

## FATE OF NITROGEN FROM A BLUE-GREEN ALGA IN A FLOODED RICE SOIL

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Received April 17, 1982

Uptake of  $^{15}\text{N}$  from a blue-green alga (*Nostoc* sp.) by rice (*Oryza sativa*) was studied in pot and field experiments. Availability of  $^{15}\text{N}$  from blue-green algae incorporated into the soil ranged between 23 and 28% for the first crop and between 27 and 36% for the first and second crops. Surface application of the algal material reduced  $^{15}\text{N}$  availability to 14-23% for the first crop and 21-27% for the first and second crops.

The pot experiment demonstrated that for the first crop algal  $^{15}\text{N}$  was less available than ammonium sulfate, but for two crops, its availability was very similar to that of ammonium sulfate. After two crops, 57% of  $^{15}\text{N}$  from blue-green algae and 30-40% of  $^{15}\text{N}$  from ammonium sulfate remained in the soil.

*Key Words:* blue-green algae,  $^{15}\text{N}$ , rice, nitrogenous fertilizers.

It has been established that blue-green algae (BGA) play a vital role in the maintenance and buildup of paddy soil fertility. Information about how much, when, and how the fixed nitrogen is made available to the rice plants, however, remains scarce (8).

The transfer of algal nitrogen to higher plants other than rice has been demonstrated qualitatively in natural ecosystems by MAYLAND and MC INTOSH (5), STEWART (9) and JONES and WILSON (2) using  $^{15}\text{N}$  tracer techniques. Tracer experiments aimed at determining the availability of algal nitrogen to wetland rice and its fate in paddy soils have been scarce and mostly qualitative (6, 10). The only available quantitative data are those from WILSON *et al.* (12) who recovered from a rice crop 37% of the nitrogen from  $^{15}\text{N}$ -labeled *Aulosira* sp. spread on the soil, and 51% of the nitrogen from the same material incorporated into the soil. These studies were conducted on a laboratory scale and did not include analysis of  $^{15}\text{N}$  remaining in the soil.

This paper reports experiments carried out at the International Rice Research Institute (IRRI) to obtain more direct information on the quantification and dynamics of the transfer of the fixed nitrogen from BGA to the rice plant.

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## MATERIALS AND METHODS

*Preparation of  $^{15}\text{N}$ -labeled *Nostoc* cells.* A unialgal *Nostoc* strain, isolated from a paddy field in Sri Lanka, was grown in 20-liter carboy bottles under continuous fluorescent light. The culture medium was a modification of the BG-11 medium (1). The composition is as follows: (mg/liter),  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 36.0;  $\text{K}_2\text{HPO}_4$ , 30.5;  $\text{Na}_2\text{EDTA}$ , 1.0;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 7.5;  $\text{Fe}^{3+}\text{-NH}_4\text{-citrate}$ , 6.0;  $\text{Na}_2\text{CO}_3$ , 100;  $\text{H}_3\text{BO}_3$ , 2.9;  $\text{MnCl}_2 \cdot 3\text{H}_2\text{O}$ , 1.8;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2;  $\text{Na}_2\text{MoO}_3 \cdot 7\text{H}_2\text{O}$ , 0.4;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.08; and  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , 0.05. The culture was continuously stirred with a magnetic stirrer and air enriched with  $\text{CO}_2$  was bubbled through it. The  $\text{CO}_2$  concentration was adjusted to reach a pH of around 7 in the culture. The pH was indirectly measured by trapping outgoing air in a measuring cell filled with the same medium (7). Sometimes, it was necessary to shake the carboy bottle to disperse the algal mass that had formed clumps on the surface or had adhered to the side of the bottle.

Every 2 or 3 days, algal cell density was determined by measuring the absorbance of phycocyanin (620–735 nm) and chlorophyll (680–735 nm) of an aliquot sonicated at 20 kHz for 4 min in an ice bath. When the algal density reached 0.2 g dry weight/liter,  $^{15}\text{N}$ -labeled  $\text{KNO}_3$  (75 atom % excess) was added at the rate of 0.14 g  $^{15}\text{N}$ /bottle (10.4 ppm  $\text{NO}_3\text{-N}$ ). Blue-green algae were grown until nitrate was consumed (10–12 days) and were harvested by decantation and moderate centrifugation (3,000 rpm). The algal material was dried in an incubator at 40°C and was ground by pestle and mortar. Because the desiccation was performed for several days at a temperature favorable for spore formation, the material was very rich in akinetes. Yields ranged from 10 to 12 g dry weight/bottle for 12 days. The algal material from different batches was mixed thoroughly before use; it was composed of 1.13% P, 0.90% K, 0.74% Ca, 0.72% Mg, 38.9% C, 7.3% N, 5.28 C/N, and 23.0 atom % excess  $^{15}\text{N}$ .

*Pot experiment.* Two kilograms (oven-dry weight equivalent) of puddled flooded soil from a field of the IRRI farm (Maahas clay {Aquic Tropudalf}: pH 6.7, clay 56%, organic matter 2.24%, N 0.18%) was put into pots 18 cm in diameter. The pots were filled to a depth of 15 cm. The soil was allowed to stand for 2 days, and then the floodwater was drained off. Four treatments were established.

- S-BGA:  $^{15}\text{N}$ -labeled *Nostoc* sp. spread on the surface of the soil at a rate corresponding to 2 g  $\text{N}/\text{m}^2$  or 50.85 mg  $\text{N}/\text{pot}$ .
- I-BGA:  $^{15}\text{N}$ -labeled *Nostoc* sp. incorporated and mixed thoroughly through the total volume of soil (2 g  $\text{N}/\text{m}^2$  or 50.85 mg  $\text{N}/\text{pot}$ ).
- S-AS:  $^{15}\text{N}$ -labeled ammonium sulfate (21.2% N, 28.55 atom % excess  $^{15}\text{N}$ ) spread on the soil surface at the same level of N as algal N.
- I-AS:  $^{15}\text{N}$ -labeled ammonium sulfate incorporated throughout the total volume of soil.

Nine replicates per treatment were used. After 1 day, the soil was submerged in about 3 cm water and 2 IR36 seedlings were transplanted per pot. Rice plants were cut just above the soil surface at panicle initiation (3 pots) and at maturing stage

(6 pots). After the harvest of the first crop, soil and plants of three pots were analyzed for total nitrogen. Soils of the three other pots were mixed and put into the pots again. The pots were kept flooded for 10 days before a second rice crop was transplanted. After harvest of the second crop, plants and soils were analyzed. The upper 0–2 cm layer of the soil in the pots was scraped off and passed through a 2-mm sieve to separate weeds. Soil remaining in the pot was mixed thoroughly and passed through a 2-mm sieve to separate the roots. Total N and  $^{15}\text{N}$  content of straw, grains, roots, weeds, and soils were determined. Total N was determined by the Kjeldahl method.  $^{15}\text{N}$  was analyzed by emission spectrometry. The Dumas combustion method modified by KUMAZAWA (3) was used for plant samples; the method of YONEYAMA and KUMAZAWA (13), for the soil.

*Field experiment.* The experiment was conducted at the IRRI farm, on soil that had been continuously flooded and had not received nitrogen fertilizer for more than 5 years. The soil in each plot was mixed with a rotary weeder three times every other day. The soil was drained, and two metal square frames, 1.5 m  $\times$  1.5 m and 40 cm in height, were placed to a depth of 15 cm from the soil surface.

Inside one frame, 65 g of labeled BGA material corresponding to 2 g N/m<sup>2</sup> or 1.245 g  $^{15}\text{N}$ /plot, mixed with a small amount of air-dried soil, was spread by hand as evenly as possible on the soil surface. In the other frame, the same amount of  $^{15}\text{N}$ -labeled *Nostoc*, mixed with a small amount of air-dried soil was incorporated by hand into the soil to a depth of 15 cm.

One day after the addition of algal material, the field was flooded. Three days after flooding, IR36 seedlings were transplanted inside and outside the metal frames at 20-cm  $\times$  20-cm spacing. Thirty-six rice hills (6 rows  $\times$  6 rows) were transplanted inside a frame. Care was taken not to disturb the soil inside the frames by handling from outside.

After the first crop had been harvested, the field was flooded for 4 weeks, then a second crop was grown similarly. At maturity of both crops, the grain and the straw just above the soil surface were harvested. Three alternating rice plants from each row were combined to make up one composite sample per row. Thus, six composite samples were made from a frame. One day after harvest, soil samples were taken by inserting glass tubes 3 cm in diameter and 20 cm long into the soil up to a depth of 15 cm. A total of 10 composite samples (3 cores each) were taken from each frame, 5 from the center of 4 hills, and 5 near (5 cm away from) the rice hills. The upper 2-cm layer of soil was separated from the lower layer of the cores. Soil nitrogen and plant nitrogen and their  $^{15}\text{N}$  content were analyzed as in the pot experiment.

## RESULTS

### *Pot experiment*

One week after transplanting, algal films developed on the surface of floodwater in the pots where *Nostoc* was spread on the soil surface. Microscopic examination

revealed the presence of *Nostoc* filaments and spores, indicating that the applied dried BGA had recovered.  $^{15}\text{N}$  content of algal films showed that  $^{15}\text{N}$  was diluted from 23 atom % excess to 9%. No quantitative measurements were made to determine the contribution of the inoculated *Nostoc* to the total algal biomass. However, because the inoculated strain was dominant, it can be concluded that the dilution of  $^{15}\text{N}$  was due mainly to  $\text{N}_2$ -fixation by the *Nostoc*. After 3 weeks, most of the BGA in the pots with surface-applied *Nostoc* disappeared and green algae became dominant. In the other treatments, only green algae developed during the first week; after three weeks, BGA other than *Nostoc* appeared.

Recovery of  $^{15}\text{N}$  from rice and weeds at the panicle initiation stage (48 days after transplanting) (Table 1) showed that nitrogen applied on the soil surface was less available to the plant than nitrogen that had been incorporated. The difference between surface-applied BGA and ammonium sulfate was not significant. In contrast, the availability of nitrogen from the incorporated ammonium was higher than that of nitrogen from incorporated BGA. The higher availability of nitrogen from the incorporated materials was confirmed by measurements performed at harvest time (Table 2a). The Duncan's Multiple Range Test (DMRT) showed no significant difference between ammonium sulfate and algal material. However, analysis of variance showed a significant effect of the source of nitrogen, indicating that algal N was slightly less available to the rice crop than ammonium sulfate.

The patterns of  $^{15}\text{N}$  distribution in grain, straw, roots, and weeds were identical in the four treatments. Because total  $^{15}\text{N}$  amount recovered from the plants was almost the same at panicle initiation and at maturity, it can be stated that most of the  $^{15}\text{N}$  was absorbed before panicle initiation and then translocated from the roots

Table 1. The recovery of  $^{15}\text{N}$  from rice shoots and roots and weeds at panicle initiation stage of the first rice crop—pot experiment.

Treatment <sup>a</sup>	Total N <sup>c</sup> (mg/pot)	Recovery of $^{15}\text{N}$ <sup>b</sup> (%)		
		Shoots	Roots and weeds	Total <sup>c</sup>
S-BGA	127 a	10.4 b	4.1 c	14.5 c
I-BGA	132 a	21.2 ab	6.9 ab	28.1 b
S-AS	145 a	13.1 b	4.3 c	17.4 c
I-AS	122 a	30.9 a	9.7 a	40.6 a
F (BGA-A)	—	—	—	4.208 ns
F (S-I)	—	—	—	23.853**
F (BGA-A)*(S-I)	—	—	—	1.612 ns

<sup>a</sup> S, surface applied; I, incorporated; AS, ammonium sulfate. <sup>b</sup> The amounts of applied  $^{15}\text{N}$  were 11.70 mg blue-green algae/pot and 14.53 mg ammonium sulfate/pot. <sup>c</sup> Each figure is a mean of 3 replicates. Means followed by a common letter are not significantly different at the 5% level. \*\* Significant at 1% level. ns: not significant.

Table 2a. Total nitrogen and recovery of  $^{15}\text{N}$  in the plants and the soil after one rice crop—pot experiment.

Treatment	Total N Plant and straw (mg/pot)	Recovery of $^{15}\text{N}$ (%)			
		In plants (1st crop)			
		Grain	Shoots	Roots and weeds	Total <sup>a</sup>
S-BGA	127 a	9.0 c	3.0 b	1.8 b	13.8±3.6 b
I-BGA	123 a	17.7 b	6.0 a	4.2 a	27.9±1.8 a
S-AS	118 a	11.4 c	3.9 b	2.4 b	17.7±6.4 b
I-AS	125 a	24.6 a	7.8 a	4.3 a	36.7±5.7 a
F (BGA-AS)					32.97**
F (I-S)					221.69**
F (BGA-AS)*(I-S)					4.5 ns

  

Treatment	Recovery of $^{15}\text{N}$ (%)			Total in plant and soil
	In soil			
	Surface (0-2 cm)	Subsurface (2-15 cm)	Total (0-15 cm)	
S-BGA	30.8 a	28.3 b	59.1±19.9 ab	72.9±22.9 b
I-BGA	24.0 a	39.6 a	63.6±7.3 a	91.4±6.0 a
S-AS	31.1 a	19.1 b	50.2±12.4 b	67.9±18.5 b
I-AS	27.3 a	25.1 b	52.4±12.9 b	88.9±7.3 a
F (BGA-AS)			9.65*	1.12 ns
F (I-S)			1.05 ns	30.27**
F (BGA-AS)*(I-S)			1.27 ns	1.14 ns

<sup>a</sup> Average±95% confidence limit. \* Significant at the 5% level. \*\* Significant at the 1% level.

Table 2b. Total nitrogen and recovery of  $^{15}\text{N}$  in the plants and the soil after two rice crops—pot experiment.

Treatment	Total N	Recovery of $^{15}\text{N}$ (%)					
		In plants (2nd crop)				In soil (total) (0-15 cm)	Total in plant and soil
		Grain	Straw	Roots and weeds	Total		
S-BGA	77.2 ab	4.4 ab	1.6 a	1.1 b	7.1±1.5 ab	57.3±4.2 a	64.4±4.3 a
I-BGA	79.3 a	4.6 a	1.5 a	2.4 a	8.5±3.2 a	57.7±15.4 a	66.2±17.7 a
S-AS	56.8 c	2.0 c	0.7 b	0.6 c	3.3±2.1 c	30.9±13.6 b	34.2±15.6 b
I-AS	65.0 abc	3.2 bc	1.1 ab	1.4 b	5.7±1.2 b	41.6±4.4 b	47.3±3.6 b
F (BGA-AS)					35.66**	71.96**	77.07**
F (I-S)					10.37*	4.81 ns	7.03*
F (BGA-AS)*(I-S)					5.79*	4.26 ns	3.72 ns

and shoots to the grains, and that the  $^{15}\text{N}$  concentration in the grains was highest at maturity. Total nitrogen content of rice did not differ among treatments. But the content of  $^{15}\text{N}$  remaining in the soil was highly variable. More  $^{15}\text{N}$  from incorporated BGA's nitrogen than  $^{15}\text{N}$  from incorporated ammonium sulfate remained in the soil after harvest (Table 2a), reflecting the plant's lower uptake of BGA's nitrogen than of ammonium sulfate nitrogen. In all treatments, total recovery was significantly lower than 100% (Table 2a). No significant difference in total  $^{15}\text{N}$  recovery was observed between BGA and ammonium sulfate applied similarly, indicating that BGA may be as susceptible to nitrogen losses as ammonium sulfate (4, 14).

Results concerning the second crop (Table 2b) indicate that, when the method of nitrogen application was identical, the total recovery of  $^{15}\text{N}$  was higher in the BGA-treated pots than in the ammonium sulfate-treated pots. This is in agreement with a higher  $^{15}\text{N}$  content, after the first crop, in soils where BGA were applied (Table 2a).

Results of the pot experiments are summarized in Table 3. The recovery of  $^{15}\text{N}$  from 2 rice crops was 20% with surface applications of either BGA or ammonium sulfate. It was about 40% with incorporation of either BGA or ammonium sulfate. The availability of BGA's nitrogen to rice was almost comparable to that of ammonium, when the two crops were taken into account. After two crops total recovery of  $^{15}\text{N}$  was higher from BGA than from ammonium sulfate, mainly because of a lower recovery of  $^{15}\text{N}$  in soils and plants after the second crop in ammonium sulfate-treated pots.

Results from both first and second crops also confirm the beneficial effect of the incorporation of fertilizers (11).

#### Field experiment

The soil used contained 0.177% N with  $0.367 \pm 0.02$  (standard deviation of 6 samples) atom %  $^{15}\text{N}$ .

Table 3. Recovery of  $^{15}\text{N}$  after two crops of rice in soil and plants—pot experiment.

Treatment	Recovery of $^{15}\text{N}$ (%)					Loss of $^{15}\text{N}$ (%)
	In plant			In soil after 2 crops	Total in plant and soil	
	1st crop	2nd crop	Total			
S-BGA	13.8±3.6 b	7.1±1.5 ab	20.9±2.9 b	57.3±4.2 a	78.2±4.4 b	21.8
I-BGA	27.9±1.8 a	8.5±3.2 a	36.4±2.8 a	57.7±15.4 a	94.1±12.0 a	5.9
S-AS	17.7±6.4 b	3.3±2.1 c	21.0±5.0 b	30.9±13.6 b	51.9±12.7 c	48.1
I-AS	36.7±5.7 a	5.7±1.2 b	42.4±4.1 a	41.6±4.4 b	84.0±5.2 a	16.0
F (BGA-A)	31.97**	35.66**		71.96**	29.0**	
F (I-S)	221.69**	10.36*		4.81 ns	50.00**	
F (BGA-AS)*	4.56 ns	5.79*		4.26 ns	5.7*	

About 10 days after the *Nostoc* application, an algal film covered almost the whole surface of both plots. Microscopic observation of algae showed *Nostoc* as the dominant BGA together with *Anabaena* and unicellular BGA as associated species, suggesting that inoculated *Nostoc* was able to establish in the field. Eighteen days after the algal application, floating BGA masses were collected from 5 sites in the frames for  $^{15}\text{N}$  analysis: contents were 3.84 atom % excess in the surface-applied treatment and 3.36 atom % excess in the soil-incorporated treatment. Outside the frames where *Nostoc* was not applied, no BGA growth was visible. After 4 weeks, *Spirulina* was the dominant BGA in the plot with surface-applied treatment while *Oscillatoria* and *Nostoc* were the associated species. In the plot with soil-incorporated treatment, *Nostoc* was still the dominant BGA. After 6 weeks, the BGA in both plots disappeared, and weeds and green algae started to grow.

About 20% of  $^{15}\text{N}$  from both surface-applied and incorporated *Nostoc* was absorbed by the first crop (Table 4a). In both treatments, 3% of  $^{15}\text{N}$  was recovered in the weeds. The  $^{15}\text{N}$  remaining in soil was on the average, the same in the 2 treatments (44 and 46%). The difference between the  $^{15}\text{N}$  remaining in the surface (0–2 cm) soil and that remaining in the subsurface (2–15 cm) soil was not significant, suggesting the homogenization of the distribution of algal material along the soil profiles in the two treatments. A downward migration of the surface-applied algal material may have been caused by the action of the soil microfauna and macrofauna

Table 4a. Recovery of  $^{15}\text{N}$  in the crop and the soil after one crop of rice—field experiment.

Treatment	Recovery of $^{15}\text{N}$ (%)						
	1st crop				Soil		
	Straw	Grain	Weeds	Total	Surface (0–2 cm)	Subsurface (2–15 cm)	Total (0–15 cm)
Surface-applied	6.7	13.3	3.1	23.1	36.8	9.7	46.5
Incorporated	8.7	12.3	2.5	23.5	32.1	11.5	43.6
95% confidence limit	2.8	2.7	—	3.3	5.8	3.3	6.7

Table 4b. Recovery of  $^{15}\text{N}$  in the crop and the soil after two crops of rice—field experiment.

Treatment	Recovery of $^{15}\text{N}$ (%)						
	2nd crop				Soil		
	Straw	Grain	Weeds	Total	Surface (0–2 cm)	Subsurface (2–15 cm)	Total (0–15 cm)
Surface-applied	0.59	1.78	1.51	3.88	23.8	7.80	31.6
Incorporated	1.01	2.02	0.26	3.29	14.1	18.7	32.8
95% confidence limit	0.36	0.70	—	1.00	3.38	4.2	5.4

Table 5. Recovery of  $^{15}\text{N}$  in crop and soil after two crops of rice—field experiment.

Treatment	Recovery of $^{15}\text{N}$ (%)					
	In plant			In soil after 2 crops (0–15 cm)	Total in plant and soil (0–15 cm)	$^{15}\text{N}$ unrecovered (%)
	1st crop	2nd crop	Total			
Surface-applied	23.1	3.9	27.0	31.7	58.7	41.3
Incorporated	23.5	3.3	26.8	32.8	59.6	40.4

and the mechanical effects of buffeting rains (mixing action and percolation) that accompanied two typhoons, one of which occurred just after the application of the algal material. The high concentration of  $^{15}\text{N}$  in the surface soil (0–2 cm) cannot be adequately explained by the motility of hormogonia from the incorporated algal material but may also be due to an imperfect mixing related to the floating property of the dried algal material. Because the  $^{15}\text{N}$  contents of algal films that developed 18 days after the application were similar in the 2 treatments, it is probable that homogenization took place early in the growth cycle.

Recoveries of  $^{15}\text{N}$  from plants and soil after 1 crop were approximately 65% in both surface-applied and incorporated BGA treatments.

Recoveries of  $^{15}\text{N}$  from the second crop of rice (Table 4b) and soil were similar in the two treatments. Recovery of  $^{15}\text{N}$  from rice and soil after 2 crops was about 60% in both treatments (Table 5). Because soil samples were not taken below 15 cm from the soil surface, and because the disturbance caused by the typhoons induced appreciable losses of algal material through leaching, some amounts of  $^{15}\text{N}$  may not have been recovered. The absence of loss through leaching from the pots is the reason for the higher  $^{15}\text{N}$  recovery from the pots particularly in the subsurface soil than from the field. The recoveries were 9.7% *versus* 28.3% for surface-applied material and 11.5% *versus* 39.6% for the incorporated material (Tables 2a and 4a).

#### DISCUSSION

Algal material spread in the field corresponded to 20 kg N/ha, 290 kg dry weight/ha, and 13 tons of fresh weight/ha. The quantity of algal material applied was selected by comparing the few data available in the literature for algal biomasses developing in paddy fields. Data recorded varied from a few kilograms to 24 tons (fresh weight)/ha (8). Hence, the quantity of algal material applied may be considered equivalent to that of a dense algal bloom.

The pot experiment conducted in a greenhouse was protected from environmental disturbances and therefore permitted a good comparison between surface-applied and incorporated treatments. However, in the pots, prevention of losses through leaching led to an overestimation of nitrogen availability to the plant. The field experiment was subjected to severe environmental disturbances, including two typhoons. There-

fore, surface-applied and incorporated material could not be compared and important losses from downward movement and seepage decreased N availability to the rice plant and the total nitrogen recovery from rice and soil up to a depth of 15 cm.

The two treatments (surface-applied and incorporated) were selected to represent two prevailing situations in the field. Under natural conditions,  $N_2$ -fixing algal blooms develop any time throughout the cultivation cycle; however, early growth is rare and BGA seem to develop preferentially during the second part and at the end of the growth cycle of rice (8).

When the algal bloom develops later in the cycle, most of the algal material will dry on the surface of the soil, be incorporated by plowing, and start to decompose only at the beginning of the next growth cycle. This sequence is similar to the situation where dried BGA were incorporated. From the results of the pot and field experiments, it appears that the availability of nitrogen from BGA incorporated into the soil ranged between 23 and 28% for the first crop and between 27 and 36% for both the first and second crops (Tables 3 and 5).

If the algal bloom develops early in the cycle, decomposition by lytic microorganisms and grazing by aquatic fauna, are likely to occur later during the same cycle, thereby making nitrogen available in the floodwater and on the soil surface. This situation, which is unfrequent under natural conditions occurs when paddy fields are inoculated by algae and is somewhat similar to that in the treatments where dried BGA were surface-applied, since an algal bloom also developed early in the cycle, but different in that the decomposition started at the beginning of the growth cycle. That may have led to an overestimation of the availability of algal nitrogen to the current rice crop. From the results of the pot and field experiments, it appears that the availability of nitrogen from BGA spread on the soil surface ranged between 14 and 23% for the first crop and between 21 and 27% for both the first and second crops.

In the field experiment, climatic disturbances in addition to a low depth of sampling did not permit balance calculations and estimation of the losses. In the pot experiment, apparently more nitrogen was lost from ammonium sulfate than from BGA after two crops. However, applied BGA multiplied causing an increase of total nitrogen in the pot and a dilution of algal  $^{15}N$ . Therefore, although  $^{15}N$  was representative of the total N balance in the ammonium sulfate-treated pots,  $^{15}N$  balance in the BGA-treated pots underestimated losses of total nitrogen as well as availability of algal nitrogen to the plant.

Availability of algal nitrogen reported by WILSON *et al.* (12), from a pot experiment, was almost twice as high as that which we measured under similar experimental conditions. The reason for this discrepancy is certainly related to the nature of the algal material, the method of preparation, and the nature of the strain. WILSON *et al.* (12) used an algal material collected directly from the flask culture and blended after resuspension in distilled water. Therefore, it can be suspected that this material was highly susceptible to decomposition because it consisted mainly of vegetative cells, a part of which may have been damaged by blending. In contrast; the material we

used consisted mainly of vegetative cells in dormancy and of akinetes and was, therefore, much less susceptible to decomposition. This hypothesis is in agreement with the results of a preliminary pot experiment (unpublished) where we used the same *Nostoc* strain directly collected from the carboy culture. When this fresh material, composed mainly of vegetative cells, was incorporated, about 38% of the  $^{15}\text{N}$  was recovered in the first crop instead of 28% when dried material was used.

In agreement with WILSON *et al.* (12), we found an enhanced availability of blue-green algae's nitrogen by incorporation. This may be due to an accelerated decomposition of BGA in the anaerobic soil layer and a better proximity of the released nitrogen to the roots. The beneficial effect of deep placement of nitrogen fertilizers due to the decrease of gaseous nitrogen losses by denitrification and volatilization (11) is well documented. Our results confirmed this observation.

The pot experiment demonstrated that for the first crop algal nitrogen was less available than ammonium sulfate but for two crops its availability was very similar to that of ammonium sulfate indicating the slow-release nature of BGA nitrogen, a finding which agrees with the cumulative effect of algal inoculation (8). However, its very low C/N ratio (from 5 to 6) gives it a better nitrogen availability than that of organic fertilizers such as farmyard manure.

#### REFERENCES

- 1) ALLEN, M.M. and STANIER, R.Y., Selective isolation of blue-green algae from water and soil, *J. Gen. Microbiol.*, **51**, 203-209 (1968)
- 2) JONES, K. and WILSON, R.E., The fate of nitrogen fixed by a free-living blue-green alga. Environmental role of nitrogen-fixing blue-green algae and symbiotic bacteria, *Ecol. Bull.*, **26**, 158-163 (1989)
- 3) KUMAZAWA, K., Optical spectrographic analysis of heavy nitrogen ( $^{15}\text{N}$ ), *Jasco Spectra*, Jasco Japan Spectroscopic Co., Ltd., **2** (1), 1-8 (1974)
- 4) MACRAE, I.C. and ANCAJAS, R., Volatilization of ammonia from submerged tropical soils, *Plant Soil*, **33**, 97-103 (1970)
- 5) MAYLAND, H.F. and Mc INTOSH, T.H., Availability of biologically fixed atmospheric  $^{15}\text{N}$  to higher plants. *Nature*, **209**, 421-422 (1966)
- 6) RENAULT, J., SASSON, A., PEARSON, H.W., and STEWART, W.D.P., Nitrogen fixing algae in Morocco, *In Nitrogen Fixation by Free-Living Microorganisms*, ed. by W.D.P. Stewart, Cambridge University Press, Cambridge, London, 1975. pp. 229-249
- 7) ROGER, P.A., Réalisation d'un dispositif de culture régulée pour algues fixatrices de  $\text{N}_2$  (An apparatus for regulating growth of  $\text{N}_2$ -fixing algae), *Biol. Sol.*, **19**, 29-32 (1974)
- 8) ROGER, P.A. and KULASOORIYA, S.A., Blue-green Algae and Rice, The International Rice Research Institute, Los Baños, Laguna, Philippines, 1980. 112 pp.
- 9) STEWART, W.D.P., Transfer of biologically fixed nitrogen in a sand dune slack region, *Nature*, **214**, 603-607 (1970)
- 10) VENKATARAMAN, G.S., Blue-green algae as a biological N-input in rice cultivation, Proc. Natl. Symp. Nitrogen Assimilation and Crop Productivity, Hissar, India, 1977. pp. 132-141
- 11) VENTURA, W. and YOSHIDA, T., Distribution and uptake by rice plants of  $^{15}\text{N}$ -labeled ammonium applied in mudballs in paddy soils, *Soil Sci. Plant Nutr.*, **24** (4), 473-479 (1978)
- 12) WILSON, J.T., ESKEW, D.L., and HABTE, M., Recovery of nitrogen by rice from blue-green algae added in a flooded soil, *Soil Sci. Soc. Am. J.*, **44**, 1330-1331 (1980)

- 13) YONEYAMA, T. and KUMAZAWA, K., A kinetic study of the assimilation of  $^{15}\text{N}$ -labeled ammonium in rice seedling roots, *Plant Cell Physiol.*, **15**, 655-661 (1974)
- 14) YOSHIDA, T. and PADRE, B., Transformation of soil and fertilizer nitrogen in paddy soil and their availability to rice plants, *Plant Soil*, **47**, 113-123 (1977)