Title

Mature beech and spruce trees under drought – higher C investment in reproduction at the expense of whole-tree NSC stores

Running head

NSC concentrations and pool sizes in mature trees under drought

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BALANCE, biomass increment, Fagus sylvatica, Picea abies, non-structural carbohydrates, carbon balance

Highlights

- A mixed beech/spruce forest was exposed to two years of recurrent summer drought.
- Under drought, NSC concentrations remained unaffected among all tree organs
- Total NSC pools size decreased by about 40% in both species under drought
- Unlike growth, investment in reproductive tissues remained stable under drought
- Carbon starvation was not evident in mature trees under two years of drought

Abstract

Under drought, potential plant death from depletion of carbon (C) stores, referred to as carbon starvation, is thought to result from negative carbon balance during (partial) stomatal closure. As evidence for C starvation is rare for mature trees, we investigated the C dynamics in mature beech and spruce under drought, focusing on non-structural carbohydrates (NSC) as an indicator of their C balance. Trees were exposed to complete exclusion of precipitation throughfall over two consecutive growing seasons. We assessed NSC concentrations during the early dormant season in leaves, twigs, stem phloem/xylem, coarse and fine roots. Up-scaling to whole-tree NSC pools was achieved using a process-based single-tree model ("BALANCE"), estimating tree biomass increment. While there were distinct differences in NSC concentration among different tissues in both beech and spruce (root < stem < twig < leaves and xylem < phloem), drought did not affect NSC concentrations. However, compared to controls, the whole-tree NSC pool size significantly decreased under drought in both beech (42 %) and spruce (36 %), in parallel to a significant growth decline of overall 52 % and 57 %, respectively. Nevertheless, drought-stressed beech and spruce invested almost twice as much C in reproductive structures relative to total C investment (i.e. 6.0 ± 3.3 and 52.3 ± 8.71 %) compared to control trees (3.1 ± 1.8 and 29.2 ± 7.8 %). This highlights the high priority of C investment into reproduction relative to growth under drought. Given that NSC concentrations are maintained even under severe drought over two growing seasons, NSC pool sizes appear to be a better proxy to assess whole-tree's carbon status in mature trees. Overall, trees maintained NSC availability, avoiding carbon starvation, by downregulating a major C sink (i.e. growth) while upholding reproduction.

1. Introduction

During drought, plants are facing several difficulties with the lack of water and decreased carbon (C) gain as severe consequences (McDowell 2011). Under harsh drought conditions, these can lead to hydraulic failure and/or carbon starvation (Sevanto et al., 2014) and eventually kill the plant. The water conducting system will be disrupted as conductive elements, i.e. vessels and tracheids, begin to cavitate and water transport is limited, resulting eventually in hydraulic failure (Barigah et al., 2013; Urli et al., 2013). With less water being transported to the leaves, stomatal conductance is often reduced and therefore less C can be assimilated (Brodribb and McAdam, 2011). Plants can follow different strategies to deal with drought. Isohydric plants close their stomata early under drought to minimize water loss and avoid hydraulic failure at the risk of carbon starvation, while anisohydric plants keep their stomata open to maximize C gain, avoiding carbon starvation at a higher risk of hydraulic failure (McDowell et al., 2008). With ongoing climate change, drought periods and heat waves will occur more frequently (Burke et al., 2006; IPCC, 2014), and such stressful conditions will be a major challenge for ecosystems, especially for long-living and slow growing organisms such as trees (Hartmann et al., 2018). Consequences of such extreme events for Central European forests have been obvious upon extreme drought years in 2003, 2015 and 2018/19 (Asner et al., 2016; Büntgen et al., 2021; Schuldt et al., 2020) with severe decreases of photosynthesis and primary production (Ciais et al., 2005), as such the C balance of trees could be critically disturbed

(Anderegg et al., 2012). During extended drought stress, C stores may become depleted and the plant could be limited in its anabolism (Palacio et al., 2014).

Carbon gained by photosynthesis is needed for many catabolic and anabolic processes. NSCs, consisting mainly of soluble sugars (e.g. sucrose) and starch are invested in five predominant sinks: storage, metabolism (e.g. growth), transport, osmoregulation and export (e.g. exudates, Hartmann and Trumbore 2016). Under drought, these sinks will be affected in different ways. One of the first consequences is a decrease of the photosynthetic rate accompanied by a depletion of C stores (Maguire and Kobe, 2015). The export of metabolites, e.g. root exudates or volatile organic compounds, also changes under drought (Gessler et al., 2017; Rennenberg et al., 2006). In mature trees, transport of photoassimilates and water, which are highly connected, are strongly decreased under drought (Adams et al., 2017; Hesse et al., 2019; Sevanto, 2018) and therefore C sinks at a longer distance from the primary source (i.e. leaves) could be affected stronger under whole-tree C limitation (Landhäusser and Lieffers, 2012). Especially in mature trees with long transport distances, this could potentially lead to a C imbalance along the tree under drought, with fine roots (longest transport distance from the source organs) being affected stronger than e.g. branches (short transport distance, Ryan and Asao, 2014). So far, most experiments on C allocation at the whole plant level under drought were carried out on juvenile trees (e.g. Hagedorn et al. 2016, Chuste et al. 2020) or under short-term drought (Anderegg et al., 2012). In this paper, we focus on long-term drought effects (i.e. two subsequent growing seasons) on the primary metabolism, in particular NSC, and its consequences for growth and investment in reproduction in a mixed forest with mature trees of Norway spruce (*Picea abies* (L.) H Karst) and European beech (*Fagus sylvatica* L.). The two species follow a rather opposing strategy, when it comes to stomatal sensitivity and regulation under drought (Pretzsch et al., 2014). The degree of isohydry can be seen as continuum with two extreme ends (Hartmann et al., 2021; Hochberg et al., 2018). Strictly isohydric species close their stomata very early under drought stress and try to avoid a sudden drop in water potential, at the expense of a limited CO_2 uptake. Conversely, more anisohydric species keep their stomata open even during severe drought conditions to further assimilate CO_2 at the expense of continued water loss (Hochberg et al., 2018). Following the literature and own observations at the experimental site of this study (Pretzsch et al. 2014), mature spruce is generally behaving more isohydric under drought (Lyr et al., 1992; Oberhuber et al., 2015; Pashkovskiy et al., 2019), although some variability in provenances exist in seedlings (Jamnická et al., 2019). Conversely, mature beech follows a more anisohydric drought strategy (Leuschner, 2020; Magh et al., 2019). We assessed NSC concentrations of different tree organs and tissues during winter early dormancy. We took advantage of the KROOF experiment, a long-term drought experiment, with retractable roofs to simulate summer drought (Grams et al. 2021). By means of the process-based single-tree model BALANCE (Grote and Pretzsch, 2002; Rötzer et al., 2010), we estimated the growth increments of tree's organs allowing for an upscaling of NSC concentrations to estimate overall NSC pools to test the following hypothesis:

H1. NSC concentration is reduced by drought, with concentrations in sink organs being more affected than in source organs and with distance from source organs, i.e. leaves > branches/twigs > stems > roots.

H2. Total NSC pools, measured during dormancy, are reduced in drought stressed trees,
  a. with the more isohydric spruce being stronger affected than the more anisohydric beech and
  b. with sink organs being stronger affected than source organs, i.e. leaves > branches/twigs > stems > roots

due to decreases in NSC tissue concentration and growth.

H3. The amount of invested C in reproductive structures decreases under drought.

2. Material and methods

2.1 Plant material and experimental design

The experimental site, a mature mixed forest composed of European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) planted around 1931 AD and 1951 AD, respectively, is part of the Kranzberg Forest Roof (KROOF) experiment in Southern Germany near Freising, Bavaria. In 2010, 12 plots were trenched to one-meter depth, allowing no further lateral water transport between the plot and outside soil (Pretzsch et al., 2016). Each plot contained between three to seven beech and spruce trees each (Grams et al., 2021). The KROOF experiment started in 2014 when six of the 12 plots were covered with a unique rainfall-activated retractable roof, which closed automatically for precipitation events during the growing season, to exclude any throughfall as well as stem run-off and retracted after the rain events passed, to minimize any other climatic effects. For further details on the experimental design, site (e.g. soil characteristics) and treatment description see Grams et al. (2021). Data presented in this publication were collected in 2013, 2014 and 2015 from two beech and spruce trees of eight plots accessible via canopy crane (four assigned to the throughfall exclusion (TE) plots and four control plots (CO)) to a total of eight TE and CO trees for each species.

The long-term average in temperature is 13.8 °C during the growing season (7.8 °C for whole year) and 750-800 mm annual precipitation. For 2013 and 2014, the precipitation was 640 mm (growing season: 444 mm) and 651 mm (growing season: 501 mm), a bit lower than the long-term average. At the same time, growing season temperature was slightly higher than average, i.e. 14.8 °C and 15.2 °C in 2013 and 2014, respectively. With only 495 mm (growing season: 330 mm) of precipitation and a growing season temperature of 17.2 °C, the year 2015 was extraordinarily warm and dry. For further details see Grams et al. (2021).

2.2 Drought stress assessment

To assess the effects of the throughfall exclusion on the trees' water status, pre-dawn leaf water potential (Ψ_{PD} in MPa) was measured with a Scholander pressure chamber (mod. 1505D, PMS Instrument CO., Albany, OR, USA) towards the end of the drought period in the middle of October of each year. Volumetric soil water content (SWC in vol.-%), was assessed weekly by Time Domain Reflectometry (TDR, TDR100, Campbell Scientific, Logan, CT, USA) in four different depths (0-7 cm, 10-30 cm, 30-50 cm and 50-70 cm) and at three positions within each plot (total of 144 TDR probes, for details see Goisser et al. 2016). Data were averaged over all four depths and three potions for each plot.

2.3 NSC sampling and concentration assessment

NSC sampling was done in early November 2013 to 2015 at the start of winter dormancy, in order to get the carbon status at the end of the vegetation period, hence one year before the onset and two consecutive year/s of experimentally induced summer drought. For each sample, the sum of soluble carbohydrates (SC, Table S1) and starch (Table S2) was assessed. Via the canopy crane, one-year old leaves of spruce (SC_L & $starch_L$) and branches/twigs of beech and spruce were sampled. For the branches/twigs (2-3 years old), the living bark (inner and outer bark; hereafter referred to as phloem, SC_{TP} & $starch_{TP}$) and xylem (SC_{TX} & $starch_{TX}$) were separated. With an increment borer and a cork borer, the xylem (SC_{SX} & $starch_{SX}$) and phloem (with the dead bark removed, SC_{SP} & $starch_{SP}$) of the stem were collected at 1.3 m above the ground, respectively. For NSC measurements in the xylem, the last 10 years of each increment core were used, combining the conductive and non-conductive portions of the xylem. Additionally, wood chippings of coarse roots (average diameter about 5 cm, SC_{CR} & $starch_{CR}$) were sampled with a driller and fine roots (with a diameter of less than 2 mm, SC_{FR} & $starch_{FR}$) of both species were directly sampled. All samples were stored on ice (0 °C) and microwaved for 30 s within 30 min after sampling, to avoid respiration and degradation losses of NSCs. In the lab, samples were dried for 72 h at 64 °C and ball milled to a fine powder (Retsch® MM200, Haan, Germany). We assessed the concentration of soluble sugars (glucose, fructose and sucrose) and starch, which account for at least 80 % of the mobile carbon pool in both species (Hoch et al., 2003). High performance liquid chromatography with pulsed amperometric detection on a Dionex® ICS 3000 ion chromatography system equipped with an autosampler was used to determine sugar/starch concentration (Raessler et al., 2010). Sample preparation for sugars followed the protocol in Hartmann et al. (2013) and for starch in Landhäusser et al. (2018).

2.4 Sampling of reproductive structures

In autumn 2015, a masting year for both species in southern Germany, reproductive structures (RS) of beech (complete beechnuts, fruits plus huskers) and spruce (cones) were sampled. For beech, a random column of 1 m^2 ground area per plot (often combining the canopy of a few beech individuals) was selected and the number of beechnuts of this area were counted ($N_{beechnut}$ in m^{-2}). Afterwards, these beechnuts were harvested and oven-dried at 64 °C and the dry mass of each beechnut was assessed ($DM_{beechnut}$, in g). In combination with the sum of the beech tree crown spread area per plot (average for CO plots: 149.1 ± 25.2 m^2 and TE plots: 147.9 ± 69.5 m^2), we then estimated the dry mass of beechnuts per plot ($DM_{beechnut\_plot}$ in kg). For spruce, five random branches from the main stem of 8 CO and 8 TE trees were selected and the number of cones were counted, afterwards the number of branches of the main stem was counted and the total number of cones was calculated (N_{cones}, unitless). Subsequently, the cones of the five branches were harvested, oven-dried at 64 °C and each cone was weighed (DM_{cones}, in g). To get the total dry mass of cones per tree ($DM_{cones\_per\_tree}$, in kg), we multiplied N_{cones} with DM_{cones} for each tree individually.

2.5 Modeling biomass increment with process-based model BALANCE

Biomass and growth of organs of individual trees were calculated using the eco-physiological growth model BALANCE (Grote and Pretzsch, 2002), which simulates the three-dimensional development of individual trees based on external environmental factors such as weather (e.g. temperature, precipitation, etc.), CO_2 concentration, soil conditions and water availability. Further on, the model describes the consequences of the individual competition for light, water and nutrients. As individual environmental conditions change with the tree development, the influence of stand structure, species mixture and management options are taken into account, too. Consequently, biomass increment is simulated in dependence of the carbon and nitrogen uptake for each segment on the basis of its energy supply and resource (e.g. water) availability (Fontes et al., 2011). Model outcomes are validated against empirical measurements of the carbon and water balance (including drought scenarios) several times (Rötzer et al., 2017b, 2013, 2012, 2010) and also for the above vs. below ground biomass ratio (Rötzer et al., 2009). For our study, BALANCE was used to generate the biomass of the fine roots (BM_{FR}), the coarse roots (BM_{CR}), the stem (BM_S), the branches/twigs (BM_T) and of the leaves (BM_L, for spruce only, Table S1). The simulation was based on meteorological data from 2013 to 2015 from the nearby weather stations in Freising with initial tree data and soil characteristics as described in Rötzer et al. (2017b, 2017a). Precipitation of the drought plots was reduced according to the throughfall exclusion experiment, i.e., rainfall was set to zero for the period when the roofs were closed (Grams et al. 2021). To validate the model outcome, we compared the modeled biomass increment of stems in 2014 and 2015 (BI_{stem}, in kg) with measured data of band dendrometers (Dendrometer D1, METER, Munich, Germany) at 1.3 m above the ground (diameter increment D_{stem}, in cm) for both species and treatments (i.e. CO and TE).

2.6 NSC pool calculations

We scaled the concentration of SC and starch in each tissue with its modeled biomass (BM in kg, Table S3) to calculate the overall C pools ($SC_{pool\_CO/TE}$ and $Starch_{pool\_CO/TE}$, both in kg, except for spruce $Starch_{pool}$ in g). For stem and branches/twigs, we additionally estimated the proportion of xylem and phloem by measuring the stem circle area at DBH (assessed via measuring tape) and the annulus area for phloem (phloem thickness was assessed with a caliper on the samples taken with the cork borer). For beech, the proportion of the stem xylem was CO: 0.989 ± 0.002 and TE: 0.990 ± 0.003, and for spruce it was CO: 0.988 ± 0.003 and TE: 0.989 ± 0.002, respectively. We then applied the following formula to calculate the stem and branch/twig pool sizes:

$$SC/Starch_{pool} = concentration_{phloem} * biomass_{phloem} * proportion_{phloem} \\ + concentration_{xylem} * biomass_{xylem} * proportion_{xylem}$$

Overall, the phloem was thicker in spruce compared to beech ($P < 0.001$). In spruce there was a significant difference between the phloem thickness, with CO trees having a thicker phloem (0.46 ± 0.05 cm) than TE trees (0.39 ± 0.06 cm) ($P < 0.05$), while in beech we did not find any statistical difference in phloem thickness ($P = 0.17$). However, in beech there appeared to be a similar trend with CO trees (0.35 ± 0.05 cm) having somewhat thicker phloem compared to TE trees (0.31 ± 0.05 cm). For beech, phloem thickness correlated strongly positive with the stem diameter regardless of treatment ($P < 0.05$, $R^2 = 0.31$), while in spruce, this correlation was only valid for TE trees ($P < 0.01$, $R^2 = 0.76$) and not for CO trees ($P = 0.91$, $R^2 = 0.00$).

2.7 Statistical analyses

Data were analyzed for statistical differences using R (version 3.6, Development Core Team 2008) in RStudio (version 1.2.1335, RStudio Team 2015). Every model was tested for normality of the residuals (Shapiro test) and data was tested for homogeneity of variances (Levene test) beforehand. For differences in Ψ_{PD}, biomass increment, NSC concentrations and pools, a linear mixed effect model (‘lme’ function) was calculated using the species (beech and spruce), year (2013, 2014 and 2015) and the treatment (CO and TE) as fixed and the tree individual nested in the plot as a random effect (package: nlme, version: 3.1-137). If the mixed effect model showed significant effects, we performed a post-hoc test with the ‘emmeans’ function with Tukey correction (package: emmeans, version: 1.3.1). For data of cones and beechnuts, the same functions were used, but with the plot as the random effect. To validate the BALANCE-data we used a linear model (package: stats, version: 3.5.0) with the BI_{stem} as the dependent variable and the D_{stem} as the test variable. Data were plotted with the ‘boxplot’ function (package: graphics, version: 3.5.2, showing the first, second (i.e., median, solid line within the box) and third quartile as a box, the whiskers indicate the 1.5 interquartile range and empty dots showing outliers), the ‘plot’ function (package: graphics, version: 3.5.2) and the ‘ggplot’ function (package: ggplot2, version: 3.1.0). Data in text and tables are given as mean ± 1 SD.

3. Results

3.1 Drought treatment effects on soil and tree water status

In 2013 prior to establishing the drought, leaf Ψ_{PD} was not different between trees in the treatment plots for both species (beech: -0.41 ± 0.09 MPa and spruce: -0.51 ± 0.06 MPa Tab. 1). Upon throughfall exclusion, TE trees of both species had significantly lower Ψ_{PD} than CO trees. In beech in 2014 and 2015, Ψ_{PD} was reduced to -0.62 ± 0.08 and -1.14 ± 0.31 MPa (Tab. 1) corresponding to reductions by 51 and 178 % compared to 2013, respectively. Ψ_{PD} in Spruce was reduction stronger to -1.33 ± 0.12 and -1.68 ± 0.13 MPa in 2014 and 2015 (Tab. 1), respectively (reductions by 160 and 229 % relative to 2013, respectively). However, during the extraordinary dry year of 2015 the CO trees of both species showed signs of drought stress in their water potential (beech: -0.76 ± 0.24 MPa and spruce: -1.38 ± 0.22 MPa), with values similar to those found in TE trees in 2014, for both species respectively (Tab. 1).

Before the onset of the drought treatment in 2013, soil water content (SWC) to a soil depth of 70 cm was very similar between CO and TE plots (mean difference between CO and TE averaged over all depths: 0.1 ± 1.8 vol.-%, p-value > 0.05, Tab. 2). During the growing seasons of 2014 and 2015, SWC was generally lower on TE compared to CO plots. In 2014, the mean difference between CO and TE was 6.0 ± 4.0 vol.-% (p-value = 0.023) and in 2015 9.5 ± 4.5 vol.-% (p-value = 0.002, Tab. 2). During winter, when roofs were permanently open, SWC on all plots was partially recharged by precipitation. In August and September 2015, the SWC on CO plots decreased due to very warm and dry conditions and reached values similar to TE plots.

3.2 Modelled biomass increments using BALANCE

We found strong overall positive linear correlations (Pearson) between the measured stem increment at 1.3 m above ground level and modeled stem biomass increment (Fig. 1), with strong positive correlations for each group (Pearson correlation: P-value/Pearson coefficient of CO spruce 2014: 0.090/0.64 & 2015: 0.011/0.83, TE spruce 2014: <0.001/0.97 & 2015: 0.027/0.76, CO beech 2014: 0.028/0.76 & 2015: 0.003/0.89 and TE beech 2014: <0.001/0.97 & 2015: 0.003/0.92, Fig. 1). Slopes and intercepts were not significantly different between treatments for beech and spruce within the single years, suggesting that our model appears to generate realistic results, with the TE trees showing significantly decreased growth for almost all tissues compared to CO trees. Overall, spruce showed a higher growth than beech, especially of stems and coarse roots (Tab. 3). Modelled biomass of spruce fine root increment (BI_{FR}) was 34% lower for both drought years in TE compared to CO trees but not significantly different (p-value 2014: < 0.1 and 2015: > 0.1), and significantly lower by 46 % in TE beech compared to CO trees (p-value 2014: < 0.05 and 2015: <0.1 0.1). Coarse root growth increment (BI_{CR}) was significantly different between the two treatments for both species only in 2015 (p-value spruce: <0.05 and beech: <0.05), with 2-times higher growth increment in CO than TE trees (Tab. 3).Stem diameter growth of both species was significantly different between CO and TE in both years, with a growth reduction in TE by more than 50 % (p-value spruce 2014: <0.001 & 2015: <0.05 and beech 2014: <0.01 & 2015: <0.01, Tab. 3). For branch/twig tissue, growth reduction was also about 30 to 40 % in TE trees compared to CO trees and significant different between treatments for both species and years (p-value spruce 2014: <0.05 & 2015: <0.05 and beech 2014: <0.01 & 2015: <0.05, Tab. 3). For leaves of spruce, there were no significant differences in both years, nevertheless reductions of 30 and 39 % under TE compared to CO were estimated in 2014 and 2015, respectively (Tab. 3).

3.3 Biomass investment in reproductive structures during a masting year (2015)

In the masting year, TE trees of spruce produced about three times more cones per tree than CO trees (N_{cones}, CO: 235 ± 66 and TE: 775 ± 349, Fig. 2), while the dry mass per cone (DM_{cone}) was on average about three times smaller in TE than in CO trees (TE: 22.0 ± 6.5 and CO: 59.0

± 17.2 g, respectively, Fig. 2). Thus, the overall invested biomass in cones per tree ($DM_{cones\_per\_tree}$) was not different between CO (13.4 ± 4.8 kg) and TE (15.7 ± 5.6 kg, Fig. 2). In beech, neither the amount of beechnuts per ground area ($N_{beechnut}$, CO: 21.0 ± 1.6 m^{-2} and TE: 19.3 ± 1.8 m^{-2}) nor the mass per beechnut ($DM_{beechnut}$, CO: 0.73 ± 0.21 g and TE: 0.82 ± 0.28 g) differed between the treatments. Therefore, also the total dry mass of fruits per area ($DM_{beechnut\_plot}$, CO: 2.27 ± 0.66 kg and TE: 2.31 ± 1.32 kg) was very similar in CO and TE (Fig. 2). Accordingly, the invested biomass of both tree species into reproductive structures was not affected by drought although the number of spruce cones was three time higher in TE compared to controls.

3.4 Biomass investment (BI) in structural sinks vs. reproductive structures in 2015

For the masting year 2015, we compared the investment of biomass (BI) into the sink tissues (sum of fine/coarse root, stem and branch/twig tissues, BI_{sum} in kg) and reproductive structures (cone-mass and whole beechnut-mass ($DM_{beechnut\_plot}$ divided by the number of trees on each plot)) per tree. For beech, the BI_{sum} of TE trees (8.4 ± 3.4 kg) was about half of CO trees (17.5 ± 4.8 kg, P-value < 0.01), while the beechnut-drymass did not change (CO: 0.5 ± 0.3 kg and TE: 0.5 ± 0.3 kg, Fig 3a). The relative biomass investment was calculated by dividing the weight of single tissues by the overall sum of all tissues (including reproductive structures). Therefore, in beech the ratio of reproductive structures compared to structural tissues (e.g. reproduction/(roots+stem+branches)) was 3 ± 1 % and 7 ± 3 % (P-value < 0.01), for CO and TE, respectively (Fig. 3b). For spruce, the BI_{sum} was reduced by 57 % (Fig. 3a) in TE (13.6 ± 3.1 kg) compared to CO (31.5 ± 5.6 kg, P-value < 0.0001), but the cone-drymass was very similar between CO (13.4 ± 4.8 kg) and TE (15.7 ± 5.7 kg, Fig. 3a). Thus, the relative ratio of the investment in reproductive structures compared to other tree tissues combined (e.g. reproduction/(roots+stem+branches)) in spruce was 44 ± 7 % and 115 ± 42 % (P-value < 0.001), for CO and TE, respectively. Most of the difference in structural tissues between CO and TE was caused by a reduced stem and coarse root growth (Figure 3a). However, the relative biomass investment of structural tissues was only significantly reduced in the stem (p-value = 0.0020) and coarse roots (p-value = 0.0475) of spruce and tendentially in stem of beech (p-value = 0.0774, Figure 3b).

3.5 NSC concentrations and NSC pools

For both species, there was no significant influence of drought on the concentration of SCs during the dormant season (P = 0.45 for beech and 0.23 for spruce) detected and thus all data points are close to the 1:1 line in Fig. 4a and 4c. However, concentrations were different among plant tissues. Structural tissues with low metabolic activity, i.e. stem xylem (SX) and coarse roots (CR), showed the lowest concentrations (mean of SX and CR: 22.0 ± 6.1 mg g^{-1} for beech and 7.4 ± 5.0 mg g^{-1} for spruce). Conversely, recently built, highly active and/or transport tissues, i.e. fine roots (FR), stem phloem (SP), branch/twig phloem/xylem (TP/TX) and spruce leaves (L), showed higher concentrations (mean of FR, SP, TP, TX and L: 40.2 ± 8.9 mg g^{-1} for beech and 50.0 ± 11.0 mg g^{-1} for spruce, Fig. 4a, c). Thus, differences in NSC concentrations between structural and active tissues (excluding spruce needles) were more pronounced in beech that in spruce.

In comparison to the SC, starch concentrations of the tissues were on average 10 and 6 times lower for spruce and beech, respectively. Similar to SC, starch concentrations did not differ between CO and TE for both species ($P = 0.408$, Fig. 4b, d). Starch concentrations of beech were highest in stem and branch/twig phloem (all years and both treatments averaged: 8.58 ± 3.48 and 11.49 ± 2.69 mg g^{-1}, respectively), lowest in the stem xylem and coarse roots (all years and both treatments averaged: 1.50 ± 0.68 and 1.62 ± 0.90 mg g^{-1}, respectively) and in between for the branch/twig xylem and fine roots (all years and both treatments averaged: 4.08 ± 1.39 mg g^{-1} and 7.30 ± 2.56 mg g^{-1}, Fig. 4d). A similar pattern was found for spruce, with high values in leaves (average over all years and both treatments: 7.76 ± 1.64 mg g^{-1}) and somewhat lower levels in stem and branch/twig phloem as well as branch/twig xylem (averaged over all years and both treatments: 4.08 ± 2.55, 4.25 ± 1.77 and 5.31 ± 2.77 mg g^{-1}, respectively, Fig. 4b). Except from the above, fine root tissue showed the highest values in spruce (averaged over all years and both treatments: 12.15 ± 4.7 mg g^{-1}). Much lower concentrations were found in the coarse roots and stem xylem (averaged over all years and both treatments: 0.59 ± 0.30 and 0.76 ± 0.71 mg g^{-1}, respectively.

In 2013, the SC pools of tissues were similar in CO and TE trees for both species (green symbols in Fig. 5a, c), but in 2014 and 2015 SC pools decreased significantly in TE, except for fine roots and leaves in spruce (Fig. 5a, c, please note logarithmic scaling of axes). Overall, drought significantly reduced the overall SC pools (for beech: $P < 0.001$ and spruce: $P < 0.0001$), with TE trees showing smaller pool sizes in 2014 and 2015 for almost all tissues (for beech, averaged for all tissues: -49 ± 8 % and spruce, averaged for all tissues (expect leaves): -45 ± 23 %). Concentration of SCs were not affected by the treatment but varied between tissues, while the SC pools were strongly reduced by in the TE treatment. However, the natural drought year of 2015 did not seem to influence the pool size of CO trees (no differences within CO between the years for both species).

For the starch pools of beech, the influence of drought was significant ($P < 0.05$, Fig. 5d). For 2014 and 2015, a strong decrease in the starch pool size of TE beech compared to CO was found for CR (by 53 %), stem tissue (by 45 %) and branch/twig tissue (by 58 %), except for fine roots which showed an increase (by 47 %, Fig. 5d). For spruce, this signal was not as clear as for beech and no significant reduction was found (Fig. 5b). Stem tissue and needle were not much affected by drought in their starch pool size and the variation in branch/twig tissue and coarse/fine roots over the different years was high (Fig. 5b).

Whole-tree pool sizes of NSCs in beech trees were 11.1 ± 4.7 and 7.0 ± 1.9 kg under CO and TE, respectively (values averaged over 2014 and 2015, Fig. 6a). Under drought, NSC pools sizes were significantly reduced by 40 % in 2014 (P-value < 0.05) and 42 % in 2015 (P-value <0.01, Fig. 6a) compared to controls. In spruce averaged over 2014 and 2015, whole-tree pool sizes of NSCs were smaller compared to beech with 7.4 ± 1.7 and 4.8 ± 0.7 kg under CO and TE, respectively. Reductions in spruce under drought were less strong compared to beech trees with 23 % in 2014 (P-value <0.01) and 36 % in 2015 (P-value <0.001, Fig. 6a). Irrespective of the drought treatment, the relative proportion of the single tissues in the NSC pool size were remarkably constant in beech with CR = 4.1 ± 0.8 %, FR = 3.1 ± 1.8 %, SX/SP = 90.7 ± 1.4 and TX/TP = 2.1 ± 0.5 % (averaged over all years and treatments, Fig. 6b). Conversely, in spruce, the relative proportion of the CR was strongly reduced under drought in both years (by 75 %, P-value < 0.0001) and strongly increased in leaves (by 55 %, P-value < 0.0001, Fig. 6b). Additionally, in 2015 the TE spruce trees showed an increase in the fine root proportion by 33 % (P-value <0.1) compared to previous years and a decrease in the stem proportion by 24 %, P-value < 0.05, Fig. 6b).

4. Discussion

Comparisons with data from mature trees in other studies for the same NSC components indicate that the trees from this study had rather low NSC levels (specially starch) (e.g. Hoch et al. 2003). This difference, could be related to site conditions and stand structure, such as high stand density with ongoing self-thinning (Gruber et al., 2012; Tsamir et al., 2019). However, the values measured during this experiment were still in the range of other studies (Oberhuber et al., 2011; Schiestl-Aalto et al., 2019) and similar to values from the same stand sampled in 2003 (Nikolova et al., 2020). The low values presented here could reflect the seasonal variation of NSC concentrations with lowest levels in early winter (Furze et al., 2019; Martínez-Vilalta et al., 2016) and the parallel hydrolization of starch to soluble sugars (Ramirez, 2017).

In response to through-fall exclusion during the growing seasons, both species showed signs of moderate to harsh drought stress in 2014 and 2015 with Ψ_{PD} as low as -1.8 MPa, while in 2013, before the start of the experiment, no differences were found between the treatments. This should be accompanied by a reduction in stomatal conductance and assimilation of CO_2 and water potential regulation at the leaf level (Hochberg et al., 2018). This reduction/regulation was stronger and earlier in spruce than in beech, reflecting the more isohydric strategy of spruce under drought compared to beech (Hesse et al., under preparation).

4.1 NSC concentration vs. NSC pools

Two subsequent years of summer drought did not significantly decrease the NSC concentrations of various tissues in both species, indicating the possibility for trees to maintain them at a steady level. The meta-analysis of He et al. (2020) revealed, that only under severe drought saplings would show a decrease in their NSC concentrations. In addition, mature trees are supposedly less strong affected than seedlings (Zhang et al., 2020). As drought did not reduce the NSC concentrations in any tissue in both species, we rejected our first hypothesis that sink organs further away from the source organs will be stronger affected by drought in their NSC concentrations. Nevertheless, we confirm the expected differences in NSC concentrations between tissues, with source and transport (e.g. phloem) structures showing higher values than predominant sink tissues, such as stem growth, which was strongly reduced under drought in the experimental trees (Tab. 3, Pretzsch et al., 2020). Even in C sinks with a transport distance of more than 25 m from the C source, e.g. from leaves to fine roots (Fig. 4) NSC concentrations remained stable, which is well in accordance with studies on juvenile trees (Hagedorn et al., 2016; Hartmann et al., 2013; Muller et al., 2011).

NSCs are needed for many other sinks next to growth and maintenance, such as defense compounds, e.g. secondary metabolites, and osmoregulation (Chaves et al., 2003; Kozlowski, 1992). NSC investments into osmoregulation, repair processes or defense are unavailable for maintenance processes, but play an important role in acclimation processes of trees to abiotic and biotic stresses. An important sink under drought is the osmoregulation of cells, mostly accompanied by the incorporation of sugars or amino acids as osmotic substances (Jamnická et al., 2019; Sevanto et al., 2014). As reported before (Tomasella et al., 2018), in the second drought summer, i.e. 2015, both beech and spruce showed osmotic adjustments at the leaf level, resulting in a decrease of the turgor loss point by 0.5 MPa, accompanied by a similar decrease of the osmotic potential at full turgor. This change is based on osmoregulation (Bartlett et al., 2012) another C sink the drought-stressed trees had to cope with. However, the increased C demand for osmoregulation under drought and the stable level of NSC concentrations in all tree organs, raises the questions whether all measured NSCs can be seen as "metabolically available" carbohydrates (e.g. for transport or respiration (Prescott et al., 2020). Further studies are needed to separate the "stored or unavailable" amount of NSCs from the "metabolically available" NSCs in different tree organs, as this could prove to be very different between well-watered and drought-stressed plants. Nevertheless, it seems reasonable that trees maintain a certain concentration of NSCs even under drought, yet not much is known about the minimum required to maintain functionality, especially not for mature trees (Adams et al., 2013).

Looking at the NSC pool sizes, all growth sinks of the tree (but especially coarse roots) showed a decrease in the total amount of NSCs under drought. Despite the stable concentration of NSCs in almost all organs, the overall carbon pool of tree individuals was reduced by 42 % in beech and 36 % in spruce after two subsequent summer droughts. Winter NSC pools were reduced under drought in both species, generally consistent with our second hypotheses. However, beech and spruce were affected to a similar extent, which is against the second part of that hypotheses assuming more isohydric spruce to be stronger affected by drought than more anisohydric beech.

However, starvation thresholds, i.e. lethal NSC concentrations or pools, are widely unknown for mature trees or long lasting drought periods and many experiments showed NSC remnants in dead tissues and for juvenile trees (Weber et al., 2018; Wiley et al., 2019, 2017). Although NSC pools are significantly reduced in drought-stressed trees of both species after two subsequent summer droughts, they can be estimated sufficient for supporting respiratory processes of trees for at least two years. Assuming that whole-tree respiration in µmol CO_2 s^{-1} is equal to 0.8 times the whole-plant mass in kg and that 1 g of NSC is needed for 6 g of respired CO_2 (Mori et al., 2010), drought stressed spruce and beech could survive for almost 2 and 4 years, respectively, if the whole NSC pools could be accessed and used for maintenance respiration to keep trees alive. While this is certainly not possible, as NSCs are needed for many other mechanisms (see above/below), it demonstrates that, neither the more anisohydric beech nor the more isohydric spruce seems therefore to be limited in their overall C availability under drought (Hoch, 2015; Körner, 2003; Leuschner, 2020). Trees keep a rather constant concentration of NSCs as a buffer by reducing selected C sinks under stress. This is also in accordance with Garcia-Forner et al. (2017), who reported that more isohydric plants are not necessarily more carbon limited than anisohydric ones.

The rather small reduction of NSC pool size in spruce fine roots (FR) might be explained by a somewhat overestimated spruce FR biomass in the model compared to the observed loss of vital fine roots under drought (Nickel et al., 2018; Zwetsloot and Bauerle, 2021). However, accumulation of NSCs in roots of drought-stressed trees has been reported previously for saplings (Hagedorn et al. 2016 and citations within) and is supported here for mature beech by the increased starch concentrations (Fig. 4d). The reduction of NSC pools in sink tissues, in particular of stem xylem (SX) and coarse roots (CR) over time, suggests that phloem transport is hindered under drought as previously reported in the same experiment (Hesse et al., 2019; Hikino et al., submitted). The impaired C transport to roots is also supported by the fact that NSC pools in spruce leaves and by that the replenishment of carbon is not affected by drought. Thus, capacity to transport carbon plays an important role in the carbon starvation scenario (Hartmann et al., 2013; Ivanov et al., 2019), as stems and coarse roots might empty their storages to maintain fine roots as the most important tissues for water uptake and exploitation of new water sources (Jackson et al., 2000). As the annual carbon allocation of the control trees was not affected by the natural drought in 2015, but as the phloem transport was reduced for the same experimental trees (measured in 2015 by Hesse et al., 2019 on the same experimental trees), short term droughts seem to be buffered by tree C stores when carbon transport decreases for a short time. However, overall both species still seem to be far from carbon starvation or even carbon limitation, even in the masting year 2015, as the remaining NSC pools can potentially sustain respiration for more than one whole year.

4.2 Carbon investment in reproduction under drought

While it has been shown that growth is one of the first C sinks to decrease under drought (Chuste et al., 2020; Muller et al., 2011), this is not clear for reproduction and might also differ among species. As the total C investment into reproductive structures in beech and spruce was unaffected even under recurrent summer drought with significant growth reductions, reproduction must be of high priority in the hierarchy of carbon sinks in mature trees (Fatichi et al., 2013; Lacointe, 2000). Accordingly, beech is known for showing signs of resource switching towards reproduction in dry masting years (Hacket-Pain et al. 2017 and citations within). Other C investments under stress, e.g. investments in defense (Huang et al., 2019a, 2019b) and osmoregulation (Morgan, 1984) are – at least partially – quick responses to stress (Hartmann and Trumbore, 2016), and as they are partially reversible, they might not be too costly for the plant after stress release. Conversely, C investments in growth or reproduction is irreversible, but seems to be regulated very different in different mature tree species under drought. C demand for reproduction might be partially met by the green inflorescence tissue itself (Hoch et al., 2013; Landhäusser, 2011; Mund et al., 2020), or largely supported by leaves of the same branchlet as reported for e.g. beech (Hoch, 2005). Knowledge about the carbon autonomy of spruce cones is scarce, but cone biomass increment dependents largely on C assimilated in the canopy (Koppel et al., 1987). Under drought, spruce trees invested similar amounts of C in reproductive and structural tissues. This might reflect a strategy to distribute seeds by zoochory (Dobrovolný and Tesař, 2010) or anemochory (Dobbs, 1976) to locations with potentially more favorable microclimatic conditions or available water. In contrast to our third hypothesis, under drought C invested in reproductive structures was not reduced in either species. Apparently, reproduction has a high priority in mature beech and spruce even under severe summer drought.

5. Conclusions

Under two-years of experimentally induced, severe summer droughts with distinct growth decline, mature beech and spruce trees maintained a stable level of NSC concentrations across all studied tissues. This preserves trees' ability to react to their environment, e.g. defense against biotic attacks. Shorter drought events, as the natural summer drought of 2015, hardly affected the C allocation of beech and spruce, as the control trees were not affected in their NSC household compared to previous years. While C pools in all tissues, except leaves and fine roots of spruce, were reduced at least by about half after two consecutive drought years, both species, even the more isohydric spruce, were apparently not carbon limited. Trees were still able to maintain functionality by preserving rather constant NSC concentrations through reducing C demand for growth. With NSC pool size in leaves in spruce being rather unaffected by drought, C transport might be critical for carbon availability in non-green tissues in mature trees, as NSC pool sizes in branch/twig, stem and coarse roots were affected the most. Conversely, C investment in reproduction remained unaffected, partial supported by carbon autonomy of inflorescence structures and the proximity to C source tissue but clearly representing a high priority among C sinks of trees. Finally, a decrease in NSC concentration would only occur once the NSC pools run completely empty. Therefore, we suggest assessing not only NSC concentrations, but also the overall amount, i.e. pool sizes, of NSCs to assess the carbon status of mature trees under drought.

Declaration of Competing Interest

None.

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Figures

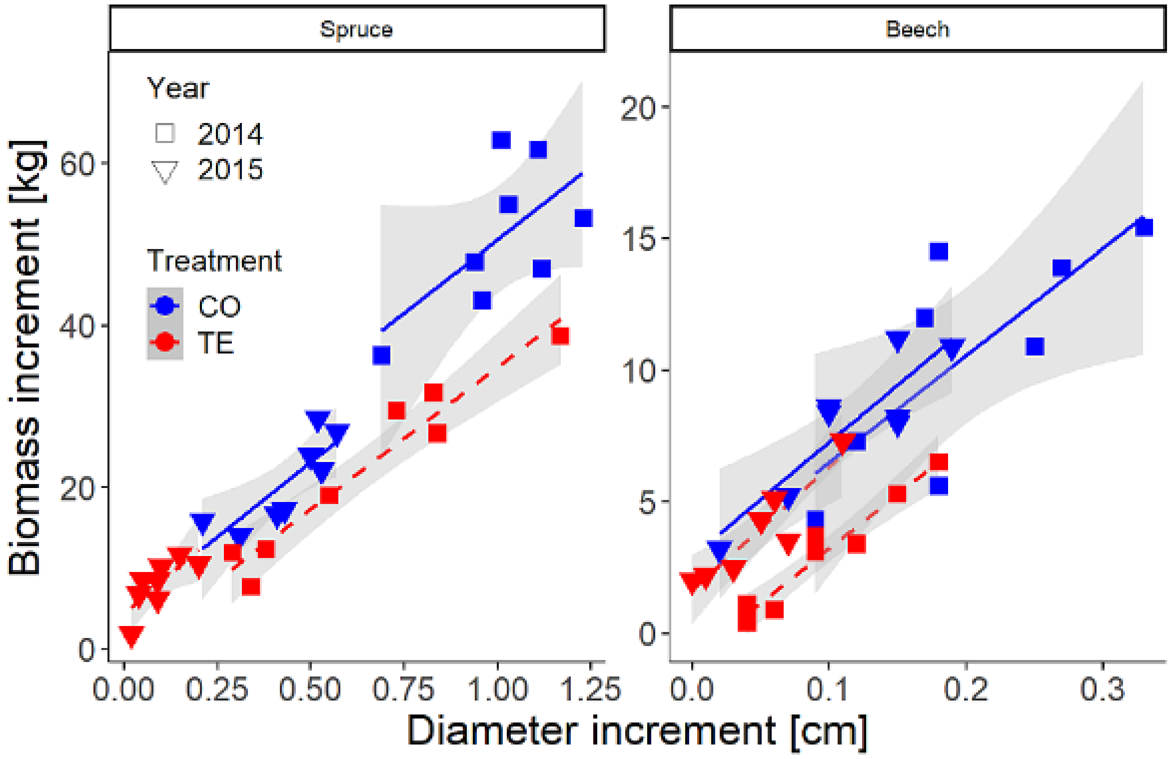

Figure 1*: Measured dendrometer increment at 1.3 m height vs modeled stem biomass increment versus; with triangles showing the values for spruce and circles for beech and in red for throughfall exclusion (TE) and blue for control (CO). Red and blue lines show Pearson correlations for each group of beech and spruce for CO (solid lines) and TE (dashed lines). Gray areas show the 95% confidence interval for each group. Please note the different scaling of the beech and spruce graph.*

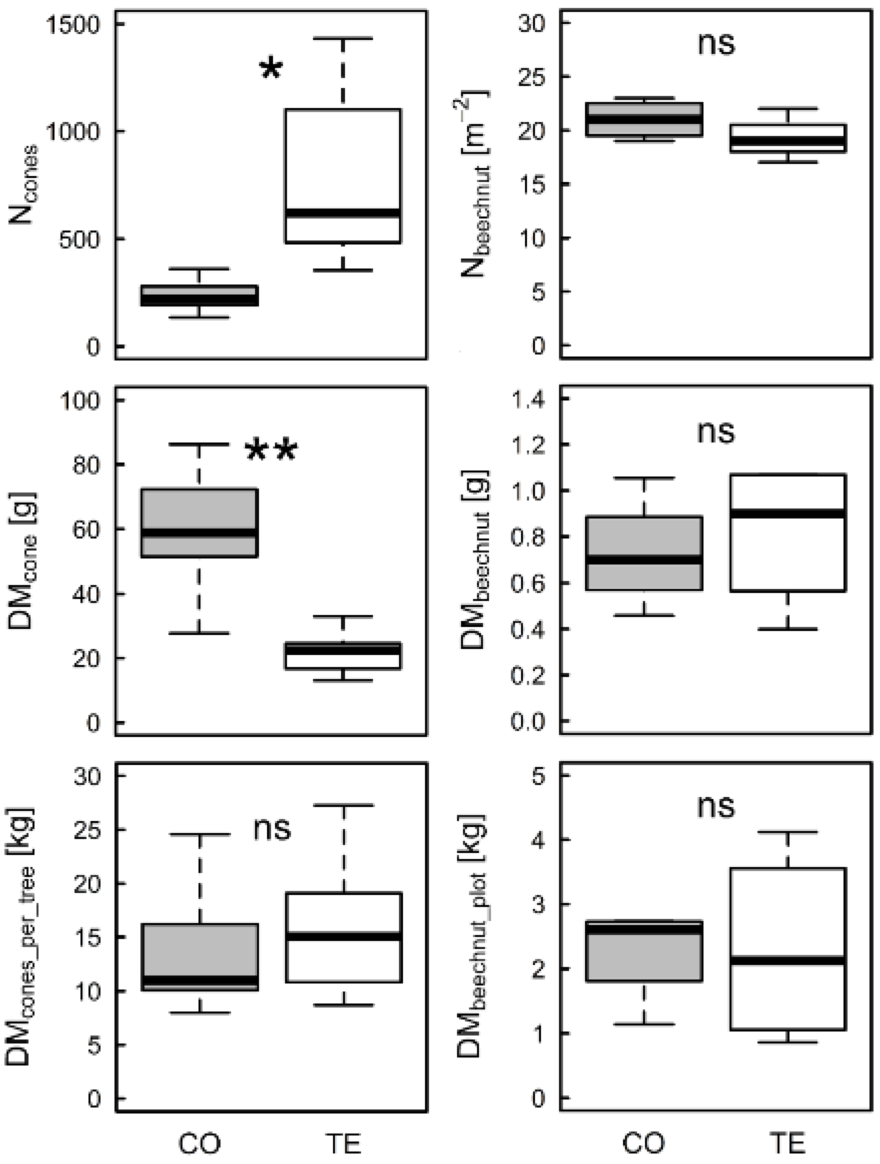

*Figure 2: Number (top), dry mass of single (middle) and accumulated mass (bottom) of reproductive structures in spruce per tree (left) and beech per plot (right; p-values: ns > 0.05, * < 0.05 and ** < 0.01).*

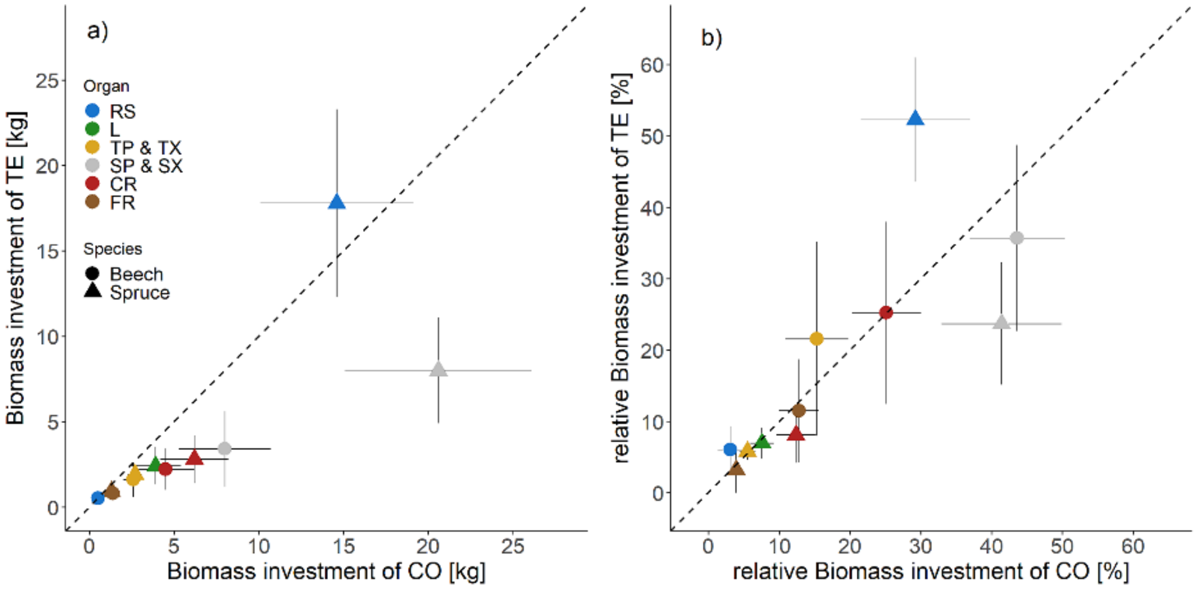

Figure 3: Total (a, data from table 3) and relative modeled biomass investment (b) in the masting year 2015. (RS = beechnut or cones (blue), leaves = L (green), branches/twigs = TP & TX (beige), stem = SP & SX (gray), coarse roots = CR (red) and fine roots = FR (brown). Data are given as the mean ± 1SD.

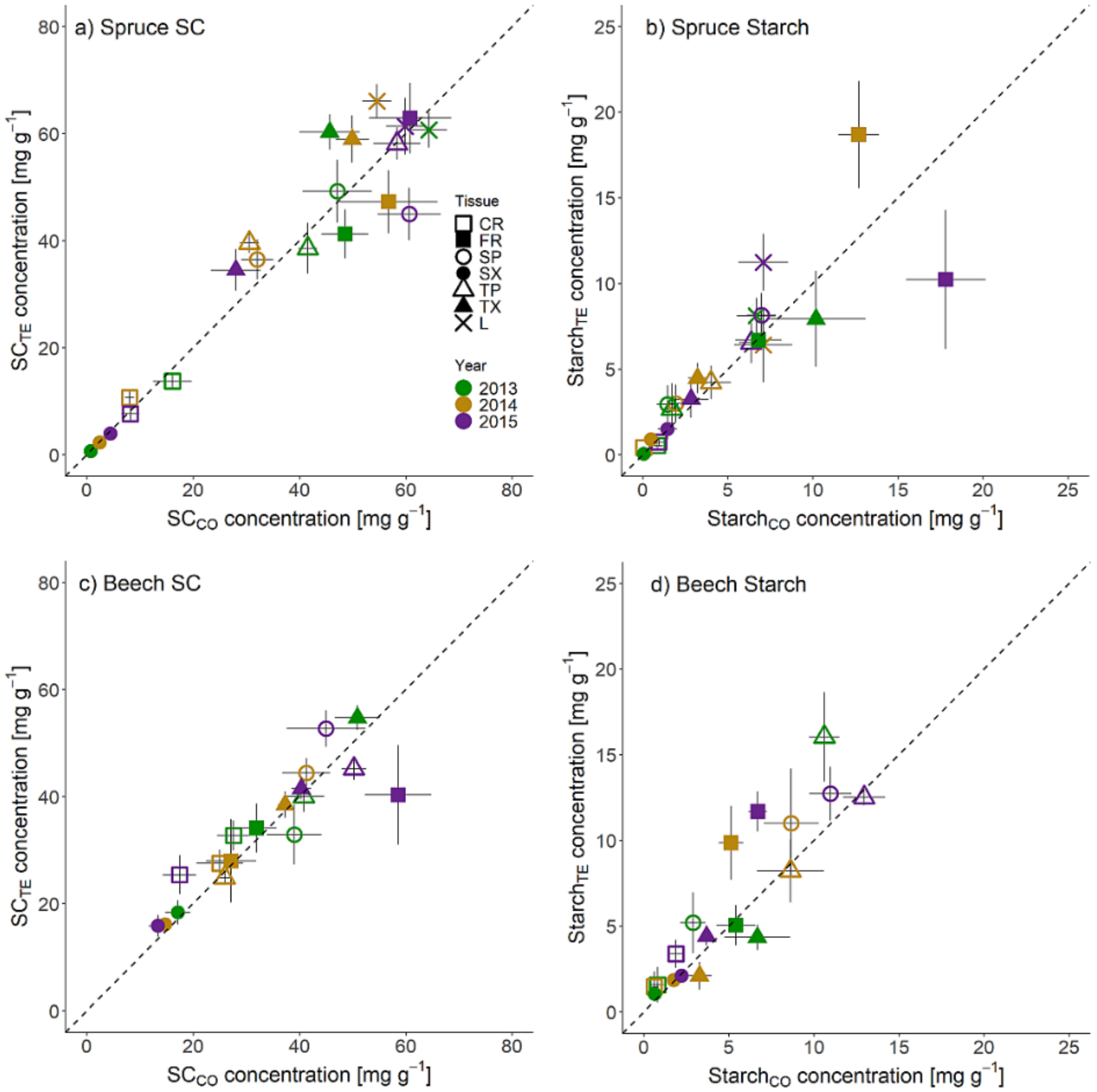

Figure 4: Mean soluble carbohydrates (SC, left) and starch (right) concentrations of spruce (top) and beech (bottom) in different tissues. X-axis is giving the values of control (CO) trees and the Y-axis of throughfall-exclusion (TE = drought stressed) trees. Coarse root = CR (open square), fine root = FR (closed square), stem phloem = SP (open circle), stem xylem = SX (closed circle), branch/twig phloem = TP (open triangle), branch/twig xylem = TX (closed triangle) and leaves = L (cross). Years are given by colors: 2013 (before drought in green), 2014 in beige and 2015 in purple. Data are given as mean ± 1 SE, dashed line = 1 to 1 line, with drought treatment: $P > 0.05$, tissue: $P < 0.0001$ and year: $P < 0.0001$ for both species).

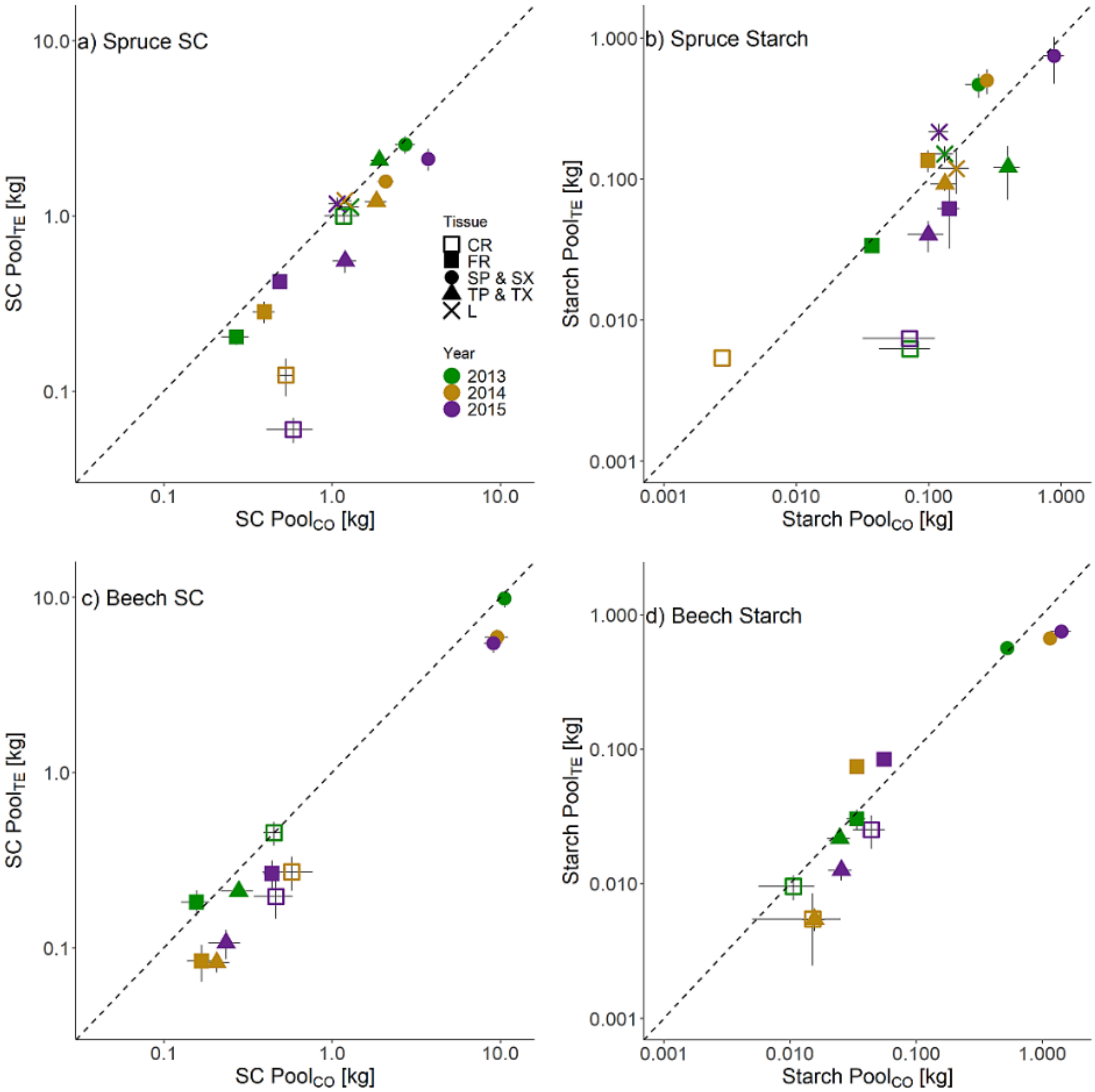

Figure 5: Mean soluble carbohydrates pool (SC, left) and starch pool (right) sizes of spruce (top) and beech (bottom) in different tissues. Coarse root = CR (open square), fine root = FR (closed square), stem phloem and xylem = SP & SX (closed circle), branch/twig phloem and xylem = TP & TX (closed triangle) and leaves = L (cross) and years (2013 (green) = before drought, 2014 (beige) = 1st year drought and 2015 (purple) = 2nd year drought) with the x-axis (in log-scale) showing the values of control (CO) trees and the y-axis (in log scale) of throughfall-exclusion (TE = drought stressed) trees. (Data are given as mean ± 1 SE, dashed line = 1 to 1 line, with treatment: $P < 0.01$, tissue: $P < 0.0001$ and year: $P < 0.0001$ for both species).

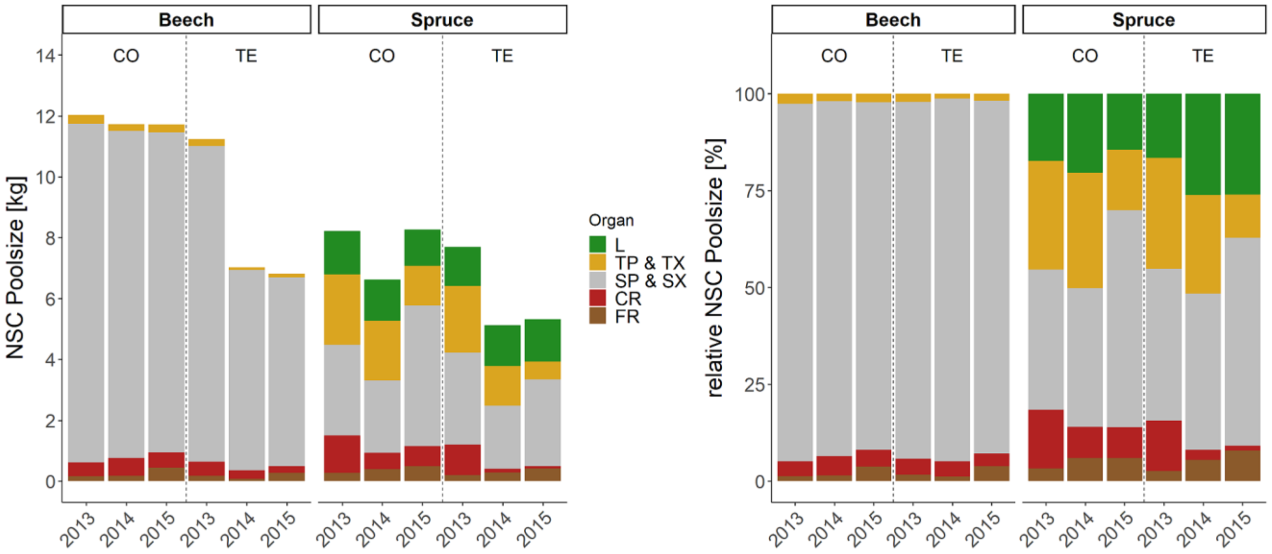

Figure 6: Total (a) and relative (b) NSC pool size of beech and spruce for different tissues (leaves = L (green), sum of branch/twig phloem and xylem = TP & TX (beige), sum of stem phloem and xylem = SP & SX (gray), coarse roots = CR (red) and fine roots = FR (brown) over three years, i.e. 2013 (before onset of drought treatment) and in 2014 and 2015 in control (CO) and drought stressed (TE) trees.

Tables

*Table 1: Pre-dawn water potential of beech and spruce at the end of the vegetation period 2013, 2014 and 2015 (Data are given as mean ± 1 SD, different letters indicate significant differences between groups (treatment*year)).*

Ψ_{PD} [MPa]	**Spruce**	
	CO	**TE**
2013	-0.52 ± 0.06 a	-0.49 ± 0.05 a
2014	-0.78 ± 0.31 a	-1.33 ± 0.12 b
2015	-1.38 ± 0.22 b	-1.68 ± 0.13 c

Ψ_{PD} [MPa]	**Beech**	
	CO	**TE**
2013	-0.41 ± 0.10 ab	-0.40 ± 0.08 ab
2014	-0.30 ± 0.04 a	-0.62 ± 0.08 bc
2015	-0.76 ± 0.24 c	-1.14 ± 0.31 d

*Table 2: Mean monthly volumetric soil water content (SWC) of control (CO) and throughfall-exclusion (TE) plots to a soil depth of 70 cm (from January 2013 to December 2015). Timespan of roof closure: from 6th of March to 12th of September in 2014 and 10th of March to 21st of November in 2015. Data are given as the monthly mean ± 1 SE per treatment and year. Statistically significant differences between CO and TE are given between each month for each year separately with p-value > 0.05 = ns, <0.05 = *, <0.01 = ** and <0.001 = ***). For further details see Grams et al. 2021.*

SWC [vol.-%]		**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
2013	**CO**	34.1 ±1.2	38.4 ±1.0	31.9 ±0.7	34.2 ±0.9	30.8 ±1.1	32.5 ±0.6	24.9 ±0.6	18.6 ±0.6	20.4 ±0.7	23.5 ±0.6	24.5 ±1.2	28.7 ±1.1
		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	TE	32.7 ±1.3	34.3 ±1.4	32.7 ±0.8	34.5 ±1.0	31.9 ±1.1	32.7 ±0.7	25.3 ±0.6	20.0 ±0.7	21.0 ±0.7	22.9 ±0.6	23.1 ±1.3	26.0 ±1.2
2014	**CO**	30.6 ±0.8	33.2 ±1.0	27.8 ±0.6	29.2 ±0.5	29.0 ±0.7	24.7 ±0.6	22.1 ±0.5	24.1 ±0.6	24.7 ±0.6	26.3 ±0.7	30.6 ±0.7	32.7 ±0.6
		ns	ns	ns	ns	*	*	*	**	**	**	***	***
	TE	27.7 ±1.0	29.1 ±1.1	25.4 ±0.7	27.8 ±0.6	23.4 ±0.7	19.5 ±0.6	17.5 ±0.5	16.5 ±0.6	16.7 ±0.5	17.0 ±0.6	17.4 ±0.6	17.6 ±0.6
2015	**CO**	35.6 ±0.5	35.0 ±0.5	33.8 ±0.5	34.2 ±0.4	35.5 ±0.5	34.6 ±0.4	24.9 ±0.5	20.4 ±0.4	20.1 ±0.5	22.5 ±0.5	24.3 ±0.5	27.7 ±0.5
		***	***	**	***	***	***	**	ns	ns	*	**	**
	TE	20.1 ±0.6	21.3 ±0.6	21.3 ±0.5	22.6 ±0.5	20.6 ±0.5	19.0 ±0.4	17.5 ±0.5	16.8 ±0.4	16.7 ±0.4	16.7 ±0.4	16.7 ±0.4	17.8 ±0.4

*Table 3: Modeled biomass increment (BI) by the model BALANCE of spruce (top) and beech (bottom) for 2014 and 2015. Data are given as mean ± 1 SD, p-values: ns > 0.1, • < 0.1, * < 0.05, ** < 0.01 and *** < 0.001.*

Spruce	**2014**			**2015**		
BI [kg]	**CO**		**TE**	**CO**		**TE**
Fine root	1.4 ± 0.4	•	0.9 ± 0.2	1.3 ± 0.2	ns	0.9 ± 0.2
Coarse root	7.0 ± 3.1	ns	7.5 ± 3.4	6.2 ± 2.0	*	2.8 ± 1.4
Stem	50.9 ± 9.1	***	22.2 ± 11.1	20.6 ± 5.5	*	8.0 ± 3.1
Branch/Twig	2.6 ± 0.7	*	1.9 ± 0.7	2.7 ± 0.4	*	1.9 ± 0.4
Leaf	4.7 ± 1.9	ns	3.3 ± 1.3	3.9 ± 1.5	ns	2.4 ± 1.1
Beech	**2014**			**2015**		
BI [kg]	**CO**		**TE**	**CO**		**TE**
Fine root	1.4 ± 0.2	*	0.7 ± 0.1	1.4 ± 0.4	•	0.8 ± 0.2
Coarse root	3.3 ± 1.8	ns	2.1 ± 1.2	4.5 ± 1.7	*	2.2 ± 1.2
Stem	10.5 ± 4.3	**	3.1 ± 2.2	8.0 ± 2.7	**	3.4 ± 2.2
Branch/Twig	2.6 ± 0.2	**	1.2 ± 0.7	2.6 ± 0.6	*	1.6 ± 1.0

Supplement

Table S1: Measured mean soluble carbohydrate (SC in mg/ mg drymass) concentrations of spruce and beech in different tissues for 2013 to 2015. Data are given as mean ± 1 SE.

Spruce	**2013**		**2014**		**2015**	
SC [mg/mg]	**CO**	**TE**	**CO**	**TE**	**CO**	**TE**
Fine root	48.59 ± 4.40	41.24 ± 4.55	56.80 ± 9.20	47.25 ± 5.91	60.85 ± 7.76	62.88 ± 6.58
Coarse root	16.13 ± 3.63	13.74 ± 1.17	8.04 ± 0.88	10.67 ± 1.39	8.20 ± 1.67	7.66 ± 1.54
Stem xylem	0.80 ± 0.04	0.70 ± 0.04	2.48 ± 0.19	2.31 ± 0.14	4.49 ± 0.41	3.95 ± 0.55
Stem phloem	47.11 ± 6.53	49.23 ± 5.86	32.06 ± 3.01	36.48 ± 3.70	60.65 ± 5.91	44.97 ± 4.88
Twig xylem	45.69 ± 5.68	60.26 ± 3.29	49.93 ± 3.14	58.94 ± 4.39	28.02 ± 4.66	34.52 ± 3.88
Twig phloem	41.58 ± 1.73	38.56 ± 4.71	30.58 ± 1.78	39.58 ± 1.87	58.33 ± 4.41	58.16 ± 3.04
Leaf	64.38 ± 3.38	60.65 ± 3.28	54.60 ± 2.73	66.04 ± 3.19	59.91 ± 3.59	61.37 ± 5.26

Beech	**2013**		**2014**		**2015**	
SC [mg/mg]	**CO**	**TE**	**CO**	**TE**	**CO**	**TE**
Fine root	31.97 ± 3.69	34.11 ± 4.56	27.16 ± 4.70	27.98 ± 7.77	58.58 ± 6.23	40.32 ± 18.60
Coarse root	27.61 ± 3.05	32.73 ± 2.80	25.01 ± 4.37	27.53 ± 2.51	17.46 ± 3.15	25.39 ± 3.62
Stem xylem	17.15 ± 2.36	18.33 ± 2.25	14.78 ± 1.25	16.08 ± 0.96	13.38 ± 1.75	15.83 ± 2.03
Stem phloem	39.02 ± 14.53	32.89 ± 5.60	41.29 ± 4.49	44.41 ± 2.72	45.03 ± 7.39	52.67 ± 3.40

Twig xylem	50.90 ± 4.23	54.74 ± 2.29	37.30 ± 1.32	38.47 ± 2.45	40.35 ± 1.84	41.47 ± 1.96
Twig phloem	40.83 ± 3.82	40.02 ± 2.91	25.94 ± 1.45	24.83 ± 0.99	50.26 ± 2.34	45.19 ± 5.45

Table S2: Measured starch (in mg/ mg drymass) concentrations of spruce and beech in different tissues for 2013 to 2015. Data are given as mean ± 1 SE.

Spruce	**2013**		**2014**		**2015**	
Starch [mg/mg]	**CO**	**TE**	**CO**	**TE**	**CO**	**TE**
Fine root	6.80 ± 1.35	6.70 ± 0.21	12.69 ± 1.19	18.68 ± 3.12	17.81 ± 2.34	10.22 ± 4.05
Coarse root	0.86 ± 0.34	0.54 ± 0.21	0.05 ± 0.04	0.42 ± 0.10	0.94 ± 0.35	0.74 ± 0.22
Stem xylem	0.06 ± 0.06	0.05 ± 0.04	0.49 ± 0.03	0.89 ± 0.12	1.45 ± 0.56	1.49 ± 0.58
Stem phloem	1.45 ± 0.63	2.95 ± 1.10	1.95 ± 0.54	2.98 ± 1.11	6.98 ± 0.85	8.15 ± 1.29
Twig xylem	10.18 ± 2.92	7.93 ± 2.80	3.21 ± 0.55	4.47 ± 0.88	2.84 ± 1.02	3.24 ± 1.09
Twig phloem	1.71 ± 0.59	4.71 ± 1.49	4.01 ± 1.16	4.22 ± 0.97	6.37 ± 0.71	6.53 ± 1.18
Leaf	6.67 ± 1.14	8.11 ± 1.05	7.08 ± 1.70	6.41 ± 2.18	7.08 ± 1.45	11.23 ±1.65
Beech	**2013**		**2014**		**2015**	
Starch [mg/mg]	**CO**	**TE**	**CO**	**TE**	**CO**	**TE**
Fine root	5.41 ± 1.14	5.04 ± 1.18	5.13 ± 0.72	9.58 ± 2.15	6.71 ± 0.54	11.68 ± 1.17
Coarse root	0.81 ± 0.37	1.57 ± 1.03	0.62 ± 0.26	1.46 ± 0.87	1.89 ± 0.52	3.37 ± 0.81

Stem xylem	0.64 ± 0.10	1.03 ± 0.16	1.76 ± 0.09	1.84 ± 0.23	2.23 ± 0.36	2.09 ± 0.30
Stem phloem	2.89 ± 0.74	5.19 ± 1.78	8.67 ± 1.60	11.00 ± 3.19	10.97 ± 1.23	12.73 ± 1.57
Twig xylem	6.67 ± 1.93	4.34 ± 0.73	3.27 ± 0.71	2.10 ± 0.80	3.69 ± 0.24	4.40 ± 0.53
Twig phloem	10.61 ± 0.89	16.04 ±2.61	8.62 ± 1.97	8.22 ± 1.84	12.95 ± 1.23	12.51 ± 0.47

Table S3: Modeled biomass (BM in kg) of spruce (top) and beech (bottom) for 2013 to 2015. Data are given as mean ± 1 SD.

Spruce	**2013**		**2014**		**2015**	
BM [kg]	**CO**	**TE**	**CO**	**TE**	**CO**	**TE**
Fine root	5.5 ± 0.4	5.0 ± 0.9	6.9 ± 0.6	5.9 ± 0.9	8.2 ± 0.7	6.8 ± 0.7
Coarse root	63.1 ± 22.6	65.2 ± 18.3	70.1 ± 24.7	72.7 ± 19.3	76.3 ± 26.2	75.5 ± 20.0
Stem	567.7 ± 141.6	563.2 ± 123.5	618.6 ± 143.6	585.4 ± 116.3	639.2 ± 144.5	593.4 ± 117.8
Branch/Twig	17.8 ± 3.3	17.3 ± 4.3	20.4 ± 3.7	19.2 ± 4.7	23.1 ± 4.0	21.1 ± 4.6
Leaf	17.5 ± 5.2	16.8 ± 5.2	22.2 ± 6.8	20.1 ± 4.9	26.0 ± 8.1	22.5 ± 5.5
Beech	**2013**		**2014**		**2015**	
BM [kg]	**CO**	**TE**	**CO**	**TE**	**CO**	**TE**
Fine root	4.8 ± 0.6	5.4 ± 0.8	6.2 ± 0.7	6.1 ± 0.9	7.6 ± 0.9	6.9 ± 0.9
Coarse root	55.3 ± 9.5	53.5 ± 10.0	58.6 ± 11.4	55.6 ± 11.1	63.1 ± 13.0	57.8 ± 12.3
Stem	463.3 ± 191.6	453.2 ± 236.4	473.8 ± 193.7	456.3 ± 237.3	481.8 ± 195.1	459.7 ± 238.6

Branch/Twig	15.5 ± 0.9	15.7 ± 3.3	18.1 ± 0.9	16.9 ± 4.0	20.7 ± 1.0	18.6 ± 5.0