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A study of drought-induced photosynthesis and stomatal limitations using carbon and oxygen isotope signals in tree-ring cellulose

Damien HERFURTH

Responsables de stage:
Jérôme Ogée: jogee@bordeaux.inra.fr
Lisa Wingate: l.wingate@ed.ac.uk

Organisme d’accueil
INRA - Unité EPHYSE
71, Avenue Edouard Boulaux
33140 Villenave d’Ornon
France

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1. Introduction

Forests contribute largely to the Earth’s climate system and to the terrestrial carbon sink strength (IPCC 2007). Due to the strong relationship between water status and forest primary productivity, severe droughts or heatwaves like the one that experienced Europe in 2003 affect the size of the terrestrial carbon sink and lead to a positive feedback on global atmospheric CO$_2$ concentrations (Ciais et al., 2005). In temperate and Mediterranean climatic regions, more intense and more frequent extreme events such as droughts or heat waves are expected in the next decades (Schar et al., 2004) that could lead, on the long run, to the dieback of large forested areas. It is therefore crucial to understand how temperate and Mediterranean forests respond to drought events and to an increase of their intensity and frequency.

Although water availability is one of the most limiting factor to plant productivity (e.g. Nemani et al., 2003), the photosynthetic response to seasonal drought still remains poorly understood. While it is well established that stomatal closure is one of the first events taking place during drought (Chaves et al., 2002), a long-standing controversy exists on which limitation is prevailing when water stress progresses (Cornic, 2000; Lawlor, 1995; Tezara et al., 1999). It has long been accepted that stomatal control is the main driver behind photosynthetic response to water stress (Cornic, 2000). Yet, the involvement of non-stomatal limitations, affecting more directly the biochemical photosynthetic apparatus or the diffusional properties of the leaf lamina mesophyll, have also been suggested (Smirnoff & Stewart, 1985; Reichstein et al., 2002). Model-data studies have shown that these non-stomatal limitations were necessary to explain drought responses of carbon and water fluxes in Mediterranean ecosystems (Reichstein et al., 2003; Keenan et al., 2009, 2010).

Gas exchange measurements can be used to understand the regulation of internal carbon pools and separate the role of stomatal and non-stomatal limitations on photosynthesis (Keenan et al., 2010; Grassi & Magnani, 2005). For example, Grassi & Magnani (2005) showed that the stomatal, biochemical and mesophyll limitations were of similar magnitude during spring and autumn but increased with drought intensity during the summer, and their relative contribution changed. The authors concluded that diffusional (stomatal and mesophyll)
limitations largely affected net assimilation during most of the year, whereas biochemical limitations occurred only during leaf development and senescence or severe droughts.

Beyond direct gas-exchange measurements, more indirect estimates of the plant ecophysiological response to drought could also be used to disentangle between stomatal and non-stomatal water limitations of forest productivity. For example, stable isotope signals in tree-rings exhibit well-defined seasonal variations that contain valuable records of past climate, leaf gas exchange and carbon allocation dynamics within the trees (e.g. Ogée et al., 2009) therefore offering retrospective insights into past environmental conditions and ecophysiological processes.

To study these isotopic signals however, process-based models that combine the present understanding of tree functioning with the associated isotopic processes are usually required (Hemming et al., 2001; Berninger et al., 2000).

The carbon isotope fractionation during photosynthesis is directly related to the intrinsic water-use efficiency, defined as the ratio of net CO\textsubscript{2} assimilation to total (stomatal and mesophyll) CO\textsubscript{2} conductance, while the oxygen isotope fractionation is an indication of evaporative demand (Barbour et al. 2000; Siegwolf et al. 1997; Sheidegger et al. 2000; Ogée et al. 2009). The combination of both signals should therefore lead to a powerful means to characterize drought-induced limitations on photosynthesis and stomatal conductance separately.

The objective of the present study was to explore this idea that stable isotope signals recorded in tree rings could be used to study the role of stomatal and non-stomatal limitations on photosynthesis and transpiration. For that we used a previously-published dataset of high-resolution tree-ring isotopes and gas-exchange measurements from Hartheim forest in Germany (Gessler et al., 2009), combined with the single-substrate model of carbon and oxygen stable isotope signals in tree-rings described in Ogée et al. (2009). This single-substrate model is forced by a canopy photosynthesis and transpiration model (Ogée et al. 2003), in which drought-induced limitations on either photosynthesis or stomatal conductance are introduced (the limitations on mesophyll conductance will be the subject of another study). The effect of these different limitations on the gas-exchange fluxes and the tree-ring isotope signals are then explored and compared to the gas-exchange and isotope measurements. The additional constraints resulting from the isotope archive are finally discussed.
2. Material and methods

2.1 Site description and soil properties

The Harteim forest is located in South-West Germany, close to the upper Rhine valley and the French border, surrounded by the Black forest to the east and the Vosges Mountains to the west. Annual precipitations amounts to 667 mm and mean annual air temperature to 9.8°C according to the nearby water station Bremgarten for the period 1951 – 1980 (Mayer et al., 2002). During the growing season (April to September) potential evaporation frequently exceeds precipitation (Tajchman, 1972). Mean temperature during the growing season is 15.4°C. Previously an alluvial mixed forest, it was progressively replaced by drought-resistant, even-aged *Pinus sylvestris* L. (Scots pine) plantations in the middle of the last century (Mayer et al. 2002).

The experimental stand used here (201m a.s.l.; 47°56’N and 7°37’E) was planted in 1964. In 2004, when gas-exchange measurements were performed (by the Meteorological Institute of the University of Freiburg), mean tree height was 14.3 m; stand density was 8000 trees ha\(^{-1}\), basal sap wood area amounted to 17.69 cm\(^2\) m\(^{-2}\), and projected LAI was 1.47 after a thinning in autumn 2003 (Brandes et al. 2007). The understorey vegetation consists mainly of *Brachypodium pinnatum*, *Carex alba* and *Carex flacca* (Wedler et al. 1996) with a leaf area index of 1.54.

The depth of the silty A\(h\) horizon varies between 15 and 80 cm with an average of 40 cm. The C horizon consists mainly of alluvial gravel that is sometimes interrupted by sandy veins (Sturm, 1996). The root zone of the pine trees extends to an approximate depth of 35 cm (Schäfer, 1977). The soil water field capacity and wilting point are respectively 31.4 m\(^3\) m\(^{-3}\) and 11.7 m\(^3\) m\(^{-3}\) (30 mm and 160 mm on a 40cm soil rooting depth basis, Garthe, 1985). Despite a high hydraulic conductivity (Sturm 1996), this low water storage capacity combined with prolonged periods without rain are at the origin of regular summer droughts when trees are subjected to water stress limitations (Sturm, 1996).

2.2 Flux measurements and partitioning

2.2.1 Meteorological parameters and fluxes measurements

Dry and wet bulb temperatures were measured at a height of 15 m on a meteorological tower. Bi-directional short-wave radiation (pyranometers, type CM21 ; Kipp & Zonen, Delft, The Nederlands) and longwave (pyranometers, type CG1 ; Kipp & Zonen Delft, The Nederlands)
radiation were measured at 16 m above ground. Photosynthetic photon flux density (LI-190A Li-Cor, Lincoln, NE, USA). Precipitation was monitored at the top of the 30 m tower. All sensor were sampled every 30 s. A data acquisition system (CR23X; Campbell, Logan, UT, USA), which calculates 10 min average values and for precipitation, 10 min totals, was stationed at the experimental site. VPD was calculated from the dry and the wet bulb temperature.

2.2.2 Flux partitioning

The measured net ecosystem exchange of CO$_2$ between the ecosystem and the atmosphere reflect the balance between photosynthesis or gross primary production (GPP) and total ecosystem respiration (TER) (Lasslop et al., 2009). NEE measured in Harteim forest was then partitioned into GPP and TER using two different methods.

**Nighttime-based estimates.** Because GPP is zero at night (defined here as global radiation < 20 W m$^{-2}$) night-time measured NEE is composed entirely of TER. The model of Lloyd & Taylor (1994) is then used to describe the temperature dependence of TER:

$$TER = R_{base} \exp\left(\frac{1}{T_{ref} - T_0} - \frac{1}{T_{air} - T_0}\right),$$

where $R_{base}$ (µmolC m$^{-2}$ s$^{-1}$) is the base respiration rate at the reference temperature $T_{ref}$ (set to 15°C), $E_0$ is a temperature sensitivity parameter, $T_{air}$ is air temperature, and $T_0$ is kept constant at -46.02°C as in Lloyd & Taylor (1994). Following Reichstein et al. (2005), $E_0$ and $R_{base}$ were estimated every 15 days using a 30-day window. TER was then extrapolated to daytime with $T_{air}$ measurements and the difference between NEE and TER estimates GPP.

**Daytime-based estimates including temperature sensitivity of respiration.** Because GPP is a saturating function of light, NEE can be fitted to a rectangular hyperbolic light response curve use (Falge et al. 2001):

$$NEE = \frac{\alpha \beta R_g}{\alpha R_g + \beta} + \gamma,$$

where NEE is net ecosystem exchange, $\alpha$ (µmolC J$^{-1}$) is the canopy light utilization efficiency and represent the initial slope of the light curve response, $\beta$ is the maximum CO$_2$ uptake rate of the canopy at light saturation, $\gamma$ (µmolC m$^{-2}$ s$^{-1}$) is the ecosystem respiration (TER), and $R_g$
(W m\(^{-2}\)) is global radiation. In practice, \(\alpha\), \(\beta\) and \(\gamma\) are fitted every 15 days using a 30-day window and GPP is recalculated using \(R_g\) measurements. The difference between NEE and GPP then yields to TER.

### 2.3 Stable isotope data

#### 2.3.1 Cellulose data

The 2004 annual rings from three individuals were cut into 40-µm sections using a microtome (Polycut E, Leica Microsystems, Brensheim, Germany). Each slice was then milled into fine particles using an ultra-centrifugal (Retsch MM 300, Haan, Germany). Cellulose was extracted using the technique described by Leavitt & Danzer (1993). Small amounts of each sample (0.7-0.12 mg for \(\delta^{13}C\) and 0.1-0.3 mg for \(\delta^{18}O\)) were transferred into silver (for \(\delta^{18}O\) analysis) or tin (for \(\delta^{13}C\) analysis) capsules. Samples were combusted in an elemental analyser (NA 2500; CE Instruments, Milan, Italy) for \(\delta^{13}C\) analysis and in high temperature conversion elemental analyser (TC/EA; Finnigan Mat GmbH, Bremen, Germany) for \(\delta^{18}O\) analysis, both coupled to an isotope ratio mass spectrometer (Deltaplus, Finnigan MAT GmbH, Bremen, Germany) (Brandes et al., 2007).

#### 2.3.2 Phloem sugars data

Phloem samples were collected every fortnight, at 11:00h Central European Time (CET). Eight trees were chosen randomly, but alternately, to avoid repetitive sampling that could produce artefacts because of wounding response (Brandes et al. 2007). Phloem exudates were obtained according to the method described in details by Gessler et al. (2004). Phloem tissues were collected from the twigs using a scalpel and from the trunks using a punch (at 1.3 m above ground). Tissues were separated from the older bark and rinsed with water to remove cellular constituents that might have been released from destroyed cells at the site of cutting (Brandes et al., 2007). Samples were incubated at room temperature in 2 ml of distilled water. Exudates were then centrifuged and 200 µl were transferred into silver (for \(\delta^{18}O\) analysis) or tin (for \(\delta^{13}C\) analysis) capsules and dried at 70°C. To improve combustion for \(\delta^{13}C\) analysis, 10 mg of Chromosorb (Sigma-Aldrich, Steinheim, Germany) were added into the tin capsules. Samples were combusted in an elemental analyser (NA 2500; CE Instruments, Milan, Italy) for \(\delta^{13}C\) analysis and in high temperature conversion elemental analyser (TC/EA; Finnigan Mat GmbH, Bremen, Germany) for \(\delta^{18}O\) analysis, both coupled to an isotope ratio mass spectrometer (Deltaplus, Finnigan MAT GmbH, Bremen, Germany) (Brandes et al., 2007).
2.3.3 Rain water data

In 2003 and 2004, rain water was collected each fortnight and analysed for oxygen isotope analysis (Finnigan MAT Delta S, Bremen, Germany). From 1977 to 2002, the oxygen isotope ratio of rain water was taken from the IAEA/WMO GINIP data base (Global Network of Isotopes Precipitation, accessible at http://isohis.iaea.org).

2.4 Model description

2.4.1 Canopy photosynthesis and transpiration model

MuSICA is a multilayer, multileaf process-based biosphere-atmosphere gas exchange model. In each vegetation layer, several leaf classes are distinguished according to their water status (wet or dry), light regime (sunlit or shaded) and age (0, 1 or 2 year-old, in the canopy only). In this model, a canopy layer therefore displays 12 ‘big shoots’ while the understorey layer, composed of annual plants, has only 4 ‘big leaves’ (Ogée et al., 2003).

For each leaf type, the CO₂ and water fluxes are computed by combining several models for leaf photosynthesis (Farquhar et al., 1982), stomatal conductance (Leuning, 1995), boundary-layer conductance (Landsberg et al., 1984) and energy budget (Gu et al., 1999). The effect of soil water limitations on the leaf stomatal conductance (gₛ) is accounted for by a limiting factor (0 < Wᶠᵃᶜ < 1) of the soil water content (Keenan et al. 2010):

\[
gₛ = \left( g₀ + \frac{mA}{(Cₛ−Γ*) (1+Dₛ/D₀)} \right) \times Wᶠᵃᶜ
\]  

where \( g₀, m \) and \( D₀ \) are parameters of the stomatal conductance model of Leuning (1995), \( A \) is the net CO₂ assimilation, \( Dₛ \) and \( Cₛ \) are the air water vapour deficit and the CO₂ concentration at the leaf surface respectively and \( Γ* \) is the CO₂ compensation point.

Alternatively, the effect of soil water deficit on photosynthesis was accounted for as in Keenan et al. (2009) by reducing both the apparent maximum carboxylation (\( V_{c,\text{max}} \)) and electron transport (\( J_{\text{max}} \)) rates using a similar multiplication factor \( Wᶠᵃᶜ \):

\[
V_{c,\text{max}} = V_{c,\text{max}} \times Wᶠᵃᶜ
\]

\[
J_{\text{max}} = J_{\text{max}} \times Wᶠᵃᶜ
\]
In all cases, $W_{fac}$ is computed as a soil moisture-dependent scalar with values between 0 and 1:

$$W_{fac} = \begin{cases} 
1, & \text{if } s(t) \geq S_{max} \\
\left( \frac{s(t)-S_{min}}{S_{max}-S_{min}} \right)^{q}, & \text{if } S(t) < S_{max}
\end{cases} \tag{5}$$

where $S(t)$ (mm) is the soil water content in the root zone at time $t$, $S_{max}$ is the soil water content below which water limitations are observed, $S_{min}$ is the wilting point and $q$ is a measure of non-linearity effects of soil water stress on physiological processes. It is worth noticing that, in MuSICA, both $J_{max}$ and $V_{cmax}$ also change with leaf age.

### 2.4.2 Pool model

The pool model (Ogée et al. 2009) is used as a transfer function to convert fluxes of photosynthesis and evapotranspiration simulated by MuSICA into carbon and oxygen isotope ratios in leaves, phloem and tree-ring cellulose. This model assumes that non-structural carbon in the tree is represented by a single and well-mixed pool of water soluble sugars that is always large enough to supply the metabolic demand (Ogee et al., 2009).

This pool of sugars is fed by net photosynthesis, ($F_{leaf}$ in kg C m$^{-2}$ ground area s$^{-1}$), and used as a substrate for maintenance and growth woody respiration ($F_{wood}$ in kg C m$^{-2}$ ground area s$^{-1}$) and wool-tree biomass synthesis ($S_{biomass}$ in kg C m$^{-2}$ ground area s$^{-1}$). The carbon budget of the pool can then be written as:

$$\frac{dC_{pool}}{dt} = F_{leaf} - F_{wood} - S_{biomass}, \tag{6}$$

where $C_{pool}$ is the size of the sugar pool (kg C m$^{-2}$ ground area). The biomass synthesis is then assumed to be source-driven and to depend linearly on $C_{pool}$:

$$S_{biomass} = K_{pool}C_{pool} \tag{7}$$

in which $k_{pool}$ (s$^{-1}$) is the pool turnover rate (Ogée et al. 2009).
Figure 1 | (a) The single-substrate pool model. The well-mixed pool is fed by photosynthesis ($F_{\text{leaf}}$) with isotopic signature $\mathcal{R}_{\text{assimilate}}$ and use as the substrate for wood respiration ($F_{\text{wood}}$) and biomass synthesis ($dC_{\text{biomass}}/dt$), with an isotopic signature $\mathcal{R}_{\text{pool}}$. A proportion of the new biomass is allocated to each ring section ($i=1,N$), with a final cellulose content $C_{\text{cellulose,}i}$ and isotopes ratio $\mathcal{R}_{\text{cellulose,}i}$. (b) The soil water pool model. The well mixed pool is fed by precipitation ($P$) with isotopic signature $\mathcal{R}_{\text{rain}}$ and use for soil evaporation ($E_{\text{soil}}$), with an isotopic signature $E_{\text{evap, canopy, canopy and understorey transpiration (E}_{\text{tot}}-E_{\text{soil}}$) or deep drainage ($D$), with an isotopic signature $\mathcal{R}_{\text{sw}}$ (Ogée et al., 2009).

The carbon and oxygen signal in the sugar pool is lead by this equation,

\[
\frac{d(C_{\text{pool}}/\mathcal{R}_{\text{pool}})}{dt} = F_{\text{leaf}}\mathcal{R}_{\text{assimilate}} - F_{\text{wood}}(1-e)\mathcal{R}_{\text{pool}} - k_{\text{pool}}C_{\text{pool}}(1-x)\mathcal{R}_{\text{pool}},
\]

where $\mathcal{R}_{\text{pool}}$ is the average isotopes ratio of sugar in the well-mixed pool (carbon and oxygen). $\mathcal{R}_{\text{assimilate}}$ is the isotope ration of currents assimilates entering the pool and $e$ and $x$ are isotopes fractionations associated with wood respiration and biomass synthesis respectively. The rate of change of the isotopes ratio in the sugar pool is driven by the difference $\mathcal{R}_{\text{pool}} - \mathcal{R}_{\text{assimilate}}$. 


and the isotopes fractionation $e$ and $x$. The recorded signal in $\mathcal{R}_{pool}$ is therefore attenuated and delayed compared with the signal in $\mathcal{R}_{assimilate}$.

Wood formation is a succession of several steps. For each tree ring sub-section (i=1,N, where N is the number of sub-section). Assuming no discrimination during cellulose synthesis (Cernuzak et al., 2005), the amount of $^{13}$C atoms contained in cellulose of tree rings sub-section is given by:

$$C_{cellulose,i} \mathcal{R}_{cellulose,i} = \int_{t_{0,i}}^{t_{0,i}+\Delta_t} \mathcal{R}_{pool} F_i S_{biomass} \, dt \quad (9)$$

where $\mathcal{R}_{cellulose,i}$ is the carbon isotope ratio of the tree ring sub-section, $t_{0,i}$ is the date at which cellulose synthesis has started, $\Delta_t$ is the duration of cellulose synthesis and deposition and $F_i$ represents the fraction of the biomass increase that is allocated to the cellulose of this particular tree ring sub-section.

For $^{18}$O the amount of $^{18}$O atoms contained in cellulose of tree rings sub-section is given by:

$$C_{cellulose,i} \mathcal{R}_{cellulose,i} = \int_{t_{0,i}}^{t_{0,i}+\Delta_t} [p_{ex} \alpha_{wc} \mathcal{R}_{sw} + (1 - p_{ex}) \mathcal{R}_{pool}] F_i S_{biomass} \, dt \quad (10)$$

where $p_{ex}$ is the proportion of oxygen atoms that have exchanges with source water during synthesis from sucrose, which is assume to be constant in time (Barbour et al., 2004; Cernuzak et al., 2005) and $\alpha_{wc} \mathcal{R}_{sw}$ is the isotopes ratio of organic matter in equilibrium with source water.

### 2.4.3 Model parameterization

Parameters for pool model and for MuSICA are shown in Table 1 and Table 2 (Annex 2) respectively.

For the equation Wfac, $S_{min}$ was fixed at 93.6 (kg H$_2$O m$^{-2}$) according to Sturm et al. (1996). $S_{max}$ was estimated using MuSICA fluxes without any limitation and fixed at 121 kg H$_2$O m$^{-2}$. A sensitive analysis of q was done for the stomatal, biochemical and both limitations at the same time (c.f. Results).
3. Results and Discussion

3.1. Identification of soil water limitation periods

Modelled and measured water (LE) and CO₂ (NEE) fluxes and relative soil water content (RSWC) are shown in Fig. 2. Over the 2003-2005 period, the relative soil water content reached a minimum from June to September in 2003 and 2005, and from August to October in 2004. As expected, the modelled NEE and LE values become most sensitive to $q$ at those times in all three scenarios. These periods of low RSWC were therefore selected in the following for our analysis on drought-induced limitations. Because we wanted to have quite some dynamics on RSWC, we also included periods when RSWC was starting to drop, as indicated by the shaded areas in Fig. 2.

Because the root zone is very shallow (ca. 40 cm), RSWC can vary very rapidly after a rain event, like in summer 2004 that was the wettest summer of the studied period (Fig. 2). These rapid variations are hard to capture by the model, given also the natural spatial variability in the field, and this explains the substantial differences observed between measured and modelled RSWC values at these periods. Modelled RSWC are also sensitive, but only slightly, to the parameter $q$. As we will see later these difficulties to measure and model accurately RSWC will complicate the study of the NEE and LE response to changes in RSWC.

![Figure 2](image)

**Figure 2** | Daily modelled and measured RSWC, GPP and LE values at Hartheim forest in 2003, 2004 and 2005 for 3 different limitation scenarios: (a) stomatal limitation, (b) biochemical limitation and (c) stomatal and biochemical limitation. Modelled values are plotted for different value of the parameter $q$. The grey zones indicate the periods when water limitations are the most pronounced.

Finally, we can see that modelled NEE and LE fluxes are most reduced during periods of low RSWC when only non-stomatal limitations are accounted for in the model (Fig. 2b and 2c).
Interestingly, when both stomatal and non-stomatal limitations are used together, modeled NEE values become intermediate between the two other scenarios, while modeled LE values resemble those obtained with the stomatal limitation alone. This unexpected behavior does not seem to be caused by feedbacks of the different limitations on the soil water content or canopy vapor pressure deficit. Note also that maximum values of LE and GPP (i.e. minimum NEE) seem underestimated by the model, emphasizing some possible issues with the model parameterization. A closer inspection of the model parameter values will be done in the coming future to try to fix this problem, but it should not affect too much the results of this study that focuses only on periods when fluxes are limited by soil water deficits.

### 3.2. GPP and LE responses to soil water deficit

![Figure 3](image)

Figure 3 | Daily values of nighttime-based (Eq. 1) and daytime-based (Eq. 2) GPP estimates as a function of relative soil water content RSWC for the periods in 2004 and 2005 when soil was drying (grey zones in Fig. 3). Also shown are theoretical curves using Eq. 5 with different values of the exponent $q$.

Nighttime-based (Eq. 1) and daytime-based (Eq. 2) GPP estimates are substantially different especially when the soil is dry (RSWC < 0.3). This is shown in Fig. 3 where daily values of these two GPP estimates and measured latent heat flux (LE) are plotted against (measured)
RSWC during periods when the soil was drying (indicated by grey zones in Fig. 2). In general, nighttime-based GPP estimates seem higher than their day-time based counterparts, especially when RSWC < 0.2 (Fig. 2). In general, the daytime-based partitioning seems to produce GPP estimates that respond to RSWC more than their night-time based counterparts and can be described by Eq. 5 with \( q \approx 0.3 \). The daily latent heat flux response to RSWC could also be reasonably described by Eq. 5 with \( q \approx 0.3 \) as well.

The theoretical curves shown in Fig. 3 are instructive but our aim is to determine parameters for Eq. 5 to be applied at the leaf level in MuSICA, not at the ecosystem scale. Following Keenan et al. (2010), sensitivity tests to the parameter \( q \) on GPP and LE predicted by the ecosystem model MuSICA were therefore performed for the three limitation scenarios (Fig. 4). It is worth noticing that, due to the feedbacks of LE on soil water content, a different scenario with a different value for \( q \) produces a different RSWC time series. A small \( q \) tends to produce higher fluxes, but also tend to dry out the soil quicker, leading to more values in the upper right corner of Fig. 4. Conversely, a large \( q \) tends to reduce the fluxes when the soil water becomes limiting, therefore preventing the soil from becoming drier, and this tends to shifts the points towards the lower right corner of Fig. 4.

From a visual inspection of Fig. 4, it is clear that a value of \( q > 0 \) is required to match the GPP and LE responses to RSWC. A value as low as \( q = 0.02 \) does not suffice but when \( q = 0.5 \) or higher, the modeled and measured GPP and LE start to display similar responses to RSWC. However, due to the shallowness of the root zone, RSWC oscillates between extremely low or high values but does not display often intermediate values, and low values are difficult to match as well. This makes the analyses of the GPP and LE response curves to RSWC more
difficult and does not allow us to disentangle between the different limitations. This was not the case in other previous studies (Keenan et al., 2010), because those were conducted over even dryer sites but with a very deep root zone (4.5 m) allowing RSWC to drop more slowly from high to intermediate and low values.

3.3. Cellulose data

Modelled and observed $\delta^{13}C$ and $\delta^{18}O$ signals in tree-ring cellulose over the 2004 growing season are shown in Fig. 5 for the different limitation scenarios and different values of $q$. The $\delta^{18}O$ of wood cellulose is more enriched early in the season with a maximum of 25.8‰ in June and then decreases below 23‰ at the end of the growing season with two noticeable secondary enrichments in August (24.5‰) and October-November (25.2‰). In contrast, the $\delta^{13}C$ of wood cellulose is depleted at the beginning of the wood formation (at about -24.5‰) then reaches a maximum in August (-23.9‰) and, from August to December, gets slowly more depleted again and reaches a minimum value in December at -25.4‰.

Unfortunately, cellulose isotope data was available only for 2004. Because year 2004 was a wet year with low soil water content occurring only occasionally during the summer, this was not ideal to study the effect of drought on photosynthesis. Despite this possible problem, the $\delta^{13}C$ signal in cellulose was still highly sensitive to any drought-induced limitation (Fig. 5b and 5c) while the $\delta^{18}O$ remain mostly unaffected. This can be explained by the fact that the $\delta^{18}O$ signal depends primarily on stomatal conductance $g_s$ while the $\delta^{13}C$ signal depends at first approximation on the ratio of photosynthesis to stomatal conductance $A/g_s$. Because $g_s$ is further assumed proportional to $A$, applying a limiting factor $W_{fac}$ on $g_s$ directly or more
indirectly on $A$ produces nearly the same $g_s$ response and therefore the same $\delta^{18}O$ values, but would produce completely different $\delta^{13}C$ signals. Comparing scenarios a and b in Fig. 2, shows that this is only an approximation because the simulated evapotranspiration fluxes do differ between the two scenarios, especially in 2003 and 2005, suggesting different $g_s$ values. This indicates that also in some other cases (i.e. dry years) the $\delta^{18}O$ signal could also be discriminating. Also, the $\delta^{18}O$ signal is always very useful to help determining the wood formation times (Ogee et al. 2009).

From the results shown in Fig. 5, it seems that biochemical limitations give the best results. However more cellulose data covering multiple years including very dry ones would probably be necessary to give a definite answer.

### 3.4. Sugars data

The comparison between the modelled sugar pool and the phloem data’s could also lead us to a better understanding of the process that could occurs on photosynthesis during a drought period. Modelled and observed stable carbon and oxygen isotope signals in the carbon pool are shown in Fig. 6. for the different limitation scenarios and only for a value of $q$ =0.5. From January to May 2004 $\delta^{18}O_{phloem}$ measured values exhibited a steady increase until it reached up a value up to 33.34 ‰, in mid may. Then they decreased slowly until December. $\delta^{13}C_{phloem}$ does not show the same profile as $\delta^{18}O$ and stay more constant. The minimum value is reached in December (-27.8‰) and the maximum value is reached in June (-25.07‰).

The seasonal pattern of the tree ring cellulose is comparable to that of phloem organic matter, albeit, showing a time lag, lower $^{18}O$ enrichment and damping of the amplitude. These observations could be attributed to the exchange of organic oxygen with unenriched xylem water during cellulose synthesis.

![Figure 6](image)

**Figure 6.** Phloem sugar isotopic composition data’s and model ( $\delta^{18}O$ and $\delta^{13}C$ ) for $q$=0.5 and stomatal limitation (a), biochemical limitation (b) and both limitation at the same time (c). The modeled $C_{pool}$ was obtained with parameters value shows in Table 2. (Annex2)
The $\delta^{13}\text{C}$ signal in the sugar pool was highly sensitive to any drought-induced limitation (Fig. 5b and 5c) while the $\delta^{18}\text{O}$ remain mostly unaffected.

From the results shown in Fig. 6. It seems that the biochemical limitation gives the best results. $\delta^{13}\text{C}$ in the sugar pool could then also be a good indicator to try to understand the limitations that occurs on photosynthesis during a drought period.

4. Conclusions

The response of global photosynthesis to drought conditions is unclear yet. Previous studies have shown that the inclusion of drought-induced limitations on the biochemistry of photosynthesis, but also on stomatal or mesophyll conductance could play an important role in regulating plant CO$_2$ uptake and water use (e.g. Keenan et al. 2010, 2009; Grassi et Magnani, 2005). Here we try to understand the driving processes behind drought-imposed changes in forest carbon and water fluxes, using both CO$_2$ and water fluxes and the stable isotope ratios in wood cellulose or phloem sugars.

Preliminary results show that the isotopic composition of carbon and oxygen in tree rings cellulose seems to be a good indicator to try to understand the influence of limited soil water content to the different limitations that could occurs on photosynthesis. The results could have an important implication on modeling leaf photosynthesis. The inclusion of biochemical limitations on leaf photosynthesis model could also lead to a better understanding of the isotopic composition of tree rings cellulose.
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Annex 1

ISOTOPES DESCRIPTION

Carbon and oxygen have more than one stable isotope. Carbon, for examples, has two stable isotopes (\(^{12}\)C and \(^{13}\)C), each with six protons, but with six or seven neutrons. Stable isotopes have almost the same chemical properties, but the difference in mass make its physical properties different. This difference in mass allows physical, chemical and biological processes to discriminate against one of them, thereby imparting an environmental signal (McCarroll, 2004). At natural abundance, the stable isotope composition of a given sample is reported as the ratio of the heavier, rare isotope to the lighter, more abundant one and is expressed in “delta” notations (\(\delta\)), i.e., relative to a standard material with known isotope ratio, in parts per thousand (‰):

\[
\delta^{13}\text{C} = \left( \frac{\Re_{\text{sample}}}{\Re_{\text{standard}}} - 1 \right) \times 1000
\]

where \(\Re_{\text{sample}}\) and \(\Re_{\text{standard}}\) are the isotope ratio of the sample and the standard, respectively. For carbon, the international standard is a fossil belemnite from the Pee Dee formation in South Carolina (PDB or VPDB) with \(\Re_{\text{standard}} = 0.0112372\), while for oxygen, the standard is the Vienna Standard Mean Ocean Water (VSMOW) with \(\Re_{\text{standard}} = 0.002005\).

DISCRIMINATION OF CARBON AND OXYGEN

ISOTOPES IN PLANTS

Carbon discrimination in plants

Plants using \(\text{C}_3\) metabolism discriminate \(^{13}\)C during net photosynthesis. According to Farquhar et al., (1982) the expression of the isotopic composition of photosynthate in \(\text{C}_3\) plants can be approximate as:

\[
\Delta^{13}\text{C} \approx a + (b - a) \frac{C_c}{C_a} - f \frac{\Gamma^*}{C_a}
\]

where \(a\) is the average kinetic fractionation factor associated with diffusion of \(\text{CO}_2\) through the leaf boundary layer, the stomata and the leaf lamina, \(b\) is the net kinetic fractionation of the enzyme-catalysed fixation of \(\text{CO}_2\) by both RuBISCO and PEP carboxylase, \(f\) is the
fractionation during photorespiration (≈11‰) (Tcherkez, 2006; Lanigan et al., 2008), $\Gamma^*$ (mol mol$^{-1}$) is the CO$_2$ compensation point in the absence of day respiration, that depends on leaf temperature (Bernachi et al., 2001) and $C_c$ and $C_a$ are the partial pressures of CO$_2$ at the carboxylation sites inside the leaf chloroplasts and in the atmosphere, respectively.

**Oxygen discrimination in plants**

Sol moisture is the source of water for trees, so the isotopic signal in trees components will come from the signature of precipitation. However, there are several potential fractionations before the water isotopes become fixed in wood components (McCarroll et al., 2004). Firstly, soil water evaporation can alter the original signal of precipitation. When tree roots take-up water, there is no fractionation (Wershaw et al., 1966) so the principal site of fractionation is the leaf, where transpiration leads to an enrichment of the heavier isotopes $^{18}$O, which can be as much as 20‰ (Saurer et al., 1998). At the site of evaporation, this enrichment $\Delta^{18}$O can be modeled as (Dongmann et al., 1975; Farquhar & Lloyd, 1993).

$$\Delta^{18}O = \varepsilon^+ + \varepsilon_k + (\Delta^{18}O_v - \varepsilon_k) \frac{W_a}{W_i}$$

Where, $\varepsilon$ (‰) is the equilibrium fractionation during the phase change from liquid to vapour, $\varepsilon_k$ (‰) is the kinetic fractionation factor caused by the diffusion of water vapour through stomata and leaf boundary layer, $\Delta^{18}O_v$ (‰) is the $^{18}$O enrichment above source water of atmospheric vapour, and $W_i$ and $W_a$ are the partial pressure of water vapour inside the leaf and in the atmosphere, respectively. However, cellulose in enriched by about 27‰ compares to water (Deniro & Epstein, 1981). This enrichment may be due to biochemical fractionation and is probably caused by an isotopic exchange of carbonyl oxygen atoms of intermediate products of photosynthesis with water (Styernberg, DeNiro & Savidge, 1986). This exchange does not occur only in leaf but also in stems during cellulose synthesis (Hill et al., 1995). The $^{18}$O signal of leaf water imprinted on sucrose may be partly lost at the time of this exchange (Saurer et al., 1997).
### Parameters of the single substrat Model

<table>
<thead>
<tr>
<th>Parameters needed to compute Cpool</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cpool, 0 (kgC tree-1)</td>
<td>0.2</td>
<td>Initial sugar pool size</td>
</tr>
<tr>
<td>Kpool</td>
<td>8</td>
<td>Whole-tree growth rate</td>
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</table>

<table>
<thead>
<tr>
<th>Parameters needed to compute Rpool (13C/12C only)</th>
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<td>Initial 12C/13C ratio of the pool</td>
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<tr>
<td>x(-)</td>
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<td>Isotopic fractionation during wood formation (lignification)</td>
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<table>
<thead>
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<th>Description</th>
</tr>
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<td>δpool,0 (VSMOW)</td>
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<td>Initial 18O/16O ratio of the pool</td>
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<tr>
<td>x(-)</td>
<td>0‰</td>
<td>Isotopic fractionation during wood formation (lignification)</td>
</tr>
<tr>
<td>pex</td>
<td>0.7</td>
<td>Proportion of exchangeable oxygen during cellulose synthesis from sucrose</td>
</tr>
<tr>
<td>εwc (-)</td>
<td>27‰</td>
<td>Fractionation factor of carbonyl oxygen exchange with water</td>
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</table>

<table>
<thead>
<tr>
<th>Parameters to compute Rassimilate (13C/12C only)</th>
<th>Value</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>g_l [mol(air)m-2s-1]</td>
<td>0.1</td>
<td>Leaf internal conductance</td>
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<tr>
<td>g_night [mol(air)m-2s-1]</td>
<td>0.01</td>
<td>Leaf night-time conductance</td>
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<tr>
<td>b(-)</td>
<td>29‰</td>
<td>Net enzymatic carbon fractionation during CO2 fixation</td>
</tr>
<tr>
<td>e(-)</td>
<td>0‰</td>
<td>Carbon isotopic fractionation during wood respiration</td>
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</tbody>
</table>

<table>
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<tr>
<th>Parameters to compute Rassimilate (18O/16O only)</th>
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<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Vlw [mol(H2O) m-2]</td>
<td>15</td>
<td>Leaf mesophyll water volume</td>
</tr>
<tr>
<td>Leff(mm)</td>
<td>15</td>
<td>Leaf mesophyll effective mixing length</td>
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**Table 2. Parameters used to compute MuSICA on Harteim Pine Forest**

<table>
<thead>
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<th>Parameters used to run MuSICA</th>
<th>Value</th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
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<tr>
<td><strong>Site Data Parameters</strong></td>
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<td></td>
</tr>
<tr>
<td>Ref_Height [m]</td>
<td>15.4</td>
<td>height of climate forcing measurements above ground</td>
<td>Stum et al. (1996)</td>
</tr>
<tr>
<td>Site latitude ['°']</td>
<td>47.93</td>
<td>latitude of the site</td>
<td>Stum et al. (1996)</td>
</tr>
<tr>
<td>SiteLongitude ['°']</td>
<td>7.6</td>
<td>longitude of the site</td>
<td>Stum et al. (1996)</td>
</tr>
<tr>
<td><strong>Soil Structure parameters</strong></td>
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</tr>
<tr>
<td>SoilSurfacePorosity [m3(air space)/m3(porous soil)]</td>
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<td>soil porosity in the top (A0-horizon) soil layer (assuming zero SOM)</td>
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</tr>
<tr>
<td>SoilBulkPorosity [m3(air space)/m3(porous soil)]</td>
<td>43</td>
<td>mean soil porosity in the entire root zone (assuming zero SOM)</td>
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</tr>
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<td>RootZoneDepth [m]</td>
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<td>maximum root zone depth</td>
<td>Stum et al. (1996)</td>
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<tr>
<td><strong>Soil hydrological parameters</strong></td>
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<tr>
<td>KsatG [kg(H2O)/m2/s]</td>
<td>1.76E-04</td>
<td>soil surface hydraulic conductivity at saturation</td>
<td>Stum et al. (1996)</td>
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<tr>
<td>bPsiG [-]</td>
<td>1.37</td>
<td>exponent used for the soil surface retention curve</td>
<td>Stum et al. (1996)</td>
</tr>
<tr>
<td>PsiSatG [m(H2O)]</td>
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<td>soil surface water potential at saturation</td>
<td>Stum et al. (1996)</td>
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<tr>
<td>C2ref [m-1]</td>
<td>3.9</td>
<td>force-restore coefficient for soil moisture</td>
<td>Stum et al. (1996)</td>
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<tr>
<td>SoilBulkFCap [m3(water)/m3(porous soil)]</td>
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<td>mean soil water content in the entire root zone at field capacity (when drainage starts)</td>
<td>Sturm et al. (1996)</td>
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<tr>
<td>SoilBulkWilt [m3(water)/m3(porous soil)]</td>
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<td>mean soil water content in the entire root zone at wilting point (when drainage stops)</td>
<td>Sturm et al. (1996)</td>
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<td><strong>Vertical structure of vegetation</strong></td>
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<tr>
<td>CanopyHeight [m]</td>
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<td>Height of the canopy</td>
<td>Holst et al. (2009) and Kodama et al. (2007, 2008)</td>
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<tr>
<td>UndHeight [m]</td>
<td>0.42</td>
<td>understorey vegetation height</td>
<td>Holst et al. (2009) and Kodama et al. (2007, 2008)</td>
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<tr>
<td><strong>Photosynthetic and stomatal conductance parameters</strong></td>
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<td></td>
</tr>
<tr>
<td>Jmax [mol(CO2)/m2(leaf)/s]</td>
<td>35 E-6</td>
<td>maximum electron transport rate (Jmax) of understory leaves [mol(CO2)/m2(leaf)/s]</td>
<td>Wedler et al. (1996)</td>
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<tr>
<td>Vcmax [mol(CO2)/m2(leaf)/s]</td>
<td>25 E-6</td>
<td>maximum carboxylation rate (Vmax) of understory leaves [mol(CO2)/m2(leaf)/s]</td>
<td>Wedler et al. (1996)</td>
</tr>
</tbody>
</table>
Abstract

Forests contribute largely to the Earth’s climate system and to the terrestrial carbon sink strength. Due to the strong relationship between water status and forest primary productivity, severe droughts or heatwaves like the one that experienced Europe in 2003 affect the size of the terrestrial carbon sink and lead to a positive feedback on global atmospheric CO$_2$ concentrations. Although water availability is one of the most limiting factor to plant productivity, the photosynthetic response to seasonal drought still remains poorly understood. While it is well established that stomatal closure is one of the first events taking place during drought, a long-standing controversy exists on which limitation is prevailing when water stress progresses.

The objective of the present study was to explore this idea that stable isotope signals recorded in tree rings could be used to study the role of stomatal and non-stomatal limitations on photosynthesis and transpiration. For that we used a previously-published dataset of high-resolution tree-ring isotopes and gas-exchange measurements from Hartheim forest in Germany (Gessler et al., 2009), combined with the single-substrate model of carbon and oxygen stable isotope signals in tree-rings described in Ogée et al. (2009). Results show that the isotopic composition of carbon and oxygen in tree rings cellulose seems to be a good indicator to try to understand the influence of limited soil water content on the different limitations that could occur on photosynthesis.

Les forêts contribuent largement au système climatique de la Terre ainsi qu’à l’importance des puits de carbone terrestre. En raison de la forte relation entre l’état hydrique des sols et de la productivité primaire des forêts, de graves sécheresses ou canicules comme celle qu’a connu l’Europe en 2003 ont une incidence sur la taille du puit de carbone terrestre et conduisent à une rétroaction positive des concentrations mondiales de CO$_2$ dans l’atmosphère. Bien que la disponibilité de l'eau soit l'un des principaux facteurs limitant la productivité des plantes, la réponse photosynthétique lors de sécheresse saisonnière reste encore mal comprise. S'il est bien établi que la fermeture des stomates est l'un des premiers mécanismes ayant lieu pendant la sécheresse, une longue controverse existe sur quelle limitation est dominante au moment où le stress hydrique progresse.

L'objectif de cette étude était d'explorer l'idée selon laquelle les signaux des isotopes stables du carbone et de l’oxygène enregistrés dans les cernes des arbres pourraient être utilisés pour étudier le rôle des limitations stomatique et non-stomatique sur la photosynthèse et la transpiration pendant des périodes de sécheresse.

Pour cela nous avons utilisé un ensemble de données sur les isotopes dans les cernes d’arbres ainsi que des mesures d'échanges gazeux de la forêt de Hartheim en Allemagne (Gessler et al., 2009), combiné avec le modèle de signaux isotopiques décrit dans Ogée et al. (2009). Les résultats montrent que la composition isotopique du carbone et d’oxygène dans les cernes de cellulose semble être un bon indicateur pour essayer de comprendre l'influence d’un stress hydrique sur les différents mécanismes de limitations qui pourraient avoir lieu au niveau de la photosynthèse.