Nitrogen Transfer and the Role of Biological Nitrogen Fixation in Paddy Fields under Intensive Rice Cultivation

By TATSUHIKO MATSUGUCHI

Department of Soils and Fertilizers, National Institute of Agricultural Sciences

A unique situation of paddy fields, being submerged throughout a major part of rice-cropping season, is assumed to be favorable not only for obtaining bases and silicates from irrigation water but also for maintaining soil nitrogen fertility. According to the recent field data using ^{15}N-tracer, a remarkable contribution of soil nitrogen fertility to rice production was clearly disclosed. As shown in Table 1, the contribution percentages of soil-N to the total-N absorbed by rice plants were 50 to 90% in the fields both in Thailand (Bangkhen) and in Japan even when the fields were dressed with nitrogen fertilizer at the rates of 4 to 15 kg N/10a[1,2]. These results confirmed the significant role of soil nitrogen fertility in rice production even under the intensive cultivation with fertilizer dressing.

Table 1. Contribution percentages of soil-N to rice plant-N (Koyama, T. 1973, 1975)

Paddy field	Amount of N dressing	Split of N dressing	Contribution %
Japan (15 fields)	7–10 kg/10a	1–4 times	65.2–87.0
	10–15	1–3	47.5–75.7
Thailand (Bang Khen)	0		100
	3.75	1 (all basal)	88
	7.5	1 (all basal)	80
	7.5	2	51.9

Abbreviations:
- NF: Biological nitrogen fixation
- ARA: Acetylene-reducing (C_2H_2-C_2H_4) activity
- $N_2[C_2H_2]$ fixed: Amount of biologically fixed nitrogen converted from ARA

Role of NF in the maintenance of soil nitrogen fertility in paddy fields

Soil nitrogen fertility in paddy fields depends not only on the artificial supply but also on the natural supply of nitrogen. Of the natural supply, NF must be most significant. Quantitative assessments of NF so far undertaken in tropical paddy fields generally without nitrogen dressing showed that 5.3 to 7.7 kg[3], 1.5 to 4.9 kg[4] and 0.1 to 5.4 kg N/10a[5] were fixed in the fields in Philippines (IRRI), India (West Bengal) and Thailand (over the country), respectively.

Though crucial it must be in rice production in the tropics, the role of NF has hitherto been ignored in Japan since the nitrogen dressing was reported to cause a conspicuous suppression of NF[6]. Recent field data with heavy dressing of nitrogen, however, indicated that the ammonium-N concentrations in paddy plough soils are generally maintained at 20 ppm or below throughout the stages after tillering despite the further top-dressing of nitrogen was applied, as shown in Fig. 1. Thus, inorganic nitrogen regime in paddy soil is assumed actually not as unfavorable for NF as has been accepted.

An indirect quantitative assessment of NF in paddy fields was carried out at the Saga

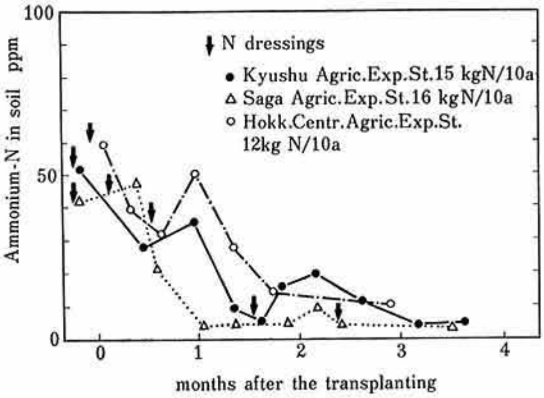
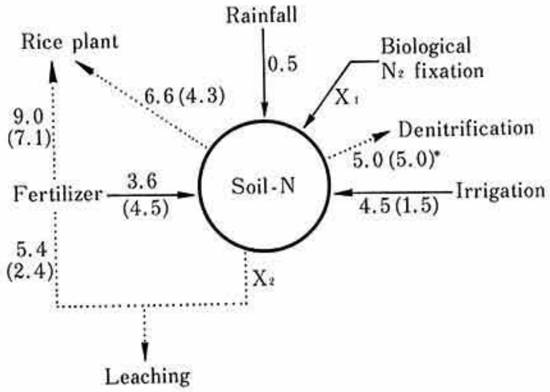


Fig. 1. Time-courses of ammonium-N concentration in paddy field soils with heavy dressing of nitrogen



1) Nitrogen quantity is shown in terms of kg/10 a/crop season. That of the transplanted field is given in parenthesis.
2) Asterisk indicates the data obtained at the Hokuriku Agr. Exp. Sta. 1974.

Fig. 2. Flow sheet of nitrogen in two paddy fields, broadcast or transplanted, of Saga Agr. Exp. Sta. (1974)

Agricultural Experiment Station, by using ^{15}N-tracer. The flow sheet of N is illustrated in Fig. 2 and the balance sheet of soil-N is given in Table 2, where the amounts of NF and the leaching from soil-N are expressed as X_1 and X_2, respectively. The net gain of N to soil-N per one cropping in the broadcast or in the transplanted plots was referred to Y_B or Y_T, where $Y_B = X_1 - (3 + X_2)$ and $Y_T = X_1 - (2.8 + X_2)$. The amounts of X_2, though not assayed, were assumed to be small in both plots. If the net gains per cropping are supposed to be nil (Y_B, $Y_T = 0$), the values of X_1 in both plots are estimated at 3 kg N/10a or more. In the second cropping with barley, however, 2.9 and 4.3 kg/10a or more of soil-N were leached out in both plots[7] while the gain by NF must have

Table 2. Balance sheet of Soil-N during one rice cropping in broadcast and transplanted fields
(Saga Agr. Exp. Sta. 1974)

Processes	Broadcast field	Transplanted field
Gain	N kg/10 a	N kg/10 a
N application	3. 6	4. 5
Irrigation water	4. 5	1. 5
Rainfall	0. 5	0. 5
Biological fixation	X_1	X_1
Loss		
Uptake by rice plant	6. 6	4. 3
Denitrification	5. 0	5. 0
Leaching	X_2	X_2
Net Gain of N to soil-N	$Y_B = X_1 - (3 + X_2)$	$Y_T = X_1 - (2.8 + X_2)$

Table 3. Estimated amounts of soil-N taken up by crops (rice-wheat rotations for 40 years) in a paddy field without nitrogen dressing (Shiga Agr. Exp. Sta, 1933–1973)

Paddy rice in summer (I)				Wheat in winter (II)				(I)+(II)	
Yield		Soil-N absorbed		Yield		Soil-N absorbed		Soil-N absorbed	
40 years	per annum	40 years	per annum	40 years	per annum	40 years	per annum	40 years	per annum
kg/10 a		kg/10 a		kg/10 a		kg/10 a		kg/10 a	
10391	260	248. 3	6. 2	2925	75	89. 2	2. 3	337. 5	8. 5

Natural nitrogen supplies per annum were; 0. 5 kg/10 a (rainfall)+1. 4 kg/10 a (irrigation)=1. 9 kg/10 a. Consequently, the estimated amount of NF per annum were; 8. 5 kg/10 a−1. 9 kg/10 a=6. 6 kg/10 a.

been insignificant. Consequently the amounts of NF(X_1) to compensate the annual loss of soil-N were estimated at 6 and 7 kg N/10a or more. Field data of a plot without nitrogen dressing for 40 years in the Shiga Agricultural Experiment Station, shown in Table 3, also give an estimation that the average amount of NF per cropping is 6.6 kg N/10a[4]. The coincidence of the estimates in these two data with or without nitrogen dressing suggests that a heavy dressing of nitrogen in paddy fields may not significantly reduce the total amount of NF per cropping.

Sites for NF in paddy field soils under the intensive cultivation

Only a few papers dealt with the role of NF in paddy fields with nitrogen dressing[8,9]. Under intensive rice cultivation, nitrogen dressings as much as 10 to 15 kg/10a together with agricultural chemicals are usually practiced. The afore-mentioned assessments of NF in Japan seem to point out a significant role of NF even under such field management.

Though the NF in the field is shared by the phototrophs (blue-green algae and photosynthetic bacteria) inhabiting in paddy water and on soil surface and the heterotrophs in rice rhizosphere and in non-rhizosphere soil, the NF due to the phototrophs will be suppressed by dressings of nitrogen fertilizers and chemicals. Fig. 3 illustrates the NF by the phototrophs and by the heterotrophs in the plots with or without nitrogen top-dressing, showing the remarkably suppressing effect of nitrogen

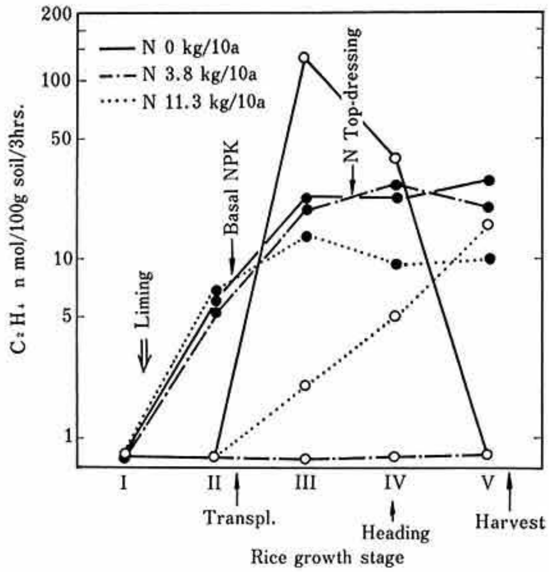


Fig. 3. Effects of nitrogen dressings on C_2H_2–C_2H_4 activities (ARA) of heterotrophs (●) and of phototrophs (○) in the plough soil of Khlong Luang Rice Experiment Station, Thailand. All the plots were dressed with P and K at the rate of 11.3 kg/10a of P_2O_5 (superphosphate) and 3.8 kg/10a of K_2O (potassium chloride). (Matsuguchi, T., et al. 1976)

on the former but only slight effect on the latter[9]. The effect of agricultural chemicals applied at field rates on the growth of blue-green algae at the submerged soil surface is also shown in Table 4, where significant inhibitions were caused by mercuric-ethyl-phosphate and PCP[10]. The paucity of phototrophic NF thus revealed witnesses an increasing share of heterotrophs in NF in the plough soil under the intensive cultivation.

Table 4. Effects of agricultural chemicals applied at the field rate on growth of blue-green algae at the surface of submerged Kōnosu alluvial soil (Ishizawa, S. et al. 1966)

Treatment	Days									
	6	11	16	22	28	32	37	43	50	58
Control	±	±	+	±	±	±	±	±	±	±
γ-BHC	+	+	+	+	+	±	—	—	—	—
1-naphtyl n-methyl carbamate	—	±	±	±	+	+			±	
Mercuric ethyl phosphate	—	±	—	±	—	—	±	±	—	±
PCP	—	—	—	—	±	±	—	—	±	±

Heterotrophic NF in paddy fields

Recently the interest has focused on the NF by the heterotrophs in rice rhizosphere. Rice roots not only excrete considerable amounts of organic matter, fructose, arabinose and glucose being most abundant[11], and molecular nitrogen[12] but also take up inorganic nitrogen from the surrounding soil. Thus a favorable environment for heterotrophic NF, being microaerophilic rich in energy sources and molecular nitrogen but poor in inorganic nitrogen, must be established around the roots.

In IRRI's fields, Yoshida, T. et al.[3] found a significantly higher ARA in the flooded plough soil planted to rice (IR-20) than in the unplanted soil. In addition, a remarkably high ARA, 15 to 20 n mol $C_2H_4 \cdot g$ fresh $root^{-1} \cdot hr^{-1}$, was obtained in the washed root at the reproductive growth stage, while no ARA was detected in the sterilized roots. They isolated from the washed roots gram-negative non-spore-forming bacteria capable of acetylene reduction. The $N_2[C_2H_2]$ fixed in the soil during a dry-season cropping was 6.3 and 2.8 kg/10a in the planted and the unplanted plots, respectively. In addition, 1.4 kg/10a of $N_2[C_2H_2]$ was fixed in paddy water in both plots. Thus the share of NF by the rhizosphere heterotrophs amounted to a half of the total NF in the fields. Dommergues, Y. et al., however, reported that the ARA in rice (IR-8) rhizosphere varied considerably with soil types, suggesting the soil factors responsible for the variation[13].

In contrast, little attention has been given so far to the heterotrophic NF working in the non-rhizosphere soil. This is attributable, as shown in Table 5, to the unrealistically high levels of available energy source required for heterotrophic NF to occur in soils; 50 to 200 kg of sugar for 1 kg of N_2 to be fixed[11]. This is the reason why we have ignored NF in the

Table 5. Genera of bacteria with known nitrogen-fixing species
(Stewart, W. D. P. 1966)

Order and Family	Genus	mg nitrogen fixed per g carbohydrate utilized
EUBACTERIALES		
Achromobacteraceae	*Achromobacter*	1
Azotobacteraceae	*Azotobacter*	10–20
	Beijerinckia	10–20
	Derxia	<25
Bacillaceae	*Bacillus*	12
	Clostridium	2–27
Enterobacteriaceae	*Aerobacter*	4–5
	Klebsiella	5
PSEUDOMONADALES		
Athiorhodaceae	*Rhodospirillum*	—
	Rhodopseudomonas	—
	Rhodomicrobium	—
Thiorhodaceae	*Chromatium*	—
Chlorobacteriaceae	*Chlorobium*	—
Pseudomonadaceae	*Azotomonas*	—
	Pseudomonas	1–4
Spirillaceae	*Desulfovibrio*	—
	Methanobacterium	—
	Spirillum or *Vibrio*	—

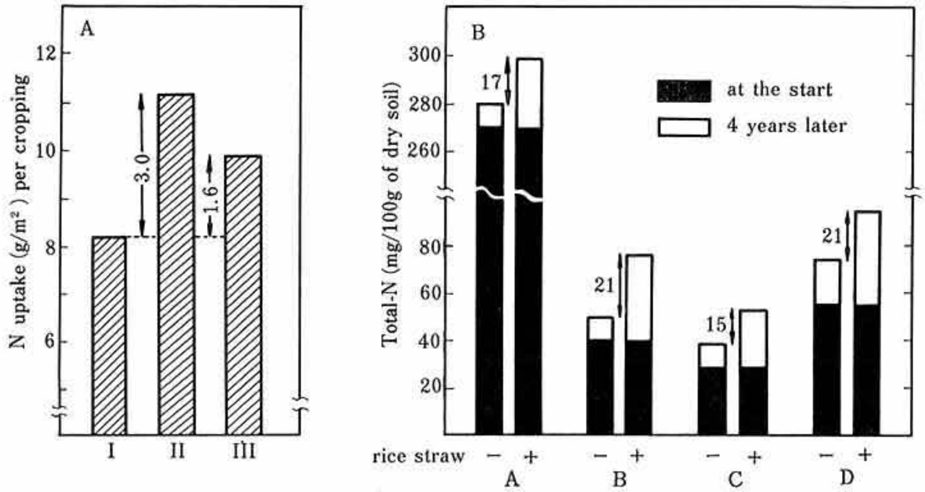


Fig. 4. Effect of rice straw application on N uptake by growing rice plants (A) and on Total-N in the soil (B).

A. Kamikawa Agr. Exp. Sta.: I; Plot without rice straw, II; Plot with rice straw applied soon after the harvest at the rate of 0.4 ton/10a, and III; Plot with rice straw applied prior to cropping. Numerals in the figure show N kg/10 a/cropping.

B. Hiroshima Agr. Exp. Sta.: A; Ando soil, B; Alluvial soil, C; Diluvial soil and D; Soil derived from Tertiary deposits. The data were obtained after the annual application for 4 years. Numerals in the figure show N kg/10 a/4 croppings.

non-rhizosphere soil.

Recently in Japan, however, the application of rice straw by the use of modern combines has been popularizing and the field data demonstrated that the straw application at the rate of 0.5 to 1.0 ton/10a resulted in a significant increase of available soil-N in general. Fig. 4 shows the data at Kamikawa in Hokkaido and at Hiroshima in the south of Mainland of Japan. At Kamikawa, the accelerated N-uptake by 3.0 and 1.6 kg/10a/cropping was attained by 0.4 ton/10a of rice straw annually applied soon after harvest (II in Fig. 4-A) or prior to cropping (III in Fig. 4-A) for 9 years. At Hiroshima, the accelerated enrichment of soil-N by 15 to 21 kg/10a/4 years was induced by the annual applications of straw at 0.75 ton/10a. Nitrogen added to the soils by the straw application amounted to 2 kg/10a/cropping at Kamikawa or 15 kg/10a/4 years at Hiroshima. Thus, the acceleration of N-uptake by rice plants at the former place and that of soil-N enrichment at the latter both obtained by rice straw applications are attributable not only to the increased efficiency of fertilizer-N absorption but also to the enhanced NF by heterotrophs. Probably, this enhancement may be most striking in the non-rhizosphere soil because NF in rice rhizosphere is assumed far less responsive to the straw application.

Response of heterotrophic NF in non-rhizosphere soil to rice straw application

Despite numerous field data supporting the effectiveness of rice straw application for maintaining soil nitrogen fertility, its effectiveness on NF in the soil which received nitrogen fertilizer has scarcely been worked out. In the soil incubated under submergence with $^{15}N_2$ but without the addition of combined nitrogen, heterotrophic NF at the rate of 1.3 to 15 or 50 to 100 kg N/10a/month was observed when the soil was applied with ground wheat straw at 1% or less or at 5 to 20%[15]. By applying ground rice straw at 1%, NF at the rate of 7 kg N/10a/month was also reported[16].

With additions of combined nitrogen, the remarkable suppressions of NF in the soil have been reported; the minimum concentrations of combined nitrogen for the suppression were 27 to 168 ppm NO_3-N[17] and 5 to 100 ppm NH_4-N[8,18]. Knowles, R. et al., however, reported that the concentration of combined nitrogen causing the suppression was roughly proportional to that of added carbohydrate[18]. These results suggest that the heterotrophic NF may occur to a considerable extent in non-rhizosphere soil even with heavy dressing of nitrogen, if rice straw is applied.

Based on the above-described approach, the author conducted a field experiment to reveal the effectiveness of rice straw application on NF in paddy plough soil with and without heavy dressing of nitrogen fertilizer. For the assessment of NF, the ARA in every 1 cm-section soil was assayed at the heading stage.

The results are shown in Fig. 5. Without nitrogen dressing (Fig. 5-A), the rice straw application at 800 kg/10a accelerated a total ARA in the plough soil (0–10 cm) by 40%. With the basal dressing at 12 kg N/10a (Fig. 5-B), however, the rice straw application remarkably accelerated the ARA in the 0–1 cm soil, resulting in an almost 90% acceleration of the total ARA. Interestingly, the rice straw application also increased the depth of soil with high ARA of heterotrophs.

This acceleration of ARA caused by nitrogen dressing in the plot with rice straw is of particular importance. Numerous papers reported the crucial suppression of nitrogenase activity due to combined nitrogen, but the accelerated activity from nitrogen dressing has not yet been reported. In our separate experiment, top-dressing of nitrogen was applied at 2 kg/10a soon after the ARA assay and again the ARA was assayed 3 and 19 days later. As shown in Fig. 6, the reduced ARA was observed 3 days later, but thereafter it began to revive. These results suggest that the nitrogen dressing stimulated the rice straw decomposition resulting in an accumulation of available energy sources for NF and in a decrease of soil Eh as well. After the rapid absorption of the applied nitrogen by rice plants, an environment favorable for NF must have been established in the soil.

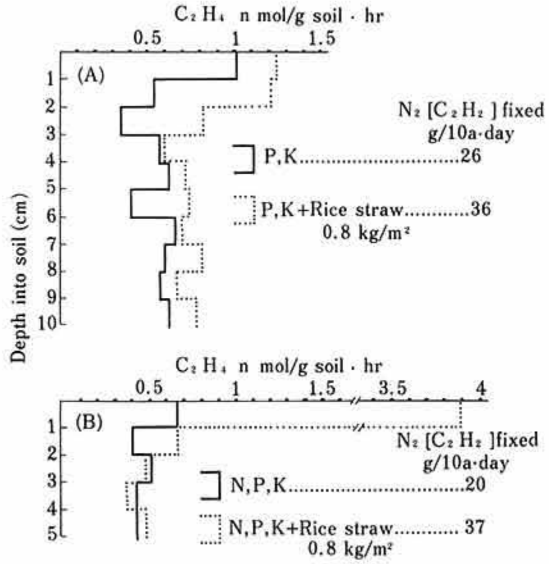


Fig. 5. Effect of rice straw application on C_2H_2-C_2H_4 activities (ARA) in paddy field soils (Arakawa alluvial soil) with P, K and N, P, K dressings.

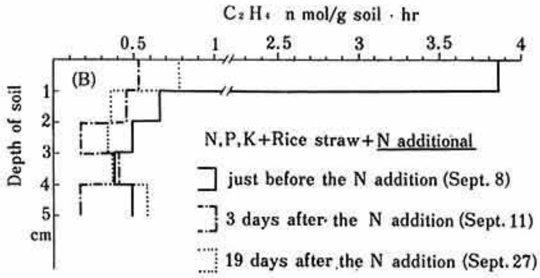


Fig. 6. The revival of C_2H_2-C_2H_4 activity (ARA) in paddy-field soil after the additional dressing of nitrogen. The additional nitrogen at 2 kg/10 a was applied to B-plot in Fig. 5 soon after the ARA assay.

Concluding remarks

The search for techniques to be used for refreshing the declining soil nitrogen fertility in paddy fields is an urgent subject in Japan. The recent studies presented in this paper have revealed that the refreshment is attainable through the rice straw application.

In paddy fields applied with rice straw, heterotrophic NF in the soil is so much accelerated as to cause a significant increase of available soil-N. The author's result that the heterotrophic NF in the soil with rice straw is remarkably enhanced by heavy dressing of nitrogen is of particular significance in this respect. Though the further studies on both NF and denitrification are required, this result suggests the effectiveness of rice straw application in increasing the soil nitrogen fertility under the intensive rice cultivation.

References

1) Koyama, T.: Practice of determining potential nitrogen supplying capacities of paddy soil and rice yield. *J. Sci. Soil and Manure, Japan*, 46, 260–269 (1975) [In Japanese].

2) Koyama, T., Chammek, C. & Niamsrichand, N.: Nitrogen application technology for tropical rice as determined by field experiments using ^{15}N tracer technique. *Tech. Bull. TARC*, No. 3, 8 (1973).

3) Yoshida, T. & Ancajas, R. R.: Nitrogen-fixing activity in upland and flooded rice soil. *Soil Sci. Soc. Amer. Proc.*, 37, 42–46 (1973).

4) Takahashi, E.: Biological nitrogen fixation —its ecological aspect. *Chemistry, Japan*, 32, 2–12 (1977) [In Japanese].

5) Matsuguchi, T., Tangcham, B. & Patiyuth, S.: Free-living nitrogen fixers and acetylene reduction in tropical rice fields. *JARQ*, 8, 253–256 (1975).

6) Konishi, T. & Seino, K., Studies on the maintenance-mechanism of paddy soil fertility in nature. *Bull. Hokuriku Agr. Exp. Sta.*, 2, 41–136 (1961) [In Japanese with English Summary].

7) Koyama, T. et al.: Studies on the efficient nitrogen application technology in Saga paddy field using ^{15}N-tracer technique. Abstract, The 1977 meeting, Soc. Sci. Soil & Manure, Japan, 142 (1977) [In Japanese].

8) Yoshida, T., Roncal, R. A. & Bautista, E. M.: Atmospheric nitrogen fixation by photosynthetic microorganisms in a submerged Philippine soil. *Soil Sci. Plant Nutr.*, **19**, 117–123 (1973).

9) Matsuguchi, T., Tangcham, B. & Patiyuth, S.: Asymbiotic nitrogen-fixing microflora and nitrogen fixation in Thai paddy fields. *Soil & Microorganisms, Japan*, 18, 7–19 (1976) [In Japanese].

10) Ishizawa, S. & Matsuguchi, T.: Effects of pesticides and herbicides upon microorganisms in soil and water under waterlogged condition. *Bull. Natl. Inst. Agr. Sci.*, **B16**, 1–90 (1966).

11) Döbereiner, J.: Nitrogen-fixing bacteria in the rhizosphere. The biology of nitrogen fixation (North-Holland Publ. Co.) 86–120 (1974).

12) Yoshida, T., Takai, Y. & Rosario, D. C. D.: Molecular nitrogen content in a submerged rice field. *Pl. & Soil*, 42, 653–660 (1975).

13) Dommergues, Y. et al.: Non-symbiotic nitrogen fixation in the rhizospheres of rice, maize and different tropical grasses. *Soil Biol. Biochem.*, 5, 83–89 (1973).

14) Stewart, W. D. P.: Nitrogen fixation in plants (Athlone Press), 72 (1966).

15) Rice, W. A. & Paul, E. A.: The role of anaerobiosis in asymbiotic nitrogen fixation. *Can. J. Microbiol.*, 13, 829–836 (1967).

16) Rajaramamohan-Rao, V.: Nitrogen fixation as influenced by moisture content, ammonium sulphate and organic sources in a paddy soil. *Soil Biol. Biochem.*, 8, 445–448 (1976).

17) Delwiche, C. C. & Wijler, J.: Non-symbiotic nitrogen fixation in soil. *Pl. & Soil*, 7, 113–129 (1956).

18) Knowles, R. & Denike, D.: Effect of ammonium-, nitrite- and nitrate-nitrogen on anaerobic nitrogenase activity in soil. *Soil Biol. Biochem.*, 6, 353–358 (1974).