Response of oak and beech to simulated summer drought and drought release: ecophysiological characteristics in leaves and tree rings

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RESPONSE OF OAK AND BEECH TO SIMULATED SUMMER DROUGHT AND DROUGHT RELEASE:
ECOPHYSIOLOGICAL CHARACTERISTICS IN LEAVES AND TREE RINGS

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»Rivers know this: there is no hurry. We shall get there some day«

Winnie-the-Pooh, A.A. Milne

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Summary

Due to increasing atmospheric concentrations of greenhouse gases, e.g. CO_2, NO_2 and CH_4, the global temperature is predicted to increase, affecting forest ecosystems worldwide. In the future, drought will most likely be a major constraint for European forests, leading to changes in forest productivity, species distribution and survival. To quantify the future state of forest ecosystems, it is crucial to investigate the species-specific responses to drought. These responses are not only determined by the ability of the trees to endure periods of drought but also by their potential to recover on the long term and overcome previous limitations. However, studies investigating the seasonal response to drought under semi-natural but controlled conditions are scarce. While European beech (*Fagus sylvatica* L.), the climax species in Europe is assumed to suffer from the expected climate change, European oak species (*Quercus robur* L. and *Q. petraea* (Matth.) Liebl.) are thought to be drought and thermo-tolerant, and therefore might benefit from the altered climate conditions. In this thesis, the seasonal, species-specific responses to drought of (i) *Quercus robur* and *Q. petraea*, imprinted in $\delta^{13}C$ and $\delta^{18}O$ in leaves and tree rings, were analysed with the dual isotope approach (*chapter 1*), and (ii) European beech was investigated with particular emphasis on the response after drought release (*chapters 2* and *3*).

In the first chapter, young trees of *Q. robur* and *Q. petraea* were analysed, which were exposed to experimental warming and a drought treatment with yearly increasing intensities in large model ecosystems over three years. Carbon and oxygen isotopic ($\delta^{13}C$ and $\delta^{18}O$) patterns were investigated in leaf tissue and tree-ring cellulose and linked to leaf physiological measures and tree-ring growth. While warming had no effect on the isotopic patterns in leaves and tree rings, drought led to an increase in $\delta^{18}O$ and $\delta^{13}C$ in both species. Under severe drought, an unexpected isotopic pattern, with a decrease in $\delta^{18}O$, was observed in tree rings but not in leaves. This decrease in $\delta^{18}O$ could not be explained by concurrent physiological analyses and is not supported by current physiological knowledge. Analysis of intra-annual tree-ring growth revealed a drought-induced growth cessation that interfered with the record of isotopic signals imprinted on recently formed leaf carbohydrates. This missing record indicates isotopic uncoupling of leaves and tree rings, which may have serious implications for the interpretation of tree-ring isotopes, particularly from trees that experienced growth-limiting stresses. However, the effect of uncoupling was higher in *Q. robur* compared to *Q. petraea*, indicating that *Q. petraea* is slightly less affected by water limiting conditions.

In the *second chapter*, the seasonal response of photosynthesis to a severe, long-lasting summer drought and drought release was followed in saplings of a mesic and xeric provenance of European beech transplanted to large model ecosystems with experimentally controlled water supply. The drought response could be divided into three phases: (i) limitation, (ii) recovery, and (iii) post-drought stimulation. Limitation of photosynthesis developed progressively and caused a major loss of integrated photosynthetic activity in both provenances, and was accompanied by impaired PSII photochemistry indicating severe stress. Recovery started rapidly after re-watering and was fully accomplished within 20 and 10 days in the mesic and xeric provenances, respectively. After recovery, photosynthesis remained at high levels while decreasing in control trees. This post-drought stimulation was sustained until the end of the vegetation period, partly counterbalancing the previous loss of photosynthetic activity. It was attributable to an altered leaf ontogeny as indicated by a delay of autumnal leaf senescence. These observed responses might be important factors when modelling seasonal carbon uptake and phenology of forest ecosystem under future climate change.

In the *third chapter*, the seasonal response of leaf physiological characteristics in young beech trees to a simulated summer drought and drought release was analysed to investigate their potential to overcome the induced stress. Water potentials (ψ_{pd}), net photosynthesis (A_N), PSII chlorophyll fluorescence (PI_{tot}), non-structural carbohydrates (NSCs) and $\delta^{13}C$ signatures in leaves were measured throughout an entire growing season. ψ_{pd}, A_N and PI_{tot} decreased as drought progressed and the concentration of soluble sugars increased at the expense of starch. Soluble sugars showed a distinct ^{13}C drought signal, while this signal was weak in leaf bulk tissue. Drought effects on leaf physiological characteristics disappeared shortly after re-watering, though full recovery of A_N and PI_{tot} was delayed compared to the very fast recovery of ψ_{pd} and NSC concentrations. The latter was also reflected by a rapid decay of the ^{13}C drought signal in soluble sugars, indicating a fast turnover of assimilates and thus recovery of leaf metabolism. Furthermore, after recovery, the previously drought exposed trees showed a trend towards a stimulation of leaf physiological activity as shown by slightly increased A_N and NSC concentrations. The results show that beech restores full leaf physiological activity shortly after drought release and overcomes the previous stress impact without persistent physiological damages indicating a high resilience to severe summer drought.

The results of this dissertation demonstrate that the dual isotope approach in tree rings should be used with caution under growth-limiting conditions. The observed uncoupling of leaves and tree rings will have large implications on the interpretation of any isotopic tree-ring chronology,

particularly in respect to severely growth-limiting conditions. Furthermore, a post-drought stimulation in beech trees was observed, counteracting the drought-induced limitations of photosynthetic activity. This highlights the need of a deeper understanding of species-specific recovery processes to drought, as, to date, this response is hardly accounted for in models predicting vegetation patterns and corresponding biogeochemical cycles in the future.

Zusammenfassung

Infolge zunehmender Konzentrationen von Treibhausgasen, wie z.B. CO_2, NO_2 und CH_4, wird die globale Durchschnittstemperatur voraussichtlich weiterhin ansteigen, und damit Waldökosysteme weltweit beeinflussen. Eine Zunahme von Trockenperioden wird dabei höchstwahrscheinlich den bedeutendsten Einfluss auf die Wälder Europas haben und zu Veränderungen in deren Produktivität, Artenzusammensetzung und Fortbestand führen. Um den Zustand von Wäldern in der Zukunft zu quantifizieren, benötigt es Kenntnisse über artspezifische Reaktionen auf Trockenheit. Dabei ist die Trockenheitsantwort von Einzelbäumen und Wäldern nicht nur durch deren Vermögen festgelegt Trockenstress zu ertragen, sondern auch von ihrem Potential, sich langfristig vom Wassermangel zu erholen und vorangegangene Limitierungen zu überwinden. Studien, welche die saisonale Reaktion von Bäumen auf Trockenheit unter naturnahen, aber kontrollierten Bedingungen untersuchen, sind jedoch selten. In den Wäldern Europas ist die Baumart des Klimaxstadiums die Rotbuche (*Fagus sylvatica* L.). Vorangegangene Studien haben diese Baumart als sensitiv gegenüber der Klimaveränderung dargestellt, während mitteleuropäische Eichenarten (*Quercus robur* L. und *Q. petraea* (Matth.) Liebl.) als trockenheits- und wärmetolerant charakterisiert wurden und dementsprechend als weniger empfindlich gegenüber Klimaveränderungen gelten. In der vorliegenden Doktorarbeit wurde die artspezifische Reaktion auf Trockenheit von (i) *Quercus robur* und *Q. petraea* mit Hilfe von $\delta^{13}C$ und $\delta^{18}O$ in Blättern und Jahrringen unter Verwendung des „dual isotope approach“ analysiert (*Kapitel 1*) und (ii) *F. sylvatica*, mit besonderem Schwerpunkt auf die Reaktion nach der Trockenheit, untersucht (*Kapitel 2* und *3*).

Im ersten Kapitel wurden junge *Q. robur* und *Q. petraea* Bäume in grossen Modellökosystemen untersucht und über drei Jahre hinweg jährlich steigender experimenteller Lufterwärmung und Trockenheit ausgesetzt. Der Einfluss dieser Bedingungen auf die Verhältnisse der Kohlenstoff- und Sauerstoffisotopen ($\delta^{13}C$ und $\delta^{18}O$) wurde in Blättern und der Zellulose von Jahrringen untersucht und mit physiologischen Messungen und dem Jahrringwachstum in Verbindung gebracht. Die Lufterwärmung hatte keinen Einfluss auf das Isotopenverhältnis in Blättern und Jahrringen, wohingegen eine Zunahme von Trockenheit zu einem Anstieg in $\delta^{18}O$ und $\delta^{13}C$ in beiden Arten führte. Bei starker Trockenheit konnte ein unerwartetes Isotopenmuster beobachtet werden, welche eine Abnahme des $\delta^{18}O$ in den Jahrringen, aber nicht in den Blättern aufzeigte. Diese Abnahme des $\delta^{18}O$ konnte durch die physiologischen Messungen nicht erklärt werden und widerspricht aktuellen pflanzenphysiologischen Erkenntnissen. Die Analyse des intraannuellen

Jahrringwachstums zeigte einen trockenheitsinduzierten Wachstumsstop, welcher das Isotopensignal der Jahrringe stört und das daher von dem Isotopenmuster der neu synthetisierten Kohlenhydrate in den Blättern abweicht. Dies weist auf eine Entkopplung des Isotopenmusters von Blättern und Jahrringen hin und könnte zu einer Fehlinterpretation der Isotopenverhältnisse in Jahrringen führen, insbesondere bei Bäumen welche wachstumslimitierenden Bedingungen ausgesetzt waren. Die Tatsache, dass diese Entkopplung in *Q. robur* deutlicher war als in *Q. petraea,* weist auf einen geringeren Einfluss von Trockenheit auf *Q. petraea* hin.

Im zweiten Kapitel wurde die saisonale Reaktion der Photosynthese von Rotbuchen auf eine starke und lang andauernde Sommertrockenheit und anschliessende Wiederbewässerung untersucht. Hierfür wurden junge Buchenprovenienzen von ursprünglich feuchten bzw. trockenen Standorten in grosse Modellökosysteme verpflanzt, in welchen die Wasserverfügbarkeit kontrolliert werden kann. Die Reaktion der Buchen auf die Trockenheit konnte in drei Phasen unterteilt werden: (i) Limitierung, (ii) Erholung und (iii) Stimulierung nach der Trockenheit. Die Limitierung der Photosynthese erfolgte fortschreitend und verursachte starke Verluste der integrierten photosynthetischen Aktivität in beiden Provenienzen. In Kombination mit einer Beeinträchtigung der PSII-Photochemie deutet dies auf ein hohes Mass an Stress für die jungen Rotbuchen hin. Nach der Wiederbewässerung erholten sich die Rotbuchen der feuchten Herkunft innerhalb von 20 Tagen, und jene der trockenen Herkunft innerhalb von 10 Tagen. Nach der Erholungsphase blieb die Photosyntheserate in den vormals trockengestressten Buchen auf einem hohen Niveau, wohingegen sie in den Kontrollbäumen abnahm. Diese Stimulierungsphase nach der Trockenheit hielt bis zum Ende der Vegetationsperiode an und steuerte so der vorangegangenen Abnahme der Photosynthese entgegen. Dies war auf eine verzögerte Blattseneszenz im Herbst zurückzuführen. Für ein besseres Verständnis und die Modellierung der saisonalen Kohlenstoffaufnahme und der Phänologie von Waldökosystemen könnten diese Resultate einen wichtigen Beitrag leisten.

Im dritten Kapitel wurde die saisonale Antwort der Blattphysiologie junger Buchen auf Sommertrockenheit mit anschliessender Wiederbewässerung untersucht, um das Potenzial dieser Bäume zur Überwindung von Trockenheit zu prüfen. Wasserpotential (ψ_{pd}), Netto-Photosynthese (A_N), PSII Chlorophyll-Fluoreszenz (PI_{tot}), die Konzentration der nicht-strukturellen Kohlenhydrate (NSCs) und die ^{13}C Signatur wurden während der gesamten Wachstumsperiode in den Blättern gemessen. Mit zunehmender Trockenheit nahmen ψ_{pd}, A_N und PI_{tot} ab und die Konzentration von löslichen Zuckern nahm auf Kosten der Stärke zu. In den löslichen Zuckern zeigte sich ein deutlicher Einfluss der Trockenheit auf das ^{13}C Signal, wohingegen der Trockenheitseinfluss auf das ^{13}C Signal im Gesamtblattgewebe gering war. Der Einfluss von Trockenheit auf die

Blattphysiologie verschwand schnell nach der Wiederbewässerung, obwohl die volle Erholung von A_N und PI_{tot}, im Vergleich zur sehr schnellen Erholung von ψ_{pd} und NSC Konzentrationen, verzögert war. Die schnelle Erholung spiegelte sich auch im raschen Abklingen des ^{13}C-Trockenheitssignals in den löslichen Zuckern wieder und weist auf eine schnelle Fluktuation der Assimilate, und somit auf eine schnelle Erholung des Blattmetabolismus, hin. Des Weiteren wiesen die zuvor dem Trockenheitsstress ausgesetzten Bäume eine tendenzielle Stimulation der blattphysiologischen Aktivitäten auf, welche durch den leichten Anstieg in A_N- und der NSC-Konzentrationen gezeigt werden konnte. Die Resultate zeigten zudem, dass trockengestresste Rotbuchen schon kurz nach der Wiederbewässerung imstande sind, die «normale», vollständige Aktivität der Blattphysiologie wiederherzustellen und damit den vorangehenden Stress bewältigen können, ohne anhaltenden Schaden zu nehmen. Dies weist auf eine hohe Resilienz dieser Bäumchen gegenüber Trockenheit hin.

Die Ergebnisse dieser Dissertation zeigen, dass der „dual isotope approach“ in Jahrringen mit Vorbehalt verwendet werden sollte, sofern die untersuchten Bäume wachstumslimitierenden Bedingungen ausgesetzt sind. Die festgestellte Entkopplung des Isotopensignals zwischen Blättern und Jahrringen hat erhebliche Auswirkung auf die Interpretation von Isotopenverhältnissen in Jahrringdatensätzen . Des Weiteren wurde in Rotbuchen eine Stimulation der Photosynthese nach Trockenheitsereignissen festgestellt, die der Limitierung der Photosyntheseaktivität durch Trockenheit entgegenwirkt. Dies zeigt die Notwendigkeit für ein tieferes Verständnis für art-spezifische Erholungsprozesse nach Trockenheit, da diese Prozesse bisher in Modellen, welche zukünftige Vegetationsmuster und zugehörige biogeochemische Kreisläufe vorhersagen, kaum berücksichtigt werden.

General Introduction

Background

Triggered by the industrial revolution, the emission of greenhouse gases, e.g. carbon dioxide (CO_2), nitrous oxide and methane, has increased significantly. For example, the partial pressure of CO_2 in the atmosphere has increased by 40% from the 1750's, reaching 390.5 ppm in 2011 (Hartmann *et al.*, 2013), mainly due to the anthropogenic use of fossil fuels. A new all-time high of 400 ppm atmospheric CO_2 concentration was recently hit on Mauna Loa observatory in 2015 (Tans, 2016). As greenhouse gases emit and absorb radiation in the range of thermal infrared radiation, the global mean surface temperature has increased by 0.85 °C as compared to 1850 (Stocker *et al*., 2013). Within the next decade, temperature is expected to increase by another 1 to 2 °C, depending on the future emission of greenhouse gases (IPCC, 2013). Additionally, summer precipitation is predicted to decrease by 21% - 28% in Europe, and the increase in temperature will additionally lead to an increase in evapotranspiration (IPCC, 2013). Global warming already has impact on all biomes of the world, e.g. by changing precipitation or fire patterns, the amount and timing of extraordinary heat or cold waves and the duration of the vegetation season (Kovats *et al*., 2014; Linderholm, 2006; Rebetez *et al*., 2006; Schär *et al*., 2004; Walther *et al*., 2002). A prominent example for recent impacts of climate change on European forest was the extraordinary hot and dry year 2003, which led to reductions in primary productivity, and therefore altered forest functioning as a carbon sink (Ciais *et al*., 2005).

One third of the Swiss area is covered with forest, providing multiple ecosystem services, e.g. the provisioning of timber, the protection against natural hazards, and the conservation of habitats for plants and animals (Brändli, 2010). Due to their relative long lifespan and generation time, forest trees are thought to be particularly vulnerable to the rapidly changing climate as the genetic adaptation to the new climatic conditions might be difficult for trees (Peters, 1990), and drought has already been shown to severely endanger tree functioning and distribution (Allen *et al*., 2010). Thus, the major environmental constraints for forests will be an increased frequency and intensity of drought, leading to changes in primary productivity, species distribution, reproduction and survival (Zhao and Running, 2010; Scholze *et al*., 2006; Boyer, 1982). The dominating tree species in Switzerland are Norway spruce (*Picea abies* (L.) H. Karst; 40%), followed by European beech (*Fagus sylvatica* L.; 18%) and silver fir (*Abies alba* Mill.; 10%) (Brändli, 2010). For sylviculturists, it

will be crucial to quantify species-specific responses to drought, so that potential risks of climate change to forest ecosystems can be extenuated.

Beech is the European climax tree species in areas with medium soil and climate conditions and competitive superior to other broadleaved tree species, primarily due to its broad habitat range, its low requirements to soil chemical properties and its tolerance to shade. Potentially, half of the forested area in Switzerland would be suitable for beech with areas providing annual precipitation and mean annual temperatures above 500 mm and 7 °C, respectively (Brändli, 2010; Leitgeb and Englisch, 2006; Felbermeier and Mosandl, 2002). Many studies have revealed a high sensitivity of beech to water shortage (Gessler *et al*., 2007; Ohlemüller *et al*., 2006; Rennenberg *et al*., 2004; Backes and Leuschner 2000), but there is a controversial debate among sylviculturists and tree biologists about the consequences of climate change for beech (Ammer *et al*., 2005; Nahm *et al*. 2006; Schütz, 2009). While the response of beech and other tree species to drought is well described (e.g Ruehr *et al*., 2009; Peuke *et al*., 2002; Heath and Kerstiens, 1997), less attention was paid to the seasonal response and recovery after drought release, although this might be a factor determining the competitiveness of trees and especially of European beech under future climate conditions.

The most common oak species, sessile oak (*Quercus petraea* (Matth.) Liebl.; dominating on 1.5% of forest area in Switzerland) and pedunculate oak (*Q. robur*; dominating on 0.5%), do potentially prefer the same habitat as beech, but can tolerate lower annual precipitation amounts of 350 to 450 mm (Köller, 2007). In addition, the later bud break of oaks compared to beech makes these species less susceptible to late frost incidents. However, due to lower growth rates and a higher demand for light, both oak species are not competitive to beech on medium sites. On the other hand, they are able to dominate at sites which are too dry for beech (Fig. 1), and therefore have the potential to become more frequent under future drier and warmer climate (Scharnweber *et al*., 2011; Friedrichs *et al*., 2009; Ohlemüller *et al*., 2006; Leuschner *et al*., 2001; Thomas, 2000; van Hees 1997).

During the last centuries, oaks were important tree species for society, providing timber for construction works, acorns for pig husbandry and bark for tannery, and thus were more abundant in Swiss forests (Bonfils, 2005). Nowadays, the economic value has decreased, and oak stands were replaced by tree species needing less care and showing higher growth rates and shorter rotation times, e.g. beech and spruce. Nevertheless, oaks are still important tree species, providing habitat for more than 1000 insects and other animals (Buβer, 2014). Sessile and pedunculate oak are thermophilic species that are more tolerant to low precipitation than beech,

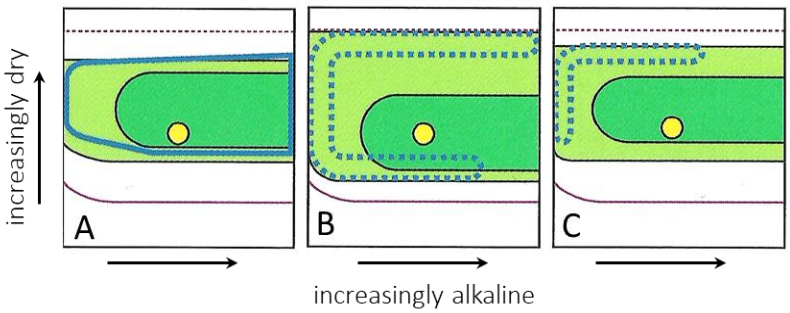

Figure 1. Ecograms of *F. sylvatica* (A), *Q. robur* (B) and *Q. petraea* (C). Fundamental niche (light green) in the absence of competition, realized niche (dark green) with competition and area of (co-) domination ((dotted) blue line). Above the dotted and below the solid black line, sites are to dry and to moist for forest vegetation, respectively. Figure adapted from Ellenberg and Leuschner, (2010) and Bartsch and Röhrig, (2016).

and are therefore thought to become more dominant in the drier areas of beech distribution. With the expected climate change, oak species are thought to stabilise forests at the dry distribution limits of beech. Hence, oaks might be able to mitigate climate effects in these forest ecosystems (Waldpolitik 2020, 2013, Pretzch *et al*. 2012).

Tree responses to drought can be studied using a wide set of variables. Tree rings, for example, provide a useful tool to study the drought response of trees, as they integrate information of different levels of plant functioning, and tree-ring width has been shown to be very sensitive to drought (Dobbertin, 2005; Dittmar *et al*., 2003). However, tree-ring width does not provide information on the underlying physiological mechanisms leading to changes in growth, and it is also determined by other factors, e.g. by intra-specific competition and nutrient availability (Speer, 2010; Kagawa *et al*., 2006). To reveal physiological changes of photosynthetic carbon fixation and assimilation in leaves and tree rings, the analyses of stable isotopes of carbon and oxygen in plant tissues have been found to be a useful tool (Farquhar *et al*., 1989; 1982). Stable isotopes are atoms of one element, displaying the same number of protons but a different number of neutrons, and therefore possess very similar chemical, but different physical properties. Stable isotopes differ in their natural abundance, and are expressed in the δ-notation given in permil (‰):

$$\delta\,{}^{x}E = \frac{R_{sample}}{R_{standard}} - 1\,, \qquad (1)$$

where E is the element of interest, x gives the mass of the less abundant isotope, and R_{sample} and $R_{standard}$ are the ratios of the light to the heavy isotope of the sample and the international standard

(Table 1). Stable isotopes of carbon and oxygen in plant tissues are sensitive to meteorological and environmental variations, can give information about ecophysiological processes and depend, besides the plant internal fractionation events, on the source of the particular molecule (Helle and Schleser, 2004; Dawson *et al*., 2000).

Table 1. Stable isotopes of carbon and oxygen, their international standard used during isotope analysis and the natural abundance percentage (Dawson *et al*., 2000)

Element	Isotope	Natural abundance (%)	Standard
Carbon	^{12}C	98.982	Vienna PeeDee belemnite (V-PDB)
	^{13}C	1.108	
Oxygen	^{16}O	99.759	Vienna Standard mean ocean water (V-SMOW)
	^{17}O	0.037	
	^{18}O	0.204	

$\delta^{13}C$ in plants, for example, is depending mainly on the isotopic ratio of atmospheric CO_2 and on the ratio of leaf internal to external CO_2 concentration (c_i/c_a). During the process of carbon fixation, a discrimination of the heavier ^{13}C isotope takes place due to fractionation events occurring during diffusion of CO_2 into the leaves and fixation of CO_2 by RubisCO. A simplified but effective equation describing the link between c_i/c_a and $\delta^{13}C$ in plant tissues was derived by Farquahr *et al*. (1989):

$$\delta^{13}C_{plant} = \delta^{13}C_{atm} - a + (b - a) \times \frac{c_i}{c_a}, \quad (2)$$

where $\delta^{13}C_{atm}$ is the ratio of ^{13}C to ^{12}C in atmospheric CO_2, a is the fractionation factor for diffusion of CO_2 in air (a = 4.4‰) and b is the fractionation factor of RuBisCO carboxylation (b = 27‰). Thus, changes in gas exchange result in changes in c_i/c_a and therefore in the incorporation of ^{13}C in plant tissues. On a physiological base, $\delta^{13}C$ is depending on both, photosynthetic capacity (A_{max}) and stomatal conductance (g_s), but it is not possible to distinguish which process had the greater influence on $\delta^{13}C$. As $\delta^{18}O$, the ratio of ^{18}O to ^{16}O, is physiologically depending only on g_s but not on A_{max}, the concurrent interpretation of $\delta^{18}O$ is a promising tool to disentangle these effects. Scheidegger *et al*. (2000) developed the conceptual dual isotope model, were both isotopes are

used to explain the influence of A_{max} and g_s on CO_2 fixation. However, the dual isotope approach was initially invented for photosynthetic tissue, and recent studies and comments have highlighted difficulties in explaining the application to tree rings (Barbour and Song, 2014; Roden and Siegwolf, 2012; Gessler *et al*., 2009; Roden *et al*., 2009).

While stable isotopes in tree rings provide a long-term archive to analyse the reaction of trees to climatic constrains and can be used to exploit climate variables during the time of growth, the direct measurement of PSII-Fluorescence and gas-exchange can provide insights into the dynamics of photosynthetic carbon fixation, leaf transpiration and stomatal conductance of a plant in situ.

In brief, when a plant is illuminated the light reaction of photosynthesis is triggered, chlorophyll molecules become excited and charge separation takes place, producing ATP and reduction equivalents in form of $NADPH_2$. The fast fluorescence transient of Chlorophyll a (Chl a) shows a specific rise in the first second after illumination onset after dark adaptation which informs about the reduction of primary electron acceptors in Photosystem II (PSII; Force *et al*., 2003; Strasser *et al*., 2000). It is therefore a good estimator of plant performance, and reveals information about the basic photosynthetic processes in plants, e.g. light harvesting, energy conversion and photoinhibition (Krause and Weis, 1991). Fluorescence measurements are widely used in ecophysiological studies to determine the photosynthetic performance of plants under different stresses like light, drought and chilling (Pflug and Brüggemann, 2012; Brüggemann *et al*., 2009; Bussotti, 2004; Krüger *et al*., 1997). Intensities of emitted fluorescence at defined reduction steps during the fluorescence transient are used to obtain information about the energy flows and probabilities of energy conversion in the photosystem (Alberts *et al*., 2010; Force *et al*., 2003). The relative quantum yield F_v/F_m, giving the probability by which a trapped electron results in a reduction of the primary electron acceptor in PSII, has been found to be poorly related to drought stress (Živčák *et al*., 2008). However, the total Performance Index of PSII (PI_{tot}), integrating the sum of changes in quantum yields and energy conversion, was shown to be tightly linked to the drought response in crop plants (Alberts *et al*. 2011, Živčák *et al*., 2008; Oukarroum *et al*., 2007).

ATP and $NADPH_2$ as products of the light reaction are used to produce carbohydrates via the dark reaction of photosynthesis. Gas exchange via the stomata of the leaves provides CO_2, which is a substrate for carbon fixation via RubisCO. Carbon uptake is also linked to water loss due to transpiration via the stomata, and therefore trees must adjust carbon uptake and water loss to fluctuating environmental conditions. Two different strategies, Isohydric and anisohydric, exist to cope with this trade off, which are characterised by a different adjustment of stomatal conductance. Isohydric trees display a constant midday water potential which is due to a strict

reduction of stomatal conductance, in turn leading to restrictions in carbon uptake. In contrast, anisohydric trees show a more variable water potential during the day as a result of a more alterable stomatal conductance, leading to optimised carbon fixation (Sade *et al*., 2012). The latter strategy is thought to be beneficial under moderate to mild drought conditions, while under severe drought this may lead to a disruption of the hydraulic system, and subsequently to a desiccation of metabolic active tree organs. While beech displays a moderate anyisohydric behaviour, oak is considered to show a strong anisohydric control of stomatal conductance (Pretzsch *et al*., 2012; Peuke *et al*., 2002).

Objectives

The main objective of this thesis was to investigate the ecophysiological responses to summer drought and drought release of young beech and oak trees under controlled, semi-natural conditions. Although tree responses to drought are well described, there are still open questions regarding the sensitivity and the recovery of beech and oak trees. Changes in the photosynthetic performance of young beech trees were assessed using net assimilation rates (A_N) and PSII fluorescence (PI_{tot}) to analyse sensitivity to drought as well as the recovery from the induced stress. Furthermore, the isotopic composition of bulk leaf material, tree-ring cellulose and leaf sugars were analysed, and concentrations of non-structural carbohydrates (NSC) were quantified to study the turnover of recently assimilated photosynthates. Furthermore, this thesis aimed to disentangle methodological questions about the applicability of the dual isotope approach in tree rings.

Structure of the thesis

The present thesis consists of three individual chapters, all dealing with the ecophysiological response of young beech and oak trees to drought and subsequent drought release. All studies were carried out at the model ecosystem facility (MODOEK) of the Swiss Federal Research Institute WSL, Birmensdorf (47°21’48’’ N, 8°27’23’’ E, 545 m a.s.l.). The *first study* is part of the multidisciplinary QUERCO project, in which growth and ecophysiological response of three European oak species (*Quercus robur* L., *Q. petraea* [Matt.] Liebl., and *Q. pubescence* Willd.) were followed during a three-year period with controlled summer drought periods and subsequently drought release. The *second* and *third* studies are associated with the BuKlim framework, an interdisciplinary project on European beech (*Fagus sylvatica* L.), aiming to assess the seasonal

drought adaptation of different Swiss marginal beech provenances. The specific aims of the three chapters are shortly summarized in the following:

Chapter 1: Growth cessation uncouples isotopic signals in leaves and tree rings of drought-exposed oak trees.

In contrast to European beech, pedunculate oak and sessile oak are commonly considered to be drought and thermo-tolerant, even though the both oak species prefer different ecological habitats in terms of soil humidity and show a different response of leaf photosynthesis to drought. During a period of three years, young oak trees were subjected to experimental warming, summer drought and a combination of both. Analysis of isotopic patterns of ^{13}C and ^{18}O in leaf tissue and tree-ring cellulose were linked to measures of leaf photosynthesis and intra-annual tree-ring growth. The following research questions were addressed:

(i) What influence do the climatic constraints have on the isotope pattern of $\delta^{13}C$ and $\delta^{18}O$ in leaves and in tree rings of sessile and pedunculate oak?

(ii) How is the isotopic signal in tree rings of sessile and pedunculate oak influenced by physiology and tree-ring growth?

(iii) Is the dual isotope approach applicable on tree ring tissue under growth limiting (drought) conditions?

Chapter 2: Seasonal photosynthetic response of European beech to severe summer drought: limitation, recovery and post-drought stimulation

European beech is a widespread and dominant tree species in European forests, but commonly thought to be endangered by climate change. In the presented study, net assimilation rates and PSII chlorophyll a fluorescence were recorded during the vegetation season to track the photosynthetic response of young European beech trees to drought and drought release under semi-natural, controlled environmental conditions. Special emphasis was given to the response of the trees after drought release, as potential post drought effects might modify the overall drought response of these beech trees. Additionally, to assess leaf ontogeny after drought release, autumnal chlorophyll content was monitored. Two beech provenances from a mesic and a xeric site in Switzerland were compared, as the physiologically drought response might to a certain degree be controlled by the beech ecotype. The following research questions were addressed:

(i) What are the effects of summer drought and drought-release on the seasonal course of leaf photosynthesis in young beech trees?

(ii) Are long-term effects detectable on photosynthesis that modify the leaf physiological status after recovery from drought?

Chapter 3: Ecophysiological response to summer drought and drought release of European beech grown under natural field conditions

While the response of beech to drought is fairly well understood, there is, however, still a lack of knowledge about the ability of these trees to recover from water-limiting stresses. This study investigated the seasonal response of leaf physiological characteristics in young beech trees to a simulated summer drought and particularly drought release. A set of ecophysiological variables, covering metabolic and photosynthetic responses, was used to track the temporal course of the drought response and the recovery process after drought release. The following research questions were addressed:

(i) At which drought level (predawn leaf water potential; ψ_{pd}) is a drought response of young beech trees detectable on the studied variables (A_N, NSC, PI_{tot}, $\delta^{13}C$)?

(ii) How fast do the trees recover after drought release?

(iii) Is a long-lasting drought effect imprinted on leaf physiology after recovery?

Chapter 1: Growth cessation uncouples isotopic signals in leaves and tree rings of drought-exposed oak trees

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Abstract

An increase in temperature along with a decrease in summer precipitation in Central Europe will result in an increased frequency of drought events and gradually lead to a change in species composition in forest ecosystems. In the present study, young oaks (*Q. robur* L. and *Q. petraea* Matt. Liebl.) were transplanted into large mesocosms and exposed for three years to experimental warming and a drought treatment with yearly increasing intensities. Isotopic patterns were analysed in leaf tissue and tree-ring cellulose and linked to leaf physiological measures and tree-ring growth. Warming had no effect on the isotopic patterns in leaves and tree rings, while drought increased $\delta^{18}O$ and $\delta^{13}C$. Under severe drought, an unexpected isotopic pattern, with a decrease in $\delta^{18}O$, was observed in tree rings but not in leaves. This decrease in $\delta^{18}O$ could not be explained by concurrent physiological analyses and is not supported by current physiological knowledge. Analysis of intra-annual tree-ring growth revealed a drought-induced growth cessation that interfered with the record of isotopic signals imprinted on recently formed leaf carbohydrates. This missing record indicates isotopic uncoupling of leaves and tree rings which may have serious implications for the interpretation of tree-ring isotopes, particularly from trees that experienced growth-limiting stresses.

Introduction

Temperatures will increase in the near future (CH2011 2011, IPCC 2012), prospectively resulting in changing species distribution and composition of forest ecosystems in Central Europe (Rebetez *et al*., 2006; Rigling *et al*., 2013). In addition, summer precipitation is predicted to decrease by 21-28% in large parts of Central Europe until the end of the century, and the frequency of extreme heat-waves and severe droughts is likely to increase (Schär *et al*., 2004; IPCC, 2012). Tree species sensitive to drought are expected to be particularly affected by these climatic constraints resulting in reduced productivities of these forest ecosystems and increased vulnerability to drought-induced mortality. The use of drought-tolerant tree species is therefore a promising strategy in future forestry to mitigate the effects of climate change on forest ecosystems.

Quantitative and qualitative analyses of tree-ring growth can provide useful information about the drought tolerance of tree species as tree rings integrate drought responses from different levels of plant organisation and functioning. Although tree-ring growth is very sensitive to different stressors (Dobbertin, 2005), it is often difficult to distinguish whether stress is due to drought, cold, air pollution, insect infestations or other factors. Here, stable isotopes of carbon

and oxygen in plant tissues, in combination with growth and leaf physiological measures, are helpful since they are good indicators for the physiological responses of plants to changes in precipitation and temperature (Dawson *et al*., 2002; Loader *et al*., 2007; Arend *et al*., 2013; Fonti *et al*., 2013). Mechanisms leading to altered isotope signatures in photosynthetic and non-photosynthetic tissues of drought-stressed plants are well described and numerous studies have shown the effects of drought on stable isotopes in plant organs (e.g. McCarroll and Loader, 2004; Brüggemann *et al*., 2011).

Under drought conditions, plants reduce their stomatal conductance to limit water loss via transpiration inevitably leading to a decreased concentration of intercellular CO_2 (c_i) relative to the concentration of ambient CO_2 (c_a). Farquhar *et al*. (1989) derived a simplified but effective equation, which describes the relationship between c_i/c_a and the carbon isotope ratio ($\delta^{13}C$) in plant tissues:

$$\delta^{13}C_{plant} = \delta^{13}C_{atm} - a + (b - a) \times \frac{c_i}{c_a} \quad (1)$$

where *a* is the fractionation factor for diffusion of CO_2 in air (a=4.4‰) and *b* the fractionation factor of RuBisCO-carboxylation (b=27‰). The strong dependency of $\delta^{13}C$ on c_i/c_a is widely used to characterize environmental effects on plant physiology, particularly on leaf photosynthesis. However, it is not possible to distinguish whether environmental constraints affect $\delta^{13}C$ via stomatal or biochemical limitations (Scheidegger *et al*., 2000). The oxygen isotope ratio ($\delta^{18}O$) can facilitate such a distinction between stomatal and biochemical limitations of photosynthesis as it depends only on the evaporative enrichment of source water at the sites of leaf transpiration (Dongmann *et al*., 1974) and the so called *Péclet* effect. The latter takes into account that advective and diffusive transport of non-enriched xylem water and enriched leaf-water inside the leaf dampens this enrichment effect (Barbour, 2007). In contrast to $\delta^{13}C$, $\delta^{18}O$ is not changed by the fractionation during carboxylation although it is tightly linked to stomatal conductance. It is therefore an ideal co-variable to determine to what degree photosynthesis or stomatal conductance modify $\delta^{13}C$ (Scheidegger *et al*., 2000). Both isotope signals are imprinted on the organic matter of plants, from leaves to tree rings and their information can be exploited to relate physiological as well as long-term responses of plants to environmental changes (e.g. Eilmann *et al*., 2010).

Current knowledge about the relationship of both isotopes to CO_2 and H_2O gas exchange has been implemented in the conceptual dual isotope approach of carbon and oxygen (Scheidegger *et al*., 2000). Numerous recent studies used the information provided by stomatal-dependent variations

in $\delta^{18}O$ to explain changes in $\delta^{13}C$ and therefore the plant's behaviour to environmental variations (Brooks and Coulombe, 2009; Gessler *et al*., 2009; Barnard *et al*., 2012; Lévesque *et al*., 2013). A considerable number of studies revealed the influence of water availability on the isotopic composition in leaf tissues and tree rings (e.g. Mc Carol and Loader, 2004; Barbour *et al*., 2007; Bowling *et al*., 2008). Furthermore, it has also been shown that changes in temperature may trigger alterations in the isotopic composition of carbon and oxygen (Libby *et al*., 1976; Loader and Switsur, 1996). So far, the influence of drought and temperature on $\delta^{13}C$ and $\delta^{18}O$ has never been examined in a single study using the dual isotope approach along with gas exchange measurements and intra-annual tree-ring growth, which could strengthen the interpretation of the isotope pattern.

Originally, the dual isotope approach was introduced for photosynthetic tissue and only recently tested conceptually for the interpretation of tree-ring data (Roden and Farquhar, 2012). The approach was valid for most of the applied treatments although there remained some unsolved issues and the authors highlighted the need for further research to make the concept operational under different scenarios. Additionally, Roden and Siegwolf (2012) listed ten concerns and suggestions regarding the application and interpretation of the dual isotope approach. The commentary aimed to prevent false conclusions and highlighted areas of caution which have to be considered before applying this approach. One of these concerns was the missing isotopic signals in tree rings during periods of wood growth cessation which, for example, may occur when trees are exposed to severe drought. While leaf organic matter can still retain a drought-related isotopic signal, tree-ring tissue may reveal an isotopic pattern representing only less stressful periods due to the shutdown of tree-ring growth. The present study will test such a constellation in which the analysis of intra-annual tree-ring growth dynamics and gas exchange measurements facilitate the uncovering of contradicting isotope responses in leaves and tree rings.

In the present study, young oak trees (*Quercus robur* and *Q. petraea*) were subjected to air warming and experimentally controlled summer droughts, with yearly increasing intensities of water shortage over a period of three years. Both oak species are commonly considered to be drought- and thermo-tolerant even though they occupy different ecological habitats in terms of soil humidity (Ellenberg, 1988) and show a different response of leaf photosynthesis to drought (Arend *et al*., 2013). Isotopic patterns of ^{13}C and ^{18}O were analysed in leaf tissue and tree-ring cellulose and linked to measures of leaf photosynthesis and intra-annual tree-ring growth to (i) study the influence of climate constraints on the coherences between isotopes, leaf physiology and tree growth and (ii) better explain the origin of isotopic signals in tree rings.

Material and methods

Experimental design

The present study was carried out within the framework of the 'Querco' experiment which was conducted from 2007-2009 in the 16 large mesocosms of the model ecosystem facility MODEOK at the Swiss Federal Research Institute WSL, Birmensdorf, Switzerland (47°21'48'' N, 8°27'23'' E, 545 m a.s.l.). Each mesocosm has a sliding roof at 3 m height allowing the exclusion of natural precipitation and encloses two concrete walled lysimeters with a depth of 1.5 m and a soil surface of 3 m^2. The lysimeters were filled with acidic (acidic Haplic Alisol; loamy sand; pH 4.1) or calcareous (Calcaric Fluvisol; sandy loam; pH 6.9) forest soil. In spring 2006, three European oak species with four provenances each were transplanted into the mesocosms. Per mesocosm, two, two-year-old saplings of the selected species and provenance were planted into each soil compartment (Arend *et al.*, 2011; Kuster *et al.*, 2013). The present study focusses on the two oaks species *Q. robur* L. and *Q. petraea* (Matt.) Liebl. with one provenance each and on acidic soil only (Table 1).

Table 1. Geographic and climatic origin of the selected oak species. Acorns were harvested at the mother stands and seeded at WSL. Elevation of the mother stands (m a.s.l.), mean annual temperature (°C) and mean annual precipitation (mm) are shown. Climate data from the period 1961–90 are from meteorological stations (MeteoSwiss) near the collections sites.

Species	Acorn origin	Altitude	Temperature	Precipitation
Q. robur L.	Tägerwilen (47°38'N, 9°08'E)	510	8.7	929
Q. petraea (Matt.) Lieb	Corcelles (46°51'N,6°41'E)	550	9.0	893

Starting in 2007, the saplings were subjected to the climate treatments drought (D), air warming (AW) and their combination (AWD) as well as a control treatment (C) for three subsequent growing seasons. Each climate treatment was replicated in four mesocosms. The water regime in the mesocosms was controlled by an automated irrigation system and the sliding glass roofs closing automatically at the onset of rain fall. During the autumn and winter months, the roofs of all mesocosms were kept open to allow full replenishment of soil water reserves with natural precipitation (Fig. 1). From spring to summer, C and AW mesocosms were irrigated with 10 mm

deionised water every 2 to 3 days using six sprinklers at a height of 1 m. Deionised water was enriched with nutrients simulating the average composition of precipitation water for this site. In D and AWD mesocosms, drought was imposed by temporary interruption of the automated irrigation. Drought intensities were increased over the three consecutive years by reducing the number and duration of the irrigation events (Fig. 1A). The air temperature inside the AW and AWD mesocosms was passively increased by varying the opening angle of the moveable mesocosm sidewalls. Air temperature and relative humidity in the control mesocosms were comparable to those of ambient stands (Fig. 1B, C).

Measurement of soil moisture, air temperature and relative humidity

Soil water content (SWC) was determined in each mesocosm manually every week using time domain reflectometry (TDR 100, Campbell Scientific Inc., Logan, Utah, USA) at a soil depth of 38 cm (Fig. 1A). Air temperature and relative humidity were measured automatically every hour at a height of 120 cm in each mesocosm using shaded EL-USB-2 data loggers (Lascar Electronics Ltd, Whiteparish, UK) (Fig. 1B, C).

Collection and preparation of leaves and tree-ring samples for isotope analyses

In early September of each experimental year (2007 - 2009), five leaves representative for the whole tree foliage (regarding flush, colour, size and location in the tree canopy) were collected from each tree per species and treatment. Drought treatment in the D and AWD mesocosms reduced the number of flushes by approximately 20% in 2008 and 2009 (Kuster *et al*., 2014). Leaf samples of two individual trees per species and mesocosm were pooled, dried at 65 °C and ground to a fine powder using a ball mill (MM400 & MM2000, Retsch, Haan, Germany) prior to isotopic analysis. At the end of the experiment in October 2009, stems were cut approximately 15 cm above ground and stem discs of 0.5 cm thickness were produced. Tree rings of the years 2007 to 2009 were dissected using a china blade under a stereomicroscope (Leica Wild M3Z, Leica Microsystems, Wetzlar, Germany). To eliminate the isotope signal of early-wood vessels, which are mostly formed from stored carbohydrates of the previous season, the first cell lines of each tree ring were discarded. Woody material was ground to a fine powder with an ultra-centrifugal mill (ZM 200, Retsch, Haan, Germany, sieve 0.5mm) before cellulose extraction. For this purpose, 10-15 mg of the powdered wood material was weighed into Teflon bags and washed for 2 hours in 5% NaOH solution at 60 °C to remove fats, hemicelluloses and phenols. Afterwards, the samples were washed with a 7% $NaClO_2$ solution (pH 4-5) for 32 hours at 60°C to remove lignins (Loader *et al*. 1997). Again, samples were dried for at least 48 hours prior to isotopic analysis.

Collection of drainage water in 2009

Drainage water was collected in closed containers from the bottom outflow of each lysimeter cumulatively at intervals of three weeks and stored at -20 °C for further analyses. At the end of the severe drought periods in the beginning of July and in the middle of August, no drainage water could be collected in AWD treatments as there was no bottom outflow in the lysimeters due to the long-lasting irrigation stop (Fig. 1A). Prior to isotopic analyses of $\delta^{18}O$, samples were filtered with a syringe filter (0.45µm, Rotilabo, Wetzlar, Germany) to remove soil particles. Drainage water consisted of two different water sources as plants were exposed to natural precipitation during the autumn and winter months and irrigated with deionised tap water enriched with nutrients during spring and summer. Deionised tap water was sampled for comparison every week and processed as described above. Precipitation water was considered to follow a clear seasonal variation with depleted $\delta^{18}O$ in winter and enriched $\delta^{18}O$ in summer months (Schürch *et al*., 2003).

Isotope analyses of plant samples and drainage water

Powdered material of leaves and tree-ring cellulose (about 0.5 mg) was weighed into tin and silver capsules (Säntis Analytical, Teufen, Switzerland) for stable C and O isotope analyses. For $\delta^{13}C$, the sample was combusted to CO_2 with excess of oxygen at 1020 °C in an elemental analyser (EA-1110, Carlo Erba Thermoquest, Milan, Italy), which was linked to a Delta S mass spectrometer with a CONFLO II (both Finnigan MAT, Bremen, Germany) operating in continuous flow mode. For $\delta^{18}O$, the samples were pyrolysed to CO at 1080 °C in an elemental analyser (EA-1108, Carlo Erba Thermoquest, Milan, Italy) linked to a DELTA plus XP mass spectrometer via a variable open-split interface (both CONFLO-III, Thermo Finnigan, Bremen, Germany). Water samples were injected into a TC/EA Analyser (Thermo Finnigan, Bremen, Germany) with a Combi-Pal autosampler (CTC, Switzerland). At a temperature of 1440 °C, the water was split into H_2 and O, while the latter reacted with glassy carbon splitters in the reaction tube to CO. The gas components were carried in a He stream to the mass spectrometer DELTA plus XP via a variable open-split interface (both CONFLO-III, Thermo Finnigan, Bremen, Germany). All isotope ratios are given in reference to their international standard in the delta notation in ‰:

$$\delta_{sample} = \frac{R_{sample}}{R_{standard}} - 1, \qquad (2)$$

with R_{sample} being the $^{13}C/^{12}C$ or $^{18}O/^{16}O$ ratio of the sample, and $R_{standard}$ being the ratio of Vienna Pee Dee Beleminite (VPDB) for C or Vienna Mean Standard Ocean water (V-SMOW) for O. Analytical precision was estimated as the standard deviation of laboratory cellulose standards, better than 0.10‰ for $\delta^{13}C$ and 0.25‰ for $\delta^{18}O$.

Measurement of leaf net photosynthesis

The seasonal course of instantaneous net photosynthesis (A_N) was measured on four trees per species and treatment with a portable photosynthesis system equipped with a broadleaf cuvette (LICOR 6400, LICOR, Lincoln, NE, USA). Conditions in the cuvette were kept constant during the measurements at 380 ppm [CO_2] and a photon flux density of 1500 μmol m^{-2} s^{-1}, while the temperature was adjusted close to conditions outside the cuvette.

Analysis of intra-annual tree-ring growth

Measurements of seasonal tree-ring growth were performed on four trees per species and treatment using the pinning technique (Wolter, 1968; Fonti *et al*., 2013). Small wound reactions to the stem cambium were induced with a pin on eleven dates from April to October 2009. After harvesting the trees, stem sections were analysed microscopically to determine the radial increment of wood tissue between the tree-ring border, formed at the end of the previous growing season, and the wound tissue formed in the growing season 2009.

Statistical analysis

Statistical calculations were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). Effects of treatments and their interactions were analysed as a three-factorial design (irrigation, temperature and species) by analysis of variance, with the factor species nested in the factors irrigation and temperature (nested ANOVA, general linear model). The factor year was highly significant; therefore datasets were analysed separately for each year. The assignment of the plants to different mesocosms had no effect on the responses of the trees. Treatments and interactions among treatments were considered as significant when $P < 0.05$. Statistical calculations were performed with four replicates per species and treatment for leaf bulk tissue (pooled leaf samples from 2 trees) and eight replicates per species and treatment for tree-ring cellulose.

Results

Climate treatments

In the first experimental year 2007, volumetric soil water content (SWC) did not drop below 0.1 m^3m^{-3} in mesocosms with the drought (D) and the combination (AWD) treatments and remained high in the well-watered control (C) and the air warming (AW) mesocosms (Fig. 1A). In 2008, soil water deficit became more severe in the drought treated D and AWD mesocosms, as SWC nearly reached values of 0.05 m^3m^{-3}. During the growing season 2009, a severe drought was

induced. SWC decreased to 0.05 m^3m^{-3} in D and AWD, while in C and AW, it remained between 0.15 and 0.20 m^3m^{-3} during the whole vegetation period (Fig. 1A). Hence, drought intensity increased over the three years due to longer periods without irrigation in the growing season 2008 and 2009, but also due to increasing water demand of the developing canopies.

During the experimental period 2007-2009, passive air warming led to an increase of mean monthly daytime temperatures (08:00-18:00 h, UTC+1) during the growing seasons of 1-2 °C in

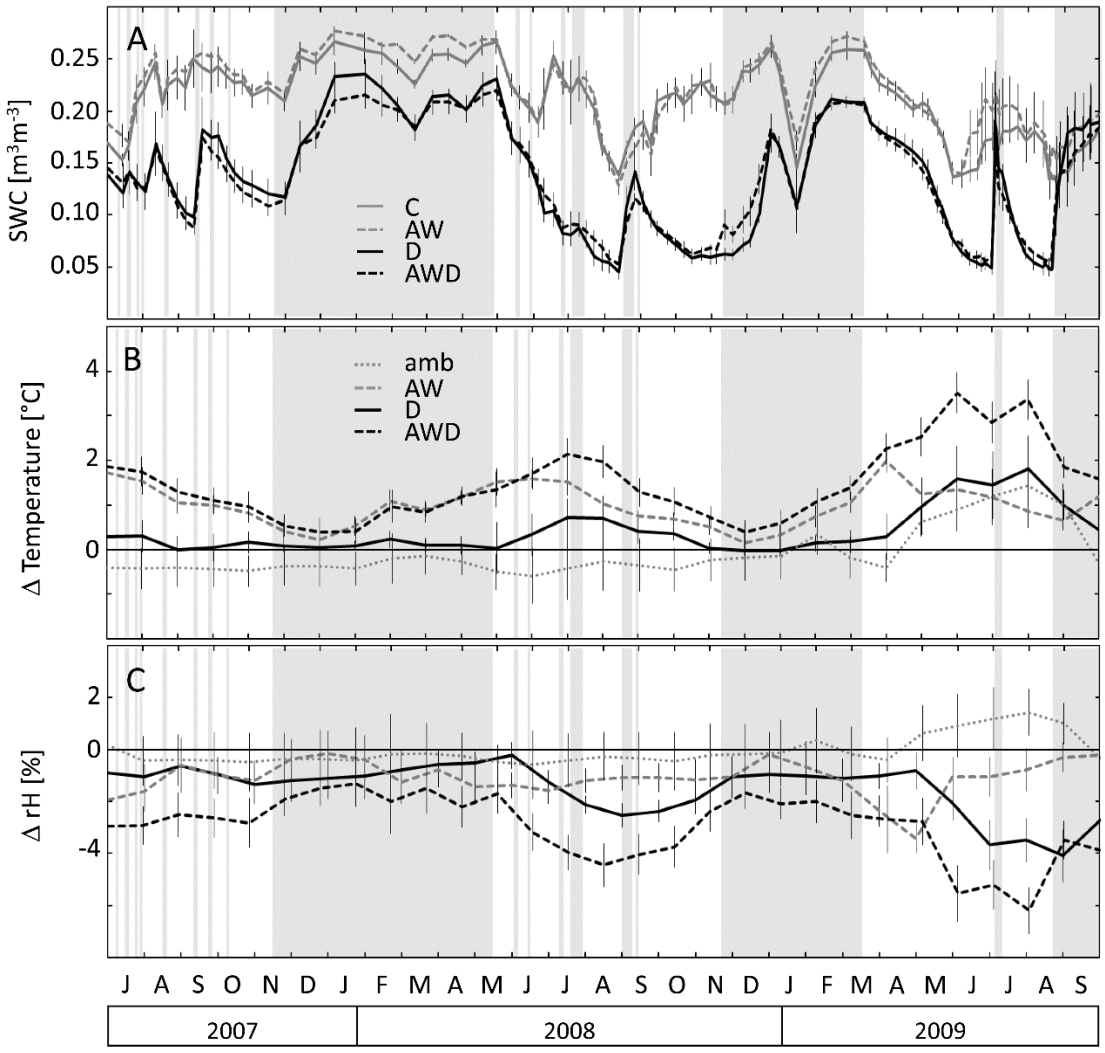

Figure 1. Soil water contents of all four treatments in 38-cm soil depth (A), differences in air temperature of the treatments and ambient plots without chamber relative to control (B) and differences in relative humidity (ΔrH) of the treatments and ambient plots without chamber relative to control (C) from 2007 to 2009. Grey shaded areas indicate water supply in the drought treatments D and AWD. Data are means of n = 4 ± SE (data of (A) and (B) taken from Kuster *et al*. 2013 with permission of the publisher).

AW and 1-3 °C in AWD mesocosms (Fig. 1B) and only small differences in relative humidity were detected among the four treatments and ambient conditions (Fig. 1C). In 2008, air warming in AWD was similar to that recorded in AW, while in 2009, the daily increase in air temperature was even higher in AWD than in AW. Moreover, in the D treatment, where no warming was applied, a drought-dependent increase in daytime temperature of nearly 2 °C in 2009 was detected, which can be attributed to a decrease in evaporative cooling due to reduced transpiration of the drought treated trees (Kuster *et al*., 2013).

Isotopic patterns of $\delta^{13}C$ and $\delta^{18}O$ in bulk leaf tissue and tree-ring cellulose

In the year 2007, $\delta^{13}C$ and $\delta^{18}O$ in leaf bulk tissue did not respond to the treatments D, AW and AWD (Fig. 2A, D; Table 2). However, *Q. robur* had lower $\delta^{13}C$ values compared to *Q. petraea*. In 2008, both species showed a highly significant difference in $\delta^{13}C$ and $\delta^{18}O$ as a response to the drought imposed in the D and AWD treated mesocosms (Fig. 2B, E; Table 2). Compared to the control, $\delta^{13}C$ increased by 1.5‰ in *Q. robur* and 1.3‰ in *Q. petraea*, while $\delta^{18}O$ increased by 1.4‰ and 1.1‰ in *Q. robur* and *Q. petraea*, respectively (Fig. 2B, E). Again, AW showed no significant effect on the isotopic ratios of C and O in leaf bulk tissue. While in 2007 the species did not differ

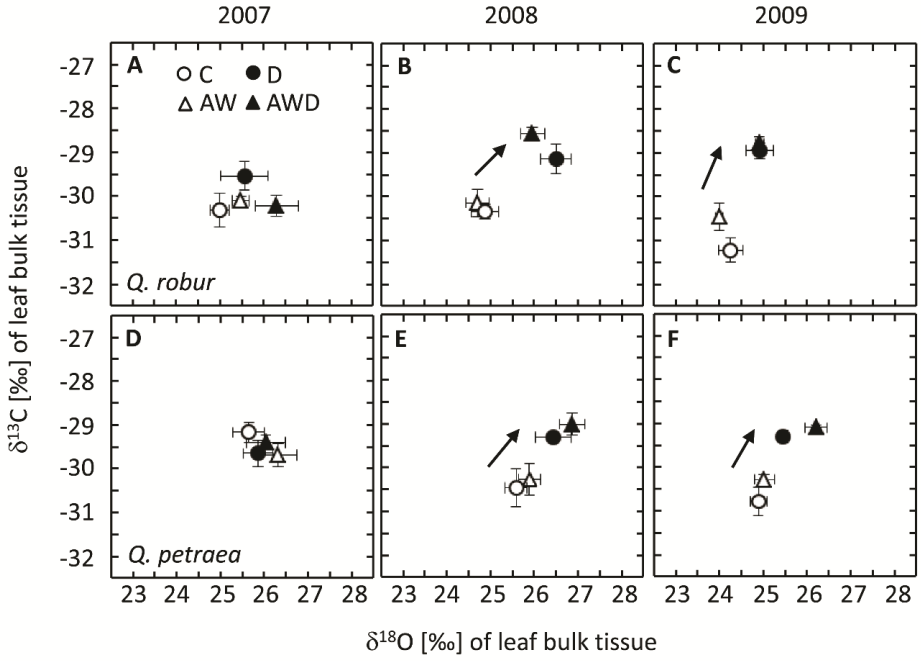

Figure 2. Patterns of carbon and oxygen isotopes in leaf bulk tissue of *Q. robur* (A–C) and *Q. petraea* (D–F) for the years 2007, 2008 and 2009. Symbols represent mean values of each treatment. Black arrows indicate the trend in isotopic patterns due to the drought treatments. Data are means ± SE, n = 4.

in $\delta^{18}O$, significant species differences were observed in 2008, with *Q. robur* showing lower $\delta^{18}O$ values compared to *Q. petraea*. In 2009, a relative increase in $\delta^{13}C$ was found in the drought treatments compared to control, exceeding the increase in $\delta^{13}C$ of the former season. Within the two oak species, *Q. robur* was more affected by the drought imposed in D and AWD treatments than *Q. petraea* (*Q. robur*: 2.4‰, *Q. petraea*: 1.8‰, Fig. 2 C, F). In contrast to $\delta^{13}C$, the drought mediated increase in $\delta^{18}O$ was less pronounced in 2009 compared to the preceding year (*Q. robur*: 0.7‰, *Q. petraea*: 0.9‰), but still highly significant (Table 2). As in 2008, *Q. robur* showed lower $\delta^{18}O$, but this species effect became highly significant in 2009. Air warming in the AW and AWD treatments had no significant effect on the isotopic ratios of C and O in leaf bulk tissue of either species in any of the experimental years. It is worth noting that in leaf bulk tissue, the relative increase in $\delta^{13}C$ observed in D and AWD mesocosms in 2008 and 2009 was related to a decrease of $\delta^{13}C$ in C and AW mesocosms.

For tree-ring cellulose, no response of the isotopic ratios of C and O to the climate treatments was detected in the first experimental year 2007 (Fig. 3A, D) but *Q. robur* showed significantly lower $\delta^{13}C$ and $\delta^{18}O$ values than *Q. petraea* in 2007 (Table 2). In 2008, differences between trees in well

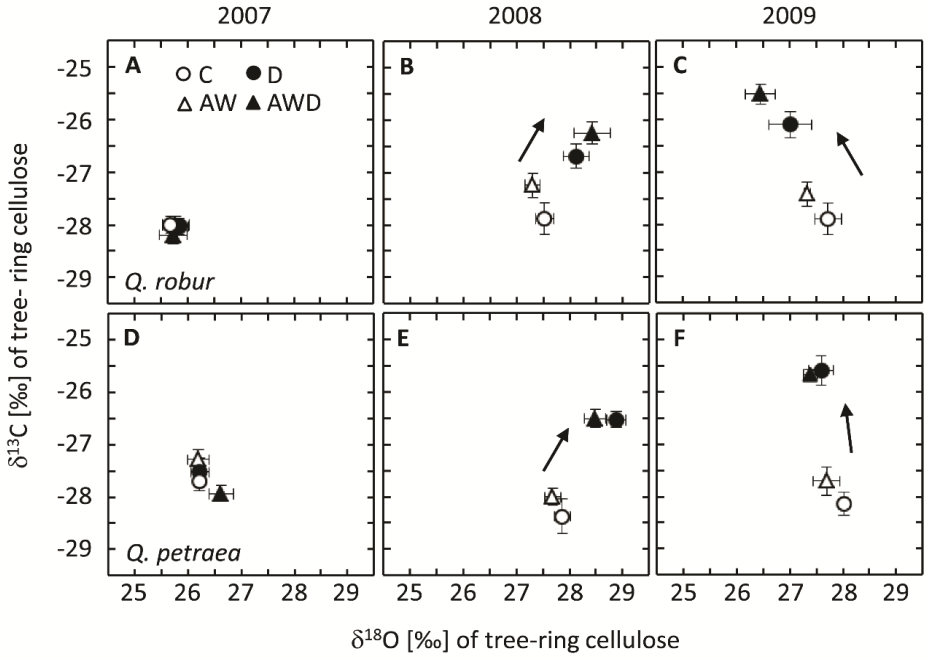

Figure 3. Pattern of carbon and oxygen isotopes in tree-ring cellulose of *Q. robur* (A–C) and *Q. petraea* (D–F) for the years 2007, 2008 and 2009. Symbols represent mean values for each treatment. Black arrows indicate the trend in isotopic patterns due to the drought treatments. Data are means ± SE, n = 8.

Table 2. Effects of the treatments air warming (AW; control vs elevated air temperature), drought (D; continuous vs discontinuous irrigation) and species on $\delta^{13}C$ and $\delta^{18}O$ of leaf bulk tissue and tree-ring cellulose in three growing seasons 2007, 2008 and 2009. Bold numbers indicate a significant *P*-value as calculated with ANOVA according to the three-factorial test design (D, AW and Species). None of the two- and three-way interactions between factors (D × AW; D × Species; AW × Species; D × AW × Species) was significant.

	Leaf bulk tissue						Tree-ring cellulose					
	2007		2008		2009		2007		2008		2009	
	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$
AW	0.703	0.171	0.270	0.245	0.102	0.101	0.665	0.755	0.052	0.413	0.061	0.064
D	0.563	0.213	**0.000**	**0.000**	**0.000**	**0.000**	0.776	0.629	**0.000**	**0.000**	**0.000**	**0.001**
Species	**0.007**	0.167	0.283	**0.003**	0.704	**0.000**	**0.011**	**0.002**	**0.040**	**0.012**	0.785	**0.001**

watered (C and AW) and drought treated (D and AWD) mesocosms became highly significant for both isotopes. As in leaf bulk tissue, $\delta^{13}C$ in tree-ring cellulose increased concomitantly with $\delta^{18}O$ for D and AWD (*Q. robur*: 1.1‰ $\delta^{13}C$ and 0.8‰ $\delta^{18}O$; *Q. petraea*: 1.1‰ $\delta^{13}C$ and 0.7‰ $\delta^{18}O$; Fig. 3B, E) in 2008. In 2009, an increase of $\delta^{13}C$ in D and AWD mesocosms was observed for both species, *Q. robur* (Fig. 3C: 2.1‰) and *Q. petraea* (Fig. 3F: 2.5‰), which was even stronger than in the former year. In contrast, $\delta^{18}O$ showed a decrease in tree-ring cellulose of -1.0‰ in *Q. robur* and -0.5 ‰ in *Q. petraea* compared to well-watered trees in 2009. In none of the years and species was a significant change in $\delta^{13}C$ and $\delta^{18}O$ values due to air warming treatment alone detected.

Seasonal net photosynthesis

Seasonal net photosynthesis (A_N) was analysed in the last experimental year 2009 (Fig. 4). A_N was strongly affected by drought in D and AWD treatments and declined in both oak species with gradually decreasing soil moisture availability (Fig. 1A). A_N in these treatments only recovered during the short re-watering period in July 2009, but decreased rapidly during the subsequent drought. Air warming in the well watered AW treatment did not lead to a significant change in A_N, neither in *Q. robur* nor in *Q. petraea*.

Intra-annual tree-ring growth

The severe drought applied in 2009 to the D and AWD mesocosms had a strong effect on the seasonal course of tree-ring growth as well as on its absolute values (Fig. 4 C, D, Table 3). While trees of *Q. robur* in the well watered C and AW mesocosms completed 50% of their inter-annual tree-ring growth until the short re-watering period in July, trees in the drought treated mesocosms already accomplished 75% of the entire annual growth and stopped tree-ring formation in early August. In contrast to *Q. robur*, *Q. petraea* maintained some growth activity in the D and AWD mesocosms until the end of the season, but grew much slower than well watered trees. Comparing absolute tree-ring width of the two species, *Q. robur* produced wider tree rings than *Q. petraea* (Table 3, $P = 0.002$). Both species suffered from the applied drought in the D and AWD mesocosms, and responded with a reduction in tree-ring width ($P < 0.001$). Compared to the control, the final reduction of intra-annual tree-ring growth was 59% and 66% for drought treated *Q. robur* and *Q. petraea*, respectively; and 74% and 57% for *Q. robur* and *Q. petraea* in the AWD treatment, respectively. For additional data on biomass, see Arend *et al*. (2013) and Kuster *et al*. (2013).

Table 3. Tree-ring width of control (C), air warming (AW), drought (D) and combination (AWD) treated trees of *Q. robur* and *Q. petraea* in 2009 (µm). Values are means ± SE; n = 4.

	C			AW			D			AWD		
Q. robur	2181	±	374	2589	±	214	908	±	238	576	±	14
Q. petraea	1552	±	65	1586	±	96	539	±	45	680	±	58

Values are means ± SE; *n*=4

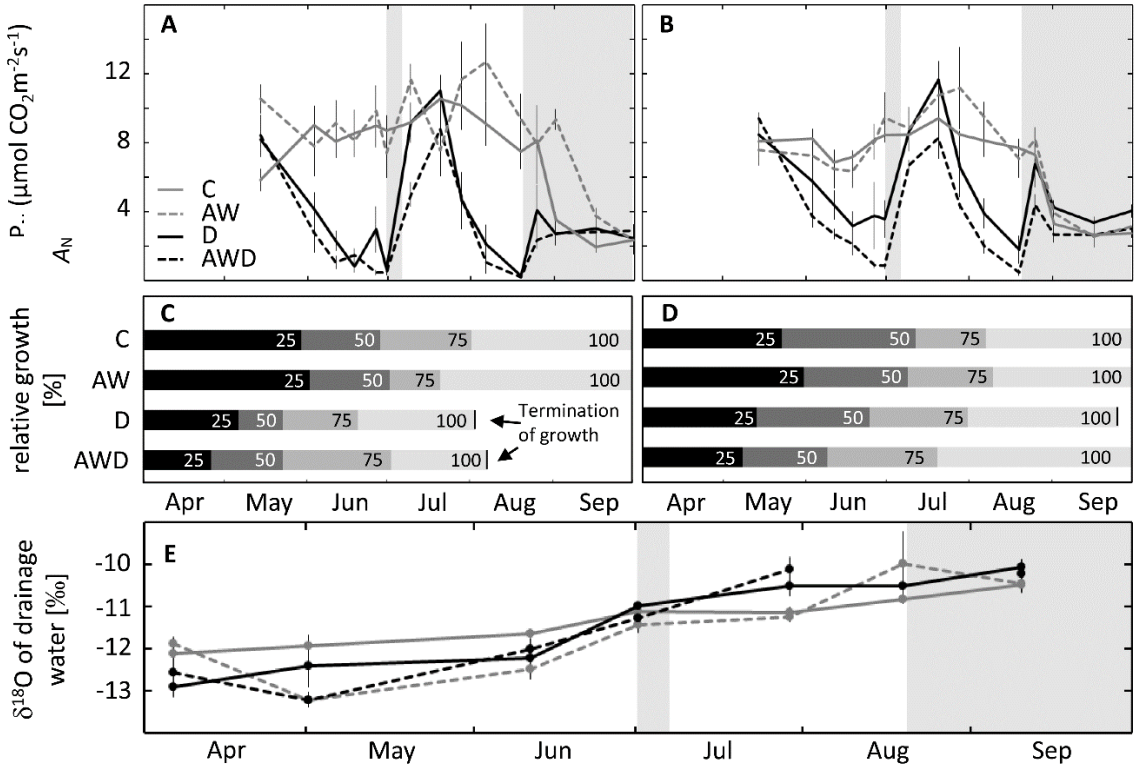

Figure 4. Seasonal course of net photosynthesis and relative tree-ring growth of *Q. robur* (A and C) and *Q. petraea* (B and D) and drainage water $\delta^{18}O$ (e) for the year 2009. Grey shadings indicate water supply in the drought treatments. (A and B) Instantaneous net photosynthesis in AW, D and AWD treatment and C trees. Data are means ± SE (n = 4). (C and D) Relative tree-ring growth during the growing season, determined with the pinning technique. Different shadings of the bars indicate 25, 50, 75 and 100% of growth as a mean value for four trees per treatment. (E) Seasonal course of $\delta^{18}O$ in drainage water in the four treatments (n = 4 ± SE; 1 July 2009: D and AWD n = 1; 19 August 2009: D n = 1 and AWD not available). Pattern of the drainage water $\delta^{18}O$ is the same for the two species (photosynthesis data taken from Arend *et al*. 2013, with permission of the publisher).

$\delta^{18}O$ in drainage water

Drainage water, collected at regular intervals from the bottom outflow of the lysimeters, showed a clear seasonal variation in $\delta^{18}O$ (Fig. 4, lower panel), with depleted $\delta^{18}O$ values in winter and enriched $\delta^{18}O$ values in summer months. The drainage water originated from two different sources with different $\delta^{18}O$ values: tap water used for irrigation in spring and summer and natural precipitation in autumn and winter. Tap water had a constant $\delta^{18}O$ value of -11‰, while natural precipitation showed temperature dependent seasonal variations, with a decrease of $\delta^{18}O$ down to -15‰ in winter (IAEA/WMO. Global Network of Isotopes in Precipitation; Schürch *et al*., 2003). This resulted in a ^{18}O depletion in the drainage water in spring by 1 to 2‰ relative to the tap water used for irrigation of all treatments. The δ^{18} values of the drainage water increased over the course of the growing season due to progressive replacement of depleted winter precipitation water by more enriched tap water and additionally increased evaporative enrichment of soil water over summer. By mid-August, drainage water of all treatments was enriched by 1.5‰ compared to irrigation water.

Discussion

Patterns of isotopic ratios of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) were analysed in leaves and tree rings of oaks (*Q. robur* and *Q. petraea*) subjected for three years to warming, drought and their combination. To better understand their physiological origin, changes in $\delta^{13}C$ and $\delta^{18}O$ were linked to leaf net-photosynthesis and inter-annual tree ring growth. The applied drought treatments did not change $\delta^{13}C$ and $\delta^{18}O$ in leaves and tree rings in the first year 2007. This is not surprising as intermediate irrigation prevented the development of a severe and physiologically effective water deficit and trees were still small, with a rather low water demand for their developing canopies. The observed $\delta^{13}C$ values in leaves and tree rings were comparable to data reported for well-watered oak trees (Picon *et al*., 1996; Zang *et al*., 2012), supporting the interpretation that our trees had not yet suffered from the applied drought. Also, the warming treatment, being rather small, did not affect $\delta^{13}C$ and $\delta^{18}O$ in leaves and tree rings in any year, probably due to the thermic plasticity of these thermo-tolerant oak species (Ellenberg, 1988). This finding contradicts earlier studies which reported effects of increased temperature on $\delta^{13}C$ and $\delta^{18}O$ (Loader and Swistur, 1996) which used $\delta^{18}O$ to detect temperature shifts of 1.5 °C in annual average temperature (Libby *et al*., 1976). However, in our experiment increased temperatures were only observed from May

to October while these former studies report a average deviation of the temperature over a whole year.

As the number of irrigation events was considerably reduced in 2008 and 2009, we could observe the first response of the trees to the applied drought treatments. In both years, an increase in $\delta^{13}C$ was observed in leaf tissue and tree-ring cellulose indicating a decrease in leaf internal CO_2 concentrations (Farquhar *et al*., 1989). This is in line with studies on carbon isotope responses to drought where reduced water availability induced a decrease in stomatal conductance leading to reduced internal CO_2 concentrations and therefore to less negative $\delta^{13}C$ ratios in photosynthetic and non-photosynthetic tissues of different oak species (Picon *et al*., 1996; Zang *et al*., 2012). Along with an increase in $\delta^{13}C$, an increase in $\delta^{18}O$ was observed in 2008 and 2009 in leaf tissue and in the year 2008 in tree-ring cellulose. According to the dual isotope approach, the increase in $\delta^{18}O$ indicates stomatal limitation of photosynthesis to be the main cause for the observed increase in $\delta^{13}C$ (Scheidegger *et al*., 2000) - which compares well with our physiological observations.

The instantaneous drought effects on photosynthetic carbon assimilation and thus leaf sugar isotope composition become permanently recorded when sugars are transported to stems and incorporated in the tree-ring cellulose matrix (West *et al*., 2006; Gessler *et al*., 2009; Gessler *et al*., 2014). It was surprising, however, that for drought treated trees of *Q. robur*, a decrease in $\delta^{18}O$ in tree-ring cellulose was observed in 2009, even below the level of well-watered control trees, while *Q. petraea* showed no clear response to the drought treatments. Applying the dual isotope approach to the observed isotope response, an increase in $\delta^{13}C$, together with a constant or decreasing $\delta^{18}O$, would suggest an increase in photosynthesis and a constant or increasing stomatal conductance despite the severe drought conditions in 2009. However, this clearly contradicts current plant physiological knowledge and is not supported by our observation of leaf gas exchange, growth data and PS II photochemistry (Arend *et al*., 2013).

Thus, here we have a situation, which Barbour and Song (2014) describe as the "*tree-ring stable isotope compositions remain somewhat unreliable as a record of tree carbon – water dynamics*". So, the question arises, what can explain these seemingly implausible results? One should note that in drought-treated oaks, 50% of the tree ring was formed until May/ June, and growth came completely to a halt (*Q. robur*) or proceeded at a low rate (*Q. petraea*) in summer. Therefore, a relatively large proportion of the tree ring in drought stressed trees was already formed in spring when the $\delta^{18}O$ of the soil source water was probably still low due to winter rain. During the summer time, when no or only little growth was observed, the $\delta^{18}O$ of the soil source water was

higher and drought reduced stomatal conductance, resulting in an increase in $\delta^{18}O$ in leaves. However, if there is no or only little growth, no isotopic signals from leaf water enrichment and from soil source water can be recorded in tree rings, probably explaining the much lower $\delta^{18}O$ signals in 2009.

This uncoupling leads to an isotope pattern in tree rings that "fakes" a counter-intuitive (and in fact non-existent) physiological response to drought. Interestingly, the $\delta^{18}O$ isotope signal in tree rings of *Q. petraea* was less affected because growth stopped a few weeks later than that in *Q. robur*. Therefore, some of the summer isotope signals could still be recorded in the tree ring of *Q. petraea*, leading to higher $\delta^{18}O$ values compared to *Q. robur*. However, although in *Q. petraea* a fake signal was recorded as well, the higher $\delta^{18}O$, together with a longer period of growth, suggests an extenuated response to drought. Thus, our results clearly show the importance of intra-annual growth and soil source water when interpreting $\delta^{18}O$ signals in tree rings, supporting other studies which provided evidence that seasonal variations in source water may have a strong impact on $\delta^{18}O$ in tree rings (Offermann *et al*., 2011).

A similar result with missing $\delta^{18}O$ signals in tree-rings was also observed by Sarris *et al*. (2013) for pine trees growing in a dry Mediterranean summer climate. In this former study, an extended period of growth cessation was observed, along with depleted ^{18}O values in tree rings. This depletion was explained by the use of different soil water sources (depleted ground water vs. enriched precipitation water), but was not explicitly related to drought-induced growth cessation. In our study, we provide clear evidence (based on tree-ring growth assessments) that a drought-induced growth cessation leads to decreased $\delta^{18}O$ values in tree rings as the isotopic signals from leaves and soil source water were not imprinted in the tree-ring matrix. Even a diminished tree-ring growth can lead to reduced $\delta^{18}O$ values as shown for *Q. petraea* and therefore bias the interpretation of isotopic patterns in tree rings. The strong and concurrent reduction in leaf photosynthesis with increasing drought is in line with the observed decrease and cessation of growth. Although photosynthesis recovered rapidly due to intermittent irrigation events, the newly fixed assimilates were not sufficient to resume growth and thus no isotopic signals from leaves were recorded in the respective annual tree rings.

Conclusion

In this study, we show that severe drought can uncouple the isotopic signals between leaves and tree rings. Once drought reaches an extent at which growth is halted or strongly reduced, e.g.

detected by intra-annual tree-ring growth analysis, the isotopic signal imprinted on freshly formed leaf carbohydrates is no longer recorded in tree rings. Instead, the signal recorded before or potentially after the growth-limiting drought can be found later on in the isotopic tree-ring chronology. Whether isotopic signals of the environment are recorded at all depends on the amount of mobile leaf carbohydrates that can be transformed to cell wall polymers in growing tree rings. Therefore, very dry (summer) conditions are most likely not recorded in tree rings and thus might get completely lost, since the major proportion of the tree ring is then probably formed in spring or autumn. As shown in our study, the dual isotope approach can be highly instrumental in detecting such missing records. This will have large implications on the physiological interpretation of any isotopic tree-ring chronology, particularly in respect to severely growth-limiting conditions, as projected for the future.

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Chapter 2: Seasonal photosynthetic response of European beech to severe summer drought: Limitation, recovery and post-drought stimulation

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Abstract

Trees experience frequent periods of severe drought during their long life and must therefore be able to recover and compensate for the limitations of previous stresses. In this study, the response of photosynthesis to a severe, long-lasting summer drought and drought release was followed in saplings of a mesic and xeric provenance of European beech (*F. sylvatica* L.) transplanted to mesocosms with experimentally controlled water supply. Photosynthesis was assessed in stressed and non-stressed saplings over the course of an entire vegetation season. The drought response was divided into three phases: (i) limitation, (ii) recovery and (iii) post-drought stimulation. Limitation of photosynthesis developed gradually and caused, in both provenances, a major loss of cumulated photosynthetic activity. It was accompanied by impaired PSII photochemistry indicating severe stress. Recovery started rapidly after re-watering and was fully accomplished within 20 and 10 days in the mesic and xeric provenances, respectively. After recovery, photosynthesis remained at high levels while decreasing in control trees. This post-drought stimulation was sustained until the end of the vegetation period, partly counterbalancing the previous loss of photosynthetic activity. It was attributable to an altered leaf ontogeny as indicated by a delay of autumnal leaf senescence. These observed responses might be important factors when modelling seasonal carbon uptake and phenology of forest ecosystem under future climate change.

Introduction

Drought is the major environmental constraint, causing physiological limitations on growth, reproduction and survival of plants worldwide (Boyer, 1982; Chaves *et al.*, 2003). It has therefore been recognized as a serious threat to forest ecosystems (e.g. Allen *et al.*, 2010). Forest trees are of particular concern as they experience frequent periods of severe drought during their exceptionally long life span. The strong reduction in primary productivity of forest ecosystems in wide parts of Europe due to the extreme summer drought in 2003 is one of the most prominent examples of the limiting effects of drought on trees (Ciais *et al.*, 2005). An increased frequency and intensity of this type of extreme climatic condition is predicted for the near future together with a temporal and spatial change in precipitation patterns (IPCC, 2012). Hence, responses of trees to drought are studied intensely at different scales of plant organisation and function (e.g. Bréda *et al.*, 2006; Rennenberg *et al.*, 2006) to provide the scientific knowledge base for estimating

the effects of climate change on forest ecosystems. However, physiological studies assessing the recovery phase and longer-term effects after drought are scarce.

Trees respond to drought with specific changes of physiological and metabolic activities constraining their functional integrity. Leaf photosynthesis is among the most seriously affected activities as itis tightly linked to tree water balances via stomatal control of CO_2 uptake (Hetherington and Woodward, 2003; Zweifel *et al.*, 2007). Recently, mesophyll conductance for CO_2 has been recognized as a further cause of photosynthetic variation that responds to environmentally driven fluctuations in the internal plant water balance (Flexas *et al.*, 2006, 2012; Hommel *et al.*, 2014). With increasing drought intensities, metabolic limitations of photochemical reactions become more and more effective disturbing the biochemistry of the photosynthetic apparatus. The degree to which stomatal control, mesophyll conductance and metabolic limitations contribute to drought-induced impairments of photosynthesis is still a matter of debate and depends largely on the severity and duration of the experienced stresses (Grassi and Magnani, 2005). Photosynthetic net CO_2 exchange is a physiological measure that integrates all these sources of photosynthetic variation and is thus a widely-used measure of stress in ecophysiological studies on drought exposed plants. On an ecological scale, it represents the plant physiological determinant that drives the seasonal course of net ecosystem fluxes of CO_2 and its climate dependent fluctuation (Baldocchi, 2003; Xu and Baldocchi, 2003).

As photosynthesis, and the underlying physiological processes and metabolic reactions, are tightly linked to plant water status they attracted much attention in terms of acclimation and adaptation to drought. A large number of studies reported effects of natural or experimentally imposed drought on photosynthetic traits of potted or field-grown trees providing basic information on stomatal and non-stomatal restrictions of photosynthesis (e.g. Epron *et al.*, 1993; Epron and Dreyer, 1993; Kubiske and Abrams, 1993; Schnaub *et al.*, 2003; Haldimann *et al.*, 2008; Mitchell *et al.*, 2013). The majority of these studies dealt with trees exposed to immediate drought while information gathered over a seasonal time scale and including processes of drought recovery is scarce. To date, there are only a few studies that addressed this fairly overlooked issue although the capacity to recovery is, in a broader sense, part of the tree's drought response (e.g. Kirschbaum, 1988; Gallé *et al.*, 2007; Gallé and Feller, 2007; Liu *et al.*, 2010; Vaz *et al.*, 2010). Most of these studies focused on the early response to re-watering while information gathered over a longer period and placed in a seasonal context is mostly missing.

Recently, seasonal drought development and recovery were studied in different European oak species, indicating that drought tolerant species resume photosynthesis faster than less tolerant

species (Arend *et al.*, 2013). There was also some indication that trees do not simply recover but retain a "memory" of drought in terms of overcompensating photosynthesis or delayed autumnal chlorophyll degradation. This idea is supported by previous studies on grassland and heath species that remained acclimated to drought and "remembered" earlier stresses when exposed to recurrent drought (Walter *et al.*, 2011; Backhaus *et al.*, 2014). These studies are included in the concept of an "ecological stress memory" describing any response of a plant after stress release that improves its response towards future stress experience (Walter *et al.*, 2013). In this sense, it refers exclusively to improved tolerance upon exposure to recurrent stress while responses occurring without recurrent stress are not considered. The need for a broader concept of an "ecological memory" was only recently stressed by Ogle *et al.* (2015) to better understand and model current plant and ecosystem processes.

Responses of trees to drought are optimally studied under natural field conditions with slowly developing soil water shortage and including the whole sequence of seasonal drought development and drought recovery. Such an approach provides a realistic picture on the tree's drought resistance and resilience but comprises some difficulties in controlling drought conditions. In the present study, provenances of European beech (*Fagus sylvatica* L.) were transplanted into large model ecosystems with controlled water supply and subjected to a long-lasting drought and re-watering treatment. This approach combines the advantage of experimentally controlled conditions with a semi-natural growth environment and allows for studying the whole seasonal sequence of tree responses to drought and re-watering. European beech was chosen as model tree as it is a key species in European forests that is commonly considered to suffer from increasing drought (Ellenberg, 1988; Ohlemüller *et al.*, 2006). Our study aims to (i) evaluate the effect of severe summer drought and re-watering on the seasonal course of photosynthesis and (ii) study the long-term effects that modify the leaf physiological status and leaf ontogeny after recovery from drought. Provenances of mesic and xeric origin were included in this study as physiological drought responses may differ among such provenances (Tognetti *et al.*, 1995; Peuke *et al.*, 2002; 2006)

Material and methods

Plant material and growth conditions

The present study was undertaken in the framework of the interdisciplinary beech experiment BuKlim: beech in a changing climate. In this experiment, saplings of twelve beech (*F. sylvatica*) provenances were excavated in natural forest stands growing along steep precipitation gradients in two Swiss inner-alpine valleys (Table 1). From each provenance, 64 saplings with a size of about 20 cm were transplanted in spring 2011 in a randomized design to the model ecosystem facility MODOEK of the Swiss Federal Institute for Forest, Snow and Landscape Research WSL. The MODOEK facility comprises 16 large mesocosms with a height of 3.5 m and a plantable area of 6 m^2. Each mesocosm is equipped with an automated irrigation system and a sliding roof closing automatically at the onset of rainfall. Belowground, each mesocosm is split into two lysimeters with a depth of 150 cm one filled with an acidic (haplic Alisol), one with a calcareous (Fluvisol) forest soil (Kuster *et al.*, 2013). In each mesocosm, 2 saplings from each provenance were transplanted on acidic and calcareous soil, respectively.

From November to April, the sliding roofs of the mesocosms were kept open to allow natural precipitation. By closing the sliding roofs from May to October, natural precipitation was excluded. The mesocosms were irrigated every second or third day with 50 l m^{-2} deionized water enriched with nutrients to simulate the average composition of ambient rainfall (see Kuster *et al.*, 2013). During hot summer periods, the irrigation intensity and frequency was increased to counterbalance higher rates of evapotranspiration and hold the soil moisture at 10 cm soil depth above 20%. With this target value, soil moisture in deeper soil layers was above field capacity as indicated by a constant outflow of drainage water at the bottom of the lysimeters. In 2014, a severe, long-lasting summer drought was imposed in half of the mesocosms by withholding irrigation from 22 May to 2 August (Fig. 1). As evapotranspirational water loss was particularly high at hot days, intermediate irrigation was applied to avoid too fast/intense soil drying and irreversible drought damage of the saplings. After the first saplings reached predawn water potentials below −2.0 MPa, the mesocosms were intensely re-watered for 1 day with 200 l m^{-2} and afterwards regularly irrigated as described above.

Table 1. Beech provenances transplanted to the model ecosystem facility MODOEK and environmental characteristics of the provenance sites. Climate data (annual mean temperature and annual sums of precipitation) are taken from nearby METEO SWISS stations (distances ≤ 10 km).

	geographic location	**annual temperature [°C]**	**annual precipitation [mm]**
Felsberg	46° 51’ N 9° 28’ E	8.9	798
Chur	46° 52’ N 9° 32’ E	8.9	798
Malans	46° 59’ N 9° 34’ E	8.9	1102
Mastrils	46° 58’ N 9° 32’ E	8.9	1102
Sargans	47° 3’ N 9° 26’ E	9.2	1325
Mels	47° 3’ N 9° 24’ E	9.2	1325
Ardon	46° 13’ N 7° 14’ E	9.2	542
Chamoson	46° 12’ N 7° 12’ E	9.2	542
Saxon	46° 8’ N 7° 11’ E	9.2	542
Martigny	46° 6’ N 7° 6’ E	9.2	843
Colombey	46° 16’ N 6° 56’ E	8.9	1055
Ollon	46° 18’ N 6° 59’ E	8.9	1055

Determination of soil and plant water balances

Volumetric soil water content and soil matrix potential were measured in each lysimeter compartment at 10 cm soil depth using PC-controlled moisture probes (5TM, Decagon, USA) and pF-sensors (Ecotech GmbH, Bonn, Germany). Tree water status was measured as predawn leaf water potentials using a Scholander pressure chamber (M 600, Mosler Tech Support, Berlin, Germany).

Measurements of net-photosynthesis and analysis of chlorophyll a fluorescence

Two provenances from a mesic (Mastrils) and xeric (Saxon) forest stand (Table 1) growing on acidic soil were selected for assessing photosynthetic responses to drought. These provenances originate from environmentally contrasting forest stands in terms of annual precipitation and differed most in phenological behaviour (Arend *et al.*, 2015). Instantaneous rates of net-photosynthesis (A_N) were measured once per sapling between 11:00 and 16:00 using a portable photosynthesis system (LI-COR 6400, Lincoln, NE, USA) equipped with a broadleaf cuvette. The conditions inside the cuvette were kept constant with 400 ppm CO_2, a photon flux density of 1000 μmol $m^{-2}s^{-1}$ and temperature adjusted close to outside conditions. Fast fluorescence kinetics were analysed once per sapling between 11:00 and 12:00 on dark adapted leaves using a portable plant efficiency analyser (Pocket PEA, Hansatech Instruments Ltd., Norfolk, UK). After a saturating light pulse of 3500 μmol quanta $m^{-2}s^{-1}$ of red light (650 nm), fluorescence rise was recorded in a high-resolution mode. The maximum quantum efficiency of PSII (ratio of variable to maximum fluorescence F_V/F_M) and the total performance index of PSII (PI_{tot}; Strasser *et al.*, 2010) were calculated from the fluorescence kinetics.

Assessment of autumnal leaf senescence

Autumnal leaf senescence was assessed by non-invasive measurements of autumnal leaf chlorophyll loss. All provenances on acidic soil were considered to reduce the statistical uncertainty in the analysis of this highly variable trait. Leaf chlorophyll loss was measured from August to November in intervals of 1 to 3 weeks using a SPAD chlorophyll meter (Konica Minolta Optics Inc., Osaka, Japan). In each sapling, 8 to 10 leaves from different tree positions were measured to account for variable senescence development within individual trees. From the resulting chlorophyll loss curves, the average date of 50% leaf chlorophyll loss (LCL_{50}) was derived as quantitative indicator of leaf senescence (Arend *et al.*, 2015).

Statistical analysis

Significance of the treatment effect on photosynthetic traits were tested by the Student's t-test for each beech provenance and time point separately. To test the significance of the treatment effect on autumnal leaf senescence, data were subjected to ANOVA with drought as fixed factor and provenance as random factor. According to the experimental design of the MODOEK facility, drought was considered as nesting factor for provenance. Treatment effects were tested with individual saplings in each treatment as replicate (n > 13) and considered to be significant for $P < 0.05$. All statistical analyses were performed with SPSS 17.0 (IBM Corporation, USA).

Results

Soil water conditions and tree water balances

Withholding natural precipitation and irrigation from the mesocosms resulted in a severe soil drought with soil moisture gradually decreasing from the end of May to early August (Fig. 1). Prior to rewatering in early August, the average soil water content was reduced to 8.2%. These values

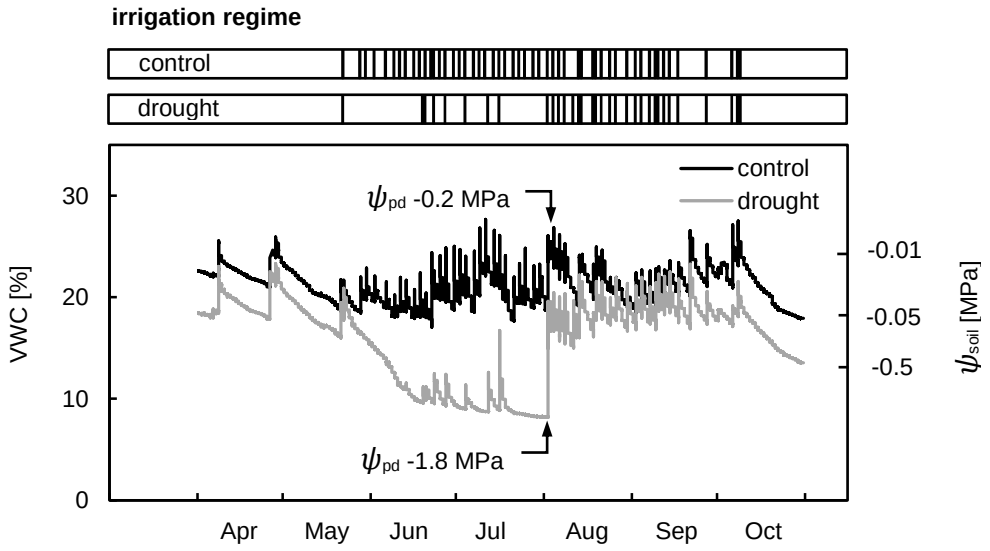

Figure 1. Soil and plant water balances in irrigated and drought-treated mesocosms. Soil moisture was measured in 10 cm soil depth as volumetric soil water content (VWC) and related to soil matrix potential (ψ_{soil}) and pre-dawn leaf water potential (ψ_{pd}, only shown before re-watering). Re-watering of drought-treated mesocosms started on 2nd August. Marks at the top indicate the temporal sequence of single irrigation events in control and drought-treated mesocosms. Soil and leaf data are means with n = 8 and n > 13, respectively.

corresponded to a soil matrix potential distinctly lower than the measurement limit of −0.5 MPa of the pF sensors. In the irrigated control mesocosms, the soil water content ranged between 18.0 and 25% throughout the vegetation period and the soil matrix potential never dropped below −0.05 MPa. After re-watering of the drought-treated mesocosms, soil water content and soil matrix potential increased immediately and reached nearly the level of regularly irrigated control mesocosms. Pre-dawn leaf water potentials were measured as plant based drought indicator prior to re-watering in the two provenances selected for leaf physiological analysis (Mastrils and Saxon; Fig. 1). Average leaf water potentials were −1.8 MPa (SE ±0.1) in drought-exposed trees of both provenances, indicating a severe internal water deficit. In irrigated control trees of both provenances, the average leaf water potentials were −0.2 MPa (SE Mas. ± 0.02/Sax. ± 0.03).

Seasonal drought response of net-photosynthesis

Net-photosynthesis (A_N) showed a distinct seasonal pattern in irrigated trees with high rates from spring to mid-summer and gradually decreasing rates from late summer towards the end of the vegetation period in autumn (Fig. 2A and B). The loss of net-photosynthetic activity coincided with the progression of autumnal leaf senescence and leaf discoloration in late October. A_N was adversely affected by the applied drought treatment resulting in an altered seasonal pattern of leaf physiological activity. The whole seasonal drought response of A_N was divided into three distinct phases: (i) drought limitation from spring to mid-summer, (ii) recovery after drought release in mid-summer, and (iii) post-drought stimulation from late-summer until the end of the vegetation period in autumn (Fig. 2A and B). Drought limitation was a gradual process proceeding along with the slowly decreasing soil moisture and finally resulting in strongly impaired rates of A_N. The two tested provenances showed nearly the same degree of drought limitation except for an earlier response of the wet provenance Mastrils to decreasing soil moisture compared to the xeric provenance Saxon.

A_N responded immediately to re-watering and increased within one day to more than 60% of the level observed in control trees. Full recovery was reached 10 days after re-watering in the xeric provenance Saxon and 20 days in the mesic provenance Mastrils. In the latter, the final step of recovery was strongly delayed as the initially fast increase of A_N slowed down before it reached the level of the respective control trees. After full recovery, A_N remained at high rates until the end of summer in the previously drought exposed trees, while it started to decrease in the control trees. As a result, A_N was relatively increased in the hitherto drought-exposed trees, an effect referred here to as “post-drought stimulation”. This effect was sustained until the end of the

vegetation period in late October when leaves lost physiological activity due to progression of autumnal leaf senescence.

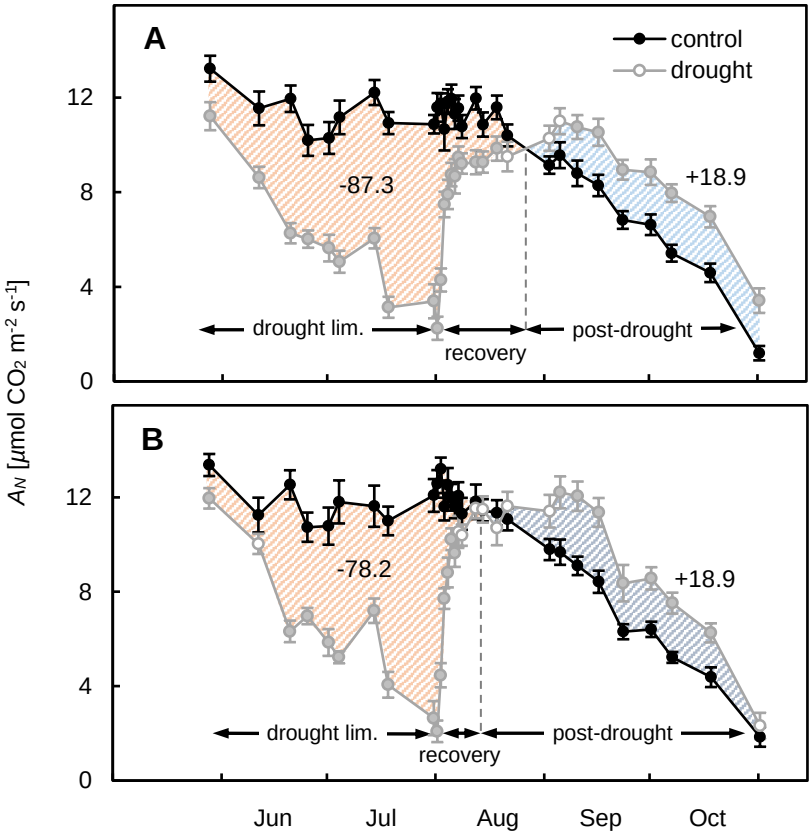

Figure 2. Seasonal course of A_N in control and drought-exposed trees of (A) the mesic provenance *Mastrils* and (B) the xeric provenance *Saxon*. The seasonal response of A_N to the applied drought treatment is divided into the three phases: limitation, recovery and post-drought stimulation. Numerical data in each figure indicate the loss and gain of photosynthetic productivity in drought-exposed trees in relation to control trees as estimated by the cumulated deviation of A_N. Closed grey circles indicate significant differences between control and drought-treated trees. Data are means ± SE with n > 13.

To better compare the different phases of the seasonal drought response in the two provenances, the deviation of A_N in drought-exposed trees from that in control trees was calculated and cumulated for each phase as a quantitative estimate of the photosynthetic response. A negative cumulated deviation was obtained in drought-exposed trees during the phases of drought limitation and recovery, with values of −87.3 and −78.2 µmol CO_2 m^{-2} s^{-1} in the provenance

Mastrils and Saxon, respectively. For the recovery phase alone, the cumulated deviation was −24 µmol CO_2 $m^{-2}s^{-1}$ in the provenance Mastrils and −14 µmol CO_2 m^{-2} s^{-1} in the provenance Saxon. The post-drought stimulation after full recovery resulted in a positive cumulated deviation of A_N that yielded the same value of 18.9 µmol CO_2 m^{-2} s^{-1} in both provenances.

Effects on chlorophyll a fluorescence

Chlorophyll a fluorescence was analysed to detect metabolic limitations of leaf photosynthesis and photochemical stress effects. Maximum quantum efficiency of PSII (F_V/F_M) did not respond to the applied drought treatment although the trees experienced a severe stress situation with water potentials decreasing to −1.8 MPa and strongly impaired rates of A_N. F_V/F_M constantly ranged between 0.7 and 0.8 in control and drought-exposed trees throughout the whole measurement period (data not shown). The total performance index of PSII (PI_{tot}), a fluorescence parameter that

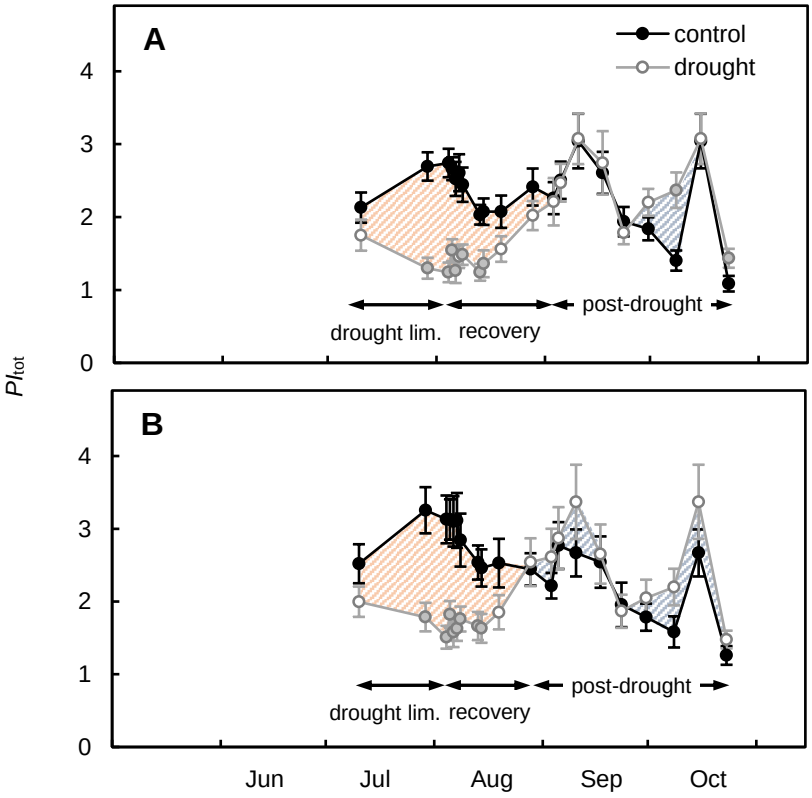

Figure 3. Seasonal changes of chlorophyll *a* fluorescence as indicated by the total performance index of PSII (PI_{tot}) in control and drought-exposed trees of (A) the mesic provenance *Mastrils* and (B) the xeric provenance *Saxon*. The seasonal response of PI_{tot} to the applied drought treatment is divided into the phases limitation and recovery as well as a tendency for a post-drought-stimulation. Closed grey circles indicate significant differences between control and drought-treated trees. Data are means ± SE with n > 13.

integrates light harvesting, electron trapping and energy conversion at PSII, was therefore used as a fluorescence indicator of higher (drought-) stress sensitivity (Strasser *et al.*, 2010; Albert *et al.*, 2011). In contrast to F_V/F_M, PI_{tot} was adversely affected in drought-exposed trees decreasing in both provenances by approx. 50% compared to control trees (Fig. 3A and B). After re-watering, PI_{tot} did not show the same immediate response as observed for A_N. Consequently, PI_{tot} recovered much more slowly reaching the level of control trees only after 3 to 4 weeks. Later in the season, there was an obvious tendency for an increased PI_{tot} in formerly drought-exposed trees, despite the large variability and strong day-to-day fluctuations of this fluorescence parameter. The two tested provenances did not substantially differ in their seasonal drought response, except an earlier recovery of PI_{tot} in the xeric provenance Saxon.

Effects on autumnal leaf senescence

As post-drought stimulation of A_N sustained until the end of the vegetation period, we additionally tested for any effects of the summer drought on autumnal leaf phenology. A first indication for altered autumnal leaf senescence was observed in October when foliage of control trees started

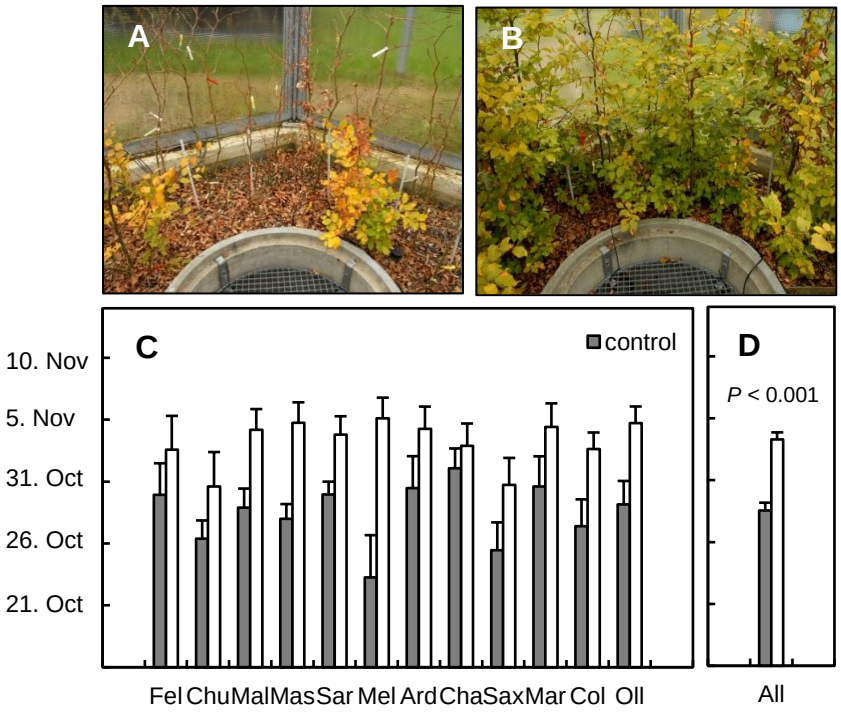

Figure 4. Onset of autumnal leaf senescence in control and formerly drought-exposed trees. Almost completed leaf senescence in (A) control trees and delayed leaf senescence in (B) previously drought-exposed trees at the end of October. Dates of LCL_{50} for (C) each provenance and (D) over all provenances. Data are means ± SE with n = 13 (C) and n > 194 (D).

turning yellow while leaf yellowing started later in hitherto drought-exposed trees. This difference became even more clearly visible when senescing leaves which exceeded the senescence threshold of LCL_{50} (see below) were successively harvested for another study of leaf growth not reported here (Fig. 4A and B). To quantify this difference, the threshold of 50% leaf chlorophyll loss (LCL_{50}) was derived from chlorophyll loss curves and used as a quantitative indicator of autumnal leaf senescence. The onset of LCL_{50} was found to be highly variable among individual trees spanning a time-period from early October to early November. Therefore, additional provenances of the experiment were considered to reduce the uncertainty of statistical analysis (Fig. 4C). All provenances showed a distinct trend for a delayed onset of LCL_{50} in formerly drought-exposed trees, though not all treatment differences were statistically significant. Over all provenances, the onset of LCL_{50} was delayed in formerly drought-exposed trees by 5.7 days (Fig. 4D; $P < 0.001$) compared to the onset of LCL_{50} in control trees.

Discussion

Trees must cope with frequent periods of severe drought during their exceptionally long life time. Responses of trees to drought have therefore been intensively studied at different scales of plant organization and function and a large body of information on the constraining effects of immediate drought has been accumulated over the past decades (Bréda *et al.*, 2006). Much less is known about the recovery from drought and studies dealing with long term effects from previous stresses are lacking. In the present study, we followed the whole seasonal response of European beech (*F. sylvatica*) to severe summer drought and drought release by analysing seasonal sequences of leaf photosynthetic activity in provenances of mesic or xeric origin. Three temporally separated phases of the seasonal photosynthetic drought response could be observed reflecting (i) drought limitation, (ii) drought recovery and (iii) post-drought stimulation.

The limitation of leaf photosynthesis is a common response of plants to drought and has also been reported for European beech (Tognetti *et al.*, 1995; Gallé and Feller, 2007). In our study, however, a severe, long-lasting drought was imposed reducing net-photosynthesis and photochemical reactions for several weeks, thus leading to a substantial loss of overall seasonal photosynthetic activity. This compares well with the situation in field-grown trees where severe summer drought causes long-lasting stress that may alter the carbon balance of forest ecosystems. Indeed, single plant and ecosystem CO_2 assimilation are closely related (Granier *et al.*, 2000) and adverse effects of long-lasting summer drought on the carbon balance of forest ecosystems have been frequently reported (e.g. Ciais *et al.*, 2005; Holst *et al.*, 2008). These studies provided ground-breaking

information about the constraining effect of drought on ecosystem carbon balances but the underlying plant physiological responses are not fully explored. This may explain the uncertainty in modelling approaches which use simplified assumptions about plant responses to drought whereas recovery processes and persistent drought effects are not considered (Verstraeten *et al.*,2006; Verstraeten *et al.*, 2008).

Trees are able to quickly restore substantial rates of photosynthesis after relief from severe drought but full recovery to pre-stress level needs a longer time as some degree of stomatal and/or photochemical limitation may persist (Gallé *et al.*, 2007; Gallé and Feller, 2007; Arend *et al.*, 2013). The underlying mechanisms are not well understood but increased sensitivity of stomata to the stress hormone abscisic acid after drought relief are likely to be involved (Loewenstein and Pallardy, 2002). A lag of full photosynthetic recovery was also observed in the present study that, beyond the immediate effect of drought, contributed to an extended loss of seasonal photosynthetic activity. Interestingly, the xeric provenance Saxon could minimize such additional losses as it reached full recovery much earlier than the mesic provenance Mastrils. This compares well with the observation that forest stands in xeric environments experienced less reduction of net ecosystem productivity in an exceptionally dry summer and restored photosynthetic carbon uptake faster than forest stands in mesic environments (Ciais *et al.*, 2005). Different rates of photosynthetic recovery have also been shown for tree species from xeric and mesic habitats and exclusively attributed to their drought tolerance (Ni and Pallardy,1992).

To date, there has been a general belief that photosynthesis is no longer affected by drought once it has recovered from this limiting stress (Flexas *et al.*, 2006). This is not necessarily true as demonstrated in the present study by the distinct post-drought stimulation of photosynthesis that occurred after recovery in previously stressed trees. As this post-drought stimulation sustained until the end of the vegetation period, the trees were able to compensate for a substantial part of the previous loss of photosynthetic activity. However, further information on respiratory activities is needed before whole plant C balances can be calculated. Also, it has to be proven to what extent the observed post-drought response can be translated to mature trees, although different leaf functioning in young and old trees is rather unlikely.

The mechanisms driving the observed post-drought stimulation of photosynthesis remain speculative but it is reasonable to assume that factors inherent to the tree's leaf phenology play a role. In fact, a leaf has a characteristic seasonal life span of photosynthetic activity after which photosynthetic activity turns down and senescence is initiated even when environmental conditions are still favourable (Quirino *et al.*, 2000; Wilson *et al.*, 2000, 2001; Grassi and Magnani,

2005; Arend *et al.*, 2013). A transient drought limitation of photosynthesis may extend a leaf's life span to compensate the previous loss of photosynthetic activity and thus provide the carbon resources required for re-growth in the following season. The assumption of an altered leaf ontology is consistent with the observation of a delayed onset of autumnal leaf senescence. Although photoperiod and temperature are commonly considered to be the main drivers of this phenological trait, there is still some uncertainty in attempts explaining the onset of autumnal leaf senescence with these environmental factors alone (Estrella and Menzel, 2006). Here, we show the influence of previous drought on this phenological trait. Together with the observed post-drought stimulation of photosynthesis, this may be an important factor to consider when modelling vegetation season length and seasonal ecosystem carbon balances with future climate change.

Conclusion

It was only recently acknowledged in ecological modelling that antecedent environmental conditions might influence and modify the effects of concurrent environmental drivers on various plant and ecosystem processes and that ecological memory might be crucial to explain ecosystem behaviour (Ogle *et al.*, 2015). Both, the observed post-drought stimulation of photosynthesis after drought release as well as the delayed autumn phenology cannot be predicted by any model that does not take into account such drought memory effects but relies on direct driver-response relationships. For a first attempt to incorporate the effects of previous drought into stochastic models it will be required to more exactly quantify the length and the strength of such memory effects as outlined by Ogle *et al.* (2015). Before mechanistic models can be extended with memory effects, a better understanding is needed on how the antecedent information is relayed.

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Chapter 3: Resilient leaf physiological response of European beech (Fagus sylvatica L.) to summer drought and drought release

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Abstract

Drought is a major environmental constraint to trees, causing severe stresses and thus adversely affecting their functional integrity. European beech (*Fagus sylvatica* L.) is a dominant, but less drought-tolerant tree species in mesic temperate forests that is commonly thought to suffer from a future climate with more intense and frequent drought events. Here, we show the seasonal response of leaf physiological characteristics in young beech trees to a simulated severe summer drought and drought release to investigate their potential to overcome the induced stress. Water potentials (ψ_{pd}), net photosynthesis (A_N), PSII chlorophyll fluorescence (PI_{tot}), non-structural carbohydrates (NSC) and carbon isotope signatures were measured throughout an entire growing season. ψ_{pd}, A_N and PI_{tot} decreased as drought progressed and the content of soluble sugars increased at the expense of starch. Carbon isotopes in soluble sugars ($\delta^{13}C_S$) showed a distinct drought signal while this isotopic signal was only weak in leaf bulk tissue ($\delta^{13}C_L$). Drought effects on leaf physiological characteristics disappeared shortly after re-watering, though full recovery of A_N and PI_{tot} was delayed compared to the very fast recovery of ψ_{pd} and NSC concentrations. The latter was also reflected by a rapid decay of the drought signal in $\delta^{13}C_S$, indicating a rapid turnover of assimilates and thus recovery of leaf metabolism. Furthermore, after recovery, the previously drought exposed trees showed a trend towards a stimulation of leaf physiological activity as shown by slightly increased A_N and NSC concentrations. Altogether, our results show that beech restores full leaf physiological activity shortly after drought release and overcomes the previous stress impact without persistent leaf damages, indicating a high resilience to severe summer drought.

Introduction

Climate models predict an increase in the annual temperature for Central Europe by 2.7 to 4°C and a decrease in summer precipitation by 21 to 28% towards the end of the 21st century (CH2011, 2011; IPCC, 2012). Additionally, climate variability is expected to increase, resulting in a higher frequency and intensity of extreme weather events, such as severe droughts, heavy rains and extraordinary cold or heat waves (Schär *et al*., 2004). This change in climate conditions may have a large impact on the physiological constitution of forest trees, thereby changing the productivity and composition of forest ecosystems (Ciais *et al*., 2005; Milad *et al*., 2010; Hanewinkel *et al*., 2012). Tree species being less tolerant to drought will face a disproportionate risk of habitat loss due to impaired competitiveness compared to drought tolerant tree species (Ohlemüller *et al*.,

2006; Czúcz *et al*., 2011). This may particularly apply to European beech (*Fagus sylvatica* L.), a main tree species in Central European forests, which is thought to be severely threatened by increasing drought (Rennenberg *et al*., 2004; Gessler *et al*., 2007; Kramer *et al*., 2010). There is, however, an ongoing controversial debate among sylviculturists and tree biologists about the consequences of climate change for European beech (Ammer *et al*., 2005; Schütz, 2009).

The responses of European beech and other tree species to drought have intensively been studied in the past. A considerable number of these studies revealed the influence of acute drought on various processes, such as photosynthesis, phenological development, wood growth, hydraulic architecture and metabolism (e.g. Tognetti *et al*., 1995; Leuschner *et al*., 2001; Peuke *et al*., 2002; Leuzinger *et al*., 2005; Nahm *et al*., 2006; Weber *et al*. 2013, Aranda *et al*., 2015; Pflug *et al*., 2015; Arend *et al*., 2016b). Considered as a moderate anisohydric species, beech has the ability to adjust stomatal conductance (g_s) and therefore optimise photosynthetic assimilation rates (A_N) under optimal or mild drought conditions (Pretsch *et al*. 2012, Peuke *et al*., 2002, Tardieu and Simonneau, 1998), while this can be a disadvantageous response under severe drought. The latter may lead to a dysfunction of the hydraulic system followed by increasing cellular water loss and damages of metabolically active tree organs. The critical cavitation resistance of beech, where the risk of irreversible hydraulic dysfunction occurs, is comparable to that of other co-occurring tree species and thus does not support an exceptional sensitivity of European beech to drought (-2.8 to -3.2 MPa; Wortemann *et al*., 2011; Gleason *et al*., 2016; Choat *et al*., 2012). Dendroecological studies, however, reveal a high sensitivity to soil water shortage (Dittmar *et al*., 2003; Gessler *et al*., 2004), which is in line with the ecological preferences of beech for mesic soils with sufficient water supply (Peters, 1997; Ellenberg, 2009) and the general view of beech as a drought sensitive tree species.

In contrast to the vast number of studies reporting instant tree responses to drought, only little is known about the recovery from this environmental stress, although this ability might be an important factor determining the tree's drought resistance on a longer time scale (Blessing *et al*., 2016). A few studies dealt with potted seedlings exposed to cycles of drought and re-watering (Gallé and Feller, 2007; Tognetti *et al*., 1995; Blessing *et al*., 2016), while others focused on the year to year variations of tree-ring growth in adult trees caused by annual fluctuations of precipitation (Dittmar *et al*., 2003; Pretzsch and Dieler, 2011). Even though these authors showed the ability of beech to resume physiological activity and growth after severe drought, they do not provide detailed information about the recovery process. In a previous study, Zang *et al*. (2014a) assessed dynamics and patterns of C allocation in beech saplings under dry and rewetted soil

conditions using a ^{13}C pulse labelling approach. This pot experiment showed not only that beech saplings recovered quickly from severe drought but also provided first insights into the different use of recent photoassimilates under severe drought and shortly after rewetting. Furthermore, recent studies reported on the direct coupling of root respiration and recently fixed photoassimilates during drought and drought release (Blessing *et al*., 2016; Hagedorn *et al*. 2016). However, experiments following drought - rewetting responses over the time scale of an entire growing season are scarce, though seasonal aspects of drought development must not be ignored (Arend *et al*., 2013; 2016a). In fact, recent studies show that trees not only recover but even increase physiological activity to counterbalance the limitations of previous drought (Hagedorn *et al*. 2016).

In this study, we followed the response of leaf physiological characteristics in young beech trees throughout the entire growing season to a severe summer drought and drought release. For this purpose, we subjected young beech trees growing in large lysimeter-based plots to a slowly developing soil water shortage and subsequent re-watering. Physiological changes were followed in leaves by weekly to biweekly measurements of leaf water potential (ψ_{pd}), net photosynthesis (A_N) and PSII fluorescence (F_V/F_M; PI_{tot}) together with an analysis of non-structural carbohydrates (NSC; soluble sugars and starch) and the isotopic composition in bulk leaf material ($\delta^{13}C_L$) and soluble sugars ($\delta^{13}C_S$). With this experimental setup, we aimed at tracking the temporal course of the drought response and the recovery process after drought release. We specifically addressed the following questions: (i) At which drought level (ψ_{pd}) is a drought response detectable? (ii) How fast do beech trees recover after drought release? and (iii) Is a long-lasting drought effect imprinted on leaf physiology?

Material and methods

Experimental design and plant material

The present study was carried out in the framework of the "BuKlim" project (detailed information is given in Arend *et al*., 2016b). In brief, beech saplings with a height of approx. 20 cm were selected in mesic and xeric forest stands of two Swiss inner-alpine valleys and transplanted to the plots of the outdoor model ecosystem facility (MODOEK) in 2011. The facility is located at the Swiss Federal Research Institute WSL, Birmensdorf, Switzerland (47°21' N, 8°27' E, 545 m a.s.l.). The plots of the MODOEK facility have a depth of 100 cm and are filled with natural forest soil (acidic Haplic Alisol; loamy sand; pH 4.6). The water regime in the plots is controlled by sliding glass roofs, closing automatically at the onset of rain fall and an automated irrigation system. In

2013, a summer drought was imposed in half of the plots by omitting the irrigation from June to mid-August, while the other half was regularly irrigated. After ten weeks, when the average ψ_{pd} in drought-treated plots reached -1.8 MPa, the plots were intensely re-watered and afterwards regularly irrigated. For this experiment, six control and six drought-treated saplings of a mesic stand in the Rhone valley (Collombey-Muraz, Valais, Switzerland, 46°16´ N 6°56´ E) were selected. Annual mean temperature at the origin site is 8.9 °C, with an average annual precipitation of 1,055 mm. The site is N-NO facing with a slope of 10-35 %.

Measurements of soil and tree water relations and sampling of leaf material

Soil water content (SWC) was measured volumetrically at 30 cm soil depth using PC-controlled soil moisture probes (Decagon 5TM; Decagon, USA). Leaf material was collected during the growing season from end of May until beginning of October in (bi-)weekly intervals. To measure pre-dawn leaf water potential (ψ_{pd}), leaves were sampled before sunrise between 4h00 and 6h00 (UTC+2). On each sampling date, four leaves of the outer crown were harvested per tree. ψ_{pd} was determined on these leaves using a Scholander bomb (M 600; Mosler Tech Support, Berlin, Germany). Afterwards, the same leaves were shortly microwaved to stop any metabolic activity. Leaves were oven-dried at 60 °C for 48 hours and milled using a ball mill (MM400, Retsch, Haan, Germany), before subjected to NSC and isotope analysis.

Measurements of PSII photochemistry and leaf net photosynthesis

Fast fluorescence kinetics were measured predawn using a plant efficiency analyser (Pocket PEA, Hansatech Instruments Ltd., Norfolk, UK). These fluorescence measurements were conducted on each sampling date prior to the analysis of ψ_{pd} in the dark-adapted state. After a saturating light pulse of 3,500 μmol quanta m^{-2} sec^{-1} of red light (650 nm), fluorescence rise was recorded in a high resolution for one second. Maximum quantum efficiency of photosystem II (F_v/F_m) and the total Performance Index (PI_{tot}) were calculated using PEA plus 1.10 (Hansatech Instruments Ltd., Norfolk, UK). On every sampling date, leaf net photosynthesis (A_N) of four sun-exposed leaves per beech sapling were measured between 11h00 and 16h00. Measurements were performed with a portable photosynthesis system using a broadleaf cuvette (LI-COR 6400; LI-COR, Lincoln, NE, USA). Conditions in the cuvette were kept constant during the measurements at 400 ppm [CO_2] and a photon flux density of 1,000 μmol m^{-2} s^{-1}, while the temperature was aligned close to outside conditions.

Quantification of non-structural carbohydrates

NSC are defined as starch and mobile low molecular weight sugars (glucose, fructose and sucrose, in the following referred to as soluble sugars). NSC were extracted according to Critchley *et al.* (2001). Approximately 100 mg of powdered leaf material was incubated for 15 min with 1.12 M perchloric acid and then centrifuged at 3000 g for 15 min. The supernatant used for quantification of soluble sugars was adjusted to pH 6 by the addition 2M KOH, 0.4 M MES and 4 M KCl. Further, the precipitated potassium perchlorate was removed by centrifugation at 3000 g for 15 min. Sucrose in the extract was broken down to glucose and fructose by Invertase (Roche, Rotkreuz, Switzerland). Free glucose and glucose originating from sucrose were then converted to gluconate-6-phostphat by Glucose-6-Phosphate Dehydrogenase (Roche, Rotkreuz, Switzerland) and determined photometrically with a 96-well microplate reader (ELx800, BioTek, Luzern, Switzerland). Afterwards, fructose was converted to glucose by phosphogluco-isomerase (Roche, Rotkreuz, Switzerland), and then measured as described above. For starch quantification, the remaining pellet was thoroughly washed with 80% EtOH, dried at room temperature and resuspended in water before starch was broken down to glucose monomers via Amyloglucosidase and α-Amylase for 2 h at 37 °C (both Roche, Rotkreuz, Switzerland) and then determined photometrically as described above. Photometric quantification was performed according to Hoch *et al.* (2003). The NSC concentrations are expressed on dry matter basis.

Extraction of soluble sugars for $\delta^{13}C$ analysis

Soluble sugars for $\delta^{13}C$ analysis were extracted and prepared according to the method of Wanek *et al*. (2001) as modified by Göttlicher *et al*. (2006) and Richter *et al*. (2009). Briefly, ground leaf material (100 mg) was extracted with 1 mL MCW (methanol, chloroform, water, 12:3:5, v/v/v) for 30 min at 70 °C. After cooling to room temperature, the sample was centrifuged at 10,000 g for 2 min, and an aliquot of the supernatant (800 μL) was used for the extraction of soluble sugars. Phase separation of the supernatant was induced by the additions of 800 μL water and 250 μL MWC and vigorously mixing. For every six samples, one blank was processed (800 μL MCW). After centrifugation at 10,000 g for 2 min, an aliquot of the upper aqueous phase (1.2 mL) was mixed with 500 μL chloroform and centrifuged for phase separation. Then, 1 mL of the upper phase was oven-dried for 24 h at 60 °C. The sample was re-dissolved in 1 mL water and separated using an ion-exchange cartridge (5 mL syringes, BD Plastipak™, Beckon Dickinson S.A., Madrid, Spain) made of 2.2 mL cation-exchange resin (DOWEX 50W X 8, 50–100 mesh, H^+-form) above 3.2 mL anion-exchange resin (DOWEX 1 X 8, 50–100 mesh, HCOO-form), separated by filter paper (Wattmann). After rinsing the columns with 30 mL water, 35 mL of the eluate, mainly consisting of soluble

sugars, was collected. The samples were lyophilised, and re-dissolved in 1 mL water. Volumes of 150 μL of each sample were pipetted into tin capsules, and oven-dried for 48 h at 60 °C before being subjected to isotopic analysis.

Measurement of $\delta^{13}C$ in leaf bulk tissue and soluble sugars

For the analysis of $\delta^{13}C$ in leaves, about 0.5 mg of the powdered leaf material or extracted leaf sugars was weighed into tin capsules (Säntis Analytical, Teufen, Switzerland). The samples were combusted to CO_2 with excess of oxygen at 1,020 °C in an elemental analyser (EA-1110, Carlo Erba Thermoquest, Milan, Italy), which was connected to a Delta S mass spectrometer with a CONFLO II (both Finnigan MAT, Bremen, Germany), performing in continuous flow mode. Isotope ratio of $\delta^{13}C$ is given in reference to its international standard Vienna Pee Dee Belemnite (VPDB) in the delta notation in ‰: $\delta_{sample} = (R_{sample}/R_{standard} -1)$, with R_{sample} being the $^{13}C/^{12}C$ ratio of the sample, and $R_{standard}$ being the ratio of VPDB. The standard deviation of laboratory cellulose standards was used as an estimation of analysis precision, and lower than 0.10‰.

Statistical analysis

Statistical analyses were executed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). In order to evaluate differences among the treated and the control saplings, a nonparametric test of variance (Mann-Whitney U test) was applied. The groups were considered significant with $P \leq 0.05$. The statistical tests were performed with three to six replicates per treatment group.

Results

Soil and tree water relations

Irrigation was prevented from the plots for 10 weeks from June to mid-August (Fig. 1A, B). Soil water content in irrigated control plots ranged from 24 to 29% throughout the entire growing season of 2013 (Fig. 1C).

In drought-treated plots, it ranged from 18 to 25% during the two irrigation periods before and after drought induction, but decreased gradually to 12% during the drought period without irrigation. Trees in control plots maintained pre-dawn leaf water potentials (ψ_{pd}) above -0.5 MPa, except at the end of the growing season in October, when leaf senescence set in and ψ_{pd} decreased to values below -1.0 MPa (Fig. 1D). For the drought exposed trees, ψ_{pd} started to decrease in mid-July, seven weeks after withholding irrigation, and reached a minimum average value of -1.8 MPa at the end of the drought period in mid-August. During re-watering, ψ_{pd}

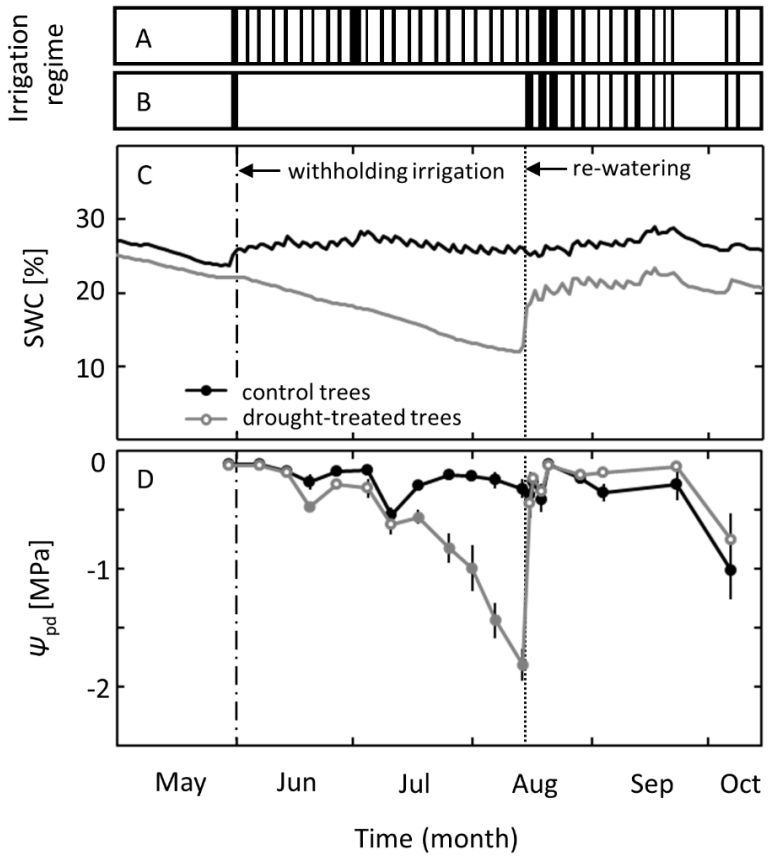

Figure 1. Irrigation regime and effects on soil and tree water relations in 2013. Timing of the irrigation events in (A) the control and (B) drought treatment. (C) Soil water content (SWC) in a depth of 30 cm and (D) pre-dawn leaf water potential (ψ_{pd}) in control and drought-treated plots. Filled grey circles indicate significant differences between the two treatments ($P \leq 0.05$). For the drought treatment, the dash-dotted and the dotted line give the timing of withholding irrigation and the re-watering event, respectively. Data are means of n = 6 ± SE.

increased within one day to the level of trees in the control plots, and reached somewhat higher ψ_{pd} values than the control trees (Fig. 1D).

Net photosynthesis and PSII photochemistry

Instantaneous net photosynthesis (A_N) showed a distinct seasonal pattern, with high rates during summer and lower rates in spring and autumn (Fig. 2). A_N in drought-exposed trees was comparable to that in control trees as long as ψ_{pd} remained above -0.6 MPa. However, when ψ_{pd} dropped below this critical value in mid-July, A_N started to decrease and was reduced by up to 60% at the end of the drought season in mid-August (Fig. 2). A_N responded fast to re-watering, reaching

85% of the photosynthetic rate in control trees after four days and recovered completely within two weeks after drought release. Afterwards, previously drought-exposed trees showed higher A_N compared to control trees, with a significant post-drought stimulation towards the end of September and the beginning of October. The relative limitation of A_N in drought treated trees, integrated over the entire drought and the subsequent recovery period, accounted for 33% and the post-drought stimulation after full recovery was 16% (Fig. 2).

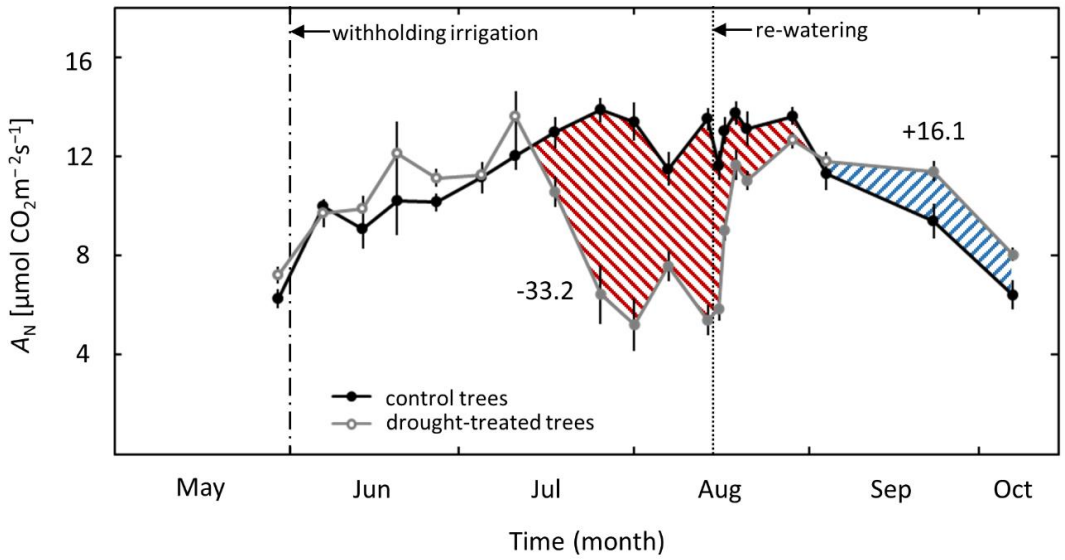

Figure 2. Seasonal course of net-photosynthesis (A_N) in 2013 in control and drought-treated beech trees (*F. sylvatica*). Numerical data indicate the integrated limitation and surplus of A_N (red and blue shaded areas, respectively). Filled grey circles indicate significant differences between the both groups ($P \leq 0.05$). For the drought treatment, the dash-dotted and the dotted line give the timing of withholding irrigation and the re-watering event, respectively. Data are means of n = 6 ± SE.

During the vegetation period from June to October, predawn maximum quantum yield of PSII (F_v/F_m) did not show any seasonal variation or response to the drought treatment (Fig. 3A). In contrast, the total performance index of PSII (PI_{tot}) showed a clear seasonal pattern (Fig. 3B). Significant differences between control and drought exposed trees were first detected at the beginning of August, when ψ_{pd} reached values of -1.44 MPa three weeks after first drought effects on A_N were observed. Recovery of PI_{tot} was completed three weeks after the re-watering, and

persisted one week longer than full recovery of A_N. Overall, the drought exposed trees showed a 35% reduction in PI_{tot} (Fig. 3B).

Concentrations of Non-Structural Carbohydrates (NSCs)

Levels of NSC were measured in leaves harvested pre-dawn. In control trees, the concentrations of sugars ranged from 10 to 25 μg mg^{-1} dry weight during the whole growing season, with no clear seasonal trend (Fig. 4A). The concentrations of starch and the total NSC level ranged from 6 to 35 μg mg^{-1} dry weight and 25 to 58 μg mg^{-1} dry weight, respectively, during the whole growing season, with a gradual decline from June to October (Fig. 4B, C). While total NSCs were not affected by the

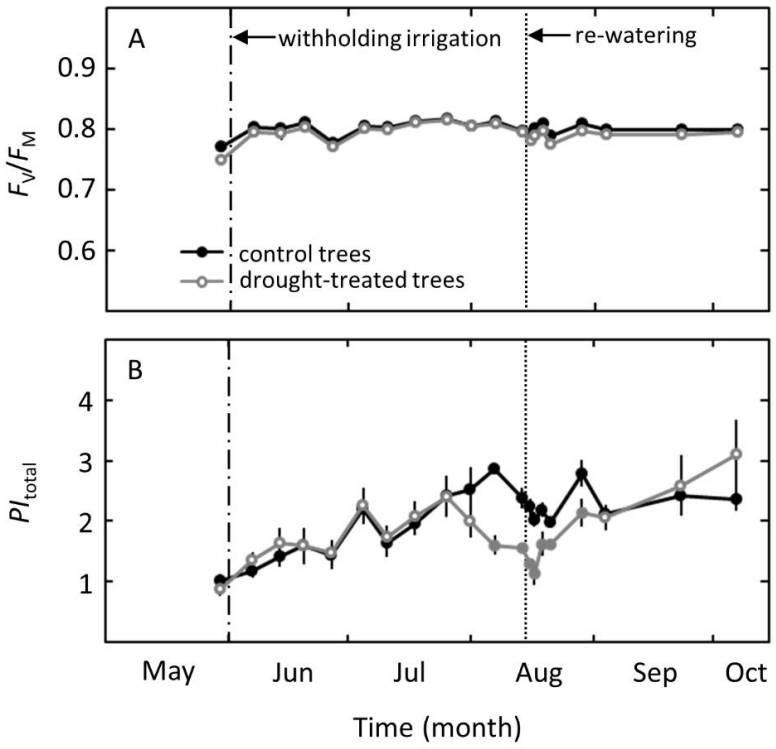

Figure 3. Seasonal course of pre-dawn PSII Chlorophyll Fluorescence parameters in young beech trees in 2013. A Seasonal course of maximum quantum yield (*F*v/*F*m) and (B) of total performance index (PI_{tot}) in control and drought-treated trees. Filled grey circles indicate significant differences between the two treatments ($P \leq 0.05$). For the drought treatment, the dash-dotted and the dotted line give the timing of withholding irrigation and the re-watering event, respectively. Data are means of n = 6 ± SE.

induced drought period, starch as well as soluble sugars showed a distinct drought response (Fig. 4).

Starch concentrations in leaves of drought-exposed trees decreased when ψ_{pd} dropped below -0.8 MPa in the end of July, one week after ψ_{pd} and A_N showed the first drought response. After re-watering in mid-August, the concentrations of starch increased within one day to the level of control trees. After full recovery, the previously drought-treated trees showed slightly higher starch concentrations than control trees, though this difference was not significant (Fig. 4B). Sugars showed an inverted pattern, increasing with the extending drought period compared to control trees (Fig. 4A). First effects on sugars were observed when ψ_{pd} reached a value of -1.0 MPa. Full recovery was completed within two days after drought release, and no post-drought stimulation was observed.

Isotope analysis

In $\delta^{13}C_L$, neither a marked seasonal variation nor a clear drought response was detected (Fig. 5A). For both treatments, $\delta^{13}C_L$ was around -30‰ in the beginning of June and decreased gradually to -31‰ in early October. Except for one measuring point in mid-August, four weeks after first drought effects on ψ_{pd} and A_N were detected, no differences between the treatments were found.

In contrast to $\delta^{13}C_L$, a strong drought-induced increase of 3.5‰ was observed in $\delta^{13}C_S$, when ψ_{pd} reached values of -0.8 MPa (Fig. 5B). $\delta^{13}C_S$ in control trees decreased gradually from -27‰ in June to -30‰ in October. In drought exposed trees, $\delta^{13}C_S$ increased with decreasing ψ_{pd} to reach a maximum value of -26‰ in mid-August, four weeks after the first drought effects were observed and ψ_{pd} was -1.8 MPa. The drought effect on $\delta^{13}C_S$ vanished within one day after the re-watering was induced.

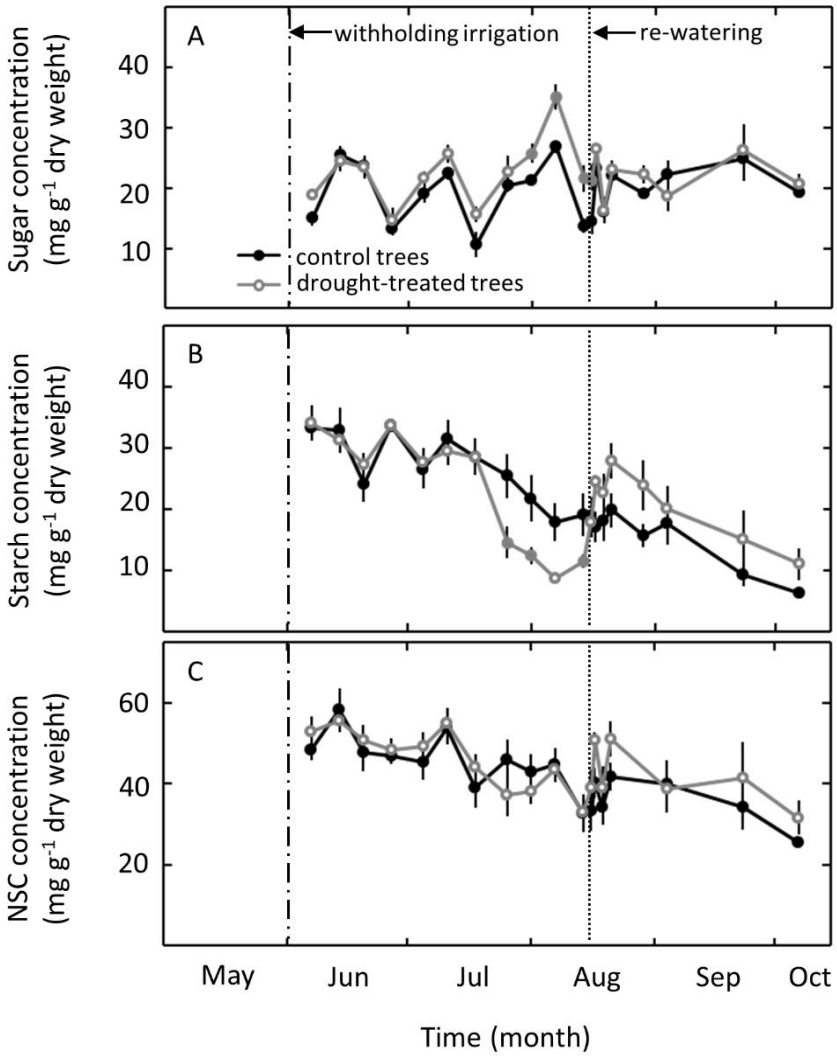

Figure 4. Seasonal course of (A) total concentrations of non-structural carbohydrates, (B) starch and (C) soluble sugars in leaves of control and drought-treated trees in 2013. Filled grey circles indicate significant differences between the two treatments ($P \leq 0.05$). For the drought treatment, the dash-dotted and the dotted line give the timing of withholding irrigation and the re-watering event, respectively. Data are means of n ≥ 5 ± SE, starch content: 7. Aug n= 3 ± SE.

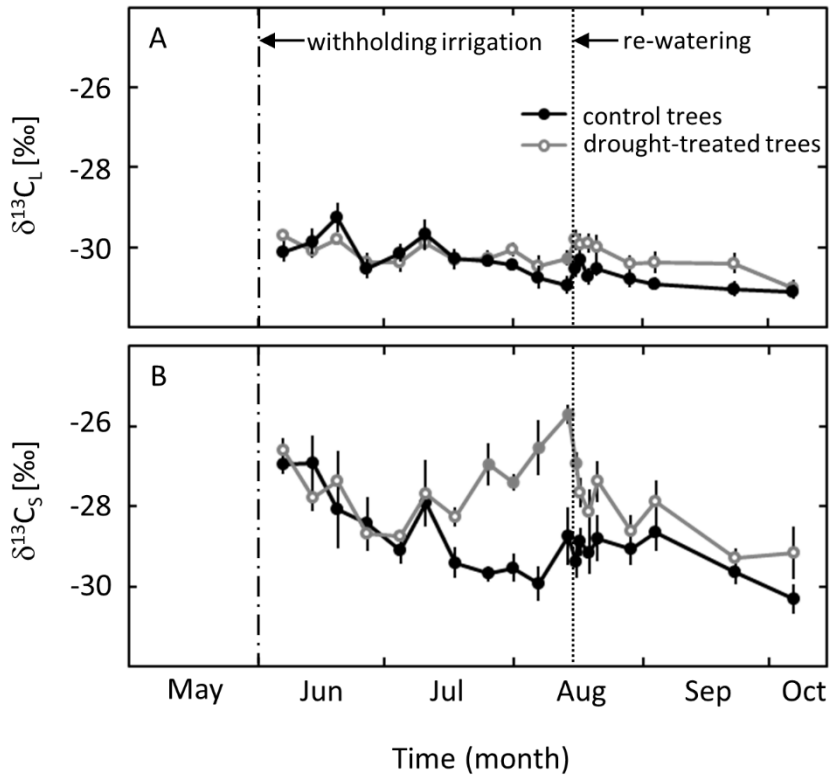

Figure 5. Seasonal course of $\delta^{13}C$ in (A) leaf bulk tissue and (B) leaf soluble sugars in control and drought-treated trees in 2013. Filled grey circles indicate significant differences between the two treatments ($P \leq 0.05$). For the drought treatment, the dash-dotted and the dotted line give the timing of withholding irrigation and the re-watering event, respectively. Data are means of n = 6 ± SE, except in B: 01. & 07. Aug n = 4 ± SE.

Discussion

Increasing drought frequency and duration in the next decade will have implications on tree competitiveness and survival. While instant drought effects on trees are fairly well understood, there is, however, a lack of knowledge on responses to drought on a longer time scale, including the recovery process after drought release and later potential post-drought effects. In this study, we aimed at investigating the entire seasonal response of leaf physiological characteristics in young beech trees (*F. sylvatica*) to a prolonged summer drought as well as to drought release.

Response to drought

The drought imposed on the young beech trees induced a well described drought response, with decreasing pre-dawn leaf water potential (ψ_{pd}), and a decrease in net photosynthesis (A_N) (Fig. 6; Tognetti *et al*., 1995; Gallé and Feller, 2007; Zang *et al*., 2014b). A_N started to decrease when ψ_{pd} dropped below -0.6 MPa. This critical value is in line with other observations on young, naturally grown beech trees under moderate drought conditions (Aranda *et al*., 2012; Cocozza *et al*. 2016). At the same time when A_N started to decrease, $\delta^{13}C_S$ revealed a strong drought mediated increase in treated beech saplings, which could either indicate stomatal or photosynthetic limitations (Farquhar *et al*., 1989). However, photosynthetic limitations are less probable in our study, as the increase in sugar concentrations does not indicate photosynthetic impairment. Soil water restriction often cause a decrease in stomatal conductance to protect plants from strong dehydration under water limiting conditions (Gallé and Feller, 2007; Peuke *et al*., 2006; Blessing *et al*., 2016). It is, therefore, likely, that the increase in $\delta^{13}C_S$ is mainly due to a decrease in stomatal conductance (Grassi and Magnani, 2005). However, concurrently measured total Performance index of PSII (PI_{tot}), a derived fluorescence parameter integrating the sum of changes in quantum yields and absorption (Strasser *et al*., 2010; Albert *et al*., 2011), indicated impaired PSII photochemistry under severe drought conditions and thus metabolic limitation of photosynthesis after a prolonged, water limiting stress. Anyhow, first drought effects on PI_{tot} were observed when ψ_{pd} dropped below -1.44 MPa and A_N was already impaired for three weeks. We therefore assume mixed drought resistance strategies with fast to slow stomatal closure in response to soil drought and metabolic limitation only at very low ψ_{pd}.

A further explanation for the higher $\delta^{13}C_S$ signal in treated trees is the use of different substrates for the synthesis of low molecular weight sugars, required for the maintenance of metabolic activity. While transitory starch synthesized in the chloroplasts is enriched in ^{13}C, cytosolic synthesized sucrose is ^{13}C depleted due to aldolase and transketolase activity in the Calvin cycle (Rossmann *et al*., 1991; Gleixner and Schmidt, 1997). Under conditions, where photosynthesis is constrained, e.g. during severe drought, a lack in newly produced sugars might lead to higher consumption of stored starch to maintain metabolic activity of the plants, which consequently would lead to a higher $\delta^{13}C$ value in the measured sugars. Indeed, starch concentrations in leaves of drought-exposed trees started to decrease at the same time as the first increase in $\delta^{13}C_S$ was detected, indicating altered starch metabolism. In contrast to $\delta^{13}C_S$, $\delta^{13}C_L$was less affected by the drought treatment. However, leaf bulk material reflects mainly the carbon pool, including structural carbon (i.e. cell walls), fixed before the drought became effective for the plants.

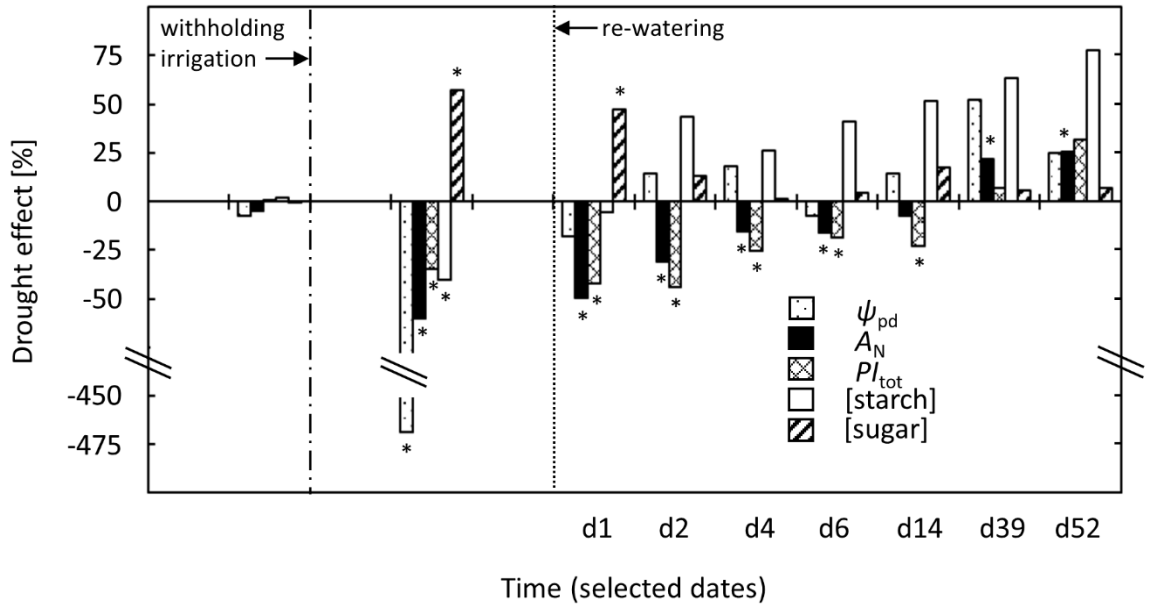

Figure 6. Comparison of drought-induced effects on pre-dawn leaf water potential (ψ_{pd}), net photosynthesis (A_N), total performance index of PSII (PI_{tot}), and concentration of starch ([starch]) and sugars ([sugar]). Effects are shown for pre-drought, drought, and re-watering conditions. For re-watering conditions, specific days after the first rewatering event (d1, d2, …, d52) were selected. Asterisk indicate significant differences between the two treatments ($P \leq 0.05$). For the drought treatment, the dash-dotted and the dotted line give the timing of withholding irrigation and the re-watering event, respectively.

Recovery after water limiting stress

An important part of the drought tolerance of trees is the ability to recover from limiting water stress (Blessing *et al*., 2016). Here, the fast recovery of plant water status, the immediate increase in starch content as a measure of available carbohydrates in leaves and the fast decay of the drought signal in $\delta^{13}C_S$ within one day indicated a prompt response of the beech saplings to re-watering (Fig. 6). However, the recovery of A_N was delayed, reaching 85% of control level after four days, and recovered completely within two weeks. Likewise, the recovery of PI_{tot} was delayed, reaching control levels two weeks after the re-watering event. This indicates some persisting stomatal or metabolic limitation of the photosystem after re-watering, which might be explained by drought-induced changes at the chlorophyll or mesophyll level (Flexas *et al*., 2004; Gallé and Fellner, 2007; Arend *et al*., 2016a). However, other studies revealed different time scales for the recovery for A_N in young beech trees. While Blessing *et al*. (2016) and Zang *et al*. (2014a) found A_N to be recovered within three days, in a study by Gallé and Fellner, (2007), a recovery period of 4

weeks was observed. These differences might be explained by different stress intensities, plant development and tree age. However, studies dealing with the recovery of trees are scarce, and generally, drought experiments are difficult to compare, owing to the heterogeneity of drought stress quantification and experimental conditions (Miyashita *et al*., 2005; Vicca *et al*., 2012). Taken together, ψ_{pd}, $\delta^{13}C_S$ and starch concentrations recovered within one day, the recovery of sugar concentrations took two days, and all measured variables reached control levels within two weeks after the rewatering event. As no permanent restriction of stomatal or metabolic processes could be observed after full recovery of A_N and PI_{tot}, we suggest a high resilience of beech to drought.

Additionally, at a longer time scale, not only the instant drought response and the subsequent recovery are crucial determinants of trees resistance strategies, but also potential post-drought effects (Walter *et al*., 2012; Arend *et al*., 2016b). Yet, we detected a post-drought stimulation on A_N, counteracting the previous drought limitation. Compared to control, A_N increased in former drought-treated trees, starting at the same time PI_{tot} had completely recovered. This is in line with recent findings, where a post-drought stimulation of A_N was observed in beech (Arend *et al*., 2016b; Hagedorn *et al*., 2016). Together with slightly increased starch content in leaves (although not significant, $P = 0.102$), these observations indicate an improved ability of these beech trees to overcome drought induced limitations. However, multiple stresses like a combination of late frost, mast fruiting and early and long lasting drought might significantly impact the here observed effects.

Conclusion

In the present study, we could highlight the sensitivity of beech to water limiting conditions. Additionally, the observed fast recovery from drought and the slight stimulation of leaf metabolic activity after release of the limiting stress points to a high resilience of these young beech trees to summer drought. The observed resilience might be an important factor determining beech's competitiveness under future climate conditions, and should be included in the recent debate about the consequences of climate change on beech. To date, data on recovery and resilience are scarce for most of the important tree species of European forests, even though this information is crucial for the reliable modelling of forest responses to changing environmental conditions. Further information on recovery and resilience of European tree species are needed, to identify suitable tree species for forestry under future climate constraints in Europe.

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Synthesis

Drought is thought to be the major constraint for European forests in the future, and an increase in the frequency and intensity of drought is likely to change tree productivity and species distribution. The species-specific response of trees to drought is not only determined by the ability of the trees to endure periods of drought but also by their potential to recover on the long term and overcome previous limitations. To cover these species-specific responses, and potential temporal changes, we analysed the whole seasonal drought response of three European trees species at different levels of plant organisation and functioning, i.e. leaves and tree rings. Here, I provide a summary of the key findings of this thesis, but also discuss methodological aspects and provide recommendations for future research.

In the *first chapter,* new insights into the isotopic response in leaves and tree rings of young oak trees (*Quercus robur* and *Q. petraea*) to severe summer drought are presented. The response of trees to drought, air warming and their combination was assessed in model ecosystems under semi-natural conditions over a period of three years. Patterns of stable carbon and oxygen isotopes ($\delta^{13}C$ and $\delta^{18}O$) in leaves and tree-ring cellulose were linked to net-photosynthesis (A_N), intra-annual tree-ring growth and $\delta^{18}O$ in drainage water to analyse the influence of the climatic constraints on the physiology of these oak species. It was also tested if the dual isotope approach according to Scheidegger *et al*. (2000) can also be used to infer physiological responses to drought from the obtained ^{13}C and ^{18}O patterns in tree-rings. However, we found that under strong drought conditions, when tree ring growth is inhibited, the dual isotope approach failed to explain physiological behaviour with the tree rings.

No effect of warming was detected in the $\delta^{13}C$ and $\delta^{18}O$ of leaves and tree rings during the three consecutive years investigated, indicating a high thermo-tolerance of both oak species. It is worth noting that the combined treatment of air warming and drought did not impose an additional effect on the isotopic composition of leaves or tree rings. This contradicts the assumption that warming increases the drought effect on trees due to increased evapotranspiration, and that drought enhances warming effects due to decreased evapotranspirative cooling.

Moderate drought in the first experimental year had no effect on carbon and oxygen isotopes neither in leaves nor in tree rings, which is in line with other experiments analysing the impact of moderate drought on oak species (Zang *et al*., 2012; Thomas and Gausling, 2000). These findings support the assumption that moderate drought has only a minor impact on the physiology of gas

exchange in *Q. robur* and *Q. petraea*. However, although the analysis of isotopes in this study gives an integrated picture of the seasonal response, as the isotopic signatures in leaves and tree rings were only determined once at the end of the season. Thus, minor, intermediate changes in physiology were not detectable. When drought levels were increased in the second and third years, a clear effect on $\delta^{13}C$ in leaves as well as in tree rings for both oak species was observed. In leaves, the dual isotope approach was conclusive, suggesting a decrease in net-assimilation, mainly due to stomatal closure rather than to a decrease in photosynthetic capacity (Scheidegger *et al*., 2000, Farquhar *et al*., 1982).

It is assumed that effects of drought on photosynthetic gas exchange become permanently recorded in tree rings when leaf sugars are translocated to the stem and incorporated in the cellulose matrix of the tree ring (West *et al*., 2005). In the presented study, the observed signals in $\delta^{13}C$ and $\delta^{18}O$ in tree rings were very similar to the signals observed in leaves as long as tree-ring growth of the trees was not affected. We did not observe an uneven dampening of the $\delta^{13}C$ and $\delta^{18}O$ signals on the way from leaves to tree rings due to xylem water exchange and dilution by stored carbohydrates as suggested by Roden and Siegwolf (2012), which would change the directional relationship of the two isotopes. However, depending on severity and duration of the drought, the conceptual dual isotope approach fails to explain the isotopic pattern observed in tree rings. Under severe drought, when tree-ring growth was constricted, an increase in $\delta^{13}C$ with constant or even decreasing $\delta^{18}O$ values was observed. According to the dual isotope model (Scheidegger *et al*., 2000), this would correspond to an increase in the photosynthetic capacity and a constant or increasing stomatal conductance, despite the severe drought conditions. However, this contradicts current physiological knowledge, and was not reflected in intra-annual recorded tree-ring growth and concurrently measured net-photosynthesis (Arend *et al*., 2013).

Recently, the issue was raised that “tree ring-stable isotope compositions remain somewhat unreliable as a record of tree carbon-water dynamics”, (Barbour and Song 2014), and possible difficulties in explaining the dual isotope approach in tree rings have been further highlighted by Roden and Siegwolf (2012). In this study, the counterintuitive results obtained from the dual isotope approach can be well explained by changes in $\delta^{18}O$ of soil source water and the drought-induced cessation of tree-ring growth, uncoupling isotopic patterns in leaves and tree rings. Although the dual isotope approach fails to reflect physiological leaf responses to drought in tree rings, it can be highly instrumental in detecting such uncoupling of leaf and tree-ring isotopic pattern which must not be ignored when interpreting isotopic signals in tree rings formed under growth-limiting drought conditions. As the decoupling was less pronounced in *Q. petraea* than in

Q. robur, this analysis does additionally show that the latter species is more affected by periods of severe drought.

While the *first chapter* provided insights into the response of oaks to drought, it did not reflect the intra-seasonal responses to drought and drought release. Paying particular attention to this, the *second* and *third chapters* provide new insights into the intra-seasonal responses of European beech (*Fagus sylvatica* L.) to drought. Young beech trees were grown in model ecosystems under semi-natural conditions and subjected to summer drought in two consecutive years. In the *second chapter*, the seasonal response of drought was investigated in two provenances of mesic and xeric origins; as the physiological drought response may differ among provenances (Peuke *et al*., 2002). The *third chapter* focussed on different leaf physiological characteristics, which were analysed in (bi-) weekly intervals over the entire growing season to display all intra-annual responses to drought and drought release. In both chapters, three distinct response phases were identified: (i) instant drought response leading to a limitation of leaf physiological activity, (ii) recovery of leaf physiological activity after drought release, and (iii) post-drought stimulation after full recovery.

(i) Instant drought response

The instant drought response was mainly reflected by a reduced net photosynthesis (A_N) due to stomatal closure. With progressing drought, disturbed photochemical processes contributed to the limitation of net photosynthesis as indicated by impaired PS II chlorophyll fluorescence (PI_{tot}). These findings are in line with other studies on the drought response of beech (e.g. Blessing *et al*., 2016; Gallé and Feller, 2007; Aranda *et al*., 2000; Tognetti *et al*., 1995). Non-structural carbohydrates (starch plus soluble sugars; NSC) and $\delta^{13}C$ of soluble sugars ($\delta^{13}C_S$) were additionally analysed and responses to the imposed drought were detected at different levels of internal plant water deficit (predawn leaf water potential; ψ_{pd}). The first parameter that reacted to the slowly developing drought was A_N at a ψ_{pd} of -0.6 MP, followed by a decrease in starch concentrations and a drought induced increase in $\delta^{13}C_S$ at a ψ_{pd} of -0.8 MPa. Increasing concentrations of soluble sugars were observed at a ψ_{pd} of -1.0 MPa, and PI_{tot}, an indicator of photochemical performance of the photosystem II, was affected late, at a ψ_{pd} of -1.4 MPa. Such a temporally differentiated response of physiological parameters was also observed for spruce saplings under controlled environmental conditions (Ditmarová *et al*., 2009), and emphasises the complex mechanisms that are involved in the drought response of trees (Ryan *et al*., 2011; Hsiao 1973).

As total NSC concentrations remained nearly constant during the experiment, a carbon limitation of the trees is not likely. However, the concentrations of soluble sugars and starch were affected

in opposite ways. On the one hand, decreasing starch concentrations and the drought mediated increases in $\delta^{13}C_S$ indicate a reduction in assimilated carbon and insufficient supply of soluble sugars for maintenance of leaf metabolism. On the other hand, increasing soluble sugar concentrations and thus constant NSC concentrations do not point to a carbon starvation.

Several studies highlight the reduction in transport velocity in trees due to water shortage (Zang *et al*., 2014a; Ruehr *et al*., 2009). Other recent studies point to a sink limitation in drought treated beech trees: while the concentrations of soluble sugars in response to drought remained more or less constant in source leaves, the concentration of NSCs increased in roots, indicating a decreased demand of carbon in these tissues (Hagedorn *et al*., 2016). Therefore, a transportation tailback is likely to occur on the way of carbohydrates from the leaves to the roots, which in turn may contribute to an inhibition of photosynthesis due to substrate limitation (e.g. Kleczkowski, 1994). However, as indicated by PI_{tot}, a metabolic limitation of photosynthesis was not observed before ψ_{pd} dropped below -1.4 MPa. Increased concentrations of sucrose and glucose in leaves, fine roots and phloem sap of beech and eucalypt species were recently related to the osmotic protection of these tissues under drought conditions (Scartazza *et al*., 2015; Cernusak *et al*., 2003; Peuke *et al*., 2002), suggesting an additional explanation for increased sugar concentrations.

(ii) Recovery after drought release

After drought release, the fast recovery of the tree water status, the immediate increase in starch concentrations as a measure of available carbohydrates in leaves and the fast decay of the drought signal in $\delta^{13}C_S$ within one day indicated a prompt response of these beech trees to re-watering. This might be, among other factors, attributed to the ability of beech to quickly utilise water independent from soil depth due to an even distribution of fine roots along the soil profile (Volkmann *et al*. 2016). In contrast, the recovery of A_N and PI_{tot} was delayed by two to three weeks, particularly in the mesic beech provenance which fully recovered one week after the xeric beech provenance. This supports the notion of a better adaptation to drought of beech provenances from xeric origins (Cavin and Jump, 2016; Peuke *et al*., 2002; Tognetti *et al*., 1995). In summary, ψ_{pd}, $\delta^{13}C_S$ and starch concentrations recovered within one day, the recovery of sugar concentrations took two days, and all other measured variables reached control levels within three weeks after the re-watering event. Even though the observed water potentials were close to the stage where hydraulic failure is observed in beech (Hacke and Sauter 1995), recovery of leaf physiological activity was fast and complete. Thus, beech responds sensitively to instant drought, but is also able to recover quickly, suggesting a high resilience to drought. The latter may indicate

a higher drought resistance than commonly expected from studies using vegetation models and dendroecological approaches, and thus contradicts studies suggesting an exceptional climate risk for European beech (Garnier *et al*., 2007; Ohlemüller *et al*., 2006; Dittmar *et al*., 2003; Fotelli *et al*., 2002).

(iii) Post-drought stimulation

After full recovery, some evidence was found for a stimulation of leaf physiological activities to counteract previous drought limitations. Indeed, photosynthesis increased after full recovery and showed higher rates compared to control trees. This post-drought stimulation seems to be an adaptive trait as beech from a xeric forest site showed a stronger increase in A_N (34.4%) than beech from mesic forest sites (21.8% and 16.1% in *chapter 2* and *3*, respectively). The post-drought stimulation of A_N was observed until the end of the growing season, when leaves lost their photosynthetic activity due to autumnal leaf senescence. Hence, the beech trees could compensate for a substantial part of the previous limitation of C assimilation, minimizing the drought impact on these trees. Additionally, in drought-treated trees, a delayed leaf senescence was observed, similar to recent findings of former drought-treated oak trees in Belgium (Vander Mijnsbrugge *et al*., 2016), indicating that leaf-inherent mechanisms might play a role in the observed post-drought stimulation. The driving mechanisms of this post-drought stimulation of A_N can be related to altered source-sink relations, as recently suggested by Hagedorn *et al*. (2016). In fact, greater assimilate allocation to and metabolisation in the rhizosphere is observed after drought, which in turn triggers higher photosynthetic activity in source leaves. This higher photosynthetic activity is also indicated by slightly higher $\delta^{13}C$ in sugars, as also observed by Blessing *et al*. (2016), and might be explained by higher RubisCO activity, which was recently found in formerly drought exposed evergreen oak species (Vaz *et al.,* 2010). The observed post-drought stimulation of photosynthesis additionally supports the view of European beech is a species with a higher resilience to drought than commonly expected.

Overall conclusions

In conclusion, this thesis gives new insights into the response of oak and beech trees to drought and drought release on different functional levels and temporal scales. In the *first chapter* it is shown that the interpretation of the dual isotope approach in tree rings can be misleading under conditions when tree-ring growth is constricted, e.g. under severe (summer) drought conditions. The strength of the observed uncoupling depends on the amount of mobile leaf carbohydrates

that can be transformed to cell wall polymers in the growing tree rings. This will have serious implications on the physiological interpretation of any tree-ring chronology, especially under growth limiting conditions. Additionally, owing to the strength of the uncoupling between leaves and tree rings, the provided data point to a higher drought tolerance of *Q. petraea* compared to *Q. robur*.

In the *second* and *third chapter*, this thesis provides evidence that European beech is able to recover quickly from drought, and even shows a post-drought stimulation of photosynthesis after drought release, pointing to a high resilience of young beech trees to summer drought. It is therefore concluded that the recovery of beech from drought is as important as the initial drought response, and should be considered for the exact modelling of forest responses to changing environmental conditions. Moreover, the observed post-drought stimulation is a promising adjustment of beech to face the emerging risks of climate change.

Methodological aspects

In all presented studies, European beech and the oak species were analysed in the lysimeter-based model ecosystem (MODOEK) facility at WSL. A great advantage of the MODOEK facility is that controlled drought treatments can be applied to larger trees under semi-natural conditions, thus combining the advantages of controlled pot experiments in greenhouses or climate chambers with small trees with the advantages of the natural conditions in field studies with larger trees. In pot experiments several environmental variables, e.g. temperature, day length, irrigation regime and relative humidity, can be precisely adjusted in accordance to the research questions and the needs of the experiment. However, pot experiments are restricted in terms of soil volume and the age of trees, as only seedlings or smaller saplings can be grown. In field studies, mature trees can indeed be analysed under natural conditions but the environmental variables of interest are difficult to control. Most studies dealing with the drought response of trees are therefore performed with potted seedlings or saplings, which can introduce a severe bias to the results, i.e. artificial plant growth, tree productivity and soil drying (Poorter *et al*., 2012). Additionally, there is evidence that results gained from experiments using seedlings or saplings cannot be transferred to the mature tree level (Sala *et al*., 2010; Grulke and Retzlaff, 2001). Thus, the experimental setup in the presented studies, with a controlled drought treatment but natural fluctuations of light and temperature, as well as trees of sufficient age and size, provides a more realistic picture of the response of trees to drought than pot experiments under artificial greenhouse conditions.

In the study with oaks presented in *Chapter 1*, the analysis of source water of the trees was a critical point in explaining the unexpected isotope pattern. It is assumed that the soil water is not fractionated when taken up by the roots and transported to the leaves (Wershaw *et al*., 1966). A more reliable way of achieving $\delta^{18}O$ values of the source water would have been the analysis of the xylem water in the leaf petioles or the stem. As these samples were also needed for the analysis of biomass and nitrogen dynamics in subsequent studies, only $\delta^{18}O$ values of the drainage water in the lysimeters was analysed. However, the seasonal changes in the $\delta^{18}O$ of the drainage water helped to explain the observed dual-isotope pattern. Compared to irrigation water, the drainage water provides a more realistic picture of the $\delta^{18}O$ in source water, since drainage water had to go through the entire soil profile, and therefore experienced all relevant fractionation events, e.g. soil-evaporation, on its way below the roots (McCarroll and Loader 2004).

For the analysis of non-structural carbohydrates (NSCs) and $\delta^{13}C$ reported in *Chapter 3*, leaf material was collected at pre-dawn. This approach allowed to analyse pre-dawn leaf water potential (ψ_{pd}) in the same leaf material though leaves or other plant tissues are usually sampled during the day for the analysis of NSC concentrations and $\delta^{13}C$, because the light dependant impact on these variables is greatest due to photosynthesis and gas exchange. However, due to the frequent measuring dates over the entire season and the need of sufficient material for all analyses, it was not possible to sample twice per measuring date. Indeed, NSC concentrations were found to be very low compared to other studies, representing minimum values or the physiological baseline for NSC concentrations. There is, however, ample evidence for a pronounced diel variation in $\delta^{13}C$ of leaf water-soluble organic matter, phloem sap sugars and canopy respired CO_2 due to varying substrates for leaf metabolism, i.e. the use of transitory starch during the night, depleted in $\delta^{13}C$, and the consumption of newly produced sugars during the day, with enriched $\delta^{13}C$ values (Gessler *et al*., 2008; Hymus *et al*., 2005; Gleixner and Schmidt, 1998). As the study compared the $\delta^{13}C$ values of drought treated trees to these of control plants, this does not introduce a bias to the study, but should be considered when comparing this study with other experiments.

Recommendation for future research

In this thesis, the seasonal response of *F. sylvatica* as well as *Q. robur* and *Q. petraea* in independent experiments is analysed. However, drought responses are not easy to compare, even if the applied treatments are similar (Miyashita *et al*., 2005; Vicca *et al*., 2012). It is therefore recommended to analyse the responses of European beech and the both oak species to drought and drought release in a single experiment. On the one hand, this would generate data for the

solid comparison of these species, with identical soil water contents. On the other hand, the response of these tree species to drought and drought release with special emphasis on species competition could be analysed. While the post-drought stimulation was exclusively observed for beech in the presented studies, a higher temporal resolution of photosynthetic and isotope data could prove if this response is species specific or if it can also be observed in the oak species. Generally, not much data are available on recovery and resilience of the important tree species of European forests, even though this information is crucial for the exact modelling of forest responses to changing environmental conditions. More data on the recovery and resilience of European tree species are needed to identify tree species appropriate for forestry under future climate constraints in Europe.

Models of terrestrial carbon cycles and vegetation dynamics (e.g. dynamic global vegetation models, DGVM's and soil-vegetation transfer models, SVATs) are a powerful and frequently used method to predict past, current and future vegetation patterns and corresponding biochemical cycles (Scheiter *et al*., 2013; Sitch *et al*., 2008; Sitch *et al*., 2003; Cramer *et al*., 1999). To date, these models mainly focus on the influence of concurrent reactions to abiotic and biotic stresses and 'only' rely on direct driver-response relationships. However, it has already been shown by Zielis *et al.* (2014) that the annual net ecosystem productivity (NEP) of a Swiss subalpine forest can better be explained by models considering not only the current but also by previous year's weather. The issue was also recently stressed by Ogle *et al*. (2015), who outlined that ecosystem memory might be crucial to explain ecosystem behaviour. Thus, including recovery processes and post-drought effects might reduce the uncertainty in these models predicting the impact of future climate change on vegetation patterns and ecosystem functions.

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