Temporal Growth Inhibition of Rice Plant and Growth Recovery Observed under Application of Anaerobically-Digested Cattle Manure

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Abstract: A field experiment assessing the effect of the annual application of anaerobically-digested cattle manure (ADM), produced at a biogas plant, on paddy rice was conducted. In plots with ADM (MF), the early growth of rice plants, from transplanting to the active tillering stage, was inhibited compared to the plots with chemical fertilizers (CF). This phenomenon was observed over many growing seasons and was especially obvious in nitrogen uptake and leaf area index (LAI). However, after panicle initiation, the growth of MF-treated plants was equal to or superior to CF-treated plants. The grain yield in all the MF plots was 96 – 105% of that in the CF plots. The inorganic nitrogen content of the soil in the MF plots was higher than that in the CF plots, which was contradictory to the growth inhibition observed in the initial growth of plants in the MF plots. In contrast, the oxidation / reduction potential and pH of the surface soil in MF plots were within the normal range, indicating that these soil factors were not associated with growth inhibition observed in MF plots. Our results implied that rice cultivars with a long growing period that are able to recover from the initial growth inhibition, such as medium or late maturing cultivars, are suitable for paddy rice production fertilized with ADM.

Key words: Digested manure, Early stage growth, Nitrogen, Organic waste, Paddy rice.

Anaerobic digestion of organic waste by large-scale biogas plants has attracted much attention since the 1990s. In western countries, such as Germany and Denmark, anaerobically-digested manure (ADM) has been considered a useful organic fertilizer and has been applied to grassland and upland crops in compliance with strict environmental standards (Angelidaki and Ellegaard, 2003; Loria et al., 2007; Holm-Nielsen et al., 2009). Similarly, in Japan ADM has been utilized in various horticultural situations (Miyata et al., 2005; Tokuda et al., 2010). However, there are few examples of ADM application to paddy fields. In Japan, actually, total acreage of the paddy field supplied with ADM is estimated to be only hundreds of hectares. Lack of the practice case has made the popularization of anaerobic digestion systems in Asian countries difficult.

The effects of ADM on rice cropping in a single season have been reported previously (Li et al., 2003a). The same experiment was continued from 2002 to 2009 to evaluate the long-term changes in both the soil and rice plant growth. The results revealed that the effects of ADM on plant growth and yield were similar to those of chemical fertilizer at standard levels of N application. Furthermore, changes in soil organic matter were relatively small (Nishikawa et al., 2012) and the effects of application of ADM on methane emissions and heavy metal input was also small (Li et al., 2003b; Nitta et al., 2009). However, a temporal growth inhibition by the ADM treatment was observed during the eight year experiment. Inhibition of initial growth by ADM has been shown to cause a serious yield decline under some cropping conditions depending on soil type, rice cultivar, fertilization and water management (Watanabe et al., 2011). It is therefore important to elucidate the growth-inhibiting factors for...
promoting normal growth and grain yield of rice plants supplied with ADM as a fertilizer.

The objectives of the present study were to characterize the specific growth pattern of paddy rice supplied with ADM and to reveal at which point of the plant growth the inhibition begins. In addition, the changes in soil chemistry throughout the growing period were assessed in an attempt to determine the cause of the growth inhibition by ADM application.

Materials and Methods

1. Description of experimental site

The study was conducted from 2002 to 2009 in a paddy field at the Experimental Farm of Kyoto University, located in Takatsuki city, Osaka Prefecture. In this paper, results of seven years of the experiment (from 2002 to 2008) were used. The soil was classified as a sandy gray lowland soil with the following particle composition: sand 68.4%; silt 17.5% and clay 14.1%. The soil bulk density was 0.86 ± 0.05 g cm⁻³. The depth of water loss during the submerged period was less than 1 cm d⁻¹. Other details on the experimental site were as reported previously (Nishikawa et al., 2012).

2. Treatments and fertilizers used

Five treatments were given, namely: non-application of N (NF), application of chemical fertilizer N (CF), and application of ADM (MF) at three different application rates. The N application rates were as follows: NF, 0 g m⁻²; CF, 10 g m⁻² (CF-10); and MF, 10, 15 and 20 g m⁻² (MF-10, MF-15 and MF-20). The application of 10 g m⁻² inorganic N was the standard application rate. Two application methods were used, namely, single application (basal : topdressing = 10:0) and split application (basal : topdressing = 7:3). Each plot was arranged within the paddy field according to a randomized blocks design with four replications. Each plot area was approximately 20 m² (5 m × 4 m), and during the growing season the plots were separated by plastic sheets to avoid the cross contamination of nutrients.

Ammonium sulfate ((NH₄)₂SO₄) was used as the N source in the CF plots, and ADM obtained from the Nantan City Yagi Bioecology Center located in Nantan city, Kyoto Prefecture was used in the MF plots. Dairy cattle excreta accounted for 75 – 80% of the organic waste (by weight) treated at this biogas plant. The composition of major nutrients in the ADM (average values over seven years) was as follows: NH₄-N, 1880 mg kg⁻¹; P₂O₅, 560 mg kg⁻¹; and K₂O, 2330 mg kg⁻¹. The content of NO₃-N within the ADM was negligible. Although ADM is often referred to as a “liquid fertilizer”, it does contain a certain amount of undigested organic matter, in this case 48.7 g kg⁻¹. We did not measure the C/N ratio of ADM used in this study, Iwashita and Iwata (2010) reported the ratio of ADM from this biogas plant to be 4.2. The correct application amount of ADM was determined annually by measuring the NH₄-N content. In the NF and CF plots, 10 g m⁻² each of P₂O₅ and K₂O (chemical fertilizer), were applied as basal application. In 2007 and 2008, 15% of ADM was replaced by corresponding amount of chemical fertilizer N in all the MF plots. This was done to evaluate the effects of a decrease in the amount of ADM applied. During this period the other experimental conditions were maintained as in previous years.

3. Field management during the growing period

In mid-June, three rice seedlings per hill were transplanted with a spacing of 33 cm by 21 cm (14.4 hills per square meter). The rice variety used was “Hinohikari”, a medium maturing variety widely planted in the warmer regions of Japan. The basal application of ADM and chemical fertilizer was made a few days after transplanting (DAT), and the topdressing was applied at the end of July, corresponding to approximately 50 DAT, approximately 25 d before heading. All the experimental plots were kept submerged at a depth of 3 – 5 cm until the heading stage, after which intermittent irrigation was implemented. The pest management was in accordance with the overall management of the farmland around the experimental site.

4. Sampling and analysis

(1) Plant sampling and analysis

Plant samples were collected annually at six growth stages, namely: active tillering, panicle initiation, reduction
division, heading, middle ripening and maturing stage. Details of the sampling are summarized in Table 1. Some of the data on plant growth are missing. The sampling at the maturing stage was conducted with four replications, but the sampling at other stages with three replications because of the limited size of the plot areas. The aboveground parts of the plant were collected from six hills in each plot that exhibited average growth. The leaf blades were separated from the other plant parts and the leaf area index (LAI) was measured using an automated area meter (AAM-9, Hayashi Denko Co. Ltd., Japan) before the leaves curled due to water loss. The entire samples were then dried at 80ºC for 48–72 hr to measure their dry biomass. The samples were ground and passed through a 2 mm sieve before N analysis, which was measured colorimetrically after H2SO4-H2O2 wet digestion as described by Mizuno and Minami (1980).

At the maturing stage, 15–20 hills of rice exhibiting average growth were harvested from all the experimental plots. After measuring total fresh weight and number of panicles, each sample was divided into two portions, the first for N analysis and the second for grain yield survey. The procedure for measuring dry weight and N analysis were the same as mentioned above. For the yield survey, threshed grains were separated into fully-ripened grains and other grains using a 1.06 g mL\(^{-1}\) saline solution. The grain yield was defined as the weight of fully-ripened brown rice at a water content of 15.5%.

(2) Soil sampling, measurements in the field, and analysis

The soil containing the applied N source was incubated for a long period to evaluate inorganic N content of the soils in the MF, CF and NF plot. In this experiment, soil samples were collected before transplanting in 2008. In each plot three soil subsamples were collected from the cultivated layer (0–15 cm depth) and mixed well. Samples were then passed through a 2 mm sieve and stored in a wet condition (moisture content 30–40%) at a low temperature. Before incubation, ADM or ammonium sulfate was added to each soil sample according to the actual application rate of N. These samples were then incubated by the standing incubation method under water-saturated conditions at 30ºC for 1–16 wk according to the protocol of Yoshino and Dei (1977).

To relate the results obtained by soil incubation to those in field conditions, we measured the temperature of the

| Year       | Treatment | Active tillering | Panicle initiation | Reduction division | Heading | Middle ripening | Maturing |
|------------|-----------|------------------|--------------------|--------------------|---------|-----------------|----------|
| 2002-2006  | NF        | 55 (0.67)        | 225 (0.70)         | 452 (0.74)         | 699 (0.79) | 946 (0.81)      | 1143 (0.82) |
|            | CF10-0    | 87               | 349                | 621                | 887     | 1173            | 1586     |
|            | MF10-0    | 73 (0.85)        | 284 (0.83)         | 588 (0.94)         | 833 (0.94) | 1098 (0.94)    | 1357 (0.98) |
|            | MF15-0    | 73 (0.89)        | 270 (0.92)         | n.a                | 846 (1.01) | n.a             | 1584 (1.00) |
|            | MF20-0    | 72 (0.88)        | 264 (0.90)         | n.a                | 839 (1.00) | n.a             | 1457 (1.05) |
|            | MF7-3     | 65 (0.88)        | 277 (0.98)         | 557 (0.93)         | 817 (0.93) | 1091 (0.94)    | 1354 (0.97) |
|            | MF10.5-4.5 | 72 (0.97)    | 263 (1.05)         | n.a                | 849 (0.98) | n.a             | 1443 (1.04) |
|            | MF14-6    | 72 (0.97)        | 284 (1.14)         | n.a                | 916 (1.06) | n.a             | 1426 (1.03) |
| Average 2002-2006 | NF | 61 (0.72) | 206 (0.70) | n.a               | 756 (0.79) | n.a             | 1137 (0.76) |
|            | CF10-0    | 89               | 291                | n.a                | 956     | n.a             | 1488     |
|            | MF10-0    | 77 (0.86)        | 291 (1.00)         | n.a                | 969 (1.01) | n.a             | 1495 (1.00) |
|            | MF15-0    | 76 (0.92)        | 293 (1.07)         | n.a                | 944 (0.94) | n.a             | 1508 (1.01) |
|            | MF20-0    | 66 (0.80)        | 254 (0.92)         | n.a                | 1010 (1.01) | n.a             | 1559 (1.05) |
|            | MF7-3     | 79               | 301                | n.a                | 963     | n.a             | 1484     |
|            | MF10.5-4.5 | 77 (1.07)    | 290 (1.01)         | n.a                | 1013 (1.03) | n.a             | 1575 (1.05) |
|            | MF14-6    | 72 (1.01)        | 337 (1.17)         | n.a                | 1086 (1.11) | n.a             | 1574 (1.05) |
| Average 2007-2008 | NF | 61 (0.72) | 206 (0.70) | n.a               | 756 (0.79) | n.a             | 1137 (0.76) |
|            | CF10-0    | 89               | 291                | n.a                | 956     | n.a             | 1488     |
|            | MF10-0    | 77 (0.86)        | 291 (1.00)         | n.a                | 969 (1.01) | n.a             | 1495 (1.00) |
|            | MF15-0    | 76 (0.92)        | 293 (1.07)         | n.a                | 944 (0.94) | n.a             | 1508 (1.01) |
|            | MF20-0    | 66 (0.80)        | 254 (0.92)         | n.a                | 1010 (1.01) | n.a             | 1559 (1.05) |
|            | MF7-3     | 74 (0.92)        | 278 (0.92)         | n.a                | 893 (0.93) | n.a             | 1486 (1.00) |
|            | MF10.5-4.5 | 77 (1.07)    | 290 (1.01)         | n.a                | 1013 (1.03) | n.a             | 1575 (1.05) |
|            | MF14-6    | 72 (1.01)        | 337 (1.17)         | n.a                | 1086 (1.11) | n.a             | 1574 (1.05) |

“n.a” denotes “no data available”. Values in parentheses denote the ratio to those in CF treatments. ** and n.s denote significant at \(p < 0.01\) and not significant, respectively. In 2007 and 2008, approximately 15% of ADM was replaced with a corresponding amount of chemical fertilizer (ammonium sulfate). Values shown in italics denote that they have some missing values (see Table 1). When there are some missing values on MF treatments, corresponding values in the CF plots were excluded from calculation of the relative ratio.
top soils with temperature data loggers (TR-52A, T&D Corporation, Japan). Then, the effective cumulative soil temperature (ECST) was calculated as follows:

\[ T_A = \Sigma(T - 15) \]

Where, \( T_A \) and \( T \) denote the ECST (°C) and daily mean temperature (°C), respectively.

It is known that application of organic materials to a paddy soil sometimes brings about marked soil reduction, and change in pH of the soil with organic materials is different from that of the soil with chemical fertilizer application. To grasp the difference in the soil chemical condition, the oxidation-reduction potential (ORP) and pH of the topsoil submerged approximately 5 cm in depth were recorded during the early growing stages of paddy rice. The ORP indicates the degree of reduced condition of submerged soils. Platinum electrodes and an AgCl comparison electrode connected to a portable soil meter (EHS-120 or RPN-41, Fujiwara Scientific, Japan) were used for the ORP measurements. The same soil meter was used for measuring soil pH in each plot by replacing electrodes with a pH electrode.

### Results

1. **Growth of rice plant and nitrogen uptake**

   The aboveground biomass of the rice plants in each treatment is shown in Table 2. In this paper, the results of the experiment are separately shown for two periods, the first period spans the five years from 2002 to 2006, when only ADM was applied in MF plots. In the second period, from 2007 to 2008, a part of the ADM in the MF plots was replaced by chemical N fertilizer. The biomass of the rice plants in the NF treatments was consistently lower than that in any of the fertilized treatments throughout the growing period.

   In the first period, 2002 – 2006, the plant biomass at the active tillering (AT) stage in the MF plots (both single and split applications) was 3–15% smaller than that in the CF plots. However, this trend persisted only until the AT stage but until the panicle initiation (PI) stage in the single application of ADM. At the maturing stage the plant biomass in the MF treatments
regardless of application rate and the experimental period although the difference of the number of stems between MF-10 and CF-10 was not significant. However, the difference in the number of stems between MF and CF plots at the PI stage was smaller with the exception of MF10-0 in the first period (2002–2006) and larger in MF-15 and MF-20 except MF20-0 in the second period (2007–2008). In the standard MF plots (MF-10), the number of panicles at the maturing stage was consistently 6–7% lower than the CF plots while the difference was not significant. The effects of higher rates of ADM application to increase eventual panicle number at the maturing stage were only seen in the split dressing treatments.

The leaf area index (LAI) in each treatment is shown in Table 4. The LAI values in the NF plot were 62–78% of those in the CF treatments. With the exception of the MF10.5-4.5 plot in 2007–2008, the LAI for all the MF treatments was lower than that for the CF plots at the AT stage, regardless of the rate of ADM application. The difference in LAI between MF-10 and CF-10 plots was significant. Furthermore, the lower LAI in the MF-10 plots relative to the CF plots persisted until the HD or middle ripening stage.

In the second period, 2007–2008, growth inhibition at the AT stage was again observed in all the MF plots, except the two plots with split dressing and the higher rate of ADM: MF10.5-4.5 and MF14-6. For these plots, the aboveground plant biomass at the AT stage was higher or comparable to that in the CF7-3 plot, although such a result was observed only in one of the two years. After this stage, plant biomass in the MF plots was larger than or comparable to that in the corresponding CF plot with some exceptions (MF7-3 and MF20-0 at the PI stage, MF7-3 and MF15-0 at the heading (HD) stage). Although some growth inhibition was observed in the both periods, difference of the plant biomass between MF-10 and CF-10 was not significant.

with a higher application rate (MF-15 and MF-20) was comparable to or slightly larger than that in the CF treatments. However that in the standard ADM treatments (MF-10) remained slightly smaller.

In the second period, 2007–2008, growth inhibition at the AT stage was again observed in all the MF plots, except the two plots with split dressing and the higher rate of ADM: MF10.5-4.5 and MF14-6. For these plots, the aboveground plant biomass at the AT stage was higher or comparable to that in the CF7-3 plot, although such a result was observed only in one of the two years. After this stage, plant biomass in the MF plots was larger than or comparable to that in the corresponding CF plot with some exceptions (MF7-3 and MF20-0 at the PI stage, MF7-3 and MF15-0 at the heading (HD) stage). Although some growth inhibition was observed in the both period, difference of the plant biomass between MF-10 and CF-10 was not significant.

The number of stems or panicles per square meter in each treatment is shown in Table 3. The values in the NF plot were on the average 74–82% of those in the CF treatments. At the AT stage, tillering was inhibited in all the MF treatments compared with the CF treatment regardless of application rate and the experimental period although the difference of the number of stems between MF-10 and CF-10 was not significant. However, the difference in the number of stems between MF and CF plots at the PI stage was smaller with the exception of MF10 in the first period (2002–2006) and larger in MF-15 and MF-20 except MF20-0 in the second period (2007–2008). In the standard MF plots (MF-10), the number of panicles at the maturing stage was consistently 6–7% lower than the CF plots while the difference was not significant. The effects of higher rates of ADM application to increase eventual panicle number at the maturing stage were only seen in the split dressing treatments.

The leaf area index (LAI) in each treatment is shown in Table 4. The LAI values in the NF plot were 62–78% of those in the CF treatments. With the exception of the MF10.5-4.5 plot in 2007–2008, the LAI for all the MF treatments was lower than that for the CF plot at the AT stage, regardless of the rate of ADM application. The difference in LAI between MF-10 and CF-10 plots was significant. Furthermore, the lower LAI in the MF-10 plots relative to the CF plots persisted until the HD or middle ripening stage.

| Year (2002–2006 and 2007–2008) | n.s | n.s | – | n.s | – |
| Treatment (MF-10 and CF-10) | * | n.s | n.s | n.s | n.s |
| Interaction | n.s | n.s | – | n.s | – |

“n.a” denotes “no data available”. Values in parentheses denote the ratio to those in CF treatments. * and n.s denote significant at $p < 0.05$ and not significant, respectively. In 2007 and 2008, approximately 15% of ADM was replaced with a corresponding amount of chemical fertilizer (ammonium sulfate). Values shown in italic denote that they have some missing values (see Table 1). When there are some missing values on MF treatments, corresponding values in the CF plots were excluded from calculation of the relative ratio.
ripening (MR) stage. The positive effects of a higher rate of ADM application on LAI at the PI stage were seen in all the MF plots with split application throughout the experimental period, and even in the plots with single application in the second period (2007–2008).

The changes in the N uptake by the rice plants in each treatment are shown in Table 5. The N content of the aboveground part of rice plants (g m\(^{-2}\)) at different stages in each treatment.

### Table 5. N content of aboveground part of rice plants (g m\(^{-2}\)) at different stages in each treatment.

| Year                | Treatment | Active tillering | Panicle initiation | Reduction division | Heading | Middle ripening | Maturing |
|---------------------|-----------|------------------|-------------------|-------------------|---------|-----------------|----------|
| NF                  | 1.7 (0.58)| 4.1 (0.55)       | 5.6 (0.55)        | 6.1 (0.59)        | 6.5 (0.59)| 7.7 (0.64)      |          |
| CF10-0              | 3.1       | 3.1              |                   |                   |         |                 |          |
| MF10-0              | 2.4 (0.80)| 6.2 (0.79)       | 8.0 (0.85)        | 8.9 (0.90)        | 9.5 (0.95)| 10.8 (0.94)     |          |
| MF15-0              | 2.3 (0.90)| 6.7 (1.04)       | n.a               | n.a               | 12.2 (1.05)|                   |          |
| MF20-0              | 2.4 (0.96)| 6.4 (0.99)       | n.a               | 10.4 (1.17)       | 12.9 (1.12)|                   |          |
| Average 2002-2006   |           |                  |                   |                   |         |                 |          |
| CF7-3               | 2.6       | 6.5              | 10.6              | 10.2              | 12.0    | 12.2            |          |
| MF7-3               | 2.2 (0.86)| 5.4 (0.88)       | 9.0 (0.85)        | 9.0 (0.91)        | 9.7 (0.84)| 11.0 (0.92)     |          |
| MF10.5-4.5          | 2.2 (0.93)| 6.4 (1.26)       | n.a               | 10.7 (1.18)       | n.a     | 14.1 (1.18)     |          |
| MF14-6              | 2.3 (0.96)| 7.8 (1.53)       | n.a               | 12.4 (1.37)       | n.a     | 15.3 (1.28)     |          |
| Average 2007-2008   |           |                  |                   |                   |         |                 |          |
| NF                  | 1.7 (0.64)| 3.6 (0.50)       | n.a               | 6.2 (0.58)        | n.a     | 6.8 (0.55)      |          |
| CF10-0              | 2.9       | 6.5              | n.a               | 9.9 n.a           |         | 11.4            |          |
| MF10-0              | 2.5 (0.85)| 6.6 (1.01)       | n.a               | 9.8 (1.00)        | n.a     | 11.5 (1.00)     |          |
| MF15-0              | 2.4 (0.88)| 8.4 (1.27)       | n.a               | 9.6 (0.97)        | n.a     | 12.4 (1.09)     |          |
| MF20-0              | 2.6 (0.93)| 8.4 (1.28)       | n.a               | 12.6 (1.27)       | n.a     | 14.6 (1.28)     |          |
| Average 2007-2008   |           |                  |                   |                   |         |                 |          |
| CF7-3               | 2.7       | 6.2              | n.a               | 11.5 n.a          |         | 12.5            |          |
| MF7-3               | 2.4 (0.86)| 5.6 (0.90)       | n.a               | 9.7 (0.84)        | n.a     | 12.0 (0.95)     |          |
| MF10.5-4.5          | 2.5 (0.95)| 7.4 (1.27)       | n.a               | 11.8 (1.08)       | n.a     | 14.1 (1.12)     |          |
| MF14-6              | 2.5 (0.96)| 8.4 (1.44)       | n.a               | 14.5 (1.32)       | n.a     | 16.4 (1.31)     |          |
| Year (2002-2006 and 2007-2008) | n.s | n.s | – | n.s | – | n.s |          |
| Treatment (MF-10 and CF-10) | * | n.s | n.s | n.s | n.s | n.s |          |
| Interaction | n.s | n.s | – | n.s | – | n.s |          |

“n.a” denotes “no data available”. Values in parentheses denote the ratio to those in CF treatments. * and n.s denote significant at \( p < 0.05 \) and not significant, respectively. In 2007 and 2008, approximately 15\% of ADM was replaced with a corresponding amount of chemical fertilizer (ammonium sulfate). Values shown in italic denote that they have some missing values (see Table 1). When there are some missing values on MF treatments, corresponding values in the CF plots were excluded from calculation of the relative ratio.

2. Grain yield and yield components

The grain yield and its components are shown in Table 6. The number of spikelets and the grain yield per square meter in the NF plot were 73 – 79\% those in the CF plots, while the ripened grain ratio and 1000 grain weight were consistently higher in the NF plot than in the CF plots.

In the MF plots with the standard application rate (MF-10 plots), the ratio of grain yield to that in CF plot was consistently below 1, namely, grain yield in MF was lower...
than in CF, regardless of the application method and the partial substitution of chemical N fertilizer for ADM (2007 – 2008). The spikelet number in 2007 – 2008, and the ripened grain ratio in 2002 – 2006 and 1000 grain weight throughout the experiment were comparable to or slightly greater than those in the CF plots. The analysis of variance (ANOVA) among plots with the standard application rate (10 g m\(^{-2}\)) indicated that none of the parameters detailed in Table 6 was significant both for experimental period and fertilizers.

The split application and higher application rates of ADM increased the spikelet number of rice plants, but decreased the ripened grain ratio and the 1000 grain weight. Over the entire period of the experiment (2002–2006 and 2007 – 2008), the highest yield was achieved in the MF10.5-4.5 plot, and interestingly not in the MF20-0 or MF14-6 plots in which the N uptake by plants was the highest.

3. Changes in soil properties

The changes in submerged soil pH in each treatment from transplanting to 50 – 55 DAT are shown in Fig. 2. The soil pH in the MF plots was higher than that of the CF and NF plots, especially during the early growth stage. The change in the pH of the soil in the NF plot was similar to that in the CF plot until 20 DAT. Thereafter the values in the CF plot were somewhat lower than those in the NF plot. In both 2006 and 2007, the difference in the pH values among treatments decreased as the growth period proceeded.

The ORP of the submerged soil measured in 2006 is shown in Fig. 3. The ORP values declined rapidly up to 10 DAT, particularly in the MF15-0 and MF14-6 plots. Thereafter, however, the ORP remained fairly stable within a range of \(-240 \sim -200\) mV for all the treatments.

The changes in the ammonium N in the flooded soils during long-term soil incubation are shown in Fig. 4. Since the N sources were added to the MF and CF plots at the beginning of the incubation, the initial ammonium N in these plots was higher than that in the NF plot. The ammonium N content of the soil generally increased with the increase in ECST up to 100ºC, although the increase in the CF10-0 soil was relatively small. Between 100ºC and 800ºC ECST, only a slight increase in ammonium N content was observed, regardless of the treatments. However, after 800ºC ECST there was a sharp increase in ammonium N content in all of the samples.

The ECST in the experimental field changed only slightly among plots during the early growing period of rice. For instance, the ECST among plots at 30 and 45
DAT, approximately corresponding to the AT and PI stages, respectively, was within the range of 301 – 309°C and 467 – 474°C, respectively (data not shown). From the results of these field ECST measurements and those in the soil incubation experiment, the inorganic N content of soil per 100 g of dry soil at approximately 400°C ECST (equivalent to the PI stage) was estimated as follows: NF, 3.52; CF10-0, 4.82; MF10-0, 7.15; MF15-0, 7.73; MF20-0, 8.46 mg, per 100 g of dry soil. Furthermore, considering the cultivated layer (15 cm) which had a soil bulk density of 0.86 g cm$^{-3}$, the N supply at around 400°C ECST (Fig. 4) was determined to be as follows: NF, 4.54; CF10-0, 3.06 (1.01); MF10-0, 3.27 (1.08); MF20-0, 3.35 (1.10), respectively. Here, inorganic N content of soils at 400°C ECST (equivalent to the PI stage) was approximately equal to that at 200°C ECST (equivalent to the AT stage) as shown in Fig. 4. Relationship between N content of rice plants (N uptake) at the AT stage and inorganic N content of soil (estimated N content of soil) at 400°C ECST is shown in Fig. 5. The N uptake by rice plants was less than the estimated N supply at the AT stage irrespective of treatments. However, the difference in the MF plots was apparently greater than that in the CF and NF plots.

Table 6. Grain yield and yield components in each treatment.

| Year          | Treatment | Grain yield (g m$^{-2}$) | Spikelet number ($10^3$ m$^{-2}$) | Ripened grain (%) | 1000 grain weight (g) |
|---------------|-----------|--------------------------|-----------------------------------|-------------------|-----------------------|
| 2002-2006     | NF        | 429 (0.79)               | 22.5 (0.73)                       | 86.6 (1.05)       | 22.4 (1.04)           |
|               | CF10-0    | 538                      | 30.4                              | 83.2              | 21.7                  |
|               | MF10-0    | 518 (0.96)               | 28.5 (0.94)                       | 84.4 (1.02)       | 22.0 (1.02)           |
|               | MF15-0    | 535 (0.99)               | 30.4 (1.00)                       | 81.9 (0.99)       | 21.8 (1.00)           |
|               | MF20-0    | 549 (1.02)               | 31.0 (1.02)                       | 82.6 (0.99)       | 21.9 (1.01)           |
| Average       | CF7-3     | 547                      | 31.3                              | 82.6              | 21.6                  |
| 2002-2006     | MF7-3     | 542 (0.99)               | 30.7 (0.98)                       | 82.6 (1.00)       | 21.7 (1.00)           |
|               | MF10.5-4.5| 569 (1.04)               | 35.2 (1.13)                       | 77.2 (0.93)       | 21.4 (0.99)           |
|               | MF14-6    | 525 (0.97)               | 36.9 (1.19)                       | 60.4 (0.84)       | 21.0 (0.97)           |
| Average       | NF        | 437 (0.77)               | 23.6 (0.75)                       | 84.4 (1.01)       | 22.0 (1.02)           |
|               | CF10-0    | 560                      | 30.5                              | 83.6              | 21.5                  |
|               | MF10-0    | 546 (0.98)               | 30.6 (1.01)                       | 82.8 (0.97)       | 21.6 (1.00)           |
|               | MF15-0    | 553 (0.99)               | 32.7 (1.08)                       | 79.4 (0.93)       | 21.4 (1.00)           |
|               | MF20-0    | 573 (1.03)               | 33.5 (1.10)                       | 79.7 (0.93)       | 21.5 (1.00)           |
| Average       | CF7-3     | 569                      | 32.3                              | 81.9              | 21.5                  |
| 2007-2008     | MF7-3     | 551 (0.97)               | 32.6 (1.01)                       | 78.5 (0.96)       | 21.6 (1.01)           |
|               | MF10.5-4.5| 598 (1.05)               | 36.4 (1.13)                       | 77.3 (0.94)       | 21.3 (0.99)           |
|               | MF14-6    | 576 (1.01)               | 36.3 (1.12)                       | 73.8 (0.90)       | 20.9 (0.97)           |
| Year (2002-2006 and 2007-2008) | n.s | n.s | n.s | n.s |
| Treatment (MF-10 and CF-10) | n.s | n.s | n.s | n.s |
| Interaction  | n.s       | n.s                      | n.s                               | n.s               | n.s                  |

Analysis of variance (ANOVA) was done for MF-10 and CF-10 treatments and “n.s” denotes “not significant”. In 2007 and 2008, approximately, 15% of ADM was replaced with a corresponding amount of chemical fertilizer (ammonium sulfate). Values in grain yield and 1000 grain weight contain 15.5% of moisture. Values in parentheses denote the ratio to those in CF treatments.

Discussion

To facilitate the widespread use of anaerobically-digested manure (ADM) in paddy rice production, it is essential to understand both the positive and negative effects of ADM application. In particular, in the case of negative effects it is important to investigate the mechanisms and to find a solution.

The growth inhibition of rice plants caused by ADM relative to chemical fertilizers was observed in all the growth parameters examined, particularly in LAI and N uptake (Tables 4 and 5). Since this inhibition was consistently observed over seven years, it is likely not to be related to some cumulative negative effects of ADM but to a general characteristic resulting from the application of ADM. Similar effects have been documented previously, for example, Li et al. (2003b) reported that ADM caused growth inhibition at the maximum tillering and heading stages, and suggested that it possibly resulted from the immobilization of inorganic N applied in MF plots during early growth of rice plants. However, results of present study indicated that the growth inhibition caused by ADM
Phenomena appear to be a very important characteristic of ADM, and it is noteworthy that rice plants fertilized with ADM have a growth pattern markedly different from those fertilized with chemical fertilizers.

Absorption of N is a particularly significant factor for plant growth. The N uptake by rice plants from transplanting to the AT stage was lower in the MF plots than in the CF plots, and growth parameters at the AT stage was temporal, and that most of growth parameters after the panicle initiation stage (approximately 45 DAT) were not inhibited. These phenomena appear to be a very important characteristic of ADM, and it is noteworthy that rice plants fertilized with ADM have a growth pattern markedly different from those fertilized with chemical fertilizers.

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stage were apparently lower than those in the CF plots. This was true not only in the plots with an equivalent rate of N application; 10 g m$^{-2}$ as inorganic N, but also for the plots with a 1.5- or 2-fold higher rate of inorganic N application (Table 5 and Fig. 1). Furthermore, in 2007 and 2008, the inorganic N (ammonium sulfate) substituted for the basal ADM did not markedly improve the plant growth and grain yield (Fig. 1, Table 6). This indicates that a 15% substitution was insufficient to remove the growth inhibition caused by ADM.

On the other hand, the N content of the rice plants did not correspond to the N supply from fertilizers and cultivating soils (inorganic N content of soil). For instance, the plants in the CF10-0 plot showed the highest N uptake at the AT stage, but the soil in this plot had the second lowest N content. The difference between the N content of the plants and the inorganic N content of soil in MF plots was greater than that in the CF and NF plots (Fig. 5). This may be partly because the inorganic N content of soil was measured in the laboratory experiments. However, it is certain that rice plants in the MF plots had restricted nutrient absorption.

These results raise a question: what is the key factor accounting for the temporal growth inhibition caused by ADM? An analysis of the soil physicochemistry in the field provided few clues. The soil ORP in all treatments had a similar pattern of decline after transplanting (Fig. 3). Similarly, little difference was noted in the soil pH. Higher pH values (around 6.5 – 7.0) were occasionally observed in the MF plots (Fig. 2), but it was still within the normal range of soil pH under submerged conditions (Ponnamperuma, 1972), and the difference in soil pH among treatments disappeared with increase in DAT.

As mentioned previously, the differences in daily soil temperature and ECST among the experimental plots during the early growth period (1 – 45 DAT) was very small, indicating that the effect of soil temperature on the rate of organic N mineralization in the soil did not differ among the plots when their soil organic N level was similar. On the other hand, immobilization of inorganic N to organic N generally occurs when organic materials with a high C/N ratio (more than 30) are applied to croplands. However, the C/N ratio of ADM is generally less than 10, implying that active N immobilization is an unlikely cause of the growth inhibition observed in this study. Indeed, the ammonium N in the soil resulting from the addition of ADM increased immediately after incubation, while the N from CF soils increased only slightly (Fig. 4). These observations also indicate that the effect of immobilization, if any, would be small.

Although it was not the subject of the current investigation, there is evidence that ADM contains inhibitory substances, especially several kinds of organic acids (Tsutsuki, 1984; Nozoe and Yasuda, 1994) that could be possible factors leading to the inhibition of rice plant growth. Mimoto et al. (1988) reported similar growth inhibition caused by Italian ryegrass cropping before rice cropping. In that study it was concluded that the growth inhibition of rice resulted from a temporal accumulation of organic acids from the Italian ryegrass applied. Tanaka and Nishida (1998) observed that several kinds of aromatic carboxylic acid are harmful to the root elongation of rice plants, and can accumulate after the application of a variety of organic materials. However it has also been reported that the standard rate of ADM application does not provide a large enough source of organic acids to cause harmful effects on plant growth (Nishikawa et al., 2012). More suspected inhibitors seem to be the volatile fatty acids produced during the digestion process of organic waste in a biogas plant. It has been shown that ADM contains several hundred mg L$^{-1}$ of these compounds (Noike et al., 2009), which is a sufficient level to damage the roots of rice plants (Takijima et al., 1960).

The polymer coagulant used for the purification of ADM might also be one of the factors responsible for the inhibition of rice plant growth. A cationic polymer coagulant was used to agglutinate the solid organic matter within ADM. The filtered water of the ADM, which contains residual traces of the coagulant, was then returned to the digestion tank. It is known that these polymer coagulants sometimes inhibit root cell elongation, which can result in reduced plant growth (Fujii, 1983). Another possible factor affecting the observed growth inhibition could be the loss of the applied N by ammonia volatilization. It is well known that ADM is weakly alkaline, having an approximate pH of 8. Win et al. (2009) reported that the ammonia volatilization can occasionally become serious in the field with intense application of ADM, namely, greater than 30 g m$^{-2}$ of ammonium N. However, in the present study, the maximum rate of N application was 20 g m$^{-2}$. The usual rate of N application to rice crops in Japan is generally limited to less than 10 g m$^{-2}$ to reduce the risk of lodging and maintain the eating quality. In this study, in the MF plots with high application rates, especially those with basal application only, ammonia volatilization could account for the loss of applied N, because the N content of rice plants in the single application plots was always lower than that in the plots with split application (Fig. 1 and Table 5). Kamioka and Kamewada (2011) found that mixing ADM with the puddled soil after application of ADM was an effective method for decreasing ammonia volatilization. Although this approach was not possible in the current study because the fertilization was conducted after transplanting of the rice seedlings, it may be an effective method to decrease ammonia volatilization.

Although the precise nature of the growth inhibition caused by ADM is still unclear, it seems likely that a combination of all the factors discussed above might cause
the inhibitory effect observed.

It is important to find measures to improve rice plant growth and produce a stable grain yield even though the inhibitory effects of ADM are still not fully understood. The correct selection of a suitable rice cultivar is an important factor in rice cropping, and is particularly important when utilizing ADM. In this study we used a medium maturing cultivar, and the growth inhibition by ADM application was substantially recouped by the PI stage (approximately 45 DAT) or by the HD stage at the latest. Even though the MF plots, with the exception of MF10.5-4.5 and MF1446 plots, produced fewer panicles than the CF plots, this deficiency was partially compensated by the improved performance for other yield components (Table 6). However, it should be noted that in early maturing rice varieties, panicle initiation starts before any substantial growth recovery. Consequently, in early maturing varieties the resulting effect on the number of panicles and spikelets could reduce the yield dramatically. Considering this, it might be better to reduce or withhold ADM from basal applications to secure early growth of early maturing cultivars.

When growing medium or late maturing cultivars, the partial substitution of chemical fertilizers for ADM, and/or split applications of ADM could be effective strategies to reduce N loss. However, the results of this study suggest that when partial substitution of chemical N for ADM is conducted in order to stabilize the initial growth of rice, a proportion of chemical fertilizer to ADM greater than 15% is required, since the 15% substitution in this study did not have any notable effects (Fig. 1). Sunaga et al. (2009) pointed out that several split applications of ADM that met the ongoing growth of rice plants could decrease the environmental impacts and increase the N recovery by rice plants. These application methods would also contribute to reduce the seasonal fluctuation of the demand for ADM and help plan the efficient use of ADM.

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