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1 **Inferring the effects of sink strength on plant carbon balance processes**

2 **from experimental measurements**

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12

13 **Abstract**

14 The lack of correlation between photosynthesis and plant growth under sink-limited

15 conditions is a long-standing puzzle in plant ecophysiology that currently severely

16 compromises our models of vegetation responses to global change. To address this puzzle,

17 we applied data assimilation of a simple carbon (C) balance model to an experiment where

18 sink strength was manipulated by restricting root volume. Our goals were to infer which

19 processes were affected by growth under sink limitation, and to attribute the overall reduction

20 in growth observed in the experiment, to the effects on component processes. Our analysis

21 was able to infer that, in addition to a reduction in photosynthetic rates, sink limitation

22 reduced the rate of utilization of non-structural carbohydrate (NSC), enhanced respiratory

23 losses, modified C allocation and increased foliage turnover. Each of these effects was found

24 to have a significant impact on final plant biomass accumulation. We also found that

25 inclusion of a NSC storage pool was necessary to capture seedling growth over time,

26 particularly for sink limited seedlings. Our approach of applying data assimilation to infer C

27 balance processes in a manipulative experiment enabled us to extract considerable new

28 information from an existing dataset. We suggest this approach could, if used more widely,

29 be an invaluable tool to develop appropriate representations of sink-limited growth in

30 terrestrial biosphere models.

31

34

1 Introduction

36 Almost all mechanistic models of terrestrial vegetation function are based on the carbon (C)

37 balance: plant growth is represented as the difference between C uptake (through

38 photosynthesis) and C loss (through respiration and turnover of plant parts). This approach to

39 modeling plant growth dates back to early crop and forest production models (McMurtrie and

40 Wolf, 1983; de Wit and van Keulen, 1987; de Wit, 1978) and now provides the fundamental

41 quantitative framework to integrate our scientific understanding of plant ecosystem function

42 (Bonan 2008).

43 However, C balance models have been criticized for being "source-focused" (Fatichi et al.,

44 2014). Most C balance models predict growth from the environmental responses of

45 photosynthesis ("source limitation"). In contrast to this assumption, many experimental

46 studies demonstrate that growth is directly limited by environmental conditions ("sink

47 limitation") rather than the availability of photosynthate. For example, growth is more

48 sensitive to water limitation than is photosynthesis (Bradford and Hsiao, 1982; Müller et al.,

49 2011; Mitchell et al., 2014); low temperatures are considerably more limiting to cell division

50 than to photosynthesis (Körner et al., 2014); nutrient limitation may slow growth without

51 reducing photosynthesis (Reich, 2012; Crous and Ellsworth, 2004); and, physical sink-

52 limitation may reduce growth with a decline in photosynthetic capacity and an accumulation

53 of leaf starch (Arp, 1991; Campany et al., 2017; Poorter et al., 2012a).

54 How can we move to models that include both source- and sink-limitation? Some C balance

55 models include a "storage" pool of non-structural carbohydrates (NSC) (Running and Gower,

56 1991; Bossel, 1996; Thornley and Cannell, 2000), but most of these models make the

57 assumption that the NSC pool acts merely as a buffer between C sources and sinks, balancing

58 out seasonally or at least over several seasons (Fatichi et al., 2014; Friend et al., 2014; De

59 Kauwe et al., 2014; Schiestl-Aalto et al., 2015). There is mounting evidence that the NSC

60 plays a more active role in tree physiology (Buckley, 2005; Sala et al., 2012; Wiley and

61 Helliker, 2012). For example, NSC accumulation can lead to down-regulation of

62 photosynthesis (Nikinmaa et al., 2014). Therefore, the need to quantify the NSC pool and to

63 better understand the prioritisation of storage vs. growth is of great importance.

64 An understanding of the dynamics of storage is also essential to correctly represent the C

65 balance in models. If, for example, a direct growth limitation is implemented into models,

66 how should the surplus of accumulated photosynthates be treated? In their proof-of-concept

67 sink-limited model, Fatichi et al. (2013) allowed reserves to accumulate indefinitely.

68 Alternatively, some models (e.g. CABLE (Law et al., 2006), O-CN (Zaehle and Friend,

69 2010)) increase respiration rates when excess labile C accumulates. Both approaches can be

70 seen as model-oriented solutions to maintain C balance that are unsatisfactory because they

71 are not based on empirical data. Experiments where sink strength is manipulated may provide

72 the key to improve our understanding of C balance processes under direct growth limitation.

73 Efforts have been made to quantify growth and understand the physiological and

74 morphological changes in response to belowground C sink limitation by manipulating rooting

75 volume in tree seedlings (Arp, 1991; Campany et al., 2017; Poorter et al., 2012a). These

76 experiments often reveal photosynthetic down-regulation and accumulation of leaf starch,

77 and reductions in growth (Arp, 1991; McConnaughay and Bazzaz, 1991; Gunderson and

78 Wullschleger, 1994; Sage, 1994; Poorter et al., 2012a; Robbins and Pharr, 1988; Maina et al.,

79 2002; Campany et al., 2017). In a recent study with Eucalyptus seedlings, Campany et al.

80 (2017) showed that the reduction in seedling growth when rooting volume was restricted

81 could not be completely explained by the negative effects of sink limitation on

82 photosynthesis, suggesting that other components of the C balance were affected in the

83 process. However, Campany et al. (2017) could not accurately quantify all components of

84 tree C balance, i.e. photosynthesis, carbohydrate storage, biomass partitioning and

85 respiration.

86 Quantifying all components of C balance is not an easy task, given that not all processes are

87 measured with equal fidelity, and data gaps will always occur. Here, we used a data

88 assimilation (DA)-modelling framework, which has been proven to be a powerful tool in

89 analyzing complex C balance problems (Williams et al., 2005; Richardson et al., 2013). For

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90   example, Richardson et al. (2013) use DA to discriminate among alternative models for the

91   dynamics of non-structural carbon (NSC), finding that a model with two NSC pools, fast and

92   slow, performed best; Rowland et al. (2014) applied DA to experimental observations of

93   ecosystem C stocks and fluxes to infer seasonal shifts in C allocation and plant respiration in

94   an Amazon forest; and Bloom et al. (2016) used DA to constrain a C balance model with

95   satellite-derived measurements of leaf C, to simulate continental-scale patterns in C cycle

96   processes.

97   Our goal in this paper was to use DA to quantify the impact of sink limitation on C balance

98   processes. We utilized data from an experiment in which sink limitation was induced by

99   restricting the rooting volume of *Eucalyptus tereticornis* seedlings over the course of 4

100   months (Campany et al., 2017). We assimilated photosynthesis and growth measurements

101   from the experiment into a simple C balance model, to infer the effects of sink limitation on

102   the main C balance processes, namely: respiration, carbohydrate utilisation, allocation, and

103   turnover. We first tested two null hypotheses:

104   H1: There is no need to consider storage in the model: growth can be adequately predicted

105   from current day photosynthate.

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

107   photosynthesis.

108   We were then interested to test the following specific hypotheses about the impact of sink

109   limitation on C balance:

110   H3: We hypothesized that the rate of utilization of carbohydrate for plant growth would be

111   lower under sink limitation, causing growth rates to slow and non-structural carbohydrate to

112   accumulate.

113   H4: We hypothesized that under sink limitation a larger proportion of C would be lost to

114   growth respiration and less used for production. We have dubbed this the "wasteful plant"

115   hypothesis; this hypothesis corresponds to the assumption embedded in some models that

116   respiration is up-regulated when labile C accumulates e.g. CABLE, O-CN (Law et al., 2006;

117   Zaehle and Friend, 2010).

118 H5: We hypothesized that foliage and root C allocation fractions would be reduced, in favour

119 of wood allocation. Sink limitation induced by nutrient and/or water stress often results in a

120 shift in C allocation away from foliage and towards fine roots (Poorter et al., 2012b).

121 However, for this experiment, the physical restriction of root growth limits the potential for

122 root allocation. Hence, we predicted that both foliage and fine root allocation would decrease.

123

124 **2 Materials and Methods**

125 **2.1 Experiment description**

126 The site and experimental setup have been described in detail by Campany et al. (2017), so

127 we only provide a brief description here. The experiment was carried out at the Hawkesbury

128 Forest Experiment site (33°37'S 150°44'E) in Richmond, NSW, Australia. Twenty-week old

129 *Eucalyptus tereticornis* seedlings in tube stock were chosen from a single local Cumberland

130 plain cohort. Ten seedlings were harvested at the start of the experiment to measure initial

131 leaf area and dry mass of foliage, woody components and roots. Forty-nine seedlings were

132 used in the main experiment, allocated to seven treatments. The plants were grown in

133 containers of differing volume set into the ground (5, 10, 15, 20, 25 or 35 L), or were planted

134 directly into soil (free seedlings, used as the control). All plants were grown in the open under

135 field conditions, but were watered regularly to avoid moisture stress.

136 The site is located in the sub-humid temperate region which experiences warm summers and

137 cool winters. The seedlings were planted on 21st January 2013 (mid-summer) and harvested

138 on 21st May 2013 (late autumn). Mean daily temperatures ranged from 22.8 to 46.4 °C

139 (monthly mean of 32.1 °C) in January 2013, which was the warmest month of the year and

140 cooled down in May 2013 with an average of 21 °C (BoM, 2017).

141 **2.2 Carbon Balance Model (CBM)**

142 We used a DA-modelling framework, similar to that used by Richardson et al. (2013). This

143 approach uses a simple carbon balance model shown in Figure 1. The model is driven by

144 daily inputs of gross primary production (GPP). Total maintenance respiration, $R_{m,tot}$,

145 (calculated as a temperature-dependent respiration rate, R_m, multiplied by plant biomass), is

146 immediately subtracted, and the remainder enters a non-structural C pool (C_n). This pool is

147 utilized for growth at a rate k (i.e. kC_n). Of the utilization flux, a fraction Y is used in growth

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148 respiration (R_g), and the remaining fraction (1-Y) is allocated to structural C pools (C_s):

149 among foliage, wood and root ($C_{s,f}$, $C_{s,w}$, $C_{s,r}$). The foliage pool is assumed to turn over with

150 rate s_f. We assume there is neither wood or root turnover as the seedlings in the experiment

151 were young.
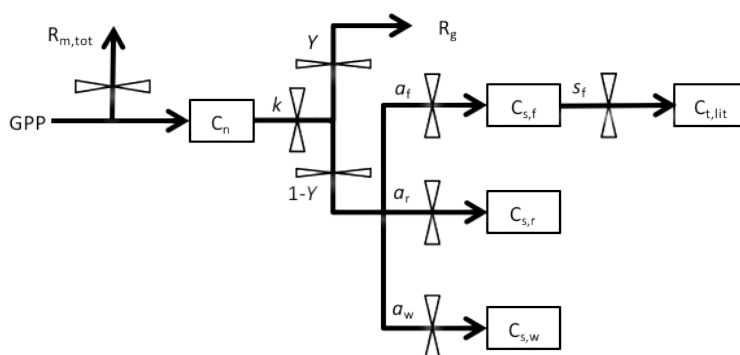
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153 **Figure 1:** Structure of the Carbon Balance Model. Pools, shown as boxes: C_n, non-structural

154 storage C; $C_{s,f}$, structural C in foliage; $C_{s,r}$, structural C in roots; $C_{s,w}$, structural C in wood.

155 Fluxes, denoted by arrows, include: GPP, gross primary production; $R_{m,tot}$, total maintenance

156 respiration; R_g, growth respiration; $C_{t,lit}$, structural C in leaf litterfall. Fluxes are governed by

157 six key parameters: k, storage utilization coefficient; Y, growth respiration fraction; a_f,

158 allocation to foliage; a_w, allocation to wood; a_r, allocation to roots; s_f, leaf turnover rate.

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

$$\Delta C_n = GPP - R_m\left(C_{t,f} + C_{t,w} + C_{t,r}\right) - k\,C_n \tag{1}$$

$$\Delta C_{s,f} = k\,C_n\,(1-Y)\,a_f - s_f\,C_{s,f} \tag{2}$$

$$\Delta C_{s,w} = k\,C_n\,(1-Y)\,a_w \tag{3}$$

$$\Delta C_{s,r} = k\,C_n\,(1-Y)\,(1-a_f-a_w) \tag{4}$$

160 Where GPP is the gross primary production (g C plant^{-1} d^{-1}); R_m is the maintenance

161 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

162 respectively (g C plant^{-1}); k is the storage utilization coefficient (g C g^{-1} C d^{-1}); Y is the

163 growth respiration fraction; a_f, a_w, a_r are the allocation to foliage, wood and root respectively;

164 and s_f is the leaf turnover rate (g C g^{-1} C d^{-1}).

165 The non-structural (storage) C pool (C_n) is assumed to be divided amongst foliage, wood and

166 root tissues ($C_{n,f}$, $C_{n,w}$, $C_{n,r}$) according to empirically-determined fractions. The sink-limited

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167   container experiment only measured leaf non-structural C ($C_{n,f}$), and therefore to estimate the

168   partitioning of the non-structural C among different organs, we used data from a different

169   experiment on similar-sized seedlings of a related species (*Eucalyptus radiata*) (Duan et al.,

170   2013). We only considered the ambient well-watered control treatments from that

171   experimental dataset, and found that mass-specific C_n was distributed in the ratio 75:16:9

172   among foliage, wood and root pools.

173   Total carbon in each tissue (C_t) is then calculated as the sum of non-structural carbon (C_n)

174   and structural carbon (C_s) for that tissue.

$$C_{t,f} = C_{n,f} + C_{s,f} \tag{5}$$

$$C_{t,w} = C_{n,w} + C_{s,w} \tag{6}$$

$$C_{t,r} = C_{n,r} + C_{s,r} \tag{7}$$

175   **2.3   Experimental data acquisition**

176   Full details of all measurements are given in Campany et al. (2017). The mass of each pool

177   (foliage, wood, root, storage) was estimated over time as follows. The initial dry mass of

178   leaves, woods and roots was measured for 10 seedlings at the start of the experiment using

179   the harvesting procedure described in Campany et al. (2017). The dry mass of all

180   experimental plants was measured at the end of the experiment following the same procedure.

181   Seedling growth was tracked during the four months of the experiment, by measuring stem

182   height (h), diameter at 15 cm height (d) and number of leaves on a weekly basis. These

183   measurements were used to estimate the time course of wood and foliage biomass: for root

184   total C we used only initial and final harvest measurements. Initial root C was estimated by

185   averaging all 10 harvested seedlings.

186   We estimated weekly total C in wood ($C_{s,w}$) from the measurements of stem height and

187   diameter, by using an allometric model fitted to initial and final harvest data.

$$\log(C_{t,w}) = b_1 + b_2 \log(d) + b_3 \log(h) \tag{8}$$

188   For each seedling, the total leaf area (LA) and foliage total C ($C_{t,f}$) over time (t) were

189   estimated based on harvested data (T = time of harvest) and weekly leaf counts (LC) over

190   time.

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$$LA\,(t) = \frac{LA\,(T)}{LC\,(T)}\,LC\,(t) \tag{9}$$

$$C_{t,f}\,(t) = \frac{M_f\,(T)}{LC\,(T)}\,LC\,(t) \tag{10}$$

192 Fully expanded new leaves were sampled for total non-structural carbohydrate (NSC)

193 concentration on a fortnightly basis. These concentrations were multiplied by leaf biomass to

194 estimate the foliage TNC pool ($C_{n,f}$) at each time point. The partitioning of the non-structural

195 C amongst foliage, wood and root tissues (75:16:9) was then used to estimate the wood and

196 root components of the total TNC pool. Structural C mass for each component was estimated

197 by subtracting non-structural C mass from total C mass.

198 We estimated daily GPP from leaf-level gas exchange measurements and a simple canopy

199 scaling scheme as described in Campany et al. (2017), and summarized below.

200 Photosynthetic CO_2 response (ACi) curves and leaf dark respiration rates (R) were measured

201 on two occasions, 13-14th March 2013 (when new leaves were first produced) and 14-15th

202 May 2013 (prior to the final harvest). The ACi curves were used to estimate photosynthetic

203 parameters (the maximum rate of Rubisco carboxylation, V_{cmax} and the maximum rate of

204 electron transport for RuBP regeneration under saturating light, J_{max}) using the biochemical

205 model of Farquhar et al. (1980) and fit with the 'plantecophys' package (Duursma, 2015) in

206 R. The parameter g_1, reflecting the sensitivity of stomatal conductance (g_s) to the

207 photosynthetic rate, was estimated by fitting the optimal stomatal conductance model of

208 Medlyn et al. (2011) to measured stomatal conductance data.

209 Daily net C assimilation per unit leaf area (C_{day}) was then estimated by using a coupled

210 photosynthesis–stomatal conductance model (Farquhar et al., 1980; Medlyn et al., 2011)

211 using mean photosynthetic parameters (J_{max}, V_{cmax}, g_1 and R_d) for each treatment and

212 meteorological data from the onsite weather station. The daily GPP was estimated by

213 multiplying C_{day}, total leaf area (LA) and a self-shading factor. The self-shading factor, which

214 is a linear function of LA, is calculated by via simulation with a detailed radiative transfer

215 model, the 'YplantQMC' R package of Duursma (2014) for individual treatment. The leaf

216 maintenance respiration rate (R_m, g C g^{-1} C plant d^{-1}) was calculated for each seedling by

217 scaling the measured rate (R) to air temperature using a Q_{10} value of 1.86 (Campany et al.,

218 2017).

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2.4 Application of Data Assimilation (DA) algorithm

219

220 DA was used to estimate the six parameters (k, Y, a_f, a_w, a_r, s_f) of the CBM for this

221 experiment. All parameters were allowed to vary quadratically with time, i.e. each parameter

222 was represented as:

$$p = p_1 + p_2 t + p_3 t^2 \qquad (11)$$

223 Quadratic variation over time was found to yield significantly better model fits than either

224 constant parameter values or linear variation over time (see supplementary section S1). We

225 executed three distinct sets of model simulations (Table 1), with the goals of (1) testing the

226 need for a storage pool; (2) determining the effect of sink limitation on model parameters;

227 and (3) attributing the overall effect of sink limitation on growth to the change in individual

228 parameters.

229 For each set of model simulations, GPP and R_m were used as inputs to the DA framework,

230 and the measurements of total C mass of each of the plant components and foliage NSC

231 concentrations were used to constrain the parameter values. The set of constraints included 18

232 measurements of $C_{t,f}$ and $C_{t,w}$, two measurements of $C_{t,r}$ (start and end of the experiment), and

233 six measurements of foliage NSC.

234 We used the Metropolis algorithm (Metropolis et al., 1953) as implemented by Zobitz et al.

235 (2011), with broad prior Probability Density Functions (PDFs) for the parameters (Table 2).

236 Values of k, a_f, a_r and s_f were allowed to vary within the maximum possible range, while

237 parameter Y was constrained according to the literature on growth respiration (Villar and

238 Merino, 2001) . Parameter a_r was calculated from a_f and a_w with a check on a_r to ensure that

239 it had reasonable values ($0 < a_r < 1$). Standard Error (SE) was used as an estimate of

240 uncertainty on the assimilated data (Rowland et al., 2014; Richardson et al., 2010), and was

241 calculated based on six replicate measurements. When combining errors (e.g. Eq. 9, 10), the

242 errors were assumed to be uncorrelated (Hughes and Hase, 2010).

243 The step size for the DA was set to a random draw from a normal distribution, with a mean of

244 0 and a SD of 0.005 in log-normal space, resulting in an acceptance rate of 35–40%. We

245 confirmed the chain convergence, having 3000 iterations to adequately explore the posterior

246 parameter space, by visual inspection of the trace plots of different parameters as suggested

247 by Van Oijen (2008).

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248   **Table 1:** Summary of the three model simulation sets

Simulation Set	Goal	Features	Addressing hypothesis
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	

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

Parameter	Minimum	Maximum	Starting value
k	0	1	0.5
Y	0.2	0.4	0.3
a_f	0	1	0.5
a_w	0	1	0.5
s_f	0	0.01	0.005
$a_r = 1 - (a_f + a_w)$, 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_s) 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.

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2.4.3 Attribution analysis

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276   We performed a sensitivity analysis to quantify the impact of the response of each individual

277   process to sink limitation on overall plant growth (Simulation Set C, Table 1). This analysis

278   consisted of forward runs of the model, including a leaf area feedback to GPP. That is, rather

279   than taking GPP based on measured LA (Eq. 9) as input, in this version of the model we

280   calculated daily GPP using the modelled LA, the photosynthesis rate and corresponding self-

281   shading factor. Adding the LA feedback to the model was necessary to quantify how the

282   treatment effect on individual model parameters affects final seedling biomass through its

283   effect on foliage mass, and consequently GPP, over time.

284   LA in each time step is estimated from NSC-free specific leaf area (SLA_{nonsc}) and the

285   predicted foliage structural carbon ($C_{s,f}$) in that time step. SLA_{nonsc} is calculated at harvest

286   discarding the foliage NSC portion and is assumed to be constant for a given treatment

287   throughout the experiment.

$$LA = SLA_{nonsc} \times C_{s,f} \tag{12}$$

288   Once the LA feedback was implemented in the CBM, we ran the model with the inputs and

289   modelled parameters from the smallest pot seedling (5 L), then changed the parameters to

290   those for the free seedling one at a time to quantify the effect of each parameter on the final

291   seedling biomass. The parameters we considered for the sensitivity test were: daily

292   photosynthetic rate per unit leaf area (C_{day}), maintenance respiration rate (R_m), C allocation

293   fractions to biomass (a_f, a_w, a_r), growth respiration rate (Y), foliage turnover rate (s_f) and

294   utilization coefficient (k).

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3   Results

3.1   Importance of storage pool

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

3.2   Sink limitation effect on C balance processes

308   We addressed our second null hypothesis (H2), that there is no effect of sink limitation on
309   carbon balance processes, by comparing BIC values obtained for model fits when all
310   treatments were combined vs separating the treatments into sub-groups. If there was no effect
311   of sink limitation, the BIC value when all treatments are fit together would be similar to that
312   obtained when treatments are separated into groups. The BIC values shown in Table 3
313   (Simulation Set B) decrease strongly as number of treatment groups increases, indicating a
314   clear effect of sink limitation on carbon balance processes. Although the BIC values continue
315   to decrease as more treatment groups are considered, we also found that interpreting
316   parameter changes became more difficult as the number of groups increased. Hence, further
317   analyses in this paper used unique parameter sets for three treatment groups: small containers,
318   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.

| Simulation Set	Model Setting	Treatment groups	BIC
Sim A	Model without storage pool	Small	459
		Large	550
		Free	182
	Model with storage pool	Small	215
		Large	338
		Free	167
Sim B	7 treatments combined	All	2768
	2 groups	Containerized	1813
		Free	170
		Total	1983
	3 groups	Small	683
		Large	457
		free	170
		Total	1310
	7 treatments individually	5 L	85
		10 L	98
		15 L	60
		20 L	63
		25 L	106
		35 L	152
		Free	170
		Total	734

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

333   experiment in freely-rooted seedlings but slowed down during the second half of the

334   experiment in containerized seedlings. In March, at the time of the first leaf NSC

335   measurements, the foliage storage pool (Figure 2D) was similar in size across all treatments,

336   but it increased over time in the free seedlings as these plants continued to grow, and

337   decreased over time in the plants in small containers.

338   Modelled C stocks for all 7 treatments closely tracked their corresponding observations

339   (Figure 2) as most of the predicted biomass values were within one standard error of the

340   measurements. The exception is the 35 L container treatment, which is underestimated

341   slightly because the grouping of 20, 25 and 35 L treatments into one group makes it difficult

342   for the model to fit all treatments in this group.
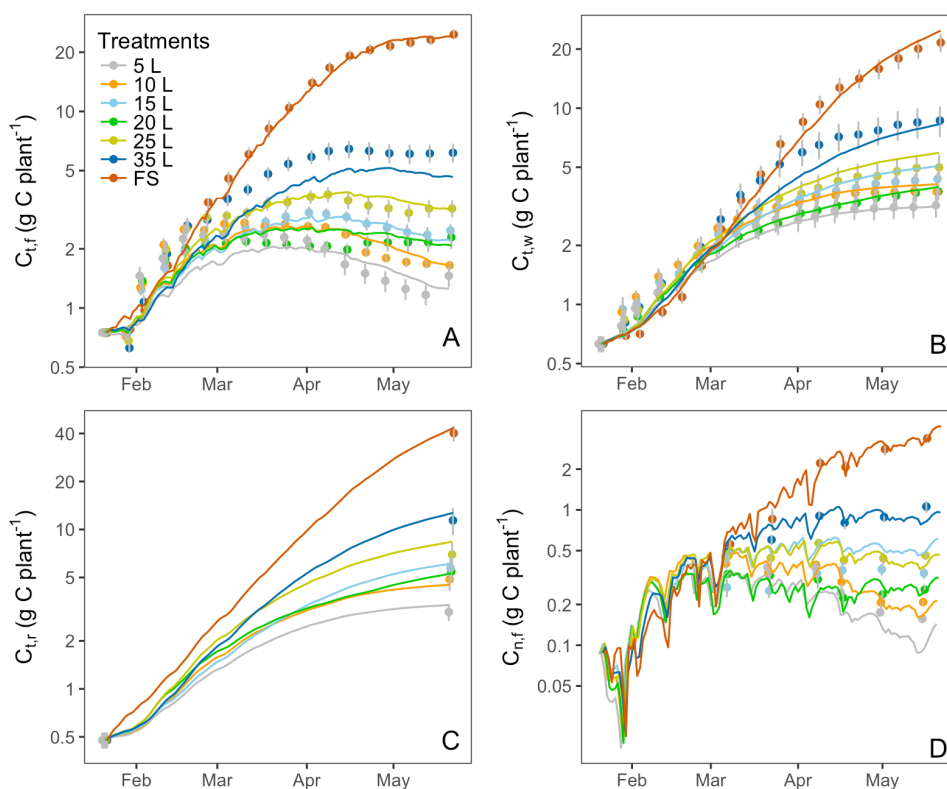
343

344   **Figure 2:** Modelled C stocks (lines) with optimum parameter settings and corresponding

345   observations (symbols): (A) total C mass in foliage $C_{t,f}$, (B) total C mass in wood $C_{t,w}$, (C)

346   total C mass in root $C_{t,r}$ and (D) total C mass in foliage NSC $C_{n,f}$. Note that the carbon pools

347 (y-axes) are plotted on log scale to visualize the changes at the beginning of the experiment.

348 Error bars (1 SE, n = 6) are shown for each observation.

349

350 **3.4   Parameter estimates**

351 Data assimilation indicated significant treatment effects on all five fitted parameters (Figure

352 3). There was a large effect of sink limitation on the utilization coefficient (k). In agreement

353 with our hypothesis H3, the free seedling had the highest k, and the seedlings in small

354 containers (most sink limited) had the lowest k (Figure 3A). As the experiment progressed,

355 the utilization rate of free seedlings began to decrease (Figure 3A). In contrast to the free

356 seedlings, the potted seedlings had relatively low utilization rates initially (k close to 0.5) and

357 the utilization rates slowed down abruptly with time, most significantly in the smallest

358 container treatments (Figure 3A).

359 In agreement with hypothesis H4, the estimated growth respiration rate (Y) varied according

360 to the sink strength of the treatment groups, and was highest in the lowest sink strength

361 treatments (Figure 3B). Moreover, Y did not vary significantly over time for the sink limited

362 treatment groups. However, the rate of growth respiration for the free seedling slowed down

363 over time.

364 The data assimilation process also indicated that the growth allocation fractions vary among

365 treatments and over time. Consistent with hypothesis H5, wood allocation fraction was

366 highest in the smallest container treatments, and lowest in the free seedlings (Figure 3D). For

367 the free seedlings, allocation was initially highest to foliage and roots (Figure 3C-E); over

368 time, the plants reduced allocation to foliage and increased it to wood and roots. In the

369 containerized seedlings, allocation was initially highest to wood and foliage; over time,

370 foliage allocation decreased to almost zero and root allocation increased.

371 The estimated leaf turnover rate, s_f was also notably higher for sink-limited treatments

372 compared to free seedlings (Figure 3F). The large value of modelled leaf litterfall for sink-

373 limited treatments is consistent with observations during the experiment that containerized

374 seedlings had relatively large leaf litterfall, beyond normal senescence. Estimated s_f increased

375 over time for all treatment groups (most notably in free seedlings), due to a combination of

376 ontogeny, seasonal change, and growth restriction in the sink-limited seedlings.

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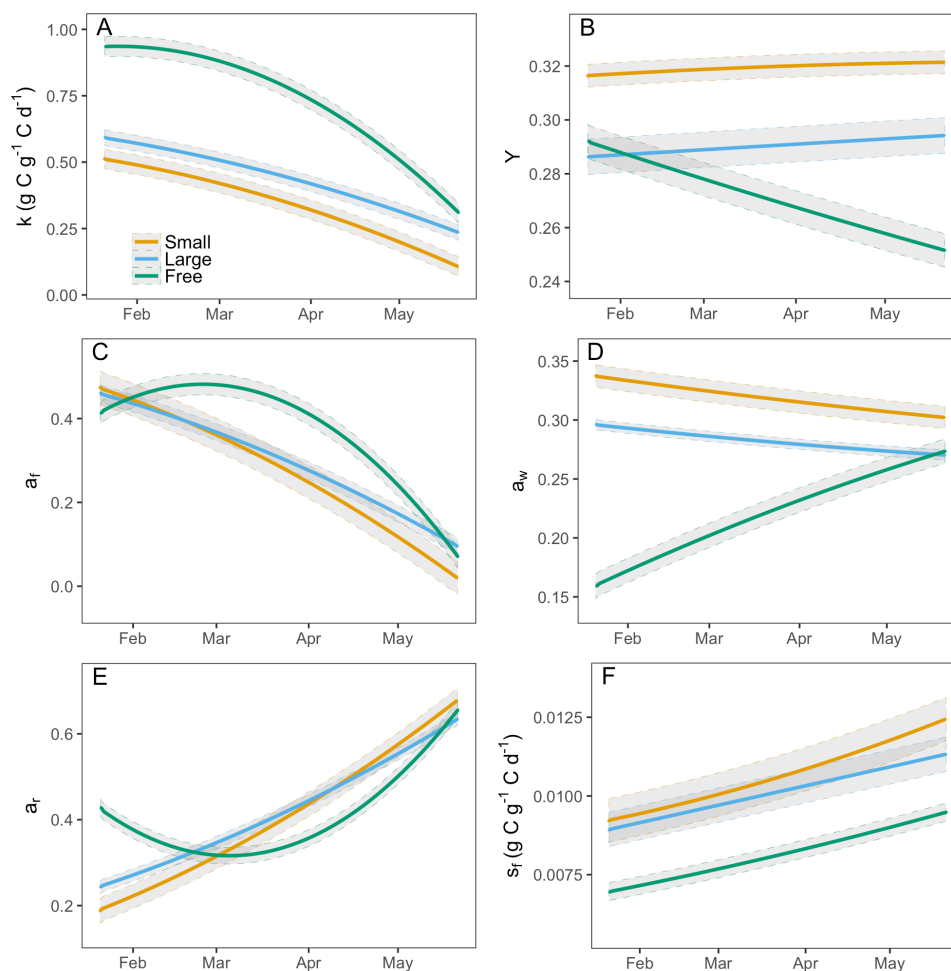
Figure 3: Modelled final parameters for three groups of treatments during the experiment period (21st Jan to 21st May 2013): (A) storage utilization coefficient, k; (B) growth respiration fraction, Y; (C) allocation to foliage, a_f; (D) allocation to wood, a_w; (E) allocation to roots, a_r and (F) leaf turnover rate, s_f. The grey shaded area shows the 95% confidence intervals of modelled parameters.

383

384 **3.5 Carbon budget**

385 The model was used to partition total GPP (g C plant^{-1}) from the entire experiment period

386 into different C pools (growth respiration, maintenance respiration, non-structural carbon,

387 structural foliage, wood, and root carbon, and litterfall) for all 7 treatments (Figure 4). Total

388 GPP was considerably lower for the containerized seedlings, owing to lower photosynthetic
389 rates per unit leaf area, C_{day} (Figure 5A), and lower total leaf area (LA) per plant. Though
390 starting with the same total LA of 0.016 m^2, the 5 L containerized and free seedlings had total
391 LA of 0.031 and 0.516 m^2 respectively after four months of treatment. Simultaneously, the
392 partitioning of GPP changed considerably across different treatments.

393 Small container seedlings (5, 10, 15 L) had a higher fraction of GPP lost in leaf litterfall
394 compared to other seedlings (Figure 4), consistent with observations during the experiment.
395 The proportion of GPP in final foliage mass was extremely low in sink limited treatments
396 (also shown in Figure 2A). Allocation of GPP to final foliage and root biomass were highest
397 in the free seedlings, although interestingly allocation to final wood biomass was similar
398 across treatments. The final allocation to storage was also higher in free seedlings. The sink
399 limited seedlings had a higher proportional C lost through maintenance respiration. Tissue
400 specific respiration rates were similar in free and containerized seedlings, so the ~35%
401 reduction in photosynthetic rate for the smallest containerized seedling, led to a higher overall
402 $R_{m.tot}$/GPP fraction. In summary, the estimated total respiration ($R_{m.tot} + R_g$) to GPP ratio was
403 considerably lower for the free seedlings compared to the sink limited treatments.

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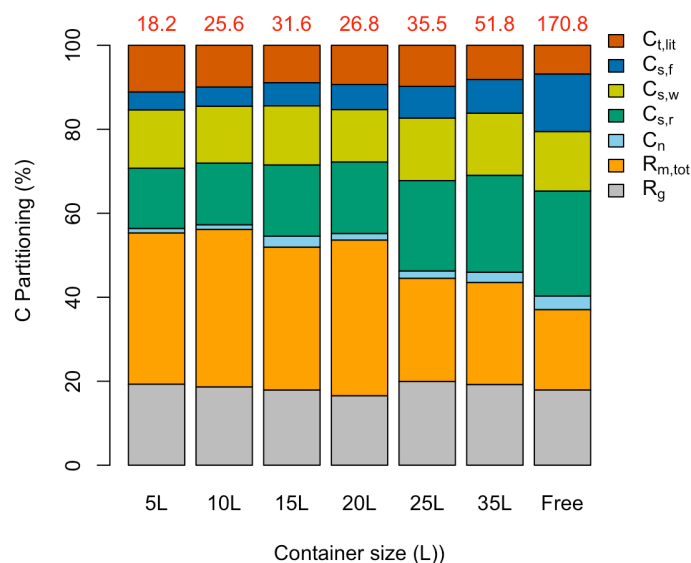
Figure 4: Proportional C partitioning for the whole experimental period. The total accumulated GPP (g C plant^{-1}) for individual treatments is shown (in red) at the top of each column. Free stands for free seedling. Different C partitions are in the colour legend: total litterfall, $C_{t,lit}$; foliage structural C, $C_{s,f}$, wood structural C, $C_{s,w}$, root structural C, $C_{s,r}$; non-structural C pool, C_n; total maintenance respiration, $R_{m,tot}$ and growth respiration, R_g.

410

411   **3.6   Attribution analysis**

412   Sink limitation affected biomass growth via a range of processes, namely reduction in

413   photosynthesis, and variation in the utilization rate, growth respiration, leaf litterfall, and C

414   allocations to foliage, wood and root across various treatment groups. We quantified the

415   contribution of each of these process responses separately by running the CBM with

416   parameter inputs changing one at a time. This analysis attempts to attribute the change in

417   biomass between the smallest container treatment (5 L) and the free seedlings to the

418   underlying mechanisms. Figure 5 shows how biomass (M_f, M_w and M_r) is predicted to change

419   due to each parameter change from 5 L container (gray line, Figure 5) to free seedling (red

420   line, Figure 5). Different colours in the figure indicate the parameter shifts (left column, A-F)

421   and their associated impacts on C budgets (right column, G-I). Final biomass values are given

422   in Table 4.

423   Daily net C assimilation per unit leaf area (C_{day}), which was 30% higher for free seedling

424   compared to 5 L container treatment (Figure 5A), had a large impact on plant growth (final

425   total biomass was increased by 11 g, Table 4 and Figure 5G-I, gray to orange). Maintenance

426   respiration rate (R_m) did not vary significantly across treatments (Figure 5B), in line with the

427   data presented in Campany et al. (2017), and consequently its impact was insignificant (the

428   final total biomass is reduced by only 0.2 g, Table 4 and Figure 5G-I, orange to light blue).

429   The modelled biomass allocation fractions (a_f, a_w and a_r) in Figure 5C had important, but

430   mixed, effects on C stocks. The final foliage mass was increased from 2.54 g to 6.62 g due to

431   the increase in C allocation to foliage (Table 4 and Figure 5G, light blue to green), which has

432   a positive feedback on GPP. Concomitant changes in C allocation to wood and root resulted

433   in smaller changes to these biomasses as shown in Figure 5H-I (1.34 g and 4.47 g rise

434   respectively, Table 4). Overall, the change in allocation pattern resulted in an increase in final

435   total biomass by 9.9 g. Growth respiration rate (Y) was ~20% lower in free seedlings (Figure

436   5D), which had a considerable impact on C budgets (the final total biomasses were increased

437   by 6.1 g, Table 4 and green to yellow, Figure 5G-I). Leaf turnover, s_f was low in the free

438   seedlings compared to the 5 L container treatment (Figure 5E) which had a large positive

439   effect on final C pools (yellow to blue, Figure 5G-I). The foliage mass was increased by 3.76

440   g; the wood and root masses were also further increased (2.27 g and 3.87 g respectively) due

441   to the increase in GPP when foliage is retained for longer. Finally, the utilization coefficient,

442   k was higher in free seedlings (Figure 5F) causing a 20-30% positive feedback on C budgets

443   (total biomass increased by 13.2 g, Table 4 and blue to red, Figure 5G-I).

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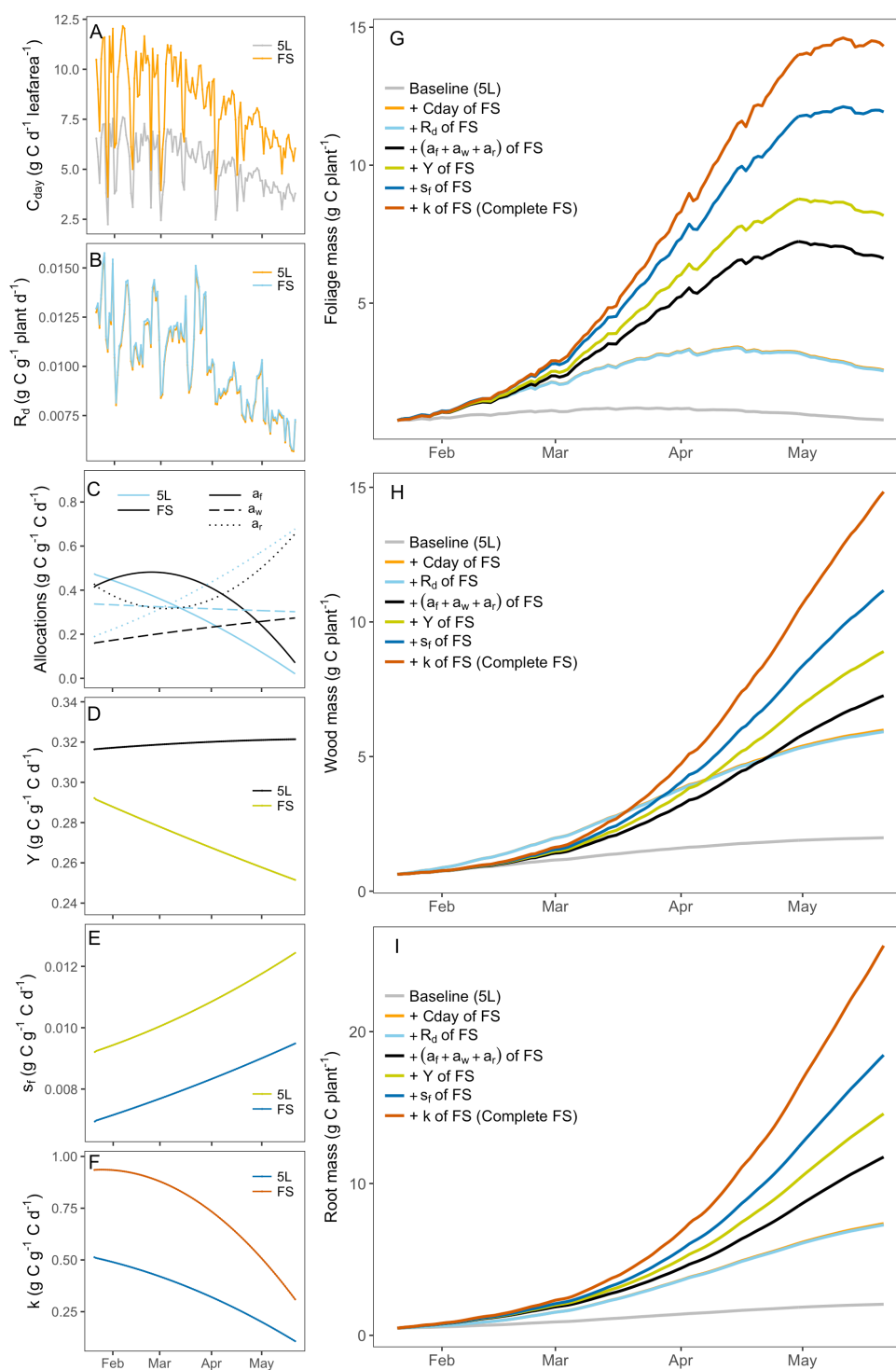
445 **Figure 5:** Attribution analysis. Left column (A-F): changes in input parameters; Right

446 column (G-I): associated impacts on C budgets (right column, G-I). Colours indicate different

447 parameters. Legend: 5L, highly sink-limited treatment with container size of 5 L; FS, Free

448 Seedling without any sink limitation. Note that the orange line is overlain by the light blue

449 line: the small change in maintenance respiration results in a very minor effect on biomass

450 growth.

451 **Table 4:** Estimates of final biomass due to individual parameter change, showing the

452 contributions to overall change in biomass. All values in g C plant^{-1}. Different columns

453 represent C partitioning to total foliage, $C_{t,f}$, total wood, $C_{t,w}$, total root, $C_{t,r}$, and the sum of

454 total plant C, C_t.

	$C_{t,f}$	$C_{t,w}$	$C_{t,r}$	C_t
5 L (Baseline)	0.77	1.98	2.05	4.8
+ C_{day}	2.57	5.98	7.36	15.9
+ R_d	2.54	5.92	7.27	15.7
+ $(a_f + a_w + a_r)$	6.62	7.26	11.74	25.6
+ Y	8.17	8.90	14.59	31.7
+ s_f	11.93	11.17	18.46	41.6
+ k	14.32	14.83	25.66	54.8

455

456 **4   Discussion**

457 **4.1   Effects of sink limitation on C balance**

458 Our DA-model analysis of this root volume restriction experiment provided significant new

459 insights in the response of key C balance processes to sink limitation. We were able to infer

460 that, in addition to a reduction in photosynthetic rates, sink limitation reduced NSC utilization

461 rates, increased growth respiration, modified allocation patterns and enhanced senescence.

462 Our attribution analysis indicated that all of these process responses contributed significantly

463 to the overall reduction in biomass observed under low rooting volume.

464 We first tested the null hypothesis (H1) that seedling growth rates could be adequately

465 predicted from current-day photosynthate. This hypothesis was rejected, with a storage pool

466 being necessary to simulate growth, particularly for containerized seedlings (Sim A, Table 3).

467   The approach of simulating growth from current-day photosynthate is commonly used in
468   models, particularly for evergreen plants (e.g. (Jain and Yang, 2005; Law et al., 2006;
469   Thornton et al., 2007)), but several authors have proposed the need for a storage pool to
470   balance the C sources and sinks in the short term, as well as simulate the effects of
471   photosynthetic down-regulation in the long-term (Pugh et al., 2016; Richardson et al., 2013).
472   Our results support the need for an NSC pool in CBMs.

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

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

499 the carbohydrate utilization rate varies with environmental conditions and ontogeny; data
500 assimilation of experiments in which photosynthesis and growth rates have been monitored
501 over time offer one means to do so.

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

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

520 We also demonstrated that the allocation fractions among organs change in sink-limited
521 conditions, with sizeable consequences for plant growth rates. There were significant
522 variations among treatments in the modelled C allocation fractions to foliage, wood and root;
523 modelled allocation fractions also varied significantly over time (Figure 3C, D and E). At the
524 beginning of the experiment, foliage allocation fractions were similar for all treatment
525 groups, but wood allocation was higher, and root allocation lower, in the containerized
526 seedlings compared to the free seedlings. Over time, allocation to foliage in the free seedlings
527 decreased, and allocation to both wood and roots increased, likely reflecting ontogenetic
528 effects. For the containerized seedlings, foliage allocation declined steeply over time, while
529 wood allocation decreased marginally and root allocation increased steeply. These allocation

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530 patterns supported our hypothesis H5 that sink limitation due to root restriction would favour

531 allocation to wood over foliage or fine roots.

532 The inferred biomass allocation pattern was also supported by the final biomass partitioning

533 from DA (Figure 4). The C partitioning to final foliage and root mass was proportional to

534 sink strength. The effect of sink limitation on foliage allocation adds further quantitative

535 information to the relatively scarce data in literature and opposes the review of Poorter et al.

536 (2012a) that summarizes no effect of foliage partitioning. On the other hand, more evidence

537 is present on root partitioning with contrasting evaluation. Our finding supports Hess and De

538 Kroon (2007), expecting root allocation to decrease in smaller containers. However, in

539 contrast NeSmith and Duval (1998) did not find any differences with sink strength and

540 Poorter et al. (2012a) reviewed that over ~80 species and experiment combinations, root

541 allocation decreased 4% on average with a doubling in sink size. This conflicting variation in

542 responses might be the result of differences in age of plants, species and even between

543 cultivars within a species considered in various experiments (Poorter et al., 2012a; NeSmith

544 and Duval, 1998). Our DA analysis shows potential drop in root allocation due to the

545 physical restriction of root development with the growth of sink limited seedlings. Moreover,

546 DA shows the proportion of C in wood biomass did not vary for sink limitation, supporting

547 the outcome of Poorter et al. (2012a). All these changes in C allocation pattern support the

548 hypothesis H5, stating both foliage and root allocations reduced in response of sink

549 limitation. However, these reductions happened not in favour of wood allocation but to

550 maintain the respiratory loses of sink limited seedlings. Overall, there was lower C utilization

551 in plant structural growth in sink limited treatments (~45%) compared to free seedling

552 (~60%).

4.2 Application of DA to infer C balance processes

554 We have demonstrated that the DA approach can be an invaluable tool for quantifying C

555 fluxes in experimental systems, enabling us to extract important new information from

556 existing datasets to inform carbon balance models, such as the rate and timing of the transfer

557 of photosynthate to and from storage pools. We applied a DA-modelling framework to a

558 belowground sink limitation experiment (Campany et al. 2017). The experimental data

559 showed that the reduction of seedling growth under sink limitation was not completely

560 explained by the drop in C assimilation, suggesting that other C balance processes were also

561 responsible for the growth reduction. However, the experimental measurements did not

562   directly quantify all the C balance components involved (Campany et al., 2017). The DA-
563   modelling approach is able to draw together the experimental data to estimate all the
564   components of C balance, including photosynthesis, respiration, NSC, biomass partitioning
565   and turnover. This approach could readily be applied to other experiments to derive new
566   information allowing better representation of C balance processes in vegetation models.

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

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

593   thus potentially include root exudation. Direct measurements of one or both processes would

594   be required to determine the role of root exudation.

595   **4.3   Implications for modelling plant growth under sink limited conditions**

596   The goal of our study was to examine how carbon balance models should be modified to

597   represent sink limitation of growth, whilst maintaining mass balance. Our results demonstrate

598   that several process representations need to be modified. Firstly, we demonstrate a clear need

599   to incorporate a carbohydrate storage pool, with a dynamic utilization rate for growth. We

600   demonstrate that the utilization rate is slowed by sink limitation, and may also vary with

601   ontogeny. Targeted experimental work is needed to better quantify this variation in utilization

602   rates. Secondly, in addition to a feedback on photosynthetic rates, other plant processes

603   including growth respiration, turnover and allocation are also affected by sink limitation.

604   Applying a DA-modelling framework to experimental data with rooting volume restriction

605   has allowed us to quantify these effects in this experiment. Applying this approach more

606   broadly would potentially allow us to identify general patterns in these responses that could

607   then be formulated for inclusion into models. Overall, this approach provides important

608   insights into the regulation of carbohydrate storage, and would significantly advance our

609   ability to predict the impacts of environmental changes on plant growth and vulnerability to

610   stress.

611

612   **Data availability**

613   The raw data are freely available on Figshare (doi:

614   https://doi.org/10.6084/m9.figshare.5125087.v3). The R source code to perform all the data

615   processing and analysis to replicate the figures is freely available as a Git repository

616   (https://github.com/kashifmahmud/DA_Sink_limited_experiment).

617

618   **Author contribution**

619   KM analyzed the data, developed the model code, performed the simulations and wrote the

620   paper. BEM conceived the idea and helped in data analysis. RAD and CC provided the

621   experimental data. BEM, RAD, CC and MGD provided in-depth editing of the manuscript.

622

623   **Competing interests**

624   The authors declare that they have no conflict of interest.

625

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