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Rice Field Cyanobacteria: Ecology, Contribution to Soil Fertility and Practical Utilisation

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INTRODUCTION

About 75% of the 143 million hectares of rice land are wetlands where rice grows under flooded conditions during part or all of the crop cycle. Flooding leads to the differentiation of macro- and microenvironments differing in redox state, physical properties, light status and nutrient status, which allows all kinds of N_2-fixing micro-organisms (aerobes, facultative and strict anaerobes, heterotrophs, phototrophs, free-living and symbiotic) to grow in wetland rice fields (Roger and Watanabe, 1986) (Fig. 12.1). This results in a unique agroecosystem in which moderate but constant yields have been obtained for centuries after continuous cropping without N fertiliser addition. A compilation of 211 balance studies in rice soils indicated that in the absence of N fertiliser application, biological N_2 fixation (BNF) averaged 31 kg N $crop^{-1}$, among which 2/3 could be roughly attributed to photodependent organisms, mostly free-living cyanobacteria (Table 12.1) (Roger and Ladha, 1992).

As nitrogen is the key nutrient in rice production, most actual and potential microbiological soil management of wetland rice refers to the N cycle. Emphasis has been on BNF and inoculation with N_2-fixing micro-organisms (Roger *et al.*, 1993).

The oldest technology employing N_2-fixing micro-organisms in rice fields is the utilisation of *Azolla* as a green manure, which dates back to the 11th century in Vietnam and at least the 14th century in China (Lumpkin and Plucknett, 1982). The causative agent of the beneficial effect of *Azolla* was identified by Strasburger (1873) as a symbiotic N_2-fixing cyanobacterium (*Anabaena azollae*) but progress in *Azolla-A. azollae* biotechnology, in particular recombination and sexual hybridisation, has only recently begun (Wei *et al.*, 1986; Lin *et al.*, 1988; Lin and Watanabe, 1988).

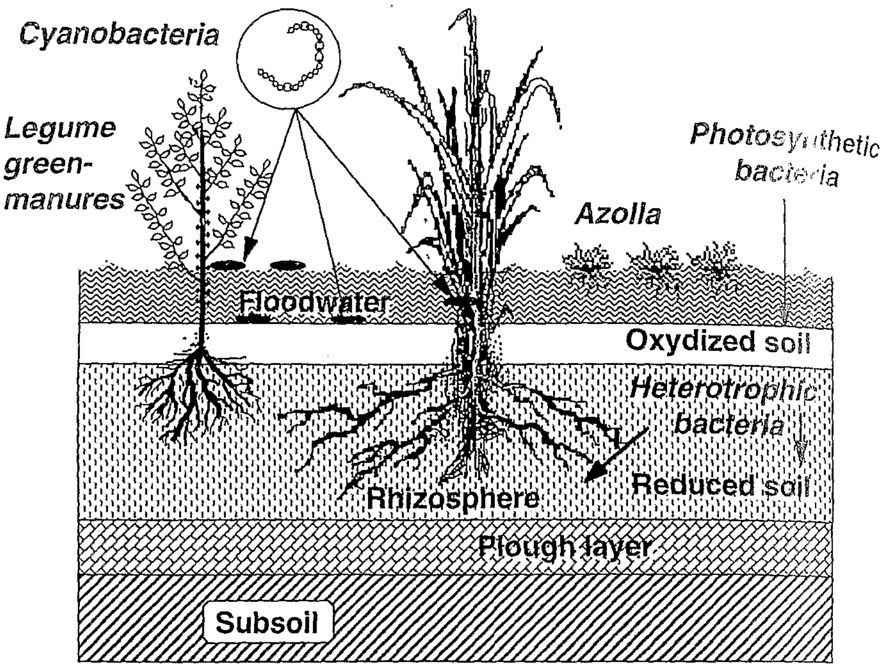


Fig. 12.1 Major environments and N_2-fixing organisms of the wetland rice field ecosystem.

The agronomic potential of free-living cyanobacteria was recognised in 1939 by De in India, who attributed the natural fertility of wetland rice fields to their N_2-fixing activity. Research on inoculation of rice fields with cyanobacteria was initiated in Japan by A. Watanabe *et al.* (1951).

FREE-LIVING CYANOBACTERIA

Cyanobacteria constitute the largest, most diverse and most widely distributed group of photosynthetic prokaryotes (Stanier and Cohen-Bazire, 1977). The N_2-fixing ability of a number of genera has implications for the maintenance of the fertility of natural and cultivated ecosystems and many trials have been conducted to increase rice yield by cyanobacterial inoculation. These aspects have been discussed in several reviews (Roger and Kulasooriya, 1980; Roger and Reynaud, 1982; Roger and Watanabe, 1986; Roger *et al.*, 1993b).

General Ecology of Cyanobacteria in Rice Fields

Occurrence and nature

Cyanobacteria are ubiquitous in rice fields, contrary to early beliefs (Watanabe, 1959; Venkataraman, 1975). Quantitative estimates of N_2-fixing forms in rice soils of 10 countries ranged from 10 to 10^7 g^{-1} dry soil, with a

Table 12.1 Bibliographic study of N-balance estimates in wetland rice fields* (apted from Roger and Ladha, 1992)

Major statistics of the set of data analysed

Number of data: 211

Unit: kg N ha^{-1} crop $cycle^{-1}$

Minimum: –102

Maximum: 171

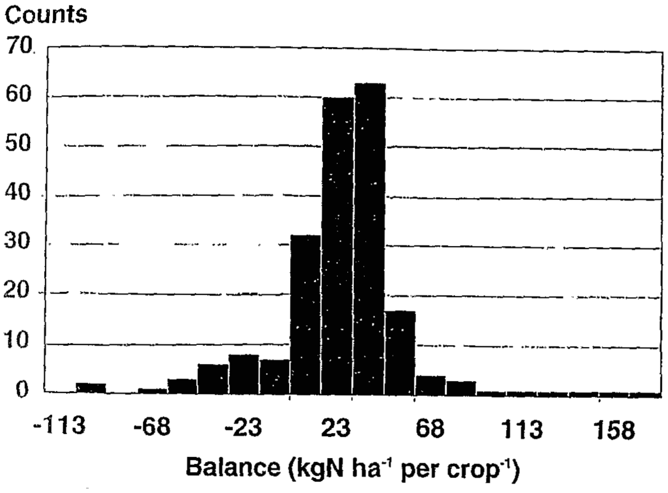
Mean: 24.2

Median: 27.0

Standard deviation: 33.1

Coefficient of variation: 136%

Histogram of the data



Effect of various factors on N-balance

Factor	Number of data	Mean (kg N ha^{-1} crop $cycle^{-1}$)	Standard error	Level of significance of the difference
N-fertiliser application				
–	166	29.7	25.4	1%
+	45	4.0	47.6	
Planted versus unplanted				
+	193	26.5	30.7	1%
–	18	–0.5	46.2	
Effect of soil exposure to light (treatments where no N-fertiliser was applied)				
+	152	31.2	25.7	1%
–	14	13.2	13.8	

*The set of data is tentatively exhaustive. Data originate from pot and field experiments reported in 15 references cited in Roger and Ladha (1992). Data from pot experiments are extrapolated in kg N ha^{-1} crop $cycle^{-1}$ based on the surface of the pots.

median of 2×10^4 from 396 measurements. In a survey of 102 rice field soils, the relative occurrence of major cultivable genera was: *Nostoc* 47%, *Aphanothece* type 27%, *Anabaena* 10%, *Calothrix* 8%, *Fischerella* 4% (Roger *et al.*, 1987).

Environmental factors

The rice field floodwater, where cyanobacteria develop, is a photic, aerobic environment subjected to large diurnal and seasonal variations in insolation (0 to 120 klux), temperature (5 to > 60°C), pH (5 to > 10), O_2 concentration (0 to > 30 ppm) and nutrient status (Roger, 1996). In arid tropics, abundant light and high temperatures may inhibit cyanobacterial growth at the beginning of the crop cycle (Reynaud and Roger, 1978). In wet monsoonal zones, they may develop early in the crop cycle during the dry/warm season (Gupta, 1966). During the wet season, light deficiency and disturbance by heavy rain may limit their growth (Ichimura, 1954; Watanabe, 1961). High water temperatures (30-35°C) favour cyanobacteria versus eukaryotic algae, but temperatures higher than 40°C may be inhibitory (Roger and Kulasooriya, 1980). Spore-forming cyanobacteria can withstand long periods of desiccation, which results in their higher relative abundance after a dry period and explains the higher incidence of *Nostoc* in dry rice field soils (80%) than in wet ones (47%) (Roger *et al.*, 1987). The abundance of N_2-fixing forms is positively correlated with soil pH and available P (Roger *et al.*, 1987) (Fig. 12.2).

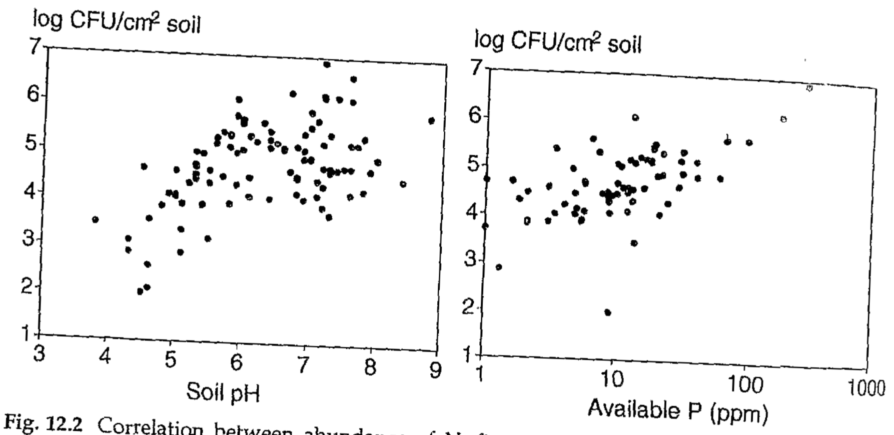


Fig. 12.2 Correlation between abundance of N_2-fixing cyanobacteria and soil pH and soil available P (Roger *et al.*, 1987).

Biotic factors

The major biotic factor limiting cyanobacterial growth is grazing by invertebrates such as ostracods, mosquito larvae and snails. A dense population of ostracods may ingest 34 kg ha^{-1} day^{-1} dry weight of algae, ingesting about 1.7 kg N and excreting 1 kg N (Grant *et al.*, 1986). Dietary preferences are exhibited, with strains forming mucilaginous colonies being less susceptible to grazing than non-colonial strains. Grazing thus leads to the dominance of mucilaginous cyanobacteria, especially *Nostoc* spp., in rice fields (Roger *et al.*, 1987), which are often less active in BNF than non-colonial forms (Antarikanonda and Lorenzen, 1982; Grant *et al.*, 1985).

Cyanophages (Singh, 1973) and myxobacteria pathogenic to cyanobacteria (Huang, 1982) have been isolated from rice fields. Antagonisms among cyanobacteria and other algae or aquatic weeds have been observed, but the mechanisms involved are not known (Saito and Watanabe, 1978; Kulasooriya *et al.*, 1980).

Effects of agrochemicals on cyanobacteria

Mineral N is known to inhibit BNF by cultures of cyanobacteria. *In situ* inhibition is less marked and depends on the method of fertiliser application. Nitrogen broadcasting often strongly inhibits photodependent BNF (Fig. 12.3) (Roger *et al.*, 1988) and shows a negative correlation between photodependent BNF and N use efficiency (kg rice produced per kg N applied) (Roger, 1991). Broadcasting N fertiliser, widely practised by farmers, not only inhibits photodependent BNF, but also causes N losses by ammonia volatilisation. In contrast, deep placement of N fertiliser decreases N losses by volatilisation and reduces the inhibitory effect of N fertiliser on cyanobacteria (Fig. 12.3) (Roger *et al.*, 1980; Roger *et al.*, 1988).

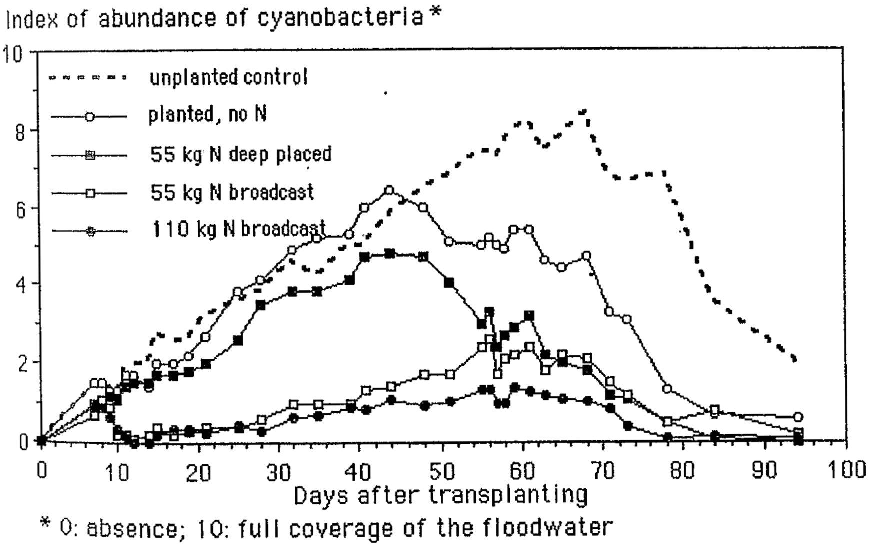


Fig. 12.3 Dynamics of cyanobacteria biomass during a crop cycle under four N fertiliser management practices (IRRI, 1991).

Phosphorus is a key nutrient for rice field cyanobacteria, which are often P deficient (Roger *et al.*, 1986). Its application stimulates photodependent BNF and cyanobacterial growth, especially in acid soils (Cholitkul *et al.*, 1980).

The impact of pesticides on rice field cyanobacteria was reviewed by Roger (1995a, b) through the analysis of 311 references. Results can be summarised as follows: Cyanobacteria are often more resistant to pesticides than eukaryotic algae, which may lead to a selective effect of some pesticides, which promotes cyanobacterial growth. This was observed with algicides (Symetryne and Algaedyn) and insecticides (BHC and PCP). Simultaneously, insecticides inhibit invertebrates that feed on algae (grazers), thus further promoting cyanobacteria and photodependent BNF. That was observed with parathion, phorate and carbofuran. Insecticide applications do not invariably increase photodependent BNF. Some inhibitory effect was reported for PCP. Also, over the long term, insecticide use might become detrimental to cyanobacteria by decreasing the diversity of aquatic invertebrates and causing algal grazers resistant to conventional pesticides to proliferate. Among pesticides not aiming at algal control, herbicides seem to be the most detrimental to cyanobacteria. They can inhibit cyanobacteria and photodependent BNF, as shown with PCP and several formulations used in rice fields. Some herbicides seem to specifically affect the N_2-fixing ability of cyanobacteria, as indicated by an inhibition observed in N-free medium, but not in the presence of inorganic N, as observed with Dichlone and Machete.

Composition, Biomass and *in situ* Productivity

The photosynthetic aquatic biomass (algae and vascular macrophytes) that develops in floodwater is usually a few hundred kg dry weight ha^{-1} and rarely exceeds 1 t ha^{-1} (10-20 kg N ha^{-1}). Reported productivity ranges from 0.5 to 1 g C m^{-2} day^{-1} (Roger, 1996) which provides an indirect estimation of the maximum productivity of cyanobacteria in rice fields. Assuming that the photosynthetic aquatic biomass is composed exclusively of N_2-fixing cyanobacteria (C/N = 7) and primary production is 0.5 t C ha^{-1} $crop^{-1}$, cyanobacteria could provide 70 kg N ha^{-1} $crop^{-1}$.

Cyanobacteria can develop impressive blooms in rice fields. Standing crops of 5-20 tons fresh weight ha^{-1} are usually recorded for growth visible to the naked eye. But because of the low and wide range of dry matter (0.2-14%) and high ash (31-71%) content of fresh cyanobacteria, a bloom usually represents less than 10 kg N ha^{-1} (Roger *et al.*, 1986). The median of 400 weekly biomass measurements in 65 plots on the IRRI farm when cyanobacteria were blooming was 4 kg N ha^{-1} and the maximum 17 kg N ha^{-1} (Roger, 1996) (Fig. 12.4). Nitrogen contribution by cyanobacteria is largely the result of nutrient turnover of the standing biomass, for which no data are yet available. However, the observation that cyanobacteria usually do not bloom more than twice during a crop cycle indicates a rough potential of 30 kg N ha^{-1} $crop^{-1}$.

Nitrogen-fixing Activity

N_2 fixation by cyanobacteria has been almost exclusively estimated from acetylene-reducing activity (ARA) measurements. Data published before 1980 vary from a few to 80 kg N ha^{-1} $crop^{-1}$ (mean 27 kg) (Roger and Kulasooriya, 1980). About 200 crop cycle measurements in experimental plots at IRRI (Roger *et al.*, 1988) show activities of the same order: 0-1200 μmol C_2H_2 for daily values and 20-500 μmol C_2H_2 for average ARA during a crop cycle (Fig. 5). Extrapolated values (assuming $C_2H_2/N_2 = 4$) ranged from 0.2 to 50

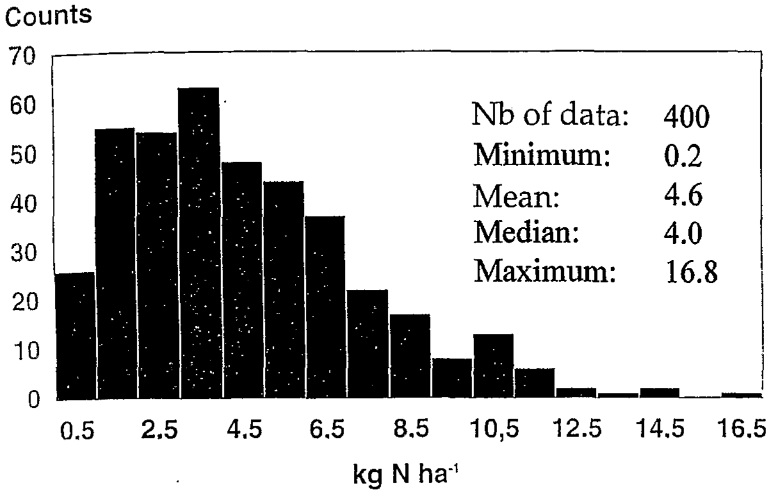


Fig. 12.4 Distribution of 400 estimates of N in cyanobacteria biomass in 65 experimental plots when growth was visible to the naked eye (after Roger, 1996).

kg N ha^{-1} $crop^{-1}$ and averaged 20 kg in no-N control plots, 8 kg in plots with broadcasted urea, and 12 kg in plots where N was deep-placed. ARA was negligible in 75% of the plots where urea was broadcast (Roger *et al.*, 1988) (Fig. 12.5). As N_2-fixing cyanobacteria usually bloom only when the photic zone is depleted of N, most of their N can be assumed to originate from BNF. Inubushi and Watanabe (1986) reported that cyanobacteria in ^{15}N-labelled plots had about 90% N derived from the air (Ndfa).

Recycling N from Cyanobacteria and Availability to Rice

The extent to which cyanobacteria contribute to the N requirement of the crop is determined by the algal standing biomass and turnover time, the rate of N_2 fixation and the extent to which N from cyanobacteria becomes available to the plant. In addition, cyanobacteria might benefit rice by mechanisms other than BNF.

No information is available on *in situ* N exudation by cyanobacteria, but because algal photosynthesis increases floodwater pH, it is foreseeable that during the build-up of the bloom, most of the excreted N will either be reimmobilised or lost by NH_3 volatilisation (Roger, 1996). Therefore, most N originating from cyanobacteria is probably made available to the crop through mineralisation after the death of the algae. Late decomposition of the bloom during the cycle and the resultant late availability of N to rice might increase N content of the grain without increasing yield (Grant *et al.*, 1985). Depending on the nature of the material (fresh vs. dried), the method of application and the presence or absence of soil fauna, recovery of cyanobacteria N by rice averages 30% and varies from 13 to 50% (Roger *et al.*, 1987) (Table 12.2). Recovery was highest with fresh cyanobacteria

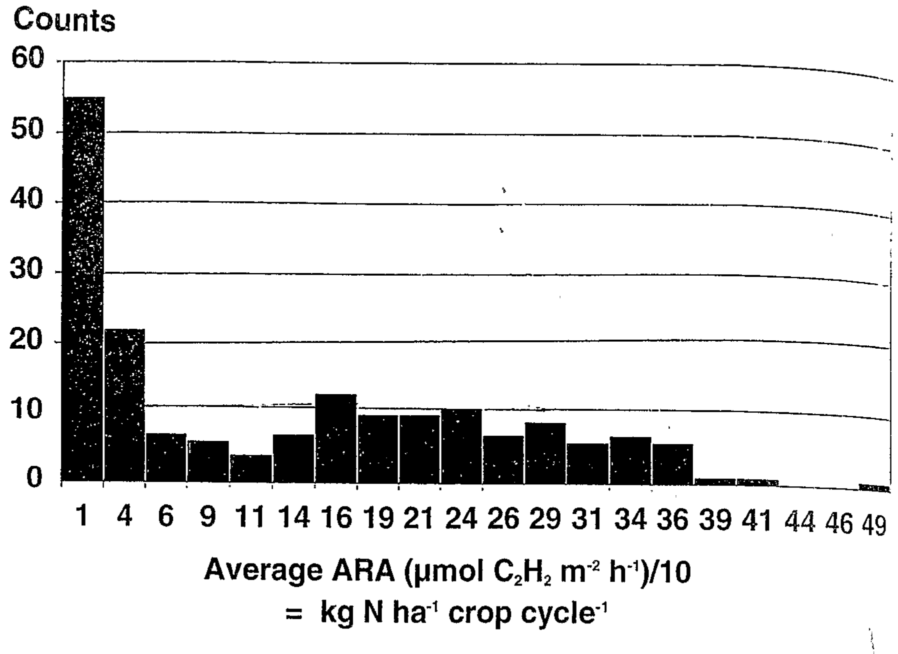


Fig. 12.5 Distribution of 180 estimates of average ARA and N_2 fixed during a crop cycle in experimental plots under 26 cultural practices (after Roger *et al.*, 1988). Each estimate is the average of 13 daily measurements performed at intervals during the crop cycle on 13 core samples comprised submersion water and the first centimetre of soil. The left part of the histogram corresponds to plots in which N fertiliser was broadcast in the floodwater; the right part corresponds to plots in which N fertiliser was neither applied nor deep-placed.

incorporated into a soil depleted of fauna (Tirol *et al.*, 1982). It was lowest with dried cyanobacteria applied on the surface of a soil rich in tubificid worms, which reduce the recovery of algal N by rice by making more soil N available through mineralisation (Grant and Seegers, 1985a).

Effects other than N on Rice

The N_2-fixing ability of cyanobacteria is the main, but probably not the only reason for increased rice yields. Other possible beneficial effects summarised by Roger and Kulasooriya (1980) include (1) competition with weeds, (2) increased soil organic matter content and improved soil aggregation, (3) excretion of organic acids that increase P availability to rice, (4) inhibition of sulphide injury in sulphate reduction-prone soils by increased O_2 content and plant resistance to sulphide, and (5) production of plant growth regulators (PGR) that enhance rice growth.

Table 12.2 Recovery in rice of N from cyanobacteria and *Azolla* (^{15}N experiments) (adapted from Roger *et al.*, 1987)

Material studied				N absorbed (%)		
Nature	State	Fauna[a]	Set-up	Broad-cast	Incor-porated	Reference
Cyanobacteria						
	fresh	?	pot	37	52	Wilson *et al.*, 1980
	dry	—	pot	14	28	Tirol *et al.*, 1982
	dry	+	*in situ*	23	23	Tirol *et al.*, 1982
	fresh	—	pot	—	38	Tirol *et al.*, 1982
	fresh	—	pot	24	44	Grant and Seegers, 1985
	fresh	+	pot	25	30	Grant and Seegers, 1985
	dry	—	pot	—	35-40	Mian and Stewart, 1985
Mean				25	36	
Azolla						
A. pinnata	fresh	+	*in situ*	—	26	Watanabe *et al.*, 1981
A. caroliniana	fresh	+	*in situ*	12/14	26	Ito and Watanabe, 1985
A. caroliniana	dry	?	pot	—	34	Mian and Stewart, 1985
A. caroliniana	fresh	+	*in situ*	—	32	Kumarasinghe *et al.*, 1986
Mean				13	29	

[a] + : present in soil; – : absent

There have been many claims that cyanobacteria can benefit rice plants by producing PGRs. Roger and Kulasooriya (1980) cited 12 references reporting that presoaking seeds or seedlings in cyanobacterial cultures or extracts enhanced germination, growth or yield. However, when Pedurand and Reynaud (1987) studied the effect of 133 unialgal strains on rice germination and growth, 70% had a negative effect on germination and only 21% had a stimulatory effect. The PGR-like effects of cyanobacterial cultures were likened to those of vitamin B12, gibberellins and amino acids but, as Metting and Pyne (1986) pointed out, despite the numerous reports on algal PGR effects, none shows the isolation and characterisation of a microalgal PGR. This statement is currently still valid. The beneficial effects of algal inoculation in rice fields might be partially due to PGRs, but the relative importance of their contribution to rice productivity is still not known.

Inoculation of Rice Fields with Cyanobacteria

Management technology

Applied research on cyanobacterial inoculation has been conducted mostly in India where the All-India Co-ordinated Project on Algae was initiated in 1977 and, to a lesser extent, in Burma, Egypt and China. A similar technique of growing cyanobacterial inocula in shallow open-air ponds has been used in India, Egypt and Burma (Venkataraman, 1981). A multistrain starter inoculum produced from laboratory cultures is propagated on the spot, in shallow trays or microplots with 5-15 cm water, about 4 kg soil m^{-2}, 100 g superphosphate m^{-2} and insecticide. When necessary, lime is added to adjust soil pH to 7.0-7.5. In 1-3 weeks, an algal mat develops which is allowed to dry. Algal flakes are then scraped from it and stored for future use at 10 kg ha^{-1} (Venkataraman, 1981).

Analysis of inoculation experiments

A compilation of 634 field experiments reported in the literature and in technical reports showed a very large variability of the yield difference between inoculated and non-inoculated plots (C.V. > 100%) (Table 12.3) (Roger, 1991). Because of the strong asymmetrical data distribution, the median grain yield difference (257 kg ha^{-1}) was considered a better index of the average effect of inoculation than the mean (337 kg ha^{-1}). While the difference in average yield between inoculated and non-inoculated plots was significant at $p < 0.01$, only 17% of the 634 individual observed differences were statistically significant. This indicates a small and variable response of yield to algal inoculation and also an experimental error frequently larger than the response.

When interpreting data from the literature, it should also be kept in mind that unsuccessful trials have often not been reported, as indicated in some reports and confirmed by the dissymmetrical data distribution.

Adoption of inoculation technology

Cyanobacterial inoculation has been used on a trial-and-error basis. Methods to estimate the chance of success of inoculation in a given agroecosystem are unavailable because the factors underlying yield increases associated with successful algal inoculation are not clearly understood or quantified. Most experiments measured only grain yield and did no estimation of inoculum quality or establishment. No published study reporting a significant increase in yield after algal inoculation includes BNF or cyanobacterial biomass estimates.

Reports on the adoption of algal inoculation are somewhat controversial, but even with the most optimistic evaluations, adoption seems to have been restricted to a limited area in a few states in India, in Egypt and possibly in Burma. In 1985, Roger *et al.* reported that algal inoculation was adopted in only two states of India (Tamil Nadu and Uttar Pradesh) where inoculated fields constituted a small percentage of the total area planted to rice. More than ten years later, inoculation of rice fields with cyanobacteria is still not a technology adopted by farmers, which is probably reflected in the low and erratic increases in yield obtained.

Reconsidering the Potential of Cyanobacteria as a Biofertiliser in Rice Fields

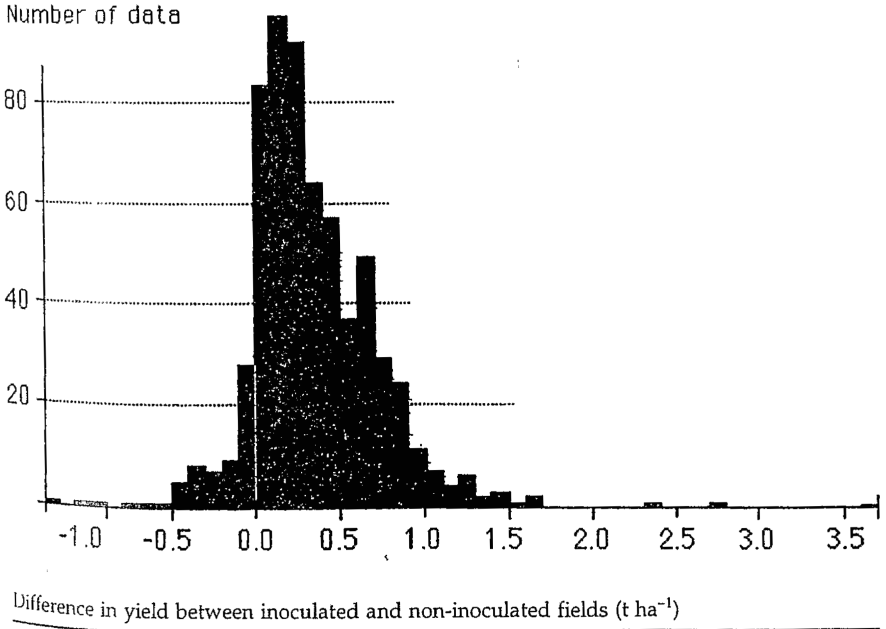
Methods for utilising cyanobacteria in rice cultivation need to be reconsidered in view of studies showing that (1) N_2-fixing forms are ubiquitous in rice soils; (2) their growth in rice fields is most commonly limited by low pH, P deficiency, grazing and broadcasting of N fertiliser; (3) non-indigenous strains

Table 12.3 Bibliographic study of the effect of cyanobacterial inoculation on rice yield* (adapted from Roger, 1991)

1. Major statistics of the data

	Difference between control and inoculated plots	
	Absolute	Relative
Number of observations (n)	634	634
Maximum	370.00 kg ha^{-1}	+168.2%
Minimum	–1280 kg ha^{-1}	–19.3%
Average	337 kg ha^{-1}	11.3%
Median	257 kg ha^{-1}	7.9%
Standard deviation	398 kg ha^{-1}	16.0%
Coefficient of variation	118%	141%

2. Histogram of the data



*Data compiled from 41 references listed in Roger, 1991.

inoculated in various soils rarely establish themselves; and (4) indigenous N_2-fixing cyanobacteria are frequently more numerous than cyanobacteria contained in the recommended dose of 10 kg of soil-based inoculum (Grant *et al.*, 1985; Reddy and Roger, 1988; Bisoyi and Singh, 1988; Reynaud and Metting, 1988; Roger *et al.*, 1987). These findings suggest that more attention should be paid to cultural practices in order to alleviate factors that limit growth and BNF by indigenous strains already adapted to the environment.

Practices known to enhance cyanobacterial growth include liming of acidic soils, P split application, grazer control and deep placement of N fertiliser (Roger and Watanabe, 1986). These practices might suffice to realise more of the potential of indigenous cyanobacteria and are a prerequisite for establishing inoculated strains, if and when inoculation is needed.

The ubiquity of heterocystous cyanobacteria in rice soils does not mean that inoculation is unnecessary. Inoculation with desired strains might be useful because the accumulation of P by the propagules of the inoculum (produced with high levels of P) gives them an initial advantage over the propagules of the indigenous cyanobacteria, which are usually P deficient (Roger *et al.*, 1986). Because spore germination is photodependent (Reddy, 1983), inoculated propagules spread on the soil surface should germinate more readily than indigenous propagules mixed with the soil. The effect of inoculation is likely to be more important after an upland crop grown before rice or after a long dry fallow, when the density of the indigenous cyanobacteria population may be low at the beginning of the rice season. Inoculation might also permit early establishment of an N_2-fixing bloom and thus the availability of more fixed N to rice.

But there are still many uncertainties about the methodological aspects of cyanobacterial inoculation and the nature of inocula to be used. Most algalisation trials have been conducted using inocula developed from a mixture of laboratory cultures, but almost none of the published inoculation experiments have paid attention to the establishment of inoculated strains. As already mentioned, there is increasing evidence that most of the blooms obtained with various treatments combined with inoculation are from indigenous strains (Grant *et al.*, 1985; Reddy and Roger, 1988; Bisoyi and Singh, 1988; Reynaud and Metting, 1988). When the inoculum is produced on the spot in small plots or trays, it is likely that the strains present in the local soil will outcompete the intended isolates even before the inoculum is added to the field. Available data are not sufficient to draw definite conclusions, but they clearly suggest that use of an inoculum produced from the soil to be inoculated should be tested whenever experiments are conducted. This strategy of local bacterial inoculation seems especially advisable where there is a long dry fallow, which often results in partial sterilisation under very warm climates. In that case inoculation should be combined either with deep placement or delayed application of N fertiliser.

One can speculate on the possibility of selecting or designing efficient strains for inoculation. Several authors have screened for high N_2-fixing activity, but their findings may not be useful because there is little reason per se why high N_2-fixing activity should correlate well with *in situ* colonisation potential. In fact, most fast-growing strains (doubling time of 5-12 hours) belong tc the genus *Anabaena*, have short filaments, and are therefore susceptible to grazing. A study of 12 strains showed that those with high N_2-fixing activity *in vitro* did not establish *in situ* (Antarikanonda and Lorenzen, 1982; Huang, 1983).

Another approach has been the selection of nitrogenase-derepressed mutants which excrete NH_4^+ into the medium. Such an *Anabaena variabilis* mutant was found to provide N to rice in an N-free gnotobiotic culture more efficiently than the parent strain (Latorre *et al.*, 1986; Thomas *et al.*, 1991). However, studies at IRRI showed that the strain is not competitive and did not multiply when inoculated in soil microplots (Roger *et al.*, unpubl.).

Biological engineering of cyanobacteria is currently limited to unicellular strains that are morphologically, physiologically and ecologically very different from the N_2-fixing strains considered for inoculating rice fields. Probably 'super N_2-fixing cyanobacteria' can be selected or designed and grown in test tubes but the characteristics that will enable them to survive, develop and fix N_2 in rice fields are still largely unknown.

It can be concluded that after several decades of experiments, inoculation is not a proven technology. Cyanobacteria are ubiquitous in rice fields and their N potential is moderate (10-30 kg N crop cycle^{-1}). A specific management may ensure the expression of this potential through the control of grazers combined with P application and deep placement of N fertiliser but is rarely economically viable. On the other hand, N-fertiliser deep placement on its own is definitely the best technology to take advantage of the potential of indigenous cyanobacteria. It avoids proliferation of unicellular eukaryotic algae, which causes floodwater alkalinisation and N losses by ammonia volatilisation. It avoids the early proliferation of predators of microalgae and vectors of human diseases (ostracods, mosquito larvae...). It allows N_2-fixing cyanobacterial growth, otherwise inhibited by competition and grazing (Fig. 12.6). A large set of experiments in non-inoculated rice fields has demonstrated the efficiency of this technology in significantly increasing rice yield by promoting BNF by cyanobacteria and ensuring a better efficiency of the N fertiliser applied (Table 12.4). A better understanding of the factors that could allow inoculated non-indigenous strains to establish *in situ* is needed before cyanobacterial inoculation could be reconsidered as an agricultural practice in rice cultivation.

Table 12.4 Average acetylene-reducing activity (ARA) during crop cycle and rice yield under different urea management practices[a] (from Roger *et al.*, 1988)

Treatment	ARA activity (μmol C_2H_2 m^{-2} h^{-1})	Grain yield (t ha^{-1})
Control (no N applied)	195 ± 14	4.08 ± 0.10
38 kg N ha^{-1} broadcast at transplanting + 17 kg N ha^{-1} at panicle initiation	80 ± 13	4.82 ± 0.12
55 kg N ha^{-1} deep placed at transplanting	116 ± 16	5.78 ± 0.09

[a] Each value is the average of 60 data.

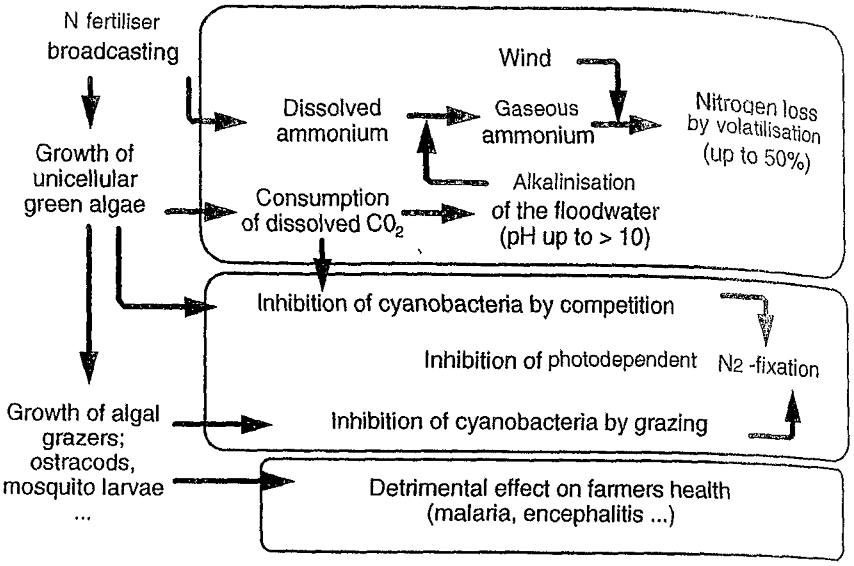


Fig. 12.6 Schematic representation of the effect of N fertiliser broadcasting in rice field floodwater on microalgae, cyanobacteria and zooplankton.

SYMBIOTIC CYANOBACTERIA: *AZOLLA*

Symbiosis

Symbiotic associations between cyanobacteria and plants exist with one or a few genera of phycomycetes, ascomycetes, green algae, diatoms, liverworts, mosses, ferns, gymnosperms and angiosperms. The only symbiosis of agronomic significance is that between *Anabaena azollae* and the genus *Azolla* Lam., a freshwater, heterosporous fern that occurs in a broad latitudinal range on five continents. The seven species of *Azolla* are taxonomically separated by their secondary reproductive structures and the branching patterns and growth habits of their sporophytes. The endophytic *Anabaena azollae* resides within a basal cavity of the dorsal lobe of each leaf of *Azolla* but its cells are also associated with the terminal meristem and the megasporocarp. Filaments of the cyanobacterium have heterocyst frequencies of 20-30% when actively fixing N_2 in the leaf cavities (Hill, 1975). Inducing N excretion by the cyanobacterium through repression of its glutamine synthetase levels, also meets the N needs of *Azolla* (Ray *et al.*, 1978). The entire association may proliferate without mineral N. *Azolla* N is released into the external environment upon decomposition and mineralisation of the fern.

Traditional Use of *Azolla* in Rice Cultivation

Because of its ability to grow rapidly together with rice in submerged soils, and its high N content, *Azolla* has been used as a green manure in rice cultivation for centuries in North Vietnam and Southern China. Its use was generally ignored by scientists in other countries until the mid-1970s. Since then, articles, reviews and information gathered from China and Vietnam have stimulated interest in and research on *Azolla* (Watanabe, 1982). Proceedings of two workshops on *Azolla* (IRRI, 1987; Silver and Shröder, 1984) provide additional information. Two issues of *The Philippine Agriculturist* (1986a, b) were devoted to free-living and symbiotic cyanobacteria in the Philippines, where a National *Azolla* Action Program was established in 1982.

China and Vietnam are the only countries with long histories of *Azolla* cultivation, extending back to the eleventh century in Vietnam and at least the Ming dynasty (1368-1644) in China (Lumpkin and Plucknett, 1982).

In China, *Azolla* has been used from 37° N (Shandong) to 19° N (Hainan). Because the strains used grow best at an average daily temperature of 25°C or below, *Azolla* is usually grown in late spring (May to June) in the North and in early spring (March to April) in the South. During summer, insect damage limits its growth. Most frequently, *Azolla* is grown for about one month, then incorporated before transplanting. To a lesser extent, wide-row transplanting permits cultivation of *Azolla* with rice and several incorporations during the crop cycle. In some places, *Azolla* is grown before and after transplanting (Liu, 1979).

In Vietnam, *Azolla* has been used in the Northern provinces for spring rice because in summer it grows poorly and insect incidence is severe. The technology described by Roger and Watanabe (1986) is as follows. About 2-3 months before transplanting (August), *Azolla* is collected from natural environments by specialists and multiplied on government *Azolla* farms. Inoculum is sold to co-operatives and farmers who propagate it in the fields from November to February. As in China, *Azolla* is grown and incorporated before and after transplanting. *Azolla* grown before transplanting is fertilised with 2.2 kg P ha^{-1} every 5 days, 4.2 kg K ha^{-1} every 10 days, and 0.5-1.0 t ha^{-1} farmyard manure every 5-10 days. When chemical fertiliser is not available, ash is substituted. Intercropped *Azolla* is usually not fertilised, but if P is available, one application of 4.4 kg P ha^{-1} is recommended. *Azolla* inoculum is applied in the field at 0.3-0.5 t fw ha^{-1} along with farmyard manure. To facilitate vegetative multiplication, fronds are broken using a special tool. *Azolla* is collected before heavy rains to prevent its being washed from the fields. If insect pests develop, *Azolla* is collected and placed under water for 15 hours to kill the larvae. Two to 3 weeks after inoculation, the field is covered with about 20 t *Azolla* fw ha^{-1}. Half of this is collected in mounds and composted by covering it with soil. The remaining half is grown for 7-10 days more, after which the field is again fully covered. Half of the second crop and the compost are incorporated in the soil. Rice is transplanted and the remaining half of the second *Azolla* crop continues to grow. Seven to 10 days after transplanting, the field is covered, and half of the third crop is incorporated by hand and foot between the rows. Sometimes a fourth crop is grown and incorporated. This technology produces an average of 40 t *Azolla* fw ha^{-1} crop^{-1}, equivalent to 80 kg N ha^{-1}. The average annual rice yield in the Red River delta is 5-7 t ha^{-1}. The winter crop, with *Azolla*, yields 3-5 t ha^{-1}, and the summer crop, without *Azolla*, yields 2 t ha^{-1} or less. The *Azolla* technology used in Vietnam is labour intensive; it might be improved by wide-row transplanting of rice for easier *Azolla* incorporation with a rotary weeder.

Ecology of *Azolla*

Occurrence

Azolla is widely distributed throughout the world, occurring in a broad latitudinal range on five continents (Lumpkin and Plucknett, 1982). The international bibliography on *Azolla* (Capaya, 1979; IRRI, 1983b) cites almost 50 articles recording *Azolla* in rice-growing countries. To develop significant biomass in rice fields, however, the fields need to be inoculated with *Azolla* and the *Azolla* cultivated.

Climatic factors

The optimum temperature for most species (20-30°C) is below the average temperature in the tropics. Cool weather is a key to successful *Azolla* cultivation in Vietnam and China. Some strains, however, can grow at temperatures higher than 35°C (Lumpkin, 1987; Watanabe and Berja, 1983). The successful growth of *Azolla pinnata* at high temperature observed in North Senegal, where humidity is low (Diara *et al.*, 1987), indicates that in the humid tropics the detrimental effect of high temperature might be mostly indirect, resulting in a high incidence of insect and fungal pests.

Light saturation for *Azolla* growth was reported to occur at 20-50% of full sunlight (Peters *et al.*, 1980). According to Lumpkin (1987), *Azolla* is not inhibited by full sunlight if other factors are not limiting. On the other hand, several reports indicate a decrease in growth or BNF at various light intensities between 60 and 120 klux (Becking, 1979; Li Zuo-Xin *et al.*, 1987; Roger and Reynaud, 1979). When *Azolla* is grown with rice, shading by the rice starts decreasing *Azolla* growth 2-3 weeks after transplanting and inhibits it after about 45 days (Lumpkin, 1987). Daylength correlates with *Azolla* growth (Lumpkin and Bartholomew, 1986), which indicates better potential for *Azolla* cultivation in higher latitudes with long, cool days than in the tropics with constant daylength.

Physicochemical factors

The pH for optimum growth of *Azolla* in culture solution lies in a range of 4.5-7.0 but *Azolla* can survive within a range of 3.5-10 (Lumpkin, 1987). The major effect of pH on *Azolla* growth is probably related to nutrient deficiencies (Fe, Zn etc.) occurring at high values, or toxicities (Al, Fe etc.) occurring at low values (Watanabe *et al.*, 1977a).

Phosphorus is most frequently the major limiting nutrient for *Azolla* growth in the field. Reported threshold values of P deficiency are 0.4% in *Azolla* (dw basis), 0.15 ppm in floodwater and 20 ppm available Olsen P in soil (Ali and Watanabe, 1986; Watanabe and Ramirez, 1984). *Azolla pinnata* can grow satisfactorily without P application when the available Olsen P in the soil is higher than 30 ppm and P sorption capacity lower than 660 mg P/100 g (Watanabe and Ramirez, 1984). Such P-rich soils are not very common. *Azolla* doubling time estimated in a growth test on 972 Philippine soils was less than 5 days (moderately suitable soil) in 40% of the samples and less than 3.5 days (highly suitable) in only 13% of the samples, showing that P fertilisation of *Azolla* would be required in many soils (Callo *et al.*, 1985).

Other nutrients seem to be much less limiting *in situ* than P. In the Philippines, total C, total N and exchangeable cations of soils did not correlate with *Azolla* productivity (Watanabe and Ramirez, 1984). Potassium, however, was necessary for *Azolla* growth in North Vietnam (Roger and Watanabe, 1986). Lumpkin (1987) cites four references reporting that the addition of Mo or Fe improved *Azolla* growth in the field.

Biotic factors

The *Azolla* mat floating on the floodwater is an environment that favours the development of insect larvae and molluscs that feed on *Azolla* and limits its growth. A tentative list of *Azolla* pests includes 30 insects (13 Diptera, 6 Coleoptera, 8 Lepidoptera, 1 Homoptera, 2 Orthoptera), 2 Arachnoidea and 9 Mollusca (Mochida, 1987). Yield losses of field-grown *Azolla* due to *Ephestiopsis vishnu* (webworm) and *Nymphula* spp. (caseworms) may reach 60% (Mochida *et al.*, 1985). Some fungi are *Azolla* pathogens that magnify the effects of insect attacks when developing on the plant wounds (Lumpkin, 1987). A negative correlation was observed between *Azolla* and algal biomass in rice fields at IRRI (unpubl. data). Algal blooms may compete with *Azolla* for nutrients and, by increasing floodwater pH, may reduce nutrient availability to *Azolla*.

Effect of N fertiliser

In the absence of competing organisms, BNF by *Azolla* is more tolerant of combined N than BNF by free-living organisms. When *Azolla caroliniana* was grown in nutrient solutions of NO_3^-, NH_4^+ and urea, all at 35 ppm N, it derived 86% of its N from BNF in NO_3^- solution, 70% in NH_4^+ solution and 60% in urea solution (Peters *et al.*, 1981). In floodwater, however, N favours the growth of competing aquatic plants that may hinder *Azolla* growth. In the presence of green algae, 1.4 mM NH_4^+ decreased *A. pinnata* growth by 60% (Watanabe *et al.*, 1977).

The *Azolla* canopy prevents light penetration into the floodwater, inhibits the growth of other phototrophs and depresses photodependent CO_2 uptake. Thus, under an *Azolla* canopy, the floodwater pH remains lower than in *Azolla*-free conditions, which may decrease N losses by NH_3 volatilisation.

Effect of pesticides

Herbicides have been shown to be toxic to *Azolla* even though one of the recognised beneficial effects of *Azolla* is its weedicide effect. Therefore, there is little interest in combining the use of *Azolla* with that of herbicides. Threshold values for toxicity are much higher for insecticides than for herbicides. Insecticides favour *Azolla* growth by decreasing insect pest incidence but some may also be phytotoxic.

Azolla as a Source of N for Rice

BNF by *Azolla* has usually been estimated from biomass measurement and the assumption that most of *Azolla* N originates from BNF. The ^{15}N dilution method and the ^{15}N method have confirmed that most N in *Azolla* originates from the atmosphere (Kumarasinghe *et al.*, 1985; Kulasooriya *et al.*, 1988). The N potential of *Azolla* was summarised by Roger and Watanabe (1986) from data obtained mostly in experimental plots. The N content in maximum standing crops ranged from 20 to 146 kg ha^{-1} and averaged 70 kg ha^{-1} (n = 17; C.V. = 58%). N_2-fixing rate ranged from 0.4 to 3.6 kg N ha^{-1} d^{-1} and averaged 2 kg N ha^{-1} d^{-1} (n = 15; C.V. = 47%). In 4-year field trials at 37 sites in 10 countries, productivity was lower than in experimental plots (Watanabe, 1987). Biomass was 5-25 t fresh weight ha^{-1} (10-50 kg N ha^{-1}) for *Azolla* grown before or after transplanting (average 15 t ha^{-1} or 30 kg N).

Nitrogen recovered by the rice plant from ^{15}N-labelled *Azolla* incorporated in soil ranged from 20 to 34% (Table 12.2). As with cyanobacteria, availability increases when *Azolla* is incorporated in the soil.

Effects other than N on Rice

Azolla not only provides additional P for the rice crop, but also enhances the use of P from mineral fertiliser (Sampaio *et al.*, 1984). The threshold concentration of K for absorption was estimated as 0.85 ppm K_2O for *Azolla* and 8 ppm for rice (Liu, 1984). Irrigation water usually contains 1-5 ppm K, which is below the level at which rice can absorb K rapidly, but enough for rapid absorption and concentration by *Azolla*, which becomes a source of K for rice when incorporated. Several reports note that *Azolla* decreases weed incidence (Diara *et al.*, 1987; Lumpkin and Plucknett, 1982). In Senegal, the *Azolla* cover decreased water evaporation by 20% (Diara *et al.*, 1987). *Azolla* improves the structure of the soil as do most organic manures (Roychoudhury *et al.*, 1979, 1983). That becomes important in fields where rice is grown sequentially with an upland crop.

Analysis of Field Experiments *in situ*

International field trials conducted for 4 years at 37 sites in 10 countries (Watanabe, 1987) showed that (1) incorporating one crop of *Azolla* grown before or after transplanting is equivalent to application of 30 kg fertiliser N

ha^{-1} and (2) incorporating two *Azolla* crops grown before and after transplanting is equivalent to split application of 60 kg N ha^{-1} (Table 12.5).

Table 12.5 International *Azolla* trials at 37 sites in 10 countries (adapted from Watanabe, 1987)

Treatment	Yield	
	($t\ ha^{-1}$)	% control*
Control: no N, no *Azolla*	3.00	100 c
30 kg N ha^{-1}, 3 split applications	3.65	121 b
60 kg N ha^{-1}, 3 split applications	4.24	141 a
Azolla incorporated before transplanting	3.73	124 b
Azolla incorporated after transplanting	3.67	122 b
Azolla inoculated after transplanting but not incorporated	3.61	120 b
Combination of treatments 2 and 4	4.15	138 a
Combination of treatments 2 and 5	4.07	135 a
Azolla incorporated before and after transplanting	4.09	136 a

*Values followed by the same letter are not significantly different at $p = 0.05$.

Current Use

In 1978, FAO (1978) estimated that *Azolla* was used on more than 6.5 million ha in China. In 1979, Liu (1979) reported an estimate of 1.34 million ha. In 1982, Lumpkin and Plucknett (1982) reported that *Azolla* was used in 2% of the 34 million ha planted to rice in China.

In Vietnam, in 1980, *Azolla* was used in the Northern and North Central provinces on about 500,000 ha or 9% of the total rice area (Roger and Watanabe, 1986).

In the Philippines, farmers adopted *Azolla* on 5000 ha in South Cotabato in 1981 (Kikuchi *et al.*, 1984); success was mainly due to a high level of available P in the soils and a short dry season. *Azolla* use extended to 26,000 ha in 1983, and 84,000 in 1985 (Mabbayad, 1987). In the proceedings of the 1985 *Azolla* Workshop (IRRI, 1987), quantitative information on the rice area where *Azolla* is used is available only for the Philippines (84,000 ha). The papers presented during the Workshop indicate that China, the Philippines and Vietnam were using *Azolla*. Brazil, India, Pakistan, Senegal, Sri Lanka and Thailand were studying the feasibility of *Azolla* adoption by rice farmers.

With the advent of available cheap sources of urea and potash, however, the area devoted to *Azolla* technology in Vietnam and China decreased dramatically during the 1980s. *Azolla* use has not spread to other countries. In Fujian province, China, *Azolla* use dropped to 5-10% of the rice hectarage, down from 20% at the beginning of the 1980s. The same trend was observed for green manures such as *Astragalus*, currently used on 10-20% of the land, down from a maximum of about 30% (Stone, 1990). On the other hand, interest in *Azolla* use as a fish and animal feed, a mineral scavenger and depollutant has increased in China (Liu, 1988). Reduction of *Azolla* use in China also stems from the changing governmental economic policy, which has led to the disbanding of many agricultural communes and the reallocation of labour. World *Azolla* use is now only a very small fraction of the estimated two million hectares of rice that were fertilised with *Azolla* in China and Vietnam in the late 1970s.

Factors Limiting *Azolla* Use and Possible Methods for Alleviating Them

Major constraints against wide use of *Azolla* by rice farmers in Asia include: difficulties in maintaining the inoculum throughout the year, particularly in the dry season; P deficiency; low tolerance to high temperature; damage by insects and fungi; poor water control and economics in relation to changing agricultural practices and policies.

Inoculum conservation and propagation

Azolla cannot withstand desiccation and requires standing water throughout its cultivation cycle. Because *Azolla* is propagated vegetatively, inoculum must be maintained in nurseries year-round and multiplied for distribution before field cultivation. Thus an irrigation network and a network for inoculum conservation, production and distribution are imperative for *Azolla* use. Further, *Azolla* adoption by farmers depends first on a government policy to establish such networks (Roger and Watanabe, 1986). A method for using sporocarps for inoculum conservation was developed in China but their growth was too slow to meet inoculum requirements in the field; 160 kg sporocarps fw ha^{-1} yielded 16 to 21 t *Azolla* fw ha^{-1} in 52 days (Lu, 1987). Conditions for sporocarp formation and germination are still incompletely understood.

Need for P fertiliser

Phosphorous application is required in most soils for growing *Azolla*. To be economically feasible, it requires a ratio of N fixed to P applied greater than the ratio of the prices of the N and P fertilisers (4 in most Asian countries). As P fertiliser is rapidly immobilised in the soil, basal application of P is often uneconomical, while split application has an efficiency of 4.6-10.0 g N_2 fixed g^{-1} P applied (Watanabe *et al.*, 1980, 1988). Phosphorus fertilisation limited to the inoculum production plot permits the P-enriched *Azolla* to multiply 6 to 7 times without P application in the main field, and ensures a high efficiency of applied P (Watanabe *et al.*, 1988).

Pest control

Although commercial pesticides effectively control *Azolla* pests, no method of field application is economical (IRRI, 1986). The possible use of commercial insecticides is limited to inoculum production (Mochida, 1987). Use of cheap pesticides of plant origin in the field might be economical in some regions.

Low temperature requirements

Temperature limitations can be reduced by selecting cold- or heat-tolerant strains (Watanabe and Berja, 1983). Among strains tested at IRRI, *Azolla* *microphylla* #418 was most tolerant of high temperature (37°C day/29°C night) (Watanabe *et al.*, 1992).

Economics

Technologies used in Vietnam and China are labour intensive and therefore have economic limitations. Kikuchi *et al.* (1984) studied the economics of *Azolla* use in the Philippines in South Cotabato, where *Azolla* spread spontaneously and no P fertiliser and little labour was necessary. Economic return from *Azolla* adoption, including cost savings in chemical fertilisers and weed control, was more than \$35 ha^{-1} at 1981 prices. Conditions in the study area were exceptionally favourable, and should be viewed realistically. The authors concluded that the economic potential of *Azolla* is greatest where the opportunity cost of labour is low, and that labour cost becomes critical where wage rates approach \$2 per day. Insect control was also an important economic limitation. If more than 200 g carbofuran active ingredient ha^{-1} was needed to control insects, benefits were eliminated. In areas of the Philippines where conditions for *Azolla* growth were not favoured by an exceptionally high level of available P, *Azolla* use was uneconomical (Rosegrant *et al.*, 1985; Rosegrant and Roumasset, 1988).

A case study in the Philippines does not suffice for drawing definite conclusions regarding *Azolla* economics, however, as this may vary according to socioagricultural systems. Economic calculations should also consider the long-term benefits of *Azolla* as an organic fertiliser with the concomitant increase in soil OM and fertility, instead of only those costs directly comparable to commercial N fertiliser prices. The economics of integrated rice-fish-*Azolla* culture might be more favourable and should be considered.

Biotechnologies for agronomic use of Azolla

Azolla strains exhibit a wide range of behaviour with regard to environmental factors, P requirement, BNF, productivity etc. (Van Hove *et al.*, 1987). The ability to combine favourable characters such as resistance to high temperature and insects, low P requirement and erect growth (permitting higher productivity), would allow strains to be designed for specific agroecological conditions. For this purpose, recombination of different algal and plant symbionts and sexual hybridisation between *Azolla* species proved feasible. *Anabaena* from *Azolla filiculoides* was recombined with *A. microphylla* and vice versa. Megasporocarps of each species were freed of their algal symbiont (Lin and Watanabe, 1988), then an indusium of the other *Azolla* species, containing the corresponding *Anabaena*, was placed on the algal-free megasporocarps and symbiosis thus established with the newly formed sporophyte (Lin *et al.*, 1988).

Formation of *Azolla* hybrids requires that macrosporocarps and microsporocarps are obtained. Sporulation of many strains can be observed under natural conditions (Payawal and Paderon, 1986), but no satisfactory method has yet been designed to induce sporulation at will. That is a major limiting factor for *Azolla* hybridisation. Hybrids of *A. microphylla* (female parent) and *A. filiculoides* have been obtained (Wei *et al.*, 1986). The IRRI biofertiliser germplasm collection contains 23 hybrid strains obtained by algal transfer and 85 obtained by sexual hybridisation (Watanabe *et al.*, 1992).

What Future for *Azolla*

Azolla is an N_2-fixing organic manure with N potential similar to that of legumes. It is easier to incorporate than other organic manure crops and grows well with rice in flooded conditions. Environmental, technological and economic factors limit its use. Problems in inoculum conservation, multiplication and transport could be solved if *Azolla* could be propagated from spores. Temperature limitations and P requirement can be reduced by selecting/constructing cold- or heat-resistant strains with low P requirement and by split application of P fertiliser, whether limited to inoculum production or not. Despite the availability of a large range of strains adapted to a broad range of environments, *Azolla* use as green manure has continuously decreased since the 1970s because of important economic limitations, together with drastic changes in agricultural policies (*Azolla* was used in China and Vietnam under a directive management of agricultural practices) and management practices, in particular a change from transplanted rice—favourable to *Azolla* growth—to direct seeded rice—where early growth of *Azolla* might be detrimental.

Azolla still has a future as green manure in areas where it can spread spontaneously and where pest incidence is low because of a dry climate. A number of rice-growing areas in Sahelian and subtropical areas and in Madagascar might be suitable for its use.

Azolla has a potential not only as a green manure, but as a multipurpose biofertiliser that can also be a weed suppressor, a K source through its ability to concentrate the element, an animal feed for pigs and chickens and a primary producer in rice-fish-*Azolla* cultivation. A rice-fish-*Azolla* system was developed in China (Liu, 1988). In field trials, average rice yield increased by 5% over the traditional system and an average fish production of 625 kg ha^{-1} was reported. About 60% of the N fixed by *Azolla* was assimilated by fish and 40% of this was excreted in faeces, equivalent to about 300 kg $(NH_4)_2SO_4$ ha^{-1} . Weed biomass and incidence of pests on rice and *Azolla* decreased (Liu, 1988). That potential may revive interest in use of *Azolla*.

The recent interest of consumers in Europe and the USA in products from 'biological' agriculture, might offer another opportunity to use *Azolla* to produce 'bio' rice (produced without agrochemicals), a label that gives it a much higher commercial value than rice produced with agrochemicals.

CONCLUSION

Nitrogen is usually the limiting factor to high yields in rice fields. Therefore, use of BNF as an alternative or supplementary source of N for rice has been the major approach in microbiological management of wetland rice. Among N_2-fixing micro-organisms present or introduced in rice fields, cyanobacteria play a major role. Free-living indigenous forms have spontaneously enabled modest but constant yields (1-2 t ha^{-1}) to be obtained for centuries after continuous cropping without N fertiliser addition. Symbiotic forms were also used for centuries in rice fields in China and Vietnam, enabling significant yields (2-5 t ha^{-1}) to be obtained through labour-intensive and quite sophisticated green manuring technology.

Research on free-living and symbiotic cyanobacteria as biofertilisers for rice reached intensive development in the 1970s and 1980s. Since then, the number of research papers dealing with these topics has markedly declined. Research on free-living cyanobacteria has shown that, as an additional source of N for rice, they have a moderate potential of about 30 kg N ha^{-1} $crop^{-1}$ which may translate to a yield increase of 300-450 kg ha^{-1} . Inoculation of rice fields with selected or transformed strains is not a proven technology. On the other hand, the knowledge acquired about the general ecology of rice-field floodwater during these studies has enabled practical recommendations to optimise its management in a way that favours BNF by cyanobacteria, improves chemical N fertiliser utilisation, and helps to control populations of detrimental invertebrates. Over the long term, biological engineering of cyanobacteria may design 'super N_2-fixing cyanobacteria' but the characteristics that will enable them to survive, develop and fix N_2 in rice fields are still not known.

Azolla has proved useful as a nitrogen biofertiliser in China and Vietnam. Currently its use has drastically declined in these countries and *Azolla* has not been adopted in most of the countries where it was tested for adoption as green manure for rice. Progress in strain collection, selection, hybridisation and recombination has opened ways to alleviate many environmental and nutritional limitations of *Azolla*. However, socioeconomic limitations are the most important and are probably increasing in relation to changes in agricultural policies and practices. But recent studies have shown that *Azolla* has a potential not only as a green manure, but as a multipurpose biofertiliser that can also decrease N losses by ammonia volatilisation. It can also be a weed suppressor, a potassium source through its ability to concentrate this element, an animal feed, and a primary producer in rice-fish-*Azolla* cultivation. The potential of *Azolla* as a multipurpose crop, which may revive interest in its use, will decide the extent of its future utilisation.

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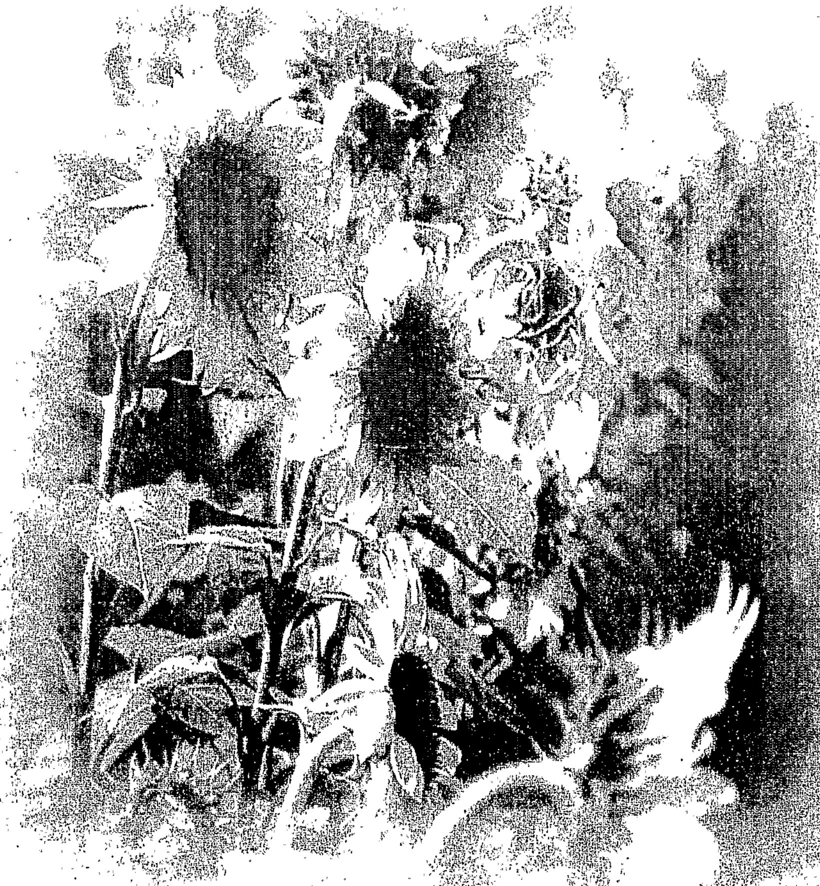

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