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RESEARCH ARTICLE

Estimation on Individual-Level Carbon Sequestration Capacity of Understory Perennial Herbs

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Abstract

The carbon sequestration capacity of plants has been used as a nature-based solution to reduce carbon emissions. Perennial herbs potentially contribute to carbon sequestration by allocating carbon to belowground parts as well as trees. As individual-level estimations have mainly been carried out for tree species, individual-level carbon sequestration for understory perennial herb species is poorly understood. To estimate the below- and aboveground carbon sequestration capacity, ten perennial herb species were planted for field experiment. Individual carbon sequestration by biomass was calculated by measuring the aboveground- and estimating belowground biomass gain at harvest. We further measured non-destructive aboveground parameters, such as photosynthesis and leaf area, to estimate the belowground biomass. Four species (*Aconitum jaluense* Kom., *Aquilegea oxysepala* Trautv. & C.A.Mey., *Disporum smilacinum* A.Gray, and *Polygonatum odoratum* var. *pluriflorum* (Miq.) Ohwi) showed the positive belowground carbon sequestration level during the experimental period. Correlation analyses indicated that the aboveground biomass and leaf area at senescence stage could be used as non-destructive estimates of belowground carbon sequestration. The perennial herb species habitat suitability for use as additional carbon sinks in urban forests and for forest restoration should be assessed based on the increase in belowground biomass.

Keywords Belowground biomass · Carbon sink · Biomass allocation · Understory layer · Urban forest

Introduction

The Climate Action Plan aims to achieve net zero CO_2 emissions by 2050 in accordance with the Zero Emission Tokyo Strategy. To achieve this goal, increasing the carbon storage capacity of the ecosystem is vital, in addition to reducing CO_2 emissions. Climate change mitigation is regarded as a major goal in environmental and ecological engineering (Seddon et al. 2021). It has been suggested that plant carbon sequestration effectively mitigates carbon emissions (Grace 2004). To use plants as carbon sinks, quantitative assessment of their carbon sequestration capacities is required (Duffy et al. 2021). The carbon sequestration processes in plants include carbon fixation via photosynthesis and carbon allocation, such as biomass (Raven and Karley 2006, Hartmann et al. 2020). The slow decomposition of dead plant material or perennial tissues slows rapid carbon dioxide emission (de Deyn et al. 2008, Conti and Díaz 2013). As well as environmental factors, plant tissues with high carbon polymer content, such as lignin, play a prominent role in carbon sequestration by plants.

Quantitative assessments of plant carbon sequestration capacity have primarily focused on trees (Conti and Díaz 2013, Hartmann et al. 2020). Intergovernmental Panel on Climate Change (IPCC) guidelines recommend determining the annual carbon sequestration of trees based on annual volumetric growth, root ratio, biomass conversion, expansion factor, and carbon fraction (IPCC 2006). This method has been primarily used to estimate carbon sinks in forest ecosystems. In contrast, individual-level estimates

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of carbon sequestration in other plant life forms, such as shrubs and herbs, have rarely been conducted (Deng et al. 2022). Although trees have been considered carbon sinks because of the relatively high lignin content in their tissues (bark and leaves), the biomass of herbs in terrestrial ecosystem is not considered a carbon sink because of their short lifespan and relatively rapid decomposition (IPCC 2006). However, the accumulated biomass in herbal species can also serve as a carbon sink (Hasegawa and Kudo 2005, Dirnböck et al. 2020, Deng et al. 2022). Herbs, as well as trees, produce biomass and rhizodeposit carbon to the soil, which delays the carbon cycle. A combination of particulate organic matter and soil bacterial and fungal necromass sequesters carbon in soil and reduces CO_2 emissions (Bai and Cotrufo 2022). In addition, storage organs for the overwintering and clonal propagation of perennial herb species have relatively longer lifespans (Klimešová et al. 2018). Thus, belowground biomass accumulation, which decomposes more slowly in perennial tissues, may serve as a viable carbon sink.

Carbon sequestration capacity by perennial herbs could be applied at diverse communities. In case of the understory layer of a forest ecosystem, most herbaceous species are perennials that emerge rapidly in early spring before canopy closure (Westerband and Horvitz 2017). Plants in the understory layer are exposed to a variable light environment with a dramatic decrease in light intensity and annual variation in light quality (Westerband and Horvitz 2017, Dirnböck et al. 2020). Plant species that exhibit vigorous growth under the understory layer can be used as nature-based solutions to enhance the ecosystem services features offered by such as urban forests. Although plant species in the understory contribute as the additional carbon sink function of forest ecosystems, the quantitative traits affecting carbon sequestration in understory layers have been underestimated.

To use perennial herb species as additional carbon sinks in forest ecosystems, it is necessary to assess their growth in understory environments and estimate their carbon sequestration capacity. A gradient of light and soil environments is found between the inner and edge areas of the understory layer (Hamberg et al. 2009, Hofmeister et al. 2019). Forest edges are commonly exposed to sunflecks and droughts more frequently than inner forests (Magnago et al. 2015, Li et al. 2018). Forest edge areas are affected by both tree canopies and neighboring areas that do not have a forest canopy, which may affect areas of up to approximately 100 m from the edge of the forest (Hofmeister et al. 2019). As urban forests are commonly fragmented by anthropogenic land use, they have a relatively higher percentage of edge areas (Hamberg et al. 2009, Li et al. 2018). Thus, perennial herb species with the vigorous growth and carbon sequestration under the environment like forest edge could be applied as additional carbon sink in fragmented forests.

We conducted a field experiment to examine carbon sequestration capacity of understory perennial herb by biomass production as additional carbon sinks for forests (Fig. 1). At first, based on the estimation method for tree species, an estimation method was developed for carbon sequestration from aboveground and belowground biomass. However, determining belowground biomass is a destructive method for the re-emergence of plants in the subsequent year. Thus, secondly, the correlation between aboveground growth traits and belowground biomass was also evaluated to estimate belowground carbon sequestration using non-destructive methods. These results could be widely applied for planting perennial herb species as additional carbon sink in diverse biota.

Materials and Methods

Field Experiment

Considering oak forests cover >20 % of Korean forest area (Lee et al. 2006), the experiment was conducted in an experimental field that simulated oak forests. The experiment was conducted in the field situated under the canopy of *Quercus* species in Chungbuk Province, Republic of Korea (36°37′31″N 127°27′10″E) from the end of March to the end of October, 2022. Several species of Aristolochiaceae, Fumariaceae, and Liliaceae (Amaryllidaceae, Asparagaceae, and Colchicaceae in APG IV), common in the understory layer, were found in the experimental field. It was relatively fertile and composed of loamy sands or sandy loams (Table S1).

Photosynthetically active radiation (PAR) at noon in experimental field were 602.2 ± 298.0 μmol m^{-2} s^{-1} (47.2 ± 23.4 % level to open space) in April, 49.8 ± 19.3 μmol m^{-2} s^{-1} (3.8 ± 1.5 % level to open space) at June, 138.1 ± 62.5 μmol m^{-2} s^{-1} (6.8 ± 3.1 % level to open space) at August, 125.8 ± 117.7 μmol m^{-2} s^{-1} (11.1 ± 10.3 % level to open space) in October. Daily variations in PAR, air temperature, relative humidity, soil temperature, and soil moisture content were monitored by loggers (Figs. S1 and S2; ATMOS 22; TEROS 12; and ZL6; Meter, NE, USA). The daily light integral (DLI) was calculated from the integral of PAR (in photosynthetic photon flux densities) at 15 min intervals daily (Poorter et al. 2019; Fig. S4).

The following ten perennial herb species commonly grow under the canopy of trees in Korea were selected for this study: *Allium microdictyon* Prokh., *Allium senescens* L., *Allium thunbergii* G.Don (Amaryllidaceae), *Polygonatum odoratum* var. *pluriflorum* (Miq.) Ohwi (Asparagaceae), *Disporum smilacinum* A.Gray (Colchicaceae), *Primula jesoana* Miq. (Primulaceae), *Aconitum jaluense* Kom. and *Aquilegia oxysepala* Trautv. & C.A.Mey. (Ranunculaceae), and *Viola*

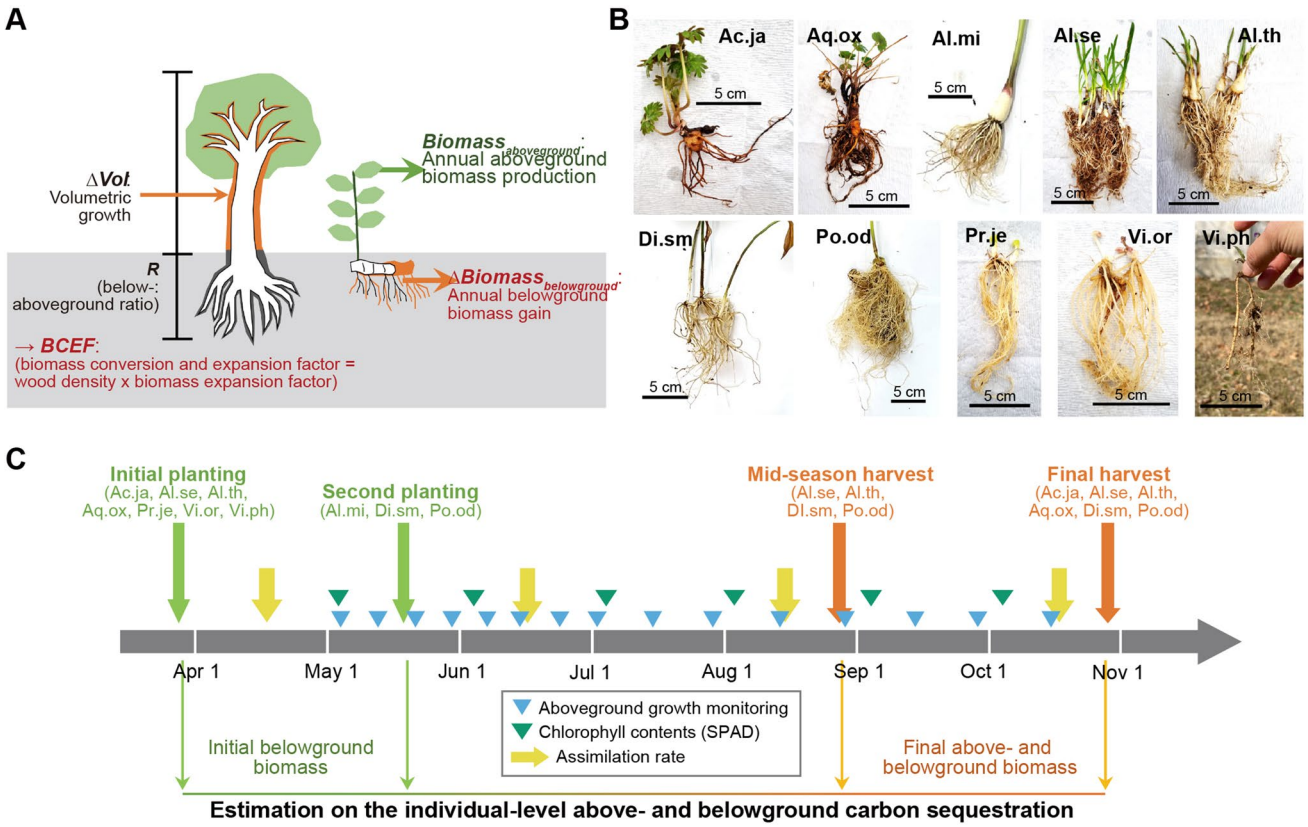

Fig. 1 Summary of the study. **A** Schematic explanation on the variables in the estimation of carbon sequestration of trees and perennial herb species. **B** Belowground organ of studied species at planting. **C** Timeline for the field experiment. Ac.ja = *Aconitum jaluense*; Al.mi = *Allium microdictyon*; Al.se = *Allium senescens*; Al.th = *Allium thunbergii*; Aq.ox = *Aquiligea oxysepala*; Di.sm = *Disporum smilacinum*; Po.od = *Polygonatum odoratum* var. *pluriflorum*; Pr.je = *Primula jesona*; Vi.or = *Viola orientalis*; Vi.ph = *Viola phillipica*

orientalis W.Becker and *Viola philippica* Cav. (Violaceae). While these ten species are common under trees in Korea and are easily planted and propagated, their carbon sequestration capacity has not been measured. Selected species were planted in the spring of 2022 to determine belowground biomass gain. All plant species were purchased from a wildflower farm (Yeoju Natural Farm, Gyeonggi Province, Republic of Korea), with the exception of *V. philippica*, which was sampled from a neighboring population ($n = 30$). Purchased or sampled plants were acclimated to the soil in the experimental field for one month after planting with adequate watering. The age of the purchased plants was mostly 3 or 4 years of age; 4 year-old *A. jaluense* ($n = 50$), 3 year-old *A. senescens* ($n = 50$), 3 year-old *A. thunbergii* ($n = 50$), 7 year-old *A. microdictyon* ($n = 40$), 2 year-old *A. oxysepala* ($n = 50$), 4 year-old *D. smilacinum* ($n = 40$), 3 year-old *P. jesona* ($n = 50$), 4 year-old *P. odoratum* var. *pluriflorum* ($n = 40$), and 4 year-old *V. orientalis* ($n = 50$). At a distance of 30 cm from each plant, the plants were planted directly in the soil after removing the existing herbs, shrubs, and litter layers from the experimental field. *Allium microdictyon*, *D. smilacinum*, and *P. odoratum* var. *pluriflorum* were already present on the field, thus the aboveground growth of the three species was measured from existing plants. The growth of the other seven species was measured in planted individuals. Weekly or biweekly measurements were taken for the survival of the aboveground parts, leaf number, and height of each individual. The chlorophyll content of three representative leaves was measured monthly for each plant (SPAD-502 Plus; Konica Minolta, Tokyo, Japan).

Using individuals with measurements of initial weight in a non-destructive way, initial biomass (dry weight) was estimated by dividing the initial fresh weight by the ratio between above- and belowground weight with moisture content. To estimate the initial ratio between the aboveground and belowground parts of each species, 10 subsamples of each species were divided into aboveground and belowground parts, and their fresh weights were measured. The fresh weight of the remaining plants was determined by measuring the whole plant and estimating their aboveground and belowground weights based on the ratio of the subsampled individuals. Initial biomass was estimated by measuring the moisture content of each plant part at harvest. In case of *A. senescens*, *A. thunbergii*, *D. smilacinum*, and *P. odoratum*

var. *pluriflorum* which showed a relatively high survival rate, half of the surviving plants of each species were harvested in late August to determine seasonal variation. The remaining individuals (including the four species) were harvested at the end of the experiment (late October). During harvest, stem diameter was measured with calipers (Mitutoyo, Kawasaki, Japan), and leaf area was calculated using ImageJ version 1.53t (Schneider et al. 2012). All belowground parts, including fine roots attached to the plant, were dug and collected to a depth of 40 cm. The fresh weights of the aboveground and belowground plants were measured. A dry weight was determined after the biomass had been dried for 48 h at 55 ℃ in a dry oven.

Carbon Assimilation Capacity Quantification

A portable infrared gas analyzer (GFS-3000, Walz, Jena, Germany) was used to measure the leaf assimilation rate in April, June, August, and October 2022. The assimilation rate measurement was performed on the fifth or sixth leaf of the apical bud. For *A. senescens* and *A. thunbergii*, which have grass-like leaves, four to five vigorous leaves that could fill the cuvette area were measured. Measurements were made at 750 μmol s^{-1} of flow, 400 ppm of CO_2 concentration, and a temperature of 25 °C. The PAR used in the assimilation rate measurements was set at 300 μmol m^{-2} s^{-1} in April and 100 μmol m^{-2} s^{-1} during the other measurement periods.

Carbon assimilation at the individual level was calculated by multiplying the leaf area by the assimilation rate. The leaf area was measured by taking a picture of each living leaf in April and June. We measured the leaf area in August and October by taking photographs of all leaves after harvest. The leaf area was quantified using ImageJ (Schneider et al. 2012).

Carbon Sequestration Capacity Estimation

For trees, annual CO_2 sequestration (CO_2 removal) calculation is generally presented as follows (IPCC 2006):

$$CO_2 removals = Vol \times BCEF \times (1 + R) \times CF \times \frac{44}{12}$$

Vol (m^3 ha^{-1} $year^{-1}$) represents the annual growth rate of each tree species. Biomass conversion and expansion factor (BCEF; [m^3 of growing stock volume]$^{-1}$) is a multiplication of *WD* (wood density; t dm m^{-3}) and *BEF* (biomass expansion factor). Species- and age-specific constants, *R* (root ratio) and *CF* (carbon fraction in biomass), are also presented.

For trees, the formula is based on the volumetric growth of perennial aboveground organs; however, for herbs, the duration of growth of aboveground and belowground organs differs. The formula for trees was used to calculate the annual individual-level CO_2 removal from perennial herb species through the summation of separately calculated values from the above- and below-ground parts:

$$CO_2 Removals = CO_2 Removals_{aboveground} + CO_2 Removals_{belowground}$$

$$CO_2 Removals_{aboveground} = Biomass_{aboveground} \times CF \times \frac{44}{12}$$

$$CO_2 Removals_{belowground} = \Delta Biomass_{belowground} \times CF \times \frac{44}{12}$$

$Biomass_{aboveground}$ is calculated from the measurement of the aboveground primary production at harvest. $\Delta Biomass_{belowground}$ is calculated from the difference between final belowground biomass of each individual and its estimated initial belowground biomass in dry weight.

For the total carbon (total-C) and total nitrogen (total-N) contents of four species that showed a positive level of belowground carbon sequestration (*A. jaluense*, *A. oxysepala*, *D. smilacinum*, and *P. odoratum* var. *pluriflorum*), aboveground- (leaves) and belowground (storage organ) tissues were sampled from planted individuals at May 2023. Frozen samples were finely ground by mortar and pestle and lyophilized. Total-C and total-N contents were measured with an elemental analyzer (Flash EA 1112, Thermo Electron, Waltham, MA, USA) at Seoul National University (Table S2). Total carbon content was used as carbon fraction (*CF*) of the four selected species. Carbon fraction of the other species was assumed as 0.4 for both parts (Garnier and Vancaeyzeele 1994, Zhang et al. 2014, Tang et al. 2018).

Statistical Analysis

Correlation analyses between aboveground traits and estimated belowground CO_2 sequestration were conducted using R version 4.2.3 (R Core Team 2023). Allometric equation for belowground carbon sequestration level from aboveground traits was calculated by linear regression ('lm()') function.

Results

Growth and Seasonal Carbon Assimilation Dynamics

The overall growth traits of surviving individuals of the 10 initially planted species were measured from one month of the acclimation period after transplanting. Of the species, no individuals of *P. jesoana*, *V. orientalis*, or *V. philippica* survived aboveground parts until the end of the experiment (Fig. S3A). Aboveground part of newly planted *A. microdictyon* individuals died after transplantation (data not shown),

and individuals from the existing clone showed a rapid decline in autumn (Fig. S3B). Therefore, growth and other traits are presented (Figs. 2, 3, 4), except for four species (*P. jesoana*, *V. orientalis*, *V. philippica*, and *A. microdictyon*).

Aconitum jaluense and *A. oxysepala* showed an initial decline after transplantation (23 of 50 *A. jaluense* and 33 of 50 *A. oxysepala* at the initial measurement of growth traits). Final survival rates from the initial measurements were 34.8 % and 57.6 % for *A. jaluense* and *A. oxysepala,* respectively (Fig. S3A). Four monocot species (*A. senescens*, *A. thunbergii*, *D. smilacinum*, and *P. odoratum* var. *pluriflorum*) exhibited a high overall survival rate at the end of the experimental period (Fig. S3B).

The shoot heights of the six species were almost consistent from May to October, with the exception of *A. jaluense* (Fig. 2A). *Aquilegia oxysepala*, *D. smilacinum*, and *P. odoratum* var. *pluriflorum* flowered before the initial measurements (April; data not shown). *Aconitum jaluense* and *A. thunbergii* flowered later in the experiment, whereas *A. senescens* did not produce flowers until the end. The other species showed a slight decrease in height during the later stages of the experiment.

Leaf numbers of *A. jaluense* and *A. oxysepala* increased during the experimental period, whereas those of *P. odoratum* var. *pluriflorum* were consistent (Fig. 2B). While the leaf number of *D. smilacinum* was consistent during the early experimental period, it decreased in early June because of herbivory. The compensatory leaf re-emergence of *D. smilacinum* was observed after this decrease (Fig. 2B). *Allium senescens* and *A. thunbergii* showed the initial increase of leaf number, whereas the decrease of leaf number by partial senescence was observed after the increase. Leaf chlorophyll content showed a trend similar to that of the leaf number, except for a dramatic decrease in October (Fig. 2C). However, the leaf chlorophyll content of *A. jaluense* and *A. thunbergii* in October was still high, although a decrease in leaf chlorophyll content occurred.

The carbon assimilation rate of the six species was the highest in April and decreased as light intensity decreased (Fig. 3A). In June, the assimilation rates of *A. jaluense*, *A. thunbergii*, and *D. smilacinum* were not measured because of senescence of the aboveground parts (*A. jaluense* and *A. thunbergii*) or herbivory damage (*D. smilacinum*). Assimilation rate of *A. oxysepala* was the highest in June. Although most of the studied species showed decreasing or consistent trends in their assimilation rates after canopy closure, the assimilation rate of *A. senescens* was higher in October than in August. Regarding the leaf area of the measured individuals, the individual carbon assimilation level in April was the highest in *P. odoratum* var. *pluriflorum* (Fig. 3B). Individual carbon assimilation levels dramatically decreased in *A. senescens*, *A. thunbergii*, *D. smilacinum*, and *P. odoratum* var. *pluriflorum* after June, whereas *A. jaluense* showed a

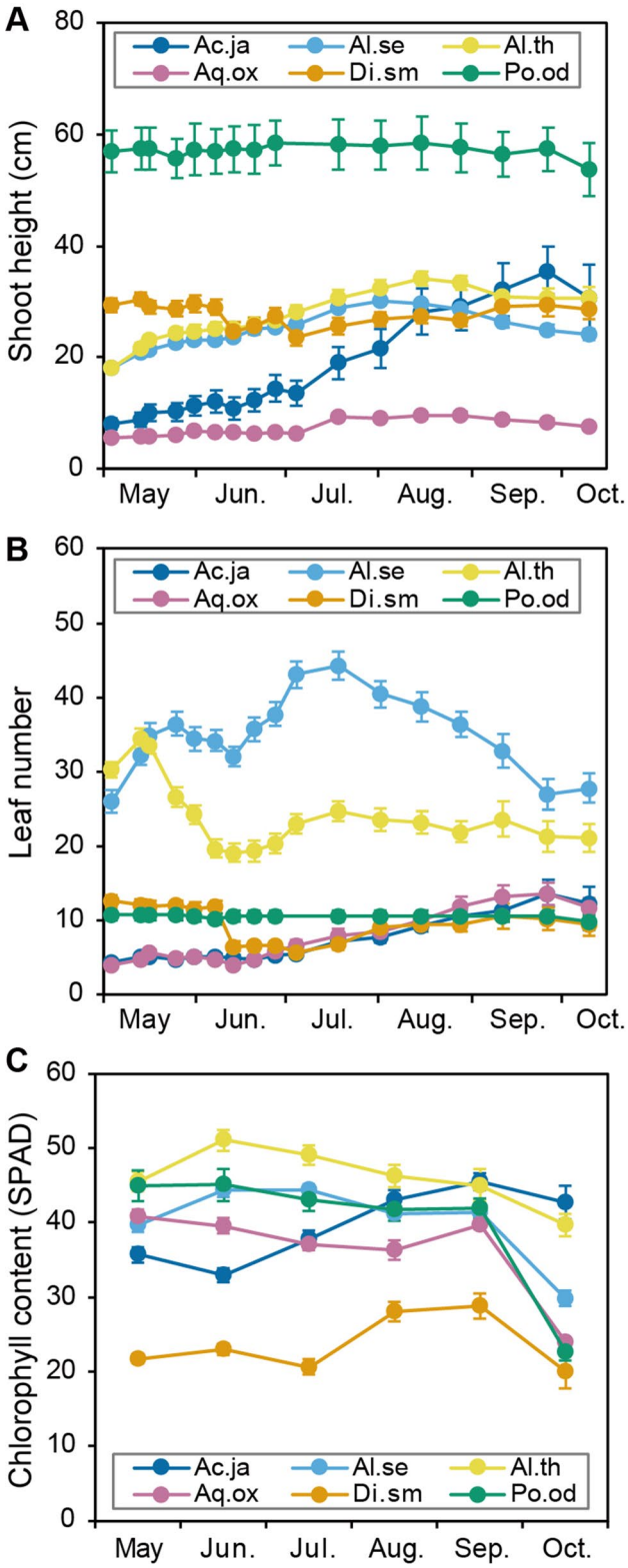

Fig. 2 The overall growth dynamics of the four studied species during the study period. **A** height (of the uppermost part of each individual); **B** leaf number; **C** chlorophyll content (average SPAD of three middle leaves). Ac.ja = *Aconitum jaluense*; Al.se = *Allium senescens*; Al.th = *Allium thunbergii*; Aq.ox = *Aquilegia oxysepala*; Di.sm = *Disporum smilacinum*; Po.od = *Polygonatum odoratum* var. *pluriflorum*

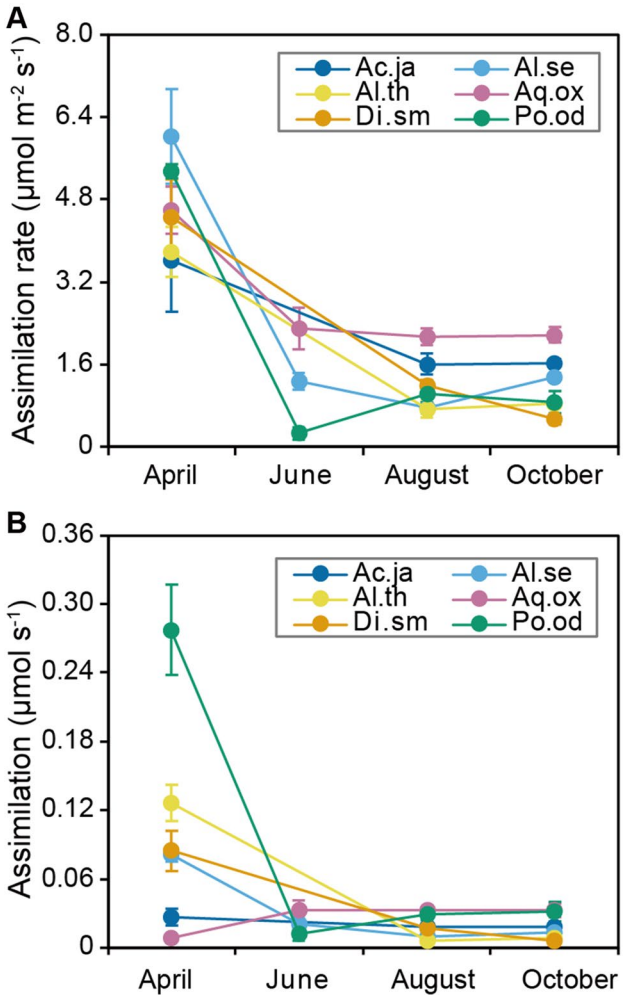

Fig. 3 **A** Assimilation rate (of CO_2) representative leaves (5th from the uppermost leaf except *Allium* spp.) and **B** estimated individual-level CO_2 assimilation from the assimilation rate and leaf area (n = 6 in April; n = 3 in June; n = 12 for Ac.ja, n = 21 for Aq.ox, and n = 25 for Al.se, Al.th, Di.sm, and Po.od in August; n = 8 for Ac.ja, n = 25 for Al.se, n = 24 for Al.th n = 19 for Aq.ox, n =18 for Di.sm, and n = 17 for Po.od in October). Ac.ja = *Aconitum jaluense*; Al.se = *Allium senescens*; Al.th = *Allium thunbergii*; Aq.ox = *Aquilegia oxysepala*; Di.sm = *Disporum smilacinum*; Po.od = *Polygonatum odoratum* var. *pluriflorum*

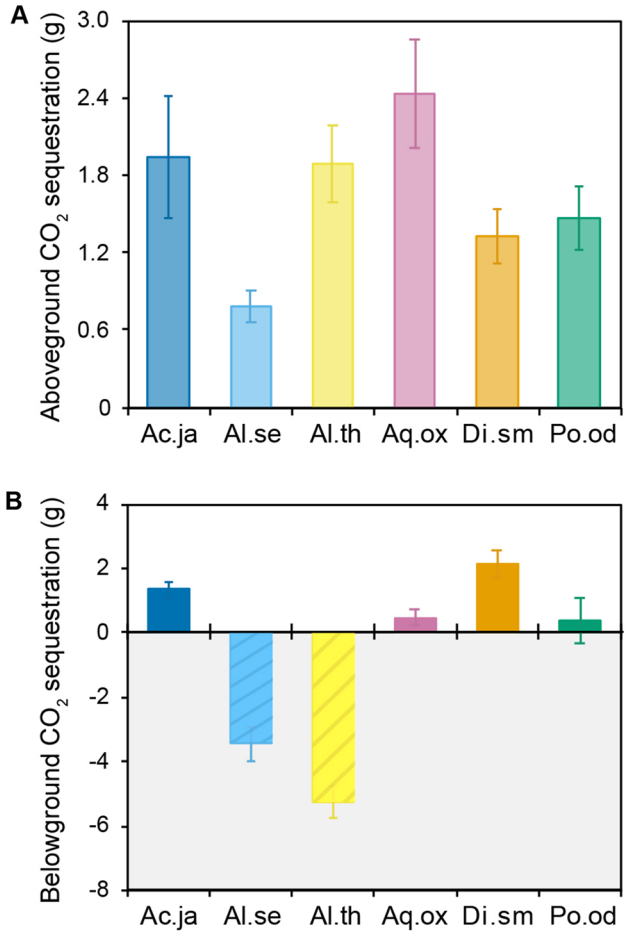

Fig. 4 Estimated individual-level **A** aboveground CO_2 sequestration from the annual aboveground primary production and **B** belowground CO_2 sequestration from the gain of belowground biomass of the four studied species at the end of the experimental period (October). n = 8 for Ac.ja, n = 25 for Al.th, n = 24 for Al.se, n = 9 for Aq.ox, n = 13 for Di.sm, and n = 15 for Po.od. Ac.ja = *Aconitum jaluense*; Al.se = *Allium senescens*; Al.th = *Allium thunbergii*; Aq.ox = *Aquilegia oxysepala*; Di.sm = *Disporum smilacinum*; Po.od = *Polygonatum odoratum* var. *pluriflorum*

consistent level, and *A. oxysepala* showed an increase after June.

Considering the light environment (as mean DLI) for 4 weeks before the measurement day (except for April in the first 2 weeks after installation date; because the logger was installed in late April), mean DLIs were 12.4 ± 3.2 mol m^{-2} day^{-1} in April, 6.7 ± 2.8 mol m^{-2} day^{-1} in June, 4.4 ± 2.3 mol m^{-2} day^{-1} in August, and 5.1 ± 2.5 mol m^{-2} day^{-1} in October (Fig. S4A). Except for the relatively high DLI in April, *A. jaluense*, *A. oxysepala*, and *A. thunbergii* exhibited nearly consistent assimilation rates regardless of DLI (Fig. S4B). In contrast, *D. smilacinum* and *P. odoratum* var. *pluriflorum* showed increased assimilation rates with decreasing DLI. At intermediate RLI levels, after canopy closure (October), *A. senescens* had the highest assimilation rate.

Individual-Level Carbon Sequestration Capacity

From the primary production of aboveground part, estimated annual CO_2 sequestration of aboveground part were highest in *A. oxysepala* (2.44 ± 1.82 g CO_2), followed by *A. jaluense* (1.95 ± 1.34 g CO_2), *A. thunbergii* (1.89 ± 1.48 g CO_2), *P. odoratum* var. *pluriflorum* (1.47 ± 0.98 g CO_2), *D. smilacinum* (1.33 ± 0.75 g CO_2), and *A. senescens* (0.78 ± 0.62 g CO_2) (Fig. 4A).

In contrast, only four of the six species showed positive values for annual growth of belowground biomass during the experimental period. Two *Allium* species showed the decrease in belowground biomass during the experiment (− 3.44 ± 2.66 g CO_2 for *A. senescens* and − 5.27 ± 2.41 g CO_2 for *A. thunbergii*; Fig. 4B and Fig. S4). Since *P. odoratum* var. *pluriflorum* showed such high variation, it was not attributed to positive belowground accumulation (0.37 ± 2.66 g CO_2). *Disporum smilacinum* showed the highest estimated belowground CO_2 sequestration level (2.13 ± 1.56 g CO_2). Estimated belowground CO_2 sequestration levels were also positive in *A. jaluense* (1.33 ± 0.63 g CO_2) and *A. oxysepala* (0.48 ± 0.95 g CO_2) (Fig. 4B).

Among the four species with positive belowground sequestration level, summation of the aboveground- and belowground-carbon sequestration level as individual-level carbon sequestration was highest in *D. smilacinum* (3.46 ± 2.15 g CO_2), followed by *A. jaluense* (3.28 ± 1.89 g CO_2), *A. oxysepala* (2.91 ± 2.60 g CO_2), and *P. odoratum* var. *pluriflorum* (1.80 ± 2.39 g CO_2).

Correlation Between Belowground Carbon Sequestration and Aboveground Traits

Since not the all studied species were subsampled in August, only the assimilation rate and individual assimilation could be used for the correlation analyses of belowground CO_2 sequestration in August (Fig. 5). Assimilation rate of *A. jaluense* showed the positive correlation with belowground CO_2 sequestration levels ($p = 0.0113$). Individual assimilation in *A. jaluense* and *A. oxysepala* showed a positive correlation ($p = 0.0013$ for *A. jaluense* and $p < 0.0001$ for *A. oxysepala*).

In October, almost all senescent aboveground parts were harvested, and the correlation was assessed. Some aboveground traits showed significant species-dependent correlations with the estimated belowground CO_2 sequestration levels (Fig. 5). Leaf area showed positive correlation with belowground CO_2 sequestration in *A. jaluense* ($p = 0.0041$), *A. oxysepala* ($p = 0.0001$), and *D. smilacinum* ($p = 0.0262$). Stem diameter showed positive correlation with belowground CO_2 sequestration in *A. oxysepala* ($p = 0.0022$). Assimilation rate did not significantly correlate with belowground CO_2 sequestration in any species. Individual assimilation showed the positive correlation with *A. jaluense* ($p = 0.0049$) and *A. oxysepala* ($p = 0.0060$).

The estimated belowground CO2 sequestration levels for three species (*A. jaluense*, *A. oxysepala*, and *D. smilacinum*) showed significant positive correlation with both leaf area and aboveground biomass. Therefore, allometric equations can be developed to determine the belowground CO_2 sequestration level for the three species using linear regression (Table 1). Statistical significance was also shown for *A. oxysepala* by linear regression of belowground CO_2 sequestration by stem diameter ($p = 0.0097$).

Discussion

In this study, we examined belowground carbon sequestration by perennial herb species in the understory layer by field experiment. Based on the annual primary production of the studied species, we estimated CO_2 sequestration in the above- and below-ground parts of the perennial herbs. Due to some limitations of the study, not all planted species showed vigorous annual growth and positive belowground carbon sequestration levels. Nevertheless, formulation of the carbon sequestration level from the biomass indicated its potential application for perennial herbs. Furthermore, we suggest that aboveground traits can be used to estimate belowground carbon sequestration levels in a non-destructive manner, such as by evaluating trees.

Assessment on the Belowground Carbon Sequestration Capacity of Perennial Herbs

From the harvest in October, not all the studied species successfully increased their belowground biomass in the experimental field. Aboveground part of *A. microdictyon*, *V. orientalis*, *V. philippica*, and *P. jesoana* did not survive in the experimental field (Figs. S1 and S3). Among the six survived species, two *Allium* species (*A. senescens* and *A. thunbergii*) showed the negative belowground carbon sequestration level. It might be considered a limitation of the study, which was conducted from late March to late October of same year. Due to the relatively short growing season of many perennial herbs in the understory (Augspurger and Salk 2017), transplanting at the beginning of the growing season could result in rapid declines. The proper planting and acclimation period should be considered in order to assess the carbon sequestration capacity of plant species with lower survival rates. In addition, at the time of harvest in October of the present study, most samples of *A. thunbergii* had flowering stalks. Although *A. senescens* flowers in September and October, it did not flower during the experimental period. Assimilation rates for *A. senescens* were highest in October, except in April (Fig. 3). Considering that belowground biomass accumulation occurs after fruiting in *Allium* species, delayed phenology may result in delayed belowground allocation (Oborny et al. 2011, Augspurger and Salk 2017). A comparison of the belowground biomass after the complete senescence of aboveground parts will allow for a more precise assessment of aboveground biomass gain.

Despite the limited experimental period, it was found that the estimation method could be applied to perennial herb species, demonstrating that the method is applicable

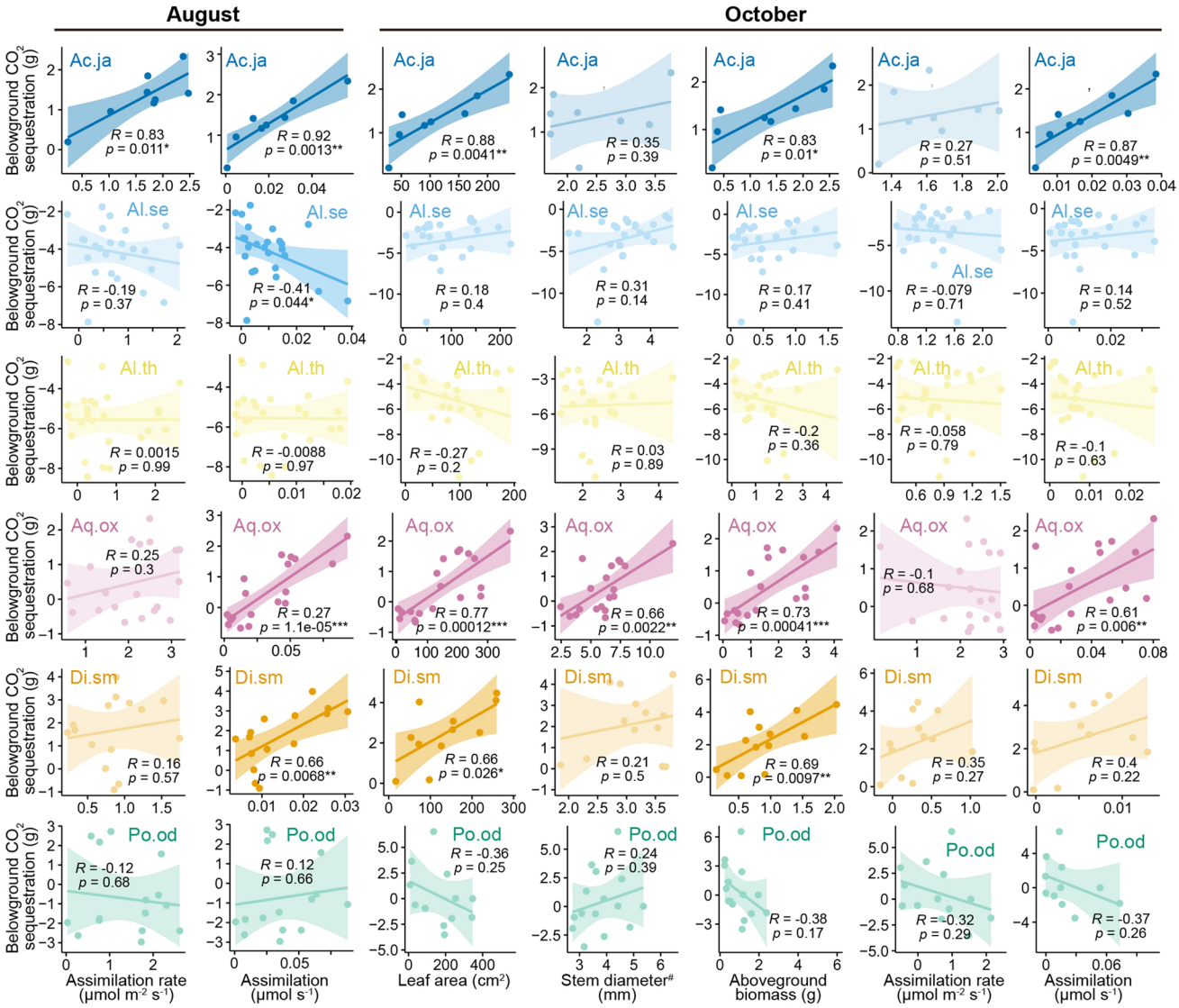

Fig. 5 Results of correlation analysis between estimated belowground CO_2 sequestration and aboveground growth traits. Shaded area represents the confidence interval in 95 % level. Ac.ja = *Aconitum jaluense*; Al.se = *Allium senescens*; Al.th = *Allium thunbergii*; Aq.ox = *Aquilegia oxysepala*; Di.sm = *Disporum smilacinum*; Po.od = *Polygonatum odoratum* var. *pluriflorum*. $^{+}$: $p < 0.1$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. $^{\#}$stem diameter: shoot diameter for Ac.ja, Di.sm, and Po.od; root collar diameter of single bulb for Al.se and Al.th; root collar diameter for Aq.ox

to perennial herb species. Most herbal carbon sequestration studies based on the annual primary production itself, which is largely composed of rapid-degrading biomass (Deng et al. 2022). Using biomass gains of herbs' belowground parts could separate carbon sequestration into relatively longer- (by belowground biomass) and shorter-term (by aboveground biomass) processes. They could be used to quantify belowground carbon sequestration itself as well as to quantify the mitigation of carbon emissions. Additionally, quantifying individual herb carbon sequestration above- and below-ground could enable comparison of the herb's carbon sequestration capacity with existing carbon emission estimates.

Typically, herb carbon sequestration is assessed at the landscape or community level as sequestered CO_2 per unit area (Chimento et al. 2016; Deng et al. 2022). Carbon sequestration capacity can be quantitatively compared by converting individual-to area-level sequestration. From the reported density of each species (or allied species) in previous studies, total area-level CO_2 sequestration were 29.5 ± 17.0 g CO_2 m^{-2} yr^{-1} for *A. jaluense* (nine individuals m^{-2}; Park et al. 2016), 46.6 ± 41.6 g CO_2 m^{-2} yr^{-1} for *A.*

Table 1 Allometric equations for estimated annual belowground CO_2 sequestration (g CO_2 individual^{-1}) of three species (*Aconitum jaluense*, *Aquilegia oxysepala*, and *Disporum smilacinum*) from linear regression by aboveground traits at October

Species	*Aboveground trait*	Allometric equation
A. jaluense	leaf area (cm^2)	$CO_2Removals_{Ac.ja,below} = 0.0007 \times Leaf.area_{Ac.ja} + 0.491$ ($p = 0.0041$)
	aboveground biomass (g)	$CO_2Removals_{Ac.ja,below} = 0.577 \times Biomass_{Ac.ja,above} + 0.562$ ($p = 0.0102$)
A. oxysepala	leaf area (cm^2)	$CO_2Removals_{Aq.ox,below} = 0.0007 \times Leaf.area_{Aq.ox} - 0.588$ ($p = 0.0001$)
	aboveground biomass (g)	$CO_2Removals_{Aq.ox,below} = 0.567 \times Biomass_{Aq.ox,above} - 0.452$ ($p = 0.0004$)
	stem diameter (mm)	$CO_2Removals_{Aq.ox,below} = 0.299 \times Stem.diameter_{Aq.ox} - 1.333$ ($p = 0.0022$)
D. smilacinum	leaf area (cm^2)	$CO_2Removals_{Di.sm,below} = 0.012 \times Leaf.area_{Di.sm} + 0.895$ ($p = 0.0262$)
	aboveground biomass (g)	$CO_2Removals_{Di.sm,below} = 2.094 \times Biomass_{Di.sm,above} + 0.244$ ($p = 0.0097$)

Ac.ja = *A. jaluense*; *Aq.ox* = *A. oxysepala*; *Di.sm* = *D. smilacinum*; $CO_2Removals_{below}$ = annual belowground CO_2 removal (g CO_2 individual^{-1}). *Leaf.area* = leaf area (cm^2); $Biomass_{above}$ = aboveground biomass (g); *Stem.diameter* = stem diameter (mm)

oxysepala (16 individuals m^{-2}; Jeong et al. 2017), 516.1 ± 320.0 g CO_2 m^{-2} yr^{-1} for *D. smilacinum* (400 shoots m^{-2}; Min 1998), and 55.2 ± 74.1 g CO_2 m^{-2} yr^{-1} for *P. odoratum* var. *pluriflorum* (30 individuals m^{-2}; Jang et al. 2001). In comparison with other trees, *D. smilacinum* had the highest carbon sequestration levels at both the individual and area levels, with area level sequestration showing 48% level of forest trees (approximately 1078 g CO_2 m^{-2} yr^{-1} for average of 30 year-old trees; Lee et al. 2019). Additionally, *D. smilacinum* grows more vigorously under closed canopies than under canopy gaps, with rapid horizontal expansion by the belowground stems (Park et al. 2010). Accordingly, *D. smilacinum*, among the species studied in the present study, could be suggested as a representative of the planting species for enhancing carbon sequestration in the understory layer of forest edge.

Estimation on the Belowground Carbon Sequestration from Aboveground Traits

Carbon sequestration in the trees was calculated based on the volumetric growth of the aboveground parts and the ratio between the aboveground and belowground parts (IPCC 2006). Therefore, it is possible to estimate the belowground biomass accumulation based on the aboveground biomass accumulation. A Tier II level estimation of carbon emissions uses all variables for the formula of tree carbon sequestration as constants, based on the subsampling of trees according to species and tree age (e.g. Lee et al. 2019). It is also possible to develop a formula for estimating carbon sequestration in herbs without destroying belowground components in the following year if aboveground growth traits have a positive correlation with belowground carbon sequestration.

However, among the four species that showed positive values for belowground carbon sequestration (*A. jaluense*, *A. oxysepala*, *D. smilacinum*, and *P. odoratum* var. *pluriflorum*), not all the aboveground traits were positively correlated with belowground carbon sequestration (Fig. 5). There appeared to be species-specific aspects of the correlation under the environment of the experimental field. Species-specific resource allocation may be attributed to the distinct belowground storage organs of the four species selected (bulb for *A. jaluense*, root for *A. oxysepala*, creeping stem for *D. smilacinum*, and rhizome for *P. odoratum* var. *pluriflorum*). Despite of that, leaf area and aboveground biomass appeared to serve as reliable indicators of belowground carbon storage, with a stronger positive correlation in three of the four species (*A. jaluense*, *A. oxysepala*, and *D. smilacinum*). In *A. oxysepala*, stem diameter positively correlated with belowground carbon sequestration. In perennial species, the storage of belowground organs determines the size and growth of aboveground sprouts in the following year under favorable environmental conditions (Schmid et al. 1995, Murphy et al. 2009).

Although the multiplication of assimilation rate and leaf area also demonstrated a positive correlation in *A. jaluense* and *A. oxysepala*, this trait also requires leaf area measurement. Thus, for the three species, the leaf area and aboveground biomass could be used as indicators of belowground carbon sequestration. While aboveground biomass can also be measured by harvesting the almost senescent parts, the destruction of aboveground parts before full senescence can also affect the storage of belowground parts (Obeso 1993). To use this estimation method in field surveys of existing plant stands, allometric equation for aboveground leaf area and/or aboveground biomass at senescence can be applied to calculate belowground carbon sequestration (Table 1). In

future studies, it may be possible to estimate the level of belowground CO_2 sequestration in a more general manner by identifying species-specific allometric relationships between nondestructively measurable aboveground traits.

Implication and Suggestion for the Carbon Sequestration of Understory Layer

In the present study, the aboveground primary production and belowground biomass gain of perennial herbs were used to quantify individual-level CO_2 sequestration. However, not all species showed the same patterns regarding above- and belowground carbon sequestration, growth, and survival. In spite of the fact that some perennial herb species in understory are able to sequester carbon both above- and belowground, our results exhibited higher deviations even in the same species (Fig. 3). It is possible that these results are due to the relatively short duration of the experimental period as opposed to the phenology of each species studied. Thus, the long-term monitoring of belowground growth would provide more precise information on the carbon sequestration of perennial herbs during the life cycle of each species (Abramoff and Finzi 2015). To assess the annual carbon sequestration in the belowground parts in a more precise and non-destructive manner, it is necessary to compare the belowground biomass between the same period in each year. In particular, the period after complete senescence of the aboveground parts may provide a more accurate reflection of the annual accumulation of belowground biomass with the exclusion of the species-specific traits.

In this study, the quantitative aspects of carbon sequestration by perennial herb species were investigated, particularly in the understory layer. As the maximum PAR was recorded before noon throughout the experimental period, our study site may represent the northeastern edge area of the forest (Fig. S1). There is a relatively high proportion of edge areas in urban forests, which is a result of either the fragmentation of existing forests or artificial planting (Hamberg et al. 2009, Li et al. 2018). In general, the edge effect occurs approximately 30–100 m from the forest edge (Hofmeister et al. 2019). Light and other microclimatic factors in urban forests may need to be considered differently from those in natural forests. Given that the carbon assimilation rates under decreasing DLI differ by species, the possible niches of each species within a forest landscape may also differ. Assimilation rates of *D. smilacinum* and *P. odoratum* var. *pluriflorum* increased with decreasing DLI, except in April, suggesting a broader range of applications from forest edges to inner areas.

Total-C of the four species (*A. jaluense*, *A. oxysepala*, *D. smilacinum*, and *P. odoratum* var. *pluriflorum*) were used as *CF* (Table S2), while *CF* of both the above- and belowground parts of the other species was assumed as 0.4 in this study (Garnier and Vancaeyzeele 1994, Zhang et al. 2014, Tang et al. 2018). Even within the same species, carbon fractions may vary depending on the environment, tissues, and plant ontogeny. Total-C content of belowground storage organ would increase at senescence stage caused by allocation for overwintering (e.g. Jeong et al. 2023). Therefore, total-C content at senescence stage might provide the more precise estimation on the carbon sequestration by biomass production. Moreover, because plant materials derived from herbs are commonly regarded as carbon sources by rapid degradation, the carbon cycle by degradation following growth should also be evaluated to determine the role of perennial herb species in the carbon cycle. Relatively slower degradation could contribute to soil carbon sequestration and reduction in soil carbon emission, which depend on the environment, soil fauna, and soil microbiome (Bai and Cotrufo 2022). The assessment of soil carbon deposition by perennial herb species by measuring soil total carbon and organic carbon content could enhance knowledge about perennial herbs' role in soil carbon cycling. In addition, similar to the estimation method used for trees, the age-dependent traits of each species can also be assessed.

The carbon sequestration capacity of trees is an age-dependent trait, increasing as they reach an earlier age (approximately 30 years) and decreasing as they reach a peak age (Iizuka and Tateishi 2015; Jo and Park 2017). The carbon sequestration capacity of young trees is also relatively low, which contributes to the time requirements. Carbon fixation in forest floor layer including understorey herbs would compensate the carbon sequestration capacity of forest age (Smith et al. 2006). Because younger trees have a smaller canopy than older trees, the microclimate under the canopy is similar to that at the edge of the mature forest. Therefore, planting herbal species under the forest edge in the understory layer improves the carbon sequestration capacity of young forests.

Conclusions

The carbon sequestration capacities of the studied perennial herb species could be used to enhance their ecological functions as carbon sinks in diverse ecosystems, particularly in forests in East Asian temperate regions. Forest edges and urban forests tended to experience more frequent fluctuations in PAR. To explore possible species in the understory layer of forest edges and urban forests, it is important to select species that are tolerant of these conditions. In the present study, the method used to estimate the accumulation of belowground biomass was used to calculate the carbon sequestration function by biomass production. Our data will facilitate the calculation of carbon emission factors

for urbanized forest areas, such as forest edges and urban forests.

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Author Contributions YJ conceived the study and designed experiments. BEN and J-MK performed experiments and data analysis. SL, YKS, and B-HL participated in the experiment and interpretation of the data. YJ, SL, YKS, and B-HL secured the funding. BEN, J-MK, and YJ wrote the paper. All authors read and approved the final manuscript.

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Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflicts of interest There are no conflicts of interest to declare.

Ethics approval N/A

Consent to Participate N/A

Consent for Publication N/A

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