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NITROGEN DYNAMICS IN STANDS DOMINATED BY SOME MAJOR COOL DESERT SHRUBS. IV. INHIBITION BY PLANT LITTER AND LIMITING FACTORS OF SEVERAL PROCESSES

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ABSTRACT

It appears that, under certain conditions, heterotrophic nitrogen fixation may be important in desert soils as determined by in vitro experiments. Ammonia volatilization was at the highest rate when soil was in the drying process. When soil is kept at a constant water potential, ammonia volatilization fluctuates and indicates an influence of nitrification. Rate and extent of denitrification are dependent on the type of available plant material. The fastest rate was obtained when the soil was amended with *Ceratoides lanata* material. Nitrite accumulates in increased quantities in *Artemisia tridentata*-amended soils due to the presence of allelopathic compounds.

Nitrogen fixation by blue-green algae-lichen crusts from south Curlew Valley, Utah, in the Great Basin Desert, was studied using the acetylene reduction technique. A molar ratio of 3 moles C_2H_4 produced:mole of N_2 fixed, was used to estimate nitrogen (N_2) fixation by acetylene reduction. *Nostoc* was found to be present in many of the lichen thalli examined microscopically. Crust nitrogen fixation decreased rapidly below -⅓ bar pressure (water potential) which indicated that nitrogen fixation occurs only when the crust is wet. This would suggest that most of the crust nitrogen fixation in the Great Basin Desert occurs during the fall and spring rainy seasons. Nitrogen fixation reached a maximum at 200 microeinsteins $m^{-2} \cdot sec^{-1}$ of incandescent light intensity, somewhat comparable to natural light with a heavy, grey cloud cover. In the winter months temperature would limit nitrogen fixation, and moisture would limit nitrogen fixation during the hot, dry summer months. Ninety grams of nitrogen (N) per hectare were fixed in situ on a typical fall day with peak in situ rates of about 14 g N fixed $ha^{-1} \cdot hr^{-1}$. When assayed in the laboratory under optimal conditions (nitrogen fixation potential), crusts could fix nitrogen at rates as high as 84 g N fixed $ha^{-1} \cdot hr^{-1}$. Since moisture and light were not limiting in in situ assays, in situ nitrogen fixation correlated with predictions made from the laboratory-determined temperature curve. There was some dark nitrogen fixation, with values varying from 5 to 30% of nitrogen fixation in the light. An estimate of 10 to 100 kg N fixed $ha^{-1} \cdot yr^{-1}$ was made, based on 120 days of fixation. The absolute value would vary from area to area depending upon the extent of blue-green algae-lichen crust cover, and with temperature, moisture availability and duration of the rainy periods. Glucose was found to potentiate crust heterotrophic nitrogen fixation with an optimum at 30 C. Soils beneath the crust exhibited no acetylene reduction, unless amended with glucose, and soils at the 40- to 50-cm depths showed no ability to reduce acetylene even when amended with glucose. Apparently the supply of available organic carbon in the soils is insufficient for significant heterotrophic nitrogen fixation to occur. Ammonium ion was found to inhibit crust nitrogen fixation, pointing toward ammonium ion regulation of nitrogen fixation. In 1973, the May versus September crust transect samples from sites dominated by *Atriplex confertifolia*, *Ceratoides lanata* and *Artemisia tridentata* were not significantly different. However, the *Ceratoides lanata* values were significantly lower and reflected the scant lichen cover in that desert shrub community. Nitrogen fixation was found to be greatly reduced under the canopies of the three desert shrubs mentioned previously, with an understory dominance by bryophytes. Aqueous leaf extracts and leaf volatile products inhibited crust nitrogen fixation, with the inhibition being particularly pronounced with *Artemisia tridentata* leaf extracts. Glucose was used to potentiate heterotrophic nitrogen fixation. Dried plant leaves, when added to soils, and the mixed soil and leaves moistened with glucose solutions, inhibited heterotrophic nitrogen fixation. The heterotrophic nitrogen-fixation potential (glucose-amended soils) was greatly reduced in soils from beneath the desert shrub canopies. Thus, shrub inhibitors may play a role in nitrogen input and blue-green algae-lichen crust distribution in desert shrub communities. There was no inhibition of nitrification in soil sampled beneath the desert shrub canopy. However, there was an increase in the organic and fixed fractions of the soil nitrogen after perfusion which was not apparent in interspace soils. No apparent inhibitors were washed out during perfusion. There does not appear to be an inhibitory effect on nitrification by *Artemisia tridentata*, *Ceratoides lanata* and *Atriplex confertifolia* when aqueous extracts or plant leachates are perfused through the soil under optimal conditions. Although there is no complete inhibition of nitrification, there seems to be suppression of the oxidation of nitrite to nitrate, thus causing an accumulation of nitrite. The high level of nitrite in our in vitro potential measurements may be a possible explanation for the high rate of denitrification that has, in previous studies, been shown to take place in situ in the desert in the crust where low oxygen tensions prevail, especially during periods of high biological activity. ATP concentration, which has been most commonly used as an index of microbial activities, may also prove to be useful as an index of biological activity.

INTRODUCTION

During 1974, ^{15}N experiments were employed to make an investigation of the nitrogen cycle in the cold desert soils of Curlew Valley, Utah. Heterotrophic fixation of N_2, ammonia volatilization, ammonification, nitrification and denitrification were studied. The previously unaccountable losses of nitrogen reflected the amount of denitrification which had occurred during the experimental period. Similarly, any gains in nitrogen are a result of heterotrophic fixation. The intermediate steps of ammonification and of nitrification were also studied.

Because of the importance of pH and water potential on the cycling of nitrogen in soil, these variables were either monitored during the experimental time period, or the water potential was held constant. In addition, the effects of fresh plant-material amendments or deletions were also investigated.

Nitrogen fixation by desert algal crusts probably constitutes a major input of nitrogen into desert ecosystems (Fuller et al. 1960, MacGregor and Johnson 1971, Mayland et al. 1966). The nitrogen fixed by the algal crust is available to desert plants (Mayland and McIntosh 1966). However, there have been no studies of nitrogen fixation in arid soils that assessed the environmental and ecological parameters affecting biological nitrogen fixation. Most of the studies of biological nitrogen fixation have been with more temperate ecosystems (Hardy et al. 1973, Hardy et al. 1968).

This study describes the moisture, temperature and light intensity parameters that govern blue-green algal-lichen crust nitrogen fixation in south Curlew Valley, Utah, in the Great Basin Desert and is an extension of previous work (Skujins and West 1974). Since its discovery by Dilworth (1966) and Schollhorn and Burris (1966), the acetylene reduction technique has proved to be a useful and valid method for estimating biological nitrogen fixation (Hardy et al. 1968, Stewart et al. 1967). The acetylene reduction technique is used here to estimate nitrogen fixation potentials in the laboratory which are compared with nitrogen fixation under field conditions. Soil heterotrophic nitrogen fixation and the potentiation of heterotrophic nitrogen fixation are also examined.

Nitrogen fixation is also examined in relation to three desert shrub communities: a sagebrush-dominated area (*Artemisia tridentata*); a winterfat-dominated area (*Ceratoides lanata*); and a shadscale-dominated area (*Atriplex confertifolia*).

Previous studies in the laboratory (Skujins and West 1973 and 1974) have shown that nitrification and ammonification experiments at lower soil humidities prove that considerable biological activity in desert soils may take place at water tensions as low as -45 atmospheres. Ammonification experiments reported herein were run at a moisture above -10 atmospheres, with the variable being the form of nitrogen compound added to the soil, i.e., plant material, egg albumin and L-leucine.

Nitrification potential was measured by an in vitro perfusion method. We have previously reported (Skujins and West 1974) that in these soils nitrification potential was greatest in spring samples and the least potential was found in fall samples. Nitrification also decreased with depth, and nitrate accumulation appeared to be decreased when there was a comparably large amount of nitrite present. The accumulation of nitrite observed in these studies suggests that perhaps some inhibition was taking place.

It was first suggested that nitrite may accumulate due to the high pH of the soils (Skujins and West 1973). The presence of sodium did not appear to be the inhibitory factor under optimum conditions (Skujins and West 1974).

Many plants produce allelopathic chemicals which are toxic to the germination and/or development of other plants. Whittaker (1970) has suggested that some chemicals released by plants are inhibitory or toxic to soil microorganisms and may exert their allelopathic effect in this fashion. For example, certain plant extracts inhibit nitrogen-fixing and nitrifying bacteria (Rice 1964 and 1965). Furthermore, allelopathics have been found to be more common in arid regions (Went 1970).

Because plant inhibitory phenomena could limit nitrogen transformation, the relationship of individual plant shrubs to nitrification was examined. Dominant shrubs of the area, which included sagebrush (*Artemisia tridentata*), winterfat (*Ceratoides lanata*) and shadscale (*Atriplex confertifolia*), were used to examine inhibition. *Philodendron* spp. and *Dactylis glomeratus* (an orchard grass) were used as biological comparisons.

Inhibitors have also been known to be leached into the soil, adhere to the clay particles and exert their effects here (Rice and Pancholy 1973). An attempt was made, therefore, to wash these possible inhibitors out of the soil and then observe if nitrification took place when they were reintroduced after building up the microbial population.

Determination of adenosine triphosphate concentration of soil has most commonly been related to microbial biomass and few studies have correlated ATP concentration to biological activity except for respiration. ATP was measured by the luciferin-luciferase bioluminescence assay and concentrations in soil sampled at various depths were measured to determine if ATP concentratons correlated better with microbial numbers or biological activity.

This report, therefore, is mainly concerned with phases of nitrogen transformation such as nitrogen fixation, nitrification, denitrification and ammonification and the factors influencing their rates in Curlew Valley desert soils.

OBJECTIVES

The main objectives in 1974 were to verify, quantify and refine most of the experimental evidence obtained in 1973:

1. To quantify the volatile NH_3 losses from the various pools supplying NH_4^+ ion in the exchangeable form.
2. To quantify the denitrification losses from the various pools supplying substrate via nitrification.
3. To quantify ammonification, nitrification and denitrification from decomposing sagebrush litter under various moisture and temperature conditions.
4. To determine the importance of fixed NH_4^+ to the desert nitrogen balance.
5. To quantify the N_2 inputs by fixation from algal crust.
6. To determine the seasonal variation in N_2 fixation in algal crust.
7. To study the allelopathic parameters that govern algal/lichen crust nitrogen fixation.
8. To determine the influence of allelopathic inhibition on nitrification potential.

METHODS

Most of the methods used in this year's studies have been described in the 1973 report (Skujins and West 1974). Additional methods or modifications of methods are given below.

The soils used in this project have been described in the 1972 and 1973 reports (Skujins and West 1973 and 1974, respectively).

^{15}N Methodologies

Studies of the Nitrogen Cycle Using Exogenously Supplied $(^{15}NH_4)_2SO_4$

One hundred grams of surface-collected soil (0-3 cm) were placed in each of the foil-wrapped 550-ml erlenmeyer flasks of the volatilization apparatus. These soils were collected in April 1974 from the *Artemisia*, *Ceratoides* and *Atriplex* sites from between the plant canopies (interspaces). Except in one case in which no amendments were made, 3 mg of N from $(^{15}NH_4)_2SO_4$ were dissolved in 25 ml of distilled water and added to the soil. The atom percent excess ^{15}N was 30.4 which corresponds to 912 μg ^{15}N added.

The soil moisture was maintained at 25% $\pm$ 2% (-1 to -2 bars) by keeping the atmospheric humidity at 100% throughout these experiments. This was accomplished by bubbling the incoming airstream through a 10-ml beaker of distilled water.

In two other experimental systems, the same procedure was followed as above, except the soil was allowed to air dry after ^{15}N amendment or maintained at 15 % $\pm$ 2% (approximately -15 bars). In the latter case, the soil moisture was maintained by keeping the atmospheric humidity at 99% throughout the experiment by passing the incoming airstream over a 10-ml beaker containing 5 ml of 0.3 M H_2SO_4. Finally, in experiments in which the effect of fresh plant material was to be studied, 1 g of ground plant material (*Artemisia*, *Ceratoides* or *Atriplex*, 20 mesh) was amended to their respective soils (5-1, 6-1, 7-1). These soils were maintained at either -1 or -15 bars, using the above procedures, throughout the duration of the experiment.

The temperature was maintained at 22 C$\pm$2 C during the experiments. The captured NH_3 was analyzed after 24 hr, and during weeks 1, 2, 3, 4 and 5 of the experiment. After the fifth week, the moisture content, bar potential and pH were determined. Forty g (wet weight) of the remaining soils were each placed in a semimicro-Kjeldahl flask which was then immersed in a dry ice-acetone bath. After the soils were quickly frozen, the flasks were then placed in the oven of a Virtis lyophilizer and lyophilized to dryness over a 24-hr period. This procedure was followed in order to analyze for the different N forms: organic N, fixed NH_4^+, exchangeable NH_4^+, NO_2^- and NO_3^-.

For the exchangeable NH_4^+, 10 g of dry soil were extracted with 50 ml of 2 N KCl solution by shaking for 1 hr. This mixture filtered through a Whatman #5 filter paper, and the total volume of the filtrate was filled up to 100 ml with distilled water. A 20-ml aliquot was used to determine the amount of NH_4^+—N by steam distillation with MgO (Bremner 1965b). The NH_3 was captured in H_2BO_3 and titrated as previously described.

Nitrite and nitrate were also determined from the same 20-ml aliquot by the addition of Devarda's alloy following the steam distillation of exchangeable NH_4^+. These samples were steam distilled and the NH_3 was captured as previously described.

Nitrate was determined by obtaining a 20-ml aliquot from the filtrate (mentioned above) and treating it with 1 ml of sulfanic acid (2 g/100 ml) to destroy the presence of NO_2^-. Following this treatment, MgO and Devarda's alloy were then added, the sample was steam distilled and NH_3 collected and titrated. This value minus the amount of exchangeable NH_4^+ gives NO_3^-—N.

Nitrite is then determined by subtracting the amount of NO_3^-—N from NO_2^- and NO_3^-.

Total NH_4^+—N (exchangeable and colloid-fixed) was determined by placing 4 g of soil in a semimicro-Kjeldahl flask and steam distilling it with 10 ml of 40% NaOH (Bremner 1965b). This value minus the value of exchangeable NH_4^+—N gives colloid-fixed NH_4^+—N.

A 1-g sample was used for total N. This sample was digested for 5 hr with 5 ml concentrated H_2SO_4, 3 ml of distilled water and 1.8 g of catalyst (100:10:1; K_2SO_4: $CuSO_4 \cdot 5H_2O$: Se). After digesting (approximately 5 hr), the solution was cooled and 10 ml of deionized distilled water were added. The cooled solution was then made alkaline with 30 ml of 40% NaOH and steam distilled as before. The calculated amount of total N less the NH_4^+ fractions gives the organic N (Bremner 1965a).

Total N of the ground plant material was determined by placing a 1-g sample of each (*Artemisia*, *Ceratoides* and

Atriplex) in a 50-ml beaker, followed by the addition of 10 ml of 0.02 M H_2SO_4. The beakers were then placed in a 110 C oven and the samples were allowed to dry over a 24-hr period. After drying, a 0.25-g sample from each of the beakers was obtained and placed in a semimicro-Kjeldahl flask and prepared for digestion with the H_2SO_4 and catalyst mixture, and analyzed for total N as described above.

In all of the above experiments, the ^{15}N samples were analyzed in duplicate, while all non-^{15}N samples were run in triplicate (DSCODES A3USQ00, 01).

Acetylene Reduction Methods (A3USQ04)

Soil Core Assays

Soil cores were assayed as described in Skujins and West (1974).

Crust Assays

Soil crust (1- to 2-mm depth) was scraped from the surface of an intact crust so as to cover the bottom surface of a 6.5-ml serum bottle (1.324×10^{-8} ha). The crust was moistened with 0.25 ml of distilled water, capped and acetylene injected to 0.1 atmospheres at zero time. After the reaction period, a 0.2-ml gas sample was withdrawn for ethylene analysis.

Soil Assays

Soil samples (½ g each) were weighed and placed in 6.5-ml serum bottles. The soils were moistened with 0.25 ml of distilled water and acetylene was injected at zero time. After the reaction period, a 0.2-ml gas sample was withdrawn for ethylene analysis. Separately, the percent moisture was determined with a sample of the soil. The soils were dried overnight at 110 C. Percent moisture was determined by weight differences.

Canopy Effect

Samples taken under the plant canopy are those soil cores collected within the 20- to 40-cm radius around each plant where the soil surface is under the influence of the shrub canopy (litter, shading, etc.). Bryophytes tended to dominate the surfaces beneath most shrub canopies, with the absolute area under the canopy varying with shrub size.

Plant Extract Inhibition

Five grams of plant leaves were ground in 20 ml of water with a mortar and pestle for 5 min. The resulting slurry was then filtered through a Whatman #1 filter. The mortar was rinsed with 80 ml of distilled water, and the solution was poured and filtered with the plant-leaf slurry. Extracts were prepared from leaves of the three desert shrubs mentioned above. Soil cores collected from the *Atriplex confertifolia* site were moistened with 1.25 ml of the extracts and incubated at 23 C for 24 hr under fluorescent light at 40 microeinsteins m^{-2} sec^{-1} for the acetylene reduction assay.

Plant Leaf-soak Filtrates

One gram of plant leaves from each of the three desert shrubs mentioned above was soaked in 10 ml of water overnight and then filtered through a Whatman #1 filter. The soil cores were moistened with 1.25 ml of the filtrate prior to the acetylene reduction assay.

Plant Leaf Volatile Inhibitors

To assess the presence of volatile inhibitors from desert shrub leaves, a 10-cm piece of rubber tubing was attached to the serum bottle containing the moistened algal crust. The rubber tubing held approximately 1 g of plant leaves, and was incubated so that only volatile plant products could come into contact with the crust. This was achieved by placing the leaves in the region of the tubing farthest from the crust. The bottles, with attached tubing, were then treated according to the acetylene reduction procedure described previously.

Volatile Inhibitors in Artemisia tridentata *Aqueous Extracts*

To assess the presence of volatile inhibitors of nitrogen fixation in *A. tridentata* extracts, 2 ml of extract were pipetted into a 15 x 135-mm test tube; the 13 x 60-mm soil core tubes (stoppered at the bottom) were placed in the larger tubes; and the larger tubes capped with injectable rubber stoppers. Thus the moistened crust would come into contact with only the vapors from the extract. A *Philodendron* extract was run as a nonaromatic control. The acetylene reduction assay was then performed in the standard fashion.

Ammonium Ion Inhibition of Nitrogen Fixation

Quadruplicate soil core tubes were moistened with 1.25 ml of solutions of ammonium sulfate at 10, 25, 50 and 100 μg NH_4^+—N per tube (per soil core). The tubes were incubated in the light at 21 C for 24 hr and then assayed for acetylene reduction.

Ar Versus N_2 Effect on Acetylene Reduction

Serum bottles containing moistened crust were flushed with a mixture of Ar, O_2, CO_2 (80%, 20%, 400 ppm, respectively) for 2 min. Serum bottles with moistened crust with a normal air atmosphere were also used. Assays were run in triplicate. Acetylene (0.6 ml) was injected at zero time. Bottles were incubated in the light for 24 hr at 21 C.

Nitrification

The following methods were followed to assess inhibition of nitrification by desert shrubs, ammonification and ATP concentration.

Nitrification Inhibition

Methods for determining nitrification potential are described in Skujins and West (1974). The following modifications were employed (A3USQ06).

In order to compare nitrification potential of soil sampled from beneath the desert shrub canopy and an interspace, soils from an *A. tridentata* and a *C. lanata* site were perfused with 0.01 M $(NH_4)_2SO_4$ for 24 days. The soils were analyzed before and after perfusion for several forms of nitrogen; total, fixed, organic, exchangeable and NO_2^- + NO_3^-. Soils were extracted with 2 N KCl (3 ml/g) and

filtered. The filtrate was brought up to a 100-ml volume and analyzed for exchangeable NH_4^+ and $NO_2^- + NO_3^-$ by steam distillation with MgO and Devarda's alloy (Bremner 1965a). The soil residue was frozen at -20 F for 30 min and then lyophilized for 24 hr in a Virtis freeze dryer. The soil was then analyzed for total, fixed and organic N according to Bremner (1965b).

Perfusates were analyzed colorimetrically for NH_4^+, NO_2^- and NO_3^-, and pH was measured periodically.

Another experiment to test the possibility of washing out any inhibitors included a setup of three flasks and controls containing sagebrush-dominated soil perfused with ammonium sulfate. One flask was perfused for 2 days after which the solution was removed, placed in a container and stored until later use. Fresh 0.01 M $(NH_4)_2SO_4$ was added to the flask and the soil was reperfused for 10 days after which the solution was discarded and replaced with the original perfusate which had been restored to the original concentration of NH_4^+. The soil was then perfused with this solution for another 10 days. The other two flasks were perfused initially for 4 and 6 days, respectively, after which the same procedure described above was followed. Gas-trap flasks containing 0.2 N H_2SO_4 were connected to the perfusion flasks to measure volatilized ammonia. The acid was analyzed for ammonium after every change in the perfusate solution and pH of the perfusates collected was measured periodically.

A typical cultivated garden soil (sampled at Hyde Park, Utah) which shows a classic example of nitrification was used to assay nitrification inhibition by desert shrubs directly.

Soil was perfused with 0.01 M $(NH_4)_2SO_4$ for 8 or 10 days. As a control, another flask was similarly prepared but distilled water was used in place of the ammonium sulfate solution.

After the initial perfusion period of 8 or 10 days, the perfusate solution was discarded and a fresh 150-ml quantity of 0.01 M $(NH_4)_2SO_4$ was added.

In flasks containing soils to be perfused with ammonium sulfate leached through plant material, a screen holding 2.5 g (1.0% leachate) or 1.25 g (0.5% leachate) of chopped plant material was placed above the soil in the flask column. Plant material was obtained from *A. tridentata*, *C. lanata*, *A. confertifolia*, *Philodendron* spp. and *Dactylis glomeratus*.

In addition, a 5% aqueous extract of each of the plants mentioned above, except *D. glomeratus*, was prepared and either 25, 24 or 23 ml (0.5, 0.48 or 0.46% extract, respectively) or 5 ml (1% extract) were brought up to a 250-ml volume and 0.3303 g ammonium sulfate was added and the solution mixed. This solution was then introduced into the perfusion apparatus.

A soil serving as a control for the experiment was perfused with 0.01 M $(NH_4)_2SO_4$ alone. *Philodendron* spp. extract and leachate and *D. glomeratus* leachate served as biological comparisons.

The fresh ammonium sulfate solution was reperfused through the soil for 20 days. The trials were run at room temperature (21 C) in the dark. Volume was maintained constant throughout the experiment and a 5-ml aliquot of perfusate was sampled every 2 days and analyzed colorimetrically for ammonium-, nitrite- and nitrate-nitrogen and referenced to a standard curve. The pH of the perfusate was measured periodically.

Ammonification

Four subsamples of soil were weighed out and 5 $\mu g/g$ N-Serve were added to each. Three subsamples were then amended with egg albumin, L-leucine and plant material, respectively. The remaining subsample was left unamended, thus obtaining three treatments and one control. The soils were mixed and saturated with water, then remixed. The soils were then allowed to air dry for three days.

After the soils were air dried, they were ground with a mortar and pestle and eight 20-g subsamples of each treatment and control were placed in 250-ml erlenmeyer flasks. Each soil subsample was brought to one-third atmospheric moisture and 2 ml 0.02 N H_2SO_4 placed in a small vial and into each flask on top of the soil or in the center well of a flask, depending on what type of erlenmeyer flask was being used. (For each experiment, however, the types of flasks used were all the same.) The acid trap was not placed in two of the flasks for each treatment and the soil was instead analyzed immediately. The flasks were capped and placed in a dark room and two samples were analyzed every 7 days.

Analysis of the soil included extraction with 2 N KCl (3 ml/g soil), filtration of the extract which was brought up to a 100-ml volume with distilled water, and analysis of the residue for total N, fixed NH_4^+, organic N and exchangeable NH_4^+, according to Bremner (1965a). The acid traps were analyzed for volatilized NH_3 by Nesslerization (Allen 1957).

Three different soils were used -- *Atriplex* dominated, *Ceratoides* dominated and garden soil. *Atriplex* plant material was used with *Atriplex*-dominated soil, *Ceratoides* plant material with *Ceratoides*-dominated soil and alfalfa with the garden soil. Ammonification data are stored under DSCODE A3USQ03.

ATP Concentration of the Soil

There exists a linear relationship between the amount of ATP added to the firefly (*Photinus pyralis*) luciferin-luciferase system and the duration of light output. The following is a summary of the mechanism of light emission as described by Ausmus (1971).

$$LH_2 + ATP + E \xrightleftharpoons{Mg^{++}} E\,LH_2\,AMP + PP$$

$$E\,LH_2\,AMP + O_2 \xrightarrow[pH]{\text{neutral}} E + \text{product} + CO_2 + AMP + \text{light}$$

LH_2 = luciferin

E = luciferase

This relationship, therefore, is the basis for the assay of microgram quantities of ATP by measuring the light intensity of the luminescence.

To prepare the luciferin-luciferase suspension, a vial of firefly lantern extract (Sigma FLE-50 or 250) was rehydrated with deionized-distilled water. The vial was left to stand at room temperature for 2 hr and then stored at 4 C. Before use, the suspension was centrifuged at 10,000 g for 10 min to remove insoluble debris, although this is not absolutely necessary for this type of enzyme preparation (Holm-Hansen 1973).

A standard stock solution was prepared initially by dissolving 10 mg of crystalline disodium ATP in 1000 ml tris buffer (0.25 M). The solution was capped and stored at -4 C and working standards were prepared by thawing and diluting stock solution with tris buffer to the desired concentration. Freezing and thawing often, however, are known to lower activity (Stanley and Williams 1969). A standard curve of concentration vs. midrange of count sequence was plotted on log paper and unknowns determined by such a curve. It is necessary to count the standards with each sample determination.

A liquid scintillation spectrometer, Packard Tri-carb Model 527, was used to determine light intensity. The settings included: 1) one channel only; 2) amplification set at 10%; 3) discriminators set at 50-1000; 4) repeat mode used for 20 sequential counts; and 5) spectrometer used out-of-coincidence.

Two grams of soil were placed in a 250-ml boiling flask and 25 ml of 1:1 boiling solution of tris buffer and 95% ethanol were added. The flask was placed on a high-vacuum, rotating evaporator so that it was partially submerged in a 55 C water bath. After 5 min, the contents of the flask were brought up to a 25-ml volume with ice-cold tris buffer. Then the soil extract was centrifuged for 10 min at 10,000 g to remove the soil particles. Aliquots of the extract were dispensed into test tubes, capped and placed in an ice bath for immediate assay. It was found that keeping the extract ice cold immediately after bringing up to volume until counting gave more reliable results. Strehler (1968) advises that any suitable method for extraction which will instantaneously or very rapidly destroy enzymes which can alter ATP concentration is applicable to the firefly assay provided that any inhibitory substances used are removed prior to assay, such as the ethanol in our case.

When determining the light intensity, exactly 1.8 ml of standard or soil extract ATP solution were pipetted into a glass, liquid-scintillation counting vial. At zero time, 0.2 ml of reconstituted enzyme extract was added and shaken. The the vial was placed on the elevator of a liquid-scintillation counter and lowered into the counting chamber. The first count was begun 15 sec after the enzyme addition for 6 sec with a 2-sec interval between counts for printout.

The analysis was conducted in semidarkness to avoid chemoluminescence and the scintillation vials were stored in the dark when not in use.

A blank was made by adding 0.2 ml of enzyme to 1.8 ml tris buffer. In addition, a soil extract blank was made by counting 1.8 ml of extract alone. The average of sequential counts was subtracted from the luminated samples.

The method described above followed the method of Utter (1972) with some modifications.

Catalase Activity in Soil

Several methods were tried for catalase activity determination in soils (Johnson and Temple 1964); these are described below.

Titrimetric Method

A 2-g, oven-dry soil sample was placed in a 125-ml erlenmeyer flask with 40 ml of distilled water and put on a shaker water bath. To this was added 5 ml of 0.3% H_2O_2 solution and the slurry was shaken for 20 min.

The remaining peroxide in the flask was then stabilized by adding 5 ml of 3 N H_2SO_4, the contents of the flask filtered through S & S No. 597 filter paper, and a 25-ml aliquot titrated with ca 0.1 N $KMnO_4$.

The initial concentration of the peroxide used was determined by titration in acid solution with 0.1 N $KMnO_4$. The $KMnO_4$ was standardized against sodium oxalate. All titrations were corrected for a blank.

The soil slurry filtrate titration values were subtracted from the amount of $KMnO_4$ needed to titrate the initial peroxide and calculated as ml of 0.1 N $KMnO_4$. Final results were expressed as ml of 0.1 N $KMnO_4$ equivalent to the peroxide decomposed per gram of oven-dry soil. The initial tiration value was: slurry titration value = ml of 0.1 N $KMnO_4$ equivalent to H_2O_2 decomposed.

Catalase Standard Curve

Stock catalase standard solution was prepared by dissolving 1 mg of catalase (Fungal, Sigma-Pure) in 100 ml of distilled water. Serial dilutions were made from the stock solution containing between 0.1 and 0.00078 mg/100 ml.

The solutions were titrated to get the ml $KMnO_4$ equal to H_2O_2 decomposed during specified periods of incubation time, i.e., 10, 15 and 20 min.

Gasometric Method

This method is based on measurement of molecular oxygen evolved as a result of the action of the enzyme on H_2O_2 during incubation.

Two, four or five g of sieved soil were weighed into 125-ml erlenmeyer flasks in duplicate. A control flask with only distilled water, plus one with sterile soil and H_2O_2 added were used with each set of sample flasks.

To the sample flasks, by injection through a rubber-capped entry tube, were added either 5, 8 or 10 ml of either 0.3% or 3.0% H_2O_2. The amount of oxygen evolved was measured in cubic centimeters of water displaced (= cc of O_2 evolved) after a specified time period.

Establishment of Blank Values

Each field-dry, sieved soil was weighed into flasks or beakers. The flasks stoppered with cotton plugs were put in an autoclave at 121 C for 2 hr. The beakers were placed in a 170 C oven for 6 hr.

After the two sets of soils were sterile a third batch of fresh soil was weighed into flasks for determination of catalase activity. The sterile soils were weighed into flasks and the procedure for measurement of catalase activity was carried through by using the titrimetric method.

Catalase Standard Time vs. Activity Curve

Two g of fresh soil were weighed into each of 18 flasks plus an equal number of flasks for blanks. The 40 ml of distilled water were added plus 5 ml of H_2O_2 to each of the flasks and started timing immediately upon addition of the peroxide (0.3%) and starting of the shaker. To each blank flask were added 45 ml of distilled water only.

Flasks, sample sets and blanks were removed from the shaker and 5 ml of the H_2SO_4 added to stabilize after different lengths of time, i.e., 2.5, 5.0, 10, 15 and 20 min. Each flask was then titrated with 0.1 N $KMnO_4$.

RESULTS

Air-Dried Soils and Soils Solely Amended with $(^{15}NH_4)_2SO_4$ (A3USQ00,01)

Table 1 shows the fraction analysis of the different forms of nitrogen and the pH for both the initial time when the experiments were begun, and for the fifth week when they were terminated. Upon inspection of the data, three distinct patterns become apparent. For the soils which were wetted to 25% and allowed to air dry, and the soils which were amended with 912 μg ^{15}N and also air dried, similar patterns of nitrogen cycling emerged; fixed NH_4^+ decreased while a substantial increase of organic nitrogen (16-57%) is observed. In addition, a 39- to 93-fold increase of NO_2^- and NO_3^- (primarily in the form of NO_3^-) is also noted. Exchangeable NH_4^+ showed slight increases in both of the experimental systems, except for the 5-1 soil treated with $(^{15}NH_4)_2SO_4$ which showed no change.

The soils treated with ^{15}N-labeled ammonium sulfate and maintained at either -1 bar or -15 bars also had similar results with the exception being the 6-1 soil at -15 bars. All of the soil types (5-1, 6-1 and 7-1) at -1 bar water tension, and the soils 5-1 and 7-1 at -15 bars water tension showed increases in fixed NH_4^+ and in NO_2^- and NO_3^- (10- to 54-fold and again in the predominant form of NO_3^-) while decreases in exchangeable NH_4^+ and organic nitrogen were noted.

Soil 6-1, which was maintained at -15 bars water tension, was the only exception. As in the other soils, exchangeable NH_4^+ had decreased, while fixed NH_4^+, and NO_2^- and NO_3^- (29-fold) had both increased. The difference was in the organic nitrogen fraction, where a 7.03% increase is observed (Table 7).

Figures 1-8 and Table 2 give the data on ammonia volatilization from the above-mentioned experimental systems. For both the untreated, air-dried soils and the ^{15}N-amended, air-dried soils, ammonia volatilization was plotted with the changes in pH, bar potential and percent moisture (Figs. 1-7). As can be seen from these graphs, and in Table 2, ammonia volatilization from the three different soils is as follows: *Artemisia* soil < *Ceratoides* soil < *Atriplex* soil. In all cases, the percent ammonia volatilized did not exceed 1.33%, this value being from the ^{15}N ammonium sulfate-amended, air-dried soil of 7-1. Finally, the soils which were maintained at -1 or -15 bars water tension (Figs. 4, 5) show fluctuations in the volatilized ammonia, thus indicating the effect of nitrification on this process when held at a constant bar potential. However, when the soils are air dried, and amended or nonamended with ^{15}N, a generally smooth curve is observed (Figs. 1-3 and 6-8), thus limiting or removing the influence of nitrification. It is also noteworthy that the nitrification process affects the soil pH as well. During the first two weeks the greatest amount of ammonia is volatilized, paralleling an increase in pH (Figs. 1-3 and 6-8) but, thereafter, the pH decreases and falls below the initial pH by 0.2-0.5. Presumably, the decrease in pH is due to the nitrification process.

^{15}N-Plant Material Amendments to Soil (A3USQ00, 01)

Table 3 lists the data concerning the fraction analysis of nitrogen in the soils amended with ^{15}N-plant material. It is observed that soils amended with ^{15}N plus plant material do not display similar patterns under identical water tension regimes as in the ^{15}N ammonium sulfate amendments. In general, the soils 6-1 and 7-1 are similar in that exchangeable NH_4^+ decreases, with increases in organic nitrogen and in NO_2^- and NO_3^-(10- to 17-fold, again primarily in the form of NO_3^-). The main difference between these two soils is that soil 6-1 experienced a decrease in fixed NH_4^+, while 7-1 had an increase, and that a 25.7% loss of nitrogen in 6-1 was seen via the denitrification process, whereas soil 7-1 had a 0% denitrification rate, with a gain in nitrogen (Table 4).

Soil 5-1 was identical to 7-1 except that the increase in NO_2^- and NO_3^- were of both nitrite (57%) and nitrate

(43%), thus indicating an allelopathic inhibition on nitrification by the fresh plant material. However, a 2.56% loss of nitrogen is observed via denitrification (Table 4).

In ^{15}N-plant material amendments to soil held at -15 bars water tension, a different set of results is obtained (Table 3). First, it should be noted that soil 6-1 has no data after the five-week period as the sulfuric acid backed up during sampling and consequently acidified some of the soil. Soils 5-1 and 7-1 are identical as both have increases in fixed NH_4^+, organic N (18.5% and 15.3%, respectively) and in NO_2^- and NO_3^- (2.5- to 31-fold). However, it is in the NO_2^- and NO_3^- data that these soils differ. In soil 5-1, only a 2.5-fold increase is noted, with most of the oxidized nitrogen in the form of nitrite (60%), while in soil 7-1, nitrate is the predominant form (85%). Also, there is a decrease in exchangeable NH_4^+ in 5-1, while an increase is observed in 7-1.

Figures 9-11 and Tables 5, 6 and 7 give the data on NH_3 volatilization and on the soil fraction analysis in the ^{15}N plant material-amended soils. Again, as in the soils treated with ^{15}N ammonium sulfate, ammonia volatilization follows the same pattern: *Artemisia* soil < *Ceratoides* soil < *Atriplex* soil. In addition, nitrification does not appear to be influencing volatilization as smooth curves are obtained in all three cases. Furthermore, the peak of volatilization is reached by the first week, as confirmed by the ^{15}N data (Fig. 10 and Table 5). Last, in the 5-1 and 7-1 soils, the pH by the fifth week had increased by 0.1 when held at -1 bar moisture tension but decreased (0.6 and .3, respectively) at -15 bars. On the other hand, the 6-1 soil experienced a 0.2 decrease in pH at -1 bar. This correlates well in that this soil had the highest percent denitrification (25.7%) thereby reflecting the highest amount of NO_2^- and NO_3^- (83.77 μg/g, Table 3). Table 4 gives the total nitrogen balance sheet for all of the experimental systems.

Table 1. Soil analysis of organic N, total NH_4^+, NO_2^- and NO_3^- for untreated soils and soils treated with $(^{15}NH_4)_2SO_4$. (All values expressed as μg N/g soil)

	Air Dry			$(^{15}NH_4)_2SO_4$ at -1 Bar		
	Soil Sample					
	5-1	6-1	7-1	5-1	6-1	7-1
	I. Initial (Time: 0)					
Exchangeable NH_4^+	2.8	0.47	3.97	8.87	5.13	8.63
Fixed NH_4^+	73.97	84.70	63.46	77.76	61.20	57.0
Organic N	709.1	712.83	824.84	1084.24	898.27	927.90
Total N	785.87	798.0	892.27	1170.87	965.60	993.53
NO_2^- and NO_3^-	1.4	0.93	1.63	7.93	1.6	0.0
pH	8.62	8.57	8.80	8.52	8.58	8.91
	II. Final (Time: 5 weeks)					
Exchangeable NH_4^+	3.85	4.9	7.0	0.7	0.0	5.95
Fixed NH_4^+	68.55	76.6	59.15	96.25	82.95	64.55
Organic N	966.4	1088.2	900.55	932.05	848.05	755.5
Total N	1038.8	1169.7	966.7	1029.0	931.0	826.0
NO_2^- and NO_3^-	54.6	87.15	68.6	129.15	86.8	38.15

Table 1, continued

	$(^{15}NH_4)_2SO_4$ at -15 Bars			$(^{15}NH_4)_2SO_4$- Air Dry		
	Soil Sample					
	5-1	6-1	7-1	5-1	6-1	7-1
NO_2^-	9.1	2.8	0.0	10.15	2.1	3.5
NO_3^-	45.5	84.35	69.65	119.0	84.7	34.65
pH	8.40	8.29	8.46	8.28	8.38	8.39
	I. Initial (Time: 0)					
Exchangeable NH_4^+	8.87	5.13	3.97	2.8	0.47	3.97
Fixed NH_4^+	77.76	61.2	63.46	73.97	84.7	63.46
Organic N	1084.24	898.27	824.84	709.1	712.83	824.84
Total N	1170.87	965.6	892.27	785.87	798.0	892.27
NO_2^- and NO_3^-	7.93	1.6	1.63	1.4	0.93	1.63
pH	8.52	8.58	8.80	8.71	8.53	8.72
	II. Final (Time: 5 weeks)					
Exchangeable NH_4^+	1.75	2.45	2.8	2.8	2.1	4.55
Fixed NH_4^+	102.9	89.75	61.9	57.7	67.5	55.1
Organic N	1067.15	927.0	751.5	862.1	979.0	996.65
Total N	1171.8	1019.2	816.2	922.6	1046.5	1056.3
NO_2^- and NO_3^-	79.1	46.55	58.1	71.05	80.15	90.65
NO_2^-	0.0	0.0	1.4	0.0	0.0	8.05
NO_3^-	79.1	47.95	56.7	71.25	80.15	90.65
pH	8.20	8.42	8.32	8.52	8.27	8.51

Table 2. Ammonia volatilization from untreated and $(^{15}NH_4)_2SO_4$ amended soils. (All values expressed as μg NH_3 volatilized from 100 g of soil)

	Air Dry			$(^{15}NH_4)_2SO_4$ at 1 Bar		
	Soil Sample					
Time	5-1	6-1	7-1	5-1	6-1	7-1
24 hrs	11.9	22.4	29.4	7.0	36.4	51.8
1 wk	22.4	26.6	81.9	18.2	44.8	77.0
2 wks	27.3	23.5	244.3	47.6	14.0	148.4
3 wks	20.3	16.8	130.9	46.2	15.4	91.0
4 wks	20.3	25.2	123.9	50.4	14.0	156.8
5 wks	21.7	30.8	69.3	29.4	36.4	75.6
Total NH_3	123.9	145.3	679.7	198.8	161.0	600.6
% volatilized	0.157	0.182	0.759	0.165	0.162	0.586
	$(^{15}NH_4)_2SO_4$ at -15 Bars			$(^{15}NH_4)_2SO_4$- Air Dry		
24 hrs	39.2	53.2	37.8	7.0	110.6	187.6
1 wk	9.8	23.8	50.4	14.0	156.8	610.4
2 wks	32.2	8.4	22.4	14.0	32.2	232.4
3 wks	23.8	25.2	32.2	8.4	21.0	58.8
4 wks	23.8	14.0	28.0	8.4	47.6	98.0
5 wks	23.8	23.8	16.8	5.6	16.8	44.8
Total NH	152.6	148.4	187.6	57.4	385.0	1232.0
% volatilized	0.126	0.149	0.203	0.07	0.469	1.33

Table 3. Soil analysis of organic N, total NH_4^+, NO_2^- and NO_3^- for $(^{15}NH_4)_2SO_4$+ plant material-amended soils. (All values expressed as μg N/g soil)

	$(^{15}NH_4)_2$ SO_4+			$(^{15}NH_4)_2$ SO_4+		
	Plant material at 1 Bar			Plant material at -15 Bars		
	Soil Sample					
	5-1	6-1	7-1	5-1	6-1	7-1
	I. Initial (Time: 0)					
Exchangeable NH_4^+	8.87	3.73	17.77	2.8	0.47	3.97
Fixed NH_4^+	96.83	83.87	62.45	73.97	84.70	63.46
Organic N	1169.7	1183.6	647.85	709.1	712.83	824.84
Total N	1275.4	1271.2	728.07	785.87	798.0	892.27
NO_2^- and NO_3^-	3.89	4.98	7.31	1.4	0.93	1.63
Plant material (mg)	11.94	13.04	13.04	11.94	13.04	13.04
Total N/100 soil (mg)	142.87	143.66	89.58	93.67	95.54	105.43
pH	8.05	8.21	8.23	8.58	8.60	8.79

Table 3, continued

	$(^{15}NH_4)_2$ SO_4+			$(^{15}NH_4)_2$ SO_4+		
	Plant material at 1 Bar			Plant material at -15 Bars		
	Soil Sample					
	5-1	6-1	7-1	5-1	6-1	7-1
	II. Final (5 weeks)					
Exchangeable NH_4^+	3.03	3.5	3.5	2.1	[a]N.D.	9.1
Fixed NH_4^+	125.77	87.93	78.5	86.95	N.D.	72.6
Organic N	1190.0	891.37	789.73	1016.95	N.D.	1129.3
Total N	1318.8	982.8	871.73	1106.0	N.D.	1211.0
NO_2^- and NO_3^-	71.63	83.77	75.83	3.5	N.D.	49.7
NO_2^-	40.83	2.1	0.0	2.1	N.D.	7.35
NO_3^-	30.8	82.6	77.35	1.4	N.D.	42.35
pH	8.13	8.01	8.31	8.52	N.D.	8.51

[a]N.D. = No data.

Table 4. N balance sheet (all values expressed in mg)

	Air-dry			$(^{15}NH_4)_2$ SO_4 at 1 Bar			$(^{15}NH_4)_2$ SO_4 at 15 Bars			$(^{15}NH_4)_2$ SO_4 - Air-dry		
	Soil sample											
	5-1	6-1	7-1	5-1	6-1	7-1	5-1	6-1	7-1	5-1	6-1	7-1
Initial Total N	78.73	79.89	89.39	121.88	99.72	102.35	121.88	99.72	92.39	81.73	82.89	92.39
Final Total N	109.46	125.83	104.21	116.01	101.94	87.02	125.24	106.72	87.62	99.42	113.05	115.93
mg of N denitrified	0	0	0	5.87 (4.83%)	0	15.33 (14.95)	0	0	4.7 (5.17)	0	0	0
mg N fixed	30.73	45.94	14.82	0	2.22	0	3.36	7.0	0	17.69	30.16	23.54
% N excess	39.0	57.4	16.8	0	2.23	0	2.27	7.03	0	21.6	36.4	25.4

Initial total N = total N + NO_2^- and NO_3^- + 3.0 mg $(^{15}NH_4)_2$ SO_4/100 g soil.
Final total N = total N + NO_2^- and NO_3^- + NH_3 volatilized/100 g soil.
N fixed = heterotrophic fixation in the form of organic N - fixed NH_4^+/100 g soil.

	$(^{15}NH_4)_2$ SO_4+ plant material at 1 Bar			$(^{15}NH_4)_2$ SO_4+ plant material at 15 Bars		
	Soil sample					
	5-1	6-1	7-1	5-1	6-1	7-1
Initial Total N	142.87	143.66	89.58	93.67	95.54	105.43
Final Total N	139.22	106.88	95.67	111.0	N.D.	121.61
mg of N denitrified	3.65 (2.56%)	36.78 (25.7%)	0	0	0	0
mg N fixed	0	0	5.5	17.33	N.D.	16.18
% N excess	0	0	5.8	18.5	N.D.	15.3

Initial total N = total N + NO_2^- and NO_3^- + 3.0 mg $(^{15}NH_4)_2$ SO_4+ 1 g plant material/100 g soil.
Final total N = total N - NO_2^- and NO_3^- + NH_3 volatilized/100 g soil.
N fixed = heterotrophic fixation in the form of organic N + fixed NH_4^+/100 g soil.
N.D. = No data.

Table 5. Ammonia volatilization from $(^{15}NH_4)_2SO_4$ + plant material-amended soils. (All values expressed as μ g NH_3 volatilized from 100 g of soil)

Time	$(^{15}NH_4)_2SO_4$ + plant material at -1 Bar						$(^{15}NH_4)_2SO_4$ + plant material at -15 Bars		
	Soil sample								
	5-1		6-1		7-1		5-1	6-1	7-1
	Total NH_3	$^{15}NH_3$	Total NH_3	$^{15}NH_3$	Total NH_3	$^{15}NH_3$			
24 hrs	45.5	3.49	67.9	2.94	60.9	5.16	5.6	71.4	295.4
1 wk	50.4	2.2	93.1	3.18	369.6	8.76	8.4	56.0	148.4
2 wks	26.6	0.36	25.2	0.72	342.3	4.18	7.0	16.8	25.2
3 wks	14.0	0.078	11.2	0.16	112.7	1.18	11.2	8.4	16.8
4 wks	14.7	0.134	4.9	0.03	16.8	0.14	4.2	8.4	30.8
5 wks	23.8	0.129	16.8	0.23	12.6	0.16	9.8	9.8	19.6
Total NH	175.0	6.39	219.1	7.26	914.9	19.58	46.2	170.8	536.2
% volatilized	0.12	0.7	0.15	0.8	1.04	2.12	0.049	0.178	0.50

Table 6. Atom percent excess ^{15}N lost as volatilized NH_3 from $(^{15}NH_4)_2SO_4$ plant material-amended soils maintained at -1 bar

Time	Soil Sample 5-1		6-1		7-1	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
24 hrs.	9.408	5.622	6.473	1.388	5.817	9.112
1 Wk.	4.554	3.972	3.170	3.591	2.626	1.870
2 Wks.	1.175	-----	3.752	1.432	1.006	1.363
3 Wks.	0.591	0.505	0.480	1.708	0.862	1.137
4 Wks.	0.933	0.833	0.750	0.506	0.882	0.737
5 Wks.	0.578	0.466	0.858	1.552	0.374	0.801

Table 7. Soil ^{15}N fractions from $(^{15}NH_4)_2SO_4$ plant material-amended soils maintained at -1 bar

Site	N fraction	μg ^{15}N per 100 g soil	Atom % excess ^{15}N*	% loss ^{15}N
Artemisia (5-1)	Exchangeable $^{15}NH_4^+$	0.42	.0013	
	Fixed $^{15}NH_4^+$	17.68	.1406	
	Organic ^{15}N	122.08	.1063	
	$^{15}NO_2^-$ **	17.52	.4290	
	$^{15}NO_3^-$	13.20	.0043	
	$^{15}NH_3$	6.39	--	
	Total	177.29	--	80.57
Eurotia (6-1)	Exchangeable $^{15}NH_4^+$	0.59	.0017	
	Fixed $^{15}NH_4^+$	14.91	.1696	
	Organic ^{15}N	132.50	.1506	
	$^{15}NO_2^-$ **	7.65	.5575	
	$^{15}NO_3^-$	40.72	.4930	
	$^{15}NH_3$	7.26	--	
	Total	203.63	--	77.68
Atriplex (7-1)	Exchangeable $^{15}NH_4^+$	0.60	.0017	
	Fixed $^{15}NH_4^+$	13.50	.1720	
	Organic ^{15}N	99.48	.1303	
	$^{15}NO_2^-$ **	4.53	.4296	
	$^{15}NO_3^-$	28.03	.3625	
	$^{15}NH_3$	19.58	--	
	Total	165.73	--	81.83

* Average of 3 determinations.
** NO_2^- was determined by subtracting the value of NO_2^- from NO_2^- and NO_3^-. Therefore, the atom % excess given is that of the NO_2^- and NO_3^- determination.

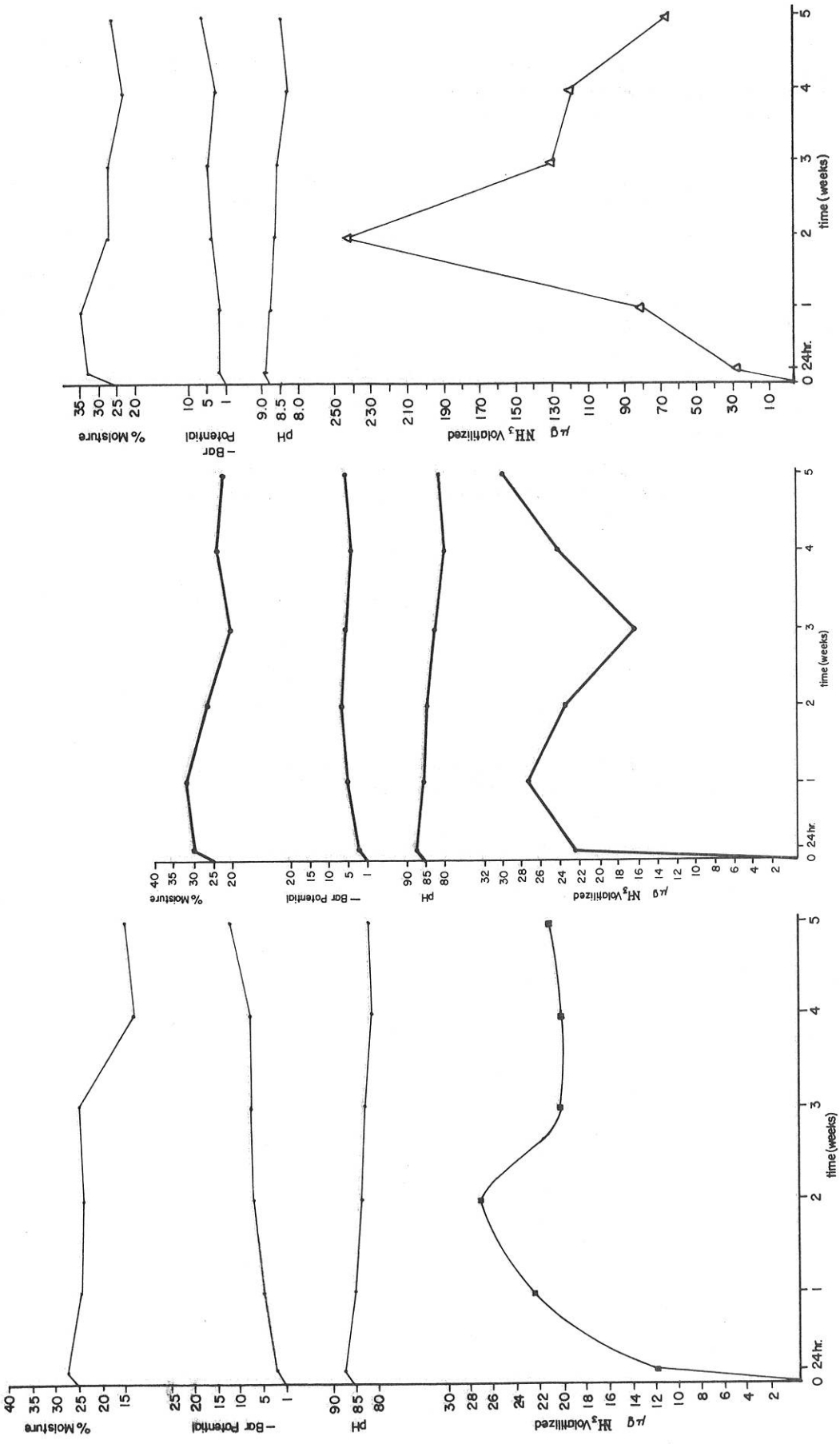


Figure 1. Ammonia volatilization of air-dried soil; 5-1, temperature = 22 C.

Figure 2. Ammonia volatilization of air-dried soil; 6-1, temperature = 22 C.

Figure 3. Ammonia volatilization of air-dried soil; 7-1, temperature = 22 C.

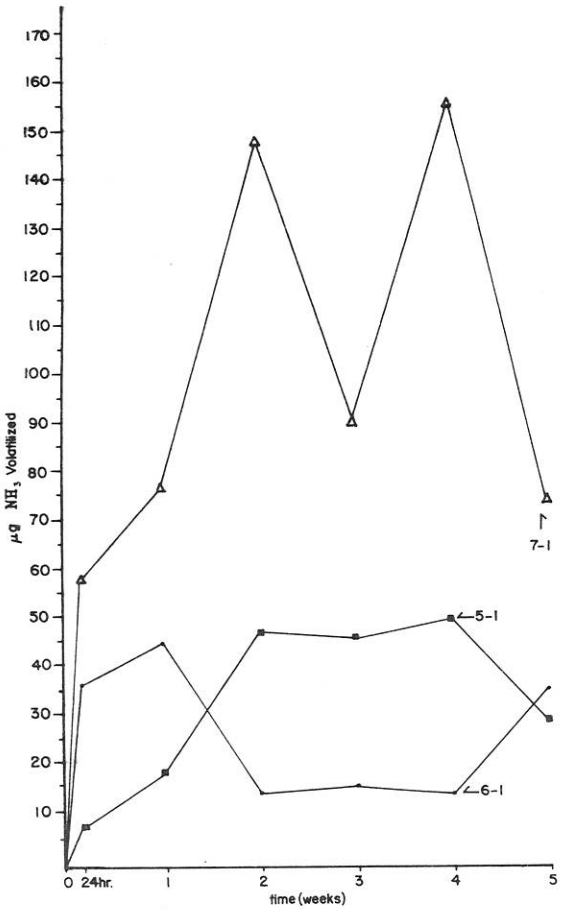


Figure 4. Ammonia volatilization of $(^{15}NH_4)_2SO_4$-amended soils; bar potential = -1, temperature = 22 C.

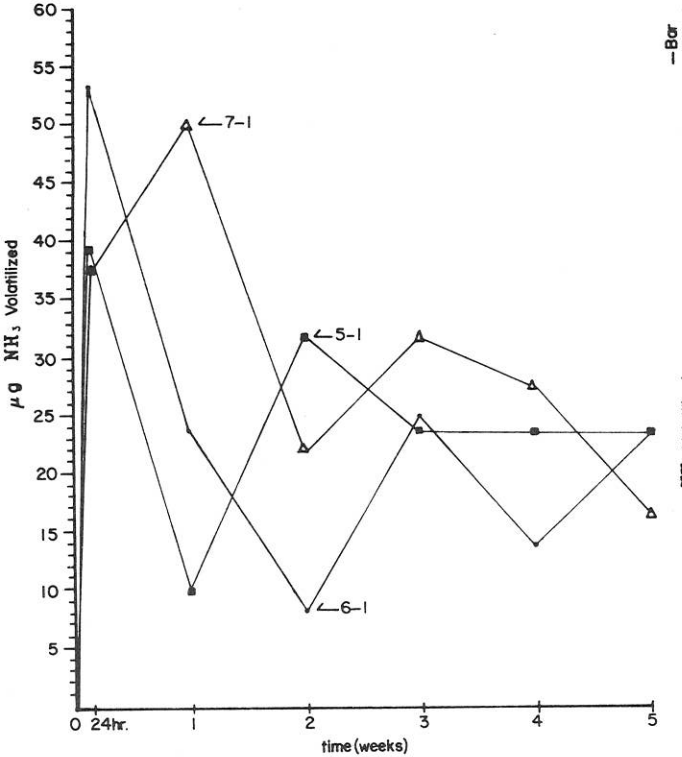


Figure 5. Ammonia volatilization of $(^{15}NH_4)_2SO_4$-amended soils; bar potential = -15, temperature = 22 C.

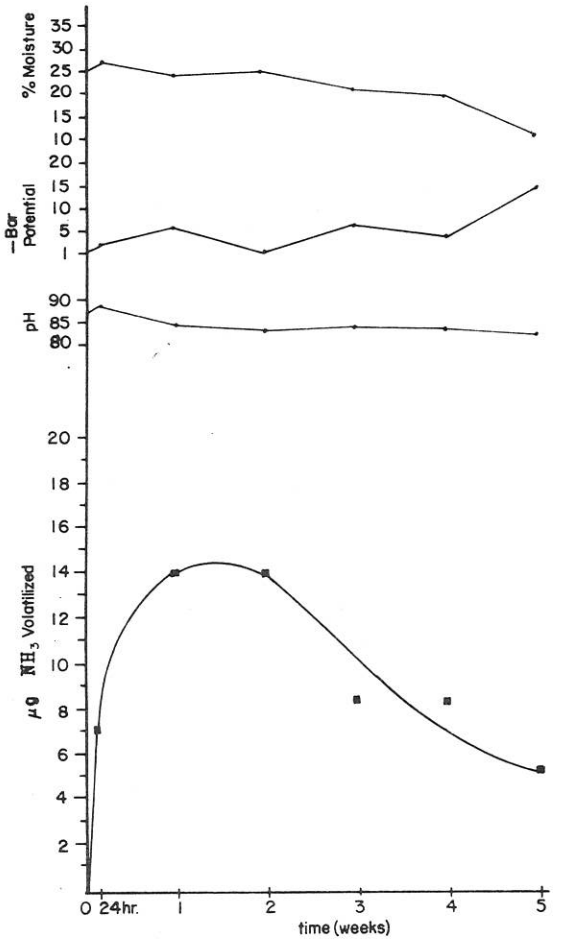


Figure 6. Ammonia volatilization of $(^{15}NH_4)_2SO_4$-amended, air-dried soil; 5-1, temperature = 22 C.

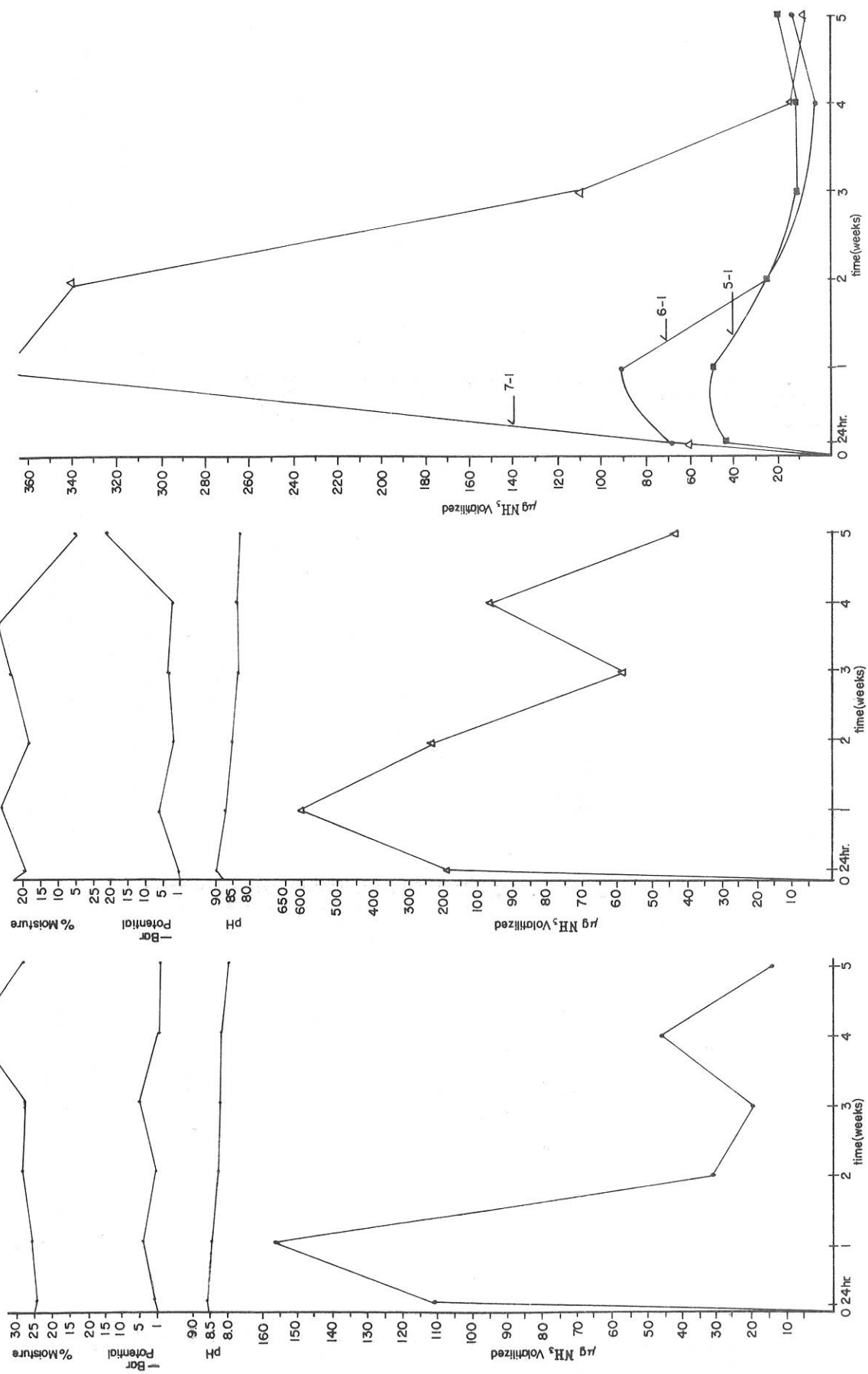


Figure 7. Ammonia volatilization of $(^{15}NH_4)_2SO_4$-amended, air-dried soil; 6-1, temperature = 22 C.

Figure 8. Ammonia volatilization of $(^{15}NH_4)_2SO_4$-amended, air-dried soil; 7-1, temperature = 22 C.

Figure 9. Ammonia volatilization of $(^{15}NH_4)_2SO_4$ plant material-amended soils; bar potential = -1.0, temperature = 22 C.

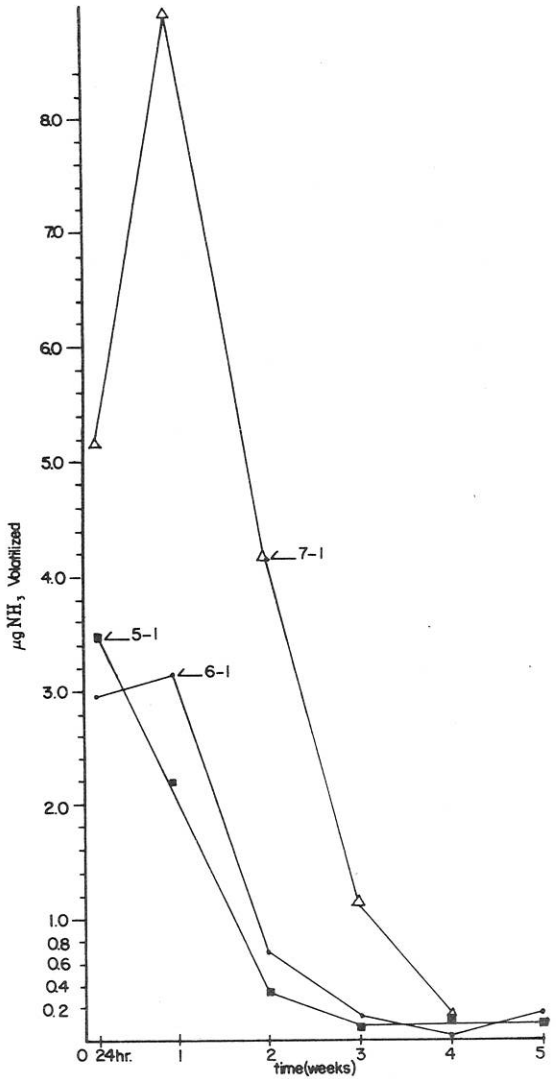


Figure 10. ^{15}N ammonia volatilization of $(^{15}NH_4)_2SO_4$ plant-amended soils; bar potential = -1.0, temperature = 22 C.

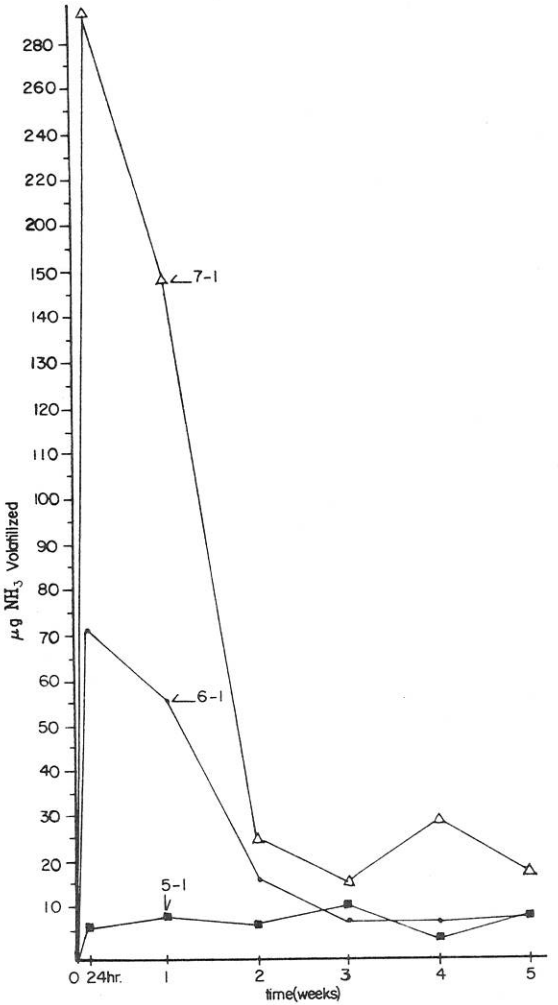


Figure 11. Ammonia volatilization of $(^{15}NH_4)_2SO_4$ plant material-amended soils; bar potential = -15, temperature = 22 C.

Acetylene Reduction Assay (A3USQ04)

Figure 12 shows the acetylene reduction assay of two relatively active soil cores with intact crust periodically sampled for ethylene over 25 hr. Both samples were collected "dry" from the *Atriplex confertifolia* site, moistened and assayed in the laboratory two weeks after collection. Both samples show that the rate of acetylene reduction is essentially linear between 2 and 25 hr. However, the sample collected in October exhibited a slight lag. The fact that there is little or no lag indicates that the blue-green algae begin fixing nitrogen almost immediately after a rainfall or moistening. Ethylene production has been detected within 5 min after moistening a crust with water. The presence or absence of lag may depend upon such factors as endogenous levels of ATP, ammonium ion or reducing substances. Those factors may in turn be a reflection of the drying conditions. For example, slower drying under lower temperatures and light intensities might have a significantly different effect than rapid drying under higher temperatures and light intensities.

Since assays were linear for at least 25 hr, a sample could be assayed at, for example, 23.8 hr, and activity extrapolated to 24 hr in order to express activity per 24 hr; or, for example, assay at 24.3 hr and extrapolate back to get activity per 24 hr. This avoids the complicating use of reagents to stop the nitrogen fixation reaction. In effect, the reaction, for analysis purposes, is stopped when the gas sample is removed from the reaction tube or bottle.

No detectable ethylene was emitted from the soil cores with intact blue-green algae-lichen crusts in the absence of acetylene, and soil beneath the crust did not reduce acetylene.

Ar Versus N_2

There was no difference in soil core acetylene reduction under Ar, O_2, CO_2 vs. the natural air atmosphere. This agrees with the results of Stewart et al. (1971).

Dark Nitrogen Fixation

Dark nitrogen fixation was 5 to 30% of nitrogen fixation in the light (Tables 8 and 9). Presumably, photosynthesis supplies the energy (ATP) and reductant necessary for dark nitrogen fixation. Table 8 shows that moistening and preincubating soil cores with crusts in the light results in much greater fixation in the dark, when compared with cores moistened and preincubated in the dark and subsequently assayed in the dark. The lower nitrogen fixation with samples moistened and preincubated in the dark is evidence of loss of reductant due to dark respiration. Samples that were not preincubated exhibited nitrogen-fixing activity slightly greater than the samples moistened and preincubated in the light, indicating that the state of the blue-green algal-lichen crust (collected "dry") had a significant pool of ATP and reductant so that should an evening or nighttime rain occur, there would follow substantial nitrogen fixation. In effect then, the crust was relatively "primed" for nitrogen fixation should moisture become available (A3USQ04).

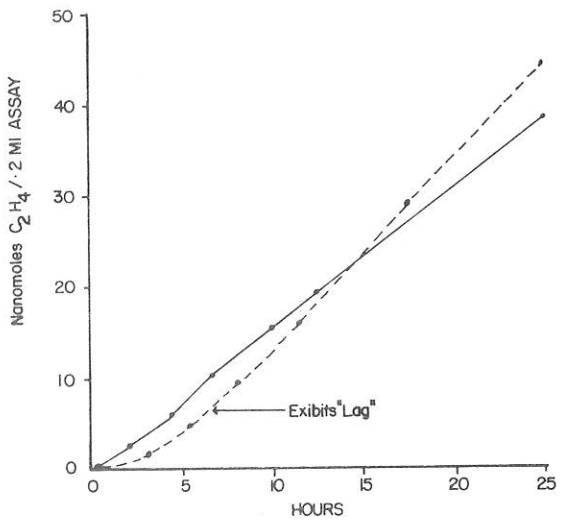


Figure 12. Rate of acetylene reduction. Broken line indicates the soil core collected in October; the solid line indicates the soil core collected in July.

Table 8. Dark nitrogen fixation

Treatment	Nanomoles C_2H_4/soil core/ 24 hours[a]
Moistened and preincubated in dark for 18 hours	6.0
Moistened and preincubated in light for 18 hours	17.6
Moistened at zero time	21.9
Moistened at zero time and incubated in light	79.7

[a]Site 7 cores; means of quadruplicate assays.

Table 9. Inhibition of autotrophic and heterotrophic nitrogen fixation by 1% extracts of *A. tridentata*

Treatment	Nanomoles C_2H_4/soil core/24 hours[a]
Dark incubation	23.9
Dark incubation/extract	20.3
Light incubation	79.7
Light incubation/extract	28.4
Dark incubation/glucose	127.5
Dark incubation/glucose/extract	40.5

[a]Site 7 soil cores; mean of triplicate assays.

Crust Heterotrophic Nitrogen Fixation Versus Temperature

Figure 13 shows that crusts, moistened with 2% glucose solutions and incubated in the dark, exhibited a temperature optimum of about 30 C for nitrogen fixation. Thus heterotrophic nitrogen fixation exhibits an optimum 10 degrees C higher than crust autotrophic nitrogen fixation. This shift may reflect the potentiation of *Azotobacter* and *Clostridium* nitrogen fixation by glucose in the crust microenvironment. Another possibility is that glucose stimulates mycobiont respiration, and thus autotrophic and/or heterotrophic nitrogen fixation is enhanced, perhaps by oxygen tension reduction.

Glucose Potentiation of Nitrogen Fixation

Table 10 shows that glucose can potentiate nitrogen fixation by crust in the light. Glucose can also potentiate nitrogen fixation of soils in the dark. Without glucose addition, there is little or no heterotrophic nitrogen fixation in soils. Thus there is a heterotrophic nitrogen fixation potential in crusts and soils beneath the crust, but nitrogen fixation is probably nil or negligible in situ due to low carbohydrate availability.

Seasonal Effects

Figure 14 shows the laboratory nitrogen fixation potential results of some acetylene reduction assays (means of triplicate assays plotted) of soil cores with intact crusts from the *Atriplex confertifolia* site. While it is difficult to draw conclusions from these few assays, a few implications may be present. Not all of the assays were performed on the same day or days after collection. Table 11 shows the dates of collection and assay and activity (nitrogen fixation) of the samples plotted in Figure 14. There does not appear to be a significant year-to-year change in the nitrogen fixation potential, particularly evidenced by the June 1973 and June 1974 values being relatively similar. However, there appear to be fall and spring peaks, which might be expected since the rainy periods occur then in the Great Basin Desert.

Diurnal fluctuations in nitrogen fixation occur (Balandreau et al. 1974), and Figure 30 in the 1973 report (Skujins and West 1974) implies this as well. Thus a diurnal fluctuation has a long-term seasonal fluctuation superimposed (A3USQ04).

Desert Shrub Canopy Effect

Nitrogen fixation was reduced under all the desert shrub canopies (Table 12). The environment under the shrub canopies is largely dominated by bryophytes. The canopy effect also involves thermal radiation effects, moisture regimes and litter organic matter. So the reduction of nitrogen fixation under the desert shrubs is due to a number of factors, but is an important aspect governing the input of nitrogen into the desert shrub communities.

Exchangeable ammonium ion and clay and organic matter-fixed ammonium ion do not appear to be significantly different under the desert shrub canopies versus the interspaces (Tables 13 and 14; A3USQ04).

Shrub Leaf Extract Inhibition of Nitrogen Fixation

Table 15 shows the effect of desert shrub leaf aqueous extracts upon algal-lichen crust nitrogen fixation. All of the extracts possess apparent inhibitors of acetylene reduction, with the leaves of *Artemisia tridentata* being particularly potent. The canopy effect involves many factors, but it is possible that desert shrub inhibitors are responsible for the scarcity of algal-lichen crusts and the dominance of bryophytes beneath the canopy. Microscopically, *Nostoc* have been observed in association with some of the lichen thalli. Significant acetylene reduction was never seen in the absence of lichens.

Some of the inhibition by extracts may be due to ammonium ion in the extracts, but this could not account for all of the inhibition (Table 16).

Leaf-soak Filtrates

Leaf-soak filtrates also showed significant inhibition of nitrogen fixation (Table 17). Perhaps plant leachate and rainwash of plants and litter can exert an inhibitory effect upon nitrogen fixation.

Inhibition of Autotrophic and Heterotrophic Nitrogen Fixation by *Artemisia tridentata* Aqueous Extracts

Aqueous extracts (1%) of *A. tridentata* leaves can significantly inhibit autotrophic nitrogen fixation (approximately 65% inhibition) and heterotrophic (glucose potentiated) nitrogen fixation (approximately 69% inhibition) (Table 9).

Potentiation and Inhibition of Heterotrophic Nitrogen Fixation

Soils, moistened with water, exhibited no measurable heterotrophic nitrogen fixation, but upon moistening soils with 10% glucose solutions there was a significant potentiation of heterotrophic nitrogen fixation (Table 18). Soils at the 40- to 50-cm depth exhibited no measurable potentiation of heterotrophic nitrogen fixation. Soils (0- to 3-cm depth), collected from beneath plant canopies, exhibited a greatly reduced heterotrophic nitrogen fixation potential compared to interspace samples. This implies that the heterotrophic nitrogen-fixing population, although low or very dormant in interspace soils, is even lower in soils beneath the shrub canopies. This indirectly supports the notion of plant microbial inhibitors.

Addition of dried leaves to soils, and moistening with water, resulted in a small potentiation of heterotrophic nitrogen fixation, with the leaf organic compounds or decomposition products presumably providing the carbon source for heterotrophic nitrogen fixation. However, dried plant leaves with soils moistened with 10% glucose solutions resulted in a significant inhibition of heterotrophic nitrogen fixation (as potentiated by glucose). This demonstrates that shrub material can inhibit heterotrophic nitrogen fixation.

Table 10. Glucose potentiation of nitrogen fixation

Sample	Conditions	Activity: nanomoles C_2H_4/g soil[a] 24 hours	48 hours	70 hours
5-crust	Light	9.9	65.0	
6-crust	Light	0.11	0.22	
7-crust	Light	2.1	1.7	
5-crust	Light/glucose	7.4	235.0	
6-crust	Light/glucose	0.46	10.0	
7-crust	Light/glucose	170.0	710.0	
5: 0-3 cm	Dark/glucose	0.0	17.5	
6: 0-3 cm	Dark/glucose	0.0	10.5	
7: 0-3 cm	Dark/glucose	6.3	290.0	
5: 5-20 cm	Dark/glucose	0.0	5.9	32.0
6: 5-20 cm	Dark/glucose	0.0	0.15	3.3
7: 5-20 cm	Dark/glucose	0.0	0.45	2.0

[a]Mean of duplicate assays.

Table 11. Nitrogen fixation; seasonal effect

Date collected[a]	Date of assay	Nanomoles C_2H_4/soil core/24 hrs[b]
14X72	27XII72	58.5
26XI72	28XI72	212.4
26III73	21V73	297.0
14IV73	26IV73	720.0
8VI73	13VI73	108.0
5IX73	11II74	132.8
12VI74	14VI74	85.5

[a]Site 7 soil cores.
[b]Mean of triplicate assays.

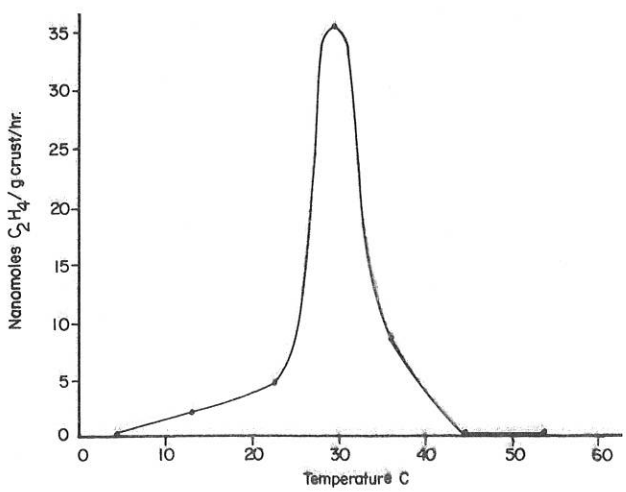


Figure 13. Effect of temperature upon crust heterotrophic nitrogen fixation.

Table 12. Effect of desert shrub canopy upon acetylene reduction

Site	Nanomoles C_2H_4/soil core/24 hours[a] Interspace	Under canopy
Atriplex confertifolia	78.7	20.7
Ceratoides lanata	4.5	2.3
Artemisia tridentata	79.5	10.8

[a]Mean of triplicate assays.

Table 13. KCl extractable NH_4^+—N; interspace versus canopy

Sample[a]	NH_4^+-N µg/g soil
5-interspace	3.6
5-interspace, no crust	1.5
5-under canopy	2.9
6-interspace	2.4
6-under canopy	2.2
7-interspace	0.0
7-interspace, no crust	0.0
7-under canopy	0.0

[a]0-3 cm soil samples collected 12V73.

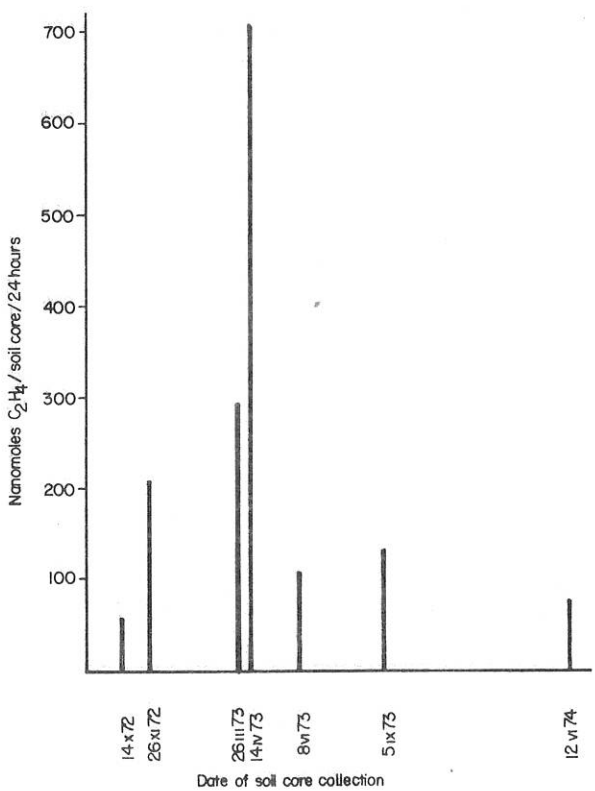


Figure 14. Nitrogen fixation seasonal effect.

Table 14. Clay and organic matter-fixed NH_4^+—N; interspace versus canopy

Sample[a]	NH_4^+-N µg/g soil
5 - interspace	29.8
5 - interspace, no crust	50.6
5 - under canopy	46.9
6 - interspace	39.4
6 - under canopy	25.7
7 - interspace	30.9
7 - interspace, no crust	29.7
7 - under canopy	27.3

[a]0-3 cm soil samples collected 12V73, kjeldahl distillation with 5 ml 40% NaOH.

Table 15. Inhibition of nitrogen fixation by desert shrub leaf extracts

Extract	Nanomoles C_2H_4/soil core/24 hrs[a]
Control (water)	24.8
Artemisia tridentata	0.0
Ceratoides lanata	5.2
Atriplex confertifolia	8.6

[a]Mean of triplicate assays.

Table 16. Ammonium ion in 5% extracts of desert shrub leaves

Plant	µg NH_4^+-N/ml extract
Atriplex confertifolia	12.3
Ceratoides lanata	9.4
Artemisia tridentata	7.3

Table 17. Inhibition of nitrogen fixation by desert shrub leaf-soak filtrates

Filtrate	Nanamoles C_2H_4/soil core/hr[a]
Control (water)	25.6
Artemisia tridentata	1.1
Ceratoides lanata	16.3
Atriplex confertifolia	14.7

[a]Mean of triplicate assays.

Table 18. Potentiation and inhibition of heterotrophic nitrogen fixation

Site	Soil sample	Amendment[a]	Nanomoles C_2H_4 /g soil, in 3 days at 30C
Artemisia tridentata	0-3 cm	--	0.0
	0-3 cm	glucose	1760.7
	0-3 cm,under canopy	glucose	4.5
	5-20 cm	--	0.0
	5-20 cm	glucose	27.0
	40-50 cm	--	0.0
	40-50 cm	glucose	0.0
Ceratoides lanata	0-3 cm	--	0.0
	0-3 cm	glucose	5740.7
	0-3 cm,under canopy	glucose	296.1
	5-20 cm	--	0.0
	5-20 cm	glucose	458.8
	40-50 cm	--	0.0
	40-50 cm	glucose	0.0
Atriplex confertifolia	0-3 cm	--	0.0
	0-3 cm	glucose	1696.5
	0-3 cm,under canopy	glucose	90.0
	5-20 cm	--	0.0
	5-20 cm	glucose	4.8
	40-50 cm	--	0.0
	40-50 cm	glucose	0.0
	0-3 cm	Artemisia leaves	2.7
	0-3 cm	Ceratoides leaves	6.3
	0-3 cm	Atriplex leaves	2.7
	0-3 cm	Artemisia leaves/glucose	3.6
	0-3 cm	Ceratoides leaves/glucose	4.5
	0-3 cm	Atriplex leaves/glucose	3.6

[a]0.5 g soil moistened with 0.25 ml water or 10% glucose solution, 200 mg dried plant material/0.5 g soil.

Biological Activities and Nitrogen Content

Biological activity of Curlew sites 5, 6 and 7 soils, including dehydrogenase activity (A3UBJJ4), proteolysis (A3USQ02) and respiration (A3UBJJ1), are given in Tables 19, 20 and 21 for April 4, June 21 and July 19, respectively.

The values given coincide with data gathered in previous years in that surface layers have the most activity, activity decreases with depth and is usually higher when the soil has more moisture. Exchangeable NH_4^+ is also reported and is present in negligible amounts.

Table 22 shows the results for chemical analysis (A3USQ01) in which the carbon:nitrogen ratio and µg/g NO_3^- is reported. Highest nitrogen content seems to have been in April samples.

Nitrification Potential

Nitrification potential (A3USQ06) of soil sampled beneath the shrub canopy and in an interspace is shown in Tables 23 and 24. *A. tridentata* soil, station 5, is shown in Table 23 and *C. lanata* soil, station 6, is shown in Table 24. The nitrite level seems to be higher in station 5 soil, both canopy and interspace, with greater potential in the soil sampled from beneath the canopy. Station 6, however, shows greater potential in the interspace sample together with a faster decrease of ammonium.

Soil nitrogen analysis for these soils before and after perfusion is shown in Table 25. Soils collected from beneath the canopy at both stations show significant increases in fixed and organic N after perfusion, whereas interspace samples show a significant decrease. There do not appear

to be significant differences between canopy and interspace in exchangeable NH_4^+ and $NO_2^- + NO_3^-$ fractions. The pH of perfusates throughout the experiment is given in Table 26. In addition, Table 27 gives the comparison of soil analyzed after extraction with KCl and lyophilization and soil analyzed immediately after extraction. There is a very slight loss of nitrogen, if any, less than one-tenth of 1% in both total and fixed nitrogen fractions.

Figures 15, 16 and 17 show results of one inhibition experiment where the inhibitors were to be supposedly washed out and then reintroduced after the microbial numbers have been built up. There were no apparent inhibitors washed out of the soil. Ammonia volatilization was measured during this experiment and results are given in Table 28. It appears that most of the ammonia volatilized is at day 4 to 6 of perfusion. Upon addition of more ammonium source, volatilization rate doubles but after the third addition the rate decreases.

A classic example of the process upon reperfusion with fresh ammonium sulfate solution is seen in Figure 18 (control). Nitrate-nitrogen increases rapidly with no lag period as ammonium-nitrogen decreases and is depleted by day 10. On the other hand, nitrite-nitrogen is oxidized at a very rapid rate and amounts become immeasurable by the eighth day of perfusion. *Philodendron* biological comparison is shown in Figures 19 and 20.

Figures 21 through 23 show results of reperfusion with 0.46, 0.48 and 0.5% plant extract. There is no significant difference between these and the control except that the decrease of ammonium is slower; although *Philodendron* 0.48% extract shows depletion of ammonium-nitrogen by day 10 as with the control.

Reperfusion with 1% leachates from *C. lanata* and *A. confertifolia* is shown in Figures 24 and 25. Nitrate continues to rise above the level reached upon initial perfusion with ammonium sulfate; however, nitrite-nitrogen is measured at twice the level found at the eighth day of initial perfusion where it had begun to decrease. Soil perfused with 1% *Atriplex* leachate, though, shows two times lower nitrate level than does the control perfused with ammonium sulfate alone.

Table 29 and Figures 26 through 34 show the results of nitrification in soil perfused initially with ammonium sulfate solution for eight days. As seen from the control (Fig. 26) at the eighth day of initial perfusion, nitrite has not decreased nor has nitrate surpassed the level of nitrite. Consequently, upon reperfusion the initial nitrite level is high but sharply decreases.

Soil perfused with a 0.1% aqueous plant extract plus 0.01 M $(NH_4)_2SO_4$ (*Artemisia* extract, Fig. 27; *Ceratoides* extract, Fig. 28; *Atriplex* extract, Fig. 29; *Philodendron* extract, Fig. 30) shows an increase in nitrite to a level which is maintained through day 12 before it begins to decrease. At day 8, however, nitrite level in sagebrush extract begins to decrease. On the other hand, soil perfused with winterfat extract shows a sharp decrease of nitrite after day 2, similar to the control. Oxidation to nitrate continues but at a lower level.

Figures 31-34 and Table 30 show results of perfusion with 0.5% leachate from *Artemisia*, *Atriplex*, *Ceratoides*, *Philodendron* and *Dactylis* leachates, respectively. Nitrite accumulation is observed in all except *Philodendron* and *Dactylis*. Nitrite level of *Atriplex* leachate surpasses nitrate level until day 16 when it begins to decrease. Nitrate level of desert shrub leachates is somewhat lower than the control.

Ammonification of *Ceratoides*-dominated soil with different nitrogen amendments is shown in Table 31. No ammonia is volatilized when soil is amended with egg albumin. *Atriplex*-dominated soil, however, does show volatilized ammonia with egg albumin (Table 30). The garden soil comparison (Table 32) contains more nitrogen and exhibits more ammonification with a plant material (alfalfa) amendment. There is no volatilization with egg albumin and leucine amendments.

When comparing soil amended with different amounts of plant material, the soil with a 3% wt/wt plant amendment has most exchangeable NH_4^+ (Table 33). Table 34 shows ammonification of a garden soil with a 2% plant amendment.

ATP Analysis

Table 35 shows ATP concentrations of June 21 samples. ATP concentrations do not exceed 4.2×10^{-7} moles/g and decrease with depth. Recovery is not exactly known. The method will be further developed and applied in 1975.

Catalase Titrimetric Method

Preliminary results showed that autoclaved soil and oven-sterilized soils gave a range of 1.1 to 4.2 and 0-2.9 ml $KMnO_4$ equal to H_2O_2 decomposed in 20 min time per 2 g dry soil. In another experiment using soils oven dried for 48 hr, the range was 0.5-2.4 ml of $KMnO_4$ to titrate (Table 36).

Catalase Standard Time and Catalase Standard Curve

Table 37 gives the results of the standard time study. It was shown by these preliminary results that catalase activity shows linear relationship from 0 to 10 min and then begins to fall off (Fig. 35). The data for the catalase standard curve are given in Table 38.

Gasometric Method for Catalase Activity

The results to date are shown in Table 39. Most attention has been given to establishment of residual activity by use of sterile blanks, and to working out a simple, yet accurate, method for our future work. The results show that the greatest peroxide decomposition takes place in the first 2 to 4 min, dropping off until 10 min when activity levels off until it reaches zero at about 30 min.

Table 19. Biological activity, April 4, 1974

Sample	pH	Moisture %	Dehydrogenase mg formazan/dry g	Proteolysis % Hydrolysis	Respiration umoles CO_2/g/min	Exchangeable NH_4^+ µg/g
5-1	8.4	13.1	.70	24.0	50.1	4.7
5-2	8.4	17.1	.10	10.1	22.3	0
5-3	8.7	19.0	.06	4.5	22.8	1.7
5-4	9.0	17.4	.01	3.5	21.9	0
6-1	8.3	10.7	.58	10.5	35.3	0
6-2	8.6	15.8	.01	5.5	16.6	0
6-3	8.7	15.4	.01	1.8	16.0	0
6-4	8.4	12.1	.01	4.8	28.3	0
7-1	8.4	18.2	1.20	36.0	56.5	0
7-2	8.6	15.9	.02	1.8	39.7	0
7-3	8.8	16.1	.02	6.0	25.9	0
7-4	8.8	16.9	.10	9.0	22.6	0

Table 20. Biological activity, June 21, 1974

Sample	pH	Moisture %	Dehydrogenase mg formazan/dry g	Proteolysis % Hydrolysis	Respiration umoles CO_2/g/min.	Exchangeable NH_4^+ µg/g
5-1	8.6	2.0	.450	14.0	71.4	5.3
5-2	8.8	4.2	.052	7.8	34.4	0.2
5-3	8.9	12.1	.023	8.8	50.1	0.2
5-4	9.0	19.5	0	4.4	47.7	2.5
6-1	8.5	1.6	.752	19.0	35.0	3.9
6-2	8.8	3.5	.155	14.0	24.7	2.2
6-3	9.1	5.9	.021	8.0	19.6	0
6-4	9.3	6.4	.043	10.0	68.2	3.9
7-1	8.8	1.3	1.500	24.0	59.8	0
7-2	9.1	7.1	.129	6.4	43.8	0.2
7-3	9.0	15.5	.060	12.6	47.1	0
7-4	8.8	22.1	0	4.4	37.4	0

Table 21. Biological activity, Curlew Valley II, July 19, 1974

Sample	pH	Moisture %	Dehydrogenase mg formazan/dry g	Proteolysis % Hydrolysis	Respiration umoles CO_2/g/min	Exchangeable NH_4^+ µg/g
5-1	8.6	1.7	.69	18	12.6	3.0
5-2	8.6	3.6	.06	0	21.2	0
5-3	8.5	8.5	.03	7	28.2	1.4
5-4	9.2	15.7	0	0	32.7	0
6-1	8.6	2.1	.61	10	6.8	5.3
6-2	8.7	2.4	.02	7	11.4	0.2
6-3	8.6	5.2	0	2	22.5	0
6-4	9.5	8.8	0	1	20.5	1.6
7-1	8.8	1.6	.93	1.5	15.3	8.4
7-2	8.4	4.7	.12	0	15.8	3.6
7-3	8.8	13.5	.12	1.4	24.2	2.2
7-4	8.7	19.8	.06	1.0	23.3	0

Table 22. Chemical analysis

Sample	% Organic C	% Total N	µg/g NO_3^-	C/N Ratio
4IV74				
5-1	.9	.14	2.5	6.4
5-2	.8	.12	1.8	6.7
5-3	.7	.11	1.7	6.4
5-4	.4	.07	1.7	5.7
6-1	.7	.11	1.8	6.4
6-2	.5	.09	2.2	5.6
6-3	.5	.08	2.1	6.3
6-4	.5	.08	1.1	6.9
7-1	.9	.13	17.1	6.9
7-2	.5	.09	1.8	5.6
7-3	.6	.09	2.4	6.7
7-4	.5	.09	.9	5.6
21VI74				
5-1	1.1	.14	1.2	7.9
5-2	.7	.07	.2	10.0
5-3	.8	.07	.7	11.4
5-4	.4	.06	.9	6.7
6-1	1.1	.10	1.1	11.0
6-2	.7	.10	.7	7.0
6-3	.6	.07	.7	8.8
6-4	.8	.09	.6	8.9
7-1	1.3	.15	2.9	8.7
7-2	.6	.07	1.0	8.6
7-3	.7	.07	1.0	10.0
7-4	.5	.08	.8	6.3
19VII74				
5-1	.9	.12	.9	7.5
5-2	.6	.07	.3	8.6
5-3	.6	.07	.6	8.6
5-4	.4	.04	.4	10.0
6-1	.7	.08	.9	8.8
6-2	.5	.09	.4	5.6
6-3	.5	.06	.4	8.3
6-4	.4	.05	.9	8.0
7-1	1.2	.13	1.4	9.2
7-2	.7	.08	1.0	8.8
7-3	.7	.06	.8	11.7
7-4	.6	.09	1.5	6.7

Table 23. Nitrification potential; canopy versus interspace (Station 5 soil)

Day	µg NH_4^+-N/g 5-C	5-I	µg NO_2^--N/g 5-C	5-I	µg NO_3^--N/g 5-C	5-I
2	1975	1975	4.5	4.8	0	0
4	----	----	----	----	--	--
6	2050	1825	10	15	19	5
8	----	----	11	17	16	14
10	1808	1700	10	9	16	0
12	----	----	17	12	16	0
14	1625	1517	43	24	7	0
16	----	----	172	34	31	32
18	----	----	----	----	--	--
20	1100	917	1267	387	62	26
22	----	----	1333	1083	47	32
24	20	20	1642	1392	93	0

* Initial NH_4^+-N added = 2333 µg/g

Table 24. Nitrification potential; canopy versus interspace (Station 6 soil)

Day	µg NH_4^+-N/g 6-C	6-I	µg NO_2^--N/g 6-C	6-I	µg NO_3^--N/g 6-C	6-I
2	1975	1975	4	4	0	4
4	----	----	---	---	--	--
6	2017	1900	14	20	0	0
8	----	----	14	30	16	0
10	1625	967	11	22	11	0
12	----	----	15	168	6	0
14	1475	1000	12	460	5	35
16	----	----	10	752	0	0
18	----	----	---	---	--	--
20	1208	142	45	1642	31	21
22	----	----	166	983	--	67
24	692	6	420	842	26	57

Initial NH_4^+-N added = 2333 µg/g

Table 25. Soil nitrogen analysis, canopy versus interspace (μg N/g)

Sample	Fixed	Organic	Exchangeable	$NO_2^- + NO_3^-$
		BEFORE PERFUSION		
5-C	102	1088	2.8	7.7
Control	102	1088	2.8	7.7
5-I	149	2231	2.1	3.4
Control	149	2231	2.1	3.4
6-C	55	955	1.5	3.7
Control	55	955	1.5	3.7
6-I	117	1583	1.3	2.8
Control	117	1583	1.3	2.8
		AFTER PERFUSION		
5-C	143	1517	5.6	92.3
Control	127	1243	8.5	0.9
5-I	88	892	22.8	105
Control	67	892	9.3	4.1
6-C	146	1254	401	38.3
Control	81	1089	11.7	4.3
6-I	50	610	5.1	106
Control	50	650	5.1	3.4

Table 26. Canopy versus interspace; pH of perfusates

Sample	Day 2	Day 10	Day 24
5-C	8.0	8.1	7.5
5-I	8.1	8.2	7.9
6-C	8.0	8.1	7.9
6-I	8.0	8.1	7.6

Table 27. Nitrogen loss upon lyophilization

Soil Analyzed After Extraction		Soil Analyzed after Extraction and Lyophilization	
mg/g		mg/g	
Total N	Fixed NH_4^+	Total N	Fixed NH_4^+
.986	.070	.930	.065

Table 28. Ammonia volatilization during perfusion (inhibition experiment)

After First Change	μg NH_4^+-N/g
Flask 1 (2 days)	81
Flask 2 (4 days)	336
Flask 3 (6 days)	332
After Second Change	
Flask 1 (10 days)	851
Flask 2 (10 days)	1167
Flask 3 (10 days)	475
After Third Change	
Flask 1 (10 days)	312
Flask 2 (10 days)	339
Flask 3 (10 days)	196

Table 29. Nitrification potential; *D. glomeratus* 0.5% leachate (cultivated garden soil)

Day	μg NH_4^+-N/g	μg NO_2^--N/g	μg NO_3^--N/g
* 2	1017	32	7
4	----	---	----
6	500	152	102
8	----	224	232
10	308	185	500
** 2	----	122	528
4	583	85	1217
6	750	75	1400
8	----	151	1992
10	67	19	2217
12	----	0	1942
14	225	0	2483
16	----	0	2250
18	17	0	2017
20	17	0	1967

* Initial NH_4^+-N added = 2333 μg/g

** Fresh 0.01 M $(NH_4)_2SO_4$ added

Table 30. Ammonification, Station 7 (mg N/g)

Time (Days)	Fixed	Organic	Exchangeable	Volatilized
		Station 7 soil + Egg Albumin		
0	.025	0.96	.006	0
7	.148	1.24	.035	.020
14	.086	1.25	.041	.105
21	.079	0.98	.042	.178

* N added = 0.1 mg/g

** Moisture <-1 bar

Table 31. Ammonification, Station 6 (mg N/g)

Time (Days)	Fixed	Organic	Exchangeable	Volatilized
		STATION 6 + EGG ALBUMIN		
0	.040	0.90	.004	0
7	.044	1.28	.017	0
14	.064	0.80	.029	0
21	.054	0.66	.031	0
		STATION 6 + L-LEUCINE		
0	.082	0.60	.003	0
7	.092	0.71	.038	0
14	.050	0.87	.032	.052
21	.065	0.68	.046	.115
		STATION 6 SOIL CONTROL (NO AMENDMENT)		
0	.084	0.73	.003	0
7	.081	0.85	.006	0
14	.050	0.93	.017	.007
21	.052	0.61	.036	.013

* N added = 0.1 mg/g

** Moisture $\leq$ -1 bar

Table 32. Ammonification, garden soil (mg N/g)

Time (Days)	Fixed	Organic	Exchangeable	Volatilized
		GARDEN SOIL + EGG ALBUMIN		
0	.065	1.30	.004	0
7	.061	1.69	.029	0
14	.098	1.58	.037	0
21	.105	1.71	.036	0
		GARDEN SOIL + L-LEUCINE		
0	.124	1.67	.004	0
7	.127	1.97	.041	0
14	.115	1.44	.040	0
21	.120	1.38	.008	0
		GARDEN SOIL + PLANT MATERIAL (ALFALFA)		
0	.124	1.96	.009	0
7	.099	2.31	.021	.012
14	.096	1.91	.027	.013
21	.102	1.61	.021	.028
		GARDEN SOIL CONTROL (NO AMENDMENT)		
0	.119	1.16	.004	0
7	.126	1.79	.017	.014
14	.079	1.61	.020	.012
21	.110	1.46	.026	.023

* N added = 0.1 mg/g

** Moisture $\leq$ -1 bar

Table 33. Ammonification, Curlew Valley II, Station 7 (0-3 cm) shadscale plant amendment (mg/g)

Time hrs	Fixed N	Organic N	Volatilized N	Exchangeable N
		1% plant amendment		
0	.059	1.167	0	.016
65	.067	1.286	.027	.390
		2% plant amendment		
0	.081	1.230	0	.114
65	.055	1.357	.059	.516
		3% plant amendment		
0	.057	1.439	0	.080
65	.071	1.439	.103	.735
		Control, no amendment		
0	.070	.850	0	.084
65	.043	.987	0	.268

* N-serve added - 7μg/g

** Added N = .188 mg N/g, 1% amendment
.375 mg N/g, 2% amendment
.563 mg N/g, 3% amendment

*** Moisture $\leq$ -10 bars

Table 34. Ammonification, cultivated garden soil with alfalfa plant amendment

mg/g

Time(hrs)	Fixed N	Organic N	Volatilized N	Exchangeable N
		2% plant amendment		
0	.097	2.353	0	.193
92	.223	2.787	0	.420
		Control, no amendment		
0	.107	1.578	0	.037
92	.078	2.132	0	.177

% N

Time (hrs)	Fixed	Organic	Volatilized	Exchanged
		plant amended		
0	3.7	89.0	0	7.3
92	6.5	81.3	0	12.2
		Control		
0	6.2	91.7	0	2.1
92	3.3	89.3	0	7.4

* N-serve added - 7μg/g

** Added N = .55 mg/g

*** Moisture $\leq$ -10 bars

Table 35. ATP concentrations, Curlew Valley II, June 21, 1974

Sample	ug ATP/g*	moles ATP/g
5-1	.1187	2.4×10^{-7}
5-2	.0500	9.9×10^{-8}
5-3	.0312	6.2×10^{-9}
5-4	.0300	5.9×10^{-9}
6-1	.1562	3.1×10^{-7}
6-2	.0912	1.8×10^{-8}
6-3	.0306	6.1×10^{-9}
6-4	.0137	2.7×10^{-9}
7-1	.2125	4.2×10^{-7}
7-2	.1250	2.5×10^{-7}
7-3	.0400	7.9×10^{-9}
7-4	.0212	4.2×10^{-9}

* Average of triplicate samples

** Standard Curve Followed:

ug/ml	count mr
.001	64
.0025	103
.005	402
.01	1019
.025	3931
.05	12758

Table 36. Catalase activity in Curlew soils after different methods of sterilization. Data in ml of 0.1 N $KMnO_4$ equal to H_2O_2 decomposed per 2 g soil per 10 min time

Soil description	Oven-dried 170C, 6 hrs	Oven-dried 170C, 48 hrs	Autoclaved
7 canopy	0	0.9	2.0
7 I	3.5	1.2	2.1
6 canopy	2.45	0.5	1.4
6 I	2.90	1.0	1.7
5 canopy	2.05	1.6	1.1
5 I	2.80	2.4	1.7
7C, fresh	4.65	4.0	4.2
7 I fresh soil	4.65	4.2	4.1

Table 37. Activity curve for catalase

Site #7-1 canopy		
Time (min)	ml of $KMnO_4$ eq. to H_2O_2 decomposed/2g soil Exp. 1	Exp. 2
0	3.5	6.1
2.5	2.3	4.1
5.0	1.4	2.6
10	0.6	1.2
15	0.3	0.3
20	0.25	0.15
25	0.12	0.05
30	0.05	0.00
35	Not measured	0.00
40	Not measured	0.16

Table 38. Data for catalase standard curve

Std. solut. conc. stock = 1 mg/100 ml	ml $KMnO_4$ eq. to H_2O_2 decomposed in 0 min	10 min	15 min
0.5 mg/100 ml	--	0	--
0.2 "	--	0	--
0.1 "	6.2	9.45	9.45
0.05 "	5.4	9.40	9.45
0.025 "	3.1	8.6	9.20
0.0125 "	2.1	6.4	8.10
0.00625 "	0.9	3.5	5.3
0.00312 "	0.8	2.5	3.1
0.00156 "	0.3	1.5	3.6
0.00078 "	0.3	1.0	--

Table 39. Gasometric catalase activity measurement

Sample description	Catalase activity in cc O_2 evolved (min) 2	4	6	8	10	15	20	25	30	cc O_2 /g/min
5 Cr[a] (4g,3%)	38	74	97	111	116	120	120	120	120	1.0
5 Cr (5g,.3%)	1.5	4	8.6		10.7	12	13	13	13	.84
5C[b] (4g,3%)	30	52	67	75	88	92	92	92	92	.77
5C (4g,3%)	4	48	73		84	86	86		86	.72
water blanks	0		0		0		0		0	0
sterile blanks										
4g,3%									5.8	.0966
2g,.3%									0.1	.0008
4g,3%									26.1	.218
5g,3%	16		20		50	66		77	88	.52
2g,.3%									0.1	.002
5C (5g,.3%)	2	3.5	9		11.2	12.4	13	13	13	.09
2g soil										
6 I[c] (3%H_2O_2)	22	35	41	45		45	45	45	45	.74
7 Cr (3% ")	31	43	44	44.5		44.5	44.5		44.5	.74
6I (.3% ")	9.8	12	12.7	13		13			13	.21
7 Cr (.3% ")	9.2	9.9	10.1	10.5		10.5			10.5	0
6I blank	0								0	0
7 Cr blank	0								0	0

[a] Cr = crust.
[b] I = interspace.
[c] C = canopy.

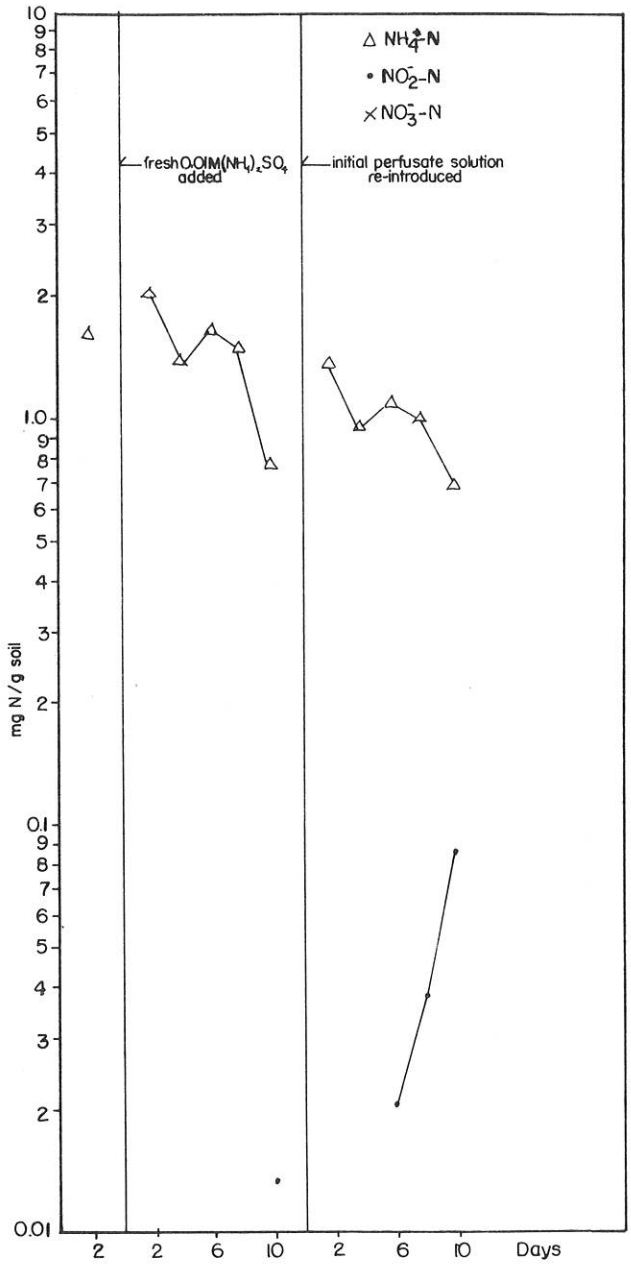


Figure 15. Effect of soil perfusate on nitrification (two-day initial perfusion).

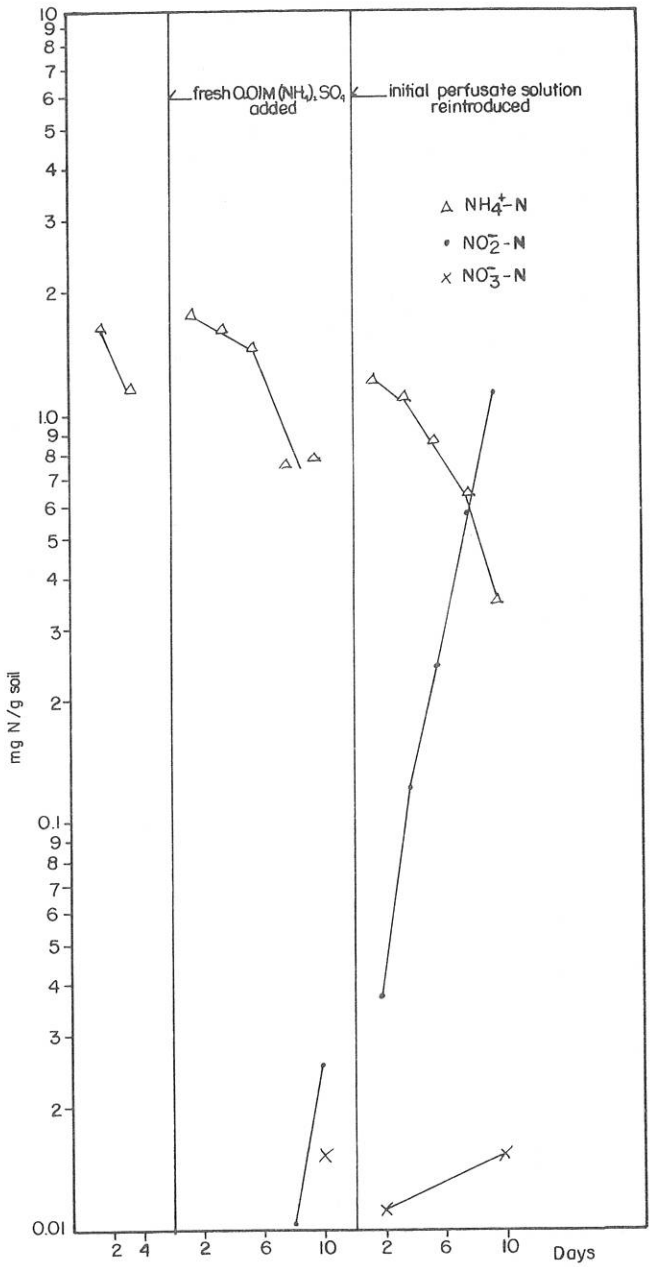


Figure 16. Effect of soil perfusate on nitrification (four-day initial perfusion).

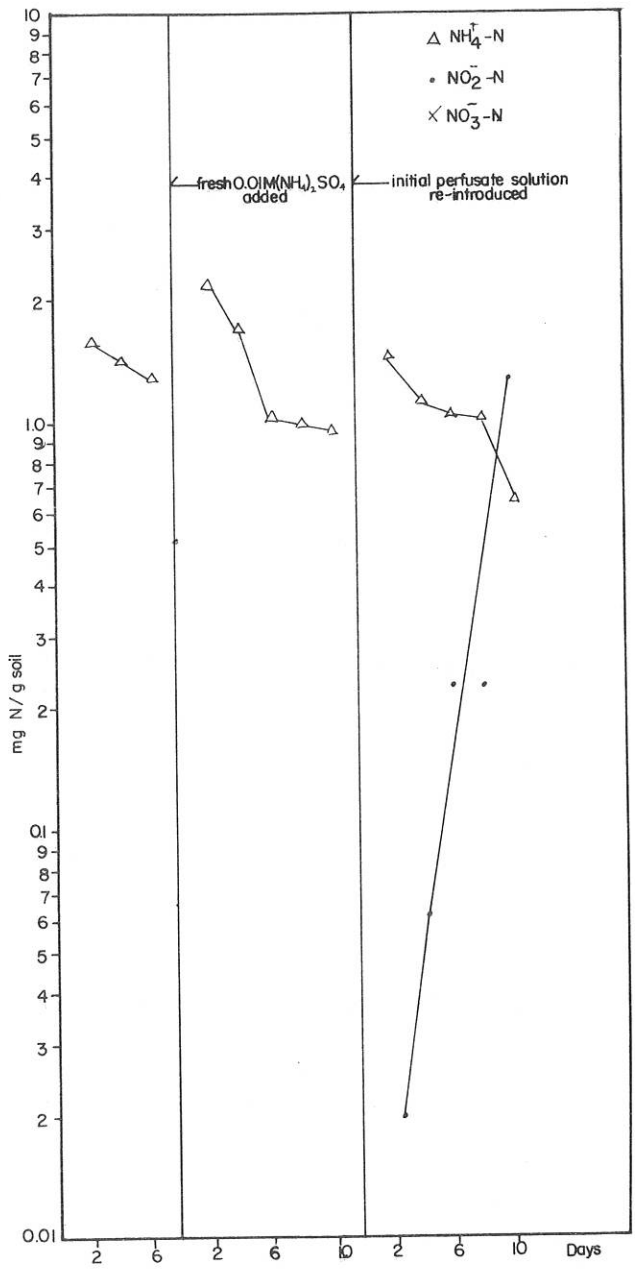


Figure 17. Effect of soil perfusate on nitrification (six-day initial perfusion).

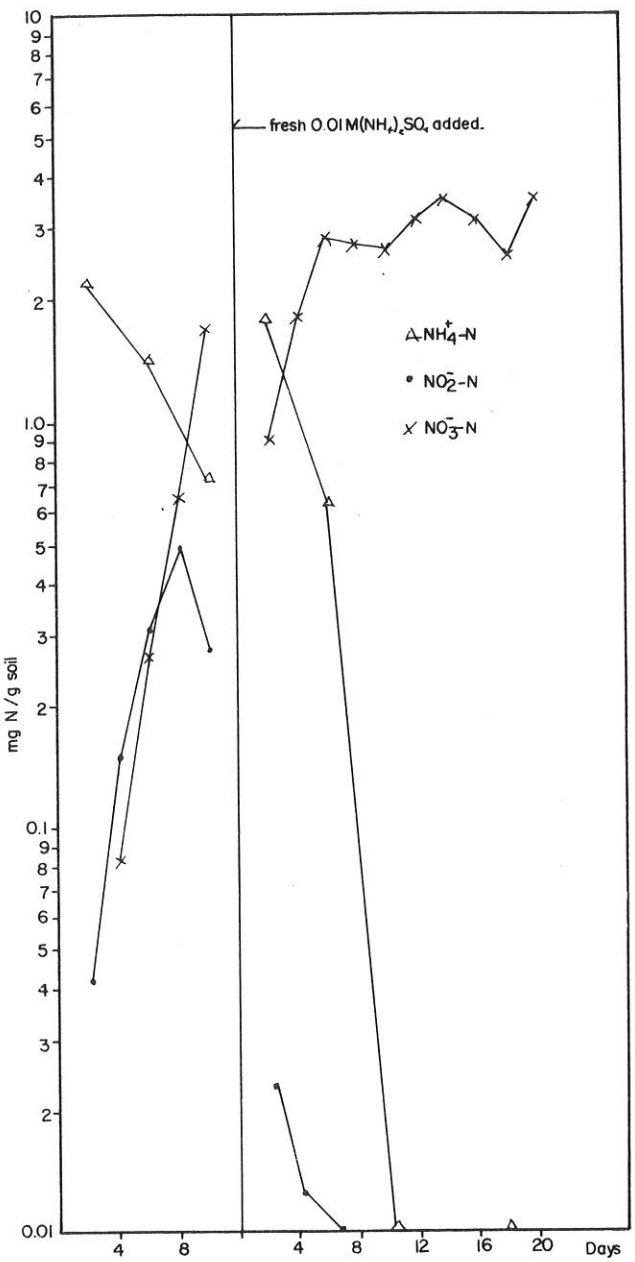


Figure 18. Nitrification; $(NH_4)_2\ SO_4$ control.

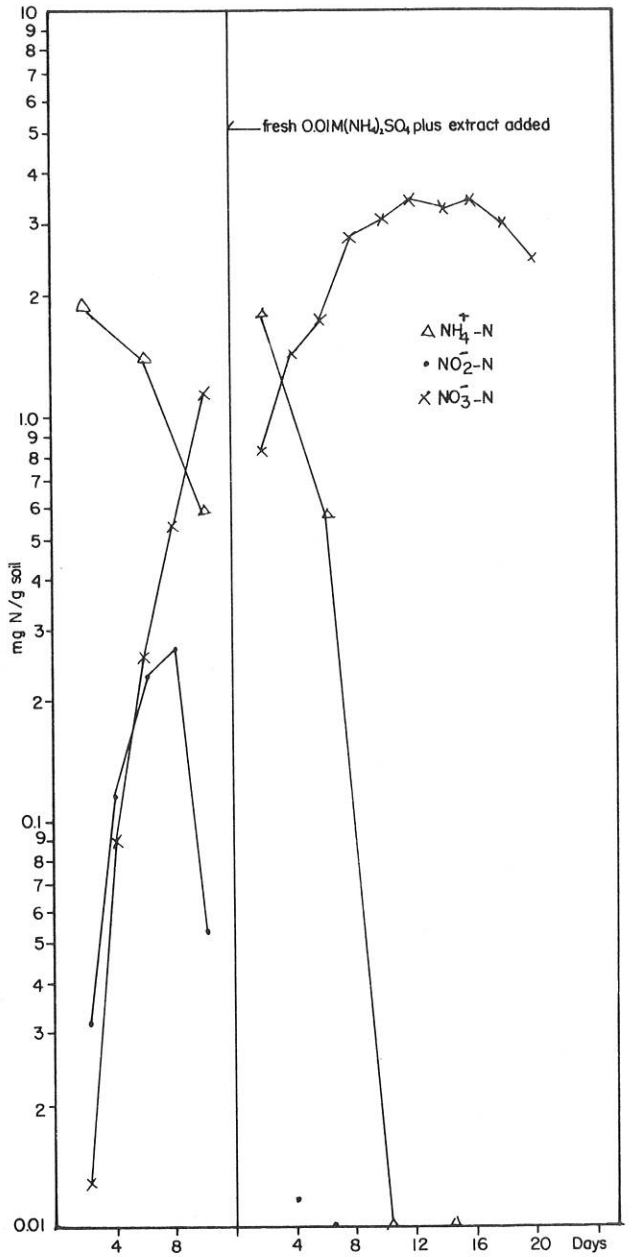


Figure 19. Effect of 0.48% *Philodendron* extract on nitrification.

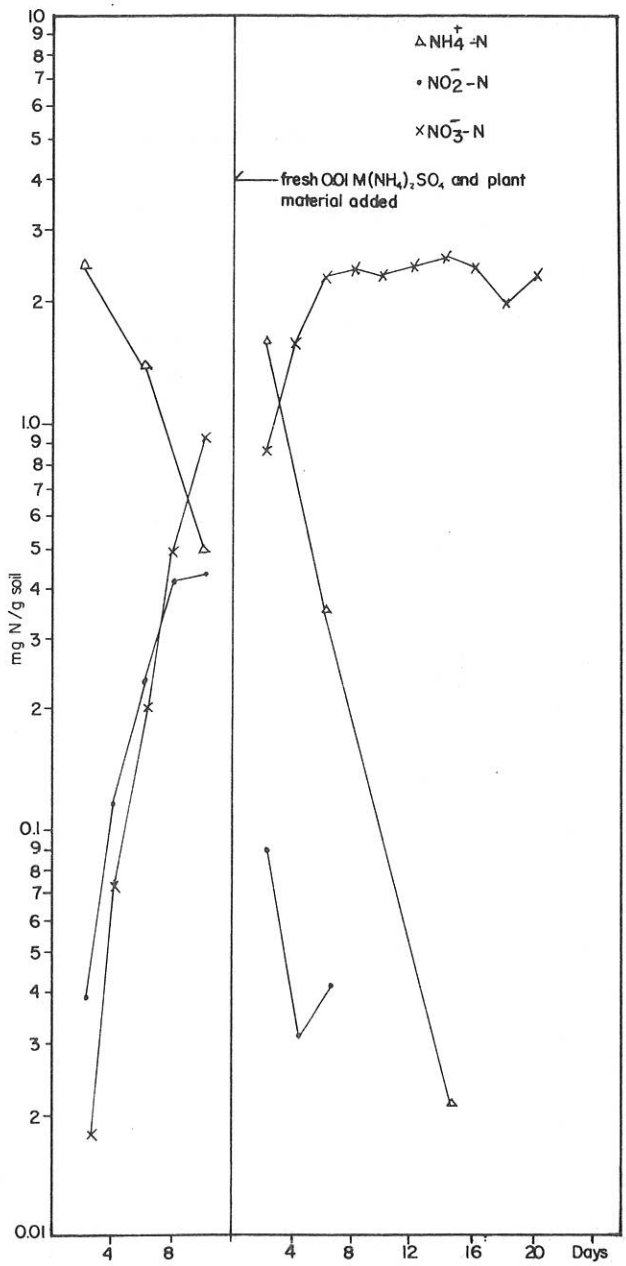


Figure 20. Effect of 1% leachate of *Philodendron* on nitrification.

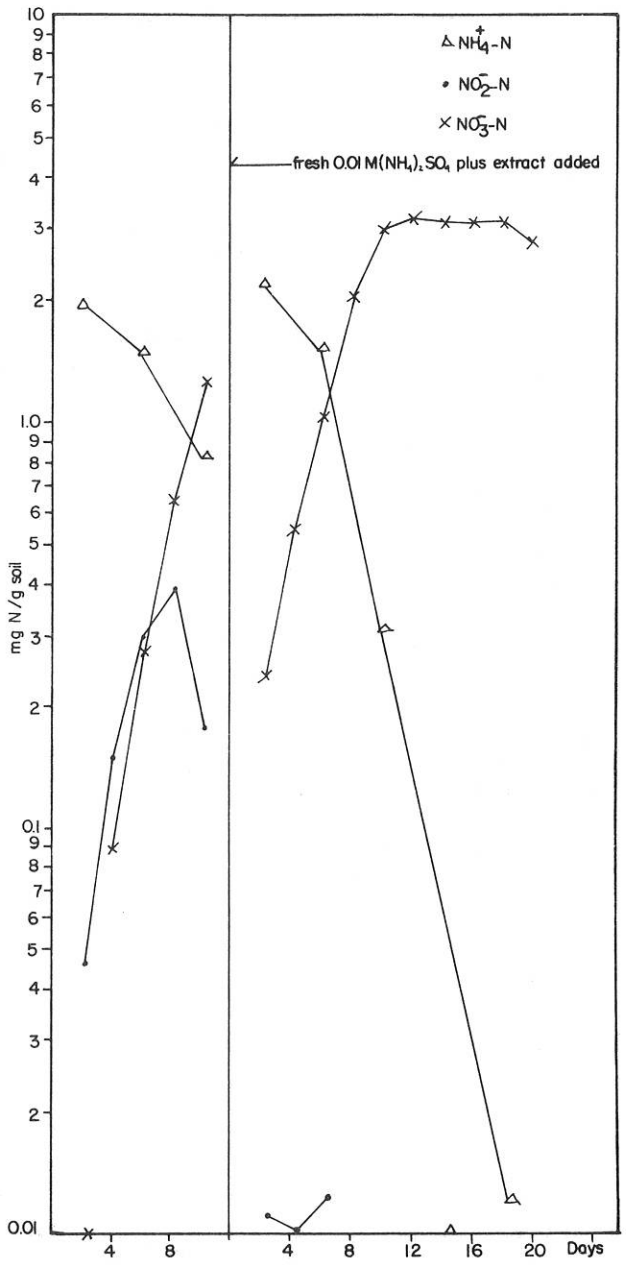


Figure 21. Effect of 0.46% *A. tridentata* extract on nitrification.

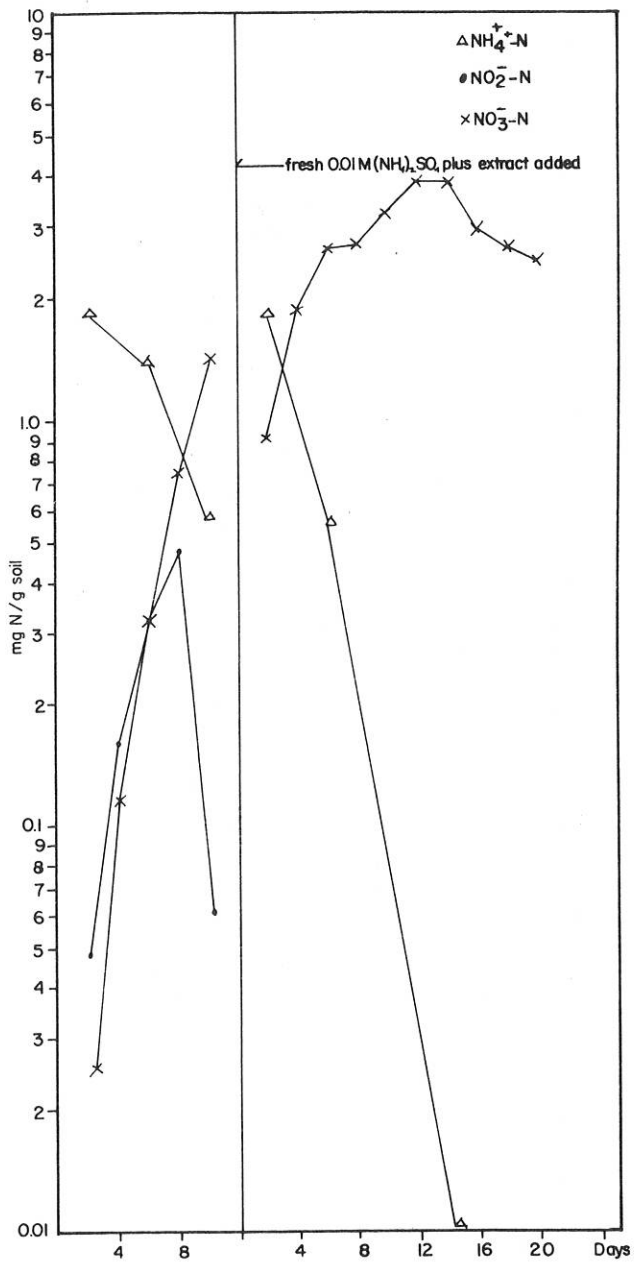


Figure 22. Effect of 0.48% *A. confertifolia* extract on nitrification.

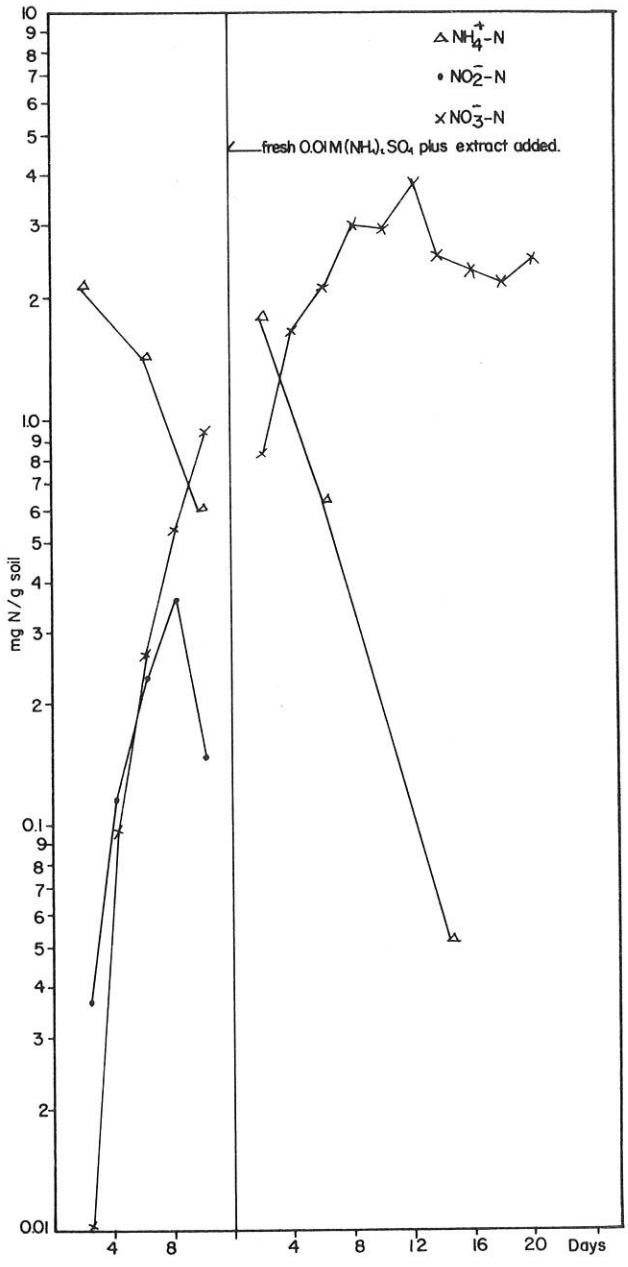


Figure 23. Effect of 0.5% *C. lanata* extract on nitrification.

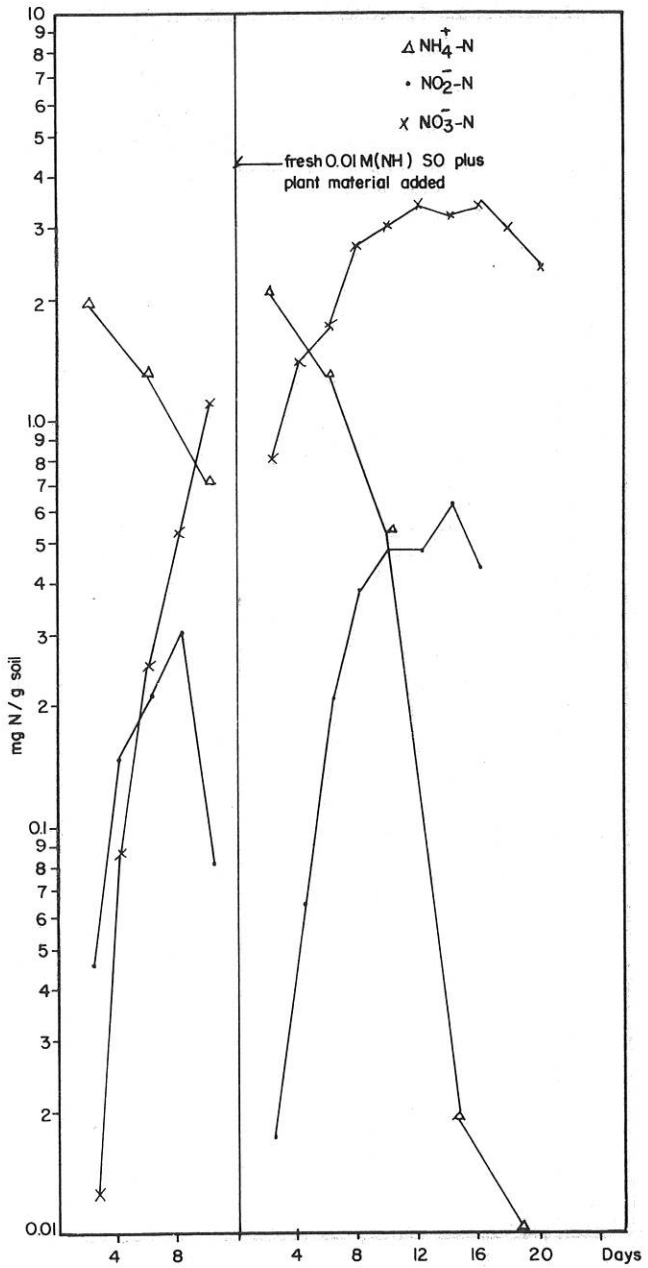


Figure 24. Effect of 1% leachate of *C. lanata* on nitrification.

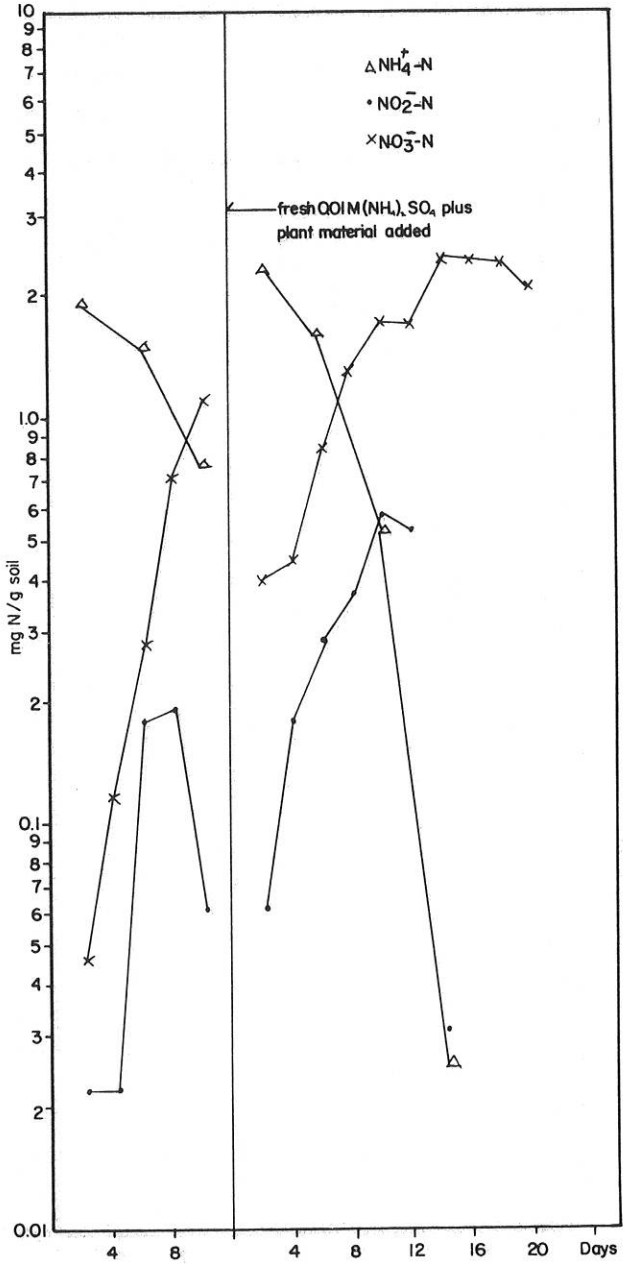


Figure 25. Effect of 1% leachate of *A. confertifolia* on nitrification.

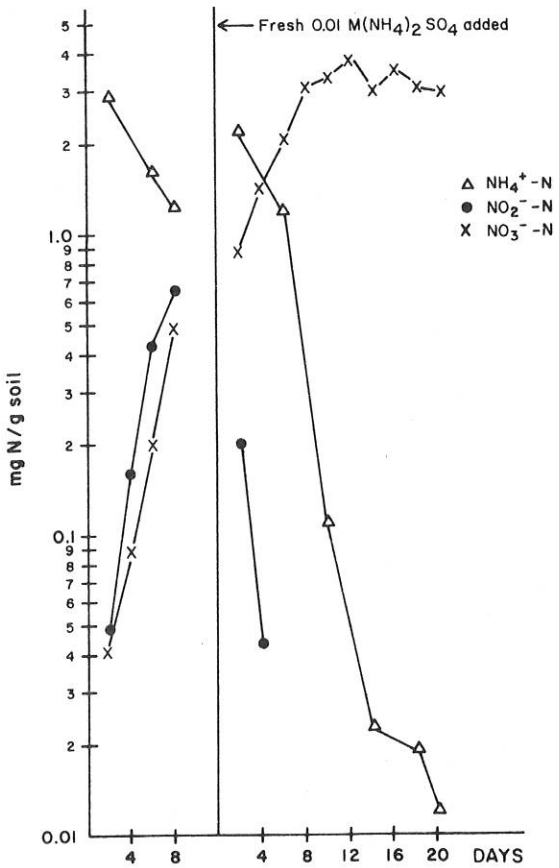
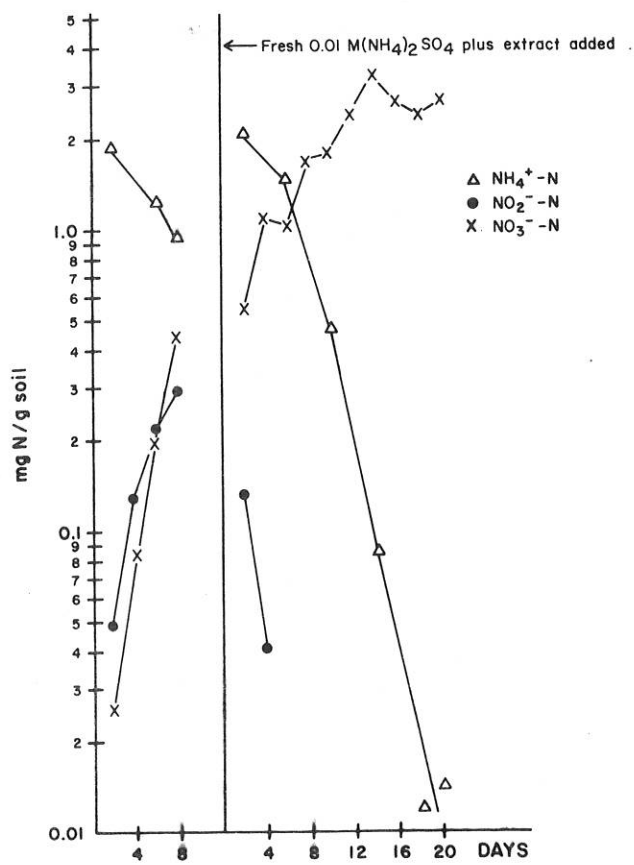


Figure 26. Nitrification; $(NH_4)_2SO_4$ control.



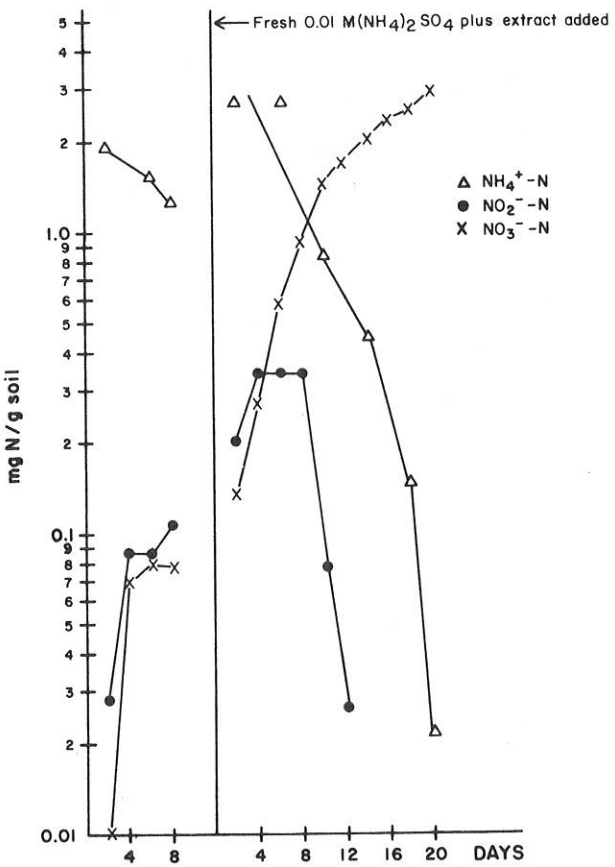


Figure 27. Effect of 0.1% *A. tridentata* extract on nitrification.

Figure 28. Effect of 0.1 % *C. lanata* extract on nitrification.

Figure 29. Effect of 0.1% *A. confertiolia* extract on nitrification.

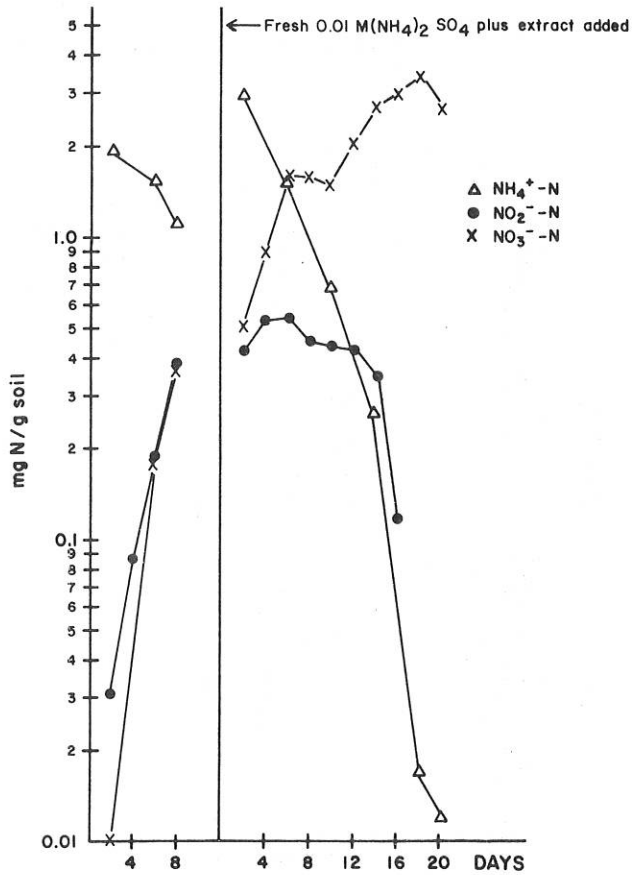
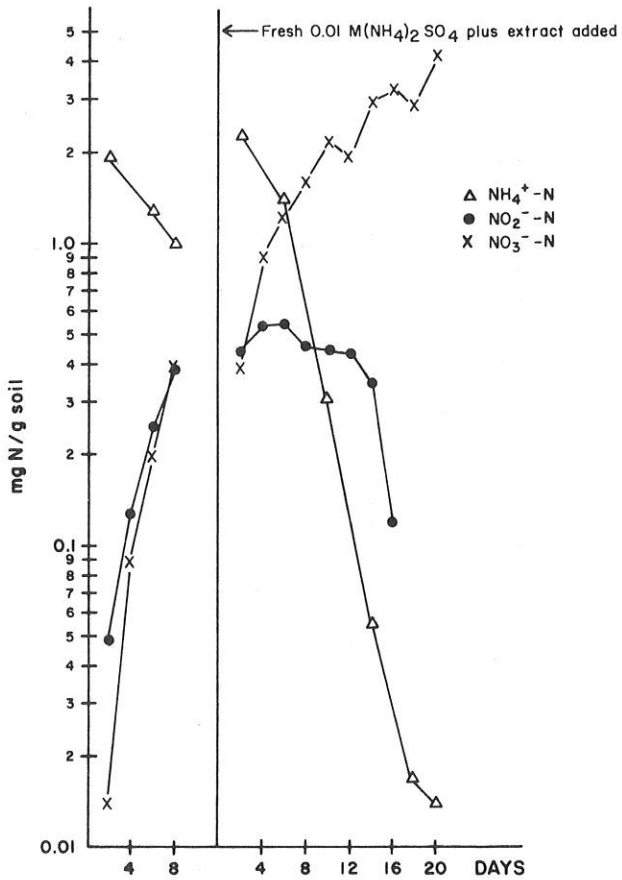


Figure 30. Effect of 0.1% *Philodendron* extract on nitrification.



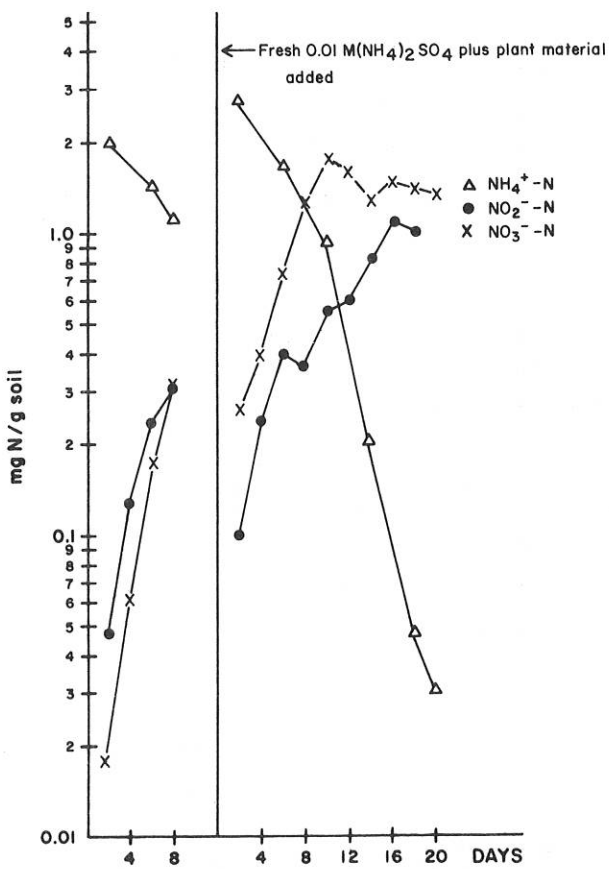


Figure 31. Effect of 0.5% *A. tridentata* leachate on nitrification.

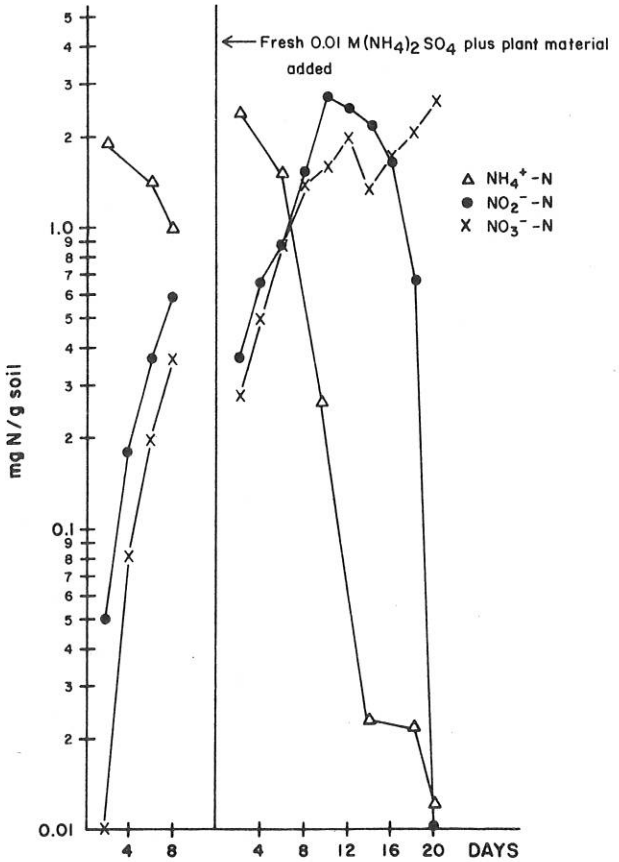


Figure 32. Effect of 0.5% *A. confertifolia* leachate on nitrification.

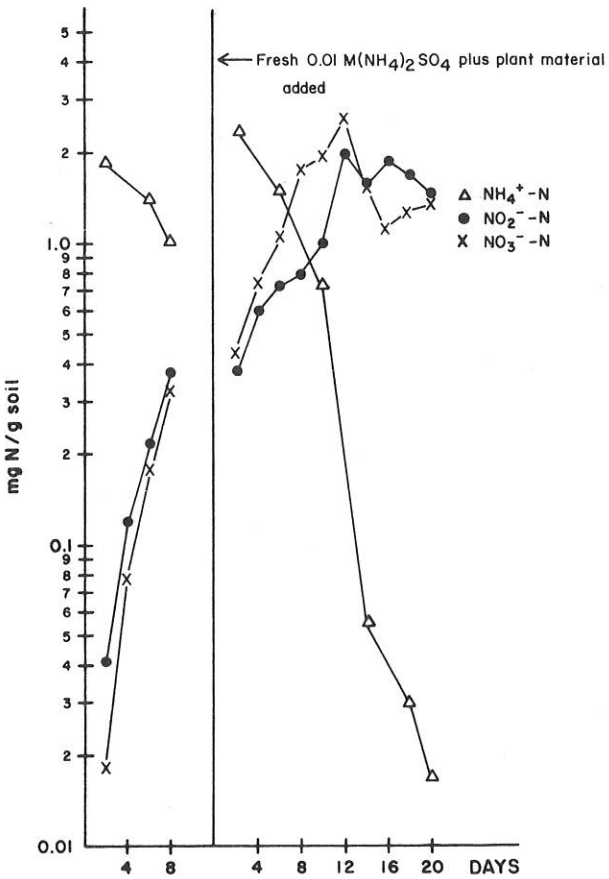


Figure 33. Effect of 0.5% *C. lanata* leachate on nitrification.

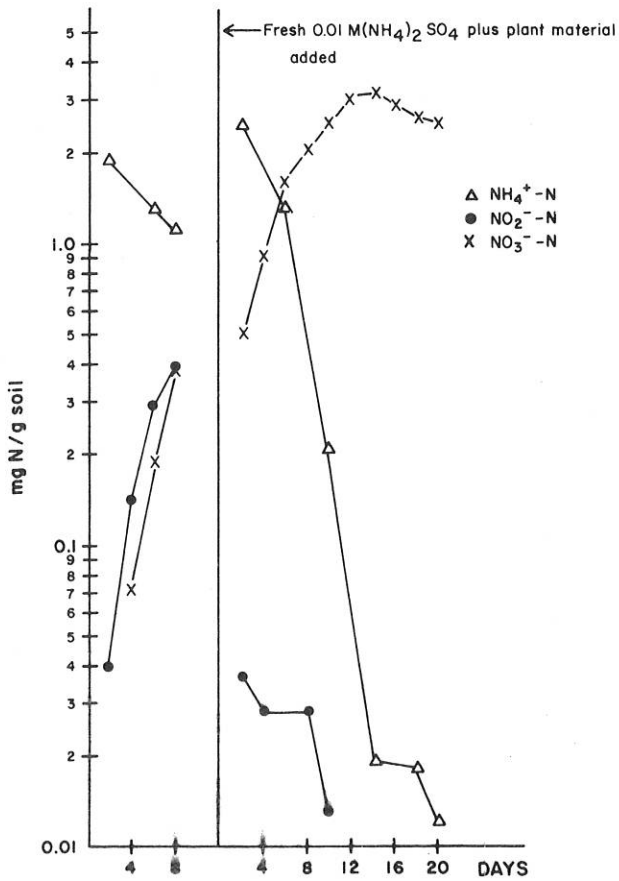


Figure 34. Effect of 0.5% *Philodendron* leachate on nitrification.

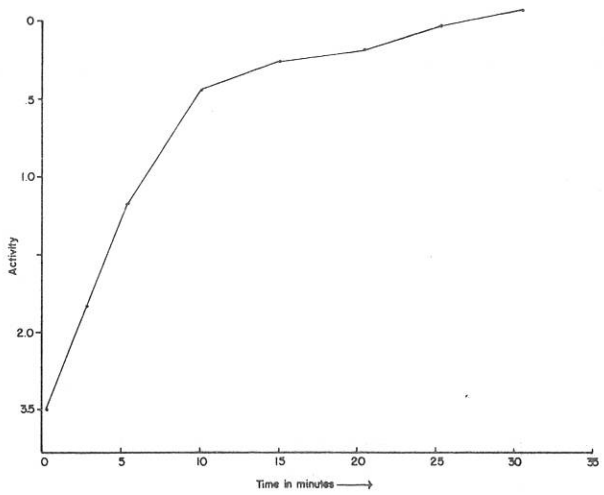


Figure 35. Rate of catalase activity. Activity is a measure of $KMnO_4$ used.

DISCUSSION

In the air-dried soils and the ^{15}N-amended, air-dried soils, the increase in organic nitrogen is significant. Because of these high values (16.8-57.4%), and since the soil chambers were wrapped in aluminum foil, heterotrophic fixation of nitrogen is the only possible explanation of these results. Furthermore, it is only when the soil is moistened to 25% and air dried that such large increases are observed. Rychert (1974), in his experiments with acetylene reduction, had suggested the algal crusts were primed for nitrogen fixation in the dark, as photosynthetic energy (ATP) and reductant were built up in pools when exposed to light. Thus, when the soil was moistened, he observed that the dark nitrogen fixation was of 5-30% of nitrogen fixation in the light.

It has also been suggested (Lynn pers. comm.) that these same reserve pools of ATP and reductant may supply the fungi (of an algal-fungal symbiotic relationship) for nitrogen fixation as well.

However, when these same ^{15}N-amended soils are held constant at -1 bar or -15 bars water tension, heterotrophic fixation is not detected, or it is of a small value. From Table 1 it is seen that except for soil 6-1 at -15 bars, the organic nitrogen content had decreased during the five-week period, thus suggesting some influence of water tension on heterotrophic fixation of N. Since the soil pores are anaerobic at -1 bar, this could conceivably block the heterotrophic fixation, assuming that such fixation is aerobic and due to the symbiotic relationship of the blue-green algae and fungi. However, at -15 bars, the pore space is under aerobic conditions and consequently allows such fixation to occur. The slight increase of nitrogen in soil 6-1 at -1 bar and soil 5-1 at -15 bars may be due to heterotrophic fixation or the margin of error in the Kjeldahl analysis, as this technique is accurate to 1 mg of nitrogen. However, since the increase in nitrogen is due to the light-fixed NH_4^+, it is difficult to say if such a gain is due to heterotrophic fixation.

Losses of nitrogen via ammonia volatilization represent less than a 10% nitrogen loss from desert soils (Skujins and West 1974) and the magnitude of this loss depends in part on the anion in the soil or fertilizer (Fenn and Kissel 1973). With respect to the ammonia volatilization in the untreated and ^{15}N ammonium sulfate-treated soils, the greatest amount of volatilization occurred when the soil was allowed to air dry. When the soils were held constant at -1 bar or -15 bars, ammonia volatilization fluctuated, indicating an influence by nitrification. In addition, the decrease in soil pH and the buildup of nitrate further supports this view as a buildup of either nitrite or nitrate, or both, in soil causes the formation of nitrous or nitric acid (Martin et al. 1942). Furthermore, since the moisture content was held constant, at -1 bar or -15 bars, this would enhance the formation of nitrous or nitric acid. However, in air-dry soils, the water present in the system is variable and, thus, may not play a significant role in nitrification and acid formation as in the experimental systems held at -1 and -15 bars. More importantly, this variability of the soil water is dependent upon the rate at which the soils dry and thus, as water vapor volatilizes off, ammonia may parallel this volatilization because of hydrogen bonding between the two compounds. Hence, the volatilization curves and the percent moisture curves closely parallel each other.

The addition of plant material plus ^{15}N treatment to the soils causes a completely different set of results. Soil 6-1, when held at -1 bar, exhibits the greatest amount of net denitrification (25.7%) in any of the experimental systems. This possibly indicates that *Ceratoides* plant material may contain readily accessible forms of carbon (such as simple sugars) which would supply an energy source for such denitrifying capabilities. Soils 5-1 and 7-1 had very little net denitrification (2.56%; 5-1) or none at all (7-1). However, the ^{15}N data indicate an $80 \pm 2\%$ denitrification loss of ^{15}N.

In soil 5-1, an inhibition of nitrification is also observed as the only buildup of both nitrite and nitrate is found to occur, nitrite being the predominant form (57%). Thus, the secondary compounds from *A. tridentata* may cause a significant but not a complete inhibition of nitrification.

In soil 7-1, no net denitrification has been observed; heterotrophic fixation of nitrogen may have played a key role since a 5.8% increase in nitrogen was noted. Furthermore, even though the amount of fixation is not as great as in the air-dry soils, its significance has merit since the greatest amount of ammonia volatilization occurs in these soils. Thus, as fixation occurs, a replenishable pool of ammonia is available, either fixed or exchangeable, for volatilization.

The soils of 5-1 and 7-1 receiving ^{15}N plus plant material amendments and maintained at -15 bars also reveal some interesting data. In both experimental systems, heterotrophic fixation of nitrogen is again significant, 18.5 and 15.3% respectively. Again this indicates the possible role of an algal-fungal symbiotic relationship in which atmospheric nitrogen is fixed aerobically and in the dark. However,

other microbial activities in the 5-1 soil are limited since this soil was maintained at the permanent wilting point. Nevertheless, a slight increase (2.5%) in nitrite and nitrate does occur, with the predominant form again being nitrite (60%), and again suggesting an allelopathic inhibition on nitrification.

In soil 7-1, nitrification does occur with a 30.5-fold increase in nitrite and nitrate, the latter being predominant (85%). Furthermore, Robinson (1957) and Justice and Smith (1962) have shown that nitrification does occur at the permanent wilting point.

In ammonia volatilization studies, the order of the total amount of volatilized ammonia at both -1 bar and -15 bars is as follows: *Artemisia* soil < *Ceratoides* soil < *Atriplex* soil. The ^{15}N data confirm the observation that the greatest amount of ammonia volatilized occurs during the first week. However, as noted before, heterotrophic fixation, nitrification and denitrification may influence the rate of ammonia loss. As a final note, the possibility of an allelopathic inhibition by *Artemisia* on ammonification may occur as the soil from site 5-1 shows the lowest amount of volatilized ammonia. In addition, it has been previously observed (Skujins and West 1974) that the amount of ammonia volatilization was least in soils under the canopy and greatest in the interspace soils.

The acetylene reduction assay was used to measure nitrogen fixation by blue-green algae-lichen crusts from Curlew Valley.

Spring in situ assays showed that 90 g of nitrogen were fixed per hectare in 24 hr. Based upon 120 days of fixation (fall and spring) at that rate, 10.8 kg N could be fixed.

Up to 14 g N may be fixed ha^{-1} hr^{-1} in situ. Based upon 120 days of fixation (12 hr per day) at that rate, 18.7 kg of N could be fixed annually.

The nitrogen fixation potential (laboratory assays) could be as high as 80 g N ha^{-1} hr^{-1}. Based upon 120 days of fixation (12 hr per day) at that rate, 115.2 kg N could be fixed annually. The previously discussed estimates would be for areas of 100% crust cover. Thus an estimate of 10 to 100 kg N fixed ha^{-1} yr^{-1} might be a reasonable one.

Based upon 120 days of nitrogen fixation at the May-September mean rate shown in Table 11, up to 7.7 kg N ha^{-1} could be fixed on the *A. confertifolia* site; up to 0.25 kg N ha^{-1} on the *C. lanata* site; and up to 6.6 kg N ha^{-1} on the *A. tridentata* site. Assuming that the transects provide a rough estimate of nitrogen-fixing crust cover, then annual nitrogen input, at least on the *Atriplex* site, would be toward the lower end of the 10 to 100 kg N range.

Nitrogen input by crust nitrogen fixation depends then upon the extent of crust cover, rainfall and temperature during the fall and spring rainy seasons, and duration of the rainy periods.

There appeared to be fall and spring peaks in the nitrogen-fixing potential of crusts, which correlates with the rainy seasons.

Soil core transect samples from the *A. confertifolia*, *C. lanata* and *A. tridentata* sites showed that there was not a significant difference in the nitrogen-fixing potential between May and September transects. The *C. lanata* potential was significantly lower and reflected the scant lichen cover in that shrub community.

Glucose was found to potentiate heterotrophic nitrogen fixation of moistened crusts and soils. The crust heterotrophic temperature optimum was found to be 30 C. No ethylene was produced in the absence of glucose. Presumably, in the field, the amount of available organic carbon is too low to significantly potentiate heterotrophic nitrogen fixation.

Blue-green algae-lichen crusts were found to be greatly reduced under the desert shrub canopies of *A. confertifolia*, *C. lanata* and *A. tridentata*. Leaf aqueous extracts and leaf volatile products inhibited crust autotrophic nitrogen fixation. Dried shrub leaves inhibited glucose potentiated, soil-heterotrophic nitrogen fixation. The heterotrophic nitrogen-fixing potential was reduced in soils collected from beneath shrub canopies. Shrub inhibitors of nitrogen fixation may then play a role in nitrogen input in desert shrub communities.

By nitrification potential (perfusion) measurements, however, there was no inhibition of nitrification (i.e., $NH_4^+ \rightarrow NO_2^-$) by the plant canopy. In fact, the nitrification rate was higher in soil sampled beneath the canopy. Upon analyzing the soil after perfusion, however, there was a significantly high level of organic and fixed nitrogen, suggesting a lower mineralization rate than the interspace soil. The pH of the perfusates always rises to a level of 8.0 to 8.5 and at the end of the experiment when nitrification has begun to take place the pH is somewhat lower.

The experiment to wash out inhibitors did not show significant results. The soil used, however, was sampled in October 1973 and was reported to have low overall biological activity because of the low moisture of the samples at that sampling date (Skujins and West 1974). This same experiment could be rerun with perhaps another more active soil and the microorganisms allowed to develop for a longer period. This perhaps would show a decrease of the nitrification taking place after the solution containing the inhibitors was introduced.

Nitrification was indeed inhibited by desert shrub plant material. As can be seen from the figures, initial perfusion, which builds up the microbial population and establishes an enrichment culture, is similar in all cases with the decrease of ammonium and concurrent increase of nitrite and nitrate. Soil reperfused with fresh ammonium solution alone shows a greater level of nitrate than with initial perfusion

and nitrite is negligible or decreases sharply by day 4. No lag period is seen since the turnover rate for *Nitrosomonas* is faster than for *Nitrobacter.*

There was no significant difference between the control and soils perfused with 0.46, 0.48 and 0.5% plant extracts except that the rate of ammonium decrease was lower. On the other hand, soils perfused with a 0.1% plant extract showed a higher level of nitrite that was maintained through day 12 or 16 with the exception of the soil perfused with *Ceratoides* extract which reacted similarly to the control. Oxidation to nitrate continued but at a lower level.

Similarly, soil perfused with 1.0 and 0.5% leachates shows an increased level of nitrite upon reperfusion, especially soil perfused with 0.5% *Atriplex* leachate in which nitrite is higher than nitrate until day 16. Indeed, oxidation of nitrite to nitrate is suppressed by plant leachates, implicating some inhibition which causes a lower rate of nitrification.

Results with *Philodendron* leachate and extract at any concentration were similar to the control. A 0.5% leachate of *D. glomeratus* as a biological comparison showed similar results to *Philodendron.*

Results obtained from the plant inhibition experiments have many implications. The inhibition may be an important competitive mechanism among plants in the desert ecosystem which have low nitrogen requirements. The stage of maturity of the plant seems to influence the inhibitory effect on the organisms with a greater inhibition occurring when the plant is at a mature stage (Rice 1964). Rice and Pancholy (1973) have suggested that climax vegetation inhibits nitrification, and soils under such vegetation are thus low in nitrate. They also suggest that ammonium ion is used in higher successional stages. The question is whether the Curlew Valley vegetation can be considered at a higher successional stage.

Plant inhibition of nitrification as it occurred under laboratory conditions does not necessarily imply that there is inhibition by plant litter in the desert. This is somewhat substantiated by the canopy vs. interspace experiment. There may be a breakdown of the inhibitory elements after the plant material has dried and fallen off the plant. Through the process of decomposition, specifically proteolysis, more nitrogen, in the form of NH_4^+, is added to the soil regime beneath the canopy which subsequently increases the substrate for the nitrifying microorganisms to develop and thus have a greater nitrification potential than the soil from the interspace.

Ammonification experiments were shallow and needed to be expanded and modified. Results obtained were rather insignificant.

Results for ATP concentration were significant; however, more replication with other soils is needed in order to sustantiate these results of ATP correlating with other biological activity rather than with microbial numbers. Strange et al. (1963) found the ATP pool was not necessarily related to the energy requirements of the organism. This does not correlate with our results.

Also, we are in the process of developing a more efficient method for determing the recovery of ATP.

Catalase experiments have shown that the bulk of the activity takes place in the first 0 to 10 min after addition of the peroxide to soils, and continues slowly up to 30 min.

Blanks are essential during catalase determination because of the residual activity caused by the mineral and organic part of the soil. According to results, 5 to 20% of the total activity is caused by this residual activity.

Using the titrimetric procedure for determining catalase activity, our results show that blank activity makes up from 20 to 50% of the total activity. Further development of the method is in progress.

EXPECTATIONS

Our studies have indicated that there are biological inhibition processes taking place in the soil which might significantly affect the various nitrogen pathways in the soil.

Detailed examination of the environmental inhibitory and regulatory factors affecting the photosynthetic nitrogen fixation and other processes of the nitrogen cycle, such as ammonification and nitrification, is expected to show that plant litter retain inhibitory substances and thus affect their own decomposition and nitrogen release rate into the soil. Examination of the microflora and biochemical activities of desert soils of various desert types would indicate a presence and dominance of a drastically different soil microflora with respect to its physiological response not only to the physical and climatological environmental factors, but also to its relationship with other biological components of the biome.

The results on nitrogen fixation and other steps in the N cycle, which are reported on here, should be verified by detailed examination and duplication during several seasons.

It is expected that most of the data will be verified also for other similar arid areas. By comparing activities of various components of the nitrogen cycle in several western deserts, the activities may be correlated with soil chemical and physical properties, especially texture, and with climatic regime. It is expected that the information developed will be of value in the construction of models for various components of the desert ecosystem.

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