Age-Related Changes in Water and Nitrogen Utilization in Crop Trees and Understory Vegetation in a Hinoki Cypress Plantation Forest in Kochi City, Southern Japan

Yoshiyuki Inagaki 1,*, Kazuki Miyamoto 2 and Atsushi Sakai 3

1 Shikoku Research Center, Forestry and Forest Products Research Institute, 2-915, Asakuranishi, Kochi 780-8077, Japan
2 Forestry and Forest Products Research Institute, 1 Matsunosato, Tukuba 305-8687, Japan; mkazuki@affrc.go.jp
3 Tohoku Research Center, Forestry and Forest Products Research Institute, 92-25 Nabeyashiki, Shimokuriyagawa, Morioka 020-0123, Japan; golgo@affrc.go.jp
* Correspondence: yinagaki@affrc.go.jp

Abstract: Age-related changes in water and nitrogen utilization of crop and understory vegetation in a hinoki cypress plantation forest were investigated from the age of 21 to 46 years in Kochi City, southern Japan. Nitrogen concentration in the leaf litter of hinoki cypress showed a decreasing trend with forest age. The leaf $\delta^{15}$N of hinoki cypress was related to a quadratic function and increased from the age of 21 to 26 years and then decreased to the age of 46 years. These results suggest that older hinoki cypress trees utilize soil nitrogen sources with lower $\delta^{15}$N values, and the competition for soil nitrogen with understory vegetation should be stronger. Carbon isotope discrimination ($\Delta^{13}$C) of hinoki cypress decreased from the age of 21 to 30 years and then increased to the age of 46 years. In contrast, the intrinsic water-use efficiency (iWUE) of hinoki cypress increased from the age of 21 to 36 years and then decreased to the age of 46 years. These findings suggest that hinoki cypress trees in the earlier time increased their iWUE by reducing stomatal opening. In the earlier time, the stomatal opening of understory vegetation increased due to higher soil water availability with decreasing stand density of crop trees. In the later time, the iWUE of hinoki cypress decreased due to lower photosynthetic capacity with nitrogen limitation. These results suggest that the increase in the iWUE of hinoki cypress in response to elevated atmospheric carbon dioxide levels should be smaller in the later time because of stronger competition with understory vegetation for soil nitrogen resources.

Keywords: hinoki cypress; nitrogen; stable isotopes; understory vegetation; water-use efficiency

1. Introduction

The development of understory vegetation in conifer plantations is crucial for improving biodiversity and ecosystem services [1–7]. The method of forest management can affect stream water chemistry [8–11]. Nitrogen concentration in stream water is often elevated by atmospheric nitrogen deposition in Japanese evergreen coniferous plantations [10,12,13]; hence, it is necessary to evaluate the effects of forest management on stream water chemistry. Nitrogen cycling in forest ecosystems can vary in relation to forest age [14–19]. The development of understory vegetation may increase the nitrogen retention capacity of forest ecosystems; however, there is still limited information on the age-related changes of nitrogen utilization in crop trees and understory vegetation [20,21].

The natural abundance of nitrogen isotope ratio ($\delta^{15}$N) is used as an index of plant nitrogen sources [22,23]. Age-related changes in nitrogen sources have been evaluated in forest ecosystems [14,24–27]. Within a forest ecosystem, some studies have demonstrated the utilization of different soil nitrogen sources by dominant trees and understory vegetation [28,29]. However, information regarding age-related changes in the leaf $\delta^{15}$N of crop trees and understory vegetation in Japanese conifer plantations is limited. The effect
of understory vegetation on forest nitrogen cycling during stand development must be clarified to conserve stream water quality.

Plant physiological properties such as water utilization are affected by elevated atmospheric CO$_2$ concentrations [30–32]. Studies on stable carbon isotope ratio in tree rings have revealed a general increase in intrinsic water-use efficiency (iWUE), and their responses were related to soil water conditions or nitrogen deposition [30,33,34]. Along a vertical profile within a forest ecosystem, iWUE increases with increasing tree height [35,36], but information about age-related changes in the iWUE of crop trees and understory vegetation in Japanese conifer plantations is limited. Leaf litterfall collected over a long-term period can provide us information about the water utilization of crop trees and understory vegetation.

This study investigated the changes in nitrogen and water utilization of crop trees and understory vegetation in a hinoki cypress (Chamaecyparis obtusa (Sieb. et Zucc.) Endlicher) forest in Kochi City, southern Japan. Hinoki cypress trees were planted in 1970, and litterfall was collected for 26 years from 1991 to 2016. Stem growth and nitrogen concentration in the leaves of hinoki cypress and understory vegetation were measured. The objectives of this study were to determine whether (1) nitrogen limitation of hinoki cypress becomes severer in later time because of stronger competition with understory vegetation for soil nitrogen resources and (2) elevated CO$_2$ concentrations increase the iWUE of hinoki cypress and understory vegetation.

2. Materials and Methods

2.1. Study Site

The site of this study was a hinoki cypress plantation forest in Shikoku Research Center, Forestry and Forest Products Research Institute (33°32′, 133°29′). The mean annual temperature of the site is 16.3 °C, and the mean annual precipitation is 2720 mm. The soil was derived from sedimentary rocks of the Pleistocene and classified as Dystrudept in Soil Taxonomy [37]. Seedlings of hinoki cypress trees were planted on the former cropland in 1970. Precommercial thinning was conducted at the age of 22, 34, and 43 years. In 1991, a study plot with an area of 265 m$^2$ was established, and the height, height at crown base, and diameter at breast height (DBH) were measured with 2- or 3-year intervals. The leaf biomass of hinoki cypress was estimated using an equation based on the pipe model theory [38,39]. For understory vegetation, the number of plant species and coverage was investigated in 2002 and 2017 [40] (Tables 1 and 2). The major understory species were shrubs such as Symplocos glauca (Thunb.) Koidz. and Ficus erecta Thunb. There were more than 80 plant species in both years, and the number of fern species was greater in 2017 than in 2002. Climate data were obtained from the Kochi Meteorological Station of Japan Meteorological Agency, located approximately 9 km east of Shikoku Research Center.

Table 1. Number of plant species of understory vegetation in 2002 and 2017 [40].

|     | 2002 |     | 2017 |     |
|-----|------|-----|------|-----|
|     | $n$  | (%) | $n$  | (%) |
| Tree | 22   | (28)| 24   | (28)|
| Shrub| 17   | (20)| 14   | (16)|
| Liana| 15   | (18)| 12   | (14)|
| Herb | 17   | (20)| 14   | (16)|
| Fern | 11   | (13)| 21   | (25)|
| Total| 83   | (100)|85   | (100)|
Table 2. Height and cover classes of major understory vegetation species.

| Species Name               | Height (m) | Cover Classes * | Height (m) | Cover Classes * |
|----------------------------|------------|-----------------|------------|-----------------|
| Symlocos glauca            | 3.1        | 2               | 7.2        | 4               |
| Daphniphyllum macropodum   | 2.4        | +               | 6.3        | 1               |
| Eurya japonica             | 3.6        | 1               | 5.6        | +               |
| Clevera japonica           | 2.6        | +               | 5.1        | 1               |
| Neolitsea sericea          | 1.2        | +               | 5.3        | 1               |
| Liquidambar formosana      | 4.5        | 1               |            |                 |
| Ficus erecta               | 2.9        | 2               | 4.0        | 1               |
| Rubus buergeri             | 0.2        | 1               | 0.2        | +               |
| Ligustrum lucidum          | 2.8        | 1               | 4.0        | 1               |
| Dryopteris erythrosora     | 0.6        | 1               | 0.8        | 1               |

* +; <1%, 1; 1–5%, 2; 6–25%, 3; 26–50%, 4; 51–75%.

2.2. Litterfall

Detailed information regarding litterfall collection has been described previously [41]. A total of 15 litter traps designed using a plastic basket covered with mesh clothes (0.14 m²) were placed in the study plot. Litterfall was collected from July 1991 to June 2017 every month. The collected samples were combined into one composite sample and separated into leaves of hinoki cypress, leaves of understory vegetation, and others. The samples were dried at 75 °C for 48 h, and dry weight was measured. Nitrogen and carbon concentrations in the samples were measured using an NC-analyzer (NC-22F; Sumika Chemical Analysis Service). Nitrogen and carbon isotope ratios were analyzed using an online elemental analyzer (NC2500; Thermo Fisher Scientific) coupled with an isotope ratio mass spectrometer (MAT252; Thermo Fisher Scientific). Results are expressed as δ values, defined as ‰ deviation from standard reference materials as follows:

\[ \delta^{13}C \text{ or } \delta^{15}N\ (\text{‰}) = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000 \]  

where \( R = \frac{^{13}C/^{12}C}{^{15}N/^{14}N} \), and values for standards are \(^{15}N\) of atmospheric air = 0 and \(^{13}C\) of Pee Dee belemnite = 0 by definition. l-Alanine, glycine, and l-histidine were used as the running standard. The precision of the measurement was less than 0.10‰ and 0.20‰ for C and N, respectively. The annual leaf fall biomass and nitrogen input were calculated by summing the monthly leaf fall between July and June of the subsequent year. The weighted average was calculated for nitrogen concentration and isotope ratios.

2.3. Plant Water Utilization

Carbon isotope discrimination, caused as a result of preferential use of \(^{12}C\) than \(^{13}C\) during photosynthesis, was defined by the following equation [29]:

\[ \Delta^{13}C = \frac{\delta^{13}Ca - \delta^{13}Cp}{1 + \delta^{13}Cp/1000} \]  

where \( \delta^{13}Ca \) and \( \delta^{13}Cp \) are the isotopic values of atmospheric CO₂ and plant, respectively.

The dependence of \( \Delta^{13}C \) on \( Ci/Ca \), the ratio of intercellular to ambient CO₂ concentrations, was described as follows [42]:

\[ \Delta^{13}C = a + (b - a) \frac{Ci}{Ca} \]  

where \( a (=4.4\text{‰}) \) is the fractionation associated with the diffusion of CO₂ through the stomata, and \( b (=27) \) is the fractionation resulting from enzymatic C fixation. iWUE is
defined as the ratio of CO$_2$ assimilation rate ($A$) to stomatal conductance for water vapor ($gw$) and related to Ca and $\Delta^{13}$C in the following equation:

$$i\text{WUE} = \frac{A}{gw} = \frac{Ca (b - \Delta^{13}C)}{(b - a)/1.6}. \quad (4)$$

Historical annual atmospheric CO$_2$ concentration and carbon isotope value were obtained from an ice-core study [31]. After 2004, they were estimated from linear functions calculated for the 1974–2003 period.

2.4. Statistical Analysis

For mass and nitrogen of the litterfall, ln-transformed values were linearly related to forest age. The annual rate of change was calculated. For other properties, the relationship with forest age was analyzed by linear or quadratic functions. For quadratic functions, the time for the maximum or minimum values was calculated. All statistical analysis was performed with JMP software (version 11.0.0., SAS Institute Japan, Osaka, Japan).

3. Results

3.1. Climate

The mean temperature during the growing season from March to October ranged from 19.7 °C to 22.2 °C and increased temporally with an annual increase of 0.03 °C ($r^2 = 0.189, p = 0.0266$, Figure 1a). Precipitation in the growing season ranged from 1400 to 4020 mm and showed no increasing or decreasing trend (Figure 1b). The maximum wind speed during the storm season from August to October was high in the ages of 23, 24, and 44 years. The maximum wind speed marginally decreased during the study period ($r^2 = 0.140, p = 0.0594$, Figure 1c).

![Figure 1. Mean air temperature (a) and precipitation (b) from March to October and maximum wind velocity from August to October (c) during the study period (1991–2016) at the Kochi Meteorological Station of Japan Meteorological Agency, located approximately 9 km east of the study site.](image-url)
3.2. Stem Growth and Leaf Litterfall

The stand density of hinoki cypress trees decreased with forest age, with the rate of annual decrease being 4.4% (Table 3). The annual stem growth rate of hinoki cypress ranged from 2.3 to 6.7 Mg ha\(^{-1}\) yr\(^{-1}\) and was lower just after the thinning practice during the ages of 35–37 and 44–46 years. The leaf biomass in the crown of hinoki cypress was 17.7 Mg ha\(^{-1}\) at the age of 22 years and decreased to 11.9 Mg ha\(^{-1}\) at the age of 46 years. The proportion of annual decrease in leaf biomass was 1.2%. The leaf litterfall of hinoki cypress decreased during the study period, with the rate of annual decline being 1.8% (Figure 2a and Table 4). The proportion of annual increase in the leaf litter of understory vegetation was 11.9%. The sum of hinoki cypress and understory vegetation litterfall remained unchanged in relation to forest age (Figure 2b). The ratio of leaf biomass to the leaf litter of hinoki cypress represents an estimate of leaf life span and ranged from 4.1 to 7.9 years (Table 3).

Table 3. Stem and leaf properties of hinoki cypress trees in the study site.

| Forest Age yr | Stand Density n ha\(^{-1}\) | Height m | DBH cm | Stem Biomass Mg ha\(^{-1}\) | Stem Production Mg ha\(^{-1}\) yr\(^{-1}\) | Leaf Biomass Mg ha\(^{-1}\) | Leaf Litterfall Mg ha\(^{-1}\) yr\(^{-1}\) | Leaf Biomass/Leaf Litterfall | Thinning |
|---------------|-----------------------------|----------|--------|-----------------------------|---------------------------------|-----------------------------|---------------------------------|---------------------------------|----------|
| 21–22         | 4264                        | 10.9     | 9.7    | 98.8                        | 5.6                             | 17.7                         | 3.3                             | 5.4                             |          |
| 23–24         | 2528                        | 12.1     | 11.4   | 82.1                        | 6.5                             | 13.7                         | 2.3                             | 5.8                             |          |
| 25–27         | 2528                        | 12.9     | 12.3   | 99.9                        | 5.9                             | 15.6                         | 2.8                             | 5.6                             |          |
| 28–30         | 2452                        | 15.3     | 13     | 113.1                       | 4.4                             | -                            | 3.4                             | -                               |          |
| 31–32         | 2302                        | 14.4     | 13.6   | 124.3                       | 5.6                             | 14.5                         | 2.9                             | 5.1                             |          |
| 33–34         | 2189                        | 15.1     | 14.3   | 135.0                       | 5.3                             | -                            | 3.0                             | -                               |          |
| 35–37         | 1774                        | 15.7     | 15.3   | 129.4                       | 2.9                             | -                            | 2.4                             | -                               | Thinning |
| 38–39         | 1623                        | 16.7     | 16.2   | 137.2                       | 3.9                             | -                            | 2.1                             | -                               |          |
| 40–41         | 1547                        | 17.3     | 16.9   | 148.4                       | 5.6                             | 12.5                         | 3.0                             | 4.2                             |          |
| 42–43         | 1472                        | 18.1     | 17.8   | 161.8                       | 6.7                             | 13.3                         | 3.3                             | 4.1                             |          |
| 44–46         | 1057                        | 19.5     | 19.6   | 147.9                       | 2.3                             | 11.9                         | 1.5                             | 7.9                             | Thinning |

Figure 2. Leaf litterfall of hinoki cypress and understory vegetation (a,b) and litter nitrogen input of hinoki cypress and understory vegetation (c,d) in relation to forest age.
Table 4. Linear and quadric models predicting litter properties. Forest age is used as an explanatory variable.

| Object Variable       | Category       | Equation                                | $R^2$ | $p$         | Annual Rate of Change (%) | Tipping Point (yr) |
|-----------------------|----------------|-----------------------------------------|-------|-------------|---------------------------|-------------------|
| Leaf litterfall       | Cypress        | $\ln(y) = -0.018x + 1.54$               | 0.183 | 0.0289      | -1.8                      |                   |
|                       | Understory     | $\ln(y) = 0.113x - 4.82$               | 0.884 | 0.00001     | 11.9                      |                   |
|                       | Total          | $\ln(y) = 0.001x + 1.09$               | 0.003 | 0.798       | 0.1                       |                   |
| Leaf litter N         | Cypress        | $\ln(y) = -0.022x + 1.25$              | 0.265 | 0.0071      | -2.2                      |                   |
|                       | Understory     | $\ln(y) = 0.104x - 4.45$              | 0.858 | 0.00001     | 11.0                      |                   |
|                       | Total          | $\ln(y) = 0.004x + 0.64$               | 0.026 | 0.435       | 0.4                       |                   |
| Leaf N concentration  | Cypress        | $y = -0.027x + 7.31$                   | 0.121 | 0.0815      |                           |                   |
|                       | Understory     | $y = -0.040x + 7.60$                   | 0.507 | 0.00001     |                           |                   |
|                       | Total          | $y = -0.0152x^2 + 0.926x - 2.14$       | 0.438 | 0.0013      | 30                        |                   |
|                       | $\delta^{15}N$ | Cypress                                |       |             |                           |                   |
|                       | Understory     | $y = -0.0037x^2 + 0.187x - 5.38$       | 0.871 | 0.00001     | 26                        |                   |
|                       | Total          | $y = -0.002x^2 + 0.098x - 4.19$        | 0.401 | 0.0027      | 28                        |                   |
|                       | $\delta^{13}C$ | Cypress                                |       |             |                           |                   |
|                       | Understory     | $y = -0.003x^2 + 0.125x - 28.62$       | 0.885 | 0.0001      | 25                        |                   |
|                       | Total          | $y = -0.003x^2 + 0.141x - 28.54$       | 0.898 | 0.0001      | 21                        |                   |
|                       | iWUE           | Cypress                                |       |             |                           |                   |
|                       | Understory     | $y = -0.006x^2 + 0.451x + 14.86$       | 0.620 | 0.0001      | 39                        |                   |

* data of high N concentration caused by storms in 2004 and 2014 were excluded.

3.3. Plant Nitrogen Utilization

The leaf litter nitrogen input of hinoki cypress decreased with forest age, with the rate of annual decrease being 2.2% (Figure 2c). In contrast, that of understory vegetation increased with forest age, with the rate of annual increase being 11.0%. The total leaf litter nitrogen input remained unchanged in relation to forest age (Figure 2d). The leaf litter nitrogen concentration of hinoki cypress was high in trees aged 34 and 44 years (Figure 3a and Table 4). During these years, leaf fall occurred earlier than the usual year due to the attack of strong typhoons. If these two years were excluded, the nitrogen concentration of hinoki cypress leaf litter was found to decrease with forest age. The leaf litter nitrogen concentration of understory vegetation was related to a quadratic function and increased from the age of 21 to 30 years and then decreased to the age of 46 years. The leaf litter nitrogen concentration of total leaf litter was not related to forest age (Figure 3b). The leaf litter $\delta^{15}N$ of hinoki cypress was lower than that of understory vegetation (Figure 3c). The leaf litter $\delta^{15}N$ of hinoki cypress was related to a quadric function and increased from the age of 21 to 26 years and then decreased to the age of 46 years. The proportion of decrease in leaf litter $\delta^{15}N$ from the age of 26 to 46 years was 1.5‰. The leaf litter $\delta^{15}N$ of understory vegetation decreased from the age of 21 to 45 years. The leaf litter $\delta^{15}N$ of total leaf litter increased from the age of 21 to 28 years and then decreased to the age of 46 years (Figure 3d). The proportion of decrease in $\delta^{15}N$ from the age of 28 to 46 years was 0.5‰.
3.4. Plant Water Utilization

The leaf litter $\delta^{13}$C of hinoki cypress increased from the age of 21 to 25 years and then decreased, whereas that of understory vegetation decreased from the age of 21 to 41 years (Figure 3e). The $\Delta^{13}$C of hinoki cypress decreased from the age of 21 to 30 years and then increased to the age of 46 years, whereas that of understory vegetation increased from the age of 21 to 39 years and then decreased (Figure 4a). The iWUE of hinoki cypress increased from the age of 21 to 36 years and then decreased, whereas that of understory vegetation decreased from the age of 21 to 37 years and then increased (Figure 4b). Leaf litter $\delta^{15}$N was linearly and negatively related to $\Delta^{13}$C in each vegetation type (Figure 5a). The leaf litter $\delta^{15}$N of hinoki cypress was not related to iWUE, whereas that of understory vegetation was linearly and positively related to iWUE (Figure 5b).
In the study site, leaf biomass and leaf litterfall of hinoki cypress decreased with forest age. Although the leaf biomass of each tree increased [21,43], the leaf biomass per stand area decreased due to the high decrease rate of stand density. Previous studies have demonstrated that the leaf biomass or leaf litterfall of Japanese coniferous plantations was constant or increased with forest age [18,44,45], and the result of the present study is different from these findings. The reduction of leaf biomass observed in this study is not common for Japanese plantations but is presumably caused by repeated thinning practices. The ratio of leaf biomass to leaf litterfall is an index of leaf life span and ranged from 4.1 to 7.9 years and is consistent with the previous study [46]. The percentage of leaf area decreased due to the high decrease rate of stand density. Previous studies have demonstrated that the leaf biomass or leaf litterfall of Japanese coniferous plantations was constant or increased with forest age [18,44,45], and the result of the present study is different from these findings. The reduction of leaf biomass observed in this study is not common for Japanese plantations but is presumably caused by repeated thinning practices. The ratio of leaf biomass to leaf litterfall is an index of leaf life span and ranged from 4.1 to 7.9 years and is consistent with the previous study [46]. The percentage of annual decrease in the leaf biomass of hinoki cypress was 1.2%, but that of leaf litterfall was 1.8%; these results suggest longer leaf life span in older ages.

The leaf litterfall of understory vegetation increased rapidly, and the proportion of understory vegetation to total leaf litter was 40% from the age of 44 to 46 years (Figure 2a). The mean proportion for understory vegetation was 15% in some hinoki cypress forests in western Japan [47]. This result suggests that the amount of leaf litter of understory vegetation in the present study is higher among hinoki cypress forests in Japan. The lower
stand density of hinoki cypress trees due to thinning is presumably a primary factor for the development of understory vegetation in this study.

4.2. Plant Nitrogen Utilization

Leaf litter nitrogen input and nitrogen concentration of hinoki cypress decreased with forest age. Similar results were reported in previous studies [16,18]. The nitrogen concentration of total leaf litter did not change with forest age, suggesting that understory vegetation compensated the lower nitrogen uptake of hinoki cypress trees. The nitrogen concentration of understory vegetation increased from the age of 21 to 30 years and then decreased. During the early period of the study, the nitrogen uptake of understory vegetation should increase in response to higher availability of soil nitrogen after the thinning practice [48], and in the later period, competition for soil nitrogen became stronger due to greater biomass of understory vegetation.

The leaf δ^{15}N of hinoki cypress in this study was within the range reported in previous studies on hinoki cypress (−7.1–0.4‰) [49,50]. The leaf δ^{15}N of hinoki cypress was lower than that of understory vegetation. Similar results were found in a Japanese cedar forest [28]. These results suggest that the nitrogen sources of crop trees were different from those of understory vegetation. Plant δ^{15}N is affected by soil nitrogen sources and mycorrhizal infection [22,23]. The leaf δ^{15}N of hinoki cypress was decreased by higher uptake of soil nitrate in the high-nitrogen-deposited forests [49]. In an experiment using ^15N tracer, it was observed that hinoki cypress preferred soil ammonium to nitrate [51]; the results suggested that the preference of nitrate is promoted in forests with high soil nitrate availability. Lower accumulation and rapid decomposition of organic horizon have been reported in hinoki cypress forest soils [47,52,53]; the results suggested rapid nitrogen release from organic horizon with a lower δ^{15}N value. These findings indicate that nitrogen released from organic horizon and soil nitrate are the two possible nitrogen sources of the lower 15N value of hinoki cypress trees.

The leaf δ^{15}N of hinoki cypress decreased in the later period, and the changes in the leaf δ^{15}N of hinoki cypress were greater than those of total leaf. Previous studies have also evaluated the changes in leaf δ^{15}N [24–27]. The leaf δ^{15}N was found to decrease by the utilization of nitrogen sources from nitrogen deposition with lower δ^{15}N [24,54,55]. In this study, the rate of nitrogen deposition was not high (8.1 kg ha\(^{-1}\) yr\(^{-1}\)) [56]. Furthermore, the changes in total leaf δ^{15}N were relatively small. These findings suggest that the contribution of nitrogen deposition toward the leaf δ^{15}N of hinoki cypress and understory vegetation is of minor importance. In Japanese cedar plantations, the leaf δ^{15}N of crop trees decreased from the age of 4 to 41 years, which corresponded to the changes in percent nitrification to mineralization [26]. In the study site, the infection rate of arbuscular mycorrhizal fungi increased with forest age [57]. Therefore, it is difficult to evaluate the effects of soil nitrate and mycorrhizal infection in the study site. Hinoki cypress has shallow fine roots in soil profiles [58]. The leaf δ^{15}N of hinoki cypress was very low in nitrogen-limited forests [50]. These findings indicate that in nitrogen-limited forests, hinoki cypress would utilize soil nitrogen released from organic horizon and the contribution of soil nitrate should be of minor importance. In this study, the leaf litter nitrogen concentration of hinoki cypress decreased with forest age, and nitrogen limitation increased with forest age. This finding implies that a higher uptake of nitrogen derived from organic horizon would be a primary factor for the lower leaf δ^{15}N of hinoki cypress.

4.3. Plant Water Utilization

During the early time of this study, the Δ\(^{13}\)C of hinoki cypress decreased, whereas iWUE increased, suggesting that stomatal opening decreased during this time. In general, the iWUE of forest ecosystems has been reported to increase with elevated atmospheric CO\(_2\) concentrations [32–34]. The extent of increase was greater in ecosystems with high nitrogen deposition and lower soil water availability [33,34]. The increase in iWUE observed in this study was relatively small because the area receives modest nitrogen deposition [56] and
has high soil water availability with high precipitation. In the later time of the study, the $\Delta^{13}$C of hinoki cypress increased, whereas iWUE decreased. Moreover, negative relationships were found between leaf $\delta^{15}$N and $\Delta^{13}$C (Figure 5a). Increased water availability with lower stand density can cause greater $\Delta^{13}$C, but there was no clear pattern of $\Delta^{13}$C in thinned hinoki cypress forests [52]. In Japanese red pine forests, the presence of understory vegetation was found to decrease $\Delta^{13}$C compared with understory-excluded forests [59], and the $\Delta^{13}$C of dominant trees would not increase with understory vegetation. These results suggest that the increase in $\Delta^{13}$C is caused by lower photosynthetic activity with severer nitrogen limitation as discussed in the previous section rather than by changes in stomatal opening. The leaf $\delta^{13}$C of understory vegetation was greater than that of hinoki cypress. Along a vertical profile of forest ecosystems, $\Delta^{13}$C decreased with tree height [35,36], and the result of the present study is consistent with these results. During the early time of the study, the $\Delta^{13}$C of understory vegetation increased, whereas iWUE decreased, with forest age. A decrease in $\Delta^{13}$C is expected with increasing plant height [35,36], but the result indicated an opposite trend. The $\Delta^{13}$C of understory vegetation is expected to increase with greater soil water availability. The water content of hinoki cypress forests increased after thinning [48], and stomatal opening would increase with decreasing stand density during forest development. Nitrogen availability is also a factor for the increased $\Delta^{13}$C of understory vegetation as discussed for hinoki cypress. However, the leaf litter nitrogen concentration of understory vegetation increased during this time (Figure 3a). Therefore, nitrogen limitation is not a factor for the increase in the $\Delta^{13}$C of understory vegetation. These results suggest that crop trees and understory vegetation compete for soil water resources and the reduction of stand density of crop trees can increase the $\Delta^{13}$C of understory vegetation in the earlier time of the study.

4.4. Implication for Forest Management

This study revealed the age-related changes in nitrogen and water utilization of crop trees and understory vegetation using carbon and nitrogen isotope ratios as an index of resource utilization. The competition of hinoki cypress and understory vegetation for soil nitrogen resources was stronger in the later time as indicated by leaf nitrogen concentration and utilization of different soil nitrogen sources. For understory vegetation, with limitation of light, water and nitrogen resources decreased with forest age due to the lower stand density of hinoki cypress trees. The abundance of understory vegetation in this study was relatively high due to the frequent thinning practice in the site. To reduce the nitrogen limitation of hinoki cypress, it is necessary to reduce the intensity of thinning. From a viewpoint of nitrogen retention in forest ecosystems, the development of understory vegetation can lead to stronger competition for soil nitrogen resources by hinoki cypress and understory vegetation. This situation results in higher capacity of nitrogen retention in forest ecosystems and lower nitrogen loss from stream water. These findings suggest that a trade-off exists between the nitrogen retention capacity and productivity of hinoki cypress trees. The amount of understory vegetation can be controlled by thinning intensity for optimizing the balance between timber production and stream water conservation. Further research is required for monitoring nitrogen leaching from soil in different thinning intensities. In this study, the long-term investigation of crop trees and understory vegetation provided us valuable information about their resource utilization. However, this study was conducted in a single site with no replications, but we consider this information highly valuable for other research. The area has a warm climate and high precipitation, which are probably suitable for the development of understory vegetation. The development of understory vegetation can vary with climate and soil conditions [5], and further studies are required to investigate the competition between crop trees and understory vegetation in different climate and soil conditions.
5. Conclusions

Nitrogen and water utilization of crop trees and understory vegetation was investigated using nitrogen and carbon isotope ratios in leaf litter. In the earlier time, the $\Delta^{13}C$ of hinoki cypress trees decreased with forest age as affected by elevated atmospheric CO$_2$ concentrations, whereas the $\Delta^{13}C$ of understory vegetation increased as affected by higher water availability after thinning practice. In the later time, the $\Delta^{13}C$ of hinoki cypress increased with forest age, whereas the leaf $\delta^{15}N$ of hinoki cypress decreased. The lower leaf $\delta^{15}N$ of hinoki cypress than that of understory vegetation and the lower leaf litter nitrogen concentration suggest that hinoki cypress utilizes soil nitrogen released from the organic horizon. Therefore, the increase in the $\Delta^{13}C$ of hinoki cypress in the later time should be caused by lower photosynthetic capacity with low nitrogen availability. Information on isotope ratio in leaf litter could provide insights into the long-term changes of resource limitation and competition between crop trees and understory vegetation in a forest ecosystem. The competition between crop trees and understory vegetation can vary with different climate and soil conditions. Further research is needed to examine the age-related changes of isotopic ratio in diverse forest ecosystems in this region for optimizing the balance between timber production and stream water conservation.

Author Contributions: Conceptualization, Y.I.; investigation, Y.I., A.S. and K.M.; formal analysis, Y.I.; writing—original draft, Y.I.; writing—review and editing, Y.I., A.S. and K.M. All authors have read and agreed to the published version of the manuscript.

Funding: This study is partly supported by Strategic International Collaborative Research Program (SICORP) (JPMJSC19C3) from the Japan Society for the Promotion of Science and by a Grant-in-Aid for Scientific Research (nos. 17K07840, 20H03034) from the Japan Society for the Promotion of Science.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data supporting reported results can be accessed by contacting yinagaki@affrc.go.jp.

Acknowledgments: We appreciate the helpful suggestions by Erik Hobbie, Keisuke Koba, and Tayoko Kubota.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Forest Agency. Annual Report on Forest and Forestry in Japan, Fiscal Year 2020. 2021. Available online: https://www.rinya.maff.go.jp/j/kikaku/hakusyo/R2hakusyo/index.html (accessed on 23 March 2022). (In Japanese)
2. Hansen, A.J.; Spies, T.A.; Swanson, F.J.; Ohmann, J.L. Conserving biodiversity in managed forests—lessons from natural forests. Bioscience 1991, 41, 382–392. [CrossRef]
3. Zhang, J.Y.; Qin, G.Z.; Zhai, Z.; Zhou, S.C.; Tang, L.Z.; Tian, Y. Diverse understory vegetation alleviates nitrogen competition with crop trees in poplar plantations. Forests 2021, 12, 705. [CrossRef]
4. Seiwa, K.; Eto, Y.; Hishita, M.; Masaka, K. Effects of thinning intensity on species diversity and timber production in a conifer (Cryptomeria japonica) plantation in Japan. J. For. Res. 2012, 17, 468–478. [CrossRef]
5. Seiwa, K.; Etoh, Y.; Hisita, M.; Masaka, K.; Imaji, A.; Ueno, N.; Hasegawa, Y.; Konno, M.; Kanno, H.; Kimura, M. Roles of thinning intensity in hardwood recruitment and diversity in a conifer, Cryptomeria japonica plantation: A 5-year demographic study. For. Ecol. Manag. 2012, 269, 177–187. [CrossRef]
6. Noguchi, M.; Okuda, S.; Miyamoto, K.; Itou, T.; Inagaki, Y. Composition, size structure and local variation of naturally regenerated broadleaved tree species in hinoki cypress plantations: A case study in Shikoku, south-western Japan. Forestry 2011, 84, 493–504. [CrossRef]
7. Miura, S.; Ugawa, S.; Yoshinaga, S.; Yamada, T.; Hirai, K. Floor Cover Percentage Determines Splash Erosion in Chamaecyparis obtusa Forests. Soil Sci. Soc. Am. J. 2015, 79, 1782–1791. [CrossRef]
8. Bormann, F.H.; Likens, G.R. Pattern and Process in a Forested Ecosystem; Springer: New York, NY, USA, 1979; p. 253.
9. Tokuchi, N.; Fukushima, K. Long-term influence of stream water chemistry in Japanese cedar plantation after clear-cutting using the forest rotation in central Japan. For. Ecol. Manag. 2009, 257, 1768–1775. [CrossRef]
10. Chiwa, M. Long-term changes in atmospheric nitrogen deposition and stream water nitrate leaching from forested watersheds in western Japan. Environ. Poll. 2021, 287, 117634. [CrossRef]
11. Takagi, M. Water chemistry of headwater streams under stormflow conditions in catchments covered by evergreen broadleaved forest and by coniferous plantation. *Landsc. Ecol. Eng.* 2015, 11, 293–302. [CrossRef]

12. Mitchell, M.; Iwatsubo, G.; Ohrui, K.; Nakagawa, Y. Nitrogen saturation in Japanese forests: An evaluation. *For. Ecol. Manag.* 1997, 97, 39–51. [CrossRef]

13. Nishina, K.; Watanabe, M.; Koshikawa, M.K.; Takamatsu, T.; Morino, Y.; Nagashima, T.; Soma, K.; Hayashi, S. Varying sensitivity of mountainous streamwater base-flow NO$_3^-$ concentrations to N deposition in the northern suburbs of Tokyo. *Sci. Rep.* 2017, 7, 7701. [CrossRef] [PubMed]

14. Tateno, R.; Fukushima, K.; Fujimaki, R.; Shimamur, T.; Oghi, M.; Arai, H.; Ohte, N.; Tokuchi, N.; Yoshioka, T. Biomass allocation and nitrogen limitation in a Cryptomeria japonica plantation chronosequence. *J. For. Res.* 2009, 14, 276–285. [CrossRef]

15. Xia, Q.; Chen, L.; Xiang, W.H.; Ouyang, S.; Wu, H.L.; Lei, P.F.; Xiao, W.F.; Li, S.G.; Zeng, L.X.; Kuzyakov, Y. Increase of soil nitrogen availability and recycling with stand age of Chinese-fir plantations. *For. Ecol. Manag.* 2021, 480, 118643. [CrossRef]

16. Sun, Z.; Liu, L.; Peng, S.; Penuelas, J.; Zeng, H.; Piao, S. Age-related modulation of the nitrogen resorption efficiency response to growth requirements and soil nitrogen availability in a temperate pine plantation. *Ecosystems* 2016, 19, 698–709. [CrossRef]

17. Fukushima, K.; Tateno, R.; Tokuchi, N. Soil nitrogen dynamics during stand development after clear-cutting of Japanese cedar (Cryptomeria japonica) plantations. *J. For. Res.* 2011, 16, 394–404. [CrossRef]

18. Inagaki, Y.; Miura, S.; Kohzu, A. Effects of forest type and stand age on litterfall quality and soil N dynamics in Shikoku district, southern Japan. *For. Ecol. Manag.* 2004, 202, 107–117. [CrossRef]

19. Wu, H.L.; Xiang, W.H.; Ouyang, S.; Xiao, W.F.; Li, S.G.; Chen, L.; Lei, P.F.; Deng, X.W.; Zeng, Y.L.; Zeng, L.X.; et al. Tree growth rate and soil nutrient status determine the shift in nutrient-use strategy of Chinese fir plantations along a chronosequence. *For. Ecol. Manag.* 2020, 460, 117896. [CrossRef]

20. Fukuzawa, K.; Shibata, H.; Takagi, K.; Nomura, M.; Kurima, N.; Fukuzawa, T.; Satoh, F.; Sasa, K. Effects of clear-cutting on nitrogen leaching and fine root dynamics in a cool-temperate forested watershed in northern Japan. *For. Ecol. Manag.* 2006, 225, 257–261. [CrossRef]

21. Masuda, C.; Morikawa, Y.; Masaka, K.; Koga, W.; Suzuki, M.; Hayashi, S.; Tada, C.; Seiwa, K. Hardwood mixture increases stand productivity through increasing the amount of leaf nitrogen and modifying biomass allocation in a conifer plantation. *For. Ecol. Manag.* 2022, 504, 119835. [CrossRef]

22. Högbom, P. Tansley review No 95 $^{15}$N natural abundance in soil-plant systems. *New Phytol.* 1997, 137, 179–203. [CrossRef]

23. Craine, J.M.; Brookshire, E.N.J.; Cramer, M.D.; Hasselquist, N.J.; Koba, K.; Marin-Spiotta, E.; Wang, L.X. Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant Soil* 2015, 396, 1–26. [CrossRef]

24. Wang, L.X.; Shen, P.J.L.; Macko, S. Foliar $^{15}$N patterns along successional gradients at plant community and species levels. *Geophys. Res. Lett.* 2007, 34, L16403. [CrossRef]

25. Peraakis, S.S.; Tepley, A.J.; Compton, J.E. Disturbance and topography shape nitrogen availability and $^{15}$N over long-term forest succession. *Ecosystems* 2015, 18, 573–588. [CrossRef]

26. Hyodo, F.; Takebayashi, Y.; Makabe, A.; Wardle, D.A.; Koba, K. Changes in stable nitrogen isotopes of plants, bulk soil and soil dissolved N during ecosystem retrogression in boreal forest. *Ecol. Res.* 2021, 10, 420–429. [CrossRef]

27. Sun, F.F.; Kuang, Y.W.; Wen, D.Z.; Xu, Z.H.; Li, J.L.; Zuo, W.D.; Hou, E.Q. Long-term tree growth rate, water use efficiency, and tree ring nitrogen isotope composition of Pinus massoniana L. in response to seasonal change and local nitrogen deposition in Southern China. *J. Soils Sediments* 2010, 10, 1453–1465. [CrossRef]

28. Koba, K.; Hirobe, M.; Koyama, L.; Kohzu, A.; Tokuchi, N.; Nadelhoffer, K.; Wada, E.; Takeda, H. Natural $^{15}$N abundance of plants and soil N in a temperate coniferous forest. *Ecosystems* 2003, 6, 457–469. [CrossRef]

29. Tateno, R.; Nakayama, M.; Yano, M.; Fukuzawa, K.; Inagaki, Y.; Koba, K.; Ugawa, S. Nitrogen source utilization in co-existing canopy tree and dwarf bamboo in a northern hardwood forest in Japan. *Trees-Struct. Funct.* 2020, 107, 1433–1444. [CrossRef]

30. Saurer, M.; Siegwolf, R.T.W.; Schweingruber, F.H. Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years. *Oecologia* 2015, 177, 191–202. [CrossRef]

31. Saurer, M.; Siegwolf, R.T.W.; Schweingruber, F.H. Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years. *Oecologia* 2015, 177, 191–202. [CrossRef]

32. Saurer, M.; Siegwolf, R.T.W.; Schweingruber, F.H. Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years. *Oecologia* 2015, 177, 191–202. [CrossRef]

33. Saurer, M.; Spahni, R.; Frank, D.C.; Joos, F.; Leuenberger, M.; Loader, N.J.; Gagen, M.; Poulter, B.; Siegwolf, R.T.W.; et al. Spatial variability and temporal trends in water-use efficiency of European forests. *Glob. Chang. Biol.* 2015, 20, 3700–3712. [CrossRef]

34. Leonardi, S.; Gentilesca, T.; Guerrieri, R.; Ripullone, F.; Magnani, F.; Mencuccini, M.; Noiije, T.V.; Borghetti, M. Assessing the effects of nitrogen deposition and climate on carbon isotope discrimination and intrinsic water-use efficiency of angiosperm and conifer trees under rising CO$_2$ conditions. *Glob. Chang. Biol.* 2017, 23, 2925–2944. [CrossRef]

35. Ehleringer, J.R.; Field, C.B.; Lin, Z.F.; Kuo, C.Y. Leaf carbon isotope and mineral-composition in subtropical plants along an irradiancecline. *Oecologia* 1986, 70, 520–526. [CrossRef]

36. Kenzo, T.; Inoue, Y.; Yoshimura, M.; Yamashita, M.; Tanaka-Oda, A.; Ichio, T. Height-related changes in leaf photosynthetic traits in diverse Bornean tropical rain forest trees. *Oecologia* 2015, 177, 191–202. [CrossRef]

37. Soil Survey Staff. *Keys to Soil Taxonomy*, 12th ed.; Natural Resources Conservation Service, USDA: Washington, DC, USA, 2014.
38. Mitsuda, Y.; Inoue, A.; Kitahara, F.; Kadota, H.; Hirota, T. Examination of factors affecting the error of aboveground biomass estimation in overcrowded planted stands: A case study using sampled felled trees from a 40-year-old Hinoki (Chamaecyparis obtusa) planted stands. *Ipn. J. For. Plant*. 2012, 46, 15–24. (In Japanese with English Summary) [CrossRef]

39. Inagaki, Y.; Nakanishi, A.; Tange, T. A simple method for leaf and branch biomass estimation in Japanese cedar plantations. *Trees-Struct. Funct*. 2020, 34, 349–356. [CrossRef]

40. Sakai, A.; Inagaki, Y. Changes of understory vegetation in a middle-aged hinoki cypress plantation forest in Shikoku Research Center. *Ann. Rep. Shikoku Res. Cent. For. For. Prod. Res. Inst*. 2018, 59, 24–25. (In Japanese)

41. Inagaki, Y.; Okuda, S.; Sakai, A.; Nakanishi, A.; Shibata, S.; Fukata, H. Inter-annual variations of leaf-fall phenology and leaf-litter nitrogen concentration in a hinoki cypress (*Chamaecyparis obtusa* Endlicher) stand. *Ecol. Res*. 2008, 23, 965–972. [CrossRef]

42. Farquhar, G.D.; Ehleringer, J.R.; Hubick, K.T. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. 1989, 40, 503–537. [CrossRef]

43. Inagaki, Y.; Nakanishi, A.; Fukata, H.; Watanabe, N. Effects of thinning on leaf biomass and stem biomass production in hinoki cypress plantations in Kochi prefecture. *Bull. For. For. Res. Inst*. 2010, 667–670. [CrossRef]

44. Ando, T.; Hatiya, K.; Doi, K.; Kataoka, H.; Kato, Y.; Sakaguchi, K. Studies on the system of density control of sugi (*Cryptomeria japonica*) stand. *Bull. Exp. For. Sta.* 1968, 209, 1–76. (In Japanese with English Summary)

45. Sumida, A.; Watanabe, T.; Miyaura, T. Interannual variability of leaf area index of an evergreen conifer stand was affected by carry-over effects from recent climate conditions. *Sci. Rep*. 2018, 8, 13590. [CrossRef]

46. Miyamoto, K.; Okuda, S.; Inagaki, Y.; Noguchi, M.; Itou, T. Within- and between-site variations in leaf longevity in hinoki cypress (*Chamaecyparis obtusa*) plantations in southwestern Japan. *J. For. Res*. 2013, 18, 256–269. [CrossRef]

47. Inagaki, Y.; Okuda, S.; Sakai, A.; Nakanishi, A.; Shibata, S.; Fukata, H. Leaf-litter nitrogen concentration in hinoki cypress forests in relation to the time of leaf fall under different climatic conditions in Japan. *Ecol. Res*. 2010, 25, 429–438. [CrossRef]

48. Inagaki, Y.; Kuramoto, S.; Torii, A.; Shinomiya, Y.; Fukata, H. Effects of thinning on leaf-fall and leaf-litter nitrogen concentration in hinoki cypress (*Chamaecyparis obtusa* Endlicher) plantation stands in Japan. *For. Ecol. Manag*. 2008, 255, 1859–1867. [CrossRef]

49. Fang, Y.; Koba, K.; Yoh, M.; Makabe, A.; Liu, X. Patterns of foliar $\delta^{15}$N and their control in Eastern Asian forests. *Ecol. Res*. 2013, 28, 735–748. [CrossRef]

50. Takebayashi, Y.; Koba, K.; Sasaki, Y.; Fang, Y.; Yoh, M. The natural abundance of $^{15}$N in plant and soil-available N indicates a shift of main plant N resources to NO$_3^-$ from NH$_4^+$ along the N leaching gradient. *Rap. Commun. Mass. Spectrom*. 2010, 24, 1001–1008. [CrossRef]

51. Inagaki, Y.; Kohzu, A. Microbial immobilization and plant uptake of different N forms in three forest types in Shikoku district, southern Japan. *Soil Sci. Plant Nutr*. 2005, 51, 667–670. [CrossRef]

52. Inagaki, Y.; Nakanishi, A.; Fukata, H. Soil properties and nitrogen utilization of hinoki cypress as affected by strong thinning under different climatic conditions in the Shikoku and Kinki districts in Japan. *J. For. Res*. 2011, 16, 405–413. [CrossRef]

53. Takahashi, M. Nutrient storage and stoichiometry of the forest floor organic matter in Japanese forests. *Soil. Syst*. 2021, 5, 51. [CrossRef]

54. Poulsom, S.R.; Chamberlain, C.P.; Friedland, A.J. Nitrogen isotope variation of tree-rings as a potential indicator of environmental change. *Chem. Geol*. 1995, 125, 307–315. [CrossRef]

55. Choi, W.J.; Lee, S.M.; Chang, S.X.; Ro, H.M. Variations of $\delta^{15}$C and $\delta^{15}$N in *Pinus densiflora* tree-rings and their relationship to environmental changes in eastern Korea. *Water Air Soil Poll*. 2005, 164, 173–187. [CrossRef]

56. Yamada, T.; Yoshinaga, S.; Morisada, K.; Hirai, K. Sulfate and nitrate loads on a forest ecosystem in Kochi in southwest of Japan. *Water Air Soil Poll*. 2001, 130, 1115–1120. [CrossRef]

57. Hishi, T.; Tateno, R.; Fukushima, K.; Fujimaki, R.; Itoh, M.; Tokuchi, N. Changes in the anatomy, morphology and mycorrhizal infection of fine root systems of *Cryptomeria japonica* in relation to stand ageing. *Tree Physiol*. 2017, 37, 61–70.

58. Karizumi, N. *Illustrations of Tree Roots*; Seibundo-Shinkosha: Tokyo, Japan, 1979. (In Japanese)

59. Kume, A.; Satomura, T.; Tsuibo, N.; Chiwa, M.; Hanba, Y.T.; Nakane, K.; Horikoshi, T.; Sakugawa, H. Effects of understory vegetation on the ecophysiological characteristics of an overstory pine, *Pinus densiflora*. *For. Ecol. Manag*. 2003, 176, 195–203. [CrossRef]