

Effects of belowground space limitation on performance of Eucalyptus seedlings: Does photosynthesis really control growth?

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Abstract

Negative feedbacks to photosynthesis are commonly interpreted as the primary factor responsible for growth limitation in plants under stress. 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 freely rooted 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 allocation 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 weak. We developed a simple seedling growth model that utilized leaf photosynthesis rates to allocate daily carbon 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 was not sufficient and the inclusion of mass allocation patterns and additional carbon pools were necessary to predict the observed growth response to increasing soil volume. Overall, we found limited evidence for sink-limitation of photosynthesis by constraining seedling growth in containers, and argue that shifts in carbon allocation are necessary to explain the experimental results. This research highlights the need to further understand adaptive strategies of plant carbon allocation, and confirms that photosynthesis and growth are not always directly related.

Key Words

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

Introduction

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 N and P 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 & Eliassaf, 1980b; Biran & Eliassaf, 1980a). 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. Each seedling was given an Imidacloprid (BAYER CropScience) insecticide tablet planted 5 cm below the roots. Containers were planted flush with the soil surface inside metal sleeves, designed to minimize excess air space between the container and outside soil while allowing container removal. This allowed for soil temperatures in containers to reflect conditions of naturally sown 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. A border of root exclusion material was buried 0.25 m deep and extended 0.25 m above the ground surface 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 collect 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 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 5th 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.), for specific root length (SRL, m m⁻¹).

Photosynthetic parameters

Leaf gas exchange measurements were performed bi-weekly at saturating light (A_{sat}) and saturating light and $[\text{CO}_2]$ (A_{max}) on new fully expanded leaves. Measurements were initiated only after 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). A_{sat} measurements were made at PPFD of 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. This choice of light level to achieve light saturation is consistent with other studies on *Eucalyptus* species (Kallarackal & 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 CO_2 assimilation rate and stomatal conductance were logged 5 times for both A_{sat} and A_{max} . Photosynthetic CO_2 response (AC_i) curves were also constructed 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). For each AC_i curve the reference $[\text{CO}_2]$ was set as follows: 400, 50, 100, 150, 230, 330, 400, 650, 900, 1200, 1500, 1600, and 1800 $\mu\text{l l}^{-1}$. From these curves the photosynthetic parameters, J_{max} and V_{cmax} , were quantified using the leaf photosynthesis model by Farquhar *et al.* (1980) within the PlantEcophys package in R v. 3.0.1 (Duursma, 2014; R Development Core Team, 2011). Leaf dark respiration rates (R_d) was measured on each seedling during the same dates as AC_i 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 $[\text{CO}_2]$ of 400 $\mu\text{l l}^{-1}$ while leaf temperature was maintained at current ambient conditions. Specific leaf area (SLA, m² g⁻¹) was calculated by measuring leaf area and dry mass for all leaves sampled during gas exchange campaigns.

Leaf water potential

Predawn (Ψ_{pd}) and midday (Ψ_l) 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 AC_i and (R_d). Ψ_{pd} and Ψ_l leaves were sampled and immediately stored inside foil covered bags and then transported to the laboratory for measurements of water potential. Ψ collections were completed before sunrise and 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 measurement and subsamples of harvested fine roots were dried to a constant mass and milled for analysis of N and C content. 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. C and N concentrations of milled samples were determined using a Carlo Erba CE1110 elemental analyzer with thermal conductivity and mass spectrometric detection (of N² and CO₂). The percentage of N and C in the sample was calculated by comparison with known standards. Leaf total non-structural carbohydrates concentration (TNC) 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.*, n.d.) and soluble sugars were determined following the anthrone method /citeebell1969variation. Full methods of the TNC assay are described in (Mitchell *et al.*, 2013).

Seedling growth model

Statistics

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 plant chemistry were performed using the lme4 and nlme packages (Pinheiro *et al.*, 2014) in R and r² values of mixed models were computed as in 'citenakagawa2013general. Tests of allometric relationships between biomass components were implemented using major axis regression in the smatr package (Warton *et al.*, 2012).

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