Fixation of Isotopic Nitrogen on a Semiarid Soil by Algal Crust Organisms

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Fixation of Isotopic Nitrogen on a Semiarid Soil by Algal Crust Organisms[1]

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ABSTRACT

Semiarid desert algal crust organisms were found to fix N_2 when exposed to an atomosphere which contained isotopically enriched N. Significant quantities of the N isotope were detected in the total crust N after 3 days of incubation under field-simulated conditions.

Net N_2 fixation rates by the algal crust organisms were 0.16 and 0.10 lb of N/acre of crust surface per day under continuous wet and cycling wet-dry conditions, respectively. Net fixation of N under field-simulated conditions adequately compensated for the removal of N by livestock. The rate of N_2 fixation under field-simulated conditions increased linearly for at least 520 days. The amount of N in the algal crust was doubled during this time. No net N change was observed in dry crusts.

Growing algal crusts contained 1% to 2% of the total N as extracellular NH_4-N. Excretion of some fixed nitrogen was suggested by the isotopic enrichment of the extracellular N fraction and uptake of labeled N by grass seedlings (*Artemesia sp.*) growing on incubated crusts.

ALGAL CRUSTS ARE growths of microorganisms which occur on the surface of noncultivated soils and under conditions where cultivated soils remain undisturbed for extensive periods. Filamentous forms of blue-green algae comprise the bulk of the microorganisms present in the crust. The algal filaments and associated fungal mycelia form a matrix in which soil particles are aggregated into crusts up to 1 cm in thickness and of varying area (Fig. 1). The crusts are found on neutral to alkaline soils and may be subject to extremes in temperature and soil moisture.

Algal-encrusted surface soils have been reported in Oklahoma, Texas, Arizona, New Mexico, Nevada, California Washington (10), and in other parts of the world (3, 10, 12) The algal crusts of semiarid desert regions have been cited as an important source of organic matter and credited with improving infiltration, decreasing erosion, and aiding in plant seedling establishment (4, 8, 9).

The possible contributions of N_2 fixation by blue-green algae on semiarid soils were discussed by Fletcher and Martin (8) in Arizona and Shields et al. (11) in other southwestern states. Later Cameron and Fuller (7) isolated several blue-green algal species (*Nostoc*, *Scytonema*, and *Anabaena*) which were capable of fixing N_2 in solution culture. Increases in soil N content were observed following short term laboratory incubation of algal crust material.

It has not been shown whether N_2 fixation occurs when the organisms grow in their natural habitat. The objective of this study was to confirm nitrogen fixation unequivocally by using the isotope N^{15}. Further study was undertaken to evaluate the potential N_2-fixation rate of algal crust organisms under

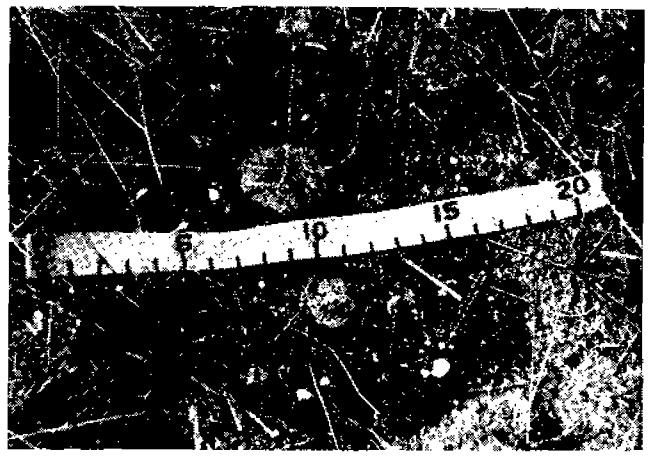

Fig. 1—Desert Algal crusts *in situ*. The scale division are in centimeters. The soil surrounding the crusts was moistened to increase contrast.

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Fig. 2 The desert grassland community where the desert algal crusts were collected.

field simulated conditions. The disposition of fixed N was investigated with respect to the potential availability to other organisms in the algal crust ecosystem.

MATERIALS AND METHODS

Algal crusts were collected from a semiarid rangeland site at an elevation of 3,700 ft (Fig. 2). This desert grassland site was located about 30 miles north of Tucson, midway between Oracle Junction and Oracle, Arizona.

Extensive investigations were made of the variation in nitrogen content of algal crusts as influenced by several sampling techniques (H. F. Mayland, 1965. Isotopic nitrogen fixation by desert algal crust organisms. *Unpubl. Ph.D. thesis, Univ. of Arizona Library, Tucson*). It was desired to have the sample variation in total algal crust N comparable to the variation usually experienced with the Kjeldahl procedure. The coefficient of variation for the N content of field crusts were found to be $> 33\%$. The method adopted allowed for the prediction of the N content of duplicate samples with a coefficient of variation of $< 4\%$. Measurements of total N content were made on surface area basis, rather than on weight. Native algal crusts were removed intact from the surface of the desert grassland soils and, if moist, allowed to air dry. Excessive adhering soil was scraped from the underside of the crust. One-centimeter diameter cores were cut from the 3- to 4-mm thick crust. A composite sample consisted of 60 random cores placed face up on a glass wool pad in a 100-mm plastic petri plate. The composite sample had a surface area of 47.1 cm^2 and contained about 20 g of crust material. The algal crust samples prepared in this manner maintained the natural organization of the algal crust components.

When N^{15}-enriched N_2 was employed, samples were incubated in gas-tight growth chambers assembled from either one liter glass resin reaction kettles or glass vacuum desiccators. An artificial atmosphere of about 5% CO_2, 20% O_2, and 75% N_2 was admitted into the incubation chamber after removal of the natural atmosphere. The crust sample was moistened with deionized water either by addition through a stopcock in the resin reaction kettle cover or by means of a cotton wick leading from the glass wool pad of the sample to a water reservoir.

Qualitative spot tests for NH_4, NO_2 plus NO_3, and NO_2 were made with Nessler's reagent, diphenylamine and Trommsdorf's reagent, respectively (1). The lower limit of detection was 0.25 ppm NH_4-N, 1.0 ppm NO_2-N or NO_3-N, and 0.1 ppm NO_2-N, respectively, for the above reagents. Free amino acids were extracted with cold water and identified by paper chromatography. A solvent system of 3:12:5 (v/v/v) of glacial acetic acid/*n*-butanol/water was used and ninhydrin employed for visualization. Identification was made by comparison with known amino acids developed on the same chromatogram.

Table 1—Total and isotopic N fixed by algal crust organisms in 21 days

Culture number	Nitrogen Atmosphere	Nitrogen		Nitrogen-15	
		Final	Fixed	Total	Excess
		mg		atom %	
0102	Control	42.4	8.4**	0.368	0.000
0105	N^{15}-enriched	41.6	7.6**	0.753	0.383
0107	Control	37.1	3.1*	0.368	0.000
0109	N^{15}-enriched	39.6	5.6**	0.752	0.384
0112	N^{15}-enriched	41.6	7.6**	0.710	0.342

* Significant and ** highly significant net increase over initial N of 34.0 mg with s = 1.29 mg

Table 2—Influence of moisture on N_2 fixation by algal crust organisms incubated in the same atmosphere

Culture number	Moisture treatment	Nitrogen		Nitrogen-15	
		Final	Fixed*	Total	Excess
		mg		atom %	
0701	Wet	42.1	5.6**	0.662	0.295
0703	Dry	39.4	3.1*	0.367	0.000
0705	Wet†	44.2	7.9**	0.684	0.317
0707	Dry†	38.0	1.7	0.379	0.012
0709	Wet	44.4	8.1**	0.679	0.312

* Significant and ** highly significant net increase over initial N of 36.3 mg with s = 1.29 mg. † Wet for final 4 days of incubation.

Quantitative analysis of NH_4-N utilized a 4-min steam distillation method (6). Total nitrogen was determined by the Kjeldahl method. A 1:3:1 sample/acid/salt ratio and a salt composition of $K_2SO_4/CuSO_4/Se$ in a 100:10:1 ratio (w/w/w) was used. Digestion was continued for 6 hr after clearing as recommended by Bremner (5). Ammonia was recovered by alkaline distillation and prepared for analysis of N isotopes by the hypobromite method (6).

Mass spectrographic analysis of sample N isotope distribution and the gaseous composition of atmospheres over incubating samples were determined on a Consolidated Electrodynamics Corporation (CEC) mass spectrometer model 21–130. Some N^{29}/N^{28} analyses were carried out on a CEC model 21-620 mass spectrometer which was equipped with a ratio readout system. Nitrogen-15 analyses of biological control samples on the CEC 21–130 had a standard deviation of 0.007 atom percent over a 15-month period. Duplicate sample data from the CEC 21–130 were compared to those of the CEC 21–620 with a difference of -0.004 to $+0.011$ atom percent N^{15}.

RESULTS AND DISCUSSION

Confirmation of Nitrogen Fixation

Crusts incubated in individual chambers in the presence of 2% N^{15} fixed at least 25 times more isotopic N_2 then was needed for significant detection of N^{15} enrichment.

Total algal crust N increased as much as 8.4 mg per sample (Table 1). These crusts were incubated at 27C for 21 days with fluorescent lighting with an illumination level of 600 to 1,000 ft-c. The fixed nitrogen was calculated as the difference between the final N content of the incubated crusts and the N content of samples not incubated. Two crusts incubated in control atmospheres exhibited a comparable increase in total N which was normally labeled with 0.368% N^{15}.

Further evidence that algal crust organisms fixed N_2 was obtained when both wet and dry crusts were exposed to the same artificial atmosphere for 47 days. Incubation conditions included fluorescent-incandescent light with an illumination level of 2,000 to 3,000 ft-c., 12-hr days at 35C, and 12-hr nights at 18C. The final nitrogen content of wet crusts was significantly higher than that of dry crusts (Table 2). A dry

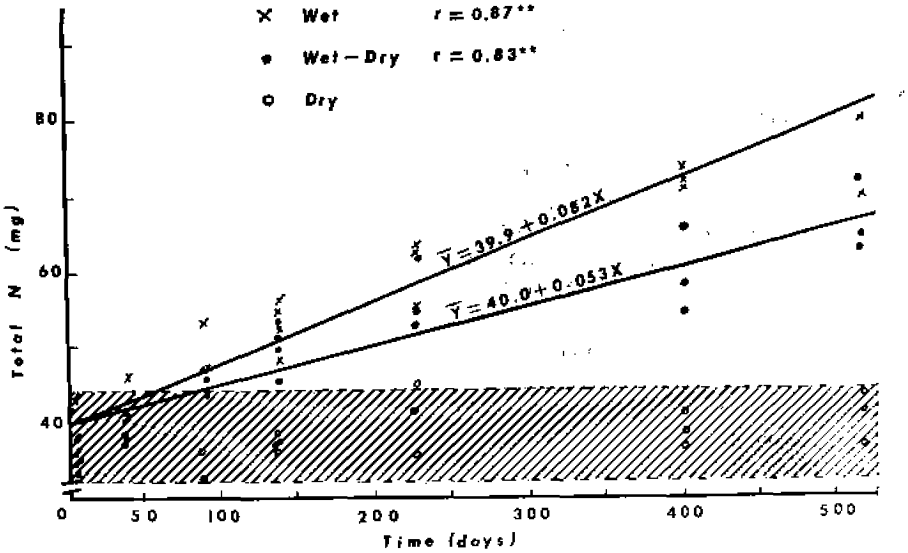

Fig. 3—Net nitrogen fixation as a function of time and moisture treatment under field simulated conditions. The cross hatched area represents the mean (±2s) total N level of all algal crusts at time zero.

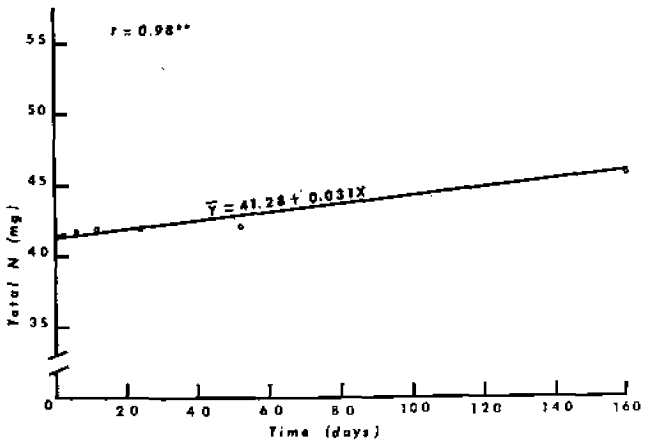

Fig. 4—Nitrogen fixation rates of algal crust organisms as deter mined by N-15 tracer.

culture, number 0707, became moist from dripping condensate during the last 4 days and fixed a very small amount of N^{15}. The data provided conclusive evidence that fixation of N_2 occurred within the algal crusts.

Physiological Investigations

Nutrient Requirements

Moistening of crusts with deionized water simulated natural conditions and eliminated the nutrient solution as a source of nitrogen contamination. Short-term studies showed that there was no apparent advantage in using an N-free nutrient solution as compared to sterile deionized water on the rates of N_2 fixation. The algal crust, which included a 3- to 4-mm thick layer of soil, served as a source of all nutrients other than C, N, O, and H_2O. The constant rate of N_2 fixation by wet crusts in long-term experiments (Fig. 3 and 4) suggested that nutrient availability within the crust did not change even when the total N was doubled.

Effects of Moistening Techniques

The incubated algal crusts were moistened either by flooding the surface of the crusts or by a wick technique whereby water moved up through the crust. Both methods produced environmental effects of salt and water movement similar to conditions expected in the field. Surface application of water resulted in the downward movement of nutrients and other salts. Evaporation of water from crusts wet by capillarity caused the accumulation of microlayers of soluble salt on the surface of crusts.

About 50 μg of NH_4-N were found in the leachates after 20 to 30 days for each of 30 cultures wet by surface application of water. Other nutrients or salts could have been leached, in addition to the ammonium salt, from the crusts. Under field conditions, these nutrients would have been eluted into the subsoil. The wick technique tended to concentrate the salts at the crust surface thus increasing the alkalinity. When crusts were subjected to upward movement of water, traces of ammonia were detected in the incubation chamber atmosphere by mass spectrometric techniques. Appreciable quantities of NH_4 were absorbed by the water reservoir and in long-term N^{15} studies showed an N^{15} enrichment (Table 3). Similar losses of fixed N by volatilization of NH_3 could occur in the field.

Incubation Gases

Circulation of atmospheric gases in solution cultures of N_2-fixing organisms has been shown to increase the rate of growth and N_2 fixation. In this study of surface growing algal crust organisms, mechanical circulation of the atmospheric gases was found unnecessary.

Photosynthetic N_2 fixation studies, which used N^{15} as a tracer were carried out in transparent chambers which provided for complete confinement of the N^{15}-labeled atmosphere. Artificial atmospheres containing approximately 5% CO_2, 20% O_2, and 75% N_2 were introduced into the incubation chambers at the initiation of each study.

Table 3—Volatilization of labeled NH_4-N from algal crusts and subsequent absorption by the reservoir water

Incubation time	Ammonia-N	Nitrogen-15*
Days	mg	atom %
12	1.75	0.3642
160	1.71	0.3771

*Data from CEC 21-620, precision of $\pm$0.0003 atom % N^{15}.

As incubation of the algal crusts began, the CO_2 composition of the atmosphere increased while O_2 decreased. After 24 days, the O_2 composition began to increase slowly towards its original concentration. This increase in the O_2 concentration was followed by a corresponding loss of CO_2 from the atmosphere. After 6 days, CO_2 fixation activity exceeded respiration. A similar biological response has been described for lichens (13).

The net biological activity of the algal crust was followed by observing changes in O_2 and CO_2 levels in the desiccator atmospheres with respect to an inert standard, A, which was introduced as a small part of the artificial atmosphere.

Field Simulated Nitrogen Fixation

Frequent late summer rains occur in the semiarid region where the crusts are found. Periods between storms may be hot with rapid drying conditions. Diurnal temperature changes of 17C are common in the regions. Experimental conditions were therefore employed which provided a 12-hr day of 35C and 12-hr night of 18C. Moisture treatments included continuously wet, continuously dry, and alternately wet 1 day followed by 3 days of drying conditions. The continuously wet treatment provided a measure of the maximum rate of N_2 fixation whereas the continuously dry treatment served as a control for the initial N content of all crusts. Continuously moist crusts fixed N_2 at a rate, which after 50 days, was significantly greater than that predicted from the initial N level (Fig. 3). The algal crusts on the cycling moisture treatment required nearly 80 days before the increase in their total N content was significantly different from the initial N content.

Nitrogen fixation continued at constant rates for both moisture treatments up to termination of the study at 520 days. The regression lines for the wet and the cycling moisture treatments indicate that 0.082 and 0.053 mg N were fixed per day, respectively. No significant change was observed in the total N of crusts which remained dry during the entire study.

The N_2 fixation rate was also determined from a series of crusts kept continuously moist while incubated in an N^{15} enriched atmosphere. The change in N content was determined from the amount of N^{15} in the crusts and was plotted as a function of time (Fig. 4). The data fit a linear regression line with an r value of 0.98 which was highly significant. The N_2 fixation rate was 0.031 mg N/day. This rate was lower than the rates of either of the two moisture treatments previously described. The decreased rate may have been caused by changes in gaseous constituents during incubation.

Plant Available Nitrogen

Water extracts made from the algal crust material in different growth stages were examined for the presence of NO_3, NO_2, and NH_4 ions. As much as 150 ppm (w/w) NH_4-N were found. Neither NO_2 nor NO_3 was detected. Attempts to isolate nitrifying organisms from the algal crusts used in this study were unsuccessful, and their probable absence could explain the lack of NO_3-N.

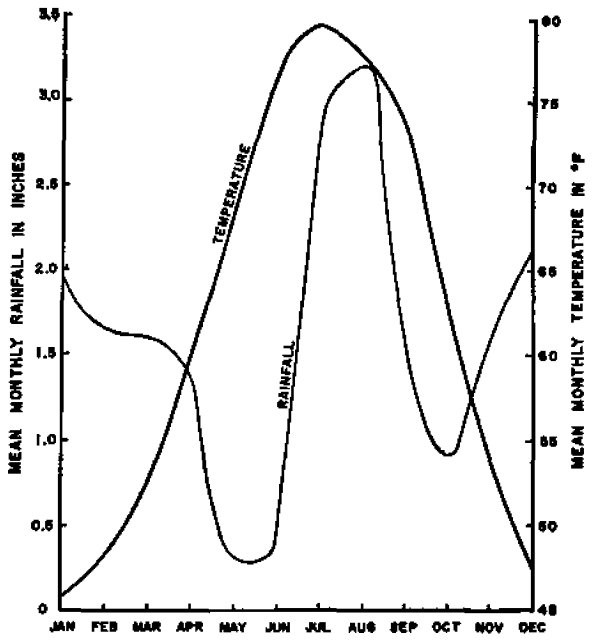

Fig. 5—Mean monthly rainfall and temperature for Oracle, Ariz.

These same extracts were analyzed for the presence of amino acids by the paper chromatographic technique. Small quantities (< 5 ppm, w/w) of glutamic and aspartic acids were identified in all extracts. Cystine and alanine may also have been present but in concentrations too low to permit unequivocal identification. Other ninhydrin reacting substances were also present. The water-extractable organic N represented as much as 0.5% of the total algal crust N. From 1% to 2% of the total crust N was found as NH_4-N in the water extracts from actively growing algal crusts. The NH_4-N in the water extracts from cultures numbered 0105, 0109, and 0112 (Table 1) contained an average enrichment of 0.737 atom percent N^{15}. Excretion of recently fixed N_2 is supported by findings of other studies (11). Grass seedlings (*Artemesia* sp.) which grew on one of the crust cultures were found to have taken up a significant amount of the N^{15} tracer (H. F. Mayland, 1965. *See* Materials and Methods).

Desiccated crusts contained about twice the amount of water-extractable NH_4-N as was found in actively growing crusts. This buildup of inorganic N could have resulted from excretion and cell autolysis. The increase of NH_4-N upon drying was similar to the observations of Birch (2).

Ecological and Economic Contribution

Properly managed, about 40 acres of rangeland in the same location where the crusts were collected will support one cow plus the production of one 400-lb calf per year. Thus the annual harvest of N is approximately 0.41 lb/acre.

Prediction of the annual contribution of N_2 fixed by algal crusts from this study can be done as follows: Rainfall and temperature patterns on the rangeland site produce conditions

conducive for algal crust and higher plant growth during about 63 days in the summer (Fig. 5).

If the rate of N_2 fixation for the continuously wet crusts (Fig. 3) is accepted as the maximum amount of fixation, about 9.6 lb of N would be fixed per acre of crusts per year. Since the algal crusts do not occupy the entire surface area under native conditions, N_2 fixation would be less *in situ*. This gain in N per year though small, compares favorably with the production capacity of the rangeland and the total N content of the soil (7). Over a long period of time the algal crusts could provide an important source of N for desert grasslands.

Since more than 20 million acres of rangeland are used for cattle production in Arizona, the economic contribution of fixed N by algal crust organisms could be of considerable significance. The economic value of the added N must include consideration that much of the rangeland is inaccessible by normal field equipment and thus distribution of fertilizer N would be expensive.

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