



Whole-Tree Storage of Non-structural Carbohydrates in Apple and Pear Trees on Size-Controlling Rootstocks

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Abstract

Size-controlling rootstocks have been one of the major innovations facilitating high-efficiency fruit production; however, biological mechanisms responsible for their size-controlling effect remain unclear. In this study we investigated if apple and pear trees grafted on dwarfing and invigorating rootstocks differ in the size and dynamics of non-structural carbohydrate (NSC) storage pools. Seasonal dynamics in NSC concentrations were assessed in current-year shoots, coarse roots, trunks, and leaves. These measurements were then upscaled to whole-organ and whole-tree NSC pools and mutually compared. Because of the small variation in the relative biomass partitioning and generally similar organ-level NSC concentrations, the size of the NSC pools scaled tightly with the overall tree biomass with vigorous trees having greater absolute storage pools compared to dwarfing trees. The magnitudes of the seasonal fluctuation in NSC pools (i.e., November to May difference) were in the range from 0.55 to 3.93 kg per tree and 20 to 50 g per kg of tree's dry weight. In absolute terms, the seasonal fluctuations in NSC pools were higher in vigorously growing trees but in relative terms and also when scaled by the tree's biomass the differences between the low and high vigor trees became negligible, suggesting that the low and high vigor trees rely on their NSC reserves to a similar extent during their annual growth cycle. Thus, our results provide no support that the observed differences in growth vigor are driven by the availability of C-reserves.

Keywords Fruit trees · Reserves · Seasonal dynamics · Starch · Storage pools · Sugars · Wood

Introduction

The use of size-controlling rootstocks is one of the major innovations in modern horticulture contributing greatly to the increased efficiency of fruit production (Atkinson and Else 2003). While the exact biological mechanisms

responsible for the size-controlling effect remain unclear, one hypothesis asserts that the dwarfing phenomena of some rootstocks might be related to their lower ability to store and/or mobilize carbohydrates (Basile and DeJong 2018). However, this potential mechanism of vigor control has not been extensively studied and is largely based on indirect evidence related to the differential growth seasonality, root anatomy, and stomatal conductance of dwarfing compared to invigorating rootstocks (Basile and DeJong 2018).

Non-structural carbohydrates (NSC) are the main carbon (C) storage resources in trees and their concentration in organs or tissues is often considered as an indicator of C balance (Körner 2003; Sala et al. 2012; Hoch 2015). Direct measurements of NSC concentrations in fruit trees on size-controlling rootstocks are scarce and the observed patterns are variable. Weibel et al. (2008) measured dormant season carbohydrate reserves in peach trees on six different size-controlling rootstocks and found the highest concentrations in the most vigorous rootstocks. In contrast, Foster et al. (2017) studied two dwarfing and one vigorous apple rootstocks and found that the dwarfing rootstocks accumulated

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large amounts of starch, whereas their concentrations of soluble sugars were very low. Based on these observations they suggested that the dwarfing rootstocks behave as “super accumulators” that hold high starch reserves at the expense of vegetative growth. Olmstead et al. (2010) measured carbohydrate profiles in the graft union and adjacent rootstock and scion tissue in sweet cherry. They found that the starch concentrations were not consistently lower or higher in dwarfed vs. vigorous trees, but rather showed distinct seasonal patterns, leading to the suggestion that the NSC reserve accumulation and mobilization were perturbed by the higher resistance to NSC translocation at the graft union.

Measurements of NSC concentrations in plant organs and tissues provide a useful but incomplete assessment of carbohydrate reserves because the magnitude of NSC reserves depends on tree size and biomass partitioning between the different organs (Furze et al. 2019; Schoonmaker et al. 2021; Fermaniuk et al. 2021). Therefore, studies scaling-up NSC concentrations to the overall size of NSC pools allow for more meaningful quantitative considerations with respect to the tree’s productivity (Bustan et al. 2011), ecological strategy (Barbaroux et al. 2003; Schoonmaker et al. 2021), or adaptation to climate (Fermaniuk et al. 2021). In addition, these studies help to assess the relative importance of different organs for storage. While roots are often believed to be the primary site for storage in trees (Loescher et al. 1990), the measurements of the whole-plant NSC pools highlighted the importance of above-ground stem and branch sapwood for C storage, mainly because of their large fraction within the total tree biomass (Bustan et al. 2011; Furze et al. 2019; Fermaniuk et al. 2021).

The whole-tree NSC pool and its dynamics are particularly important for understanding growth of dwarfed vs. invigorated trees because of their implicit difference in size. In addition, trees on size-controlling rootstocks may also differ in the relative biomass partitioning among their various organs. For instance, high relative proportions of roots, which are likely to contain high concentrations of carbohydrates, can make for a substantial contribution to the overall NSC budget. Similarly, greater allocation of biomass to leaves might be associated with higher photosynthetic gain which is the ultimate source of NSC. As in case of the measurements of NSC concentrations, biomass partitioning in trees on size-controlling rootstocks is currently poorly documented especially for large field-grown trees. Greater relative biomass allocation to roots has been found in peach trees on dwarfing rootstocks, likely as a compensation for the greater hydraulic resistance of their roots (Solari et al. 2006). In contrast, Tworokski et al. (2016) reported greater relative biomass allocation to roots compared to leaves in vigorously growing apple trees grafted on MM.111 rootstock compared to the trees on semi-dwarfing M.9 rootstock. Vigorous apple trees on MM.106 rootstock also had fewer

leaves per unit sapwood compared to trees on semi-dwarfing M.9 rootstock. Higher number of leaves per sapwood area resulted in a better water supply-to-water loss ratio in semi-dwarfing trees (Cohen and Naor 2002) but potentially also in a lower photosynthetic gain of those trees. Taken together, the differences in the overall tree size, differential biomass allocation, and NSC concentrations of individual organs or tissues all combine and affect the overall size of the NSC storage pool.

Another issue is if the entire NSC pool of a tree can indeed be remobilized and used as C source for metabolism and growth or if a proportion of NSC remain unused and become sequestered or utilized only in the case of an extreme event, such as severe defoliation or drought (Landhäuser and Lieffers 2012; Carbone et al. 2013; Richardson et al. 2015). Also, the adequacy of storage capacity needs to be evaluated relative to the overall growth. It is possible that dwarfing trees have smaller storage pools, but as they grow less, this capacity might be sufficient (Basile and DeJong 2018). To resolve these issues, quantifying seasonal fluctuations in the whole-tree NSC pool would be insightful, but, to the best of our knowledge, has not been done so far with respect to rootstock-induced control of growth vigor.

Thus, the aim of this study was to evaluate if apple trees on dwarfing rootstocks have lower NSC reserves and/or reduced capacity to mobilize NSC compared to trees on invigorating rootstocks (Basile and DeJong 2018). We address this question in terms of both organ-level NSC concentration as well as the size of the whole-tree NSC pool and its seasonal fluctuation.

Materials and Methods

Plant Material

All measurements were done on 29-year-old trees of *Malus × domestica* Borkh. var. ‘Jonagold’ and 27-year-old trees of *Pyrus communis* L. var. ‘Williams’ grafted onto rootstocks that induced either low or high growth vigor (Table 1). The low vigor rootstocks were J-TE-G for apple trees and S1 for pear trees. The high vigor rootstocks were J-TE-H for apple trees and pear seedling (PS) for pear trees. The trees were selected from a larger rootstock field trial so that they provided the greatest differences in growth vigor in the two studied species (Jupa et al. 2021). The trees were grown on the experimental fields of the Research and Breeding Institute of Pomology in Holovousy, the Czech Republic (50.37N, 15.57E; 283 m.a.s.l.). The site experiences a temperate climate with a mean annual temperature of 8.4 °C and a mean annual precipitation of 664 mm. The site is on a loamy brown soil (22.2% clay, 69.1% silt, and 8.7% sand) with neutral pH and a medium fertility. The trees

Table 1 Growth and yield characteristics of apple var. ‘Jonagold’ (J) and pear var. ‘Williams’ (W) grafted onto rootstocks inducing low (J-TE-G, S1) or high (J-TE-H, pear seedling) growth vigor

Scion	Rootstock	Abbrev	TCSA (cm ²)	Shoot length (cm)	Fruit yield (kg/tree)
Apple var. ‘Jonagold’	Apple J-TE-G	J-low	113.9 ± 7.2	41.4 ± 2.3	41.2 ± 5.4
Apple var. ‘Jonagold’	Apple J-TE-H	J-high	298.8 ± 10.6	62.2 ± 3.4	83.8 ± 18.3
Pear var. ‘Williams’	Quince S1	W-low	159.5 ± 13.2	41.3 ± 2.1	10.9 ± 3
Pear var. ‘Williams’	Pear seedling	W-high	277.4 ± 14.3	58.3 ± 3.1	11.3 ± 5.4

Trunk cross-sectional area (TCSA) and shoot length data were taken from Jupa et al. (2021)

Means ± SD ($n=3$) are reported

were planted with a spacing of 4.5 × 2.3 m and 5.0 × 3.0 m for apple and pear trees, respectively. Trees were trained as freely growing hedgerows with a short stem height with the tip of the central leader being removed in the fifth year. Supplemental fertilization and pest management practices were applied according to local recommendations for commercial orchards. The weed control in 1.5-m-wide strips was maintained by herbicides and grass grown in inter-rows was periodically removed. No irrigation was supplied and no hand or chemical thinning of the fruits was conducted. For the measurements, three healthy individuals for each scion/rootstock combination were used.

Collection of Samples for NSC and Carbon Isotope Analyses

Sample for the NSC analyses were taken from coarse woody roots, trunks, 1-year-old shoots and leaves. The coarse woody roots (5–10 mm in diameter, 10–20 cm in length) were excavated from the soil depth of 15–50 cm and at the distance of 50 cm from the root collar. The trunk cores were extracted at the height of 30–50 cm, which was well above the graft union and well below the insertion of the lowest scaffold branches. A sharp increment borer (Mora Coretax, Switzerland) was used for the coring. The samples taken consisted of the outermost 3 cm of sapwood and the bark. The repeated coring throughout the season was conducted in a spiral fashion around the trunk to minimize the potential influence of the wounding caused by the previous sampling. The 1-year-old proleptic shoots (10–15 mm in diameter and 15–30 cm in length) were cut from a sun-lit part of the crown at the height of 1.5 m and the leaves were stripped from the woody axis. The sampling took place at the following five dates in 2019: 20th March (before bud break), 16th April (at bud break), 17th May (at full bloom), 19th July (at peak summer, approx. two weeks after the termination of shoot extension growth), and 4th November (beginning of winter dormancy). At each date, we collected six roots, six shoots (i.e., two specimens from each individual tree), and one trunk core per each tree. Immediately after the collection, the samples were transported to the laboratory where they were shortly microwaved

(30 s at 600 W) to deactivate the NSC-modifying enzymes according to Popp et al. (1996). The samples were then oven dried at 80 °C for 5 days, homogenized using a centrifugal grinding mill (ZM 100, Retsch, Haan, Germany) and sent to the Plant Ecophysiology Laboratory at the University of Basel, Switzerland, for NSC analyses and to Stable Isotope Laboratory at the Crop Research Institute, the Czech Republic for the analysis of carbon isotopes.

NSC Analyses

Concentrations of NSC were analyzed using the enzymatic-photometric method described in Landhäusser et al. (2018). Briefly, low-molecular weight sugars were extracted from the plant powder with 80% ethanol at 90 °C for 10 min, the supernatant was collected, the pellet was washed three more times with ethanol, and the supernatants were mixed. After evaporation of the ethanol and resuspension of the extracts in deionized water, the total amount of glucose was determined photometrically after the enzymatic conversion of fructose and sucrose to glucose in a multiplate photometer (HR 700, Hamilton, Reno, NE, USA) at 340 nm by converting glucose-6-P to gluconate-6-P. To break down the starch in the remaining pellet, the pellet was resuspended in deionized water and first treated with α -amylase (from *Bacillus licheniformis*) for two hours at 85 °C. After centrifugation of the samples, an aliquot of the supernatant was treated with amyloglucosidase (from *Aspergillus niger*) at 55 °C for two hours for complete conversion of the starch to glucose. The total amount of glucose, corresponding to the initial amount of starch, was determined photometrically as given above. To control the reproducibility of the extraction, standard plant powder (Orchard leaves, Leco, St. Joseph, MI, USA) and pure starch, glucose, fructose, and sucrose solutions were included in the analysis. Glucose standard (1 mg mL⁻¹) was used to calculate the glucose concentrations of the extracts. All chemicals and enzymes were purchased from Sigma-Aldrich, St. Louis, MO, USA. NSC concentrations were expressed as % dry mass of starch and the

three quantitatively most important soluble sugars (glucose, fructose, and sucrose).

Carbon Isotope Analyses

Approximately 1 mg of powdered bulk sample of 1-year-old shoots and leaves was weighted into tin capsules and the isotopic ratio was measured using an elemental analyzer (Vario PYRO Cube, Elementar, Germany) coupled to an isotope mass spectrometer (Isoprime precision, Elementar, UK) at Crop Research Institute, Prague. The carbon isotopic composition ($\delta^{13}\text{C}$) was expressed relative to the international standard (Vienna Pee Dee Belemnite, VPDB) according to the following formula $\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{VPDB}} - 1)$, where R is the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample or the VPDB standard.

Tree Harvesting and Biomass Estimation

The whole-tree biomass and the biomass of different tree parts including fruits, leaves, 1-year-old shoots, scaffold branches, trunk, stump, and coarse roots > 3 mm in diameter were assessed using destructive sampling at the end of the growing season. Fruits were manually picked, the leaves were stripped and the woody portions of the current-year shoots were clipped with a pair of hand pruners. Subsequently, the trees were felled with a chain saw and separated into trunks and scaffold branches. Tree stumps, including the root collar, the graft union, and the lowest 10 cm of the trunk, were then excavated with a mechanical digging machine. In place of the excavated stumps, we dug pits with the diameter of 2 m and a depth of 0.5 m (*M. × domestica*) and 0.8 m (*P. communis*) and sieved the soil to extract the coarse roots (> 3 mm in diameter). The dimensions of the pits were selected to represent the volume with the majority of root biomass. The fresh weight of all harvested tree parts was measured immediately using a portable scale with the accuracy of 0.01 kg. The measured fresh weights were converted to the dry weights (DW) using the conversion factor determined on smaller sub-samples. At least 20 sub-samples were measured for each tissue type.

Estimation of Whole-organ and Whole-tree NSC Pools

We used the dry weight biomass estimations and the measured NSC concentrations to calculate the size of NSC pools in different tree organs and in the whole trees. Considering the observed seasonal dynamics of NSC concentrations, the calculations were made for two contrasting periods of the growing season. First, we used May NSC data to characterize the situation when NSC reserves are at their minimums during the spring flush. This situation was contrasted with the conditions in November at the onset of winter dormancy, when the NSC

reserves reached their maximal concentrations. The NSC concentrations and biomass DW for roots, shoots, and leaves were directly paired and multiplied, except that in May we reduced the biomass of shoots and leaves to 25% because the shoot extension growth and leaf unfolding were just at their beginning. For tree parts that were not directly monitored for NSC concentrations (e.g., scaffold branches, stumps) the NSC concentrations were calculated based on the measurements of the adjacent parts. Thus, we assumed that the NSC concentration of the stump was an average between the concentrations measured in the trunk and the roots. Similarly, the NSC concentrations of older scaffold branches were estimated as an average of the trunk and the current-year shoots. Furthermore, we assumed that heartwood contributes little to NSC storage pool due to the generally low NSC concentrations (Hoch et al. 2003). Therefore, we reduced the biomass of trunks, stumps, and scaffold branches by multiplying them with a factor of 0.7. The average sapwood depth in our trees was between 2 and 5 cm, which approximately correspond to a 70% of the total wood cross-sectional area, hence the factor 0.7. To obtain the estimation of the NSC pool for the whole tree, we summed the organ-level estimates of NSC contents.

Statistical Analyses

Differences in biomass ratios, sizes of the NSC pools, and seasonal fluctuation in NSC pools were analyzed with one-way ANOVA with scion/rootstock combination as the fixed factor. The means were then separated using Tukey HSD test. Differences in starch, soluble sugars, total NSC concentrations, and $\delta^{13}\text{C}$ were analyzed separately for each species and tissue type using a linear mixed-effects model. The models were fitted in R (R Development Core Team 2010) using lmer function from lme4 package (Bates et al. 2015). Homogeneity of variance and normality of the data were evaluated by visual inspection of model residuals. In these models, rootstock and sampling date (incl. the interaction term) were implemented as fixed factors and tree_id was included as random factor to account for the repeated sampling. The significance of the fixed effect factors was evaluated using F test with Satterthwaite approximation for the degrees of freedom implemented in the lmerTest package (Kuznetsova et al. 2017). For significant fixed effects, differences between pairs of means were evaluated using Tukey-adjusted pairwise comparisons. The results were considered significant at $\alpha = 0.05$.

Results

NSC Concentrations and Their Seasonal Dynamics

In both species, the NSC concentrations in all studied tissue types varied seasonally with the lowest values observed

during the May or July sampling and the highest values being reached in November (Figs. 1, 2). The trunk cores had the lowest total NSC concentrations of all measured organs, except for the leaves of pear trees that had NSC concentrations lower than 3% (d.w.) throughout the entire season. Despite broadly similar seasonal dynamics of NSC concentrations, there were some significant differences between the trees of contrasting vigor in both species. For instance, the roots of low vigor apple trees had significantly lower starch concentrations, but higher concentrations of soluble sugars early in the season than vigorously growing apple trees (Fig. 1a, e). At some sampling dates, the low vigor apple trees also showed significantly lower starch concentrations than high vigor apple trees in their shoots and leaves (Fig. 1c, d). In pear trees, low vigor trees had significantly higher starch and total NSC concentrations in trunks throughout the whole sampling period compared to vigorously growing trees (Fig. 2 b, j). Starch and total NSC concentrations were also higher in the roots of low vigor pear trees early in the growing season, but the opposite

pattern was observed in November (Fig. 2 a, i). In November, low vigor pear trees also had significantly higher NSC concentrations in shoots than their high vigor counterparts (Fig. 2 c, k).

Tree Biomass Components

The difference in overall growth vigor for trees on size-controlling rootstocks was greater in apple trees than pear trees (Table 1, 2). In apple trees, trunk cross-sectional area (TCSA) of low vigor trees was 62% lower than TCSA in high vigor trees, while the difference was only 43% in pear trees (Table 1). Similarly, low vigor apple trees had about half the fruit yield in comparison with the high vigor rootstock-scion combination, while the difference in yield was minimal for pear trees (Table 1). The estimated mean total tree biomass (excluding fruits) was 27.7 kg in low vigor, 82.5 kg in high vigor apple trees, 39.6 kg in low vigor, and 78.9 kg in high vigor pear trees (Table 2). Thus, the low vigor trees had about 66% (in the case of apple trees) and 50% (in the case of pear trees) less biomass

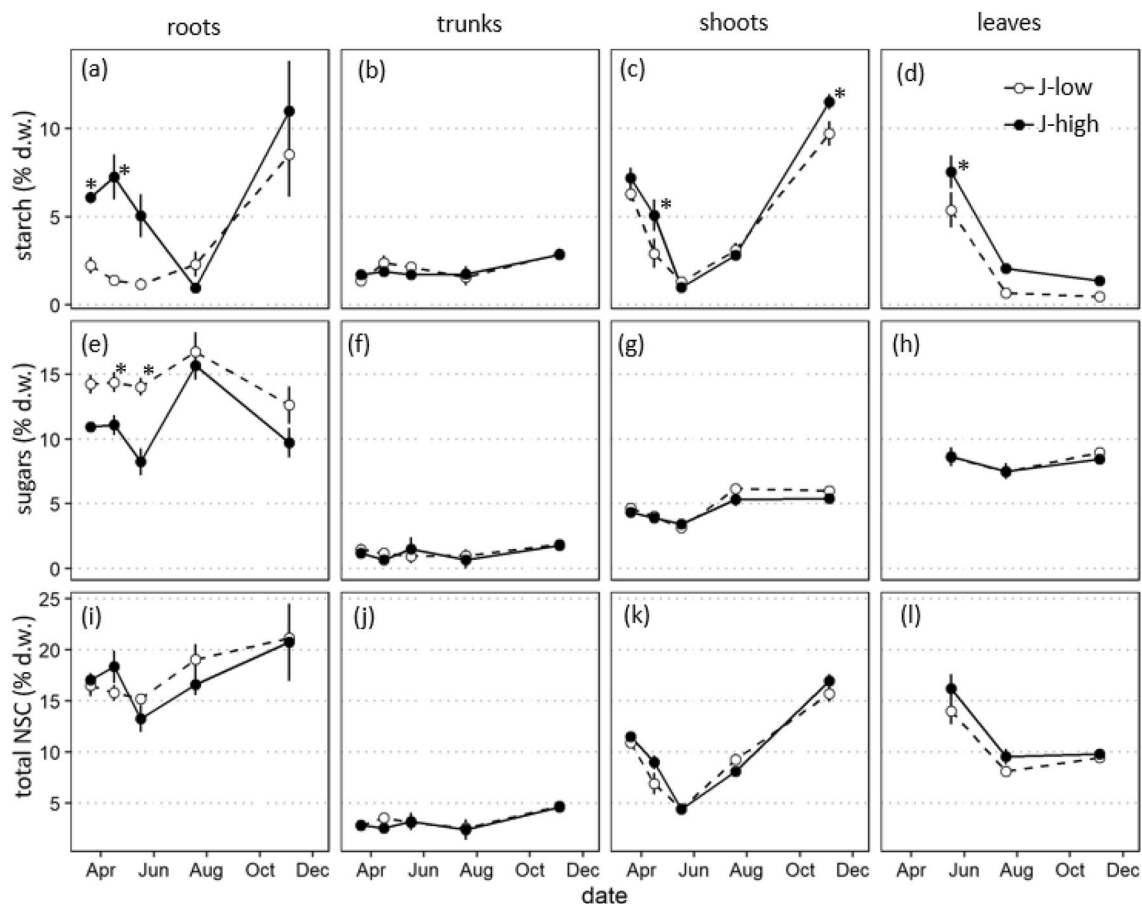


Fig. 1 Seasonal concentrations of starch, soluble sugars, and total NSC measured in coarse roots, trunk cores, 1-year-old shoots, and leaves of apple tree var. ‘Jonagold’ (J) grafted on rootstocks inducing low or high growth vigor. The data are means \pm SE ($n=3$ for trunk

cores, $n=6$ for other tree parts). The asterisk indicates significant differences between the rootstock means within the sampling date as evaluated with the Tukey-adjusted pairwise comparison

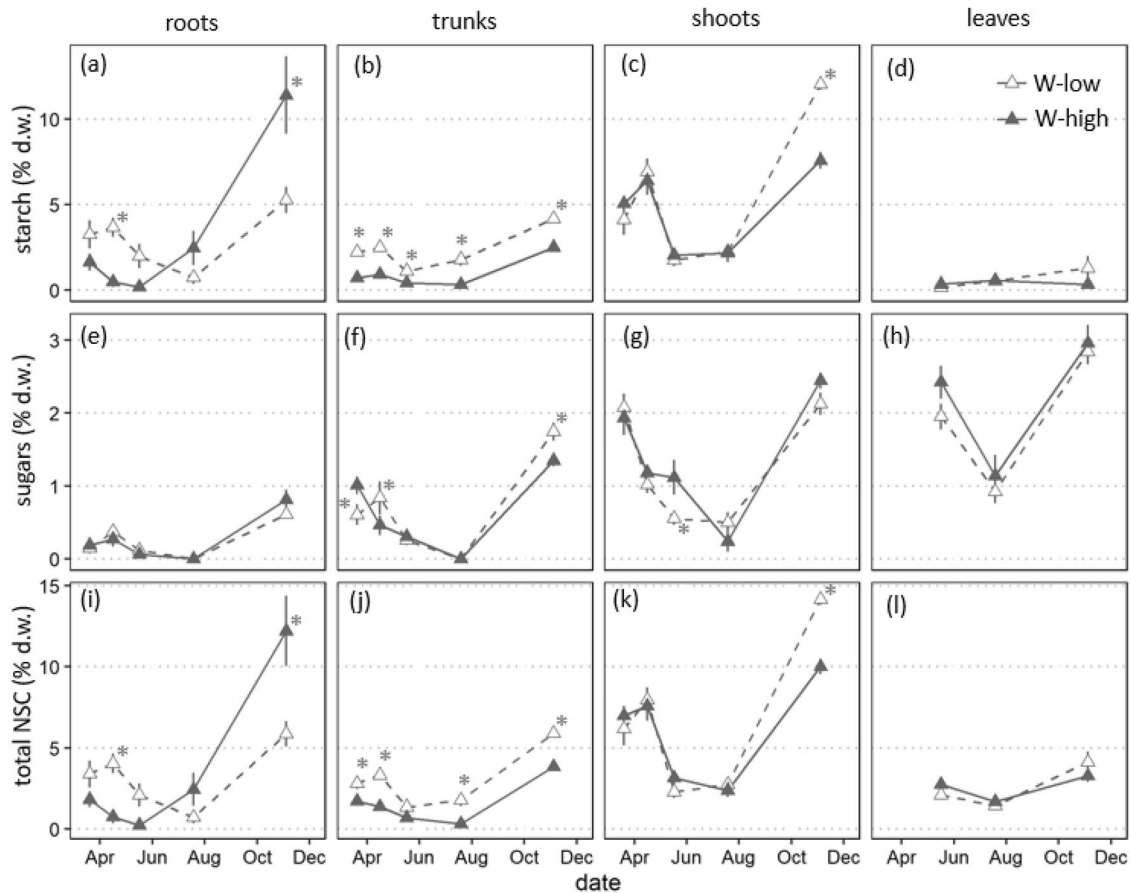


Fig. 2 Seasonal concentrations of starch, soluble sugars, and total NSC measured in woody roots, trunk cores, 1-year-old shoots, and leaves of pear var. ‘Williams’ (W) grafted on rootstocks inducing low or high growth vigor. The data are means \pm SE ($n=3$ for trunk cores,

$n=6$ for other tree parts). The asterisk indicates significant differences between the rootstock means within the sampling date as evaluated with the Tukey-adjusted pairwise comparison

Table 2 Estimated biomass dry weights (kg tree^{-1}) of various tree parts in apple tree var. ‘Jonagold’ (J) and pear tree var. ‘Williams’ (W) grafted onto rootstocks inducing low or high growth vigor

Organ	J-low	J-high	W-low	W-high
Roots	3.1 ± 0.7 (9.5%)	4.7 ± 0.9 (5%)	3.8 ± 0.1 (9.2%)	7.1 ± 1.1 (9.3%)
Stump	7.4 ± 0.8 (22.6%)	22.1 ± 5.3 (23.6%)	7.4 ± 0.6 (18.2%)	21 ± 5.7 (26%)
Trunk	9.6 ± 1.8 (29.2%)	37.6 ± 2 (40.7%)	12.7 ± 2.8 (31.2%)	24.8 ± 5.1 (31.1%)
Scaffold branches	6.1 ± 0.5 (18.7%)	12.7 ± 2.7 (13.7%)	11.9 ± 2.4 (29.1%)	17.9 ± 4.5 (22.1%)
1-year-old shoots	0.3 ± 0.1 (0.9%)	1.6 ± 0.5 (1.7%)	1.6 ± 0.4 (3.8%)	4.1 ± 2.2 (4.8%)
Leaves	1.3 ± 0.3 (3.9%)	3.8 ± 0.5 (4.1%)	2.2 ± 0.3 (5.3%)	4 ± 1.3 (4.9%)
Fruits	5 ± 0.7 (15.3%)	10.1 ± 2.2 (11.1%)	1.3 ± 0.4 (3.2%)	1.4 ± 0.7 (1.8%)
Total	32.7 ± 3 (100%)	92.6 ± 8.6 (100%)	40.9 ± 2.9 (100%)	80.3 ± 15.9 (100%)

The values in brackets refer to the percent fraction of a given tree part relative to the total tree biomass
Means \pm SD ($n=3$) are reported

than their high vigor counterparts. The majority of tree biomass consisted of older woody parts (trunk, scaffold branches, and stump). All together these tissues comprised 72–88% of total standing tree biomass. 1-year-old shoots and leaves represented between 4.8 and 9.7% of the total tree biomass. Across all trees, leaf biomass, and correspondingly the estimated leaf

area (LA), scaled tightly with the TCSA ($r=0.954$, $P < 10^{-4}$), resulting in a similar LA-to-TCSA ratio (Fig. 3a). Root biomass represented less than 10% of the total tree biomass. However, the fraction of below-ground biomass increased, if parts of the stump were considered as below-ground component. When half of the stump weight was attributed to the

below-ground biomass, while the second half was considered above-ground, estimated above- to below-ground biomass ratios ranged between 3:1 and 5:1 (Fig. 3b). Fruits accounted for 15.3 and 11.1% of total biomass in low and high vigor apple trees, while this fraction was much lower (between 1.8 and 3.2%) in pear trees. The ratios of reproductive to vegetative biomass were not significantly different between low vs. high vigor trees, but this ratio was higher for apple than pear trees (Fig. 3c). In contrast, pear trees had greater biomass of 1-year-old shoots than apple trees (4% vs 1.5% in pear vs apple trees, respectively).

Estimated NSC Storage Pools

The whole-tree NSC pools estimates excluding fruits were 1.89 kg for low vigor apple, 4.77 kg for high vigor apple, 0.64 kg for low vigor pear, and 0.75 kg for high vigor pear in May. For November, the whole-tree NSC pool estimates were 2.44 kg for low vigor apple, 6.54 kg for high vigor apple, 2.47 kg for low vigor pear, and 4.68 kg for high vigor pear (Table 3, Fig. 4a). Thus, in absolute terms high vigor apple trees had the greatest NSC pool size from all measured trees, while the low vigor pear trees had the smallest pool size. The seasonal fluctuations expressed as the absolute difference between the November and May pool size was highest (3.93 kg) in high vigor pear trees and lowest (0.55 kg) in low vigor apple trees (Fig. 4b). The difference in the seasonal fluctuation between trees on low vs. high vigor rootstocks was significant ($P < 0.05$) in pear trees, while the difference was even larger, but due to higher variation not statistically significant in apple trees. When expressed as a fraction of the November (i.e., maximal) pool size, the magnitude of the seasonal fluctuation accounted for 22.5% in low vigor apple, 27.1% in high vigor apple, 74.1% in low vigor pear, and 84% in high vigor pear. Finally, when scaled to the tree's biomass, the fluctuation was higher in pear trees than apple trees with no significant difference between low and high vigor trees (Fig. 4c).

Variation in $\delta^{13}\text{C}$

The $\delta^{13}\text{C}$ values of 1-year-old shoots and leaves varied between -26 and -30‰ . In apple trees, no significant differences in $\delta^{13}\text{C}$ were found between low and high vigor trees, whereas in pear trees low vigor trees had significantly higher $\delta^{13}\text{C}$ values in shoots and leaves in at least some sampling dates (Fig. 5).

Discussion

The main aim of our study was to compare the size of NSC pools and NSC dynamics in trees grafted on low vs. high vigor rootstocks (Table 1), while considering differences in

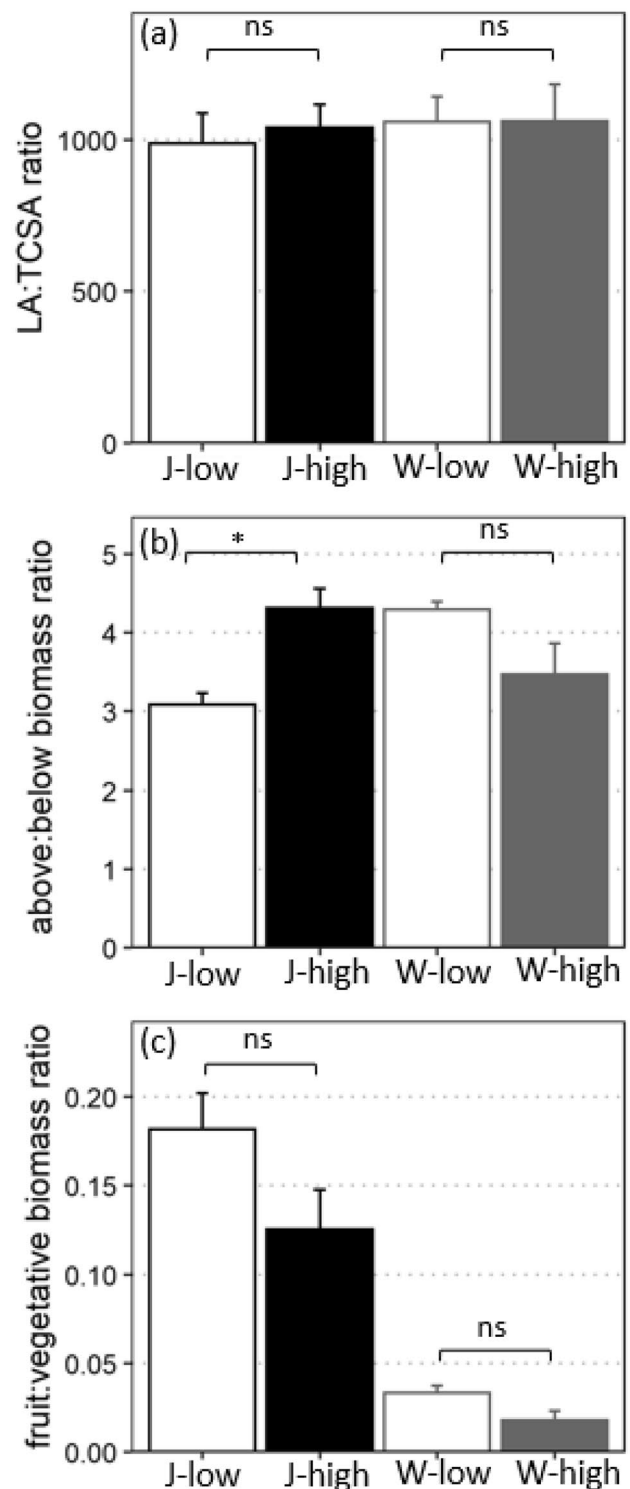


Fig. 3 **a** Ratio between leaf area (LA) and trunk cross-sectional area (TCSA), **b** ratio between above- and below-ground biomass, **c** ratio between reproductive (i.e., fruit) and vegetative biomass in apple tree var. ‘Jonagold’ (J) and pear tree var. ‘Williams’ (W) grafted onto rootstocks inducing low or high growth vigor. The bars represent means \pm SE ($n=3$). The results of Tukey-adjusted pairwise comparisons of means are labeled either with asterisk (significant differences at $\alpha=0.05$) or “ns” (non-significant)

Table 3 Estimated NSC pool size (kg) in May (during spring flush) and November (the onset of winter dormancy) of various tree parts in apple tree var. ‘Jonagold’ (J) and pear tree var. ‘Williams’ (W) grafted onto rootstocks inducing low or high growth vigor

NSC pool in May (kg)				
Organ	J-low	J-high	W-low	W-high
Roots	0.48 ± 0.11 (25.1%)	0.63 ± 0.2 (13.3%)	0.08 ± 0.06 (12.4%)	0.02 ± 0.01 (2.4%)
Stump	0.82 ± 0.1 (43.5%)	2.24 ± 0.91 (46.9%)	0.13 ± 0.08 (20.8%)	0.09 ± 0.03 (11.3%)
Trunk	0.31 ± 0.13 (16.1%)	1.23 ± 0.61 (25.8%)	0.18 ± 0.08 (28%)	0.18 ± 0.09 (24.2%)
Scaffold branch	0.24 ± 0.03 (12.7%)	0.51 ± 0.07 (10.6%)	0.23 ± 0.08 (35.5%)	0.41 ± 0.07 (54.6%)
1-year-old shoots	0.004 ± 0.002 (0.2%)	0.02 ± 0.002 (0.4%)	0.009 ± 0.002 (1.4%)	0.03 ± 0.01 (4.1%)
Leaves	0.05 ± 0.02 (2.4%)	0.15 ± 0.01 (3.1%)	0.012 ± 0.004 (1.9%)	0.03 ± 0.004 (3.4%)
Total	1.89 ± 0.29 (100%)	4.77 ± 1.69 (100%)	0.64 ± 0.28 (100%)	0.75 ± 0.19 (100%)
NSC pool in November (kg)				
Organ	J-low	J-high	W-low	W-high
Roots	0.58 ± 0.23 (23.9%)	0.94 ± 0.22 (14.4%)	0.25 ± 0.06 (10.1%)	0.87 ± 0.06 (18.6%)
Stump	0.81 ± 0.24 (33.2%)	2.5 ± 0.54 (38.2%)	0.34 ± 0.11 (13.9%)	1.56 ± 0.43 (33.3%)
Trunk	0.32 ± 0.05 (12.9%)	1.22 ± 0.07 (18.6%)	0.53 ± 0.12 (21.4%)	0.66 ± 0.1 (14.2%)
Scaffold branch	0.57 ± 0.09 (23.2%)	1.24 ± 0.23 (18.9%)	1.04 ± 0.24 (42.1%)	1.05 ± 0.28 (22.5%)
1-year-old shoots	0.05 ± 0.01 (1.9%)	0.27 ± 0.07 (4.1%)	0.22 ± 0.07 (9%)	0.41 ± 0.24 (8.8%)
Leaves	0.12 ± 0.03 (4.9%)	0.37 ± 0.06 (5.7%)	0.09 ± 0.03 (3.5%)	0.12 ± 0.02 (2.6%)
Total	2.44 ± 0.57 (100%)	6.54 ± 0.83 (100%)	2.47 ± 0.38 (100%)	4.68 ± 0.94 (100%)

The values in brackets refer to the percent fraction of a given tree part relative to the total tree pool size.

Means ± SD ($n=3$) are reported.

tree’s overall size and biomass allocation into different tree parts. Because of the small variation in the relative biomass partitioning (Table 2) and generally similar organ-level NSC concentrations (Fig. 1, 2), we found that the size of the NSC pools scaled tightly with the overall plant biomass with vigorous trees having greater absolute storage pools compared to low vigor trees (Fig. 4a). The magnitudes of the seasonal fluctuation (i.e., the difference in NSC pool size between November and May) in the range from 0.55 to 3.93 kg were similar as those estimated for forest trees in the boreal zone (Schoonmaker et al. 2021; Fermaniuk et al. 2021). In the absolute terms, the seasonal fluctuation was higher in vigorously growing trees. However, in relative terms and also when scaled by the tree’s biomass, the differences between the low and high vigor trees became negligible (Fig. 4c). This finding suggests that the low and high vigor trees rely on their NSC reserves to a similar extent during their annual growth cycle. In other words, the size of NSC storage pool and the NSC demands vary in accordance with each other in low and high vigor trees, which indicates that a mechanism other than the ability to accumulate or utilize NSC is driving growth differences observed in these rootstocks.

The relative magnitude of seasonal fluctuation of NSCs differed between apple and pear trees with apple trees showing smaller relative fluctuation. This was because the apple trees maintained high NSC pool in May (Fig. 4a), mainly due to high soluble sugar concentrations in their roots (Fig. 1e).

The differences in the root NSC pool size between the two species could originate from their different root morphology and anatomy (Jupa et al. 2021). Apple trees have relatively shallow root systems and may need high concentrations of soluble sugars to adjust osmotically to frequently drying upper soil horizon (Davies and Lakso 1979). Also, apple tree roots have thick bark and abundant ray parenchyma accentuating their role in storage (Jupa et al. 2021). In contrast, pear trees have deep sinker roots, thin bark, and high vessel density on the root cross-sections, which has been related to their high water-conducting capacity (Jupa et al. 2021). Based on our estimates, roots and stump accounted for about a half of the total tree NSC pool in apple trees and high vigor pear trees (Table 3). Our estimates also indicate that the stump that consisted of the root crown, graft union, and the lowest portion of the trunk could be an important site for storage because of its large biomass and presumably high NSC concentrations at least in the root structures. On the other hand, the graft union and the associated outgrowth tissue can represent a severe restriction for the long-distance xylem and phloem transport (Soumelidou et al. 1994; Olmstead et al. 2010).

Based on our data, trunk and scaffold branches also harbor a substantial portion of the NSC reserves (Table 2), particularly in low vigor pear trees. The importance of above-ground woody tissue for NSC storage, which originates mainly from its large total biomass, agrees well with the previous results on forest trees (Hoch et al.

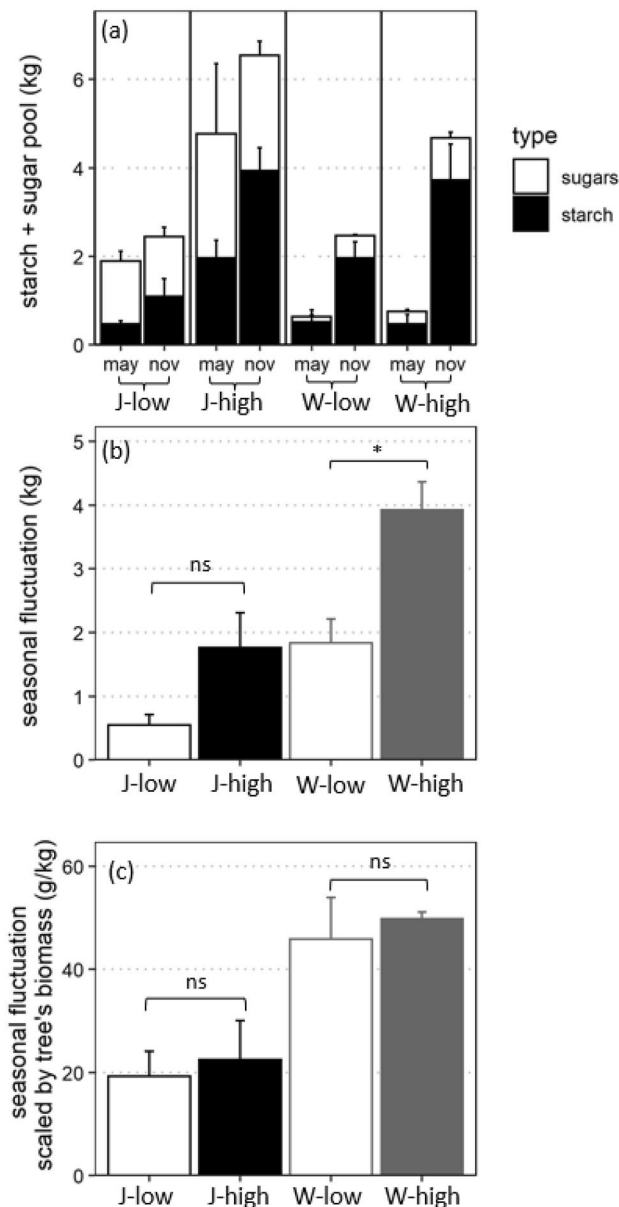


Fig. 4 **a** Mean estimated pool sizes of starch and soluble sugars in May (during spring flush) and November (during the onset of winter dormancy) in the whole trees of apple tree var. ‘Jonagold’ (J) and pear tree var. ‘Williams’ (W) grafted onto rootstocks inducing low or high growth vigor. **b** Difference in the estimated pool sizes between November and May in the four studied scion/rootstock combinations. **c** Difference in the estimated pool sizes scaled by the tree’s biomass. The data are means \pm SE ($n=3$). The results of Tukey-adjusted pairwise comparisons of means are labeled either with asterisk (significant differences at $\alpha=0.05$) or “ns” (non-significant)

2003; Würth et al. 2005; Furze et al. 2019). In contrast to other measured organs, trunk tissue NSC concentrations were low (Fig. 1, 2) and not very dynamic suggesting that trunk sapwood is large but not a very active NSC pool (Carbone et al. 2013). Interestingly, the high vigor

pear trees had consistently lower starch concentrations in their trunks compared to low vigor trees (Fig. 2b). This finding could indicate that NSC reserves were used to fuel rapid cambial growth or to support stem suckering which was quite intense in high vigor pear trees. Compared to the perennial woody organs, the biomass of current-year shoots, leaves, and fruit was relatively small, accounting for 11.5 to 20.1% of the total biomass (Table 2). The ratio of reproductive (i.e., fruit) to vegetative above-ground biomass was higher for apple trees than pear trees and did not differ significantly between low vs. high vigor trees (Fig. 3c), although the trend for higher yield efficiency in low vigor trees was apparent and consistent with the previous findings in this rootstock trial (Kosina 2004) as well as broader horticultural literature (Atkinson and Else 2003). If we consider that the fruit represents a major sink for carbohydrates (DeJong and Grossman 1995), it is likely that a higher yield efficiency related with lower leaf to fruit ratio may further compete with the vegetative growth.

Total leaf area is an important determinant of photosynthetic gain and overall growth (Watson 1958). Differences in the relative proportion of leaf area were anticipated between low versus high vigor trees as a consequence of architectural changes during canopy development. For instance, low vigor trees typically have shorter shoot internodes and different proportions of sympodial to monopodial branching (Seleznyova et al. 2008), which is in pome fruit closely related to different proportions between vegetative and reproductive wood (Costes et al. 2006). We found that leaf area scaled allometrically with TCSA with a common exponent which resulted in a similar leaf area-to-TCSA ratio across all studied trees (Fig. 3a). The scaling between leaf and sapwood cross-sectional area presumably reflects the functional balance between water loss, C gain, and water transport toward the leaf evaporating sites (Petit et al. 2018). Thus, our results indicate that, from the structural point of view, this balance was roughly the same for low and high vigor trees. However, the measurements of leaf and shoot carbon isotopic composition (Fig. 5) suggest that low vigor pear trees likely had lower stomatal conductance compared to high vigor pear trees and both scion/rootstock combinations of apple trees, which might have translated into lower photosynthetic gain and lower growth vigor. In agreement with our results, less negative $\delta^{13}\text{C}$ values were associated with reduced vigor in ‘Honeycrisp’ apple tree scion grafted onto a series of size-controlling rootstocks (Casagrande Biasuz and Kalcsits 2022). Less-depleted $\delta^{13}\text{C}$ can also be caused by a greater contribution of stored carbohydrates relative to the current-year assimilates (Han et al. 2016). This interpretation would be supported by the less negative $\delta^{13}\text{C}$ values of spring compared to summer leaves. To disentangle these possibility measurements of compound-specific $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$ composition or leaf gas exchange rates could be helpful (Hartmann and Trumbore 2016).

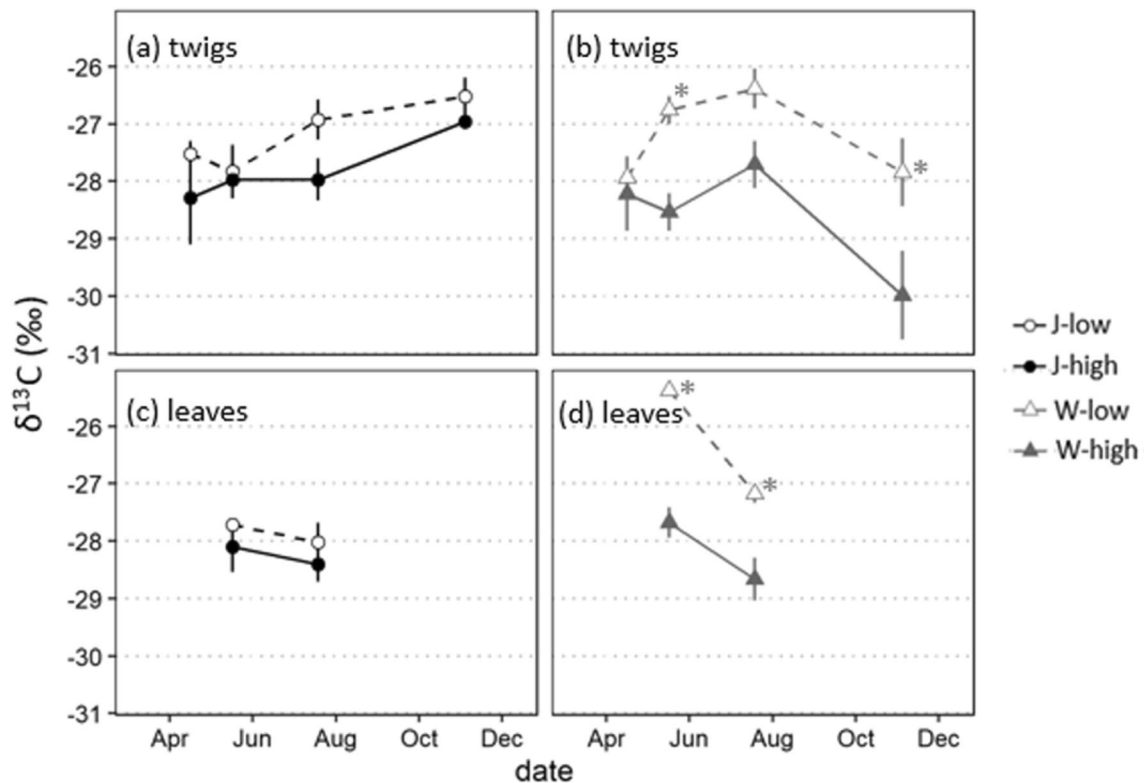


Fig. 5 Seasonal carbon isotope ($\delta^{13}\text{C}$) variation in 1-year-old shoots and leaves of apple tree var. 'Jonagold' (J) and pear tree var. 'Williams' (W) grafted onto rootstocks inducing low or high growth vigor.

The data are means \pm SE ($n=3$). The asterisk indicates significant differences between the rootstock means within the sampling date (Tukey-adjusted pairwise comparison)

To the best of our knowledge, this is the first study that tested the carbohydrate reserve theory of rootstock-induced control of growth vigor (Basile and DeJong 2018) from the perspective of whole-tree NSC storage. We found that the size of the NSC storage pool as well as the magnitude of seasonal fluctuation varied proportionally with tree size. Thus, from the whole tree perspective, the trees seem to rely on NSC reserves to a similar extent and there was no evidence for a generally lower NSC storage capacity in low vigor trees compared to their high vigor counterparts. Nevertheless, there were some nuances in the dynamics of NSC concentrations across organs, indicating that NSC use and demands were locally perturbed by the different size-controlling rootstocks. It is unlikely that these small differences would be the ultimate cause of differential growth vigor. Instead, other mechanisms such as disturbed water, hormonal, or nutritional relations were likely to affect the trees' growth and subsequently C use.

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Author Contributions LP: conceived the study, LP, RJ, and MM: carried out the field work and biomass measurements. GH: carried out the NSC analyses. LP: analysed the data and wrote the text of the manuscript with all co-authors contributing.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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