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3. Technologies for utilizing biological nitrogen fixation in wetland rice: potentialities, current usage, and limiting factors

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Abstract. Almost all types of N_2-fixing microorganisms are found in lowland rice fields. The resulting N fertility has permitted moderate but constant productivity in fields where no N fertilizer is applied. Current and potential technologies for utilizing biological N_2 fixation in lowland rice production are reviewed in terms of potential, current usage, and limiting factors.

Legumes and azolla have been traditionally used as green manure in parts of Asia, permitting yields of 2–4 t/ha. To a limited extent, straw incorporation favors heterotrophic biological N_2 fixation. Recently, inoculation with blue-green algae has been claimed to increase yields by about 10%. Using non-symbiotic N_2-fixing systems is still experimental.

Utilization of biological N_2 fixation as an alternative or additional N source for rice is severely limited by technological, environmental, and socioeconomical factors.

Rice is the staple food of approximately one half of the world's people. About 75% of the 143 million hectares of ricelands are lowlands where rice grows in flooded fields during all or part of the cropping period.

Lowland rice can be grown on the same land year after year without N fertilizer and produce moderate but constant yields. In contrast, upland rice yields decline over time if no N fertilizer is applied. The continuing N fertility in lowland rice fields has been attributed to higher N_2 fixing activity coupled with slower decomposition of organic N compounds under poorly aerated conditions [15].

N is usually the limiting factor to produce high yields. The green revolution in rice production is based on fertilizer--responsive rice varieties. In Asia, one of the constraints to high yields is the limited availability and high prices of N and P fertilizers. In 1978 fertilizer use in tropical Asian countries averaged 30–55 kg NPK/ha arable land [92]. The idea of utilizing biological N_2 fixation (BNF) as an alternative or supplementary source of N for rice is not new. N_2-fixing green manures have been used for centuries in some rice growing areas, and research on biofertilizers, including algal and bacterial inoculants, began in the early 1930s.

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Biological N_2 fixation in flooded soil systems was reviewed by Buresh et al. [15]. Processes and ecology of BNF in rice soils were reviewed by Watanabe [147] and Watanabe and Brotonegoro [152]. An extensive study of quantitative data was presented in Lowendorf's review [75]. New knowledge of BNF in flooded rice fields was summarized by Watanabe and Roger [159].

This paper intends to describe the potential for practical utilization of BNF technology by rice farmers rather than to discuss mechanisms and N_2-fixing agents. After a short summary of the properties of the lowland rice field ecosystem as a site for BNF, emphasis is placed on technologies, their potential and current usage, and the technological, environmental, and socio-economical factors that limit adoption. Although most legume green manures are grown in upland conditions before lowland rice, we found it appropriate to include legume green manuring in our discussion. Straw incorporation, seldom considered in previous reviews, is also discussed.

I The wetland rice field ecosystem as a site for BNF

A Agronomic characteristics of lowland rice fields

Wetlands (lowlands) were defined by Cowardin et al. [23] as 'lands where saturation with water is the dominant factor determining the nature of soil development and the types of plant and animal communities. Flooding the soil has many advantages for rice production:

- it provides a continuous water supply to the crop;
- it changes the pH of alkaline and acidic soils towards neutrality or slight acidity which is favorable for rice growth;
- it diminishes the incidence of soil sickness and outbreak of soil borne diseases usually observed under continuous monocropping in upland soils;
- it depresses weed growth, especially C_4 type grasses;
- it favors BNF, giving flooded soils a higher spontaneous fertility than upland soils;
- irrigation water supplies nutrients such as Ca, Si, and K; and
- bunded rice fields act as water reservoirs and prevent soil erosion.

B Different sites for BNF in rice fields

Principal environmental characteristics of lowland rice fields are determined by: flooding, the presence of rice plants, and agricultural practices.

Flooding of the soil soon creates anaerobic conditions in the reduced layer, a few millimeter beneath the soil surface. Flooding and rice plants lead to the differentiation of five major environments differing by their physico-chemical and trophic properties and the energy sources for BNF: floodwater, surface oxidized soil, reduced soil, rice plants (submerged parts and rhizosphere), and subsoil (Fig. 1).

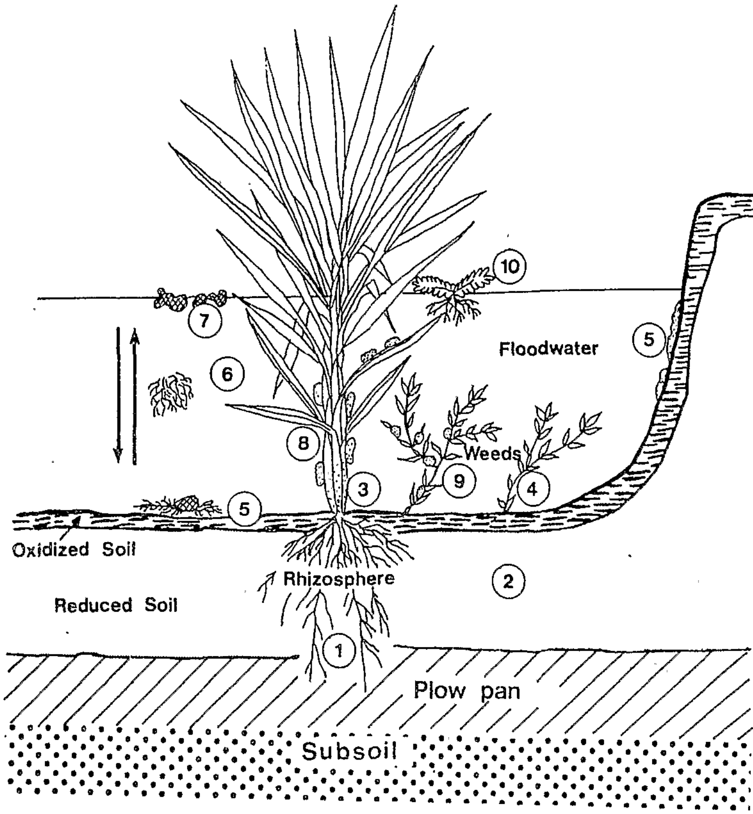

Figure 1. Diagram of environments and N_2-fixing components in a rice field ecosystem. *N_2 fixing bacteria:* 1) associated with the roots, 2) in the soil, 3) epiphytic on rice, 4) epiphytic on weeds. *blue-green algae:* 5) at soil-water interface, 6) free floating, 7) at air-water interface, 8) epiphytic on rice, 9) epiphytic on weeds. *azolla:* 10

The floodwater is a photic, aerobic environment where aquatic communities of producers (algae and aquatic weeds) and consumers (bacteria, zooplankton, invertebrates, etc.) provide organic matter to the soil and recycle nutrients.

The oxidized soil layer is a photic aerobic environment with a positive redox potential [161], a few millimetres thick, where NO_3^-, Fe^{+3}, SO_4^{-2}, and CO_2 are stable and where algae and aerobic bacteria predominate.

The reduced soil layer is nonphotic anaerobic environment where Eh is predominantly negative [161], reduction processes predominate, and where microbial activity is concentrated in soil aggregates containing organic debris [145].

The rice plant comprises two major subenvironments: submerged plant parts and the rhizosphere. In floodwater, basal portions of rice shoots and aquatic weeds are colonized by epiphytic bacteria and algae. Epiphytism is important in deepwater rice where the submerged plant biomass is very high [115]. The rhizosphere is a nonphotic environment where redox conditions are determined by the balance of oxidizing and reducing capacities of rice roots and where production of carbon compounds by roots provides energy sources for microbial growth.

The soil beneath the plow pan is aerobic in well-drained soils and anaerobic in poorly drained soils. It has microbial activity in the upper layer and its role in providing N to rice should not be underestimated [143].

Although those five major environments can be macroscopically differentiated, they are more or less continuous and heterogeneous. Floodwater and surface soil can be considered as a continuum where algae become benthic in night and float in day and epipelic organisms such as chironomids migrate between the two. The activity of soil fauna induces the formation of microaerophilic sites in anaerobic soil, whereas organic matter debris may provide some anaerobic microenvironments in floodwater. Agricultural practices dedifferentiate these environments mainly through mechanical disturbances. As Dommergues [32] wrote, flooded rice soil is far from being uniformly reduced and should be regarded as a complex system formed by the juxtaposition of microenvironments that are oxidation reaction sites or reduction sites.

C N_2-fixing microorganisms in wetland rice fields

As a result of the differentiation of macro- and microenvironments that differ by redox state, physical properties, light status, and nutritional sources for the microflora, all major N_2-fixing groups can and do grow in the lowland rice field ecosystem. Those are free living and symbiotic autotrophs, symbiotic heterotrophs, and aerobic, facultative anaerobic, and anaerobic free living heterotrophs (Table 1). The floodwater, the submerged plant biomass, and the aerobic soil layer are sites of photodependent N_2 fixation. Heterotrophic N_2 fixation develops preferentially in nonphotic environments: the soil aggregates that contain organic debris and the rhizospere.

From an ecological point of view, N_2-fixing organisms in rice fields can be classified as:

- three groups of autotrophs comprising photosynthetic bacteria, free living blue-green algae (BGA), and azolla, and
- three groups of heterotrophs comprising N_2-fixing bacteria in the soil, N_2-fixing bacteria associated with rice, and legume green manures.

D Evidence and quantification of BNF in lowland rice fields

A summary of data on N uptake by rice crops in nonnitrogen plots of long-term fertility trials [154] indicates that in the absence of N fertilizer, a rice crop uses an average 40–50 kg N/ha. In most of those experiments soil N did not decrease, indicating that used N is compensated for by mechanisms among which BNF is most important.

Table 1. Major groups of N_2-fixing microorganisms in lowland rice fields

Photoautotrophs					
	Free living				
		Photosynthetic bacteria			*Rhodopseudomonas*
		Blue-green algae (cyanobacteria)			*Nostoc, Anabaena*
	Symbiotic				*Anabaena azollae* in Azolla sp.
Heterotrophs					
	Free living				
		In the soil			
			Oxidized soil:	obligate aerobes	*Azotobacter, Beijerinckia*
				microaerobes**	*Methylomonas*
			Reduced soil:	obligate anaerobes	*Clostridium, Desulfovibrio*
		In association with rice			
			On submerged parts:	facultative anaerobes*	*Klebsiella*
			In the rhizosphere:	facultative anaerobes	*Enterobacter*
				microaerobes**	*Flavobacterium, Pseudomonas*
					Azospirillum
	Symbiotic				*Rhizobium* in legumes

*Growth in presence of O_2 but fix N only in absence of O_2
**Fix only in the presence of low O_2 concentrations

Quantification of BNF during the crop cycle has been tried, unfortunately the methods were inaccurate and controversial. Nitrogen balance experiments give only the sum of N gains and losses. In field experiments the contribution of subsoil is generally not estimated [154], and in pot experiments the contribution of phototrophs may be overestimated [115]. The limitations of the acetylene reduction assay and ^{15}N methods were summarized by Watanabe and Roger [159].

Long term fertility experiments show that N balance between losses and inputs through BNF and other minor N sources range from 20 to 70 kg N/ha and per crop in plots receiving no N fertilizer [154]. Evaluation of photo-dependent BNF, compiled by Roger and Kulasooriya [115], ranged from very little to 80 kg N/ha per crop and averaged 27 kg N/ha per crop. An extensive compilation of quantitative estimates of BNF in lowland rice fields is in Lowendorf's [75] review. He summarized the data as ranges of fixed N/ha per crop as: legumes, 25–61 kg/ha; BGA, 0.2–39; azolla green manure, 25–121; azolla dual crop, 2–75; and soil and rhizosphere fixers, 1.2–18.3.

E Available and potential technologies

Potential for a significant agronomical N contribution has been proved for five of the six major groups of N_2-fixing organisms in lowland rice fields. The potential of photosynthetic bacteria has not been assessed. Among those five groups, only N_2-fixing legume and azolla green manures are being used purposefully as a N source for rice. Heterotrophic N_2 fixers are favored by straw incorporation but that technology is not used to increase BNF. A technology for rice fields inoculation with BGA has been developed in some countries but is not used by farmers to a noticeable extent. Technology to enhance the activity of heterotrophs associated with rice roots is unavailable.

II Technologies used by farmers

A Legume green manures

The potential of legume green manures for rice was early recognized. In 1936 the International Institute of Agriculture [49] reported that 'application of green manure may involve great progress in rice growing by ensuring yields higher than those at present attained'. Similar statements were recorded by Pandey and Morris [93] from the proceedings of international meetings in 1952, 1953, and 1954. Since then, less attention has been given to legumes in rice production than to other sources of BNF. Lowendorf [75] wrote that legumes were mentioned in only a few paragraphs of the proceedings of the International Rice Research Institute (IRRI) symposium on *Nitrogen and Rice* [69, 94]. However, emphasis on legumes was increased at the recent IRRI symposium on *Organic Matter and Rice* [66, 106, 126, 139, 160].

1 Potentialities. Based on reported N contents in legume green manure crops, (Table 2) it appears that one crop accumulates on average 100 kg N/ha. Highest values have been reported for *Sesbania* spp: 146 kg N/crop for *S. sireceda* [40], 202 kg for *S. sesban* [51], and 267 kg in 52 d for *S. rostrata* [114]. *Sesbania rostrata* forms N_2-fixing nodules on the roots and the stems, and has 5–10 times more nodules than most legumes. Because of its stem nodules, it can fix N under waterlogged conditions and when the N content of the medium is high, giving it exceptionally high potential for BNF [34, 35]. Assuming that between 50 and 80% of N accumulated in legumes originates from BNF [39] it appears that legume green manures could provide 50–80 kg N to a rice crop.

Because N availability to rice may vary with the kind of green manure, a better evaluation of green manure potential is to compare them with N fertilizers. Incorporating one legume crop is equivalent to applying 30 to 80 kg fertilizer N (Table 3).

Table 2. Legume green manures as a N source. [Data from 11, 40, 51, 52, 149].

Species	N content of a crop (kg/ha)	(% fresh weight)
Astragalus sinicus	108–123	0.35–0.47
Canavalia ensiformis	98	0.47
Cassia mimosoides	97	0.44
Crotalaria anagyroides	98	0.33
Crotalaria juncea	129	0.30
Crotalaria juncea	105	–
Crotalaria quinquefolia	88	0.19
Dolichos biflorus	89	0.58
Gycine koidzumii	71	0.42
Phaseolus sp.	–	0.28
Phaseolus lathyroid	90	–
Phaseolus calcaratus	42	0.22
Sesbania aculeata	122	0.32
Sesbania aculeata	96	0.36
Sesbania rostrata	267	–
Sesbania sesban	100	0.39
Sesbania sesban	202	–
Sesbania microcarpa	87	0.50
Sesbania sirececa	146	–
Average value	114	0.37
c.v. %	43	29

The effects of incorporating a legume green manure on rice yield and soil properties have been thoroughly documented by field experiments in many countries [93]. The relative efficiency of different legume species and comparisons with chemical fertilizers were discussed by Singh [126]. In China, which has a long history of green manuring, Wen Qi-Xiao [160] reported that on average, applying 1 ton (fresh weight) of winter green

Table 3. Experiments comparing legume green manures and N fertilizers

Incorporated green manure (1 crop)	N fertilizer needed for the same yield (kg/ha)	Reference
Crotalaria juncea	75	11
Sesbania aculeata	50	11
Sesbania (leaves)	40	20
Sesbania (20 kg N)	30	136
Sesbania aculeata	60	10
Sesbania aculeata	65–70	133
Vigna radiata	80	87
Green gram	60	85
Daincha	75	26
1 crop green manure (average)	30–80	93

manure to rice will increase yield by 30–80 kg, depending on soil fertility, rate of application, and rice cultivar. The N utilization rate was estimated to be about 30%. Pandey and Morris [93] estimated legumes potentialities to be 100 kg grain yield increase per ton of green manure incorporated (winter, spring, and summer green manures). Rinaudo et al. [114] reported a record yield increase from *S. rostrata.* Incorporated as a 52-d-old crop before transplanting, it increased rice yield by 3.7 t/ha over the control. Applying 60 kg N as ammonium sulfate increased yield by 1.7 t/ha over the control. About 33% of the N accumulated by *S. rostrata* was transferred to the crop.

Tiwari et al. [133] reported that wheat crop following a rice crop with green manure registered a 54% increase in grain yield compared with the wheat crop that followed rice with no green manuring, indicating residual effects.

Besides increasing rice yield and N content of the grain [133], incorporating legume green manures is thought to have additional beneficial effects such as increasing soil N and organic matter content [122], available Zn [48], hydraulic conductivity, water holding capacity, and aggregate stability of the soil [12]. Another possible beneficial effect is trying up mineralized soil N, thus preventing its loss by denitrification, volatilization, or leaching when land is fallow [75].

2 *Methods of utilization.* In areas where rainfall is the primary water source in wet season, legumes may be intersown with rice shortly before harvest, grown through dry season, and incorporated when soil is prepared for the next crop [16, 43]. Although those legumes must be drought tolerant, the economic value of legume green manures is less affected by drought than that of grain legumes because green manure is not strongly dependent on drought sensitive reproduction processes.

Where two rice crops are grown or where rainy season is long enough, a green manure can be grown just before rice and incorporated before transplanting. In North Vietnam, *Sesbania* is planted about 60 d before harvesting spring rice and is incorporated, with rice straw, $2\frac{1}{2}$ mo later before the summer rice crop. *Sesbania* biomass is 5–7 t/ha, fresh weight, equivalent to 25–35 kg N. For spring rice, farmers prefer azolla, which supplies more N [50].

Leaves and cuttings of perennial plants grown along field borders, and even wild legumes collected nearby have been used as green manure and applied at or after planting [126]. Devoting fields to green manure production is uncommon. However, Chari [16] wrote that 1 ha of *Sesbania* could provide 10 ha of rice with 6.7–9.0 t of green manure or 40–50 kg N/ha. Growing a cash crop that is harvested for grain and then incorporated into the soil is another green manure alternative [139]. However, under such conditions, soil N may be depleted if more N is removed in the harvest than was fixed [75].

Legume utilization may be improved in integrated management as reported by Wen Qi-Xiao [160]. Using 1,250 kg (fresh weight) of milk vetch (*Astragalus sinicus*) as swine fodder increased the swine's weight by 26 kg. Applying the resulting excreta to rice as fertilizer increased grain yield by 27 kg. Applying milk vetch directly similarly increased rice yield. In that combination, 2-step green manure utilization was more profitable.

3 Present usage Despite their potential to increase yield, use of leguminous green manures has decreased in recent years.

China is the only country where legumes are still widely used. In a review of utilization of organic materials in rice production in the tropical and subtropical zone south of the Huai River (the center of rice production in China), Wen Qi-Xiao [160] wrote that the total area of leguminous green manure crops and azolla in that region had increased from 2.45 million ha in 1952 to 8.35 million ha in 1979. representing an additional 346 000 t N. In 1980, Chen [21] reported that green manure was planted on 20- 50% of the total farmland in Jiangsu, Zhejiang, Jiangxi, Huuan, and Husei provinces. Total green manured area was estimated to be 10 million ha. Although total green manure used for rice production in China has increased since 1949, their relative contribution to N fertilization decreased [160]. The higher the level of intensive agriculture the lower is the ratio of organic manure to chemical fertilizers and the higher the proportion of farm yard manure in organic manure. Hectarage of green manure tends to increase where soil fertility is low and decreases where soil fertility is high.

In other countries, green manure use seems to have become incidental. About 100,000 ha of *S. sesban* are estimated to be grown annually for summer rice in northern Vietnam, when azolla cannot be used because of high pest incidence and high temperatures [50].

In India, Singh [126] reported that 20–30%of several rice growing areas were planted to legume green manure at mid century but that recently green manuring has decreased with increased cropping intensity and ready fertilizer availability. Although green manuring and green leaf manuring are well-known to farmers, they have not been extensive in India since organic fertilizers were introduced [139].

Since 1955 a spectacular decline in green manuring has also been observed in Japan [149].

4 Limiting factors. Many reasons have been given to explain the decline and the non acceptance of green manuring by farmers in South and Southeast Asia.

In temperate climates some detrimental effects of green manuring have been reported. Watanabe [149] attributed the decline of green manures in Japan to:

- possible rice growth reduction caused by anaerobic decomposition of green manure;
- lack of synchronization between N release and plant N needs which depresses growth at early stages and causes excessive growth, detrimental to yield at later stages;
- soil degradation, and;

unpredictability of the amount of N applied.

An important limiting factor is the bulkiness of green manures and resulting incorporation difficulties. Nitrogen content in legumes varies from 0.2 to 0.6% (Table 2) therefore the fresh weight corresponding to 50 kg N/ha varies from 10 to 26 t. Large scale incorporation of such a large and more or less lignified biomass using animal draft power and traditional implements is difficult. In developed countries high wage rates and lacking manpower may be limiting. One of the reasons for the abandonment of organic manure in Japan is the decreasing labor available for farming in combination with the fact that about 90% of rice farmers are part-time farmers [66].

There are other socioeconomic limitations. Legume green manures are not appealing because they do not directly yield food or cash. Also, green manures are poorly competitive or not competitive where commercial N fertilizer is available. Assuming an average yield of 15 kg grain/kg applied N, the cost of inorganic N fertilizer relative to rice price is very favorable [163]. Furthermore, many governments have a fertilizer subsidy policy and made cheap credit available for farmers to buy N fertilizer. The cost of green manure seed and necessary land preparation are unfavorable, given the limited yield response obtained from the green manure [93]. If there is residual soil moisture after rice harvest, farmers prefer to grow food legumes, peanut, maize, millet, onion etc. and apply to inorganic fertilizers for the next rice crop [139]. Economics often favor a food cash crop over green manure.

If N fertilizer is unavailable it is most frequently to subsistence farmers, with small land holdings, who cannot afford to release land to green manure production and prefer growing a food crop. In some areas where no N fertilizer is available and organic manure was traditionally applied to rice, green manure is now applied to vegetable cash crops rather than to rice [134].

There are also incidental limitations. In certain areas of India indiscriminate cattle grazing because of inadequate social control makes farmers reluctant to grow a green manure crop [139].

5 Conclusion. In terms of fixed N, legumes have tremendous potential and there is a large range of drought or submergence adapted green manure species. Within the aquatic legumes, stem-nodulated species (*S. rostrata, Aeschynomene indica, Neptunia oleracea*) represent a further step in adaptation to waterlogging and nitrogen fixation in soil with high level of combined nitrogen [42]. Despite this potential, green manuring is not widespread and has even declined in recent years. As indicated by Pandey and Morris [93] that trend should evoke cautious and thoughtful research.

The same authors concluded that green manuring has realistic potential where the subsistence component of the farm-household complex is high. Such areas are frequently rainfed with no available N fertilizer. They wrote that BNF by a green manure, and conservation of NO_3^- mineralized in dry season may be economically viable if production costs can be kept low, and if the green manure does not compete with marketable or subsistence crops. This finding indicates two possibilities – one is to grow a drought tolerant green manure early in dry season – the other is to grow, early in rainy season, a fast-growing excess-water tolerant green manure species.

Because legume green manures must be cultivated under very different agroclimatic conditions, selection should be done for diverse environmental tolerance, rapid growth, spontaneous nodulation, and high N_2-fixing ability. Additionally, technologies should be developed for specific crop cycles. For example, optimum time for incorporation before flooding is required to avoid denitrification losses. However, this time must also coordinate N demand of rice with green manure N release, which varies by the nature and state of green manure. Therefore, factors such as green manure species, C:N, lignin content, age at incorporation, and delay between incorporation and planting, must be considered.

As pointed out by Morris and Pandey [87] total reliance on organic fertilizers is unrealistic when considering modern, high yielding, N responsive rice varieties. Inorganic N must be applied to obtain desirable high yields. Under such conditions the inorganic N component is of major significance because application timing is more flexible.

It appears that despite a high N_2-fixing potential, green manure usage, known to many farmers, has been abandoned for various reasons. However, green manuring with fast growing, naturally nodulating species, adapted to specific environmental conditions may be important in some cropping patterns. Therefore, considering their present usage, legumes may be considered as an underutilized N source in rice production.

B Azolla

Azolla is a symbiosis between an aquatic fern of the *Azolla genus* and a N_2-fixing blue-green alga, *Anabaena azollae.* Because of its rapid growth and high N content azolla has been used as green manure in rice culture for centuries in northern Vietnam and southern China. Utilization of azolla in those areas was generally ignored by scientists in other countries until the mid-1970s. Since then information gathered by visitors to and from China and Vietnam, articles and reviews [76, 86, 148] have stimulated interest and research on azolla. A book on azolla as a green manure was written in 1982 by Lumpkin and Plucknett [77] and a Primer on azolla by Khan in 1983 [67]. Besides its utilization as green manure, azolla also is used as swine and poultry feed [74]

1 Potential. Growth rate, maximum biomass, N_2-fixing activity, and rice yields in experiments comparing azolla utilization with fertilizer application estimate the potential of azolla for rice production. Growth rates were summarized by Becking [9]. He showed that doubling time varies between 2 and 10 d for most species. Growth follows an approximate logistic curve until maximum biomass is reached. Growth rate declines as plant density increases [4]. Maximum biomasses and N_2-fixing rates were summarized by Kikuchi et al. [68]. Maximum biomasses ranged from 0.8 to 5.2 t dry matter/ha and averaged 2.1 t dry matter/ha and averaged 2.1 t/ha (n = 13; c.v. = 57%). N content ranged from 20 to 146 kg N/ha and averaged 70 kg N/ha (n = 17; c.v. = 58%). N_2-fixing rate ranged from 0.4 to 3.6 kg N/ha per day and averaged 2 kg N/ha per day (n = 15, c.v. = 47%).

In 1 yr, N fixed by continuous azolla culture, repeatedly harvested, can be as high as 500 (*A. pinnata*) [151] and 1200 kg N/ha (*A. filiculoides*) [72]. When grown dual cropped with rice, azolla can accumulate from 25 to 170 kg N/ha in 60 d [68] which may exceed the N requirement of rice. Azolla N_2-fixing activity per unit area is similar or higher than that of legume pastures.

International azolla field trials conducted for 4 consecutive years in 19 sites and 9 countries by the International Network on Soil Fertility and Fertilizer Evaluation for Rice (INSFFER) network [53, 54, 55, 57] have shown that:

1 Incorporating one crop of azolla grown before or after transplanting was equivalent to split application of 30 kg fertilizer N.
2 Incorporating two crops of azolla grown before and after transplanting was equivalent to split application of 60 kg fertilizer N.

3 Increase in rice yield per unit weight of azolla incorporated was roughly proportional to the response of rice to chemical fertilizer at the same site.

4 Spacing of rice plants (20 x 20 cm vs 10 x 40 cm) did not significantly affect azolla growth and rice yield.

2 Methods of utilization. China and Vietnam are the only countries with a long history of azolla cultivation extending back to the 11th century in Vietnam [25] and at least to the Ming dynasty (1368–1644 A.D.) in China [77].

In China, azolla is used from 37 °N (Shandong) to 19 °N (Hainan). Because strains used grow best at a 25 °C average daily temperature, azolla usually is grown in late spring (May to June) in the north and early spring (March to April) in the south. Most frequently it is grown for about 1 mo, then incorporated before transplanting. To a lower extent, the wide-narrow row spacing for transplanted rice [74] is used to grow azolla with rice, which permits several incorporations during the crop cycle. In some places, azolla is grown before and after transplanting.

In Vietnam [50] azolla is used in the northern provinces where spring and summer rice are grown with adequate irrigation. Azolla only is used for spring rice because in summer it grows poorly and insect incidence is severe. Straw incorporation and legume green manuring are used for summer rice.

About 2–3 mo before transplanting, azolla is collected from natural environments by specialists, and is multiplied in government azolla farms at regional and district levels. Inoculum is sold to cooperatives and farmers who propagate it in the fields from November to February, when conditions are favorable for its multiplication. As in China, azolla production and utilization is done two ways; as green manure incorporated before transplanting, and as an intercrop incorporated after transplanting. Azolla grown before transplanting is fertilized with P, K, and farmyard manure. Recommended applications are: 2.2 kg P/ha every 5 d, 4 kg K/ha every 10 d, and 500–1000 kg/ha farmyard manure every 5–10 d. When chemical fertilizers are unavailable, ash is substituted. Intercropped azolla usually is not fertilized but if superphosphate is available one application of 4.5 kg P/ha per crop is recommended.

Azolla is inoculated in the field at 300–500 kg fresh weight/ha with farmyard manure. To facilitate vegetative multiplication, fronds are broken using a special tool. Azolla is collected before heavy rains to protect it from being washed from the fields. When attacked by insects, azolla is collected and placed under water for 15 h to kill insect larvae. Sixteen to 20 d after inoculation the field is covered with about 20 t of azolla, half of which is collected in mounds and composted by covering it with soil. The remaining half is grown for 7–10 more days 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 keeps on growing. Seven to ten days after transplanting, the field is covered and again half of the third crop is hand-and-foot incorporated between the rows. Sometimes a 4th crop is grown and incorporated.

This technology produces, on average, 40 t/ha per rice crop (fresh weight) of azolla, equalling 80 kg N/ha. It requires application of 0.5 t fresh azolla inoculum, 2–3 t farmyard manure, 20–30 kg P, and | 20 kg K/ha. Average annual rice yield in the Red River Delta is 5–7 t. The winter crop, with azolla, yields 3 to 5 t/ha, and the summer crop, without azolla, yields 2 t or less/ha.

The azolla technology used in Vietnam is labor-intensive and might be improved by changing the transplanting method, thus permitting easy azolla incorporation with a rotary weeder.

3 *Current usage.* Data concerning the extent of azolla use in China are controversial. FAO [38] estimated that azolla is grown in over 6.5 million ha of rice lands in China. In contrast, Liu Chung-Chu [74] reported that 1.34 million ha were planted to azolla in 1979, which was slightly less than 5% of the total rice area. According to Liu Chung-Chu, azolla is used in rice-growing regions from the South Yantze River to northeast China (40 °N) and in the Hanchung Basin of Shensi in the west. Data reported by Lumpkinand Plucknett [77] indicate that azolla is cultivated as a green manure on only 2% of China's harvested rice area and for about 5% of its spring rice crop.

In Vietnam, in 1980, azolla was used in the northern and north central provinces on about 500 000 ha, about 14% of the irrigated rice growing area and 9% of the total rice area [50]. On the Red River Delta, azolla is grown for 40–60% of the irrigated spring rice [77].

In countries other than China and Vietnam, azolla use is incidental and limited. In the Philippines, farmers adopted azolla as green manure on more than 5 000 ha in South Cotabato in 1981 [68]. In 1983 it was used on 26 000 ha, although all the areas are not necessarily suitable for azolla growth. In Gambia, spontaneously growing azolla is incorporated as a green manure [108].

4 *Limiting factors.* A first requirement for azolla use is water availability and control. Azolla cannot withstand desiccation. Water should be in the field throughout cultivation. Additionally, because azolla grows from vegetative multiplication, inoculum must be maintained in nurseries all year and multiplied for distribution before field inoculation and multiplication. Those two requirements imply that an irrigation network and a network for inoculum conservation, production, and distribution are prerequisites for azolla utilization. This also imply that azolla adoption by farmers first depends on a governmental policy to establish such networks.

High temperatures retard azolla growth. Cool weather is a key to successful azolla utilization in Vietnam and China. However, successful growth of *A. pinnata* was observed at high temperatures in north Senegal [138]. Although azolla growth slows at temperatures higher than 30 °C, the major detrimental effect in relation to high temperature and humidity is the resulting high incidence of insect and fungus pests.

Among nutrients, P is most important. Success in spreading *A. pinnata* in South Cotabato, Philippines, was mainly due to a high level of available P in the soil. Watanabe and Espinas [157] reported that 25 ppm (Olsen P) in the soil is optimum for azolla and that P absorbing capacity of the soil should be less than 440 mg P/100 g soil. Such conditions are quite rare in rice soils and P fertilization for azolla growth usually is needed.

Economics of azolla use are very important. The technology used in Vietnam and China is labor intensive and could not be adopted in most rice growing countries. Kikuchi et al. [68] studied the economics of azolla use in the Philippines in South Cotabato, where azolla spreaded spontaneously and no P fertilizer and little labor were needed. They concluded that in that area economic return from azolla adoption, including cost savings in chemical fertilizers and weed control, was more than $35/ha at 1981 prices. Economic return from savings on inorganic fertilizer was about $10.

However, the authors clearly indicated that conditions in the studied area were exceptionally favorable and should be viewed realistically. They indicated that the economic potential of azolla is greatest where the opportunity cost of labor is low, and calculated that labor cost for azolla use becomes critical where agricultural wage rates approach $2/d. Insect control was also an important economic limitation. If more than 200 g carbofuran/ha (active ingredient) is needed to control insects, economic benefits are eliminated.

5 Conclusion. Azolla is N_2-fixing organic manure with similar N potential to that of legume green manures. It is easier to incorporate than other organic manures and grows well with rice in flooded conditions. Environmental and technological limitations are important but can be managed. Problems arising from inoculum conservation, multiplication, and transport could be solved to a large extent if azolla could be propagated from spores. Azolla sporulates but no method is knownl to induce sporulation. Possible use of *A. filiculoides* sporocarps for oversummering is being tested in China [72]. Temperature limitations and P requirements can be reduced by selecting cold or heat tolerant strains with low P requirements and by using P fertilizer. Labor costs do not apply in many rice growing countries. Among green manures, azolla is still less utilized than legumes but, contrary to legume use, azolla use is reported to be increasing, and many countries are evaluating it for popularization.

C Straw application

Straw incorporation is a traditional agricultural practice which has been primarily used to add nutrients and organic matter to the soil. A large volume of field data elucidates the effectiveness of organic amendments for increasing N fertility of rice soils. Beneficial effects on BNF were first demonstrated in small scale laboratory experiments [8, 100, 112, 162]. During the first *Nitrogen and rice* symposium at IRRI, Matsuguchi [81] presented data indicating that the root-free soil layer was the most important site for N fixation in a Japanese rice field. Additional research suggested that organic debris were an important site for heterotrophic BNF [145] and that straw application benefited photodependant BNF [83].

As a cultural practice, straw incorporation should be considered as an integrated management in which the beneficial effect on N fixation is only one of the components.

Rice straw contains about 0.6% N, 0.1% P, 0.1% S, 1.5% K, 5% Si, and 40% C. Because it is available on site in amounts varying from 2 to 10 t/ha, it is a convenient source of plant nutrients. Ponnamperuma [97] wrote that total nutrient content of straw in India, Philippines, and Sri Lanka in 1979 was more than twice that of chemical fertilizers used on rice in those countries. Straw also contains sugar, starches, celluloses, hemicelluloses, pectins, lignins, fats, and proteins (about 40%, as C, of the dry matter) that provide substrates for microbial metabolism (including N_2-fixing microorganisms) in the soil.

Long-term experiments indicate that straw incorporation in lowland rice fields increases organic C and N, and available P, K, and Si. Straw is a slow release source of nutrients for rice. Undecomposed straw releases less N initially but more N later, than decomposed straw [20]. Peak absorption of N from incorporated straw was at middle plant growth stage. Plants recovered 25% N from straw after 130 d [20].

The yield advantage from straw incorporation rather than straw burning or removal is about 0.4 t/ha per season, and increases with time as soil fertility improves [97].

1 Potential for BNF

a Evidence of the process

In a 5 yr drum study with 3 soils, N increase from straw incorporation was computed to be 40 kg/ha per season, about 10 kg/ha per season more than the straw's N content. The extra N probably came from BNF by heterotrophs and phototrophs [97, 56].

b Effect on heterotrophic anaerobic BNF

In the early 1940s Jensen [62] wrote that when cellulosic material is added to soil 'a certain degree of anaerobiosis is necessary to bring about an active fixation (of N). This was confirmed by Mayfield and Aldworth [84]. Using 1 cm diameter, sandy clay loam soil aggregates amended with 2% wheat straw, they found that the only N_2-fixing bacteria that developed were anaerobes or facultative anaerobes, whether the aggregates were incubated under aerobic or anaerobic conditions, and that acetylene reducing activities (ARA) were similar under both conditions. Yoneyama et al. [162] reported that ARA of waterlogged soils was stimulated by incorporating rice straw at a relatively early stage of decomposition. Straw decreased the inorganic N and redox potential, making the environment favorable for anaerobic N_2-fixing bacteria. BNF was stimulated only when soils were waterlogged.

c Effect on other N_2-fixing organisms

The beneficial effect of straw applications is not limited to anaerobic heterotrophic fixers, but also applies to aerobic heterotrophs and phototrophs.

Straw application (5 and 10t/ha) was reported to enhance *Azotobacter* populations in submerged rice soil [101]. Magdoff and Bouldin [79] using a waterlogged sand matrix supplemented with a small amount of soil and mineral nutrients and enriched with cellulose, found fixation to be 10 to 15 times greater in the upper 2 to 3 mm of soil than in the lower layers. They suggested that products of anaerobic decomposition may be an energy source for aerobic BNF when brought under aerobic conditions by diffusion. Reddy and Patrick [105] observed a four fold increase of N_2-fixing activity in soils incubated under light after straw incorporation (0.4%). Similar results did not occur in darkness. However, the authors indicated that the increase in BNF in light incubated straw-enriched samples was unrelated to algal growth.

Matsuguchi and Yoo [83] measured ARA of straw fraction, root fraction and soil 7 w after transplanting in a soil with 8 t incorporated straw/ha. In the 0- 1 cm surface layer, photodependent and photoindependent ARA of straw were roughly 1000-fold and 100-fold higher than those in the soil fraction. In deeper layers the trend was similar. The same authors compared the effect on BNF of deep placement and topdressing of 8 t rice straw/ha in two soils. In all cases straw stimulated BNF. Stimulation was more marked for photodependent ARA in the 0–1 cm surface layer than for photoindependent ARA in the whole profile. Topdressing rice straw induced higher photodependent ARA and better rice growth than deep placement. Photodependent ARA in the topdressed straw was 10^3-fold more than in the soil fraction and was enhanced by N and herbicide application [83].

d Effect in the presence of N fertilizers

There are reports that straw incorporation may significantly reduce the inhibitory effect of N fertilizer on BNF.

Ammonium sulfate suppressed BNF with 5 t incorporated straw/ha but was not inhibitory with 10 t/ha [102]. Rhizosphere soil from plots receiving 3–6 t rice straw/ha exhibited more pronounced nitrogen fixing activity than the control. Forty to 80 kg mineral N/ha did not inhibit BNF with 6 t straw/ha [17]. Kalininskaya et al. [65] reported a maximum rate of BNF (ARA and ^{15}N) with N fertilizer and high straw application rates. They also reported that applying 100 to 200 kg N/ha did not inhibit BNF in the presence of straw [63].

On the other hand, Charyulu and Rao [19] reported that the inhibitory effect of ammonium sulfate increased with N concentration in small scale experiments with four soils enriched with 1% cellulose (Table 4). Similar results were presented by Rao [100].

e Quantification of fixed N

When analyzing experiments conducted to evaluate BNF in soils after straw (and other carbon sources) incorporation, it appears that all experiments are laboratory incubations of small samples of a few grams of soil (Table 4). In many experiments the quantity of straw incorporated was much higher than incorporation in the field. Assuming 300 t soil/ha, 3 t of straw incorporated per hectare equals 0.1% whereas 1–2% were commonly used in the experiments and sometimes as much as 20% (w/w) was incorporated [111].

Such experiments are hardly representative of the field conditions and seem to substantially overestimate amount of fixed N. For example, Charyulu et al. [18], using 5 g soil samples enriched with 0.5% rice straw, reported values ranging from 200 to 740 μg N fixed/g of soil in 100 d. Assuming 3000 t soil/ha of rice field, the above values correspond to 600 to 2220 kg N fixed/ha in 100 d. Under waterlogged conditions with 1 to 2% straw, 10 to 70 μg N/g soil were found to be fixed in 28 d [112], which equals 30–210 kg N fixed/ha in 28 d. High activity (45 mmol C_2H_2/g dry soil per h) were reported by Durbin and Watanabe [36] 4 d after incorporating 14 mg rice straw/g dry soil.

Results of larger scale expeiments show that a few mg N are fixed per gram of straw incorporated. Using ^{15}N, Rao [102] estimated that BNF by free-living bacteria in a flooded rice field was 7 kg N/ha in unamended soil and 25 kg N/ha in amended soil (5 t straw added; 3.6 mg N fixed per gram of straw). In saline takyr soils of Kazakh, BNF productivity was estimated as 5–10 mg N/g of applied straw [64]. Balance experiments by App and collaborators showed that about 5.5 mg N was fixed per gram of straw added [56].

When pooling data from Table 4 it appears that efficiency of heterotrophic BNF varies from 0.08 to 7.07 mg N fixed per gram of substrate (mainly straw) added. The average value of the 35 listed data is 2.39 mg N fixed per gram of substrate added, in about 1 mo.

2 Straw management in rice growing countries. Rice straw can be utilized or disposed by:

– direct return to the soil either as organic matter, with or without preliminary composting, or as ash after burning;

Table 4. Summary of the effects of carbon substrate incorporation on biological N_2 fixation

Soil	Substrate: Nature	Substrate: Concentration (%)	Incubation: Conditions[a]	Incubation: Duration (days)	Method[b]	Efficiency: mg N fixed per g of substrate: Consumed	Efficiency: mg N fixed per g of substrate: Added	Reference
Dryland (5g)	Barley straw	5	Subm.	28	Kj.		1–2	8
		10	Subm.	28	Kj.		2.2–2.5	
		20	Subm.	28	Kj.		0.8–1.0	
		40	Subm.	28	Kj.		1–2	
Dryland (5g)	Straw	5	Subm.	30	Kj.		0.08	14
		16	Subm.	30	Kj.		2.15	
		32	Subm.	30	Kj.		1.26	
Soil dilutions								
nonamended	Glucose				Kj.		2–3	18
previously amended							3–6	
5 g soil	Cellulose	1	Subm.	20	^{15}N		1–2	19
	straw						3–4	63
50 g of straw	Wheat							
inoculated	straw	100	Aero.	56		11.5	5.0	78
not inoculated		100	Aero.	56		8.8	2.8	
	Glucose	1.5	Anaero.			12.3		91
	Glucose	2.5	Anaero.			14.2		
	Glucose	3.0	Anaero.			10.6		
	Mannitol	1.5	Anaero.			10.8		
	Mannitol	2.0	Anaero.			10.3		
	Mannitol	3.0	Anaero.			7.7		
5 g of soil with different pretreatments	Cellulose	1.0	Subm.	30	^{15}N		2–7	102

Table 4. Contd.

Soil	Substrate		Incubation		Method[b]	Efficiency: mg N fixed per g of substrate		Reference
	Nature	Concentration (%)	Conditions[a]	Duration (days)		Consumed	Added	
Meadow chernozem	Rice	1.0		30	^{15}N		0.53	100
rice soil	straw	2.0		30	^{15}N		0.91	
		1.0	Subm.	30	^{15}N		1.72	
	Cellulose	1.0	Anaero.		^{15}N	4.2–20		103
Dark brown	Wheat straw	1	Subm.	28	^{15}N		6.7	112
chernozenic soil		5	Subm.	28	^{15}N & Kj.		4.4	
(0.6 g)		20	Subm.	28	Kj.		2.3	
2 g soil	Wheat straw	20	Subm.	21	Kj.	16.1	2.2	111
2 g sand-clay + 0.1 g soil	Wheat straw	19	Subm.	21	Kj.	12.8	2.1	
2 g soil	Hemicellulose	5.0	Subm.	21	Kj.		2.4	
2 g soil	Straw residue	20	Subm.	21	Kj.		1.1	
2 g sand-clay + 10^8	Glucose	0.5	Subm.	21	Kj.		1.5	
cell *C. pasteurianum*	Glucose	3.0	Subm.	21	Kj.		1.1	

[a]Subm.: submerged; Aero.: aerobiosis; Anaero.: anaerobiosis
[b]Kj.: Kjeldahl

- indirect return to the soil after such uses as bedding for cattle, mushroom culture, or mulch for a crop after rice;
- use as feed, fuel, or household roofing material; or
- industrial uses such as for paper, rope, bags, mats, or packing material.

The status of straw handling in rice growing countries was reviewed by Tanaka (131) who distinguished four major groups:
- countries where the major concern is to dispose of straw with
- minimum labor and where it is burned or incorporated – (USA, Southern European countries, Japan);
- countries where there is a belief that recycling organic matter makes soil more productive and where straw is harvested at the ground level and returned to the soil mainly as compost (China, Vietnam);
- countries where straw is mainly used as feed or fuel (India, Egypt); and
- countries where rice is frequently harvested at a rather high level and where much of the straw is returned to the soil, but although unintentionally (Malaysia, Indonesia, Philippines).

Tanaka's [131] survey indicated that burning is the most frequent straw removal-use practice. Araragi and Tangcham [3] reported that in Thailand, Burma, Philippines, Indonesia, and Malaysia, most rice straw is burned before starting the next cultivation. Straw incorporation is widely practiced only in China and before the summer rice crop in Vietnam. In some areas the straw is spread on the field and partially decomposed on moist soil or under water to facilitate incorporation. Heaping straw in mounds at threshing sites in the field is common in the Philippines and Indonesia. Straw decomposes slowly, largely aerobically, and is spread and easily incorporated at the beginning of the next season. This practice, however, reduces planted area and causes large nutrient losses by N denitrification, leaching, and C mineralization.

3 Limiting factors. Straw incorporation is an uncommon agricultural practice. Reasons are comprised of detrimental effects and socioeconomic factors.

a Detrimental effects

Adding straw to soils with high SO_4^{-2} and/or low in active Fe and Mn, such as acid sulfate soils and degraded saline soils may favor formation of H_2S, which is highly toxic to rice. Adding straw to neutral to alkaline soils, may markedly depress water soluble Zn, probably because of increased pCO_2, which leads to the formation of insoluble Zn CO_3. An increased HCO_3^- concentration in the soil solution also affects Zn root-to-shoot transport [162]. Yield of rice varieties susceptible to Zn deficiency has decreased after straw incorporation [58].

There are few reports on the effect of straw incorporation on BNF in relation to the release of toxic compounds such as organic acids and phenolic compounds. Phenolic compounds produced by decomposing rice straw inhibited the growth of and acetylene reduction by *Anabaena cylindrica* [109].. Such compounds also inhibited strains of *Rhizobium leguminosarum* and *R. japonicum* and reduced BNF by legumes *in situ*. This may explain the great reduction in soybean yields following rice that are observed in Taiwan when rice stubble is left in the field [110].

b Socioeconomic factors

Straw management is traditional, as are other agricultural operations. Where straw is utilized for definite purposes (feed, fuel, etc.) management changes cannot be expected without a demonstrated economic advantage. Straw is bulky and requires substantial labor to incorporate. Additionally, uniform incorporation with low power equipment is difficult. Where two rice crops are grown, straw disposal should be rapid and burning is the easiest method. Economics do not always favor incorporation of undecomposed straw. In a survey of straw management in the Philippines, Marciano et al. [80] observed that farmers do not follow practices that maximize the benefits of straw incorporation, despite the demonstrated beneficial effect on yield and soil fertility. Because incorporation of undecomposed straw is labor and power intensive in peak labor periods of land preparation and because the necessary time for decomposition before transplanting may delay crop establishment, Philippine farmers adopt management practices that require little labor, i.e., burn, stack/decompose, and stack/burn. Benefits of incorporating undecomposed straw were perceived by farmers to be uncertain and generally limited. Marciano et al. [80] calculated that 7-17% yield increase is necessary to make undecomposed straw incorporation more attractive to farmers than current practices. Incremental costs of incorporating undecompsed straw probably frequently exceeded the expected benefits, therefore farmers had little financial incentive for adoption.

4 Conclusion. Straw incorporation is an agronomic practice that returns significant quantities of nutrients to the soil and increases heterotrophic and autotrophic BNF. Data indicate that 2–3 kg N can be fixed per ton of straw incorporated. Limitations are primarily socioeconomic.

III Developing technologies

A Free living BGA

Among the N_2-fixing microorganisms, only BGA can generate photosynthate from CO_2 and water. This trophic independence makes BGA especially attractive as a biofertilizer. The agronomic potential of BGA was recognized in 1939 by De [28] who attributed the natural fertility of tropical rice fields to N_2-fixing BGA. Since then many trials have been conducted to increase rice yield by algal inoculation. Literature on BGA and rice was reviewed by

Roger and Kulasooriya [115] and BGA in tropical soils was reviewed by Roger and Reynaud [117].

1 Potential. Potential of BGA as a N source for rice can be described in three different ways:

- evaluation of BGA biomass and N content,
- measurement of N_2-fixing activity, and
- field experiments measuring BGA productivity and rice yield.

Records of BGA biomasses were summarized by Roger and Kulasooriya [115]. Fresh weight estimates range from a few kg to 16 t/ha and dry weight estimates from a few kg to 480 kg/ha. However, because of the variable dry matter, 0.5–5%; ash, 5–70%; and N, 2–13% contents of BGA field samples [58] such data are of little significance. Recent evaluations of artificially produced BGA blooms indicated standing biomass of N_2-fixing strains culminating at 150–250 kg dry weight/ha on an ash free basis, equalling 10–20 kg N/ha [59]. Those values may be considered to be the maximum standing biomass that can be expected in a rice field at blooming time. However, they underestimate the value for BNF, which is the result of the activity of a standing biomass and its turnover. No data are available on nutrient turnover rate from field-growing BGA.

Another way to roughly estimate BGA potential is to assume that all C input in the floodwater and surface soil is through BGA (which is an overestimation). Saito and Watanabe [120], estimated an input of 0.6 t C in phytoplankton/crop per ha. Using that estimate and assuming a BGA C:N ratio ranging from 4 to 16 [58], the potential contribution of N_2-fixing BGA could be 37 to 150 kg N/ha per crop.

BNF by BGA has been most frequently studied by the acetylene reducing activity method, which may provide erroneous results [75]. ARA variations during the day and the growing cycle can be rapid and important, and moreover, ARA has a log-normal distribution [118]. Therefore, many replicates and very frequent measurements are needed to satisfactory measure total ARA.

However, this tedious work will provide an imprecise evaluation of the N_2-fixing activity (NFA) because the conversion factor of acetylene-N is not constant and must be determined [95]. Few reliable estimations of ARA have been published, and the number of measurements and replicates have been generally too low. Reported data on BNF related to BGA varied from a few to 80 kg N/ha and averaged 27 kg/ha per crop [115].

Field experiments on algal inoculation (algalization) provide indirect information on the overall potential of BGA. The most frequently used criterion for assessing the effects of algalization has been grain yield. Results of field experiments conducted mainly in India report an average 14% yield increase over the control corresponding to about 450 kg grain/ha per crop, where algal inoculation was effective. A similar increase was observed with and without N fertilizers. Because BNF is known to be inhibited by inorganic N the beneficial effect of algalization in the presence of N fertilizers was most frequently interpreted as resulting from growth-promoting substances produced by algae or by a temporary immobilization of added N, followed by a slow release through subsequent algal decomposition that permitted more efficient crop N utilization. Such interpretations have yet to be demonstrated.

Grain yield measurements suggest that algalization produces both a cumulative and residual effect. This was attributed to a build-up of organic N content and the number of BGA propagules in the soil, which facilitated the reestablishment of the BGA biomass. Several reports indicate an increase in organic matter and organic N. Algalization was also reported to increase aggregation status of the soil [125], waterholding capacity [127], and available P, total microflora, *Azotobacter, Clostridium,* and nitrifiers [47].

Thus, it appears that the potential of BGA has not been clearly quantified. Biomass measurements indicate that BGA have less potential than legumes and azolla. Comparison with N fertilizers indicated that algal inoculation may be equivalent to the application of 25–30 kg N/ha [142] but nothing is known about the relative importance of fixed N and other possible effects (auxinic effect, P solubilization, effects on soil properties and microflora, etc.) in the reported yield increase.

A classical statement in the reports on BGA inoculation is 'although the yields obtained in inoculated plots were higher, the difference between the yields of plots using and not using BGA was not significant'. This indicates that: the response to algal inoculation varies, the response is small, and the experimental error is larger than the response. The most common design for BGA inoculation experiments has been 4 x 4 m plots with 4 replicates which usually gives a coefficient of variation higher than 10% and a minimum detectable difference of 14.5% [44]. Such a value agrees with the average increase in yield reported after algal inoculation and confirms that a relatively low yield increase can be expected from this practice.

2 Methods of utilization. Research on methods for using BGA in rice cultivation, emphasizes algal inoculation (algalization) alone or together with agricultural practices favoring the growth of inoculated strains. This arose from the earlier belief that N_2-fixing strains were not normally present in many rice fields. It appears now that results concerning the occurrence of N_2-fixing BGA in rice fields are controversial. Watanabe and Yamamoto [146] found that only 5% of 911 soil samples from Asia and Africa harbored N_2-fixing species. Venkataraman [141] reported that 33% of 2213 soil samples from rice fields in India contained N_2-fixing strains. Okuda and Yamaguchi [90] reported the presence of N_2-fixing strains in 71% of the samples they collected in Japan. Reynaud and Roger [107] found N_2-fixing strains in 95% of the samples they collected in Senegal. In a survey of 40 rice fields in Thailand, Matsuguchi et al. [82] found BGA in all soils. In an ongoing survey of the Phillipine rice soils, we found N_2-fixing strains in all of the 79 samples collected (unpublished). N_2-fixing strains most probably are more common in rice fields than was previously thought. Unsuitable survey methodology, especially sampling method, probably caused the low values recorded [117]. Therefore, research should equally emphasize inoculation and indigenous strain enhancement.

a Algal inoculation

The methodology of BGA inoculum production was reviewed by Watanabe and Yamamoto [146] and Venkataraman [140]. Methods of field application were reviewed by Venkataraman [142]. Inoculum production in artificially controlled conditions was developed mainly in Japan where algalization is not used. Inoculum production under artificially controlled conditions is efficient but expensive. Open air soil culture, developed in India, is more simple, less expensive, and easily adoptable by the farmers. It is based on the use of a multistrain starter inoculum of *Aulosira, Tolypothrix, Scytonema, Nostoc, Anabaena,* and *Plectonema* provided by the 'All India Coordinated Project on Algae' [1]. The inoculum is multiplied by the farmer in shallow trays or tanks with 5–15 cm water, about 4 kg soil/m^2, 100 g triple superphosphate/m^2, and insecticide. If necessary, lime is added to correct the soil pH to about 7.0–7.5. In 1 to 3 w, a thick mat develops on the soil surface and sometimes floats. Watering is stopped and water in the trays is allowed to evaporate in the sun. Algal flakes are scraped off and stored in bags for use in fields. Using that method, the final proportion of individual strains in the algal flakes is unpredictable, but it is assumed that, because the inoculum is produced in soil and climatic conditions similar to those in the field, dominant strains will be the best adapted to the local conditions. The recorded rates of production of algal flakes in the open air soil culture range from 0.4 to 1.0 kg/m^2 in 15 d, indicating that in 2–3 mo a 2 m^2 tray can produce enough algal material to inoculate a 1 ha rice field. For transplanted rice, the algal inoculum is generally applied 1 w after transplanting. When rice is direct-seeded, seeds can be coated by mixing the algal suspension and 2–3 kg calcium carbonate per 10–20 kg seed and air-dried in the shade.

Recommendations for field application of dried algal inoculum (algal flakes) given by the All India Coordinated Project on Algae [1] indicate that:

- 8–10 kg of dry algal flakes applied 1 w after transplanting is sufficient to inoculate 1 ha, a larger inoculation will accelerate multiplication and establishment in the field;
- algalization can be used with high levels of commercial N fertilizer, but N application should be reduced by 30%;
- to benefit from the cumulative effect of algalization the algae should be applied for at least three consecutive seasons; and
- recommended pest-control measures and other management practices do not interfere with BGA establishment and activity in the fields.

b Methods to enhance indigenous BGA growth

The growth of N_2-fixing BGA in rice fields is most commonly limited by low pH, P deficiency, and grazer populations. Application of P and lime has frequently increased growth, particularly in acidic soils [153]. Increased BGA biomass has also been reported after insecticide application [46].

Recently, surface straw application was reported to benefit BGA growth and photodependent ARA [119]. This may be due to an increase of CO_2 in the photic zone, a decrease of mineral N and O_2 concentration in the floodwater, and the provision of microaerobic microsites by the straw. Increased CO_2 availability and low N concentration favor the growth of N_2-fixing BGA. Low O_2 concentration in the photic zone may increase specific N_2-fixing activity.

3 Current usage. Most of applied research on algal inoculation is conducted in India where a national program has been developed, the All-India Coordinated Project on Algae. To a lesser extent, applied research is also conducted in Burma, China, and Egypt.

Reports on the adoption of algal technology are controversial, but even considering the most optimistic evaluations, use of algal inoculation is restricted to very limited hectarage in a few Indian states and in Burma.

In a review on adoption of biofertilizers in India, Pillai [96] wrote: 'Apart from the work carried out at Research Stations very little organized work on development of the material for being adopted by the farmers has been taken up, especially in areas where it could be of potential benefit.'

In a review on biofertilizers, Subba Rao [129] wrote that the production capacity of BGA flakes in India was around 40 t/yr, which was approximately 0.01% of the total inoculum requirement for the country (40 t will inoculate 4 000 ha).

From the most recent extensive report on BGA field trials published by the Agricultural Economics Research Center of the University of Madras [135], it appears that despite an official radio and print publicity campaign, BGA use remains at the trial level and that in many cases inoculated algae did not multiply. Therefore, it seems appropriate to consider that this technology is more at an experimental level of large scale field testing than at a popularization stage.

4 Limiting factors. The major limiting factor to adopt algal inoculation is the lack of reliable technology for recommendation to farmers. Inoculum establishment is sporadic and the reasons for failure are frequently not known.

In reviewing BGA literature, it is surprising to observe the imbalance between the different topics. Taxonomy, morphology, micromorphology, physiology, and enzymology are highly documented and test tube BGA growth has been studied extensively. However, field studies are rare, most probably limited by lack of suitable methodology. Therefore, BGA ecology is still poorly understood.

The physiological characteristics of N_2 fixation desirable for strains suitable for field inoculation are known [128], but the selection of 'Super N_2-Fixing Strains' is meaningless unless they survive, develop, and fix N_2, as programmed, in rice fields.

As indicated by Gibson [41], virtually nothing is known of the attributes permitting introduced strains to colonize the various hostile environments to which they will be exposed. Similarly, the factors permitting the establishment of an N_2-fixing bloom of inoculated or indigenous strains still are unknown.

Low pH, low temperatures, and P deficiency limit BGA growth. However, because in some soils algalization is inefficient despite the addition of lime and phosphate [90], pH and available P are not the only limiting factors, but texture, organic matter content, CEC of saturated extracts, and total N are probably not important [130]. Grazing by invertebrate populations is an important biotic limiting factor [45]. Other possible limiting mechanisms such as antagonism, competition, etc. have been suggested, but their role is unclear. Low temperatures, heavy rains, and cloudy weather also have been reported to limit the inoculum establishment [115].

Inoculum quality also may be a limiting factor. In published methods of inoculum production, no tests of composition and viability have been included. We have shown that the density of colony-forming unit in BGA inocula may vary from 10^3 to 10^7/g of dry inoculum and that in some cases N_2-fixing strains are not dominant [59]. Some commercial inoculants also have been reported to have limited potential for BGA population enhancement [132]. Therefore, special attention must be paid to inocula quality.

Economics apparently do not limit BGA utilization. In a study of the economics of BGA use of 40 farmers in Tamil Nadu [135], no significant difference was found in the average per hectare cost of cultivation between crops using ($247) and not using ($246) BGA. The average return of BGA utilization was $4/ha.

When needed, grazer population can be controlled with inexpensive natural insecticides [46].

5 *Conclusion.* Blue-green algae seems to be a possible N source in neutral to alkaline soils with moderate to high P availability and low grazer incidence. BGA have less potential in terms of N_2-fixed than legumes or azolla, but need very limited additional inputs. Their usage is limited by technological problems governing inoculum quality and establishment and by generally low yield increases attributable to algal inoculation.

B Heterotrophic BNF associated with the rice plant

In 1929, Sen [124] reported the presence of N_2-fixing bacteria in rice roots, but his observation was overlooked. In 1961, Dobereiner and Ruschel [31] studied the growth stimulation of N_2-fixing bacteria in the rhizosphere of lowland rice. Their observations led to Dobereiner's idea that non-nodulated, non-leguminous plants can fix N through bacteria associated with roots [30]. The association between roots and bacteria was called rhizocoenosis.

In 1971, Rinaudo and Dommergues [113] and Yoshida and Ancajas [164] demonstrated N_2-fixing activity of wetland rice roots by using sensitive acetylene reduction assays. It was confirmed by $^{15}N_2$ incorporation [37, 60, 166] and N balance studies [2]. The basal portion of shoots also is a site of heterotrophic BNF [156]. Various groups of N_2-fixing bacteria were isolated from the same root samples using a non-selective medium [150] and a spermosphere model [5]. Ohta and Hattori [89] described an oligotrophic N_2-fixing bacteria that is abundant in rice roots. Bacteria genera isolated from rice roots are *Azospirillum* [70], *Beijerinkia* [31], *Pseudomonas* [7], *Enterobacteria* [71], *Klebsiella* [71], *Flavobacterium* [5], *Alcaligenes* [99], and *Agromonas* [89]. The N_2-fixing ability of the association has been proved, some major N_2-fixing organisms have been identified, and there have been some field and laboratory studies. However, there are many uncertainties about heterotrophic BNF in the rice rhizosphere. Major concerns of current research are: quantification of N_2 fixation, interactions between plant and bacteria, and varietal differences in the ability of rice to enhance associative BNF. Heterotrophic BNF in tropical soils was reviewed by Yoshida and Rinaudo [165].

1 Potential. In early studies, excised root assays were used to estimate potential N_2 fixation rate [144]. However, that technique was neither quantitative nor semiquantitative [137]. In situ or undistribed core assays are now used to measure ARA associated with plant [167].

In many assays ARA was highest at or near rice heading stage [6, 13, 121] and ranged from 0.3 μmol C_2H_4/plant per hour in temperate regions [121, 165] to 2 μmol C_2H_4/plant per hour in the tropics [6, 162]. Assuming (1) that ARA measured at heading stage continues for 50 d, (2) an acetylene/N conversion rate of 3:1, and (3) a plant density of $25/m^2$, the estimated N_2-fixing rate would be 0.8- 6 kg N/ha per cropping season. Extrapolation from ^{15}N incorporation experiments ranges from 1.3 to 7.2 kg N/ha per cropping season [37, 60, 166].

A rough estimation of the maximum value of heterotrophic N_2-fixation in the rhizosphere can be calculated using estimated C flow from the roots, but no data are available for rice. Sauerbeck and Johnen [123], growing wheat from the seedling to maturity under $^{14}CO_2$, estimated that C respired by microorganisms in the rhizosphere and converted to microbial biomass accounted for 4–5 times the remaining root C at harvest. Using this value and

and 0.2 t C/ha of roots at harvest [120], 1.0 t C/ha is estimated to pass through the microbial biomass in the rhizosphere. Assuming that all C is used for N_2-fixation (which does not happen) and 40 mg N as fixed/g C consumed, 40 kg N/ha would be the theoretical maximum of associative BNF.

Based on actual and potential estimates on N_2 fixation, it may be said that the potential of associative BNF is the least among N_2-fixing agents discussed in this paper.

2 Possible utilization methods. Manipulations of rice varieties and root-associated bacteria are possible methods to enhance heterotrophic BNF.

a Varietal differences

There are several reports on the varietal differences in the ability to support associative BNF (Nfs character). The differences were genetically analyzed by Iyama et al. [61]. Nothing is known, however, about the physiological basis of the apparent varietal differences. Dommergues [33] reported mutants with higher Nfs character than parents. In a pot experiment, 90 rice varieties grown in flooded conditions, including wild *Oryza* species, were screened for the ability to stimulate N gains by Kjeldahl assays [58]. The methodology used estimated gains due to heterotrophs and phototrophs. Varietal differences were found, and maximum N gains were 8 times the minimum gains. Correlation coefficients of N gains were high with total N uptake, total dry matter production, and daily dry matter production. This indicated that vigorously growing rice plants stimulated more BNF (character NFs).

b Microflora manipulation

Inoculation of N_2-fixing bacteria has been reported to increase growth and yield of rice [104]. As with BGA, interpreting the results is difficult in the absence of experimental data on inoculum establishment and its N_2-fixing activity. Here again the relative importance of N_2 fixed and other possible inoculation effects must be evaluated. Results obtained by O'Hara et al. [88] who inoculated Nif^+ and Nif^- strains of *Azospirillum* on maize, indicated that observed growth stimulation was not because of N_2 fixation. Watanabe and Lin [158], using the ^{15}N dilution method, observed no difference in ^{15}N abundance between rice plants inoculated with *Azospirillum* or *Pseudomonas* sp. and non inoculated plants despite growth stimulation by inoculation.

3 Prospect. The idea of breeding varieties higher in Nfs is attractive because it would enhance BNF without additional cultural practices. However, a prerequisite to Nfs breeding is the availability of a rapid screening technique. Acetylene reducing activity was used for most of varietal screenings, however, it is time consuming because it needs several measurements during the plant growth cycle. Additionally, the high variability of the measurements may mask varietal differences. ^{15}N dilution may be used for screening and genetic studies, but for that purpose, reference varieties with low N_2 fixation stimulation ability must be identified.

N balance studies have shown that vigorously growing varieties tend to have high associative N_2-fixation ability. If this trait, a characteristic of traditional tall indica rices, is essential, the dilemma comes in choosing between the high yielding character associated with short satured plant types and the N_2 fixation stimulating trait associated with tall stature. However, IR42, a high yielding variety, high in stimulating N_2-fixation gives hope of possible selection for high yielding, high N_2-fixing varieties [58].

Another approach is microflora manipulation. If the positive effect of inoculation on plant growth is at least partly because of enhanced BNF, the selection or the improvement of N_2-fixing strains is the first possibility. N_2-fixing ability of bacteria isolated from rice may be enhanced by recombinant DNA techniques. The idea to develop derepressed N_2-fixing bacteria in which BNF is not suppressed by NH_4^+ appears to be attractive. However, it is not known if such derepressed bacteria can compete with non N_2-fixing ammonium utilizing microflora. A related problem is how to select competitive strains with the ability to live in the rhizosphere of inoculated plants. A better knowledge of root bacteria relationships is a prerequisite to this work.

No cultural practice are known to enhance the associative BNF process. Associative BNF seems to be lower in acidic soils [22, 27] and to be less sensitive to N fertilizer application than other N_2-fixing systems [155].

In the next few years, associative BNF may be understood by using a simple system like monoaxenic culture. Enormous effort will be needed to develop intentional utilization and enhancement of the process. As Postgate [98] stated, 'yet it would be as foolish to abandon the project of establishing productive rhizocoenoses with cereals as it was to expect such an association to mature in just a couple of years of research.'

IV General conclusion

Spontaneous BNF has permitted moderate but constant rice yield, around 2 t/ha, without N fertilization. Through traditional management practices such as green manuring with legumes or azolla, BNF contribution to soil N fertility has been substantially enhanced, thus producing higher yields (2–4 t/ha). Although all groups of N_2-fixing microorganisms have been shown to inhabit rice fields, and their possible agronomic use has been demonstrated, technologies available for popularization are still limited to practices based on the production of a large biomass followed by its incorporation, namely legumes, azolla, and straw incorporation.

Legumes and azolla have best known potential as N_2-fixers in rice fields, with recorded 50–100 kg N fixed/ha per crop. Current usage of separate-crop legumes is decreasing. Use of azolla, grown with rice, is increasing. Legume use is limited by socioeconomic factors whereas azolla technology is limited by socioeconomic and environmental factors. Straw incorporation, besides providing soil nutrients, increases heterotrophic BNF by 2–4 kg N/t of straw incorporated. Its use is limited by socioeconomic factors.

Other N_2-fixing systems are unused or little-used by farmers. Free living BGA have less potential than legumes and azolla (around 30 kg N/ha per crop) but their use is promising because little additional labor is required. However, algal inoculation is still at a research level in most of the rice growing countries. Factors involved in yield increase reported after algal inoculation, factors leading to the establishment of a bloom, and the general ecology of BGA in rice fields are still poorly understood. Therefore, algal inoculation cannot be confidently recommended yet.

There are evidences that some rice varieties promote heterotrophic BNF. However, no technology has yet been developed to utilize heterotrophic N_2 fixation associated with rice. The overall impression from experiments design to enhance the process by inoculation is not one of a great success.

Biological N_2 fixation has potential where N fertilizer is unavailable. Data on hectarage of rice grown without fertilizer are not available, but assuming that there is a correlation between water management and fertilizer use and considering that only about 30% of riceland in Southeast Asia is irrigated, it appears that the hectarage of nonchemically fertilized rice may represent a very large area.

A common characteristic of the BNF technologies currently adopted by farmers is intensive labor use. They are most often used under socio-economic conditions where labor intensive practices are economically feasible or where economics is not a major factor. In the future it is unlikely that BNF could be an exclusive N source for producing high yields under economically feasible conditions.

However, this does not mean that BNF technology has potential only where N fertilizer is unavailable or unaffordable, and that BNF will only produce low to moderate yields. Most probably the future of BNF in rice cultivation is in integrated management. A better knowledge of the microbiology and the ecology of rice fields will encourage high rice yields through a more efficient usage of chemical fertilizers and the simultaneous utilization of BNF.

When considering that:

– the efficiency of nitrogenous fertilizers is 30–50% or less [24];
– a large part of N not recovered in rice is lost; and that
– N fertilizer is applied so that it inhibits major components of the N_2-fixing biomass,

it appears that it is not a fine tuning of N fertilizer management in lowland rice that is needed but drastic changes in present fertilization concepts. Urea deep placement [29], which significantly decreases losses of N by volatilization and does not inhibit photodependent BNF [116] is a good example of the kind of technology that must be developed for integrated management of BNF and chemical fertilizers.

When considering current usage by farmers, it appears that BNF is purposefully used in only a small percentage of rice fields in a few countries and that rice farmers are far from realizing its potential. This underutilization is due to ecological and socioeconomic factors and lack of technology development and knowledge. On a short-to medium-term basis, BNF has underexploited potential where N fertilizers are not available or affordable. On a long term basis, BNF integrated management should permit high yields with lower N fertilizer application.

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