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Effects of Temperature, Water Regime, Light, and Soil Properties on $^{15}N_2$ Fixation Associated with Decomposition of Organic Matter in Paddy Soils

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Abstract

Effective utilization of the biological nitrogen fixation (BNF) activity is important for enhancing the N fertility of paddy soils and for developing sustainable rice cultivation systems. To analyze the soil factors that affect BNF in paddy soils, in this study, the effects of the temperature, water regime, and long-term soil management on $^{15}N_2$ fixation were examined in relation to the decomposition of organic matter in incubation experiments. Within the range of 15–30ºC, heterotrophic $^{15}N_2$ fixation under dark conditions changed almost proportionally to the formation of CO_2+CH_4 with glucose and straw as C sources by increasing the temperature, while the C use efficiency (N_2 fixed/(CO_2+CH_4)) was relatively higher at low temperatures in the presence of cellulose. The examination of the effect of the water regime on heterotrophic $^{15}N_2$ fixation indicated that flooding after aerobic conditions promoted heterotrophic $^{15}N_2$ fixation as well as the decomposition of cellulose. Among the soils with different types of management, the soils amended with manure and rice straw showed the largest photodependent $^{15}N_2$ fixation. On the other hand, the soils with a lower content of mineralizable-N tended to depend more on heterotrophic $^{15}N_2$ fixation. Soils from paddy fields converted into upland fields (hereafter referred to as "converted upland soils"), particularly showed a high heterotrophic $^{15}N_2$ fixation. Overall, it appeared that the management of organic matter application and the water regime may result in significant variations in BNF in paddy soils. The results obtained suggested that further studies should be conducted on the C and N metabolism involved in BNF during the decomposition processes of organic matter along with the changes in the soil redox status to identify methods for efficient soil management to promote BNF.

Discipline: Soils, fertilizers and plant nutrition
Additional key words: heterotrophic N_2 fixation, photodependent N_2 fixation,

Introduction

For the development of sustainable rice cultivation systems with minimum inputs, it is important to effectively utilize indigenous soil microbial activities such as biological N_2 fixation (BNF) and recycled organic matter for maintaining the N fertility. Our previous observations in fields[9,18] suggested the importance of BNF for the enrichment of soil N along with the decomposition of rice straw and cellulose in the Tohoku region, which is one of the major rice-producing areas of Japan under cool temperate conditions. Many soil factors are known to affect the BNF activity in paddy soils such as the contents of available-P, NH_4, pH, and redox potential[2,10,12,17]. Soil management related to the application of N, P and organic matter considerably affected N enrichment in fields[9,18]. However, the effects of soil management and properties on BNF by various heterotrophic and photodependent N_2 fixers have not been fully documented. In addition, information about the relationship between the decomposition processes of organic matter and the magnitude of BNF is limited. BNF activity can be affected by soil properties and site-specific environmental factors

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such as temperature and the water regime directly and indirectly through their effects on the decomposition of organic matter in soil. In this paper, we investigated the effects of the temperature, water regime, and long-term soil management on $^{15}N_2$ incorporation associated with the decomposition of organic matter, with emphasis placed on the relation with the formation of CO_2 and CH_4.

Materials and methods

1. Soil properties

The paddy soils (Fluvaquents, SiCL) used for the experiments were sampled from the plow layer in long-term field trials at the experimental farm of the National Agricultural Research Center for Tohoku Region in Omagari, Akita, Japan (N39° 29', E140° 30', altitude 30 m). The details of soil management of the field trials were reported elsewhere[9]. The properties of the soils are presented in Table 1.

2. Experiment 1: Effects of temperature and C source on heterotrophic BNF

The effects of the temperature and C source on heterotrophic N_2 fixation were examined by $^{15}N_2$ incorporation under dark conditions. Fresh soil (6 g dry weight) from the NPK plot was amended with cellulose powder (300 mesh, Toyo Co., Ltd.), rice straw, and glucose at a ratio of 1% w/w, put in 40 mL glass tubes (base area 3.1 cm^2) and flooded at a 0.5 cm depth. The tubes were closed with a butyl rubber stopper and the gas phase was replaced with $^{15}N_2$ (44.7 atom %):O_2 (80:20 v/v). The tubes were incubated under dark conditions at 15, 20, 25, 30ºC for 4 weeks except for the soil with glucose amendment that was incubated at 30ºC due to the failure of incubation. After the incubation, the amounts of CO_2 and CH_4 in the gas phase were determined by using a gas chromatograph equipped with FID (Shimadzu GC-14A, Shimadzu Co.) and TCD (Hitachi 263-70, Hitachi Co., Ltd.), respectively. The formation of CO_2 and CH_4 was calculated by summing the amounts in the gas phase and estimated amounts in the liquid phase[16]. After the gas analysis, the soil was subjected to the determination of the ^{15}N ratio and total N content by using a mass spectrometer (VG Micromass 622 interfaced to a Roboprep-CN, Europa Scientific Co., Ltd.) for the estimation of $^{15}N_2$ fixation.

3. Experiment 2: Effect of alternate water regimes on heterotrophic BNF

Effect of alternate water regimes on heterotrophic $^{15}N_2$ fixation was examined under dark conditions. Fresh soil (4 g dry weight) from the NPK plot was amended with cellulose powder (1% w/w) and put in 40 mL Erlenmeyer flasks. The flasks were incubated at different alternate cycles with 3 water regimes, submerged (water at 0.2 cm depth), wet (water content 75% w/w), and dry (water content 50% w/w), for 4 weeks under dark conditions at 25ºC to determine the $^{15}N_2$ fixation and formation of CO_2 and CH_4. The gas phase during the incubation and the procedures for the determination of the formation of CO_2 and CH_4 and $^{15}N_2$ fixation were the same as those used in Experiment 1.

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

Effect of long-term soil management related to the application of organic matter, N, and P and water regime

Table 1. Properties of the soils used for the experiments

Experiment	Treatment	Total C (%)	Total N (%)	C/N ratio	Mineralizable-N* (mg N kg^{-1})	Available-P Bray-2 (mg P kg^{-1})
Experiments 1,2	NPK	2.31	0.179	12.9	ND**	172
Experiment 3	NPK	2.08	0.167	12.4	24.4	131
	no P	1.89	0.153	12.3	14.2	23
	no N	1.48	0.119	12.4	15.3	110
	no NPK	1.47	0.117	12.6	12.8	38
	Compost 30 t ha^{-1}	3.23	0.287	11.2	43.9	190
	Compost 10 t ha^{-1}	2.11	0.179	11.8	19.5	141
	Straw 10 t ha^{-1}	2.24	0.168	13.3	31.1	111
	Manure 36 t ha^{-1}	2.72	0.198	13.7	36.4	263
	Converted upland soil	1.80	0.140	12.8	11.7	173
	Converted upland soil+compost 20 t ha^{-1}	2.26	0.203	11.2	25.1	203

*NH_4-N formed after incubation of fresh soils under flooding conditions[14] for 4 weeks at 25ºC.
**Not determined.

on heterotrophic and photodependent BNF was examined based on $^{15}N_2$ fixation. For the measurement of heterotrophic BNF under dark conditions, fresh soil (4 g dry weight) subjected to various treatments (Table 1) was amended with cellulose powder (0.5% w/w) and put in 40 mL glass tubes (base area 3.1 cm^2). All the tubes were incubated at 25ºC under flooding conditions (0.5 cm depth), including the converted upland soils, under dark conditions for 4 weeks. The incubation methods for the measurement of photodependent BNF were similar to those for heterotrophic BNF except that the soil was put in 40 mL Erlenmeyer flasks (base area 12.6 cm^2) and the incubation was carried out under light conditions (10,000 lx). The gas phase during the incubation and the procedures for the determination of CH_4 formation and $^{15}N_2$ fixation were the same as those used in Experiment 1. All the measurements in these experiments were replicated 3 times.

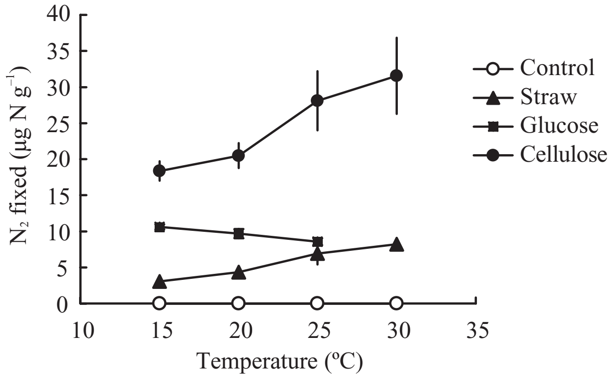

Fig. 1. Effect of temperature and C source on $^{15}N_2$ fixation (Experiment 1)
Bars indicate standard deviation.

Results

1. Effects of temperature and C source

Among the C sources, $^{15}N_2$ fixation was highest in the presence of cellulose at all the temperatures (Fig. 1). The response of $^{15}N_2$ fixation to the temperature differed among the C sources. $^{15}N_2$ fixation increased at higher temperatures in the presence of cellulose and rice straw while there was a slight decrease of $^{15}N_2$ fixation with increasing temperature in the presence of glucose. The formation of CO_2 increased sharply as the temperature increased without C source and in the presence of cellulose and rice straw (Table 2). The formation of CO_2 in the presence of glucose was much higher than that with other C sources at 15ºC and it was almost constant as the temperature increased up to 25ºC. Temperature dependency of CH_4 was observed with all the C sources. Notably, there was a strong response to increasing temperature in CH_4 formation for cellulose. The N_2 fixed/(CO_2+CH_4) ratio in the presence of cellulose was higher than that in the presence of glucose and rice straw, particularly at 15ºC (Table 2). The N_2 fixed/(CO_2+CH_4) ratio was similar at all the temperatures for glucose and rice straw.

Table 2. Effect of temperature and C source on the formation of CO_2 and CH_4 and ratio of N_2 fixed/(CO_2+CH_4) (Experiment 1)

C source	Temperature (ºC)	CO_2 (mg C g^{-1})	CH_4 (μg C g^{-1})	N_2 fixed/(CO_2+CH_4) (mg N g^{-1}C)
Control	15	0.15	0.1	0.0
	20	0.27	0.0	0.0
	25	0.47	0.1	0.0
	30	0.56	0.1	0.0
Glucose	15	2.59	1.1	4.1
	20	2.57	2.3	3.8
	25	2.69	3.0	3.4
Rice straw	15	0.96	23.0	3.2
	20	1.61	33.9	2.7
	25	1.92	39.3	3.5
	30	2.41	43.2	3.4
Cellulose	15	0.61	3.1	30.1
	20	1.22	27.0	16.7
	25	1.99	37.0	14.1
	30	2.60	46.2	12.1
LSD (5%)		0.12	2.61	1.4

Table 3. Effect of water regime on $^{15}N_2$ fixation, formation of CO_2 and CH_4 and ratio of N_2 fixed/(CO_2+CH_4) (Experiment 2)

Treatment	Water regime		N_2 fixed (μg N g^{-1})			CO_2+CH_4 (mg C g^{-1})			N_2 fixed/(CO_2+CH_4) (mg N g^{-1} C)		
	0–4 week	4–8 week	0–4 week	4–8 week	0–8 week	0–4 week	4–8 week	0–8 week	0–4 week	4–8 week	0–8 week
W1	Flooding	Flooding	37.1	31.2	68.2	1.34	1.21	2.55	27.6	25.7	26.7
W2	Flooding	Wet	37.1	28.0	65.1	1.34	1.26	2.60	27.6	22.2	25.0
W3	Wet	Flooding	4.8	55.0	59.8	0.79	1.58	2.37	6.1	34.8	25.2
W4	Wet	Wet	4.8	10.6	15.4	0.79	0.86	1.66	6.1	12.3	9.3
W5	Dry	Flooding	0.2	51.0	51.2	0.71	1.39	2.10	0.3	36.7	24.4
W6	Dry	Dry	0.2	0.1	0.2	0.71	0.57	1.28	0.3	0.1	0.2
LSD (5%)			4.0	10.6	9.9	0.05	0.16	0.15	2.3	7.0	4.1

2. Effect of water regime (Experiment 2)

In Experiment 2, $^{15}N_2$ fixation was larger with a higher water content both during the first and second 4 weeks in general (Table 3). The difference in $^{15}N_2$ fixation among the water regimes was well correlated with the formation of CO_2+CH_4. Flooding after wet conditions (W3 in Table 3) promoted most notably $^{15}N_2$ fixation as well as the formation of CO_2+CH_4. The ratio of N_2 fixed/(CO_2+CH_4) was also highest under flooding conditions, especially the flooding period after the wet period.

3. Effect of soil properties on $^{15}N_2$ fixation (Experiment 3)

$^{15}N_2$ fixation under dark conditions ranged from 18.3 mgN g^{-1} for manure to the highest value of 30.5 mgN g^{-1} for the converted upland soil in Experiment 3 (Table 4). The soil without NPK showed a higher $^{15}N_2$ fixation than that with NPK. Different trends in $^{15}N_2$ fixation were observed among the treatments under light conditions compared with dark conditions. The soil amended with manure showed the highest $^{15}N_2$ fixation followed by that with rice straw. The soil without P, N, and NPK showed a lower $^{15}N_2$ fixation than the soil with NPK. The formation of CH_4 under dark conditions was highest in the soil with rice straw followed by compost (30 t ha^{-1}) and lowest in the converted upland soil.

As for the relation of N_2 fixation with the soil properties, $^{15}N_2$ fixation under dark conditions was negatively correlated with the amount of mineralizable-N, with the converted upland soils exhibiting a higher $^{15}N_2$ fixation relative to mineralizable-N than other soils (Fig. 2A). Conversely, $^{15}N_2$ fixation under light conditions was positively correlated with the amount of mineralizable-N (Fig. 2B). In addition, $^{15}N_2$ fixation under dark conditions was negatively correlated with CH_4 formation (–0.77**). On the other hand, $^{15}N_2$ fixation under light conditions was positively correlated with the amounts of available-P (0.77**) and total-C (0.75*). The ratio of $^{15}N_2$ fixation under dark conditions to that under light

Table 4. Effect of long-term soil management on $^{15}N_2$ fixation under dark and light conditions and CH_4 formation under dark conditions (Experiment 3)

Treatment	N_2 fixed (μg N g^{-1})		A/B	CH_4 (μg C g^{-1}) Dark
	Dark (A)	Light (B)		
NPK	19.6	210.3	0.09	40.4
no P	21.9	129.3	0.17	43.1
no N	23.9	147.7	0.16	35.9
no NPK	26.8	122.6	0.22	34.2
Compost 30 t ha^{-1}	20.8	224.7	0.09	48.8
Compost 10 t ha^{-1}	22.1	176.1	0.13	37.9
Rice straw 10 t ha^{-1}	22.1	254.9	0.09	52.6
Manure 36 t ha^{-1}	18.3	298.8	0.06	39.7
Converted upland soil	30.5	164.0	0.19	16.9
Converted upland soil+compost 20 t ha^{-1}	25.0	213.9	0.12	34.4
LSD (5%)	6.0	34.0	0.04	14.5

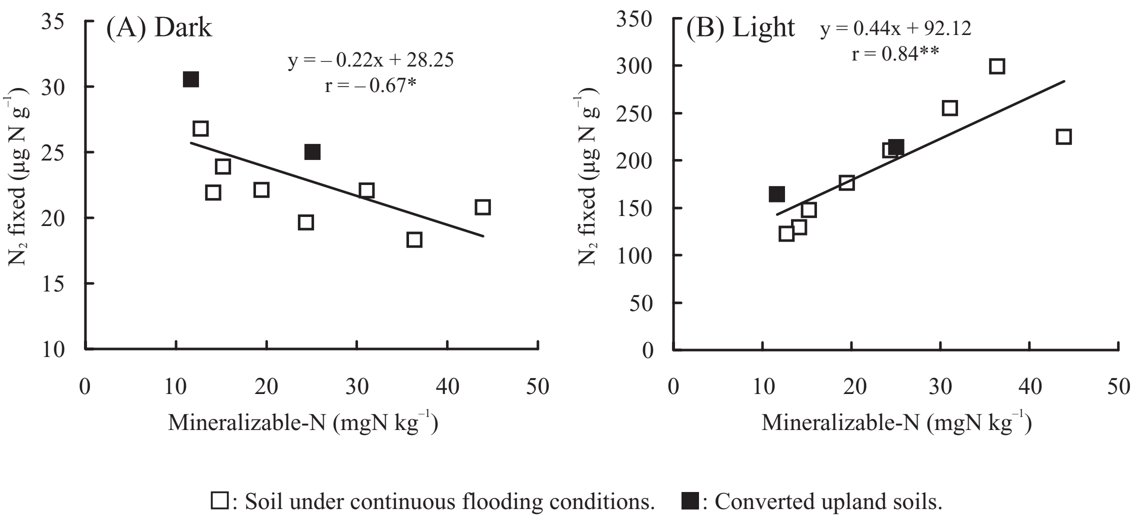

Fig. 2. Relationship between $^{15}N_2$ fixation and amount of mineralizable-N in soil under dark (A) and light (B) conditions (Experiment 3)
* and ** indicate a significance at $p = 0.05$ and 0.01, respectively.

conditions ranged from 0.06 to 0.22 (Table 4) and the soils with lower levels of total-C and mineralizable-N, such as those without NPK, the converted upland soils, the soils without P and no N showed higher values.

Discussion

1. Effects of temperature and C source on heterotrophic BNF

Temperature dependency of N_2 fixation was observed in the presence of cellulose and rice straw, but not with glucose addition. Glucose did not induce any increase of $^{15}N_2$ fixation at higher temperatures, which was probably due to the active decomposition even at low temperatures. The temperature dependency of $^{15}N_2$ fixation was similar to that of the formation of CO_2+CH_4 in the presence of rice straw and glucose, which indicated that increasing temperatures promoted $^{15}N_2$ fixation proportionally to the decomposition of these organic materials. The ratio of N_2fixed/(CO_2+CH_4) was higher in the presence of cellulose than glucose and rice straw particularly at 15ºC. At 15ºC, CH_4 formation was very low for cellulose, probably because of the limited amount of electron acceptors and methanogenic biomass associated with the lower temperature[3,7]. The relatively high ratio of $^{15}N_2$ fixed/(CO_2+CH_4) at low temperatures in the presence of cellulose may be ascribed to a higher availability of substrates for N_2 fixation, such as volatile fatty acids and alcohols, because the accumulation of these intermediate products from the decomposition of cellulosic substrates increased at low temperatures due to the limited level of methane formation[4,16].

As most of other microbial activities, BNF activity in cellulosic substrates is generally enhanced by higher temperatures and the optimum temperature seemed to range from 20 to 45ºC [6,13]. The results obtained in this study indicated that heterotrophic BNF is maintained to an extent similar to or higher than the decomposition rate of organic matter at a temperature as low as 15ºC. These findings indicated the possible important contribution of BNF to the enrichment of soil N under cool temperate conditions.

2. Effect of water regime

Among the water regime cycles, flooding conditions after aerobic conditions showed the highest $^{15}N_2$ fixation. Heterotrophic BNF activity associated with the decomposition of cellulosic materials is particularly high at the aerobic-anaerobic interface[1,5], which can be due to the supply of products from the aerobic decomposition of cellulosic materials to anaerobic or facultative anaerobic N_2-fixing bacteria. In this study, the largest $^{15}N_2$ fixation and the highest ratio of N_2 fixed/(CO_2+CH_4) under flooding after aerobic conditions indicated that the chronological succession from aerobic to anaerobic conditions promoted the decomposition of cellulose and led to efficient N_2 fixation.

3. Effect of soil management

The results obtained in Experiment 3 showed that the effect of soil management on $^{15}N_2$ fixation markedly differed between light and dark conditions. The importance of available-P has been recognized for both heterotrophic and photodependent BNF in paddy soils[2,10,12].

It appeared that photodependent N_2 fixation was more affected by available-P levels than heterotrophic N_2 fixation in this study.

The positive relation of $^{15}N_2$ fixation with the total-C and mineralizable-N contents under the light conditions indicated the importance of the role of decomposable organic matter in the increase of the populations of phototrophic N_2 fixers. Among the organic materials, fresh organic materials such as manure and rice straw were found to be more effective in enhancing $^{15}N_2$ fixation than the compost, which is in agreement with the trends in N enrichment in field evaluation[9].

On the other hand, soils with lower total-C and mineralizable-N contents tended to depend more on heterotrophic BNF, presumably due to the alleviation of the adverse effect of NH_4 on heterotrophic N_2 fixation in part[11]. Converted upland soils showed a relatively higher $^{15}N_2$ fixation than other soils under continuous flooding paddy conditions. A similar trend was observed in N enrichment under field conditions when the C source was applied[9,16]. Because of the large consumption of soil organic matter under aerobic conditions during upland periods[3], converted upland soils showed a low level of mineralizable-N, which was one of the factors for the high heterotrophic N_2 fixation. However, since converted upland soils showed a higher $^{15}N_2$ fixation than other soils in relation to the amount of mineralizable-N, it appeared that the low NH_4 level could not fully account for the enhancement of heterotrophic BNF in these soils. Converted upland soils showed a low CH_4 formation indicating a lower reduction of the soil. Heterotrophic BNF activity becomes highest under moderately low redox potentials, and not under excessively aerobic or extremely low redox potentials[8,15]. The negative relationship between $^{15}N_2$ fixation and CH_4 formation suggested that the BNF activity was promoted when the methanogenic activity was limited. The higher heterotrophic BNF potentials in the converted upland soils were possibly related to the differences in the redox status and anaerobic microflora between the soils under upland and continuous flooding conditions.

The results obtained in this study illustrated the effects of the temperature, water regime, and management of organic matter on BNF in paddy soils. It was suggested that further studies on the variation of the BNF activity with the changes in the redox status should be conducted to understand the role of N_2 fixation in the processes of decomposition of organic matter in paddy soils. In the closed incubation system used in this study, oxygen supply did not remain constant during the incubation and might not be sufficient to support the maximum decomposition rate of added C substrates. Further studies should be carried out on the metabolism of C and N involved in BNF by using oxygen supply conditions relevant to the field conditions to identify efficient management practices of organic matter to enhance BNF.

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