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NITROGEN FIXATION IN FLOODED SOIL[1]

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ABSTRACT

The rate of N_2 fixation was evaluated in four experiments using Crowley silt loam soil incubated under flooded conditions. When a flooded soil not planted to rice was incubated under light for a period of 2 yr, the N_2 fixation rate, as determined by ^{15}N tracer, was 57 µg/g/yr, whereas the N_2 fixation rate measured by the increase in total N content was 61 µg/g/yr. Dinitrogen fixation rate, measured by the acetylene reduction method, was increased considerably when N-poor rice straw was added to the soil. In undisturbed soil cores taken from a rice field, ^{15}N fixation was relatively low in relation to the plant's need, occurring only under light and only in the surface layer of soil. Aquatic weeds growing in these soil cores contained appreciable amounts of ^{15}N. Measurement of N_2 fixation by the acetylene reduction method in plexiglass enclosures in the field also showed a low fixation of N_2.

INTRODUCTION

Dinitrogen fixation is an important process in flooded soils planted to rice. In areas where no chemical fertilizers are used, the N requirement of rice mainly depends on N_2 fixation, which apparently provides a low but dependable supply of N year after year.

The most common method of measuring N_2 fixation activity in soils is the acetylene reduction method, which involves indirect measurement of N_2 fixation by assessing nitrogenase activity. However, accurate measurements of N_2 fixation can be obtained only by using $^{15}N_2$ gas as a tracer. This is a direct method not subject to the correction factor required to convert acetylene reduction to potential N_2 fixation. The factor for converting acetylene reduction to N_2 fixation appears to be especially variable for flooded soils (Hauck and Bremner 1976).

In addition to the apparent advantage of direct measurement of N_2 fixation, the reliability of N_2 fixation measurements can also be improved by incubating the soils for longer periods, which is possible only when using ^{15}N-labeled N_2. The acetylene reduction method has the advantages of simpler analytical requirements and lower cost. Both methods are used in the present study.

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The objectives of the experiments reported here were (1) to evaluate N_2 fixation by measuring the increase in ^{15}N content and total N content of a flooded soil incubated under natural light for 2 yr, (2) to determine the effect of N-poor crop residue incorporation on N_2 fixation, as measured by the acetylene reduction method, (3) to determine ^{15}N fixation directly in undisturbed flooded soil cores obtained from a rice field during the growing season, and (4) to measure N_2 fixation in a rice field using the acetylene reduction method.

MATERIALS AND METHODS

The soil used was Crowley silt loam (Typic Albaqualfs) at the Rice Experiment Station, Crowley, Louisiana. The soil had a total C content of 0.70 percent and a total N content of 0.076 percent. The pH of the soil was 5.6 (1:1 soil:water ratio).

Experiment 1: effect of long-term incubation on N_2 fixation as measured by increase in total N content and fixation of ^{15}N

Five grams of soil (oven-dried basis) was weighed into eight long test tubes (1.5-cm OD and 30-cm length) containing 5 ml of water. The test tubes were then sealed with rubber serum caps (see Fig. 1). The atmosphere in each of the test tubes was displaced with 0.75 atm ^{15}N-labeled N_2 (20.683 atom percent ^{15}N) and 0.25 atm O_2. In this experiment and the one described below, in which ^{15}N-labeled N_2 was used, a mass spectrometer spectrum was run to ensure that labeled N_2O was not present in the gas mixture because of the possiblity that this compound would react with the soil. The only CO_2 supply was that released from the soil organic matter and that recycled from microorganisms during the long incubation period. After the labeled N_2 atmosphere was established in the test tubes, the serum caps were covered with epoxy glue to ensure a complete seal. The tubes were then incubated for 2 yr in an air conditioned laboratory window exposed to afternoon sunlight.

At the end of the incubation period, the atmosphere in the flasks was analyzed again for labeled N content, using a Du Pont 21-621 mass spectrometer, to ensure that the labeled N_2 had not leaked from the tubes and that the O_2 level had remained adequate (O_2 level remained between 0.21 to 0.25 atm). No leakage occurred. The soil from each flask was transferred into 250-ml extraction flasks by washing with 50 ml 2 *N* KCl solution. The samples were then shaken for 1 h and filtered. The filtrates were further analyzed for inorganic N (NH_4-N + NO_3-N) content. The residual soil was dried at 40°C and analyzed for organic N using a modified Kjeldahl method (Bremner 1965a). All samples were further analyzed for labeled N content (Bremner 1965b).

Experiment 2: effects of rice straw application and light on N *fixation in a flooded soil as measured by the acetylene reduction method*

Five hundred-gram samples of soil were incubated with and without 0.4 percent rice straw in either darkness or natural light. Three replications of each of the four treatment combinations were used. The N-deficient straw (0.41 percent total N content) was mixed with air-dry soil, and the samples were placed in 1-liter capacity wide-mouth bottles and flooded with 500 ml water.

At the end of a 30-day incubation period, the atmosphere above floodwater was purged with air (21 percent O_2) for a period of 30 min, and then the bottles were sealed with rubber stoppers fitted with serum caps for injection and removal of gas. Each of the bottles was injected with acetylene to provide approximately 10 percent of the atmosphere, and the samples were further incubated for 2 days. At the end of this period the air space above the floodwater was sampled and analyzed for ethylene by flame ionization gas chromatography, and the N_2 fixation activity was calculated.

Experiment 3: dinitrogen fixation in undisturbed soil cores obtained from a rice field during the growing season

Soil cores were obtained from field plots that had received no N, at midseason (51 days after planting) and late season (70 days after planting). The samples were taken by driving sharpened thin-wall PVC cylinders (length 20 cm, diameter 11.6 cm) between drill rows to a depth of 15 cm and bringing them to the laboratory intact for incubation under labeled N_2. The soil cores were sealed at the bottom with paraffin-petroleum jelly, using the procedure described by Reddy and Patrick (1976). The plastic pan was covered with a clear, plastic dome provided with a serum cap, and the container was then sealed with epoxy glue and plastic rubber. The system is illustrated in Fig. 1. About 300 ml of 99.9 atom percent ^{15}N-enriched N_2 gas was injected into the container after an equal amount of air had been removed, which resulted in a measured ^{15}N content of 3.75 atom percent. Soil cores were incubated under light or in the dark for 30 days in a growth chamber where light (2000 foot candles) and temperature (25°C) were controlled. For the soil cores incubated in dark, the plastic dome was painted with several coats of aluminum paint so that no light entered the incubation chamber. Three replications of each treatment were used. At the end of the incubation period the aquatic weeds that had developed in the cores were harvested and analyzed for total N and labeled N. The soil cores were sectioned horizontally (Reddy and Patrick 1976) and also analyzed for total and labeled N (Bremner 1965b).

Experiment 4: measurement of N *fixation in a rice field*

Dinitrogen fixation was estimated, using the acetylene reduction method, during the growing season in field plots that had received no N fertilizer. The study was carried out with 'Vista' variety on Crowley silt loam at the Rice Experiment Station, Crowley, Louisiana, in 1976. Measurements of acetylene reduction were begun 75 days after seeding when the rice was in the panicle initiation stage.

A plexiglass chamber 2845 cm^2 × 54 cm tall was sealed onto a plexiglass base that had been previously inserted in the soil. A diagram of the

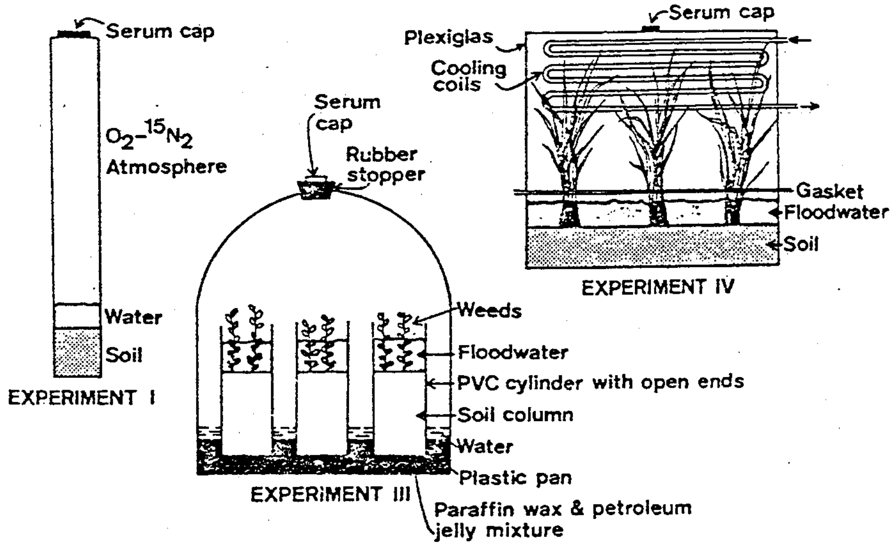

FIG. 1. Experimental systems used to measure N fixation.

apparatus is shown in Fig. 1. Rice plants were growing normally in the container. Ten percent of the atmosphere was replaced with acetylene gas. The chamber contained cooling coils through which ice was pumped for the duration of the measurements to prevent the temperature from rising. Gas samples were collected daily and analyzed for ethylene as already described.

RESULTS AND DISCUSSION

Experiment 1

The data on increase in ^{15}N content of the soil after a 2-yr incubation under an $^{15}N_2$-O_2 atmosphere are shown in Table 1 for each of the eight replications. After 2 yr the average increase in ^{15}N concentration of the soil was 2.62 atom percent. The total amount of labeled N_2 fixed is also shown in Table 1. An average of 114 $\mu g/g$ of labeled N_2 was fixed over the 2-yr period, which amounts to an average daily fixation of 0.16 ppm. Much of this N_2 fixation could have been due to the activity of blue-green algae, since algae growth was evident in all of the tubes, both on the top and sides of the soil. The amount of fixed N represents over 10 percent of the total soil organic N and indicates that a significant part of the organic N pool was involved in relatively short-term N turnover in the soil.

Dinitrogen fixation was also estimated from the increase in total N over the 2-yr incubation period, since the amount fixed was large enough to be easily detected by an increase in Kjeldahl N. The increase in N was 112 $\mu g/g$ for the 2-yr period. This value is not significantly different from that determined from labeled N_2 fixation. The actual amount of N_2 fixed during the 2-yr period, as determined by both methods, was probably higher than the measured amount, since some of the fixed N_2, as well as some of the original soil N, was probably cycled back to the N_2 form during the long incubation period. This process occurs in flooded soils as a result of the aerobic-anaerobic zones that support nitrification-denitrification reactions (Reddy et al. 1976).

Experiment 2

Little or no N_2 was fixed in the samples incubated without light (Table 2). In the soils incubated under light, N fixation activity was higher (about fourfold) in the soil with added rice straw compared to the soil not treated with rice straw. Algae and weed growth occurred in the flasks without added rice straw but none was evident where rice straw had been added. The high nitrogenase activity where rice straw was added was apparently not related to algae growth. Application of glucose, cellulose, and straw have been shown to significantly increase the rate of N_2 fixation in submerged soils (Rice and Paul 1972; Okafor and MacRae 1973; Mayfield and Aldworth 1974; Rao 1976). Dinitrogen fixation due to incorporation of N-poor crop residues may be due to the N stress created in the soil system and probably also to increased activity of microorganisms (anaerobic N_2-fixing bacteria) due to addition of an energy source. The amount

TABLE 1

Effect of long-term incubation (2-yr) on N fixation in flooded soil, as measured by fixation of ^{15}N-labeled N_2 and by an increase in total Kjeldahl nitrogen

Sample No.	Total nitrogen[a]		N_2 fixation		
	Org N	Inorg N	Calculated by increase in total N	Calculated from labeled N	Increase in ^{15}N content of the soil, atom %[b]
	μg N/g soil/2 yr				
1	869	4.8	111	152	3.55
2	827	1.9	66	69	1.70
3	884	3.6	125	93	2.13
4	955	4.4	196	133	2.83
5	863	4.5	105	111	2.63
6	852	4.4	93	115	2.75
7	894	4.0	135	122	2.76
8	898	7.5	142	115	2.60
Mean	880	4.4	122	114	2.62
SD ±	36	1.4	39	25	0.54

[a] At the beginning of the experiment the organic N content of the soil was 756 μg/g, and the ammonium N content was 8 μg/g.

[b] The ^{15}N content in the atmosphere was 20.68 atom percent, and the background ^{15}N level in the soil was 0.36 atom percent.

TABLE 2

Nitrogen fixation in a flooded soil with and without added rice straw and with and without light, as measured by the acetylene reduction method

Treatment[a]	Acetylene reduced in 2 days, μmol	N_2 fixed, μg/cm^2/day[b]
Soil columns incubated under light		
No rice straw added	58.5	4.29
Rice straw added	211.4	15.51
Soil columns incubated in dark		
No rice straw added	0	0
Rice straw added	2.0	0.15

[a] Each soil column had an area of 63.6 cm^2 with an overlying floodwater layer of 2.5 cm.

[b] (μg/cm^2)/10 = kg/ha.

of N_2 fixed, equivalent to 1.5 kg/ha/day, suggests that this mechanism should be further evaluated because of its possible value in increasing the N content of rice soils between rice crops where there is a large amount of plant residue available and where another crop is not immediately planted.

Experiment 3

The data on N_2 fixation in undisturbed soil cores obtained from a rice field at midseason (51 days after planting) and late season (70 days after planting) are presented in Table 3. Soils incubated under light developed algal growth and aquatic weeds. An increase in ^{15}N concentration was observed in the surface 0.5-cm layer of the soil incubated under light, and no increase was observed in the soil columns incubated in the dark. This finding suggests that N_2 fixation in a flooded soil occurs mostly in the surface soil layer, probably due to the presence of blue-green algae and other N_2 fixing organisms, although soils treated with N-poor residues in Experiment 2 showed greater N_2 fixation where no algae were evident. Also, in flooded soils in which rice is growing, some N_2 fixation apparently occurs in the root rhizosphere (Yoshida and Ancajas 1971). Maximum N_2 fixation was observed in the soil cores obtained at late season (0.75 μg N/cm^2/day) followed by midseason (0.58 μg N/cm^2/day). This is equivalent to an N_2 fixation rate of about 1.7 to 2.3 kg N/ha/month, which is not a significant amount in relation to the crop requirement. The amount of N_2 fixed in the surface layer of soil was about the same at both sampling dates, but the aquatic weeds in the soil columns obtained at late season contained more fixed labeled N_2 compared to the plants developed in the soil cores obtained at midseason (Table 4). Watanabe et al. (1978) observed the highest peak of acetylene reduction activity late in the growing season of rice. The enrichment of the weed plants with labeled N is surprising, since the most likely pathway of uptake is fixation of N_2 by microorganisms and the subsequent release of available N through decompo-

TABLE 3

Nitrogen fixation in soil cores obtained from a rice field and incubated under light in a growth chamber

Date of sampling	Soil depth, cm	Atom % ^{15}N in the soil[a]	Labeled N fixed, $\mu g/cm^2$	Labeled N fixed (soil + plants), $\mu g/cm^2/day$[b]
July 19, 1976 (midseason sampling)	0–0.5	0.428 ± 0.044	30.4 ± 19.4	0.58
	0.5–1.5	0.366 ± 0.007	0	0
	1.5–2.5	0.361 ± 0.005	0	0
Aug. 19, 1976 (late-season sampling)	0–0.5	0.448 ± 0.037	32.2 ± 13.3	0.75
	0.5–1.5	0.363 ± 0.007	0	0
	1.5–2.5	0.363 ± 0.007	0	0

[a] No ^{15}N increase was detected in the soil cores incubated in the dark.
[b] $(\mu g/cm^2)/10 = kg/ha$.

TABLE 4

Labeled N in aquatic weeds growing in the soil cores after incubation under light for 30 days

Date of sampling	Weight of the dry plant material/core, mg	Atom % ^{15}N	Labeled N in plant material, μg
July 19, 1976 (midseason sampling)	916	0.395	232
Aug. 19, 1976 (late-season sampling)	530	0.549	683

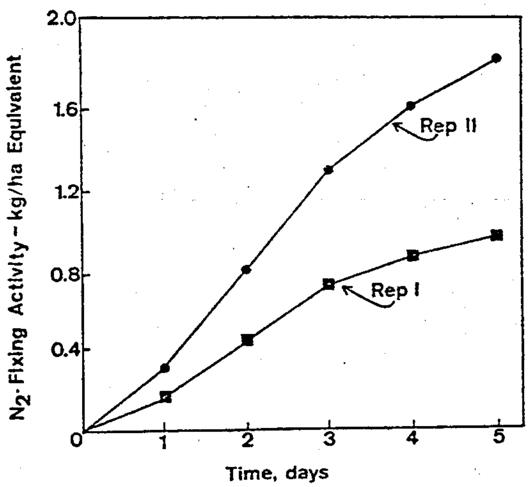

FIG. 2. N_2 fixation rates based on acetylene reduction measurements in the field.

sition. The 1-mo incubation period was probably not long enough for this process to have been significant.

Experiment 4

Dinitrogen fixation based on the amounts of ethylene present in air over the floodwater is shown in Fig. 2. The two replicates showed daily N_2 fixation of about 0.2 and 0.4 kg/ha/day equivalent, respectively. The reason for the difference in fixation is not known. The measured values may not represent all the acetylene reduced, since ethylene may have been produced in the soil and not diffused to the atmosphere. Also, ethylene produced in the root rhizosphere would probably not move rapidly to the atmosphere. These conditions indicate that the measured value probably underestimates the actual fixation. Although the amount of N_2 fixed was not enough to fulfill the crop's needs, the amount was appreciably greater than was measured in the undisturbed soil cores and suggests a role of the plant in increasing N_2 fixation.

SUMMARY

Four different experiments were used to gain some additional insight into factors affecting N_2 fixation in flooded soils. A long-time incubation under labeled N in natural light showed a significant fixation of atmospheric N_2 with more than 10 percent of the soil organic N consisting of labeled N. A second experiment, using the acetylene reduction method, showed that apparent N_2 fixation was much greater where a large quantity of rice straw was incorporated in the soil. In a third experiment the measurement of N_2 fixation in undisturbed soil cores from a rice field showed labeled N in the surface 0.5 cm of soil and in aquatic weeds growing in the soil. The amount of N_2 fixed in the cores (equivalent to 2.3 kg/ha for the 1-mo period) was not enough to provide a significant part of the crop's requirement. A field experiment using the acetylene reduction method showed a higher fixation rate.

A low but significant fixation of N_2 apparently occurs in flooded Crowley silt loam soil. The amount of N_2 fixed is not enough to support high yields of rice but may make an important con-

tribution to the N economy of the soil. Fixation is greater under light than in the dark, indicating a role of photosynthesis by plants. The apparent higher fixation where high C:N ratio plant material was added suggests that management practices that involve incorporating crop residues and flooding between crops could possibly increase the amount of N_2 fixed.

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