

Effects of below-ground space limitation on performance of Eucalyptus seedlings: Does photosynthesis really control growth?

Courtney E. Company^{1,3}, Belinda E. Medlyn² and Remko A. Duursma¹

¹Hawkesbury Institute for the Environment, University of Western Sydney,
Richmond, NSW 2753, Australia

²Department of Biological Sciences, Macquarie University, North Ryde, NSW 2109,
Australia

³Corresponding author (c.company@uws.edu.au)

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Abstract

Interpreting limitations to plant growth requires understanding of the balance between carbon (C) source and sink activity in order to assess carbon allocation and biomass partitioning. This study used manipulations of soil volume to test how growth is coupled to physiology, allocation, and sink activity in *Eucalyptus tereticornis* seedlings. We grew seedlings in a large range of container sizes and planted containers flush to the soil alongside naturally sown seedlings (free). Reduced soil volume was expected to induce rapid negative effects on growth and physiology compared to free seedlings. It was hypothesized that soil volume effect would be largest in the smallest containers, resulting in physical constraints to growth independently of photosynthesis. Photosynthesis would then become sink-limited, resulting in the build-up of leaf nonstructural carbohydrates eventually leading to photosynthetic down regulation. We observed a negative container effect on aboveground growth soon after the experiment started. Although growth was consistently different across soil volumes mass, partitioning to leaves, stems, roots was conserved after 120 days. Photosynthetic capacity was also significantly reduced in containers, and was related to both leaf nitrogen content and starch accumulation, however starch effects were weaker. We developed a simple seedling growth model that utilized leaf photosynthesis rates to allocate daily C uptake towards mass growth of stems, leaves and roots. We then asked whether the observed reductions in photosynthesis explained the observed differences in seedling biomass. We found that photosynthesis reductions alone were not sufficient and the inclusion of additional carbon and mass allocation strategies were necessary to better predict the observed growth responses to decreasing soil volume. Overall, we found limited evidence for sink-limitation of photosynthesis by constraining seedling growth in containers, and argue that shifts in C allocation are necessary to explain the experimental results. This research highlights the need to further understand adaptive strategies of plant C allocation, and confirms that photosynthesis and growth are not always directly related.

Key Words

photosynthesis, sink regulation, growth, carbon allocation, soil volume

Introduction

N C (Körner, 2006; Fourcaud et al., 2008) *A*

Understanding plant growth requires knowledge of the mass balance that must be achieved between carbon uptake and subsequent allocation to growth, storage, and respiration. It is commonly assumed plant growth is limited by carbon availability, yet it has long been demonstrated that correlations between photosynthesis and growth are weak or seldom present. Körner (2013) argues that growth instead controls photosynthesis, as it is the norm for sink activity to feedback on source activity. This is supported by evidence that growth limitation of plants under environmental stress is not limited by the supply of photoassimilates (Palacio et al., 2014). As woody plants have highly integrated systems of competing carbohydrate sinks (Kozłowski, 1992), growth should principally depend on the transport of these photoassimilates between different tissues and organ sinks. Despite a wealth of studies, however, large uncertainties still remain regarding the coordination of carbon supply, via photosynthesis, and growth of woody species.

In woody species, how photosynthesis controls growth has been studied with manipulations of carbon source activity. Examples included elevated CO₂ experiments, for example FACE (reviewed in Ainsworth and Long (2005), and partial defoliation experiments. Elevated CO₂ has been shown to increase photosynthesis rates (Drake et al., 1997; Ainsworth and Rogers, 2007) and across four FACE experiments this resulted in a conserved increase in forest production (Norby et al., 2005). Evidence from elevated CO₂ experiments, however, also reveals that the growth response tends to be much smaller than the photosynthetic enhancement (Kirschbaum, 2011). In defoliation experiments, compensatory increases in photosynthesis are commonly shown yet are attributed to variable mechanisms, including reduction in end product inhibition (Iglesias et al., 2002; Zhou and Quebedeaux, 2003; Handa et al., 2005), enhanced biochemical activity (Ovaska et al., 1993a; Layne and Flore, 1995), increased stomatal conductance (Layne and Flore, 1995), leaf nutrient status (Turnbull et al., 2007), and regulatory sugar signaling (Eyles et al., 2013). However, increases in photosynthesis did not always produce increased growth due to reductions in meristem sink strength (Palacio et al. 2012), carbon limitation to mycorrhizal colonization (Markkola et al. 2004), or an overall decrease in whole plant carbon gain (Ovaska et al., 1993b). These manipulations of C source activity expose unresolved issues with how changes in photosynthesis do not always infer similar responses in growth.

Alternatively, tissue sink activity can restrict biomass production when limited by environmental or developmental constraints (Körner, 2003). This is because metabolic signaling networks, relaying information on carbon and nitrogen status of different tissues, can down regulate photosynthetic activity (Paul and Foyer, 2001). Whether this down regulation, via sink inhibition, exists in woody species has been tested through fruit removal, girdling, and low temperatures. In these studies, down regulation of photosynthesis was frequently correlated to carbohydrate accumulation resulting from reduced tissue sink strength (Iglesias et al., 2002; Hoch et al., 2002; Urban and Alphonsout, 2007; Haouari et al., 2013). However, reductions in photosynthesis were also attributed to biochemical limitations prior to carbohydrate accumulation (Nebauer et al., 2011), irreversible photo-oxidative damage (Duan et al., 2008), and stomatal limitation (Li et al., 2005). These mixed results are not surprising as we still know little about the pathways in which plants achieve balance between assimilation, storage, and growth across temporal scales (Smith and Stitt, 2007). As these manipulations likely impact source as well as sink activity simultaneously, affect water transport, are very extreme, or are specific to large annual fruiting sinks, they tell us little about source-sink coordination in normal field-grown woody species. This coordination is further confounded by the fact that under normal growing conditions, photosynthesis is not always correlated to photosynthate accumulation, as with fir needles (Little and Loach, 1973). Unfortunately, there is still limited understating of the physiological roles of carbohydrates in photosynthetic regulation and the elements triggering the down-regulation process (Nebauer et al., 2011).

An alternative approach is to lower belowground carbon sink strength in tree seedlings by manipulating rooting volume, by varying the container size. The advantage of this approach is it

allows a large range of manipulations, can be easily compared to naturally planted seedlings and mimics natural conditions as seedlings compete for space or reach bedrock. Seedlings undergo many physiological and morphological changes in response to rooting volume, including biomass partitioning, photosynthesis, water relations, nutrient uptake and respiration (NeSmith and Duval, 1998, and references therein). The rooting volume available to plants can decrease carbon sink strength by reducing root growth. Container size studies frequently have photosynthetic down-regulation, likely as a result of sink limitation (Arp, 1991; McConnaughay and Bazzaz, 1991; Gunderson and Wulfschleger, 1994; Sage, 1994; Maina et al., 2002; Ronchi et al., 2006). A meta-analysis by Poorter et al. (2012a) concluded that photosynthesis is the process likely to be the strongest affected by pot size and may best explain the observed effect on biomass seen in the large number of studies where containers are used. This conclusion arises because plants grown in small containers are shown to accumulate leaf starch while having lower carbon exchange and assimilate export rates (Robbins and Pharr, 1988). However, evidence in support for a trade-off between carbon storage and growth in trees is, to date, inconclusive (Palacio et al., 2014). Based on these previous studies, using container size as a sink-strength manipulation can be used to empirically test how growth and photosynthesis are coordinated.

Another barrier in understanding the coordination between photosynthesis and growth is a lack of knowledge regarding the allocation of carbon in woody species. Understanding the fate of assimilated carbon is crucial when source or sink manipulations do not directly lead to equivalent changes in growth. As programmed plasticity in carbon allocation naturally occur as plants growth and develop, this must be accounted for when evaluating induced variation in growth (Wright and McConnaughay, 2002). Thus, when carbon allocation is altered by a treatment, it should be shown whether these allocation patterns differ from common sized untreated plants (Reich et al., 2002; Poorter et al., 2012b). Only then can we test if functional balance preservation or optimal foraging occurs from changes in carbon uptake. For example, seedlings in containers may shift allocation to leaves if root restriction occurs or alter fine root morphology to increase nutrient uptake. In woody species, shifts in allometric relationships of leaves, stems and roots can reveal if growth is ontogenetically constrained or actively adjusted when photosynthesis is affected. This then allows additional pools of carbon, such as root exudation or changes in tissue respiration, to be accounted for and evaluated.

This study utilizes a novel field design to investigate the coordination between growth and photosynthesis in *Eucalyptus tereticornis* Sm. seedlings, by manipulating container size and thus rooting volume. Seedlings were maintained under well watered conditions in order to evaluate only the effect of restricted soil volume and the limited nutrient resource pool. We used freely-rooted seedlings as a control for the container size treatments. Empirical results were combined with a simple plant growth model to simulate seedling growth with a carbon mass balance approach, which was then compared to observed harvested seedling mass. The model used whole-plant carbon gain, scaled from instantaneous rates of leaf photosynthesis, to quantify seedling production over 120 days.

1). The manipulations of container size were hypothesized to induce a belowground sink limitation in these seedlings which was expected to be largest in the smallest containers, resulting in physical constraints to growth independently of photosynthesis.

2). Reducing soil volume was expected to induce rapid negative effects on growth and physiology compared to free seedlings (container effect), with accumulation of leaf nonstructural carbohydrates triggering photosynthetic down regulation through time as a function of available soil volume.

3). Last, the growth model was expected to find agreement between simulated and observed seedling mass, through direct correlation of the effects of soil volume on rates of leaf photosynthesis.

Methods

Experimental Design

This experiment was located on the Hawkesbury Forest Experiment site in Richmond, NSW, Australia. Plots were located in open cover with a site history that consists of a paddock that was converted from native pasture grasses. Top soils at this site, used for the study, are an alluvial formation of low-fertility sandy loam soils (380 and 108 mg kg⁻¹ total nitrogen and phosphorus respectively) with low organic matter (0.7 %) and low water holding capacity. At this site a soil hard layer exists at ~1.0 m with a transition to heavy clay soils. The climate for the region is classified as sub-humid temperate.

Eucalyptus tereticornis seedlings, 20 weeks old and approximately 40 cm tall in tube stock, were chosen from a single local Cumberland plain cohort. Previous experiments have confirmed that species with tap roots (similar to *E. tereticornis*) use the center of the container as the medium for thick roots leaving the periphery of the soil as the most active sites for fine root proliferation (Biran and Eliassaf, 1980a,b). This is generally hypothesized to be a different response than seedlings with no taproot. By using a species with tap root growth and manipulations of container length rather than width, it is believed that a more realistic test of inhibition of growth through constrained soil volume would be achieved. Six seedlings were harvested before planting to measure initial leaf area and dry mass of leaves, stems and roots.

Six container volumes were used ranging from 5 l to 3 l, with a 22.5 cm diameter, and lengths ranging from 15 to 100 cm. Containers were constructed of PVC pipe and were filled with local top soil (described above). Soil in each container was packed to achieve a target soil bulk density of 1.7 g m⁻³. A Imidacloprid (BAYER CropScience) insecticide tablet was planted 5 cm below the roots of each seedling. Containers were planted flush with the soil surface inside metal sleeves, designed to minimize excess air space between the container and outside soil while also allowing for container removal. This allowed for soil temperatures in containers to reflect conditions of naturally sown (free) seedlings. Each experimental block (n=7) contained a complete replicate set of container volumes as well as one free seedling, with 1 m² spacing. For each free seedling, a 1 m² subplot was excavated to 0.5 m and replaced with the same soil used in each container. For free seedlings a border of root exclusion material was buried 0.25 m deep and extended 0.25 m above the ground surface around each subplot to exclude local vegetation.

Plants were watered bi-weekly or when needed, accounting for natural precipitation, to maintain soil moisture at field capacity (13-15 %). To achieve field capacity all soil filled containers were weighed and soil moisture was measured (Time Domain Reflectometer at 10 cm depth) before watering. Derived equations based on container weight at planting (when soil moisture was 3 %) and at field capacity was used to calculate watering requirements for each individual plant. Drain systems were built into each pot to prevent pooling of water in containers before root expansion, from reduced root uptake, or from large rainfall events. These conditions could lead to an anaerobic environment around the root that could hinder the uptake of water through reduced root conductance (Poorter et al., 2009), an undesired experimental artifact. A collection compartment in the bottom of containers, containing gravel covered by root exclusion mesh, was used to collect excess water for 20, 25, and 35 l containers. Plastic tubing (6 mm diameter) was inset into the gravel layer and extended through the top of the container. A lysimeter pump was then used to suction excess water, through the tubing, as needed. As small containers (5, 10, and 15 l) have a larger irradiation effect a simple bottom plug was used to drain excess water from the gravel compartment.

Growth and morphology metrics

Seedlings were planted on January 21st 2013 and stem height, diameter at 15 cm and leaf count were measured weekly thereafter. Once the growth rate of individual plants had significantly de-

187 clined a full biomass harvest was completed (May 21st 2013). Dry mass of leaves, stems, roots
 188 and cumulative leaf area (LI-3100C Area Meter; LI-COR, Lincoln, NE, USA) was measured for each
 189 seedling. Mean individual leaf area for each harvested seedling was calculated by dividing cu-
 190 mulative leaf area by total leaf count of only fully expanded leaves. This value was then used to
 191 interpolate cumulative leaf area through time with weekly leaf counts. Root mass was collected by
 192 passing soil from each container through a 1 mm sieve, washing, separating into fine and coarse
 193 roots (<2 mm and >2 mm diameter, respectively) and then drying to a constant mass. Roots from
 194 the free seedlings were collected by excavating each 1 m² subplot to 0.5 m depth. 25 g fresh weight
 195 subsamples of washed fine roots were analyzed, using WhinoRhizo software (Regent Instruments
 196 Inc.), for specific root length (SRL, cm m⁻¹).

197 ***Photosynthetic parameters***

198 Leaf gas exchange measurements were performed bi-weekly at saturating light (A_{sat}) and saturat-
 199 ing light and $[\text{CO}_2]$ (A_{max}) on new fully expanded leaves. Measurements were initiated only after
 200 sufficient new leaf growth occurred (March 17th, 2013), approximately 6 weeks following planting,
 201 and continued until the biomass harvest. Leaf level gas exchange was measured with a standard
 202 leaf chamber equipped with blue-red light emitting diodes using a portable gas exchange system
 203 (LI-6400, LI-COR, Lincoln, NE, USA). A_{sat} measurements were made at PPFD of 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$
 204 and $[\text{CO}_2]$ of 400 $\mu\text{l l}^{-1}$ and A_{max} with $[\text{CO}_2]$ of 1600 $\mu\text{l l}^{-1}$ and PPFD of 1800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.
 205 This choice of light level to achieve light saturation is consistent with other studies on *Eucalyptus*
 206 species (Kallarackal and Somen, 1997; Pinkard et al., 1998; Crous et al., 2013; Drake et al., 2014).
 207 These measurements were conducted during midday (10:00-14:00 h) with leaf temperature main-
 208 tained at 25°C. After leaves acclimated to the chamber environment, net CO_2 assimilation rate
 209 and stomatal conductance (g_s) were logged 5 times for both A_{sat} and A_{max} . Photosynthetic CO_2 re-
 210 sponse (AC_i) curves were also developed at 25°C on a random subset of each container size ($n=3$)
 211 after new leaves were first produced and immediately prior to the final harvest (May 23rd 2013).
 212 Each AC_i curve began at the reference $[\text{CO}_2]$ of 400 $\mu\text{l l}^{-1}$ and then consisted of 12 additional steps
 213 from $[\text{CO}_2]$ of 50 to 1800 $\mu\text{l l}^{-1}$ at 25°C and saturating light (above). From these curves the pho-
 214 tosynthetic parameters, J_{max} and V_{cmax} , can be quantified using biochemical model of Farquhar
 215 et al. (1980). Leaf dark respiration rates (R_d) was measured on each seedling during the same
 216 dates as AC_i curves using detached leaves inside a conifer chamber attached to the Licor 6400
 217 at least 1 hour after sundown. Measurements were taken at a reference $[\text{CO}_2]$ of 400 $\mu\text{l l}^{-1}$ while
 218 leaf temperature was maintained at current ambient conditions. Specific leaf area (SLA, $\text{m}^2 \text{kg}^{-1}$)
 219 was calculated by measuring leaf area and dry mass for individual leaves sampled during gas
 220 exchange campaigns.

221 ***Leaf water potential***

222 Predawn (Ψ_{pd}) and midday (Ψ_i) leaf water potentials were measured for each seedling using a
 223 PMS 1505D pressure chamber (PMS Instruments, Albany, OR, USA) on fully expanded leaves dur-
 224 ing the same time period as AC_i and R_d . Leaves were collected, immediately stored inside foil
 225 covered bags and then transported to the laboratory for measurements of water potential. Ψ_{pd}
 226 was measured before sunrise and Ψ_i at midday 13:00-14:30 h. These measurements were used
 227 as a measure of static water stress on the seedlings (Sellin, 1999), and to ensure that the bulk
 228 soil water availability was high enough for plants as they became larger and roots filled the soil
 229 volume.

230 ***Leaf, root and soil chemistry***

231 Leaves used in each gas exchange measurements and subsamples of harvested roots were dried
 232 to a constant mass and milled for analysis of N content. Pre-planting soil samples ($n=6$) and sub-
 233 samples of soil from each container following harvest were sieved to remove organic material,

air dried and milled for analysis. N concentrations of milled samples were determined using a Carlo Erba CE1110 elemental analyzer with thermal conductivity and mass spectrometric detection (of N_2 and CO_2). The percentage of N in the sample was calculated by comparison with known standards. Leaf TNC concentration was analyzed using a total starch assay kit (Megazyme International 303 Ireland Ltd., Wicklow, Ireland) on gas exchange leaves (above) and represents the sum of starch ($mg\ g^{-1}$) and soluble sugar ($mg\ g^{-1}$) concentrations. Starch was quantified using a thermostable α -amylase and amyloglucosidase assay (McCleary et al., 1997) and soluble sugars were determined following the anthrone method (Ebell, 1969). Full methods of the TNC assay are described in (Mitchell et al., 2013).

Seedling growth model

We developed a simple seedling growth model that utilized leaf photosynthesis to allocate daily Assimilate towards mass production of stems, leaves and roots. The model begins with the mean initial component mass ($leaf_i$, $stem_i$ and $root_i$) and a starting leaf area (LA_i) measured prior to planting. Biomass production for each day of the experiment was estimated by multiplying the whole tree seedling carbon uptake gain per day (C_{day}) by a C use efficiency parameter (Y_C , Mäkelä (1997)), allocating new carbon to the previous days biomass and then accounting for carbon loss via tissue respiration. C_{day} was calculated for each seedling by fitting a coupled photosynthesis - stomatal conductance model (Farquhar et al., 1980; Medlyn et al., 2011) in the 'plantecophys' package in R (Duursma, 2014) to the mean photosynthetic parameters (R_d , J_{max} , V_{cmax} , and g_1) for each treatment and meteorological data from an onsite weather station. Examples of the photosynthesis model are described in Medlyn et al. (2002) and the approach of the coupled leaf gas exchange model are described in Duursma et al. (2014). The g_1 parameter was generated by fitting observed stomatal conductance values into the optimal stomatal conductance model from (Medlyn et al., 2012). Combined with the meteorological parameters PPFD, air temperature, and relative humidity, at 15 m intervals, photosynthesis rates ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$) were then predicted for each soil volume treatment. Rates were assumed to be representative of the entire 15 min meteorological interval. C_{day} was calculated by converting predicted rates to mass C gain over 15 min ($g\ m^{-2}$) and then summed for 24 h. Additionally, it is necessary to calculate a self-shading parameter (M) when scaling flat leaf photosynthesis to seedling cumulative leaf area. This was accomplished by utilizing 61 previously digitized Eucalyptus seedlings, covering 5 total species which include *E. tereticornis*, from Duursma et al. (2012) to run in 'YplantQMC' package in R (Cieslak, Mik. Uses code by Robert Percy and Medlyn.) to build a 3d plant structure based on digitized metrics of plant allometry and crown structure. Inputting the same physiological parameters listed above, 'YplantQMC' outputs total photosynthesis, using total leaf area, for seedlings assuming self-shading as well as for a full sun large horizontal leaf. The ratio of self-shading to flat leaf total photosynthesis was then used to calculate M , which was then applied to modeled rates of photosynthesis when scaling to seedling cumulative leaf area. All default parameters used in model simulations are reported in Table. 3.

We then utilized this model to investigate whether the effects of soil volume on photosynthesis were enough to accurately predict overall seedling production after 120 days or whether changes in C allocation or mass partitioning were also necessary to predict biomass. First, the model was simulated using only changes in photosynthesis rates across treatments combined with mean values of mass partitioning and either published or local data of stem and root respiration rates. Next, different mass partitioning and C allocation scenarios were simulated to investigate sources of missing C from the initial model simulation. This included testing model sensitivity by altering leaf C allocation, root respiration rates, and fine root C allocation. For all cases, mass production was compared between model output and harvested seedlings to investigate what C allocation strategies beyond reduction in photosynthesis aid in prediction of seedling growth responses induced by limiting soil volume.

Data Analysis

Differences in experimental parameters with soil volume were analysed by one-way analysis of variance (ANOVA) in R with individual containers as random effects and soil volume as a categorical fixed effect. Tukeys post-hoc tests were performed in conjunction with ANOVA to determine which specific paired comparisons among soil volumes were different. Mixed model ANOVAs of A_{\max} and leaf chemistry were performed using the 'nlme' package (Pinheiro et al., 2014) in R and r^2 values of mixed models were computed as in Nakagawa and Schielzeth (2013). Tests of allometric relationships between biomass components were implemented using major axis regression in the 'smatr' package (Warton et al., 2012). Results were considered significant at $P \leq 0.05$.

Results

Growth and morphology metrics

In this open field study, colder temperatures and reductions in cumulative PPFD per day (Fig. 1) most likely lead to the reduced growth in the free seedlings in the final weeks of the experiment (Fig. 2). Combined with severe growth reductions in the smallest container volumes the experiment was chosen to be harvested after 120 days. Over the 120 days height, diameter, and leaf area diverged between container volumes (Fig. 2). First, seedling leaf area significantly diverged between soil volumes ($P < 0.026$) during the 5th week of the experiment. Following this period both height (8th week) and then diameter (9th week) significantly deviated across soil volumes ($P < 0.002$ & 0.001 , respectively). Negative growth effects then manifested as severely reduced height gain and declining leaf area through time with small soil volumes across the final two months of the experiment. Seedlings maintained diameter growth throughout the experiment, although marginal with smaller soil volumes in the final month. Final seedling height significantly increased with increasing soil volume ($P < 0.001$). Increases in both final stem diameter ($P < 0.001$) and cumulative leaf area (both $P < 0.001$) were found with increasing soil volume and these differences were driven mainly by the largest container and the free seedling.

Total plant biomass at harvest was significantly different across container volumes ($P < 0.001$) and with free seedlings ($P < 0.001$, Table. 1). We analyzed the relationship between biomass growth with each fold increase in soil volume and found an increase of 34 % with a doubling of pot size, consistent with the meta-analysis of Poorter et al. (2012a). Additionally, plant biomass was highly correlated with total leaf area across all treatments ($r^2 = .97$, $P < 0.001$). Differences in biomass partitioning to leaves, stems, and roots were not different across soil volumes when variation in seedling biomass within treatments was factored in the analysis. Across all treatments, the final harvested root:shoot was conserved in these seedlings, with a slightly higher shoot than root mass on average ($\bar{x} = 0.904$).

SRL of harvested fine roots was not different across soil volumes (Table. 1). Over the duration of the experiment SLA was higher in free seedlings but was not different across containers sizes (Table. 1, $P < 0.001$) and this pattern was evident in the first gas exchange measurement campaign ($P < 0.001$).

Leaf and root chemistry

Leaf N % was significantly higher in free seedlings and the largest container volume at the onset of gas exchange measurements (6th week, $P < 0.001$). Over the remaining duration of the experiment the smallest container volume had a significant reduction in leaf N % compared to other soil volumes, while the free seedling maintained a greater leaf N % (Table. 1, $P < 0.001$). Additionally, mean leaf starch content in the smallest container was double that of free seedlings ($P = 0.042$), while leaf soluble sugars did not differ across treatments throughout the experiment (Table. 1).

328 Differences in leaf starch between the free seedling and the smallest container were also evident
329 during the first gas exchange campaign ($P=0.0013$).

330 ***Gas exchange and photosynthetic parameters***

331 A_{sat} (Fig. 3) and A_{max} (Table. 2) were both significantly higher in the largest container volume
332 and the free seedling at the first measurement campaign (both $P<0.001$). Across measurement
333 campaigns A_{sat} was consistently higher in free seedlings than in containers (Figure 3, $P<0.001$).
334 The interaction between photosynthetic capacity, leaf starch, and leaf N , on a mass basis, was
335 marginally significant ($P=0.0584$) but A_{max} was highly correlated to both leaf N content and leaf
336 starch (both $P<0.001$). Across all measurement campaign A_{max} was higher when foliar N was also
337 higher, usually associated with low levels of leaf starch (Fig. 3a). A_{max} was also lower when leaf
338 starch was high as higher leaf N often did not coincide with high leaf starch (Fig. 3b)

339 The photosynthetic parameters J_{max} and V_{Cmax} were not different across measurement campaigns,
340 therefore the parameter means per volume are reported here (Table. 2). Overall, both J_{max} and
341 V_{Cmax} were significantly higher in free seedlings with little variation between container volumes
342 ($P=0.0012$ & 0.0021 , respectively). Leaf dark respiration rates were not significantly different across
343 soil volumes (Table. 2). The g_1 parameter, generated for each seedling from the Medlyn et al.
344 (2012) optimal stomatal conductance model, was not different across soil volumes (Table. 2). Pre-
345 dicted values of g_s , using the g_1 parameter, were highly correlated with observed values ($r^2= .74$,
346 $P<0.001$, data not shown).

347 Neither Ψ_{pd} nor Ψ_{I} were different across treatments, with mean values of -0.27 and -1.2 mPa across
348 all seedlings, respectively. Although g_s in free seedlings was generally higher than those in con-
349 tainers (Table. 2, $P=0.0023$), the mean rates for all seedlings were high at 0.37 mol $\text{H}_2\text{O m}^{-2} \text{ s}^{-1}$ and
350 did not decline significantly across the experiment duration. Additionally, leaf $\delta^{13}\text{C}$ at final harvest
351 was not different across treatments (Table. 1). Combined these indices provide strong evidence
352 that water stress was not apparent on these well-watered seedlings throughout the experiment.
353 Soil N % at harvest was not different across soil volumes ($\bar{x}=04.5$ %) and decreased approximately
354 3 % across all containers over the experiment duration. This indicates that nutrient leaching from
355 free seedlings or from draining of containers following natural rainfall events did not differ be-
356 tween treatments.

357 ***Modelling seedling biomass***

358 The initial model simulation, testing only treatment specific photosynthesis rates, was unable to
359 accurately predict the responses of actual harvested seedling biomass to reduced soil volume.
360 When both C_{day} and biomass of modeled and harvested seedlings were scaled to to the free
361 seedling the initial model design overestimated biomass for all treatments (Fig. 6a). This sug-
362 gests that reductions in photosynthesis alone could not realistically capture the reduced growth
363 response of these seedlings. Next, the model was re-run using the mean mass fractions of
364 leaves, stems and roots from the final harvest, per treatment, to drive daily mass partitioning
365 and seedling production. Combined with treatment specific photosynthesis rates this model still
366 overestimated seedling production for all treatments (Fig. 6b).

367 To test whether potential sources of missing C could improve the modeled response of seedling
368 mass production the model was run with 3 different C allocation scenarios that could not be tested
369 within the framework of this experiment. This was accomplished by testing the sensitivity of the
370 model to adjustments of C allocation to leaves or fine roots or root respiration rates by ± 50 %
371 of default values. The model output of these scenarios was then compared to both the original
372 model output and with harvested seedling biomass. Increasing C allocation to either leaves or fine
373 roots, in coalescence with photosynthesis rates, did improve the model but neither were sufficient
374 to accurately predict the actual biomass response (Fig. S1a,c). Altering respiration rates of fine and
375 coarse roots did not have a noticeable deviation from the original model (Fig. S1b).

376 **Discussion**

377 **Tables**

Table 1: Responses of plant and leaf characteristics of *Eucalyptus tereticornis* seedlings to soil volume treatments. Each value reflects the mean (standard error) of each treatment.

Volume (L)	Seedling mass (g)	SLA (m ² kg ⁻¹)	Leaf Nitrogen (%)	Leaf Sugars (%)	Leaf Starch (%)	SRL (cm m ⁻¹)	Root Nitrogen (%)	δ ¹³ C (‰)
5	14.8 (1.82)	9.5 (0.23)	1.1 (0.02)	6.4 (0.28)	12.7 (0.97)	39.1 (5.47)	0.82 (0.05)	-30.1 (0.26)
10	20.0 (2.38)	9.8 (0.24)	1.3 (0.04)	6.7 (0.25)	9.4 (0.75)	34.2 (5.83)	0.75 (0.02)	-30.2 (0.25)
15	25.4 (2.49)	11.0 (0.47)	1.4 (0.06)	7.2 (0.28)	7.3 (0.73)	37.6 (4.63)	0.71 (0.02)	-30.3 (0.36)
20	23.4 (1.63)	9.8 (0.28)	1.4 (0.05)	6.6 (0.26)	9.5 (0.88)	45.3 (5.50)	0.76 (0.04)	-29.7 (0.28)
25	30.4 (5.49)	10.4 (0.37)	1.3 (0.06)	6.9 (0.24)	9.8 (0.71)	47.0 (7.10)	0.74 (0.02)	-29.7 (0.25)
35	52.2 (9.55)	11.3 (0.44)	1.5 (0.08)	6.8 (0.22)	9.8 (0.65)	50.6 (11.61)	0.77 (0.03)	-30.6 (0.38)
Free	174.5 (18.02)	13.0 (0.44)	2.4 (0.09)	7.4 (0.25)	6.8 (0.65)	43.7 (6.24)	0.87 (0.04)	-30.0 (0.34)

Table 2: Responses of leaf level gas exchange parameters of *Eucalyptus tereticornis* seedlings to soil volume treatments. Each value reflects the mean(standard error) of each treatment. Units for A_{\max} and R_{dark} are $\mu\text{mol m}^{-1} \text{s}^{-1}$ and g_s are $\text{mol m}^{-1} \text{s}^{-1}$.

Volume (L)	A_{\max}	R_{dark}	J_{\max}	$V_{C\max}$	g_s	g_1
5	21.2 (0.9)	2.8 (0.3)	103.7 (5.8)	62.9 (2.8)	0.30 (0.01)	5.1 (0.1)
10	22.3 (1.4)	2.7 (0.4)	116.5 (5.9)	69.4 (2.9)	0.36 (0.01)	5.4 (0.1)
15	23.3 (1.2)	1.4 (0.1)	123.1 (15.4)	79.4 (10.0)	0.45 (0.01)	6.2 (0.2)
20	26.1 (0.7)	1.7 (0.1)	130.0 (14.1)	81.4 (6.0)	0.38 (0.01)	5.2 (0.2)
25	23.9 (0.9)	1.2 (0.1)	131.9 (5.9)	78.6 (2.6)	0.32 (0.01)	4.8 (0.2)
35	25.0 (1.0)	1.5 (0.2)	121.2 (3.7)	78.0 (3.5)	0.33 (0.02)	4.7 (0.2)
Free	33.1 (0.7)	1.3 (0.1)	171.7 (18.6)	101.2 (5.7)	0.49 (0.02)	5.0 (0.2)

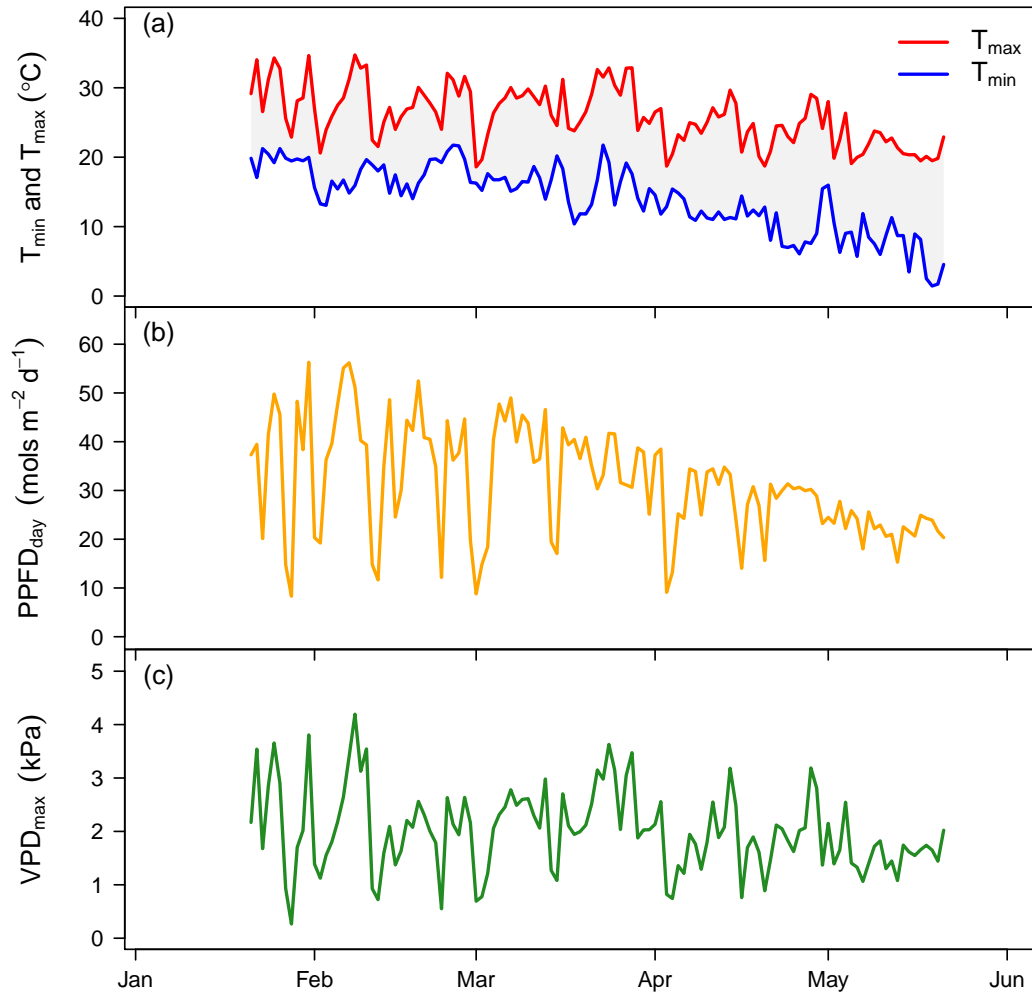


Figure 1: Daily maximum and minimum temperature (a), cumulative daily PPFD (b), and daily maximum vapour pressure deficit (c) across the experiment duration in 2013.

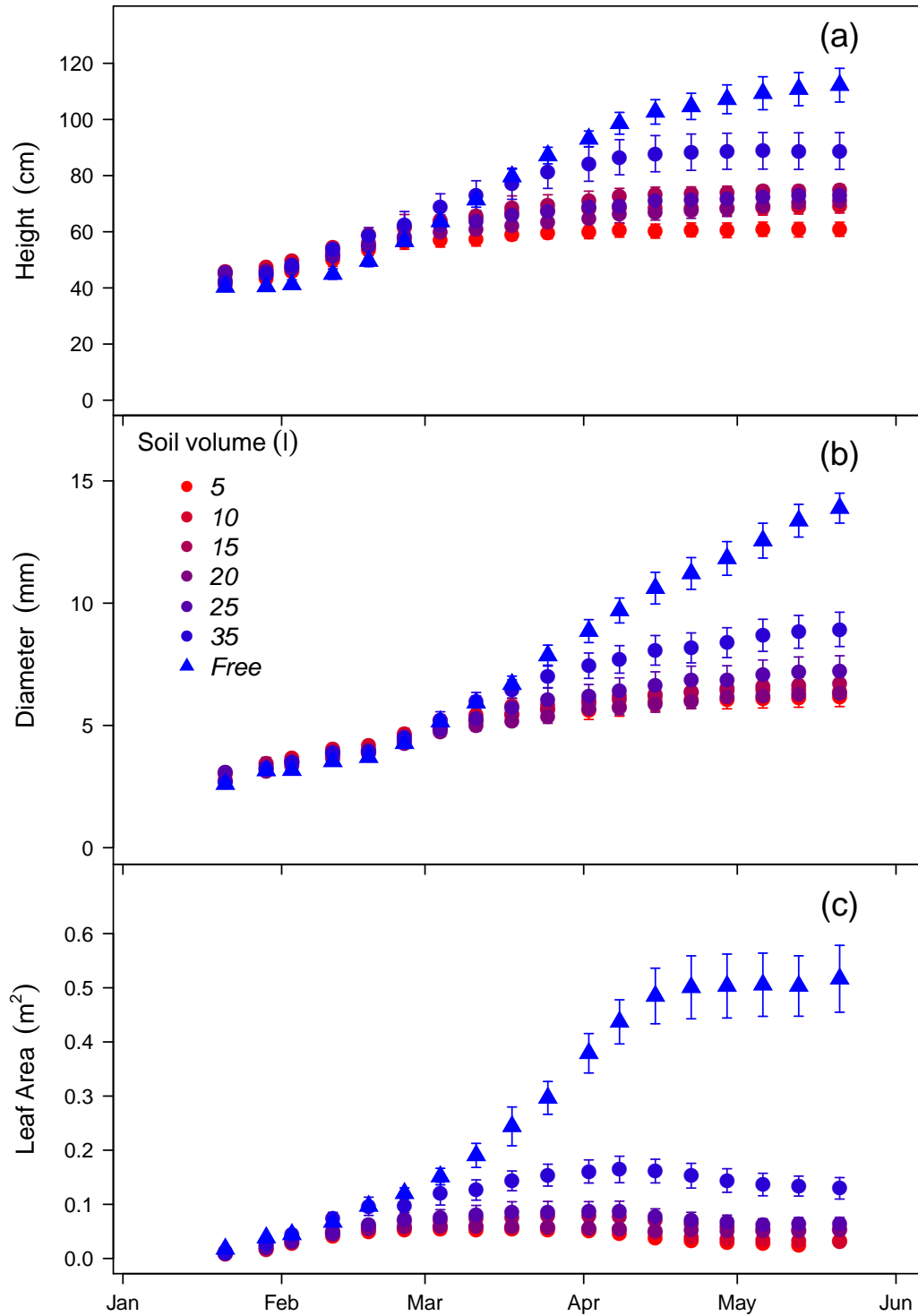


Figure 2: Soil volume treatment means \pm SE (n=7) of height growth (a), diameter growth (b), and interpolated seedling leaf area (c) measured weekly of *Eucalyptus tereticornis* seedlings across the experiment duration in 2013.

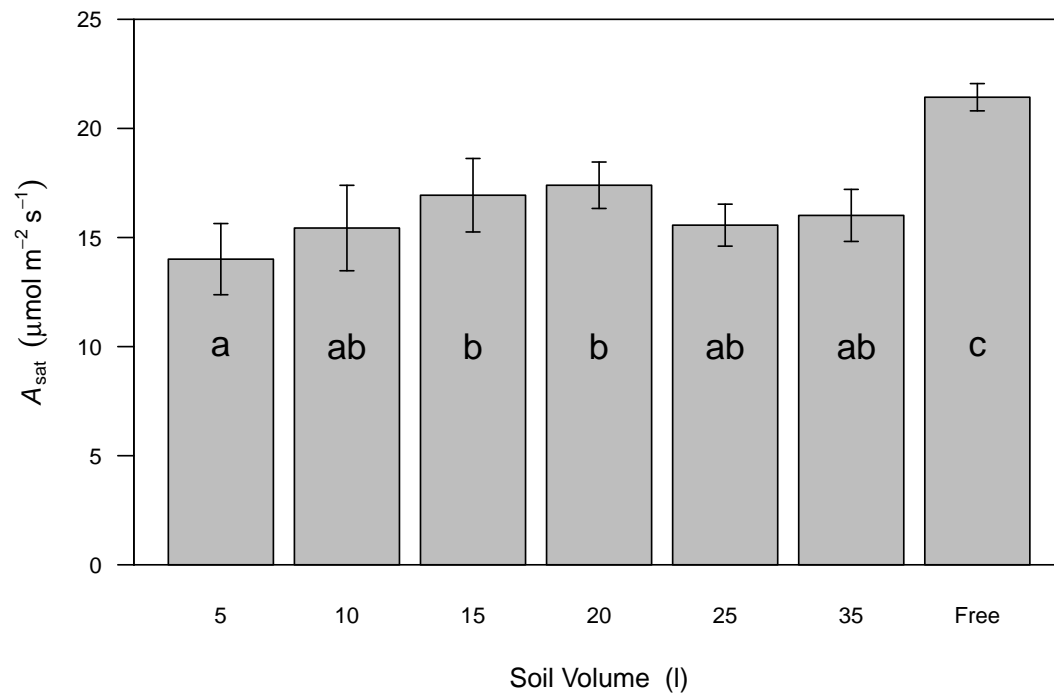


Figure 3: Soil volume treatment means \pm SE (n=7), across all measurement dates (n=6), of light saturated rates of photosynthesis at 25°C. Different letters represent significant differences between treatments.

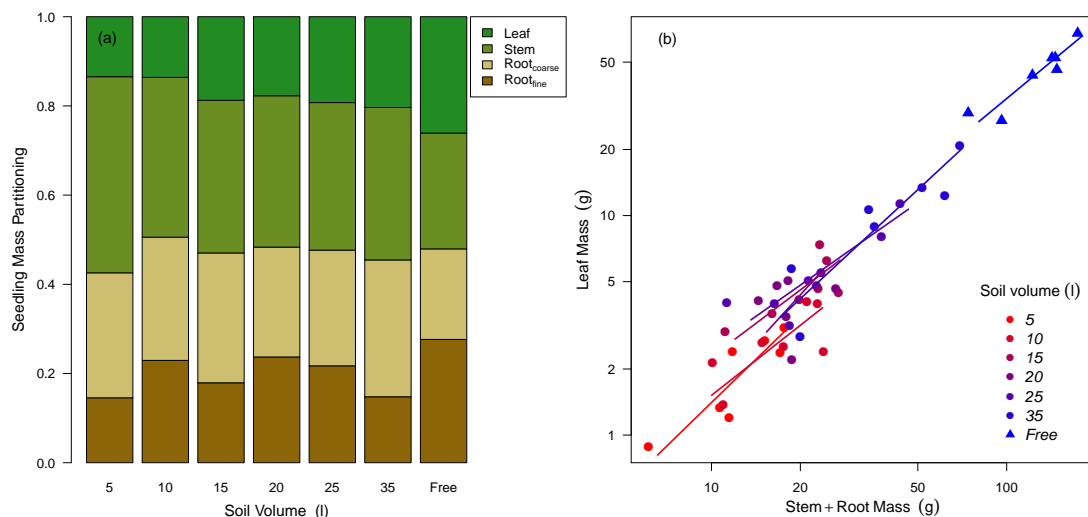


Figure 4: Soil volume treatment means (n=7) of mass partitioning to leaves, stems, and roots (a) and bi-variate relationships between mass allocation to leaves and stems + roots (b). Lines represent standardized major axis fitting of the log transformed allometric relationships of leaf mass fraction by treatment.

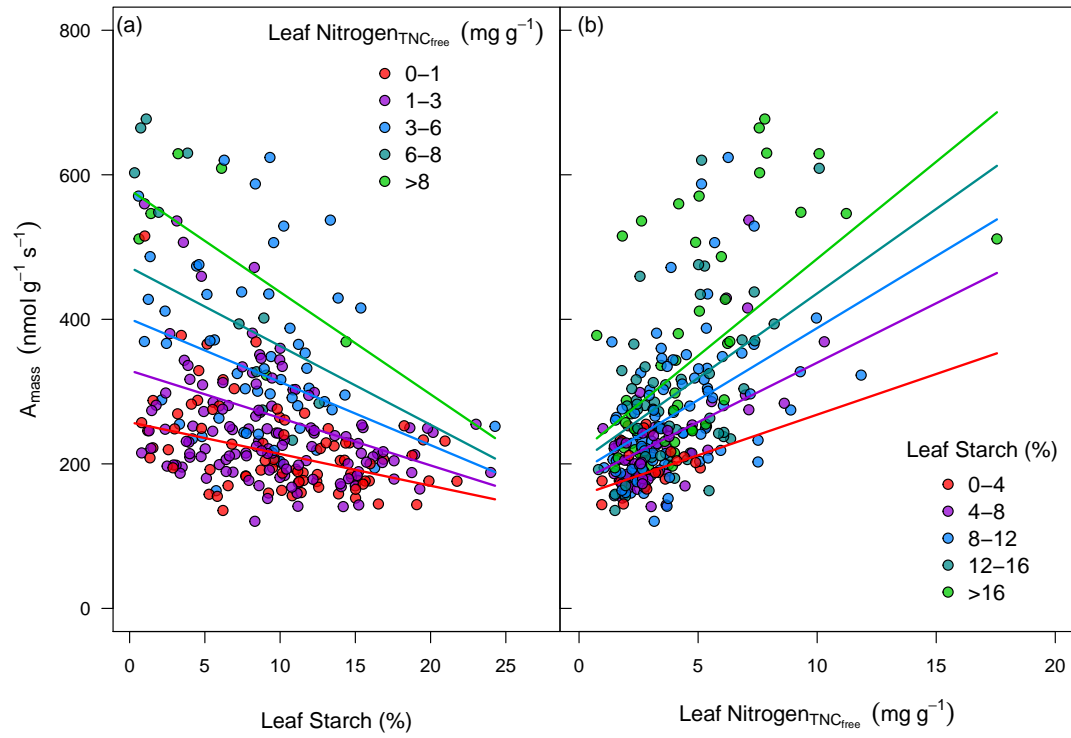


Figure 5: Photosynthetic capacity, on a leaf mass basis, as a function of accumulation of leaf starch (a) and leaf nitrogen content without TNC (b). Colors represent bins levels ($n=5$) of both leaf starch and nitrogen grouped from low to high. Lines represent predictions, for each bin level, from the linear mixed effects model equation of A_{max} as a function of starch and nitrogen. The marginal r^2 (fixed effects only) was 0.37 and the conditional r^2 (fixed and random effects) was 0.48 for the complete model.

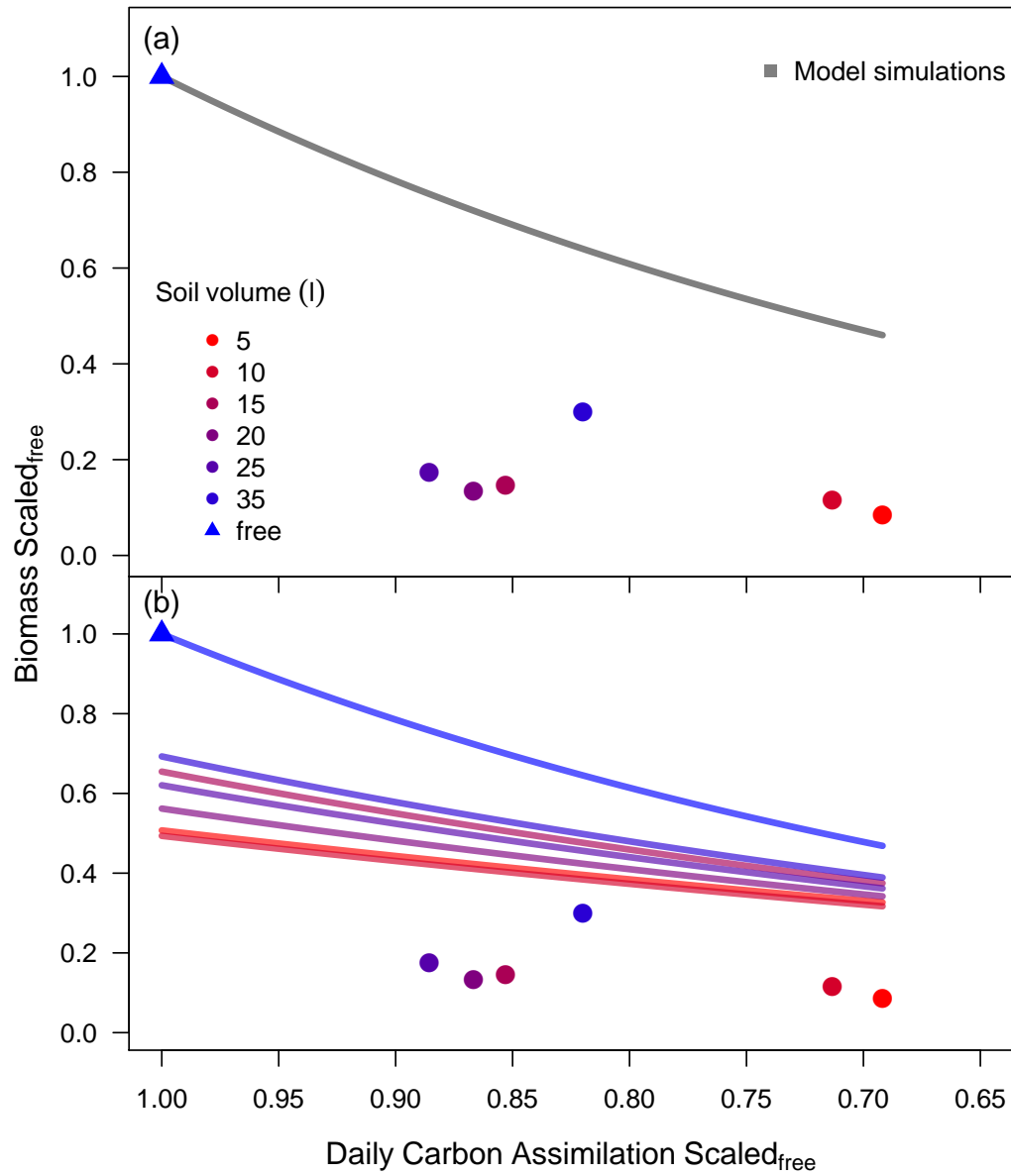


Figure 6: Modeled and harvested seedling biomass (g) versus the measure range of daily assimilated carbon gain (g m^{-2}) across all treatments (a) and then with treatment specific plant component mass partitioning included (b). Values of biomass and carbon gain are scaled to the free seedling with unlimited soil volume.

1 Supporting Information Figures

Table 3: Seedling Growth Model Default Parameters

Variable	Default Value	Units	Source
Leaf area _i	0.035	m ²	this study
Leaf mass _i	3.45	g	this study
Stem mass _i	1.51	g	this study
Root mass _i	0.99	g	this study
Y _c	.65	g C g mass	Mäkelä (1997)
R _{coarse root}	0.00124	g C g root ⁻¹	Marsden et al. (2008)
R _{fine root}	0.010368	g C g root ⁻¹	Ryan et al. (2010)
R _{stem}	0.00187	g C g stem ⁻¹	Drake 2014 (unpublished)
C _{day}	4.7-6.8	g C m ²	this study
M	0.80-0.87		this study

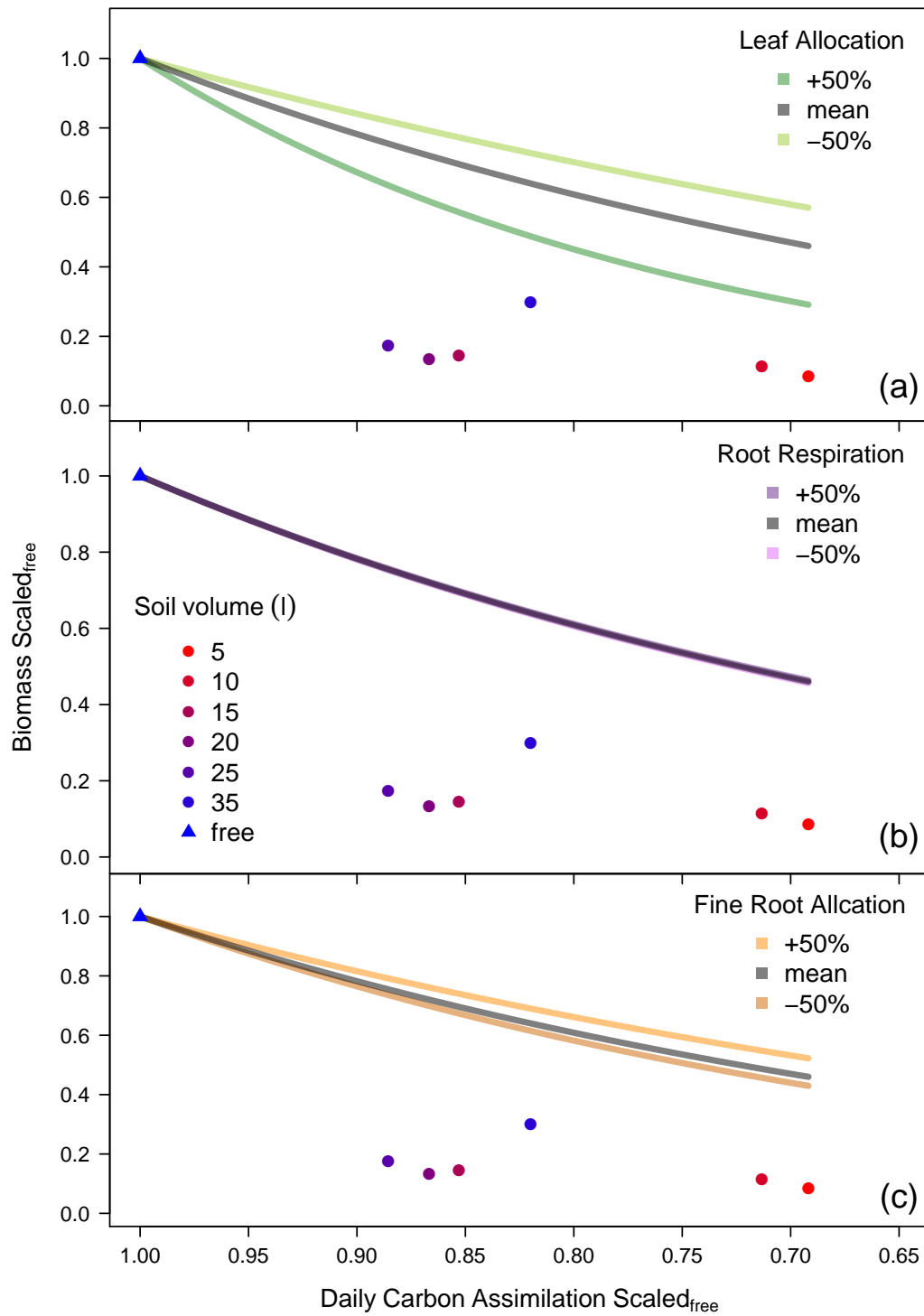


Figure S1: Modeled and harvested seedling biomass (g) versus daily assimilated carbon gain (g m^{-2}) including sensitivity testing for unmeasured carbon allocation scenarios. Model parameters of leaf allocation (a), root respiration (b), and fine root allocation (c) were increased or decreased by 50%.

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