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Carbon and oxygen dual-isotopes in tree rings indicate alternative physiological responses opted by European beech trees to survive drought stress

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ABSTRACT

Poor drought tolerance of European beech trees raised concerns in Europe. We hypothesized that beech could show an opposite physiological response to the same level of climatic drought with change in edaphic drought. We performed a combined analysis of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in tree rings to reveal retrospective temporal physiological responses of trees to drought. The edaphic drought was assessed by quantifying the capacity of soil to store water in plots (classified as “dry” and “less-dry”) near the drought limit of the species in three near-natural oak-beech ecotones in Germany and Switzerland. Neighbourhood competition was quantified. A climatic drought index was calculated from meteorological records and related to the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of the trees. Trees from dry plots showed a higher response to drought and climatic dependency than less-dry plots. Neighbourhood competition increased $\delta^{18}\text{O}$ values significantly. Dual isotope analysis shows a tendency of greater stomatal resistance in dry plots and higher stomatal conductance in less-dry plots. We conclude that beech trees belonging to the same population under changing soil water availability can show different physiological responses under climatic drought stress. Our finding indicates the high plasticity of the beech trees to survive drought stress with changing site conditions.

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Introduction

Global climate change-induced heatwaves and precipitation anomalies increase the frequency and intensity of summer droughts worldwide (IPCC 2007, 2014). In central Europe, intensive drought harshly impacts the biota (Schar 2004; Allen et al. 2010). Drought affects physiological processes like photosynthesis, transpiration, carbon allocation, etc., followed by increasing susceptibility to insect or pathogen attacks causing growth decline and dieback in many European tree species (Breda et al. 2006). A detailed climatic study from the south-west Germany showed an increase of 0.22°C/decade for the mean annual temperature, with frequent droughts, decreasing precipitation during summer, and higher precipitation during winter from 1950 to 2000 (Mayer et al. 2005). These climate projections implicate a need for a better understanding of the impacts of droughts on the health and ecology of European tree species. Earlier studies indicate that drought is one of the main drivers impacting the vitality and growth of trees through a complex cascade of changes involving interconnected plant

systems like hydraulic function, carbohydrate and defense metabolism over multiple timescales (McDowell 2011; Anderegg et al. 2012).

European beech (*Fagus sylvatica* L.) is a widely distributed ecologically and economically significant tree in Europe (Ellenberg and Leuschner 2010). Some previous studies claimed beech as a drought-sensitive species and susceptible to decline under climate change due to decreasing growth and competitive ability (Gessler et al. 2007), compared to other drought-resistant trees like oaks, particularly after starking drought events (Rubio-Cuadrado et al. 2018; Meyer et al. 2020). However, some studies contradicted this claim and suggested that beech trees can overcome drought impacts because of their wide ecological amplitude and higher competitiveness over other trees and ability to quick recovery (Ammer et al. 2005; Leuschner et al. 2006; Bolte et al. 2007; Vanhellefont et al. 2019; Tegel et al. 2014). The latter may help a species overcome stress through an increased number of survival strategies that exist in geno/phenotypes of the species. A tree's survival is a complex species character, which includes structural and functional alteration of an

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individual, and could be studied by assessing the autecology of the concerned species and its physiological stress responses (Schulze et al. 2005; Camarero et al. 2015). Existing experimental studies revealed many morphological and physiological coping strategies of beech trees to overcome drought stress (Fotelli et al. 2001, 2003; Zang 2014; Aranda et al. 2015, 2017; Tomasella 2019; Gebauer et al. 2020).

Although, most of the earlier studies focused on a limited number of beech trees growing under simulated drought conditions and for a relatively short period which are common ways for laboratory experiments. Furthermore, these experimental studies are primarily based on seedlings. Hence, the findings obtained in controlled experiments can often not be fully transferred to mature trees; as adult trees are grown under uncontrolled conditions, including interactions with neighbouring trees and other plants.

The intrinsic water use efficiency of the trees in natural conditions will be different because of the variability of soil and rooting system, and with more varied climatic conditions following regular drought events. To overcome this problem, one probable solution is a large forest-scale experimental study which can be costly and time-consuming. However, in a very recent forest-scale large experimental study (Grams et al. 2021) authors measured the response of mature beech trees after simulated drought conditions in the forest for 2014–2018. At the same time, there is an urge for long-term physiological experimental studies comparing drought impacts on beech trees growing under natural conditions although challenging to conduct due to the feasibility limitations. For example, beech trees growing in naturally dry sites facing permanent drought may have different adaptations to drought stress when compared to beech trees that are only affected by periodic droughts (Saure et al. 1995; Saurer et al. 1997a; Tegel et al. 2014; Chakraborty et al. 2021). In this context one important thing to mention is how the beech forest stands are naturally grown under this stressed condition. It is common that beech trees growing in natural condition in dry sites are found mostly as co-dominant class under another drought resistant species growing as pre-dominant class (Kohler et al. 2006; Gärtner et al. 2008) but has potential to overcome drought stress with preferable site condition (Reif et al. 2017). Hence it is trivial to study these specific group of co-dominant stressed trees to elaborate further knowledge on the ecological behaviour of the species under drought condition that could fulfil the aim of this study.

Many dendroecological studies relate the annual growth increment of the trees to climatic drought conditions and reported growth depression in beech trees due to drought (Lebourgeois et al. 2005; Piovesan et al. 2008; Hackett-Pain et al. 2016; Meyer et al. 2020). Nevertheless, the drought resistance strategies of a tree cannot be fully understood by assessing only the growth performance, rather additional assessment of physiological and environmental responses through natural archives and relating it with tree growth could narrate the species-specific adaptation strategy (Breda and Badeau 2008; Gessler et al. 2014).

In this context, observational studies combined with a physiological assessment using stable isotopes extracted

from the wood of tree rings can help to retrospectively reveal environmental interactions and growth responses of trees growing under drought stress (Schleser et al. 1999; Dawson et al. 2002; McCarroll and Loader 2004; Leavitt 2010; Dawson and Siegwolf 2011). The discrimination of ^{13}C against ^{12}C by photosynthetic fixation of the enzyme Rubisco in C_3 plants is related to the ratio of leaf internal to leaf external (atmosphere) and CO_2 partial pressure (C_i/C_a) which is mainly controlled by stomatal conductance (g_s) and changes in carbon assimilation rates (A) (Farquhar et al. 1982). $\delta^{13}\text{C}$ of assimilates translates into the $\delta^{13}\text{C}$ of wood of annual tree rings and thus, can be related to the differences in A/g_s , which indicates the so-called intrinsic water use efficiency for the given growing period. It is commonly associated with changes in soil water availability (Farquhar et al. 1989; Feng and Epstein 1995; McCarroll and Loader 2004). However, stomatal conductance and assimilation rates and hence the intrinsic water use efficiency of a plant can be influenced by various abiotic environmental and meteorological parameters like light, temperature, precipitation, or relative humidity (which all influence soil water availability) but also nutrient availability (Cowan 1982; Saurer et al. 1995; Livingston and Spittlehouse 1996; Hemming et al. 1998; Leavitt et al. 2002; Saurer et al. 2004; Ferrio and Voltas 2022).

Yet, it is not trivial to deduce information about physiological responses and tree interactions from stable carbon isotopes alone. In this context, a probable solution is to adopt “dual-isotope analysis” by including the oxygen isotope ratio in addition to the carbon isotopic ratio of the same tree rings. This can help to separate the effects of carbon assimilation (A , which is the demand for CO_2) and stomatal conductance (g_s , which is the supply for CO_2) on Rubisco discrimination (through C_i/C_a) (Farquhar et al. 1982; Saurer et al. 1997b; Scheidegger et al. 2000; Barbour et al. 2002). However, the interpretation of the stable oxygen isotope ratio ($\delta^{18}\text{O}$) in tree rings is not very straightforward as several independent mechanisms control the variation (McCarroll and Loader 2004; Gessler et al. 2014). First of all $\delta^{18}\text{O}$ in plants' organic matter is influenced by the isotopic composition of water taken by trees from soil ($\delta^{18}\text{O}$ source for the trees) modified by partial water loss through leaf transpiration, and cause enrichment of water at the evaporative sites (leaf stomata) (Yakir and Sternberg 2000; Barbour 2007). Another important mechanism is the exchange rate of ^{18}O between oxygen in sucrose and water during phloem transport and cellulose synthesis of stems (Barbour 2007). Subsequently, the oxygen isotope signature of mesophyll cell water is imprinted on assimilated sugars as well (Barbour et al. 2000a; Lehmann et al. 2020), even post-photosynthetic and post-evaporative oxygen atom exchange processes could affect the initial oxygen isotope signal in leaf (Geßler et al. 2014).

Roden and Siegwolf (2012) mention “dual isotope analysis with tree rings” as an important technique to explain retrospective physiological responses of the trees despite multiple associated sources of errors. Several existing studies have proved that oxygen isotopic signature in the wood of the beech tree rings could be linked to the sensitivity of plants to evaporative conditions, hence, it can be designated as a

proxy for the changes in stomatal conductance (Offermann et al. 2011; Barnard et al. 2012; Klesse et al. 2018). Hence, the combined analysis of carbon and oxygen isotope ratios could reveal a plant's long-term photosynthetic performance (i.e. A_{\max}) concerning its water use (i.e. g_s) as there is evidence that plants growing under differing humidity conditions show a positive correlation between both isotope ratios ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) in their organic matter (Saurer et al. 1997b). This approach helps to interpret whether the reduction in intercellular CO_2 concentration (C_i) is a result of reduced stomatal conductance (at a constant A_{\max}), or increased photosynthetic capacity (at a constant g_s) (Scheidegger et al. 2000). Past studies demonstrated that isotopic signals are sensitive to the local site conditions, both biotic and abiotic environmental conditions which can cause spatially distinctive stress responses of trees of the same species (Saurer et al. 1997a; Grams and Matyssek 2010; Metz 2016). Therefore, following the latter, it could be assumed that trees growing under the same climatic conditions getting the same source of water but on sites with different amounts of available soil water supply and neighbourhood competition would create different isotope signals in their wood. Therefore, the trees' physiological mechanism response to cope with climatic drought stress can be analysed by comparing the isotopic signals of the tree rings growing under different available soil water conditions.

Previous studies focusing on $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in beech tree rings mainly aimed to study the influence of aspect and topography on the relationships between climatic variables and isotopic composition (Saurer et al. 1995; Saurer et al. 1997a; Saurer et al. 1997b; Hartl-Meier et al. 2014). However, the influence of soil water availability at the micro-site level under the climatic drought conditions on $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in tree rings at the dry distribution limit of the beech trees had not yet been done. This study aims to bridge these gaps by analysing the carbon and oxygen stable isotopes from wood material collected from the tree rings of drought-stressed beech trees growing in near-natural forests on sites close to the drought limit of beech trees. Here we want to mention that this study focus on the drought limit of the beech trees that could be created due to site constraints, not due to geographical distribution limitations often found in range edge. Moreover, this study could improve our understanding of plant-plant interaction in natural forest conditions by assessing the influence of within-stand competition on $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in tree rings.

We hypothesized that soil water availability would alter the physiological responses through stomatal behaviour and control over acclimatization of the beech trees growing under the same climatic drought condition in non-controlled environments. Under this hypothesis, we formulated four research questions:

- (1) How do the temporal responses in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of beech trees from neighbouring sites change with the increase of available soil water storage capacities?
- (2) How does the soil water storage capacity, neighbourhood competition and location of plots influence the responses of the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of beech trees?

- (3) How are the different climatic variables related with $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of beech trees?
- (4) How does the relationship between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in beech trees vary in sites with different levels of available water storage capacities, as per the conceptual model of Scheidegger et al. (2000)?

Materials and methods

Study areas and soil analysis

The study was conducted in three forest areas (namely Badenweiler: BAD, Klettgau: KLET, and Schwäbische Alb: SBA; Figure 1) of Southern Germany and Northern Switzerland (geographic, topographic, and climatic data of the study areas are given in Table 1). These forests were situated on south-facing rocky limestone hill slopes with low water storage capacity. Historically these forests experienced intense land-use pressure (managed as oak coppice forests) until the early twentieth century but remained unmanaged from the 1950s onwards due to the change in the forest management policies (Sayer 2000; Gärtner et al. 2008; Chakraborty et al. 2017, 2021). Because of the abandonment, old senescence oaks (*Quercus petraea* (Matt.) Liebl., *Quercus pubescens* Schwarz) are dominating the canopy layer of the forests where broadleaf trees (*Acer campestre* L., *Acer pseudo-platanus* L., *Carpinus betulus* L., *Fagus sylvatica* L., *Fraxinus excelsior* L., *Sorbus aria* Crantz, *Sorbus torminalis* (L.) Crantz) and woody perennial shrubs (*Cornus sanguinea* L., *Crataegus laevigata* (Poir.) DC., *Hedera helix* L., *Ilex aquifolium* L., *Ligustrum vulgare* L., *Viburnum lantana* L.) are found at the understorey layer (Sayer 2000; Chakraborty et al. 2017). Previous studies (Kohler et al. 2006; Gärtner et al. 2008) at these forest areas showed how oak and beech trees create a transitional forest area. In the lower hill slopes with deep soils, beech-dominated forests are found, and oak-dominated forests are found at the adjacent sites of the upper hill slopes with the shallow soils. In between these beech- and oak-dominated forested areas, a transitional forest zone is formed due to the soil water availability controlled by the topographic and solar irradiation effects on the aspect of the hills, where occurrence and establishment of naturally regenerated beech trees are limited due to the edaphic drought (Chakraborty et al. 2017). These three oak-beech transitional forest zones were selected as the sampling area for this study. The transition areas had heterogeneous soil conditions due to varying soil depths and rock contents. This caused a gradual change in the available soil water storage capacity (AWC). We applied a systematic sampling to capture this soil water heterogeneity and laid transects from beech-dominated forests at one end to oak-dominated forests at the other (Mueller-Dombois and Ellenberg 1974). Each transect was subdivided into continuous 10×10-meter quadrat plots, which allowed us to capture the heterogeneous soil conditions.

A one-meter-wide soil profile was dug at the centre of each 10×10-meter plot for the total 17 plots, until the compact bedrock was reached to record soil properties. AWC was calculated using soil texture, soil depth, horizon

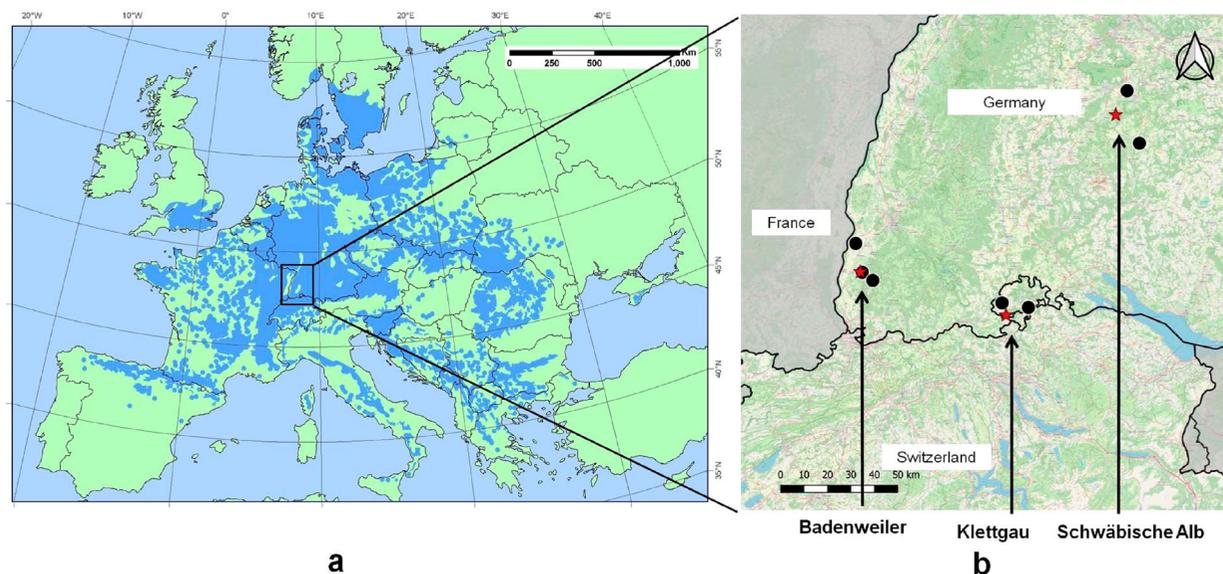


Figure 1. (a) Natural distribution area of European beech (*Fagus sylvatica* L.) (reproduced from the distribution map of European beech (*Fagus sylvatica* L.) by the European Forest Genetic Resources Program-EUFORGEN (2009) – beech occurrences shown in dark blue in front of green background) marked with the location of the study area; (b) study locations and corresponding climate stations in Southern Germany and Northern Switzerland- three study sites are shown by red asterisks and climate stations corresponding to the study sites are shown by black dots, the map was created using data from OpenStreetMap in QGIS.

thickness, skeleton content and humus content for each plot following the Food and Agriculture Organization of the United Nations (FAO 2006; Chakraborty 2010; Chakraborty et al. 2013). The plots were classified as “dry” (AWC < 60 mm) and “less-dry” (AWC > 60 mm) based on the values of the AWC, following the aridity classification of the German Soil Classification Working Group on Forest Site Mapping (Standortskartierung 2016). The AWC ranged from 34 to 103 mm within all plots, and we classified nine plots as “dry” (three from each forest) and eight plots as “less-dry” (three each from KLET and SBA, and two from BAD). From the soil profile, rooting depth was measured (maximum up to 1 m), and the trees had developed their fine roots mostly at the upper 30–40 cm of the soil.

Tree sampling, neighbourhood competition and dendroecological assessments

During 2012 and 2013, we selected co-dominant beech trees as our sample trees from the three study locations. The main reason for choosing co-dominant beech trees for this study is the lacking of dominant beech trees in the transitional zones

where the ecological drought limit of beech is found (Gärtner et al. 2008; Reif et al. 2017). Furthermore, stable isotope study involving this crown class of beech trees are rare but has ecological importance. Existing study (Chakraborty 2010; Chakraborty et al. 2013) showed that trees in lower height cohorts in dry sites had higher drought stress compared to the bigger trees in a stand as the resource acquisition capacity can be higher in the bigger trees due to size-asymmetric competition. Eight trees were selected from each study location: four each from dry and less-dry plots. While selecting trees, the height and the age of the trees were considered to standardize the difference of within-stand competition effects among trees and to overcome variations in stable isotope fractionations among crown dominance classes as the efficiency of resource utilization differs among different crown classes (Oliver and Larson 1996; Barnard et al. 2012). Furthermore, the distance among the trees of the same group was not more than 50 m to ensure the comparable source of water for a group of trees. In that way, we selected in total of 24 trees for this study from three locations. Neighbourhood competition was assessed for each individual tree. The sample stem discs were collected at the 1.3 m height of the felled trees

Table 1. Topographic and climatic characteristics of three oak-beech forest stands on Jurassic limestone (rendzina soil) from three locations of southern Germany and Northern Switzerland.

Region/ locality/ stand	Country	Geographic location (Long. E, Lat. N)	Elevation (m a.s.l.)	Size (ha)	MAT (°C) ²	MTAP (mm) ²	MTAPET (mm) ^{2,3}	Days > 10°C ¹	Slope angle (°)	Aspect (°)
Badenweiler (in Markgräflerland)	Germany	7.67, 47.81	524	0.9	9.2	1000	1085	177	23	155–180
Schwäbische Alb	Germany	9.12, 48.42	781	0.8	5.3	831	912	143	31	205–243
Klettgau	Switzerland	8.50, 47.65	584	0.5	7.7	966	992	164	24	251–280

Note: m a.s.l.: metres above sea level, ha: hectare, MAT: mean annual temperature, MTAP: mean total annual precipitation, MTAPET: mean total annual potential evapotranspiration, °: degree; ¹ = total days per annum with average temperature > 10°C, gathered after Gauer and Aldinger (2005); ² = Average and sums were calculated from the climatic data collected from German (DWD 2015)⁶³ and Swiss (MeteoSwiss 2015) weather services for the period of 65 years (1947–2011); ³ = after Turc (1961).

Table 2. Plot characteristics and descriptive statistics of the sampled trees based on dry and less-dry plots.

Parameter	Badenweiler		Klettgau		Schwäbische Alb	
	Dry plots	Less-dry plots	Dry plots	Less-dry plots	Dry plots	Less-dry plots
Number of trees (N)	4	4	4	4	4	4
Mean DBH (cm)	14.2	9.4	8.3	12	9.3	10.8
St. Dev. Of DBH	1.4	0.5	2.0	4.0	1.1	0.5
Mean height (m)	11.9	9.4	8.6	11.7	9.5	9.8
St. Dev. Of height	0.6	0.5	1.1	1.3	1.1	1.2
Mean age (years)	76	61	93	89	51	51
St. Dev. Of age	9.5	6.1	1.2	5.7	2.3	2.4
Mean AWC (mm)	54	80	47	61	54	87
St. Dev. Of AWC	3.4	20	11.7	2.3	1.4	17.8

Note: DBH, diameter at the height of 1.3 m; AWC, available soil water storage capacity, St. Dev., standard deviation. Age was calculated from the discs for the entire life span of the sampled trees.

to gather wood material for the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analysis. North direction was marked on each wood disc. A detailed description of the sampled trees with their dendrometric data and related plot level AWC values is given in Table 2. A comparison between the plot level AWC, and the trees' DBH, height, age and competition between dry and less-dry plots were carried out (Figure 2).

Neighbourhood competitions were assessed for each target beech tree by using Hegyi's competition index (Hegyi 1974) to assess plant-plant interaction. The Hegyi's index is independent of stand age and size, and depends only on the relative spatial pattern of the trees. The competitor trees were selected and measured applying the searching cone method described by Pretzsch (2009) so that we could include all potential competitors for light, nutrients, water and space to capture size-asymmetric competition.

Tree-ring width (TRW) and age were calculated for all sampled stem discs with the software WinDENDRO (2009). As we used whole discs, TRW was measured in four radial directions for each stem, and an average of the four radii was used for each year. Raw TRW data were cross-dated visually and statistically using the software COFECHA (Holmes 1983). We used the last 16 years of TRW (1995–2010) for isotope analysis to avoid the possible "juvenile effect" of isotope composition in wood (McCarroll and Loader 2004). Moreover, these years contained an extensive range of year-to-year climatic variation with the drought year of 1998 and an extreme drought event with severe summer heatwaves during 2003. The wood samples were collected from each tree ring after cross-dating for consistency and were never pooled by mixing the rings. Finally, we had 384 annual tree rings by following 3 study locations: BAD, KLET and SBA, 2 contrasting habitats of dry and less-dry plots in each location, 4 trees from each habitat, and 16 years of tree rings for each sampled trees.

Stable isotope sample preparation and data treatment

Tree-ring widths of sampled discs were very narrow for all three sites since the study sites were under permanent edaphic drought stress. Therefore, we chose the UV-laser-based microscopic dissection method for isotopic sample preparation following (Schollaen et al. 2014) as it was very difficult to collect the annual wood sample by cutting

tangential slices manually by fixed-blade sledge or microtome without mixing the year rings. This approach allowed us to get wood samples from each annual increment without contamination (for a detailed methodological approach see section S2 in the supplementary document). We prepared samples from the whole annual ring without separating earlywood and latewood as the transition of earlywood and latewood in beech is not "unambiguous" and is "unlikely" to represent the exact date of the year (Dupouey et al. 1993). Only tree ring boundaries, resin pockets, and rays were avoided under the microscopic dissection process. Thus we were able to collect enough wood material from the narrow annual rings for the analysis. We used whole wood instead of cellulose as the narrow annual rings, particularly of the drought years, could not supply enough sample material if only cellulose had been used. Although it gives a slight systematic error (Livingston and Spittlehouse 1996), previous studies (Geßler et al. 2001; Mölder et al. 2011; Offermann et al. 2011) found a strong correlation in isotopic values between woods and cellulose for beech trees.

Samples were collected semi-automatically and packed in a tin capsule for carbon isotope analysis and a silver capsule for oxygen isotope analysis. The sample masses of 130–220 μg of whole wood were used for Isotope Ratio Mass Spectrometry (IRMS) analyses. The carbon isotope ratios were measured by combustion at 1080°C by using an elemental analyzer (Model NA 1500; Carlo Erba, Milan, Italy) coupled online to an IRMS (Isoprime Ltd, Cheadle Hulme, UK). The oxygen isotope ratios were measured using a high-temperature TC/EA pyrolysis oven at 1340°C coupled online to an IRMS (Delta V Advantage; Thermo Fisher Scientific, Bremen, Germany). All isotope measurements were conducted at a precision of $\pm 0.1\text{‰}$ for carbon and $\pm 0.3\text{‰}$ for oxygen values. The isotope ratios are expressed using the conventional delta (δ) notation:

$$\delta = \left(\frac{R_p}{R_s} \right) \times 1000$$

where R_p and R_s are the isotope ratios ($^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$) of the plant material and standard, respectively, carbon isotope ratios ($\delta^{13}\text{C}$) are expressed relative to the Vienna Pee Dee Belemnite (VPDB) and oxygen isotope ratios ($\delta^{18}\text{O}$) relative to the Vienna Standard Mean Ocean Water (VSMOW) standards.

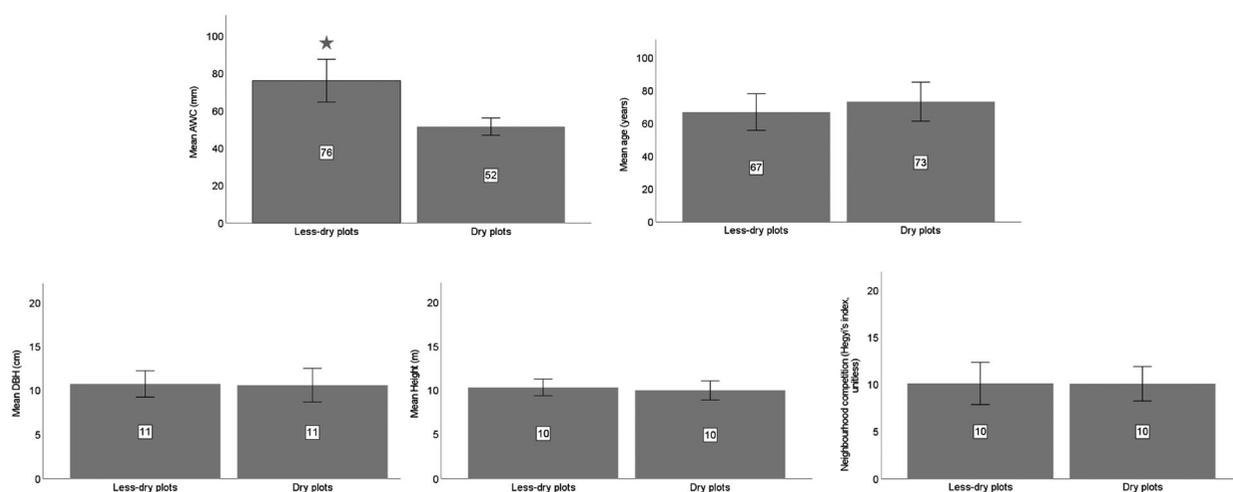


Figure 2. Comparison of mean AWC, age, DBH, height and competition between dry and less-dry plots of the sampled beech trees used in this study. The confidence intervals (95%) of the standard errors of the means were given in thin bars. The significant difference (as per *t*-test) was marked with asterix.

The $\delta^{13}\text{C}$ of the whole wood was corrected for the ^{13}C Suess effect (Keeling 1979), i.e. the decline of $\delta^{13}\text{C}$ in atmospheric CO_2 by increasing anthropogenic combustion of fossil fuel depleted in ^{13}C . This correction was done with data from the Mauna Loa Observatory, Hawaii (Keeling 2005), following the methodology suggested by McCarroll and Loader (McCarroll and Loader 2004). The Suess effect has been reported in most century-long $\delta^{13}\text{C}$ series of tree rings during the last 150 years since industrialization as additive fractionation (McCarroll and Loader 2004); and recommended to correct the $\delta^{13}\text{C}$ values of tree rings for ecophysiological analysis (Belmecheri and Lavergne 2020). Therefore, all further analyses were done by using the corrected atmospheric CO_2 , termed $\delta^{13}\text{C}$.

To assess the potential impact of source water $\delta^{18}\text{O}$ on $\delta^{18}\text{O}$ in wood during the study period of 1995–2010, we depicted $\delta^{18}\text{O}$ of precipitation of corresponding years (Brandes et al. 2007). This assumption was justified as the rooting depth at the study sites were low, fine roots were mostly accumulated at the upper soil horizons where soil water is stored mainly from the recent precipitation, and since the sites were located at hill slopes, access to the groundwater was negligible, and finally, trees of the same group were located at the proximity having same source water. Here we want to mention that our $\delta^{18}\text{O}$ data set had few missing values as few samples were not been analysed due to technical problems in the laboratory. But we did not miss any yearly mean value in our data set as we had enough replicates.

Climate data and quantification of drought

We used a climatic water balance model for each study location to quantify drought stress by using various climatic parameters like daily mean temperature, cloud cover, relative humidity and precipitation from German and Swiss weather stations (DWD; MeteoSwiss) and daily solar radiation calculated by using RayMan software (Matzarakis et al. 2007) and Potential evapotranspiration (PET) calculated by Turc's formula (Turc 1961). Finally the monthly and annual climatic drought index was calculated from the deviation of

precipitation-potential evapotranspiration (P-PET) to depict the water balance model, for each study location. The water balance model was prepared for 65 years, starting from 1947 to 2011 (Figure S1 in the supplementary document) that showed the climatic trend of the study locations. The water balance model estimates surplus water in forest soil when precipitation exceeds PET, and when precipitation is less than PET, the stored water in the soil begins to be depleted. Therefore, high values in the water balance model indicate a less-dry condition, whereas low values denote dry conditions. The temperature, precipitation, PET, and drought index were calculated monthly, annually, and for the growing season (May to September) (for detailed calculation see section S1 in the supplementary documents).

Application of the Scheidegger conceptual model for dual-isotope analysis

For applying dual-isotope analysis, we used the model by Scheidegger et al. (2000) (for detailed conceptual application see Figure S2 in the supplementary documents) with our $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values from the tree rings of beech trees. Scheidegger's model interprets changes in $\delta^{18}\text{O}$ because of leaf water enrichment, assuming due to a change in air humidity mainly, whereas changes in $\delta^{13}\text{C}$ are related to the intrinsic water use efficiency of the plants. Past studies (Barbour and Farquhar 2000; Barbour 2007; Grams et al. 2007; Roden and Siegwolf 2012) already proved that if the source water for trees remains the same or the trees are in close proximity, which is our case, then inter-tree variation in $\delta^{18}\text{O}$ is due to tree physiological process. Considering this fact, we can assume that when trees have same source of water, the variation in $\delta^{18}\text{O}$ is strongly correlated with relative humidity and evaporative demand which influence stomatal conductance (Barnard et al. 2012). It means the model could explain the deduction of changes of A_{max} and g_s in the scenario of different isotopic reaction patterns. It also assumes a negative relation between $\delta^{13}\text{C}$ and intercellular CO_2 concentrations (C_i). We wanted to identify changes of patterns in isotopes between two groups of beech trees based on their AWC

classes (dry vs. less-dry plots) over the 16 years (from 1995 to 2010). The different soil moisture conditions in the two groups, i.e. dry and less-dry plots, can result in higher, lower, or similar values in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of tree rings. The central assumption of this model is that when trees have access to a similar source of water $\delta^{18}\text{O}$, then the majority of $\delta^{18}\text{O}$ variation could be explained by evaporative enrichment, which was the case for our study. Furthermore, considering the same source of water for comparing plant groups, we have assumed that $\delta^{18}\text{O}$ variation would be altered based on soil water storage capacity for each group. The model's prediction was used to explain the physiological shifts between dry and less-dry plots over 16 years and was then matched with the current ecophysiological understandings to interpret the data. A positive correlation between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of tree rings was interpreted as a result of decreasing g_s , but constant A_{max} or increasing A_{max} at constant g_s depending on the position of the input arrow ($\delta^{13}\text{C}$ vs $\delta^{18}\text{O}$) in the model with the reference of our data. Conversely, a negative correlation between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of tree rings was interpreted as decreasing A_{max} at constant g_s or increasing g_s at constant A_{max} , again depending on the position of the input arrow in the model with our data.

The original conceptual model of Scheidegger et al. (2000) was prepared in the leaves of the C3 plants but could also be applied for the tree rings (Barnard et al. 2012) with careful pre-requisite (Roden and Siegwolf 2012) as the tree rings also carry the carbon and oxygen isotopic signatures of the leaves.

Statistical analyses

For comparison of different variables, we always performed the analysis separately for three study areas as case studies. According to the Shapiro–Wilk test, the data did not follow the Gaussian distribution for at least one of each comparing group. Hence, the nonparametric Mann–Whitney U test was performed to compare the difference of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ between dry and less-dry plots. The influence of plot level AWC (i.e. less-dry vs. dry plots), neighbourhood competition (Hegyí's index) and location of the plots on average $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of each trees were studied in a generalized linear mixed model (GLMM) analysis. The plot level AWC and competition were considered as fixed effect and location of plots were taken as random effect. Above-mentioned statistical analyses were done by using the SPSS (Version 20.0 SPSS 2011).

The relationship between temperature, precipitation, and drought index (P-PET) with mean $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ was analysed annually and by growing season (May to September). The Pearson's product-moment correlation analysis (data with Gaussian distribution) and the Spearman's rank correlation analysis (data with non-Gaussian distribution) was done to study the relationship between stable isotope ratios and climatic variables. A total of 1000 bootstrap replicates were used to quantify the significant level ($p < 0.05$) at the 95% confidence intervals by using the SPSS software version 20.0 (IBM Corp. 2011).

Then the relationship analysis of both previous and current year's $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ with climatic variables was done. A total of 17 months, starting from the previous year's May to the current year's September, climatic data (temperature, precipitation and drought index) was related to annual mean $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values. This timespan was selected as the growth of beech trees is dependent on the weather of both current and previous years' growing seasons (Hackett-Pain et al. 2016; Chakraborty et al. 2021). Moreover, it was proven that temperate deciduous trees use the previous year's carbohydrate storage (Kozłowski and Pallardy 1997). The correlation analysis was done using the DENDROCLIM 2002 software package (Biondi and Waikul 2004). With DENDROCLIM 2002, we calculated a series of coefficients from Pearson's product-moment correlation functions and used them for further correlations of the monthly climate with $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ chronologies. In this analysis, a total of 1000 bootstrap replicates were used, with 95% of the confidence interval for the standard errors during the significance testing (see Biondi and Waikul 2004 for the details). Finally, pair-wise comparisons of chronologies were performed between dry and less-dry plots. The interpretations of the correlations are as follows: (a) positive correlation between monthly temperature and $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$ means negative growth, (b) negative correlation between monthly precipitation and $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$ means positive growth, and (c) negative correlation between monthly drought index and $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$ means positive growth (Saurer et al. 2008; Hartl-Meier et al. 2014).

For the dual-isotope analysis adapted to the Schiedegger conceptual model, we have normalized the isotope data by using a grand mean to evaluate the shift in the isotopes (Barnard et al. 2012). This grand mean was calculated by combining all the trees of a site (8 for each site) for the total period (16 years for our case). Then a new normalized series was prepared for each of the dry and less-dry groups of each site by subtracting the grand mean from the individual $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of each year for each sampled tree. Three grand means were prepared for three study sites: Badenweiler, Klettgau and Schwäbische Alb. By using these normalized values, we were able to combine data of all three sites to find a general trend of physiological responses among the trees, only based on soil water storage capacity as dry and less-dry plots.

Results

$\Delta^{13}\text{C}$ and $\delta^{18}\text{O}$ signatures in tree rings

$\delta^{13}\text{C}$ values ranged from -29.96 to -25.58 ‰ for the whole study, including all individuals of both dry and less-dry plots. Ranges were from -29.96 to -25.58 ‰ in Badenweiler (standard deviation: 0.6 and 0.8 for dry and less-dry plots respectively), -28.81 to -26.91 ‰ in Klettgau (0.4 and 0.4 for dry and less-dry plots respectively), and -28.32 to -26.02 ‰ in Schwäbische Alb (0.5 and 0.6 for dry and less-dry plots respectively) (Figures 3a–c).

Greater mean $\delta^{13}\text{C}$ values were found in dry plots than less-dry plots for both Badenweiler and Schwäbische Alb,

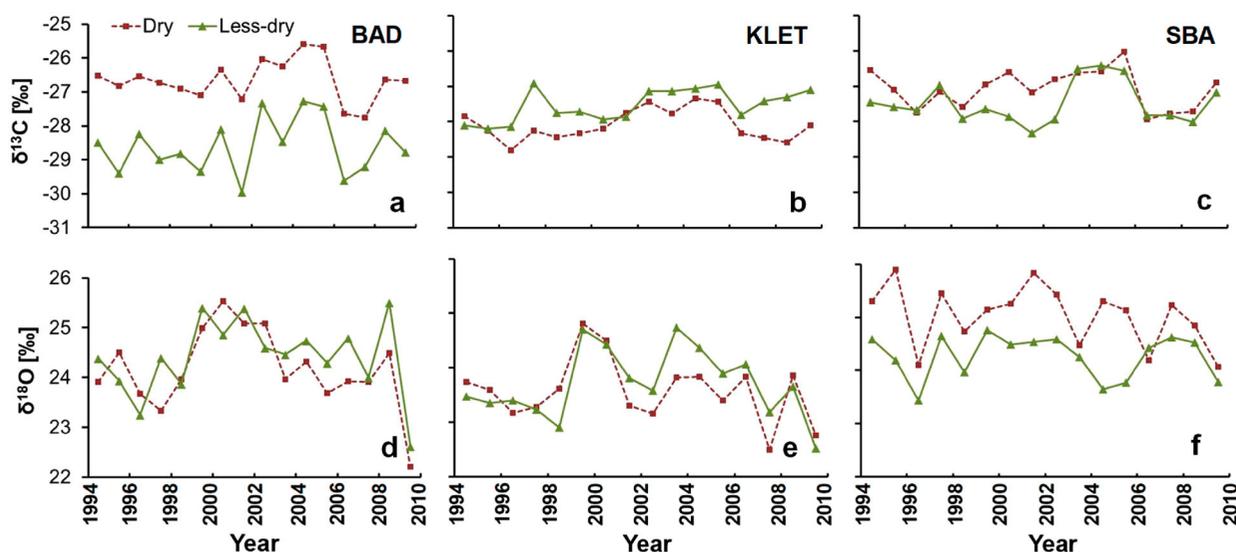


Figure 3. Time series of carbon ($\delta^{13}\text{C}$, plates a, b, c) and oxygen ($\delta^{18}\text{O}$, plates d, e, f) isotope data measured in wood samples taken at 1.3 m height for the beech trees of dry (brown line with symbol) and less-dry (green line with symbol) plots separately from Badenweiler (BAD), Klettgau (KLET) and Schwäbische Alb (SBA) regions². Please see Table 2 for the sample size, tree DBH, tree height, age and AWC.

where the difference was significant for Badenweiler only (Mann–Whitney $U = 6.0$, $p < 0.05$, $N_{\text{total}} = 32$, $N_{\text{dry plots}} = 16$, $N_{\text{less-dry plots}} = 16$). In contrast, Klettgau showed the opposite result with significantly greater mean $\delta^{13}\text{C}$ values in less-dry plots (Mann–Whitney $U = 45.0$, $p < 0.05$, $N_{\text{total}} = 32$, $N_{\text{dry plots}} = 16$, $N_{\text{less-dry plots}} = 16$) (Figure 4a).

$\delta^{18}\text{O}$ values ranged from 22.21–25.90‰ for the whole study, including all individuals of both dry and less-dry plots. The values were from 22.21–25.51‰ in Badenweiler (standard deviation: 0.8 and 0.7 for dry and less-dry plots respectively), from 22.50–24.82‰ in Klettgau (standard deviation: 0.6 and 0.6 for dry and less-dry plots respectively), and from 23.44–25.90‰ in Schwäbische Alb (standard deviation: 0.6 and 0.4 for dry and less-dry plots respectively) (Figures 3d–f).

The values for $\delta^{18}\text{O}$ were commonly higher in the dry plots than the less-dry plots in Schwäbische Alb. However, in Badenweiler and Klettgau, only a few years had shown

higher $\delta^{18}\text{O}$ values in dry plots than less-dry plots (Figure 3d–f).

Dry plots had significantly higher mean $\delta^{18}\text{O}$ value than less-dry plots in Schwäbische Alb (Mann–Whitney $U = 42.0$, $p < 0.05$, $N_{\text{total}} = 32$, $N_{\text{dry plots}} = 16$, $N_{\text{less-dry plots}} = 16$). Both Badenweiler and Klettgau had the opposite trend than Schwäbische Alb. The $\delta^{18}\text{O}$ values were higher in less-dry plots than dry plots in these two sites, but the differences were not significant (Figure 4b).

Influence of soil water availability, neighbourhood competition, and plot location on $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$

The influence of neighbourhood competition, water availability status of the plots (i.e. less-dry vs. dry plots), and plot locations on $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were investigated in generalized

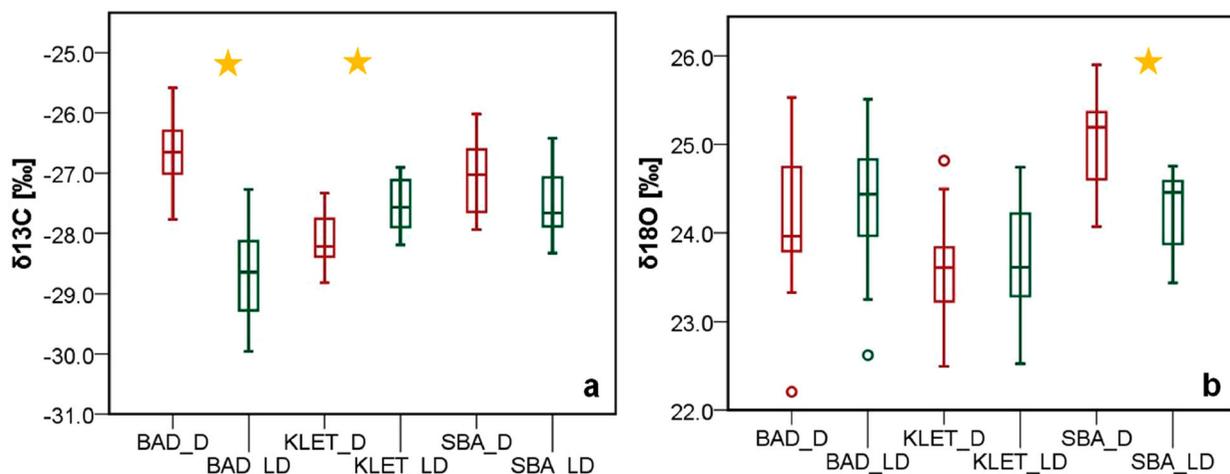


Figure 4. Comparison of $\delta^{13}\text{C}$ (a) and $\delta^{18}\text{O}$ (b) values of dry and less-dry plots from three study sites based on the mean isotopic values for the period of 1995–2010. Significant results are marked with yellow stars. BAD_D: Badenweiler dry, BAD_LD: Badenweiler less-dry, KLET_D: Klettgau dry, KLET_LD: Klettgau less-dry, SBA_D: Schwäbische Alb dry and SBA_LD: Schwäbische Alb less-dry plots. Please see Table 2 for the sample size, tree DBH, tree height, age and AWC.

Table 3. Results from the Generalized Linear Mixed Model/GLMM showing the relationships among $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ with soil water storage capacity, neighbourhood competition and plot location as explanatory variables.

$\delta^{13}\text{C}$ (target variable)	Model Term	Coefficient	Std. Error	t values	p values
Fixed effects					
	Intercept	-27,3941	0,8117	-33,7488	0,0000
	Neighbourhood competition (Hegyí's index)	-0,0017	0,0423	-0,0400	0,9684
	AWC (less-dry plots vs. dry plots)	-0,5674	0,2512	-2,2590	0,0346
Residual effects					
	Estimate		Std. Error	Z values	p values
	Tree id 5	0,4951	0,8726	0,5674	0,5704
	Tree id 8	0,5189	1,3327	0,3893	0,6970
	Tree id 10	0,6744	1,3079	0,5156	0,6061
	Tree id 15	0,2929	1,8543	0,1580	0,8745
	Tree id 16	0,5785	1,4070	0,4112	0,6810
	Tree id 17	0,8081	1,3949	0,5793	0,5624
Random Effect					
	Random effect covariance	Estimate	Std. Error	Z values	p values
	Plot id	0,3332	1,8573	0,1794	0,8576
$\delta^{18}\text{O}$ (target variable)	Fixed effects				
	Model Term	Coefficient	Std. Error	t values	p values
	Intercept	23,1069	0,7546	30,6210	0,0000
	Neighbourhood competition (Hegyí's index)	0,0962	0,0371	2,5930	0,0170
	AWC (less-dry plots vs. dry plots)	0,2020	0,1633	1,2368	0,2298
Residual effects					
	Estimate		Std. Error	Z values	p values
	Tree id 1	0,2684	0,8013	0,3350	0,7376
	Tree id 3	1,0535	1,5133	0,6962	0,4863
	Tree id 4	0,1857	0,6357	0,2921	0,7702
	Tree id 8	0,6904	0,7792	0,8860	0,3756
	Tree id 9	3,2933	3,4484	0,9550	0,3396
	Tree id 15	0,2231	0,8725	0,2557	0,7981
	Tree id 17	0,4902	1,0770	0,4552	0,6490
	Tree id 18	1,1518	1,3956	0,8253	0,4092
	Tree id 20	0,4939	2,0593	0,2398	0,8105
	Tree id 21	0,3807	0,7422	0,5129	0,6080
	Tree id 22	0,7106	1,1768	0,6038	0,5460
Random Effect					
	Random effect covariance	Estimate	Std. Error	Z values	p values
	Plot id	0,7461	1,0078	0,7404	0,4591

Note: The GLMM was run on 24 trees. The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values are average values of 16 years from 1995 to 2010. The fixed effects variable such as neighbourhood competition and AWC were measured once in 2012 and 2013. Significant coefficients and p-values are marked in bold.

linear mixed model or GLMM analysis (Table 3). The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were the mean annual values for each tree's observation period. The competition and AWC were treated as fixed effects, whereas the locations of the plots were considered a random effect. The trees belonged to less-dry plots over dry plots had a significantly lower level of $\delta^{13}\text{C}$, but, that trend was not significant for $\delta^{18}\text{O}$. The $\delta^{18}\text{O}$ values increased with an upsurge of competition although this was not found in case of $\delta^{13}\text{C}$. The location of the plot and residual effects of the GLMM analysis were not significant (see Table 3).

Relationships between climatic variables, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$

All three study sites showed increasing temperature, decreasing precipitation, increasing PET, and decreasing P-PET trends from 1947 (Figure S1 in the supplementary document). These trends were more prominent since 1995 (study period) (Figure 5). Although SBA showed opposite trend for PET and drought index. Climate data of the growing period

specifically showed that the combination of higher temperatures, lower precipitation, and higher PET has led to a situation of continuous drought stress in all study sites (Figure 5).

The correlation of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ with temperature and drought index had a few significant values where precipitation did not show any significant relation for any of the isotopes (Table 4). Mean annual $\delta^{13}\text{C}$ showed a negative relationship with temperature in less-dry plots of Schwäbische Alb where positive relation was found during the growing season in less-dry plots of Badenweiler. Looking at the relation of $\delta^{13}\text{C}$ and drought index, only less-dry plots of Klettgau showed a negative relation. With $\delta^{18}\text{O}$, the annual temperature of dry plots of Badenweiler and less-dry plots of Klettgau had a significant positive relationship, whereas the less-dry plots of Schwäbische Alb had a positive relation.

The correlation of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ with climatic variables (temperature, precipitation, and drought index) was significant at both dry and less-dry plots for

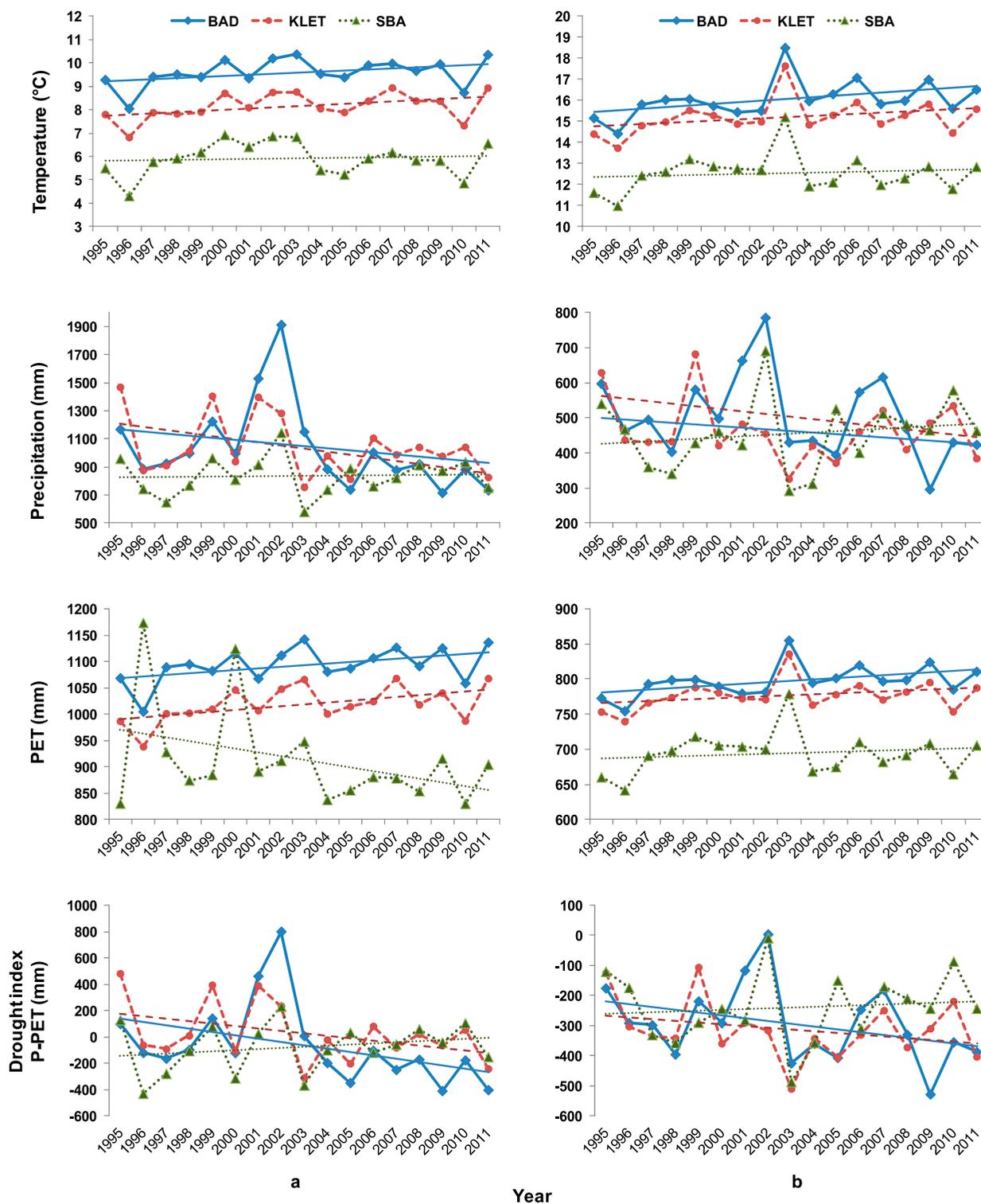


Figure 5. Mean annual temperature, measured in degree centigrade (°C), total annual precipitation, total annual PET (i.e. potential evapotranspiration), and total annual P-PET (i.e. precipitation–potential evapotranspiration), all data in millimetre (mm) for the three study locations from 1995 to 2011 with the respective linear trend lines. All graphs at the left-hand side, denoted by (a), are for the entire year, whereas all graphs at the right-hand side, denoted by (b), are for the growing season (May–September).

the preceding and current ring forming year (Figures 6 and 7).

Mainly looking at the correlation of the $\delta^{13}\text{C}$ and the summer months, dry plots showed higher relations for temperature, precipitation, and drought index. In general, the current months of the tree ring forming year showed

higher relations than the preceding months. Nevertheless, summer months from the preceding year of ring formation had shown few significant relations for all climatic variables. In this context, the trees at the sites of Klettgau and Schwäbische Alb showed higher relations in less-dry plots for the preceding months. (Figure 6).

Table 4. Significant correlation coefficients between 16-years mean values of $\delta^{13}\text{C}$ ($N = 12$), $\delta^{18}\text{O}$ ($N = 12$) and annual, growing season temperature and drought index for dry (D) and less-dry (LD) plots of Badenweiler (BAD), Klettgau (KLET) and Schwäbische Alb (SBA) are shown below.

$\delta^{13}\text{C}$	Temperature		Drought index	
	Annual	Growing season	Annual	Growing season
BAD_D				
BAD_LD		0.6		
KLET_D				
KLET_LD			-0.4	
SBA_D				
SBA_LD	-0.5			
$\delta^{18}\text{O}$				
BAD_D	0.6			
BAD_LD				
KLET_D				
KLET_LD	0.4			
SBA_D				
SBA_LD	0.5			

Temperature and $\delta^{13}\text{C}$ had both positive and negative relations. Summer temperature showed positive relations, which means the temperature causing higher $\delta^{13}\text{C}$ discrimination. At the same time, the spring temperatures are showing negative relations. Significant negative relation with precipitation and drought index support the crucial role of water availability for $\delta^{13}\text{C}$ discrimination during the summer months (Figure 6).

For $\delta^{18}\text{O}$, we found higher climatic associations in dry plots than less-dry plots in both current and preceding summer months for Badenweiler. This result was consistent in Schwäbische Alb, for both precipitation and drought index. On the other hand, Klettgau showed the opposite trend with the higher climate-growth association in less-dry plots (Figure 7).

Dual isotope: Scheidegger model

According to the Scheidegger model, the correlation between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ can be interpreted as the performance of stomatal conductance (g_s) and photosynthetic capacity (A_{max}). Applying our $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ data in the model, less-dry plots showed higher stomatal conductance; whereas, dry plots had shown opposite response, reduction in stomatal conductance, or higher stomatal resistance (Figure 8).

$\Delta^{18}\text{O}$

Looking after the yearly values (Figure 9), our observational data under the Scheidegger model interpretation had showed a higher stomatal conductance (7 times) in the less-dry plots. In contrast, dry plots showed higher yearly values with reduction in stomatal conductance (8 times). The yearly series of graphs with all data points are given in the supplementary document (Figure S3).

Discussion

This study assessed retrospective physiological and climatic responses and the consequences of that by using the example of European beech trees. The beech trees of this study grew under permanent and periodic drought, quantified by edaphic and climatic parameters, respectively. We hypothesized that soil water availability would alter the physiological responses through stomatal behaviour and control over the acclimatization of the beech trees growing under

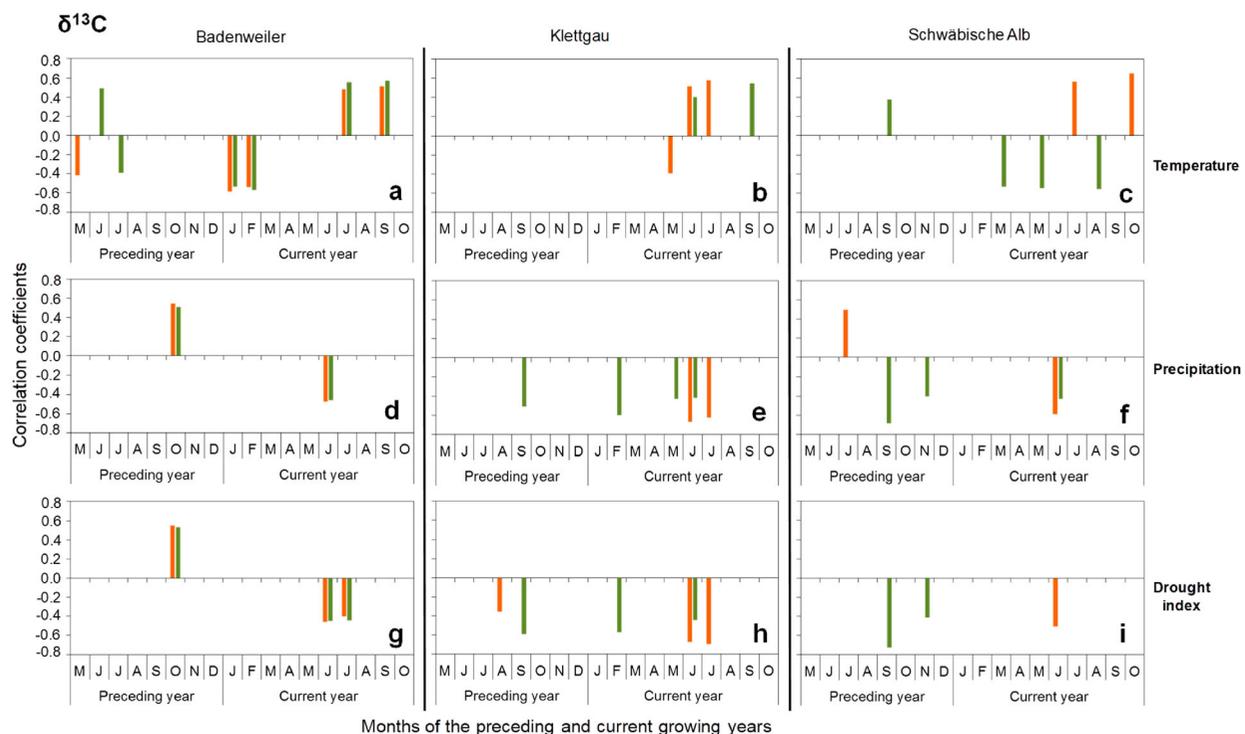


Figure 6. Significant correlation of yearly mean $\delta^{13}\text{C}$ with the preceding (May to December) and current (January to October) year's monthly temperature (plates a, b, c), precipitation (plates d, e, f), and drought index (plates g, h, i) of Badenweiler, Klettgau, and Schwäbische Alb. Orange bars indicate dry plots ($N_{\text{trees}} = 12$) whereas green bars indicate less-dry plots ($N_{\text{trees}} = 12$). The period of observation was between 1995 and 2010.

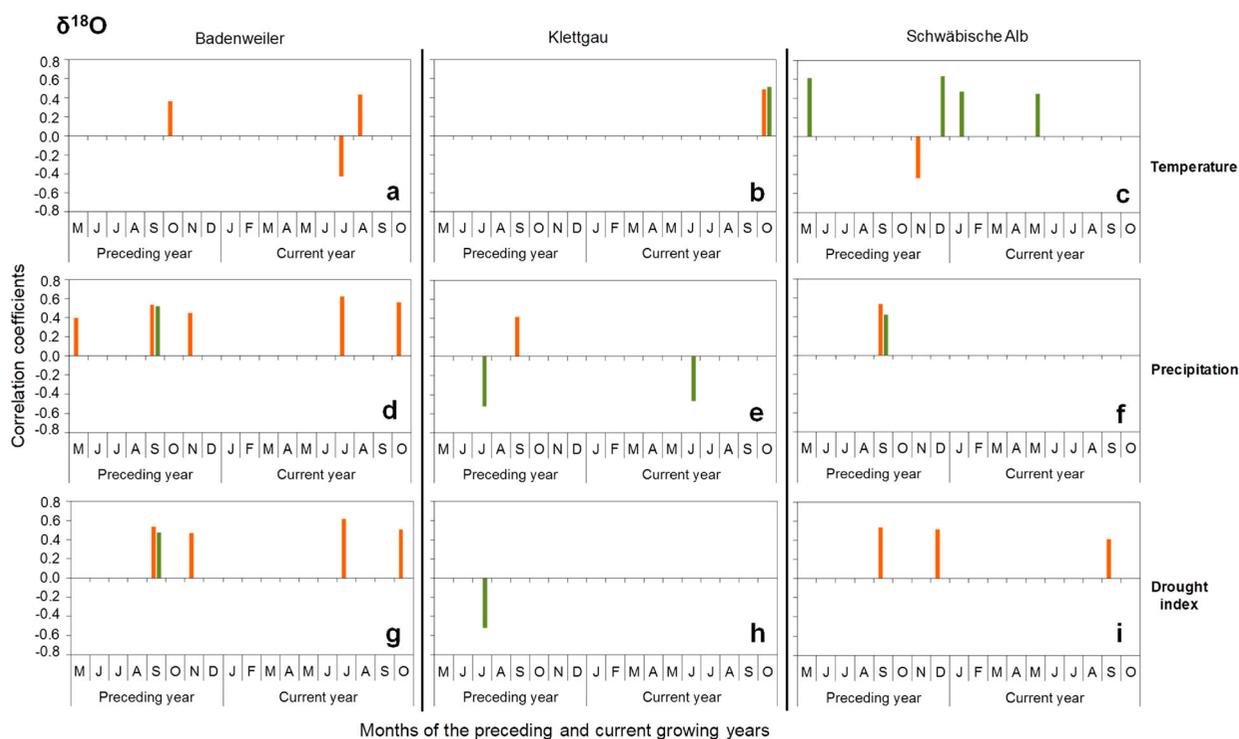


Figure 7. Significant correlation of yearly mean $\delta^{18}\text{O}$ with the preceding (May to December) and current (January to October) year's monthly temperature (plates a, b, c), precipitation (plates d, e, f), and drought index (plates g, h, i) of Badenweiler, Klettgau, and Schwäbische Alb. Orange bars indicate dry plots whereas green bars indicate less-dry plots; $N = 16$, years (1995–2010).

the same climatic drought condition in non-controlled environments. This hypothesis was corroborated through our analysis.

$\Delta^{13}\text{C}$ and $\delta^{18}\text{O}$ signatures in tree rings and influence of different environmental variables

The sampled trees of this study was co-dominant beech trees, and we were successfully able to get isotopic signal in all tree rings. General practice is to use dominant trees as sample tree for the stable isotope analysis with tree rings. But due to the natural forest stand condition, this study had notable deviation for sampling trees. In one earlier study authors (Barnard et al. 2012) found isotopic signal in different lower crown classes of beech trees in natural forest condition and discussed about the variation of water use efficiency of the trees in various size classes. Nevertheless, our study did not aim to look difference of water use efficiency among various size classes, rather we focused only on one size class (co-dominant) to assess the performance.

In this study, we found that the trees' physiological responses to environmental and climatic variables in the two groups (dry vs. less-dry) were different, especially when there was a higher difference in soil water between dry and less-dry plots (in the case of Badenweiler and Schwäbische Alb for $\delta^{13}\text{C}$ and only Schwäbische Alb for $\delta^{18}\text{O}$). The forests at those two locations had higher variation in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ between the trees of dry and less-dry plots. One reason for this variation is that the trees of all study sites

have suffered from recurrent drought situations during the last decades because of increasing temperature, higher irradiation, and decreasing precipitation (Gärtner et al. 2008; Kohler et al. 2006). The trees growing in dry plots have been regularly affected by drought stress as the soil in those plots had low AWC (Chakraborty et al. 2017). Recurrent drought stress resulted in higher $\delta^{13}\text{C}$ values in temporal data of stem wood material which is consistent with the isotopic fractionation model prepared in the greenhouse experiments (Farquhar and Richards 1984). The findings were in line with the studies dealing with tree-ring data in experimental setups where $\delta^{13}\text{C}$ values were found sensitive to soil moisture and drought stress (Saurer et al. 1995; Pollastrini 2010).

The increase in $\delta^{13}\text{C}$ could be related to stomatal conductance, which is lowered more than photosynthesis and causes the increase of intrinsic water use efficiency of trees under stressed conditions during drought (Adams and Kolb 2004; Egilla et al. 2005). The variation of $\delta^{18}\text{O}$ between dry and less-dry plots may attribute to the evaporative enrichment occurring in the leaf, which was previously proved by the oxygen isotope ratio from the organic plant materials (Roden and Ehleringer 1999; Barbour et al. 2000). Although Roden and Ehleringer (1999) found that between 96 and 99% of the variation of $\delta^{18}\text{O}$ in the wood cellulose of temperate trees could be explained by the variation of the source water of the growing area, in our study, trees have access to the same source water because the samples are from small forest stands (0.5–0.9 ha), under the same precipitation patterns, and have no accessibility to groundwater. Their growth depends mainly on the precipitation of the growing season as the soil water storage capacity is meagre in these

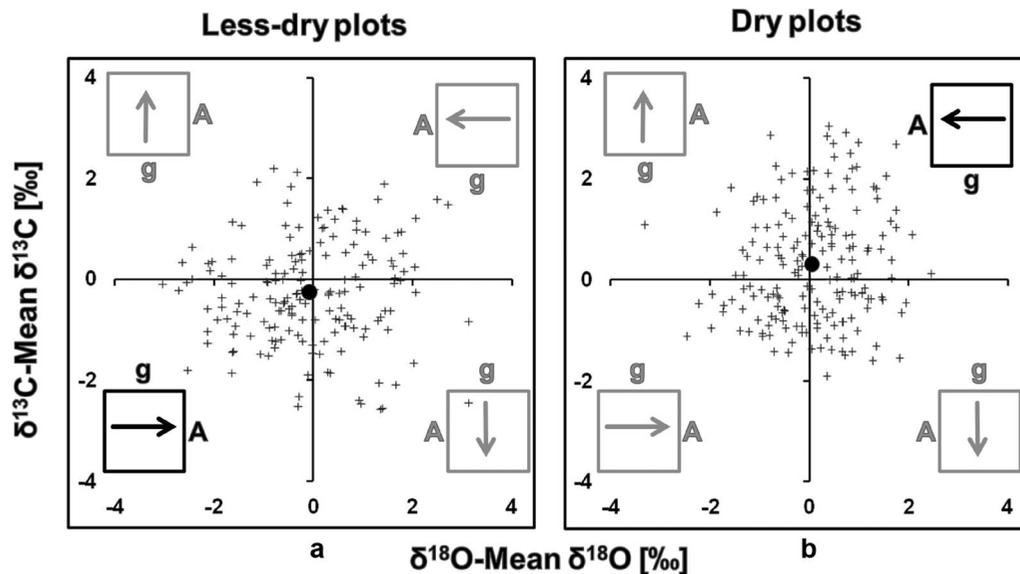


Figure 8. The relationship between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ for all samples from each site for the period of 1995–2010 in (a) less-dry and (b) dry plots. The x-axis denotes the difference between a corresponding $\delta^{18}\text{O}$ value for a tree of a particular year and the grand mean value of $\delta^{18}\text{O}$. The y-axis denoted the same for $\delta^{13}\text{C}$. The grand mean was calculated for three different sites and described in method section 2.6. The “+” marks are 192 in total (4 trees each from dry as well as less-dry plots from 3 sites with $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of 16 years). The bold black circles indicate the mean of the 192 values. In the inset, corresponding photosynthetic capacity (A) to stomatal conductance (g) trends are indicated by the arrows in the box at the corner of each block. Those trends were derived after the synthesis of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values following the conceptual model by Scheidegger et al. (2000), section 2.5 of the method and Figure S2 of the supplementary document. The responses in black shade is significant where responses in grey shade is not significant.

sites. So, the variation of $\delta^{18}\text{O}$ should hardly be influenced by differences in the source water.

Previous studies (Saurer et al. 1995; Hartl-Meier et al. 2014) found isotopic variation due to soil moisture content from dry and moist site conditions of differing aspects, topography, or bioclimate. However, we have shown the change in stable isotopic signature from the same population of beech trees within a forest stand of the same slope and aspect due to intra-stand differences in soil water storage capacity. The study site Klettgau showed the opposite trend of higher $\delta^{13}\text{C}$ in less-dry plots that tend to show higher drought impact in the trees of less-dry plots, and one probable cause of this finding is the less pronounced difference in AWC between dry and less-dry plots in this site. This result also supports that the importance of AWC is high to characterize the isotopic behaviour of the trees. Although only this explanation might not enough to justify the opposite trend we found in Klettgau, therefore we could assume that the hidden cause of this finding could not be detected fully which warrants further investigation,

Looking after the influence of different stresses (drought and competition), $\delta^{13}\text{C}$ had shown higher discrimination in dry plots over less-dry plots under drought stress. The competition did not show any significant influence on $\delta^{13}\text{C}$. Fernández-de-Uña et al. (2016) did a study with three different Mediterranean species and concluded that under the scenario of drought and competition, drought is the main driver for the change in $\delta^{13}\text{C}$ (in terms of intrinsic water use efficiency), and not the competition. Previous studies discussed the facilitation effect on the vitality (Chakraborty et al. 2017) and the negative impact of competition on growth (Chakraborty et al. 2021) on the beech trees in these study areas. Metz (2016) analysed $\delta^{13}\text{C}$ of beech tree rings and showed that overall impact (as the response of growth decline and less recovery) was higher in beech trees than those trees which were facing both stresses (drought and competition), but mixed forest could mitigate this stress. Overall competition between the two groups (dry vs less-dry) did not differ (Figure 2). It means in our case this result also explains that the beech trees in dry plots are

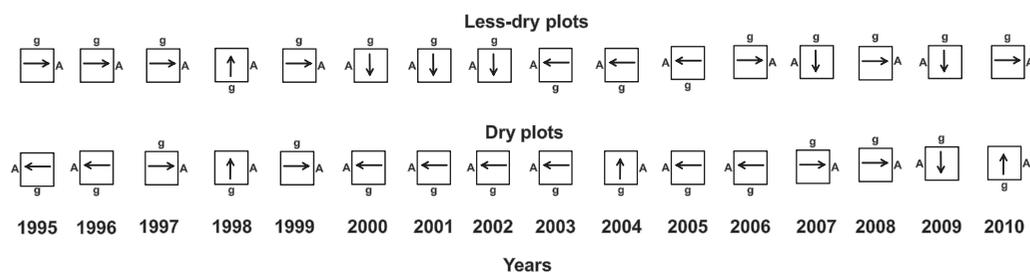


Figure 9. Yearly trends in photosynthetic capacity (A) and stomatal conductance (g) for the study period (1995–2010). The trends are derived after synthesizing $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values for each as described in Scheidegger et al. (2000), in the method section 2.5 and Figure S2 in the supplementary document. A detailed description of relations between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ for each year has been provided in Figure S3 in the supplementary document.

facing higher pressure due to both drought and competition stresses, but, trees from less-dry plots are more capable of overcoming imposed drought stress than trees from dry plots just because of the intensity of imposed stress differs in between two groups. A positive relation between competition and $\delta^{18}\text{O}$ discrimination was found, which could result from high size-asymmetric competition faced to the target beech trees. Because an asymmetric mode of competition for light can influence the conditions for evaporation, leading to the enrichment of $\delta^{18}\text{O}$ in leaves and reducing tree growth. For example, Moreno-Gutiérrez et al. (2015) showed that size-asymmetric competition from dominant *Pinus halepensis* trees can increase the $\delta^{18}\text{O}$ in understory woody shrub *Rhamnus lycioides* in the semi-arid Mediterranean ecosystem. However, more physiological experimental studies should be carried out at the field condition to understand the underlying processes behind the influence of competition on $\delta^{18}\text{O}$ in beech trees with changing soil water storage capacity.

Climate and tree physiological interactions

All three study areas have very high PET causing strong climatic drought conditions. In this scenario, soil moisture plays a crucial role in mitigating drought. Those trees growing in higher AWC plots benefitted during the climatic drought condition as we found that the trees growing in less-dry areas with comparatively higher soil water storage capacity had a lower physiological response from the climatic variables (at least for two study areas). A strong correlation with local climatic variables for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ indicate a strong climate signal and climate moisture dependency of the trees, which is natural because the trees are growing at their ecological drought limit (due to site constraints). This finding is supported by the following evidence. For example, climate sensitivity of the isotopic signatures expressed by significant correlations between carbon and oxygen isotopes and several climatic variables, like temperature, precipitation, and drought index for beech trees were found. by Hiltunen and Berninger (2010) in Finnish oaks. We found a relationship between temperature and $\delta^{18}\text{O}$ for all three study areas (Table 3), which corroborates previous work of Hartl-Meier et al. (2014). This relation was true for both the current and preceding year of ring formation, a finding also reported by the previous researchers (Skomarkova 2006; Mölder et al. 2011). Beech trees use the stored carbohydrate from the previous years to grow, so a longer carry-over effect of dry periods was found (Czajkowski et al. 2005; Chakraborty et al. 2013). The higher correlation between $\delta^{13}\text{C}$ with temperature and precipitation during the summer months reveals a higher chance of drought impact on the fractionation of $\delta^{13}\text{C}$ during the growing season (Saurer et al. 1995; Skomarkova 2006). The trees in dry plots showed higher magnitude and frequency of climate dependency compared to those from less-dry plots. Saurer et al. (1995) found a dominant correlation between $\delta^{13}\text{C}$ and climatic parameters of the dry sites compared to the wet sites. This higher association of both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ with climatic variables in dry plots states that the trees are

more climate-dependent and sensitive than the trees in less-dry plots. Higher association during summer months also indicates higher hydrological dependency following higher risk for the trees, particularly under the summer droughts, which supports the finding of the companion study showing higher mortality risk for the trees growing in dry plots (Chakraborty et al. 2017).

However, the climate correlation and microsite variation (based on soil water storage capacity) were weaker for $\delta^{18}\text{O}$ values compared to $\delta^{13}\text{C}$ values in all study sites, showing a minor association of $\delta^{18}\text{O}$ with climatic parameters. Moreover, several fractionation variations might occur within different plant tissue before storing oxygen isotopes from leaf to wood. This could be a reason for lower climatic signals found in $\delta^{18}\text{O}$ of wood. However, this assumption warrants further research on fractionation pathways of $\delta^{18}\text{O}$ within the tissues of trees.

Data interpretation under dual-isotope conceptual model

According to the Scheidegger conceptual model reproduced by our data, a difference between the two groups (trees from dry vs. less-dry plots) were found: dry plots tend to have stomatal closure earlier, and less-dry plots tend to keep stomata open for more extended period. In the time series between 1995 and 2010, a higher temporal frequency of stomatal conductance was observed in less-dry plots than in dry plots supports our hypothesis that beech trees from the same population would react differently to drought growing on soil with different levels of water storage capacity.

Some previous experimental studies (Hiltunen and Berninger 2010) found intra-specific variations in drought mechanisms in diverse beech populations. For this study an explanation is that the trees from less-dry plots have higher available soil water, which supports them in keeping stomata open and continuing photosynthesis for a more extended period, whereas because of less available soil water, the trees from dry plots of these sites have grown a tendency of closing stomata earlier. Earlier Ramesh et al. (1985) discussed that intra-specific differences in C and O isotopes might occur in the trees growing at different microclimatic levels, affecting macro-climatic reconstruction. According to previous companion studies (Chakraborty et al. 2017, 2021), the trees from dry plots have a drought avoidance mechanism as an acclimatization strategy by reducing their growth rate and allowing higher partial crown dieback to maintain water status and root/shoot ratio as the soil water reduces very early during the drought. However, this acclimatization is causing severe damage to the trees, sometimes forcing to death. Whereas the trees from less-dry plots still can grow by keeping their stomata open with the support of more soil water. A mortality threshold in respect of crown dieback was observed in the study sites for the beech trees from dry and less-dry plots (Chakraborty et al. 2017), and that finding supports the possible risk of dieback of beech trees due to more extended drought period for both dry and less-dry plots (Breda and Badeau 2008). This might be one reason for a minor

difference in our adapted Scheidegger conceptual model (Figure 8), as frequent droughts with higher magnitudes are common in the study sites (Figure S1 in the supplementary documents).

Nevertheless, in this study, we have demonstrated that beech trees growing naturally on forest soil with contrasting water storage capacity show difference in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ and that might cause difference in the physiological responses as a growth adaptation strategy during climatic drought events which is a novel finding of this study.

Comments

A few non-significant results, an opposite result in one of the study sites, some limitation of the $\delta^{18}\text{O}$ analysis, and the application of Scheidegger conceptual model have to be discussed. We want to point out that a big challenge was finding beech trees at least at codominant size class. We have tried to follow the height class comparability so that we can have trees from the same growing environment. Nevertheless, to follow this, we had less difference in AWC class (Klettgau had very low difference between mean dry and less-dry values). And we can not overlook the fact that this study was designed under natural condition where following the prerequisite for “dual isotope study” under the Scheidegger conceptual model of having the same growing environment and source was not always possible. We want to remark on another important point: dendrochemical studies with stable isotopes are expensive and time-consuming in our case, leading to comparably small sample sizes. Moreover, due to having narrow tree rings, the main challenge was getting enough wood samples to get a proper signal of the isotopes, which allows relating them with environmental variables. Fortunately, we were able to derive both carbon and oxygen isotopic signals but sometimes had less representation due to missing values, particularly with $\delta^{18}\text{O}$, as analysing oxygen isotopes is always challenging (Saurer et al. 1997a; Barbour 2007).

Recent studies showed that $\delta^{18}\text{O}$ variation in precipitation might change over the years (Vystavna et al. 2020, 2021). For example, Vystavna et al. (2021) used data from 20 weather stations worldwide from 1960 to 2016 to find the influence of temperature and total precipitation on a continuous time series of $\delta^{18}\text{O}$ variation. That study included Stuttgart weather station, which was the nearest station to the study area (Vystavna et al. 2021). The study concluded that $\delta^{18}\text{O}$ variation in Stuttgart was related to a higher contribution of recycled moisture to precipitation and a higher impact of North Atlantic Oscillation (NAO) on $\delta^{18}\text{O}$ values compared to other stations from the same climatic zone like Vienna (Vystavna et al. 2021). However, we only observed $\delta^{18}\text{O}$ for a short time series from 1995 to 2010 in a relatively small geographic area. Nevertheless, how long-term change in $\delta^{18}\text{O}$ in precipitation can influence $\delta^{18}\text{O}$ concentration in annual tree rings should be researched in the future.

Use of the Scheidegger conceptual model was helpful but challenging to interpret the physiological mechanisms during the drought scenario assessments with tree ring isotope. However we have to be very conservative to interpret tree

ring isotopic signals based on this model as the model is based on some assumptions, all of which was difficult to follow in the scenario of working in field condition, but with careful study design this technique was used to interpret isotopic signals in tree rings (Barbour 2007; Roden and Siegwolf 2012). The critical factor is that the model was prepared based on leaf water isotopic data. When tree ring wood isotopes are used, it has some mixture of evaporated leaf water and unevaporated xylem water, which dilute the $\delta^{18}\text{O}$ signals compared to the leaf water signals (DeNiro and Epstein 1979). Remobilization of starch from the previous year to the current year of ring growth also changes the signals in the wood (DeNiro and Epstein 1979; Farquhar and Lloyd 1993) although current studies proved remobilization plays a minor role in latewood development (Roden and Farquhar 2012). One of the model assumptions is sites with high humidity (>80%) are of poor choices for using this model due to undetectable changes in g_s (Roden and Siegwolf 2012), our study sites had <80% relative humidity conditions for the whole year and <75% during the growing season which again could support this assumption. These limitations need to be considered in a study involving wood materials. Perhaps using some more micrometeorological data with isotopic source data and in situ physiological measurements (like stem water $\delta^{18}\text{O}$) would have helped to interpret the isotopic signature in tree rings more efficiently, which could not be done due to financial and time constraints.

Conclusions

Our findings indicate that combining carbon and oxygen stable isotopes from narrow tree rings could reveal the variation of drought impact on the beech trees growing asco-dominant trees in near-natural forest stands at their drought limit. We found that the beech trees of the same population grew under different micro-climatic (due to the variation of soil water storage capacity), but the same macro-climatic conditions showed variation in physiological responses to drought. Beech trees growing on drier soil suffer from higher drought impact than the trees from less dry soil at a lower spatial scale as competition is creating additional stress on dry plots. Moreover, the climate-growth dependency was found to be higher in the trees growing on drier plots. Dual isotope analysis showed a tendency of higher stomatal resistance in the trees from dry plots where the trees from less-dry plots tend to have the opposite physiological response with comparatively higher stomatal conductance. We conclude that beech trees have high acclimatization ability under the drought condition. We emphasize that may be the trees in dry plots are more drought sensitive, whereas a more drought-tolerating physiological strategy can be opted by trees in less-dry plots. This difference might diminish with frequent and severe drought events as mortality risks increase for both groups which requires experimental physiological studies in the natural forest condition with soil water control mechanism. The findings from this study enrich our knowledge on retrospective physiological responses of the beech trees to

drought growing at the drought limit or the ecotones with oak forests within their geographical distribution range. The forest administration should preserve such near-natural forest ecotones to study beech trees' long-term response and adaptation to drought events and allow beech trees to struggle for survival which is crucial for creating genetic diversity and fostering evolution. Such near-natural ecotones may also play a crucial role for future seed source and in forest regeneration and restoration programmes.

Contributions by authors

T.C., A.R., and S.S. developed the concept of this study. T.C. and S.S. did the field data collections. T.C. did all laboratory and statistical analyses. A.M. contributed to the quantification of drought. T.C. wrote the original draft of the manuscript. A.R. and A.M. provided doctoral supervision to T.C. G.H. contributed to the isotope laboratory analysis. F.F. and S.S. reviewed and edited the manuscript. T.C. acquired the necessary funding for this research. All authors approved the final manuscript and contributed in writing.

Data availability

The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

Experimental research and field studies on plants

We want to declare that this study followed all the national and international regulations on field studies involving plant. The permissions from the Swiss and German forest authorities were taken before European beech trees were felled for the purpose of scientific research.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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