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

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ABSTRACT

Semiarid desert algal crust organisms were found to fix \( N_2 \) when exposed to an atmosphere 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.

Fig. 1—Desert Algal crusts in situ. The scale division are in centimeters. The soil surrounding the crusts was moistened to increase contrast.
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 was 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 cm² 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 N⁰-enriched N₂ was employed, samples were incubated in gas-tight growth chambers assembled from either one liter glass resin reaction kettles or glass vacuum desiccanors. An artificial atmosphere of about 5% CO₂, 20% O₂, and 75% N₂ 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 NH₄, NO₃ plus NO₂, and NO₂ were made with Nessler’s reagent, diphenylamine and Trommsdorf’s reagent, respectively (1). The lower limit of detection was 0.25 ppm NH₄-N, 1.0 ppm NO₃-N or NO₂-N, and 0.1 ppm NO₂-N, respectively, for the above reagents. Free amino acids were extracted with cold water and identified by paper chromatography. A solvent system of 6:1:25 (v/v/v) of glacial acetic acid, butanol, and water was used and ninhydrin employed for visualization. Identification was made by comparison with known amino acids developed on the same chromatogram.

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

Quantitative analysis of NH₄-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 K₂SO₄/CuSO₄/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 spectographic 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 N²⁰/N²¹ analyses were carried out on a CEC model 21-130 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 N²¹.

RESULTS AND DISCUSSION

Confirmation of Nitrogen Fixation

Crusts incubated in individual chambers in the presence of 2% N²¹ fixed at least 25 times more isotopic N₂ than was needed for significant detection of N¹⁹ enrichment.

Total algal crust N increased as much as 8.4 mg per sample (Table 1). These crusts were incubated at 27°C 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% N²¹.

Further evidence that algal crust organisms fixed N₂ 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 35°C, and 12-hr nights at 18°C. The final nitrogen content of wet crusts was significantly higher than that of dry crusts (Table 2). A dry

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

<table>
<thead>
<tr>
<th>Culture number</th>
<th>Nitrogen atmosphere</th>
<th>Nitrogen N¹⁵</th>
<th>Nitrogen excess</th>
<th>Nitrogen N¹⁵ excess</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fixed</td>
<td>Fixed</td>
<td>Total</td>
<td>Fixed</td>
</tr>
<tr>
<td>0102</td>
<td>Control</td>
<td>42.4</td>
<td>6.4 **</td>
<td>6.268</td>
</tr>
<tr>
<td>0105</td>
<td>N²¹-enriched</td>
<td>41.6</td>
<td>7.4 **</td>
<td>6.753</td>
</tr>
<tr>
<td>0107</td>
<td>Control</td>
<td>37.1</td>
<td>5.1 **</td>
<td>6.368</td>
</tr>
<tr>
<td>0108</td>
<td>N²¹-enriched</td>
<td>38.8</td>
<td>6.0 **</td>
<td>6.752</td>
</tr>
<tr>
<td>0112</td>
<td>N²¹-enriched</td>
<td>41.6</td>
<td>7.6 **</td>
<td>6.716</td>
</tr>
</tbody>
</table>

* Significant and ** highly significant net increase over initial N of 34.6 mg with

\[ n = 1.29 \text{ mg} \]

Table 2—Influence of moisture on N₂ fixation by algal crust organisms incubated in the same atmosphere

<table>
<thead>
<tr>
<th>Culture number</th>
<th>Mollecular treatment</th>
<th>Nitrogen N¹⁵</th>
<th>Nitrogen excess</th>
<th>Nitrogen N¹⁵ excess</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fixed</td>
<td>Fixed</td>
<td>Total</td>
<td>Fixed</td>
</tr>
<tr>
<td>0701</td>
<td>Wet</td>
<td>42.1</td>
<td>6.0 **</td>
<td>6.562</td>
</tr>
<tr>
<td>0703</td>
<td>Dry</td>
<td>30.4</td>
<td>5.3 **</td>
<td>6.378</td>
</tr>
<tr>
<td>0705</td>
<td>Wet</td>
<td>46.2</td>
<td>7.9 **</td>
<td>6.784</td>
</tr>
<tr>
<td>0707</td>
<td>Dry</td>
<td>38.0</td>
<td>4.7 **</td>
<td>6.370</td>
</tr>
<tr>
<td>0708</td>
<td>Wet</td>
<td>44.4</td>
<td>5.1 **</td>
<td>6.781</td>
</tr>
</tbody>
</table>

* Significant and ** highly significant net increase over initial N of 36.5 mg with

\[ n = 1.29 \text{ mg} \]
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\(_2\)O. 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 \(\mu\)g 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.
As incubation of the algal crusts began, the CO₂ composition of the atmosphere increased while O₂ decreased. After 24 days, the O₂ composition began to increase slowly towards its original concentration. This increase in the O₂ concentration was followed by a corresponding loss of CO₂ from the atmosphere. After 6 days, CO₂ 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 O₂ and CO₂ 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 17°C are common in the regions. Experimental conditions were therefore employed which provided a 12-hr day of 35°C and 12-hr night of 18°C. 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 N₂ fixation whereas the continuously dry treatment served as a control for the initial N content of all crusts. Continuously moist crusts fixed N₂ 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 N₂ fixation rate was also determined from a series of crusts kept continuously moist while incubated in an N₁₄ enriched atmosphere. The change in N content was determined from the amount of N₁₄ 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 N₂ 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 NO₃, NO₂, and NH₄ ions. As much as 150 ppm (w/w) NH₄-N were found. Neither NO₂ nor NO₃ 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 NO₃-N.

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 NH₄-N in the water extracts from actively growing algal crusts. The NH₄-N in the water extracts from cultures numbered 0105, 0109, and 0112 (Table 1) contained an average enrichment of 0.737 atom percent N₁₄. Excretion of recently fixed N₂ 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 N₁₄ tracer (H. F. Mayland, 1965. See Materials and Methods).

Desiccated crusts contained about twice the amount of water-extractable NH₄-N as was found in actively growing crusts. This buildup of inorganic N could have resulted from excretion and cell autolysis. The increase of NH₄-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 N₂ 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.

ACKNOWLEDGEMENTS

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LITERATURE CITED