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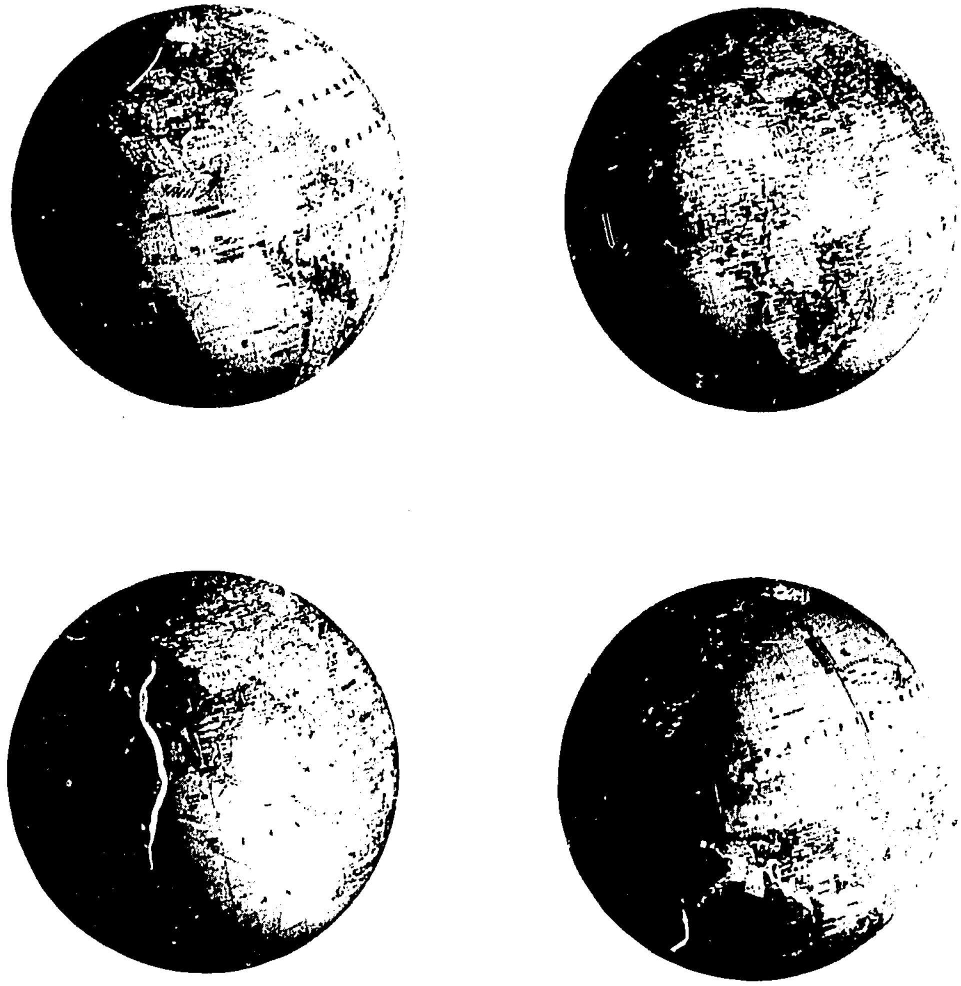

BIOLOGICAL NITROGEN FIXATION IN FLOODED RICE FIELDS

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CONTENTS

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One third of the world's population depends on rice for the major portion of its nutrition (Anon, 1973, 1974), and tens of millions of families depend on growing rice for a subsistance living. In many of the developing countries, particularly in the tropics and subtropics, rice is, and for the foreseeable future will continue to be, the staple food. In these lands, rice yields average less than half that of the more industrialized countries, from less than 0.5 to about 2.5 metric tons of grain per hectare compared to 5.5 metric tons or more in Japan, both Koreas, and Spain (FAO, 1977).

Using statistics from the Food and Agriculture Organization of the United Nations, Stangel (1979) found a strong positive correlation between rice yields, irrigation rates, use of modern high-yielding rice varieties, and the use of nitrogen fertilizer. Additional constraints on rice yields included insect damage and poor fertilizer management. Although this paper will focus on one parameter, nitrogen fertilization, and only a single aspect of that, biological nitrogen fixation, it would be a mistake to ignore or underestimate the important interrelationships among all the above factors.

That nitrogen is a major nutrient limiting rice production is generally acknowledged. In modern high yielding rice cultivars, the response to nitrogen fertilizers is dramatic (de Geus, 1967; Patnaik and Rao, 1979; Sanchez _et al._, 1973). For example, rice grain yields of variety IR8 were increased from about 3500 kg ha^{-1} to approximately 11,600 kg ha^{-1} by fertilizing with 480 kg N ha^{-1} as urea (Sanchez _et al._, 1973). In addition, the results of these experiments imply that more nitrogen would further enhance yields.

Subsequent to petroleum price increases in 1972-73, the cost of nitrogen fertilizer worldwide rose substantially, making it even less accessible to small, subsistence farmers (Stangel, 1979). One result of the fertilizer-price squeeze was a renewed interest in biological nitrogen fixation as a means of reducing the economic limitation to nitrogen use. Attention was directed at the multitude of small farmers, who have been historically unable to afford industrially produced nitrogen fertilizers (either fixed by the Haber-Bosch process or mined). Biologically fixed nitrogen could potentially provide a source of fertilizer for those farmers that were unable to obtain industrial nitrogen after the price inflation, and also for those who had been excluded even before the price rise. In addition, biologically fixed nitrogen could substitute for industrially fixed nitrogen to lessen demand on fossil fuels, to decrease dependence on industrial development prior to increasing agricultural productivity, and to lessen the environmental impact of production and utilization of fertilizers.

Biological fixation was initially thought to offer an alternative to industrial nitrogen in rice production because of reports that constant, albeit low, yields had been obtained from vast areas for hundreds of years with no manure or fertilizer nitrogen (Howard, 1924; Matsuo, 1961, as reported in Grist, 1975; Yamaguchi, 1979) or with only weeds as green manures (Van Breemen et al., 1970). For long term cropping of unfertilized rice, contemporary evidence indicates that nitrogen recovered in the rice crop is not accompanied by equivalent decreases in soil nitrogen (Yamaguchi, 1979; Koyama and App, 1979). Hence, a net input of nitrogen must at least equal that removed as grain and straw and lost by leaching, volatilization of ammonia, and denitrification.

The amount of nitrogen removed in a crop, of course, depends largely on the rice yield, the nitrogen content of the rice, and whether or not the rice straw is returned to the field. For example, Tanaka et al. (1964) determined that the total amount of nitrogen removed by an average rice crop yielding 4740 kg ha^{-1} at the International Rice Research Institute (IRRI) in the Philippines was 90 kg N ha^{-1}. When the straw was left, only 48 kg N ha^{-1} was removed. The nitrogen content of the raw rice grain in this case was about 1 percent.[1] The highest present average yields of paddy rice (not yet cleaned) are found in the Republic of Korea: almost 7 metric tons per hectare. To replace the nitrogen removed by this quantity of rice requires about 70 kg N ha^{-1} or more. If yields of 12 to 13 tons ha^{-1} as recorded in special contests (deGeus, 1967) are to be approached regularly, then no less than 120 kg N ha^{-1} must be returned to the paddy ecosystem to allow continuous rice culture and to maintain soil nitrogen in a steady state. These values of nitrogen required to provide high rice yields do not take into account losses or inefficiencies, rather they represent a minimum replacement amount. These figures will also have to be inflated as the N content of rice is improved by breeding and selection for varieties with higher protein content. Thus a reasonable estimate of the net amount of nitrogen that must be supplied to a high yield crop of rice is from 50 to 120 kg ha^{-1}.

Among the relevant contemporary questions are how much nitrogen can be supplied by biological fixation for high rice yields and what are the various costs. This paper will focus on the former question. It will evaluate sources and sinks of nitrogen in flooded rice, their amounts, and agronomic significance. It will present evidence on the ability of biological

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[1]Although different varieties have been found to contain from 0.9% to 2.4% nitrogen in the milled rice (Anon, 1973), to simplify calculations in this paper, grain-nitrogen content will be estimated as 1% of grain weight.

nitrogen fixers to maintain or increase rice yields in the field. Finally, it will examine constraints on the fixation of nitrogen and its transfer to the rice plant.

With few exceptions, the overall costs of utilizing biologically fixed nitrogen for rice have not been determined.

Comment on Methods

Methods for measuring biological nitrogen fixation and nitrogen cycling have been reviewed (Hauck, 1979; Bremner, 1977; Hardy and Holsten, 1977; Burns, 1974; Burris, 1972). These methods include the Kjeldahl, ^{15}N, and acetylene-reduction assays. A few, brief comments are in order regarding the sensitivity of each method and the interpretation of results presented in this paper.

The Kjeldahl analysis is the classical method. It provides a direct measure of the total nitrogen content in a given sample of rice, nitrogen fixing organism, or soil. Nitrogen gains or losses in a system are determined by differences in Kjeldahl nitrogen values before and after a given treatment. However, the value found for total nitrogen in a nitrogen fixing organism such as azolla or a legume is not equal to the amount of N_2 fixed by that organism but rather is the sum of nitrogen fixed and mineral nitrogen absorbed from soil and flood water, less any losses to the environment. A simple Kjeldahl value for these organisms probably overestimates the newly-fixed nitrogen. For the purposes of this paper, half the Kjeldahl nitrogen values have been taken to represent nitrogen fixed. This proportion was previously used by Moore (1969).

For measurements of changes in soil nitrogen content, the Kjeldahl method is also limited because of the large amount of nitrogen inherent in most soils. For example, Hauck (1979) pointed out that the expected variability of the Kjeldahl assay limits measurements of soil nitrogen to a precision of about 60 to 120 kg N ha^{-1} for soil containing 4000 kg N ha^{-1} per 15-cm furrow slice. This uncertainty covers the range of values necessary to replace the nitrogen removed by a single rice crop and may be overcome by making soil measurements over the course of several cropping seasons.

Use of ^{15}N-N_2 is the most sensitive and reliable method for measuring nitrogen fixation in the flooded rice system. When samples are analyzed by mass spectrometry, this method may be more sensitive than the Kjeldahl assay by 60-fold or more depending on the enrichment level of ^{15}N-N_2 (Hauck, 1979). The utility of this technique, however, is limited by the comparatively high cost of ^{15}N and of mass spectrometers. In addition, the necessarily short-term incubations must be repeated over the course of a cropping season in order to provide meaningful nitrogen-fixation data.

The measurement of the reduction of acetylene to ethylene by the nitrogenase-enzyme system is by far the simplest, least expensive, and most widely used method to estimate nitrogen fixation. It is also the least direct and the one most liable to misinterpretation. Burris (1974) and Bremner (1977) discuss criticisms of this method and prerequisites for using it properly. For example, the theoretical conversion factor of 3.0 moles of acetylene reduced per mole of N_2 fixed is often used in investigating nitrogen fixation in flooded rice systems. Yet, only occasionally have scientists actually correlated ethylene production to $^{15}N_2$ reduction under identical conditions. Hauck (1979) reported acetylene to dinitrogen ratios to range from 3.0 to 25 in flooded or anaerobic soils.

As is the case with the ^{15}N assay, acetylene reduction must be measured repeatedly in order to extrapolate nitrogen fixation value over the course of a cropping season.

Nitrogen Gains and Losses in the Rice Field

Few studies have been carried out to measure the changes of net nitrogen occurring in the rice paddy environment during the cropping year. No single study has attempted to measure the individual inputs and losses for a particular paddy ecosystem, especially when nitrogen was provided from biological sources. As a result, we have little gauge of the relative importance of various sources and sinks except as pieced together from separate measurements made at disparate locations. The following is meant to be a representative summary of these reports.

Exogenous Nitrogen Sources

In Table 1 are listed sources of exogenous nitrogen, that is, nitrogen which enters the rice field from elsewhere. Excluded from this list are industrially produced nitrogen fertilizers. Although sources of nitrogen such as rain, floodwater, silt and atmospheric ammonia are not readily manipulated by agriculturists, they must utimately be included in considerations of nitrogen balance. These sources, it can be seen, may provide considerable fixed nitrogen. Rain presumably brings down nitrogen naturally volatilized from the earth's surface and fixed by lightning as well as by industrial processes. Additionally, ammonia volatilized from a fertilized rice field may be deposited in another rice field downwind. Data on these exogenous sources of fixed nitrogen are few. These sources have been considered for constructing a nitrogen balance in fertilized fields (Yatazawa, 1977), but are rarely included in estimates of overall nitrogen needed to crop rice in the absence of industrial fertilizers. Underestimating the amount of nitrogen brought into the rice ecosystem by rain, flood water, etc., when taken in a total nitrogen balance may lead to overestimating the amount of nitrogen fixed by biological sources. Such an overestimate could then lead to an unwarranted dependence on certain fixers, especially if the conclusions from data were applied to fields away from the research plots receiving less exogenous nitrogen.

Animal manures and composts may also serve as exogenous sources of fixed nitrogen. If these materials contain about 0.5 percent N (Yatazawa, 1977), then 10,000 to 20,000 kg ha^{-1} must be applied to achieve 50-100 kg N ha^{-1}. Less may be added to complement other sources of nitrogen. Grist (1975) pointed out that even when most of the nitrogen has been lost because of improper storage of manure, its application improved rice yields. This effect complicates any analysis of results with manures as measured by yield increases, and indeed with any source of biologically-fixed nitrogen. The above problem of distinguishing stimulation by nitrogen from stimulation by other factors is infrequently considered in reports on biological nitrogen fixation.

By taking from Table 1 the lowest and highest value for each exogenous source of fixed nitrogen, and assuming that half of the absorbed ammonia is revolatilized, a summation of the values for a cropping season of one third of a year leads to a range of 15 to 61 kg N ha^{-1} yr^{-1}. These figures are not insignificant compared to the nitrogen removed by a crop of rice. In fact the nonbiological nitrogen might readily account for enough nutrient to maintain continual cropping of rice as described above.

Endogenous Nitrogen

Included in the catagory of endogenous sources of nitrogen are paddy soil and nitrogen fixed biologically *in situ*. By far the greatest reservoir of nitrogen is the soil itself. In rice soils with a total nitrogen content of between 0.1 and 0.6%, from 2000 to 12,000 kg N ha^{-1} is estimated in the 15-cm plow layer (2 x 10^6 kg soil). How much is mineralized and made available to the growing rice under different management techniques is beyond the scope of this paper but is considered by Broadbent (1979). In long-term field experiments on rice to which no nitrogen fertilizer was added, the soil relinquished a net amount of nitrogen in some cases, gained in others, and underwent no net change in a third set. After 21 crops of rice, nitrogen in the soil at the Aomori Experiment Station, Japan, had decreased by 15% and after 21 crops of a rice-rice-rye rotation, while nitrogen in the soil of the Kagawa Experiment Station, Japan, had dropped by 37% (Koyama and App, 1979). Yet, in the Philippines, soil nitrogen increased by up to 17% after 17 or 24 crops of rice which had received no nitrogen fertilizer (Koyama and App, 1979). Taking into account changes in soil nitrogen, 15 to 38 kg N ha^{-1} yr^{-1} in the Japanese experiments and 75 to 100 kg N ha^{-1} yr^{-1} in the Philippines had been added to the rice ecosystems beyond that nitrogen known to be removed. In other long-term experiments in Japan, no significant change in soil nitrogen was reported (Yamaguchi, 1979), although up to fifty crops of rice were grown on these soils and the incorporated nitrogen removed. Despite possible short-term net losses of soil nitrogen to rice crops, long-term cropping obviously cannot continuously depend on a limited source such as the soil and requires renewable inputs.

The remaining nitrogen sources are the agents of biological fixation: legumes, blue-green bacteria (also called blue-green algae or cyanobacteria), the water fern Azolla, rhizosphere or soil bacteria, and photosynthetic bacteria.

Legumes

Farmers have used legumes for many centuries to improve soil fertility. For less than one century, it has been known that legumes are capable of doing this through their symbiosis with bacteria of the genus Rhizobium. Legumes can be used as a green manure in rotation with rice. In India a common green manure, Sesbania aculeata (dhaincha), has provided 96 kg N ha^{-1} for a subsequent rice crop (Ghose et al., 1956). A wild relative of dhaincha, S. sirecea, provided 146 kg N ha^{-1}. An even higher value has been recorded in the Philippines for S. sesban of 202 kg N ha^{-1} (Anonymous, 1964). These and some other high values are listed in Table 2.

One important question which presently remains unanswered is how much of the nitrogen in the green manure comes directly from N_2 and how much represents assimilation by the legume of nitrogen mineralized from the soil. Only that which is newly fixed should be considered in accounting for nitrogen input as biological fixation and not that which is transferred from one compartment in the paddy environment to another. In addition, legumes as well as nonlegume crops in rotation with rice may play a role in tying up mineralized soil nitrogen so that it is not denitrified, volatilized, leached or lost by runoff, processes which could occur were the plants not grown. It is not yet possible to assign a value to these losses.

Assuming half the nitrogen in the legumes listed in Table 2 is newly fixed and that one of these high nitrogen-producing varieties is grown as a green manure for rice, it can be estimated that from 25 to 101 kg N ha^{-1} can be made available to subsequent rice crops. Thus, a considerable fraction of the nitrogen required by rice could be supplied by legumes.

Blue-green Bacteria

Blue-green bacteria are photosynthetic organisms and, as such, can reproduce in the presence of water, inorganic salts, CO2, and sunlight. A general description of these organisms is found in the book by Fogg _et al_. (1973). Some blue-greens are capable of fixing nitrogen. Stewart _et al_. (1978) reported that more than 125 strains fixed nitrogen in pure culture. Blue-green bacteria, along with nonnitrogen-fixing algae, are common inhabitants of paddy fields. The significance of blue-green bacteria in rice cultivation has been analyzed by Roger and Kulasooriya (1980).

Cultures of blue-greens are already being inoculated into flooded fields, primarily in India in order to increase rice yields (Venkataraman, 1979). Amounts of nitrogen estimated to be fixed by blue-greens associated with rice are listed in Table 3. Amounts of nitrogen fixed in laboratory experiments often extrapolate to very high field values (Rinaudo _et al_., 1971; De and Mandal, 1956). Field measurements, although difficult to obtain, have provided more conservative estimates. Furthermore, most field measurements were made on indigenous blue-green populations. Only a few data have been gathered on the incremental nitrogen provided by inoculation of blue-greens into flooded fields (Hirano, 1958; Watanabe and Cholitkul, 1979; App _et al_., 1980). In fact, very little information is available on the subsequent growth and establishment of the inoculated blue-green species (Roger and Kulasooriya, 1980).

Several specific difficulties exist in measuring nitrogen fixation by blue-green bacteria in the field. These include 1) the patchy or uneven distribution of the organisms in a given field, 2) the rapidity of appearance and disappearance of a blue-green bloom, which might be easily missed without frequent observations, and 3) the problem of excluding non-fixing organisms from a measurement of total nitrogen-fixing biomass. In addition to these is the more general problem of determining how much of the total nitrogen of the blue-greens represents absorption of mineral nitrogen. Problem 3) tends to lead to an overestimate of fixation; problems 1) and 2) could lead to either underestimates or overestimates, depending on sampling error. By assuming that one-half the nitrogen measured represents newly fixed nutrient (from Table 3), a range of values of 0.2 to 39 kg N ha^{-1} per cropping season results. The upper end of this range covers that suggested by Venkataraman (1979). He estimated that inoculation with blue-greens could benefit farmers in an amount equivalent to the application of 25 to 30 kg N ha^{-1} of industrial nitrogen. It thus appears that a large fraction, or perhaps all, of the nitrogen required by low-yielding rice in the poorly to moderately efficient farming systems could be provided by nitrogen-fixing blue-green bacteria.

Azolla

The small, fresh-water fern Azolla is found in temperate and tropical climates worldwide (Peters, 1977). It is often considered to be a weed growing in paddies because it can cover young rice shoots or appears to compete with the rice for nutrients (Moore, 1969). In each fern leaf may be found a chamber containing nitrogen-fixing, blue-green bacteria thought to be of the genus Anabaena. Present evidence indicates that the azolla and its enclosed blue-green bacteria form a symbiotic relationship in which the fern provides photosynthetic products to the microsymbiont in return for fixed nitrogen. Lumpkin and Plucknett (1980) summarized botanical, physiological, and agricultural work on Azolla.

Long before any understanding of the Azolla-Anabaena symbiosis, the Vietnamese in the 11th century and the Chinese in the 17th century cultivated the water fern in paddy water to improve rice yields (Dao and Tran, 1979; Liu, 1979). Azolla can be grown in the rice field during a flooded fallow period, in a separate field in order to be used later as a green manure. or along with the rice as a dual crop. The amount of nitrogen potentially available for one rice crop appears prodigious (Table 4). In some field trials, the amount of nitrogen harvested in azolla in one year, up to 1250 kg ha^{-1} in India (Singh, 1979a) and 675 kg ha^{-1} in China (Liu, 1979), far exceeded the present requirements of rice for the same area. However, the amount of inoculum was also prodigious: for example, Singh applied 13.6 metric tons ha^{-1} of fresh azolla each month for an annual total of 164 tons of inoculum containing 410 kg N. Thus, about one-third the final nitrogen yield was supplied as inoculum. As a general solution to nitrogen requirements for growing rice, such methods seem impractical.

More modest inocula and more modest yields of azolla have provided more impressive percentage increases of nitrogen contained in the azolla. an 8-fold increase in azolla nitrogen over the amount inoculated with _A. pinnata_ in 106 days (Watanabe _et al._, 1977a); an 18- to 28-fold increase in nitrogen with _A. pinnata_ to between 24 and 37 kg N ha^{-1} in 25 days (Singh, 1979b); and nearly a 30-fold increase with _A. mexicana_ to 41 kg N ha^{-1} in 46 days (Rains and Talley, 1979). In the last mentioned experiments, only 500 kg ha^{-1} of fresh azolla was introduced into the field (Rains and Talley, 1979). For comparison, one would have to add 245 kg of ammonium sulfate or 111 kg of urea to obtain the equivalent of 52 kg N. Thus, the work to spread azolla in the flooded rice fields may not be much greater than that expended in applying fertilizer nitrogen. Of course, any economic comparison of the costs and benefits of different sources of nitrogen must include many other factors as well.

How much of the nitrogen in azolla represents N_2 fixation has not been determined under field conditions. Assuming as before that only half of the nitrogen in azolla grown in the field represents new nitrogen fixation, the amount fixed in one-third of a year by azolla as a green manure ranges from 25 to 121 kg ha^{-1} (Dao and Tran, 1979; Rains and Talley, 1979) and by azolla dual cropped with rice from 8 to 75 kg ha^{-1}. Using the highest values of nitrogen fixed for a green manure given here assumes that only small inocula are practical and that it is reasonable to extrapolate to an annual nitrogen increment from growth of azolla during the spring of the year. More labor-intensive management could lead to far greater nitrogen inputs. Whether or not this is feasible, the extraordinarily large values of nitrogen fixed by the Azolla-Anabaena complex, even during short time periods, have prompted interest in the important present and potential role it has in rice culture and encouraged the recent upsurge in research.

Soil and Rhizosphere Microorganisms

Values for determinations of the contribution of soil and rhizosphere bacteria to nitrogen fixation in the paddy field are shown in Table 5. Except for the Kjeldahl data of Rinaudo et al. (1971) from laboratory studies and the ^{15}N results of Eskew et al. (1981), the reported values are derived from acetylene-reduction assays. Many authors do not convert their data from ethylene produced to nitrogen fixed, because of the many untested assumptions involved. Rather, they prefer to use the acetylene assay only to assess seasonal changes or to determine the effects of various treatments on activity in rhizosphere versus bulk soil. Nonetheless, it is important to make field estimates and to assess the importance to rice cropping of the production of fixed nitrogen within the soil.

An important complication in measuring the amount of nitrogen fixed by rhizosphere microorganisms has been excluding fixation by contaminating blue-greens in the flood water, on the soil surface or on the plants. One recently developed method is to eliminate the blue-greens by using selective bactericides (Habte and Alexander, 1980). Yoshida and Ancajas (1973b) and Watanabe et al. (1977b) attempted to avoid fixation by blue-greens in the flood water by removing surface water and replacing it with fresh water. Watanabe et al. (1977b) found 18-fold higher acetylene reduction activity if blue-greens were left in the soil-plant-water assay system than if removed. These results suggest that microbial nitrogen fixation in the soil itself is but a small fraction of total fixation.

However, the field data suggest a rather broad range of 0.8 to 63 kg N fixed per hectare per one-third year season (Watanabe and Cholitkul, 1978; Yoshida and Ancajas, 1973b). Independent confirmation of nitrogen fixation in the rhizosphere may be seen from the nitrogen balance data of De and Sulaiman (1950b) and App et al. (1980). In the latter report, 6-10 kg N per hectare per crop could be ascribed to rhizosphere fixation (see below).

Photosynthetic Bacteria

Photosynthetic bacteria are generally thought to make no significant contribution to nitrogen fixation in paddy fields, although experimental evidence is lacking. However, these organisms are relatively abundant and as many as 10^5 to 10^7 have been found per ml of floodwater or per gram of dry soil (Materowski and Balloni, 1965; Kobayashi et al., 1967; Watanabe et al., 1978b). Arguments against the importance of the photosynthetic bacteria invoke the observation that fixation by these organisms is very slow (Alexander, 1977), that fixation is inhibited by oxygen (Stewart, 1977) and that flood waters of rice fields are likely to be aerobic, especially where blue-green bacteria or algae are photosynthesizing. Kobayashi and Haque (1971), however, reported considerable aerobic nitrogen fixation, two thirds as high as anaerobic fixation, by photosynthetic bacteria under light in culture with pyruvate as a carbon source. Although these authors provided no absolute figures for rates of fixation, they felt that excretion of pyruvate by heterotrophic bacteria in paddy fields might allow significant nitrogen fixation by photosynthetic bacteria. The results of Habte and Alexander (1980) suggest that fixation by photosynthetic bacteria is increased when blue-greens are killed by chemical treatments. However, information on photosynthetic bacteria in rice fields is still insufficient to extrapolate to an amount of nitrogen fixed in a rice field.

Comparing Sources of Fixed Nitrogen

According to the foregoing, potential sources of "exogenous" and biologically fixed nitrogen *in situ* for paddy rice are considerable. Table 6 summarizes estimates of these sources. Of the sources that can be agriculturally manipulated, the limited data suggest that *Azolla* and legumes stand out. The blue-greens and then soil and rhizosphere fixers follow in quantity of N_2 fixed. Yet, this is but one side of the nitrogen balance sheet. An inquiry into the magnitude of losses reveals fewer studies and much less information, particularly regarding losses in the absence of added industrial nitrogen fertilizer.

Nitrogen Sinks

As mentioned in the introduction, the rice grain harvested from one hectare of land may contain over 100 kg N. The rice straw may also be removed from the rice growing area or returned to the soil. In rice with a grain yield of 4.74 tons ha^{-1}, the straw contained 43 kg of nitrogen (Tanaka *et al.*, 1964; Table 7). Thus, the straw held a substantial quantity of nutrient nearly equal to the 47 kg of nitrogen removed in the grain. Incorporation of straw into paddy soil has increased in Japan from an average 1 kg ha^{-1} in 1955 to over 500 kg ha^{-1} by 1970 and, in some areas, to nearly

1.4 tons (Stangel, 1979). However, the latter value is still a small fraction of the total rice straw and represents roughly 7 kg N.

Losses of nitrogen from ammonia volatilization and denitrification have been measured mainly after addition of nitrogen fertilizer. The topic has been reviewed by Craswell and Vlek (1979) and Prasad and de Datta (1979). Denitrification in the absence of nitrogen fertilizer addition has been measured as 46.4 kg N ha^{-1} per season (Watanabe, 1965, cited in Yatazawa 1977). Leaching losses in the absence of industrial nitrogen ranged from 6.2 to 28.0 kg N ha^{-1} per season (Yatazawa, 1977). These sinks and others, such as runoff or temporary fixation of ammonium by clay, have drawn slight attention in rice soils receiving nitrogen only from biological sources.

Although it is generally believed that nitrogen losses are significantly less from organically amended than from inorganically fertilized soils, such may not hold for the flooded rice environment. De and Digar (1954) incorporated oil cake or water hyacinth at a rate of 135 kg N ha^{-1}, or added ammonium sulfate or sodium nitrate (67 kg N ha^{-1}) to flooded fallow soils in a closed vessel and determined nitrogen losses in drainage water or as gas. They found that the equivalent of about two-thirds or more of the added nitrogen was lost. In terms of losses, no advantage was gained by adding nitrogen as organic manure compared to the inorganic fertilizers. In soils cropped with rice, about 25% less nitrogen gas was lost than in uncropped soils when either oil cake or ammonium sulfate was the source of nitrogen. However, in the cropped soil, nitrogen losses were slightly greater if the form of fertilizer was oil cake than if it was ammonium sulfate.

Broadbent and Tusneem (1971) confirmed that significant losses of nitrogen take place from flooded soil amended with organic nitrogen labelled with ^{15}N. When immature rice straw (3.02% N) was incorporated into soil, as much as half of the total nitrogen was progressively lost in 120 days. With more mature straw (1.19% N), however, no loss of nitrogen from the system was recorded.

These results indicate substantial losses of nitrogen can occur in unamended or manured, flooded soils in the laboratory, depending on the source and age of manure. Losses were reduced when soils were planted to rice. The magnitude of such nitrogen losses under field conditions needs to be determined in order to make a proper assessment of the value of green manures to the nitrogen economy in rice cultivation, and to develop efficient management schemes.

Nitrogen Balance

In the previous discussion, individual inputs and losses of nitrogen in the rice environment have been reported for laboratory and field conditions. In no single study have all of the individual influxes and effluxes of nitrogen been totaled, although Yatazawa (1977) summarized various estimates (Table 8). However, net changes of nitrogen in the paddy environment have been measured in a few laboratory and greenhouse studies. These investigations demonstrated significant net increases in nitrogen content of the system over the course of a single growing season (Willis and Green, 1948), 5 years (De and Sulaiman, 1950b), and 4 or 6 seasons (App et al., 1980) (Tables 9, 10, and 11). Despite different methods and experimental conditions, the results of these 3 studies revealed some important similarities (Table 12). In the presence of a crop and with sunlight reaching the soil, a nitrogen increase was found in the soil-plant system in all cases, and an increase was found in the soil as well in two of the three systems. Thus, under these experimental conditions, the sum of increases was greater than total losses of nitrogen from the system. When black cloth was used to exclude light from the soil surface, nitrogen gains were still recorded in the soil-plant system, but the soil lost N. Thus, one can conclude that nitrogen was being fixed even in the absence of light, implying fixation by heterotrophic rhizosphere and soil bacteria. Depletion of soil nitrogen in the presence of a crop indicates that the rate of transfer from soil to crop (plus any losses) was greater than the rate of nitrogen fixation.

From the results of App et al. (1980), the relative amount of phototrophic fixation may be estimated as slightly more than twice that for heterotrophic fixation. However, because both nitrogen-fixing and non-fixing phototrophs may scavenge ammonia and nitrate which might be lost from flood waters to the atmosphere or denitrified, one should consider that eliminating these organisms may eliminate a pathway for nitrogen recycling within the paddy environment. Thus, under black cloth conditions a source of fixed nitrogen is removed, and a sink is created or expanded. By underestimating the expanded nitrogen losses, one also underestimates the contribution to the pool of fixed nitrogen by rhizosphere bacteria and overestimates the contribution to newly fixed nitrogen by phototrophs such as blue-green bacteria.

In cropped soil, De and Sulaiman (1950b) measured increases of nitrogen in the light and decreases in the dark. Similarly, App et al. (1980) found a greater nitrogen loss in the darkened soils than in the lighted soils. These results provide evidence that phototrophs help maintain the levels of soil nitrogen in flooded soils.

Consistent with the above, App et al. (1980) found no significant change in nitrogen in uncropped soils in the light and a significant loss in the dark. These authors argued that nitrogen lost from lighted, fallow soils was normally replaced by phototrophically fixed nitrogen, which could not take place in the dark. Willis and Green (1948), on the other hand, obtained inconsistent results on nitrogen changes in uncropped soils.

When the effect of cropping of rice on soil nitrogen is considered, the results are conflicting. Willis and Green (1948) found the gain of soil nitrogen greater in planted than in fallow soils. App et al. (1980), to the contrary, reported that losses of nitrogen in planted soils were greater than losses in fallow soils. In both sets of experiments, some of the nitrogen from cropped soil, of course, was transferred to the rice. From the former study, one concludes that cropping the soil left it with more nitrogen than allowing it to remain fallow, but the opposite conclusion is drawn from the latter results. Reasons for this qualitative difference remain unclear.

Treatments intended to increase nitrogen fixation in the balance experiments were successful. Amendments with nutrients and inoculation of blue-greens or azolla all increased the net gain of nitrogen (see Table 11).

Long-term cropping of rice without industrial nitrogen inputs and with constant yields requires that the nitrogen content of the soil remain in a steady state. This condition did not hold in the nitrogen balance studies. The nitrogen content of the soil either increased 0.3-5.8 percent for one crop (Willis and Green, 1948) or 0.7-5.7 percent per year (De and Sulaiman, 1950b), or decreased 1.4 percent per crop (App et al., 1980) when rice was grown and the soil exposed to light. Similarly, the nitrogen content of the rice crops was not constant. In successive crops in the nutrient-amended soils of De and Sulaiman, crop nitrogen apparently rose, and it fell in the experiments of App et al. Thus, for both soils and crops, variances were reported from steady-state conditions. Yet, the sum of individual accumulations of nitrogen to the rice-soil ecosystem must at least equal the sum of losses and removals if rice is to be grown year after year with no decrease in yield or loss of soil nitrogen. The imbalances found in these experiments in the greenhouse must be accounted for if the results are to be extrapolated to real farm conditions.

Despite some differences in results among the three studies, the nitrogen-balance experiments have provided convincing evidence of net nitrogen increases in systems of flooded rice closed to all nitrogen gains or losses save gaseous exchange. These experiments also pointed strongly to the fact that nitrogen fixed by heterotrophic bacteria can form a sizable fraction of total fixation in unamended soils.

Yield Increases From Nitrogen Fixers

Clearly, large quantities of nitrogen may be made available to rice through biological fixation. For the farmer, however, the key question is can he manipulate nitrogen fixers to increase yields. An indication of the answer is recorded in Tables 13-15. Considerable evidence has accumulated that the use of legumes, blue-green bacteria, and azolla can significantly raise rice yields.

Legumes incorporated into rice soils as a green manure have been shown to increase rice yields by over 100 percent in India (Ghose *et al*., 1956) (Table 13). Percentage increases in rice yields were found to be greater on the less fertile, sandy soils, which gave the lowest yields of rice receiving no manures, than on the clay soils. Investigations need to be made on how soil fertility might affect the stimulation of rice yields by green manures.

Ghose *et al*. (1956) also presented evidence that non-legumes such as *Ipomea carnea* and *Cannabis sativa* used as green manures also stimulate rice yields. When grown on the same fields as the rice, these crops presumably recycle nitrogen that was already fixed, a point to be remembered before ascribing yield increases resulting from green manuring to net nitrogen fixation.

Inoculation of paddy fields with blue-green bacteria has frequently led to increases in rice yields varying from slight to over 50% (Table 14). Stimulation of yields by many species of blue-greens has been found with different varieties of rice under diverse methods of soil and crop management. Even in the presence of considerable quantities of alternative nitrogen as green manure (Venkataraman and Goyal, 1968) or industrial nitrogen fertilizers (Venkataraman, 1979), inoculation with blue-greens has stimulated rice yields. Consideration of such findings prompted Venkataraman (1975, 1979) to propose that the blue-greens also produced hormones or plant regulators that encourage rice growth. Evidence to support this proposal, i.e. stimulation of rice by excretions of blue-greens, is minimal (Venkataraman and Neelakantan, 1967; Roger and Kulasooriya, 1980). In addition, one would expect growth substances to stimulate rice yields as well when nitrogen is not limiting, but this possibility has not been tested.

Absence of phosphorus may limit rice-crop development and is often thought to limit blue-greens. Stimulation of nitrogen fixation and yields of rice by this element has been tested in the field (Jha et al., 1965). However, distinguishing stimulation of rice directly by phosphorus from a stimulation resulting indirectly from increased growth and nitrogen fixation of blue-greens is difficult, and conclusive experiments have yet to be carried out.

The economic benefits to the farmer of inoculating blue-greens instead of using the equivalent amounts of nitrogen fertilizer have been determined on the basis of many field trials in India (Venkataraman, 1979; Roger and Kulasooriya, 1980). The return in value of increased rice yield was found to be ten-fold the cost of producing and inoculating blue-greens.

Although inoculation with blue-greens has frequently led to positive results in India, such is not the case in Japan (Watanabe, 1973) and in Taiwan (Huang, 1978), where yields in the field have often not improved. Watanabe (1973) attributed these findings to the fact that development of the blue-green bacteria is inhibited in the acid, Japanese soils. Indeed, liming of these acid soils has improved the growth of blue-greens and also the yields of rice (Watanabe, 1962; Konishi and Seino, 1961). Limiting factors for blue-greens will be dealt with in the next section of this paper.

Recent experimental evidence has revealed substantial yield increases in rice upon applying azolla as a green manure, by growing it as a dual crop or both (Table 14). This evidence suggests that use of azolla has increased grain yields by more than 100% with an average of about 30%. In China, Liu (1979) reported that yields of double crops of rice averaged 13,200 kg ha^{-1} (6,600 kg ha^{-1} per crop) where azolla was cultivated most of the year. At the same time, the average azolla yield was 109,000 kg ha^{-1} (54,500 kg ha^{-1} per rice crop). Yields of rice grown in the absence of azolla were not given. The yield increases clearly depend upon how much azolla is used, the method of application and N content of the azolla, and apparently on the relative fertility of the soil (Singh, 1977, 1979b; Rains and Talley, 1979). As with the application of legumes, the poorer the yield of uninoculated rice, the greater the stimulation following application of azolla. The variety of rice used may also be a factor in the response to azolla application.

Because azolla can be grown away from the rice field, transported to the paddy and added as a green manure, very large amounts of fresh matter and nitrogen may be incorporated into the soil, as indicated earlier. In fact, azolla presumably can be grown for this purpose in localized industries distributed throughout rice growing regions. Thus, even though azolla provides biologically fixed nitrogen, it offers the potential for industrial production (Singh, 1979 a,b). More pertinent to the farmer, however, is the cultivation of azolla in flooded rice fields during the fallow period, in dual culture along with the rice or both, or perhaps in nearby shallow ponds, ditches or tanks.

Watanabe et al. (1977a) found that puddling (i.e., disrupting the physical structure of flooded rice soil) resulted in the same rice-yield increases as did incorporation into soil of azolla grown in dual culture. Puddling has been shown to stimulate the mineralization of soil nitrogen (Patnaik and Rao, 1979). This fact complicates the interpretation of yield increases following incorporation of azolla. On the other hand, the data of Singh (1979b) and Rains and Talley (1979) indicate substantial benefit from unincorporated azolla and dual culture of azolla and rice, pointing to a possible release of nitrogen by the fern into flood waters.

It is worth noting that Singh (1979b) found that grain yields decreased at the highest quantity of azolla incorporated (20 tons ha^{-1}). Thus, an upper limit to stimulation may occur. However, in parallel experiments, Singh (1979b) found depression of yields by high levels of ammonium sulfate (80 kg N ha^{-1}) as well. These depressions occurred in the wet season but not in the dry. The possibility exists that adding large amounts of organic carbon to rice leads to phytotoxicity, yet the reports of high rice yields (6,600 kg ha^{-1} per crop) in China upon application of enormous amounts of azolla (55 ton ha^{-1} per crop) (Liu, 1979) suggests that management plays a significant role in the response of the rice crop.

Results are lacking which demonstrate that inoculation of photosynthetic bacteria or heterotrophic nitrogen fixers into rice fields increase yields, although one report (Subba Rao et al., 1978) stated that inoculation of rice seedlings with heterotrophic bacteria of the species Spirillum lipoferum (Azospirillum lipoferum?) with an additional 40 kg N ha^{-1} as fertilizer gave a rice yield equivalent to the application of 60 kg ha^{-1} of nitrogen fertilizer. Whether such results can be ascribed to nitrogen fixation or some other mode of stimulation and whether they can be reproduced in the field remains to be seen.

At present, experimental evidence shows that legumes, azolla, and blue-green bacteria can contribute to significant increases in grain yield. In particular, utilization of legumes or azolla has led to greater increases in grain yields than inoculation of blue-greens. This is partly so because a greater mass of legumes and azolla can be spread onto a field than is presently the case with blue-greens.

For both agronomic and economic reasons, it is of considerable value to compare the effect of different forms of nitrogen on rice yields. Few data are available relating the stimulation by industrial fertilizers to that by biological fixers. As mentioned, Venkataraman (1979) concluded that inoculation with blue-greens could provide the equivalent stimulation of 25-30 kg N ha^{-1} added as industrial fertilizer. Singh (1977, 1979b) and Rains and Talley (1979) found that nitrogen in azolla was equivalent to that of ammonium sulfate in stimulating rice yields. Watanabe et al. (1977a), however, concluded that not all the azolla nitrogen was available to the rice crop. Further investigation is needed to determine how much of the applied nitrogen in the form of biological material ultimately becomes available to the rice crop.

Limits to the Growth of Nitrogen Fixers, to Nitrogen Fixation, and to The Transfer of Nitrogen to Rice

The following section treats some important parameters that limit the effectiveness of nitrogen fixers in providing nitrogen to rice.

Legumes

As an already important group of food, forage and green manure crops, legumes have generally received considerable attention. Presently, however, less attention has been given to the use of legumes in rice production compared to other sources of biologically fixed nitrogen, as evidenced by the fact that legumes were mentioned in only a few paragraphs in the whole symposium on Nitrogen and Rice held at IRRI (Patnaik and Rao, 1979; Koyama and App, 1979). This should not detract from their tremendous importance in rice growing regions of the world.

Most rice in Asia is grown in fields whose primary source of water is rainfall during a wet season. In these areas, legumes such as *Tephrosia purpurea* may be intersown with rice shortly before harvest, grown through the dry season, and incorporated into the soil when water is let into fields for the next rice crop (Chari, 1957; Gomez and Zandstra, 1977). Other legumes, for example *Sesbania* spp., may be planted on the borders of a field simultaneous with rice planting or in separate fields to provide green manure for the next crop (Chari, 1957). Clearly, for cultivation in different seasons, some legumes must be drought tolerant, and others must be able to grow under waterlogged conditions. Some legumes are suitable only for dryland cultivation, such as *Crotolaria*, while *Sesbania* species tolerate waterlogging and are more suitable to lowland conditions. Different legume varieties may be required for specific conditions of elevation, soil texture, season length, etc. (Ghose et al., 1956; Staker, 1958; Crist, 1975). An extensive list of characters and performances of green manures has been compiled by Ghose et al. (1956; appendix v, pp. 380ff).

It may be difficult to convince farmers to grow a green manure crop which is not going to bring them an immediate return in the form of food or cash, but is rather supposed to raise the yield of the next rice crop. They may prefer to grow grain legumes such as soybeans even though soil nitrogen may be depleted if more nitrogen than was fixed is removed in the harvest. However, Chari (1957) pointed out that one hectare of Sesbania could provide 10 hectares of rice with 6.7 to 9.0 tons of green leaf per hectare. This comes to about 38 to 50 kg N ha^{-1} (using figures of 80 percent moisture content and 2.8 percent nitrogen on a dry basis (Ghose et al., 1957)). In addition, whatever nutrients have to be purchased to maximize legume productivity, such as phosphorus, are also available to a subsequent manured rice crop and thus do not represent a major extra expense.

Although little research has been carried out on the symbiosis between Rhizobium and tropical legumes that are used as green manures for rice, in some cases inoculation was found to be necessary or useful (Wu et al., 1968; Chen, 1978). In others, it was not (ten Have, 1959). Flooding of soils does not seem significantly to inhibit rhizobial survival (Wu et al., 1968; Osa-Afiana and Alexander, 1979). Further research is needed to determine total nitrogen increase, above and below ground, resulting from the cultivation of different legumes under the conditions of rice culture. So far, only the nitrogen in the plant tops has been reported under rice-growing conditions.

The optimum time for incorporation of green manures into soils is thought to be shortly before flooding. Methods and timing of incorporation have received some attention (Rodrigo, 1974; Grist, 1975; Patnaik and Rao, 1979). Plowing in green manures under semi-dry soil conditions allows the formation of nitrate to take place. The nitrate, upon flooding and puddling of the soil, is lost in surface water or denitrified and lost as N_2 (Rodrigo, 1974; Grist, 1975). Tender or fresh plants apparently release nutrients more readily than dried material. Perhaps this difference may be used in applying green manures to coordinate nitrogen release with the nitrogen demand of the rice. But caution is needed because much of the nitrogen from fresh manures may also be lost from the soil-rice system (Broadbent and Tusneem, 1971).

What may be limiting farmers' use of legumes as green manure for rice is sufficient land area to grow the legume, as well as information and availability of varieties that grow well and fix generous amounts of nitrogen under the specific field conditions. The potential for use of legumes is great, and there is a need to reconsider the priority given to legumes as a source of nitrogen for rice.

Blue-green Bacteria

Blue-green bacteria have not been found in every rice soil. Although 71 percent of paddy soils sampled from various parts of Japan contained nitrogen-fixing blue-greens (Okuda and Yamaguchi, 1956), in only a third of the samples from India (Venkataraman, 1975) and in only 5 percent of samples from Asia and Africa were these organisms found (Watanabe and Yamamoto, 1971). The reasons for finding an uneven and limited distribution of blue-greens are obscure. They may include inadequate methodology for culturing the organisms.

One environmental limitation seems to be soil acidity. In culture and in natural environments, blue-greens grow better at neutral to alkaline pH (Fogg *et al.*, 1973; Brock, 1973). Blue-greens have been found in acid soils (below pH 5.0) by some investigators (Okuda and Yamaguchi, 1956) but not by others (Garcia et al., 1974). A significant correlation was found in Senegal rice fields between soil pH and biomass of nitrogen fixers (Carcia et al., 1974; Roger and Reynaud, 1977, 1979). Similarly, in laboratory experiments, neutral or alkaline soils showed greater acetylene reduction activity than soils with pH less than 6.5 (Wilson and Alexander, 1979). Furthermore, liming acid soils of India raised the population density of blue-greens (Amma et al., 1966) and increased nitrogen fixation (De, 1936). Watanabe (1973) considered the fact that inoculation of blue-greens, such as Aulosira fertilissima, raised rice yields in India but not in Japan to be a consequence of the greater acidity of Japanese soils. Both he and Konishi and Seino (1961) recommended the liming of those soils. An alternative to the liming of soils to encourage the proliferation of blue greens might be to isolate strains which grow rapidly under acid conditions and use these as inocula for acid soils. The acid tolerance of strains found in acid soils has not yet been compared with that of strains from neutral soils.

Roger and Reynaud (1979) and Roger and Kulasooriya (1980) recently reviewed the literature on chemical and physical limits to the growth of blue-green bacteria. In addition to excessive acidity, they concluded that the supply of phosphorus, calcium and possibly carbon were limiting. Wilson and Alexander (1979) reported that in 10 of 12 soils tested phosphorus stimulated acetylene reduction activity, and that in the presence of phosphorus CO_2 further enhanced this activity. However, extensive evidence on these factors is still lacking. Too much or too little light, low temperatures, and even wind and rain, which may cause clumping or raise the level of nutrients in the flood water, may limit growth of blue-greens. Increased nutrient level is thought to encourage competition with the blue-green populations by other organisms, such as green algae or diatoms. Roger and Reynaud (1977) observed a pattern of sequential eucaryotic algal and blue-green bacterial blooms in Senegal rice fields during a cultivation cycle. Nonnitrogen-fixing organisms were dominant until heading (flowering) of the rice. After that, the mass of nitrogen-fixing blue-greens began to dominate. Consistent with this pattern are the observations that a dense rice-plant canopy significantly encouraged blue-green proliferation, whereas a sparse plant cover favored nonfixing forms. However, light intensities in Senegal are high (greater than 80,000 lux at 1300 h), and in monsoon areas of India, blue-greens dominate much earlier in the course of the rice crop (Gupta, 1966). If fixation took place only late in the season, then nitrogen from these organisms would be of little value to the rice crop growing at the same time, because rice normally requires nitrogen at earlier stages for maximum yields (Murayama, 1978). However, the nitrogen fixed during the end of a rice cultivation cycle may be available to subsequent crops (Watanabe, 1962; Roger and Kulasooriya, 1980). Management of blue-green blooms for maximum productivity and fixation activity remains to be tried.

In addition to competition, blue-green populations may be limited by pathogens such as viruses, bacteria, and fungi, and predators such as ostracods, daphnids, and fish (Roger and Reynaud, 1979; Wilson _et al._, 1979; Osa-Afiana and Alexander, 1981; Grant and Alexander, 1981). Feeding by ostracods in laboratory experiments severely reduced the biomass of blue-greens and acetylene reduction activity in flooded soils, while _Anabaena_ was more resistant to predation than were _Tolypothrix_ or _Aulosira_ (Osa-Afiana and Alexander, 1981; Grant and Alexander, 1981). Hirano _et al._ (1955) reported that indigenous green algae and blue-greens outcompeted blue-greens that had been newly introduced into flooded fields. They also noted severe predation of these introductions. Thus, inoculant blue-green species should be chosen not only for ability to fix nitrogen, but also for ability to withstand predation.

Hirano et al. (1955) and Watanabe (1962) used pesticides to eliminate micropredators in field trials. Osa-Afiana and Alexander (1981) and Grant and Alexander (1981) reported similar results in the laboratory. It is conceivable that with an understanding of their ecological role micropredators might be manipulated, along with the choice of inoculated species of blue-green, to restrict competition by indigenous forms and to allow maximum nitrogen-fixing activity. Presently, however, such possibilities are academic.

The nitrogen-fixing activity of blue-green bacteria is proportional to their biomass and growth rate but it also reflects environmental influences. For example, molybdenum is required for nitrogenase activity and, to avoid deficiency, it has often been added to inoculated fields in India (Venkataraman and Goyal, 1968; Subrahmanyan et al., 1964). It is conceivable that lack of other nutrients, such as Fe or CO_2, also limits nitrogenase activity, but this has not been shown in the field. Combined nitrogen depresses acetylene-reduction activity by blue-greens in culture. In several studies, fertilizer nitrogen reduced acetylene-reduction activity in the field. NPK (60:30:30) in the wet season or NPK (100:30:30) in the dry season reduced the acetylene-reduction activity of (mostly) blue-greens to 77 and 33%, respectively, of the activity of unfertilized plots at IRRI (Watanabe et al., 1978b). In measurements by Alimagno and Yoshida (1977), maximum acetylene-reduction activity of blue-green bacteria in a fertilized (55 kg N ha^{-1}) loamy sand was only 13% of that in an unfertilized clay soil; an obvious problem with this comparison is that two different soils were used. In view of the thesis by Venkataraman (1979) that blue-green bacteria produce nitrogen for a crop independent of the amount of nitrogen fertilizer, the effects of such fertilizers on the nitrogenase activity of blue-greens deserve further attention.

Nitrogen fixation by blue-green bacteria is often stimulated in culture by the presence of other microorganisms (Becking, 1971), which presumably provide growth promoting substances to the blue-greens. Blue-greens in turn have been found to stimulate the growth of heterotrophic nitrogen fixers such as Azotobacter (Perminova, 1964). Whether the assisting or commensal relationships have any significance in rice fields or can be manipulated has not been studied.

Once fixed, nitrogen must somehow become available to the growing rice plant. The processes involved in transfer of combined nitrogen are largely a mystery. When the nitrogen fixed by blue-green bacteria becomes available to the rice is also a matter of debate. Stewart (1970) reviewed laboratory evidence suggesting the release of nitrogenous compounds by blue-greens while they are growing, but it is clear that the large amounts found may be an artifact resulting from resuspending the organisms in fresh medium (Fogg and Patnaik, 1966) or physically damaging the cells. However, after growth of the blue-green Anabaena variabilis in a nitrogen-free culture medium, as much as 44 percent of the total nitrogen fixed was found outside the cells (De, 1939). The amount of nitrogen released during growth of blue-greens under flooded soil conditions has not been measured. Also unknown is the fate of released nitrogen in flood water or on the soil surface. Some nitrogen may be volatilized as ammonia, especially when the water pH rises during the day as photosynthetic processes remove CO_2. Some of the nitrogen may be assimilated by other microorganisms.

Nitrogen from blue-green bacteria may find its way to rice plants by way of digestive tracts of predators feeding on the blue-greens or following death and decay of the latter. Hirano et al. (1955) and Hirano (1958) measured the accretion of organic matter in the top 0.5 cm layer of flooded soil as a function of the presence of blue-green bacteria. The difference in humus nitrogen over the course of one year between plots inoculated with T. tenuis and uninoculated plots was 26 kg N ha^{-1} (71 kg less 45 kg).

Because stimulation of the growth of blue-greens and their nitrogen fixing-activity did not increase total nitrogen in the rice crop, App et al. (1980) considered that nitrogen from blue-greens replaced more easily assimilable soil nitrogen and was only slowly available to the rice. The decomposition of blue-greens in the presence of soil has been determined to vary widely among species (Gunnison and Alexander, 1975). Some species were visibly decomposed within one week, others not until after 4 weeks or more. Wilson et al. (1980) recovered from an initial rice crop grown in pots 36 percent of the nitrogen from ^{15}N-labeled Aulosira spread on the soil or 51 percent of the nitrogen when the blue-green was incorporated into the soil. Thus, it appears in this direct measurement that nitrogen in blue-green bacteria is readily available to rice.

Given the many alternate pathways that nitrogen from blue-green bacteria might take, it is conceivable that significant quantities do not directly reach the rice roots. However, much of what is not recovered in one growth cycle may be available to a subsequent crop.

Azolla

The genus Azolla is represented by 6 extant species found in different regions of the world (Becking, 1979). Unlike the case with blue-green bacteria, however, reports on the presence or absence of azolla in samples of paddy-field water are lacking.

The presence of abundant water is a requirement for survival of the vegetative plant. It can grow on wet mud, prefers shallow water, and dies upon complete drying of the soil (Becking, 1979). The biology and some agricultural features of Azolla have been recently reviewed (Becking, 1979; Lumpkin and Plucknett, 1980).

Several investigators have reported that azolla responded to phosphorus in the field (Singh, 1977; Talley et al., 1977; Watanabe et al., 1977a). Large quantities of phosphorus, however, were found to stimulate competing green algae and blue-green bacteria in dual culture of A. mexicana with rice (Rains and Talley, 1979). Potassium did not stimulate growth but did increase azolla-chlorophyll content in Indian field plots (Singh, 1977). Azolla was stimulated in the presence of added iron in field trials (Talley et al., 1977), and the requirement for iron seemed to rise with pH (Watanabe et al., 1977a; Olsen, 1970). Requirements for macro- and micronutrients doubtless depend on the characteristics of the soil in question, and precise amounts will have to be determined according to specific conditions.

Watanabe et al. (1977a) found that Azolla (pinnata ?) grew optimally at pH 5.5 in water culture, but they considered that the optimum pH for growth in flood water may be different. Dao and Tran (1979) reported the optimal pH for A. pinnata to vary from 4.5 to 7.5 depending on temperature, light intensity, and the presence of soil and soluble iron.

Summer temperatures greater than 30° are considered detrimental to the growth and survival of azolla (Watanabe et al., 1977a; Becking, 1979; Dao and Tran, 1979). Factors complicating this interpretation are the increase in salinity and pH of the paddy water as it evaporates. Continued cultivation of azolla during the summer has been carried out in ponds where water temperature and salinity may be moderated (Dao and Tran, 1979) or in water that has been carefully acidified (Galston, 1975).

In temperate climates, cold weather may limit azolla growth. Talley et al. (1977) found that A. filiculoides was frost hardy but that A. mexicana was not. On the other hand, Becking (1979) reported that both A. filiculoides and A. caroliniana were sensitive to long periods of frost.

The maximum growth rate of azolla has been reported at light intensities ranging from 500 to 58,000 lux (about 60 percent of full sunlight) and maximum nitrogen fixation at 5,000 to 47,000 lux (Lumpkin and Plucknett, 1980). High light intensity was shown to inhibit growth of A. filiculoides (Rains and Talley, 1979). However, high light intensities were found to be favorable to growth of A. pinnata at pH 5.0, and only inhibited growth at pH values above 6.0 (Dao and Tran, 1979). Clearly, the interaction of various physical and chemical factors needs to be explored in drawing conclusions on the effects of any given factor.

Wind may displace an azolla cover leading to clumping, decreased growth, and decomposition of the water fern. Rains and Talley (1979) recommended leaving rice stubble in fallow fields and Singh (1979a) raised embankments to limit clumping.

According to Singh (1977b, 1979b), insects, if not controlled, can damage an entire azolla crop in a few days, but he also observed that after a plant mat of azolla had formed, the pests could aid in decomposition. How to control pests to aid in the management of an azolla crop requires extensive study (Liu, 1979; Singh, 1979 a,b; Lumpkin and Plucknett, 1980).

Little information is available on nitrogen fixation by Azolla and factors limiting it in flooded fields. The amount of nitrogen fixed is generally taken as proportional to the yield of the fern. Moore (1969) estimated that newly fixed nitrogen is equal to one-half the total azolla nitrogen, and the same fraction has been used in this paper. However, no reports have been published in which the amounts of nitrogen fixed and of combined nitrogen assimilated from the paddy soil or water have actually been determined. Growth of azolla on combined nitrogen in the laboratory has been found to lead to a 15-30 percent reduction in nitrogenase activity over a short period of time and a 90 percent reduction when the azolla was grown on nitrate or urea for 6-7 months (Peters and Mayne, 1974; Becking, 1979). How combined nitrogen affects azolla's nitrogen-fixing activity under field conditions is unknown. Deficiencies of micronutrients needed specifically for nitrogen fixation, such as cobalt or molybdenum, have not been reported in paddy fields.

Comparisons of the rate or extent of fixation between species are few. Talley *et al*. (1977) measured a higher midday acetylene-reduction activity but lower nitrogen content for *A. mexicana* relative to *A. filiculoides*. This was atrributed to the possibility that *A. mexicana* excreted more nitrogen as it grew (since the rice yielded better when dual-cropped with *A. mexicana* than with *A. filiculoides*). Becking (1979) compared the acetylene-reduction activity of two species of *Azolla* over a wide temperature range. Under the experimental conditions used, *A. filiculoides* showed slightly greater activity than *A. pinnata* and had a broad temperature maximum from about 20° to 35°C, whereas the latter species showed a narrower maximum range of 25° to 35° C, but had greater activity at 40° C than did *A. filiculoides*. Exploiting these differences in fixation may depend also on the relative growth rates of species tested and their sensitivities to other environmental factors.

The above results point to the need for a) careful assessments of nitrogen fixation under field conditions, b) accounting for the effects of combined nitrogen on the rate and amount of growth and fixation, and c) establishing the pathways and amounts of nitrogen lost from the azolla.

What influences transfer of azolla nitrogen to rice plants has barely been studied. When incorporated into rice soil as a green manure, azolla is presumed to decompose and release nitrogen as does any other green manure. If so, the fresher the azolla, presumably the faster the release, and possibly the greater the loss of nitrogen from the paddy environment. Based on their nitrogen balance results, App et al. (1980) concluded that transfer to rice of the nitrogen from azolla incorporated into soil was quite rapid, although they presented no direct evidence. Other evidence of transfer of nitrogen from azolla to rice is also indirect and is based on increases in rice yields following azolla application (Singh, 1977, 1979a, 1979b; Talley et al., 1977; Watanabe et al., 1977a). Singh (1977a) found that azolla incorporated into flooded soils raised rice yields no more than azolla left unincorporated. He felt that incorporation of the fern could not be done properly in flooded fields and, when tried, led to large variations in rice yield. Very little information has been published on the residual effects of azolla incorporation on subsequent crops of rice (Singh, 1979b).

Growth of azolla in dual culture with rice has been advocated especially when a flooded fallow is not possible. Some evidence for excretion of nitrogen by azolla has been reported for A. mexicana but the absence of excretion seems to be the case for A. filiculoides and A. pinnata (Talley et al., 1977; Singh, 1979b). The possibility of nitrogen excretion needs further study in order to determine the agronomic value of dual culture.

Azolla and blue-green bacteria are often treated as no more than alternative sources of nitrogen fertilizer rather than as separate crops, as are the legumes. There is a need for comprehensive management procedures which include the use of varieties optimal for specific climates and soils, development of pest and disease resistant varieties, control of pests, and rotation cropping of rice with azolla.

Heterotrophic Bacteria

Presently, no way has been found to manipulate heterotrophic nitrogen-fixing bacteria to benefit the nitrogen nutrition of the rice plant.

Surveys of heterotrophic nitrogen fixers in rice fields have been carried out (Matsuguchi, 1979; Dommergues and Rinaudo, 1979). Watanabe et al. (1978b) found that NPK fertilization had no effect on numbers of heterotrophic fixers in rice soils, whereas Matsuguchi (1979) reported that applying phosphorus and lime together to a phosphorus-deficient, acid-sulphate soil in Thailand raised the numbers of these organisms. Matsuguchi also found that populations of nitrogen fixers often increased with soil fertility.

Compost and rice straw which stimulate acetylene-reduction activity by heterotrophs (Matsuguchi, 1979) probably also stimulate their reproduction. Carbon, the major limiting nutrient for heterotrophs, can be supplied by rice as root exudates, dead cells, dead roots, or straw. Carbon may also come from weeds and microfauna. In the attempt to exploit possible variations in amounts of carbon provided, many rice varieties have been compared for differences of acetylene-reduction activity in the root zone. Hirota et al. (1978) analyzed 50 varieties, and Habte and Alexander (1980) tested 16 varieties of rice. In both studies, substantial differences were found among varieties, and the high values for acetylene-reduction activity correlated with plants having a large root mass. In another study, Watanabe and Cholitkul (1979) found no superior varieties among 70 tested. These results suggest the possibility that rice may be bred for the support of high levels of nitrogen fixation in the rhizosphere. Evidence that nutrients other than carbon stimulate heterotrophic fixation is lacking.

The availability of nitrogen gas is thought not to limit fixation (Yoshida et al., 1975), but this has not been directly tested. Reports on the effect of combined nitrogen on acetylene-reduction activity in the rhizosphere are contradictory (Balandreau et al., 1975; Trolldenier, 1977; Watanabe and Cholitkul, 1979). Thus, it is unclear how nitrogen fertilizers or the mineralization of soil nitrogen might affect heterotrophic nitrogen-fixing activity.

If rhizosphere-nitrogen fixers actually live external to the rice root, then the transfer of nitrogen to the rice probably follows the same pathways as nitrogen from any organic source. If these organisms actually invade the root, however, the movement of nutrient into the rice plant may well be much simpler and more direct than the incorporation of nitrogen from a green manure. Indirect evidence for such an intimate relationship in grasses has been found (van Berkum and Bohlool, 1980).

Conclusions

1. Biologically fixed nitrogen can provide as much as 100 kg N ha^{-1} or more per crop of rice. Such large amounts of nitrogen have been obtained by growing legumes or the water fern azolla as green manures on separate land or in rotation with rice and incorporating these manures into paddy fields, or by growing azolla as a companion crop with the rice. As much as a third of the amount of nitrogen available from the green manures has been provided by inoculating rice fields with small quantities of nitrogen-fixing blue-green bacteria.

2. One hundred kg N is sufficient to replace the nitrogen contained in about 10,000 kg of grain. However, yields as high as 10,000 kg ha^{-1} per crop have not yet been achieved simply using biological sources of nitrogen. Yields of grain of about 5,000-6,000 kg ha^{-1} per crop or more have been achieved using legumes, azolla, or blue-greens. How these yields depend on soil fertility, whether they can be maintained or improved upon over the long term, and the social and ecological costs of managing the crops of nitrogen fixers in conjunction with rice remain to be examined. In India, the economics of inoculating blue-green bacteria into flooded rice fields appear to be favorable.

3. Other potential sources of biological nitrogen fixation, such as heterotrophic, rhizosphere bacteria and the photosynthetic bacteria, have not yet been knowingly manipulated to affect rice production. Evidence indicates that heterotrophic nitrogen-fixing bacteria may provide as much as 30 kg N ha^{-1} per rice crop, and that rice varieties with the largest root systems probably maintain the most active populations of these bacteria. How these microorganisms might be managed other than by choice of rice variety is unknown.

4. Losses of nitrogen from flooded rice fields by runoff, leaching, denitrification, or ammonia volatilization limit the efficiency of added industrially- or biologically-produced fertilizer. Some data are available on losses when ammonium sulfate or urea are used. Almost no information exists on nitrogen lost from rice fields following introduction of green manures or inoculation of azolla or blue-green bacteria. Limited evidence from laboratory experiments indicates substantial losses of nitrogen from flooded soil amended with manures or rice straw. Determination of management practices which reduce these losses is critical for the practical utilization of biologically fixed nitrogen for rice.

5. Factors limiting the growth of legumes and fixation of nitrogen by the legume-Rhizobium symbiosis have received some study in farming systems involving flooded rice. For example, species of legumes can be chosen for cultivation based upon tolerance to flood or drought stress. Rhizobial populations do not seem to be seriously damaged by flooding.

6. Factors which may limit growth and nitrogen fixation by blue-green bacteria and azolla include phosphorus, molybdenum, soil pH, wind, light intensity, predators and competitors. However, information on the influence of these factors under field conditions and the interaction among them is sparse.

7. Even less information is available on what may influence the transfer of nitrogen from the fixing organisms to the rice plant and how the farmer can control this transfer for maximum crop yield.

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Table 1. Sources of exogenous fixed nitrogen in flooded rice fields (kg N ha^{-1}).

Source	Location	Amount	Time	Reference
Rain	Ceylon	14.8	yr	Grist, 1975
	Gambia	13.4-47	yr	Grist, 1975
	India	33.4	yr	Vijayalakshmi and Pandalay, 1962
	Japan	3.4-17[a]	yr	Yatazawa, 1977
	Nigeria	56.9	yr	Jones, 1960
	Nigeria	2.5-4.6	yr	Jones and Bromfield, 1970
	Philippines	6	yr	Koyama & App, 1979
	Vietnam	61.5	yr	Eriksson, 1952
Flood water	Java	10	Crop	Grist, 1975
	Japan	16.5	yr	Yatazawa, 1977
Silt	USSR	1.1	yr	Grist, 1975
	Japan	18.8	Crop	Grist, 1975
Atmospheric NH_3	USA	50-67[b]	yr	Hanawalt, 1969

[a]The average was 5 kg N ha^{-1}.

[b]This line is included for comparison. The values represent atmospheric ammonia absorbed by dry New Jersey soils and are estimates from Hanawalt's figures by the present author for average world ambient NH_3 levels.

Table 2. Legumes as nitrogen source in flooded rice fields (kg N ha^{-1} $crop^{-1}$).

Location	Plant	Amount	Reference
India	*Sesbania sirecea*	146	Ghose et al., 1956
	Crotolaria juncea	105	
	Sesbania aculeata	96	
	Vigna sinensis	50	
Philippines	*Sesbania sesban*[a]	202	Anon., 1964
	Crotolaria juncea[a]	129	
	Sesbania aculeata[a] No. 72	122	
	Sesbania aculeata[a] No. 71	104	
Philippines	*S. sesban*[b]	100	Anon., 1965
	S. aculeata[c]	75	
	S. aculeata[a]	100	

[a]Grown under dry land conditions.

[b]Flooded or dry land conditions.

[c]Flooded conditions.

Table 3. Blue-green bacteria as nitrogen source in flooded rice fields (kg N ha^{-1}).

Location	Treatment	Method	Amount fixed Uninoc.	Amount fixed Inoc.	Time	Reference
India	Field inoculated with *Aulosira fertilissima*	?	-	53	1 crop	Singh, 1961
India	Soils cropped in laboratory--Sonarpur soil	Disappearance of N_2 gas	15	-	5-6 wk	De and Mandal, 1956
	Chinsura soil		79	-	5-6 wk	
India	Scraping of algal incrustations on rice field	Kjeldahl	14	-	1 crop	Prasad, 1949
Ivory Coast	Flooded soils in laboratory[a]	ARA[b]	9-16.6[c]		1 day	Rinaudo et al., 1971
		Kjeldahl	14-14.7		1 day	
Ivory Coast	Rice field	ARA	0.33		1 day	Balandreau et al., 1974
Japan	Rice field inoculated with *Tolypothrix tenuis*	Kjeldahl	45	71	1 crop	Hirano, 1958
Mali	Rice field	ARA	56-78		1 crop	Traore et al., 1978
Nigeria	In culture	ARA	-	3-30	year	Stewart et al., 1978
Philippines	Rice fields	ARA			1 crop	Alimagno and Yoshida, 1977
	Puro soil		18.5-33.3			
	Santo Domingo soil[d]		2.3-5.7			
Philippines	Rice fields					Watanabe et al., 1978
	Wet season	ARA[g]			216 days	
	Unfertilized		14.0			
	Fertilized[e]		10.8			
	Dry season	ARA[g]			119 days	
	Unfertilized		11.1			
	Fertilized[f]		3.7			
	Dry season	Biomass estimate[h]	10		119 days	

Table 3.--continued

Location	Treatment	Method	Amount fixed		Time	Reference
			Uninoc.	Inoc.		
Philippines	Rice fields ± NPK fertilizer	ARA	0.034[i]	0.17[i]		Watanabe & Cholitkul, 1979
Philippines	Greenhouse	Kjeldahl	20[j]	27[j]	1 crop	App et al., 1980
Thailand	Rice fields	ARA			1 day	Watanabe & Cholitkul, 1979
	No NPK fertilizer		0.0065-0.019[i]	0.047-0.12[i]		
	+ NPK fertilizer		0.011-0.021[i]	0.10-0.23[i]		
Senegal	Soil cores	Biomass estimate[k]	0.4-18.0[l]		1 crop	Reynaud & Roger, 1978

[a]2 grams each of three soils were incubated for 30 days.

[b]ARA is acetylene reduction activity. To estimate the equivalent amount of nitrogen fixed, the ratio three molecules ethylene produced to one molecule N_2 fixed was used unless otherwise indicated.

[c]Calculated by present author from investigators' figures using 2 X 10^6 kg soil ha^{-1}.

[d]Santo Domingo soil received 55 kg ha^{-1} of fertilizer N.

[e]NPK was added at 60:30:30 kg ha^{-1}.

[f]NPK was added at 30:30:100 kg ha^{-1}.

[g]Ratio of ethylene produced to N_2 fixed was 4:1.

[h]Biomass estimated at 2400 kg ha^{-1} fresh, and total N estimated as 0.04% of that. All N was assumed to result from nitrogen fixation.

[i]In this case blue-greens were not inoculated, but rather the figures in the inoculated column represent nitrogen fixed by the natural population, and in the uninoculated column represent fixation when blue-greens had been physically removed.

[j]Calculated by present author by averaging relevant extrapolations from Table 10 and subtracting the average amount of nitrogen fixed in rhizosphere.

[k]Total N estimated as 0.8% of fresh weight.

[l]Range of 17 cores.

Table 4. Azolla as a source of nitrogen in flooded rice fields (kg ha^{-1})[a]

Location	Treatment	Method	Amount fixed	Time	Reference
China	Field, A. pinnata				Liu, 1979
	Dual cropped	ARA[b]	300	year	
	Green manure	Biomass estimate[c]	540-675	year	
India	Field, A. pinnata				Singh, 1979a
	Monthly inoculation with weekly azolla harvest	ARA	840[d]	year	
	Spring inoculation	Biomass estimate[e]	45[f]	23 days	
Indonesia	Laboratory, A. pinnata[g]	ARA	103-162	year	Becking, 1976
Philippines	Field, Azolla sp.	Biomass estimate[h]	117[i]	106 days	Watanabe et al., 1977
U. S.	Field	Kjeldahl N			Talley et al., 1977
	Standing crop			(?)	
	A. mexicana		29		
	A. filiculoides		58-105		
	Inoculum			35 days	
	A. mexicana		39.6[j]		
	A. filiculoides		50.8[k]		
U. S.	Field inoculum, A. filiculoides	Kjeldahl N	50.5-91.5[l]	46 days	Rains and Talley, 1979
Vietnam	Field, A. pinnata as green manure	Kjeldahl N	25	2 months	Dao and Tran, 1979

[a] In a review of azolla in the rice system, Moore (1969) gave the range of nitrogen actually fixed as 90-310 kg ha^{-1} per season calculated mainly on fresh weight.

[b] ARA: acetylene reduction activity. See footnote "b", Table 3

[c] Total N estimates as 0.3% of fresh weight of azolla.

[d] Nitrogen in total harvest (1250 kg ha^{-1}) less nitrogen in sum total inoculum (410 kg ha^{-1}).

[e] Total N estimated as 0.2-0.3% of azolla fresh weight.

[f] Nitrogen in total harvest (50 kg ha^{-1}) less nitrogen in the sum total inoculum (5 kg ha^{-1}).

[g] Measurements made on log phase azolla corrected for field conditions.

[h] The sum of 5 harvests. Total N estimated as 0.17-0.27% of fresh weight of azolla.

[i] Nitrogen in total harvest (133 kg ha^{-1}) less nitrogen in total inoculum (16.3 kg ha^{-1}).

[j] Nitrogen in total harvest (41 kg ha^{-1}) less nitrogen in total inoculum (1.4 kg ha^{-1}).

[k] Nitrogen in total harvest (52 kg ha^{-1}) less nitrogen in total inoculum (1.2 kg ha^{-1}).

[l] The range of nitrogen harvested in azolla less nitrogen in total inoculum (1.5 kg ha^{-1}).

Table 5. N_2 fixation in soil and rhizosphere of rice fields (kg ha^{-1}).

Location	Treatment	Method	Amount fixed	Time	Reference
Ivory Coast	Soil in laboratory[a]			day	Rinaudo et al., 1971
	Rhizosphere	ARA[b]	3.7-10.2[c]		
		Kjeldahl	11.2-18.2		
	Nonrhizosphere	ARA	0.002-0.004		
		Kjeldahl	7.4-16.6		
Ivory Coast	Field	ARA	64[d]	100 days	Balandreau et al., 1975; Balandreau, 1975
Philippines	Field	ARA			Yoshida and Ancajas, 1973b
	Wet season				
	Flooded			17 weeks	
	Planted		57		
	Unplanted		22		
	Unflooded			19 weeks	
	Planted		7		
	Unplanted		3		
	Dry Season				
	Flooded			17 weeks	
	Planted		63		
	Unplanted		28		
	Unflooded			19 weeks	
	Planted		5		
	Unplanted		3		
Philippines	Field[e]	ARA	0.1	day	Watanabe and Lee, 1977
Philippines	Field[e]	ARA	0.052	day	Watanabe et al., 1977b
Philippines	Field[e]	ARA			Watanabe & Cholitkul, 1979
	IR26		5.9	107 days	
	IR107		9.8	95 days	
Philippines	Greenhouse	Kjeldahl	6-[illegible][f]	crop	App et al., 1980
Thailand	Field	ARA	0.0065-0.021	day	Watanabe & Cholitkul, 1979
United States	Growth chamber, Planted	$^{15}N_2$	2.6	13 days	Eskew et al., 1981

Table 5.--continued

[a]Six grams soil in test tubes for 2-3 weeks.
[b]ARA: acetylene reduction activity; see footnote "b", Table 3.
[c]Extrapolation of investigators' figures by present author using 2×10^{6} kg ha^{-1} soil.
[d]Includes blue-greens.
[e]Planted to rice, blue-greens removed.
[f]Calculated by present author by extrapolating from experiments A and B of App et al., (1980) (Table 10).

Table 6. Summary of exogenous and biologically fixed nitrogen for paddy fields[a]

Source	Range (kg ha^{-1} per rice crop)
Exogenous[b]	15-61
Legumes	25-101
Blue-greens	0.2-39
Azolla	
Green manure	25-121
Dual crop	8-75
Soil and rhizosphere N_2 fixers	1.2-18.3

[a]For purposes of comparison, the amount of nitrogen fixed was calculated for one-third of a year (as an average rice-cropping season), unless the original authors reported values of nitrogen fixed for a rice crop.

[b]Exogenous nitrogen as in Table 1 includes rain, floodwater, silt, and atmospheric ammonia.

Table 7. Sinks for nitrogen in flooded rice fields (kg N ha^{-1} per season).

Sink	Location	Treatment	Method	Nitrogen removed or lost	Reference
Rice crop	India?	Grain[a]	Total N	23	Grist, 1975
		Straw		22	
	Indonesia, Japan, Philippines, Thailand	Grain plus straw			Watanabe, 1978
		No N added	Total N	37-113	
		Labeled N fert.	^{15}N	30-113	
	Philippines	Grain[b]	Total N	47	Tanaka et al., 1964
		Straw		43	
	Philippines	Grain plus straw	Total N	40-60	Watanabe et al., 1977b
Denitrification	Japan	No fertilizer N added	^{15}N	46	Yatazawa, 1977
Ammonia volatilization	Japan	Nitrogen fertilizer[c]	^{15}N	68	Yatazawa, 1977
Leaching	Japan	No fertilizer	Lysimeter	6.2-28	Yatazawa, 1977

[a]Grain yield, 2.4 tons ha^{-1}.

[b]Grain yield, 4.74 tons ha^{-1}.

[c]50 kg ha^{-1} nitrogen.

Table 8. Nitrogen balance experiments of Willis and Green (1948) in Louisiana, United States, using pots with 6 kg soil, flooded, in greenhouse for one season.

Soil and amendment	N_o (mg)	Nitrogen change (mg pot^{-1})[a]							
		Unplanted Soil		Planted soil					
		Soil		Soil		Plants		Plants + Soil	
		$+CuSO_4$	$-CuSO_4$	$+CuSO_4$	$-CuSO_4$	$+CuSO_4$	$-CuSO_4$	$+CuSO_4$	$-CuSO_4$
Silt loam									
unfertilized	5030	+43	+50	+68 C	+132 B	+205 C	+166 C	+273	+298
+PK[b]	5030	+184	+166	+219 A	+210 A	+254 B	+265 B	+473	+475
+NPK[c]	5320	-98	-273	+53 C	+16 C	+367 A	+333 A	+420	+349
Clay loam									
unfertilized	4310	+50	+41	+113 B	+224 A	+122 B	+132 B	+235	+356
+PK[b]	4310	-36	-58	+249 A	+169 B	+166 B	+144 B	+415	+313
+NPK[c]	4590	-245	-77	+243 A	+135 B	+244 A	+229 A	+457	+364

[a]Nitrogen changes for a given soil or for plants within a given soil followed by a common letter do not differ significantly at $P < 0.05$ (present author's calculations). For nitrogen increases in plants + soil, 5 mg (silt loam) or 6 mg (clay loam) should be subtracted for seedling N. $CuSO_4$ was added at 1.5 ppm to eliminate blue-greens. N_o is the initial soil nitrogen.

[b]PK fertilization was 897 kg ha^{-1} soil of 0-12-12 or 48 mg kg^{-1} soil each of P_2O_5 and K_2O.

[c]NPK fertilization was 897 kg ha^{-1} soil of 12-12-12 or 48 mg kg^{-1} soil each of N, P_2O_5 and K_2O.

Table 9. Nitrogen balance experiments of De and Sulaiman (1950b) in India using wide-mouthed bottles with 2.27 kg of soil. Values represent the average of 5 years.

Soil	N_o (mg)	Nitrogen change (mg $bottle^{-1}$ $year^{-1}$)					
		Soil		Grain + straw		Soil + grain + straw[b]	
		Dark[a]	light	Dark	Light	Dark	Light
Faridpur	1480						
H_2O		-34.0	+13.6	+40.0	+43.0	+6.0	+56.6
Nutrients[c]		-20.4	+84.4	+45.7	+71.9	+25.3	+156.
Tippera	2590						
H_2O		-56	+18.2	+73.9	+82.3	+17.1	+101
Nutrients		+36.4	+99.8	+82.2	+126.	+45.8	+226

[a]"Dark" soil was covered with black paper.

[b]Present author's calculations made assuming N was 0.6% of grain plus straw (see Grist, 1975).

[c]Nutrient solution: 1500 ml of 0.5 g K_2KPO_4, 0.2 g $Mg_4 \cdot 7H_2O$, 1.0 g $Ca_3(PO_4)_2$, 0.1 g $CaSO_4$, 0.1 g $FePO_4$ per liter of water.

Table 10. Nitrogen balance experiments of App. et al. (1980) in the Philippines using pots with 10 kg soil continuously flooded.

Experiment number	Crops	Treatment	Nitrogen change (mg pot^{-1} crop^{-1})[a]							
			Soil		Grain+straw+root		Misc. inputs		Soil+grain+straw+root	
			Dark[c]	Light	Dark	Light	Dark	Light	Dark	Light
1	4	Planted	-199a	-	+249a	-	5.3	-	+45a (18%)[d]	-
		Planted, stubble removed	-219	-	+261a	-	5.3	-	+37a (14%)	-
		Fallow	-61b	-	0b	-	0.0	-	-61b	-
2	6	Planted	-160b	-101a	+191a	+196a	4.3	4.5	+27b (14%)	+91a (46%)[e]
		Fallow	-	+32c	-	-		4.0	-	+28b
3	6	Planted	-	-132a	-	+201b		4.5	-	+65c (32%)
		Planted + Fe + P	-	-149b	-	+202b		15.3	-	+121b (60%)
		Planted + Fe + P + blue-greens[f]	-	-44b	-	+212b		16.3	-	+152ab (72%)
		Planted + Fe + P + azolla[g]	-	-70b	-	+280a		17.7	-	+192a (69%)

[a] Nitrogen changes for a given experiment followed by a common letter do not differ significantly at $P < 0.05$.

[b] Nitrogen increases for soil + grain + straw + root are all significantly different from zero except experiment 2--fallow.

[c] "Dark" soil was covered with black cloth.

[d] Values in parentheses are the ratio of the increased nitrogen in the total system to total crop N times 100%.

[e] Investigators calculated that the incremental amount in this case would be equivalent to approximately 2.1 kg nitrogen per hectare-furrow slice.

[f] *Anabaena*, *Gloeotrichia*, and *Rivularia*.

[g] *Azolla pinnata*.

Table 11. Nitrogen balance sheet for lowland rice, compiled by Yatazawa (1977) from many studies done in Japan (in kg $ha^{-1} yr^{-1}$).

Input		Amount
Manure		20
Fertilizer		96
Fixation		40
Irrigation H_2O		17
Dry and wet deposition		5
	Total	178
Removal		
Denitrification		70
Leaching		20
Runoff		1
Plant		96
	Total	187
	Net	-9

Table 12. Qualitative comparison of nitrogen balance experiments in flooded rice[a].

		Change of N in					
		Flooded soil			Soil + plant		
Crop	Light[b]	Willis & Green	De & Sulaiman	App et al.	Willis & Green	De & Sulaiman	App et al.
+	+	Inc.[c]	Inc.	Dec.	Inc.	Inc.	Inc.
+	-	ND	Dec.	Dec.	ND	Inc.	Inc.
-	+	Var.	ND	NSD	Var.	ND	NSD
-	-	ND	ND	Dec.	ND	ND	Dec.

[a]These are from experiments of Willis and Green (1948); De and Sulaiman (1950b); App et al. (1980).

[b]Light allowed to reach soil or not.

[c]The following abbreviations are used: Inc. = increase; Dec. = decrease; ND = not done; NSD = no significant difference; Var. = variable results.

Table 13. Rice yields (kg ha^{-1}) as influenced by incorporation of green manures (amount added kg ha^{-1}).

Location	Green manure	Amount added	Soil amendment	Rice grain yield No manure	Rice grain yield + manure	Reference
Ceylon	?	6300		1900	2300	Staker, 1958
India	<u>Crotolaria juncea</u>	6700		3010	3220	Ghose et al., 1956
	<u>Ipomea carnea</u>[a]	?		2970	3760	
	<u>C. juncea</u>	6700		942	2020	
	<u>Sesbania aculeata</u>	?		2060	2570	
India	<u>S. aculeata</u>	12 wks' growth		3140	3520	Staker, 1958
India	<u>S. aculeata</u>	?	None	2470[c]	3580	Relwani & Ganguli, 1959
			$(NH_4)_2SO_4$ (22.4)[b]	3030	3640	
			$(NH_4)_2SO_4$ (44.8)	3210	3680	
Java	<u>C. juncea</u>	1520		3840	4570	Staker, 1958

[a] <u>Ipomea carnea</u> is a non-legume.

[b] In parentheses: kg ha^{-1} of nitrogen.

[c] Calculated by the present author from the investigators' values using 181 kg per maund.

Table 14. Rice yields as influenced by inoculation with blue-green bacteria (kg ha^{-1}, except as noted).

Location	Blue-green	Rice variety	Field or pots	Amendment or treatment	Rice grain yield: No blue-greens	Rice grain yield: Blue-greens	Reference
Egypt	Tolypothrix tenuis[a]		Field[b]	None	20.8[c]	23.1	Abou Fadl et al., 1967
				P (15)[d]	21.3	23.7	
				N (10)	22.7		
				NP (10;15)	24.8		
				N (20)	22.4		
				NP (20;15)	25.3		
	Tolypothrix tenuis		Field[e]	None	13.1	17.5	
				P (15)	17.9	17.0	
				N (10)	16.8		
				NP (10;15)	19.5		
				N (20)	17.2		
				NP (20;15)	18.4		
India	Aulosira fertilissima		Pots		(100%)	(468%)	Singh, 1961
			Field		(100%)	(215%)	
India	Aulosira fertilissima	NP130	Pots	None	0.28[f]	1.2	Sundara Rao et al., 1963
				$(NH_4)_2SO_4$ (44.6)	3.57	11.3	
India	Nostoc sphaericum + N. amplissimum + Tolypothrix campylonemoides + Westiella sp.	T141	Field	Basal lime, P, Mo (500; 20; 0.28)	2620	3400	Subrahmanyan et al., 1964
				Farmyard manure at N (20)	3390	3540	
				Green manure, Sesbania speciosa at N (20)	3910	3900	
				$(NH_4)_2SO_4$ (20)	3430	3472	
				Urea (20)	3370	3590	

Table 14.--continued

Location	Blue-green	Rice variety	Field or pots	Amendment or treatment	Rice grain yield: No blue-greens	Rice grain yield: Blue-greens	Reference
India	Nostoc spp.	T141 &	Field	None	1540	1810	Subrahmanyam
	+ Tolypothrix sp.	Ptb. 10		Rabbing[h]	2060	2160	et al., 1965[g]
	+ Westiella sp.			Lime,P,Mo (1000;20 0.28)	2240	2800	
				Rabbing+lime,P,Mo	2560	1860	
				$(NH_4)_2SO_4$ (?)	2100		
India	Tolypothrix tenuis[i]		Field	None[j]	2190	3010	Jha et al., 1965
				P (45)	2270	3190	
				None[k]	1830	2490	
				P (45)	1830	2860	
India	Aulosira fertilissima	ASDS	Field	Basal green manure[l]	5860		Venkataraman &
	+ Tolypothrix tenuis			P,Mo (112;0.25)	6450	7470	Goyal, 1968
	+ Cylindrospermum muscicola						
	+ Nostoc sp.						
India	Aulosira fertilissima	IR8	Field	Basal PK (39.7; 50.5)	2760	3370	Venkataraman &
	+ Tolypothrix tenuis						Goyal, 1968
	+ Cylindrospermum muscicola			$(NH_4)_2SO_4$ (112)	3570	4010	
	+ Nostoc sp.						
India	Aulosira fertilissima	ADT27	Pots	Basal N (100)	15.8[f]	21.2	Venkataraman &
	+ Tolypothrix tenuis	IR8			13.9	18.9	Goyal, 1970
	+ Cylindropermum muscicola	TN1			13.5	21.6	
	+ Nostoc sp.	TKM6			13.2	22.7	

Table 14.--continued

Location	Blue-green	Rice variety	Field or pots	Amendment or treatment	Rice grain yield No blue-greens	Blue-greens	Reference
India	*Anabaena torulosa* or Nostoc-4	D6-22	Field	Basal PK + *Anabaena*[i]	2180	2290	Thomas, 1977
				Nostoc[i]		3170	
				Anabaena+Nostoc[i]		2640	
Japan	*Tolypothrix tenuis*		Fields[m]	Basal slaked lime (75)	2890	3580	Watanabe, 1962
Japan					no increases		Watanabe, 1973
Japan			Field	None	7.54[n]	7.76	Yamaquchi, 1978
				Nitrogen (?)	9.82	9.64	
Taiwan	*Anabaena cylindrica*	Tainan	Field	Basal PK(148;148)	14.4[o]	14.1	Huang, 1978
				N (198)	17.6	16.3	
		Chin Hsin		Basal PK(148;148)	15.1	15.5	
				N (198)	19.1	18.5	

[a]0.1 kg feddan^{-1}.

[b]A pH 7.4 soil. Rice crop followed horsebean.

[c]Yield given in units of ardeb feddan^{-1}.

[d]In parentheses is given the amount of amendment. In experiments of Abou Fadl et al., the units are kg feddan^{-1}; in all the rest units are kg ha^{-1}.

[e]A pH 8.3 soil. Rice crop followed wheat.

[f]Units of gram per pot.

[g]These data are essentially the same as in Sankaram et al., 1967.

[h]Rabbing is burning of topsoil.

[i]Incorporated into soil

[j]1962-1963.

[k]1963-1964.

[l]9075 kg ha^{-1} dry weight.

[m]Summary of results from 8 agricultural experiment stations and 3 universities. Average of 5 years.

[n]Given in units of kg per plot.

[o]Units of gram per plant.

Table 15. Rice yield (kg ha^{-1}) as influenced by inoculation or incorporation of azolla (amount added as fresh weight in tons ha^{-1}).

Location	Season	Rice variety	Azolla species	Amount added[a]	How applied	Soil amendment	Rice yield No azolla	Rice yield + azolla	Reference
China			A. pinnata	21.4	Green manure & dual incorp.		3720	5000	Liu, 1979
India			A. pinnata		Green manure				Singh, 1977a
	Dry	IR8		10(20-25)[b]	incorp.		4720	5920	
		Supriya		10	incorp.		3490	5130	
		Kalinga-2		10	incorp.		1720	2420	
				10	unincorp.			2400	
				20(40-50)	incorp.			2620	
India			A. pinnata		Green manure				Singh, 1979b
	Wet	Vani		5	incorp.		2300	2500	
				10	incorp.			2780	
				15	incorp.			3000	
				20	incorp.			2560	
				3	unincorp.			3560	
				4.5	unincorp.			3140	
				6	unincorp.			2930	
				5	incorp.	N(20)[c]	2500	2590	
				10	incorp.	N(40)	2720	2830	
				-		N(60)	3140		
				-		N(80)	2830		
	Dry			5	incorp.		2620	3230	
				10	incorp.			4030	
				15	incorp.			4200	
				20	incorp.			4420	
				3	unincorp.			3350	
				4	unincorp.			3380	
				6	unincorp.			3240	
				5	incorp.	N(20)	3420	4030	
				10	incorp.	N(40)	4140	5020	
				-		N(60)	4560		
				-		N(80)	5410		

Table 15.--continued

Location	Season	Rice variety	Azolla species	Amount added[a]	How applied	Soil amendment	Rice yield: No azolla	Rice yield: + azolla	Reference
Philippines		IR30	*A. pinnata*?	0.5	dual culture				Watanabe et al., 1977a
					unincorp.		1480	1850	
					incorp.		2360	2160	
					unincorp.	P(32)[c]	1860	2020	
					incorp.	P(32)	2250	2530	
Vietnam,			*A. pinnata*						Le Van Kan & Sobachkin, 1963, in Mishustin & Shil'nikova 1971
Hai Duong	1958						2670	3130	
Ha Tinh	1960						2690	3470	
Haiphong	1961						3410	3610	
Nam Dinh	1960						1640	1750	
	1961						2330	2660	
United States		Calrose 76	*A. filicu.*[d]	(30)	dual culture		1270	1560	Talley et al., 1977
			A. mex.[e]	(38)	dual culture			2120	
			A. filicu.	(60)	incorp.[f]			2740	
			A. filicu.		incorp.[f] & dual			3970	
			A. filicu.+ *A. mex.*		incorp. & dual			3470	
United States			*A. filicu.*	(40)	incorp.[f]		1300[g]	2900	Rains & Talley, 1977
			A. filicu.		dual culture			1630	
			A. mex.		dual culture			3900	
			–			N(40)	2900		
			–			N(80)	4800		

Table 15.--continued

[a]In parentheses is N content of azolla in kg ha^{-1}.

[b]Nitrogen content of azolla was 0.2-0.25% of fresh weight, or about 20-25 kg N per 10 ton fresh weight of azolla.

[c]Nitrogen fertilizer applied as $(NH_4)_2SO_4$. In parentheses amount of N (or P) in kg ha^{-1}.

[d]*A. filiculoides*

[e]*A. mexicana*

[f]Azolla grown separately as a green manure.

[g]These values are the present author's estimates from the investigator's graph.