

Reactions of three European oak species (*Q. robur*, *Q. petraea* and *Q. ilex*) to repetitive summer drought in sandy soil

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ABSTRACT

Young trees of deciduous *Quercus robur* and *Q. petraea* and evergreen *Q. ilex* were grown together in a competition lysimeter experiment to assess i) – whether the observed growth differences between evergreen *Q. ilex* and the deciduous *Q. robur* and *Q. petraea* on sandy soil in the field and ii) – whether the different natural distribution of *Q. robur* and *Q. petraea* could be attributed to physiological differences between the species under experimental drought stress (DS). Half of the plants were subjected to long-term DS in two consecutive years and monitored for physiological and growth parameters. In the first year, water withholding for more than three months did not lead to significant drought stress, probably because of a sufficient residual water volume in the lysimeter for the relatively small plants. However, in the second year, 2018, which was warmer, the bigger plants now competed for the residual water and clear drought stress symptoms developed for more than two months in all trees in the DS lysimeter basin. Growth was only moderately (and mostly not significantly) affected by the DS in the second year, except for a smaller total leaf area in DS *Q. ilex* as compared to DS *Q. robur* and *Q. petraea* and smaller root collar diameter in DS *Q. ilex* compared to DS *Q. robur*. Under DS, the deciduous species revealed significant decreases in ΔV_{IP} , indicating a negative effect on electron transport through PS I. P_n , PI_{abs} and water relations parameters (Ψ_{PD} and LWC) all decreased to various extents under DS in all three species, leading to clear separation of the deciduous from the evergreen species by PCA. However, PCA did not separate the two deciduous species from each other. It is concluded that longer root growth in the two deciduous species as compared to *Q. ilex* ameliorates DS effects in *Q. robur* and *Q. petraea* and may be the key to understand the better performance of deciduous oaks on sandy soil in the field.

1. Introduction

The extremely hot and dry summer weather in Central Europe in 2003 and 2018, resulting in unprecedented losses of viable forest trees across all forest types (Schuldt et al., 2020), has highlighted the need for a proactive restructuring of the European forest landscape. Hanewinkel et al. (2012) pointed already to the shifts in the potential distribution ranges of main European forest tree species by 2100 in the course of climate change and predicted that for large parts of Central and Southeastern Europe a shift from beech- to oak-dominated forests must be taken into account, including Mediterranean oak species. But migration is a slow process in trees, because of their long generation cycles. Many tree species, particularly zoochory ones those with heavy seeds, like oaks, cannot spread at the time scale of climate change (McKenney et al., 2007). One option to counter this, is to use assisted migration in managed forests (Bussotti et al., 2015). To evaluate which species could be used for this management option it is important to test

their behavior in different setups with various water availability, soil composition and climate conditions.

In this context, we have previously studied the behavior of the Mediterranean *Quercus pubescens* and *Q. ilex* in a competitive lysimeter setup together with the local species, *Q. robur*, under controlled drought conditions (Früchtenicht et al., 2018a, 2018b) in a nutrient-rich, loamy soil. In this study, *Q. pubescens* and *Q. ilex* revealed very similar growth – measured as stem diameter increase – during a severe, long-term summer drought and under well-watered control conditions, in contrast to *Q. robur*, which showed a strong growth decline under drought. This preliminarily indicated that the Mediterranean species had a growth advantage over the local species, *Q. robur*, under drought. The better performance of *Q. ilex* over *Q. robur*, however, contrasted our findings in field trials on a sandy soil in non-controlled forest plantations in Southern Germany, where during 10 years with frequent summer drought conditions, size growth of French *Q. ilex* was only half the growth observed in *Q. robur* (Holland, Brüggemann et al., unpublished results).

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Since during recent heat waves (2003 and again 2018–2020) *Q. robur* growing on sandy soil in floodplain forests e.g. along the Rhine river was particularly susceptible to combinations of heat and drought (cf. e.g. Süßel and Brüggemann, 2021), we therefore repeated our experiment with sandy soil collected near the site of our field trial and included *Q. petraea* from Southwest Germany in the study, to address the following hypothesis:

I: The better performance of *Q. robur* on sandy soil in field trials over *Q. ilex*, contrasting their behavior in loamy soil, may be attributed to soil conditions rather than to other differences in abiotic or competition factors between our previous lysimeter experiment and the field plot.

II: The different distribution patterns of *Q. robur* (stronger in floodplain forests and in wetter stands) and *Q. petraea* (stronger in warmer and dryer conditions and in hill slope stands, cf. e.g. Arend et al., 2011; Kuster et al., 2013; Skiadaresis et al., 2019), may – at least in part – be attributed to physiological differences in the drought response of the two species.

To evaluate the fitness of the species we conducted a setup with controlled water supply. In addition to classic biomass parameters and sap flow measurements, we assessed the efficiency of photosynthesis via net photosynthetic rate (P_n) and prompt chlorophyll a fluorescence (ChlF). Here we focus on two main parameters which are proxies for energy conservation from photons absorbed by PSII to the reduction of inter-system electron acceptors and for the efficiency of electron transport from intersystem carriers to PSI end acceptors (Ceppi et al., 2012).

2. Material and methods

2.1. Plant material, experimental site and setup

Two-year old plants of *Q. robur* L. (provenance: Oberrheingraben, Germany; D-81707), *Q. petraea* (Matt.) Liebl. (provenance: Oberrheingraben, Germany; D-81809) and *Q. ilex* (provenance F-qil Languedoc) were provided bare-rooted with intact taproots by Darmstädter Forstbaumschule, Darmstadt, Germany. Six trees of uniform size from all species were grown together in two identical lysimeter basins ($6 \times 4.5 \times 2 \text{ m}^3$) filled with sand (sand: 87,32%, silt: 7,78%, clay: 4,9%; field capacity: 30.35% volumetric water content; pH value 5.57) collected from the fluvial terrace of the Main river in the Frankfurt city forest (50°02'14.87"N; 8°36'10.34"E). The basins were established in the Scientific Garden of the University of Frankfurt (50°10'N and 8°37'E). Trees were evenly distributed and allowed to develop root systems under well-irrigated conditions for one vegetation periods in the basins prior to the experiment (groundwater level -1.7 m). Tree spacing is given in Fig. 1. The gaps between the trees were filled with six *Q. pubescens* from Hungary (provenance Ausn.-Erl. 213qpu12018, Pilismarot-Dömos), which were outperformed before

starting the drought experiment. Data on this species were therefore not included in the present paper.

The groundwater level was monitored by floaters connected to drainage pipes at the bottom of the basins. The two basins were shielded against precipitation by a foliar semi-cylindrical greenhouse (6.2 m long, 9.5 m width and 3.9 m maximum height; FiLCLAIr greenhouse technology, Venelles, France) with 60–70 % light transmission. From April to November, the lower 2.5 m of the north and south sides of the greenhouse were kept 90° open to allow air circulation. Temperature was recorded continuously by EASYLOG USB 2 data loggers (Lascar Electronics Ltd., Salisbury, UK). In both basins five trees per species were randomly selected for measurements (Fig. 1). One basin was selected as control (CO) and watered throughout the experiment, maintaining a constant groundwater level of -1.7 m . In the other basin, groundwater was extracted at the start of the drought treatment and the basin was not watered from Jun 06th to Sep 07th 2017 (first water deficit period, then groundwater level was adjusted to -1.7 m again) and from May 05th to Sep 05th 2018 (second water deficit period).

2.2. Volumetric soil water content (VWC), predawn (branch) water potential (Ψ_{PD}) and relative leaf water content (LWC)

The VWC was monitored periodically with a Theta probe ML2x (Delta-T Devices, Cambridge, UK) in 50 cm depth. Ψ_{PD} was measured with a Scholander pressure chamber on-site immediately after cutting Ø 2–4 mm two-year-old branches from the main stem (SKPM 1400 pressure chamber, SKYE Instruments, Powys, Wales, UK). We used two north-facing branches close to the ground (maximum height 40 cm) per tree and measured one to three hours before dawn.

For leaf water content (LWC), ten fully developed south exposed leaves per treatment at breast height were cut predawn and transported to the lab in an airtight plastic bag. After cutting off the leaf stalk, the fresh weight (FW) was recorded. After full saturation (achieved in a Petri dish with a moist tissue for 26 h) the turgor weight (TW) was measured. Thereafter, leaves were dried in the oven for two to three days at 70 °C for the measurement of the dry weight (DW). Relative leaf water content was calculated according to Smart and Bingham (1974) [$\text{LWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW})$]. Due to an experimental error, LWC data of the control and the recovered *Q. ilex* on 3rd Sep. 2018 were not collected.

2.3. Biomass parameters

Height and root collar diameter (i.e. stem diameters 5 cm above ground) were measured right after planting to ensure uniform size of the trees across all three species, before and after each water deficit period, and at the end of the experiment. Maximum attained root length ($> 1 \text{ cm}$ diameter) was measured after completion of the experiment and excavating one representative tree per species with a stem length close to the average of the respective species/treatment group. This approach was chosen due to limited manpower for carefully excavating the 2 m deep basins. Leaf Area Index (LAI) was measured with a Delta-T SunScan Canopy Analysis System SS1 (U.K.).

2.4. Leaf gas exchange

A portable gas exchange analyzer (model GFS-3000 with standard head, Heinz Walz GmbH, Effeltrich, Germany) with an eight-cm² leaf chamber (model 3010-2 × 4) was used to measure net photosynthetic rate (P_n [$\mu\text{mol m}^{-2} \text{ s}^{-1}$] = $[[u_e * (c_e - c_o)] / \text{LA}] - E * c_o$; c_e = CO₂ mole fraction at the outlet of the cuvette; c_o = CO₂ mole fraction at the inlet of the cuvette.) Measurements were performed *in situ* under the following conditions: 900 $\mu\text{E m}^{-2} \text{ s}^{-1}$, 400 ppm CO₂, 25 °C and 50% relative humidity within the cuvette. All measurements were conducted between 8 am and 3 pm. For each tree, we selected two south exposed leaves at breast height. First one leaf per tree was measured alternating between the control (CO) and water withholding (DS) trees with five val-

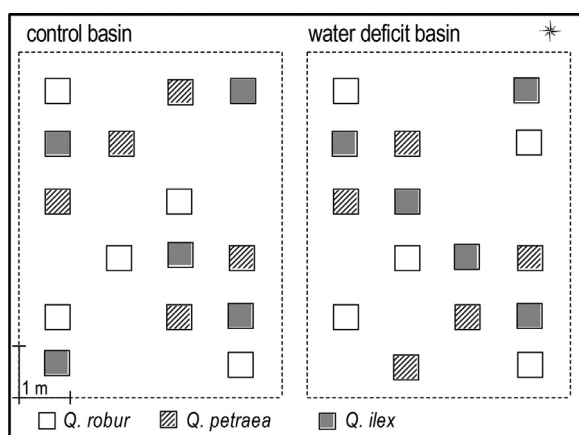


Fig. 1. Setup of the *Quercus robur*, *petraea* and *ilex* plants in the lysimeter.

Table 1

Abbreviations. Calculated fluorescence parameter according to Strasser et al. (2010) and Ceppi et al. (2012).

ChlF	Prompt chlorophyll a fluorescence	
	$PI_{\text{abs}} [RC/ABS] * [\varphi PO / (1 - \varphi PO)] * [\psi E0 / (1 - \psi E0)]$	Performance index on absorption basis; for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors
	$\Delta V_{IP} (1 - VI)$	Relative amplitude of I-P phase, proxy for efficiency of electron transport from ISC to PSI end acceptors
CO	Control treatment	
DS	Water withholding treatment	
HRM	Heat ratio method	
LWC	Leaf water content	
PCA	Principal Component Analysis	
P_n	net photosynthetic rate [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	$[[u_e * (c_e - c_o)]/LA] - E * c_o$ $c_e = \text{CO}_2$ mole fraction at the outlet of the cuvette; $c_o = \text{CO}_2$ mole fraction at the inlet of the cuvette
PS I	Photosystem I	
PS II	Photosystem II	
VWC	Volumetric water content	
Ψ_{PD}	Predawn water potential	

ues recorded per leaf after reaching stable values, to generate a mean for each leaf. When all trees were measured once, i.e. around 11 am, the second leaf of each tree was measured in the same order. We could not detect significant different values between leaves, which were measured in the morning and early afternoon (regarding to midday depression), hence the values of the two measuring rounds were merged.

2.5. Chlorophyll fluorescence measurement

The prompt chlorophyll a fluorescence (ChlF) was recorded using the JIP test with a portable fluorimeter (Pocket-PEA, Hansatech Instruments Ltd, Norfolk, UK) between 3 and 1 h before dawn to guarantee fully dark-adapted leaves. The measurements were taken and values were calculated according to Strasser et al. (2010) with 50 μs as F_0 (see Table 1). The JIP test parameters deduced from the original induction curves were further calculated with the Biolyzer 4HP v.4.0.30.03.02 software and Excel 2016.

2.6. Sap flow measurement

The heat ratio method (HRM) (Burgess et al., 2001) commercial system SFM1 Sap flow meter (ICT International Pty Ltd., Armidale, NSW, Australia) was used for continuous measurements (every 15 min) of sap flow. Eastward-oriented, unshaded or little shaded branches with 11–21 mm diameter of three individuals per species (1 sensor CO, 2 sensors DS) were used. Flow rates were related to calculated final leaf area per branch. For leaf area calculation, total number of leaves per measured branch was counted and multiplied with the average area per leaf. Average leaf area was determined by scanning 30 randomly selected leaves per tree with a flatbed scanner (Epson perfection V750 PRO), using Adobe Photoshop Elements 9.0 for determining pixel numbers. Daily flow rates reported the 5-day running average of the sum of hourly flow rates per day from 9 am to 3 pm to dampen effects of shortterm temperature and radiation oscillations.

2.7. Statistical analysis and graphic program

Most obtained data were analyzed and illustrated with the GraphPad Prism 5.04 software (GraphPad Software Inc., La Jolla, USA). Normality tests were done with Kolmogorow–Smirnow-Test.

The Principal Component Analysis (PCA) was calculated and illustrated in PAST 4.03 (Oyvind Hammer, Freeware: <https://www.softpedia.com/get/Science-CAD/PAST.shtml>). The following settings were used: Matrix: correlation; Groups: between-groups; Missing values: Iterative imputation; Scatter plot in Eigenvalue scale.

3. Results

3.1. Environmental features

The time courses of temperature in 2017 and 2018 inside the foliar greenhouse are presented in Fig. 2. The data loggers inside the greenhouse revealed higher temperatures compared to an outside climate station close by (night/during dawn: + 0.7 °C; cloudy/rainy days (solar radiation up to 400 Wh/m²): + 1.8 °C; sunny days (solar radiation above 400 Wh/m²): + 4.4 °C). The elevated temperature especially under full sunny conditions, can be considered as an (additional) stressor in both basins. In both years, the maximum daily temperature reached 30 °C more than 100 times (2017: 104; 2018: 107). The minimum daily temperature remained above 20 °C 9 times in 2018 and not once in 2017.

Outside the water withholding periods and in the control basin the groundwater level stayed around -170 cm. During the water withholding times, the level dropped to -200 cm (2017: Jul 28th; 2018: Jun 06th) in the DS basin, which means that no residual water was visible in the drainage pipes. Prior to the first water withholding period in 2017 the soil moisture in 50 cm depth stayed around 19% VWC. Until Sep 04th the value in the DS basin dropped to 13.3% VWC. After rewatering the soil in both basins contained around 19% VWC again. In 2018 the values in the water deficit basin dropped down to 5.8% VWC by Aug 28th and after rewatering the values approached 19% again.

3.2. Water relations

Ψ_{PD} did not show significant differences before the drought periods and after recovery comparing the three taxa and CO and DS, with all values above -0.3 MPa (Fig. 3 A, B). Even during the water withholding times in 2017 the statistical analyzes could not detect significant differences between the species within one basin, both for the DS and the CO basins. In 2018, again no statistical interspecific differences were obtained under drought conditions (ST1). Considering the intraspecific differences between the DS and CO groups, all three taxa showed significant differences during the water withholding times in 2018 (Fig. 3). In 2017, intraspecific differences between CO and DS groups were not significant and no real drought stress could be verified, with values around -0.3 to -0.4 MPa on Sep 05th (Q. robur: -0.37 ± 0.11 MPa; Q. petraea: -0.31 ± 0.03 MPa; Q. ilex: -0.41 ± 0.04 MPa; means \pm SEM [standard error of mean]). Contrastingly, in 2018, all species showed significant intraspecific differences during the DS treatment. Minimum values were reached on Aug 29th (Q. robur: -2.84 ± 0.22 MPa; Q. petraea: -2.87 ± 0.12 MPa; Q. ilex: -2.59 ± 0.06 MPa; means \pm SEM). (Fig. 3A, B).

The LWC values in 2017 stayed above 90% LWC in all species and both basins (Fig. 3C, D). In 2018, trees in the control basin revealed a decline in the course of the summer but did not show significant differences

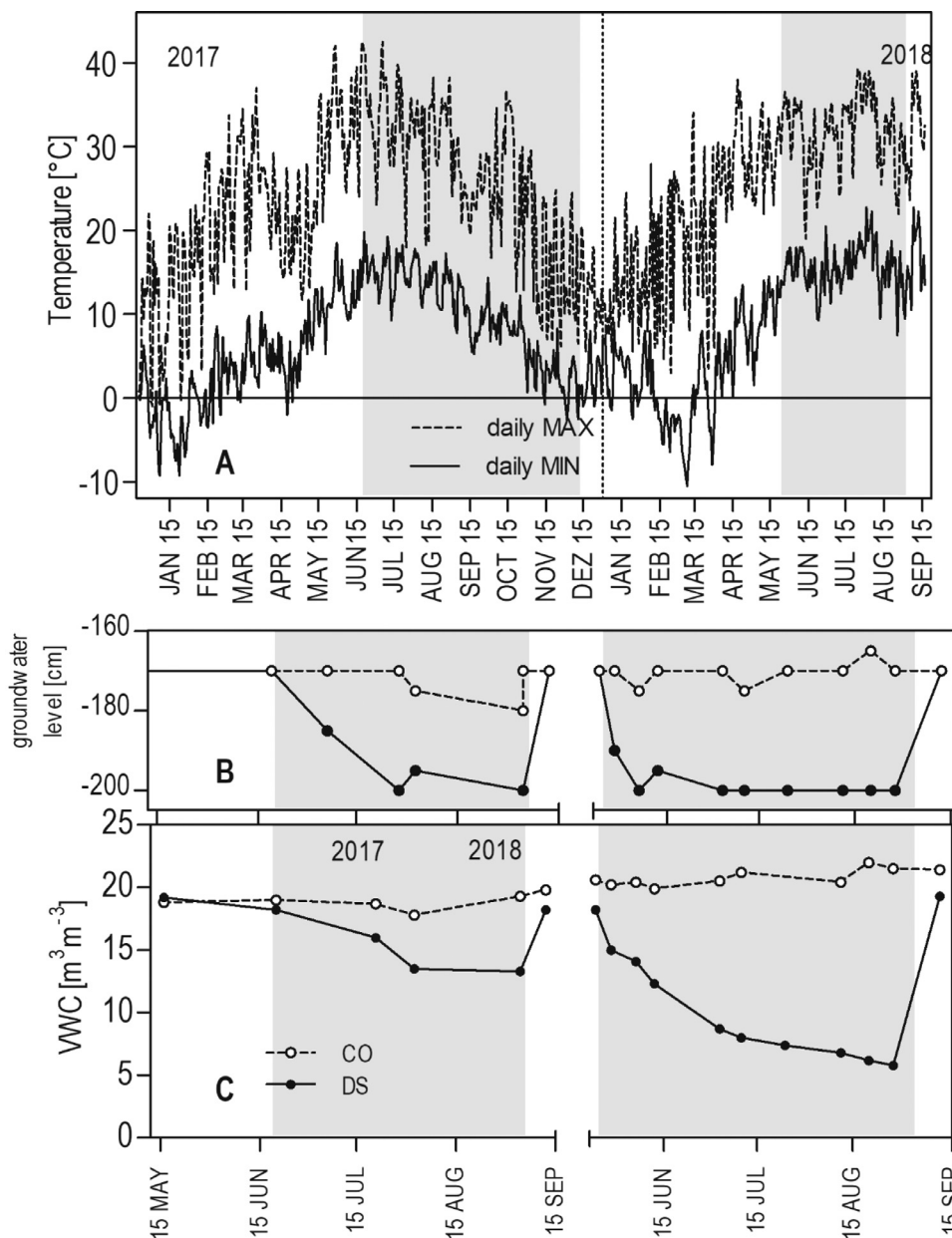


Fig. 2. Daily minimum and maximum temperatures (2A), groundwater level in the basins (2B) and volumetric water content at 50 cm depth (2C) in the lysimeter.

among the species (all values $> 85\%$ LWC). Under DS, *Q. robur* showed significantly higher values than *Q. petraea* and *Q. ilex* under DS on Aug 01st and Aug 10th, but, significant intraspecific differences between the trees in the control and the DS basins could only be detected towards the end of the DS treatment (cf. Supplementary data Table ST1). The minimum values were reached in the DS plants on Aug 29th (*Q. robur*: $77.0 \pm 5.5\%$; *Q. petraea*: $77.8 \pm 6.2\%$; *Q. ilex*: $77.1 \pm 1.0\%$) (Fig. 3D).

Since the young trees were hardly affected by withholding water from mid-June to begin of September in 2017, the water deficit period started much earlier in 2018 and was prolonged, to simulate not only a summer drought, but a dry spring and a dry full summer period. In the following, we will focus on data sets obtained in 2018, since data from 2017 in general revealed little or insignificant effects between trees from the two basins. This time, during the DS period, trees reacted strongly with sap flow values decreasing in all three species (Fig. 4). However, while DS *Q. robur* and *Q. ilex* developed a decrease of the sapflow rates compared to the controls already from roughly DOY 180 (*Q. robur*) and 190 (*Q. ilex*) on, DS *Q. petraea* maintained sapflow rates similar to the control tree until DOY 220.

3.3. Effects of drought intensity and duration on photosynthesis-related parameters

In 2017, with the minor effects of the drought treatments on plant water relations, the photosynthetic rates (P_N) showed only slightly, but significantly lower values in the DS plants of *Q. petraea* and *Q. ilex* than in the CO, but not between *Q. robur* CO and DS plants on August 12 (cf. Supplementary data Table ST2).

To account for the effects of leaf development, we measured P_N and ChlF parameters at the beginning (JUN 13; DOY 164) and at the end (AUG 29; DOY 241) of the DS treatment in 2018, both in control and in DS plants, and calculated the ratios (end of DS/begin of DS) for each individual plant to obtain unit-free and specimen-independent data describing a potentially positive effect of leaf development (i.e. ratio > 1) or a potentially negative effect of DS (ratio < 1). In 2018, all three species showed significantly lower ratios of P_N in DS than in control plants (Fig. 5A).

In Fig. 5 (B, C), we compare the ChlF parameters PI_{ABS} and ΔV_{IP} in the same manner as ratios before and during the water withholding

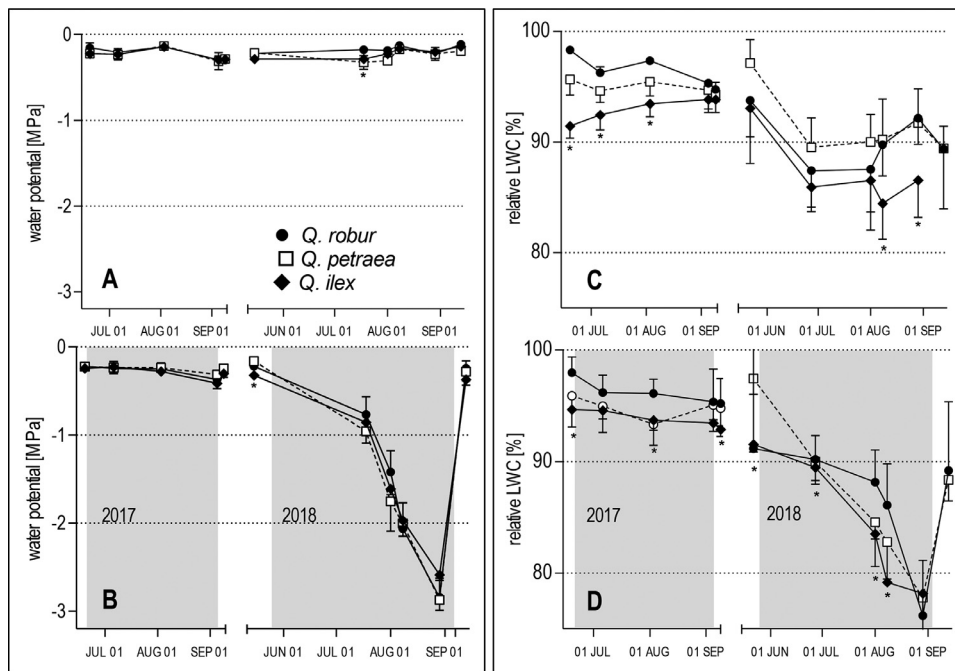


Fig. 3. Leaf water potential (3A, 3B) and relative leaf water content LWC (3C, 3D) in *Quercus robur*, *Q. petraea* and *Q. ilex* leaves during the experiment. Top: control basin, bottom: Water deficit basin. Means \pm SD: Ψ_{PD} $n = 5$ with 2 technical replicates per tree and LWC $n = 5$ with 10 technical replicates per tree. Significant differences between the species are indicated by asterisks (*: $P < 0.05$, Kruskal-Wallis test).

time in 2018 (AUG 29/ JUN 13) between control and DS trees to account for potential effects of leaf development (i.e. potential deviations from a value of 1.0 in the control plants). The PI_{ABS} (Fig. 5B) ratio reached significant lower values in DS *Q. ilex* compared to the control. The ΔV_{IP} ratios showed a significant reduction in the DS trees compared to the controls in *Q. robur* and *Q. petraea*, but not in *Q. ilex* (Fig. 5C).

PCA analysis of the ChlF and the gas exchange parameters clearly clustered DS from CO plants and *Q. ilex* vs the deciduous species, except for one outlier tree in each group (Fig. 6). The principal axes explained 67 and 23% of the variance, respectively. Attempts to separate the two deciduous species by PCA were unsuccessful.

3.4. Biomass parameters

Right after planting, there were no significant differences between the species with respect to biomass parameters. All species revealed nearly the same values in root length, mass, height and root collar diameter (cf. Supplementary data Table ST3).

While *Q. petraea* did not show significant differences in the biomass parameters over the treatments (Fig. 7), and a similar maximum root length at the end (CO: 180 cm; DS: 190 cm), *Q. robur* showed generally (not significant) lower growth values in the drought treatment than in control (Fig. 7), but the maximum root length at the end of the study was higher in the drought treatment (CO: 150 cm; DS: 190 cm). In *Q. ilex*, again a tendency towards lower values in height and total leaf area of the DS plants were visible, yet they were not significantly different from the control plants (Fig. 7). Final root length was nearly identical (150–155 cm) in DS and CO *Q. ilex*.

As a cumulative effect of the tendency towards decreasing total leaf areas in DS plants in all three species (Fig. 7), even if not significant on species level, the total leaf area index was significantly ($P < 0.05$) higher in the control basin (5.44 ± 1.64) as compared to the DS basin (4.22 ± 1.08 , means \pm SD, $n = 16$).

4. Discussion

4.1. Experimental setup, drought development and physiology

The experiment described in this contribution was similar to the one described in Früchtenicht et al. (2018a,b); however, the soil was quite

different this time to study the hypothesis that, in a sandy soil, *Quercus ilex* performs worse than *Q. robur* while, under good mineral and water conditions in loamy soil, growth differences between these species can be neglected. Furthermore, in the present study, we wanted to find out whether, under low mineral conditions, differences may be obtained between *Quercus petraea* and *Q. robur*. The two latter species co-occur in various conditions throughout western Europe in cultivation, whereas, under (semi-) natural conditions, *Q. petraea* rather occupies drier stands (e.g. slopes), while *Q. robur* rather occupies floodplain stands, often with groundwater access (e.g. Ponton et al., 2001; Skiadaresis et al., 2019), suggesting that the former could be more drought tolerant under field conditions. In a thorough study comparing four different Swiss provenances of each of the two species, together with *Q. pubescens*, and exposing them to a similar drought treatment on a loamy sand substrate, Arend et al. (2011) found that repetitive long-term drought over three subsequent years inhibited growth in both species significantly with provenance-specific responses. However, their data revealed no clear species-specific pattern of the growth response towards the consecutive long-term drought events. By using a sandy substrate with low water holding capacity, we supposed to find physiological and/or growth response differences between these species under more severe drought exposure than used in the Arend et al. (2011) and in our previous study.

In the first year of the experiment, i.e. one year after the plantation, the trees were apparently still too small to efficiently compete for the residual water in the DS basin, as evidenced by the lack of a decline of Ψ_{PD} (Fig. 3B, left panel). Thus, even if they experienced water shortage during daytime, as reflected in the slightly decreased photosynthesis rates in the DS plants of *Q. ilex* and *Q. petraea*, they were all able to refill their water reservoirs overnight again from residual (interstitial) water in the basin (cf. Fig. 2C). However, in the second year, with the trees being bigger and warmer conditions (2018 was an exceptionally hot summer in Germany: compare spring temperatures in Fig. 2A between 2017 and 2018), drought developed faster and more uniformly in the sandy soil in the DS basin than it had done in the previous study (Früchtenicht et al., 2018a,b), where *Q. pubescens* and *Q. ilex* trailed behind *Q. robur* in the development of strongly negative Ψ_{PD} values. This time, with soil water content decreasing sharply in the second year, also *Q. ilex* developed a Ψ_{PD} well below -2.5 MPa in parallel to the deciduous species. The more uniform development of Ψ_{PD}

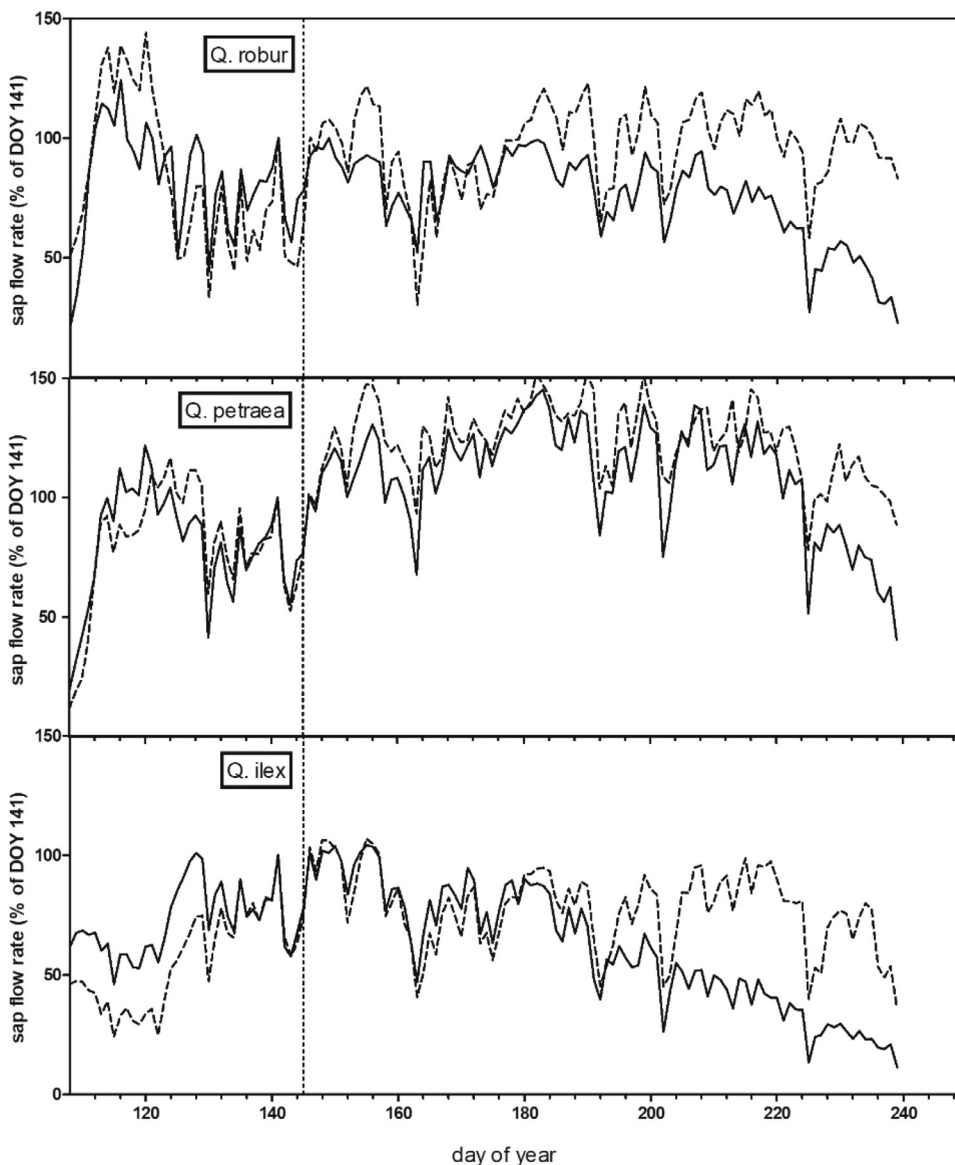


Fig. 4. Daily sapflow volumes in control (dotted lines) and DS (solid lines) trees of *Q. robur*, *Q. petraea* and *Q. ilex* in 2018, normalized for the last peak before the onset of the DS treatment on DOY 141. 100% values on DOY 141 reached between 100 and 120 ml/dm² sap wood * day in *Q. robur* and *Q. petraea* and 200–250 ml/dm² sap wood * day in *Q. ilex*, respectively.

also indicated that the trees exploited the limited water resources more uniformly now than their counterparts had done in the previous experiment. In *Q. robur* and *Q. petraea*, leaf water content decreased in parallel to Ψ_{PD} in 2018. In contrast, in a field study in a mixed 30-year-old *Q. robur* / *Q. petraea* stand in a forest in Northern France on silty clay loam, Bréda et al. (1993) had observed significantly more negative Ψ_{PD} in *Q. robur* under DS than in *Q. petraea* and suggested this could be due to different root lengths (*Q. petraea* developing the deeper root system) under competing field conditions, which was not the case in our study (both species developed 1.90 m long roots under DS). *Q. ilex*, in contrast, retained a higher LWC for several weeks, indicating a better control of leaf water loss (Fig. 3D). Interestingly, in 2018, LWC of *Q. robur* and *Q. ilex* also decreased to some extent in the control basin in all species (Fig. 3C). We suppose that this may be an effect of the higher temperatures compared to 2017, which could lead to increased cuticular transpiration. In the course of the DS, *Q. ilex* showed an earlier decline of sap flow than the deciduous species (Fig. 4). Since *Q. ilex* and *Q. robur* developed similar minimum stomatal conductance under prolonged DS in a previous study (Koller et al., 2013; Urli et al., 2015), we presume that this correlates with two other factors: first, DS *Q. ilex* had lower leaf area at the end of the experiment, this being either the result of partial leaf shed-

ding, lower leaf development and/or a combination of both. Secondly, the excavated representative specimen of *Q. ilex* developed a shorter taproot than those of the deciduous species, thus experiencing more problems to reach the latest water reserves at the bottom of the basin (see also Fig. 2). In a 40-d-drought experiment with potted 3-year-old *Q. ilex*, *Q. frainetto* and *Q. cerris*, Manes et al. (2006) reported that *Q. ilex* developed a much smaller root system than the deciduous species, with a further strong reduction under drought conditions (Chiatante et al., 2005). Similarly, a 100-d drought treatment of *Q. ilex* seedlings resulted in a strong reduction of fine roots in the lowest soil compartment. However, that despite the general assumption that xerophytic Mediterranean tree and shrub species, including *Q. ilex*, often rely on deep root systems as part of a drought avoidance strategy (e.g. Manes et al., 1997; Fotelli et al., 2000; Bantis et al., 2018), *Q. ilex* root growth may follow a different pattern when growing in sandy soil like in the floodplains on the coast of Tuscany, where real drought stress hardly occurs due to groundwater availability (cf. Bussotti et al., 2002).

Both factors will contribute to lower whole-plant transpiration, which was then manifested in the lower sap flow values. However, once the last reserves of water in the DS basin were used up, sap flow decreased in the deciduous species as well (Fig. 4). The gradual decline

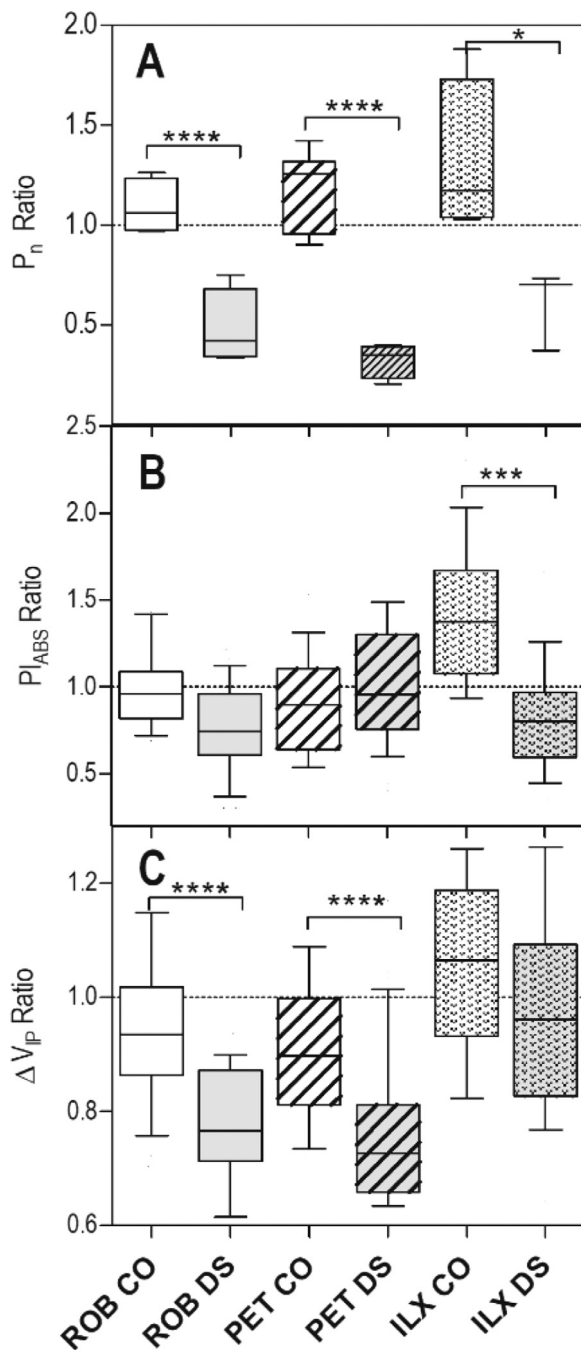


Fig. 5. Development of net photosynthesis, performance index and ΔV_{IP} during the DS period in control and DS trees in 2018. Data are ratios of values obtained by dividing the values measured on DOY 241 by those obtained in the early DS phase (DOY 164) for each individual tree. Box plots with 10–90% whiskers; $n = 3-7$ (5A) and 35–40 (5B, 5C, respectively). Significant differences between control and DS plants within one species are indicated by asterisks (*: $P < 0.05$; ***: $P < 0.001$; ****: $P < 0.0001$; unpaired t -test).

observed in *Q. ilex*, as opposed to the maintenance of higher sap flow over a prolonged period in *Q. robur* and *Q. petraea* until the last reserves are used up, also resembles the behaviour of g_s in the plants in the Manes et al. (2006) experiment and points to a better control of water use under DS in the evergreen species.

As expected and shown in numerous previous studies (e.g. Epron and Dreyer, 1990; Arend et al., 2013; Früchtenicht et al., 2018a; Bréda et al., 2006 review), prolonged DS resulted in significant declines of P_N in all

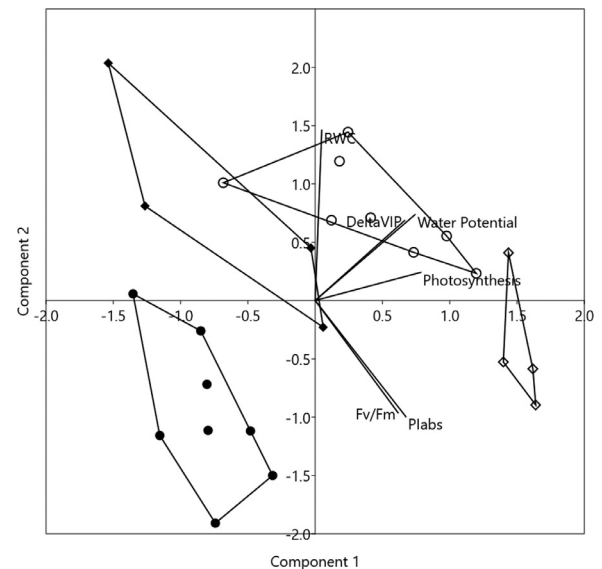


Fig. 6. PCA of PI_{abs} , ΔV_{IP} , P_N , RWC, F_v/F_m and Ψ_{pd} in DS (filled circles and diamonds) and control trees (open circles and diamonds) of pooled *Q. robur* and *Q. petraea* (circles) vs *Q. ilex* (diamonds) in 2018.

three species (Fig. 5A). However, as far as non-stomatal effects are concerned, our ChlF analysis revealed that in the two deciduous species the initial steps in photosynthetic electron transport were not or hardly affected (Fig. 5B), this was only the case in *Q. ilex*, when DS plants were compared to control plants. However, the transport of electrons from the intersystem carriers to the PSI acceptor side was hampered by prolonged DS especially in the deciduous species, as visualized in decreasing ΔV_{IP} ratios (Fig. 5C). A similar finding had been observed in the previous study (Früchtenicht et al., 2018b). Combination of the water relations and the photosynthesis-related parameters in a PCA confirmed the very similar behavior of the deciduous species, but clearly separated them from the evergreen species (Fig. 6). As described by Pollastrini et al. (2016) and Bussotti et al. (2020) under field conditions, stress during summer may lead to an uncoupling of responses of PS II and PS I. This was reflected in the fact that the PI_{abs} and the ΔV_{IP} vectors in Fig. 6 were perpendicular to each other.

4.2. Growth

Tree growth is sensitive towards suppressed photosynthetic carbon sequestration by drought (Bréda et al., 2006) and this had also been observed in our previous experiment on loamy soil with respect to root collar diameter in *Q. robur* (Früchtenicht et al., 2018b). Similar results were reported in the experiment of Arend et al. (2011) for shoot length and stem diameter in different provenances of *Q. robur* and *Q. petraea*. Nevertheless, in the present study no significant responses to the prolonged DS were observed (Fig. 7), despite the clear effect of the DS treatment on water status and photosynthetic activity of the trees.

Arend et al. (2011, 2013) showed a moderate decrease of root biomass formation under prolonged DS and a shift towards increased relative root length growth as compared to shoot growth in 3-year-old trees under prolonged DS in both *Q. robur* and *Q. petraea*, resulting from the smaller DS effect on total root length, but a strong (inhibiting) effect of DS on shoot length. Similarly, maximum attainable root length of *Q. robur* in loamy soil decreased from 170 to 100 cm under DS in our previous study, while *Q. ilex* rooted down to 130–150 cm only (Früchtenicht et al., 2018a). Consistently, Fort et al. (1997), Teskey and Hinchley (1981) and Kuhns et al. (1985) reported a decline of root growth at water potentials below -0.3 MPa. However, in the present study on sandy soil, and in contrast to its behavior in loamy soil

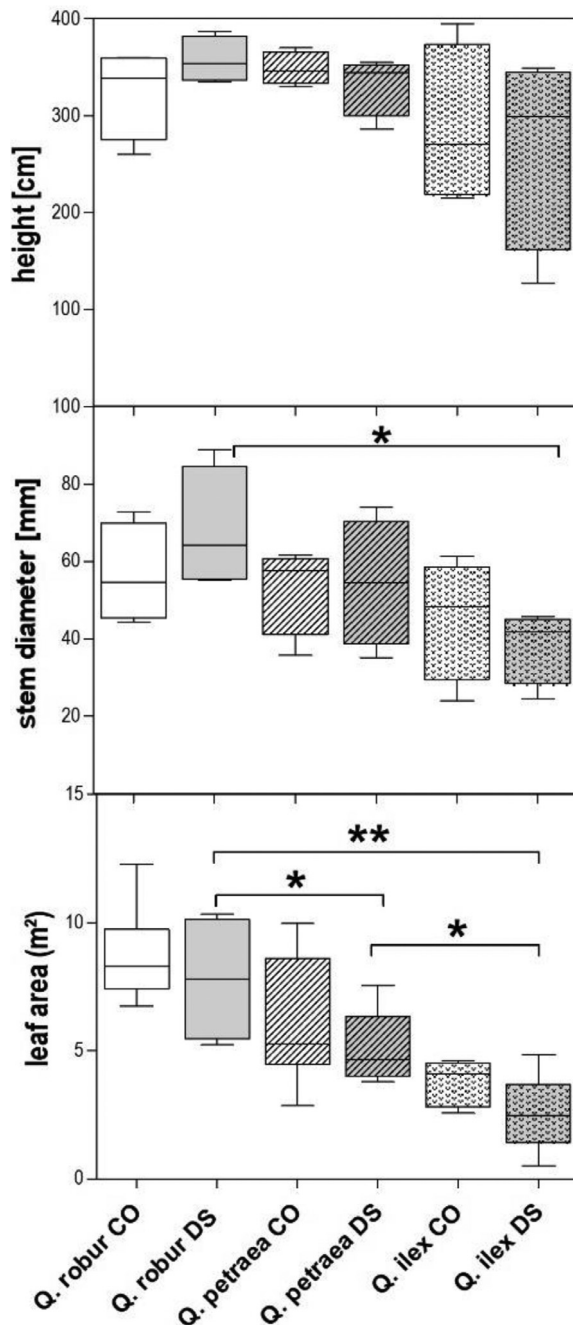


Fig. 7. Mean tree height, stem diameter (5 cm above ground) and leaf area in control and DS trees at the end of the DS period in 2018; $n = 5$. Data are presented as box plots with 10–90% whiskers. Within species, data for DS and CO plants were not significantly different. Significant differences between DS plants of different species indicated by asterisks (*: $P < 0.05$; **: $P < 0.01$, unpaired t -test).

(Früchtenicht et al. 2019a), *Q. robur* developed an increased root length in representative specimens under DS (190 cm vs 150 cm in a control tree), allowing access to the last residual water at the bottom of the basin, while representative *Q. petraea* specimens developed sufficiently long roots (180 cm) also under control conditions. In this context, it is interesting to note that adult *Q. robur* trees in a floodplain forest on deep sandy soil had groundwater/capillary water access during the 2018 heat and drought wave in central Germany with groundwater tables decreasing beyond -4 m (Süßel und Brüggemann, 2021). We therefore hypothesize that the long root development of *Q. robur* in the DS basin

occurred already in 2017 (i.e. under moderate DS), and that the trees then benefitted in 2018 from this increment. The striking difference in root length under DS between the former experiment (100 cm), which resembled the findings of Arend et al. (2011, 2013) in drought-treated *Q. robur*, and the current one (180 cm) can explain the drastic difference in the overall performance of *Q. robur* between the two different experiments: while, in the previous study, *Q. robur* was unable to reach residual water resources deep in the basin and suffered significantly both in terms of photosynthetic activity and growth, this time the trees were apparently able to reach the last water resources and could maintain water transport over a longer period of DS treatment than the shorter-rooted *Q. ilex* (Fig. 4), resulting in a similar decrease of P_N (presumably through stomatal closure and probably less so through decreasing PS I end acceptor reduction capacity – see ΔV_{IP} in Fig. 5) as *Q. ilex*. In the previous experiment on loamy soil, *Q. robur* had shown lower P_N than *Q. ilex*. However, in combination with the higher total leaf area (Fig. 7) than *Q. ilex*, the photosynthetic capacity of the DS trees was sufficient to keep track with the unstressed control trees with respect to above-ground biomass accumulation (Fig. 7). Thus, the restricted root growth of *Q. ilex* can be one major cause for the less efficient growth performance of this species in the sandy forest stand as compared to *Q. robur* in the long run (i.e. accumulated over several years), observed under field conditions (Holland, Brüggemann et al. unpublished).

Concerning the presumed differences between *Q. robur* and *Q. petraea*, our hypothesis that *Q. petraea* might be more effective under DS, could not be corroborated. Although it maintained the best water supply throughout the DS period (Fig. 4), its photosynthesis-related parameters resembled those of *Q. robur*, it did not separate with respect to its physiological reaction to DS in the PCA, and the only observed significant difference between the two species was with respect to its leaf area (Fig. 7). Similarly, Arend et al. (2011, 2013) could not find consistent differences between the two species when comparing provenances from origins with different average rainfall and growing them in loamy soil under prolonged DS.

In conclusion, our study indicates that mesic, deciduous European oaks (*Q. robur* and *Q. petraea*) tend towards an avoidance strategy to cope with potential drought stress on sandy soil by establishing deep root systems, provided water supply during the initial growth phase allows for it. In contrast, the evergreen species, *Q. ilex*, with a less developed root system, must rely on drought avoidance-strategy, possibly a consequence of lower whole-plant photosynthetic carbon sequestration due to lower P_N and leaf area than in the deciduous species.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tfp.2021.100093.

References

- Arend, M., Brem, A., Kuster, T.M., Günthardt-Goerg, M.S., 2013. Seasonal photosynthetic responses of European oaks to drought and elevated daytime temperature. *Plant Biol.* 15 (Suppl 1), 169–176. doi:10.1111/j.1438-8677.2012.00625.x.
- Arend, M., Kuster, T., Günthardt-Goerg, M.S., Dobberrin, M., 2011. Provenance-specific growth responses to drought and air warming in three European oak species (*Quercus robur*, *Q. petraea* and *Q. pubescens*). *Tree Physiol.* 31 (3), 287–297. doi:10.1093/treephys/tpr004.
- Bantis, F., Radoglou, K., Brüggemann, W., 2018. Differential ecophysiological responses to seasonal drought of three co-existing oak species in northern Greece. *Plant Biosyst.* – Int. J. Deal. Asp. Plant Biol. 153 (3), 378–384. doi:10.1080/11263504.2018.1492990.
- Bréda, N., Cochard, H., Dreyer, E., Granier, A., 1993. Water transfer in a mature oak stand (*Quercus petraea*): seasonal evolution and effects of a severe drought. *Can. J. For. Res.* 23 (6), 1136–1143. doi:10.1139/x93-144.

- Bréda, N., Huc, R., Granier, A., Dreyer, E., 2006. Temperate forest trees and stands under severe drought. A review of ecophysiological responses, adaptation processes and long-term consequences. *Ann. For. Sci.* 63 (6), 625–644. doi:10.1051/forest:2006042.
- Burgess, S.S.O., Adams, M.A., Turner, N.C., Beverly, C.R., Ong, C.K., Khan, A.A.H., Bleby, T.M., 2001. An improved heat pulse method to measure low and reverse rates of sap flow in woody plants. *Tree Physiology* 21 (9), 589–598. doi:10.1093/treephys/21.9.589.
- Bussotti, F., Bettini, D., Grossoni, P., Mansuino, S., Nibbi, R., Soda, C., Tani, C., 2002. Structural and functional traits of *Quercus ilex* in response to water availability. *Environ. Exp. Bot.* 47 (1), 11–23. doi:10.1016/S0098-8472(01)00111-3.
- Bussotti, F., Gerosa, G., Digrao, A., Pollastrini, M., 2020. Selection of chlorophyll fluorescence parameters as indicators of photosynthetic efficiency in large scale plant ecological studies. *Ecol. Indic.* 108, 105686. doi:10.1016/j.ecolind.2019.105686.
- Bussotti, F., Pollastrini, M., Holland, V., Brüggemann, W., 2015. Functional traits and adaptive capacity of European forests to climate change. *Environ. Exp. Bot.* 111, 91–113. doi:10.1016/j.envexpbot.2014.11.006.
- Ceppi, M.G., Oukarroum, A., Çiçek, N., Strasser, R.J., Schansker, G., 2012. The IP amplitude of the fluorescence rise OJIP is sensitive to changes in the photosystem I content of leaves. A study on plants exposed to magnesium and sulfate deficiencies, drought stress and salt stress. *Physiol. Plant.* 144 (3), 277–288. doi:10.1111/j.1399-3054.2011.01549.x.
- Chiantante, D., Di Iorio, A., Scippa, G.S., 2005. Root responses of *Quercus ilex* L. seedlings to drought and fire. *Plant Biosyst. – Int. J. Deal. Asp. Plant Biol.* 139 (2), 198–208. doi:10.1080/11263500500160591.
- Epron, D., Dreyer, E., 1990. Stomatal and non stomatal limitation of photosynthesis by leaf water deficits in three oak species. A comparison of gas exchange and chlorophyll a fluorescence data. *Ann. For. Sci.* 47 (5), 435–450. doi:10.1051/forest:19900503.
- Fort, C., Fauveau, M.L., Muller, F., Label, P., Granier, A., Dreyer, E., 1997. Stomatal conductance, growth and root signaling in young oak seedlings subjected to partial soil drying. *Tree Physiol.* 17 (5), 281–289. doi:10.1093/treephys/17.5.281.
- Fotelli, M.N., Radoglou, K.M., Constantinidou, H.I., 2000. Water stress responses of seedlings of four Mediterranean oak species. *Tree Physiol.* 20 (16), 1065–1075. doi:10.1093/treephys/20.16.1065.
- Früchtenicht, E., Klein, N., Brüggemann, W., 2018a. Response of *Quercus robur* and two potential climate change winners – *Quercus pubescens* and *Quercus ilex* – to two years summer drought in a semi-controlled competition study. II — Photosynthetic efficiency. *Environ. Exp. Bot.* 152, 118–127. doi:10.1016/j.envexpbot.2018.03.019.
- Früchtenicht, E., Neumann, L., Klein, N., Bonal, D., Brüggemann, W., 2018b. Response of *Quercus robur* and two potential climate change winners—*Quercus pubescens* and *Quercus ilex*—To two years summer drought in a semi-controlled competition study. I—Tree water status. *Environ. Exp. Bot.* 152, 107–117. doi:10.1016/j.envexpbot.2018.01.002.
- Hanewinkel, M., Cullmann, D.A., Schelhaas, M.-J., Nabuurs, G.-J., Zimmermann, N.E., 2012. Climate change may cause severe loss in the economic value of European forest land. *Nat. Clim. Change* 3 (3), 203–207. doi:10.1038/nclimate1687.
- Koller, S., Holland, V., Brüggemann, W., 2013. Effects of drought stress on the evergreen *Quercus ilex* L., the deciduous *Q. robur* L. and their hybrid *Q. × turneri* Willd. *Photosynthesis* 51 (4), 574–582. doi:10.1007/s11099-013-0058-6.
- Kuhns, M.R., Garrett, H.E., Teskey, R.O., Hinckley, T.M., 1985. Root growth of black walnut trees related to soil temperature, soil water potential, and leaf water potential. *For. Sci.* 31 (3), 617–629. doi:10.1093/forestscience/31.3.617.
- Kuster, T.M., Arend, M., Günthardt-Goerg, M.S., Schulin, R., 2013. Root growth of different oak provenances in two soils under drought stress and air warming conditions. *Plant Soil* 369 (1–2), 61–71. doi:10.1007/s11104-012-1541-8.
- Manes, F., ASTORINO, G., Vitale, M., LORETO, F., 1997. Morpho-functional characteristics of *Quercus ilex* L. leaves of different age and their ecophysiological behaviour during different seasons. *Plant Biosyst. – Int. J. Deal. Aspects Plant Biol.* 131 (2), 149–158. doi:10.1080/11263504.1997.10654176.
- Manes, F., Vitale, M., Donato, E., Giannini, M., Puppi, G., 2006. Different ability of three Mediterranean oak species to tolerate progressive water stress. *Photosynthesis* 44 (3), 1136. doi:10.1007/s11099-006-0040-7.
- McKenney, D.W., Pedlar, J.H., Lawrence, K., Campbell, K., Hutchinson, M.F., 2007. Potential impacts of climate change on the distribution of North American trees. *BioScience* 57 (11), 939–948. doi:10.1641/B5711106.
- Pollastrini, M., Holland, V., Brüggemann, W., Bussotti, F., 2016. Chlorophyll a fluorescence analyses in forests. *Ann. Bot.* (6) 23–37. doi:10.4462/annbotm-13257.
- Ponton, S., Dupouey, J.-L., Bréda, N., Feuillat, F., Bodénès, C., Dreyer, E., 2001. Carbon isotope discrimination and wood anatomy variations in mixed stands of *Quercus robur* and *Quercus petraea*. *Plant Cell Environ.* 24 (8), 861–868. doi:10.1046/j.0016-8025.2001.00733.x.
- Schuldt, B., Buras, A., Arend, M., Vitasse, Y., Beierkuhnlein, C., Damm, A., et al., 2020. A first assessment of the impact of the extreme 2018 summer drought on Central European forests. *Basic Appl. Ecol.* 45 (5/6), 86–103. doi:10.1016/j.baae.2020.04.003.
- Skiadreas, G., Schwarz, J.A., Bauhus, J., 2019. Groundwater extraction in floodplain forests reduces radial growth and increases summer drought sensitivity of pedunculate oak trees (*Quercus robur* L.). *Front. For. Glob. Change* 2, 267. doi:10.3389/ffgc.2019.00005.
- Smart, R.E., Bingham, G.E., 1974. Rapid estimates of relative water content. *Plant Physiol.* 53 (2), 258–260. doi:10.1104/pp.53.2.258.
- Strasser, R.J., Tsimilli-Michael, M., Qiang, S., Goltsev, V., 2010. Simultaneous in vivo recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*. *Biochim. Biophys. Acta* 1797 (6–7), 1313–1326. doi:10.1016/j.bbabi.2010.03.008.
- Süßel, F., Brüggemann, W., 2021. Tree water relations of mature oaks in southwest Germany under extreme drought stress in summer 2018. *Plant Stress* 1, 100010. doi:10.1016/j.stress.2021.100010.
- Teskey, R.O., Hinckley, T.M., 1981. Influence of temperature and water potential on root growth of white oak. *Physiol. Plant* 52 (3), 363–369. doi:10.1111/j.1399-3054.1981.tb06055.x.
- Urli, M., Lamy, J.-B., Sin, F., Burrell, R., Delzon, S., Porté, A.J., 2015. The high vulnerability of *Quercus robur* to drought at its southern margin paves the way for *Quercus ilex*. *Plant Ecol.* 216 (2), 177–187. doi:10.1007/s11258-014-0426-8.