Nitrogen Uptake by the Rice Plant and Changes in the Soil Chemical Properties in the Paddy Rice Field during Yearly Application of Anaerobically-Digested Manure for Seven Years

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Abstract: This study investigated the changes in the chemical properties of the plow soil during a 7-year period with yearly application of anaerobically-digested manure (ADM), and the effect of the different ADM application methods on nitrogen (N) uptake by rice plants and the apparent N balance (input N minus output N). Among the treatments significant differences were observed in the pH, total N, and available N in the plow soil. The cumulative N uptake by rice plants in the ADM split application plots was significantly higher than that in single application plots. Consequently, the apparent N balance (input N minus output N) in the single ADM application plots was more positive. This result suggests that split application of ADM is more favorable for N uptake by rice plants. Although the apparent N balance was negative in the plots without N fertilizer, with chemical N and with low ADM, there was no observable decline in the total N and available N in the plow soil. This result implies that input and output of N in these plots were well balanced by additional N supply other than fertilization. On the other hand, the apparent N balance during the 7-year study under standard and heavy application of ADM was positive, indicating the preservation of soil fertility.

Key words: Digested manure, Long-term experiment, Method of fertilizer application, Nitrogen, Paddy rice, Soil fertility.

The methane fermentation process is an effective method for treating organic wastes because the process also generates “biogas” that contains approximately 60% methane. In Japan, large biogas plants for organic waste, such as animal manure, food waste and sewage sludge, have been in operation since the 1990s. The anaerobically-digested manure (ADM) from a biogas plant, which is a liquid by-product of the fermentation process, contains several kinds of nutrients that are essential for plant growth. In European countries, ADM as well as other anaerobic digestate have been used as liquid fertilizer for pasture grass and upland crops. We previously investigated the feasibility of ADM application in paddy rice cropping and found that ADM can be used as an alternative to chemical fertilizers (Nishikawa et al., 2012).

Reduction in environmental load such as application of excess fertilizers and agrochemicals under agricultural production has been recently suggested all over the world for sustainable agriculture (Horrigan et al., 2002; Spiertz, 2010). In paddy rice production, the appropriate nutrient application, improvement of nutrient efficiency, utilization of organic materials instead of chemical fertilizers, and so on are possible choices for the sustainable agriculture. The application of ADM to arable land contributes to the utilization of organic material. In Japan, the amount of organic material applied to the fields has been declining (MAFF, 2009). It is essential to apply organic material corresponding to approximately 10 – 15 tons ha\textsuperscript{-1} as straw compost in order to maintain soil fertility (MAFF, 2008). However, instead of this organic application, rice straw is chopped and returned to the paddy field during the harvesting operation, which somewhat contributes to the preservation of soil fertility. Rice straw, however, is often retrieved for pasture and bedding materials for livestock or mulching material for other agricultural production. In these situations, where there is no application of organic...
matter, the decline in soil fertility is inevitable. Unlike the application of chemical fertilizers, the application of ADM can aid soil fertility because it contains a certain amount of organic matter.

The assessment of nutrient flow, especially nitrogen (N) and its balance (input N minus output N), aids in the comprehension of changes in soil fertility and environmental impacts by fertilization. However, it is difficult to discuss the effects of ADM on soil fertility from the results of short-term experiments because the associated changes in soil fertility are often small. Most studies on N loss following the application of ADM in paddy fields have been conducted only over year-long periods, and/or only using excess application rates (Li et al., 2003; Hou et al., 2007; Sunaga et al., 2009). Experiments on the long-term N balance at relatively normal N application rates of ADM have been scarcely reported.

The objectives of this study are to evaluate changes in the plow soil chemical properties under yearly application of ADM and to evaluate the effects of different application methods of ADM on N uptake by rice plants and N balance.

Materials and Methods

1. Description of the experimental site and treatments

The field experiment commenced in 2002 and ADM has been applied every year for 7 yr. The experimental site is a paddy field in the Experimental Farm of Kyoto University, located in the Osaka Prefecture. The soil at the site was characterized as a sandy gray lowland soil, classified as a Gleysol according to the Food and Agriculture Organization of the United Nations (FAO’s) soil taxonomy, with a particle composition of 68.4% sand, 17.5% silt, and 14.1% clay. The N treatments were as follows: no application of N (NF), application of chemical N (CF) and application of ADM (MF). Inorganic N was applied every year at the following rates: 0 g m⁻² in NF, 10 g m⁻² as CF (designated as CF-10) and 5, 10, 15 and 20 g m⁻² as MF (designated as MF-5, MF-10, MF-15 and MF-20), respectively. In this study, two methods of N application were employed; single application (only basal application) and split application (basal N, 70%; topdressing N, 30%). In total, eleven treatments with four replicates per treatment were arranged in the paddy field in a randomized block design. Each plot area was approximately 20 m² (5 m by 4 m) and plots were separated with plastic sheets to avoid cross-contamination of nutrients among plots. The rice variety Hinohikari, widely cultivated throughout southwestern Japan, was used in this study. The recommended N application rate for cultivation of this variety around the region where the study was undertaken is approximately 10 g m⁻².

In the MF plots, ADM from the Nantan City Yagi Bioecology Center, Nantan city, Kyoto Prefecture, was used. Dairy cattle excreta accounted for 75–80% of the organic waste (in weight) treated at this biogas plant. Discarded milk and soybean curd refuse (okara) from food factories around the biogas plant were also disposed together. These organic wastes were fermented under thermophilic condition (55°C), for approximately 33 d. The major nutrients in the ADM (average values over 7 yr) are as follows: NH₄⁻N, 1.88 g L⁻¹; P₂O₅, 0.56 g L⁻¹; and K₂O, 2.33 g L⁻¹. Total N in the ADM was 2.80 g L⁻¹, while NO₃⁻-N was 0.012 g L⁻¹, being negligibly small. The ADM contained undigested solid organic matter, and its average concentration (over 7 yr) before application was 48.7 g L⁻¹. Ammonium sulfate ((_NH₄)_₂SO₄) was applied as the N source in the CF plots. In both NF and CF plots, P and K (chemical fertilizer) were applied as a basal application, at an application rate of 10 g m⁻² as P₂O₅ and K₂O, respectively. On the other hand, annual application rate of P₂O₅ and K₂O in MF-10 plots was 3.0 and 12.4 g m⁻², respectively.

2. Field management during the rice growing period

In mid-June, three rice seedlings per hill were transplanted at a planting density of 33 cm by 21 cm (14.4 hills m⁻²). A few days after transplanting, the basal application of ADM and chemical fertilizers was conducted. This was followed by topdressing at the end of July; approximately 25 d before the heading of rice. All the experimental plots were kept submerged at 3–5 cm water depth until the heading stage. Intermittent irrigation was then conducted until the maturation stage. Pest management practices were conducted in accordance with neighboring farmers’ practices. In yearly harvest operation, all the rice plant biomass, excluding the stubble and underground portions, was removed from the field, which was then kept fallow until the next cropping season.

3. Plant and soil sampling and analysis

(1) Plant biomass sampling and analysis

Every year, plant samples were collected at maturation stage, corresponding to approximately 120 d after transplanting. In each plot, 15–20 hills of rice showing average growth were harvested. Approximately a quarter of each sample was used to measure N content, while the rest of it was used to determine grain yield (in this study yield data is not shown). Samples were dried at 80°C for 48 hr and then ground to pass through a 2 mm sieve. Total N content in these ground samples was measured colorimetrically following H₂SO₄-H₂O₂ wet digestion, as described by Mizuno and Minami (1980).

(2) Soil sampling and analyses

In all the treatments soil the plow layer (0–15 cm depth) was sampled before transplanting in 2002 and 2009, the first and eighth year of the experiment. These
soil samples were air-dried, ground to pass through a 2 mm sieve and stored at room temperature under dark conditions until analysis.

Soil pH was measured with a pH meter. In measuring the pH, the ratio of soil to deionized water was adjusted to 1 : 2.5 (in weight). Total N and carbon (C) content were measured by the dry combustion method using a CN coder (Fisons, EA1108). Available N content was determined by the anaerobic soil incubation method described by Yoshino and Dei (1978). This N was calculated from the difference between the extracted NH$_4$-N content before and after anaerobic incubation of the soil samples at 30°C for 4 wk, and the NH$_4$-N content before incubation was defined as “initial ammonium N”. The concentration of NH$_4$-N extracted with 10%-KCl solution was determined using the indophenol-blue method.

### 4. Statistical analysis

Statistical analysis was performed using the software STATISTICA for Windows ver. 5.0j (StatSoft Inc., Japan). Analysis of covariance (ANCOVA) was used to detect significance between regression coefficients. Fisher’s least significant difference (LSD) test was used to detect significant difference among treatments.

### Results

#### 1. Changes in soil properties

Changes in soil pH, total C, total N and C/N ratio in the plow layer soil from 2002 to 2009 are shown in Table 1. In 2002, the soil pH values in all the treatments fell within a relatively narrow range, from 6.25 to 6.40. On the other hand, in 2009, soil pH significantly declined in all the treatments and the difference in the soil pH among treatments became wider, from 5.58 to 5.87. The difference in the soil pH between 2002 and 2009, shown as “Δ” in the table, tended to be larger in CF treatments than in NF and MF treatments.

| Treatments | pH(H$_2$O) | Total C g kg$^{-1}$ | Total N g kg$^{-1}$ | C/N ratio |
|------------|------------|---------------------|---------------------|------------|
|            | 2002  | 2009 | Δ   | 2002  | 2009 | Δ   | 2002  | 2009 | Δ   |
| NF         | 6.27  | 5.81 | -0.46 a | 22.3  | 25.4 | 3.1 a | 1.95  | 2.14 | 0.19 b | 11.4  | 11.8 | 0.4 a |
| CF10-0     | 6.28  | 5.59 | -0.69 e | 21.4  | 24.5 | 3.1 a | 1.97  | 2.12 | 0.15 b | 10.9  | 11.5 | 0.6 a |
| CF7-3      | 6.27  | 5.58 | -0.69 e | 19.3  | 22.2 | 2.8 a | 1.76  | 1.97 | 0.21 b | 11.0  | 11.2 | 0.2 a |
| MF5-0      | 6.30  | 5.80 | -0.50 abc | 18.7  | 22.2 | 3.5 a | 1.70  | 1.97 | 0.28 b | 11.1  | 11.3 | 0.2 a |
| MF3.5-1.5  | 6.33  | 5.72 | -0.61 de | 21.1  | 26.2 | 5.1 a | 1.92  | 2.28 | 0.36 ab | 11.0  | 11.5 | 0.5 a |
| MF10-0     | 6.25  | 5.74 | -0.51 abcd | 19.8  | 24.2 | 4.4 a | 1.84  | 2.15 | 0.31 ab | 10.8  | 11.2 | 0.5 a |
| MF7-3      | 6.40  | 5.85 | -0.55 abcd | 20.2  | 24.6 | 4.5 a | 1.85  | 2.14 | 0.29 ab | 11.0  | 11.5 | 0.6 a |
| MF15-0     | 6.36  | 5.76 | -0.61 de | 22.9  | 29.1 | 6.2 a | 2.08  | 2.46 | 0.38 ab | 11.1  | 11.8 | 0.7 a |
| MF10.5-4.5 | 6.30  | 5.77 | -0.54 abcd | 23.5  | 27.9 | 4.4 a | 2.09  | 2.39 | 0.30 ab | 11.6  | 11.7 | 0.1 a |
| MF20-0     | 6.36  | 5.87 | -0.49 ab  | 22.5  | 27.8 | 5.3 a | 2.05  | 2.32 | 0.27 b  | 11.0  | 11.9 | 0.9 a |
| MF14-6     | 6.32  | 5.73 | -0.59 bcde | 20.7  | 26.8 | 6.1 a | 1.91  | 2.46 | 0.55 a  | 10.8  | 10.9 | 0.1 a |

Numbers after kinds of fertilizer (CF and MF) denote annual application rate of inorganic N (g m$^{-2}$) as basal application and topdressing application, respectively. In the “Δ” columns, values denote subtraction of the value in 2002 from that in 2009, and values with different letters are significantly different at $p < 0.05$ by Fisher’s LSD test.
and CF treatments, because growth of the rice plants was poor due to a severe cold summer. Regardless of application method of N, the N uptake in CF-10 plots was 14 – 40% larger than in the MF-10 plots in 2003 and 2004. The difference in N uptake between MF-10 and CF-10 in these 2 yr affected the difference in total N uptake for 7 yr. Rice plants under split N application tended to take up more N than those under single application, both in MF and CF.

The cumulative N input by fertilization, cumulative N output by rice plants and apparent N balance (input N minus output N) during the 7 yr of experimental treatment are shown in Table 3. For the standard N plots (CF-10 and MF-10), N input in the MF-10 plots was approximately 1.5 times higher than that in the CF-10 plots because 34.3 g m$^{-2}$ of organic N as well as 70.0 g m$^{-2}$ of ammonium N has been applied together. However, the cumulative N uptake by rice plants was less in the MF-10 treatments than that in the CF-10 treatments, irrespective of the application method. Although the amount of the cumulative N absorbed by rice plants related to the application N rate, more N was utilized by rice plants in split application than in single application, regardless of the fertilizer used. In the MF plot, the difference in the N absorbed between application methods (single application and split application) was larger in high N application treatments (MF-15 and MF-20) than in low and standard N application treatments (MF-5 and MF-10).

The apparent N balance in the NF, CF-10 and MF-5 treatments was negative, whereas the value was positive for the MF-10, 15 and 20 treatments. The most negative N balance was observed in NF plot: 51.9 g m$^{-2}$ of N was taken out from the field during 7 yr of the experiment. On the other hand, the most positive N balance was observed in the MF20-0 plot, where 115.0 g m$^{-2}$ of N was apparently remained. In MF-5, MF-15 and MF-20, the values of the apparent N balance in the split application plots were significantly larger than in the corresponding single application plots.

### Table 2. Changes in mineral N property of plow soil at the first and eighth year (2002 and 2009) of the experiment.

| Treatments   | Initial ammonium N mg kg$^{-1}$ | Available N mg kg$^{-1}$ |
|--------------|---------------------------------|--------------------------|
|              | 2002 | 2009 | Δ    | 2002 | 2009 | Δ    |
| NF           | 4.8  | 5.3  | 0.5 c | 105.3| 108.4| 7.9 c |
| CF10-0       | 3.6  | 6.3  | 2.7 bcd| 92.0 | 123.7| 35.2 ab|
| CF7-3        | 4.1  | 5.9  | 1.9 bcd| 84.7 | 112.9| 32.3 ab|
| MF5-0        | 4.5  | 6.1  | 1.6 bcd| 87.1 | 111.1| 28.5 bc|
| MF3.5-1.5    | 4.7  | 9.7  | 5.0 a  | 95.3 | 125.7| 35.2 ab|
| MF14-6       | 4.1  | 7.6  | 3.5 abc| 94.2 | 143.3| 53.3 a |
| MF10-0       | 2.2  | 5.9  | 3.7 ab  | 92.2 | 131.9| 41.8 ab|
| MF7-3        | 4.6  | 6.7  | 2.1 bcd| 87.6 | 116.6| 33.5 ab|
| MF15-0       | 5.4  | 8.5  | 3.0 abc| 97.8 | 117.2| 24.8 bc|
| MF10-5-4.5   | 4.9  | 6.2  | 1.3 cde| 102.1| 131.4| 34.2 ab|
| MF20-0       | 4.1  | 6.4  | 2.3 bcd| 94.8 | 117.7| 27.0 bc|
| MF14-6       | 4.1  | 7.6  | 3.5 abc| 94.2 | 143.3| 53.3 a |
| MF10.5-4.5   | 4.9  | 6.2  | 1.3 cde| 102.1| 131.4| 34.2 ab|
| MF15-0       | 5.4  | 8.5  | 3.0 abc| 97.8 | 117.2| 24.8 bc|
| MF14-6       | 4.1  | 7.6  | 3.5 abc| 94.2 | 143.3| 53.3 a |

For abbreviations see Table 1.

Fig. 1. Yearly nitrogen uptake by rice plants at maturation stage. For abbreviations see Table 1.
3. Correlation of N input with N balance and soil chemical properties

Fig. 3 shows the correlation of the amount of total N application with the difference between 2002 and 2009 in total C, total N and C/N ratio of the plow layer soils. The difference in total C positively related to the amount of total N application: the correlation coefficient including all the treatments was \( r = 0.701 \) (\( p < 0.05 \)). This trend was also seen in MF treatments alone: \( r = 0.550 \), though not significant at \( p < 0.05 \). However, this trend was not significant in MF treatments alone: \( r = 0.418 \). The relationship between the amount of total N application and the difference in C/N ratio between 2002 and 2009 was not clear: \( r = 0.099 \).

The relationship between the cumulative amount of total N application (inorganic- and organic-N) and cumulative N uptake by rice plants is shown in Fig. 2A. In MF, the regression equations in single application and split application are as follows,

- **Single application:** \( y = 0.224x + 49.4 \) (\( R^2 = 0.948 \))
- **Split application:** \( y = 0.295x + 49.1 \) (\( R^2 = 0.991 \))

Where, \( x \) and \( y \) denote cumulative application N (g m\(^{-2}\)) and cumulative N uptake by rice plants (g m\(^{-2}\)), respectively. The regression coefficient of the equation for the split application of MF was significantly larger than that for the single application at \( p < 0.05 \).

Similarly, the relationship between the cumulative amount of total N application and the cumulative apparent N balance is shown in Fig. 2B. In MF, the regression equations in single application and split application are as follows,

- **Single application:** \( y = 0.776x - 49.4 \) (\( R^2 = 0.995 \))
- **Split application:** \( y = 0.705x - 49.1 \) (\( R^2 = 0.998 \))

Where, \( x \) and \( y \) denote cumulative application N (g m\(^{-2}\)) and apparent N balance (g m\(^{-2}\)), respectively. The regression coefficient of the equation for the single application of MF was significantly larger than that for the split application at \( p < 0.05 \).

For abbreviations see Table 1. In the “N balance” column, difference between application methods was compared. **, * and n.s denote significant at \( p < 0.01 \), significant at \( p < 0.05 \) and not significant, respectively.

The correlation of the apparent N balance and the difference between initial ammonium N and available N content in the plow layer soil (between 2002 and 2009) are shown in Fig. 4. The relationships between the apparent N balance with the difference of both the initial ammonium N and available N were not significant at \( p = 0.05 \): \( r = 0.225 \) and 0.444, respectively. Similarly, these relationships were not significant when only the values in the MF plots were considered: \( r = -0.203 \) and 0.134, respectively.
In paddy soils, organic matter is more liable to accumulate than in upland soils due to the submerged conditions during the growth period of rice plants (Kyuma, 2004). With respect to the long-term utilization of organic materials, changes in soil fertility are an important issue. ADM contains a considerable amount of organic N that results from undigested organic matter and dead bacterial cells, as well as inorganic form N. In order to assess the behavior of N that is applied to agricultural crops, it is necessary to examine the balance between input and output of N.

In this study, the effects of yearly application of ADM for 7 yr on plow soil were unclear, irrespective of the application rates of ADM (Table 1). This result is different from that obtained in the long-term experiments on application of compost and manure with relatively high C/N ratio; higher than 15. However, the application of chemical N fertilizer tended to reduce soil pH. It seems that this reduction in soil pH is due to the yearly application of ammonium sulfate defined as a potentially acidic fertilizer, because the degree of pH decline in the NF plots was significantly smaller than in the CF plots. Because the appropriate soil pH for paddy rice is from 5.5 to 6.5 (JSA, 2011), the soil pH measured in 2009 is still within the normal range for all the treatments. However,

Fig. 3. Correlation of total amount of N application with (A) total C content, (B) total N content and (C) C/N ratio in the plow soils (0 – 15 cm depth) between 2002 and 2009.
Total N application denotes sum of annual input of inorganic N and organic N. Bars denote standard error (n = 4).

Fig. 4. Correlation of apparent N balance with the difference in (A) initial ammonium N and (B) available N of the plow soils (0 – 15 cm depth) between 2002 and 2009.
the soil pH in the CF treatment plots may fall below 5.5 if chemical N application is repeated.

The increase in total C in the plow soil was related to the cumulative amount of N. Since soil total C in the NF and CF plots also increased by approximately 3 gkg⁻² during the 7 yr of rice production (Table 1, Fig. 3A), the presence of the other factors that increased soil C is suggested. However, in MF plots, more soil C, 4.8 g kg⁻² on the average, was accumulated. This result implies that C input by application of ADM. In particular, the larger increase in total C observed in the high application MF treatment plots implies that there is preservation of the soil fertility. In addition, the change in soil total C generally relates to the change in soil total N. In fact, in this study, the increase in soil total C strongly related to that in soil total N: r = 0.844 (p < 0.01). Therefore, in the plots with a greater increase in total C, the N supply from the plow soil during the rice growth period can increase, which also means that it is necessary to decrease the N application rate to avoid excess N absorption by rice plants which increases the risk of lodging and pest outbreak. On the other hand, the relationship between the available N content and apparent N balance was not significantly positive (Fig. 4). This result is reasonable because the value of available N relates to quickly-decomposable soil organic N, rather than slowly-decomposable soil organic N. Gutser et al. (2005) have found that the degradability of organic matter in biogas slurry (equivalent to ADM) is small.

In the NF plot, 51.9 g m⁻² of N was taken out of the field during the 7 yr of this experiment (Table 3). This N seems to be “natural supply” from soil N mineralized, N contained in irrigation water and biological N fixation (Yoshida et al., 1973; Ohta et al., 1986). This is an adequate value because the average amount of N as natural supply in Japan has been estimated to be 5 – 8 g m⁻² yr⁻¹ (Yanagisawa and Takahashi, 1964). The NF plots may have a considerable amount of N from origins other than the paddy soil because the decline of total N and available N content of soil was small (Table 1). In the CF-10 and MF-5 plots that showed negative N balance, N supply other than applied N also contributed to the growth of the rice plants and the maintenance of soil fertility.

Generally, N fertilizer is applied using a split application method to improve efficiency of N utilized by rice plants. This is because inorganic N is liable to escape from the paddy soil into the environment as gaseous N₂ by denitrification, as nitrate by leaching and run off and sometimes as ammonia by volatilization. Therefore N efficiency of basal fertilizer N is generally lower than that of topdressing fertilizer N because of low N absorption capacity of rice plants during early growth stage. In this study, there was a significant difference in the cumulative N uptake by rice plants between the different application methods of ADM (Figs. 1, 2A). Furthermore, the cumulative N uptake by rice plants in MF-10 plots was less than that in the corresponding CF-10 plots. The sandy soil in the experimental paddy field and the temporal growth stagnation from application of basal fertilizer to active tillering stage of rice plants observed in the MF plots (Nishikawa et al., 2013) would result in the lower N efficiency. As in the case of chemical N application, it is more appropriate to apply split fertilization method in using ADM for improving applied N efficiency.

The apparent N balance in single application MF plots is higher than that in split application plots because more N has been absorbed by rice plants in the split application plots (Table 1, Fig. 3A). If the unutilized inorganic N was successively converted into organic N, more accumulation of N into the soil would be possible in the single application plots. However, a significant increase in the soil total N in the single application plots of MF was not observed (Table 1). Furthermore, the relationships between the apparent N balance and the increase in N in the MF plots was not significant (Fig. 4). In this study, the net N balance and the amount of N loss by vertical movement as nitrate cannot be considered because of the absence of available data. After this study, Izawa et al. (2011) investigated the chemical properties of deeper subsoil (12 – 60 cm depth) in NF, CF10-0, MF10-0 and MF200 plots, but significant changes in the subsoil among plots was not observed. Although the possibility remains that unutilized N leached out to much deeper soil layers, it seems more likely that a part of the unutilized N has been lost by denitrification and/or ammonia volatilization.

We previously suggested that difference in grain yield between MF-10 and CF-10 plots was not significant (Nishikawa et al., 2012). Results of this study implied that the application rate of ADM corresponding to the same inorganic N as in chemical fertilizer N led to the same or slightly lower N uptake by rice plants. In using ADM as liquid fertilizer, it is necessary to consider its chemical properties. In particular, most of the inorganic N within ADM is ammonium N, and the pH of ADM is often weakly alkaline, indicating the possibility of ammonia volatilization. To gain the same N uptake as CF-10 treatments, it may be necessary to apply ADM corresponding to 11 – 12 g m⁻² of ammonium N application (Fig. 2A). Furthermore, appropriate application rate and more topdressing-emphasized application of ADM as well as split application mentioned above seem to be practical measures to minimize the N loss.

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** In Japanese with English summary.
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