>	<i>Ceratoides</i>	<i>Atriplex</i>
7 days	1.02	27.21	32.63
14 days	2.66	2.53	2.48
21 days	0.38	0.56	0.27
Total Volatilized	2.03	30.3	35.38
% Volatilized	0.22	3.32	3.38

**Table 13.** Soil  $^{15}\text{N}$  fractions from  $(^{15}\text{NH}_4)_2\text{SO}_4$  plus plant material plus N-Serve amended, air dried soils ( $-7$  to  $-35$  bars): 21 days

N fraction	$\mu\text{g } ^{15}\text{N}/100 \text{ g soil}$	Atom % excess $^{15}\text{N}$	% Loss $^{15}\text{N}$
<b>Site: <i>Artemisia</i></b>			
Exchangeable $^{15}\text{NH}_4^+\text{-N}$	5.22	1.49	
Fixed $^{15}\text{NH}_4^+\text{-N}$	35.79	0.552	
Organic $^{15}\text{N}$	94.61	0.1373	
$^{15}\text{NO}_2^-\text{-N}$	1.23	0.330	
$^{15}\text{NO}_3^-\text{-N}$	1.43	3.04	
$^{15}\text{NH}_3\text{-N}$	18.2	--	
Total	156.5	--	82.8
<b>Site: <i>Ceratoides</i></b>			
Exchangeable $^{15}\text{NH}_4^+\text{-N}$	20.71	5.38	
Fixed $^{15}\text{NH}_4^+\text{-N}$	7.22	0.092	
Organic $^{15}\text{N}$	58.91	0.072	
$^{15}\text{NO}_2^-\text{-N}$	8.03	0.604	
$^{15}\text{NO}_3^-\text{-N}$	5.61	0.067	
$^{15}\text{NH}_3\text{-N}$	41.9	--	
Total	142.4	--	84.4
<b>Site: <i>Atriplex</i></b>			
Exchangeable $^{15}\text{NH}_4^+\text{-N}$	2.48	0.786	
Fixed $^{15}\text{NH}_4^+\text{-N}$	23.56	0.315	
Organic $^{15}\text{N}$	57.96	0.0769	
$^{15}\text{NO}_2^-\text{-N}$	0.0	0.0	
$^{15}\text{NO}_3^-\text{-N}$	43.69	0.814	
$^{15}\text{NH}_3\text{-N}$	51.7	--	
Total	179.4	--	80.3

**Table 14.** Soil  $^{15}\text{N}$  fractions from  $(^{15}\text{NH}_4)_2\text{SO}_4$  plus plant litter amended, air dried soils ( $-7$  to  $-10$  bars): 21 days

N fraction	$\mu\text{g } ^{15}\text{N}/100 \text{ g soil}$	Atom % excess $^{15}\text{N}$	% Loss $^{15}\text{N}$
<b>Site: <i>Artemisia</i></b>			
Exchangeable $^{15}\text{NH}_4^+\text{-N}$	23.1	6.60	
Fixed $^{15}\text{NH}_4^+\text{-N}$	26.57	0.238	
Organic $^{15}\text{N}$	80.42	0.109	
$^{15}\text{NO}_2^-\text{-N}$	0.0	0.0	
$^{15}\text{NO}_3^-\text{-N}$	165.35	5.08	
$^{15}\text{NH}_3\text{-N}$	2.03	--	
Total	297.47	--	67.4
<b>Site: <i>Ceratoides</i></b>			
Exchangeable $^{15}\text{NH}_4^+\text{-N}$	8.36	1.29	
Fixed $^{15}\text{NH}_4^+\text{-N}$	48.64	0.657	
Organic $^{15}\text{N}$	89.49	0.1046	
$^{15}\text{NO}_2^-\text{-N}$	0.68	0.020	
$^{15}\text{NO}_3^-\text{-N}$	101.39	1.66	
$^{15}\text{NH}_3\text{-N}$	30.3	--	
Total	278.86	--	69.4
<b>Site: <i>Atriplex</i></b>			
Exchangeable $^{15}\text{NH}_4^+\text{-N}$	8.69	1.21	
Fixed $^{15}\text{NH}_4^+\text{-N}$	33.64	0.463	
Organic $^{15}\text{N}$	59.68	0.080	
$^{15}\text{NO}_2^-\text{-N}$	3.86	0.49	
$^{15}\text{NO}_3^-\text{-N}$	41.97	2.16	
$^{15}\text{NH}_3\text{-N}$	35.38	--	
Total	183.22	--	79.9

Tables 13 and 14 list the  $^{15}\text{N}$  analysis of the plant material plus N-Serve and plant litter amended soils. The organic nitrogen fraction of the plant material plus N-Serve treated soils has the greatest amount of  $^{15}\text{N}$ , although it has the lowest percent enrichment.  $^{15}\text{NO}_3^-$  has the highest percent enrichment in the *Artemisia* and *Atriplex* soils but not in the *Ceratoides* soil. Rather,  $^{15}\text{NO}_2^-$  is greater than  $^{15}\text{NO}_3^-$  in both amount and concentration of  $^{15}\text{N}$ , and the exchangeable  $\text{NH}_4^+$  fraction has the greatest percent enrichment. The average percent loss of the applied  $^{15}\text{N}$  due to denitrification was 82.5.

The  $^{15}\text{N}$  analysis of the plant litter amended soils shows that the greatest amount and concentration of  $^{15}\text{N}$  is in the nitrate. However, the percent enrichment of the exchangeable  $^{15}\text{NH}_4^+$  is greater than  $^{15}\text{NO}_3^-$  in the *Artemisia* soil. Organic  $^{15}\text{N}$  follows the nitrate fraction, but again has the lowest percent enrichment. The average percent loss of  $^{15}\text{N}$  in this treatment scheme was 72.2.

Table 15 is the total nitrogen balance sheet for all of the 21-day  $^{15}\text{N}$  experiments. As in the previous nitrogen balance sheets (Tables 31 and 35, Skujins 1976) the initial total nitrogen and the final total nitrogen are given. The amount of biologically fixed or denitrified nitrogen and their corresponding percentages are also included. In all cases the reported values for fixation and denitrification are significant as the standard deviation,  $\bar{s}$ , for the determination of total nitrogen is either added to or subtracted from the difference between the initial and final total nitrogen.

Table 15, set 1. Total N balance sheet for untreated soils: 35 days (all values expressed in mg N)

	Air Dry			-1 Bar			-15 Bars		
	Site			Site			Site		
	Art.	Cer.	Atp.	Art.	Cer.	Atp.	Art.	Cer.	Atp.
Initial total N	78.7	79.9	89.4	72.4	79.2	79.5	72.4	79.2	79.5
Final total N	109.5	125.8	104.2	84.2	89.0	88.1	73.8	84.3	99.7
mg of N denitrified	0	0	0	0	0	0	none	none	0
% denitrified	0	0	0	0	0	0	none	none	0
mg N <sub>2</sub> fixed	23.3	38.4	7.3	4.3	2.3	1.1	none	none	12.7
% N <sub>2</sub> fixed	29.6	30.5	8.2	5.9	2.9	1.4	none	none	16.0

Initial total N = Organic N + clay-fixed NH<sub>4</sub><sup>+</sup> + exchangeable NH<sub>4</sub><sup>+</sup> + NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup> per 100 g soil.

Final total N = Organic N + clay-fixed NH<sub>4</sub><sup>+</sup> + exchangeable NH<sub>4</sub><sup>+</sup> + NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup> per 100 g soil.

N<sub>2</sub> fixed = Heterotrophic nitrogen fixation.

Values reported for denitrification or nitrogen fixation are corrected by subtraction of  $\bar{s}$  (7.5 mg N/100 g soil) from the difference between the initial and final total N.

Art. = *Artemisia*; Cer. = *Ceratoides*; Atp. = *Atriplex*.

Table 15, set 2. Total N balance sheet for untreated and (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> treated soils: 21 days (all values expressed in mg N)

	Air Dry (approx. -15 bars)			<sup>15</sup> NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> - Air Dry (approx. -15 bars)		
	Site			Site		
	Art.	Cer.	Atp.	Art.	Cer.	Atp.
Initial total N	75.93	79.42	75.87	90.78	90.90	87.87
Final total N	89.77	90.24	93.37	100.15	100.84	83.39
mg of N denitrified	0	0	0	0	0	none
% denitrified	0	0	0	0	0	none
mg N <sub>2</sub> fixed	6.4	3.3	10.0	1.9	2.4	none
% N <sub>2</sub> fixed	8.4	4.2	13.2	2.1	2.6	none

Initial total N = Organic N + clay-fixed NH<sub>4</sub><sup>+</sup> + exchangeable NH<sub>4</sub><sup>+</sup> + NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup> + 3.0 mg (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. Plant material amendments include 1 g plant material per 100 g soil.

Table 15, set 3. Total N balance sheet for (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> treated soils: 35 days (all values expressed in mg N)

	<sup>15</sup> NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> - Air Dry			<sup>15</sup> NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> at -1 Bar			<sup>15</sup> NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> at -15 Bars		
	Site			Site			Site		
	Art.	Cer.	Atp.	Art.	Cer.	Atp.	Art.	Cer.	Atp.
Initial total N	80.7	82.9	92.4	120.9	99.7	102.4	120.9	99.7	92.4
Final total N	99.4	113.0	115.9	116.0	101.9	87.0	125.2	106.7	87.6
mg of N denitrified	0	0	0	none	none	7.9	none	none	none
% denitrified	0	0	0	none	none	7.7	none	none	none
mg N <sub>2</sub> fixed	11.2	22.6	16.0	none	none	0	none	none	none
% N <sub>2</sub> fixed	13.9	27.3	17.3	none	none	0	none	none	none

Initial total N = Organic N + clay-fixed NH<sub>4</sub><sup>+</sup> + exchangeable NH<sub>4</sub><sup>+</sup> + NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup> per 100 g soil + 3.0 mg (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.

Table 15, set 4. Total N balance sheet for (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> plus plant material plus N-Serve, and (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> plus plant litter treated soils: 21 days (all values expressed in mg N)

	<sup>15</sup> NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + plant material + N-Serve			<sup>15</sup> NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + plant litter		
	Site			Site		
	Art.	Cer.	Atp.	Art.	Cer.	Atp.
Initial total N	89.4	92.9	105.7	89.9	100.7	114.2
Final total N	76.4	101.0	91.8	89.9	103.9	86.3
mg of N denitrified	5.5	0	6.4	none	none	20.4
% denitrified	6.2	0	6.1	none	none	17.9
mg N <sub>2</sub> fixed	0	0.6	0	none	none	0
% N <sub>2</sub> fixed	0	0.7	0	none	none	0

Initial total N = Organic N + clay-fixed NH<sub>4</sub><sup>+</sup> + exchangeable NH<sub>4</sub><sup>+</sup> + NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup> + 3.0 mg (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. Plant material or plant litter amendments include 1 g plant sample per 100 g soil.

The 21-day air dried (approximately —15 bars) soils again show significant gains in nitrogen. It is also interesting to note that the amount of nitrogen gain in these soils is less than the air dried (approximately —15 bars) soils incubated for 35 days. This represents a 72.5% reduction for the *Artemisia* soil and a 91% reduction for the *Ceratoides* soil. As in the 35-day experiments, the nitrogen gain in the ammonium sulfate treated soils is less than in the untreated, air dried (approximately —15 bars) soils. The percent reduction in nitrogen gain for the *Artemisia*, *Ceratoides* and *Atriplex* treated soils is 70, 27 and 100, respectively. In the plant material and N-Serve treated soils, a net loss of nitrogen occurs in all cases. In addition, greater losses are observed to have occurred in the N-Serve treated soil. In the plant material plus N-Serve treated soils, losses in nitrogen are again found to occur in the *Artemisia* and *Atriplex* soils. The *Ceratoides* soil, however, shows a slight gain. The plant litter amended soils show only a net loss of nitrogen in the *Atriplex* soil, while the *Artemisia* and *Ceratoides* soils show neither significant gains nor losses in nitrogen.

#### $^{15}\text{N}_2$ FIXATION BY SOIL CRUSTS

Table 16 gives the  $^{15}\text{N}_2$  fixation data by the Curlew Valley and Pine Valley crusts. In this particular test the Curlew Valley *Ceratoides* site crust had fixed the greatest amount of  $^{15}\text{N}$  (5.15  $\mu\text{g}$ ), while the Pine Valley crust had fixed the least, 0.30  $\mu\text{g}$   $^{15}\text{N}$  per sample.

#### $^{15}\text{N}$ FLUX IN SOIL CRUST DECOMPOSITION

Table 17 gives the  $^{15}\text{N}$  analysis of the crusts following a postfixation incubation in the dark. Most of the fixed  $^{15}\text{N}$  resides in the organic fraction, while the presence of  $^{15}\text{N}$  in the  $\text{NO}_2^-$  and  $\text{NO}_3^-$  fractions was undetectable. However, the Curlew Valley *Artemisia* site soil experienced the greatest  $^{15}\text{N}$  loss due to denitrification (38.4%), while the Pine Valley soil had the least (13.3%).

#### PHOTOAUTOTROPHIC NITROGEN FIXATION (ACETYLENE REDUCTION)

Table 18 summarizes the photoautotrophic fixation potential by soil crusts. As can be seen, most of the photoautotrophic fixation potentials occur at 22 C from crust sampled during the wet season. Little activity, however, is detectable when the crusts are incubated at 37 C.

#### IN SITU NITROGEN FIXATION (ACETYLENE REDUCTION)

Tables 19 through 22 illustrate the in situ acetylene reduction data for Curlew Valley and Pine Valley. Considerable activity occurs during the night, while the daytime measurements vary. Pine Valley did not have measurable activity during the day, while each of the Curlew Valley sites demonstrated daytime activities at different time intervals. The *Artemisia* site had its maximum ethylene production from 0630 to 0800 hr; *Ceratoides* from 0830 to 1000 hr; and *Atriplex* from 1030 to 1200 hr.

#### GLUCOSE POTENTIATED HETEROTROPHIC FIXATION (ACETYLENE REDUCTION)

The glucose potentiated nitrogen fixation data are summarized in Table 23. In all three Curlew Valley soils,

Table 16.  $^{15}\text{N}_2$  fixation by soil crust

Site	$\mu\text{g } ^{15}\text{N}_2 \text{ fixed/g soil}^{-1} \cdot 9.62 \text{ cm}^{-2} \cdot 24 \text{ hr}^{-1}$
Curlew Valley - <i>Artemisia</i>	1.33
Curlew Valley - <i>Ceratoides</i>	5.15
Curlew Valley - <i>Atriplex</i>	0.41
Pine Valley	0.30

Table 17.  $^{15}\text{N}$  flux in soil crust decomposition

N fraction	$\mu\text{g } ^{15}\text{N/g soil}$	% Loss $^{15}\text{N}$
Site: Curlew Valley - <i>Artemisia</i>		
Organic $^{15}\text{N}$	0.69	
Total $^{15}\text{NH}_4^+$	0.13	
$^{15}\text{NO}_2^- + ^{15}\text{NO}_3^-$	0.0	
Total	0.82	38.4
Site: Curlew Valley - <i>Ceratoides</i>		
Organic $^{15}\text{N}$	4.07	
Total $^{15}\text{NH}_4^+$	0.29	
$^{15}\text{NO}_2^- + ^{15}\text{NO}_3^-$	0.0	
Total	4.36	15.3
Site: Curlew Valley - <i>Atriplex</i>		
Organic $^{15}\text{N}$	0.23	
Total $^{15}\text{NH}_4^+$	0.10	
$^{15}\text{NO}_2^- + ^{15}\text{NO}_3^-$	0.0	
Total	0.33	19.5
Site: Pine Valley		
Organic $^{15}\text{N}$	0.21	
Total $^{15}\text{NH}_4^+$	0.05	
$^{15}\text{NO}_2^- + ^{15}\text{NO}_3^-$	0.0	
Total	0.26	13.3

greater activity occurs at 22 C than at 37 C. However, the reverse is true for the Pine Valley soil.

#### ACETYLENE REDUCTION: LIGHT VS. DARK AEROBIC INCUBATION

The effect of a postfixation incubation in the dark on soil crusts under aerobic conditions is given in Table 24. None of the soil crusts reduced acetylene when incubated under aerobic conditions.

#### ACETYLENE REDUCTION: LIGHT VS. DARK ANAEROBIC INCUBATION

Table 24 summarizes the data for a post-light fixation incubation of soil crusts in the dark. It is noted that in both of the *Ceratoides* and *Atriplex* soils, considerable acetylene reduction had occurred during the dark incubation period. However, the *Artemisia* soil demonstrated no nitrogen fixation capabilities during the same incubation period.

Table 18. Photoautotrophic nitrogen fixation by soil crust (all values expressed in  $\text{nm C}_2\text{H}_4 \cdot 1.33 \text{ cm}^{-2} \cdot 48 \text{ hr}^{-1}$ )

Site	22 C		37 C
	Dry season*	Wet season*	
Curlew Valley - <u>Artemisia</u>	0.353	2.99	0
Curlew Valley - <u>Ceratoides</u>	1.96	38.9	0.004
Curlew Valley - <u>Atriplex</u>	0.203	11.9	0.021
Pine Valley	0.148	--	0.015

\*Indicates time of sampling.

Table 19. In situ acetylene reduction at the Artemisia site, Curlew Valley, Utah, July 19-20, 1976

Time	nm C <sub>2</sub> H <sub>4</sub> Produced			Soil Temp. °C	Air Temp. °C
	Wetted soil	Field moisture control	Formaldehyde control		
2030-0630	0	0.742	0	31	21
0630-0800	0	2.992	0	20	15
0800-1000	0	0.203	0	19	28
1000-1200	0	0.075	0	22	34
1200-1400	0	0.083	0	31	37
$\text{gN}_2 \text{ fixed} \cdot \text{ha}^{-1} \cdot 24 \text{ hr}^{-1}$	0	86.570	0		

Table 20. In situ acetylene reduction at the Ceratoides site, Curlew Valley, Utah, July 30-31, 1976

Time	nm C <sub>2</sub> H <sub>4</sub> Produced			Soil Temp. °C	Air Temp. °C
	Wetted soil	Field moisture control	Formaldehyde control		
2000-0630	0.033	0	0	32	28.5
0630-0830	0	0	0	20	15
0830-1000	0.006	0	0	24	25
1000-1200	0.003	0	0	24.5	32
1200-1400	0	0	0	35	36
$\text{gN}_2 \text{ fixed} \cdot \text{ha}^{-1} \cdot 24 \text{ hr}^{-1}$	0.89	0	0		

Table 21. In situ acetylene reduction at the Atriplex site, Curlew Valley, Utah, August 4-5, 1976

Time	nm C <sub>2</sub> H <sub>4</sub> Produced			Soil Temp. °C	Air Temp. °C
	Wetted soil	Field moisture control	Formaldehyde control		
2030-0630	0.61	0.008	0	27	19
0600-0830	0.01	0.019	0.015	15	6
0830-1030	0.002	0.019	0.027	18	23
1030-1200	0.831	0	0	24	29
$\text{gN}_2 \text{ fixed} \cdot \text{ha}^{-1} \cdot 24 \text{ hr}^{-1}$	30.71	0.97	0.89		

Table 22. In situ acetylene reduction at Pine Valley, Utah, May 14-15, 1976

Time	nm C <sub>2</sub> H <sub>4</sub> Produced			Soil Temp. °C	Air Temp. °C
	Wetted soil	Field moisture control	Formaldehyde control		
2030-0600	0.902	0	0	26	26
0600-0800	0	0	0	10	10
0800-1000	0	0	0	15	18
1000-1200	0	0	0	23	19
1200-1400	0	0	0	30	22
$\text{gN}_2 \text{ fixed} \cdot \text{ha}^{-1} \cdot 24 \text{ hr}^{-1}$	3.04	0	0		

**Table 23.** Glucose potentiated heterotrophic nitrogen fixation (all values expressed as  $\text{nm C}_2\text{H}_4 \cdot 2 \text{ g soil}^{-1} \cdot 24 \text{ hr}^{-1}$ )

Site	22 C		37 C	
	glucose	- glucose	glucose	- glucose
Curlew Valley - <u>Artemisia</u>	0.339	0.021	0.024	0
Curlew Valley - <u>Ceratoides</u>	0.40	0.019	0.167	0
Curlew Valley - <u>Atriplex</u>	0.924	0.015	0.772	0.004
Pine Valley	0.018	0	0.75	0

**Table 24.** Acetylene reduction by algal crust

Curlew Valley Site	$\text{nm C}_2\text{H}_4 / 1.33 \text{ cm}^2$			
	Light, 24 hr		Dark, 7 days	
	Aerobic		Anaerobic	
<u>Artemisia</u>	11.64	0	0.965	0
<u>Ceratoides</u>	6.38	0	0.067	0.613
<u>Atriplex</u>	1.71	0	0.0003	0.237

#### ACETYLENE REDUCTION: LIGHT VS. DARK, AEROBIC/ANAEROBIC INCUBATION

The results from an aerobic/anaerobic postfixation incubation of soil crusts in the dark are summarized in Table 25. Limited acetylene reduction occurred in the first week, but significant activity did occur during the second and third weeks, particularly with the *Ceratoides* soil. The *Artemisia* and *Atriplex* soils, however, showed little to no activity by the third week.

#### ACETYLENE REDUCTION BY GROUND SOIL CRUST DURING A DARK INCUBATION PERIOD

Table 26 illustrates the data for the acetylene reduction potential by ground soil crust when incubated in the dark for 3 wk. Considerable activity has occurred in each of the three soils. It was estimated that 1-2  $\mu\text{g N}$  per 100 g of soil may be potentially fixed.

#### INHIBITION OF GLUCOSE POTENTIATED FIXATION BY PLANT MATERIAL EXTRACTS

The allelopathic inhibition on glucose potentiated nitrogen fixation is apparent in Table 27. For the Curlew Valley soils, a 46.8-90.8% inhibition of nitrogen fixation is observed to have occurred at 22 C, while a 76-97% inhibition occurred at 37 C. However, the *Artemisia* soil failed to show any reduction in fixation potential at this temperature. The Pine Valley soils show a 44.4-100% inhibition at 22 C but a 43.9-97.3% reduction at 37 C. Again, the soil receiving treatment with *Artemisia* extract experienced less inhibitory influences at 37 C than at 22 C.

#### INHIBITION OF PHOTOAUTOTROPHIC NITROGEN FIXATION BY PLANT MATERIAL EXTRACTS

The effect of plant material extract on nitrogen fixation

by soil crust is given in Table 28. It is apparent that a greater inhibition by the plant material extract has occurred with crust sampled during the dry season. However, a 46.9% inhibition occurred due to the *Atriplex* extract on the crust sampled from the wet season.

#### INHIBITION OF PHOTOAUTOTROPHIC NITROGEN FIXATION BY PLANT LITTER EXTRACTS

The influence of plant litter extract on the fixation potential by soil crusts is given in Table 29. It is observed that no inhibitions have occurred in the dry season crust, but a 16.1 and a 43.9% inhibition are found to occur in the wet season crust by *Ceratoides* and *Atriplex* litter, respectively.

#### INHIBITION OF PHOTOAUTOTROPHIC NITROGEN FIXATION BY N-SERVE

The effect of N-Serve on the nitrogen fixation potential by soil crusts is given in Table 30. A greater inhibition on acetylene reduction has occurred with the dry season crust than for the wet season crust. The percent inhibition for the former ranges from 63.2-95.8%, while the latter is negligible.

#### INHIBITION OF PHOTOAUTOTROPHIC NITROGEN FIXATION BY N-SERVE PLUS PLANT MATERIAL EXTRACT

The combined effect of N-Serve plus plant material extract is summarized in Table 31. A greater inhibition by the N-Serve plus plant material extract occurred with the dry season crust than with the wet season crust. For the former the inhibition is 73-93.2%, and for the latter, 0-81.7%.

**Table 25.** Acetylene reduction by algal crust: light vs. dark, aerobic/anaerobic incubation

NM C <sub>2</sub> H <sub>4</sub>	Site: Curlew Valley		
	<i>Artemisia</i>	<i>Ceratooides</i>	<i>Atriplex</i>
24 hours	0.153	1.820	0.327
7 days	0.06	0.063	0.042
14 days	0.07	0.11	0.054
21 days	0.003	0.173	0

**Table 26.** Acetylene reduction by ground soil crust incubated in the dark for 3 wk

Sample	nm C <sub>2</sub> H <sub>4</sub> /2 g	µg N <sub>2</sub> Fixed/100 g
<u><i>Artemisia</i></u>	0.699	0.98
<u><i>Ceratooides</i></u>	0.969	1.36
<u><i>Atriplex</i></u>	1.278	1.79

**Table 27.** Inhibition of glucose potentiated heterotrophic nitrogen fixation by plant material extract

Site	22° C		37° C	
	nm C <sub>2</sub> H <sub>4</sub>	% Inhibition	nm C <sub>2</sub> H <sub>4</sub>	% Inhibition
Curlew Valley - <u><i>Artemisia</i></u>	0.059	82.6	0.037	0
Curlew Valley - <u><i>Ceratooides</i></u>	0.213	46.8	0.04	76
Curlew Valley - <u><i>Atriplex</i></u>	0.085	90.8	0.023	97
Pine Valley - <u><i>Artemisia</i></u>	0	100.0	0.421	43.9
Pine Valley - <u><i>Ceratooides</i></u>	0.01	44.4	0.325	56.7
Pine Valley - <u><i>Atriplex</i></u>	0.002	88.9	0.02	97.3

**Table 28.** Inhibition of photoautotrophic nitrogen fixation by plant material extract

Site	Dry Season		Wet Season	
	nm C <sub>2</sub> H <sub>4</sub>	% Inhibition	nm C <sub>2</sub> H <sub>4</sub>	% Inhibition
Curlew Valley - <u><i>Artemisia</i></u>	0	100	13.49	0
Curlew Valley - <u><i>Ceratooides</i></u>	0	100	74.24	0
Curlew Valley - <u><i>Atriplex</i></u>	0	100	6.32	46.9
Pine Valley - <u><i>Artemisia</i></u>	0.015	89.9	N.D.*	N.D.
Pine Valley - <u><i>Ceratooides</i></u>	0.022	85.0	N.D.	N.D.
Pine Valley - <u><i>Atriplex</i></u>	0.077	50.0	N.D.	N.D.

\*N.D. = No data.

**Table 29.** Inhibition of photoautotrophic nitrogen fixation by plant litter extract

Site	Dry Season		Wet Season	
	nm C <sub>2</sub> H <sub>4</sub>	% Inhibition	nm C <sub>2</sub> H <sub>4</sub>	% Inhibition
Curlew Valley - <u><i>Artemisia</i></u>	1.67	0	10.99	0
Curlew Valley - <u><i>Ceratooides</i></u>	2.13	0	32.64	16.1
Curlew Valley - <u><i>Atriplex</i></u>	1.25	0	6.68	43.9

**Table 30.** Inhibition of photoautotrophic nitrogen fixation by N-Serve

Site	Dry Season		Wet Season	
	nm C <sub>2</sub> H <sub>4</sub>	% Inhibition	nm C <sub>2</sub> H <sub>4</sub>	% Inhibition
Curlew Valley - <u><i>Artemisia</i></u>	0.13	63.2	5.22	0
Curlew Valley - <u><i>Ceratooides</i></u>	0.083	95.8	38.41	1.3
Curlew Valley - <u><i>Atriplex</i></u>	0.013	93.6	13.74	0

**Table 31.** Inhibition of photoautotrophic nitrogen fixation by N-Serve plus plant material extract

Site	Dry Season		Wet Season	
	nm C <sub>2</sub> H <sub>4</sub>	% Inhibition	nm C <sub>2</sub> H <sub>4</sub>	% Inhibition
Curlew Valley - <i>Artemisia</i>	0.024	93.2	7.79	0
Curlew Valley - <i>Ceratoides</i>	0.53	73.0	7.12	81.7
Curlew Valley - <i>Atriplex</i>	0.053	73.9	8.05	32.4

#### INHIBITION OF GLUCOSE POTENTIATED FIXATION BY PLANT LITTER EXTRACT AND N-SERVE

The influences of plant litter extract and N-Serve on the heterotrophic nitrogen fixation potentials are summarized in Table 32. The litter extracts from *Artemisia* and *Atriplex* produce a greater inhibition on heterotrophic fixation than N-Serve. However, the reverse is true for the *Ceratoides* soil.

#### PLATE COUNTS OF HETEROTROPHIC NITROGEN- FIXING BACTERIA

The plate counts of heterotrophic nitrogen fixers at the initial time and after 24-hr and 3-wk incubation periods are given in Table 33. Significant increases in the plate counts occur after the 24-hr light incubation period of the soil crusts. However, no significant gains or losses in the count occur when the crusts are incubated in the dark for 3 wk.

#### ALGAL COMPONENT OF CRYPTOGAMIC CRUSTS

The results of soil crust examination for algal components are shown in Table 34. Both Curlew Valley and Pine Valley share common genera of blue-green algae: *Nostoc*, *Lyngbia*, *Phormidium* and *Oscillatoria*. *Microcoleus* was not observed in the Pine Valley soils. However, the green alga, *Chlorococcum*, was frequently encountered on rock surfaces at this site. For both sites, *Nostoc* was found to be the blue-green symbiont in association with the fungal hyphae.

#### C:N RATIOS OF CURLEW VALLEY SOIL CRUSTS

To quantify the amount of carbon and nitrogen in the surface soil crusts, the percent organic carbon and total nitrogen were determined. Table 35 summarizes these results. The organic carbon in the cryptogamic crusts varies around 2%, while the percent nitrogen varies from 0.18-0.26%. Thus, the C:N ratio for all three Curlew Valley soil crusts varies between 9 and 12.

#### TOTAL N OF PLANT MATERIAL AND PLANT LITTER EXTRACTS

Table 36 presents the total N analysis of the plant material and plant litter extracts. Except for the Curlew Valley *Artemisia* and Pine Valley *Ceratoides* extracts, the total N of the plant material extracts varies from 1.68-1.99 mg N/g. For the Curlew Valley *Artemisia* extract, 6.51 mg N/g was determined, while the Pine Valley *Ceratoides* extract had 4.37 mg N/g.

The plant litter extracts in the Curlew Valley samples were lower than the plant material extracts, ranging from 0.80-1.38 mg N/g.

## DISCUSSION

### HETEROTROPHIC NITROGEN FIXATION

The data given in the total nitrogen balance sheets (Table 31, Skujins 1976; Table 15, this report) demonstrate net gains in nitrogen. All of the experimental systems were incubated in the dark from 3-5 wk, thus strongly suggesting that the net gain in nitrogen is due to heterotrophic fixation. Upon further inspection of these data, it is observed that greater gains in nitrogen are achieved by the air dried (approximately -15 bars) soils incubated for 5 wk over those soils incubated 3 wk. Thus, a 3.6-fold gain in nitrogen is observed for the *Artemisia* soil, and an 11.6-fold increase for the *Ceratoides* soil. The *Atriplex* soil, however, was the exception, showing no increase in nitrogen.

If these observed increases in nitrogen are biological, rather than by an abiotic influx, then NH<sub>4</sub><sup>+</sup> would be expected to inhibit or reduce nitrogenase activity. The rate of application of (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> in all of the experimental systems was 30 μg N per gram of soil and the inspection of data shows definite inhibition or reduction in the net gain of nitrogen. In the 5-wk experiments, the net gain in nitrogen for the ammonium sulfate treated *Artemisia* and *Ceratoides* air dried soils represents a 52 and 41% reduction in nitrogen fixation when compared to their respective untreated soils. Again, the *Atriplex* soil was the exception with no reduction in nitrogen gain. The ammonium sulfate plus plant material amended air dried soils show greater reductions in nitrogen gains; in this case, a 73% reduction for *Artemisia* soil, a 58% reduction for *Ceratoides* soil and a 100% reduction for the *Atriplex* soil. Possibly both NH<sub>4</sub><sup>+</sup> and some allelopathic inhibitor(s) are repressing nitrogenase activity. All of the ammonium sulfate treated and most of the ammonium sulfate plus plant material amended soils maintained at -1 bar and -15 bars show a 100% reduction in nitrogen gain. The plant material amended *Artemisia* soil maintained at -15 bars shows no reduction in nitrogen when compared to its untreated counterpart. The *Ceratoides* soil under similar conditions shows a 55% reduction.

**Table 32.** Inhibition of glucose potentiated heterotrophic nitrogen fixation by plant litter extract and N-Serve

Site	Plant litter extract		N-Serve	
	nm C <sub>2</sub> H <sub>4</sub>	% Inhibition	nm C <sub>2</sub> H <sub>4</sub>	% Inhibition
Curlw Valley - <u>Artemisia</u>	0.234	31	0.074	21.8
Curlw Valley - <u>Ceratoides</u>	0.431	0	0.133	33.3
Curlw Valley - <u>Atriplex</u>	0.626	32.3	0.073	7.9

**Table 33.** Plate counts of heterotrophic nitrogen-fixing bacteria, Curlw Valley

Time of sampling	Site		
	<u>Artemisia</u>	<u>Ceratoides</u>	<u>Atriplex</u>
0	119 × 10 <sup>4</sup> s̄ = 36	131 × 10 <sup>4</sup> s̄ = 26	151 × 10 <sup>4</sup> s̄ = 39
After 24 hours, light	64 × 10 <sup>5</sup> s̄ = 18	50 × 10 <sup>5</sup> s̄ = 9	144 × 10 <sup>5</sup> s̄ = 93
After 21 days, dark	54 × 10 <sup>5</sup> s̄ = 10	40 × 10 <sup>5</sup> s̄ = 14	196 × 10 <sup>5</sup> s̄ = 144

**Table 34.** Algae of Curlw Valley and Pine Valley cryptogamic soil crusts

Site	Cyanophyta	Chlorophyta
Curlw Valley	<u>Nostoc</u> <u>Lynobia</u> <u>Microcoleus</u> <u>Phormidium</u> <u>Oscillatoria</u>	
Pine Valley	<u>Nostoc</u> <u>Lynobia</u> <u>Phormidium</u> <u>Oscillatoria</u>	<u>Chlorococcum</u>

**Table 35.** C:N ratios of Curlw Valley cryptogamic crusts (0-2 cm)

Sampling Data	% C	% N	C:N
Site: <u>Artemisia</u>			
20 April 1976	2.04	.21	9.7
9 July 1976	2.21	.24	9.2
19 July 1976	1.92	.18	10.7
4 August 1976	2.80	.23	12.2
25 August 1976	1.70	.18	9.4
Site: <u>Ceratoides</u>			
20 April 1976	2.15	.20	10.8
9 July 1976	2.06	.22	9.4
19 July 1976	1.90	.19	10.0
4 August 1976	2.10	.22	9.6
25 August 1976	1.80	.18	10.0
Site: <u>Atriplex</u>			
20 April 1976	2.33	.25	9.3
9 July 1976	2.36	.21	11.2
19 July 1976	2.30	.23	10.0
4 August 1976	2.70	.26	10.4
25 August 1976	1.90	.21	9.1

**Table 36.** Total N of plant material and plant litter extracts (values expressed in mg N/g plant sample)

Sample	Plant Material Extract	Plant Litter Extract
Curlw Valley - <u>Artemisia</u>	6.51	0.80
Curlw Valley - <u>Ceratoides</u>	1.75	1.38
Curlw Valley - <u>Atriplex</u>	1.99	1.05
Pine Valley - <u>Artemisia</u>	1.80	N.D.
Pine Valley - <u>Ceratoides</u>	4.37	N.D.
Pine Valley - <u>Atriplex</u>	1.68	N.D.

N.D. = No Data.



Inhibition of dinitrogen fixation or reduction in nitrogen gain is also observed in the ammonium sulfate treated soils incubated for 3 wk. In these soils, a 70% reduction in nitrogen fixation has occurred in the *Artemisia* soil, a 27% reduction in the *Ceratoides* soil and a 100% reduction in the *Atriplex* soil. Further, when these same soils are compared to their 5-wk counterpart experiments, reductions in nitrogen gain are also observed. These comparisons represent an 83, 89 and 100% reduction in nitrogen gain for the *Artemisia*, *Ceratoides* and *Atriplex* soils. Thus, the inhibitory influence of  $\text{NH}_4^+$  on nitrogenase activity is greater after 3 wk than after 5 wk. Except for the plant material plus N-Serve treated *Ceratoides* soil, all of the other 3-wk experiments demonstrate a total inhibition on nitrogen gain when compared to their untreated counterparts. For the *Ceratoides* soil (Table 15, last set) the gain in nitrogen represents a 79% decline when compared to its untreated soil (Table 15, second set).

These data suggest, then, that the available ammonium in soils is preferentially utilized as a source of nitrogen rather than relying on the more energy-expensive dinitrogen fixation process. This is true for any heterotrophic nitrogen-fixing bacterium. Furthermore, the additions of plant material, plant litter or N-Serve restrict the cycling of nitrogen by the allelochemic inhibition of nitrogen fixation (plant material), the immobilization of nitrogen (plant litter) and the inhibition of nitrification (N-Serve). Thus, the observed reduction in nitrogen gain (reduced heterotrophic nitrogen fixation) is feasible as the heterotrophic fixation process is inhibited by either the presence of an allelochemic inhibitor, the presence of ammonium-nitrogen (applied, or retained by plant litter, or N-Serve) or both.

Other evidence supporting the occurrence of heterotrophic nitrogen fixation is also found in Skujins (1976). Whenever a net gain in nitrogen occurred, an increase in the organic nitrogen fraction was always noted.

The observed acetylene reduction (Table 26), by ground soil crust when incubated in the dark for 3 wk, also provides additional evidence for the presence of heterotrophic nitrogen fixation. Under these conditions, 0.699-1.278 nm of ethylene was produced. Furthermore, glucose potentiated acetylene reduction verifies the existence of these microorganisms (Table 23), as well as the plate count numbers on a nitrogen-free medium (Table 33).

It is of interest to note that from the colonies appearing on the nitrogen-free medium, a gram-variable, subterminal, spore-forming bacillus was observed to reduce acetylene after 12 days. Based on colonial and cell morphologies, the organism is similar to the nitrogen-fixing bacterium *Bacillus polymyxa*.

Heterotrophic nitrogen fixation has been indicated by us before (Table 10, Skujins 1975) in these same Curlew Valley soils. In glucose potentiated soils, up to 290 nm  $\text{C}_2\text{H}_4/48$  hr were produced in the dark.

Knowles and Denike (1974) have reported on the effect of  $\text{NH}_4^+$  on nitrogenase activity in soil. In a 1% glucose

amended sandy loam soil, nitrogenase activity was partially repressed by a concentration of 50  $\mu\text{g NH}_4^+\text{-N/g}$  soil. However, when the glucose amendment was lowered to 0.1%, 5  $\mu\text{g NH}_4^+\text{-N}$  partially suppressed nitrogenase activity. Thus, the concentration of nitrogen required to inhibit nitrogenase activity was dependent upon the concentration of the carbohydrate. That is, the lower the amount of an available energy source, the lower the fixation potential, and thus a lower amount of  $\text{NH}_4^+$  would be required to inhibit nitrogenase activity (Knowles and Denike 1974).

In the soil crusts of Curlew Valley, the carbon content is approximately 2%. Based on the data of Knowles and Denike (1974), a carbohydrate concentration of 2% would require 100  $\mu\text{g NH}_4^+\text{-N/g}$  soil. However, because the carbohydrate content of these soils is not in the form of glucose, a partial repression of nitrogenase activity by 30  $\mu\text{g NH}_4^+\text{-N}$  would be realistic.

One might also consider the possibility of the observed increases in soil nitrogen being due to dark fixation by the blue-green algae. The data presented in Table 23 tend to discourage this suggestion, as very little to no ethylene production occurs in the glucose unamended soils after 24-hr incubation in the dark.

Fay (1976) has reported on factors influencing dark nitrogen fixation by the blue-green alga *Anabaenopsis circularis*. It was observed that cultures transferred from the light to the dark retained acetylene reduction activity for up to 72 hr only when supplemented with 0.03 M glucose. Non-glucose supplemented cultures were found to retain activity for only 6 hr.

In the experiments reported herein, the soils were incubated in the dark for 3-5 wk. Thus, it would be unlikely that the observed gains in nitrogen are due to dark fixation by the blue-green algae. Furthermore, since ethylene production was detected after a 3-wk dark incubation period, it is suggested that the available organic matter must first be degraded and made available to the organisms before nitrogenase activity can occur.

The total nitrogen balance sheets (Table 15) also define a threshold value for heterotrophic nitrogen fixation based on the initial total nitrogen of an experimental system. If the initial total nitrogen was below 90 mg N/100 g soil, nitrogen fixation was followed with an increase in soil organic nitrogen (see also data in Skujins 1976).

In order for heterotrophic nitrogen fixation to occur, an energy source must be available in the form of organic carbon. The source of this carbon may be the organic matter produced by the observed blue-green algae *Lyngbia*, *Microcoleus*, *Phormidium* and *Oscillatoria*. Sorensen (1975) and Lynn and Cameron (1972) have also observed these genera in Curlew Valley soils. Brock (1975) has also reported on massive bacterial association with the sheaths of *Microcoleus*; the alga was isolated from Great Basin Desert crust near Mud Lake, Idaho.

Significant increases in the bacterial plate counts on nitrogen-free media indicate the possibility of the bacterial population responding to the input of carbon by the photosynthetically active soil algae. Hence, a phycosphere effect may be created in the environment of the algae. The input of carbon by the primary production of the algae would allow heterotrophic nitrogen fixation to occur.

We have demonstrated (Tables 27 and 32) the existence of potential allelopathic inhibitory effects on heterotrophic nitrogen fixers. The chemical agent, N-Serve, was also found to be inhibitory (Table 32). The inhibition varied from 7.9-33.3%. This compares favorably to the inhibitory effects produced by the plant litter extract where up to 32.3% of the acetylene reduction capability was inhibited. Possibly the inhibition of nitrification in the one case, and the immobilization of nitrogen in the other case, might cause such inhibitory influences. However, Table 36 shows that the litter extracts have a total nitrogen content of 0.80-1.38 mg N/g. This is equivalent to 15.9-27.5  $\mu\text{g N/ml}$  of added extract. We have demonstrated (Skujins and West 1974) that 25  $\mu\text{g NH}_4^+\text{-N}$  produced a 78% inhibition on nitrogenase activity in soil crusts. The plant material extracts have total nitrogen content of 1.68-6.5 mg N/g. Since these extracts may be easily degraded in the soil, their inhibitions should be classified as potential allelopathic inhibitors, as no clear-cut differentiation could be made between a truly allelopathic inhibitor or an inhibition due to  $\text{NH}_4^+$  release.

At present it is difficult to assign absolute values for the N input by heterotrophic fixation into this ecosystem on an area and annual basis. If it is in the range of, say, 5-10% of the total  $\text{N}_2$ -fixation input, however, it may be a significant contribution to the total nitrogen balance in a xeric ecosystem, and the currently published annual balance sheets (West and Skujins 1977) might require revision.

#### NITROGEN LOSS BY DENITRIFICATION AND VOLATILIZATION

The loss of  $^{15}\text{N}$  from  $(^{15}\text{NH}_4)_2\text{SO}_4$  amended soils was discussed in the previous Research Memorandum (Skujins 1976). The currently reported results on  $^{15}\text{NH}_4^+$  and  $^{15}\text{NO}_3^-$  treated soils support our previous conclusions.

Additions of plant litter failed to prevent the loss of the applied  $^{15}\text{N}$ . The results in Table 10 show the presence of both nitrite and nitrate nitrogen, and in Table 12 most of the  $^{15}\text{N}$  is in the nitrate form. Apparently, the addition of organic matter from the plant litter did not immobilize the nitrogen but rather supplied an accessible energy source for denitrification.

The total nitrogen balance sheets define a possible threshold value for denitrification. Whenever the initial total nitrogen exceeded 110 mg N/100 g soil, net denitrification occurred. When the initial total nitrogen of an experimental system is below 90  $\mu\text{g N/g}$  soil, nitrogen fixation occurs with an increase in organic nitrogen. When the initial total nitrogen lies between these two threshold values, heterotrophic fixation or net denitrification may or may not occur. This suggests a dependence on the soil crust C:N ratio by the nitrogen fixers and denitrifiers. Thus, if the

C:N ratio is high, nitrogen would be limiting and fixation would occur. As the level of organic nitrogen increases, the C:N ratio would then decline until nitrogen is no longer immobilized. Assuming a loss in carbon due to microbial respiration, the nitrogen would then be denitrified and nitrogen fixation would continue to be repressed until nitrogen again becomes immobilized. Thus, a higher C:N ratio would subsequently occur.

The C:N ratios of the soil crusts reported in Table 35 vary between 9.1 and 12.2. Such ratios in cultivated soils are considered to be low, thus allowing nitrogen to be mineralized. Table 2 indicates nitrate assimilation of the applied  $\text{K}^{15}\text{NO}_3$ . These values represent a 5.1-17.8% assimilation rate.

Kai et al. (1969) have proposed that immobilization is a constant and ongoing process regardless of the C:N ratio. In view of the assimilated  $^{15}\text{NO}_3\text{-N}$ , this appears to be true. Therefore, does a C:N ratio of 10 necessarily imply that the mineralization of nitrogen will occur? Skujins and West (1974) have reported the C:N ratios of these surface soils to be as low as 6.7 and as high as 11.1. In an arid system such as Curlew Valley, a ratio above 10 might be considered high, particularly at the crust microenvironment.

Skujins and West (1974) have also shown that as  $^{15}\text{N}_2$  is fixed, it is released into the soil within a few hours as  $^{15}\text{NH}_4^+$  and is subject to nitrification and denitrification. In their study, the predominant form of nitrogen lost to the atmosphere was  $^{15}\text{N}_2\text{O}$ , and accounted for a total loss of 13% after 7 days of incubation at room temperature. The results in Table 17 agree with this reported value, as 13.3-38.4% of the fixed  $^{15}\text{N}_2$  was also lost due to denitrification.

Since we are considering heterotrophic nitrogen fixation and denitrification, the soil carbon assumes importance as it represents the energy source. The source of this carbon may be from the primary production by the blue-green and green algae, and from litter fall. Table 34 lists sheathed blue-green algae isolated from Curlew Valley and Pine Valley soil crusts, which are taxonomically recognized as slime producing (Prescott 1970). Data presented in Tables 10 and 12 indicate the inability of ground plant litter to prevent denitrification by the immobilization of nitrogen.

Verma and Martin (1976) have described the decomposition of algal cells and their components. One of the genera in question was *Microcoleus* which was shown to have 36% of its cell wall components decomposed in 2.5 days. Only 12% of these components were incorporated into humic acid, and 10% into fulvic acid. The carbohydrate content of the extracellular polysaccharides was found to be 68%.

As mentioned, Brock (1975) has reported massive bacterial associations with the *Microcoleus* sheaths. Hence, in the Great Basin soil crusts, a phycosphere-like effect is suggested, allowing denitrification to occur.

#### AMMONIFICATION

The ammonification potentials of Curlew Valley and Pine

Valley soils have been summarized in Tables 1 and 17. The values reported for the total  $^{15}\text{NH}_4^+$ -N in Table 17 represent a 48.1% ammonification potential for the *Artemisia* soil, 21% for the *Ceratoides* soil, 48.8% for the *Atriplex* soil and 30% for the Pine Valley soil. The values for the *Artemisia* and *Atriplex* soils compare favorably well with the potentials given in Table 1. The values given in Table 1 show a 47.4-62.2% ammonification rate, suggesting that the casein is hydrolyzed more easily than the labeled  $^{15}\text{N}$  organic nitrogen. This implies that the ammonification process may be considered as the rate-limiting step for denitrification. This is supported by the data in Tables 5-7, and in Table 17. Approximately 50-60% of the applied  $(^{15}\text{NH}_4)_2\text{SO}_4$  is lost during a 7-day incubation, while 13.3-38.4% of the fixed  $^{15}\text{N}_2$  is denitrified during the same time span.

#### $\text{N}_2$ FIXATION (ACETYLENE REDUCTION) BY SOIL CRUSTS

The in situ nitrogen fixation results are summarized in Tables 19-22. The experiments were conducted during a dry season and little acetylene reduction was found to occur. It is interesting, however, that each site had its maximum rate of fixation occurring at different diurnal time intervals. At the Pine Valley site, the only measurable activity occurred during the night. At Curlew Valley, the *Artemisia* site displayed its greatest activity from 0630 to 0800 hr; the *Ceratoides* site at 0830 to 1000 hr; the *Atriplex* site at 1030 to 1200 hr. Except for the *Artemisia* site, little to no acetylene reduction activity was detected by the field moisture controls. At the *Artemisia* site, the reverse is true with all of the activity occurring in the field moisture cores, and no activity in the wetted cores. These data suggest an important role played by the soil moisture in regard to the inputs of nitrogen.

Skujins and West (1974) have also shown the effect of soil moisture on nitrogen fixation in in situ measurements at Curlew Valley. Measurements with  $^{15}\text{N}$  showed that the greatest rate of  $^{15}\text{N}_2$  fixation occurred between 0800 to 1000 hr in both field dry soils and soils maintained at  $-1/3$  bar. In addition, little fixation was found to occur in the dry crust during the afternoon hours. It was therefore suggested that greater nitrogen fixation may occur in the morning hours when the soil surface is cool and damp with dew. The importance of soil moisture on the inputs of both carbon and nitrogen has also been emphasized by Lynn and Vogelsberg (1974) and Sorensen and Porcella (1974).

The importance of soil moisture during the time of sampling the soil crusts is also reflected in Tables 18 and 28-31. The presented data are divided into two parts, measuring the differences between the crusts sampled during the spring and summer. Table 18 indicates greater nitrogen fixation potentials by the wet season crust over the dry at 22 C, but no differences were observed at 37 C.

The results in Table 28 also demonstrate differences in the crusts' response to plant material extracts. One hundred percent inhibition was observed to occur in the Curlew Valley dry season crust, while their wet season counterparts showed a 46.9% inhibition by the *Atriplex* samples.

Similar data are also reported in Tables 30 and 31. N-Serve is observed to bring about a 63.2-95.8% inhibition on the dry season crusts, while only a 1.3% inhibition on the *Ceratoides* wet season crust is reported. When both plant material and N-Serve are added to the crust, greater inhibition (in general) occurs with the dry season crusts. However, Table 29 shows that a divergence and inconsistency in the inhibitory phenomena may exist, pointing to other possible factors involved in the process. Perhaps the differences between the total nitrogen content of the plant material and litter extracts are involved.

All of the above data suggest that the physiological state of the soil crust organisms is a determinant factor in nitrogen fixation. The dry season crusts demonstrate low activity. When plant material extracts, N-Serve or both were added, the acetylene reduction activity was essentially reduced to zero. Thus, if the blue-green algae are physiologically in a low state, their ability to resist any chemical agent would be low. If the algae are physiologically active, their ability to resist chemicals or any other deleterious influence may be expected. However, this does not exclude the possibility of the accumulation of the allelochemic inhibitor(s) in the soil crust, thus causing the dry season samples to show lower fixation abilities.

#### CONCLUSIONS

The following conclusions may be drawn from the current phase of study:

1. The algal population in the Curlew Valley and Pine Valley soils appears to be dominated by the sheathed blue-green algae.
2. The association of bacteria with the sheathed blue-green algae suggests a phycosphere-like effect, thus allowing heterotrophic nitrogen fixation and denitrification to occur.
3. Measurements of heterotrophic nitrogen fixation in the laboratory have been found to be as high as 10 mg N/100 g soil for 3-wk incubated soils, and 38.4 mg N/100 g soil in 5-wk incubated soils. Additions of ammonium sulfate or ammonium sulfate plus plant material reduce the net gain in nitrogen.
4. The ammonification of fixed nitrogen is the apparent rate-limiting step in nitrogen loss.
5. Fifty to sixty percent of the applied  $^{15}\text{NH}_4^+$  or  $^{15}\text{NO}_3^-$  is denitrified during the first week of incubation. In longer incubation periods, over 80% of the applied  $^{15}\text{NH}_4^+$  may be denitrified.
6. Ammonia volatilization represents a nitrogen loss of less than 5% of the applied  $^{15}\text{NH}_4^+$ .
7. Allelopathic inhibition on nitrogen fixation and nitrification has been observed. However, the physiological state of the microbial population may play a role in the effectiveness of the allelochemic inhibitor(s).

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