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Seasonal Changes in N_2 Fixation Activity and N Enrichment in Paddy Soils as Affected by Soil Management in the Northern Area of Japan

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Abstract

Enhancing the indigenous ability of biological N_2 fixation (BNF) in paddy soils to increase N fertility is important to develop sustainable rice cultivation systems. This study aimed at evaluating the effect of light conditions and long-term soil management on the seasonal changes in the BNF activity of paddy soils associated with the decomposition of rice straw and cellulose, by determining the acetylene reduction activity (ARA) and N enrichment in the Tohoku region of Japan under cool temperate conditions. ARA of the straw applied on the soil surface showed a peak in the mid-cropping season in the summer. The ARA of the straw applied in autumn was 30% of that in spring application. Carbon use efficiency for N enrichment with cellulose was about 3 times higher under light than dark conditions. Among the long-term soil management practices, continuous application of fresh organic materials such as rice straw and manure considerably enhanced N enrichment and ARA. In the soil without P, the inhibition of N enrichment by the low P availability appeared in the early cropping season. Soils from paddy fields converted to upland fields (hereafter referred to as "converted upland soils") showed the highest level of N enrichment when the soils were amended with cellulose. The highest level of N enrichment with and without cellulose amendment was estimated to be 1.6–2.0 g m^{-2} in one cropping season. The results indicated the important contribution of BNF even under cool temperate conditions and the significant effect of soil management related to organic matter application and the water regime on the magnitude of BNF and, therefore, possibly on the N cycling of the paddy soil systems.

Discipline: Soils, fertilizers and plant nutrition
Additional key words: cellulose, straw, Tohoku region

Introduction

Development of sustainable rice cultivation systems requires a proper understanding and utilization of the indigenous ability of paddy soils to conserve and increase N fertility. Submerged conditions in a rice paddy field provide suitable conditions for biological N_2 fixation (BNF) by both phototrophic and heterotrophic microorganisms[9]. Numerous semi-quantitative determinations of BNF attempted mainly in tropical Asia and southern Japan strongly indicated the significance of BNF for N enrichment in the paddy soil systems[6,9,10]. In the Tohoku region in northern Japan under cool temperate conditions, the significance of BNF was also implied by the increase in the amount of available and total N in the shallow soil layer during the cropping season[14,15]. However, information about the dynamics of the heterotrophic and phototrophic BNF activity throughout the year and the soil factors affecting the contribution to N fertility of the soils in this region is limited. This study aimed at clarifying the seasonal changes in the BNF activity associated with the decomposition of straw and cellulose in the soil surface layer and to estimate the contribution to N enrichment under field conditions in the Tohoku region. Soils under different conditions of long-term management related to organic matter, N and P application, and the water regime were compared for

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BNF to analyze the effect of soil management on N enrichment.

Materials and methods

1. Experimental conditions

Three trials, Experiments 1 to 3, were conducted to determine the changes in the acetylene reduction activity (ARA) and N content in the soil of a paddy field (Fluvaquents, SiCL) during the 1993 and 1994 rice cropping seasons at the experimental farm of the National Agricultural Research Center for Tohoku Region in Omagari, Akita, Japan (N39° 29', E140° 30', altitude 30 m). Long-term soil management of the soils used for Experiments 1 to 3 is presented in Table 1. Mean temperature during the rice cropping season (May to September) was 18.3ºC and 21.1ºC in 1993 (Experiments 1 and 2) and 1994 (Experiment 3), respectively (Fig. 1). The field remained flooded with standing water at a depth of about 5 cm during the cropping season and was drained during the off-cropping season from October to April. The field was fully covered by snow from the end of December to mid-March.

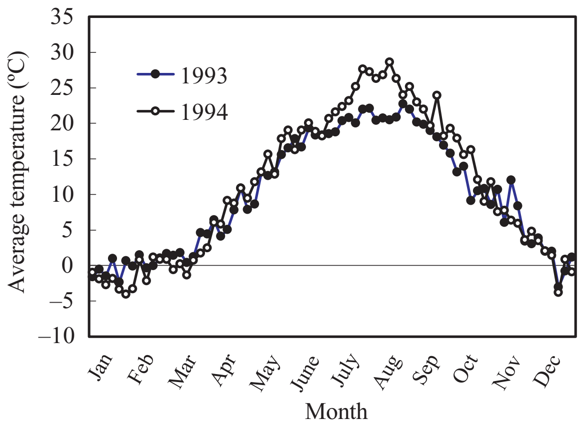

Fig. 1. Changes in daily average temperature in 1993 and 1994

2. Experiment 1: Seasonal changes in ARA of rice straw

ARA of rice straw applied at different locations was monitored during the period 1993 to 1994. Air-dried straw (5.22 g dry weight) in the form of cut pieces (3–4 cm length) in a plastic fine mesh bag (11 × 18 cm) was placed on the soil surface or incorporated into the soil at a 5 cm depth in the NPK plot on May 19 for the spring application and November 18 for the autumn application in 1993. Rice straw was placed at a distance from the rice plants to avoid shading by the rice canopy. Mesh bag was periodically sampled to determine the changes in the dry weight and ARA until harvest time in the second cropping season in September 1994. For the ARA measurement, the rice straw was carefully detached from the mesh and most of the soil particles attached to the surface of rice straw were gently removed with running water. The rice straw was then put in 50 mL Erlenmeyer flasks (base area 12.6 cm^2) by avoiding the overlapping of pieces of rice straw and closed with a butyl rubber stopper. Procedures for the measurement of ARA were similar to those for soils[5,7]. Flasks filled with He: Air: C_2H_2 (87:3:10) were incubated for 24 h at the temperature equivalent to the soil temperature in the field at the sampling time. The amount of C_2H_4 generated during the incubation was determined by using a gas chromatograph (Shimadzu GC14A, Shimadzu Co., Ltd.) equipped with a flame ionization detector using Porapak R (80–100 mesh). ARA of the rice straw applied onto the soil surface was measured both under light (10,000 lx) and dark conditions. ARA of the rice straw incorporated at a 5 cm

Table 1. Long-term management of soils used for the experiments

Experiment	Treatment	N (kg N $ha^{-1}y^{-1}$)	P (kg P $ha^{-1}y^{-1}$)	K (kg K $ha^{-1}y^{-1}$)	Organic matter (t $ha^{-1}y^{-1}$)	Water regime during cropping season	Duration (y)
Experiments 1, 2	NPK	90	31	29	0	Flooding	11
Experiment 3	NPK	80	36	33	0	Flooding	31
	no P	80	0	33	0	Flooding	31
	no N	0	36	33	0	Flooding	31
	no NPK	0	0	0	0	Flooding	31
	Compost 30 t	80	36	33	Compost* 30	Flooding	31
	Compost 10 t	80	36	33	Compost* 10	Flooding	31
	Rice straw 10 t	100	31	29	Rice straw 10	Flooding	12
	Manure 36 t	60	27	24	Manure** 36	Flooding	27
	Converted upland soil	30	31	29	0	Upland for soybean	12

*Made from rice straw amended with urea (N 0.85% and P 0.15% on the average).
**Made from cowdung and sawdust (N 0.42% and P 0.14% on the average).

depth was measured under dark conditions.

3. Experiment 2: Effect of light and cellulose addition on N enrichment

Fresh soil (NPK plot in Table 1) (10 g dry weight) with and without cellulose amendment (cellulose powder 300 mesh, Toyo Co. Ltd.) at 0.94% w/w was put in 100 mL Erlenmeyer flasks (base area 38.5 cm^2). The flasks were placed in the plough layer under light or dark conditions in the field on May 19, 1993 so that the soil surface in the flask was at the same level as the surface of the field. The flasks were flooded and placed at a distance from rice plants to avoid shading by the rice canopy. The outlet of the flasks was covered with aluminum foil to prevent the penetration of large soil particles into the flask. Flasks were taken from the field on October 5 for the determination of N enrichment. Nitrogen enrichment in the soil was determined by measuring the changes in the N content by the Kjeldahl method. Decomposition rate of cellulose in the soil was estimated from the changes in the C content in the soil as follows:

Decomposition rate (%) = 100 × {1 – (Cfin1 – Cfin2) / Ccell}

where Cfin1 and Cfin2 are the C contents (mg C $flask^{-1}$) in the soil after incubation with cellulose and without cellulose, respectively. Ccell is the C content in added cellulose (mg C $flask^{-1}$). Total C content in the soil was determined by using a CN analyzer (Yanaco Co., Ltd.).

4. Experiment 3 : Effect of long-term soil management on N enrichment

Changes in the ARA and N content in soils under various conditions of long-term management were compared during the 1994 cropping season. Fresh soil (10 g dry weight) from the plots differing in long-term management (Table 1) with and without cellulose (1% w/w) was put in 50 mL Erlenmeyer flasks (base area 12.6 cm^2) placed in the field on May 23 for the measurement of ARA and N enrichment until 105 days after the placement. All the flasks including that with converted upland soils were flooded and placed in the field in the same manner as in Experiment 2. For the ARA measurement, surface water in the flasks was removed carefully before the exchange of the gas phase. The incubation for ARA followed the same procedure as that in Experiment 1 under light conditions (10,000 lx). All the measurements in these experiments were replicated 4 times.

Results

1. Experiment 1: Seasonal changes in ARA of rice straw

Based on the decrease in dry weight, 44 and 57% of the rice straw applied in spring was decomposed before the end of September in the first cropping season in surface application and incorporation, respectively (Fig. 2). During the second cropping season, a slight decomposition persisted (6–11%). In the autumn application, 26%

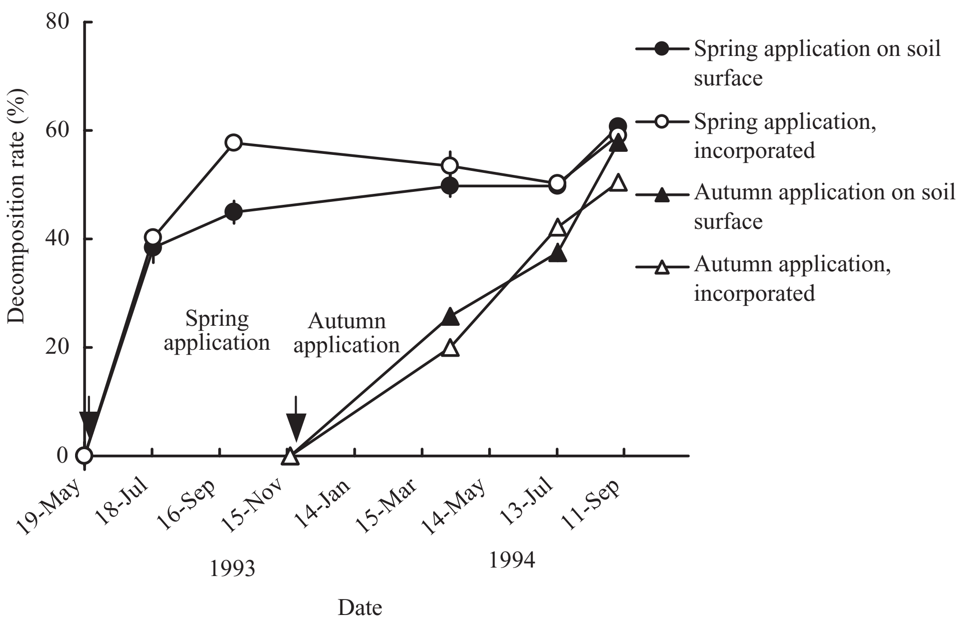

Fig. 2. Decomposition rate of rice straw applied on the soil surface and incorporated into the soil in spring and autumn (Experiment 1)

Bars indicate standard deviation.

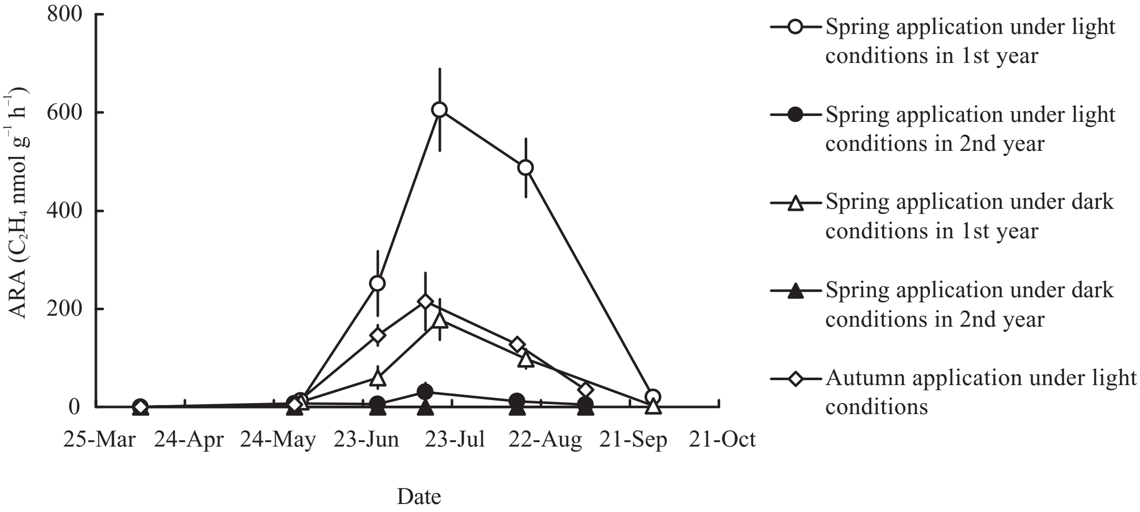

Fig. 3. Changes in ARA of rice straw applied on the soil surface in spring and autumn (Experiment 1)
Bars indicate standard deviation.

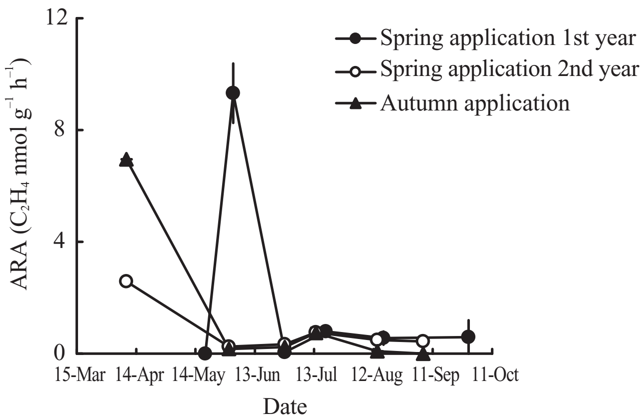

Fig. 4. Changes in ARA of straw incorporated into the soil in spring and autumn (Experiment 1)
Bars indicate standard deviation.

of the rice straw was decomposed in the surface application and 20% in the incorporation before April in the following year. Cumulative decomposition rates during the cropping season were 32 and 30% in the surface application and the incorporation, respectively.

ARA of the rice straw applied in the spring on the soil surface increased substantially from May to July and August, then decreased until September both under light and dark conditions (Fig. 3). ARA measured under dark conditions remained lower than that under light conditions. The cumulative ARA during the first year under dark conditions was 25% of that under light conditions. In the succeeding second cropping season, the cumulative ARA under light conditions throughout the cropping season decreased to 9% of that in the first year with a small peak in July, as in the first year. The rice straw applied in autumn on the soil surface under light conditions also showed a peak of ARA in July and the cumulative ARA during the first cropping season was equivalent to 30% of that in spring application. The range of the maximum ARA values of the rice straw incorporated into the soil under dark conditions was substantially lower, compared with surface application (Fig. 4). ARA of the rice straw incorporated into the soil increased in the early cropping season in June and then decreased. ARA again increased slightly in April before submergence of the field in the second year in the spring application. Similarly, ARA of the rice straw incorporated in autumn showed the highest value in April before submergence.

2. Experiment 2: Effect of light and cellulose addition on N enrichment

Nitrogen enrichment was largest under light conditions in the presence of cellulose (Table 2). There was a slight N enrichment under light conditions without cellulose and under dark conditions with cellulose. Decomposition rate of cellulose was higher than 80% both under light conditions and dark conditions. Apparent C use efficiency for N enrichment under light conditions was 14.9 mg N g^{-1} cellulose consumed, a value much higher than that under dark conditions for 5.2 mg N g^{-1} cellulose consumed.

3. Experiment 3 : Effect of long-term soil management on N enrichment

The mean ARA during the cropping season (32 and 67 days after placement) was well correlated with the N enrichment at the end of the cropping season (105 days

Table 2. N enrichment, decomposition rate of cellulose and apparent C use efficiency for N enrichment (Experiment 2)

Treatment		N enrichment (μg N g^{-1} soil)	N enrichment increased by cellulose (μg N g^{-1} soil)	Cellulose decomposition rate (%)	C use efficiency for N enrichment	
					mg N g^{-1} cellulose consumed	mg N g^{-1} cellulose added
Light	No cellulose	71 (16)	–	–	–	–
Light	Cellulose	187 (15)	116 (15)	82.9 (5.4)	14.9 (1.2)	12.3 (1.0)
Dark	No cellulose	15 (13)	–	–	–	–
Dark	Cellulose	58 (1)	42 (1)	86.7 (2.0)	5.2 (0.5)	4.5 (0.5)

Values in parentheses denote SE.

after placement) across treatments in the presence and absence of cellulose (Fig. 5), suggesting that N enrichment during the rice cropping season occurred mainly through BNF. Large variations in N enrichment during the 105-day period were observed among the soil managements in the absence of cellulose amendment (Fig. 6). High N enrichment was obtained in the treatment with manure and rice straw, especially during the period of 0 to 32 days after the placement. Nitrogen enrichment with cellulose addition was considerably higher than that in the absence of cellulose in most of the treatments except for the treatment with manure and rice straw. With cellulose addition, the treatment of the converted upland soil showed the highest level of N enrichment during the

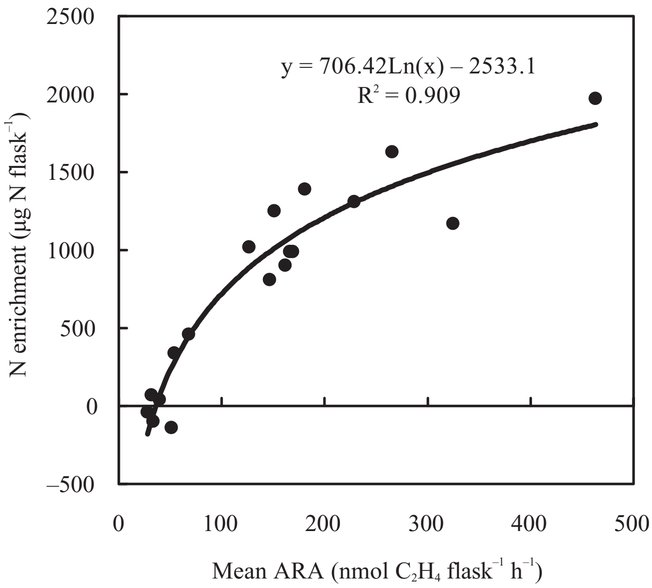

Fig. 5. Relationship between mean ARA at 35 and 65 days after placement and N enrichment at 105 days after placement (Experiment 3)

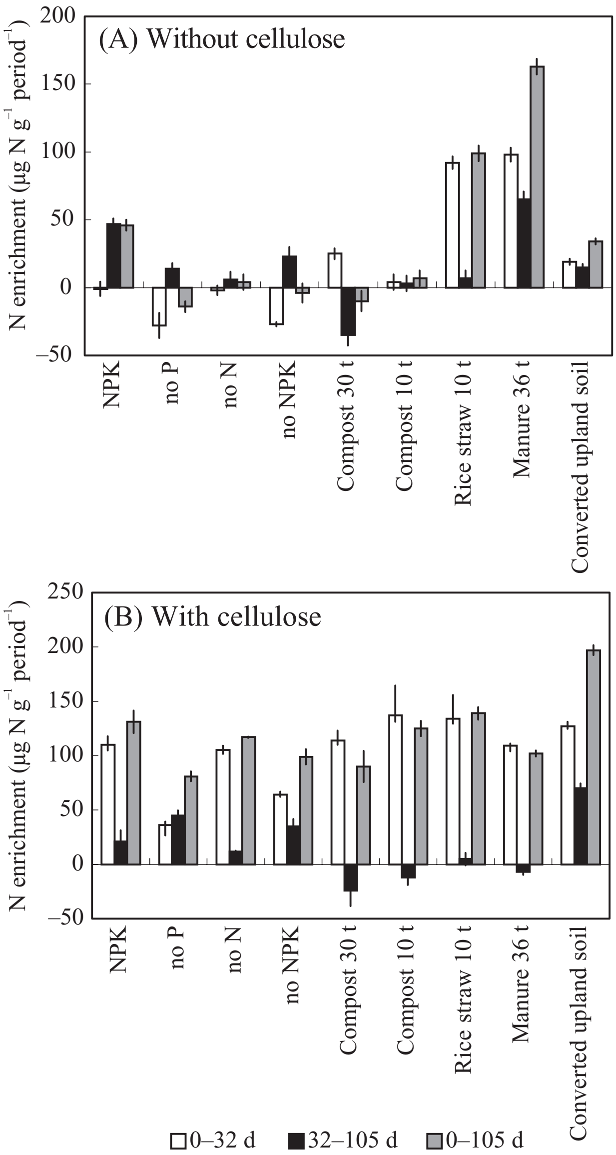

Fig. 6. Effect of long-term soil management on N enrichment without (A) and with (B) cellulose amendment (Experiment 3)

Bars indicate standard error.

whole period. The absence of treatment with P and NPK in the presence of cellulose led to a lower N enrichment during the period of 0 to 32 days after placement than the other treatments, which showed a relatively high N enrichment during the period of 32 to 105 days after the placement.

Discussion

1. Decomposition and ARA of rice straw

ARA of the rice straw applied on the soil surface was much higher than that in the case of incorporation of straw into the soil, which is in agreement with the results obtained in an other report showing that the addition of rice straw stimulated BNF by phototrophs on the soil surface and in flood water[4]. Although the contribution of cyanobacteria and phototrophic bacteria to N enrichment was not separately quantified, phototrophic bacteria seemed to be the major N_2-fixing agents since the proliferation of cyanobacteria was recognized only after August based on visual observation. The sharp peak of ARA in the summer during the period of July to August under light conditions in the rice straw applied on the soil surface indicated the high sensitivity of photodependent ARA to the changes in the light intensity and temperature. In the spring application, ARA decreased to a much lower level in the second year than in the first year, which was associated with the low decomposition rate in the second year.

Rice straw applied in autumn was still a significant source for photodependent BNF, accounting for 30% of cumulative ARA compared with the spring application. This could be partly attributed to the delay in the decomposition of rice straw during the off-cropping season in the winter, compared with a warmer rice-growing area. ARA in rice straw was maintained depending on the decomposition rate of rice straw.

In rice straw incorporated into the soil in spring, a peak of ARA was detected in the very early period in the first cropping season, which was probably associated with the vigorous decomposition of readily decomposable substrates such as soluble sugars and hemicellulose[11,16]. On the other hand, the second slight increase of ARA of rice straw during the drainage period in April before transplanting in the following season was possibly related to the presence of microaerophilic or aerobic-anaerobic interfacial conditions that may stimulate BNF[2,4].

2. Effect of light on BNF

Based on the difference in the N enrichment under light and dark conditions recorded in Experiment 2, it was estimated that photodependent BNF contributed to 79% and 69% of total BNF in the absence and presence of cellulose, respectively. Similarly, the contribution of photodependent ARA of the rice straw on the soil surface was estimated to be 75% in Experiment 1. These values were similar to those obtained in the tropics and temperate area[10].

3. Effect of long-term soil management

The results of ARA without cellulose amendment obtained in Experiment 3 indicated that the accumulation of readily decomposable C substrates by continuous application of fresh organic materials (rice straw and manure) was very effective in enhancing BNF. On the other hand, with cellulose amendment, in the converted upland soil, a relatively high BNF activity was maintained in terms of N enrichment even after 32 days of placement. The high BNF in this soil could be partly attributed to the reduced inhibition of BNF by inorganic N[8] due to the low amount of mineralizable-N associated with the high consumption of readily decomposable organic matter during the upland period. However, since in the soil of the no-N plot that showed a similar low level of mineralizable-N, N enrichment and ARA as high as those in the converted upland soil were not observed, other factors than mineralizable-N were likely to be involved in the high BNF in the converted upland soil, such as the predominance of aerobic or facultative anaerobic microflora. It is important to further clarify the factors stimulating BNF in converted upland soil and the contribution of phototrophic and heterotrophic BNF. Lower BNF in the absence of P treatment than that with NPK indicated the importance of P availability for BNF which was pointed out in other reports[1,3,17], particularly at the early stages. N enrichment in the absence of treatment with P resumed after 32 days to some extent, which suggested a reduction of the inhibition associated with the low P availability at the middle to later stages, possibly due to a longer period of submergence and higher temperature.

4. Utilization of BNF in paddy soils

The results obtained in Experiment 2 indicated that C use efficiency for N enrichment was about 3 fold higher under light than dark conditions. The value of 5.2 mg N g^{-1} cellulose consumed (or 4.5 mg N g^{-1} cellulose added) under dark conditions was comparable to the reported values of 2 to 7 mg N g^{-1} cellulose added[8] and 0.1 to 7.1 (2.4 on the average) mg N fixed g^{-1} C substrate (mainly straw)[9]. The highest N enrichment in the soil with and without amendment of cellulose was extrapolated to 1.6 to 2.0 g N m^{-2}. N balance in the field and pot experiments was positive, averaging 24–29 kg N ha^{-1} cropping $season^{-1}$ in the tropical area and southern area of Japan[6,10]. The values recorded in this study appeared to be relatively lower than these reported values. The discrepancies in the values may be partly due to the differences in the experimental methods and scales used. Measurement system for N enrichment in this study enabled only semi-quantitative estimations due to the differences between the experimental conditions and actual field conditions, e.g. the lack of water percolation and light conditions.

The results obtained indicated that N enrichment by BNF is important in affecting N cycling even in the cool temperate area. In addition, long-term soil management, particularly in relation to organic matter application and the soil water regime, was found to exert a significant impact on the modification of the BNF and hence N enrichment. Application of fresh organic materials enhanced BNF, but may increase the risk of inducing the inhibition of rice growth during the early growth stage, presumably due to the accumulation of toxic compounds such as organic acids[12,13]. Further studies on the chemical and microbial associations of BNF and decomposition processes of organic matter should be conducted to develop methods for effective utilization of BNF, while minimizing deleterious effects on rice growth.

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