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Research paper

Long-term effects of drought on tree-ring growth and carbon isotope variability in Scots pine in a dry environment

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Drought frequency is increasing in many parts of the world and may enhance tree decline and mortality. The underlying physiological mechanisms are poorly understood, however, particularly regarding chronic effects of long-term drought and the response to increasing temperature and vapor pressure deficit (VPD). We combined analyses of radial growth and stable carbon isotope ratios ($\delta^{13}\text{C}$) in tree rings in a mature Scots pine (*Pinus sylvestris* L.) forest over the 20th century to elucidate causes of tree mortality in one of the driest parts of the European Alps (Pfywald, Switzerland). We further compared trees that have recently died with living trees in a 10-year irrigation experiment, where annual precipitation was doubled. We found a sustained growth increase and immediate depletion of $\delta^{13}\text{C}$ values for irrigated trees, indicating higher stomatal conductance and thus indeed demonstrating that water is a key limiting factor for growth. Growth of the now-dead trees started declining in the mid-1980s, when both mean temperature and VPD increased strongly. But growth of these trees was reduced to some extent already several decades earlier, while intrinsic water-use efficiency derived from $\delta^{13}\text{C}$ values was higher. This indicates a more conservative water-use strategy compared with surviving trees, possibly at the cost of low carbon uptake and long-term reduction of the needle mass. We observed reduced climatic sensitivity of raw tree-ring $\delta^{13}\text{C}$ for the now-dead in contrast to surviving trees, indicating impaired stomatal regulation, although this difference between the tree groups was smaller after detrending the data. Higher autocorrelation and a lower inter-annual $\delta^{13}\text{C}$ variability of the now-dead trees further indicates a strong dependence on (low) carbon reserves. We conclude that the recent increase in atmospheric moisture demand in combination with insufficient soil water supply was the main trigger for mortality of those trees that were weakened by long-term reduced carbon uptake.

Keywords: climate change, tree decline, tree mortality, vapor pressure deficit, water-use efficiency.

Introduction

Drought-related climate change is increasingly affecting many ecosystems worldwide (Allen et al. 2010, 2015). Summer 2003 was recorded as the hottest summer in Europe in the last 500 years (Schaer et al. 2004), and 2015 was reported as the Earth's warmest year since start of instrumental measurements (NOAA 2016). Moreover, model-based climate projections for the 21st century show further anticipated increases in average global temperatures, whereas precipitation is predicted to vary

regionally, increasing in some parts of the globe and decreasing in others (Stott et al. 2006, IPCC 2013). Consequences of these changes include an increasing frequency and duration of extreme weather events, particularly of drought. The latter affects forest ecosystems in many climatic zones and may lead to decreased tree vigor and growth (Martinez-Vilalta et al. 2002), reduction of primary productivity (Ciais et al. 2005) and ultimately forest decline, mortality and vegetation shifts (Allen et al. 2010, 2015, Choat et al. 2012). To better predict such changes in the future,

it is crucial to deepen our understanding of the mechanisms that allow trees to survive and adapt to drought (McDowell et al. 2008, 2011, Sala et al. 2010).

Some of the key mechanisms for tree survival under drought are the capacity to accumulate sufficient carbon reserves to maintain metabolism (Breshears et al. 2005, Sala 2009, McDowell et al. 2011), lower vulnerability to hydraulic failure (Bréda et al. 2006, Meinzer et al. 2009, 2010, McDowell et al. 2011), or a combination of both (Mueller et al. 2005, McDowell et al. 2011). Hydraulic failure occurs when plant tissues are desiccated due to cavitation, which leads to the disruption of water transport in a large number of vessel elements or tracheids. Carbon starvation develops when stomata close to prevent water loss, leading to reduced photosynthetic carbon uptake, while plant metabolism is in need of freshly produced carbohydrates, whose availability may be hindered also by phloem transport limitation (McDowell et al. 2008). Many other factors such as pathogens and insect attacks may accelerate decline and possibly lead to death, particularly of weakened trees. The complex interplay of the different factors and their combined effects still remain poorly quantified, and the lack of a solid mechanistic understanding makes it difficult to predict tree mortality (McDowell et al. 2011).

Several studies have found that increasing atmospheric moisture demand is becoming more important for plant performance and survival as temperature increases globally (Breshears et al. 2013, Eamus et al. 2013, Williams et al. 2013, Allen et al. 2015). Increasing trends in vapor pressure deficit (VPD) amplify evaporation and plant transpiration (McVicar et al. 2012). Thus, the combination of increasing temperature and VPD is likely to cause more droughts or make them more severe and may have radical effects on plants and forest ecosystems (Eamus et al. 2013). However, the role of specific climate parameters for forest decline is still not well understood (Williams et al. 2013) and there are only few studies that have explicitly addressed the relative importance of VPD for tree decline (Eamus et al. 2013).

Stable carbon isotope ratios ($\delta^{13}\text{C}$) are very useful for unraveling the mechanistic links between growth and gas exchange, as well as determining stomatal responses to VPD. They are particularly useful for gaining insights into the ecophysiological changes associated with drought and to differentiate between the mechanisms that cause decline and mortality (Leavitt and Long 1989, Saurer et al. 2004, Moreno-Gutiérrez et al. 2012, Herrero et al. 2013, Gessler et al. 2014, Hereş et al. 2014). The carbon isotope ratio of plant tissue is mainly controlled by stomatal conductance during carbon fixation (g_s) and the rate of photosynthesis (A), both of which are driven by environmental conditions (Farquhar et al. 1982, 1989). When applied to tree rings, $\delta^{13}\text{C}$ can be used to study long-term aspects of tree physiology in addition to sensitivity to climatic parameters (Saurer et al. 2004, Treydte et al. 2007). For instance, an increase in $\delta^{13}\text{C}$ could indicate stomatal closure and reduced conductance

to prevent water loss during drought (Leavitt and Long 1989, Saurer et al. 1995, Treydte et al. 2007, Kress et al. 2010) or could be due to changes in photosynthetic rates affected by irradiance during cool and wet periods (Voelker et al. 2014). One of the main drivers of stomatal regulation, which is often reflected in tree-ring $\delta^{13}\text{C}$ values in both broadleaved and conifer trees, is VPD (Treydte et al. 2007, Ferrio and Voltas 2011, Voelker et al. 2014). Furthermore, intrinsic water-use efficiency (WUE_i) can be derived from $\delta^{13}\text{C}$ values in plant tissue, indicating the ratio of the photosynthesis to stomatal conductance (Seibt et al. 2008, Lévesque et al. 2014). Climate change and increasing atmospheric CO₂ are leading to changes in WUE_i of various tree species around the globe, observed particularly in the northern hemisphere (Keenan et al. 2013, Saurer et al. 2014, Frank et al. 2015, van der Sleen et al. 2015).

Combined studies of tree-ring growth and water-use efficiency can provide a better understanding of how plants in general and trees in particular adapt their water-use strategies to secure survival. For instance, recent studies have shown that trees that died later had higher growth rates (Bigler and Veblen 2009) as well as higher WUE_i (Levanič et al. 2011) several decades prior to death compared with trees that survived. These authors concluded that dying trees were hydraulically under-equipped for dry conditions (Levanič et al. 2011). In this context, it would be important to have century-long, annually resolved tree-ring growth and isotope records of dying and surviving trees, which are still rare or absent in the literature. Such studies would enable analysis of the causal links between drought and tree mortality and the related physiological mechanisms in the long-term.

The goal of our study is to better understand long-term ecophysiological mechanisms that cause altered growth behavior and sensitivity to climatic conditions, comparing Scots pine trees that died recently with still living individuals over the last ~100 years in a dry inner alpine valley. In this region, high Scots pine mortality rates were already reported by Dobbertin et al. (2005) and Bigler et al. (2006), similar to other dry regions in southern Europe (Galiano et al. 2010, Hereş et al. 2014, Aguadé et al. 2015, Benavides et al. 2015). Scots pine is a key species, both ecologically and commercially, due to its extended geographic range, high longevity and ability to grow in marginal conditions, whereas small changes in temperature and/or water availability can strongly affect its growth and physiology. By providing well replicated, annually resolved carbon isotope records of dying and surviving Scots pine trees, climate correlation analysis can provide insights into the relationship and sensitivity of gas-exchange to past climate changes. Furthermore, we can make use of a long-term irrigation experiment in a natural Scots pine forest (Pfywald, Valais, Switzerland). This study was set up more than a decade ago, and provides an ideal platform to investigate the physiological effects of water availability in this ecosystem (Brunner et al. 2009, Dobbertin et al. 2010, Eilmann et al. 2010, Herzog et al. 2014) as there are still very few such

controlled studies on mature trees and long time-scales (Beier et al. 2012). Specifically, we wanted to address the following questions:

- What are the effects of the 10-year long irrigation on tree-ring growth and tree-ring carbon isotope patterns?
- How does tree-ring growth differ between still living and recently deceased trees over the past ~100 years in terms of climatic sensitivity and carbon isotope variability?
- What is the role of increasing VPD for tree decline in the study region?
- Can the combined analysis of tree-ring growth and $\delta^{13}\text{C}$ -derived water-use efficiency help to elucidate the mechanisms underlying the observed tree decline?
- Can we identify physiological key indicators that characterize trees with low and high mortality risk?

Materials and methods

Study site

The study site is a xeric mature Scots pine forest in the canton of Valais, Switzerland (46° 18' N, 7° 36' E, 615 m above sea level), where an irrigation experiment has been running since June 2003. The dominant species is Scots pine (*Pinus sylvestris* L.). The forest is characterized as uneven-aged *Erico-Pinetum sylvestris* (Dobbertin et al. 2010), with an approximate average stand age of 100 years and a stand density of 730 stems ha⁻¹. The area is known to be the driest part of Switzerland, with a mean annual temperature of 10.1 °C (15.8 °C for June–August) and mean annual precipitation of 605 mm (169 mm for June–August) for the period 1981–2010. The soil is shallow and characterized by low water retention (Brunner et al. 2009). The 1.2 ha experimental area is divided into eight plots of 1000 m² each. Trees on four randomly selected plots were irrigated each growing season (April–October) over the period of 2003–14. The irrigation water was taken from a water channel next to the experimental area. The amount of irrigation water was set to roughly double the annual precipitation compared with the control plots. Trees in the other four plots are growing under naturally dry conditions and were used as controls.

Sampling and tree-ring width measurements

After the end of the growing season in October/November 2014, we sampled increment cores from a total of 75 living trees from the control and irrigated plots (two to three cores per tree). Trees were cored below breast height (at ~1 m) using a 5 mm increment borer (Haglöf, Långsele, Sweden) to avoid any bias of subsequent circumference measurements. Stem disks from 12 trees that had recently died were cut out at breast height in November 2013. We defined dead trees as individuals that were still standing, but with no green needles left in the crowns. Recently died, standing trees were only found in the control area (not in irrigated

plots) and we sampled also some dead trees outside the experimental area (with the same conditions as in control area). All deadwood stem disks were dried for 48 h at 40 °C. Stem disks were then sanded with progressively finer sandpaper (60–400 grit). The surface of the tree cores were cut with a core-microtome to improve ring visibility (Gärtner and Nievergelt 2010). Tree-ring width (TRW) was measured using a Lintab system with a precision of 0.01 mm, using the TSAP-Win software V.3.5 (Rinntech, Heidelberg, Germany), and dated with existing TRW chronologies from the study site (Eilmann et al. 2010, 2011) using the software COFECHA (Grissino-Mayer 2001). Three TRW chronologies were developed and referred to in the following as 'now-dead', 'now-living' or, when referring to the irrigation experiment, 'control' and 'irrigated'. All analyses were done with raw as well as detrended TRW data. For detrending, indices from a negative exponential curve and 30-year splines, respectively applied to the individual TRW series were calculated (Cook 1985). The negative exponential detrending is well-known as the most classical one and retains much low-frequency variance, whereas the 30-year spline removes the low-frequency variance more rigorously (Fritts 1976, Cook 1990, Schweingruber 1996).

Carbon isotope measurements

Five trees from each group ('dead', 'control' and 'irrigated') were selected for $\delta^{13}\text{C}$ analysis of cellulose based on good agreement of their individual TRW series with the site chronologies. Individual rings were separated using a surgical scalpel under a Wild M8 stereomicroscope. In the case of the now-dead trees, isotope measurements started with the tree ring of the year 1900, and the outermost rings of the selected trees varied between 2005 and 2012 AD. Thus, the $\delta^{13}\text{C}$ chronology of the now-dead trees was established for the period 1900–2005, which is the period of common overlap of all trees. In the case of the now-living trees, the isotope analysis was done for the common overlap period 1900–2014, but for the irrigated trees for the 1980–2014 period only because in prior decades the behavior of control and irrigated trees is expected to be indistinguishable. The separated rings were cut into small pieces and packed into teflon filter bags for subsequent chemical treatment (Ankom Technology, Macedon, NY, USA).

Cellulose extraction was performed based on Boettger et al. (2007), with an ultrasonic bath modified for larger numbers of samples that allows for the extraction of up to 400 samples in one batch (Roden et al. 2009). Homogenization of the cellulose material was done following Laumer et al. (2009) using an ultrasonic treatment with a HD3100 sonotrode (Hielscher, Berlin, Germany).

For the $\delta^{13}\text{C}$ analysis, the samples from the now-dead trees were packed into tin capsules, with a sample weight ranging from 0.4 to 0.6 mg. The cellulose samples of the now-living and

the irrigated tree groups were packed into silver capsules, with a sample weight ranging from 0.45 to 1.3 mg. The samples of the now-dead trees were measured using the combustion method and subsequent analysis by isotope-ratio mass spectrometry (IRMS; delta S; Thermo, Bremen, Germany; instrument precision 0.1‰). The samples of the now-living and the irrigated trees were measured using a recently developed pyrolysis method for the IRMS (delta Plus XP, Thermo; instrument precision 0.2‰). The combustion and pyrolysis methods yielded identical results for $\delta^{13}\text{C}$ within the instrument precision. The values obtained by pyrolysis needed a correction due to the addition of a small amount of carbon from the reactor to the sample gas, which results in a dampened isotope signal. We therefore measured a subset of the same samples that cover the whole range of expected $\delta^{13}\text{C}$ values using the conventional combustion method in addition to pyrolysis method to identify a linear correction curve (Woodley 2012, Weigt et al. 2015). Carbon isotope ratios are reported against Vienna Pee Dee Belemnite (VPDB). Individual tree-ring $\delta^{13}\text{C}$ values were corrected back to pre-industrial conditions to account for the Suess effect (McCarroll and Loader 2004). However, we did not apply an additional correction for the potential physiological response to changes in atmospheric CO_2 , due to still existing uncertainties with respect to the correction procedures (McCarroll et al. 2009, Treydte et al. 2009).

Climate data

The meteorological station closest to the study site is Sion (distance ~20 km). Monthly data of mean temperature and precipitation sums were obtained for the period 1900–2014 from MeteoSwiss (Begert et al. 2005). We used homogenized data, where artifacts in the series were removed, meaning that the historical measured values were adapted to current measuring conditions and non-climatic influences were excluded (Begert et al. 2005). Reliable relative humidity (RH) data are often difficult to obtain due to measurement uncertainties and small-scale heterogeneity (Fatichi et al. 2015), and, hence, such measurements were available for Sion for the 1960–2014 period only. Therefore, also VPD data were calculated for this period only (1960–2014) using temperature and the reliable RH data. The VPD is the difference between the saturation vapor pressure (e_s) at air temperature, minus the actual (e_a) vapor pressure:

$$\text{VPD} = e_s - e_a, \quad (1)$$

where

$$e_s = 0.611 \times 10^{\wedge}(7.5 \times T / (237.3 + T)) \quad (2)$$

and

$$e_a = (\text{RH} / 100) \times e_s. \quad (3)$$

T is mean temperature in degrees Celsius ($^{\circ}\text{C}$) and RH is relative humidity in %. Additionally, we used the global 0.5° gridded Standardized Precipitation-Evapotranspiration Index (SPEI) dataset at different time scales (from 1 to 48 months) for the 1901–2013 period based on CRU TS3.22 (Harris et al. 2014). The SPEI data were originally from IPE-SCIC and obtained via the KNMI Climate Explorer (Beguería et al. 2010, Vicente-Serrano et al. 2010, Trouet and Van Oldenborgh 2013).

Mean temperature for the spring (March–May) and summer (June–August) seasons have increased significantly during recent decades (1960–2003, spring: slope = $0.04 \text{ }^{\circ}\text{C year}^{-1}$, $R^2 = 0.27$, $P < 0.001$; summer: slope = $0.05 \text{ }^{\circ}\text{C year}^{-1}$, $R^2 = 0.39$, $P < 0.001$, $n = 44$, Figure 1). The main increase occurred after the mid-1980s. Vapor pressure deficit also increased significantly for both seasons (1960–2003, spring: slope = $0.02 \text{ hPa year}^{-1}$, $R^2 = 0.29$, $P < 0.001$; summer: slope = $0.03 \text{ hPa year}^{-1}$, $R^2 = 0.17$, $P < 0.01$, $n = 44$). Consistent with the VPD increase, though less pronounced (only spring, not summer), RH showed a negative trend (1960–2003, slope = $-0.07\% \text{ year}^{-1}$, $R^2 = 0.12$, $P < 0.05$, $n = 44$).

In contrast, the trends of precipitation amount and the SPEI index, calculated at time-scales of 3 and 6 months, had no significant trends. Considering the full period, time series of mean temperature (1903–2003, spring: slope = $0.01 \text{ }^{\circ}\text{C year}^{-1}$, $R^2 = 0.18$, $P < 0.001$; summer: slope = $0.02 \text{ }^{\circ}\text{C year}^{-1}$, $R^2 = 0.27$, $P < 0.001$, $n = 101$ years), 3-month SPEI (1903–2003, summer: slope = 0.01 year^{-1} , $R^2 = 0.07$, $P < 0.01$, $n = 101$ years) and 6-month SPEI (1903–2003, spring: slope = 0.01 year^{-1} , $R^2 = 0.05$, $P < 0.05$; summer: slope = 0.01 year^{-1} , $R^2 = 0.08$, $P < 0.01$, $n = 101$ years) had significant increasing trends (see Figure S1 available as Supplementary Data at *Tree Physiology* Online).

Data analysis

To characterize the response of tree-ring growth and $\delta^{13}\text{C}$ values to climate, Pearson's correlation coefficients were calculated based on monthly climate data (from previous January to current December) and combinations of several months (March–May and June–August) between the mean series of each climate variable and TRW and $\delta^{13}\text{C}$ chronologies, respectively. To test the significance of the correlation coefficients, two-tailed Student's t -tests were applied. The strength and significance of two independent correlation coefficients were compared based on Fisher's Z transformation. This test provides an asymptotic confidence interval, if there are at least four complete pairs of observation. Temporal autocorrelation was taken into account by calculating the 'effective' sample size based on the sample size and first-order autocorrelation for each time series of the TRW chronologies and individual climate data (Dawdy 1964):

$$N' = N \times \frac{(1 - r_1 \times r_2)}{(1 + r_1 \times r_2)}, \quad (4)$$

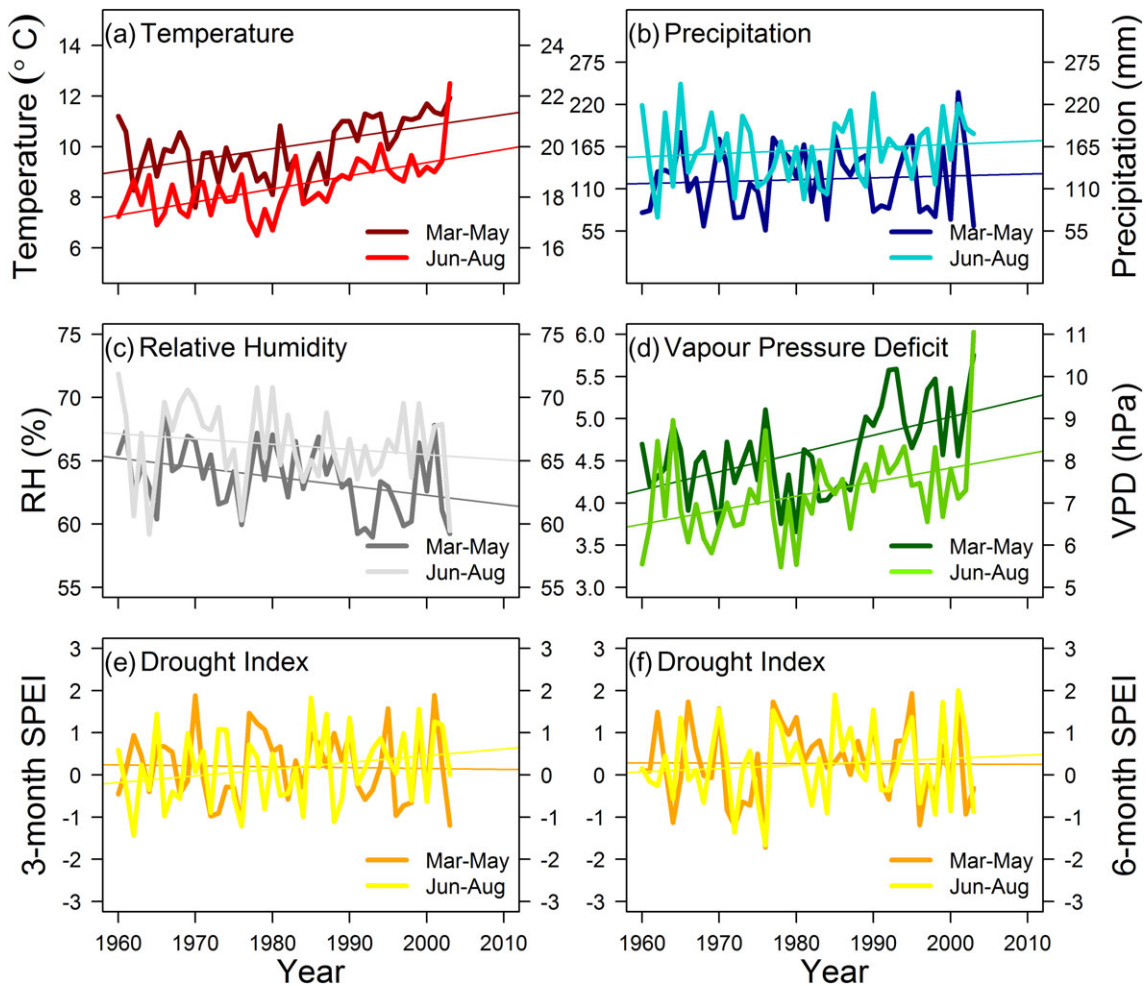


Figure 1. Spring and summer meteorological variables at the Sion meteorological station for 1960–2003: (a) mean surface temperature; (b) precipitation sum; (c) relative humidity; (d) VPD; (e) 3-month SPEI drought index; (f) 6-month SPEI drought index. Dark colors are for spring (March–May; y-axis to the left) and light colors for summer data (June–August; y-axis to the right). Linear regression lines are also indicated.

where N is sample size, N' is effective sample size, and r_1 and r_2 are the first-order sample autocorrelation of the first and second time series, respectively. Two-tailed Student's t -tests were applied to test for significant differences between the means of two groups.

Intrinsic water-use efficiency (WUE_i) for each group of trees was derived from tree-ring $\delta^{13}\text{C}$ values; it is defined as the ratio of the rate of carbon assimilation (A , photosynthesis) and stomatal conductance (g_s), and can be calculated as:

$$WUE_i = A/g_s = c_a \times \frac{[b - (\delta^{13}\text{C}_{atm} - \delta^{13}\text{C}_{plant})]}{[(b - a) \times 1.6]} \quad (5)$$

where c_a is atmospheric CO_2 concentration, $\delta^{13}\text{C}_{atm}$ is the isotopic ratio of atmospheric CO_2 , $\delta^{13}\text{C}_{plant}$ is the isotopic ratio of the plant, a (4.4‰) is the fractionation due to diffusion and b (27‰) is the biochemical fractionation (Farquhar et al. 1982, 1989).

Average Pearson's correlation coefficients between individual series for each group (r_{bar}) and the expressed population signal (EPS) were calculated to quantify the strength of common variation within a group. This common variation is considered to be strong when EPS is equal or above 0.85, which usually indicates strong climate forcing (Wigley et al. 1984). In addition, the 95% confidence limits ($\bar{x} \pm 1.96 \times \text{SE}$, SE – standard error) were calculated for both groups. The F -test for equality of variance was used to identify if the true ratios of variances are equal or not to one between the control and dead groups for two periods (1900–79 and 1980–2005). Mann–Whitney U -test was additionally applied as a non-parametric test for comparing two groups of values. To test the temporal stability of the climate–growth and isotope relationships, 30-year moving correlations between mean temperature, precipitation and TRW and $\delta^{13}\text{C}$ chronologies on a seasonal basis (spring and summer) were calculated for the 1903–2003 period.

Results

Effects of irrigation on tree-ring width and $\delta^{13}\text{C}$

We determined averages of the main parameters investigated over the period 2003–14, when the irrigation experiment was carried out. Irrigation resulted in an approximate doubling of available water from 543 ± 87 mm (mean \pm SD) to 1139 ± 197 mm (Figure 2a, difference significant at $P < 0.001$). Mean TRW increased up to 1.11 ± 0.28 mm for the irrigated trees, while it was only 0.69 ± 0.15 mm for the control trees (Figure 2b, $P < 0.001$).

The mean $\delta^{13}\text{C}$ values of the irrigated trees were significantly lower ($-23.67 \pm 0.65\text{‰}$) than those of the control trees ($-21.87 \pm 0.48\text{‰}$), with depletion starting already in the first growing season after the beginning of irrigation in 2004 (Figures 2c and 3b). Intrinsic water-use efficiency (WUE_i) calculated by Eq. (5) was strongly reduced from 143.67 ± 4.80 $\mu\text{mol mol}^{-1}$ to 124.38 ± 6.31 $\mu\text{mol mol}^{-1}$ (Figure 2d).

Individual tree-ring $\delta^{13}\text{C}$ series of the control trees did not show any trend after the Suess correction, while the values of the irrigated trees showed a step-like change with persistently low values from 2004 on and a stronger common variability compared with the control trees (Figure 3, control trees, 1980–2014: $r_{\text{bar}} = 0.63$, $P < 0.001$; irrigated trees, 1980–2014: $r_{\text{bar}} = 0.73$, $P < 0.001$). A relatively high $\delta^{13}\text{C}$ value during the irrigation period was observed during a year with a dry summer (2011). A step-like increase in TRW was observed for the same trees after irrigation, with a stronger common variability compared to control trees (see Figure S2 available as Supplementary Data at *Tree Physiology* Online, control trees, 1980–2014: $r_{\text{bar}} = 0.62$, $P < 0.001$; irrigated trees, 1980–2014: $r_{\text{bar}} = 0.70$, $P < 0.001$).

Long-term tree-ring width and tree-ring $\delta^{13}\text{C}$ data

Although relative variations in TRW of the now-living and the now-dead trees were quite similar during their early life phase

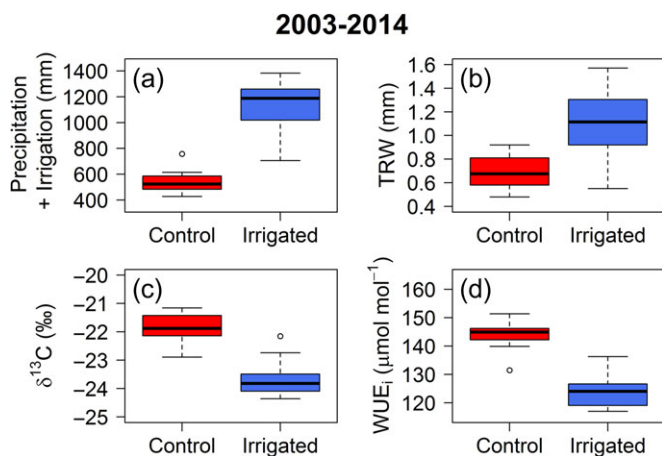


Figure 2. Effect of irrigation on mean TRW and $\delta^{13}\text{C}$ values for the period 2003–14. All differences between control and irrigated tree groups are significant at $P < 0.001$.

until the mid-1980s, absolute growth rates of the now-dead trees were significantly lower than those of the now-living trees already during that period (Figure 4, Student's t -test for differences between groups, 1900–79: -0.158 mm \pm 0.047 mm (mean difference \pm SD), $P < 0.01$). Afterwards, the growth rates of the now-dead trees started to deviate more strongly from the now-living trees and continuously declined until the trees eventually died (Figure 4, Student's t -test for differences

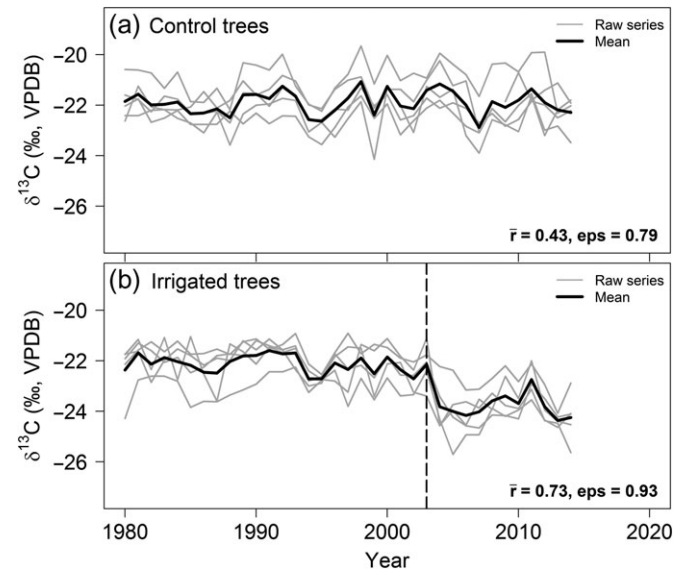


Figure 3. Individual series (gray lines) and mean curves of tree-ring $\delta^{13}\text{C}$: (a) control trees and (b) irrigated trees. The dashed vertical line indicates the start of irrigation in 2003.

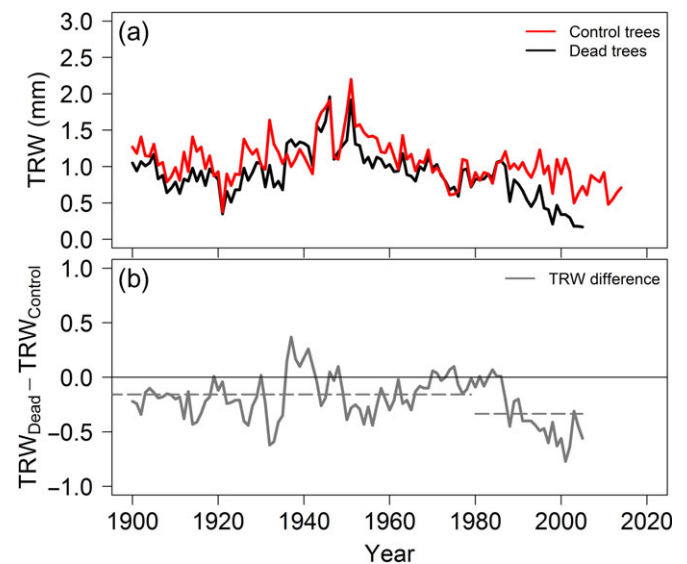


Figure 4. Tree-ring width chronologies of now-living and now-dead trees (a) and their differences (b) for the period 1900–2014. The dashed lines indicate the mean of the differences between now-dead and now-living trees for 1900–79 and 1980–2005, respectively. Note: the chronology of the now-dead trees covers the period 1900–2005.

between groups, 1980–2005: $-0.334 \text{ mm} \pm 0.066 \text{ mm}$, $P < 0.001$). The growth decline started approximately at the same time as the increasing trends in mean temperatures and VPD (Figure 1a and d) were recorded. The detrended TRW indices mainly keep the high-frequency, thus reducing the offset between the now-living and the now-dead trees (Figure 4, see Figure S3 available as Supplementary Data at *Tree Physiology* Online).

Individual $\delta^{13}\text{C}$ series of the now-dead trees had lower variability, expressed as average SD between trees compared with the now-living individuals, particularly during the period of decline (Figure 5, control trees: SD = 0.68 (1900–79), SD = 0.73 (1980–2005); dead trees: SD = 0.60 (1900–79), SD = 0.55 (1980–2005)), and this difference in variability was significant (1900–79: $F = 1.23$, $P < 0.05$; 1980–2005: $F = 1.74$, $P < 0.01$). Furthermore, the first-order autocorrelation was much higher for the now-dead compared with the now-living trees (see Figure S5 available as Supplementary Data at *Tree Physiology* Online). The different trees showed a rather coherent behavior with the 95% confidence limits for the dead and control groups below 0.95‰ and 1.04‰, respectively. The average correlation between all trees and the EPS was within the same range (Figure 5). For TRW data, the now-dead trees had also slightly lower SD values compared with the now-living trees (see Figure S6 available as Supplementary Data at *Tree Physiology* Online, control trees: SD = 0.63 (1900–79), SD = 0.47 (1980–2005); dead trees: SD = 0.58 (1900–79), SD = 0.43 (1980–2005)).

The average tree-ring $\delta^{13}\text{C}$ series of both groups varied within a similar range, but in fact they were significantly higher for the now-dead trees before the onset of growth decline in the

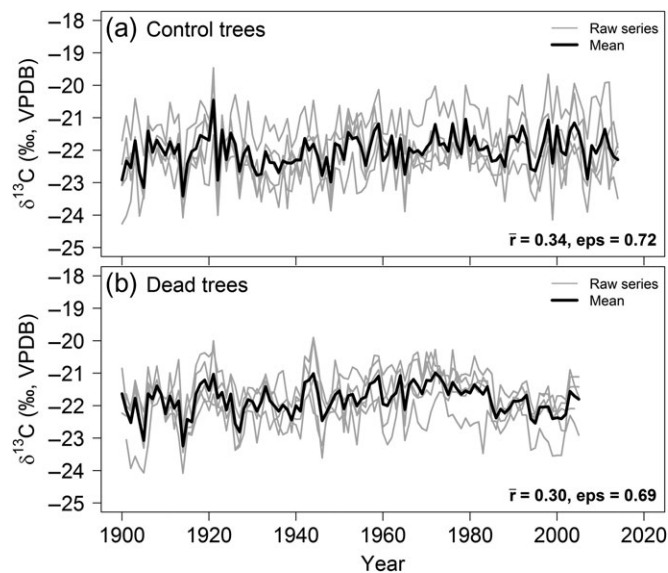


Figure 5. Individual series (gray lines) and mean curves of tree-ring $\delta^{13}\text{C}$: (a) control trees and (b) now-dead trees. Note: chronology of now-dead trees covers the period 1900–2005.

mid-1980s (Figure 6a, Student's t -test for differences between groups, 1900–79: 0.269 ± 0.078 , $P < 0.001$). This difference disappeared afterwards (1980–2005: -0.104 ± 0.112 , n.s.). Comparison of the two groups for each individual year indicated that the difference between groups was significant for a few years only due to the tree-to-tree variability (Mann–Whitney U -test). A strong increase in WUE_i over the 20th century derived from $\delta^{13}\text{C}$ series was observed for both groups (Figure 6b). Again, WUE_i of the now-dead trees was higher from the 1930s until the mid-1980s, but then fell below the values of now-living trees later (Figure 6c, Student's t -test for differences between groups, 1900–79: 2.329 ± 1.252 , $P < 0.001$; 1980–2005: -1.117 ± 2.106 , n.s.).

Tree-ring widths and $\delta^{13}\text{C}$ response to climate

Raw TRW chronologies were significantly and negatively correlated with summer temperatures and VPD (Figure 7c, summer: T, dead trees: $r = -0.66$, $P < 0.001$, control trees: $r = -0.37$, $P < 0.05$; VPD, dead trees: $r = -0.51$, $P < 0.001$, control trees: -0.47 , $P < 0.01$). Correlation coefficients of the dead trees with these same variables were even more pronounced and significant in spring in contrast to the control trees (Figure 7a, spring: T, dead trees: $r = -0.62$, $P < 0.001$, control

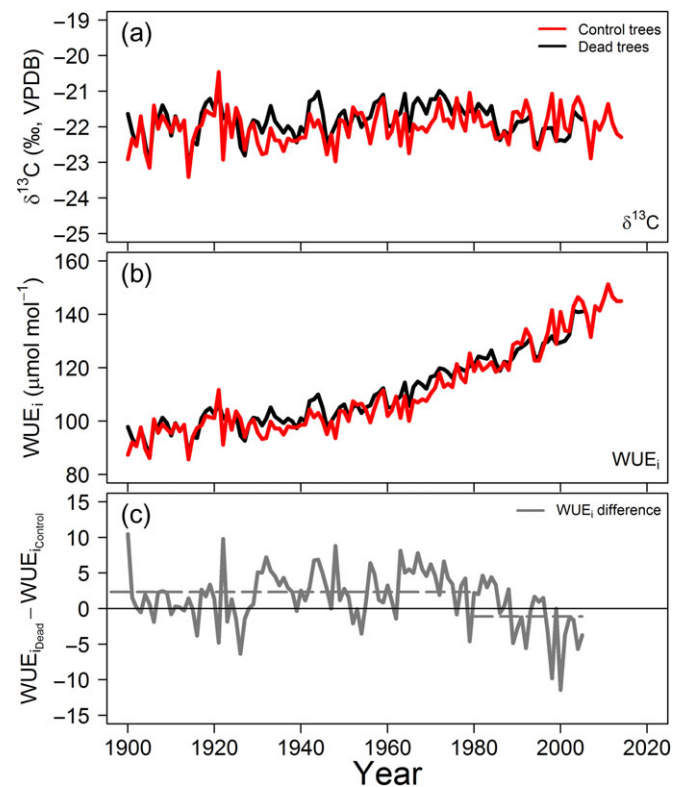


Figure 6. Tree-ring $\delta^{13}\text{C}$ chronologies of now-living and now-dead trees (a), WUE_i chronologies (b) and WUE_i differences (c). The dashed lines indicate the mean of the differences between now-dead and now-living for 1900–79 and 1980–2005, respectively. Note: the chronology of the now-dead trees covers the period 1900–2005.

trees: -0.03 , $P > 0.05$; VPD, dead trees: $r = -0.70$, $P < 0.001$, control trees: $r = -0.27$, $P > 0.05$). However, the now-dead trees did not show any significant correlation with precipitation and SPEI (Figure 7a and c). Furthermore, the now-dead trees reacted more sensitively to temperature in the previous year than the now-living trees (see Figure S7 available as Supplementary Data at *Tree Physiology Online*). Correlation results of the TRW chronologies detrended with negative exponential curve did not substantially differ from the results derived from raw TRW chronologies (see Figure S8 available as Supplementary Data at *Tree Physiology Online*). However, the difference in the correlation between the control and dead trees was much weaker, when all the climate and TRW data were more rigorously detrended with 30-year splines (see Figure S9 available as Supplementary Data at *Tree Physiology Online*).

Carbon isotope variations of the now-living trees were highly significantly negatively ($P < 0.01$) correlated with precipitation, SPEI and RH (Figure 7b and d), positively with temperature and significantly positively ($P < 0.05$) with VPD (Figure 7b and d) for both, spring and summer. For the now-dead trees, correlations were generally weaker or not significant (Figure 7b and d) and for temperature and VPD even in opposite direction compared with living trees. These differences in correlation between the groups were mainly due to low-frequency trends in the data, as these differences also were much smaller, when all the climate and $\delta^{13}\text{C}$ data were detrended with 30-year splines (see Figure S9 available as Supplementary Data at *Tree Physiology Online*). A weaker $\delta^{13}\text{C}$ response of the now-dead trees to precipitation and drought was still present. Moving correlations in 30-year windows between growth and temperature as well as $\delta^{13}\text{C}$ and temperature showed similar values for the now-dead trees and the survivors for the first part of the record, but values for the now-dead trees started diverging after the mid-1970s, for both, spring and summer (Figure 8). Due to the availability of

VPD data only for 1960–2014, this analysis was restricted to temperature.

Relationships between tree-ring widths and $\delta^{13}\text{C}$ values

For the irrigated trees TRW and $\delta^{13}\text{C}$ values were strongly negatively correlated ($R^2 = 0.42$, $P < 0.001$, $n = 35$), for the control group they were still significantly correlated ($R^2 = 0.16$, $P < 0.001$, $n = 115$), but for the now-dead trees there was no relationship at all (Figure 9). Results of correlation analysis over the various time periods showed that the now-dead trees did not exhibit any relationship between tree-ring growth and tree-ring $\delta^{13}\text{C}$ values before (1900–79, Table 1) as well as after the growth decline (1980–2005, Table 1). During the common period of analysis (1980–2005), the relationship between growth and tree-ring $\delta^{13}\text{C}$ values was stronger for the now-living trees compared with the other two groups (Table 1). However, in this period, there are only 3 years of irrigation

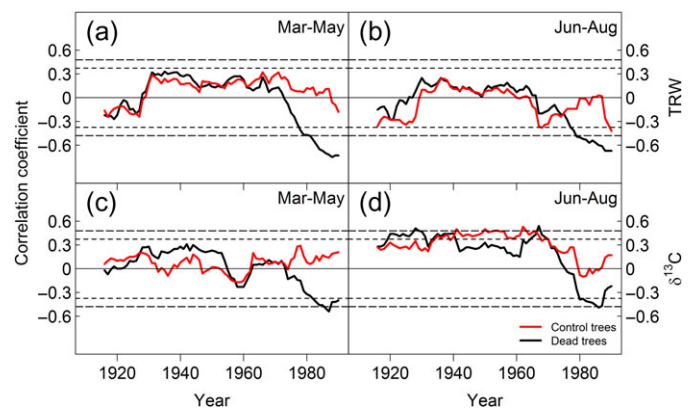


Figure 8. Thirty-year moving correlation coefficients between TRW and temperature (a, b) and $\delta^{13}\text{C}$ chronologies and temperature (c, d) for the period of 1902–2005. The short dashed line indicates the significance level at 95% and the long dashed line at 99%.

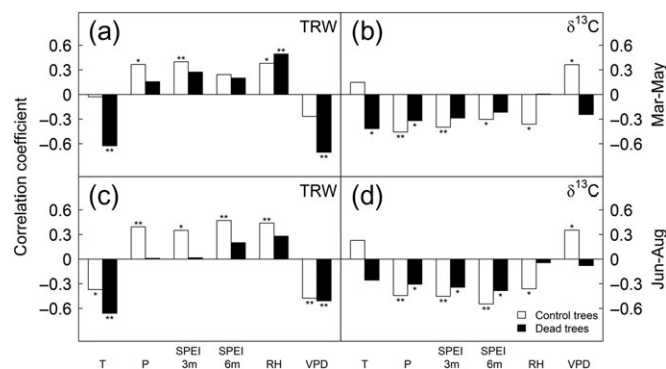


Figure 7. Pearson's correlation coefficients between TRW and $\delta^{13}\text{C}$ chronologies and climate variables for the period of 1960–2003 for the spring (March–May; upper panel) and summer (June–August; lower panel). Black bars refer to the now-dead trees and white bars to the control trees. Significant correlations are marked by * $P < 0.05$ and ** $P < 0.01$.

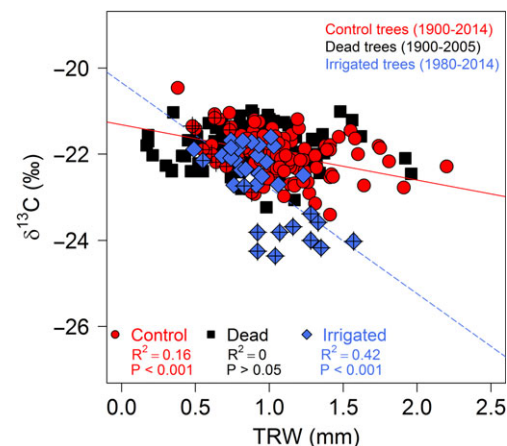


Figure 9. Relationships between tree-ring growth and $\delta^{13}\text{C}$ for now-dead, control and irrigated trees. Symbols marked by crosses indicate the values for the irrigation period (2003–14).

Table 1. Results of correlations between raw tree-ring and $\delta^{13}\text{C}$ data for all groups over the various time periods. NA = not available.

Time period	Now-dead trees	Now-living trees (control)	Irrigated trees
1900–79	$r = -0.18, P > 0.05$	$r = -0.36, P < 0.001$	NA
1980–2005	$r = 0.10, P > 0.05$	$r = -0.68, P < 0.001$	$r = -0.53, P < 0.01$
2003–14	NA	$r = -0.52, P > 0.05$	$r = -0.60, P < 0.05$

(2003–05). During the entire period of irrigation (2003–14), the strongest relationship was found for the irrigated trees (Table 1).

Discussion

Effects of irrigation

Water supply is typically assumed to be a key limiting factor for Scots pine in dry inner alpine valleys (Dobbertin et al. 2005, Bigler et al. 2006, Rigling et al. 2013). This hypothesis can be tested directly using the results from the long-term irrigation experiment (Dobbertin et al. 2010) and was indeed confirmed at our study site. Irrigation almost doubled TRW compared to the control trees. A strong response was also observed by the depletion of tree-ring $\delta^{13}\text{C}$ values of the irrigated trees, which occurred immediately after the start of irrigation. This recovery was not only a short-term response, but was sustained throughout the period of irrigation, reflected also in the absence of recently deceased trees in the irrigation area. The fast and distinct growth and isotope response to the irrigation is consistent with results of Eilmann et al. (2010). They observed a decrease in $\delta^{13}\text{C}$ of the irrigated trees independent of crown transparency in the year after the irrigation start, for both early- and latewood as well as for intra-annual $\delta^{13}\text{C}$ patterns. They interpreted this decrease in $\delta^{13}\text{C}$ as increased stomatal conductance due to improved water availability. The fast isotope response in their and our cases indicates that freshly synthesized assimilates with relatively low isotope values are immediately and directly transported to the sites of xylem cell production, showing a strong carbon demand in the stem. This indicates either a low availability of long-term stored carbohydrates or that the trees are not able to access stored carbon under drought (McDowell and Sevanto 2010).

Relationship between tree-ring width and $\delta^{13}\text{C}$

Increasing growth along with decreasing carbon isotope values were often reported for sites under water-limiting conditions (McDowell et al. 2010, Hereş et al. 2014). As observed also in our irrigation experiment for Scots pine, an inverse relationship indicates that trees take advantage of the favorable conditions, reduce the water-use efficiency and greatly enhance stomatal conductance. Similar relationships were observed for various tree species in the Mediterranean region and in North America, particularly during drought periods (McDowell et al. 2010, Voltas et al. 2013, Hereş et al. 2014, Voelker et al. 2014). In

our study, however, this relationship was weaker for the control and absent for the now-dead trees. This may indicate a de-coupling between gas-exchange and growth for severely damaged trees. Comparing co-habiting now-dead with now-living Scots pines in northeastern Spain, Hereş et al. (2014) also found that the WUE_i response of the now-dead trees was weaker than of the now-living ones and it was not associated with a growth increase. In contrast, the growth– WUE_i relationship was significantly stronger for now-dead ponderosa pines (*Pinus ponderosa*) compared with surviving trees along an elevational transect in northern New Mexico (McDowell et al. 2010). Such apparent contradictions could be related to species-specific differences. Our findings could also be related to recent observations, where under severe drought conditions the isotope signal generated in the leaves is no longer represented in the tree-rings, because growth is halted under the driest summer conditions (Sarris et al. 2013, Pflug et al. 2015). Such a weakened response of the tree-ring isotopic variation to environmental conditions has also been observed in slow-growing trees in unthinned stands of ponderosa pines compared with fast-growing thinned stands in Arizona, USA (Sohn et al. 2014). The authors of this study assumed that such a response of control trees and their extremely low growth throughout the entire experimental period are most likely due to chronic water stress. The latter might have suppressed within-tree carbon sink activity rather than source activity (Hartmann 2015). The lack of a relationship between growth and WUE_i in any case seems to be an indication of severe drought stress.

Long-term weakening processes

Tree-ring studies are particularly useful for providing a retrospective view on growth performance and physiological characteristics long before an actual tree decline may have started. This can help to understand why some trees succumb to drought, while others do not (McDowell et al. 2008). We considered different phases of tree life, particularly an early phase, when growth of the now-dead and the now-living trees differed only to a small extent (1900–79), and a later or a 'final' phase, when differences augmented. Our results show that the now-dead trees had slightly lower growth and higher WUE_i already early in their life, i.e., several decades before the distinct decline, as found also for pinyon (*Pinus edulis*) and Scots pine (Macalady and Bugmann 2014, Camarero et al. 2015). In Macalady and Bugmann (2014), the dying pinyon pines also appeared to have generally lower average growth rates than the surviving trees,

but growth prior to drought-induced mortality was rather variable depending on site conditions (Macalady and Bugmann 2014). Our findings indicate that the now-dead trees were exerting strong stomatal control and were more conservative in their water-use strategy over many decades, showing stronger isohydric behavior compared with the now-living trees. Stomatal closure then probably led to lower photosynthesis and reduced production of fresh carbohydrates. The effect of lower conductance and photosynthesis in the long-term can as a consequence result in multiple other physiological changes. In fact, not only stomatal conductance, but also total needle mass and needle specific area were reduced due to water limitation at this site (Dobbertin et al. 2010). Further, lower growth could result in smaller tree height, a competitive disadvantage compared with neighboring trees. Overall, the distinct decline that started in the mid-1980s was most likely a result of the previous long-term weakening process due to reduced carbon uptake.

Carbon starvation or hydraulic failure?

Our results point to an important role of storage depletion in dying trees caused by a long-term water-conservation strategy, although additional factors in the final decline phase when trees are already weak may be important (McDowell et al. 2011). However, actual reduction of carbon reserves (non-structural carbohydrates; NSC) in all parts of a tree (branch, stem, roots) is rarely observed (Gruber et al. 2012) and the role of carbon starvation therefore has been debated for over a decade now (Körner 2003, 2015, Hartmann 2015). Several studies of Scots pine and also other genera indicated a different water-use strategy compared with our site, as dying/declining trees grew significantly better and had higher WUE_i in the earlier life phase than living/surviving trees (Levanič et al. 2011, Morán-López et al. 2014, Hentschel et al. 2014). These studies hypothesized that the divergent patterns of survival and mortality within a stand were mainly due to hydraulic acclimation prior to drought. The faster growth in the earlier phase predisposed trees to become more vulnerable to drought. These studies indicated that trees that grow fast and build large xylem vessels are not able to adapt their water-use strategy during drought conditions, thus rendering them more vulnerable to severe drought and predisposing them to cavitation and hydraulic failure, rather than carbon starvation. Such patterns were observed for Scots pines in Switzerland as well in the eastern Pyrenees (Morán-López et al. 2014). On the other hand, carbon limitation may be more frequent than assumed from stem NSC measurements, because stored carbon may not be available for growth anymore (Sala et al. 2012). Results from the literature, including our study, therefore demonstrate a varying, but non-negligible role of carbon depletion for tree mortality.

Final phase and role of VPD

Remarkably, the final tree-ring decline after the mid-1980s occurred when mean temperatures and VPD started to increase,

although there were no significant trends in precipitation and drought index at that time. Furthermore, the weakened trees that died later did not maintain their conservative water-use strategy by increasing WUE_i further but reduced it, in contradiction to expected behavior during drought conditions. This unusual behavior of growth and WUE_i also resulted in the absence of a correlation between them, reflecting the de-coupling between gas-exchange and growth mentioned above. Our findings indicate that VPD may be a critical factor, triggering decline and mortality at our site. Higher temperatures and increased VPD have been suggested as possible drivers of the recent large-scale die-off event in the southwestern USA (Breshears et al. 2005, Williams et al. 2010, 2013). Higher rates of potential water loss from soils substantially reduce water supply for plants due to rising temperatures and associated increases in VPD (Breshears et al. 2013, Eamus et al. 2013). Increased VPD leads to stomatal closure and reduced carbon metabolism as well as transpiration, the net result of which is likely a further intensification of plant water stress (Breshears et al. 2013, Eamus et al. 2013). Studies analyzing variations in $\delta^{13}C$ and TRW may be particularly helpful in this respect, as shown also for bur oaks in North America (Voelker et al. 2014). Studies explicitly using VPD are still rare (Eamus et al. 2013). Reliable local RH data may further be needed rather than large-scale extrapolated data due to the high spatial variability of this variable (Fatichi et al. 2015). Our results indicate that the recent increase in VPD for trees growing under chronically low water supply may be detrimental for the most weakened individuals.

Climate sensitivity

For a detailed analysis of the influence of different climate variables on tree physiology and for understanding the sensitivity to drought, a correlation analysis is very useful (McDowell et al. 2010, Levanič et al. 2011, Voltas et al. 2013). We found that the growth of both tree groups was strongly associated with spring and summer temperatures as well as VPD. Some correlations, particularly for spring, were significant for the now-dead trees only, indicating a more sensitive response to temperature as reported previously (McDowell et al. 2010, Voltas et al. 2013, Macalady and Bugmann 2014), but in contrast the influence of precipitation and drought (SPEI) was less important for the now-dead trees compared with the control trees. The results of this analysis, however, depended quite strongly on the statistical treatment of the data, i.e., detrending. When removing only the age-related TRW trends by negative exponential fitting, the correlation results were still similar, but differences between groups largely disappeared after more rigorously detrending TRW as well as climate data. This shows that now-dead trees still responded to year-to-year climate fluctuations in a similar way to control trees. Nevertheless, we consider the complete removal of low-frequency signals not adequate for investigating the causes of tree decline, where actually the low-frequency trends

are most important to understand. Regarding carbon isotopes, we observed a diverging response between mean temperatures, VPD and tree-ring $\delta^{13}\text{C}$ values between the two groups of trees: tree-ring $\delta^{13}\text{C}$ of the now-dead trees was negatively correlated with spring and summer temperatures, as well as with VPD, but it was positively correlated with both temperature variables and VPD for the control trees. As mentioned above, this divergence was mainly observed for analysis with raw data. A positive relationship between tree-ring $\delta^{13}\text{C}$ values and increasing temperatures as well as VPD, as observed for the control trees, is an expected response during drought conditions (Treydte et al. 2007, Ferrio and Voltas 2011, Saurer et al. 2014, Voelker et al. 2014). The divergent isotope response to temperature and VPD of the now-dead trees may be explained once more by impaired stomatal regulation of the weakened trees (McDowell et al. 2010). Growth of the now-dead trees was more strongly coupled to RH, VPD, maximum temperatures and drought as observed also in other studies (McDowell et al. 2010, Macalady and Bugmann 2014). These findings may indicate that mortality is associated with greater climate sensitivity of stem growth, rather than leaf gas exchange (Seibt et al. 2008), i.e., a decoupling of leaf- and stem-level processes under drought (Sala et al. 2012, Fatichi et al. 2014, Palacio et al. 2014, Gessler and Treydte 2016).

Indicators of tree decline

Some characteristics of tree-ring data may be indicative for trees that later die and therefore signify 'early-warnings of tree-dieback' (Camarero et al. 2015). Possibly related to the lack of stomatal response to climate variations, we found that the first-order temporal autocorrelations were positive and high for the now-dead trees in both TRW and $\delta^{13}\text{C}$, but very low for the now-living trees. This is consistent with the fact that the climate–growth relationships of the months of previous years were significant for dying trees only (see Figure S5 available as Supplementary Data at *Tree Physiology* Online). Furthermore, the variance of tree-ring $\delta^{13}\text{C}$ values was lower for the now-dead trees during the period of distinct decline (1980–2005). This may be explained as follows: during decline, the trees shed some of their needles and with a reduced needle mass the potential difference between years becomes down-scaled and the variance smaller. The low year-to-year variability also indicates the influence of old, well-mixed reserves. These findings therefore suggest that the growth and physiology of the now-dead trees were strongly dependent on the environmental conditions of the previous growing season, and that these trees relied mainly on carbon reserves, although they were already reduced (Eilmann et al. 2010). Focusing on growth alone, Camarero et al. (2015) observed a loss of synchronicity and decreases in the autocorrelation and variance for declining Scots pine in northeastern Spain, but opposite patterns in other species at the same site. Consideration of autocorrelation and variability prior to death

may improve our knowledge of tree vitality at least for some species (Fritts 1976, Camarero et al. 2015). Our results indicate that several factors in combination may help to identify trees that are potentially threatened and to elucidate the role of different physiological processes under long-term, chronic drought. Specifically, we suggest as indicators of impending mortality the lack of correlation between growth and isotope data, a high autocorrelation and low variance as well as a reduced gas-exchange response to high T/VPD of the now-dead trees.

Conclusions

A multitude of inter-related factors influence tree decline, which are difficult to disentangle. The irrigation experiment in a mature Scots pine forest therefore was very useful to quantify the isolated effect of water on tree physiology. The growth response as well as the strong relationship to water-use efficiency has demonstrated the limiting effect of water for non-irrigated trees. The long-term perspective provided by the tree-ring analysis indicates that trees were subject to a weakening process over several decades, which slowly reduced their vigor and led to the mortality of the most isohydric individuals. Lower stomatal conductance and reduced photosynthetic activity most likely resulted in insufficient carbon reserves of now-dead trees compared with the still living trees, visible also in reduced needle length and specific needle area. Freshly produced assimilates may not have been sufficient to support metabolic processes. Although there is a general lack of water at the site, we could show that recent increases in temperature and elevated atmospheric moisture demand (increased VPD) are most likely the triggers that are responsible for the final decline. This is concerning as climate models predict further increases in both temperature and VPD in many parts of the world. In our study area it was already proposed that due to the mechanisms outlined above, pine forests might be eventually substituted by oak forests, particularly at low elevations (Rigling et al. 2013). Therefore, possible improvement of the water balance by thinning has been suggested (Giuggiola et al. 2016). Our analysis also showed that high-resolution growth and carbon isotope data are very useful for extracting indicators that characterize weakened trees, such as the lack of correlation between growth and isotope data and a high autocorrelation. Such growth–carbon isotope-related indicators may help to identify trees predisposed to decline and could be useful for detecting threatened trees also for other species and sites.

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Supplementary Data

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

Conflict of interest

None declared.

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