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NPK Accumulation and Use Efficiencies of Manawthukha Rice (*Oryza sativa* L.) Affected By Pretransplant Basal and Split Applications of Nitrogen

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We investigated the effects of split applications of nitrogen (N) on N, phosphorus, and potassium (NPK) uptake and use efficiency of rice under basal surface-application and incorporation methods. Different amounts of N were applied at the basal, tillering, and panicle initiation stages in five N split treatments. Basal incorporation provided greater NPK uptake than basal surface application until initiation of the panicle. In basal surface application, N2 (25:50:25) resulted in the greatest total NPK uptake, use efficiency, and N recovery efficiency. In basal incorporation, N1 (50:25:25) resulted in greater values for all parameters. The N5 (50:0:50), which included omitting N at tillering, resulted in low N recovery efficiency and uptake, both under basal incorporation and basal surface application. These results emphasize that split applications of N influence N recovery efficiency and total NPK uptake and use efficiency of rice.

Keywords Basal application method, NPK uptake, N recovery efficiency, N split application, rice

Introduction

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population (Khush 2004). In particular, it is the most important food grain in the diets of hundreds of millions of Asians, Africans, and Latin Americans living in tropical and subtropical areas (Julino 1993). Rice provides 21% of the global human per capita energy and 15% of per capita protein (IRRI 2002). Furthermore, rice cultivation and postproduction activities provide food and income and also create employment opportunities for farmers.

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Nitrogen (N) is a limiting nutrient during rice cultivation. Loss of N can affect the growth and quality of rice, and N applied to rice accounted for approximately 50% of the worldwide N fertilizer used in 2009 (IFA 2009). The N use efficiency (NUE) of rice is usually poor due to volatilization, runoff, denitrification, and leaching losses (Modgal, Singh, and Gupta 1995). Nitrogen losses are estimated to be 10–65% of applied N (Cassman et al. 1998). The N recovery by rice is also low, ranging from 20 to 40% depending on the source and timing of N fertilizer, crop and water management, and agroecological conditions (Vlek and Craswell 1981).

Low NUE results from inefficient split application of N, including use of excess N. Split applications of N fertilizer improve the synchrony between the N demand of crops and the supply from soil and/or applied N fertilizer (Win 2003). Rao et al. (1995) reported that split application of N fertilizer improved crop productivity by improving water control. Generally, a strategy of multiple split applications of mineral N fertilizer reduces N losses and increases NUE (Cassman, Kropff, and Zhen-De 1994). Increasing the number of splits from two to four results in greater N recovery, total N uptake (NU), and agronomic NUE (AUE) but hardly affects grain yield. Belder et al. (2005) demonstrated that increasing the number of splits to six had no effect. This finding was in line with those of Singh et al. (1991), who found that increasing the number of split doses of urea from 3 to 10 did not enhance the efficiency of urea-N use in permeable soil in lowland India. Therefore, in the present study, different split application patterns were applied to rice only at critical growth stages (basal, active tillering, and panicle initiation). Although increasing the number of split applications results in a greater NUE, it is essential to determine an economical number of split applications for Asian farmers.

Moreover, the amount of N demanded by crops may vary at each critical growth stage of rice, and thus the amount and timing of N application are important to consider in order to match the supply with the crop's demand. Murty, Dey, and Jachuk (1992) reported that N management should coincide with crop demand and that it is important to ensure that N absorbed by the plant is transferred to grain production. Optimum uptake of N, as a primary nutrient, is key to acquire high uptakes of P and K. Rice plants suffering from low NU produce fewer leaves, show poor growth of shoots and roots, and thus absorb less P and K. The timing of N application to rice should be balanced to fulfill crop requirements before and after anthesis to achieve optimum nutrient uptake, growth, and yield (Mahajan et al. 2011). In the present study, different amounts of N were applied at the critical growth stages of rice to determine the demand of each nutrient at each stage and to investigate whether omitting N at the active tillering and panicle initiation stages would affect NPK uptake and use efficiency.

Improper split application can also lead to a low NUE. In Asia, urea, the main N fertilizer source, is not used efficiently by rice farmers, and the rice crop only recovers about 30–40% of the applied N (De Datta, Magnaye, and Moomaw 1968). Most Asian farmers broadcast urea directly into the floodwater 2–4 weeks after transplanting the rice (De Datta et al. 1979). Vlek and Fillery (1984) reported that such broadcast applications result in high concentrations of urea and/or ammonium in floodwater and lead to major N loss via ammonia volatilization, nitrification-denitrification, and surface runoff. They suggested several ways to potentially reduce the amount of N fertilizer in floodwaters, including using slow-release fertilizer, nitrification inhibitors, and/or urease inhibitors; incorporating N into the soil; and/or via split application of the fertilizer dose.

Thus, several techniques have been formulated to improve nutrient recovery and uptake. De Datta (1981) demonstrated in a field experiment in the Philippines that NUE

is greater when fertilizer is infused into soil at a depth of 10 cm. An instrument called a liquid urea injector has been used to inject dissolved urea at a 5–6 cm deep (Schnier et al. 1993). These techniques are effective for improving NUE in rice but are costly to farmers.

Hence, there is a need to find alternative, more economical methods that can simultaneously improve NUE and minimize N losses and ammonia N concentrations in floodwater. In the present study, we investigated the split application of N in different amounts and at different stages of rice growth. In particular, applying N at the basal stage of growth could be a promising technique. In addition, as ammonia gas is an environmental pollutant, using an incorporation method at this stage might be beneficial. Therefore, different N split application patterns were investigated to achieve optimal NPK accumulation and use efficiency of rice via two methods of application: surface application and an incorporation method immediately before transplantation. We investigated the proper split application patterns for improving NUE and examined crop uptake and use efficiency of the major nutrients (N, P, and K) under the different basal application methods.

Materials and Methods

Experimental Site

A field experiment was carried out at a Kyushu University farm in Fukuoka Prefecture, Japan (33° 37′ N, 130° 25′ E) from June to October 2012 to evaluate the effects of split application patterns and different basal application methods of N fertilizer (immediately before transplanting rice) on NPK accumulation and use efficiency of Manawthukha rice (Myanmar variety).

Experimental Design

A split-plot design with three replications was used in this experiment. Each experimental plot was 4.5×1 m. Two basal fertilizer application methods were used on main plots: the basal incorporation method (BIC) and the basal surface application method (BSF). On subplots, split N fertilizer application at the critical growth stages of rice was carried out as described in Table 1.

Description	of N-spin treatmen	its in the spin-plot design of	neia experiment
N treatment	Basal	Active tillering	Panicle initiation
N0 (control)	0%	0%	0%
N1	50%	25%	25%
N2	25%	50%	25%
N3	25%	25%	50%
N4	50%	50%	0%
N5	50%	0%	50%

 Table 1

 Description of N-split treatments in the split-plot design of field experiment

Note. The percentage of N application at each stage were based on 120 kg N ha^{-1} .

Treatments

The land was irrigated so as to be easily plowed, subsequently harrowed, and divided into two main plots: one using BIC and the other using BSF. Bunds were made to prevent seepage into and from adjacent plots, using a plastic liner installed to a depth of 15 cm between drains. Split N (as urea), 60 kg phosphorus pentoxide (P_2O_5) ha⁻¹ (as superphosphate), and 60 kg dipotassium oxide (K_2O) ha⁻¹ (as potash muriate), and potassium chloride (KCl) were incorporated into the soil at the last harrowing and leveling stage for the BIC treatment; for BSF, the same mixture was broadcast onto the soil surface. The two application methods were conducted 2 days prior to transplanting.

In the subplots, split N fertilizer was applied at a rate of 120 kg N ha⁻¹ except for the control treatment (N0), in which no N fertilizer was applied. The percentage of N applied was varied in five different treatments as follows: N1 (50:25:25%) at the basal, active tillering, and panicle initiation stages, respectively), N2 (25:50:25%), N3 (25:25:50%), N4 (50:50:0%), and N5 (50:0:50%).

Soil Sampling and Analysis

Initial soil samples were collected from six locations in the experimental field at 0–20 cm deep, using a soil sampling tube (5 cm in diameter) before conducting the field experiment. Samples were spread out, air dried at room temperature, crushed by hand, sifted through a 2-mm mesh sieve, and stored for further analysis. The water content of soil samples was determined by comparing the weights of each sample before and after drying them at 105 °C in an oven for 5 h.

The soil texture was clay loam. Soil pH_{H2O} [1:2.5 soil/water (H₂O)] and soil pH_{KC1} (1:2.5 soil/KCl) were measured using a pH meter (Beckman 360 pH/Temp/mV Meter; Beckmann Coulter, Brea, CA, USA). The nutrient content of soil was determined by extracting nutrients using the salicylic acid–sulfuic acid (H₂SO₄)–hydrogen peroxide (H₂O₂) digestion method (Ohyama et al. 1991). Then total N was analyzed using the indophenol method (Cataldo, Schrader, and Youngs 1974), total P was analyzed using an atomic absorption spectrophotometer (Z-5300, Hitachi, Tokyo, Japan). Cation exchange capacity and exchangeable cations were determined using the ammonium acetate shaking extraction method (Muramoto, Goto, and Ninaki 1992) followed by atomic absorption spectrophotometry (Z-5300, Hitachi). Analysis of mineralizable N was performed using the soil incubation method (Sahrawat 1983) followed by the indophenol method (Cataldo, Schrader, and Youngs 1974). The available P of soil samples was analyzed using Truog's method (Truog 1930) followed by the ascorbic acid method (Murphy and Riley 1962).

Crop Management

The rice cultivated in this study was a high-yielding Indica variety from Myanmar (Manawthukha). The adaptability of this variety to the Japanese environment has been verified (Myint 2011). Good seeds were chosen by using an air blower. Then the collected seeds were sterilized by immersing them in hot water and shaking them at 58 °C and 74 rpm for 15 min. The sterilized seeds were washed with distilled water and stored in a 25 °C incubator for 48 h in the dark.

The incubated seeds were sown as two seeds per cell on seed beds using commercial seedbed soil (Kokuryu Baido, Seisin Sangyo Co., Kitakyushu, Japan). The seed beds were placed in a pond maintained at a shallow water level. Then 21-day-old seedlings were transplanted into the plots at two seedlings per hill with a spacing of 25×15 cm. Irrigation was provided through a common management plan together with the surrounding area.

Plant Sampling and Nutrient Uptake Analysis

At the active tillering, panicle initiation, grain filling, and harvest stages, two hills from each plot were harvested as destructive samples to determine the uptake of nutrients (N, P, and K). The rice plants were cut at 2–3 cm aboveground, oven dried at 70 °C for 48 h, and ground to a fine powder using a Cyclotec 1093 Sample Mill (100–120 mesh, Tecator AB, Hoedanaes, Sweden). Nutrients that accumulated in plant parts such as straw, filled grains, and unfilled grains were digested separately using the salicylic acid–H₂SO₄–hydrogen peroxide (H₂O₂) digestion method (Ohyama et al. 1991) followed by analysis of total N, P, and K using the methods mentioned previously. The total uptakes of each nutrient were calculated from the sum of the products of the biomass and the concentration of each nutrient of the different plant parts (straw, filled grains, and unfilled grains).

Computation of NUE and Related Parameters

After analyzing the total NPK uptake of rice plants, NUE (Peng et al. 1996), P use efficiency (PUE), and K use efficiency (KUE) were calculated as follows:

$$NUE = \frac{\text{Grain yield (kg ha^{-1})}}{\text{Applied N (kg ha^{-1})}}.$$

The N harvest index (NHI) (Witt et al. 1999) was calculated as follows:

$$NHI = \frac{N \text{ accumulation in grain } (kg \text{ ha}^{-1})}{\text{Total N uptake } (kg \text{ ha}^{-1})}.$$

Internal efficiency (IE) (Witt et al. 1999) was calculated as follows:

$$IE = \frac{\text{Grain yield } (\text{kg ha}^{-1})}{\text{Total N uptake}(\text{kg ha}^{-1})}.$$

The AUE (Novoa and Loomis 1981) was calculated as follows:

$$AUE = \frac{\text{Grain yield in N fertilized plot} - \text{Grain yield in N control plot } (\text{kg ha}^{-1})}{\text{Applied N in N fertilized plot } (\text{kg ha}^{-1})}.$$

The N recovery efficiency (NRE) (Dilz 1988) was calculated as follows:

 $NRE(\%) = \frac{N \text{ uptake in N fertilized plot} - N \text{ uptake in N control plot } (kg \text{ ha}^{-1})}{\text{Applied N in N fertilized plot } (kg \text{ ha}^{-1})} \times 100.$

Statistical Analysis

Data were summarized and subjected to analysis of variance (ANOVA). Means among treatments were compared to Tukey's highly significant difference (HSD) test at the 5% probability level using STATISTIX 8 (Analytical Software, Tallahassee, FL, USA).

Simple regression and correlation analyses were performed following the procedures outlined by Gomez and Gomez (1984).

Results

Soil Analysis

Table 2 shows the physical and chemical properties of the soil profile (0-20 cm) at the experimental site.

NU Affected by N Split Application Patterns at Critical Growth Stages. Significant differences in NU were observed at the 0.05 probability level between BSF and BIC at the active tillering and panicle initiation stages (Figure 1). Greater NU was observed when using the BIC method at both stages but no further differences occurred at later stages. The different N split application patterns significantly influenced NU throughout the crop period at the 0.05 probability level (Figure 1). NU at the active tillering (NU_{AT}) stage increased with the percentage of N applied in both treatment methods. High doses of basal N produced greater NU_{AT} in the N1 (50:25:25), N4 (50:50:0), and N5 (50:0:50) treatments at the 0.05 probability level, but no significant differences were observed among them. N2 (25:50:25) and N3 (25:25:50) treatments led to lower NU_{AT}.

With the BSF method at the 0.05 probability level, NU at panicle initiation (NU_{Pl}) was maximized (88.38 kg ha⁻¹) in treatment N4 (50:50:0), in which the full percentage of N had already been applied before panicle formation. However, a similar NU_{Pl} (86.14 kg ha⁻¹) was also found in the N2 treatment (25:50:25). That 63.88 kg ha⁻¹ of

Table 2
Physical and chemical properties of the surface (0-20 cm)
soil profile at the experimental site

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Physicochemical property	Value
Soil pH (soil/H ₂ O;1:2.5)	6.4
Soil pH (soil/KCl;1:2.5)	5.2
Total N (%)	0.13
Total P_2O_5 (%)	0.24
Total K_2O (%)	0.48
Available N (mg N / 100 g soil)	3.05
Available P (mg P ₂ O ₅ / 100 g soil)	16.79
CEC (c mol _c kg ^{-1})	13.18
Exc. Ca (c $mol_c kg^{-1}$)	3.60
Exc.Mg (c $mol_c kg^{-1}$)	0.80
Exc. K (c mol _c kg ^{-1})	0.40
Exc. Na (c $mol_c kg^{-1}$)	0.95

Notes. CEC, cation exchangeable capacity; Exc., exchangeable.



Figure 1. The N uptake (kg ha⁻¹) of Manawthukha rice affected by pretransplant basal application methods and split application patterns of nitrogen at the active tillering, panicle initiation, and grain filling stage. The histograms with the same letter in the same cases at each stage are not significantly different by the Tukey HSD test (p < 0.05). * Significant difference at 5% probability. BSF, basal surface application method; BIC, basal incorporation method.

N3 (25:25:50) was greater than that of N5 (50:0:50), which had the lowest NU_{PI} (45.97 kg ha⁻¹). Although the same amount of N had been applied up until the panicle initiation stage in treatments N1 and N2, N1 (50:25:25) had a lower NU_{PI} (69.82 kg ha⁻¹) than did N2 (25:50:25).

In the BIC plots, N2 (25:50:25) obtained the greatest NU_{PI} (105.98 kg ha⁻¹) at the 0.05 probability level, but the value was similar to that of N4 (50:50:0). Again, even though the same amount of N had been applied up until the panicle initiation stage in treatments N1 and N2, N1 (50:25:25) had a lower NU_{PI} (80.41 kg ha⁻¹) than did N2.

The greatest NU at the grain filling stage (NU_{GF}) occurred in N3 (25:25:50) for both basal application methods, but the values were similar to those for treatment N2 (25:50:25). The lower panicle application of N in treatment N1 (50:25:25) resulted in lower NU_{GF} values (98.26 and 106 kg ha⁻¹) in both application methods but they were similar to those of N4 (50:50:0). Despite the high doses of N applied at panicle initiation in treatment N5 (50:0:50) it did not achieve optimum NU_{GF}, resulting in 88.69 and 97.71 kg ha⁻¹ with the BSF and BIC methods, respectively.

At the time of harvest, the highly significant differences in NU in straw (NU_S), grains (NU_G), and the entire rice plant (TNU) occurred at the 0.01 probability level among the N split applications (Table 3). With BSF, the greatest NU_S (51.67 kg ha⁻¹) was found in N2 (25:50:25), but it was similar to that of N4 (50:50:0). The high level of N applied at the active tillering and panicle initiation stages resulted in greater NU_G in the N2 (25:50:25) and N3 (25:25:50) treatments. N2 (25:50:25) achieved the greatest TNU (162.09 kg ha⁻¹), but it was similar to N3 (25:25:50). Generally, the high doses of basal surface N generated lower TNU values at harvest in N1 (50:25:25), N4 (50:50:0), and N5 (50:0:50). Omitting N at the active tillering stage, N5 (50:0:50) did not produce optimum TNU under either application methods. With the BIC method, N1 (50:25:25) resulted in the greatest TNU values, being 59.98, 107.09, and 167.07 kg ha⁻¹ in straw, grain, and the whole rice plant, respectively. Similar TNU values were observed among other N split applications except N5 (50:0:50), in which no N was applied at the active tillering stage.

	Tot	al N uptake (kg ha-	(1	Total P.O. untake (TPII)	Total K.O untake (TKII)
Treatment	Straw (NU _S)	Grain (NU _G)	Total (TNU)	$(kg ha^{-1})$	$(kg ha^{-1})$
Basal surface applica	tion method				
N0 (control)	21.13 c	55.38 c	76.50 c	41.02 c	95.40 d
N1 (50:25:25)	36.42 b	91.85 b	128.27 b	63.57 a	142.20 b
N2 (25:50:25)	51.67 a	110.42 a	162.09 a	68.86 a	153.06 ab
N3 (25:25:50)	42.43 ab	105.85 a	148.28 ab	65.31 a	143.76 ab
N4 (50:50:0)	45.79 ab	92.21 b	138.00 ab	61.57 b	156.67 a
N5 (50:0:50)	36.84 b	94.82 b	131.66 ab	61.48 b	126.46 c
Basal incorporation	method				
N0 (control)	22.84 c	55.39 d	78.22 c	39.27 c	92.16 c
N1 (50:25:25)	59.98 a	107.09 a	167.07 a	65.88 a	166.56 a
N2 (25:50:25)	50.91 ab	112.55 a	163.46 a	64.48 a	163.98 a
N3 (25:25:50)	48.90 ab	105.11 ab	154.02 ab	61.71 a	149.43 b
N4 (50:50:0)	45.47 b	99.45 b	144.92 ab	62.87 a	165.58 a
N5 (50:0:50)	38.65 b	88.60 c	127.25 b	60.48 b	142.83 b
Source of variance (1	$v_r > F$)				
Methods	ns	ns	su	ns	ns
N split	< 0.0001	< 0.0001	<0.0001	< 0.0001	<0.0001
Methods \times N split	su	ns	su	su	ns

Table 3



Figure 2. The P_2O_5 uptake (kg ha⁻¹) of Manawthukha rice affected by pretransplant basal application methods and split application patterns of nitrogen at the active tillering, panicle initiation, and grain filling stage. The histograms with the same letter in the same cases at each stage are not significantly different by the Tukey HSD test (p < 0.05). * Significant difference at 5% probability. BSF, basal surface application method; BIC, basal incorporation method.

Phosphorus Uptake Affected by N Split Application Patterns at Critical Growth Stages.

The basal application method significantly influenced P_2O_5 uptake (PU_{AT}) of rice at the active tillering stage at the 0.05 probability level, but no other differences were observed between the application methods at later stages (Figure 2). The BIC method generated greater PU_{AT} values than those of BSF. The most significant differences in PU_{AT} were among the N split applications. With BSF, greater PU_{AT} values of 10.17, 10.93, and 11.33 kg ha⁻¹ were found in treatments N1 (50:25:25), N4 (50:50:0), and N5 (50:0:50), respectively. The lower basal N applications in treatments N2 (25:50:25) and N3 (25:25:50) achieved lower PU_{AT} values of 8.39 and 7.05 kg ha⁻¹, respectively. Similar results were found among the BIC N split treatments.

The greatest P uptake at panicle initiation (PU_{Pl}) were obtained (37.76 and 37.19 kg ha⁻¹) for treatment N4 (50:50:0) in both basal application methods but they were similar to those of N2 (25:50:25) at the 0.05 probability level. N1 (50:25:25) resulted in a lower PU_{Pl} than that of N2 (25:50:25). The values for N3 (25:25:50) were 27.92 and 27.55 kg ha⁻¹ with BSF and BIC, respectively. N5 (50:0:50) obtained the lowest values (20.92 and 27.36 kg ha⁻¹) in both methods.

The greatest P uptakes at the grain filling stage (PU_{GF}) were observed (59.62 and 55.18 kg ha⁻¹ using the BSF and BIC method, respectively) in the N3 treatment (25:25:50). N5 (50:0:50) did not generate optimal PU_{GF} values (41 and 46 kg ha⁻¹) in either methods. In general, the two N split treatments N4 (50:50:0) and N5 (50:0:50) led to lower PU_{GF} values than those of the N1 (50:25:25), N2 (25:50:25), and N3 (25:25:50) treatments.

At harvest, the maximum total P_2O_5 uptake (TPU) was observed (68.86 kg ha⁻¹) in treatment N2 (25:50:25) with BSF at the 0.01 probability level but similar results were observed in N1 (50:25:25) and N3 (25:25:50) (Table 3). As they all received the same amounts of N, just at different stages, N4 (50:50:0) and N5 (50:0:50) had the lowest TPU values. The N1 treatment (50:25:25) using the BIC method produced the greatest TPU



Figure 3. The K₂O uptake (kg ha⁻¹) of Manawthukha rice affected by pretransplant basal application methods and split application patterns of nitrogen at the active tillering, panicle initiation, and grain filling stage. The histograms with the same letter in the same cases at each stage are not significantly different by the Tukey HSD test (p < 0.05). * Significant difference at 5% probability. BSF, basal surface application method; BIC, basal incorporation method.

(65.88 kg ha⁻¹) but it was similar to those of N2 (25:50:25), N4 (50:50:0), and N3 (25:25:50). Omitting N at the active tillering stage, N5 (50:0:50) resulted in lower TPU values of 61.48 and 60.48 kg ha⁻¹ with BSF and BIC, respectively.

Potassium Uptake Affected By N Split Application Patterns at Critical Growth Stages.

The K uptake (KU) was affected by the different basal application methods at the active tillering and panicle initiation stages at the 0.05 probability level (Figure 3) but similar results were observed between BSF and BIC at later stages. At both earlier stages, rice plants absorbed more K when the BIC method was used than when BSF was used. Highly significant differences in KU were observed among the different N split applications throughout the crop period. The K uptake at the active tillering stage (KU_{AT}) was greatest (59.18 and 75.32 kg ha⁻¹ with BSF and BIC, respectively) for treatment N4 (50:50:0) but it was similar to the values of N1 (50:25:25) and N5 (50:0:50), both of which also had high levels of N applied at the basal stage. KU_{AT} was lower in N2 (25:50:25) and N3 (25:25:50), both of which had lower amounts of N applied at the basal stage.

When using the BSF method, the greatest K uptake at panicle initiation (KU_{PI}) was obtained (149.99 kg ha⁻¹) in treatment N4 (50:50:0); N2 (25:50:25) achieved a similar value (138.25 kg ha⁻¹), where that of N1 was low (118.37 kg ha⁻¹), similar to that of N3 (25:25:50). When using the BIC method, the high levels of N applied during the active tillering stages of treatments in N2 (25:50:25) and N4 (50:50:0) resulted in greater KU_{PI} values of 155.22 and 148.77 kg ha⁻¹, respectively. The values of N1 (50:25:25) and N3 (25:25:50) were lower, but greater than that of N5 (50:0:50).

When using the BSF method, the greatest K uptake at the grain filling stage (KU_{GF}) was observed (172.46 kg ha⁻¹) in treatment N3 (25:25:50), but was similar to that of N4 (50:50:0). Both N1 (50:25:25) and N2 (25:50:25) had lower values (154.09 and

153.31 kg ha⁻¹, respectively). Despite the high input of N at the panicle initiation stage in treatment N5 (50:0:50), the KU_{GF} remained low (116.87 kg ha⁻¹). Similar results were observed when using the BIC method. The greatest KU_{GF} (180.64 kg ha⁻¹) was achieved by N3 (25:25:50), but the difference was not significant compared to N2 (25:50:25). N1 (50:25:25) and N4 (50:50:0) had lower values, which did not differ significantly from each other but were greater than that (146.83 kg ha⁻¹) of N5 (50:0:50).

When using the BSF method, the high doses of N applied during the active tillering stage in treatments N2 (25:50:25) and N4 (50:50:0) led to the greatest total K uptake (TKU) values of 153.06 and 156.67 kg ha⁻¹, respectively, at the 0.01 probability level (Table 3), but the value for N3 (25:25:50) was only slightly lower. N1 (50:25:25) resulted in a significantly lower TKU and N5 was lowest. When using the BIC method, the greatest TKU (166.56 kg ha⁻¹) was obtained for N1 (50:25:25), but it was similar to those for treatments N2 (25:50:25) and N4 (50:50:0). TKU was again lowest in N5 (50:0:50), in which no N was applied at the active tillering stage.

The NHI, NRE, IUE, and AUE Affected by N Split Applications. The NHI ranged from 0.60 to 0.69 in both basal application methods (Table 4), but were generally lower when using BIC than when using BSF. There were significant differences among the different N split applications at the 0.05 probability level.

The NRE was generally greater with the BIC method than with BSF (Table 4). With BSF, the greatest value (71.33 kg additional grain kg⁻¹ N applied) was obtained in N2 (25:50:25) at the 0.05 probability level, but it was similar to those of N3 (25:25:50) and N4 (50:50:0). High losses of N occurred in N5 (50:0:50) and N1 (50:25:25) resulting in a lower NRE (45.96% and 43.14%, respectively). With BIC, the maximum NRE (74.04%) was obtained in N1 (50:25:25) but it was not significantly different from those of N2 (25:50:25) and N3 (25:25:50). N4 (50:50:0) and N5 (50:0:50) had the lowest values.

Internal N use efficiencies (IUEs) did not differ by basal application method, but did differ significantly at the 0.05 probability level according to different N split applications (Table 4). When using the BSF method, N0 (control) produced the maximum IUE (60.08 kg grain kg⁻¹ NU) with low NU compared to grain yield. Similar results were found in treatments N1 (50:25:25), N3 (25:25:50), and N4 (50:50:0). The IUE of N2 (25:50:25) was lower (48.96), and similar to that of N5 (50:0:50). When using BIC, the greatest IUE (58.70) was again found in N0 (control) but similar results (52.56 and 53.60, respectively) were obtained for N4 (50:50:0) and N5 (50:0:50). N1 (50:25:50), N2 (25:50:25), and N3 (25:25:50) had lower IUEs that were not significantly different from each other at the 0.05 probability level.

The same overall trends were seen for AUE (Table 4), which ranged from 15.60 to 28.42 with BSF. The maximum value of 28.42 kg was obtained in N2 (25:50:25) at the 0.05 probability level, but it was similar to N3 (25:25:50) and N1 (50:25:25) (24.50 and 23.02, respectively). With BIC, AUE was greater in N1 (50:25:25) and N4 (50:50:0) (24.54 and 25.08, respectively) but it was similar to that of N2 (25:50:25). Treatment N5 (50:0:50) resulted in the lowest AUE under both methods (15.60 and 18.32).

The NUE, PUE, and KUE Affected by N Split Application Patterns. The basal application method did not influence NUE and no interaction effect between application method and N split application was observed (Table 4). However, highly significant differences in NUE were observed among N split applications at the 0.01 probability

Table 4

ferent split application patterns and basal application methods of nitrogen at harvest				
Treatment	NHI	NRE (Kg additional grain kg ⁻¹ N applied)	IUE (Kg grain kg ⁻¹ N uptake)	AUE (Kg additional grain kg^{-1} N applied)
Basal surface appli	ication m	ethod		
N0 (control)	0.66 ab		60.08 a	
N1 (50:25:25)	0.68 a	43.14 b	57.10 ab	23.02 ab
N2 (25:50:25)	0.63 b	71.33 a	48.96 b	28.42 a
N3 (25:25:50)	0.68 a	59.82 ab	50.62 ab	24.50 a
N4 (50:50:0)	0.62 b	51.25 ab	52.93 ab	22.99 b
N5 (50:0:50)	0.69 a	45.96 b	48.70 b	15.60 c
Basal incorporation method				
N0 (control)	0.64 a		58.70 a	
N1 (50:25:25)	0.60 b	74.04 a	45.18 b	24.54 a
N2 (25:50:25)	0.63 a	71.03 a	45.47 b	23.82 a
N3 (25:25:50)	0.65 a	63.16 ab	45.63 b	20.51 b
N4 (50:50:0)	0.64 a	55.58 bc	52.56 a	25.08 a
N5 (50:0:50)	0.66 a	50.76 c	53.60 a	18.32 b
Source of variance $(Pr > F)$				
Methods	ns	ns	ns	ns
N split	0.0221	0.0113	0.0023	0.0244
Methods \times N split	ns	ns	ns	ns

Nitrogen harvest index (NHI), nitrogen recovery efficiency (NRE), internal use efficiency (IUE), and agronomic nitrogen use efficiency (AUE) of Manawthukha rice among different split application patterns and basal application methods of nitrogen at harvest

Notes. Means followed by the same letter in each column and in each method are not significantly different in Tukey's HSD tests (P < 0.05). Numbers in parentheses show the amount of N applied as a percentage based on 120 kg N ha⁻¹ at the basal, active tillering, and panicle initiation stages; ns, nonsignificant difference.

level. When using BSF, the greatest NUE (66.27 kg grain kg⁻¹ N applied) was obtained in N2 (25:50:25) followed by N3 (25:25:50) (62.35). Similar values were observed in N1 (50:25:25) and N4 (50:50:0). With BIC, the greatest NUE (63.00) was in N4 (50:50:0) followed by N1 (50:25:25) and N2 (25:50:25). N3 (25:25:50) obtained a lower NUE value of 58.55. N5 (50:0:50) did not generate an optimal NUE result with either application method (53.45 and 56.37, respectively).

The PUE and KUE were the same at the time of harvest, when the same rate of 60 kg ha⁻¹ N was applied just before transplanting (Table 5). The PK use efficiency did not differ significantly by application method but showed highly significant differences among N split applications at the 0.01 probability level. With BSF, the value was greatest (132.55 kg grain kg⁻¹ PK applied) in N2 (25:25:50) followed by N3 (25:25:50). Similar values were found in N1 (50:25:25) and N4 (50:50:0). N5 again had the lowest value of 106.90. With BIC, the greatest value (126.25) was obtained in N4 (50:50:0) followed by N1 (50:25:25). Similar values were obtained by N2 (25:25:50), N3 (25:25:50), and N5 (50:0:50).

Table	5
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Nitrogen use efficiency (NUE), phosphorus use efficiency (PUE), and potassium use efficiency (KUE) of Manawthukha rice is affected by different N split application patterns and basal application methods at harvest

Treatment	NUE (kg grain kg^{-1} N applied)	PUE (kg grain kg ⁻¹ P applied)	KUE (kg grain kg ⁻¹ K applied)
Basal surface applica	tion method		
N0 (control)	_	75.70 c	75.70 c
N1 (50:25:25)	60.86 ab	121.73 a	121.73 a
N2 (25:50:25)	66.27 a	132.55 a	132.55 a
N3 (25:25:50)	62.35 ab	124.69 a	124.69 a
N4 (50:50:0)	60.85 ab	121.70 a	121.70 a
N5 (50:0:50)	53.45 c	106.90 b	106.90 b
Basal incorporation n	nethod		
N0 (control)		76.09 c	76.09 c
N1 (50:25:25)	62.59 a	125.17 a	125.17 a
N2 (25:50:25)	61.87 a	123.73 a	123.73 a
N3 (25:25:50)	58.55 b	117.10 ab	117.10 ab
N4 (50:50:0)	63.12 a	126.25 a	126.25 a
N5 (50:0:50)	56.37 b	112.74 ab	112.74 ab
Source of variance (P	Pr > F)		
Methods	ns	ns	ns
N split	< 0.0001	< 0.0001	< 0.0001
Methods × N split	ns	ns	ns

Notes. Means followed by the same letter in each column and in each method are not significantly different in Tukey's HSD tests (P < 0.05). Numbers in parentheses show the amount of N applied as a percentage based on 120 kg N ha⁻¹ at the basal, active tillering, and panicle initiation stages; ns, nonsignificant difference.

Discussion

Our experimental plots were slightly acidic (pH 6.4) and under yearly rice cultivation. The soil has a low level of exchangeable calcium (Ca) and magnesium (Mg), a moderate level of K, and a high level of sodium (Na) (Metson 1961). Total N (0.13%) is generally low (Enwezor et al. 1989), and there is also a low level of available N and a moderate level of available P (FPDD 1990). Thus, the soil (clay loam) is of poor quality.

In Asia, most farmers directly broadcast urea N onto their rice fields within 2–3 weeks after transplanting. This leads to high ammonia N concentrations in floodwaters and high N losses via ammonia volatilization at the soil surface (De Datta et al. 1979). In the present study, we applied N immediately before transplanting (using two different application methods) to solve these problems. Significant differences in NPK uptake were observed between the BSF and BIC method until the panicle initiation stage. The BIC was effective and reduced N losses at the time of transplanting. The BSF led to lower NPK uptake, particularly in treatments in which a larger amount of N was applied at the basal stage. Both Catchpoole (1975) and Terman (1979) reported that gaseous losses of ammonia (NH₃) equal to 50% of the fertilizer N applied can occur when using the BSF method. In

contrast, incorporating urea fertilizer into deep soil layers (using the BIC method) reduces N losses and enhances nutrient uptake by rice plants. This is because urea is hydrolyzed by urease to form NH_3 and carbon dioxide (CO₂) in soil and is converted into ammonium (NH_4^+) with the help of water. This method is also effective for lowering the ammonia concentration in floodwaters, and thus ammonia volatilization (Vlek and Craswell 1981), and for reducing air pollution by decreasing NH_3 and nitrite (N₂O) emissions from paddy fields (De Datta et al. 1989).

In the present study, basal application method did not affect NPK accumulation and use efficiency at the time of harvest. That is, after the panicle initiation stage, no additional differences occurred in each parameter between BSF and BIC. There were often rain showers just after fertilizer was applied during the growth period. Consequently, N losses were likely the same using both methods. De Datta et al. (1981) suggested that ammonia volatilization from surface-applied urea depends on soil pH, the temperature of the flood-water, algal and aquatic weed growth, crop growth, wind speed, and soil properties.

However, in the present study, different N split applications affected total NPK uptake at each stage. Positive correlations were observed between NU and PU at each stage (Table 6). This relationship was highly significant and positively correlated when using either BSF or BIC. Despite the lack of differences between the two basal application methods at harvest, greater correlations were observed in each N split treatment of the BIC. Our results showed that sufficient N supply is essential for optimum growth of rice plants as well as for better utilization of P. In our experiments, P was applied only at the basal growth stage before transplanting the rice, which is the method used by farmers in Myanmar. Although equal amounts of P (60 kg ha⁻¹) were applied in all treatments, PU varied and depended on the amount of NU in each treatment. Qiao et al. (2011) emphasized that applying N improves PUE in rice.

The correlation coefficients (R^2) between NU and KU at each stage were significant at the 0.01 probability level (Table 7) but were slightly greater with the BIC than with BSF method. TNU was also positively correlated with TKU at each stage. Many researchers have recommended split application of K fertilizers during rice cultivation. In Japan, K fertilizers are split-applied at the basal and panicle initiation stages. However, only the basal dose tends to remain in fields in Myanmar and was

Table 6

Regression equations and coefficients of determination between N uptake and P_2O_5 uptake of Manawthukha rice at the critical growth stages under two pretransplant basal application methods

Basal incorporation
$= 0.2823x + 1.7038$ $x^{2} = 0.9312^{**}$
= 0.3351x + 2.8214 $x^{2} = 0.9289^{**}$
= 0.4066x + 5.1332 $x^2 = 0.9482^{**}$
$= 0.305x + 15.618$ $x^{2} = 0.9127^{**}$
 _2

Notes. $y = P_2O_5$ uptake, $\times = N$ uptake. ** indicates significance at P = 0.01.

Regression equations and coefficients of determination between N uptake and K ₂ O
uptake of Manawthukha rice at the critical growth stages under two pretransplant
basal application methods

Table 7

Growth stage	Basal surface application	Basal incorporation
Active tillering	$y = 1.5892x + 4.6622$ $R^2 = 0.9663^{**}$	y = 01.5617x + 5.2894 $R^2 = 0.9807**$
Panicle initiation	y = 1.5966x + 7.2978 $R^2 = 0.9509**$	$y = 1.3137x + 19.213$ $R^2 = 0.9147**$
Grain filling	y = 1.2234x + 24.514 $R^2 = 0.8003**$	y = 1.1637x + 22.284 $R^2 = 0.87**$
Harvest	y = 0.7384x + 39.681 $R^2 = 0.7736^{**}$	$y = 0.7988x + 35.595$ $R^2 = 0.8104^{**}$

Notes. $y = K_2O$ uptake, x = N uptake. ** indicates significance at P = 0.01.

used in this study. According to the correlation results, the KU by rice plants in each treatment depended on the NU at each stage. Thus, split application of N led to a greater NU of rice and consequently greater KUs.

Moreover, different split applications of urea N were effective for synchronizing crop N demand and N supply from soil or fertilizer. For example, when using BSF, treatment N2 (25:50:25) showed the greatest NPK uptake and use efficiencies as well as N recovery. This finding coincides with the plant's N demand and N supply throughout the crop growth period. The low basal surface applied N and synchronized split applications might result in optimum nutrient uptake in the early growth stages. However, tight water management with a shallow water level was essential so as not to lose basal N through surface runoff. When using the BIC method, treatment N1 (50:25:25) was best, resulting in greater total NPK uptake, use efficiencies, and NRE. The incorporation of high basal N prior to transplanting reduced N losses and allowed for the slow release of available nutrients over the entire period. However, incorporating N into deep soil layers tends to cause leaching below the root zones of rice plants. Thus, split applications of N with high but not extreme amounts of N applied at the basal growth stage were effective for achieving high NPK uptake of rice and ensuring a greater NRE.

In contrast, optimum doses of N split applied at critical growth stages were essential for better N accumulation. A nonsynchronized supply of N during critical growth stages can lead to N loss and decreased total NPK uptake. Liu et al. (2007) reported that nutrient absorption varies at different stages of rice growth. Despite using the same N rate, the different patterns of N application led to different nutrient uptake results. The N2 (25:50:25) results revealed that the N requirement at the active tillering stage was high and that N top dressing was necessary to augment vegetative growth and increase NPK uptake. Doberman and Fairhurst (2000) reported that top dressing N is essential when a crop has a great demand for N and when the rate of NU is high. Omitting N at the active tillering stage caused a severe N deficiency in the N5 (50:0:50) treatment and affected the total NPK uptake and use efficiencies of rice. This finding is in agreement with Wang et al. (2011), who reported the greatest NU during the active tillering stage followed by the young panicle stage. Duan et al. (2005) also reported that the key period for N absorption by rice plants is

from the tillering to flowering stages, as the absorption of soil N is at its maximum during this period. This would explain our results for treatment N5. However, when insufficient N was supplied during the vegetative growth stage, avoiding N fertilization at the panicle stage did not severely affect the total NPK uptake and use efficiencies of the rice. The N4 (50:50:0) treatment resulted in optimal total NPK uptake, use efficiencies, and NRE. In rice, most of the absorbed N is stored in the leaves at the vegetative stage and is transported to the grains during the grain filling stage (Jiang et al. 2004; Duan et al. 2005).

Significant differences in NHI were observed among the different N split applications at the time of harvest. The NHI values in this study were relatively lower than the 0.68 reported by Peng et al. (1996) but relatively greater than the 0.59 reported by Witt et al. (1999). Maximum N recovery and AUE were obtained in the N2 (25:50:25) treatment with BSF even though the same N rate was used among the N split application patterns. This indicates that the N2 treatment synchronized the N supply from soil and/or fertilizer with the crop demand at each critical growth stage. In contrast, the high amount of N supplied during the basal stage in the N1 (50:25:25) treatment did not coincide with the crop demand, thus leading to N losses. Omitting N at the active tillering stage seriously affected NRE and AUE in the N5 (50:0:50) treatment. Cassman, Dobermann, and Walters (2002) found that it is possible to achieve a high NRE with relatively high N fertilizer rates, but only when N demand is much greater than the indigenous supply. According to Bruesh et al. (2001), an AUE of 25 can be obtained by dynamically timing the amount of N fertilizer applied to match the real-time N requirements of the rice crop. Treatments N2 (25:50:25) with the BSF method and N1 (50:25:25) and N4 (50:50:0) with BIC achieved greater AUE values than those of Bruesh et al. (2001).

When using the BIC method, the greater NRE and AUE values in treatment N1 (50:25:25) may have delayed the conversion of urea to ammonium, leading to the slow release of N and a better match between supply and demand. Indeed, Craswell et al. (1981) reported that infusing urea deep into the soil prevents rapid conversion of ammonium into nitrate and is an effective way to reduce the concentration of ammonia in floodwaters. However, incorporating low levels of basal N might happen to the low N uptake. This point was explained by the results of N2 (25:50:25) and N3 (25:25:50).

In our experiments, N fertilizer was incorporated into the soil manually, by pressing it in with the feet or mixing it in with a metal rake. This method can be readily performed by harrowing at the final leveling of the field and is very economical. It is essential for Myanmar farmers to have an efficient and cheap way to apply N fertilizer, although mechanized farming is spreading in Myanmar.

Based on our results, a low amount of surface N can be applied at the basal growth stage just before transplanting the rice, but then it is essential to maintain a shallow water level so that the applied fertilizers do not leak into the field. The soil type should be considered when applying basal surface N. From an ecological viewpoint, the incorporation of basal N prior to transplanting is effective not only for decreasing N losses but also for reducing the release of ammonia gas into the atmosphere. In rice fields, some ammonia from surface-applied urea is lost to the air. Basal N applied just before transplanting may help avoid this problem and could work well for sustainable agriculture.

For these reasons, we conclude that split application of N fertilizer is effective for rice cultivation not only for improved nutrient uptake but also for a cleaner environment. Nevertheless, further evaluation of basal application methods is necessary on a wide range of soil types and difference fertilizer sources. Further studies on the split application of N should develop guidelines for specific rice varieties tailored to specific recommended N rates.

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