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# Nitrogen Accumulation and Initial Growth Response in Lettuce Planted at Different Periods After Hairy Vetch Incorporation

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**Abstract:** One-month-old lettuce seedlings were planted in the field with and without prior hairy vetch (*Vicia villosa* L.) incorporation (HV and Fallow, respectively). The periods between hairy vetch incorporation and lettuce planting were 1 day, 8 days and 15 days. The inorganic nitrogen concentration in the soil was higher after hairy vetch incorporation, as well as the nitrogen concentration in the lettuce leaves from the HV plot compared to the Fallow plot, at any planting period. When lettuce seedlings were planted 8 days after hairy vetch incorporation, the leaf dry weight in the HV plots was 11% lower than that in the Fallow plots 4 days after planting; however, leaf growth recovered and the dry weight was 24% higher 12 days after planting, which could be due to enhanced leaf growth as the result of additional accumulated nitrogen provided by the hairy vetch. In the incubation experiment, it was suggested that the inhibitory effect of hairy vetch decomposition was not due to allelopathic substances. Therefore, in the hairy-vetch-incorporated field, growth suppression in the lettuce plants occurred early and continued for a short period after hairy vetch incorporation, but the damage due to the growth suppression effect was minor. More importantly, nitrogen supplied from the hairy vetch promoted lettuce growth after the inhibition period.

**Keywords:** hairy vetch; cover crop; incorporation; nitrogen; lettuce



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## 1. Introduction

A cover crop is a plant that is used to conserve or improve soil fertility, rather than for its harvested product. Cover crops can provide multiple benefits, including carbon sequestration in the soil, the prevention of soil erosion, weed suppression and nutrient supply [1]. Recently, the integration of cover crops has been recommended not only in row cropping systems, but also vegetable cropping systems [2–4]. This is because winter cover crops utilize residual nitrate nitrogen in the soil, thereby reducing environmental burdens [5] and supplying available nutrients as green manure, thus improving soil fertility [6]. They are also recommended as vegetables have a shorter growing season than row crops, making it easier to introduce winter cover crops into vegetable cropping systems [7]. In addition, for vegetables with a shallow root system, such as lettuce, it is important for nutrients to be present in the surface layer of the soil and cover crops are an effective way to collect nutrients from the soil and return them to the surface layer [8].

When it comes to the usage of cover crops as green manure, it is recommended to start the cultivation of main crops more than 2 to 4 weeks after the incorporation of cover crops, as the germination and growth of the main crops are inhibited during residue decomposition [9–11]. There are several possible reasons for this phenomenon, including promoting the growth of *Pythium* species during residue decomposition [12], nitrogen starvation caused by the decomposition of high C/N residues, allelopathic chemicals [13,14] and infection from cover-crop-derived pathogens [15]. However, waiting a long time for the sufficient decomposition of residue leads to a delay in main crop production following the use of cover crops, where late planting reduces the main crops' growth period.

Hairy vetch (*Vicia villosa* L.) is a leguminous cover crop [16], which is often used in cold regions because of its high winter hardiness [17]. It generates an allelopathic substance, cyanamide [14,18], in its body which, as it covers the ground, inhibits the growth of other plants for a few weeks after it is incorporated. However, some previous studies have observed high yields of lettuce and tomato in plots with hairy vetch residues without a sufficiently long interval between the incorporation of hairy vetch and the planting of a subsequent crop [6,19]. As hairy vetch is known to supply nitrogen quickly to the soil and to subsequent crops during its decomposition, it is possible that the nitrogen from the hairy vetch promotes growth in the lettuce after the growth inhibition effect subsides [19]. The confirmation of such information will allow for the cultivation of main crops to begin without a long interval (e.g., a few weeks) after the incorporation of hairy vetch, thus preventing delayed cultivation of main crops; however, it has not yet been investigated.

In this study, we hypothesize that hairy vetch residue releases cyanamide, inhibiting the growth of subsequently planted lettuce, but also releases nitrogen, thus accelerating growth in the plants when incorporated into the soil. To determine a suitable timeframe for planting a main crop after hairy vetch incorporation, we investigated the growth performance of lettuce seedlings and nitrogen uptake in lettuce at three different intervals, from the incorporation of hairy vetch to planting the lettuce in a field experiment. We also investigated the duration of the growth-inhibiting effect and the involvement of allelopathic substances through incubation experiments.

## 2. Materials and Methods

### *2.1. The Field Experiment*

#### 2.1.1. Experimental Field Design

The field experiment was conducted at the Experimental Farm (43°07′34.5″ N, 141°33′73.6″ E, 12.8 m elevation) located at the Field Science Center for Northern Biosphere, Hokkaido University, Japan, in 2021. The soil type was Greysol [6]. The chemical properties of the soil at a depth of 0–10 cm were the following: pH = 5.80, EC = 42.1 mS $m^{-1}$, total C = 53.1 g $kg^{-1}$ soil, total $n$ = 3.42 g $kg^{-1}$ soil, P = 60 mg $kg^{-1}$ soil, K = 360 mg $kg^{-1}$ soil, Ca = 1460 mg $kg^{-1}$ soil and Mg = 370 mg $kg^{-1}$ soil, according to Muchanga et al. [6]. Lettuce plants were grown with and without previously cultivated hairy vetch, a leguminous cover crop. Precipitation and air temperature were monitored every hour during the experimental period (June–August) by using an AMeDAS (Automated Meteorological Data Acquisition System, Japan Meteorological Agency, Japan) [20], which was located in Sapporo, Hokkaido, Japan.

A split-plot design was arranged for the experiment. The main factor was cover-crop treatment, and the following two types of fields were prepared: (1) a plot where hairy vetch was grown and its residue was incorporated (HV plot) and (2) a bare field where hairy vetch was not grown (Fallow plot). The secondary factor was the period between hairy vetch incorporation and lettuce planting, of which there were three levels: 1 day (1-DAI), 8 days (8-DAI) and 15 days (15-DAI). We prepared 6 plots with these combinations and the experiments were replicated 4 times, for a total of 24 plots. The area of each plot was 8 $m^2$ (4 × 2 m).

#### 2.1.2. The Cultivation of Hairy Vetch and Determination of Nitrogen and Carbon Content

An overwintering variety of hairy vetch (*Vicia villosa* L. cv. Kantaro) (Snow Brand Seed Co., Ltd., Sapporo, Japan) was sown on 1 October 2020, at a density of 10 g $m^{-2}$ (209 seeds $m^{-2}$). On 16 June 2021, the hairy vetch was cut using a straw chopper and was incorporated using a rotary at a depth of about 15 cm the next day (17 June). Just before cutting, the above-ground biomass of the hairy vetch was determined using a 0.25 $m^2$ quadrate (0.5 × 0.5 m). The fresh weight was measured in each plot, and then the samples were dried at 60 °C for at least one week to measure their dry weight. The dried samples were ground and 10 mg of the samples were weighed in Sn foil capsules. The total nitrogen and carbon content were determined via combustion at 950 °C by means of an elemental analyzer (Vario EL III, Elementar, Hanau, Germany) (1 sample per plot with hairy vetch × 4 replications) [21].

#### 2.1.3. The Decomposition of Hairy Vetch Residue Under Field Conditions

To examine the decomposition pattern of hairy vetch, a litter bag assay was conducted. A 3 g portion of fresh hairy vetch residue (equivalent to 0.59 g of dry weight) cut into a 5 cm length was wrapped with a double layer of cotton gauze and placed in a 10 $cm^2$ litter bag (2 mm mesh). Eight bags per plot were embedded at a depth of 10–15 cm at the edge of three of the four hairy vetch replication plots on the same day as hairy vetch residue incorporation (17 June). Two bags per plot were taken 5, 12, 19 and 26 days after embedding and dried at 60 °C for at least 1 week to determine their dry weight. The dried samples were ground and the total nitrogen content in the samples was determined using an elemental analyzer (2 samples per plot × 3 replications) [21]. From the obtained nitrogen content, the nitrogen release rate was calculated as follows:

$$\text{Nitrogen release rate (\%)} = \text{(Nitrogen content of hairy vetch at beginning)} - \text{(Nitrogen content of hairy vetch sampled at 4 periods after embedding)}/\text{(Nitrogen content of hairy vetch at beginning)} \times 100.$$

#### 2.1.4. Lettuce Growth Performance

Red leaf lettuce (*Lactuca sativa* L. var. crispa cv. red fire) (Takii Seed Co., Tokyo, Japan) was sown in 1526 $cm^2$ plastic trays (54.5 × 28 cm) with 128 holes (3.0 cm in diameter and 4.5 cm in depth) on 19 May, 26 May and 2 June 2021, and grown in a greenhouse (Table 1). Thirty days after sowing (18 and 25 June, and 2 July), 64 lettuce seedlings with 5–7 true leaves were planted in each plot area (8 $m^2$; 4 × 2 m) with 45 cm between the rows and 25 cm between the plants. The planting dates were 1, 8 and 15 days after incorporation, respectively. When hairy vetch was incorporated, chemical fertilizers, 6 g of ammonium sulfate as nitrogen, 12 g of calcium superphosphate as phosphate ($P_2O_5$) and 14 g of potassium sulfate as potassium ($K_2O$) per $m^2$ were added to both the HV and Fallow plots. For the first week after planting in each plot, hand watering was provided daily. Weeds were pulled out by hand.

**Table 1.** Field operation in the present experiment.

| Year | Date | Period of Lettuce Planting After the Hairy Vetch Incorporation | | |
|---|---|---|---|---|
| | | 1-DAI | 8-DAI | 15-DAI |
| 2020 | 1 October | Sowing of hairy vetch (10 g $m^{-2}$) | | |
| 2021 | 19 May | Sowing of lettuce | | |
| | 26 May | | Sowing of lettuce | |
| | 2 June | | | Sowing of lettuce |
| | 16 June | Sampling of hairy vetch and cutting with a straw chopper | | |
| | 17 June | Incorporation of hairy vetch with a rotary | | |
| | 18 June | Fertilization<br>Planting of lettuce | | |
| | 22 June | Sampling of soil<br>1st sampling of lettuce | | |
| | 25 June | | Fertilization<br>Planting of lettuce | |
| | 26 June | 2nd sampling of lettuce | | |
| | 29 June | | Sampling of soil<br>1st sampling of lettuce | |

**Table 1.** *Cont.*

| Year | Date | Period of Lettuce Planting After the Hairy Vetch Incorporation | | |
|---|---|---|---|---|
| | | 1-DAI | 8-DAI | 15-DAI |
| | 30 June | 3rd sampling of lettuce | | |
| | 2 July | | | Fertilization<br>Planting of lettuce |
| | 3 July | | 2nd sampling of lettuce | |
| | 6 July | | | Sampling of soil<br>1st sampling of lettuce |
| | 7 July | | 3rd sampling of lettuce | |
| | 10 July | | | 2nd sampling of lettuce |
| | 14 July | | | 3rd sampling of lettuce |

To examine initial growth in the lettuce, leaf biomass was collected from each plot 4, 8 and 12 days after planting (6 plants per plot × 4 replications), dried at 60 °C for at least 1 week, and measured for its dry weight. A portion of the dried samples were ground and the total nitrogen content of the samples was determined using an elemental analyzer (2 plants per plot × 4 replications) [21].

2.1.5. The Collection and Analysis of the Soil Samples

Soil samples were collected from two points and mixed for analysis in each plot 4 days after planting (1 sample per plot × 4 replications) in order to evaluate the chemical properties in the soil affecting lettuce growth. Soil samples were collected at a depth of 0–30 cm from the ground's surface and coarse organic matter was removed with a 2 mm sieve within 1 day of collection. The soil samples were used to measure pH, EC and inorganic nitrogen concentration (ammonium nitrogen and nitrate nitrogen).

To determine pH and EC, 4.0 g of soil was put in a plastic tube, to which 10 mL of milliQ water was added, then shaken for 1 h and measured with a pH meter. In addition, 10 mL of milliQ water was added to the sample again, shaken by hand and the EC measured using an EC meter [6]. To determine the inorganic nitrogen concentration, 4.0 g of soil was put in a plastic tube, to which 40 mL of 2 mol $L^{-1}$ KCl solution was added and shaken for 1 h. The solution was then filtered through a filter paper (No. 5C, Advantec) to obtain a soil extract. The soil extracts were analyzed using a flow injection analyzer (FIA, AQLA-700, Aqualab, Tokyo, Japan) to determine their ammonium nitrogen and nitrate nitrogen content [19].

*2.2. Incubation Experiment*

We investigated the allelopathic effect derived from hairy vetch decomposition on lettuce seedlings with varying levels of below-ground segregation (Figure 1), as reported by Kong et al. [22]. We used a series of pots, each with a central cylinder, covered with either a 2000 µm polyethylene mesh (allowing for chemical and microbial interactions), a 0.45 µm nitrocellulose membrane (Bio-Rad Laboratories, Inc., preventing common fungal hyphae while allowing for chemical and bacterial interactions), or plastic (a complete separation). The pots contained 50 g of soil inside the central cylinder and an additional 50 g in the surrounding area. A total of 1.03 g of hairy vetch residue was incorporated with the soil in the central cylinders, after which 10 lettuce seeds were sown in the surrounding area with below-ground segregation at each of the following intervals: 1 day (1-DAI), 8 days (8-DAI) and 15 days (15-DAI). Deionized water (40 mL) was added to the central cylinder to make the soil moisture equal to the field water capacity. In the plastic segregation treatment, 20 mL of deionized water was added to the central cylinder and another 20 mL was added to the surrounding area. All pots were placed in an incubator under a 14 h/10 h (15 °C/10 °C) day–night cycle. The ots were watered daily to keep the soil moisture at field water capacity. For each interval treatment, from the incorporation of hairy vetch to the lettuce sowing and for each segregation treatment, three pots were selected for each culture date (i.e., 4, 8 and 12 days after sowing) and the length of the shoots and roots were destructively measured. Dead seedlings were removed and the median values of the measured shoot and root lengths of the surviving lettuce seedlings were chosen as representative values of each pot and analyzed statistically.

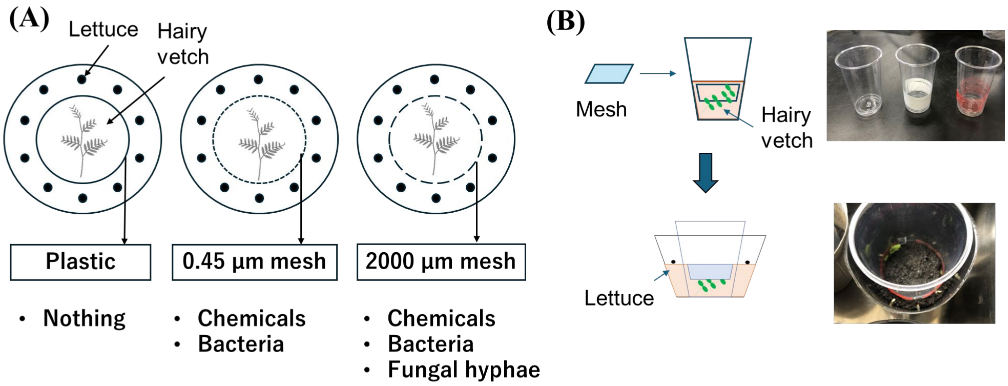


**Figure 1.** Schematic diagram of experimental design with below-ground segregation in incubation experiment (**A**) and illustrations and pictures of central cylinder and whole system of a pot (**B**).

### 2.3. Statistical Analysis

Statistical analysis was performed using R, version 4.1.2 (R core team, Vienna, Austria) [23]. To compare the HV and Fallow plots from the field experiment, *t*-tests were used to analyze the effect of hairy vetch residue incorporation on soil chemistry, lettuce dry weight and nitrogen concentration at each sampling time. To compare between the segregation treatments from the incubation experiment, a one-way ANOVA was used to detect significant differences between the segregation treatments. In cases where the $p$-value was < 0.05, a Tukey HSD test was used to analyze the effect of segregation at each sampling time.

## 3. Results

### 3.1. The Climatic Conditions During the Experimental Period

Precipitation and temperature during the lettuce cultivation periods are shown in Figure 2. In 2021, the total precipitation from 16 June to 14 July was only 26.5 mm, which was extremely low compared to the average precipitation (583 mm) over the past 30 years during the same period. The temperature tended to be higher than the average during the cultivation period.

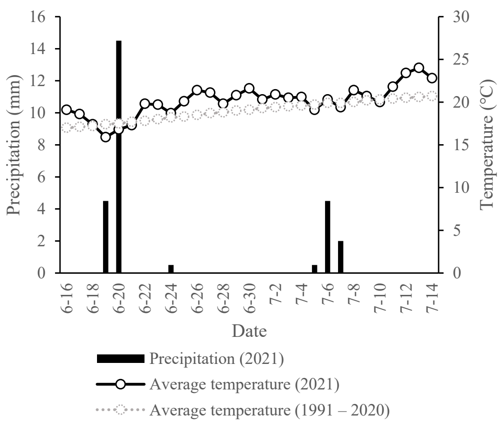


**Figure 2.** Precipitation and average temperature during cultivation. Average temperature (2021) is a daily average. Average temperature (1991–2020) is the average daily temperature on the given date over the past 30 years.

### 3.2. Hairy Vetch Growth and Decomposition

The characteristics of the incorporated hairy vetch residue are shown in Table 2. The biomass, C, and N concentration in the above-ground part of the hairy vetch at the time of incorporation were the following: a dry weight of 668.7 g $m^{-2}$, a carbon concentration of 42.38%, a nitrogen concentration of 4.10% and a C/N ratio of 10.34.

**Table 2.** Characteristics of hairy vetch residue before incorporation.

| | Fresh Weight (g $m^{-2}$) | Dry Weight (g $m^{-2}$) | C (%) | N (%) | N Content (g $m^{-2}$) | C/N |
|---|---|---|---|---|---|---|
| Mean | 4597.8 | 668.7 | 42.38 | 4.10 | 27.43 | 10.34 |
| SD | 1268.8 | 184.6 | 0.10 | 0.03 | 7.57 | 0.07 |

Mean values and SD values are shown ($n$ = 12).

The decomposition and nitrogen release rates of hairy vetch residue in the soil are shown in Figure 3. The dry weight of the hairy vetch residue decreased rapidly over the 12 days after the litter bag was embedded in the soil, followed by a gradual decrease. As for the nitrogen release rate, about 80% of the nitrogen was released 12 days after embedding, followed by a gradual increase.

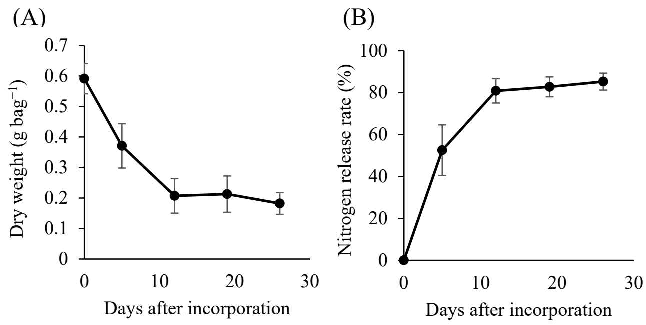


**Figure 3.** Changes in dry weight of hairy vetch residue (**A**) and nitrogen release rate (**B**) in litter bag. Points represent mean values ± SD ($n$ = 6).

### 3.3. Chemical Properties of the Soil

The chemical properties of the soil in each plot 4 days after planting are shown in Table 3. The soil pH was lower in the HV plots than in the Fallow plots 8-DAI. The soil EC was 54% higher 8-DAI and 30% higher 15-DAI in the HV plots than in the Fallow plots. The ammonium nitrogen concentration 1-DAI and 8-DAI tended to be higher in the HV plots (+95% and +43%, respectively), and the nitrate nitrogen concentration 1-DAI, 8-DAI, and 15-DAI was significantly higher in the HV plots than in the Fallow plots (+34%, +110% and +60%, respectively).

**Table 3.** Soil chemical properties 4 days after planting the lettuce.

| Plot | Cover Crop | DAI | pH ($H_2O$) | | EC (mS $m^{-1}$) | | Ammonium Nitrogen (mg $kg^{-1}$ Soil) | | Nitrate Nitrogen (mg $kg^{-1}$ Soil) | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1-DAI | Fallow | 5 | 5.68 ± 0.05 | | 23.1 ± 2.8 | | 12.4 ± 4.2 | | 14.0 ± 0.7 | * |
| | HV | | 5.73 ± 0.06 | | 25.9 ± 3.3 | | 24.1 ± 4.0 | | 18.7 ± 1.8 | |
| 8-DAI | Fallow | 12 | 6.15 ± 0.05 | ** | 24.8 ± 3.5 | ** | 16.3 ± 2.7 | | 15.1 ± 1.7 | ** |
| | HV | | 5.94 ± 0.04 | | 38.3 ± 2.3 | | 23.3 ± 4.2 | | 31.7 ± 1.5 | |
| 15-DAI | Fallow | 19 | 6.25 ± 0.02 | | 20.2 ± 2.2 | * | 14.5 ± 3.3 | | 15.5 ± 1.0 | ** |
| | HV | | 6.13 ± 0.06 | | 26.3 ± 1.5 | | 14.2 ± 1.0 | | 24.8 ± 1.8 | |

Mean values ± SE are shown ($n$ = 4). A $t$-test was performed to detect significant differences between the Fallow plots and the HV plots of 5% (*) and 1% (**). DAI indicates the number of days after the incorporation of hairy vetch residue.

### 3.4. The Dry Weight and Nitrogen Concentration in the Lettuce

The leaf dry weight and nitrogen concentration in the lettuce plants are shown in Figure 4. There was no significant difference in leaf dry weight between the HV plots and the Fallow plots in the 1-DAI group (Figure 4A). In the 8-DAI group, the dry weight was 11% less in the HV plots than in the Fallow plots 4 days after planting; however, the dry weight was 24% greater in the HV plots than in the Fallow plots 12 days after planting (Figure 3B). In the 15-DAI group, the dry weight was greater in the HV plots than in the Fallow plots 8 and 12 days after incorporation (+28% and +22%, respectively) (Figure 4C).

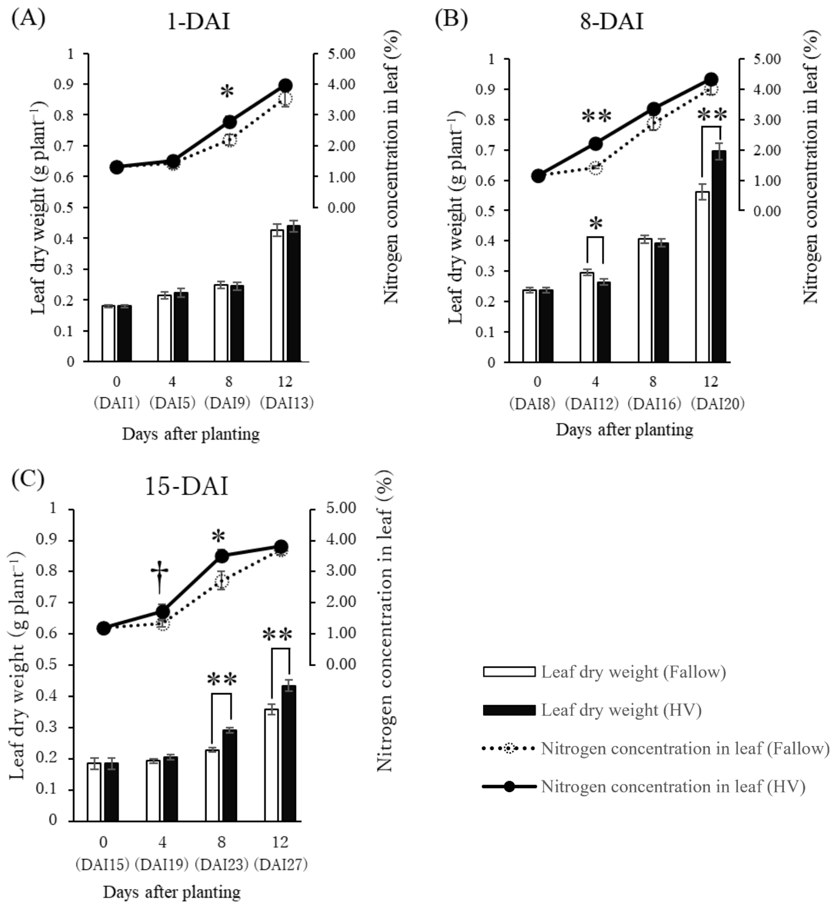


**Figure 4.** The changes in leaf dry weight and nitrogen concentration in lettuce planted on different dates after the incorporation of hairy vetch (**A**–**C**). Bars and points represent mean values ± SE ($n$ = 24 and 8, respectively). A $t$-test was performed to detect significant differences between the Fallow plots and the HV plots at 10% (†), 5% (*) and 1% (**). DAI indicates the number of days after the incorporation of hairy vetch residue.

The nitrogen concentration in the leaves was generally higher in the HV plots than in the Fallow plots in all 1-DAI, 8-DAI and 15-DAI groups. In particular, the concentration in the HV plots 8 days after planting was 28% higher than in the Fallow plots in the 1-DAI group and 55% higher 4 days after planting in the 8-DAI group (Figure 4A,B). In the 15-DAI group, the concentration was 44% and 30% higher in the HV plots than in the Fallow plots 4 and 8 days after planting, respectively (Figure 4C).

### 3.5. The Shoot and Root Lengths of the Lettuce in the Incubation Test

Shoot and root lengths are shown in Figure 5. In the 1-DAI group, the shoot length was 36% smaller and the root length was 79% smaller 12 days after sowing under the 2000 µm segregation treatment compared to the plastic segregation treatment. In the 8-DAI group, shoot and root growth were suppressed under the 2000 µm segregation treatment

4 days after sowing and their lengths were 39% and 65% smaller, respectively, 12 days after sowing compared to the plastic segregation treatment. In the 15-DAI group, shoot length and root length did not differ among the segregation treatments 12 days after sowing.

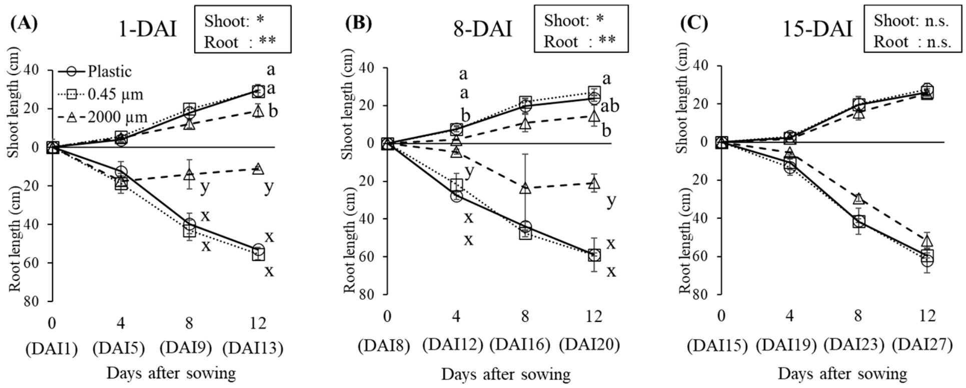


**Figure 5.** The changes in shoot and root length from different below-ground segregation methods at different periods after incorporation of hairy vetch (**A**–**C**). Points represent mean values ± SE ($n$ = 3). In the box in the upper right-hand corner are the results of the one-way ANOVA, performed to detect significant differences in shoot and root length 12 days after sowing of 5% (*) and 1% (**). "n.s." denotes a $p$-value > 0.05. The effects of segregation treatment were clarified at each sampling time according to Tukey HSD, in cases of a $p$-value < 0.05 ($n$ = 3). The different letters indicate a significant difference between the treatments. The days without an accompanied letter denote non-significant effects. DAI indicates the number of days after the incorporation of hairy vetch residue.

## 4. Discussion

### *4.1. Growth Response in Lettuce at Different Periods After Hairy Vetch Residue Incorporation*

The dry weight of the lettuce plants showed different responses depending on the date on which they were planted (Figure 4). Although the nitrogen concentration in the leaves was higher, there was no significant difference in the leaf dry weight between the HV and Fallow plots in the 1-DAI group (Figure 4A) and was rather low in the HV plots compared to the Fallow plots in the 8-DAI group 4 days after planting (Figure 4B). These results suggest that the increase in dry weight was suppressed even with sufficient nitrogen acquisition due to the growth inhibition effect occurring just after hairy vetch residue incorporation. As nitrogen is mainly supplied to plants by mass flow [24], the higher nitrogen accumulation in leaves from the HV plots was attributed to the higher inorganic nitrogen concentration in the soil (Table 3). In other words, a higher nitrogen concentration enhances photosynthesis in leaves [25] which leads to a greater dry weight in plants. In our results, however, a greater dry weight of lettuce in the 1DAI group and 4 days after planting in the 8-DAI group was not observed, indicating the occurrence of an inhibitory effect on growth. This may have been caused by the allelopathic effects of cyanamides in hairy vetch residue [14]. Furthermore, the rapid residue decomposition could have led to an increase in microbes, including *Pythium* species [12], which may have inhibited growth in the lettuce plants. On the other hand, the leaf dry weight of lettuce was greater in the HV plots than in the Fallow plots 12 days after planting in the 8-DAI group (Figure 4B) and 8 days after planting in the 15-DAI group (Figure 4C). Considering that the growth inhibitory effect of hairy vetch residue did not continue for more than 2 weeks (Figure 5C) in the incubation experiment, these results suggest that the additional nitrogen accumulation in the lettuce from the HV plots accelerated their growth after the inhibition period associated with hairy vetch decomposition. Consequently, the lettuce seedlings recovered and grew, and those in the HV plots had a greater dry weight than those in the Fallow plots in the 8-DAI group. From an agronomic point of view, planting lettuce seedlings immediately after hairy vetch residue incorporation may be a practical cultivation technique, as the inhibitory effect on growth was not severe and the accumulated nitrogen supplied from the hairy vetch can be expected to accelerate growth in the lettuce plants at a later stage.

### 4.2. The Identification of Causal Factors in Growth Inhibition and Timing After Incorporation

The results of the incubation test indicated that the allelopathic substance released during hairy vetch residue decomposition did not directly inhibit the growth of the lettuce. The growth of lettuce under the 0.45 μm mesh segregation treatment, through which chemicals and bacteria could pass, was similar to that in the plastic segregation treatment at any period between the incorporation of hairy vetch to sowing the lettuce (Figure 5). On the other hand, the growth of lettuce in the 2000 μm mesh treatment was significantly inhibited in the 1-DAI and 8-DAI groups. These results suggest that the inhibition of lettuce growth occurred 5 days after the incorporation of hairy vetch residue, due, not to allelopathic chemicals, but instead to pathogenic fungal species that cannot pass through 0.45 μm pores. Acharya et al. [26] have reported that the immediate planting of corn after the incorporation of rye residue causes root rot due to *Pythium* and *Fusarium* species suppressing germination and early growth. As for cyanamide, Kamo et al. [14] have reported that cyanamide suppresses lettuce growth. Soltys et al. [18] also observed growth inhibition in lettuce treated with a cyanamide solution. However, Geddes et al. [27] indicate that allelopathic substances derived from hairy vetch do not inhibit plant growth in soil, which may imply that cyanamide degrades quickly and has a limited inhibitory effect in soil.

Growth inhibition in the incubation experiment occurred at a similar time period to the increased nitrogen concentration in the lettuce in the field experiment. In the 1-DAI group, growth inhibition in the incubation experiment was observed 8 days after sowing while an increase in the nitrogen concentration was observed 8 days after planting in the field experiments (Figures 4A and 5A). In the 8-DAI group, growth inhibition was observed 4 days after sowing in the incubation experiment while an increase in the nitrogen concentration was observed 4 days after planting in the field experiment (Figures 4B and 5B). Considering the decomposition of hairy vetch residue, it is reasonable to conclude that the effects of growth inhibition and nitrogen supply occurred simultaneously. Growth in the lettuce was more severely inhibited in the incubation experiment than in the field experiment, which could be due to the different growth stages of the lettuce seedlings used; namely, a nursery plant in the field experiment and a seedling just after germination in the incubation experiment. Kruidhof et al. [4] insist that plants at a more advanced growth stage are less sensitive to growth inhibition after residue decomposition. Therefore, even when a subsequent crop is planted immediately after hairy vetch residue incorporation, the effect of growth inhibition by the hairy vetch residue can be alleviated by planting crops of an advanced growth stage and the growth of the plants can be accelerated by absorbing nitrogen supplied by the hairy vetch residue.

## 5. Conclusions

The lettuce planted after hairy vetch incorporation were affected by both growth inhibition and nitrogen supply due to residue decomposition. The inhibition effect occurred early, within a short period after planting the lettuce in the field, and its negative effect was not severe. After the growth inhibition period, enhanced nitrogen accumulation in the crops contributed to a subsequent increase in leaf dry weight. Therefore, planting lettuce seedlings immediately after hairy vetch incorporation can be considered a practical agronomical technique, enabling an early start to cultivation. In future research, it is necessary to investigate the applicability of this regime of hairy vetch incorporation to other vegetable crops. It is also necessary to investigate the potential of not only hairy vetch, but also other leguminous cover crops.

**Author Contributions:** Conceptualization, H.U. and T.H.; validation, T.H.; formal analysis, H.U.; investigation, H.U.; writing—original draft preparation, H.U.; writing—review and editing, T.S. and T.H.; supervision, T.H. and T.S. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** Data available in a publicly accessible repository.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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