Research paper

Root carbon and nutrient homeostasis determines downy oak sapling survival and recovery from drought

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The role of carbon (C) and nutrient uptake, allocation, storage and especially their interactions in survival and recovery of trees under increased frequencies and intensities of drought events is not well understood. A full factorial experiment with four soil water content regimes ranging from extreme drought to well-watered conditions and two fertilization levels was carried out. We aimed to investigate whether nutrient addition mitigates drought effects on downy oak (*Quercus pubescens* Willd.) and whether storage pools of non-structural carbohydrates (NSC) are modified to enhance survival after 2.5 years of drought and recovery after drought relief. Physiological traits, such as photosynthesis, predawn leaf water potential as well as tissue biomass together with pools and dynamics of NSC and nutrients at the whole-tree level were investigated. Our results showed that fertilization played a minor role in saplings' physiological processes to cope with drought and drought relief, but reduced sapling mortality during extreme drought. Irrespective of nutrient supply, *Q. pubescens* showed increased soluble sugar concentration in all tissues with increasing drought intensity, mostly because of starch degradation. After 28 days of drought relief, tissue sugar concentrations decreased, reaching comparable values to those of well-watered plants. Only during the recovery process from extreme drought, root NSC concentration strongly declined, leading to an almost complete NSC depletion after 28 days of rewetting, simultaneously with new leaves flushing. These findings suggest that extreme drought can lead to root C exhaustion. After drought relief, the repair and regrowth of organs can even exacerbate the root C depletion. We concluded that under future climate conditions with repeated drought events, the insufficient and lagged C replenishment in roots might eventually lead to C starvation and further mortality.

Keywords: carbon storage, drought intensity, nitrogen availability, non-structural carbohydrates (NSC), *Quercus pubescens*, recovery.

Introduction

Increasing frequency and severity of drought as a result of global climate change threatens the structure and functioning of forest ecosystems (Allen et al. 2010). Forest decline and tree mortality related to drought have been observed across large forested regions of the globe (Allen et al. 2015, Choat et al. 2018), and consequently, a better understanding of the physiological mechanisms driving drought-induced loss of tree functioning are of increasing importance across scales, from the individual plant level to the global scale (McDowell et al. 2008, Adams et al. 2017). Moreover, as future climate scenarios predict higher precipitation variability, where extreme drought periods alternate with intensive rainfall events (IPCC 2013), the mechanisms of plant recovery from drought are unclear and need to be considered for a conceptual mortality-resilience framework (Ruehr et al. 2019).

Hydraulic failure is assumed to be the one of the main causes of drought-induced tree mortality (Adams et al. 2017). In addition, depletion of non-structural carbohydrates (NSC) leading to carbon (C) starvation also frequently couples to hydraulic failure, contributing to tree mortality (McDowell 2011). Moreover, pathogens and insect outbreaks are also involved in tree die back (Sala et al. 2010). Non-structural carbohydrates (NSC), mainly consisting of soluble sugars and starch, play a central role in plant functioning (Hartmann and Trumbore 2016). Carbon starvation is presumed to occur when stomatal closure prevents excessive water loss under drought, and restricts photosynthesis to a level that the continued metabolic demand for carbohydrates cannot be met any more (McDowell et al. 2008). However, contrasting findings of either C depletion at the time of mortality but also of increases in NSC concentration have fueled the debate about the significance of the NSC levels, as they depend on the mechanisms that drive partitioning of new assimilates between growth and storage under stress (Adams et al. 2017). Increased NSC storage during drought might be (i) due to active storage strategies in drought-stressed trees causing prioritized C allocation to storage at the expense of growth (Wiley and Helliker 2012, Li et al. 2018) or (ii) a result of the inability to use the NSC due to sink-driven control of the tree C balance, where the activity of growth and respiration is reduced earlier during a drought than photosynthesis (Körner 2015). Huang et al. (2019) found in a C limitation (i.e. CO_2 concentration reduction) experiment in Norway spruce that a strong reduction of belowground C allocation leading to C restriction in the root was able to compensate the aboveground C depletion. They indicated that reduced C supply that may occur during drought can strongly alter the plant C partitioning pattern. In contrast to this finding, however, Hommel et al. (2016) observed increased C allocation to roots in drought-exposed beech. After drought and upon re-watering, Hagedorn et al. (2016) found that beech prioritized the investment of new assimilates belowground to recover the root functions. Thus, there are still uncertainties about allocation of new assimilates and reserves use under drought and after drought relief.

Nutrients play a critical role in forest primary production via their close interplay with the C cycle (LeBauer and Treseder 2008, Kreuzwieser and Gessler 2010, Norby et al. 2010, Thomas et al. 2010). Higher soil nutrient and especially nitrogen (N) availability promotes photosynthesis through increased formation of photosynthetic enzymes such as Rubisco (Evans 1989). In the long-term, elevated N availability could increase the shoot-to-root ratios (Aaltonen et al. 2017), widen vessel diameters and lower the ratio of sapwood-to-leaf area (Hacke et al. 2010), predisposing plants to hydraulic failure once a drought event occurs (Beikircher et al. 2019). Drought as such is known to reduce soil nutrient mobilization and microbial activity, and consequently impede nutrient uptake by the plants (Jensen et al. 2003, Andresen et al. 2010). In this respect, drought-induced changes in N supply might be particularly important for the CO_2 uptake and assimilate allocation between growth and NSC storage due to the key role that N plays in photosynthesis and stomatal regulation (Millard and Grelet 2010). Moreover, reduced N availability might impair general metabolic function and cell level structural integrity due to reduced N supply for N-containing osmoprotectants (Yancey 2005). Furthermore, large N reserves in the plant might facilitate the recovery from drought, as larger soil N pools might allow a faster replenishment of the plant nutrient demand after drought relief (Brödlin et al. 2019). While many experimental and long-term studies have focused on forest nutrition and how it affects tree functioning (Binkley and Högberg 2016, Högberg et al. 2017, Simon et al. 2017), there is a lack of studies on how it interacts with water availability and C, which could affect the plant's responses to drought and recovery after drought (Gessler et al. 2017).

To study the effect of interaction between water availability and soil nutrient on plant C-nutrient balance during drought and the recovery after drought, we performed experiments with downy oak, *Quercus pubescens* Willd. *Quercus pubescens* is a deciduous broadleaf species that is distributed from the Caspian Sea in the east to the northern Spanish Atlantic coast in the west. It is a drought-resistant tree species, which is assumed will cope well with the changing climate in Central Europe and become increasingly competitive in this region compared with other tree species (Hanewinkel et al. 2013, Früchtenicht et al. 2018). During two consecutive years, we exposed 3-year-old *Q. pubescens* saplings to four soil water content regimes from extreme drought to well-watered conditions and two fertilization regimes. During the third growing season, we ended the drought treatments by maintaining all saplings under well-watered conditions. We monitored gas exchange and predawn leaf water potential, and determined the biomass allocation as well as N and NSC concentrations of different tree organs (leaves,

stems and roots) during and after drought. We hypothesized that (hypothesis 1) higher nutrient availability would mitigate negative effects of mild drought by enhancing C assimilation rates but also promoting allocation of assimilates to the roots; (hypothesis 2) fertilized trees would be more sensitive to extreme drought as the higher biomass leads to a greater water demand; (hypothesis 3) moderate to severe drought would stimulate *Q. pubescens* saplings to allocate relatively more C into storage pools at the expense of growth; and (hypothesis 4) a high proportion of new assimilates would be prioritized to storage pools even after drought relief to secure future survival.

Material and methods

Experimental set-up

The study was conducted in the Model Ecosystem Facility at the Swiss Federal Institute for Forest, Snow and Land-scape Research WSL, Switzerland (47°21′48″ N, 8°27′23″ E, 545 m above sea level). In brief, the aboveground compartment of each of the 16 hexagonal units consisted of open-top glass chambers with 3-m height and 1.6-m side length. The chambers were covered by mobile glass roofs that were kept closed during the whole experiment. The glass walls and roofs reduced photosynthetic active radiation available for the saplings only by about 10%. The belowground compartment consisted of two lysimeters of 1.5-m depth with a plantable area of 1.5 m² each. The lysimeters were filled with a 1-m deep layer of gravel for fast drainage, covered with a fleece layer that is impermeable for roots but permeable for water, with a 40-cm layer of calcareous sandy loam soil on the top (Kuster et al. 2013). Air temperature and humidity inside and outside the open-top chambers, as well as soil moisture and soil temperature in the lysimeters (5, 20, 35 cm below ground), were automatically monitored (5TM soil moisture and temperature logger, Meteogroup, Munich, Germany). Six sprinklers (1 m high) per lysimeter were evenly distributed, and irrigation was programmed for every lysime-ter separately. In March 2015, a total of 320 3-year-old *Q. pubescens* saplings of similar height and identical provenance were planted in the 16 chambers (10 trees per lysimeter, 20 trees per open-top chamber).

Treatments

A split–split plot design was applied in this study. Each open-top chamber was assigned to one of four different water regimes as a whole plot treatment. There were consequently four chambers as replicates for one regime arranged as a Latin Square (Schönbeck et al. 2020). The amount of water to be applied was controlled via automated soil moisture measure-ments until May 2018. Due to technical issues, soil moisture measurements were performed with a manual soil moisture probe (Delta-T Devices, Wet-2 Sensor Kit, Cambridge, UK) thereafter. Automated soil moisture measurements were taken

with 5TM sensors (Decagon Devices, Pullman, WA, USA) at 20-cm soil depth. Every chamber contained two sensors (one per lysimeter). Field capacity (FC) and wilting point (WP) were determined by pF curves (Figure S1 available as Supplementary data at *Tree Physiology* Online), obtained with the Hyprop mea-suring system (Metergroup, Munich, Germany). These curves show the soil volumetric water content (VWC) plotted against the logarithm of the soil matric potential (pF). For the first 1.5 years, VWC was adjusted to the following four treatment levels: close to FC (approximately 23% VWC; referred to as D0 or well watered), close to the WP (approximately 6% VWC; achieved by no irrigation at all; D100 or extreme drought) and two intermediate levels, corresponding to approximately 60% (D30 or mild drought) and 30% (D60 or severe drought) of FC equaling 18 and 13% VWC (Figure 1).

The soil water content regimes started 1 year after planting the trees and ran from April 2016 to August 2018. To avoid excessive mortality, the D100 treatment was re-watered to field capacity in mid-July 2017 until the end of growing season and then not watered at all again in 2018. We intensified water shortage in the D30 and D60 treatments to reach values close to WP in the 2018 growing season. For that reason, both treatments then also received no water at all, causing the soil moisture drop from May 2018 to the early August 2018 (Figure 1). A second, overall rewetting treatment was applied on 6 August 2018, to reach soil water content close to field capac-ity in all treatments. The irrigation regimes thus comprised a highly intensive drought for 1.5 years, followed by 3 months re-watering to field capacity and an additional intensive drought for one spring and early summer (D100), two intermediate drought levels with intensifying drought over a period of 2.5 years (D60, D30) and a well-watered level (D0). Fertilization treatments were conducted in April 2016 and 2017. Liquid fertilizer, diluted in 3-l water (Wuxal, Universaldünger, NPK 4:4:3), equal to 2.5 g N m⁻² year⁻¹ was added to one of two lysimeters in each chamber. The amount of N addition approached to the maximum N deposition (2.9 g N m⁻² year⁻¹) in Swiss forests observed on long-term forest monitoring plots (Thimonier et al. 2005). Lysimeters treated without additional fertilizer were irrigated with 3-l water to prevent soil water differences between fertilization treatments. The applied irrigation of 3-l water was equal to 2-mm precipitation. Fertilization and non-fertilization treatments will be referred to as HN and LN, respectively, from now onwards.

Harvests, biomass and mortality determination

Tree harvests took place shortly after 1.5 years of drought, on mid-July 2017 (one tree per lysimeter), and at the end of the experiment, on 3 September 2018 (full harvest of all surviving saplings), to assess biomass during drought and after rewetting. The whole individual sapling was separated into leaf, shoot and root and organs were dried until stable weight at 60 °C,
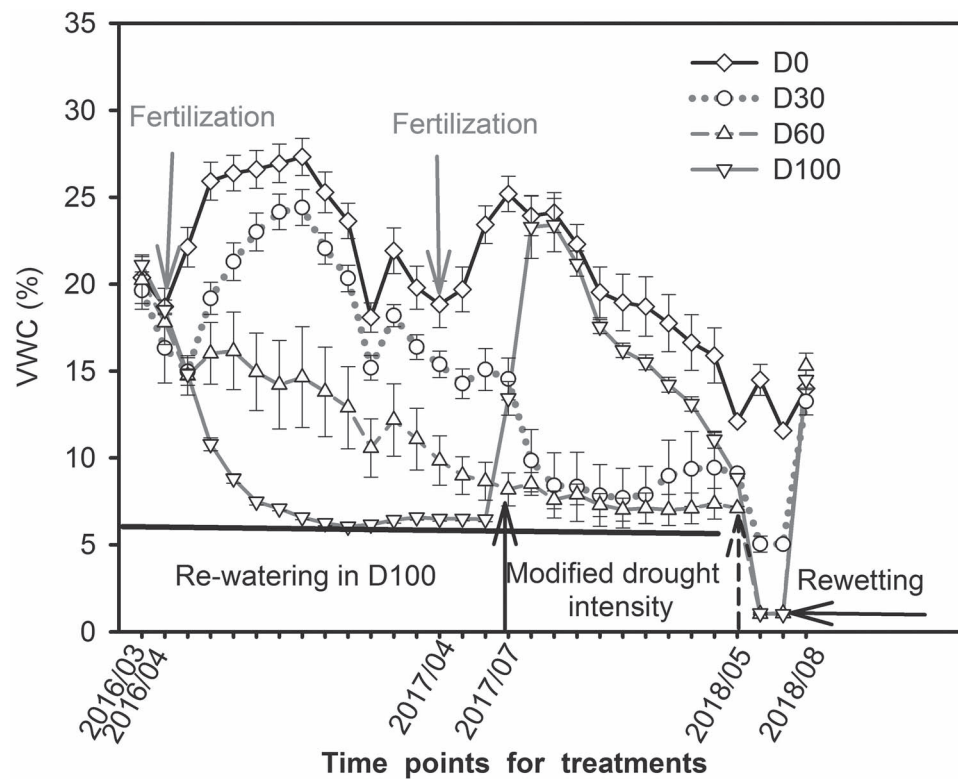
Figure 1. Mean monthly soil volumetric water content (VWC) in the experiment from March 2016 to September 2019. Soil volumetric water content treatments consisted of D0 (well watered; black solid line), D30 (mild drought; gray dotted line), D60 (severe drought; gray dashed line) and D100 (extreme drought; gray solid line). The D100 treatment was re-watered (black solid vertical arrow) during June to August 2017 to avoid excessive mortality. The black horizontal arrow represents the final rewetting event for all drought treatments in August 2018. The dashed vertical arrow indicates intensified drought stress in all drought stressed treatment from 1 May 2018 to 6 August 2018. The two gray solid arrows represent fertilizer application in April 2016 and 2017. The horizontal line represents the VWC at the wilting point (6.1%). From May 2018, VWC was manually measured by soil moisture probe (Delta-T Devices, Wet-2 Sensor Kit, UK) at the soil depth of 5 cm. Error bars represent the standard error (mean $\pm$ 1 SE, $n = 8$). The sensitivity of manual measures differed from the fixed installed soil moisture probes, causing the soil moisture drop below wilting point (WP).

dry weight was determined, and the samples were stored until further analysis. Additionally, during the rewetting period, leaves, shoots and fine roots of oak saplings were collected 1, 3, 7 and 28 days after rewetting (corresponding to 7, 9, 13 August and 3 September 2018). Four branches with green leaves were cut from living saplings in each lysimeter to form leaf and twig samples. For saplings without green leaves, (e.g. in the severe and extreme drought treatments), only branches were collected. At the last sampling date, newly flushed leaves that had appeared after 2 weeks of rewetting, were mixed with previously flushed leaves to form leaf samples. Around 1–2 g of fresh root samples (Ø < 2 mm) were collected manually from the soil by digging carefully with a small shovel in each lysimeter. All plant samples described above were dried at 60 °C and stored until laboratory analyses of NSC and N concentration. Individuals without any green leaves and without re-flushing after re-watering in 2017 are regarded as dead saplings. The mortality rate (%) in the D100 treatment in July 2017 was calculated based on the numbers of dead saplings accounting for the total number of seedlings at the beginning of the experiment. The mortality

calculation for the D100 and D60 treatments in September 2018 (after rewetting all treatments) was done as in July 2017. Refoliation rate was assessed as the number of saplings that showed a new leaf flush after rewetting in relation to the total amount saplings before drought in each chamber.

Gas exchange and predawn leaf water potential

Net photosynthesis (A_{leaf}) was measured during drought (13 July 2018) and 1, 3, 7, 14 and 28 days after rewetting using a LiCor 6400 system (LI-COR, Lincoln, NE, USA). Six trees per chamber with three trees per fertilization treatment were measured. Three representative leaves were selected from an individual sapling. The first-flush leaves of 2018 were used for the photosynthesis and leaf-predawn water potential measurement during drought and the first four times after rewetting. Either first-flush leaves or a mixture of first-flush and new, second-flush leaves (which grew after rewetting in a part of the saplings in D60 and D100) were used to assess gas exchange and predawn leaf water potential at the last time of measuring on 3 September 2018. Only living saplings were measured. Due to the loss of leaves in saplings and failure leaf flushing in spring

(after the intermittent irrigation) in the D100 treatment, the replicate of three trees per fertilization treatment per chamber could not always be met. Detailed information about the number of individuals selected for gas exchange measurement at each time point in the different treatments is given in Table S1 available as Supplementary data at *Tree Physiology* Online. A_{leaf} was measured with 400 µmol mol^{-1} CO_2, 1200 µmol m^2 s^{-1} photosynthetically active radiation, approximately 65% relative humidity and 25 °C air temperature. Predawn leaf water potential (ψ_{leaf}) was measured between 03:00 and 05:00 CET, at the same dates as gas exchange measurements on living individuals using a Scholander bomb (Model 600 pressure bomb; PMS Instrument Company, Albany, Oregon, USA).

Non-structural carbohydrates and N measurement

Leaves, stems and roots were ground to a fine powder with a ball mill (Retsch MM 400, Retsch GmbH, Haan, Germany). Samples collected in mid-July 2017 and on 13 July (during drought), 3 and 28 days after rewetting in 2018 were used for N analysis. Samples collected on 13 July, as well as 1, 3, 7, 28 days after rewetting in 2018 were used for NSC analysis. To determine NSC concentrations, we applied the method of Wong (1990) modified by Hoch et al. (2002). For soluble sugar extraction, approximately 10 mg of the homogenized material was boiled in 2-ml distilled water for 30 min. After centrifugation, a 200-µl aliquot of the extract was taken and invertase and isomerase (Sigma-Aldrich, St Louis, MO, USA) were added to degrade sucrose and convert fructose into glucose. The amount of glucose was then determined photometrically with the glucose-hexokinase assay. In this assay, glucose is converted via hexokinase in the presence of ATP to glucose-6-P. In a second step, glucose-6-P and NAD are converted to gluconate-6-phosphate and NADPH by glucose-6-phosphate dehydrogenase. In the reaction, the concentration of NADPH (equating to the concentration of glucose) was determined photometrically at 340 nm. The total amount of NSC (soluble sugars plus starch) was determined by adding amyloglucosidase from *Aspergillus niger* (Sigma-Aldrich) to a 500-µl aliquot of the plants extract and incubating it at 49 °C for 15 h for starch digestion. Total glucose was determined as described above and the starch concentration was calculated as the difference between total NSC and soluble sugars. Pure starch, glucose, fructose, sucrose and standard plant powder (Orchard leaves; Leco, St Joseph, MI, USA) were used as standards. Five to six milligrams of ground plant material was weighted into tin capsules that were combusted in an Element analyzer (CE Instruments NC2500, ThermoFisher, Lancashire, UK) for N analysis.

Soil nutrients

Topsoil samples (0–5 cm) were taken from each lysimeter during drought (13 July 2018) and 7 days after rewetting (13 August 2018). For determining soil inorganic N, an equivalent of

7.5 g dry soil was weighed into centrifugation tubes, extracted with 30 ml 1 M KCl and filtered with filter paper (DF 5895, Albet LabScience, Dassel, Germany) into 50-ml PE bottles. Ammonium-N (NH_4^+-N) concentration in the extracted solution was measured photometrically with flow injection (FIAS-400) and UV/VIS spectrometer (Lambda 2 s, Perkin-Elmer, Schwerzenbach, Switzerland). Nitrate-N (NO_3^-N) was analyzed by colorimetric analysis (Cary-UV50 spectrophotometer, Santa Clara, CA, US) by setting the absorption wavelength of nitrate at 210 nm (Navone 1964).

Statistical analysis

Statistical tests were computed independently for different plant tissues. Photosynthesis, leaf predawn water potential, dry weight, shoot:root ratio, NSC and N concentration were all analyzed using general linear models, followed by multiple-comparison post hoc tests for pairwise comparisons. Fixed factors were drought (four levels) and nutrients (two levels), time and their interaction. Chamber and lysimeter were included as random factors to control for the split-plot design of the experiment, using the four chambers of each water treatment as replicates, thus resulting in $n = 4$. Row and column number of the chambers were included in the complete model to check for possible spatial patterns, but were never significantly different and afterwards omitted from the model. Mortality and refoliation as dependent on fertilization were separately assessed for the D60 and D100 treatment applying a Student's *t*-test. All statistic calculations were performed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Significant differences were considered when $P < 0.05$.

Results

Drought-caused mortality and refoliation after rewetting

After 1.5 years (i.e. in 2017) of extreme drought (D100), mortality of saplings under fertilization was lower than in the unfertilized treatment (32 vs 50%, respectively, Table 1). Intensified drought caused higher mortality rate of saplings—nearly 13 and 65% of saplings died in D60 and D100 ($P < 0.05$), respectively. Under D100 (extreme drought), sapling mortality rate was 79% at LN and 52% at HN (Table 1, $P < 0.05$), respectively, while in the D60 treatment (severe drought) sapling mortality rate was similar between the fertilization regimes (14% at LN, 11% at HN, Table 1). A second-leaf flush occurred in 2018 after 2 weeks of rewetting under severe and extreme drought conditions (Figure S2 available as Supplementary data at *Tree Physiology* Online).

Responses of leaf water potential, photosynthesis and biomass during and after drought

Predawn leaf water potential and net photosynthesis of surviving oaks significantly decreased with increasing drought intensity (Figures 2 and 3A). This confirmed the increasing drought

Table 1. Biomass for organs (g individual^{-1}), the shoot:root ratio and mortality (%) during drought in July 2017 and after 28 days of rewetting in September 2018 and refoliation (%) after rewetting.

	Time	D0	D30	D60	D100
Leaf biomass	2017	23.2 ± 3.1a	21.3 ± 2.1a	11.1 ± 2.0b	1.1 ± 0.7c
	2018	28.0 ± 2.1a	14.4 ± 1.0b	7.1 ± 1.0c	7.2 ± 2.4c
Stem biomass	2017	57.0 ± 11.3ab	61.8 ± 13.1a	29.6 ± 6.0b	33.6 ± 5.7b
	2018	94.5 ± 7.9a	66.9 ± 5.3b	28.1 ± 3.6c	30.2 ± 4.4c
Root biomass	2017	84.3 ± 10.9a	74.8 ± 7.9a	50.5 ± 6.4b	30.5 ± 3.1b
	2018	141.6 ± 12.8a	91.5 ± 7.6b	52.4 ± 3.2c	39.6 ± 5.9c
Aboveground biomass	2017	80.2 ± 14.0a	83.1 ± 15.0a	40.6 ± 7.5b	34.6 ± 5.2b
	2018	122.6 ± 26.7a	81.2 ± 15.7b	35.3 ± 11.0c	37.4 ± 6.3c
Total biomass	2017	164.5 ± 23.1a	157.9 ± 18.5a	91.1 ± 12.6b	65.9 ± 6.1b
	2018	264.1 ± 21.3a	172.8 ± 10.5b	87.6 ± 6.6c	77.0 ± 12.0c
Shoot: root	2017	0.94 ± 0.12a	1.15 ± 0.17a	0.81 ± 0.13a	1.22 ± 0.29a
	2018	0.88 ± 0.04a	0.92 ± 0.09a	0.67 ± 0.04b	0.95 ± 0.08a
Mortality	2017 LN				50.0 ± 14.9A
	2017 HN				32.4 ± 17.1A
	2018 LN			14.3 ± 10.1a	78.6 ± 9.2A
	2018 HN			11.3 ± 7.0a	51.2 ± 8.3B
Refoliation	2018 LN			50.0 ± 20.6a	14.3 ± 8.3A
	2018 HN			59.5 ± 2.4a	24.6 ± 9.2A

Different c indicate significant differences ($P < 0.05$) among the drought treatment, based on the ANOVA analysis with post hoc multiple comparison test in 2017 and 2018. Data represents the mean ± SE ($n = 8$). D0, D30, D60 and D100 represent well-watered, mild drought, severe drought and extreme drought treatment, respectively. LN and HN mean non-fertilized and fertilized treatment, respectively. For mortality and refoliation, different lowercase and uppercase letters indicate the significant difference ($P < 0.05$) between nutrient treatments in D60 and D100, respectively, with Student's t-test. Data represents the mean ± SE ($n = 4$). The mortality of D100 treatment in 2018 represent the overall mortality during the experiment.

exposure across four soil water content treatments. Water-use efficiency tended to increase in the drought treatments compared with the control (Figure 3C). Drought significantly reduced the biomass of plants in severe and extreme drought treatments (D60 and D100) (Figure 3E), while shoot:root ratio did not differ among the four drought treatments (Figure 3G). Fertilizer application had no significant effects on the all measured saplings' physiological indictors during drought, such as photosynthesis, water-use efficiency, biomass allocation and leaf N concentration (Figure 3, $P > 0.05$).

Rewetting resulted in an increase of the predawn leaf water potential in previously drought-exposed saplings, returning to control level after 3, 14 and 14 days of rewetting in D30, D60 and D100, respectively (Figure 2A). Leaf net photosynthesis also increased in all drought treatments during the first week following rewetting and recovered 7, 28 and 28 days after rewetting in D30, D60 and D100, respectively (Figure 2B). Four weeks after drought relief, photosynthesis in the D30 treatment even significantly exceeded the values of controls ($P < 0.05$), which showed a continuous decline with time (Figure 2B). Leaf biomass in the D100 treatment was only 5% of the control in July 2017, but increased sixfold until the end of the experiment in September 2018 (Table 1, Table S2 available as Supplementary data at *Tree Physiology* Online, $P > 0.05$) and root biomass increased by more than 30% (Table 1, Table S2 available as Supplementary data at *Tree*

Physiology Online, $P < 0.05$). Leaf net photosynthesis was significantly higher in the LN (unfertilized) than that in the HN (fertilized) treatment after rewetting (Figure 3B, Table 2). In addition, fertilization significantly enhanced shoot:root ratio biomass in the D30 treatment (mild drought) after rewetting (Figure 3H).

Responses of plant N during and after drought

Leaf N concentration was slightly increased in drought-exposed saplings compared with that in well-watered plants (Figure 3I, $P < 0.05$). In contrast, whole-tree N pool in D60 and D100 was lower than that in D0 and D30 because of reduced biomass in those two treatments during drought (Figure S3A and Table S3 available as Supplementary data at *Tree Physiology* Online, $P < 0.05$). No significant effect of fertilization was found in N pools of leaves, stems and roots from the four drought treatments (Figure S3A and Table S3 available as Supplementary data at *Tree Physiology* Online).

Drought relief significantly increased leaf N concentration in D60 and D100, stem N concentration in D60 and root N concentration in D30 (Figure S4 available as Supplementary data at *Tree Physiology* Online, $P < 0.05$). Whole-tree N pools in D60 and D100 were significantly lower than those in D0 and D30 (Figure S3B and Table S3 available as Supplementary data at *Tree Physiology* Online), while fertilization had no significant effect on tissues N pools across the drought treatments after
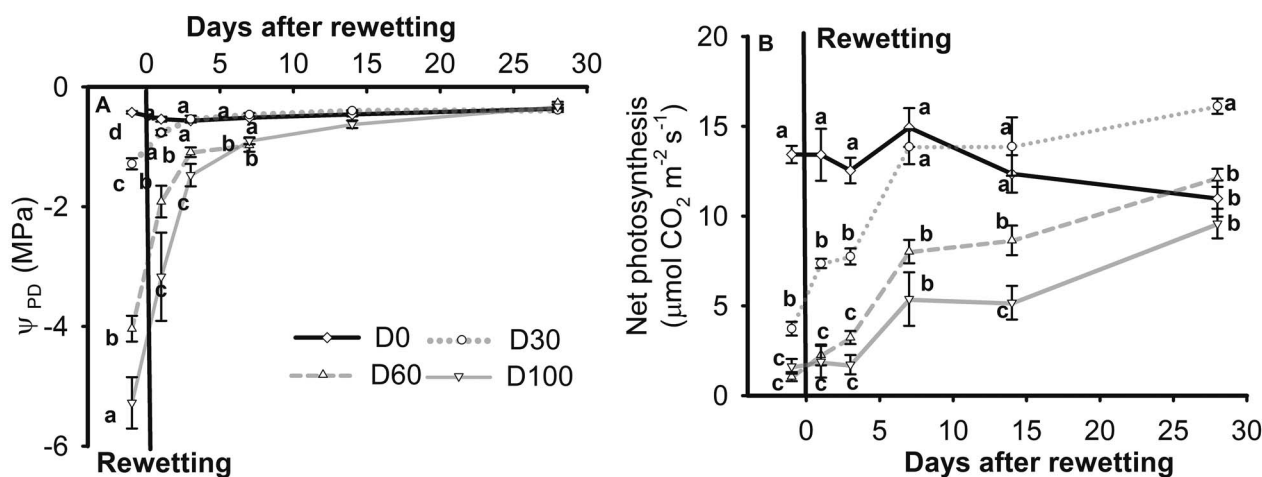
Figure 2. Dynamics of predawn leaf water potential (Ψ_{PD}, panel A) and net photosynthesis (panel B) in *Q. pubescens* saplings during drought and after rewetting. The vertical solid lines denote the time point of rewetting on 6 August 2018. The *x*-axis before number zero represents the drought period and measurement performed on 13 July 2018. D0, D30, D60 and D100 represent former drought stress conditions as described in Figure 1. Error bars represent standard error (mean $\pm$ 1 SE, *n* = 8). The letters indicate significant differences (*P* < 0.05) among drought treatments at a given timepoint.

Table 2. Three-way ANOVA statistical analysis of time, drought, nitrogen and interaction effects on plant physiological indicators.

	A		PWP		Leaf NSC		Stem NSC		Root NSC		WUE	
	F	P	F	P	F	P	F	P	F	P	F	P
T	39.40	<0.001	99.13	<0.001	10.28	<0.001	51.45	<0.001	2.67	<0.05	9.35	<0.001
D	166.36	<0.001	77.78	<0.001	94.63	<0.001	381.58	<0.001	26.82	<0.001	7.31	<0.001
N	5.10	<0.01	0.28	0.60	3.70	0.06	15.49	0.10	1.78	0.18	0.89	0.35
T × D	9.88	<0.001	22.85	<0.001	4.24	<0.001	22.56	<0.001	0.73	0.72	1.03	0.43
N × T	0.88	0.51	0.10	0.99	0.21	0.93	4.97	0.48	0.91	0.46	2.65	<0.05
N × D	0.01	1.00	0.69	0.56	4.94	<0.01	25.48	<0.01	5.08	<0.01	0.28	0.84
T × D × N	0.34	0.99	0.33	0.99	0.05	1.00	3.30	0.85	0.69	0.76	1.46	0.12

A, PWP and WUE represent net photosynthesis, predawn leaf water potential and water-use efficiency, respectively. T, D, and N indicate time, drought and nutrient treatments, respectively.

rewetting (Figure S3B, Table S3 available as Supplementary data at *Tree Physiology* Online, *P* > 0.05).

Responses of plant NSC during and after drought

During drought, leaf NSC was highest under well-watered conditions (D0), while the lowest values were found in the mild drought treatment D30 (Figure 3K). Soluble sugars in leaves, stems and roots were higher in the drought treatments compared with the well-watered, most likely at the expense of starch in leaves and stems (Figure 4, *P* < 0.05).

Twenty-eight days after rewetting, soluble sugars in all tissues of previously drought-exposed oak saplings were significantly reduced (Figure 4, *P* < 0.05), independent of the severity of the previous drought treatment. In stems, starch concentration tended to increase or at least to remain stable (Figure 4E–H). In the roots of D100 trees, sugars decreased strongly to a concentration as low as 0.48% after 28 days of rewetting, with starch values ranging even below detection limit (Figure 4L); in comparison, leaves and stems showed rather stable NSC concentrations (Figure 4D and H). The NSC pools, concentration

multiplied by biomass, showed comparable patterns as the concentration for stem and root NSC, while in the leaves, the highest leaf biomass in the D0 and D30 treatment resulted in higher NSC pools than in D60 and D100 (Figure S5 available as Supplementary data at *Tree Physiology* Online).

Responses of soil inorganic-N during and after drought

Soil inorganic-N concentrations in the fertilized treatment were significantly higher than in the non-fertilized during drought, and increased after 7 days of rewetting in D60 under non-fertilized and D100 (Table 3). In the well-watered soil (D0), soil inorganice-N concentration showed less changes over time and between nutrient treatments and concentration were significantly lower than in the rewetted soil (Table 3).

Discussion

Role of nutrient addition during and after drought

In contrast to our hypothesis 1, nutrient addition showed only minor or non-significant effects on gas exchange and

Table 3. Inorganic-N concentration (mg N kg^{-1} dry soil) in the top soil (0–5 cm) during drought and after seven days of rewetting.

	D0		D30		D60		D100	
	LN	HN	LN	HN	LN	HN	LN	HN
During drought	19.02 (2.58)Ba	19.82 (1.48)Aa	19.64 (4.55)Ba	52.08 (1.31)Bb	30.15 (5.02)Ca	58.98 (11.20)Bb	11.07 (0.65)Aa	26.87 (5.00)Ab
After 7 days of rewetting	14.55 (1.77)Aa	16.12 (1.66)Aa	23.35 (4.25)Ba	25.61 (2.93)Ba	61.02 (10.29)Da	64.73 (14.28)Da	49.78 (14.96)Ca	45.87 (5.39)Ca

Letters indicate significant differences ($P < 0.05$) between nutrient (lower case) and drought (upper case) treatments based on the ANOVA analysis with post hoc multiple comparison test in the given time point. Data in the parentheses represent the standard error ($n = 4$).

biomass partitioning between aboveground and belowground tissues under the four drought regimes. Soil N concentrations were higher under drought and fertilization compared with well-watered and unfertilized conditions, respectively. Drought stressed saplings could not benefit from the additional soil nutrients and this finding strongly suggest that drought limited the mobility of inorganic N in the soil. We might also infer that the downy oak saplings were not nutrient limited in our experiment under well-watered and drought conditions. Rather than the often observed positive effect of increased N availability (N deposition, Guerrieri et al. 2011), our result indicated that increased soil N negatively affected photosynthesis during drought recovery. After rewetting, inorganic N in the soil increased in the D60 without fertilization and D100 treatments 7 days after rewetting (Table 3), which might be responsible for the significantly increased leaf N and stem N concentration, thus supporting the recovery process in those treatments.

Nutrient addition and its interaction with drought did not significantly affect the shoot:root ratio in July 2017 after 1.5 years of drought treatment. We may thus infer that *Q. pubescens* is not highly sensitive to drought, which is in accordance with the previous findings (Damesin and Rambal 1995, Weber et al. 2007). In contrast to our results, studies on European beech and sessile oak showed that N addition during drought enhanced drought sensitivity of young trees (Dziedek et al. 2016). However, in our experiment, we only added half of the amount of N compared Dziedek et al. (2016) and did not observe increases in shoot:root ratios, which is often attributed to increased drought sensitivity.

After drought relief, saplings that were exposed to mild drought before and not fertilized, slightly increased belowground biomass investment, while this was not observed in fertilized saplings. This could make fertilized plants more susceptible in future drought events (Gessler et al. 2017). Nevertheless, lower sapling mortality under extreme drought (D100) and at least a trend to higher ratio of refoliation after drought with fertilization indicated that higher nutrient availability reduces adverse impacts of extreme drought. Thus, our hypothesis 2– that fertilized trees are more sensitive to extreme drought as the higher biomass leads to a greater water demand–can be rejected. We might assume that fertilization changes hydraulic

architectures of plants (Faustino et al. 2013), and higher nutrient availability might reduce embolism risk by decreasing stomatal conductance under drought (Bucci et al. 2006, Goldstein et al. 2013). Meanwhile, higher nutrient content in the soil during drought might have still provided some protection possibly due to N-based osmoprotectants (Lei et al. 2006), giving these trees a benefit over non-fertilized trees leading to a reduced mortality and improving the recovery process after drought relief.

Non-structural carbohydrate storage during drought

In agreement with our hypothesis 3, drought-exposed *Q. pubescens* saplings increased their NSC concentration in stems (except D30) and roots (except D100) compared with the well-watered saplings, while growth significantly decreased. This result is consistent with previous findings from drought-exposed saplings, which reduced growth and no depletion in NSC (*Quercus faginea* and *Pinus halepensis*; Sanz-Pérez et al. 2009) or an increase in NSC (*Populus tremuloides* and *Populus balsamifera*; Galvez et al. 2013; *Quercus* spp., Li et al. 2013). The increase in NSC concentration as observed here was also found for Scots pine, which accumulated NSC in the roots and stems but also in needles under short-term drought (Galiano et al. 2017). While needles in evergreen conifers are a central organ for long-term C storage (Kramer and Kozlowski 1979, Vanderklein and Reich 1999, Hoch and Körner 2003), deciduous leaves are mainly transitory stores for starch over the short-term (Vanderklein and Reich 1999, Fajardo et al. 2013).

The contrasting responses of soluble sugars (mainly increased) and starch (mainly decreased) to drought in leaves, stems and roots in the D100 treatment can be attributed to the different functional roles of these components. Low-weight sugars have multiple functions acting as intermediate metabolites, C transport compounds, osmolytes, and substrates for growth and respiration (Hoch 2015, Hartmann and Trumbore 2016, Martínez-Vilalta et al. 2016). Starch in contrast, is purely a storage compound, and can be mobilized and re-allocated for metabolic processes when the availability of new photosynthates is lower than the actual metabolic demand (McDowell et al. 2008, Sala et al. 2012, Hartmann et al. 2018). The fact that soluble sugars and not starch were accumulated
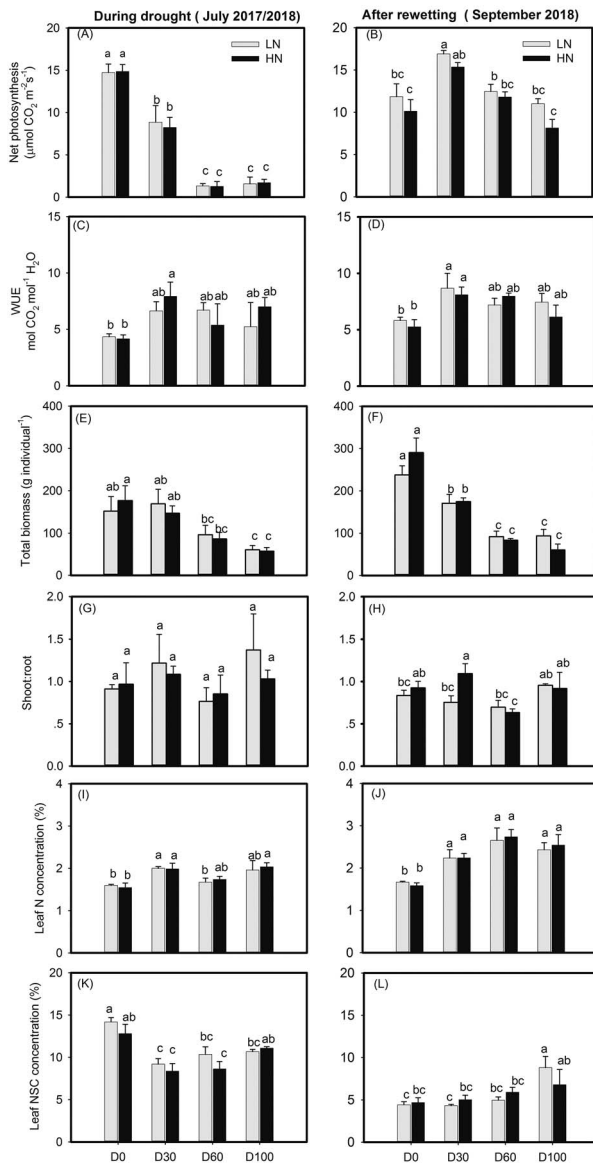
Figure 3. Net photosynthesis, water-use efficiency (WUE), total biomass of individual, shoot:root ratio, leaf N and NSC concentration of *Q. pubescens* saplings under fertilization (HN) and non-fertilization (LN) conditions during drought (left panels) and after 28 days of rewetting (right panels). Water supply conditions were well-watered (D0), two intermediate drought levels (mild drought D30, severe drought D60) and extreme drought (D100). (E) Total biomass measured in mid-July 2017 (before re-watering). Other indicators under drought condition were measured in July 2018. Different letters indicate significant differences between treatments combinations within a time point ($P < 0.05$). Error bars represent standard errors (mean ± 1 SE, $n = 4$).

in stems and roots under drought indicates that soluble sugars can be actively accumulated in two growing seasons as an osmotic response to maintain cellular and vascular integrity as water availability decreases (Woodruff and Meinzer 2011, Salmon et al. 2015). Our results are consistent with a long-term precipitation manipulation experiment with 100-year-old Scots

pine trees, where a switch to increased sugar concentration was also observed at different levels of soil water availability (Schönbeck et al. 2018).

Only in the roots were NSC concentrations not stabilized, and either slightly higher than controls in the mild and severe drought-stressed saplings or lower under extreme drought conditions, indicating a threshold for drought-stress where our hypothesis 3 is not valid. The reduced root NSC in extreme drought (D100) could on one hand be due to the temporary re-watering in the D100 treatment in 2017 to avoid excess mortality. As a consequence, root starch storage was utilized to support new tissues' growth after re-watering, leading to low starch concentration before the re-initiation of drought in spring of 2018. On the other hand, an almost complete exhaustion of starch as a consequence of extreme drought is in agreement with the findings of Huang et al. (2019) in Norway spruce. These authors induced C limiting conditions by reducing the CO_2 concentration and observed a rather constant supply of aboveground organs with new assimilates at the expense of a reduced root allocation. We thus suggest that Norway spruce and downy oak have the similar conservative strategy to respond with source limitation of C through preferentially allocating more C to aboveground tissues for regrowth and metabolites production, even at the expense of reduced root growth from C exhaustion (see Gessler and Grossiord 2019). Klein et al. (2014) hypothesized that NSC imbalances in different tissues can occur during drought, the restrained phloem transportation lowered availability of assimilates to root leading to passive root C starvation at first. In this case, our results indicate that under long-term extreme drought, C exhaustion begins in the roots (Adams et al. 2017) and indicates specific organ C starvation caused by a limited transport among tree compartments (Ruehr et al. 2009, Yang et al. 2016).

Plant responses after rewetting

After rewetting, trees in the mild drought treatment recovered within 1 week, and an overshoot in photosynthesis was observed compared with control saplings, as previously described by Arend et al. (2016) for beech. Such overshoot might partially compensate for losses of photosynthetic activity during drought.

In contrast to our expectations and to hypothesis 4, we did not observe an overall increasing trend for allocation of C to storage pools after drought relief as it was observed in leaf starch pool of *Tilia platyphyllos* and in stem and root pools in *Pinus sylvestris* (Galiano et al. 2017). Galiano et al. (2017) exposed plants only to a short drought period, and thus, recovery and allocation of new assimilates to storage most likely occurred much faster. The impairment (as indicated by the water potentials at the end of the drought treatment in 2018) and consequently the repair of hydraulic function were most likely determined by the previous drought intensity (Gessler et al. 2020). This might lead
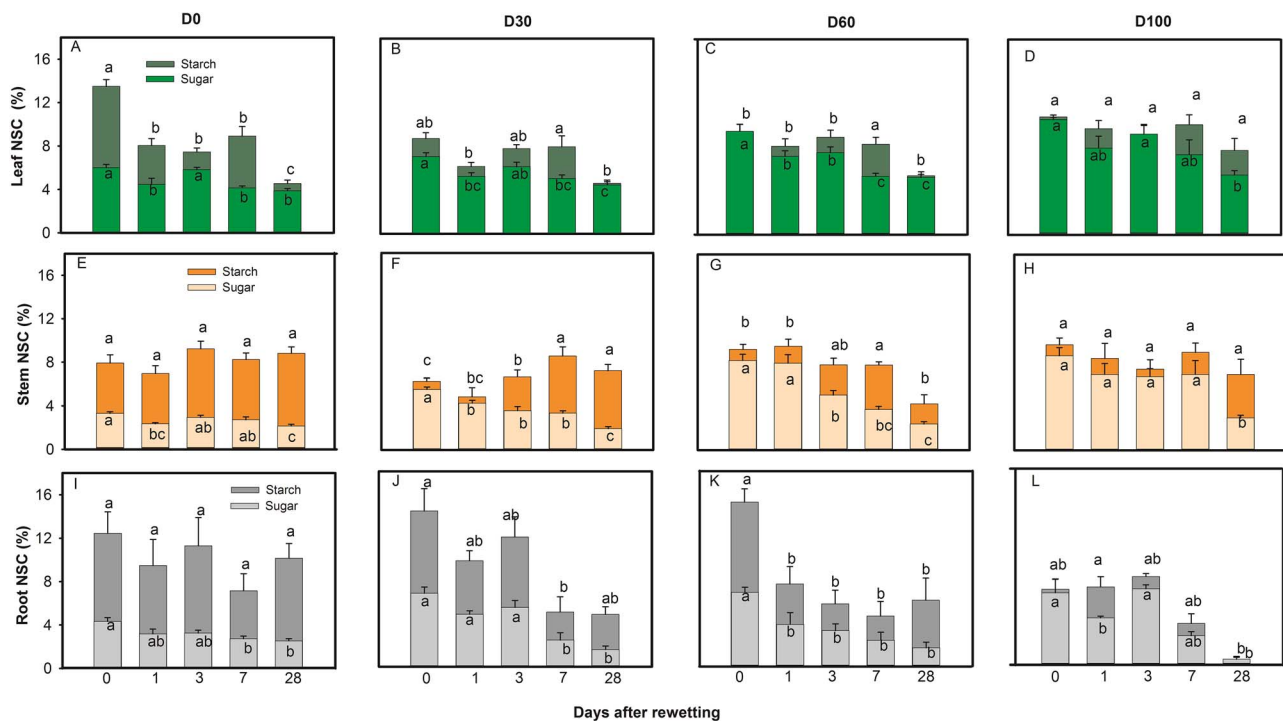
Figure 4. Temporal dynamics of soluble sugars and starch concentration in leaves, stems and roots of *Q. pubescens* saplings during recovery from four drought conditions. The number zero on the *x*-axis indicates sampling performed during the period of drought (13 July 2018). The four panels at the top, in the middle and on the bottom indicate dynamics of soluble sugars and starch in leaves, stems and roots, respectively, during drought and in the duration of rewetting. Green, light orange and light gray represent soluble sugars; deep green, dark orange and dark gray indicate starch. Error bars represent standard errors (mean ± 1 SE, *n* = 8). Different letters indicate significant differences of sugars and starch among the time points during drought and after rewetting (*P* < 0.05).

to the observed differences in NSC assimilation and partitioning between sugars and starch. In the D100 treatment, NSC was made up mostly of soluble sugars until day 7. Stem starch in that treatment increased only at Day 28 after rewetting when the photosynthesis and predawn water potential recovered to the well-watered level. The less stressed saplings (D30 and D60) increased stem starch earlier than D100, corresponding to their earlier photosynthesis recovery. We speculate that C investment into storage after drought relief could have been postponed in D100 plants due to the delayed photosynthesis and hydraulic recovery. The general conversion of soluble sugars towards starch during recovery as observed in our study are similar to the findings in stems of European beech saplings (Tomasella et al. 2019) and in two dwarf forest species (Yoshimura et al. 2016). All tissues' NSC concentrations showed no increase in D60 and D100 during the 4 weeks following rewetting. This pattern indicates that the C demand for the re-establishment of metabolic processes and repair of organs after drought exceeds the supply from the recovering assimilation during the observation period.

In roots, NSC concentrations decreased after drought relief in plants previously exposed mild and severe drought (D30 and D60) (compared with the control treatment), and this decrease was attributed to both, starch and soluble sugars.

The reduction in sugar concentration might be related to the fact that the demand for osmotic adjustment was reduced after rewetting (Salmon et al. 2015), while the general decrease in NSC might be caused by a strong increase in root metabolic activity (Ruehr et al. 2019), which leads to a higher demand than assimilate supply in the time after drought. Hagedorn et al. (2016) showed that root metabolic activity in beech after drought relief increases even before photosynthesis reacts, and that the C demand for restoration of root functioning strongly increases. Another probable reason might be that phloem transport, impaired during drought, did not completely recover, resulting in a higher proportion of recent assimilates retained aboveground to support repair and regrowth (Zang et al. 2014) and a decrease of NSC in the roots.

In the extreme drought treatment (D100), root soluble sugar and starch concentrations were completely exhausted 28 days following rewetting in 2018, leading to root NSC pool depletion. The extensive production of new leaves in this treatment strongly suggests that the root NSC pool (but not the stem pool) was almost completely depleted (i) to support new leaf growth (Wiley et al. 2019), (ii) to keep leaf NSC levels higher to sustain leaf functioning (iii) to repair hydraulic structure. Moreover, the root NSC depletion might in addition be due to root growth after re-watering in 2017 as root biomass

increased between July 2017 (drought) and September 2018 (after rewetting). However, we need to acknowledge that we cannot unambiguously attribute the increase in root biomass to the rewetting in 2018 or to the intermittent drought relief (to avoid excess mortality) in 2017. Galiano et al. (2011) showed that the amount of NSC storage determines the recovery of trees from long-term drought. We might assume that the ability to supply roots on the long-term with sufficient C might be decisive to regain the capability in taking up water and nutrients, but apparently *Q. pubescens* prioritized maintaining or restoring NSC pools in aboveground organs in previously extreme drought-stressed plants, which may increase the risk of mortality in case of repeated drought periods.

Conclusions

Our results suggest that increased nutrient availability did not lead to increased biomass allocation belowground in *Q. pubescens* under different drought intensities. The stabilized biomass partitioning under drought and fertilization was contrary to *P. sylvestris* and *Fagus sylvatica*, and this points to species-specific responses to nutrient supply in maintaining root metabolic function and C sink strength under drought stress. However, under extreme drought, fertilization reduced the mortality rate of saplings and the underlying mechanisms might be related to an altered hydraulic architecture. The reason for the decrease in mortality rate still needs further investigation. Even after relief of extreme drought, fertilization had no compensating effects on leaf C assimilation and root nutrient uptake. We found increased tissue NSC concentration under mild and severe drought mainly due to high sugar concentrations. This suggests that starch is converted to sugars for osmotic adjustment, instead of an active allocation of C to storage. Strong C depletion occurred in plant roots under extreme drought, mainly as a result of starch exhaustion, and C reserves were not replenished after drought relief even though photosynthetic rates increased, confirming findings from Hartmann et al. (2013) in roots of Norway spruce. Drought relief even aggravated the depletion of root NSC, when—as in our case—root storage pools were used to support production of new tissues. We assume that repeated drought events that force trees to re-establish their leaves in multiple years in a row might eventually lead to C starvation and further mortality.

Conflict of interest

The authors declare that they have no conflicts of interest.

Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* Online.

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