# Abstract

Plants must utilize external resources including light, CO2, water and mineral nutrients to fuel photosynthetic carbon gain. This photoassimiliate is then allocated within the plant as the essential C resource for growth, maintenance and storage. Theory and observation suggest that C allocation and leaf physiology are optimized to maintain functional balance for external resource capture and to maximize C gain. However, the impacts of a changing climate may disrupt the proposed balance of C allocation between above and belowground pools. Variation in resource distribution and leaf physiology within tree canopies is also not fully understood, thus all canopy leaves may not follow theories of leaf optimal behaviour. These unanswered questions regarding C uptake and fate of assimilated C inhibit our ability to precisely test the coordination between canopy A and growth. Therefore, this PhD research was designed to investigate how strongly resource allocation in Eucalyptus species, including external plant resources and photosynthetic C assimilate, are correlated to whole tree biomass growth. My goal was to measure aspects of resource allocation and C uptake across different scales, from leaf to whole tree, to generate a greater physiological understanding of the processes which define tree growth and the sensitivity of these processes to changing environments.

Firstly, I determined whether manipulations of soil volume would limit growth in *Eucalyptus tereticornis* seedlings by disrupting the balance between source and sink activity. Seedlings were grown in a large range of container sizes and planted containers flush to the soil alongside naturally sown seedlings. Aboveground growth of seedlings in containers was negatively affected soon after the experiment started. Despite large reductions in growth across soil volume treatments, dry mass partitioning to leaves, stems and roots was similar for all seedlings after 120 days. Leaf photosynthetic capacity decreased in containers compared to free seedlings, and was correlated to both leaf nitrogen content and starch accumulation. Although belowground sink limitation resulted in down regulation of A, a mass balance seedling growth model concluded that these reductions were not large enough to explain observed growth responses. As A and growth were not tightly coordinated, excess photosynthetic C not attributed to biomass resulted in seedlings with soil volume restriction. Quantifying the fate of this excess C will be essential in evaluating feedbacks between sink strength and leaf C uptake in future studies.

Secondly, I investigated how light gradients within *Eucalyptus tereticornis* tree canopies affect the distribution of plant resources that define photosynthetic capacity of sun and shade leaves. Additionally, I measured the capacity of shade leaves to adjust their physiological behaviour and increase C uptake when sunflecks occur. Trees were grown in climate controlled whole tree chambers under prevailing ambient and warmed (+3 °C) treatments and leaf gas exchange was coupled with online carbon isotope discrimination to measure photosynthesis (A), stomatal conductance (gs) and mesophyll conductance (gm) of sun and shade leaves. Photosynthesis rates were reduced by ca. 40 % in shade leaves associated with a 75 % reduction in photosynthetically active radiation compared to sun leaves. Photosynthetic capacity (ca. 20 % lower Vcmax and Jmax) and leaf nitrogen were also lower in shade leaves than sun leaves however, similar rates of gs were observed between leaf types. Leaf Ci, estimated from both leaf 13C and gas exchange, was higher in shade leaves than sun leaves. Here, the optimization theory hypothesis that Ci should be optimized throughout the canopy was rejected because shade leaves used more water with lower rates of A, as compared to sun leaves. When light intensity was increased from low light to high light for shade leaves both gs and gm increased rapidly, leading to increases in A greater than sun leaves at the same high light environment. This rapid response of gm with light enables shade leaves to respond quickly to sunflecks and represents a new mechanism underpinning leaf gas exchange responses to light. These findings reveal that plant resources within a canopy may be distributed to utilize sunflecks and the dynamic physiological responses of shade leaves to altered light environments must be accounted for when upscaling leaf level measurements to predict whole canopy carbon gain.

Lastly, I examined how whole tree C flux correlated to tree biomass growth and whether elevated [CO2] and drought treatments altered C allocation patterns above or belowground in *Eucalyptus saligna* trees. Trees were grown in climate-controlled whole tree chambers (WTCs) over a period of two years with interacting treatments of two [CO2] (380 ppm and 620 ppm) and two watering regimes (well-watered and a four month drought). Additionally, we utilized a novel approach to calculated total belowground C allocation (TBCA) for each WTC as the residual between the aboveground net CO2 flux and aboveground C mass. Measured cumulative aboveground net CO2 flux correlated positively to whole tree C mass production and leaf area over the final eleven months of the experiment. Contrary to previous studies, biomass partitioning to roots and cumulative TBCA were unaffected by either elevated CO2 or drought treatments. As a fraction of total aboveground net C flux, TBCA was found to remain relatively stable across daily time steps for all trees. Increases in C allocation to leaves were detected in elevated CO2 treatments, while the effects of a 4 month drought were negligible on C allocation in aboveground tissues. These results reveal how climate change factors impact the investment of photosynthetic C in a *Eucalyptus* tree species and provide evidence that belowground processes may not be as sensitive to global change factors as previously thought.

In conclusion, this PhD research addressed essential questions regarding the

# Chapter 1 General Introduction

## Overview

### Resource allocation in plants

Plants need to extract resources including light, CO2, water and mineral nutrients to support growth, reproduction and fitness. To accomplish this each plant must concentrate resources from dilute surroundings, which requires energy, appropriate tissues for uptake and a transport system to deliver resources to their required destination (Grace 1997). The uptake of nutrients from roots is necessary for leaf growth and leaves then fix the carbon, via photosynthesis, required for growth of the entire plant. This assimilated C from source leaves, in the form of simple sugars, then becomes the essential C resource that must be allocated to the growth and maintenance of tissues or as a storage pool of carbohydrates. As a result, growth is driven by several simultaneous processes, including photosynthesis, C investment among organs, resource acquisition and metabolic costs (Körner 2006, Fourcaud et al. 2008).

### Resource allocation theories

Theoretically, growth will be maximized when investment into the acquisition of any single resource from the environment leads to an equivalent increase in growth (Bloom et al. 1985). The two critical assumptions of allocation theory are that the resource in question is in fixed supply and that allocation among competing functions is mutually exclusive (Bazzaz et al. 2000). Thus, trade-offs between different tissue C sinks will exist as resources are invested within the plant. Allocation of newly acquired resources to different tissues will then affect the subsequent rates of capture of CO2 and soil resources (Shipley and Meziane 2002). In resource saturated environment plants should maximize growth by allocating resources to leaves to increase C acquisition (Monsi and Saeki 2005) source availability, however, is rarely saturated in natural ecosystems. As a result, differential allocation of nutrients and assimilated C to different tissue or ecosystem components is commonly observed in plant species.

Differential patterns of assimilate allocation within plants has led to two main theories regarding resource allocation strategies. First, the balanced growth hypothesis suggests that individual plant tissues should provide a ‘balanced internal economy’ as each component supplies resources for the other (Davidson 1969). This functional equilibrium between tissues can then be adaptive if conditions limit A or soil nutrient uptake (Cannell et al. 1985). A balanced growth strategy infers that a plant needs to allocate resources to the organ that is harvesting the resource most limiting growth (Shipley and Meziane 2002). Alternatively, differential resource allocation in plant tissue is theorized to be a function of allometric trajectories of plant development related to plant size, independent of changes in nutrient supply (Müller et al. 2000). In this strategy, investment into leaf mass increases with both stems and root, but not proportionally, as a greater allocation of biomass to the stem exists as a simple function of plant size (Zens and Webb 2002). In this strategy, plants are more likely to adjust tissue morphology, chemistry, metabolism or turnover to alter resource uptake or loss, instead of adjusting allocation to biomass components (Reich et al. 2002).

### Allocation of external resources

Incident PPFD declines exponentially with cumulative leaf area index, creating a steep light gradient within tree canopies (Monsi and Saeki 2005) and changing irradiance strongly affects rates of photosynthesis (Evans 1995). As costs and limitations of light harvesting prevent plants from exposing all leaves to full sun (Niinemets 2010), it follows that a substantial portion of canopy C assimilation should occur in leaves with the highest exposure to light. Consequently, the distributions of resources required for A are partially defined by these canopy light gradients. As the photosynthetic capacity of leaves is related to its N content (Field and Mooney 1986) a larger investment in N to the upper canopy should yield a larger return from whole-canopy C assimilation (Ellsworth and Reich 1993). The supply of water also imposes limits photosynthetic C gain through direct limitations of leaf level physiology. The stomatal resistance to CO2 uptake is a function of the balance between transpiration losses and leaf water potentials (Farquhar and Sharkey 1982) and sun leaves frequently experience greater water limitations in the upper canopy [Sellin et al. (2008); niinemets2012optimization]. Thus, photosynthetic N investment in the upper canopy will be ineffective in enhancing A if water supply is insufficient (Niinemets 2012, Peltoniemi et al. 2012). Overall, the allocation of soil resources within the canopy constrains leaf physiology and photosynthetic capacity, thereby limiting the efficiency of light and CO2 uptake within the canopy.

### Fate of assimilated C

Carbon allocation represents the fraction of net primary productivity distributed to different tissue and ecosystem components above and belowground. In trees, C allocation encompasses investment into tissue biomass production as well as fluxes including respiration, exudation and turnover rates (Litton et al. 2007). A shift in C allocation to any of these components likely represents an adaptive response of trees when environmental conditions change. The fate of this assimilated C is also regulated by the delicate balance between source leaf activity and the C sink strength of the different biomass pools. For example, the sensitivity of C source and sink activities to water and nutrient controls could lead to an imbalance between C supply and C used for tissue growth and respiration (Fatichi et al. 2014). Additionally, imbalances between source and sink activity can led to investment into carbohydrates synthesis as a transient C storage sink (Paul and Foyer 2001). When shifts in carbohydrate storage, tissue respiration or turnover rates occur rates of C assimilation may not necessarily reflect biomass production at a given time point (Rocha et al. 2006, Litton et al. 2007, Gough et al. 2008). This makes separating of the investment of C between biomass production and other flux components vital when assessing temporal and spatial patterns of tree growth.

### Tree C balance with mass balance approaches

As woody plants have highly integrated systems of competing carbohydrate sinks, growth should principally depend on the allocation of leaf C assimilate among different sink organs (Kozlowski 1992, Lacointe 2000). Due to the law of conservation of mass, the fates of this assimilate are theoretically able to be tracked across scales from leaf C uptake to their eventual destination in above and belowground pools. Gaining an understanding of these sensitivity of these processes are to changing environments is crucial for predicting future terrestrial C cycling [friedlingstein1999toward], as there is currently little consensus on how C allocation should be modelled (Franklin et al. 2012, De Kauwe et al. 2014). These mass balance principles also allow the coordination between canopy C assimilation and whole tree growth to be investigated over long time scales. Success of this approach, however, depends on the ability to account for within canopy variation when estimating net canopy C gain (De Pury and Farquhar 1997, Linderson et al. 2012) or to empirically measure whole tree flux (Medhurst et al. 2006, Barton et al. 2010). Although mass balance approaches can be used to quantitatively asses tree C allocation, so far few studies have been able to provide direct empirical measurements of C allocation among component pools (Klein and Hoch 2015).

### Eucalpytus tree species as model for research

Research on Eucalyptus trees is ecologically important for Australia as it is the most dominant tree genus in Australia (Boland et al. 2006). Eucalyptus forests are the continent’s most common forest type (92 million hectares), covering about three-quarters of Australia’s native forests and occurring in all but the continent’s driest regions (Australia’s State of the Forests Report 2013). Eucalyptus tree species are also economically important in worldwide as they are commonly cultivated in plantations due to fast growth. Despite the fact that only a few Eucalypt species have natural ranges outside continental Australia (Pryor and Johnson 1981), Eucalyptus species are grown in plantations in more than 90 countries (Booth 2013). This is because Eucalyptus species have been shown to exhibit both adaptive plasticity and genetic specialisation to spatial variation in climate (Byrne et al. 2013). Currently, the global plantation area of Eucalypts totals nearly 20 million hectares, accounting for around 15% of the world’s total plantation forests (International Union of Forestry Research Organizations 2015). Consequently, this iconic Australian tree species is an excellent model to investigate strategies of resource allocation in trees facing global climate change.

## Current Knowledge Gaps

### Carbon allocation (trees, resource availability, climate change)

The distribution of assimilated C is a primary determinant of plant growth (Friedlingstein et al. 1999), yet our knowledge of the mechanisms by which allocation is regulated is poor (Poorter et al. 2012). A key issue with drawing generalized conclusions about the plasticity of C allocation in trees is with the inconsistency in terminology used to define C allocation to specific tissue or ecosystem components (Litton et al. 2007.). As changes in resource availability can lead to changes in C allocation among tissue components, it is essential to differentiate shifts in biomass production from other potential fates of assimilated C (respiration, exudation, storage and turnover). Biomass distribution should not be confused with the allocation of newly fixed photosynthates to different organ systems, as the observed biomass distribution at any time points is the cumulative result of dynamic carbon allocation over time including loss through respiration and turnover (Poorter et al. 2015). As C allocation integrates all of these processes, it is extremely difficult using current methods to assess C allocation in whole trees. Disentangling the effects of resource supply on shifts in C allocation is often tested in plants across “snapshots” in time, which should be done with caution (Reich et al. 2002). This is because of plants have developmental shifts in biomass partitioning, independent of resource supply, as they age (**???**). Additionally, the supply of light and soil resources fluctuate continuously, which makes an equilibrium with allocation at any given time point highly unlikely (Shipley and Meziane 2002).

The allocation of photosynthetic C above and belowground is an important factor in terrestrial C cycling yet our knowledge of how global change impacts C allocation is incomplete (Litton et al. 2007, Warren et al. 2012). As accurately measuring tree C allocation remains a difficult task, especially belowground, drawing conclusions which are applicable to whole plants or ecosystems are challenging. Currently, the representation of C allocation lags behind photosynthesis (A) in applied forest models (Friedlingstein et al. 1999, Franklin et al. 2012, Iversen and Norby 2014). This inefficiency in representing C allocation accurately in trees is of major concern due to the potential for forest ecosystems to sequester C in a changing climate. This deficiency needs to be filled with more empirical data measuring patterns of tree C allocation under multi-factor global change manipulations. However, this will require novel experiments and approaches to better quantify changes in C allocation above and belowground moving forward.

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