Inferring the effects of sink strength on plant carbon balance processes from experimental measurements

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Abstract

The lack of correlation between photosynthesis and plant growth under sink-limited conditions is a long-standing puzzle in plant ecophysiology that currently severely compromises our models of vegetation responses to global change. To address this puzzle, we applied data assimilation of a simple carbon (C) balance model to an experiment where sink strength was manipulated by restricting root volume. Our goals were to infer which processes were affected by growth under sink limitation, and to attribute the overall reduction in growth observed in the experiment, to the effects on component processes. Our analysis was able to infer that, in addition to a reduction in photosynthetic rates, sink limitation reduced the rate of utilization of non-structural carbohydrate (NSC), enhanced respiratory losses, modified C allocation and increased foliage turnover. Each of these effects was found to have a significant impact on final plant biomass accumulation. We also found that inclusion of a NSC storage pool was necessary to capture seedling growth over time, particularly for sink limited seedlings. Our approach of applying data assimilation to infer C balance processes in a manipulative experiment enabled us to extract considerable new information from an existing dataset. We suggest this approach could, if used more widely,
be an invaluable tool to develop appropriate representations of sink-limited growth in terrestrial biosphere models.

Keywords: Non-structural carbohydrate, carbon allocation, data assimilation, mass-balance, photosynthesis, plant growth, sink regulation

1 Introduction

Almost all mechanistic models of terrestrial vegetation function are based on the carbon (C) balance: plant growth is represented as the difference between C uptake (through photosynthesis) and C loss (through respiration and turnover of plant parts). This approach to modeling plant growth dates back to early crop and forest production models (McMurtrie and Wolf, 1983; de Wit and van Keulen, 1987; de Wit, 1978) and now provides the fundamental quantitative framework to integrate our scientific understanding of plant ecosystem function (Bonan 2008).

However, C balance models have been criticized for being “source-focused” (Fatichi et al., 2014). Most C balance models predict growth from the environmental responses of photosynthesis (“source limitation”). In contrast to this assumption, many experimental studies demonstrate that growth is directly limited by environmental conditions (“sink limitation”) rather than the availability of photosynthate. For example, growth is more sensitive to water limitation than is photosynthesis (Bradford and Hsiao, 1982; Müller et al., 2011; Mitchell et al., 2014); low temperatures are considerably more limiting to cell division than to photosynthesis (Körner et al., 2014); nutrient limitation may slow growth without reducing photosynthesis (Reich, 2012; Crous and Ellsworth, 2004); and, physical sink-limitation may reduce growth with a decline in photosynthetic capacity and an accumulation of leaf starch (Arp, 1991; Campany et al., 2017; Poorter et al., 2012a).

How can we move to models that include both source- and sink-limitation? Some C balance models include a “storage” pool of non-structural carbohydrates (NSC) (Running and Gower, 1991; Bossel, 1996; Thornley and Cannell, 2000), but most of these models make the assumption that the NSC pool acts merely as a buffer between C sources and sinks, balancing
out seasonally or at least over several seasons (Fatichi et al., 2014; Friend et al., 2014; De Kauwe et al., 2014; Schiestl-Aalto et al., 2015). There is mounting evidence that the NSC plays a more active role in tree physiology (Buckley, 2005; Sala et al., 2012; Wiley and Helliker, 2012). For example, NSC accumulation can lead to down-regulation of photosynthesis (Nikinmaa et al., 2014). Therefore, the need to quantify the NSC pool and to better understand the prioritisation of storage vs. growth is of great importance.

An understanding of the dynamics of storage is also essential to correctly represent the C balance in models. If, for example, a direct growth limitation is implemented into models, how should the surplus of accumulated photosynthates be treated? In their proof-of-concept sink-limited model, Fatichi et al. (2013) allowed reserves to accumulate indefinitely. Alternatively, some models (e.g. CABLE (Law et al., 2006), O-CN (Zaehle and Friend, 2010)) increase respiration rates when excess labile C accumulates. Both approaches can be seen as model-oriented solutions to maintain C balance that are unsatisfactory because they are not based on empirical data. Experiments where sink strength is manipulated may provide the key to improve our understanding of C balance processes under direct growth limitation.

Efforts have been made to quantify growth and understand the physiological and morphological changes in response to belowground C sink limitation by manipulating rooting volume in tree seedlings (Arp, 1991; Campany et al., 2017; Poorter et al., 2012a). These experiments often reveal photosynthetic down-regulation and accumulation of leaf starch, and reductions in growth (Arp, 1991; McConnaughay and Bazzaz, 1991; Gunderson and Wullschleger, 1994; Sage, 1994; Poorter et al., 2012a; Robbins and Pharr, 1988; Maina et al., 2002; Campany et al., 2017). In a recent study with Eucalyptus seedlings, Campany et al. (2017) showed that the reduction in seedling growth when rooting volume was restricted could not be completely explained by the negative effects of sink limitation on photosynthesis, suggesting that other components of the C balance were affected in the process. However, Campany et al. (2017) could not accurately quantify all components of tree C balance, i.e. photosynthesis, carbohydrate storage, biomass partitioning and respiration.

Quantifying all components of C balance is not an easy task, given that not all processes are measured with equal fidelity, and data gaps will always occur. Here, we used a data assimilation (DA)-modelling framework, which has been proven to be a powerful tool in analyzing complex C balance problems (Williams et al., 2005; Richardson et al., 2013). For
example, Richardson et al. (2013) use DA to discriminate among alternative models for the dynamics of non-structural carbon (NSC), finding that a model with two NSC pools, fast and slow, performed best; Rowland et al. (2014) applied DA to experimental observations of ecosystem C stocks and fluxes to infer seasonal shifts in C allocation and plant respiration in an Amazon forest; and Bloom et al. (2016) used DA to constrain a C balance model with satellite-derived measurements of leaf C, to simulate continental-scale patterns in C cycle processes.

Our goal in this paper was to use DA to quantify the impact of sink limitation on C balance processes. We utilized data from an experiment in which sink limitation was induced by restricting the rooting volume of Eucalyptus tereticornis seedlings over the course of 4 months (Campany et al., 2017). We assimilated photosynthesis and growth measurements from the experiment into a simple C balance model, to infer the effects of sink limitation on the main C balance processes, namely: respiration, carbohydrate utilisation, allocation, and turnover. We first tested two null hypotheses:

H1: There is no need to consider storage in the model: growth can be adequately predicted from current day photosynthate.

H2: There is no effect of sink limitation on C balance processes other than via a reduction of photosynthesis.

We were then interested to test the following specific hypotheses about the impact of sink limitation on C balance:

H3: We hypothesized that the rate of utilization of carbohydrate for plant growth would be lower under sink limitation, causing growth rates to slow and non-structural carbohydrate to accumulate.

H4: We hypothesized that under sink limitation a larger proportion of C would be lost to growth respiration and less used for production. We have dubbed this the “wasteful plant” hypothesis; this hypothesis corresponds to the assumption embedded in some models that respiration is up-regulated when labile C accumulates e.g. CABLE, O-CN (Law et al., 2006; Zaehle and Friend, 2010).
We hypothesized that foliage and root C allocation fractions would be reduced, in favour of wood allocation. Sink limitation induced by nutrient and/or water stress often results in a shift in C allocation away from foliage and towards fine roots (Poorter et al., 2012b). However, for this experiment, the physical restriction of root growth limits the potential for root allocation. Hence, we predicted that both foliage and fine root allocation would decrease.

2 Materials and Methods

2.1 Experiment description

The site and experimental setup have been described in detail by Campany et al. (2017), so we only provide a brief description here. The experiment was carried out at the Hawkesbury Forest Experiment site (33°37'S 150°44'E) in Richmond, NSW, Australia. Twenty-week old Eucalyptus tereticornis seedlings in tube stock were chosen from a single local Cumberland plain cohort. Ten seedlings were harvested at the start of the experiment to measure initial leaf area and dry mass of foliage, woody components and roots. Forty-nine seedlings were used in the main experiment, allocated to seven treatments. The plants were grown in containers of differing volume set into the ground (5, 10, 15, 20, 25 or 35 L), or were planted directly into soil (free seedlings, used as the control). All plants were grown in the open under field conditions, but were watered regularly to avoid moisture stress.

The site is located in the sub-humid temperate region which experiences warm summers and cool winters. The seedlings were planted on 21st January 2013 (mid-summer) and harvested on 21st May 2013 (late autumn). Mean daily temperatures ranged from 22.8 to 46.4 °C (monthly mean of 32.1 °C) in January 2013, which was the warmest month of the year and cooled down in May 2013 with an average of 21 °C (BoM, 2017).

2.2 Carbon Balance Model (CBM)

We used a DA-modelling framework, similar to that used by Richardson et al. (2013). This approach uses a simple carbon balance model shown in Figure 1. The model is driven by daily inputs of gross primary production (GPP). Total maintenance respiration, $R_{m, tot}$ (calculated as a temperature-dependent respiration rate, $R_{m}$, multiplied by plant biomass), is immediately subtracted, and the remainder enters a non-structural C pool ($C_{n}$). This pool is utilized for growth at a rate $k$ (i.e. $kC_{n}$). Of the utilization flux, a fraction $Y$ is used in growth.
respiration ($R_g$), and the remaining fraction (1-$Y$) is allocated to structural C pools ($C_s$):

among foliage, wood and root ($C_{s,f}$, $C_{s,w}$, $C_{s,r}$). The foliage pool is assumed to turn over with rate $s_f$. We assume there is neither wood or root turnover as the seedlings in the experiment were young.

**Figure 1**: Structure of the Carbon Balance Model. Pools, shown as boxes: $C_n$, non-structural storage C; $C_{s,f}$, structural C in foliage; $C_{s,w}$, structural C in roots; $C_{s,r}$, structural C in wood.

Fluxes, denoted by arrows, include: GPP, gross primary production; $R_{m,tot}$, total maintenance respiration; $R_g$, growth respiration; $C_{l,lit}$, structural C in leaf litterfall. Fluxes are governed by six key parameters: $k$, storage utilization coefficient; $Y$, growth respiration fraction; $a_f$, allocation to foliage; $a_w$, allocation to wood; $a_r$, allocation to roots; $s_f$, leaf turnover rate.

The dynamics of the four carbon pools are described by four difference equations:

\[
\begin{align*}
\Delta C_n &= GPP - R_m (C_{t,f} + C_{t,w} + C_{t,r}) - k C_n \\
\Delta C_{s,f} &= k C_n (1 - Y) a_f - s_f C_{s,f} \\
\Delta C_{s,w} &= k C_n (1 - Y) a_w \\
\Delta C_{s,r} &= k C_n (1 - Y) (1 - a_f - a_w)
\end{align*}
\]

Where GPP is the gross primary production (g C plant$^{-1}$ d$^{-1}$); $R_m$ is the maintenance respiration rate (g C g$^{-1}$ C d$^{-1}$); $C_{t,f}$, $C_{t,w}$, and $C_{t,r}$ are the total C in foliage, wood and root respectively (g C plant$^{-1}$); $k$ is the storage utilization coefficient (g C g$^{-1}$ C d$^{-1}$); $Y$ is the growth respiration fraction; $a_f$, $a_w$, $a_r$ are the allocation to foliage, wood and root respectively; and $s_f$ is the leaf turnover rate (g C g$^{-1}$ C d$^{-1}$).

The non-structural (storage) C pool ($C_n$) is assumed to be divided amongst foliage, wood and root tissues ($C_{n,f}$, $C_{n,w}$, $C_{n,r}$) according to empirically-determined fractions.
container experiment only measured leaf non-structural C \( (C_{n,f}) \), and therefore to estimate the partitioning of the non-structural C among different organs, we used data from a different experiment on similar-sized seedlings of a related species \( (Eucalyptus radiata) \) (Duan et al., 2013). We only considered the ambient well-watered control treatments from that experimental dataset, and found that mass-specific \( C_n \) was distributed in the ratio 75:16:9 among foliage, wood and root pools.

Total carbon in each tissue \( (C_t) \) is then calculated as the sum of non-structural carbon \( (C_n) \) and structural carbon \( (C_s) \) for that tissue.

\[
C_{t,f} = C_{n,f} + C_{s,f} \\
C_{t,w} = C_{n,w} + C_{s,w} \\
C_{t,r} = C_{n,r} + C_{s,r}
\]

\[ (5) \quad (6) \quad (7) \]

### 2.3 Experimental data acquisition

Full details of all measurements are given in Campany et al. (2017). The mass of each pool (foliage, wood, root, storage) was estimated over time as follows. The initial dry mass of leaves, woods and roots was measured for 10 seedlings at the start of the experiment using the harvesting procedure described in Campany et al. (2017). The dry mass of all experimental plants was measured at the end of the experiment following the same procedure.

Seedling growth was tracked during the four months of the experiment, by measuring stem height \( (h) \), diameter at 15 cm height \( (d) \) and number of leaves on a weekly basis. These measurements were used to estimate the time course of wood and foliage biomass: for root total C we used only initial and final harvest measurements. Initial root C was estimated by averaging all 10 harvested seedlings.

We estimated weekly total C in wood \( (C_{s,w}) \) from the measurements of stem height and diameter, by using an allometric model fitted to initial and final harvest data.

\[
\log(C_{t,w}) = b_1 + b_2 \log(d) + b_3 \log(h)
\]

\[ (8) \]

For each seedling, the total leaf area \( (LA) \) and foliage total C \( (C_{t,f}) \) over time \( (t) \) were estimated based on harvested data \( (T = time \ of \ harvest) \) and weekly leaf counts \( (LC) \) over time.
\[ LA(t) = \frac{LA(T)}{LC(T)} LC(t) \]  \hspace{1cm} (9)

\[ C_{t,f}(t) = \frac{M_f(T)}{LC(T)} LC(t) \]  \hspace{1cm} (10)

Fully expanded new leaves were sampled for total non-structural carbohydrate (NSC) concentration on a fortnightly basis. These concentrations were multiplied by leaf biomass to estimate the foliage TNC pool \( C_{n,f} \) at each time point. The partitioning of the non-structural C amongst foliage, wood and root tissues (75:16:9) was then used to estimate the wood and root components of the total TNC pool. Structural C mass for each component was estimated by subtracting non-structural C mass from total C mass.

We estimated daily GPP from leaf-level gas exchange measurements and a simple canopy scaling scheme as described in Campany et al. (2017), and summarized below. Photosynthetic CO\(_2\) response (AC\(_i\)) curves and leaf dark respiration rates (R) were measured on two occasions, 13-14\(^{th}\) March 2013 (when new leaves were first produced) and 14-15\(^{th}\) May 2013 (prior to the final harvest). The AC\(_i\) curves were used to estimate photosynthetic parameters (the maximum rate of Rubisco carboxylation, \( V_{\text{cmax}} \) and the maximum rate of electron transport for RuBP regeneration under saturating light, \( J_{\text{max}} \)) using the biochemical model of Farquhar et al. (1980) and fit with the ‘plantecophys’ package (Duursma, 2015) in R. The parameter \( g_1 \), reflecting the sensitivity of stomatal conductance \( (g_s) \) to the photosynthetic rate, was estimated by fitting the optimal stomatal conductance model of Medlyn et al. (2011) to measured stomatal conductance data.

Daily net C assimilation per unit leaf area \( (C_{\text{day}}) \) was then estimated by using a coupled photosynthesis–stomatal conductance model (Farquhar et al., 1980; Medlyn et al., 2011) using mean photosynthetic parameters \( (J_{\text{max}}, V_{\text{cmax}}, g_1 \text{ and } R_d) \) for each treatment and meteorological data from the onsite weather station. The daily GPP was estimated by multiplying \( C_{\text{day}} \), total leaf area \( (LA) \) and a self-shading factor. The self-shading factor, which is a linear function of LA, is calculated by via simulation with a detailed radiative transfer model, the ‘YplantQMC’ R package of Duursma (2014) for individual treatment. The leaf maintenance respiration rate \( (R_m \text{ g C g}^{-1} \text{ C plant d}^{-1}) \) was calculated for each seedling by scaling the measured rate (R) to air temperature using a Q\(_{10}\) value of 1.86 (Campany et al., 2017).
2.4 Application of Data Assimilation (DA) algorithm

DA was used to estimate the six parameters \((k, Y, a_f, a_w, a_r, s_f)\) of the CBM for this experiment. All parameters were allowed to vary quadratically with time, i.e. each parameter was represented as:

\[
p = p_1 + p_2 t + p_3 t^2
\]  

Quadratic variation over time was found to yield significantly better model fits than either constant parameter values or linear variation over time (see supplementary section S1). We executed three distinct sets of model simulations (Table 1), with the goals of (1) testing the need for a storage pool; (2) determining the effect of sink limitation on model parameters; and (3) attributing the overall effect of sink limitation on growth to the change in individual parameters.

For each set of model simulations, GPP and \(R_m\) were used as inputs to the DA framework, and the measurements of total C mass of each of the plant components and foliage NSC concentrations were used to constrain the parameter values. The set of constraints included 18 measurements of \(C_{t,f}\) and \(C_{t,w}\), two measurements of \(C_{t,r}\) (start and end of the experiment), and six measurements of foliage NSC.

We used the Metropolis algorithm (Metropolis et al., 1953) as implemented by Zobitz et al. (2011), with broad prior Probability Density Functions (PDFs) for the parameters (Table 2). Values of \(k, a_r, a_t\) and \(s_f\) were allowed to vary within the maximum possible range, while parameter \(Y\) was constrained according to the literature on growth respiration (Villar and Merino, 2001). Parameter \(a_t\) was calculated from \(a_r\) and \(a_w\) with a check on \(a_r\) to ensure that it had reasonable values \((0 < a_r < 1)\). Standard Error (SE) was used as an estimate of uncertainty on the assimilated data (Rowland et al., 2014; Richardson et al., 2010), and was calculated based on six replicate data points. When combining errors (e.g. Eq. 9, 10), the errors were assumed to be uncorrelated (Hughes and Hase, 2010).

The step size for the DA was set to a random draw from a normal distribution, with a mean of 0 and a SD of 0.005 in log-normal space, resulting in an acceptance rate of 35–40%. We confirmed the chain convergence, having 3000 iterations to adequately explore the posterior parameter space, by visual inspection of the trace plots of different parameters as suggested by Van Oijen (2008).
Table 1: Summary of the three model simulation sets

<table>
<thead>
<tr>
<th>Simulation Set</th>
<th>Goal</th>
<th>Features</th>
<th>Addressing hypothesis</th>
</tr>
</thead>
</table>
| A              | Test importance of storage pool | • DA applied to estimate parameters for model without storage pool and model with storage pool  
• Three treatment groups  
• Not constrained with NSC data  
• No leaf area feedback | H1 |
| B              | Identify effect of sink limitation on model parameters | • DA applied to estimate parameters for model with storage pool  
• Data divided into one, two, three or seven treatment groups  
• Constrained with NSC data  
• No leaf area feedback | H2-H5 |
| C              | Attribute overall effect on growth to changes in individual parameters | • Forward model runs to quantify impact of individual processes on overall plant growth  
• 5L & free seedlings considered  
• Parameters changed one at a time  
• Leaf area feedback |  |

Table 2: Prior parameter PDFs (with uniform distribution) and the starting point of the iteration for all parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Starting value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k$</td>
<td>0</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>$Y$</td>
<td>0.2</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>$a_f$</td>
<td>0</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>$a_w$</td>
<td>0</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>$s_f$</td>
<td>0</td>
<td>0.01</td>
<td>0.005</td>
</tr>
</tbody>
</table>

\[ a_r = 1 - (a_f + a_w), \text{ where } 0 < a_r < 1 \]
2.4.1 Importance of storage pool

We tested the hypothesis (H1) on the importance of including a non-structural C storage pool in CBM by contrasting fits of the full model with fits of a simplified model without the non-structural C pool (Simulation Set A, Table 1). The simplified model omits the non-structural C pool ($C_n$) from the full model (Figure 1) and assumes that all available C is utilized for growth each day. We applied the DA framework to both model options and calculated the Bayesian Information Criterion, BIC (Schwarz, 1978) to determine the better model structure. BIC measures how well the model predicts the data based on a likelihood function and compare model performance taking into account the number of fitted parameters, with the lowest BIC number indicating the best model setting. For this comparison, both models were fit to the biomass data only, not leaf NSC data, in order to ensure that both models were fit to the same number of data points.

2.4.2 Effects of sink limitation on model parameters

The effects of sink limitation on C balance were investigated by applying the DA framework to data from all treatments combined, and then subsets of treatments (Simulation Set B, Table 1). Considering all treatments pooled together gives same parameters for all the treatments and effectively assumes no effect of sink limitation. On the other hand, taking more subsets of treatments produces more parameter sets (one for each subset) and allows for parameters to vary according to the degree of sink limitation. We first fitted the model to all data, ignoring treatment differences; then considered 2 treatment groups (free seedling / 5-35 L containerized seedlings), 3 groups (free / 5–15 L / 20–35 L) and 4 groups (free / 5-10 L / 15-20 L / 25-35 L). We also fitted the model to each of the 7 treatments individually, where the parameter set for each treatment is unique. The BIC values were compared across treatment groupings.
2.4.3 Attribution analysis

We performed a sensitivity analysis to quantify the impact of the response of each individual process to sink limitation on overall plant growth (Simulation Set C, Table 1). This analysis consisted of forward runs of the model, including a leaf area feedback to GPP. That is, rather than taking GPP based on measured LA (Eq. 9) as input, in this version of the model we calculated daily GPP using the modelled LA, the photosynthesis rate and corresponding self-shading factor. Adding the LA feedback to the model was necessary to quantify how the treatment effect on individual model parameters affects final seedling biomass through its effect on foliage mass, and consequently GPP, over time.

LA in each time step is estimated from NSC-free specific leaf area ($SLA_{nonsc}$) and the predicted foliage structural carbon ($C_{s,f}$) in that time step. $SLA_{nonsc}$ is calculated at harvest discarding the foliage NSC portion and is assumed to be constant for a given treatment throughout the experiment.

\[
LA = SLA_{nonsc} \times C_{s,f}
\]

Once the LA feedback was implemented in the CBM, we ran the model with the inputs and modelled parameters from the smallest pot seedling (5 L), then changed the parameters to those for the free seedling one at a time to quantify the effect of each parameter on the final seedling biomass. The parameters we considered for the sensitivity test were: daily photosynthetic rate per unit leaf area ($C_{day}$), maintenance respiration rate ($R_m$), C allocation fractions to biomass ($a_t$, $a_w$, $a_r$), growth respiration rate ($Y$), foliage turnover rate ($s_f$) and utilization coefficient ($k$).
3 Results

3.1 Importance of storage pool

First, we tested the null hypothesis (H1) that there is no need for a non-structural carbohydrate storage pool in the carbon balance model. We compared BIC values for model structures with and without a storage pool. Table 3 (Simulation Set A) shows the results for model fits with the optimal grouping strategy (three treatment groups). BIC values were consistently lower for the model including the storage pool; the improvement in model fit is most noticeable for the containerized seedlings. This analysis demonstrates that the model does need to include a storage pool to correctly represent the experimental data. In all remaining analyses, the full CBM (with non-structural C pool) is applied to data from all four plant C pools (NSC, foliage, wood and root biomass).

3.2 Sink limitation effect on C balance processes

We addressed our second null hypothesis (H2), that there is no effect of sink limitation on carbon balance processes, by comparing BIC values obtained for model fits when all treatments were combined vs separating the treatments into sub-groups. If there was no effect of sink limitation, the BIC value when all treatments are fit together would be similar to that obtained when treatments are separated into groups. The BIC values shown in Table 3 (Simulation Set B) decrease strongly as number of treatment groups increases, indicating a clear effect of sink limitation on carbon balance processes. Although the BIC values continue to decrease as more treatment groups are considered, we also found that interpreting parameter changes became more difficult as the number of groups increased. Hence, further analyses in this paper used unique parameter sets for three treatment groups: small containers, large containers, and free seedlings.
Table 3: BIC values from model fits. The lowest BIC values (in bold) indicate the best performing parameter settings for any particular simulation. Note that, for Sim A, leaf NSC data were not used to constrain either model, to ensure that both models were fit to the same dataset, resulting in lower BICs compared to Sim B. Treatment groups are: ‘Small’ - 5 L, 10 L and 15 L containers; ‘Large’ - 20 L, 25 L and 25 L containers; ‘Free’ – freely rooted seedlings; ‘All’ - all data; ‘Containerized’ - all plants in containers.

<table>
<thead>
<tr>
<th>Simulation Set</th>
<th>Model Setting</th>
<th>Treatment groups</th>
<th>BIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model without storage pool</td>
<td>Small</td>
<td>459</td>
</tr>
<tr>
<td>Sim A</td>
<td></td>
<td>Large</td>
<td>550</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Free</td>
<td>182</td>
</tr>
<tr>
<td></td>
<td>Model with storage pool</td>
<td>Small</td>
<td>215</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Large</td>
<td>338</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Free</td>
<td>167</td>
</tr>
<tr>
<td>Sim B</td>
<td>7 treatments combined</td>
<td>All</td>
<td>2768</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Containerized</td>
<td>1813</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Free</td>
<td>170</td>
</tr>
<tr>
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<td>Total</td>
<td>1983</td>
</tr>
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<td>2 groups</td>
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<td>Free</td>
<td>170</td>
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<tr>
<td></td>
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<td>Total</td>
<td>1983</td>
</tr>
<tr>
<td></td>
<td>3 groups</td>
<td>Small</td>
<td>683</td>
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<tr>
<td></td>
<td></td>
<td>Large</td>
<td>457</td>
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<tr>
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<td>Total</td>
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<td>7 treatments individually</td>
<td>5 L</td>
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<td>15 L</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 L</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 L</td>
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</tr>
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<td></td>
<td></td>
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</tr>
<tr>
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3.3 Analysis of carbon stock dynamics

Figure 2 shows the correspondence between modeled C pools and data. The model reproduced the key features of biomass growth over time in response to treatment. Biomass growth (Figure 2A, B and C) and the foliage storage pool (Figure 2D) were very clearly impacted by sink limitation: biomass growth was strongly reduced for containerised seedlings, which was very well mimicked by the model. Foliage growth in the free seedlings slowed towards the end of the experiment. Wood and root growth continued throughout the
experiment in freely-rooted seedlings but slowed down during the second half of the experiment in containerized seedlings. In March, at the time of the first leaf NSC measurements, the foliage storage pool (Figure 2D) was similar in size across all treatments, but it increased over time in the free seedlings as these plants continued to grow, and decreased over time in the plants in small containers.

Modelled C stocks for all 7 treatments closely tracked their corresponding observations (Figure 2) as most of the predicted biomass values were within one standard error of the measurements. The exception is the 35 L container treatment, which is underestimated slightly because the grouping of 20, 25 and 35 L treatments into one group makes it difficult for the model to fit all treatments in this group.

**Figure 2:** Modelled C stocks (lines) with optimum parameter settings and corresponding observations (symbols): (A) total C mass in foliage $C_{t,f}$, (B) total C mass in wood $C_{t,w}$, (C) total C mass in root $C_{t,r}$ and (D) total C mass in foliage NSC $C_{n,f}$. Note that the carbon pools...
(y-axes) are plotted on log scale to visualize the changes at the beginning of the experiment. Error bars (1 SE, n = 6) are shown for each observation.

3.4 Parameter estimates

Data assimilation indicated significant treatment effects on all five fitted parameters (Figure 3). There was a large effect of sink limitation on the utilization coefficient ($k$). In agreement with our hypothesis H3, the free seedling had the highest $k$, and the seedlings in small containers (most sink limited) had the lowest $k$ (Figure 3A). As the experiment progressed, the utilization rate of free seedlings began to decrease (Figure 3A). In contrast to the free seedlings, the potted seedlings had relatively low utilization rates initially ($k$ close to 0.5) and the utilization rates slowed down abruptly with time, most significantly in the smallest container treatments (Figure 3A).

In agreement with hypothesis H4, the estimated growth respiration rate ($Y$) varied according to the sink strength of the treatment groups, and was highest in the lowest sink strength treatments (Figure 3B). Moreover, $Y$ did not vary significantly over time for the sink limited treatment groups. However, the rate of growth respiration for the free seedling slowed down over time.

The data assimilation process also indicated that the growth allocation fractions vary among treatments and over time. Consistent with hypothesis H5, wood allocation fraction was highest in the smallest container treatments, and lowest in the free seedlings (Figure 3D). For the free seedlings, allocation was initially highest to foliage and roots (Figure 3C-E); over time, the plants reduced allocation to foliage and increased it to wood and roots. In the containerized seedlings, allocation was initially highest to wood and foliage; over time, foliage allocation decreased to almost zero and root allocation increased.

The estimated leaf turnover rate, $s_l$ was also notably higher for sink-limited treatments compared to free seedlings (Figure 3F). The large value of modelled leaf litterfall for sink-limited treatments is consistent with observations during the experiment that containerized seedlings had relatively large leaf litterfall, beyond normal senescence. Estimated $s_l$ increased over time for all treatment groups (most notably in free seedlings), due to a combination of ontogeny, seasonal change, and growth restriction in the sink-limited seedlings.
Figure 3: Modelled final parameters for three groups of treatments during the experiment period (21st Jan to 21st May 2013): (A) storage utilization coefficient, $k$; (B) growth respiration fraction, $Y$; (C) allocation to foliage, $a_f$; (D) allocation to wood, $a_w$; (E) allocation to roots, $a_r$ and (F) leaf turnover rate, $s_f$. The grey shaded area shows the 95% confidence intervals of modelled parameters.

3.5 Carbon budget

The model was used to partition total GPP (g C plant$^{-1}$) from the entire experiment period into different C pools (growth respiration, maintenance respiration, non-structural carbon, structural foliage, wood, and root carbon, and litterfall) for all 7 treatments (Figure 4). Total
GPP was considerably lower for the containerized seedlings, owing to lower photosynthetic rates per unit leaf area, $C_{\text{day}}$ (Figure 5A), and lower total leaf area (LA) per plant. Though starting with the same total LA of 0.016 m$^2$, the 5 L containerized and free seedlings had total LA of 0.031 and 0.516 m$^2$ respectively after four months of treatment. Simultaneously, the partitioning of GPP changed considerably across different treatments.

Small container seedlings (5, 10, 15 L) had a higher fraction of GPP lost in leaf litterfall compared to other seedlings (Figure 4), consistent with observations during the experiment. The proportion of GPP in final foliage mass was extremely low in sink limited treatments (also shown in Figure 2A). Allocation of GPP to final foliage and root biomass were highest in the free seedlings, although interestingly allocation to final wood biomass was similar across treatments. The final allocation to storage was also higher in free seedlings. The sink limited seedlings had a higher proportional $C$ lost through maintenance respiration. Tissue specific respiration rates were similar in free and containerized seedlings, so the $\sim$35% reduction in photosynthetic rate for the smallest containerized seedling, led to a higher overall $R_{\text{m,tot}}/\text{GPP}$ fraction. In summary, the estimated total respiration ($R_{\text{m,tot}} + R_g$) to GPP ratio was considerably lower for the free seedlings compared to the sink limited treatments.
3.6 Attribution analysis

Sink limitation affected biomass growth via a range of processes, namely reduction in photosynthesis, and variation in the utilization rate, growth respiration, leaf litterfall, and C allocations to foliage, wood and root across various treatment groups. We quantified the contribution of each of these process responses separately by running the CBM with parameter inputs changing one at a time. This analysis attempts to attribute the change in biomass between the smallest container treatment (5 L) and the free seedlings to the underlying mechanisms. Figure 5 shows how biomass (M_f, M_w, and M_r) is predicted to change due to each parameter change from 5 L container (gray line, Figure 5) to free seedling (red line, Figure 5). Different colours in the figure indicate the parameter shifts (left column, A-F).
and their associated impacts on C budgets (right column, G-I). Final biomass values are given in Table 4.

Daily net C assimilation per unit leaf area ($C_{\text{day}}$), which was 30% higher for free seedling compared to 5 L container treatment (Figure 5A), had a large impact on plant growth (final total biomass was increased by 11 g, Table 4 and Figure 5G-I, gray to orange). Maintenance respiration rate ($R_m$) did not vary significantly across treatments (Figure 5B), in line with the data presented in Campany et al. (2017), and consequently its impact was insignificant (the final total biomass is reduced by only 0.2 g, Table 4 and Figure 5G-I, orange to light blue).

The modelled biomass allocation fractions ($a_f$, $a_w$ and $a_r$) in Figure 5C had important, but mixed, effects on C stocks. The final foliage mass was increased from 2.54 g to 6.62 g due to the increase in C allocation to foliage (Table 4 and Figure 5G, light blue to green), which has a positive feedback on GPP. Concomitant changes in C allocation to wood and root resulted in smaller changes to these biomasses as shown in Figure 5H-I (1.34 g and 4.47 g rise respectively, Table 4). Overall, the change in allocation pattern resulted in an increase in final total biomass by 9.9 g. Growth respiration rate ($Y$) was ~20% lower in free seedlings (Figure 5D), which had a considerable impact on C budgets (the final total biomasses were increased by 6.1 g, Table 4 and green to yellow, Figure 5G-I). Leaf turnover, $s_f$ was low in the free seedlings compared to the 5 L container treatment (Figure 5E) which had a large positive effect on final C pools (yellow to blue, Figure 5G-I). The foliage mass was increased by 3.76 g; the wood and root masses were also further increased (2.27 g and 3.87 g respectively) due to the increase in GPP when foliage is retained for longer. Finally, the utilization coefficient, $k$ was higher in free seedlings (Figure 5F) causing a 20-30% positive feedback on C budgets (total biomass increased by 13.2 g, Table 4 and blue to red, Figure 5G-I).
**Figure 5:** Attribution analysis. Left column (A-F): changes in input parameters; Right column (G-I): associated impacts on C budgets (right column, G-I). Colours indicate different parameters. Legend: 5L, highly sink-limited treatment with container size of 5 L; FS, Free Seedling without any sink limitation. Note that the orange line is overlain by the light blue line: the small change in maintenance respiration results in a very minor effect on biomass growth.

**Table 4:** Estimates of final biomass due to individual parameter change, showing the contributions to overall change in biomass. All values in g C plant\(^{-1}\). Different columns represent C partitioning to total foliage, \(C_{t,f}\), total wood, \(C_{t,w}\), total root, \(C_{t,r}\), and the sum of total plant C, \(C_t\).

<table>
<thead>
<tr>
<th></th>
<th>(C_{t,f})</th>
<th>(C_{t,w})</th>
<th>(C_{t,r})</th>
<th>(C_t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 L (Baseline)</td>
<td>0.77</td>
<td>1.98</td>
<td>2.05</td>
<td>4.8</td>
</tr>
<tr>
<td>+ (C_{\text{day}})</td>
<td>2.57</td>
<td>5.98</td>
<td>7.36</td>
<td>15.9</td>
</tr>
<tr>
<td>+ (R_d)</td>
<td>2.54</td>
<td>5.92</td>
<td>7.27</td>
<td>15.7</td>
</tr>
<tr>
<td>+ ((a_f + a_w + a_r))</td>
<td>6.62</td>
<td>7.26</td>
<td>11.74</td>
<td>25.6</td>
</tr>
<tr>
<td>+ (Y)</td>
<td>8.17</td>
<td>8.90</td>
<td>14.59</td>
<td>31.7</td>
</tr>
<tr>
<td>+ (s_f)</td>
<td>11.93</td>
<td>11.17</td>
<td>18.46</td>
<td>41.6</td>
</tr>
<tr>
<td>+ (k)</td>
<td>14.32</td>
<td>14.83</td>
<td>25.66</td>
<td>54.8</td>
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4 Discussion

4.1 Effects of sink limitation on C balance

Our DA-model analysis of this root volume restriction experiment provided significant new insights in the response of key C balance processes to sink limitation. We were able to infer that, in addition to a reduction in photosynthetic rates, sink limitation reduced NSC utilization rates, increased growth respiration, modified allocation patterns and enhanced senescence. Our attribution analysis indicated that all of these process responses contributed significantly to the overall reduction in biomass observed under low rooting volume.

We first tested the null hypothesis (H1) that seedling growth rates could be adequately predicted from current-day photosynthate. This hypothesis was rejected, with a storage pool being necessary to simulate growth, particularly for containerized seedlings (Sim A, Table 3).
The approach of simulating growth from current-day photosynthate is commonly used in models, particularly for evergreen plants (e.g. (Jain and Yang, 2005; Law et al., 2006; Thornton et al., 2007)), but several authors have proposed the need for a storage pool to balance the C sources and sinks in the short term, as well as simulate the effects of photosynthetic down-regulation in the long-term (Pugh et al., 2016; Richardson et al., 2013).

Our results support the need for an NSC pool in CBMs.

We then tested the second null hypothesis (H2) that there was no effect of treatment on the parameters of the C balance model. This hypothesis was also rejected: fitting the DA-model framework simultaneously to all treatments with one set of parameters (ignoring sink limitation effect) gave a low goodness of fit (Sim B, Table 1). This result is consistent with the finding of Campany et al. (2017) that the observed effects of sink limitation on photosynthesis in this experiment were insufficient to explain the large reduction in biomass. Instantaneous photosynthetic rates were reduced 20-30% by sink limitation. The C balance model was driven with daily photosynthesis values derived from these measurements. Had the reduction in growth been due solely to photosynthesis, other parameters to the model would have been similar across treatments. Instead, our DA analysis indicated that several other processes contributed to the reduction in biomass growth, including carbohydrate utilization, growth respiration, allocation patterns, and turnover.

Our results suggested a significant effect of sink limitation on the carbohydrate utilization rate, k (Figure 3A). The modelled k values were approximately twice as high in free seedlings compared to the small containers. This result supports the hypothesis (H3) that plants would have the lowest utilization rate under sink-limited conditions. At the start of the measurement period, the free seedlings were utilizing almost all C produced immediately in growth (k close to 1.0, Figure 3A), partially supporting the hypothesis H2. However, introducing a storage pool in the model improved the DA-modelling results for the entire experimental period, emphasizing the importance of C partitioning to storage and rejecting the hypothesis H2. The utilization coefficient of the free seedlings decreased over time, causing a build-up of C storage (Figure 2D). This decrease in utilization rate could potentially be an ontogenetic effect, with free seedlings initially allocating all carbon to growth during establishment but increasing storage with increasing size. However, ontogenetic effects are confounded with season in this experiment, such that decreasing utilization rates over time could also be a result of decreasing temperatures moving into autumn. There is a real need to quantify how
the carbohydrate utilization rate varies with environmental conditions and ontogeny; data assimilation of experiments in which photosynthesis and growth rates have been monitored over time offer one means to do so.

Although the carbohydrate utilization rate was highest in the free seedlings, leaf carbohydrate concentrations were not lower in these plants at the end of the experiment. As shown in the final C budget analysis (Figure 4), there was a higher total C allocation to the NSC pool in free seedlings than sink-limited treatments. Final carbohydrate storage was high in free seedlings despite high $k$ because the carbohydrate pool was recharged throughout the experiment (Figure 2D), as the free seedlings had high photosynthetic rates but no higher maintenance respiration requirement. In contrast, NSC was depleted for the smallest pot treatments after mid-March (Figure 2D) when demand exceeded supply due to both limited production of photoassimilates and enhanced leaf litterfall (Figure 3F).

The modelled rate for growth respiration, $Y$ was larger for sink limited treatments than the free seedling (Figure 3B). This finding supports the “wasteful plant” hypothesis H4. Inferred $Y$ remained constant over time for the containerized treatments, implying a fixed portion of C loss due to growth respiration despite seasonal variation. However, a reduction in $Y$ over time was inferred for the free seedling, suggesting a possible ontogenetic effect. However, it is important to note that we have inferred growth respiration from the CBM framework. Therefore, these estimates could possibly also include C losses via other pathways. Direct measurements of growth respiration rates would be useful to confirm the inferred effects of sink limitation and investigate potential underlying mechanisms.

We also demonstrated that the allocation fractions among organs change in sink-limited conditions, with sizeable consequences for plant growth rates. There were significant variations among treatments in the modelled C allocation fractions to foliage, wood and root; modelled allocation fractions also varied significantly over time (Figure 3C, D and E). At the beginning of the experiment, foliage allocation fractions were similar for all treatment groups, but wood allocation was higher, and root allocation lower, in the containerized seedlings compared to the free seedlings. Over time, allocation to foliage in the free seedlings decreased, and allocation to both wood and roots increased, likely reflecting ontogenetic effects. For the containerized seedlings, foliage allocation declined steeply over time, while wood allocation decreased marginally and root allocation increased steeply. These allocation
patterns supported our hypothesis H5 that sink limitation due to root restriction would favour allocation to wood over foliage or fine roots.

The inferred biomass allocation pattern was also supported by the final biomass partitioning from DA (Figure 4). The C partitioning to final foliage and root mass was proportional to sink strength. The effect of sink limitation on foliage allocation adds further quantitative information to the relatively scarce data in literature and opposes the review of Poorter et al. (2012a) that summarizes no effect of foliage partitioning. On the other hand, more evidence is present on root partitioning with contrasting evaluation. Our finding supports Hess and De Kroon (2007), expecting root allocation to decrease in smaller containers. However, in contrast NeSmith and Duval (1998) did not find any differences with sink strength and Poorter et al. (2012a) reviewed that over ~80 species and experiment combinations, root allocation decreased 4% on average with a doubling in sink size. This conflicting variation in responses might be the result of differences in age of plants, species and even between cultivars within a species considered in various experiments (Poorter et al., 2012a; NeSmith and Duval, 1998). Our DA analysis shows potential drop in root allocation due to the physical restriction of root development with the growth of sink limited seedlings. Moreover, DA shows the proportion of C in wood biomass did not vary for sink limitation, supporting the outcome of Poorter et al. (2012a). All these changes in C allocation pattern support the hypothesis H5, stating both foliage and root allocations reduced in response of sink limitation. However, these reductions happened not in favour of wood allocation but to maintain the respiratory loses of sink limited seedlings. Overall, there was lower C utilization in plant structural growth in sink limited treatments (~45%) compared to free seedling (~60%).

4.2 Application of DA to infer C balance processes

We have demonstrated that the DA approach can be an invaluable tool for quantifying C fluxes in experimental systems, enabling us to extract important new information from existing datasets to inform carbon balance models, such as the rate and timing of the transfer of photosynthate to and from storage pools. We applied a DA-modelling framework to a belowground sink limitation experiment (Campany et al. 2017). The experimental data showed that the reduction of seedling growth under sink limitation was not completely explained by the drop in C assimilation, suggesting that other C balance processes were also responsible for the growth reduction. However, the experimental measurements did not
directly quantify all the C balance components involved (Campany et al., 2017). The DA-modelling approach is able to draw together the experimental data to estimate all the components of C balance, including photosynthesis, respiration, NSC, biomass partitioning and turnover. This approach could readily be applied to other experiments to derive new information allowing better representation of C balance processes in vegetation models.

Applying this approach requires a range of measurements to constrain the key C balance processes. Here, we used estimated daily C assimilation and maintenance respiration rate as model inputs and constrained the model with measurements of biomass pools (foliage, wood, root) and foliage NSC concentrations. We used fortnightly foliage and wood biomass measurements; the DA framework would work with fewer data observations, but parameters would be estimated with less accuracy. Informal exploration of the model suggested that measurements of foliage turnover would have been particularly useful to better constrain the model. Any experiment having estimates of GPP, maintenance respiration, and structural biomass could potentially be investigated with this framework. However, additional measurements of storage and turnover would be highly beneficial for the performance of the simulation. Repeated observations over time are also useful, particularly for young plants, to account for variations in parameter values over time. We found significant changes in parameter values during the course of the 4-month experiment, which may be linked to both ontogeny and seasonal variation in temperature.

One major caveat on our results is that below-ground carbon cycling processes were not well characterized. For practical reasons, processes such as root growth, respiration, turnover, and exudation are rarely well quantified in empirical studies. Here, we had access to initial and final estimates of root biomass. Root respiration was estimated; root turnover and exudation were assumed to be zero. There is evidence that stress can increase rates of root exudation: for example, Karst et al. (2016) demonstrate increased exudation rates in seedlings exposed to cold soils. They also showed that stressed plants may exude C beyond that predicted by simple concentration gradients in NSC between root and soil. The loss of C independent of NSC in roots suggests that exudation may be actively enhanced once plant growth is limited (Hamilton et al., 2008; Karst et al., 2017). As our CBM does not include this process, it would attribute any C loss through root exudation to another process removing C from the plant, such as growth respiration. The increase in growth respiration that we inferred may
thus potentially include root exudation. Direct measurements of one or both processes would be required to determine the role of root exudation.

4.3 Implications for modelling plant growth under sink limited conditions

The goal of our study was to examine how carbon balance models should be modified to represent sink limitation of growth, whilst maintaining mass balance. Our results demonstrate that several process representations need to be modified. Firstly, we demonstrate a clear need to incorporate a carbohydrate storage pool, with a dynamic utilization rate for growth. We demonstrate that the utilization rate is slowed by sink limitation, and may also vary with ontogeny. Targeted experimental work is needed to better quantify this variation in utilization rates. Secondly, in addition to a feedback on photosynthetic rates, other plant processes including growth respiration, turnover and allocation are also affected by sink limitation. Applying a DA-modelling framework to experimental data with rooting volume restriction has allowed us to quantify these effects in this experiment. Applying this approach more broadly would potentially allow us to identify general patterns in these responses that could then be formulated for inclusion into models. Overall, this approach provides important insights into the regulation of carbohydrate storage, and would significantly advance our ability to predict the impacts of environmental changes on plant growth and vulnerability to stress.

Data availability

The raw data are freely available on Figshare (doi: https://doi.org/10.6084/m9.figshare.5125087.v3). The R source code to perform all the data processing and analysis to replicate the figures is freely available as a Git repository (https://github.com/kashifmahmud/DA_Sink_limited_experiment).

Author contribution

KM analyzed the data, developed the model code, performed the simulations and wrote the paper. BEM conceived the idea and helped in data analysis. RAD and CC provided the experimental data. BEM, RAD, CC and MGD provided in-depth editing of the manuscript.

Competing interests

The authors declare that they have no conflict of interest.
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