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Nitrogen use efficiency and recovery from N fertilizer under rice-based cropping systems

M. Motior Rahman[1], Takahisa Amano[2] and Tatsuhiko Shiraiwa[3]

[1]Central Agricultural Research Center, Montana State University, 52583 US Hwy 87, Moccasin, MT 59462, USA
[2]Laboratory of Plant Production Systems, Graduate School of Agriculture, Kyoto University, Japan
[3]Crop Science Laboratory, Graduate School of Agriculture, Kyoto University, Japan

[1]Corresponding author: mmotiorrahman@yahoo.com

Abstract

Broad bean-rice, hairy vetch-rice, naked barley-rice and fallow-rice cropping systems was investigated at Kyoto University Farm, Takatsuki, Japan during 2001-2002 and 2002-2003 to determine the effects of broad bean and hairy vetch on seasonal crop N accumulation, fertilizer N use efficiency (FNUE) and N recovery from rice-based cropping systems using ^{15}N-labeled fertilizer. Cropping systems used as main plot treatments and N fertilizer at rates of 0, 40, 80 and 120 kg ha^{-1} as subplot treatments. Hairy vetch added 6.4 to 13.1 g N m^{-2} of which 1.4 to 5.4 g fixed N m^{-2} while broad bean added 32.0 to 35.2 g N m^{-2} of which 18.1 to 27.5 g fixed N m^{-2} to the soil when incorporated as green manure. Broad bean-rice accumulated 19.4 to 20.4 and 21.4 to 22.6 g N m^{-2} and hairy vetch-rice accumulated 12.9 to 18.7 and 13.2 to 16.4 g N m^{-2} in 2002 and 2003, respectively. In 2002, FNUE was highest in hairy vetch-rice with N 40 kg ha^{-1} while in 2003 FNUE was highest in naked barley-rice with N 40 kg ha^{-1}. In broad bean-rice, plant nitrogen accumulation at maturity always increased and FNUE decreased in spite of increased grain yield of rice. The highest (94 %) recovery of ^{15}N-labeled fertilizer was achieved in rice after broad bean with N 40 kg ha^{-1} and the recoveries of ^{15}N-labeled fertilizer were higher in the first year than the second year.

Key words: cropping systems, fertilizer, legume, N-use efficiency, recovery, rice

Introduction

Nitrogen fertilization is widely adopted to enhance grain production and improve nitrogen utilization in rice all over the world (Jiang et al., 2005). Rice is produced under both upland and lowland ecosystems with about 76% of the global rice produced from irrigated-lowland rice systems (Fageria et al., 2003). Improved nitrogen use efficiency, particularly for N, is an important goal in cropping system development. Determination of N use efficiency in cereal based agro-ecosystems enabled broad assessment of agronomic management and environmental factors related to N use, Grain yield and N accumulation, N in aboveground, N harvest index, and grain N accumulation are the key indicators of N use efficiency (Huggins and Pan, 2003). Soil N and biological nitrogen fixation (BNF) by associated organisms are major sources of N for lowland rice. Soil organic N is continually lost through plant removal, leaching, denitrification and ammonia volatilization. An additional concern is that the capacity of soil to supply N may decline with continuous intensive rice cropping under wetland conditions, unless it is replenished by biological N fixation (Kundu and Ladha, 1995). More than 50 % of the N used by flooded rice receiving fertilizer N is derived from the combination of soil organic N and BNF by free-living and rice plant-associated bacteria (Roger and Ladha, 1992). The remaining N requirement is normally met with fertilizer. Legumes are used commonly in agricultural systems as a source of N for subsequent crops and for maintaining soil N levels (Glasener et al., 2002) and reducing energy requirements by adding significant amounts of N to the soil (Entz et al., 2002). Reducing fertilizer N use in lowland rice systems while maintaining the native soil N resource and enhancing crop N output is desirable from both environmental and economic perspectives. This may be possible by obtaining N on

Table 1. Aboveground dry matter, nitrogen accumulation of winter crops and estimates of the proportion of plant N derived from N_2 fixation of broad bean and hairy vetch determined by N difference method 2001-2002

Winter Crop	N fertilizer (Kg ha^{-1})	Dry matter (g m^{-2})	Nitrogen (g m^{-2})	††Legume N (g m^{-2})	†Plant N derived from N_2 fixation (%) by N difference method	†††Soil N fixed (g m^{-2})
Column1	Column2	Column 3	Column 4	Column 5	Column 6	Column7
Broad bean	0	1082	35.2	27.5	78	7.7
Hairy vetch	0	335	13.1	5.4	41	7.7
Naked Barley	0	817	7.7	-	-	-
Fallow	0	-	-	-	-	-

ANOVA

Source of variation	Degrees of freedom	Mean squares				
		Dry matter	Nitrogen	-	-	-
Crops	3	1721.1**	254.8**	-	-	-
Error	9	123.5	5.6	-	-	-

** Significant at the 0.01 level. † In 2001–02, N fixed by legumes was calculated based on N difference method. Naked barley used as reference plants for estimated by N difference method. †† Data collected from average percentage of total N derived from N_2 fixation (%Ndfa) values derived from columns 4th and 6th columns of table 1 as N fixed = 1/100(% Ndfa X total N). [e.g. ††Legume N. = {1/100(78x35.2)} = 27.5 (g m^{-2})]. ††† Soil N fixed (7th column) is the difference between the 4th and 5th columns of table 1

the land through legume BNF, minimizing soil N losses, and by improved recycling of N through plant residues. Sustainable cropping systems are essential for agronomic, economic, and environmental reasons (Camara et al., 2003). Thus the management of indigenous soil N and N derived in situ through legume BNF poses potentials for enhancing the N nutrition and N use efficiency of crops and total N output from a lowland rice-based cropping system (George et al., 1998). The ability of legumes to fix N and their residual impact on soil N status has long been recognized, but many farmers also realize that the accrued N benefits will vary between different legume systems (Rochester and Peoples, 2005). To date the fate of N in green manure and productivity of dual-purpose dry season legumes and their effects on soil N dynamics and their contributions to the yield and N uptake of the following rice crop has been studied only a few instances (George et al., 1998). Cropping systems that include legumes have the potential for contributing N to following crops and may moderate NO_3 levels in the soil to avoid potential for NO_3 leaching (Grant et al., 2002). Grain and forage legumes grown in dry season and their residues could supplement some sort of N source for succeeding crop (Muhammad et al., 2003). Broad bean is used as a winter or spring cover crop, green manure, vegetable and an expensive food legume. It is capable of producing large amounts of dry matter and accumulating large quantities of nitrogen (N) and fixed substantial quantities of N for subsequent crops (Evans et al., 2001). Several international studies suggest vetches are efficient N-fixers (Mueller and Thorup, 2001) and accumulate large amounts of N during growth (Cho, 2003). Hairy vetch not only supply N fixed by leguminous bacteria to the soil but also inhibits the weed growth and decrease the density of insect pest by allelopathy (Kamo et al., 2003). Broad bean and hairy vetch are used in the rice-based cropping systems in Japan, but scientific information is very little. Therefore the present study was undertaken to the following objectives: (i) to determine N accumulation and quantify N fixed by broad bean and hairy vetches using the ^{15}N natural abundance and N difference method (ii) to quantify N recoveries from rice after broad bean and hairy vetch systems and inorganic fertilizer sources using ^{15}N labeled fertilizer and (iii) to determine the amount of fertilizer N required to optimize rice yield when broad bean and hairy vetch are included in the system

Materials and Methods

Experimental site and plan

This study was conducted at the experimental plots of Kyoto University Farm, Takatsuki, Japan ($34^{0}51^{'}$ N, $135^{0}37^{'}$ E) during 2001-2002 and 2002-2003. The top 20-cm soil layer had an air dried pH (1:5 w/v water) of 5.97±0.20, cation-exchange capacity of 15 ($cmol_c$ kg^{-1} soil), and contained 1.85±0.48 % organic carbon, 0.17±0.04 % total N, NH_4-N 6.67±1.27 (mg 100^{-1} g soil), exchangeable CaO 173.0±21.18 (mg 100^{-1} g soil), exchangeable MgO 11.8±2.79 (mg 100^{-1} g soil) and exchangeable K_2O 14.9±9.06 (mg 100^{-1} g soil). The soil texture of experimental plot was clay loam

Table 2. Aboveground dry matter and N accumulation (NA) of winter crops and estimates of the proportion of plant N derived from N_2 fixation of broad bean and hairy vetch determined by N difference and ^{15}N natural abundance methods 2002-2003.

Cropping systems (CS)	N fertilizer (NF) (Kg ha^{-1})	Dry matter (g m^{-2})	NA (g m^{-2})	††Legume N (g m^{-2})	† N_2 fixation (%)			†††Soil N fixed (g m^{-2})
					†N diff.	†^{15}N	†Mean	
Col. 1	Col. 2	Col. 3	Col. 4	Col. 5	Col. 6	Col. 7	Col. 8	Col. 9
Simple effects of CS								
Broad bean-Rice		1001	32.0	18.1	82.0	31.0	56.5	13.9
Hairy vetch-Rice		209	6.4	1.4	24.0	21.0	22.5	5.0
Naked barley-Rice		487	4.9	-	-	-		-
Fallow-Rice		-	-	-	-	-		-
Simple effects of NF								
	0	511	16.3	9.5	58.0	-	58.0	6.8
	40	535	17.1	7.8	57.0	34.0	45.5	9.3
	80	551	17.6	6.8	52.0	25.0	38.5	10.8
	120	559	17.9	6.0	47.0	20.0	33.5	11.9
CS X NF								
Broad bean-Rice	0	980	31.3	26.0	83.0	-	83.0	5.3
	40	1003	32.1	20.7	84.0	45.0	64.5	11.4
	80	1007	32.2	17.7	83.0	27.0	55.0	14.5
	120	1015	32.5	16.3	78.0	22.0	50.0	16.2
Hairy vetch-Rice	0	192	6.0	1.9	32.0	-	32.0	4.1
	40	211	6.5	1.7	29.0	22.0	25.5	4.8
	80	211	6.6	1.4	20.0	22.0	21.0	5.2
	120	222	6.5	1.1	15.0	18.0	16.5	5.4
Naked barley-Rice	0	440	4.1	-	-	-	-	-
	40	472	4.6	-	-	-	-	-
	80	512	5.3	-	-	-	-	-
	120	523	5.5	-	-	-	-	-
Fallow -Rice	0	-	-	-	-	-	-	-
	40	-	-	-	-	-	-	-
	80	-	-	-	-	-	-	-
	120	-	-	-	-	-	-	-

ANOVA

Sources of variation	DF	Mean squares	
		Dry matter (g m^{-2})	Nitrogen (g m^{-2})
CS	3	23070307.5**	15066**
Error (a)	9	32700.8	2.8
N fertilizer	3	41286.67 ns	10.35 **
CS X NF	9	6694.17 ns	3.05**
Error (b)	36	14639.72	0.26

** Significant at the 0.01 level; ns = non significant. † In 2002–03, N fixed by legumes was calculated based on N difference and ^{15}N natural abundance methods. Naked barley used as reference plants for estimated by N difference and ^{15}N natural abundance methods. †† Data collected from average percentage of total N derived from N_2 fixation (%Ndfa) values derived from columns 4th and 8th (8th column=average of 6th and 7th columns) of table 2 as N fixed = 1/100(% Ndfa X total N). ††† Soil N fixed (9th column) is the difference between the 4th and 5th columns of table 2.

belongs to the loamy gray lowland soil series. Meteorological data such as rainfall and temperature data are presented in Table 1. In the 2001-02 winter season broad bean (*Vicia fava*), hairy vetch (*Vicia villosa*), naked barley (*Hordeum vulgare*) and a weedy fallow treatment were randomly assigned to plots, each of 10 by 26 m size, in RCB design with four replications. In 2002 summer season for rice crops with four rates of N fertilizer (0, 40, 80 and 120 kg N ha-1) were superimposed onto the fallow plots and each of the winter crop plots, and each of the sixteen main plot was subdivided into four each of 5 by 10 m size and fertilizer N rates were randomly assigned then the field layout had a split plot design. In the 2002-03 winter season broad bean, hairy vetch, naked barley and a weedy fallow treatment were randomly assigned to sub plots, and then the field layout was considered as split plot design although no fertilizer N was applied in winter crops. The field layout in 2003 summer season was also a split plot design. Ammonium sulfate was used as source of fertilizer N. Treatments were replicated four times. Land was prepared by rotovator. Winter crops (cover crops) viz. broad bean, hairy vetch, naked barley (non-N_2-fixing reference plant) and summer crop rice were grown during the month of November to May and June to October, respectively. No fertilizer was applied to the cover crops to observe the contribution of legumes for subsequent rice crop. Nitrogen fertilizer (ammonium sulfate) was used only in first year rice crop and no fertilizer N and other chemical fertilizer was applied in second year rice crop. In 2001-2002, after land preparation micro-plots (2m x 2m) were established in each subplot. In 2002-2003, micro-plots were prepared manually by spading. Micro-plots were fertilized with equivalent N rates such as 0, 40, 80 and 120 kg ha^{-1} supplied as ^{15}N-labeled ammonium sulfate [$(NH_4)_2SO_4$] at 3 atom % ^{15}N in 2001-2002. No ^{15}N-labeled ammonium sulfate was applied in 2002-2003 to observe the fate of residual effect of ^{15}N for second year rice crop. To prevent lateral movement of the labeled ^{15}N, wooden barriers surrounding the micro plots were inserted into the soil to a depth of approximately 20 cm (Eagle et al., 2001).

Cultivation and sampling of winter season crops

The winter season broad bean (cv. Minpo), hairy vetch and naked barley (cv. Ichiban Boshi) was grown on tilled land with residual soil moisture after harvest of wetland rice. In early November of 2001 and 2002, broad bean was planted in 60 cm rows and 30 cm plant spacing and seeded at a rate of 150 kg ha^{-1}. Hairy vetch was broadcast seeded at a rate of 30 kg ha^{-1}. Naked barley was planted on 30 cm rows at a seeding rate of 100 kg ha^{-1}. No fertilizer was applied in winter crops for both years. Weeds were allowed to grow in the weedy fallow but were removed from the other plots by manual weeding. About 21 d before transplanting rice, physiological mature broad bean and hairy vetch was harvested by harvester machine and above ground residues were incorporated into the top soil with rolling cultivators for both years. In micro plots, the aboveground legume residues were cut and chopped into 10-to 12-cm pieces manually and uniformly spread onto the plots and incorporated to a depth of about 20 cm into soil with spading following flooding of the field in preparation for rice planting. Plant samples were taken from a 2 m^{-2} areas in each micro plots for above ground dry matter and N determination for each winter crop at final harvest.

Estimation of biological nitrogen fixation

The contributions of biological nitrogen fixation (BNF) to total N accumulation in legume were estimated by the N difference method and the ^{15}N natural abundance technique (Peoples and Herridge, 1990; Peoples et al., 2002). Crops grown in micro plots were used for the BNF estimation. Plant materials were dried at 70^{0}C for at least 48 h, weighed, milled and total N concentration was determined by Kjeldahl digestion (IAEA, 2001). Sources of N for non-fixing and fixing crops are different (IAEA, 2001). It is assumed that sources of N for non-fixing crops are soil and fertilizer. For non-fixing crop, the proportions of N from all available sources can be expressed (IAEA, 2001):

$\%\ Ndff_{NF} + \%\ Ndfs_{NF} = 100\%$; Where, Ndff $_{NF}$ stands for nitrogen derived from fertilizer for non fixing crops, Ndfs $_{NF}$ stands for nitrogen derived from soil for non fixing crops. On the contrary, sources of N for fixing crops (F) are soil, fertilizer and atmosphere and it can be expressed:

$\%\ Ndff_F + \%\ Ndfs_F + \%\ Ndfa_F = 100\ \%$

$\%\ Ndfa = 100 - (\%\ Ndff_F + \%\ Ndfs_F)$

Where, Ndff $_F$ stands for nitrogen derived from fertilizer for fixing crops, Ndfs $_F$ stands for nitrogen derived from soil for fixing crops and Ndfa $_F$ stands for nitrogen derived from atmosphere for fixing crops.

Estimates of the proportion of legume N derived from N_2 fixation (% Ndfa) with the N difference procedure were calculated by comparing N accumulated in the legume with the non legume reference as follows:

$$\%\ Ndfa = \frac{100[(\text{Legume N} - \text{Reference N})]}{(\text{Legume N})}$$

The ^{15}N-labeled plants were analyzed for the concentration of N and atom percent of ^{15}N using a combustion continuous flow isotope ratio mass spectrometer (IAEA, 2001). The proportion of N derived from ^{15}N-labeled fertilizer in the plant and soil and percent ^{15}N recovered by the plant and that remaining in soil were calculated with the following equation:

$$\% \text{ Ndff} = \frac{^{15}\text{N atom \% excess of sample}}{^{15}\text{N atom \% excess of fertilizer}} \text{ X } 100$$

$$\% \text{ Ndfa} = 1 - \frac{^{15}\text{N atom \% excess}_{\text{F}}}{^{15}\text{N atom \% excess}_{\text{NF}}} \text{ X } 100$$

$$\text{N}_2 \text{ fixed (kg ha}^{-1}\text{)} = \frac{\text{\% Ndfa X total N in fixing crop}}{100}$$

Cultivation of summer season rice

In both years three-week-old seedlings of rice (cv. Hinohikari) were transplanted at 20 by 20 cm spacing during first week of June. In 2002, first year rice crop was fertilized with only N fertilizer applied at rates of 0, 40, 80 and 120 kg ha^{-1} in three splits: one third at transplanting, one third at tillering and one third at panicle primordial initiation stages, respectively. In 2003, second year rice crop did not fertilize with N and other chemical fertilizers. In both years rice was harvested during the second week of October. Four ^{15}N-labeled plants were selected at random and harvested at each sampling time. The plants were washed to remove soil from roots; separated into culms, leaves and panicles (when present); dried at 70^{0}C (Ladha et al., 1996); weighed; and ground to powder in a ball mill. The ^{15}N-labeled plants were then analyzed for the concentration of N and atom percent of ^{15}N using a combustion continuous flow isotope ratio mass spectrometer (TRACER MAT EA 1108, Thermo Quest Co. ltd, Tokyo, Japan).

Total biomass and grain yield (2 m^2) were determined from both inside and outside of the ^{15}N micro plot. Yields from the zero-N-fertilizer micro plot were used for calculating fertilizer use efficiency by N difference (FUE-ND) method. Rice grain, culm and leaf were dried to constant weight at 70^{0}C and analyzed for total N by the micro-Kjeldahl method (Bremner and Mulvaney, 1982 and Ladha et al., 1996). The proportion of N derived from ^{15}N-labeled fertilizer in the plant and soil and percent ^{15}N recovered by the plant and that remaining in soil were calculated with the following equation (IAEA, 2001):

N recovery from ^{15}N-labeled fertilizer =

$$\frac{(\text{atom \% }^{15}\text{N excess}_{\text{plant}})}{(\text{atom \% }^{15}\text{N excess}_{\text{fertilizer}})} \text{ X } \frac{(\text{N}_{\text{plant}})}{(\text{N}_{\text{fertilizer}})} \text{ X100}$$

Where atom % ^{15}N excess $_{\text{plant}}$ = atom % ^{15}N excess (over back ground levels) in the plant, atom % ^{15}N excess $_{\text{fertilizer}}$ = atom % ^{15}N excess in the labeled fertilizer N, N $_{\text{plant}}$ = total plant N (kg ha^{-1}), and N $_{\text{fertilizer}}$ = fertilizer N applied (kg ha^{-1}).

Soil sampling and analysis

In both years periodic sampling for NH_4-N analysis in the soil at 0-30 cm depths were done in each treatment for each winter crops. During the first month following incorporation of winter crop residues and soil submergence, soil was sampled to a 30-cm depth five times in each rice growing season to determine available N under all treatments. Soil samples were collected with a 4-cm-diam augur, and each sample represented a mixed composite from four cores taken in micro plot. Soil NH_4-N content was analyzed by semi micro Kjeldahl method after extraction with 2N KCL solution in a 1:5 soil: solution ratio. At the end of flooded rice crop NH_4-N dominated in the soil with negligible amounts of NO_3-N (George et al., 1992) Determination of soil nitrate-N was not included because of the negligible concentrations apparent in flooded rice soils (Diekmann et al., 1993) and large amounts of accumulated soil NO_3 may be lost from rice lowlands upon the flooding of aerobic soil for rice production and puddle before transplanting rice (Ladha et al., 1996).

Statistical analysis

The data were analyzed using a Statistical Analysis System (SAS, 1989). Following the analysis of variance procedures, differences among treatment means were determined using the least significant differences (LSD) comparison method.

Results and Discussion

Dry matter and nitrogen accumulation by winter crops

Dry matter accumulation of winter crops was affected significantly by type of crops but not by N fertilizer or their interaction effects. Plant N accumulation had significant influence by using different winter crops

Table 3. The NH_4-N availability in the 0- to 30-cm soil layer as influenced by fallow or winter cropping in 2001-2002

Winter crops	NH_4-N (mg 100^{-1} g soil)			
	2001	2002		
	Nov 10	March 20	April 20	May 15
Broad bean	7.59	8.08	8.76	9.50
Hairy vetch	7.60	7.91	8.46	8.69
Naked barley	7.58	7.44	7.07	7.51
Fallow (weedy)	7.59	7.57	7.67	7.73

ANOVA					
Sources of variation	Degrees of freedom	Mean squares for NH_4-N (mg 100^{-1} g soil)			
		2001	2002		
		Nov. 10	March 20	April 20	May 15
Crops	3	0.0002 ns	0.970 **	7.082 **	10.191 **
Error (a)	9	0.0002	0.006	0.002	0.006

** Significant at the 0.01 level; ns = non significant

and N fertilizer in 2002-2003 (Table 2) but no effect of N fertilizer was observed in 2001-2002 due to blank application of N fertilizer (Table 1). In 2001-2002, average dry matter accumulation of cover crops at harvest was 335 g m^{-2} for hairy vetch and 1082 g m^{-2} for broad bean with corresponding N accumulations of 13.1 g m^{-2} for hairy vetch and 35.2 g m^{-2} for broad bean, respectively. In 2002-2003, dry matter accumulation at harvest was 209 for hairy vetch and 1001 g m^{-2} for broad bean and contained 6.4 g m^{-2} of N in hairy vetch and 32 g m^{-2} of N in broad bean, respectively (Table 2). In both years, broad bean produced consistently more dry matter with greater N accumulation compared to hairy vetch and naked barley. Crop type and legume species was the main source of variation for total dry matter and N accumulation. In addition, the higher dry matter production of winter crops in 2001-2002 was largely achieved because the favorable rainfall received during that growing season (414 mm) compared with the higher rainfall (695 mm) received in 2002-2003. This higher precipitation in the latter growing season negatively affected hairy vetch germination and growth. Nitrogen production from legumes is a key benefit of growing cover crops and green manures. Nitrogen accumulations by leguminous cover crops ranged from 45 to 225 kg N ha^{-1} (Evans et al., 2001). The amount of N available from legumes depends on the species of legumes grown the total biomass production and the percentage of N in the plant tissue (Sullivan, 2003). The total N inputs from faba bean crop residues (116 to 199 kg ha^{-1}) which was lower than those achieved by green manure vetch (164 to 264 kg ha^{-1}) (Rochester and Peoples, 2005). In our study, total N inputs from broad bean residues were significantly higher than hairy vetch residues due to higher dry matter production.

Biological nitrogen fixation (BNF) by broad bean and hairy vetch

Broad bean fixed 27.5 g N m^{-2} (Table 1) and 16.3 to 26.0 g N m^{-2} (Table 2) in 2002 and 2003, respectively. Nitrogen fixed from broad bean was appreciably higher when N fertilizer was not applied. Legume contributions from BNF were lowest in treatments with the highest level of N fertilizer (120 kg N ha^{-1}) applied to the preceding rice crop. Nitrogen fixed by hairy vetch was significantly lower than broad bean due to lower biomass and N accumulation. In this study the average plant N derived from N_2 fixation (% Ndfa) in hairy vetch was 41% and broad bean was 78% of total plant N in 2001-2002 in N-difference method (Table 1). In 2002-2003, N_2 fixation of hairy vetch was 24% in N-difference method and 21% in ^{15}N natural abundance method while broad bean was 82% and 31% in N-difference method and ^{15}N natural abundance method, respectively (Table 2). The estimate of plant N derived from N_2 fixation was higher when the N difference method was used compared to ^{15}N natural abundance method in broad bean and hairy vetch. Nitrogen fixation determined by the ^{15}N natural abundance method was higher in broad bean with N 40 kg ha^{-1}. Application of N fertilizer at the lower rate was associated with the highest N_2 fixation in both hairy vetch and broad bean (Table 2).

Broad bean provided the highest quantity of N to the soil contributed from its above and below ground residues. Rochester and Peoples (2005) found that faba bean produced low to moderate grain yields 1.46 to 3.20 t ha^{-1} which removed 59 to 120 kg N ha^{-1}. Our observations suggest that legumes incorporated into rice-based cropping systems contribute not only to increased productivity but also to the maintenance and improvement of soil fertility in winter legumes by virtue of their capacity to fix large amounts of

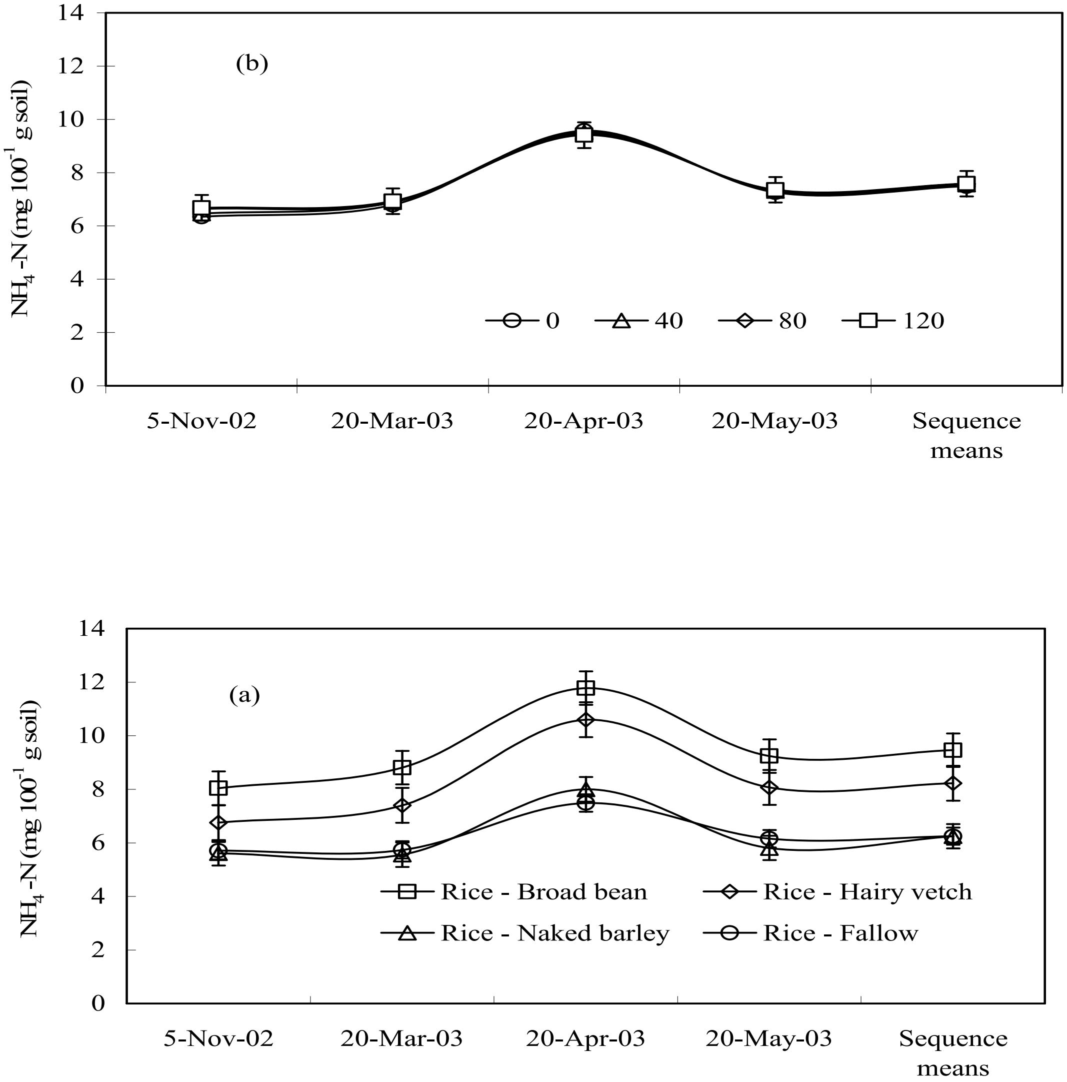


Fig 1. The NH_4-N availability in the 0- to 30-cm soil layer as influenced by (a) fallow or winter cropping (b) N fertilizer in 2002-2003. Error bars are LSD value at 0.01 level of probability

atmospheric N. Legumes can play a positive role in enhancing soil N fertility; however, they must leave behind more N from N_2 fixation than the amount of soil N they remove (Sullivan, 2003).

Estimates of the proportion of legume N derived from the atmosphere (% Ndfa) were 31-64% for soybean, 37-44% for mungbean and 42-48% for black gram (Ali et al., 2002). Our study coincides with the findings of Lopez et al., (2006) and they found that nitrogen derived from the atmosphere (Ndfa) percentages ranged between 70 and 96%, and N_2 fixed between 39 and 144 kg N ha^{-1}in faba bean. Environmental conditions also have a strong influence on BNF and the sensitivity of legumes to various climatic stresses determines the amount of N_2 fixed. The larger amounts of N_2 fixed in the 2001-2002 broad bean resulted primarily from better growth. The larger amounts of N_2 fixed in broad bean resulted from its much greater biomass production.

Soil NH_4-N status At the commencement of the experiment, NH_4-N in the 0-30 cm profile averaged 7.6 mg 100^{-1}g soil (Table 3). In 2002, after harvesting of the winter crops NH_4-N status increased up to 9.5 mg 100^{-1}g soil in broad bean, 8.7 mg 100^{-1}g soil in hairy vetch, 7.7 mg 100^{-1}g soil in fallow weedy plots and 7.5 mg 100^{-1}g soil in naked barley plots, respectively. The soil NH_4-N status of naked barley plots was slightly reduced over time (Table 3). After harvesting of first year rice, the soil NH_4-N contents were significantly reduced compared to end of the

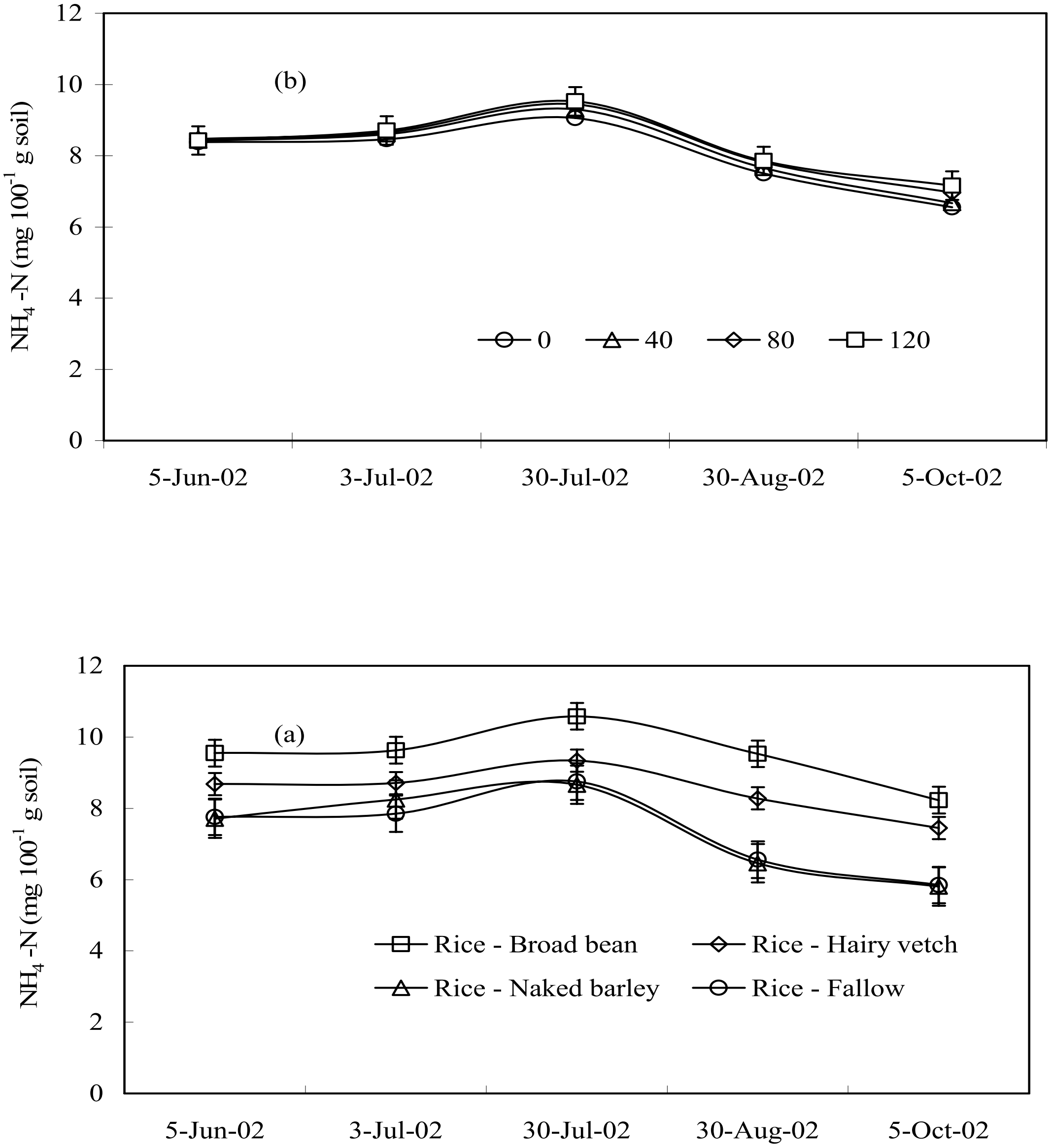


Fig 2. The NH_4-N availability in the 0- to 30-cm soil layer as influenced by (a) rice cropping (b) N fertilizer in 2002. Error bars are LSD value at 0.01 level of probability

first year winter crop soil status. In 2003, after harvesting of winter crops (May 20, 2003), soil NH_4-N ranged from 5.8 to 9.3 mg 100^{-1}g soil (Figure 1). Regardless of cropping systems and N fertilizer application, NH_4-N status in soil reached at peak in April 20, 2003 (Figure 1). Significantly higher soil NH_4-N (9.2 mg 100^{-1}g soil) was found in broad bean-rice followed by hairy vetch-rice and fallow weedy-rice systems while naked barley-rice received lower soil NH_4-N compared to the first year winter crops (Figure 1). In 2002 and 2003, the highest soil NH_4-N was recorded in rice after broad bean followed by rice after hairy vetch plots. Identical soil NH_4-N was recorded in rice after naked barley and fallow weedy plots. Regardless of cropping systems, plots having higher N application received higher status of soil NH_4-N (Figures 2 and 3). Regardless of N fertilizer, soil NH_4-N concentration was appreciably higher in rice after broad bean followed by rice after naked barley, rice after hairy vetch and rice after fallow weedy systems in both 2002 and 2003. In 2003 rice crops, the pattern of soil NH_4-N concentration was a

Table 4. Recovery of ^{15}N added in rice plants at harvest as affected by N fertilizer and cropping systems in Japan

Cropping systems X N fertilizer (Kg ha^{-1})	Recovery of ^{15}N-labeled fertilizer (% of total fertilizer N applied) 2002			2003		
	Soil	Plant	Total	Soil	Plant	Total
Broad bean-Rice X 0	-	-	-	-	-	-
Broad bean-Rice X 40	46	48	94	22	8	30
Broad bean-Rice X 80	32	45	77	23	5	28
Broad bean-Rice X 120	23	45	68	17	4	21
Mean	34	46	80	21	6	27
Hairy vetch-Rice X 0	-	-	-	-	-	-
Hairy vetch-Rice X 40	35	46	81	17	8	25
Hairy vetch-Rice X 80	33	47	80	14	4	18
Hairy vetch-Rice X 120	24	42	68	13	4	17
Mean	31	45	76	15	5	20
Naked barley-Rice X 0	-	-	-	-	-	-
Naked barley-Rice X 40	35	41	76	17	7	24
Naked barley-Rice X 80	34	43	77	14	4	18
Naked barley-Rice X 120	26	44	70	16	2	18
Mean	32	43	74	16	4	20
Fallow (weedy)-Rice X 0	-	-	-	-	-	-
Fallow (weedy)-Rice X 40	22	43	65	15	4	19
Fallow (weedy)-Rice X 80	20	54	74	17	2	19
Fallow (weedy)-Rice X 120	22	56	78	17	2	19
Mean	21	51	72	16	3	19
Analysis of variance			Statistics			
Cropping systems	**	**	**	**	**	**
N fertilizer (NF)	**	**	**	**	**	**
CS X NF	**	**	**	**	**	**

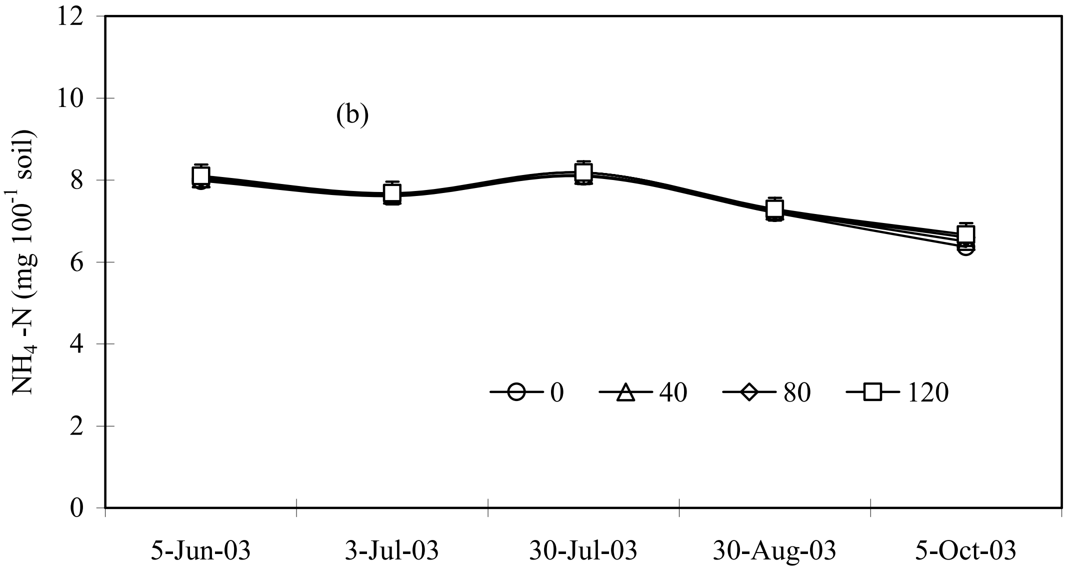
** Significant at the 0.01 level

bit different (Figure 3). The soil NH_4–N uptake pattern differed among different cropping systems. The increased level of mineral N in legume compared with that in weedy fallow plots could be due to enhanced mineralization of soil N caused by soil disturbance during sowing of legume seeds. Increased availability of soil N resulting from reduced immobilization or enhanced remineralization of immobilized N in the legume rhizospheres (Ali et al., 2001); N contributions by leaf litter or from nodulated roots, decreased denitrification losses resulting from improved soil structure and smaller water-filled pore space, promoted by the legumes or reduced utilization of soil mineral N by legumes (Dobermann et al., 2000; Cassman et al., 2002).

The larger early concentrations of NH_4-N measured in 2003 could partly be explained by contributions from plant residues incorporated into the soil in the previous year. The increase in NH_4-N was likely attributed to mineralization of soil organic matter and plant residues. The enhanced levels of mineral N observed in the soil after a legume than after fallow or naked barley can be credited to the larger amounts of plant N returned to those plots. The subsequent decline in mineral N presumably reflected uptake and assimilation by growing rice plants and possible losses through various transformation processes such as leaching, volatilization, and denitrification (Cassman et al., 2002).

N-recovery from ^{15}N-labeled fertilizer by rice cropping systems

Plant, soil and total N recovery was significantly influenced by the cropping systems and N fertilizer. In 2002, rice recovered 65 to 94 % of total N among applied ^{15}N-labeled fertilizer and legume residue incorporation. Rice after broad bean had the highest (68 to 94 %) total N recovery and rice after fallow had the lowest (65 to 78 %) total N recovery, respectively (Table 4). In 2003, recovery of applied ^{15}N-labeled fertilizer and legume residue incorporation ranged from 19% in rice after fallow to 21-30 % in rice after broad bean, respectively (Table 4).



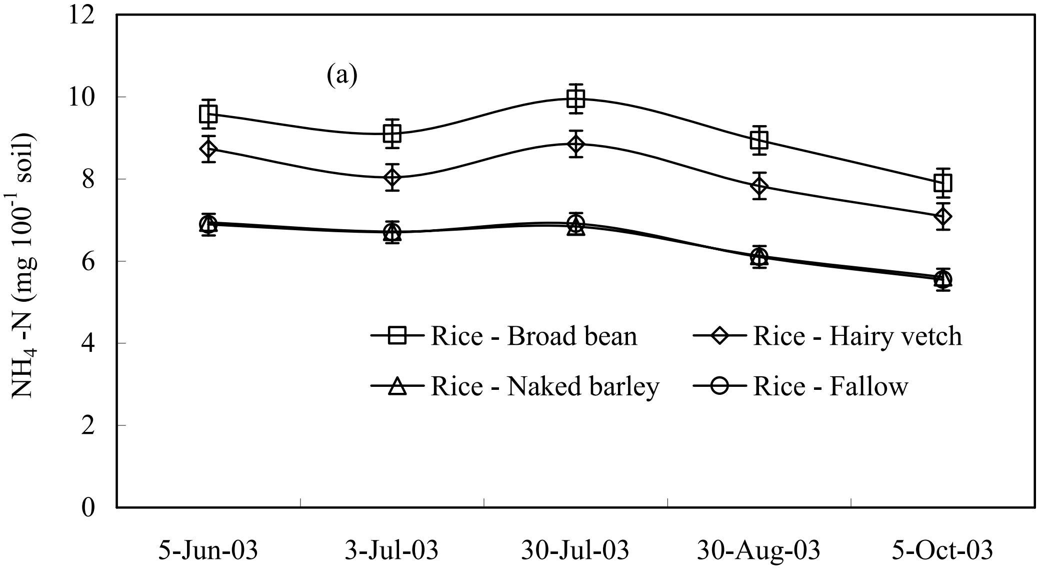


Fig 3. The NH_4-N availability in the 0- to 30-cm soil layer as influenced by (a) rice cropping (b) N fertilizer in 2003. Error bars are LSD value at 0.01 level of probability

In 2002, plant N recovery was highest in rice after fallow while in 2003 N recovery was highest in rice after broad bean followed by rice after hairy vetch or naked barley. In 2002, rice plant N recovery of ^{15}N-labeled fertilizer (% of N applied) were in the range of 41 to 56%. Rice after broad bean with N 40 kg ha^{-1} and hairy vetch systems with N 80 and N 120 kg ha^{-1} achieved moderate plant N recovery. Minimum plant N recoveries of ^{15}N-labeled fertilizer were obtained from rice after naked barley with N 40 kg ha^{-1} (Table 4). In 2003, rice plant N recovery of ^{15}N-labeled fertilizer was in the range of 2 to 8 %. Higher plant N recovery from ^{15}N-labeled fertilizer was achieved in rice after broad bean. Rice after broad bean and rice

Table 5. Fertilizer N use efficiency (FNUE) determined by ^{15}N dilution (FUE-^{15}N) and N difference method (FUE-ND) at harvest of rice as affected by N fertilizer and cropping systems in Japan

Cropping systems X N fertilizer (Kg ha^{-1})	2002 FUE-^{15}N	 FUE-ND	2003 FUE-^{15}N	 FUE-ND
Broad bean-Rice X 0	-	-	-	-
Broad bean-Rice X 40	48	16	8	19
Broad bean-Rice X 80	45	10	4	16
Broad bean-Rice X 120	45	10	4	12
Mean	46	12	5	16
Hairy vetch-Rice X 0	-	-	-	-
Hairy vetch-Rice X 40	46	98	6	17
Hairy vetch-Rice X 80	47	64	5	18
Hairy vetch-Rice X 120	42	50	2	26
Mean	45	71	4	20
Naked barley-Rice X 0	-	-	-	-
Naked barley-Rice X 40	41	72	9	47
Naked barley-Rice X 80	43	22	4	25
Naked barley-Rice X 120	44	9	2	20
Mean	43	34	5	31
Fallow (weedy)-Rice X 0	-	-	-	-
Fallow (weedy)-Rice X 40	43	68	4	29
Fallow (weedy)-Rice X 80	54	61	2	23
Fallow (weedy)-Rice X 120	56	75	2	18
Mean	51	68	3	23
		Statistics		
Analysis of variance				
Cropping systems (CS)	**	**	**	**
N fertilizer (NF)	**	**	**	**
CS X NF	**	**	**	**

** Significant at the 0.01 level

after hairy vetch with N 40 kg ha^{-1} achieved superior plant N recovery from ^{15}N-labeled fertilizer. The lowest plant N recoveries of ^{15}N-labeled fertilizer were obtained from rice after fallow with N 80 and N 120 kg ha^{-1} and rice after naked barley with N 120 kg ha^{-1} (Table 4). In 2002, rice after broad bean with 40 kg N ha^{-1} obtained the highest (46%) soil N recoveries while in 2003, rice after broad bean with N at rates of 40 and 80 kg ha^{-1} recorded maximum (22-23%) soil N recovery (Table 4). The poor soil N recoveries were recorded by fallow-rice with N 40 and naked barley-rice with 80 kg ha^{-1}. These results suggest that soil N uptake was greater from organic sources than from inorganic N fertilizer. Nitrogen recovery was poor when rice crop received both unfertilized legume residue and higher rate of N fertilizer regardless of winter legume crops (Table 4). Thus, based on total ^{15}N recovery, N losses from the soil-plant system in rice-fallow systems were appreciably higher than those in rice-broad bean and rice-hairy vetch systems. Therefore, recovery of ^{15}N-labeled fertilizer of rice-broad bean and rice-hairy vetch systems indicate a positive contribution of biological nitrogen fixation on enhancing soil N fertility for rice production. The greater recovery of ^{15}N-labeled fertilizer (% of N applied) in rice after fallow when compared to the legume-based systems. The substantial amount of N unaccounted from labeled fertilizer was probably due to ammonia volatilization losses and denitrification caused by anaerobic conditions predominant during the time fertilizer was applied. Incorporation of legume residues in rice after broad bean and rice after hairy vetch increased the soil N availability when compared with rice after naked barley and rice after fallow. This is likely due to an increase in net N mineralization and corresponding addition of fertilizer ^{15}N. The increase in fertilizer N uptake appears to be associated with an increase in soil N uptake when broad bean or hairy vetch was incorporated. Consequently, the rate of fertilizer N application can be reduced when broad bean and hairy vetch residues are available. Crops grown in previous years impact the amounts of residual nutrients available for subsequent plant growth (Gan et al., 2003). Substantial amount of N returned in the crop residue for use by subsequent

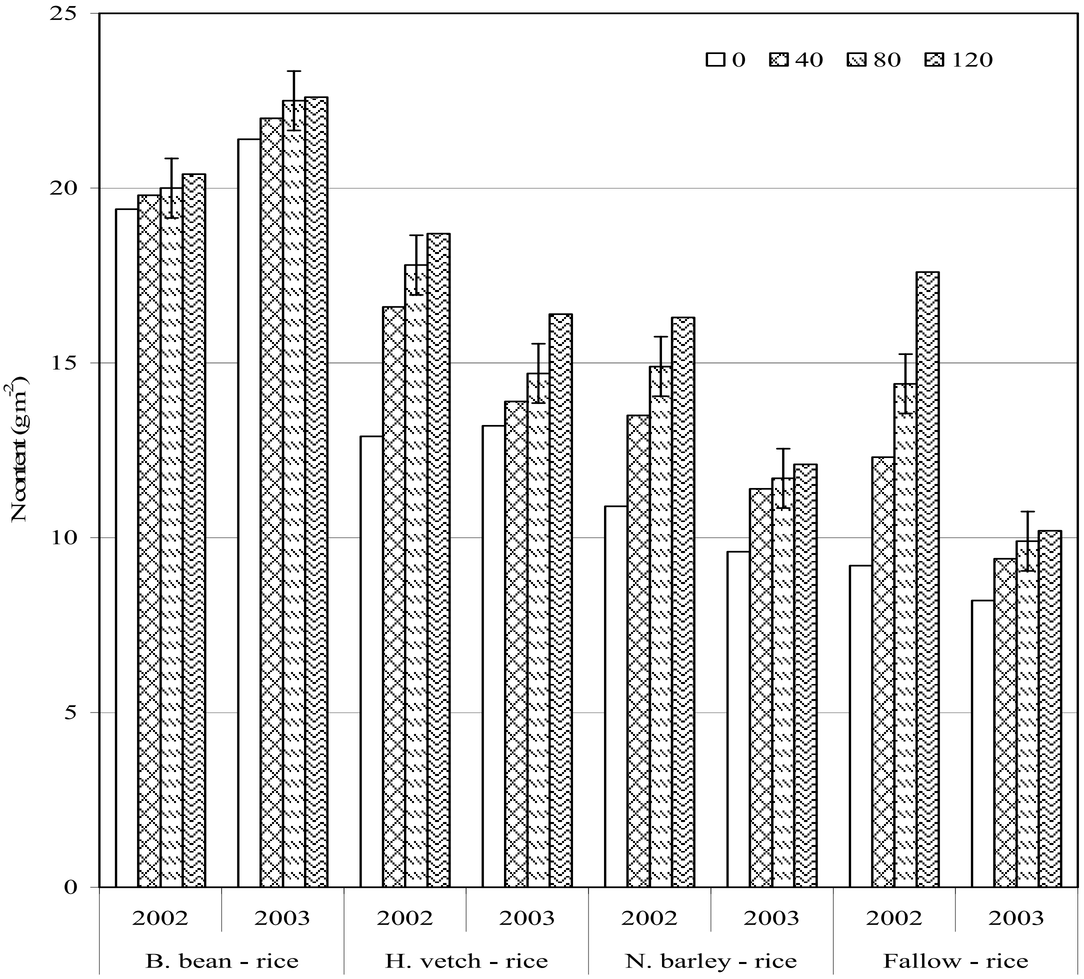


Fig 4. Nitrogen accumulation of rice as affected by cropping systems and N fertilizer

crops primarily due to the quantity of crop residue and N concentration of residue (Grant et al., 2002). A considerable amount of applied ^{15}N–labeled fertilizer remained in the soil after harvest of winter crops and rice. The amount of ^{15}N remaining in the soil at crop maturity from basally applied N fertilizer was significantly lower than that from basally applied legume residues. Presumably because some legume residue remained undecomposed, ^{15}N was retained in the microbial biomass, and N losses were lower from green manure. Most of the decomposable C fraction of added residues was mineralized within one crop-growing season and application of N fertilizer and GM further accelerated this process (Milkha et al., 2001). A combined application of unfertilized legume with 40 kg labeled N fertilizer ha^{-1} resulted in significantly higher recov-eries of N in the soil than that of labeled N fertilizer applied alone at the same N rates (Table 4).

Fertilizer nitrogen use efficiency (FNUE)

Fertilizer nitrogen use efficiency measured with either ^{15}N natural abundance or the N-difference method which was significantly influenced by the cropping systems and levels of N fertilizer for both years (Table 5). The FNUE-^{15}N values measured in this study are comparable to the 41 to 56 % in 2002 and 2 to 9 % in 2003. Fertilizer nitrogen use efficiency by the ^{15}N natural abundance method over the rice season in 2002 was significantly greater in rice after fallow systems with N 120 kg ha^{-1} while in 2003 rice after broad bean and rice after naked barley

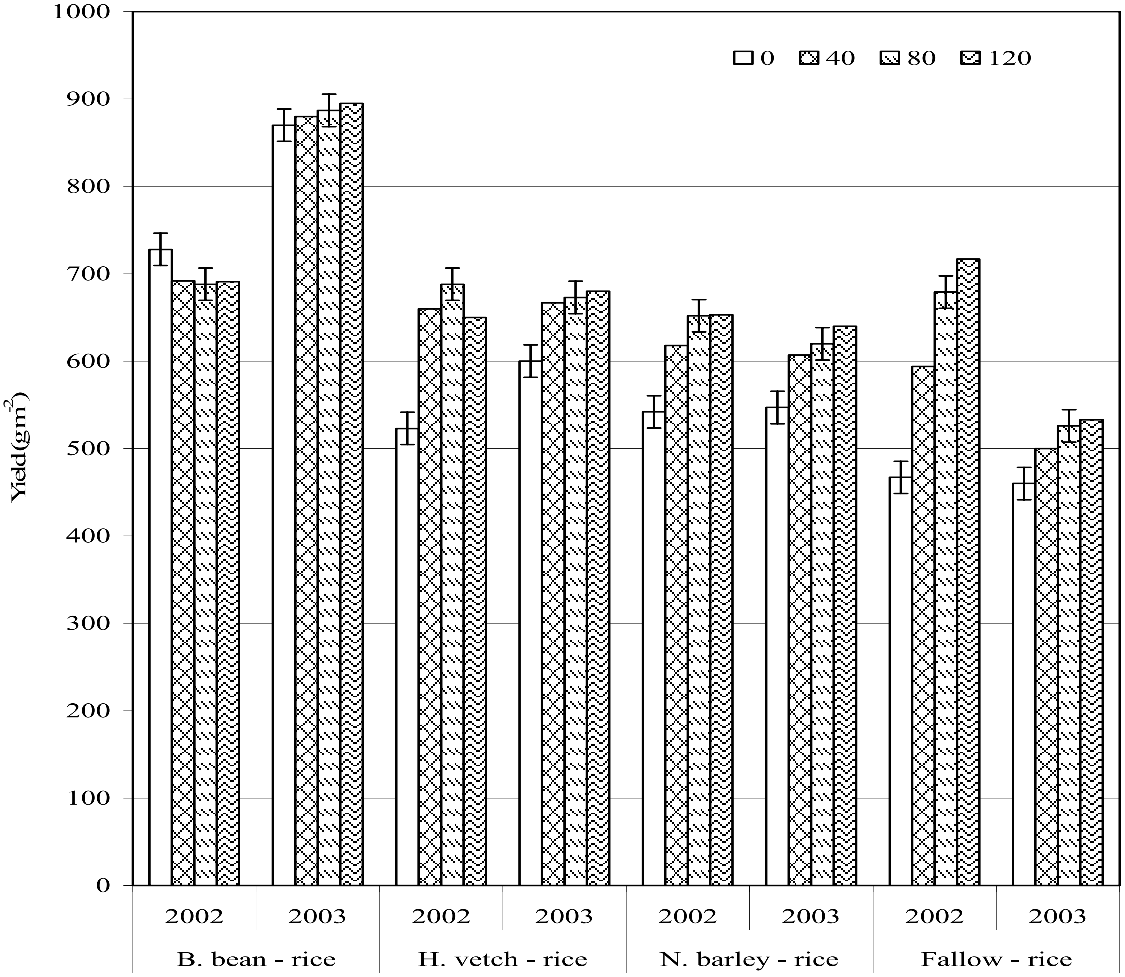


Fig 5. Grain yield of rice as affected by cropping systems and N fertilizer

with N 40 kg ha^{-1} recorded maximum FNUE. The FNUE using the ND method was higher than that obtained from the ^{15}N natural abundance method except in rice after broad bean in 2002. In 2002, FNUE by ND method was highest in rice after hairy vetch with N 40 kg ha^{-1} while in 2003, rice after naked barley with 40 kg N ha^{-1} obtained the superior FNUE (Table 5). Our findings suggest that the rice–fallow system largely depends on inorganic N fertilizer. The greater FNUE in the rice-legume systems after the first year crops appears related to the important quantities of residual N accumulated during the first year. In general, the FNUE found in this study was comparatively lower than those of other studies where fertilizer N was applied later in the growing season. Bronson et al., (2000) did not observe any differences in fertilizer use efficiency (FUE)-^{15}N between split fertilizer applications at different times. The average N-fertilizer uptake efficiency achieved by rice farmers is 31% of applied N based upon on-farm measurements in the major rice production regions of Asian countries. In contrast, recovery efficiency of nitrogen (RE_N) for rice in well managed field experiments typically range from 50 – 80 % (Dobermann et al., 2000). Therefore, need to improve RE_N is to be emphasized because N fertilizer is the largest source of N input to and losses from cereal cropping systems.

Nitrogen accumulation and grain yield of rice

Total N accumulation at rice maturity was influenced by the cropping systems, N fertilizer application, and their interaction. Both years, the incorporation of legume residue significantly increased N accumulation of wetland rice. Regardless of N fertilizer maximum N accumulation was obtained by rice after broad bean and in rice after hairy vetch with N 120 kg ha^{-1}. Moderate N accumulation was measured in rice after hairy vetch using N 40 and 80 kg ha^{-1} and rice after naked barley using N 120 kg ha^{-1}, and rice after fallow using N 120 kg ha^{-1}. The lowest N accumulation occurred in rice after fallow and rice after naked barley both without N fertilizer application (Figure 4). Grain yield of rice was influenced by the incorporation of legume residue and N fertilizer application in both years. In 2002, regardless of N fertilizer rice after broad bean, rice after naked barley with N 80 and N 120, rice after hairy vetch with N 40, N 80 and N 120, and rice after fallow with N 120 kg ha^{-1} produced maximum yields. Minimum yield was obtained in rice after fallow, rice after hairy vetch, and rice after naked barley without applied N. Rice after hairy vetch with N 40, N 80 and N 120 kg ha^{-1} gave similar yields. Rice after naked barley with N 80 and N 120 kg ha^{-1} produced identical yield. No appreciable difference was observed on rice after fallow with N 80 and N 120 kg ha^{-1} (Figure 5). In 2003, irrespective of N fertilizer significantly highest rice grain yield was obtained from broad bean – rice systems and minimum yield was recoded by fallow weedy – rice and naked barley – rice systems with no N application. In both years, rice yield showed that broad bean was more effective than applied N fertilizer with other cropping systems. Hairy vetch was same effective as N 40 kg ha^{-1} in producing rice grain yield compared with fallow-rice and naked barley-rice with highest rate of N application. The increased plant N following broad bean and hairy vetch incorporation could possibly be attributed to the N contribution from above ground biomass incorporation and below ground residues of the legumes. A rapid increase in soil microorganisms occurs after a young, relatively lush green manure crop is incorporated into the soil. The soil microbes multiply to attack the freshly incorporated plant material. During microbial breakdown, nutrients held within the plant tissues are released and made available to the following crop (Sullivan, 2003). In our study both broad bean and hairy vetch was incorporated into soil at physiological mature stage. Presumably this was because of slow decomposition of legume residue resulting less volatile loss of N during rice after broad bean and rice after hairy vetch growing period. On the contrary in rice fallow systems with fertilizer, a substantial part of the applied urea presumably was lost by ammonia volatilization. This is probably due to the fact that N was applied up to panicle initiation stage. N applied as urea up to the sampling stage (plant sampling done before top dressing) a substantial loss of N had occurred by ammonia volatilization. The primary determinants of total plant-available N supply are the net rate of N release from soil organic matter and incorporated crop residues, which is controlled by the balance between N immobilization and mineralization as mediated by soil microbes, the contributions from applied organic and inorganic N sources, and losses from the plant-available N pool (Cassman et al., 2002). Faba bean had positive effect on grain yield and soil nitrate at sowing in the wheat crop compared with fallow, chickpea, sunflower, and continuous wheat (López-Bellido, 2001).

Conclusion

This study revealed that that N derived from broad bean and hairy vetch is readily available and can be used efficiently by wetland rice crop. No fertilizer was needed when rice was grown after broad bean but rice required only 40 kg N ha^{-1} when hairy vetch was grown preceding rice crop. Broad bean and hairy vetch residues incorporated into the soil supplied N to wetland rice and produced benefits comparable with that of 40 to 80 kg fertilizer N ha^{-1}. Rice after broad bean and hairy vetch systems became more N fertile over the course of the experiment and produced higher rice yields than the comparative fallow-rice and non-legume systems (rice-naked barley). This superior performance of rice after broad bean or hairy vetch is likely linked to greater N fixation and may be a viable alternative N source to enhance soil fertility. Such winter legumes that improve annual productivity of rice might be attractive to farmers who are generally resource-poor farmers. Since the benefit of a steady increase in soil N and soil fertility is clear. Thus, broad bean and hairy vetch has the potential to substitute or supplement for chemical/inorganic N fertilizer.

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