ESTIMATION OF BIOLOGICAL N_2 FIXATION AND ITS CONTRIBUTION TO NITROGEN BALANCE IN WETLAND RICE FIELDS

P. A. Roger* and J.K. Ladha

International Rice Research Institute (IRRI), PO Box 933, Manila, Philippines
* Institut Français de Recherche Scientifique pour le Developpement en Cooperation (ORSTOM), Visiting Scientist at IRRI

INTRODUCTION

Flooding ricefields leads to the differentiation of a range of macro- and micro-environments differing by their redox, physical properties, light status, and nutrient sources for the microflora. As a result, all N_2-fixing groups can grow in ricefields: photosynthetic bacteria and blue-green algae (BGA), indigenous heterotrophic bacteria in soil and associated with rice, and introduced *Azolla* and legumes for green manure. Biological N_2 fixation (BNF) in ricefields and its use were reviewed by Watanabe & Roger (1984) and Roger & Watanabe (1986). Specific reviews deal with BGA (Roger 1990), heterotrophs (Yoshida & Rinaudo 1982), BNF associated with straw (Ladha & Bonkerd 1989), rice genotypic differences in stimulating BNF (Ladha et al. 1988c), *Azolla* (Watanabe 1982), and legume green manures (Ladha et al. 1988b). This paper reviews recent improvements and new methodological approaches to estimate BNF in wetlands, summarizes earlier and recent quantitative estimates, and briefly discusses research needs.

IMPROVEMENTS AND NEW METHODOLOGICAL APPROACHES

Acetylene reducing activity (ARA)

Recent studies confirm limitations that may make quantitative extrapolations risky. ARA was linear with time with aquatic legumes (Ladha et al. 1988a) but not with associative (Barraquio et al. 1986) and algal BNF (Roger et al. in press). C_2H_2/N_2 conversion factor varied from 1.6 to 7.9 with *Azolla*, depending on species, P_{N_2}, assay duration, and age of culture (Eskew 1987). It varied from 3.9 to 30 with dense algal mats, mostly depending on P_{N_2} used for incubation under ^{15}N, but when P_{N_2} was close to that of air and all other factors were close to those used for C_2H_2 exposure, C_2H_2/N_2 ratio was close to the theoretical value of 4 (Roger et al. in press). Most incubations are done under 10% C_2H_2 in air, but ARA increases with up to 25% C_2H_2 in air with thick BGA blooms (Roger et al. in press) and associative BNF (Barraquio et al. 1986). *In situ*, the greenhouse effect in enclosures used for incubation reduced photodependent ARA of soil (Roger et al. in press) and *Azolla* (Li et al. 1987). Despite its limitations, ARA was used in about 2/3 of the 38 quantitative BNF studies related to rice published since 1985.

To overcome some limitations and obtain reproducible values, methods have been developed where composite and/or standardized samples collected *in situ* are incubated under controlled laboratory conditions (Roger et al. in press; Barraquio et al.

1986). In field studies, emphasis has been on sampling methods. Using 400 groups of four and five replicated ARA measurements in plots with various agronomic [illegible] than air, is advantageous because of the stable isotopic composition of N sources. But *in situ*, a ^{15}N gradient observed with soil depth is a serious source of error. Growing plants in pots avoids this problem.

QUANTIFICATION OF BNF BY VARIOUS AGENTS

Heterotrophic BNF

Total heterotrophic BNF estimated from the N balance in unfertilized planted pots covered with black cloth averaged 36 mg N crop^{-1} pot^{-1} or 7 kg N ha^{-1} App et al. (1980). In similar trials, Trolldenier (1987) found balances negatively correlated with the amount of N applied and ranging from - 440 to + 418 mg N crop^{-1} pot^{-1}. Extrapolated values averaged 19 kg N ha^{-1} crop^{-1} with 65 kg N ha^{-1}, - 0.3 with 112 kg N, and -14 with 146 kg N. Using available N of a stabilized ^{15}N-labelled soil as control, Zhu et al. (1986) estimated that, when no N-fertilizer was applied and photodependent BNF was

[redacted]

Blue-green algae

[redacted]

N'Doye & Dreyfus (1988) for 53- to 63-d-old *S. rostrata*, probably because they used an uninoculated *S. rostrata* as control. A few estimates of BNF by *S. rostrata* as a prerice LGM are available from small-scale balance studies. Rinaudo et al. (1988) reported a gain of 267 kg N ha^{-1} after incorporating a 52-d crop. In a [45-d Sesbania-rice (WS)/55-d *Sesbania*-rice (DS)] sequence, Ladha et al (1988a) estimated that *Sesbania* fixed 303 kg N ha^{-1} year^{-1} when uninoculated, and 383 kg N when inoculated with *Azorhizobium*.

N BALANCE AND BNF CONTRIBUTION IN WETLAND RICE

N balances in long-term fertility experiments listed by Greenland & Watanabe (1982) range from 19 to 98 kg N ha^{-1} crop^{-1} (average 51) in 9 fields with no N fertilizer. In 4 fields with N fertilizer, the average is -1.5 kg N ha^{-1} crop^{-1}. With rice grown in alternation with an upland crop, the average was 44 kg N ha^{-1} year^{-1} in 3 fields with no N fertilizer and -29 kg N in 2 fields with applied urea. At two Philippine sites, App et al. (1984) found no decrease in total soil N after 24 and 17 crops. Calculations based on yields and known inputs suggested that two crops year^{-1} resulted in balances equivalent to 79 and 103 kg N ha^{-1} year^{-1}.

Balance studies in the field encounter additional difficulties, as compared with pot experiments, because of sampling errors, unaccounted subsoil contribution, and losses by leaching. Therefore, there is renewed interest in pot studies (App et al. 1986, Santiago Ventura et al. 1986, Singh & Singh 1987, Trolldenier 1987). In a 4-crop experiment comparing organic N (*Azolla* and BGA) and urea, Singh & Singh (1987) found N gains ranging from 13 to 163 mg N crop^{-1} pot^{-1}. Gains were highest (133-163 mg N crop^{-1} pot^{-1}) in pots that received 60 kg organic N ha^{-1}, and lowest (13-29) in pots that received 30-60 kg N ha^{-1} as urea. Gain in the control was 51 mg N crop^{-1} pot^{-1}. In a second experiment comparing the effects of soil exposure to light, presence of rice, and flooding in nonfertilized plots, N gains ranged from 78 to 103 mg crop^{-1} pot^{-1} in fallow pots not exposed to light and from 243 to 277 mg crop^{-1} pot^{-1} in planted pots exposed to light. The N gains reported by Singh & Singh in flooded pots exposed to light (51 and 277 mg N crop^{-1} pot^{-1}) cover a similar range than 89 values reported by App et al. (1986) (70-260 mg N crop^{-1} pot^{-1}, average 153). Extrapolating values of App et al. (1986) shows N gains ranging from 16 to 70 kg N ha^{-1} crop^{-1} (average 38) in unfertilized planted pots exposed to light. Santiago Ventura et al. (1986) reported balances of about 100 mg N crop^{-1} pot^{-1} in pots exposed to light and receiving none or low levels of N fertilizer. With high levels of N fertilizer, balance was unsignificant.

CONCLUSION

Recent methodological progress in measuring BNF in ricefields includes improved strategies for sampling and a better understanding of the potential of the ^{15}N dilution methods (labeled substrate and natural abundance). ^{15}N dilution, using available soil N as control, is promising for screening rice varieties for ability to utilize biologically fixed N.

BNF by individual systems (Table 1) can be estimated more or less accurately. Estimates for introduced green manures (*Azolla* and legumes) are based on biomass measurements combined with Ndfa determination and are probably more reliable than estimates for indigenous fixers based mostly on indirect methods (ARA) or balance in small-scale trials. However, total BNF in a ricefield has not yet been estimated by measuring simultaneously the activities of the various components *in situ*. As a result, the relation between the different N_2-fixing systems, especially indigenous ones, are not fully understood and it is not clear if their activities are independent or related. A method to estimate *in situ* the contribution of N_2 fixed to rice nutrition is still not available. Dynamics of BNF during the crop cycle is known for indigenous agents but the pattern of fixed N availability to rice is known only for a few green manure crops. As a result, BNF in models of N cycling in wetlands, is either not taken into account or taken into account as a non-dynamic input.

Table 1. Range of estimates of N_2 fixed by various agents in wetland ricefields (kg N ha^{-1} crop $^{-1}$) and theoretical maximum potential (assumptions and value).

BNF associated with rice rhizosphere: 1-7 kg N ha^{-1} crop $^{-1}$
If all rhizospheric bacteria are N_2 fixers, C flow through the rhizosphere is 1 t ha^{-1} crop^{-1}, and C efficiency is 40 mg N fixed g C^{-1}: 40 kg N ha^{-1} crop $^{-1}$
BNF associated with straw: 2-4 kg N t^{-1} straw applied
If 5 t of straw is applied and 7 mg N are fixed g^{-1} of straw: 35 kg N ha^{-1} crop $^{-1}$
BNF heterotrophic (total): 1-31 kg N ha^{-1} crop $^{-1}$
If all C input (2 t crop^{-1}) is used by N_2-fixers: 60 kg N ha^{-1} crop $^{-1}$
Blue-green algae: 0-80 kg N ha^{-1} crop $^{-1}$
If the photosynthetic aquatic biomass is composed exclusively of N_2-fixing BGA ($C/N = 7$) and primary production is 0.5 t C ha^{-1} crop^{-1}: 70 kg N ha^{-1} crop $^{-1}$
Azolla: 20-150 kg N ha^{-1} crop $^{-1}$ in experimental plots, 10-50 in field trials.
If *Azolla* maximum standing crop is 140 kg N ha^{-1}, two *Azolla* crops are grown per rice crop, and Ndfa is 80%: 224 kg N ha^{-1} crop $^{-1}$
Legume green manures: 20-190 kg N ha^{-1} crop $^{-1}$
If 265 kg N ha^{-1} is accumulated in 50-60 d and Ndfa is 80%: 212 kg N ha^{-1} crop $^{-1}$

Acknowledgments: The authors thank Drs I. Watanabe (IRRI) and R. Buresh (International Fertilizer Development Center) for their very useful comments.

REFERENCES

App A.; Santiago T.; Daez C.; Menguito C.; Ventura W.; Tirol A.; Po J.; Watanabe I.; De Datta S.K.; Roger P.A. Estimation of the nitrogen balance for irrigated rice and the contribution of phototrophic nitrogen fixation. Field Crops Research 9: 17-27; 1984.

App A.; Watanabe I.; Alexander M.; Ventura W.V.; Daez C.; Santiago T.; De Datta S.K. Nonsymbiotic nitrogen fixation associated with the rice plant in flooded soils. Soil Sci. 130: 283-289; 1980.

App A.; Watanabe I.; Santiago T.; Bravo M.; Daez J. The effect of cultivated and wild rice varieties on the nitrogen balance of flooded rice. Soil Sci. 141: 448-452; 1986.

Barraquio W.; Daroy M.; Tirol A.; Ladha J.K.; Watanabe I. Laboratory acetylene reduction assay for relative measurement of N_2-fixing activities associated with field-grown wetland plants. Plant Soil 90: 359-372; 1986.

Eskew D.L. Use of ^{15}N in N_2 fixation and N-cycling studies of *Azolla* pp. 233-239 in Azolla utilization. pp. 233-239 in Azolla utilization. International Rice Research Institute; 1987.

Greenland D.J.; Watanabe I. The continuing nitrogen enigma. Trans. Internat. Congress Soil Sci., 5: 123-137; 1982.

Inubushi K.; Watanabe I. Dynamics of available nitrogen in paddy soils. II Mineralized N of chloroform-fumigated soil as a nutrient source for rice. Soil Sci. Plant Nutr. 32: 561-577; 1986.

Kulasooriya S.A.; Senviratne P.R.G.; De Silva W.S.A.G.; Abeysekera S.W.; Wijesundara C.; De Silva A. P. Isotopic studies on N_2-fixation in *Azolla* and the availability of its nitrogen to rice. Symbiosis 6: 151-166; 1988.

Ladha J.K.; Bonkerd N. Biological nitrogen fixation by heterotrophic and phototrophic bacteria in association with straw. pp 173-192 in Proc Internat Symp on Paddy Soil Fertility. ChiangMai, Thailand, Dec 6-13. ISSS pub.1079 pp.;1989.

Ladha J.K.; Miyan S.; Garcia M. *Sesbania rostrata* as green manure for lowland rice: Growth, N_2 fixation, *Azorhizobium* sp. inoculation, and effects on succeeding crop yields and nitrogen balance. Biol. Fertil. Soils 7 : (in press); 1988 a.

Ladha J. K.; Tirol A.; Punzalan G.; Watanabe I. Nitrogen-fixing (C_2H_2 reducing) activity and plant growth characters of 16 wetland rice varieties. Soil Sci. Plant Nutr. 33: 187-200; 1987.

Ladha J.K.; Padre A.T.; Punzalan G.; Watanabe I.; De Datta S.K. pp 747-752 in Bothe, de Bruijn, Newton eds., N_2 fixation: hundred years after. G. Fischer pub; 1988b.

Ladha J.K.; Watanabe I.; Saono S. Nitrogen fixation by leguminous green manure and practices for its enhancement in tropical lowland rice. p. 165-183 in Green manure in rice farming, International Rice Research Institute; 1988c.

Li Z.X.; Zu S.X.; Mao M.F.; Wang F.L.; Zhao B.B. Determination of amount of N_2 fixation and change in N_2-fixing activity of Azolla in natural environment. pp 223-231 in Azolla utilization, International Rice Research Institute; 1987.

N'Doye I.; Dreyfus B. N_2 fixation by *Sesbania rostrata* and *Sesbania sesban* estimated using ^{15}N and total N difference methods. Soil Biol. Biochem. 20: 209-213; 1988 management. p 827 in Nitrogen fixation: hundred years after. Bothe H, de Bruijn FJ, Newton WE eds., G. Fischer pub.; 1988.

Roger P.A.; Watanabe I. Technologies for utilizing biological nitrogen fixation in lowland rice : potentialities, current usage, and limiting factors. Fertilizer Research 9: 39-77; 1986.

Santiago Ventura T.; Bravo M.; Daez C.; Ventura W.; Watanabe I.; App A.A. Effect of fertilizers, straw and dry fallow on the nitrogen balance of a flooded soil planted with rice. Plant Soil 93: 405-411; 1986.

Singh A.L.; Singh P.K. Nitrogen fixation and balance studies of rice soil. Biol. Fert. Soils 4: 15-19; 1987.

Tirol-Padre A.; Ladha J.K.; Punzalan G.; Watanabe I. A plant sampling procedure for acetylene reduction assay to detect rice varietal differences in ability to stimulate N_2 fixation. Soil Biol. Biochem. 20: 175-183; 1988.

Trolldenier G. Estimation of associative nitrogen fixation in relation to water regime and plant nutrition in a long-term pot experiment with rice. Biol. Fert. Soils 5: 133-140; 1987.

Ventura W.; Watanabe I. ^{15}N dilution technique of assessing the contribution of nitrogen fixation to rice plant.Soil Sci. Plant Nutr. 29: 123-131; 1983.

Watanabe I. pp169-185 in Microbiology of tropical soils and plant productivity. YD Dommergues & HG Diem eds., M NijHoff & W Junk pub. 328p; 1982.

Watanabe I. pp 197-205 Summary report of the *Azolla* program of the International Network on Soil Fertility and Fertilizer Evaluation for Rice in Azolla utilization, International Rice Research Institute; 1987.

Watanabe I.; Roger P.A. Nitrogen fixation in wetland rice fields. pp 237-276 in Current developments in biological nitrogen fixation. Subba Rao NS ed., Oxford & IBH Pub. Co, New Delhi, 351pp; 1984.

Watanabe I.; Roger P.A. Use of ^{15}N in the study of biological nitrogen fixation in paddy soils at the International Rice Research Institute. pp. 81-98 in Proc. Consultant Meeting. FAO/IAEA Joint Project on Azolla and Blue-green algae. IAEA technical