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# Effects of Cover Crops on Soil Inorganic Nitrogen and Organic Carbon Dynamics in Paddy Fields

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**Abstract:** Rice is a staple food in Asia, and its impact on the environment is considerable, such as chemical input concerns. Organic rice farming represents an alternative approach to reducing environmental concerns throughout rice production. However, the precise nutrient management to optimize organic rice production while recovering soil residual nitrogen (N) for the subsequent crops remains unclear. This study aims to: (1) assess nutrient recovery in soil cultivated with cover crops, including Italian ryegrass and hairy vetch, and (2) investigate the optimization of nutrient management in organic rice farming using cover crops. An experiment was conducted in a paddy field adopting cover crop plots and fallow (FA) plots in four replicates each from 2021 to 2023. In addition, incubation studies were conducted in 2021 and 2022. The incubation study included various treatments: (1) soil from cover crop or FA plots, (2) with or without cover crop residues, (3) with or without weed input (2021). In 2022, fertilizer input replaced weed input. The field study indicated cover crop biomass was larger than that of weeds. Furthermore, it can determine cover crops have more recyclable plant N compared to weeds when incorporated into the soil. In contrast, there was no noticeable difference in soil inorganic N and soil total organic carbon (C) contents between cover crop and FA plots at the 0–90 cm depth. In the incubation study, we found the soil of cover crop plots and cover crop input show less inorganic N than the soil of FA plots and cover crop input during the incubation period. However, the soil of the cover crop plots and cover crop input showed a high inorganic N content after setting the flooded condition. It indicates the soil of cover crop plots, and cover crop input provides N to the soil for a longer period. Overall, our results show that winter cover crop application in paddy fields contributes to N recovery and helps maintain soil fertility. Specifically, the occasional cultivation of a combination of Italian ryegrass and hairy vetch as winter cover crops can contribute to reducing the reliance on chemical fertilizers. This practice also promotes sustainable rice farming in paddy fields.

**Keywords:** cover crop; paddy fields; soil nitrogen recovery; soil carbon stock; sustainable rice farming



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## 1. Introduction

Improving the cycling of organic matter in farmland is not only a factor that affects crop production, but also a key element contributing to sustainable agriculture. Cover crops are generally defined as crops that serve as a source of fertilizer for cash crops and improve soil productivity [1]. Therefore, cover crop cultivation and incorporation into fields is one of the cyclical uses of organic matter that can reduce reliance on chemical fertilizers. Komatsuzaki [2] highlighted some merits of cover crops, such as soil conservation, water quality conservation, and biodiversity conservation, in addition to the effect of their nutrient function.

In Japan, paddy fields cover an area of 2,352,000 ha, which constitutes 54.4% of the total agricultural land [3]. Initially, the potential of paddy fields is recognized for their multiple functions. For example, Sekiya [4] noted their groundwater recharge capacity and water purification capacity, etc., and defined paddy fields as a determining factor in environmental conservation agriculture. Nevertheless, while most of the rice farming in Japan is practiced by conventional farming, organic rice farming occupies only 3360 ha of the total paddy field area [5]. Conventional rice farming is mostly dependent on chemical fertilizers and pesticides. These chemical materials have contributed to the improvement of agricultural productivity as essential materials of modern agriculture. However, there are concerns that farming practices that rely heavily on chemical fertilizers and pesticides have had an impact on soil fertility, damage to continuous cropping, salinity, and groundwater pollution [6]. Another concern about using chemical materials is their origin. Most of the chemical fertilizers applied in Japanese conventional farming are mainly produced in foreign countries. The nitrogen (N)-derived chemical fertilizer changes into nitrate nitrogen ($NO_3$-N) in the soil. The $NO_3$-N then reaches the groundwater, increasing its concentration and causing pollution [7]. Conventional farming practices, mainly chemical fertilizer utilization, need to be reviewed from the perspective of stabilizing crop productivity and economic efficiency [8].

In recent years, the use of cover crops in paddy fields is expected to become one of the most important technologies to meet consumer demands for safe and secure food [9]. Leguminous cover crops were known to be an important source of N for paddy fields before modern agricultural technology was adopted in Japan, China, and India [1]. In Japan, Astragalus Sirius (leguminous cover crop) had been widely grown after rice harvest and used as an organic N fertilizer incorporated into the paddy field since the 1700s [10]. Leguminous cover crops fix and use atmospheric nitrogen (N) through the function of rhizobium bacteria, which live symbiotically in their roots. As a result, it leads to saving fertilizer input. Additionally, because leguminous cover crop residues decompose with a low carbon(C)-to-nitrogen (C/N) ratio, they release N early, benefiting subsequent crops [11]. However, the use of Astragalus Sirius in Japanese paddy fields has declined since the 1960s, largely due to the increased availability of inexpensive chemical fertilizers [10]. On the other hand, hairy vetch has recently drawn attention to upland farming in Japan as an alternative to chemical fertilizer [12]. Asagi [8] showed that the hairy vetch-derived N can be applied to paddy fields at the same rate as chemical fertilizer and increase yield compared to other cover crops. Nevertheless, these leguminous cover crops frequently degrade the nutritional quality of rice by enhancing the protein content of the grain. This is because nitrogen released from the cover crops affects the nitrogen uptake of rice during the panicle formation stage [13].

The application of gramineous cover crops in paddy fields has been attempted in Japan in the recent past. Unlike leguminous cover crops, Kakar et al. [14] showed that gramineous cover crops (Italian ryegrass) and organic fertilizer (Bokashi) treatments reduced amylose and protein contents of rice grains contrary to chemical fertilizer application. Additionally, as the gramineous cover crop has a high N uptake function, an efficient technique of using the gramineous cover crop is required to maintain the environmental conservation function of the paddy fields [15]. However, the high C/N ratio of gramineous cover crops is more likely to result in immobilization in the early growth stages of the following crops as the cover crop decomposes [11]. Therefore, the C/N ratio is a very important consideration in the incorporation of cover crops into fields to avoid a reduction in food value or immobilization.

Mixed seeding of gramineous cover crops and leguminous cover crops has also been attempted, especially in upland farming. Hirsh et al. [16] examined the capacity of cover crops, including mixed seeding, to take up N from deep soil levels and showed mixed seeding resulted in higher $NO_3$-N in the 0–30 cm topsoil than gramineous cover crops (winter cereals) and was just as effective as gramineous cover crops at reducing $NO_3$-N from 30 to 210 cm depth. However, a study is needed to verify whether similar results are expected in paddy fields. To fully harness the potential of cover crops as a soil management tool, it is crucial to know their capacity to effectively use them for managing paddy soil ecosystems [13].

The use of organic fertilizers also plays a crucial role in improving soil nutrient levels. Continuous application of bokashi, a type of organic fertilizer, may enhance microbial activity in the soil, potentially increasing the protozoa population [17]. These soil microbes contribute to improving the soil environment, which has led to a growing number of farmers in Japan adopting bokashi fertilizer to promote sustainable farming [18]. Further research is needed to verify the role of bokashi application when combined with cover crop application in organic rice farming systems.

In the current study, we investigated a 3-year field study consisting of winter cultivation and incorporation of cover crops into a paddy field. The main objective was to verify nutrient dynamics under flooded conditions. Similarly, we conducted incubation studies consisting of adding cover crops into paddy soil under flooded conditions. The aim was to evaluate the effects of cover crop application on N release during their decomposition in paddy soil. This study also sought to optimize nutrient management for organic rice farming.

## 2. Materials and Methods

### *2.1. Field Study*

#### 2.1.1. Study Site and Design

The field study was conducted on a paddy field at the Center for International Field Agriculture Research & Education, Ibaraki University, Japan, from 2021 to 2023. The field was 3600 $m^2$ (50 m × 72 m) and has been managed organically since 2019. The field was divided into 8 plots, and each plot size was 450 $m^2$ (50 m × 9 m). A combination of cover crop plot and fallow (FA) plot was prepared with four replications in this field. In 2023, cover crop plots and FA plots were further divided into tillage and no tillage. Therefore, a pattern of tillage and no-tillage cover crop plots and FA plots with four replications was installed in the year 2023.

Mean monthly air temperatures at the study site ranged from 3.9 to 26.9 °C (average: 15.6 °C) in 2021, from 3.2 °C to 26.8 °C (average: 15.3 °C) in 2022, and from 4.2 °C to 29.0 °C (average: 16.7 °C) [19]. The soil is a Typic Endoaquand in the Kanto region of Japan [13].

Italian ryegrass as a cover crop was sown in four cover crop plots in early November 2020 and 20 November 2021. Regarding the cover crop growing for the year 2023, Italian ryegrass and hairy vetch were sown in the cover crop plots on 20 October 2022. The seed rate per ha of each cover crop was 100 kg for Italian ryegrass and 50 kg for hairy vetch. In April of the following year, Bokashi, which comprised rice bran, rice husks, oil meal, and rapeseed meal, was applied (1000 kg $ha^{-1}$) to all plots in 2021 and 2022. In 2023, an organic fertilizer, Yuuki Agret 666, was applied (500 kg $ha^{-1}$, Asahi Agria Co., Ltd., Saitama, Japan) to all plots as an organic fertilizer. Subsequently, the Italian ryegrass grown in the cover crop plots and the weeds grown in the FA plots were cut and incorporated into each plot on 16 April 2021 and 18 April 2022, respectively. In 2023, Italian ryegrass and hairy vetch in the cover crop tillage plots and weeds in the FA tillage plots were cut on 21 April 2023 and incorporated into each plot on 1 May 2023. In contrast, cover crops and weeds in the no-tillage plots were mowed down by a roller crimper and not incorporated into the plots. The field was flooded on 6 April 2021, 22 April 2022, and 2 May 2023.

Rice seedlings were transplanted on 2 June 2021, 20 May 2022, and 29 May 2023. A liquid fertilizer (fish extract and rice bran, Taiseinozai, Japan) was applied several times to the rice grown in a part of the experimental paddy field by foliar application in July and August in 2021 and 2022. We did not apply liquid fertilizer in 2023. The water in the field was temporarily drained and left to dry for approximately 3 weeks from 19 July in 2021 and from 7 July in 2023. This temporarily drying was not carried out in 2022. The rice was harvested on 17 September 2021, 15 September 2022, and 27 September 2023.

2.1.2. Crop and Weed Sampling

Before transplanting the rice seedlings, cover crops and weeds were sampled by the quadrat method (50 cm × 50 cm) [20] on 15 April in 2021 and 2022 and on 19 April in 2023. These sampled plants were oven-dried at 65 °C for 3 days to measure biomass weight. The biomass of each plant was powdered in a blender, and its C and N concentrations were measured using the CN analyzer (JM3000CN, J-Science Lab, Kyoto, Japan) according to the improved Dumas method [21] (Table 1).

**Table 1.** Parameters of the CC and the weeds of the paddy field from 2021 to 2023.

| | Plants | C (%) | N (%) | C/N |
|---|---|---|---|---|
| 2021 | Italian ryegrass | 42.5 ± 1.1 | 0.6 ± 0.0 | 73.7 ± 4.5 |
| | Weed | 43.4 ± 0.5 | 0.8 ± 0.2 | 63.8 ± 17.1 |
| 2022 | Italian ryegrass | 43.6 ± 0.2 | 0.7 ± 0.0 | 65.3 ± 4.4 |
| | Weed | 44.5 ± 0.2 | 1.3 ± 0.0 | 35.0 ± 1.1 |
| 2023 | Italian ryegrass | 43.3 ± 1.3 | 1.7 ± 0.3 | 28.0 ± 4.8 |
| | Hairy vetch | 46.6 ± 0.3 | 3.4 ± 0.1 | 13.9 ± 0.4 |
| | Weed | 44.4 ± 0.2 | 1.4 ± 0.1 | 33.6 ± 3.2 |

CC: cover crop, C: carbon, N: nitrogen. ±: standard error. The C/N ratio was calculated as the average of the total C/N ratios across all samples for each plant type.

2.1.3. Soil Sampling

Paddy soil was sampled from the surface to a depth of 90 cm in each plot of the field twice a year in April and December in 2021, 2022, and 2023. The soil sampled in April was collected on 15 April in both years. In 2023, the soil was sampled on April 18. The other soil samples were taken on 21 December 2021, 1 December 2022, and 29 November 2023, a few days after sowing Italian ryegrass each year.

Each soil sample was divided into six layers: 0–5, 5–10, 10–15, 15–30, 30–60, and 60–90 cm. 5 g of fresh soil from each sample was extracted in 40 mL of 1 N KCl solution. The $NH_4^+$ and $NO_3^-$ contents were measured with a flame photometer (SPCA-6210, Shimadzu, Kyoto, Japan) using ultraviolet absorption spectrophotometry [22].

Similarly, 10 g of fresh soil from each layer was oven-dried at 105 °C for 3 days. These oven-dried samples were then weighed to determine the water content. The soil bulk density of the soil was calculated based on the water content of the soil sample in each layer.

The remaining fresh soil samples were air-dried in a room and then passed through a 2 mm mesh sieve. The sieve-passed samples were oven-dried at 105 °C for 1 day and analyzed with a C/N analyzer to determine their C and N concentrations.

*2.2. Incubation Study*

2.2.1. Soil Used

Soil samples were collected from the same field used in the field study. Soil samples were randomly taken from the surface of four cover crop plots and four FA plots on 16 April 2021, and 21 April 2022, respectively. The soil samples were mixed with plot type and air-dried. Ten grams of three soil samples from cover crops (Italian ryegrass) and FA plots were oven-dried at 105 °C for 1 day and weighed to determine the water content.

2.2.2. Plant Residue Used

While cover crops and weeds were used in 2021, only cover crops were applied in 2022. These plant residues were sampled in the same field as the one where the plants were collected for the field study. Cover crops were randomly sampled from four cover crop plots each. Similarly, weeds were collected from four FA plots without a definite pattern. Each plant residue was air-dried, powdered in a blender, and thoroughly mixed.

#### 2.2.3. Study Design

Water was added to the air-dried soil samples to rewet them to a water content of 30%. In the study in 2021, the rewetted soil samples from each plot were placed in 100 mL Erlenmeyer flasks, with each sample containing 60 g of soil. The treatment consisted of cover crop input, weed and control (no plant residue input) with 3 replications per plot soil sample. Each sample contained 6 g of either cover crop or weed.

In the study in 2022, the rewetted soil samples from each plot were transferred to 120 mL plastic cups. Soil quantity was 50 g per sample. While the plant residue used was only cover crop, Bokashi was applied in some samples to measure fertilizer's effect on soil N. The treatments comprised cover crop, Bokashi, and liquid fertilizer as top dressing, cover crop, Bokashi, cover crop and no fertilizer, no cover crop residue, Bokashi, liquid fertilizer, no plant residue, Bokashi, Control (no plant residue + no fertilizer application) with 3 replications per soil samples from cover crop plots and soil samples from FA plots. Cover crop input was 5 g. Bokashi and liquid fertilizer applications were 0.2 g and 0.05 g, respectively, in the samples to measure fertilizer effect.

All samples were thoroughly mixed, weighed, and incubated in an incubator (CN-25C, Mitsubishi Electric Engineering, Tokyo, Japan) at 23 °C. All samples were weighed on a weekly basis. If they lost weight, distilled water was added to maintain the soil volumetric water content at 30%. The day after sample weighing, 5 g of soil was taken from each sample and extracted in 40 mL of 1 N KCl solution. This process was repeated for 4 weeks. All samples were flooded and weighed in the 4th week after collecting that week's soil. Samples were weighed the following week, and distilled water was added if they lost weight from the 5th to 7th week. The soil was not sampled for these 3 weeks. In the 7th week, a liquid fertilizer was added as a top dressing. In the 8th week, 5 g of soil was collected again from each sample and extracted in 40 mL of 1 N KCl solution.

#### 2.2.4. Plant and Soil Analysis

Plant C and N concentrations of cover crops and weeds were determined based on the plant analysis of the field study in 2021 and 2022. These plants' C and N concentrations were measured with the C/N analyzer as described in Section 2.1.2. Soil $NH_4^+$ and $NO_3^-$ contents from the 1st week to 4th week and 8th week were measured using a flame photometer as described in Section 2.1.3. Similarly, the soil total C and total N of the soil were quantified with the same C/N analyzer as used for the soil analysis of the field study as presented in Section 2.1.3.

### *2.3. Statistical Analysis*

Statistical analysis was conducted using Stat View 5.0 (version 5.0, SAS Institute Inc, Cary, NC, USA). A *t*-test was applied to compare the means of two populations, while ANOVA was used to compare the means of three or more populations to assess the effects of the main factors in the study. For the field study, plant biomass and plant N were analyzed using a *t*-test, while soil inorganic N and soil total C were analyzed using ANOVA. In the 2021 incubation study, the factors included soil type, plant input, and their interaction. In the 2022 incubation study, the analysis focused on soil type, cover crop input, fertilizer input, and their interactions. A two-way ANOVA in Stat View 5.0 was used to assess these interactions. Statistical significance was set up at $p < 0.05$.

## 3. Results

### *3.1. Field Study*

#### 3.1.1. Cover Crops and Rice Growth in the Field

The biomass of cover crops exceeded that of weeds in every year of the study. Particularly, the biomass of the cover crop in 2021 was significantly greater than that of the weeds, being 3.5 Mg $ha^{-1}$ and 1.5 Mg $ha^{-1}$, respectively (Figure 1). Both plants' biomass in 2022 was less than that in 2021, with cover crops at 1.3 Mg $ha^{-1}$ and weeds at 0.7 Mg $ha^{-1}$. However, the biomass of cover crops was still significantly higher than that of weeds. In 2023, the combined biomass of cover crops (hairy vetch and Italian ryegrass) was considerably higher than that of weeds, 2.6 Mg ha$^{-1}$ for cover crops and 0.7 Mg ha$^{-1}$ for weeds.

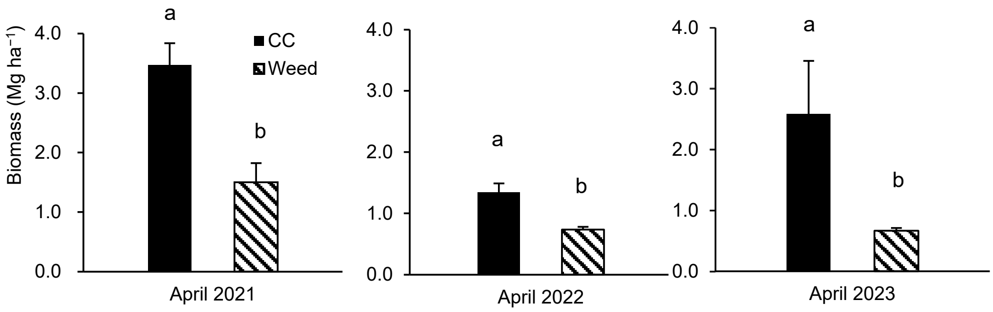


**Figure 1.** Comparison of CC (cover crop) biomass and weed biomass in a paddy field. Vertical bars mean the standard error. Different letters indicate significant differences ($p < 0.05$). The biomass of the CC in 2023 is the sum of the Italian ryegrass and the hairy vetch.

In 2021, cover crops had higher plant N than weeds (Figure 2), with cover crops and weeds containing 19.9 kg and 12.6 kg ha$^{-1}$, respectively. In 2022, both plants had nearly equal N. However, a large difference was observed between cover crops and weeds in 2023. The cover crop in 2023 consisted of hairy vetch and Italian ryegrass, with plant N of the hairy vetch (60.1 kg ha$^{-1}$) and Italian ryegrass (11.8 kg ha$^{-1}$).

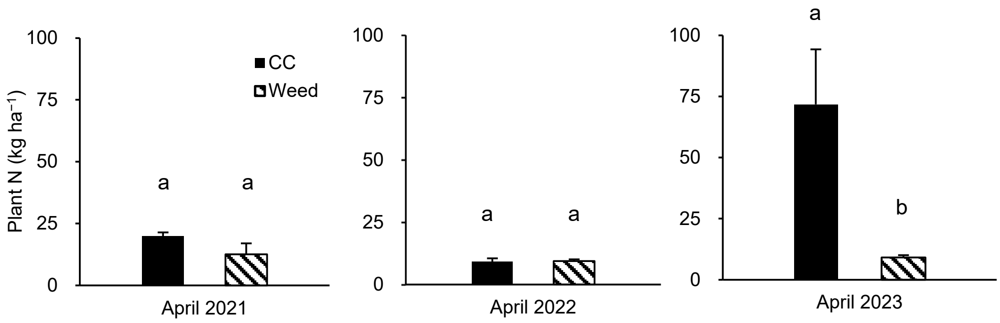


**Figure 2.** Comparison of cover crop (CC) N and weed N in a paddy field. Vertical bars mean the standard error. Different letters indicate significant differences ($p < 0.05$). Plant N of the CC in 2023 is the sum of the Italian ryegrass and the hairy vetch.

Rice grain yield responses between the cover crop plots and FA plots were 4.54 Mg ha$^{-1}$ versus 4.8 Mg ha$^{-1}$ in 2021 and 4.8 Mg ha$^{-1}$ versus 4.8 Mg ha$^{-1}$ in 2022 on a paddy basis (Figure 3). This indicates that the N contribution from the cover crop was not significant. However, yield responses between the Italian ryegrass and hairy vetch mixture and the FA were 7.2 Mg ha$^{-1}$ versus 6.0 Mg ha$^{-1}$, showing that the cover crop mixture significantly improved rice yield in 2023.

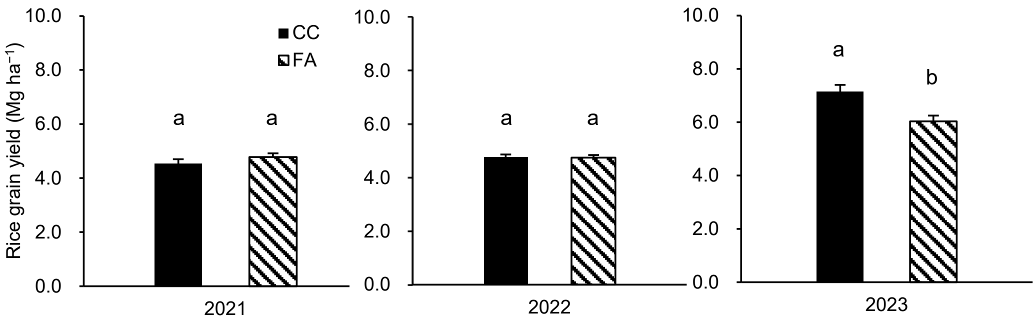


**Figure 3.** Comparison of rice grain yield in a paddy field. Yield is on a paddy basis. Vertical bars mean the standard error. Different letters indicate significant differences ($p < 0.05$). CC: cover crop. FA: fallow.

3.1.2. Soil Inorganic N in the Field upon Cover Crop and Fallow

The dynamics of soil inorganic N (the sum of $NH_4$-N and $NO_3$-N) in the paddy field did not show a clear effect of cover crop application compared to the trend observed in plant biomass and plant N. In April 2021, soil inorganic N in the cover crop plot was higher than in the FA plot below 15 cm (Figure 4A). In December 2021, soil inorganic N levels up to 30 cm were nearly the same; however higher inorganic N was exhibited in cover crop plots in lower than 30 cm depth. (Figure 4B).

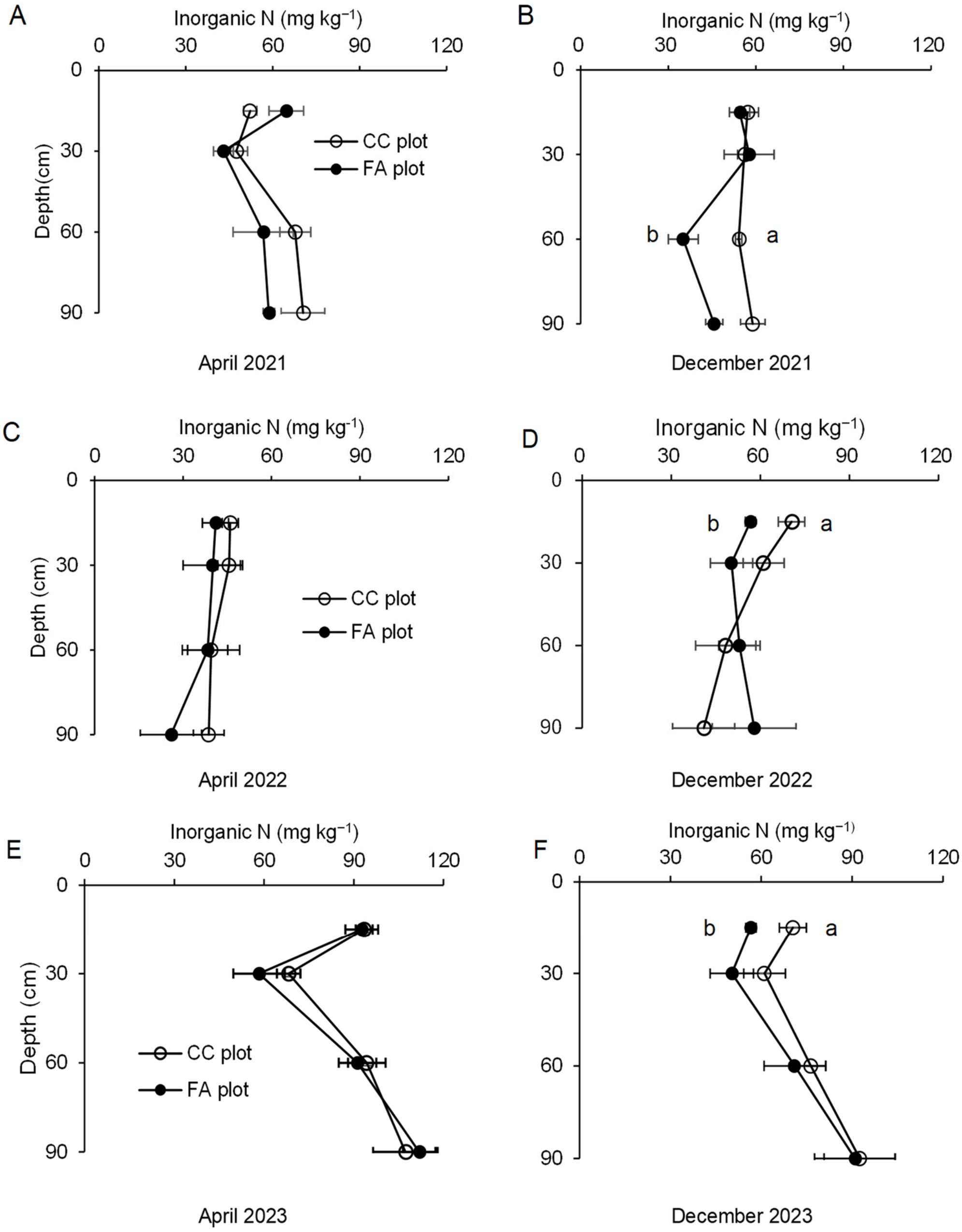


**Figure 4.** Soil inorganic N dynamics (mg $kg^{-1}$) in 0–90 cm soil profile of the paddy field in April 2021 (**A**), December 2021 (**B**), April 2022 (**C**), December 2022 (**D**), April 2023 (**E**) and December 2023 (**F**). Inorganic N: sum of $NH_4$-N and $NO_3$-N. CC: cover crop. FA: fallow. Inorganic nitrogen at a depth of 15 cm at each sampling period was calculated by dividing the nitrogen storage in the 0–15 cm soil layer by the soil mass in the same layer. Horizontal bars mean the standard error. Different letters indicate significant differences ($p < 0.05$).

In April 2022, N was higher in the cover crop plot than or equal to the FA plot across all layers (Figure 4C). By December 2022, soil inorganic N in the cover crop plot was more than that in the FA plot down to 30 cm, but below 60 cm, the FA plot had higher N levels than the cover crop plot (Figure 4D).

In 2023, N dynamics showed similar trends in both sampling periods. Soil inorganic N decreased at 30 cm layers from the 15 cm level, followed by an increase in the deeper layers (Figure 4E,F).

Soil inorganic N stock at 0–30 cm depth indicated that FA plots were generally higher than cover crop plots at each sampling period until 2022, with the exception of April 2022 (Figure 5). Across sampling times, N stock was lower in April than in December for both plots in 2021 and 2022. However, this trend was different in 2023. In that year, N stock in the cover crop plots was higher than in the FA plots at both sampling times, with April values exceeding those in December. While a significant difference was observed across sampling periods, no notable differences were found for soil type or the interaction between soil type and sampling period (Table 2).

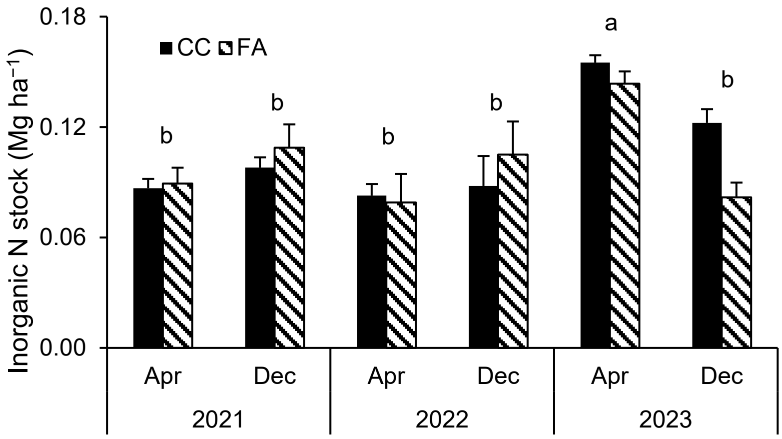


**Figure 5.** Soil inorganic N stock (Mg ha$^{-1}$) in the 0–30 cm soil profile of paddy fields in April and December from 2021 to 2023. CC: cover crop. FA: fallow. Vertical bars mean the standard error. Different letters indicate significant differences by sampling period ($p < 0.05$).

**Table 2.** Effect of soil type, sampling period, and their interaction on soil inorganic N stocks over all sampling periods.

| Effect | *p*-Value |
|---|---|
| Soil type [1] | NS |
| Sampling period [2] | *** |
| Soil type × Sampling period | NS |

*** $p < 0.001$, NS: no significant difference ($p > 0.05$). [1] CC plot soil, FA plot soil, [2] April and December from 2021 to 2023. CC: cover crop. FA: fallow.

### 3.1.3. Soil Total Organic C upon Cover Crop and Fallow

The incorporation of cover crop residues also indicated a random trend in soil C content among the six sampling times. In April 2021, C concentration in the cover crop plot was higher at a depth of up to 30 cm (Figure 6A). From that point until April 2023, however, C concentration was generally higher in the FA plot, except in the surface layer during these sampling periods. In December 2022, C concentration in the FA plot was significantly higher at the 30 cm depth (Figure 6B–E). In December 2023, the cover crop plot showed a trend of higher C concentration across all layers, with a particularly significant increase at the 15 cm depth (Figure 6F).

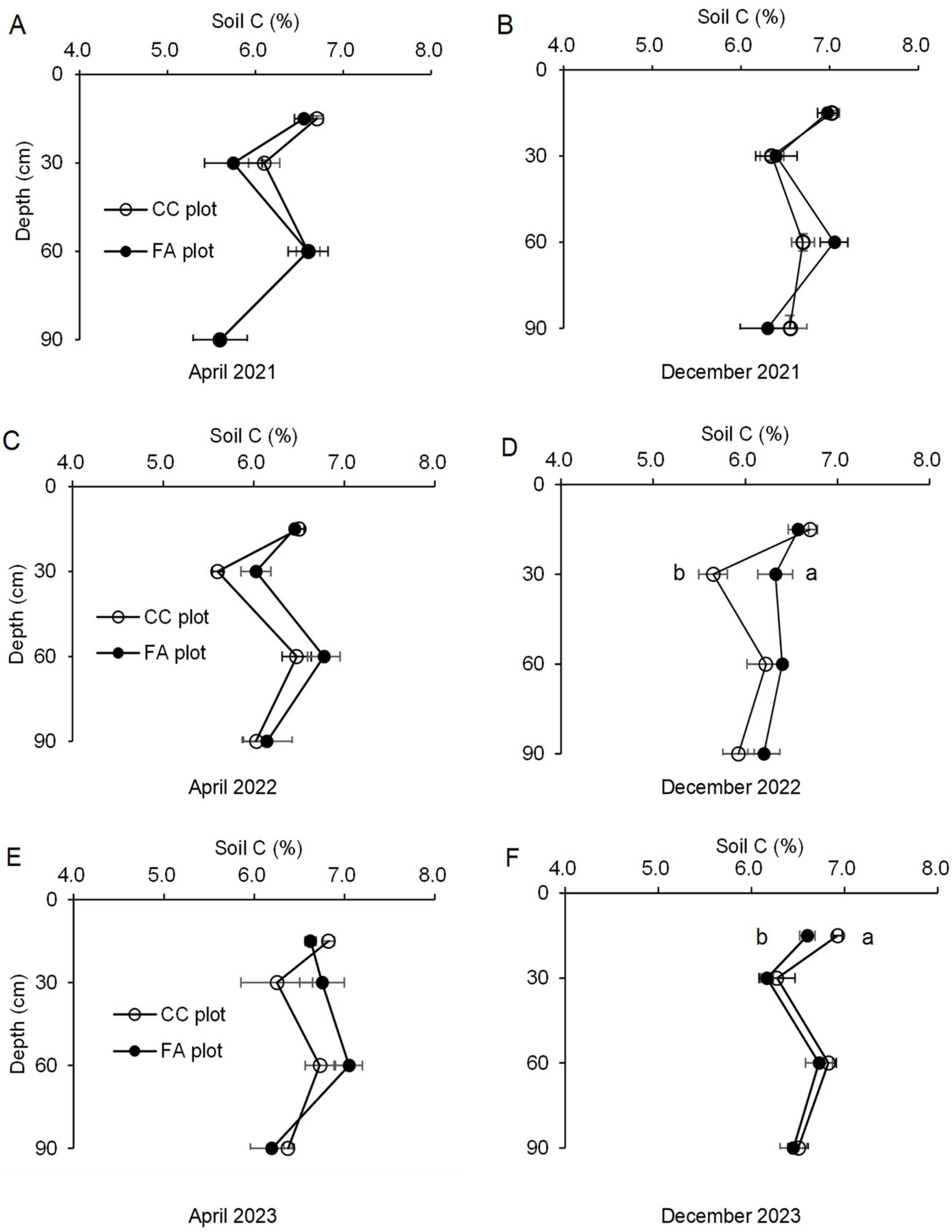


**Figure 6.** Soil total organic C content (%) in the 0–90 cm soil profile of paddy fields in April 2021 (**A**), December 2021 (**B**), April 2022 (**C**), December 2022 (**D**), April 2023 (**E**) and December 2023 (**F**). CC: cover crop. FA: fallow. Soil total C at a depth of 15 cm at each sampling period was calculated by dividing the C stock in the 0–15 cm soil layer by the soil mass in the same layer. Horizontal bars mean the standard error. Different letters indicate significant differences ($p < 0.05$).

Soil C stock at 0–30 cm depth showed a similar trend to soil inorganic N stock until 2022 (Figure 7). Although soil C stock in the cover crop plots was higher than in the FA plots in April 2021, FA plots showed higher C stocks than cover crops in the other three sampling times. In 2023, however, C stock in the cover crop plots was lower than that of the FA plots in April, but higher in December. Notably, C stock in December (cover crop plots: 124.3 Mg ha$^{-1}$, FA plots: 101.0 Mg ha$^{-1}$) was lower than in April (cover crop plots: 126.5 Mg ha$^{-1}$, FA plots: 130.0 Mg ha$^{-1}$) in both plots, contrary to the trend observed in 2021 and 2022. Soil C stock at 0-30cm depth was significantly affected by the single effect of sampling period as well as its interaction with soil type (Table 3).

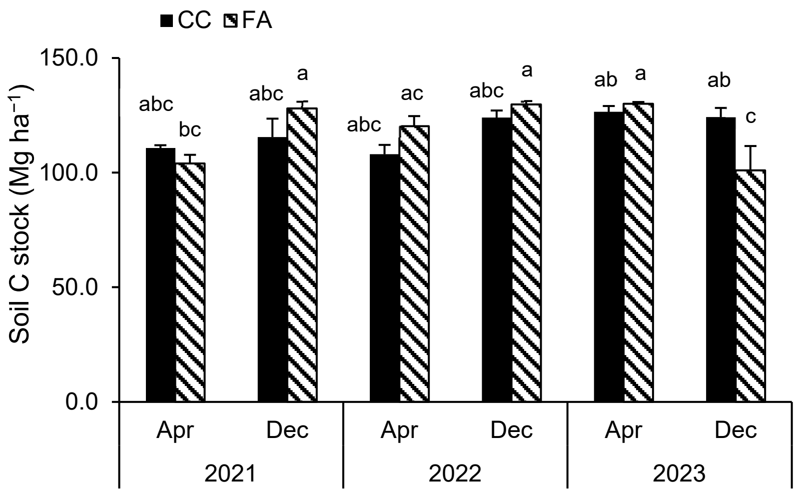


**Figure 7.** Soil C stock (Mg $ha^{-1}$) in the 0–30 cm soil profile of paddy fields in April and December from 2021 to 2023. CC: cover crop. FA: fallow. Vertical bars mean the standard error. Different letters indicate significant differences by interactive effect between soil type and sampling period ($p < 0.05$).

**Table 3.** Effect of soil type, sampling period, and their interaction on soil total C stocks over all sampling periods.

| Effect | *p*-Value |
|---|---|
| Soil type [1] | NS |
| Sampling period [2] | *** |
| Soil type × Sampling period | ** |

** $p < 0.01$, *** $p < 0.001$, NS: no significant difference ($p > 0.05$). [1] CC plot soil, FA plot soil, [2] April and December from 2021 to 2023. CC: cover crop. FA: fallow.

### *3.2. Incubation Study: Measuring the Effect of Soil Management, Cover Crop Input, and Fertilization on Soil N Mineralization*

The 2021 study examined the effects of cover crop or weed input on soil inorganic N under three treatments. ANOVA showed a significant difference in soil inorganic N between CC and FA plots at week 4 (Table 4). Additionally, as the interaction between soil type and plant input exhibited a notable trend with a *p*-value of 0.056, a simple main effect test was performed. It identified a significant difference between the CC and FA plots under the weed input treatments (Figure 8).

**Table 4.** Effect of soil management and cover crop on soil inorganic N availability.

| | *p*-Value | | | | |
|---|---|---|---|---|---|
| **Treatment** | **Week 1** | **Week 2** | **Week 3** | **Week 4** | **Week 8** |
| Soil type [1] | NS | NS | NS | * | NS |
| Plant input [2] | *** | *** | *** | *** | *** |
| Soil × Plant input | NS | NS | NS | NS [3] | * |

* $p < 0.05$, *** $p < 0.001$, NS: no significant difference ($p > 0.05$). [1] CC plot soil, FA plot soil, [2] with Italian ryegrass input, with weeds input, no plant input. [3] *p*-value of week 4 is 0.056. CC: cover crop. FA: fallow.

Samples with or without plant (Italian ryegrass or weeds) input show significant differences during the incubation period. Nevertheless, no differences were observed between the samples with cover crop input and weed input apart from week 8 (Figure 9). Soil N in soil with cover crop input was nearly the same as that in soil with weed input until week 4. After that, it gradually decreased, and by week 8, it was close to the level of soil with no plant input.

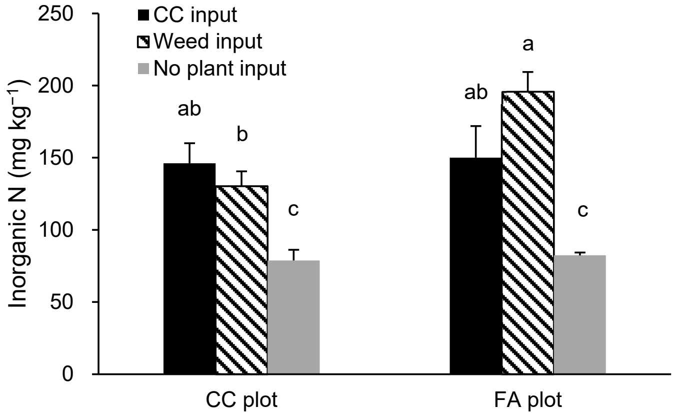


**Figure 8.** Comparison of soil inorganic N between cover crop plot and FA plot in paddy field at week 4. CC: cover crop. FA: fallow. Vertical bars mean the standard error. Different letters indicate significant differences ($p < 0.05$).

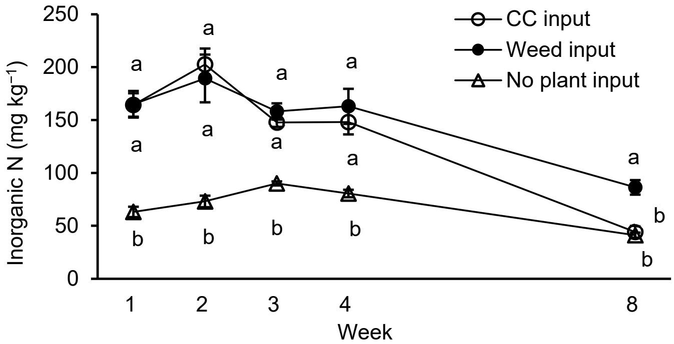


**Figure 9.** Changes in soil inorganic N during the 8 weeks of incubation of paddy soil with or without plant residues input. Vertical bars mean the standard error. CC: cover crop. Different letters indicate significant differences ($p < 0.05$). Significant differences were compared by week.

We also found an interactive effect between soil and plant input in week 8. Contrary to our expectations, samples with weed input exhibited higher soil inorganic N compared to those with cover crop input (Figure 10). Samples were ranked in treatments in the order FA plot and weed input > cover crop plot and weed input > cover crop plot and cover crop input > cover crop plot and no plant input > FA plot and no plant input > FA plot and cover crop input.

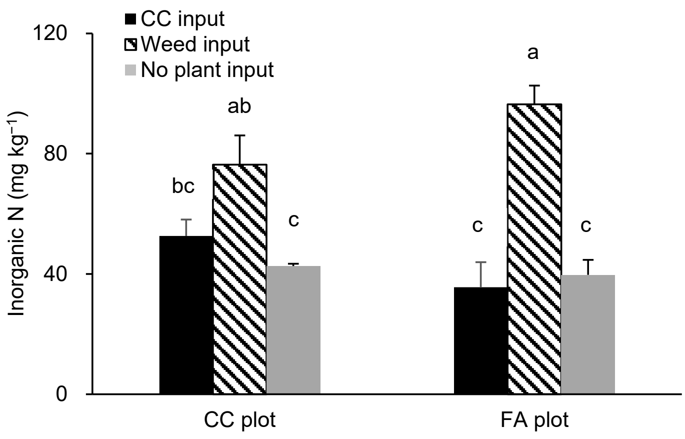


**Figure 10.** Interactive effect of cover crop plot or FA plot soil and with or without plant residues input on soil inorganic N in week 8. CC: cover crop. FA: fallow. Vertical bars mean the standard error. Different letters indicate significant differences by treatment ($p < 0.05$).

The effects of cover crop application were found across various soil treatments, including those with or without cover crop input and with or without fertilizer input. The results also revealed an interactive effect between soil and cover crop (Table 5). In soil level, the difference in soil inorganic N between the cover crop plots and FA plots appeared in week 1 and week 4.

**Table 5.** Effect of soil management, cover crop input, and fertilization on soil inorganic N availability.

| | *p*-Value | | | | |
|---|---|---|---|---|---|
| **Treatment** | **Week 1** | **Week 2** | **Week 3** | **Week 4** | **Week 8** |
| Soil type [1] | * | NS | NS | *** | NS |
| CC input [2] | NS | NS | NS | NS | *** |
| Fertilizer input [3] | * | * | ** | *** | *** |
| Soil × CC input | NS | * | NS | *** | NS |
| Soil × Fertilizer input | NS | NS | NS | NS | NS |
| CC input × Fertilizer input | NS | NS | NS | NS | NS |
| Soil × CC input × Fertilizer input | NS | NS | NS | NS | NS |

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS: no significant difference ($p > 0.05$). [1] CC plots and FA plots, [2] with or without Italian ryegrass input, [3] Bokashi and liquid fertilizer, only Bokashi and no fertilizer. CC: cover crop. FA: fallow.

Significant differences were also observed in the effect of fertilizer input during the incubation period (Table 5). Especially, samples treated with Bokashi and liquid fertilizer showed a significant effect against samples with nonfertilizer input across the experiment (Figure 11). On the other hand, we did not find a significant difference between samples with Bokashi input and nonfertilizer input in week 1. As liquid fertilizer was applied to the samples with Bokashi and liquid fertilizer in week 7, there was no difference in samples with fertilizer input.

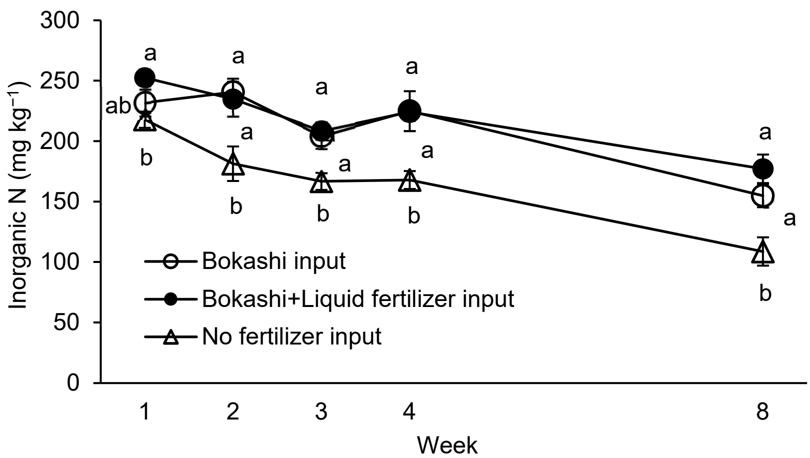


**Figure 11.** Changes in soil inorganic N during the 8 weeks of incubation of paddy soil with or without fertilizer input. Vertical bars mean the standard error. Different letters indicate significant differences ($p < 0.05$). Significant differences were compared by week.

We found an interactive effect between soil and cover crop input in week 2 and week 4 (Table 5). Further analysis of this interactive effect showed significant differences after week 4 (Figure 12). Contrary to our expectation, we did not find an interactive effect between soil and cover crop input on soil inorganic N until week 3. Significant differences among the treatments appeared in week 4; however, the soil inorganic N of FA plot samples was higher than that of cover crop plots, in the order of FA plots with cover crop input > FA plots with no cover crop input > cover crop plots with no cover crop input > cover crop plots with cover crop input. A notable shift in this trend was observed between weeks 4 and 8. Although FA plots with cover crop input maintained the highest soil inorganic N levels through 8 weeks, cover crop plots with cover crop input exhibited the 2nd highest levels of soil inorganic N. The ranking of soil inorganic N levels is as follows: FA plots with cover crop input > cover crop plots with cover crop input > FA plots with no cover crop input > cover crop plots with no cover crop input.

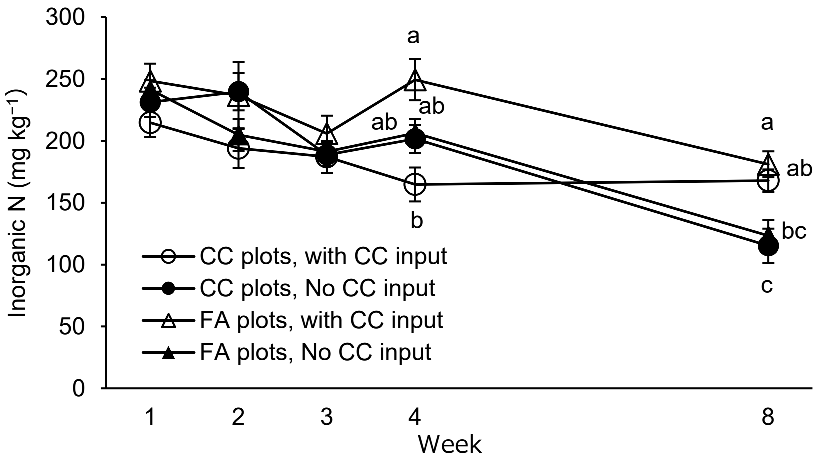


**Figure 12.** Changes in soil inorganic N during the 8 weeks of incubation of a paddy soil with or without cover crop input. CC: cover crop. FA: fallow. Vertical bars mean the standard error. Different letters indicate significant differences ($p < 0.05$). Significant differences were compared by week.

## 4. Discussion

### *4.1. Biomass Production and the Impact of Precipitation on Cover Crop Growth*

Our study demonstrated that cover crops consistently produced higher biomass than weeds over the three-year period (Figure 1). The biomass of cover crops was more than two times that of weeds in 2021, with a threefold increase noted in 2023 (cover crop: 2.6 Mg ha$^{-1}$, weed: 0.7 Mg ha$^{-1}$). Our finding agrees with previous research by Komatsuzaki [2], which highlighted that cover crops can readily return mass organic matter to farmland. The reason for the low plant growth in 2022 remains unclear. In particular, cover crops (Italian ryegrass) in 2022 were not grown well compared to 2021 and 2023. The low growth of Italian ryegrass in the winter of 2021 may have been due to the precipitation levels. The total precipitation at the study site from December 2021 to April 2022 was 459 mm, whereas the average rainfall between 1991 and 2020 at the site was 337.6 mm [19]. This result implies that precipitation should be considered when selecting Italian ryegrass as a cover crop, especially in regions with high winter precipitation.

### *4.2. Legacy Effect of Long-Term Italian Ryegrass Cultivation on Cover Crop and Soil N Accumulation*

The legacy effect of long-term Italian ryegrass cultivation under the same plot design may have affected N content of cover crops and soil N accumulation. Notably, cover crops exhibited higher N content than weeds, particularly in 2023. This result suggests that cover crops, with a combination of Italian ryegrass and hairy vetch, play a crucial role in N accumulation in their tissue. Contrary to our expectations, we did not find significant differences in plant N between Italian ryegrass and weeds in 2021 and 2022 (Figure 2). Gramineous cover crops are generally considered to have high N uptake capacity, and previous studies support Italian ryegrass as an option to reduce N leaching loss. He et al. [23] showed the high C/N ratio of nonleguminous cover crops facilitates available N uptake in paddy fields. Similarly, Zebarth et al. [24] demonstrated that increased N fertilization resulted in N uptake of cover crops in harvested forage and in stubble tissue in upland farming. Our result of low plant N in 2021 and 2022 in cover crops may relate to its C/N ratio. The C/N ratio of nonleguminous cover crops is affected by the growing period and soil N levels, with low soil fertility often resulting in a high C/N ratio [6]. In our study, the C/N ratio of Italian ryegrass in 2021 and 2022 was 73.7 and 65.3, respectively, and it was much higher than the 2023 ratio of 28.0.

The soil inorganic N content in the experimental field highlights a correlation between soil fertility and the C/N ratio of nonleguminous cover crops. This study also found the maximum soil inorganic N at a depth of 90 cm was 70.5 mg $kg^{-1}$ in the cover crop plot and 64.7 mg $kg^{-1}$ in the FA plot during the April 2021 and April 2022 sampling periods (Figure 4). In contrast, in December 2022 and April 2023, the maximum soil inorganic N reached 107.3 mg $kg^{-1}$ in the cover crop plots and 111.9 mg $kg^{-1}$ in the FA plots, respectively. The mixed seeding of gramineous cover crops and leguminous cover crops may have enhanced soil N levels in the cover crop plots, leading to increased plant biomass and plant N contents in 2023. This study suggests that long-term, single-crop cultivation of Italian ryegrass during winter may reduce soil inorganic N levels in the upper layers of paddy fields. In contrast, leguminous cover crops, either used alone or in combination with gramineous cover crops, are valuable in restoring soil fertility after extended periods of residual N uptake by gramineous cover crops.

### *4.3. Effect of Cover Crop Residues on Soil C Stocks in Paddy Fields*

Cover crop residues contributed to increased soil organic matter and C retention in the upper soil layer. At the 0–15 cm depth, cover crop plots consistently exhibited higher soil C compared to FA plots (Figure 6). The incorporation of cover crop and weed residues in April, combined with rice straw residues harvested in October, provided substantial organic matter inputs. Relatively greater cover crop biomass, in particular Italian ryegrass, led to increased soil C concentration in the upper layer during both sampling periods. However, below the depth of 15 cm, FA plots generally showed higher soil C concentration than cover crop plots. As shown by Lei et al. [1], the reducing zone of paddy fields is a significant source of greenhouse gas emissions, primarily $CO_2$ and $CH_4$. Organic matter decomposition by methanogens in this zone can lead to substantial greenhouse gas emissions. Zhou et al. [25] demonstrated that residue incorporation noticeably increased C loss as $CO_2$ and $CH_4$, and that plant residues with higher C/N ratios produced more $CH_4$. Our finding indicates that cover crop residues, especially Italian ryegrass, had a higher C/N ratio, particularly in 2021 and 2022 (Table 1). This higher C/N ratio may have accelerated C release into the atmosphere, resulting in lower soil C concentration in cover crop plots below the 15 cm depth.

### *4.4. N Mineralization Resulting from the Decomposition of Cover Crop*

In the incubation study to investigate the effect of soil management and cover crop input (study in 2021), soil with plant input exhibited significantly higher inorganic N than soil with no plant input (Figure 9). However, notable differences between cover crop input and weed input were observed only at week 8. As shown in Figure 2, no significant differences were found in plant N between cover crops and weeds in 2021; thus, N released from both plant types may have been at nearly equivalent levels. In addition, the C/N ratio of the two plant residues may have influenced their respective N release rates. The addition of plant residues with an equal quantity of C to the soil reduces N availability when the residues have a higher C/N ratio [25]. The lower C/N ratio of weeds compared to cover crops may have facilitated its decomposition.

We also observed that flooded conditions affected soil N mineralization. The inorganic N in soils with cover crops and weeds decreased from week 4 (Figure 9). This result suggests that flooding inhibited the nitrification of N derived from cover crops or weeds in the reducing layer, leading to denitrification. This finding is consistent with other studies (e.g., [26]).

In the incubation study to examine the effect of soil management, cover crop input, and fertilization (study in 2022), we observed that Bokashi fertilizer input enhanced soil inorganic N levels (Figure 11). Soils with Bokashi input or Bokashi and liquid fertilizer input showed higher soil inorganic N compared to soils without fertilizer input throughout the incubation period. In contrast, the 2021 incubation study showed a decline in inorganic N in soils with plant input, especially with cover crop residues, beginning in week 4. Our 2022 study implies that Bokashi input may have a prolonged effect on maintaining soil fertility.

An interactive effect of soil and cover crop input showed a different trend from the 2021 incubation study. Significant differences in soil inorganic N were not observed among the soils with and without cover crop input until week 3, with differences appearing from week 4 onward (Figure 12). This trend may be explained by immobilization caused by the soil microbes. Residues with a high C/N ratio can cause immobilization of soil N, reducing N mineralization during the early plant growth stage [27]. The C/N ratio of cover crops in 2022 was 65.3, which may have reduced soil inorganic N. On the other hand, the inorganic N in soils of cover crop plots with cover crop input gradually increased from week 4 to week 8, even under flooded conditions. Our finding suggests that cover crop decomposition began around week 4.

## 5. Conclusions

Our results suggest that cover crop application contributes to providing organic matter and recovering soil N in paddy fields through its incorporation into the field. In particular, combining gramineous cover crops and leguminous cover crops can be included intermittently between rice cultivation and winter cover crop cultivation cycles. This approach effectively takes up residual soil nitrogen while enhancing soil N supply. Furthermore, the use of organic fertilizers such as Bokashi will support rice during its initial growth stage. Implementing this approach in rice farming will reduce the dependence on chemical fertilizers, promoting the sustainability of rice farming practices in paddy fields. However, the C/N ratio of gramineous cover crops should be considered as a critical factor that influences their decomposition rates and nutritional utilization in the soil. In comparison to weeds, the effect of cover crops on soil organic C stock could not be observed clearly in this study. Further research is needed to evaluate the effective methods for enhancing the impact of cover crops on soil C stock. This may include investigating the potential benefits of mixed-species cover cropping and determining the optimal timing for incorporating cover crops into paddy fields.

**Author Contributions:** M.K. and N.A. designed the field study. J.S., N.T., K.M., T.K. and H.N. conducted the study preparation, sample collection, and sample analysis. M.K. also designed the incubation study, with J.S., N.A. and K.M. responsible for preparation, sample collection, and analysis. J.S. drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

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