Nitrogen Balance in Forage Rice (Oryza sativa L. cv. Tachisuzuka) Cultivation in Pots with Animal Manure Application

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Abstract: Experiments were conducted to evaluate the nitrogen (N) balance in forage rice cultivation using animal manure in 1/2,000a Wagner pots in a greenhouse. The cattle manure and poultry manure were applied at 3 levels of N (0, 14, 28 g available N m⁻²) without additional chemical fertilizer application. The pots were designed to simulate the fluid percolation in the paddy field. The results indicated increasing levels of N input improved plant height, tiller number, SPAD value and biomass (straw, grain and root) production, however, N leaching from soil (Andosols) due to percolating water also increased. The planting of rice plants proved to reduce 30% of the N leaching loss. N use efficiency, the ratio of N uptake by plant per unit N application, decreased in higher N application. The N uptake by the above-ground parts occupied about 66% of the whole plants.

Key words: Animal manure, Biomass production, Forage rice, N balance, N leaching.

In Japan, increasing numbers of farmers are producing forage rice (Oryza sativa L.), which not only improves the country’s self-sufficiency with respect to feed but also contributes to the rural economy. New rice cultivars for whole crop silage (WCS) have been released by the Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan (Sakai et al., 2003; Ookawa et al., 2010). As a result of subsidies from the Japanese government, the area for WCS rice cultivation has been increasing, reaching 26,000 ha in 2013 (MAFF, 2013; Asada et al., 2013). Cultivation of forage crop in excess paddy fields is considered a promising way to enhance feed supply (Sakai et al., 2003; Kato, 2008; Matsushita et al., 2011). Whole plants of forage rice are generally harvested at the yellow-ripe stage, at which point the plants exhibit maximum dry matter and nutrient yield (Nakano and Morita, 2009), better fermentative quality and greater voluntary intake by beef cattle, as well as a lower proportion of grain undigested by beef cattle (Nakui et al., 1988). Tachisuzuka is a new rice cultivar for WCS use and the agronomic characteristics of Tachisuzuka have potential to increase above-ground parts of biomass production; the straw yield is high and the grain yield is low, and the sugar content is high (Sakai et al., 2003; Ookawa et al., 2010; Matsushita et al., 2011, 2012; Nakano et al., 2011, 2012).

Cultural practices for whole crop rice are similar to those for normal rice. Forage rice, comprising a series of new cultivars of rice, requires and tolerates higher nitrogen (N) loading than most rice cultivars for human consumption (Kyaw et al., 2005; Zhou and Hosomi, 2008). N is the most important nutrient for plants, which plays a role in regulating growth and affects photosynthesis as well as dry matter and grain production of paddy field rice. The level of available N in paddy soils substantially impacts rice yield. In the past, N has been applied to satisfy the nutritional demands of rice and to improve rice yield (Wada and Cruz, 1989; Wada et al., 1991; Ntamatungiro et al., 1999). Farmers often apply larger quantities of N fertilizers than strictly required for achieving maximum yield, to ensure potential yield every year. At the same time, N is considered one of the major pollutants in terrestrial ecosystems. Excessive use of N fertilizer causes lodging and disease, resulting in increased input costs and net yield reduction (Kyaw et al., 2005; Kamiji and Sakuratani, 2011). Some of the applied N fertilizer is easily lost through a variety of processes including leaching and denitrification. High nitrate (NO₃⁻) concentrations may substantially increase NO₃⁻ leaching, which can potentially contaminate...
Forage rice is grown intensively with the application of large amounts of animal manure, which is a by-product of a prosperous livestock industry that, after composting, can be used as a highly effective N fertilizer, and the manure type and application timing is important (Liu et al., 2008; Hara et al., 2009; Nakano et al., 2011; Ooya et al., 2013). We have been studying biomass production and environmental load of forage rice cultivation under different levels of compost in the paddy field (Itani et al., 2012; Gusmini et al., 2013a, b; Shigeuchi et al., 2013).

In this study, our objective was to estimate the N balance in forage rice (cv. Tachisuzuka) cultivation system with application of animal manure to soil in pot experiments. The N balance was predicted by estimating N input derived from animal manure (no chemical fertilizer was applied) and N output through leaching, plant uptake, and retention in the soil after harvest. Special attention was paid to the N-reducing effects of rice plants and the N-uptake by rice roots.

### Materials and Methods

#### 1. Experimental design

The pot experiments were conducted from 18 June to 17 October 2012, using 1/2,000a Wagner pots in a greenhouse located on the campus of the Prefectural University of Hiroshima (PUH), Shobara, Japan. Mixed animal manure, fermented cattle manure (CM, sawdust compost with cow excreta) and fermented poultry manure (PM, chicken feces dried and pelletized), was applied as the organic N source. CM and PM are widely used in the Hiroshima area and are commercially produced in Hiroshima Prefecture (Table 1). Treatments comprised three levels of manure application based on N content (0, 14, and 28 g N m⁻²) applied as a one-time basal fertilizer with no additional chemical fertilizer, with (+) or without (−) rice plants, expressed hereafter as 0N+, 14N+, 14N−, 28N+ and 28N−, respectively. The manure was mixed in soil 0 to 10 cm depth and the amendment levels were set based on the estimated N availability of manure as 15% and 70% of total N content in CM and PM, respectively, to ensure that similar available N was supplied for both manure treatments. In each pot, three 25-day-old seedlings of forage rice (cv. Tachisuzuka) were transplanted in a single hill to the center of each pot with different manure treatments. Five treatments in total (Table 2) were repeated three times.

To determine the amount of N leached, we used the pots designed to simulate the percolation conditions in paddy fields (Fig. 1). Each pot was filled with 10 kg of air-dried soil (passed through a 5-mm sieve) from a paddy field on the PUH campus classified as Andosols. The bottom of each pot was filled with 5 cm of gravel, and a filter cloth was placed above the gravel. A ceramic drainage (percolation tube) was placed inside the gravel and connected to a pinch cock via a rubber stopper and plastic hose. The pots were flooded with water immediately after amending the soil with manure and held for 3 d prior to transplantation of the rice seedlings. Tap water was added...

#### Table 1. Chemical composition of animal manure (cattle and poultry manure).

| Chemical composition (mg g⁻¹ DW) | Cattle manure | Poultry manure |
|----------------------------------|---------------|----------------|
| Total N                          | 16.8          | 33.8           |
| Total P                          | 18.2          | 51.0           |
| Total C                          | 411.0         | 293.5          |
| K₂O                              | 26.2          | 40.7           |
| CaO                              | 7.9           | 191.7          |
| MgO                              | 7.9           | 12.4           |
| NO₃⁻N                            | 0.006         | 0.088          |
| NH₄⁻N                            | 0.415         | 2.980          |
| Total inorganic N                | 0.421         | 3.068          |

NO₃⁻N, NH₄⁻N was analyzed by 15% KCl extraction; K₂O, CaO, MgO were analyzed by dry basis analysis.

#### Table 2. Pot experimental design.

| Treatment | Available N application (g m⁻²) | Animal manure (g pot⁻¹) | With or without forage rice |
|-----------|---------------------------------|-------------------------|---------------------------|
|           |                                 | Cattle manure (CM)      | Poultry manure (PM)       |                           |
| 0N+       | 0                               | 0                       | 0                         | With                      |
| 14N+      | 14                              | 100                     | 31.6                      | With                      |
| 14N−      | 14                              | 100                     | 31.6                      | Without¹                 |
| 28N+      | 28                              | 100                     | 68.0                      | With                      |
| 28N−      | 28                              | 100                     | 68.0                      | Without                  |

¹Control to observe N-movement resulting from water percolation.

The water contents of cattle and poultry manure are 63.7 and 18.6 %, respectively. Availability of N was estimated as below; CM 15% and PM 70% of total N.

Treatment groups: 0N to 28N, available N in animal manure (g N m⁻²); +, with rice plant; −, without rice plant.
to the pots, and the water was maintained at 3 cm deep until the plants reached maturity. Water was allowed to percolate (leaching) every day by draining 500 mL of water every morning and 500 mL every evening. One liter of percolation water (leachate) per day corresponds to a 2-cm decrease in water level per day in the field. This leaching treatment was continued until heading because soil became clogged up gradually. Leachate samples (100 mL) were collected from each pot on 1, 2, 3, 7, 10, 20, 30, 40, 50, 60 and 70 days after transplanting (DAT). A small quantity of the surface water at 0 DAT was collected.

2. Analysis of rice growth characteristics
During the rice growth stage (7 to 70 DAT), plant height, tiller number and leaf color index (SPAD values) were recorded weekly. SPAD values were determined on the topmost fully expanded leaf with a hand-held chlorophyll meter (Soil Plant Analysis Development Chlorophyll Meter, SPAD-502, Konica Minolta Sensing Inc., Osaka, Japan).

3. Nitrogen analysis of water, soil and plant samples and biomass production
The total N levels in the leached water samples were determined by the persulfate digestion method with a Hach DR/2800 spectrophotometer. The total N contents of plant and soil samples were determined with a CN Corder instrument (Macroorder JM 1000CN, J-Science, Kyoto, Japan). Upon reaching maturity, rice plants were cut at 2 – 3 cm above ground, and the dry-matter weight (biomass) of the whole plant, straw, grain (unhulled rice grain) and root, were recorded. The rachis and rachis-branches from the panicles were ignored. Root samples were carefully rinsed to remove any soil. All plant components were air-dried at 65°C for 72 h to a constant weight and ground before being evaluated for total N content. Soil samples were taken from each pot and passed through a 2-mm sieve before air dried.

4. Nitrogen balance in forage rice cultivation
N balance was estimated between the amount of N input (manure (CM + PM), and soil N) and N output (N-uptake by plant, N-remained in soil, and N-leaching).
5. Statistical analysis
Data were analyzed following standard procedures for the analysis of variance (ANOVA), followed by Tukey’s test ($P < 0.05$) to test differences.

Results and Discussion
1. Rice growth characteristics
The changes in plant height, tiller number, and SPAD value of rice plants are shown in Fig. 2. Growth characteristics of rice (plant height and tiller number) increased with increasing N input. The influence of N input level on growth characteristics was observed throughout the growth period. The number of tillers increased rapidly after 40 DAT (middle of the growing period) in 28N+. The percentage of productive tillers was almost same in the 0N+, 14N+ and 28N+ treatments (89, 92 and 90%, respectively). SPAD value, which indicates the relative chlorophyll content of rice plant leaves and is arguably the most important plant growth characteristic, also increased with increasing N input. In all treatments, the SPAD value increased until 40 DAT, declining slightly and stabilizing through the remainder of the growing period.

Table 3. Biomass production, N content and N uptake by forage rice plants for level of animal manure application.

| Treatment | Biomass (g pot$^{-1}$) | N content (g 100g$^{-1}$) | N uptake by plant (g pot$^{-1}$) |
|-----------|-------------------------|---------------------------|---------------------------------|
|           | Straw | Grain | Root | Top | Top/whole (%) | Straw | Grain | Root | Top | Top/whole (%) |
| 0N+       | 35.0 a   | 25.8 a | 24.4 a | 60.8 a | 71 a | 0.51 a | 1.13 a | 0.97 a | 0.18 a | 0.29 a | 0.24 a | 0.47 a | 67 a |
| 14N+      | 48.6 b   | 29.2 ab | 34.9 ab | 77.8 ab | 69 a | 0.62 a | 1.19 a | 0.85 a | 0.30 b | 0.35 ab | 0.30 ab | 0.65 b | 63 a |
| 28N+      | 55.4 b   | 36.5 b | 47.6 b | 91.9 b | 66 a | 0.62 a | 1.22 a | 0.98 a | 0.34 b | 0.45 b | 0.46 b | 0.79 b | 64 a |

Top: Above-ground parts (straw + grain), whole: straw + grain + root.
Within each column means with the same letter are not significantly different at $P < 0.05$ (by Tukey’s test).

Fig. 3. Changes in the concentration of total N in the surface water and leachate during the forage rice growing stage.
0 DAT, surface water; 1 to 70 DAT, leachate. Treatment groups: 0N to 28N, available N in animal manure (g N m$^{-2}$); +, with plant; –, without plant. Symbols and error bars represent the mean and standard deviation of three pots.

2. Biomass production, N content and N-uptake
Table 3 shows the effect of level of animal manure application on the biomass production, N content, and N uptake by forage rice. It can be seen that increasing levels of N input resulted in significant differences in those at harvest. For the most part, the greatest increase in biomass production and N uptake relative to the non-amended control were observed in the 28N+ treatment. The N content of straw, grain and root were not significantly increased from 0N+ to 28N+. The weight and N uptake of the straw and grain was 66 – 71% and 63 – 67% of that of the whole plant, respectively. The N uptake by the above-ground parts occupied about 66% of the whole plant in this experiment. Zhou et al. (2005) reported that the root occupied about 20% of N uptake by whole the rice plant. The above-ground part of forage rice is carried out and the rice straw is not returned to the rice field, thus N and other minerals should be applied.

In this study, we used two kinds of animal manure (CM and PM, Table 1) typically used for forage rice cultivation that were rich in various nutrients, including N, which had a substantial impact on rice growth and nutrient uptake.
CM and PM were found to contain substantial amounts of inorganic N and to have a substantial impact on the N uptake by plants as well as amount of biomass production (Table 3). In addition, both types of manure were found to uptake by plants as well as amount of biomass production, inorganic N and to have a substantial impact on the N content of manure. Nitrogen in the form of NH$_4$-N, making the CM + PM manure combination a suitable alternate basal fertilizer in rice cropping systems. Rice plants prefer NH$_4$-N over NO$_3$-N (Oji and Izawa, 1974; Zhou et al., 2005, 2011; Riya et al., 2012). High levels of NH$_4$-N input caused rapid increases in plant growth parameters (Fig. 2).

### 3. Change in concentration of total N in leachate by percolation

Changes in the concentration of total N in the surface water and leachate during rice plant growing period are shown in Fig. 3. In the surface water, the concentration of total N at 0 DAT (3 d after watering) was highest in 28N followed by 14N and 0N, but most of them were cleaned by percolation through soil (see 1 DAT). In the leachate, the concentration of total N tended to increase with the time until the middle period (40 DAT). In 0N+, however, the total leached N concentration was low and it tended to be at the same level throughout the growing stage. In 14N+ to 28N+ with rice plant, total leached N concentration tended to decrease after 40 DAT, but it tended to increase until the late growing stage in 14N– and 28N– without rice plant.

These results are similar to those reported by Sahu and Samant (2006) where the larger the amount of percolation water, the larger the N leaching. Application of N fertilizer at higher doses causes greater leaching loss. The NO$_3$-N leaching was higher than NH$_4$-N in the percolation water during the cultivation of forage rice periods (Gusmini et al., 2013b). The biomass (straw and grain) production at 30 days after heading has been shown to be almost maximum in 14N and its N contents increased with the N level in field experiments with 0N, 7N, 14N, 21N and 28N (Itani et al., 2012; Gusmini et al., 2013a, b; Shigeuchi et al., 2013). The highest NO$_3$-N leaching was found in N28 (6.3mg L$^{-1}$), indicated on the polluted level.

### 4. N balance in forage rice cultivation in pots with animal manure application

N input and N output in forage rice cultivation was shown in Table 4. N uptake by whole plant was calculated by summing the N contents of straw, grain, and root. The value of change, N output minus N input, were from -5.6% to +2.0%. They could be assumed due to higher amount of N fixation in planted plot (14N+ and 28N+) and higher amount of N denitrification (gaseous N) in unplanted plot (14N– and 28N–) despite the fact that heterogeneity of soil or manure was not denied. High levels of N input can increase the N content and N uptake by individual plants, while at the same time, potentially resulting in increased N loss from soil due to leaching via percolation water. The ratio of N uptake by plant per unit N application, that is N use efficiency, was 64.9% and 50.8% in 14N+ and 28N+, respectively, decreasing with the applied N rate. The ratio of N leached out per N applied was 20.3, 29.1, 23.4 and 33.5% in 14N+, 14N–, 28N+ and 28N–, respectively. The planting of rice plants proved to reduce 30% of the N leaching loss. Greatest leaching was observed for 28N–. In 0N+, no fertilized plot, N retained in soil was lower (30.8g) than in original soil (33.4 g), leached out N was small and the change showed minus.

The results indicated that increasing levels of N input improved plant height, tiller number, SPAD value and biomass (straw, grain and root) production, however, N leaching from soil (Andosols) due to percolating water also increased. Tachisuzuka is characterized by the higher dry weight of straw with small panicles. The specificity of biomass production and N uptake of this variety compared to normal varieties is not clear in this experiment, however the earlier decrease in N uptake during ripening stage could be assumed because the sink size is limited in Tachisuzuka. The forage rice is studied on N removal ability to clean polluted river water and liquid cattle waste (Zhou et al., 2005, 2011; Riya et al., 2012).

### Table 4. N input and N output in forage rice cultivation.

| Treatment | Cattle manure | Poultry manure | Original soil | Total | Taken up by plants$^{1}$ | Retained in soil | Leached out$^{1}$ | Total | Change$^{3}$ | Ratio of N uptake by plant per N applied | Ratio of N-leached out per N- applied |
|-----------|---------------|----------------|---------------|-------|--------------------------|-----------------|----------------|-------|-------------|------------------------------------------|----------------------------------------|
| 0N+       | 0             | 0              | 33.4          | 33.4  | 0.71 ± 0.03 a            | 30.8 a          | 0.11           | 31.6  | -1.8 (−5.3%) | --                                      | --                                     |
| 14N+      | 0.61          | 0.87           | 33.4          | 34.9  | 0.95 ± 0.07 b            | 34.3 b          | 0.30           | 35.6  | +0.7 (+2.0%) | 0.649                                   | 0.203                                  |
| 14N–      | 0.61          | 0.87           | 33.4          | 34.9  | -                        | 32.6 b          | 0.43           | 33.0  | -1.9 (−5.4%) | --                                      | 0.291                                  |
| 28N+      | 0.61          | 1.87           | 33.4          | 35.9  | 1.25 ± 0.07 c            | 34.6 b          | 0.58           | 36.4  | +0.5 (+1.4%) | 0.508                                   | 0.234                                  |
| 28N–      | 0.61          | 1.87           | 33.4          | 35.9  | -                        | 33.1 b          | 0.83           | 33.9  | -2.0 (−5.6%) | --                                      | 0.335                                  |

1) Calculated from Table 3  2) Calculated from Fig. 3  3) Change in total N output from total N input (ratio to total N input). Within each column means with the same letter are not significantly different at $P<0.05$ (by Tukey’s test).
Conclusions

It is indicated that the practice of amending soil with CM and PM manure is in accordance with the agricultural development policy of promoting environmentally friendly agriculture in the sense that it reduces environmental contamination while maintaining soil fertility and utilizing (rather than disposing of) livestock waste as manure (Kumazawa, 2002; Yoshida, 2008; Myint et al., 2011). Based on the results of these pot studies with Tachisuzuka, a new rice cultivar bred for WCS, we conclude that increased application of animal manure may increase the crop production, but the risk of N leaching may also be increased. We assume that rice plants can reduce the N leaching loss from soil due to percolating water. However, excess application of N is not only inefficient for promoting biomass production but also increases the amount of N leaching out of the root zone and is harmful for the environment. Even if we use animal manure in rice production, we should consider application levels and timing.

Acknowledgements

We would like to express our deepest gratitude to the Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima, Japan and the Directorate General of Higher Education (DGHE) in the Republic of Indonesia for their financial support.

References

Asada, K. et al. 2013. Paddy Water Environ. 11: 559-571.
Gusmini et al. 2013a. Jpn. Jour. Crop Sci. 82 (Extra issue 1): 50-51.
Gusmini et al. 2013b. J. Tropical Soils 18: 209-216.
Hara, Y. et al. 2009. Jpn. J. Soil Sci. Plant Nutr. 80: 241-249**.
Itani, T. et al. 2012. Jpn. Jour. Crop Sci. 81 (Extra issue 2): 66-67*.
Kamiji, Y. and Sakuratanri, T. 2011. J. Agric. Sci., Tokyo Univ. Agric. 56: 93-102.
Kato, H. 2008. JARQ 42: 231-236.
Keeney, D.R. 1982. In F.J. Stevenson ed., Nitrogen in Agricultural Soils. Agron. Monogr. 22 ASA, CSSA, and SSSA, Madison. Wisc. 605-649.
Kumazawa, K. 2002. Nutr. Cycl. Agroecosyst. 63: 129-137.
Kyaw, K.M. et al. 2005. Biol. Fertil Soils 42: 72-82.
Liu, J. et al. 2008. Plant Prod. Sci. 11: 271-277.
Matsushita, K. et al. 2011. Breed. Sci. 61: 86-92.
Matsushita, K et al. 2012. Bull. Natl. Agric. Res. Cent. West Reg. 11:1-13.
Ministry of Agriculture, Forestry and Fisheries (MAFF). 2013. Accessed 25 December 2014.
http://www.maff.go.jp/tokai/seisan/tikusan/siryou/pdf/urakawa
Myint, A.K. et al. 2011. Commun. Soil. Sci. Plant Anal. 42: 457-474.
Nakano, H. and Morita, S. 2009. Plant Prod.Sci. 12: 351-358.
Nakano, H. et al. 2011. Plant Prod. Sci. 14: 190-197.
Nakano, H et al. 2012. Crop Sci. 52:345-350.
Nakui, T. et al. 1988. Bull. Tohoku Natl. Agric. Exp. Stn. 78: 161-174**.
Nishikawa, T. et al. 2012. Plant Prod. Sci. 15: 284-292.
Numatungiro, S. et al. 1999. Agron. J. 91: 676-685.
Oji, Y. and Izawa, G. 1974. Jpn. J. Soil Sci. Plant Nutr. 45: 341-351*.
Ookawa, T. et al. 2010. Plant Prod. Sci. 13: 58-66.
Ooya, M. et al. 2013. Jpn. J. Soil Sci. Plant Nutr. 84: 437-446.
Riya, S. et al. 2012. Kagaku Koukagu Rombunshu (The Society of Chemical Engineers, Japan) 38: 290-298**.
Sahu, S.K., and Samant, P.K. 2006. Orissa Review. December 2006, India: 34-36.
Sakai, M. et al. 2003. Breed. Sci. 53: 271-275.
Shigeuchi, Y. et al., 2013. Rep. Chugoku Branch Crop Sci. Jpn. 53: 27-28*.
Wada, G. and Cruz, P.C.S., 1989. Jpn. J. Crop. Sci. 58: 732-739.
Wada, G. et al. 1991. Jpn. J. Crop. Sci. 60: 101-106.
Yoshida, N. 2008. Beef Cattle 11: 22-23. In Japanese translated by JAICAF.
Zhou, S and Hosomi, M. 2008. Ecol. Eng. 32: 147-155.
Zhou, S. et al. 2005. J. Jpn. Soc. Water Environ. 28: 697-703**.
Zhou, S. et al. 2011. J. Chem. Eng. Jpn. 44: 713-719.

* In Japanese.
** In Japanese with English summary.