Microbial Nitrogen Fixation and Its Availability to Rice Plants as Revealed with the Use of ^{15}N in Japan

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Introduction

Nitrogen is one of the most important factors affecting rice production. About 60–70% of nitrogen absorbed by rice plants during the growth in a field plot with N fertilization is derived from the native soil N. On the other hand, no significant decrease in soil N has been recognized in paddy fields without N application. Such long-term maintenance of N fertility in paddy fields is mainly dependent on the natural N supply. Among the factors affecting natural supply of N to paddy fields, the biological N_2 fixation (NF) is the most important one. From the nitrogen balance in paddy fields in Japan, it was pointed out that about 40–45 kg N/ha was brought by biological NF. However, because of the heavy supply of chemical N fertilizer to rice (100–150 kg N/ha/crop), little attention has been paid to biological NF in paddy fields until recent years.

From the development of the acetylene reduction technique for estimating biological NF in various ecosystems and the finding of N_2-fixing microorganisms, heterotrophic bacteria, in rice rhizosphere by Yoshida and Ancajas, IRRI (1973)*, the research in this area was significantly stimulated and developed in the world. Furthermore, in view of saving resource materials and energy and of reducing the environmental pollution which is partly attributed to the excessive use of chemical fertilizer, the effective utilization of biological NF deserves more attention. The number of research works on NF in Japanese paddy fields, however, is not so many as compared with that in the tropics. In addition, the study using ^{15}N-labelled compounds in this particular field is considerably restricted due to higher cost of ^{15}N substances and related equipments and lower sensitivity of measuring directly the activity of NF with the use of ^{15}N as compared with the acetylene reduction method. As a result ^{15}N-works are few, particularly those on *in situ* studies.

On the other hand, a lot of works on the N fertility in paddy soils have been carried out in Japan. From the studies on the mineralization of newly immobilized N in microbial cells and the readily decomposable organic N as a source of N fertility in paddy soils, it was found that the immobilized N in soil microorganisms (microbial biomass N) significantly contributed to the N absorbed by rice plants.

Research works which have been done in Japan using ^{15}N-labelled substances on microbial N_2 fixation, microbial immobilization of soil N, and their availability to rice plants are reviewed in this paper.

Microbial NF in paddy soils and its availability to rice plants

The N_2-fixing microorganisms in paddy soils include both phototrophs and heterotrophs. The phototrophs, especially blue-green algae, are predominant in irrigated water and surface soil. The algal NF is appreciable in fields without N fertilizer, but significant depression of algal growth and phototrophic NF has been observed in tropical

* Yoshida, T. & Ancajas, R. R.: Nitrogen fixing activity in upland and flooded rice fields. *Soil Sci. Soc. Am. Proc.*, **37**, 42-45 (1973).

soils that received heavy doses of N and pesticides. Though the NF by photosynthetic bacteria (Okuda et al. 1965),[12] azolla or blue-green algae was indicated by several workers, in the intensive agriculture system with heavy doses of N fertilizer, herbicides and pesticides under the temperate climatic condition, the role of phototrophs as N_2-fixing microorganisms in paddy fields is not so great as compared to heterotrophs.

Matsuguchi and Shimomura (1973)[11] reported that the NF rate was about 60–70 kg N/ha in paddy plots treated with heavy doses of ^{15}N-fertilizer in Saga Prefecture. Yoshida and Yoneyama (1980)[13] determined the atmospheric dinitrogen fixation *in situ* in the submerged rice rhizosphere using the ^{15}N isotope technique and a specially designed $^{15}N_2$-exposed gas-tight growth chamber. They found that the amount of N fixed by the rice rhizosphere would be av. 11.6 kg/ha/month calculated from the $^{15}N_2$ exposure for a period of 7 to 13 days, and that 19–25% of the total atmospheric N fixed was found even in the ears at a fairly short time after the $^{15}N_2$ exposure (Table 1). They suggest that the N fixed by heterotrophic bacteria in the rice rhizosphere can be utilized rapidly by the rice plant. In addition, Yoshida et al. (1983)[14] examined the NF by N-fixing bacteria in rice rhizosphere using the $^{15}N_2$-exposure with the similar growth chamber. They indicated that the amount of biological NF was larger at the later growth stage than at the early growth stage of rice plants. The amount of NF at the flowering stage of rice was the highest and about 20% of fixed N was absorbed by rice plants (Table 2).

Factors affecting the microbial NF

Many works on the factors affecting the microbial NF in paddy soils were conducted by using acetylene reduction techniques, but there are only few works using the ^{15}N isotope method. Heterotrophic microorganisms require considerable amounts of carbohydrates for the NF on anaerobic soils. Therefore their growth and activities of NF are naturally affected by the amount of available carbon supplied into rice rhizosphere. Yoshida et al. (1983)[14] observed that the activity of NF in rhizosphere varied with the growth stage of rice plants, and that its peak occurred at the flowering stage (about 15 weeks after transplanting) along with the development of anaerobic condition and increase of organic materials excreted by plant roots in soils.

Fujii and Sano (1984)[7] reported that the rice plants with higher activity of acetylene reduction showed the higher amount of NF and that the amount of N fixed in the plant which was infected by *K. oxytota* was larger than that of uninfected one in the experiment using the ^{15}N-dilution method.

Table 1. Atmospheric nitrogen fixation in the rice rhizosphere and the uptake of fixed nitrogen by a rice plant under *in situ* conditions — First experiment

	Sample materials	Dry weight (g)	Total N (%)	^{15}N atom % excess	Fixed N content (μg N)
Plant	Ears	3.39	0.941	0.026	57
	Leaves and stems	9.53	0.529	0.026	91
	Roots	3.39	0.443	0.110	113
Soil	Root zone	400	0.182	0.022	1,105

Isotope N-15 labelled nitrogen gas was added to the atmospheric phase of the lower compartment of the growth chamber in which the rice plant was kept for 13 days under greenhouse conditions. The upper part of the plant was left exposed to the air. The initial and final N-15 isotope abundances in the $^{15}N_2$-labelled atmospheric phase were 22.7 and 6.35 atom % excess, respectively (Yoshida and Yoneyama 1980).

Table 2. Dry weight, total-N and fixed-N content of rice plant samples taken at different growth stages

Rice plant samples	Pot No.	Dry weight (g)	Total N (mg N)	^{15}N atom % excess	Fixed N content (μg N)
41 days after transplanting					
Ears	1	3.91	34.0	0.001	—
	2	4.99	35.8	0.011	110
Leaves and stems	1	9.91	37.3	0.000	—
	2	7.93	26.5	0.008	59
Roots	1	3.43	23.8	0.000	—
	2	3.97	26.0	0.007	51
Soil	1	300	2250	0.000	—
	2	300	2316	0.002	1310
75 days after transplanting					
Ears	3	1.94	21.0	0.028	27
	4	2.05	19.7	0.016	17
Leaves and stems	3	7.39	34.0	0.055	87
	4	5.73	23.9	0.026	33
Roots	3	3.34	24.5	0.082	94
	4	2.62	17.6	0.089	84
Soil	3	300	2130	0.005	497
	4	300	2100	0.006	675
103 days after transplanting					
Ears	5	4.74	41.3	0.013	39
	6	5.18	45.9	0.016	36
Leaves and stems	5	5.32	18.6	0.052	71
	6	5.42	17.8	0.072	62
Roots	5	2.75	19.9	0.090	131
	6	4.00	28.4	0.081	112
Soil	5	300	2330	0.004	682
	6	300	2120	0.011	1140

(Yoshida et al. 1983)

Microbial biomass N and its availability to rice plants

When mineral N is added to soils, a portion of it is usually immobilized by microorganisms and thereby converted into organic forms. Asami (1970–1971)[2–6] and Kai et al. (1973–1977)[1,8,9] examined the immobilization and mineralization of N in paddy soils using the ^{15}N isotope techniques. From the distribution[1,6,8,9] of different chemical forms of organic N derived from native and applied N in paddy soils, it was presumed that a major origin of the amino acid N contributing to the mineralization process might be peptide complex substances such as mucopeptides and structural proteins, which originate from the microbial cells and cell walls remaining in the soil as a part of newly immobilized N (Table 3).[9] Ahmad et al. (1973)[1] found that the newly immobilized N by microorganisms was more susceptible to degradation than the native soil N (Table 4). Asami (1971a)[3] indicated some differences in the mineralization pattern between newly immobilized N and native soil organic N (Fig. 1). The mineralization of soil organic N under flooded conditions may continue at a steady rate for a long period, wheras the remineralization of immobilized N proceeds rapidly and almost ceases at an early stage of incubation, and a quarter to half of newly immobilized N was remineralized within 1-2 weeks of incubation. Ahmad et al.[1] also found that the newly immobilized N became more decomposable than the native organic N, during the incubation after the successive treatment of

Table 3. Distribution of different forms of organic nitrogen derived from native-N and applied-N in Kasuya soil

Incubation period		Organic-N	Nonhydrolyzable N	Hydrolyzable N			
				Ammonium	Amino sugar	Amino acid	Unidentified
				(mg N per 100g dry soil)			
2 weeks	Native-N	232.5 (97.0)	50.3 (21.0)	26.4 (11.0)	20.4 (8.5)	92.3 (38.5)	43.1 (18.0)
	Applied-N	7.8 (78.0)	0.4 (4.0)	0.9 (9.0)	0.6 (6.0)	4.6 (46.0)	1.3 (13.0)
20 weeks	Native-N	214.8 (89.6)	53.0 (22.1)	35.5 (14.8)	21.0 (8.8)	83.7 (34.9)	21.6 (9.0)
	Applied-N	4.2 (42.0)	0.4 (4.0)	0.7 (7.0)	1.0 (10.0)	1.5 (15.0)	0.6 (6.0)

Figures in parentheses show the percentage to the total amount of native-N or applied-N (Kai and Kawaguchi 1977).

Table 4. Index numbers of the susceptibilities of different forms of the native- and the immobilized-N to mineralization at 6-weeks incubation of either the air-dried or the oven-dried and remoistened soil samples

Pretreatment		Nonhydrolyzable N	Hydrolyzable N			
			Ammonium N	Hexosamine N	Amino acid N	Unidentified N
Air-dried	Native-N	153	−169	30	363	112
	Immobilized-N	100	−443	200	127	300
Oven-dried	Native-N	133	603	187	342	204
	Immobilized-N	137	323	320	143	309

Index numbers were calculated on the basis of the mineralized amount of each N fraction of the native- and the immobilized-N at 6-weeks incubation of the nontreated soil samples (Ahmad et al. 1973).

air-drying or oven-drying and remoistening of soil (Table 5).

Marumoto et al. (1984)* pointed out that the N immobilized as microbial cells (microbial biomass N) significantly contributed to the mobile plant N pool in paddy soils, and that about 66% of N in that pool was derived from biomass N. However, this is not the result obtained by the use of ^{15}N, and, up to now, no research has been conducted on this point using ^{15}N with Japanese paddy fields.

The amount and rate of N immobilization and mineralization are mainly affected by the environmental conditions such as temperature, soil reaction, moisture, aeration and susceptibility of the organic materials to microbial decomposition. Kai and Kawaguchi (1977)[9] reported the influence of

* Marumoto, T., Kai, H., Yoshida, T. & Harada, T.: Drying effect of mineralization of microbial cells and their cell walls in soil and contribution of microbial cell walls as a source of decomposable soil organic matter due to drying. *Soil Sci. Plant Nutr.*, **23**, 9-19 (1977). Marumoto, T.: Mineralization of C and N from microbial biomass in paddy soil. *Plant and soil*, **76**, 165-173 (1984).

Table 5. Percentage mineralization of the native- and the immobilized-N of the nontreated, the air-dried and remoistened, and the oven-dried and remoistened soil samples at 6-weeks incubation

	Nontreated	Air-dried	Oven-dried
Native-N	3.4	5.9	10.7
Immobilized-N	14.0	28.0	36.0

(Ahmad et al. 1973)

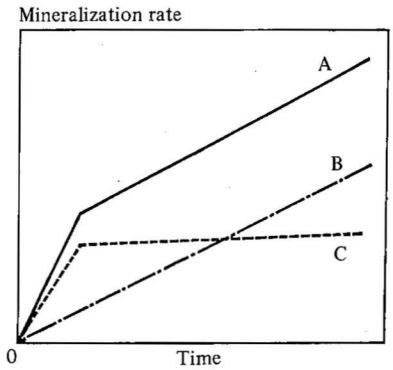

Fig. 1. A schematic presentation of mineralization of organic N in paddy soil (Asami 1971a, modified)
A: Total amount of N mineralized
B: Amount of N mineralized from native soil organic N
C: Amount of N mineralized from immobilized N

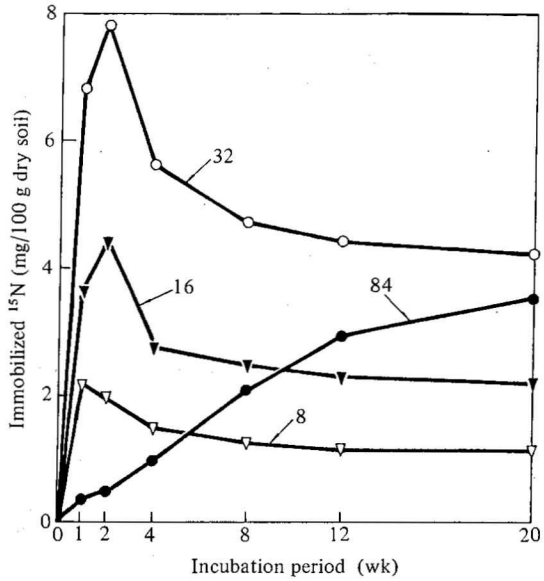

Fig. 2. Immobilization and release of N in paddy soil amended with rice straw and $(^{15}NH_4)_2SO_4$ at different carbon-nitrogen ratios (Kai and Kawaguchi 1977).

the C/N ratio of a mixture of rice straw and ^{15}N-labelled fertilizer on N transformation. The higher the C/N ratio, the larger the amount of N immobilized. The rate of N remineralization in the soil is largest after the maximum immobilization and decreases thereafter (Fig. 2). Asami (1970)[2] indicated that the immobilization of added ammonium-N was active in order of 37° > 30° > 26.5°C, and that the activity of microorganisms involved in the reaction was increased at higher temperature. Furthermore, ammonium-N was used for the synthesis of cell substances by the addition of glucose, i.e. easily decomposable organic matter. Addition of rice top or root also increased the immobilization of added ammonium-N, especially in the first 1-2 weeks, and the increased amount was larger under upland conditions than under submerged conditions.[5]

Recently, Kai et al. (1982)[10] reported the fate of ^{15}N-tagged fertilizer applied to Kasuya paddy soil (in Fukuoka Prefecture) and indicated a balance sheet of N for cropping and non-cropping ecosystems in the paddy soil (Fig. 3).

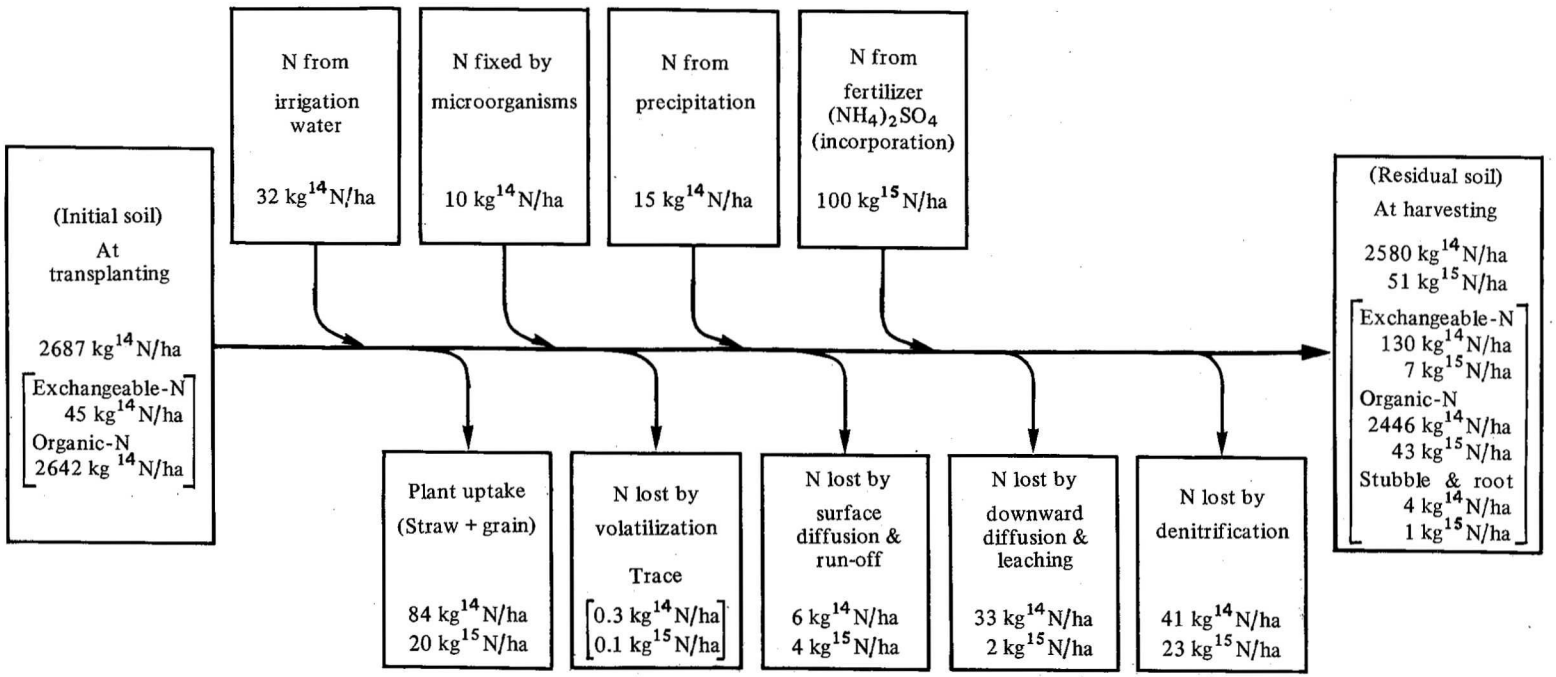

Fig. 3. Balance sheet of N in paddy soil under rice cropping (Kai et al. 1982)

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