New Phytologist Supporting Information

Article title: Explicitly accounting for needle sugar pool size crucial for predicting intra-seasonal dynamics of needle carbohydrates $\delta^{18}O$ and $\delta^{13}C$

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The following Supporting Information is available for this article:

- **Fig. S1** Measured needle sucrose $\delta^{13}C$ against measured needle sugar (sucrose+glucose+fructose) $\delta^{13}C$ in current-year needles and one-year-old needles.
- **Fig. S2** Measured isotopic composition of needle water-soluble carbohydrates in current-year needles and one-year-old needles, and a combined data series over the two needle generations.
- **Fig. S3** Measured $\delta^{18}O$ of atmospheric water vapor against corresponding values predicted by IsoGSM.
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- **Fig. S7** Modeled and measured diurnal course of needle water $\delta^{18}O$.
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Fig. S1  Measured needle sucrose $\delta^{13}$C against measured needle sugar (sucrose+glucose+fructose) $\delta^{13}$C in current-year needles (0N) and one-year-old needles (1N) of Scots pine. r denotes Pearson’s correlation and error bars indicate the standard deviation of the five sampled trees.

Fig. S2  Measured isotopic composition of needle water-soluble carbohydrates (WSC) in current-year needles (0N) and one-year-old needles (1N) of Scots pine, and a combined data series over the two needle generations. (a) $\delta^{18}$O of bulk WSC, (b) $\delta^{13}$C of bulk WSC, (c) $\delta^{13}$C of sucrose, and (d) $\delta^{13}$C of pinitol. Error bars indicate the standard deviations of the five sampled trees.
**Fig. S3** δ¹⁸O of atmospheric water vapor measured at the experimental site against corresponding values predicted by IsoGSM (Yoshimura et al., 2008). The dashed line is 1:1 and MAE denotes the mean absolute error between the datasets.

**Fig. S4** (a) Time course of modeled and measured source (twig) water δ¹⁸O of Scots pine and (b) modeled against measured values. The dashed line is 1:1 and the solid line the linear least squares regression. $R^2$ and MAE denote the coefficient of determination and mean absolute error, respectively. Error bars present standard deviation of measurements from five trees. The range of observed soil water δ¹⁸O between 0.02 m and 0.1 m is shown as grey shaded area.
**Fig. S5** (a) Measured concentrations of needle water-soluble carbohydrates (WSC) and (b) measured ratio of needle pinitol to needle sugar (sucrose+glucose+fructose) concentrations in current-year needles (0N) and one-year-old needles (1N) of Scots pine. The dotted lines indicate the values used in the modeling. Error bars indicate the standard deviations of the five sampled trees.

**Fig. S6** Water content of one-year-old needles of Scots pine obtained by measuring the fresh weight of needle samples and the dry weight of needle samples after oven-drying. The dotted lines indicate the value used in the modeling. Error bars indicate the standard deviations of the five sampled trees.

**Fig. S7** Modeled diurnal course of needle water $\delta^{18}$O during 22 to 24 May 2019 and measured values for Scots pine sampled during 23 May 2019.
Fig. S8  Modelled relationship between CO₂ mole fraction in chloroplast (Ci) and intercellular spaces (Ci) when using (a) mesophyll conductance defined by Eq. S9, (b) constant mesophyll conductance and (c) a constant ratio between stomatal and mesophyll conductance. The dashed line is 1:1 and the solid line the linear least squares regression.

Table S1  Parameter values applied for shoot gas exchange modeling

| Parameter | Unit¹ | Description | Value | Source |
|-----------|-------|-------------|-------|--------|
| Vcmax25² | µmol m⁻² s⁻¹ | maximum carboxylation capacity at 25°C | 32 | calibrated |
| Jmax25² | µmol m⁻² s⁻¹ | maximum electron transport rate at 25°C | 2 × Vcmax25 | Kattge and Knorr (2007) |
| rd25² | µmol m⁻² s⁻¹ | mitochondrial respiration rate at 25°C | 0.014 × Vcmax25 | night-time shoot chamber data |
| θ | ° | curvature of the response of electron transport to irradiance, Eq. S6 | 0.7 | Launiainen et al. (2015) |
| I | µmol m⁻² s⁻¹ | photosynthetically active radiation (PAR) effectively absorbed by photosystem II, Eq. S6 | 0.05 × PAR | calibrated (note that PAR given by ground area) |
| Γs, Kc, Ko | µmol m⁻² s⁻¹ | Michaelis–Menten constants for CO₂ and O₂ | - | Bernacchi et al. (2001) |
| T0 | °C | threshold for delayed temperature, Eq. S7 | -3.1 | Mäkelä et al. (2008) |
| Smax | °C | level at which delayed temperature reaches saturation, Eq. S7 | 14.2 | Mäkelä et al. (2008) |
| τ | days | time constant for delayed temperature | 18 | calibrated |
| gb | mol m⁻² s⁻¹ | boundary layer conductance for CO₂ | 1.5 | Wingate et al. (2007) |
| ĝs,0 | mol m⁻² s⁻¹ | stomatal model parameter, Eq. S8 | 0.001 | shoot chamber data |
| ĝs,1 | kPa⁰.⁵ | stomatal mode parameter, Eq. S8 | 2.0 | shoot chamber data |
| ĝs,night | mol m⁻² s⁻¹ | night-time stomatal conductance | 0.003 | night-time shoot chamber data |
| ĝm,1 | - | mesophyll conductance parameter, Eq. S9 | 4.5 | calibrated |
| ĝm,night | mol m⁻² s⁻¹ | minimum value for mesophyll conductance | 0.01 | |
| b0 | - | water stress parameter for ĝs,1 and Vcmax25, Eq. S10 | 0.31, 0.39 | Launiainen et al. (2022) |
| b1 | - | water stress parameter for ĝs,1 and Vcmax25, Eq. S10 | 3.0, 0.83 | Launiainen et al. (2022) |

¹all area-based units refer to all-sided leaf area; ²parameters of temperature response functions (Medlyn et al., 2002) adopted from Tarvainen et al. (2013); ³temperature response function (Bernacchi et al., 2001) fitted to night-time shoot chamber data
Methods S1 Modeling shoot gas exchange

The exchange of water vapor (\(E\), mol m\(^{-2}\) s\(^{-1}\)) and \(\text{CO}_2\) (\(A_n\), \(\mu\)mol m\(^{-2}\) s\(^{-1}\)) between the shoot and air follow

\[
E = 1.6g_b(w_s - w_a) = 1.6g_s(w_i - w_s)
\]

\[
A_n = g_b(C_s - C_i) + g_s(C_s - C_t) = g_m(C_i - C_c)
\]

where \(g_b\), \(g_s\), and \(g_m\) (mol m\(^{-2}\) s\(^{-1}\)) are boundary layer, stomatal, and mesophyll conductance for \(\text{CO}_2\), respectively; 1.6 is the ratio of molecular diffusivity of water vapor to that of \(\text{CO}_2\) in air; \(w_s\), \(w_a\), and \(w_i\) (mol mol\(^{-1}\)) are mole fractions of water vapor in the atmosphere, at the leaf surface and inside the leaf, respectively; \(C_s\), \(C_s\), and \(C_t\) are \(\text{CO}_2\) mole fractions in the atmosphere, at the leaf surface, inside the leaf, and in the chloroplast, respectively. In the model, we assume vapor pressure inside the leaf is saturated and that the leaf is at air temperature.

Following the photosynthesis model of Farquhar et al. (1980), net leaf \(\text{CO}_2\) exchange is given as

\[
A_n = \min(A_c, A_j) - r_d
\]

where \(A_c\) and \(A_j\) (\(\mu\)mol m\(^{-2}\) s\(^{-1}\)) are Rubisco- and RuBP regeneration-limited assimilation rates, respectively, and \(r_d\) (\(\mu\)mol m\(^{-2}\) s\(^{-1}\)) is mitochondrial respiration. The Rubisco-limited rate is defined as

\[
A_c = \frac{(1 - \Gamma_c/C_c) V_{c,max} C_c}{C_c + K_c(1 + O/K_o)} - \Gamma_s/C_c J C_c \frac{C_c}{4(C_c + 2\Gamma_s)}
\]

where the irradiance response of the rate of electron transport \(J\) (\(\mu\)mol m\(^{-2}\) s\(^{-1}\)) is modeled by

\[
\theta J^2 - (I + J_{max})J + I J_{max} = 0
\]

where \(\theta\) (-) is the curvature of the response of electron transport to irradiance, \(J_{max}\) (\(\mu\)mol m\(^{-2}\) s\(^{-1}\)) is the potential electron transport rate, and \(I\) (\(\mu\)mol m\(^{-2}\) s\(^{-1}\)) is the photosynthetically active radiation (PAR) effectively absorbed by photosystem II. In Eqs. S4–S5, the term \((1 - \Gamma_c/C_c)\) is used to account for \(\text{CO}_2\) release through photorespiration, i.e. carboxylation \(V_c = \min(A_c, A_j)/(1 - \Gamma_c/C_c)\) and photorespiration \(F = V_c \Gamma_c/C_c\).

The temperature dependency of \(V_{c,max}\) and \(J_{max}\) have the general form given in Medlyn et al. (2002) and \(r_d\) that in Bernacchi et al. (2001). Additionally, maximum carboxylation capacity \(V_{c,max25}\), maximum electron transport rate \(J_{max25}\), and mitochondrial respiration rate \(r_{d25}\) at a reference temperature of 25°C are assumed to vary with seasonal cycle of photosynthetic capacity following a delayed temperature model (Mäkelä et al., 2008)

\[
f_{pheno} = \min \left( \max \left( \frac{S - T_0}{S_{max} - T_0}, 1 \right), 0 \right)
\]

where \(S_{max}\) and \(T_0\) (°C) are parameters, and the delayed temperature \(S\) (°C) is solved from \(dS/dt = (T_{daily} - S)/\tau\),
where \( T_{daily} \) (°C) is mean daily ambient temperature and \( \tau \) (days) is the time constant of the delay process.

As defined by Eq. S2, \( A_n \) is additionally limited by stomatal and mesophyll conductance. Stomatal conductance \( (g_s, \text{mol m}^{-2} \text{s}^{-1}) \) is defined by the unified stomatal model relying on the principal that stomata act to minimize the amount of water used per unit carbon gained (Medlyn et al., 2011)

\[
g_s = g_{s,0} + \left( 1 + \frac{g_{s,1}}{\sqrt{D}} \right) \frac{A_n}{C_s} \tag{S8}
\]

where \( g_{s,0} \) (mol m\(^{-2}\) s\(^{-1}\)) and \( g_{s,1} \) (kPa\(^{0.5}\)) are parameters and \( D \) (kPa) is vapor pressure deficit. Eq. S8 describes stomatal behavior when photosynthesis is active, therefore an additional parameter \( g_{s,\text{night}} \) (mol m\(^{-2}\) s\(^{-1}\)) is defined as a lower limit for \( g_s \). \( g_{s,\text{night}} \) has an important role in the non-steady-state solution of leaf water \( \delta^{18}\text{O} \) (Ogée et al., 2009).

The behavior of mesophyll conductance \( (g_m, \text{mol m}^{-2} \text{s}^{-1}) \) is less well understood, but its role in \(^{13}\text{C}\)-discrimination is widely recognized (Medlyn et al., 2017). Here, we used a \( g_m \) description suggested by Dewar et al. (2018) and applied successfully for Scots pine by Schiestl-Aalto et al. (2021)

\[
g_m = g_{m,1} \frac{A_n}{C_c - \Gamma_s} \tag{S9}
\]

where \( g_{m,1} \) (·) is a fitted parameter. Again, Eq. S9 describes only conditions when photosynthesis is active, thus \( g_{m,\text{night}} \) (mol m\(^{-2}\) s\(^{-1}\)) is defined as a lower limit for \( g_m \).

To account for water stress (Zhou et al., 2013; Kellomaki and Wang, 1996), \( V_{cmax25} \) and \( g_{s,1} \) (Eq. S8) were adjusted as non-linear functions of relative plant extractable water (Launiainen et al., 2022)

\[
f_w = \min \left( 1.0, \left( \frac{Rew}{b_0} \right)^{b_1} \right) \tag{S10}
\]

where \( Rew = (\theta - \theta_s)/(\theta_s - \theta_e) \) and \( b_0 \) and \( b_1 \) are fitting parameters. The soil water content \( \theta \) (m\(^3\) m\(^{-3}\)) was measured at ca. 5cm depth in the mineral soil, where field capacity and residual water content were \( \theta_s = 0.30 \text{ m}^3\text{m}^{-3} \) and \( \theta_r = 0.03 \text{ m}^3\text{m}^{-3} \), respectively. \( b_0 \) and \( b_1 \) were adopted from Launiainen et al. (2022), who fitted the parameters to shoot chamber data from the same site during drought year 2006.

All parameters values applied in the modeling of shoot gas exchange at the study site are listed in Table S1.

Methods S2 Derivation of model for \(^{13}\text{C}\)-discrimination of net CO\(_2\) exchange (Eq. 8)

The starting point is Eq. A2.6 in Appendix II of Wingate et al. (2007):

\[
^{13}\Delta = \frac{k C_a}{k (C_a - \Gamma_s) - r_d} \left( a_s + (b - a_s) \frac{C_i}{C_a} - \left( ^{13}\Delta + f \right) \frac{c}{C_a} - \frac{r_d}{k C_a} \left( 1 - \frac{R_{sug}}{R_s} (1 - e) \right) \right) \tag{S11}
\]

where \( R_{substrate} \) of Wingate et al. (2007) is replaced by \( R_{sug} \) as that is our substrate for mitochondrial respiration. Solving Eq. S11 for \(^{13}\Delta\), which now appears on both sides of equation, results in:

\[
^{13}\Delta = \frac{k C_a}{k C_a - r_d} \left( a_s + (b - a_s) \frac{C_i}{C_a} - \frac{\Gamma_s}{C_a} - \frac{r_d}{k C_a - r_d} \left( 1 - \frac{R_{sug}}{R_s} (1 - e) \right) \right) \tag{S12}
\]

Here, the first term accounts for diffusion through stomata, carboxylation and photorespiration. However, as in Wingate et al. (2007) this part can be easily extended to cover also diffusion through the leaf boundary layer and
The oxygen isotope ratio of source water \( R_{\text{tot}} \) was modeled based on a mass balance approach for the soil rooting zone (Ogéé et al., 2009). The rooting zone water budget is expressed as:

\[
\frac{dW_{\text{soil}}}{dt} = P - E_{\text{tot}} - D
\]  

(S16)

where \( W_{\text{soil}} \) (kg m\(^{-2}\)) is rooting zone water storage, and \( P, E_{\text{tot}} \) and \( D \) (kg m\(^{-2}\) s\(^{-1}\)) are precipitation, total evapotranspiration and drainage. We applied Eq. (S16) at a daily timescale to solve \( D \), which was the only unknown as \( dW_{\text{soil}}/dt \) was derived from soil moisture measurements assuming a root zone depth of 0.2 m, \( E_{\text{tot}} \) was available from eddy covariance, and \( P \) was measured. \( R_{s} \) can then be solved from the budget of the rooting zone water \(^{18}O\):

\[
\frac{dR_{s}W_{\text{soil}}}{dt} = R_{\text{rain}}P - R_{s}E_{\text{tot}} - R_{s}D
\]  

(S17)

where \( R_{\text{rain}} \) is the oxygen isotopic ratio of rainfall (measured on a monthly basis). Eq. (S17) assumes that all \( E_{\text{tot}} \) occurs without fractionation (cf. Ogée et al., 2009).
The resulting source water $\delta^{18}O$ composition was compared against the values observed for twig water suggesting 73% of the variation of twig water $\delta^{18}O$ was captured with the approach (Fig. S4). Generally measured twig water $\delta^{18}O$ lie within the range measured for soil water at depths 0.02 m and 0.1 m. During times when this was not true (e.g., late September 2019), modeled values typically also deviated from the measured twig water values. Plausible reasons for this are that root uptake is from deeper layers or that twig water is not in equilibrium with soil water due to low transpiration rates.

**Methods S4** Derivation of Eqs. 10–12

When the sugar pool size is taken as a constant over time and $q = A_n$ (see section 3.2), the implicit solution of Eq. 6 (or Eq. 9 when neglecting $e$) is:

$$S_{sug}(R_{sug} - R_{sug}^{t-1}) = (A_n + r_d)^t R_{assimilates}^t - (A_n + r_d)^t R_{sug}^t$$

(S18)

where $\Delta t (s)$ is the time interval between $t - 1$ and $t$. Solving for the isotopic ratio of sugars at time $t$ yields:

$$R_{sug}^t = \frac{(A_n + r_d)^t R_{assimilates}^t + (S_{sug}/\Delta t) R_{sug}^{t-1}}{S_{sug}/\Delta t + (A_n + r_d)^t}$$

(S19)

Defining $\alpha = (A_n + r_d)/(S_{sug}/\Delta t + A_n + r_d)$ further simplifies the formulation to that presented in Eq. 10:

$$R_{sug}^t = \alpha^t R_{assimilates}^t + (1 - \alpha^t) R_{sug}^{t-1}$$

(S20)

Inserting the same formulation for the isotopic ratio of sugars at time $t - 1, t - 2, ...$ into Eq. S20 yields:

$$R_{sug}^t = \alpha^t R_{assimilates}^t + (1 - \alpha^t) (\alpha^{t-1} R_{assimilates}^{t-1} + (1 - \alpha^{t-1}) (\alpha^{t-2} R_{assimilates}^{t-2} + (1 - \alpha^{t-2}) (...)))$$

(S21)

which can be written as a weighed mean of past time instances $R_{assimilates}$

$$R_{sug}^t = \sum_{n=0}^{\infty} (w_n R_{sug}^{t-n})$$

(S22)

where $w_n$ is the weight of the signal at time $t - n$:

$$w_n = \alpha^{t-n} \times \prod_{j=0}^{n-1} (1 - \alpha^{t-j})$$

(S23)

To enable the calculation of weighted signals the sum in Eq. S22 was cut off at $\tau$ so that $\sum_{n=0}^\tau w_n \approx 0.95$. With this cut-off, the sum of the weights is less than unity unlike in Eq. S22 and therefore we need to add the sum to the denominator resulting in the formulation of Eq. 12:

$$R_{sug}^t = \frac{\sum_{n=0}^\tau (w_n R_{sug}^{t-n})}{\sum_{n=0}^\tau w_n}$$

(S24)
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