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Fast response of Scots pine to improved water availability reflected in tree-ring width and $\delta^{13}C$

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ABSTRACT

Drought-induced forest decline, like the Scots pine mortality in inner-Alpine valleys, will gain in importance as the frequency and severity of drought events are expected to increase. To understand how chronic drought affects tree growth and tree-ring $\delta^{13}C$ values, we studied mature Scots pine in an irrigation experiment in an inner-Alpine valley. Tree growth and isotope analyses were carried out at the annual and seasonal scale. At the seasonal scale, maximum $\delta^{13}C$ values were measured after the hottest and driest period of the year, and were associated with decreasing growth rates. Inter-annual $\delta^{13}C$ values in early- and latewood showed a strong correlation with annual climatic conditions and an immediate decrease as a response to irrigation. This indicates a tight coupling between wood formation and the freshly produced assimilates for trees exposed to chronic drought. This rapid appearance of the isotopic signal is a strong indication for an immediate and direct transfer of newly synthesized assimilates for biomass production. The fast appearance and the distinct isotopic signal suggest a low availability of old stored carbohydrates. If this was a sign for C-storage depletion, an increasing mortality could be expected when stressors increase the need for carbohydrate for defence, repair or regeneration.

Key-words: climate change; drought; forest decline; pinning; seasonal $\delta^{13}C$ variation; stable carbon isotopes; storage depletion.

INTRODUCTION

Tree mortality in response to extreme climate events can lead to rapid vegetation shifts and forest decline (McDowell *et al*. 2008). Drought in particular has been discussed as an important trigger for forest decline and tree mortality (e.g. Allen & Breshears 1998; Breshears *et al*. 2005; Bréda *et al*. 2006; Gitlin *et al*. 2006), and is expected to gain in importance in the future. Increasing frequency and severity of drought are suggested by most climate change scenarios for Central Europe (e.g. Schär *et al*. 2004; IPCC 2007), with still unknown consequences for tree performance and ecosystem dynamics.

Ecosystems such as the dry forests at low elevation in the inner Alpine valleys in Europe are excellent field localities to analyse the role of drought on tree performance. Here, precipitation is generally low because of the rain shadow of the surrounding high mountain ranges and frequent drought years (Rebetez & Dobbertin 2004). During the last three decades, climatic conditions have continually shifted towards increasing summer droughts caused by a changing seasonality of precipitation (Weber, Bugmann & Rigling 2007) and increasing evapotranspiration (Rebetez & Dobbertin 2004), leading temporarily to a more negative water balance of the trees (Zweifel *et al*. 2006).

Recently, the sub-boreal Scots pine (*Pinus sylvestris* L.), naturally dominating the dry forest at low elevation, shows high mortality rates in the main inner-Alpine dry valleys [e.g. in the Aosta Valley (Vertui & Tagliaferro 1998), in the Inn Valley (Oberhuber 2001) or in the Swiss Rhone Valley (Dobbertin *et al*. 2005)]. Locally in the Swiss Rhone Valley, almost half of the Scots pine population has died since 1995 (Rebetez & Dobbertin 2004), with the highest mortality rates on dry sites following drought years (Dobbertin *et al*. 2005).

Continuous depletion of stored carbohydrates was discussed as one mechanism leading to drought-induced tree mortality (Bréda *et al*. 2006; McDowell *et al*. 2008). The theory is based on the fact that drought reduces photosynthetic carbon assimilation, which is reflected in a decreased primary production of forests (Ciais *et al*. 2005; Granier *et al*. 2007; Reichstein *et al*. 2007), but also leads to a diminished amount of carbohydrate storage (Bréda *et al*. 2006). Reduced photosynthesis under drought may lead to a negative carbon balance when the demand for carbohydrates for the maintenance of metabolic or repair processes exceeds the assimilatory supply. Thus, to meet the tree's carbon needs, an increased amount of stored carbohydrates are used. Increasing frequency and/or severity of drought events may lead to tree death as neither the C-assimilation nor the stored carbohydrates suffice to cover the metabolic demands for carbohydrates.

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Tree-ring widths (*TRW*s) yield direct information on tree productivity, which is used as a proxy for climatic impacts on tree growth (e.g. Jacoby, D'Arrigo & Davaajamts 1996; Fritts 2001; Briffa, Osborn & Schweingruber 2004). The combination with stable isotopes in tree-ring analysis allows the distinction between drought situations or very cold growth seasons (e.g. Francey & Farquhar 1982; McCarroll & Loader 2004) because the carbon isotope ratio in tree rings is more directly linked to the plant carbon water relations. During photosynthesis, plants discriminate against the heavier $^{13}CO_2$ in favour of the lighter $^{12}CO_2$, leaving plant biomass depleted in ^{13}C. During warm weather conditions, the photosynthetic rate is usually higher than when it is cold. Consequently, higher isotope ratios (less negative $\delta^{13}C$ values) are a strong indicator for warm and dry (leading to a reduction in stomatal conductance) weather conditions, and even more expressed for drought periods, with limited water supply.

A further aspect to consider is the isotopic difference in early- and latewood. Carbon isotope values in the latewood show a clear response to current climate condition (Porte & Loustau 2001; Jäggi *et al*. 2002). Generally, $^{13}C/^{12}C$ isotope ratio in earlywood is not well correlated with the current climatic parameters, because earlywood is expected to be predominantly formed from old stored carbohydrates, which were assimilated in the previous years (Smith & Paul 1988; Kagawa, Sugimoto & Maximov 2006). Therefore, $^{13}C/^{12}C$ isotope values in earlywood are poorly related to the ongoing climate because of the mixing of old and new assimilates (von Felten *et al*. 2007; Keel *et al*. 2007). Whether this is also true for trees on dry sites with potentially low and eventually exhausted carbohydrate reserves because of chronic drought (Bréda *et al*. 2006; McDowell *et al*. 2008) must be examined.

Isotope studies at the seasonal scale provide higher temporal resolution between the impact of environmental conditions and the $^{13}C/^{12}C$ ratio (e.g. Barbour, Walcroft & Farquhar 2002). A prerequisite is, however, that wood can be dated within the season. A precise technique to determine the seasonal xylem increment is the pinning method (Wolter 1968). However, until now no combination of seasonal $^{13}C/^{12}C$ ratio and the pinning method has been undertaken.

We installed an irrigation experiment in a site where the Scots pine trees were exposed to drought since their germination. These pine trees growing under contrasting water supply treatments were compared by analyzing tree growth and $^{13}C/^{12}C$ ratio values on the inter- and seasonal scale using the pinning method.

The goal of this study was to answer the following questions: (1) Is the current water availability [calculated as a seasonal drought index (*DI*)] recorded in early- and latewood $\delta^{13}C$ in equal measure? (2) How fast do trees respond to an improved water supply after a chronic drought stress, and to what degree is it reflected in the width and the carbon isotope ratio of tree rings?

MATERIALS AND METHODS

Experimental site

The experimental site (46°18′ N, 7°36′ E, 615 m a.s.l.) is located in a xeric mature Scots pine forest (average age 90 years) at the valley floor in the driest central part of the Swiss Rhone valley (Valais). The climate is continental with a mean annual temperature of 9.2 °C and mean annual precipitation of 599 mm (standard period 1961–1990, Meteo Swiss, weather station Sion, at 20 km distance from the experimental site).

The forest of the experimental site can be described as *Erico-Pinetum sylvestris*. The soil type is a pararendzina, which is shallow and skeletal (soil depth 60 cm) characterized by low water retention. The experimental site (about 1 ha) was split up into eight plots of 1000 m^2 each, separated by a 5 m buffering strip. Four randomly selected plots were irrigated since June 2003, using water from an adjacent water channel, fed by the river Rhone. Irrigation, corresponding to a supplementary rainfall of about 700 mm per year, was applied at night during the vegetation period (April to October) using sprinklers of about 1 m height. Irrigation earlier than April was not possible because of the risk of equipment damages by late frost. Site conditions differ only in soil moisture because other factors, such as radiation or soil properties, were similar on all plots. Because of the small plot size and the constantly high wind speeds, vapour pressure deficit (VPD) is the same between the plots.

Pinning technique and sample preparation

Three dominant trees per treatment (irrigated and control) were included in this study. To allow a pairwise statistical analysis, the selected trees were classified by crown transparency estimated by visual rating using reference photographs [range: 0% = a fully foliated tree, to 100% = a dead tree; for details on the method of crown transparency estimation, see Dobbertin, Hug & Mizoue (2004)]. The first estimation for the rating of the sample trees took place in March 2003 before the new shoots emerged. To assess the development of foliage density caused by irrigation, the rating was repeated in March 2006. In this study, we concentrated on trees with low defoliation rates with very low to mid crown transparency (see Table 1) to assure that the tree rings were wide enough for seasonal analysis of $\delta^{13}C$.

The pinning method was used for dating the seasonal tree ring formation. Pinning is a technique using the wound reaction resulting from regularly marking the cambium by inflicting micro-injury to date wood formation (e.g. Wolter 1968; Yoshimura *et al*. 1981; Nobuchi, Fujisawa & Saiki 1993). Trees were wounded at breast height according to Schmitt, Jalkanen & Eckstein (2004) on a biweekly basis during the growth period 2005 (13 April to 13 October) using a small needle (diameter 0.6 mm).

In spring 2006, the trees were cut, and the stem sections with the pinning wounds and two additional stem discs from above the pinning stem sections were taken and stored until

Table 1. Principal characteristics of the six analysed pine trees

		Crown transparency (%)		Age (years)	Tree-ring width (*TRW*) (mm)	Tree height (m)	Stem diameter (cm)	Crown length (m)	Crown diameter (m)
		Before	After						
Irrigated	Tree$_1$	5	10	118	1.2	11.8	31.0	4.3	6.8
	Tree$_2$	10	5	61	1.9	10.8	21.0	5.0	4.4
	Tree$_3$	25	30	71	0.6	12.9	25.0	3.6	3.5
Control	Tree$_1$	5	10	114	1.2	9.0	24.5	2.9	4.3
	Tree$_2$	15	50	74	0.7	11.6	19.5	4.5	3.5
	Tree$_3$	25	60	74	1.4	11.4	26.0	3.9	4.3

TRW was calculated for the 10 years before irrigation started (1993–2002). Crown transparency value shows the estimates of March 2003, before the start of irrigation. Tree height, stem diameter, crown length and crown diameter were measured during the experiment (spring 2006).

further processing at low temperature (–20 °C) to avoid fungal infestation of the samples.

Intra- and inter-annual increments

To assess the variation in annual increment, one stem disc per tree was sanded (35 μm particle diameter), and *TRW* was measured on two radii using a Lintab digital positioning table and the software TSAP (both from RINNTECH, Heidelberg, Germany). The two measurements per disc were averaged to obtain a mean value per tree.

To study the seasonal increment, cores (19 mm) including the pinning wound were extracted from the stem section and stored in Strasburger solution [one-third ethanol (95%), one-third glycerol (99%) and one-third distilled water] at –20 °C. Using a sliding microtome (Reichert, Vienna, Austria), thin sections (thickness of 10 μm) were cut from the cores including the pinning wound. The thin sections were stained with safranin (1% solution) and astrablue (2% solution) for a better contrast between cell wall and cell lumen. Subsequently, the sections were dehydrated with an alcohol concentration gradient (70, 95% and absolute ethanol), then in xylol (>98%) and permanently embedded in Canada balsam. Pictures of the thin sections were taken (100× magnification, microscope: Olympus BX41, Tokyo, Japan; camera: ColorView III, Soft Imaging system, Muenster, Germany), and the seasonal increments as well as the corresponding *TRW* were measured on the same thin section using the software IMAGE PRO PLUS (Media Cybernetics, Silver Spring, MD, USA). The seasonal increment was measured as the distance between the last tree-ring border (between year 2004 and 2005) and the last mature cell below the pinning wound (= a cell with a secondary wall, determined by polarized light).

The timing of wood formation was analysed by identifying three phases of tree ring formation: (1) the first mature tracheid found below the wound tissue was regarded as a sign for the beginning of wood formation; (2) early-/latewood transition was reached if the last mature cell was surrounded by latewood cells; and (3) the tree ring was regarded as finalized, if a continuous band of flattened latewood cells was visible, completing the current tree ring.

Sample preparation for isotope analysis

The second stem disc from each tree was dried in the laboratory, and a radial bar, including the last 10 tree rings, was cut from each disc using a band saw. Early- and latewood between the years 1996 and 2005 were separated with a scalpel under a stereomicroscope (Leica Wild M3B, Wetzlar, Germany; 40× magnification), and homogenized using a mill (Ultra Centrifugal Mill ZM 200, Retsch, Haan, Germany).

To analyse the seasonal variation of δ^{13}C values, an additional bar was cut from the disc, and the last tree ring (2005) was sliced by cutting tangential thin sections (thickness of 15 μm) of the specimen using a sliding microtome. Depending on the *TRW* (0.7–2.1 mm), between 43 and 138 thin sections per tree ring were obtained. To combine the tissue built in the same period of time, we used the relative increment, giving the percentage of the total annual increment built between the pinning dates. It was calculated by relating the seasonal increment (increment$_{\text{seasonal}}$) to the corresponding *TRW*, measured near the wound (increment$_{\text{relative}}$ = increment$_{\text{seasonal}}$/*TRW*). To assign the thin section to the time scale, the total number of thin sections per tree ring was regarded as 100%, and all sections representing the tissue built between two pinning dates were pooled. For example, if 5% of the annual increment was developed since the date of the first pinning, then the first 5% of thin sections were pooled as the first sample. If 20% of the tree ring developed until the second pinning, then the next 15% of thin sections were pooled, and so on. All thin sections belonging to the same pinning date were crushed with an ultrasonic processor (UP100S, Hielscher, Teltow, Germany) as milling was not possible because of the small sample size.

The wood powder (about 0.6 mg) of the early- and latewood samples and the thin sections was weighed into tin cups, combusted to CO_2 in an elemental analyser (EA-1110, Carlo Erba Thermoquest, Milan, Italy). The elemental analyser was linked to an isotope ratio mass spectrometer (Delta-S Finnigan MAT, Bremen, Germany), which was operating in the continuous flow mode for the determination of the C-isotope ratio. All δ^{13}C values were expressed relative to the international standard Vienna Pee Dee Belemnite (VPDB): $\delta^{13}C = (R_{sample}/R_{VPDB} - 1)$, with R being the $^{13}C/^{12}C$ ratio of the sample or the VPDB standard. The measured isotope ratio was expressed in the δ notation in ‰. To refer tree-ring $\delta^{13}C$ data relative to the pre-industrial standard, data were corrected by adding the difference between the current atmospheric $\delta^{13}C$ value at the time when the respective tree ring was formed and the standard (pre-industrial) value to the current $\delta^{13}C$ value of each tree ring (McCarroll & Loader 2004).

Climatic data

To compare inter-annual $\delta^{13}C$ values with climatic conditions, a *DI* was calculated as estimate for water availability. The *DI* was computed as $DI = P - PET$ using monthly data of precipitation sums (P) and potential evapotranspiration (PET) according to Thornthwaite (1948), with climate data coming from the weather station in Sion. For the climate correlation with $\delta^{13}C$ values, the monthly *DI* was inverted and summed up for the 2 months before and the month of early- or latewood formation (earlywood formation = mid-April to mid-June; therefore, climate data were summed up for the period February to June; latewood formation = mid-June to the end of September; therefore, climate data were summed up for the period April to September). For the seasonal comparison between $\delta^{13}C$ values and climatic conditions, daily data of temperature (weather station in Sion) and soil moisture [measured on the experimental site at 10 cm depth using Time Domain Reflectometry (1502B cable tester; Tektronix, Beaverton, OR, USA)] were smoothed by a moving window of 15 d.

RESULTS

Inter-annual $\delta^{13}C$ signal

Inter-annual variation of *TRW* showed a rather high conformity to the seasonal *DI* in the pre-irrigation period until 2002 (Fig. 1). However, because of the strong variation among the trees, the mean correlation averaged over the individual correlation between the tree ring series and the seasonal *DI* was not significant ($r = 0.44$). Regarding $\delta^{13}C$ values, the conformity with water availability was more pronounced (Fig. 2). The correlation between $\delta^{13}C$ and the inverse seasonal *DI* was highly significant for early- and latewood. In contrast to the correlation between earlywood $\delta^{13}C$ and the previous earlywood (see autocorrelation in Table 2) or the previous latewood $\delta^{13}C$ (see 'latewood$_{previous}$' in Table 2) was not significant at all. As $\delta^{13}C$ values in early- and latewood both strongly responded to the water availability (high correlation with the seasonal *DI*) under naturally dry conditions, the linear regression between $\delta^{13}C$ in both tissues was highly significant (see 'non-irrigated' in Fig. 3; $P < 0.001$, $R^2 = 0.81$).

The irrigation experiment started late in June 2003, which was an unusual hot and dry year. As the bulk of the tree ring (especially earlywood) was already formed at this date, *TRW* in all analysed trees, even in the irrigated ones, decreased in this dry year (Fig. 1). But, in 2004, a clear differentiation according to the treatment was observed, as *TRW* clearly increased in all irrigated trees, while it remained low in the control, non-irrigated trees.

For the $\delta^{13}C$ values, we found an immediate response to irrigation as $\delta^{13}C$ values in latewood decreased already in 2003, the very first year of the experiment (Fig. 2). Earlywood $\delta^{13}C$ values also responded immediately to irrigation, as detected by a decrease of the isotopic signature in 2004, the first earlywood formation under improved water supply. Only in tree$_2$ the response to irrigation in earlywood and latewood was delayed by 1 year. However, in control trees, $\delta^{13}C$ values increased and remained constantly high in and after the drought year 2003.

Seasonal variation in $\delta^{13}C$

The 2005 seasonal $\delta^{13}C$ values showed a common pattern with increasing isotope ratios to a maximum in mid-summer and decreasing values until the end of the growth period (Fig. 4a). The shape of the seasonal $\delta^{13}C$ curves is rather smooth with small deviations at the sub-seasonal (bi-weekly) scale.

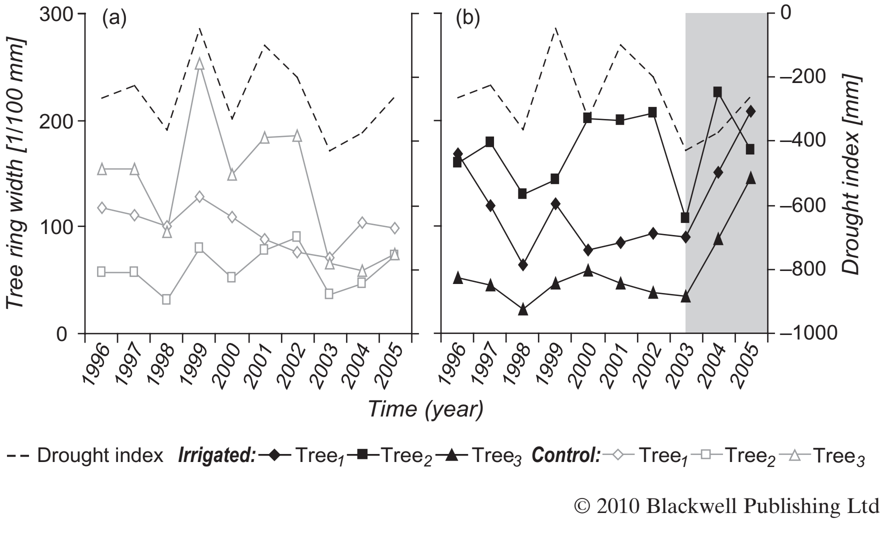

Figure 1. Tree-ring width (*TRW*) of control pine (a) and irrigated trees (b) with the drought index (*DI*) over time (hatched line; summed up for February to September). The irrigation period (June 2003–2005) is highlighted as shaded area.

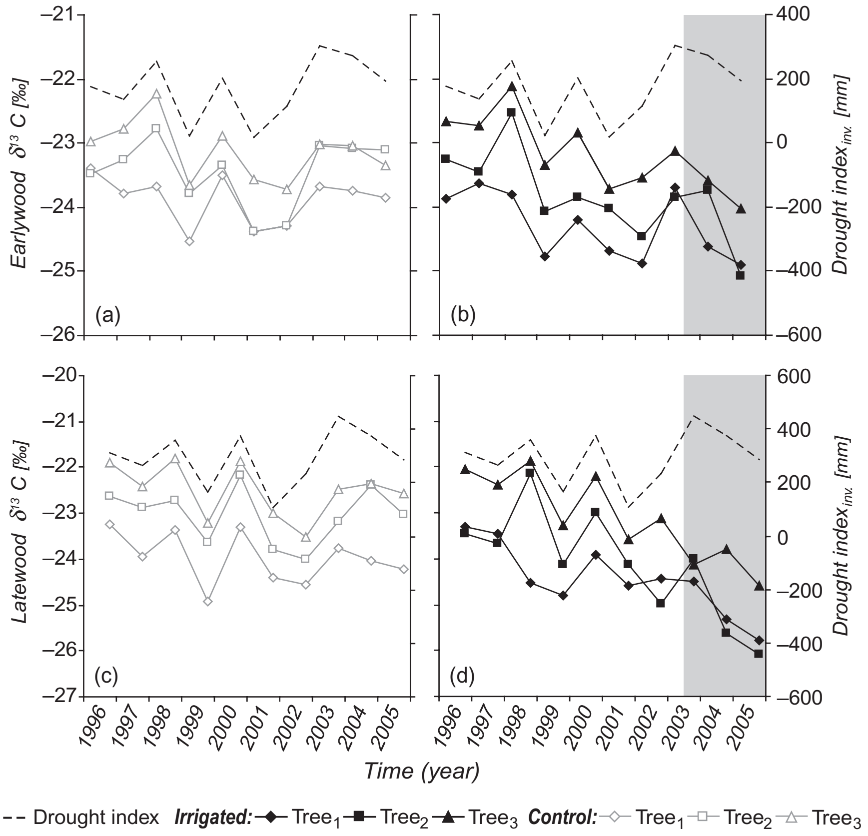

Figure 2. δ^{13}C values in early and latewood of control (a,c) and irrigated Scots pine (b,d). Top δ^{13}C in earlywood (a,b) together with the drought index (*DI*) (summed up for February to June). Bottom δ^{13}C in latewood (c,d) together with the *DI* (summed up for April to September). δ^{13}C in earlywood and latewood are shifted against each other in the graph and plotted with the respective time period in which they were built. The irrigation period (June 2003–2005) is highlighted as shaded area.

The irrigated trees showed significantly lower δ^{13}C values over the entire growth period compared to the control trees (Wilcoxon signed rank test, $P < 0.001$) because of clearly higher soil moisture (Fig. 4c). In addition, the maximum of the seasonal δ^{13}C curve was shifted because of irrigation and occurred 2 weeks earlier compared to the control trees.

For the control trees, maximal δ^{13}C values were observed after the hottest and driest days of the year (Fig. 4a versus c) concurrently with early/latewood transition and a drop in the growth rate (see slope in Fig. 4b).

In irrigated trees, the highest δ^{13}C values were reached in the week prior to the hottest and driest period of the year.

Table 2. Autocorrelation coefficient (Auto) and correlation coefficients between the tissues (earlywood versus current or the previous latewood) and with climate conditions [drought index (*DI*)] of δ^{13}C values in earlywood and latewood

	Auto	Latewood$_{current}$	Latewood$_{previous}$	$DI_{seasonal}$
Earlywood	0.07	0.84**	0.11	0.76**
Latewood	0.09			0.71**

δ^{13}C in earlywood was correlated with the *DI* summed up for the months February to June. δ^{13}C in latewood was correlated with the *DI* summed up for the months April to September.
**$P < 0.01$.

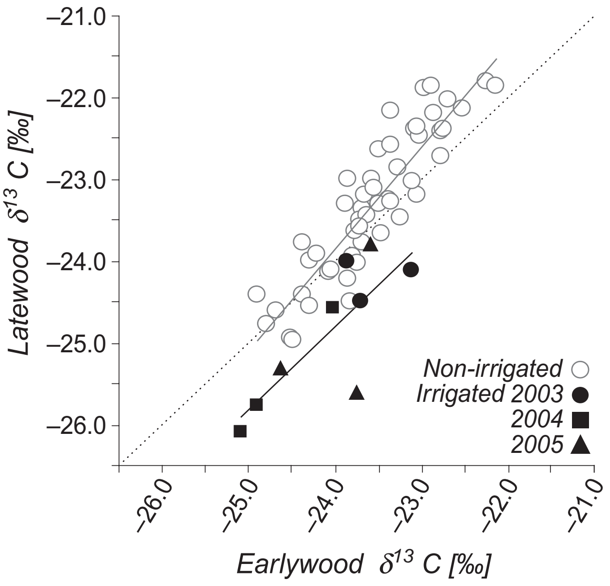

Figure 3. δ^{13}C values in latewood versus earlywood. The open symbols comprise δ^{13}C values of the pre-irrigation period (1996–2002) and control trees for the years 2003–2005. Closed symbol marks δ^{13}C values of irrigated trees in the years 2003, 2004 and 2005. The regression for these pre-irrigated and control trees was $y = 1.2438x + 6$, $R^2 = 0.81$, $n = 51$, $P < 0.001$. The regression for the irrigated period (2004 and 2005, only, as during earlywood formation in 2003 no irrigation took place) was $y = 1.0276x - 0.13$, $R^2 = 0.64$, $n = 9$ and not significant.

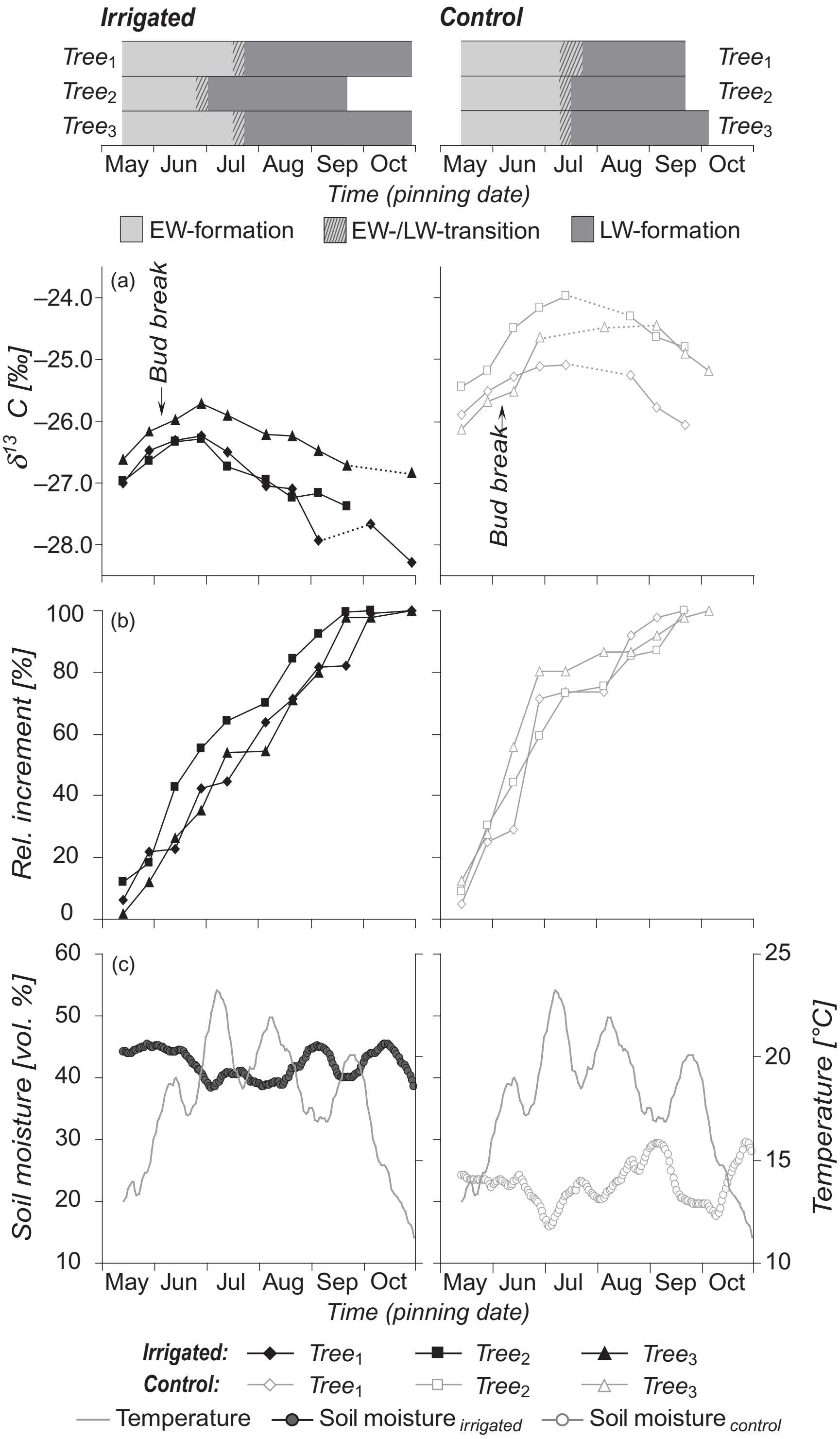

Figure 4. Timing of earlywood (EW) and latewood (LW) formation, and (a) the intra-annual variations of the seasonal δ^{13}C signal, and (b) the relative increment in irrigated and control Scots pines together with (c) a running mean (15 d) of temperature and soil moisture on irrigated and control sites within the growth period in the year 2005. If data were missing in the δ^{13}C data set, adjacent points were jointed with dashed lines. Bud break occurred between the second and the third pinning, and was marked with an arrow in diagram (a).

The maximum in δ^{13}C values were not associated with the early/latewood transition or an early decrease of the growth rate. In contrast to control trees, no growth depression or stop was observed in irrigated trees before the end of the growing season. In contrast to the control trees, no clear correlation with growth or climatic conditions was observed in the occurrence of the highest δ^{13}C values in irrigated trees.

DISCUSSION

Inter-annual time-scale

Previous studies showed that earlywood formation strongly depends on previously stored carbohydrates (Smith & Paul 1988; Kagawa *et al.* 2006). Thus, $\delta^{13}C$ in earlywood is expected to reflect a climate impact, which is more related to the previous year(s) than to the weather conditions of the current year (see Porte & Loustau 2001 for *Pinus pinaster*). In contrast, our data showed a high sensitivity of early- and latewood $\delta^{13}C$ to instantaneous (current year) water availability (see Fig. 2 and Table 2 correlation with the seasonal *DI*, $P < 0.01$) and low correlations with the climate conditions of the previous year (see Table 2, autocorrelation and correlation between early- and the previous latewood). As $\delta^{13}C$ in early- and latewood reflects the current climate conditions, the relationship between $\delta^{13}C$ in the two tissues is very high ($P < 0.001$; Fig. 3). These results suggest a tight coupling of wood formation and currently produced photosynthates. The assumption of a rapid and direct use of currently produced assimilates for biomass production was also supported by the immediate response to irrigation observed in early- and latewood $\delta^{13}C$ (Fig. 2). Hence, an intensive mixing of old and new carbohydrates masking the current year's climate signal in earlywood is very unlikely for the trees we studied. This is in contrast to the other observations for deciduous trees in a temperate forest (Keel, Siegwolf & Körner 2006; Keel *et al.* 2007) and for conifer trees at the upper tree line (von Felten *et al.* 2007). We assume that the low mixing of new assimilates with stored photosynthates indicated by our data is caused by a low amount of stored carbohydrates. During the preceding years of chronically dry conditions in the Valais, the plants could not synthesize sufficient carbohydrates to sustain growth and the ongoing metabolic processes, which resulted in a depletion of C-storage. The deviating response pattern of $tree_2$ could be caused by the following: compared with the other irrigated trees, $tree_2$ showed a premature switch to latewood formation and an earlier cessation of wood formation in 2005 (Fig. 4). The year 2003, the first year of irrigation, was particularly dry. As earlywood latewood transition and the cessation of wood formation are expected to occur even earlier under drought (Dünisch & Bauch 1994), it is very likely that $tree_2$ was not able to response to the start of irrigation as the bulk of latewood was already formed. This could have caused the increase in $\delta^{13}C$ for the latewood in 2003 leading to a carry-over effect in the $\delta^{13}C$ of the earlywood of the following year (2004), which rather increased instead of decreased, contrary to trees 1 and 3. As for the reason, we can only assume that the irrigation was not as effective for $tree_2$, either because the irrigation water did not reach the roots as abundantly and fast as for the other two trees. The reason for the decreasing *TRW* in 2005 in $tree_2$ might be a result of natural variation in radial increment or because of the generally high increment rate in $tree_2$, allowing no strong increase in the radial increment after the maximal *TRW* in 2004.

However, our results suggest that trees growing under constantly dry conditions are source limited. According to the source limitation theory (see Berman & DeJong 1996), carbon availability is reduced under drought if photosynthetic activity is reduced, but carbon demand remains constantly high. Most recent modelling results support this theory: photosynthesis was shown to be more sensitive than respiration to the influence of drought at the stand level (Schwalm *et al.* 2010). The resulting storage depletion might explain the long-lasting growth depression in Scots pine after severe drought events (Eilmann *et al.*, unpublished results), which can also be observed in $tree_2$ and $tree_3$ of the control plot after the drought year 2003 (Fig. 1).

However, according to the sink limitation theory, drought would reduce tree growth first. This lack of sink activity would lead to carbohydrate accumulation in the leaves inhibiting photosynthesis. The direct effects of drought on photosynthesis are expected to be rather low (Körner 2003; Bacelar *et al.* 2006). Following this theory, carbohydrate supply should never be limited. In case of a sink limitation, we would not have seen such an immediate response to irrigation in $\delta^{13}C$. The new assimilates would have been mixed with the large pool of stored carbohydrates, and the change in signal would have been delayed and dampened, which is in contrast to the results of our experiment. The sink limitation theory is based on studies with species that are well-adapted to drought, like *Olea europaea* or *Pistacia lentiscus*. In contrast, we analysed the sub-boreal Scots pine growing at the edge of its distribution, which have to cope with meteorological conditions that are changing towards a sub-Mediterranean climate regime. Therefore, the conclusion lays at hand that if a species is growing beyond their ecological optimum, an ongoing drought situation can lead to storage depletion as a result of a persistent carbon imbalance. Low carbon pools reduce trees' resistance against stressors (Bréda *et al.* 2006). Hence, increasing mortality must be expected, when a stressor either increases the need (parasitism) or decreases the production (drought) of carbon reserves.

The strong impact of drought on tree growth in the Valais is underlined by the high $\delta^{13}C$ values observed in trees growing under the prevailing dry conditions. The mean values of –23.6‰ for earlywood and –23.3‰ for latewood (calculated for the non-irrigated period in irrigated trees and for the whole period in control trees) were among the highest ever found in the wood of pine species (e.g. the following values for different trees from different regions were found: –23.9‰ for wood in *Pinus halepensis* in the east Iberian Peninsula (Ferrio & Voltas 2005); <–24‰ analysed for wood in *P. sylvestris* from Southern Germany (Gessler *et al.* 2009); –25.77‰ analysed for wood in *P. sylvestris* from Norway to Sibiria (Saurer, Siegwolf & Schweingruber 2004). These high $\delta^{13}C$ values reflect highly reduced stomatal conductance as a consequence of a chronic drought situation for Scots pine in the Valais.

Seasonal time-scale

In this study, we present exactly dated seasonal δ^{13}C values referring to the timing and the quantity of wood formation. Seasonal variations in micrometeorology and especially altering soil moisture were discussed as main factors responsible for seasonal changes in δ^{13}C (e.g. Leavitt 1993; Barbour *et al*. 2002; Schulze *et al*. 2004). This is in agreement with our data, as maximal δ^{13}C values in control trees were observed after the hottest and driest period of the year (Fig. 4a versus c). The continuous decrease in δ^{13}C values until the end of the vegetation period might be caused by decreasing VPD and increasing relative humidity in the second part of the vegetation period. At the same time, as δ^{13}C was at its maximum, noticeable reductions in wood formation occurred. Trees strongly reduced the growth rate or even stopped growing (see slope in Fig. 4b). This underlines the strong limitation of tree growth under the prevailing dry conditions. Besides the amount of produced wood, its structural constitution changed. Wood formation turned towards latewood production, forming smaller cells with thicker cell walls. This earlier switch to latewood formation is again a reaction to the very dry conditions (Dünisch & Bauch 1994).

In irrigated trees, maximal δ^{13}C values occurred previous to the hottest and driest period of the year (Fig. 4a versus c). In contrast to the early maximum in the seasonal δ^{13}C curve of the irrigated trees, the transition from early- to latewood formation was delayed by improved water supply (Dünisch & Bauch 1994; Rigling *et al*. 2003; Eilmann *et al*., unpublished results). The increment curve showed a late flattening and constantly high growth rates throughout the growing season, a rather common pattern for conifers (Rossi, Deslauriers & Morin 2003). Thus, a substantial limitation of tree growth by climatic conditions could not be observed in irrigated trees. Regarding the level of seasonal δ^{13}C values, we observed significantly lower δ^{13}C values throughout the entire growing season in irrigated trees (Fig. 4a). This is in agreement with the results on the inter-annual level (Fig. 2) and is in line with previous findings (e.g. Livingston & Spittlehouse 1993; Warren, McGrath & Adams 2001; Ferrio & Voltas 2005).

To summarize: in irrigated trees, the point of maximal δ^{13}C values seemed to be decoupled from changes in wood formation or varying climatic conditions, while trees, growing under prevailing dry conditions, show a strong coupling between climate, tree growth and maximal δ^{13}C values.

CONCLUSION

We found a strong relationship between early- and latewood δ^{13}C as both mainly reflect the current climate conditions. Accordingly, the irrigation signal was immediately recorded in the δ^{13}C values. These data, indicating a rapid and direct incorporation of current photosynthates, support the source limitation theory with chronic storage depletion as important mechanism leading to drought-induced mortality. This phenomenon of restricted tree growth as a result of source limitation might only be valid for trees at the edge of their distribution growing under changing climate conditions. However, an ongoing climate change will substantially alter the growth conditions for various species and in different ecosystems. Thus, tree mortality because of preceding storage depletion as a result of continuous drought-induced source limitation is expected to become increasingly relevant.

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