

Root carbon resources determine survival and growth of young trees under long drought in combination with fertilization

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Abstract

Current increases in not only the intensity and frequency but also the duration of drought events could affect the growth, physiology, and mortality of trees. We experimentally studied the effects of drought duration in combination with fertilization on leaf water potential, gas exchange, growth, tissue levels of non-structural carbohydrates (NSCs), tissue NSC consumption over winter, and recovery after drought release in oak (*Quercus petraea*) and beech (*Fagus sylvatica*) saplings. Long drought duration (> 1 month) decreased leaf water potential, photosynthesis, and NSC concentrations in both oak and beech saplings. Nitrogen fertilization did not mitigate the negative drought effects on both species. The photosynthesis and relative height increment recovered in the following rewetting year. Height growth in the rewetting year was significantly positively correlated with both pre- and post-winter root NSC levels. Root carbon reserve is critical for tree growth and survival under long-lasting drought. Our results indicate that beech is more sensitive to drought and fertilization than oak. The present study, in a physiological perspective, experimentally confirmed the view of Ellenberg (2009) that the European beech, compared to oak, may be more strongly affected by future environmental changes.

1 Introduction

Global climate change has led to and is continuously resulting in increases not only in drought intensity but also in the frequency and duration of drought events globally (IPCC, 2013). Two main hypotheses have been proposed and are currently debated to explain the mechanisms for the widespread forest dieback caused by increased drought events (Hartmann *et al.*, 2018; Spinoni *et al.*, 2018): trees would die (1) due to hydraulic failure or (2) as a result of carbon starvation (McDowell & Allen, 2015; Gessler *et al.*, 2018). The hydraulic failure hypothesis proposes that the tree mortality results from the embolism of the xylem vessels under high evaporative demand and restricted soil water availability (Nardini *et al.*, 2013) whereas

the carbon starvation hypothesis suggests that tree mortality would be caused by a carbon supply limitation due to stomatal closure and thus reduced photosynthesis that cannot cover the carbon and energy demand for maintenance processes (McDowell *et al.* , 2008; Rowland *et al.* , 2015). Concerning carbon starvation, several studies have shown that the contents of starch, a compound that serves as carbon storage and is build up when assimilation exceeds plant's demand for carbon, were strongly reduced by severe soil water deficit (McDowell, 2011), implying carbon limitation (Reinhardt *et al.* , 2015). Previous studies also suggested that hydraulic perturbation could prevent phloem transport (Sevanto, 2014) and consequently constrains carbon accessibility (Sala *et al.* , 2012; Hartmann & Trumbore, 2016), even though the carbon availability in the tree crown area might not be restricted. Thus, hydraulic failure and carbon starvation are often seen to be associated with each other in the cascade of drought events leading to tree mortality (Adams *et al.* , 2017).

Droughts, however, are very tougher to be defined, and thus a universally accepted drought definition considering both water deficit intensity and duration is still lack (Buitink *et al.* , 2021). Previous drought-related forest and agricultural studies, especially manipulation experiments, mainly focused on the effects of drought intensity (e.g. various levels of watering such as very limited, limited and optimum watering) on trees or plants (Allen *et al.* , 2010; Yuet *et al.* , 2021), while less is known about the effects of drought duration on trees' physiology, growth and mortality. A long-lasting drought event, may cause irreversible changes in plant physiology which are different from those found in relatively short-term severe droughts. For example, recurrent or short-term lasting drought events may allow trees to recover and even acclimate to water restriction (Gessler *et al.* , 2020), and thus, permit them to survive in the long-term. However, longer term lasting drought events may strongly affect the recovery ability of trees. Currently, unexpected whole-season drought occurs more frequently in many regions around the world. For instance, since the beginning of the 21st century Europe already experienced severe drought summer of 2003, 2010, 2013, 2015, and 2018 (Hane *et al.* , 2018; Brunner *et al.* , 2019). Therefore, mechanistic understanding of tree and forest responses to various drought duration is particularly critical for sustainably forest management under future climate change.

Theoretically, trees' resilience and resistance to drought stress and the recovery and thus survival ability should be associated with the resource storage and availability. Non-structural carbohydrates (NSC = soluble sugars + starch) among other resources (e.g. nutrients, see Gessler *et al.* 2017) are known to contribute to tree resilience after stress (Li *et al.* , 2002; Li *et al.* , 2008b). For many years, reserve storage was considered as a passive process resulting from an accumulation of resources when uptake and assimilation exceeded growth demand (Sala *et al.* , 2012; Wiley & Helliker, 2012). An alternative hypothesis was proposed in which reserves storage would be rather an active process during the growing season and would act as a sink competing with other sinks (e.g. growth and reproduction) for available resources (Wiley & Helliker, 2012). Several studies investigated the NSC levels of trees during and at the end of the growing season following a growing-season-long drought (Liet *et al.* , 2013; Schönbeck *et al.* , 2018; Schönbeck *et al.* , 2020a; Schönbeck *et al.* , 2020b). They found a drought-induced growth reduction but did not observe a drought-induced NSC decrease of trees, suggesting an active NSC storage under drought at the expense of growth (Wiley & Helliker, 2012; Li, M-H *et al.* , 2018). Recent evidence indicates that stress actively induces NSC transfer from aboveground tissues to roots stored (Kannenber *et al.* , 2018; Li, W *et al.* , 2018). In contrast, Li W. *et al.* (2018) analyzed 27 case studies and found that drought decreased NSC concentration by 17.3% in roots, while it did not change NSC in aboveground tissues in the current season. To our knowledge, even less is known about the changes of NSC over winter (post- vs. pre-winter) in trees previously stressed by drought.

Recently, it was proposed that nutrient addition (i.e. fertilization) will affect the fitness of trees under dry conditions, showing intensifying or mitigating effects on trees' tolerance to drought (Kreuzwieser & Gessler, 2010; Gessler *et al.* , 2017; Schönbeck *et al.* , 2020b). Nitrogen (N) deficiency can increase the sensitivity of stomata to low leaf water potential (Radin & Ackerson, 1981; Ghashghaie & Saugier, 1989), which further increases the risk of drought-induced carbon starvation (McDowell, 2011). N itself is a main growth limiting nutrient in temperate terrestrial ecosystems and is also a major component of Rubisco and other photosynthetic enzymes and structures which regulate the photosynthetic activity and thus carbon gain and the NSC level of trees in response to environmental factors such as drought (Bond *et al.* , 1999;

Meng *et al.*, 2016). Schönbecker *et al.* (2020a) found that negative effects of moderate drought intensity (but not of severe drought) could be compensated by increased nutrient availability in Scot pine saplings. In contrast, Jacobs *et al.* (2004) reported that fertilization with blended fertilizer impaired the root system development and drought avoidance ability of drought-stressed Douglas-fir seedlings. Similarly, Dziedek *et al.* (2016) found that nitrogen addition increased the drought sensitivity of saplings of several deciduous tree species (Dziedek *et al.*, 2016). Despite these studies, there is a strong knowledge gap about drought and nutrient interaction and especially about whether and to what extent nutrient addition affects winter NSC consumption and thus growth recovery in the season following drought.

The species, *Quercus petraea* (Matt.) Liebl. (oak) and *Fagus sylvatica* L. (beech), are two coexisting species in European forests. According to (Ellenberg, 2009), oak will become more competitive than beech at sites where as July-temperatures increase to $>18^{\circ}\text{C}$ and precipitation decreases to <600 mm/year as a result of climate change. We carried out a greenhouse experiment to explore the effects of different drought duration in combination with fertilization on the physiology, mortality and growth of these two species. We mainly focused on the following research questions: 1) How does drought duration influence trees' performance; 2) How does various drought duration in previous growing season affect tree's winter NSC consumption and thus the over-winter NSC change? 3) Does post-winter NSC level (at the early beginning of next growing season) rather than pre-winter NSC level determine growth recovery of previously drought-stressed trees? 4) Can fertilization mitigate the negative drought effects on trees as proposed by Gessler *et al.* (2017)?

2 Materials and Methods

2.1 Experimental design and treatments

The experiment was carried out in the greenhouse of the Swiss Federal Institute for Forest, Snow and Landscape Research WSL ($47^{\circ}21'48''$ N, $8^{\circ}27'23''$ E, 545 m a.s.l.), Birmensdorf, Switzerland. On February 27, 2018, 3 years old sessile oak and European beech saplings (~ 30 -40 cm in height) were planted into 10-liter plastic pots (26 cm in diameter). After transplanting into the pots, the plants were grown for 4 weeks under well-watered conditions (irrigation every 2-3 days) in the greenhouse to recover from the transplant shock. The cultivation soil consisted of semi-decomposed humus and commercial potting soil. The initial soil nitrogen and carbon contents were: 39 mg N kg^{-1} soil for $\text{NH}_4^{+}\text{-N}$, 573 mg N kg^{-1} soil for $\text{NO}_3^{-}\text{-N}$, 22.42% for soil C, and 0.82% for total soil N. The greenhouse temperature and humidity during the period of drought duration treatment are shown in Fig. S1.

A split-plot experimental design with three blocks was employed in this study. Each block was divided into two main plots, one of which was randomly assigned for oak (48 individuals) and the other one for beech (48 individuals) (Fig. S2). Each plot was then divided into two sub-plots, one of which was randomly assigned for fertilization (24 individuals) and the other one for the non-fertilization treatment (ambient, 24 individuals) (Fig. S2). Each sub-plot was further divided into four sub-sub-plots (rows) randomly assigned for one of the four drought duration treatments (6 individuals each). Therefore, a total of 288 plant individuals (144 individuals for each species) was included (Fig. S2).

The fertilization treatment was conducted on 10 June 2018 (just prior to the drought duration treatment) (Fig. S2). The fertilizer (Osmocote Exact 3-4M Standard, 7.0 % $\text{NO}_3^{-}\text{-N}$, 9.0% $\text{NH}_4^{+}\text{-N}$, 9 % P_2O_5 , ICL, Suffolk, UK), equal to 1.68 g N kg^{-1} dry soil (0.945 g N kg^{-1} dry soil $\text{NH}_4^{+}\text{-N}$, 0.735 g N kg^{-1} dry soil $\text{NO}_3^{-}\text{-N}$) was added to each pot assigned for fertilization.

After the N fertilization, plants were exposed to four drought duration treatments for 4 months in 2018 (Fig. S3). Plants in the well-watered treatment (D0) were watered thoroughly once a week. There were two moderate drought duration treatments, one of which was watered thoroughly biweekly (D1), and the other one was watered thoroughly once a month (D2) (Fig. S3). The treatment of the longest drought duration in the present study was watered thoroughly once two months (D3) (Fig. S3). We focused on the duration between two watering events and thus did not measure the soil water condition. After the harvest occurred on 1 October 2018, all remaining plants were treated (e.g. well-watering) in the same way until the end of the experiment (Fig. S3).

2.2 Leaf water potential, gas exchange, height measurement and mortality record

To detect the effects of drought duration, all measurements and sampling were always carried out directly before the next watering (Fig. S3).

Three plants from both nutrient and all drought treatments were randomly selected from each sub-sub-plot and three mature leaves from each selected plant were used for water potential and gas-exchange measurements during the drought treatment in 2018. The midday leaf water potential (ψ_{leaf}) was measured between 12:00 and 14:00 h on 5 August 2018 (Fig. S3), with a Scholander bomb (Model 600 pressure bomb; PMS Instrument Company, Albany, NY, USA). Net-photosynthesis (A_{leaf}) was measured on 6 August and 28 September 2018 (Fig. S3), and after rewetting in the following year on 24 June 2019, with a LiCor 6400 system (LI-COR, Lincoln, USA). A_{leaf} was measured at 400 $\mu\text{mol mol}^{-1} \text{CO}_2$, 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation, ca. 65% relative humidity and 25°C air temperature.

Plant height of each individual was measured on 30 May 2018 (initial height), on 1 October 2018 (end of the treatment), and on 8 October 2019 (after one recovery season) (Fig. S3), and height increments were calculated.

2.3 Harvest and sampling

Destructive sampling (harvest) was conducted twice, one on 1 October 2018 as pre-winter samples, and the other one on 2 April 2019 as post-winter samples. One plant was randomly selected from each sub-sub-plot for each harvest time, and 48 individuals (24 individuals for each species; 3 for each nutrient and drought treatment) were harvested for each time (Fig. S2). The whole plant was harvested, the roots were carefully washed, and the plants were separated into leaves (Oct. 2018 only), shoots, and belowground tissue and separately sampled (leaves, shoots, mixed roots (i.e., fine and coarse roots). The samples were immediately put in an oven at 105°C for half an hour to stop the metabolic activity, and then were dried at 65°C until stable weight. The dried samples were ground to fine powder, using a ball mill (MM 400; Retsch, Haan, Germany), for NSC analysis.

2.4 Mortality record

At the beginning of the experiment on 31 May 2018, there were 6 individuals for each treatment in each sub-sub-plot (Fig. S2). During the experiment period, one plant out of the 6 individuals in each sub-sub-plot was destructively harvested on each sampling date of 1 October 2018 and 2 April 2019 (Fig. S2). Thus, each species had, theoretically, 4 individuals (= 6 - 2) left in each sub-sub-plot (Fig. S2), and each species had 12 individuals (4 x 3 blocks = 12) for each treatment across the three blocks available at the end of the recovery season 2019. However, in some sub-sub-plots all the 4 plants died by the end of 2019. Therefore, it was not possible to statistically analyze the treatment effects on the mortality. Instead, we recorded the total dead individuals (A) that did not sprout new leaves or shoots from any part of a plant during the recovery season across the three blocks, and calculated the total mortality rate with $(A / 12) \times 100\%$.

2.5 Non-structural carbohydrate analysis

The NSC concentrations were measured according to the method by Wong (Wong, 1990) as modified by Hoch et al. (Hoch *et al.*, 2002). NSC refers to the sum of mobile sugars (mainly glucose, fructose and sucrose) and starch. First, 10-12mg sample powder was boiled in 2 ml distilled water for half an hour. Then, 200 μl aliquot mixed with Invertase (Sigma-Aldrich, Buchs, Switzerland) were extracted to degrade sucrose to glucose and fructose. After centrifugation, glucose hexokinase and phosphogluconate isomerase (Sigma-Aldrich, Buchs, Switzerland) were added. The concentration of sugars was obtained as the total amount of glucose that was determined by 340nm photometry (HR 7000, Hamilton, Rone, NE, USA) in a 96-well microplate photometer (Sigma-Aldrich, Buchs, Switzerland). 500 μl extract were taken from the sample aliquot and reacted with amyloglucosidase (Sigma-Aldrich, Buchs, Switzerland) for 15h at 49°C, to break down starch to glucose, and to measure the total NSC concentration. The starch concentration was calculated as total NSC minus soluble sugars. NSC concentrations are expressed on a dry mass (d.m.) basis.

2.6 Calculation and statistical analysis

The relative height increment rate (RHI) was calculated based on height measured on 30 May, 2018 (A), on 10 October 2018 (B), and on 8 October 2019 (C), using $[(B - A) / A] \times 100\%$ for the 2018 growth, and $[(C - B) / B] \times 100\%$ for the 2019 recovery growth. The NSC concentration was calculated as the sum of the concentration of soluble sugars plus that of starch for each sample.

All data were checked for normality with the Kolmogorov-Smirnov test and for homogeneity of variance with Levene's test. The effects of species, drought duration, N-fertilization, and their interactions on the parameters measured on each date were analyzed using linear mixed effect models, with block and main plot as random effects. Within each species, the effects of drought duration, N-fertilization, and their interactions on the parameters measured on each date were also analyzed using linear mixed effect models, with block and main plot as random effects, followed, if significant, by Tukey's post-hoc test. Regression analysis was used to test the relationship between 2019 relative height increment rate and tissue NSC levels (both pre- and post-winter). All analyses are carried out by the package "LME" in R v.3.2.5 (R Core Team).

3 Result

3.1 Mortality

After a recovery growing season that followed the drought duration treatment in combination with N-fertilization, the mortality of beech was higher than oak, and the mortality rate seemed to show a tendency to increase with drought duration for the two species (Fig. 1). Especially, the N-fertilized beech saplings had much higher mortality rate within each drought duration treatment category (Fig. 1b).

3.2 Leaf water potential and photosynthesis during drought duration treatment (2018)

Drought duration significantly decreased leaf water potential (Table 1, Fig. 2a,b) in both species. A significant S x D interaction ($P < 0.001$; Table 1) indicated that the leaf water potential of the two species responded to drought duration significantly differently, showing a ψ_{leaf} order of $D0 = D1 > D2 = D3$ for oak (Fig. 2a), and of $D0 > D1 > D2 = D3$ for beech (Fig. 2b). Neither N-fertilization nor any interaction of N with other factors affected leaf water potential (Table 1). In beech, N-fertilization significantly decreased ψ_{leaf} in D0 plants (Fig. 2b).

Species, drought duration, N-fertilization, as well as their two-way and three-way interactions significantly affected the A_{leaf} of plants two months after the onset of the drought treatment on 6 August 2018 (except for non-effects of N and S x N interaction) and after four months on 28 September 2018 (Table 1). Oak's A_{leaf} significantly decreased with drought duration for the two measurement dates (Fig. 2c,e), while in beech A_{leaf} significantly increased from D0 to D1, and then decreased in D2 and D3 (Fig. 2d,f) on both dates. N-fertilization seemed to decrease A_{leaf} of oak under D1 and D2 (Fig. 2c,e), whereas it significantly increased A_{leaf} of beech under D1 (Fig. 2d,f). Photosynthesis almost ceased in D3 plants after four-month of treatment (Fig. 2e,f), especially in beech (Fig. 2f).

During the drought duration treatment, the relative height increment (RHI) differed significantly only between species (Table 1), showing higher RHI in oak than in beech (Fig. 2g,h). Neither N and D, nor their interactions affected RHI (Table 1, Fig. 2g,h).

3.2 Pre-winter NSC after drought duration treatment

The pre-winter NSC levels in leaves differed significantly with species (Table 2). The shoot NSC levels, however, did not vary with species but were significantly affected by both drought treatment and N-fertilization (Table 2). The root NSC was significantly influenced by species and drought duration (Table 2). In addition, N-fertilization significantly interacted with species to affect the NSC levels in both shoots and roots (Table 2). Compared to the D0, D1 and D2 treatments, oaks in D3 had significantly higher leaf NSC levels (Fig. 3a), mainly caused by higher sugar concentration (Fig. S4a), but significantly lower shoot NSC (Fig. 3b) mainly resulting from significantly lower starch levels (Fig. S4e). The root NSC of oak tended to decrease with increasing drought duration (Fig. 3c), due to both decreasing sugar and starch levels (Fig. S4c,f). For

beech, both leaf and root NSC levels did not change with drought (Fig. 3f,h) but shoot NSC decreased with increasing drought duration (Fig. 3g) caused by both decreased sugars (Fig. S5b) and starch (Fig. S5l). N-fertilization significantly increased the shoot NSC in oak (Fig. 3b), whereas it significantly decreased NSC levels in shoots (Fig. 3g) and roots (Fig. 3h) of beech.

3.3 Post-winter NSC after drought duration treatment last growing season

The post-winter NSC levels in both shoots and roots were significantly affected by drought duration treatment in the previous growing season (Table 2). The post-winter root NSC was species-dependent ($P < 0.05$; Table 2), and species interacted with N-fertilization to influence the post-winter shoot NSC ($P < 0.05$; Table 2). There was no direct effect of N on NSC for both species (Table 2). Both shoots and roots of D3 oak had significantly lower post-winter NSC levels compared to the other drought treatments (Fig. 3d,e), mainly caused by both lower sugars and starch levels for shoots (Fig. S6a,c) and by lower sugar levels for roots (Fig. S6b). In beech only shoots of D2 and D3, due to both lower sugar and starch levels (Fig. S7a,c), showed significantly lower post-winter NSC levels compared to the other drought treatments (Fig. 3i,j). N-fertilization significantly increased shoot NSC in oak (Fig. 3d) but it had no effects on shoot (Fig. 3i) and root NSC (Fig. 3j) in beech.

3.4 Changes in NSC level over winter

Only N-fertilization significantly affected the over-winter NSC consumption (post-winter level minus pre-winter level) in shoots but not in roots (Table S1). Neither D nor N and their interactions changed the tissue NSC consumption in oak over-winter (Fig. 4a,c), while N-fertilized beech significantly decreased the over-winter NSC consumption in both shoots and roots (Fig. 4b,d). The changes in sugar/starch ratio were significantly influenced by both, species and N-fertilization (Table S1). N-fertilization tended to decrease the sugar/starch ratio in beech, especially in shoots of D3 plants (Fig. 4f,h), while the unfertilized oak saplings seemed to increase the tissue sugar/starch ratio over winter, especially the D3 plants (Fig. 4e,g).

3.5 Recovery responses to past drought and rewetting

After rewetting for one growing season, both the recovery photosynthesis measured on 10 June 2019 and recovery height growth measured on 8 October 2019 significantly responded to species only (Table 1), showing that both photosynthesis (Fig. 5a vs. 5b) and height growth (Fig. 5c vs. 5d) were greater in oak than in beech. Otherwise, previous season drought duration and N-fertilization treatment did not affect recovery responses of the two species (Fig. 5).

3.6 Recovery height growth in relation to pre- and post-winter NSC level

Within each species, no correlations between recovery growth and NSC storage were found. The pooled data of the two species showed that the relative height increment of recovery growth during the whole rewetting season following the drought duration treatment was marginally significantly correlated with pre-winter root NSC ($P = 0.07$; Fig. 6a) and significantly correlated with post-winter root NSC ($P = 0.01$; Fig. 6b), but not with both pre- and post-winter NSC levels in shoots (Fig. 6a,b).

4 Discussion

With increasing drought duration, tree mortality increased (Fig. 1), leaf water potential and photosynthesis decreased for the two species (Fig. 2), which is similar to those results found in drought intensity experiments with trees (Schönbeck *et al.*, 2018; Lauder *et al.*, 2019; Archambeau *et al.*, 2020; Schönbeck *et al.*, 2020a; Ouyang *et al.*, 2021). For instance, extreme drought was found to significantly decrease predawn water potential and net-photosynthetic rates and to increase the mortality for both *Pinus sylvestris* (Schönbeck *et al.*, 2020a) and *Quercus pubescens* saplings (Ouyang *et al.*, 2021). Drought, both severe drought intensity and long drought duration, decreases soil water availability and plant leaf water potential, and thus results in stomatal closure to prevent transpiration exceeding root water uptake capacity, which caused declined photosynthesis and CO₂ uptake (Li *et al.*, 2020). Duan *et al.* (2019) found that severe drought intensity with short duration led to a stronger decrease in leaf water potential and photosynthesis of three tree species

(*Syzygium rehderianum*, *Castanopsis chinensis* and *Schima superba*) than moderate drought with longer duration. The water potential of *Robinia pseudoacacia* exhibited a linear decline with increasing drought duration, while *Quercus acutissima*'s water potential remained relatively stable during the first month of mild drought (Li *et al.* , 2020), and thus Liet *al.* (2020) concluded that the two tree species differ in their sensitivity to drought (Bhusal *et al.* , 2021), which confirmed that *Quercus* species are anisohydric plants (Sade *et al.* , 2012).

However, in spite of decreased photosynthesis (Fig. 2c-f) and NSC levels (Fig. 3b, g, c, h) with increasing drought duration, the relative tree height increment of the two species did not differ among the drought treatments in our study (Fig. 2g, h). Li *et al.* (2013), Schönbeck *et al.* (2018, 2020a), and Ouyang *et al.* (2021) found that drought-stressed trees maintained relatively stable NSC levels at the expense of growth, implying an active process of NSC storage (Li, M-H *et al.* , 2018). For example, drought declined the growth but did not decrease tissue NSC level in *Quercus faginea* and *Pinus halepensis* (Sanz-Perez *et al.* , 2009). The present study, however, seemed to support the view of Martínez-Vilalta (2016) that NSC storage is mainly a passive process following the growth priority, because the growth did not vary with drought duration (Fig. 2g,h) while the NSC levels in storage tissues, especially in shoots, decreased with increasing drought duration (Fig. 3b,c,d,e,g,h,i,j). In this case, for example, the mortality (Fig. 1b) of fertilized beech under D2 and D3 is thus mainly a result of carbon limitation that was confirmed by very low leaf photosynthetic rate (Fig. 2d, f) and near-zero NSC level at the end-season (Fig. 3g, h). McDowell *et al.* (2011) proposed that NSC concentrations can increase initially under drought due to the faster decline of growth than photosynthesis, but NSC concentrations may decline later on due to the prolonged suppression of photosynthesis and the utilization of stored C for meeting C demands especially under extreme drought.

Similar to results gained from most drought intensity experiments with trees (Li *et al.* , 2013; Schonbeck *et al.* , 2018; Schonbeck *et al.* , 2020a; Zhang *et al.* , 2020; Ouyang *et al.* , 2021), the present study found that the longer drought duration treatments (D2, D3) did not decrease the end-season leaf NSC (pre-winter) levels (Fig. 3a,f), although the D2 and D3 treatment significantly decreased leaf photosynthesis of the two species (Fig. 2c-f). This might be explained by the osmoregulation strategy of plants suffering from drought stress on the one hand (O'Brien *et al.* , 2014; Dickman *et al.* , 2015), and on the other hand, it may be a result of basipetal carbon translocation failure (Rowland *et al.* , 2015), if the phloem function becomes impaired and carbon translocation gets limited or stopped by hydraulic failure caused by severe or long drought stress (Griffin-Nolan *et al.* , 2021). In this case, lower NSC levels in the sink tissues of shoots, and especially roots, and thus carbon limitation may be expected. Recently, this expectation has been repeatedly confirmed in severe drought-stressed trees in controlled drought intensity experiment (e.g. Schonbeck *et al.* , 2020a; Ouyang *et al.* , 2021), and also in trees under longer drought duration (D2, D3) treatment in the present study (Fig. 3b,g,c,h). Therefore, it may be speculated that a hydraulic failure induced carbon limitation seems to be the physiological mechanism underlying the high mortality of beech saplings, particularly the N-fertilized D2 and D3 beech (Fig. 1b) which had very low end-season shoot and root NSC levels close to zero (Fig. 3g,h). These results also seem to exclude that limited sink activity, e.g. in root tissues as a result of drought is responsible for reduced sugar transport from the leaves to the sink tissues as in that case increased NSC concentrations are to be expected in both, roots and shoots (Hagedorn, F. *et al.* , 2016; Gessler & Grossiord, 2019).

We found that the responses of end-season NSC level to drought duration seemed to be both species- and tissue type-dependent (Fig. 3a-b, Fig. 3f-h). For instance, leaf NSC increased (Fig. 3a) but shoot NSC decreased (Fig. 3b) in oak with increased drought duration, while they did not change in beech (Fig. 3f,g). Similar to beech, root NSC reserve of aspen (*Populus tremuloides*) seedlings didn't change over a 3-month period of severe drought (Galvez *et al.* , 2011). However, moderate drought was found to increase NSC in stems and roots of *Quercus pubescens* saplings (Ouyang *et al.* 2021). Experiments with more vs. less precipitation found that extreme drought (no irrigation for two consecutive years) reduced shoot and root NSC, whereas intermediate drought levels did not affect shoot and root NSC for *Pinus sylvestris* saplings (Schonbeck *et al.* 2020a).

Less is known about winter NSC consumption of trees previously exposed to drought of various intensities or duration. Trees, as exemplified by the deciduous species in the present study, consume NSC storage for maintenance respiration over winter (Sperling *et al.*, 2015). Therefore, we found that the post-winter NSC levels were lower than the pre-winter level in each tissue for both species (Fig. 3b vs. 3d; Fig. 3c vs. 3e; Fig. 3g vs. 3i; Fig. 3h vs. 3j). The winter temperature was beyond 5degC in the greenhouse of the present study (Fig. S1), but even near freezing winter temperatures were found to significantly increase stem respiration by 10% to 170% in 13 out of 15 species studied in the western US, according to Sperling *et al.* (2015). Sperling *et al.* (2015) further calculated that “frost-induced respiration accelerated stem NSC consumption by 8.4 mg (glucose eq.) cm⁻³ yr⁻¹ on average (cm⁻³ stem wood basis) in the western US, a level of depletion that may continue to significantly affect spring NSC availability”. This is agreement with findings that in temperate deciduous trees, tissue NSC concentrations decline during winter dormancy. This decrease is more pronounced in stem than in roots as observed for aspen (*Populus grandidentata*) and oak (*Quercus rubra*) (Gough *et al.*, 2010). The present study, for the first time, indicated that the over-winter NSC consumption was not affected by drought duration for the two species but it was significantly decreased by N-fertilization for beech across the four drought treatments (Table S1; Fig. 4a – d). This result, may indicate on the one hand a common response of winter NSC consumption of tree species that is independent on the previously imposed drought duration. On the other hand, our results suggest a species-specific sensitivity of winter NSC consumption to other environmental change such as nutrient availability. We can only speculate why the NSC consumption was lower in fertilized beech but it is known that free amino acids and soluble proteins can increase stress resistance of beech (Stajner *et al.*, 2013). Thus, an increased N availability might reduce stress-induced respiration in this species under winter temperature conditions (Fig. S1).

The over-winter changes (post-winter vs. pre-winter) in the sugar/starch ratio (Fig. 4e – h) indicated that starch to sugar conversion occurred in oak saplings (Fig. 4e, g; Figs. S4 and S6), whereas a strong sugar consumption and depletion were the main reasons for decreased tissue sugar/starch ratio in beech saplings (Fig. 4f – h; Figs. S5 and S7). Similarly, starch concentrations were reduced and soluble sugars increased in *Prunus dulcis* during winter, and the NSC concentration (starch + sugars) were only slightly reduced (Sperling *et al.*, 2019). In winter, increased sugar concentrations in the xylem are important to avoid or reduce the number of freeze-thaw embolization cycles, because sugars increase the osmotic potential of xylem and thus lowering its freezing point (Sauter *et al.*, 1973; Thierry *et al.*, 2004; Li *et al.*, 2018).

Previous season drought duration treatment did not affect photosynthesis of the two species after rewetting in the next year (Table 1). The decreased photosynthesis determined in the longer duration drought treatments in 2018 (Fig. 2c – f) recovered and all treatments showed the same level of photosynthesis in June 2019 (Fig. 5a, b). This recovery indicates that there is no legacy of previous drought duration on photosynthetic carbon assimilation. Previous studies found that drought stress can result in incomplete and lagged growth recovery (Anderegg *et al.*, 2013; Pederson *et al.*, 2014; Huang *et al.*, 2018). Extreme drought caused drought legacy response with reduced growth for deep-rooted forests for up to 4 years (Wu *et al.*, 2018), and negative drought legacy was found to last about one year for different plant functional types in Tibetan Plateau (Li *et al.*, 2020). However, fast recovery of carbon acquisition and allocation to different plant organs after drought release was also observed in different tree species (Hagedorn, Frank *et al.*, 2016; Joseph *et al.*, 2020). In the present study, small saplings with large plasticity may be one reason for the quick recovery after rewetting leading to a lack of legacy of past drought. In addition, the longest drought duration (2 months) applied here may be still not long (or severe) enough to impair the physiological processes on the longer term. For example, an open top chamber experiment with 40 cm soil depth but without any watering for 2 years resulted only in a mortality rate of 60% for *Pinus sylvestris* (Schonbeck *et al.* 2020a) and 50% for *Quercus pubescens* saplings (Ouyang *et al.* 2021). In line with the findings of Schonbeck *et al.* (2020a) for pine, we found a fertilization-induced higher mortality rate for beech but not for oak saplings (Fig. 1). Ouyang *et al.* (2021) showed a lower mortality rate (32%) in fertilized compared to non-fertilized (50%) *Q. pubescens* saplings under extreme drought. In oak species fertilization might thus not enhance drought effects but genus and species-specific mechanism still need to be elucidated.

The height growth of the two species was not correlated with shoot NSC storage but significantly positively

correlated with both pre-winter ($P = 0.07$) and post-winter ($P = 0.01$) root storage (Fig. 6). The small values of R^2 (Fig. 6) suggest that root NSC is not the only or the most important factor determining the recovery growth. However, this result (Fig. 6) in conjunction with low photosynthetic rate (Fig. 2c-f), low pre- and post-winter NSC levels in both shoots and roots of D2 and D3 saplings suggests a root carbon limitation that determines the high mortality rate of D2 and D3 saplings for the two species, particularly for beech (Fig. 1). This result, i.e. root carbon limitation, supports a recent hypothesis that the alpine climatic treeline is determined by a winter root carbon limitation as proposed by Li *et al.* (2018) recently. Our result is also supported by data for *P. sylvestris* (Schonbeck *et al.*, 2020a) and *Q. pubescens* saplings (Ouyang *et al.*, 2021) under extreme drought that also showed low root NSC. Indeed, root carbon shortage has been widely found in various tree and shrub species in stressed conditions (Shi *et al.*, 2006; Li *et al.*, 2008a; Li *et al.*, 2008b; Genet *et al.*, 2011; Zhu *et al.*, 2012a; Zhu *et al.*, 2012b; Ouyang *et al.*, 2021; Wang *et al.*, 2021).

Interactions between drought duration and N-fertilization were found only for gas exchange rate during the treatment period (Table 1), indicating that the effects of N-fertilization vary in direction (or magnitude) with drought duration only for photosynthesis but not on other parameters studied (Tables 2 and S1). Schonbeck *et al.* (2020a) found that a mitigating effect of N-fertilization on the negative drought effects on *P. sylvestris* saplings occurred only when the drought stress was relatively mild. In a summer drought experiment (no rainfall for 2 summer months during two consecutive years) it was found that the negative effects of drought on beech growth were amplified by N fertilization (Dziedek *et al.*, 2016), which is similar to our results that drought-induced mortality of beech was amplified by N-fertilization (Fig. 1b). Theoretically, increases in N availability may promote the formation of xylem structures that transport water more efficiently in humid conditions (Borghetti *et al.*, 2017) but may also easily lead to xylem embolism – due to larger cross section and bigger tracheids or vessels – in dry conditions, and therefore, further studies are needed to clarify the N-fertilization effects (e.g. addition rate, amount and frequency) in relation to drought intensity or duration not only on seedlings and saplings but also adult trees.

Now we can go back to our research questions to see whether they have been answered. We found that longer drought duration decreased the physiological performance (e.g. leaf water potential, photosynthetic capacity, NSC levels) but not the growth rate (question 1). We, therefore, speculate that growth is a higher priority than resource storage for the saplings of the two species stressed by long-lasting drought below a certain threshold, as the longest drought duration in the present study was 2 months only. Previous growing season drought seems to not affect the tissue NSC over winter, but the over-winter NSC is considerable in saplings for both species (question 2). The post-winter root NSC level (Fig. 3i, j) plays a more important role in determining the growth (Fig. 6) and survival (Fig. 1) for both species (question 3), suggesting a root carbon limitation in severe drought-stressed saplings, particularly for beech. In line with recent findings (Schonbeck *et al.*, 2018; Schonbeck *et al.*, 2020a; Ouyang *et al.*, 2021), N-fertilization did not play a role to mitigating the negative drought effects on saplings of the two species (question 4). Compared to oak, beech had lower levels of physiological parameters and growth (Figs. 2, 3, 5) but showed higher winter NSC consumption (Fig. 4) and especially higher mortality rate with increasing drought duration in combination with fertilization (Fig. 1), indicating that beech is more sensitive to drought and N-deposition. The present study, in a physiological perspective, experimentally confirmed the view of Ellenberg (2009) that the European beech, compared to oak, may be more strongly affected by future environmental changes.

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Author Contribution

Yue Y. wrote the manuscript. MH. Li designed the research. Ouyang S. collected the samples. Risu N. and Xiaoyu W. conducted the data analysis. Gessler A., HongS. H., ZhengF. W. and MH. Li put forward some constructive suggestions for the manuscript. All authors contributed to the interpretation of the results and the final version of the manuscript.

Data Availability

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

Conflict of Interest

The authors declare there is no conflict of interest.

References

- Adams HD, Zeppel MJB, Anderegg WRL, Hartmann H, Landhausser SM, Tissue DT, Huxman TE, Hudson PJ, Franz TE, Allen CD, et al. 2017.** A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. *Nat Ecol Evol* **1** (9): 1285-1291.
- Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Venetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH, et al. 2010.** A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* **259** (4): 660-684.
- Anderegg WRL, Plavcova L, Anderegg LDL, Hacke UG, Berry JA, Field CB. 2013.** Drought's legacy: multiyear hydraulic deterioration underlies widespread aspen forest die-off and portends increased future risk. *Global Change Biology* **19** (4): 1188-1196.
- Archambeau J, Ruiz-Benito P, Ratcliffe S, Frejaville T, Changenet A, Castaneda JMM, Lehtonen A, Dahlgren J, Zavala MA, Garzon MB. 2020.** Similar patterns of background mortality across Europe are mostly driven by drought in European beech and a combination of drought and competition in Scots pine. *Agricultural and Forest Meteorology* **280**.
- Bhusal N, Lee M, Lee H, Adhikari A, Han AR, Han A, Kim HS. 2021.** Evaluation of morphological, physiological, and biochemical traits for assessing drought resistance in eleven tree species. *Science of The Total Environment* **779** : 146466.
- Bond BJ, Farnsworth BT, Coulombe RA, Winner WE. 1999.** Foliage physiology and biochemistry in response to light gradients in conifers with varying shade tolerance. *Oecologia* **120** (2): 183-192.
- Borghetti M, Gentilesca T, Leonardi S, van Noije T, Rita A, Mencuccini M. 2017.** Long-term temporal relationships between environmental conditions and xylem functional traits: a meta-analysis across a range of woody species along climatic and nitrogen deposition gradients. *Tree Physiology* **37** (1): 4-17.
- Brunner MI, Liechti K, Zappa M. 2019.** Extremeness of recent drought events in Switzerland: dependence on variable and return period choice. *Natural Hazards and Earth System Sciences* **19** (10): 2311-2323.
- Buitink J, van Hateren TC, Teuling AJ. 2021.** Hydrological system complexity induces a drought frequency paradox. *Frontiers in Water* **3** : 42.
- Dickman LT, McDowell NG, Sevanto S, Pangle RE, Pockman WT. 2015.** Carbohydrate dynamics and mortality in a pinon-juniper woodland under three future precipitation scenarios. *Plant, Cell and Environment* **38** (4): 729-739.
- Dziedek C, Hardtle W, von Oheimb G, Fichtner A. 2016.** Nitrogen addition enhances drought sensitivity of young deciduous tree species. *Frontiers in Plant Science* **7** : 1100.
- Ellenberg HH. 2009.** *Vegetation ecology of central Europe. Fourth edition.* : Cambridge University Press.

- Galvez DA, Landhausser SM, Tyree MT. 2011.** Root carbon reserve dynamics in aspen seedlings: does simulated drought induce reserve limitation? *Tree Physiology* **31** (3): 250-257.
- Genet M, Li M, Luo T, Fourcaud T, Clement-Vidal A, Stokes A. 2011.** Linking carbon supply to root cell-wall chemistry and mechanics at high altitudes in *Abies georgei*. *Annals of Botany* **107** (2): 311-320.
- Gessler A, Bottero A, Marshall J, Arend M. 2020.** The way back: recovery of trees from drought and its implication for acclimation. *New Phytologist* **228** (6): 1704-1709.
- Gessler A, Cailleret M, Joseph J, Schonbeck L, Schaub M, Lehmann M, Treydte K, Rigling A, Timofeeva G, Saurer M. 2018.** Drought induced tree mortality—a tree-ring isotope based conceptual model to assess mechanisms and predispositions. *New Phytologist* **219** (2): 485-490.
- Gessler A, Grossiord C. 2019.** Coordinating supply and demand: plant carbon allocation strategy ensuring survival in the long run. *New Phytologist* **222** (1): 5-7.
- Gessler A, Schaub M, McDowell NG. 2017.** The role of nutrients in drought-induced tree mortality and recovery. *New Phytologist* **214** (2): 513-520.
- Ghashghaie J, Saugier B. 1989.** Effects of nitrogen deficiency on leaf photosynthetic response of tall fescue to water deficit. *Plant, Cell & Environment* **12** (3): 261-271.
- Gough CM, Flower CE, Vogel CS, Curtis PS. 2010.** Phenological and Temperature Controls on the Temporal Non-Structural Carbohydrate Dynamics of *Populus grandidentata* and *Quercus rubra*. *Forests* **1** (1): 65-81.
- Griffin-Nolan RJ, Mohanbabu N, Araldi-Brondolo S, Ebert AR, LeVonne J, Lumsden-Pinto JJ, Roden H, Stark JR, Tourville J, Becklin KM, et al. 2021.** Friend or foe? The role of biotic agents in drought-induced plant mortality. *Plant Ecology* **222** (5): 537-548.
- Hagedorn F, Joseph J, Peter M, Luster J, Pritsch K, Geppert U, Kerner R, Molinier V, Egli S, Schaub M. 2016.** Recovery of trees from drought depends on belowground sink control. *Nature Plants* **2** (8): 1-5.
- Hagedorn F, Joseph J, Peter M, Luster J, Pritsch K, Geppert U, Kerner R, Molinier V, Egli S, Schaub M, et al. 2016.** Recovery of trees from drought depends on belowground sink control. *Nature Plants* **2** (8).
- Hanel M, Rakovec O, Markonis Y, Maca P, Samaniego L, Kysely J, Kumar R. 2018.** Revisiting the recent European droughts from a long-term perspective. *Scientific Reports* **8** (1): 1-11.
- Hartmann H, Moura CF, Anderegg WR, Ruehr NK, Salmon Y, Allen CD, Arndt SK, Breshears DD, Davi H, Galbraith D. 2018.** Research frontiers for improving our understanding of drought-induced tree and forest mortality. *New Phytologist* **218** (1): 15-28.
- Hartmann H, Trumbore S. 2016.** Understanding the roles of nonstructural carbohydrates in forest trees—from what we can measure to what we want to know. *New Phytologist* **211** (2): 386-403.
- Huang MT, Wang XH, Keenan TF, Piao SL. 2018.** Drought timing influences the legacy of tree growth recovery. *Global Change Biology* **24** (8): 3546-3559.
- IPCC. 2013.** Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. *Cambridge, UK: Cambridge University Press*.
- Jacobs DF, Rose R, Haase DL, Alzugaray PO. 2004.** Fertilization at planting impairs root system development and drought avoidance of Douglas-fir (*Pseudotsuga menziesii*) seedlings. *Annals of Forest Science* **61** (7): 643-651.

- Joseph J, Gao DC, Backes B, Bloch C, Brunner I, Gleixner G, Haeni M, Hartmann H, Hoch G, Hug C, et al. 2020. Rhizosphere activity in an old-growth forest reacts rapidly to changes in soil moisture and shapes whole-tree carbon allocation. *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* **117** (40): 24885-24892.
- Kannenbergs SA, Novick KA, Phillips RP. 2018. Coarse roots prevent declines in whole-tree non-structural carbohydrate pools during drought in an isohydric and an anisohydric species. *Tree Physiology* **38** (4): 582-590.
- Kreuzwieser J, Gessler A. 2010. Global climate change and tree nutrition: influence of water availability. *Tree Physiology* **30** (9): 1221-1234.
- Lauder JD, Moran EV, Hart SC. 2019. Fight or flight? Potential tradeoffs between drought defense and reproduction in conifers. *Tree Physiology* **39** (7): 1071-1085.
- Li M-H, Cherubini P, Dobbertin M, Arend M, Xiao W-F, Rigling A. 2013. Responses of leaf nitrogen and mobile carbohydrates in different *Quercus* species/provenances to moderate climate changes. *Plant Biology* **15** (s1): 177-184.
- Li M-H, Hoch G, Korner C. 2002. Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. *Trees* **16** (4-5): 331-337.
- Li M-H, Jiang Y, Wang A, Li X, Zhu W, Yan C-F, Du Z, Shi Z, Lei J, Schonbeck L, et al. 2018. Active summer carbon storage for winter persistence in trees at the cold alpine treeline. *Tree Physiology* **38** (9): 1345-1355.
- Li M-H, Xiao W-F, Shi P-L, Wang S-G, Zhong Y-D, Liu X-L, Wang X-D, Cai X-H, Shi Z-M. 2008a. Nitrogen and carbon source-sink relationships in trees at the Himalayan treelines compared with lower elevations. *Plant, Cell & Environment* **31** (10): 1377-1387.
- Li M-H, Xiao W-F, Wang S-G, Cheng G-W, Cherubini P, Cai X-H, Liu X-L, Wang X-D, Zhu W-Z. 2008b. Mobile carbohydrates in Himalayan treeline trees I. Evidence for carbon gain limitation but not for growth limitation. *Tree Physiology* **28** (8): 1287-1296.
- Li PL, Zhu D, Wang YL, Liu D. 2020. Elevation dependence of drought legacy effects on vegetation greenness over the Tibetan Plateau. *Agricultural and Forest Meteorology* **295** : 108190.
- Li W, Hartmann H, Adams HD, Zhang H, Jin C, Zhao C, Guan D, Wang A, Yuan F, Wu J. 2018. The sweet side of global change—dynamic responses of non-structural carbohydrates to drought, elevated CO₂ and nitrogen fertilization in tree species. *Tree Physiology* **38** (11): 1706-1723.
- McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* **178** (4): 719-739.
- McDowell NG. 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiology* **155** (3): 1051-1059.
- McDowell NG, Allen CD. 2015. Darcy's law predicts widespread forest mortality under climate warming. *Nature Climate Change* **5** (7): 669-672.
- Meng S, Zhang C, Su L, Li Y, Zhao Z. 2016. Nitrogen uptake and metabolism of *Populus simonii* in response to PEG-induced drought stress. *Environmental and Experimental Botany* **123** : 78-87.
- Nardini A, Battistuzzo M, Savi T. 2013. Shoot desiccation and hydraulic failure in temperate woody angiosperms during an extreme summer drought. *New Phytologist* **200** (2): 322-329.
- O'Brien MJ, Leuzinger S, Philipson CD, Tay J, Hector A. 2014. Drought survival of tropical tree seedlings enhanced by non-structural carbohydrate levels. *Nature Climate Change* **4** (8): 710-714.

- Ouyang S-N, Gessler A, Saurer M, Hagedorn F, Gao D-C, Wang X-Y, Schaub M, Li M-H, Shen W-J, Schonbeck L. 2021. Root carbon and nutrient homeostasis determines downy oak sapling survival and recovery from drought. *Tree Physiology* .
- Pederson N, Dyer JM, McEwan RW, Hessl AE, Mock CJ, Orwig DA, Rieder HE, Cook BI. 2014. The legacy of episodic climatic events in shaping temperate, broadleaf forests. *Ecological Monographs* **84** (4): 599-620.
- Radin JW, Ackerson RC. 1981. Water relations of cotton plants under nitrogen deficiency: III. Stomatal conductance, photosynthesis, and abscisic acid accumulation during drought. *Plant Physiology* **67** (1): 115-119.
- Reinhardt K, Germino MJ, Kueppers LM, Domec J-C, Mitton J. 2015. Linking carbon and water relations to drought-induced mortality in *Pinus flexilis* seedlings. *Tree Physiology* **35** (7): 771-782.
- Rowland L, da Costa ACL, Galbraith DR, Oliveira R, Binks OJ, Oliveira A, Pullen A, Doughty C, Metcalfe D, Vasconcelos S. 2015. Death from drought in tropical forests is triggered by hydraulics not carbon starvation. *Nature* **528** (7580): 119-122.
- Sade N, Gebremedhin A, Moshelion M. 2012. Risk-taking plants: anisohydric behavior as a stress-resistance trait. *Plant signaling & behavior* **7** (7): 767-770.
- Sala A, Woodruff DR, Meinzer FC. 2012. Carbon dynamics in trees: feast or famine? *Tree Physiology* **32** (6): 764-775.
- Sanz-Perez V, Castro-Diez P, Joffre R. 2009. Seasonal carbon storage and growth in Mediterranean tree seedlings under different water conditions. *Tree Physiology* **29** (9): 1105-1116.
- Schonbeck L, Gessler A, Hoch G, McDowell NG, Rigling A, Schaub M, Li MH. 2018. Homeostatic levels of nonstructural carbohydrates after 13 yr of drought and irrigation in *Pinus sylvestris*. *New Phytologist* **219** (4): 1314-1324.
- Schonbeck L, Gessler A, Schaub M, Rigling A, Hoch G, Kahmen A, Li M-H. 2020a. Soil nutrients and lowered source:sink ratio mitigate effects of mild but not of extreme drought in trees. *Environmental and Experimental Botany* **169** : 103905.
- Schonbeck L, Li M-H, Lehmann MM, Rigling A, Schaub M, Hoch G, Kahmen A, Gessler A. 2020b. Soil nutrient availability alters tree carbon allocation dynamics during drought. *Tree Physiology* **41** (5): 697-707.
- Sevanto S. 2014. Phloem transport and drought. *Journal of Experimental Botany* **65** (7): 1751-1759.
- Shi PL, Korner C, Hoch G. 2006. End of season carbon supply status of woody species near the treeline in western China. *Basic and Applied Ecology* **7** (4): 370-377.
- Sperling O, Earles JM, Secchi F, Godfrey J, Zwieniecki MA. 2015. Frost Induces Respiration and Accelerates Carbon Depletion in Trees. *Plos One* **10** (12): e0144124.
- Sperling O, Kamai T, Tixier A, Davidson A, Jarvis-Shean K, Raveh E, DeJong TM, Zwieniecki MA. 2019. Predicting bloom dates by temperature mediated kinetics of carbohydrate metabolism in deciduous trees. *Agricultural and Forest Meteorology* **276** : 107643.
- Spinoni J, Vogt JV, Naumann G, Barbosa P, Dosio A. 2018. Will drought events become more frequent and severe in Europe? *International Journal of Climatology* **38** (4): 1718-1736.
- Stajner D, Orlovic S, Popovic BM, Kebert M, Stojnic S, Klasnja B. 2013. Chemical Parameters of Oxidative Stress Adaptability in Beech. *Journal of Chemistry* **2013** .
- Wang X, Yu F-H, Jiang Y, Li M-H. 2021. Carbon and nutrient physiology in shrubs at the upper limits: a multispecies study. *Journal of Plant Ecology* **14** (2): 301-309.

Wiley E, Helliker B. 2012. A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. *New Phytologist* **195** (2): 285-289.

Wu XC, Liu HY, Li XY, Ciais P, Babst F, Guo WC, Zhang CC, Magliulo V, Pavelka M, Liu SM, et al. 2018. Differentiating drought legacy effects on vegetation growth over the temperate Northern Hemisphere. *Global Change Biology* **24** (1): 504-516.

Yu LY, Zhao XN, Gao XD, Jia RH, Yang MH, Yang XL, Wu Y, Siddique KHM. 2021. Effect of natural factors and management practices on agricultural water use efficiency under drought: A meta-analysis of global drylands. *Journal of Hydrology* **594** : 125977.

Zhang P, Zhou X, Fu Y, Shao J, Zhou L, Li S, Zhou G, Hu Z, Hu J, Bai SH. 2020. Differential effects of drought on nonstructural carbohydrate storage in seedlings and mature trees of four species in a subtropical forest. *Forest Ecology and Management* **469** : 118159.

Zhu W-Z, Cao M, Wang S-G, Xiao W-F, Li M-H. 2012a. Seasonal dynamics of mobile carbon supply in *Quercus aquifolioides* at the upper elevational limit. *Plos One* **7** (3): e34213.

Zhu W-Z, Xiang J-S, Wang S-G, Li M-H. 2012b. Resprouting ability and mobile carbohydrate reserves in an oak shrubland decline with increasing elevation on the eastern edge of the Qinghai-Tibet Plateau. *Forest Ecology and Management* **278** : 118-126.

Table 1 Results of linear mixed models with species (S), drought duration (D), nitrogen fertilization (N) as factors, for water potential (ψ_{leaf}), net photosynthetic rate during the drought duration treatment in 2018 and in the recovery growing season in 2019, relative height increment rate (RHI) in 2018 (drought) and 2019 (recovery). Numbers of degrees of freedom (df), F - and P -values are given.

df	Leaf water potential		Photosynthesis (6 Aug 2018)		Photosynthesis (28 Sep 2018)		Photosynthesis (10 June 2019)		Relative height increment (2018)	
	F	P	F	P	F	P	F	P	F	P
Species (S)	0.312	0.580	77.622	<0.001	132.315	<0.001	49.840	<0.001	17.219	<0.001
D-duration (D)	13.826	<0.001	108.001	<0.001	233.175	<0.001	0.505	0.681	0.316	0.574
Nitrogen (N)	1.553	0.221	0.004	0.952	13.158	<0.001	0.310	0.580	2.332	0.127
S×D	6.982	<0.001	17.820	<0.001	9.902	<0.001	1.474	0.231	0.141	0.910
S×N	0.211	0.649	0.004	0.952	8.300	<0.05	0.972	0.328	0.569	0.456
D×N	0.781	0.513	9.317	<0.001	19.339	<0.001	0.381	0.767	0.561	0.476
S×D×N	0.047	0.986	11.456	<0.001	6.825	<0.001	1.006	0.397	0.290	0.859

Table 2 Results of linear mixed models with species (S), drought duration (D), nitrogen fertilization (N) as factors, for tissue NSC concentrations after the drought duration treatment in the growing season 2018 (pre-winter) and before the growing season 2019 (post-winter). Numbers of degrees of freedom (df), F - and P -values are given.

df	Leaves	Leaves
	F	P

	<i>df</i>	Leaves	Leaves
October 2018 (Pre-winter)	October 2018 (Pre-winter)	October 2018 (Pre-winter)	October 2018 (Pre-winter)
Species (S)	1	11.339	<0.05
D-duration (D)	3	0.954	0.428
Nitrogen (N)	1	0.540	0.469
S×D	3	1.864	0.158
S×N	1	1.932	0.175
D×N	3	0.156	0.925
S×D×N	3	0.869	0.468
April 2019 (Post-winter)	April 2019 (Post-winter)	April 2019 (Post-winter)	April 2019 (Post-winter)
Species (S)	1	(No leaves)	(No leaves)
D-duration (D)	3		
Nitrogen (N)	1		
S×D	3		
S×N	1		
D×N	3		
S×D×N	3		

Fig. 1 Mortality rate of oak (*Quercus petraea*) and beech (*Fagus sylvatica*) saplings at the end of a recovery growing season following a growing season with 4 drought duration treatment (D0 = watering weekly, D1 = watering biweekly, D2 = watering monthly, and D3 = watering bimonthly) combined with N-fertilization (1.68 g N kg⁻¹ dry soil fertilized vs. unfertilized).

Fig. 2 Midday water potential (ψ_{leaf}) after two-month of drought treatment on 5 August 2018 (a, b), leaf net-photosynthesis (A_{leaf}) after two- (6 August 2018) (c, d) and four- (28 September 2018) (e, f) months of drought duration treatment in 2018, and the relative height increment (g, h) between 30 May (initial) and 1 October 2018 (after 4-months of drought treatment) for oak (*Quercus petraea*) and beech (*Fagus sylvatica*) saplings. D0 (watering weekly), D1 (watering biweekly), D2 (watering monthly), and D3 (watering bimonthly) represent the drought duration. Different letter indicates significant difference in parameters among D0, D1, D2, and D3.

Fig. 3 Non-structural carbohydrate (NSC) concentrations in leaves, shoots and roots of oak (*Quercus petraea*) and beech (*Fagus sylvatica*) saplings after four months of drought duration treatment in 2018 (pre-winter) and before the growing season 2019 (post-winter). D0 (watering weekly), D1 (watering biweekly), D2 (watering monthly), and D3 (watering bimonthly) represent the drought duration. Different letters indicate significant differences in parameters among D0, D1, D2, and D3.

Fig. 4 Over-winter changes in non-structural carbohydrate (NSC) concentrations (post-winter values minus pre-winter values) and the sugar/starch ratio in shoots and roots of oak (*Quercus petraea*) and beech (*Fagus sylvatica*) saplings treated with four drought durations during the previous growing season. D0 (watering weekly), D1 (watering biweekly), D2 (watering monthly), and D3 (watering bimonthly) represent the drought duration.

Fig. 5 Leaf net-photosynthesis (A_{leaf}) and relative height increment of oak (*Quercus petraea*) and beech (*Fagus sylvatica*) saplings treated with four drought durations in 2018 and well-watered in 2019. D0 (watering weekly), D1 (watering biweekly), D2 (watering monthly), and D3 (watering bimonthly) represent the drought duration. Different letters indicate significant difference in parameters among D0, D1, D2, and D3.

Fig. 6 2019 recovery growth rate (measured at the end-season 2019) in relation to pre-winter and post-winter NSC in shoots and roots of oak (*Quercus petraea*) and beech (*Fagus sylvatica*) saplings treated with four drought durations in 2018 and well-watered in 2019. In case there is a significant ($p < 0.05$) relationship between growth and NSC level for the pooled data, a fitting line is given.

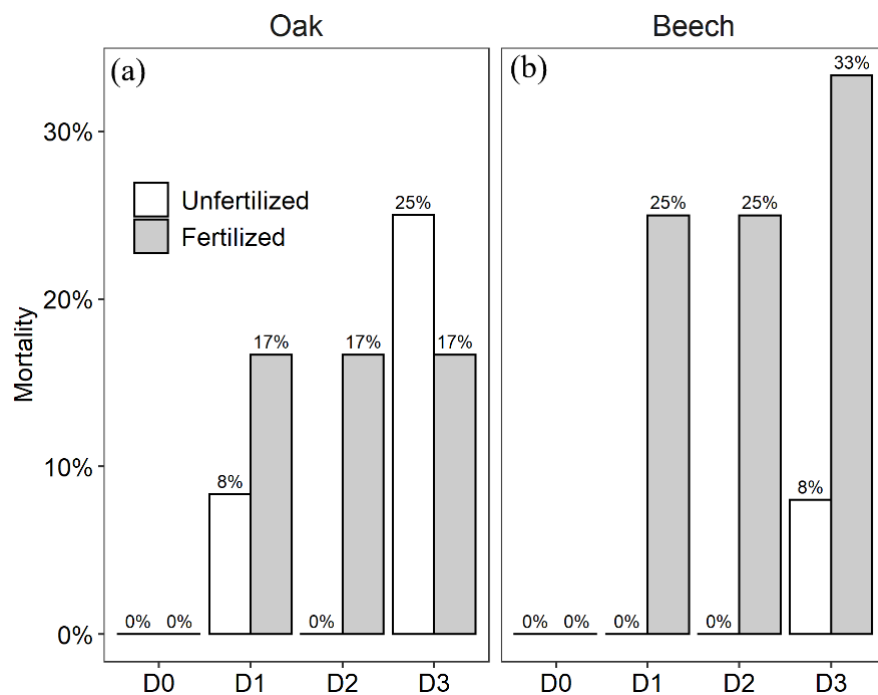


Fig. 1 Mortality rate of oak (*Quercus petraea*) and beech (*Fagus sylvatica*) saplings at the end of a recovery growing season following a growing season with 4 drought duration treatment (D0 = watering weekly, D1 = watering biweekly, D2 = watering monthly, and D3 = watering bimonthly) combined with N-fertilization (1.68 g N kg⁻¹ dry soil fertilized vs. unfertilized).

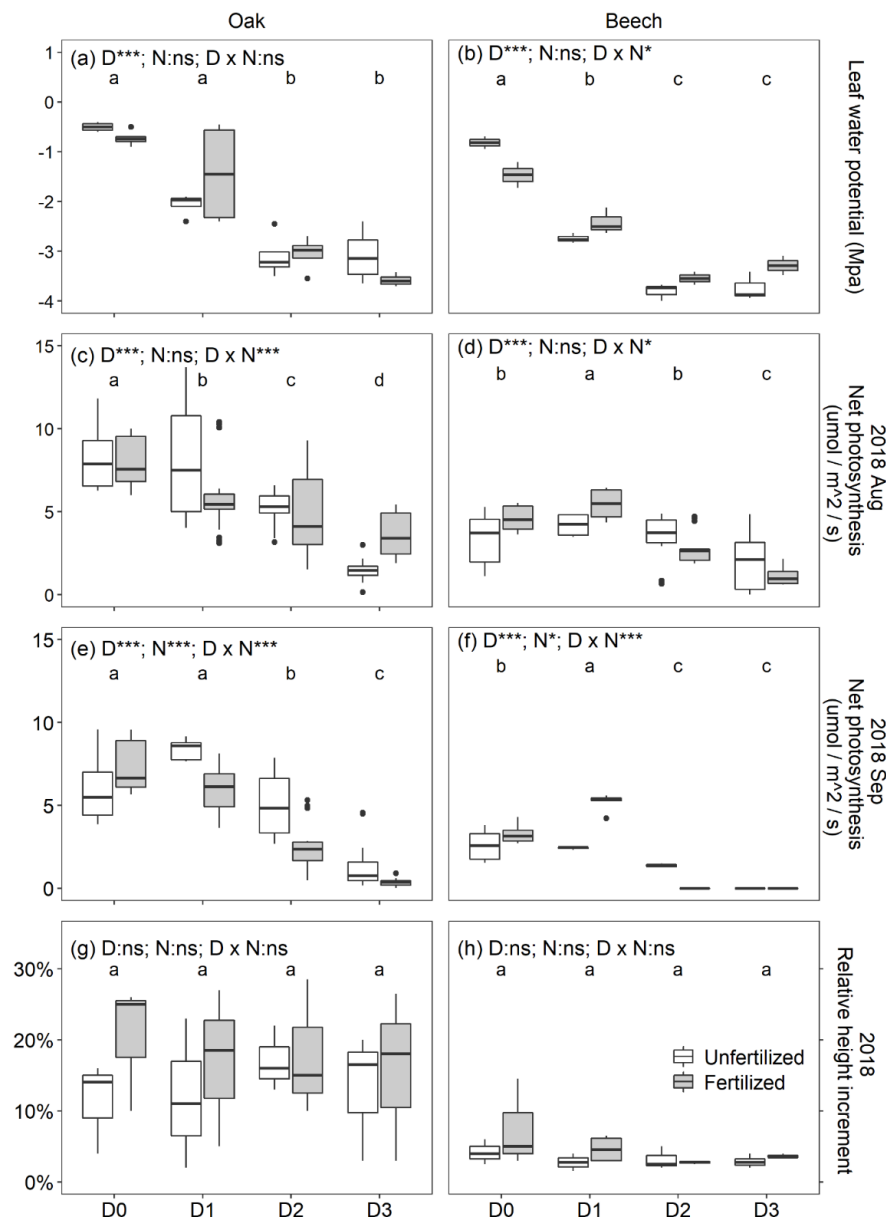


Fig. 2 Midday water potential (ψ_{leaf}) after two-month of drought treatment on 5 August 2018 (a, b), leaf net-photosynthesis (A_{leaf}) after two- (6 August 2018) (c, d) and four- (28 September 2018) (e, f) months of drought duration treatment in 2018, and the relative height increment (g, h) between 30 May (initial) and 1 October 2018 (after 4-months of drought treatment) for oak (*Quercus petraea*) and beech (*Fagus sylvatica*) saplings. D0 (watering weekly), D1 (watering biweekly), D2 (watering monthly), and D3 (watering bimonthly) represent the drought duration. Different letter indicates significant difference in parameters among D0, D1, D2, and D3.

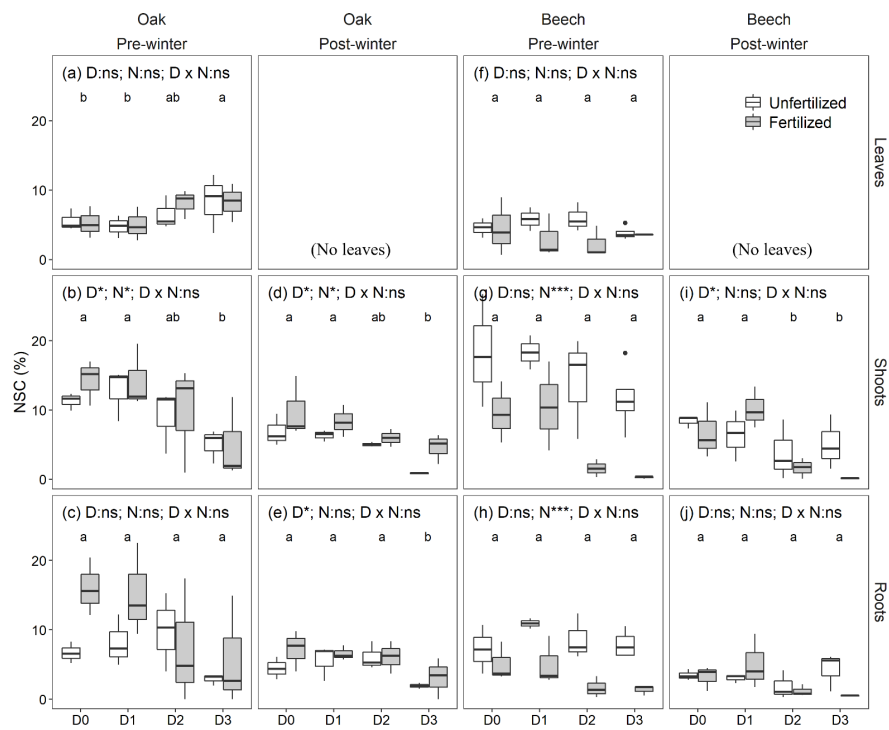


Fig. 3 Non-structural carbohydrate (NSC) concentrations in leaves, shoots and roots of oak (*Quercus petraea*) and beech (*Fagus sylvatica*) saplings after four months of drought duration treatment in 2018 (pre-winter) and before the growing season 2019 (post-winter). D0 (watering weekly), D1 (watering biweekly), D2 (watering monthly), and D3 (watering bimonthly) represent the drought duration. Different letters indicate significant differences in parameters among D0, D1, D2, and D3.

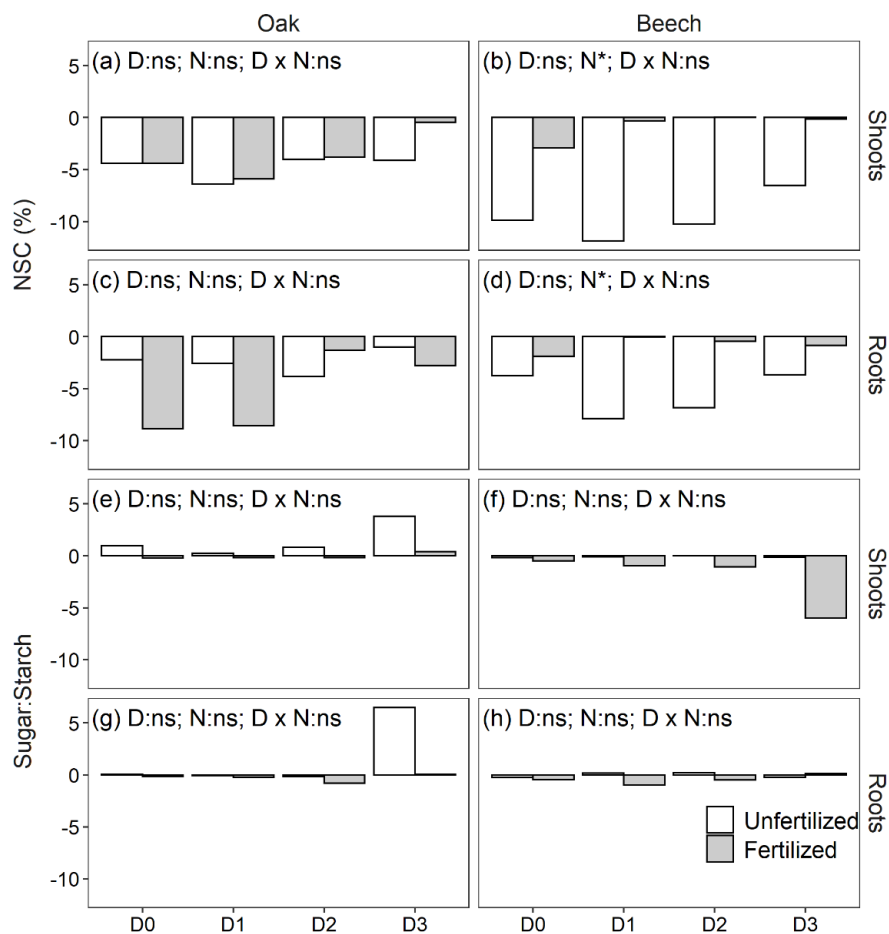


Fig. 4 Over-winter changes in non-structural carbohydrate (NSC) concentrations (post-winter values minus pre-winter values) and the sugar/starch ratio in shoots and roots of oak (*Quercus petraea*) and beech (*Fagus sylvatica*) saplings treated with four drought durations during the previous growing season. D0 (watering weekly), D1 (watering biweekly), D2 (watering monthly), and D3 (watering bimonthly) represent the drought duration.

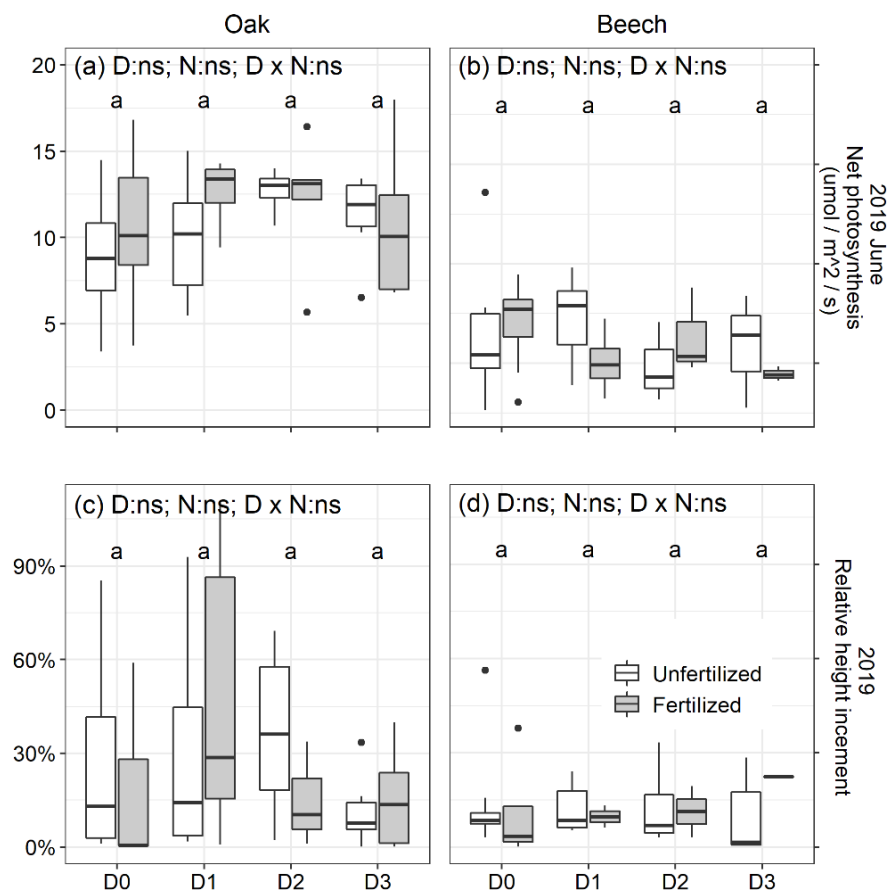


Fig. 5 Leaf net-photosynthesis (A_{leaf}) and relative height increment of oak (*Quercus petraea*) and beech (*Fagus sylvatica*) saplings treated with four drought durations in 2018 and well-watered in 2019. D0 (watering weekly), D1 (watering biweekly), D2 (watering monthly), and D3 (watering bimonthly) represent the drought duration. Different letters indicate significant difference in parameters among D0, D1, D2, and D3.

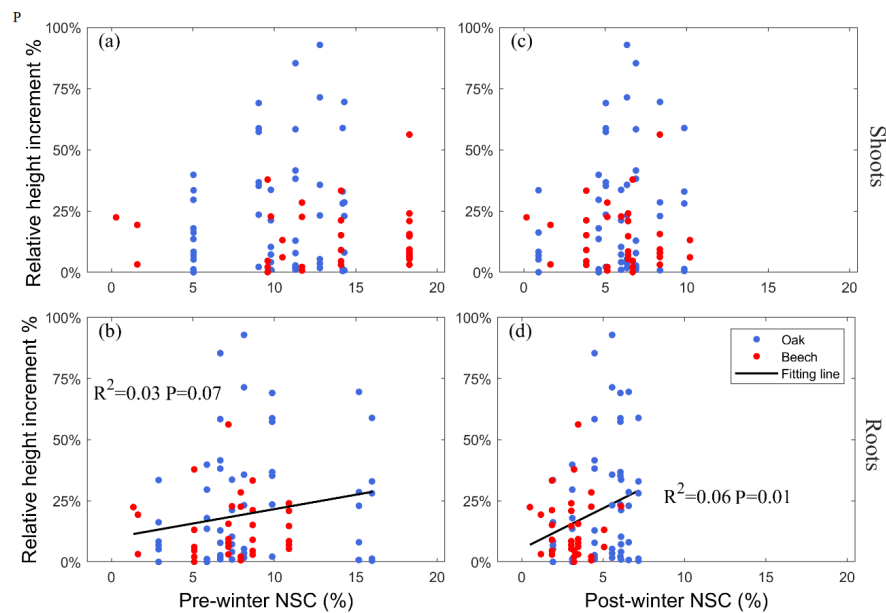


Fig. 6 2019 recovery growth rate (measured at the end-season 2019) in relation to pre-winter and post-winter NSC in shoots and roots of oak (*Quercus petraea*) and beech (*Fagus sylvatica*) saplings treated with four drought durations in 2018 and well-watered in 2019. In case there is a significant ($p < 0.05$) relationship between growth and NSC level for the pooled data, a fitting line is given.