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Effect of Irrigation Regime and Soil Nutrients on the Growth of the Paddy Weed *Heteranthera reniformis* and Rice Grain Yield

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Abstract: The growth of *Heteranthera reniformis*, an invasive alien paddy weed, can be affected by cultivation practices. The experiments were conducted using herbicide-free soil to understand the effects of irrigation regimes and nutrient treatments on the growth of *H. reniformis*, as well as yield parameters while competing with a pre-existing seedbank. The pot experiments were conducted in a randomized complete block design (RBCD) with three replicates and twelve treatments. The four irrigation regimes (IRs): continuous irrigation (CI), soil condition at near saturation (non-puddled) (S), alternate wetting and drying (AWD) irrigation under two conditions [rewatered when the soil water potential reached −25 kPa (25P) and −35 kPa (35P)], and three nutrient treatments (NTs) of 0–0–0 NPK (NT_0), 40–25–30 NPK (NT_1), 80–50–60 NPK (NT_2), kg ha^{-1} were established. The IRs had a significant effect on the growth of *H. reniformis* and other paddy field weeds, and the growth of *H. reniformis* was suppressed in the AWD regimes. NT_2 resulted in more rice panicles, higher grain yield, and increased irrigation water use efficiency (IWUE). The highest grain yield and protein content were observed in S–NT_2 and 25P–NT_2 treatments. The IR and NT can be maintained to prevent yield penalties and reduce the invasiveness of weeds.

Keywords: invasive alien plants; irrigation; paddy weeds; grain yield; weed seedbanks

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1. Introduction

Heteranthera reniformis (Ruiz and Pavon), also called kidneyleaf and mud-plantain, originates from the freshwater wetlands of North, Central, and South America [1,2]. However, this plant has been naturalized in non-native countries such as Hawaii, Australia, and several European countries [3–5]. In Japan, *H. reniformis* was first observed in a flood bypass in Shizuoka City in 1996. Subsequent surveys conducted in 2013 and 2014 revealed its proliferation to 73 sites, including the pesticide-free rice fields and waterways of the town of Kawajima [6]. The invasive nature of this plant is evident, as even the fragments of its stem can develop into a new plant, and its seeds can remain viable for many years [1,3]. The invasive nature of this plant can affect biodiversity and ecosystems. Paddy fields are considered a wetland ecosystem in which various plants and animals coexist during rice cultivation [7], And the infestation of paddy fields by *H. reniformis*, which forms dense floating mats, could significantly impact rice yield [8].

The quality of crops can be degraded because weeds compete with crops for crucial growth factors, including water, sunlight, nutrients, and space [9]. Generally, farmers use mechanical and chemical methods to remove weeds from their fields. Herbicides are only a short-term solution as many weeds develop resistance genes [10]. Additionally, chemicals can harm nontarget organisms, the environment [11–13], and ultimately human health [14,15]. On the other hand, mechanical methods such as mulching and weed pulling are tedious and ineffective for perineal weeds and in large-scale fields. Likewise, the change in irrigation pattern also affects the diversity and density of weed communities, weed infestation, and bacterial compositions [16,17]. Unsaturated soil conditions favor the dominance of grasses, whereas continuous irrigation (CI) favors the growth of aquatic broadleaves and sedges. Moreover, non-puddled saturated irrigation (S) and alternate wetting and drying (AWD) irrigation have been implemented to increase irrigation water use efficiency (IWUE), reduce greenhouse gas emission, and prevent nutrient leaching [16,18–22]. The rice yield in response to AWD irrigation has varied across past studies: some showed similar or improved grain yield in AWD irrigation compared with CI, whereas some showed reduced yield [22,23].

Seedling establishment is affected by many parameters, such as soil pH, temperature, light, flooding time, soil moisture, depth of planting, and soil nutrients. Integrated weed control programs involve management strategies that impede growing and establishing weeds based on individual requirements. AWD studies have a high degree of variation in obtaining the optimal threshold for maximizing rice yield while minimizing irrigation input, in which dryness up to −30 kPa could probably be safe [24,25]. In this study, we hypothesized that changes in irrigation regimes and optimizing nutrient management will affect the growth of *H. reniformis* and crop parameters. This study aimed to determine the effects of *H. reniformis* infestation on grain yield, crop parameters, and water use efficiency in response to different irrigation regimes and nutrient treatments.

2. Materials and Methods

2.1. Field Visits, Sample Collection, and Seed Harvesting

H. reniformis were collected from an infested paddy field in the town of Kawajima, Saitama [35°59′20″ N, 139°29′21″ E], in May 2020, and cultivated in a glass house at Saitama University for seed collection. The plants started flowering in August, and the fruits matured and formed seeds after two weeks. After collecting mature seeds, they were air-dried and kept at 5 °C for two months to break dormancy, and pot experiments were conducted.

2.2. Site Description and Soil Conditions for Pot Experiments

Pot experiments with herbicide-free soil were conducted at Saitama University [35°51′41″ N, 139°36′30″ E] during the rice growing seasons of 2022 (June–October). The soil was clay with 47%, 30%, and 23% clay, silt, and sand, respectively, and the field capacity was 45.3% by volume (gravimetric method). No herbicide was applied to simulate herbicide-free field conditions. The values of the soil's physiochemical properties, such as soil bulk density, pH, electrical conductivity (EC), total carbon (TC), total nitrogen (TN), soil extractable potassium (K), available soil phosphorus (P), and loss on ignition percentage (LOI %), are shown in Table 1.

Table 1. Physiochemical properties of soil before experiment.

Soil Type	Bulk Density g cm^{-3}	pH	EC [mS m^{-1}]	TC [g kg^{-1}]	TN [g kg^{-1}]	K [mg kg^{-1}]	P [mg kg^{-1}]	LOI [%]
Clay	1.09	6.3	107.6	32.3	3.3	449	35.77	6.19

2.3. Plant Material and Cultivation Practices

The experimental setup involved using four irrigation regimes (IRs), three nutrient treatments (NTs), and three replications, resulting in 36 pots. The IRs were continuous irrigation (CI) at a 3 cm depth of water, S (non-puddled, water given every day), and two AWD irrigation techniques [rewatering after reaching soil water potentials of −25 kPa (25P) and −35 kPa (35P)] (Figure 1, Table 2). As mentioned in Table 2, each IR was applied with three nutrient treatments (NTs): 0–0–0 NPK (NT_0), 40–25–30 NPK (NT_1), and 80–50–60 NPK (NT_2), kg ha^{-1}. To maintain the paddy field condition according to the protocol of crop fertilization established by Saitama prefecture, basal fertilizers of 50–60–50% NPK were added to the soil before transplanting, followed by the addition of 25–40–25% NPK on 14 days after transplanting (DAT). Later, a top dressing of 25–25% NK was added 18 days before the heading stage of the rice. A randomized complete block design (RCBD) with three replications was established. Three kilograms of soil was placed into 4.3–L-sized pots (18.6 cm long, 20.5 cm top diameter, and 17.5 cm base diameter). Tensiometers were used to monitor the soil water potential, and the required amount of water was added every evening. Two rice seedlings of Koshihikari with 30 seeds of *H. reniformis* were sowed in June 2022. A total of 100 *H. reniformis* seeds were placed in glass vials with distilled water and kept inside a glass house to evaluate their viability; 95% of the seeds germinated. No weeding or herbicide application was applied throughout the cultivation to emulate an herbicide-free paddy culture.

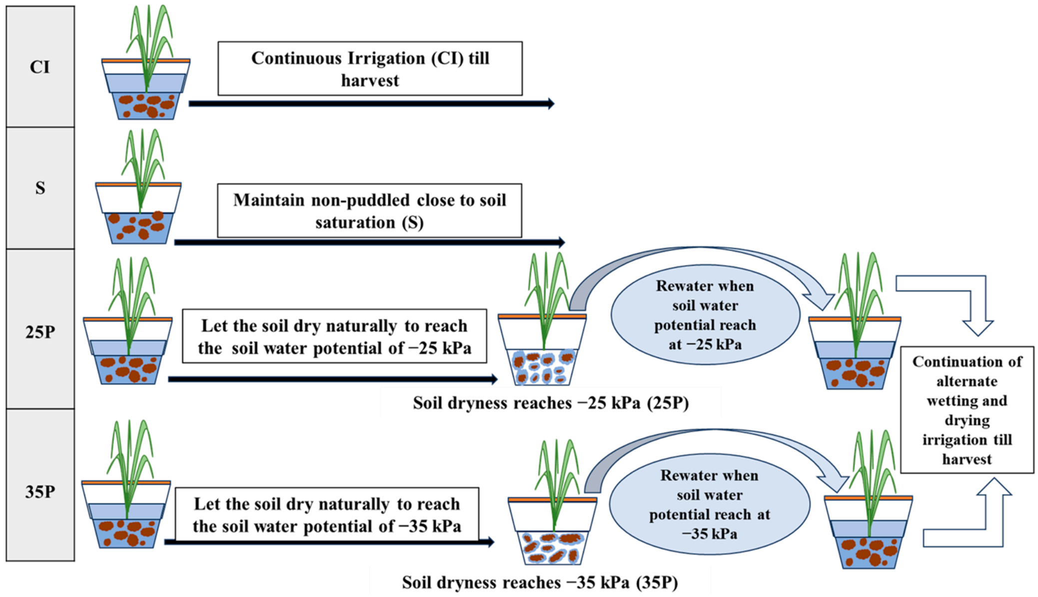

Figure 1. Experimental set-up for irrigation management.

Table 2. Experimental set-up for irrigation regimes, nutrient treatments, application amount, and time.

Irrigations (IRs)	Applied Nutrients (NTs)	Nutrient Amount and Application Time
CI—Continuous flooding at a depth of 3 cm of water	No application (NT_0)(kg ha^{-1}) Nitrogen—0 Phosphorous—0 Potassium—0	1st application Nitrogen—50% Phosphorous—60% Potassium—50% (Before transplanting)
S—Near saturation but non-puddled with daily watering	Half of the recommended quantity (NT_1) (kg ha^{-1}) Nitrogen—40 Phosphorous—25 Potassium—30	2nd application Nitrogen—25% Phosphorous—40% Potassium—25% (14DAT)
25P—Alternate wetting and drying; rewatering after reaching soil water potential of −25 kPa	Recommended quantity (NT_2) (kg ha^{-1}) Nitrogen—80 Phosphorous—50 Potassium—60	3rd application Nitrogen—25% Potassium—25% (18 days before the heading stage of rice)
35P—Alternate wetting and drying; rewatering after reaching soil water potential of −35 kPa		

2.4. Weeds and Crop Parameters Measurement

The number of plants of *H. reniformis* was counted monthly during the experiment. Additionally, all weed species that emerged during the experiments were recorded. All the weeds were collected and cleaned on 120 DAT, the types of weeds were recorded, and their dry weight was analyzed. To achieve a dry weight, they were kept in the oven for 4 h at 105 °C and then kept at 70 °C until we obtained a constant weight. The efficiency of *H.*

reniformis control was calculated by comparing the dry weight of *H. reniformis* in CI with those in other IRs.

2.5. Crop Parameters, Yield, and Water Use Efficiency Assessment

The height of the rice plants and the number of tillers were recorded monthly during rice growth. Plant height was measured manually from the bottom to the tip of the highest leaf. Rice was harvested in the first week of October. After harvest, the numbers of panicles and spikelets were counted. The spikelets were dried at 105 °C for 1 h, then dried at 80 °C until a constant weight was obtained for calculating crop parameters such as grain yield, filled grain percentage, and 1000-grain weight. Grain yield was then adjusted to 14% moisture content (the standard moisture content). The dry weight of roots and straw was obtained after oven drying at 70 °C until a constant weight was obtained. The harvest index (HI) was calculated as the ratio of the total grain yield to the total dry shoot mass. Irrigation water use efficiency (IWUE) was determined by calculating the ratio of grain yield to the amount of irrigation water applied throughout the rice cultivation period. The nitrogen uptake by the rice grains was obtained by multiplying the nitrogen content of rice by rice grain yield and dividing it by 100. The protein content of the rice was generalized by nitrogen percent multiplied by 6.25.

2.6. Soil Analysis

Soil carbon and nitrogen content were determined by using a C–N corder (Yanaco, MT-500, Yanagimoto Co. Ltd, Kyoto, Japan). Soil K was determined by inductively coupled plasma optical emission spectrometers (Shimadzu, Kyoto, Japan) after extraction using the 3050B method [26]. P was extracted using 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5) and analyzed using a QuAAtro39 continuous segmented flow analyzer (BL-Tec, Osaka, Japan; SEAL Analytical, Norderstadt, Germany). LOI% was analyzed by igniting moisture-free samples in a muffle furnace for 2 h at 550 °C. The pH and EC of the soil were determined by preparing a suspension of 1:2.5 and 1:5 (soil–deionized water), respectively, and measured using a portable meter.

2.7. Data Analysis

A two-way ANOVA test was conducted to test the main and interactive effects of IR and NT (IR × NT) on paddy field weeds and crop parameters. The Tukey test separated means for significant treatment effects with significant differences at the $p < 0.05$ probability level. The Kruskal–Wallis test ($p < 0.05$) was applied to non-normal data that included the contents of carbon, nitrogen, and available phosphorous in the soil after the harvest (Wallis, 1952). All statistical analyses were conducted using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Heteranthera Reniformis Infestation and the Emergence of Other Rice Weeds

The study indicated that the dry weight of *H. reniformis* exhibited significant variation among different IRs ($p < 0.001$). However, soil NTs did not have any discernible impact on the dry weight of *H. reniformis*. Even though the seedlings of *H. reniformis* appeared under 25P and 35P at 30 DAT, growth was notably inhibited in later days, which persisted until harvest (Figures 2 and 3).

Various weeds appeared as we did not use herbicides to simulate field conditions (Figure 4). The weeds that emerged during the experiments were *H. reniformis*, *Monochoria vaginalis*, *Lindernia procumbens* [krock.], *Rotala indica* var. *uliginosa*, *Ludwigia* spp., *Ammannia multiflora*, *Cyperus difformis*, *Echinochloa* spp., *and Fimbristylis littolaris*. The IR markedly affected the dry weight of the weeds ($p < 0.001$), whereas NT and IR × NT had negligible effects ($p > 0.05$). Among the weeds that primarily emerged, six species were broadleaf, three were sedges, and one was a grass. We found that the weeds were most diverse in the S regime, with more than ten species. The diversity of the emerged species can be ranked as

S > 25P > CI > 35P. Furthermore, we observed that the total dry weight of the weeds was the highest in the CI and S regimes, with an average weight exceeding 8 g per pot. In contrast, 35P exhibited the lowest dry weight, averaging 4 g per pot. In addition, CI notably impacts the proliferation of aquatic weeds such as *H. reniformis* and *M. vaginalis*. Our findings, as illustrated in (Figure 3), indicate that the control efficiencies of *H. reniformis* in the 25P and 35P regimes were over 80% and almost 100%, respectively, compared with the CI regime, irrespective of NT. Likewise, the control efficiencies of the S regime for *H. reniformis* were 68.04%, 63.3%, and 44.35% with NT_0, NT_1, and NT_2, respectively.

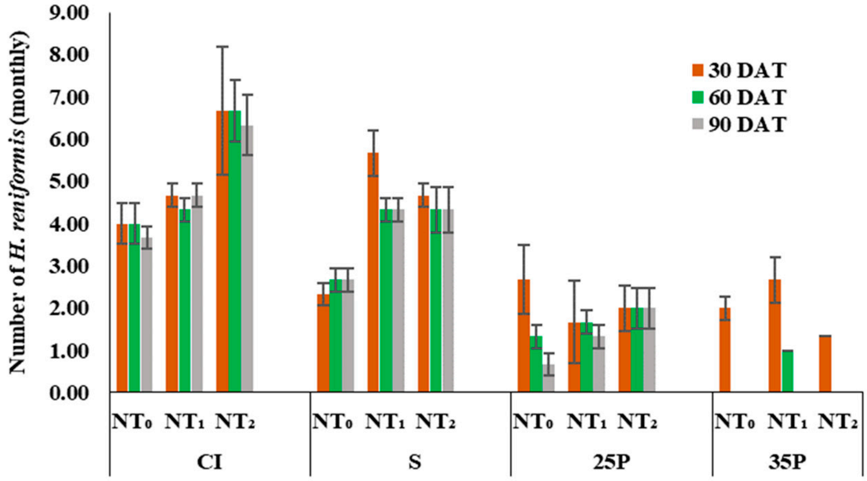

Figure 2. Number of *H. reniformis* monthly (based on the visual inspection on 30 DAT, 60 DAT, and 90 DAT). The error bar represents a standard error (n = 3).

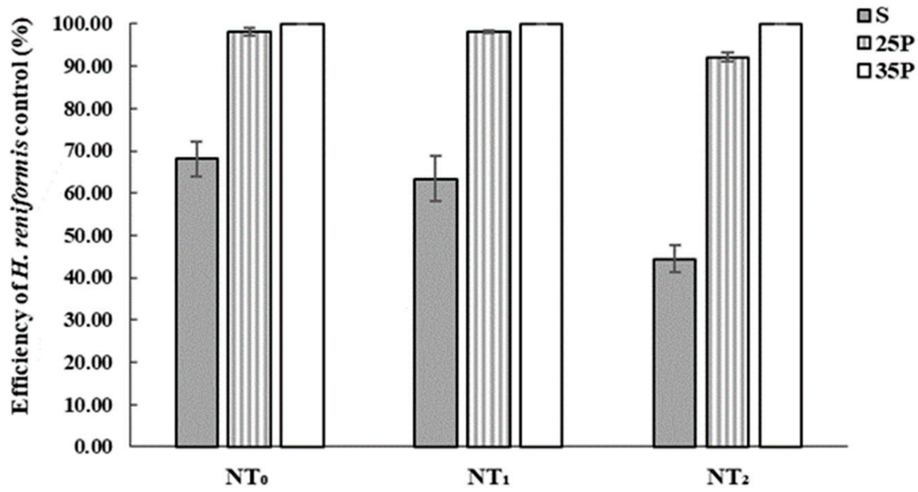

Figure 3. Efficiency of *H. reniformis* control in percentage compared with CI (based on the dry weight on 120 DAT). The error bar represents a standard error (n = 3).

3.2. Crop Parameters, Yield, and Water Use Efficiency

Changes in IR and NT affected the rice growth and yield parameters. It was observed that, during the early vegetative stage, there was a significant difference in plant height among the treatments, as indicated in Table S1 in the Supplementary Material. However, no significant difference in height was observed from the later stage of vegetative growth (90 DAT) onwards. The mean number of tillers was significantly increased at the initial stage in the CI and S regimes (Table S2, Supplementary Material). Nonetheless, it recovered in the tillering phase in 25P and 35P, significantly promoting tiller development. The mean numbers of tillers and panicles during harvest were affected considerably by NTs ($p < 0.001$), with larger numbers observed in NT_2 (Table 3). However, IR and IR $\times$ NT did not affect the numbers significantly. The largest and smallest numbers of tillers were observed in 35P–NT_2 and 35P–NT_0, with mean numbers of 13 and 7.67, respectively. Similarly, the number of panicles was larger in 35P–NT_2, with a mean number of 12.3, whereas the lowest mean number was 7.3 in 35P–NT_0. Moreover, the dry weight of the straw was affected by IRs ($p < 0.05$), NTs ($p < 0.001$), and their interaction (IR × NT, $p < 0.05$). The dry weight of straw was significantly higher in CI–NT_2, with a weight of 34.34 g, whereas the lowest was in S–NT_0, with 21.16 g (Figure 5e).

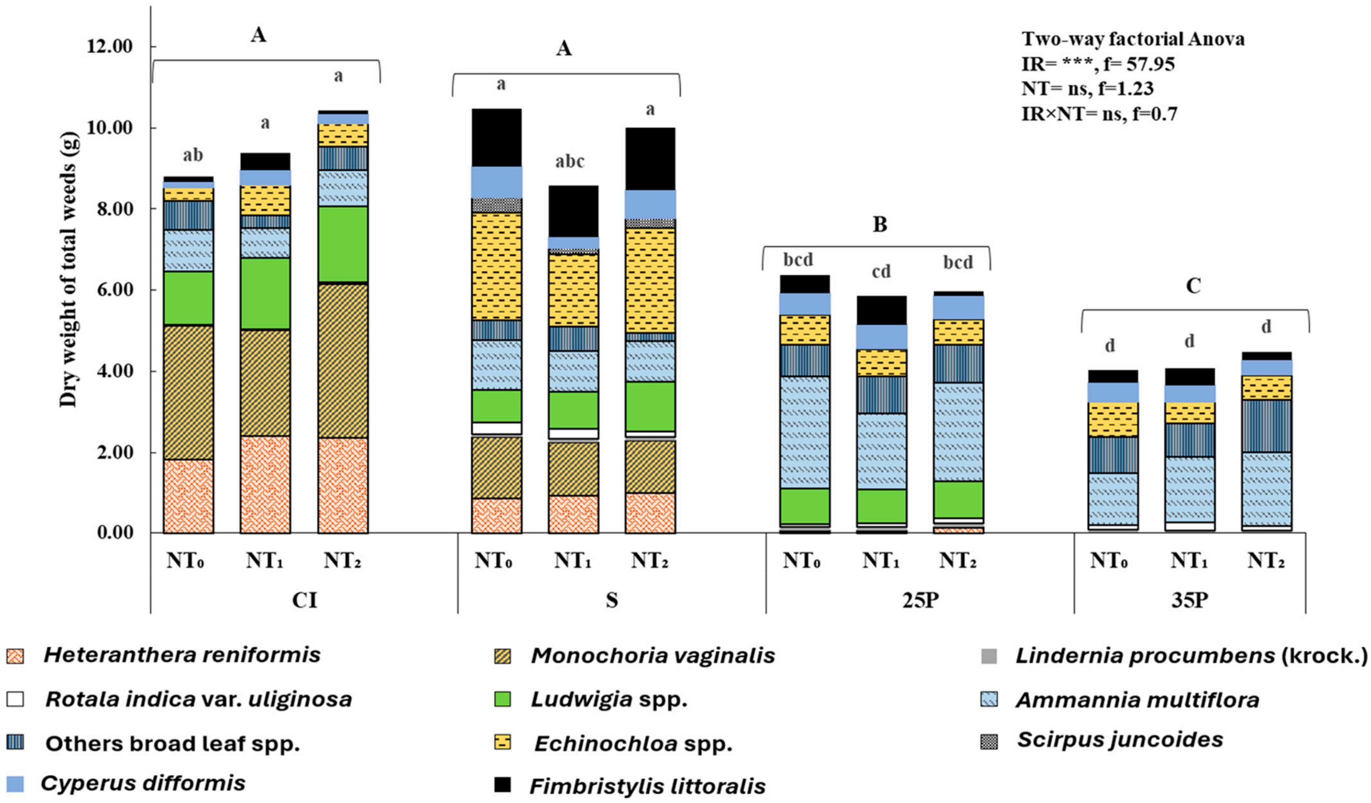

Figure 4. Mean dry weight of emerged weeds in different IRs and NTs (g pot^{-1}). According to the Tukey test, different lowercase letters indicate that means are significantly different ($p < 0.05$) among all individual treatments, whereas different uppercase letters indicate that means are significantly different ($p < 0.05$) among different IRs. ns—not significant and *** $p < 0.001$ in two-way ANOVA test.

Table 3. Mean values of crop parameters under different IRs and NTs.

Irrigation [IRs]	Nutrient [NTs]	No. of Tillers	No. of Panicles	No. of Spikelets $Panicles^{-1}$	1000 Grains Weight [g]	Harvest Index [%]	IWUE [kg m^{-3}]
CI							
	NT_0	10.3 ^{abc}	10.3 ^{ab}	87.1 ^{a}	20.36 ^{a}	30.02 ^{ab}	0.34 ^{ab}
	NT_1	11.3 ^{ab}	10.7 ^{ab}	85.5 ^{a}	20 ^{a}	30.96 ^{ab}	0.41 ^{ab}
	NT_2	12 ^{ab}	11.3 ^{a}	82.1 ^{a}	19.88 ^{a}	28.15 ^{ab}	0.41 ^{ab}
S							
	NT_0	9 ^{bc}	9 ^{ab}	87 ^{a}	19.03 ^{a}	38.37 ^{a}	0.45 ^{ab}
	NT_1	9.3 ^{bc}	9.3 ^{ab}	90.3 ^{a}	19.39 ^{a}	34.97 ^{a}	0.5 ^{ab}
	NT_2	11.7 ^{ab}	11.7 ^{a}	91.1 ^{a}	19.56 ^{a}	36.04 ^{a}	0.64 ^{a}
25P							
	NT_0	9.3 ^{bc}	9 ^{ab}	85.5 ^{a}	18.77 ^{a}	27.64 ^{ab}	0.39 ^{ab}
	NT_1	10 ^{abc}	9.7 ^{ab}	90 ^{a}	19.76 ^{a}	35.25 ^{a}	0.56 ^{ab}
	NT_2	11 ^{ab}	10.7 ^{ab}	82.3 ^{a}	20.07 ^{a}	39.47 ^{a}	0.61 ^{a}
35P							
	NT_0	7.67 ^{c}	7.3 ^{b}	85.5 ^{a}	19.16 ^{a}	18.02 ^{b}	0.2 ^{b}
	NT_1	10.3 ^{abc}	10 ^{ab}	87 ^{a}	20.16 ^{a}	32.19 ^{ab}	0.55 ^{ab}
	NT_2	13 ^{a}	12.3 ^{a}	87.1 ^{a}	20.35 ^{a}	30.55 ^{ab}	0.6 ^{a}

Table 3. *Cont.*

Irrigation [IRs]	Nutrient [NTs]	No. of Tillers	No. of Panicles	No. of Spikelets Panicles^{-1}	1000 Grains Weight [g]	Harvest Index [%]	IWUE [kg m^{-3}]
	f-value						
	IR	2.5 ns	1.11 ns	1.2 ns	1.4 ns	5.26 **	2.46 ns
	NT	21.9 ***	12.2 ***	0.75 ns	1.7 ns	3.09 ns	8.81 ***
	IR × NT	2.2 ns	1.5 ns	0.6 ns	0.39 ns	2.17 ns	1.13 ns

According to the Tukey test, different lowercase letters within each column indicate that means are significantly different ($p < 0.05$) among all individual treatments. ns—not significant, ** $p < 0.01$, and *** $p < 0.001$ in two-way ANOVA test.

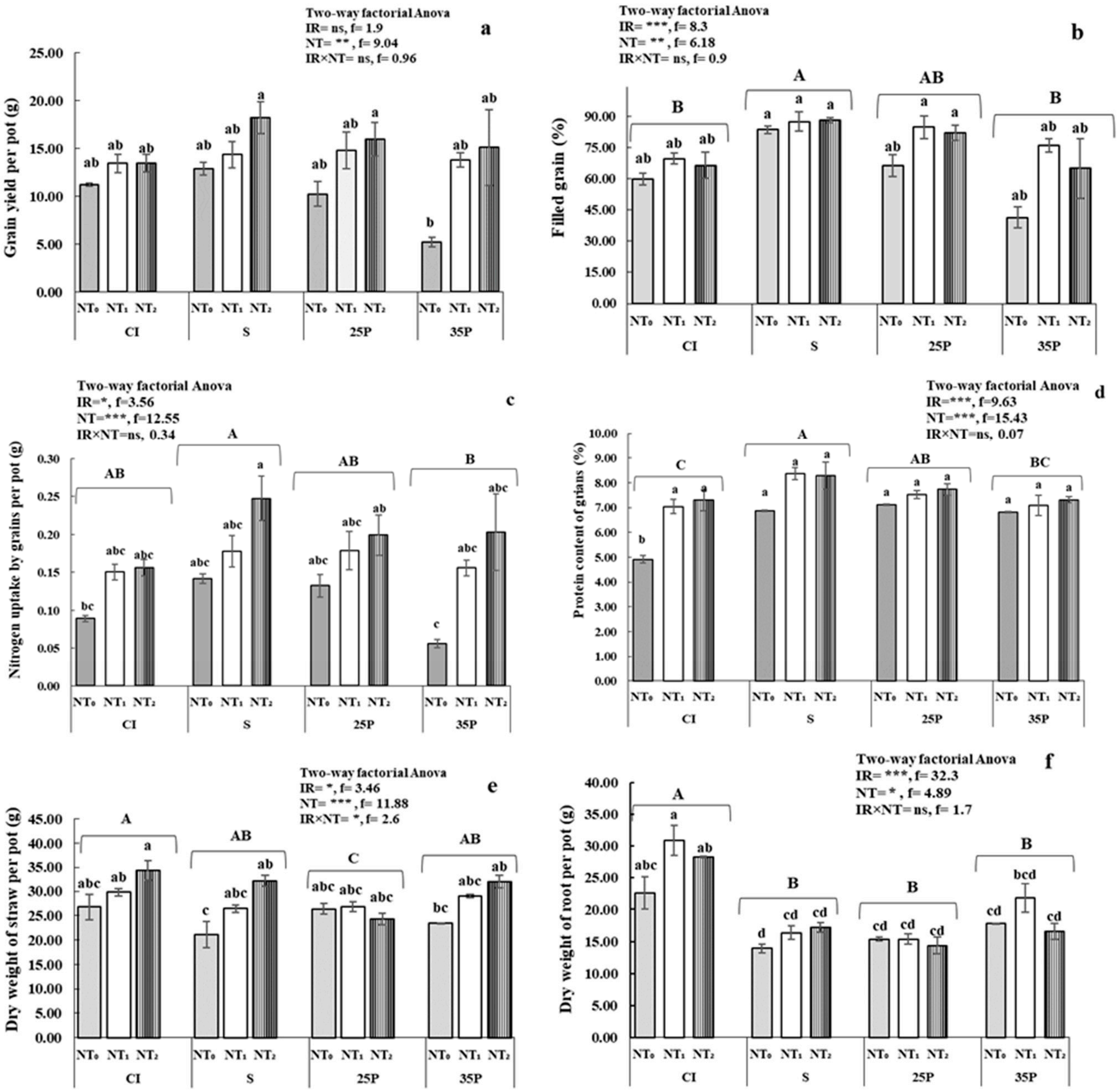

Figure 5. Crop parameter values under different IRs and NTs: (**a**) grain yield per pot; (**b**) filled grain percentage; (**c**) Nitrogen uptake by grains; (**d**) protein content in grains; (**e**) dry weight of straw; (**f**) dry weight of root. The error bar represents a standard error ($n = 3$). According to the Tukey test, different lowercase letters indicate that means are significantly different ($p < 0.05$) among all individual treatments, whereas different uppercase letters indicate that means are significantly different ($p < 0.05$) among different IRs. ns—not significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ in two-way ANOVA test.

When comparing IRs in each NT, a significantly higher straw weight was observed in the CI regime; when comparing NTs in each IR, the straw weight was higher in NT_2 and lowest in NT_0. Likewise, Figure 5f shows that the dry weight of the roots was significantly affected by IRs ($p < 0.001$) and NTs ($p < 0.05$), whereas IR × NT had no consistent impact. Comparatively, the maximum and minimum dry root weights were 30.09 g and 13.9 g in CI–NT_1 and S–NT_0, respectively. When comparing IRs in each NT, the dry weight of roots was highest in CI; when comparing NTs in each IR, it was higher in NT_1. Furthermore, the study showed that applying NTs significantly impacted grain yield ($p < 0.01$) (Figure 5a). As the amount of NTs used decreased, the yield also decreased. The maximum and minimum grain yield were 18.21 g and 5.18 g in S–NT_2 and 35P–NT_0, respectively. Figure 5b indicates that changes in IR and NT significantly impacted the filled grain percentage, while their interaction did not affect it. The S regime showed a significantly higher filled grain %, whereas CI and 35P showed the lowest. Furthermore, nitrogen uptake by the grains was substantially higher in the S regime and was lowest in 35P (Figure 5c). The protein content in brown rice was markedly influenced by the applied NTs ($p < 0.001$) and IRs ($p < 0.001$). Figure 5d shows that the protein content was highest in the S and was lowest in the CI regime. The applied NTs affected both nitrogen uptake by the rice grains and the protein content of the brown rice, which were highest in NT_2 and lowest in NT_0 (Figure 5c,d). The 1000-grain weights and the number of spikelets per panicle did not differ significantly among the treatments (Table 3). The HI was considerably higher in the S regime and was lowest in the 35P regime (Table 3). Regarding the IWUE (i.e., the relationship between water use and crop production), the amount of water consumed in the S, 25P, and 35P regimes was reduced by 13%, 20%, and 23%, respectively, compared to that in the CI regime (32.8 L, Table 3). The IWUE (water use efficiency) was highest in NT_2 among the NTs. IWUE was significantly highest in S–NT_2, 25P–NT_2, and 35P–NT_2, but lowest (0.2 kg m^{-3}) in 35P–NT_0.

3.3. Soil Parameters

Soil is a primary factor affecting the paddy ecosystem and vice versa. Among the treatments, the highest carbon content of 23.3 g kg^{-1} was observed in CI–NT_2 and the lowest in 35P–NT_2 with 18.53 g kg^{-1} (Figure 6a). However, differences in nitrogen content were insignificant among the treatments (Figure 6b). Both soil carbon and nitrogen contents in post-harvest were lower than in the initial soil condition. Similarly, Figure 6c indicates that the soil available phosphorous was higher in 35P and lower in CI. The highest and lowest mean content were 28.17 mg kg^{-1} and 12.81 mg kg^{-1} observed in 35P–NT_2 and CI–NT_0, respectively. Moreover, Figure 6d shows no significant difference in the amount of extractable potassium except in 35P–NT_0 (557.7 mg kg^{-1}), which was the significantly highest, and lowest in S–NT_2 (310.8 mg kg^{-1}) and in CI–NT_1 (262.7 mg kg^{-1}). It was observed that the soil pH after the harvest varied considerably across the different treatments. IRs affected soil pH ($p < 0.001$) significantly, where CI showed a higher pH, and 35P showed the lowest pH (Table 4). The minimum soil pH of 6.07 was observed in 35P–NT_2, whereas the maximum of 6.37 was in CI–NT_0. On the other hand, no significant differences were observed in EC, which was within the range from 332 to 420 mS m^{-1}. Furthermore, LOI % was found to be influenced by IRs ($p < 0.05$), but was not affected by NTs and IR × NT. Even though there was no significant differences among the treatments, LOI % was highest in CI–NT_2 (38.93%) and lowest in 35P–NT_2 (15.9%).

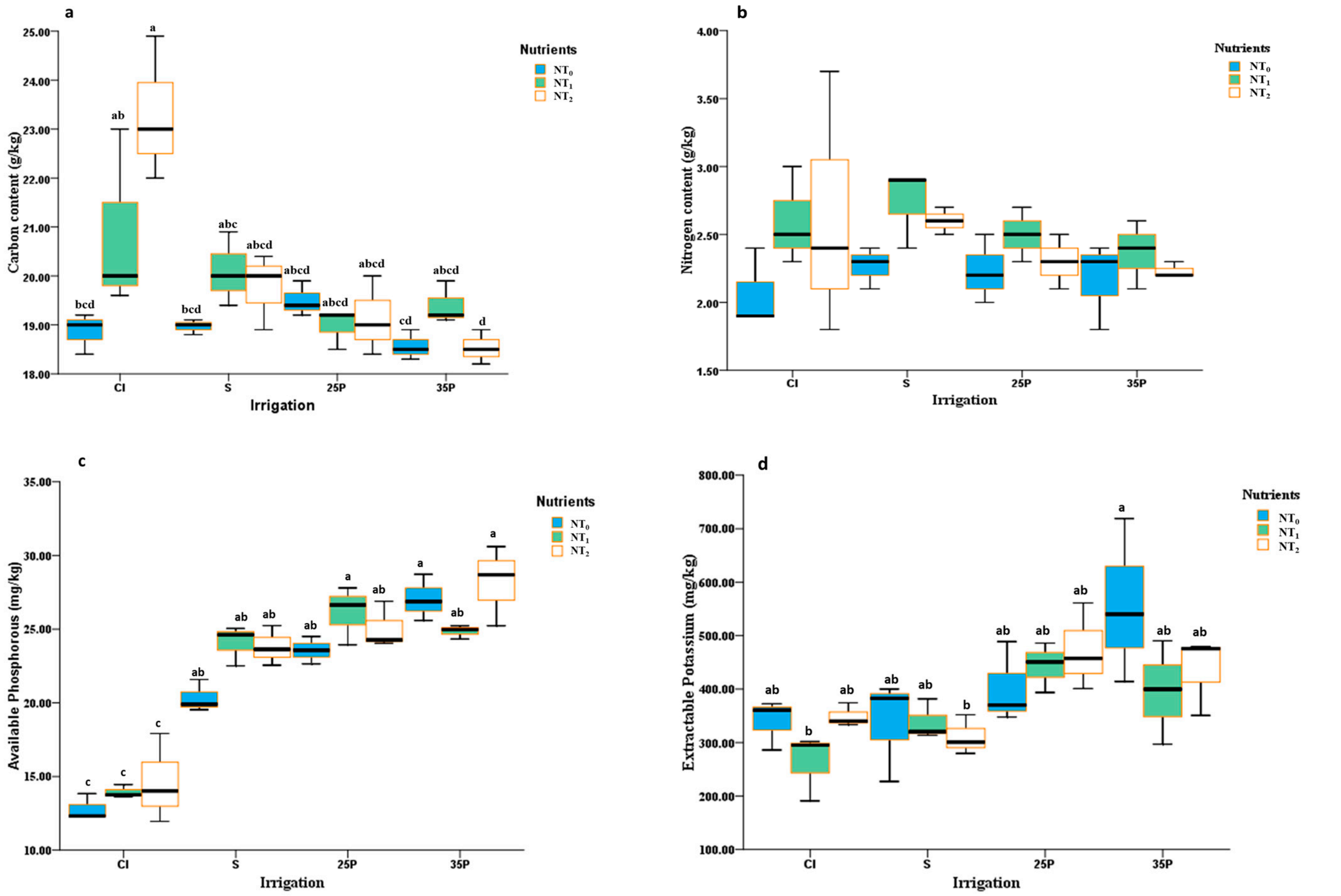

Figure 6. Box plot showing (**a**) carbon content, (**b**) nitrogen content, (**c**) available phosphorous, and (**d**) extractable potassium content of the soil under different IRs and NTs. The upper and lower sides of the box plot are 75% and 25% quantiles. The line in the middle of the box represents the median of the data. Different lowercase letters indicate that means are significantly different ($p < 0.05$) among all individual treatments (Kruskal–Wallis test for carbon, nitrogen, and available phosphorous, Tukey test for extractable potassium).

Table 4. Mean values of soil physiochemical properties after harvest.

Irrigation [IRs]	Nutrient [NTs]	pH	EC [mS m^{-1}]	LOI [%]
CI				
	NT_0	6.37 ^{a}	386.67 ^{a}	29.03 ^{a}
	NT_1	6.35 ^{a}	394.33 ^{a}	28.47 ^{a}
	NT_2	6.36 ^{a}	373.33 ^{a}	38.93 ^{a}
S				
	NT_0	6.36 ^{a}	390.33 ^{a}	29.12 ^{a}
	NT_1	6.29 ^{ab}	389.33 ^{a}	21.94 ^{a}
	NT_2	6.24 ^{ab}	331.67 ^{a}	24.51 ^{a}
25P				
	NT_0	6.19 ^{bc}	406.33 ^{a}	19.82 ^{a}
	NT_1	6.23 ^{bc}	332 ^{a}	21.85 ^{a}
	NT_2	6.33 ^{ab}	437.01 ^{a}	23.68 ^{a}

Table 4. *Cont.*

Irrigation [IRs]	Nutrient [NTs]	pH	EC [mS m^{-1}]	LOI [%]
35P				
	NT_0	6.15 ^{bc}	420.67 ^{a}	16.63 ^{a}
	NT_1	6.09 ^{c}	389.9 ^{a}	22.75 ^{a}
	NT_2	6.07 ^{c}	360.33 ^{a}	15.9 ^{a}
	f-value			
	IR	25.04 ***	0.46 ns	4.25 *
	NT	0.5 ns	1.3 ns	0.23 ns
	IR × NT	2.24 ns	2.2 ns	0.8 ns

According to the Tukey test, different lowercase letters within each column indicate that means are significantly different ($p < 0.05$) among all individual treatments. Value with letter 'a' has the highest mean, significantly different from letter 'b', The letters 'ab' represents no significant difference, and value with letter 'b' has a significantly lower mean than 'a'. ns—not significant, * $p < 0.05$, and *** $p < 0.001$ in two-way ANOVA test.

4. Discussion

Presumably, different weeds emerged along with *H. reniformis* as the soil was from herbicide-free fields. Menalled et al. [27] also reported that agricultural practices affect the abundance of and variation in weeds in the long term. In some cases, invasive weeds proliferate, becoming dominant and affecting the ecosystem [28,29]. Ismail et al. [30] illustrated that although the CI regime was put in place to suppress the growth of weeds, these weeds also adapted to flooding owing to the evolution of flood tolerance genes. This makes seed banks a crucial factor affecting an ecosystem. In our study, the diversity and dry weight of the weeds were affected by the IRs, but we did not find any impact of NTs on them. Moreover, the dominance of aquatic broadleaf weeds, such as *H. reniformis* and *M. vaginalis*, was higher than that of other weeds in CI (Figure 4). The reasons may be the faster growth, the allelopathic potential of the weeds, or the coverage of the surface by broad leaved weeds, thereby preventing light passage, which could have impeded the germination and growth of other plants [31–33]. Fogliatto et al. [34] also found a higher weed density in CI, which included *H. reniformis* in an Italian rice field. *H. reniformis* can multiply in aquatic habitats, facilitated by its broad, kidney-shaped leaves that enable efficient nutrient absorption, gas exchange, and photosynthesis [2–4]. On the other hand, S, a non-puddled rice cultivation, might contribute to different weed germination and growth rates. Past reports mentioned that S could increase the number and density of weed species, mainly grass, and sedges. However, in the case of AWD (25P and 35P), due to periodic water irrigation, competition for water occurs among weeds, which suppresses the growth of aquatic and semi-aquatic plants. Samoy-Pascual et al. [23] also demonstrated that the dry weight of *Echinochloa crusgalli* was 11% less under AWD than in CI in the USA. Dossou-Yovo and Saito [35] also found that AWD reduced weed density in lowland weed-dominated areas than in CI. Soil moisture is vital for the germination and establishment of *H. reniformis*. Thus, insufficient water moisture might impede the emergence and establishment of *H. reniformis* seedlings. Apparently, the lack of moisture and competition between different weeds and rice could have obstructed the emerging seedlings of *H. reniformis* and other weeds.

In our present results, IRs and NTs and the composition of weeds influenced the crop parameters and grain yield. Although IR did not affect grain yield significantly in the current experiments, it affected other crop parameters, such as the filled grain percentage, the dry weights of straw and roots, the nitrogen uptake and protein content of the rice, and HI. The highest grain yield in S–NT_2 and 25P–NT_2 can be attributed to the higher filled grain percentage owing to the applied nutrients and available water (Figure 5a,b). Some studies in the past had already shown the higher yield and water use efficiency in S regime [36]. Kato et al. [37] also illustrated that some Japanese rice cultivars have good grain yield and reported that aerobic conditions had more than 10 t ha^{-1} grain yield for the Takanari cultivar. Aerobic conditions with enough available water in the S regime could have improved N uptake efficiency and promoted beneficial soil microbial activity, enhancing crop growth [38]. Also, MacLaren et al. [39] explained that the more diverse the community of weeds, the lower the competition with crops due to competition between each other. The application of recommended nutrients increased the yield parameters, such as the numbers of tillers, grain yield, and filled grain percentage, which could be associated with the higher rate of available nutrients. However, the excessive and continuous application of chemical fertilizers can lead to soil-related issues such as acidification, loss of organic matter, and reductions in biological activities and fertility, causing a decline in crop yield and a rise in environmental issues [40,41]. Thus, managed S or AWD and nutrients can reduce the amount of water consumed and maintain grain yield with increased water-use efficiency by preventing superfluous water reduction through evaporation, leakage, and percolation [42]. Although the number of tillers was higher in CI, the ineffective tillers and unfilled grains could have caused a yield reduction [43]. The resulting ineffective tillers and unfilled grains may be due to the weeds that emerged, such as *H. reniformis* and *M. vaginalis*, along with other paddy field weeds. This can be compared to the results of Ferrero and Zhang et al. [8,17], who showed that the amount of rice yield reduction by *H. reniformis* and *M. vaginalis* was very high. Even though the competition with weeds was less in 35P, the yield declined compared with S and 25P. A considerable loss can be observed in the NT_0 treatment, resulting in the lowest number of tillers (7.7) with 5.18 g of yield, 18.02% HI, and 0.2 kg m^{-3} IWUE. Filled grain percentages in 35P were low, which agrees with the results of Davatgar et al. [44], in which unfilled grains under severe AWD resulted in a reduction in grain filling. Water restrictions could have led to competition among the plants. Water and nutrient supply during the critical growth stages could have affected the factors contributing to growth and yield. Owing to a lack of water, rice plants may be unable to absorb available nutrients, resulting in decreased photosynthesis, growth retardation, and yield loss [45].

Although LOI % was not significantly different among the treatments, it was highest in CI, and lowest in 35P. This may be because CI can promote higher accumulation of decaying plant material owing to flooding throughout the crop growth cycle. CI has an anaerobic condition under which the microbial activity responsible for the decomposition of organic matter [OM] is restricted, resulting in the slower decay of plant material than in well-aerated soils and the accumulated plant residues remain without decaying [46]. However, anaerobic microbial respiration may occur in CI and increase pH [47]. In addition, the redox conditions that arise in CI may increase soil pH due to the release of hydroxide ions [OH^-] during the reduction of some oxide compounds in the soil [47]. Conversely, microorganisms enhance the rapid decomposition of OM, which can be facilitated under aerobic conditions in S and dry periods in AWD and has a significant role in N mineralization. However, there is a possibility that immobilized nitrogen losses from the soil can occur during dry periods through volatilization [48]. Effective nutrient management is critical to minimizing these losses and maintaining soil fertility. Safe AWD can alter the rhizosphere environment and soil microbial community, favoring both aerobic and anaerobic bacteria and stimulating soil NT cycling, enhancing NT uptake by the rice [49,50]. Organic P and K in the soil can be bound to OM, and inorganic P and K can be present as soluble form or attached to soil particles (nonlabile forms). The anaerobic condition in CI can release P and K ions during flooding, which could become available to plants during cultivation as affected by redox conditions [51]. The release of P and K ions could promote the absorption of nutrients in CI and S, resulting in the growth of both the rice crop and the weeds. However, OM decomposition is limited owing to the reduced microbial activity, limiting the release of P and K from OM. On the other hand, the water deficit in 25P and 35P can reduce nutrient uptake by roots because water is the transporter of nutrients to the rhizosphere [45]. This could have contributed to the higher P and K contents in the soil in 35P after harvest.

CI can impact rice production sustainability and global food security due to excessive water usage, methane emissions, invasive weed proliferation, and soil fertility degradation [18,27,52]. Hence, searching for alternative methods becomes necessary. In our study, although weed growth was managed, AWD negatively affected crop parameters, especially with 35P treatment, making it unsafe to recommend dryness up to this level. Thus, it is necessary to determine the threshold level of AWD to maintain the rice yield. Carrijo et al. [53] suggested that mild AWD practices (soil water potential > −20 kPa) do not reduce yield compared to CI, with some proposing a threshold between −15 kPa and −30 kPa [24,25,37]. Furthermore, the optimum requirements of NPK varies with rice cultivar, climate, region, and cultural practices [54–58]. Field surveys conducted by Khem et al. [59] over two years revealed that fields subjected to heavy manure application showed markedly higher residual nutrient levels than those where chemical fertilizers, consisting of N–P–K in a ratio of 82–24–66, kg ha^{-1}, were applied. So, it is important to understand and appropriately manage nutrient inputs to maintain soil fertility and environmental sustainability. Our study found that S–NT_2 treatment increased grain yield and enhanced flora diversity, while 25P–NT_2 treatment led to higher grain yield with weed growth suppression. Further field studies are needed to understand how to effectively inhibit invasive weed growth without yield penalties under both CI and AWD irrigation. Moreover, understanding the hydraulic parameters of soil can aid in quantifying water movement, predicting infiltration rates, and optimizing irrigation and drainage practices [60–62].

5. Conclusions

NTs in the paddy field did not affect the growth of weeds but, in combination with the IR, they did affect the crop parameters. The most yield-influencing traits and grain yield were higher in S among the four IRs and in NT_2 among the applied NTs. The dual goal of increasing grain yield and water productivity was achieved with the combination of S and NT_2 treatment. Proper IR and NT management practices are crucial for preserving soil fertility and maintaining crop yield. A water-saving IR can be modified to minimize the negative impact of weed flora. The main difficulty in adopting this practice includes the pre-existing seed banks, efficiency, and reliability. There is a current concern about decreasing biodiversity due to herbicide usage and removing weed flora from the field. Maintaining the biodiversity of weed flora and inhibiting the dominance of invasive flora is necessary. To increase the rice yield in herbicide-free rice cultivation, improving practices to increase the number of panicles and the filled grain percentage and understanding pre-existing seed banks could be a reliable approach. Therefore, integrated cultural systems such as the use of weed-competitive cultivars, crop rotation, sowing time, and IR and NT management are also required.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments11030056/s1, Table S1: Mean height of rice during growth (monthly); Table S2: Mean number of tillers observed during growth (monthly).

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