

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



Matthias Arend^{a,*}, Krunoslav Sever^c, Ellen Pflug^{a,b}, Arthur Gessler^a, Marcus Schaub^a

^a Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Zurich, Switzerland

^b Department of Environmental Sciences, Federal Institute of Technology Zurich, Zurich, Switzerland

^c Department of Forest Genetics, Dendrology and Botany, Faculty of Forestry, University of Zagreb, Zagreb, Croatia

ARTICLE INFO

Article history:

Received 24 June 2015

Received in revised form 7 September 2015

Accepted 12 January 2016

Available online 22 January 2016

Keywords:

Photosynthesis

Drought

Recovery

Post-drought

Phenology

Beech

ABSTRACT

Trees experience frequent periods of severe drought during their long lives 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 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: (1) limitation, (2) recovery and (3) 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.

© 2016 Elsevier B.V. All rights reserved.

1. 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

organization 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 it is tightly linked to tree water balances via stomatal control of CO₂ uptake (Hetherington and Woodward, 2003; Zweifel et al., 2007). Recently, mesophyll conductance for CO₂ 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

* Corresponding author. Tel.: +41 447392436.

E-mail address: matthias.arend@wsl.ch (M. Arend).

net CO₂ exchange is a physiological measure that integrates all these sources of photosynthetic variation and is thus a widely used measure of stress in eco-physiological 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 CO₂ 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; Schaub 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 time 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).

2. Material and methods

2.1. 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 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 m². 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.

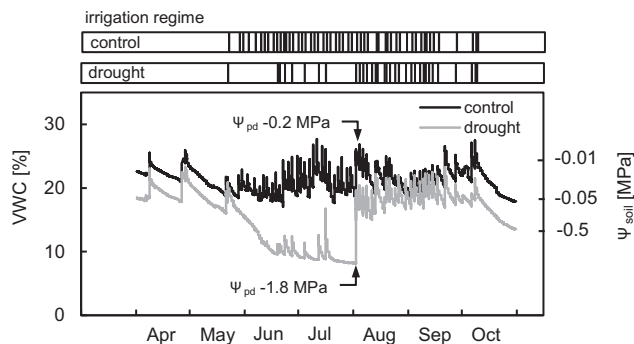


Fig. 1. Soil and plant water balances in irrigated and drought-treated mesocosms. Soil moisture was measured in 10 cm soil depth as volumetric soil water content (VWC) and related to soil matrix potential (Ψ_{soil}) and pre-dawn leaf water potential (Ψ_{pd} ; only shown before re-watering). Re-watering 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.

2.2. 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).

2.3. 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 behavior (Arend et al., 2015). Instantaneous rates of net-photosynthesis (P_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 $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and temperature adjusted close to outside conditions. Fast fluorescence kinetics were analyzed once per sapling between 11:00 and 12:00 on dark adapted leaves using a portable plant efficiency analyzer (Pocket PEA, Hansatech Instruments Ltd., Norfolk, UK). After a saturating light pulse of $3500 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ 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.

2.4. 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).

2.5. 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).

3. Results

3.1. 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 re-watering in early August, the average soil water content was reduced to 8.2%. These values 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 ($\text{SE} \pm 0.1$) 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 ($\text{SE Mas.} \pm 0.02/\text{Sax.} 0.03$).

3.2. Seasonal drought response of net-photosynthesis

Net-photosynthesis (P_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. P_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 P_N was divided into three distinct phases: (1) drought limitation from spring to

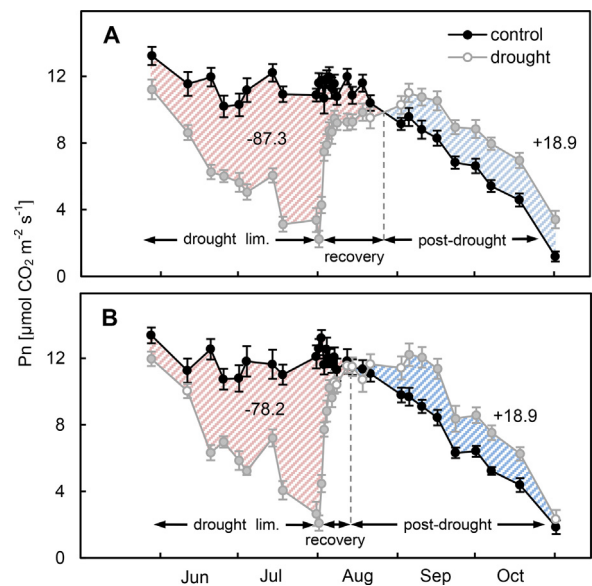


Fig. 2. Seasonal change of P_N in control and drought-exposed trees of (A) the mesic provenance *Mastrils* and (B) the xeric provenance *Saxon*. The drought response of P_N is divided into the three phases: limitation, recovery and post-drought stimulation. Numerical data indicate the loss and gain of cumulated photosynthetic activity in drought-exposed trees in relation to control trees as estimated by the cumulated deviation of P_N . Closed grey circles indicate significant differences between control and drought-treated trees. Data are means \pm SE with $n > 13$.

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) taken from nearby METEO SWISS stations (distances ≤ 10 km).

Provenance	Geographic location	Elevation [m a.s.l.]	Ann. temperature [$^{\circ}$ C]	Ann. precipitation [mm]
<i>Ardon</i>	46° 13' N 7° 14' E	750-850	9.2	542
<i>Chamoson</i>	46° 12' N 7° 12' E	750-850	9.2	542
<i>Saxon</i>	46° 8' N 7° 11' E	700-800	9.2	542
<i>Martigny</i>	46° 6' N 7° 6' E	500-700	9.2	843
<i>Collombey</i>	46° 16' N 6° 56' E	550-650	8.9	1055
<i>Ollon</i>	46° 18' N 6° 59' E	600-700	8.9	1055
<i>Felsberg</i>	46° 51' N 9° 28' E	650-800	8.9	798
<i>Chur</i>	46° 52' N 9° 32' E	700-800	8.9	798
<i>Malans</i>	46° 59' N 9° 34' E	600-700	8.9	1102
<i>Mastrils</i>	46° 58' N 9° 32' E	550-650	8.9	1102
<i>Sargans</i>	47° 3' N 9° 26' E	650-750	9.2	1325
<i>Mels</i>	47° 3' N 9° 24' E	650-750	9.2	1325

mid-summer, (2) recovery after drought release in mid-summer, and (3) 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 P_N accounting for less than 20% of that in control trees. 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*.

P_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 P_N slowed down before it reached the level of the respective control trees. After full recovery, P_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, P_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.

To better compare the different phases of the seasonal drought response in the two provenances, the deviation of P_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 $-78.2 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in the provenance *Mastrils* and *Saxon*, respectively. For the recovery phase alone, the cumulated deviation was $-24 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in the provenance *Mastrils* and $-14 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in the provenance *Saxon*. The post-drought stimulation after full recovery resulted in a positive cumulated deviation of P_N that yielded the same value of $18.9 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in both provenances.

3.3. Effects on chlorophyll a fluorescence

Chlorophyll *a* fluorescence was analyzed 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 P_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

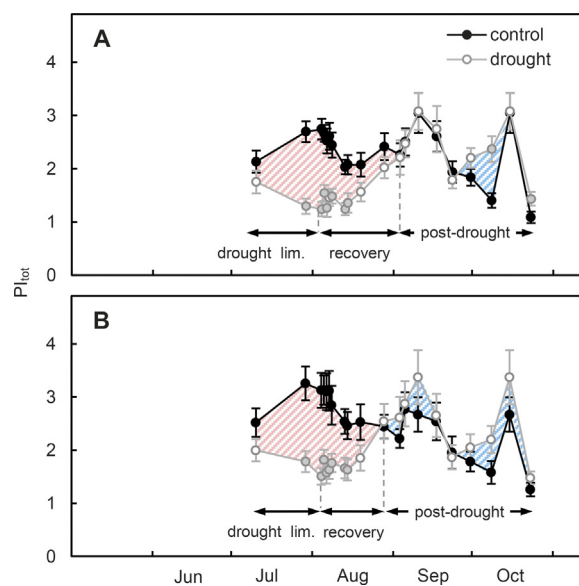


Fig. 3. Seasonal changes of chlorophyll *a* fluorescence as indicated by the total performance index of PSII (PI_{tot}) in control and drought-exposed trees of (A) the mesic provenance *Mastrils* and (B) the xeric provenance *Saxon*. The seasonal response of PI_{tot} to the applied drought treatment 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 \pm 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 P_N . As a consequence, 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*.

3.4. Effects on autumnal leaf senescence

As post-drought stimulation of P_N sustained until the end of the vegetation period, we additionally tested for any effects of the

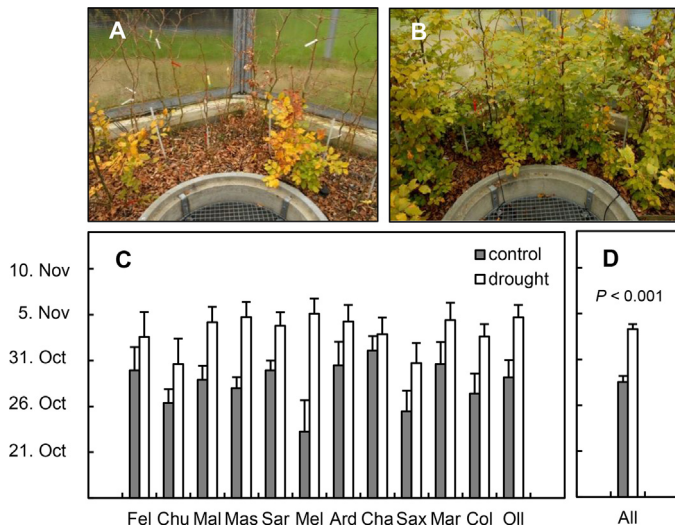


Fig. 4. Onset of autumnal leaf senescence in control and formerly drought-exposed trees. Almost completed leaf senescence in (A) control trees and delayed leaf senescence in (B) previously drought-exposed trees at the end of October. Dates of LCL₅₀ for (C) each provenance and (D) over all provenances. Data are means \pm SE with $n = 13$ (C) and $n > 194$ (D).

summer drought on autumnal leaf phenology. A first indication for altered autumnal leaf senescence was observed in October when foliage of control trees started 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 LCL₅₀ (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 (LCL₅₀) was derived from chlorophyll loss curves and used as a quantitative indicator of autumnal leaf senescence. The onset of LCL₅₀ 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 LCL₅₀ in formerly drought-exposed trees, though not all treatment differences were statistically significant. Over all provenances, the onset of LCL₅₀ was delayed in formerly drought-exposed trees by 5.7 days (Fig. 4D; $P < 0.001$) compared to the onset of LCL₅₀ in control trees.

4. 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 to severe summer drought and drought release by analyzing 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 (1) drought limitation, (2) drought recovery and (3) 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 CO₂ 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 favorable (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.

5. 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 behavior (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.

Funding

This study was financially supported by the Swiss Federal Office for the Environment FOEN and the Swiss Federal Institute for Forest, Snow and Landscape Research WSL.

Acknowledgements

We thank Peter Bleuler for his valuable technical assistance and the maintenance of the research facility MODOEK. We also thank the internship students Florian Schreyer, Pascal Buri, David Hanimann and Jonas Schuler for helping us with the physiological measurements.

References

- Albert, K.R., Mikkelsen, T.N., Michelsen, A., Ro-Poulsen, H., van der Linden, L., 2011. Interactive effects of drought, elevated CO₂ and warming on photosynthetic capacity and photosystem performance in temperate heath plants. *J. Plant Physiol.* 168, 1550–1561.
- Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., et al., 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For. Ecol. Manage.* 259, 660–684.
- Arend, M., Gessler, A., Schaub, M., 2015. The influence of the soil on spring and autumn phenology in European beech. *Tree Physiol.*, <http://dx.doi.org/10.1093/treephys/tpv087>.
- Arend, M., Brem, A., Kuster, T., Günthardt-Goerg, M., 2013. Seasonal pattern of photosynthetic responses to drought and air warming in *Quercus robur* Q. *petraea* and *Q. pubescens*. *Plant Biol.* 15, 169–176.
- Backhaus, S., Kreyling, J., Grant, K., Beierkuhnlein, C., Walter, J., Jentsch, A., 2014. Recurrent mild drought events increase resistance toward extreme drought stress. *Ecosystems* 17, 1068–1081.
- Baldocchi, D.D., 2003. Assessing the eddy covariance technique for evaluating carbon dioxide exchange rates of ecosystems: past, present and future. *Global Change Biol.* 9, 479–492.
- Boyer, J.S., 1982. Plant productivity and environment. *Science* 218, 443–448.
- Bréda, N., Huc, R., Granier, A., Dreyer, E., 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Ann. For. Sci.* 63, 625–644.
- Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003. Understanding plant responses to drought—from genes to the whole plant. *Funct. Plant Biol.* 30, 239–264.
- Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogee, J., Allard, V., et al., 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* 437, 529–533.
- Ellenberg, H., 1988. *Vegetation Ecology of Central Europe*, fourth ed. Cambridge University Press, New York, NY.
- Epron, D., Dreyer, E., 1993. Long-term effects of drought on photosynthesis of adult oak trees [*Quercus petraea* (Matt.) Liebl. and *Quercus robur* L.] in a natural stand. *New Phytol.* 125, 381–389.
- Epron, D., Dreyer, E., Aussenac, G., 1993. A comparison of photosynthetic responses to water stress in seedlings from 3 oak species: *Quercus petraea* (Matt.) Liebl., *Q. rubra* L. and *Q. cerris* L. *Ann. For. Sci.* 50, 48–60.
- Estrella, N., Menzel, A., 2006. Responses of leaf colouring in four deciduous tree species to climate and weather in Germany. *Clim. Res.* 32, 253–267.
- Flexas, J., Bota, J., Galmés, J., Medrano, H., Ribas-Carbó, M., 2006. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiol. Plant.* 127, 343–352.
- Flexas, J., Barbour, M.M., Brendel, O., Cabrera, H., Carriqui, M., Díaz-Espejo, A., Warren, C.R., 2012. Mesophyll diffusion conductance to CO₂: an unappreciated central player in photosynthesis. *Plant Sci.* 193, 70–84.
- Gallé, A., Feller, U., 2007. Changes of photosynthetic traits in beech saplings (*Fagus sylvatica*) under severe drought stress and during recovery. *Physiol. Plant.* 131, 412–421.
- Gallé, A., Haldimann, P., Feller, U., 2007. Photosynthetic performance and water relations in young pubescent oak (*Quercus pubescens*) trees during drought stress and recovery. *New Phytol.* 174, 799–810.
- Granier, A., Ceschia, E., Damesin, C., Dufrière, E., Epron, D., Gross, P., et al., 2000. The carbon balance of a young Beech forest. *Funct. Ecol.* 14, 312–325.
- Grassi, G., Magnani, F., 2005. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant Cell Environ.* 28, 834–849.
- Haldimann, P., Gallé, A., Feller, U., 2008. Impact of exceptionally severe summer stress conditions on photosynthetic traits in oak (*Quercus pubescens*) leaves. *Tree Physiol.* 28, 785–795.
- Hetherington, A.M., Woodward, F.I., 2003. The role of stomata in sensing and driving environmental change. *Nature* 424, 901–908.
- Holst, J., Barnard, R., Brandes, E., Buchmann, N., Gessler, A., Jaeger, L., 2008. Impacts of summer water limitation on the carbon balance of a Scots pine forest in the southern upper Rhine plain. *Agric. For. Meteorol.* 148, 1815–1826.
- Hommel, R., Siegwolf, R., Saurer, M., Farquhar, G.D., Kayler, Z., Ferrio, J.P., Gessler, A., 2014. Drought response of mesophyll conductance in forest understorey species—impacts on water use efficiency and interactions with leaf water movement. *Physiol. Plant.* 152, 98–114.
- IPCC, 2012. Summary for policymakers: managing the risks of extreme events and disasters to advance climate change adaptation. In: Field, C.B., Barros, V., Stocker, T.F., Qin, D., Dokken, D.J., Ebi, K.L., et al. (Eds.), A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, pp. 1–19.
- Kirschbaum, M.U.F., 1988. Recovery of photosynthesis from water stress in *Eucalyptus pauciflora*—a process in two stages. *Plant Cell Environ.* 11, 685–694.
- Kubiske, M.E., Abrams, M.D., 1993. Stomatal and non-stomatal limitations of photosynthesis in 19 temperate tree species on contrasting sites during wet and dry years. *Plant Cell Environ.* 16, 1123–1129.
- Kuster, T.M., Arend, M., Bleuler, P., Günthardt-Goerg, M.S., Schulin, R., 2013. Water regime and growth of young oak stands subjected to air-warming and drought on two different forest soils in a model ecosystem experiment. *Plant Biol.* 15, 138–147.
- Liu, C.C., Liu, Y.G., Guo, K., Zheng, Y.R., Li, G.Q., Yu, L.F., Yang, R., 2010. Influence of drought intensity on the response of six woody karst species subjected to successive cycles of drought and re-watering. *Physiol. Plant.* 139, 39–54.
- Loewenstein, N.J., Pallardy, S.G., 2002. Influence of a drying cycle on post-drought xylem sap abscisic acid and stomatal responses in young temperate deciduous angiosperms. *New Phytol.* 156, 351–361.
- Mitchell, P.J., O'Grady, A.P., Tissue, D.T., White, D.A., Ottenschlaeger, M.L., Pinkard, E.A., 2013. Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. *New Phytol.* 197, 862–872.
- Ni, B.-R., Pallardy, S.G., 1992. Stomatal and nonstomatal limitations to net photosynthesis in seedlings of woody angiosperms. *Plant Physiol.* 99, 1502–1508.
- Ogle, K., Barber, J.J., Barron Gafford, G.A., Bentley, L.P., Young, J.M., Huxman, T.E., Loik, M.E., Tissue, D.T., 2015. Quantifying ecological memory in plant and ecosystem processes. *Ecol. Lett.* 18, 221–235.
- Ohlemüller, R., Gritti, E.S., Sykes, M.T., Thomas, C.D., 2006. Quantifying components of risk for European woody species under climate change. *Global Change Biol.* 12, 1788–1799.
- Peuke, A.D., Schraml, C., Hartung, W., Rennenberg, H., 2002. Identification of drought-sensitive beech ecotypes by physiological parameters. *New Phytol.* 154, 373–387.
- Peuke, A., Gessler, A., Rennenberg, H., 2006. The effect of drought on C and N stable isotopes in different fractions of leaves, stems and roots of sensitive and tolerant beech ecotypes. *Plant Cell Environ.* 29, 823–835.
- Quirino, B.F., Noh, Y.S., Himelblau, E., Amasino, R.M., 2000. Molecular aspects of leaf senescence. *Trends Plant Sci.* 5, 278–282.
- Rennenberg, H., Loreto, F., Polle, A., Brilli, F., Fares, S., Beniwal, R.S., Gessler, A., 2006. Physiological responses of forest trees to heat and drought. *Plant Biol.* 8, 556–571.
- Schaub, M., Skelly, J.M., Steiner, K.C., Davis, D.D., Pennypacker, S.P., Zhang, J., et al., 2003. Physiological and foliar injury responses of *Prunus serotina* Fraxinus

- americana and *Acer rubrum* seedlings to varying soil moisture and ozone. *Environ. Pollut.* 124, 307–320.
- Strasser, R.J., Tsimilli-Michael, M., Qiang, S., Goltsev, V., 2010. Simultaneous *in vivo* recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopsis*. *Biochim. Biophys. Acta* 1797, 1313–1326.
- Tognetti, R., Michelozzi, M., Borghetti, M., 1995. The response of European beech (*Fagus sylvatica* L.) seedlings from two Italian populations to drought and recovery. *Trees—Struct. Funct.* 9, 348–354.
- Vaz, M., Pereira, J.S., Gazarini, L.C., David, T.S., David, J.S., Rodrigues, A., et al., 2010. Drought-induced photosynthetic inhibition and autumn recovery in two Mediterranean oak species (*Quercus ilex* and *Q. suber*). *Tree Physiol.* 30, 946–956.
- Verstraeten, W.W., Veroustraete, F., Feyen, J., 2006. On temperature and water limitation of net ecosystem productivity: implementation in the C-Fix model. *Ecol. Modell.* 199, 4–22.
- Verstraeten, W.W., Veroustraete, F., Heyns, W., VanRoey, T., Feyen, J., 2008. On uncertainties in carbon flux modelling and remotely sensed data assimilation: the Brasschaat pixel case. *Adv. Space Res.* 41, 20–35.
- Walter, J., Nagy, L., Hein, R., Rascher, U., Beierkuhnlein, C., Willner, E., Jentsch, A., 2011. Do plants remember drought? Hints towards a drought-memory in grasses. *Environ. Exp. Bot.* 71, 34–40.
- Walter, J., Jentsch, A., Beierkuhnlein, C., Kreyling, J., 2013. Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. *Environ. Exp. Bot.* 94, 3–8.
- Wilson, K.B., Baldocchi, D.D., Hanson, P.J., 2001. Leaf age affects the seasonal pattern of photosynthetic capacity and net ecosystem exchange of carbon in a deciduous forest. *Plant Cell Environ.* 24, 571–583.
- Wilson, K.B., Baldocchi, D.D., Hanson, P.J., 2000. Quantifying stomatal and nonstomatal limitation to carbon assimilation resulting from leaf aging and drought in a mature deciduous tree species. *Tree Physiol.* 20, 787–797.
- Xu, L., Baldocchi, D.D., 2003. Seasonal trends in photosynthetic parameters and stomatal conductance of blue oak (*Quercus douglasii*) under prolonged summer drought and high temperature. *Tree Physiol.* 23, 865–877.
- Zweifel, R., Steppe, K., Sterck, F.J., 2007. Stomatal regulation by microclimate and tree water relations: interpreting ecophysiological field data with a hydraulic plant model. *J. Exp. Bot.* 58, 2113–2131.