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Dynamics of Carbon and Nitrogen Accumulation during Stand Development in Japanese Oak (*Quercus crispula*) Chronosequence Stands

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1. Introduction

In terrestrial ecosystems, carbon and nitrogen cycling play critical roles in influencing global climate change and promoting the sustainability of ecosystems (He et al., 2022). Therefore, understanding the dynamics of carbon and nitrogen accumulation during stand development is essential for assessing the contribution of secondary forests to the terrestrial carbon cycle as well as for interpreting the long-term interactions between carbon and nitrogen in terrestrial ecosystems (Yang et al., 2011). The interactions between carbon and nitrogen in terrestrial ecosystems have engaged significant attention due to their important role in determining the long-term sustainability of the carbon sink in land ecosystems, such as the accumulation of carbon in secondary forests (Luo et al., 2006; Reich et al., 2006). It has been suggested that the dynamics of nitrogen play a key parameter in regulating the long-term carbon sequestration in terrestrial ecosystems. Therefore, in order to enhance our comprehension of the interaction between carbon and nitrogen within terrestrial ecosystems, it is crucial to use long-term data series to assess the transformations taking place in the ecosystem (D. W. Johnson, 2006; Rastetter et al., 1997).

Additionally, there is a limited understanding of the dynamics of soil carbon within the secondary forests (Yanai et al., 2003) even though it is widely acknowledged that vegetation serves as a carbon sink during the stand development (C. M. Johnson et al., 2000; Silver et al., 2000; Yang et al., 2011). Soil stores a significant amount of organic carbon and nitrogen in terrestrial ecosystems (Dixon et al., 1994; Yang et al., 2007). Soil carbon is the major terrestrial carbon pool and plays a vital role in the overall carbon cycle on a global scale (Lal, 2004; Song et al., 2016). Similarly, soil nitrogen plays a crucial role in enhancing ecosystem productivity and facilitating carbon sequestration by interacting with soil carbon (Reich et al., 2006). The interrelation between these two elements can be described in terms of the CN ratio which is a sensitive soil quality indicator and has a critical impact on the cycles of soil carbon and nitrogen (Zhang et al., 2016). In addition, soil carbon and nitrogen play a key role as an indicator of soil quality and are frequently used as indexes in a forest ecosystem (Dong et al., 2021; Gong et al., 2022). The dynamics of carbon and nitrogen in forest soils are influenced by climate (Barnes et al., 1998), topography (Enoki et al., 1996), disturbance history (Goodale & Aber, 2001), past land use patterns (Guo & Gifford, 2002; D. Li et al., 2012) and tree species (Finzi et al., 1998; Vesterdal et al., 2008). The effect of time, including factors such as the successional stage and the time elapsed after harvesting is also important in determining the carbon and nitrogen cycling in forest soils (Gower et al., 1996; Yang et al., 2011).

The temporal dynamics of soil carbon and nitrogen stocks have been examined over decades to centuries to understand the factors contributing to the decline in forest productivity as stand age (Gower et al., 1996). Studies have shown that soil carbon and nitrogen stocks can exhibit various trends over time, including increased carbon and nitrogen stocks in the organic layer and mineral soil (0-5 cm) following afforestation (H. Sakai et al., 2010), increased nitrogen stocks in the forest floor but almost no changes in mineral soil after afforestation (Ritter et al., 2003), increased carbon accumulation in the surface layer (0-20 cm) but no significant changes in soil nitrogen storage during stand development (Wang et al., 2011), decreased total carbon and nitrogen in the soil (0-30 cm) with stand age (Alberti et al., 2008; Tremblay et al., 2006), remained stable in the soil organic carbon pools over stand development (Bruckman et al., 2011), no substantial changes in the soil nitrogen content with stand age but with the horizontal depth (Bond-Lamberty et al., 2006). Yang et al (2011)

conducted a global synthesis on the temporal dynamics of carbon and nitrogen during stand development and reported that the carbon accumulated with stand age in the aboveground vegetation, while the accumulation of carbon and nitrogen in the mineral soil (0-100 cm) does not exhibit significant changes in most cases. The authors also emphasized the necessity for additional observations with a higher temporal resolution to develop a precise model that accurately interprets the response of soil carbon storage to changes in stand age.

The majority of investigations on the soil carbon and nitrogen dynamics over stand age were either site-specific experimental findings e.g., (Covington, 1981) or local/regional scale of the model estimations e.g., (Peltoniemi et al., 2004). On the other hand, the belowground organs are also considered to be important components of material dynamics as well as the aboveground organs (Ohtsu et al., 2015). In particular, fine roots decompose more slowly than leaves (Fujii & Takeda, 2010) and accumulate more readily, and are known to play an important role in carbon and nitrogen accumulation in soil (McClaugherty et al., 1982; Tateno & Takeda, 2010). Furthermore, few studies have investigated the effects of forest age on carbon and nitrogen cycling as well as on the understory (Ohtsu et al., 2015). It has been reported that the dynamics of fine roots are related to the development of forest structure, and it has been shown that the fine root biomass is maximum just before canopy closure in cedar forests (Fujimaki et al., 2007) and just after canopy closure in Douglas-fir (conifer) forests (Vogt et al., 1987). In addition to aboveground conditions, the dynamics of fine root biomass with stand age may be important for understanding soil carbon and nitrogen accumulation.

Therefore, to accurately assess their role in the global carbon cycle of terrestrial ecosystems, it is imperative to conduct a comprehensive evaluation of carbon and nitrogen dynamics in both vegetation and soil within secondary forests worldwide (Houghton et al., 2009; Law et al., 2001). In the past few decades, extensive research has been conducted to assess the dynamics of both carbon e.g., (Gough et al., 2007; Law et al., 2001) and nitrogen e.g., (Knops & Tilman, 2000; Zak's' et al., 1990) accumulation during the stand development. However, the experimental results across the individual studies are highly variable, indicating that a comprehensive understanding of the dynamics of carbon and nitrogen accumulation over stand age during the stand development remains exclusive (Yang et al., 2011). In this present study, a chronosequence approach was applied to the analysis of the dynamics of carbon and nitrogen accumulation during stand development in *Quercus crispula* experimental stands in the Ashoro Research Forest, eastern Hokkaido, Japan.

The Ashoro Research Forest of Kyushu University is located in eastern Hokkaido, Japan. The natural vegetation is a cool-temperate deciduous broad-leaved forest, and the understory is dominated by dwarf bamboo, *Sasa nipponica*. In the research forest, the plantation has been planted with Japanese oak, *Quercus crispula*, every year since 1972 (Imada, 1996), and as of 2022, the age of the stand ranged from 0 to 50 years. The area of each plantation is averaging about 0.6 ha (Imada, 1976) and the understory is mainly covered by *Sasa nipponica*. In this study, the effects of climate, parent material, and topography were eliminated, and the dynamics in vegetation structure, soil carbon, and nitrogen dynamics over stand age could be grasped, and the relationships among them could be investigated. The objectives of this study were (i) to evaluate the dynamics of carbon and nitrogen accumulation in vegetation and soil during stand development in *Quercus crispula* chronosequence stands in the Ashoro Research Forest, (ii) to clarify the effects of vegetation structure and stand age on soil carbon and nitrogen accumulation and (iii) to assess the goodness of fit in prediction models (Ohtsu et al., 2015) against the observed values and the predicted values. In addition, I also investigated the stand development from the mean diameter at breast height (DBH) and stem density for the predicted values (Imada, 1996) and the actual values (2022) of the chronosequence stands in the research forest.

2. Materials and Methods

2.1 Study site

The study site was located in the Ashoro Research Forest (ARF) of Kyushu University, central Ashoro, eastern Hokkaido Island, Japan (Figure 1; 43° 15′ N, 143° 33′ E) with a forest area of 3,713 hectares and the topography is hilly with an altitude ranging from 114 m to 471m above sea level (Ashoro Research Forest, 2022). The climate of the ARF is inland with low precipitation, cool temperatures, and temporal soil freezing during winter by being covered with snow from late December to mid-April (Hishi et al., 2014). The mean annual precipitation and temperature for the past five years from 2017 to 2021 were 796.8 mm and 6.88 °C, respectively (Japan Meteorological Agency, 2022). The natural forests of this region are dominated by the species *Quercus crispula*, *Acer pictum* subsp. *mono*, *Tilia japonica* and *Ulmus dividiana* var. japonica (Okano, 1994). In this study area, *Larix kaempferi* is mainly planted and other species such as *Quercus crispula*, *Abies sachalinensis*, *Picea glehni*, *Picea abies*, and other broad-leaved species are also planted (Ashoro Research Forest, 2022).

The study was conducted in the stand age of 0-50 years old *Quercus crispula* plantations of the ARF (Fig. 1). The elevation of the study area ranged from 350-400 m, and the slope was 2.9°-22.7° (average 11.6°±5.1) (Ohtsu et al., 2015). Each forest division is located on a small ridge (Imada, 1996), and the spatial variation in SRI (Solar Radiation Index) is about 5% in coefficient of variation (Ohtsu et al., 2015). The nature of the soil is almost the tuff strata derived from the Neogene Period and the horizontal alternate strata of sandstone and shale (Ashoro Research Forest, Kyushu University, 2022). The soils are classified as Cambisols (IUSS working group WRB, 2014), and the soil parent material is volcanic ash with a black surface soil layer. The understory is mainly covered with *Sasa nipponica* and the vegetation before afforestation was a secondary broad-leaved forest dominated by *Quercus crispula*.

In this study area, the plantation has been established every year from 1972 to the present, and the management system was based on the clear-cutting system in tongue-shaped blocks surrounded by shelterbelts and rotations for 150 years (Imada, 1973). The plantations were established by the clear-cutting in blocks that were naturally seeded with seeds fallen from the just-harvested trees but in years with limited seed availability, planting was used instead of this natural seeding system (Imada, 1996). The process of reforestation is as the following procedures: Before seed dispersal in the designated cutting area (early September), all of the non-seed trees and weeds are systematically cleared. Additionally, line scarification of the forest floor beneath the targeted stand is performed either by the cultivator on gentle slopes or manually by hoe on steep slopes. After the completion of seed dispersal (mid-October), the dispersed seeds that present on the non-regenerated strips (1 m wide) are relocated to the designated regeneration strips (0.5 m wide). Subsequently, after seed supply, the dispersed seeds on the regeneration strips are immediately covered with soil by the same methods used for the site preparation. The detailed regeneration process is described in Imada (1996). Since the plantations has been planted in 1972, the age of the stands was 0-50 years old in 2022 while the survey was carried out in this study area.

2.2 Stand sampling design

A chronosequence of seven *Quercus crispula* stands, which were established in years 2014 (8-year-old), 2003 (19-year-old), 1996 (26-year-old), 1991 (31-year-old), 1987 (35-year-old), 1980 (42-year-old) and 1974 (48-year-old) were included in this study. In August 2022, a total of seven plots was set up in the stands and the size of the sample plots were 5m

× 5 m in the 8-year-old stand and 10 m × 10 m in the other six stands because the density of *Quercus crispula* was high in the young stands.

2.3 Estimation of the aboveground biomass

In August 2022, a vegetation survey was conducted in each study plot to estimate the biomass using the allometric models. Stand parameters such as girth at breast height (GBH) was measured at 1.3 m above the ground of the individual trees with a height limit of 2 m and above, and five trees per plot were randomly selected for height measurement. For all trees under 2 m in height, species with height were recorded.

The aboveground biomass of the stands was estimated by dividing the biomass into two categories: the overstory and the understory. The overstory was the tree species that currently consist of or could make up the forest canopy in the future, while the understory was the herbaceous, grasses, and shrubs that grow on the forest floor. In addition to *Quercus crispula*, other species classified in the overstory were *Lespedeza bicolor*, *Rubus crataegifolius*, *Aralia elata*, *Betula platyphylla*, *Toxicodendron trichocarpa*, *Cerasus sargentii*, *Staphylea bumalda*, *Acer pictum*, *Cerasus maximowiczii*, *Celastrus orbiculatus*, *Betula maximowicziana,* and *Kalopanax septemlobus*. The mean stem density and the biomass of *Quercus crispula* in the overstory were 69.9% and 78.0%, respectively (Table 1).

To estimate the aboveground biomass of the individual trees, the following allometric equations which were parameterized using *Betula ermanii*, *Quercus crispula*, and *Abies sachalinensis* in Hokkaido (Ohtsu et al., 2015; Takagi et al., 2010) were used:

$$\ln W = 2.8325 \ln (H) - 1.1843 \qquad \text{(Equation 1)}$$

$$\ln W = 2.428 \ln (DBH) - 2.282 \qquad \text{(Equation 2)}$$

where W is the aboveground biomass (kg), DBH is the diameter at breast height (cm), and H is the tree height (m). The first equation (Ohtsu et al., 2015) was used for trees under 2 m in height, and the second equation (Takagi et al., 2010) was used for trees with 2 m and above in height.

In order to determine the aboveground biomass of the understory using a destructive method, a harvesting operation was performed in August 2022. A quadrat, 1 m × 1 m was placed at a point on the forest floor in each study plot where there was no apparent disturbance recently. All of the plants inside the quadrat were destructively harvested using a hand saw. The harvested plants were separated into *Sasa nipponica*'s leaves, culms, and others (herbaceous, grasses, shrubs, etc.) and placed in the brown paper bags, and all the samples were then oven-dried at 70°C for 48 hours and weighed. In this study area, the aboveground part of the *Sasa nipponica* can be replaced in about one year (Ohtsu et al., 2015), so the biomass can be regarded as the litter supply from the understory.

2.4 Estimation of leaf biomass of the overstory

In order to evaluate the development of the tree canopy, the leaf biomass of the overstory was estimated from the Leaf Area Index (LAI) and Leaf Mass per unit Area (LMA). While conducting a vegetation survey in August 2022, a RICOH Theta-V 360 spherical camera connected to a smartphone was used to take hemispherical photographs. The photographs were taken near the center of the study plots at a height of 100 cm above the forest floor. The LAI was calculated using the Gap Light Analyzer version 2.0 (Simon Fraser University, Canada Institute of Ecosystem Studies, USA) from the hemispherical photographs taken in each study plot.

To calculate Leaf Mass per unit Area (LMA), unshaded fresh leaves from the uppermost part of the canopy of *Quercus crispula* were collected using a pole pruner in each study plot. Fresh leaves were collected from five trees per plot, one branch per tree, and three leaves per branch, and therefore, a total of fifteen leaves per plot were sampled. The collected fresh leaves were scanned with a scanner (EPSON GT-X980) and the leaf area was measured by Image J (National Institutes of Health, USA) to calculate LMA (g/m^2). All of the fresh leaves were then oven-dried at 70°C for 48 hours and weighed after the drying process. Dried-leaf materials were then ground into a fine powder by using a mill for the carbon and nitrogen analysis. The leaf biomass of the overstory (Mg/ha) was estimated from the product of LAI and LMA. In this study area, the overstory consisted of deciduous trees, so the amount of leaf could be regarded as the amount of litter supplied by the overstory.

2.5 Soil sampling and collection of fine roots

In October 2022, soil samples were collected in three layers: organic layer (i.e., soil O horizon), and mineral soil at depths of 0-5 cm and 5-10 cm at the four corners of each study plot. A quadrat (internal hole size: 20 cm × 20 cm) was placed on the forest floor at the sampling point where there was less affected by human activities. The organic layer samples inside the quadrat were then cut and collected by a sharp knife at each corner of the study plot. After the removal of the organic layer, the first mineral soils were sampled from 0 to 5 cm soil depth using a 100 ml stainless sampling tube with a supplemental soil sampler and hammer. The second mineral soils were then sampled from 5 to 10 cm soil depth of the nearest area. This sampling was repeated in every study plot of the stands.

The collected organic layer samples and mineral soil samples were then placed in brown paper bags and plastic bags respectively and transported to the laboratory. Samples were then oven-dried at 70°C for 48 hours and weighed. Once in the laboratory, each sample of the organic layer from four corners of the study plot was homogenized and randomly selected and then ground using a mill prior to chemical analysis. The mineral soil samples from four corners of the study plot were also homogenized, crushed manually, and sieved through a 2 mm mesh sieve and divided into a fine soil fraction (< 2 mm) and a coarse fraction (≥ 2 mm). The fine roots (< 2 mm in diameter), coarse roots (≥ 2 mm), litter, and gravel (≥ 2 mm) were then wet-sieved from the coarse fraction of soil and manually picked up with forceps. The collected fine roots, coarse roots, litter, and gravel were washed and cleaned from soil particles and then dried at 70°C for 48 hours and weighed. The dry weight of the fine roots was determined by dividing the samples into two categories, i.e., *Sasa nipponica* and non-*Sasa nipponica* (mainly *Quercus crispula*) to estimate the fine root biomass.

2.6 Chemical analysis of carbon and nitrogen concentration

The carbon and nitrogen concentrations (%) of the organic layer, fine soil obtained from the two layers of mineral soils (0-5 cm and 5-10 cm), and fresh leaf samples were determined using a CN analyzer (Yanaco CN corder MT-700, Yanaco Co., Japan). The carbon and nitrogen contents were then calculated from the product of the concentration and dry weight in the organic layer and the concentration and fine soil weight in the mineral soils and then converted to the amount per hectare.

2.7 Statistical analysis

Changes in vegetation structure and soil with stand age vary widely, and model comparison methods have been used (Yang et al., 2011). In 2010, Ohtsu et al (2015) surveyed to assess the effect of the change in stand structure including understory vegetation

(*Sasa nipponica*) on soil carbon and nitrogen accumulation from 0 to 40-year-old Japanese oak (*Quercus crispula*) plantations in this study area by model comparison methods.

In this study, a least-squares regression analysis was performed using the vegetation components (aboveground biomass of the overstory and understory, leaf biomass, fine root mass, carbon, and nitrogen concentration, and CN ratio in fresh leaves) and soil components (amount and concentration of carbon and nitrogen, and CN ratio) as response variables and stand age as an explanatory variable to clarify the changes with stand age.

The following four models (Ohtsu et al., 2015) were used in the analysis to fit with the observed values and predicted values from the model: the logistic model (Equation 3), the linear model (Equation 4), the exponential function (Equation 5), and the null model (Equation 6).

$$y = \frac{a}{1 + exp\,(b - ct)} \quad \text{(Equation 3)}$$

$$y = at + b \quad \text{(Equation 4)}$$

$$y = at * exp^{bt} + c \quad \text{(Equation 5)}$$

$$y = a \quad \text{(Equation 6)}$$

where *t* is the age of the stand, and a, b, and c are the regression coefficients.

The exponential function is a function that has been used to represent changes in understory vegetation with stand age (Lewis, 1989; White et al., 2004). In this study area, the amount of understory vegetation present does not decrease to zero over stand age, so a constant was added to the equation used in the analysis (Equation 5) (Ohtsu et al., 2015). The exponential function is a model that draws a curve with a maximum or minimum (Ohtsu et al., 2015). The null model is a model in which the response variable cannot be explained by the explanatory variable, and it is an expression with only an intercept (Ohtsu et al., 2015). These models were selected based on the Akaike Information Criterion (AIC), and the one with the smallest value of AIC was selected as the optimal model (Ohtsu et al., 2015).

In order to assess how well a regression model fits the observed values, the root mean square error (RMSE) which indicates the absolute fit of the model to the observed values, was calculated for all of the observed values in 2010 and 2022, and the changes between the initial survey (2010) and the present study (2022).

In addition, the multiple regression analysis was conducted using the observed values from the initial survey (2010) and the present study (2022). The leaf biomass of the overstory, the aboveground biomass of the understory, and stand age were used as the independent variables, and the carbon accumulation, nitrogen accumulation, and CN ratio in the organic layer and mineral soil layers (0-5 cm and 5-10 cm) were used as the dependent variables to determine which independent variables have a significant impact on the dependent variable and to quantify the strength and direction of relationships between them simultaneously.

All the statistical analyses were performed using R statistical software version 4.2.3 (R Core Team, 2023).

3. Results

3.1 Stand age and aboveground stand structure

The models developed by Ohtsu et al (2015) to fit the observed values with stand age and the results of the Root Mean Square Error (RMSE) between the predicted values and the observed values for each model are presented in Table 2. In this study, I compared the changes between the observed values of the initial survey (2010) and the present study (2022)

to evaluate a measure of how accurately the model predicts the response. For the aboveground biomass of the overstory, a model that increases linearly with stand age (Ohtsu et al., 2015) was used to fit with the observed values (Table 2). All the observed values followed a general trend of monotonous increase of the overstory biomass over stand age and therefore, it was relatively fit with the model (Figure 2a; RMSE = 0.289).

For the aboveground biomass of the understory, the exponential function (Ohtsu et al., 2015) was used to fit with the observed values (Table 2). The observed values showed that the aboveground biomass in the understory initially (in the young stands) decreased, and thereafter tended to stabilize over stand age following the same trend as the predicted model (Figure 2b; RMSE = 0.453).

The aboveground biomass of the overstory and the understory showed that the understory biomass was higher than that of the overstory in the initial stage of the stands after the start of afforestation, but after seven years, the aboveground biomass of the overstory exceeded that of the understory in all stands.

3.2 Changes in fresh leaves of the overstory with stand age

The logistic model (Ohtsu et al., 2015) was used to fit the observed values of the leaf biomass of the overstory (Table 2). According to the results of the initial survey (2010) in this study area, (Ohtsu et al., 2015) explained that in the model, the canopy closure ratio is defined as the ratio of the leaf biomass of the overstory at each stand age to 2.74 Mg/ha, which represents the steady state after 20-year-old stand, was less than 0.15 until the 11-year-old stand, then it became 1.31 at the 12-year-old stand and remained at 1.0 ± 0.2 except for the 14-year-old stand and therefore the canopy closure started in the 12-year-old stand after afforestation. In the present study (2022), the ratio of the leaf biomass of the overstory at each stand age to 2.66 Mg/ha and then tended to a steady state from the 23-year-old stand. The observed values showed that the leaf biomass increased during the initial stage of the stands, later, reached its peak, and tended to stabilize after twenty years over stand age therefore, it was relatively fit with the model (Figure 4a; RMSE = 0.585).

The null model (Ohtsu et al., 2015) was used for the carbon concentration of the fresh leaves (*Quercus crispula*) in response to the stand age with the observed values (Table 2). When comparing the observed values of the initial survey (2010) and the present study (2022), it showed that the carbon concentration of *Quercus crispula* leaves did not change any noticeable amount and remained in a steady state (mean = 45.6%), after that, stabilized over stand age and therefore, there was no relative change with stand age following the model (Figure 4b; RMSE = 1.359).

For the nitrogen concentration of *Quercus crispula* leaves, a monotonically decreasing linear model, which did not differ from the null model (Ohtsu et al., 2015) was used to fit with the observed values (Table 2). The observed values in the nitrogen concentration of the present study tended to fluctuate with stand age and represent the mean value of 2.41%, remaining at ± 0.2 in all stands. On the other hand, when comparing the observed values of the initial survey (2010) and the present study (2022), the nitrogen concentration of *Quercus crispula* leaves slightly increased with stand age but no substantial changes were observed in all stands (Figure 4c; RMSE = 1.356).

A monotonically increasing linear model (Ohtsu et al., 2015) was used for the CN ratio of the *Quercus crispula* leaves to fit with the observed values (Table 2). The observed values of the CN ratio also tended to fluctuate with stand age in the present study (2022), however, a comparison of the observed values in the initial survey (2010) and the present

study (2022) showed that the CN ratio slightly decreased over stand age, but no significant changes were observed (Figure 4d; RMSE = 1.359).

3.3 Stand age and fine root biomass

For the total fine root biomass in mineral soil (0-10 cm), the null model (Ohtsu et al., 2015) was used to fit with the observed values with stand age (Table 2). The total fine root biomass observed in the initial survey (2010) for the six stands was found to be smaller than those of the predicted model. This discrepancy in values was further confirmed in the present study (2022), where smaller total fine root biomass values were also observed. All the observed values of the total fine root biomass in the six stands did not significantly change during the period from 2010 to 2022 and tended to stabilize over stand age following the trend of the model (Figure 5a; RMSE = 0.961).

For the fine root biomass of the *Sasa nipponica*, the exponential function (Ohtsu et al., 2015) was used, which is a similar trend to the understory biomass (Table 2). The decrease in the fine root biomass of *Sasa nipponica* with stand age was shown by the model using the initial survey (2010). A comparison showed a decrease in the values of the fine root biomass of the *Sasa nipponica* from 2010 to 2022, following the same trend as the predicted model (Figure 5b; RMSE = 0.898). On the other hand, the fine root biomass of *Sasa nipponica* decreased while the other fine root biomass increased over stand age during the period from 2010 to 2022.

A linear model (Ohtsu et al., 2015) that monotonically increased with stand age was used for the other fine root biomass (mainly *Quercus crispula*) other than *Sasa nipponica* (Table 2). The increase in the other fine root biomass with stand age was shown by the model using the initial survey (2010). A comparison showed an increase in the values of the other fine root biomass from 2010 to 2022 (Figure 5c; RMSE = 1.330).

3.4 Changes in soil organic layer with stand age

The exponential function(Ohtsu et al., 2015) was used to fit the observed values of the accumulation in the organic layer (Table 2). During the period between the initial survey (2010) and the present study (2022), all the observed values showed not much significant change and tended to stabilize over stand age following the model (Figure 6a, RMSE = 1.057). For the carbon concentration of the organic layer, the logistic model (Ohtsu et al., 2015) was used to fit with the observed values (Table 2). The carbon concentration in the organic layer showed no significant change and generally stabilized over stand age following the model in all the observed values of the initial survey (2010) and the present study (2022) (Figure 6b; RMSE = 1.060). The exponential function (Ohtsu et al., 2015) was used for the nitrogen concentration in the organic layer accumulation to fit with the observed values (Table 2). The nitrogen concentration in the organic layer also did not significantly change and generally stabilized over stand age following the predicted model (Figure 6d; RMSE = 1.136).

The exponential function (Ohtsu et al., 2015) was used for both the carbon accumulation (Figure 6c; RMSE = 1.018) and nitrogen accumulation (Figure 6e; RMSE = 1.179) in the organic layer to fit with the observed values (Table 2). All the observed values did not significantly change and showed a tendency to stabilize over stand age in all stands. For the CN ratio, the exponential function (Ohtsu et al., 2015) was used to fit with the observed values (Table 2). The CN ratio in the organic layer did not show a significant change and tended to stabilize over stand age with the model in all stands (Figure 6f; RMSE = 1.151). The data from the initial survey (2010) and the present study (2022) showed that there was not much chronological change in the organic layer with stand age.

3.5 Changes in mineral soil with stand age

The relationship between stand age and the properties of mineral soil (0-5 cm) is shown in Figure 7. For fine soil content at 0-5 cm in depth, the linear model which did not differ from the null model (Ohtsu et al., 2015) was used to fit the observed values (Table 2). There was no significant change in the amount of fine soil content with stand age in all stands, however, a slight increase was observed from the period of the initial survey (2010) to the present study (2022) in some stands (Figure 7a; RMSE = 1.339). The exponential function (Ohtsu et al., 2015) was used for both the carbon concentration (Figure 7b, RMSE = 1.269) and nitrogen concentration (Figure 7d; RMSE = 1.285) in the mineral soil (0-5 cm), to fit with the observed values (Table 2). All the observed values did not significantly change and showed a tendency to stabilize over stand age in all stands. For the carbon accumulation (Figure 7c; RMSE = 1.490) and nitrogen accumulation (Figure 7e, RMSE = 1.848), the exponential function (Ohtsu et al., 2015) was used to fit with the observed values (Table 2) and the trend was like the carbon and nitrogen concentrations. For the CN ratio, the null model (Ohtsu et al., 2015) was used to fit with the observed values (Table 2). The CN ratio in the mineral soil (0-5 cm) also remained in a steady state with stand age in all stands following the predicted model (Figure 7f, RMSE = 0.961).

The change in mineral soil properties (5-10 cm) with stand age is shown in Figure 8. The null model (Ohtsu et al., 2015) was used to fit the observed values of the amounts of fine soil content (Figure 8a, RMSE = 0.961), carbon concentration (Figure 8b, RMSE = 0.961), carbon accumulation (Figure 8c, RMSE = 1.359), nitrogen concentration (Figure 8d, RMSE = 0.961), nitrogen accumulation (Figure 8e, RMSE = 0.961), and CN ratio (Figure 8f, RMSE = 1.359) with stand age (Table 2). All the observed values followed the same trend as the models and remained in a steady state, and there was no significant change with stand age. Likewise, the data from the initial survey (2010) and the present study (2022) showed that there was not much chronological change in the mineral soil layers (0-5 cm and 5-10 cm) with stand age in all stands.

3.6 Effect of vegetation structure and stand age on soil carbon and nitrogen accumulation

To determine which independent variable (the overstory leaf biomass, the understory biomass, and stand age) has a significant impact on the dependent variable (carbon and nitrogen accumulation in the organic layer and mineral soil layers at depths of 0-5 cm and 5-10 cm), a multiple regression analysis was conducted from the observed values in 2010 and 2022 and the results are presented in Table 3, 4, and 5.

For the carbon accumulation in the organic layer, overall, the results showed that the predicted model was significant, $F = 7.42$, $R^2 = 0.74$, $P < .01$. All the predictors explain a significant amount of variance between the variables (74%). The results showed that the leaf biomass of the overstory significantly affected the carbon accumulation in the organic layer, $\beta = 0.60$, $t = 2.18$, $P < .05$ (Figure 9a). However, the understory biomass and stand age did not significantly affect the carbon accumulation in the organic layer (Table 3).

In addition, the nitrogen accumulation in the organic layer, overall, the results showed that the predicted model was significant, $F = 7.94$, $R^2 = 0.75$, $P < .01$. All the predictors explain a significant amount of variance between the variables (75%). The results also showed that the leaf biomass of the overstory significantly affected the nitrogen accumulation in the organic layer, $\beta = 0.04$, $t = 2.77$, $P < .05$ (Figure 9b) while the understory biomass and stand age did not significantly affect the nitrogen accumulation in the organic layer (Table 3).

Furthermore, the CN ratio in the organic layer, overall, the results showed that the predicted model was significant, $F = 11.4$, $R^2 = 0.81$, $P < .01$. All the predictors explain a significant amount of variance between the variables (81%). The results showed that the leaf biomass of the overstory significantly affected the CN ratio in the organic layer, $\beta = -2.70$, $t = -3.14$, $P < .05$ (Figure 9c) while the understory biomass and stand age did not significantly affect the nitrogen accumulation in the organic layer (Table 3).

All the variables of the leaf biomass of the overstory, the understory biomass, and stand age did not show significant predictors for the carbon accumulation, nitrogen accumulation, and CN ratio in the mineral soil layers, 0-5 cm, and 5-10 cm (Table 4, 5).

4. Discussion

4.1 The growth dynamics of the stand structure

The aboveground biomass of the overstory in all stands during this study followed a general trend of monotonous increase over stand age. It is known that the biomass of the deciduous broadleaf trees per unit area in a forest ecosystem increases with the forest stand age after establishment, but eventually remains in a steady state (Barnes et al., 1998). A recent study on the aboveground biomass increments of cool-temperate deciduous forests in northern Japan has shown that the aboveground biomass increased steadily over 26 years with stand age in all topographic units (Noguchi et al., 2022). Previous studies on Japanese cedar plantations have also revealed that the aboveground biomass of the cedar trees approaches to stop increasing between the stand age of 30 to 60 years after the plantation establishment (Shutou & Nakane, 2004; Tateno et al., 2009). On the other hand, the aboveground biomass of the deciduous broadleaved beech (*Fagus crenata*) in a secondary forest continued to increase throughout 75 years of stand age (Kawaguchi & Yoda, 1986). In agreement with the previous study (Ohtsu et al., 2015) conducted in this study area in 2010, the overstory biomass continued to increase for 48 years in this study. These results are consistent with reports that the secondary tropical forests continuously gain biomass over the long term and are positively related to forest age (Jones et al., 2019).

In addition, the target DBH of the research forest is expected to be 55 cm at a 150-year-old stand and the expected DBH at a 48-year-old stand is 17.7 cm (Imada, 1996). In the present study, the mean DBH in the 48-year-old stand was 14.2 cm and the maximum DBH was 24.1 cm which is close to the expected size. Additionally, this study observed a significant trend as the stand matures, leading to a decrease in stem density and a concurrent increase in the mean DBH (Table 1), which is consistent with the intended trajectory outlined in the plan proposed by Imada (1996) for this study area. On the other hand, the relationship between the mean DBH and stem density for the predicted values (Imada, 1996) and actual values (2022) suggests that the stand development was delayed than predicted and the actual stem density was also larger than predicted (Figure 3a) indicating the self-thinning had not occurred well yet in the stands.

According to Imada (1996), the first thinning was intended to commence in the 35-year-old stand. However, thinning was not done as planned in the research forest and therefore, the delayed stand development might potentially be attributed to the absence of thinning. To assess the effect of thinning, the data simulation was performed by using the largest ones of the actual data of DBH based on the predicted stem density (Imada, 1996) for each stand. The simulation results of DBH for the 35-year-old stand was 10.8 cm (predicted value = 13 cm), the 42-year-old stand was 16.1 cm (predicted value = 15.5 cm) while the 48-year-old stand was 18.3 cm (predicted value = 17.7 cm) considering the ones after thinning. This suggests that if thinning takes place from the 35-year-old stand, the stands developed as

predicted and the delayed stand development might be recovered even if the stand development was delayed in the early phase of the plantation in this study area (Figure 3a, 3b).

On the other hand, the results suggest that thinning operation may not be necessary if the stem density was maintained according to Imada (1996) from the early phase of the plantation, the stand structure developed as predicted (Figure 3b). Even though the actual stand was not thinned, a growing well substantial population of larger trees was observed, and the biomass was expected to be as much as the prediction of Imada (1996), which was originally planned for thinning operations. In this study, the oldest stand, which was 48 years old, was still in the young stage from the perspective of the long-term plan for 150 years, therefore, it is important to monitor according to the long-term plan to improve the understanding of the stand structure with stand age.

The understory biomass initially (in the young stands) decreased, and thereafter tended to stabilize within a certain range is consistent with the results of previous studies on the relationship between the understory vegetation and stand age (Maclean & Wein, 1977; Ohtsu et al., 2015; White et al., 2004) suggesting that the temporal changes in the abundance of the understory vegetation reflect the development process of the forest canopy (Figure 4a). On the other hand, the understory biomass decreased over the stand age when comparing the results of the initial survey (2010) and the present study (2022). One of the possibilities is that it might be an overabundant sika deer (*Cervus nippon*) population and its impacts on overbrowsing in the research forest. During the last several decades, populations of sika deer have drastically increased throughout Japan (Miura & Tokida, 2009; Takatsuki, 2009) and it has become an issue because overabundant and excessive overbrowsing deer populations had profound effects with direct and negative impacts on forest vegetation especially on the survival of tree seedlings (Enoki et al., 2016; Uno et al., 2019). Furthermore, overbrowsing by sika deer alters patterns of relative abundance and vegetation dynamics, affecting the growth and survival of herb, shrub, and tree species and denuding the forest floor vegetation and reducing species cover and diversity (Côté et al., 2004; Murata et al., 2009).

Sika deer mainly rely on dwarf bamboo, *Sasa nipponica*, as an exclusively key winter forage in Hokkaido (Akashi et al., 2021) and food habits of sika deer in eastern Hokkaido have revealed that sika deer were dependent on *Sasa nipponica* throughout the year, particularly as much as 77.7 % of the diet in winter, 33.1 % in spring, 45.6 % in summer and 12.2 % in autumn respectively. Besides *Sasa nipponica*, large amounts of other graminoids (66-96.7%) were also accounted for (Campos-Arceiz & Takatsuki, 2005). Therefore, it had shown that the exclusion of deer increased the aboveground biomass of dwarf bamboo (*Sasa nipponica*) and forest floor vegetation (Itô & Hino, 2005). A study that was conducted in Akan National Park, eastern Hokkaido, Japan to examine the effects of decreasing deer density on the understory vegetation including *Sasa* species has shown that the understory biomass was increased where hunting and culls were conducted in enclosure sites than control sites and therefore, they suggested that population control of sika deer is an effective measure to recover the understory vegetation by reducing browsing pressure (Inatomi et al., 2012).

In addition, another potential factor contributing to the loss of understory vegetation (specifically *Sasa nipponica*) in the study area might be the effects of the caterpillars (larvae of moths). Recent observations in the research forest have frequently performed the presence of caterpillars; however, the specific species has yet not been identified. These caterpillars have been observed consuming the leaves of *Sasa nipponica*, leading to a significant reduction in leaf coverage. While the caterpillar species did not reach outbreak levels, their

population was found to be substantial within the study site. Consequently, in combination with previous studies and considering other potential factors, it is possible to suggest that the combined influences of overbrowsing deer populations and the presence of caterpillars might contribute to the loss of understory biomass in this particular study area.

4.2 Patterns of leaf biomass, carbon and nitrogen concentration, and CN ratio

In this study, the leaf biomass increased during the initial stage of the stands after afforestation, later, reached its peak maximum after the canopy closure, and tended to stabilize over stand age. This is consistent with the fact that the leaf biomass reaches a peak maximum in the early stage of forest development than other organs, thereafter close to a constant level over a relatively short period (Barnes et al., 1998; Ogawa, 2017; Ohtsu et al., 2015). A study showed that LAI was high in a 12-year-old stand and then remained gradually constant after canopy closure until a 75-year-old stand in the secondary beech forests. The results of this study are also consistent with those of (Kawaguchi & Yoda, 1986), indicating that the leaf biomass and LAI remain relatively stable following the canopy closure over considerable periods of time during the stand development.

The carbon concentration of fresh leaves (*Quercus crispula*) was stable and no significant change (mean = 45.6 %) with stand age in all of the stands in this study area which was similar results to previous studies (H. Li et al., 2017; Ohtsu et al., 2015). These results are also consistent with a chronosequence study of the evaluation of age-related variation in carbon allocation of *Quercus* species (Genet et al., 2009) and the global synthesis results for the arithmetic mean of carbon concentration in leaves was 45.6% among woody plants (Ma et al., 2018), indicating that the carbon balance between the growth and storage remained a consistent equilibrium in oak species across age classes.

Nitrogen concentration in the leaves of this study area followed a decreasing trend with increasing stand age, and only slight changes in fluctuation with stand age were observed. Similar findings were observed that leaf nitrogen concentrations were highest in the young stage of the stands while lowest in the old stands (Li et al., 2017), and leaf nitrogen concentrations can fluctuate with stand age in the broadleaved forests (Chang et al., 2022). The amount of leaf, which is considered for most of the nutrient demand of *Quercus crispula*, increased rapidly at the time of canopy closure and remained constant thereafter, indicating that the nitrogen nutrient demand was low before canopy closure, and tended to increase rapidly at the time of canopy closure and then decreased slightly or remained constant after canopy closure, which is followed the results of the previous study (Ohtsu et al., 2015).

The CN ratio of the mineral soil is a major indicator for controlling the rate of soil nitrogen mineralization (Hishi et al., 2014). Therefore, assuming that the nutrient supply from the mineral soil does not change with stand age because there was no significant change in the CN ratio of mineral soil (Figure 7f and Figure 8f), the change in nitrogen concentration of fresh leaves with stand age in this study area can be attributed to the change in nitrogen absorption by *Quercus crispula*. Considering that the decrease of nitrogen concentration in the leaves was almost the same as that in the null model (Ohtsu et al., 2015), the nitrogen demand of the vegetation and the nitrogen supply of the soil may be balanced at the time of canopy closure in this study area (Ohtsu et al., 2015). According to a meta-analysis of the many old-growth forests worldwide, a long-term trend of nitrogen deficiency has been observed (Yang et al., 2011). In addition, in many studies on secondary forests of various ages, there are many cases of species conversion from old forests to pioneer species, or from natural broadleaved forests to coniferous plantations, whereas, in this study site, there was no species conversion because there were the secondary forests of *Quercus crispula* before the establishment of *Quercus crispula* plantations, which may be another reason for the low

impact on soil. Therefore, considering that the decrease in nitrogen cycling is generally observed in long-term secondary forests and that the range of age in this study may not be a sufficient time for the change in *Quercus crispula* stands, long-term observations should be conducted to determine whether nitrogen limitation will occur in the future.

The CN ratio of the leaves also fluctuated with an increasing trend over stand age and there was no significant change among the stands, which is similar to the results of (Chang et al., 2022) indicating that leaf CN ratios may differ markedly with different ages of the stands. Generally, the leaf CN ratio increased with growth (H. Li et al., 2017), and they also pointed out that the variations in the leaf CN ratio may significantly be affected by stand age.

4.3 Fine root biomass

The total fine root biomass was relatively constant and there was no significant change with the stand age (Figure 5a), while the other fine root biomass (mainly *Quercus crispula*) other than *Sasa nipponica* increased with the stand age (Figure 5c). In addition, the total fine root biomass observed in the initial survey (2010) for the six stands was smaller than the predicted model and the observed values were also smaller in the present study (2022). The results suggest that these six stands had smaller fine root biomass than other stands in this study area. The fine root biomass of *Sasa nipponica* had maximum values after the start of afforestation and then gradually decreased until it decreased levels off over stand age (Figure 5b), showing a similar trend to the understory biomass. This result is in agreement with previous studies (Fukuzawa et al., 2006; Ohtsu et al., 2015), which showed that fine root biomass of *Sasa nipponica* increased rapidly after clear-cutting and thereafter gradually decreased over stand age. In addition, the decreased fine root biomass of *Sasa nipponica* can be related to the consequences of understory (*Sasa nipponica*) biomass loss due to the impacts of overbrowsing deer and the abundance of the caterpillars (larvae of moth) in this study area. Some studies showed that the abundance of earthworms had a negative effect on the belowground plant biomass and a decreasing occurrence of fine root biomass was observed with an increasing abundance of earthworms (Agapit et al., 2017; Bertrand et al., 2015; Castellanos Suarez et al., 2014). Furthermore, a chronosequence study on the effect of stand age on fine root biomass in different age classes of three European forests has shown that the fine root biomass increased rapidly to its maximum after the clear-cutting, thereafter, decreased during the stand development and remained in a steady state in the mature stands (Claus & George, 2005).

On the other hand, the fine root biomass of *Sasa nipponica* decreased while the other fine root biomass (mainly *Quercus crispula*) increased over stand age during the period from the initial survey (2010) to the present study (2022). A study that examined the effects of snow removal and the soil freeze-thaw on the fine root biomass of the oak (*Quercus crispula*) and the dwarf bamboo (*Sasa nipponica*) in eastern Hokkaido has shown that the fine root biomass (annual mean) of *Quercus crispula* was significantly larger than that of *Sasa nipponica* (Hosokawa et al., 2020). The dispersion of the genus *Sasa* is influenced by whether or not its height is covered by snow (A. Sakai & Larcher, 1987) while *Quercus crispula* is resilient to frost and is predominantly found in regions of Japan that experience reduced snowfall (Yagihashi et al., 2003). This suggests that the *Sasa nipponica* is more susceptible to snow freezing compared to other trees that coexist in the same environment. In addition, the sampling error can result in either overestimating or underestimating the fine root biomass, highlighting its high sensitivity to the accuracy of estimation (Kurz & Kimmins, 1987) because the fine root biomass is highly variable spatially (i.e., incomplete sampling) and temporally (i.e., incomplete core processing) (Berhongaray et al., 2013). In the laboratory, fine roots were picked up by hand manually using forceps from the soil samples

to separate the roots from the soil and therefore, it might also be associated with personal as one of the limiting factors in root sampling. Therefore, the results suggest that the rapid increase of fine roots during the initial phase of development plays a crucial role in preserving fine roots in the stands and the methodology of root sampling is relatively important in the assessment of fine root biomass.

4.4 Soil carbon and nitrogen accumulation with stand age

In this study, the accumulation of carbon and nitrogen from the organic layer to the mineral soil layers (0-5 cm and 5-10 cm) showed not much chronological change with stand age and remained in a steady state in all stands (Figure 6-7). This is consistent with the previous study, indicating that the change in carbon and nitrogen accumulation with increasing stand age over forty years became smaller as the stand age increased from the organic layer to the mineral soil layers (Ohtsu et al., 2015). In addition, a global synthesis of previous studies has also shown that the carbon and nitrogen accumulation from the surface to the mineral soil layers did not change significantly in most cases (Yang et al., 2011). Previous studies indicated that the period required for the organic layer to stabilize ranged from as early as 12 years to as late as 60 years or even more (Covington, 1981). In this study area, both carbon and nitrogen accumulation reached a steady state in a short timeframe and carbon accumulation took less time to stabilize than nitrogen accumulation, which agrees with the previous study in this study area (Ohtsu et al., 2015).

The organic layer accumulation was stabilized over stand age in a relatively short period during stand development in this study (Figure 6a). (Kawaguchi & Yoda, 1986; Ohtsu et al., 2015) pointed out that the accumulation of the organic layer decreased at first and then gradually increased and tended to stabilize over stand age. The reason for the decrease of forest floor organic matter after afforestation has been explained on the basis that the decomposition is accelerated by the increase of soil temperature or soil moisture after logging (Covington, 1981; Shutou & Nakane, 2004). Furthermore, it is clear that in the early stages of stand development, the amount of litter produced by the young stands is typically lower compared to mature stands, and therefore, the reduced litter inputs result in a decreased accumulation of organic matter in the forest floor.

Carbon and nitrogen accumulation in the organic layer did not significantly change and showed a tendency to stabilize over stand age (Figure 6c, 5e). A positive correlation between leaf biomass and carbon and nitrogen accumulation in the organic layer (Table 3) suggests that the increase in organic matter accumulation after a disturbance can be attributed to the restoration of litter supply from the canopy layer, which had been disrupted by the loss of the overstory vegetation. However, the recovery of carbon stocks was much faster than that of the litter supply of the canopy, as it took about five years after the plantation establishment in this study area (Ohtsu et al., 2015). This suggests that the carbon and nitrogen accumulation in the organic layer reached a stable state in a relatively short period of time due to the rapid increase of the understory vegetation, *Sasa nipponica*, and the recovery of litter supply after clear-cutting in this study area (Ohtsu et al., 2015). The CN ratio in the organic layer also did not show a significant change and tended to stabilize with stand age during stand development (Figure 6f). A study has observed that the values of CN ratio decreased during the 8 to 15-year-old stands, thereafter, tended to a steady state and this suggested that the decreasing CN ratio indicated increasing mineralization (Olsson et al., 1996). In addition, the CN ratio showed a peak initially after afforestation and gradually decreased before stabilizing (Figure 6f), reflecting the difference in the number of years required for carbon (Figure 6c) and nitrogen (Figure 6e) storage to reach a steady state. Previous studies on Japanese cedar plantations have shown that the nitrogen demand of

vegetation increases before and after canopy closure, and the CN ratio of litter increases after canopy closure and remains high for decades (Fukushima et al., 2011; Tateno et al., 2009). The CN ratio of the organic layer did not show a monotonous increase pattern as seen in past studies but had a peak at the very early stage when the stand structure mainly changed from the understory vegetation to the overstory (Ohtsu et al., 2015). In other words, the change in the CN ratio of this study was not caused by the long-term nitrogen deficiency of the vegetation, but by the difference in the type and amount of litter between the overstory and understory vegetation in the initial stage (Ohtsu et al., 2015).

In the mineral soil at a depth of 0-5 cm, there was no significant change with stand age, and remained in a steady state during the stand development (Figure 7). On the other hand, a slight increase in the fine soil content in the mineral soil (0-5 cm) was observed in some stands during the period from 2010 to 2022 (Figure 7a). In connection with previous studies, these results might be related to the overbrowsing of sika deer and its impacts on soil physical properties. A recent study that investigated the legacy effects of sika deer (*Cervus nippon*) on ground vegetation and soil properties of Japanese natural broad-leaved forests and cedar plantations has revealed that the sika deer have negative indirect effects on soil physical properties in the present, such as the increased bulk density and decreased coarse porosity (Harada et al., 2020). Other studies have also pointed out that there were some crucial changes in soil characteristics at 0-5 cm depth due to deer grazing. As a result, the soil was significantly compacted, and the bulk density and fine soil contents were significantly higher in breeding areas (Kumbasli et al., 2010; Lai & Kumar, 2020). The carbon and nitrogen accumulation and CN ratio (Figure 7c, 6e, 6f) did not show significant changes and remained in a steady state over stand age, which is consistent with the results of the previous studies (Ohtsu et al., 2015; Yang et al., 2011).

Soil properties of the mineral soil at a depth of 5-10 cm also did not change with stand age (Figure 8) which is also consistent with the previous study (Ohtsu et al., 2015). In many cases, the carbon and nitrogen accumulation in mineral soil does not change with stand age when the dynamics of carbon and nitrogen accumulation are evaluated from the surface to the deep soil layers during the stand development (Georgiadis, 2011; Yang et al., 2011). On the other hand, there are many cases of changes with stand age in mineral soil relatively close to the surface, such as at a depth of 5-10 cm (Bruckman et al., 2011; Gough et al., 2007; Wang et al., 2011). In this study area, the natural vegetation before afforestation was a secondary forest of *Quercus crispula*, which is the same species as the plantations, and clear-cutting was carried out when the soil was frozen (Ohtsu et al., 2015), which may have made the area less susceptible to species conversion by afforestation and disturbance caused by operations. Therefore, there was no species conversion in this study area, which may be one of the reasons for the low impact on soil properties.

Furthermore, in this study, a multiple regression analysis was conducted to examine the factors influencing the accumulation of soil carbon and nitrogen with respect to litter supply from the vegetation layer, encompassing both the overstory and understory, as well as the stand age. The results of this investigation indicated that the leaf biomass of the overstory had a significant impact on the accumulation of carbon and nitrogen in the organic layer, however, neither the biomass of the understory nor the stand age exhibited a significant effect (Table 3). The accumulation of the organic layer is influenced by the quantity of litterfall, given an equivalent decomposition rate (Nelson, 1999). It is known that in deciduous trees, the accumulation of the organic layer is influenced by the quantity of leaf biomass, as the amount of litterfall corresponds to the leaf biomass. Therefore, these results are consistent in that the increase in litterfall production results in the strengthening of the accumulation of organic matter on the forest floor (Kim et al., 2011; O'connell & Grove, 1993).

Consequently, it suggests that the carbon and nitrogen accumulation in the organic layer was not dependent on the time after the plantation establishment but dependent on the overstory leaf biomass.

On the other hand, the leaf biomass of the overstory, understory biomass, and the stand age did not exhibit significant impacts on the accumulation of carbon and nitrogen in the mineral soil layers 0-5 cm and 5-10 cm (Table 4, 5). These results indicate that carbon and nitrogen accumulation in the mineral soil (0-5 cm and 5-10 cm) did not depend on the overstory leaf biomass, understory biomass, and stand age, and the accumulation pattern was constant within the stand age in the mineral soil layers (Yang et al., 2011).

In fact, it can be interpreted that the accumulation pattern of carbon and nitrogen was independent of the time elapsed since the plantation establishment, however, it is dependent on the aboveground structure (overstory leaf biomass). To clarify the mechanisms of carbon and nitrogen accumulation in soils, it will be necessary to examine how the production and decomposition of understory vegetation, litter, and fine roots contribute to the accumulation of carbon and nitrogen.

5. Conclusion

This study investigated the dynamics of carbon and nitrogen accumulation during stand development in Japanese oak (*Quercus crispula*) chronosequence stands in the Ashoro Research Forest. The overstory biomass monotonously increased while the understory biomass initially decreased and thereafter, tended to stabilize over stand age. However, there was not much chronological change in the organic layer and mineral soil layers (0-5 cm and 5-10 cm) with stand age in all stands. This study showed that carbon accumulated in aboveground vegetation during stand development while the soil carbon and nitrogen did not exhibit significant changes over stand age. The accumulation pattern of carbon and nitrogen was independent of the time elapsed since the plantation establishment; however, it is dependent on the aboveground structure. In addition, the models developed by Ohtsu et al (2015) proved effective in the prediction of carbon and nitrogen accumulation with stand age and the observed values followed the predicted trends, indicating a relatively good fit. The findings emphasize the importance of considering the differential responses of overstory and understory vegetation and the interactions between the aboveground and belowground processes. These results contribute to our understanding of forest ecosystem functioning and have practical implications for forest management strategies aimed at maximizing carbon sequestration, nutrient cycling, and overall ecosystem health. Interestingly, the observed delay in stand development of the research forest, coupled with the sustained growth and biomass accumulation, suggested that early management practices focused on stem density maintenance can effectively guide stand development without the need for additional thinning operations. These findings provide insights into optimizing effective forest management strategies for promoting ecosystem resilience and sustainability of the Ashoro Research Forest. Further studies should focus on the long-term monitoring and modeling approaches to better understand the underlying mechanisms driving carbon and nitrogen dynamics in forest ecosystems and refine management practices accordingly.

Table 1. General characteristics of the experimental plots of the stands.

Stand No.	Plot ID	Year planted	Stand age (years)	No. of *Q. crispula* (n/ha)	Density of *Q. crispula* (%)	Biomass of *Q. crispula* (%)	Mean DBH (cm)	Mean height (m)	Slope aspect
1	FII-2	2014	8	21600	9.09	19.61	1.73	1.19	NE
2	CI-8	2003	19	11700	60.68	64.96	3.77	2.15	E
3	CI-5	1996	26	3200	96.97	97.84	7.75	7.52	SE
4	CI-4	1991	31	2900	82.86	93.87	8.41	10.16	SE
5	DI-7	1987	35	6600	80.49	90.61	6.42	7.48	SW
6	CI-2	1980	42	2500	59.52	78.88	9.94	10.12	S
7	CI-1	1974	48	1600	100.00	100.00	14.24	14.08	SE

Stand age (years): age of the stands as of August 2022

Table 2. Results of RMSE from the observed values and predicted model values of the initial survey (2010) and the present study (2022).

	Regression model	RMSE		
		2010	2022	2010-2022
Overstory biomass	Li: $y = -12.54 + 2.79t$	0.288	0.289	0.289
Understory biomass	Exp: $y = 1.49t*e^{-0.18t} + 1.47$	0.295	0.591	0.453
Leaf biomass of the overstory	Lg: $y = 2.67/ (1 + e^{(6.82 - 0.58t)})$	0.465	0.700	0.585
Fresh leaf: C concentration	Li: $y = 44.84$	1.713	0.758	1.359
Fresh leaf: N concentration	Li: $y = 2.14 - 0.0051t$	1.335	1.381	1.356
Fresh leaf: C:N ratio (fresh leaf)	Li: $y = 20.9 + 0.065t$	1.382	1.331	1.359
Fine root mass (Total)	N: $y = 343$	1.039	0.861	0.961
Fine root mass (*Sasa nipponica*)	Exp: $y = 48.0t*e^{-0.16t} + 73.4$	1.028	0.717	0.898
Fine root mass (Other spp:)	Li: $y = 82.85 + 1.52t$	1.624	0.870	1.330
Organic layer: accumulation	Exp: $y = -9.07t*e^{-0.40t} + 14.5$	0.908	1.207	1.057
Organic layer: C concentration	Lg: $y = 38.39/ (1 + e^{(1.40 - 1.02t)})$	0.521	1.455	1.060
Organic layer: N concentration	Exp: $y = 0.064t*e^{-0.030t} + 0.98$	1.454	0.574	1.136
Organic layer: C accumulation	Exp: $y = -6.46t*e^{-0.054t} + 5.44$	0.793	1.230	1.018
Organic layer: N accumulation	Exp: $y = 0.026t*e^{-0.042t} + 0.022$	1.357	0.930	1.179
Organic layer: CN ratio	Exp: $y = 3.23t*e^{-0.15t} + 21.32$	1.493	0.519	1.151
0-5 cm: fine soil content	Li: $y = 419.39 - 1.28t$	1.152	1.529	1.339
0-5 cm: C concentration	Exp: $y = -2.55t*e^{-0.41t} + 9.95$	0.671	1.721	1.269
0-5 cm: N concentration	Exp: $y = -0.17t*e^{-0.38t} + 0.63$	0.606	1.775	1.285
0-5 cm: C accumulation	Exp: $y = -4.53t*e^{-0.56t} + 18.11$	0.818	2.007	1.490
0-5 cm: N accumulation	Exp: $y = -0.33t*e^{-0.49t} + 1.15$	1.799	1.903	1.848
0-5 cm: CN ratio	N: $y = 16.06$	0.795	1.123	0.961
5-10 cm: fine soil content	N: $y = 492.92$	1.101	0.766	0.961
5-10 cm: C concentration	N: $y = 6.14$	0.815	1.107	0.961
5-10 cm: N concentration	N: $y = 0.39$	0.769	1.145	0.961
5-10 cm: C accumulation	N: $y = 14.32$	1.244	1.481	1.359
5-10 cm: N accumulation	N: $y = 0.91$	0.694	1.199	0.961
5-10 cm: CN ratio	N: $y = 16.08$	1.531	1.125	1.359

Exp: exponential function, Lg: logistic model, Li: linear model, N: null model, y: response variable, *t*: stand age

Table 3. Results of multiple regression analysis for the relationship between the overstory leaf biomass, the aboveground biomass of the understory, stand age and carbon accumulation (Cm), nitrogen accumulation (Nm), and CN ratio (n=12) in the organic layer. Bold letters represent a significant relationship ($P < .05$).

	Organic layer		
	Cm	Nm	CN ratio
Leaf biomass of the overstory	**0.599**	**0.042**	**-2.696**
Aboveground biomass of the understory	0.029	0.003	0.916
Stand age	0.041	0.001	0.064

Table 4. Results of multiple regression analysis for the relationship between the overstory leaf biomass, the aboveground biomass of the understory, stand age and carbon accumulation (Cm), nitrogen accumulation (Nm), and CN ratio (n=12) in the mineral soil (0-5 cm). Bold letters represent a significant relationship ($P < .05$).

	Mineral soil (0-5 cm)		
	Cm	Nm	CN ratio
Leaf biomass of the overstory	0.190	0.060	-0.171
Aboveground biomass of the understory	0.348	0.054	-0.582
Stand age	0.068	0.009	-0.138

Table 5. Results of multiple regression analysis for the relationship between the overstory leaf biomass, the aboveground biomass of the understory, stand age and carbon accumulation (Cm), nitrogen accumulation (Nm), and CN ratio (n=12) in the mineral soil (5-10 cm). Bold letters represent a significant relationship ($P < .05$).

	Mineral soil (5-10 cm)		
	Cm	Nm	CN ratio
Leaf biomass of the overstory	-0.038	0.015	-0.171
Aboveground biomass of the understory	0.567	0.058	-0.583
Stand age	0.048	0.010	-0.138

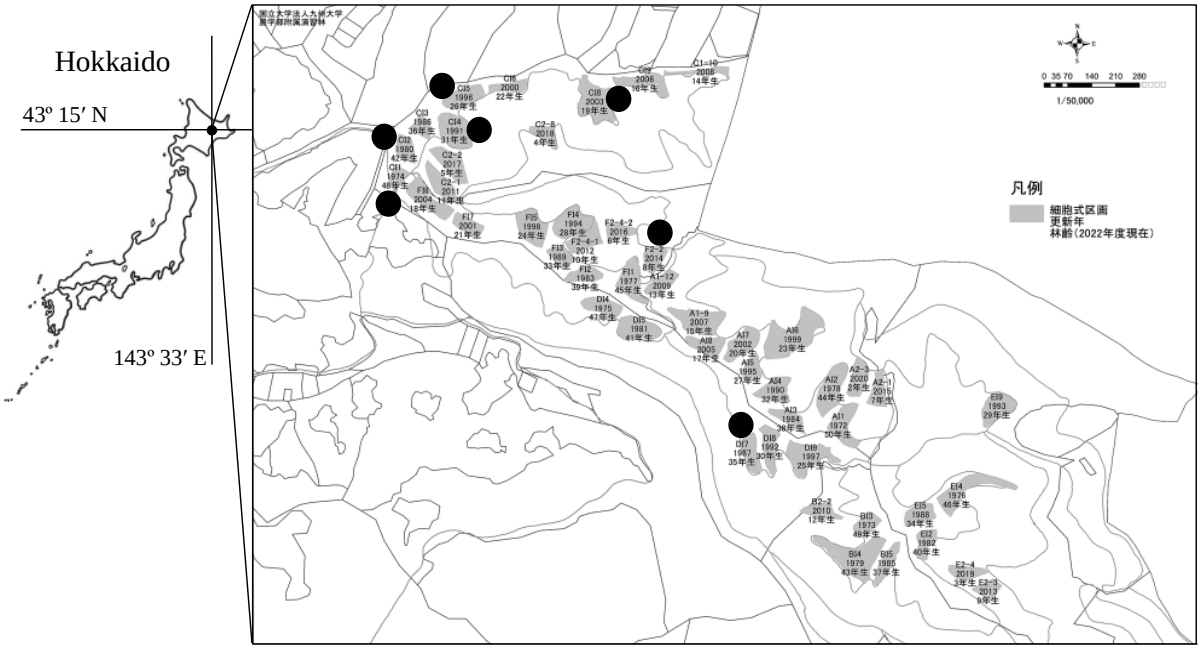


Figure 1. The geographical location map of the Ashoro Research Forest and distribution of the study plots (close circles) of the stands. (Map modified from ARF)

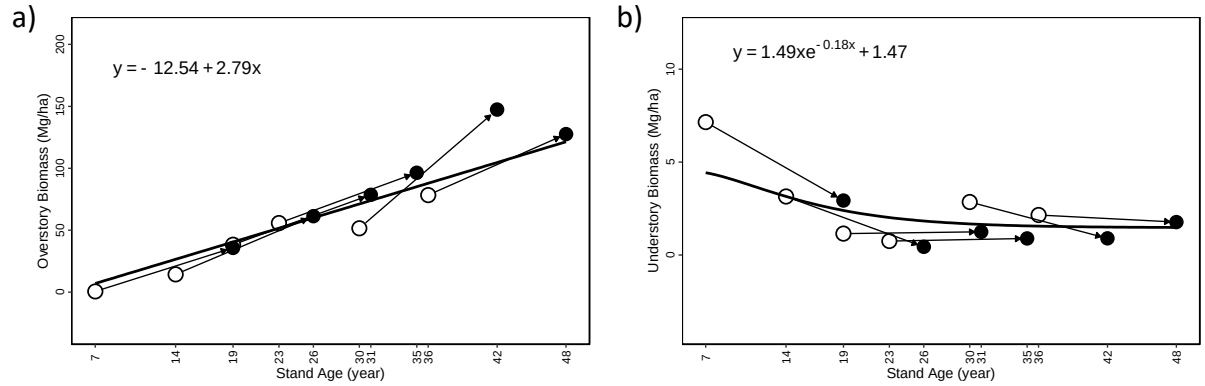


Figure 2. Relationship between stand age and aboveground biomass a) overstory biomass, b) understory biomass. The open circles represent the observed values in 2010 and the closed circles represent the observed values in 2022. Regression lines represent the models as shown in each figure.

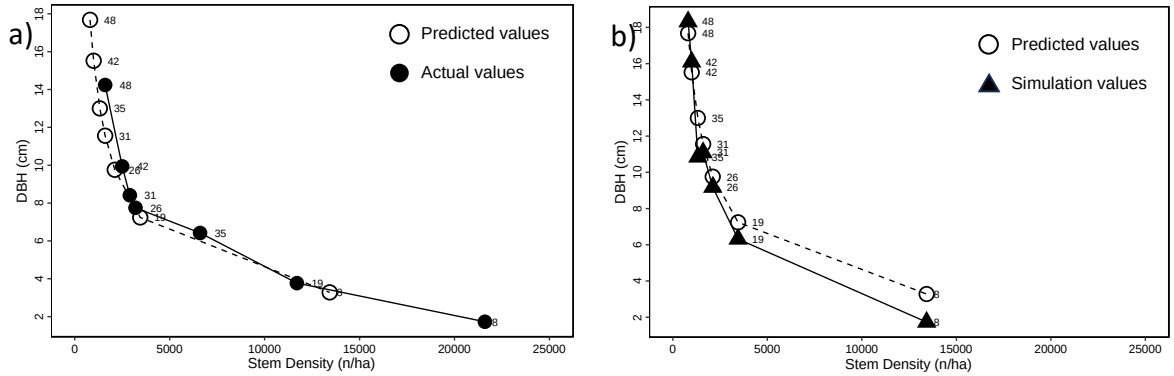


Figure 3. Relationship between mean DBH and stem density a) actual data and prediction (Imada et al.,1996), b) prediction (Imada et al.,1996) and simulation in all stands

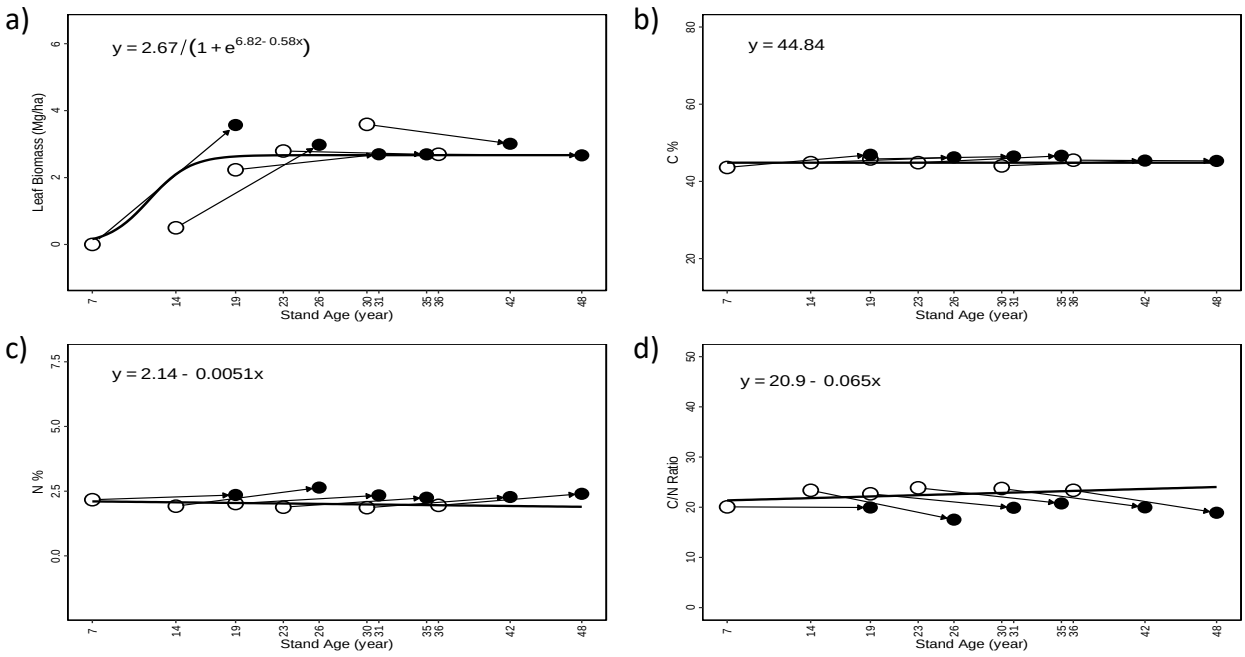


Figure 4. Relationship between stand age and a) leaf biomass, b) carbon concentration, c) nitrogen concentration, d) CN ratio of the fresh leaf. The open circles represent the observed values in 2010 and the closed circles represent the observed values in 2022. Regression lines represent the models as shown in each figure.

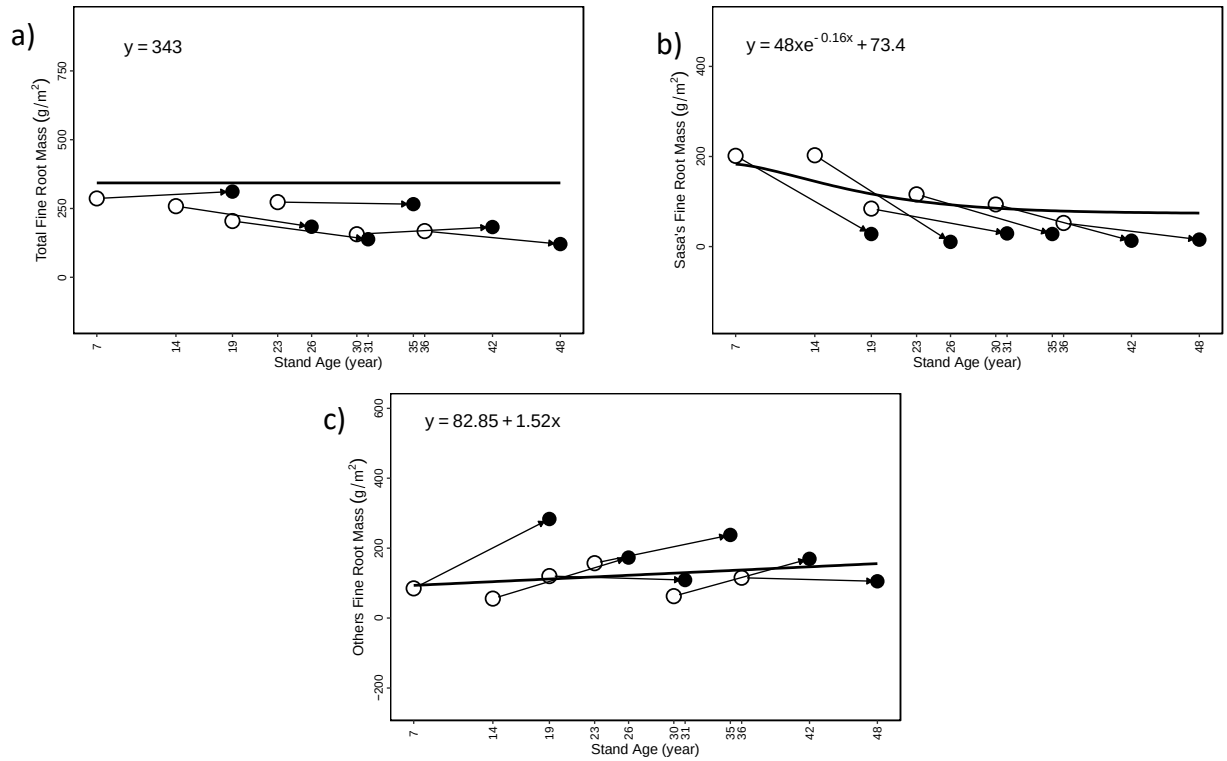


Figure 5. Relationship between stand age and fine root biomass (mineral soil 0-10 cm) a) total fine root biomass, b) fine root biomass of *Sasa nipponnica*, c) fine root biomass of other species (mainly *Quercus crispula*). The open circles represent the observed values in 2010 and the closed circles represent the observed values in 2022. Regression lines represent the models as shown in each figure.

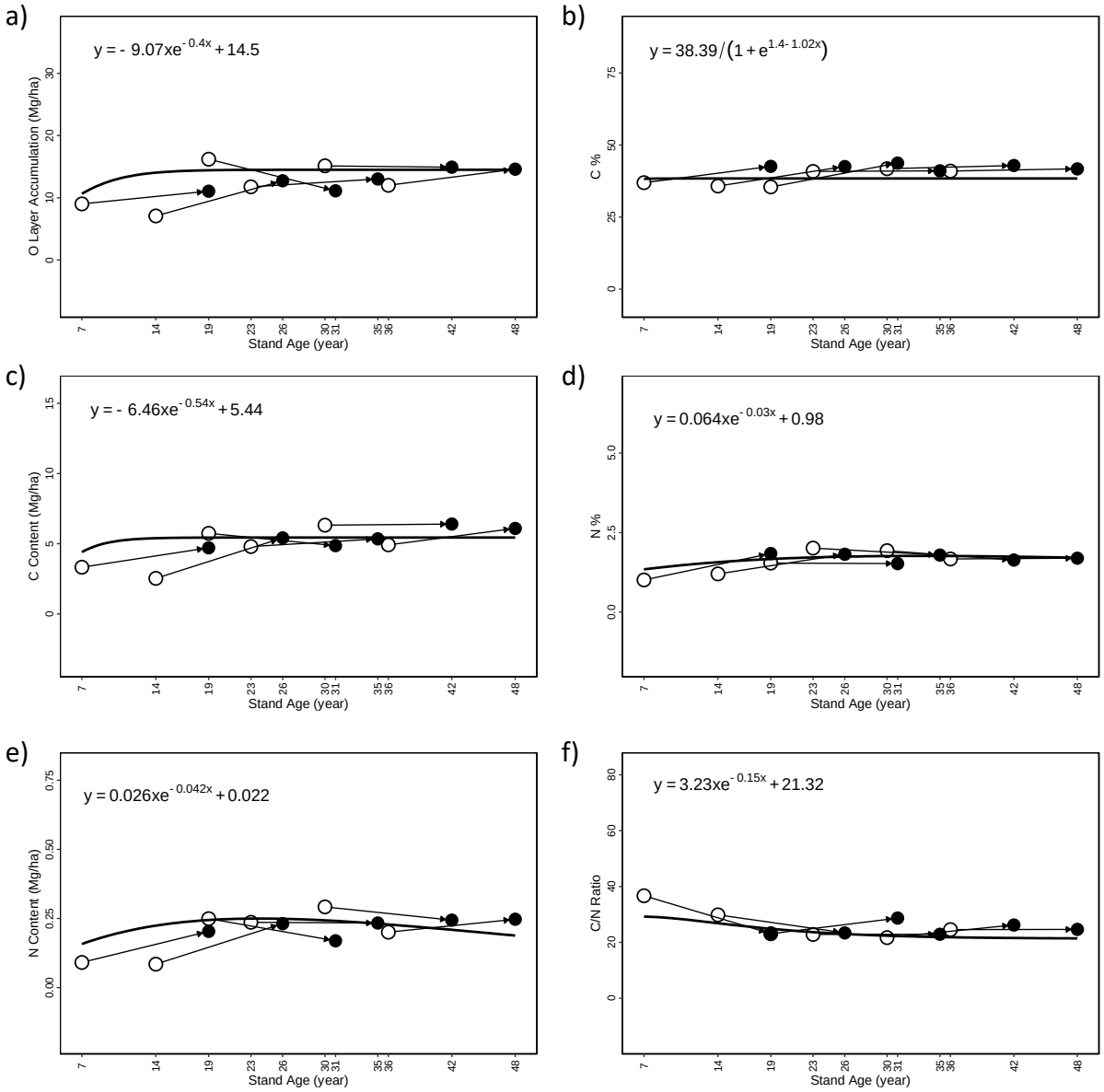
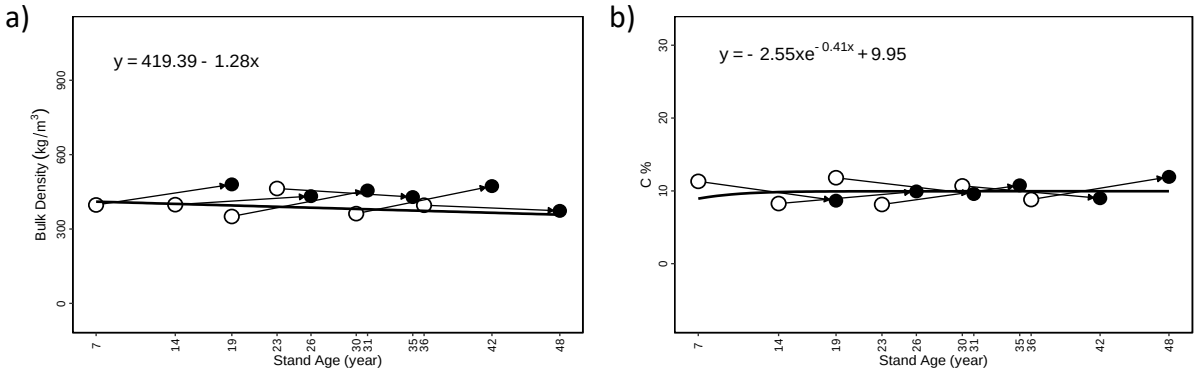


Figure 6. Relationship between stand age and organic layer a) amount of organic layer accumulation, b) carbon concentration, c) carbon accumulation, d) nitrogen concentration, e) nitrogen accumulation, f) CN ratio. The open circles represent the observed values in 2010 and the closed circles represent the observed values in 2022. Regression lines represent the models as shown in each figure.



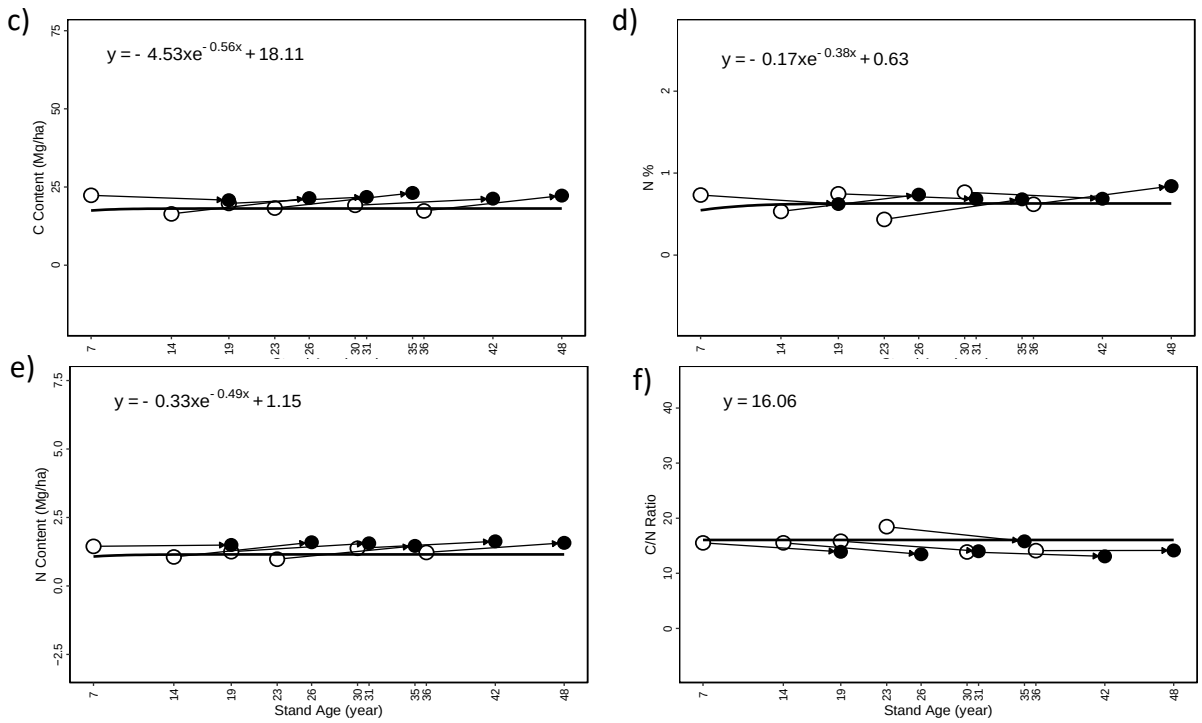
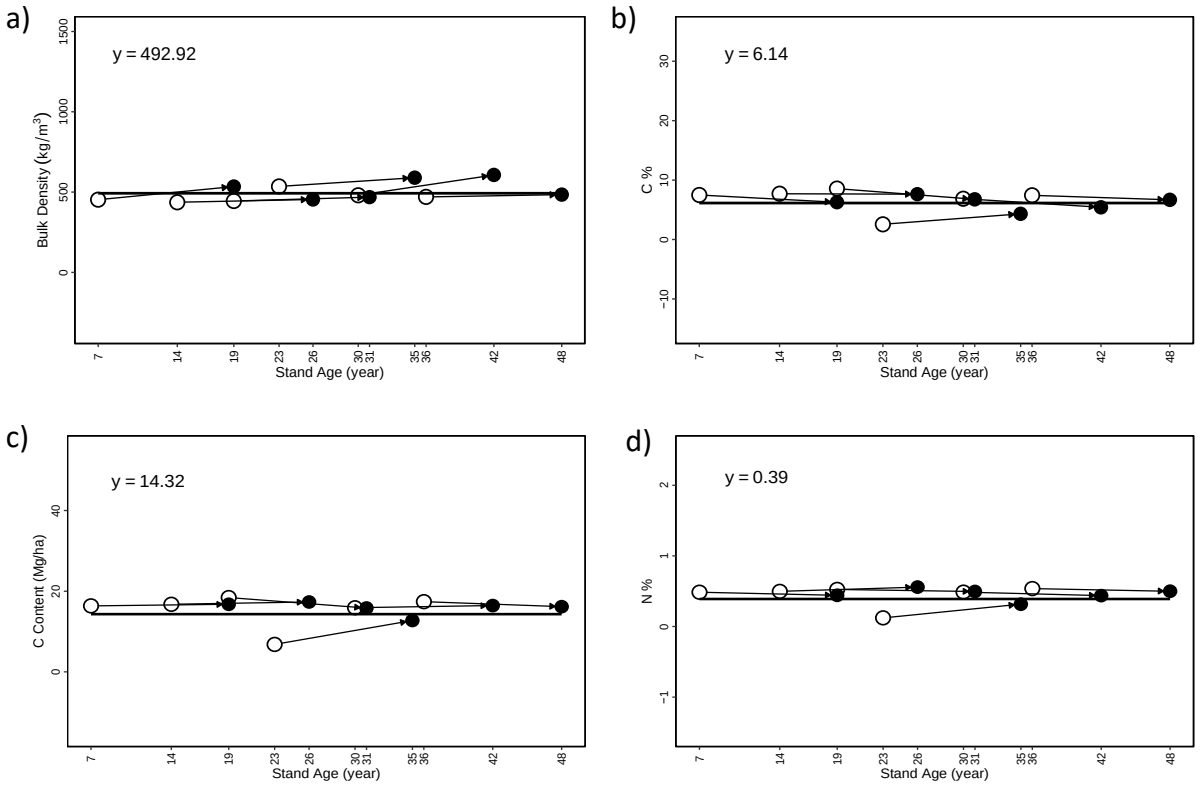


Figure 7. Relationship between stand age and mineral soil (0-5 cm) a) fine soil content, b) carbon concentration, c) carbon accumulation, d) nitrogen concentration, e) nitrogen accumulation, f) CN ratio. The open circles represent the observed values in 2010 and the closed circles represent the observed values in 2022. Regression lines represent the models as shown in each figure.



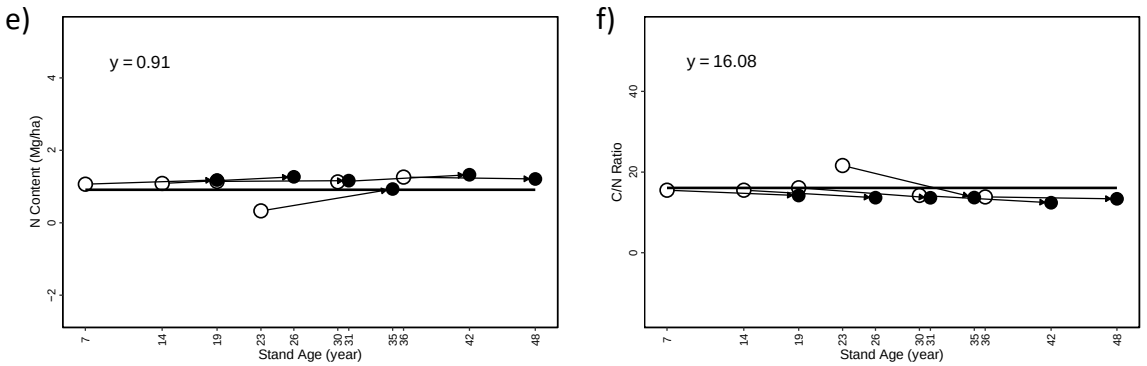


Figure 8. Relationship between stand age and mineral soil (5-10 cm) a) fine soil content, b) carbon concentration, c) carbon accumulation, d) nitrogen concentration, e) nitrogen accumulation, f) CN ratio. The open circles represent the observed values in 2010 and the closed circles represent the observed values in 2022. Regression lines represent the models as shown in each figure.

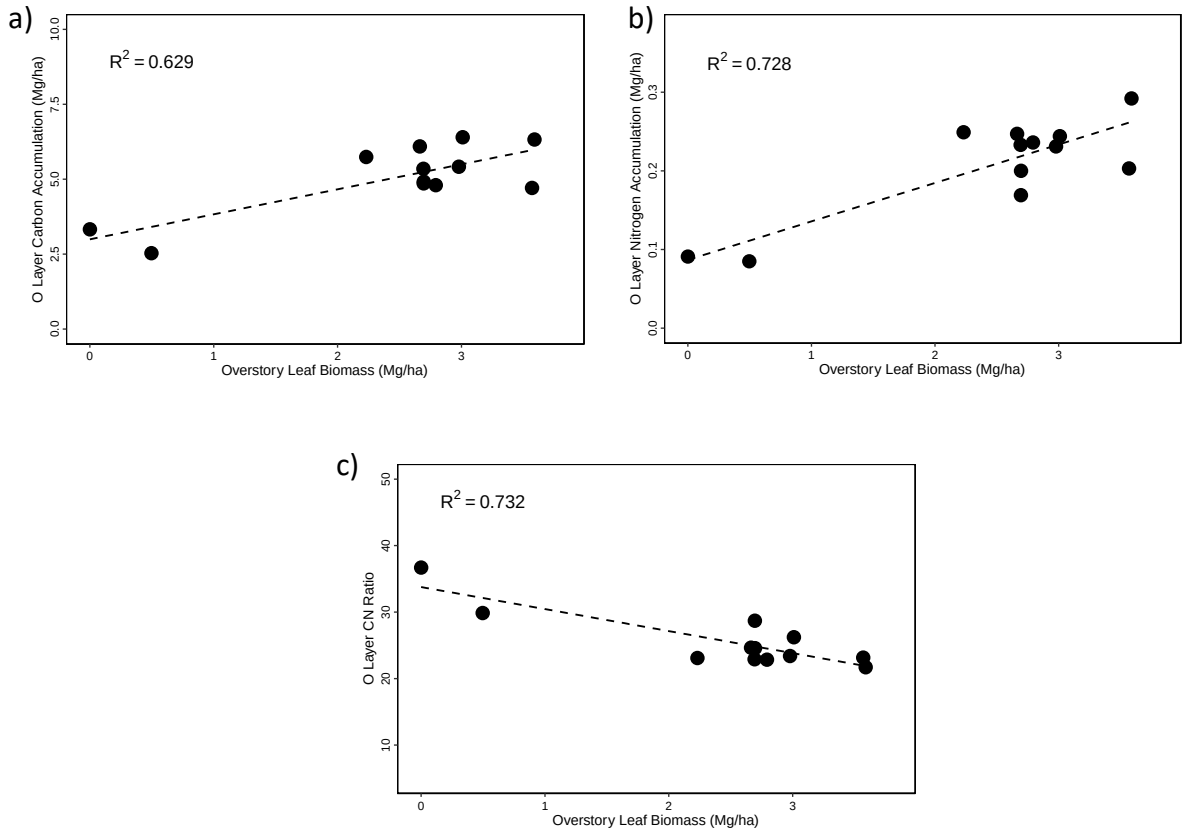


Figure 9. Relationship between the leaf biomass of the overstory a) carbon accumulation, b) nitrogen accumulation, and c) CN ratio in the organic layer of the observed values (2010-2022).

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