Effects of below-ground sink limitation on growth and carbon balance of Eucalyptus seedlings

Courtney E. Campany1, Belinda Medlyn1, Remko A. Duursma1.

1 Hawkesbury Institute for the Environment, University of Western Sydney, Locked Bag 1797, Penrith, NSW, Australia

*Corresponding author*: Courtney Campany E: [courtneycampany@gmail.com](mailto:courtneycampany@gmail.com)

# Abstract

Interpreting limitations to plant growth requires understanding of the balance between carbon (C) source and sink activity in order to assess C 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, negatively impacting mass partitioning belowground. The accumulation of leaf non-structural carbohydrates, resulting from reduced sink strength, was expected to correlate to reductions in photosynthetic capacity. 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 and 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. We developed a seedling growth model that utilized leaf photosynthesis (A) rates to allocate daily C uptake towards mass growth of stems, leaves and roots. We then asked whether the observed reductions in A explained the observed differences in seedling biomass. We found that although belowground sink limitation resulted in the down regulation of A, these reductions were not significant enough to explain observed growth responses. Thus, as A and growth were not coordinated an excess pool of non-biomass C resulted in seedlings with soil volume restriction. This research highlights the need to further utilize mass balance approaches when evaluating plant C allocation and confirms that A and growth are not always directly related.

# Keywords

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

# Introduction

Understanding plant growth requires knowledge of the mass balance that must be achieved between C uptake and subsequent allocation to growth, storage, and respiration. As woody plants have highly integrated systems of competing carbohydrate sinks (Kozlowski 1992), growth should principally depend on the allocation of photosynthate among different tissues and organs. At long enough time scales photosynthesis (A) and growth must be correlated to maintain mass balance, however, at shorter temporal scales growth is not necessarily limited by the availability of recent photosynthate. This has led to the current debate over how strongly plant growth is controlled by either source of sink activity. Consequently, plant growth cannot always be simply determined by the photosynthesis rate, making it complex to understand and challenging to model (Fourcaud et al. 2008). Despite a wealth of studies large uncertainties still remain regarding the coordination of C supply and growth of woody species.

In woody species, the coordination of A and growth has been studied with manipulations of C 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 A rates (Drake et al. 1997, Ainsworth and Rogers 2007) and across four FACE experiments this resulted in a conserved increase in forest biomass production (Norby et al. 2005). Evidence from elevated CO2 experiments, however, also reveals that even with average photosynthetic enhancement of over 30 % the growth rate only increases by around 10 % (Kirschbaum 2011). In defoliation experiments, increases in A of the remaining foliage 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, Sari, et al. 1993, 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 A did not always produce increased growth due to reductions in meristem sink strength (Palacio et al. 2012), C limitation to mycorrhizal colonization (Markkola et al. 2004), or an overall decrease in whole plant C gain (Ovaska, Walls, et al. 1993). These manipulations of C source activity expose unresolved issues with how changes in A do not always infer similar responses in growth.

Alternatively, manipulating plant tissue C sinks is often used to investigate the correlation of A and growth. This is because metabolic signaling networks, relaying information on C and N status of different tissues, can down regulate photosynthetic activity (Paul and Foyer 2001). If this sink inhibition of A occurs then a close coordination between declines in A and growth should be expected. Whether this photosynthetic down regulation is evident in woody species has been tested through fruit removal, girdling, and low temperatures. In these studies, down regulation of A was frequently correlated to carbohydrate accumulation resulting from reduced tissue sink strength (Hoch et al. 2002, Iglesias et al. 2002, Urban and Alphonsout 2007, Haouari et al. 2013). However, reductions in A 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 typical field conditions for woody species.

An alternative experimental approach is to lower belowground C 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 may mimic 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, A, water relations, nutrient uptake and respiration (NeSmith and Duval 1998, and references therein). Inadequate rooting volume may decrease C sink strength by progressively restricting root growth (Thomas and Strain 1991). Container size studies frequently have photosynthetic down-regulation, likely as a result of sink limitation (Arp 1991, McConnaughay and Bazzaz 1991, Gunderson and Wullschleger 1994, Sage 1994, Maina et al. 2002, Ronchi et al. 2006). A meta-analysis by Poorter et al. (2012) concluded that A 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 C exchange and assimilate export rates (Robbins and Pharr 1988). However, evidence in support for a trade-off between C 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 A are coordinated.

This study utilizes a novel field design to investigate the coordination between growth and A 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 C mass balance approach, which was then compared to observed harvested seedling mass. The model used whole-plant C gain, scaled from instantaneous rates of leaf A, to quantify seedling production over 120 days.

Our hypotheses were as follows:  
1). The manipulations of container size were expected to induce a belowground sink limitation compared to free seedlings. We hypothesized that declines in seedling growth would be largest in the smallest containers.

2). As the finite pool of rooting volume and soil nutrients will decline faster in small containers we expected decreases in partitioning to fine root mass with decreasing container size.

3). Reduced sink strength was expected to lead to accumulation of leaf non-structural carbohydrates, which is known to inhibit A. We therefore expected a correlation between carbohydrate accumulation and photosynthetic capacity as a function of soil volume.

4). Last, the growth model was expected to find agreement between observed seedlings mass and mass predicted from a simple carbon balance model taking into account measured rates of photosynthesis.

# Materials and Methods

## Experimental design

This experiment was located at 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 N 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, 1980b). 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 35 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, used as the control, a 1 m2 subplot was excavated to 0.5 m and replaced with the same soil used in each container. 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 weekly or when needed, accounting for natural precipitation, to maintain soil moisture at field capacity (13-15 %). 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 declined a full biomass harvest was completed (May 21st 2013). Dry mass of leaves, stems, roots and cumulative leaf area (LI-3100C Area Meter; LI-COR, Lincoln, NE, USA) was measured for each seedling. Mean individual leaf area for each harvested seedling was calculated by dividing cumulative leaf area by total leaf count of only fully expanded leaves. This value was then used to interpolate cumulative leaf area through time with weekly leaf counts. Root mass was collected by passing soil from each container through a 1 mm sieve, washing, separating into fine and coarse roots (<2 mm and >2 mm diameter, respectively) and then drying to a constant mass. Roots from the free seedlings were collected by excavating each 1 m2 subplot to 0.5 m depth. 25 g fresh weight subsamples of washed fine roots were analyzed, using WhinoRhizo software (Regent Instruments Inc., Quebec, QC, Canada), for specific root length (SRL, m g-1).

## Photosynthetic parameters

Leaf gas exchange measurements were performed bi-weekly at saturating light (Asat) and saturating light and [CO2] (Amax) on new fully expanded leaves. Measurements were initiated only after sufficient new leaf growth occurred (March 17th, 2013), approximately 6 weeks following planting, and continued until the biomass harvest. Leaf level gas exchange was measured with a standard leaf chamber equipped with blue-red light emitting diodes using a portable gas exchange system (LI-6400, LI-COR, Lincoln, NE, USA). Asat measurements were made at PPFD of 1800 mol m-1 s-1 and [CO2] of 400 l l-1 and Amax with [CO2] of 1600 l l-1 and PPFD of 1800 mol photons m-1 s-1. This choice of light level to achieve light saturation is consistent with other studies on *Eucalyptus* species (Kallarackal and Somen 1997, Pinkard et al. 1998, Crous et al. 2013, Drake et al. 2014). These measurements were conducted during midday (10:00-14:00 h) with leaf temperature maintained at 25 °C. After leaves acclimated to the chamber environment, net CO2 assimilation rate and stomatal conductance (gs) were logged 5 times for both Asat and Amax.

Photosynthetic CO2 response (ACi) curves were also developed at 25 °C on a random subset of each container size (n=3) after new leaves were first produced and immediately prior to the final harvest (May 23rd 2013). Each ACi curve began at the reference [CO2] of 400 l l-1 and then consisted of 12 additional steps from [CO2] of 50 to 1800 l-1 at 25 °C at saturating light (above). From these curves the photosynthetic parameters, Jmax and Vcmax, were quantified using the biochemical model of (Farquhar et al. 1980).

Leaf dark respiration rates (Rd) was measured on each seedling during the same dates as ACi curves using detached leaves inside a conifer chamber attached to the Licor 6400 at least 1 hour after sundown. Measurements were taken at a reference [CO2] of 400 l l-1 while leaf temperature was maintained at current ambient conditions. Reported values of Rd are standardized rates at 25 °C using a Q10 value (1.86) developed for these seedlings in a separate experiment (Drake et al. unpublished). Leaf area and dry mass were recorded for each leaf during gas exchange campaigns.

## Leaf water potential

Predawn (pd) and midday (l) leaf water potentials were measured for each seedling using a PMS 1505D pressure chamber (PMS Instruments, Albany, OR, USA) on fully expanded leaves during the same time period as ACi and Rd. Leaves were detached and immediately stored inside foil covered bags before water potential measurements were performed. pd was measured before sunrise and l at midday 13:00-14:30 h. These measurements were used as a measure of static water stress on the seedlings (Sellin 1999) and to ensure that the bulk soil water availability was high enough for plants as they became larger and roots filled the soil volume.

## Leaf, root and soil chemistry

Leaves used in each gas exchange measurements and subsamples of harvested roots were dried to a constant mass and milled for analysis of N content, 13C, and total non-structural carbohydrates (TNC). Pre-planting soil samples (n=6) and subsamples of soil from each container following harvest were sieved to remove organic material, air dried and milled for analysis. Nitrogen concentrations of leaf and soil samples were determined using a Carlo Erba CE1110 elemental analyzer with thermal conductivity and mass spectromic detection (of N2 and CO2). The percentage of N in the sample was calculated by comparison with known standards. Leaf 13C was analyzed with an Delta V Advantage coupled to a Flash HT and Conflo IV isotope ratio mass spectrometer. Leaf samples were flash combusted at 1000°C to convert to CO2, feed to the mass spectrometer and isotopic signatures are reported relative to the VPDP scale.

Leaf TNC concentration was analyzed using a total starch assay kit (Megazyme International, Wicklow, Ireland) and includes the starch (mg g-1) and soluble sugar (mg g-1) concentrations. Starch was quantified using a thermostable -amylase and amyloglucosidase assay (McCleary et al. 1997) and soluble sugars were determined following the anthrone method (Ebell 1969). Complete methods of the TNC assay are described in (Mitchell et al. 2013). Specific leaf area (SLA, m2 kg-1), for leaves sampled during gas exchange campaigns, was then calculated by first subtracting the TNC content from individual dry leaf mass before dividing leaf area by leaf mass.

## Seedling growth model

We developed a simple seedling growth model that utilized leaf A rates to allocate daily C assimilate towards biomass production of stems, leaves, fine roots and coarse roots. The model begins with mean initial tissue component biomass (leafi, stemi and rooti) and a starting leaf area (LAi measured prior to planting. The initial biomass of roots was divided evenly between fine and coarse roots. The daily net biomass production of seedlings (Pi) is then given by

(1)

where L is total plant leaf area (m2), Cday,i is the predicted daily carbon assimilation (g d-1), s is a self shading parameter, c is a biomass conversion efficiency parameter and R is the mass based total respiration of all tissue components. Total respiration was calculated as

(2)

where Rc is tissue respiration of fine roots, coarse roots or stems (g C g mass-1) and Mc is the standing biomass of each component (g). Rleaf is represented in the calculation of Cday (described below). The change in individual component biomass (Mc), here solved on a daily time step, is given by

(3)

where Ac is the component specific biomass partitioning to whole plant biomass (%) and c is component specific turnover rate. Due to the duration of the experiment stem was assumed to equal 0. Total seedling biomass, per time step, was then equal to the sum of all biomass components; leaves, stems, fine roots and coarse roots.

Cday was predicted by using a coupled photosynthesis - stomatal conductance model (Farquhar et al. 1980, Medlyn et al. 2011) in the 'plantecophys' package (Duursma 2014) in R (R Development Core Team 2011) with the mean photosynthetic parameters (Jmax, Vcmax, Rd and g1) for each treatment and meteorological data from an onsite weather station. Jmax and Vcmax were estimated from ACi curves (explained above), Rd was empirically measured and the g1 parameter was generated by fitting the optimal stomatal conductance model from (Medlyn et al. 2012) with observed gs values. 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). Combined with the meteorological parameters; PPFD, air temperature, and relative humidity, at 15 min intervals, leaf A rates (mol CO2 m-2 s-1) were then predicted for each soil volume treatment. Cday was calculated by converting predicted rates to mass C gain over 15 min time steps (g m-2) and then summed for 24 h. This resulted in 120 unique values of Cday for each soil volume treatment, one value for each day of the experiment. Thus, each daily time step for model runs included a value of Cday that represented both treatment specific photosynthetic parameters and meteorological constraints across the duration of the experiment.

It was further necessary to calculate a self-shading parameter (s) when scaling leaf A with total plant 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 (fix cite YplantQMC) to build a 3D plant structure based on digitized metrics of plant allometry and crown structure. Inputting the same treatment specific physiological parameters listed above, 'YplantQMC' outputs total A, using total leaf area, for seedlings assuming self-shading as well as for a full sun large horizontal leaf. The ratio of total A with self-shading to horizontal leaf was then used to calculate s for each of the 61 digitized seedling, independently for each treatment. Next, the linear relationship between s and total leaf area was for determined across digitized seedlings, within each treatment. For the growth model, s was then predicted for each daily time step using the previous days cumulative leaf area and this value was then applied to Cday,i. All default parameters used in model simulations are reported in Table S1.

We then utilized this model to test the hypothesis that the effects of belowground sink limitation on rates of leaf A where sufficient to accurately predict overall seedling biomass production after 120 days. Each model run utilized changes in A and leaf mass fraction (LMF), with published or local values of stem and root respiration rates, to generate total seedling mass and leaf area after 120 days. Cumulative net leaf C gain for each treatment was equal to the sum of each value of Cday,i over 120 days and final seedling C was assumed to equal half of the final mass for both modeled and observed seedlings. First, a default model was optimized to produce a final LMF that correctly predicted both the final leaf mass and total biomass of the harvested free seedling controls (M0). This optimized LMF was then applied to model runs with treatment specific Cday to determine if changes in leaf A alone could accurately predict biomass (M1). Next, model sensitivity to different C allocation scenarios, including non-optimized treatment specific LMF and up regulation of non-leaf tissue respiration by 50 % of default values (M2,3, respectively), was used to improve predictions of initial model simulations from measured harvest biomass. For all cases, seedling production was compared between model output and harvested seedlings with treatment specific mean daily C assimilation by first scaling values to the free seedling control.

## 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. Tukey's post-hoc tests were performed in conjunction with ANOVA to determine which specific paired comparisons among soil volume treatments were different. Mixed model ANOVAs of Amax and leaf chemistry were performed using the 'nlme' package (Pinheiro et al. 2014) in R and R2 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 in R (Warton et al. 2012). Results were considered significant at P ≤ 0.05.

# Results

## Growth and morphology metrics

In this field study, colder temperatures and reductions in cumulative PPFD per day (Figure 1) most likely lead to the reduced growth in the free seedlings in the final weeks of the experiment (Figure 2). Combined with severe growth reductions in the smallest container volumes the experiment was chosen to be harvested after 120 days. Over this duration height, diameter, and leaf area diverged between container volumes (Figure 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 seedling 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. (2012). Additionally, plant biomass was highly correlated with total leaf area across all treatments (R2 = 0.97, P < 0.001). Differences in biomass partitioning to leaves, stems, and roots were not different across soil volumes after variation in seedling biomass within treatments was factored in the analysis (Figure3a,b). Across all treatments, the final harvested root:shoot was conserved in these seedlings, with a slightly higher shoot than root mass on average ( = 0.904, Figure 2c).

Overall, SRL was higher in seedlings in containers compared to free seedlings but not significantly in every soil volume treatment (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 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 free seedlings maintained the highest leaf N % (Table 1, P < 0.001). Leaf starch content in the smallest container was double that of free seedlings (P=0.039), while leaf soluble sugars did not differ across treatments throughout the experiment (Table 1). Differences in leaf starch between the free seedling and the smallest container were also evident during the first gas exchange campaign (P = 0.001).

## Gas exchange and photosynthetic parameters

Asat and Amax were both significantly higher in the largest container volume and the free seedling at the first measurement campaign (both P < 0.001). Across all measurement campaigns Asat (Figure 4) and Amax (Table 2.) were consistently higher in free seedlings than in containers (both P < 0.001). The interaction between photosynthetic capacity, leaf starch, and leaf N on a mass basis was marginally significant (P = 0.058) but Amax was highly correlated to both leaf N content and leaf starch (both P < 0.001). Across all measurement campaign Amax was higher when foliar N was also higher, usually associated with low levels of leaf starch (Figure 5a). Amax was also lower when leaf starch was high as higher leaf N often did not coincide with high leaf starch (Figure 5b)

The photosynthetic parameters Jmax and Vcmax were not different within treatments at the beginning and end of gas exchange campaigns, therefore the parameter means per treatment are reported here (Table 2). Overall, both Jmax and Vcmax were significantly higher in free seedlings with little variation between soil volume treatments (P = 0.001 & 0.002, respectively). Leaf dark respiration rates were not significantly different across soil volumes (Table 2). The g1 parameter, generated for each seedling from the Medlyn et al (2012) optimal stomatal conductance model, was lowest in the free seedling and was marginally different across soil volume treatments (Table 2). Predicted values of gs, using the g1 parameter, where highly correlated with observed values (R2 = .74, P < 0.001, data not shown).

Neither pd nor l were different across treatments, with mean values of -0.27 and -1.2 mPa across all seedlings, respectively. Although gs in free seedlings was generally higher than those in containers (Table 2. P=0.001), the mean rates for all seedlings were high at 0.37 mol H20 m-2 s-1 and did not decline significantly across the experiment duration. Additionally, leaf 13C at final harvest was not different across treatments (Table 1). Combined these indices provide strong evidence that water stress was not apparent on these well-watered seedlings throughout the experiment. Soil N % at harvest was not different across soil volumes ( = 04.5 %) and decreased approximately 3 % across all containers over the experiment duration. This indicates that nutrient leaching from free seedlings or from draining of containers following natural rainfall events did not differ between treatments.

## Modelling seedling biomass

Model M0, was able to converge on an optimum LMF (21.6 %) which predicted the mean harvest total biomass of free seedlings within 1.2 %. Using this optimized LMF, the total biomass of modeled seedlings with soil volume restriction (M1) was on average 23±2.4 g C more than measured seedlings when comparing against predicted total net leaf C gain (Figure 6a). Thus, seedling C mass was overestimated by an average of 50±8.7 % in modeled seedlings across the reductions in daily C assimilation per treatment (Figure 6b). As a result, the reductions in leaf A were not sufficient enough to explain the reduction in harvested seedling biomass with soil volume restriction. This resulted in an unexplained pool of excess C, generated from A, which was not allocated to biomass.

The remaining model simulations tested possible C allocation scenarios to account for this excess pool of C. Constraining the model by treatment-specific LMF from the final harvest (M2) still overestimated seedling total C by 32±11.1 %, but provided the most improved model predictions (Figure S1a). Using harvested LMF, however, does not capture the increase in senescence of seedlings in small containers (Figure 2c). Thus, the 7-61 % reduction in measured harvested leaf mass fraction from large to small soil volumes compared to the optimized LMF (M0) represents an underestimation of realized leaf C allocation. Increases of 50 % in non-leaf tissue respiration (M3) improved biomass estimates slightly but overestimated mass C by an average of 46±9.3 % in seedlings with soil volume restriction (Figure S1b). It was further determined that non-tissue respiration rates in modeled seedlings would need to increase by ca. 250 % to account for this entire pool of C.

# Discussion

This study utilized a simple but novel field design to manipulate belowground sink limitation and physically restrict *Eucalyptus tereticornis* seedling production. We then addressed questions regarding the coordination of A and growth by complementing empirical results with modelling approaches. We found that reductions in leaf A rates were not sufficient to explain observed reductions in total plant biomass production. We thus encourage the utilization of mass balance approaches and provide direction for future studies when testing factors that control plant growth under environmental change.

## Reductions in growth and physiology under sink limitation

As soon as seedlings became established both height and diameter growth were negatively affected by decreasing soil volume. This lead to the large reductions in harvested biomass in small containers when compared to free seedlings. These growth reductions were expected, as the impedance of root growth can cause reductions overall plant growth and activity (McConnaughay and Bazzaz 1991, Young et al. 1997). It has been shown that roots undergoing difficult conditions may send inhibitory signals to the shoots that affect gs, cell expansion, cell division and the rate of leaf appearance (Passioura 2002). Here, this was evident in a large divergence in leaf area between seedlings in containers and free seedlings through time, with the eventual cessation of new leaf growth in seedlings in small containers.

Decreases in Asat paralleled the reductions in allometric growth parameters of seedlings in containers. This initially suggests a strong link between growth and an apparent down regulation of A. However, there are several possible mechanisms that can explain reduced A in small pots including nutrient content, water or reduced sink strength (Poorter, Bühler, et al. 2012). It was therefore necessary to examine each of these factors to distinguish if the induced belowground sink limitation actually triggered photosynthetic down regulation.

With high rates of gs, non-limiting leaf water potential and consistent leaf 13C across soil volume treatments there was little evidence of water stress inducing a limitation to A. This finding is consistent with other container size studies without drought treatments. For example, reduced Amax in cotton seedlings grown at elevated CO2 was attributed to sink-limited feedback inhibition from inadequate rooting volume, not decreased gs (Thomas and Strain 1991). Additionally, severe reductions in A in coffee plants was not attributed to impacts of container size on leaf water potentials or gs (Ronchi et al. 2006). It is likely that reductions in A of these well watered seedlings was instead the result of limiting soil nutrients on belowground sink strength.

Although the soil N pool declined evenly across all treatments leaf N was lowest in the smallest containers, suggesting sink limitation was the greatest in these containers. This makes sense as small containers may reduce N uptake, either from physical root restriction or decreased supply, which affects growth, Rubisco limitation, sugar metabolism, and carbohydrate partitioning between source and sink tissues (Stitt 1991, Hermans et al. 2006). Mycorrhizal colonization could also have been affected in containers. Whether unrestricted mycorrhizal recruitment facilitated the increase in leaf N uptake in free seedlings is unknown. Regardless of whether sink limitation occurred from root or mycorrhizal restriction, the stark contrast in leaf N between free seedlings and seedlings in containers was significantly correlated to reductions in A.

As both rooting space and resources were finite in containers, the inability of seedlings to increase the capacity of the belowground C sink resulted in the buildup of C assimilate in leaves. The feedback inhibition of A from starch accumulation has been proposed, yet it is still not known whether there is a starch threshold that triggers the down-regulation process (Nebauer et al. 2011). Here, declines in Amax were correlated with higher starch content throughout the experiment. This agrees with findings from Equiza et a. (2006) where photosynthetic downregulation from reduced sink strength was more correlated with starch than sugar content in a deciduous conifer. As starch content in the smallest containers was nearly double that of free seedlings this suggests the response of A to sink inhibition was regulated by this accumulation, as hypothesized.

## Mass partitioning under sink limitation

As mass partitioning is likely controlled by the source and sink strength of all organs (Poorter, Niklas, et al. 2012) it was important to determine what tissue components were most affected by the sink limitation. It was necessary to distinguish if growth was affected beyond ontogenetic constraints, by correcting for size, as biomass distribution is strongly size-dependent (Gould 1966, Lleonart et al. 2000). In this study, there were no significant changes in root, leaf, or stem mass fractions to reduced soil volume compared to free seedlings, outside of ontogenetic drift. This is significant as shifts in allocation have been noted specifically for nutrient limitation (McConnaughay and Coleman 1999, and references therein). Surprisingly, fine root to leaf mass ratio was conserved across all treatments suggesting a functional partitioning response to optimize resource gain did not occur.

As partitioning to fine roots did not change this provides evidence against an optimal foraging strategy for seedlings in containers. This could be because lateral root development is affected by inanimate obstacles and avoiding growth towards container walls could improve the efficiency of resource allocation (Falik et al. 2005). Also, the sensitivity of roots to their own exudates near obstructions may be used to adjust growth before stressful conditions occur (Semchenko et al. 2008). Alternatively, physical restriction of root proliferation could have impacted root development and morphology prior to shifts in mass partitioning. Here, increases in root length where detected in several of the soil volume treatments . This is not surprising as plants in containers have been shown to have different root morphology to field grown plants as roots essentially compete with themselves for nutrients (NeSmith and Duval 1998). Poor soil quality and root restriction, however, likely decreased the capacity of this morphological response to increase N uptake.

## Do reductions in photosynthesis explain reductions in seedling growth?

Our growth model used a simple but conventional approach to drive seedling growth with fluctuations in A while treating carbon use efficiency, respiration and carbon allocation as fixed processes. Contrary to expectation, the model consistently overestimated seedling growth in containers when parameterized with an optimized LMF. This provides further evidence that links between sink limitation and A do not necessarily imply the same coordination between reduced A and growth. These findings are important as this model reflects classical approaches in tree growth and production modelling. Our results indicate a need to evaluate the use of fixed processes in models which distinguish the fate of assimilate C within a plant. Doing so will provide valuable input to future models as assimilate allocation is a key component in functional-structural tree models, yet C partitioning remains a weak point (Lacointe 2000). To address this issue we utilized the flexibility of this model to test plausible fates of the extra pool of non-biomass carbon unaccounted for with mass balance. Similar to Lohier et al. (2014) we manipulated processes contributing to seedling C mass balance, including changes to leaf C allocation or non-leaf tissue respiration, to quantitatively test their respective influences on model predictions.

Altering the LMF from that of the optimized seedling controls (M2) improved biomass predictions and provided insight into how sink limitation can impact leaf C allocation beyond A. The observed sub-optimal decrease LMF with increasing soil volume restriction has several possible explanations. Shifts in LMF could represent changes in senescence or leaf production that could not be explicitly quantified in this field study. As TNC accumulation can lead to accelerated leaf senescence (Paul and Foyer 2001), this could explain the large declines in total leaf area of seedlings in small containers. Alternatively, seedlings in containers could have reduced leaf production to maintain laws of stoichiometry (leaf C:N ratio). The two-fold decrease in leaf N % in small containers from free seedlings provides support to this possible scenario. Future empirical and modelling studies should focus on how feedbacks from sink activity affect both rates of A and the fate of C allocated to leaves. It will be the interactions between these two components that will determine the total C gain available for plant growth.

Increasing rates of non-leaf respiration (M3) improved biomass predictions but to a far lesser extent than changes to leaf C allocation. This is still noteworthy as it shows that sink limitation could differentially affect respiration of component tissues. Additionally, keeping rates of respiration fixed in growth models may underestimate impacts of environmental change on tissue respiration above and belowground. The fraction of photosynthate used in respiration varies substantially among species and environments and is sensitive to changes in growth rates (Lambers et al. 2008). As C balance is a delicate equilibrium between fluxes of A and respiration, partial accounting of C dynamics can easily lead to erroneous conclusions (Valentini et al. 2000).We agree with Delucia et al. (2007) that it is likely inappropriate to assume that respiration is a constant fraction of gross primary production in models. Our findings reveal that a combination of different mechanisms, beyond A, is likely at play in driving the observed seedling biomass response to sink manipulation. However, the degree to which these mechanisms will regulate a growth will undoubtedly shift across different experimental manipulations and plant species.

## Conclusions

It has long been know that conditions which affect the photosynthetic process affect growth but that there is also an interrelationship between growth and A (Sweet and Wareing 1966). Here, the fate of assimilated C available for plant growth varied between naturally sown seedlings and seedlings with belowground sink inhibition. First, this is important as manipulations of plants grown in containers are often used to draw conclusions about growth and physiological principles but how these results actually reflect field-grown plants has seldom been studied. Second, our finding add more evidence that A and growth are not always entirely synced, an important distinction often missed in studies that manipulate source/sink activity. Korner (2013) suggests that it is the norm for sink activity to feedback onto source activity, causing growth to control A through the demand for C. Although this may be true, we argue that attempts to quantify or at least predict the fate of assimilated C into known pools of growth, storage and C loss are needed prior to addressing this debate. Our modelling results agree with conclusions from Valentine and Mäkelä (2005) where the problem with predicting tree growth is a problem in forecasting the assimilation and allocation of C and other constituents. A lack of knowledge regarding C allocation restricts our ability to achieve mass balance and is a major obstacle in understanding the coordination between A and growth. The approach used here has the flexibility to account for multiple drivers of C allocation and provides an avenue to address future questions regarding the impact of environmental change on plant growth.

# List of Tables

**Table 1**. Responses of plant and leaf characteristics of *Eucalyptus tereticornis* seedlings to soil volume treatments. Each value reflects the mean(standard error) for each treatment. Seedling mass, SRL, root nitrogen and leaf ^13C values are from final harvest. Values of leaf starch, sugars, nitrogen and SLA represent overall means across measurement campaigns (n=6). Different letters represent significant differences between treatments. The container effect P value represents the overall difference between seedlings with soil volume restriction and the control seedlings.

**Table 2**. Responses of leaf level gas exchange parameters of *Eucalyptus tereticornis* seedlings to soil volume treatments. Each value reflects the mean(standard error) for each treatment. Units for Amax and Rdark are mol m-2 s-1 and gs are mol m-2 s-1, each at at 25 °C. Values of Amax, gs and g1 represent overall means across measurement campaigns (n = 6). Rdark, Jmax and Vcmax values are means of two measurement campaigns at beginning and end of gas exchange measurements. Different letters represent significant differences between treatments. The container effect P value represents the overall difference between seedlings with soil volume restriction and the control seedlings.

**Table S1**. Seedling growth model default parameters.

# List of Figures

**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 ± standard error 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 of mass partitioning to leaves, stems, and roots at harvest (a), bi-variate relationships between mass allocation to leaves and stems + roots (b) and leaf mass as a function of fine root biomass with ± standard error (c). For (b) lines represent standardized major axis fitting of the log transformed allometric relationships of leaf mass fraction by treatment. For (c) the dashed line is the 1:1 relationship and the solid line represents the significant linear model fit (R2=0.81).

**Figure 4**. Soil volume treatment means ± standard error, across all measurement campaigns (n=6), of light saturated rates of photosynthesis at 25°C. Different letters represent significant differences between treatments.

**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 represents predictions, for each bin level, from the linear mixed effects model equation of Amax as a function of starch and nitrogen. The marginal R2 (fixed effects only) was 0.37 and the conditional R2 (fixed and random effects) was 0.48 for the complete model.

**Figure 6**. Total carbon mass for harvested and modeled seedlings versus predicted total carbon gain after 120 days (a) and reductions in final seedling carbon mass, both modeled and observed, as a function of the reduction in leaf photosynthesis across treatments (b). For (a) the dashed 1:1 identifies the difference between net total leaf carbon gain and gross seedling production. For (b) both seedling carbon mass and daily carbon assimilation were first scaled to the free seedling control.

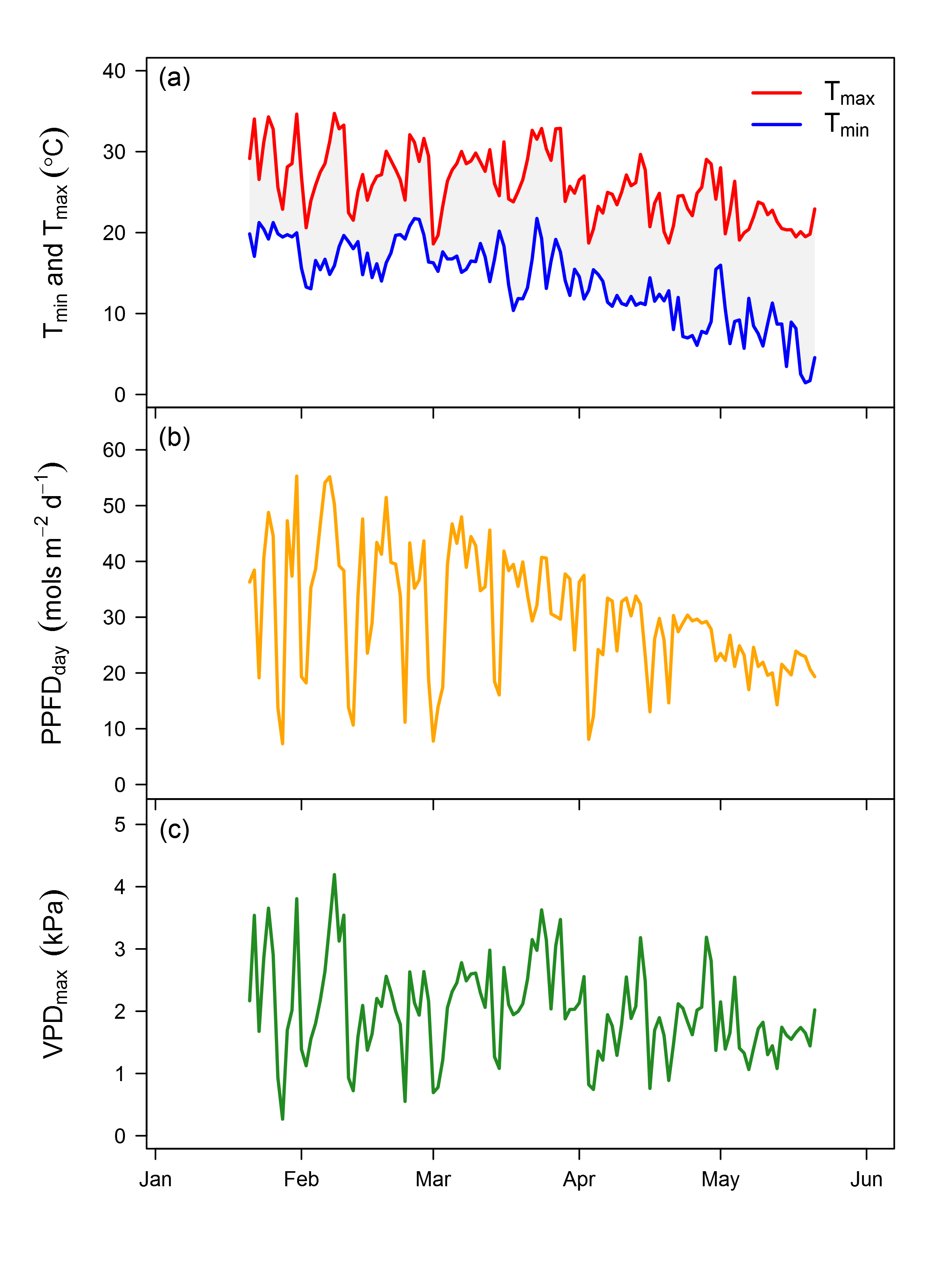
**Figure S1**. Sensitivity testing of seedling growth model to different carbon allocation strategies including; constraints of leaf mass fraction to treatment specific final harvest values (a) and increases in respiration of non-leaf tissue components by 50 % (b). Open and filled symbols represent default model and harvest values, while shaded symbols represent model sensitivity to each scenario by soil volume treatment. Both seedling carbon mass and daily carbon assimilation were first scaled to the free seedling control.

# Tables

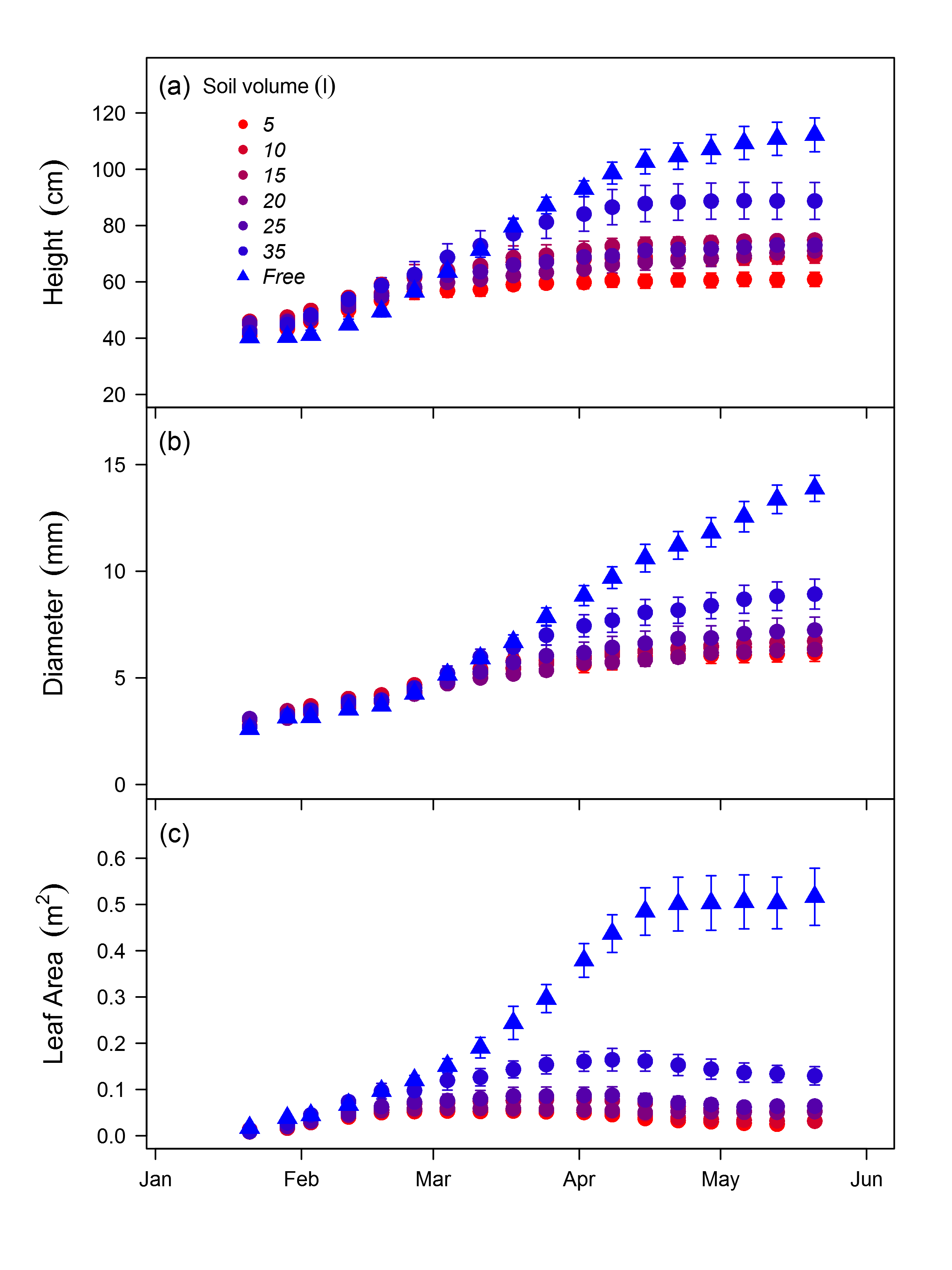
**Table 1**.

**Table 2**.

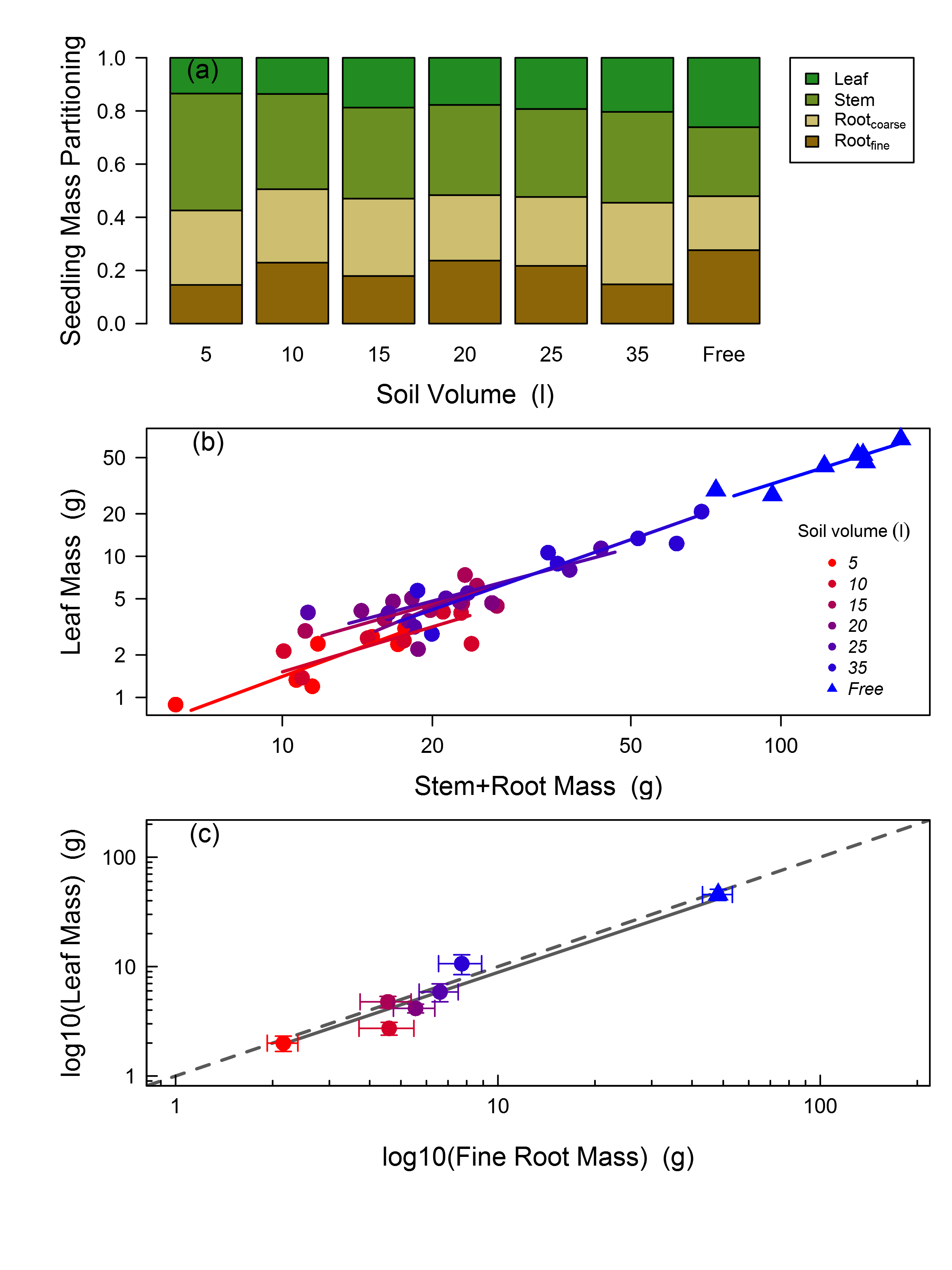
# Figures



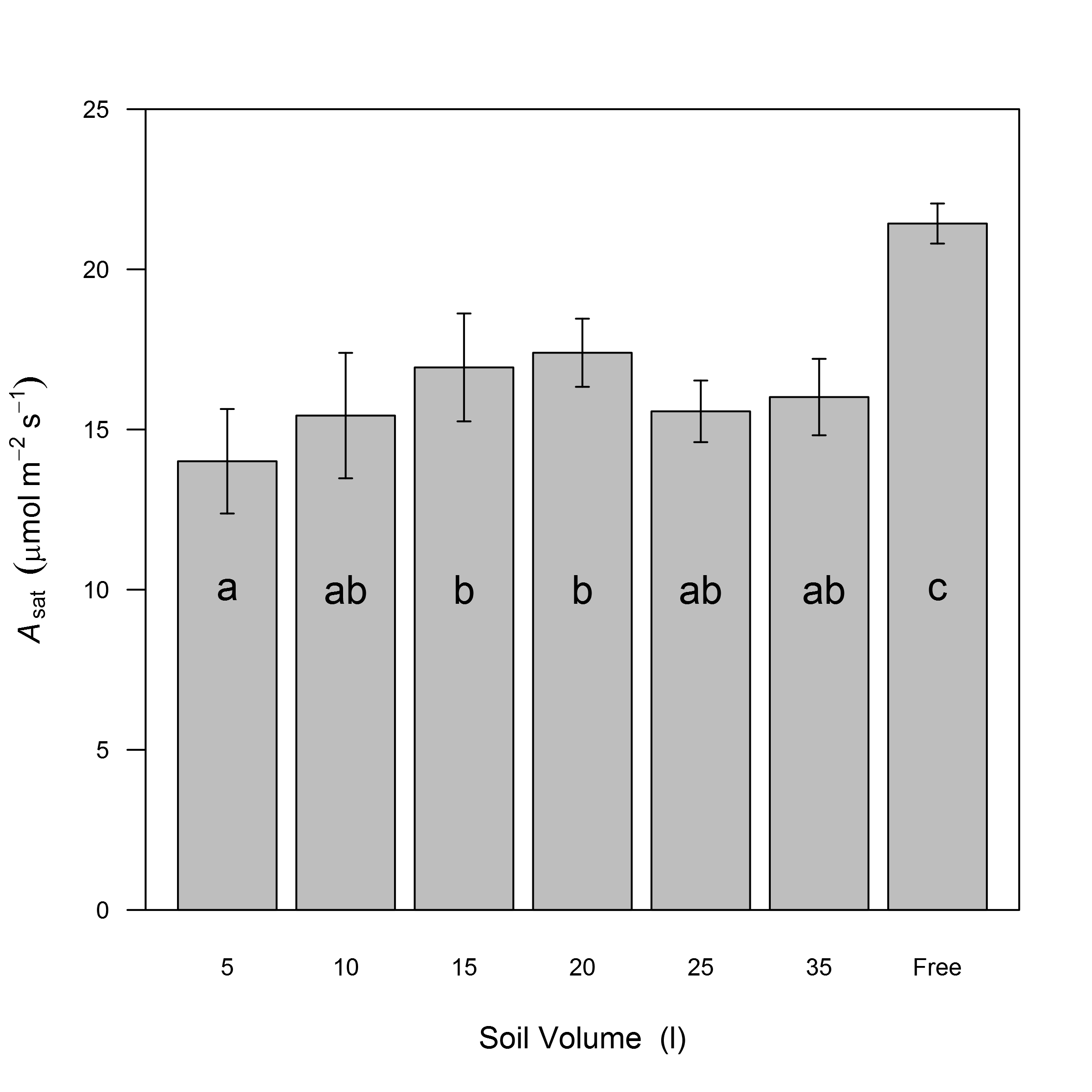
**Figure 1**.



**Figure 2**.

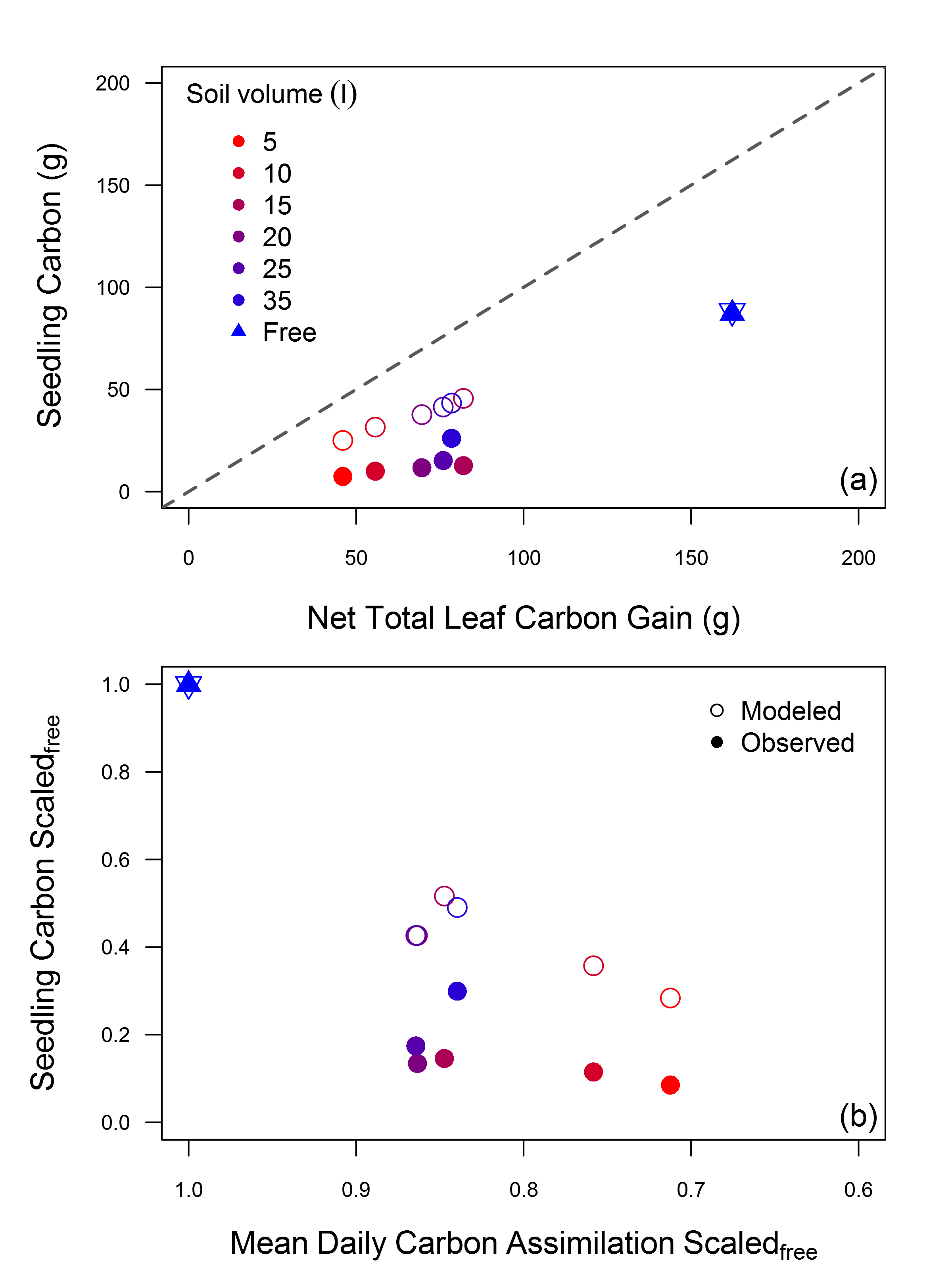


**Figure 3**.



**Figure 4**.

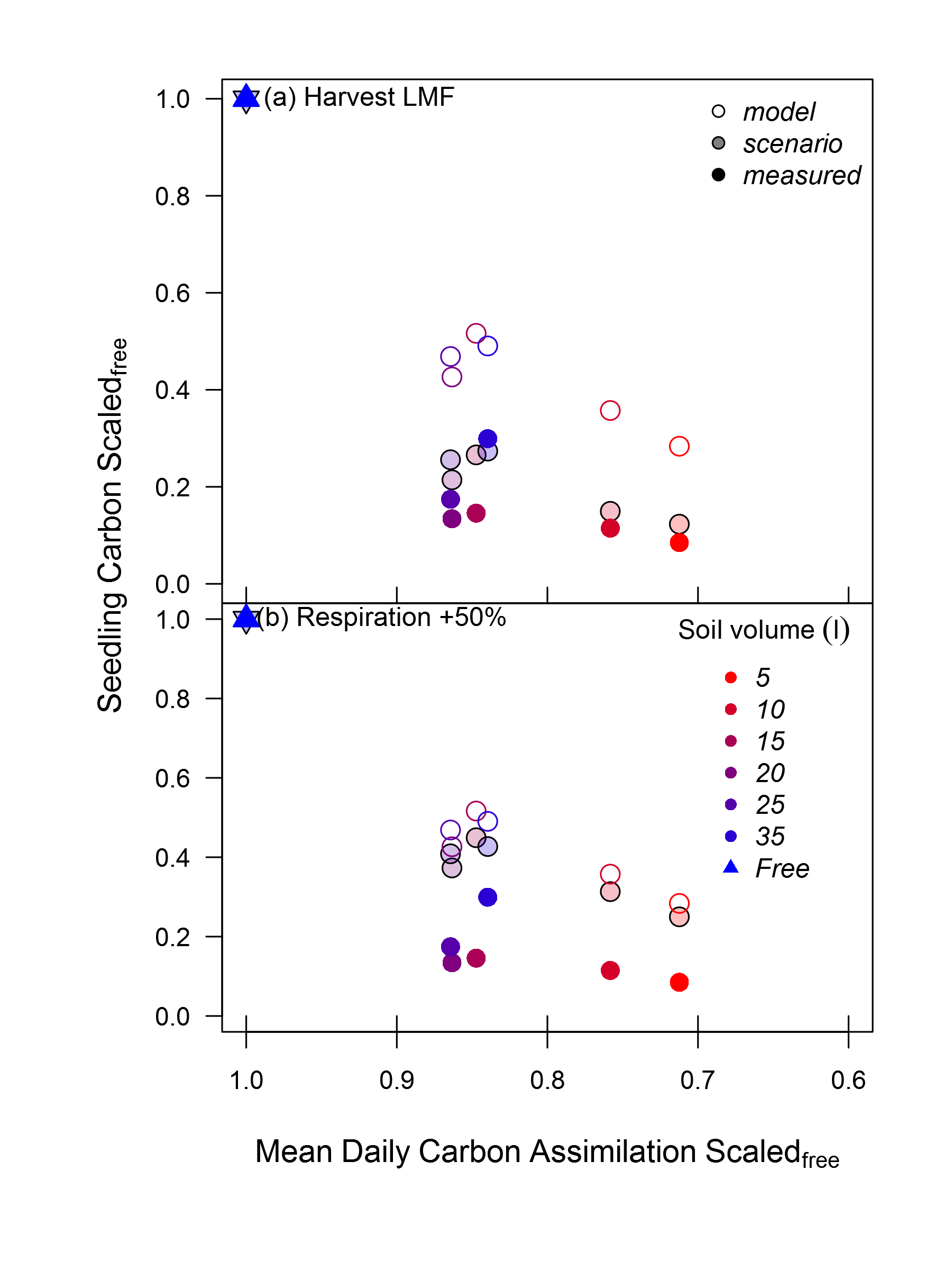
**Figure 5**.



**Figure 6**.

# Supporting Information

**Table S1**.



**Figure S1**.

# References

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO2. New Phytologist 165:351–372.

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO2]: mechanisms and environmental interactions. Plant, cell & environment 30:258–270.

Arp WJ (1991) Effects of source-sink relations on photosynthetic acclimation to elevated CO2. Plant, Cell & Environment 14:869–875.

Biran I, Eliassaf A (1980a) The effect of container size and aeration conditions on growth of roots and canopy of woody plants. Scientia Horticulturae 12:385–394.

Biran I, Eliassaf A (1980b) The effect of container shape on the development of roots and canopy of woody plants. Scientia Horticulturae 12:183–193.

Crous KY, Quentin AG, Lin Y-S, Medlyn BE, Williams DG, Barton CVM, Ellsworth DS (2013) Photosynthesis of temperate Eucalyptus globulus trees outside their native range has limited adjustment to elevated CO and climate warming. Global change biology 19:3790–3807.

DeLucia E, Drake JE, Thomas RB, Gonzalez-Meler M (2007) Forest carbon use efficiency: is respiration a constant fraction of gross primary production? Global Change Biology 13:1157–1167.

Drake JE, Aspinwall MJ, Pfautsch S, Rymer PD, Reich PB, Smith RA, Crous KY, Tissue DT, Ghannoum O, Tjoelker MG (2014) The capacity to cope with climate warming declines from temperate to tropical latitudes in two widely distributed Eucalyptus species. Global change biology

Drake BG, Gonzàlez-Meler MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO2? Annual review of plant biology 48:609–639.

Duan W, Fan PG, Wang LJ, Li WD, Yan ST, Li SH (2008) Photosynthetic response to low sink demand after fruit removal in relation to photoinhibition and photoprotection in peach trees. Tree physiology 28:123–132.

Duursma R (2014) plantecophys: Modelling and analysis of leaf gas exchange data.

Duursma RA, Barton CVM, Lin Y-S, Medlyn BE, Eamus D, Tissue DT, Ellsworth DS, McMurtrie RE (2014) The peaked response of transpiration rate to vapour pressure deficit in field conditions can be explained by the temperature optimum of photosynthesis. Agricultural and Forest Meteorology 189:2–10.

Duursma RA, Falster DS, Valladares F, Sterck FJ, Pearcy RW, Lusk CH, Sendall KM, Nordenstahl M, Houter NC, Atwell BJ, Others (2012) Light interception efficiency explained by two simple variables: a test using a diversity of small-to medium-sized woody plants. New Phytologist 193:397–408.

Ebell LF (1969) Variation in total soluble sugars of conifer tissues with method of analysis. Phytochemistry 8:227–233.

Eyles A, Pinkard EA, Davies NW, Corkrey R, Churchill K, O’Grady AP, Sands P, Mohammed C (2013) Whole-plant versus leaf-level regulation of photosynthetic responses after partial defoliation in Eucalyptus globulus saplings. Journal of experimental botany 64:1625–1636.

Falik O, Reides P, Gersani M, Novoplansky A (2005) Root navigation by self inhibition. Plant, Cell & Environment 28:562–569.

Farquhar GD, Caemmerer S von von, Berry JA (1980) A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. Planta 149:78–90.

Fourcaud T, Zhang X, Stokes A, Lambers H, Körner C (2008) Plant growth modelling and applications: the increasing importance of plant architecture in growth models. Annals of Botany 101:1053–1063.

Gould SJ (1966) Allometry and size in ontogeny and phylogeny. Biol Rev 41:587–640.

Gunderson CA, Wullschleger SD (1994) Photosynthetic acclimation in trees to rising atmospheric CO2: a broader perspective. Photosynthesis research 39:369–388.

Handa IT, Körner C, Hättenschwiler S (2005) A test of the treeline carbon limitation hypothesis by in situ CO2 enrichment and defoliation. Ecology 86:1288–1300.

Haouari A, Van Labeke M-C, Steppe K, Mariem FB, Braham M, Chaieb M (2013) Fruit thinning affects photosynthetic activity, carbohydrate levels, and shoot and fruit development of olive trees grown under semiarid conditions. Functional Plant Biology 40:1179–1186.

Hermans C, Hammond JP, White PJ, Verbruggen N (2006) How do plants respond to nutrient shortage by biomass allocation? Trends in plant science 11:610–617.

Hoch G, Popp M, Körner C (2002) Altitudinal increase of mobile carbon pools in Pinus cembra suggests sink limitation of growth at the Swiss treeline. Oikos 98:361–374.

Iglesias DJ, Lliso I, Tadeo FR, Talon M (2002) Regulation of photosynthesis through source: sink imbalance in citrus is mediated by carbohydrate content in leaves. Physiologia Plantarum 116:563–572.

Kallarackal J, Somen CK (1997) An ecophysiological evaluation of the suitability of Eucalyptus grandis for planting in the tropics. Forest Ecology and Management 95:53–61.

Kirschbaum MUF (2011) Does enhanced photosynthesis enhance growth? Lessons learned from CO2 enrichment studies. Plant Physiology 155:117–124.

Kozlowski TT (1992) Carbohydrate sources and sinks in woody plants. The Botanical Review 58:107–222.

Körner C (2013) Growth controls photosynthesis–mostly. Nova Acta Leopoldina 114:273–283.

Lacointe A (2000) Carbon allocation among tree organs: a review of basic processes and representation in functional-structural tree models. Annals of Forest Science 57:521–533.

Lambers H, Chapin FS, Pons TL (2008) Plant physiological ecology, 2nd edn. Springer New York, New York. <http://dx.doi.org/10.1007/978-0-387-78341-3\_5>

Layne DR, Flore JA (1995) End-product inhibition of photosynthesis in Prunus cerasus L. in response to whole-plant source-sink manipulation. Journal of the American Society for Horticultural Science 120:583–599.

Li WD, Li SH, Yang SH, Yang JM, Zheng XB, Li XD, Yao HM (2005) Photosynthesis in response to sink-source manipulations during different phenological stages of fruit development in peach trees: regulation by stomatal aperture and leaf temperature. Journal of horticultural science & biotechnology 80:481–487.

Lleonart J, Salat J, Torres GJ (2000) Removing allometric effects of body size in morphological analysis. Journal of Theoretical Biology 205:85–93.

Lohier T, Jabot F, Meziane D, Shipley B, Reich PB, Deffuant G (2014) Explaining ontogenetic shifts in root–shoot scaling with transient dynamics. Annals of botany:mcu128.

Maina GG, Brown JS, Gersani M (2002) Intra-plant versus inter-plant root competition in beans: avoidance, resource matching or tragedy of the commons. Plant Ecology 160:235–247.

Markkola A, Kuikka K, Rautio P, Härmä E, Roitto M, Tuomi J (2004) Defoliation increases carbon limitation in ectomycorrhizal symbiosis of Betula pubescens. Oecologia 140:234–240.

McCleary BV, Gibson TS, Mugford DC (1997) Measurement of total starch in cereal products by amyloglucosidase--amylase method: Collaborative study. Journal of AOAC International 80:571–579.

McConnaughay KDM, Bazzaz FA (1991) Is physical space a soil resource? Ecology:94–103.

McConnaughay KDM, Coleman JS (1999) Biomass allocation in plants: ontogeny or optimality? A test along three resource gradients. Ecology 80:2581–2593.

Medlyn BE, Dreyer E, Ellsworth D, Forstreuter M, Harley PC, Kirschbaum MUF, Le Roux X, Montpied P, Strassemeyer J, Walcroft A, Others (2002) Temperature response of parameters of a biochemically based model of photosynthesis. II. A review of experimental data. Plant, Cell & Environment 25:1167–1179.

Medlyn BE, Duursma RA, Eamus D, Ellsworth DS, Colin Prentice I, Barton CVM, Crous KY, Angelis P, Freeman M, Wingate L (2012) Reconciling the optimal and empirical approaches to modelling stomatal conductance. Global Change Biology 18:3476.

Medlyn BE, Duursma RA, Eamus D, Ellsworth DS, Prentice IC, Barton CVM, Crous KY, Angelis P de, Freeman M, Wingate L (2011) Reconciling the optimal and empirical approaches to modelling stomatal conductance. Global Change Biology 17:2134–2144.

Mitchell PJ, O’Grady AP, Tissue DT, White DA, Ottenschlaeger ML, Pinkard EA (2013) Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. New Phytologist 197:862–872.

Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4:133–142.

Nebauer SG, Renau-Morata B, Guardiola JL, Molina R-V (2011) Photosynthesis down-regulation precedes carbohydrate accumulation under sink limitation in Citrus. Tree Physiology 431:169–177.

NeSmith DS, Duval JR (1998) The effect of container size. HortTechnology 8:495–498.

Norby RJ, DeLucia EH, Gielen B, Calfapietra C, Giardina CP, King JS, Ledford J, McCarthy HR, Moore DJP, Ceulemans R, Others (2005) Forest response to elevated CO2 is conserved across a broad range of productivity. Proceedings of the National Academy of Sciences of the United States of America 102:18052–18056.

Ovaska J, Sari R, Rintamäki E, Vapaavuori E (1993) Combined effects of partial defoliation and nutrient availability on cloned Betula pendula saplings II. Changes in net photosynthesis and related biochemical properties. Journal of Experimental Botany 44:1395–1402.

Ovaska J, Walls M, Vapaavuori E (1993) Combined effects of partial defoliation and nutrient availability on cloned Betula pendula saplings I. Changes in growth , partitioning and nitrogen uptake. Journal of Experimental Botany 44:1385–1393.

Palacio S, Hernández R, Maestro-Martínez M, Camarero JJ (2012) Fast replenishment of initial carbon stores after defoliation by the pine processionary moth and its relationship to the re-growth ability of trees. Trees 26:1627–1640.

Palacio S, Hoch G, Sala A, Körner C, Millard P (2014) Does carbon storage limit tree growth? New Phytologist 201:1096–1100.

Passioura JB (2002) Soil conditions and plant growth. Plant, cell & environment 25:311–318.

Paul MJ, Foyer CH (2001) Sink regulation of photosynthesis. Journal of experimental botany 52:1383–1400.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2014) {nlme}: Linear and Nonlinear Mixed Effects Models. <http://cran.r-project.org/package=nlme>

Pinkard EA, Beadle CL, Davidson NJ, Battaglia M (1998) Photosynthetic responses of Eucalyptus nitens (Deane and Maiden) Maiden to green pruning. Trees 12:119–129.

Poorter H, Bühler J, Dusschoten D van, Climent J, Postma JA (2012) Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. Functional Plant Biology 39:839–850.

Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytologist 182:565–588.

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytologist 193:30–50.

R Development Core Team R (2011) R: A Language and Environment for Statistical Computing Team RDC (ed). R foundation for statistical computing 1:409. <http://www.r-project.org>

Robbins NS, Pharr DM (1988) Effect of restricted root growth on carbohydrate metabolism and whole plant growth of Cucumis sativus L. Plant physiology 87:409–413.

Ronchi CP, DaMatta FM, Batista KD, Moraes GABK, Loureiro ME, Ducatti C (2006) Growth and photosynthetic down-regulation in Coffea arabica in response to restricted root volume. Functional Plant Biology 33:1013–1023.

Sage RF (1994) Acclimation of photosynthesis to increasing atmospheric CO2: the gas exchange perspective. Photosynthesis research 39:351–368.

Sellin A (1999) Does pre-dawn water potential reflect conditions of equilibrium in plant and soil water status? Acta Oecologica 20:51–59.

Semchenko M, Zobel K, Heinemeyer A, Hutchings MJ (2008) Foraging for space and avoidance of physical obstructions by plant roots: a comparative study of grasses from contrasting habitats. New Phytologist 179:1162–1170.

Smith AM, Stitt M (2007) Coordination of carbon supply and plant growth. Plant, cell & environment 30:1126–1149.

Stitt M (1991) Rising CO2 levels and their potential significance for carbon flow in photosynthetic cells. Plant, Cell & Environment 14:741–762.

Sweet GB, Wareing PF (1966) Role of plant growth in regulating photosynthesis. Nature 210:77–79.

Thomas RB, Strain BR (1991) Root restriction as a factor in photosynthetic acclimation of cotton seedlings grown in elevated carbon dioxide. Plant Physiology 96:627–634.

Turnbull TL, Adams MA, Warren CR (2007) Increased photosynthesis following partial defoliation of field-grown Eucalyptus globulus seedlings is not caused by increased leaf nitrogen. Tree Physiology 27:1481–1492.

Urban L, Alphonsout L (2007) Girdling decreases photosynthetic electron fluxes and induces sustained photoprotection in mango leaves. Tree Physiology 27:345–352.

Valentine HT, Mäkelä A (2005) Bridging process-based and empirical approaches to modeling tree growth. Tree Physiology 25:769–779.

Warton DI, Duursma RA, Falster DS, Taskinen S (2012) smatr 3–an R package for estimation and inference about allometric lines. Methods in Ecology and Evolution 3:257–259.

Young IM, Montagu K, Conroy J, Bengough AG (1997) Mechanical impedance of root growth directly reduces leaf elongation rates of cereals. New Phytologist 135:613–619.

Zhou R, Quebedeaux B (2003) Changes in photosynthesis and carbohydrate metabolism in mature apple leaves in response to whole plant source-sink manipulation. Journal of the American Society for Horticultural Science 128:113–119.