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Nitrogen fixation in some rice soils in Sri Lanka

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Introduction

Biological nitrogen fixation is the major source of fixed nitrogen in traditional tropical agriculture (Döbereiner *et al.* 1978). For rice, it is one of the main causes for sustained production throughout centuries (De 1936; Becking 1971; Yoshida & Ancajas 1973; Stewart 1977; Watanabe *et al.* 1978a,b).

In many developing countries like Sri Lanka, chemical fertilizers and newly bred high-yielding rice varieties are now being exploited to meet the demands of increasing food production for a rapidly growing population. However, owing to the escalation in the cost of the fossil fuel-based nitrogenous chemical fertilizers much less is being used than is recommended. Although these fertilizers are highly subsidised for the farmers there are problems with import, transport, storage and distribution, implying that they are not always available at the required time. Broadcast application of the commonly used N-fertilizers, urea and ammoniun sulphate, into highly organic soils, such as in the low-lying lands (34 000 ha) in south-west and west Sri Lanka, may also be of reduced value owing to the relatively high soil pH. Large doses of N-fertilizers often result in great losses of ammonia by volatilization and of N_2 by nitrification with concomitant risks for leaching and thereby water pollution (Craswell & Vlek 1979). Concentrations of N as NO_3^- of 40 ppm or more are not uncommon in organic paddy soils of Sri Lanka (G. Jayaweera, personal communication). The potential for denitrification losses at such levels is certainly appreciable under the highly reducing conditions that prevail in these soils (histosols). In heavily N-fertilized rice fields in Japan (Matsuguchi & Shimomura 1977) denitrification losses have been estimated at a rate of 50 kg N ha^{-1}.

It is therefore highly desirable to use and optimize the natural nitrogen cycle, which is energy-conserving and environmentally preferable (Döbereiner 1977). In this cycle, the greatest inputs of nitrogen occur through biological nitrogen fixation. These inputs are basically organic, which can be advantageous for crop fertilization and control of pollution (Benemann 1979; Granhall 1981). Furthermore, biologically-fixed N is released slowly (Stewart *et al.* 1979) and other biologically-active extracellular

products, formed by the N_2-fixing organisms, may enhance the growth of other organisms, e.g. rice (Jacq & Roger 1977; Mohan & Mukerji 1979; Roger & Kulasooriya 1980).

The principal agents of nitrogen fixation in wetland rice fields are the cyanobacteria (blue–green algae), free-living or in symbiosis with the water fern *Azolla* and bacteria associated with rice roots and in the bulk of soil (Watanabe 1979). The potentials for nitrogen fixation in rice fields may be considerable, especially by cyanobacteria, as they do not need exogenous energy sources (Watanabe 1979; Roger & Kulasooriya 1980). Values as high as 80 kg N ha^{-1} y^{-1} for free-living cyanobacteria and 840 kg N ha^{-1} y^{-1} for *Azolla* have been reported by Traore *et al.* (1978) and Singh (1977) respectively. Rhizosphere fixation in the order of 5–10 kg N ha^{-1} y^{-1} have also been reported (Anon. 1979) and fixation in the non-rhizosphere can sometimes be substantial, especially after addition of rice straw (Kimura *et al.* 1979).

In order to assess the potentials for nitrogen fixation in rice fields in Sri Lanka a cooperative research programme was initiated in 1977 between the Swedish Agency for Research Cooperation with Developing Countries (SAREC), the National Science Council (NSC) of Sri Lanka, the Swedish University of Agricultural Sciences, Uppsala, and the University of Peradeniya, Peradeniya, Sri Lanka. The first *in situ* measurements of nitrogenase activities in rice fields in Sri Lanka, by means of the acteylene reduction technique, were performed during these investigations (1977–1980). Some of the original data, along with subsequent observations, are summarized in this paper. Comparative assays between the test sites were conducted at harvest time in order to minimize seasonal differences in soil conditions.

Materials and methods

Rice soils of Sri Lanka—general information

The total area in Sri Lanka under rice cultivation in 1980/81 was about 700 000 ha. Based on rainfall, vegetation, soils and present land use, the country can be divided into the main agroclimatic zones (Fig. 1) of wet, intermediate and dry (Panabokke 1978).

The three sites studied represent each one of these zones. About 50% of the rice lands are situated in the dry zone, 20% are in the intermediate zone and 30% are in the wet zone. The main rice soils are distributed over five soil orders and consequently exhibit great diversity in their pedology. Low humic gley soils and reddish brown earths predominate.

Experimental sites and field treatments

Bentota Agricultural Research Station. The site is situated in the low country of the wet zone, in south-west Sri Lanka (Fig. 1). Annual rainfall is 1500–1900 mm and generally only one crop of rice is grown here each year (Maha season, from August to January/February), owing to extreme soil problems. The soils in this region are highly organic histosols (bogs, half-bogs or gleyic–alluvial) with 10–30% organic matter. They are phosphorus-, iron- and manganese-deficient, slightly acid (pH 6–7), have a high groundwater table and are poorly drained. Soil conditions are heterogeneous but, generally, strongly reducing activities prevail. The soils are flooded by seasonal rains (Yala flood, April/May; Maha flood, October/November). The soils are also saline

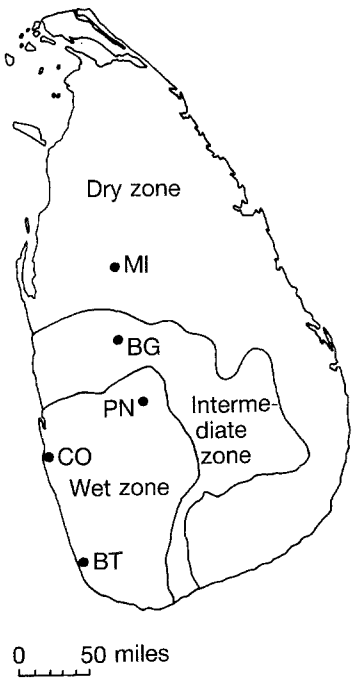

Fig. 1 Map of Sri Lanka showing different climatic zones. CO: Colombo; BT: Bentota; PN: Peradeniya; BG: Bathalagoda; MI: Maha Illuppallama.

owing to periodic sea-water influx. Altogether this results in very high risks for rice cultivation and very low yields. There are, however, very few alternatives to rice cultivation on these soils. Ratooning rice varieties are of interest in this region as they may give a second rice crop and thereby increase annual yields by 60–70% (G. Jayaweera, personal communication).

The recommended N-fertilization level is only 40 kg N, as higher levels result in lodging problems, besides other risk factors. The soil investigated was a gleyic–alluvial soil.

Field assay and treatment

Field assays were conducted just after harvest (Maha season) on stubbles at the initial ratooning stage of the common high-yielding rice variety 'BG 11-11', initially broadcasted. Plots investigated received varying N-additions (urea; 0, 30 and 90 kg N ha^{-1}, as one basal and two top dressings) and basal P and K (60 kg ha^{-1} of each element, in the form of superphosphate and KCl (muriate of potash) respectively. No plot had received any nitrogen the previous season.

Bathalagoda Agricultural Research Station. Bathalagoda is situated in the low country of the intermediate zone with an undulating or flat terrain (Fig. 1). Annual rainfall is 1000–1500 mm and two crops of rice are grown annually. Red–yellow podzolic soils, low humic gley soils and regosols dominate. Organic matter content is generally around 2% and soil pH between 5.8 and 6.7. In our study, however, the gley soil

investigated had an organic matter content of almost 10%. The recommended N-fertilization level is 120 kg N per crop. No specific fertility problems occur.

Field assay and treatment
Field assays were conducted at harvest time, 91–92 days after transplanting in plots of a yield trial, planted with a modern, brown plant-hopper resistant rice variety ('BG 379'), with urea at recommended fertilizer levels (120 kg N cop^{-1}, as basal and two top dressings). P and K (60 kg of each) were given as basal dressing in the form of superphosphate and KCl (muriate of potash) respectively.

Maha Illuppallama Agricultural Research Station. This site is situated in the low country of the dry zone (Fig. 1). Annual rainfall is 750–900 mm and where irrigation facilities are not available only one crop of rice can be grown annually (Maha season). Soils are of the low humic gley type, approximately neutral and with an organic matter content between 4 and 6%.

The recommended N-fertilization level is, on average, 90 kg N per crop. The particular site investigated had a high content of sand and gravel, causing seepage and percolation problems, and the fields needed daily flooding for cultivation of wetland rice.

Field assay and treatment
Field assays were conducted at harvest at the end of the 1978/79 Maha season in periodically flooded fields of transplanted rice, variety 'BG 11-11'. The investigated plots were part of a randomized complete block experiment with four replicates. The field had dried out 10 days before investigation, but was irrigated and kept flooded for one or two days before assays to revive dormant surface organisms and to create soil assay conditions more similar to the other sites. Triplicate plots of the following treatments were assayed:

(a) Plots with varying N-additions (0, 20, 40 or 90 kg N ha^{-1}) in the form of urea which was applied one-third of each total quantity at a time, as basal, at maximum tillering and at primordia initiation, and with 22 kg P and 62 kg K ha^{-1}, in the form of superphosphate and KCl (muriate of potash).
(b) Zero-N (0-N) plots with basal P and K plus Mo (0.3 kg Mo ha^{-1} as sodium molybdate sprayed at the time of cyanobacterial inoculation).
(c) 0-N PKMo plots inoculated one week after transplanting with cyanobacterial biofertilizers, either with 10 kg dry weight ha^{-1} of an indigenous axenic strain of *Scytonema simplex* (A_s) or with 12.5 kg dry weight ha^{-1} of a soil-based mixed cyanobacterial inoculum (A_i) recommended for field use in India. This mixture contained approximately equal amounts of *Aulosira*, *Tolypothrix*, *Scytonema*, *Anabaena*, *Nostoc* and *Plectonema* spp (Venkataraman 1977, 1979). Both biofertilizers were in an actively-growing stage, i.e. healthy surface growth was harvested from aqueous cultures in shallow trays kept outdoors (Venkataraman 1979).
(d) Untreated control plots (without NPK or Mo) were also included.

In order to reduce insect damage to rice plants, Furadan (carbofuran) was added to all plots at a recommended field concentration of 2.6 g m^{-2}. This pesticide is unlikely to

have had any negative effects on cyanobacterial acetylene reduction at Maha Illuppallama, since soil pH was relatively high (6–8) and oxidizing conditions prevailed (see Kar & Singh 1979; Nayak & Rajaramamohan Rao 1980).

General experimental protocols

Field acetylene reduction activity (ARA) assays. Three principal types of experiments were performed for the measurement of ARA at each site: (a) ARA of flood water and/or surface soil between rice hills; (b) ARA associated with rice stem bases; and (c) total ARA of rice hills (plant and soil, including the rhizosphere).

Assays were conducted at ambient gas conditions with addition of 10% (v/v) acetylene. All ARA measurements were conducted on randomly selected triplicate samples per treatment. Controls for endogenous ethylene formation were always included (light-incubated). Light and temperature conditions were followed during incubations (in the case of chamber assays, both inside and outside bags). The change in flood-water pH during the assay period was followed in one case (Bentota).

Flood water, soil and rice stem ARA. Water and soil samples, taken with disposable syringes or with an auger (diameter 1.6 cm, pushed down to 5 cm) respectively, were incubated in 30 ml McCartney bottles for a few hours (see below). Cut rice stem pieces (basal 5 cm of each hill) were incubated likewise. From these bottles, 1-ml gas samples were withdrawn once, or at intervals and stored as described below.

Rice hill (ARA-chamber) assays. Total ARA was measured in gas-tight plastic bags attached to metal cylinders (diameter 13 cm) inserted into the soil as described by Lee & Yoshida (1977) with some modifications (see below). All chamber assays were performed throughout a 24-h cycle and 3-ml gas samples were withdrawn after suitable periods of incubations (in the morning, midday, afternoon and night) and injected into pre-evacuated 7-ml glass bottles fitted with screw caps and rubber serum liners. The latter were stored in the refrigerator until analysis (within a couple of days). At the end of each 24-h experiment the water-saturated soil phase of each assay chamber was stirred for 2 min, with a previously inserted cobra-shaped iron wire or a bamboo-stick, to release ethylene absorbed in soil and water (Watanabe *et al.* 1978a,b). Gas samples were removed prior to, and after, stirring as a check of the effect of this procedure.

At the Bentota and Bathalagoda sites, chamber assays were conducted after separate filling and evacuation with a hand vacuum pump. Filling with air (9 l) and acetylene (1 l) was done manually from large gas cylinders. At Maha Illuppallama, a more convenient method was used: to purge the evacuated assay chamber at the start of incubations with the correct amount of acetylene (10% v/v) and thereafter fill them to full expansion size (24 l) with air from a gas cylinder. With this method the plants were prevented from touching the walls of the bags onto which evaporated water vapour sometimes condensed. In any such case, the water droplets were repeatedly removed by gently tapping the bags.

Three types of plastic bags: 'IRRI' Saran Bags (Lee & Watanabe 1977), hand-made (24 l) Cryovac three-layered barrier bags (Grace Duncan Co., USA) and Japanese 'Flek-samplers' (obtained from Dr Watanabe, IRRI) were compared with respect to

gas leakage. The two former types were also checked with respect to light penetration and temperature conditions *in situ*.

The comparison between the Cryovac bags and the 'IRRI' bags under *in situ* conditions at Bentota showed that light intensity (total range 5–108 klux) was only 9.6% lower inside the Cryovac bags than outside, compared to an 18.3% reduction in the 'IRRI' bags ($n = 3$). Temperature conditions measured during 24-h assays both at Bentota and Bathalagoda varied between 21.1 °C and 43.9 °C but were only 0.9 °C ($n = 17$) higher inside the Cryovac bags than outside. Environmental conditions could thus be regarded as relatively unchanged at the rice canopy within the Cryovac chambers. Previous investigations have shown that 'IRRI' bags (Lee and Watanabe 1977) and Cryovac bags (T. Lindberg, unpublished) show low or negligible gas leakage and that they do not change in volume during a 24-h assay. Flek-samplers, however, showed a leakage of ca. 10% during the same time. Cryovac bags were therefore judged as being the best and were used subsequently.

Separation between phototrophic and heterotrophic activity. In order to separate phototrophic from heterotrophic activity, half the bottles/assay chambers were incubated under prevailing daylight conditions and half under constant dark, after preincubations in the dark or by water replacement. In the latter case, the surface soil was scraped off and replaced with clean tap water (Watanabe *et al.* 1978a,b).

Since surface scraping could disturb the micro-habitat the following alternative methods were also tested: subsites chosen randomly within each plot with inserted iron cylinders (later replaced by assay chambers) were covered with black plastic bags during the preceding night and morning (ca. 20 h), or water replacement was made without surface scraping but with dark-covering of the rice stems and surface soil. In both cases subsequent incubations were made under dark-cover.

Sampled materials (in closed bottles) were preincubated in the dark (wrapped in aluminium foil), soil samples for 20 h and stem bases for 5 h, momentarily exposed to ambient gas conditions and dark incubated with acetylene. Nitrogenase activity (ARA) of soil or rice stems estimated by the above methods is ascribed to heterotrophic activity in general, whereas activity in the soil–plant system represents mainly rhizosphere activity, because the rice plant acts as an efficient transporting system for various gases whereas gas diffusion into the bulk of submerged soils is comparatively slow (Lee & Watanabe 1977; Becking 1978).

ARA analysis. Acetylene and ethylene were analysed on a Perkin-Elmer Sigma 4 gas chromatograph with an H_2-flame ionization detector, fitted with a 1.8-m stainless-steel column packed with Porapak T (mesh size 100–120), run at 80 °C and with N_2 as carrier gas at a flow rate of 30 ml min^{-1}. In all estimations of N_2/fixation, the conversion rate for acetylene reduction to nitrogen fixation was assumed to be 4:1 (Burns & Hardy 1975; Peterson & Burris 1976; Watanabe *et al.* 1978a,b; Basilier 1980).

Environmental factors. Light intensity and water pH were measured with portable standard instruments (Diffusion Systems Ltd photoelectric photometer and Corning Model 3 pH-meter, respectively). Temperature was checked with maximum–minimum thermometers before and after each period of sampling.

Chemical analysis. The pH of soils was measured with the portable pH-meter in equilibrated 1:2.5 soil–water suspensions. Organic matter content was determined after oxidization at 350–400 °C for 7–8 h (Jackson 1962). Total-N and NH_4^+-ions were also determined (Jackson 1962). NH_4^+-ions were extracted with 10% NaCl-solution, acidified to pH 2.5 and nesslerized before analyses. Most soil analyses were made on triplicate core samples (15-cm deep) from each plot.

Bacterial and cyanobacterial counts. Heterotrophic bacterial counts were made from triplicate 5-cm soil cores from each plot diluted 10^{-3}, 10^{-4}, 10^{-5} and plated on an N-free salts medium (Henriksson 1951) with sucrose (20 g l^{-1}) instead of glucose to include *Azospirillum* (Okon *et al.* 1976). Plates were incubated at room temperature (ca. 27 °C) and counted after growth for 7 days at pH 6 or at both pH 4 and 7.

Cyanobacteria at the surface (0.5-cm depth) of soil cores were prefixed in Lugol's iodine solution, diluted, agar smears prepared on slides and the organisms counted directly under the light microscope. Five slides were prepared from each sample 20 fields (diameter 0.36 mm) counted from each slide under × 400 magnification (Saito & Watanabe 1978).

Results and discussion

(A) Bentota

In situ ARA values of light incubated chambers, with and without water replacement, and of dark incubated chambers are shown in Fig. 2A. As the ARA measured after removal of flood water and surface organisms was the same as under dark incubations, only mean values of all heterotrophic activities, representing mainly rhizosphere fixation (see Methods), are therefore shown in Fig. 2A. This also means that bacterial N_2-fixation in the flood water and on the surface of the soil could generally be neglected.

Elimination of the bulk of phototrophic activity greatly decreased total nitrogenase activity. Heterotrophic (rhizosphere) fixation was generally less than 10% of the total. Total fixation responded well to changed light conditions during the different incubation periods, whereas dark-incubated chambers and such with water replacement showed much lower values correlated mainly to changes in temperature. Both phototrophic and heterotrophic fixation rates were highest during the midday period (10.00–14.00 h). The appreciable total activities during the night (about 35% of total) minus heterotrophic fixation (Fig. 2A) may well be ascribed to the numerous cynaobacteria (see below) as it is well known that these organisms can use previously-formed photosynthates for endogenous N_2-fixation in the dark up to at least 6 h (Granhall & Lundgren 1971; Lännergren *et al.* 1974).

Light intensities in the flood water at 5-cm depth (total depth 15–20 cm) were only about one-third (28.9% at 10.40 h) of that at the rice canopy. As the bags decreased the light to some extent (see Methods) and some shading also occurred inside the cylinders (owing to difficulties in inserting them totally into the boggy soil) incident light intensities were clearly lower in the flood water inside the chambers than outside. This means that the measured photrophic fixation at Bentota might be a slight underestimation of the actual activity.

During all incubation periods, total N_2 fixation was higher in 0-N plots compared to 90-N plots (Fig. 2A). Total daily (24 h) ARA values were 2.5 times higher in 0-N plots

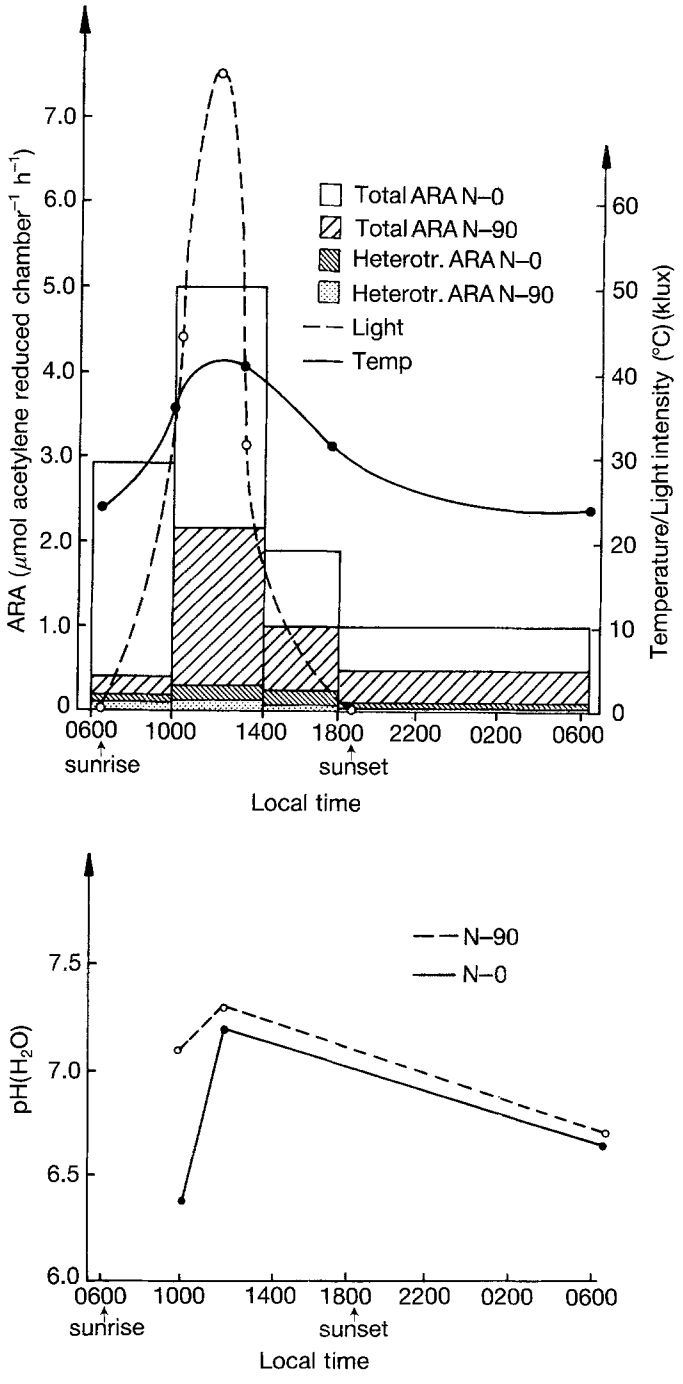

Fig. 2 Daily *in situ* nitrogenase activity at Bentota of cyanobacteria and rhizosphere bacteria (see text) of ratooning stubbles of rice variety 'BG 11-11' (end of Maha season 1977/78) at 0- and 90-N fertilizer levels. Temperature inside chambers, and light intensity in surface waters inside chambers (ca. 70% of incident light) are also shown. Mean values of 2–6 chambers per treatment.

Fig. 2B Daily *in situ* changes in flood-water pH at Bentota at 0- and 90-N fertilizer levels.

compared to 90-N ones (Table 1). Total ARA in 30-N plots is not shown, owing to leakage in two of the light incubated chambers. As photosynthetic bacteria were not observed microscopically in any soil or water sample, total fixation minus heterotrophic fixation can probably be attributed to cyanobacteria alone. Cyanobacterial nitrogen fixation thus accounted for 94 and 87% of the total values in 0-N and 90-N plots respectively. There are reports (Watanabe *et al.* 1979a,b) of a corresponding value of 88% in unfertilized long-term fertility plots at IRRI. The dominating organism in all treatments and plots was *Scytonema simplex* (Bharadwaja), a heterocystous cyanobacterium which covered the soil surface and also occurred as an epiphyte on submerged weeds and as floating 'hairy' macro-colonies. This organism was noted, both in the field and in the laboratory, to move up and down in the flood water in a daily cycle in relation to light. This buoyancy regulation could be a means to avoid excess light intensities at midday (cf. Reynaud & Roger 1979). The very prominent bleached upper side of the macro-colonies may further act as a light screen (cf. Stewart *et al.* 1978).

Filamentous, non-heterocystous cyanobacteria (Oscillatoriaceae) also occurred and might have contributed to the measured phototrophic fixation (Stewart *et al.* 1978)

Table 1 Comparison between acetylene reduction activity (ARA), numbers of aerobic, potentially nitrogen-fixing bacteria, and soil characteristics in planted rice fields at Bentota and Bathalagoda at the end of 1977/78 Maha season in relation to different fertilizer levels of urea. Mean values of duplicates or triplicates

Assay	Treatment: fertilizer-N (kg ha^{-1})			
	Bentota			Bathalagoda
	0-N	30-N	90-N	120-N
Total ARA (μmol acetylene reduced $chamber^{-1}$ day^{-1})*	51.7 ± 5.2	Nd	20.4 ± 13.9	4.5 ± 0.5
Phototrophic ARA (value and per cent of total)	48.5** (94)	Nd**	17.7** (87)	0.0*** (0)
Heterotrophic ARA§ (value and per cent of total)	3.2 (6)	2.6 (Nd)	2.7 (3)	4.5 (100)
Bacterial counts ($\times 10^{-6}$)g^{-1} on N-free medium, pH 6§§	7.5 ± 2.4	5.0 ± 2.5	3.4 ± 0.7	4.2 ± 1.1
Total soil N (per cent Kjeldahl -N)	0.89	0.44	0.40	0.60
Extractable NH_4^+-N (μg g^{-1} soil, dry weight)	53.4	29.4	26.8	43.7
Combustible organic matter (%)	–	8.05	8.25	9.60

*10 μmol acetylene reduced correspond to 19.4 g N ha^{-1} day^{-1} with a 4:1 conversion rate according to Watanabe (1976)
***Scytonema simplex* dominating
***Only green algae and non-heterocystous cyanobacteria present
§Mainly rhizosphere fixation (see Watanabe *et al.* 1979)
§§Aerobic, potentially N_2fixing bacteria
Nd Not determined

because of the near anaerobic field conditions (Eh between −100 and −200 mV; G. Jayaweera personal communication) in the boggy soil. Besides cyanobacteria, green algae (filamentous and colonial) and diatoms were also present. Floating cyanobacteria and algae were collected from each plot (with a 0.25 m^2 bamboo-frame placed at random). These values (not shown), however, only gave qualitative indications that cyanobacterial biomass was higher in the 0-N plots in comparison with the 30-N and 90-N ones as the relation between the biomass of floating organisms versus soil surface organisms was not studied.

Heterotrophic (rhizosphere) N_2-fixation was somewhat higher without than with N-fertilization (Table 1). Heterotrophic bacterial counts of aerobic non-rhizosphere potential N_2-fixers decreased with increasing N-levels. Bacterial colonies were consistently of one type, being whitish and slimy. No further identification was made. The procedure adopted gave one to two orders of magnitude higher counts than those reported by other workers (Watanabe *et al.* 1979) using a glucose medium (pH 7.0) for counts of aerobes in rice soils in general. Considerably higher counts have, however, been reported from the rice rhizoplane and rhizosphere (Nayak and Rao 1977; Watanabe *et al.* 1979; Roussos *et al.* 1980) with media allowing growth also of facultative anaerobes.

Total soil-N and extractable NH_4^+-N of combined samples were about twice as high in 0-N plots at harvest compared to 90-N plots (Table 1). These differences appear too big to be a result of treatments only. More interesting is that although soil NH_4^+-N levels at harvest were as high as 53 ppm in 0-N plots; N_2-fixation was considerable, which seems to contradict the results at Maha Illuppallama (see below) and of Venkataraman (1979), who stated that reduction of activity starts at 20 ppm and that inhibition occurs above 40 ppm. Owing to the high soil organic matter content, however, much of the ammonia might have been absorbed meaning that the cyanobacteria in the flood water and at the soil surface were not directly exposed to such high concentrations.

The flood-water pH increased clearly (from 6.4 to 7.2; Fig. 2B) around noon in all plots and most in 0-N plots, whereafter it dropped in all cases during the night. The pH changes in the 30-N plots (not shown) were intermediate to those in the 0-N and 90-N plots. These changes in pH during daytime can be attributed to the photosynthesis of both the dominating cyanobacteria and the macrophytes (*Chara*, *Nymphea*, *Cyperus* spp. etc.) present (Mikkelsen & de Dutta 1979).

(B) Bathalagoda

N_2 fixation activities at this site were low (Fig. 3 and Table 1) and obviously confined to the rhizosphere, as assay chambers in the light did not show higher values than chambers with water replacement and surface soil removed or chambers with stems wrapped with black plastic sheets. No heterocystous cyanobacteria could be detected.

Although the light intensity was greatly decreased (82 ± 2% decrease, $n = 30$) at the water surface below the dense rice canopy, the midday light intensity (up to 10 klux) was not too low for cyanobacterial growth, as shown by the occurrence of some green algae and non-heterocystous cyanobacteria. The absence of heterocystous species, however, indicates the high N-fertilization level to be the main cause of growth depression of the N_2-fixing species.

Heterotrophic bacterial counts (Table 1) were similar to those in N-fertilized plots at Bentota, whereas rhizosphere activity was slightly higher. Total soil-N, extractable

NH_4^+-N and combustible organic matter levels were not significantly different from those noted at Bentota.

Rhizosphere fixation of the mature plants (Fig. 3) showed an interesting two-peak pattern during the 24-h cycle with relatively higher activities in the morning and afternoon. This might be owing to the exceedingly high temperatures achieved during the midday period (46.1 °C inside chambers), as significant drops in rice rhizosphere fixation above 40 °C have been reported (Dommergues & Rinaudo 1979). A less likely explanation would be disturbance in energy supply from the host plant.

(C) Maha Illuppallama

Chemical fertilization experiments. The results are presented in Fig. 4A and 4B and

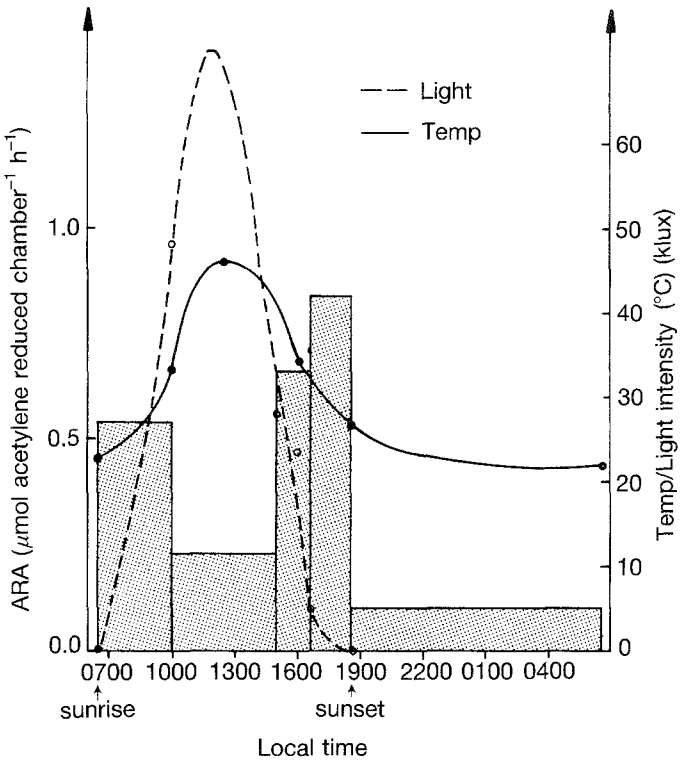

Fig. 3 Daily *in situ* nitrogenase activity at Bathalagoda of rice hills of rice 'BG 379' at end of 1977/78 Maha season. Mainly rhizosphere fixation (see text). Temperature inside chambers and light intensity at rice canopy (ca. 110% of incident light) are also shown. Symbols as in Fig. 2A. Mean values of six chambers.

Tables 2, 3 and 4A. The major conclusions are that both total N_2 fixation (ARA of light-incubated samples) around noon (Table 2) and daily rates of N_2 fixation (Fig. 4A) in surface soil between rice hills and that associated with rice stems (Table 3) appeared to be affected by the degree of N-fertilization. All showed maximal values at 20 kg N ha^{-1} level. Rice yields also tended to be optimal at the 20-N level, followed by the 90-N level (Fig. 4A and Table 4A). Cyanobacterial filament numbers at the soil surface and on rice stems increased up to the 40-N level (Tables 2 and 3), but specific

nitrogenase activity (Fig. 4B) was significantly lower at the two highest fertilizer levels (40-N and 90-N) compared with the two lowest (0-N and 20-N) (see below).

As total soil nitrogen fixation (ARA) – except rhizosphere fixation – in all these treatments was greatly dominated (78–94%) by phototrophic cyanobacteria (Table 2), cyanobacterial nitrogen fixation *per se* can be concluded to be relatively more important at lower N-fertilization levels, in agreement with results at Bentota,

Table 2 Results of assays on soil between rice hills at Maha Illuppallama in rice fields of 'BG-11-11' at harvest time of 1978/79 Maha season in relation to different fertilizer levels of urea. Mean values of triplicates ± S.E.

	Treatment: fertilizer-N (kg ha^{-1})			
Assay	0-N	20-N	40-N	90-N
Total midday (11.00–13.00 h) ARA between rice hills (μmol acetylene reduced m^{-2} h^{-1})	4.9 ± 1.8	6.2 ± 2.9	3.7 ± 31	4.0 ± 0.5
Phototrophic* ARA (value and per cent of total)	4.6 (94)	5.8 (94)	3.3 (90)	3.1 (78)
Filaments of cyanobacteria in surface soil (× 10^{-8} m^{-2})	150 ± 10	200 ± 10	230 ± 20	220 ± 20
Heterotrophic ARA	0.3 (6)	0.4 (6)	0.4 (10)	0.9 (22)
Bacterial counts** (× 10^{-6} g^{-1} soil, dry weight) on N-free medium				
at pH 7	4.9 ± 0.6	6.2 ± 0.9	5.3 ± 0.6	2.9 ± 0.4
at pH 4	4.1 ± 0.9	4.0 ± 0.5	4.7 ± 0.7	2.8 ± 0.3

*Calculated as the differences between total and heterotrophic ARA
**Potentially N_2-fixing aerobes

Table 3 Results of assays on rice stem bases ('BG 11-11') at Maha Illuppallama at harvest time of 1978/79 Maha season. Mean values of triplicates ± S.E.

	Treatment: fertilizer-N (kg ha^{-1})			
Assay	0-N	20-N	40-N	90-N
Total midday (11.00–13.00 h) ARA of rice stem bases (μmol acetylene reduced m^{-2} h^{-1})	2.4 ± 0.4	2.4 ± 1.1	0.7 ± 0.3	0.5 ± 0.1
Phototrophic ARA* (value and per cent of total)	1.9 (81)	2.3 (92)	0.0 (0)	0.0 (0)
Filaments of cyanobacteria on rice stem bases (× 10^{-8} m^{-2})	0.60 ± 0.06	0.53 ± 0.03	0.83 ± 0.06	0.73 ± 0.08
Heterotrophic ARA (value and per cent of total)	0.5 (19)	0.2 (8)	0.7 (100)	0.5 (100)

*Calculated as the difference between total and heterotrophic ARA

Bathalagoda and elsewhere (Matsuguchi & Shimomura 1977; Watanabe *et al.* 1978a,b). The diurnal curves of total soil ARA (results not shown) varied between different N-treatments, but generally increased and decreased in relation to the light curve. Both light intensity (above 60 klux) and temperature (45–46 °C) were extremely high during the midday period.

The indigenous cyanobacterial flora at initial stages (1 month) was dominated by

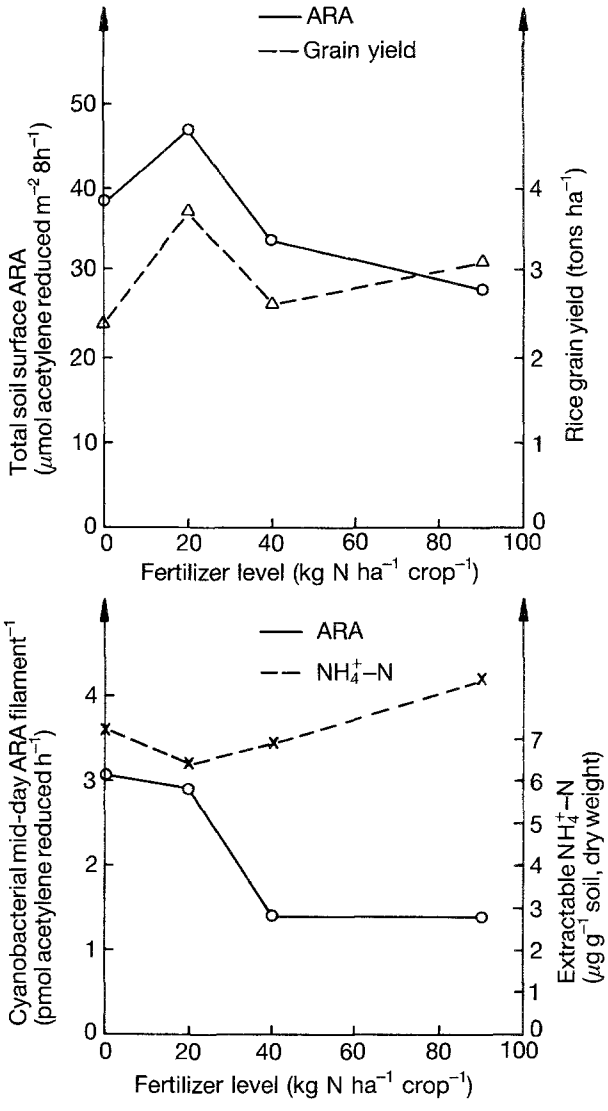

Fig. 4A Total daily (09.00–17.00h) *in situ* soil surface ARA at harvest time and grain yields of rice ('BG 11-11') at Maha Illuppallama at end of 1978/79 Maha season in relation to various fertilizer levels. Mean values of triplicates.

Fig. 4B Specific nitrogenase activity of cyanobacteria at Maha Illuppallama and extractable NH_4^+-N at harvest time in relation to various fertilizer levels. Mean values of triplicates.

Anabaena, *Nostoc*, *Calothrix*, *Oscillatoria*, *Cylindrospermum* and *Lyngbya*. At later stages *Anabaena* and *Scytonema* (as at Bentota) became dominant.

Heterotrophic N_2 fixation in the top soil also varied throughout the day, but this generally decreased in the afternoon. In relation to corresponding cyanobacterial activity (Table 2) it was of significance (0.9 μmol acetylene $m^{-2}h^{-1}$) only at the 90-N level (22% of total ARA around noon). Actual numbers of aerobic, potentially N_2-fixing bacteria in non-rhizosphere soil were, however, lowest at this level (Table 2). High fertilizer levels may have reduced numbers, but as the available NH_4^+-N at harvest (Table 4A) was not in excess in any treatments, the actual activity at harvest could be relatively high, as low concentrations of added N may stimulate heterotrophic nitrogen fixation (Nayak & Rajaramamohan Rao 1977; Charyulu & Rajaramamohan Rao 1981). Bacterial counts were similar on media at pH 7 and pH 4 and of the same order of magnitude as those (pH 6) at Bentota and Bathalagoda (Tables 1 and 2). The tolerance of these aerobic bacteria to such low pH values and the copious slime production suggests the presence of *Beijerinckia* (cf. Becking 1978; Diem *et al.* 1978).

Rice stem ARA. Heterotrophic activity was slightly higher (Table 3) on stems than between rice hills. In contrast to cyanobacterial activity it was highest at the 40-N level and minimal at 20-N (cf. Watanabe 1976). Cyanobacterial nitrogen-fixation activity associated with rice stems, on the other hand, was clearly higher than heterotrophic activity at low fertilizer levels, but totally absent above the 20-N level although the highest numbers of cyanobacteria were found at the 40- and 90-N levels (cf. Kulasooriya *et al.* 1981). The presence of inactive quiescent cells or propagules of cyanobacteria epiphytic on rice stems at maturity have been reported (Roger *et al.* 1979) and these may have stimulated bacterial activity at higher N-levels.

Soil factors. Soil organic matter content was not much affected by different N-treatments and soil-pH was similar in all treatments (Table 4A). Raised levels of total soil N were, however, found at harvest time at the two highest N-levels (40- and 90-N). Specific nitrogenase activities of the surface cyanobacteria (pmol acetylene $filament^{-1}\ h^{-1}$), mainly of *Scytonema* (i.e. filament numbers are approximately equal to heterocyst numbers), were decreased at fertilizer levels above the 20-N levels (Fig. 4B). This also partly coincides with increased levels of available NH_4^+-N at harvest in the top soil.

Table 4A Soil characteristics and grain yields in relation to various fertilizer concentrations of urea at Maha Illuppallama at harvest time. Mean values of triplicates ± S.E.

	Treatment: fertilizer-N (kg ha^{-1})			
Analysis	0-N	20-N	40-N	90-N
Organic matter (%)	5.5 ± 0.4	4.5 ± 0.7	5.1 ± 0.2	5.2 ± 0.2
Total soil-N (%)	0.21 ± 0.00	0.21 ± 0.00	0.25 ± 0.01	0.27 ± 0.01
Extractable NH_4^+-N (μg g^{-1} soil, dry weight)	7.2 ± 0.6	6.4 ± 0.7	6.9 ± 0.8	8.4 ± 0.6
Soil pH	6.8 ± 0.1	7.0 ± 0.1	7.0 ± 0.1	6.8 ± 0.1
Grain yield (tons ha^{-1})	2.44 ± 0.64	3.77 ± 0.31	2.66 ± 0.80	3.20 ± 0.42

Biofertilization experiments

The total ARA of rice hills, including rhizosphere fixation, was studied in plots the day before the above experiment with the chamber technique was carried out. The results are presented in Fig. 5A and Tables 4B and 5. Owing to some gas leakage in several of the light-chambers at the end (probably as a result of the coarse soil texture), only results from the first incubational period (around noon) are considered.

Heterotrophic rhizosphere activity was substantial but decreased throughout the day in relation to temperature. Total ARA was not significantly different between all the treatments but cyanobacterial activity *per se* (P-ARA per chamber) had increased owing to inoculation, especially by the Indian mixture (Fig. 5A).

Cyanobacterial ARA of surface soil was also studied in the biofertilization experiment (Fig. 5B; Table 5). At midday both A_i and A_s showed considerably higher cyanobacterial activity (2–4 times) than the control. Cyanobacterial numbers (Table 5), organic matter content and total soil N (Table 4B) also were higher in these two treatments. Extractable NH_4^+-levels were not raised owing to cyanobacterial development *per se*, but Mo-addition appeared to increase soil available N considerably, possibly as a result of preceding higher N_2-fixation (Tables 4A,B; Fig. 5). Preliminary experiments had shown that soil additions with Mo stimulated soil colonization by the actual cyanobacteria at this site.

Significant differences in rice yields were not observed, either in plots with increased cyanobacterial growth or in plots with chemical N-fertilizers versus the controls (Tables 4A,B), although grain yields ranged from 2.2 to 3.7 tons ha^{-1}. This was because of large heterogeneity among plots in relation to seepage and percolation. Rapid nitrification in this coarse aerobic soil, with alternating dry and flooded conditions, causing irregular nitrate losses as well as ammonia volatilization (a pH often over 8 at mid-season), could altogether account for the weak response of rice yields to N-fertilization. Much of the phosphate must also have been leached out as there was no increase in rice yields when different amounts of phosphate were applied (data not shown).

Among the different treatments, highest rice yields, as well as maximum soil surface ARA and cyanobacterial numbers, were indicated at the 20-N level in the N-fertilization experiment (Fig. 4A). This suggests that low levels of N-fertilizers

Table 4B Soil characteristics and grain yields in cyanobacterial biofertilization experiments (Mo-applied 0-N plots) at Maha Illuppallama at harvest time. A_i: Indian mixture. A_s: Local *Scytonema*. Mean values of triplicates ± S.E.

	Treatment: cyanobacterial inoculation		
Analysis	Control	A_i	A_s
Organic matter (%)	3.3 ± 0.2	4.4 ± 0.3	4.1 ± 0.2
Total soil N (%)	0.21 ± 0.01	0.24 ± 0.01	0.24 ± 0.01
Extractable NH_4^+-N ($\mu g\ g^{-1}$ of soil, dry weight)	14.3 ± 1.1	12.4 ± 1.7	14.0 ± 0.3
Soil pH	6.9 ± 0.1	6.9 ± 0.1	6.6 ± 0.2
Grain yield (tons ha^{-1})	2.38 ± 0.47	2.19 ± 0.27	2.43 ± 0.55

Table 5 Results of microbial analysis in biofertilization experiment at Maha Illuppallama in rice fields of 'BG 11-11' at harvest time of 1978/79 Maha season. All plots had received P,K,Mo. A_i: Indian mixture. A_s: Local *Scytonema*. Mean values ± S.E.

Analysis	Treatment: cyanobacterial inoculation		
	Control	A_i	A_s
Filaments of cyanobacteria in surface soil (0–2 cm) between rice hills ($\times 10^{-8}$ m^{-2})	810 ± 70	1350 ± 100	1250 ± 100
Filaments of cyanobacteria on rice stem bases (rice hills) ($\times 10^{-8}$ m^{-2})	0.71 ± 0.15	0.50 ± 0.08	0.33 ± 0.07
Heterotrophic bacterial counts* between rice hills ($\times 10^{-6}$ g^{-1} soil, dry weight)			
at pH 7	4.6 ± 0.7	4.5 ± 0.9	5.2 ± 0.7
at pH 4	5.8 ± 0.5	4.5 ± 0.7	4.4 ± 0.6

*Potentially N_2-fixing aerobes in surface soil (0–5 cm)

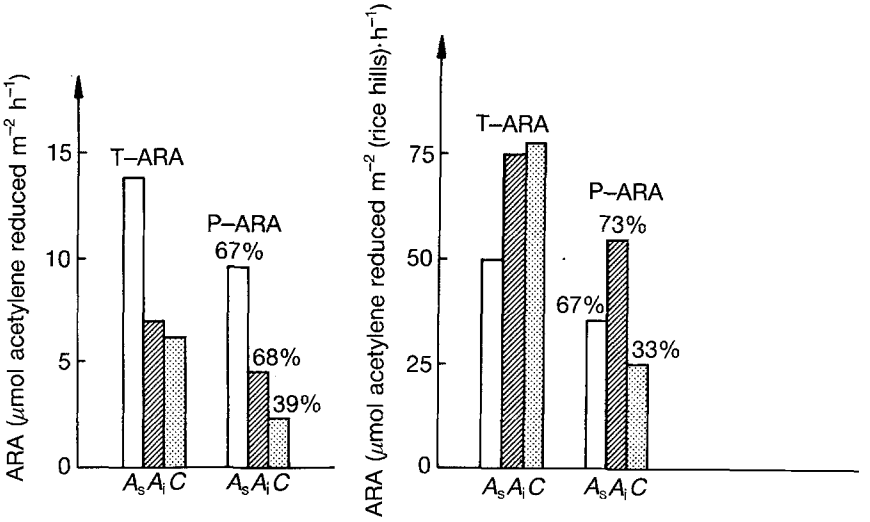

Fig. 5A Total (T-ARA) and phototrophic (P-ARA) *in situ* midday (11.30–13.40 h) soil surface ARA at end of 1978/79 Maha season in rice fields fertilized with cyanobacteria ('BG 11-11') at Maha Illuppallama. A_i: Indian mixture; A_s: local *Scytonema* sp.; C = control. Percentage activity of total is also shown. Mean values of triplicates.

Fig. 5B Total and phototropic *in situ* midday (10.00–13.00 h) ARA of rice hills (chambers) at end of 1978/79 Maha season at Maha Illuppallama. Total values minus phototrophic activity may be ascribed to the rhizosphere. Symbols as in Fig. 5A. Mean values of triplicates.

stimulate total cyanobacterial activity, which could enhance rice yields under more homogeneous soil conditions.

The varying soil moisture conditions may also have adversely affected the proper development of cyanobacteria. Other factors such as competition with the indigenous flora (Wilson *et al.* 1979) and grazing by the soil fauna (Watanabe *et al.* 1955; Roger & Kulasooriya 1980) were not investigated. Nevertheless inoculated cyanobacteria were observed to establish themselves in the field (data not shown) and inoculation increased their total numbers (Table 5) and ARA (Fig. 5A,B).

Conclusions

Cyanobacterial nitrogen fixation is evidently potent in certain rice soils in Sri Lanka (cf. results at Bentota and Maha Illuppallama) and results in all three sites and at IRRI (Watanabe *et al.* 1978a,b) show that significant heterotrophic bacterial fixation may also occur in the rice rhizosphere. Cyanobacterial nitrogen fixation in rice fields at low N-fertilizer levels is, however, generally much more important than rhizosphere fixation (cf. Watanabe *et al.* 1978a,b). Under Indian conditions it has been shown (Venkataraman 1977) that cyanobacterial biofertilizers can be used as a biological input in rice fields and reduce chemical N-fertilizer use by 30%. Such use of biofertilizers at Maha Illuppallama with the local *Scytonema* spp. (same as at Bentota) seemed more efficient in terms of soil colonization and activity than that by the Indian cyanobacterial mixture (this species became dominant along with *Anabaena* even in plots not inoculated with it). Addition of P and Mo generally stimulates cyanobacterial nitrogenase activity, but only Mo appeared to be effective in this respect at Maha Illuppallama. 0-N minus P plots showed values similar to those in 0-N plus P plots. Owing to the heterogeneous soil conditions no significant effects were, however, noticed on grain yield of either biofertilizer.

ARA rates at Bentota in 0-N plus P,K plots converted into fixed N, indicate an input of 30 to 35 kg N per crop, taking light reduction inside chambers (ca. 30%) and estimates of the amounts of ethylene in soil and water into account, and assuming similar seasonal variations as at IRRI (Watanabe *et al.* 1978a,b). This calculation indicates that the cyanobacterial contribution might almost meet (75–90%) the N-fertilizer demand (40 kg N ha^{-1}) for this area.

The results at Bathalagoda, generally agree with those at Bentota and Maha Illuppallama in terms of a great reduction in cyanobacterial growth and total nitrogen fixation at high N-fertilizer levels (cf. Watanabe *et al.* 1981).

Seasonal nitrogen inputs from N_2-fixation at Maha Illuppallama cannot be estimated owing to the heterogeneous soil conditions and the varying soil moistures during the cropping season. The data presented, however, show that 65% of the daily fixation occurred between 09.00 and 17.00 h and 15% at midday (between 11.30 and 13.30 h) at harvest. Using these values the distribution of fixation at harvest in, e.g. the 0-N plus P plots, assuming rhizosphere fixation as in 0-N plus P,Mo plots, were as follows: (a) cyanobacterial nitrogen fixation between rice hills 8%; (b) cyanobacterial fixation on rice stems 3%; (c) heterotrophic bacterial fixation in the top soil 1%; (d) bacterial fixation on rice stems 1%; and (e) bacterial rhizosphere fixation 87%. Rhizosphere fixation at Maha Illuppallama at harvest time was thus substantial and much more important than cyanobacterial fixation at low N-levels. It should therefore not be ignored in relation to cyanobacterial fixation, especially as others (Anon. 1979)

have reported that it may be in the order of 5 to 10 kg N ha^{-1} $^{-1}$ with a possible potential for increasing it 3 to 4 fold by appropriate amendments, genotype selection of the rice and cultural practices.

As regards phototrophic fixation in paddy fields, cyanobacterial activity plays an important role in ammonia volatilization by increasing the pH of the flood water during daytime (cf. Fig. 2B and IRRI results; Mikkelsen & de Datta 1979). As urea is rapidly converted into ammonia in paddy soils by microorganisms, the pH increases considerably and volatilization losses may become highly significant when combined with high temperatures (Bouldin & Alimagno 1976). Broadcast application of urea or $(NH_4)_2SO_4$ at any stage in flooded rice soils with appreciable cyanobacterial growth is thus rather ineffective in terms of fertilizer-use (Prasad & de Datta 1979). It also evidently decreases biological N_2-fixation (Table 1 and Watanabe *et al.* 1978a,b). Deep placement of fertilizers can, however, markedly improve the efficiency of N use (Mitsui 1954; de Datta *et al.* 1968; Anon. 1980). Experiments in the form of urea supergranules put into the rice root zone have shown considerably less disturbance of cyanobacterial N_2-fixation (Roger *et al.* 1981).

In summary, many rice fields in Sri Lanka harbour a rich cyanobacterial flora (Kulasooriya *et al.* 1980). In the present study it has been shown that they are highly adapted to local climatic conditions and able to fix substantial amounts of N *in situ* under flooded low N-fertilized field conditions. Such organisms, as well as local strains of *Azolla pinnata*, which appear to be capable of tolerating high light and temperature conditions (Kulasooriya *et al.* 1980), may therefore be used as two potent ways to reduce the need of costly chemical N-fertilizers in rice production in certain areas of the wet zone in Sri Lanka. Finally attention should also be paid to rhizosphere fixation which appears to be of importance under some dry zone conditions.

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Summary

In situ measurements of nitrogenase activities in some rice soils, representing three different agroclimatic zones of Sri Lanka, demonstrated that there is a great potential for nitrogen fixation in these paddy soils, provided that they are continuously flooded and that nitrogenous fertilizer levels are relatively low. Under such conditions cyanobacterial (blue–green algal) fixation predominates. In certain areas of the wet zone, with highly organic soils, cyanobacterial fixation could probably meet a great part of the N-fertilizer input recommended. Heterotrophic rhizosphere fixation may also be significant, especially in the dry zone. Low concentrations of fertilizer-N were found to stimulate cyanobacterial numbers and total nitrogen fixation, at one site (dry zone), whereas high levels negatively affected total fixation at all sites investigated. In the absence of N-fertilizers, inoculation as well as additions of Mo, increased cyanobacterial numbers, nitrogen fixation activity and available ammonia levels substantially at the investigated site (dry zone). Rice grain yields did not vary significantly between treatments, because of too large a heterogeneity in the field with respect to water management.

Resumé

Fixation d'azote dans quelques sols de rizière au Sri Lanka

Des mesures *in situ* d'activité de nitrogénase dans quelques sols de rizière, représentant trois zones agro-climatiques différentes du Sri Lanka, ont démontré qu'il y a une grande potentialité pour la fixation d'azote dans ces sols, pourvu qu'ils soient continuellement inondés et que le niveau de fertilisation azotée soit relativement bas. Dans ces conditions, la fixation par les

cyanobactéries (algues bleu-vertes) prédomine. Dans certaines régions de la zone humide, aux sols à forte teneur en matière organique, la fixation par les cyanobactéries peut probablement satisfaire une grande partie du besoin recommandé en fertilisant azoté. La fixation par la rhizosphère hétérotrophe peut, elle aussi, être significative, surtout dans la zone sèche. Des concentrations faibles en fertilisant azoté ont révélé stimuler le nombre de cyanobactéries et la fixation totale d'azote, sur un site (zone sèche), tandis que des teneurs élevées affectaient négativement la fixation totale sur tous les sites étudiés. En absence de fertilisant azoté, l'inoculation ainsi que l'ajout de Mo ont augmenté le nombre de cyanobactéries, l'activité fixatrice d'azote et le niveau d'ammoniaque disponible, de manière substantielle sur le site étudié (zone sèche). Le rendement en graines de riz n'a pas varié de manière significative d'un traitment à l'autre, à cause d'une hétérogénéité trop grande sur le champ en matière de gestion d'eau.

Resumen

Fijación de nitrógeno en algunos suelos arroceros de Sri Lanka

Medidas 'in situ' de las actividades nitrogenàsicas de algunos suelos arroceros representativos de tres zonas agroclimáticas distintas de Sri Lanka demostraron que existe un potencial considerable para la fijación de nitrógeno en estos suelos encharacados siempre y cuando permanezcan bajo el agua y la fertilización nitrógenada se mantenga relativamente baja. En estas condiciones la fijación predominante es la realizada por cianobacterias (algas verde-azules). En algunas areas de la zona húmeda con suelos muy orgánicos la fijación mediante cianobacterias podría suministrar una gran parte del aporte en nitrógeno recomendado. La fijación heterotrófa en la rizosfera puede también representar un aporte significativo, especialmente en la zona seca. Bajas concentraciones de fertilizante nitrogenado estimularon el número de cianobacterias y la fijación de nitrógeno total en uno de los suelos estudiados (zona seca) mientras que niveles elevados afectaron negativamente a la fijación en todos los suelos estudiados. En ausencia de fertilizantes nitrógenados la inoculación así como adiciones de Mo incrementaron sustancialmente el número de cianobacterias, la actividad fijadora de nitrógeno y los niveles de amonio disponibles en el suelo estudiado (zona seca). La cosecha de arroz no varió significativamente entre los distintos tratamientos debido a la excesiva heterogeneidad en el uso del agua.