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Effects of organic fertilizer applied using the estimated mineralizable nitrogen method on nitrogen uptake, growth characteristics, yield, and yield components of Genkitsukushi rice (*Oryza sativa*)

Kyi Moe[a,b], Aung Zaw Htwe[a,b], Doan Cong Dien[a], Yoshinori Kajihara[c], and Takeo Yamakawa[d]

[a]Plant Nutrition Laboratory, Graduate School of Bioresource and Bioenvironmental Sciences, Faculty of Agriculture, Kyushu University, Fukuoka, Japan; [b]Department of Agronomy, Yezin Agricultural University, Yezin, Nay Pyi Taw, Myanmar; [c]University Farm, School of Agriculture, Kyushu University, Fukuoka, Japan; [d]Plant Nutrition Laboratory, Division of Molecular Biosciences, Department of Biosciences & Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka, Japan

ABSTRACT

The nitrogen (N) uptake, growth characteristics, and yield of the Genkitsukushi rice variety were evaluated over 2 years (2017 and 2018) of field experiments involving the application of poultry manure (PM), cow manure (CM), and compost (CP). Organic fertilizers were quantified as the estimated-mineralizable-nitrogen (EMN) based on the total N content. Compared with chemical fertilizer (CF_{100}), $CF_{50}PM_{50}$ resulted in greater growth characters, N uptake, dry matter, and yield in both years. The PM (total N > 4%) had a higher N mineralization, a greater N availability to rice and thus, a greater contribution to yield. More N was available from CM and CP containing ~2% of total N in the second year. In conclusion, an organic fertilizer with a higher total N (>4%) was compatible with the EMN method and let to increase N availability and yield of Genkitsukushi japonica rice.

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KEYWORDS
chemical fertilizer; estimated mineralizable nitrogen; organic fertilizer; rice; yield

Introduction

Rice serves as a staple crop for 50% of the world's population. About 35–60% of dietary calories are supplied by rice for more than 3 billion people worldwide (Fageria 2003). Farmers will need to produce more rice of better quality to meet the demands of consumers in the coming years (Peng and Yang 2003). However, the average rice yield has been lower than the potential yield, due mainly to low nitrogen (N) use efficiency in rice; this efficiency rarely exceeds 50% and usually ranges from 15% to 35% (de Datta 1986). The improvement of N use efficiency is also essential for the economic sustainability of cropping systems (Amanullah, Almas, and Shah 2010). Proper N fertilizer management, good N sources, and optimum N rates enhance N use efficiency and yield and create the opportunity to reduce the cost of production and environmental pollution (Fageria, Baligar, and Jones 2011). Decreased yield and soil fertility are the primary consequences of unbalanced use of N fertilizers in modern rice cultivation (Yadav 2008).

The soil system should be highly fertile and rich in organic matter; these properties form the backbone of long-term sustainability of rice systems (Sahrawat 2004). The N condition of the soil can be sustained by maintaining a balance between N removal through crop harvest and N gain

CONTACT Kyi Moe kyimoeyau@gmail.com Plant Nutrition Laboratory, Graduate School of Bioresource and Bioenvironmental Sciences, Faculty of Agriculture, Kyushu University, 744 Motooka Nishi-ku, Fukuoka 819-0395, Japan.

from applied organic and inorganic fertilizers (Ladha and Peoples 1995). However, this balance has been disturbed with high inputs of chemical fertilizers (CFs) under the continuous and exhaustive rice mono-cropping system (Ladha et al. 2000). The organic matter content and the native N status gradually decrease in soils under yearly application of large amounts of CF without an organic source (Fu et al. 2014; Pei et al. 2015). Consequently, yields are not sustained over years, even with the cultivation of high-yield varieties. The excessive and imbalanced use of CF reduces soil fertility and rice yield by 38% (Singh, Chaure, and Parihar 2001). The application of CF is expensive and progressively leads to environmental problems; these issues have led to growing interest in the use of organic manures or waste. The application of organic manure or waste, which contains mineral and organic N, is useful for the maintenance and improvement of soil fertility and rice production (Takahashi, Uenosono, and Nagatomo 2004). Cow manure (CM) is an excellent source of soil organic matter, as it is rich in micro- and macronutrients and can be used in crop production systems as a source of nutrients and organic matter (Butler et al. 2001). Poultry manure (PM) contains mainly nitrogenous compounds, which are readily mineralized to ammonia and nitrate (Eghball et al. 2002). Organic waste as compost (CP) made from kitchen garbage with bamboo powder has excellent potential in rice cultivation, where it can serve as a soil fertilizer supplying nutrients for uptake by plants (Binh and Shima 2018). The use of organic manures can increase crop productivity for sustainable agriculture (Suzuki 1997; Myint et al. 2011). Organic manures not only provide nutrients to the soil, but also improve water holding capacity and contribute to the soil's ability to maintain better aeration for seed germination and root development (Zia, Baig, and Tahir 1998). Organic manures with high nutrient contents can serve as adequate and less-expensive substitutes for CFs (Masarirambi et al. 2012).

Although organic manures have beneficial effects, the use of organic manures alone might not meet plant requirements, as these manures have low-nutrient contents. The application of organic manure with CF enhances the activity of microbes, increases nutrient use efficiency (Narwal and Chaudhary 2006), and promotes the availability of native nutrients to plants, resulting in greater nutrient uptake. Many studies have shown that a sole application of inorganic or organic fertilizer alone does not result in sustainable crop productivity (Satyanarayana et al. 2002; Jobe 2003).

The proper application of organic manure in the cultivation of rice remains a challenge. In contrast to those in CF, the nutrients in manure must undergo mineralization after application. Plants can only utilize mineralizable N (i.e., NH_4-N and NO_3-N converted from organic N by different soil microbes). In our previous studies, organic manures were applied on a material weight basis to hybrid rice (Palethwe-1) (Moe et al. 2017a, 2017b). However, adjustment of the N supply from manure according to the N demand of rice using this technique is difficult. Ohyama et al. (1998) reported that weight-based organic matter application significantly affected the growth and yield of japonica rice only after 3–5 years. In this study, we applied PM, CM, and CP based on the estimated mineralizable nitrogen (EMN) method for the cultivation of rice (*Oryza sativa* L.). The EMN method entails the application of organic manure according to the amount of mineralizable N, estimated from the total N content of the manure based on Nishio (2007). In the pot and field experiments, Nishio (2007) measured the amount of mineralized N from manures of different N concentration over 12 weeks. The mineralized N was calculated as a percentage that is the proportion of mineralized N to organic N from different manures. The results revealed that from manure containing <2%, 2–4%, and >4% of total N, the 20%, 30%, and 50% of mineralizable N of organic N applied, respectively, is mineralized. The mineralization rate of nutrients from applied manure depends mainly on total N content. We supposed that the EMN from PM, CM, and CP would meet the target N rate and supply sufficient N to Genkitsukushi japonica rice during the growing season. In our previous reports, the treatment with 50% of the recommended CF application rate combined with 5 t ha^{-1} PM (weight basis) performed the best regarding N uptake, growth characteristics, and yield of rice. The treatment with 75% of the recommended CF application rate combined with CM (5 t ha^{-1}, weight basis) is the next best alternative for the reduction of CF use

(Moe et al. 2017a, 2017b). Therefore, we prepared treatments of 50% of the recommended CF application rate plus 50% EMN from PM, CM, and CP ($CF_{50}PM_{50}$, $CF_{50}CM_{50}$, and $CF_{50}CP_{50}$, respectively) in this study to optimize the amounts of CF and organic fertilizers. Whether organic manure application based on the EMN method combined with CF application can synchronize the N demand of rice and enhance rice N uptake, growth characteristics, and yield is of interest.

The effects of CF on N uptake, growth characteristics, and yield of rice have been well documented in many studies. Importantly, few studies have considered the application of organic manures using the EMN method. Hence, the objective of this study was to evaluate the effects of organic manure application using the EMN method combined with CF on the N uptake, growth characteristics, yield, and yield attributes of the high-yield, heat-tolerant Genkitsukushi rice variety, compared with the individual application of CF.

Materials and methods

Experimental site, design, and treatments

Field experiments were conducted from June to October in 2017 and 2018 at the Kyushu University farm in Fukuoka Prefecture, Japan (33°37′ N, 130°25′ E). The experimental soil had a clay-loam texture, pH_{H2O} of 6.12, 0.15% total N, 5.20 mg N 100 g^{-1} mineralizable N, 0.25% total P_2O_5, 15.37 mg P 100 g^{-1} available P, 0.48% total K_2O, and 0.37 $cmol_c\ kg^{-1}$ exchangeable K. Its cation exchange capacity was 15.70 $cmol_c\ kg^{-1}$.

The experiment had a randomized complete-block design with three replications. The land was initially irrigated and prepared by disc plowing and harrowing. Puddling was conducted 3 days before transplanting. Then, the land was divided into three replicate blocks using corrugated plastic sheeting (45 cm height, 0.5 mm thickness). Each replicate block was subdivided into six plots by the insertion of plastic sheeting (30 cm height, 0.5 mm thickness) to a 15-cm depth to prevent seepage of soil/irrigation water between adjacent plots. Irrigation water and drainage water were separated in each block. Each individual plot in a block was irrigated independently, and water drained out to the main outlet. Large plastic sheets inserted between blocks prevented irrigation water from entering adjacent plot inlets and drain outlets. The experiment involved the application of six treatments: control (N_0), 50% CF (CF_{50}), 100% CF (CF_{100}), CF_{50} + PM_{50}, CF_{50} + CM_{50}, and CF_{50} + CP_{50}. The 100% CF treatment was equivalent to 85 kg N ha^{-1} (as urea) that is the recommended N application rate for the tested rice variety in the study area. The control (N_0) plot did not receive N fertilizer. The treatments were applied to the same plots in both years. In this study, as we focused mainly on N application, the phosphorus (P) and potassium (K) levels in the manure were not adjusted. P and K were applied at rates of 60 kg P_2O_5 ha^{-1} (as superphosphate) and 85 kg K_2O ha^{-1} (as potash muriate, KCl), respectively. The individual plot size was 4.5 × 1 m. Urea (containing N) and potash muriate (containing K_2O) were applied in three splits: 60% was incorporated into the soil 1 day before transplantation, 20% was applied at the active tillering stage, and the remaining 20% was applied at the panicle initiation stage. The full amount of superphosphate (containing P_2O_5) was applied as basal dressing.

PM, CM, and CP were incorporated manually into the soil with a metal rake in the respective treatment plots as basal dressing 1 day before transplanting. We took care that the organic fertilizer did not to move to either side or the corner of each plot. The quantities of N applied from PM, CM, and CP (Table 1) were calculated as EMN based on the total N content (%) of each manure type according to Nishio (2007) using the following equation:

$$\text{EMN (kg ha}^{-1}) = \text{Wt. organic fertilizer (DW) (kg ha}^{-1}) \times \frac{\text{Total N (\%)}}{100} \times \frac{\text{Mineralizable N (\%)}}{100}$$

Table 1. Weight and nitrogen (N), phosphorus (P) and potassium (K) applied (kg ha^{-1}) from organic and inorganic fertilizers.

		Organic fertilizers					Inorganic fertilizers		
No.	Treatments	DW	N	P_2O_5	K_2O	EMN	N	P_2O_5	K_2O
1	N_0	–	–	–	–	–	0.0	60.0	85.0
2	CF_{50}	–	–	–	–	–	42.5	60.0	85.0
3	CF_{100}	–	–	–	–	–	85.0	60.0	85.0
4	CF_{50} PM_{50}	1745.3	85.0	79.5	37.3	42.5	42.5	60.0	85.0
5	CF_{50} CM_{50}	5927.4	141.6	113.2	90.1	42.5	42.5	60.0	85.0
6	CF_{50} CP_{50}	6558.6	141.6	148.8	93.1	42.5	42.5	60.0	85.0

Subscript numbers of treatments show the amount of N applied as a percentage based on 85 kg N (or EMN) ha^{-1}.
EMN: estimated mineralizable N; PM: Poultry manure; CM: Cow manure; CP: Compost; DW: Dry weight.

Table 2. Chemical compositions of PM, CM, and CP.

				Total % (dry basis)							
No.	Sample	C:N	Moisture (%)	N	NH_4-N	NO_3-N	P_2O_5	K_2O	Ca	Mg	Na
1.	PM	6.80	21.86	4.87	0.75	0.00	4.56	2.14	8.85	0.75	0.28
2.	CM	15.23	46.40	2.39	0.19	0.00	1.91	1.52	1.09	0.55	0.34
3.	CP	17.71	41.02	2.16	0.04	0.02	2.27	1.18	5.28	0.21	0.85

Analysis of organic manure and soil

Soil samples (0–15 cm) were taken with a sampling tube (5 cm in diameter) from nine locations in the experimental field before the field experiment in 2017. The physical and chemical properties of these samples were analyzed. Soil pH_{H2O} (1:2.5 soil: H_2O) was measured using a pH meter (HM-10P; DKK-TOA Corp., Tokyo, Japan). The soil samples were digested using the salicylic acid–H_2SO_4–hydrogen peroxide method (Ohyama et al. 1991), total N was analyzed using the indophenol method (Cataldo, Schrader, and Youngs 1974), and total P was analyzed using the ascorbic acid method (Murphy and Riley 1962). Cation exchange capacity and exchangeable cations in the soil were determined using an ammonium acetate shaking extraction method (Muramoto, Goto, and Ninaki 1992) and measured by atomic absorption spectrophotometry (Z-5300; Hitachi, Tokyo, Japan). The analysis of mineralizable N was performed using the soil incubation method (Sahrawat 1983) at 30 °C for 4 weeks, followed by the indophenol method (Cataldo, Schrader, and Youngs 1974). The available P in the soil samples was determined using Truog's (1930) method, followed by the ascorbic acid method (Murphy and Riley 1962).

Total N and P contents of the manures were analyzed using the same methods as for the soil analysis (Table 2). Total K, Ca, and Mg contents of the manures were analyzed using a digesting solution and an atomic absorption spectrophotometer (Z-5300; Hitachi). In addition, mineral N (NH_4-N and NO_3-N) and Na were extracted from the manures using the hot water method (Curtin et al. 2006).

Crop management

Genkitsukushi rice (a heat-tolerant, high-yield Japanese variety) was cultivated in this study. The Kyushu University farm provided the seeds. Healthy rice seeds were chosen by NaCl solution with a specific gravity of 1.13, according to Yoshida (1981). The seeds were washed three times in distilled water and sterilized by shaking in 10% ethanol at 150 rpm for 3 min. Then, the seeds were rewashed at least three times in distilled water. The seeds were shaken again in 5% NaOCl solution at 150 rpm for 30 min. The sterilized seeds were washed in distilled water and germinated in an incubator at 25 °C for 48 h in the dark.

The germinated seeds were sown homogenously on seedbeds (100 g/tray) prepared with commercial seedbed soil (Kokuryu Baido; Seisin Sangyo Co., Kitakyushu, Japan). The 21-day-old seedlings were transplanted with a hill spacing of 25 × 15 cm and planting of two seedlings per hill on 22 June 2017 and 21 June 2018. The university farm was irrigated according to the standard method. The rice plants were harvested 94 days after transplanting (DAT) on 25 September 2017 and 24 September 2018.

Plant growth characteristics

Five representative hills in each plot were marked with poles to measure plant height (cm), the number of tillers hill^{-1}, and the soil-plant analysis development (SPAD) values throughout the crop period. These plant growth characteristics were measured weekly from 10 DAT to 50% flowering and at 2-week intervals after flowering. The SPAD values of the top fully expanded leaves were measured with a SPAD-502 chlorophyll meter (Konica Minolta, Inc., Osaka, Japan) before the panicle initiation stage, and the flag leaf was used after that stage.

Determination of dry matter weight and N uptake

At each of the active tillering, panicle initiation, flowering, and harvest stages, two representative hills from each plot were cut 2 cm above the ground. The samples were separated into sheaths, leaves, panicles, and seeds, oven dried at 70 °C for 48 h, and then weighed immediately. The dry matter (DM) accumulation was determined by summing all plant parts and expressed in tons per hectare (t ha^{-1}).

The oven-dried samples were ground to a fine powder using a Cyclotec 1093 Sample Mill (100–120 mesh; Tecator AB, Hoedanaes, Sweden). At each stage, the N accumulated in each plant part (sheath, leaf, panicle, and seed) was digested separately using the salicylic acid–H_2SO_4–hydrogen peroxide (H_2O_2) method (Ohyama et al. 1991) followed by the analysis of total N using the method described for the soil analysis. Total N uptake was calculated as the sum of the products of the biomass and the N concentration of each plant part (sheath, leaf, panicle, and seed).

Determination of yield and yield components

At harvest time, 10 hills were used to measure DM, yield, and yield components [number of panicles per hill, spikelet number per panicle, filled grain percentage, 1000-grain weight (g), and maximum panicle length (cm)]. The rice seeds were separated into filled and unfilled seeds using a wind blower machine. The filled seeds were immersed in NaCl solution (specific gravity 1.13) to separate heavy from light seeds. Then, the rice seeds were washed three times with deionized water. Rice yield was determined using the weight of the heavy seeds, which was adjusted to 15% moisture content. The harvest index (HI) was calculated as the ratio of economic yield (seed weight) to biological yield (total DM weight) (Yoshida 1981).

The yield of Genkitsukushi rice derived from manure (Y-dfM; %) was calculated as follows:

$$\mathrm{Y-dfM}\ (\%) = \frac{\mathrm{Yield}\ [(\mathrm{PM_{50} or\ CM_{50} or\ CP_{50}} + \mathrm{CF_{50}}) - \mathrm{CF_{50}}]\ \mathrm{t\ ha^{-1}}}{\mathrm{Yield}\ (\mathrm{PM_{50} or\ CM_{50} or\ CP_{50}} + \mathrm{CF_{50}})\ \mathrm{t\ ha^{-1}}} \times 100$$

where PM_{50} = 50% EMN applied from poultry manure, CM_{50} = 50% EMN applied from cow manure, CP_{50} = 50% EMN applied from compost, CF_{50} = 50% N applied from chemical fertilizer.

Incubation experiment

To understand the pattern of N mineralization from organic fertilizers, an incubation experiment was conducted in the laboratory for 12 weeks. Four treatments (control, PM, CM, and CP) were tested using a completely randomized design with three replications. Air-dried soil (equivalent to 25 g dry soil) and organic fertilizers were added to the 30-mL centrifuge tube (Sarstedt AG & Co., Nümbrecht, Germany). The amount of N level from organic fertilizer was calculated for 25 g of soil based on the rate of field experiment. Ten milliliters of distilled water were added and stirred with a glass rod. The air trapped inside the soil was removed using a suction pump at 0.09 MPa. Then, distilled water was added to achieve a water level 10 cm above the surface of the soil in the tube. Finally, the centrifuge tubes were incubated at 30 °C for 4 weeks. The contents of each incubated centrifuge tube were transferred to a 500-mL glass bottle by vortexing several times with 100 mL 2 M KCl solution. Then, the glass bottles were covered with rubber stoppers and shaken at 137 rpm for 30 min. The soil suspensions were filtered through no. 131 filter paper (Adventec Co., Tokyo, Japan). After 0, 2, 4, 8, and 12 weeks, mineralizable N (NH_4-N and NO_3-N) for each treatment was determined by the indophenol method (Cataldo, Schrader, and Youngs 1974). The N mineralization (%) from each organic fertilizer was calculated as the equation

$$\begin{aligned} \text{N mineralization (\%)} &= \text{Mineralized N } (\mathrm{NH_4 - N + NO_3 - N}) \text{ [treatment – control]} \\ &\div \text{Applied N} \times 100 \end{aligned}$$

Statistical analysis

The data were subjected to analysis of variance. Mean values were compared among treatments using Tukey's honestly significant difference test at a 5% probability level. The data were analyzed using Statistix software (ver. 8.0; Analytical Software, Tallahassee, FL, USA).

Results

Weather conditions

The climate of the study area is temperate and humid, with quite mild winters and hot, moist, and rainy summers. The average maximum temperature was 34.3 °C and the average minimum temperature was 14.1 °C from June to October in 2017 and 2018 (Figure 1). The mean monthly temperatures did not differ between years. However, the pattern of rainfall differed considerably between 2017 and 2018 in the experimental region. Rainfall was high during crop cultivation in 2017. In 2018, rainfall was high in June and July, but significantly lower during the grain filling stage in later months. The minimum monthly rainfall was 53.5 mm and the maximum was 423.0 mm. Solar radiation did not differ between years.

Plant growth characteristics

Plant height was similar among the treatments until the active tillering stage (24 DAT) in 2017 and 2018 ($p < 0.05$). After that stage, the $CF_{50}PM_{50}$ treatment produced taller plants in both years (Figure 2). At the time of harvest, the maximum plant heights were obtained by $CF_{50}PM_{50}$ (102.49 cm in 2017, 100.2 cm in 2018). Of the treatments with N supplied, the $CF_{50}CM_{50}$ and $CF_{50}CP_{50}$ treatments attained the lowest plant heights, which did not differ from those attained by CF_{100}, in both years and were higher than N_0.

Although similar tiller numbers were observed among the treatments before 24 DAT, significant differences were observed at later stages in both years ($p < 0.01$; Figure 3). At 30 DAT,

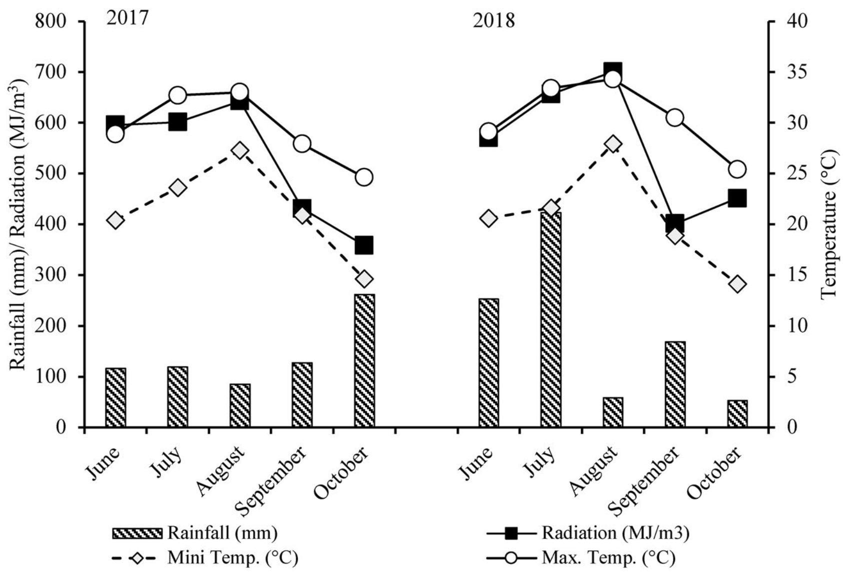

Figure 1. Monthly average maximum (max.) and minimum (mini.) air temperature, rainfall, and solar radiation during exprimental period of 2017 and 2018 rice seasons. Source: Weather station, University Farm, Kyushu University.

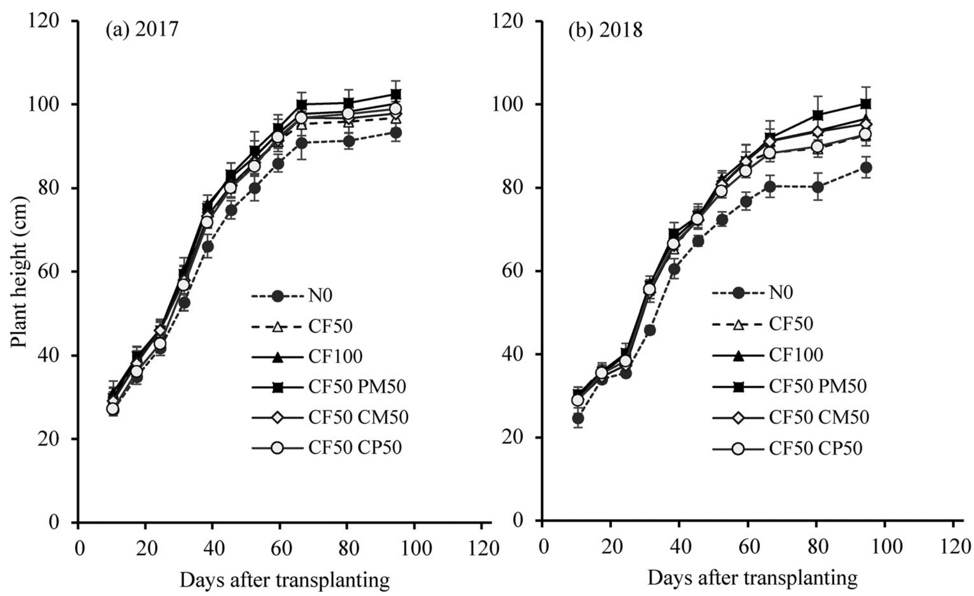

Figure 2. Plant height (cm) of Genkitsukushi rice affected by organic fertilizers. Number followed by treatment shows the amount of N applied as a percentage based on 85 kg N (or EMN) ha^{-1}. CF = chemical fertilizer, PM = Poultry manure, CM = Cow manure, CP = Compost. Error bar represents the standard deviation. ($n = 15$).

$CF_{50}PM_{50}$ yielded the maximum tiller numbers (18.00 in 2017, 23.67 in 2018). After that stage, tiller numbers declined in all treatments. At harvest, the $CF_{50}PM_{50}$ treatment achieved maximum tiller numbers of 16.80 in 2017 and 16.67 in 2018. Of the treatments with N supplied, the $CF_{50}CM_{50}$ and $CF_{50}CP_{50}$ treatments produced the smallest tiller numbers in 2017, but tiller numbers similar to those produced by CF_{100} in 2018. Generally, the rice plants in all treatment groups produced more tillers in 2018 than in 2017.

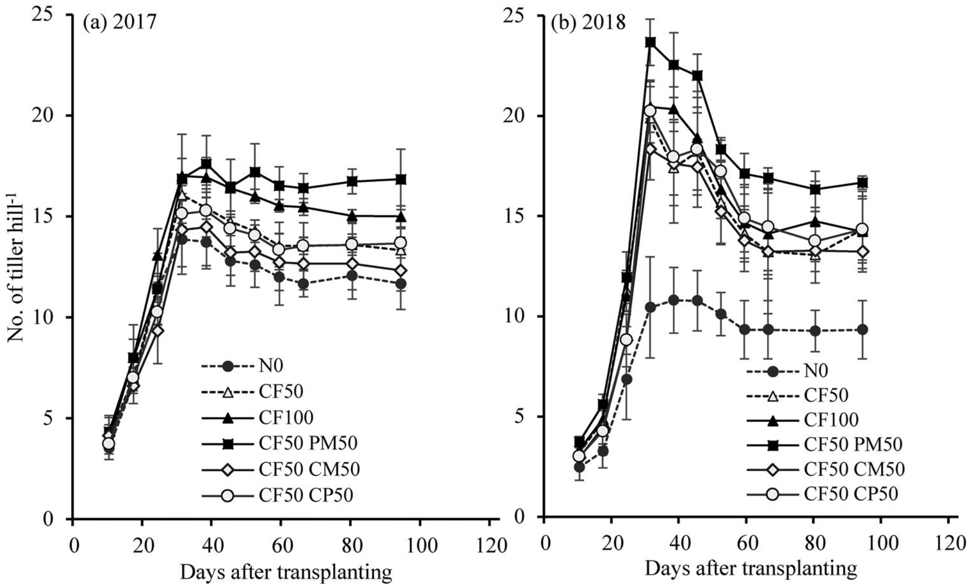

Figure 3. Tiller number of Genkitsukushi rice affected by organic fertilizers. Error bar represent standard deviation ($n = 15$).

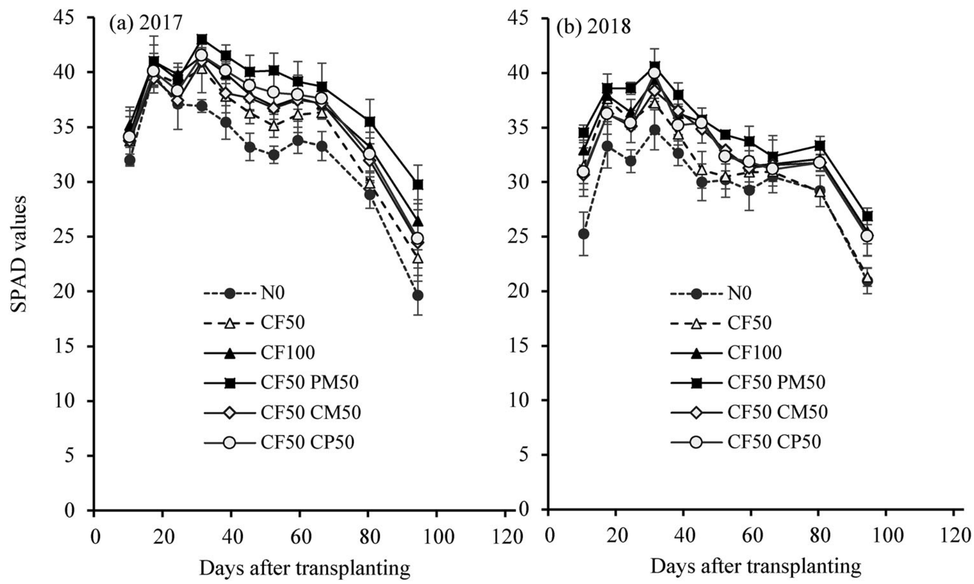

Figure 4. Changes in SPAD values of Genkitsukushi rice affected by organic fertilizers as EMN method. Error bar represents standard deviation ($n = 15$).

Before the active tillering stage (24 DAT) in both years, SPAD values increased rapidly with all treatments, with no significant difference between any treatment groups ($p < 0.01$; Figure 4). At the panicle initiation stage (38 DAT), the SPAD values peaked for all treatments. The $CF_{50}PM_{50}$ treatment yielded the highest SPAD values (43.03 in 2017, 40.59 in 2018). The SPAD values decreased gradually for all treatments at the later stages. However, the $CF_{50}PM_{50}$ treatment yielded the highest SPAD values throughout the cultivation period. The SPAD values for the $CF_{50}CM_{50}$ and $CF_{50}CP_{50}$ treatments were similar to those for the CF_{100} treatment in both years.

There were no differences of DM among CF treatments and organic fertilizer treatments at the active tillering and panicle initiation stages in either year ($p <$ 0.05; Figure 5). However, at the flowering stage, the $CF_{50}PM_{50}$ treatment resulted in greater DM production, similar to that attained with the CF_{100} treatment. At this stage, DM weights did not differ among the PM, CM, and CP treatments in either year. In particular, the $CF_{50}PM_{50}$ treatment yielded the greatest DM production at harvest (13.02 t ha^{-1} in 2017, 13.86 t ha^{-1} in 2018). In both years, the DM weights were similarly low for the CF_{100}, $CF_{50}CM_{50}$, and $CF_{50}CP_{50}$ treatments.

N uptake of rice at critical growth stages

A high N uptake value of 24.97 kg N ha^{-1} at the active tillering stage was obtained with CF_{100} in 2017 (Table 3), followed by CF_{50}. However, the N uptake values for all organic fertilizer treatments were similar to those for the CF treatments ($p < 0.05$). At the panicle initiation and flowering stages, the $CF_{50}PM_{50}$ treatment produced higher N uptake values. The N uptake values achieved with the CF_{100} treatment were significantly lower at these stages. At the time of harvest, the $CF_{50}PM_{50}$ treatment achieved the greatest N uptake (120.60 kg N ha^{-1}). The N uptake values were similar among the CF_{100}, $CF_{50}CM_{50}$, and $CF_{50}CP_{50}$ treatments.

In the initial growth stage in 2018, the $CF_{50}PM_{50}$ treatment produced the maximum N uptake of 15.80 kg N ha^{-1} (Table 3) but no apparent difference was seen among treatments ($p < 0.05$). Thereafter, the $CF_{50}PM_{50}$ treatment maintained the greatest N uptake throughout the crop period and obtained the maximum N uptake (110.42 kg N ha^{-1}) at harvest. Similar N uptake values were observed among the $CF_{50}CM_{50}$, $CF_{50}CP_{50}$, and CF_{100} treatments in 2018. Due to the low rainfall in the experimental area, the N uptake values for all treatments were slightly lower in 2018.

Harvest index, yield, and yield parameters

The highest HI values in 2017 were 0.61 for $CF_{50}PM_{50}$ and 0.61 for $CF_{50}CM_{50}$ ($p < 0.05$; Table 4). However, these HI values were similar to that achieved with CF_{100}. In 2018, the $CF_{50}CM_{50}$ treatment produced the highest HI value of 0.57, which did not differ from those of $CF_{50}PM_{50}$, $CF_{50}CP_{50}$, and CF_{100}.

In both years, the $CF_{50}PM_{50}$ treatment produced the largest numbers of panicles (16.00 and 15.33 per hill, respectively; $p < 0.05$, Table 4). In 2017, the $CF_{50}CM_{50}$ and $CF_{50}CP_{50}$ treatments provided fewer panicles per hill than did the CF_{100} treatment. However, in 2018, the panicle numbers for $CF_{50}CM_{50}$ and $CF_{50}CP_{50}$ were larger than that for CF_{100}. But these values were not significantly different among the treatments. The $CF_{50}CP_{50}$ treatment yielded the maximum numbers of spikelets per panicle (80.16 in 2017, 84.03 in 2018; Table 4). The CF_{100}, $CF_{50}PM_{50}$, and CF_{50} treatments yielded similar numbers of spikelets per panicle in both years. Smaller numbers were produced by $CF_{50}CM_{50}$ in 2017 and by $CF_{50}CP_{50}$ in 2018.

The $CF_{50}PM_{50}$ treatment produced the highest filled grain percentages (85.62% in 2017, 84.81% in 2018; Table 4). The CF_{100} achieved a similar value of 82.32% in 2017. The $CF_{50}CM_{50}$ and $CF_{50}CP_{50}$ treatments resulted in lower filled grain percentages than that of CF_{100} in 2017. Nevertheless, their values were higher than that of the CF_{100} treatment in 2018. The 1000-grain weight was unaffected by the application of organic fertilizer using the EMN method ($p > 0.05$).

The $CF_{50}PM_{50}$ treatment resulted in the highest yields (7.93 t ha^{-1} in 2017, 7.77 t ha^{-1} in 2018; Table 4). These yields were similar to those of CF_{100}, $CF_{50}CM_{50}$, and $CF_{50}CP_{50}$ in 2017. In 2018, the yield of $CF_{50}CM_{50}$ increased slightly and that of $CF_{50}CP_{50}$ was stagnant. The rice yield of CF_{100} decreased from 7.32 in 2017 to 6.68 t ha^{-1} in 2018. However, these yields did not differ from those of the CM and CP treatments. The yields of the N_0 treatment were low in 2017 and 2018.

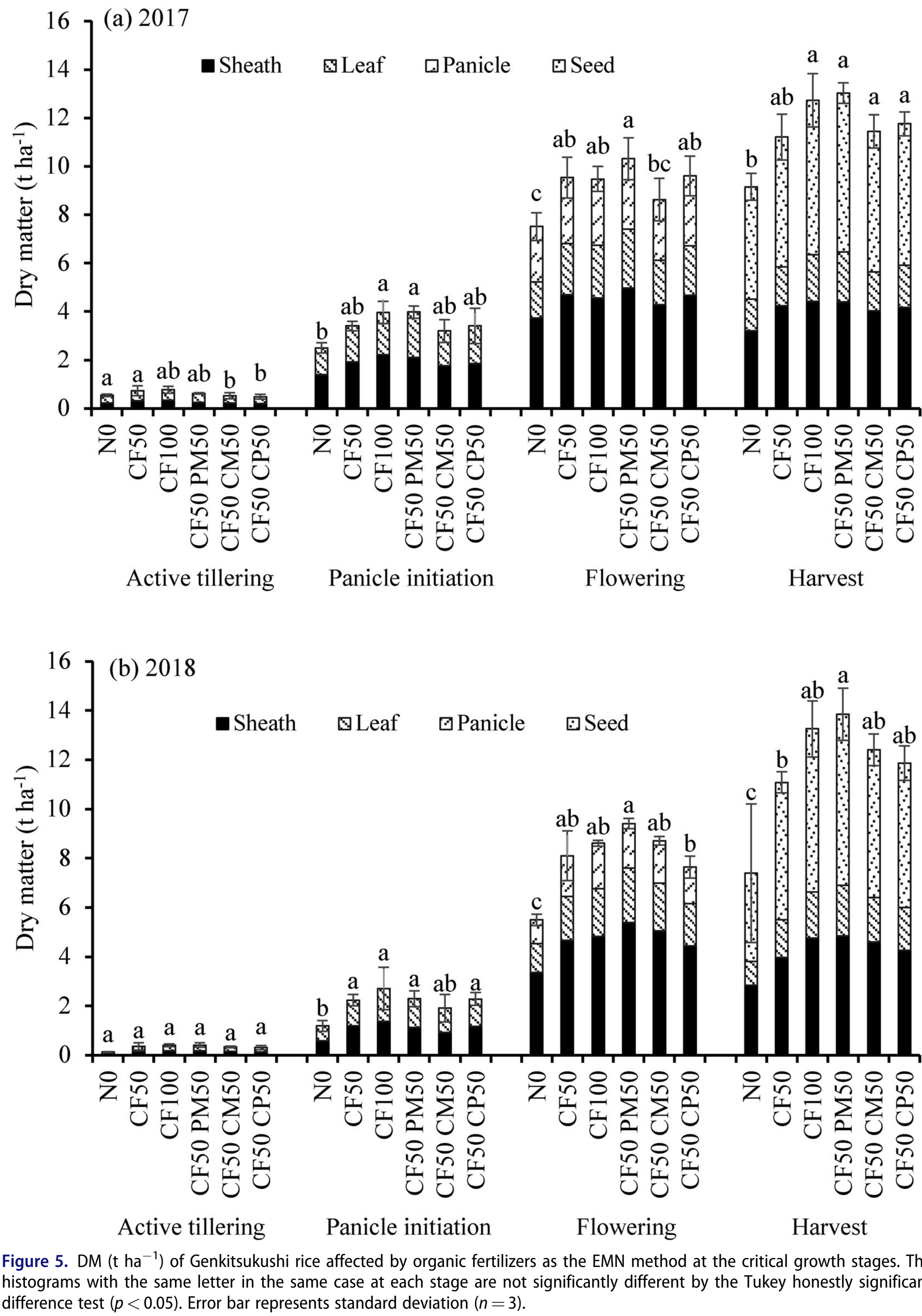

Figure 5. DM (t ha^{-1}) of Genkitsukushi rice affected by organic fertilizers as the EMN method at the critical growth stages. The histograms with the same letter in the same case at each stage are not significantly different by the Tukey honestly significant difference test ($p < 0.05$). Error bar represents standard deviation ($n = 3$).

Table 3. N uptake (kg ha^{-1}) of Genkitsukushi rice affected by organic fertilizer as the EMN method at the critical growth stages.

Treatment	N uptake (kg ha^{-1}) Active tillering	Panicle initiation	Flowering	Harvest
Year 2017				
N_0	16.05 a	36.79 d	57.71 f	70.19 c
CF_{50}	23.54 a	54.78 c	80.96 d	87.66 b
CF_{100}	24.97 a	70.83 b	94.76 b	114.53 a
CF_{50} PM_{50}	22.45 a	82.01 a	107.99 a	120.60 a
$CF_{50}CM_{50}$	16.45 a	54.34 c	74.11 e	95.72 ab
CF_{50} CP_{50}	15.65 a	58.75 c	88.17 c	97.75 ab
Year 2018				
N_0	4.69 B	25.98 B	43.41 C	55.76 C
CF_{50}	13.09 A	43.27 AB	62.38 B	81.75 B
CF_{100}	14.67 A	60.04 A	73.32 AB	101.83 AB
CF_{50} PM_{50}	15.80 A	61.16 A	83.34 A	110.42 A
CF_{50} CM_{50}	12.15 A	44.60 AB	73.70 AB	98.90 AB
CF_{50} CP_{50}	11.54 A	51.07 A	65.04 B	93.13 AB

Means followed by the same case letter in the same year in each column are not significantly different in Tukey's honestly significant difference test ($p < 0.05$). Number followed by treatment shows the amount of N applied as a percentage based on 85 kg N (or EMN) ha^{-1}.
CF: chemical fertilizer; PM: Poultry manure; CM: Cow manure; CP: Compost; EMN: Estimated mineralizable N.

Table 4. HI, yield and yield components of Genkitsukushi rice affected by organic fertilizer as the EMN method.

Treatment	HI	No. panicle hill^{-1}	No. spikelets panicle^{-1}	Filled grain (%)	1000 grain weight (g)	Yield (t ha^{-1})
Year 2017						
N_0	0.60 a	11.50 d	69.61 c	73.06 de	27.53 a	4.51 b
CF_{50}	0.52 c	12.33 cd	77.30 ab	71.84 e	26.81 ab	5.89 b
CF_{100}	0.57 ab	14.50 ab	79.46 a	82.32 ab	27.09 a	7.32 ab
CF_{50} PM_{50}	0.61 a	16.00 a	77.96 a	85.62 a	27.20 a	7.93 a
CF_{50} CM_{50}	0.61 a	13.33 bc	75.43 b	79.19 bc	27.75 a	7.06 ab
CF_{50} CP_{50}	0.55 b	13.50 bc	80.16 a	76.76 cd	27.12 a	6.55 ab
Year 2018						
N_0	0.45 B	8.33 C	72.59 B	78.36 A	26.43 AB	3.38 C
CF_{50}	0.49 B	12.66 B	75.70 B	82.94 A	26.21 AB	5.49 B
CF_{100}	0.50 AB	13.33 AB	84.06 A	73.90 B	26.80 A	6.68 AB
CF_{50} PM_{50}	0.56 A	15.33 A	83.03 A	84.81 A	27.92 A	7.77 A
CF_{50} CM_{50}	0.57 A	14.83 A	82.03 A	74.16 B	27.11 A	7.14 A
CF_{50} CP_{50}	0.54 A	13.33 AB	73.87 B	77.98 AB	28.10 A	6.50 AB

Means followed by the same case letter in the same year in each column are not significantly different in Tukey's honestly significant difference test ($p < 0.05$). EMN = Estimated mineralizable N.

N mineralization in the incubation experiment

At the 0 week (Figure 6), the PM (total N > 4%) measured the highest N mineralization 29.17% but the CM (total N < 4%) and the CP (total N < 4%) had the lower values 12.20% and 1.43%, respectively. After 2 weeks, the PM released rapidly the higher mineralizable N while the CM and CP steadily released the mineralizable N. At 12 weeks, the maximum N mineralization 72.81% was recorded by the PM (total N > 4%) treatment. The CM (total N < 4%) obtained 27.45% of N mineralization. However, the CP (total N < 4%) achieved the lower N mineralization 16.16%.

Discussion

Effects of organic fertilizer application using the EMN method on N uptake, growth characteristics, and DM production at various growth stages

The application of organic fertilizer using the EMN method influenced the N uptake and growth characteristics of Genkitsukushi rice during cultivation. Before the active tillering stage (24 DAT), the organic fertilizers produced lesser responses, resulting in lower N uptake. As a result, plant

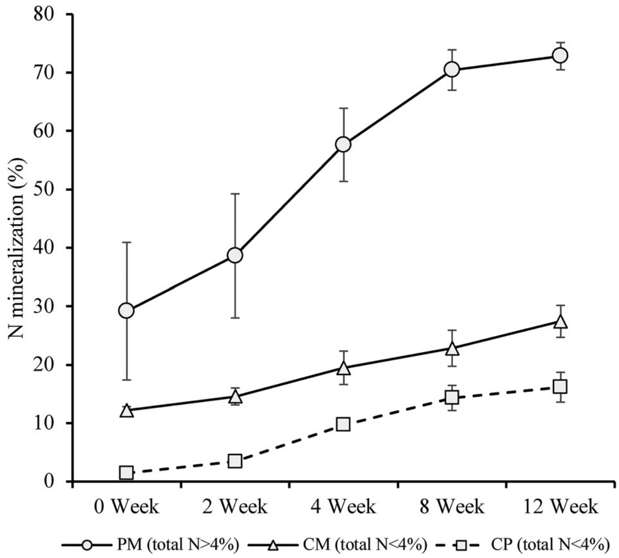

Figure 6. N mineralization from manure as the EMN method in the incubation experiment at 30 °C. Error bar represent standard deviation ($n = 3$).

height and tiller number produced by the PM, CM, and CP treatments were low, but not significantly different from those produced by the CF treatment. Therefore, the organic fertilizer treatments achieved low DM production during the early stage of rice cultivation. This finding is in agreement with the results of Myint et al. (2011), who reported that organic fertilizer releases nutrients slowly, resulting in little plant growth during the initial stage of rice cultivation, but enhanced growth during the later stages. At the same time, the CF resulted in higher N uptake, SPAD values, and growth characteristics. The CF dissolved readily in the floodwater after application and provided nutrients immediately during the early growth stage (Sarker et al. 2004). Therefore, DM production was greater for the CF_{100} and CF_{50} treatments than for the PM, CM, and CP treatments.

The N supply from the $CF_{50}PM_{50}$ treatment was greater at the panicle initiation stage (38 DAT), resulting in the highest N uptake and SPAD values among treatment groups. Consequently, the plants were taller and the tiller number was increased significantly by the PM treatment. The application of organic manure in combination with CF has been shown to increase plant height (Masarirambi et al. 2012). However, the $CF_{50}CM_{50}$ and $CF_{50}CP_{50}$ treatments produced fewer tillers compared with CF_{100}, particularly in 2017. The CM and CP treatments achieved low N uptakes and SPAD values at this stage. Eghball et al. (2002) reported that CM and CP, which have low N contents and high C: N ratios, release only small portions of total N and other nutrients during the first year of application. The effects of CM and CP improved significantly in 2018, with larger tiller numbers, similar to that achieved with CF_{100}. This result supports the findings of Eghball and Power (1999), who reported that about 60% N from CM and 80% N from CP become available to plants in the consecutive year.

The $CF_{50}PM_{50}$ treatment maximized the N uptake and resulted in the largest SPAD value among treatments at the flowering stage. Consequently, taller plants and larger tiller numbers were observed compared with the CF_{100} and other treatments, as well as the greatest DM production. Rakshit, Sarkar, and Sen (2008) stated that PM application provides a sufficient and continuous N supply. At the time of harvest, the $CF_{50}PM_{50}$ treatment resulted in the highest N uptake and the greatest DM production (13.02 t ha^{-1} in 2017, 13.86 t ha^{-1} in 2018). The DM yields of the CM and CP treatments were low, but similar to that of CF_{100}. The 50% EMN from PM (total $N > 4\%$) supplied more N than did those from CM and CP (total $N < 4\%$). This finding is in agreement with the results of Belay et al. (2001), who stated that PM combined with CF supplies continuous N and improves the growth and dry weight of rice plants by reducing nutrient loss.

Effects of organic fertilizer applied using the EMN method on N uptake, yield, and yield components of rice at harvest

The effects of organic fertilizer application using the EMN method on N uptake, yield, and yield components of rice were examined and compared with those of a sole application of CF_{100}. The supply of EMN from the PM treatment synchronized well with the crop N demand throughout the growth period. It performed well not only in terms of growth characteristics, but also in terms of yield. The highest yields were attained by the $CF_{50}PM_{50}$ treatment (7.93 t ha^{-1} in 2017, 7.77 t ha^{-1} in 2018). Myint et al. (2011) reported that the main advantage of PM is that it releases nutrients slowly throughout the growing season, synchronized to the N demand of rice plants. This characteristic explains the achievement of the maximum N uptake with the $CF_{50}PM_{50}$ treatment. The other CM and CP treatments yielded low N uptakes, similar to those obtained with CF_{100}. Nishio (2007) reported that the N supply depends on the amount of mineralizable N from organic fertilizer, which depends on the total N content of organic manure. According to that report, manure with a total N content $\geq$4% releases 50% mineralizable N of total N applied. The PM had a greater total N content (4.87%) than did CM and CP. It also had moderate P_2O_5 (4.56%) and K_2O (2.14%) contents. The total N contents of CM and CP were <4%.

The $CF_{50}PM_{50}$ treatment significantly influenced the panicle number $hill^{-1}$, spikelet number $panicle^{-1}$, filled grain percentage, and panicle length. These parameters were significantly greater than those for CF_{100} in both years. Lungmuana et al. (2016) reported that organic and inorganic fertilizers significantly influence the number of panicles, or fertile tillers producing panicles, per hill. The achievement of the maximum yield with $CF_{50}PM_{50}$ was due mainly to the greater number of productive tillers containing panicles. Chaturvedi (2005) reported that a larger number of tillers, particularly fertile tillers, leads to a higher yield. Arif et al. (2014) determined that the maximum number of fertile tillers per hill is produced when PM (5 t ha^{-1}) is combined with 50% of the recommended amount of CF. In the present study, we applied PM at only 1.75 t ha^{-1} according to the EMN method, combined with 50% CF.

The positions of the treatment plots did not change between years, to obtain continuous effects of the organic and inorganic fertilizers. With the continuous application of CF_{100}, the N uptake and yield decreased slightly in 2018 due to decreased rainfall in the grain filling period. The cultivation of rice using only CF might be sensitive to climate change. Under dry weather conditions, the sole application of CF could result in high N loss and low N recovery, which tends to produce a low rice yield (Liu et al. 2016). The $CF_{50}PM_{50}$ treatment resulted in greater N uptake and yield, and $CF_{50}CM_{50}$ and $CF_{50}CP_{50}$ provided stable N uptakes and yields in both years. The CM and CP treatments also produced yields and yield components as high as those attained by CF_{100} in both years. These results indicate that 50% of recommended CF rate could replace with the continuous application of 50% EMN by organic fertilizers plus 50% CF in rice. Zhang et al. (2018) also reported that 5 t ha^{-1} organic fertilizer combined with 75% CF results in a greater yield and N recovery efficiency than does organic fertilizer (5 t ha^{-1}) combined with 100% CF over 3 years. In this study, the application of EMN via PM (total N > 4%) was effective in terms of N uptake, the growth characteristics, and yields in both years. The application of CM and CP (total N < 4%) using the EMN method was more effective in 2018. N mineralization differed among the manure types. Mineralization of organic N is expected to be low for manure with a low total N content in the first year, and residual amounts become available for the crop in the second, third, and fourth years (Eghball et al. 2002). Therefore, the successive use of organic

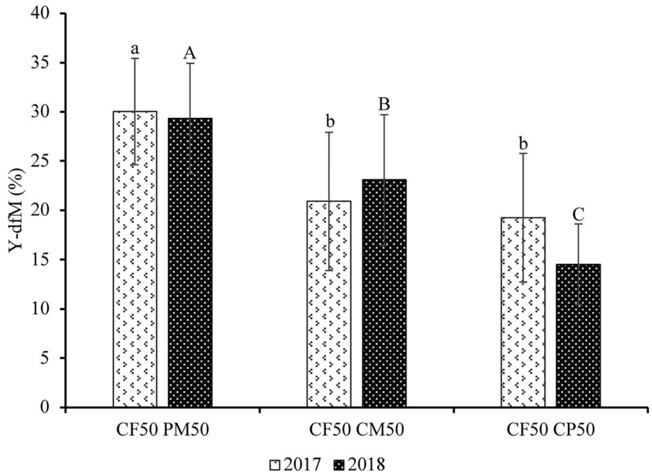

Figure 7. Yield of Genkitsukushi rice derived from manure (Y-dfM; %) as affected by organic fertilizers as the EMN method. The histograms with the same letter in the same case in the same year are not significantly different by the Tukey honestly significant difference test ($p < 0.05$). Error bar represents standard deviation ($n = 3$).

fertilizer creates the opportunity to reduce the amount of CF applied. In a future experiment, the continuous application of organic fertilizers using the EMN method should be optimized by reducing the proportion applied. Nevertheless, manure combined with CF produces significantly greater yields and improved yield parameters compared with CF alone (Akter et al. 1993). Thus, the application of organic manures based on the EMN method combined with CF is highly beneficial for a sustainable rice yield.

To clarify the effects of EMN from the individual manure types, we calculated the percentages of yield derived from manure (Figure 7). The yields of Genkitsukushi rice derived from PM (total N > 4%) were 30.02% and 29.31% in 2017 and 2018, respectively. Yields of 20.91% and 23.08% were derived from CM, and yields of 19.27% and 14.53% were derived from CP, in 2017 and 2018, respectively. Khalil, Hossain, and Schmidhalter (2005) emphasized that manures with high C:N ratios or low total N contents provide slower mineralization due to slower decomposition and nitrification. However, the yield contribution potential was greater for PM combined with 50% CF than for CM and CP applied based on the EMN method.

N mineralization and availability derived from organic fertilizer

According to the results of incubation experiment (Figure 6), the PM (total N > 4%) generated the higher N mineralization throughout the period. Consequently, the maximum N mineralization 72.81% was recorded after 12 weeks. The CM (total N < 4%) obtained 27.45% of N mineralization. However, the CP (total N < 4%) achieved the lower N mineralization 16.16%. Therefore, the N mineralization from organic fertilizer depends on its total N content. The finding was coincided with Nishio (2007) who reported that the organic fertilizer with total N > 4% releases 50% of mineralized N and the organic fertilizer with total N 2 ∼ 4% releases 30% of mineralized N.

In addition, the results were consistent between the incubation experiment and field experiments. In the field experiments, the PM (total N > 4%) applied a lower amount of N than the CM (total N < 4%) and the CP (total N < 4%) by using the EMN method. However, the PM (total N > 4%) provided the higher N availability (77.49 and 67.44%) than the CM (total N < 4%) and the CP (total N < 4%) in both years (Figure 8). It is clear that the organic fertilizer with total N (≥4%) creates a higher N availability to japonica rice variety in both years. The more N was available from CM and CP with total N (<4%) in 2018, and it can be due to the mineralization

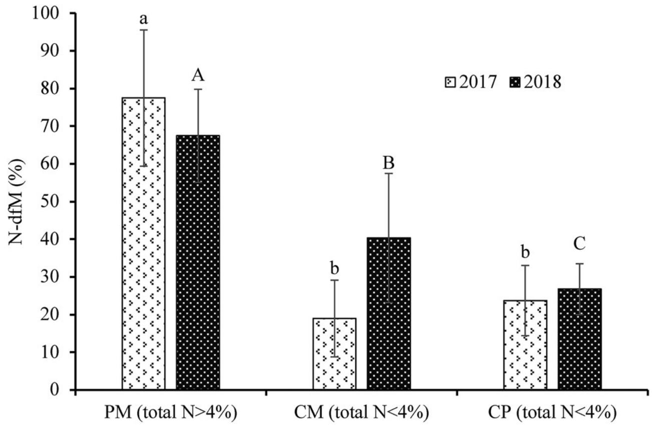

Figure 8. N availability derived from manure (N-dfM; %) as the EMN method. The histograms with the same letter in the same case in the same year are not significantly different by the Tukey honestly significant difference test ($p < 0.05$). Error bar represents standard deviation ($n = 3$). N availability derived from manure (N-dfM %) = N uptake [(50% organic + 50% inorganic) − 50% inorganic] ÷ N apply (50% organic) × 100.

of N by the end of the crop growth in 2017. But it cannot be definitively proven in this study. This would be usefully investigated as a further study. The findings have coincided with the report of Eghball et al. (2002), Aulakh, Khera, and Doran (2000), and Kaupa and Rajashekhar (2014).

Advantages of the EMN method for the application of organic fertilizer

In this study, we developed a practical method for the application of organic fertilizers (PM, CM, and CP). Organic fertilizer application based on the EMN method depends on the total N content of the fertilizer (Nishio 2007). We developed this method to synchronize the mineralizable N available from organic fertilizer with the crop N demand. We did not adjust the weight of the organic fertilizer, but adjusted the EMN to achieve a target rate of 42.5 kg N ha^{-1} for rice. The PM (total N > 4%) treatment performed well, resulting in greater N uptake, better growth characteristics, and better yields. Several researchers have reported general application rates for organic fertilizers combined with the recommended CF amount that increase yield [e.g., PM 5 t ha^{-1} (Biswas et al. 2016), CM 10 t ha^{-1} (Sudarsono, Melati, and Aziz 2014), CP 10 t ha^{-1} (Hussain, Arshadullah, and Mujeeb 2001)]. The EMN method was effective compared with the results achieved with the general application rates, indicating that the 50% EMN applied from PM (1.75 t ha^{-1}) combined with 50% N (42.5 kg ha^{-1}) from CF synchronized the N supply with the crop N demand, resulting in a higher yield than that attained with 100% N from CF. The $CF_{50}PM_{50}$ treatment increased N uptake by 5.03% and 7.78%, rice yields by 7.69% and 14.02%, and DM production by 2.26% and 4.34% in 2017 and 2018, respectively, compared with the CF_{100} treatment. The 50% EMN applied via CM (5.9 t ha^{-1}) and CP (6.5 t ha^{-1}) combined with 50% N (42.5 kg ha^{-1}) via the recommended CF provided a yield similar to that attained with CF_{100}.

The EMN method saved organic fertilizer and CF without decreasing the yield of Genkitsukushi rice. Therefore, instead of applying organic fertilizer at the general application rate, we recommend that farmers apply it using the EMN method, which synchronizes the available EMN with the crop N demand and supplements the use of inorganic fertilizer.

Conclusion

The application of organic fertilizer using the EMN method significantly increased and sustained rice yields, but responses varied among of organic fertilizer types with different total N contents.

Organic fertilizer containing ≥4% total N had a higher N availability, provided the highest N uptake and yield compared with a sole application of CF_{100}. Manure with ∼2% total N provided a lower N availability and achieved a similar yield in the first year and enhanced N availability and a greater yield in the second year. The results of this study indicate that the use of organic manure, such as PM, which has a high total N content (≥4%) and moderate P and K contents, provided excellent Genkitsukushi yields. The 50% EMN applied from organic fertilizer combined with 50% N from the recommended CF increased N availability, efficiently enhanced the growth characteristics, and sustained the yield of Genkitsukushi japonica rice.

Disclosure statement

We have disclosed that there is no conflict of interest regarding publication of this article.

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