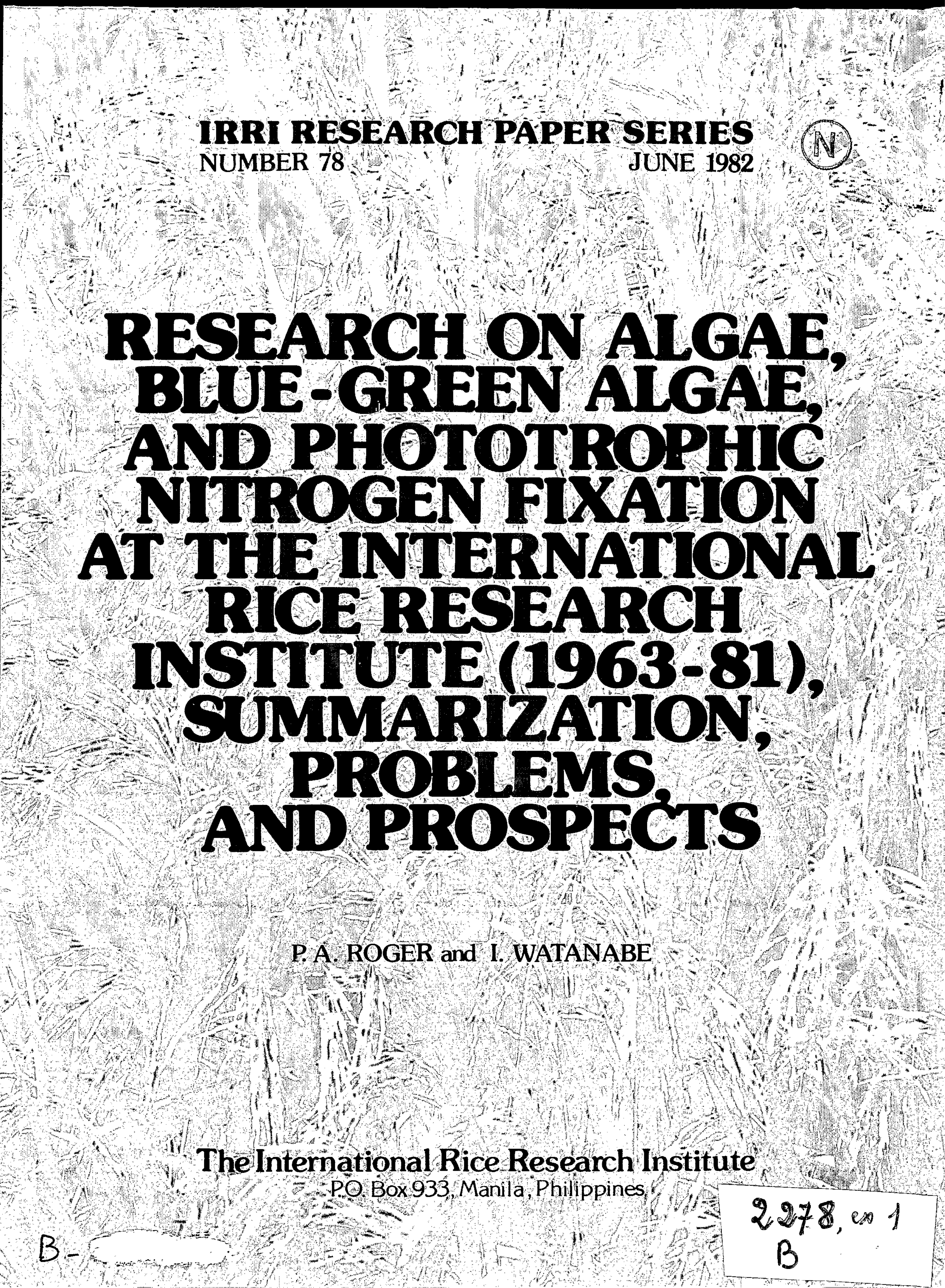

IRRI RESEARCH PAPER SERIES
NUMBER 78 JUNE 1982

RESEARCH ON ALGAE, BLUE-GREEN ALGAE, AND PHOTOTROPHIC NITROGEN FIXATION AT THE INTERNATIONAL RICE RESEARCH INSTITUTE (1963-81), SUMMARIZATION, PROBLEMS, AND PROSPECTS

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RESEARCH ON ALGAE, BLUE-GREEN ALGAE, AND PHOTOTROPHIC NITROGEN FIXATION AT THE INTERNATIONAL RICE RESEARCH INSTITUTE (1963-81), SUMMARIZATION, PROBLEMS, AND PROSPECTS[1]

ABSTRACT

This paper provides general information on one of the major research areas of IRRI's Soil Microbiology Department. Conclusions made after an extensive survey of the literature on the role of blue-green algae in rice cultivation are summarized. Microbiological research at IRRI on algae, blue-green algae, and phototrophic nitrogen fixation is reviewed. The paper also provides information on problems encountered and prospects for future research.

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It has long been recognized that flooded rice soils maintain a degree of nitrogen fertility. Long-term fertility trials have indicated that there is a major input of nitrogen (35-50 kg N/crop) into flooded rice soils. Much of this input is attributed to nitrogen fixation by:

- heterotrophic microorganisms associated with rice roots,
- azolla,
- BGA, and
- photosynthetic bacteria.

Nitrogen fixation has been a major research area since the establishment of soil microbiology research at IRRI in 1963. Equal importance has been given to the study of the different groups of nitrogen fixers. This paper summarizes research conducted on the photosynthetic free-living nitrogen fixers.

Early IRRI studies of biological nitrogen-fixing activity (NFA) used ^{15}N isotope. In 1968 the inexpensive and sensitive acetylene reduction assay (ARA) method to detect nitrogen fixation was introduced, stimulating research activities on NFA. As a result of discussions during the 1978 Nitrogen and Rice Symposium at IRRI, research priority was given to NFA by azolla, BGA, and heterotrophic microorganisms in the rice root zone. IRRI was asked to compile all relevant information on the BGA that relates to rice.

The information was compiled in 1979 and Blue-green algae and rice was published in 1980.

Since 1979, the relative importance given to IRRI research on BGA and phototrophic NFA has increased. Trials for evaluating phototrophic NFA have continued and research on relations between BGA and the rice plant and on factors limiting BGA has started.

BLUE-GREEN ALGAE AND RICE

Based on the literature review presented by Blue-green algae and rice (Roger and Kulasooriya 1980) the following general statements can be made.

- The wetland rice environment is favorable for the growth of blue-green algae (BGA). The relative occurrence of BGA, however, varies greatly and they are not always present in rice soils. Reasons for their heterogenous and sometimes limited distribution are still not well known. No systematic analysis has correlated their presence or absence with environmental factors.

- Ecological studies of BGA in submerged soils are limited by problems in methodology, primarily in estimating algal biomasses quantitatively. Fragmentary quantitative measurements indicate that N_2-fixing BGA population densities vary from a few to 10^7/g dry soil; biomasses vary from a few kilograms to 24 t (fresh weight)/ha. In a favorable environment a N_2-fixing algal bloom may contribute 30-60 kg N/ha to the ecosystem.

- Among the physical factors affecting the seasonal fluctuations of the phytoplankton, light is the most important. It affects the algal biomass qualitatively and quantitatively. BGA may be regarded as a low-light species; in areas of high incident light intensities, BGA develop only when protected by a sufficiently dense rice canopy. In areas of moderate incident light intensities, during rainy or cloudy weather, light deficiency under a dense rice canopy may limit BGA growth. To a lesser extent temperature and water regime may also influence BGA growth in the wetland field.

- Among the biotic factors capable of limiting BGA growth in rice fields, only grazing by invertebrates has been documented. Evidence exists that pathogenicity and antagonisms may affect BGA in the field but those have not yet been demonstrated.

- Among soil properties, pH is the most important factor determining the algal flora composition. BGA prefer environments that are neutral to alkaline. A positive correlation between pH and occurrence of BGA is common. Next to pH, available phosphorus content of the soil is the most decisive factor favoring BGA growth. Little information is available on the effect of other soil properties on BGA.

- Agronomic practices for growing rice influence BGA growth.
  - Land preparation and management have only incidental effects.
  - Pesticides -- depending on their nature, their concentration, and the algal strains -- can have inhibitory, selective, or stimulatory effects. BGA are

generally more resistant to pesticides than other algae and tolerate pesticide levels recommended for field application. Insecticides are generally less toxic to BGA than other pesticides and have the secondary beneficial effect of suppressing the population of algae grazers.

- Among chemical fertilization practices, phosphorus application and liming of acidic soils have a beneficial effect on BGA growth. The effect of nitrogenous fertilizers is not well known. BGA growth was inhibited by mineral nitrogen in flask cultures but that may not occur to the same extent in the field. The effect of nitrogen fertilizers on BGA in the field has received little attention. This is surprising in view of the observation that BGA inoculation produced an increase in grain yield even at high levels of fertilizer N. From experiments conducted without algal inoculation, a depressive effect of nitrogen fertilizer on algal NFA has been established. Other nutrients (Mo, Fe, Mg, K, etc.) are required for optimal growth of BGA, but their ecological implications as liming factors, or as factors affecting the composition of the algal community in wetland rice fields, have not been documented.

- Organic manure, depending on the nature and mode of application, may favor or depress BGA growth. Plant residue incorporation, which produces by-products of anaerobic decomposition toxic to algae, seems less beneficial to BGA than surface application.

- In physiological studies on BGA in wetland rice fields, much emphasis has been placed on the NFA, whereas the study of in situ productivity and photosynthetic activity has been neglected. A conceivable role of CO_2 depletion as a limiting factor for a ARA when large biomasses occur, and the influence of the concurrent pH increase on nitrogen losses by volatilization, need to be documented.

- Algal NFA has most frequently been studied by ARA measurement. This method is liable to give misinterpretation of quantitative results, but it is convenient and reliable for qualitative studies when the measurements are brief, the problems of gas diffusion and greenhouse effects are minimize minimized, and statistically valid sampling methods are adopted.

Estimates of the amounts of nitrogen fixed by BGA vary from a few to several kilograms per crop. The average value of the reported estimates (30 kg/crop) seems to constitute a satisfactory reference value when environmental factors favor BGA growth. The relative contribution of BGA as a percentage of the total nitrogen fixed in the rice field varies within large limits and seems to be more affected by nitrogen fertilizers than heterotrophic nitrogen fixation. BGA epiphytism makes a limited contribution to the nitrogen input in shallow-water rice, but this contribution has agronomic significance in deepwater rice.

- BGA have benefited rice plants by the production of growth-promoting substances. The additive effect of algalization in the presence of a high level of nitrogen fertilizer was interpreted as an index of this growth-promoting effect, but such an interpretation has not been demonstrated in the field and should be treated with caution. Beneficial effects of BGA on rice, such as increasing phosphorus availability, decreasing sulfide injury, and preventing the growth of weeds, have also been reported.

- Because BGA are recognized as one of the most important nitrogen-fixing agents in flooded rice soils, many trials have studied the increase in rice yield by algal inoculation (algalization).

  - Most of the experiments were on a <u>black box</u> basis and examined only the <u>grain yield</u> effect of an agronomic practice (algalization); the intermediate effects were not studied. Little information is available on the qualitative and quantitative evolution of the nitrogen-fixing algal flora, the evolution of the photosynthetic NFA, and the nitrogen balance in inoculated paddy soils. Pot experiments may be suitable for qualitative studies, but they overestimate the effects of algal inoculation. On the other hand, most of the field experiments have been conducted over one growing season only and may have underestimated the effects of algalization. The advantages of a slow nitrogen release might not be apparent in the first crop after algal inoculation.

  - Algalization has been reported to have a beneficial effect on grain yield in several countries. There are also reports, however, that indicate failure of algalization in widely different agroclimates. Little is known about the limiting factors for algalization. Among the soil properties, a low pH and low available phosphorus content are the only ones well documented. Knowledge of the relation between soil properties and the establishment of the algal inoculum is certainly a major gap. Among detrimental biotic factors, only grazing by zooplankton has been studied. Low temperature, heavy rains, and cloudy weather have also been reported to limit the establishment of the algal inoculum.

- Algalization, when effective, has been reported to increase the size of the rice plant, its nitrogen content, and the number of tillers, panicles, spikelets, and filled spikelets per panicle. The better grain yield has been used to assess the effect of algal inoculation. From the reports on field experiments, mainly in India, it appears that algal inoculation, where effective, causes about 14% relative increase in yield, corresponding to about 450 kg grain/ha per crop.

- A higher increase in grain yield was observed when algalization was done in combination with lime and phosphorus, and sometimes with molybdenum application. It appears, however, that there is no significant difference in yield increase strictly due to algalization in the presence or absence of non-nitrogen fertilizers and that the increase in yield due to non-nitrogen fertilizers is generally higher than that due to algalization.

- Results concerning the effects of algalization in the presence of nitrogen fertilizers are controversial. Several reports indicate failure of algalization in the presence of nitrogen fertilizer. A large-scale experiment in India, however, indicates a beneficial effect of algalization even at high levels of nitrogen.

- There is evidence that algalization produces both a cumulative and residual effect attributed to a buildup of the soil nitrogen, organic matter, and the algal flora. However, little is known about the effects of algalization on soil properties and soil microflora.

- Another knowledge gap concerns the comparison between algalization and management practices that enhance the growth and activity of indigenous natural populations of nitrogen-fixing BGA. In some cases, management can make algalization unnecessary but it is necessary where efficient strains are absent in the soil. The search for highly efficient strains is still at a theoretical level and the recommended inoculum remains a soil-based mixture of strains.

- In India algalization in rice fields has proceeded a little beyond the stage of fundamental research. A method of producing algal inoculum, easily adoptable by farmers, has been developed and recommendations for field inoculation have been developed. Inoculum production and algalization technology indicated a cost-benefit ratio of 1 to 10 and an additional income of about 300 Indian rupees/ha per crop in 1979. To our knowledge, such trials are still confined to India.

As a general conclusion, a beneficial role of BGA in wetland rice fields appears positive. The abundance of a sometimes repetitive literature on this subject clearly indicates that researchers have felt the importance and the potentialities of BGA in rice cultivation. Unfortunately the ecology of BGA in rice fields and their modes of action on the plant are still poorly understood. This limits the use of BGA as a biofertilizer.

PHOTOTROPHIC NITROGEN FIXATION RESEARCH AT IRRI (1963-81)

IRRI work with BGA since 1964 has ranged from ecological studies to field tests of cultural practices. A summary of papers on algae, blue-green algae, and photosynthetic nitrogen fixation published by IRRI authors is presented in Appendix 1.

Ecological and physiological studies on algae

Studies on the changes in soil algae and BGA after flooding of the soil were done in 1963 and 1964. Populations were assessed by recording growth after incubation in a liquid medium and applying the most probable number estimation. Studies with a soil with pH 6.6 (Table 1) indicated that Chlorophyceae, mainly unicellular, were dominant. Among the BGA, Oscillatoriaceae were generally more abundant than Nostocaceae. The population of Nostocaceae reached its peak 48 days after flooding but was only of the order of 1,700/g of soil. Similar results were obtained with an acidic soil (pH 4.7). From these data it was concluded that a major role of BGA in nitrogen fixation in the soils studied was doubtful. However, because no measurement of the NFA was done and the methodology used for enumeration was demonstrated to underestimate algal populations (Reynaud and Roger 1977), this conclusion may be erroneous, particularly in the case of the soil with a pH of 6.6.

Table 1. Algal populations developing in flooded clay soil (pH 6.6; OM 2.0%; total N 0.14%). (From IRRI /1965/.

No. of days submerged	Chlorophyceae	Oscillatoriaceae	Nostocaceae
0	500	200	200
13	1900	1900	200
48	1700	1700	1700
69	1300	0	0
90	1200	200	100

Occurrence and abundance of total algae and BGA in flooded Maahas clay at different stages of rice growth were studied in the greenhouse in 1968 (Fig. 1). High numbers of algae and BGA in the 2- to 7-cm soil layer indicated a high concentration of spores in the soil. Algal population was generally highest at maximum tillering. Application of

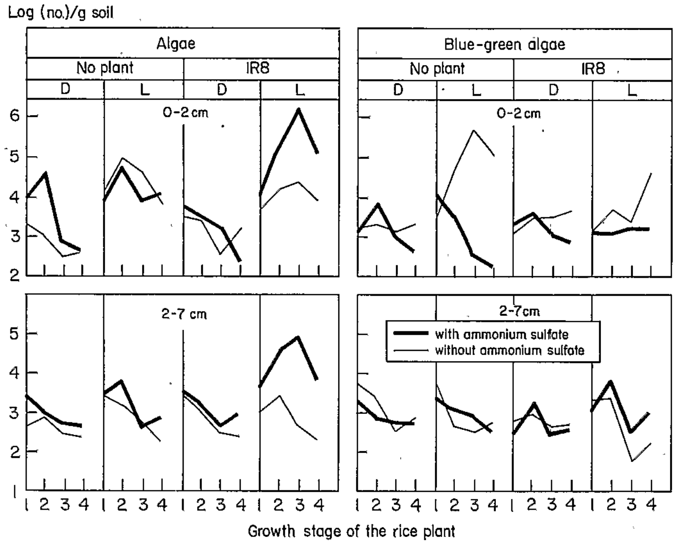

Fig. 1. Algal population during the rice-growing period (L = light, D = dark; depth of soil 0-2 cm and 2-7 cm; stages of rice: 1 = transplanting, 2 = maximum tillering, 3 = panicle initiation, 4 = maturity (IRRI 1968).

ammonium sulfate had a depressive effect on nitrogen-fixing BGA. Of 308 nitrogen-fixing isolates, 267 belonged to the Nostoc species, 38 to Anabaena, 1 to Stigonema, 1 to Tolypothrix, and 1 to Scytonema. Gloeotrichia and Aphanothece, which are common at present on IRRI farm, were not recorded.

Measurements of specific ARA among 12 strains gave values ranging from 0.5 to 5.4 nmol C_2H_4/min per mg protein with an average value around 2 (IRRI [1968]). These values are in agreement with those reported in the literature (see Reynaud and Roger 1979).

A laboratory study of the effects of light intensity on BGA showed that regardless of the age of the culture, the maximum NFA was obtained at 10 klux. At 5 klux NFA was about 80% of the maximum. Activity of a field-grown Gloeotrichia was saturated at about 10 klux. At 5 klux, 87% of the maximum NFA was obtained, suggesting that NFA under 5 klux is similar to that under light-saturated conditions (IRRI 1976).

The possibility of a detrimental effect of algae on direct-seeded rice was reported in 1964 (IRRI [1965]). Algae may form a soil surface mat, which germinating rice seedlings have difficulty penetrating. Seedlings that do penetrate the mat are often uprooted when the mat breaks free from the soil and floats to the surface of the water.

Among the algae that are detrimental to rice, BGA can be considered incidental. The most harmful genera are the filamentous or reticulated colonial types, most frequently Chlorophyceae (Roger and Kulasooriya 1980). Copper sulfate is the most commonly used algicide. A search for more effective chemicals that are less phytotoxic to rice plant was undertaken in 1969. Of the commercially available algicides and herbicides, monoquat and dichlone, tested at the rate of 0.5 and 2.5 kg a.i./ha, were effective at both rates (IRRI 1970). Dichlone 50 WP was found efficient (IRRI 1971) and much less harmful to rice plant than copper sulfate.

Further experiments (IRRI 1972) indicated that fungicides like benomyl and maneb can also effectively control algae. The best control resulted from Dichlone 50 WP at 6 kg/ha.

Evaluation of phototrophic NFA in paddy fields

The first IRRI trial to estimate phototrophic NFA was in 1968. Soil from pot experiments was incubated in test tubes under an atmosphere enriched with ^{15}N. The data (Table 2) show that phototrophic NFA was dominant in this soil and that the addition of nitrogen fertilizer remarkably depressed the amount of nitrogen fixed. Soils without nitrogen fertilizer had an estimated NFA corresponding to 30 kg N/ha per month when exposed to light (IRRI [1968]). It has been reported, however, that small-scale experiments favor the growth of BGA and may largely overestimate phototrophic NFA (Roger and Kulasooriya 1980).

The acetylene reduction assay (ARA) was used to estimate NFA in the floodwater during the 1970 wet season. ARA was generally higher in nonplanted fields than in planted fields (Fig. 2). This was probably due to the shading effect of rice plant when incident light intensities were low because of the cloudy weather of the wet season. This hypothesis was supported by measurement of ARA of the floodwater during the 1971 dry season which showed a higher activity than during the wet season (Fig. 3) and no significant difference between the planted and nonplanted fields (IRRI 1972).

Diurnal variations of phototrophic NFA were studied in 1973. A bell-shaped curve was observed in a soil inoculated with Aulosira fertilissima 3 weeks before measurement (Fig. 4a). Measurement of the activity of laboratory-grown A. fertilissima and Anabaena spiroides placed in the field showed a dissimetrical curve with a maximal activity in the morning and a negligible activity in the afternoon (Fig. 4b). Such curves are characteristic of algal cultures not adapted to high light intensities and placed under direct sunlight (Roger and Kulasooriya 1980).

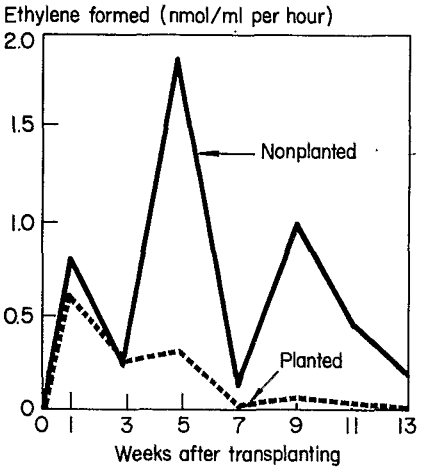

Fig. 2. Nitrogen-fixing activity, measured by C_2H_2-C_2H_4 assay, in floodwater of planted IR20 and nonplanted fields, 1970 wet season (IRRI 1971).

ARA assays were made in 1974 in rice fields at two sites in Albay province, Philippines. The sites represented 2 distinct soil types -- Puro clay loam (pH: 5.2, O.M: 3.08%, total N: 0.19%, available P: 11.5 ppm, no nitrogen fertilizer applied) and Santo Domingo loamy sand (pH: 6.7, O.M: 2.6%, total N: 0.14%, available P: 11.7 ppm, nitrogen fertilizer applied). A peak of nitrogenase activities equivalent to 48 g N_2/ha per hour for the Puro soils and 6.4 g N_2/ha per hour for the Santo Domingo soils were obtained at 44 and 41 days after transplanting, respectively. Thereafter the activity decreased until harvest. The absence of NFA in samples covered with black cloth suggested that BGA were the principal agent of nitrogen fixation in these soils. The lower rate of NFA in the less acidic soil (Santo Domingo) was probably due to an inhibitory effect of nitrogen fertilizers. The estimated amount of nitrogen fixation by BGA ranged from 18.5 to 33.3 kg N/ha per crop season for Puro soils and from 2.3 to 5.7 kg N/ha per cropping season for Santo Domingo soils (IRRI 1975).

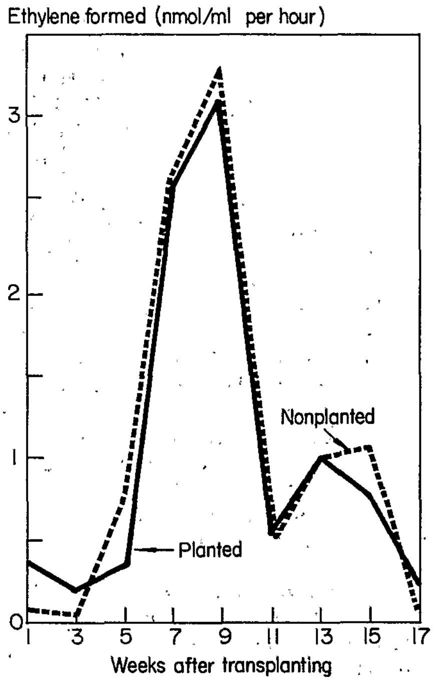

Fig. 3. Nitrogen-fixing activity, measured by C_2H_2-C_2H_4 assay, in floodwater of planted IR20 and nonplanted fields, 1971 dry season (IRRI 1972).

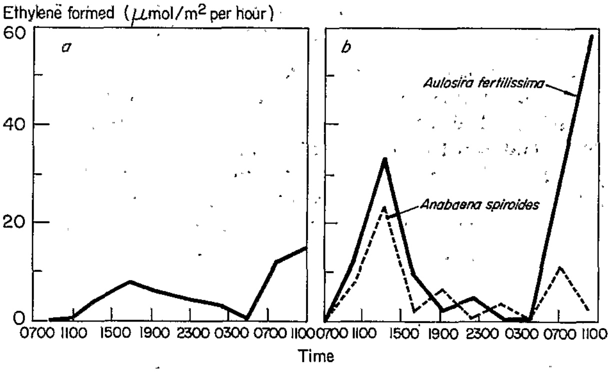

Fig. 4. Diurnal changes of acetylene reduction in a field; a, acetylene reduction in an Aulosira fertilissima-treated plot planted to IR20 (activity determined 3 weeks after inoculation); b, acetylene reduction by laboratory-grown N_2-fixing algae placed in the field (IRRI 1974).

In situ ARA measurement in long-term fertility trials of IRRI (IRRI 1976) showed that photosynthetic NFA was important in nonfertilized plots, particularly during the later stages of rice growth where values corresponding to 0.1-0.2 kg N fixed/ha per day were recorded. Continuation of the measurements during the dry season (IRRI 1977) indicated that phototrophic NFA was higher in the dry season than in the wet season (Fig. 5). At the end of the dry season, when algal biomass was high, total in situ NFA was 5,700 nmol C_2H_4/m^2 per day. This activity decreased to 660 nmol C_2H_4/m^2 per day when floodwater and BGA were removed and replaced by deionized water. This indicated that photodependent NFA in floodwater was the major component. In both seasons, the removal of algae greatly reduced NFA (Fig. 6). By extrapolating measurements, daily algal ARA was estimated to be 200 mmol C_2H_4/m^2 in the wet season (163 days) and 300 mmol C_2H_4/m^2 during the dry season (168 days). Daily ARA associated with the rice plant was 90 mmol C_2H_4/m^2 in the wet season (IR26) and 50 mmol C_2H_4/m^2 in the dry season (IR36) (IRRI 1978). The relative contribution of BGA, photosynthetic bacteria, and heterotrophic bacteria was assessed using propanil, a herbicide that acts as a potent inhibitor of BGA. Unfortunately, propanil very significantly enhanced the population of N_2-fixing purple sulfur bacteria. Both photosynthetic bacteria and BGA had a substantially higher activity than rhizosphere microflora. The results also suggested that the potential contribution of photosynthetic bacteria may even approach that of active BGA (IRRI 1979).

A nitrogen balance technique was also used to evaluate phototrophic nitrogen fixation. Total nitrogen content of the soil was measured after two crops in a pot experiment (IRRI /1968/). A larger amount of nitrogen was measured in the soils kept in the light than in those kept in the dark.

Nitrogen balance studies conducted in 1977 suggested an input of 37 to 50 kg N/ha per crop into flooded paddies. From greenhouse experiments it was suggested that most of the net nitrogen fixation was due to the phototrophic microorganisms and that the rice plant stimulated NFA (IRRI 1978).

An attempt was made to ascertain if there is an accumulation of nitrogen in the surface (0-3 mm) layer of flooded soil during a rice crop. Any accumulation detected would presumably represent nitrogen derived from phototrophic nitrogen fixation at soil surface. Greenhouse experiments proved the feasibility of this principle. Covering pots with black cloth eliminated the surface accumulation of nitrogen. In the field, frames (1 m x 1 m) were installed and 3 treatments -- without black cloth cover and insecticide, without black cloth but with insecticide, and with insecticide and black cloth -- were compared. Weeding was manual with the least disturbance of the surface soil. The plots that received insecticide and were exposed to light had a statistically significant increase of total nitrogen content of the surface 3 mm of soil, amounting to 7 kg N/ha. This accumulation represents the residue of newly fixed nitrogen, which had not undergone downward movement, plant uptake, or loss (IRRI 1981).

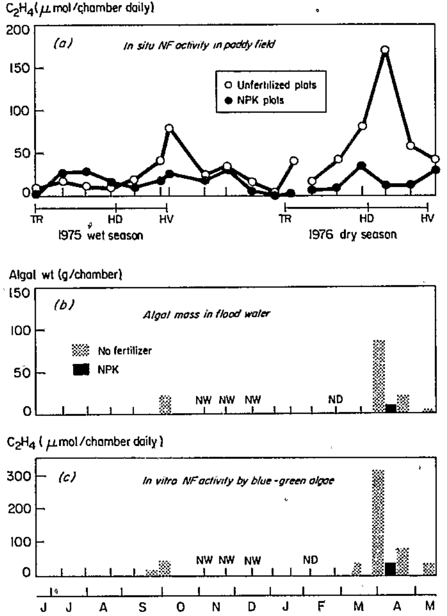

Fig. 5. Nitrogen-fixing (NF) activity and algal mass in long-term fertilizer experiments at IRRI, 1975-76. NW = no water, ND = no determination, TR = transplanting, HD = heading, HV = harvest (IRRI 1977).

Table 2. Nitrogen fixation in various soil treatments (IRRI /1968/).

Pretreatment[a]	Incubated in light		Incubated in dark	
	Atom % excess ^{15}N [b]	Estimated amount of fixed nitrogen (kg/ha per month)	Atom % excess ^{15}N [b]	Estimated amount of fixed nitrogen (kg/ha per month)
PKL, no plant[c]	.395	32.0	.038	3.2
PKD, no plant[d]	.493	32.8	.020	1.2
PKL IR8	.459	32.6	.026	1.9
PKD IR8	.514	36.0	.006	0.5
PKL Peta	.517	38.1	.047	3.4
PKD Peta	.410	27.2	.027	1.8
NPKL No plant	.043	5.7	.048	4.6
NPKD No plant	.020	2.0	.000	0.0
NPKL IR8	.075	6.4	.017	1.5
NPKD IR8	.070	5.1	.024	1.8
NPKL Peta	.151	13.3	.031	2.5
NPKD Peta	.089	5.6	.000	0.0

[a] Treatment of Maahas clay soil in greenhouse pots from which soil samples were obtained 2 months after transplanting IR8 and Peta. [b] Determined after incubation of soil in glass tubes for 1 month in the greenhouse. [c] L = light (good growth of indigenous algae). [d] D = dark (no algal growth observed).

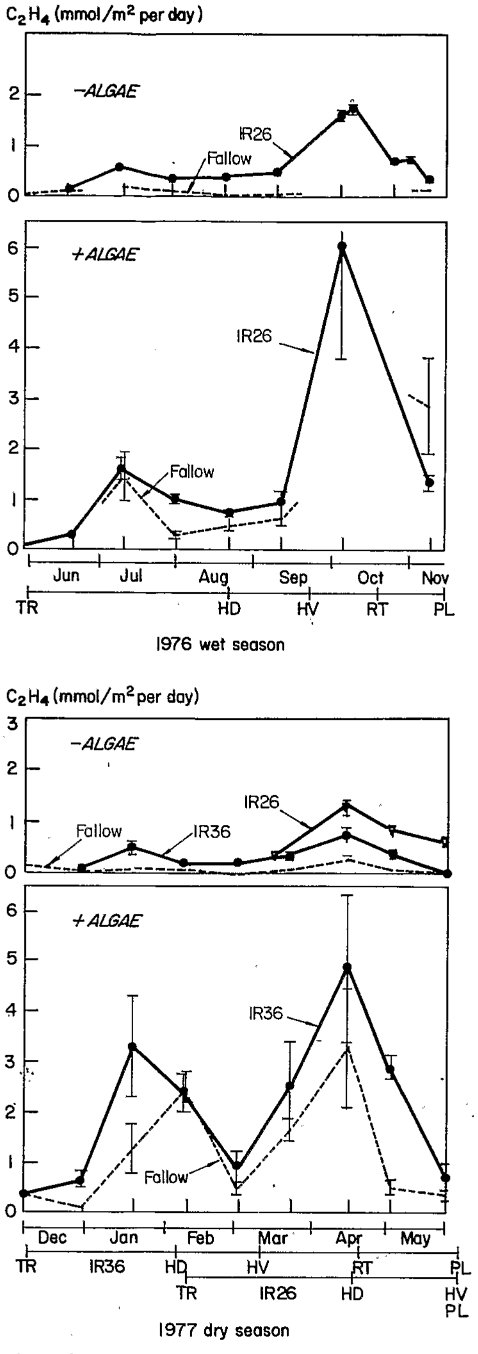

Fig. 6. Acetylene reduction rate in rice plots at IRRI, with and without algae. TR = transplanting, HD = heading, HV = harvesting, RT = ratoon, PL = plowing (IRRI 1978).

Blue-green algae and the rice plant

Compared with the bacteria-plant association, the BGA-plant association has been less studied. Observations of algal colonies on submerged portions of the rice shoot led us to study the possible association between BGA and rice. A second topic has been the availability to the rice plant of nitrogen fixed by BGA.

Epiphytic BGA and epiphytic phototrophic NFA on wetland rice, aquatic weeds, and deepwater rice. Observations in 1976 on deepwater rice roots indicated a significant NFA (ARA) on aquatic roots. Specific ARA ranged from 37 to 260 nmol C_2H_4/h per g fresh weight and increased with the diameter (age) of the roots. A preferential colonization around the axils of the emerging lateral root by Nostoc sp. was observed (IRRI 1977).

Detailed studies on epiphytism were initiated in 1979. Visual and microscopic observations, algal and bacterial counts, and ARA assays were made on shallow-water rice, submerged weeds, and deepwater rice to study epiphytic growth of BGA.

Nature of algal epiphytism. Comparing the three different hosts, it was found that epiphytism and the associated ARA on wetland rice at seedling, tillering, and heading stages, and on the submerged weed Chara was predominantly due to Gloeotrichia colonies that were visible to the naked eye. On the other hand, the epiphytic algae on wetland rice at maturity, on the submerged weed Najas, and on deepwater rice could be observed only under the microscope and the dominant algae were Nostoc, Calothrix, and Anabaena. On deepwater rice plants, Anabaena and Calothrix were found on the aquatic roots and leaf sheaths; Nostoc colonies were frequently present at the points of lateral branches of roots.

A unique finding in these studies was that BGA also exist inside the cavities of senescent leaf sheaths. Algal growth inside the cavities of dead or senescent leaf sheaths was also found in a Thailand deepwater rice area, not only on deepwater rice but also on submerged grasses. This "endophytism", however, in addition to being not confined to rice, was not present in living, healthy tissues.

A frequent observation was that older parts of the hosts supported more numerous epiphytic BGA. Older parts appear to have more rough surface. Also Chara supported more epiphytic algae than Najas, probably because of the rough corticated surface of Chara leaves. Even old, rough nylon strings, but not new, smooth ones, supported epiphytic algal growth.

From these facts, it was concluded that both epiphytism and "endophytism" are possibly related to abiotic effects, of which a mechanical effect in relation to the roughness of the host surface appears to be of major importance.

Epiphytic nitrogen-fixing activity. Rates of light ARA on wetland rice gradually diminish from seedling to maturity, mainly due to the concomitant decrease of Gloeotrichia epiphytism and the reduction of available light (Table 3). In deepwater rice, also, there was a decrease in specific ARA (nmol/g fresh weight of host) in the light from heading to maturity, but this was compensated by an increase in the host biomass, so that a constant activity per plant was observed at both these stages.

Table 3. Epiphytic ARA on rice and weeds (Kulasooriya et al 1980).

Sample	Dominant nitrogen-fixing BGA	nmol/g fresh weight per hour		µmol/m^2 per hour (total)
		Light	Dark	
Rice at seedling stage	Gloeotrichia Nostoc Calothrix	614.0	n.d.[b]	51.0
Rice at tillering stage		37.5	10.1	15.0
Rice at heading stage		1.9	0.67	1.2
Rice at maturity		1.1	1.1	2.5
Deepwater rice at heading	Nostoc Calothrix Anabaena	15.9	4.9	125
Deepwater rice at maturity		9.7	1.0	125
Chara	Gloeotrichia Nostoc Calothrix	36.6	3.3	8.0[a]
Najas	Nostoc Calothrix	26.7	1.8	5.9[a]
Monochoria		1.8	1.3	0.6[a]
Cyperus		4.4	2.5	1.4[a]

[a] For a weed biomass of 2 t/ha. [b] n.d. = no data.

Table 4. The ^{15}N - ^{14}N ratio in deepwater and shallow-water rice (IRRI 1981).

Site	Water depth	Light exposure	Total N (mg/pot) A	^{15}N (mg/pot) B	$^{15}N/^{14}N$ ratio ($\frac{B}{A}$ x 100)
Bangkhen	Shallow	+	718	111	15
Huntra	Semideep	+	979	86	9
Bangkok	Deep	+	952	97	10
IRRI	Shallow	+	502	194	39
	Shallow	-	504	230	46
	Deep	+	737	201	27.2
	Deep	-	530	114	21.5

The results indicated that the contribution by epiphytic N_2-fixing microorganisms on wetland rice fields is low but the epiphytic BGA play an important role in inoculum conservation, from which the regeneration of periodic algal blooms can take place. Epiphytic nitrogen fixation on deepwater rice, on the other hand, makes a substantial contribution to this ecosystem (10-20 kg N/ha), mainly due to the greater biomass available for colonization by epiphytic algae.

Role of epiphytic BGA in the nitrogen nutrition of deepwater rice. To evaluate the importance of epiphytic nitrogen fixation in deepwater rice, and the availability of fixed nitrogen for the plant, ^{15}N experiments were conducted at IRRI and in Thailand.

In one trial (IRRI 1981) ^{15}N dilution technique was used in the field. Pots containing soil with ^{15}N ammonium salt were placed in shallow water (5 cm) and deep water. Some pots in shallow water were covered with black cloth to prevent algal growth on the soil surface. In deep water, the submerged portion of some plants was covered with black cloth to prevent epiphytic algal growth on the rice plant. Total N and ^{15}N uptake to the aboveground portions are shown in Table 4. The $^{15}N/^{14}N$ ratio was always lower in shallow water, indicating that in deep water more nitrogen from a source other than soil was absorbed by the plant than in shallow water. If the nitrogen in the plant comes partly from biological nitrogen fixation by epiphytic BGA, the deepwater rice, of which the submerged portion was covered with black cloth, must have a lower $^{15}N/^{14}N$ ratio. The ratio of measurements on the whole plants was not in agreement with that prediction. No significant difference was observed, perhaps because of an abnormal growth of the deepwater rice plant when covered with black cloth. But the comparison of $^{15}N/^{14}N$ ratio of various parts of deepwater rice was indicative. The least enrichment of ^{15}N in the deepwater rice which had submerged portions exposed to light was found in the aquatic roots and submerged leaf sheaths. These portions were heavily colonized by epiphytic BGA. The ^{15}N dilution data of these portions are, therefore, consistent with the observation of epiphytic growth of BGA in deepwater rice that was reported previously.

The transfer of ^{15}N fixed by epiphytic BGA to other tissues was examined by direct feeding of ^{15}N to deepwater rice (IRRI 1982). For 9 days exposure at the heading stage, a deepwater rice plant fixed almost 8 mg·N, of which about 40% was translocated to aerial parts (leaves and grains) until maturity. High enrichment in ^{15}N was found in the aquatic roots and leaf sheaths under water which are highly active sites for epiphytic phototrophic NFA.

Availability of algal nitrogen to the rice plant. Nutrients fixed by BGA are released either through exudation or through microbial decomposition after the cells die. The yields of one crop of IR8 and one crop of Peta in pots without nitrogen were similar when the soil surface was exposed to light or kept in the dark despite the large potential of the soil to fix nitrogen in the light (IRRI /1968/). This may indicate that algal nutrients become available to the plant mainly after the death of the algal biomass and its incorporation into the soil. In a preliminary experiment (IRRI 1980) a Gloeotrichia bloom was grown in a small pond where ^{15}N nitrate was added. Algal material obtained had 8.95% dry matter, 1.2% N in dry matter, and 8.89 atom % excess ^{15}N. The fresh algal material was incorporated in a 1-m^2 plot in which IR26 was grown in flooded soil. Recovery of ^{15}N in the grain, straw, and root of the first crop was 14.7% of ^{15}N applied as algal mass. In the second crop, the recovery was only 2.26%. Although ^{15}N remaining in soil was not analyzed, this preliminary experiment suggested a low availability of algal nitrogen to a rice crop. Because the algal mass used in this experiment had an unusually low nitrogen content and high carbon-nitrogen ratio, the results had a limitation.

A device for producing ^{15}N-labeled algae under controlled condition was, therefore, constructed. An axenic Nostoc strain was grown in 20-liter carboys under artificial light. The medium was buffered with Na_2CO_3 to maintain a slightly alkaline pH by bubbling air enriched with 0.5% CO_2. Chemical compositions of the algal material was 1.13% P, 0.90% K, 0.74% Ca, 0.72% Mg, 38.9% C, 7.3% N, 5.3 C/N, and 23.0 atom % excess ^{15}N (IRRI 1981). Uptake of ^{15}N from this material by rice was studied in pot and field experiments.

Availability of nitrogen from dried BGA incorporated in the soil was between 23 and 28% for the first crop and between 27 and 36% for 2 crops (Table 5). Surface application of the algal material reduced the availability to 14-23% for the first crop and 21-27% for 2 crops. Availability of nitrogen from fresh algal material was similar to that of dried material when surface-applied (14%) but much higher (38%) when incorporated.

The ^{15}N balance in plants and soil after two crops (pot experiment, dried algae) showed that losses from ^{15}N ammonium sulfate were more than twice that from BGA regardless of the mode of application.

Availability of algal nitrogen to the current and following crop was almost equal to the availability of ammonium sulfate to the first crop.

Due to its organic nature, BGA material is less susceptible to nitrogen losses than inorganic fertilizers. However, its low C-N ratio (5-6) gives it a better nitrogen availability than those of an organic fertilizer like farmyard manure.

Cultural practices to increase phototrophic NFA

Phosphorus application. Phosphorus is perhaps one of the most effective factors known to promote nitrogen fixation by BGA but research results at IRRI are controversial.

Laboratory experiments (IRRI 1976) indicated that application of phosphorus (50 kg P_2O_5/ha) enhanced NFA during the initial stages of a rice crop. Response to phosphorus varied with the soil type (Fig. 7). In the most responsive soils the increase in nitrogen fixation was estimated to be 0.7-1.2 g N/g P_2O_5 applied.

Table 5. Recovery of ^{15}N (%) after two crops of rice in soil and plants (IRRI 1982).

	BGA incorporated				BGA surface-applied			
	1st crop	2d crop	Soil	Unaccounted	1st crop	2d crop	Soil	Unaccounted
Fresh algae pot experiment	38	5	53	4	13	8	73	6
Dried algae pot experiment	28	8.5	58	55	14	7	57	22
Dried algae field experiment	23.5	3.5	33[a]	40	23	4	32	41[a]

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[a] BGA were incorporated in the 0- to 15-cm layer of the soil. Because of climatic disturbances (typhoon), a downward movement of the algal material occurred which led to an overestimation of the loss.

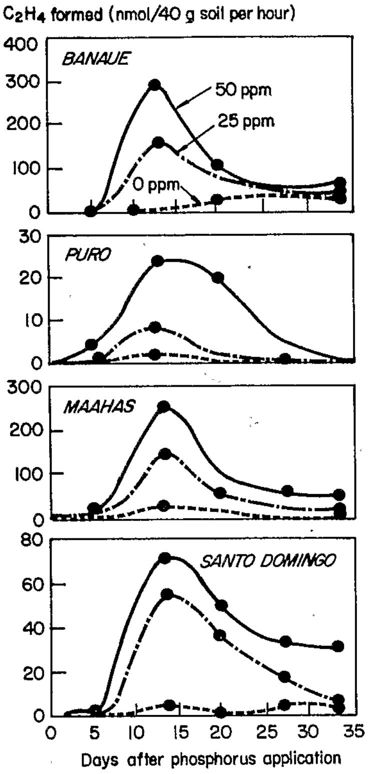

Fig. 7. Effect of phosphorus on the acetylene-reducing activity of four Philippine wetland rice soils (IRRI 1976).

Greenhouse experiments in 1978 assessed the influence of lime and phosphate on phototrophic NFA. Luisiana clay (pH:4.8, OM: 2.2%, total N: 0.160%) and Maahas clay (pH: 7.0, OM: 1.4%, total N: 0.100%) were treated with combinations of 2 levels of phosphate and lime and then kept submerged with and without light. Neither lime nor phosphorus produced a significant increase in nitrogen (Soil Chemistry Department).

Effect of nitrogen fertilizer management on phototrophic nitrogen fixation and algal flora. Nitrogen fertilizers have a depressive effect on NFA. This was observed with ammonium sulfate, which reduced both the number of N_2-fixing BGA present in the soil (Fig. 1) and their NFA (Table 2). In tests of the effect of various fertilizers on NFA, inhibition was observed on both phototrophic and heterotrophic NFA (Table 6). Inhibition was almost complete at 160 ppm N. Algae growth was luxuriant in pots that received ammonium nitrogen but nitrogen fixed was not appreciable. This indicated, besides an inhibitory effect on nitrogen, that a stimulation of the growth of nonfixing algae by mineral nitrogen can limit N_2-fixing BGA growth by competition and antagonistic effects (IRRI /1968/). From ARA measurements in IRRI research plots about 1 month after submergence, NFA was estimated to be 38.7 g N/ha per hour in a nonfertilized plot and 25.7 g N/ha per hour in a plot that received 90 kg N/ha (IRRI 1975).

The effect of different methods of nitrogen fertilizer application on the algal flora and phototrophic NFA in a wetland rice soil was studied in pot and field experiments. Results, partially summarized in Tables 7 and 8, indicated that surface broadcast application of nitrogen fertilizer, which is widely practiced by farmers, not only inhibits NFA but also encourages the growth of green algae. These deleterious algae immobilize the nitrogen fertilizer, making it temporarily unavailable to the plant. A profuse growth of green algae also increases the pH of the floodwater, which increases fertilizer loss by ammonia volatilization. Deep placement of urea supergranules, in contrast, decreased the losses of nitrogen by volatilization and did not disturb the natural algal N_2-fixing system.

Table 6. Effect of ammonium and urea fertilizers on biological nitrogen (IRRI /1968/).

Fertilizer	Level of N application (ppm)	Atom % excess ^{15}N Incubation in light	Incubation in dark
No fertilizer	0	0.150	0.100
Ammonium sulfate	80	0.042	0.036
	160	0.009	0.000
Ammonium chloride	80	0.005	0.015
	160	0.000	0.000
Urea	80	0.024	0.043
	160	0.000	0.000

Effect of surface application of straw on phototrophic NFA. A preliminary field experiment during the 1980 dry season indicated that surface application of straw (3 t/ha) induced earlier growth of N_2-fixing BGA and a significantly higher ARA at the beginning of the growth cycle of rice. Averaged ARA along the cycle was 180 mmol C_2H_4/m^2 per hour in the plots where straw was applied and 57 mmol C_2H_4/m^2 per hour in the control. A second experiment in 60 cm x 60 cm plots with transplanted rice had 3 treatments -- no straw, surface-applied straw, and incorporated straw. Figure 8 shows the change of photodependent aerobic ARA and the number of BGA propagules per cm^2 of soil. In the control and plots with straw incorporated, phototrophic ARA showed sporadically high values, but in the surface-applied-straw plot, higher phototrophic ARA was maintained from 34 days to 64 days after the application. Respiratory activity of floodwater, estimated by dissolved O_2 content and pH at night, was highest in the surface-applied-straw plots throughout the experimental period. Differences among plots became smaller, however, beyond 34 days after straw application. Photosynthetic activity of floodwater (approximated by the difference between the dissolved oxygen content at nighttime and that at daytime) in the surface-applied-straw plot surpassed that of the other plots 34 days after the application. During this period, algal nitrogen fixation was also stimulated. Thus, it was shown that surface application of straw stimulated the growth of algae, particularly BGA, and phototrophic NFA.

Insecticide application in relation to populations of algae grazers. Phenomena such as poor growth of BGA despite favorable soil and climate conditions, failure of algal inoculation, and rapid destruction of algal blooms may be due to crustaceans that graze on algae.

A role of grazers in the paddy was first suggested in 1964 when field observations indicated that gamma BHC (Lindane) had a pronounced effect on the algal population (IRRI [1965]). Greenhouse experiments showed that soil treated with BHC exhibited a dense algal growth and clear water after 10 days of incubation whereas nontreated soil showed scanty algal growth and a turbid floodwater. This was related to the development, in the untreated plot, of large populations of microcrustaceans that graze on algae and probably caused the turbidity of the floodwater by their mechanical action on the soil-water interface (IRRI [1965]). Further experiments (IRRI [1966]) confirmed that application of the insecticide at usual field rates (5 kg/ha) resulted in a marked increase in algal populations. Higher doses (50 kg/ha) were not inhibitory to algae although algal number was generally less than in soils treated at the recommended rate.

Among algae, BGA gave the greatest response to application of γBHC. This selective enhancement was confirmed by measurements of NFA (^{15}N) in Maahas clay and Luisiana clay where an application of γBHC (6 kg/ha) increased NFA by 91 and 62%, respectively. A third soil (Calabanga clay) did not respond favorably to γBHC application.

Algal growth was used as an index for the persistence of the insecticide in soils. The beginning of the decline in algal populations in treated soils started about 6-7 weeks after insecticide application and coincided with the recolonization of the floodwater by the crustaceans. The time elapsing between the second application of BHC and the recolonization by the crustaceans was shorter than that noted after the first application. Recolonization after a third application was even more rapid. This was interpreted as the progressive

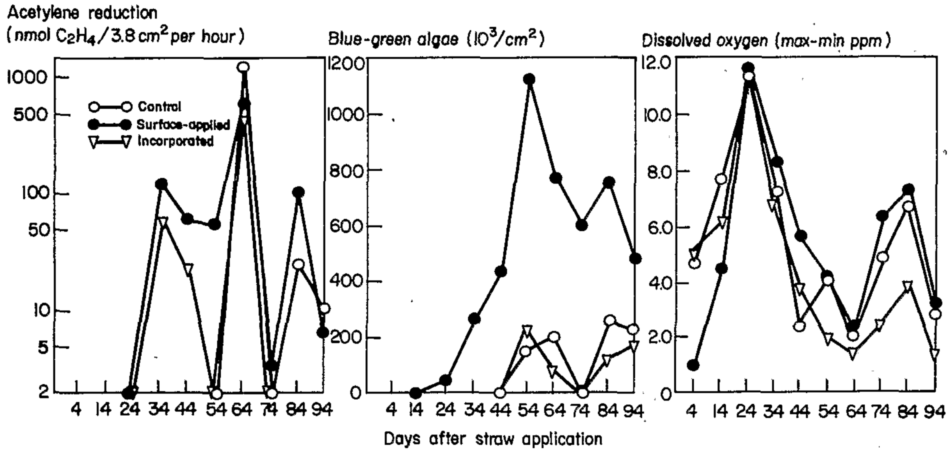

Fig. 8. Nitrogen fixation rate, BGA counts, and photosynthetic rate as affected by straw application (IRRI 1982).

Table 7. Effects of fertilizer placement on the algal flora and nitrogen fixation in a field experiment 28 days after treatment (Roger et al 1980).

Treatment	Control	Urea supergranule (deep placement)	Urea (broadcast)
ARA	70	48	0
µmol C_2H_4/m^2 per hour (% of the control)	100	69	0
Chlorophyll a (µg/cm^2)	12.4	12.3	21
Number of nitrogen-fixing blue-green algae/cm^2	2.0×10^5	1.7×10^5	7.0×10^4
Number of green algae/cm^2	$<10^4$	5.0×10^5	1.0×10^7

establishment of populations of microorganisms active in the degradation of BHC. The comparison of the persistence of the insecticide in sterilized and nonsterilized soils was in agreement with this interpretation (IRRI 1966). The possible development of a strain resistant to the insecticide was not evocated.

Diazinon had a similar but less marked effect as γBHC on the algal flora (IRRI 1967).

Feeding rates of Cypris on BGA were determined in relation to the animal's body size. Rates varied with the BGA species and a preferential grazing was evidenced. Feeding rates increased with the size of the animal. Younger cultures (Anabaena) were more readily ingested than old ones. Lindane at 0.01 ppm stimulated feeding but 0.1 ppm was inhibiting (IRRI 1980).

Field observations showed that two important groups of predators -- Gastropoda and Ostracoda -- had the potential to prevent or limit algal growth and nitrogen fixation (IRRI 1981).

The biomass of snails (mainly Lymnaea sp.) at IRRI ranges from a few kg to 1.5 t fresh weight/ha. A greenhouse pot experiment (Table 9) demonstrated the immense reduction in ARA as a result of established algal biomass being grazed by an average field biomass of snails. After 2 days, ARA decreased to 25% of the control.

In a field experiment, the population of snails (Lymnaea viridis and T. riquetti) was slightly depressed by insecticide (carbofuran) but not completely eliminated.

Field densities of ostracods (subclass Crustacea) in IRRI's small field plots where experiments on surface accumulation of nitrogen were conducted were consistently high, ranging between a few hundred to 10,000/m^2. The densities of ostracods in 250-ml water samples were 115, 4, 6, and 253 in insecticide, insecticide plus black cloth, no insecticide, and insecticide plus straw treatments, respectively. Insecticide application encouraged the population of ostracods. It is probable that repeated application of insecticide (mainly carbofuran) establishes a tolerant microfauna. Alternately, the elimination of the secondary consumers by the insecticide may encourage the biomass of the primary consumers (ostracods).

Table 8. Effect of fertilizer nitrogen management in a pot experiment on phototrophic nitrogen fixation (ARA 15 days after transplanting, in µmol/m^2 per hour) (IRRI 1980).

	Luisiana soil (pH 5)	Maahas soil (pH 7)	Tiaong soil (pH 8)
Control	3.5	5.1	43.1
Urea supergranule deep-placed	2.7	88.7[a]	33.1
Urea broadcast	1.4	0.5	5.5

[a] Value largely higher than that of the control because of a profuse growth of Cylindrospermum in half of the pots -- Pot experiment artifact, but this clearly demonstrates that deep placement does not inhibit BGA growth and nitrogen-fixing activity.

A pot experiment (Table 10) demonstrated the detrimental effect of spontaneously developing ostracod populations on ARA in submerged soils (IRRI 1981).

Table 9. Pot experiment to demonstrate the effect of snails on algal nitrogen fixation (IRRI 1981).

Treatment (6 replications)	ARA (nmol C_2H_4/pot) Before treatment	48 hours after treatment	10 days after treatment
No snails	129.0	254.5	106.5
Snails added (750 kg/ha)	137.5	59.0	11

Table 10. Pot experiment with nonplanted soil to determine the effect of ostracods on algal ARA (IRRI 1981).

		µmol C_2H_4/m^2 per hour		Ostracods (no.)/m^2	
Soil	pH	14 days after submersion	28 days after submersion	14 days after submersion	28 days after submersion
Luisiana	4.0	1.8	3.7	None	None
Maahas	6.7	22.7	5.1	+	10,000
Tiaong	7.9	19.7	0.3	+	8,500

Both snails and ostracods are extremely resistant to conventional pesticides. In the absence of their natural predators, they are capable of exploiting the available niches, which typically results in high biomass of few species.

Trials to control grazer populations with selective pesticides were undertaken in 1981. In 1-m^2 paddy subplots surrounded by a metal frame, various pesticides -- carbofuran (3 kg a.i./ha), clonitralid (1 kg a.i./ha), ethylan (2.2 kg a.i./ha), and crushed seed of neem tree (Azadirachta indica), 100 kg/ha, were applied 10, 24, 44, and 60 days after puddling and flooding. Ethylan was combined with clonitralid. The population of ostracods and molluscs, chlorophyll contents, and photodependent ARA were monitored throughout rice cultivation. Clonitralid successfully controlled

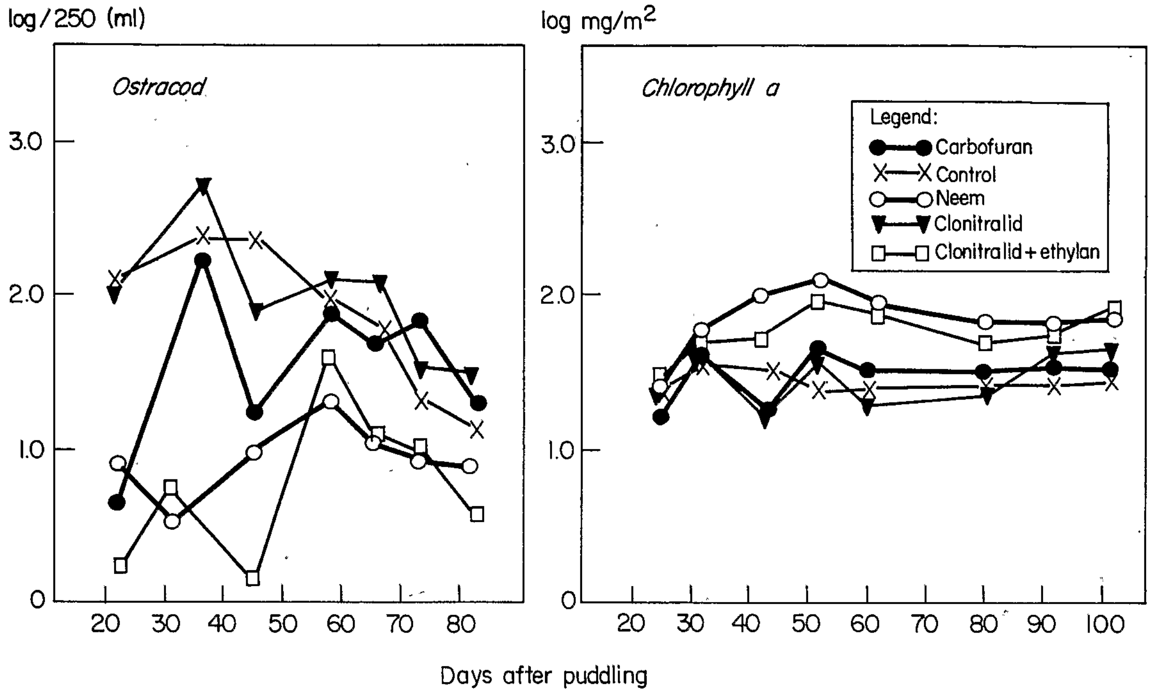

Fig. 9. Ostracod population and chlorophyll content as affected by pesticides (IRRI 1982).

populations of molluscs and ethylan controlled ostracods. Crushed neem seed also successfully controlled ostracods. Depression of mollusc population did not result in the enhancement of algal biomass and photodependent ARA because the molluscs were replaced by ostracods. When populations of ostracods were successfully reduced by ethylan + clonitralid and neem seed treatments, algal biomass and its nitrogen fixation were successfully enhanced from about 30 to 60 days after puddling and flooding (Fig. 9).

The oil extraction residue of neem seeds (neem cake) is known to have insecticidal and insect-repellant properties. Its effects on phototrophic NFA were tested in greenhouse and field experiments. The addition of neem cake at the rate of 6-12 g/m^2 maintained the population of BGA and the photodependent NFA (ARA) much longer than in the control. The field experiment was severely disturbed by a typhoon and wide variation between replications was observed. However, 9 weeks after transplanting, larger algal biomass was recorded in the treated plot than in the nontreated plots (IRRI 1982).

Algalization. A first trial on algalization was conducted in 1965 in the laboratory. Three soils, inoculated with Nostoc culture, were incubated under an atmosphere (O_2, N_2, He) enriched with $^{15}N_2$. After 28 days of incubation, increases in nitrogen fixation, through algal inoculation, of 56 and 28% were obtained for Luisiana and Maahas soil, respectively. No significant change in nitrogen fixation was found when Calabanga clay soil was inoculated with the alga.

An increase in NFA, evaluated by ARA, was also observed in a nonplanted Maahas clay soil after inoculation with a Nostoc strain. This effect was measurable for more than 10 weeks after inoculation (IRRI 1974).

The effect of algalization on rice yield (grain and straw) was tested in Maahas clay by spreading a Nostoc strain on the floodwater and applying lime (1 t/ha) and molybdenum (0.28 kg/ha) to provide a more suitable environment on the BGA. No significant increase in yield was observed, perhaps due to the strain chosen (IRRI 1966).

Similar results were observed in 1968 from a pot experiment in which the soil was inoculated with Nostoc muscorum and Tolypothrix tenuis. Algae growing in the light stimulated rice tillering and increase panicle numbers, but the total nitrogen uptake of the plant and the yield did not significantly differ between the soil inoculated with algae and the control.

Balance experiments conducted in pots in the greenhouse indicated a stimulation of nitrogen fixation when BGA were inoculated and phosphorus and iron were simultaneously added (IRRI 1978). However, no significant difference was obtained when a (P, Fe, algae) treatment and a (P, Fe) treatment were compared (IRRI 1979). Apparently the addition of phosphorus and iron increased molecular nitrogen fixation by stimulating the indigenous populations and inoculation with BGA did not significantly improve NFA.

LIMITING FACTORS FOR THE RESEARCH ON BLUE-GREEN ALGAE AND PHOTOTROPHIC NITROGEN FIXATION

Studies on BGA in submerged soils are limited by problems in methodology, primarily in estimating algal biomass qualitatively and quantitatively. In addition, problems in sampling techniques in relation to spatial distribution of BGA and their NFA increase the inaccuracy of quantitative measurements. The significance of measurement conducted in an experimental farm also needs some critical examination.

Our purpose is not to describe extensively the different techniques used but to point out their limitations. For more information on methods for evaluating algal abundance and algal NFA, see the recent reviews such as those by Fogg et al (1973), Roger and Kulasooriya (1980), and Stewart (1980). Problems in sampling strategy have been considered by Roger et al (1977) and Roger and Reynaud (1978).

Techniques for evaluating algal abundance in soils

Three techniques for evaluating algal abundance in soils are generally cited: plating of soil suspension, direct count, and pigment analysis.

Pigment analysis gives no indication of species and, because of contaminations by humic acid and chlorophyll degradation products, can be used only to compare roughly the effects of different treatments on the total flora in the same soil.

Direct examination and counting by light microscopy, although time-consuming and boring, is theoretically the most satisfactory method. Moreover because a homogenized soil suspension is used, algal filaments are more or less broken in pieces and the determination of species may be impossible in some cases.

A dilution and plating technique permits the simultaneous enumeration, identification, and even isolation of the different algae of the sample. Accuracy of the plating method can be improved by using selective media for eucaryotic, procaryotic, and N_2-fixing algae. The results of enumerations can be expressed in terms of biomass by determining the mean volume of each count unit (cell, filament, or colony, according to species) by directly examining the first dilution and multiplying the results by the corresponding volume unit.

The main disadvantage of the plating method is that it does not ensure that all species present in the soil develop on the plates and even if they grow their relative frequency may change. It also does not distinguish between active and inactive forms. Despite its disadvantages the plating method is the most widely used. It provides an index of the importance of algal populations, which can be useful as an adjunct to the measurement of ARA.

With the plating method, however, enumeration of the algal flora of 1 soil sample requires the preparation of 45 Petri dishes, 3 media, and 6 soil dilutions. After plating, the Petri dishes are incubated under artificial light for 3 weeks. Of the 45 Petri dishes, about 18 will be used for enumeration under a stereoscopic microscope. Enumeration and characterization of the colonies from 1 dish take between 1 and 5 minutes. It is, therefore, easily understandable that such a method can be used for enumerating simultaneously algae from a restricted number of soil samples only and that replications are rarely done.

Techniques for evaluating nitrogen-fixing activity

Nitrogen analysis by the Kjeldahl technique is used in nitrogen balance studies. The use of this method to distinguish between phototrophic NFA by parallel light and dark treatment is suitable only for long-term trials for gross measurement. Balance studies with planted pots corresponding to 10 different treatments needed 2,650 analyses (IRRI 1979); that indicates how tedious this method becomes for obtaining accurate results.

Acetylene reduction activity is now widely used for measuring phototrophic NFA. It is the most useful technique for routine studies on the nitrogenase activity. Measurement of algal ARA is reliable when the incubation is brief, the problem of gas diffusion and greenhouse effects are minimized, and statistically valid sampling methods are adopted; however, the method is generally considered suitable only for qualitative estimates.

Sampling problems in relation to the distributional ecology of BGA

The validity and accuracy of algal enumerations and ARA measurements in situ depend principally on the density of sampling. The density of sampling for a given degree of accuracy varies with the distribution law of variables. BGA populations have an uneven distribution that approximates a log-normal pattern, therefore, a high density of sampling is needed.

As a sample, the mean value of Anabaena biomass based on 40 samples of 10 core each, taken in a 2,500-m^2 paddy had a confidence interval of +32% and -27% of the mean. Evaluation of the algal population in that field, made on 3 selective media, required 1,800 Petri dishes.

A similar problem occurs with ARA measurement. As a sample, Table 11 shows values of replicate measurement done in 1-m^2 plots along a growth cycle of rice. Results varied greatly. The small size of the plot together with the irregular distribution of algae and the fact that the plots were protected from inoculation by the water or the soil of

Table 11. Acetylene reduction assay (μmol C_2H_4/m^2 per hour) during a rice crop (App et al 1982).[a]

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

[a] Figures in parenthesis are replicate values. Average values in a column followed by a common letter are not significantly different by DMRT. DT = days after transplanting. [b] Carbofuran at 3 kg a.i./ha was applied every 2 weeks. [c] Assays were done 1 day ~~every~~ after a heavy rain (80 mm). [d] Assays were done after harvest.

the surrounding field by a continuous frame may have prevented simultaneous growth of N_2-fixing BGA in the different replications of a treatment.

In addition to an uneven distribution, BGA exhibit rapid variations in their growth and their activity. Therefore, frequent measurements are needed. For this reason most of the experiments are conducted near the laboratory, which limits experiments conducted to an experimental farm.

Significance of measurements conducted on an experimental farm

In BGA and rice (Roger and Kulasooriya 1980) it was pointed out that pot experiments are hardly representative of field plots and overestimate the effect of BGA on rice. The better growth of BGA in a pot than in the field is probably due to less disturbance than in the field, better control of the experimental conditions, and better care than in the field. A similar difference may exist between the fields of a sophisticated experimental farm like IRRI's and those of the average rice farmer. We do not have, at present, quantitative data to compare BGA populations and phototrophic NFA on the IRRI farm and in the surrounding farmer fields. However, there is evidence that year after year the IRRI farm ecosystem develops some peculiar characteristics. In 1968 a record of the BGA occurring at different stages of the rice plant in Maahas soil did not show the presence of Gloeotrichia and Aphanothece, which are now frequently dominant on the IRRI farm and also develop in pot experiments. The dominance of these strains is probably an indirect result of the constant use of insecticide at IRRI, which has caused the establishment of a desequilibrated zooplankton population dominated by a Cyprinotus sp. strain resistant to carbofuran. This strain feeds on BGA and exhibits a preferential grazing. Among BGA, strains forming mucilaginous massive colonies like Gloeotrichia and Aphanothece are not ingested probably because of the morphology of the colony.

Thus, it appears that studies on the ecology of BGA and phototrophic NFA are limited by methodological problems. Available methods are either inaccurate and tedious (enumeration) or qualitative and open to criticism (ARA). The number of replications and the density of sampling requested for a satisfactory accuracy are frequently not compatible with the practical realization of the measurements. Although IRRI farm provides excellent facilities for experiments, its ecosystem may differ significantly from that in farmers' fields in the same area.

OPPORTUNITIES FOR RESEARCH ON PHOTOTROPHIC NITROGEN FIXATION IN RICE FIELDS

After an extensive study of the literature on BGA and rice in 1979 (Roger and Kulasooriya 1980) it was concluded that increasing the efficiency of indigenous or inoculated nitrogen-fixing BGA by cultural practices is an efficient way to provide an alternative source of nitrogen for rice cultivation.

From the reported field measurements, BGA can fix as much as 80 kg N/ha per crop -- the average of 38 tests was 27 kg N/ha per crop. Taking into account the algal biomasses recorded in the fields and the ARA values measured in the laboratory, it appears that BGA certainly have a higher potentiality than that measured in the field and that limiting factors are involved.

From the compilation of the literature three major gaps in our knowledge were pointed out:

- ecology of BGA on rice paddies,
- mode of action of BGA on rice, and
- limiting factors for development of either indigenous or inoculated BGA.

Recent studies at IRRI have filled some of these gaps:

- The availability of algal nitrogen to the rice plant has been quantified, depending on the nature of the algal material, and the mode of application values ranging from 16 to 36% for 2 crops has been obtained.
- An extensive study has shown that epiphytic BGA make a low nitrogen contribution to shallow wetland rice. The contribution becomes of value with deepwater rice and the transfer of fixed nitrogen from the algae to the rice plant has been demonstrated.
- Several cultural practices favoring phototrophic NFA have been identified. These are deep placement of nitrogen fertilizers, surface application of straw, and surface application of neem cake.
- Populations of grazers have been identified as an important limiting factor for BGA growth.

Agronomical use of BGA is, however, still severely limited by a lack of knowledge on the ecology of indigenous and inoculated BGA in the paddy fields. Some research priorities are, therefore, recommended:

1. There should be a compilation and a review of the literature on BGA in freshwater ecosystems to complete the information obtained from the compilation of literature on BGA and rice.
2. Ecological studies on BGA in long-term field experiments are needed to measure algal biomasses and phototrophic ARA in relation to the main environmental parameters. Such experiments have to be as cooperative research, in fields where balance studies and fertilizer management studies are conducted (INSFFER). Special attention has to be given to:
   - the role of photosynthetic N_2-fixing processes in replenishing available soil nitrogen in wetland rice soil (including photosynthetic bacteria);
   - the limiting factors for growth of BGA and expression of the NFA; and
   - the cultural practices favoring growth of N_2-fixing BGA. Integrated nitrogen management practices by inoculation of algae and the use of straw amendment, together with deep placement of nitrogen fertilizer, can be tested. Sources of stimulants for algal growth, such as neem cake or, possibly, rock phosphate, have to be identified and tested.

A network for the collection of a large number of soil samples, to relate the presence or absence of N_2-fixing strains to physicochemical properties of soil and climatic conditions, should be established. That should also provide an opportunity to constitute a strain collection.

Long-term field inoculation experiments conducted on ecological bases are needed to:

- quantify the nitrogen fixed by inoculated algae,
- determine the relative importance of growth-promoting substances in increasing grain yield compared to fixed nitrogen, and
- study the influence of BGA on soil properties and the effect of changes in soil properties on rice growth.

Experiments on the use of BGA will be initiated within the INSFFER network. Likewise emphasis will be placed on identifying land characteristics where BGA will provide the highest benefit.

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