Reprinted from SOIL SCIENCE SOCIETY OF AMERICA PROCEEDINGS
Vol. 30, No. 5, September–October 1966, pages 606–609
677 South Segoe Road, Madison, Wisconsin 53711 USA

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Distribution of Nitrogen Fixed in Desert Algal-Crusts[1]

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ABSTRACT

Fixation of isotopically labeled N_2 by desert algal crust organisms was very rapid after initial wetting of crusts. After 8 days of incubation, a steady rate of fixation was reached which continued for 152 days.

The initial distribution of N was 4% extracellular NH_4, 16% acid-hydrolyzable NH_4 (included extracellular NH_4), 41% α amino acid, 4% hexosamine, 29% acid-hydrolyzable but unidentified, and 11% nonhydrolyzable. After 21 days of incubation the distribution of total N was 2, 18, 42, 2, 28 and 11%, respectively. The distribution of excess N^{15} after 21 days of incubation was trace, 12, 51, 2, 29 and 7% for the respective fractions. Continued incubation had little effect on the relative distribution of N in the several fractions.

ENTRY OF N_2 into the soil N pool via the process of nonsymbiotic N fixation has not been critically investigated. Studies of N_2 fixation by desert algal crust organisms provided an opportunity to observe the fixation rate and N forms entering into the soil N pool (9).

Previous investigations of the distribution of organic N forms in soil have been limited to partition of soil N into acid or alkaline soluble or insoluble classes following hydrolysis (1, 11, 12). Recently refined chemical techniques permit identification and isotope ratio analysis of specific organic N forms such as hexosamines, α amino acids, and acid-hydrolyzed NH_4 (2, 4). Such techniques have permitted specific identification of about 50% of the total soil N.

Knowledge of specific organic N forms is needed for propeevaluation of cultural practices on mineralization, immobilizar tion, fixation and the subsequent availability of soil N to higher plants. Intensive use of a soil for cultivated crops resulted in a decrease in the amount of N found in the several N fractions (10) but may have had little effect on the relative proportion of N in the several fractions (6). Immobilization and subsequent mineralization have been studied with isotopically labeled straw and fertilizer N (13, 14). Immobilization reached a maximum within 5 to 10 days; however, the subsequent increase of inorganic N may not have resulted from an equivalent remineralization of the added tracer N (3, 15). About 75% of the N absorbed by plants was derived from the acid-hydrolyzable, nondistillable class of N compounds. However, the labeled fertilizer N in this acid-hydrolyzable fraction was utilized at a faster rate by plants than the N initially present in the fraction (13).

A more detailed knowledge is needed of the specific forms of N in soil and the pathways of transformation between the several N forms. The study reported here evaluates the disposition of isotopically labeled N_2 fixed by algal crust organisms under field-simulated conditions.

MATERIALS AND METHODS

Algal crusts were removed intact from the surface of a soil supporting desert-grassland vegetation. Excess soil was removed and 1-cm-diameter cores were cut from the crusts. Composite samples consisting of 60 random cores were placed in a desiccator containing about 75% N_2 enriched with 1 to 2 atom % N^{15}, 5% CO_2, 20% O_2 and 0.3% A by volume.

The samples were moistened with deionized water by means of a cotton wick leading from a water reservoir to the sample. Incubation was in a plant growth chamber with a fluorescent-incandescent light source of 2,000 ft-c at the bench top. The 12-hr day temperature was 35C and the 12-hr night temperature was 18C.

Crusts, after incubation, were rapidly frozen, lyophilized and then ground to pass a 100-mesh sieve. Analysis of total N, and of extra-cellular NH_4-N were made by semimicro-Kjeldahl digestion followed by alkaline steam distillation and by steam distillation in the presence of MgO, respectively.

The NH_4 released when algal crust material is steam distilled 4 min with MgO is termed extracellular NH_4 and includes NH_4 in the soil solution and on the exchange complex but not cellular N since alkali labile organic N is not released under these conditions (2). The acid hydrolysis and subsequent analyses of various N forms were essentially those of Bremner (2) with the following modifications. Eight grams of material were hydrolyzed with 25 ml of 6*N* H_2SO_4 at 121C for 5 hr. The hydrolyzed material was filtered under vacuum through a sintered glass, F porosity, filter. The residue was washed 4 times with about 3 ml of water/washing. The washings were combined with the filtrate, neutralized to pH 6.5 and made up to 100 ml volume. Aliquots from this stock solution were used for the various N analyses.

Mass spectrographic analysis of the N isotopic distribution was made with a precision of $s = \pm 0.0003$ atom % N^{15}.

[1] Journal Paper No. 1075 of the Arizona Agr. Exp. Sta., Tucson. This research was supported in part by the Cooperative Western Regional Research Project W-85. Presented before Div. S-3, Soil Science Society of America, Nov. 1965, Columbus, Ohio. Received Jan. 24, 1966. Approved April 27, 1966.

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RESULTS AND DISCUSSION

Rate of N_2 Entry

The rate of N_2 entry into the soil N pool via biological fixation was determined by the use of N^{15}. The exponential accumulation of excess N^{15} (Fig. 1) was very rapid for 8 days of incubation, followed by a less rapid increase in N fixed. The first 8-day accumulation of N^{15} appeared as a period of activation of dormant N_2-fixing organisms. An increase in numbers of N_2-fixing organisms may have occurred during the first 8 days and consequently a high N demand followed by high N fixation rates. After 8 days, cell growth (increase in size and number) probably occurred at a different and rather constant rate during the present study.

The initial rapid increase shown in Fig. 1 was not detectable in an earlier study (9). The small quantity of N fixed in the first few days and the lower sensitivity may have been the reason for not previously detecting the shape of the curve. Certain unknown environmental peculiarities in the incubation chambers may have induced the exponential fixation rate. The rate is, however, many times slower than the mean doubling time of 8 hr for *Nostoc muscorum*, one of the more rapid N_2-fixing algae (7).

Distribution of Nitrogen

Nitrogen fractionation data are presented in Table 1 for algal crusts incubated air dry, alternately wet one day followed by 3 days of drying, and continually wet to field capacity. Incubation was for 147 days. The means of five samples are given for each moisture treatment.

Extracellular NH_4 (MgO distillable) was composed of exchangeable and soluble NH_4. Dry crusts contained more extracellular NH_4 than actively growing crusts. This may have resulted from active excretion of N and by cell autolysis. Recycling of N was blocked when insufficient moisture was available for cell growth.

Nonhydrolyzable N accounted for about 10% of the total algal crust N regardless of moisture treatment. This value compares to the range of 10 to 20% reported by Ferguson and Sowden (5) and Stevenson (11). The smaller amount of insoluble N found in the algal crusts in our study was probably due to the presence of higher concentrations of active cell material. As is the case with acid-hydrolyzable residue, little is known about the identity of N forms in the nonhydrolyzable fraction. Cyclic N compounds such as found in nucleoproteins may be stable against complete acid hydrolysis.

Acid-hydrolyzable N accounted for 90% of the total algal crust N. This amount was larger than the 70 to 83% reported by Cheng and Kurtz (4), and others (5). The difference again is likely due to the greater amount of cellular N in crusts as compared to predominately residual soil organic N examined by others (4, 5).

The NH_4 released following acid hydrolysis included the extracellular plus the NH_4 obtained by deamination of compounds such as amides. The reason for differences in the relative amount of hydrolyzable NH_4 for the three moisture treatments was not known. The relative amount of NH_4-N found in the algal crusts was about one-half that found in other soils (4, 5, 11).

About 3 to 20% of the total soil N has been found in the hexosamine form (4, 5, 11). Algal crust samples contained about 3% of the total N in this form. Analyses were made of the hexosamine fraction of soils to which known quantities of standard glucosamine hydrochloride had been added. Recovery of 86 to 88% of the added amino sugar was obtained. This compared with Bremner's recovery (2) of 86.9% from refluxing with 6*N* HCl for 6 hr. Treatment differences may not be real. Errors of this magnitude could have been caused by the presence of large amounts of free iron oxides. Little is known about the amino sugars in algae (8). Chitin, commonly found as a structural component of insects and fungi, has not been found in the few algal species investigated (8). The small amount of amino sugar may have been derived from the chitin in the fungal component of the algal crusts.

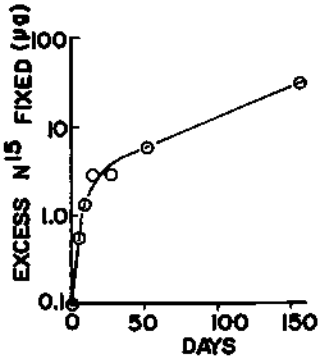

Fig. 1—Accumulative rate of fixed excess N^{15} in algal crusts as a function of time (1.000 atom % N^{15} excess in atmosphere).

The α amino acid N content of algal crusts was found to be about 41%. Acid hydrolysis of soil released from 20 to 40% of the total N as α amino acid N (2). The large amount of amino N found in the crusts was probably due to the high amount of cellular material present and may have included some free amino acid N. Amino acids such as tryptophan, and to a lesser extent cystine, serine and threonine, are known to be destroyed by acid hydrolysis and the N subsequently recovered as NH_4 (2).

The oxidative deamination by ninhydrin (triketohydrindene hydrate) is fairly specific for α amino acid N but has been shown upon acid hydrolysis to yield about 9% of the proline N and 5% of the β alanine N to the α amino acid fraction (2).

Recent investigations have shown that the N yielded by the ninhydrin reaction was approximately equal to the total amino acid N (5). It was, however, suspected that either the ninhydrin or the H_2O_2 used to destroy excess ninhydrin, released NH_4 from some N source other than the α amino acids.

Table 1—Distribution of algal crust N as affected by incubation-moisture treatment after 147 days

Moisture treatment	Extracellular NH_4	Nonhydrolyzable N	Acid-hydrolyzable N				
			Total	NH_4*	Hexosamine	α Amino	Unidentified
	% of total						
Dry	4.2	10.6	89.4	16.2	3.6	41.0	28.8
Wet-dry	2.0	9.0	91.0	15.5	2.4	40.8	32.6
Wet	1.9	10.7	89.3	17.0	2.8	41.9	27.5

* Hydrolyzable NH_4 fraction included extracellular NH_4 N.

The acid-hydrolyzable but unknown N fraction from the algal crusts constituted about 30% of the total N. The reason for the variation in unidentified N for the different moisture treatments, if real, was not known. Cheng and Kurtz (4) concluded that the N in several hydrolyzed soil N fractions, similar to the soluble residue reported here, was probably derived from nonprotein sources. However, the chemical nature of the N in this acid-hydrolyzable but unidentified fraction is still obscure.

Crusts contained a higher proportion of viable cellular tissue than normally found in mineral soils. The hydrolyzable NH_4 and α amino-N fractions accounted for a higher proportion of the total N found in the crusts compared to those same fractions in the soil. Moisture treatments had little effect on distribution of algal crust N, except that dry crusts contained more extracellular NH_4 than either wet-dry or wet-treated crusts.

Distribution of Nitrogen-15

The distribution of N in various fractions obtained from crusts incubated for 21 and 45 days is presented in Table 2. When incubation time was lengthened from 21 to 45 days, the relative amount of N found in the soluble but unknown form increased by an amount approximately equal to the combined loss in the hydrolyzable NH_4 and α amino fractions. Otherwise, small differences in N distribution resulted.

Table 2—Distribution of N forms in algal crusts incubated for 21 and 45 days

Culture number	Non-hydrolyzable N	Acid-hydrolyzable N				
		Total	NH_4	Hexosamine	α Amino	Unidentified
	% of total					
		21-day Incubation				
0105	10.5	89.5	16.1	3.4	42.3	27.6
0109	11.5	88.5	18.7	2.8	40.4	26.5
0112	10.2	89.8	16.2	2.3	43.0	28.4
0102*	10.4	89.6	19.3	0.7	41.1	28.5
$\bar{X}$	10.6	89.4	17.6	2.3	41.7	27.7
		45-day Incubation				
0701	10.7	89.3	15.2	2.8	40.6	30.6
0705	10.0	90.0	14.2	3.4	39.9	32.6
0709	10.1	89.9	13.3	4.0	37.9	34.7
2008*	10.0	90.0	15.6	3.4	36.4	34.5
$\bar{X}$	10.2	89.8	14.5	3.4	38.7	33.1

* Control crusts, incubated in nonenriched-N^{15} atmosphere.

Table 3—Distribution of excess N^{15} in various N forms of algal crusts incubated 21 and 45 days

Culture number	Non-hydrolyzable N	Acid-hydrolyzable N				
		Total	NH_4	Hexosamine	α Amino	Unidentified
	% of total					
		21-day Incubation				
0105	7.5	92.5	11.6	1.1	52.0	27.8
0109	7.7	92.3	12.0	3.1	50.7	26.6
0112	6.2	93.8	11.4	0.3	49.2	32.9
$\bar{X}$	7.1	92.9	11.7	1.5	50.9	29.1
		45-day Incubation				
0701	7.4	92.6	7.1	3.3	54.0	28.2
0705	7.1	92.9	7.9	1.9	49.4	33.6
0709	7.3	92.7	6.1	3.2	47.8	35.6
$\bar{X}$	7.3	92.7	7.0	2.8	50.4	32.5

Excess N^{15} in the partitioned N samples is shown in Table 3. It was determined by subtracting the atom percent N^{15} of partitioned N of control crusts from that found in crusts incubated in the presence of enriched atmospheres. The product of atom percent N^{15} difference and absolute quantity of N then yielded the quantity of excess N^{15}. Little appreciable difference was experienced in any fraction by the increased incubation time. A small decrease was observed in the amount of N in the acid-hydrolyzed NH_4 form and a corresponding increase in the acid-hydrolyzable, but unidentified fraction. A portion of N detected as hydrolyzable NH_4 apparently became more resistant with incubation time to deamination by acid hydrolysis.

The relative distribution of N^{15} in the nonhydrolyzable form, even after 45 days, had not completely equilibrated with the total N found in that fraction. Conversely, the N^{15} in the acid-hydrolyzable fraction had equilibrated and slightly diluted the native N in that fraction. Labeling of the acid-hydrolyzable NH_4 decreased with time.

Initially, the N^{15} content of the acid-hydrolyzable NH_4 fraction contributed greatly to labeling of the entire acid-hydrolyzable fraction. As incubation continued, the proportionate labeling of the acid-hydrolyzable NH_4 decreased. This decrease was likely due to the incorporation of NH_4-N into other compounds and by dilution of the acid-hydrolyzable NH_4 fraction with native N. The decrease with time of excess N^{15} in the acid-hydrolyzable NH_4 fraction is partly explained by a small decrease in the relative distribution of total N in the same fraction.

The N^{15} content of the hexosamine fraction had not equilibrated with native N in 45 days of incubation; however, the small values and wide sample variation in the hexosamine fraction preclude any definite conclusions.

The percent of excess N^{15} in the α amino fraction was nearly the same for both incubation periods. A noticeable concentration of the isotope appeared in the amino fraction since 50% of the isotope was detected in the fraction compared with only 40% of the total N.

Equilibrium had been achieved in the acid-hydrolyzable but unidentified fraction within 21 days and was maintained in the crusts incubated for as long as 45 days.

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