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IRRI/ORSTOM RESEARCH PROJECT ON THE PHOTOSYNTHETIC AQUATIC BIOMASS IN WETLAND RICEFIELDS

P. A. ROGER, May 1989

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1. STATUS OF THE PROJECT.

Project Title:

Contribution of the photosynthetic aquatic biomass to nitrogen fertility of rice soils through nitrogen fixation and nutrient recycling.

Main collaborators:

- International Rice Research Institute (IRRI),
- Institut Français de Recherche Scientifique pour le Développement en
Cooperation (ORSTOM).

Other major collaborators (with signed protocol of agreement):

- University of Florida (Dr Shanmugham),
- ODNRI (England) (Dr I. Grant).

Project Leader:

Dr P. A. Roger

Personel:

2 Research Assistants, 1 Collaborative Research Fellow (6month/year), 1 Laboratory Aide,
1 Field Aide.

Duration:

2 years (June 1989 - June 1991)

Financing:

French contribution: \$ 45. 000/ year provided by the French grant. Salary and related benefits
of P. Roger paid by ORSTOM

IRRI contribution: 2 research assistants. Laboratory and experimental field facilities.
Perquisites of P. Roger.

Other contributions:

\$ 10. 000/year from a 2-year subcontract USAID with the University of Florida (1989-1990).
One Collaborative Research Fellow (6 months/year) provided by ODNRI (England) under a 2-
3-year collaborative project (1989-1991).

2. BACKGROUND AND DESCRIPTION :

This project derives from a previous one established between IRRI and ORSTOM to study of
the ecology of N₂-fixing blue-green algae (BGA) in ricefields and estimate their potential as
biofertilizer.

Studies on BGA have shown that:

- (1) contrarily to earlier belief, BGA are ubiquitous in wetland ricefields and the absence of development of N₂-fixing blooms of BGA is rather due to environmental or agronomic limiting factor than the absence of N₂-fixing strains,
- (2) inoculation with BGA has somewhat been overemphasised in the literature and the inoculation of foreign strains might not be needed/effective in many soils,
- (3) some agricultural practices, especially P application, grazer control, and deep-placement of N fertilizer favor the growth of indigenous BGA and might be sufficient for taking advantage of their N₂-fixing potential,
- (4) BGA have a moderate potential as biofertilizer (30 kg N ha⁻¹ crop⁻¹). This potential can be useful in subsistence farming or in an integrated system of fertilization, but is insufficient on its own to produce high yield,
- (5) environments responsive to BGA management/inoculation need to be further defined, and
- (6) more attention should be paid to the management of the photosynthetic aquatic biomass (algae and aquatic plants) as a whole, with regard not only to its role in nitrogen fixation but also in causing nitrogen losses by volatilization and in replenishing available soil nitrogen.

Those aspects have been included in the new research project which has two main goals:

- (1) to achieve the studies on the potential of N₂-fixing blue-green algae as biofertilizer initiated during the previous phases of the IRRI-ORSTOM project, and ensure the transfer of this knowledge to national systems interested in testing BGA utilization in rice cultivation
- (2) to assess the contribution of the photosynthetic aquatic biomass to the nitrogen fertility of wetland soils and the impacts of the factors of crop intensification, especially agrochemical use, on its productivity, nutrient turnover, and contribution to soil microbial biomass and available N.

3. CURRENT RESEARCH ACTIVITIES, THEIR STATUS AND PLACE IN IRRI'S WORK PLAN

The project currently comprises 8 research activities listed thereafter with their status:

1. Methods for field studies of BGA (almost achieved)
2. BGA collection (active phase)
3. Occurrence of N₂-fixing BGA in ricefield soils (almost achieved)
4. Establishment of inoculated BGA in ricefield soils (active phase).
5. Field study of BGA inoculation (in collaboration with national programs)
6. Role of algae in nitrogen losses by ammonia volatilization (low emphasis)
7. Estimation of the nutrients contribution and recycling by the photosynthetic aquatic biomass, effects on microbial biomass and available N (active phase)
8. Interactions between agrochemicals, photosynthetic aquatic biomass, soil and water fauna, and soil fertility (active phase).

The IRRI/ORSTOM research project is part of IRRI's Irrigated Rice Program.

The first 7 research activities are included in the Subprogram "Crop, resource, and pest management" under the Output "Understanding nitrogen cycling and nitrogen-fixing agents, improved fertilizer formulations, and more efficient management of nutrients in submerged soils", which reads :

Experimental and simulation research on nitrogen cycling, transformation and losses will seek ways to predict soil nitrogen availability and yield response, and ensure efficient plant uptake of fertilizer nitrogen, soil nitrogen, and biologically fixed nitrogen. We also will develop and evaluate improved formulations for nitrogen fertilizer, improved N₂-fixing agents for submerged soils, and adaptable methods for their management...

Research activity n^o 8 is included in the Subprogram " Livelihood and production environments " under the Output " The effects of high-input cropping systems and changing climate on ricefield ecology and productivity." which reads:

We will examine the effects of intensive cropping on fieldwater ecology and soil microbial biomass.....

Time allocation for the different research activities is as follows:

IRRIGATED RICE, Subprogram 1

Collaborative field experiments on BGA inoculation	0.2
Establishment of nitrogenase derepressed mutants of BGA in rice soils.	0.2
Nutrients contribution by the photosynthetic aquatic biomass, effects on microbial biomass and available N	0.2
BGA collection	0.1

IRRIGATED RICE, Subprogram 2

Interactions between agrochemicals, photosynthetic aquatic biomass, soil and water fauna, and soil fertility	0.3
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Information on the research activities is provided in the annexed summaries.

5. SPECIFIC RESEARCH OBJECTIVES FOR 1989-1991

- (1) to publish of a booklet on methods for field studies of algae in wetland ricefields.
- (2) to conduct field experiments on BGA inoculation in collaboration with IIRR and WARDA and study the possibility to establish nitrogenase derepressed mutants of BGA in ricefield soils (in collaboration with the University of Florida).
- (3) to achieve the description and computerization of the collection of BGA isolated from ricefields.
- (4) to quantify the nutrient contribution by the photosynthetic aquatic biomass and the related effects on microbial biomass and soil available N under various levels of agrochemical inputs (this data will also be of value for estimating potential methane production in ricefields).
- (5) to study, in the long term experimental design already establish in the IRRI farm and in a representative sample of farmer fields, the interactions between agrochemicals, photosynthetic aquatic biomass, soil and water fauna, and soil fertility (in collaboration with other IRRI departments, UPLB, and ODNRI).

Research activity summary

METHODS FOR FIELD STUDIES OF BLUE-GREEN ALGAE

GENERAL INFORMATION:

Project : Contribution of the photosynthetic aquatic biomass to nitrogen fertility of rice soils through nitrogen fixation and nutrient recycling.

Year started: 1984

Expected year of completion: 1989

Personnel:

Research project leader: P.A.Roger

IRRI Soil Microbiology personnel:

Susan Santiago Ardales (RA)

Ruby Remulla Jimenez (RA)

Collaborators:

Other IRRI departments involved: none

Outside of IRRI: ORSTOM (France)

Protocol of agreement: IRRI/ORSTOM (June 1989 - June 1991)

DESCRIPTION AND GOAL:

To develop methods for quantitative estimate of BGA populations and their activities (N₂-fixation and photosynthesis) in wetland soils with emphasis on inoculation experiments.

RESULTS:

Methods for the study of blue-green algae (BGA) in ricefields have been modified from existing ones, or designed and standardized. They include:

- Isolation, culture, and conservation of unialgal BGA from ricefields
- Sampling in wetland ricefields
- Qualitative and quantitative evaluations of BGA present in a field, a soil sample or an inoculum.
- Evaluation of photodependant acetylene reducing activity and photosynthetic activity in floodwater.
- Designing field experiments and statistical analysis of the data

With regard to the very uneven distribution of algae in ricefields, special attention has been paid, when standardizing field methods, to (1) the distributional ecology of BGA and their activities, (2) the implications for sampling procedures, and (3) the intra- and inter- plot variability of the measurements.

The large number of replicated field measurements on BGA populations and photodependant acetylene reducing activity that we performed during the last years, allowed to draw general conclusions regarding the accuracy of such measurements and the limitations related to number of replicates and subsamples.

The log-normal law is a model that fit with most of the measured variables.

Composite sampling usually permits to obtain satisfactory measurements within a plot, while average values among replicated plots usually have a very poor accuracy because the number of replicated plots that can be used is frequently low. On an average, 5 replicated plots will only permit to find significant differences between values whose ratio is about 3. Ten replicates would permit to separate values whose ratio is two. Most field experiments on rice have usually 3 or 4 replicates which is adequate for normally distributed data such as rice yield or data that varies exponentially such as bacterial counts but might be often too low for studies of BGA biomass, photodependant N₂-fixation, and, in general, many measurements of microbial activities.

BGA counts by plating have a moderate accuracy and suffers from an inherent bias mostly due to competition among BGA colonies on petri dished. Plating is useful for characterizing BGA populations in a soil or an inoculum, but, in inoculation experiments, direct measurement of BGA biomass is much more reliable and is almost always usable when the algal biomass is of agronomic significance. Utilization of visual estimates of algal abundance is convenient and efficient for many field studies.

ARA measurements *in situ* are of little quantitative value because of a greenhouse effect unavoidable without sophisticated incubation devices that would not be compatible with the large numbers of replicated measurements required. ARA measurements under standardized temperature and light intensity permit to obtain a more reliable index of photodependant N₂-fixation but extrapolation to N fixed still remains quite questionable.

Many of the results of these studies are not restricted to algae but are applicable directly or after some modifications to microbial populations and zooplankton.

PUBLICATIONS AND REPORTS : *

Remulla-Jimenez R, Roger PA, Watanabe I (1985) Standardization of acetylene reducing activity measurements for photodependant N₂-fixing microorganisms in wetland rice fields. The International Rice Research Institute , Po Box 933, Manila, Philippines. Saturday Seminar, June 15 1985, 39 p.

Santiago-Ardales S, Roger PA (1987) Characterization, quantification, isolation and conservation of N₂-fixing BGA from rice soils. IRRI Saturday Seminar October 3, 1987. The International Rice Research Institute Po Box 933, Manila, Philippines. 32p.

WORK PLAN:

Compilation of the methods as a book has been undertaken. The purpose is not to provide the reader only with a "cookbook" of ready made recipes but also with extensive information on the various factors that affect the measurements and the limitations of these methods in order to avoid misuse and allow adaptation of the methods to specific requirements.

* only material published since 1985 is listed in the summaries

Research activity summary

BLUE-GREEN ALGAE COLLECTION

GENERAL INFORMATION:

Project : Contribution of the photosynthetic aquatic biomass to nitrogen fertility of rice soils through nitrogen fixation and nutrient recycling.

Year started: 1979

Expected year of completion: not relevant

Personnel:

Research project leader: P. A. Roger

IRRI Soil Microbiology personnel:

Susan Santiago-Ardales (RA. 50%)

Collaborators:

Other IRRI departments involved: none

Outside of IRRI: ORSTOM (France).

Potential collaborators:

University of Durham (Dr B. Whitton)

Indian Facility for BGA (Dr Venkataraman)

Protocol of agreement:

IRRI/ORSTOM (June 1989 - June 1991)

DESCRIPTION AND GOAL:

To collect, characterize, conserve, and provide on request unialgal strains of N₂-fixing BGA collected from rice fields. The collection is a "working collection" and not a "germplasm". However it is currently the largest collection of N₂-fixing BGA collected from rice fields and one of the largest collections in the world.

METHODS:

Strains are conserved as stock cultures in liquid medium and as dry material.

RESULTS:

About 210 strains of N₂-fixing BGA from 19 countries, mostly isolated from ricefields, are maintained as unialgal cultures in liquid medium and as dried material. Information on the strains is partially computerized in a specially designed program written in "Hypertalk" language. Besides classical information on the strains, the program allows direct access to taxonomical keys, a glossary, information on the sites the strains were collected from, and on methodologies. Information stored in fields on cards can be compiled and merged as a text directly transferable in a word processing program. Strains are provided free on request.

Conservation as soil-based inoculum (70 strains), dry powder from mass culture (12 strains), and dried cultures deposited on paper stripes (170 strains) has been tested. Conservation on paper stripes is convenient for short term conservation and mailing but most of the strains could not be regrown after 2 years of storage. Storage as powdered material from mass cultures seems to be more efficient (survival > 56 month) but need testing on a larger number of strains.

The elementary composition of 32 strains of the collection has been determined and compared with that of field samples. Laboratory strains have a higher N and P content and a lower ash content than field samples usually deficient in P. There is little relationship between the taxonomical position of the strains and their elementary composition.

Isozymes of 18 enzymes have been studied on a representative sample of 9 genera and 15 strains of a single genus (*Anabaena*) grown under 3 environmental conditions and collected at 6 different ages, to evaluate the possible use of isozymes in identifying BGA strains in soil inoculation experiments. Four enzymes were selected. The method is highly discriminatory at genus and species levels and has a satisfactory replicability which allows its use for strain characterization. More data are needed before conclusions on taxonomical use can be drawn.

PUBLICATIONS AND REPORTS :

- Glaszmann JC, Roger PA (1985) Isozymes, possible markers for blue-green algae identification. Int. Rice Res. News. 10(4) 29-30.
- Roger PA, Tirol A, Ardales S, Watanabe I (1986 a) Chemical composition of cultures and natural samples of N₂-fixing blue-green algae from rice fields. Biol Fert Soils 2 : 131-146.
- Santiago-Ardales S, Roger PA (1987) Characterization, quantification, isolation and conservation of N₂-fixing BGA from rice soils. IRRI Saturday Seminar October 3, 1987. International Rice Research Institute Po Box 933, Manila, Philippines. 32 pp.

WORK PLAN:

Depends 1) upon the facilities that will be establish for the soil microbiology Department and 2) further information on the need for a BGA germplasm. In the current stage, addition of strains to the collection will be minimal and will take geographical diversity as the major criteria.

The work plan is limited to:

- completion of the characterization of the strain by description and photographs of material and colonies from liquid and solid medium
- study of isozymes of few sets of strains of the same genus from various locations and from the same location with regard to the morphology of the colonies on solid medium
- standardization of a method of preservation in dry state of material from mass cultures.

When larger facilities become available and more information of the feasibility of inoculating foreign strains becomes available the possible establishment a BGA germplasm shall be considered.

Research activity summary

OCCURENCE OF N₂-FIXING BGA IN RICEFIELDS

GENERAL INFORMATION:

Project : Contribution of the photosynthetic aquatic biomass to nitrogen fertility of rice soils through nitrogen fixation and nutrient recycling.

Year started: 1980

Expected year of completion: 1990

Personnel:

Research project leader: P.A.Roger

IRRI Soil Microbiology personnel: Susan Santiago Ardales (RA)

Collaborators:

Other IRRI departments involved: none

Outside of IRRI: ORSTOM (France)

Protocol of agreement: IRRI/ORSTOM (June 1989 - June 1991)

DESCRIPTION AND GOAL:

To estimate N₂-fixing BGA populations in rice soils and assess the need for BGA inoculation with regard to the abundance of indigenous strains in relation with soil properties and some environmental parameters.

METHODS:

Estimation of BGA populations in a wide range of rice soils, by plating techniques. Study of the correlation between soil properties and BGA abundance.

RESULTS:

An extensive survey of the algal flora in 120 ricefield soils from six countries has contradicted the earlier belief that N₂-fixing BGA are absent in many rice soils. Taking advantage of other field studies, this survey has now been extended to about 200 ricefields from 8 countries.

N₂-fixing strains have been found in all studied ricesoils, where they can be considered ubiquitous. Densities range from a few dozen to more than 10⁶ colony-forming units (CFU)/cm² of soil; the median is about 5 x 10⁴ CFU/cm².

Positive correlations were found between the abundance of heterocystous BGA and (1) pH, (2) available P, and (3) CEC of the soils.

Field surveys and bibliographic study shows that *Nostoc* spp., *Anabaena* spp. and *Calothrix* spp. are present in 80% or more of the studied soils. *Nostoc* is most frequently the dominant genus. The dominance of *Nostoc* is partly due due to its ability to form mucilaginous macrocolonies resistant to grazing.

Indigenous propagules of N₂-fixing BGA in the first cm of rice soils are frequently more abundant than propagules contained in the recommended dose of soil-based inoculum for application.

Results show that the absence of development of N₂-fixing blooms of BGA in wetland soils is rather due to environmental or agronomic limiting factor than the absence of N₂-fixing strains.

PUBLICATIONS AND REPORTS :

Roger PA, Grant IF, Reddy PM (1985) Blue-green algae in India : a trip report. The International Rice Research Institute , Po Box 933, Manila, Philippines. 93 pp.

Roger PA, Santiago-Ardales S, Watanabe I (1986) Nitrogen-fixing blue-green algae in rice soils of northern Luzon (Philippines). *Phil. Agric.* 69:589-598.

Roger PA, Voggesberger M, Margraf J (1986) Nitrogen fixing phototrophic organisms in Ifugao rice terraces (Philippines). *Phil. Agric.* 69:599-609.

Roger PA, Santiago-Ardales S, Reddy P M, Watanabe I (1987) The abundance of heterocystous blue-green algae in rice soils and inocula used for application in rice fields. *Biol Fert Soils* 5: 98-105.

WORK PLAN:

To complete this study, additional observations are needed to establish if long periods of dry fallow, succession of wetland and dryland cultures, and straw burning may cause a decrease in indigenous populations of N₂-fixing BGA sufficiently marked to justify special attention, with regard to BGA inoculation, to agrosystems where such practices are used .

Research activity summary

ESTABLISHMENT OF INOCULATED BGA IN RICE SOILS

GENERAL INFORMATION:

Project : Contribution of the photosynthetic aquatic biomass to nitrogen fertility of rice soils through nitrogen fixation and nutrient recycling.

Year started: 1984

Expected year of completion: 1990

Personnel:

Research project leader: P. A. Roger

IRRI Soil Microbiology personnel:

Susan Santiago Ardales (RA)

Collaborators:

Other IRRI departments involved: None

Outside of IRRI:

ORSTOM (France),

University of Florida (Dr Shanmugham)

Protocols of agreement:

IRRI/ORSTOM (June 1989 - June 1991)

University of Florida (Dec 1988 - Dec 1990)

DESCRIPTION AND GOAL:

The main objective is to establish if foreign strains of BGA inoculated in a soil can establish and what are the factors responsible for their establishment.

The research project was initiated in 1984 as part of the IRRI/ORSTOM project on BGA. First experiments were conducted in 1984, together with a study of grazing with I. F. Grant (Boyce Thompson Institute). Further studies in microplots were conducted in 1986-87 in collaboration with P. M. Reddy.

In 1988, the University of Florida has proposed IRRI a collaborative research activity on the study of the establishment of nitrogenase derepressed mutants of BGA in rice soils. Such mutants excrete a major part of the nitrogen they fix and may be of special interest for inoculation. This activity will last 2 years (1989-90).

METHODS:

Visual and microscopic observations, biomass measurements, counts and recovery tests by plating on media with low or high selectivity, and acetylene reducing activity measurements are used in studies conducted in microplots and *in situ*.

RESULTS:

Microplot and field experiments have shown that nonindigenous strains do not easily establish in a field. When practices ensuring the formation of a BGA bloom were applied in various soils inoculated with nonindigenous strains, the bloom was most frequently composed of indigenous strains.

In field experiments, no foreign strain established.

In microplot experiments, foreign strain inoculated as dry soil-based inoculum at a level compatible with agronomic utilization rarely established, while grazer control and P application permitted in most cases the development of N₂-fixing blooms of indigenous BGA. Among combinations of 5 foreign strains and 5 soils only one (*Aulosira fertilissima* x alkaline soil) produced a bloom of inoculated BGA. The persistence of the inoculated strains in the various soils was more related to the nature of the strains than to that of the soil.

When large quantities of foreign strains were inoculated in soil microplots, either as fresh culture or, in some cases, as dry powdered cultures, and if grazers were controlled, it was possible to produce blooms of foreign strains, but the quantity of inoculum used was too high to allow agronomic utilization.

PUBLICATIONS AND REPORTS :

Grant IF, Roger PA, Watanabe I (1985) Effect of grazer regulation and algal inoculation on photodependent N₂ fixation in a wetland rice field. Biol. Fertil. Soils 1: 61-72.

Reddy PM, Roger PA (1986) Studies on algal inoculation in microplots and field experiments. IRRI Saturday Seminar, April 26 1986, 34 pp.

Reddy PM, Roger PA (1988) Dynamics of algal populations and acetylene reducing activity in five soils inoculated with blue-green algae. Biol Fert Soils 6:14-21.

WORK PLAN:

The first step to study the fate of a NH₄-excreting strain inoculated into soil will be a screening in microplots of a wide range of factors (soil type, N and P fertilization, grazer control), taking advantage of the relative easiness for studying the fate of the mutant strain by semi-quantitative plating on a very selective MSX medium.

The experiments will also be used to study the effect of P fertilization on indigenous BGA biomass production, P accumulation in algal biomass, and the ratio between P applied and N fixed by BGA in inoculum production plots.

Establishment of field studies will depend upon the results of microplot experiments.

Research activity summary

EFFECT OF BLUE-GREEN ALGAL INOCULATION ON RICE YIELD

GENERAL INFORMATION:

Project : Contribution of the photosynthetic aquatic biomass to nitrogen fertility of rice soils through nitrogen fixation and nutrient recycling.

Year started: 1984

Expected year of completion: 1991

Personnel:

Research project leader: P.A.Roger

IRRI Soil Microbiology personnel:

Susan Santiago Ardales (RA)

Ruby Remulla Jimenez (RA)

Collaborators:

Other IRRI departments involved: none

Outside of IRRI: ORSTOM (France). University of the Philippines at Los Baños (Dr Martinez). Institute of Water and Soil, Egypt (Dr Ghazal).

International Institute for Rural Reconstruction, Cavite (IIRR) (Dr J.

Gonzalves), WARDA, (Senegal) (Dr Diara)

Potential collaborators:

Thai institute of scientific and technological research (Dr Antarikanonda).

Protocol of agreement: IRRI/ORSTOM (June 1989 - June 1991)

DESCRIPTION AND GOAL:

To study the effect of algal inoculation on photodependant biological N₂ fixation and rice yield. To understand factors involved in the increase in yield that have been reported in the literature, after algal inoculation.

METHODS:

Direct measurements of BGA biomass, plate counts, ARA measurements, yield and yield components measurements. Compilation and analysis of bibliographic data.

RESULTS:

A thorough review of the literature in the book " BGA and rice ", of which more than 3,000 copies have been distributed, showed adequate evidence to establish that BGA significantly contributed to the nitrogen required by the rice crop; however results varied considerably. An updated analysis of the literature in 1988, compiling all available published papers and reports, shows an average increase in yield of 340 kg grain/ha over 634 inoculation experiments. Data are very variable and exhibit some surprising extreme values (Average increase in yield : 337 kg/ha; maximum: 3700 kg/ha; minimum: -1280 kg/ha; median: 250 kg/ha; standard deviation 398; C.V. 118%). Less than 30% of the differences are statistically significant.

Six experiments at IRRI, including three consecutive experiments in an experimental design of 65 plots, and one experiment outside IRRI have shown no statistically significant increase in yield due to algal inoculation, but some significant effects on ARA and grain protein content were recorded.

Some agricultural practices applied to favor the establishment of inoculated BGA were found to favor ARA and growth of indigenous BGA. Those include N fertilizer deep-placement, grazer control, and phosphorus application.

Numerous ARA and biomass measurements we performed during the three last years shows that BGA have a N potential of about 30 kg N ha⁻¹ crop⁻¹.

PUBLICATIONS AND REPORTS :

- Grant IF, Roger PA, Watanabe I (1985) Effect of grazer regulation and algal inoculation on photodependent N₂ fixation in a wetland rice field. *Biol. Fertil. Soils* 1: 61-72.
- Roger PA, Grant IF, Reddy PM (1985) Blue-green algae in India : a trip report. The International Rice Research Institute , Po Box 933, Manila, Philippines. 93 pp.
- Grant IF, Roger PA, Watanabe I (1986) Ecosystem manipulation for increasing biological N₂ fixation by blue-green algae (Cyanobacteria) in lowland rice fields. *Biol. Agric. Hort.* 3 : 299-315.
- Reddy PM, Roger PA (1986) Studies on algal inoculation in microplots and field experiments. IRRI Saturday Seminar, April 26 1986, 34 pp.
- Reddy PM, Roger PA, Ventura W, Watanabe I (1986) Blue-green algal treatment had no significant effect on rice yield in an acidic wetland soil. *Phil. Agri.* 69:629-632.
- Roger PA, Watanabe I (1986) Technologies for utilizing biological nitrogen fixation in lowland rice : potentialities, current usage, and limiting factors. *Fert. Res.* 9 : 39-77.
- Roger PA, Reddy PM, Remulla-Jimenez R (1988) Photodependant acetylene reducing activity (ARA) in ricefields under various fertilizer and biofertilizer management. p 827 in *Nitrogen fixation: hundred years after*. Bothe et al. eds, Gustav Fischer ed..
- Roger PA (1989) Blue-green algae in agriculture. pp 000-000 in Dart P, Dawson JO eds, *Microorganisms that promote plant productivity*. M Nijhoff/W Junk pub. (in press)
- Roger PA (198-) Reconsidering the utilization of blue-green algae in wetland rice cultivation. Paper presented at the International Symposium " Biological N₂ fixation associated with rice production and rice culture" Cuttack, India, December 26 1988-January 2nd 1989 (in press).
- Roger PA, Watanabe I (198-) Recent studies on free-living blue-green algae and Azolla at the International Rice Research Institute. Paper presented at the 2nd Conference of the African Association of Biological N₂ Fixation. Cairo, Egypt. Dec. 1986 (in press).

WORK PLAN:

Our studies shows that algal inoculation with foreign strains has a moderate potential and is probably not useful in many ricefields (see also "Occurrence of BGA in rice soils" and "Establishment of inoculated BGA in rice soils"). Additional information on environments responsive to algal management/inoculation is still needed. This requires multilocation trials and does not need sophisticated methods but need time consuming replicated measurements and a manpower not available in our department. IRRI has no comparative advantage for conducting inoculation studies which can better be handled by national programs with, if required, a methodological support by IRRI. We have collaborative projects with the Philippines, Egypt, IIRR, and WARDA.

ROLE OF ALGAE IN N LOSS BY AMMONIA VOLATILIZATION

GENERAL INFORMATION:

Project : Contribution of the photosynthetic aquatic biomass to nitrogen fertility of rice soils through nitrogen fixation and nutrient recycling.

Year started: 1986

Expected year of completion: 1989

Personnel:

Research project leader: S K De Datta/IFDC scientists

IRRI Soil Microbiology personnel: P. A. Roger, R. Remulla-Jimenez (RA)

Collaborators:

Other IRRI departments involved: Agronomy

Outside of IRRI: ORSTOM (France), IFDC

Protocol of agreement: IRRI/ORSTOM (June 1989 - June 1991)

DESCRIPTION AND GOAL:

Estimation of algal populations in experiments conducted by the IFDC/IRRI program on nitrogen losses by ammonia volatilization.

METHODS:

Microscopic examinations, plate counts, ARA and dissolved oxygen measurements

RESULTS:

Small algal biomasses of a few hundred kg fresh weight ha⁻¹ are sufficient to increase floodwater pH to values causing very significant losses of ammonia by volatilization. On the average, a photosynthetic activity sufficient to saturate the floodwater to a value of 20 ppm O₂ increases floodwater pH by 2.25 units.

Application of algicide terbutryn to control algal growth at the beginning of the crop reduces losses by volatilization, has no long-term effect on total algal populations, but might affect photodependant nitrogen fixation. However the effect on rice yield is very moderate (+ 3-5%), and the method might have little economic feasibility.

PUBLICATIONS AND REPORTS :

Fillery IFG, Roger PA, De Datta SK (1986) Ammonia volatilization from nitrogen sources applied to rice fields, Part 2 : Floodwater properties and submerged photosynthetic biomass. Soil Sci. Soc Am. J. 50 : 86-91.

Muirhead WA, De Datta SK, Roger PA, Gusto RM (198*) Effect of algicides in reducing NH₃ loss from urea applied to transplanted rice. 1. Floodwater chemistry and biota. Fert. Research (Submitted).

WORK PLAN:

No specific experiments are planned on this topic. Measurements and observations are conducted upon request by interested scientists.

Research activity summary

**CONTRIBUTION OF THE PHOTOSYNTHETIC AQUATIC BIOMASS
TO SOIL MICROBIAL BIOMASS
AND AVAILABLE N IN WETLAND RICE.**

GENERAL INFORMATION:

Project : Contribution of the photosynthetic aquatic biomass to nitrogen fertility of rice soils through nitrogen fixation and nutrient recycling.

Year started: 1988

Expected year of completion: 1991

Personnel:

Research project leaders: P.A.Roger/I. Watanabe

IRRI Soil Microbiology personnel:

Ruby Remulla Jimenez (RA)

Collaborators:

Other IRRI departments involved: Soils

Outside of IRRI: ORSTOM (France)

Protocol of agreement: IRRI/ORSTOM (June 1989 - June 1991)

DESCRIPTION AND GOAL:

This research activity is part of a larger study of the role of microbial biomass in contributing to the nitrogen fertility of wetland soils.

Goals related to the study of the photosynthetic aquatic biomass are:

- to develop a standardized methodology to estimate microbial biomass in the oxidized and reduced soil layers.
- to estimate nutrient turnover rate of the phytoplankton
- to estimate the nutrient contribution of the photosynthetic aquatic biomass to the ricefield ecosystem and its role in replenishing soil microbial biomass.

METHODS:

Estimation of standing crops of the photosynthetic aquatic biomass and chemical analysis of the components. ARA measurements. Estimation of the productivity through 24 h monitoring of O₂ and temperature in the floodwater.

For estimating microbial biomass, the chloroform fumigation method, heat treatment in microwave oven and freezing/thawing methods are being tested.

RESULTS:

We have established a long term experimental design of 65 plots (16 m² each) under 3 nitrogen fertilizer managements (none, split, deep placed), 2 photosynthetic aquatic biomass managements (incorporation, removal), and with planted, dark control microplots, of 1 m² included in each of the

65 plots. This design has been used to estimate standing crops of algae and weeds in terms of biomass and composition (C, N, P) and the contribution of the photosynthetic aquatic biomass to soil microbial biomass.

Average ARA during a crop cycle has been estimated in each of the plots for three crops. Average value during a crop is about $200 \mu\text{mol C}_2\text{H}_2/\text{m}^2$ per h in plots where a significant ARA developed.

Estimates of floodwater productivity indicates that values around $2 \text{ g C}/\text{m}^2$ per day may occur in plots where algal growth is visible to the naked eye.

The absence of photosynthetic activity in the ricefield floodwater (dark controls) caused a decrease in the soil microbial biomass by about one fourth after one year. First results show that the photosynthetic aquatic biomass constitute a major component and source of nutrients for the microbial biomass in the upper soil layer and a significant source of nutrients for the microbial biomass in the deeper soil.

PUBLICATIONS AND REPORTS :

Roger PA, Santiago-Ardales S, Watanabe I (1985) Unicellular mucilaginous blue-green algae: impressive blooms but deceptive biofertilizers. *Int. Rice Res. News.* 10: 27-28.

Roger PA (1987) Effect of algae and aquatic macrophytes on nitrogen dynamics in wetland rice fields. pp 783-793 in Vol 6 of the Transactions of the 13th Congress of the International Society of Soil Science Hamburg, Germany, August 13-21 1986 .

Roger PA, Grant IF, Reddy PM, Watanabe (1987) The photosynthetic biomass in wetland rice fields and its effect on nitrogen dynamics. pp 43-68 in Efficiency of nitrogen fertilizers for rice. The International Rice Research Institute, Po Box 933, Manila, Philippines.

Watanabe I, De Datta SK, Roger PA (1988) Nitrogen cycling in wetland rice soils. pp 239-256 in Advances in Nitrogen Cycling in Agricultural Ecosystems. Wilson JR ed, C.A.B. International ed.

Roger PA, Kurihara Y (1988) Floodwater Biology of Tropical Wetland Ricefields. Pages 275-300 in Proceedings of the First International Symposium on Paddy Soil Fertility, 6-13 Dec 1988, ChiangMai, Thailand.

WORK PLAN:

We will study the variations of the soil microbial biomass in relation with the photosynthetic aquatic biomass in an already established 65 plot experimental design.

We will conduct field surveys in farmers fields (see "Interactions between agrochemicals, photosynthetic aquatic biomass, soil and water fauna, and soil fertility")

Research activity summary

INTERACTIONS BETWEEN AGROCHEMICALS, PHOTOSYNTHETIC AQUATIC BIOMASS, SOIL AND WATER FAUNA, AND SOIL FERTILITY

GENERAL INFORMATION:

Project : Contribution of the photosynthetic aquatic biomass to nitrogen fertility of rice soils through nitrogen fixation and nutrient recycling.

Year started: 1989

Expected year of completion: 1991
(the project might get further extension by ODNRI)

Personnel:

Research project leader: P. A. Roger (IRRI), I.F. Grant (ODNRI)

IRRI Soil Microbiology personnel:

Bert Official (temporary research aide)

Collaborators:

Other IRRI departments involved:

Soils (H.U. Neue)

Plant Pathology (Nematodes) (J.C. Prot),

Outside of IRRI:

ORSTOM (France)

Ian Simpson (Collaborative research Fellow, ODNRI)

Protocol of agreement:

IRRI/ORSTOM (June 1989 - June 1991)

IRRI/ODNRI (January 1989-January 1990, renewable for one year)

DESCRIPTION AND GOAL:

Tripartite collaborative research activity (IRRI, ORSTOM, ODNRI) to study:

- 1) how agrochemical utilization affect the productivity of the photosynthetic aquatic biomass (C and N fixation) and the soil and water fauna, especially in their role of nutrient recycling, and
- 2) how the effects on these two key components of the ricefield ecosystem affect the microbial biomass in soil.

METHODS:

Classical methods of soil microbiology and hydrobiology. Effects on soil fertility are estimated through microbial biomass measurements.

RESULTS:

Preliminary surveys in the IRRI farm have shown significant differences in soil fauna among plots with different organic matter management. The preserved quantitative samples were brought to ODNRI in August 88 for identification. Most frequent soil invertebrates in wetland rice soils are

tubificids worms. Their populations, estimated in a preliminary survey at the end of the dry season 1988-89 in 23 farmer fields, range from nil to about 15 000 m⁻². This corresponds to biomasses ranging from 0 to 300 kg ha⁻¹.

PUBLICATIONS AND REPORTS :

- Roger PA, Watanabe I (1988) Some microbiological and hydrobiological environmental effects of new rice technology. Paper presented at the Workshop " Expert consultation for identifying issues and developing methodologies for measuring the environmental and socioeconomic and the interactive impacts of new rice technology" IRRI, Los Baños. December 1-4, 1987.
- Roger PA, Bhuyian SI (1989) Ricefield ecosystem management and its Impact on Disease Vector. Paper presented at the Seminar on Environmental Management For Vector Control 18-19 April 1989, WHO/FAO/ UNEP Panel of Experts on Environmental Management. Moscow, USSR.

WORK PLAN:

A preliminary study is conducted at IRRI in a long term experimental design of 65 plots where floodwater properties, photosynthetic aquatic biomass productivity and composition, soil and water fauna, and soil microbial biomass are monitored.

A survey of the population of tubificidae with regard to fertilizer management is being conducted in the IRRI farm. Another survey is made in farmers fields with known history, within the project on "Cost of pesticides use" which involves the collaboration of several IRRI departments and UPLB. The same experimental plots and farmer fields are used for a survey of nematods populations (Dr J. C. Prot).

Both studies will be continued in the wet season of 1989 and in 1990.

Greenhouse experiments, in collaboration with the Soils Department, will be conducted in the facilities ("artificial ricefield" with controlled temperature) of this department, to complete the study.