Nitrogen Cycling In South-East Asian Wet Monsoonal Ecosystems

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Proceedings of a Regional Workshop arranged by the SCOPE/UNEP International Nitrogen Unit of the Royal Swedish Academy of Sciences and the Chiang Mai University, sponsored by the Man and the Biosphere (MAB) programme of UNESCO and the National Research Council of Thailand, and held at Chiang Mai, Thailand, 5–10 November, 1979.

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AUSTRALIAN ACADEMY OF SCIENCE,
CANBERRA 1981

Published by
The Australian Academy of Science,
P.O. Box 783, Canberra City, 2601

ISBN 0 85847 097 7

Suggested citation:

Author's name. 1981. Title of paper.—In: Wetselaar, R., Simpson, J. R., & Rosswall, T. (eds) Nitrogen Cycling in South-East Asian Wet Monsoonal Ecosystems, pp. 000–000. Canberra: Australian Academy of Science, 1981.

Printed at Griffin Press Limited,
Netley, South Australia

PREFACE

The Scientific Committee on Problems of the Environment (SCOPE) of the International Council of Scientific Unions, together with the United Nations Environmental Program (UNEP), established the SCOPE/UNEP International Nitrogen Unit in 1978 at the Royal Swedish Academy of Sciences in Stockholm. The two main objectives of this Unit were to collect and evaluate existing knowledge of the biogeochemical nitrogen cycles in major regions and ecosystems throughout the world and to draw attention to regional environmental problems related to these cycles.

As part of the former objective, an international workshop was held in Sweden in 1979 [F.E. Clark & T. Rosswall (eds.) 1981. Terrestrial Nitrogen Cycles. Processes, Ecosystem Strategies and Management Impact. - Ecol. Bull. (Stockholm) 33]. With respect to the regional objective, three workshops have been held. The first one was in West Africa in 1978 [T. Rosswall (ed.) 1980. Nitrogen Cycling in West African Ecosystems. Proceedings of a Workshop, Ibadan, Nigeria, December 1978]. The second one was in south-east Asia at Chiang Mai, and the third one was in Latin America [Robertson, G.P., Herrera, R., Sylvester-Bradley, R. & Rosswall, T. (eds.) 1982. Nitrogen Cycling in Ecosystems in Latin America and the Caribbean. Proceedings of a Workshop, Cali, Colombia, March 1981].

The south-east Asian meeting was held in November 1979 in the Central Library of the Chiang Mai University, Chiang Mai, Thailand. Most of the papers presented at this workshop are included in these Proceedings. The fact that half of these are related to rice, emphasizes the importance of this food crop in the region. The paper by Watanabe *et al.* is listed first, as it gives a comprehensive review of existing data on the different aspects of nitrogen cycling in wetland rice. The following eight papers reflect the importance of dinitrogen fixation processes in a paddy field, although later papers stress the need for nitrogen fertilizers if high grain yields are to be achieved.

The second half of the papers covers, appropriately, a wide variety of ecosystems such as forests, mangrove, rubber and oil palm. In addition, attention is given to nitrogen cycling in a catchment context, environmental problems associated with terrestrial nitrogen transformations, methods for studying nitrogen cycle processes under field conditions, and a modelling approach to nitrogen exchanges. The importance of nitrogen input *via* precipitation is underlined by a bibliography on this subject.

The Work Groups covered the four areas of Irrigated Wetland Rice, Forests and Plantation Crops, Catchments, and Shifting Cultivation. In addition, one group specifically addressed itself to how the Man and the Biosphere (MAB) programme of UNESCO could contribute in the area of the biogeochemical nitrogen cycle. Nearly half of the participants joined the Work Group on the first area, reflecting again the importance of the rice crop. This Group was the only one that managed to present N inputs and outputs in a tabulated form. All Work Groups' Reports point out the problems associated with obtaining reliable data on nitrogen balance sheets, and all list the research priorities needed to overcome these problems.

Because the number of participants of the Workshop had to be restricted, not all study areas related to nitrogen cycling in the ecosystems of the region concerned could be covered adequately. Only one paper, by Chee Yan Kuan & Devendra, dealt with the important role of animals in an ecosystem, in this case a rubber plantation. Perhaps, this gap is a reflection of the nature of the region, wherein the nitrogen cycles of the ecosystems involving animals are, in general, a very complex mixture of cycles concerning man, animal, food, and feed. Other areas that might need more attention are food crops other than rice, and the role of legumes in crop rotations.

In spite of these shortcomings, these Proceedings are important, because for the first time a collection of papers has been produced that reflects our current knowledge of nitrogen cycling in south-east Asian ecosystems, and indicates research priorities through the Work Group Reports. This book is meant as a catalyst for individual scientists concerned with nitrogen cycling in the natural and managed ecosystems of the region, and as an encouragement to such scientists to make contact with each other and to assist their cooperation towards a common goal.

The actual workshop and the publication of the proceedings were financially supported by UNEP, SCOPE of the International Council of Scientific Unions (ICSU) and the MAB programme. We are particularly indebted to the National Research Council of Thailand for its co-sponsoring of the Workshop, to the Chiang Mai University, especially Professor Manu Seetisarn of the Faculty of Agriculture, for co-sponsoring the Workshop and for making its staff and facilities available at such a generous level, and to the Australian team of the Thai-Australian Highland Agricultural Project for their help during the preparatory phase, and above all to Dr Niwat Hirunburana of the Faculty of Agriculture of the Chiang Mai University, who was in charge of the overall preparation for, and organization of, the Workshop and its associated social events. Without Dr Niwat, this Workshop could not have been so successful. We are

also indebted to CSIRO's Division of Land Use Research in Canberra for its administrative support and assistance with postage during the preparation of the proceedings.

Personally, I should like to thank my colleague and co-editor Dr J.R. Simpson for his continuous editorial support, my friend and co-editor Professor T. Rosswall, who made it all possible in the first place, for his editorial cooperation in spite of all other pressures, and Mrs A. Clugston for her highly competent and efficient typing of all manuscripts.

We will all remember the warm hospitality, kindness and charm of our Thai colleagues and of the people of Chiang Mai in general, and all are still grateful to the Ping river for having kept our burning candles afloat for so long.

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WELCOME ADDRESS

Professor Dr Tawan Kangwanpong

Rector of the Chiang Mai University, Chiang Mai, Thailand

Your Excellency, Deputy Minister of Agriculture and Cooperatives,
Distinguished Participants and Observers,
Honourable Guests,
Ladies and Gentlemen,

It is a great pleasure and privilege for me to welcome you all to Chiang Mai University, the first regional university of Thailand, and to this beautiful and ancient city of Chiang Mai. In my capacity as rector, I feel greatly honoured that this university has been chosen as a venue for this important meeting.

Chiang Mai University has grown from three faculties, with some 200 students in 1964, to 11 faculties and a graduate school, with more than 8 000 students now. I am especially pleased that this academic institution is able to take part in this regional workshop.

As I understand that your workshop covers a wide range of topics relating to the nitrogen cycle in different ecosystems, SCOPE and UNEP must be commended on their leadership in providing a timely needed study programme in this area. As you may already know, the success of agriculture after World War II has depended on the availability of cheap and abundant energy and fertilizer. But fertilizer, especially nitrogenous fertilizer, is no longer cheap and abundant. In this situation, an increasing use of large quantities of nitrogenous fertilizer assumes great importance. This workshop has provided a forum for the interchange of ideas and experiences relating to the nitrogen cycle, and will surely generate further valuable ideas and research guidelines that will be beneficial to all. Although agricultural technology may be specific for local conditions, and thus not transferable, the scientific principles on which sound agricultural research is based are always transferable.

On behalf of the Chiang Mai University, I sincerely wish the workshop an unqualified success and wish you all that your stay in Chiang Mai will be a very pleasant and memorable one.

Thank you.

WELCOME ADDRESS

Dr. Thomas Rosswall

Project Coordinator, SCOPE/UNEP International Nitrogen Unit,
Royal Swedish Academy of Sciences, Stockholm, Sweden

Your Excellency, Deputy Minister of Agriculture and Cooperatives,
The Rector, Chiang Mai University, Professor Tawan,
Professor Manu,
Dear Colleages,

At the time of the 4th SCOPE General Assembly in Stockholm in June of this year, Dr. M.K. Tolba, Executive Director of United Nations Environment Programme (UNEP), and Professor G.F. White, President of the Scientific Committee on Problems of the Environment (SCOPE) of ICSU, issued a joint statement on global life support systems. They "stressed the fundamental scientific importance of understanding the biogeochemical cycles, which link and unify the major chemical and biological processes of the earth's surface and the atmosphere". They called for stronger support by governments and closer cooperation between governments, international agencies and researchers to stimulate a comprehensive research programme on biogeochemical cycles.

SCOPE and UNEP have collaborated with the Man and the Biosphere (MAB) programme of UNESCO concerning an international cooperative project on the global biogeochemical cycles of carbon, nitrogen, phosphorus and sulphur. As part of this programme, the SCOPE/UNEP International Nitrogen Unit has been established at the Royal Swedish Academy of Sciences. The unit has acted as a catalyst and hopefully stimulated further interest in and research on the biogeochemical nitrogen cycle. The unit was established at an appropriate time, because interest in nitrogen as a limiting factor for ecosystem productivity and as a possible environmental pollutant, had prompted a number of research projects and cooperative efforts. The unit has not conducted, planned or supervised any specific field- or bench-work. It has, however, concentrated on a critical evaluation of present knowledge on the nitrogen cycle, and to this effect an international symposium on terrestrial nitrogen cycles was arranged in September of this year (Clark, F.E. & Rosswall, T. (eds) Terrestrial Nitrogen Cycles. Ecol. Bull. (Stockholm) Vol. 33, 1981).

A major emphasis has also been placed on sub-tropical and tropical areas, and a first regional workshop was held in Nigeria in 1978. The present workshop is the second regional meeting and our attention is now turned to south-east Asia with its ecologically and economically important ecosystems. An impressive group of scientists is assembled here and it is our hope that this meeting will give an opportunity for researchers to present their own research work, critically evaluate present knowledge of natural and managed ecosystems in the region and that you will freely and constructively contribute with your expertise in the discussions.

This meeting has been made possible through a generous grant from UNEP, and is also financially supported by SCOPE. The meeting has been planned in close cooperation with the MAB secretariat in Paris. On behalf of the organizers and the participants I would like to express our gratitude for this support.

The National Research Council of Thailand and the University of Chiang Mai have generously offered to host the meeting at this beautiful campus of the University of Chiang Mai and put these excellent facilities at our disposal. I am sure that our hosts will make everything possible to make our stay here a memorable one.

Your Excellency, Ladies and Gentlemen, on behalf of SCOPE and UNEP I wish you very welcome to this regional workshop. We are here because we know that a detailed understanding of the behaviour of nitrogen in our environment is of utmost importance. Let us make sure that our discussions during the meeting and recommendations at the end will make our conclusions perfectly clear to those who are not able to be with us during this week. Our deliberations should take us closer to a better management of our nitrogen resources, which are vital for the well-being of mankind.

OPENING ADDRESS

H.E. Professor Rapee Sagarik

Deputy Minister of Agriculture and Cooperatives, Bangkok, Thailand

The Rector, Chiang Mai University, Professor Tawan,
Distinguished Participants and Observers,
Honourable Guests,
Ladies and Gentlemen,

It is an honour and a great pleasure for me to be invited to preside over the Opening Ceremony for this workshop. Let me take this opportunity to welcome you all to Thailand and wish you a pleasant stay in Chiang Mai.

The Royal Thai Government is greatly honoured and privileged that Thailand has been chosen as a venue for the workshop on "Nitrogen Cycling in South-East Asian Wet Monsoonal Ecosystems". The topic of your workshop has a very important bearing in our attempts to increase agricultural production and conserve natural resources of the region. This is especially true when the decline in resources, caused partly by population increase and by increasingly higher fertilizer costs, are making the difficult task of agricultural development even more formidable. Our society is therefore obliged to search for ways and means of reducing fertilizer inputs without sacrificing production substantially. In the past, in order to alleviate immediate problems, projects were often undertaken with limited prior research on their feasibility and ecological soundness. We are now aware that such undertakings can easily aggravate the problems they are intended to solve. Today, the tasks facing us are more complex, and if we are to avoid repeating costly mistakes, sound information based on proper research is needed.

Workshops such as this one are valuable in delineating problems in the light of previous experience as well as in giving guidelines for future studies. In planning for future research, we ought to keep in mind the feasibility of transferring the research findings to development schemes. We are often faced with a lack of adequate facilities and, to a lesser extent, of a sufficient number of trained researchers, for investigating all aspects of a complex theme, such as nitrogen cycling. These constraints can be partially alleviated with the cooperation of international, regional and national research institutes. In this way it is possible to avoid any unnecessary duplication and to benefit from each others experience. I would also venture to state that the facilities and conditions necessary to promote effective participation in research are often not available to the trained scientists in our countries. There is a need for facilities and incentives to commensurate with the training of our scientists if we wish to avoid being relegated to an observer position in research problems of importance in our region.

I am convinced that the SCOPE/UNEP units and other institutions can help in paving the way for research cooperation based on unity of purpose and the common good. May this workshop be a step towards this worthy goal. I am certain that if the spirit of cooperation for research and development is maintained in the region, support and assistance from outside the region will not be difficult to obtain.

Ladies and Gentlemen, I am pleased and honoured to be addressing this distinguished gathering, but since you have a long agenda before you, I shall not take more of your time. May I at this auspicious moment declare the workshop open and wish you every success in your deliberations.

Thank you.

NITROGEN CYCLING IN WETLAND RICE FIELDS IN SOUTH-EAST AND EAST ASIA

I. Watanabe[1], E.T. Craswell[2], and A.A. App[3]

[1] The International Rice Research Institute, Los Banos, Laguna, Philippines.

[2] The International Fertilizer Development Center, Muscle Shoals, Alabama, U.S.A.

[3] Boyce Thompson Institute, Cornell University, Ithaca, New York, 14853, U.S.A.

ABSTRACT

Soil submergence to grow wetland rice produces a dramatically different nitrogen cycle in a flooded soil-rice ecosystem. Many processes of the nitrogen cycle in this system cannot be measured directly because adequate techniques are not available.

The total nitrogen balance studies in long term-fertility trials reveal a net positive balance when nitrogen fertilizer is not applied. This positive balance varies from 20 to 70 kg N ha^{-1} per crop and is assumed to be due to biological nitrogen fixation. From the limited number of ^{15}N balance studies done in the field, total losses of fertilizer nitrogen range from 10 to 96% of added nitrogen.

In the total nitrogen balance method, possible nitrogen inputs by rain, irrigation water, ammonia absorption and others, are subtracted in order to estimate biological nitrogen fixation. This fixation ranges from 5 to 50 kg N ha^{-1} per crop. Although acetylene reduction is a useful tool for nitrogen fixation studies, the limited data from published field acetylene reduction assays do not account for the nitrogen gain estimated by nitrogen balance.

Nitrogen fixation appears to take place in fields where nitrogen fertilizer is applied.

The incorporation of rice straw would increase nitrogen gain by decreasing nitrogen removal and enhancing nitrogen fixation.

Nitrogen fertilizers are being used in increasing quantities for rice production. The amount of applied nitrogen lost depends largely on the fertilizer management. Surface applications in areas with poor water control are likely to induce large gaseous losses and surface runoff. Better techniques are needed for measuring gaseous losses in the field so that the relative importance of denitrification and ammonia volatilization can be determined.

Because about 60% of the nitrogen taken up by wetland rice comes from soil nitrogen, cultural practices to enhance soil nitrogen supply are of agricultural significance.

INTRODUCTION

Wetland rice is grown under flooded conditions during most of its growing season, thus creating a unique ecosystem in the field (Moorman & van Breemen, 1978). Soil submergence has a dramatic effect on the nitrogen cycle in this system, so it is appropriate to consider the wetland rice-flooded soil ecosystem separately from other agricultural ecosystems. Wetland rice (paddy) fields can be grouped into: (a) irrigated, (b) rainfed bunded and (c) rainfed unbunded (deep water). Unfortunately, although 75% of the paddy fields in south-east Asia are rainfed, most available information on nitrogen cycling and transformations have been obtained from irrigated fields.

BRIEF DESCRIPTION OF THE SYSTEM

Fig. 1 shows the nitrogen cycle in the wetland rice-flooded soil ecosystem. This ecosystem has five major components: (a) floodwater, (b) oxidized-surface soil, (c) reduced plough layer, (d) oxidized or partly oxidized subsoil beneath the ploughpan layer, and (e) rice plant and oxidative rice rhizosphere.

Nitrogen input (gain) sources are: (1) fertilizer, (2) dry and wet precipitation, (3) irrigation water, (4) seepage, (5) ammonia absorption, (6) seedlings, and (7) biological nitrogen fixation.

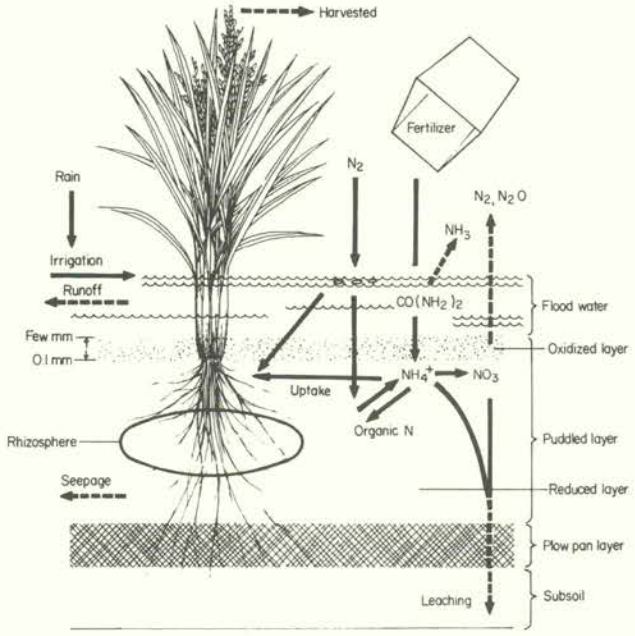

Fig. 1. Diagram of N cycling in soil-rice ecosystem.

Output (loss) pathways are: (1) harvest of grain and straw, (2) runoff, (3) seepage and leaching, (4) ammonia volatilization, and (5) denitrification as nitrous oxide (N_2O) and dinitrogen (N_2).

Chemical and biological immobilization of inorganic nitrogen in the soil nitrogen pool and the mineralization of soil nitrogen represent internal cycles. Nitrogen compounds in the rice canopy may be returned to the soil through leaching by dew and rain (Tanaka & Navasero, 1964); straw nitrogen may be returned if the straw is ploughed into the soil. Both can be regarded as internal cycles.

Many of the processes in Fig. 1 cannot be measured directly because adequate techniques are not available. Therefore, before considering the processes individually, we discuss information obtained using nitrogen balance techniques, namely, the total nitrogen balance, which has largely been used as an indirect measure of biological nitrogen fixation, and the ^{15}N balance technique which has been used to measure fertilizer nitrogen losses.

TOTAL NITROGEN BALANCE OF THE SYSTEM

One of the best ways to determine whether the system gains or loses nitrogen is to make a nitrogen balance calculation on data from long-term fertility trials. Most of such data originate from Japan, where long term fertility experiments have been conducted on most provincial agricultural experiment stations. Nevertheless we have little quantitative data on nitrogen balance in paddy fields (Koyama & App, 1979). Table 1 illustrates some nitrogen balance data, and the following conclusions may be drawn:

. In non nitrogen-fertilized plots, gains of nitrogen from 20 to 70 kg N ha^{-1} per rice crop were obtained (except for a peat soil at Sorachi);

. the addition of phosphorus plus potassium increased nitrogen gains;

. in non N-fertilized plots, more nitrogen was absorbed by wetland rice than by dryland crops; and,

. in most of the nitrogen fertilized treatments, net losses of nitrogen were obtained.

Nitrogen balance data, particularly data on soil nitrogen changes, are subject to many errors, because the total soil nitrogen constitutes such a large proportion of the total nitrogen in the system. A 10% error in soil total nitrogen analysis (mainly due to sampling errors) corresponds to 100-200 kg N ha^{-1}. However, most of the Japanese data on soil nitrogen appear to be significant because the experiments have run for such long periods.

Table 1. N-balance in long-term fertility experiments

Site	Cropping (yr^{-1})	Duration (yr)	Treatment	kg N ha^{-1} yr^{-1} Input	Soil change	Plant uptake	Balance
Aomori[a] Japan (41°N)	Wetland rice	21	PK	0	- 20	45	+ 25
			NPK	57	- 35	66	- 25
Kagawa[b] Japan (34°N)	Wetland rice and barley	21	PK	0	- 42	80(55)*	+ 38
			NPK	157	- 18	154(96)	- 21
Sorachi[c] Japan (45°N)	Wetland rice	12	PK	0	- 44	142	+ 98
			NPK	39	- 51	136	+ 46
Ishikawa[d] Japan (36°N)	Wetland	22	Unfertilized	0	- 34	53	+ 19
			PK	0	- 30	64	+ 34
			CaPK	0	- 34	72	+ 38
			NPKCa	100	-15	119	+ 4
Shiga[e] Japan (35°N)	Wetland rice and wheat	40	Unfertilized	0	- 1.7	41(30)	+ 39
			PK	0	- 13.1	67(51)	+ 55
			NPK	152	+ 2.2	112(74)	- 37
Los Banos[f] Philippines (14°N)	2 wetland rice	12	Unfertilized	0	+ 30	116	+146
Maligaya[f] Philippines (16°N)	2 wetland rice	8.5	Unfertilized	0	+ 30	91	+121
Chainat[g] Thailand (15° N)	2 wetland rice	2	- N	0	+ 47	58	+ 63
		2	+ N	240	+ 39	139	- 62
	Wetland rice and fallow	2	- N	0	+ 28	36	+ 57

[a] Koyama & App (1979); [b] Ando (1975); [c] Inatsu & Watanabe (1969); [d] Konishi & Seino (1961); [e] Takahashi (pers. comm.); [f] Koyama & App (1979); [g] Firth *et al.* (1973).

* Figures in parentheses are N uptake by rice grain.

Nitrogen balance experiments in the tropics have a limited value towards understanding the changes in soil nitrogen. In most nitrogen balance sheet calculations, soil nitrogen changes below the plough layer are not taken into consideration. Sekiya & Shiga (1977) estimated the contribution to paddy rice of nitrogen from soil below the plough layer by putting plastic sheets below this layer. The contribution was 22-30%, except for volcanic ash soil (5%). The high nitrogen gain in peat soils (Sorachi) appears to be from the underlying peat layer rich in organic matter.

Nitrogen uptake in the plots where nitrogen fertilizers have not been applied for a long period can give an approximation of the nitrogen input (natural supply), although soil nitrogen changes must be taken into consideration as shown in Table 1.

Table 2 gives some examples of results from long term fertility trials in Japan, Philippines and Thailand. Yanagisawa & Takahashi (1964) also gave the nitrogen uptake in some fertility trials in Japan; the average for 15 experiment stations was 64 kg N ha^{-1}. About 50 kg N ha^{-1} per crop must be gained to compensate for nitrogen losses through plant harvest (Table 2).

FERTILIZER N BALANCE

Of all the nitrogen inputs into the system, fertilizer nitrogen is the easiest to quantify. Its use in south-east Asia is increasing rapidly, particularly in those areas where new high-yielding rice varieties are grown. Nitrogen fertilizer production in the region is expected to increase from 354 000 t in 1978 to 2 755 000 t in 1983 (Stangel, 1979). Much of this increased production is intended for wetland rice and its impact on nitrogen cycling in this ecosystem will be dramatic. The fact that 85% of this fertilizer will be urea is also important, because much of our knowledge of the fate of nitrogen applied to rice is based on research done with ammonium sulphate (Craswell & Vlek, 1979a).

Table 2. Grain yield and N uptake in long term fertility plots in the absence of fertilizer nitrogen

Countries	Sites	Number of crops and (number of crops yr^{-1})	Treatment	Yield (t ha^{-1})	Per crop uptake (kg N ha^{-1})
Japan	Shiga[a]	40(1)	PK	3.5	51
	Miyagi[b]	33(1)	- NPK**	1.6	37
			PK	2.1	42
	Kagawa[c]	21(1)	- NPK	2.8	53
	Aomori[d]	21(1)	- NPK	2.3	45
	Sorachi[e] (peat)	12(1)	- NPK	5.6	142
	Konosu[f]	50(1)	- NPK	2.0	40*
	Hokkaido[f]	41(1)	- NPK	2.1	41*
			PK	2.3	46*
	Aichi[f]	41(1)	- NPK	1.4	29*
			PK	2.3	46*
	Hyogo[f]	26(1)	- NPK	3.2	63*
			PK	3.4	67*
	Saitama[f]	47(1)	- NPK	1.8	37*
			lime only	2.3	47*
	Fukui[f]	48(1)	- NPK	2.5	50*
			lime only	3.3	66*
				Average	49 (except 142)
Philippines	Los Banos[g]	24(2)	- NPK	3.9	78*
	Maligaya[g]	17(2)	- NPK	3.1	62*
Thailand	Chainat[h]	20(2)	- NPK	2.7	55*
			PK	3.1	62*
	Supanburi[h]	20(2)	- NPK	2.1	42*
			PK	2.5	50*
	Klong Luang[h] (acid sulfate)	20(2)	- NPK	0.8	16*
		20(2)	PK	1.7	34*
				Average	51 (except 16)

[a]Takahashi (pers. comm.); [b]Yomogita (1971); [c]Ando (1975); [d]Koyama & App (1979); [e]Inatsu & Watanabe (1969); [f]Yamaguchi (1979); [g]Koyama & App (1979); [h]Cholitkul *et al.* (1980).

*Estimated.

** Without N, P and K.

Statistics on currently-used rates of nitrogen applied to wetland rice are different to find. Stangel (1979) estimated that nitrogen use in the region varies from 4 kg N ha^{-1} in Burma to 115 kg N ha^{-1} in Malaysia, but these figures were calculated using the total area of arable land. In Japan, the average use for rice is 96 kg N ha^{-1} (Yatazawa, 1977).

The rate of fertilizer nitrogen in the soil-plant system can be traced using ^{15}N-labelled materials. By constructing a balance of ^{15}N in the system, the total gaseous losses can be estimated indirectly if the soil is sampled deep enough to take leaching into account. This method does not, however, identify the mechanisms of loss. Nevertheless, because suitable methods for estimating gaseous losses directly in the field are extremely difficult to use, or are simply not available, the ^{15}N balance technique is a valuable tool. Unfortunately, there are many problems associated with the ^{15}N technique in the field, and its application has therefore mostly been restricted to soil-plant systems in the greenhouse (Craswell & Vlek, 1979a).

Table 3. Field measurements of fertilizer N losses using the ^{15}N balance technique

Location and authors	Fertilizer Material	Application	N loss (% added ^{15}N)
Australia Wetselaar *et al.* (1973)	$(NH_4)_2SO_4$	Surface	51
		Deep	37
	$NaNO_3$	Surface	66
		Deep	96
USA Patrick & Reddy (1976)	$(NH_4)_2SO_4$	Broadcast	22
		Deep	25
Japan Koyama *et al.* (1977)	$(NH_4)_2SO_4$	Surface	18
	NH_4Cl	"	10
	$NH_4{}^*NO_3$	"	20
	$NH_4NO_3{}^*$	"	72
	$NH_4{}^*NO_3{}^*$	"	47
	$CO(NH_2)_2$	"	47

* Position of ^{15}N label.

The results of a few published studies of the ^{15}N balance of rice-soil systems in the field are shown in Table 3. Unfortunately, none of the experiments was conducted in southeast Asia. Total losses of fertilizer nitrogen range from 10 to 96%, depending on the form of the fertilizer and its mode of application. The extensive loss of nitrate-nitrogen shows the large potential for denitrification when soils containing fallow-accumulated nitrate are flooded (see further discussion in the section on denitrification). The only figure for urea loss is 47% (Koyama *et al.*, 1977). In a greenhouse experiment with ^{15}N-labelled urea fertilizers, Craswell & Vlek (1979b) recently measured losses of 30-50% of broadcast urea-nitrogen- but found losses of only 0-4% from supergranule urea-nitrogen placed 8 cm below the soil surface.

In general, the results in Table 3 show that applied nitrogen is subject to extensive losses, particularly when the fertilizer is broadcast. This suggests that these large losses - presumed to be caused by denitrification and ammonia volatilization - are associated with high concentrations of ammonium-nitrogen in the floodwater and surface soil layer. Mineralized native soil nitrogen reaches these zones only by the slow process of diffusion, and is therefore unlikely to be lost at such a high rate. This is important when using nitrogen balance methods to calculate biological nitrogen fixation rates in unfertilized fields (see below).

Table 4. Input of N by rain and irrigation water

Rain	2-15 kg N ha^{-1} yr^{-1} (Fried & Broeshart, 1967)
Irrigation water	Japan average 0.51 ppm (Yoshida, 1961) Japan average 1.1 ppm (Yatazawa, 1977) Thailand 0.26 ppm (Kobayashi, 1958) Assuming 1500 mm irrigation ha^{-1} $crop^{-1}$ → 4-8 kg N ha^{-1} crop-
Analytical examples	. Ibaragi, Japan (Takamura *et al.* 1977) Rain 12.8 kg N ha^{-1} yr^{-1} Irrigation 15.3 kg N ha^{-1} yr^{-1}
	. Los Banos, Philippines (Singh, 1978; IRRI unpublished) Rain 1-4 kg N ha^{-1} (half year)$^{-1}$ (1250 mm) Irrigation 5-4 kg N ha^{-1} $crop^{-1}$

NITROGEN GAINS

Biological nitrogen fixation is the most difficult source of nitrogen to quantify. Indirect estimates can, however, be made by quantifying other sources of nitrogen gain to the system. These will be discussed below.

Rain and irrigation

Table 4 gives an approximation of nitrogen input from rain and irrigation water, with an annual average of 10-20 kg N ha^{-1}.

Irrigation water in Japan contains a high nitrogen content probably due to pollution. Singh (1978) also observed a high nitrogen content in irrigation water in the Philippines due to pollution. In Japan, Yatazawa (1977) estimated the average nitrogen content in irrigation water as 1.1 ppm, assuming an average of 0.32 ppm nitrogen in non-polluted areas and a 6% area polluted by 12.5 ppm nitrogen.

Seedlings

The contribution will be 1-2 kg N ha^{-1} per crop if 2-3 week old seedlings are used.

Ammonia absorption from the atmosphere

Porter *et al.* (1972) gave evidence that corn plants absorb ammonia from the atmosphere. Based on their data of 0.07 µg nitrogen uptake day^{-1} cm^{-2} leaf area of corn seedlings at 1 ppm of ammonia in the atmosphere, we can estimate the contribution from atmospheric ammonia. At IRRI, 0.13 - 0.04 ppm ammonia nitrogen was recorded just above the rice canopy in fertilized plots. With an average leaf area index of 3.5 for 120 days, 0.35-0.1 kg N ha^{-1} might be absorbed by the rice plant canopy. This amount is negligible.

Approximations of the net annual nitrogen gain by biological nitrogen fixation are calculated from the difference between nitrogen gain and possible sources other than biological nitrogen fixation (Table 5).

Table 5. Calculated net, annual, biological nitrogen fixation, in the absence of fertilizer nitrogen

		Japan (1 rice crop)	Tropics (2 rice crops)
		(kg N ha^{-1})	
A.	Total nitrogen gain	40	120
B.	Inputs other than biological fixation		
	Rain and irrigation water	10-20	10-16
	Assuming 5-30% of gain originated from subsoil	2-12	6-36
	Seedlings	1	2
	Others	1	2
A-B (net nitrogen fixation)		26-6	100-64

Net and gross nitrogen-fixing rate

The nitrogen-fixing rate estimated by nitrogen balance is likely to be an under-estimate, because it only gives the net contribution to the system. To estimate the gross nitrogen fixing rate, it is presumed that the percentage loss of available soil nitrogen is much smaller than that of fertilizer nitrogen (see section on fertilizer nitrogen balance). Experiments with ^{15}N showed that $^{15}N_2$ once immobilized to soil organic nitrogen, becomes less susceptible to loss than inorganic ^{15}N (Yoshida & Padre, 1975; Ventura & Watanabe, 1978). Both publications indicate that about 90% of the immobilized nitrogen was recovered after one or two rice crops.

Nothing is known about the fate of nitrogen fixed by autotrophs living in floodwater. Algal nitrogen may be more susceptible to losses than nitrogen fixed by heterotrophs because algal nitrogen accumulates on the soil surface where conditions are conducive to nitrogen losses.

At present, we assume 20% of the biologically fixed nitrogen may be lost each year or during a rice crop.

Estimation of nitrogen fixing rate by acetylene reduction assays

In addition to the nitrogen balance Kjeldahl method, acetylene reduction techniques, $^{15}N_2$ gas feeding techniques and, ^{15}N dilution techniques (Rennie *et al.*, 1978) can all be used to estimate nitrogen fixing activities. Use of $^{15}N_2$ gas is more direct than others, but too expensive for field experiments. The acetylene reduction technique is the most feasible one for field assays.

Table 6. N_2 fixation measured by acetylene reduction assay and percentage contributions by floodwater, soil and rhizosphere

Author and place	Treatment	Fixation (kg N ha^{-1} $crop^{-1}$)	Contributions F	S	R*	Method
			(%)			
Matsuguchi (1979), Japan	NPK	11	0	60	40	*in vitro*
	NPK + compost	17	0	70	30	
	NPK + straw	19	5	80	15	
Panichsakpatana *et al.* (1979) Thailand	No fertilizer	1.1	<5	>85	<10	*in vitro*
	Green manure	3.8	<5	>85	<10	
Yoshida & Ancajas (1973), Philippines	Flooded wet season	60	5	95	NE	*in vitro*
	Flooded dry season	77	18	82	NE	
Watanabe *et al.* (1978b); IRRI, (unpublished), Philippines	- NPK** dry season	33	70	15	15	*in situ*
Watanabe *et al.* (1978a) Philippines	- NPK	14	65	NE	35	*in situ*
	+ NPK	7	30	NE	70	
	mean of dry and wet seasons					
Cholitkul *et al.* (1980), Thailand	Acid sulfate soil					
	- NPK	5	83	NE	17	*in situ*
	+ NPK	10	75	NE	25	
	Alluvial soil					
	- NPK	17	93	NE	7	
	+ PK	21	80	NE	20	

* F: Floodwater, S: Soil, R: Rhizosphere.
** Without N, P and K.
NE: Not estimated.

The disadvantages of this technique are reviewed by Watanabe & Cholitkul (1979). Although not quantitative, the technique is useful for comparing nitrogen fixing activities among various soils and nitrogen fixing agents. Table 6 summarizes examples of acetylene reduction activity (ARA) assays. The relative contributions of nitrogen fixing agents, algae (probably photosynthetic bacteria are partly included) in the floodwater and at the soil surface, and heterotrophs associated with rice and in soil are shown.

Even when a ratio of 3:1 nitrogen reduction/acetylene reduction ratio is assumed, the estimates are too low to account for the nitrogen gain estimated by nitrogen balance. Probably, the incomplete access of acetylene and the incomplete recovery of ethylene may partly explain low ARA values. Much improvement is needed in our field ARA assay techniques.

Nitrogen fixation and nitrogen fertilizers

Usually, in the laboratory, application of mineral nitrogen to the soil greatly depresses nitrogen fixation by heterotrophs (Knowles & Denike, 1974), photoautotrophs (Yoshida *et al.*, 1973), and bacteria associated with the rice plant (Balandreau *et al.*, 1975, Watanabe & Cabrera, 1979). The addition of small amounts of ammonium sometimes accelerates nitrogen fixation (Balandreau *et al.*, 1975). Under field conditions, the rice plant absorbs the applied nitrogen and thus, at later growth stages, the ammonium content in nitrogen-fertilized plots almost equals that in plots without fertilizer (Shiga & Ventura, 1976). The effect of nitrogen fertilizer on nitrogen fixation in the field may therefore not be as depressive as in the laboratory. Surveys at IRRI suggest that nitrogen fertilizer (60-100 kg N ha^{-1} $crop^{-1}$) does not depress heterotrophic nitrogen fixation in soil and in the rhizosphere. In contrast, nitrogen fertilizer is clearly depressive to phototrophic nitrogen fixation when applied on

the surface (Watanabe *et al.*, 1978a).

There is still a shortage of data on nitrogen fixation in nitrogen fertilized plots. Agronomic practices are needed which maintain nitrogen fixation in the presence of nitrogen fertilizers. Preliminary trials at IRRI suggest that the use of deep placement or slow release fertilizers may make nitrogen fertilizers compatible with phototrophic nitrogen fixation.

Enhancement of nitrogen fixation by additional sources

Straw incorporation. In most of south-east Asian countries, rice straw is used as fodder or burnt in the field and seldom incorporated (Tanaka, 1978). If rice straw is incorporated, we can expect nitrogen gains due to decreased crop removal of nitrogen (5 kg N t^{-1} rice) and nitrogen fixation associated with straw decomposition. Laboratory experiments with powdered rice straw showed a nitrogen fixation rate of 2-7 kg t^{-1} of straw (Watanabe, 1978b). Pot experiments with flooded rice done recently at IRRI showed that heterotrophic nitrogen fixation was enhanced by straw (Table 7).

Table 7. Effect of straw incorporation on nitrogen balance of flooded rice

Treatment[a]		Nitrogen balance ± S.E. (mg N per pot)*	N increase by straw (mg N (g straw)$^{-1}$)
Exposed to light	Straw Incorporated		
+	-	390 ± 69 b	
+	+	586 ± 31 a	2.2
-	-	162 ± 20 c	
-	+	519 ± 55 ab	4.0

[a] Three crops of IR28 were grown. Straw was added at 30 g (10 kg dry soil)$^{-1}$ crop^{-1}. Pots were either covered with black cloth or exposed to light.

* Means followed by a common letter not significantly different at 5% level. Balance significant at 1% level.

A. App, T. Santiago, C. Daez, W. Ventura, & I. Watanabe (unpublished data).

Assuming a similar efficiency of nitrogen fixation in the field (3 kg N t^{-1} straw), the incorporation of 3 tonnes of rice straw increases the gain by 24 kg nitrogen (5 x 3 + 3 x 3). Satisfactorily quantitative data to calculate the efficiency of straw addition on nitrogen balance in the field are still lacking, although soil nitrogen enrichment by straw in the field has been reported (Nishio *et al.*, 1978; Saito *et al.*, 1975).

Inoculation of blue-green algae. Watanabe *et al.* (1951) reported that the inoculation of blue-green algae in paddy rice fields in Japan added approximately 20 kg N ha^{-1} yr^{-1} and increased rice yield by 2-10%. Hirano (1958) reported that inoculated rice fields accumulated 26 kg N ha^{-1} $crop^{-1}$ more nitrogen on the surface soil than in the uninoculated control.

In the Philippines, algalization by *Nostoc* increased rice yields by 22% for three crops and nitrogen accumulated on the surface soil (Pantastico & Gonzales, 1976). More quantitative data are needed on the effects of algalization on the nitrogen balance.

Use of Azolla-Anabaena complex. This water fern can accumulate nitrogen fixed by symbiotic algae in its leaf cavities. Watanabe (1978a) reported that in 330 days, 22 crops of *Azolla* could accumulate 450 kg N ha^{-1}. The daily nitrogen fixing rate is 1.4 kg N ha^{-1}. In China and Vietnam, *Azolla* is widely used as a green manure for rice. The incorporation of *Azolla* before or after transplanting was confirmed to increase yield in the Philippines (Watanabe *et al.*, 1977), and Thailand (Sawatdee *et al.*, 1978).

The addition of phosphorus is essential for free living and symbiotic processes of autotrophic nitrogen fixation.

Heterotrophic nitrogen fixation associated with the rice plant

Heterotrophic nitrogen fixation associated with rice has been confirmed by acetylene reduction (Watanabe *et al.*, 1978b), $^{15}N_2$ gas incorporation (Ito *et al.*, 1980), and nitrogen balance (Koyama & App, 1979). However, we need more research to enable the further exploitation of this association.

NITROGEN LOSSES

Plant nitrogen removal

Nitrogen in rice grain is invariably removed from the ecosystem. The amount varies widely with the amounts of soil and fertilizer N available to the crop. After reviewing data from a large number of south-east Asian experiments, van Keulen (1977) found that at low levels of nitrogen supply, 1000 kg of unhusked rice contained 14 kg N. Using the mean country data for rice yields given by Stangel (1979), nitrogen removal in harvested grain in the region could, therefore, vary from 18 to 40 kg N ha^{-1} in Laos (1.2 t grain ha^{-1}) and Malaysia (2.8 t ha^{-1}), respectively.

While nitrogen in the rice roots re-cycles through the soil nitrogen pool, the straw nitrogen is lost in many areas of south-east Asia where stubble is burnt. In modern short stature varieties, the straw nitrogen content is assumed to be half the grain nitrogen, so the total nitrogen removal by grain and straw would be 27-60 kg N ha^{-1}.

Nitrogen loss in surface runoff

Only fertilizer nitrogen is likely to be lost substantially by surface runoff. This will become more significant as fertilizer usage increases. Takamura *et al.* (1977) found runoff losses ranging from 4 to 16 kg N ha^{-1} in Japan. In one of the few such studies conducted in south-east Asia, Singh (1978) found runoff losses as high as 5.6 kg N ha^{-1} from irrigated rice fields in the Philippines. The loss was highest with poor management of fertilizer nitrogen and water. The form of nitrogen applied was ammonium sulphate which, because of its greater adsorption by soil clay, is probably much less mobile than urea. Urea nitrogen, broadcast in the monsoon season on rice crops grown in low-lying areas with poor water control, is likely to be lost extensively. Further research will be needed to determine the potential environmental pollution from runoff losses.

Nitrogen loss in percolating water

Leaching losses of soil nitrogen from wetland rice soils are probably small because the predominant form of mineralized nitrogen is ammonium which would normally be adsorbed by the soil colloids. Nevertheless, Fe^{2+} and Mn^{2+} can displace NH_4^+ from the exchange complex of some waterlogged soils; 29 and 56 kg N ha^{-1} was leached in 12 weeks from unfertilized Luisiana and Maahas clays respectively when the percolation rate was 10 mm day^{-1} (IRRI, 1967). Fertilizer nitrogen additions are even more likely to exceed the adsorptive capacity of some light-textured soils and can therefore be lost if the water percolation rate is high. Losses are very site- and season-specific and only a few field studies have been reported. Koshino (1975), reviewing the field lysimeter experiments conducted in Japan between 1928 and 1971, reported applied nitrogen losses ranging from 3 to 25%. Using 4-115 kg N ha^{-1}, leaching losses would vary from nearly 0 to a maximum of 30 kg N ha^{-1} yr^{-1}. Losses might be even greater with expanded use of urea which is only weakly adsorbed by soil colloids. Furthermore, recent research under controlled conditions (Vlek *et al.*, 1980), shows that the deep placement of urea as a supergranule - a method designed to reduce volatilization losses of nitrogen - causes leaching losses of 10 to 90% of applied nitrogen at water percolation rates of 4 and 17 mm day^{-1} respectively. Percolation losses of broadcast urea were negligible under the same conditions.

Nitrification-denitrification losses

Losses of nitrogen can occur from continuously flooded soil due to nitrification in the surface-oxidized soil layer or rhizosphere and subsequent denitrification in the anaerobic soil layer (Shiori, 1941, see review by Mitsui, 1977). In intermittently flooded soil, nitrogen that is nitrified in aerobic soil is denitrified during subsequent waterlogging; this can lead to substantial losses of soil nitrogen in extreme conditions (Patrick & Wyatt, 1964). Ponnamperuma (1978) estimated that an average of 26 kg N ha^{-1} would be lost from wetland-rice soils in the Philippines when nitrate nitrogen, accumulated in the soil between harvesting and replanting, is denitrified during subsequent flooding.

Gaseous losses due to denitrification are extremely difficult to measure directly because N_2, the main product, already constitutes about 80% of the atmosphere. Japanese workers (Kosuge, 1979) using ^{15}N have, however, measured N_2 losses of 12 kg N ha^{-1} (96% of the added ^{15}N was recovered in the soil, plant, and $^{15}N_2$ gas). Further measurements of this kind are needed.

The other gaseous product of denitrification - N_2O - is relatively easy to measure. However, research in Australia (Denmead *et al.*, 1979) suggests that N_2O is by far the minor product of denitrification in flooded soils. Similar studies using their infra-red gas

analyzer technique in the Philippines at IRRI have also shown only small N_2O fluxes from flooded soils. Unfertilized rice soils evolve negligible quantities of N_2O (less than 0.3 g N ha^{-1} h^{-1}). Broadcast applications of urea or ammonium sulphate cause a delayed but brief flush of N_2O production which, with a strong diurnal fluctuation, peaks at 3.0 g N ha^{-1} h^{-1} in the middle of the day and lasts only 4-5 days. However, in contrast to the Australian results, additions of 100 kg NO_3-N ha^{-1} to a flooded IRRI Maahas clay (an aquic Tropudalf) caused a large N_2O flux of 72 g N ha^{-1} h^{-1} without the need for added organic substrate. Thus NO_3^--N which accumulates during periods when the rice-growing soils are aerobic may produce relatively large N_2O fluxes during subsequent flooding (Denmead *et al.*, 1979). Expanded use of irrigation and intensified cropping may therefore increase N_2O emissions from flooded soils in Asia by increasing the area of soil subject to intermittent flooding. On the other hand, these vast areas of paddy soils may have an important role as a sink for N_2O. Garcia (1975) has shown that rice soils have a large potential for N_2O reduction to N_2. Much more research is needed in this area which has very important environmental implications.

Ammonia volatilization

Traditionally, nitrification-denitrification was considered the major mechanism of nitrogen loss from waterlogged soils. Recently ammonia volatilization has been the subject of increasing research, particularly since D. Bouldin & B. Alimagno (unpublished data, IRRI) showed that algae can cause the pH of floodwater to rise to as high as pH 9-10 in the middle of the day. Table 8 shows some of the recent work on this loss mechanism. Extensive losses (0.3 to 60% of added nitrogen) have been reported. Unfortunately, the values obtained vary widely and depend too much upon the technique chosen by the particular workers. This is currently a controversial area of research.

Table 8. Ammonia volatilization losses from broadcast applications of fertilizers to flooded soils

Authors	Type of Experiment	Fertilizer	NH_3-N loss (% added N)
MacRae & Ancajas (1970)	Laboratory	$(NH_4)_2SO_4$	0.5 - 7
		$CO(NH_2)_2$	0.3 -19
Ventura & Yoshida (1977)	Field	$(NH_4)_2SO_4$	4
		$CO(NH_2)_2$	8
Bouldin & Alimagno* (1976)	Field	$(NH_4)_2SO_4$	30 - 60
Wetselaar *et al.* (1977)	Field	$(NH_4)_2SO_4$	5 - 17
Sahrawat (1978)	Field	$(NH_4)_2SO_4$	5 - 50
Mikkelsen *et al.* (1978)	Field	$(NH_4)_2SO_4$	1 - 16
		$CO(NH_2)_2$	4 - 19
Vlek & Craswell (1979)	Greenhouse-gas lysimeter	$(NH_4)_2SO_4$	1 - 15
		$CO(NH_2)_2$	10 - 50

* Unpublished report, IRRI.

The importance of ammonia volatilization as a loss mechanism should now be re-appraised using techniques which do not disturb the natural environment - e.g. Denmead *et al.* (1977). Getting reliable field data on ammonia loss will be increasingly important with the expanded use of urea. Theoretically, urea has a high potential for ammonia loss because its hydrolysis to ammonium carbonate increases floodwater alkalinity (Vlek & Stumpe, 1978). Furthermore, the broadcasting of urea directly into the floodwater - the method presently used by most Asian rice farmers - causes greater ammonia losses than modern methods such as the deep placement of urea supergranules or the use of sulfur-coated urea (Mikkelsen *et al.*, 1978, Vlek & Craswell, 1979).

Soil nitrogen can also be lost through ammonia volatilization, but Wetselaar *et al.* (1977) found a loss of only 1.5 kg N ha^{-1} during one cropping season. Ammonia can also be lost from senescing plant leaves. Wetselaar (1981) estimated these losses to be only 0.15 kg N ha^{-1} during one cropping period.

INTERNAL CYCLING OF NITROGEN

Immobilization

Inputs to the system of inorganic fertilizer nitrogen are subject to chemical or biological immobilization. About 20-30% of nitrogen fertilizer applied at the time of transplanting remains in the soil at harvest (Broadbent & Nakashima, 1970; Yoshida & Padre, 1975; Maeda & Onikura, 1976; Kai & Wada, 1979). Most of the residual nitrogen exists as organic or chemically fixed nitrogen and equals 20-30 ppm (Ventura & Watanabe, 1978). Algae growing in the floodwater may have a major role in immobilizing nitrogen from broadcast fertilizer applications (Mitsui, 1954; Craswell & Vlek, 1979b).

Mineralization

Mineralization of soil organic nitrogen normally exceeds immobilization under flooded conditions, although both processes take place simultaneously.

As already shown in Table 1, paddy rice absorbs more soil nitrogen than dryland crops. Table 9 shows the contribution of soil nitrogen to nitrogen uptake by paddy rice using ^{15}N fertilizer. In most cases, the uptake of soil N was 10-20 kg ha^{-1} higher in fertilized than in non-N fertilized plots (Koyama, 1975).

Table 9. Contribution of soil nitrogen to the nitrogen absorbed by plant, measured by ^{15}N

Place	Soil nitrogen uptake (kg N ha^{-1})	Soil nitrogen uptake (% total N uptake)	Grain yield (t ha^{-1})
Japan (Koyama, 1975)			
Gifu	75	71	4.6
Hokuriku	72	67	6.3
Honoshu (early rice)	51	47	6.2 (3.0)*
Konoshu (late rice)	50	51	6.1 (3.5)
Omachi	96	62	8.7
Misato	102	58	10.0
Nagano	108	58	10.5
Ageo	67	65	3.5
Thailand (Koyama, 1975)			
Bangkhen (1st crop)	52	61	4.8 (3.2)
Bangkhen (2nd crop)	43	57	3.2 (1.5)
Philippines (Yoshida & Padre, 1977)			
Los Banos	97 - 113	78 - 83	6.8 - 8.5 (5.9)
Los Banos	102 - 113	80-- 87	4.9 - 5.9 (4.5)

* Grain yield of control plot (no N applied).

Thus the application of chemical fertilizers appears to accelerate soil nitrogen decomposition and its uptake by the plant. This increased uptake of soil N due to fertilizer addition would probably be compensated by the immobilized residue of the fertilizer nitrogen.

Even when 20-30 kg N ha^{-1} from fertilizer nitrogen remains in the soil after a rice crop, the plants absorb 60-70 kg N ha^{-1} from soil nitrogen (Table 9). If no nitrogen input other than fertilizer N occurs, the soil nitrogen reserves become depleted. However, soil analyses after long term fertility trials show little difference in the soil N content between fertilized and unfertilized plots (Yamaguchi, 1979). This suggests that nitrogen gain, probably nitrogen fixation, must take place in nitrogen fertilized plots, as discussed already.

PERSPECTIVE

An increase in rice production is expected due to the introduction of improved rice varieties, use of chemical fertilizers, pesticides, and improved cultural practices. A simultaneous increase is expected in cropping intensity with changing cropping patterns. What is the impact of these innovations on the nitrogen cycle?

The pressure to increase food production allows little time to investigate the impact of modern methods and inputs on the environment of flooded rice fields. It is reasonable to assume that in most cases their use will not result in permanent damage. However, we cannot adequately assess the risk since the basic data on the nitrogen cycle in flooded rice are incomplete. We should not be complacent about this issue. The indiscriminate application of technology to agriculture in advanced nations has not always been environmentally sound and serious damage has occurred. The problem is compounded by the fragile economies in the developing world and a miscalculation in rice production could be very serious. The limited

manpower and support currently used for research to increase rice production in developing areas cannot be diverted from this urgent task.

From an agricultural viewpoint, an interest in enhancing biological nitrogen fixation and reducing fertilizer nitrogen losses is increasing. Our ignorance of the loss mechanisms operating in the nitrogen cycle of flooded rice fields and of how the relative magnitude of each mechanism is affected by soil, water, and fertilizer management is seriously retarding further progress in improving nitrogen fertilizer efficiency.

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STIMULATION OF PHOTOTROPHIC N_2 FIXATION IN PADDY FIELDS THROUGH RICE STRAW APPLICATION

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Effects of various methods of rice straw application to paddy fields on soil ARA (C_2H_2-reducing activity) were investigated. Effects of soil types, soil pretreatments and N applications on N_2-fixing populations and ARA in applied straw were also studied in the laboratory.

The stimulating effect of straw application at the rate of 8000 kg ha^{-1} was more marked on the photo-dependent ARA in the surface layer (0-1 cm) than on the photo-independent ARA. Topdressing of rice straw induced higher photo-dependent ARA and better growth of rice than deep placement. Photo-dependent ARA in the topdressed straw was 1000-fold higher than that in the soil fraction, and was enhanced by an application of N and herbicide.

Laboratory results from submerged soil incubations showed that top-dressed straw promoted growth of Rhodopseudomonas *in a 3-week incubation; thereafter there was a cyanobacterial bloom despite the straw pH being around 5.5. The dominant genera were* Anabaena, Cylindrospermum *and* Nostoc *in the absence of N, whereas with N, up to 100 ppm,* Calothrix *dominated. As they bloomed, photo-dependent ARA in the straw reached 2 to 8 μmole C_2H_4 g^{-1} h^{-1}. The maximal ARA was significantly higher in the alluvial soils than in the andosol. Soils which received rice straw annually showed a greater photo-dependent ARA in the currently topdressed straw than soils which did not receive straw annually. An addition of 100 ppm N to the former soils increased the straw ARA, while the addition to the latter gave an adverse effect; this indicates higher CO_2-producing and more N-tolerant N_2-fixing populations in the annually treated soils.*

INTRODUCTION

Recent data in Japan suggest that biological N_2 fixation plays an appreciable role in maintaining paddy soil fertility. The ^{15}N field data indicate that annual N_2 fixation can reach 60-70 kg ha^{-1} (Matsuguchi & Shimomura, 1977). Research on field techniques for promoting natural soil N supply is still needed in order to reduce the requirements for chemical fertilizers.

Soil N balance studies in paddy soils, annually treated with rice straw for several years, indicated considerable increases in soil N and N absorbed by rice. These were attributable to an increased activity in biological N_2 fixation (Matsuguchi & Shimomura, 1977). Although evidence that a straw application to water-logged soil promotes heterotrophic N_2 fixation has been reported (Barrow & Jenkinson, 1962; Rice & Paul, 1967; Kalininskaya *et al.*, 1973; Rajaramamohan-Rao, 1976; Yoneyama *et al.*, 1977), the effect of the application on phototrophic N_2 fixation is still uncertain.

Fogg (1949) found that glucose and organic acids stimulated the formation of cyanobacterial heterocysts. Compost application to water-logged soils stimulated growth and N_2 fixation of *Tolypothrix tenuis* (Konishi & Seino, 1961). A similar effect was obtained by rice planting and carbon dioxide aeration of water-logged soils (De & Sulaiman, 1950). These results indicate that rice straw applied to paddy soils should accelerate phototrophic N_2 fixation during its decomposition. In our previous experiments, a deep placement of rice straw to the paddy plough layer significantly stimulated both heterotrophic and phototrophic ARA (C_2H_2-reducing activity) particularly in the surface layer (0-1 cm), but caused a reduction in rice growth; this implies that top-dressing of straw is better than incorporation (Matsuguchi, 1979).

This paper presents recent results of field and laboratory experiments which investigate in more detail the effects of rice straw application on N_2 fixation in paddy fields as influenced by application methods, soil types, soil pretreatments and N_2-fixing populations present.

EFFECTS OF DEEP PLACEMENT AND TOPDRESSING OF RICE STRAW TO PADDY FIELD ON C_2H_2-REDUCING ACTIVITY IN THE PLOUGH LAYER

Materials and Methods

Three paddy plots, each 1.5 x 3 m, were prepared on Arakawa alluvial soil and also on Tochigi andosol. Plot A was a control without straw, plot B was topdressed with rice straw, and plot C received a deep placement of straw. Plot B and plot C received 8000 kg ha^{-1} of 5-cm-segment straw one month before transplanting (TP). One week before TP the straw in plot C was deep-placed by ploughing, then all the plots were flooded and puddled. Two days before TP, all the plots were treated with ammonium sulfate, superphosphate and potassium sulfate at the rate of 40 kg N, 87 kg P (Arakawa soil) or 262 kg P (Tochigi soil), and 83 kg K, each per hectare, and then puddled. Two days after TP, half the area of each plot was treated with a herbicide, MO (2,4,6-trichlorophenyl ether), at the commercial rate. At maximum tillering stage, each plot was supplemented with ammonium sulfate at 40 kg N ha^{-1}.

Measurement of ARA in the soil-rice root system. Four randomized core samples (7 cm diameter and 10 cm deep) of the soil-root system were taken from each plot at intervals during a rice growing season, and separated into three sections (0-1, 1-3 and 3-10 cm depth). Photodependent and photo-independent ARA's in each section were measured by the following procedure (Matsuguchi *et al.*, 1979). Ten-gram undisturbed samples were taken from each section and placed in 100-ml side-arm vacuum flasks together with 2 ml of distilled water. After being stoppered with gas-tight rubber caps, the flasks were attached to a gas-exchange apparatus equipped with a gas manifold, assay gas tank, manometer and vacuum pump. The flasks were then evacuated gradually, kept for one minute under 0.01 atm pressure, and de-evacuated gradually up to 1 atm pressure by filling with assay gas (10% C_2H_2 and 0.04% CO_2 in Ar). For the blank test, gas not containing C_2H_2 was used instead of the assay gas. This gas-exchange procedure was repeated four times. Then, the flasks were incubated in the light (6000 lx) or in the dark for three hours. After incubation, the flasks were strongly vibrated for 1 min, and 1-ml portions of the headspace gas were taken by gas-tight syringes to measure the amounts of C_2H_4 using a gas chromatograph (Shimazu GC-6AM with hydrogen flame ionization detector). All the assays were duplicated.

To measure ARA in the applied straw, rice root and soil fractions separately, duplicate samples of a large volume (20 x 30 cm in area and 5 cm in depth) of the soil-root system were taken from each plot at panicle formation stage. These were sectioned into two layers (0-1 cm and 1-5 cm in depth), and separated carefully into the above-described three fractions before use.

The ARA's are expressed as nmole or μmole C_2H_4 (g dry weight)$^{-1}$ h^{-1}.

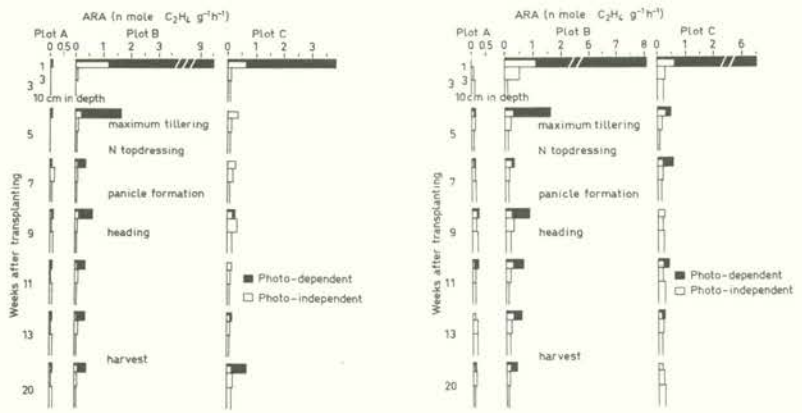

Fig. 1. Effect of deep placement and topdressing of rice straw on ARA in the plough layer; at left: Arakawa alluvial soil; at right: Tochigi andosol.

Results

Time courses of ARA in the soil-rice root system (Fig.'s 1, 2) indicate that rice straw application strikingly stimulated ARA in the surface layer (0-1 cm) during the rice tillering stage, and the stimulation was several times greater on photo-dependent ARA than on photo-

independent ARA. The ARA in the applied straw, soil and rice root fractions at panicle formation stage (Table 1) proved that photo-dependent and photo-independent ARA in straw from the surface layer were 1000-fold and 100-fold higher respectively, than those in the soil fraction. Activity in the rice roots was about 10-fold higher than in the soil.

Effects of the herbicide on ARA in the straw from the surface (0-1 cm) and the lower (1-5 cm) layers are shown in Table 2. The herbicide stimulated the photo-dependent ARA by 30% and 90% for Arakawa and Tochigi soils respectively, while it was ineffective or inhibitive to the photo-independent ARA.

Table 1. Photo-dependent and photo-independent ARA in the applied straw, soil and rice root fractions measured at panicle formation stage

Sampling site	Photo-dependence	Fraction	ARA (nmole C_2H_4 g^{-1} h^{-1})	
			Arakawa soil	Tochigi soil
0-1 cm	Dependent[a]	straw	370	499
		soil	0.54	0.35
		root	23.0	-
	Independent[b]	straw	17.4	47.1
		soil	0.27	0.18
		root	8.14	-
1-5 cm	Dependent	straw	6.29	11.2
		soil	0.22	-
		root	7.41	-
	Independent	straw	3.72	6.03
		soil	0.19	0.17
		root	7.35	-

[a] ARA measured in the light (6000 lx), [b] ARA measured in the dark.

Topdressing and deep placement of rice straw had different effects on rice growth. With topdressing, growth was promoted throughout the growing season, whereas with deep placement it was significantly depressed at tillering stage and the gradual recovery thereafter did not compensate for the early depression, even at maturity.

Table 2. Effects of a herbicide application on ARA in the straw applied to the surface (0-1 cm) and the lower (1-5 cm) plough layers measured at panicle formation stage

Sampling site	Photo-dependence	ARA (nmole C_2H_4 g^{-1} h^{-1})			
		Arakawa soil		Tochigi soil	
		Herbicide application[a]			
		(-)	(+)	(-)	(+)
0-1 cm	Dependent	281	370	265	499
	Independent	21.2	17.4	45.6	47.1
1-5 cm	Dependent	10.9	6.3	10.0	11.2
	Independent	6.7	3.7	4.6	6.0

[a] Granular MO (2,4,6-arichlorophenyl ether) at 36 kg ha^{-1} was topdressed 2 days after transplanting.

CUMULATIVE EFFECTS OF ANNUAL APPLICATIONS OF RICE STRAW TO A RICE PADDY ON ARA IN CURRENTLY TOPDRESSED STRAW

The mechanism through which annual applications of rice straw to a paddy field tend to increase the soil available-N is still obscure. In this laboratory experiment, cumulative effects of the annual applications on the soil N_2-fixing characteristics were investigated by examining the phototrophic N_2-fixing microflora and its ARA as developed in currently topdressed rice straw.

Materials and methods

Plough layer soils from two plots in a long-term fertility trial (Aomori Agricultural Experiment Station) were used; one plot had received NPK fertilizer annually for 47 years and the other, of the same origin, had received NPK and rice straw annually for the last 13 years. Fresh, 2-mm screened samples of each soil were treated with potassium diphosphate (87 ppm P) and various rates (0, 33, 100 and 300 ppm N) of ammonium sulfate. Portions of these prepared samples, equivalent to 100 g of dry soil, were packed into Neubauer pots (11 cm in diameter), and the uppermost layer (0-5 mm) was amended with 2 g of 5-mm-segment rice straw and then carefully flooded with distilled water. The pots were incubated for 50 days in a growth chamber, alternatively for 14 hours at 30 C in the light (6000 lx) and for 10 hours at 25 C in the dark. During incubation, the straw segments were sampled at intervals so that growth of phototrophic N_2-fixers and ARA in the straw could be measured.

Photosynthetic bacteria (Rhodospirillaceae). Two segments of the straw were transferred to each of five anaerobic test tubes filled with liquid medium, sealed with a rubber stopper and incubated as just described, for one week. The liquid medium contained: NH_4Cl, 1 g; $NaHCO_3$, 1 g; K_2HPO_4, 0.2 g; CH_3COONa, 2 g; $MgSO_4.7H_2O$, 0.2 g; yeast extract, 0.1 g; NaCl, 1 g; and 10 ml of salts solution ($FeCl_3.6H_2O$, 5 ppm; $CuSO_4.5H_2O$, 50 ppb; H_3BO_3, 1 ppm; $MnCl_2.4H_2O$, 50 ppb; $ZnSO_4.7H_2O$, 1 ppm; $Co(NO_3)_2.6H_2O$, 0.5 ppm) in 1000 ml of distilled water, at pH 7. After incubation, the growth of pink-coloured bacteria on the straw was examined with the naked eye. After purification, the morphology of the bacteria was examined under an electron microscope (6000 x).

Cyanobacteria. Loopful samples of algal bloom on the straw were examined under a microscope to quantify and identify heterocystous cyanobacteria.

ARA measurement. Portions of twenty straw segments were sampled at intervals from each pot, placed in assay flasks, and the photo-dependent and photo-independent ARA's were measured as described above.

pH values of the straw. At each sampling, pH values of five segments per pot of the straw were measured by both methyl-red and bromocresol-green indicator papers.

Table 3. Growth[a] of Rhodospirillaceae *on rice straw topdressed onto submerged Aomori andosols*

Soil pretreatment	N added (ppm)	Days incubated 10	18	25	32	40	50
Annually applied with NPK	300	+	++	±	±	±	±
	100	±	±	+	±	±	+
	33	-	++++	+	±	±	+
	0	-	++++	+	±	±	±
Annually applied with NPK and rice straw	300	+	±	++	±	+	±
	100	±	+	+++	±	+	++
	3	-	+	+++	±	+	++
	0	-	+	++	++	+	+

[a] Degree of growth: -, none; ± to ++++, poor to abundant.

Results

Growths of *Rhodospirillaceae* and heterocystous cyanobacteria on the topdressed straw are shown in Tables 3 and 4, respectively. *Rhodospirillaceae* attained their maximal growth in the third week of incubation; thereafter the growth declined gradually or rapidly for the soils pretreated with or without straw, respectively. The maximal growth levels for the two pretreatments were almost equal, though this stage was retarded with the straw pretreatment. Electron microscopy identified the bacteria as *Rhodopseudomonas*.

Heterocystous cyanobacteria appeared on the straw after two-week incubation. The soil pretreated with straw produced a vigorous bloom of cyanobacteria on the currently topdressed straw, while the other treatment did not. Thus, there was an obvious cumulative effect of annual applications of rice straw on the growth potential of N_2-fixing cyanobacteria. In addition, an application of ammonium-N, as much as 33 ppm, to the soil significantly enhanced

the blooming. Thus the straw-pretreated soil seemed to have achieved a cyanobacterial flora more N-tolerant than that in the soil not treated with straw. This trend was also observed in *Rhodopseudomonas*. The results in Table 4 also indicate that an application of ammonium-N caused a change in the cyanobacterial population of the bloom. Without N, *Anabaena*, *Cylindrospermum* and *Nostoc* dominated while, with increasing amounts of N, *Calothrix* became dominant.

Table 4. Growth[a] and dominant genera[b] of heterocystous cyanobacteria blooming on rice straw topdressed onto submerged Aomori andosols

Soil pretreatment	N added (ppm)	Days incubated 10	18	25	32	40	50
NPK	300	-	-	-	-	-	Cy
	100	-	-	± Ca	± Ca	+ Ca	+ Ca,Cy
	33	-	± Ca	+ Ca	+ Ca	+ Ca	+ Ca,Cy
	0	-	± A,N	+ N,Cy	+ N,Ca	+ Ca,N,A	+ Ca,N
NPK and rice straw	300	-	-	-	-	-	-
	100	-	± Ca	+ Ca	+ Ca	+ Ca	+ Ca
	33	-	++ Ca	++++ Cy,Ca	++++ Cy,Ca	++++ Cy,Ca	++++ Cy,Ca
	0	-	+ A,Cy	+++ A,Cy	+++ A,Cy	+++ A,Cy	+++ A

[a] Degree of growth: -, none; ± to ++++, poor to abundant.

[b] Dominant genera: A = *Anabaena*, Ca = *Calothrix*, Cy = *Cylindrospermum*, N = *Nostoc*.

The pH values of the straw proved that, even during the vigorous blooming of cyanobacteria, the straw remained acidic (pH ranged from 5.4 to 5.6). Though it has been reported that pH ranges within 7.5 to 9.0 are optimal for the growth and N_2 fixation of most cyanobacteria (Gerloff *et al.*, 1950; Kratz & Myers, 1955) and acidic reactions are depressive (Allison *et al.*, 1937), our results suggest that an acidic condition may have less importance in the paddy ecosystem.

Time courses of photo-dependent and photo-independent ARA's in the topdressed straw are illustrated in Fig.'s 2 and 3 respectively. The photo-dependent ARA gradually increased during the initial 3-week incubation, then was markedly enhanced through cyanobacterial blooming, reaching maxima as high as 2 to 4 µmole C_2H_4 g^{-1} h^{-1} at about one month of incubation. A cumulative effect of annual applications of straw was obvious in the cyanobacterial ARA. Extrapolating from greenhouse data, approximately 40% (w/w) of rice straw applied to submerged soils is exponentially decomposed during the first 50 days (Komoto & Sakai, 1970). Thus total ARA's in the straw during a 50-day incubation with 0, 33, 100 and 300 ppm of ammonium-N were estimated as 1117, 1168, 599 and 52 µmole C_2H_4 per gram of applied straw, respectively, in the straw pretreated soil, while they were 732, 575, 297 and 46 µmole C_2H_4, respectively in the soil pretreated with NPK only.

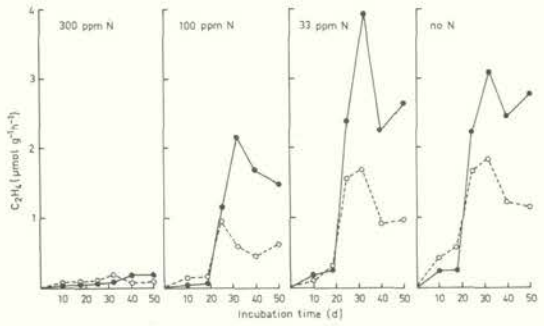

Fig. 2. Time courses of light-dependent ARA in rice straw topdressed onto submerged Aomori andosol pretreated with (●—●) and without (o---o) annual applications of rice straw.

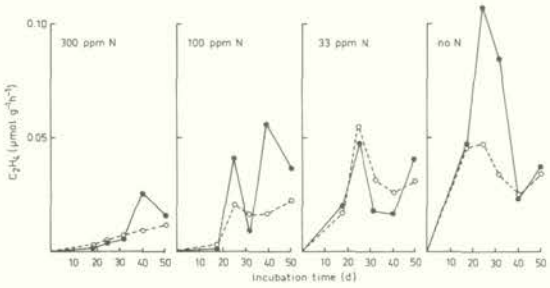

Fig. 3. Time courses of light-independent ARA in rice straw topdressed onto submerged Aomori andosol pretreated with (●——●) and without (o----o) annual applications of rice straw.

The cumulative effect also appeared in N tolerance of the photo-dependent ARA. An addition of 33 ppm N to the straw-pretreated soil increased the ARA in the currently topdressed straw, while the addition was depressive in the other treatment. This effect was more marked for alluvial soils from Niigata, Hiroshima and Saga Agricultural Experiment Station, as shown in Fig. 4. Here, responses of the photo-dependent ARA in the topdressed straw to an addition of 100 ppm of ammonium-N were measured after a 1-month incubation. The addition of N to the soil treated annually with rice straw caused a marked increase in ARA, while the effect was adverse on soils not pretreated with straw. Photo-independent ARA's in the straw (Fig. 3) were comparatively low, and the cumulative effect of the soil pretreatments was obscure.

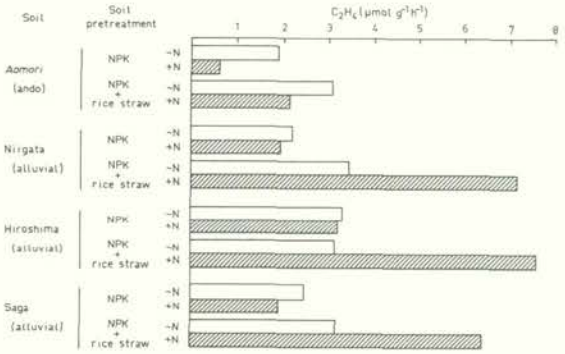

Fig. 4. Responses of light-dependent ARA in the topdressed rice straw to an addition of 100 ppm ammonium-N.

DISCUSSION

Despite a considerable volume of papers reporting a stimulating effect of straw application on heterotrophic ARA or NFA (N_2-fixing activity) in water-logged soils, few works have studied its effects on phototrophic NFA. Our field and laboratory results in the present paper indicate that rice straw applied to paddy soils provides niches far more beneficial to phototrophic NFA than to the heterotrophic one. To optimize the effect, therefore, topdressing of rice straw is more advantageous than deep placement (Fig.'s 1 and 2, and Table 1). This must be due to the aerobic and/or microaerophilic environments of the submerged soil surface in which the applied straw is efficiently decomposed, and where organic acids (mainly acetic acid) and carbon dioxide are supplied as substrates for the growth and N_2 fixation of *Rhodospirillaceae* and especially cyanobacteria.

It is noteworthy that vigorous growth and extremely high ARA of cyanobacteria in the

topdressed straw were maintained even in the presence of 33 ppm applied N (Table 4 and Fig. 2). A similar, but more conspicuous trend was observed in alluvial soils (Fig. 4), where photo-dependent ARA in the topdressed straw was higher, and where an application of ammonium-N as much as 100 ppm markedly enhanced the ARA when straw was topdressed to the soils treated annually with straw, while it was depressive in soils which had not been pretreated. To explain these conflicting results, the following possibilities should be taken into account: the soils treated annually with rice straw must have attained either a N-tolerant N_2-fixing community or higher potential activities for aerobic microbial respiration and N immobilization. Field data at Hokuriku Agricultural Experiment Station showed that a field application of N-fertilizer, even to a plot which had not been pretreated with straw, induced a 40% stimulation of carbon dioxide evolution from freshly deep-placed straw at a rate of 10^4kg ha^{-1}. The amount of carbon dioxide evolved, however, corresponded to less than 20% of total C content in the applied straw. A cumulative effect of annual applications of straw to a rice paddy on CO_2-evolving and N-immobilizing activities in the soil could be responsible for the present conflicting results.

The modification of the cyanobacterial flora, blooming on the topdressed straw, by applying ammonium-N should also be mentioned. As shown in Table 4, *Anabaena*, *Cylindrospermum* and *Nostoc* dominated in the absence of applied N, while in the presence of 100 ppm N these genera were mostly replaced by *Calothrix*. The same trend was observed in the alluvial soils used in this experiment. Taha (1964) reported that NFA of *Calothrix elenkinii* culture was stimulated by adding as much as 58.2 ppm of nitrate-N or 7.5 ppm of ammonium-N, though NFA of the other cyanobacteria tested were depressed. Our results shown in Fig. 4, however, demonstrated that an addition of 100 ppm ammonium-N, corresponding to 100 kg N ha^{-1}, strikingly enhanced photo-dependent ARA, mostly ARA of cyanobacteria. This result suggests that even the rates of N-fertilizer used in intensive rice cultivation (100-200 kg N ha^{-1} by split application) should not be depressive but beneficial to NFA in rice paddies if topdressed with straw. A similar trend was obtained with a herbicide applied at a recommended rate (Table 2), which resulted in a stimulated photo-dependent ARA in the topdressed straw. Recently it has been reported that N_2 fixation of some cyanobacterial strains can be stimulated not only at low concentrations of pesticides but also in the presence at high levels. This indicates that there could be a wide range of tolerance limits of cyanobacterial N_2 fixation for pesticides (Venkataraman, 1975). To optimize NFA in paddy fields, more studies should be conducted on N- and pesticide-tolerant N_2-fixing populations associated with freshly added organic materials in paddy ecosystems.

The fact that cyanobacteria can vigorously bloom on straw with an acidic reaction (Table 4) is also noteworthy. Conflicting with a commonly accepted concept of cyanobacterial response to pH conditions, this result may cause discussion whether these organisms were either acid-tolerant or more responsive to carbon dioxide concentration in the environment. The results suggest that topdressing of rice straw can promote N_2 fixation in rice paddies on the acidic soils which are widely distributed in southeast and east Asian countries.

The extremely high ARA in the topdressed rice straw and its tolerances for ammonium-N, herbicide and acidic reaction, demonstrated in the present paper, should be of practical value as clues to promoting biological N_2 fixation both in extensive and intensive rice cultivation systems.

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NITROGEN FIXATION BY *AZOTOBACTER* IN TROPICAL SOILS

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ABSTRACT

Soil samples were collected from rice fields in the central region of Thailand and from the dry evergreen and dry dipterocarp forests of Sakaerat experiment station, Nakorn Ratsima province. Samples from rice fields were taken both while the soils were submerged and when they were in a dry condition. Fifty-nine isolates of Azobacter *were obtained from these samples by the dilution plating technique. Acetylene reduction assay was used for screening efficient strains of the fifty nine isolates. Distinctively higher nitrogenase activities were obtained from six isolates. In order to estimate the maximum attainable nitrogen fixation by these efficient strains, total numbers of* Azotobacter *in the field samples were determined by using a dilution plating method. The estimated maximum attainable nitrogen fixation by the most effective isolate was 43.7 kg* ha^{-1} yr^{-1}.

INTRODUCTION

The most intensively investigated representative of the non-symbiotic nitrogen fixing bacteria group is *Azotobacter*, because it is commonly present in soils. The application of *Azotobacter* in the field has not been successful because the amount of nitrogen fixed by the tested organism is in most cases not high enough. This is presumably due to the fact that the screening method for effective strains of *Azotobacter* used in the past has not been sufficiently sensitive. In recent years, more effective techniques such as the acetylene reduction test (Hardy *et al.*, 1968) have been introduced for screening efficient strains of nitrogen-fixing organisms. The present study was carried out primarily to screen effective strains of *Azotobacter*, using these techniques.

MATERIALS AND METHODS

Isolation and identification of Azotobacter *strains*

Twelve soil samples were collected from rice fields in Ayuthaya, Arngthong and Singhburi provinces; seven samples were collected under submerged conditions and five samples from dry soils. At Sakaerat experiment station 16 samples were collected from study plots of 1 km^2 within the dry dipterocarp and the dry evergreen forests. All samples were kept in plastic bags and isolations were made within 24 hours. Soil suspensions of different dilutions were prepared for the dilution counting technique described in this paper. Aliquots of 0.2 ml of soil suspensions were spread on the surface of N-free agar in petri-dishes. The petri-dishes were then incubated at 30 C for 5 days. From colonies with different characteristics, a series of *Azotobacter* strains were collected. Studies were made on the morphological, cultural, and physiological characteristics of the organisms. Identification of *Azotobacter* colonies was based on characteristics described by Buchanan & Gibbons (1974).

Examination of nitrogen-fixing capacity

Each of the isolates obtained was transferred to 5-ml slants of N-free agar in test tubes of 20.5 ml volume. The tube was plugged with cotton wool and incubated at 30 C for 5 days. The cotton plug was then replaced with a rubber stopper. Three ml of acetylene gas were injected into each test tube and the tube was then incubated at 30 C for one hour. After incubation, two 1-ml portions of gas in the test tube were removed for determination of ethylene by gas chromatography. The total number of *Azotobacter* in the test tube after this test was assessed using a haemocytometer (Hardy *et al.*, 1968).

Total count examination

Thirteen soil samples, representing soils under different land uses, were collected for total count assessment. These included eleven samples from rice fields in Ayuthaya, Arngthong and Singhburi provinces, one from the dry dipterocarp forest and one from the dry evergreen forest of Sakaerat experiment station. Ten of the samples from rice fields were collected while the soil was submerged and the rice plants were growing. One sample was collected from a rice field in Arngthong province while the land was dry and fallowed. All the samples were kept in plastic bags and the total count was performed within 24 hours.

A weighed soil sample was mixed with a known amount of sterile distilled water and shaken for a few minutes. Serial dilution was then carried out and the diluted suspensions were transferred to petri-dishes containing solid N-free medium. The plates were then incubated for 5-7 days at 28-30 C, *Azotobacter* colonies were counted, and the numbers of organisms per gram of soil were calculated.

RESULTS AND DISCUSSION

Identification of Azotobacter

Fifty nine isolates of *Azotobacter* were obtained. These included 13 isolates from rice fields, 31 from the dry dipterocarp forest and eight from the dry evergreen forest. By morphological, cultural, and physiological characteristics, the 59 isolates could be divided into 13 groups. Four of the groups were identified as *Azotobacter chroococcum*, *A. beijerinckia*, *A. vinelandii*, and *A. paspali*. The rest was unidentifiable at the species level.

Table 1. Total ethylene produced, total number of cells found after nitrogenase test, and nitrogenase activity of Azotobacter *found to have high nitrogen-fixing ability*

Isolate[a] number	Source of isolate	C_2H_4 produced ($\mu l\ ml^{-1}$)	Total number of cells found after nitrogenase test ($culture^{-1}$)	Nitrogenase activity (nmole C_2H_4 10^{-9} $cell^{-1}hr^{-1}$)
R29	Rice field	0.1400	3.8×10^9	24
R20/3	Rice field	0.1432	7.2×10^8	133
DD5	Dry dipterocarp forest	0.1642	4.0×10^6	27392
DD4	"	0.0011	6.4×10^5	1158
DD3	"	0.0017	1.6×10^5	6973
DD14	"	0.0018	1.0×10^6	1169
DE4	Dry evergreen forest	0.0019	2.0×10^6	625

[a] Isolate no R29 was identified as *Azotobacter chroococcum*.
Isolate no DD3 was identified as *Azotobacter beijerinckia*.
Isolate no R20/3, DD4, DD5, DE4 were unidentifiable.

Screening for efficient isolates

Nitrogenase activity and total C_2H_4 produced were used for screening the isolates of *Azotobacter*. It was found that 6 out of the 59 isolates showed higher N_2-fixing activity (Table 1). The nitrogenase activity per cell of *Azotobacter* from the dry dipterocarp forest was very much higher than that of the strains from the dry evergreen forest and rice fields. However, total counts of cells in the medium (Table 1) showed that the strains isolated from rice fields grew much better than the strains from forests. This growth difference resulted in comparable total C_2H_4-production per culture for most efficient strains from the dry dipterocarp forest. The growth of the efficient strains from the dry dipterocarp forest was low, only 10^5-10^6 cell $culture^{-1}$.

The poor growth of *Azotobacter* isolated from dry dipterocarp might indicate that the growth medium was not optimum for the isolates and therefore resulted in low acetylene reduction activities. However, the total counts in the soil samples (Table 3) suggest that the poor growth was a common characteristic for this organism.

Maximum attainable nitrogen fixation

The numbers of *Azotobacter* in the dry soil from rice fields were higher than those in the submerged soils (Table 2). This was probably due to the better aeration in the dry soils. The total numbers in soils from submerged rice fields varied from 10^4 to 10^5 CFU g^{-1} whereas those for soils in the dry condition varied from 10^5 to 10^6 CFU g^{-1}.

Table 2. Total counts of Azotobacter *in soil samples collected from different areas*

Area	Total count (CFU[a] x 10^4 (g soil)$^{-1}$
Rice field, submerged	16 - 80
Rice field, dry	1280
Dry dipterocarp forest	54
Dry evergreen forest	11

[a] CFU = colony forming unit.

The total numbers of *Azotobacter* in the samples from the dry dipterocarp forest and from the dry evergreen forest were in the order of 10^5 CFU g^{-1}. The number of *Azotobacter* in dry dipterocarp forest was a little higher than in dry evergreen forest. This is probably due to the pH of the soils. The pH values of the soil from dry evergreen forest were in the range of 4.4-4.6 and those from the dry dipterocarp forest in the range of 5.2-6.2.

From the estimated total counts of *Azotobacter* in soil and the C_2H_2-reducing activities obtained, the maximum attainable rates of nitrogen fixation were calculated using the conversion ratio 3:1 (C_2H_4:N_2) (Hardy *et al.*, 1973) and assuming 1950 t ha^{-1} of soil. The maximum attainable amount of nitrogen fixation for the most efficient isolate was 43 kg ha^{-1} yr^{-1} (Table 3).

Table 3. Specific N_2-fixation activities, total counts of cells in soil, and estimated potential amounts of N_2 fixation by selected Azobacter *isolates*

Isolate number	Rate of N_2-fixation (nmole N 10^{-9}) cell hr^{-1})	Total count in soil used for estimation (CFU[a] g^{-1})		Estimates of potential of N_2 fixation (kg ha^{-1} yr^{-1})	
		S[b]	D[b]	S	D
R29	8	10^4	10^6	0.4	3.9
R20/3	44	10^4	10^6	2.1	21.2
DD5	9181		10^4		43.7
DD4	386		10^4		1.8
DD3	2324		10^4		11.1
DD14	390		10^4		1.9
DE4	208		10^4		1.0

[a] CFU = colony forming unit.

[b] S = submerged.
D = dry.

It is generally accepted that the acetylene reduction assay should be measured *in situ*. In the present study, however, the assay was done in culture medium in order to screen the effective strains. The medium used for culture was a N-free medium, and under such conditions, the micro-organisms would show a high N_2-fixing efficiency. In the real situation they might not fix as much nitrogen as in the culture medium. There are many factors that might affect the nitrogen fixing ability of the *Azotobacter*, such as environmental factors and growth of other micro-organisms in the soil. However, results of this study show that the different organisms themselves vary widely in their capacity to fix nitrogen. Inoculation with the most effective strains should therefore achieve a higher rate of fixation than with the least effective strains.

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BLUE-GREEN ALGAE AND THE FERTILITY OF LOWLAND RICE FIELDS IN THE PHILIPPINES

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ABSTRACT

*The effectiveness of nitrogen-fixing algae (*Nostoc *and* Gloeotrichia*) was compared with a chemical fertilizer (NPK, 14-14-14) for growth of lowland, irrigated rice in a field experiment in the dry season, 1976.*

Yields (grain and straw) and nitrogen uptake by plants were consistently higher in plots inoculated with algae than in non-inoculated plots, with algae contributing about 19-28 kg N ha^{-1} *crop*$^{-1}$*. The number of algal* N_2*-fixers decreased with time and growth of the rice plant, while non-*N_2*-fixing algae increased in population up to tillering stage. NPK fertilization and low-light intensity conditions appeared to have depressed growth of the diazotrophs.*

INTRODUCTION

The abundance of nitrogen-fixing blue-green algae in the tropics is well established (Singh, 1961; Watanabe & Yamamoto, 1971). Their importance in contributing to the nitrogen fertility of the soil, especially in paddy fields, is also well-documented. Rice in south-east Asia has been grown for many years without yield decline in the absence of artificial fertilizers. This is exemplified by experiments of Watanabe *et al.* (1978) at the International Rice Research Institute (IRRI), where crops grown for 12 continuous years in unfertilized plots did not show a decline in yield. The use of an *in situ* acetylene reduction assay, for 24 hours in these plots, further suggested that the blue-green algae contributed more to nitrogen fixation than the bacterial nitrogen-fixers. Data on the nitrogen contribution of N_2-fixing blue-green algae show some variations. Findings by Singh (1961) suggested a contribution of 70 kg N ha^{-1} yr^{-1} in India. However, lower values, e.g. 1-30 kg N ha^{-1} yr^{-1}, have been obtained elsewhere (Reynaud & Roger, 1978; Watanabe *et al.*, 1978).

To assess the potential agricultural use of algae as a fertilizer source, attempts have been made to inoculate field crops with algae. Singh (1961) inoculated Indian paddy fields with *Aulosira fertilissima* and observed an increase in crop yield of 114%. Watanabe (1962, 1973) using the same alga in Japan, did not obtain any positive response, but when another alga, *Tolypothrix tenuis*, was used, it gave an increase in yield. In the Philippines, Pantastico & Gonzales (1976) reported an increase in grain yield of 23% over the unfertilized plots when *Nostoc commune* was used as a biofertilizer. There was also a positive response when mixed species of N_2-fixers were added in the field (Subrahmanyan *et al.*, 1965).

The soil-algae-rice ecosystem is a dynamic and complex one that does not guarantee the performance of the inoculum. This paper describes the periodic changes, both qualitative and quantitative, of the algal flora during one cultivation cycle of rice. Soil and plant N analyses are presented to give an accurate assessment of the nitrogen contribution of a specific alga, or group of algae, and to further our understanding of N cycling within this ecosystem.

MATERIALS AND METHODS

Field experiment

The experimental field is located near the IRRI lowland ricefields in College, Laguna, Philippines between 14°10'N latitude and 121°15' E longitude, at about 60 km south-east of Manila. It has been used for algae-fertilization experiments on rice since 1974. The soil is Maahas clay that may approximate the chemical properties reported by Watanabe *et al.* (1978) with pH 6.6, organic matter 2.04%, and total nitrogen 0.18%.

The area was subdivided into 32 plots of 2.5 x 5 m each. Two-week old plants of rice varieties IR-32 and IR-34, were transplanted equidistantly in each plot. There were four treatments for each variety used, distributed in a randomized split-plot design. The treatments, replicated four times and based on the previous study of Pantastico & Gonzales (1976) were

T_1 = control (unfertilized plots).
T_2 = NPK (14,14,14) with N at 60 kg ha^{-1}.
T_3 = NPK (14,14,14) + algae, with N at 30 kg ha^{-1}.
T_4 = algae.

The algae-fertilized plots (T_3 and T_4) were inoculated with *Nostoc commune* Vauch. (4.02-4.88% N, dry wt, *in vitro* culture). However, abundant floating algal masses in the field, identified also as a N_2-fixer, (*Gloeotrichia* sp.), were removed from the other treatments except those that were algae-fertilized. *Gloeotrichia* had 4.26% N content based on dry weight after 21 days incubation in Fogg's (minus N) medium (Fogg, 1949). Split fertilizer application was used for NPK, the first application being given one week after transplanting and the next one four weeks later. The algal inoculum was added only once, i.e., one week after transplanting. The effects of the various treatments were determined by following the vegetative and reproductive growth parameters of IR-32 rice plants. Yield components for IR-34 variety were not measured, because these plants were damaged by typhoon "Didang" in May, 1976. An insecticide, Sevin (1-napthyl-N-methyl carbamate), was applied in the field as needed at the recommended dose of 2 kg active ingredient ha^{-1}.

For two of the treatments (T_3 and T_4), the qualitative and quantitative distribution of the algae was measured before transplanting, and at approximately monthly intervals after transplanting. Algal enumeration was not done in T_1 (unfertilized) and T_2 (NPK) plots, since these were cleared of floating algal masses during the course of the experiment.

Sampling was done from five 1-m^2 quadrats, four located at the corners, and the fifth at the centre, of each plot. Total algae gathered from each plot was mixed, concentrated by centrifugation at 1200 rpm for 5 min, and preserved in vials with Lugol's iodine (2 drops per 10 ml). Two plots per treatment served as the replicates. Algal enumeration was done with the aid of a haemacytometer and a compound microscope as described in a previous paper (Martinez *et al.*, 1975). The cell density of algae occurring in mats or colonies was determined by noting their relative occurrence in the five quadrats per plot. The mean percentage occurrence for a plot was calculated before doing the cell count. For example, if the mean relative occurrence of *Gloeotrichia* within a plot was 10%, then 10-g fresh weight of the colony was added into 90-ml water. In this instance, the clathrate colonies were broken down by shaking in a stoppered tube with glass beads.

The field experiment was conducted during the dry season from February 27 to July 14, 1976.

Chemical analyses

Soil samples of the 0-15 cm layer were taken monthly and were analyzed using a macro-Kjeldahl method (Bremner & Keeney, 1966). For sampling of plant N, the harvested straw and rice grain were separately dried, ground, and analyzed for total nitrogen using the modified macroKjeldahl method (AOAC, 1950).

The soil N data are averages of two plots planted with IR-32 and IR-34, while the plant N analyses are mean data taken from plots planted with IR-32. The amount of N fixed was calculated by taking the differences between N uptake of the algae-fertilized plants and those of the unfertilized plants.

RESULTS AND DISCUSSIONS

Algal population

A total of 21 genera of algae were observed in the algae-fertilized plots (T_3 and T_4) for one cultivation cycle of rice during the dry season of 1976 (Table 1). Among the nitrogen fixing algae (NFA) observed were : *Anabaena*, *Cylindrospermum*, *Gloeocapsa*, *Gloeotrichia* and *Nostoc*. *Gloeocapsa*, a non-heterocystous form, is considered to be an N_2-fixer, based on work by Stewart *et al.* (1979) which demonstrated that strains of this genus showed positive acetylene reduction activity even under aerobic conditions. However, another chroococcacean, *Synechococcus*, while forming an important component of the total number of algae in our sampling (ave. 20.7%), did not show any nitrogen fixing activity (S.A. Kulasooriya, pers. comm.). Other possible N_2-fixers, such as *Oscillatoria* and *Lyngbya*, have so far been established as N_2-fixers only under anaerobic and microaerophilic conditions (Stewart *et al.*, 1979), so they are grouped here among the non-N_2-fixers (NNFA).

Table 1. Relative abundance of some algae observed in the paddy field in one cultivation cycle of rice varieties, IR-32 and IR-34, dry season, 1976

Algae	0[a]	12 DAT[b]	52 DAT	82 DAT	94 DAT
Cyanophyta					
N_2 fixers					
Anabaena	++[c]	++	++		
Cylindrospermum	++	++			
Gloeocapsa	++	++	++	++	
Gloeotrichia	++++	+++	+++	++++	+++
Nostoc		++			
Non-N_2-fixers					
Synechococcus	++	+++	++	+++	++++
Chroococcus					++
Lyngbya				++	
Oscillatoria	+++	++++	++++	+++	++
Spirulina			+	+	
Euglenophyta					
Euglena	+		+		
Trachelomonas		+	++	+	++
Chlorophyta					
Actinastrum				+	
Chlorella				+	
Cladophora				++	
Closterium				+	+
Coelosphaerium		+			
Pandorina			+		
Scenedesmus		+			+
Selenastrum	+				
Spirogyra	+				
Bacillariophyta					
Centric species				+	+
Pennate species	++	++	++	++	++

[a] At 26 days before transplanting. [b] Days after transplanting.
[c] ++++ comprised 30% and above of total algae +++ comprised 10-29% of total algae, ++ comprised 2-9% of total algae, + comprised 1% or less of the total algae.

The total population of the algae tended to decrease in time (Fig. 1). One factor that may have reduced the number of photoautotrophs was the decreasing light intensity due to shading by the growing rice plants (IRRI, 1975).

However, when the algae were grouped into N_2-fixers (NFA) and non-N_2-fixers (NNFA), the former followed also a decreasing trend with growth of the plants while the latter showed an increasing population up to tillering initiation 12 days after transplanting (12 DAT), and then declined. Also, the NPK amended plots (T_3) reached a maximum population for NNFA (744 x 10^3 cells ml^{-1}) at 52 DAT. The non-diazotrophs consisted mainly of blue-green algae and diatoms. Among the abundant species observed were *Oscillatoria* and *Synechococcus*, which comprised on average 54 and 32% respectively of the total NNFA.

These results conform to the pattern of algal succession in wet monsoonal areas, where light intensity is not so high (Gupta, 1966), and the pH of the paddy water is higher (Watanabe, 1973). Such conditions existed in the experimental field, where the total algal density and the density of nitrogen fixers each varied inversely with rice growth. The mean total solar radiation and air temperature during the experimental period (February to July) ranged from 50 666 to 71 415 lx and from 24 to 27 C, respectively (Dept. Agric. Engr., 1977). The values may be lower under the rice canopy where the algae are located. Thus, light intensity may have been a limiting factor depressing the growth of the N_2-fixers but not of the non-N_2-fixers. This is especially apparent where NPK was not added (T_4, Table 2).

A contrasting situation is reported from Senegal by Roger & Reynaud (1977) wherein there is a positive correlation between the biomass of the N_2-fixing algae and the increased number of rice tillers under conditions of very high light intensity (70 000-80 000 lx). This may mean that with higher light intensities the amount of light that penetrates through the rice canopy is nearer optimum for the growth of the nitrogen fixing algae.

A study of the numbers of N_2-fixing algal cells in samples from the + NPK + algae fertilized plots (T_3) and - NPK + algae fertilized plots (T_4) showed a decline in both populations with time (Fig. 1). Initially, the T_4 treatment had a higher NFA population than the T_3 one. However, this margin widened at 12 DAT, 3 days after NPK application. This supports

an earlier report that the population of N_2-fixers is depressed by N-fertilization probably due to inhibition of their N_2-fixing ability (Yoshida *et al.*, 1973). However, the NPK treatment must have sustained the growth of the NFA in the longer term, even under a light-limiting environment. In fact, at 82 DAT (42 days after fertilizer application) their population comprised about 57.8% of the total population (Table 2).

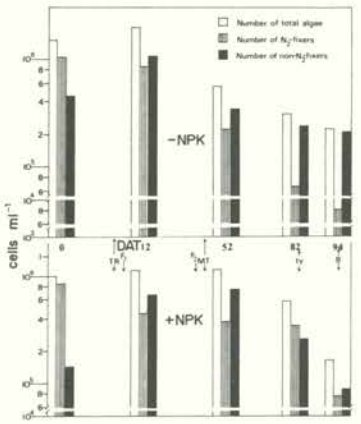

Fig. 1. Total number of algae, N_2-fixers and non-N_2-fixers in the algae-fertilized plots without or with NPK (N supplied at 30 kg ha^{-1}) at different times after transplanting.
TR = time of transplanting.
DAT = days after transplanting.
MT = maximum tillering stage.
B = booting stage.
F_1, F_2 = first and second NPK application.
ty = typhoon.

Table 2. A comparison of the relative abundance of the N_2-fixers and the non-N_2-fixers in the two treatments inoculated with algae (mean for two rice varieties)

DAT[a]	T_3 (NPK + algae)		T_4 (algae only)	
	N_2-fixers	Non-N_2-fixers	N_2-fixers	Non-N_2-fixers
0[b]	85.8	14.2	70.3	29.7
12[c]	40.4	59.6	44.3	55.7
52[d]	33.4	66.6	39.3	60.7
82	57.8	42.2	21.6	78.4
94	45.4	54.6	3.6	96.4

[a] Days after transplanting. [b] Sampling done 26 days before transplanting.
[c] Sampling done 3 days after first NPK application. [d] Sampling done 12 days after second NPK application.

In both plots the initial population of the N_2-fixers was higher than of the non-N_2-fixers, but at 12 DAT a reverse situation occurred, and continued up to maximum tillering stage of the plants (52 DAT). At 82 DAT, in the T_3 plots, the population of the N_2-fixers again increased over the non-N_2-fixers. The highest population for the total algae was attained in T_4 plots at 12 DAT (i.e. 1.9×10^6 cells ml^{-1}) when the non-N_2-fixers constituted 56% of the total (Table 2). The cell density of non-fixers then equalled the maximum attained by the N_2-fixing algae (1.1×10^6 cells ml^{-1}) in the initial population in T_4 plots.

Gloeotrichia sp., occurring as a gelatinous, brownish, clathrate algal mass, formed the greatest bulk of the algal population and therefore it was the most important N_2-fixer in

both treatments. Of the total N_2-fixers (ave. 4.3 x 10^5 cells ml^{-1}) it constituted about 81%, and of the total algae (ave. 8.5 x 10^5 cells ml^{-1}) 41%, in one season for both treatments. The highest *Gloeotrichia* population recorded was 10 x 10^5 cells ml^{-1} sampled in T_4 plots prior to rice cultivation, but on average about 3.5 x 10^5 cells ml^{-1} were counted throughout one sampling season. Assuming that the alga can occupy a water depth of up to 5 cm, then it attained a maximum biomass of 32 t fresh weight ha^{-1} with an average value of 11 t ha^{-1}. These values are close to that obtained by Watanabe *et al.* (1978) at IRRI, (24 t ha^{-1}), when the alga almost fully covered the unfertilized plots. Furthermore, assuming that *Gloeotrichia* had 0.04% N (fresh weight) (Watanabe *et al.* 1978) during the experimental period, then, on average the alga contributed 4.4 kg N ha^{-1} with a peak of 12.8 kg N ha^{-1}. However, since this value represents only a 60% coverage of the alga in the plot, it may still be a conservative estimate of the N contribution of the alga to the soil, accomplished either through lysis or microbial decomposition after the cells died (Roger & Reynaud, 1979; Stewart *et al.*, 1979).

The other N_2-fixers noted, in their decreasing order of abundance, were *Gloeocapsa*, *Anabaena* and *Cylindrospermum*, respectively.

The distribution of algae may be due to the interaction of several physico-chemical and biotic factors, such as the presence of grazers, flow of water from one plot to another, the occurrence of typhoons, etc., which were not considered. Nevertheless, it is apparent that the addition of NPK caused a decrease in the total algal biomass and number of N_2-fixers, but sustained the population of the latter for a longer period compared to the plots without NPK.

It seems that the results of the inoculation of the same algal species may not only vary with site (Watanabe, 1973) but also within one site. The earlier success of *Nostoc commune* Vauch., as an inoculant in a series of fertilizer experiments (Pantastico & Gonzales, 1976) was later hampered by infestation of some pests, i.e., chironomid larvae (Martinez *et al.*, 1977). Its inability to float, unlike *Gloeotrichia*, makes it also a less successful competitor. These are only two of many possible explanations to account for the relative abundance of a given algal species within an ecological niche, or its relative success or failure as an inoculum.

Plant analyses and nitrogen fixation

There were significant differences in the yield components (total and straw) of IR-32 plants between the algae-plots (T_4) and the unfertilized plots (T_1) (Table 3). The percentage increase in the yield components in the T_4 plots over those in the T_1 plots was as high as 11.1% for rice straw, 4.9% for grain yield and 21.1% for total dry matter.

Table 3. Dry matter, N concentration and N yield of above-ground parts of IR-32, dry season 1976

Treatments	Straw		Grain		Total	
	DM(kg ha^{-1})	N(%)	DM(kg ha^{-1})	N(%)	DM(kg ha^{-1})	N(kg ha^{-1})
T_1 (control)	3620*a*	0.99*a*	2490*a*	1.88*b*	6120*b*	83.4*c*
T_2 (NPK)	4370*ab*	0.97*a*	2830*a*	o.70*b*	7200*a*	92.8*b*
T_3 (NPK + algae)	4220*ab*	0.98*a*	2770*a*	1.77*b*	6990*ab*	92.5*b*
T_4 (algae only)	4960*b*	1.11*a*	2860*a*	1.99*a*	7750*a*	111.8*a*

Figures followed by the same letter(s) are not significantly different at P = 0.05 level.

When the N analyses of the tissues were considered, the T_4 plants had higher N values than the plants in T_1, an increase of 10.6 and 5.5% over T_1, for straw and grain yield, respectively. There were statistical differences in N uptake between the plants in three treatments (T_2, T_3, T_4) and the control (T_1). The rice plants in the algae-fertilized plots (T_4) contained 28.4 kg ha^{-1} more nitrogen than plants in unfertilized plots (T_1), or about a 25% increase. The N uptake in T_1 was statistically different from T_2 and T_3 values, but there was no difference between T_2 and T_3 treatments. This may mean that the N taken up by these plants came from the soil and/or through nitrogen fixation by the heterotrophs, and not through nitrogen fixation by algae. This suggestion is supported by the data of Yoshida *et al.* (1973), indicating that algal N_2fixation was suppressed when NPK was added (T_3), but that heterotrophic N_2fixation probably was not. A gradual build-up of available soil N may occur in Maahas clay soil due to algal fertilization and/or the presence of native algal and bacterial N_2-fixers (Alimagno & Yoshida, 1975). During a study on N mineralization of Maahas

clay Shiga & Ventura (1976) estimated that as much as 60-80 kg N ha^{-1} can be released in one cropping season of rice, and it is probable that some of this came from algal N_2 fixation.

We can compare the amounts of nitrogen taken up by the plants in T_4 and T_2 plots. The difference (T_4-T_2) was also statistically significant (19 kg N ha^{-1}), but the higher value of 28 kg N ha^{-1} (T_4-T_1) is the better estimate of the amount of N_2-fixed by algae. These values are higher than those obtained in the experimental plots of IRRI, where the values calculated for the dry season using *in situ* acetylene reduction assay were 11.1 kg N ha^{-1} in the unfertilized plots and 3.7 kg N ha^{-1} in the fertilized plots (Watanabe *et al.*, 1978). Alimagno & Yoshida (1975) estimated from N uptake that 14.1 kg N ha^{-1} was fixed by algae in association with the rice variety, IR-20. The higher values obtained in our experiment may be due to the combined effect of nitrogen fixation by the inoculated algae and/or by the native algae and bacteria.

CONCLUSION

In one cropping season in an irrigated rice paddy, *Gloeotrichia* sp. was the dominant algal species. It had an average biomass of 11 t ha^{-1} or a potential yield of as much as 4.4 kg N ha^{-1}. Rice plants in algal plots took up 28 kg more N ha^{-1} than did plants in non-algal, non-NPK-fertilized, plots and 19 kg more N ha^{-1} than the NPK-fertilized plots. This amount is approximately equivalent to the N in three bags of ammonium sulfate fertilizer, whose current market price (1979) is about ₱240 (US$32).

ACKNOWLEDGEMENT

This work was made possible through the financial support from UPLB-PCARR Project Nos. 34-13 and 618. The suggestions of Drs S.N. Tilo, I.J. Dogma, Jr, S.A. Kulasooriya and P. Roger, the technical assistance in isolating *Gloeotrichia* provided by S.Y. Ng and N. Dulay, and the editing done by J.T. Berdach are acknowledged.

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NITROGEN CYCLING IN LOWLAND RICE FIELDS WITH SPECIAL ATTENTION TO N_2 FIXATION

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ABSTRACT

A nitrogen balance study in a lowland rice field near Bogor, Indonesia, showed that as much as 60 kg N ha^{-1} $crop^{-1}$ from outside sources contributed to the nitrogen balance on non-N-fertilized plots. An average of about 50 kg N ha^{-1} $crop^{-1}$ was removed by the rice plants, and on non-N plots the nitrogen content of the flooded soils increased by up to 50 kg N ha^{-1} $crop^{-1}$. Some of the nitrogen input came from irrigation water (~ 9.5 kg ha^{-1} $crop^{-1}$) and rainfall (~ 2.5 kg ha^{-1} $crop^{-1}$). The source of the remaining nitrogen (~ 47 kg N ha^{-1} $crop^{-1}$) is generally believed to be biological nitrogen fixation.

On the other hand, the measurements of N_2 fixation on the same plots using the acetylene reduction technique gave an average rate of only 3.5 kg N ha^{-1} $crop^{-1}$. Similar measurements conducted under pot culture conditions in a greenhouse gave a somewhat higher average rate of 9.1 kg N ha^{-1} $crop^{-1}$. Possible causes for these differences are discussed. It is concluded that a better and more reliable method must be found for assessing the rate of nitrogen fixation in flooded rice fields.

INTRODUCTION

Up to several years ago, rice has been cultivated for centuries on the same lands in south-east Asia, year after year, with hardly any addition of fertilizers. Although the yields were generally rather low, rice planting continued without showing any decline in yields. The maintenance of soil fertility in flooded rice fields has been ascribed mainly to biological N_2 fixation.

Since approximately forty years ago (De, 1939), many research workers have investigated the occurrence of nitrogen fixation in rice fields. However, most of these investigations were mainly qualitative in character. Since the development of the acetylene reduction technique, about twelve years ago, for measuring nitrogen-fixing activities of different systems (Hardy *et al.*, 1968), several papers have reported quantitative studies on the extent of N_2 fixation in lowland rice fields (Watanabe & Brotonegoro, 1980). Some of those studies were carried out by taking samples of soil or water from the rice fields and measuring the nitrogenase activities in the laboratory. Such measurements could introduce artifacts by giving better aeration and/or light, thus producing artificially high rates of N_2 fixation.

In the present investigation, measurements were carried out in the field to estimate the rate of biological nitrogen fixation *in situ*. Similar measurements were carried out in pot cultures under laboratory conditions in a greenhouse. This paper compares the results with estimates derived from a nitrogen balance study carried out on the same plots.

MEASUREMENTS IN THE FIELD

Measurements of nitrogen fixing activities of lowland rice fields were carried out in several plots belonging to a larger, long-term, fertility trial of the Central Research Institute of Agriculture, at the Muara Experimental Station near Bogor. Its soil has been classified as a latosol clay with an average pH of 5.8, a total organic carbon content of 1.97% and a total nitrogen content of 0.21%. To measure the acetylene-reducing activities of rice rhizospheres, bottomless bottles, each of one litre capacity, were inserted into the soil in the vicinity of rice plants. After being closed and injected with 0.2 atm. of acetylene, the production of ethylene was measured within 24 hours using a gas chromatograph. The theoretical conversion factor of 1/3 mole of N_2 fixed per one mole of acetylene reduced was used to convert the rate of acetylene reduction into that of nitrogen fixation. The proper conversion factor could not be determined because of lack of facilities for conducting measurements with $^{15}N_2$.

Table 1. Rates of nitrogen fixation in situ measured at the vicinity of rice plants grown on field plots receiving different amendments

Condition of light	Soil amendment[a]	Weeks after transplanting							
		1	2	3	5	7	9	11	13
		(g N ha^{-1} day^{-1})							
Light	N_0 S_0	4.58	13.35	47.92	83.39	16.50	41.52	20.04	3.21
	N_0 S_1	16.60	18.39	16.80	3.64	1.40	2.48	7.98	12.30
	N_1 S_0	4.58	6.31	4.96	6.28	2.69	0.71	2.79	0.55
	N_1 S_1	9.80	19.43	7.00	3.49	1.38	1.26	1.13	0.84
Dark	N_0 S_0	-	3.75	2.28	5.70	4.26	1.97	5.66	2.07
	N_0 S_1	-	2.19	1.84	0.90	1.79	1.61	3.23	2.43
	N_1 S_0	-	0.60	1.68	0.90	0.72	1.29	2.61	0.68
	N_1 S_1	-	3.03	2.99	1.26	1.02	0.97	0.83	0.77

[a] All plots received P (in the form of triple superphosphate) which was equivalent to 13 kg P ha^{-1};

N_0 - received no N fertilizer; N_1 - received urea equivalent to 120 kg N ha^{-1} split into 40 kg as a basal application, 40 kg at tillering and 40 kg at panicle initiation stages.

S_0 - received no rice straw; S_1 - straw from the previous crop (about 2.5 t ha^{-1}) was mixed with the topsoil during the preparation of the plots.

The results of these measurements (Table 1) show the fluctuations in N_2-fixing activity in the soil around the rice hills. The cause of these fluctuations is not known, but may be related to the age of plants. In plots receiving only phosphorus fertilizer, the activity increased considerably in the second week after transplanting and reached a maximum in the fifth week. In the eleventh week the activity decreased and reached a very low level by the end of the growing season.

Contrary to some findings reported by other research workers (Matsuguchi, 1979) in the present experiment, addition of rice straw did not enhance nitrogen fixation in lowland rice fields. The straw did stimulate the activity somewhat in the first three weeks after transplanting, but also depressed the process during most of the rest of the growing period of the rice plants. The cause of this depression is not known.

In agreement with the findings obtained in a study of nitrogen fixation by pure cultures of nitrogen fixers in the laboratory (Brotonegoro, 1974; Mulder & Brotonegoro, 1974), the nitrogen-fixing activity in flooded rice fields was almost totally inhibited by the addition of urea. This inhibition was only partially alleviated by the addition of rice straw which may have caused immobilization of the urea N.

Results obtained using darkened assay chambers showed that the rate of nitrogen fixation in lowland rice fields in the dark was considerably lower than that in the light.

From data presented in Table 1 it can be calculated that the nitrogen fixation in flooded rice fields at Muara reached an average of 3.5 kg N ha^{-1} $crop^{-1}$ in plots receiving P fertilizer only. This amount is quite small compared with the average of 50 kg N ha^{-1} $crop^{-1}$ removed by rice plants.

MEASUREMENTS IN THE GREENHOUSE

Equal-sized, three-week old rice seedlings were transplanted into plastic pots, each of 20 l capacity, containing 20 kg of soil taken from the N_0 S_0 plots of the field experiment at Muara. Prior to rice planting, the soil had been amended with rice straw and/or mineral fertilizers as described in the footnote of Table 2, and flooded with rainwater to 2 cm above the soil surface. All these pots were then placed in a greenhouse. The rates of nitrogen fixation were measured weekly by means of an acetylene reduction technique, similar to that used in the field.

Table 2 shows the fluctuations in N_2-fixing activity in the soils at the vicinities of rice plants in the pots. In the pots receiving only P fertilizer the activity increased in the third week after transplanting. In the sixth week the activity reached a high level and remained high until the tenth week. In the eleventh week the activity was decreasing consid-

erably and reached a very low level at the end of the growing period of the rice plants.

As in the field, the addition of rice straw to the soil stimulated nitrogen fixation during the first five weeks of rice growth. However, during the rest of the growth period, straw depressed the nitrogen-fixing activities in the flooded soils. The inhibition of nitrogen fixation by urea was much higher in the pot experiment than in the field. This was probably due to the absence of surface run-off and leaching in the pots which created a higher concentration of inorganic N in the pots. The addition of rice straw to the urea-amended pots did not alleviate this inhibition.

Table 2. Rates of nitrogen fixation in flooded soil, measured at the vicinity of rice plants grown in pots receiving different soil amendments

Condition of light	Soil amendment[a]	Weeks after transplanting									
		1	3	5	6	7	8	9	10	11	12
		($g\ N\ ha^{-1}\ day^{-1}$)									
Light	$N_0\ S_0$	9.50	55.94	22.10	151.51	96.34	120.72	100.56	163.22	32.38	10.63
	$N_0\ S_1$	76.63	117.24	119.30	47.83	35.09	20.52	26.62	63.31	11.45	6.24
	$N_1\ S_0$	0.22	0.34	0.43	0.43	0.72	0.60	0.34	0.26	0.38	0.28
	$N_1\ S_1$	4.01	1.58	0.70	0.72	0.70	0.55	0.38	0.38	0.38	0.48
Dark	$N_0\ S_0$	28.20	44.86	15.38	32.04	77.28	18.29	25.42	48.12	12.26	4.73
	$N_0\ S_1$	59.21	47.83	29.33	30.46	11.47	0.86	0.70	1.30	4.03	0.55
	$N_1\ S_0$	0.22	0.26	0.55	0.48	0.86	0.77	0.55	0.26	0.65	0.50
	$N_1\ S_1$	2.11	2.18	0.82	0.65	0.77	0.72	0.91	0.82	0.65	0.94

[a] All pots received P at a rate of 3.10 g triple superphosphate pot^{-1}
N_0 - received no N fertilizer; N_1 - received urea at a rate of 6.33 g pot^{-1}
S_0 - received no rice straw; S_1 - received rice straw at a rate of 0.5 kg fresh weight pot^{-1}.

As in the field, the rate of nitrogen fixation in the dark, measured by using darkened assay chambers, was considerably lower than those measured in the light.

Nitrogen fixation in flooded P-fertilized rice soils in the greenhouse reached an average level equivalent to 9.1 kg N ha^{-1} $crop^{-1}$. This amount is more than twice the average rate obtained in the field. This was probably due to the better water control procedures in the greenhouse. We noted that the replicates which had received less water in the field, and thus had shallower water layer, generally gave higher activity than those receiving a greater depth of water. Thus the thinner water layer of soil in the pots might have contributed to the higher rate of nitrogen fixation in the greenhouse.

NITROGEN BALANCE IN A LOWLAND RICE FIELD

As stated by Koyama & App (1979), nitrogen balance studies are usually long-term, expensive and time consuming. The longer the experiment and the greater the number of the crops, the better the estimates that will be obtained. A long experimental period is required to get a reliably large change in total soil N. Therefore, such studies have been rejected in favour of shorter-term research on crop productivity in tropical areas.

Long-term fertility-trial plots have been established for more than twenty years at the Muara Experimental Station. Unfortunately, these experiments have not always been accompanied by nitrogen analysis of the soil, crop, irrigation water or rainfall. Therefore, a proper nitrogen balance could not be obtained. In the present study, a nitrogen balance has been made on the plots where the measurements of nitrogen fixation *in situ*, reported in the preceeding section, had been carried out in 1975.

As had been found in some experiments in the Philippines (Koyama & App, 1979), we found that the total soil N value after some cropping exceeded the original value. The original total soil N for the 0-15 cm depth was 0.18% in 1971, when the experiment was commenced. In 1975 the soil N value was 0.20-0.21% in non-N-fertilized plots, and 0.23% in N-fertilized ones. The increase of soil N during one crop was calculated by dividing these differences in total soil N values by twelve (four years, with maximum of three crops for each year).

Table 3. Nitrogen balances in a lowland rice field at Muara Experimental Station, Bogor, Indonesia

Soil amendments[a]	Inputs (A)				Outputs (B)			Balances (B-A)
	Fertilizer N	Straw N	Irrigation + Rain-N	Total	Increase in Soil-N	Crop N	Total	
	(kg N ha^{-1} $crop^{-1}$)							
N_0 S_0	-	-	13	13	50	30	80	67
N_0 S_1	-	12.7	13	25.7	33.3	46	79.3	53.6
N_1 S_0	120	-	13	133	83.3	64	147.3	14.3
N_1 S_1	120	12.7	13	145.7	83.3	61	144.3	-1.4

[a] For explanations of the symbols used for soil amendments see Table 1.

The amount of N found in irrigation water was calculated by multiplying the average water requirement of one crop (from three successive crops), that amounted to 6276 m^3 ha^{-1} (Partohardjono & Suzuki, 1978), with the average content (1.5 ppm) of forty samples of water obtained from an upper level supply ditch, as reported by Newton (1962). The result is approximately 9.5 kg N ha^{-1} $crop^{-1}$, which is almost twice the estimates reported by Koyama & App (1979). The amount of N which can be expected from rainfall was calculated by multiplying the annual precipitation (3730 mm) with the average N content of rainwater of 0.2 ppm (Brotonegoro & Sukiman, unpublished). When divided by three, it will give the amount of N contained in rainwater of 2.5 kg N ha^{-1} $crop^{-1}$, which is similar to that reported by Koyama & App (1979).

Table 3 shows the nitrogen balances obtained in the present study. The values for non-N-fertilized plots were positive and varied from 53 to 67 kg N ha^{-1} $crop^{-1}$. As is frequently reported in N balance studies on dryland soils (Allison, 1965), a lower balance was found where inorganic fertilizer was applied with straw.

Table 4. Average amount of nitrogen fixed in flooded soils, measured at the vicinities of rice plants, compared to a nitrogen balance of the same plots

Soil amendments[a]	Nitrogen fixation[b]		Nitrogen balance[c]
	Field	Greenhouse	Field
	(kg N ha^{-1} $crop^{-1}$)		
N_0 S_0	3.46	9.13	67
N_0 S_1	1.20	6.30	53.6
N_1 S_0	0.44	0.06	14.3
N_1 S_1	0.66	0.17	-1.4

[a] For the explanations of the symbols, see Table 1.

[b] Measured by using the acetylene reduction technique with a conversion factor of three.

[c] See Table 3.

DISCUSSION AND CONCLUSIONS

Table 4 shows a comparison between the average amount of N_2 fixed in flooded rice soils and the nitrogen balance of the same soils. If it is assumed that the nitrogen balance in non-N-fertilized plots indicates the minimum amount of nitrogen fixed in these plots (since N losses due to denitrification, volatilization and leaching were not yet taken into account in the outputs), it can be concluded that the rate of N_2 fixation derived from the N balance study is much higher than that obtained by direct measurements with the acetylene reduction method. This difference may be due to some of the following factors:

(1) *Azolla* and other small aquatic plants that may harbour N_2 fixers in their leaves or roots had been excluded from direct measurement of N_2 fixation *in situ*; the contribution of these plants, especially *Azolla*, to the nitrogen economy of rice fields could be much larger than those of free-living N_2 fixers in soils;
(2) in flooded soils, the transfer of acetylene to the nitrogen fixing sites in soil water systems, and the release of the evolved ethylene to its gas phase in the assay chamber may limit the apparent acetylene reducing activity; as much as 52% of the evolved ethylene was reported to remain in the water and soil, and could be released partially by stirring (Watanabe *et al.*, 1977);
(3) the evolved ethylene could be oxidized by some soil bacteria (de Bont, 1976) and therefore give an underestimate of acetylene reducing activity of the soils;
(4) some gas leakage could lead to apparently lower acetylene reducing activity.

On the other hand, the following factors could eliminate the negative factors mentioned above:
(1) in flooded soils, certain anaerobic sporeformers could produce ethylene (Smith & Cook, 1974); the concentration of ethylene produced in soils under waterlogged conditions could sometimes be very significant in causing a higher acetylene reducing activity of the soils (Smith & Restall, 1971);
(2) as mentioned above, in the present report the theoretical conversion factor of three had been used to convert the moles of acetylene reduced into the moles of N_2 fixed, but values as high as 25 have been reported for flooded soils (Hauck, 1979).

In conclusion, it is suggested that a better and more reliable method, possibly by using $^{15}N_2$, is required for assessing the rate of nitrogen fixation in flooded rice fields.

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GROWTH AND NITROGEN FIXATION CAPACITY OF SOME BLUE-GREEN ALGAE IN WEST MALAYSIA

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ABSTRACT

Thirteen heterocystous blue-green algae species, namely Anabaena *(3 species),* Nostoc *(4 species),* Fischerella, Gloeotrichia, Hapalosiphon, Tolypothrix, Cylindrospermum *and* Microchaete *were isolated from paddy soils in West Malaysia. Growth rate and nitrogen fixation activity of unialgal cultures of these species were studied under different temperature and light intensity conditions. Different species reacted differently to these conditions. In general, 28-30 C and 5000 lx light intensity supported better growth. High temperature and greenhouse full sunlight suppressed growth.* Gloeotrichia, Nostoc *and* Anabaena *were fast growing species. High rates of nitrogen fixation were found at 28-30 C under 5000 lx continuous light or at variable temperature of 24-33 C under natural light conditions in a Greenhouse.* Anabaena, Cylindrospermum *and* Hapalosiphon *showed the highest nitrogen fixation capacity followed by* Nostoc, Fischerella *and others.*

INTRODUCTION

Blue-green algae are considered to be the most important contributors to natural nitrogen fixation in paddy fields. Stewart (1969) has shown that fifty heterocystous species of blue-green algae were capable of fixing atmospheric nitrogen. However, information on the blue-green algae in Malaysia is scanty. Johnson (1969) isolated four species of blue-green algae from Malaysian paddy fields. Ang (1975) and Broughton *et al.* (1976) tested the nitrogen fixation capacity of three blue-green algae species isolated from a paddy field in the Gombak area. In this work, more nitrogen fixing blue-green algae were isolated in West Malaysia. The growth rate and nitrogen fixation capacity of these local species were studied under different temperature and light conditions.

MATERIALS AND METHODS

Thirteen unialgal cultures of blue-green algae isolated in West Malaysia were purified to bacteria-free, using the method suggested by Wieringa (1968). Absence of turbidity in the culture media was taken as the criterion for purity of culture.

To ensure that all algae were in an actively growing condition before starting an experiment, all unialgal cultures were grown for four days in a nitrogen-free medium (Allen & Arnon, 1955), which was diluted fourfold to avoid precipitation during autoclaving. Three ml of each culture was transferred with a sterile pipette into a 250 ml conical flask containing 100 ml of the medium. The flasks were stoppered with cotton plugs and were shaken at 150 r.p.m. in Gallenkamp Illuminated Cooled Orbital Incubators at 20-22 C, 28-30 C or 36-38 C under constant illumination of 5000 lx. For high light intensity treatments, cultures were shaken on a New Brunswick shaker in a greenhouse.

One ml samples, taken from these cultures at different times, were placed in venoject tubes (10 ml capacity) fitted with rubber stoppers. Air in the tubes was replaced with a 1:9 mixture of acetylene and argon at the beginning of the nitrogenase activity assay. After one hour of incubation in the same incubator, the reaction was stopped by introducing 0.4 ml of 4N NaOH with a syringe through the rubber stopper. Production of ethylene from acetylene was determined with a Varian Aerograph Series 1400 Gas Chromatograph with a Porapak T column (152.4 by 0.3175 cm). Chlorophyll *a* was determined by the method of Arnon (1949) and the optical density at 645 nm and 663 nm was measured with a Hitachi 101 spectrophotometer.

RESULTS AND DISCUSSION

It is evident from the results of this work that all thirteen cultures of *Nostoc*, *Anabaena*, *Fischerella*, *Cylindrospermum*, *Gloeotrichia*, *Tolypothrix*, *Hapalosiphon* and *Microchaete* are capable of fixing nitrogen, as has been reported by Stewart (1969). However, great differences were found in the rate of growth and the activity of nitrogen fixation between different species. Different species also reacted differently to the growth conditions. Generally, 28-30 C and 5000 lx supported better growth (Fig. 1). High temperature and full sunlight suppressed growth for some species. *Gloeotrichia*, *Nostoc* species and *Anabaena* species 1 and 2 showed higher growth rates (Fig. 1). Most of these species continued to grow up to the 10th day of the experimental period.

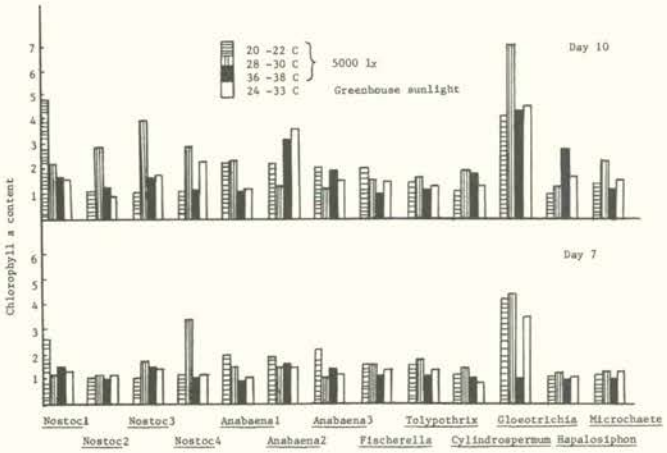

Fig. 1 - Comparison of growth of blue-green algae under different light and temperature conditions. Growth is expressed as chlorophyll a content on day indicated, divided by chlorophyll a content on the third day of culturing.

Fig. 2 shows that high rates of nitrogen fixation are generally found under greenhouse high light conditions or at temperatures of 28-30 C under 5000 lx. There was usually a decline in the rate of nitrogen fixation after the seventh day of culture. *Anabaena* species 2, *Cylindrospermum*, *Hapalosiphon*, *Fischerella*, *Nostoc* species 3 and *Gloeotrichia* showed a higher rate of N_2 fixation among these species.

It is difficult to compare our results with the various reports made on the rate of nitrogen fixation in these algae, because most of the published data are not shown on a chlorophyll basis. Pankow & Martens (1964) showed that the nitrogen fixing capacity of *Nostoc sphaerium* was 0.615 mg nitrogen fixed in 90 days per 10 ml culture solution. Stewart (1962) found that the percentage of total nitrogen fixed per dry weight of *Nostoc entophytum* was 5.83. Laloraya & Mitra (1970) have shown that the amount of nitrogen fixed by *Nostoc puncitforme* and *Fischerella muscicola* was 2.43 and 2.23 per 100 ml culture in 35 days respectively. Further work on the effect of pH and other ecological factors on the growth and nitrogen fixation of selected blue-green algae is in progress.

In general, *Tolypothrix*, *Nostoc* and *Anabaena* are the most frequently found species in West Malaysian paddy fields. During the enrichment culture period, *Nostoc* was the only species which grew fast enough to form visible colonies on a soil surface. *Tolypothrix* grew fast on agar plate. In this work, blue-green algae were cultured in liquid medium under artificial conditions. It is not known whether the growth or nitrogen fixation rate measured under these conditions correlates with the performance of these algae in the field. According to our data, *Gloeotrichia* is a fast growing species and has a high rate of nitrogen fixation. However, it was isolated from only one place (Butterworth) and was seldom found in

the other sampled areas. Therefore, studies on the performance of these species, after being inoculated back to the paddy field soils, are needed.

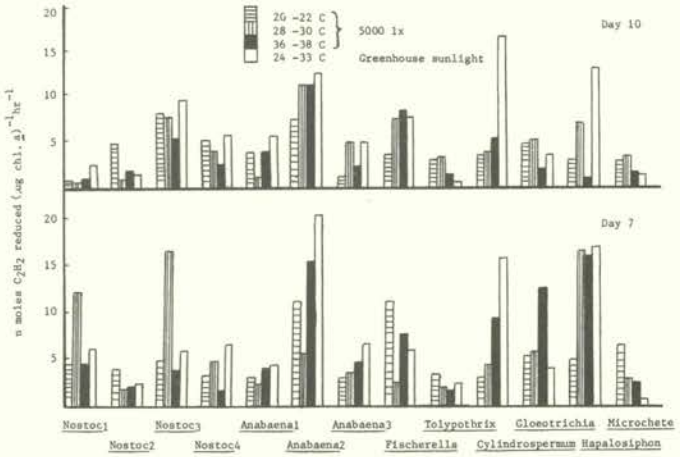

Fig. 2 - Comparison of nitrogenase activity of blue-green algae cultures under different light and temperature conditions.

Henriksson (1970) showed that N_2 fixation in Swedish soils by blue-green algae occurred at the same level during day and night and seemed independent of the temperature fluctuations. Renaut *et al.* (1975) have suggested that in a terrestrial habitat, temperatures higher than 56 C and light intensities higher than 58 000 lx were inhibitory to nitrogenase activity. In this work, high rates of nitrogen fixation were found at 28-30 C under 5000 lx or at 24-33 C under full sunlight in a greenhouse. These light intensity and temperature conditions are similar to those in a field covered by a fully grown rice crop in Malaysia during daytime. This suggests that the shading by a growing rice crop in the field may not inhibit nitrogen fixation by blue-green algae.

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STUDIES ON ALGAL NITROGEN FIXERS AT THE MUDA IRRIGATION CANAL SYSTEM, MALAYSIA

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ABSTRACT

A survey on nitrogen fixing blue-green algae in 11 areas of the MUDA irrigation system of Malaysia indicated the presence of the following species: Anabaena affinis, Chroococcus turgidus, Oscillatoria curviceps *and* Microcystis incerta. *The nitrogenase activities of these organisms could be ranked in the order* M. incerta > A. affinis > C. turgidus > O. curviceps *with values for both light-dependent and dark reactions.*

Biofertilizer studies indicated most of the organisms to be comparatively effective in producing rice grain compared to controls amended with appropriate amounts of N and P. M. incerta, *inoculated at 5 mg chlorophyll (100 g soil)$^{-1}$ in a N-free experimental pot 20 days prior to transplanting of rice seedlings, produced a grain yield increase comparable with the +N control, while with* A. affinis, C. turgidus *and* O. curviceps *the grain yields were 4, 8 and 17% respectively less than control. However, similar inoculations of these algal forms together with the normal level of N supplement produced grain yields in the range 5-38% greater than the +N controls.*

In a pot experiment, amendments with slag, lime, potassium and phosphorus in non-productive acid sulfate soils of the Guar series from the MUDA area culminated in the blooming of Chroococcus *and* Microcystis. *For the 20 t ha^{-1} slag and lime treatments, this resulted in 90 and 100% increases in the dry matter yield of rice. These results can be attributed to the effects of slag and lime and the increased nitrogen availability due to the presence of the algae.*

INTRODUCTION

The nitrogen fixing blue-green algae are ubiquitously distributed throughout the world, from arctic (Fogg & Stewart, 1968) and temperate regions (Granhall & Henriksson, 1969; Henriksson *et al.*, 1972) to the desert regions (Snyder & Wullstein, 1973). However, they are relatively abundant in soils from below 30° latitude (Watanabe & Yamamoto, 1971).

Blue-green algae release organic compounds such as polypeptides, amino acids, ammonia and other growth substances, as products of exosmosis. The death of blue-green algal cells would also be beneficial because of their rapid mineralization, with a release of nutrients (Sweeney, 1976). Their production of mycopolysaccharides also facilitates the propagation of nitrogen-fixing bacteria. It should also be noted that through their extracellular production of certain toxic substances they act as suppressors of the growth of mosquitoes and could therefore have a beneficial effect in the control of malaria (Griffin & Rees, 1956).

Based on these characteristics, there is the possibility of replacing chemical nitrogen fertilizers by the inoculation of blue-green algae as biofertilizers in paddy fields to increase grain yields in the tropics (Watanabe, 1951; Nawawy *et al.*, 1958; Singh, 1951; Iha *et al.*, 1965; Hardy & Havelka, 1975; Sweeney, 1976).

Research in Malaysia on biofertilizers is scanty except for work by Broughton *et al.* (1976). Their study indicated that the major period of nitrogen fixation occurred during the panicle stage (308 g N ha^{-1} d^{-1}). However, the study was confined to the Tanjong Karang district where the rice varieties grown are Matchandi (a strain of *Oryza sativa*), Sekinchan and Sri Malaysia. The blue-green algae identified from this area were two *Nostoc* spp. and two *Anabaena* spp. (Ang Cheng Gek, 1975). The report by Johnson (1975) on nitrogen fixation in Singapore soils is also of interest on a regional basis.

Little work has been done on blue-green algae in the important Muda irrigation area, which produces 37% of the rice of Malaysia. This paper therefore reports on the distribution of blue-green algal communities in this area and the efficiency of isolates as biofertilizers. Their rates of nitrogen fixation were also studied.

The MUDA irrigation area includes about 7 400 ha of low productivity due to the presence of acid sulphate soils known commonly as the Telok and Guar soil series. The paper reports results of an experiment on ways of increasing productivity of the Guar soils through

the application of slag, lime, potassium and phosphorus. Effects on the growth of blue-green algae and paddy yields were studied.

MATERIALS AND METHODS

Survey of existing blue-green algal forms in paddy fields of the MUDA scheme

Soil and water samples were collected from waterlogged paddy fields in 10 different localities as indicated in Fig. 1, during the rice growing seasons of 1977-1978. Prior to identification of the existing blue-green algal forms under a high-powered optical microscope, the samples were incubated for two weeks in a "NIKKO TRON" culture chamber at 30 C using a light source of about 30 000 lx. The density of the identified blue-green algae were categorized in arbitrary values of very low, low, moderate, and high.

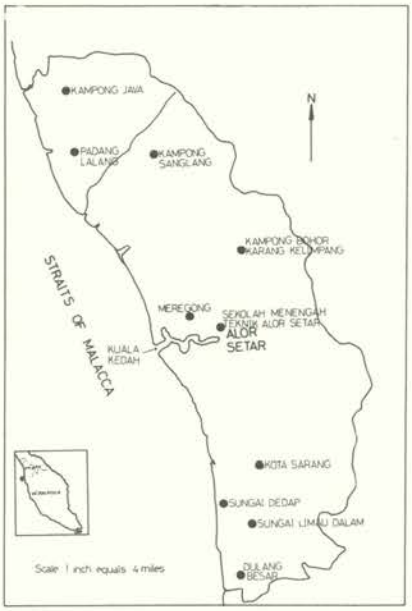

Fig. 1. Map showing location of sampling sites for identification of blue-green algae in paddy fields of the MUDA irrigation area.

Isolation of blue-green algae and their propagation

The identified blue-green algal forms were isolated with the aid of fine capillary tubes under an optical microscope and subcultured in the following media: *Anabaena* spp. in De medium, *Croococcus* spp. in Emerson-Lewis medium, *Microcystis* spp. in the modified medium of Fitzgerald, and *Oscillatoria* spp. in the Cambridge Culture Collection medium (Tamiya & Watanabe, 1965). After isolation, all these culture media were replaced by flood water, obtained from the specific fields where the algae existed, instead of distilled water. The culture media with the specific inoculum were then continuously bubbled with CO_2 and cultured at 30 C under an actinic light intensity of 30 000 lx.

Rate of acetylene reduction by the identified blue-green algal species

The subcultured, almost pure, blue-green algae were evaluated separately for their rates of nitrogen fixation through the acetylene-ethylene reduction technique (Sivalingam, 1979; Stewart *et al.*, 1967). Five ml of algae, equivalent to 0.3 mg chlorophyll ml^{-1}, were used to determine the specific acetylene reduction activity at 30 C and under 30 000 lx actinic light in triplicates. Control samples were incubated similarly and concurrently in darkness. Prior to the initiation of the reduction experiment the reaction vessel was evacuated to 0.9 atm. and the air replaced with pure acetylene (C_2H_2) (Matheson, Co., U.S.A.) at a partial pressure of 0.1 atm. Termination of the reaction was achieved by the addition of 0.5 ml of 5% NaOH.

The concentration of the evolved ethylene both in the light and dark was determined with a Shimadzu GC-5 gas chromatograph fitted with a hydrogen flame ionizing detector and a 2 m long column of Porapak R. Nitrogen was used as the carrier gas at a flowrate of 50 ml min^{-1}. The column temperature was 60 C and injection port and detector temperatures were 170 C. The average amount of ethylene produced in the experimental samples was determined by comparison with standard gas chromatograms.

Pot trials on the effects of various isolated blue-green algae on yields of rice plants

To study the effects of blue-green algae as a biofertilizer on plant growth and yield of rice, trials were performed in pots, each containing 4 kg of soil from paddy fields around the Meregong area. The soil was air-dried, pounded and sieved through a 2.0 mm mesh prior to experimentation. Plain soil without any further amendment was used as the control while triplicate experimental pots each received N, P, and algae amendments as indicated in Table 3. Normally, the amendment with algae isolates was done 20 days prior to direct sowing of 10 rice seeds (Mashuri variety), followed by thinning to three seedlings per pot after two weeks. After 14 weeks of plant growth the dry weights of the straw and grain yields of rice in each treatment were measured. The P + N treatment was taken as control (100%).

Effects of slag, lime, P and K on growth of blue-green algal forms and rice plants on an acid sulphate soil

An infertile, acid sulphate soil of the Guar series was subjected to slag, lime, K, and P in order to improve rice growth and grain yield. In addition, periodic monitoring of the blooming of blue-green algae was followed in the various treatments indicated in Table 5, in plastic pots accommodating 4 kg of experimental soil.

Rice, Mashuri variety, was sown at 10 seeds pot^{-1} and thinned to three seedlings pot^{-1} after two weeks. The experiment was carried out for a period of seven weeks and in the course of the experiment the rate of tillering, pH change, Fe and Mn elution were determined according to Thiagalingam & Zulkifli (1977). In addition, the blue-green algal growth was periodically determined for each treatment, and was related to rice straw yield.

RESULTS

The identified blue-green algal species were *Anabaena affinis*, *Chroococcus turgidus*, *Microcystis incerta* and *Oscillatoria curviceps* (Table 1). Fields in the northern districts allowed greater proliferation of *A. affinis*, *M. incerta* and *O. curviceps* than those further south. Sites in the central district, close to the city of Alor Setar, contained moderate amounts.

Table 1. Blue-green algal nitrogen fixers present in the MUDA irrigation-system paddy fields

Location	Field condition	pH	Algal forms	Density[a]
Sekolah Menengah Teknik Alor Setar	Poorly irrigated	5.7	*Oscillatoria curviceps* C.A. Agaroh	+
			Chroococcus turgidus (Kutzing) Naegeli	+
			Microcystis incerta (Lammermann)	+
Meregong	Poorly maintained field	5.5	Nil	-
Kota Sarang	Newly planted field	5.75	Nil	-
Kampong Java	Well established field	6.5	*Oscillatoria curviceps* C.A. Agardh (Mud Bank)	++++
Sungai Dedap	Experimental field of MUDA Agricultural Development Authorities	6.45	Mud Bank	
			Anabaena affinis Lemmermann	+++
			Oscillatoria curviceps C.A. Agardh	+++
			Floating algal forms	
			Anabaena affinis Lemmermann	++
			Oscillatoria curviceps C.A. Agardh	++
Padang Lalang	Established field with high productivity	6.5	*Oscillatoria curviceps* C.A. Agardh	++++
			Microcystis incerta Lemmermann	++++
Sungai Limau Dalam	Low productivity	6.3	*Anabaena affinis* Lemmermann	+
Dulang Besar	Low productivity	6.03	Nil	-
Kampong Bohor Karang Kelimpang	Established field	8.13	Surface soil	
			Microcystis incerta Lemmermann	++++
			Anabaena affinis Lemmermann	++++
			Oscillatoria curviceps C.A. Agardh	++++
Kampong Sanglang	-	6.82	Nil	-

[a] + = very low, ++ = low, +++ = moderate, ++++ = high.

The rates of acetylene reduction activity of the isolated blue-green algal forms are shown in Table 2. The ranking of light-dependent fixation rates is in the order of *M. incerta* > *C. turgidus* > *A. affinis* > *O. curviceps* while dark fixation is in the order *A. affinis* > *O. curviceps* > *M. incerta* and *C. turgidus*. After summing light and dark fixation rates, the order is *M. incerta* > *A. affinis* > *C. turgidus* > *O. curviceps*.

Table 2. Acetylene reduction activity by blue-green algae at 30 C[a]

Algal species	Ethylene (nM sample^{-1} hr^{-1})	
	Light	Dark
Anabaena affinis Lemmermann	213	153
Chroococcus turgidus (Kutzing) Naegeli	269	45
Oscillatoria curviceps C.A. Agardh	108	95
Microcystis incerta Lemmermann	453	69

[a] Each sample contained *c* 0.3 mg chlorophyll ml^{-1} in 5 ml.

The effect of blue-green algae as biofertilizers is shown in Table 3. The yields of rice straw and grain for algal-amended soils without added N are comparable to the yields of the P + N treatment. On the other hand, algal treatments with the addition of $(NH_4)_2SO_4$ all increased in straw and grain yield above those of the P + N treatment.

Table 3. Effects of blue-green algae on paddy plant growth and yield of rice

Experimental treatment[a]	Dry weight			
	Straw (g pot^{-1})	%[b]	Grain (g pot^{-1})	%[b]
Control - nil	35	43	24	39
P	48	59	38	62
P + N	82	100	61	100
P + *Anabaena affinis*	79	96	59	96
P + *A. affinis* + N	97	118	72	117
P + *Oscillatoria curviceps*	68	83	51	83
P + *O. curviceps* + N	86	104	64	105
P + *Microcystis incerta*	80	98	62	102
P + *M. incerta* + N	110	134	85	138
P + *Chroococcus turqidus*	75	91	56	92
P + *C. turqidus* + N	87	106	66	108

[a] P addition 13 mg (100 g soil)$^{-1}$, N addition 25 mg (100 g soil)$^{-1}$, algal addition 5 mg chlorophyll (100 g soil)$^{-1}$.

[b] % of P + N treatment.

Results on the studies of effects of slag, lime, potassium, and phosphorus treatments on acid sulfate soil (chemical composition, Table 4) are given in Table 5. Evidently the 10 and 20 t ha^{-1} slag and lime treatments are the most effective, with maximum yields in the 20 t ha^{-1} lime and slag treatments. It should also be noted that the blooming of *C. turgidus* and *M. incerta* was highest in the 20 t ha^{-1} treatments.

DISCUSSION

It is obvious from this investigation that four types of nitrogen fixing blue-green algae, viz. *Anabaena affinis*, *Chroococcus turgidus*, *Oscillatoria curviceps* and *Microcystis incerta*, which are in the *Nostocaceae*, *Chroococcaceae* and *Oscillatoriaceae* families, exist in the MUDA irrigation area. In comparison with some other areas the number of general species found is very low. Jutono (1973) reports for soils of Central Java the presence of 7 genera and 12 species of the *Nostocaceae*, 8 genera and 18 species of the *Oscillatoriaceae*, 3 genera and 3 species of the *Rivulariaceae*, 3 genera and 2 species of the *Scytonemataceae*, and 6 genera and 9 species of the *Chroococcaceae*. It should also be noted that the blue-green algal species of *Tolypothrix tenuis* and *Calothrix brevissima*, as reported by Watanabe (1959), were not detected in the MUDA area.

The distribution density shows a trend of northern district areas > central areas (around the city of Alor Setar) > southern district areas. This could probably be due to the influences of the chemical characteristics of the soil and water of these regions.

Based on the acetylene reduction activities of the blue-green algal species and their effects on grain yields, it is evident that *M. incerta* is the most effective (38% increase in grain yield) followed by *A. affinis* (17.3%); *C. turgidus* (8%), and *O. curviceps*. These results are comparable to those reported for *Aulosira fertilissima* and *Tolypothrix tenuis* in Japan and India (Subrahmanyam & Sahay, 1964; Subrahmanyan *et al.*, 1965; Watanabe, 1973).

Table 4. Chemical properties of the 0-15 cm layer of the Guar series soils[a]

Parameter	Value
pH (wet)	3.5
pH (dry)	2.9
Organic matter (%)	4.75
Mechanical Characteristics	
Sand (%)	15
Silt (%)	26
Clay (%)	59
Exchangeable cations (meq (100 g soils)$^{-1}$)	
Ca	2.42
Mg	3.15
Na	0.10
K	0.19
Base saturation (%)	27.1
Soluble sulphate (%)	0.071

[a] Data from Thiagalingam *et al.* (1979).

Table 5. Effects of slag, lime, potassium, and phosphorus treatments on acid sulfate soil from MUDA irrigational system area on the bloom of blue-green algal forms and the plant growth

Treatment[a]	pH		Blue-green algal species	Chlorophyll content (mg ml^{-1})	Dry wt. plant (g pot^{-1})	% of overall max. yield	% of max. yield within treatments
	Initial (1st week)	Final (7th week)					
Control	3.55	4.10	-	-	0.29	0.56	
Slag (t ha^{-1})							
0	3.53	3.73	-	-	16.9	33	36
5			-	-	33.8	65	72
10			Trace of *Chroococcus turgidus* & *Mycrocystis incerta*	0.0006	40.2	76	86
20	4.25	4.71	*Chroococcus turgidus* & *Microcystis incerta*	0.0066	47.0	91	100
Lime (t ha^{-1})							
0	3.53	3.73	-	-	16.9	33	33
5	3.90	4.72	-	-	36.5	70	70
10	4.35	4.79	*Chroococcus turgidus*	0.0008	37.9	73	73
20	5.35	5.60	*Chroococcus turgidus* & *Microcystis incerta*	0.0046	51.9	100	100
Potassium (mg (100 g soil)$^{-1}$)							
0	3.90	4.75	-	-	27.5	53	75
21	4.05	4.43	-	-	33.9	65	93
42.5	3.90	4.72	-	-	36.5	70	100
Phosphorus (mg (100 g soil)$^{-1}$)							
0	3.88	4.05	-	-	1.1	2	3
15 (TSP)	3.85	4.21	-	-	28.1	54	77
30 (TSP)	3.90	4.72	-	-	36.5	70	100
15 (RP)	3.93	3.88	-	-	3.83	7	10
30 (RP)	3.93	3.98	-	-	8.04	16	22
10 (TSP) + 20 (RP)	3.93	4.26	-	-	19.8	38	54
15 (TSP) + 15 (RP)	3.85	4.28	-	-	27.8	53	75

[a] Slag and lime treatments also contained N, P, and K, applied at the rates of 25, 13, and 42.5 mg (100 g soil)$^{-1}$ respectively. Potassium treatments also contained 25 and 13 mg (mg soil)$^{-1}$ of N and P respectively. Phosphorus treatments also contained 25 and 42.5 mg (mg soil)$^{-1}$ of N and K respectively.
TSP = triple super phosphate, RP = rock phosphate.

Amendments of 10 and 20 t ha^{-1} of slag and lime to the acid sulphate soil produced highest yields and algal blooms of *C. turgidus* and *M. incerta*. Blooming of these algal forms could be attributed to the change in pH from 4.25 to 4.71 and 5.36 to 5.60 for the 20 t ha^{-1} slag and lime treatments, respectively. Further, in the same experiment the levels of iron and manganese ions in the soil solution at tillering and harvest were the lowest in the highest yielding treatment (20 t ha^{-1} lime) on this acid sulphate soil (Thiagalingam & Zulkifli 1977). This could have facilitated the observed proliferation of algae. It was also reported that tillering was highest in the forementioned treatments. In this connection, Aiyer *et al.* (1972) demonstrated experimentally that blue-green algae increased the number of productive tillers per unit area by about 30%, although the application of lime and molybdenum had no significant effects. Hence, it can be concluded that slag and lime treatments of acid sulphate soil might facilitate a regulatory effect on Fe and Mn availability, acidity-basicity balance and Ca availability for plant growth. This would prevent Fe or Mn toxicity and improve soil structure. In addition, algae blooms of *C. turgidus* and *M. incerta*, due to the high application of lime or slag, might enhance plant growth and increase grain yield.

It should be noted that the blue-green algal species of *C. turgidus* and *M. incerta* were only noticeable during the tillering stages of the rice plants. The probable reason for this phenomenon is the increase in iron concentration in the culture medium from 352 to 812 ppm for the 20 t ha^{-1} slag treatment and 232 to 421 ppm for the 20 t ha^{-1} lime treatment during the period of experimentation (Thiagalingham & Zulkifli, 1977). Nevertheless, this aspect requires confirmation because blue-green algae are believed to withstand high concentrations of iron. The effects of pesticides should also be considered because extensive spraying normally takes place after the tillering stage.

The results also indicate that, prior to the application of blue-green algal species, the soil should be amended to provide optimal soil conditions for nitrogen fixation. For each soil series these conditions should be thoroughly investigated, and methods for mass culture, preservation, and transportation of cultured species for dispensing should be defined precisely. If these conditions are established, it appears that the use of biofertilizers would not only cut down cost on chemical nitrogenous fertilizers but also result in better paddy yields in the MUDA rice growing area.

ACKNOWLEDGEMENTS

The author wishes to express his deepest gratitude to the School of Biological Sciences, University of Science Malaysia, Minden, Penang, for all the aid rendered during the course of this study and to the Royal Swedish Academy of Sciences for kindly financing his participation in the regional workshop on "Nitrogen cycling in south-east Asian wet monsoonal ecosystems", Chiang Mai, Thailand, 5-11 November, 1979.

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GROWTH AND NITROGEN FIXATION BY AN *AZOLLA-ANABAENA* COMPLEX IN PENINSULAR MALAYSIA

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ABSTRACT

A local species of Azolla (A. pinnata) *grown on flooded soil under the natural light and temperature conditions of Peninsular Malaysia doubled its fresh weight in 5-6 days. Its rate of growth was limited to a large extent by the paucity of nutrients in the floodwater rather than by the tropical environmental conditions it experienced, because the fern, when grown under the same light and temperature conditions but in a nitrogen-free nutrient medium, could achieve a doubling time of 2.8 days, in terms of its fresh weight. The activity of the enzyme nitrogenase in the* Anabaena azollae *was related to the growth of the fern. Addition of phosphate to the floodwater markedly enhanced the growth of the fern and the nitrogenase activity of the algal symbiont.*

INTRODUCTION

Azolla is a genus of small, aquatic, heterosporous fern, all species of which live in symbiosis with a heterocystous blue-green alga *Anabaena azollae* (Smith, 1955). Because of the ability of the algal symbiont to fix atmospheric nitrogen, the aquatic fern in association with the alga can assimilate the fixed atmospheric nitrogen and grow in a nitrogen-free medium. The fern-alga complex is abundant not only in the tropics but also in temperate regions (Moore, 1969). Because it can cover a water surface in a very short time, *Azolla* has been considered a weed (Sculthorpe, 1967). However, it is also used as a green manure for rice crops in Vietnam and South China (Lumpkin, 1977; Watanabe *et al.*, 1977) and as feed for pigs and ducks in Indochina and Taiwan (Moore, 1969).

In Peninsular Malaysia, *Azolla* is widely distributed in rice growing areas. However, there is still no published work on the use of *Azolla* as a source of nitrogen in our paddy fields. One of the common species of *Azolla* growing in Peninsular Malaysia is *Azolla pinnata* and, in the following report, we present results from our preliminary investigation on the growth and nitrogen fixation of the *A. pinnata-Anabaena azollae* complex on flooded soil. These studies are pertinent to the potential utilization of *A. pinnata* as a source of nitrogen and minerals in paddy fields.

MATERIALS AND METHODS

The growth of *A. pinnata* on artificial medium (Watanabe *et al.*, 1977), or on flooded soil under natural light and temperature conditions, was followed in cement tanks at the Botany Department, University of Malaya. The tanks (103 x 72.5 x 30.5 cm) were filled with culture medium to a depth of 10 cm or were half-filled with black garden soil and flooded with water to a depth of 10 cm. The soil and water were thoroughly mixed by repeated stirring. The floodwater was allowed to settle for a day before *Azolla* was introduced on to the surface. Initial pH of the floodwater was 5.8, and mineral elements were added as required according to nutritional studies. All growth studies were replicated, usually in triplicate.

Nitrogenase activity of the *A. pinnata-Anabaena* complex was assayed with the acetylene-reduction technique. The uses and limitations of this technique have been discussed (Stewart *et al.*, 1967; Hardy *et al.*, 1973). Usually 1 g fresh weight of the fern was introduced into 50-ml calibrated Erlenmeyer flasks containing 3 ml of the growth medium and sealed with a gas-tight serum cap. The flasks were incubated under natural light and temperature conditions in a large water bath, to avoid temperature rise due to exposure to sunlight. The assay was started by injecting acetylene gas into the flask to give a final acetylene partial pressure of 0.125 atm. The gas phase was analysed after 30 minutes and after 1 hour. Under our experimental conditions acetylene at a partial pressure of 0.1 atm and above is saturating for acetylene reduction (Fig. 1). The incubation temperature varied from 30-32 C and light intensity from 30 to 65 klx.

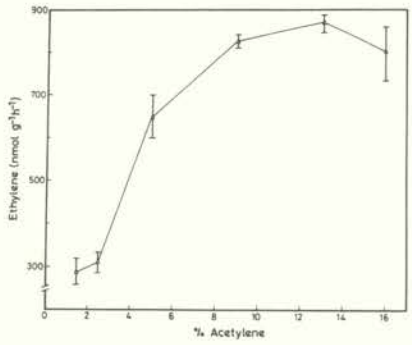

Fig. 1. The effect of acetylene concentration on nitrogenase catalyzed ethylene production by the A. pinnata-Anabaena *complex.*

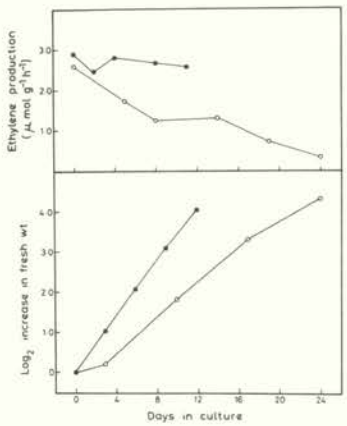

Fig. 2. Growth and acetylene reduction activity of A. pinnata *cultured in floodwater (O) and in a nitrogen-free medium (●). The growth of the fern was followed in cement tanks under natural light and temperature conditions.* Azolla *plants grown in a nitrogen-free medium under natural light and temperature conditions were used as the starting material.*

RESULTS AND DISCUSSION

The concentrations of the main macroelements in floodwater and those used in the nitrogen-free medium are given in Table 1. Except for sodium, the concentrations in the floodwater were less than 20% of those present in the nutrient medium used. Magnesium and sulphur in particular, were present at a very low concentration.

Table 1. Concentration of major nutrients in culture media

Macroelements[a]	Floodwater	N-free medium
Ca	2.93 ± 0.67 ppm	40 ppm
Mg	0.31 ± 0.05 ppm	40 ppm
Na	6.42 ± 0.43 ppm	15 ppm
K	7.75 ± 1.98 ppm	40 ppm
P	3.78 ± 0.05 ppm	20 ppm
S	ND[b]	69 ppm

[a] The metal ions (Ca, Mg, Na, K) were determined by atomic absorption spectrometry. P was assayed by the ammonium molybdate method in sulphuric acid system (Ozbun *et al.*, 1973) and S by turbidimetric determination (Jackson, 1958).

[b] ND = not-detectable by method used.

Results (Fig. 2) show that the *Azolla-Anabaena* complex on floodwater doubled its fresh weight in 5-6 days. The rate of growth was constant for 2 weeks after an initial period of adaptation. Over the entire culture period of 24 days the *Azolla* had doubled its fresh weight four times. During that period the pH change of the floodwater was from 5.80 to 6.95. That the growth in floodwater may be limited by the low concentration of mineral elements is indicated by the much faster growth of the complex in an artificial nitrogen-free nutrient medium, identical to that used by Watanabe *et al.* (1977). From Fig. 2, it is seen that the fern-alga complex grown in the nitrogen-free medium doubled its fresh weight in less than 3 days. This doubling time is in agreement with the optimum growth rates reported for *A. pinnata* (Brontonegoro & Abdulkadir, 1978; Watanabe, 1978) and for other *Azolla* species (Watanabe *et al.*, 1977; Holst & Yopp, 1979). However, even in floodwater, the growth rate of *A. pinnata* is comparable to that reported for a slower growing species, *A. caroliniana* (Peters, 1977).

Pigment formation in *A. pinnata*, grown in floodwater, occurred after 17 days in culture, while the fern grown in a nitrogen-free nutrient medium did not develop the red pigment at all. It appears therefore, that pigment formation in *A. pinnata* is associated with the growth conditions of high light intensity coupled with low nutrient levels.

Nitrogenase activity (measured as acetylene reduction) of the *A. pinnata-Anabaena* complex reflects its growth rate (Fig. 2). The acetylene reduction activity of the fern-alga association grown in a nitrogen-free medium is higher than that grown in floodwater. The nitrogenase activity of *Azolla*, grown in a nutrient medium, remained more or less constant throughout a 2-week culture period and only started to decline slightly subsequently, when the medium was not renewed. On the other hand, the nitrogenase activity of *A. pinnata-Anabaena* complex on floodwater was high at the beginning (because the starting material used was an *Azolla* culture grown in nitrogen-free medium) but stabilized at a much lower value after one week's culture. At the end of the second week of culture, the activity of the nitrogenase enzyme decreased again. This decrease in activity was correlated with the decline in the rate of growth of the fern over the same period of time.

An experiment was carried out to determine the nitrogenase activity of the *Azolla-Anabaena* complex on floodwater enriched with supplements of major and minor elements of the nitrogen-free medium used in the experiment mentioned above. The results (Table 2) confirmed the earlier findings that growth and nitrogen-fixing ability of the *Azolla-Anabaena* complex are limited by the paucity of nutrients in the floodwater. However, it can be seen from Table 2 that floodwater supplemented with minor elements increased neither the rate of growth nor the nitrogenase activity of the fern-alga association. In contrast, addition of major elements increased its growth and nitrogenase activity markedly, although the best growth was obtained in the treatment where the floodwater was supplemented with the full complement of major and minor elements.

Table 2. Effect of nutrients on the growth and acetylene reduction of A. pinnata-Anabaena[a]

Treatment	Increase in fresh[b] weight (%)	Acetylene[c] reduction (%)
Floodwater (FW) (control)	213	100
FW + major elements	322	382
FW + minor elements	217	71
FW + major and minor elements	478	710

[a] *Azolla* plants growing on flooded soil under natural light and temperature conditions were used as the starting material in this experiment. Each treatment was triplicated. Fresh weight and acetylene reduction measurements were taken on the sixth day.

[b] Increase in fresh weight is expressed as % of the initial weight.

[c] Acetylene reduction is given as % of the acetylene reduction in the control.

One of the major elements which has been reported to influence the growth and nitrogenase activity of *Azolla* is phosphorus (Cohn & Renlund, 1953; Talley *et al.*, 1977; Watanabe *et al.*, 1977; Watanabe, 1978). In fact, the growth and development of high nitrogen-fixing ability in *Azolla* depends on a suitable concentration of phosphorus. Our observations indicate that an addition of 20 ppm Phosphorus to floodwater either as monobasic calcium phosphate

(Fig. 3) or as sodium dihydrogen phosphate (Table 3) increased the rate of growth and the nitrogenase activity in the alga-plant association. The addition of other macroelements to the floodwater (Table 3) did not affect nitrogen fixation in *A. pinnata*. From Fig. 3, it is of interest to note that when the floodwater was stirred regularly during the experimental period to facilitate the release of soil nutrients into the floodwater, the growth and acetylene reduction rates were enhanced compared to the growth and acetylene reduction of the fern growing on the surface of an undisturbed body of floodwater. Our results also indicate that in shallow water where the roots could reach the soil surface, the growth and acetylene reduction rates for *A. pinnata* did not improve. Hence, the suggestion that root tips must touch the soil surface for good growth (Schaede, 1947) appears to be incorrect as was pointed out by Moore (1969). We noticed that the roots never penetrated into the soil.

Table 3. Comparison of effects of individual salts[a] on acetylene reduction of A. pinnata-Anabaena

Treatment	Acetylene reduction[b] (%)
$NaH_2PO_4.2H_2O$	278
$MgSO_4.7H_2O$	114
K_2SO_4	103
$CaCl_2.2H_2O$	80
Full nutrient (major & minor elements)	251
Control floodwater)	100

[a] Individual salts were added to the floodwater at concentrations equal to those in the N-free medium.

[b] Acetylene reduction was determined after four days, and its activity is shown as % of the control.

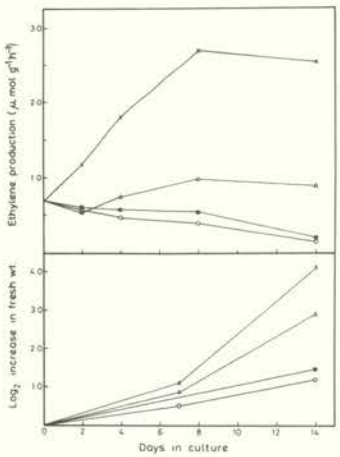

Fig. 3. Growth and acetylene reduction activity of A. pinnata *cultured under different conditions:- (O) Floodwater; (x) floodwater + $Ca(H_2PO_4)_2$; (Δ) frequently stirred floodwater; (●) shallow floodwater. The starting material used for all treatments were* Azolla *plants grown on floodwater under natural light and temperature conditions. Other experimental details are as given in Fig. 2.*

From our observations, it is concluded that *A. pinnata-Anabaena* can grow well under the high temperature and light conditions of the tropics. In floodwater, the release of mineral nutrients, especially phosphorus, from the soil appears to be the factor limiting its growth and nitrogen fixation. However, with a suitable application of phosphate not only does the complex double its fresh weight in three days but its nitrogen fixing ability is also greatly enhanced.

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EPIPHYTIC NITROGEN FIXATION ON WEEDS IN A RICE FIELD ECOSYSTEM

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ABSTRACT

*Epiphytic N_2 fixation on submerged (*Chara *sp., *Najas *sp.) and non-submerged (*Monochoria *sp., *Cyperus *sp.) weeds in a paddy field was studied by:*

- *evaluating the weed biomass in planted and fallow fields;*
- *measuring specific dark and light-dependent acetylene reducing activity (ARA);*
- *enumerating and identifying epiphytic N_2-fixing micro-organisms.*

Submerged weeds produced a mean biomasses of 1 t ha^{-1} at rice tillering and 3 t ha^{-1} at rice harvest stage; under fallow they reached 7.5 t ha^{-1}. Corresponding biomasses of non-submerged weeds were 1.7 t ha^{-1} under rice and 7.7 t ha^{-1} under fallow at rice harvest stage.

Dominant N_2-fixing Cyanobacteria were Gloeotrichia *sp.,* Nostoc *spp. and* Calothrix *spp. Epiphytism by* Gloeotrichia *was predominantly on* Chara *whereas that by other Cyanobacteria did not exhibit any host selectivity. Submerged weeds harboured both aerobic and micro-aerophilic N_2-fixing bacteria. Growth on glucose medium showed the presence of acid-gas-producing organisms (probably Enterobacteriaceae), while growth on malate revealed* Azospirillum*-like organisms. Light ARA on the submerged weeds (29-35 nmole C_2H_4 (g fresh weight)$^{-1}$ h^{-1}) was about ten times higher than that on the non-submerged ones (1.8-4.4 nmole C_2H_4 (g fresh weight)$^{-1}$ h^{-1}). Dark activity was about the same for all the weed types studied (0.9-2.5 nmole C_2H_4 (g fresh weight)$^{-1}$ h^{-1}). Relating specific ARA to weed biomass measurements it was found that the non-submerged weeds exhibit a very low activity (0.4-2.3 g N ha^{-1} d^{-1}) while the activity on submerged weeds (5-34 g N ha^{-1} d^{-1}) makes an appreciable N_2 input into this ecosystem.*

INTRODUCTION

Biological nitrogen fixation contributes significantly to the fertility of rice soils. Microorganisms operative in this process and their spatial distribution within a rice field ecosystem are illustrated in Fig. 1. Studies have been conducted in most of the components depicted in this Figure and have been recently reviewed by Dommergues & Rinaudo (1979) on the rhizosphere; by Matsuguchi (1979) on heterotrophic bacteria; by Roger & Reynaud (1979) and Venkataraman (1979) on the blue-green algae (BGA), and by Watanabe (1978) and Becking (1979) on *Azolla*. There are a few reports on nitrogen-fixing bacteria associated with rice stems (Watanabe *et al.*, 1979; Watanabe & Barraquio, 1979) and nitrogen fixation by blue-green algae epiphytic on fresh water macrophytes (Finke & Seeley, 1978), but we are unaware of any studies on nitrogen fixation by epiphytic BGA in rice fields.

The epiphytic microflora appears to occupy an ecological niche with certain distinctive features. Being attached in a somewhat permanent submerged position, BGA are protected from dessication and inhibitory effects of high solar radiation (Reynaud & Roger, 1979). This epiphytic habit is advantageous to the heterotrophic bacteria, which may obtain nourishment from their hosts, but no nutritive association between the algae and the host plants has yet been found.

The submerged weed population in a rice field can develop into a considerable biomass (Saito & Watanabe, 1978) and the nitrogen fixed by their epiphytic microflora could then make a significant contribution to the total nitrogen input. Studies were therefore undertaken to investigate the epiphytic N_2 fixation on weeds in a rice field.

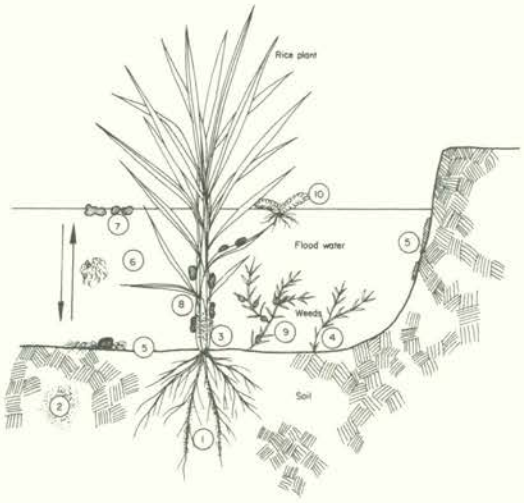


Fig. 1. Diagram of N_2-fixing components in a rice field ecosystem.

Bacteria	*Cyanobacteria*
1) rhizosphere	*5) Soil water interface*
2) soil	*6) free floating*
3) epiphytic on rice	*7) water air interface*
4) epiphytic on weeds	*8) epiphytic on rice*
10) Azolla	*9) epiphytic on weeds*

MATERIALS AND METHODS

Experimental

An experiment was conducted in 1.5 m^2 plots with four treatments in triplicate, distributed on a randomized block design. Each plot was planted separately with either submerged weeds (*Chara* sp. or *Najas* sp.) or non-submerged weeds (*Monochoria vaginalis* or *Cyperus iria*). The plots with submerged weeds were sampled by harvesting the total plant material in a plot, mixing them together and removing random triplicate 10-g samples for subsequent analysis. In the case of non-submerged weeds, the root system and the aerial parts above the flood water level were first cut off, and the remaining material was mixed together before sampling. The samples were studied in regard to their specific acetylene reducing activity (ARA) and their epiphytic algal and bacterial flora.

As it was observed that more algae were present on the older parts of submerged weeds, old and young parts were separated and aliquot samples were analyzed for ARA and N_2-fixing microflora. To extrapolate to the field from the specific ARA measurements (expressed in terms of activity per gram of host), an assessment of the weed biomass and its variability in the field was done at two stages of the growth of rice and in fallow plots.

Acetylene reducing activity

ARA measurements were conducted in 250-ml Erlenmeyer flasks, under an atmosphere of 10% acetylene in air. Incubation was done either under 800 lx provided by fluorescent lights, or in the dark by wrapping the flask with aluminium foil. Plant material destined for the dark incubation was covered *in situ* with a black cloth the day before harvesting, in order to eliminate any residual algal activity. Gas samples were removed after 0.5, 1, 2, 4, and 6 hours of incubation and analysed by gas chromatography.

Algal counts

Algae were enumerated by plating on BG II medium (Allen & Stanier, 1968) with and without combined nitrogen to estimate respectively the total and the N_2-fixing algal populations. After incubating for three weeks at 30 C under continuous fluorescent light (800 lx) the plates were observed under a stereoscopic microscope, algal colonies were identified and separately counted.

Bacterial counts

Aerobic heterotrophic N_2-fixing bacteria were enumerated by the most probable number (MPN) technique as described by Watanabe *et al.* (1979). Inoculation was done into semi-solid glucose-yeast extract medium, which usually gives higher counts than malate medium (Watanabe *et al.*, 1979) and into malate-yeast-extract medium to detect the presence of *Azospirillum* (Day & Döbereiner, 1976). After incubating for two days at 30 C, the tubes were exposed to

10% acetylene in air for 24 hours and the ethylene formed was measured. The tubes with ethylene values twice that of the uninoculated controls were considered as positive.

Total aerobic heterotrophic bacteria were enumerated by spreading on tryptic-soy (0.1%) agar (1.5%) plates (Watanabe & Barraquio, 1979). After one week of incubation at 30 C, the colonies were counted on the plates containing 30 to 300 colonies.

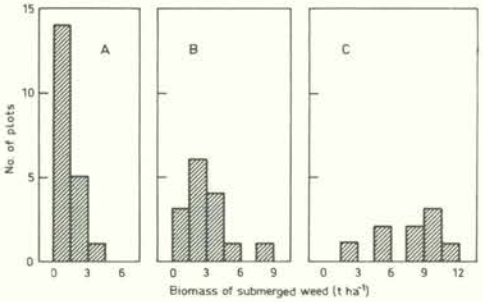


Fig. 2. Distribution of submerged weeds biomass (t ha^{-1} fresh weight) among
A: 20 plots at end of tillering; the plots had been handweeded four weeks before the measurement,
B: 15 plots at harvesting stage; no weeding was performed,
C: 9 fallow plots at harvesting stage of rice.

RESULTS

Biomass of weeds

The distribution of the biomass of submerged weeds (*Chara* and *Najas*) is shown in Fig. 2 at tillering stage, harvesting stage and in a fallow plot at harvesting stage of rice. This Figure shows that the submerged weed population under a rice crop at the end of tillering had a mean biomass of about 1 t ha^{-1} within a range of 0.4 to 3 t ha^{-1} and that it had increased at maturity to a mean of 3 t ha^{-1} within a range of 0.2 to 4.5 t ha^{-1}. The highest values, which ranged from 2.7 to 12 t ha^{-1} with a mean of 7.5 t ha^{-1}, were recorded in the fallow plots. Twenty field measurements of non-submerged weed biomass under rice cropping gave a mean value of 1.7 t ha^{-1} and a maximum of 4.1 t ha^{-1}. In fallow plots, completely covered either with *M. vaginalis* or *C. iria*, the values obtained were 7.7 t ha^{-1} and 2.8 t ha^{-1} respectively, of which about 10% was found to remain submerged. These figures give an idea of the weed biomass that is available for colonization by epiphytic microorganisms in a rice field.

Epiphytic microorganisms and their distribution

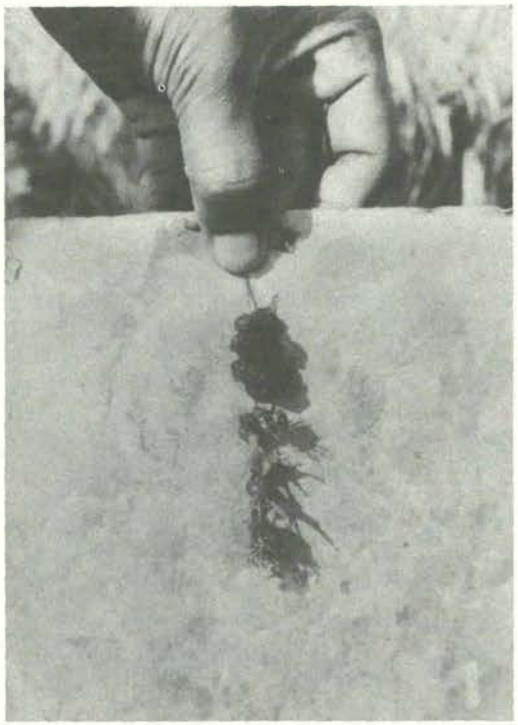
Two types of algal epiphytism, (1) visible to the naked eye and (2) observable only under the microscope, were noticed, specially in the case of the submerged weeds. In the first type, globose gelatinous colonies of *Gloeotrichia* (2-10 mm in diameter) were attached to the *Chara* filaments (Plate 1a). The distribution of the colonies on the host was frequently unequal, the older parts being more heavily colonized (Plate 1a, 1b). The second epiphytic habit, which could be seen only under the microscope, was predominantly due to *Nostoc*, *Calothrix* and *Anabaena* sp., whose filaments grew firmly attached to the host surface. Even in this case, colonization by the epiphytes became progressively higher from apex to base of the host and this was quite apparent among the young, intermediate and old leaves of *Najas*. These observations were confirmed by algal enumerations done separately on old and young parts of *Chara* and *Najas*. Table 1 shows that the total algal population on the old parts was four times that on the young parts.

Submerged weeds harboured both aerobic and micro-aerophilic N_2-fixing bacteria. Growth on glucose medium showed the presence of acid-gas-producing organisms (probably Enterobacteriaceae), while growth on malate revealed *Azospirillum*-like organisms.

Based on the MPN method the number of N_2-fixing bacteria was in the order of 10^5 cell (g fresh weight)$^{-1}$ of host. There was very little difference in the cell numbers on the different weeds and between old and young parts (Table 1) except, on old parts of *Chara*, where the N_2-fixing bacterial population was approximately three times that on the young parts.

ARA measurements in the light, carried out separately (Table 2), showed that the activity on young parts of *Chara* was much higher than on old parts, while on *Najas* both old and young parts had the same activity. ARA measurements in the dark (Table 2) also showed higher

activities on young parts, specially on *Chara*, despite the fact that the populations of N_2-fixing bacteria on the older parts were higher (Table 1).



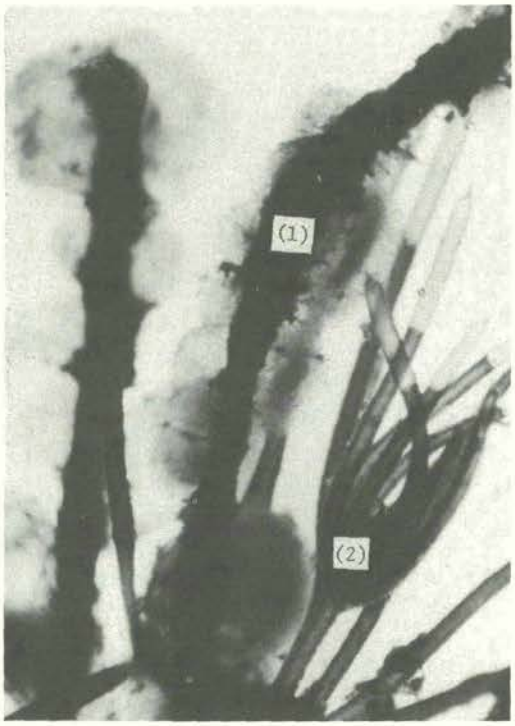


Plate 1

a) Gloeotrichia *colonies epiphytic on* Chara.

b) Gloeotrichia *epiphytism on* Chara *(x 20).*
- *present on old parts: (1)*
- *absent on young parts: (2)*

Table 1. Enumeration of epiphytic microorganisms on submerged weeds

	Chara		*Najas*	
	Old parts	Young parts	Old parts	Young parts
	(Number g^{-1} fresh weight of host)			
N_2-fixing blue-green algae	$78 \cdot 10^4$	$19 \cdot 10^4$	$13 \cdot 10^4$	$4 \cdot 10^4$
Bacteria on:				
glucose[a]	$70 \cdot 10^4$	$16 \cdot 10^4$	$6 \cdot 10^4$	$5 \cdot 10^4$
malate[b]	$40 \cdot 10^4$	$25 \cdot 10^4$	$25 \cdot 10^4$	$11 \cdot 10^4$
tryptic soy agar[c]	$140 \cdot 10^6$	$210 \cdot 10^6$	$32 \cdot 10^6$	$99 \cdot 10^6$

[a] N_2-fixing Enterobacteriaceae.
[b] N_2-fixing *Azospirillum*-like.
[c] Total aerobic heterotrophs.

Table 2. ARA[a] by epiphytes on old and young parts of submerged weeds, in the light and in the dark

	Chara		*Najas*	
	Light	Dark	Light	Dark
	(nmole C_2H_2 h^{-1} g^{-1} fresh weight)			
Old parts	23.6	1.51	27.2	1.97
Young parts	49.6	5.05	26.2	1.57

[a] Difference between 60 at 30 minutes measurements.

Acetylene-reducing activity

Time course of incubation. Cumulative production of ethylene by epiphytes on *Chara* and *Najas* incubated in the light exhibited a non-linear increase after one hour of incubation, as reported by David & Fay (1977). Therefore, specific activities were calculated using the difference between 60 and 30 minutes measurements.

Specific activities and extrapolation to the field scale. Results presented in Table 3 show that the activity per unit fresh weight in the light on the submerged weeds is much higher than that on the non-submerged ones. Compared to the light activities, the dark activities are very low and are of the same order among the different weeds. The light activities measured under laboratory conditions were multiplied by 1.8 to extrapolate them to the field, as it was found that the ARA measured in the laboratory was, on an average, 55% of the outdoor activity. Relating the specific ARA to the biomasses of the weeds, it was found that epiphytic N_2 fixation on the submerged weeds could contribute 11 to 24 g N ha^{-1} d^{-1} under rice and 41 to 63 g N ha^{-1} d^{-1} under fallow, whereas the activity on the non-submerged weeds contributes only negligible quantities of nitrogen to this ecosystem.

Table 3. Specific ARA on weeds and extrapolation of NFA to field level using mean and maximum (in parentheses) values of weed biomass recorded

Habitat	Weed type	ARA under lab. conditions (nmole C_2H_4 h^{-1} (g^{-1} fresh weight)		Biomass of submerged host material (t ha^{-1})		ARA values extrapolated to field condition (g N ha^{-1} d^{-1})[a]	
		Light	Dark	Under rice crop	Fallow	Under rice crop	Fallow
Submerged	*Chara*	35	0.9			7 (13)	22 (34)
				2.0 (4.5)	7.5 (11.8)		
	Najas	29	1.7			5 (11)	19 (29)
Non-submerged	*Monochoria vaginalis*	1.8	1.3		7.7 (n.d.)[b]	0.4 (1.0)	2 (n.d.)
				1.7 (4.1)			
	Cyperus iria	4.4	2.5		2.8 (n.d.)	1.0 (2.3)	1.6 (n.d.)

[a] Assumes C_2H_2 : N_2 = 3:1, [b] not determined.

DISCUSSION AND CONCLUSION

Results indicate that both N_2-fixing algae and bacteria were present on weeds but most of the activity was due to blue-green algae. Although higher densities of these organisms were observed on old parts of submerged weeds, ARA measurements showed that the activity on young parts was either higher (*Chara*) or of the same order (*Najas*). This perhaps indicates a higher concentration of quiescent or less active populations on the old parts.

Among the different weeds studied, only submerged ones exhibited a significant activity, approximating to an input of 2 kg N ha^{-1} $crop^{-1}$ under rice and 4 kg N ha^{-1} under fallow (C_2H_2:N_2 = 3).

Another important role of the submerged weeds, mainly *Chara*, is to offer a substratum

suitable for the attachment of *Gloeotrichia* sp. This blue-green alga forms floating, flobose, colonies that could develop considerable biomasses of several t ha^{-1} (Watanabe *et al.*, 1978), but are frequently washed out of the field by heavy rains or bleached by high light intensities. Epiphytic *Gloeotrichia* are protected from these adverse conditions and provide an inoculum from which regeneration of the bloom is possible. It is therefore clear that in the nitrogen cycle of a rice field, the submerged weeds play a positive role in the N_2 fixation process.

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EPIPHYTIC NITROGEN FIXATION ON LOWLAND RICE PLANTS

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ABSTRACT

Epiphytic nitrogen fixation on the submerged part of the rice stems was examined by:

- *studying the distribution of acetylene-reducing activity (ARA) and epiphytic algae among the hills at tillering stage;*
- *enumerating and identifying epiphytic microorganisms on the outer and inner leaf sheaths;*
- *measuring ARA and evaluating algal populations at seedling, tillering, heading and maturity stages of rice growth.*

Dark and light ARA (nmole C_2H_4 h^{-1} $hill^{-1}$) exhibited a log-normal distribution (L-shaped histogram; mean = standard deviation) while the total algal flora had an assymmetrical histogram, indicating the presence of several dominant epiphytic species.

Total and N_2-fixing algal populations on the outer parts of the stems (3.5×10^5 and 1.2×10^5 cells (g fresh weight)$^{-1}$ respectively) were about twenty times higher than those of the inner parts. A similar distribution was observed with N_2-fixing bacteria (outer parts: 2.9×10^7 cells (g fresh weight)$^{-1}$; inner parts: 1.0×10^5 (g fresh weight)$^{-1}$) where the dominant types were related to the Enterobacteriaceae, associated with Azospirillum*-like organisms. A macroscopic epiphytism by* Gloeotrichia *sp. was observed at seedling (2 t ha^{-1}, fresh weight) and tillering stage (0.5 t ha^{-1}), whereas only a microscopic epiphytism was present at heading and maturity stage, with* Nostoc *spp. as dominant species.*

Light ARA declined along the cultivation cycle from 51 µmole C_2H_4 m^{-2} h^{-1} at seedling stage to 2.5 µmole C_2H_4 m^{-2} h^{-1} at maturity whereas dark ARA remained low throughout (0.3 - 2.5 µmole C_2H_4 m^{-2} h^{-1}). This corresponds to an input of 2 kg N ha^{-1} $crop^{-1}$.

INTRODUCTION

Epiphytic nitrogen-fixing activity in a rice field ecosystem can develop on rice plants and weeds within a submerged habitat, in which the epiphytic microorganisms are protected from certain adverse environmental factors like desiccation and high light intensities. In the previous paper (Kulasooriya *et al.*, 1981), we have dealt with epiphytic nitrogen fixation associated with weeds. Watanabe & Barraquio (1979) and Watanabe *et al.* (1979) have reported on bacteria associated with rice stems. This paper reports on nitrogen fixation by blue-green algae and bacteria epiphytic on lowland rice.

MATERIALS AND METHODS

Experiments were conducted on field-grown rice plants (IR26) without algal inoculation and fertilization. Epiphytic microorganisms and their nitrogen-fixing activities (NFA) were examined by:

- studying the distribution of acetylene-reducing activity (ARA) and epiphytic algae among the hills at tillering stage;
- enumerating and identifying epiphytic microorganisms on the outer and inner leaf sheaths;
- measuring ARA and evaluating algal populations at seedling, tillering, heading and maturity stages of rice growth.

Assessment of the epiphytic microbial populations

Algae. Depending on the quantity of algae present on the host, different methods were used for their evaluation. At seedling stage, when a very dense growth was observed the direct

fresh weight was determined of the epiphytic algae dislodged from their host. At tillering, a visible growth was still present but insufficient for direct weighing. Biomass was calculated from chlorophyll measurements on algal material removed from the stems. Chlorophyll was measured after acetone extraction using MacKinney's (1941) specific absorption coefficient. Fresh weight was calculated using a ratio of 30.5 mg chlorophyll-*a* per gram fresh weight determined from the same algal material. These measurements were done separately on 35 hills, harvested from the same plot, in order to study the variability of algal epiphytism among rice hills.

At heading and maturity stages where epiphytism was not observable by the naked eye, algal enumerations were done on BG11 media (Allen & Stanier, 1968) with and without combined nitrogen for total and N_2-fixing algae respectively, as described earlier (Kulasooriya *et al.*, 1981).

Bacteria. Bacterial enumerations were conducted by the MPN method as described by Watanabe *et al.* (1979) for N_2-fixing Enterobacteriaceae and *Azospirillum*-like organisms. Total heterotrophic bacteria were enumerated by plating according to Watanabe & Barraquio (1979).

Host biomass measurements

After harvesting the whole plant, the root system and the aerial parts above the flood water level were cut off; the remaining material was used for ARA and fresh weight measurements and algal enumeration.

ARA measurements

Light and dark ARA measurements were carried out in the laboratory as previously described (Kulasooriya *et al.*, 1981) using cut rice stems. At seedling stage, parallel measurements were done *in situ* and in the laboratory to compare ARA under these different conditions.

At tillering stage, cut stems of 35 rice hills from the same plot were separately incubated to study the variability of the ARA among rice hills.

At heading and harvesting stages ARA measurements were done on 10 g triplicates randomly selected from the mixed material from the entire harvest of a plot of 35 hills.

At heading stage, the outermost leaf sheaths (outer parts) were separated from the inner parts of the tillers. Samples from these two sets were used separately for ARA measurements and enumerations of epiphytic microorganisms.

RESULTS

Epiphytic organisms

Of the epiphytic algae, *Gloeotrichia* sp. produced a visible growth on the rice stems at seedling and tillering stage. This growth could be observed irrespective of whether the host material was living or dead. Furthermore *Gloeotrichia* colonization was also observed on synthetic material such as nylon strings.

Gloeotrichia epiphytism decreased from seedling to tillering stage, mainly due to algal masses getting detached from their hosts as a result of gas bubble formation within the colonies. It was also noticed that colonies attached to the living parts were more easily dislodged than those attached to the dead parts.

At heading and harvesting stages, algal epiphytism was observable only under the microscope and during these stages the dominant N_2-fixing species was *Nostoc*, together with *Calothrix*, *Tolypothrix* and *Gloeotrichia* as associated species. At heading stage, N_2-fixing blue-green algae constituted 36% of the total epiphytic algal flora (Table 1).

Bacterial enumerations done at heading showed the presence of N_2-fixing acid-gas producing bacteria (Enterobacteriaceae) as well as *Azospirillum*-like organisms. The presence of these bacteria on rice has been already reported by Watanabe *et al.* (1979).

Results of the comparison of epiphytism on outer and inner leaf sheaths (Table 1) indicated that both ARA and microbial colonization of the outer parts was much higher than on the inner parts irrespective of the type of microorganisms. Experiments using labelled N_2-gas have also shown a higher N_2-fixing activity on the outer surface of stems than on the inner parts (Ito *et al.*, in press).

In the case of algae this may be related to light availability. N_2-fixing algae present on the inner leaf sheaths (5.3×10^3 (g fresh weight)$^{-1}$) were mainly spores or inactive forms as demonstrated by the negligible difference between dark and light ARA measurements on the inner leaf sheaths. The much higher density of bacteria on the outer parts may be interpreted on the basis that outer parts contain partially decomposing material that provides suitable substrates for bacterial growth.

Table 1. Distribution of ARA (nmole C_2H_4 (g fresh weight)$^{-1}$ h^{-1}) and epiphytic microorganisms (number (g fresh weight of host material)$^{-1}$) between outer and inner parts of rice stem at heading stage

	Outer sheath	Inner sheath	Whole stem (leaf sheaths + culm)
ARA Light	2.5	0.14	1.9
Dark	0.5	0.11	0.47
Total algal flora	3.5×10^5	1.7×10^4	1.4×10^5
N_2-fixing algae	1.2×10^5	5.3×10^3	4.8×10^4
Total aerobic heterotrophs	4.7×10^8	3.0×10^6	1.8×10^8
N_2-fixers on glucose (Enterobacteriaceae)	2.0×10^7	9.5×10^4	7.5×10^6
N_2-fixers on malate (*Azospirillum*-like)	9.5×10^6	9.5×10^3	3.7×10^6

Variation of epiphytism among rice hills

Light and dark ARA among 35 hills from the same plot are depicted in Fig. 1A and B, in the form of histograms. Both histograms exhibited a characteristic L shape; mean and standard deviation of the variables were very close to one another. These features indicate a log-normal distribution of ARA in the light and in the dark. Similar results have been reported for ARA by soil algae and bacteria (Roger *et al.*, 1977).

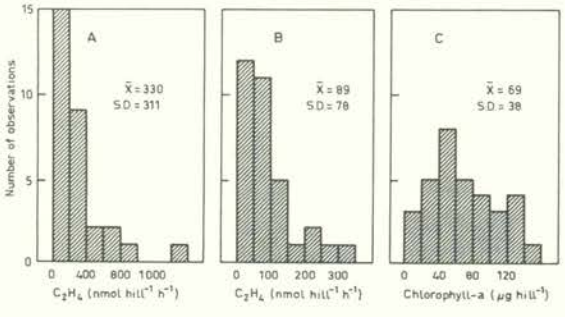

Fig. 1. Histograms showing the variations of: (A) light ARA; (B) dark ARA and (C) epiphytic algal chlorophyll, among 35 hills from a rice field at tillering stage.

This large variability of ARA among the hills implied that subsequent measurements should be done on replicates obtained from mixed material from the complete harvest of a plot and not on a few randomly selected hills. The distribution of epiphytic algae on the rice plants, determined as chlorophyll-*a* per hill was not log-normal (Fig. 1C). The assymmetrical histogram indicates that algae other than *Gloeotrichia* had also contributed to these pigment measurements. This was confirmed by plating dislodged algal material, which showed the presence of several associated blue-green algae, mainly *Oscillatoria*, *Pseudoanabaena* and *Nostoc*.

Variations of epiphytism and ARA along the cultivation cycle

A remarkable change was found in the algal epiphytism along the developmental cycle of the rice plant, with a corresponding change in the light ARA (Table 2). At seedling stage, when the rice stems had an epiphytic *Gloeotrichia* biomass of about 2 t fresh weight ha^{-1}, ARA in the light was 51 µmole C_2H_4 m^{-2} h^{-1}. At tillering, when this biomass had diminished to 0.5 t fresh weight ha^{-1} it still had an activity of 15 µmole C_2H_4 m^{-2} h^{-1}. The algae exhibited the same specific activity at these two stages (about 2.4 nmole C_2H_4 (mg protein)$^{-1}$ min^{-1}). A similar specific activity was reported by Finke & Seeley (1978) for *Gloeotrichia* epiphytic on *Myriophyllum*. At heading and maturity, algal epiphytism was not visible to the

naked eye and the light ARA had decreased to low values: 1.2 and 2.5 µmole C_2H_4 m^{-2} h^{-1}, respectively. Nevertheless, enumerations done on the rice stems showed the presence of several epiphytic N_2-fixing algae with *Nostoc* as dominant species.

These results show that the algae, though present during these stages, probably existed to a large extent as quiescent cells or propagules and contributed very little N_2 to the crop.

Table 2. Acetylene reduction activity (ARA), biomass and rate of N_2-fixation of blue-green algae[a] on rice stems, at different stages of crop growth

		Growth Stage			
		Seedling	Tillering	Heading	Maturity
ARA					
Light	C_2H_4 (µmole m^{-2} h^{-1})	51.0	15.0[b]	1.2[b]	2.5[b]
	C_2H_4 (µmole (g fresh weight of stem)$^{-1}$)	614.0	37.5[b]	1.9[b]	1.1[b]
Dark	C_2H_4 (µmole m^{-2} h^{-1})	-	2.2	0.3	2.5
	% of light ARA	-	27	25	100
Biomass					
	Fresh weight (kg ha^{-1})	2037	553	-	-
	Number (g fresh weight of stem)$^{-1}$	-	-	4.8×10^4	5.9×10^4
N_2-fixation					
	(nmole C_2H_4 (mg protein)$^{-1}$ min^{-1})	2.3	2.5	-	-

[a] dominant species: *Gloeotrichia* sp. and *Nostoc* spp.

[b] *in situ* values extrapolated on the basis of an activity under artificial light, equal to 55% of the *in situ* activity.

Along the crop cycle, dark ARA remained low (0.3 to 2.5 µmole C_2H_4 m^{-2} h^{-1}) and relatively unchanged from tillering to maturity. The range of dark ARA on rice stems (bacterial activity) was in agreement with the results reported by Watanabe *et al.* (1979).

DISCUSSION AND CONCLUSION

Among the epiphytic bacteria, N_2-fixing Enterobacteriaceae and *Azospirillum*-like forms corresponded to 8% of the total aerobic heterotrophs and their contribution to the epiphytic NFA was low.

Epiphytic NFA was primarily due to a visible growth of *Gloeotrichia*, which was predominant during the early stages of rice growth. The ARA decrease observed from seedling to tillering and thereafter was mainly due to a decrease of the epiphytic *Gloeotrichia* that detached from their host and became floating.

Towards the latter part of the crop cycle a "microscopic epiphytism" mainly due to *Nostoc* and *Calothrix* had a very low activity. This decrease in algal biomass and its activity was possibly related to a dramatic decrease in light availability due to the start of the rainy season and an increased rice canopy. Results obtained are insufficient to explain fully the relationship between the algal epiphytes and their host, but certain inferences can be drawn.

Gloeotrichia has been reported to be epiphytic on aquatic plants (Fremy, 1930; Finke & Seeley, 1978). However, according to our experience, it does not exhibit any selectivity between dead and living, organic or inorganic material, but seems to grow preferentially on rough surfaces as indicated by the following observations:

- epiphytism on *Chara*, which has a rough corticated surface was much more than on

Najas (Kulasooriya *et al.*, 1981).

- colonies on living, smooth rice stems get detached more easily than those on dead plant material which has rough surfaces as demonstrated by Howard-Williams *et al.* (1978).
- colonization was observed even on old, rough nylon strings but not on new smooth ones placed into the flood water. Similar colonization on polyethylene strips has been reported by Finke & Seeley (1978).

In the case of "microscopic epiphytism" it was also observed that most of the isolated epiphytic strains grew adherent to the surface of the culture vessels and rarely formed floating colonies. The results obtained do not permit confirmation of either the existence or the absence of biotic relationships between the algae and the host, but indicate that both a mechanical effect in relation to the roughness of the support and an ability of certain strains to grow attached to a support are involved in algal epiphytism. From the ARA measurements of these experiments the N_2 input by organisms epiphytic on rice can be evaluated as a few (2-3) kg ha^{-1} $crop^{-1}$, mainly due to the activity of *Gloeotrichia*.

In terms of nitrogen supply, algal epiphytism may appear to be of little value, but it has an important role in providing an inoculum potential for the regeneration of N_2-fixing algal blooms which are affected periodically by adverse conditions.

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NITROGEN CYCLING IN PADDY FIELDS: A COMPARISON BETWEEN THAILAND AND JAPAN

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ABSTRACT

Transformations of nitrogen in a Bangkhen paddy field, Thailand, were investigated using a ^{15}N tracer technique, and the results were compared with ones obtained in Japan.

The uptake of nitrogen by the rice plants was frequently higher than the fertilizer N input. The apparent increase in soil-N uptake due to the application of nitrogen was most likely due to an exchange between soil and fertilizer N.

Estimates of inputs and outputs of N are given for the Bangkhen soil and for some Japanese soils. The problems in estimating these gains and losses are discussed, and the need for more nitrogen balance studies in the field are emphasized.

INTRODUCTION

Throughout the rice growing areas of the world, nitrogen is generally the most important nutrient limiting crop growth. In tropical rice growing areas, soil nitrogen content is generally low, partly because rice has been grown for many years without any use of nitrogen fertilizers, and partly because the climatic conditions cause rapid transformations in the soil and high nitrogen losses from it. An understanding of these transformations and an assessment of the losses is therefore required. To this end, experiments were conducted in a Bangkhen field, Thailand, using ^{15}N-labelled fertilizers. Some of the results of these experiments are given below and are compared with similar results from Japan.

RESULTS

Transformation of soil nitrogen in a Bangkhen field

Chemical nature of soil nitrogen. Soil nitrogen is predominantly in the organic form, the inorganic forms constituting only about 1% of the total. The organic nitrogen in Bangkhen soil was separated into three fractions by hydrolysis with strong acid and subsequent fractionation of the hydrolysate (Stewart *et al.*, 1963). The results are compared with those from Japanese soils treated in the same way (Fig. 1).

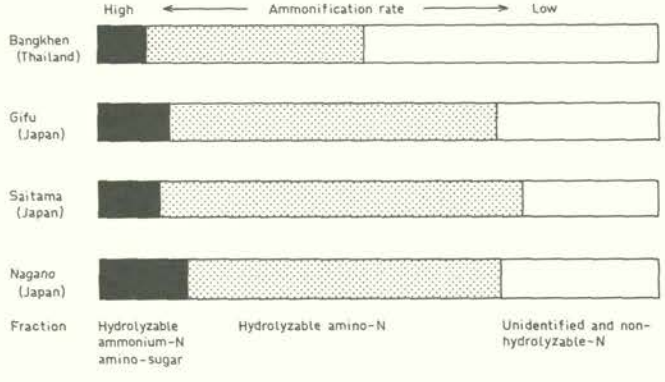

Fig. 1. Fractionation of organic nitrogen in flooded rice soils (Koyama et al., *1973).*

There is a clear difference between the soil from Thailand and the soils from Japan in the amounts of nitrogen that were converted during hydrolysis to either ammonium, amino sugar, or amino acid and the amounts of the resistant, non-hydrolysable, fraction. This agrees with the observation that Bangkhen soil produces less mineral nitrogen than the Japanese soils (Kawaguchi & Kyuma, 1969). Motomura (1973) also showed that the ammonification rates of Thai soils were noticeably lower than those observed in soils from other south-east Asian countries.

Mineralization of soil nitrogen. A major source of nitrogen for rice is the mineralization of soil organic nitrogen, even in cases where fertilizer nitrogen is applied at high rates (Koyama, 1975). For Bangkhen paddy soil 50-80% of the plant nitrogen was derived from soil organic nitrogen.

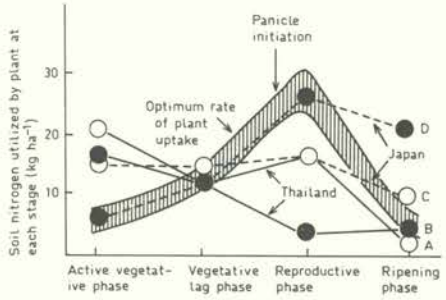

Fig. 2. Difference in mineralization rates of soil N between Thai and Japanese paddy fields, based on different plant uptake patterns (Koyama, 1971).
A, B: Bangkhen paddy field, fertilized (A) and no N applied (B),
C, D: Japanese paddy field, south-east (C) and north-east (D).

The mineralization process of soil nitrogen in tropical regions is somewhat controversial. It has already been shown (Koyama, 1971) that the soil nitrogen in Bangkhen soil becomes available at an earlier stage of rice growth than in Japanese soils (Fig. 2), but Shiga & Ventura (1976) could not find this trend in the Philippines. Ventura & Watanabe (1978) claimed that the early release of soil nitrogen was most probably due to the effect of soil drying before transplanting. Since the initial release of nitrogen from soil organic matter is known to be enhanced by drying the soil prior to waterlogging (Shioiri *et al.*, 1941), a similar effect was expected in Bangkhen soil. However, a preliminary field experiment showed no significant difference in nitrogen utilization between plants on plots which had been dry and those that had been continuously flooded during the dry season. It was concluded that water was supplied by capillary movement to the dry surface soil in the field soil, thus decreasing the drying effect.

The effects of fertilizer nitrogen on the mineralization and immobilization of soil nitrogen. The increase in soil nitrogen uptake induced by the addition of fertilizer nitrogen to Bangkhen soil amounted to as much as 15-29% of the total nitrogen ocntained in plants (Table 1). There was no appreciable difference in the additional mineralization between Bangkhen and Japanese soils.

The phenomenon of apparent additional soil nitrogen mineralization due to applied N, known as the priming effect, could be due to

1. stimulation of the mineralization of soil organic nitrogen due to the application of nitrogen fertilizer,
2. enhanced root growth, due to the application of nitrogen fertilizer, to the extent that more native soil nitrogen can be taken up,
3. exchange between ^{15}N-labelled fertilizer nitrogen and native soil nitrogen.

Results from 13 experiments (Koyama, 1975), in which the magnitude of the priming effect was assessed, gave an average value of 17% of the applied nitrogen. At the same time, the amount of fertilizer-N immobilized was 20%. Such a close correspondence between apparent additional mineralization and immobilization was also found by Yohino & Dei (1978). Similar values were reported by Maeda & Shiga (1978), who showed that the ratio between apparent additional mineralization and immobilization of added nitrogen was about 0.6 for soils with a high N content. These results suggest that, in general, the priming effect is likely to be due to an exchange between fertilizer- and soil-N. Thus, the net effect of nitrogen application on soil nitrogen availability is small or non-existent.

Table 1. Additional mineralization of soil nitrogen caused by the application of fertilizer nitrogen

Location	Treatment	A	B	A-B	
		Soil-N removed by plants from fertilized plot (kg ha^{-1})	Soil-N removed by plants from check plot(no N) (kg ha^{-1})	Additional mineralization of soil-N (kg ha^{-1})	(% of N applied)
Bangkhen (Thailand)	First cropping (improved variety)	52.1	37.1	15.0	20
	Second cropping	43.3	26.8	16.5	22
	Early planting (local variety)	35.9	39.7	-3.8	-5
	Late planting	63.3	33.4	29.9	40
Hokuriku[a] (Japan)	Fertilizer (NPK)	66.2	54.2	12.0	17
	Fertilizer (NPK) + manure	70.6	58.2	12.4	18
Konosu[b] (Japan)	Early planting	51.1	38.9	12.2	10
	Late planting	50.7	43.2	7.5	6
Miyagi[c] (Japan)	Early planting low N	116.2	99.3	16.9	24
	Early planting high N	122.6	99.3	23.3	16
	Ordinary planting low N	113.7	100.2	13.5	19
	Ordinary planting High N	120.1	100.2	19.9	13
Average value		75.0	60.5	14.5	17

[a] Iimura *et al.* (1972), [b] Yoshino & Dei (1978), [c] Shoji *et al.* (1971).

Nitrogen balance in a Bangkhen rice field

The soil could be considered as the centre of nitrogen cycling in an ecosystem. The nitrogen input for paddy soils comes from rainfall, seedlings, fertilizers, manure, biological nitrogen fixation and inflowing irrigation water. Nitrogen output results from crop removal, denitrification, leaching, ammonia volatilization and outflowing irrigation water. Some of these inputs and outputs can be measured better than others. Biological nitrogen fixation, denitrification, ammonia volatilization and leaching are most difficult to measure or, in certain cases, even to estimate. With the standard fertilizer application, i.e. 37.5 kg N ha^{-1} as a basal dressing and 37.5 kg N ha^{-1} as a top dressing, the rice plants removed 82 kg N ha^{-1} of which 30 kg was derived from fertilizer, while the balance, 52 kg N ha^{-1}, originated from the soil nitrogen (Table 2).

The amount of soil nitrogen mineralized, and thus made available for the use of plants (the 'A-value'), was determined by the isotope dilution technique of Fried & Dean (1952). The 'A-value' amounted to 131 kg N ha^{-1} in Bangkhen paddy soil, while in Japan this value averaged 175 kg N ha^{-1} ranging from 98 to 249 kg N ha^{-1} (Koyama & Shibuya, 1975).

The extent to which added nitrogen was immobilized in the soil was not determined, but as already mentioned above, the quantity of added nitrogen immobilized in the soil was almost equivalent to the quantity of additional mineralization of soil nitrogen utilized by plants. The amount of added nitrogen immobilized was estimated to be around 15 kg N ha^{-1} in Bangkhen soil.

The inflow and outflow of water to and from the paddy field have potentially an important influence on the balance of nitrogen under submerged conditions. Although certain waters carry a high content of nitrogen due to pollution, the amount of nitrogen in irrigation water is usually small. In general therefore, it may be considered that substantial losses or gains of nitrogen are not imposed by irrigation water.

The amount of nitrogen precipitated annually in rainfall is usually estimated at below 10 kg N ha^{-1}, although high values (25-57 kg N ha^{-1} yr^{-1}) have been reported for the humid tropics (Hauck, 1971).

Table 2. The fate of nitrogen applied to paddy soils

Location / Method	Bangkhen (Thailand)				Nagano (Japan)	Saitama (Japan)
	basal	basal	split	No N control	basal	split
	(kg N ha^{-1})					
(a) Applied fertilizer-N	37.5	75.0	75.0	0	82.5	75.0
(b) Soil-N in rice plant	44.8	58.1	51.9	37.1	77.9	65.5
(c) Applied-N in rice plant	6.2	14.0	29.8	0	25.8	32.0
Total N in rice plants (b + c)	51.0	72.1	81.7	37.1	103.7	97.5
Applied-N immobilized or lost from soil-plant system (a-c)	31.3	61.0	45.2	0	56.8	43.0

Since rice has been cultivated continuously over hundreds of years without application of fertilizers, and since natural sources of nitrogen such as rainfall and irrigation water do not account for the amount of nitrogen used by the rice plants, scientists have long recognized that there must be substantial biological nitrogen fixation occurring in paddy soils.

Using the acetylene reduction method, Matsuguchi & Tangcham (1974) reported estimates of total nitrogen fixation ranging from 0.5 to 54 kg N ha^{-1} (mean 7 kg N ha^{-1}) for different soils in Thailand. The value was approximately 10 kg N ha^{-1} in marine alluvial soil. Koyama & App (1979) estimated the amount of N being biologically fixed in a paddy field to be 15-50 kg N ha^{-1}. Kawaguchi & Kyuma (1977) pointed out that if there was no such mechanism to compensate for the nitrogen loss by cropping, it was inevitable that the nitrogen status of a paddy field must decrease because traditionally most rice straw is burnt after harvest. However, it is not wise to overestimate the contribution of biological nitrogen fixation in Bangkhen soil, since available soil nitrogen was appreciably depleted by adopting double-cropping in each rainy season (Koyama *et al.*, 1971).

The most important loss mechanism for nitrogen in a paddy soil is the denitrification process. It was considered that large amounts of nitrogen were lost through denitrification in Bangkhen soil, because recovery of ^{15}N by plants was appreciably increased by deep placement or addition of a nitrification inhibitor (Koyama, 1971). Arraragi & Tangcham (1974) reported that volatilized nitrogen was 1.9 kg N ha^{-1} in the check plot during 64 days after transplanting, whereas in the plot with rice straw this loss increased by up to 18.6 kg N.

Direct measurements in the field are essential to our understanding of denitrification in agricultural ecosystems. However, my view is that the most reliable estimates of nitrogen losses from soil could be obtained from accurate nitrogen balance studies. As was evidenced from a tracer study (Koyama *et al.*, 1973), the unaccounted for ^{15}N in Bangkhen paddy field ranged from 16 to 46 kg N ha^{-1} (mean 23.8 kg N ha^{-1}).

The amount of nitrogen lost by leaching through the soil is generally unknown. It seems likely that during the rice growing season this amount is not so large, because percolation is mostly poor. In recent years there has been much interest in the nitrogen loss during the post harvest period. Maeda & Onikura (1976) and Koyama *et al.* (1977) showed that considerable amounts of nitrogen were removed with the draining water, possibly due to favourable conditions for nitrification and absence of plant nitrogen uptake.

The best estimates for the inputs and outputs for the Bangkhen soil are given in Table 3 and are compared with those for some areas in Japan. The variation among the results obtained at different locations seems to be largely due to the different conditions under which the experiments were carried out. No distinctive differences between Thailand and Japanese paddy soils are found.

CONCLUSIONS

During the past decade substantial progress has been made on the studies of the dynamic behaviour of nitrogen in rice fields. However, reliable assessments of nitrogen losses or gains are still not possible, specifically of biological nitrogen fixation, denitrification and leaching.

More intensive, long-term, nitrogen balance studies in the field are badly needed in order to improve our understanding of the nitrogen cycling in rice ecosystems.

Table 3. Inputs and outputs of nitrogen in flooded rice soils, and rates of two internal processes, for one rice crop per year, except for Saga (two rice crops per year)

Component	Thailand	Japan			
	Bangkhen	Saga (Koyama *et al.*, 1977)	Hokuriku (Kosuge & Iimura, 1977)	Ibaraki (Takamura *et al.*, 1976)	Aomori (Aomori Agric. Exp. Stn., 1960)
			(kg N ha^{-1} yr^{-1})		
Input					
Fertilizer	75	140	50	69	56
Manure	-	-	-	-	58
Flow-in irrigation water	(10)[a]	15	-	7	10
Rainfall	(7)	5	-	13	-
Biological fixation	10	(30)	-	-	-
Output					
Crop removal [fertilizer]	30	71	15	78	81
Crop removal [soil]	52	43	84		
Denitrification [fertilizer]	2 ~ 19	23.8	12	-	50
Denitrification [soil]			56	-	
Leaching-runoff	-	24	-	5	
Ammonia volatilization	-		0.05	-	
Flow-out irrigation water	(10)		-	17	
Mineralization of soil-N (A-value)	131	84	139	-	-
Immobilization of fertilizer-N	(15)	45	23	-	-

[a] Figures in brackets indicate estimates.

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NITROGEN CYCLING AND THE FATE OF FERTILIZER NITROGEN IN RICE FIELDS OF THE SUCHOW DISTRICT, JIANGSU PROVINCE, CHINA

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ABSTRACT

The Suchow district is one of the high-yielding rice areas in the country, with an average grain yield of 9 t ha^{-1} in 1978. The major cropping system within one year is rice-rice-winter crop, requiring a high application of chemical fertilizer-nitrogen (246 kg ha^{-1} yr^{-1}).

The total, district-averaged, N input of 343 kg ha^{-1} yr^{-1} (not including non-symbiotic fixation) is approximately balanced by an output of 337 kg ha^{-1} yr^{-1}, suggesting a stable cropping system with relation to nitrogen.

The efficiency of soil-derived and fertilizer nitrogen is generally lower for the first crop in the rice-rice-winter crop system than in the rice-winter crop system. A higher fertilizer-N efficiency could be achieved by deep placement of supergranular urea or ammonium bicarbonate.

INTRODUCTION

The Suchow district is situated in the lower Yangtze Delta at a latitude of 31-32°N and a longitude of 120-122°E, with an average annual temperature of 16 C and a precipitation of 1231 mm. The soils are developed on alluvial lacustrine deposits.

The total area under cultivation is 486 000 ha, of which 89% is used for rice. This district, with a mean annual grain yield of 9 t ha^{-1} in 1978, is one of the highest yielding regions in the country. However, for some production brigades, yields of up to 12 t ha^{-1} have been reported under the three-cropping system rice-rice-winter crop (barley, rape, wheat, or milk vetch) in one year. The traditional cropping system of rice-wheat, or rice-milk vetch, still occurs in small areas. These two systems will be referred to as the RRO and RO systems respectively.

NITROGEN UPTAKE AND NITROGEN RESPONSES BY THE RICE PLANT

The soils of the Suchow district are reasonably fertile and productive. In general, they have a clay loamy texture, containing 20-30% particles smaller than 0.001 mm, an organic matter content of 2 to 3.5%, and a pH of about 6.0.

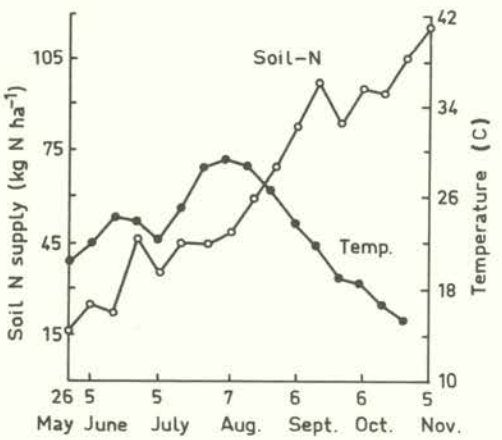

Fig. 1. Soil N supply (N assimilated by rice plus ammonium-N in the soil) and 10-day mean air temperature during the rice-growing season in the Suchow District, without nitrogen fertilizer application.

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The amount of nitrogen supplied by the soil in the Suchow District of the Jiangsu Province (Fig. 1) indicates a marked increase in the rate of soil nitrogen mineralization in August. Therefore, the percentage and total contribution of nitrogen by the soil is higher for the rice crop in the RO system than for the first rice crop in the RRO system (Table 1).

Table 1. Soil, plant- and fertilizer-nitrogen efficiency for the rice crop in the two cropping systems

	First rice crop in RRO system	Rice crop in RO system
Percentage of soil nitrogen mineralized during rice growing period (%)	1.8±0.3 (n=7)	3.2 (n=2)
Contribution of soil nitrogen supply to the total demand of nitrogen (%)	57.8±1.3 (n=5)	75.9±4.0 (n=13)
Efficiencies		
kg grain per kg N assimilated	47.4±6.3 (n=29)	55.1±5.9 (n=23)
kg grain per kg N applied	12.5±2.0 (n=3)	22.0±4.5 (n=4)

References: Liu *et al.* (1965); Suchow Institute of Agriculture (1963) unpublished results; Wu-siang Institute of Agriculture (1977) unpublished results; Z.L. Zhu (1974-1975) unpublished results.

Furthermore, this increase in soil N supply during August matches the nitrogen requirement of the rice crop in the RO system, which is then at the panicle formation stage. For the first rice crop in the RRO system, on the other hand, the nitrogen supply by the soil is too low at the early stages of growth (Hsi *et al.*, 1978). During the later stages (end of June and early August), the higher soil N supply usually gives a higher straw N content, but a lower percentage of filled grain (Chu *et al.*, 1978). This results in a lower grain production per unit N taken up by the plant as compared with the rice crop in the RO system (Table 1). For similar reasons, the amount of grain produced per kg N applied is also lower for the first rice crop in the RO system (Table 1).

NITROGEN FERTILIZER PLACEMENT

Urea and ammonium bicarbonate are the major nitrogen fertilizers in the district. When 1-g urea supergranules are placed at 6 cm depth a recovery of 74.5% can be achieved, with the overall balance showing only a 13% deficit at the full heading stage (Table 2; Zhu *et al.*, 1979). Cheng & Fan (1978) found similar results for ammonium bicarbonate.

Surface broadcasting of powdered urea or incorporating it into the top 6 cm of the soil, both applied at transplanting, gave a higher N uptake than deep placement at the early stages of growth (Fig. 2). This uptake pattern reversed dramatically later, resulting in a higher N uptake and a lower deficit for the deep-placed fertilizer-N (Table 2).

Chu *et al.* (1977) could not detect fertilizer-N in the 15-30 cm layer when ^{15}N-labelled ammonium sulphate was surface broadcast to an early rice crop. It can therefore be assumed that under the prevailing experimental conditions a fertilizer-N loss *via* percolating water is negligible. The causes for the deficits given in Table 2 are likely to be denitrification and ammonia volatilization. Confirmation of these loss pathways by actual measurements in the field is still required.

NITROGEN RECOVERY FROM ORGANIC SOURCES

Farmyard manure, compost, azolla, and milk vetch are used in the district as organic nitrogen fertilizers for rice. The use of azolla is at present restricted to only 50 000 ha. This is probably a reflection of its slow decomposition rate, which is unable to match the nitrogen demand for high yielding rice varieties. Other aquatic plants, such as common

Table 2. Fate of ^{15}N-labelled fertilizer-N at full heading stage of rice, applied by different methods[a] at 75 kg N ha^{-1}

	N in plant[b] (%)	N in soil (%)	Deficit (%)
Urea			
Surface broadcast	27.5	18.6	53.9
incorporated with top 6 cm	37.2	27.7	40.1
spread at 6 cm	37.6	18.9	43.5
1 g supergranule, at 6 cm	74.5	12.4	13.1
top-dressed[c]	64.7	5.4	29.9
Ammonium bicarbonate surface broadcast	24.0	18.6	57.4
Ammonium sulphate surface broadcast	50.1	21.4	28.5
LSD			
P = 0.05	4.7	5.3	7.4
P = 0.01	6.3	7.2	10.1

[a] All fertilizer was applied at planting except top-dressed urea.

[b] Tops plus roots.

[c] Applied 17 days before full heading.

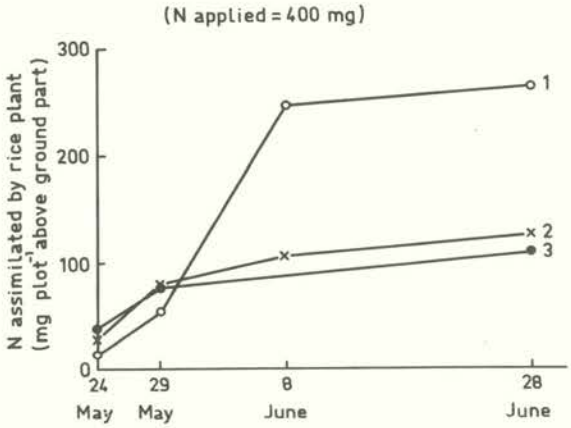

Fig. 2. Uptake of urea-N as affected by method of application of urea to early-grown rice in cylindrical microplots (29 cm diameter), each receiving 400 mg of ^{15}N-labelled urea-N.

1. Deep placed supergranule (0.9 g).
2. Incorporated.
3. Surface broadcast.

water hyacinth and water lettuce, cover up to 30 000 ha in the district, but are mainly used as fodder.

The percentage recovery in the rice plant of the N from the different organic sources is given in Table 3. It seems that in addition to the C/N ratio, the lignin content also governs the nitrogen release from these organic materials (Shi *et al.*, 1978).

Table 3. Recovery of nitrogen by rice plants from organic sources

Source	N recovery (%)	
Azolla	26.3 - 45.8	Pot experiment[b]
Milk vetch	52.4 - 70.8	
Farmyard manure	16.7 ± 9.0[a]	Field experiment[c]
Compost	16.7 ± 5.6[a]	

[a] Standard deviation.

[b] Shi *et al.* (1978).

[c] Nanjing Institute of Soil Science (1978).

NITROGEN BALANCE SHEET

On the basis of the results presented above a nitrogen balance sheet has been drawn (Table 4). Since the inputs and outputs are more or less in balance, the nitrogen status of the soils in the district as a whole should not deteriorate.

Chemical fertilizers provide 60-70% of the total input. This is a reflection of the high nitrogen demand by the rice crop in order to fulfil the food requirements.

Table 4. Nitrogen balance sheet, averaged over all agricultural fields of the Suchow district, for 1978

Input	(kg N ha^{-1} yr^{-1})	Output	
Chemical fertilizers	246	Harvest	204
Symbiotic fixation,		Leaching + runoff	2
milk vetch	12	Estimated loss from	
azolla	2	chemical fertilizers	123
Non-symbiotic fixation	?	Organic manures losses	8
Aquatic plants	1		
Rice straw	11		
Night soil	10		
Pig manure	28		
Seeds	7		
Irrigation water	3		
Precipitation	23[a]		
Total	343+?		337

[a] Lu & Shi (1979).

Symbiotic nitrogen fixation, using azolla, is confined to small areas, and its contribution to the district is therefore small. Also, the input *via* the return of straw is low due to local fuel shortage. Since straw could play an important role in the stimulation of non-symbiotic nitrogen fixation in rice fields, alternatives to the fuel shortage need to be investigated.

The output *via* chemical fertilizers is estimated at 123 kg ha^{-1} yr^{-1}, probably through denitrification and ammonia volatilization.

It can be concluded that, over the district as a whole, the agricultural systems involving the growing of high-yielding rice are reasonably stable as far as nitrogen is concerned.

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LONG TERM STUDIES ON NITROGEN FERTILIZER USE AND NITROGEN STATUS OF RICE SOILS IN TAIWAN

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ABSTRACT

During the last three decades, from 1948 to 1977, the average annual application rate of chemical nitrogenous fertilizers on permanent paddy soils, for double cropping of rice, was 210 kg N ha^{-1}. The amount of nitrogen contained in the biomass of rice plants was almost the same as fertilizer N applied. Furthermore, during the last thirty years, the N content of the soil remained nearly constant. At first glance, it seems that the nitrogen requirement of paddy fields was maintained by the input through fertilizer nitrogen. In fact, both by the difference method and by a ^{15}N tracer method, it was established that no more than 45% of fertilizer nitrogen was absorbed by each rice crop. Every rice crop obtained a large part of its nitrogen from the soil.

The results of in situ *acetylene reduction measurements suggest that the balance of nitrogen may be obtained through nitrogen fixation by free living organisms, using crop residues and root exudates as an energy source under water-logged conditions. This process seems more favoured in fields cultivated with* japonica *varieties. The loss of nitrogen from the soil is mainly through denitrification and possibly also through ammonia volatilization. Overall, more than one half of the fertilizer nitrogen was lost, but the actual extent depended on the fertilization practices and soil conditions.*

RESULTS AND DISCUSSION

Most of the area discussed here has been under permanent paddy fields for more than a century. During the last fifty years, the area under rice has remained practically unchanged. The average annual area during 1953 to 1977 was 771 742 ha. This consisted of 102 000 ha under single cropping and a further 335 000 ha under double cropping. The annual area under cultivation, the yield of brown rice, nitrogen fertilizer rate, and estimated N yield from 1948 to 1977 are given in Table 1.

The N yield was estimated from the yield of brown rice, the available records of mean grain to straw ratios, and the N content of each component for corresponding crop seasons. For the double cropping fields, about 210 kg N ha^{-1} has been applied each year from 1948 to 1977, while on the other hand, about 220 kg N ha^{-1} had been removed each year from the rice fields.

The available records of soil nitrogen content for the period 1948 to 1977 show that the figures have remained practically the same, with only slight fluctuations, the coefficients of variance for each soil group being smaller than 10%. The N content of the soil depends firstly on the soil texture; in general the heavier soils contain more nitrogen (Table 2).

A 48-year long field trial (Lin *et al.*, 1971) conducted in Taipei has demonstrated that the soil nitrogen content was not affected by fertilization practices (Table 3). It was found that, over the past thirty years, the N content of the soil in the rice fields remained nearly constant. At first glance, it seems that the nitrogen cycle was maintained by the nitrogen input through fertilizer nitrogen. In fact, the utilization of fertilizer nitrogen by the rice plant is very low as can be seen from Table 4.

An investigation using ^{15}N-labelled fertilizer revealed an uptake of fertilizer nitrogen similar to the results cited above. The utilization of basal nitrogen fertilizer was only 20% or less and that of N top dressed at heading stage was 60-69%. In total, no more than 44-46% of the fertilizer nitrogen was absorbed by each rice crop (Houng & Liu, 1979).

The fact that the soil nitrogen content was not significantly affected by fertilizer application suggests that every rice crop may have obtained a large part of its nitrogen from sources other than the soil. An *in situ* acetylene reduction experiment with rice, in pot culture at panicle initiation stage, suggests that the gain of nitrogen may be due to

nitrogen fixation by free living organisms using root exudate and crop residues as energy sources under water-logged conditions (Lin & Wen, 1980). This process seems to be favoured more in fields cultivated with *japonica* varieties (Table 5).

Table 1. Annual cultivation area, yield of brown rice, nitrogen fertilization rate and yield of nitrogen by rice crop[a]

Year	Area of rice crop (ha)	Yield of brown rice (kg ha^{-1})	N-fertilizer rate (kg ha^{-1})	Estimated yield of N by rice crop (kg ha^{-1})
1948	717 744	1489	28.0	
1949	747 676	1624	28.5	
1950	770 262	1845	60.3	
1951	789 075	1882	68.4	
1952	785 729	1998	63.6	
1953	778 334	2109	67.0	89.9
1954	776 660	2183	83.4	92.6
1955	750 739	2151	83.7	88.5
1956	783 629	2284	85.3	91.2
1957	783 767	2340	87.2	117.2
1958	778 189	2434	90.0	86.1
1959	776 050	2392	89.0	82.7
1960	766 409	2495	96.3	88.3
1961	782 510	2577	102.3	89.4
1962	794 228	2660	116.1	100.5
1963	749 220	2815	121.5	96.9
1964	764 935	2937	136.9	107.7
1965	772 918	3038	136.1	111.2
1966	788 635	3017	149.2	154.0
1967	787 097	3067	154.1	104.9
1968	789 976	3188	160.6	162.4
1969	786 592	2952	115.0	150.3
1970	776 139	3173	79.3	156.6
1971	753 451	3071	112.1	119.7
1972	741 570	3291	88.0	118.3
1973	724 164	3114	138.5	
1974	777 849	3153	133.8	
1975	790 248	3156	142.8	
1976	786 343	2450	145.7	99.8
1977	779 487	3406	163.3	
Mean 1948-1977			105.0	110.0

More exact figures for the recent 25 years from 1953 to 1977 are:

Total aceage 19 293 569 ha = 771 742 ha yr^{-1}.

Total brown rice yield 54 788 855 t = 2 191 554 t yr^{-1}.

Mean brown rice yield = 2840 kg ha^{-1} $crop^{-1}$.

Total nitrogen consumption 227 683 t = 115.5 kg N ha^{-1} $crop^{-1}$.

Estimated nitrogen yield = 115.6 kg N ha^{-1} $crop^{-1}$.

[a] Source: Food Bureau, Taiwan Provincial Government.

The rest of the fertilizer N must have been lost, presumably *via* denitrification and/or ammonia volatilization. The rate at which ammonia is lost depends upon the pH, temperature, and ammonium concentration in the floodwater. As heavy surface application of ammoniacal fertilizer is still a common practice in Taiwan, it is quite likely that some losses occur as ammonia.

Nitrate can be formed in the zone near the soil-water interface, due to the presence of oxygen through diffusion from the atmosphere and algal photosynthesis. When this nitrate diffuses into deeper, anaerobic layers it can be denitrified. Yen (1964) has shown that such denitrification is enhanced by the presence of an energy source.

To avoid such losses, techniques for deep placement of nitrogen fertilizers have been developed successfully in Taiwan.

Table 2. Present status of soil nitrogen in various paddy soils

	Texture[a]	pH	C.E.C. (meq 100 g^{-1})	Organic matter (%)	Total nitrogen (%)	C/N
Red Soils						
Taoyuan Yangmei	SCL	6.1	8.64	2.86	0.144	11.52
Taichung Hsitun	L	6.4	5.40	1.54	0.072	12.41
Pingtung Tapu	CL	4.8	7.77	2.19	0.111	11.44
Sandstone and shale alluvial soils						
Taipei Shulin	CL	7.1	0.94	3.34	0.089	21.77
Tainan Hsuehchia	SL	7.3	6.39	1.24	0.079	9.10
Slate alluvial soils						
Changhua Huatan	SCL	7.6	9.97	4.92	0.216	13.21
Ilan Lotung	SC	5.4	6.78	4.15	0.189	12.74
Pingtung Neipu	SCL	5.7	8.31	5.52	0.235	13.62
Volcanic ash alluvial soils						
Taipei Yangmingshan	CL	6.3	23.74	5.21	0.158	19.13
Hualien Fengpin	L	6.4	27.41	4.07	0.107	22.06
Schist alluvial soil						
Hualien Shoufeng	SL	7.5	2.62	2.22	0.076	16.94

[a] SCL = silty clay loam, L = loam, CL = clay loam, SL = silty loam, SC = sandy clay.

Table 3. Final nitrogen status of soils after the 48-year long field trial

Treatment	pH	C (%)	N (%)	C/N
NPK	5.3	2.26	0.17	13.6
N	5.2	2.16	0.17	13.1
P	5.5	2.16	0.16	13.2
K	5.4	2.26	0.16	14.6
Control	5.4	2.11	0.15	14.0

Table 4. Recovery of fertilizer nitrogen[a] by rice plants in two crops, Chiu, 1976

Crop	1st Crop			2nd Crop		
Location	N100[b]	N120	Mean	N100	N120	Mean
			(%)			
Ilan	35.9	38.6	37.3	41.4	39.9	40.7
Taoyuan	28.8	28.8	28.8	26.4	30.7	28.6
Sulin	12.0	10.5	11.3	8.0	8.6	8.3
Mean	25.6	26.0	25.8	25.3	26.4	25.9

[a] $\text{Recovery} = \dfrac{\text{N uptake}_{(\text{NPK treatment})} - \text{N uptake}_{(\text{PK control})}}{\text{N rate applied}} \times 100.$

[b] rate of fertilizer N application (kg ha^{-1}).

Table 5. Acetylene reduction by in situ paddy soil, growing rice in 5 kg pots

Rice crop varieties	Ethylene produced (μmole pot^{-1})		
	Incubation period (hr) 4	20	48
TN-5 (*japonica*)	1.0	8.0	13.5
TCS-3 (*indica*)	0.5	2.5	2.0

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TRANSFORMATION OF INDIGENOUS AND ADDED NITROGEN IN SOME FLOODED MALAYSIAN SOILS

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ABSTRACT

Studies on mineralization of N in flooded soils showed that after four weeks of incubation at 30 C, the amount of NH_4^+-N extracted by 1N KCl ranged from 1.4 to > 100 ppm. While a significant relationship existed between contents of organic carbon and total N of soils (r = 0.90), NH_4^+-N extracted was not correlated with organic carbon and only weakly related to total N. The amount of NH_3-N volatilized using urea as the N source ranged from zero in some heavy textured soils to 37% in the light textured soils. Volatilization of NH_3, however, was effectively and significantly reduced in some soils by incorporation of 200 ppm Cu^{2+} or Ag^+ even when a high rate of urea was used (ca. 1.4% urea, soil basis). Use of sulfur-coated urea (SCU) also dramatically reduced the amount of NH_3 volatilized compared to uncoated commercial urea.

INTRODUCTION

It is recognized that rice requires a high level of nitrogen nutrition in order to produce high yields. This is especially true for the new hybrid varieties which have replaced the older traditional varieties in Malaysia.

Because the contribution of native or indigenous soil N is generally low and inadequate for sustained high yields, fertilization with nitrogenous materials is essential. Urea is becoming an important source of N for rice as well as other crops (Harre *et al.*, 1971), although comparisons of yield responses to urea and to other N sources reveal inconsistent results in certain regions (Tomlinson, 1970). The popularity of urea as a fertilizer may be attributed to its apparent cost advantages.

Many changes occur to fertilizer N after its addition to a submerged soil. Some of the fertilizer may be taken up by the rice plant, a considerable part of it may be immobilized (Broadbent & Nakashima, 1970; Yoshida & Padre, 1975; Patrick & Reddy, 1976), some of it may be nitrified in the thin oxidized zone and subsequently denitrified when leached to the reduced zone (Broadbent & Tusneem, 1971; Yoshida & Padre, 1974), while the rest of it may be lost as NH_3 through volatilization (MacRae & Ancajas, 1970; Ventura & Yoshida, 1977; Vlek & Craswell, 1979). Other researchers have also shown, using isotopic techniques for evaluating fertilizer N recovery under submerged conditions, that a certain amount of added N was not accounted for (Patrick & Reddy, 1976; Yoshida & Padre, 1977). This portion may be presumed lost into the atmosphere as NH_3 or other gaseous forms of N. Losses of N through volatilization of NH_3 from nitrogenous fertilizers have been known as early as the 1930's, but to date the problem has not been completely solved without incurring extra costs of incorporating fertilizers into the soil as opposed to broadcasting, or use of inhibitors of urea hydrolysis. Denitrification which is active near the interface of oxidized and reduced layers of submerged soil may also be responsible for loss of N from ammonium fertilizers when surface applied (Broadbent & Tusneem, 1971; Yoshida & Padre, 1974). Some researchers have found that greater amounts of NH_3 volatilized under flooded conditions than under upland conditions (Mitsui *et al.*, 1954; Blasco & Cornfield, 1966). Deep placement of urea or $(NH_4)_2SO_4$ was found to be superior to other methods of application for rice (Patrick *et al.*, 1967; Broadbent & Tusneem, 1971).

Nor (1979b), in a study with several Malaysian soils, showed that significant correlations existed between urease activity of soils and amounts of urea hydrolyzed after two days of incubation, and between urease activity and amounts of NH_3 volatilized. Therefore, if urease activity could be controlled and inhibited, volatilization of NH_3 might be prevented. It has been known that many compounds, both organic and inorganic, could act as inhibitors of urease activity (Kiss *et al.*, 1975). Thus, these compounds could be used to prevent urea hydrolysis and increase efficiency of N utilization by crops. Among the inorganic compounds, Ag^+ and Cu^{2+} have been shown by Nor (1979a) to be effective inhibitors of urease activity in some Malaysian soils.

The purpose of this laboratory investigation was to study mineralization of native N and volatilization losses from urea and sulfur-coated urea (SCU) under flooded conditions. The effect of urease inhibitors (Cu^{2+} and Ag^{+}) on volatilization of NH_3 was also evaluated.

MATERIALS AND METHODS

The soils used in this study consisted of surface (0-15 cm) samples with a wide range of physical and chemical characteristics. Total N ranged from 0.065-0.292%; organic carbon (Allison, 1965), 0.83-3.01%; pH (1:2.5, soil:H_2O), 3.4-7.1; cation exchange capacity by an ammonium acetate method, 3.8-25.3 meq $100g^{-1}$ soil; and texture (Day, 1965), clay to sandy loam.

Nitrogen mineralized was determined by steam distillation of an aliquot of 1N KCl extract from a 10 g soil sample kept flooded with water to 2 cm above the surface and incubated at 30 C for various times.

For volatilization studies, a 100 g sample of soil was placed in a 250-ml conical flask and sufficient water added to keep the soil submerged. Uncoated commercial urea or SCU (obtained from TVA, Muscle Shoals, Alabama) was applied at a rate of 500 µg N g^{-1} (oven-dry soil). The SCU contained 37.6% N and had a dissolution rate of 21.0% in seven days, according to the manufacturer. A 10-ml beaker containing 5% boric acid and a mixed indicator (methyl red + bromocresol green) was introduced into the conical flask and suspended from its mouth by a string. The mouth of the flask was then plugged with a rubber stopper. Ammonia evolved was absorbed by the boric acid solution, and was determined periodically by back titrating with H_2SO_4 of known strength. Results were expressed as percent of added N volatilized.

RESULTS AND DISCUSSION

Nitrogen mineralization from soils

Fig. 1A shows NH_4^+-N mineralized from five soils over a period of four weeks. Bumbung Lima and Keranji soils showed a continuing production, while in Renggam and Selangor soils production levelled off after four weeks of incubation. Chuping soil, however, showed very low NH_4^+-N production.

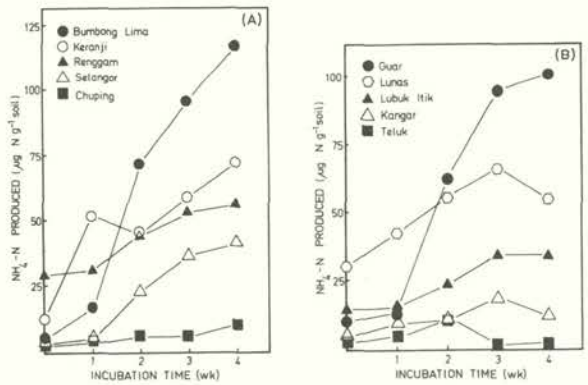

Fig. 1. Production of ammonium-N in ten soils, when incubated under flooded conditions.

Fig. 1B shows mineralization from another five soils. A decrease in NH_4^+-N produced was observed in three of the soils (Lunas, Kangar, Teluk) after three weeks of incubation, whereas in Guar and Lubuk Itik soils the quantity levelled off after four weeks.

The soils showed greatly different mineralization capacities, the quantity of NH_4^+-N produced ranging from only a few ppm in Teluk soil to about 115 ppm in Guar soil. The amount of NH_4^+-N extracted, however, was only weakly correlated to total N and not correlated to organic carbon content, although a significant correlation between total N and organic carbon existed in the soils. Miyake (1964) in a study of mineralization of NH_4^+-N from Malaysian padi soils obtained a range of 50-500 ppm with more than 70% of the soils studied possessing a range of 50-150 ppm N mineralized. Thus, with three exceptions, the soils fall in this category. According to Ponnamperuma (1972), almost all the mineralizable N in a

soil is converted to ammonium within two weeks of submergence under favourable temperatures. Results of this study, however, suggest that in some soils, NH_4^+-N production continued even after four weeks of submergence when incubated at 30 C. Organic soil N has a vital role for the rice plant because in the later part of the growing season it is the major source of available N (Patrick & Reddy, 1976; Yoshida & Padre, 1977).

Volatilization of NH_3 from urea and SCU

Table 1 shows results of volatilization of NH_3 from soils with urea as the N source. The soils used consisted of three upland and six rice soils. More NH_3-N was volatilized from upland soils (25-38% of added N) than from rice soils (0-8%). This observation was perhaps coincidental. The phenomenon was probably more related to soil texture and cation exchange capacity (CEC). The correlation coefficient between CEC and NH_3-N volatilized after four weeks was 0.841 which was significant at $P < 0.01$. Purushothaman & Joseph (1975), in another laboratory study, also showed that loss of NH_3 from urea under aerobic conditions was largely a function of CEC of soils.

Table 1. Volatilization of NH_3 from soils under flooded conditions using urea as the N source

Soil[a]	CEC[b]	% of added N volatilized after			
		1 week	2 weeks	3 weeks	4 weeks
Renggam	5.1	6.8	11.6	20.1	37.6
Cuping	4.4	15.6	21.4	26.0	30.4
Lunas	3.8	8.3	13.5	19.0	25.0
Bumbung Lima	17.5	3.2	4.8	6.4	8.1
Kangar	25.3	1.8	2.3	4.5	7.4
Lubuk Itik	15.0	0.6	1.1	1.1	1.1
Guar	19.0	10.0	0.0	0.0	0.0
Telok	16.0	0.0	0.0	0.0	0.0
Selangor	23.3	0.0	0.0	0.0	0.0

[a] The first three soils are upland soils, while the rest are soils normally cultivated with rice.

[b] meq $100g^{-1}$ soil.

It should be noted here that data from laboratory studies can only show relative effects and differences between soils. In the field, the absolute amounts of NH_3 lost could be quite different although the relative effects could persist.

Some successful attempts have been made to reduce volatilization losses using coated N materials under aerobic conditions (Allen *et al.*, 1971; Matocha, 1976; Nor, 1979b); but few studies have reported on NH_3 volatilization from coated urea under waterlogged conditions. Table 2 presents effects of N source (urea or SCU) on volatilization loss in four soils. It is obvious that use of SCU drastically reduced losses of NH_3 from all soils. The N loss averaged only 8.5% with the SCU treatment, whereas that of the urea treatment averaged 29.4%. The results obtained agreed well with those reported by Vlek & Craswell (1979) who found 5% loss of NH_3-N from soils fertilized with SCU after three weeks of treatment.

Table 2. Volatilization of NH_3 from soils under flooded conditions when treated with urea or SCU as the N source

Soil	Treatment[a]	% of added N volatilized after			
		1 week	2 weeks	3 weeks	4 weeks
Kranji	Urea	13.2	23.6	29.6	35.3
	SCU	1.8	4.0	6.9	9.4
Hutan	Urea	11.7	19.9	25.0	30.0
	SCU	1.7	4.3	5.2	6.0
Relau	Urea	10.4	18.4	24.0	27.0
	SCU	5.7	8.9	10.8	12.0
Kodiang	Urea	6.4	14.0	21.8	25.3
	SCU	3.6	5.3	6.0	6.9

[a] SCU, sulfur-coated urea or urea added to soils at a rate of 0.05% N.

Effect of Cu^{2+} and Ag^{+} on volatilization

Table 3 shows results of the study with four soils in which 200 ppm Cu^{2+} or Ag^{+} (dry soil basis) had been incorporated with urea-N applied at a rate of 1.4%. The high rate of urea was deliberately selected in order to really evaluate the effectiveness of the inhibitors. In three of the soils, Ag^{+} was 100% effective in controlling volatilization, while Cu^{2+} was only effective in two of the soils (Hutan and Lubuk Itik), and marginally effective in Gajah Mati soil. In Kundor soil, however, both Cu^{2+} and Ag^{+} failed to provide any control

Table 3. Volatilization of NH_3 from incorporated urea (1.4% on dry soil basis) under flooded conditions as affected by Cu^{2+} and Ag^{+}

Soil	Treatment[a]	% of added N volatilized after				
		2 days	4 days	6 days	14 days	21 days
Hutan	Control	1.6	19.7	26.8	36.4	37.6
	Cu^{2+}	0.0	0.0	0.0	0.0	0.0
	Ag^{+}	0.0	0.0	0.0	0.0	0.0
Gajah Mati	Control	0.0	0.0	0.3	17.8	18.8
	Cu^{2+}	0.0	0.0	0.0	5.0	10.0
	Ag^{+}	0.0	0.0	0.0	0.0	0.0
Lubuk Itik	Control	0.0	0.0	0.9	5.7	6.3
	Cu^{2+}	0.0	0.0	0.0	0.9	0.9
	Ag^{+}	0.0	0.0	0.0	0.0	0.0
Kundor	Control	0.0	1.8	3.5	12.7	17.8
	Cu^{2+}	0.0	1.7	6.1	14.3	17.1
	Ag^{+}	0.0	0.6	4.0	13.4	16.4

[a] Cu^{2+} or Ag^{+} added at a rate of 200 ppm on soil basis.

over volatilization. It is possible that the cations were inactivated in this soil, thus permitting urea hydrolysis to proceed resulting in similar amounts of volatilization to that of the control. It can be seen that while inhibitors of urease activity (Cu^{2+} and Ag^{+}) provide either complete or partial control over volatilization in some soils, in others their impact was minimal. The data of this study imply that in theory urease inhibitors have potential in reducing NH_3 loss from urea applied to soils. For practical application, however, the cost benefit-ratio will have to be assessed first in view of the added cost of inhibitors and their application to the field. Also, there is a need to investigate the response in crop yield relative to any potential health hazards resulting from the use of these chemicals.

ACKNOWLEDGEMENTS

The senior author wishes to express his gratitude to the Vice-Chancellor, Universiti Sains Malaysia for permission to present this paper and financial support through the short-term research grant. Thanks are due also to Puan Hajar Abd. Rani for typing the manuscript.

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RECOVERY BY RICE PLANTS OF FERTILIZER NITROGEN APPLIED AT DIFFERENT GROWTH STAGES*

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ABSTRACT

Two crops of rice were grown successively in pots. Ammonium sulfate was applied in four equal doses at different growth stages, one of which was enriched with ^{15}N-labelled nitrogen. The results obtained may be summarized as follows:

1. Recovery of fertilizer nitrogen was lowest when applied as a basal dressing, being 22-26% in the spring crop and 19-22% in the summer crop. It was highest when applied at the panicle initiation stage, being 64% in the spring crop and 60-69% in the summer crop. When applied at the heading stage, the recovery was high but had no effect on yield.

2. Recovery of fertilizer nitrogen applied to the previous crop by the following crop was very low, amounting only to about 2%. It contributed only 3.5% of the total nitrogen uptake to the following crop.

3. The amount of soil nitrogen available to rice plants was significantly reduced after one crop, and the recovery of fertilizer nitrogen applied to the following crop was also slightly reduced, resulting in a significant decrease in grain yield.

INTRODUCTION

It has been postulated that rice plants respond to fertilizer nitrogen to a lesser degree than most upland crops, their growth depending more on nitrogen supplied by the soil (Harada, 1974; Houng, 1976). Generally speaking, rice plants grown without addition of N fertilizer will give as much as 75% of the yield obtained from the use of a complete NPK fertilizer (Dei, 1975). The recovery of fertilizer nitrogen by rice plants is correspondingly low. Wada *et al.* (1971a) showed that recoveries of fertilizer nitrogen applied as a basal dressing were 22% in 1969 and 26-27% in 1970. Those applied as top dressings may range from 36% to 67%, depending on the growth stages and the type of season (Wada *et al.*, 1971b). Reddy & Patrick (1976) showed that the recovery from deep placement was 47% in 1974, and 49% in 1975. Recoveries from topdressings ranged from 31% to 38% in 1974, depending on the time and number of applications; it was 61% in 1975. We present here the results of our study on the uptake of fertilizer nitrogen by rice plants from applications at various growth stages, using ^{15}N as the tracer.

MATERIALS AND METHODS

Two crops of rice were grown successively in pots, using Taipei silt loam with a pH of 4.4, 3.4% organic matter and 0.17% total nitrogen. It is an alluvial soil derived from sandstone and shale. Chlorite and illite are the major clay minerals. Its cation exchange capacity is 6.5 meq $(100g)^{-1}$.

Summer crop season, 1977

Experiment 1977-A. A series of Wagner pots (200 cm^2 surface area) was filled with 4 kg pot^{-1} of soil; 2.0 g of superphosphate and 0.7 g of potassium chloride were applied. Ammonium sulfate, at the rate of 105 mg N pot^{-1}, was applied at four growth stages: a basal dressing and topdressings at tillering, panicle initiation and heading stages. One or two of the applications were given as ^{15}N-enriched ammonium sulfate, as indicated in Table 1.

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* The full details of this paper are given in

Houng Kun-Huang & Liu Tien-Pin (1979). Absorption rate of fertilizer nitrogen applied at different growth stages and its effect on the grain yield of rice. - Memoirs College of Agriculture, National Taiwan University, Taipei, Taiwan, China 19(1), 18pp. (in Chinese, English summary).

Table 1. Treatments of experiments 1977-A and 1977-B

Treatments	Basal dressing	Top dressing at		
		Tillering	Panicle initiation	Heading
	Aug. 4	Aug. 16	Sept. 24	Oct. 19
I	*[a]	-	-	-
II	-[b]	*	-	-
III	-	-	-	*
IV	-	*	*	-
V	-	-	*	*
VI	-	-	-	-

[a] 1977-A series: ^{15}N-labelled ammonium sulphate applied
1977-B series: no nitrogen applied.

[b] 1977-A and 1977-B series: ordinary, fertilizer grade, ammonium sulphate applied.

Experiment 1977-B. All the basic treatments were similar to the 1977-A series. The ammonium sulfate was applied at the four growth stages as described above, but one or two of the four application doses were omitted, as indicated in Table 1.

Rice varieties used were: Taipei 309 (*japonica*) and NTU No. 43 (*indica*).

Table 2. Treatments of experiments 1978-A and 1978-B

Treatments	Basal dressing	Tillering	Panicle initiation	Heading
	Mar. 14	Aug. 16	Sept. 24	Oct. 19
I	No[a]	No	No	No
II	*[b]	-	-	-
III	-[c]	*	-	-
IV	-	-	*	-
V	-	-	-	*
VI	-	-	-	-

[a] No nitrogen applied.

[b] 1978-A series: ^{15}N-labelled ammonium sulphate applied.
1978-B series: no nitrogen applied.

[c] 1978-A and 1978-B series: ordinary, fertilizer grade, ammonium sulphate applied.

Spring crop season, 1978

Experiment 1978-A. Experiment 1977-A was repeated, using a new lot of soil kept dry during the 1977 crop season. The treatments were adjusted as shown in Table 2.

Experiment 1978-B. Experiment 1977-B was repeated, using a new lot of soil kept dry during the 1977 crop season. Treatments were adjusted as shown in Table 2. One treatment without the addition of ammonium sulfate was included.

Experiment 1978-C. The soil used for 1977-B was mixed thoroughly and returned to each pot. Same treatments as 1978-A were given.

Experiment D. All four doses of unlabelled sulfate were applied to the pots of the 1977-A series.

Varieties used were Tainung No. 67 (*japonica*) and NTU No. 43 (*indica*).

All pots were placed in the open. After harvest, grain and straw were separately dried, ground and total nitrogen contents determined by a modified Kjeldahl method (Batey, *et al.*, 1974). ^{15}N atom percentage was determined by the emission spectrographic method described by Karino *et al.* (1974), using a JASCO NIA-1 N-15 analyzer. Although the method has been improved considerably by Yamamuro & Kono (1977), we could not obtain a satisfactory

precision in the determination of ^{15}N concentration close to the natural abundance, so that the fertilizer nitrogen remaining in the soil was not determined.

Table 3. Fertilizer nitrogen recovery by ^{15}N method, summer crop, 1977-A

Treatments[a]	N uptake (mg pot^{-1})	^{15}N[b] (%)	^{15}N added (mg pot^{-1})	^{15}N recovered (%)
		Taipei No. 309		
I	482	4.2	105	19
II	545	7.7	105	40
III	544	12.0	105	62
IV	444	19.4	210	41
V	493	24.8	210	58
VI	546	-	-	-
		NTU No. 43		
I	539	4.3	105	22
II	576	7.5	105	41
III	497	8.9	105	42
IV	501	19.5	210	46
V	442	24.8	210	52
VI	548	-	-	-

[a] Treatments are described in Table 1.

[b] ^{15}N enrichment of the rice plants.

Table 4. Fertilizer nitrogen recovery by difference method, summer crop, 1977-B

Treatments[a]	N uptake (mg pot^{-1})	Fert. N added (mg pot^{-1})	Red. in N uptake[b] (mg pot^{-1})	Fert. N recovered by diff.[c] (%)	Grain Yield (g pot^{-1})
			Taipei No. 309		
I	500	315	32	30	25.1
II	403	315	129	123	19.8
III	424	315	108	103	25.2
IV	394	210	138	66	17.8
V	361	210	171	81	22.3
VI	532	420	-	-	25.4
			NTU No. 43		
I	503	315	38	36	23.0
II	471	315	70	67	25.1
III	430	315	111	106	27.8
IV	-	210	-	-	19.8
V	371	210	170	81	24.6
VI	541	420	-	-	28.4

[a] Treatments are described in Table 1.

[b] Red. in N uptake = N uptake of VI - N uptake of Y, Y being I, II, III, IV, or V.

[c] Fert. N recovered by diff. = Red. in N uptake x 100/ (420-fert. N added).

RESULTS AND DISCUSSION

The results shown in Tables 3 and 4 indicate that nitrogen recovered by the rice plants was grossly over-estimated by the difference method as compared with the ^{15}N method. Tables 3 to 6 also indicate the following.

1. Recovery of ^{15}N applied as basal dressing was slightly higher in the spring rice crop than in the summer one, being 22-26% in the former and 19-22% in the latter.
2. Recovery of fertilizer nitrogen applied at heading stage varied rather widely, presumably depending on the root activity of the rice crop at this stage. Though the recoveries were in general the second highest, they did not contribute to the increase in

yield (compare grain yields of Treatments III and VI in Table 4, with those of Treatments V and VI in Table 6) in most cases.

3. Recovery of fertilizer nitrogen applied at the tillering stage was within a narrow range at about 40%, regardless of rice variety and crop season (compare ^{15}N recovered in Treatments II in Table 3 with Treatments III in Table 5).

4. Recovery of fertilizer nitrogen was the highest when applied at the panicle initiation stage in both crop seasons. It may be estimated as about 60-69% from the results in Table 3 in the summer crop of 1977 and 64% in the spring crop of 1978 (Table 5, Treatment IV).

Table 5. Fertilizer nitrogen recovery by ^{15}N method, spring crop, 1978-A

Treatments[a]	N uptake (mg pot^{-1})	^{15}N (%)	^{15}N added (mg pot^{-1})	^{15}N recovered (%)
		Tainung No. 67		
II	608	3.7	105	22
III	553	7.4	105	39
IV	599	11.2	105	64
V	534	11.5	105	59
		NTU No. 43		
II	609	4.5	105	26
III	634	6.6	105	40
IV	608	11.1	105	64
V	573	9.8	105	54

[a] Treatments are described in Table 2.

Table 6. Fertilizer nitrogen recovery by difference method, spring crop, 1978-B

Treatments[a]	N uptake (mg pot^{-1})	Fert. N added (mg pot^{-1})	Red. in N uptake[b] (mg pot^{-1})	Fert. N recovered[c] (%)	Grain Yield (g pot^{-1})
			Tainung No. 67		
I	305	0	269	-	19.7
II	499	315	75	71	29.4
III	468	315	106	101	27.4
IV	455	315	119	113	25.7
V	447	315	127	121	25.4
VI	574	420	-	-	28.5
			NTU No. 43		
I	318	0	289	-	18.4
II	570	315	37	35	31.4
III	524	315	83	79	30.0
IV	468	315	139	132	27.2
V	527	315	80	76	31.2
VI	607	420	-	-	31.0

[a] Treatments are described in Table 2.

[b] Red. in N uptake = N uptake of VI - N uptake of Y, Y being I, II, III, IV or V.

[c] Fert. N recovered = Red. in N uptake x 100/fert. N added.

The results of Experiment 1978-D were used to calculate the recoveries by the spring crop of 1978, of fertilizer nitrogen applied to the summer crop of 1977 and are shown in Table 7. It shows that

1. the residual effect of fertilizer nitrogen applied at any growth stage of the previous crop was very small, comprising less than 1% of the total nitrogen of the succeeding crop,

2. as a whole, fertilizer nitrogen contributed less than 3.5% of the total uptake, corresponding to about 2% of all fertilizer nitrogen applied to the previous crop.

Table 7. Recovery of ^{15}N-labelled fertilizer nitrogen applied to the summer crop of 1977-A by the spring crop of 1978-D

Treatments[a]	N uptake (mg pot^{-1})	^{15}N (%)	^{15}N added (mg pot^{-1})	^{15}N recovered (%)
			Tainung No. 67	
I	326	0.5	105	1.5
II	292	0.8	105	2.3
III	244	0.9	105	2.1
IV	300	1.6	210	2.2
V	263	1.6	210	2.0
			NTU No. 43	
I	347	0.5	105	1.5
II	304	0.6	105	1.6
III	267	0.5	105	1.3
IV	302	1.1	210	1.6
V	270	1.6	210	2.0

[a] Treatments of 1977-A are described in Table 1.

The results of Experiment 1978-C were used to determine the effect of continuous cropping on the recovery of fertilizer nitrogen. The results are shown in Table 8, and the contributions of soil and fertilizer nitrogen to the total uptake are evaluated and listed in Table 9. They indicate the following:

1. Over-all recovery of fertilizer nitrogen was slightly reduced, because of the general tendency for decreased recovery of fertilizer nitrogen applied at various growth stages, as the result of continuous cropping. Only the fertilizer nitrogen applied at the heading stage was absorbed with a higher efficiency.
2. Although the recovery rates were lower in the 1978-C experiment, nevertheless the fertilizer nitrogen constituted nearly 50% of the total nitrogen absorbed by the rice plants. This was probably due to the fact that the amount of soil nitrogen available to the rice plants had been reduced by the absorption of the previous crop, as shown in Table 9.

Table 8. Fertilizer nitrogen recovery by ^{15}N method in the rice plants of 1978-C, grown on soils which were used in 1977-B experiment

Treatments[a]	N uptake (mg pot^{-1})	^{15}N (%)	^{15}N added (mg pot^{-1})	^{15}N recovered (%)
			Tainung No. 67	
II	331	4.1	105	13
III	333	8.4	105	27
IV	337	16.5	105	53
V	315	20.2	105	61
			NTU No. 43	
II	372	6.0	105	21
III	364	10.2	105	35
IV	339	17.0	105	55
V	341	17.2	105	56

[a] Treatments are described in Table 2.

Table 9. Amounts of nitrogen recovered by the rice plants from fertilizer and soil sources (mean of all treatments)

Sources	(mg N pot^{-1})								
	1977-A			1978-A			1978-C		
	309[a]	43[b]	Av.	67[c]	43	Av.	67	43	Av.
Fertilizer N	184	179	182	194	194	194	166	178	172
Soil N	325	338	332	380	412	396	163	176	170
Total	509	517	513	574	606	590	329	354	342

[a] Taipei No. 309, [b] NTU No. 43, [c] Tainung No. 67.

With the limited amount of soil used and under the conditions of these experiments, apparently the mineral nitrogen supply could not be maintained at its original level during the three months of the cold inter-crop season. The decrease in nitrogen uptake resulted in a significant reduction in the grain yields: 28.5 and 31.0 g pot^{-1} for Tainung No. 67 and NTU No. 43, respectively, when not cultivated continuously (1978-A), as compared with 14.6 and 17.8 g pot^{-1} for respective varieties when cultivated continuously (1978-C).

ACKNOWLEDGEMENTS

This work was supported by the National Science Council, China, under Project No. NSC-66B-0409-02(11).

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SOURCES OF NITROGEN AND CROP RESPONSES TO FERTILIZER NITROGEN IN RICE DOUBLE-CROPPING SYSTEMS IN MALAYSIA

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ABSTRACT

In Peninsular Malaysia about 65% of the rice land is double-cropped. The source of water for the wet season crop is rainfall, while in the dry season irrigation is provided from river catchments. The nitrogen supply in the irrigation water is generally low, and substantial loss of applied nitrogen fertilizer occurs in drainage water. The incorporation of moderate amounts of crop residues can contribute to maintaining the nitrogen content of the soil.

An attempt has been made to understand the role of native soil nitrogen in maintaining crop yield, and the response to nitrogen application. It is observed that in a rice double-cropping system, a lower grain yield is obtained in the non-nitrogen plots during the wet season. A period of soil drying prior to the dry season crop seems to increase the availability of soil nitrogen.

In the rice double-cropping system, the source of irrigation water, method of straw disposal, the length of fallow period, and soil drying between crops have a marked influence on the nitrogen supply to the soil, the crop yield and response to applied nitrogen.

INTRODUCTION

In Peninsular Malaysia about 65% of the rice land is under double-cropping. The wet season crop depends on the high rainfall that adequately floods the fields, while the dry season crop depends on irrigation. In the double-cropping system there is a regular pattern in crop yields, and in responses to nitrogen fertilization, in the two seasons. Nitrogen is the main nutrient deficiency of rice in this system, other nutrients giving only marginal responses in grain yield in specific localities.

In the system discussed above, the important sources of nitrogen input, other than fertilizer, are the irrigation water and biological nitrogen fixation. The availability of soil nitrogen and the rice cultural practices determine the balance of the natural nitrogen supply. This supply, however, is not sufficient to meet the nutrient requirements of the current high yielding rice varieties under double-cropping conditions, and nitrogen fertilizers have to be applied. Nitrogen fertilizers in flooded rice soils are subject to losses, and it is estimated that only about 30-40% of the applied nitrogen is effectively used by the rice plant.

In this paper the intention is to identify the various inputs of nitrogen in the rice double-cropping system and to describe the pattern of crop yields and response to nitrogen in the two planting seasons.

CROP YIELDS IN RICE DOUBLE CROPPING

In the Muda Irrigation Scheme (96 000 ha), the rainfall pattern and the water distribution system essentially determine the cropping schedule for the double-cropping of rice. The average grain yields in the Scheme, since the beginning of double-cropping, are given in Table 1. The grain yield in the dry season is generally higher than in the wet season. The average solar radiation in the dry and wet seasons are not very different - 679 W m^{-2} d^{-1} (474 cal cm^{-2} d^{-1}) and 633 W m^{-2} d^{-1} (441 cal cm^{-2} d^{-1}) respectively (Nozaki *et al.*, 1977). The dry season crop is grown from March to August with irrigation. Dry weather with high temperatures, high solar radiation and low precipitation prevails for 1 to 2 months before transplanting commences, but thereafter precipitation increases, reaching an average of about 200 mm $month^{-1}$ during the ripening period. The wet season crop is grown from September to February. The average precipitation of 250-300 mm $month^{-1}$ persists for the period from sowing to about heading, after which the weather is dry.

The dry season crop is harvested during the rainy season and the cut straw is to a large extent removed from the field. The wet season crop is harvested during the dry season and

the crop residues are generally burned and partially ploughed into the soil. In the double-cropping system there is a definite fallow and soil drying period prior to the dry season crop, while there is only a very short fallow period prior to the wet season crop when the soil remains submerged or wet. The methods of straw disposal, and the types of fallow period between crops,appear to have a substantial influence on nitrogen availability and crop yield.

Table 1. Average grain yield trend in the Muda Irrigation Scheme since the beginning of double-cropping

Year	Dry season	Wet season
	(t ha^{-1})	
1968	-	3.2 (single-crop)
1969	-	3.3 (single-crop)
1970	3.8	3.6
1971	3.9	3.7
1972	4.0	3.9
1973	4.0	3.9
1974	4.2	3.9
1975	4.2	3.7
1976	4.7	4.0
1977	4.0	3.7

Table 2. Grain yield of rice as related to the clay and silt content and C.E.C. of soils

	Soil Series		
	Chengai series	Tualang series	Hutan series
Nitrogen applied: (kg ha^{-1})	Grain yield (t ha^{-1})		
0	4.38	3.66	3.06
80	5.50	4.59	4.45
Soil characteristics:			
Clay (%)	75	60	41
Silt (%)	20	30	18
C.E.C. (mg (100 g soil)$^{-1}$)	32	19	17

Studies in the Muda Irrigation Scheme indicate that rice grain yield is positively related to the clay, silt and cation exchange capacity (C.E.C.) of the soil (Table 2).

SOURCES OF NITROGEN SUPPLY

Biologically fixed nitrogen

In water-logged rice soils the active autotrophic nitrogen fixers are *Azolla*, blue-green algae and photosynthetic bacteria, while the heterotrophic rhizosphere bacteria can also be important. The amount of biologically fixed nitrogen in a rice field is estimated to be 13 to 99 kg ha^{-1} (Hauck, 1971).

Plant residues

In Peninsular Malaysia, an immediately available source of organic matter in rice cropping is the straw. Rice straw incorporated into the soil in moderate amounts is beneficial for nitrogen fixation and would contribute about 30-50 kg N ha^{-1} (Kanapathy, 1976). The incorporation of straw also assists in temporary immobilization of nitrogen and reduction in losses of nitrogen by seepage and percolation. Vamadevan & Samy (unpublished) found that leaching losses of N were 25% when straw was incorporated and 37% when all crop residues were removed. About 60-80% of the straw was decomposed within three weeks after incorporation, without any adverse effect on crop growth.

Soil organic nitrogen

Soil organic matter is a major source of nitrogen for the rice crop. Rice soils contain 1000 to 6000 kg N ha^{-1}, mostly in organic forms (T. Yoshida, pers. comm.). In the Muda Irrig-

ation Scheme, nitrogen uptake by the rice plant in the non-nitrogen plots is 53 to 71 kg N ha^{-1} (Samy, 1977).

Fertilizer nitrogen

Fertilizer studies in the Scheme have shown that native soil nitrogen plays a role in determining the response of the crop to applied nitrogen. The response pattern of three rice varieties in three different soil series is illustrated in Fig. 1. In spite of the varietal differences, it is evident that the Chengai soil series gives the highest grain yield in the non-nitrogen plots followed by the Tualang and Hutan soil series. This is related to the plant uptake of nitrogen in the non-nitrogen plots, which is 71, 69 and 53 kg N ha^{-1} in the Chengai, Tualang and Hutan soil series respectively. These values reflect differences in both available soil nitrogen and relative fertility of the three soil series.

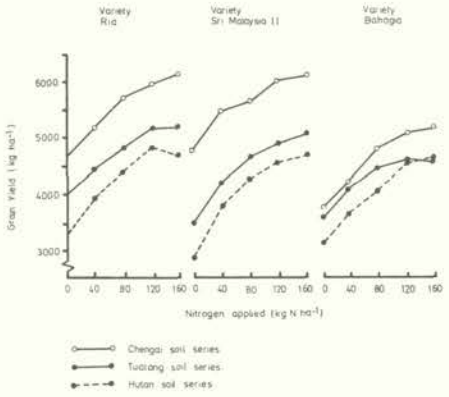

Fig. 1. Grain yield responses of three rice varieties to nitrogen application in three soil series.

Studies by Nozaki *et al.* (1977) have shown that the grain yield in the dry season without the application of nitrogen fertilizer is higher than in the wet season (Table 3).

In Japan it has been recognised for a long time that drying of rice soil before submergence improves the growth of the rice plant in comparison to fields that are not dried (Mitsui, 1960). This is due to the accumulation of inorganic nitrogen in the soil by microbial mineralization of soil organic nitrogen. The higher soil temperature in the dry season would also favour the mineralization process.

Table 3. Effect of nitrogen application on grain yield in the dry and wet season (after Nozaki et al., *1977)*

Variety	Dry season crop			Wet season crop		
	Without nitrogen	With nitrogen	Difference	Without nitrogen	With nitrogen	Difference
	(t ha^{-1})					
Jaya	3.05	4.06	1.01	2.04	3.12	1.07
Bahagia	2.97	3.52	0.55	2.42	3.29	0.87

Rainfall and irrigation water

The nutrient content of rain water is low and the nitrogen supply is only in the range of 3 to 10 kg ha^{-1} yr^{-1} (Kanapathy, 1968). The nutrient content of irrigation water depends on its origin and the area over which it flows. Irrigation water in Peninsular Malaysia often contains adequate amounts of plant nutrients, except for nitrogen.

The nitrogen concentration of irrigation and drainage canals in several rice growing areas in Peninsular Malaysia is given in Table 4. In general, the drainage water contains as high a concentration of nitrogen as the irrigation water. This would indicate that there is a considerable loss of nitrogen from the rice fields, particularly in the irrigation systems with gravity flow of water from plot to plot which finally discharges into drainage canals.

The nitrogen loss would possibly also include losses from applied nitrogen fertilizers. It is not surprising from the analyses that luxuriant growth of aquatic weeds like water hyacinth is frequently observed in drainage canals.

Table 4. Average analyses of irrigation water and drainage water in some selected areas during the 1962-63 seasons (after Kanapathy, 1968)

Location	pH	Total N (ppm)	NH_4^+-N (ppm)
Kedah			
Inlets	7.1	1.60	0.58
Drainage	6.8	1.25	0.34
Province Wellesley (Bumbong Lima)			
Inlet	7.0	1.73	0.47
Outlet	6.2	2.01	0.59
Kelantan			
Inlets	6.4	1.40	0.36
Drainage	6.4	1.45	0.40

In the dry season, the total water consumption for rice cultivation in the Muda Irrigation Scheme is estimated to be 1344 mm (Sugimoto, 1971). With an average of 2.7 ppm of total nitrogen content, the irrigation water would contain 31 kg N ha^{-1}. In the wet season the rice crop would derive very little nitrogen from the flood water as the main water supply is rainfall.

CONCLUSION

In the rice double-cropping system, the methods of straw disposal and the types of fallow period between crops have a marked influence on the availability of soil nitrogen and on crop yields. Soil organic nitrogen plays an important role in the nitrogen nutrition of the crop.

ACKNOWLEDGEMENTS

The authors are grateful to the Director-General of MARDI for permission to present this paper. Thanks are also due to Encik Ramli Saad, Encik Ishak Nayan and Encik Idris Ismail for their assistance in conducting the field trials.

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SOME KEY PROCESSES IN THE NITROGEN CYCLE OF RICE-BASED MULTIPLE CROPPING SYSTEMS

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ABSTRACT

Studies of key processes in the nitrogen cycle of rice-based multiple cropping systems in the Chiang Mai Valley are presented. The topics include the nitrogen requirements of the wet season rice crop, survival of rhizobia under paddy rice cultivation, and a possible role of nitrogen fertilizer in a decline in soil fertility under increasing cropping intensity.

For soils with a low N content, the grain yield potential and harvestable N are high: 4.5 t ha^{-1} and 90 kg N ha^{-1} respectively. The possible contribution from biological fixation of nitrogen and differences between rice varieties in their ability to take up nitrogen from unfertilized soils are noted.

The number of rhizobia in soil decreased markedly upon flooding, but some survived until the end of the rice season. The growth of the rhizobial population from the time the field was drained to the time of legume germination is suggested as the next critical step in the symbiotic development.

Experimental evidence and field observations suggest that under "improved technology" - with new improved crop cultivars, increased inputs of fertilizers, chemical pest control measures and intensive cropping with up to three crops per year - drastic reductions in yields of paddy and other crops may have resulted from a decline in soil fertility and stability. Soil acidification is thought to be one of the factors involved and this is discussed in relation to the role of nitrogen fertilizers.

INTRODUCTION

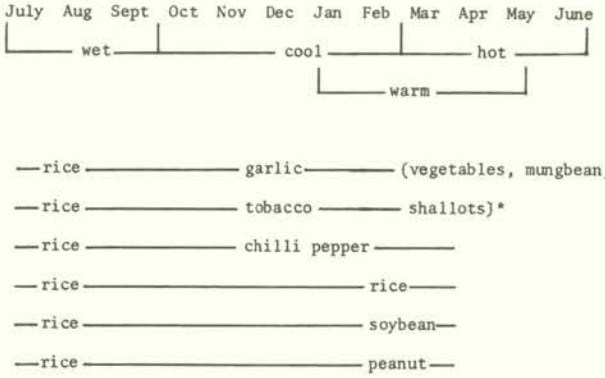
Multiple cropping in the Chiang Mai Valley is an established practice. Cropping patterns are built around rice, which is grown in submerged soil. The seasons in Chiang Mai, in common with the rest of northern Thailand, can be divided into: wet (July-October), cool (November-February) and hot (March-June). Most of the 1100 mm of annual rain falls in the wet season and irrigation is essential for cropping during the cool and hot seasons. Virtually all arable land in the valley is planted to paddy rice in the wet season. In some areas the wet season rice crop is followed by one or two crops, i.e., double or triple cropping. Currently the cropping intensity index over the whole valley is 163%. The common cropping patterns are shown in Fig. 1.

The Multiple Cropping Project (MCP) of the Faculty of Agriculture, Chiang Mai University, is an interdisciplinary group concerned with problems of multiple cropping in the Chiang Mai Valley. As in most other agroecosystems, nitrogen supply is one of the biggest concerns. This paper presents some of the MCP work on processes involved in the nitrogen cycle in rice-based multiple cropping.

NITROGEN FOR THE WET SEASON RICE CROP

Most of the rice cultivars planted in the wet season are of the glutinous, photosensitive type, normally with no nitrogen fertilizer. The yield, nitrogen uptake and response to nitrogen for one typical variety, Niew San Patong, are shown in Table 1 in comparison with a new improved variety, RD1.

Without nitrogen fertilizer the average paddy yield was about 4.5 t ha^{-1} for both varieties. The amount of nitrogen harvested was 89.9 kg N ha^{-1} for Niew San Patong and 60.5 kg N ha^{-1} for RD1. The higher straw yield accounted for most of the greater nitrogen uptake in Niew San Patong, but its nitrogen concentrations were also higher. The nitrogen content of the soil is very low (0.08-0.10%); rain and irrigation water possibly contributed 20 kg N ha^{-1} to the amount of nitrogen harvested (A.D. Brown, pers. comm.). Other nitrogen sources are biological fixation and residual nitrogen from preceding crops. The difference in N



* any of the hot season crops may follow a cool season crop in triple cropping.

Fig. 1. Seasons and cropping patterns in the Chiang Mai valley.

Table 1. Grain yield, N uptake and grain yield response to N in farmers fields[a]

Rice yield without nitrogen fertilizer	Grain (g ha^{-1})	Straw (t ha^{-1})	Total N-uptake (kg ha^{-1})
Niew San Pa Tong (traditional variety)	4.55	9.82	89.9
RD 1 (new improved variety, an early progeny of IR 8)	4.63	4.47	60.5

Responses to nitrogen fertilizer	Paddy (g ha^{-1})			
	N_0[b]	N_{30}	N_{60}	N_{120}
Niew San Pa Tong	4.55	4.59	4.73	-
RD 1	4.63	-	6.16	6.43

[a] Total soil N = 0.08 - 0.10%, [b] kg N ha^{-1} applied.

uptake between Niew San Patong and RD1 deserves to be investigated further. In this experiment Niew San Patong did not respond to 60 kg N ha^{-1} of nitrogen fertilizer; therefore nitrogen deficiency was not likely to be the factor limiting yield of this variety. On the other hand the extra 30 kg N ha^{-1} that was taken up by Niew San Patong did not appear to be available to RD1, since the latter strongly responded to 60 kg N ha^{-1}.

SURVIVAL OF *RHIZOBIUM* IN SUBMERGED SOIL

Legumes play a prominent role in the cycling of nitrogen in multiple cropping. Legume nodulation is highly dependent on the number of rhizobia present in the rhizosphere during germination, particularly under adverse conditions, e.g., in acid soils. Survival of the strictly aerobic rhizobia under flooded rice conditions largely determines the success of symbiotic development of legumes grown as components of rice-based multiple cropping systems. In one experiment, the number of rhizobia decreased about 10-fold in two weeks after flooding. From an original population of some 10^4 cells g^{-1} soil about 10-100 cells g^{-1} remained viable after 60 days under water. Therefore, it would seem that although the number of rhizobia in rice soil decreases under rice cultivation, some can survive through the rice growing season. This was confirmed by the number of rhizobia counted in soil samples taken from under a rice crop in the field just before the water was drained to prepare for harvest. The number of

viable rhizobia found ranged from 10 to over 10^4 cells g^{-1} soil. However, these numbers of rhizobia are rather small for successful nodulation. Therefore, the next critical step would be the growth of the rhizobial population from the time the field is drained to when the legume germinates. In addition, the other aspect of survival that needs to be considered is variability among strains of *Rhizobium* in their ability to survive in flooded soil and the correlation between this ability and symbiotic efficiency in nitrogen fixation.

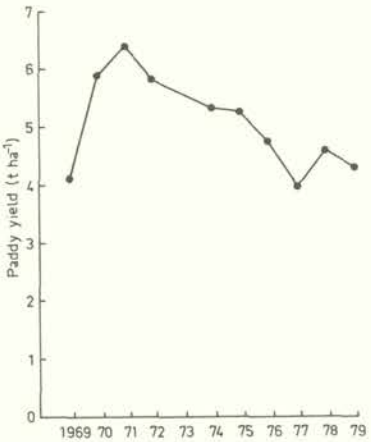


Fig. 2. Paddy yields in intensive systems from 1969 to 1979 (each year represents mean from 16 plots, except for 1979).

DETERIORATION OF SOIL UNDER RICE-BASED MULTIPLE CROPPING SYSTEMS: POSSIBLE ROLE OF NITROGEN FERTILIZERS

Under intensive cropping, with up to three crops a year, and high inputs of fertilizers and chemical pesticides, a declining trend in the yield of rice (Fig. 2) and other crops suggests that the stability of the system may have been disturbed.

One of the changes that has been monitored is soil acidification. The remainder of this paper deals with the nature of pH changes under rice-based multiple cropping systems and the possible role of nitrogen fertilizers. The cropping patterns chosen were rice-garlic-sweetcorn and rice-soybean-mungbean. A brief description of nitrogen fertilizer input and land preparation in these systems is given in Table 2.

Table 2. Nitrogen inputs, as ammonium sulphate, and land preparation for two cropping systems used to study pH changes

System:	Rice	RT[a]	Garlic	RT	Sweet corn
kg N ha^{-1}	90		105		40
System	Rice	NT[b]	Soybean	NT	Mungbean
kg N ha^{-1}	90		nil		nil

[a] Rototilled and bedded up, [b] No tillage.

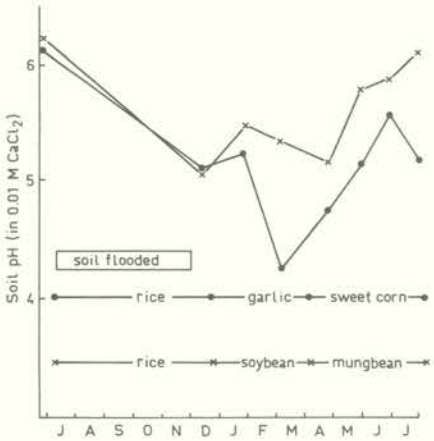


Fig. 3. Soil pH as affected by time and by different cropping systems. All samples were taken when soil was dry; in case of rice, before the soil was flooded and after drainage was completed (S. Penchan & B. Rerkasem, unpublished results).

Soil under rice-based multiple cropping exhibits marked fluctuation in acidity within a year (Fig. 3). Cropping patterns have a strong influence on these changes. From an original pH of about 6.2 (in 0.01 M $CaCl_2$ at a soil to solution ratio of 1:5) a decrease to pH 5.0 was observed after one crop of rice. When rice was followed by soybean, with neither tillage nor fertilizer input, the soil pH gradually increased, reaching close to the original value of pH 6.1 at the end of the cropping cycle. In the system of rice-garlic-sweetcorn, soil acidity continued to increase further under garlic to about pH 4.3. Towards the end of the garlic season the pH began to increase and continued to increase under sweet corn to just above pH 5, when the cropping cycle ended. Similar trends were observed in farmers' fields. Changes in soil pH under rice-peanut resembled those under rice-soybean, those under rice-tobacco were similar to those under rice-garlic (Table 3).

Table 3. Changes in soil pH[a] under farmers' cropping systems

7.2	rice	6.4	peanut	6.9
6.4	rice	5.8	soybean	6.4
6.3	rice	5.8	tobacco	5.6
6.9	rice	6.0	garlic	5.8

[a] Each line denotes a cropping sequence with the pH values found by sampling before and after each crop. Each value is the average of measurements from 5-8 farms.

The implication of this to multiple cropping is clear: unless lime is applied, crops following paddy rice will be subjected to some acidity problems. Other processes in the soil may also be affected by acidity after the rice crop. For example, manganese toxicity problems in crops following rice may be accentuated, since oxidation of manganous ions released under rice, is solely microbiological and is inhibited at a pH lower than 5.8 (Leeper, 1970). This dynamic aspect of soil acidity deserves to be considered more critically by those involved in rice-based multiple cropping.

To assess the possibility of nitrogen fertilizers playing a role in soil acidification, the effects of source of nitrogen on soil acidity were examined under waterlogged (rice) and upland (sweet corn) conditions. The results are shown in Fig. 4.

With sweet corn, an upland crop, the effect of ammonium sulphate and ammophos (ammonium sulphate and ammonium phosphate mixture) were as expected, i.e., these nitrogen sources, at

the rate of 80 kg N ha^{-1}, caused a decrease in soil pH of 0.3-0.5 pH units. In comparison there were slight increases in the pH when the same amount of nitrogen was applied as urea or when none was applied. The slight increase in soil pH may be explained by the dynamic nature of soil acidity discussed above.

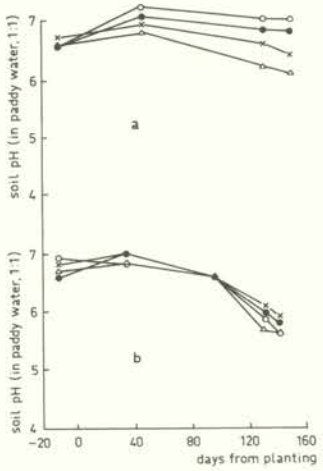


Fig. 4. Effects of nitrogen fertilizers on soil pH changes in time, under upland (sweet corn) (a) and waterlogged (rice) (b) conditions.
o = *nil nitrogen,* ● = *urea,* x = *ammonium sulphate, and* Δ = *ammophos. All applied N at 80 kg* ha^{-1} *for (a) and 120 kg* ha^{-1} *for (b) (B. Lerssupavithnapa & B. Rerkasem, unpublished results).*

The effect of nitrogen fertilizer on pH change under rice was rather unexpected. In a submerged soil a drop of about one unit of pH occurred, regardless of nitrogen fertilizer treatment. No similar results or any explanation of the effect, has been found in the literature. Accumulation of organic acids is suggested as one possibility, loss of cations through leaching and plant uptake is another. The cation exchange capacity of the soil is very low, at 4 meq 100 g^{-1}.

The main objective of the Multiple Cropping Project is to increase food production at the least cost, while maintaining a system which is ecologically as well as socio-economically stable. Nitrogen is one of the most critical inputs to the system, and how it is utilized has a crucial impact on the stability of the system. The results presented above cover some aspects of the key processes, which are being studied in an attempt to understand the system, and in order that it may be modified to meet farmers' needs.

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NITROGEN CYCLE IN RUBBER *(HEVEA)* CULTIVATION

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ABSTRACT

The paper presents data on nitrogen depletion from the soil, through uptake and immobilization in trees, and removal in crop. Nitrogen needs, based on estimates and confirmed by trials, are discussed in relation to nitrogen in the soil, returns from leaf litter, and nitrogen input from rain, leguminous covers or fertilizers; leaching losses are also considered.

The paper also evaluates in detail, the role of leguminous covers as an important source of nitrogen in Hevea *cultivation.*

Generally, in a stand of rubber trees, over a period of about 30 years, the trees immobilize about 1700 kg N ha^{-1}*, while a further 500 kg N* ha^{-1} *is removed in the latex harvested. Thus the tree's requirement for N could be assessed at 2200 kg* ha^{-1}*. In well-maintained fields where leguminous covers are established, 550 kg N* ha^{-1} *of fertilizer N is sufficient to meet the tree's needs, while in non-legume cover areas, about 1500 kg N* ha^{-1} *is needed. Thus it is estimated that legumes could account for a supply of about 1000 kg N* ha^{-1}*. In addition, the annual leaf fall from rubber trees recycles about 50 kg N* ha^{-1} yr^{-1}*. This recycling contributes to the total requirement for N by the trees.*

INTRODUCTION

Rubber (*Hevea brasiliensis*), which occupies about two million hectares or over 55 percent of the cultivated land in Malaysia, has an economic life span of thirty years. It requires a well-drained and deep soil with fairly good physical characteristics and a soil pH of 4.2 to 5.5 for optimal growth.

The major nutrient requirements of the crop are nitrogen, phosphorus, potassium and magnesium. The contents of these nutrients in a soil vary with agronomic practices and the time lapse since the conversion of the land from jungle to rubber. The total consumption of nitrogen by the rubber industry is about 25 000 t N yr^{-1} (Khoo, 1979).

In the cultivation of *Hevea*, the trees are planted in rows spaced at 9 to 10 m, and a planting distance within a row of about 2 to 2.5 m. During the initial stages after establishment, a small circular area at the base of the tree is kept free of weeds. This area is gradually enlarged with growth of the canopy. Towards the end of the second year of growth, the weeded circles merge to form a clean strip along the tree rows. By the time the canopies of the trees close over, this weed-free zone on which the fertilizers are applied, would be about 2 m wide. However, at this stage, the root zone can extend laterally to beyond 5 m on either side of the tree. The interrow space between two weeded strips can support varying vegetation depending on cultural practices. The type of interrow vegetation considerably affects the nitrogen status of the soil.

This paper discusses the nitrogen needs of rubber in relation to the tree's requirement, the fertilizer practice, and changes that take place in the nitrogen cycle in the interrow area.

NITROGEN REQUIREMENT OF THE TREE

For growth or tree immobilization

By destructive sampling, Shorrocks (1965) estimated the total amount of nutrients that are immobilized in the tree during its 30 years of economic life. The nutrients immobilized as assessed by Shorrocks (1965) were adjusted, based on the work of Lim (1978). The latter worked with clone RRIM 600 and confined his sampling to the first few years after establishment of rubber. The total amount of nitrogen required for growth or immobilized, as reassessed, is given in Fig. 1. This shows that during the thirty years of economic life the trees remove about 1700 kg N ha^{-1} to satisfy their growth function.

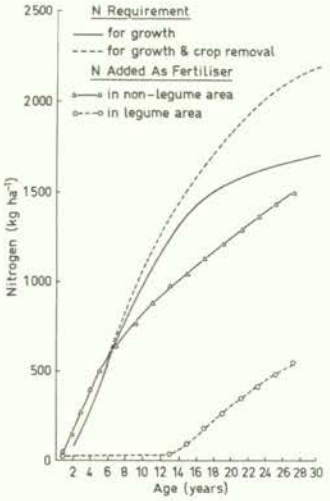

Fig. 1. N requirement for growth and yield of rubber, and fertilizer N added to crops without and with legumes, over a 30-year growth period.

Table 1. Nitrogen added, drained in latex and returned in leaf litter

Age (yr)	Stand (trees ha^{-1})	N added (kg ha^{-1})[a] Legume	Non-legume	Dry rubber yield[b] (kg ha^{-1})	N removed in rubber[c] (kg ha^{-1})	Dry wt[d] leaves (kg ha^{-1})	Litter-N[e] (kg ha^{-1})
1	395	27.1	36	-	-	-	-
2	380	-	110	-	-	-	-
3	370	-	120	-	-	-	-
4	360	-	133	-	-	-	-
5	350	-	103	-	-	3500	48
6	345	-	67	620	6.1	3800	52
7	340	-	67	1030	10.2	4250	58
8	335	-	67	1360	13.5	4850	66
9	330	-	60	1580	15.6	4980	68
10	320	-	60	1960	19.4	5150	70
11	315	-	55	1970	19.5	5355	73
12	310	-	50	2000	19.8	5210	71
13	305	-	45	2100	20.8	5030	68
14	300	35	35	2300	22.8	4920	67
15	300	35	35	2010	19.9	4800	65
16	295	35	35	1860	18.4	4570	62
17	290	50	50	1730	17.1	4400	60
18	285	45	45	2260	22.4	4270	58
19	280	45	45	2500	24.8	4120	56
20	275	40	40	3000	35.7	3850	52
21	275	40	40	2700	32.1	3800	52
22	270	35	35	2500	29.8	3650	50
23	260	35	35	2100	25.0	3380	46
24	260	35	35	1900	22.6	3250	44
25	260	35	35	1800	21.4	3120	42
26	260	30	30	1600	19.0	3040	41
27	255	30	30	1600	19.0	2860	40
28	255	-	-	1600	19.0	2670	36
29	255	-	-	1400	16.7	2600	35
30	255	-	-	1200	14.3	2500	34

[a] Partly based on Pushparajah & Mahmud Wahab (1977).

[b] Assumed commercial yield of RRIM 600 - 85% of trial yield (Rubber Research Institute of Malaysia, 1977) and stimulation introduced from 20th year.

[c] Based on Pushparajah *et al.* (1972).

[d] Based on Shorrocks (1965).

[e] Mean N in litter = 1.36% (Tan 1977).

For production or removal in crop

From the sixth year after establishment of the trees, harvesting (i.e. tapping) commences. The nitrogen losses through latex removal vary from 6.1 to 35.7 with a mean of 20.2 kg ha^{-1} yr^{-1} (Table 1 and Fig. 1). The variation in removal of nitrogen in the latex is mainly due to the different latex yields. However, the use of yield stimulants from the twentieth year, in addition to increasing the yield and hence the total N removed, also results in an increase in concentration of N removed per unit weight of yield (Pushparajah *et al.*, 1972).

The sum of nitrogen immobilized in the trees and that removed in the latex (Fig. 1) may be considered as the tree's total requirement. This amounts to about 2200 kg ha^{-1}.

NITROGEN FERTILIZATION

Various investigations, as reported by Pushparajah (1977), have shown that the amount of nitrogenous fertilizers required by rubber varies with ground cover, soil conditions, clonal material used, age of the tree and the yield or exploitation system. However, for purposes of the current study, a common high yielding clone RRIM 600, growing on a common soil, Rengam series (an Ultisol), is considered. The fertilizer needs under two cover conditions during the initial 15 years (Pushparajah, 1977; Pushparajah & Mahmud, 1978) and the estimated requirements during the latter phase, are given in Table 1 and Fig. 1. This clearly shows that the amount of nitrogen added (based on response in fertilizer trials) falls short of the total nitrogen requirements of the trees. In addition, a part of the applied nitrogen will be lost from the soil through leaching and surface runoff. The balance of the tree's needs must therefore be obtained from the soil or from outside the ecosystem. The total deficit of nitrogen, which must be furnished from elsewhere during a 30-year period, ranges from 1650 kg ha^{-1} in an area where a legume cover was maintained to 720 kg ha^{-1} in a non-legume cover area.

NITROGEN IN THE SOIL

The nitrogen contents of most soils in replanted areas are low. The value for total nitrogen in the upper 45 cm of soil (Table 2) varies from a low 2720 kg ha^{-1} in the Durian series to a high 22 180 kg ha^{-1} in the Selangor series soil. The top 45 cm of soil has been used to assess the total N present, as Soong *et al.* (1971) have shown that most of the feeder roots are present in this zone and hence this zone is the most active in nutrient supply. The total nitrogen data have been used, as Tan (1972) showed that this parameter related best with uptake of nitrogen by rubber.

Table 2. Estimate of total nitrogen in the top soil (0-45 cm)

Soil series	Soil order	Bulk density (g cc^{-1})	Range of N (kg ha^{-1})
Rengam	Ultisol	1.15	4190 - 11300
Serdang	Ultisol	1.30	4090 - 10690
Durian[a]	Ultisol	0.95	2720 - 13870
Malacca[b]	Oxisol	1.07	2800 - 10100
Holyrood	Entisol	1.25	5150 - 13560
Selangor	Inceptisol	0.69	4650 - 22180

[a] Fifteen % of soil is laterized nodules more than 2 mm.

[b] Thirty % of soil is laterite and of fraction more than 2 mm.

Even when the total nitrogen is considered, it is obvious that at least in some situations the soil will not be able to support the tree's needs; especially if a second replanting is made without supplementary nitrogen applications. At each replanting, the old trees are felled, most of the timber is removed and the residue burnt. Thus, at each replanting, about 1700 kg N ha^{-1} is removed from the area.

Furthermore, the nitrogen in the soil would be available only very slowly. This was demonstrated by Guha & Watson (1958), who showed that about 7.8 percent of the nitrogen in Rengam series and 4.1 percent in Selangor series soils were mineralized annually. This is low in relation to the tree's needs. The addition of ammonium sulphate at rates equivalent to 115 kg N ha^{-1} did not affect the rate of mineralization. Similarly, the addition of senescent leaves of rubber did not alter the mineralization rate or amount. However, the

addition of litter from leguminous covers such as *Pueraria phaseoloides* or *Centrosema pubescens* increased the mineralization rate by two to three fold.

EFFECT OF COVERS ON SOIL NITROGEN

The type of cover influences the nitrogen level of the soil. In various investigations (Watson *et al.*, 1964c; Pushparajah & Chellapah, 1969), it was observed that a rapid depletion of the nitrogen level in the topsoil would result if the soil surface were bare, even under the canopy of rubber. The total soil N content fell from the original 0.130% to 0.092% by the eighth year when the soil surface was bare (Table 3). However, when a legume cover was maintained, the soil nitrogen level increased continuously until the fourth year and thereafter declined to a level of 0.115% by the eighth year. However, the final level was still lower than the inherent value under forest conditions. The effect of the other types of cover on soil nitrogen content was intermediate.

Table 3. Effect of covers on soil nitrogen status[a]

Type of cover	Nitrogen in soil (%)		
	2nd yr.	4th yr.	8th yr.
Legumes[b]	0.140	0.144	0.115
Grass[c]	0.130	0.123	0.109
Naturals[d]	0.119	0.124	0.106
Bare	0.099	0.095	0.092
L.S.D. (P = 0.05)	0.012	0.017	0.009

[a] At clearing soil N was 0.130%; results of year 2 and 4 from Watson *et al.* (1964c) for year 8 from Pushparajah & Chellapah (1969).

[b] *Pueraria phaseoloides, Calopogonium mucunoides, centrosema pubescens.*

[c] *Ischaemum muticum* (90%) and *Ottochloa nodosa* (10%).

[d] *Trema, Hornstedtia, Solanum, Macaranga, Ficus* etc.

Watson *et al.* (1964c) report that in the second and third year the mineralization rate under bare conditions was rapid. The NO_3^--N content in a bare soil was about 30 to 40 ppm as compared to levels of 10 to 15 ppm under the other cover conditions. On the other hand, the level of NH_4^+-N in the bare soil was only about 2 ppm, while under covers this ranged from 5 to 10 ppm. Under bare conditions, a considerable proportion of the nitrogen had leached to the subsurface horizons as NO_3^--N (about 40 ppm in the 60-75 cm and 75-105 cm layers), but in the areas with inter-row covers there was no appreciable downward movement of nitrogen.

This clearly indicates that legume covers, besides replenishing the soil nitrogen, can also suppress the rate of N losses. It is emphasized that the levels of N observed in the soils under legume covers do not reflect the full returns of N by these covers because a high proportion of the litter still remains on the surface of the soil.

ADDITION BY RAINWATER AND INTERNAL CYCLES

Rainfall

With an annual rainfall of 2500 mm, Shorrocks (1965) estimated a return of about 20 kg N ha^{-1} yr^{-1}. Subsequent work has shown that in the vicinity of some industrial areas the amount of nitrogen returned through rain could be as high as 38 kg ha^{-1} yr^{-1}.

Annual leaf fall

From the fifth year after establishment (if not earlier), the trees shed their leaves annually and form new leaves. An estimate (Table 1) shows that the annual leaf fall returns nitrogen ranging from 30 to 70 kg ha^{-1} yr^{-1} depending on the age of the tree, the mean over the 25 years being about 56 kg N ha^{-1} yr^{-1}. However, Tan (1977) has shown that the rate of decomposition of this litter is very slow. During decomposition, there was little or no change in the percent nitrogen of the residual litter, but there was a loss in dry matter and

thus a loss or release in N. Based on this, he estimated that about 60 and 80% of the litter would decompose in about four and ten months respectively. However, an estimate of the gaseous losses of nitrogen during decomposition is not available.

Ground vegetation

The ground vegetation or covers in the inter-row areas play a role in the nitrogen cycle. Initially, they would compete with the trees for available nitrogen and render some of the native soil nitrogen unavailable to the trees. However, nutrients would be eventually returned to the soil as plant litter and would then become available to the trees and covers.

Watson *et al.* (1964b) found that, at the end of two years of growth, legume creepers immobilized 128 kg N ha^{-1} while covers of grasses, *Mikania cordata* and 'naturals', immobilized 45 and 84 kg N ha^{-1} respectively. Simultaneously during this period, the covers also returned some nitrogen to the soil in their litter. The amounts of nitrogen so returned were: creeping leguminous covers, 140; grasses, 63; *Mikania*, 68 and 'naturals', 64 kg ha^{-1}. Watson *et al.* (1964b) have shown that during the period of four to five years when the covers were able to persist, they returned the following amounts of nitrogen: creeping conventional legumes, 226-335; grasses, 24-65; *Mikania*, 74-119 and 'naturals', 13-117 kg ha^{-1}.

Subsequently, Pushparajah & Tan (1979) showed that estimates of nitrogen returns on the basis of sampling and analysing covers and litter at periodic intervals, as done earlier by Watson *et al.* (1964b), were in fact underestimates. Pushparajah & Tan (1979) found that in the absence of creeping legume covers about 860 to 1000 kg N ha^{-1} had to be applied to rubber trees to obtain growth and yields similar to those observed when legume covers were present. They therefore suggested that the effective contribution by legumes could be about 800 to 1000 kg N ha^{-1} over the total tree growth period of 30 years. Further, Pushparajah & Mahmud (1978) showed that the residual effect of covers on the nitrogen status and hence the needs of the trees, persists until the thirteenth year of cover and rubber establishment. This long-term residual effect of legume covers could in part be due to the early high build-up and partly to the very high leaf nitrogen status initially sustained in the trees, which later could have acted as reserve in the tree. Watson *et al.* (1964a) showed that at the sixth year after establishment the annual leaf fall from trees in the legume cover areas returned twice as much nitrogen as trees did in the non-legume cover areas, thus ensuring a larger annual cycling of nitrogen. Further, they also showed that the density of feeder roots of rubber in the legume plots was much higher than that in the non-legume area, allowing for greater exploitation of the soil nitrogen.

Table 4. Annual nitrogen return from mixed cover[a]

Years after establishment	N returned in litter (kg ha^{-1}) *Calopogonium caeruleum*	*Pueraria phaseoloides*	Total
1	-	151.0	151.0
2	16.0	117.0	133.0
3	101.4	165.9	267.3
4	127.3	35.8	163.1
5	101.4	21.4	122.8
6	75.5	7.0	82.5
7	45.4	0.3	45.7
8	12.6	-	12.6
1st to 8th	479.6	498.4	978.0

[a] Data for 3rd to 8th year from Tan *et al.* (1976); for the 1st and 2nd year are from a new study (E. Pushparajah, unpublished data).

Tan *et al.* (1976) showed that the use of a shade-tolerant legume cover, *Calopogonium caeruleum*, in combination with the more conventional legumes, allowed for increased returns of nitrogen from the covers. This was because the *C. caeruleum* was able to persist longer, even after canopy closure. The estimated amount of nitrogen returned between the third and eighth year was 694 kg ha^{-1}. A subsequent estimation has shown that this is an underestimate, since the returns during the first and second year had not been included in the earlier study. When these returns are also considered (Table 4), then the total nitrogen returned from the mixed legume cover is 978 kg ha^{-1}. The high N returns could in part be due to the proper maintenance and regular application of phosphate to the covers. The leaf nitrogen

concentration of the rubber trees at 15 years after establishment are over 3.70%; a level at which no nitrogen needs to be applied (Pushparajah & Tan, 1972). The residual effect of this cover is therefore expected to continue even longer.

Another important reason for the longer residual effect of *C. caeruleum* is the rate of decomposition of the leaf litter. Tan *et al.* (1976) showed that the rate of decomposition of the litter of *C. caeruleum* is much slower than that of *Pueraria*. Whereas *Pueraria* releases over 90% of the nitrogen from its litter in about 30 weeks, only about 50% of the nitrogen is released from the litter of *C. caeruleum* during the same period.

Thus, the use of *C. caeruleum* could result in a considerable saving in nitrogenous fertilizers, which would be more than when the conventional creeping legumes, discussed earlier, are used.

LEACHING AND AMMONIA VOLATILIZATION LOSSES FROM APPLIED NITROGEN

Earlier, it was shown that, under different cover conditions, losses of native soil nitrogen occur. These losses are considerably higher under bare conditions than when covers are present. Further, as indicated earlier, a weed free zone is maintained around the tree and the fertilizers are applied on this zone (Pushparajah *et al.*, 1977). During the first year the fertilizers are applied on a circle with a diameter of about 30-40 cm around the tree. Thus the area for fertilizer application in a 1 ha stand of trees is only 0.006 ha, and one application of fertilizer at 170 g per tree to a stand of 460 trees ha^{-1} would on an effective soil area basis, amount to 13 000 kg NPKMg fertilizers ha^{-1}. Ammonium sulphate accounts for 40 percent of the NPKMg formulation. Thus the effective rate of N ha^{-1} would amount to about 1090 kg N ha^{-1}. At this rate, depending on rainfall intensity and soil conditions, leaching losses could be as high as 50% of the applied N (Pushparajah *et al.*, 1977). Further, most of the N lost by leaching was in the NH_4^+-N form.

With a view to overcoming or reducing such losses, more frequent applications of fertilizers at lower rates have been recommended (Pushparajah *et al.*, 1974). However, based on the findings of Soong (1974) and Sivanadyan (1972), it can be deduced that even at the new recommended frequencies and levels, leaching losses of N would still range from 12 to 28% depending on the soils and the intensity of rainfall. For practical purposes, a mean leaching loss of 15% may be assumed.

Losses of N from ammonium nitrate were found to be more than that from ammonium sulphate; the losses occurring were about 60% of the losses in the sandier soils (Pushparajah *et al.*, 1977). On the other hand, when urea was used as a fertilizer and broadcast applied, as is the practice in rubber cultivation, volatilization losses of up to 24% have been measured in laboratory investigations (Watson *et al.*, 1962), this being an underestimate. This loss is in addition to the leaching loss. Pushparajah (1977) found in field investigations on a soil similar to the Rengam series that in order to obtain growth and yields similar to those observed under treatments receiving ammonium sulphate, twice as much nitrogen had to be used when the source of N was urea. This implies that total losses of N from urea can be assessed as more than 50% of the amount added under normal field practice. On the other hand, Pushparajah (1964) showed that on the marine coastal clay soils with high water tables (sulfic Tropaquepts) urea was equally as efficient as ammonium sulphate.

Ammonium sulphate and ammonium nitrate are the commonly used nitrogenous fertilizers in rubber cultivation. Thus, for general estimate purposes, it is assumed that 15% of the N added is lost by leaching.

NITROGEN BUDGET

It has been shown that during the first six years of growth, the fertilizer nitrogen supplied to rubber trees in non-legume areas is higher than that used by the trees (Fig. 1). In the case of legume cover areas, it has been shown clearly that the returns of nitrogen from the legumes is sufficient to meet the trees' needs.

From the seventh to the thirtieth year, the trees' needs for growth and removal in latex amount to about 67 kg N ha^{-1} yr^{-1}. During the same period, the amount of N fertilizer added in a non-legume area, after allowing for a 15% leaching loss, amounts to 32 kg ha^{-1} yr^{-1}. The balance of 35 kg N ha^{-1} yr^{-1} has to come from elsewhere.

Though the total N in rainfall is 20 kg ha^{-1}, after allowing for runoff and leaching losses only about 15 kg ha^{-1} could be assumed to be available to the trees. This return is distributed throughout the year, but Pushparajah & Tan (1972) have shown that after the annual leaf fall and regrowth of new leaves, the maximum uptake of N occurs when the leaves are less than four months old. This means that a high rate of uptake occurs in February to May, during refoliation and active leaf growth, which coincides with the periods of low precipitation. Thus, most of the nitrogen returned from the rain which falls outside this period may not be available to the rubber, as the N not used would be leached away.

Leaf litter fall from the trees returns an average of 56 kg N ha^{-1} yr^{-1}. However, the rate of decomposition is slow, with about 60% of the N being released in four months after leaf fall. As this release occurs during the active uptake period, the nitrogen will be utilized by the tree. Assuming that there is negligible gaseous loss during leaf decomposition, it can be assumed that leaf litter supplies about 30 kg N ha^{-1} yr^{-1} to the trees, while the remainder, which is released subsequently, would be lost by leaching. The balance of the nitrogen needs (about 5 kg ha^{-1}) could originate from the native soil reserves or residual build-up through the covers. The estimate shows that very little soil N is used by trees and this could explain the relatively stable level of soil nitrogen from about the eighth year after establishment of the trees.

Where conventional creeping legume covers are established, the N value of these legumes to the trees is about 1000 kg ha^{-1} over a whole growth cycle of 30 years, with most of the benefits being obtained in the first thirteen years. Nevertheless even under these cover conditions, from the sixth to the thirtieth year, the nitrogen added amounts to an average of only 21 kg ha^{-1} yr^{-1}.

Current investigations show that the use of a shade tolerant legume cover would be more beneficial. Though the present study has covered only about 15 years, yet it may be predicted that nitrogen application to mature rubber may not be required for some years in areas with shade tolerant covers.

Even using the evidence with the conventional legume covers, Ti *et al.* (1972) showed that the savings in nitrogenous fertilizers can be large and economically important. With a replanting cycle of 30 years, they assessed that there would be a an average of 56 300 ha of rubber being replanted each year. Likewise, there would be a similar area with rubber for each age from one to fourteen years after replanting; thus giving 788 200 ha of rubber in this age group. In all such areas, N need not be applied. They then assessed that the total amount of nitrogen saved by the rubber industry in Malaysia through the use of legume covers would be 64 665 t of N or 307 928 t of ammonium sulphate per year - a considerable economic saving.

ACKNOWLEDGEMENTS

I would like to thank the Swedish Academy of Sciences for sponsoring my participation, and the Director and Board of the Rubber Research Institute of Malaysia for permission to attend the Workshop and present this paper.

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THE ROLE OF LEGUMES AND ANIMALS IN THE NITROGEN CYCLING IN RUBBER CULTIVATION

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ABSTRACT

The common legume covers in rubber cultivation are Calopogonium caeruleum, Calopogonium mucunoides, Centrosema pubescens *and* Pueraria phaseoloides. *Recently* Mucuna cochinchinensis *has also been introduced as a vigorous legume cover. The advantages and deleterious effects of cover crops are discussed. The plant characteristics of each pasture legume are described and data are presented on the nutritive value in terms of dry matter, crude protein, crude fibre, ether extract, ash, nitrogen-free extract and gross energy.*

The herbage under rubber can be (i) a natural cover (weeds and ferns), (ii) a natural cover and legumes or (iii) pure legumes with dry matter yields of 500, 1400 and 2600 kg ha^{-1} *respectively. These yields of dry matter can support 2, 6 and 12 sheep* ha^{-1} *respectively. Calculations are presented on the amounts of unutilized nitrogen that are returned as dung and urine by sheep.*

INTRODUCTION

The establishment of a legume cover crop is a standard practice in the replanting and newplanting of rubber trees. Mainstone (1961, 1963) established that rubber trees grown in association with leguminous cover became tappable twelve months earlier and yielded 20% more than those grown with natural cover over the 10 years of tapping experiments. This was confirmed by Pushparajah & Chellapah (1969). Ti *et al.* (1971) and Lim & Chai (1979) have shown that it is economic to establish legume covers in rubber cultivation. According to the Rubber Research Institute (RRIM, 1972b) the agronomic and beneficial effects of legume cover are the following:

(a) They protect the soil surface and reduce erosion,
(b) add organic matter to the soil and thus improve soil structure, water infiltration and retention, reduce soil temperature and decomposition rate of organic matter, and minimize leaching losses of nutrients,
(c) fix atmospheric nitrogen and return it to the soil,
(d) are a source of animal feed and legume seeds, and,
(e) recycle nutrients.

The deleterious effects are as follows:

(a) They compete with rubber for moisture and nutrients,
(b) reduce the availability of nutrients when the plant litter has a high carbon/nitrogen ratio,
(c) act as intermediate or subsidiary host for pests and diseases, and,
(d) alter the micro-climate and make it more conducive for the activity of pests and diseases.

Considering the advantages and disadvantages, the positive effects of a legume cover policy outweigh the negative ones. This paper discusses the legume species, their nutritive value and the nitrogen cycle involving animals in the rubber and cover crops ecosystem.

THE LEGUMES AND THEIR NUTRITIVE VALUE

In rubber cultivation, a mixture of creeping legumes is usually planted, and the most common species are *Calopogonium caeruleum, Calopogonium mucunoides, Centrosema pubescens, Mucuna cochinchinensis* and *Pueraria phaseoloides*. A mixture of the five leguminous creepers are recommended to counter the pest and disease problems, which might endanger a pure stand of one species, and also for ecological succession of the legume covers. *Calopogonium mucunoides* develops rapidly during the first few months after sowing, but it does not persist as a strong component for more than twenty months. *Pueraria phaseoloides* provides the bulk of the cover during the second and third year after planting, whilst *Calopogonium caeruleum, Centrosema pubescens* and *Desmodium ovalifolium* persist longer than the other two covers under

the developing rubber trees.

The nutritive values of these pasture legumes (Table 1) are much higher than those of cultivated grasses such as *Panicum maximum*, *Pennisetum purpureum* and *Brachiaria mutica*. These data are typical for similar legumes grown elsewhere in the tropics.

Table 1. Nutritive value of leaves plus stems of legumes used as cover crops under rubber in Malaysia (Devendra, 1979)

Legume species	Dry matter (% of fresh plant)	Crude protein	Crude fibre	Ether extract	Ash	Nitrogen-free extract	Gross energy (MJ kg^{-1})
		(% of dry matter)					
Calopogonium mucunoides	25.6	15.6	31.5	2.3	6.2	44.4	10.9
Centrosema pubescens	24.3	22.2	30.9	2.5	9.5	34.9	15.2
Desmodium ovalifolium	24.0	9.2	40.0	2.1	9.2	39.5	13.0
Mucuna utilis	16.6	35.0	14.5	3.0	9.0	38.6	-
Pueraria phaseoloides	19.1	19.9	28.8	2.1	7.9	48.4	15.5
Mean	21.9	20.4	29.1	2.4	8.4	41.2	13.7

UTILIZATION OF HERBAGE UNDER RUBBER

In rubber plantations three systems of herbage cover are feasible under rubber trees less than three years old (Table 2). System 1 (natural cover) is found mostly in small holdings (about 2 ha), while Systems 2 and 3 are found in estates (more than 45 ha of rubber trees).

The legume cover derives a substantial part of its nitrogen from the atmosphere; estimates over a five year period vary from 50-80 (Watson *et al.*, 1964) to 150 kg ha^{-1} yr^{-1} (Broughton, 1977) of nitrogen being fixed biologically. In addition, rainfall supplies another 20 kg N ha^{-1} yr^{-1} (RRIM, 1972a).

Table 2. The plant species, dry matter production and stocking capacities of three ground cover systems under rubber in Malaysia

System	Vegetation	Plant species	Dry matter (kg ha^{-1})	Number of goats or sheep (ha^{-1})	Reference
1	Natural cover	*Axonopus compressus*, *Paspalum conjugatum*, *Ottochloa nodosa*, *Mikania cordata*, *Nephrolepis biserrata*, *Gleichaenia linearis*	500	2	Devendra (1976)
2	Natural cover and legumes	- as above - and *C. pubescens*, *C. mucunoides*, *P. phaseoloides*	1400	6	Tan Keh Huat (pers. comm.)
3	Pure legumes	*Cajanus cajan* and *C. pubescens*	2600	12	Devendra & Chee (1979)

The vegetation under the rubber tree can support goats or sheep (Devendra, 1978; Wan Mohamad, 1977). On the basis of a 3% intake of dry matter on a body weight of 20 kg for indigenous sheep, the daily intake is 0.6 kg (Devendra, 1976). Thus, from Table 2, equivalent carrying capacity is 2, 6 and 12 animals for Systems 1, 2 and 3 respectively, i.e. three and six times more animals can be carried due to the increased availability of feed under natural cover plus legumes, and pure legumes respectively.

Table 3 shows that the amount of nitrogen that is unutilized by the sheep increases from 8.6 kg ha^{-1} in System 1 (natural cover) to 64.4 kg ha^{-1} in System 3 (pure legumes). This nitrogen, in the dung and urine, will eventually be mineralized and partly taken up by the rubber trees and legumes, while another part is probably volatilized or denitrified. Thus, in a pure legume system, the herbage helps in supporting more animals per unit area of land and also returns more nitrogen to the soil.

Table 3. Utilization of three systems of ground vegetation by indigenous sheep under rubber in Malaysia

System	Vegetation	Dry matter (kg ha^{-1})	Crude protein (%)	Crude protein	N Content	N[1] retained	N unutilised
					(kg ha^{-1})		
1	Natural cover	500	11.4	57	9.1	0.5	8.6
2	Natural cover and legumes	1400	15.0	210	33.6	1.6	32.0
3	Pure legumes	2600	24.4	619	99.0	4.9	64.4

[1] A 70% digestibility of crude protein at a 7% retention of nitrogen by the grazing animal is assumed.

Because of the varied nature of the mixed herbage, especially in Systems 1 and 2, it is possible that goats are better suited than sheep, since the former are known to make fuller use of mixed herbage than the latter.

CONCLUSIONS

The integration of animals, especially ruminants, with rubber is a relatively new venture in Malaysia. The objective of recent research by the Rubber Research Institute of Malaysia is to utilize the legumes in the interrow of rubber. Studies on these aspects suggest the following:

(1) Pure covers of legumes are very important in the nitrogen cycle in rubber cultivation. They benefit the growth and yield of rubber in terms of nutrient return and also reduce the cost of nitrogen fertilization of the rubber.

(2) Further investigations are necessary on shade tolerant legumes so that when *C. pubescens* and *P. phaseoloides* die off due to shading of rubber after 5 to 6 years of age, other species can continue to provide a source of feed for the animals.

(3) The high nutritive values of legumes grown in rubber are comparable to or greater than some of the cultivated grasses (*P. maximum*, *B. mutica* and *P. purpureum*).

(4) The integration of animals, especially ruminants, with rubber provides a means for more complete utilization of the total herbage.

(5) A pure stand of legumes in rubber can support the rearing of goats and sheep, and thus provide a source of extra income to the farmers.

(6) The integration of various classes of animals with rubber cultivation and the nutrient cycle involved need further investigation.

ACKNOWLEDGEMENTS

The authors wish to thank Dr Ani bin Arope, Director, Rubber Research Institute of Malaysia, for permission to present this paper and Dr P.D. Abraham, Head, Smallholders Project Research Division, for his valuable comments.

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NITROGEN CYCLING IN A LEGUME-OIL PALM ECOSYSTEM IN MALAYSIA

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ABSTRACT

Nitrogen cycling was studied within a developing legume-oil palm system in Malaysia. Legume cover crops (a mixture of Centrosema pubescens *and* Pueraria phaseoloides*) were sown between young palms. Over a period of two and a half years, development of both legumes and palms was studied, especially in relation to nitrogen cycling. At the end of the experiment, soils under legumes contained 460 to 920 kg ha^{-1} more N than soils under "natural" covers or under "bare" soil. The bulk of this excess stemmed from effective exploitation of the soil by legumes, from nitrogen fixation, and from a reduction in leaching losses. Nitrogen fixation averaged about 150 kg ha^{-1} yr^{-1} under the palms. Two and a half years after legume establishment the covers returned 250 to 300 kg N ha^{-1} yr^{-1} through litter decomposition. Lysimeter studies showed that the rate of leaching of nitrogen from "bare" soil was about 100 kg ha^{-1} yr^{-1}, and that legumes reduced this loss by about 60%. As soils in the experimental area were extremely acidic (pH 4.3), loss of nitrogen through denitrification was regarded as negligible. Leaching of nitrate was greater than of ammonium. The amounts of nitrate and ammonium leached from the soil were related to rainfall. We conclude that the significant increase in soil nitrogen due to the presence of legumes accounts for the better growth of associated palms.*

INTRODUCTION

Tropical soils are generally deficient in nitrogen (Date, 1973). In young oil palms (*Elaeis guineensis* Jacq.) nitrogen deficiency results in paling of the seedlings, yellowing, and finally necrosis (Bull, 1957). In the mature palm, nitrogen deficiency causes significant increases in floral abortion and hence a reduction in yield (Broeshart *et al.*, 1957). This may be remedied by applying fertilizers, of which the most commonly used is ammonium sulphate (Hartley, 1969). Other sources of nitrogen available to the palm include soil nitrogen and nitrogen from decaying legume litter. Sowing leguminous covers concomittantly with the palms seems to be the most effective way of boosting soil nitrogen (Broughton, 1976; 1977). Given proper husbandry, fertilizer application does not increase fruit yields above those obtainable from legume plots (Broughton, 1976). Simple economic arguments suggest that legume benefits do not stem directly from nitrogen fixation by the legumes, but rather from a long lasting effect that pre-conditions the plants to higher yields (Broughton, 1977). As one possible explanation of this phenomenon concerns nutrient cycling within the legume-oil palm ecosystem *in toto*, we decided to measure various agronomic and physiological parameters of the system, especially with regard to nitrogen. This communication summarizes our findings.

MATERIALS AND METHODS

Experimental site

Clearing of 92 ha of old rubber land at the Damansara Estate, Batu Tiga, Selangor began in September, 1975. Of this, 6.5 ha was set aside for special attention, while the rest of the field was planted with legumes and oil palms in the normal estate manner. One-year old oil palm seedlings, raised in large polyethylene bags in the nursery, were planted in the experimental block in October, 1975. Each was set 8.9 m apart on an equilateral triangle pattern. Then the entire experimental area was divided into 32 plots, the boundaries marked, and treatments randomly allocated. Details of the soil type and climate experienced in the experimental area are given in Agamuthu (1979).

Experimental design

Randomized blocks of eight treatments replicated four times and with fully guarded plots were used throughout. Each plot covered an area of 0.17 ha, and contained 25 palms.

Only the central nine palms of each plot, or the area they comprised, were used for measurements. Five of the eight treatments relevant to this communication were:

(a) naturally regenerating cover - "Natural";
(b) bare ground - "Bare";
(c) planted legume cover, hand-weeded - "Control";
(d) planted legume cover, hand-weeded with extra fertilizer - "Fertilizer";
(e) planted legume cover, sprayed with the pre-emergence herbicide "Oxyfluorfen" at 0.25 kg a.i. ha^{-1} - "Oxyfluorfen 0.25".

Clean-weeded circles of 2 m diameter were established around each palm, and maintained in this condition throughout the experiment by a combination of hand-weeding and prophylactic herbicide application (Paraquat at 90 ml (15 l water)$^{-1}$). Fertilizers were applied to palms in all the plots according to Table 1.

Table 1. Fertilizer types, rates and timing for all treatments

Date	Fertilizer	Rate (kg ha^{-1})
March, 1976	CIRP[a]	100
June, 1976	CIRP[a]	100
August, 1976	CCM-11[b]	125
September, 1976	CIRP[a]	100

[a] Christmas Island rock phosphate (15% P)

[b] A commercial fertilizer containing 11% nitrogen and 8% P. Palms in "Fertilizer" plots received twice the fertilizer dosages listed here.

Treatments

(a) "Natural" regeneration consisted mostly of the grass *Paspalum conjugatum*, but later the creeper *Mikania cordata* and the fern *Nephrolepis biserrata* invaded the plots. *Pueraria phaseoloides* also occurred sporadically. Woody shrubs and noxious weeds (e.g. *M. cordata*) were regularly removed by hand.

(b) "Bare". This treatment was used mainly to quantify nutrient cycling in the system. Except for the oil palms, the land was maintained free of vegetation by a combination of hand-weeding and Paraquat application (see above).

(c) "Control". After clean-weeding each legume plot, three parallel drills, 2.1 m apart, were dug between the rows of palms with a hoe. Scarified *Centrosema pubescens* and *P. phaseoloides* seeds (2:3 by weight) were mixed with the appropriate amount of *Rhizobium* (UMKL44 - see Broughton & John, 1979) and sown into the drills (at a seed rate of 8.0 kg ha^{-1}). Loose soil was then pressed back over the seeds. Plots were maintained free of weeds by repeated hand-weeding (at roughly monthly intervals).

(d) "Fertilizer". Plots were prepared and maintained exactly as described for "Control", except that palms received twice the normal fertilizer dosage.

(e) "Oxyfluorfen 0.25". Oxyfluorfen or "Goal" or RH-2915 - 2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene (Rohm and Haas Company), was applied at the rate of 0.25 kg a.i. ha^{-1} to legumes, sown as described in (c) above, the day after planting. The herbicide was applied in water (560 l ha^{-1}) with a 9-l pump operating at about 10^5 Pa. A series of strips 2 m wide were sprayed over the entire area using a 1.6 mm internal diameter nozzle.

Analytical

Assessment of herbicide effects, rate of legume cover growth and elemental analysis were made by periodically sampling the vegetation from ten randomly selected quadrats (each 0.25 m^2) per plot. Leaves, litter and roots were cut into small pieces (1 to 2 cm) and mixed thoroughly at the site. A portion was oven dried at 70 C for four days, ground into a powder and used for the analysis of N, P, K, Mg, Mn and Ca (RRIM, 1970).

Palm leaflets collected from the nine central palms of each plot were cleaned, cut into 1 to 2 cm pieces and oven dried at 70 C for 48 hours before grinding through a 1 mm sieve. The ground material was used for mineral analysis (RRIM, 1970).

Lysimeter Studies

The lysimeters employed were cylindrical containers of 1 m^2 surface area and 1 m deep with one outlet at the bottom, connected to a plastic jerrycan. After measuring the volume of the leachate, bulked samples of 120 ml were collected monthly for the determination of ammonium-N and nitrate-N (Department of Agriculture, 1975). One and a half years later, the soil and the vegetation in the lysimeters were analysed for various forms of nitrogen.

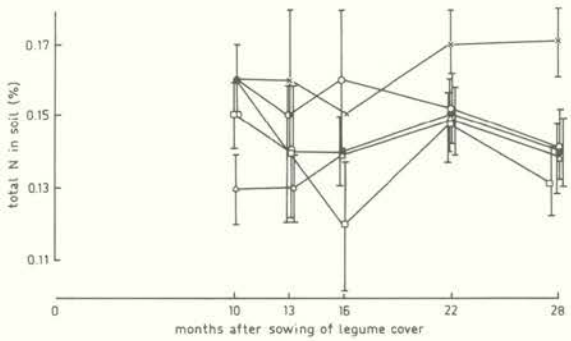

Fig. 1. Percent nitrogen in the top 15 cm of soil under the various soil covers at different times after legume cover crop planting. x *Oxyfluorfen 0.25,* o *fertilizer,* ● *control,* △ *natural,* □ *bare soil (vertical bars are L.S.D. values at P = 0.05).*

Soil Analysis

Ten soil samples were collected from each plot (top 15 cm) using a hollow boring device of 1.25 cm internal diameter and about 30 cm long. The soil was air dried at 70 C for 6 days, powdered, sieved through a 250 µ sieve and analysed for N (Bremner, 1965).

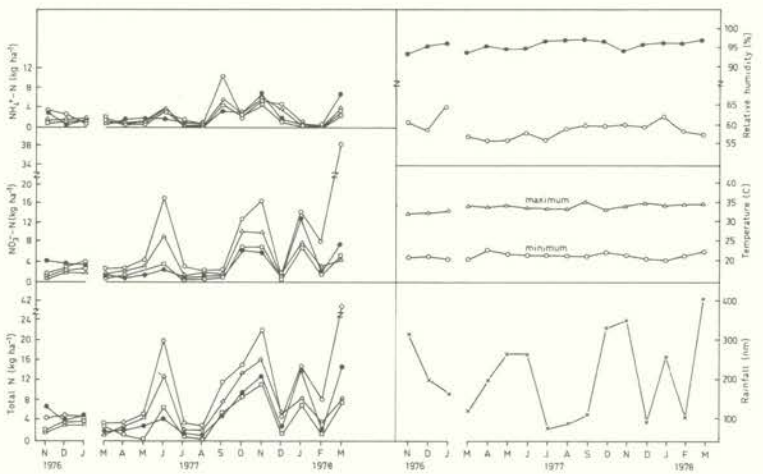

Fig. 2. Climatic parameters and leaching of ammonium and nitrate nitrogen from the lysimeters with different soil cover types. ● *Control,* △ *natural,* o *bare,* □ *oxyfluorfen 0.25.*

RESULTS

Generally, soils under legume covers had more nitrogen compared to soils under "natural" covers or under the "bare" treatment (Fig. 1). Ten months after legume establishment, soils from the legume cover treatment averaged 0.16% nitrogen. The corresponding figures for "natural" soil were 0.13% and for "bare" soil 0.15%. Soils under legumes established with the aid of "Oxyfluorfen 0.25" contained more nitrogen than soils from any other treatment.

Leaching of nitrogen was evident from the "bare" soil, which, 16 months after initiation of the experiment, contained only 0.12% nitrogen. The difference between nitrogen fixation and litter decomposition in legumes on the one hand, and increased leaching from "bare" soils on the other is about 300 kg N ha^{-1} yr^{-1} (Agamuthu *et al.*, 1980). Unlike legumes, "natural" covers accumulated little litter, and what was accumulated turned over slowly.

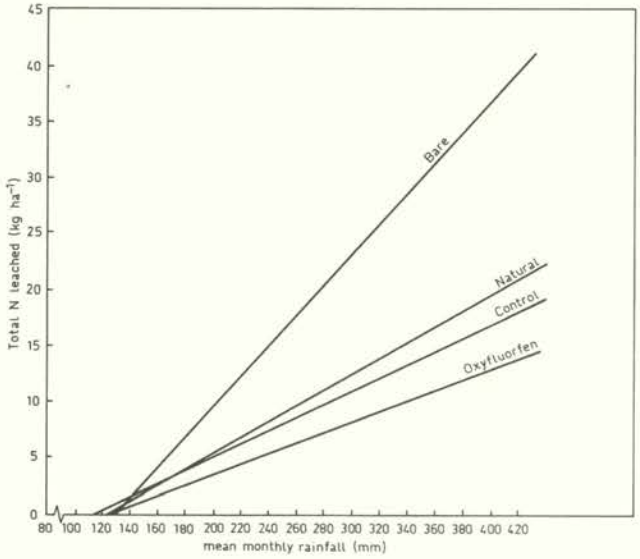

Fig. 3. Relation between mean monthly rainfall and total nitrogen leached for different soil covers. Equations of the regressions are:
"Bare": $y = 0.12\ x - 12.24$ ($r = 0.76^a$).
"Natural": $y = 0.07\ x - 8.52$ ($r = 0.69^a$).
"Control": $y = 0.6\ x - 7.00$ ($r = 0.81^a$).
"Oxyfluorfen 0.25": $y = 0.45\ x - 5.44$ ($r = 0.75^a$).
where y = total amount of nitrogen leached (kg ha^{-1} $month^{-1}$) x = monthly rainfall (mm), and a indicates significant at P = 0.001.

Legumes contribute nitrogen to the soil in two ways, directly through litter decomposition, and indirectly through reducing leaching losses. The extent of soil nitrogen preservation is shown in Fig. 2. Generally, more nitrate than ammonium was leached from the soil (Fig. 2), and the pattern of leaching followed the rainfall (Fig. 3). Heaviest losses of nitrogen (at the rate of 41 kg ha^{-1} $month^{-1}$) were recorded in March, 1978, when rainfall was highest (404 mm) (Fig. 3). Nitrogen lost from the "bare" soil was about 100 kg ha^{-1} yr^{-1} and legumes prevented the loss of about 60 kg ha^{-1} yr^{-1} of this. Legumes established using the herbicide "Oxyfluorfen 0.25" were superior in retaining both ammonium and nitrate (Table 2). One and a half years after the experiment was initiated, soils under "Oxyfluorfen 0.25"-

Table 2. Amount of nitrogen in soils and vegetation in the lysimeters nineteen months after planting

	Exchangeable NH_4^+-N and NO_3^--N remaining in the soils		Total N in vegetation	Total
	(a)	(b)	(c)	(a + b + c)
Treatments	NH_4^+-N (kg ha^{-1})	NO_3^--N (kg ha^{-1})	(kg ha^{-1})	(kg ha^{-1})
Control (hand-weeded)	193 ± 60	43 ± 4	217 ± 26	453
Natural	171 ± 10	30 ± 2	92 ± 8	293
Bare	77 ± 8	25 ± 0	-	102
Oxyfluorfen 0.25	205 ± 21	90 ± 17	368 ± 11	663

established legume covers contained 205 kg ammonium nitrogen ha^{-1} and 90 kg nitrate nitrogen ha^{-1}. Corresponding figures for soils under "natural" covers and "bare soil were substantially less.

DISCUSSION

We have attempted to provide a preliminary balance sheet of nitrogen inputs and outflows in the legume cover crop-oil palm ecosystem (Fig. 4). Major omissions include that consumed and returned to the soil by animals, as well as losses due to denitrification and NH_3 volatilization. Denitrification is unlikely to be large, however, as the soils at the experimental site were acidic (pH σ 4.3) (Agamuthu, 1979). Despite these limitations, a picture of nitrogen cycling between both the legumes and oil palms is emerging (Fig. 4). Legumes contribute about 150 kg nitrogen ha^{-1} yr^{-1} to the system through nitrogen fixation (Agamuthu *et al.*, 1980). In the early stages of oil palm growth, they also absorb large quantities of nitrogen from the soil (149 kg ha^{-1} yr^{-1} in our experiments), and reduce leaching to 41 kg N ha^{-1} yr^{-1}. Therefore the legumes store about 300 kg N ha^{-1} yr^{-1} more than do "natural" covers. As competition for light and nutrients reduce the vigour of the legume, this stored nitrogen is gradually made available to the oil palm (Broughton, 1977). Consequently, during the establishment of the plantation, enough nitrogen is available from this source to account for better oil palm growth. Later, fertilizer nitrogen and the contribution from rain (high in this case as the experimental site is in the approaches to a major airport and is surrounded by industry), provide the total nitrogen requirements of the crop. By this time, the benefits of having had a legume cover have set the palms along the road to greater vigour which lasts for most of their reproductive life (Broughton, 1977).

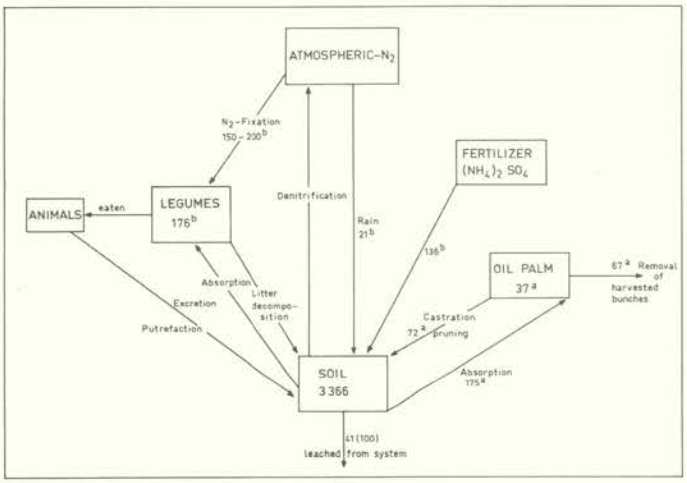

Fig. 4. Nitrogen cycling within the developing legume-oil palm ecosystem. Figures within the rectangles represent the amount of nitrogen (kg ha^{-1}) within the system at maximum growth. Data when accompanied by the superscript a, as reported in Corley et al. *(1976), or b, as in Agamuthu & Broughton (1981).*

ACKNOWLEDGEMENTS

We wish to thank Khoo Khee Ming, Han Siew King, Chan Yik Kuan, Rolf Jesinger, Paravathy, Jodhy, Kokilam and Rama Devi for their help with many aspects of this work, and the laboratory staff of the Soil & Analytical Service Division of the Department of Agriculture for their help in some of the analyses. Financial assistance was provided by Sime Darby Plantations Ltd., Rohm and Haas Asia Inc. and the University of Malaya.

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NITROGEN CYCLING IN TROPICAL FORESTS

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ABSTRACT

Litter fall and litter decomposition in forests vary from 1.0 to 10.9 t ha^{-1} yr^{-1} and from 1 to 50% yr^{-1} respectively, according to climatic zones, the rates being higher in warmer climates. The accumulation of N in the topsoil is similarly affected and ranges from 4 to 11.5 t ha^{-1}, being lowest in the humid tropics. The amount of soil N has been found to be dependent on topography in some areas.

The inputs and outputs and internal cycles of N are given for a sugi (Cryptomeria japonica) *forest in Japan, together with results of some plant nutrition studies.*

It is concluded that an interruption of the N cycle in a tropical forest by clear cutting is likely to result in a rapid soil degradation except in dry or water-logged areas, and research is needed on management systems which will maintain an adequate rate of nutrient cycling.

INTRODUCTION

In the tropical forest zone, especially in the wetter regions, the decomposition of organic matter in soil is very rapid. This has an important bearing on tropical forest management.

Forests can still exist in some parts of the world, because the land in these regions could not be developed further for agricultural crops. For instance, in the boreal zone it is too cold to grow crops, and alpine regions like those in Japan are too steep for cultivation. Recently, we have noticed that in large areas of forest land in the tropical zone an infertile soil may result after cultivation. This is not only due to structural degradation but also to poor nutrient status resulting from disturbance.

The future development of forest land will be very important both for timber resources and for food production after cultivation. Nitrogen and phosphorus play a dominant role in the problems associated with the development of these forests. Some aspects of the cycling of nitrogen in tropical forest will be discussed.

Table 1. Decomposition rate of litter in different climatic zones (Yoda & Kira, 1969)

Type of forest	Decomposition rate (% yr^{-1})
Sub-arctic coniferous forest	1 - 3
Cool temperate, deciduous hardwood forest	2 - 6
Warm temperate, laurel-leaved forest	4 - 9
Sub-tropical, laurel-leaved forest	7 - 15
Tropical rain forest	10 - 50

INTERNAL CYCLES AND INPUTS AND OUTPUTS OF NITROGEN

In general, the annual amounts of litter fall vary according to the climatic zone. These are, for the arctic-alpine, 1.0; for the cool temperate, 3.5; for the warm temperate, 5.5; and for the equatorial zone, 10.9 t ha^{-1} yr^{-1} (Bray & Gorham, 1964). The decomposition rate is similarly affected (Table 1), reflecting its dependence on temperature. The higher the temperature the higher the decomposition rate and therefore the lower the accumulation of nitrogen (Tsutsumi, 1973) and carbon (Yoda & Kira, 1969).

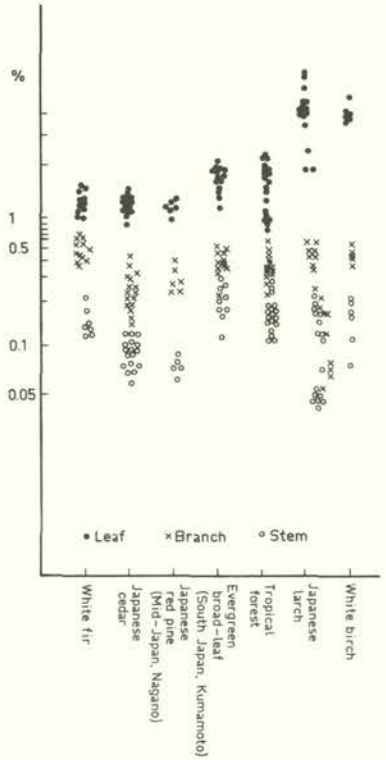

Fig. 1. Nitrogen concentration in different plant parts for different species or forests (Tsutsumi et al., *1968).*

Table 2. Amount of nitrogen accumulated in the top 7 cm of the soil for different forests (Tsutsumi, 1973)

Region or climatic zone	Type of forest	Nitrogen (t ha^{-1})	Reference
South Thailand	Rain	5.4	Tsutsumi *et al.* (1966)
Sub-tropical	Rain	4 - 5	Nomura & Sato (1963)
Warm temperate	Laurel-leaved	8.5	Ogawa (1974)
Cool temperate	Beech	11.5	Kawahara (1971)
Sub-arctic	Coniferous	6 - 8	Shidei & Tsutsumi (1962)

Nitrogen concentrations in different plant parts, especially in leaves, differ according to tree species (Fig. 1) (Tsutsumi, 1973).

The accumulation of nitrogen in forest soils is affected by differences in forests types and regions (Table 2), while this accumulation for both nitrogen and carbon has been shown to be dependent on topography in Japan and N.E. Thailand (Table 3). This topographic effect has yet to be confirmed for tropical forests in other areas.

According to Kawada (1978), the sugi *(Cryptomeria japonica)* forests in Japan produce 7 t ha^{-1} yr^{-1} of timber dry matter, containing 50 kg ha^{-1} yr^{-1} of nitrogen. The needles contain about 20 t ha^{-1} of dry matter and the litter fall is 4-6 t ha^{-1} yr^{-1}. The amount of N held in the Ao horizon of the soil is 55-280 kg ha^{-1} and the total nitrogen content in the soil (to 60 or 70 cm depth) is 4800-14 700 kg ha^{-1}.

In sugi forests in Japan bole weight increases proportionally with age, but other parts such as needles reach their maximum at crown closure, and thereafter a slight decrease occurs, Haibara (1980). The nitrogen content in the total above ground part of the tree follows a similar pattern, with a maximum at nine years of tree age (Fig. 2).

Table 3. Accumulation of carbon and nitrogen as affected by topography in forest soils (Tsutsumi, 1973)

	South Japan (Kyushu)[a]						NE Thailand		
	Slope lower	Slope middle	Slope upper	Gentle ridge	Concave slope	Main ridge	Slope lower D.E.F.[b]	Slope middle D.D.F.[c]	Slope upper D.D.F.[c]
Ao-layer (t ha^{-1})	16.5	15.7	14.9				3.85	0.78	1.04
Soil organic matter (t ha^{-1})	193	156	151	125	149	82	155	79.8	47.8
Total	210	172	166				159	80.5	48.8
Soil nitrogen (t ha^{-1})	8.7	7.5	5.9	5.4	7.2	3.4	9.04	3.64	2.61
C/N	13.0	12.1	15.0	13.4	12.0	14.0	10.0	12.7	10.6
Surface soil C/N ratio	14.7	14.3	20.9	16.1	15.6	21.4	13.2	16.5	17.1
Litterfall (t ha^{-1})		5.82		5.60		5.00			

[a] Laurel-leaved forest. [b] Dry evergreen forest. [c] Deciduous dipterocarp forest.

In stands younger than four years old the Ao horizon contains 3-4 t ha^{-1} of dry weight, which is equal to the dry matter weight of the undergrowth. This suggests that a major portion of the decomposing undergrowth must reach soil layers well below the Ao horizon. The nitrogen content of the Ao horizon increases due to the supply of organic matter by pruning (Fig. 3). According to Salas (1979) the dry matter and nitrogen of the biomass increase by 5.8-15.3 t ha^{-1} yr^{-1} and 57-225 kg ha^{-1} yr^{-1}, respectively, in tropical forests. In commercial plantations these rates are about 25% lower.

The input of nitrogen by precipitation is 3.0-8.2 kg ha^{-1} yr^{-1} in Japan and other countries in temperate zones (Kawada, 1978).

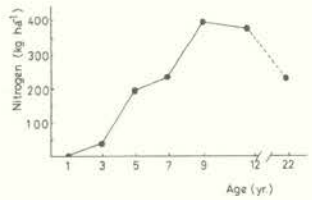

Fig. 2. Relation between stand age and total amount of N above ground of a sugi forest (Haibara, 1980).

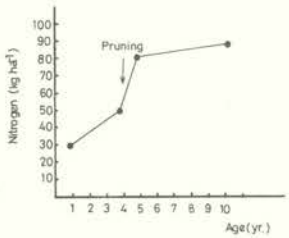

Fig. 3. Relation between stand age and nitrogen content of Ao horizon in a Sugi forest (Haibara, 1980).

Due to interception by the canopy, about 60-80% of the precipitation reaches the forest floor in the form of throughfall and stemflow. In general, the total amount of nitrogen and its concentration are increased in these two forms due to canopy leaching and washing of aerosols adhering to the surface of plant bodies. According to Kawada (1978) throughfall and stemflow contribute 4.4-9.0 and 0.3-1.7 kg N ha^{-1} yr^{-1} respectively of inorganic N in Japan, and the output from forests to streams is in the order of 2-3 kg N ha^{-1} yr^{-1}.

Arimitsu (1974), using tension-free lysimeters, found that 50-70% of the throughfall is retained in subsurface flow, while 10-30% is found in the subsoil.

CONCLUSIONS

The biomass and the rate of litter fall are highest in tropical forests except for dry and very wet areas, but due to the high rate of decomposition of litter in the tropics the accumulation of N and C on the forest floor is relatively low. This results in a high rate of recycling of nutrients. As long as this recycling can be sustained, a high production rate of the forest can be maintained.

When this recycling is interrupted, e.g. by clear cutting, soil degradation is likely to occur rapidly and problems will be encountered in trying to restore soil productivity.

Research is needed to develop rational management systems of tropical forests in which the high rate of nutrient cycling, especially of nitrogen, can be maintained.

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CYCLING OF NITROGEN IN A TROPICAL DECIDUOUS FOREST

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ABSTRACT

A detailed analysis was made of the stocks and cycling of nitrogen in producer compartments and soil of an Anogeissus-Diospyros *dominated tropical deciduous forest. The nitrogen stocks have been estimated in both trees and shrubs (bole, branch, major root, small root, leaf compartments), herbs (above- and belowground), litter and soil. The trees and shrubs represent summations of separate determinations for 12 species. The total stock of nitrogen in the ecosystem was 3680 kg* ha^{-1}*; 81% was contained in the soil, 17% in trees, 0.6% in shrubs, 0.3% in herbs and 1% in litter.*

Twentyone flows, representing net annual transfers between compartments, have been quantified. Total uptake of nitrogen by the vegetation was 173 kg ha^{-1} yr^{-1}*; 82% accounted for by trees, 13% by herbs and 5% by shrubs. The deciduous leaf compartment exhibited the greatest turnover of nitrogen; trees and shrubs returned 65% and 47% of the annual uptake of this compartment via leaf litter, and the rest was translocated to non-photosynthetic compartments. Total transfer through leaf and non-leaf litterfall in the ecosystem was 82 kg* ha^{-1} yr^{-1}*. The immobilization of nitrogen in small roots was 29 kg* ha^{-1} *during the rainy season, the whole being released into the soil during the following dry period. Total nitrogen release into the soil was 104 kg* ha^{-1} yr^{-1} *(72% by litter decomposition and 28% by root disappearance), resulting in net retention of 62 kg* ha^{-1} yr^{-1} *in vegetation and 6 kg* ha^{-1} yr^{-1} *in litter. Rain input to the system was 23 kg* ha^{-1} yr^{-1}*, 22.7% of which was absorbed by the vegetation, the balance moving with throughfall and stemflow to the ground. The loss of nitrogen in run-off from the ecosystem was 5.2 kg* ha^{-1} yr^{-1}*. Thus, the forest stand exhibited a net positive accretion of nitrogen each year.*

INTRODUCTION

Approximately 50% of the total forest land area of the world is tropical forest, which provides 69% of the world forest net primary production (Bolin, 1970; Whittaker, 1970; Brunig, 1971). Deciduous forests, ranging from moist to dry types, constitute 66% of the tropical forest area of the world (Brazier *et al.*, 1976) and 70% of the total forest area in India. An understanding of nutrient cycling in this ecosystem is critical for successful long term forest management. Nitrogen is of particular nutritional significance, and its availability depends on complex interactions within and between the compartments in the air, soil and biota.

A number of recent studies (Kawahara & Tsutsumi, 1972; Rochow, 1973; Johnson & Risser, 1974; Hirose, 1975; Henderson & Harris, 1975; Rosswall, 1976; Foster & Morrison, 1976; Turner *et al.*, 1976; Tsutsumi, 1971, 1977; Westman, 1978a; Schlesinger, 1978; Rolfe *et al.*, 1978; Katagiri *et al.*, 1978) are concerned with nitrogen cycling in temperate forests. Such studies in tropical forests mostly pertain to evergreen forests (Ovington & Olson, 1970; Jordan, 1978, 1979), except a few on sub-tropical eucalypt forest (Westman & Rogers, 1977; Westman, 1978b) and deciduous forest (Greenland & Kowal, 1960; Singh, 1974). The present study attempts to quantify the stocks and cycling of nitrogen in a tropical dry deciduous forest. This study is a part of the Indian Man and the Biosphere research project entitled 'Structure and functioning of natural, modified and silvicultural ecosystems of Eastern Uttar Pradesh'.

DESCRIPTION OF STUDY AREA

The study area is within the Chandraprabha sanctuary (24°52' to 24°58' N lat. and 83°3' to 83°12' E long.), spread over the Vindhyan plateau, about 85 km southeast of Varanasi. The red, sandy loam soil, developed from sandstone, is shallow with frequent rock outcrops.

The climate is monsoonal, with three distinct seasons: a warm, wet rainy season (July-October); a cool, dry winter (November-February); and a hot, dry summer (March-June). The long-term mean annual rainfall is 1057 mm, about 93% being received during the rainy season. The ranges of mean monthly temperature during different seasons are (min.-max.): rainy, 23.9-26.2 C; winter, 13.0-22.9 C, and summer, 26.3-37.5 C.

The vegetation is a deciduous forest of mixed species (5B/C2 of Champion & Seth, 1968). The total species number of trees, shrubs and herbs were 21, 4 and 33, respectively. The dominant tree species, each contributing more than 10% of the standing biomass were: *Anogeissus latifolia* Wall., *Diospyros melanoxylon* Roxb., *Buchanania lanzan* Spreng., and *Pterocarpus marsupium* Roxb., while the dominant shrub was *Holarrhena antidysenterica* Wall. The total densities of trees and shrubs (> 10 cm girth at breast height) were 1174 and 132 ha^{-1} respectively, and basal areas 18 and 0.5 m^2 ha^{-1} respectively. The height of canopy trees varied from 9-15 m and the most frequent girth range was 20-50 cm. Tree and shrub saplings of almost all the species could be found, but those of shrubs predominated. The herbaceous vegetation, mostly developed during the rainy season, was dominated by *Oplismenus burmannii* P. Beauv. and *Cyperus aristatus* Rottb. In most of the tree and shrub species, rapid leaf expansion takes place after the onset of rains, coinciding with the growth of the herbaceous layer. While this layer starts drying up by the end of the rainy season, the trees and shrubs remain green till December-January after which major leaf fall occurs.

METHODS

Field sampling

Within a sample area of 100 m x 150 m, located in a typical forest stand, six permanent plots (each 20 x 25 m) were established in two rows about 20 m apart. During June 1976 all the > 10 cm girth individuals (291, distributed amongst 16 tree and two shrub species), rooted within permanent plots, were identified, numbered and tallied by girth at 1.3 m height. Girth measurements were repeated during June 1977 and 1978. Measurable girth increments occurred in 97% individuals. Prior to leaf fall during the 1976-77 growing season, 11-24 individuals, representing the entire girth range in each of 11 tree and one shrub species, were harvested outside the sample area to develop girth and component biomass regressions (Singh & Misra, 1978). Small roots (< 25 mm diameter) of trees and shrubs were recovered from six soil monoliths (each 25 x 25 cm and 50 cm deep) during August and December 1976 and April 1977. Tree and shrub saplings (< 10 cm girth) were sampled separately during November 1977 from six plots (each 4 x 5 m) located at 20 m intervals along a transect parallel to the sample area length. For herbaceous vegetation, four soil monoliths (25 x 25 cm and 30 cm deep) were excavated every month from June to October 1976 and later bimonthly till June 1977 to determine above- and below-ground biomass. Small plant samples were dried and saved for analysis.

Litter and soil sample collections were made at monthly intervals through July 1976 to June 1977. Litterfall from trees and shrubs was collected from 18 randomly distributed 1-m^2 permanent litter plots. The litter was separated into species (leaf portion) and components (leaves, twigs, barks, flowers and fruits) and weighed. Variations in litter layer were evaluated from six plots (1 m^2 each) laid randomly each month, by weighing total accumulation on the forest floor. Soil samples were collected every month from three depths (0-10, 10-20 and 20-30 cm) at three random locations. Small sub-samples of litter and soil were dried for analysis.

Chemical analysis

All samples were ground; plant samples passed through a 44-mesh and soil samples through a 5-mesh sieve. Total nitrogen in the plant sample was analysed by a micro-Kjeldahl method (Paech & Tracey, 1956; Misra, 1968) and in the soil by a Kjeldahl method (Jackson, 1958). Nitrate-nitrogen in the soil was estimated by 2, 4-phenol-disulphonic acid colorimetric method (Jackson, 1958).

Using girth biomass regressions, the biomass of the standing crop of trees and shrubs in the experimental plots was estimated from 1976-78 girth measurements. To calculate the stock of nitrogen, the separate component biomass values of 12 species in different girth classes were multiplied with corresponding nitrogen concentrations. For rarer tree species, mean nitrogen concentrations of 11 species were used, and for rarer shrubs, that of *H. antidysenterica*. Nitrogen stocks of trees and shrubs are 3-year means. The nitrogen content in soil horizons was estimated from annual mean nitrogen concentration and bulk density. Nitrate nitrogen, which formed less than 0.5% of total nitrogen, was added. Nitrogen contents of tree and shrub saplings were added to the respective components of trees and shrubs on account of their small amount. Where time series data were available, as in herbs, litter

layer, small roots and soil, monthly nitrogen stocks were averaged over the year.

Table 1. Nitrogen stock in vegetation, litter and soil compartments

	Bole	Branch	Major root	Small root	Leaf	Total	%
			(kg ha^{-1})				
Trees	178	259	82	65	59	643	17.5
Shrubs	6.0	6.2	3.3	2.6	3.5	22	0.6
Herbs				4.9[a]	6.6[b]	11	0.3
Litter						36	1.0
Soil						2970	80.7
Total						3682	100.0
Vegetation aboveground						519	
Vegetation belowground						157	

[a] Total belowground.

[b] Total aboveground.

RESULTS AND DISCUSSIONS

Nitrogen stocks

Total nitrogen content in the ecosystem was 3680 kg ha^{-1}, of which vegetation, litter and soil contributed 18.4, 1.0 and 80.7%, respectively (Table 1). Thus, the major portion of nitrogen in the forest was contained in the soil, as has also been reported for several other forests. Data from a tropical moist deciduous forest (Greenland & Kowal, 1960), a tropical dry deciduous forest (Singh, 1974), a sub-tropical forest (Westman & Rogers, 1977) and two temperate deciduous forests (Johnson & Risser, 1974; Rolfe *et al.*, 1978) indicate that the range for nitrogen distribution in these forests is: ecosystem total 3688 - 10 700 kg ha^{-1}, fractionated in vegetation 8-32%, litter 0.5-5%, soil 69-90%. In tropical situations, due to heterogeneity of vegetation and soil, large spatial variability is inherent in such estimates. In the present study, separate estimates of the nitrogen content of trees and shrubs located in six plots permitted the evaluation of the variability in terms of coefficient of variation (CV). The CV for the stock of nitrogen in the woody vegetation was 13.5%, which can be considered reasonably low for such studies. For the herbaceous vegetation and the litter layer, both varying significantly through the year ($P < 0.01$), the CV for nitrogen content at most sampling dates varied from 46 to 89% and 6 to 15%, respectively. On the other hand, soil nitrogen content did not vary significantly through the year and the range for CV at different samplings was 3-21%. Therefore, it is believed that the extent of sampling for the present study was reasonable.

According to life forms, 95.1% of the total vegetation nitrogen was in trees, 3.2% in shrubs, and 1.7% in herbs. The vegetation nitrogen was apportioned 77% aboveground and 23% belowground. This nitrogen was shared by several species, without strong dominance by any one of them. Amongst the tree species, together contributing > 80% biomass, the decreasing order by percentage nitrogen share was: *A. latifolia* 29.6, *P. marsupium* 20.5, *D. melanoxylon* 9.5, *E. officinalis* 8.8, *B. lanzan* 7.9, *M. tomentosa* 4.4, *F. indica* 3.9, *G. tiliaefolia* 3.5 *L. paryiflora* 3.1, *A. catechu* 2.5 and *E. hookeriana* 1.8. In the shrub layer strong dominance was shown by *H. antidysenterica*, which contributed 82.1% of the nitrogen. Due to successional status and moderate tree density, most of the trees have spreading crowns, resulting in higher nitrogen content in the branches (40%) than the boles (28%). In shrubs, however, the nitrogen content was comparable in boles and branches (28%). Evidently, the nitrogen content proportion in leaves of shrubs was greater than for those of trees. In both woody life forms about half of the belowground nitrogen content was in the small roots. These were richer in nitrogen concentration than the major roots. The herbaceous vegetation nitrogen content increased from a minimum (3.8 kg ha^{-1}) in June to a maximum (26.9 kg ha^{-1}) in September, and later declined gradually.

Cycling of nitrogen

The nitrogen cycle in the stand has been represented as flows of nitrogen from soil to vegetation, from vegetation to litter layer and from litter to soil. The flows represent net amounts transferred from one compartment to another within one year.

Uptake by vegetation. The nitrogen uptake by vegetation was assumed to be the sum of annual net dry matter production in vegetation compartments multiplied by the respective nitrogen concentration. The flows through vegetation were considered to move from soil to leaves and the total flow in each life form was partitioned from compartment to compartment. The net uptake was computed as the sum of: annual increment in bole, branch and major root of trees and shrubs, peak leaf crop plus before-peak leaf litterfall in trees and shrubs, annual non-leaf litterfall in trees and shrubs, maximum minus minimum amount in small roots of trees and shrubs, and sum of successive positive increments in herbs. The nitrogen uptake was mainly derived from reliable estimates of nitrogen stocks of woody vegetation at yearly intervals and that of herbs at monthly intervals. The CV for nitrogen uptake by trees and shrubs, which have a major share, was 9%.

Table 2. Nitrogen uptake and net increment by vegetation compartments

	Flows			Net increment in last compartment		
	Trees	Shrubs	Herbs	Trees	Shrubs	Herbs
	(kg $ha^{-1}yr^{-1}$)					
Soil to small root	142.0	8.1	23.1[a]	21.1	1.6	6.0[a]
Small root to major root	120.9	6.5	-	4.6	0.4	-
Major root to bole	116.3	6.1	17.1[b]	10.1	0.8	17.1[b]
Bole to branch	106.2	5.3	-	32.0[c]	1.3[c]	-
Branch to leaf	74.2	4.0	-	74.2	4.0	-
Total				142.0	8.1	23.1

[a] Soil to belowground.

[b] Belowground to aboveground.

[c] Annual non-leaf litter fall+net increment in branch.

Table 2 shows annual flows and net increment of nitrogen in different vegetation compartments. Total uptake was 173 kg ha^{-1} yr^{-1}; 82% in trees, 5% in shrubs and 13% in herbs. The magnitude of total nitrogen uptake is undoubtedly higher than in most temperate forests; however, these values are much less than the available estimates for tropical rain forests (430 kg ha^{-1} yr^{-1} by Rodin & Bazilevich, 1967; 136-242 kg ha^{-1} yr^{-1} by Bernhard-Reversat *et al.*, 1978). Of the total uptake by the trees 30% is accounted for by *A. latifolia*, 15% by *P. marsupium* and 10% each by *B. lanzan* and *D. melanoxylon*. The enrichment ratio, defined as the ratio of nitrogen stock to net annual uptake (Westman, 1978b), was highest for trees (5.1), followed by shrubs (3.3) and herbs (0.6). The low enrichment ratio for herbs reflects a high turnover rate of nitrogen, and this decreased through the shrubs to the trees. The enrichment ratio of trees exceeded that of herbs approximately eight times, whereas the nitrogen stock of the former was 56 times greater than the latter. Although the stock of nitrogen in the herbs was small, the net annual uptake of nitrogen was more concentrated relative to that for trees, because of the leafier character of the herbs. The more rapid turnover of the leaf component of plants relative to wood in turn led to a higher ratio of uptake to nitrogen content for the herbaceous vegetation. On average, based on enrichment ratios, the turnover rate of nitrogen in the herbs was eight times greater than that in the trees.

About 80% of the total annual uptake was directed to above-ground parts of the vegetation (Table 2). Most of the uptake occurred during the rainy season, when moisture status of the soil was optimum (11.4-19.8% by dry weight) and nitrogen was rapidly released from the litter layer. The deciduous compartments of trees and shrubs shared a high proportion of the annual uptake (leaves 49.4-52.2%, small roots 14.8-19.7%). In this stand a significant fraction of leaf nitrogen uptake appeared to be translocated into thin branches, which showed a distinct increase in nitrogen concentration during summer. This translocation was estimated to be 25.7 and 2.1 kg ha^{-1} yr^{-1} for trees and shrubs, respectively, corresponding to 18.1 and 25.9% of total uptake and 34.6 to 52.5% of net increment in leaves. The major roots, however, did not show a summer nitrogen increase; therefore, the post-rainy season decrease in the small root compartment was probably due to decomposition loss.

Recycling through litterfall. Nitrogen flow from vegetation to litter layer was computed from the weight and concentration of nitrogen in leaf and non-leaf litterfall of trees and shrubs and the uptake in aboveground parts of herbs, which completely dry up in the winter season.

Table 3. Transfer of nitrogen from vegetation and litter to soil

	(kg N $ha^{-1}yr^{-1}$)	(%)
1. Vegetation to litter		
Tree leaf litter	48.5	58.8
Shrub leaf litter	1.9	2.3
Herb leaf litter	17.1	20.8
Non-leaf litter	14.9	18.1
Total	82.4	100.0
2. Litter to soil	75.8	
3. Small root to soil		
Tree small roots	21.1	73.5
Shrub small roots	1.6	5.6
Herb roots	6.0	20.9
Total	28.7	100.0

Total nitrogen recycling from vegetation to litter layer through litterfall was 82.4 kg ha^{-1} yr^{-1}; 58.8% by tree leaf litter, 2.3% by shrub leaf litter, 18.1% by non-leaf litter and 20.8% by herb litter (Table 3). Earlier workers (Singh, 1968; Desh Bandhu, 1971; Singh, 1974) have assumed the peak litterfall nitrogen during summer to be the total annual transfer from vegetation to the litter layer. However, our observations indicate that only 41.2% of total tree and shrub litter nitrogen was cycled during summer, 33.1% in winter and 25.7% in the rainy season. A large fraction (65.3%) of total non-leaf litter nitrogen was contributed by twigs and the remaining fraction by bark, flowers and fruits.

Nitrogen release from the litter and small roots into the soil. Nitrogen release from the litter layer was computed as the sum of June litter layer and annual litterfall contents less succeeding May litter layer content. From the litter layer 75.8 kg N ha^{-1} yr^{-1} was released into the soil, which was 92.1% of annual litterfall nitrogen. A leaf litter decomposition study under field conditions, using a nylon net containing leaves of *A. latifolia, D. melanoxylon* and *B. lanzan* as test species, indicated that on average about 63% of the nitrogen was released during one year. The nitrogen release value calculated from litter dynamics was distinctly higher than from the nylon bag study, possibly due to faster decomposition of mixed litter (including that of leguminous trees and herbs) under unconfined conditions.

After rapid growth during the rainy season, the biomass of small roots of trees and shrubs decreased to a minimum in summer. Therefore, if small root growth is assumed to be equal to its decomposition during the year, 22.7 kg N ha^{-1} yr^{-1} was released by root decomposition into the soil (Table 3). By the post-rainy season, decomposition of belowground herb parts released 6.0 kg N ha^{-1}. Thus, total release of nitrogen from the belowground vegetation component to soil was estimated at 28.7 kg ha^{-1} yr^{-1}, 73% being contributed by tree small roots, 6% by shrub small roots and 21% by herb roots. Thus, total release by above- and below-ground plant material decomposition into soil was 101 kg N ha^{-1} yr^{-1} (72% from litter and 28% from roots), corresponding to 60.1% of the total uptake.

Rainfall, throughfall, stemflow and runoff. Data on nitrogen input through rainfall, stemflow and throughfall and output via runoff, collected by S.N. Tewari have been given by Singh & Misra (1978). Rainfall nitrogen content was 23.4 kg ha^{-1} yr^{-1}, 22.7% being absorbed by vegetation aboveground, and the balance moving with throughfall (17.2 kg ha^{-1} yr^{-1}) and stemflow (0.9 kg ha^{-1} yr^{-1}) to the soil. The rainfall nitrogen content was found to be within the range indicated in several recent publications (23.7 kg N ha^{-1} yr^{-1} by Ulrich, 1977; 21 kg N ha^{-1} yr^{-1} by Bernhard-Reversat *et al.*, 1978; 60 kg N ha^{-1} yr^{-1} by Westman, 1978b). The absorption of rainfall nitrogen by the tree canopy, although quite small (5.3 kg ha^{-1} yr^{-1}), was clearly significant, as the nitrogen concentration in rainfall generally exceeded that in the throughfall collected from all the three tree species tested. Moreover, the rainy

season coincides with an active growth period of the leaves when such absorption could be expected. Such negative leaching of nitrogen by the canopy has also been reported by several authors (Carlisle *et al.*, 1966; Foster & Morrison, 1976; Killingbeck & Wali, 1978).

The effect of biological nitrogen fixation is not known. The runoff loss from the stand was 5.2 kg ha^{-1} yr^{-1}. The difference between throughfall+ stemflow and runoff was considered as the gain by the soil and litter layer (12.9 kg ha^{-1} yr^{-1}).

The underground leaching loss from the soil was not estimated, but was assumed to be negligible. Large quantities of small roots, both from woody and herbaceous species proliferated during rainy season, would also reduce such loss. Crude computation, based on generally known infiltration rates and nitrate concentration in deep well water, suggests the leaching loss to be less than 0.5 kg N ha^{-1} yr^{-1}.

Table 4. Nitrogen budget of the stand

	Input	Output	Balance	Turnover time[e]
	(kg N $ha^{-1}yr^{-1}$)			(yr)
Vegetation	178.5[a]	111.1[b]	67.4	3.78
Litter	82.4	75.8	6.6	0.44
Soil	122.6[c]	178.4[d]	-55.8	24.22
Stand	23.4	5.2	18.2	157.35

[a] Vegetation input = total uptake + absorption from rain.

[b] Vegetation output = litterfall + root decay.

[c] Soil input = litter release + root decay + throughfall + stemflow.

[d] Soil output = vegetation uptake + runoff.

[e] Turnover time = stock/input.

Nitrogen budget of stand

Table 4 and Fig. 1 show the nitrogen balance of the stand, which was found to be positive for vegetation and litter and negative for soil. The input into vegetation was calculated as the sum of uptake and absorption from rain, and the output was obtained as sum of litterfall and root decay. No net leaching loss from the canopy was indicated, as the nitrogen concentrations in the throughfall and stemflow were lower than that in rainfall. The vegetation and litter layer exhibit a positive balance of 67.4 and 6.6 kg N ha^{-1} yr^{-1}, which is 10.0 and 18.2% of the nitrogen stock, respectively.

The input to the soil was the sum of output from litter layer, small root decay and throughfall+stemflow, and the output from soil was estimated as the sum of vegetation uptake and runoff. On this basis, the soil compartment had a deficit of 55.8 kg N ha^{-1} yr^{-1} (Table 4), which could be met by nitrogen fixation input. Greenland & Nye (1959) have reported the fixation input to range between 20 and 55 kg N ha^{-1} yr^{-1} in tropical forests. It has been estimated by Bremner (1967) that 1-3% of the soil nitrogen content is mineralized each year. If a value of 2% is accepted for this stand, 59.4 kg N ha^{-1} yr^{-1} is expected to be released within the soil. Soil release plus litter release (135 kg N ha^{-1} yr^{-1}) fell short of the vegetation uptake by 38 kg N ha^{-1} yr^{-1}, an amount well within the nitrogen fixation possibility.

This stand exhibits rapid nitrogen cycling, evident from comparatively low turnover time for all components (Table 4). For several elements in wet tropical forests, Golley *et al.* (1975) have calculated the following ranges for turnover time: stems 11.7-15.7 yr, litter 0.2-1.2 yr. The nitrogen turnover rates in this stand are in the order: litter > vegetation > soil. Despite relative dryness, the litter layer had very rapid turnover, so that its nitrogen release was equivalent to about half of the vegetation uptake. The stand as a whole had a net annual increase in nitrogen content which is about 0.5% of the total nitrogen stock.

ACKNOWLEDGEMENTS

We are thankful to Professor R. Misra for encouragement, to the Head of the Botany Department for facilities, and to the Department of Science and Technology, Govt. of India, for financial support including a Junior Research Fellowship to one of us (O.N.P.).

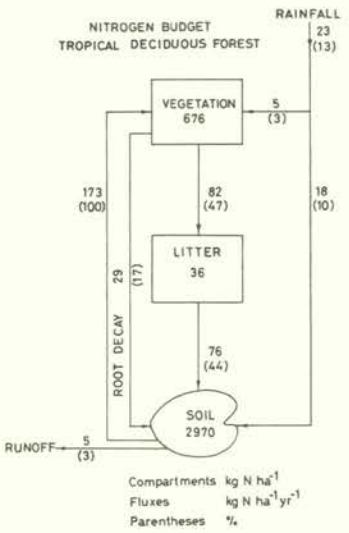

Fig. 1. Nitrogen contained in vegetation, litter and soil (kg ha^{-1}), nitrogen fluxes (kg ha^{-1} yr^{-1}) and their percentages of influx or efflux (given in brackets) for a tropical deciduous forest.

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NITROGEN FIXATION AND AMMONIA VOLATILIZATION IN A PHILIPPINE MANGROVE SWAMP

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ABSTRACT

An investigation designed to determine the contribution of nitrogen fixation in situ *and losses of nitrogen via volatilization was conducted in a swamp in Pagbilao, Quezon Province, Philippines under four predominant mangrove species:* Avicennia alba, Rhizophora apiculata, Sonneratia caseolaris, *and* Nypa fruticans. *The effect of roots and light energy on the rate of nitrogen fixation was also determined to evaluate the contribution from heterotrophic bacteria.*

The amounts of acetylene reduced to ethylene in situ *corresponded to 23.8, 21.7, 21.1 and 11.1 g N fixed* ha^{-1} d^{-1}, *for* Avicennia, Rhizophora, Sonneratia *and* Nypa, *respectively. The rate of acetylene reduction activity (ARA) was closely related to the amount of available soil organic matter. Thus, ARA in root-associated sediment under* Rhizophora apiculata *was approximately twice as great as the activity in non-root sediment from the same site. Higher ARA under dark than under light conditions indicated the importance of heterotrophic bacteria in nitrogen fixation.*

The rapid disappearance of nitrate added to the surface 2.5 cm layer of swamp soils placed in jars demonstrated the high biological activity of this layer. The nitrate removed from the top layer partially showed up as floodwater ammonium under Sonneratia *sp.*, Rhizophora *sp., and to a certain extent* Nypa *sp. The rates of ammonium volatilization* in situ *averaged over a 17-day incubation, were in the order:* Rhizophora > Nypa > Sonneratia > Avicennia. *The values were 27.3, 22.5, 17.6, 9.9 g N volatilized* ha^{-1} d^{-1}, *respectively. The low magnitude of* NH_3 *volatilized was probably due to the soil pH, low temperature, high soil organic matter and alternate drying and wetting as occurs in diurnal tide. The results indicate the possible harmful consequences of excessive harvesting of mangrove tree species on the nitrogen cycle.*

INTRODUCTION

While mangroves comprise a productive ecosystem, this productivity is rather paradoxical because coastal waters are often nutrient limited, particularly in nitrogen (Rhyther & Dunston, 1971). Except for the atmospheric deposition of nitrogen compounds, there is no discernible flow of combined nitrogen into the swamp ecosystem under study. Thus, it is assumed that the main source of nitrogen input is through biological nitrogen fixation. However, some of the nitrogen gain by this process may be lost via ammonia volatilization and denitrification. Therefore there is a need to understand the importance of these processes in determining the nitrogen balance if management strategies are to be developed for this rather fragile ecosystem.

There are very few reports on biological nitrogen fixation in mangrove areas of the subtropics, and probably none on the tropics. Rodina (1964) reported the presence of several types of diazotrophs in soils of mangrove thickets in the Gulf of Tonkin, Vietnam. These diazotrophs fix nitrogen in the rhizosphere of marine angiosperms (Patriquin & Knowles, 1972; Zuberer & Silver, 1974, 1978, 1979; Kimball & Teas, 1974).

The acetylene reduction activity of plant-associated sediments in a Florida mangrove community was slightly higher than that of plant-free sediments, probably due to organic materials given off by the plants (Zuberer & Silver, 1974, 1978). Gotto & Taylor (1976) reported that nitrogen fixation was associated with decaying leaves of *Rhizophora mangle*. Zuberer & Silver (1978) observed marked response in nitrogenase activity after the addition of various carbon sources, indicating the existence of energy limitation in mangrove sediment. Such a limitation has been observed also in salt marshes (Hanson, 1977a, 1977b).

Certain transformations of nitrogen such as denitrification and ammonia volatilization affect the soil nitrogen balance and lead to a N loss. Of these, denitrification is probably the major mechanism of nitrogen loss in mangrove swamps considering the conditions pertaining in the swamps.

This report assesses the contribution of biological nitrogen fixation and N loss via ammonia volatilization and/or by denitrification on the general state of the mangrove ecosystem. A study of nitrogen mineralization in mangrove soils was presented in an earlier report (Pahm & Aspiras, 1979).

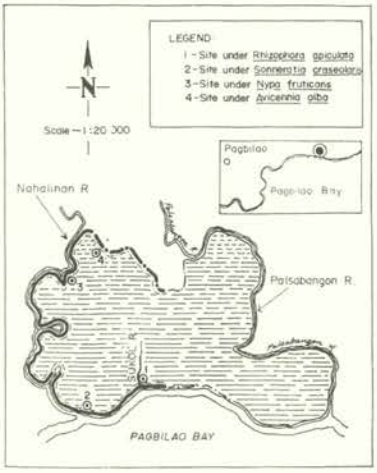

Fig. 1. Sampling sites in the Mangrove Research Center, Pagbilao and its location in relation to Pagbilao town (insert).

MATERIALS AND METHODS

Location of experimental sites

There is a definite pattern of vegetational distribution in mangrove swamps (Arroyo, 1977). Different species occupy different areas. This zonation is controlled by the interaction of tidal flooding, salinity and drainage of the soil. The location of the experimental sites (Fig. 1) was based on the zonation pattern described by Brown (1918).

The physico-chemical characteristics of soils under the different mangrove species at the experimental sites are presented in Table 1. The NH_4^+-N concentration in the soil is weakly correlated (r = 0.82) with soil organic matter; the NO_3^--N concentration was equally low under all four vegetation types.

Table 1. Physico-chemical characteristics of soil samples taken from under different vegetation types of the mangrove swamp (values are means of 4 replicates)

Vegetation types	pH (1:1 soil: water)	Organic matter (%)	Moisture (% oven-dry)	NH_4^+-N (ppm)	NO_3^--N (ppm)	Ambient temp. (C at 10 a.m.)	Soil
Sonneratia craseolaris	7.0	4.79	109.6	28.2	5.2	28.0	Sandy loam
Nypa fruticans	7.1	2.52	81.8	28.4	7.5	26.8	Silty clay
Rhizophora apiculata	6.8	7.94	126.8	33.2	5.1	26.0	Sandy loam
Avicennia alba	6.5	9.42	159.1	51.5	4.1	24.8	Silty clay

Acetylene reduction studies

In situ *determination at four sites.* The assay technique of Lee & Yoshida (1977), using plastic bags for *in situ* determination of nitrogen fixation by acetylene reduction, was employed in this investigation.

The assay chamber was driven into the soil and evacuated. Two l of air and 2 l of acetylene were introduced into the 8-l volume assay chamber using a 1000-ml syringe. The incubation period was 24 hours.

The gas inside the reaction chamber was stirred for five minutes just before sampling. Gas samples were withdrawn directly into pre-evacuated B-D vacutainer tubes using a two-way needle, and the gas samples were analyzed immediately by gas chromatography using the method of Alimagno (1974). Ethylene and acetylene were separated by a 1-m column of Porapak N at 45 C with nitrogen as the carrier gas and with a flow rate of 15 ml min^{-1}. The hydrogen flame ionization detection was operated at 55 C.

The ethylene standard was prepared by mixing a known volume of ethylene (The Matheson Co., Inc., New Jersey, 99.6%) with ordinary atmosphere in a 100-ml volumetric flask, calibrated by water displacement, using glass beads (5 mm) to facilitate gas mixing in the flask.

The results are expressed in µmole ethylene $chamber^{-1}$ hr^{-1} or nmole ethylene $flask^{-1}$ hr^{-1}, depending on the kind of sample used for assay and incubation time.

Effects of Rhizophora *roots.* Soil samples attached closely to the roots of *Rhizophora* sp. were collected and analyzed for nitrogenase activity. The samples contained some rootlets which could not easily be separated from the soil. Twenty ml of distilled water was added to 20 g of the soil sample (wet weight) in a 50-ml flask and mixed well. One ml of acetylene was added to each flask. Ethylene present at zero time was measured, after which the samples were incubated for 24 hours prior to another ethylene measurement.

Effect of light. Two sets of three assay chambers each were installed side by side under similar conditions. One set was covered completely with a black cloth to determine N_2 fixation in the dark. The soil under *Avicennia* sp. was chosen as the experimental site, since in the previous study soils under this vegetation gave a higher level of N_2 fixation than soils from any of the other vegetation types studied.

Denitrification in soils supporting different mangrove species

Soil samples were collected from the 0-5 cm and 5-10 cm layers at the four experimental sites. These layers were reconstructed from sieved subsamples in 10-cm diameter glass jars of 16 cm height. After the soil was transferred to the jars, 150 ml of distilled water was added to each jar to simulate the moisture conditions in the field.

For each vegetation type 20 ml of 50-ppm KNO_3-N was applied to each of three jars, while two jars served as control (no nitrate application). The jars were then covered with aluminium foil secured with a rubber band and incubated at room temperature.

The first sampling was done three hours after the nitrate addition; thereafter sampling was done every two days for six days. At the end of an incubation period all the floodwater in the jar was collected and 2 ml of chloroform added to arrest microbial activity.

To study denitrification in the top soil layer, soil samples from 0-2.5 cm depth were taken at three different points in the jar. Ten-g subsamples were shaken with 50 ml of 2N KCl for one hour and centrifuged at 2000 rpm for 10 minutes. The supernatant was collected into a separate container, after which another 50 ml of 2N KCl was added for another centrifugation at the same speed and duration. The extract solution was finally filtered, using another 25 ml of 2N KCl to rinse the container.

The floodwater collected at the end of the incubation period, and the soil extracts, were analyzed for ammonium and nitrate nitrogen by steam distillation using MgO and Devarda's alloy (Bremner, 1965). A 75-ml distillate was collected into a 20-ml boric acid-indicator solution prior to titration with standard H_2SO_4.

The values obtained at any sampling time are expressed as the amount of nitrogen in the 0-2.5 cm soil depth.

In situ *determination of ammonia volatilization*

The ammonia trapping method of Ventura & Yoshida (1977) was employed in this study, any ammonia evolved being trapped in the glass wool and determined by steam distillation.

The results are expressed as µg NH_4^+-N l^{-1}. The values are averages of four determinations. Samples were taken on the 3rd, 8th, 13th, and 17th day of incubation and the results of these determinations have been averaged to get the values on a per day basis.

RESULTS AND DISCUSSION

Acetylene reduction studies

The amount of acetylene reduced to ethylene in µmole chamber^{-1} d^{-1} was 4.68 for *Avicennia* site, 4.28 for *Rhizophora* site, 2.18 for *Nypa* site, and 4.16 for *Sonneratia* site after 24 hours of incubation. These values correspond to 23.8, 21.7, 11.1, and 21.1 g N fixed ha^{-1} d^{-1} respectively assuming a conversion factor of three (it was not proper to use any other factor considering the wide diversity of conditions present in a mangrove swamp).

The rates of nitrogen fixation in the various sites studied followed the same order as for soil organic matter: *Avicennia* > *Rhizophora* > *Sonneratia* > *Nypa*. Zuberer & Silver (1978) observed that nitrogenase activity in sediments increased greatly upon the addition of various carbon sources. They likewise noted that surface litter consisting of mangrove leaves (*Rhizophora mangle*) and sea grass material transported into the site exhibited high rates of acetylene reduction. In another study, Gotto & Taylor (1976) have shown that N_2 fixation was associated with decaying leaves of *Rhizophora mangle*.

The diffusion coefficient of acetylene in water-saturated soil is approximately 10^{-6} to 10^{-5} cm^2 sec^{-1} (Lee & Watanabe, 1977) and acetylene can therefore be expected to reach only the top few mm of the surface soil during the 24-hour assay. Thus, the data presented in the present study may be indicative only of the surface activity of the air-soil-surface root interface.

The amount of ethylene formed from acetylene added was 105 nmole (20 g wet weight)$^{-1}$ (24 hr)$^{-1}$ in root-associated sediment of *Rhizophora* sp. and 55 nmole (20 g wet weight)$^{-1}$ (24 hr)$^{-1}$ in non-root sediment. These values correspond tc 11.9 and 6.2 nmole (g dry sediment)$^{-1}$ (24 hr)$^{-1}$, respectively. This difference could be due to the availability of high organic matter content in the vicinity of *Rhizophora* roots. In a similar study, Zuberer & Silver (1978) showed that higher rates of nitrogenase activity were associated with excised roots of *Rhizophora mangle* L., *Avicennia germinans* (L) Stern, and *Laguncularia racemosa* Gaertn. These observations and our results indicate that diazotrophs in the mangrove rhizoplane are able to use root exudates and/or sloughed off cell debris as energy sources for nitrogenase activity. A comparison of nitrogen fixation in clipped and unclipped *Spartina* plots (Hanson, 1977a) also indicated that root exudation promotes nitrogen fixation.

To determine whether nitrogen fixation in mangrove swamps was due primarily to heterotrophic bacteria or to blue-green algae, an *in situ* experiment was conducted with the treatment involving the shielding of the nitrogen fixation chamber from sunlight with the use of black cloth 24 hours prior to gas sampling. In the *Avicennia* site the amount of acetylene reduced was 6.2 µmole chamber^{-1} (24 hr)$^{-1}$ without black cloth cover and 8.2 µmole chamber^{-1} (24 hr)$^{-1}$ with black cloth cover. This confirms that nitrogenase activity in mangrove areas was conducted by non-photosynthetic bacterial diazotrophs. In a study of nitrogen fixation in a salt marsh, Hanson (1977b) has also shown that their surface nitrogen fixation proceeds at a relatively constant rate in the light and in the dark, except in areas where heterocystic nitrogen-fixing blue-green algae were present.

Table 2. Recovery of 1000 µg NO_3^--N, after addition to the floodwater, in jars at two incubation times for four soils

Soil	After 3 hr (%)	After 6 days (%)
Sonneratia site	59	0.9
Rhizophora site	68	0.6
Nypa site	93	3.2
Avicennia site	102	3.8

Denitrification

Recovery of added nitrate in the floodwater. The recovery of the nitrate added to the floodwater of the soils collected at the different sites was already low after 3 hours for soils collected from the *Sonneratia* and *Rhizophora* sites (Table 2). This could have been due to the mild stirring of the floodwater that was done during addition of the nitrate to achieve a uniform distribution. Apparently, this stirring effect was much more marked for the two lighter-textured soils (Table 1).

After six days nearly all added nitrate had disappeared from the floodwater from all soils, presumably due to denitrification.

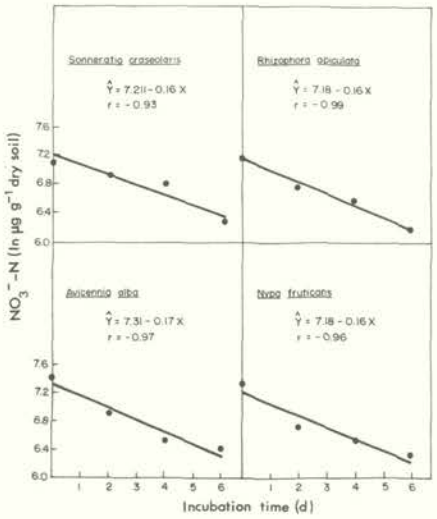

Fig. 2. Relation between incubation time and nitrate concentration in soil plus floodwater in jars to which 1000 µg NO_3^--N had been added at start of incubation, for the four vegetation sites as indicated.

Rate of nitrate disappearance. The linear relationship between the logarithm of the nitrate concentration in the soil plus floodwater and incubation time (Fig. 2) indicates that the rate of nitrate disappearance followed first order kinetics, the rate of loss being 16% d^{-1} for soils under *Sonneratia*, *Rhizophora* and *Nypa* sp., and 17% d^{-1} under *Avicennia* sp. This constant rate of denitrification in all samples suggests stability in the ecosystem from which these samples were taken.

Fate of nitrate-nitrogen in the floodwater. The losses of added nitrate could involve two major processes i.e., downward movement to the lower layers or microbial reactions that would possibly lead to losses. Nitrate in the top 2.5 cm layer did not show any increase within six days (results not presented) so that it was assumed that the NO_3^--N had probably undergone denitrification or nitrate reduction to ammonia. The first process leads to conversion of nitrate to gaseous products, N_2 and N_2O, but the other transforms NO_3^--N to NH_4^+-N. In this investigation gaseous products were not determined, but, by following the trend in floodwater NH_4^+, it is possible to examine the extent of nitrate reduction (P.G.L. Vlek & E.T. Crasswell, unpublished; Vlek & Stumpe, 1978).

In soils from under *Sonneratia* sp. and *Rhizophora* sp. the floodwater NH_4^+ increased during 6 days of incubation with added nitrate (Fig. 3). In soil from the Sonneratia site the floodwater NH_4^+ (after deducting control values) showed a slow increase from 0 to 4 days and a rapid rise on the 6th day accounting for nearly 40% of the NO_3^--N applied. This brings out the strong possibility that nitrate has undergone reduction to ammonium. The amount of NH_4^+-N in the untreated floodwater was usually lower than the treated floodwater, but showed some increases especially toward the end of incubation. This could have been brought about by the natural diffusion of NH_4^+ from the soil where ammonification of soil organic matter was presumed to be active.

In the case of *Avicennia* and *Nypa* soils, the level of NH_4^+ in the treated floodwater was about the same as that of the untreated floodwater (Fig. 3). It is apparent that nitrate was not transformed to NH_4^+ in these cases. This observation suggests that losses of nitrate in soils under *Avicennia* sp. and *Nypa* sp. are predominantly via denitrification.

Ammonia volatilization

The amount of NH_3 volatilized was least in *Avicennia* soil, 10 g N ha^{-1} d^{-1}, followed by 18 g N in *Sonneratia*, 23 g N in *Nypa* and 27 g N in *Rhizophora* soils, calculated on the surface area of the jar. The cumulative amount of NH_3 volatilized over 17 days is shown in Fig. 4.

The ammonia volatilization in the mangrove area studied is understandably low since the soil pH values ranged from 6.6 to 7.2, and the amount of ammonia volatilized is known to increase at higher pH (Ernst & Massey, 1960; Du Plessis & Kroontje, 1964; Ventura & Yoshida,

1977). The low levels of volatilization, despite high levels of NH_4^+-N formed, are probably also due to relatively low ambient temperature and high cation exchange capacity of the soils.

The escape of ammonia could increase as the soil dries, especially if the ammonia is formed near the surface. This may become significant because the *Rhizophora* site, unlike that of the *Avicennia* site, is completely inundated during high tide and then becomes exposed during low tide.

A summary of the *in situ* N_2 fixation and *in situ* ammonia volatilization estimates at the different experimental sites is presented in Table 3.

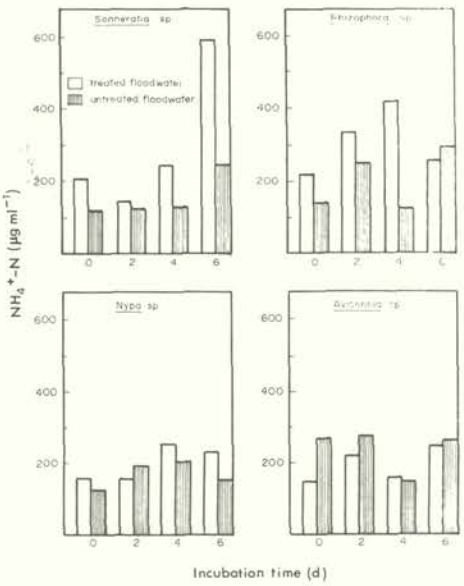

Fig. 3. Relation between incubation time and ammonium concentration in the floodwater above the soil, to which nil or 1000 µg NO_3^--N had been added at start of incubation, for the four vegetation sites as indicated.

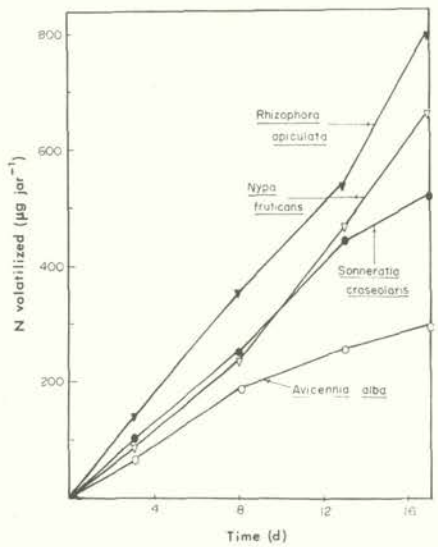

Fig. 4. Cumulative ammonia volatilization in situ at the four vegetation sites as indicated.

Table 3. Summary of in situ N_2 *fixation and* in situ *volatilization in soils under various mangrove species*

Predominant vegetation type	Fixed N (g N ha^{-1} d^{-1})	Volatilized N
Nypa fruticans	11	23
Sonneratia craseolaris	21	18
Rhizophora apiculata	22	27
Avicennia alba	24	10

A full discussion of the N balance in the mangrove area studied is limited by the fact that N_2 fixation and ammonia volatilization were conducted at different times. Therefore differences between the amount of N fixed and N volatilized could not be properly represented as gains or losses, respectively. Nevertheless, indications are that *Avicennia* sp. tend to promote accumulation of plant biomass and soil N, *Rhizophora* sp. and *Sonneratia* sp. tend to maintain their soil N status, while *Nypa* sp. appears to allow more N losses. These results may partly explain the wide differences in soil organic matter (organic N) accumulations (Table 1), which are greatest for the *Avicennia* site, intermediate for *Rhizophora* and *Sonneratia* sites, and lowest for the *Nypa* site.

SUMMARY AND CONCLUSIONS

An investigation was conducted to measure the rates of nitrogen fixation *in situ* in pre-selected sites of Pagbilao swamps covered by different predominant vegetation types such as *Avicennia alba* (Blm.), *Rhizophora apiculata* (Blm.), *Sonneratia craseolaris* (Linn.), and *Nypa fruticans* (Wurmb.). The levels of acetylene reduction activity (ARA) in these areas correspond to 23.8, 21.7, 21.1 and 11.1 g N fixed ha^{-1} d^{-1} respectively. The rate of nitrogen fixation was well correlated with the amount of soil organic matter and at the *Rhizophora* site activity was released to the presence of plant roots. Heterotrophic bacteria appeared to be the source of activity.

The rate constant for nitrate disappearance in the flood-water was approximately 16% per day in all the samples, suggesting a certain stability for the ecosystem. Determination of ammonium in the floodwater indicated the partial conversion of the added nitrate to ammonium soils from *Sonneratia* and *Rhizophora* sites.

The magnitude of NH_3 loss by volatilization *in situ* was in the order: *Rhizophora* > *Nypa* > *Sonneratia* > *Avicennia*, amounting to 27.3, 22.5, 17.6 and 9.9 g N ha^{-1} d^{-1}, respectively. Although N_2 fixation and ammonia volatilization experiments were not conducted simultaneously, *Avicennia* sp. and *Sonneratia* sp. appeared to be more N-conserving communities than *Nypa* sp. and *Rhizophora* sp..

While the rate of N_2 fixation in mangrove areas appears to be low, the contribution of this process to the nitrogen budget of mangrove communities could be important. Moreover, nitrogen fixation takes place in soil/sediment, in root-associated soil/sediment, and even in organic litter brought into the area (Zuberer & Silver, 1978). The N incorporated by N_2 fixation might be the most significant N input into this ecosystem. While it is also important to consider the extent of N losses in this particular ecosystem, it should be noted that many of the factors that favour N losses do not become active unless ecosystem perturbations occur. For instance, removal of the mangrove vegetation from this community has resulted in decreases of soil organic matter and mineral N (Pahm & Aspiras, 1979).

ACKNOWLEDGEMENTS

The senior author is particularly grateful to the National Science Development Board for the study grant toward an MS in Microbiology at UP at Los Banos and to the Philippine Council for Agriculture and Resources Research for thesis funding support. Both authors wish to thank the Mangrove Research Center for the use of a portion of the mangrove area.

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PEAT DEPOSITION, AN IDLE STAGE IN THE NATURAL CYCLING OF NITROGEN, AND ITS POSSIBLE ACTIVATION FOR AGRICULTURE

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ABSTRACT

Peats in south-east Asia comprise about 20.5 x 10^6 ha and contain about 9.2 x 10^{11} kg of N. Peat is the result of delicate interactions among the components of a specific ecosystem. Therefore, the dynamic equilibrium in peats is easily disturbed by even a small change in one of the components; this is especially true for peats of tropical regions.

This paper tries to deal with the practical side of the temporary mobilization of N in peats, and increasing the availability of N for the benefit of agricultural crops.

INTRODUCTION

In the tropics the rate of production of phytomass is generally high, but because of an equally high rate of organic matter breakdown it was generally believed that peats would not be formed over large areas in the tropics (Visscher, 1949).

Recent soil surveys have dismissed that conjecture. South-east Asia has close to 20.5 x 10^6 ha of peat land, which is mainly concentrated around the Sunda Flat. Indonesia has a total peat area of about 17 x 10^6 ha, covering some 9% of the country's total land surface; 2.3 x 10^6 ha are found in west and east Malaysia, while the southern part of Vietnam has more than 1 x 10^6 ha peat (Pons, 1974; Soepraptohardjo & Driessen, 1976). With an average thickness of 2 m, a bulk density of 0.15 g cm^{-3} and a total N content of 1.5%, the total amount of N stored in those peats is approximately 9.2 x 10^{11} kg.

This paper concentrates on discussing the practical implications of the temporary storage of N in peats. Peasant agriculture, which is still prevailing in developing countries, is heavily dependent on the natural productivity of the habitat. While ambitious plans are being drawn and executed to develop large portions of peat lands for agriculture, it will be most appropriate to define ways of activating the potential N deposits in peats and at the same time to indicate the ecological pre-requisites for sustained yields. The present study has been undertaken in the framework of peatland advancement in Indonesia.

OCCURRENCE AND FORMATION OF PEAT

In tropical regions where the bioclimate favours the production of large quantities of phytomass, the critical factor in peat formation is the edaphic environment that determines the rate of organic matter destruction. The most important factors that hamper the ready decomposition of organic matter are (i) an impeded drainage which develops a deficiency in free oxygen within the weathering zone and (ii) an oligotrophic condition. Oligotrophy tends to produce low quality plant material, consisting more of cellulose and lignin than of starch, sugars and proteins (Pons, 1974). A low quality organic matter will be more resistant to microbial attack. A medium poor in nutrients is also unfavourable for the growth and development of decomposers.

The majority of the Indonesian peats are woody ombrogenous, formed in mixed swamp forests with mosses and ferns, or in mangrove forests. These dome-shaped peats may reach thicknesses of well over 10 m in the summit. Many ombrogenous peats were formed on top of minerotrophic peats, the latter being frequently richer in plant nutrients than the former. This does not necessarily mean, however, that all dome-shaped peats are low in potential fertility. Those which are subjected to periodic intrusions by brackish or fresh water, or river overflow, are richer and much less acid (or even neutral) in reaction.

The principal peat areas of Indonesia are the deltaic and estuarine plains, and the downstream interfluves of big rivers. Peat deposits cover vast areas of the eastern coastal plain of Sumatera, the western and southern coastal plains of Kalimantan, and the southwestern coastal plain of Irian Jaya.

The rate of peat decomposition in Kalimantan was determined by carbon dating. The results are presented in Table 1. Anderson (Pons, 1974) dated an ombrogenous peat in Sarawak (Table 2). Both results show similar trends in several respects. Age increases

with depth and this is parallelled by an increase in the rate of accumulation. Age increasing with depth along a peat profile indicates a sedentary formation. At comparable depths the Kalimantan peats seem slightly older than the Sarawak peat, showing a slower average growth rate of the former than that of the latter. The relations between depth and age and between depth and rate of accumulation are given in Fig.'s 1 and 2 respectively, assuming no difference between the Sarawak and Kalimantan peats.

Table 1. Age determination by carbon dating on two peat samples from Kalimantan[a]

Sample	Old method[b] (yr)[d]	New method[c] (yr)[d]	Probable age (yr)[d]	Rate of accumulation[e] (m (100 yr)$^{-1}$)
Pontianak, West Kalimantan: ombrogenous peat at 2.5 m depth.	2820 ± 70	2910 ± 80	2870 ± 80	uc 0.09 c 0.13
Barambai, South Kalimantan: minerotrophic peat at 1 m depth, at transitional layer between peat and underlying mineral layer.	2310 ± 90	2380±100	2350±100	uc 0.04 c 0.05

[a] In collaboration with Prof. E.C.A. Runge, University of Missouri, Columbia, USA, and Dr R.C. McGill, Institute of Nuclear Sciences, DSIR, New Zealand.

[b] Old $T^1/2$ (5568 yr).

[c] New $T^1/2$ (5730 + 40 yr).

[d] Years before present.

[e] uc = uncorrected, c = corrected by a compaction factor.

Subagjo & Driessen (1974) and Driessen & Rochimah (1976) noted a progressive increase in bulk density with depth in an ombrogenous peat profile in West Kalimantan. This is due to the heavier load imposed on deeper layers. There is also a temporal relationship of land use to peat compaction. Considering the vertical distribution of bulk density, a correction factor may be applied to the measured thickness of peat to obtain an "undisturbed" or "unconfined" thickness from which the average rate of peat accretion can be calculated (Table 3). Walker (Moore & Bellamy, 1976) reported rates of 0.18 m to 1.03 m (100 yr)$^{-1}$ with a mode of 0.64 m (100 yr)$^{-1}$ at a variety of British and Irish sites. Apparently, the rate of peat accumulation in the tropics does not differ significantly from that in temperate zones.

Table 2. Age and rate of accumulation of an ombrogenous peat in Sarawak (Pons, 1974)

Sampling depth (m)	Age by C-dating (yr)[a]	Rate of accumulation (m (100 yr)$^{-1}$)
5	2255	0.22
10	3850	0.31
12	4270	0.48

[a] years before present.

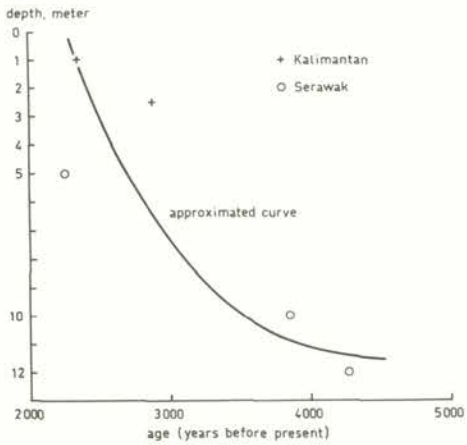

Fig. 1. Relationship between age and depth of peat in Kalimantan and Sarawak.

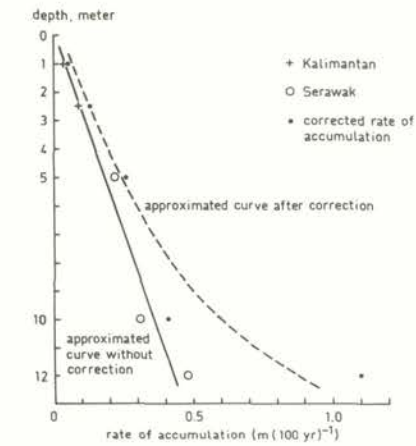

Fig. 2. Relationship of rate of accumulation to depth of peat in Kalimantan and Sarawak.

If the magnitude of growth of the Sarawak peat at a depth of 12 m can be used as a bench-mark of peat growth in wet monsoonal regions of the tropics, the growth deficiency of the younger deposits becomes evident on the basis of rate of weight accretion (Table 3). There is a growth deficiency of 88% of the Pontianak peat and 95% of the Barambai peat. This means that recent peat formations are 5 to 12% effective only.

Table 3. Calculation of corrected rate of peat accretion, assuming a given correction factor

	Thickness			Corrected mean rate of peat accretion	
	Measured	Correction factor	Corrected		
	(m)		(m)	(m(100yr)$^{-1}$)	(10^3kg ha^{-1} yr^{-1})[a]
Pontianak	2.5	1.5	3.75	0.13	2.3
Barambai	1.0	1.2	1.2	0.05	0.9
Sarawak	12.0	2.3	27.6	1.10	19.8

[a] Assumes a bulk density of 0.18 g cm^{-3}.

NITROGEN RELATIONS IN PEATS

Detailed information about nutrient dynamics in peat is very limited. To quantify the changes brought about by decomposition, an index constituent should be selected which can be assumed to have remained appreciably unchanged during the alteration processes. Lignin can be used properly (Alexander, 1961; Kononova, 1966; Čižek, 1967; Hurst, 1967; Robert-Géro *et al.*, 1967; Mohr *et al.*, 1972), or ash (Driessen & Soepraptohardjo, 1974), provided that no substantial leaching occurs and that the ash content of microbial tissues is insignificant. Peat environments are not conducive to leaching. Besides, organic compounds are more leachable than ash, the relative leachability of ash to organic compounds being 1:1.7 (Volobuev, 1964). On average, the bio-population in soil organic matter is 1.5% by weight (Volobuev, 1964) and the ash content of microbial cells is around 8% of the dry weight (Rippel-Baldes, 1952). Thus, the average addition of ash from microbial tissues will be only 0.12% by weight, which can indeed be disregarded.

Brotonegoro & Abdulkadir (1978) found a total ash content of 14% in fresh mangrove leaf fall on the peat island of Pulau Rambut, Jakarta Bay. The ash content of the peat from the same locality was 29.5%. Using ash as index, the mass reduction of leaf fall to peat is 53%, which means that the present peat mass is 47% of the original mass of leaf fall. This figure agrees well with the decomposition rates on the surface of tropical upland soils in Colombia and Costa Rica, which range between 40 and 65% (Jenny *et al.*, as cited by Mohr *et al.*, 1972; Volobuev, 1964).

From the figures presented by Longman & Jenik (1974) and Moore & Bellamy (1976), the average N content in the vegetative matter of a tropical rain forest can be calculated being 0.87%. Brotonegoro & Abdulkadir (1978) found a N content of 0.55% in the mangrove leaf fall of Pulau Rambut. If there were no change in the N content during the transformation of forest organic matter into peat, and assuming a 53% mass reduction along that transformation, then the peat should contain 1.87% N when derived from tropical rain forest, or 1.17% when it was formed in a mangrove forest. The average N content in peat which came from the primary source of phytomass is then 1.52%. This is exactly what has been found actually in peats.

With 1.5% N and a bench-mark rate of accumulation for the Sarawak peat of 19.8×10^3 kg ha^{-1} yr^{-1}, the rate of N storage in peat from the primary source is 300 kg ha^{-1} yr^{-1}. The rates in the Pontianak and Barambai peats are 35 and 14 kg ha^{-1} yr^{-1}, respectively. Thus the average rate of N storage in the younger peats is 25 kg ha^{-1} yr^{-1}. The mid rate between older and younger deposits is then 163 kg ha^{-1} yr^{-1}.

Table 4. Calculated amount of nitrogen added through rainfall annually and in the dry and wet seasons[a]

Constituents	Average content[b] (mg l^{-1})		Equivalent N (mg l^{-1})		Total increment of N (kg ha^{-1})		
	July	October	July	October	Dry season	Wet season	Annual
NH_4^+	1.6	0.4	1.2	0.3	Total	Total	
NO_3^-	0.02	0.00	0.005	0.00	rainfall[c]	rainfall[c]	
NO_2^-	0.00	0.03	0.00	0.01	276.2 mm	2133.2 mm	
OM[d]	24.2	28.5	1.0	1.1			
Total content of N			2.205	1.41	6.1	16.4[e]	22.5

[a] Barambai and Banjarmasin stations, South Kalimantan.

[b] R. Harijoto (pers. comm.).

[c] S. Wisnubroto (pers. comm.).

[d] Organic matter, assumed average N content 4%.

[e] Mean of maximum (October) figure and minimum (0.1 x October) figure; see text.

From the data given by Brünig (1975), Moore & Bellamy (1976) and Longman & Jenik (1974), the probable net production of tropical forests can be estimated at 25×10^3 kg ha^{-1} yr^{-1}. With a N content of 0.87%, the annual increments of N in the phytomass is 218 kg ha^{-1} yr^{-1}. The data of Srivastava & Sani bin Shaffie (1979) suggest a productivity rate for a *Rhizophora* dominated mangrove forest in Malaysia of 50×10^3 kg ha^{-1} yr^{-1} fresh weight, or 15×10^3 kg ha^{-1} yr^{-1} dry matter (assuming a 30% dry weight). With a N content of 0.55%, the annual increments of N in a mangrove phytomass will be 83 kg ha^{-1} yr^{-1}. The mid value between a

tropical rain forest and a tropical mangrove is 151 kg ha^{-1} yr^{-1}. The discrepancy of 12 kg ha^{-1} yr^{-1} between the observed value of 163 and the calculated value of 151 is immaterial as it is less than 10%. It is an acceptable error when seen from the complexity and scale of the problem. Thus the rate of N accumulation in peat of 163 kg ha^{-1} yr^{-1} can be assumed to have come from the primary source.

Table 4 shows the influx of N via atmospheric precipitation. The dry season rains, represented by the July figure, are more concentrated than the wet season rains as depicted by the October figure. The same tendency has been reported also by Horne (1978). The July figure may represent generally the whole dry season of 2 to 3 months. If a factor of 0.1 can be applied to the October figure to approximate the concentration by the end of the wet season (Wetselaar & Hutton, 1963), while the October figure represents the concentration by the beginning of the wet season, then the average for the wet season is 0.77 mg l^{-1}. The yearly total will be 22.5 kg ha^{-1} yr^{-1}, which is expected to be temporarily stored in the pore water of peat.

Table 5. N concentration in different waters in Indonesia

	N (mg l^{-1})	Reference
Estuary waters	2.5 - 2.6	a
	1.3 - 2.5	a
	1.6 - 50.0	van Wijk (1951)
River waters	0.8 - 1.9	van Wijk (1951)
Canal waters	2.5 - 4.9	a
Ground waters	2.4 - 4.0	a
	2.3 - 31.7	van Wijk (1951)

[a] Test Farm Report, Gadjah Mada University, 1971 (unpublished); Coordinated Teams, Faculty of Geography and Agriculture, Gadjah Mada University, 1979 (unpublished Report).

N concentrations in different waters in Indonesia vary considerably (Table 5) with time of the day, season and place of sampling. The average contents of N in mg l^{-1} can be calculated: river waters 1.6, canal waters 3.7, inundating or surface stagnant waters 14.2, drainage waters 2.6 and ground waters 10.1. N is concentrated in stagnant and ground waters. Unless the volume of water involved in each case is known, the N dynamics through the tidal activities cannot be determined.

It has been shown that a biological N_2 fixation in the surface layer of peats is possible, especially by free living organisms. The population is denser in the vicinity of canals than farther away. This suggests that river water could be an important nutrient supplier in peat ecosystems (Test Farm Report Gadjah Mada University, 1972, unpublished).

There are still uncertain transfers in the N relations in a peat ecosystem, especially across the interface of peat and streams, peat and ground water, and streams and ground water. These are likely to be the most intricate facets of the entire N dynamics in peats, and they are precisely the most crucial components in controlling the stability of peat ecosystems, in particular those which occupy the interfluves of big estuaries. There are no clues yet to knowing how much of the N is actual gain, how much is real loss, and what portion is just recycled within the peat body itself. Considering the hydrometric behaviour of canal and ground waters, and the enormous capacity of peats to retain water, there is a strong probability that part of the N just shuttles between peat and water. As a properly constructed canal network is fundamental to the advancement of peat lands for agricultural settlements, the understanding of the interchange of matter across the interface of peat and water is basic.

AGRICULTURAL SIGNIFICANCE OF PEAT ECOSYSTEMS

It has been shown that the rate of peat growth declines progressively with lesser depths. When the surface of the peat becomes more elevated by growth, the higher oxidation potential will accelerate the breakdown of organic matter. Elevation of the peat surface also promotes water percolation which results in stronger leaching and eventually leads to the impoverishment of the peat as a habitat. Both activation of organic matter destruction and declining productivity will slow down the rate of peat deposition. Human involvements

are apt to speed up this degradation process. The thickness of peat correlates inversely with degree of decomposition and also with its potential fertility (Notohadiprawiro, 1975; Notohadiprawiro, *et al.*, 1979). An ultimate situation can therefore be envisaged, in which production balances destruction. This should be the ecological maturity of peat ecosystems.

From the point of view of land development, however, this same situation denotes metastability of the most delicate kind. A slight increase in degradation, or a slight decrease in productivity, will result in the collapse of the whole peat ecosystem. The very factors that cause the extremely high productivity of tropical terrestrial ecosystems are also agents that make these ecosystems highly vulnerable. These factors, high rainfall and high temperatures, both accelerate the processes of weathering and decomposition.

A natural peat ecosystem acts like a buffer against the detrimental effects of the tropical environment. It stores nutrients and protects them from harmful leaching, chemical fixation, and erosion. When organic litter is undergoing partial decomposition to form peat, its chemical constituents become more concentrated as the breakdown of the organic tissues progresses. The ash content also increases (Brotonegoro & Abdulkadir, 1978). It may be said that organic matter in general, and peat in particular, contain the extract of mineral substances drawn by plants from the weathering zone of the lithosphere. Peat is a huge storage bin of plant nutrients, especially N, P and K, accumulated by thousands of years of uninterrupted biological activities.

There are two opposing forces operating in the agricultural utilization of peats: (1) the provision of an adequate drainage for optimum crop yields, and (2) the maintenance of as high a water table as practicable to prolong the life of the peat soil. The dissecting canals enhance drainage and leaching. But the same canals will facilitate the supply of plant nutrients through better quality river water during flood-tides. Drainage will also encourage organic matter decomposition and mineralization, leading to (1) the formation of physically more advantageous humus for plant growth, and (2) a higher availability of N and other plant nutrients.

Observations made in Kalimantan revealed a 3- to 4-fold increase in microbial population in surface peat following peat cultivation (Test Farm Report Gadjah Mada University, 1972, unpublished). N_2 fixation is likely to be activated too. Thus the import of N from the atmosphere and streams is augmented. It is true that peat growth comes to a halt. But this does not necessarily mean that N accumulation also stops.

The appearance of the ecosystem and the functional inter-relationships between the ecotopes are changed. An ecotope is the basic component of an ecosystem (Klink, 1974). From the point of view of peat as peat, these changes are truly called disruption. But on the account of peat as "soil", the phenomena should be called "development" or "maturation". Organic debris or raw peat is unsuitable for agriculture, but well decomposed peats are rated as the best soils in the world, provided that water control is effective and the fertility management is well designed, including the application of trace elements (Cu, Zn, Mo).

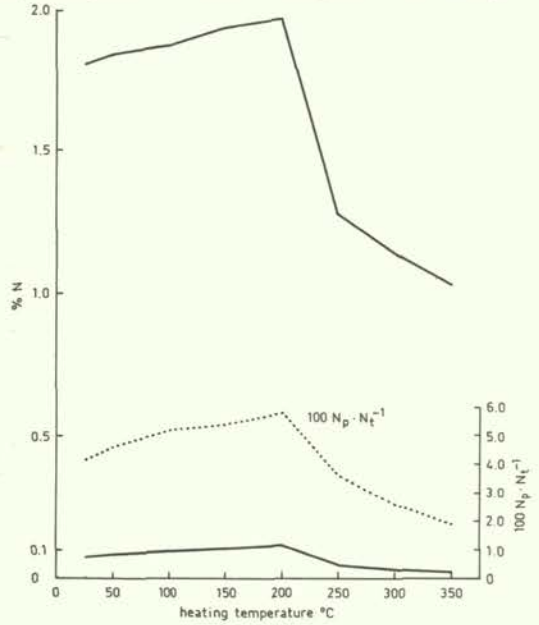

Fig. 3. The effect of heat on the N content of peat under laboratory conditions.
Top solid line = Nt (% total peat N), bottom solid line = Np (% potentially available peat N), (see also Table 7).

One way to release N quickly from its organic structures in peat, or making it more transformable into simpler compounds, is by applying heat. Besides increasing the availability of N, this measure also increases the availability of the other nutrients, notably P and K, and corrects pH. But excessive heat brings about great losses in total as well as in available N, in addition to deteriorating the moisture characteristics of peat. The peat becomes distinctly hydrophobic.

Andyantoro (1978) treated peat with heat in the laboratory for 2 hours, after which it was incubated for 30 days at ambient temperature and at saturated moisture content to determine the rate of N mineralization or "potential N" (Prasad, 1956). Heating up to 200 C steadily increased the total as well as the potentially available N (Fig. 3). Above this temperature both forms of N decreased abruptly until the contents reached much lower values than the untreated samples. The increase in total N with heating below 200 C may be only an apparent one being caused by the decrease in total weight of peat. Heating causes (1) the release of hygroscopic and, later on also, chemically bound water, and (2) the partial charring of the peat material. At these temperatures the degree of N volatilization, if any at all is less than that corresponding to the decrease in the total weight of the peat.

Table 6. Amount of N removed by rice and maize grown on peat and the volume of peat required to supply that amount of N, assuming a 40% recovery in the plant tops of N made available in the peat

	Rice	Maize
Length of cropping season (d)	120	90
Yield (t ha^{-1})		
grain	1.5	0.6
straw	1.5	1.0
total	3.0	1.6
N recovered (kg ha^{-1})		
grain	26	11
straw	9	8
total	35	19
Required volume of peat[a]		
untreated (m^3)	31.3	16.7
(cm ha^{-1})	0.31	0.17
optimum treatment (200 C) (m^3)	20.8	12.5
(cm ha^{-1})	0.21	0.13

[a] Bulk density of peat 0.15 g cm^{-3}.

Table 6 shows the representative yields of rice and maize grown on recently developed peat areas of Indonesia. The N removed by the crops is approximated, using the N content in the plant tissues given by Sanchez (1976), International Potash Institute (1974), and Sankaram (1970) for the respective crops grown in tropical and subtropical regions. On the assumption that all N can be transformed into its available form in due time, the untreated peat can supply enough N for 24 rice crops before its reserve, equivalent to a surface layer of 7.5 cm, gets exhausted. Twenty four maize crops can be supported by a 4 cm surface layer. By preheating the peat at 200 C, a thinner layer of peat will suffice. For rice the equivalent layer is reduced to 67% and for maize it is 75%.

The above calculations are based on a conservative approach, as only the native N was taken into account and the remaining 60% N was assumed not retrievable due to leaching. A restricted leaching by keeping the ground water level at a shallow depth is likely to extend greatly the useful life of the rooting layer of peat. N inputs from rainfall, biological fixation and river water intrusion will obviously increase the life expectancy of peat even further, as also will the returning of straw of stubble into the peat soil.

In the field, controlled burning has been shown to be practical and effective in raising the availability of N in peat (Table 7). By keeping the ground water level close enough to the surface (some 40 cm will be adequate) with ditches laid out all around each land parcel (average size of parcels 0.1 ha), the fire could be reasonably confined. The shallow ground water acts like a barrier against the downward advancement of fire. The water in the ditches serves a dual purpose: as a depth regulator of the ground water table and as a corridor against the lateral spread of fire.

Table 7. Total and potentially available N content of peat during the growth of a crop of maize, when the peat was locally preheated before sowing (Andyantoro, 1978)

Treatment and location	Np[a] (%)	Nt[b] (%)	100 Np Nt^{-1}
Before preheating	0.06	0.98	6.1
At border between preheated and original	0.11	1.07	10.3
Preheated:			
in rhizosphere	0.13	1.16	11.2
attached to roots	0.05	0.64	7.8

[a] Potentially available peat-N.

[b] Total peat-N.

A field scale demonstration on thick peat of over 4 m at Pinang Luar, West Kalimantan, has shown that without preburning no crop could be grown. But with fire treatment a corn crop can be established yielding between 200 and 600 kg ha^{-1} of grain (Test Farm Report Gadjah Mada University, unpublished). It has been said previously that the thicker the peat the less fertile the surface layer is. Although the yield is still poor, it is nevertheless a promising start. Never before, at least in Indonesia, has agriculture been possible on a very unripe peat right after land clearing.

CONCLUDING REMARKS

Many facets of the dynamics of elements in peat ecosystems are still obscure. This is particularly so with the ever increasing involvement of Man. The transitional phases from natural to artifactual condition are critical to the other phases to come. Since the intrinsic balance of such a unique ecosystem as peat is very delicate, a well planned monitoring scheme on a long term basis is imperative. For instance, a rapid release of mineral N from peat, although beneficial for agriculture, may cause harmful eutrophication of neighbouring waters, such as lakes, rivers and canals. N becomes also more subjected to leaching and loss by denitrification.

It has been implicitly expressed that the N problem in peat ecosystems is different from those of other tropical ecosystems. In peat ecosystems it is more a matter of a controlled activation of the N cycle rather than correcting gross deficiencies.

The most decisive factor of peat ecology is hydrology. The problem of hydrology gets much more complicated in estuary flats. In estuaries there are a multitude of flows. Beside the landward and seaward flows, there are the up and down movements, the lateral flow, and a variety of combinations of the different flows and movements. The nature and behaviour of ground water are so much more important in peat ecosystems than in other ecosystems.

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NITROGEN FIXATION IN ROOT NODULES OF *NEPTUNIA OLERACEA* LOUR. IN WATER CULTURE

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ABSTRACT

Neptunia oleracea Lour. is a well known legume species grown in wet or aquatic habitats in south-east Asia and is used as a vegetable by many people. Selection for nitrogen fixation ability was made among the Rhizobium *strains isolated from root nodules of this plant. One of the effective strains isolated supported growth of the experimental plant as good as the control supplied with calcium nitrate in pot experiments. Many farmers in Thailand cultivate* N. oleracea *and practise chemical fertilization in the culturing ponds or flooded fields. The fertilizer (usually urea), which is applied once every five days, amounts to about 7600 kg N* ha^{-1} yr^{-1}, *while the nitrogen removal in terms of the harvested young shoots is about 368 kg N* ha^{-1} yr^{-1}, *an extremely low apparent N recovery (4.8%). Field inoculation of the crop with an effective nodule bacterium, if successful, would save fertilizer cost and reduce the pollution effects of excess nitrogen.*

INTRODUCTION

Neptunia oleracea Lour., a species of legume herb, is found in wet or aquatic habitats in many countries of south-east Asia (Baeker *et al.*, 1963; Ridley, 1922). The plant is usually described as a perennial aquatic herb. In natural growth, root nodules of the nitrogen fixing type are occasionally found. Details on the bacterium of the root nodules and nodulation of this plant were described by Schaede (1940). The succulent young tops or shoots, the length of which depends on the rapidity of growth, are used as vegetables. In Thailand, chemical fertilization is practised by many farmers and vegetable growers culturing this crop in ponds or flooded fields, the depth of water being 0.5-1.0 m.

This paper describes some of the attempts to isolate nitrogen fixing bacteria from root nodules of *N. oleracea*, and to select effective nitrogen fixing strains. It also describes some observations on the agricultural practices of Thai farmers in raising this crop, with emphasis on the nitrogen input and output of this culture system.

MATERIALS AND METHODS

N. oleracea root nodules were collected from natural growth in various places, and pure cultures of the nodule bacteria (*Rhizobium* sp.) were isolated using mannitol-yeast extract-congo red agar medium (Skerman, 1967).

Because the vegetative method of propagation of *N. oleracea* was employed, clean cuttings were prepared for use as planting material in the experiments testing the effectiveness of the bacterial strains isolated. Top cuttings of about 30 cm length, selected from the fields, were planted in small concrete containers, about 1.20 m in diameter and 50 cm high, filled with a 15 cm layer of tap water on top of a 20 cm layer of clean sand. Care was taken that the base of each cutting was buried in the sand to promote rapid growth. The containers were placed in the nursery with access to sunlight. The top ends of the new growing shoots were mechanically supported so that they remained above the water surface. The new shoots, with two leaves and new roots just appearing, were cut and used as planting material for the subsequent inoculation experiments. These selected cuttings were washed twice in 10% sodium hypochlorite solution and then twice in sterile water. Each cutting was placed in a sterile planting glass jar (wide mouth, cylindrical, 6 cm diameter, 15 cm height) containing washed sand (6 cm deep) and 250 ml of nutrient solution (Sloger, 1966). The base of the cutting was pushed under the sand by using a glass rod. The mouth of the jar was covered with aluminium foil so that contamination and excessive loss of moisture were avoided. After two weeks, each of the planted jars was inoculated with a bacterial strain isolated as described. Inoculation was accomplished by adding 50 ml of a water suspension of cells grown in mannitol yeast extract broth. The concentration was adjusted so that the 250 ml planting solution in each jar contained 10^9 cells ml^{-1}. Control jars were included without bacterial inoculation,

some without bacterial inoculation but with addition of 100 mg N l^{-1} in the form of calcium nitrate. Forty five days after inoculation the plants were harvested, and the number and weight of the nodules, and the weight and length of the plants, were determined.

RESULTS, DISCUSSIONS AND CONCLUSIONS

Some of the results are shown in Table 1. The first six strains in the Table are the best of the total of the fifty-nine strains isolated from natural growth. The best strain of those could promote vegetative growth comparable to, if not better than, the control supplied with the nitrogen fertilizer under the experimental conditions.

Table 1. A selection of the data obtained, from tests of 59 bacterial isolates from root nodules of N. oleracea, *in washed sand culture*

Isolates	No. of nodules per plant	Dry wt. of nodules (g)	Dry wt. of whole plant (g)	Crown length of plant (cm)
D3	61	15.76	3.33	81.4
A13	109	25.75	3.21	93.8
A11	107	23.69	3.09	78.3
A18	164	56.07	2.90	77.6
E1	46	7.29	2.74	76.4
A10	64	9.77	2.63	68.7
B24	79	20.51	2.34	64.4
F1	29	2.66	1.73	48.8
E2	18	1.02	1.38	53.5
F2	3	0.03	1.25	46.3
B25	20	0.75	1.05	46.3
A31	11	0.12	0.75	39.1
D5	11	0.22	0.50	34.2
A16	3	0.03	0.46	33.4
C10	6	0.04	0.34	30.8
A6	0	0	0.41	26.8
control[1]	0	0	0.38	20.2
control[2]	0	0	3.97	92.0

[1] Without bacterial inoculation.

[2] Without bacterial inoculation but with 100 mg N l^{-1} in the form of $Ca(NO_3)_2$.

N. oleracea has become a cultivated crop in Thailand and many growers apply fertilizers rather heavily and at frequent intervals. Fertilizer is applied by spreading it onto the body of water at the rate of 144 kg N ha^{-1} (usually as urea) every five days during each growing period of three months. At the end of the period the field is cleaned to be ready for the next crop. A farmer usually manages three growing periods a year. He starts harvesting young shoots two weeks after planting and every week thereafter until the end of that growing period. About 31 250 shoots are cut, weighing 1140 kg ha^{-1} per harvest. With a dry matter content of 22.8% and a total N content of the dry material of 4.56%, about 12 kg N ha^{-1} is removed with each harvest.

The total fertilizer N input per annum is 7600 kg ha^{-1}, while only 368 kg N ha^{-1} yr^{-1} is removed by harvesting, giving an apparent fertilizer N recovery of 4.8%. This recovery is exceptionally low by normal agronomic standards and suggests a very low efficiency of nitrogen fertilizer usage.

Some of the unrecovered N might have been used by other aquatic organisms and some of it might have been lost due to denitrification or ammonia volatilization.

A more efficient and less costly system might be the stimulation of symbiotic nitrogen fixation. Our results indicate that this might be possible, but their application to the field situation needs to be studied.

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LOSS OF NITROGEN FROM DECOMPOSING NODULES AND ROOTS OF THE TROPICAL LEGUME *CENTROSEMA PUBESCENS* TO SOIL

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ABSTRACT

Samples (20 g) of an air-dry, sieved (< 2mm) silty loam with or without (controls) the incorporation of 200 mg root portions of Centrosema pubescens, *were moistened to 50% moisture holding capacity at 0.33 bar (33 kPa). The root portions were healthy, freshly harvested, clean samples of nodules, laterals or radicles; treatments* Nod, Lat *and* Rad *respectively. Soil samples were incubated aerobically at 30 C for 16 weeks. The radicles, laterals and nodules respectively contained 1.65, 3.46 and 6.91 mg N per sample and their C to N ratios were 16.5, 7.0 and 4.2.*

The root fragments disappeared rapidly and less than 40% and 10% of their initial dry weight remained after 1 and 16 weeks respectively; nodules decomposed more rapidly than laterals and laterals more quickly than radicles. At 16 weeks the total N contents of the controls had not changed but Rad, Lat *and* Nod *soils respectively showed gains over the controls from week 1 onwards and by week 16, these amounted to 8.2, 17.1 and 31.8%. Concentrations of mineral N increased in all soils from week 4 onwards and by week 16, the amounts of mineral N that had accumulated in* Nod, Lat *and* Rad *were 1.62, 1.16 and 1.14 times greater than in the controls (201 μ N g^{-1} oven-dry soil).*

Under the conditions of this experiment, the legume root and nodule tissues decomposed rapidly and all of the legume root N (except an unaccounted for 5.6% of the nodule N) was transferred to the soil without any apparent net loss or gain of volatile N.

INTRODUCTION

When legumes decompose, much of the symbiotically-fixed nitrogen contained in their tissues becomes transferred to the soil by the normal process of mineralization. For a variety of reasons legume roots and root nodules can die, become diseased, decay or slough off during the life of the plant. But knowledge of the biology of nodule decomposition and the rate of mineralization of the nitrogen contained in decomposing nodules is lacking. Indeed, Waid (1974) commented that there is a need for information on when, where, how and in what form nitrogen is released from decomposing roots and nodules of legumes.

Our purpose was to study the rates of decomposition of radicle roots, lateral roots, and root nodules of the leguminous cover crop *Centrosema pubescens* Benth. and to measure the accumulation of legume-derived nitrogen in the soil in which the decomposing roots and nodules had been incorporated.

MATERIALS AND METHODS

Roots and nodules

Centrosema pubescens, grown from seed inoculated with an effective strain of *Rhizobium* (RRIM 968), was grown in a weeded plot on Serdang Series soil at the Universiti Pertanian. When 3 months old, 600 plants were harvested by carefully removing their root systems from soil. Root systems with well developed radicles and laterals were selected, and from them the following healthy root portions were removed, washed free of soil and rinsed in sterile water.

Nodules. Yellow-white nodules, presumed to be effective (see Broughton *et al.*, 1978), 2 to 3 mm diam., containing 4.7% N and a C to N ratio of 4.2.

Laterals. Pale yellow-brown laterals, 1 to 1.5 mm diam., 2.7% N, C/N 7.0. They were trimmed of root hairs and cut into segments from 2 to 5 cm long.

Radicles. Light-brown radicles, 2 to 3 mm diam., 1.3% N, C/N 16.5, were cut into segments from 2 to 5 cm long.

Soil. The Serdang Series soil used is a loamy siliceous isohyperthermic Typic Paleudult, pH in water 4.9, clay 24%, silt 3%, total N 0.101%, C to N ratio 12.3 (Paramanathan, 1978). Larger mineral and organic particles were removed from a soil sample which was then air-dried, sieved (< 2mm) and 20 g samples were weighed into McCartney bottles. Each sample contained 129 μg NH_4^+- and 10.5 μg NO_3^--N g^{-1} oven-dry soil.

Conditions of Incubation

There were four soil treatments: no addition (control), radicles, laterals, or nodules. The fresh plant tissues were mixed into soil at the rate of 200 mg per 20 g soil sample; radicles, laterals and nodules supplying 1.65, 3.46 and 6.91 mg N per sample respectively. All soil samples were then moistened and maintained at 50% moisture holding capacity (m.h.c. determined at a water tension of 33 kPa = 0.33 bar) by the addition of 3 ml sterile water which distributed itself evenly through capillary movement. The bottles were loosely capped to allow aeration and were incubated in the dark at 30 C. Moisture losses (*c* 1 ml wk^{-1}) were determined weekly by weighing, and replaced.

Sampling and Chemical analyses

At predetermined times 12 bottles for each treatment were removed from the incubator. Legume tissues remaining in the sample tubes were removed by sieving (> 2mm) or manually, using fine forceps, brushed free of soil with a fine camel-hair brush, oven-dried and weighed.

The following measurements were made on the soil samples: oven-dry weight (50 C overnight); total N (micro-Kjeldahl) and NH_4^+-N both on an Autoanalyser II (Technicon 1971, Industrial Method 218 - 72A); NO_3^--N (Myers & Paul, 1968); pH in a 1:5 soil-water slurry. Each measurement was based on at least three replicates.

Ammonia volatilization from soil containing roots or nodules was measured by two standard methods but the amounts of NH_3 released were < 60 ng N g^{-1} oven-dry soil after 8 weeks' incubation of nodule tissue. Consequently NH_3 losses were not measured.

Nitrite was not detected in any of the soil samples.

Table 1. Disappearance of Centrosema *root and nodule tissue in soil*

Week	1	2	4	8	12	16
	(% of initial oven-dry weight)					
Radicles	35.4a*	26.0a	20.5a	15.0a	9.8a	7.9a
Laterals	25.8b	19.5b	17.2b	10.2b	8.6b	6.8b
Nodules	18.4c	15.0c	8.2c	6.8c	4.8c	3.4c
Significance (P)	<0.001	<0.001	<0.01	<0.001	<0.001	<0.001

* Values for any particular week that differ significantly are followed by a different letter.

RESULTS

Disappearance of legume roots and nodules

The bulk of the *Centrosema* tissues disappeared during the first week of incubation with the loss of 64, 74 and 82% of the radicle, lateral and nodule tissues respectively (Table 1). Thereafter, the rate of loss was less rapid, but at each sampling time there were more radicle residues than lateral, and more lateral than nodule residues.

Transfer of nitrogen from legume roots and nodules to soil

Nitrogen accumulated in the soils containing decomposing portions of legume roots and nodules throughout the 16 weeks of the experiment, except during the first week of radicle decay (Table 2). By week 16 there was an apparent complete net transfer of N from the portions of radicles and laterals that had decayed and a 94.4% net transfer of nodule N to soil. But the pattern of N transfer was not directly related to the pattern of disappearance of the root and nodule fragments. There were delays of 4 weeks in soil in which radicle or lateral root fragments were decomposing before concentrations of total N increased significantly. Such delays could have been caused by decomposer organisms immobilizing N in the decaying residues. Where nodules were decomposing, N was transferred to soil in significant amounts throughout the experiment. Even so, the bulk of the N had not been transferred until week 8 (Table 2).

Table 2. Changes in the total and inorganic nitrogen contents of soil in which Centrosema *tissues decomposed (initial concentrations were 1010 µg total N, 129 µg NH_4^+-N and 10.5 µg NO_3^--N g^{-1} oven-dry soil)*

Week	1	2	4	8	12	16	16* (% recovery)
			(µg total N g^{-1} oven-dry soil)				
Control	- 10a**	- 10a	- 10a	0a	- 3a	0a	-
Radicles	- 10a	- 23a	+ 73ab	+ 80b	+ 76b	+ 83b	100.6
Laterals	+ 15b	+ 40a	+ 73ab	+ 90b	+ 96b	+173c	100.0
Nodules	+107c	+120b	+222b	+293c	+300c	+315d	91.2
Significance (P)	<0.001	<0.1	<0.1	<0.001	<0.001	<0.001	
			(µg NH_4^+-N g^{-1} oven-dry soil)				(% recovery)
Controls	- 9	- 7	+ 71a	+121a	+159a	+171a	-
Radicles	- 6	- 4	+121b	+146b	+184b	+196b	29.4
Laterals	0	- 4	+121b	+134ab	+171ab	+196b	14.5
Nodules	- 9	+ 19	+184c	+221c	+259c	+271c	28.9
Significance (P)	NS	NS	<0.001	<0.001	<0.001	<0.001	
			(µg NO_3^--N g^{-1} oven-dry soil)				(% recovery)
Control	- 4.7	- 7.2a	- 6.4	- 2.5a	+ 4.3a	+30.1a	-
Radicles	- 4.6	- 7.1ab	- 6.5	- 2.1a	+ 4.0a	+32.8b	2.1
Laterals	- 5.7	- 6.8b	- 6.0	- 0.5b	+ 7.9b	+37.8c	4.5
Nodules	- 4.6	- 4.7c	- 6.2	+ 2.0c	+14.5c	+55.0d	7.2
Significance (P)	NS	<0.001	NS	<0.001	<0.001	<0.001	

* Apparent percentage recovery in the soil of the applied tissue-N at 16 weeks allowing for control values; radicles, laterals and nodules, if fully decomposed would supply the equivalent of 82.5, 173.0 and 345.5 µg N g^{-1} oven-dry soil.

** Values for any particular week that differ significantly are followed by a different letter.

Changes in inorganic nitrogen

In the control soils, concentrations of NH_4^+-N fell during the first two weeks but thereafter they rose and NH_4^+ accumulated. NO_3^--N concentrations also fell and did not increase until week 8 when nitrate began to accumulate (Table 2). These results suggest that organic residues in the soil began to decompose following remoistening and as a result inorganic-N was immobilized but later organic N was mineralized with perhaps some breakdown of native soil organic matter. Changes of total soil organic carbon (which fell from 1.23 to 1.08%) and C to N ratios (12.2 to 10.7) by the end of the experiment support this interpretation.

Similar patterns of change of concentrations of inorganic N were measured in the soils containing legume roots or nodule residues (Table 2), but significantly more inorganic N accumulated than in soil alone. Soils containing nodule residues accumulated significantly more NH_4^+-N and NO_3^--N than the soils containing root residues.

DISCUSSION

Under the conditions of this experiment with optimum soil moisture and initially high soil mineral N contents, the legume root and nodule tissues decomposed rapidly, probably more rapidly than in the field where conditions such as those imposed in this experiment are very unlikely to prevail immediately following the harvesting of a legume crop. Decay was so rapid that by 16 weeks an amount of N equivalent to all of the N originally present in the decayed fractions of the legume radicles and laterals had accumulated in the soil. Most of the N of the decayed nodules was transferred; the unaccounted portion (5.6%) may represent experimental or analytical errors, or loss of volatile NH_3-N or a denitrification loss. But the soil pH, which did not exceed 5.8 in any treatment throughout the experiment, was probably too acid to permit NH_3 volatilization, and the moisture regime, which should have permitted good soil aeration, would not have favoured denitrification.

If the organic N that became mineralized in the control soils (N derived from breakdown

of organic residues and native soil organic matter) is allowed for, then not all of the inorganic N that had accumulated by week 16 in the soils treated with legume residues would have been derived from legume roots or nodules. But it is not known if in the presence of fresh legume residues the soil organic N would decompose at the same rates, or release the same amounts of inorganic N as the controls. Nor is it known if in the presence of the decaying roots and nodules there was fixation of nitrogen by free living nitrogen-fixing bacteria. Further studies are needed to elucidate these problems of interpretation of what is at first sight a simple experimental system.

ACKNOWLEDGEMENTS

Azizah Chulan thanks the Australian Development and Assistance Bureau and the Universiti Pertanian Malaysia for their support of her studies in Australia and Malaysia respectively. John Waid thanks the Australian Research Grants Committee for financial support and the Universiti Pertanian for hospitality and facilities.

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THE ROLE OF *CASUARINA* UNDER SHIFTING CULTIVATION - A PRELIMINARY STUDY

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ABSTRACT

Casuarina trees are planted in food gardens as a fallow crop or as a shade tree for coffee in the highlands of Papua New Guinea. Preliminary studies suggest that soil N content increases with age of Casuarina. *The N content under* Casuarina *was higher than that under* Albizzia *or* Crotalaria. *Thereafter, soil N declined with annual cropping. The planting of* Casuarina *is therefore suggested as a means of maintaining a high soil nitrogen status.*

INTRODUCTION

Some 60-80% of the population of Papua New Guinea depends on subsistence agriculture. Shifting cultivation has been practised for thousands of years. In this system, the land is cleared and cropped for 2-3 years with sweet potato; the soil is then allowed to rest for a number of years under bush fallow to restore soil fertility. In the highlands of Papua New Guinea, *Casuarina* trees are planted in subsistence food gardens during the second or third cropping cycle (Barrie, 1956; Parfitt, 1976). Once the yields decline, the land is allowed to remain under *Casuarina* for 10-20 years before it is cleared for replanting of sweet potato.

Casuarina has also been used as a shade tree along with legumes, such as *Leucaena* sp, *Albizzia stipulata* and *Crotalaria*, in commercial and smallholder plantations. Approximately 70% of the coffee in Papua New Guinea is produced by 205 000 small-holders or village producers (Munnul & Densley, 1977). In most cases, coffee is planted under shade and, except in commercial plantations, without any fertilizer. *Casuarina* trees act as a windbreak and as a source of timber for fencing the gardens.

Several species of *Casuarina* are found in Papua New Guinea. *Casuarina oligdon* is the most common species in the subsistence gardens (Paijmans, 1976). Many species were found to have nodules, the greatest number of these occurred where the soil pH was neutral (Bond, 1957). It is reported that some *Casuarina* species fix about 60 kg N ha^{-1} yr^{-1} (Dommergues, 1966), but it is not known whether *Casuarina oligdon* is capable of fixing nitrogen from the atmosphere.

EXPERIMENTAL

Soil samples were collected from various locations in the Highlands of Papua New Guinea. Groups of cores, usually four or five, were taken to 15-cm depth at each location and bulked for analysis. Carbon was determined by the Walkley-Black method and total nitrogen by a Kjeldahl digestion.

Table 1. Soil N and C under old and young Casuarina *in coffee plantations*

Site	%C	%N
Old *Casuarina* (with litter)	9.0	0.73
Old *Casuarina*	6.1	0.56
Young *Casuarina*	2.5	0.31
Young coffee	2.8	0.28

Soil nitrogen under old and young Casuarina

Soil samples were collected from old and young *Casuarina* trees in coffee plantations. The results are presented in Table 1. The soil nitrogen was high under old *Casuarina* trees. This high N content was associated with a high organic matter content, probably due to litter fall.

Parfitt (1976) determined the N content of soil samples collected under *Casuarina* trees of different ages among food gardens in different locations. His results showed that soil nitrogen gradually accumulates under *Casuarina*, increasing at 0.015-0.018% yr^{-1}. Assuming a bulk density of 1.4 and a soil layer of 15 cm, this would represent an annual increase of 315-378 kg N ha^{-1}. Except for this study there are no quantitative data available on N accumulation or N_2 fixation by *Casuarina* in shifting cultivation or under coffee plantations. When the amount of nitrogen accumulated during a certain period is known, it might be possible for farmers to shorten the fallow period, giving them more land for cropping.

Table 2. Nitrogen and carbon contents of surface soils under different shade trees

Tree Species	%C	%N
Albizzia stipulata	2.6	0.25
Crotalaria	2.3	0.21
*Casuarina**	5.7	0.58
*Casuarina***	3.9	0.43

* Number of *Casuarina* trees ha^{-1} high.
** Number of *Casuarina* trees ha^{-1} low.

Nitrogen status under different shade trees in unfertilized coffee

Surface soil samples were collected in the Highlands under two leguminous shade trees and *Casuarina* trees. The soils were analysed for carbon and nitrogen and the results are presented in Table 2.

Under *Casuarina* the N and C content was twice as high as under *Albizzia* and *Crotalaria*, suggesting that a high soil N content can be maintained under *Casuarina*. It also suggests that *Casuarina* leaves may have a high N content through high N_2 fixation. A detailed comparitive study on the mineralization of organic matter produced under these trees is necessary to evaluate the usefulness of the shade trees under shifting cultivation or smallholder coffee plantations.

DISCUSSION AND CONCLUSIONS

The increase in population in the Highlands of Papua New Guinea increases the demand for land to establish subsistence food gardens. The high cost of fertilizer prohibits its recommendation at the subsistence level or smallholder level. *Casuarina*, a tree which is accepted by the people in the Highland gardens, can be used as a timber, windbreak, shade tree and apparently as a soil improver through N_2 fixation and N-release from litter.

It is evident from the preliminary data presented in this paper that *Casuarina* increases soil N more than *Albizzia* or *Crotalaria*. Thus it may not be necessary to have the fallow period for 10-20 years as practised by farmers in the Highlands. There is very little information on the value of *Casuarina* as a soil improver. It is essential therefore that the rate of nitrogen accumulation under this species should be studied in detail. Such studies should include:

1. Assessment of the interaction between species and soil and climatic factors on effective nodulation.
2. Determination of the rate of litter production in relation to species, planting density and climate.
3. Determination of the rates of nitrogen cycling at different ages of *Casuarina* in coffee crops and food gardens.
4. Comparison of *Casuarina* with legumes as covers and shade trees, with relation to soil N accumulation and soil N release.
5. Assessment of the compatibility of *Casuarina* with other crops that could be grown under subsistence agriculture.
6. Research into other uses of *Casuarina*.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the facilities provided by the Department of Agriculture, University of Papua New Guinea and the Department of Primary Industry. They also acknowledge the assistance of Mr M.R. Bourke of the Highlands Agricultural Research Station.

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ENVIRONMENTAL PROBLEMS ASSOCIATED WITH TERRESTRIAL NITROGEN TRANSFORMATIONS IN AGROSYSTEMS IN THE WET MONSOONAL TROPICS

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ABSTRACT

High soil temperatures and high soil moisture contents are significant climatic features of the wet-monsoonal tropics. Their effects on the production and transformations of NH_3, NH_4^+, NO_x, N_2O and NO_3^- in agrosystems are discussed. The evidence is that the rates of production and transformation of all these nitrogen products increase as soil temperature and soil moisture increase, and it is concluded that accumulations of nitrogenous end products in various biospheric pools are likely to be higher in the wet-monsoonal tropics than in drier or cooler climatic zones. The expected large increase in the use of nitrogen fertilizers in tropical regions is seen to be an important contributing factor.

Possible deleterious effects resulting from accumulations of nitrogen products in the biosphere are discussed, but we can only speculate on their importance in the wet-monsoonal tropics because of a general lack of knowledge of processes of the terrestrial nitrogen cycle in tropical regions. The accumulation of N_2O in the stratosphere and its possible reaction with ozone appears to be the most serious environmental problem.

INTRODUCTION

It is generally assumed that before Man interferred with natural ecosystems, they were part of a 'closed' nitrogen cycle, i.e. gains by and losses from the systems were approximately in balance. Although major, natural catastrophes such as earthquakes, landslides, and volcanic eruptions might have caused temporary disturbances, a new balance was probably restored soon afterwards. Agricultural activity by Man has led to continuous and often permanent interference with some ecosystems, with a consequent disturbance of the nitrogen balance, even to the extent that accumulation of harmful nitrogen constituents in the environment must now be considered seriously.

In the following, we discuss the likely directions and rates of disturbances due to agricultural activities in the wet-monsoonal tropics, and what their consequences might be. It is timely to consider this topic since authorities predict that the world use of nitrogen fertilizers will increase by a factor of about three in the next twenty years (e.g. Council for Agricultural Science and Technology, 1976), and a good part of this use will be in developing countries in tropical regions.

Harmful accumulations of nitrogenous products result mostly from nitrogen transformations in the soil. We discuss these first.

THE MAJOR NITROGEN TRANSFORMATIONS

The major nitrogen transformations and the directions in which they can contribute to accumulation of nitrogen compounds in the environment are depicted in Fig. 1. These transformations and the factors affecting them are described in detail by Myers *et al.** Their model assumes a hypothetical intermediate, $H_2N_2O_2$, although its presence has never been established.

Two notable characteristics of the wet-monsoonal tropics are high soil moisture contents

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* Myers, R.J.K., Simpson, J.R., Wetselaar, R. & McKinney, G.T. 1979. Problems in modelling the environmental aspects of the nitrogen cycle in agro-ecosystems. SCOPE Workshop on "Dynamic aspects of nitrogen cycling in Australian ecosystems", Aspendale, Vic., Australia.

and high soil temperatures. Our initial discussion is therefore centred around their effects on the transformations given in Fig. 1.

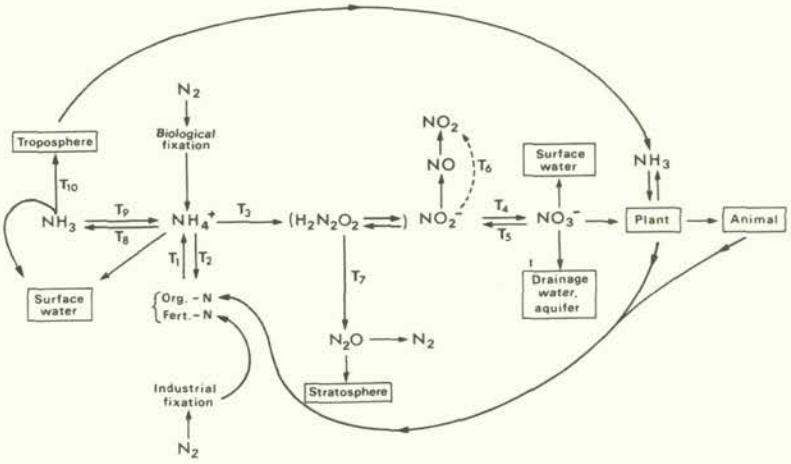

Fig. 1. The major terrestrial nitrogen transformations and pools (boxes) in which accumulation and/or detrimental effects of nitrogenous products can occur.

Effects of temperature

All the transformations appear to be highly temperature dependent; e.g. ammonification (T_1) and immobilization (T_2), increase linearly with temperature between 0 and 60 C (Myers, 1975). When organic nitrogen is applied as urea, T_1 represents the hydrolysis of that compound. This transformation is also temperature dependent, the activity increasing with temperature between 2 and 45 C (Gould *et al.*, 1973).

Transformations T_3 + T_4, which comprise nitrification, increase linearly with temperature to about 25 C (Sabey *et al.*, 1959), exhibit a marked peak at 35 C, and decrease linearly above 40 C to near zero at 60 C (Myers, 1975).

Transformations involving anaerobic N_2O formation, T_5 and T_7, are approximately linearly related to temperature between 10 and 60 C, above which they decrease sharply to zero at 85 C (Nommik, 1956). Aerobic N_2O formation (via T_3 and T_7) increases slowly with temperature up to 18 C, but rapidly above that (Freney *et al.*, 1979).

The formation of NO as a result of chemical decomposition of NO_2^-, transformation T_6, is postulated to occur only at temperatures above 25 C (Laudelout *et al.*, 1977). However, the few field observations of NO release in soils made so far show little temperature dependence for this process (Galbally & Roy, 1978, and unpublished data).

Temperature and pH affect the relative proportions of NH_4^+ and NH_3 present in the soil solution (transformations T_8 and T_9). At a given pH, the higher the temperature, the greater the proportion of NH_3 in the solution and the greater the potential for NH_3 volatilization (Vlek & Stumpe, 1978; Freney *et al.*, 1981a). Further, the partial vapour pressure of the NH_3 in solution increases exponentially with temperature (Linke, 1965) so that, as Vlek & Stumpe (1978) found, when other conditions such as pH, titratable alkalinity and buffering capacity are favourable, volatilization (T_{10}) also increases exponentially with temperature.

Effects of soil moisture

Reliable data exist for ammonification (T_1), but not for immobilization (T_2). The former transformation has a maximum at a much lower water potential (-50 bars) than nitrification (T_3 + T_4). Nitrification has a maximum at about -0.15 bars (Robinson, 1957; Wetselaar, 1968; Myers, 1974). Of course, the influence of soil water on the transformations involving nitrification is a combination of the influence of water *per se* and its influence on aeration (Myers, 1974). At very high soil water contents oxygen becomes limiting and denitrification

(T_5 + T_7) dominates. Aerobic N_2O formation (T_3 + T_7) appears to increase with increasing soil moisture content up to field capacity (Freney *et al.*, 1979).

For NH_3 to be formed from NH_4^+ in the soil (transformation T_8), NH_4^+ must be present in the soil solution rather than be absorbed on the exchange complex. The higher the soil water content, the more the equilibrium $[NH_4^+]_{ads} \rightleftarrows [NH_4^+]_{sol}$ will shift to the right (Wiklander, 1965), permitting the formation of more NH_3 in the soil solution, provided other conditions, mentioned earlier, are appropriate.

To sum up, the rates of production of NH_3, NH_4^+, NO, N_2O or NO_3^- tend to increase with higher soil temperatures and higher soil moisture contents, and it can therefore be expected that in general, agricultural activities will lead to higher accumulations of nitrogenous end products in the wet tropics than in drier or cooler climatic zones.

Effects of agricultural practices

During periods of active crop growth and in most natural ecosystems, plants take up NH_4^+ and/or NO_3^- from the soil at about the rate at which they are formed, so that soil accumulations are small. However, in some agricultural systems, particularly those involving annual cropping, periods exist during which plants are absent or their rate of nitrogen uptake is lower than the rates of formation or addition of NH_4^+ and NO_3^-. Extreme examples are periods of bare fallowing when mineral nitrogen may accumulate from the breakdown of some of the organic nitrogen in the soil through ammonification (T_1). Under most circumstances, nitrification (T_3 + T_4) proceeds more rapidly than ammonification (T_1) (Myers, 1975), so that nitrate tends to accumulate in the soil. Wetselaar (1967), for instance, found that after four years of bare fallowing, the nitrate accumulation in the top 2 m of the soil profile was as high as 370 kg N ha^{-1}.

When fertilizers are applied at sowing, the nitrogen uptake rate by the developing seedlings is extremely low for several weeks. If a nitrate fertilizer is applied, leaching of nitrate towards the subsoil can occur, or, with very high soil moisture contents, denitrification might prevail. If any ammonium fertilizer is applied, losses by volatilization can occur *via* transformations T_8 and T_{10}. The ammonium may also be nitrified to nitrate at a rate higher than the seedlings can take it up, leading again to losses by leaching or denitrification. This is particularly likely in the wet tropics. For these reasons, there is a need to develop fertilizer types and methods of placement more appropriate for tropical conditions.

THE FATE OF ACCUMULATED NITROGEN PRODUCTS AND THEIR EFFECTS ON THE ENVIRONMENT

Organic-, ammonium-, and fertilizer-nitrogen

In catchments, run-off of water occurs when the rate of precipitation is higher than the average rate of infiltration of the water into the soil. The run-off water can dissolve plant nutrients such as inorganic nitrogen compounds, and can transport with it suspended particles containing organic nitrogen fractions and clay particles onto which ammonium is absorbed.

In most natural ecosystems, nitrogen losses by run-off are usually small, because the continuously present plant cover permits little accumulation of soluble nitrogen constituents, and the plants, litter, and other obstructions on the soil surface filter out suspended materials. These filtering mechanisms are absent in ploughed land and consequently runoff losses, including those from surface-applied nitrogen fertilizer, can be high, particularly on steep slopes.

When run-off water with high nitrogen content enters streams or lakes it may stimulate algal blooms. Their eventual decomposition may cause a depletion in dissolved oxygen, which can lead to death of fish (Klein, 1972).

Ammonia

Under certain conditions, described earlier, ammonia can be emitted into the atmosphere. Substantial emissions from paddy fields have been reported (Craswell & Vlek, 1978), and these can account for up to 60% of the fertilizer-nitrogen applied, although much lower losses seem to be more general (Wetselaar *et al.*, 1977; Freney *et al.*, 1981b). The residence time of ammonia in the troposphere is only a matter of days as it is leached out by rain or diffuses back to the ground surface (Wetselaar & Hutton, 1963; Eriksson, 1966; Denmead *et al.*, 1978) where it can be readily absorbed by soils (Malo & Purvis, 1964; Hanawalt, 1969), plants (Hutchinson *et al.*, 1972; Denmead *et al.*, 1976; Farquhar *et al.*, 1980) and water bodies (Hutchinson & Viets, 1969). In the last case eutrophication may result. Denmead *et al.* (1978), working in Canada, found that the accruals of nitrogen to a corn crop by absorption

of atmospheric ammonia were relatively small. However, in the tropics where ambient ammonia concentrations appear to be higher than elsewhere (Lodge *et al.*, 1974), gains of nitrogen in this way may be more important.

When ammonia has dissolved in rainwater in the presence of SO_2 or other gases, aerosols such as ammonium sulphate and ammonium nitrate can be formed, but the mechanisms are not completely understood (Ayers *et al.*, 1979). Ammonia can also be destroyed in the troposphere by its reaction with hydroxyl radicals to form NO.

The aerosols are eventually removed by precipitation or particulate deposition, but while in the atmosphere they may have a significant effect on the climate; they affect the earth's radiation balance (by intercepting incoming solar radiation and outgoing terrestrial radiation) and they affect cloud-forming and precipitation processes (by acting as cloud condensation nuclei), which may in turn also affect the radiation balance (Twomey, 1974; Twomey & Wojciechowski, 1969). It should be said that the relative importance of any of these removal mechanisms in the wet tropics is not yet known, although it is likely that sulphate aerosols will be of less significance in the tropics than in the more industrialized temperate regions of the world where large amounts of sulphur are emitted to the atmosphere from combustion processes.

Nitrate

Nitrate is very mobile in the soil. Run-off losses may lead to eutrophication of water bodies, as described previously. Excessive rainfall or irrigation can leach nitrate to the sub-soil (Wetselaar, 1962) and transfer it to aquifers.

A health hazard can arise if aquifer water high in nitrates is used for drinking purposes. First, an excessive nitrate intake can cause the disease methemoglobinemia in infants under 6 months of age (Maynard *et al.*, 1976) or in Livestock (Lorenz, 1978). Second, it is believed that high nitrate intake may lead to the formation of carcinogenic nitrosamines in the stomach and intestines of humans (Wolff & Wasserman, 1972), although the evidence is not yet conclusive. The U.S. Public Health Service and the World Health Organization have set a concentration of 10 mg NO_3^--N l^{-1} as the safe limit for drinking water.

A high nitrate intake, with hazards similar to those described above, can also occur by ingestion of vegetables, fruits, and forages. Nitrate can accumulate in different plant parts when the uptake of the nitrate ion is in excess of its reduction and subsequent assimilation within the plant. This may be the case when high amounts of fertilizer-nitrogen are applied to crops. Fresh and processed vegetables have been cited most often as the major source of dietary nitrate intake. The fatal adult dose in humans is of the order 18-68 mg NO_3^--N per kg body weight and for animals 70-140 (Burden, 1961). It is generally agreed, however, that, except for infants below 3 months of age, the levels of nitrate currently found in food pose no major health hazard, provided sanitary preparation, proper refrigeration, and timely consumption are observed (Maynard *et al.*, 1976; Lorenz, 1978).

Other detrimental side effects of high nitrate contents in plant material are (1) the possible formation of nitrous oxides when the plant material is stored anaerobically and (2) detinning when this material is stored in cans (Seale, 1973; Wetselaar, 1974).

Nitrate can be formed in the atmosphere from oxides of nitrogen emitted from soils and from domestic, agricultural, and industrial combustion processes, or it may be carried into the atmosphere on dust and dried vegetation (Wetselaar & Hutton, 1963). The nitrate may be absorbed onto hygroscopic particles or react with atmospheric NH_3 to form nitrate salt aerosols (NH_4NO_3 for instance). It is removed either by precipitation or by dry deposition. The effects of nitrate aerosols on climate have been referred to previously when discussing ammonia in the atmosphere.

As for ammonia, the chemistry of atmospheric nitrates in the wet tropics remains speculative. One might guess that in the wet monsoon period the air will be relatively free of aerosols and that deposition through gaseous diffusion and solution in rainwater will prevail. In the dry period, aerosol formation and particulate deposition are likely to be more important.

Nitrous oxide

Most of the N_2O in the atmosphere is believed to originate from microbial transformations in the soil: either under anaerobic conditions from the reduction of nitrate (T_5 + T_7), or under aerobic conditions from the oxidation of ammonium (T_3 + T_7) - see Freney *et al.*, 1979. The relative importance of the two pathways depends on the moisture and oxygen contents of the soil. However, as conditions in the wet tropics are conducive to the formation of high levels of both ammonium and nitrate in the soil (see earlier discussion), we might expect that N_2O emissions might also be high in these regions.

Very few sinks have been postulated for N_2O in the lower atmosphere. Observations so

far show an unvarying atmospheric N_2O content (Roy, 1979), but detailed monitoring of atmospheric N_2O is only just beginning. The main sink is believed to be in the stratosphere where N_2O is broken down photochemically to nitrogen oxides which in turn react with ozone (McElroy & McConnell, 1971; Nicolet & Peetermans, 1972). What is concerning many people now is the prospect that a sharp increase in the use of nitrogen fertilizers (and legumes) might result in the liberation of sufficient N_2O to the atmosphere to significantly reduce or redistribute the stratospheric ozone content (see e.g., Council for Agriculture Science and Technology, 1976; Crutzen & Ehhalt, 1977; Galbally & Freney, 1978). Ozone effectively filters out ultraviolet radiation from the sun. An increase in ultraviolet radiation at the earth's surfaces could have detrimental effects on living organisms and possibly have effects on climate.

Attempts have been made to model the likely effects of increased N_2O emissions on stratospheric ozone. In all this, there are still great uncertainties in the understanding of the atmospheric transport process, the atmospheric chemistry, the location and magnitudes of terrestrial sources, and the soil chemistry of N_2O.

Since a good deal of the extra fertilizer use is likely to be in developing countries in tropical areas, it would seem that work on N_2O production in tropical soils and agricultural systems is particularly needed. Rice growing, a major agricultural activity in the tropics and one in which soil conditions are favourable for NO_3^- reduction, is a pertinent example. Some preliminary work by Denmead *et al.* (1979) in a flooded rice field indicated that 1% of the nitrate lost during denitrification was emitted as N_2O, but that figure cannot be taken as general; higher rates of N_2O have been anticipated (Council for Agricultural Science and Technology, 1976; Söderlund & Svensson, 1976). Freney *et al.* (1981b) found a loss of < 0.1% was N_2O of the fertilizer nitrogen applied to a lowland rice field.

Nitric oxide and nitrogen dioxide

Nitric oxide and nitrogen dioxide (NO_x) accumulate in the lower atmosphere as the result of exhalation of these gases from the soil (Kim, 1973; Galbally & Roy, 1978) and from the effluent of domestic, agricultural and industrial combustion processes (e.g. Evans *et al.*, 1977). This nitric oxide and nitrogen dioxide can be transformed by various chemical reactions into nitric acid, nitrates, and perhaps, according to recent suggestions, into nitrosamines (National Research Council, 1977, 1978).

The possible environmental effects of release of large quantities of nitrogen oxides into the atmosphere from the various combustion sources are summarized below.

Nitrogen dioxide can have a direct effect on health. Studies in Japan and the United States (Anon., 1976; Koizumi, 1976; National Research Council, 1977) have shown that increased respiratory disease occurs in populations exposed to elevated yearly average nitrogen dioxide concentrations (0.05-0.31 ppm) in the presence of other pollutants. Similar elevated nitrogen dioxide levels may occur in other industrial centres.

NO_x can be transformed by chemical reactions in the lower atmosphere to nitric acid. The nitric acid can be absorbed into cloud water and rainwater, causing a decrease in the pH of the rain, which is termed "acid rain". Acid rain can cause health problems and can have detrimental effects on plant and animal life (Anon., 1976, 1977). Acid rain has been observed in Europe, United States and Japan. However, recent studies in Europe and Japan (Anon., 1977; Söderlund, 1977) have shown that nitrogen (as nitrate) contributes less acid to the rain than sulphur (as sulphate), indicating that sulphur dioxide emissions are, in the studied areas, presently the major cause for the acidity in rain. Most of these emissions are due to industrial activities (Kellogg *et al.*, 1972), and such activities are much less intense in the wet-monsoonal tropics. On the other hand, the density of the number of volcanoes is much higher in these regions, but they contribute about two orders of magnitude less sulphur to the atmosphere than man's activities do (Kellogg *et al.*, 1972).

Nitrogen oxides can have an indirect effect on the quality of the environment, when, in conjunction with hydrocarbons and sunlight, they form oxidants in photochemical smog. These oxidants, ozone and PAN's (peroxyacylnitrates), are far more phytotoxic and injurious to health than the nitrogen oxide and hydrocarbon precursors. Photochemical smog occurs in Australia in Melbourne and Sydney (Galbally, 1971; Environment Protection Authority of Victoria, 1979; Ferrari *et al.*, 1979), in Japan (Air Quality Bureau, 1973), and perhaps in other large south-east Asian cities.

Recently it has been suggested that the carcinogenic nitrosamines might be present in polluted atmospheres and in fact may be formed by various reactions within the air. Amines have been measured in the atmosphere near cattle feed lots (Mosier *et al.*, 1973). These could combine with nitrogen oxides, from combustion sources or soil exhalation, and form nitrosamines (National Research Council, 1978), but it needs yet to be established whether the process does actually happen in the atmosphere.

CONCLUSIONS

The high soil temperature and soil moisture conditions in the wet-monsoonal tropics appear to be conducive to the accumulation of NH_3, NH_4^+, NO_x, N_2O or NO_3^- in pools such as plants, animals, surface and drainage waters, and the atmosphere. However, because of the high turnover rates at the relatively short atmospheric lifetime of a number of the gaseous products, many of the detrimental effects due to accumulation of nitrogenous compounds in the different pools are likely to be temporary. Some can be avoided with proper management of the agrosystem. The main challenge appears to be the development of fertilizer types and management strategies that keep these effects to a minimum.

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GROUNDWATER TRANSPORT OF NITROGEN IN RICE FIELDS IN NORTHERN THAILAND

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ABSTRACT

Some paddy fields in Chiang Mai Province are underlain by relatively permeable soil layers which may conduct large quantities of water. First estimate calculations show that these shallow, perched aquifers are capable of transporting measurable quantities of dissolved nitrogen which has leached from the surface soil. Several sets of wells have been observed for one year (three crops) at three sites under the auspices of the Multiple Cropping Project at Chiang Mai University. The nitrogen concentration in the groundwater was generally well below 5 mg N l^{-1} and was distributed between organic and ammonium forms. The final estimate of nitrogen transport depends strongly on the assumptions and observations made of the physical characteristics of the aquifer and the chosen boundaries. The results suggest that less than 1% of the applied N is exported from the catchment with the groundwater.

INTRODUCTION

Some nitrogen compounds in soils are quite mobile. Nitrate movement has been studied in environments where chemical conditions in the soil favor its formation. However, in flooded soils it is unstable and tends to be reduced to nitrogen gas while ammonium and other reduced compounds tend to accumulate (Ponnamperuma, 1976). In places where the soil water of the paddy field is continuous with or close to the underlying groundwater there is potential for movement of nitrogen out of the soil and into the underlying aquifer.

The Chiang Mai basin is filled with deep alluvium composed of poorly sorted clay, silt, coarse sand, gravel, pebbles, cobbles and clay lenses. The Ping River has shifted back and forth across the valley many times, leaving braided stream deposits all along the valley (Chuamthaisong, 1971). The result of such deposits is multiple-layered aquifers; shallow, perched aquifers very close to the surface in some instances and very deep aquifers as well. According to a survey by Chuamthaisong (1971), nitrate concentrations in some shallow wells reached 59.4 mg N l^{-1} with an average of 2.89 mg N l^{-1}. Rienvatana (1975) also noted high concentrations of nitrate in well waters near Chiang Mai.

An experiment was conducted to investigate whether fertilized paddy fields contribute significantly to the concentration of nitrogen in shallow aquifers.

MATERIALS AND METHODS

Two villages, Ban Han Kaeo (HK) (see Fig. 1) and Ban Mae Kung (MK) were selected on the basis of their prior connection with Chiang Mai University through the Multiple Cropping Program. One site with easily defined hydrologic boundaries was chosen at each village. A third site was the multiple cropping experimental fields (MCP) on the university campus in Chiang Mai. The USDA Classification for soils at all sites is typic Tropaqualfs. The national classification is low humic grey soils. Ban Han Kaeo soils are of the Hang Dong series.

Well sites were chosen at the upper and lower ends of fields and 10-cm diameter plastic pipes were used to line the wells, which were dug by hand. The pipes were perforated from 1 m below the surface to reduce seepage along the sides of the pipe. The pipes were covered with iron lids. Existing wells were also sampled when possible, although these wells were not covered and hence open to gross contamination.

Water table levels were recorded approximately monthly at the time of water sampling. Irrigation water was collected from ditches and canals adjacent to sampling sites. Rainwater was collected occasionally in distilled-water-rinsed glass beakers of various sizes placed in the courtyard of the Faculty of Agriculture during rainstorms. Water samples were collected and preserved on site with mercuric chloride or by refrigeration. Organic nitrogen was determined by a modified Kjeldahl method, ammonium by direct Nesslerization, nitrate by the brucine method and nitrite by azo-dye reaction. Methods were based on those of Taras *et al.* (1971).

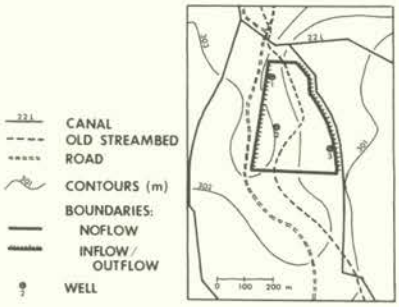

Fig. 1. Location of hydrologic boundaries and well sites in relation to the contours.

Surface soil samples were collected several times during the experiment and analyzed by the Soil Science and Conservation Department, Faculty of Agriculture, Chiang Mai University. Hydraulic conductivity was measured on several occasions by the auger-hole method. Rainfall, evaporation, and crop data were obtained from the Multiple Cropping Project, Faculty of Agriculture, and irrigation data and maps from the Royal Thai Irrigation Department.

RESULTS

Table 1 shows the average concentrations of various compounds in well water samples collected at Ban Han Kaeo during the rainy season (June to November). Using the Wilcoxon test for paired variates (which assumes data are not necessarily normally distributed), significant differences (at P = 0.05) in total nitrogen were found between the two upper wells and the lower well (HK 1 and HK 3, and HK 2 and HK 3, see Fig. 1). Han Kaeo well 2 (HK 2) is an open, bricklined well in the middle of the most intensively cultivated field at that site. Although there is no significant difference in the total nitrogen found in well HK 2 compared with well HK 1 (located closer to the edge of the field), the average level of nitrate nitrogen was considerably higher in HK 2. Perhaps this was a result of greater access of oxygen to the well water in HK 2.

Table 1. Average concentrations of various forms of nitrogen in groundwater during the rainy season 1978

	($mg\ N\ l^{-1}$)				
Well	Nitrate	Nitrite	Ammonium	Organic	Total
HK 1	0.059	0.001	0.166	0.306	0.52
HK 2	0.290	0.002	0.387	0.397	1.14
HK 3	0.099	0.007	0.896	0.790	1.79

Fig. 2 shows the monthly variation in concentration of compounds of nitrogen at Han Kaeo wells 1 and 3. There is a peak in concentration in August followed by a sharp drop for the last two months of the rainy season. The change in distribution of nitrogen compounds in time demonstrates a shift in response to the reduced conditions in the flooded soil. Nitrate disappears as organic nitrogen, and ammonium predominates at the end of the rainy season. Average concentrations of nitrogen in well water were higher at the other two sites. Total-N at the Ban Mae Kung wells was 1.46 and 1.96 mg N l^{-1} for the MK 1 and MK 2 wells respectively. At the MCP wells, MCP 1 and MCP 2, it was 3.29 and 3.44 mg N l^{-1} respectively.

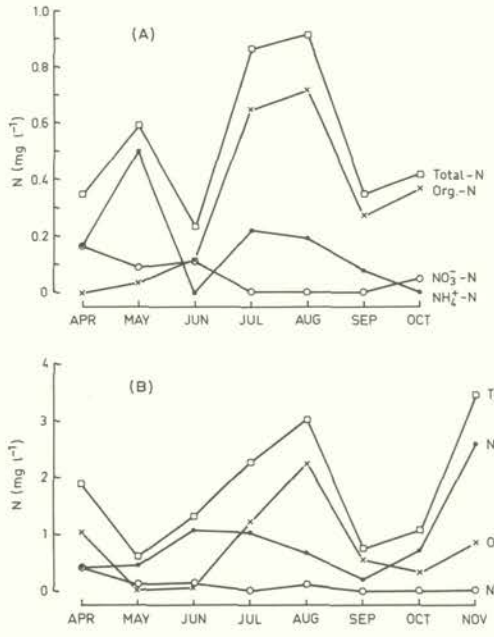

Fig. 2. Concentration of different N fractions at different times for well 1 (Fig. 2A) and well 3 (Fig. 2B). Note differences in scale between (A) and (B). The nitrite-N concentration was always smaller than 0.01 mg l^{-1}, except in April for well 3 (0.04 mg l^{-1}).

Table 2 shows the average concentrations of various compounds and total-N observed in irrigation and rainwater collected during the rainy season 1978. The amount of irrigation water provided at Ban Han Kaeo during that season was 506 mm. This yields a nitrogen input due to irrigation of 6.4 kg N ha^{-1}. Ban Mae Kung had a higher average concentration of nitrogen in irrigation water (2.27 mg N l^{-1}) and a higher total input of about 13 kg N ha^{-1}. This agrees with the estimate of 10 kg N ha^{-1} by Takahashi (1964). Nitrogen in rainfall was calculated by month for a total wet precipitation of 13 kg N ha^{-1} during the rainy season. The Department of Agricultural Chemistry (1976) estimated precipitation of nitrogen as ammonium and nitrate at 8.1 kg N ha^{-1} yr^{-1} in Bangkok.

Table 2. Average concentrations of various forms of nitrogen in irrigation and rainwater during the rainy season 1978

	(mg N l^{-1})				
Location	Nitrate	Nitrite	Ammonium	Organic	Total
		Irrigation water			
HK	0.020	0.002	0.217	1.02	1.26
		Rain water			
MCP	0.517	0.015	0.838	0.176	1.55

Transport of N in groundwater

The flux of nitrogen transported in groundwater is estimated from the concentration of nitrogen in the water and the rate of discharge of groundwater. The latter is estimated by the product of the hydraulic conductivity of the soil, the hydraulic gradient (slope of the water table) and the cross-sectional area through which the water passes the boundary of interest. The measured hydraulic conductivity ranged from 0.6-3.8 cm hr^{-1}, which is in close agreement with the estimates of the Royal Thai Irrigation Department (1971). Their report lists values for a detailed profile study in Han Kaeo (core 23-8) ranging between 0.5 and 2.0 cm hr^{-1} for the most permeable layers. High and low values for hydraulic conductivity were used to indicate a range of possible values for discharge. The slope of the water table between wells HK 2 and HK 3 was used as an estimate of the hydraulic gradient. From auger holes, soil cores, the Royal Thai Irrigation Department (1971), and farmer's observat-

ions it can be assumed that the permeable layer extends from 0.5 or 1.0 m to 4 m below the surface. On this basis, the depth of the aquifer was estimated at 3.5 m. The boundaries were derived from contour maps showing the edges of the fields of the study site (Fig. 1). The lengths of the boundaries thus obtained were for inflow 400, outflow 500 and noflow 300 m. Hence, for example on April 17 the estimate of minimum hydraulic conductivity was 0.5 cm hr^{-1} (0.12 m d^{-1}). The gradient was 0.3% (Table 4), and the cross-sectional area of the inflow boundary was 3.5x400 m. The calculated flow was 0.504 m^3 d^{-1}. The resulting groundwater flows are shown in Table 3.

Table 3. Groundwater discharge at Ban Han Kaeo 1978

Date	Elevation of water table (m above sea level) HK2	HK3	Gradient (%)	Discharge (inflow) (m^3 d^{-1})	(outflow)
Apr. 17	301.6	301.2	0.3	0.504-2.02	0.63-2.52
May 9	301.6	300.9	0.35	0.588-2.35	0.735-2.94
Jun. 19	301.6	300.9	0.35	0.588-2.35	0.735-2.94
Jul. 24	301.9	301.5	0.2	0.336-1.34	0.42-1.68
Aug. 21	302.8	301.5	0.65	1.09-4.37	1.37-5.46
Sep. 25	302.8	301.5	0.65	1.09-4.37	1.37-5.46
Oct. 19	302.6	301.5	0.65	1.09-4.37	1.37-5.46
Nov. 27	302.3	301.3	0.5	0.84-3.36	1.05-4.2

The groundwater transport of nitrogen in Table 4 is estimated as the product of the groundwater discharge in Table 3, the number of days represented by the sample dates (during which the discharge was assumed constant), and the concentration of nitrogen found in the well water at the time of sampling (Fig. 3), divided by the area of the field (8.47 ha). The period represented by a sample date was considered to be the time half-way after the last sample and half-way before the next one. For instance, the April 17 sampling represented a flow period of 23.5 days. With an inflow of 0.504-2.02 m^3 d^{-1} and a total N concentration in the water at HK 1 of 0.346 mg l^{-1}, the inflow of N was 0.48-1.94 g ha^{-1} over that flow period, using wells HK 1 and 3 for inflow and outflow concentrations respectively. For outflow of N, similar calculations were made and the results are given in Table 4.

Table 4. Groundwater transport of nitrogen at Ban Han Kaeo 1978

Date	Number of days	Discharge (inflow)	(outflow) (g N ha^{-1})	Difference (net outflow)
Apr. 17	23.5	0.484-1.94	3.32-13.3	2.84-11.4
May 9	31.5	1.28-5.13	1.63-6.52	0.350-1.39
Jun. 19	38	0.604-2.41	4.29-17.2	3.69-14.8
Jul. 24	31.5	1.07-4.29	3.53-14.1	2.46-9.81
Aug. 21	31.5	3.70-14.8	15.5-61.7	11.8-46.9
Sep. 25	29.5	1.31-5.25	3.58-14.3	2.27-9.05
Oct. 19	32	1.69-6.77	5.42-21.6	3.73-14.8
Nov. 27	34.5	8.14-32.6[a]	14.7-58.9	6.56-26.3[a]
Total		18.3-73.2	52.0-208	33.3-134

[a] N-concentrations from HK 2 used in place of HK 1.

Variations in the physical characteristics of the aquifer, used to estimate groundwater flow, can greatly affect the nitrogen outflows estimated. The range of hydraulic conductivities used was two orders of magnitude. Combined errors in estimating depth and length of boundaries of the aquifer could possibly add another order of magnitude of difference to the flux estimates. Hence, results obtained must be considered more qualitative than quantitative. Based on these data a reasonable estimate of groundwater transport of nitrogen during the rainy season would be of the order of 0.1 kg N ha^{-1}.

Improvement of this nitrogen flux model would require a thorough hydrologic study of the perched aquifer in question, including geologic and water table mapping. An increase in the number of sampling wells and the addition of wells at varying depths would allow a more precise estimate of actual groundwater nitrogen concentrations. Addition of ion exchange processes would also improve the model.

Table 5. Nitrogen flow for a paddy field in Northern Thailand, Ban Han Kaeo 1978

Inflow	(kg N ha^{-1})	Source
Rainfall	13	This paper
Irrigation	6.4	This paper
Fertilizer	40-95	(San Pa Tong; RD 7) MCP data 1978
Fixation	2-10	(low humic gley soils) Matsuguchi *et al.* (1974), Sangtong *et al.* (1976)
Groundwater	0.01-0.07	This paper
Total:	61-124	
Outflow		
Volatilization	0.4-1.0	(1% of fertilizer N) Mikkelsen *et al.* (1978), Ventura & Yoshida (1977), Basdeo & Gangwar (1976)
Denitrification	2.2-7.2	(low humic gley soils) Araragi & Tangchan (1978)
Rice uptake	90-110	(San Pa Tong; RD 7) MCP data 1978.
Groundwater	0.05-0.2	This paper
Total:	93-118	

A crude nitrogen balance compares the calculated nitrogen transport in groundwater with other fluxes (Table 5). Other inputs and outputs of nitrogen were obtained from the sources indicated. Two levels of N fertilization and N uptake by rice were shown for two of the common varieties of rice used in the areas studied. RD 7 is a non-photosensitive, fertilizer-responsive variety and San Pa Tong is a local, photosensitive variety of glutinous rice which is not responsive to N fertilizer, although it takes up 82% as much N as the RD 7 variety.

The data of Table 5 indicate that the groundwater export of nitrogen (0.1 kg N ha^{-1}) is roughly 0.1-0.5% of the nitrogen fertilizer used. This is not a large quantity in terms of agricultural losses. Because of low gradients and low soil permeability, common in rice cultivation of this type, high amounts of leaching of nutrients is not expected. It should be noted, however, that measurable increases in concentrations of nutrients in groundwater occur over fairly short distances: a few hundred meters at the Ban Han Kaeo and the MCP plots. The observed differences in total-N concentration are attributed to leaching of fertilizer. The highest nitrogen concentrations in groundwater were found at the MCP experimental plots (3.3 and 3.4 mg l^{-1}), suggesting a relationship between multiple cropping and leaching of nitrogen. However, large accumulations of nitrogen as found by Chuamthaisong (1971) and Rienvatana (1975) were not found in this study.

ACKNOWLEDGEMENTS

I wish to thank Dr Niwat Hirumburana and Mr Chavalit Chalothorn for their work in initiating this project; Mrs Navarat Sivisilp, Miss Asanee Chindawongse, and Miss Wichitra Tiencharoen for their assistance in laboratory work. The project was supported by funds from the Soil Science and Conservation Department and the Multiple Cropping Project, Faculty of Agriculture, Chiang Mai University, Thailand, and the Shansi Memorial Association, Oberlin College, Ohio, U.S.A.

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IDENTIFICATION OF ALGAL SPECIES IN JADEE BUCHA CANAL, NAKORN PATHOM PROVINCE, THAILAND

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ABSTRACT

Jadee Bucha is part of a canal that runs through the centre of Nakorn Pathom Province, approximately 60 km southwest of Bangkok. An annual algal bloom is observed from December to April when the water is dark green. The water is black and odorous for the remainder of the year. As a first phase of the study on eutrophication in Jadee Bucha Canal, phytoplankton in the algal bloom were identified. The dominant species were found to be two Anabaena *spp. In total, 11 species were identified comprising four Cyanophytes, four Chlorophytes and three Euglenophytes.*

INTRODUCTION

Jadee Bucha is part of a canal that runs through the commercial section of Nakorn Pathom province. Two weirs were built at each end of the canal to prevent the drying up of water in summer. The canal's dimensions are 22 m wide, 50 m long and 4.5 m deep. The weirs are opened in rainy seasons. Domestic wastes are discharged directly into the canal. It was observed that from December, 1976, to April, 1977, the water in the canal was dark green while it was black and odorous for the rest of the year. From December, 1977, the water became dark green again and the pattern was repeated. As a first phase towards the study of this periodic eutrophication process the phytoplankton in the algal bloom were identified. The second phase of the study will involve measurements of various physical parameters and determinations of the system's ammonia, nitrate and phosphate contents in order to pinpoint the causes of the eutrophication. This report presents results of the first phase of study.

MATERIALS AND METHODS

Samples were collected by trailing a 20 μ pore size plankton net along the surface water. They were preserved in a 6-3-1 preservative as described by Prescott (1970). The preserved and live specimens were studied under the Olympus microscope model BHA equipped with an Olympus model PM-10 camera. The specimen's size was determined with an ocular and a stage micrometer. The phytoplankton species were identified by comparing the specimens' size and morphology with those published in keys and other related literature.

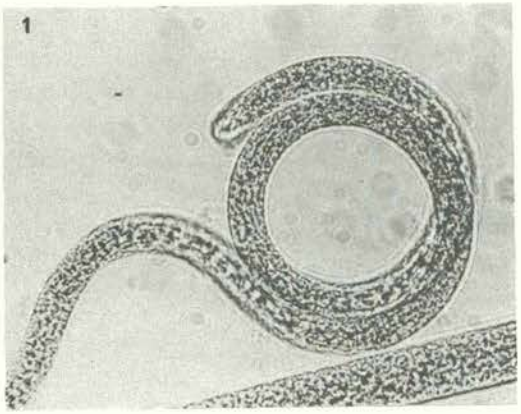
RESULTS

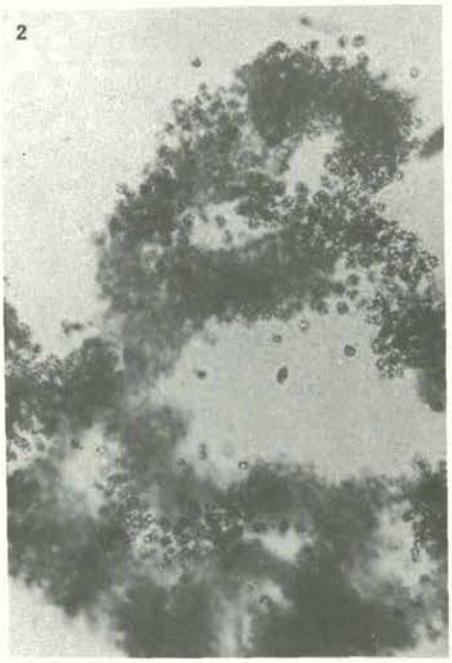
Cyanophyta

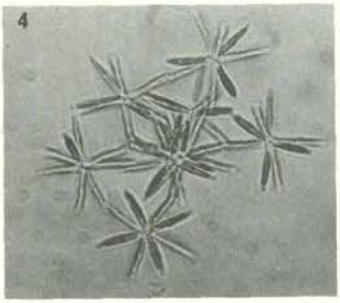
Anabaena spp. (Fig. 1). There are two types of *Anabaena* spp. which are the dominant ones. The cell diameter is 10-14 μ. They could not be identified at the species level, because neither heterocysts nor akinetes were found. It is interesting to note that Yamagishi & Hirano (1973) recorded a similar *Anabaena* sp., *Anabaena circinalis*, with straight and coiled trichromes, from Cambodia.

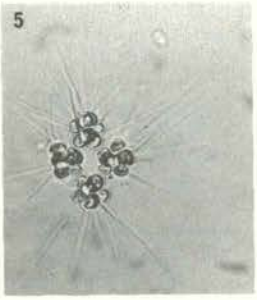
Microcystis aeruginosa Keutzing (Fig. 2) (Yamagishi & Hirano (1973), pl. 8, Fig. 1). Cell diameter is 3-4 μ. This species is a frequent component of water blooms, especially in lakes with eutrophic characteristics (Prescott, 1962).

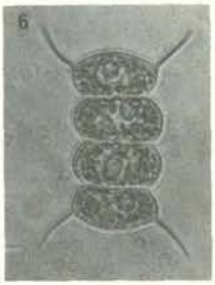
Oscillatoria sp. (Fig. 3). Cell width 5-7 μ.

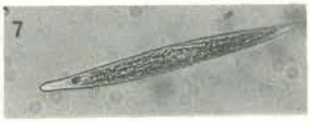












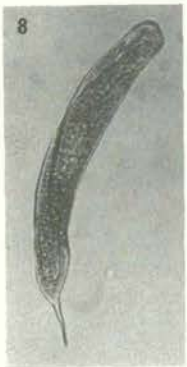


Fig. 1–8. For explanation see text.

Chlorophyta

Actinastrum hantzschii (Fig. 4) (Viyakornvilas (1974), pl. 3, Fig. 5). Cell length 10-20 µ, width 2-5 µ.

Dictyosphaerium pulchellum Wood (Yamagishi & Hirano (1973) Pl. 7, Fig. 10). Cell diameter 10-12 µ. No sheath was observed.

Micractinium pusillum Fres. (Fig. 5) (Viyakornvilas (1974) Pl. 2, Fig. 18). Cell diameter 3-5 µ.

Scenedesmus quadricauda (Turpin) Breb. (Fig. 6) (Yamagishi & Hirano (1973) Pl. 2, Fig. 8). Cell length 3-4 µ, width 10-12 µ.

Euglenophyta

Euglena acus Ehrenb. (Fig. 7) (Yamagishi & Hirano (1973) Pl. 3, Fig. 1). Cell length 136-150 µ, width 9-13 µ.

Euglena charkowiensis Swir. (Fig. 8) (Hirano (1975) Pl. 2, Fig. 7). Cell length 130-195 µ, width 20-30 µ.

Euglena sp. Cell length 3-37 µ, width 20-25 µ.

DISCUSSION

From the available literature, lists of phytoplankton species contributing to algal blooms in south-east Asian aquatic systems could not be found. In the investigated canal of Nakorn Pathom province, the algal blooms appear to be dominated by two *Anabaena* spp. The above results, combined with author's preliminary surveys of freshwaters with green coloration in Bangkok and Nakorn Pathom, suggest that species composition varies from place to place. It also suggests that eutrophication can be caused by a wide variety of species.

Such eutrophication is mainly caused by high inputs of nitrogen and phosphorus (Goering, 1972; Stumm & Stumm-Zollinger, 1972) *via* domestic waste disposal. There is therefore an urgent need for a detailed study of the cycles of these elements in eutrophying waters, in order to devise appropriate solutions to the eutrophication problem.

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A MODELLING APPROACH TO NITROGEN CYCLING IN AGRO-ECOSYSTEMS

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ABSTRACT

The processes leading to nitrogen losses from ecosystems to outside waters and to the atmosphere are part of a complex nitrogen cycle. The relative rates of nitrogen flow between the various pools in the cycle are affected by the interaction of a number of environmental factors. In order to minimize losses from an ecosystem, or maximize the recovery of nitrogen in a crop, the interacting effects of environmental changes on the rate of each process in the nitrogen cycle must be known.

It is not possible to measure all the relevant effects of environmental changes in many different ecosystems. Thus some technique is required which will enable the limited data from a few 'benchmark' experiments to be applied to a wide range of situations. Mathematical modelling seems to offer the best approach to this problem. Some of the limitations of currently available data, which retard the development of nitrogen cycle models, are discussed, together with suggestions on field techniques for obtaining appropriate data.

As an example of the type of approach suggested, a brief account is presented on the development of a sub-system model to describe all of the processes from hydrolysis of urea in soil to the release of ammonia into the open atmosphere. This model has focussed attention on the important factors controlling the rate of ammonia flux. These include the soil cation exchange capacity, the vapour pressure of ammonia at the soil surface and the windspeed near to the surface.

INTRODUCTION

Although much is known about the mechanisms of nitrogen transformations and transfers in soils, plants, animals and waters, much more study is needed before the effects of disturbances (such as forest clearing, cultivation or fertilizer N application) on nitrogen cycling and the losses of nitrogen from whole ecosystems can be predicted quantitatively. Without such knowledge, we cannot ensure that nitrogen will be utilized efficiently in the ecosystem, and that losses to the outside environment are kept to a minimum.

One of the main limitations to progress in nitrogen cycling research is the complexity of interactions between biological, chemical and physical processes which occur in natural ecosystems. The component reactions have been studied analytically in the laboratory, but it is much more difficult to fit them all together and to establish their respective quantitative importance in the ecosystem.

Many research groups have now realised that a systems modelling (or simulation) approach may facilitate the quantitative description of the complex nitrogen cycling in ecosystems. In this concept, each nitrogen transformation or transfer process is considered as part of a network of interdependent reactions, of which the relative rates are controlled by environmental factors, such as temperature, moisture content and pH, as well as by substrate concentration. Inputs of nitrogen by biological fixation, losses of ammonia to the atmosphere and nitrate movement to groundwater can be regarded as additional processes controlled from within the system.

The intention of this paper is to discuss what can be gained from a modelling approach to problems of nitrogen cycling in ecosystems, and the types of measurements required before we can verify and apply models successfully.

THE USEFULNESS OF NITROGEN CYCLE MODELS

Aspects of nitrogen cycling on which information is urgently required are the rates of biological input, crop uptake and losses from the system as they are affected by changes in agricultural management, crop species, physical environment or climatic events. Advantages

of the modelling approach to this problem are: (a) it induces study of whole ecosystems, (b) the component processes where information is most urgently needed are identified, (c) a successful model has predictive value so that the results from a few experimental sites may be applied in many different situations.

A number of nitrogen cycle models have been conceived for soils and whole ecosystems (e.g., Ruess & Innis, 1977) and some of them have been reviewed recently (Tanji & Gupta, 1978). They differ in their approaches - some are based heavily on microbial population dynamics and require time-consuming measurements, while others are quite empirical. Generally, progress has been slow in the application of these models, largely because the relationships contained in them are beyond our present capacity to verify them. Thus the models cannot be applied easily to predict events in a variety of field situations.

Simpler models must be developed, verified, and modified where necessary, using suitable experimental data (Davidson *et al.*, 1978). It may be helpful in many cases to develop models of separate sub-systems, e.g., for denitrification, for nitrogen fixation and for nitrate leaching, then to link these together at a later stage. The properly designed model should be:

(a) scientifically correct, using known principles in chemical reaction or physical exchange mechanisms;
(b) as simple as possible so that it is verifiable from actual data obtained from feasible experiments, or from the literature;
(c) sufficiently detailed and versatile to predict how rates of nitrogen flow and pool sizes (including losses and input from outside the system) will be affected by changes in soil characteristics, climatic events (or geographic locations) and agronomic management, e.g., cultivation, cropping and fertilizer practices.

THE MEASUREMENTS REQUIRED FOR VERIFYING MODELS OF NITROGEN FLOW IN ECOSYSTEMS

If useful models of nitrogen flow in ecosystems are to be developed, there is an obvious need for collaboration, and for standardizing field measurements, between research teams at different locations. Tanji & Gupta (1978) concluded that "it appears that we do not have a single field experiment in the literature by which one can adequately document a nitrogen simulation model as a whole for hydrologic (physical), biologic, and chemical considerations. A concerted joint effort is of high priority for the design, monitoring, and evaluation of information and data taken under field conditions".

Separate measurements are required on the rates of every important biological or chemical transformation or transport process in the system. We can only hope to make all these measurements in a few carefully located 'benchmark' experiments but, knowing the effects of environmental variables on the rate of each process it should be possible to interpolate and apply the results to a variety of locations and soils. To achieve this, the measurements on the 'benchmark' experiments must be comprehensive. All aspects of soil processes may need to be measured, including biological factors (e.g., organic matter, microbial biomass, urease activity) chemical factors (e.g., pH, cation exchange capacity, buffering capacity) and physical factors (e.g., soil temperature fluctuations, moisture characteristics, oxygen diffusion rate).

There are special problems in determining rates of N_2 input and outflow for ecosystems by direct measurements. Methods involving ^{15}N isotope are the best available for the measurements, but problems remain for field use (Freney & Denmead, 1981). For N_2 fixation by legumes, the ^{15}N dilution techniques (Rennie *et al.*, 1978), which involve labelling any soil nitrogen absorbed by the legume, are promising. For N_2 loss by denitrification, spatial variability in soils is a major problem. Perhaps the best compromise is still, regrettably, an accurate N balance method using ^{15}N-labelling in monolith lysimeters (Burford, 1977; Hauck, 1979).

More precisely, the requirements for verifying a model are:

(a) short-term rates of nitrogen flows (min^{-1}, h^{-1} d^{-1} as appropriate) between the known pools in the system,
(b) the kinetics of each process, i.e., the effect of substrate concentration on the reaction rate in the biological transformations, so that a *rate constant* can be calculated. First order kinetics are usually assumed but sometimes without justification,
(c) the effects of temperature, moisture, pH, O_2 tension, or any other relevant factors, on the rate constant,
(d) estimates of pool sizes in the field for each nitrogen form in the model at some reference time or standard condition.

The rate constants must be determined *under the conditions existing in the field.* Because of the difficulties of simulating field conditions in the laboratory, it is usually preferable to make the measurements in the field. In the case of

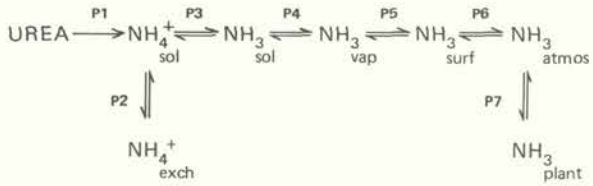
ammonia volatilization, for example, there are complications of reabsorption in vegetation, effects of wind speed, and dew formation (Denmead *et al.*, 1976) which are most difficult to simulate in the laboratory. Exact amounts of surface wetting or drying can greatly affect the accumulation of nitrate in undisturbed field soils (Simpson, 1962). Thus great care is required to determine rates of nitrogen flow between particular pools with the minimum disturbance.

One great disadvantage of working in the field, rather than on small mixed samples in the laboratory, is the problem of spatial variations in soil nitrogen, vegetation, litter and hydrology from one point to another within an experimental site. The problem is magnified when a large number of intensive measurements on nitrogen transformations and flows are required. However, recent developments in techniques are beginning to provide ways of overcoming this problem. For example, measurements of gaseous losses of nitrogen and leaching losses from catchments can sometimes be taken by techniques which integrate over large areas and so overcome the problems of spatial variability involved in soil sampling.

AN EXAMPLE OF A MODELLING APPROACH : AMMONIA LOSS

It may be useful to describe the progress of the CSIRO groups in Canberra and Brisbane towards modelling the loss of ammonia from agro-ecosystems to the atmosphere. We realised about 10 years ago, from N balance experiments on grazed pastures, that over 50% of fertilizer N applied, or legume N estimated to be fixed could not be recovered. About the same time it was observed that urea applied to pastures could be ineffective when dry weather occurred after application. We adopted the hypothesis that much of the urea N, from either fertilizer or animal urine, was hydrolysed to ammonia and lost to the atmosphere. Experiments with ^{15}N-labelled urea applied to undisturbed cores of pasture soils confirmed this theory (Simpson, 1968). In 1973-74 it was demonstrated for the first time by direct measurement that ammonia loss from grazed pastures is an important aspect of N cycling in the field environment. Losses of ammonia were up to *c.* 0.3 kg N ha^{-1} d^{-1} (Denmead *et al.*, 1974). Development of our field techniques has enabled us to show that the rate of ammonia volatilization is related to the evaporation of soil water, ammonia partial pressure near the soil surface and, in some circumstances, to the wind speed and rate of air exchange near the surface. By collaboration, a conceptual model was developed of the sub-system from urea in soil to ammonia in the atmosphere (Myers *et al.*, 1979).

Briefly, the model follows the scheme:



(sol = in solution; exch = exchangeable, adsorbed; vap = vapour in soil air; surf = at soil surface; atmos = open atmosphere; plant = reabsorbed by plants).

We have now completed field experiments on ammonia loss under a variety of agricultural systems from wetland rice to grazed pasture. This has provided an opportunity to test some aspects of the model. Our main observations apply at various stages:

(a) The first stage (P1) is the hydrolysis of urea to ammonium by the enzyme, urease. The rate of this reaction determines the intensity of the ammonia source and thus the rate of volatilization. The rate constant for P1 can either be determined by incubation or estimated from published relationships between urease activity and organic carbon. Corrections for urea concentration, temperature and soil moisture tension are made according to existing data.

In laboratory simulation experiments on the fate of urea applied to soils under controlled physical environments, losses of nitrogen to the atmosphere were related to the urease activity of the surface soil (1 cm layer) as shown in Table 1.

However, field experiments with urea applied during irrigation showed that hydrolysis is rapid, usually with a half-life of a few hours, especially at higher temperatures. Thus the accumulation of ammonium/ammonia in the soil reaches a maximum before the main efflux to the

atmosphere occurs. In such cases, urease activity of the surface soil appears to be a less important variable in the rate of volatilization than the subsequent processes in the system. In other situations, e.g., in the floodwater of rice fields, it is likely that the low urease activity can be a limiting factor controlling the rate of ammonia accumulation and hence its diffusion into the atmosphere (Freney *et al.*, 1981a).

Table 1. The effect of urease activity in the surface soil (0-1 cm) on the loss of nitrogen to the atmosphere from urea applied to soil cores in controlled environments

Urease activity[a]	% N loss[b]
115	60.3
29	46.1
21	37.6
11	32.6
10	31.4

[a] µg urea-N hydrolysed (g soil)$^{-1}$ in 3 h at 25 C, from a 200 µg solution.

[b] Mean loss after application of 112 kg N ha^{-1} at 18 g N l^{-1} to soils at 10, 20, 30% moisture and temperatures of 10, 20 C.

(b) The next stage is comprised of chemical equilibria (P2, P3) which, although they are assumed to be instantaneous, have to be quantified to obtain the pool size for $NH_{3_{sol}}$, and a third group of processes (P4, P5) which are principally physical. All of these processes are difficult to quantify in soil, and present the main barrier to further development of the model. The concentration of ammonium ion in solution is controlled by the exchange equilibrium (P2), and the rate of diffusion of ammonia vapour to the surface (P5) depends on the diffusion coefficient (D) according to Fick's Law -

$$P5 = 0.6\ \alpha\ f\ D \cdot dc/dx \ ,$$

where α is a tortuosity factor, f is soil porosity and dc/dx is the change in NH_3 concentration with depth.

Both the exchange equilibrium and the diffusion coefficient depend on the cation exchange capacity of the soil (CEC). The initial pH of the soil and its buffering capacity control the equilibrium (P3) between $NH_4^+{}_{sol}$ and $NH_{3_{sol}}$ (Freney *et al.*, 1981b). Initial pH has been of minor significance in the soils we have studied, as they quickly became buffered at pH 9-9.2 during the urea hydrolysis. Buffering capacity is usually related to CEC. The remaining soil process (P4) is the equilibrium between ammonia in solution and ammonia vapour. This we assume to obey Henry's Law -

$$[NH_{3_{sol}}] = K_h \cdot [NH_{3_{vap}}] \ ,$$

where the partial pressure of ammonia vapour is related to the concentration in solution by Henry's constant, K_h. This constant increases with absolute temperature (T), according to

$$\log_{10} K_h = \frac{1477.8}{T} - 1.6937$$

(Subcommittee on Ammonia, 1979).

Essentially then, an increase in CEC results in a lower $[NH_4^+{}_{sol}]$, less $NH_{3_{vap}}$ and slower diffusion to the surface. Table 2 shows the striking effect of cation exchange capacity on $[NH_4^+{}_{sol}]$ and losses of ammonia in the field after the application of solutions of urea to two contrasting soils. It appears probable that an empirical function based on CEC can be developed to approximate the equilibria and processes represented in P2, P3, P4, P5, with appropriate coefficients for temperature, moisture content and depth of urea placement.

(c) The flux of ammonia into the atmosphere (P6) is related to windspeed and partial pressure of ammonia near the soil or water surface (Denmead *et al.*, 1981) and can be described by the equation

$$F = [D + f(u)](P_o - P_z)$$

where F is the flux rate into the atmosphere,
D is the diffusion coefficient of NH_3 in still air,
f(u) is a function of windspeed, u at height, z,
and $P_o - P_z$ is the difference in partial pressure of NH_3 between the surface and height, z.

Table 2. Retention of ammonium ion in solution, after equilibration of ammonium chloride solutions with two contrasting soils; a comparison with the losses of ammonia measured from these soils in the field

	Retention of NH_4^+ in solution (% of addition)						NH_3 loss[c] in the field
Concn. added ($\mu g\ N\ ml^{-1}$)	100		200		400		(% of added N)
Soil : soln. ratio	1:1	1:2	1:1	1:2	1:1	1:2	
MIA soil[a]	2	5	5	12	10	19	2
ACT soil[b]	43	55	57	68	64	73	50

[a] Soil from the Murrumbidgee Irrigation Area (pH 8.5; CEC 37.0 meq 100 g^{-1}).

[b] Soil from the Australian Capital Territory (pH 5.5; CEC 6.5 meq 100 g^{-1}).

[c] Losses after surface applications of urea at $\underline{c}$ 100 kg N ha^{-1}.

Over flooded rice fields, F increases linearly with u and f(u) = k.u, where k is a constant, according to Freney *et al.* (1981b). In some crop canopies, the ammonia flux increases markedly at higher windspeeds, as shown in Figure 1, and F varies approximately as u^2 (Denmead *et al.*, 1981).

On the other hand, over bare soil surfaces with moderate to high windspeeds, diffusion through the soil is more likely to limit the ammonia flux than air movement above the soil.

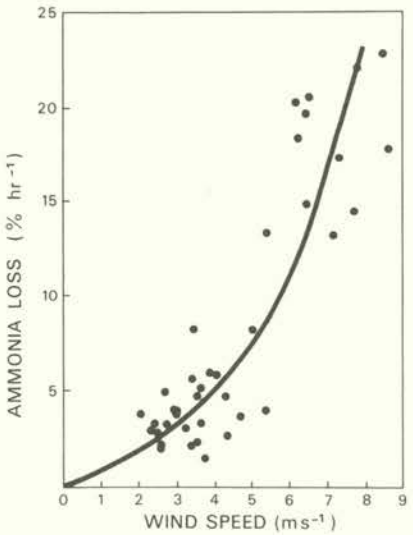


Fig. 1. The effect of wind speed on ammonia volatilization from irrigation water after applications to a maize crop of 90 cm height.

(d) Exchange of ammonia between plants and the atmosphere (P7) is the final factor in the net loss. Studies of ammonia formation and loss in plant canopies have revealed an important influence of crop density in preventing the loss. This resolves into two aspects: (a) the crop canopy reduces windspeed and air movement around the source of ammonia vapour, thus reducing the transport of ammonia out of the crop; (b) the presence of plant leaves allows the reabsorption of ammonia into leaf tissue at rates proportional to the increase in ammonia

concentration in the air (Farquhar *et al.*, 1980). This absorption rate is greatly increased when the leaf surfaces are wetted by dew or rain, and can be sufficient to prevent the loss of ammonia almost completely (Denmead *et al.*, 1976).

Thus substantial progress has been made in developing a model of the processes in ammonia volatilization. More experimental data and field verification are required before the model can be applied to predict losses in a variety of soils, crops or vegetation systems. So far, the achievements of the modelling approach to ammonia loss are: (a) it has focussed attention on the soil processes which appear to control ammonia release, e.g., cation exchange and ammonia diffusion through the soil, (b) the concept has helped to identify important nitrogen forms, such as $NH_{3_{sol}}$, which cannot readily be measured in soils but which must be quantified indirectly in 'benchmark' experiments if the potential predictive value of the model is to be realised.

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NITRATE MOVEMENT IN CATCHMENTS

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ABSTRACT

The main inorganic nitrogen constituents in soil and groundwater are nitrate (NO_3^--N) and ammonium (NH_4^+-N). Nitrate is potentially very mobile. Its vertical displacement through the soil profile to a water table, and lateral movement through groundwater bodies to surface outlets, is studied, using simple conceptual models. Movement from below the root zone to a water table is treated by considering dispersion of a non-exchangeable ion in a homogeneous soil. Lateral movement is treated as steady, convective flow through groundwater bodies of different geometry. Model results are presented in terms of solute travel times and changes in groundwater outflow concentration; these agree with experimental data, where they have been collected in sufficient detail. This is not always possible due to the generally long travel times in the larger catchments. Model modification is necessary where denitrification, anion-adsorption or preferential flow occur.

INTRODUCTION

Intensification of cropping in wet, monsoonal ecosystems has involved the introduction of nitrogen responsive crop varieties such as dry-season rice, and improvement of drainage and irrigation to achieve better control over traditional environments. As the same time, concern has arisen in established intensive cropping areas, as well as in less intensive agricultural systems such as forest clearing for shifting cultivation, about possible nutrient losses by leaching, and subsequent deterioration of drainage- or stream-water.

In this paper we consider therefore a 'catchment' or 'watershed' in a wide context, to include both the traditional water catchment areas that provide streamflow in the upper tributaries and main stream, and the much smaller irrigation-drainage schemes in the river basins, developed for intensive agriculture. Nitrogen accessions to catchments and N-transformations in the root zone are not considered; these subjects are partly covered elsewhere in this symposium and have also been reviewed recently by Khanna (1981). Our interest here is firstly in vertical transport of the most mobile nitrogen component, NO_3^--N (e.g. Steenvoorden, 1976; Khanna, 1981) and secondly in its lateral transport through groundwater to streams or drainage outlets. Movement of the less mobile ion, NH_4^+-N, is not considered in detail, but can in principle be treated in the same way.

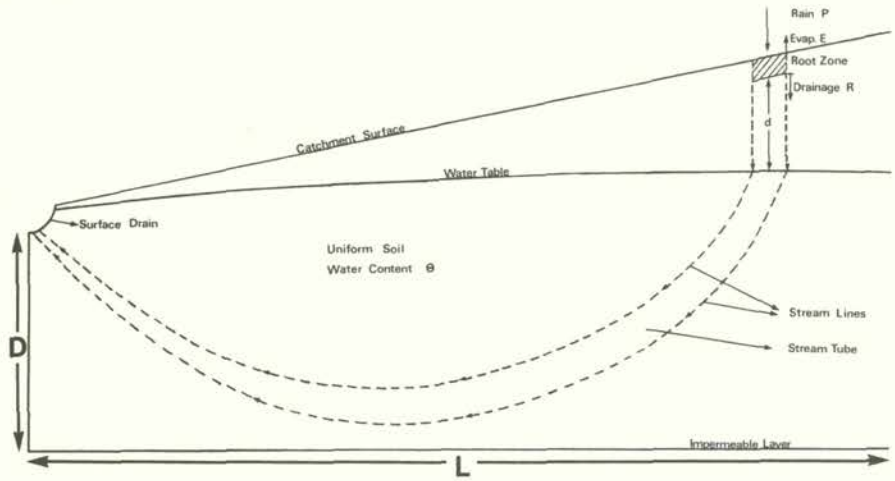

Fig. 1. Schematic presentation of soil- and groundwater flow geometry.

For a unified modelling approach to groundwater flow, all such catchments may be characterized by two dimensions; their spacing, L, between catchment boundary and effluent stream (for individual cropped fields, the half-spacing, L, between field boundary drains), and the depth, D, of permeable soil (Fig. 1). A conceptual model based on steady state water transport, that considers most of the mechanisms operating in nitrate transport, and which has given adequate agreement with observed data, is discussed below. We will indicate where and how this model needs modification. The essential elements of the model are shown in Fig. 1. Sample calculations using this model will be made by assuming, for south-east Asian monsoonal areas, an annual rainfall, P = 1500 mm, and an evapotranspiration, E = 1000 mm. This results in an annual drainage flux of 500 mm, or a daily rate R = 1.37 mm d^{-1}.

NITRATE LEACHING

That part of any nitrogen fertilizer applied to, or available near the surface, that is not taken up by plants, and remains as nitrate in the root zone, will be gradually removed by excess rain or irrigation to increasing depths beyond the root zone. As long as there is no shallow water table or impermeable sub-surface layer, such movement will be mainly vertically downward. Sub-surface impermeability results in either surface run-off or prolonged ponding; these processes are not considered in detail. Impermeable plough pans sometimes occur in wet puddled fields used for transplanted rice cultivation. For example Talsma (1974) reported severe restriction to water movement in silty loam and clay loam soils in the Central Plain of Thailand. In relation to the daily flux of 1.37 mm d^{-1} considered here, no other soils in Thailand had such restrictions to vertical leaching (Talsma, 1974; Perroux *et al.*, 1974).

It is well known that soil water containing nitrate does not usually move downward as a distinct front of concentrated nitrate but mixes with the fresher sub-soil water. When there is no solute-soil interaction (exchange), this occurs through both diffusion, from regions of high to regions of low concentration, and dispersion by flow distribution irregularities in the soil pore space. A detailed analysis of these processes has been given by Gardner (1965) and will not be fully repeated here.

Briefly the (more correct) equation considering both diffusion and dispersion (eq. 21 of Gardner, 1965):

$$\partial C/\partial t = D_a \, \partial^2 C/\partial x^2 - v \, \partial C/\partial x \qquad (1)$$

can often be simplified to a dispersion equation (eq. 24 of Gardner, 1965):

$$\partial C/\partial t = \kappa \partial^2 C/\partial X^2 \qquad (2)$$

where C = solute concentration (mg l^{-1}), D_a = 'apparent diffusion' or dispersion coefficient (cm^2 d^{-1}), v = average pore water velocity (cm d^{-1}), x = distance in flow direction (cm), t = time (d), κ = dispersion coefficient (cm^2 d^{-1}) and X = (x-vt) in cm.

Solutions to either equation (1) or (2) are available for a wide range of initial and boundary conditions (Gardner, 1965), but their use in predicting solute behaviour in field soils requires that D_a or κ and v be measured or assessed. A simpler, approximate, equation which obviates this difficulty has been given by Burns (1975):

$$f = [Rt/(Rt+\theta)] \qquad (3)$$

where the fraction f of surface applied nitrate leached below any depth x is a function of accumulated drainage Rt (cm) and volumetric soil moisture content, θ, at field capacity. Burns (1975) found good agreement with observed data of several authors.

Gardner (1965) showed that equation (2) predicted the movement of nitrate observed by Wetselaar (1962) quite accurately (Fig. 2). We observe that the peak concentration decreases with increasing quantity of applied water. This is typical when water movement through the soil is quite slow. Higher flow velocities usually result in smaller dispersion coefficients, especially where pore size is uniform (see e.g. Nielsen & Biggar, 1963), and a displacing solution may then move with a distinct front. This 'piston' flow concept is used in models, including the one used here, for calculation of travel time of contaminants in groundwater.

With reference to the model (Fig. 1) and the assumed drainage rate of R = 1.37 mm d^{-1}, an application of 200 kg NO_3^--N ha^{-1}, evenly distributed in the top 20 cm of soil, which at field capacity is assumed to have a moisture content of 40% (θ = 0.4), will result in an initial concentration C_i = 250 mg l^{-1}. An assumed recovery rate by the crop of 60% will leave 100 mg l^{-1} for downward movement through the soil profile. The average drainage flux

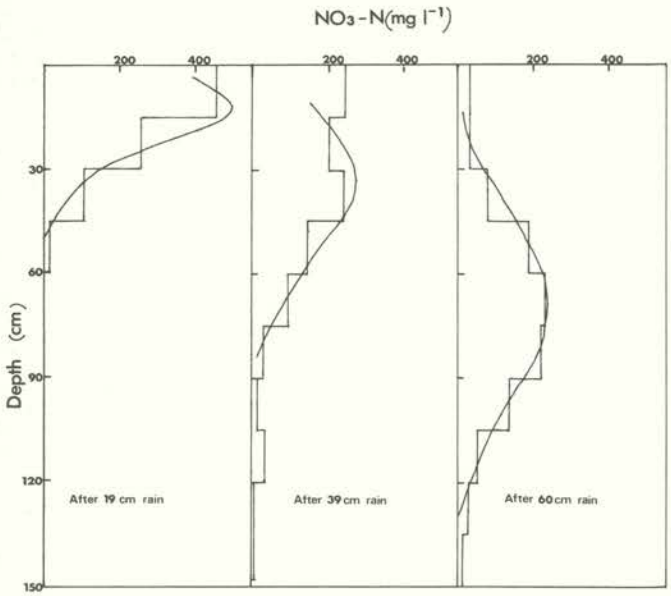

Fig. 2. Nitrate distribution observed by Wetselaar (1962), compared with calculated distribution (smooth curves); after Gardner (1965). Redrawn by permission of the American Society of Agronomy.

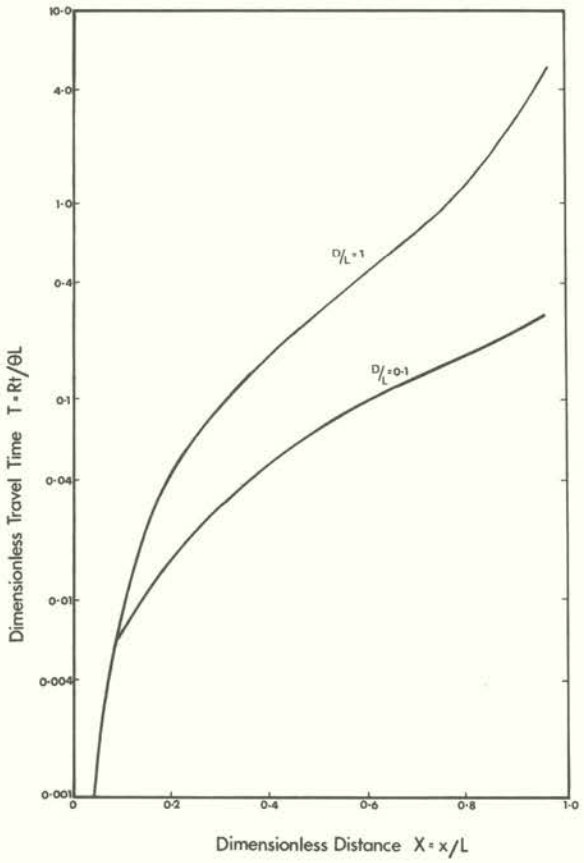

Fig. 3. Dimensionless travel time between water table and drain as a function of a lateral distance from drain line, for two flow geometries; after Jury (1965a).

$R = 1.37$ mm d^{-1} will result in a mean pore velocity, $v = R/\theta$, $\simeq 5$ mm d^{-1} (assuming a lower value, $\theta = 0.3$, for field capacity in the normally denser sub-soil layers). At such low flow velocities, dispersion should be expected (see Fig. 2) and we assume that the nitrate-contaminated water arrives at the water table with a concentration C_o of approximately 50 mg l^{-1}, at an approximate travel time for the peak concentration of $t = q/v$. If d, the distance from the root zone to the water table, is 500 mm, and $v = 5$ mm d^{-1}, $t = 100$ days. We next consider lateral movement of the nitrate-containing water from the water table, through groundwater bodies of different geometries, to drains.

TRANSPORT OF NITRATE IN GROUNDWATER

Steady state drainage theory includes many analyses that have yielded the distribution of streamlines for a variety of flow geometries. Such streamlines represent the actual flow paths followed by moving fluid particles. Two such streamlines, and the enclosed stream tube, are shown in Fig. 1.

We use here an anlysis of Kirkham (1958) as adapted by Jury (1975a), suitable for the flow geometry shown in Fig. 1. This analysis gives a general solution for the calculation of solute travel time, t, for combinations of characteristic catchment width, L (m), and depth to impermeable subsoil, D (m), soil moisture content, θ, and steady drainage rate, R (m d^{-1}). The model assumes 'piston' flow, i.e. no mixing by diffusion or dispersion below the water table. Results of such an analysis, for a 'deep' catchment (D/L = 1) and for a 'shallow' catchment (D/L = 0.1) are shown in Fig. 3 as a plot of dimensionless travel time, $T = Rt/\theta L$, *versus* dimensionless distance, $X = x/L$. Here, x is the horizontal distance (m) from the drain outlet.

Table 1. Solute travel time, t (days), in various catchments; assumed moisture content, θ=0.3, R=0.00137 m d^{-1}

Catchment description	D (m)	L (m)	Travel times (d) from X = 0.10	X = 0.30	X = 0.70
			x = 1[a]	x = 3	x = 7
Small, shallow	1	10	15	68	289
Small, deep	10	10	20	191	1445
			x = 5	x = 15	x = 35
Medium, shallow	5	50	73	339	1445
Medium, deep	50	50	99	953	7227

[a] Distance from drain (m).

Solute travel times from near and distant parts of a catchment (Table 1) and effluent concentration curves (Fig. 4) can easily be derived from relationships such as those presented in Fig. 3. Table 1 contains travel times for both small and medium sized catchments. For example, in the small, shallow catchment (L = 10 m, D = 1 m) we have at X = 0.3 (x = 3 m from the drain) T = 0.031 (Fig. 3). This gives a travel time $t = T\theta L/R = 0.031 \times 0.3 \times 10/0.00137$ = 68 days (see Table 1).

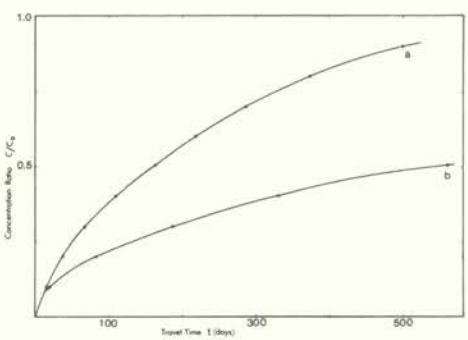

Fig. 4. Nitrate effluent curves for two catchments, $\theta = 0.3$.
(a) L = 10 m, D = 1 m, R = 0.5 m yr^{-1}.
(b) L = 10 m, D = 10 m, R = 0.5 m yr^{-1}.

Large increases in solute travel time are found at distances remote from drain outlets in the larger catchments, especially where these are permeable to great depth. The change of the solute concentration ratio C/C_o, where C is the concentration in the effluent water and C_o is nitrate containing leaching water arriving at the water table (C_o = 50 mg l^{-1} in our example), is given in Fig. 4 as a function of time for the shallow and deep, small catchments. The nitrate concentration in the effluent drainage water in the stream, or drain, reaches 50% (25 mg l^{-1} in the example) of the nitrate concentration reaching the water table after 162 days of continual steady removal for the shallow catchment; for the deep catchment this requires 560 days.

It has been assumed, in the sample calculations, that a concentration of 50 mg l^{-1} arrives continually at the water table. This would entail continuous nitrate application or availability at the soil surface. In practice, fertilizer is normally applied during the growing season only, say once or twice during a 3 months period, and subsequent removal occurs during the remainder of the year. Both single and repeated periodic inputs, as well as partially treated catchments, can be analysed using Fig. 3, as shown fully in Jury (1975a)*. Because of space limitations such realistic examples are not treated here. The main purpose of our simple example calculation is to note the rather long travel times involved in groundwater flow to surface outlets. This necessitates lengthy monitoring of groundwater and surface water quality. Successful comparison of experiments with this model should also include measurements, or close estimation, of L, D, θ and R.

MODEL VALIDITY

Model calculations made by Jury (1975b) agreed closely with experimental observations on continual chloride leaching (Sadler *et al.*, 1965) and cyclic chloride leaching (Talsma, 1967). Good agreement was also observed for nitrate leaching studied by Calvert & Phung (1971). In all these studies, transit times were short (narrow drain spacing, large drainage rates), and all parameters were sufficiently well known. These findings imply that the assumption of steady, piston-type displacement flow was reasonable under rather variable field conditions, where neither soil homogeneity nor steady state flow was strictly obtained. For large travel times (low drainage rate, large drain spacing) model verification is often difficult, since solute outflow should be related to surface applications made several years ago. Such data are often not available (e.g. Steenvoorden, 1976; Jury, 1975b). For example, Devitt *et al.* (1976) found little relation between short-term fertilizer application and either concentration or quantity of nitrate removed by several tile drainage systems with mean travel times of about 10 years.

Also, it is likely, particularly at large travel times, that nitrate may be reduced either chemically (reaction with Fe) or biochemically by denitrifying bacteria. The latter process requires low oxygen concentration, denitrifying organisms and the presence of an energy source for the microbes along the waterflow paths. Devitt *et al.* (1976) monitored chloride as well as nitrate; their study also included measurements of redox-potentials and Mn-concentrations as indicators of oxygen concentration. They concluded that denitrification did not occur in sandy soils but was likely in clay soils that had generally lower redox-potentials (<300 mV) and higher Mn-concentrations (>0.1 mg l^{-1}). Steenvoorden (1976) reached very similar conclusions. The study of Yimprasert *et al.* (1976) on sandy soils of north-east Thailand showed identical chloride and nitrate distributions; hence denitrification was unlikely. For some finer textured soils, or those containing organic matter, the model used here may need modification to include these interaction processes. Omission of such effects should not seriously change the time for NO_3^--N to appear in drainage water, but would affect its concentration.

Much shorter travel times and different solute distributions may be expected in soils containing preferential flow paths, such as occur in cracking clays and in many forest soils. The latter often have many continuous, large pores. During intense rainfall or flood irrigation, surface saturation may occur, resulting in very rapid water and solute movement through large continuous pores or planar voids. This is well illustrated by Kanchanasut *et al.* (1978) who found the critical minimum cylindrical pore diameter for preferential solute flow to be about 0.2 mm. Preferential flow was also found in a study (Sidle & Kardos, 1979) of nitrate movement following sludge application on forest soil plots. At lower water application rates the soil surface may not saturate and preferential flow would not occur.

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* Figure captions in Jury (1975a) are in error. The caption for Fig. 4 is for Fig. 6, the Fig. 5 caption is for Fig. 4, and the Fig. 6 caption is for Fig. 5.

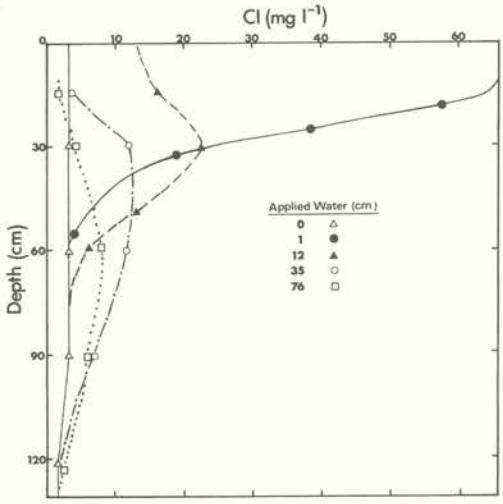

Fig. 5. Soluble chloride distribution after application of 90 kg ha^{-1} KCl to an oxisol as a function of applied water (T. Talsma, unpublished data).

Some soils (e.g. Gebhart & Coleman, 1974; Keng & Uehara, 1974) are anion adsorbers, so that NO_3^--N, like cationic NH_4^+-N, undergoes exchange reactions with the soil during downward leaching and lateral transport through groundwater. Equation (1) may then be expanded (e.g. Gardner, 1965) to include an adsorption term, to allow the prediction of leachate concentration through the soil profile. However, such predictions tend to become difficult. Investigations in Australia on oxisols and ultisols (Black & Waring, 1979; T. Talsma, unpublished data) indicate that anion adsorption increases with soil depth, and that adsorption isotherms are generally non-linear. The distribution of solute concentration in soil water may then be very different from those shown in Fig. 2. This is evident in Fig. 5, where we note in particular the rapid decay of the peak concentration as more water is applied. Only 24% of the initially applied chloride remained in solution in the soil water after applying 35 cm of water. There was no increase in soluble chloride at 150 cm depth (not shown in Fig. 5), although 10 cm out of the 76 cm applied water passed beyond this depth. In such soils, effluent concentrations of anions such as nitrate and chloride in open streams will change gradually, due to subsequent desorption, without exhibiting sharp peak concentrations.

CONCLUDING REMARKS

For many natural catchments it has been observed that outflow concentrations change exponentially with time, or with the amount of applied water. This is approximately as predicted for the shallow flow system in our model (Fig. 4). A recent review by Raats (1977), of solute transport by steady flow, predicts for shallow flow systems (when $D \to 0$) that the outflow concentration-time relation is exponential as a result of the distribution of solute transport arrival time at the outflow (drainage) surface. Earlier work (e.g. Ericson, 1971; Peck, 1973) assumed thorough mixing, such as by dispersion or preferential flow, to predict this result. It would appear then, if the convection flow model assumed here is correct, that shallow catchments represent actual field conditions better than catchments that are assumed permeable to great depth. Exceptions may be very deep, uniform, sands.

Prediction of surface water contamination by nitrate for larger catchments or agricultural basins are obviously extremely difficult. Sample calculations given here, however, are in general agreement with time scales for hydrological disturbances. These are of the order of decades for medium sized irrigation areas, and centuries for large scale catchments (Holmes, 1971).

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RECENT DEVELOPMENTS IN METHODS FOR STUDYING NITROGEN CYCLE PROCESSES IN THE FIELD

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ABSTRACT

Significant advances have been made during the past five years in methodologies for studying nitrogen cycle processes in the field. Perhaps the major achievement has been the development of techniques for measuring the exchange of nitrogenous gases between the atmosphere and the plant-soil system, the unknowns in earlier nitrogen cycle studies.

Fixation of dinitrogen by legumes can now be measured under actual growing conditions in the field using an isotopic dilution technique that does not require labelled dinitrogen gas. Instantaneous gains or losses of nitric oxide, nitrous oxide and ammonia by plant, soil or water surfaces can be measured accurately in the field with minimum disturbance to the natural environment. Methods suitable for each of these measurements are outlined.

INTRODUCTION

Considerable interest has been generated recently in the study of nitrogen cycle processes in the biosphere. This has been due partly to the need for increased food and fibre production to meet the needs of a rapidly expanding population, and partly to the recognition that increased use of fertilizer nitrogen and biologically fixed nitrogen can have detrimental effects on the environment. The increase in research effort has resulted in a significant improvement in the methodology available for studying various nitrogen cycle processes.

There have been some advances in laboratory techniques and instrumentation over the last five years; e.g., the development of the high temperature electron capture detector and its use for the analysis of small quantities of nitrous oxide. However, the most significant advances in the study of nitrogen cycle processes have been made in the development of techniques for measuring what were previously missing elements in the field situation, the exchange of nitrogen gases between the atmosphere and the plant-soil system; e.g., the measurement of dinitrogen fixation using isotope dilution techniques (Rennie *et al.*, 1978) and the measurement of nitrous oxide and ammonia loss to the atmosphere. Lack of knowledge of these processes greatly inhibited progress for many years.

Adequate laboratory techniques now exist for the study of nitrogen transformations, including the use of ^{15}N-enriched and -depleted materials, and analytical procedures for the assay of the various nitrogen species in plant materials, soils and waters. These have been reviewed by Bremner (1965a, 1965b, 1965c and 1965d), Edwards & Hauck (1974), Fiedler & Proksch (1975), Hauck (1973, 1980), and Hauck & Bremner (1976), and already are well known to most researchers interested in nitrogen.

For these reasons, discussion will be restricted to techniques developed during the last five years for studying nitrogen cycle processes in the field.

ESTIMATION OF FERTILIZER NITROGEN RECOVERY BY PLANTS

Recovery of fertilizer nitrogen by crops has been measured in the field by a number of methods. In some of these, ^{15}N-enriched fertilizers have been used, but their high cost prohibits their general use (Westerman & Kurtz, 1974). Recent attempts to increase the availability of ^{15}N-enriched compounds by the U.S. Department of Energy has resulted in large quantities of ^{15}N-depleted materials (< 0.009 atom % ^{15}N) being available at relatively low cost for research purposes (Bremner, 1977; Hauck, 1978a). The availability of highly sensitive mass spectrometers permits the use of ^{15}N-depleted materials, even when their nitrogen has been diluted about 2 000 times with natural nitrogen (Bremner, 1977).

^{15}N-depleted ammonium sulphate has been shown to be quite effective for measuring the recovery of fertilizer nitrogen by a number of different crop plants (Broadbent & Carlton, 1978; Edwards & Hauck, 1974; Patrick *et al.*, 1974; Starr *et al.*, 1974). Broadbent & Carlton (1978) showed that the depleted material was also suitable for following the movement of nitrogen derived from the fertilizer through the soil profile, down to at least 3 m.

ESTIMATION OF NITROGEN FIXATION

Detection of nitrogen increases in natural and cropped systems in the field due to atmospheric nitrogen fixation by leguminous plants is difficult, and objections can be found with nearly all of the methods that have been used. Some of these objections have been discussed by Rennie *et al.* (1978).

Several new methods have been proposed recently for the quantitative assessment of dinitrogen fixation by legumes using the ^{15}N-isotope. These are discussed below, together with the acetylene reduction method.

"A"-value

Fried & Broeshart (1975) showed that fixation could be measured using the "A"-value concept, and additions of ^{15}N-labelled fertilizer at low rates to a legume crop and at normal rates to a non-legume crop. The "A"-value is defined as the amount of available nitrogen in the soil and is calculated from the relationship

$$\text{"A"-value} = \frac{\%\ \text{N derived from soil}}{\%\ \text{N derived from fertilizer}} \times \text{rate of fertilizer applied (kg ha}^{-1}\text{)}.$$

The amount of nitrogen fixed is then determined as follows:

$$N_2 \text{ fixed (kg N ha}^{-1}\text{)} = [\text{"A"-value (fixing system) - "A"-value (non fixing system)}] \times [\text{fraction of fertilizer N used by the fixing system}].$$

This technique should be most valuable because it will provide fixation data integrated over a whole growing season (Phillips & Bennett, 1978), and the values for nitrogen fixed are obtained under the actual growing conditions (see Rennie *et al.*, 1978).

When the rate of application of fertilizer nitrogen is the same for both fixing and non-fixing crops, the calculations are simplified to:

$$N_2 \text{ fixed} = (1 - \frac{\text{atom \% } ^{15}N \text{ excess in legume crop}}{\text{atom \% } ^{15}N \text{ excess in reference crop}}) \times (\text{N uptake by legume});$$

(Fried & Middleboe, 1977).

This approach still requires further testing, as the amount fixed may be influenced by the amount of fertilizer nitrogen added, and it is important that the legume and reference crop have similar rooting patterns (Ham, 1977; Hauck, 1980; Phillips & Bennett, 1978).

Legg & Sloger (1975) used a similar approach but they added energy sources at the same time as the ^{15}N-fertilizers to ensure rapid microbiological turnover, and uniform labelling of at least the pool from which the plant absorbs nitrogen. This method involves a modification of the natural soil-plant system, and requires large amounts of ^{15}N and lengthy incubation periods (Rennie *et al.*, 1978).

Natural ^{15}N abundance

The second approach attempts to make use of variations in the natural abundance of ^{15}N in legumes and non-legumes (Amarger *et al.*, 1977; Edmeades & Goh, 1979; Rennie *et al.*, 1976). It is essentially similar to the methods given above, but there are problems in interpreting the data (Hauck, 1980). Bremner (1977) recommends that this method not be used until convincing evidence is provided that it will work.

Acetylene reduction

The acetylene reduction method, which has been in use for a number of years, is still the most sensitive method available for determining nitrogenase activity. However, during the intervening years since it was first proposed (Dilworth, 1966; Schollhorn & Burris, 1966), it has been shown to have a number of deficiencies when used for the quantitative estimation of nitrogen fixation in the field. Some of the problems arise from the extrapolation of acetylene reduction to nitrogen reduced by the use of a conversion factor without suitable calibration with ^{15}N for each of the incubation procedures used (Bergersen, 1970; Bremner, 1977; Burris, 1972, 1974; Gibson & Turner, 1979; Ham, 1977).

Other problems arise from the need to measure nitrogen fixation over a growing season. Nitrogenase activity varies with temperature, light intensity and moisture, and thus there are diurnal and seasonal variations in acetylene reduction activity. These variations make the calculation of nitrogen fixation over an entire growing season from limited short term measurements of acetylene reduction of doubtful value (Gibson & Turner, 1979; Goh *et al.*, 1978; Halliday & Pate, 1976; Sinclair *et al.*, 1976).

Problems also arise because of different solubilities of gases in water, transformations of ethylene by activities unrelated to nitrogen fixation, spatial distribution of clover plants within a pasture, etc. (Bremner, 1977; Goh *et al.*, 1978; Ham, 1977; Hauck, 1980; Witty, 1979).

ESTIMATION OF NITROGEN LOSS

The most significant advances during the last five years have been made in the measurement of gaseous emissions to the atmosphere, the unknowns in early nitrogen cycle studies. Methods are now available for the determination of nitric oxide (NO), nitrous oxide (N_2O) and ammonia (NH_3) fluxes from soils with a minimum disturbance of the plant-soil environment.

Nitric oxide

Emission of nitric oxide, and other nitrogen oxides (NO_x) from soil can be determined by placing an open-ended box over the soil and measuring the rate of increase in concentration of these gases in the box during the following few minutes (Galbally & Roy, 1978). The box used by Galbally and Roy is a perspex cube (0.8 m per side) internally lined with Mylar polyester film (Dupont) to prevent gas uptake on the walls of the box. The contents of the box are stirred by a 0.2 m diameter stainless steel fan driven by an external motor.

The NO and NO_x concentrations in the sampled air are determined by a gas-phase chemiluminescent technique. In the analyzer, the incoming air stream is mixed with a stream of air or oxygen containing 0.5-1% ozone. Any NO present is oxidized to nitrogen dioxide (NO_2) in an excited state. Red light is then emitted due to the decay of a certain fraction of the excited NO_2. A photomultiplier detector senses the light emitted and generates a current, which is linearly related to the light output. To measure NO_x (which may include NO, NO_2, alkyl nitrites and nitrates, and peroxyacetylnitrate), the air stream is diverted through a molybdenum converter which reduces the NO_x to NO and the subsequent analysis is as described above (Fontijn *et al.*, 1970; Galbally, 1977). Basic nitrogen compounds such as ammonia and amines interfere if high temperature catalytic converters are used, otherwise no interferences have been documented (Spicer, 1977). The detection limit for the system is 0.15 ppb (Galbally, 1977).

Nitrous oxide

A number of methods have been proposed recently for measuring emissions of N_2O from soils in the field (Denmead, 1979; Lemon, 1978; Rolston *et al.*, 1976; Roy, 1979; Ryden *et al.*, 1978).

The technique described by Rolston *et al.* (1976) is based on calculating the flux of N_2O at the soil surface from measurements of the N_2O concentration gradient in the soil profile and the diffusion coefficient for N_2O in soil. There are difficulties with the method. It is unlikely to be successful if N_2O production is transitory, if there are distributed sources and/or sinks in the soil (because of the slowness of the soil profile to equilibrate by diffusion), or if the production of N_2O is close to the surface (because of the difficulty in measuring the N_2O concentration gradient there). Other problems occur in gas sampling and in determining the diffusion coefficient for N_2O. Excellent discussions of these difficulties and the errors they may introduce into the flux calculations have been given recently by Kimball (1978), Rolston (1978), and Smith (1978). Rolston estimates an uncertainty of 114% in the flux estimate, and Kimball 160%.

Lemon (1978) suggests the use of micrometeorological methods for measuring the vertical flux of N_2O in the air layers above the soil. In principle, such methods are highly desirable since they do not disturb the soil or aerial environments and they integrate the flux over a large area, thus smoothing out small-scale variability in N_2O emission at the soil surface. In practice, they are unlikely to be successful because of the difficulty in measuring the very small gradients in N_2O concentration that develop in the turbulent atmosphere. Lemon (1978), for instance, calculates that even with relatively light winds (3 m s^{-1} at 10 m height) and large N_2O fluxes (300 ng m^{-2} s^{-1}, about 100 kg ha^{-1} yr^{-1}) the differences in N_2O concentration between heights of 0.5 and 1 m above the surface would be about 3 ppb, close to the limits of detection of the best available N_2O measurement systems.

However, conditions in the field are unlikely to be as favourable for the success of the method as those in Lemon's example. Data from Matthias *et al.* (1979) illustrate the point. They made measurements of N_2O concentration at different heights above the soil surface with a gas chromatograph having a resolution of ±1 ppb. Chamber measurements showed 'substantial' emissions, and gradients in N_2O concentration of as much as 10 ppb m^{-1} could be detected in calm conditions at night. By day, however, no significant differences could be detected in the N_2O concentration of the air at heights up to 6 m above the ground.

It thus seems that micrometeorological methods, requiring measurements of very small differences in the N_2O concentration of air at different heights, are not yet suitable for routine use. Trapping techniques, permitting N_2O enrichment, might eventually overcome the difficulties.

The methods described by Denmead (1979), Roy (1979) and Ryden *et al.* (1978), make use of enclosures placed over the soil to collect the emitted N_2O. Ryden *et al.* (1978) sweep air slowly through the enclosure and trap the N_2O emitted into the air stream on a 5-Å molecular sieve. The N_2O is subsequently displaced from the molecular sieve and its concentration determined by gas chromatography. The collection period extends over 3 or 4 hours and the subsequent displacement of N_2O requires another 15 hours.

Roy (1979) draws air slowly from the collection chamber while allowing its replacement with outside air drawn into the chamber through a small vent. The increase in the N_2O concentration of the withdrawn air is measured at intervals with a gas chromatograph. The experiment is terminated before the N_2O concentration reaches 50 ppb above ambient, which generally limits its duration to 30 or 60 minutes.

A novel feature of the chamber systems described by Denmead (1979) is the use of an infrared gas analyzer for measurement of the N_2O enrichment of the chamber air, which, when employed with an open air flow system, permits continuous, automatic measurement of the N_2O flux with a time resolution of minutes and a discrimination of less than 2 ng N m^{-2} s^{-1}. Outside air is drawn continuously through the chamber to one measurement cell of the gas analyzer while another air stream is drawn from outside the chamber to the second measurement cell. The analyzer thus measures the instantaneous difference between the two streams. Differences as small as 6 ppb can be measured accurately.

Variations of this system have been used successfully to measure the N_2O emission from a grass sward (Denmead *et al.*, 1979a) and from a flooded rice field (Denmead *et al.*, 1979b). The technique has been found to be sufficiently sensitive to detect the small amounts of N_2O emitted from dry soils and N_2O emissions during the nitrification of ammonium (Freney *et al.*, 1978). In more recent experiments in a grass-clover pasture (C.R. Roy, O.T. Denmead & J.R. Freney, unpublished), we compared the chamber technique with a similar one which employed gas chromatography instead of infrared gas analysis for measurement of N_2O enrichment, in addition to simultaneous comparisons with the enclosure method of Roy (1979). All systems indicated a surprisingly large point to point variability in N_2O emission (more than 100% at sites only a few meters apart), but on average, good agreement was obtained between the three methods.

In all enclosure methods certain precautions are necessary: (1) to minimize pressure deficits inside the chamber (which can induce a mass flow of N_2O from soil in addition to the normal diffusive flow), (2) to ensure that the physical conditions for the exchange of N_2O between the ground surface and the chamber air are close to those in the free atmosphere, and (3) to avoid the development of abnormally high concentrations in the chamber and/or soil air, which affect the rate of diffusion of N_2O within the soil. These problems are discussed by Denmead (1979), Focht (1978), Lemon (1978), Roy (1979) and Ryden *et al.* (1978).

Lemon (1978) also points out that long-term atmospheric pressure changes and shorter-term pressure fluctuations associated with turbulent winds might have significant influences on gas diffusion in soils in the field. He quotes some examples where the rates of emission of gases from the soil surface have been found to increase in high (gusty) winds. The extent to which these effects are simulated inside enclosures of various designs is not known, but warrants further investigation.

Ammonia

Ammonia is very reactive and very soluble. Hence, enclosure methods should not be used for the determination of NH_3 emissions from soil. The gas is likely to be retained on the walls of the enclosure and air pipes and to be dissolved by free water anywhere in the system. As well, NH_3 volatilization depends very strongly on environmental conditions: temperature, evaporation rate, and perhaps wind speed. All these will be greatly affected by the presence of the enclosure (Freney *et al.*, 1981a). Although enclosure methods have been used for measuring NH_3 emissions in the field, e.g. Hargrove *et al.* (1977), we cannot recommend them.

Micrometeorological methods offer an attractive alternative, although they do require precise measurements of atmospheric variables. They do not disturb the natural environment, they integrate the flux over a large area, and they enable the exchange process to be studied over short periods, thus facilitating the investigation of environmental effects. We describe the use of appropriate methods below.

Large areas. Where there is a uniform source of NH_3 at the ground surface over a large area, a gradient in the atmospheric concentration of the gas is developed in the air above the surface, and if conditions are steady in time, the upward transport of ammonia can be calculated from measurements of the gradient through the relationship:

$$F = -K.dc/dz. \qquad (1)$$

In eq. (1), F is the flux density of NH_3, K is the diffusivity for NH_3 in the air, c is atmospheric NH_3 concentration, and z is height.

In the atmosphere, K is very much higher than the molecular diffusivity. Its magnitude varies with height and atmospheric conditions, and it must therefore be measured in place during each determination of F. Several micrometeorological methods are available for measuring K, but need not be detailed here. Denmead & McIlroy (1971) describe procedures appropriate for use in gas exchange work. Versions of those procedures have been employed by Denmead *et al.* (1974, 1976b) to measure the flux of NH_3 over pastures, by Freney *et al.* (1981b) to study the loss of NH_3 from ammonium sulphate fertilizer applied to paddy fields, and by Denmead *et al.* (1978) to measure NH_3 exchange over a corn crop. Lemon (1978) describes approaches via the wind profile, which include corrections for the effects of temperature stratification in the atmosphere. Methods for measuring atmospheric NH_3 concentrations are given in Denmead *et al.* (1976b).

A limitation of all these methods is that eq. (1) is valid only when the flux is constant with height in the air layers above the surface. This requires that the experimental area be large and uniform. Roughly, the depth of the air layer in which the flux is constant with height is between 1/100 and 1/200 of the fetch (i.e. the distance upwind), so that practical applications of eq. (1) can only be made successfully in fields of several ha.

Small areas. Recent developments in the micrometeorological techniques suitable for 'small' areas promise greater flexibility. Their application to measurements of NH_3 emissions in various situations has been described by Beauchamp *et al.* (1978) and Denmead *et al.* (1976a, 1977). Whereas the methods described previously require large areas and uniform surface fluxes, these techniques are most successful when the experimental area is small and the surface flux in it is quite different from that in the surroundings. Their principle is described below.

When NH_3 is emitted from the soil, it is spread vertically by turbulent diffusion and is convected horizontally with the wind. Consider a perpendicular plane normal to the wind and downwind of the experimental area. The rate of transport of NH_3 across a unit area of the plane at height z, due to emission from the area, will be the product of windspeed u and the atmospheric ammonia concentration in excess of the background C. Then Q, the total flux of emitted NH_3 across a face of unit width in the plane, will be given by:

$$Q = \int_0^Z u(z)\, C(z)\, dz, \qquad (2)$$

where Z is the height of the air layer affected by the emission. The mean emission of NH_3 per unit area of soil surface is obtained by dividing Q by the distance (X) that the wind has travelled over the experimental area.

Successful application of the method requires that measurements of u and C extend to the top of the NH_3 cloud so as to account for all the transported gas. As a rough rule, $Z \simeq 0.1\ X$, but Z increases by day when thermal convection enhances upward diffusion and decreases by night when temperature inversions suppress vertical transport. From the foregoing it is evident that the method will be most successful when X is small (so that Z is a conveniently small height) and there are no significant emissions of NH_3 outside the experimental area (so that corrections for background are also small).

In order to evaluate the integral in eq. (2), the profiles of u and C must be precisely defined. This will usually require measurements at four heights at least, preferably more. As well, X must be known precisely. If the experimental area is the usual agronomist's rectilinear plot, X will vary with the direction of the wind and frequent measurements of wind direction must be made - see, for instance, Denmead *et al.* (1977). However, this complication can be overcome by working with a circular plot and measuring u and C at its centre.

Regardless of compass direction, the wind will always blow towards the centre along some radius of the circle so that X is always equal to the plot radius. Circular plots of 36 m radius were used by Beauchamp *et al.* (1978), and 25 m radius by Denmead *et al.*1976a).

Finally, it should be noted that unlike some of the large area techniques, this method requires no special form for the wind profile or any corrections for thermal stratification. If, however, the wind does have a log- or power law-profile, theory exists which may make it possible to predict the surface flux from only one measurement of gas concentration. The problem is being studied by one of us (O.T.D.), but some first steps are outlined in Denmead *et al.* (1976a).

Reliability of micrometeorological methods. Comparisons between fluxes of NH_3 or other soil-formed nitrogenous gases, as measured by micrometeorological and by other methods, is hardly possible at present, either because alternative direct methods of flux measurement do not exist, or the traditional difference methods based on soil and plant sampling are too inaccurate. However, given that the large-area micrometeorological methods have been found to give reliable estimates of other mass fluxes such as those of water vapour and carbon dioxide (e.g. Denmead & McIlroy, 1970; Verma & Rosenberg, 1975), there is no theoretical reason why they should be unreliable for NH_3.

Some confirmation of the reliability of the small-area methods was obtained by Denmead *et al.* (1977) who found good agreement between their measurements of NH_3 loss following application of anhydrous NH_3 fertilizer, using eq. (2), and the average loss calculated from total nitrogen analysis of soil samples. Uncertainties in the latter calculations, however, were 15 times the estimated loss. More recently, O.T. Denmead, J.R. Freney and J.R. Simpson (unpublished) obtained satisfactory agreement between similar micrometeorological measurements of NH_3 loss from NH_3 dissolved in irrigation water for maize and the change in NH_3 concentration of the water.

Dinitrogen. The most serious deficiency in this list is the lack of a suitable method for direct measurement of dinitrogen (N_2) loss from soil. Rolston *et al.* (1976) have used diffusion theory to calculate the flux of N_2 at the soil surface in the same way as for N_2O (discussed earlier). Unfortunately, the same problems occur and the same uncertainties apply (Kimball, 1978; Rolston, 1978; Smith, 1978). Ryden *et al.* (1979) present a technique based on the inhibition of N_2O reduction to N_2 by acetylene. While this approach to the problem is commendable, the published method cannot be recommended for general use because acetylene inhibits the nitrification of ammonium and thus removes one of the sources of N_2O (Walter *et al.*, 1979). However, the exploitation of alternative inhibitors of nitrous oxide reductase which do not interfere with other steps in the nitrogen cycle may make this method more useful in the future.

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NITROGEN IN PRECIPITATION IN SOUTH-EAST ASIA AND ADJOINING AREAS : A BIBLIOGRAPHY

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A collection of references on nitrogen in precipitation in south-east Asian countries and some adjoining regions is presented. These references have been inspected by the authors except where marked by an asterisk (*). Two bibliographies on the chemistry of precipitation are referenced along with two articles having extensive bibliographies on atmospheric nitrogen studies (Rigby & Sinha, 1961; Asman & Conrads, 1976; Eriksson, 1952; Böttger *et al.*, 1978).

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NITROGEN BALANCE IN IRRIGATED WETLAND RICE

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INTRODUCTION

Rice is one of the world's most important food crops, forming nearly 20 percent of the total world food grain production (Stangel 1979). Rice crops are mainly rainfed and their characteristic environment is in the tropics where they are usually grown in flooded paddies (Evans & Wardlaw, 1976). More than 90 per cent of all rice grain produced is grown in Asia where it is the dominant food crop.

Plant scientists suggest that modern rice varieties have the potential to produce 13 to 15 tonnes of grain ha^{-1} in the tropics, which is 3 to 10 times the average yield obtained by farmers (IRRI, 1978). One of the reasons for the gap between potential and actual yield is inadequate nutrition of the rice plant; nitrogen being the main nutrient limiting production. Where no other limitations exist, yield is strongly related to the amount of nitrogen applied and the efficiency of its utilization, i.e. it is related to the amount of nitrogen in the plant.

The nitrogen requirements of rice crops differ considerably depending on plant variety, soil type and climate and can be as high as 175 kg N ha^{-1}. However, the rice crop seldom recovers more than 30-40 per cent of the nitrogen applied as fertilizer and the remainder is lost. Because of its nutritional importance and its increasing cost, nitrogen fertilizer must be utilized more efficiently in rice production in the future. Before this increase in efficiency can be realized more background information is required on the nitrogen economy of the rice crop.

There have been many nitrogen balance studies on dryland soils (Allison 1955, 1965), but no such studies have been reported for rice in tropical regions (Koyama & App, 1979). For these reasons a working group was organized to draw up a nitrogen balance sheet for a rice field in the south-east Asian wet monsoonal region. During this exercise the gaps in our knowledge of the nitrogen cycle became apparent and some priorities for future research were developed.

Table 1. General characteristics of model and actual rice ecosystems for the south-east Asian wet monsoonal region

Characteristics	Rice ecosystems					
	Model[a]		Actual[b]			
	Range	Average	1	2	3	4
Climate						
Mean annual temperature (C)		28	27	23	16	24.5
Annual precipitation (mm yr^{-1})	800-4800	2800		2000	1231	1480
Rainy season (d yr^{-1})		150	10	100	133	160
Growing season for annuals (d yr^{-1})		365	365	260		365
Growing season for perennials + trees (d yr^{-1})				365	240	365
Growing season for cultivated spp (d yr^{-1})			365	230	340	275
Land Use						
Cultivated land (% of total area)		90		10	70	96
Unused land (% of total area)		10		0	0	0.5
Soil Data						
Carbon (%C)	0.5-3.5	3.0		1.5	1.6	0.39
Nitrogen (%N)	0.03-0.5	0.3	0.025	0.20	0.15	0.03
Phosphorus (%P)	0.012-0.037	0.025	0.0015	0.02	0.035	0.001
Potassium (mq 100 g^{-1})	0.2-0.4	0.3	0.100		1.4	
pH	4-9	7.0	5.60	6.00	6.85	6.35
Distribution of N in the soil-plant system (kg N ha^{-1})						
Cultivated spp.	0-100	50		220	110	98
Soil organic N	300-5000	2660	3500	2500	3000	640

[a] Derived from workgroup discussion.

[b] Actual data for four regions in south-east Asia. 1. Paddy rice, Philippines. 2. Paddy rice, two crops per year, China. 3. Paddy rice in a rice-rice-winter crop rotation, China. 4. Multiple cropping, rice-soybean-vegetables (fruit trees) rotation, Thailand.

SYSTEM

The model system under study was defined in the following way:

(a) Region: south-east Asian wet monsoonal region as a whole.

(b) Crop: (i) : irrigated wetland rice,
(ii) : one crop per year,
(iii): wet season crop only.

(c) Paddy field: bunds were not to be included, although they can amount to 10 per cent of the total land area.

The main characteristics of the region relating to climate, land use and chemical composition are given in Table 1 along with some actual values for defined ecosystems within that region. Estimates are given for the distribution of nitrogen in the soil-plant system. Since inorganic nitrogen varies with time and fertilizer practice no value is given for this fraction.

BALANCE SHEET

Estimates were also made for nitrogen fluxes in and out of the model system, and these are compared with values for defined ecosystems within the region in Table 2. Values are given for each of the possible fluxes, with the exception of dry deposition, as no reliable data were available for this flux in this region.

Table 2. Estimated rates of inputs and outputs of nitrogen for model and actual rice systems

Flux	Rice ecosystems						
	Model[a]		Actual[b]				
	Range	Average	1	2	3	4	5
			(kg N ha^{-1} yr^{-1})				
Fixation							
by rhizobia		0			12	57	
by algae	0-80	27	17.5	5	2	5	26
by bacteria							
rhizosphere	1-5	2	35	30		15	22
free living	1-5	2		20		0	16
Atmospheric deposition							
wet	1-25	10		10.5	23	40	5.5
dry	?	?		0.5		15	1
Manure, crop residues, etc.	0-30	5	5	60	60	58	27
Fertilizer	0-90	50	120	228	248	180	0
Irrigation water	5-10	7					
Total in		90	177.5	354	343	370	97.5
Harvest	10-75	40	90	150	204	285	51
Leaching	0-20	10	15	44	2	25	17.5
Ammonia volatilization	10-90	50	10	45		44	1.5
Denitrification			30	90	131	24	18
Erosion	0-10	5	5	5		50	5.5
Burning	0-25	10	70	20		60	0
Grazing (animals, birds, etc.)	0-10	5	0			80	6.5
Total out		120	220	354	337	568	100

[a] Derived from work group discussion.

[b] 1-4 as described in Table 1, 5 estimated by Wetselaar (1981).

Little reliance can be placed on any of the flux values given because of technical problems, and because the few data available are not fully representative for the various countries within the region. For example, fixation values for algae and bacteria were obtained by the acetylene reduction method, which is very useful for comparing microbial nitrogenase activity over short time periods, but is not satisfactory for measuring nitrogen fixation in the field over a whole growing season (Freney & Denmead, 1981). Major reasons for this are uncertainties in the conversion factors for the calculation of nitrogen fixation from acetylene reduction in different situations, and the variation in acetylene reduction activity, both diurnally and over longer periods, which necessitates numerous assays.

It should also be noted that fixation by algae varies with differences in rates of nitrogen application; the amount fixed varies from approximately 30 kg N ha^{-1} yr^{-1} for a paddy field without added nitrogen down to a few for a fertilized field (Roger & Kulasooriya, 1980).

Even fluxes which are apparently simple to measure, such as wet deposition, cannot be satisfactorily characterized. This is because it is not known whether the deposition is a real accession to the region or whether the nitrogen is merely being recycled within the region.

Again, no satisfactory figures could be assigned to ammonia volatilization or denitrification because of lack of reliable data. No direct method exists for the determination of denitrification losses, and the method used in the past for the assessment of ammonia volatilization from paddy fields either underestimate or overestimate the flux rate, depending on the conditions of the experiment and equipment used (Freney & Denmead, 1981).

Because of the large error terms placed on the values for fluxes, there seemed to be no point in preparing separate balance sheets for nitrogen fertilized and unfertilized paddies.

The input and output figures for this model ecosystem (Table 2) suggest that it is losing more nitrogen than it gains, but the variability in estimates is so large that it is not possible to draw any firm conclusion.

GAPS IN KNOWLEDGE

By considering only a single crop of irrigated wet season rice to formulate the nitrogen balance sheet the following gaps in our knowledge were highlighted.

(a) (i) The effect of poor water control on the nitrogen cycle. This is especially important, because 70 per cent of the rice grown in the region is rain-fed.

(ii) The effect of other crops on the nitrogen cycle. Rice is grown in rotation with many different crops, but our knowledge of the nitrogen cycle in rice-based cropping systems is sparse.

(b) Most of our knowledge on the fate of fertilizer nitrogen is based on research done with ammonium sulfate, not urea; the latter is the major form of fertilizer nitrogen now produced in this region and likely to be used in the future.

(c) Good baseline information is lacking, such as farmer's fertilizer use and recovery data, nitrogen accession in rain water and dry deposition,and scale of rice culture in different countries.

(d) Reliable data are needed for gaseous fluxes into and out of the system. Loss pathways via denitrification and ammonia volatilization need to be distinguished and quantified.

(e) We need a better understanding of the role of organic matter in rice production, and the relative roles of immobilization and mineralization of nitrogen, especially in certain problem areas, or where adverse soil conditions affect the behaviour of soil and fertilizer nitrogen.

(f) The environmental effects of increased nitrogen fixation or increased nitrogen fertilizer use.

PRIORITIES FOR FUTURE RESEARCH

While it is desirable that we strive to gain a better understanding of all the processes in the nitrogen cycle in the irrigated wetland rice system, there are some areas of research that must be attacked promptly because of pressure of circumstances, viz. the likely sixfold increase in nitrogen fertilizer use in south-east Asia in the next decade and its increasing cost.

For many of the component nitrogen fluxes adequate measuring techniques do not exist or are in the course of development, so that investigation of these processes constitutes a long-term expensive project. Fortunately, measuring *net* gains or losses is possible, with a reasonable degree of accuracy, so that it is possible to monitor the effects of various treatments e.g. fertilizer form, method of application, timing, etc. on the efficiency of nitrogen fertilizer use. Research in this area should aim at the development of management practices (i) to increase plant uptake efficiency, (ii) to increase gains of nitrogen by nitrogen fixation and (iii) to decrease losses of fertilizer nitrogen.

At the same time, methods for measuring nitrogen fixation and denitrification in the field need to be developed, and the relative roles of denitrification and ammonia volatilization in the loss of nitrogen from rice fields assessed. A method should also be developed for the prediction of nitrogen released from animals, green manures, and soil organic matter, so that the most efficient use can be made of supplementary nitrogen.

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NITROGEN BALANCE IN FORESTS AND PLANTATION CROPS

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INTRODUCTION

The Work group decided to restrict its considerations to the following ecosystems:

1. Natural forests.
2. Artificial forests.
3. Plantation crops, confined to rubber, oilpalm, and coffee.

NATURAL FORESTS

Forest ecosystems in south-east Asia vary widely in terms of species, types and composition, occur under a wide variety of climatic and edaphic conditions, and are subject to different management practices. All these variations cannot be covered in one nitrogen budget. It was therefore agreed that some subgrouping should be made, based mainly on climatological consideration, as follows:

1.1. Tropical forest zone, including
- 1.1.1. lowland type
- 1.1.2. hill type
- 1.1.3. peat swamps
- 1.1.4. mangrove swamps

1.2. Tropical monsoonal forest zone, including
- 1.2.1. deciduous type
- 1.2.2. evergreen type
- 1.2.3. savanna type.

Preferably, separate N-budgets should be worked out for different soil orders such as Oxisols, Ultisols, etc. Before any nitrogen budgeting is undertaken, a thorough compilation of published data should be made.

ARTIFICIAL FORESTS

The work group was of the opinion that nitrogen cycling in an artificial forest is highly dependent on the way these forests are managed. Since the management practices are in particular influenced by rate of growth, shade tolerance, and length of the rotation period, it was decided to propose budgeting for the subtypes classified on the following basis:

Shade intolerant species)	x	(Fast growing, short rotation
Shade tolerant species)		(Slow growing, long rotation

Each of these subtypes should be considered for conifers and broad-leaf forests separately.

PLANTATION CROPS

Rubber

The nitrogen cycling in, and a nitrogen budget of, 26-year old rubber plantation was presented comprehensively at the Workshop by Pushparajah (see these proceedings). However, it became clear during the preparation of this budget that separate budgets should have to be made for the active vegetative period (a 3-5-year old crop) and the peak biomass period (a 20-35-year old crop). In both cases, any cover crop and other ground vegetation such as grasses, shrubs and woody species should be taken into account. Chee & Devendra (see these proceedings) have shown that grazing animals can play an important role economically, and where present, the effect such grazing has on the nitrogen cycling and nitrogen budget should be taken into account. In addition, the different soil types on which rubber plantations exist or are being raised should be considered.

For Malaysia, most of the data are available for the construction of nitrogen balances covering the various situations discussed above. However, they have yet to be compiled. The Workgroup was not sure how relevant such Malaysian budgets would be to other areas in the region.

Oil palm

A good example of a nitrogen budget was given during the Workshop by Agamuthu & Broughton (see these proceedings), but more data are needed to prepare a complete balance sheet, covering the whole life cycle of this crop. The Work group was of the opinion that most of such data would be available in the literature, but they would have to be compiled yet.

Coffee

As for oil palm and rubber, it was thought that sufficient data would be available for the construction of a nitrogen balance.

RECOMMENDATIONS

All members of the Work group agreed on the following recommendations:

1. Through a representative of the Work group, one or two research scientists should be contacted, who are actively engaged in nutrient cycling in forests and plantation crops in each of the countries in south-east Asia.
2. These scientists should be asked to compile all relevant data on N cycling for their country within a stipulated period and make these data available to those given the responsibility for one crop.
3. A workshop should be convened on forests and plantation crops in order to
   (i) identify the gaps in our knowledge of nitrogen cycling in the ecosystems concerned,
   (ii) determine research priorities to fill the major gaps,
   (iii) plan a comprehensive and integrated research programme on nitrogen cycling in south-east Asia,
   (iv) identify sources of funds for implementing such a research programme, and
   (v) standardise procedures and methodology.

NITROGEN CYCLES IN CATCHMENT SYSTEMS

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INTRODUCTION

Catchment studies of nitrogen cycling are highly convenient because of the relative ease with which the boundaries of the system may be defined, (assuming a non-leaky catchment) and also because of the advantages of averaging over large areas. Such a system is self-contained and readily analysed in terms of classical balance methods. Because a catchment study includes all parts of the nitrogen cycle along with inputs and outputs, it is necessarily a multicrop, long-term approach which is a step beyond field plot studies.

The methodology used in catchment studies is general, in that it may be applied to some extent to studies of different elements besides or in addition to nitrogen (e.g. phosphorus, sulfur, etc.). The measurements made in a comprehensive catchment study are applicable to many substudies of components of the nitrogen cycle. Indeed the catchment approach is a methodology for integrating many levels of research in nitrogen cycling.

Catchment studies have been conducted in many parts of the world. The experiments by Likens *et al.* (1977) at Hubbard Brook is a well known example. In their publication (loc. cit.) reference is given to an additional 16 catchment studies, out of which *only one* refers to tropical areas.

Catchment studies are known to be underway in a number of locations in the monsoon regions of south-east Asia, including Thailand, Malaysia, the Philippines, and Japan. Unfortunately, the experts associated with these studies were not present at this meeting, probably because nitrogen cycling is only peripheral to the main purpose of most catchment studies in watershed management.

Catchment studies are a unique way to study some of the effects of ecological changes in a region. The understanding derived from such studies is uniquely valuable for decision making in environmental management.

This report defines catchments and some of the problems encountered in selection of study sites. The relevant processes, inputs, and outputs in groundwater, surface water, the soil-plant zone, and atmosphere are outlined briefly. Finally some research priorities are listed.

DEFINITION OF A CATCHMENT

A catchment is generally an area of land (terrain) where (at least) surface drainage is toward one stream (≡ primary catchment) or one drainage network of streams (≡ composite catchments). It does not necessarily follow that all underground drainage is towards the same outlet.

We note that boundaries of catchment systems are not so well defined for the atmospheric fluxes of fixed nitrogen. Also the validity of analyses of a catchment system (and we include systems which might be described elsewhere as watersheds) depends on the proper recognition of the time delays inherent in the system. These factors must be duly considered in such studies.

Studies of groundwater within catchments require detailed knowledge of the hydrogeology of the aquifer. This requires extensive geological and hydrological survey and aquifer testing, which needs to be accomplished before the definition of catchment boundaries can be considered complete. It is important to be aware of impermeable and leaky formations and their effect on groundwater movement.

1. *Large catchments:* 10^4 - 10^6 ha. Selection of area, and location of measuring sites should be done with care. Points to be considered are:
   - (i) The purpose of the study: monitoring land use changes? intensified fertilizer application? urbanization (domestic disposal)? or industrialization (using water for its processes)?
   - (ii) Catchment uniformity, slope, topography, geology, soil, vegetation and land use.
   - (iii) Selection of the best location so that (nearly) all flow (out) is measured at the monitoring station for water quantity and quality.
   - (iv) Large catchments have large residence and pollutant (N) transit times if they are permeable. Monitoring over long times is needed (∿100 years).
2. *Small catchments:* 1 - 100 ha. The criteria are the same as for large catchments, but problems are not so severe for site selection. More intensive monitoring is possible

and should be contemplated. Also, more precise modelling is often possible - using physically meaningful parameters that can be measured. Residence and transit times are shorter (∿1 yr), especially on slowly permeable catchments, but if such catchments are on deep, permeable soils residence times can still be long (∿10 yrs).

Notes:

(a) In many catchment studies calibration times (to monitor prior state) have been too short, or have not been undertaken. This makes a subsequent study on the same catchment very difficult.
(b) Pairing catchments is a valuable technique, where calibration time is inadequate. However, similarity of biomass composition does not always reflect the physical state of a catchment (soils, geology).
(c) Transfer of catchment information to surrounding areas is valid where analysis is physically based. Where "black box" modelling is used (often the only means for analysis of large catchment data), transfer of catchment information, is more doubtful.
(d) Constant time and proportional flow-sampling for water quality are the two most commonly used methods for monitoring outflow versus concentration relationships.

SOIL-PLANT NITROGEN

In the soil-plant nitrogen subsystem we consider that part of the process composed of plants and the soil horizon down to the root zone, which may be a few meters in depth. The process involved can be summarized thus:

(a) Atmospheric input, e.g. rainfall and fallout. This is further elaborated in the section dealing with atmospheric exchange.
(b) Inputs from irrigation water, fertilizer and any external sources such as pollutants.
(c) Fixation by plant - root association and by non-symbiotic N_2 fixation.

OUTPUTS

(a) Volatilization losses from the surface, e.g. denitrification, NH_3 volatilization.
(b) Net surface runoff, (including solution and erosion).
(c) Leachate and seepage transfer out of the root zone.
(d) Removal of biomass.
(e) Losses associated with burning (i.e. transfer to the atmosphere).

In assessing the N fluxes, one of the areas with limited information is considered to be the soil-plant zone. The processes involved (biological, physical and chemical) in this zone are complex and relatively not well studied. It would be desirable to carry out intensive studies on these processes in small catchments, where possible.

The measurements needed are:

(a) Specifications of the state conditions. These include soil characteristics, (e.g. N content, organic matter, texture, moisture characteristics), other N pools (and forms), plant growth, and human activities in the area of study.
(b) Dynamic aspects.

Chemical and biological transformations and exchanges between solute, gaseous, and solid phases.

Comprehensive measurements of these are needed in order to facilitate the transfer of findings of such studies to other areas where intensive studies can not be conducted.

ZONE BELOW THE ROOTING BY PLANTS

This zone is the area of relatively lower biological activity. At its upper boundary it interacts with the soil-root zone while in the lower boundary it may reach the ground water.

In the transition zone, it is necessary to monitor the amount of nitrogen leached, if possible with methods that involve minimum disturbance. Such measurements are needed, particularly in view of the annual cycles of saturation and drying of the surface soil. At the lower boundary, it is necessary to evaluate the fluxes of N in and out of the ground water. Parameters of interest are the aquifer characteristics, i.e. water storage and transmission capacity of the aquifer, as well as nitrogen concentrations, exchange and transformations in the aquifer.

It should be noted that all the processes are interactive to varying degrees. Hence, the delineation of zones is diffuse. Any measurements of parameters need to account for space and time variations adequately so as to enable estimation of net nitrogen fluxes.

Groundwater quality surveys are needed in order to point out irregularities in the nitrogen content of groundwater and to pinpoint areas with potential health problems. Because

of the long residence times for groundwater, surveys need not be conducted very frequently but need to be conducted systematically in order to identify problem areas before they develop. Environmental damage to aquifers will not be easily reversed.

EXCHANGE PROCESSES FROM AND TO THE ATMOSPHERE IN A CATCHMENT

The processes involved in this exchange are:

(1) uptake of gaseous NO, NO_2, NO_3, dry particulate nitrate, ammonium and organic N, rainwater containing nitrate, ammonium and organic N, and N_2 for fixation,
(2) release of gaseous N_2, NO, NO_2 and NH_3 and dust rise including nitrate ammonium and organic N,
(3) the role of fire in the N balance. This needs careful examination, especially where a forest is cleared and burned for agriculture.

We think that when the horizontal scale of a catchment system is of the order of 100 km or larger, the uptake and release rates of NO, NO_2, NH_3 and Organic N probably balance each other over the catchment. This balance does not include the net N_2 exchange (fixation minus denitrification).

For smaller catchments of a horizontal scale of the order of 10 km or less, the uptake and release of these forms of fixed nitrogen need to be measured or estimated, because there may be horizontal changes of the concentration of these species in the atmosphere and this could lead to net inflow or outflow of fixed nitrogen through the atmosphere above the catchment system.

Because of the long time scale of groundwater storage and transmission, the atmospheric inputs need to be adequately sampled at a number of sites throughout the catchment and measurements need to be made over a number of years to obtain a suitable average atmospheric input into the catchment.

There are a number of problems with atmospheric inputs that concern the group. In particular the measurement of horizontal transport of particulate nitrogen (in all forms) and its rise and deposition within a catchment, whether the latter be by interception on vegetation, or dust fall on the ground. These fluxes are most difficult to measure. It is difficult to assess how important this transport is compared with other inputs.

RECOMMENDATIONS

(1) Existing catchment studies in the region should, where possible, make nitrogen studies as part of their overall programme.
(2) In intensive studies of nitrogen in catchment systems, there must be quantitative evaluation of soil characteristics and studies of the transformations of soil-N in the rhizosphere, e.g. mineralization, exchange, denitrification, fixation.
(3) Further studies of leaching of N from the soil-plant zone are needed, particularly in view of the annual cycles of saturation and drying of the surface.
(4) Systematic surveys of the nitrogen content of groundwater, river water, rainwater, and irrigation water are needed at various locations in the catchment.
(5) There is a need for further research on chemical reactions and exchange reactions of nitrogen within the aquifer over long-time periods of greater than 100 years.
(6) Studies of the gaseous uptake/release by plants and the soil of fixed nitrogen with the atmosphere are needed.
(7) Development of a method for measuring the dust rise, horizontal transport and deposition of particulate nitrogen within catchment systems is highly desirable.
(8) Studies on transfers to the atmosphere are vital in the tropics, i.e. on denitrification and the effects of burning.

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NITROGEN CYCLING IN SHIFTING CULTIVATION

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INTRODUCTION

Shifting cultivation systems vary considerably between different locations and between different ethnic groups. The workgroup decided to define shifting cultivation as: "a cyclical system involving a period of cropping followed by a period of natural revegetation". This includes not only the slash and burn systems commonly found in highland areas of south-east Asia, but also other types of swidden agriculture where natural vegetation is cut and removed, crops are grown from one to several years and then the land is left idle. The regrowth after cropping may be secondary forest, grassland or weeds, depending on various factors including farming practices, the prevalence of fire and grazing animals, and ecological aspects such as climate, soil and natural vegetation.

Initially the group intended to investigate two systems, one involving a swidden type of system in the uplands of north-east Thailand and another system of slash and burn agriculture in a highland setting. However, there was insufficient time to deal with the latter system and the group instead concentrated on attempting to describe changes in soil nitrogen associated with swidden cultivation of Cassava. This system was chosen because it was well known to some members within the group and some information on it was available. It is not necessarily representative of shifting cultivation in other parts of Asia and is certainly different from highland shifting cultivation systems such as described by Zinke *et al.* (1973) and Nakano (1978).

DESCRIPTION OF THE SYSTEM

Climate

The north-east region of Thailand is a slightly elevated plateau of 17 x 10^6 ha, approximately 100 to 300 m above sea level. The area lies between 14-19°N latitude and experiences a tropical savannah climate (Koppen "AW") with pronounced seasonal distribution of rainfall. At Khon Kaen (central north-east Thailand), more than 85% of the annual total of 1255 mm falls in the six months from mid-April to mid-October, due to the influence of the south-west monsoon. The duration of humid months is slightly longer in the border areas. Mean annual temperature is 27 C.

Soils and vegetation

A series of alternate sedimentation and erosion phases, due primarily to the action of the Mekong and its tributaries, have created four sedimentation levels designated the alluvial plains, low terrace, middle terrace and high terrace (Moorman *et al.*, 1964). The upland soils which have formed on these terraces are largely grey podzolics, red-yellow podzolics and red-yellow latosols; these soils are characterized by sandy texture, acid reaction, low organic matter contents (0.4-0.7% carbon), low cation exchange capacity and a low level of plant nutrients. In particular, upland soils of the north-east appear to be deficient in nitrogen, phosphorus and sulphur.

Vegetation is characterized by an open dipterocarp forest with an understory of bamboo grass (*Arundinaria* spp.).

During the dry season an area of forest is cut, timber is then removed and burnt. Remaining stumps are cut and removed from the swidden, which is then cultivated and planted to cassava usually before the first rain. After about ten months, the cassava is harvested and normally both tops and roots are removed. The same swidden will be recropped with cassava for another two or three years and then abandoned. This land then reverts to woody perennial weeds such as *Eupatorium odoratum* or *Hispis* spp. and grasses particularly *Digitaria* species.

NITROGEN BALANCE

Insufficient data were available to accurately assess the nitrogen balance of the system. Based on the results of Zinke *et al.* (1973), it was estimated that 150 to 200 kg N ha^{-1} would be lost when the forest was cut and burnt or removed. When cassava is harvested 60 to 70 kg N ha^{-1} is removed each year. However, no data were available to estimate losses due to leaching, denitrification or erosion.

On the input side, no fertilizer is applied and the major influx of nitrogen would appear to come from rain. However, this has not been measured in this area and, in addition, the gains from nitrogen fixation were unknown. Rough estimates of the unknown parameters suggested that there was a net loss of nitrogen from the system, but it was impossible to state the magnitude of this loss with any confidence. It has been observed both in this area, and also in West Africa where similar practices are used (Nye & Greenland, 1960), that yields of cassava decline with each successive crop. This would be consistent with a depletion of soil nitrogen, but whether or not this is the cause of the decline in yields could not be determined from our data.

RECOMMENDATIONS

The following recommendations were agreed upon:

1. An international survey should be undertaken of which the results should yield a proper definition and description of the different shifting cultivation systems and their distribution over south-east Asia.
2. On the basis of this survey a selection of particular systems should be made for further research on the stability of the systems.
3. Such research should include at least measurements (i) of net changes in nitrogen stored in the biomass and the soil in the virgin state and at various intervals during the cropping and revegetation phase, and (ii) of as many nitrogen fluxes as possible to explain these net changes.
4. An attempt should be made to standardize techniques for sampling and for measurement of the difficult fluxes.
5. Based on the results obtained, existing and alternative management practices should be examined to be able to advocate an economically and ecologically sound system.

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RELEVANCE OF NITROGEN CYCLING STUDIES TO THE MAB (MAN AND THE BIOSPHERE) RESEARCH PROGRAMME

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The Work Group recognized the significance of the basic theme of global MAB research, the evaluation of human activities on structure and functioning of natural and derived ecosystems for better management and conservation, and particularly for south-east Asia, the ecosystem studies in relation with socio-economic aspects of the region (as outlined in MAB Report, Proceedings of Kuala Lumpur Meeting, 1974). It should be stressed that, among nutrients, nitrogen appears to be the key element that limits the primary production of the vegetation, the fertility of the soil and growth of animals, including Man. Nitrogen may also have deleterious environmental consequences. As evident from the presentations at this workshop, too little is known about the storages and transfers of nitrogen in forests, plantations, rice fields and other cropping systems that are main resources in south-east Asia. Details of the MAB projects (on-going or proposed) in south-east Asia were not available. However, from the information submitted by participants, it appears that nitrogen is not included in several MAB projects, such as sedimentation loss studies in rivers, logging studies in forests, etc. The Work Group felt that studies on nitrogen are especially relevant in the following MAB projects for this region: Project 1 on tropical and sub-tropical forests, Project 3 on grazinglands and savanna, Project 5 on lakes, marshes, rivers, deltas, estuaries and coastal zones, Project 6 on mountain ecosystems, Project 9 on assessment of pest management and fertilizer use and Project 14 on environmental monitoring.

In view of the above considerations the Work Group recommends to the International Coordinating Council of the MAB programme to:

1. initiate action to urge the participating countries to include nitrogen balance sheet studies in their programmes, particularly in MAB projects 1, 3, 5, 6 and 9; where appropriate, nitrogen studies should also be included in project 14,
2. support the training and exchange of research personnel for wide application of techniques for different nitrogen studies,
3. sponsor periodical workshops to assess the state of knowledge of nitrogen in diverse ecosystems, and on the earth as a whole.

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