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RESEARCH ARTICLE

Mechanical weeding frequency enhanced rice growth by competing with weeds for N absorption in an organic field in northeastern Japan

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ABSTRACT

Organic rice fields face a variety of problems, such as nutrient deficiency, weed growth, and pest infection. Weeds have a greater impact on organic than on conventional rice cultivation. Mechanical weeding with a rotary weeder machine is a common practice employed by Japanese organic farmers. Here, a two-year field experiment was conducted in one organic rice plot located at Yamagata University Farm, Tsuruoka, Japan. This study aims to improve the competitiveness of rice to weeds and the effective application of mechanical weeding in organic rice fields. ‘Sasanishiki’ rice was planted from May to September 2020 and 2021. Different weeding frequencies (0, 2, 4, 6, and 8 times) were employed from 0 to 49 DAT (days after transplanting). Rice and weeds were sampled five times to determine their biomass, density, and nitrogen (N) uptake. The findings showed that weeding 8 times induced the highest biomass, tiller number, N concentration, and N uptake in rice. In contrast, these were significantly suppressed in weeds. For instance, during eight weedings, rice biomass recorded was 2 and 2.6 times greater than that in the control in 2020 and 2021, respectively. Also, the N uptake in the former was 2.5- (2020) and 3-fold (2021) higher than that in the latter. Biomass and N uptake elevated as the weeding frequency increased from 2 to 8 times. This was probably due to the enhanced competitiveness of rice. Additionally, while considering efficient weeding frequency, although six weedings produced biomass lower than eight weedings, it did not vary markedly from the biomass and N uptake of eight weedings. Additionally, the harvest index was slightly higher at 6 times. These findings show that a weeding frequency of 6 times was the most economical and effective with potentially the greatest organic rice yield.

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N absorption; plants competitiveness; organic rice farming; paddy soil; weeds management

1. Introduction

The worldwide population keeps growing and is predicted to reach ~9.1 billion in 2050 (Parker 2012). Under these conditions, food production must meet the world's demand sustainably (Wezel et al. 2014). As an alternative to conventional farming, organic farming is claimed to be more sustainable by offering several benefits. Organic farming can maintain environmental quality by preserving biodiversity and improving the soil and water quality per unit area by avoiding the use of chemicals in the field (Cheng, Okamoto, et al. 2015; Seufert, Ramankutty, and Mayerhofer 2017). Although it represents only 1% of the world's agricultural area, organic farming is known for high-quality products (Seufert, Ramankutty, and Mayerhofer 2017). However, the yields tend to be lower ranging from 5% to 34%, than those in conventional farming, depending on the system employed and site characteristics (Reganold and Wachter 2016; Seufert, Ramankutty, and Foley 2012).

Weeds are a major biotic constraint on crop production, contributing up to 45% in yield loss (Dass et al. 2017; Korav et al. 2018; Ramesh et al. 2021). Following a significant decrease

in crop productivity due to weed pressure, effective and sustainable weed control is necessary to meet the global food demand while conserving ecosystems and biodiversity (Cheng, Takei, et al. 2015; Jabran et al. 2015; Maclaren et al. 2020; Sardana et al. 2017). The widespread paradigm of weed control in developed countries has recently centered on two major tools: herbicides and tillage for weed removal. However, these methods negatively affect the environment, one example being the common occurrence of herbicide resistance (Maclaren et al. 2020). In organic agriculture, the impacts of culture techniques, e.g., fertilization and weed management on crop – weed interactions typically appear more slowly than those in conventional agriculture. Direct physical weed control techniques, such as harrowing, hoeing, or raking, are less effective than herbicides (Bärberi 2002). However, a previous study introduced mechanical weed control methods using autonomous weeding machines in rice fields as an alternative to herbicide use. This method can reduce the chemical load on the environment, loosen the soil, and promote rice growth (Liu et al. 2023).

Despite the evident risk that weeds bring to organic crop production, little focus has been dedicated to weed control (Bàrberi 2002). This study aimed to elucidate the impacts of weeding frequency on rice growth and its competitiveness with weed growth. Thus, experiments were conducted in the organic fields in Japan over two consecutive years, 2020 and 2021. Our previous study noted that a higher frequency of mechanical weeding provides notable benefits not only in suppressing weeds but also in improving rice growth, especially in increasing nitrogen (N) uptake (Maimunah et al. 2021). In this study, we hypothesized that more frequent mechanical weeding in organic farming would strengthen the competitiveness of rice against weeds. As a result, rice could absorb more nutrients, especially N. Thus, organic rice would grow better and potentially produce a higher yield.

2. Materials and methods

2.1. Site description

A two-year field experiment was conducted using rice (*Oryza sativa* L.) cv. 'Sasanishiki' during the growing season from May to September 2020 and 2021 at the Yamagata University Farm, Takasaka, Tsuruoka, Yamagata prefecture, northeastern Japan (38°42' N; 139°49' E). Study plots were established in a 30 × 100 m paddy field that was managed organically without any fertilizer or external organic material input except the residues of plants (rice straw and weeds) originally grown in the plot. Based on the Japan Meteorological Agency database of the Tsuruoka Meteorological Observatory (<http://www.data.jma.go.jp/obd/stats/etrn/index.php>). The annual mean temperature and precipitation during the rice growth seasons were 22.4°C and 1004 mm for 2020 and 21.9°C and 697 mm for 2021, respectively (Figure S1). The soil, classified as inceptions, contained 20.1 g kg⁻¹ organic carbon (C) and 1.80 g kg⁻¹ total nitrogen (TN), with a pH of 5.43 (H₂O, 1:5 w/w), respectively. The soil texture was categorized as sandy loam consisting of silt:clay:sand at 29.5%:13.6%:56.9%.

2.2. Treatments and management

Rice was transplanted on May 26 and May 25 and harvested at 119 and 120 days after transplanting (DAT) in 2020 and 2021, respectively. Mechanical weeding was applied by employing a rotary machine weeder between 7 and 49 DAT (Table S1). The five weeding frequencies applied were 0 (non-weeding), 2, 4, 6, and 8 times. For each frequency, four sub-plots each of 20 × 2.4 m were set as replications.

2.3. Sampling and analysis

Rice plants were sampled at five development stages: tillering (29 DAT), stem elongation (52 DAT), panicle initiation (72 DAT), flowering (97 DAT), grain filling, and maturation (119 and 120 DAT) in 2020 and 2021, respectively (Table S1). Rice and weeds were randomly sampled together, with a 30 × 15 cm metal frame sampler. The plants were washed with tap water to remove the soil. Rice and weeds were separated and placed inside labeled paper bags and then oven-dried at 70°C to

a constant weight to ascertain the biomass. The roots, stems + leaves, and ears of rice were analyzed separately. Meanwhile, the weed parts were not separated likewise. The oven-dried samples were finely ground with a Force Mill YDK Y-308 B (Osaka Chemical Co. Ltd., Osaka, Japan) to determine the TN content. A dry combustion method was applied to identify the TN content with a Sumigraph NC 220F Analyzer (Sumika Chemical Analysis Service Ltd., Osaka, Japan). Lastly, the N uptake was calculated by multiplying the plant biomass (g m⁻²) with the N concentration (%). The total N concentration in rice was determined by calibrating the N levels of each part (roots, stems, and ears) to the total biomass. Meanwhile, the total weed N concentration was ascertained by calibrating the N contents of each species to the total biomass.

2.4. Statistical analysis

Data were analyzed with SPSS 22.0 for Windows (IBM Corp., NY, U.S.A.). The two-year data for rice and weed biomass, number, and harvest index were subjected to a two-way analysis of variance (ANOVA), while the notation of significance with letters was based on one-way ANOVA in 2020 and 2021. The N concentration and uptake data were subjected to one-way ANOVA. The means of each year were compared employing Tukey's honestly significant difference test at $p < 0.05$. Correlation and regression analyses utilized Excel 365 (Microsoft Corp., WA, U.S.A.).

3. Results

3.1. Rice and weed growth

The accumulative rice (i.e., roots, stems, and ears) and total weed biomass at harvest during the 1st and 2nd years of the rice growing period are shown in Figure 1(a,b). The eight weeding treatments induced the highest rice biomass at 1032.81 and 1145.73 g m⁻² in 2020 and 2021, respectively. Weeding for six times produced the second-highest rice biomass at 17.77% and 27.23% but did not vary significantly with that at eight times in 2020 and 2021, respectively. The least biomass was recorded in the non-weeded plots at 508.49 and 438.85 g m⁻² in 2020 and 2021, respectively. Rice biomass at zero to six weedings was greater in 2020 than in 2021. Weeding for eight times resulted in higher rice biomass in 2021. However, rice biomass did not vary remarkably between six and eight times in 2021. As expected, weeding frequencies negatively affected weed biomass, with eight times demonstrating the maximal suppression at harvest in both years (Figure 1(b)). At harvest time, compared with non-weeding, eight weedings reduced the weed biomass by 80.4% and 54.1% in 2020 and 2021, respectively. Weed biomass was relatively higher at five times of sampling for all treatments in 2021 (Table S3). Statistical analysis showed that the weeding frequency remarkably ($p < 0.001$) influenced rice biomass since the 2nd sampling time, whereas the weed biomass was significantly ($p < 0.001$) affected by the weeding frequency since the 1st sampling time (Table S2 and S3). These findings indicate that weeding frequencies had a marked impact on the total

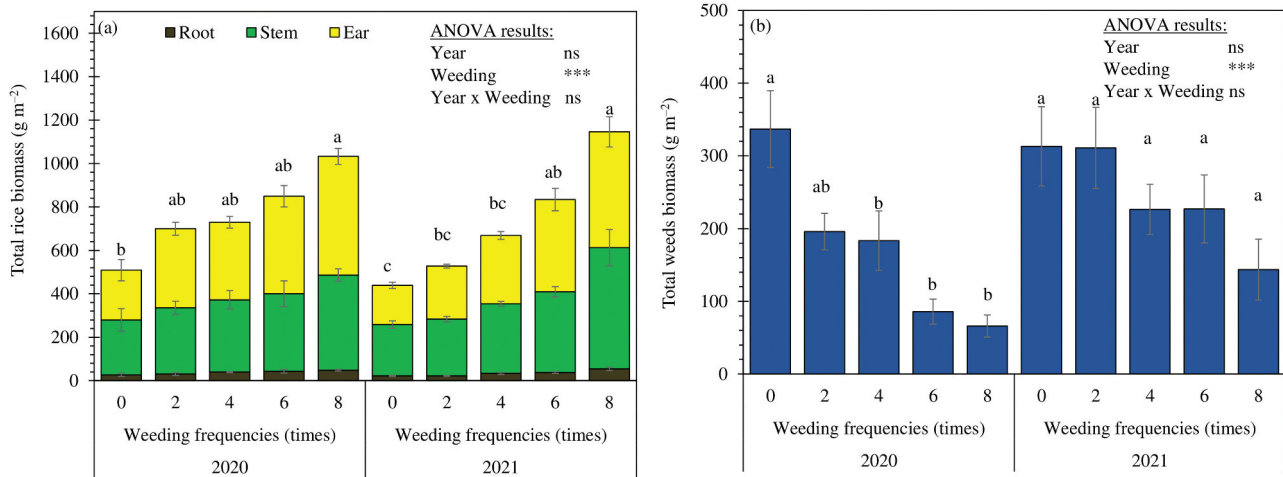


Figure 1. Effect of weeding frequencies on rice (a) and weeds (b) biomass at harvest time in 2020 and 2021. The number of 0, 2, 4, 6, and 8 were the frequencies of mechanical weeding until 49 DAT. The bars represent the standard error ($n = 4$). ANOVA results meaning, ns: no significance; ***, $p < 0.001$. Different letters indicate a significant difference among the five treatments at each year by Tukey's HSD at $p < 0.05$.

biomass of rice and weeds at harvest time (Table S4), especially in 2021 (Figure S2). The total biomass of rice and weeds elevated from 0 to 8 times. In general, the harvest index was slightly greater in 2020 than in 2021 (Table S5). Interestingly, the highest proportion of harvest index at 56.24% and 52.86% was reached at six weeding in 2020 and 2021, respectively.

3.2. Fractions of plant number and biomass between rice and weeds

In terms of density, the percentage of plant number/tiller and biomass between rice and weeds were compared at the harvest stage, which is presented in Figure 2. The major weed varieties were *Echinochloa* weeds, *Monochoria vaginalis* (Burm. f.) Kunth (*M. vaginalis*), *Schoenoplectus juncooides* (Roxb.) Palla (= *Scirpus juncooides* Roxb. var. *ohwianus* T. Koyama) (*S. juncooides*), and *Eleocharis kuroguwai* Ohwi (*E. kuroguwai*). The *Echinochloa* weeds can include *Echinochloa crus galli* (L.) Beauv. var. *crus-galli*, *Echinochloa*

oryzicola Vasing. (= *Echinochloa oryzoides* (Ardo) Fritschare) and/or others. However, this study did not confirm which species of *Echinochloa* weed(s) corresponded to the plant(s) shown here.

Without weeding, weeds were 88.38% and 85.35% over rice in 2020 and 2021, respectively (Figure 2(a)). Mechanical weeding from two to eight times enhanced the dominance of rice in the field. The highest proportions of rice tiller numbers among the total plant numbers reached 49.6% in 2020 and 48.35% in 2021 at eight weeding. Weeds dominated the plant density even at eight times, whereas rice dominated the weeds in biomass at 0–8 times, ranging from 60.15% to 94.0% in 2020 and 58.37% to 88.86% in 2021 (Figure 2(b)). The increment in rice biomass at eight weeding was 56.3% and 52.2% in 2020 and 2021, respectively. Concerning the plant number and biomass at harvest time, weeding frequencies markedly affected all parameters (Table S2 to Table S7), while the differences between years affected weed number and biomass (Table S3 and S7).

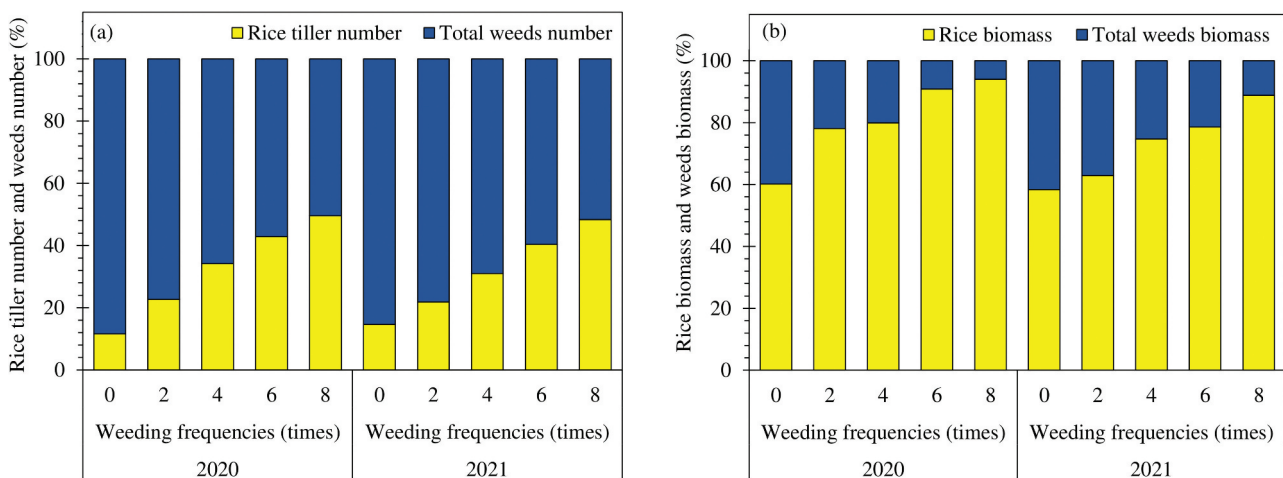


Figure 2. Effect of weeding frequencies on rice:weed percentage on (a) plant number and (b) biomass at harvest time in 2020 and 2021. The number of 0, 2, 4, 6, and 8 were the frequencies of mechanical weeding until 49 DAT.

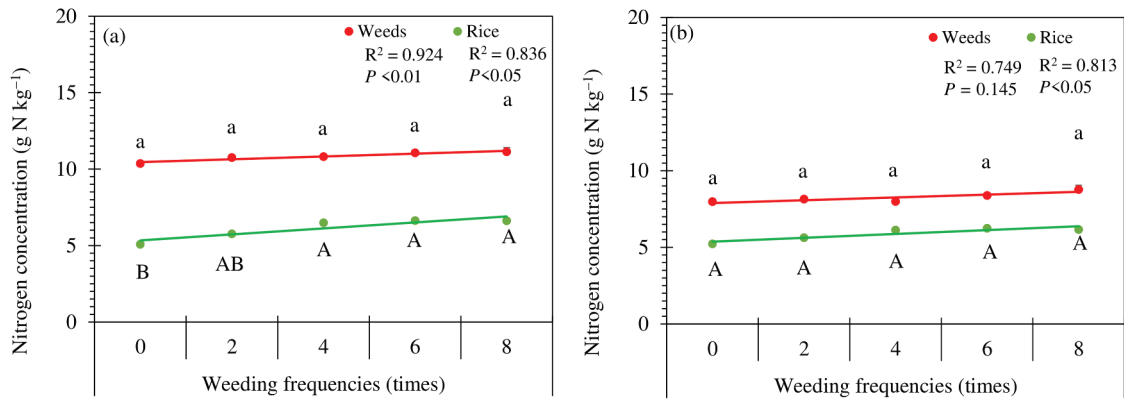


Figure 3. Effect of weeding frequencies on rice and weeds nitrogen concentration at harvest time in (a) 2020 and (b) 2021. The number of 0, 2, 4, 6, and 8 were the frequencies of mechanical weeding until 49 DAT. The bars represent the standard error ($n = 4$). Different letters indicate a significant difference among the five treatments at each year by Tukey's HSD at $p < 0.05$.

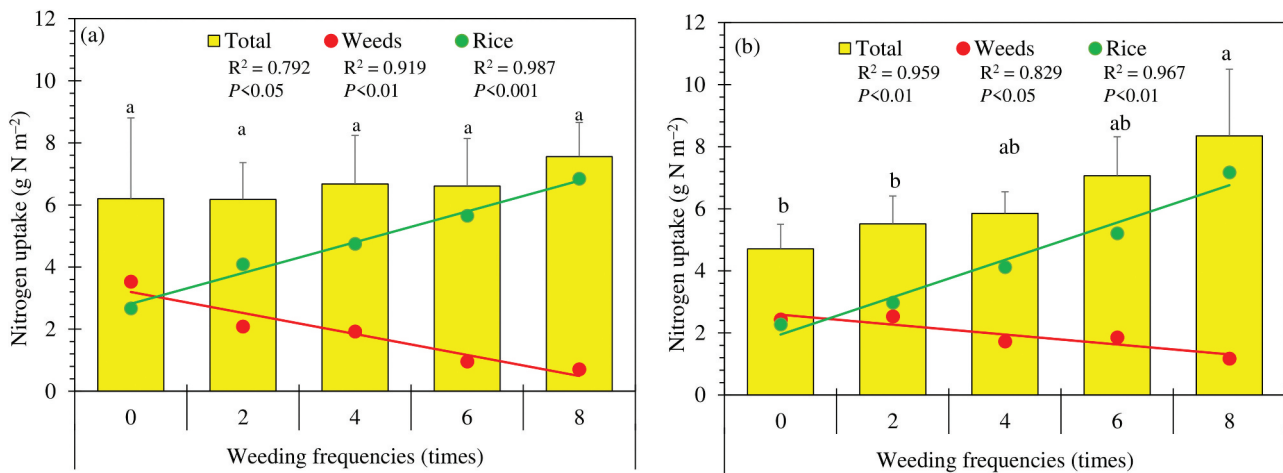


Figure 4. Effect of weeding frequencies on each of rice and weeds nitrogen uptake, and total nitrogen uptake of rice and weeds at harvest time in (a) 2020 and (b) 2021. The number of 0, 2, 4, 6, and 8 were the frequencies of mechanical weeding until 49 DAT. The bars represent the standard error ($n = 4$). Different letters indicate a significant difference among the five treatments at each year by Tukey's HSD at $p < 0.05$.

3.3. N concentration and uptake in rice and weeds

A comparison of the rice and weed N concentrations at harvest indicated that a higher frequency of mechanical weeding enhanced these levels in both years (Figures 3 and 4). The N concentration in weeds was higher than that in rice, ranging from 10.36 to 11.13 g N kg⁻¹ in 2020 and from

7.97 to 8.77 g N kg⁻¹ in 2021. Meanwhile, the rice N levels ranged from 5.08 to 6.62 g N kg⁻¹ in 2020 and from 5.22 to 6.16 g N kg⁻¹ in 2021. N concentrations in rice and weeds were similar in the 1st and 2nd samplings (Table S8 and S9). However, rice had lesser N than weeds from the 3rd sampling (72 DAT).

Table 1. Nitrogen concentration and uptake of rice and weeds at harvest time in 2020 and 2021. Values are given as mean \pm standard error (SE).

Year	Weeding frequencies	Rice		Weeds	
		N Concentration (g N kg ⁻¹)	N Uptake (g N m ⁻²)	N Concentration (g N kg ⁻¹)	N Uptake (g N m ⁻²)
2020	0	5.08 \pm 0.28	2.67 \pm 0.67	10.36 \pm 0.32	3.53 \pm 0.64
	2	5.77 \pm 0.33	4.09 \pm 0.61	10.75 \pm 0.57	2.08 \pm 0.24
	4	6.49 \pm 0.45	4.75 \pm 0.55	10.81 \pm 0.53	1.93 \pm 0.38
	6	6.63 \pm 0.17	5.65 \pm 0.80	11.06 \pm 0.51	0.96 \pm 0.22
	8	6.62 \pm 0.15	6.85 \pm 0.53	11.13 \pm 1.41	0.71 \pm 0.16
2021	0	5.22 \pm 0.35	2.27 \pm 0.14	7.97 \pm 0.53	2.43 \pm 0.30
	2	5.62 \pm 0.23	2.98 \pm 0.20	8.14 \pm 0.67	2.54 \pm 0.51
	4	6.12 \pm 0.46	4.12 \pm 0.47	7.98 \pm 0.80	1.73 \pm 0.14
	6	6.24 \pm 0.19	5.21 \pm 0.48	8.38 \pm 0.61	1.85 \pm 0.32
	8	6.16 \pm 0.26	7.17 \pm 1.28	8.77 \pm 0.78	1.17 \pm 0.28
ANOVA					
Year		ns	ns	***	ns
Weeding		**	***	ns	***
Year x Weeding		ns	ns	ns	ns

ANOVA results meaning, ns: no significance; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$ based on the Tukey's HSD test.

N absorption showed the same trend as the N concentration in rice, with more weeding enhancing N absorption (Table 1). Concerning 5-time sampling, the N uptake in rice elevated from the first sampling to the harvest day (Table S10). Meanwhile, the opposite trend was observed in N uptake in weeds, which was inversely proportional to the weeding frequency. Rice N uptake at harvest time ranged from 2.67 to 6.8 g N m⁻² and 2.27 to 7.17 g N m⁻² (0–8 times weeding) in 2020 and 2021, respectively. The weed N uptake at harvest time varied from 3.53 to 0.71 g N m⁻² and from 2.43 to 1.17 g N m⁻² (0–8 times) in 2020 and 2021, respectively. Statistical analysis results indicate that weeding frequencies significantly impeded N uptake in weeds (Table S11).

The different responses in rice and weeds to N uptake were apparent (Figure 4). Weeding positively and robustly affected rice N uptake in both years ($R^2 = 0.987$, $p < 0.001$ in 2020 and $R^2 = 0.967$, $p < 0.01$ in 2021), and total N uptake in rice and weeds in 2021 ($R^2 = 0.959$, $p < 0.01$). Conversely, a significant negative impact of weeding frequencies on N uptake in weeds was observed in 2020 ($R^2 = 0.919$, $p < 0.01$) and 2021 ($R^2 = 0.829$, $p < 0.05$).

4. Discussion

4.1. Effect of mechanical weeding frequencies on the biomass of rice and weeds

The increase in rice biomass was followed by a decrease in weed biomass at higher weeding frequencies consistently observed in the two-year experiment. The existence of weeds significantly decreases rice biomass due to the competition between the two (Namuco, Cairns, and Johnson 2009; Galal and Shehata 2015; Hosoya and Sugiyama 2017). Positively, the more frequently the weeds were removed from the field, rice growth was supported, indicated by 2- and 2.6-fold higher biomass at eight weeding than those in the non-weeded plots in 2020 and 2021, respectively. In line with our result, Hosoya and Sugiyama (2017) reported that the average rice yield was 1.6-times more in the absence of weeds, which was equivalent to six times of weeding in our study; 1.67- and 1.89 times higher than in the non-weeded plots in 2020 and 2021, respectively. Furthermore, mechanical weeding after 5 weeks of rice transplanting effectively controls various weeds, whereas it increases rice biomass by 45% (Liu et al. 2023). Compared with our study, a higher rice biomass increment was reached at six weeding, which was achieved 6 weeks after transplantation (67.02% and 89.97% in 2020 and 2021, respectively). However, at eight times, the enhancement was 103% and 161% in 2020 and 2021, respectively. This result indicates that frequent weeding also multiplies the positive impact on rice biomass.

During the two consecutive years, except for eight weeding, rice biomass was higher in 2020, which could be attributed to enhanced precipitation. Precipitation, soil organic components, and fertilizer application affect rice biomass (Zheng et al. 2023). However, the impact of weed interference is more intense in reducing crop biomass accumulation. As the biomass of *Echinochloa* weeds, *S. juncooides*, and *E. kuroguwai* increased in 2021, they

contributed to an enhancement in the total weed biomass in 2021 (Table S13).

The eight weeding improved rice biomass more in 2021 than in 2020. This result proved that frequently removing weeds in the early rice growing periods prevents their dominance in the field. Adeux et al. (2019) mentioned that specific weeds within the same niche as the crops would decrease productivity the most. Weed domination altered from 2020 to 2021; with eight weeding, the weed population was equal to that in 2021 but with the lowest total weed biomass. This finding was in line with that of Adeux et al. (2019), who concluded that wider weed diversity was related to lower weed biomass and competition with crops.

However, compared with the vegetative stage, the reproductive stage of rice is the most sensitive to both biotic and abiotic stresses. Booting and flowering during this stage affect panicle formation (Fageria 2007). Higher weeding frequencies were beneficial to rice growth during both the vegetative and reproductive stages due to lesser competition. Nevertheless, the harvest index at six weeding was the maximal that could potentially be reached along with the highest yield. Laza et al. (2003) reported that the harvest index is more applicable than biomass when estimating grain yield under suboptimal growing environments.

4.2. The effect of mechanical weeding on the plant number and biomass fractions between rice and weeds

Regarding, plant density, weeds were more dominant than rice due to their large numbers and varieties growing in the field, even on harvest day (Table S7). As the weeding frequencies enhanced, the tiller amount in rice increased, even though it did not outnumber the weed population. Without weeding, the decline in rice tiller numbers reached 20% and 50% at eight weeding in 2020 and 2021, respectively. This result was in line with a previous study (Tian et al. 2020) which stated that the increment in weeds density decreased rice spikes by $\leq 20\%$. Furthermore, filled grains per panicle and rice yield declined markedly due to these weeds. Liu et al. (2023) also found that the rice tiller number is enhanced by 7–23% with mechanical weeding. Thus, more frequent weeding would influence rice competitiveness by increasing its density.

4.3. The effect of mechanical weeding on the competition for N uptake between rice and weeds

N, the most limiting nutrient factor in crop production, has a critical role in influencing the final protein contents of brown and white rice (Blumenthal et al. 2008). N is imperative for rice and weed growth. Competition for N uptake between rice and weeds is inevitable, especially in organic farms. Based on the alterations in N concentration, weeds absorbed more N than rice during the 2 years (Figure 4). Similar results were reported by (Maimunah et al. 2021). In particular, compared to other rice growth stages, rice required lesser N concentration at the grain-filling stages (Burgos et al. 2006; Sun et al. 2019). The N uptake in weeds was inversely proportional to the weeding frequency, unlike that of rice (Figure 4(a)). These findings indicate that high

weeding frequencies can effectively reduce weed biomass and enhance productivity in high-yield rice. It also demonstrates the contribution of the weeding effect; i.e., greater weeding frequencies can suppress weed biomass and competition, thereby allowing rice plants and weeds left in the field to absorb more N (Maimunah et al. 2021).

Pratap, Verma, and Dass (2023) pointed out that substantial weed biomass in the field enhances the depletion of available nutrients in soils. Thus, weed removal is crucial in organic farms. Furthermore, when the biomass residues are incorporated into the soil, benefits such as improving water-holding capacity, alleviating soil acidification, enhancing organic C content, and stimulating microbial activities are obtained (Goswami, Mondal, and Mandi 2020). Weeds and rice plant residues can be an N source in paddy fields (Hayashi 2022; Utami et al. 2020). The incorporation of fallow weeds for several consecutive years supports the sustainable and stable yield in organic rice farming by contributing 16.9% of the N uptake by rice plants (Toriyama, Amino, and Kobayashi 2020). Kautsar et al. (2022) also suggested that immature weeds showed greater C decomposition and N mineralization potential than mature weeds. Therefore, higher weeding frequencies would eliminate more interrow weeds, reducing competition for solar radiation and nutrient uptake. Moreover, weed residues have the potential to be a source of N and other nutrients in organic farms.

Variations in weed biomass trends over time are responsible for those in total biomass, which is one reason for the distinct N uptake tendencies between 2020 and 2021. However, rice competitiveness against weeds is urgently needed in Japanese organic rice fields due to large-scale interventions being applied against weeds. In this study, weeding frequencies were proved to statistically impact weed population, biomass, and N uptake negatively. Furthermore, weeding frequencies could increase rice competitiveness with weeds by supporting rice growth and N uptake in the organic field.

5. Conclusions

Increasing rice competitiveness against weeds in the soil is an extremely effective approach for enhancing organic rice yield. This study revealed that increasing the frequency of mechanical weeding can improve rice competitiveness, as evidenced by the elevation in rice biomass and the maximum N absorption at eight weedings. However, no significant change was observed after six weedings, which, however, slightly enhanced the harvest index than at eight weedings. Thus, six weedings can be a reasonably efficient frequency for achieving maximum rice yields in the organic fields of Northeastern Japan.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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