Seasonal accumulation of photoassimilated carbon relates to growth rate and use for new aboveground organs of young apple trees in following spring

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Deciduous trees accumulate carbon (C) in woody parts during the growth season which is subsequently used for the initial development and growth of newly formed organs in the following season; however, it is unclear which period during the growth season contributes to C accumulation. Three-year-old potted Malus domestica (apple) trees were grown in controlled growth chambers during the growth season and exposed to $^{13}$CO$_2$ in an exposure chamber at seven different periods of the growth season, including vegetative and reproductive growth periods. Approximately half of the trees were harvested in late autumn, and the remaining trees were grown in a field in the following year. The $^{13}$C accumulation in the different organs in late autumn, and its concentration in the new aboveground growth during the following growth season, was determined. The concentration of the photoassimilated $^{13}$C in woody parts (shoots, trunk, rootstock and coarse roots) in the late autumn was higher in the trees labeled during the period of vigorous vegetative growth than in those labeled during other periods of growth. Furthermore, $^{13}$C concentration in the leaves, annual shoots, flower buds and flowers in the following early spring was also high in the trees labeled during this period. The concentration of $^{13}$C in the flower buds and flowers was positively correlated with that in the woody parts in the late autumn and old shoots in the following spring. Hence, the seasonal accumulation of photoassimilated C in woody parts in late autumn is related to growth rates during the growth season and its use for the initial development of newly formed organs in the following spring. These results suggest that under non-stressed conditions, C accumulated during the period of vigorous vegetative growth largely contributes to the C reserves that are used for the development of new organs in the following year.

Keywords: $^{13}$CO$_2$, early spring, growth season, newly formed organs, photoassimilation, woody parts.

Introduction

Carbon (C) accumulation is an important process related to environmental tolerance, growth and long-term survival of trees (Sala et al. 2012). C photoassimilated from atmospheric CO$_2$ is distributed within the plant body throughout the growth season. A fraction of the photoassimilated C is accumulated in woody parts of trees in the form of non-structural carbohydrates (NSCs), such as starch, sugars and other C compounds, which serve as stored C reserves to meet variable demands for resources under environmental stresses, such as drought, flood, wind and frost, as well as disturbances throughout the year (Palacio et al. 2014). C reserves are available in early spring for use in metabolism, development and growth of new organs, such as leaves and fine roots (Hoch et al. 2003, Sala et al. 2012). In deciduous trees, stored C is essential for the initial development and growth of newly formed organs in the following season, particularly during early spring (Hansen 1967b, Kandiah 1979). However, although trees maintain high levels of NSC in woody parts throughout the growth season (Furze et al. 2019, Hoch et al. 2003), it remains unclear which period in the
growth season contributes to the allocation of assimilated C to the storage reserves, which is then available for use in the early spring.

Photoassimilated C accumulates as stored C reserves in woody parts of the trees. Although the age of stored C used in early spring varies with tree species (Kuptz et al. 2011) and forests (Gaudinski et al. 2009), it is likely that C storage pools contain a higher proportion of photoassimilated C accumulated in the previous season. Previous studies using stable (13C) and radioactive C isotope (14C) have revealed that stored C aged less than 1–2 years was used for the growth of leaf buds and fine roots in temperate deciduous forests (Gaudinski et al. 2001, 2009) and that aged 1–2 years was used for stem respiration prior to leaf development in mixed hardwood forests (Carbone et al. 2013). Richardson et al. (2015) reported that the outer tree ring (i.e., younger tissues) had young NSC pools that were not likely to be mixed with old pools, suggesting that trees would use younger stored C for growth and metabolism after dormancy. In contrast, Muhr et al. (2016) reported that relatively older stored C (three–five years old) was used for leaf growth, based on the analysis of the xylem sap of a sugar maple (Acer saccharum); however, it is unclear whether the trees use only stored C in the xylem sap (Gessler and Treydte 2016).

The concentration of stored C in trees fluctuates during the growth season (Furze et al. 2019, Hoch et al. 2003) and among successive years (Weber et al. 2019). Previous studies have reported that NSCs are maintained at a high level throughout the growth season and do not deplete even during periods of vigorous vegetative and reproductive growth in deciduous and evergreen trees (Furze et al. 2019, Hoch et al. 2003). Such seasonal patterns in NSC concentrations are considered to occur because the supply of photoassimilated C does not necessarily coincide with C demands for growth, respiration, and tolerance (Sala et al. 2012). Furthermore, NSC concentration in the storage organs of deciduous fruit trees decreases around bud break, reaches a minimum at the time of vigorous vegetative growth, and reaches a maximum around leaf fall (Mochizuki and Hanada 1956, Wardlaw 1990, Pallas et al. 2018, Tixier et al. 2019), although a clear trade-off relationship was not detected between NSC concentration in aboveground woody parts and trunk growth in Mediterranean tree crop species (Tixier et al. 2019). Seasonal patterns in the accumulation of C in woody parts of fruit trees can largely be influenced by changes in fruit sink strength (Wardlaw 1990, Rosati et al. 2018, Ryan et al. 2018) since fruit is a dominant sink for photoassimilates (Hansen 1967a, Monerri et al. 2011). The periods of vegetative and reproductive growth differ in fruit trees; fruit growth rate increases after shoot growth decreases in apple (Malus domestica Borkh) (Wardlaw 1990, Imada et al. 2017, 2021). Thus, fruit trees can be a good model for understanding the process of C accumulation in storage organs during vegetative and reproductive growth (Ryan et al. 2018).

To determine the seasonal patterns in C accumulation and its distribution during initial development in the following season, we exposed young apple trees to 13CO2 during different growth periods and determined the accumulation of photoassimilated 13C in woody parts in late autumn and in newly formed organs in the following growth season. Since trees generally maintain high levels of NSC in woody parts throughout the growth season (Furze et al. 2019, Hoch et al. 2003), a portion of the accumulated 13C in woody organs, regardless of the assimilation periods, could transfer to the newly formed organs in the following year. Based on our previous findings that allocation of photoassimilated 13C in apple shoots is related to growth rates (Imada et al. 2017, 2021), we hypothesized that C storage increases during the period of higher growth in woody organs.

**Materials and methods**

**Study site and plant materials**

This study was conducted in the field, as well as in controlled growth chambers at the Institute for Environmental Sciences (IES), Aomori, Japan (40° 57’ N, 141° 21’ E, 27 m elevation) during two successive growth seasons in 2017 and 2018. Before the experiment, 106 three-year-old M. domestica ‘Fuji’ trees grafted on JM.1 stocks were individually transplanted in plastic pots (25 L, 38 cm diameter, 31 cm tall) filled with a soil mixture, consisting of black soil, peat moss, vermiculite, pearlite, zeolite and soluble phosphatic manure at volume ratios of 4:2:1:2:1:2:0.5, respectively. The pH of the soil mixture was adjusted to approximately 6 by adding magnesia lime powder at a mass ratio of 0.01. The potted trees were stored in a refrigerator at 2 °C and >80% relative humidity.

**Growth conditions**

All trees in the refrigerator were transported to the field in the IES on 28 April 2017 and grown until 19 May 2017, to allow for selection of trees with flower buds for the exposure experiments. A fertilizer (15-6-9 NPK) was added to the soil surface at 3 g N m⁻² in each pot. The trees in the field were fully irrigated when the surface soil was dry and sprayed with pesticides, according to standard cultivation management protocols. The average dates of bud break (BBCH scale: 07, Meier 1997) and onset of leaf extension (BBCH scale: 11) were May 2 and May 8, respectively. The mean air temperature was 12 °C during the growth period in the field.

Seventy-two trees with flower buds selected based on their trunk diameter and 36 trees grown in the field for 21 days were transferred to two controlled growth chambers (5 m x 8 m x 2.3 m). The trees were aligned and placed in four rows in each growth chamber and grown until harvest. On July 24 and July 25, the trees were moved outside the facility for pesticide application. Additionally, 15-6-9 NPK fertilizer was added to the soil surface at 2 g N m⁻² on June 21. The placement
of the trees within the rows was changed at 2 or 3 week intervals and the trees were arranged in opposite directions when changing the rows to account for the non-uniformity of environmental conditions in the growth chambers. The average dates of flowering (BBCH scale: 60) and full bloom (BBCH scale: 65) were May 24 and May 25, respectively.

Air temperature of the photoperiod and dark period in the controlled growth chambers was changed at approximately ten-day intervals with reference to the values of ten-year average temperature in Aomori city (Japan Meteorological Agency) to simulate natural conditions. Meanwhile, the lengths of the photoperiod were also changed based on the sunrise and sunset times in the region, ranging between 10 and 15 h. The average air temperatures were monitored with 16 evenly distributed temperature sensors in each growth chamber; the average temperature in the photoperiod and dark period during the growth period, were 20.4 and 17.4 °C, respectively (Figure S1 available as Supplementary Data at Tree Physiology Online). Metal halide lamps (n = 78 in each chamber) (1 kW, MF1000LE/BUH, Iwasaki Electroc Co., Ltd, Tokyo, Japan) were used for lighting. The photosynthetic photon flux density was approximately 600 μmol photon m⁻² s⁻¹ from May 19 to November 5 and was measured with a quantum sensor (Apogee Instruments, Inc., Logan, USA) around the canopy height. The number of lamps was reduced to 54 in each chamber (the lighting was weakened to approximately 400 μmol photon m⁻² s⁻¹) from November 6 to 17 to decrease the air temperature. The total CO₂ concentration in the growth chambers was usually controlled between 400 and 500 μL L⁻¹ during the photoperiods.

Forty-one trees were harvested during November 13 or 17, 2017 (BBCH scale: 89). The remaining 31 trees were stored in the refrigerator after the leaves senesced. The trees were transported to the field on April 9, 2018, and randomly placed at 50–100 cm distances between the adjacent trees, and a fertilizer (6–40-6 NPK) was added to the soil surface at 2 g N m⁻². Additionally, urea (2 g L⁻¹) was sprayed on the trees on June 11 and 22, and a fertilizer (15–6-9 NPK) was added to the soil surface at 2 g N m⁻² on August 23. The average dates of bud break (BBCH scale: 07), onset of leaf extension (BBCH scale: 11), flowering (BBCH scale: 60) and full bloom (BBCH scale: 65) of the trees were April 22, April 29, May 17 and May 21, respectively. The trees were harvested on 16 November 2018 (BBCH scale: 89).

¹³C labeling experiment

C isotope (¹³C) labeling was conducted by exposing the trees to ¹³CO₂ in an exposure chamber, similar in size to the growth chamber with a function for controlling the total CO₂ concentration and ¹³C fraction [x (¹³C), in %] of CO₂ using a CO₂-free air supplier. In addition, it also had ¹³CO₂ cylinder, mass flow controllers, an isotope mass spectrometer (ARCO-2000, Arco System Inc., Chiba, Japan) for monitoring ¹²CO₂ and ¹³CO₂ concentrations with multiple sampling air selectors, and a computer system for control and data acquisition (Ohnishi Netsugaku Co., Ltd, Tokyo, Japan). The labeling experiments were performed seven times in 2017: May 31 (6 days after full bloom), June 14 (increased shoot growth, Figure 1), July 5 and July 20 (decreasing shoot growth), August 2 and August 16 (increased fruit growth) and October 11 (decreased fruit growth). Nine trees in a row were moved to the exposure chamber one day before labeling and were moved back to the growth chamber a day after labeling and grown until mid-November. The lighting system was not functional on August 2 for approximately 25 min during exposure due to system failure during the labeling experiment. Nine unlabeled trees were also grown as controls in growth chambers throughout the growth period.

The ¹²CO₂ and ¹³CO₂ concentrations in the exposure chamber were regulated using a computer system during the labeling experiments. The ¹²CO₂ concentration in the exposure chamber was reduced to 340 μL L⁻¹ by gas exchange until 9:00 on the days of the labeling experiment. ¹³CO₂ gas (99%, SHOKO SCIENCE Co., Ltd, Yokohama, Japan) was intermittently injected from 9:00 onwards at a rate of 450 mL min⁻¹ with a mass flow controller (ACE Inc., Kanagawa, Japan) until it reached an atmospheric x (¹³C) of approximately 15% (30–75 min). The ¹²CO₂ and ¹³CO₂ gases were added at different rates with mass flow controllers (ACE Inc., Kanagawa, Japan) until 17:00 to maintain the total ¹²CO₂ concentration and atmospheric x (¹³C) at approximately 340 μL L⁻¹ and 15%, respectively. Subsequently, the ¹²CO₂ concentration was adjusted to approximately 400 μL L⁻¹ from 17:00 to the end of the photoperiod. Thereafter, the air in the exposure chamber was ventilated by the outside air. The atmospheric x (¹³C) in the exposure chamber was confirmed to be close to its natural abundance before moving the labeled trees from the chamber. ¹³C/¹²C molar ratio of the air in the exposure chamber was determined by pumping the air out and monitoring it at three min intervals using an isotope mass spectrometer (ARCO-2000) calibrated using sensitivity calibration (N₂ + O₂: 21%) and reference gases (N₂ + O₂ + Ar + CO₂, O₂: 19%, Ar: 1%, CO₂: 500–1000 ppm, and ¹³CO₂: approximately 10 atom %) before and during the labeling experiments.

The total CO₂ concentration and atmospheric x (¹³C) concentrations in the exposure chamber from 9:00 to the end of the photoperiod were similarly regulated across the different labeling days and ranged from 400 to 405 μL L⁻¹ and from 0.11 to 0.13, respectively (Table S1 available as Supplementary Data at Tree Physiology Online). The ventilation duration (from 17:00 to the end of the photoperiod) was slightly shorter on August 16 and October 11 (3.25 and 2 h, respectively) than on the other days (3.5 h) due to differences in the duration of the photoperiod. However, the different ventilation durations were not expected to influence the labeling conditions as the
Growth measurements

The sizes of all fruits, leaves, annual shoots, and old shoots of five out of the nine control trees were measured at approximately 3- to 4-week intervals during the 2017 growth season. The horizontal and vertical diameter of fruit, the length and width of leaves, the length and basal diameter of annual shoots, the diameter of old shoots with different ages, as well as the diameter of trunks were measured. The length of old shoots (of varying ages) and trunks was measured once. Leaves and annual shoots were measured during the same time. Details of the measurement analyses are available in Table S2 (available as Supplementary Data at Tree Physiology Online).

The volumes of fruits, annual shoots, old shoots and trunks of the control trees were estimated using the formulas of ellipsoid, cone, circular truncated cone and cylinder forms, respectively. The total leaf area was estimated from the leaf size data and the relationship between leaf size and leaf area approximated by the least square method (single leaf area = 0.672 × length × width, n = 303; Figure S2 available as Supplementary Data at Tree Physiology Online). The absolute growth rates of the estimated total leaf area (AGR\(_{\text{LA}}\)), volumes of fruits (AGR\(_{\text{FV}}\)), annual shoots (AGR\(_{\text{CV}}\)), old shoots (AGR\(_{\text{PV}}\)) and trunks (AGR\(_{\text{TV}}\)) of the five control trees were calculated between the successive measurements of all the organs. The AGRs for the leaves, fruits and annual shoots were calculated from the time of emergence (i.e., the date of bud break or flowering). The number of annual shoots used for the calculations was 60 ± 17 (mean ± S.D., n = 5). The relative growth rates for trunk diameter (RGR\(_{\text{TD}}\)) between the first (May 12) and last (October 26) measurements were also calculated for all the trees.

Sampling and biomass determination

Six out of the nine control trees (including the five measurement trees) and five out of nine labeled trees (from each labeling date) were harvested on 13 November and 17 November 2017, respectively. The harvested trees were cut above the ground and separated into fruits, leaves, annual shoots, old shoots, trunk, grafting part and rootstock samples. Leaves on five long shoots (reproductive or vegetative) of each control tree (21.1 ± 6.2 cm, mean ± S.D., n = 25) were measured using a leaf area meter (LI-3100C; LI-COR Inc., Lincoln, NE, USA). The belowground organs were washed with tap water and divided into belowground rootstock, coarse root and fine root (≤ 1 mm) samples. All organs were dried at 60 °C for more than 72 h and weighed for dry mass. The data of one harvested control tree were not used and were not measured in the following analyses.

Branches of all the trees were pruned before bud break (April 12, BBCH scale: 03) in 2018. The biomass of the pruned branches was 38.4 ± 23.7 g DW tree\(^{-1}\) (mean ± S.D., n = 31). The pruned branch samples were divided into terminal buds and old shoots of different ages. At the pink stage (just before flowering, May 14, BBCH scale: 59), branch samples were...
collected and separated into leaves, annual shoots, flower buds and older branches of different ages. Flowers and fruits were collected on May 17 (flowering, BBCH scale: 60), June 6–7 (BBCH scale: 71), July 4 (BBCH scale: 74) and August 15 (BBCH scale: 77).

The trees were harvested on 16 November 2018 and separated according to the organs, as mentioned above. All plant organs were dried at 60 °C for more than 72 h and weighed for dry mass.

**Chemical analysis**

Oven-dried organs were ground into a powder using a vibration sample mill or a grinder mill. The ground samples were analyzed for total C content and $^{13}C/^{12}C$ ratio using an NCH analyzer (NCH-22F, Sumika Chemical Analysis Service, Ltd, Tokyo, Japan) and mass spectrometer (DELTA V Advantage, Thermo Fisher Scientific K.K., Kanagawa, Japan), respectively. The stable C isotope composition is expressed in the δ notation (%). Since the total C content and $^{13}C/^{12}C$ ratio were almost the same between the rootstock and belowground rootstock in 2017, the measurements were not performed for the belowground rootstock in 2018, and the values of the rootstock were used for the calculation of the belowground rootstock.

The $^{13}C$ fraction, $x$ ($^{13}C$), was calculated using equation 1 (Dawson et al. 2002).

$$x \left( ^{13}C \right) = \left( \delta^{13}C/1,000 + 1 \right) \times 0.0112372 \left[ \left( \delta^{13}C/1,000 + 1 \right) \times 0.0112372 + 1 \right] \times 100. \quad (1)$$

The $x$ ($^{13}C$) for the control trees varied among plant organs in mid-November of 2017 and 2018, as well as among new aboveground parts in 2018 (Figure S3 available as Supplementary Data at *Tree Physiology* Online), likely due to isotope discrimination (Ghashghaie and Badeck 2014). The excess atomic fraction of $^{13}C [x^\delta(13C)$, in %], and the total $^{13}C$ recovered from plant parts, were calculated using Eqs 2 and 3, respectively.

$$x^\delta(13C) = x \left( ^{13}C \right)_e - x \left( ^{13}C \right)_c \quad \text{(2)}$$

Total $^{13}C$ recovered = $x^\delta(13C) \times 100 \times M \text{ (g DW)}$, \text{(3)}

where $x \left( ^{13}C \right)_e$ and $x \left( ^{13}C \right)_c$ were the $x \left( ^{13}C \right)$ in the exposed trees and the average value in the control trees, respectively. Since the $x^\delta(13C)$ of the trunk, grafting zone and rootstock were similar, the weighted average of the values was determined and presented as trunk.

The mean residence time (MRT) was estimated for flowers and fruits using the following equations:

$$F(t) = a \times \exp (-k \times d) \quad \text{(4)}$$

$$\text{MRT (d)} = 1/k, \quad \text{(5)}$$

where $a$, $k$ and $d$ were the $x^\delta(13C)$ of flower buds (at the pink stage on May 14), the decay ratio of the $x^\delta(13C)$ and the days after the pink stage, respectively. $k$ was estimated by least-squares fitting method.

**Statistical analysis**

One-way analysis of variance (ANOVA) was used to determine if the RGR$_{TD}$ in 2017, as well as the total C mass and that of woody parts in mid-November of 2017 and 2018 differed among the treatments. Differences in the AGRs of the plant organs between the different measurement periods were assessed using one-way repeated measures ANOVA using the ’rstatix’ package. A one-way ANOVA followed by Tukey HSD multiple comparison test, or a Kruskal–Wallis test followed by Dunn’s multiple comparison test were performed using packages ‘FSA’ and ‘rcompanion,’ respectively, to determine if the $x^\delta(13C)$ and total $^{13}C$ recovered in each organ, as well as whether MRT differed among the trees labeled in the different periods in 2017 and 2018. Data were logarithmically transformed to follow the assumptions of normality and homogeneity of variance by Shapiro–Wilk test and Levene’s test, respectively, using ‘car’ package wherever necessary. Pearson correlation analysis was conducted to evaluate the relationships among the $x^\delta(13C)$ of plant organs between 2017 and 2018, between woody parts in late November 2017 and plant organs in 2018, as well as among the $x^\delta(13C)$ of plant organs within each individual year. A linear regression analysis was conducted between woody parts in late November 2017 and one-year-old shoots in early spring (mid-April or mid-May) or flower buds in mid-May 2018 and between one-year-old shoots in early spring (mid-April or mid-May) and flower buds in mid-May 2018. The abovementioned analyses were performed using R (version 4.1.2).

**Results**

**Growth and C mass**

Trunk growth during the growth season and C mass (dry weight × C content) in mid-November 2017 did not vary among the control and labeled trees from different periods. The RGR$_{TD}$ did not differ significantly (ANOVA, $P = 0.99$, $n = 9$; Figure S4 available as Supplementary Data at *Tree Physiology* Online). The C mass in the whole plants and woody parts in mid-November of 2017 (ANOVA, whole plants: $P = 0.31$, $n = 5$; woody parts: $P = 0.50$, $n = 5$) and 2018 (ANOVA, whole plants: $P = 0.97$, $n = 3–4$; woody parts: ANOVA, $P = 0.70$, $n = 3–4$; Figure S5 available as Supplementary Data at *Tree Physiology* Online) did not differ significantly among the control and labeled trees from the different periods, indicating that the experimental trees showed similar growth and C mass during the growth seasons in both the years.

The leaf area, annual shoot volume, fruit volume, old shoot volume and trunk volume were estimated at the whole-tree
level for the control trees (Figure 1). The leaf area and annual shoot volume increased between late May and early July (Figure 1a and b). Meanwhile, the fruit volume increased between early August and late September (Figure 1c) and the old shoot increased between early June and early July (Figure 1d). Hence, the absolute growth rates of leaves and branches were higher during the previous growth season. Moreover, the trunk volume increased gradually during the growth season (Figure 1e). Temporal changes in their absolute growth rates were also calculated between the measurement dates during the 2017 growth season (Figure S6 available as Supplementary Data at Tree Physiology Online). The differences between AGRLA, AGRALV, AGRASV, and AGROSV of the control trees were significant (repeated measured measures ANOVA, \( P < 0.001 \), \( n = 5 \)). The AGRLV was higher between mid-May and early June; however, the difference was not significant (repeated measures ANOVA, \( P = 0.26 \), \( n = 5 \)).

**\( x^E(13C) \) in plant organs**

The \( x^E(13C) \) in each plant organ was calculated as a \( 13C \) fraction in excess of the \( 13C \) fraction relative to the control trees (Figure 2, Figure S3 available as Supplementary Data at Tree Physiology Online). The \( x^E(13C) \) in each plant organ, during mid-November 2017 and 2018, was influenced by different periods of labeling (Figure 2). Indeed, significant variations were observed in the \( x^E(13C) \) of each plant organ among the trees labeled in the different periods (one-way ANOVA or Kruskal–Wallis test, \( P < 0.001 \); Figure 2a). Moreover, the \( x^E(13C) \) was higher in the aboveground parts (leaves, annual shoots, old shoots and trunk) in the trees labeled in late May and mid-June (one-way ANOVA or Kruskal–Wallis test, \( P < 0.001 \)). Meanwhile, the \( x^E(13C) \) of fruits was higher in mid-August (one-way ANOVA, \( P < 0.001 \)). The timing of higher \( x^E(13C) \) in the aboveground parts corresponded to the periods with higher growth rates (Figure 1). In fact, positive correlation was detected between the \( x^E(13C) \) in leaves and woody parts (Pearson correlation, \( r = 0.77–0.88, P < 0.001, \ n = 35 \); Table S3 available as Supplementary Data at Tree Physiology Online). In contrast, the \( x^E(13C) \) in the belowground rootstock and coarse roots was higher in the trees labeled in mid-June (one-way ANOVA, \( P < 0.001 \)). Similar to that in the leaves and aboveground woody parts, while that in the fine roots was higher in early July (Kruskal–Wallis test, \( P < 0.001 \); Figure 2a).

Negative correlations were found between the \( x^E(13C) \) in leaves (Pearson correlation, \( r = -0.80, P < 0.001, \ n = 35 \)) or woody parts and fruits (Pearson correlation, \( r = -0.50, P < 0.01 \) to \( -0.90, P < 0.001, \ n = 35 \); Table S3 available as Supplementary Data at Tree Physiology Online).

Variations in the \( x^E(13C) \) of woody parts harvested in mid-November 2018 appeared to not differ from the corresponding organs in 2017 (e.g., one-year-old branch in 2018 vs. annual shoot in 2017), although the values were lower in 2018 (Figures 2a and b). The \( x^E(13C) \) in each woody organ in 2017 was strongly and positively correlated with that in 2018 (Pearson correlation, \( r = 0.87, P < 0.05 \) to 0.98, \( P < 0.001, \ n = 7 \); Table 1). In contrast, the \( x^E(13C) \) in the fruits, leaves and annual shoots in 2018 was negligible compared to that in 2017 (Figures 2a and b), most likely due to \( 13C \) dilution by the current-year photoassimilates during the 2018 growth season.

**Correlation between \( x^E(13C) \) in the woody parts and newly formed organs**

Before the bud break (in mid-April) in 2018, the \( x^E(13C) \) in the 1- and 2-year-old shoots had relatively higher values in the trees labeled in late May and mid-June (Kruskal–Wallis, \( P < 0.001 \); Table 2), while that in the terminal buds had higher values in the trees labeled in early July (ANOVA, \( P < 0.01 \)). The \( x^E(13C) \) in 1- and 2-year-old shoots was not significantly related with that in terminal buds (Table 3). At the pink stage, just before flowering (mid-May), \( x^E(13C) \) in the leaves, annual shoots, flower buds and 1-year-old branches peaked in the trees labeled in mid-June (ANOVA or Kruskal–Wallis, \( P < 0.05 \), Table 2). Moreover, the \( x^E(13C) \) in the flower buds correlated with that in the 1- (Pearson correlation, \( r = 0.41, P < 0.05, \ n = 28 \)) or 2-year-old shoots in mid-April (Pearson correlation, \( r = 0.46, P < 0.05, \ n = 28 \), Table 3). Positive correlation was also observed between \( x^E(13C) \) in woody organs in mid-November 2017 with that in 1-year-old shoots in mid-April (Pearson correlation, \( r = 0.88, P < 0.01, \ n = 7 \)) and mid-May (\( r = 0.82, P < 0.05, \ n = 7 \)), as well as flower buds in mid-May 2018 (\( r = 0.78, P < 0.05, \ n = 7 \)); however, not with that in leaves or annual shoots (Table S4 available as Supplementary Data at Tree Physiology Online). Linear regression analyses further revealed that the \( x^E(13C) \) in woody organs in late autumn 2017 was significantly associated with that in 1-year-old shoots (adjusted \( R^2 = 0.97, P < 0.001, \ n = 7 \)) and flower buds (adjusted \( R^2 = 0.53, P < 0.05, \ n = 7 \); Figure 3a). Similarly, the \( x^E(13C) \) in 1-year-old shoots in mid-April (adjusted \( R^2 = 0.14, P < 0.05, \ n = 28 \)) or mid-May was associated with that in flower buds (adjusted \( R^2 = 0.28, P < 0.01, \ n = 28 \); Figure 3b).

**Total \(^{13}C\) recovered**

The excess \(^{13}C\) in whole plants and woody parts in mid-November 2017 and 2018 was higher in the trees labeled in mid-June (ANOVA, \( P < 0.001, \ n = 5 \)). The excess \(^{13}C\) in whole plants and woody parts was consistently lower in 2018 than that in 2017 (Figure 4). This is partly due to biomass loss caused by pruning branches before bud break in 2018 (10–28% for C mass and 17–48% for excess \(^{13}C\) in the old shoots in mid-November). Meanwhile, strong positive correlations were observed in the concentration of photoassimilated \(^{13}C\) in each woody organ between 2017 and 2018.
Figure 2. Mean $\delta^{13}C$ of the fruits (F), leaves (L), annual shoots, 1-, 2-, 3- and 4-year-old shoots (1OS, 2OS, 3OS and 4OS), trunk (T), belowground rootstock (BRS), coarse roots (CR) and fine roots (FR) in mid-November of 2017 (a) and 2018 (b) for the trees labeled in the different periods of the 2017 growth season. One-way ANOVA followed by Tukey HSD test, or Kruskal–Wallis test followed by Dunn’s multiple comparisons test was performed for each plant organ ($P < 0.05$). The error bars denote $\pm$ SD ($n = 5$ in 2017, $n = 4$ in 2018).

Table 1. Pearson correlation analyses of the $\delta^{13}C$ in each organ of the exposed trees between mid-November of 2017 and 2018

|       | F       | L       | AS      | 1OS     | 2OS     | 3OS     | 4OS     | T       | GZ      | RS      | CR      | FR      |
|-------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 2017  |         |         |         |         |         |         |         |         |         |         |         |         |
| F     | 0.34    | −0.64   | −0.92** | −0.85*  | −0.91** | −0.88** | −0.75*  | −0.77*  | −0.82*  | −0.67   | −0.67   | −0.03   |
| L     | −0.17   | 0.86*   | 0.89**  | 0.97**  | 0.94**  | 0.81*   | 0.81*   | 0.92**  | 0.89**  | 0.83*   | 0.86*   | 0.18    |
| AS    | −0.05   | 0.91**  | 0.83*   | 0.98*** | 0.89**  | 0.72    | 0.75    | 0.93**  | 0.91**  | 0.91**  | 0.93**  | 0.31    |
| 1OS   | −0.22   | 0.62    | 0.95**  | 0.89**  | 0.98*** | 0.97*** | 0.88**  | 0.86*   | 0.85*   | 0.64    | 0.67    | −0.1    |
| 2OS   | −0.2    | 0.69    | 0.95**  | 0.92**  | 0.99*** | 0.96*** | 0.89**  | 0.89**  | 0.88**  | 0.69    | 0.72    | −0.03   |
| 3OS   | −0.12   | 0.78*   | 0.94**  | 0.96*** | 0.99*** | 0.91**  | 0.87*   | 0.93**  | 0.91**  | 0.78*   | 0.81*   | 0.07    |
| T     | −0.06   | 0.84*   | 0.87*   | 0.98*** | 0.96*** | 0.83*   | 0.86*   | 0.96*** | 0.95**  | 0.87*   | 0.90*   | 0.24    |
| GZ    | −0.02   | 0.89**  | 0.83*   | 0.99*** | 0.91**  | 0.75    | 0.78*   | 0.95*** | 0.95**  | 0.93**  | 0.94**  | 0.34    |
| RS    | 0.00    | 0.94**  | 0.81*   | 0.97*** | 0.86*   | 0.67    | 0.68    | 0.92**  | 0.89**  | 0.92**  | 0.93**  | 0.32    |
| BRS   | 0.13    | 0.93**  | 0.80*   | 0.95**  | 0.86*   | 0.67    | 0.72    | 0.94**  | 0.88**  | 0.89**  | 0.91**  | 0.31    |
| CR    | −0.07   | 0.89**  | 0.58    | 0.82*   | 0.68    | 0.46    | 0.62    | 0.79*   | 0.77*   | 0.87*   | 0.89**  | 0.52    |
| FR    | −0.16   | 0.36    | −0.12   | 0.12    | −0.02   | −0.19   | 0.10    | 0.11    | 0.24    | 0.45    | 0.40    | 0.87*   |

The measurement was not performed for the BRS in 2018 (see the Materials and Methods section).
In 2018, the excess $^{13}$C in woody parts was slightly lower than that in the whole plants, unlike in 2017, due to lower excess $^{13}$C in the newly developed organs, particularly the fruits (Figure 4, Table S5 available as Supplementary Data at Tree Physiology Online). In contrast, the excess $^{13}$C in the fine roots was similar in 2017 and 2018 (Table S5 available as Supplementary Data at Tree Physiology Online).

**Temporal changes in $\chi^{13}(^{13}$C) in the flowers and fruits**

The $\chi^{13}(^{13}$C) was the highest in the flowers in mid-May, and young fruits in early June in the trees labeled in mid-June (ANOVA, $P < 0.001$ and 0.01, respectively, $n = 4$; Table 2), with patterns similar to those in the flower buds. The $\chi^{13}(^{13}$C) in the flowers and young fruits was also positively correlated with that in the 1- (Pearson correlation, $r = 0.56, P < 0.01$ and $r = 0.41, P < 0.05$, respectively, $n = 28$) or 2-year-old shoots (Pearson correlation, $r = 0.57, P < 0.01$ and $r = 0.48, P < 0.05$, respectively, $n = 28$; Table 3). The $\chi^{13}(^{13}$C) in the fruits in early July did not differ significantly among the trees labeled during different periods (Kruskal–Wallis, $P = 0.128, n = 4$; Table 2) and was not significantly correlated with that in the 1- (Pearson correlation, $r = 0.34, P = 0.07, n = 28$) or 2-year-old shoots before bud break (Pearson correlation, $r = 0.33, P = 0.08, n = 28$; Table 3), possibly due to the dilution via translocation of the current-year photoassimilates. The $\chi^{13}(^{13}$C) in the flower buds, flowers and fruits decreased exponentially, following an exponential decay function (Figure 5) and decreased to negligible levels in mid-August (Table 2). The average values of MRT in the trees labeled in the different periods ranged from 13.3 to 18.9 days after the pink stage (May 14). Although the initial $\chi^{13}(^{13}$C) at the pink stage, $n = 4$) differed significantly among the trees labeled in the different periods (Kruskal–Wallis, $P < 0.01, n = 4$), the MRT did not differ significantly among the trees.

**Discussion**

In the present study, the trees labeled in the period of vigorous vegetative growth accumulated more $^{13}$C in woody parts (annual shoots, old shoots, trunk, rootstock and coarse roots) by late autumn. Moreover, the concentration of photoassimilated $^{13}$C in newly formed organs (leaves, annual shoots, flower buds and flowers), during the early periods of the following season, was high in the trees labeled during vigorous vegetative growth. The concentration of photoassimilated $^{13}$C in the woody parts was positively correlated with that in the flower buds and flowers in the following early spring. These results strongly suggest that the accumulation of C, which is used in the initial development of new organs, is associated with the growth (biomass increment) rates of the woody parts during C assimilation. This may be due to a certain fraction of the photoassimilated C being allocated to a long-term storage pool (Wiley and Helliker 2012) throughout the growth season.

**Accumulation of photoassimilated C in late autumn**

In late autumn, a higher concentration and accumulation of $^{13}$C was observed in the woody parts of the trees labeled in mid-June, when the growth rate of leaves and aboveground woody
Table 2. Mean $\delta^{13}C$ in organs of the trees labeled in the different periods in 2018

| Dates of samples | Dates of labeled in 2017 | May 31 | June 14 | July 5 | July 20 | August 2 | August 16 | October 11 |
|------------------|--------------------|--------|--------|--------|--------|--------|--------|----------|
| April 12, 2018 (before bud break) | TB | 0.0423 ± 0.0158 | 0.0679 ± 0.0263 | 0.0951 ± 0.0295 | 0.0830 ± 0.0158 | 0.0748 ± 0.0070 | 0.0587 ± 0.0090 | 0.0518 ± 0.0019 |
| | 10S† | 0.175 ± 0.041 | 0.142 ± 0.053 | 0.0976 ± 0.0283 | 0.0691 ± 0.0176 | 0.0405 ± 0.0098 | 0.0241 ± 0.0063 | 0.0253 ± 0.0059 |
| | 20S† | 0.0926 ± 0.0276 | 0.0819 ± 0.0167 | 0.0317 ± 0.0080 | 0.0460 ± 0.0115 | 0.0246 ± 0.0110 | 0.0146 ± 0.0036 | 0.0166 ± 0.0099 |
| May 14 (pink) | L | 0.0280 ± 0.0028 | 0.0472 ± 0.0072 | 0.0423 ± 0.0085 | 0.0303 ± 0.0008 | 0.0350 ± 0.0137 | 0.0263 ± 0.0076 | 0.0370 ± 0.0081 |
| | AS | 0.0422 ± 0.0055 | 0.0678 ± 0.0112 | 0.0576 ± 0.0106 | 0.0442 ± 0.0030 | 0.0459 ± 0.0223 | 0.0400 ± 0.0084 | 0.0477 ± 0.0106 |
| | FB† | 0.0430 ± 0.0037 | 0.0703 ± 0.0119 | 0.0461 ± 0.0051 | 0.0365 ± 0.0020 | 0.0427 ± 0.0176 | 0.0283 ± 0.0065 | 0.0455 ± 0.0135 |
| | 10S† | 0.152 ± 0.068 | 0.209 ± 0.054 | 0.133 ± 0.030 | 0.0674 ± 0.0137 | 0.0361 ± 0.0091 | 0.0213 ± 0.0071 | 0.0136 ± 0.0045 |
| May 17 (flowering) | FL | 0.0432 ± 0.0071 | 0.0682 ± 0.0132 | 0.0446 ± 0.0055 | 0.0329 ± 0.0097 | 0.0369 ± 0.0128 | 0.0257 ± 0.0077 | 0.0347 ± 0.0071 |
| June 6 or 7 | F | 0.0094 ± 0.0037 | 0.0132 ± 0.0119 | 0.0076 ± 0.0051 | 0.0055 ± 0.0020 | 0.0075 ± 0.0176 | 0.0077 ± 0.0076 | 0.0088 ± 0.0081 |
| July 4 | L | 0.0014 ± 0.0005 | 0.0017 ± 0.0041 | 0.0013 ± 0.0010 | 0.0005 ± 0.0003 | 0.0009 ± 0.0008 | 0.0008 ± 0.0006 | 0.0010 ± 0.0004 |
| August 15 | F† | 0.0004 ± 0.0005 | 0.0005 ± 0.0002 | 0.0002 ± 0.0008 | 0.0000 ± 0.0003 | 0.0000 ± 0.0005 | 0.0000 ± 0.0005 | 0.0005 ± 0.0004 |

Different letters indicate significant differences among the treatments for each plant organ ($P < 0.05$, ANOVA followed by Tukey HSD multiple comparison test, or †Kruskal–Wallis test followed by Dunn’s multiple comparison test).

TB: terminal buds, FB: flower buds. See Table 1 for the other abbreviations.
Table 3. Pearson correlation analyses of the $x^2(13\text{C})$ in plant organs on April 12, 2018 (before bud break) with those on May 14 (pink), 17 (flourishing), June 6 or 7, and July 4.

|          | May 14 (pink) | May 17 (flourishing) | June 6 or 7 | July 4 |
|----------|---------------|----------------------|-------------|--------|
|          | L | CB | FL | F | F | L | CB | FL | F | F | L | CB | FL | F | F |
| Apr. 12 (before bud break) | | | | | | | | | | | | | | | |
| TB       | r | 0.37 | 0.34 | 0.12 | 0.08 | 0.23 | -0.06 | -0.03 |
|          | P | 0.054 | 0.074 | 0.55 | 0.68 | 0.24 | 0.75 | 0.89 |
| 1OB      | r | 0.21 | 0.32 | 0.41 | 0.78 | 0.56 | 0.41 | 0.34 |
|          | P | 0.28 | 0.092 | 0.029 | <0.001 | 0.0019 | 0.031 | 0.074 |
| 2OB      | r | 0.17 | 0.26 | 0.46 | 0.64 | 0.57 | 0.48 | 0.33 |
|          | P | 0.40 | 0.17 | 0.015 | <0.001 | 0.0014 | 0.011 | 0.084 |

$n = 28$

TB: terminal buds, FB: flower buds. See Table 1 for the other abbreviations.

Figure 5. Mean remaining $x^2(13\text{C})$ during the course of the growth season of 2018 after the pink stage (day 0, just before flowering, May 14) in the flower buds, flowers, and fruits of the trees labeled in the different periods of the growth season of 2017. Replicated values and average data are presented in Table 2 and Figure 3a. The $x^2(13\text{C})$ values were fitted with the least square methods with a nonlinear equation $F(t) = a \times \exp(-k \times d)$, where $a$ is the $x^2(13\text{C})$ at the pink stage and $k$ is the decay rate of $x^2(13\text{C})$. The values of $F(t)$ were fitted to $F(t) = 0.0672 \times \exp(-0.0617 \times d)$ labeled on May 31, 2017, $F(t) = 0.112 \times \exp(-0.0665 \times d)$ on June 14, $F(t) = 0.0750 \times \exp(-0.0697 \times d)$ on July 5, $F(t) = 0.0599 \times \exp(-0.0752 \times d)$ on July 20, $F(t) = 0.0676 \times \exp(-0.0722 \times d)$ on August 2, $F(t) = 0.0412 \times \exp(-0.0576 \times d)$ on August 16, and $F(t) = 0.0699 \times \exp(-0.0743 \times d)$ on October 11.

Use of photoassimilated C for initial development and growth in the following spring

The photoassimilated $13\text{C}$ from all periods of labeling was detected in new leaves, annual shoots and flower buds in the following early spring, showing that the photoassimilated C at any period of the previous growth season was incorporated into the new organs. This indicates that regardless of the assimilation period of the growth season, the photoassimilated C would be used for the initial development of new organs in the following spring. This also suggests that some of the photoassimilated C were stored in woody parts throughout the growth season, even during vigorous vegetative and reproductive growth, to be used in the following spring, which may explain why stored NSC in trees does not deplete over the year, and increases during the growth season (Furze et al. 2019, Hoch et al. 2003). However, the nature of the compounds that accumulate in the woody parts during late autumn remains unclear. Moreover, whether the compounds change according to differences in the assimilation periods is yet to be determined. According to Pollock (1986),
C compounds in cell wall structures, such as fructans, may degrade and retranslocate to new organs; however, evidence regarding the role of cell wall materials as reserves in fruit trees is lacking (Loescher et al. 1990).

Similar to the concentration of photoassimilated $^{13}$C in woody organs in late autumn, higher concentrations of photoassimilated $^{13}$C were observed in the new organs in the following early spring, in the trees labeled in mid-June. The photoassimilated $^{13}$C in the flower buds and flowers in the following season was positively correlated with that in the woody organs in late autumn, as well as in the old shoots before bud break. These results indicate that photoassimilated C during the period of higher vegetative growth contributes more toward C storage for use in the initial growth in the following spring, although it has been shown that NSC concentration decreases in woody organs during vigorous vegetative growth (Mochizuki and Hanada 1956, Loescher et al. 1990, Hoch et al. 2003, Tixier et al. 2019). Klein et al. (2016) reported that the breakdown of starch in the branchlets of temperate deciduous tree species is the major C source for bud break and leaf development. In the present study, the concentration of photoassimilated $^{13}$C in the new organs correlated with that in the shoots from the previous year, but not with the terminal buds. Hence, the stored C in woody organs, particularly in old shoots, could be used for the development of new organs. Additionally, roots are also considered important sources of stored NSC for use in the development of new organs in the following spring (Loescher et al. 1990, Dois et al. 2014); however, carbohydrate transportation from distant locations during winter and spring is not well understood (Tixier et al. 2019).

While stored C in woody parts is used for the development of new organs in the following early spring, it may not significantly contribute to subsequent growth. That is, the photoassimilated $^{13}$C in leaves and annual shoots during the pink stage did not correlate with that in the woody parts during late autumn, or old shoots before bud break. This is likely attributable to the differences in the onset time of the shoot and fruit growth in the trees, as the day of leaf expansion onset was 18 days earlier than that of flowering. Thus, the photoassimilated $^{13}$C in the leaves and annual shoots may be diluted by the translocation of current-year photoassimilates between leaf onset and flowering. Indeed, it has been reported that assimilation of CO$_2$ occurs in developing leaves in early stages of leaf expansion in deciduous tree species (Keel and Schädel 2010).

The photoassimilated $^{13}$C in flower buds, flowers, and fruits decreased exponentially during the following growth season. The photoassimilated $^{13}$C reduced largely in early June (16–17 days after full bloom) and reached a negligible level in mid-August, probably because of the dilution by photoassimilate translocation from newly developing leaves and a negligible amount of redistribution of stored C from other organs. The MRT of the remaining photoassimilated $^{13}$C in flower buds, flowers, and fruits was estimated in our study under the assumption that C does not reallocate to other plant organs once it is incorporated into the flowers and fruits. The MRT of the remaining photoassimilated $^{13}$C did not vary among the trees labeled in the different periods, while the flower buds of the trees had different concentrations of photoassimilated $^{13}$C. This suggested that the use of photoassimilated C in the flowers and fruits would not be influenced by the assimilation periods of stored C of the previous year.

Conclusions

The current study investigated seasonal patterns in C storage in young apple trees for use in the initial development and growth of newly developed organs in the following spring. Our results showed that the trees labeled in the period of vigorous vegetative growth (mid-June) accumulated more photoassimilated $^{13}$C in woody parts in late autumn, as well as in new organs during the following early spring. This supports the hypothesis that C storage, for use in the initial development and growth of new organs, increases in the woody organs during their period of higher growth rate. Our results also show that the use of stored C in the development and growth of new organs is limited to early spring. However, the trees were grown under non-stressed conditions and, therefore, the allocation patterns of photoassimilated C to storage organs for use in new organs under stressed conditions warrant further investigation.

Authors' Contributions

S.I. conceived and designed the research; Y.T. operated the growth and exposure chambers; S.I. and Y.T. performed the exposure experiments; S.I. performed the data collection, analyzed the data, and wrote the manuscript; both authors have read the manuscript and commented on it.

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Conflict of interest

None declared.
References

Artacho P, Bonomelli C (2016) Changes in fine-root production, phenology and spatial distribution in response to N application in irrigated sweet cherry trees. Tree Physiol 36:601–617.

Carbone MS, Czimczik CI, Keenan TF, Murakami PF, Pederson N, Schaberg PG, Xu X, Richardson AD (2013) Age, allocation and availability of nonstructural carbon in mature red maple trees. New Phytol 200:1145–1155.

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant ecology. Annu Rev Ecol Syst 33:507–559.

Dovis VL, Machado EC, Ribeiro RV, Magalhaes Filho JR, Marchiori PE, Sales CR (2014) Roots are important sources of carbohydrates during flowering and fruiting in ‘Valencia’ sweet orange trees with varying fruit load. Sci Hortic 174:87–95.

Furze ME, Huggett BA, Aubrecht DM, Stolz CD, Carbone MS, Richardson AD (2019) Whole-tree nonstructural carbohydrate storage and seasonal dynamics in five temperate species. New Phytol 221:1466–1477.

Gaudinski J, Badeck FW (2014) Opposite carbon isotope discrimination during dark respiration in leaves versus roots—a review. New Phytol 201:751–769.

Hansen P (1967a) 14C-studies on apple trees I. The effect of the fruit on the translocation and distribution of photosynthesis. Physiol Plant 20:382–391.

Hansen P (1967b) 14C-studies on apple trees III. The influence of season on storage and mobilization of labelled compounds. Physiol Plant 20:1103–1111.

Hoch G, Richter A, Körner C (2003) Non-structural carbon compounds in temperate forest trees. Plant Cell Environ 26:1067–1081.

Imada S, Tako Y, Tani T, Takaku Y, Hisamatsu S (2017) Translocation and distribution of photosynthetically assimilated 13C to ‘Tsuchar’ apple fruits. J Agric Meteorol 73:187–194.

Imada S, Tani T, Tako Y, Moriya Y, Hisamatsu S (2021) In situ experimental exposure of fruit-bearing shoots of apple trees to 13CO2 and construction of a dynamic transfer model of carbon. J Environ Radioact 233:106595.

Kandiah S (1979) Turnover of carbohydrates in relation to growth in apple trees. II. Distribution of 14C assimilates labelled in autumn, spring and summer. Ann Bot 44:185–195.

Keel SG, Schädel C (2010) Expanding leaves of mature deciduous forest trees rapidly become autotrophic. Tree Physiol 30:1253–1259.

Klein T, Vitasse Y, Hoch G (2016) Coordination between growth, phenology and carbon storage in three coexisting deciduous tree species in a temperate forest. Tree Physiol 36:847–855.

Kuptz D, Fleischmann F, Matyssek R, Grams TE (2011) Seasonal patterns of carbon allocation to respiratory pools in 60-yr-old deciduous (Fagus sylvatica) and evergreen (Picea abies) trees assessed via whole-tree stable carbon isotope labeling. New Phytol 191:160–172.

Lareau MJ (1988) Some growth regulator effects on the carbohydrate content of apple leaves and stems. Can J Plant Sci 68:229–232.

Loescher WH, McCamant T, Keller JD (1990) Carbohydrate reserves, translocation, and storage in woody plant roots. HortScience 25:274–281.

Meier U (1997) Growth stages of mono- and dicotyledonous plants. Blackwell Wissenschafts-Verlag, Berlin.

Mohchizuki T, Hanada S (1956) The seasonal changes of the constituents of young apple trees (part 1) total sugars and starch. Soil Sci Plant Nutr 2:115–122.

Moncrier C, Fortunato-Almeida A, Molina RV, Nebauer SG, Garcia-Luis A, Guardiola JL (2011) Relation of carbohydrate reserves with the forthcoming crop, flower formation and photosynthetic rate, in the alternate bearing ‘Salustiana’ sweet orange (Citrus sinensis L.). Sci Hortic 129:71–78.

Muhr J, Messier C, Delagrange S, Trumbore S, Xu X, Hartmann H (2016) How fresh is maple syrup? Sugar maple trees mobilize carbon stored several years previously during early springtime sap-ascent. New Phytol 209:1410–1416.

Munneke AE (1929) Hemicellulose as a storage carbohydrate in woody plants, with special reference to the apple. Plant Physiol 4:251–264.

Naschitz S, Naor A, Genish S, Wolf S, Goldschmidt EE (2010) Internal management of non-structural carbohydrate resources in apple leaves and branch wood under a broad range of sink and source manipulations. Tree Physiol 30:715–727.

O’Leary MH (1981) Carbon isotope fractionation in plants. Phytochemistry 20:553–567.

Palacios S, Hoch G, Sala A, Körner C, Millard P (2014) Does carbon storage limit tree growth? New Phytol 201:1096–1100.

Pallas B, Bly S, Ngao J, Martinez S, Clément-Vidal A, Kelner JJ, Costes E (2018) Growth and carbon balance are differently regulated by tree and shoot fructifying contexts: an integrative study on apple genotypes with contrasting bearing patterns. Tree Physiol 38:1395–1408.

Pollock CJ (1986) Fructans and the metabolism of sucrose in vascular plants. New Phytol 104:1–24.

Psarras G, Merwin IA, Lakso AN, Ray JA (2000) Root growth phenology, root longevity, and rhizosphere respiration of field grown ‘Mutsu’ apple trees on ‘Malling 9’ rootstock. J Am Soc Hort Sci 125:596–602.

Richardson AD, Carbone MS, Huggett BA, Furze ME, Czimczik CI, Walker JC, Xu X, Schaberg PG, Murakami P (2015) Distribution and mixing of old and new nonstructural carbon in two temperate trees. New Phytol 206:590–597.

Rosati A, Paolletti A, Al Harir A, Morelli A, Fiamini F (2018) Resource investments in reproductive growth proportionately limit investments in whole-tree vegetative growth in young olive trees with varying crop loads. Tree Physiol 38:1267–1277.

Ryan MG, Oren R, Waring RH (2018) Fruiting and sink competition. Tree Physiol 38:1261–1266.

Sala A, Woodruff DR, Meinerz FC (2012) Carbon dynamics in trees: feast or famine? Tree Physiol 32:764–775.

Tixier A, Gambetta GA, Godfrey J, Orozco J, Zwieniecki MA (2019) Non-structural carbohydrates in dormant woody perennials; the tale of winter survival and spring arrival. Front For Glob Change 2:18.

Wardlaw IF (1990) The control of carbon partitioning in plants. New Phytol 116:341–381.

Weber R, Gessler A, Hoch G (2019) High carbon storage in carbon-limited trees. New Phytol 222:171–182.

Wiley E, Helliker B (2012) A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. New Phytol 195:285–289.