Rice yields and the effect of weed management in an organic production system with winter flooding

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ABSTRACT
Winter flooding of paddy rice production is characterized by flooding of paddy fields during the winter fallow season. It has attracted attention in terms of its ecological function, supporting the biodiversity of paddy field ecosystems. Therefore, recent winter flooding in Japan has been conducted by organic farming. However, it is not well documented how organic farming with winter flooding affect the productivity of rice. We conducted field experiments to examine changes in rice yield in organic farming with winter flooding compared with those in conventional and organic farming over the period of 5 and 9 years. The yields in organic farming with winter flooding were lower than those in conventional farming but were almost the same as those in organic farming. We found a trend that shows the relative yield of organic farming with winter flooding compared to that of conventional farming increased during the first several years after the conversion from conventional farming to organic farming with winter flooding, but that it decreased after several years. This trend may be caused by the flourishing of weeds during continuous organic farming with winter flooding. Therefore, we tested four weed control methods: rice bran application, mechanical weeding, repeated puddling, and side-row fertilizer application. Although each method suppressed weeds to certain extents, some combinations of different weeding methods more effectively suppressed weed growth, thereby resulting in enhanced rice yields. We found a trend in rice yield and a possibility of combining several weed control methods in organic farming with winter flooding.

Introduction
Winter flooding paddy rice production systems (winter flooding), which keep water in the fields during the winter non-cropping season, have traditionally been found throughout the world. For instance, winter flooding has been conducted for preventing the collapse of fields and retaining spring water in terraced fields in Yunnan, China for 1300 years (Nakamura & Morishita, 2009); used for desalination for more than 200 years in Valencia, Spain (Martinez-Eixarch et al., 2017); used for maintaining waterfowl populations for hunting in the Camargue, France, and in California and Mississippi, in the USA (Pernellet et al., 2015); used for preserving biodiversity, including waterfowl, in the Ebro Delta of Spain (Martinez-Eixarch et al., 2017); and used to promote the decomposition of rice straw (in response to the regulation of straw burning in the 1990’s) in the USA (Linquist et al., 2006; Pernellet et al., 2015). In Japan, winter flooding was widely used as a technique to maintain the fertility of paddy fields from the 1600s to the 1800s (Arizono, 2013), but it almost disappeared in the 1900s when chemical fertilizers became widely used and most paddy fields have been well-drained. However, winter flooding has recently been rediscovered as an alternative wetland for the conservation of waterfowl (Kurechi, 2007) and aquatic organisms (Kurechi, 2007; Mineta et al., 2009). In addition, in winter flooding, the purification and recharge of groundwater are enhanced (Ogura, 2016; Takahashi, 2016). Accordingly, winter flooding events are recognized as beneficial farming practices for biodiversity conservation both in Japan and other countries.

The productivity of rice in winter flooding systems has not been well-documented. Cheng-fang et al. (2008) reported a decrease in weed seeds due to waterfowl foraging, and the increase of fertility by the addition of waterfowl wastes and by promoting the decomposition of rice straw. Furthermore, Linquist et al. (2006) and Nira and Miura (2019) reported an increase in available nitrogen in soil with winter flooding. Ito et al. (2011), (2015)) found that the density of Tubifex tubifex, an aquatic oligochaete, increased in paddy fields with winter
flooding practices and that this resulted in the increase of ammonium nitrogen content in the soil and in suppression of weed seeds by burying them into soil. Conversely, Niang et al. (2016) indicated the demerits of winter flooding on rice productivity: the prevention of double cropping, the deterioration of soil structure, the decrease of workability due to the delay of soil drying in spring, and the higher risk of rice lodging caused by softening of the soil.

Recent winter flooding in Japan has been conducted by environmentally friendly agriculture, especially on organic farming. The Japanese government subsidizes environmentally friendly farming practices combined with the winter flooding of paddy fields for biodiversity conservation. However, the productivity of organic farming is unstable. Reports on the productivity of rice in organic farming systems in Japan showed inconsistent trends; the yield increased with age (Tamaki et al., 2002), and also increased after 3–5 years from the conversion from conventional farming to organic farming (Asai et al., 2016; Saitoh et al., 2001). In contrast, the yield decreased as the number of weeds increased over the years (Oomori, 2015). In other countries, the yield difference between conventional and organic farming has been increasing (Switzerland, Mäder et al., 2002) or decreasing (USA, Gliessman et al., 1996; Switzerland; Mäder et al., 2002). In particular, when the yield levels of conventional farming were high, the yield difference between conventional and organic farming was large (De Ponti et al., 2012; Stanhill, 1990).

Weed damage is one of the major factors contributing to the low yield of organic farming. Several promising weed control methods in organic paddy fields have been proposed: puddling 2 times (Hasegawa, 2010), rice bran application (Komori, 2007; Nozoe et al., 2016), deep water management (Onuma, 1952; Yatsuyanagi et al., 1952), and side-row fertilizer application (Kaneda et al., 2006). Puddling 2 times removes weeds that emerge in the interval between the puddling, before the rice planting, and as a result it reduces weeds after rice planting (Miyake, 1999; Sugawara et al., 2004). In rice bran application, Nozoe et al. (2016) showed to drastically the number of Monochoria vaginalis, one of the major weeds, in field experiments. Nakai et al. (2011) and Miura et al. (2015) reported that rice bran application suppressed weeds efficiently. However, the growth of rice plants is also suppressed (retarded and inhibited) by rice bran application (Nakai & Toritsuka, 2009; Nakayama et al., 2002). The effect on weed control of rice bran application often varies depending on soil conditions, application period, and the amount used (Nozoe et al., 2012). Therefore, the effects of rice bran application are inconsistent.

However, although weed control in organic rice farming has been widely reported; weed growth and weed control in winter flooding systems have not been well investigated. Changes in the yield of winter flooding systems in comparison with conventional and organic farming systems over the years has not been reported. Oomori (2015) reported that a higher rice yield and lower weed abundance were found only in the first year of the conversion from conventional farming to organic farming with winter flooding. Arai et al. (2010) and Kaneko and Nakamura (2011) reported weed density in the 2 or 3 years from the shift to winter flooding.

In this study, we investigated the yield in an organic farming system with winter flooding compared with a conventional farming system over 9 years in Experiment 1 (Exp. 1) and compared with conventional and organic farming without winter flooding over 5 years in Experiment 2 (Exp. 2). Furthermore, we evaluated the effect of rice bran application and mechanical weeding in Experiment 3 (Exp. 3) and, in addition to those two methods, the effect of repeated puddling and local fertilizer application in Experiment 4 (Exp. 4).

Materials and methods

Experimental site

Field experiments were conducted in five paddy fields (A–E) at the Kawatabi Field Science Center, Graduate School of Agricultural Science, Tohoku University, located in Osaki, Miyagi Prefecture, northeastern Japan (38°7′N, 140°7′E, 178 m above sea level). Exp. 1 was conducted from 2009 to 2017 in fields A, B, and C, Exp. 2 was conducted from 2013 to 2017 in field D, Exp. 3 was conducted in 2009 and 2010 in field E, and Exp. 4 was conducted in 2011 and 2014 in field E. The different experiments are summarized in Table 1.

Climate and soil conditions

This region has a temperate climate. During the rice growing season from April to September, the 30-year (1981–2010) average daily air temperature was 19.9°C and the monthly sunshine duration was 143.0 h (Kawatabi Meteorological Observatory, Japan Meteorological Agency database). During the non-growing season, the fields were left fallow and were covered with snow from late December to March. The soil is an Anthraquic Hapludand. The soil properties of
the experimental fields (A–E) were estimated before planting in 2014: clay loam texture; pH 5.9; total carbon, 31–37 g C kg⁻¹; total nitrogen, 3.1–3.6 g N kg⁻¹.

**Experimental setup and management**

We cultivated rice (*Oryza sativa* ‘Hitomebore’) using three management practices: organic farming with winter flooding (WOF), organic farming (OF), and conventional farming (CF). In each management regime, 10 t ha⁻¹ of cow compost was applied in November and rice straw was removed from the field at harvest every year. WOF and OF were managed according to the guidelines for organic rice farming (Oyama, 2007). Rice seedlings were grown in greenhouses and transplanted into the fields. Nursery management of rice seedlings was the same for both WOF and OF. Similarly, we adopted the following management practices for these two systems during the growing season: in mid-April, 40 kg N ha⁻¹ of a commercial organic fertilizer made from fish meal, rapeseed oil cake, and ricinus oil cake, ‘Yuki Agret 6-6-6’ (N₃P₃₀₅K₂O: MgO = 6:6:6:1%, Asahi Industries Co. Ltd., Japan) was applied, and rice bran was applied 1 to 2 days after transplanting (DAT) except in field E in 2009 for Exp. 3; no herbicides, pesticides, fungicides, or insecticides were applied, and fields were mechanically weeded at 10-day intervals from 10 to 40 DAT. The difference between WOF and OF was in the water management of the field; the paddy field in OF flooded during the growing period, from a week before transplanting until a few weeks before harvest, while the field in WOF was flooded not only during the growing period but also during the winter, from December to April. CF plots were managed according to local guidelines (Miyagi Prefecture, 2005): commercial chemical fertilizer, insecticide, and fungicide were applied at the time of transplanting, then herbicide, fungicide, and insecticide were applied at 7–10 DAT, 60–70 DAT, and 80–90 DAT, respectively. Water and field management treatments are summarized in Figure 1, and management details are provided in the supplementary information (Table S1).

**Measurement of seedlings**

At the time of transplanting in 2008, 2009, 2010, 2012, 2013, and 2017, we measured plant height, number of

| Experiment | Field | Year          | Management practice | Replication | Major objective                                                      | Measurements                                                                 |
|------------|-------|---------------|---------------------|-------------|---------------------------------------------------------------------|----------------------------------------------------------------------------|
| Exp. 1     | A, B, C | 2009–2017    | CF, WOF             | 3           | Comparison of yields                                                 | Machine-harvested yield                                                    |
| Exp. 2     | D     | 2013–2017    | CF, OF, WOF         | No replication | Comparison of yields                                                 | Rice yield and growth, Weed density and dry weight, Dissolved oxygen concentration, Turbidity of paddy surface water |
| Exp. 3     | E     | 2009, 2010   | WOF                 | 3           | Effects of rice bran application and mechanical weeding             | Rice yield and growth, weed density and dry weight, Soil inorganic nitrogen |
| Exp. 4     | E     | 2011, 2014   | WOF                 | 3           | Effects of repeated puddling and local fertilizer application       |                                                                           |

**Table 1. Outline of the experiments in this study.**

**Figure 1. Outline of water and field management practices of organic farming with winter flooding, organic farming, and conventional farming in experiments 1 (2009–2017), 2 (2013–2017), 3 (2009, 2010), and 4 (2011, 2014). Grey parts indicate the flooding period.**
leaves, shoot dry weight, and leaf color of 40–60 seedlings; these were not measured in 2011, 2014, 2015, and 2016.

**Exp. 1: Yields of organic farming with winter flooding compared with conventional farming**

Prior to the commencement of the present study, experimental fields A (0.21 ha), B (0.22 ha), and C (0.22 ha) were managed as conventional farms. In 2008, each field was split into two separate plots by making a ridge in the center of the field. One plot from each field was managed with WOF, while the other plot was managed with CF. Fields A, B, and C were the three blocks in the randomized block design. Seedlings for WOF and CF were grown under greenhouse conditions according to the respective guidelines mentioned above. From 2009 to 2017, rice plants were continuously cultivated under these management conditions in fields A, B, and C. All rice plants in each plot were harvested using a rice combine harvester. After mechanically hulling and thickness grading the rice grains, the yield of brown rice was determined as the seed weight (15% moisture) of grains that were more than 1.9 mm thick.

**Exp. 2: Yields of organic farming with winter flooding compared with conventional and organic farming**

The experimental field D (0.26 ha) was managed as a conventional farm until 2007. In 2008, the field was split into six plots by constructing ridges, with pairs of adjacent plots being assigned to WOF, OF, or CF. In 2013, the field was rearranged into three plots of sizes 670, 720, and 830 m² for WOF, OF, and CF, respectively, and rice plants were continuously cultivated using these management practices until 2017. Eighteen hills of rice plants were manually harvested from 3 or 4 sites selected randomly within each plot, and yield of brown rice was determined based on these samples. After air-drying, rice grains obtained by manual threshing were hulled, and the yield of brown rice was estimated as in Exp. 1.

**Exp. 3: Effect of rice bran application and mechanical weeding**

The effect of rice bran application in combination with mechanical weeding on weeds and rice yield was examined in 2009 and 2010 in field E (0.14 ha) that had been managed with WOF since 2008. The experimental plots were set in three replicates according to a randomized block design. Each 150 m² block was divided into three treatments. In 2009, treatments were as follows: no weeding (control treatment), rice bran application (RB), and rice bran application with mechanical weeding (RB+MW). Rice bran was broadcast at a rate of 80 g fresh weight m⁻² a day after transplanting. Mechanical weeding was performed using three passes of a hand-pushed weeder, every 10 days after transplanting. The surface water of paddy fields was collected at 1–3 p.m. on days 7, 9, 15, and 49 after transplanting. Dissolved oxygen concentration (DO) and turbidity of water samples were measured using a DO meter (YSI Co. Ltd) and turbidity meter (YSI Co. Ltd). In 2010, new experimental plots were set in three replicates according to a randomized block design. As with the 2009 experiment, each 150 m² block was divided into three treatments. Plot treatments were as follows: rice bran application (RB), rice bran application with mechanical weeding (RB+MW), and rice bran application with hand weeding (RB+HW). Mechanical weeding was conducted as in 2009, and hand weeding was repeated twice at intervals of about 30 days after transplanting. A control treatment without rice bran application was not used in 2010 because weeds had thrived under this treatment in 2009. Brown rice yields were determined using the same method as in Exp. 2.

During rice sample harvest (141 DAT in 2009 and 132 DAT in 2010), weeds were collected from the same sampling area as the rice plants. Weed sample species were identified, and the number of plants of each species was counted. After washing with tap water, the aboveground part of the weeds was oven-dried (70°C, 72 h) and the dry weight was recorded. Weed plants were counted using the same method at 51 DAT in 2009.

**Exp. 4: Effect of repeated puddling and side-row fertilizer application**

In addition to the above two weed control methods (RB and MW), the effect of repeated puddling before transplanting in combination with the fertilizer application method on weeds and rice yield was examined in 2011 and 2014 in field E. In 2011, three treatments (2, 4, and 6 puddling events) were set, with three replicates of each treatment in a randomized block design. The first puddling event took place at the end of April after flooding (Table S1). Puddling events were produced 2, 4, and 6 times, at approximately 25, 8, and 5-day intervals after the previous pudding, respectively. In 2014, repeated puddling treatments (2, 4, and 6 times) were combined with two fertilizer application methods: broadcasting before puddling (B), and application to the side-row at transplanting (SR). Since, in contrast to B, fertilizer components in SR should only diffuse to the limited soil volume near the rice plants, we expected that SR would not facilitate weed growth compared with B. The amount of applied fertilizer was the same as in Exp. 1–3. The experimental plot was 50 m² with three replicates in each puddling plot. The soil from the plow
layer of each experimental plot was collected one day before transplanting. Ammonium nitrogen concentration in the soil was measured after extraction with 10% KCl using an automatic analyzer (BL Tech Co.). At the maximum tillering stage (65 DAT), shoot dry weight was measured by sampling two hills that showed average growth. Brown rice yields were determined using the same method as in Exp. 2. In addition, the weed density and weight were measured at harvest in 2011 and 2014 using the same method as in Exp. 3.

**Statistical analysis**

For seedling growth analysis, one-way ANOVA was applied with the years as replications. In Exp. 1, two-way analysis of variance (ANOVA) and Tukey’s test in each management practice were applied to the yield. Management practices and replications were nested by years. Therefore, years are considered a fixed variable in two-way ANOVA. In Exp. 3, two-way ANOVA was used to test whether the weeding treatments and blocks affected rice growth, density, and dry weight. In Exp. 3, if the result of the ANOVA of weeding treatments was significant, post hoc analysis was conducted using Tukey’s test. In Exp. 4, one-way and two-way ANOVA were used to analyze the effects of puddling and fertilization in 2011 and 2014, respectively. In addition, the linear relationships between DO, turbidity, and weed density in Exp. 3 were analyzed. The linear relationship between the number of panicles and soil inorganic nitrogen was also analyzed in Exp. 4. All the analyses were performed in R 4.0.2 (R Core Team, 2020).

**Results**

**Weather data during cultivation**

The mean temperature during the field growing season from June to September in 2009–2017, was 20.7°C. In 2009, it was cooler and cloudy, while in 2010 it was hotter and sunny. Monthly mean temperature and sunshine duration are shown in the supplementary information (Table S2).

**Growth parameters of the seedlings**

Table 2 shows the average growth parameters of rice seedlings at transplanting time in WOF and CF during the experimental period (2008–2017). Seedlings for OF in Exp. 2 were managed in the same way as they were for the WOF plot. Plant height and leaf color index were significantly higher in CF than in WOF, while the number of leaves was slightly higher in WOF. As a result, the quality of seedlings (the ratio of shoot dry weight to plant height), which is widely used in Japan for the index of healthy seedlings, was higher in the WOF plots. The plant dry weight of seedlings did not show any difference with these treatments. No significant yearly differences in these parameters were found.

**Exp. 1: Trend of yearly change in brown rice yield in winter flooding in comparison with conventional farming**

The yearly change in the yield of brown rice is shown in Figure 2. Average brown rice yields over 9 years were 3.38 t ha⁻¹ and 5.29 t ha⁻¹ in WOF and CF, respectively. The annual variations in yield may depend largely on weather conditions. For example, in 2013, we found that the yield in CF plots was the lowest in the 9 years of the study, which could be attributed the lower amount of sunshine in July (a critical period for rice growth), which was less than one-third that recorded in other years (Table S2). Significant differences in farming management, year, and interactions were found by ANOVA. The yield in WOF was significantly lower than that in CF, except in 2011, while the degree of decrease in the yield of WOF changed year by year. The relative yield of WOF compared to conventional farming indicates an increase for the initial 3–5 years followed by a decrease after 5 to 6 years.

**Exp. 2: Yearly change of brown rice yield in winter flooding in comparison with organic and conventional farming**

The change in yield by quadrat sampling in Exp. 2 was shown in Figure 3. The average brown rice yield over 5 years was 4.44 t ha⁻¹, 4.20 t ha⁻¹, and 6.03 t ha⁻¹ in WOF, OF, and CF, respectively. The yields in plots subjected to WOF and OF treatments were lower by 26% and 30%, respectively, compared to that under CF. Compared with CF, the relative yield trend under WOF was found to be similar to that observed for first several years in Exp. 1.

**Exp. 3: Effect of rice bran application and mechanical weeding**

In this experiment, 16 species of weeds were found in the experimental plot. Weed and rice growth parameters are listed in Table 3. In 2009, the dominant weed species were Echinochloa oryzicola, Monochoria vaginalis var. plantaginea, and Rotala indica var. uliginosa, which formed a major part of the weeds in terms of their density and shoot dry weight. Both weeding treatments, RB and RB+MW, reduced weed density at 51 DAT, while total weed biomass at harvest (141 DAT) was not reduced. In 2010, rice bran was applied to all plots because the control plot in 2009 was seriously devastated by weeds. We compared mechanical weeding
and hand weeding treatment (RB+MW, RB+HW). Since weed density was much lower than that in 2009, weeds were examined only at the harvest (132 DAT). In 2009, we detected no significant increases in the tiller number or yield of rice (Table 3), whereas in 2010, we observed a significant increase in tiller number in plots that had been hand weeded, although not in plots that had been mechanically weeded. Notably, however, the combination of rice bran application and either mechanical or hand weeding was found to double the yield of rice (Table 3). In addition, we examined the DO and turbidity of surface water in both years. In 2009, RB decreased DO at 9 DAT and increased turbidity at 51 DAT. The density of M. vaginalis positively correlated with DO and R. indica negatively correlated with turbidity (Figure 4). A similar trend was found in 2010 (data not shown).

**Exp. 4: Effect of repeated puddling and side-row fertilizer application**

Weed density, biomass, and rice growth are shown in Table 4. In 2011 and 2014, mechanical weeding after transplanting and rice bran application were effective. Therefore, the emergence of weeds was not as serious as in Exp. 3. The dominant weed species were M. vaginalis, Sagittaria trifolia L., and Eleocharis kuroguwai Ohwi. In particular, M. vaginalis was the most dominant weed. Puddling 4 and 6 times before transplanting tended to reduce weed biomass in 2011, but the treatment did not show a statistically significant reduction in density and biomass of weeds, except for the biomass of S. trifolia in 2011. In 2014, neither repeated puddling nor side-row fertilizer application affected rice yield. However, repeated puddling increased shoot dry weight at the maximum tillering stage (Table 4). Soil mineral nitrogen immediately after transplanting was found to correlate positively with the panicle number (Figure 5).

**Discussion**

We investigated the changes in rice yields of organic farming with winter flooding compared with conventional and organic farming in Exp. 1 and 2, over periods of 9 and 5 years, respectively. In Exp. 1, the yield gap between organic farming with winter flooding and conventional farming became smaller in the first 3–5 years after the conversion from conventional farming to organic farming with winter flooding, but became larger after 5 to 6 years. A similar trend was observed during the first few years in Exp. 2. The yield trends in the first several years in the present study were consistent with several previous studies on organic farming (Asai et al., 2016; Saitoh et al., 2001; Tamaki et al., 2002). However, most studies on the changes in yield in organic farming, including organic farming with
winter flooding, were conducted only for the first several years. Furthermore, few studies have been compared with conventional cultivation (Gliessman et al., 1996; Mäder et al., 2002; Neera et al., 1999). In this study, we observed yield trends for 9 and 5 years in Exp. 1 and 2, respectively, which are longer periods than those in previous studies. Although the factors underlying the effects of organic farming with winter flooding on rice yield are not entirely clear in this study, the findings of previous studies on organic farming may provide some insights. The yield gap becoming greater several years after the conversion is considered to be caused by weed damage (Oomori, 2015; Saitoh et al., 2001). Indeed, we observed an abundance of weeds in Exp. 1 and 2 but did not take measurements.

In Exp. 3, we found that the total weed density decreased in response to the application of rice bran and tended to decrease when this treatment was performed in conjunction with mechanical weeding (Table 3). Although we failed to detect any significant increases in the tiller number and yield of rice in 2009, a reduction in weed biomass and doubling of rice yield were observed in 2010 in response to the combined application rice bran and mechanical weeding (Table 3). These results could be linked by the fact that the weed density in 2010 was much lower than that in 2009, suggesting that rice bran application and mechanical weeding may be effective in the field where weed density is not so high, while the efficacy of these weeding methods may be of limited applicability where weed density is very high. Rice bran

Figure 2. Rice yield in experiment 1 (2009–2017). Bars indicate standard errors (n = 3). ANOVA indicates the significance of the farming methods, years, and interaction effects. **p < 0.01. Different letters indicate significant differences among years. (Tukey’s test, p < 0.05). CF: Conventional farming, WOF: Organic Farming with winter flooding.

Figure 3. Rice yield in experiment 2 (2013–2017). Bars indicate the standard error of years. CF: Conventional farming, OF: Organic farming, WOF: Organic Farming with winter flooding.
Table 3. Weed density, dry weight, tiller number of rice, and brown rice yield in rice bran application and mechanical weeding in experiment 3 (2009, 2010).

| Sampling date (year) | Treatments | Weed density m⁻² | Weed dry weight g m⁻² | Rice | Tiller number m⁻² | Brown rice yield g m⁻² |
|---------------------|------------|------------------|------------------------|------|------------------|------------------------|
|                     |            | M. vaginalis     | E. oryzicola           | R. indica | Other 13 species | Total | M. vaginalis | E. oryzicola | R. indica | Other 13 species | Total |                      |
| 51 DAT (2009)       | Control    | 212.0 a          | 21.3 ab                | 824.0 a | 105.3 a          | 1162.7 a | -           | -           | -         | -           | 342    | -                      |
|                     | RB         | 114.7 b          | 53.3 a                 | 57.3 b  | 32.0 b           | 257.3 b  | -           | -           | -         | -           | 350    | -                      |
|                     | RB+MW      | 104.0 b          | 13.3 b                 | 173.3 b | 37.3 b           | 172.0 b  | -           | -           | -         | -           | 316    | -                      |
|                     | Treatment  | ns               | ns                     | ns      | ns               | ns      | ns          | ns          | ns        | ns          | ns     | ns                      |
|                     | ANOVA      |                   |                        |         |                  |         |             |             |           |             |        |                         |
| 141 DAT (2009)      | Control    | 125.6            | 7.8 a                  | 740.0 a | 203.8 a          | 1077.6 a | 112 a       | 20.6 a     | 158.7 a   | 119.8       | 350    | -                      |
|                     | RB         | 73.7             | 40.0 a                 | 143.9 b | 16.5 b           | 274.2 b  | 142.3       | 84.3 ns    | 32.0 b    | 142.3       | 316    | -                      |
|                     | RB+MW      | 51.7             | 2.4 a                  | 36.8 b  | 16.4 b           | 107.3 b  | 106.4       | 47 ns      | 113.6 ns  | 106.4       | 316    | -                      |
|                     | Treatment  | ns               | ns                     | ns      | ns               | ns      | ns          | ns          | ns        | ns          | ns     | ns                      |
|                     | ANOVA      |                   |                        |         |                  |         |             |             |           |             |        |                         |
| 132 DAT (2010)      | RB         | 74.1 a           | 9.8                    | 0.0     | 5.8              | 89.7 a  | 29.2 a      | 113.7 a    | 1.0 test   | 102.2       | 153.0 a | 138 b       |
|                     | RB+MW      | 67.4 ab          | 0.0                    | 0.0     | 0.5              | 67.9 ab | 0.0        | 64.6 b     | 0.0        | 0.2         | 64.8 b  | 345 a       |
|                     | RB+HW      | 16.2 b           | 0.0                    | 1.0     | 0.0              | 17.2 b  | 0.0        | 4.8 b      | 0.1        | 0.0         | 4.9 b   | 387 a       |
|                     | Treatment  | ns               | ns                     | ns      | ns               | ns      | ns          | ns          | ns        | ns          | ns     | ns                      |
|                     | ANOVA      |                   |                        |         |                  |         |             |             |           |             |        |                         |

Control: without weeding; RB: rice bran application; MW: mechanical weeding; HW: hand weeding.
ANOVA indicates the significance of the treatment effect. ***p < 0.001; **p < 0.01; *p < 0.05; †p < 0.1; ns not significant (n = 3).
Different letters indicate significant differences among the treatments. ns indicates not significant (Tukey’s test p < 0.05, n = 3).
application is known to be effective in weed control (Nakai & Toritsuka, 2009; Nakayama et al., 2002; Nozoe et al., 2016, 2012), while the combination of rice bran application and mechanical weeding may enhance the efficacy of weeding. In Exp. 3 in 2010, the weed density and biomass in plots with hand weeding and rice bran application were lower than in plots with mechanical weeding. However, since hand weeding was highly labor-intensive and no significant difference in yield was found between hand weeding and mechanical weeding (Table 3), mechanical weeding should be preferred for practical reasons. Recently, various weeding machines, including a weeding robot, have been developed (e.g., Yasuda et al., 2017); therefore, mechanical weeding will be more effective.

The efficacy of rice bran application may differ depending on the weed species. In 2009, rice bran application suppressed two of the three dominant weed species, but not E. oryzae. Rice bran application decreased the dissolved oxygen level of paddy surface water during the early growth season of rice (Figure 4A), and thus, its application may suppress the emergence of weed seedlings because oxygen is required by most weed seeds (Kataoka & Kim, 1978). Rice bran application also increased the turbidity of paddy surface water (Figure 4B). When the surface water became turbid, light was prevented from reaching the soil, and weed growth was inhibited. Nozoe et al. (2012), Nozoe et al. (2016) found that M. vaginals was suppressed with high EC in soil solution and higher settled soil volume of the surface layer. These findings are closely related to the development of reductive conditions in soil with rice bran application. Such changes in physicochemical properties of the surface soil layer with rice bran application may cause unfavorable conditions for weed emergence. E. kuroguwai germinates from the deep layer of the plowed soil layer, so it is difficult to control with rice bran application and mechanical weeding, which only affects the shallow layer of the plowed soil layer. To control E. kuroguwai, it is recommended to dig the seeds up by deep ploughing and then to expose the seeds to dry and cold conditions (NARO, 2016). It may be difficult to adopt such deep ploughing during winter flooding. Further studies are needed.

On the other hand, mechanical weeding is a physical process; i.e., digging weeds up. Mechanical weeding also increases turbidity. By combining various weed control mechanisms, weeding becomes more effective. Therefore, farmers should apply multiple weed control methods rather than a single method.

In addition to rice bran application and mechanical weeding, we tested the other two weed control methods: repeated puddling and side-row fertilizer application. In contrast to the findings of previous studies (Kaneda et al., 2006; Miyake, 1999; Sugawara et al., 2004), our results had no significant effect on weeds, except for S. trifolium in 2011 (Table 4). However, the shoot dry weight at the maximum tillering stage and panicle number at harvest increased with puddling (Table 4). Puddling of paddy soil often increases soil nitrogen mineralization by destroying macro-soil aggregates (Sakanoue & Matsubara, 1967). With this previous study, the result may indicate that levels of soil inorganic
Table 4. Weed density, dry weight, and rice growth in repeated puddling and fertilizer application methods in experiment 4 (2011, 2014).

| Year | Fertilization method | Puddling number of times | Treatments | Weed density m⁻² | Weed dry weight g m⁻² | Rice Shoot dry weight at Maximum tillering stage g m⁻² | Number of panicles m⁻² | Brown rice yield g m⁻² |
|------|----------------------|--------------------------|------------|-------------------|-----------------------|---------------------------------------------------|-----------------------|------------------------|
| 2011 | Broadcasting (B)     | 2                        | 5.7        | 1.6               | 1.8                   | 9.1                  | 6.6                   | 1.5                  | 10.1               | -                     | 271                   | 503                   |
|      |                      | 4                        | 5.7        | 0.6               | 2                     | 8.3                   | 1.4                  | 0.4                  | 1                  | 2.8                  | -                     | 280                   | 533                   |
|      |                      | 6                        | 5.0        | 0.1               | 0.8                   | 5.9                   | 0.8                  | 0.4                  | 0.2              | 1.4                  | -                     | 318                   | 550                   |
| ANOVA|                      |                          | ns         | ns                | ns                   | ns                   | ns                   | ns                   | ns                | ns                  | ns                    | ns                    | ns                    |
| 2014 | Broadcasting (B)     | 2                        | 53.9       | 4.2               | 29.2                  | 87.3                 | 16.3                 | 4.4                  | 22                | 42.7                 | 465                   | 359                   | 507                   |
|      |                      | 4                        | 25.5       | 4                 | 21.1                  | 50.7                 | 7.5                  | 0.8                  | 8.9              | 17.2                 | 549                   | 428                   | 551                   |
|      |                      | 6                        | 57.1       | 3.4               | 8.7                   | 69.2                 | 23.4                 | 3.2                  | 5.3              | 31.8                 | 532                   | 363                   | 527                   |
|      | Side-row (SR)        |                          | 31.8       | 0.4               | 24.9                  | 57                   | 18.7                 | 0                   | 10.4             | 29.2                 | 525                   | 362                   | 499                   |
|      |                      | 4                        | 39.1       | 7.8               | 21.3                  | 68.3                 | 6.3                  | 4.1                  | 13.2             | 23.5                 | 587                   | 402                   | 574                   |
|      |                      | 6                        | 28.4       | 0.7               | 36.6                  | 65.6                 | 12.1                 | 0.9                  | 24.4             | 37.5                 | 473                   | 355                   | 501                   |
| ANOVA|                      |                          | ns         | ns                | ns                   | ns                   | ns                   | ns                   | ns                | ns                  | ns                    | ns                    | ns                    |
|      | Fertilization        |                          | ns         | ns                | ns                   | ns                   | ns                   | ns                   | ns                | ns                  | *                     | †                     | ns                    |
|      | Puddling number      |                          | ns         | ns                | ns                   | ns                   | ns                   | ns                   | ns                | ns                  | †                     | ns                    | ns                    |
|      | interaction          |                          | ns         | ns                | ns                   | ns                   | ns                   | ns                   | ns                | ns                  | †                     | ns                    | ns                    |

Except for rice shoot samples at maximum tillering stage (65 DAT, 2014), all weeds and rice samples were collected at the rice harvesting stage (125 DAT at 2011, 129 DAT at 2014). ANOVA indicates the significance of the treatment effect. *p < 0.05; †p < 0.1; ns means not significant (n = 3)
nitrogen are increased by repeated puddling, which in turn has the effect of enhancing panicle production and shoot dry weight. Our results also indicate that the number of tillers is correlated with soil inorganic nitrogen, and the level of soil inorganic nitrogen was partially increased by repeated puddling (Figure 5). The findings of several studies have indicated larger rice plants can suppress weed growth by competing for light and nutrients (Garrity et al., 1992; Gibson et al., 2001; Graf et al., 1990; Koarai, 2004). Thus, if repeated puddling and application of fertilizer can effectively promote rice plant growth, these methods might represent promising approaches for weed control.

In this study, we investigated the yield changes of organic farming with winter flooding compared with conventional and organic farming and tested the combination of several promising weed control methods. These results show that the yield of organic farming with winter flooding is lower than that of conventional farming and is similar to the yield of organic farming. The yield of organic farming with winter flooding for the first several years after conversion from conventional farming to organic farming with winter flooding tended to increase over the years, but after that the yield decreased, which could be caused by the flourish of weeds. The promising weed control methods in our testing methods were the combination of rice bran application and mechanical weeding. In addition to these two methods, repeated puddling and side-row fertilizer application were effective for weed control to some degree. As the next step, the more detailed quantitative analysis of each method for reducing weed abundance should be investigated. Our results help to promote ecologically friendly agriculture that supports biodiversity, such as winter flooding paddy rice production system.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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