Research paper

The influence of increasing temperature and CO_2 concentration on recent growth of old-growth larch: contrasting responses at leaf and stem processes derived from tree-ring width and stable isotopes

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Time series of tree-ring growth show significant increases since the early 1970s at the alpine tree line, with simultaneously increasing temperatures and atmospheric CO_2 concentration. For a comprehensive understanding of this growth change, the physiological response patterns at both the leaf and stem level need to be separately analyzed and identified, and can be retrieved from tree-ring growth and isotope ($\delta^{13}C$, $\delta^{18}O$) series. In this study, we assessed the relative contribution of environmental factors to interannual tree-ring variability by multivariate linear mixed-effects models and the dual isotope approach on a dataset of tree-ring records of ~400-year-old larch (*Larix decidua* Mill.) from a non-water-limited high-elevation site in the Swiss Alps. The models suggest that summer temperatures and the recent lack of larch budmoth outbreaks were most important for explaining growth variations and trends, while a significant direct effect of the continuously increasing CO_2 concentration could not be confirmed. In contrast, $\delta^{13}C$ and $\delta^{18}O$, which are strongly influenced by fractionation changes in the leaf, clearly reflected the impact of air humidity (precipitation and vapor pressure deficit) and CO_2 concentration: the increase in ($\delta^{13}C$-derived) intrinsic water-use efficiency over the second half of the 20th century suggests an increase in carbon assimilation as a result of enhanced CO_2 concentration. The tree-ring $\delta^{18}O$ largely reflected recent precipitation as source water, thus indicating a low variability in stomatal conductance, which was confirmed by the dual isotope approach. These leaf-level effects were not reflected in stem growth as they may have been masked by the temperature-caused growth limitation controlling the allocation of increased amounts of photosynthates into wood growth. Our approach demonstrates that the identification of different roles of environmental factors on leaf and stem processes helps to improve the assessment of site-specific changes of carbon fluxes and growth performance under future environmental conditions.

Keywords: [13]C, climate response, dual isotope approach, *Larix decidua*, mixed-effects model, ^{18}O, tree growth.

Introduction

Since around the early 1970s, increased tree growth has been observed at non-water-limited regions, particularly at high altitude and high latitude sites (e.g., Boisvenue and Running 2006, Bolli et al. 2007, Bošela et al. 2014). This increase is synchronous mainly with increasing growing temperatures, atmospheric CO_2 concentration (Hartmann et al. 2013), and in the vicinity of rural and industrial regions nitrogen emissions (Reay et al. 2008). At high-elevation sites, where trees grow at their climatic and edaphic limits within short growing seasons, sensitivity to climate variability is particularly pronounced (Tranquillini 1979), although trees do not seem to be carbon-limited (Körner 2003). Tree-ring based studies including the isotope signature of $\delta^{13}C$ and/or $\delta^{18}O$ in these high-altitude ecosystems may reveal medium- to long-term-changes in response and sensitivity to climate change (e.g., Carrer and Urbinati 2006, Salzer et al. 2009,

Kress et al. 2010, Gomez-Guerrero et al. 2013, Wieser et al. 2016). Yet, it remains challenging to draw conclusions about the underlying ecophysiological response mechanisms based on the information derived from tree rings. The combined impacts of the driving factors (i.e., temperature, water availability and elevated CO$_2$) controlling the water balance (stomatal conductance, g_s), net carbon assimilation (A_N) and allocation to net stem wood production are far from being fully understood (Schleser et al. 1999, Gessler et al. 2014).

Interannual tree-ring growth variability provides information on growth conditions. For instance, moderate temperatures, high humidity and non-limiting water supply are ideal conditions for biomass production resulting in wide tree rings. Usually this corresponds with high assimilation rates at high stomatal conductance. Small tree rings can be the result of either low temperatures or drought, while in both cases assimilation rates are low. Photosynthesis and growth are not strictly coupled, as trees may assimilate carbohydrates that are not invested into growth. At temperatures between 2 and 7 °C still considerable assimilation rates can be observed, whereas the rate of cell division during growth is drastically reduced (Körner 2015). As soon as temperatures increase, growth is resumed under inclusion of assimilates, which contain the isotope signatures that correspond to milder weather conditions.

Stable carbon and oxygen isotopes are considered as a more direct indicator of tree physiological responses to environmental impacts than tree growth, since those signals are mainly affected by environmental conditions during photosynthesis. Farquhar et al. (1982, 1989) showed that the carbon isotope fractionation (Δ) is inversely proportional to photosynthesis and directly correlated with g_s, while the oxygen isotope ratio is inversely proportional to g_s (Farquhar and Loyd 1993, Cernusak et al. 2002, 2007).

While the cellulose signature of ^{13}C is mainly a function of the ratio of the partial pressure of CO$_2$ in the substomatal cavities of the leaf to the partial pressure of CO$_2$ in the atmosphere (c_i/c_a), which is determined by possible changes in (i) atmospheric CO$_2$ concentration, (ii) photosynthetic capacity and (iii) stomatal conductance, the ^{18}O signature is rather a function of the ratio of atmospheric and intercellular vapor pressure (e_a/e_i) as influenced by (i) relative humidity and (ii) stomatal conductance, but also determined by possible variation in soil water such as the primary water source and vapor ^{18}O (e.g., Barbour 2007, Cernusak et al. 2016). Changes in environmental conditions, such as temperature, precipitation, vapor pressure deficit, CO$_2$ concentrations and irradiation, may thus differently affect the isotope values of ^{13}C and ^{18}O in the plant tissue (e.g., Ferrio and Voltas 2005, Treydte et al. 2007, Knorre et al. 2010, Mutlu et al. 2012). The variation of both, δ^{13}C and δ^{18}O, of tree-ring cellulose over time reflects the tree's responses to environmental changes and facilitates the identification of possible adaptation mechanisms (Saurer and Siegwolf

2007). Thus the stable isotope ratio serves as a suitable proxy representing the response of physiological processes to changes in the environment. Derived from biomass, i.e., leaves or tree rings, it represents the integral of the immediate responses of A_N and g_s, imprinting the corresponding isotopic fingerprint on assimilates that are ultimately deposited in the biomass/tree rings throughout the growing period.

The isotope ratio of the leaf tissue can therefore be considered as an integral of the whole prevalent climatic spectrum during its expansion (structural tissue) and life span (non-structural carbohydrates). However, the 'link' between leaf-level carbon gain and stem wood production, the 'carbon allocation', shows a different response pattern to environmental conditions than leaves. For example, while increased carbon assimilation under elevated CO$_2$ concentrations is observed (Streit et al. 2014), tree (stem) growth may be insensitive to additional carbon supply (Bader et al. 2013, 2016, Klein et al. 2016), particularly if other environmental conditions, such as drought or low temperatures, limit growth (Andreu-Hayles et al. 2011, Gomez-Guerrero et al. 2013).

This discrepancy between leaf gas exchange processes (determining stable isotope rensponses) and tree growth indicates two different physiological domains, namely leaf CO$_2$ uptake and stem growth. The latter often occurs within a limited period and under environmental conditions, which are more stringent than for photosynthesis. Thus, tree-ring growth and isotope signatures are not directly linked, neither temporally during their genesis nor physiologically. The isotopic composition of the incorporated assimilates can therefore reflect climate conditions that are distinct from those of the actual stem growth period.

In this study, we present a concept to combine measurements of stem growth with isotope signatures (δ^{13}C, δ^{18}O) of tree rings by linear mixed-effects models and the conceptual dual isotope model (Scheidegger et al. 2000) applied at the tree-ring level (Saurer and Siegwolf 2007), to identify environmental drivers for tree physiological processes from carbon assimilation to stem production. Although it is difficult to fully disentangle the impact of multiple factors that simultaneously affect plant growth, such multivariate statistical approaches provide a powerful tool to quantitatively investigate the relative contribution of a variety of factors on tree-ring time series, but have rarely been applied in combined growth/stable isotope studies (Martínez-Vilalta et al. 2008, Martin-Benito et al. 2011, Kint et al. 2012). As a case study, a dataset of five ~400-year-old European larch (*Larix decidua* Mill.) trees at a high-altitude site in the Swiss Alps (Kress et al. 2009a, 2010) was investigated to determine the possible contributions of increasing CO$_2$ concentrations and temperature on physiology and stem growth between 1900 and 2004, considering also other interfering factors like budmoth (larch budmoth, LBM) outbreaks. This dataset contains tree-ring growth and isotope data, and has not been evaluated in view of relating leaf-level physiology to stem growth.

In particular, the following questions were addressed: (i) Which are the main environmental factors affecting stem growth and leaf physiological processes of high-elevation larch trees? (ii) Do old-growth larch trees at high elevation increase tree growth due to increasing CO_2 concentrations and/or temperature? (iii) Does responsiveness to climate factors, e.g., with respect to carbon allocation patterns, change in more recent decades, i.e., before and since the sharp increase in global CO_2 concentrations in the 1960s and the subsequent increase in temperature?

Materials and methods

Study site and tree-ring data

The study is based on a dataset of five individual European larch trees (*L. decidua* Mill.) with ages between 390 and 460 years, growing at ~2100 m above sea level in the region of the Lötschental, Swiss Alps (Kress et al. 2009*a*, 2010). The site has a mean annual temperature of 6.4 °C and annual precipitation of 1161 mm (1900–2004). It is located at the tree line within mixed European larch-Swiss Stone pine (*Pinus cembra*) forests. Within the site, three of the five studied trees were sampled from a SSE-facing slope (46°26′ N; 7°48′ E) dominated by podzolic cambisols, while two of the trees were sampled from a NNW-facing slope (46°23′ N; 7°47′ E) on ferric podzols (Treydte et al. 2001, Kress et al. 2010). Despite these differences in microclimatic conditions, all trees were exposed to the same regional climate. Tree-ring width (TRW), as well as $\delta^{13}C$ and $\delta^{18}O$ of tree-ring cellulose were used from a time period between 1900 and 2004. Sample preparation and measurements are presented in Kress et al. (2010). The TRW data from the investigated time period did not show any age trend for the old-growth trees at the established stand. Therefore, no detrending of a juvenile effect was performed. The $\delta^{13}C$ of tree-ring cellulose was corrected for the isotopic shift of the $\delta^{13}C$ in the atmosphere according to Leuenberger (2007). Due to the increasing release of CO_2 from fossil fuel combustion the atmospheric CO_2 is progressively depleted in $\delta^{13}C$ (Suess effect; Keeling 1979).

Climate data were derived from the public databases HISTALP (for both precipitation and temperature using a regional average; Kress et al. 2010) and from KNMI (http://climexp.knmi.nl; CRU TS3 data of vapor pressure). CO_2 concentrations were taken from the NOAA records (http://www.esrl.noaa.gov/gmd). Monthly vapor pressure deficit (VPD) was calculated via mean monthly temperature and vapor pressure as

$$\text{VPD (hPa)} = c_1^{(c_2 T/(c_3+T))} - \text{Vap} \qquad (1)$$

where T is the average monthly temperature, Vap is the average monthly vapor pressure, c_{1-3} are constants with $c_1 = 6.1078$, $c_2 = 17.08085$ (at $T > 0$) or $= 17.84362$ (at $T < 0$), and $c_3 = 234.175$ ($T > 0$) or $= 245.425$ ($T < 0$), according to Germany's National Meteorological Service (Deutscher Wetterdienst (DWD) 1979).

Statistical models

Multivariate linear mixed-effects models (combining both, fixed and random factors for hierarchical data structures) were applied to study TRW and isotope variations over time as a function of environmental and tree-specific factors (Table 1). Predictor variables were chosen following screening of a variety of candidate variables and their combinations including previously described climate correlations for the respective site (Kress et al. 2009*a*). Those variables that showed significant relationships ($P < 0.05$) with TRW, $\delta^{13}C$ or $\delta^{18}O$ according to simple linear regression analysis were included in the initial models (Table 1). For example, while the CO_2 concentration was positively correlated with annual mean temperature ($r = 0.7$) due to their common long-term trend (but not in interannual variability), it did not show any significant correlation with TRW or isotopes, in contrast to temperature. In addition, CO_2 was not considered as imposing a direct effect on ring growth or $\delta^{18}O$, rather acting through intrinsic water-use efficiency (iWUE), which was included as an additional factor (see below). CO_2 was therefore only included in the initial $\delta^{13}C$ model to account for a possible effect in combination with other factors.

Since environmental factors may show collinearity among each other, a careful selection of biologically most relevant independent factors and/or a combination of factors as interaction term should be considered (e.g., Dormann et al. 2013). In support of this procedure, the variance inflation factor (VIF) was applied as a selection criterion and variables with the most direct consequences for physiology and growth retained in the final model (Zuur et al. 2010). For example, temperature was considered a more direct factor influencing TRW than the (correlated) VPD, while for $\delta^{13}C$, VPD was preferred over temperature due to its strong influence on stomatal conductance. A VIF <3 was used as threshold for factor selection.

Continuous predictor variables (Table 1, except for 'LBM' and 'Time period') were standardized by subtracting the mean and dividing by twice the standard deviation (SD) to obtain a mean of 0 and SD of 0.5. This standardization allows for direct comparison between model output estimate values, i.e., effect sizes, of both continuous and binary predictors (Gelman 2008, Schielzeth 2010). Note that factor effects estimate values can only be compared within models and not between models. To account for the interdependency in the time series of each tree and the variability among them, individual tree was defined as a random effect. Autocorrelation structures (detected from the residuals of initial models for both ring growth and isotopes) were integrated into the full models as autoregressive-moving-average functions ARMA(1,1) for $\delta^{13}C$ or ARMA(2,1) for TRW and $\delta^{18}O$, respectively. This approach may adequately describe tree-ring time series, accounting for the first and/or second previous year's growth as well as for a 'random shock' as moving average terms of the order 1 (Monserud and Marshall 2001).

Table 1. Environmental variables used as covariates (i.e., fixed factors) in linear mixed-effects models for tree-ring growth, $\delta^{13}C$ and $\delta^{18}O$.

Covariate	Selection criteria
LBM and LBM_{lag1} (larch budmoth event of current and previous year)	Outbreaks of the gray larch budmoth (*Zeiraphera diniana* Gn.) can cause significant reductions in tree growth; such events were detected at the study site (northern and/or southern slope) for the years 1908, 1912, 1915, 1923, 1937, 1945, 1954, 1963, 1972, 1974 and 1981 (Kress et al. 2009*a*); although three types of LBM event intensity can be distinguished (Weber 1997, Weidner et al. 2010), a simple binary variable 'present/absent' appeared to be most suitable; previous year's larch budmoth event accounted for severe and/or late seasonal outbreaks
T_{summer} and T_{lag1} (temperature of current year's summer and previous year's annual mean)	Influence of current year's temperature on tree-ring growth was best represented by the summer temperature as a mean of June–August monthly mean temperatures, indicating the season of highest physiological activity; in addition, average temperature conditions of the previous year may influence regulations (genes, phytohormones, etc.) related to physiological adaptations; 'lag1' refers to previous year mean including winter season, i.e., $(pNov-Oct)_{lag1}$
P_{summer_JA} and $P_{\_JA\_lag1}$ (sum of July–August precipitation of current and previous year)	Summer precipitation of July and August correlated best with isotopic signatures, indicating the most significant time of water availability for stem growth
VPD_{summer_JA} and $VPD_{\_JA\_lag1}$ VPD_{summer} and VPD_{lag1} (mean vapor pressure deficit of current year's summer and previous year's annual mean)	VPD of July and August showed correlated best with both $\delta^{13}C$ and $\delta^{18}O$, indicating direct influence on stomatal conductance controlling gas exchange; for TRW, the previous year's annual mean (previous growing season including winter season before) was included as a potential driver of seasonal drought stress
iWUE	Since CO₂ was considered as an indirect factor influencing tree-ring growth, its effects were tested via iWUE. This is a proxy for stomatal conductance and CO₂ assimilation, calculated by yearly CO₂ concentrations and annually measured $\delta^{13}C$ (see below).
CO₂ concentration (annual mean)	While mean annual CO₂ concentration did not show any significant relationship to TRW or isotopic signature, it was included in the initial $\delta^{13}C$ model to check for a possible 'fertilizer effect' on photosynthesis
Time period (before 1962 and since 1962)	The two periods of 1900–61 and 1962–2006 were separated because atmospheric CO₂ concentration has increased sharply since approximately 1962, which may have changed the tree growth responses to increasing CO₂; in addition, mean annual temperature has also risen since ca the 1960s and may have led to an altered growth response
Tree size^2	The squared tree radius showed a significant relationship with TRW and $\delta^{18}O$ and was used as additional tree characteristic

Starting with a 'full' model that contained all the defined environmental variables, the model was then reduced by stepwise selection of non-significant factors and the best model was chosen based on the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) using the maximum likelihood method (ML) (Pinheiro and Bates 2000, Zuur et al. 2009). Both model comparison criteria depend on the number of factors used in the model, while BIC also accounts for the number of observations (Zuur et al. 2009). The final models, inspected for homogeneous distribution of residuals, were fitted using the restricted maximum likelihood method (REML). The R package nlme (version 3.1-113, Pinheiro et al. 2013) was used to calculate the models.

The dual isotope theory and water-use efficiency

Results of the mixed-effects models were compared with the dual carbon and oxygen isotope approach that serves to provide additional insight into plant physiological processes (Scheidegger et al. 2000, Saurer and Siegwolf 2007). At the leaf level, plants respond actively to environmental conditions by regulating gas exchange of CO₂ and H₂O via stomata and photosynthesis. This affects the signature of both, $\delta^{13}C$ and $\delta^{18}O$ in plant material as a result of physical and biochemical fractionation processes during gas exchange and incorporation into plant material. Thereby, the fractionation of ^{13}C by photosynthesis is well described according to Farquhar et al. (1982) by

$$\delta^{13}C_{plant} = \delta^{13}C_{air} - a - (b - a)c_i/c_a \qquad (2)$$

where a is the fractionation factor for $^{13}C/^{12}C$ of CO₂ diffusing in air (4.4‰), b is the fractionation factor for $^{13}C/^{12}C$ during carboxylation in C3 plants (27‰), c_i is the partial pressure of CO₂ in the substomatal cavities of the leaf and c_a is the partial pressure of CO₂ in the atmosphere. Note that this simplified

model does not account for potential fractionation related to boundary layer as well as mesophyll conductance and respiration (Farquhar et al. 1982).

The iWUE of the individual trees over time was then calculated from measured $\delta^{13}C$ data of tree-ring cellulose as an average value for each year, as

$$iWUE = [CO_2]_{air} / 1.6 (1 - (\Delta^{13}C - a)/(b - a)) \quad (3)$$

where $[CO_2]_{air}$ is the atmospheric CO_2 concentration of the respective year, and $\Delta^{13}C$ is the discrimination ($\approx$ difference between the atmospheric $\delta^{13}C$ (derived from Leuenberger 2007 and tree-ring $\delta^{13}C$ raw value), as calculated by

$$\Delta^{13}C = (\delta^{13}C_{air} - \delta^{13}C_{cellulose})/(1 + \delta^{13}C_{cellulose}/1000) \quad (4)$$

The fractionation of ^{18}O in the plant is described according to Dongmann et al. (1974), as

$$\delta^{18}O_{leaf} = \delta^{18}O_{sw} + \varepsilon_k + \varepsilon_e \\ + (\delta^{18}O_{vapor} - \delta^{18}O_{sw} - \varepsilon_k)e_a/e_i \quad (5)$$

and adapted for tree-ring cellulose (Saurer et al. 1997a), as

$$\delta^{18}O_{cellu} = \delta^{18}O_{sw} + f\,[\varepsilon_k + \varepsilon_e \\ + (\delta^{18}O_{vapor} - \delta^{18}O_{sw} - \varepsilon_k)e_a/e_i] + \varepsilon_c \quad (6)$$

where $\delta^{18}O_{sw}$ is the isotopic signature of source water taken up by the trees, $\delta^{18}O_{vapor}$ is the signature of the water vapor in the air, ε_k represents the kinetic fractionation during diffusion of water vapor (26.5 ‰), and ε_e the equilibrium fractionation due to phase change (9.8‰), e_a/e_i is the ratio of the atmospheric and the intercellular vapor pressure, ε_c refers to the biochemical fractionation during cellulose formation (27‰) and f describes a factor of dampening of the leaf water $\delta^{18}O$ signal in stem cellulose. The proportion of the leaf $\delta^{18}O$ signal may be further influenced by the 'Péclet effect', i.e., the dilution of the ^{18}O-enriched leaf water used for sugar synthesis through increased xylem water flow under high transpiration (Farquhar and Lloyd 1993, Barbour et al. 2004). Due to these effects, a signal of stomatal conductance is also recorded in the wood isotope signal. This, however, holds only if other important factors in Eq. (5), such as the $\delta^{18}O$ of source water and of water vapor, do not vary strongly over time. Further, the signal is influenced not only by stomatal conductance but also to some degree by leaf temperature (increase in e_i). During the assimilate transport between leaf and wood the isotopic signal is dampened, through oxygen exchange between the phloem and xylem water (Roden et al. 2000) and diluted with stored assimilates. In a natural system where these factors are beyond control, physiological interpretations of changes in the $\delta^{18}O$ signal should be made with care as outlined in Roden and Siegwolf (2012).

Results

Temporal trends and variability between trees

Yearly variations in radial stem increment across the different trees showed a rather uniform pattern during the first half of the 20th century (Figure 1). From around 1970 on, TRW increased in most trees, while variability between trees also increased. In this recent period, a sharp short-term decline in growth was observed in all trees in 2004, indicating effects of the severe European-wide summer drought in 2003 (Ciais et al. 2005). In contrast, both, $\delta^{13}C$ and $\delta^{18}O$ of stem cellulose did not show a clear long-term trend, but also varied between years and individual trees. In particular, differences (offsets) between trees were high for $\delta^{13}C$ although showing a rather uniform pattern over time. This suggests tree-individual physiological performance perhaps related to canopy size and whole-tree leaf area. In contrast, $\delta^{18}O$ varied strongly between years, but with fewer differences between individual trees except for some time periods, e.g., 1930s, 1950s and the 1980s. Dry conditions in 2003 were partly reflected by high $\delta^{18}O$.

Sudden growth reductions were observed across all trees, e.g., 1909, 1946, 1964 and 1972/73. These reductions can be partly attributed to LBM outbreaks in the respective or the previous year, but they did not affect the isotopic signature as previously described for the same study trees (Kress et al. 2009a). However, the $\delta^{18}O$ appeared to be partly lower during years of LBM outbreaks (see below).

While CO_2 concentrations have increased globally since approximately 1962, the mean annual temperature in the southern Swiss Alps increased from the 1980s to present (Figure 1). Depending on air temperature, the VPD also increased slightly from the 1990s to present. It showed a peak in the exceptionally dry year 2003. Annual precipitation in this region, however, remained unchanged across the 20th century, with the exception of individual years of very low (1921, 2003) or high (1965, 1999, 2002) precipitation.

Tree-ring growth and environmental impact factors

The linear model describing TRW variability as influenced by environmental conditions did not show any effect of the two distinguished time periods (i.e., different slopes in CO_2 increase), thus resulting in the best model M2 obtained from a 'full' model (M1; Table 2). The two models were not significantly different from each other, thus we can consider the smaller model as appropriate to describe TRW patterns. Comparing the effects estimates within each model, the most important drivers for tree-ring growth of these trees appeared to be temperature and LBM, acting in opposite directions. The negative effect of LBM events on TRW was even stronger in the following year than in that of the outbreak as shown by the higher estimate value of LBM_{lag1} compared with LBM. In contrast, current and previous year's temperatures contributed almost equally to positive growth trends. The iWUE, a 'proxy' for
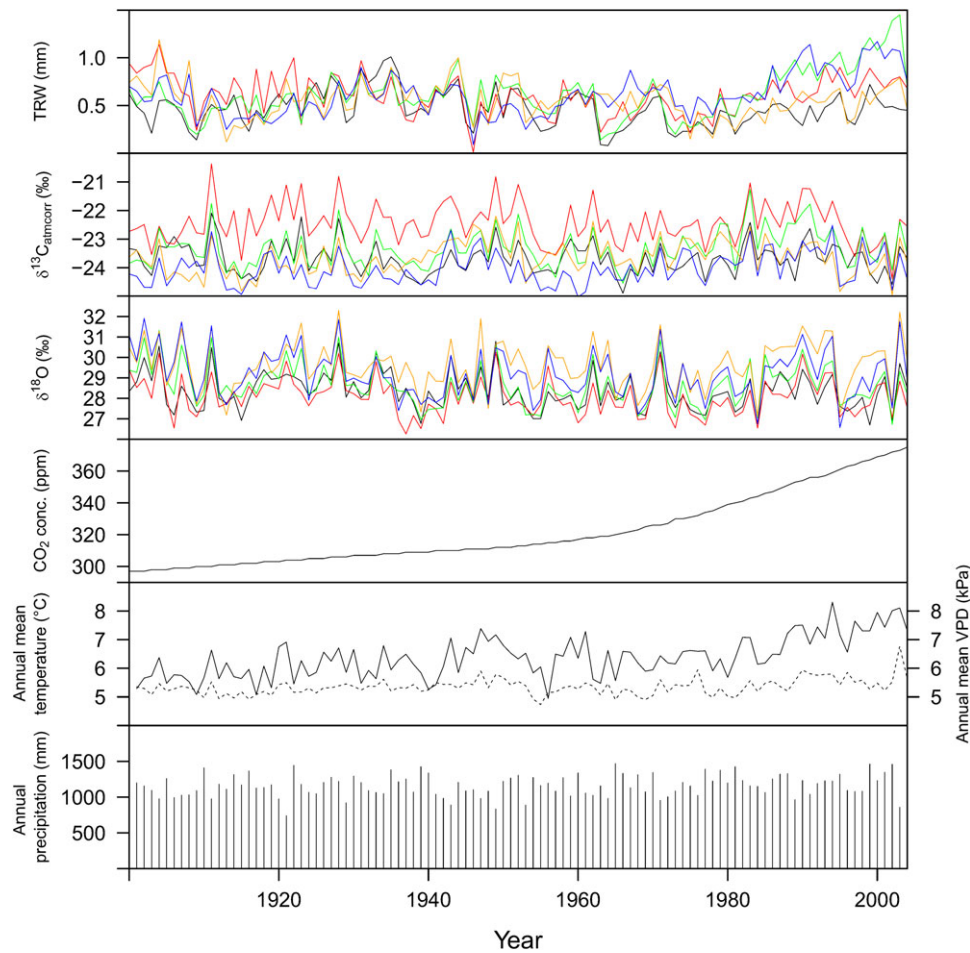
Figure 1. Time series of tree-ring width, δ^{13}C and δ^{18}O of five individual larch trees (based on Kress et al. 2009*a*), as well as CO$_2$ concentration, mean annual temperature, vapor pressure deficit (dashed line) and annual sum of precipitation throughout the 20th century; 'δ^{13}C$_{atmcorr}$' indicates that δ^{13}C was corrected for the atmospheric decrease over time (Suess effect).

stomatal control and VPD, played a minor, but still significant role by slightly restricting TRW. In addition, the tree size significantly affected TRW, although at a relatively high variation (standard error). The autocorrelation structure identified for TRW demonstrated that the two previous years' of ring growth influenced actual TRW, although the second previous year to a much lower extent (Phi1, Phi2; Table 2). Overall, the general pattern of the modeled TRW largely followed the observed growth of the five trees, as shown by reasonably high correlations per each individual tree (Table 2) and depicted in Figure 2. However, the year-to-year variability was lower in the modeled time series than in the observed data, indicating that other individual tree factors were not captured by the model. In particular, the strong but variable effect of LBM events seemed to be partly underestimated, while years of enhanced growth due to beneficial conditions and/or the capacity of recovery were not fully reflected.

Tree-ring δ^{13}C and δ^{18}O

Similar to TRW, variability of δ^{13}C and δ^{18}O in response to environmental factors was not affected by the two distinguished time

periods before or since the stronger increase in CO$_2$ (Table 3; full models given in Table S1 available as Supplementary Data at *Tree Physiology* Online). In the case of δ^{13}C, the best model indicated a strong negative effect of summer (July–August) precipitation and a slightly smaller positive effect of summer VPD. The significant interaction of precipitation and VPD exhibited a negative effect on δ^{13}C, probably emphasizing the generally high precipitation and therefore rather non-limiting soil water availability at this site, resulting in a weaker response to VPD. The previous year's summer precipitation and VPD showed a much smaller, although significant influence compared with the current year's climate. CO$_2$ concentration and tree size did not show any significant effect and were therefore excluded in the reduced model.

For δ^{18}O, the combination of environmental factors appears to be more complex. The iWUE was strongly reflected in δ^{18}O, emphasizing stomatal control on transpiration. Also, summer VPD positively affected δ^{18}O. To a similar extent, precipitation and the previous year's LBM negatively influenced the signal. In addition, tree size (radius^2) showed a strongly negative effect on the δ^{18}O value.

Table 2. Mixed-effects models describing the influence of environmental factors on tree-ring width. M1 represents the 'full' model and M2 the 'reduced' model following stepwise selection. Models include 'individual tree' as random factor, and ARMA(2,1) as autocorrelation structure. Significant fixed effects ($P < 0.05$) are indicated in bold. Continuous covariates (fixed effects) were centered and scaled for comparison of effect estimate values (i.e., 'Estimate'). Models were fitted by restricted maximum likelihood method (REML), but model comparison was done by AIC/BIC via the maximum likelihood method (ML). Modeled values for each tree were correlated to the respective measured values by Pearson correlation.

Covariates, standardized	Tree-ring width (M1)			Tree-ring width (M2)		
	Estimate	Std error	P-value	Estimate	Std error	P-value
Intercept	0.621	0.044	**<0.001**	0.612	0.036	**<0.001**
LBMlag1	−0.138	0.021	**<0.001**	−0.143	0.020	**<0.001**
LBM	−0.103	0.024	**<0.001**	−0.090	0.020	**<0.001**
Timeperiod	−0.012	0.048	0.802			
Tannual_lag1	0.137	0.022	**<0.001**	0.122	0.018	**<0.001**
Tsummer	0.149	0.021	**<0.001**	0.129	0.017	**<0.001**
iWUE	−0.075	0.034	**0.027**	−0.049	0.024	**0.043**
VPDannual_lag1	−0.032	0.023	0.179	−0.036	0.015	**0.016**
Tree size^2	−0.122	0.075	0.102	−0.137	0.067	**0.043**
Timeperiod × LBM	0.035	0.040	0.378			
Timeperiod × Tannual_lag1	−0.035	0.039	0.366			
Timeperiod × Tsummer	−0.057	0.035	0.110			
Timeperiod × iWUE	0.068	0.048	0.157			
Timeperiod × VPDannual_lag1	−0.012	0.032	0.705			
Stdev of random effect intercept, (i.e., tree variability)	0.000			0.000		
Stdev of random effect residual	0.201			0.196		
AIC/BIC/logLik (ML)		−497/−416/268			−504/−449/265	
Autocorrelation structure						
Phi1	1.212			1.226		
Phi2	−0.247			−0.259		
Theta1	−0.766			−0.786		
Pearson's r for individual trees (r fitted-measured)						
LOE05	0.48			0.46		
LOE09	0.53			0.61		
LOE14	0.69			0.72		
LOE24	0.56			0.54		
LOE28	0.40			0.47		
Number of observations	515			515		

For both isotopes, the most adequate autocorrelation structure indicated the partial imprint of the previous 1–2 years' isotopic signals, presumably captured in the early wood built upon reserves.

Although the variability in $\delta^{13}C$ and $\delta^{18}O$ differed between trees and time, a positive correlation between isotope ratios of both elements, i.e., their year-to-year variability, was observed for all five trees, ranging between $r = 0.6$ and 0.77 (Figure 3). This strong correlation suggests that the isotope signal is strongly influenced by environmental drivers that impact stomatal conductance as common controlling factor for both isotopes, such as, e.g., VPD, rather than affecting variability in photosynthesis (reflected by $\delta^{13}C$) or source water ($\delta^{18}O$).

While the absolute isotope values have not changed strongly with increasing CO_2 concentrations and VPD in the long-term, the iWUE increased, particularly since the late 1960s, remaining relatively high from the 1980s onwards (Figure 4). Comparing the average isotope values of the two distinguished time periods

for each tree, a slight but significant increase in $\delta^{13}C$ for the trees LOE14 and LOE28 and a similar tendency for LOE24 was observed (Figure 5). In contrast, no significant changes were found for $\delta^{18}O$. From these slight changes, the following physiological scenarios can be suggested via the dual isotope approach (Scheidegger et al. 2000): the increase in $\delta^{13}C$ (corrected for the Suess effect) resulted from a rather constant c_i, which suggests at increasing atmospheric CO_2 concentrations (c_a), c_i/c_a must have decreased. Thus, the difference D ($c_a − c_i$) —which is proportional to the iWUE—should have increased. Thereby, the higher CO_2 supply probably stimulated photosynthesis (A_N). Correspondingly, these trees (LOE14 and LOE28) also showed the highest TRW in the later time period (see above). Since $\delta^{18}O$ remained unchanged when comparing the two time periods, the observed increase in $\delta^{13}C$ can only be explained by an increase in A but not by a decrease in g_s, according to the model based on Scheidegger et al. (2000).
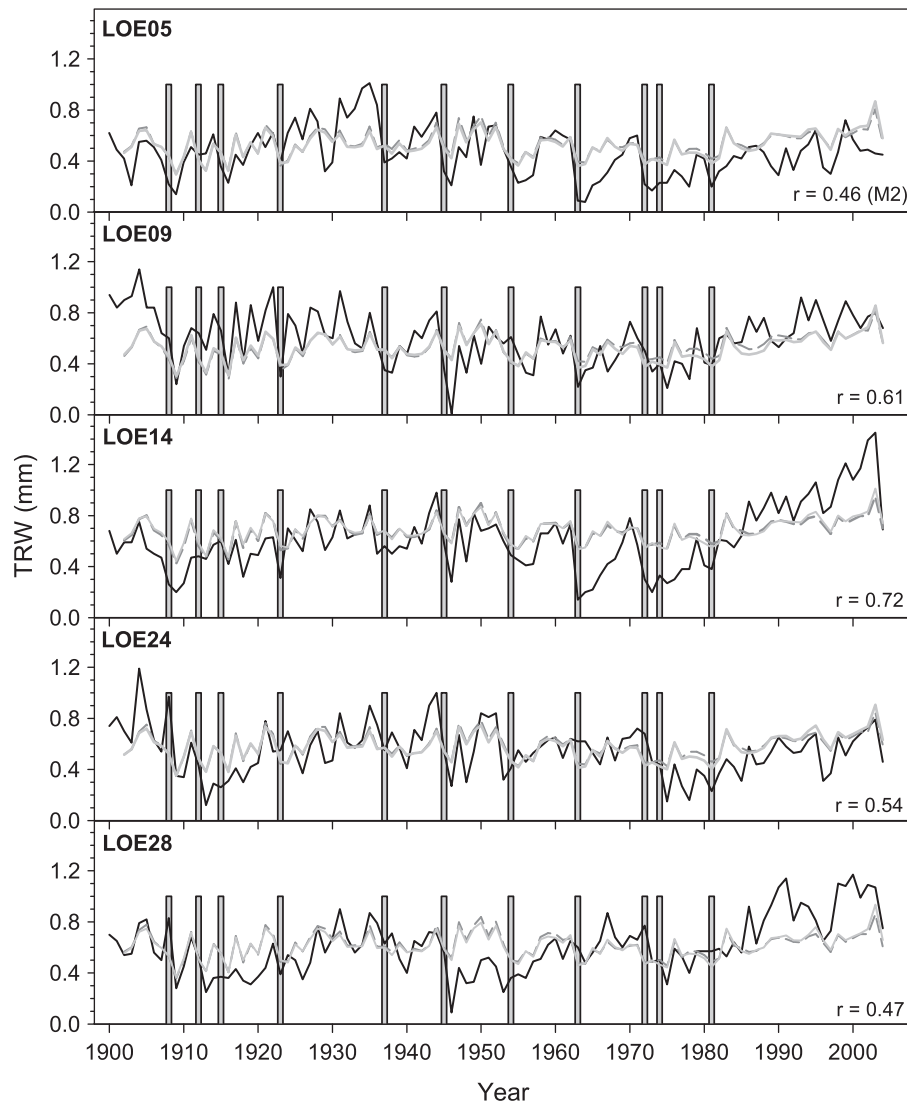
Figure 2. Measured tree-ring width (black) and model predictions of the 'full' M1 (dark gray, dashed) and the 'reduced' model M2 (light gray) for each of the five trees. The model fit of the reduced model is given as Pearson's r correlation of measured vs modeled data for each tree separately. Larch bud-moth events are indicated as gray bars.

Discussion

Growth patterns of old-growth larch trees

Estimated relative importance of environmental factors based on the mixed-effects model demonstrated that TRW at this site was most strongly influenced by temperature and to a similar extent by LBM outbreaks, supporting previous findings on the role of temperature (e.g., Büntgen et al. 2006, Carrer and Urbinati 2006) and LBM (Baltensweiler et al. 1977, Weber 1997) on larch growth at high-elevation sites. The strong effect of current and previous year's LBM events on growth is likely related to the budmoths' defoliation activity cutting the carbon supply of fresh photosynthetic assimilates (Baltensweiler et al. 1977), with direct consequences on the buildup of tissue and carbon storage. Local differences in the LBM outbreak intensity and time between the northern and southern slope of the site (Kress et al.

2009a), together with the tree's capacity to cope with this stress probably explained the tree-individual variability in growth. The absence of the LBM in this alpine region since the 1980s has been also found to be related to increasing temperatures, particularly during summer (Esper et al. 2007, Kress et al. 2009a). The growth responses to environmental factors did not change between the two time periods (i.e., levels of CO₂ increase), suggesting that carbon allocation into stem growth followed rather consistent mechanisms linearly related to the main important environmental factors, i.e., temperature and insect infestation. The pattern of increasing growth towards recent decades is in line with tree-ring chronologies of larch in the same region based on a large number of trees (Büntgen et al. 2006). Similarly, strong growth release from the LBM effects since the late 1970s were observed on other larch trees

Table 3. Mixed-effects models describing the influence of environmental factors on tree-ring $\delta^{13}C$ and $\delta^{18}O$. Reduced models with significant fixed effects ($P < 0.05$) are displayed (for full models see Supplementary Data at *Tree Physiology* Online). Models include 'individual tree' as random factor, and ARMA(1,1) and ARMA(2,1) as autocorrelation structure for $\delta^{13}C$ and $\delta^{18}O$, respectively. Continuous covariates were centered and scaled for comparison of effect estimate values (i.e., 'Estimate'). Models were fitted by restricted maximum likelihood method (REML). Modeled values for each tree were correlated to the respective measured values by Pearson correlation.

Covariates, standardized	$\delta^{13}C_{atmcorr}$			$\delta^{18}O$		
	Estimate	Std error	*P*-value	Estimate	Std error	*P*-value
(Intercept)	−23.49	0.267	**<0.001**	28.816	0.489	**<0.001**
LBMlag1				−0.417	0.111	**<0.001**
P_JAlag1	−0.149	0.048	**0.002**			
VPD_JAlag1	−0.122	0.051	**0.017**	−0.327	0.075	**<0.001**
P_JA	−0.525	0.047	**<0.001**	−0.411	0.067	**<0.001**
VPD_JA	0.365	0.054	**<0.001**	0.434	0.098	**<0.001**
P_JA × VPD_JA	−0.321	0.082	**<0.001**			
iWUE				0.988	0.150	**<0.001**
TRW				0.206	0.087	**0.018**
Tree size^2				−1.746	0.226	**<0.001**
Stdev of random effect intercept, (i.e., tree variability)	0.586			1.088		
Stdev of random effect residual	0.467			0.807		
Autocorrelation structure						
Phi1	0.863			−0.690		
Phi2				0.19		
Theta1	−0.641			0.978		
Pearson's *r* for individual trees (*r* fitted vs measured)						
LOE05	0.67			0.65		
LOE09	0.71			0.58		
LOE14	0.77			0.69		
LOE24	0.60			0.68		
LOE28	0.67			0.69		
Number of observations	515			515		

within the same region, while such a pattern was absent in Norway spruce (S. Klesse, unpublished).

Summer temperature of current and previous years exhibited the second most important environmental factor driving tree growth, while there was no difference in the effective strength between the two time periods. Strong signals of summer temperatures in larch have been previously reported from other alpine sites (e.g., Carrer and Urbinati 2004, Hafner et al. 2011). Also, recent increase in TRW of old-growth bristlecone pines at the treeline appeared to be related to increased temperatures rather than to elevated CO_2 concentrations (Salzer et al. 2009). For trees growing at the lower temperature limit for growth, generally positive effects of increasing temperature on physiological processes can be expected, such as the onset of cambial activity and cell formation (Tranquillini 1979, Körner 1998), that ultimately determine stem growth. Furthermore, we can assume that a temperature-driven increase in soil mineralization under sufficient water supply (Aerts 2006) increased the nutrient availability and hence also contributed to tree growth. Since precipitation did not show any effect on tree growth and remained constant over the last ~100 years, water supply was not limiting at this site.

In contrast to temperature, we did not find a significant direct positive effect of CO_2 concentration on growth, although a smaller contribution of CO_2—as well as other unknown factors —may not fully be excluded, since we applied a rather simple linear model. The trees' increase in iWUE, likely driven by higher CO_2 concentrations, did not promote tree growth rather showing a slightly negative effect on TRW. This indicates that the trees were not limited in carbon for stem growth. Recent studies on different aged stands at alpine sites suggested that increasing forest productivity was related to increased iWUE via CO_2-stimulated photosynthesis (Giammarchi et al. 2017). However, increased photosynthesis in response to elevated CO_2 is not necessarily related to tree growth, both for juvenile (Resco de Dios et al. 2016) and old-growth trees (e.g., Andreu-Hayles et al. 2011, Klein et al. 2016). At the same study area at the alpine treeline, Simard et al. (2013) also found that cambial activity during radial stem growth is not limited by carbon, i.e., sugar availability, suggesting that effective growth is rather determined by factors controlling conversion of sugars into cell formation. Exposure of larch trees to experimentally elevated CO_2 concentrations at a high-elevation treeline site resulted in increased basal area growth, but mainly pronounced in warm years with relatively early snowmelt (+33% TRW summed over the nine FACE treatment years; Dawes et al. 2011, 2013). Yet, at this experimental site, unchanged concentrations of woody
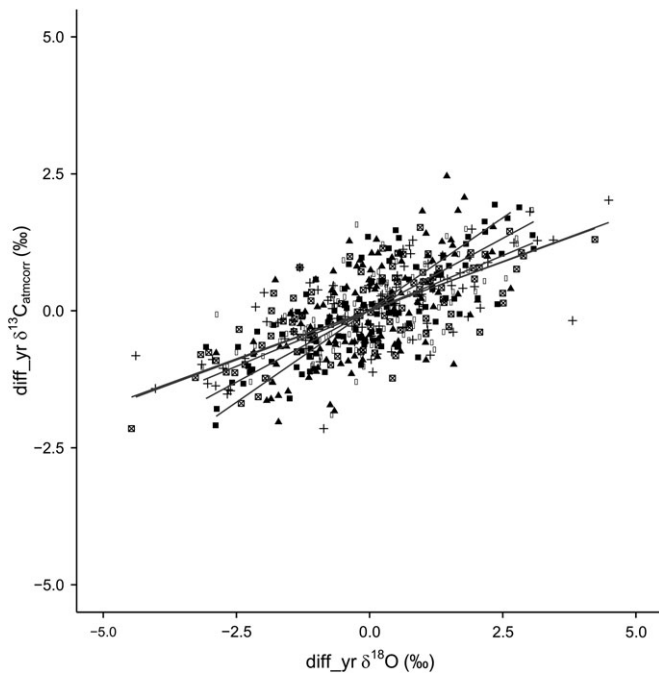
Figure 3. Correlation between year-to-year differences in $\delta^{13}C_{atmcorr}$ and $\delta^{18}O$ in tree rings of five larch trees across the time span 1900–2004. The individual trees are represented by different symbols and orthogonal regression lines.
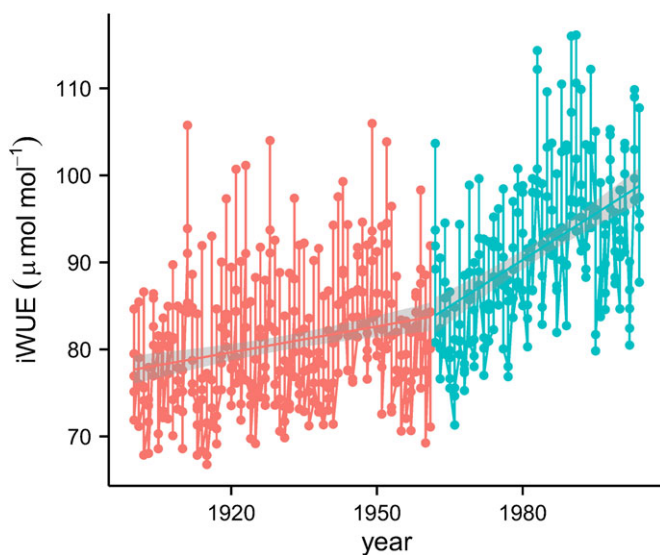

Figure 4. Intrinsic water-use efficiency of the larch trees across the time span 1900–2004 as based on measured $\delta^{13}C$. Mean of five trees ± 1 SE with linear fit and 95% confidence interval for the periods 1900–61 and 1962–2004.

non-structural carbohydrates did not indicate higher sink strength after 9 years of CO_2 exposure (Streit et al. 2013).

In addition to the above-mentioned external factors, tree size and the two previous years' growth were strongly related to TRW patterns. While increasing stem area can generally decrease radial growth increment, the strong positive effect of previous year's ring width on current year TRW—a well known pattern of tree growth (e.g., Monserud 1986, Meko et al. 1995)—indicated a strong influence of storage size and capacity (Kozlowski and Pallardy 1997, Skomarkova et al. 2006). Favorable growing conditions (i.e., higher temperatures and sufficient water supply) may promote the formation of larger cells and storage of starch and other carbohydrates as well as nutrients that might be available for the next growing season (but see below).

Environmental effects on isotope patterns and related physiological mechanisms

Both, $\delta^{13}C$ and $\delta^{18}O$ were not correlated to TRW, suggesting that a direct relationship between gas exchange and carbon allocation to annual stem growth was unlikely. Similarly to tree-ring growth, isotope signatures responded to environmental factors independent of the time period. In contrast to TRW, both isotopes were clearly affected by summer precipitation, confirming findings from the same (Kress et al. 2010) and from other sites and tree species (e.g., Saurer et al. 1997b, Treydte et al. 2007). Both $\delta^{13}C$ and $\delta^{18}O$ also strongly responded to summer VPD and were closely correlated in their year-to-year variation. This suggested a strong control of stomatal conductance according to the model by Scheidegger et al. (2000), which was also emphasized by the large effect of iWUE on the $\delta^{18}O$ signal (cf. Table 3). The strong negative effect of tree size on $\delta^{18}O$ but not on $\delta^{13}C$ may be an indication of size-related root development, leading to shifts in rooting depth towards deeper (more depleted) soil water pools (Marshall and Monserud 2006). Besides stem diameter, possible effects of tree height on $\delta^{13}C$ with respect to light exposure and hydraulic conductance (Monserud and Marshall 2001) could also be excluded here, since these old-growth trees of the rather shade-intolerant species had already reached their maximum height.

The large effect of precipitation amounts on the isotope signatures likely demonstrates that recent rain water—due to water runoff along the slope—represented the main source water at a rather shallow soil cover. This assumption is supported by the high correlation between the tree-ring $\delta^{18}O$ of the individual study trees and the average $\delta^{18}O$ of precipitation water of June–August for the period 1973–2004, ranging between $r = 0.6$ and 0.72 (precipitation data derived from the alpine monitoring station Grimsel, 1950 m above sea level, Global Network of Isotopes in Precipitation (GNIP) database; compare also Saurer et al. 2012). In the same study region, high resolution intra-seasonal data of different tree compartments and soil water within the same study region revealed that seasonal variation in $\delta^{18}O$ largely reflected recent precipitation and soil water pools (Treydte et al. 2014).

The autocorrelation structure in the variability of both isotopes further indicated the partial contribution of stored carbohydrates and/or previous seasons' soil water reserves to recent ring growth (Monserud and Marshall 2001, Marshall and Monserud 2006).
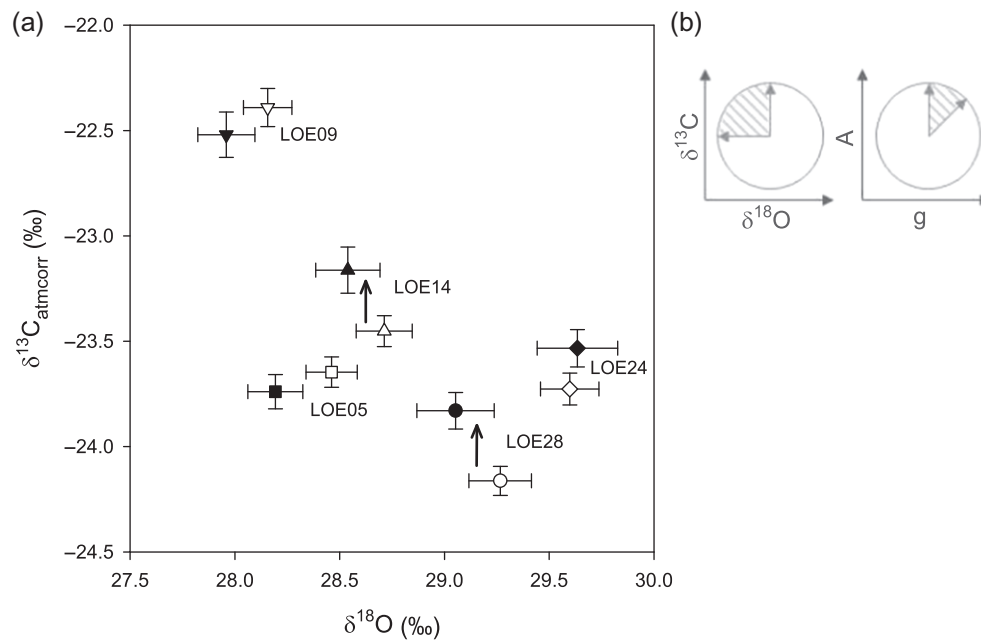
Figure 5. (a) Average isotopic signature of tree-ring cellulose for each of the five trees of the time periods 1900–62 (white symbols) and 1962–2004 (black symbols), ±1 SE. Arrows indicate a signficant shift in $\delta^{13}C$ between time periods at $P < 0.05$. (b) Corresponding shifts in photosynthesis and stomatal conductance according to the qualitative dual isotope concept (Scheidegger et al. 2000).

In contrast to findings at the same site by Kress et al. (2009a) but consistent with Weidner et al. (2010), the linear model showed a negative effect of LBM events on $\delta^{18}O$, although to a smaller degree than those of the climate parameters. The negative effect may be related to strong defoliation followed by disturbance of the transpiration flow, such as an increase in stomatal conductance of remaining needles (Handa et al. 2005), which may in turn induce a shift in the contribution of the source $\delta^{18}O$ signal.

Indications of physiological adaptation to environmental changes

The small shifts in isotope values between the two distinguished time periods derived from the dual-isotope approach further supports the outcome of the mixed-effects models indicating no general change in the effect strength of environmental factors. The dual-isotope model was employed here in addition to the mixed-effects models to (qualitatively) elaborate links to biological processes, although model interpretation should be handled with care in such natural ecosystems (Roden and Siegwolf 2012, see also Materials and methods). Yet, for example, the model assumption of constant influences of environmental factors on evaporative enrichment in ^{18}O appears to be supported here by: (i) strong effects of precipitation on isotope variation and a high correlation between rain water ^{18}O and tree-ring ^{18}O (see above), thus, the potential contribution of deeper source water on ^{18}O variation for the investigated two time periods was reduced; (ii) no change in temperature effects or VPD between the two time periods; and (iii) in general relatively high relative

humidity (low VPD) due to sufficient precipitation maintaining a rather unchanged responsiveness of stomatal conductance.

The temperature rise obviously did not strongly enhance the effects of VPD likely because of still rather high levels of precipitation and relative humidity (no change in $\delta^{18}O$ between the two time periods, cf. Figure 5). Similarly, experimental studies on tree saplings showed no significant effect on leaf $\delta^{18}O$ in response to elevated temperature when relative humidity was high (Roden and Farquhar 2012). Thus, a clear physiological adaptation to increasing temperatures at the leaf level could not be expected for the old-growth larch trees.

In contrast to various climate conditions affecting photosynthesis and stomatal conductance, the increasing atmospheric CO_2 concentrations have so far not been described by the dual-isotope model. Possible scenarios may comprise increasing photosynthesis at reduced or unchanged g_s, resulting in increased $\delta^{13}C$ at simultaneously decreased or unchanged $\delta^{18}O$ (as to some extent observed here), given that above-mentioned assumptions (Roden and Siegwolf 2012) are met. Further experimental studies would be helpful to clearly identify the variety of such scenarios with respect to CO_2 effects on the isotope pattern and hence physiological mechanisms.

Carbon dynamics under elevated CO_2

In the present study, the strong increase in iWUE during, and the slight shift in average $\delta^{13}C$ over the second half of the 20th century suggested an increase in photosynthetic activity in response to either elevated CO_2 and/or temperature at rather constant stomatal conductuance over time (unchanged $\delta^{18}O$). In the

same region as the presented study site, Norway spruce trees showed a similar but more pronounced shift in the isotopic signature between the two distinguished time periods, towards increased $\delta^{13}C$ and partly increased $\delta^{18}O$ (unpublished data). Such an increase in net photosynthetic rates at unchanged stomatal conductance has been found in studies on young larch trees at similar site conditions throughout 9 years of experimentally elevated CO_2 exposure (Dawes et al. 2013, Streit et al. 2014). Similar responses across many sites and a wide range of CO_2 concentration may point towards an optimization of leaf gas-exchange and stomatal control (Voelker et al. 2016).

Generally, higher non-structural carbohydrate concentrations but slower carbon transfer to sink tissues was reported from high-elevation sites compared with lowland sites, thus indicating limitation in sink activity rather than shortage of available carbohydrates (Hoch and Körner 2012, Streit et al. 2013). Accordingly, for the old-growth larch trees, this suggests that assimilation and sugar production may have increased in response to CO_2 rise, but incorporation of additional photosynthates into wood production was ultimately dependent on the increasingly warmer summer temperatures at this high-elevation site. Additionally, the absence of LBM in recent decades supported increased growth in terms of, e.g., less effort of recovering and more assimilates available for building more and larger cells throughout the growing season and perhaps enlarging the carbon storage pools. The variability of storage capacity was also reflected in the autocorrelation structure of the isotopes, suggesting partly imprint of previous year's sugar isotope signals in the recent cellulose. Consistent with stem growth, this indicated the use of stored carbohydrates for current year's growth (Monserud and Marshall 2001, Helle and Schleser 2004, Kagawa et al. 2006, Skomarkova et al. 2006, von Felten et al. 2007). However, Kress et al. (2009b) observed strong common $\delta^{13}C$ patterns in early and late wood at the same study site, suggesting rather small reserves of these treeline trees with a rather short growing season, and/or a partly mixing of old and new carbon pools. Nevertheless, climate effects on tree-ring growth, starting earlier in the season (temperature of June–August) than on $\delta^{13}C$ (July–August climate), indicate a contribution of stored carbohydrates on current year's ring growth, as also suggested from similar results of Hafner et al. (2011).

Growth conditions determining carbon allocation patterns

Taken together, these multiple effects of environmental factors indicate that several physiological processes, differently influenced by external and internal factors, need to be distinguished from the way of carbon gain until incorporation into tree stem growth (Figure 6). At the leaf level, increasing temperatures and CO_2 concentrations may have enhanced the photosynthetic capacity of the old-growth trees at the investigated site. Since water supply was sufficient, increasing VPD in combination with CO_2 towards the end of the 20th century did not clearly change stomatal
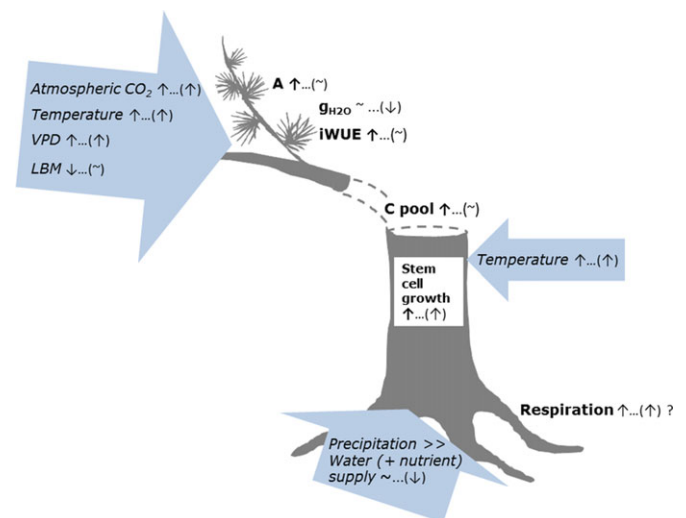

Figure 6. Concept of the interaction of changing environmental conditions, physiological responses and growth of old-growth larch trees at a temperature limited study site; arrows indicate increase or decrease, sinuous line unchanged conditions, symbols in brackets indicate potential future trends.

conductance. Both, increased photosynthesis and constant stomatal conductance thereby led to a CO_2-stimulated increase in iWUE. At the stem growth level, increasing temperatures at this temperature limited site and the absence of the LBM from around the 1980s onwards allowed for increasing carbon allocation into stem growth, although to a variable extent between the individual trees. Future changes in environmental conditions may alter the relative importance of the driving factors for tree growth. For example, more frequent extreme summer temperatures will increase the effects of VPD on gas exchange and may diminish the stimulating effect of temperature (and CO_2) on tree growth, as evident from a lowland mesic larch stand (Lévesque et al. 2013, 2014).

Current observations of a stabilizing iWUE of larch trees during recent years (Churakowa Sidorova et al. 2016) suggest that carbon assimilation may not increase further in the future. Prolonged and warmer growing seasons may still promote tree growth at high elevation, but other carbon sinks may also become more important, e.g., belowground biomass production, turnover and respiration (Dawes et al. 2013). The impact of severe insect attacks with their large effect on tree performance currently appeared to decline in this study area, but possible future infestations could overrule the beneficial climate conditions.

Conclusions

Based on the combined study of tree-ring growth and stable isotope signatures, this case study aimed at disentangling the impacts of environmental factors for growth performance of old-growth larch trees at the levels of assimilation (leaf level) and ring growth (whole-tree level). Thereby, the trees' age allowed for studying physiological responses independent of stand dynamics.

While precipitation and VPD predominantly influenced assimilation, as shown by isotope signals, growth variability was influenced to a similar strength by LBM outbreaks and summer temperature across the 100-year time period. These response patterns did not differ between the earlier and later time period (different slopes of CO_2 increase). Yet, along with increasing CO_2 and temperature from the 1960s on, the shift in $\delta^{13}C$ together with increasing iWUE indicated higher net photosynthesis over time under sufficient water supply. The absence of a direct relationship between CO_2 and growth, confirming former reported experimental outcomes, showed that trees at high-elevation sites are not carbon limited. Therefore, increased carbon sink strength (resulting in enhanced stem growth) is driven to a higher degree by temperature, rather than by increased carbon assimilation due to elevated CO_2 concentration.

Our study reveals that the response patterns on the leaf level are not necessarily fully reflected in the stems, as the transformation from assimilates to stem biomass is predominantly temperature controlled. This multivariate approach represents a more quantitative assessment to identify the relative contribution of the controlling environmental factors for different physiological processes (from carbon assimilation until allocation and stem growth), facilitating the evaluation of their relevance in climate change scenarios for site-specific tree growth.

Supplementary Data

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

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Conflict of interest

None declared.

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