Biol Fertil Soils (1993) 15:241–248

Biology and Fertility of Soils

Green manure production of *Azolla microphylla* and *Sesbania rostrata* and their long-term effects on rice yields and soil fertility

W. Ventura and I. Watanabe

International Rice Research Institute (IRRI), Division of Soil Microbiology, P.O. Box 933, Manila, Philippines

Received July 21, 1992

Summary. *Azolla* spp. and *Sesbania* spp. can be used as green manure crops for wetland rice. A long-term experiment was started in 1985 to determine the effects of organic and urea fertilizers on wetland rice yields and soil fertility. Results of 10 rice croppings are reported. *Azolla* sp. was grown for 1 month and then incorporated before transplanting the rice and 3–4 weeks after transplanting the rice. *Sesbania rostrata* was grown for 7–9 weeks and incorporated only before transplanting the rice. *Sesbania* sp. grew more poorly before dry season rice than before wet season rice. *Aeschynomene afraspera*, which was used in one dry season rice trial, produced a larger biomass than the *Sesbania* sp. The quantity of N produced by the *Azolla* sp. ranged from 70 to 110 kg N ha^{-1}. The *Sesbania* sp. produced 55–90 kg N ha^{-1} in 46–62 days. Rice grain yield increases in response to the green manure were 1.8–3.9 t ha^{-1}, similar to or higher than that obtained in response to the application of 60 kg N ha^{-1} as urea. Grain production per unit weight of absorbed N was lower in the green manure treatments than in the urea treatment. Without N fertilizer, N uptake by rice decreased as the number of rice crops increased. For similar N recoveries, *Sesbania* sp. required a lower N concentration than the *Azolla* sp. did. Continuous application of the green manure increased the organic N content in soil on a dry weight basis, but not on a area basis, because the application of green manure decreased soil bulk density. Residual effects in the grain yield and N uptake of rice after nine rice crops were found with a continuous application of green manure but not urea.

Key words: Organic matter – *Azolla* spp. – *Sesbania* spp. – *Aeschynomene afraspera* – Fertility trials – Flooded rice soil – Green manuring – Biofertilizer – Decomposition

Organic matter management and biological N_2 fixation are the major components of a sustainable rice agro-ecosystem. With the increasing costs of inorganic fertilizers and the growing concern in long-term soil fertility and environmental protection, interest in N_2-fixing green manure has recently increased.

The application of green manure adds N, adds (or recycles) other nutrient elements, increases the organic matter content of the soil, and affects the soil physical and biological properties. The application may also increase the long-term fertility of rice soils. Green manure has an advantage over other organic manures in that it can be grown directly in the field and can be incorporated during land preparation or regular weeding operations. Although this type of manure has long been known as a source of plant nutrients, very little is known about the effect of a continuous application of green manure in flooded rice soils. It is therefore necessary to study long-term effects of green manure on the wetland rice-soil system.

In the tropics, green manure can be grown between two rice crops, but available irrigation or rainwater limits the duration of its growth to about 30–60 days. Fast-growing and N-accumulating plants are therefore needed. Among several types of aquatic leguminous green manure studied, *S. aculeata* (Ladha et al. 1988), *S. rostrata* (Ventura et al. 1987, Becker et al. 1990a, b; Ladha et al. 1989), and *Aeschynomene afraspera* (Becker 1990; Becker et al. 1990a) were found to be promising, because they fixed large amounts of N_2 and grew quickly under both dryland and flooded soil conditions. *Azolla* spp., a small aquatic fern, which lives in association with N_2-fixing cyanobacteria, has also proved to be a valuable green manure for wetland irrigated rice because it has a high N_2-fixing ability and rapid growth and can be grown before and during the rice crop (Lumpkin and Plucknett 1982; Watanabe 1987; Ventura et al. 1987; Watanabe et al. 1989; Ventura et al. 1992).

Our first report (Ventura et al. 1987) focused on the suitability of *Azolla* spp. and *Sesbania* spp. as biofertilizer for wetland rice. The aim of the present study was

---

Correspondence to: W. Ventura

to examine the long-term effects of *A. microphylla* and *S. rostrata* on the yield of irrigated wetland rice and on soil fertility compared with urea fertilizer and a no-N input control.

Materials and methods

A long-term field experiment was started in March 1985 in 7-×7-m plots at an International Rice Research Institute (IRRI) field. The aim was to demonstrate the feasibility of replacing industrially produced N sources with farm-grown fertilizers. The soil (Andaqueptic Haploquoll), a silty clay, had a pH of 6.3, total N of 1.4 g kg^{-1}, and a cation exchange capacity of 335 mEq kg^{-1} air-dried soil. The treatments were as follows:

1. Urea fertilizer was applied at 50 kg N ha^{-1} (first, second, and third rice crops) or 60 kg N ha^{-1} (fourth and succeeding rice crops) in a split application (30 kg N basal, before the last harrowing, and 20–30 kg N as topdressing at about panicle initiation stage).

2. *Azolla* sp. was inoculated at 0.3 kg fresh weight m^{-2} after the first plowing and harrowing of plots (30 days before transplanting the rice). When the plot was fully covered with *Azolla* sp., it was drained for a day, and then the fern was incorporated manually or by rotary weeder. About 60–70% of the total *Azolla* sp. biomass was incorporated. The remaining 30–40% was allowed to grow for 12–18 days or until saturation coverage was reached. A day before transplanting, 85–90% of the *Azolla* sp. biomass was submerged, coinciding with the final harrowing and leveling. The remaining 10–15% *Azolla* sp. was allowed to grow or, if there was insufficient fern, *Azolla* sp. at 0.3 kg fresh weight m^{-2} was inoculated 14–16 days after transplanting and then incorporated during the first weeding operation at about 30 days after transplanting.

A. microphylla 4018 (from Paraguay) was used alone up to the seventh crop. A mixture of *A. microphylla* 4018 and hybrid strain 4028 (from IRRI, *A. microphylla* 4018×*A. filiculoides* 1001, Do Van Cat et al. 1989) in equal proportions was used as the inoculum for the eighth and ninth rice crops. A water solution of triple superphosphate was applied by sprinkler at 0.87–2.17 kg water-soluble P ha^{-1} every week during the *Azolla* sp. growth. *Azolla* spp. insect pests were controlled by spraying triazophos at the manufacturer's recommended rate.

3. *S. rostrata* (West Africa, Dreyfus and Dommergues 1981) seeds were scarified, using concentrated H_2SO_4 for 20 min, then rinsed five times with water, and dried in the shade. The seeds were broadcast at a seed rate of 30–40 kg ha^{-1} after plowing and draining the plots. No irrigation water was allowed in the plots for 10–14 days. Then floodwater was introduced, and the field was kept submerged throughout the *Sesbania* sp. growth, until its incorporation into the flooded soil 1 day before transplanting the rice. For the eighth rice crop, *Aeschynomene afraspera* (accession no. 14054, from Senegal) was grown in place of *S. rostrata*, using the same management.

No bacterial inoculation was made because the soil used in this study contained an adequate population of rhizobial strains for both *Sesbania* sp. and *Aeschynomene* sp. and good spontaneous nodulations were observed in roots up to 14 days old and in stems at later stages of growth.

4. In a control treatment, no N fertilizer was applied. With the tenth rice crop, no fertilizer was applied in any treatment, in order to determine the residual effects on rice growth of the urea and the green manure treatments.

Treatments 1, 2 and 3 had four replicates and treatment 4 had three replicates. Plots were assigned to four blocks. The experimental plots were irrigated carefully to avoid contamination among plots. Irrigation water was allowed to flow continuously in the canals to the drainage outlet for about 15 min to clean the irrigation water of algae and weeds before allowing it to enter the plots. The plots were drained 2 weeks before the rice harvest.

The *Azolla* sp. biomass was estimated before incorporation by collecting samples in 25-×25-cm frames from 10 locations in every plot. The *Sesbania* sp. biomass was determined from two 1-m^2 subsamples in every plot before incorporation. Dried and ground (passing through 40-mesh screen) samples were analyzed for N by the micro-Kjeldahl technique (Yoshida et al. 1972).

The sampling area for rice yield determination was 8 m^{-2}, taken from two opposite sites (five rows with 20 plants per row) in the plot, starting from the fifth plant row. The plants were cut 3 cm from the ground, gathered together, and threshed. Then the base of the straw was washed in running water to remove adhering soil particles. The plants remaining in the plot were cut in the same way and taken out of the field. The entire bulk of grain or straw from each plot was mixed, and then three composite samples were taken for analysis by the micro-Kjeldahl method (Yoshida et al. 1972).

Just before sowing the green manure for the first rice crop, soil bulk density was determined at a depth of 0–20 cm with the use of a 20-×20-×20-cm (width×length×depth) box, inserted into the desired depth of the puddled soil (three sites in every plot). Sampling sites were randomly selected across the experimental site. The entire volume of soil was weighed and then soil samples were taken from the mixed soil. Dry weight and total N as wet soil were determined (macro-Kjeldahl, Bremner and Shaw 1958). After the seventh rice crop was harvested, soil samples were taken from the 0–20 cm and 20–50 cm layers. Bulk density and N content were determined from the wet soil. A portion of the soil sample was air-dried, ground, and passed through a 2-mm sieve. Organic C (chromic acid method of Walkley and Black as described by Jackson 1958), pH (1 : 1 soil: water ratio) and other soil chemical analyses were made.

The decomposition of *Aeschynomene afraspera* (62 days old) in wetland rice soil was studied during the eight rice crop and that of *S. rostrata* (50 days old) during the ninth rice crop. The newly harvested green manure plants were separated into leaves and stem-root stubble. The stem-root stubble was cut into 2- to 3-cm pieces. Ten grams of the stem or leaves were put into a nylon net bag (5×10 cm, 0.7-mm mesh). Just after the rice was transplanted, a number of these bags were buried at a depth of 10 cm, each in the middle of four plant hills. At periodic intervals, 10 bags of either leaves or stems were taken out and the bags were rinsed by gently soaking them five times in distilled water. The dry weight and ash content were measured. The organic matter content was determined (ignition at low temperature by Mitchell, as described by Jackson 1958).

Results and discussion

The rice cropping seasons, rice varieties, and growth duration of legumes and *Azolla* sp. are shown in Table 1.

Green manure production

The *Azolla* sp. biomass production was lower for the first rice crop than for the others (Table 1), because the phosphate fertilizer applied had a low level of water-soluble P (less than 1% of available P). The application of water-soluble triple superphosphate significantly improved the percentage N content and biomass of the succeeding rice crops. About 65–80% of the *Azolla* sp. N was produced within 30 days before rice transplanting, and 20–35% within 15–20 days after rice transplanting, with the *Azolla* sp. grown together with the rice plants (data not shown). Inoculating the eighth and ninth rice crops with an equal proportion of *A. microphylla* 4018 and hybrid 4028, instead of *A. microphylla* 4018 alone, increased both the *Azolla* sp. percentage N content (4.2–5.2) and N production.

Table 1 shows the amount of aquatic legume produced, the percentage N content, and the amount of accumulated N. The *Sesbania* sp. growth period was 50–58 days for the wet season rice crop and 53–60 days for the dry season rice crop. The leaves comprised about 25% of

Table 1. Amount of green manure incorporated, N added, fertilizer N recovery, and fertilizer N efficiency of urea, *Azolla* sp. Sesbania sp. in nine rice crops, International Rice Research Institute field, 1985 – 1989

Crop no.	Cropping period/ variety	Fertilizer material	Green manure added		N (%)	Amount of N incorporated (kg ha^{-1})	Apparent N recovery (%)	Fertilizer efficiency (kg grain·kg^{-1} N added)	N use efficiency (kg grain·kg^{-1} N absorbed)
			t dry weight ha^{-1}	Days of growth					
1	May – Aug 1985	Urea			46.30	50	30	22	60
	(IR54)	*Azolla* sp.	1.5	57	1.87	28	32	7	22
	W	*Sesbania* sp.	3.2	46	2.31	74	63	19	30
2	Oct 1985 – Feb 1986	Urea			46.30	50	60	24	40
	(IR54)	*Azolla* sp.	2.6	58	3.70	96	77	22	28
	D	*Sesbania* sp.	2.7	60	2.37	64	75	25	20
3	Jun – Sept 1986	Urea			46.30	50	46	20	43
	(IR54)	*Azolla* sp.	3.0	56	2.90	87	54	17	32
	W	*Sesbania* sp.	4.0	53	1.98	79	61	18	29
4	Dec 1986 – Apr 1987	Urea			46.30	60	53	30	56
	(IR54)	*Azolla* sp.	2.8	50	3.43	96	61	34	56
	D	*Sesbania* sp.	3.2	61	1.72	55	67	31	46
5	Jul – Oct 1987	Urea			46.30	60	48	18	38
	(IR54)	*Azolla* sp.	2.2	53	3.18	70	67	24	37
	W	*Sesbania* sp.	5.0	53	1.80	90	40	14	36
6	Jan – Apr 1988	Urea			46.30	60	51	43	74
	(IR66)	*Azolla* sp.	2.8	51	3.14	88	69	38	55
	D	*Sesbania* sp.	3.6	57	1.81	65	68	34	50
7	Jul – Oct 1988	Urea			46.30	60	59	22	36
	(IR68)	*Azolla* sp.	3.1	53	3.12	97	71	17	25
	W	*Sesbania* sp.	6.0	67	1.37	82	56	13	23
8	Feb – May 1989	Urea			46.30	60	48	33	69
	(IR66)	*Azolla* sp.	2.6	57	4.23	110	82	35	43
	D	*Aeschynomene* sp.	3.4	62	2.50	85	45	25	58
9	Jul – Nov 1989	Urea			46.30	60	60	18	31
	(IR72)	*Azolla* sp.	2.1	60	5.19	109	85	11	12
	W	*Sesbania* sp.	2.7	50	2.26	61	82	23	28
	Average	Urea			46.30	57	51	26	50
		Azolla sp.	2.5		3.42	87	66	23	34
		Sesbania sp.	2.7		2.06	73	62	22	34

D, Dry season; W, wet season. *Sesbania* sp. N includes recycled soil N as well as fixed N_2

the total green manure dry weight and the stems 75%. Sixty percent of the total N content was in the leaves. Except for the ninth rice crop (1989 wet season), N production was higher during April-June than during October-January. *Sesbania* sp. has photoperiod-sensitive (short day) flowering characteristics. When the plant flowered, growth was retarded, but other climatic factors may be related to the poor growth in the October-January period. *Aeschynomene afraspera*, an aquatic legume less sensitive to the photoperiod, was grown from December 1988 to February 1989 (short day season) and had a higher N concentration and production than the *Sesbania* sp. With the seventh rice crop, *Sesbania* sp. growth was stunted and therefore the duration of growth was extended to 67 days. Because of this longer growth period, the biomass and amount of N added was increased, but the N content decreased to 1.4%.

Green manure decomposition

The decomposition of different *Azolla* spp. has been reported earlier (Ito and Watanabe 1985; Watanabe et al. 1991; Ventura et al. 1992). The decomposition of *S. rostrata* and *Aeschynomene afraspera* in flooded soils is shown in Fig. 1. The organic matter content in the nylon bags decreased rapidly during the first 10 days of decomposition, and then gradually decreased. By 10 days after the incubation, more than 50% of the leaves of both *Sesbania* sp. (initial N 5.0%) and *Aeschynomene* sp. (initial N 4.7%) were already decomposed. Decomposition of the stems and root stubble of both plants (initial N: *Sesbania* sp. 2.6%; *Aeschynomene* sp. 1.4%) occurred more slowly, reaching about 30 – 46% after 10 days. Thereafter, decomposition slowed down. The half-life of organic material decay, after the initial rapid decay for 10 days, was 35 days in *Sesbania* sp. leaves and 22 days in *Aeschynomene* sp. leaves. The decomposition of stems and root stubble was also slower than that of leaves, with a half-life of 110 days for the *Sesbania* sp. and 94 days for the *Aeschynomene* sp. The stem bark decomposed first, so that after 2 or 3 weeks, only the woody portion remained. This hard part of the stem then decomposed very slowly and persisted in the soil, even after 1 year from the time of incorporation.

Decomposition and mineralization studies conducted earlier (Ventura et al. 1987; Nagarajah et al. 1989) showed that N is released rapidly from green manure during the first 2 – 3 weeks after incorporation, and then slows

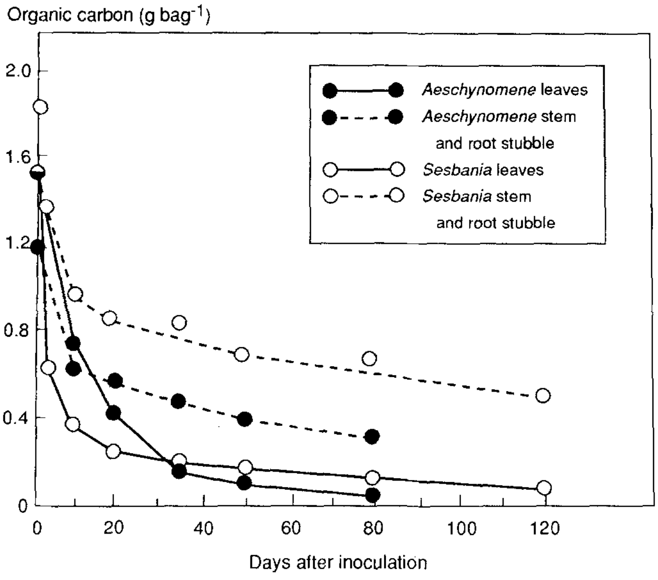

Fig. 1. Decomposition of *Aeschynomene afraspera* and *Sesbania rostrata* in wetland rice, International Rice Research Institute field, 1989 dry season (*Aeschynomene* sp.) and wet season (*Sesbania* sp.)

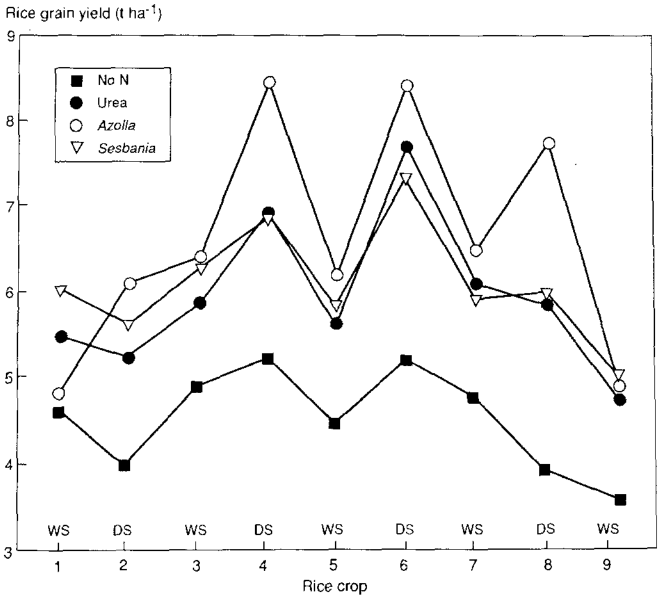

Fig. 2. Effect of *Azolla* sp., legumes, and urea on the yield of wetland rice, International Rice Research Institute field, 1985–1989. SE (four replicates) of a treatment mean was 0.27 for crop 1 (*1*), 0.15 (*2*), 0.28 (*3*), 0.19 (*4*), 0.21 (*5*), 1.0 (*6*), 0.27 (*7*), 0.24 (*8*), 0.12 (*9*). *WS*, Wet season; *DS*, dry season

down. N mineralization rates and N uptake by rice plants are related to N and lignin contents (Watanabe et al. 1991), which are in turn directly determined by the health and age of the green manure. It is important, therefore, to know the best time to incorporate green manure. For *Azolla* sp. the best time is when the ferns are healthy and have just covered the plot with no overlapping fronds (Watanabe et al. 1991; Ventura et al. 1992). For *Sesbania* sp., it is about 45–55 days after emergence when 55–90 kg N has accumulated (Table 1). In the present study, incorporation of more mature plants (with maturing pods) resulted in a low N content (< 1.8%) which, in turn, affected decomposition and fertilizer efficiency.

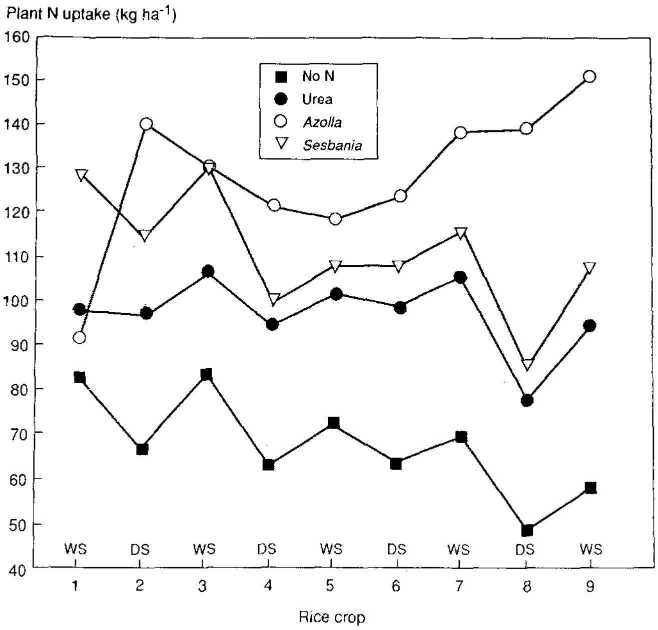

Fig. 3. Effect of *Azolla* sp., legumes, and urea on the N uptake of wetland rice, International Rice Research Institute field, 1985–1989. SE (four replicates) of a treatment man was 6.9 for crop 1 (*1*), 2.9 (*2*), 5.8 (*3*), 3.6 (*4*), 2.8 (*5*), 4.8 (*6*), 5.8 (*7*), 4.8 (*8*), 1.5 (*9*). *WS*, Wet season; *DS*, dry season

Rice grain yield and N uptake

Figure 2 shows that grain yield and grain yield response to fertilizer N were higher during the dry season than during the wet season. Despite the large amount of *Azolla* sp. N that was added, the rice plants were able to withstand lodging. Grain yields from the fourth crop went up to 7.9–8.5 t ha^{-1} during the dry season, and yield increases over control ranged from 3.3 to 3.9 t ha^{-1}. During the same cropping periods, the rice grain yield increased by 1.8–2.5 t ha^{-1} with the application of urea or aquatic legumes. In the wet season, there was no differnce among *Azolla* sp., *Sesbania* sp., and urea treatments, and the grain yield increase over the control was up to 1.7 t ha^{-1}. The larger yields in the organic matter treatments were primarily due to the higher levels of N application with the organic fertilizers. The grain production per unit amount of N added or N absorbed did not differ with N source.

The rice N uptake (Fig. 3) increased with the application of *Azolla* sp., aquatic legumes and urea. In the first rice crop, the uptake of N in *Azolla* sp. plots was lower than that in plots treated with urea or the aquatic legume, because a substantially lower amount of *Azolla* sp. N was added (Table 1). The N uptake in plots with the legume green manure was higher than that in the urea treatment (50 kg N ha^{-1}) for the first three crops, and was similar to that in the 60 kg N ha^{-1} urea treatment from the fourth crop.

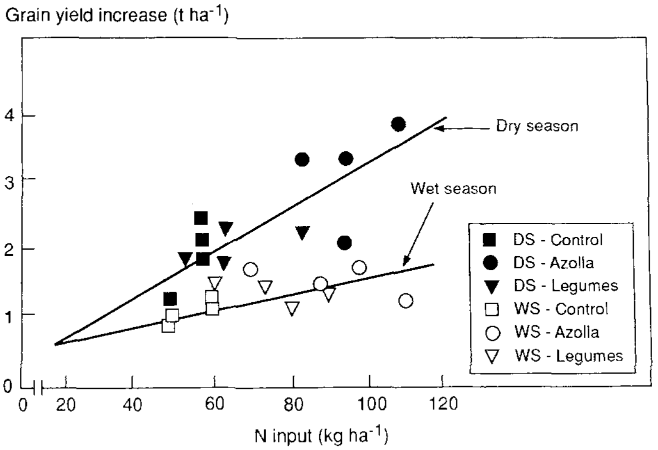

Fig. 4. Linear relationship between N and grain yield increase. Dry season (*DS*): $y = -0.08 + 0.032x (R^2 = 0.63)$; wet season (*WS*): $y = 0.4 + 0.012x (R^2 = 0.44)$

In all fertilizer treatments, the grain yield increase was proportional to the level of N applied (Fig. 4). The grain production response to the application of N was higher in the dry season (32 kg grain per kg N) than in the wet season (12 kg per kg N).

Figure 3 shows that the plant N uptake in no-N plots decreased with the number of rice crops. This may be an indication of decreasing soil fertility. The N uptake in the *Azolla* sp. plots increased from the fifth cropping because of the increase in added N, but those in the urea and legume treatments did not show an increasing tendency.

Except in the *Azolla* sp. plot, N uptake (grain + straw) by rice was higher during the wet season than during the dry season (Table 2). With the exception of the *Azolla* sp. plot, the N in grain did not differ between the wet and dry season, but there was more N in straw during the wet season in all treatments. The ratio of N in grain and straw was lower during the wet season, and was always lowest in the *Azolla* sp. plot. Clearly, the amount of N absorbed by the plant reflected the amount of N added to the soil and its rate of availability for plant use. As indicated by the decomposition study on aquatic legumes and by *Azolla* sp. mineralization reports (Ventura et al. 1987; Watanabe et al. 1989; Watanabe et al. 1991; Ventura et al. 1992), green manure N is mineralized gradually even at later stages of rice growth. The incorporation of *Azolla* sp., especially more than 4 weeks after rice transplanting, would give a very low grain: straw N ratio.

The apparent N recovery in grain and straw of *Azolla* sp. N was greater than that of urea, except in the first rice crop (Table 1). The apparent recovery of legume N was greater than or similar to that of urea. The value of this apparent N recovery was sometimes relatively too high because the basis for the estimate was the difference in N uptake between a given N treatment and the control plots. The soil N-supplying capacities of the control and N-treated plots, however, changed over time, as shown in Fig. 3, so that there is no fully reliable control treatment for calculating the N uptake by difference in a long-term fertilizer experiment.

Table 2. Grain and straw N averages in dry and wet seasons as affected by application of urea or green manure, International Rice Research Institute field, 1985–1989

Treatment	N uptake (kg ha^{-1})			
	Dry season (four crops)		Wet season (five crops)	
	Grain	Straw	Grain	Straw
Control	45 ± 5.9	15 ± 2.2	50 ± 7.8	23 ± 4.2
Urea	72 ± 11.8	23 ± 1.7	67 ± 6.2	40 ± 12.8
Azolla sp.	96 ± 3.7	35 ± 7.6	75 ± 11.1	51 ± 13.2
Aquatic legume	75 ± 9.8	26 ± 3.9	71 ± 6.2	45 ± 8.1

Average ± SE (among crops)

There was a direct relationship between the green manure N content and the amount of N recovered by rice (Fig. 5); the correlation coefficients were 0.70 for *Sesbania* sp. and 0.89 for *Azolla* sp. The *Sesbania* sp. clearly needed a lower N content than the *Azolla* sp. for the same N recovery. The average N recovery in urea-treated plots was 51%. For this level of recovery, *Azolla* sp. needed a 2.6% content of N and *Sesbania* sp., 1.5%. This may be partly explained by the lignin content of green manure, which is about 10% in 42- to 56-day-old *Sesbania* sp. (Becker 1990) and 18–30% in *Azolla* sp. (Watanabe et al. 1991).

The N efficiency (kg grain increase kg N applied), as defined by Van Keulen and Van Heemst (1982), did not differ with N source (Table 1). Although the recovery of organic N was generally greater than that of chemical fertilizer N, the N efficiency was similar to that of urea (except in the case of poor *Azolla* sp. growth for the first rice crop) (Table 1). The N use efficiency [(grain yield in treatment plot-grain yield in control)/(N uptake in treatment plot-N uptake in control)] was higher in the urea treatment than in the two organic manure treatments, and it was often lower in the *Sesbania* sp. plots than in the

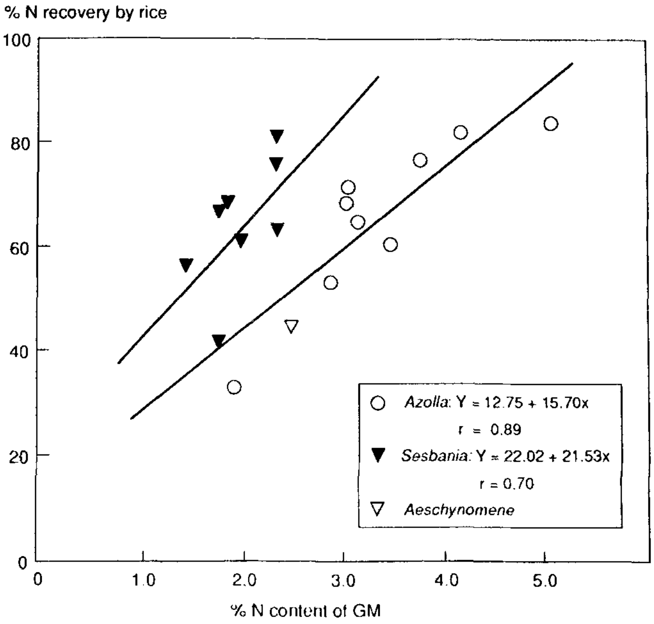

Fig. 5. Relationship between percentage N content of *Azolla* sp. and of *Sesbania* sp. and percentage N recovery in rice, International Rice Research Institute long-term experiment, 1985–1990. *GM*, Green manure

Table 3. Soil chemical analysis after the seventh rice crop, International Rice Research Institute field, November 1988

Soil analysis	Treatment					SE
	Soil depth (cm)	Control	Urea	*Azolla* sp.	*Sesbania* sp.	
Bulk density (g cm^{-3})	0–20	0.57	0.53	0.50	0.47	0.014
	20–50	0.75	0.72	0.69	0.69	NS
N content (%)	0–20	0.147	0.149	0.171	0.174	0.004
	20–50	0.118	0.121	0.133	0.132	NS
N content (kg ha^{-1})	0–20	1680	1571	1699	1625	NS
	20–50	2472	2519	2725	2642	NS
pH	0–50	6.2	6.3	6.3	6.2	NS
EC (dS m^{-1})	0–50	0.81	0.86	0.98	0.86	NS
Organic C (%)	0–50	1.17	1.20	1.33	1.30	NS
Organic C (t ha^{-1})	0–50	37.8	37.6	40.2	38.1	NS
Available P (Olsen, μg g^{-1})	0–50	17	15	25	14	1.7
Exchangeable cations (mEq 100 g^{-1} airchied soil)						
Na	0–50	1.9	1.8	1.8	2.1	0.08
K	0–50	0.82	0.74	0.74	0.78	NS
Mg	0–50	17.2	17.4	17.8	18.1	NS
Ca	0–50	28.1	26.7	26.0	27.3	NS
Available B (μg g^{-1})	0–50	4.5	4.5	5.1	5.1	0.15

SE, Standard error of a treatment mean; NS, no significant difference among treatments; EC, electrical conductivity

Azolla sp. plots (Table 1). Values were generally higher in the dry season than in the wet season. The N obtained from organic N was less efficient for rice grain production. A part of *Azolla* sp. N became available to rice at a later stage, but had no effect on grain production. This may be a reason why the use of *Azolla* sp. N in grain production was lower than that of urea. We observed that the rice in the *Azolla* sp. plots remained green even at harvest-time. Probably, N obtained from *Azolla* sp. grown and incorporated after transplanting was not effectively used for grain production.

A comparison of the urea and *Sesbania* sp. treatments poses some problems, because the *Sesbania* sp. was added only once, before the rice was transplanted, in large amounts, while the urea was applied in a split dose, half before transplanting and the other half at panicle initiation. Thus, urea N was made more efficient for grain production.

Table 4. Residual effect of green manure and urea on grain yield and N uptake by the tenth rice crop, International Rice Research Institute field, 1990 dry season

Treatment	Grain (t ha^{-1})	Straw (t ha^{-1})	Total N uptake (kg ha^{-1})
Control	2.9	2.0	35.8
Urea	2.9	2.0	36.3
Azolla sp.	3.4	2.6	47.1
Sesbania sp.	3.4	2.2	46.8
SE	0.32	0.13	1.5

SE, Standard error of a treatment mean

Soil chemical analysis

The bulk density of soil samples at 0–20 cm taken from seven different plots just before sowing the green manure for the first rice crop (March 1985) was 0.548 ± 0.027 g cm^{-3}, and their N content was $0.137\% \pm 0.008\%$, and the level of soil N was 1531 ± 91 kg ha^{-1}. Standard deviations indicated that the field was fairly uniform in bulk density and soil N content.

Analysis of the 20-cm upper soil layer was repeated after harvest of the seventh rice crop, and soil samples were also taken from the 20–50 cm layer. A comparison of the soil analyses before the first crop and of control plots after the seventh crop showed no measurable change in the N content of the upper 20-cm layer (Table 3). This measured N includes both labile and large non-labile soil N pools. The cumulative amount of N taken up in the grain and straw of seven rice crops in the control plots was 497 kg ha^{-1} (71 kg N per crop). Of this amount, 60% was estimated to come from the upper 20-cm layer (Ventura and Watanabe 1984), and therefore about 300 kg N ha^{-1} for seven crops or 43 kg N ha^{-1} per crop was taken up from this soil layer. A rough estimate showed a positive balance or 449 kg N ha^{-1} in 7 years or 128 kg N ha^{-1} per year. This is close to the estimated N balance reported for 17–24 irrigated rice crops (App et al. 1984).

N uptake in the control plots decreased from 82 kg ha^{-1} in the first crop to 69 kg ha^{-1} in the seventh crop (Fig. 3) and 36 kg ha^{-1} (Table 4) in the tenth crop. Although no decrease in total soil N was observed at 0–20 cm, the results suggest that the labile or available soil-N pool was depleted without the addition of fertilizer N or N from outside sources. A depletion in labile N, which occupies only 2% of total soil N per crop, would not be measurable in the analysis of total soil N.

Table 3 shows that the N concentration (N g^{-1} dry soil) in the upper 20-cm soil layer was significantly higher in *Azolla* sp. and *Sesbania* sp. plots than in the control and urea plots. Soil bulk density, however, decreased significantly. Consequently, the amount of N per unit area was not significantly increased by the addition of organic matter during seven rice crops. Table 3 shows that the N content in the soil below the 20 cm of puddled soil did not significantly differ with fertilizer treatment.

The present results show that measuring the soil N content may not be sufficient to determine changes in soil fertility. The quantity per unit area is needed to monitor changes. Soil bulk density is not constant, and careful and periodic measurement in experimental plots to determine the effects of organic matter on soil properties is needed.

Green manure is considered to have long-term positive effects on soil fertility. These effects can be calculated from the amount of N added every cropping period. A total of 550 kg N ha^{-1} was added in *Azolla* sp. in seven rice crops (Table 1). Of this amount, 384 kg was absorbed by the rice grain and straw. Unfortunately, the rate of N loss was not determined in this study, but it can be estimated from related ^{15}N study (Ventura et al. 1990, unpublished data) in concrete plots containing a 30-cm depth of soil. The *Azolla* sp. N content was 3.1% – 3.7%, and at a rate of 45 kg N ha^{-1} basal, N losses of 5% – 12%, average 8.3%, were obtained. With this value, a loss of 45.7 kg N ha^{-1} was estimated, and therefore 120 kg N ha^{-1} (or 17.2 kg N ha^{-1} per crop) should have remained in the soil. Total N (kg ha^{-1}) in the whole puddled soil layer of the *Azolla* sp. and control plots did not differ statistically after seven rice crops (Table 3), because the estimated N accumulation from *Azolla* sp. was still too small to be detected by soil analysis.

The addition of green manure did not affect soil pH, electrical conductivity, organic C content (as a percentage or in kg ha^{-1}), or the exchangeable cations (Table 3). As for N, it may take more years before a significant effect on the organic matter content of the soil can be detected. The available soil P content was higher in the *Azolla* sp. plots because water-soluble P was added to promote growth of the *Azolla* sp. This experimental field, however, is known to contain high levels of available P and rice plants do not respond to P fertilizer. The B content increased with the addition of organic matter, probably because B is absorbed by legumes and by *Azolla* sp.

Residual effects

No fertilizer was applied to the tenth rice crop. The grain yield and total N uptake decreased in all treatments, including the control (Table 4). The grain yield was reduced by about 1 t and N uptake by 12 kg in the control compared with those in the two previous crops.

Grain yields from the *Azolla* sp. and *Sesbania* sp. plots were 0.5 t ha^{-1} higher than in the control and urea plots (Table 4). N uptake from the green manure plots was 11 kg ha^{-1} higher than that in the urea and control plots, indicating that the green manure had positive residual effects. This amount accounted for 18 (*Sesbania* sp. plots) or 10% (*Azolla* sp. plots) of N added in the previous crop. Grain yield and N uptake were the same in the urea and control plots, indicating that there was no residual effect from the urea fertilizer application after nine consecutive rice crops.

N uptake decreased in the non-N treatments during the residual experiment (tenth cropping) compared with the N uptake during previous croppings, where N fertilizers were applied in adjacent plots. Varied environmental factors that affect plant performance must be considered. In a long-term fertilizer experiment, there is also a need to look at the possibility of N contamination from adjacent treated plots by seepage through old dikes and bunds, or from irrigation water. This would put in question the N balance of 128 kg N ha^{-1} per crop estimated earlier for the present experiment. Floodwater N monitoring in the different plots and irrigation canals is therefore required. Many long-term soil fertility trials are made on relatively small plots, surrounded by fertilized plots, and N balances in no-N plots obtained by long-term fertility plots (Koyama and App 1979; Watanabe and Roger 1984) may therefore be overestimated. The decline in N uptake in the no-N plots did not reflect a measurable change of total N in the soil.

The present results suggest that it may take many years before soil organic matter and N are significantly increased by green manuring. The need for long-term trials to monitor soil fertility changes in flooded rice soils was suggested by Suzuki et al. (1990). They reported the effects of a continuous application of inorganic or organic fertilizers on rice soil fertility and rice yields in the temperate region by using a 60-year, long-term field experiment at the National Agricultural Research Center Farm in Konosu, Saitama Prefecture, Japan. The yields in the organic fertilizer plot, in which 60% of the N was derived from rice straw compost, were clearly lower than those in the inorganic fertilizer plot for the first 10 years. Afterwards, the yields in the organic fertilizer plot reached the levels in the inorganic fertilizer plot, eventually becoming higher after 30 years. Total C, total N, and the amount of N mineralized annually in the organic fertilizer plot gradually increased over the 60 years.

The importance of the more labile fractions of soil organic N was emphasized by Glendining and Powlson (1990) in analyzing the N balance in long-term fertility plots at Rothamsted Experiment Station. Because a large fraction of soil N is inert, another measure such as microbial biomass N would be useful for monitoring changes in available N (Inubushi and Watanabe 1986).

References

App A, Santiago T, Daez C, Menguito C, Ventura W, Tirol A, Po J, Watanabe I, De Datta Sk, Roger PA (1984) Estimation of the nitrogen balance for irrigated rice and the contribution of phototrophic nitrogen fixation. Field Crops Res 9:17 – 27

Becker M (1990) Potential use of the stem-nodulating legumes *Sesbania rostrata* and *Aeschynomene afraspera* as green manure for lowland rice (*Oryza sativa* L.). Doctoral dissertation, Justus-Liebig-Universität Giessen, Germany

Becker M, Ladha JK, Ottow JCG (1990a) Growth and N_2-fixation of two stem nodulating legumes and their effect as green manure on lowland rice. Soil Biol Biochem 22:1109 – 1119

Becker M, Diekmann KH, Ladha JK, DeDatta SK, Ottow JCG (1990b) Effect of NPK on growth and nitrogen fixation of *Sesbania rostrata* as a green manure for lowland rice (*Oryza sativa* L.). Plant and Soil 132:149–158

Bremner JM, Shaw K (1958) Denitrification in soil. I. Methods of investigation. J Agric Sci 51:22–39

Do Van Cat, Watanabe I, Zimmerman WJ, Lumpkin TA, De Waha Baillonville T (1989) Sexual hybridization among *Azolla* species. Can J Bot 67:3482–3485

Dreyfus BL, Dommergues YR (1981) Non inhibition de la fixation d'azote par l'azote combine chez une légumineuse à nodules caulinaires. C R Acad Sci Paris 291:767–770

Glendining MJ, Powlson DS (1990) 130 years of inorganic nitrogen fertilizer applications to the Broadbalk Wheat Experiment: The effect on soil organic nitrogen. In: Trans 14th Int Congr Soil Sci, Vol IV, Commission IV. Int Soil Sci Soc, Kyoto, Japan, pp 9–13

Inubushi K, Watanabe I (1986) Dynamics of available nitrogen in paddy soils. II. Mineralized N of chloroform-fumigated soil as a nutrient source for rice. Soil Sci Plant Nutr 32:561–577

Ito O, Watanabe I (1985) Availability to rice plants of nitrogen fixed by azolla. Soil Sci Plant Nutr 31:91–104

Jackson MI (1958) Soil chemical analysis. Prentice Hall, Inc., Eaglewood Cliffs, New Jersey

Koyama T, App AA (1979) Nitrogen balance in flooded rice soils. In: Nitrogen and Rice. Int Rice Res Inst, Los Baños, Philippines, pp 95–104

Ladha JK, Watanabe I, Saono S (1988) Nitrogen fixation by leguminous green manure and practices for its enhancement in tropical lowland rice. In: Sustainable agriculture: Green manure in rice farming. Int Rice Res Inst, Los Baños, Philippines, pp 165–183

Ladha JK, Miyan S, Garcia M (1989) *Sesbania rostrata* as green manure for lowland rice: Growth, N_2 fixation, *Azorhizobium* sp. inoculation, and effects on succeeding crop yields and nitrogen balance. Biol Fertil Soils 7:191–197

Lumpkin TA, Plucknett DL (1982) *Azolla* as a green manure: Use and management in crop production. Westview Press, Boulder, Colorado

Nagarajah S, Neue HU, Alberto MCR (1989) Effect of *Sesbania, Azolla* and rice straw incorporation on the kinetics of NH_4, K, Fe, Mn, Zn and P in some flooded soils. Plant and Soil 116:37–48

Suzuki M, Kamekawa K, Sekiya S, Shiga H (1990) Effect of continuous application of organic or inorganic fertilizer for sixty years on soil fertility and rice yield in paddy field. In: Trans 14th Int Congr Soil Sci, vol IV, Commission IV. Int Soil Sci Soc, Kyoto, Japan, pp 14–19

Van Keulen H, van Heemst HDJ (1982) Crop response to the supply of macronutrients. Centre for Agricultural Publishing and Documentation, Wageningen

Ventura W, Watanabe I (1984) Dynamics of nitrogen availability in lowland rice soils: The role of soil below plow layer and effect of moisture regimes. Philipp J Crop Sci 9:135–142

Ventura W, Mascariña GB, Furoc RE, Watanabe I (1987) *Azolla* and *Sesbania* as biofertilizers for lowland rice. Philipp J Crop Sci 12:61–69

Ventura W, Watanabe I, Mascariña GB (1992) Mineralization of *Azolla* N and its availability to wetland rice. II. Fertilizer effect and ^{15}N uptake by rice from different species of Azolla with varying N contents Soil Sci Plant Nutr 38:505–516

Watanabe I (1987) Summary report of the *Azolla* Program of the International Network on Soil Fertility and Fertilizer Evaluation for Rice. In: *Azolla* utilization. Int Rice Research Inst, Los Baños, Philippines, pp 197–201

Watanabe I, Roger RA (1984) Nitrogen fixation in wetland rice field. In: Current developments in biological nitrogen fixation. Subba Rao NS (ed) Oxford & India Book House, New Delhi Bombay Calcutta, pp 237–276

Watanabe I, Ventura W, Mascariña G, Eskew DL (1989) Fate of *Azolla* spp. and urea nitrogen applied to wetland rice (*Oryza sativa* L.). Biol Fertil Soils 8:102–110

Watanabe I, Padre B Jr, Ramirez C (1991) Mineralization of *Azolla* N and its availability to wetland rice. Part I. Nitrogen mineralization of different *Azolla* species as affected by their chemical composition. Soil Sci Plant Nutr 37:575–584

Yoshida S, Forno Do, Cock J, Gomez K (1972) In: Laboratory manual for physiological studies of rice. Int Rice Res Inst, Los Baños, Philippines, pp 11–12