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ESTIMATION OF THE NITROGEN BALANCE FOR IRRIGATED RICE AND THE CONTRIBUTION OF PHOTOTROPHIC NITROGEN FIXATION

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ABSTRACT

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The total N content of soils from long-term fertility plots in two sites in the Philippines was measured by Kjeldahl analysis. One site had grown 24 and the other 17 crops of irrigated rice (*Oryza sativa* L.). It appears that the total soil N at each site did not decrease during the cropping period. There was little evidence that N, P, or K fertilization affected the total soil N content. Nitrogen (NH_4^+ and NO_3^-) input by rainfall varied between 0.6 and 2.4 kg of N/ha per year. Calculations based on crop yields and known N inputs suggest that the two flooded rice crops grown each year resulted in a positive N balance equivalent to 79 and 103 kg N/ha per year.

An attempt was made to measure the accumulation of N that may occur in the oxidized surface layer of the soil in the field as a result of N fixation by phototrophic microorganisms during a rice crop. No acetylene reduction activity or accumulation of N (Kjeldahl analysis) was observed in the surface soil when the light was not allowed to penetrate to the water and soil surface. Plots open to the light accumulated the equivalent of approximately 6—8 kg N/ha in the surface soil between transplanting and heading.

INTRODUCTION

Soils used for flooded rice production maintain a moderate degree of nitrogen fertility without the application of N (Yamaguchi, 1979). It was previously assumed that nonsymbiotic N fixation by blue-green algae was responsible for the observed N fertility (De, 1936). More recent research has suggested that heterotrophic bacteria, and perhaps photosynthetic bacteria, are also important (Kobayashi et al., 1967; Rinaudo et al., 1971; Yoshida and Ancajas, 1971).

Although rice is a very important food crop in the world, there are few reports of field research on the N balance of flooded rice (Firth et al., 1973;

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Koyama and App, 1979; Watanabe et al., 1981). However, there are reports of N-balance studies in pots under greenhouse conditions in the tropics (App et al., 1980) and temperate regions (Willis and Green, 1948). Published estimates of biological nitrogen fixation by blue-green algae (based on Kjeldahl and acetylene reduction data) have been summarized by Roger and Kulasooriya (1980).

The present study on the N balance of flooded rice in the tropics was undertaken to obtain an approximation of the quantity of N that may be fixed under field conditions. Total N measurements were obtained for plots of two long-term fertility experiments established in 1966 and 1968 in the Philippines. Yield data from one of these experiments on rice were reported earlier (De Datta and Gomez, 1975). Field experiments were also conducted to detect the possible accumulation of N by phototrophic N_2-fixing microorganisms on the soil surface.

MATERIALS AND METHODS

Long term fertility plots

The long-term fertility plots were established by the International Rice Research Institute's (IRRI) Agronomy Department at Los Baños, Philippines, in 1966 and at the Maligaya Rice Research and Training Center in Nueva Ecija, Philippines, in 1968. The plots were arranged in a randomized complete block design. Treatments were repeated three times at Maligaya and four times at IRRI. The rice (*Oryza sativa* L.) varieties planted in each of the variety trials (designated A, B and C) varied through the years. Two crops were grown each year. The following is a list of the varieties grown each year. Different varieties were employed at IRRI and Maligaya in 1968 and 1969. The list is by year followed by the variety used in Trial A, B and C. 1964, Chianung 242, Milfor 6(2), BPI-76; 1965, PI 215936, 6993 (B5580), Taichung (Native) -1; 1966, Chianung 242, IR8, H-4; 1967 and 1968, IR8, IR262-43-8-11 (Early short Peta), IR262-43-8-11 (Early tall Peta); 1968 (Maligaya), IR8, IR5, C4-63; 1969, IR8, C4-63, IR305-4-12-1-3; 1969 (Maligaya), IR8, IR5, C4-63; 1970, IR8, IR22, IR773A1-36-2-1; 1971, 1972 and 1973, IR8, IR20, IR22; 1974, IR8, IR20, IR26; 1975, IR8, IR26, IR30; 1976, IR8, IR26, IR36.

The Maligaya plots and their operation have been described (De Datta and Gomez, 1975). The IRRI soil is a Maahas clay (Aquic Tropudalf). The plots (21 m^2) were sampled to the traffic pan level (at an average depth of 30 cm) after 24 crops at IRRI and after 17 crops at Maligaya. Three composite samples (five borings each) were obtained from each plot. The soil samples were stored frozen.

Sampling of the IRRI long-term plots in 1980 was done by preparing a composite sample from seven random borings. The soil samples were air dried and ground before analysis.

Rain collection

An automatic rain collector (Wong Laboratories, Cincinnati, OH, USA, Mark V Model) was installed on the roof of a building at IRRI. The collection drum was protected with a cover that opened when the sensor head was moistened and closed after the moisture had evaporated. All components that came in contact with the rain water were acid washed and thoroughly rinsed with deionized water. Water was removed from the collector reservoir on an average of four to five times during the 3-year period. The volume of water was measured and the sample frozen. The collected rain water seldom had any suspended particulate matter.

Accumulation of N *in the soil surface*

Soil (Maligaya clay, pH 7.0) was obtained from a rice field at the Maligaya Rice Research Training Center. The flooded soil was sieved through a 20-mesh screen, thoroughly mixed, and then placed in 25-cm diameter porcelain pots as previously described (App et al., 1980). The pots contained 8.5 kg dry weight of soil and were kept continuously flooded with deionized water. Treatments were replicated six times. Two-week old IR26 seedlings were transplanted into each pot. Where indicated, light penetration to the surface of the soil and water was prevented by covering the pots with two layers of black cloth. A small hole permitted the plants to grow through the cloth. The temperature of these pots was found to vary less than 1°C from the open pots, and the covered pots had no visible algal growth. In the open pots, care was taken to reduce the recycling of nitrogen from the lower depth of the soil profile by weeding, removing dying rice leaves from the plants before they fell onto the soil surface, and sampling before flowering. At the heading stage, 94 days after transplanting (DAT), soil samples were taken at two locations in each pot. A plastic ring (diameter 10 cm, length 15 cm) was carefully inserted into the upper 3 cm of the soil surface and the floodwater and the surface oxidized layer of the soil removed by aspiration. A long aluminum tube (diameter 8 cm) was then inserted inside the plastic ring and a sample of the entire soil profile taken. Results are expressed as change in the difference in the percentage N of the surface soil and entire soil profile to minimize variability in soil N between sampling sites.

A similar experiment was conducted under field conditions at IRRI during the 1980 dry season. The plots had received no N fertilizers for 5 years and were continuously flooded. Metal frames (1 m^2) in each plot served as subplots. There were three replicates for every treatment. Sixteen hills of IR36 were transplanted per subplot at a 20 cm × 20 cm spacing. Carbofuran was added at the rate of 3 kg active ingredient per ha every 2 weeks. Styrofoam frames supported the black cloth. Hand weeding was done with a minimum disturbance to the surface soil. Soil samples from the surface oxidized layer and the soil profile (approximately 20 cm) were taken at three locations in each treatment as described for the pot experiment one DAT and at the heading stage (62 DAT). Acetylene reduction activity (ARA) was measured 26, 41, 61, 84, and 98 DAT. An attempt to repeat this experiment during the wet season was unsuccessful because of excessive flooding during several typhoons.

Chemical and statistical analysis

Total nitrogen content of the soil samples was analyzed by the Kjeldahl method of Bremner and Shaw (1958) and Rinaudo et al. (1971) as previously described (App et al., 1980). Kjeldahl assays on each soil sample were run in duplicate with the exception of the data in Table IV (single digest per sample). The ammonium and nitrate-N contents of the rain water samples were determined by the modified phenol hypochlorite method and phenoldisulfonic acid method respectively (Liddicoat et al., 1975; Am. Public Health Assoc., 1965). Where indicated, data were subjected to analysis of variance (ANOV) and the Duncan multiple range test (DMRT).

Measurement of the acetylene reduction activity (ARA)

Seven soil-core samples were taken randomly from each 1-m^2 plot by inserting glass tubes (diameter 2 cm, length 12 cm) into the soil to a depth of about 5 cm. The tubes were plugged at the bottom and placed inside an airtight plexiglass cylinder, 7.2 cm in diameter and 32 cm long. Incubation was performed in an atmosphere of 10% acetylene in air under sunlight (45—50 klx). The temperature inside the cylinders was maintained at 30—35°C by placing them in a water bath. Gas samples were taken after 15 min, 1 h and 3 h of incubation. Before each sampling, the atmosphere of the cylinders was circulated using a 50-ml syringe. The amount of ethylene produced was determined by gas chromatography (Lee and Yoshida, 1977).

RESULTS

The data in Table I suggest that the total amount of mineral N (as NH_4^+ or NO_3^-) contained in the rain at Los Baños is approximately 0.6 to 2.4 kg N/ha per year. This is a minimum estimate of N because it was observed that if the rainwater was allowed to remain in the collector for 3 days (over a weekend), the NH_4^+-content was sometimes substantially reduced (relative to rain water removed daily). The NO_3^--content remained unchanged. This loss of NH_4^+ was not always observed, and did not appear related to a high pH since the rain water was consistently around pH 5. These observations indicate that NH_4^+ can be a very transient component of rain water under some conditions.

The values for the total soil N content of the long-term plots at IRRI and Maligaya after 24 and 17 crops, respectively, were higher than the origi-

TABLE I

Mineral N content (kg/ha) of rain water (kg/ha per year)

Year	Rainfall (mm)	NO_3—N	NH_4—N	Total N
1978	2312	0.57	1.88	2.45
1979	1702	0.16	0.46	0.62
1980	1751	0.68	0.28	0.96
1981	1503	0.21	0.52	0.73

TABLE II

Total soil N-content of long term fertility plots[a,b] in 1976

Fertilizer treatment	Variety trial		
	Trial A	Trial B	Trial C
IRRI (24 crops)			
Original soil value[c]: 0.178			
0-0-0	0.194 ± 0.004 a	0.188 ± 0.004 b	0.192 ± 0.002 a
60-0-0 (dry season) 140-0-0 (wet season)	0.202 ± 0.007 a	0.216 ± 0.004 a	0.199 ± 0.011 a
0-30-0	0.206 ± 0.003 a	0.203 ± 0.004 ab	0.199 ± 0.005 a
0-0-30	0.203 ± 0.007 a	0.199 ± 0.008 b	0.193 ± 0.003 a
Maligaya (17 crops)			
Original soil value[c]: 0.08			
0-0-0	0.088 ± 0.004	0.094 ± 0.003	0.091 ± 0.002

[a]N content as % N ± standard error.
[b]Means in a column followed by a different letter are significantly different at 5% level by DMRT.
[c]Analysis by Agronomy Department.

nal values (Table II). Since a different laboratory performed the sampling and Kjeldahl analysis at the start of the trial, the significance of the apparent increase in the total soil N content cannot be statistically tested.

A higher N content was observed in IRRI trial B when nitrogen fertilizer was applied. There is much evidence that continued application of inorganic N fertilizers to flooded rice soils does not normally increase the soil N content (Matsuo et al., 1976) and the data reported here are not sufficiently conclusive to challenge this conclusion.

Phosphorus fertilization can enhance growth of blue-green algae and increase the total soil N content (Roger and Kulasooriya, 1980). This effect was not evident in the data in Table II. Several different rice varieties were grown in each of the Maligaya and IRRI variety trials over the years. However, the total N content of the soil was significantly different from the identical treatment in another trial only in the N treatment, IRRI variety trial B (0.216 was significantly different from 0.199 by DMRT). Therefore, there is little evidence that the sequence of varieties used in each of the trials at IRRI and Maligaya affected the total N content of the soil.

Table III is an estimation of the total N balance for the long-term fertility plots (0-0-0) at IRRI and Maligaya. Average yields of the crop were 3.88 and 3.06 t/ha, respectively. For this calculation the soil N content is assumed to remain constant. There was a positive N balance of 103 and 79 kg N/year (2 crops) respectively at the two sites. If the soil N in the plow layer (assuming the total N content was uniform to a 35-cm depth) was the sole source of this N, the total soil N at IRRI would be reduced from 0.178 to 0.158% and the reduction at Maligaya would be 0.080 to 0.065%.

TABLE III

Estimation of N balance (kg N/ha per year) for long term fertility plots (0-0-0)

	IRRI	Maligaya
Crop removal[a] (A)		
Grain (1.1% N)	1024	572
Straw (0.6% N)[b]	368	206
Miscellaneous inputs (B)		
Rain (2.5 kg N/year)[c]	30	21
Irrigation (10 kg N/year)[d]	120	85
Balance (A - B)	+1242 (103 kg N/year)	+672 (79 kg N/year)

[a]N content of grain and straw are approximations and not based on Kjeldahl determinations.
[b]Straw weight is an approximation (ratio straw : grain, 1:1), 1/3 of straw is assumed returned to soil.
[c]Rain N content is an approximation based on rain analysis.
[d]Irrigation water N content based on data of Singh et al. (in press).

TABLE IV

Total nitrogen content (% dry soil) of soil profile in IRRI long-term fertility plots (0-0-0), variety trial A. Soil samples taken after 33rd crop, 1980

Depth (cm)	Replicate I	II	III	IV	Mean
0–25	0.188	0.206	0.201	0.188	0.196
25–40	0.173	0.176	0.197	0.145	0.173
40–70	0.070	0.083	0.096	0.067	0.079
70–85	0.045	0.027	0.060	0.018	0.038

There is a possibility that some rice roots penetrated through the traffic pan and absorbed N from the subsoil. Therefore, root pits were dug in the IRRI long-term plots at the end of the 33rd crop. Very few roots were observed below the traffic pan. The subsoil was also sampled and the total nitrogen content determined (Table IV). Although 28% of the total N in the soil profile is below an average sampling depth of 30 cm, the N concentration is low.

A certain proportion of the N fixed by phototrophic microorganisms during a crop of rice may accumulate in the surface layer of the soil (Hirano, 1958; Alimagno and Yoshida, 1975). Evidence from N balance experiments in pots suggests that N fixed by phototrophic agents accumulates in the soil and is not rapidly mineralized (App et al., 1980). The results of the preliminary greenhouse experiment indicated that accumulation of N in the surface-oxidized layer could be detected by Kjeldahl analysis. Pots open to the light had accumulated N in the surface-oxidized layer of soil, but preventing light from falling on the surface soil and water prevented the accumulation of N. The differences in percent N between the surface and the entire soil profile were 0.027 (open to light) and 0.005 (covered). The difference between the treatments (light vs. dark) was statistically significant at the 5% level.

In the field experiment, the N content at transplanting of the surface soil and subsoil for the three treatments (Table V) did not differ. At heading, there was no statistically significant accumulation of N in the surface soil if light was prevented from falling on the soil and water. In the two other treatments, a highly significant increase in the N content of the surface soil was observed. N accumulation measured in the surface soil of the insecticide treated plots (to control blue-green algal grazers) was higher but not statistically different from that of plots without insecticide. The amount of N accumulated in the surface oxidized layer is equivalent to 6—8 kg N/ha. This presumably represents the residue of newly fixed N which has not undergone plant uptake, loss, and downward movement.

A second method of estimating the N accumulation in the surface soil is to compare the total N content for each treatment at transplanting and

TABLE V

Accumulation of N (%) in the surface soil under field conditions. All treatments were planted and weeded

Treatment		Transplanting			Heading		
Application of insecticide	Surface covered with black cloth	Surface soil	Subsoil	Difference	Surface soil	Subsoil	Difference
–	–	0.155[a][1]	0.142[a]	0.013[ns][2]	0.161[a]	0.136[a]	0.025**
+	–	0.147[a]	0.139[a]	0.008[ns]	0.165[a]	0.135[a]	0.030**
+	+	0.147[a]	0.141[a]	0.007[ns]	0.142[b]	0.136[a]	0.005[ns]

[1]Means in a column followed by a common letter are not significantly different by DMRT.
[2]ns and **; nonsignificant and significant at 1% level by ANOV.

heading. This method of calculation is less desirable since it does not minimize sampling variability (the variability in the surface N content was three times that of the subsoil). The total N content of the surface layer at transplanting and heading was significantly different for only the treatment open to light and including insecticide. A similar analysis of the total N contents of the subsoils indicated that the differences were insignificant for all treatments. A third method to calculate the accumulation of N is to compare the surface total N content of the black cloth treatment with that of the treatments open to light. As shown in Table V, the total N contents were only significantly different at the heading stage.

Blooms of blue-green algae appeared at irregular intervals in treatments exposed to light, but were not present in all replicates of a given treatment at any given time. This observation is in agreement with the results of ARA measurements made at various stages of the growth cycle (Table VI) and characterized by a very large variability between replicates. ARA was negligible when light was excluded. Activities measured in the insecticide-treated plots were the same (26, 41, 93 DAT) or significantly higher (61, 84 DAT) than in nontreated plots. This suggests a beneficial effect of insecticides on ARA.

TABLE VI

Acetylene reducing activity (μMol C_2H_4 m^{-2} h^{-1}) *in plots during the 1980 dry season*

Treatment		Days after transplanting[1]					
Insecticide	Black cloth	26	41[2]	61	84	98[3]	Average
		46 a	0 a	9 a	0 a	71 a	
–	–	(5, 5, 128)	(0, 0, 0)	(17, 7, 7)	(0, 0, 0)	(24, 104, 86)	25
		32 a	0 a	115 b	27 b	113 ab	
+	–	(80, 8, 8)	(0, 0, 0)	(60, 157, 128)	(1, 80, 3)	(4, 320, 13)	57
		0 b	0 a	1 c	1 ab	4 b	
+	+	(0, 0, 0)	(0, 0, 0)	(0, 3, 0)	(2, 0, 2)	(5, 4, 3)	1

[1] Figures in parenthesis are replicate values. Average values in a column followed by a common letter are not significantly different by DMRT.
[2] Assays performed one day after a heavy rain (80 mm).
[3] Assays performed after harvest.

DISCUSSION

This research was undertaken to obtain field data on the amount and sources of nitrogen that enters the flooded soils, used for rice production. Nitrogen balance data are the sum of all N gains and losses; they do not measure biological N fixation. The merits and disadvantages of nitrogen balance studies have been discussed and it is evident that there are serious limitations (Allison, 1955; Koyama and App, 1979; Watanabe et al., 1981). Methodology that will permit the accurate measurement of all inputs and losses of nitrogen from a plant-soil system under field conditions (and especially for one or more crops) is not available. For example, we have not measured surface runoff and lateral movement of N through the soil, denitrification, dry precipitation of N, and absorption and volatilization of ammonia by the plant-soil system (Malo and Puvris, 1964; Denmead et al., 1976; Mikkelsen and De Datta, 1978). Although we know the recent nitrogen content of the lower depths of the soil profile (Table IV), no data on the N content of these depths at the beginning of the fertility trials are available. It is, therefore, not possible to assess the extent of participation of the deeper layers of soil as sources or sinks for N or their impact on the N balance calculations. It has been noted that the use of a plastic sheet to separate the upper 15 — 20 cm of soil from the remainder of the soil profile can reduce crop N uptake by 5—50% (Sekiya and Shiga, 1977; Ventura and Watanabe, unpublished). Finally, no consideration has been given to the effect of the fallow periods between crops. The soil may dry up during this period and weeds are frequently present. The wetting and drying cycle could increase N losses, and addition of organic matter may enhance heterotrophic N fixation.

Results of ARA measurements, conducted to assess phototrophic nitrogen fixation, were very variable and probably due to a non-uniform growth of blue-green algae in the replicate plots. It has been previously reported that blue-green populations have a very uneven distribution that approximates a log-normal pattern (Roger et al., 1977). The small size of the plots, together with the irregular distribution of algae and the fact that the plots were protected from inoculation by the water or the soil of the surrounding field by a continuous frame, may prevent simultaneous growth of the N_2-fixing blue-green algae in the different replicates of a treatment. Although we conclude that the acetylene reduction activity data reported in this manuscript are too variable to use for an estimation of phototrophic nitrogen fixation, it is possible that very frequent sampling (with several replicates) over the growth cycle may greatly improve the estimate.

It is likely that the observed accumulation of N in the surface oxidized layer of soil is primarily the result of phototrophic N_2-fixing organisms. This is consistent with the observation that the accumulation was light dependent. The ARA data (insecticide vs. control) suggest that fauna which graze on blue-green algae may be a constraint on phototrophic N_2-fixation. There are several earlier reports on grazing of blue-green algae. Roger and Kulasooriya (1980) and Tirol et al. (1982) have shown that only a fraction of algal N remains on the surface of flooded soil. It is noted that the N accumulation in the surface oxidized layer is only a small fraction of the positive N balance calculated for a crop in the long term fertility trials. The accumulation figures only represent the time period from transplant to heading. Furthermore, a certain fraction of the newly fixed nitrogen must be mineralized and absorbed by the rice roots, leached, or lost to the atmosphere. We suspect that the N accumulation data considerably underestimate phototrophic N_2-fixation during a crop of rice. It is of course not appropriate to use the surface accumulation data as an estimate of total phototrophic fixation and compare this with the total N balance estimates from the long-term plots.

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