# This document contains all the text from thesis to generate a master reference file

Plants must utilize external resources including light, CO2, water and mineral nutrients to support photosynthetic carbon (C) gain. This photoassimilate is then allocated within the plant as the essential C resource for growth, maintenance and storage. Theory and observations 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 behavior. 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 improve physiological understanding of the processes which define tree growth and the sensitivity of these processes to changing environments.

First, 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 flush to the soil alongside naturally planted seedlings ('free'). Aboveground growth of seedlings in containers was negatively affected compared to free seedlings soon after the experiment started. Despite large reductions in growth across soil volume treatments, relative partitioning of mass 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 (N) content and starch accumulation. Although belowground sink limitation resulted in a reduction of A, a mass balance model concluded that these reductions were not large enough to explain observed growth responses. As A and growth were not tightly coordinated, the model predicted excess photosynthetic C not attributed to biomass in potted seedlings. Quantifying the fate of this excess C will be essential in evaluating feed-backs between sink strength and leaf C uptake in future studies.

Second, I investigated how light gradients within *Eucalyptus tereticornis* tree canopies affect the distribution of resources that define photosynthetic capacity of sun and shade leaves. 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 C isotope discrimination to measure 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 N were also lower in shade leaves than sun leaves however, gs was similar. 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 water use efficiency was lower in shade leaves, 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. This capacity of shade leaves to adjust their physiological behavior and increase C uptake when sunflecks occur likely plays significant role in whole tree C uptake for some tree species. 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 up-scaling leaf level measurements to predict whole canopy C gain.

Finally, 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 calculate 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, cumulative TBCA was unaffected by either elevated CO2 or drought treatments. As a fraction of total aboveground net C flux, TBCA was also 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 diverse questions regarding resource allocation in *Eucalyptus* tree species by linking leaf physiology to whole canopy C gain and allocation of photosynthetic C to whole tree growth. This study confirms that the distribution of photosynthetic resources constrain canopy C uptake, yet within canopy leaf physiology does not follow prevailing optimal theory. Results from this work reveal how quantifying the fate of photosynthetic C among tissue and ecosystem pools, beyond biomass production, is imperative to accurately assess the impacts of environmental change on tree productivity. This research offers critical empirical data needed to refine process based models which predict canopy C gain from rates of A and forest growth models where C allocation is represented. Ultimately, this work contributes valuable information regarding the physiological and growth responses of iconic *Eucalyptus* tree species essential for reconciling the impacts of resource availability and global climate change on fragile Australian ecosystems and the productivity of *Eucalyptus* plantation forests.

*Resource allocation in plants*

Plants need to extract resources including light, CO2, water and mineral nutrients to support growth and reproduction. To accomplish this requires energy, appropriate tissues for uptake and a transport system to deliver resources to their required destination (**???**). The uptake of nutrients from roots is necessary for leaf growth. Leaves then fix the C, via net photosynthesis (A), required for growth of the entire plant. This assimilated C from source leaves, in the form of simple sugars, is in itself an essential C resource that must be allocated to the growth and maintenance of tissues or is diverted to a storage pool of carbohydrates. As a result, growth is driven by several simultaneous processes, including A, C investment among organs, resource acquisition and metabolic costs (**???**, **???**). Gaining an understanding of the sensitivity of these processes to environmental change is crucial for predicting future terrestrial C cycling (**???**), as there is currently little consensus on how C allocation should be modeled (**???**, **???**).

*Resource allocation theory*

Theoretically, growth under resouce limitation will be maximized when investment into the acquisition of any single resource from the environment leads to an equivalent increase in growth (**???**). 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 (**???**). 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 subsequent rates of capture of CO2 and soil resources (**???**). In resource saturated environments plants should maximize growth by allocating resources to support leaf growth to increase C acquisition (**???**). Resource availability, however, is rarely saturated in natural ecosystems. As a result, shifts in allocation of external resources and assimilated C to different tissue or ecosystem components can occur.

Shifts in resource allocation within plants have led to two main theories regarding 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 (**???**). This functional equilibrium between tissues can then be adaptive if conditions limit A or soil nutrient uptake (**???**), such that plants should allocate resources to the organ that is capturing the resource most limiting growth (**???**). Changes in plant resource allocation is also theorized to be a function of allometric trajectories of plant development related to plant size, independent of changes in nutrient supply (**???**). 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 (**???**). When constrained by ontogeny, plants are more likely to adjust tissue morphology, chemistry, metabolism or turnover to alter resource capture (**???**).

*Tree canopy resource gradients*

Incident PPFD declines exponentially with cumulative leaf area index from the top of the tree downward, creating steep light gradients within tree canopies (**???**). A responds strongly and non-linearly to irradiance (**???**). As costs and limitations of light harvesting prevent plants from exposing all leaves to full sun (**???**), 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 canopy light gradients. As the photosynthetic capacity of leaves is related to its N content (**???**), a larger investment in N to the upper canopy should yield a larger return from whole canopy C assimilation (**???**). The supply of water also imposes limits photosynthetic C gain through direct limitations on leaf level physiology. The stomatal resistance to CO2 uptake is a function of the balance between transpiration losses and leaf water potentials (**???**) and sun leaves frequently experience greater water limitations in the upper canopy (**???**, **???**). Thus, photosynthetic N investment in the upper canopy will be ineffective in enhancing A if water supply is insufficient (**???**, **???**). Overall, the allocation of soil resources within the canopy constrains leaf physiology and photosynthetic capacity, thereby regulating the efficiency of CO2 uptake.

*Fate of assimilated carbon*

Carbon allocation represents the fraction of net primary productivity distributed to different tissue components above and belowground. In trees, C allocation encompasses investment into tissue biomass production as well as fluxes including respiration, exudation and turnover rates (**???**). The fate of this assimilated C is regulated by the delicate balance between leaf C uptake (source) and the C sink strength of the different biomass pools. For example, the sensitivity of C source and sink activities to water and nutrient availability could lead to an imbalance between C supply and C used for tissue growth and respiration (**???**). Additionally, imbalances between source and sink activity can lead to investment into carbohydrates synthesis as a transient C storage sink (**???**).  
As woody plants have competing tissue carbohydrate sinks, growth should principally depend on the allocation of leaf C assimilate among different sink organs (**???**, **???**). In response to changing environnmental conditions, however, trees may adaptively shift tissue C allocation to balance growth, storage and C loss. Due to conservation of mass, it is theoretically possible to track the fates of this assimilate from leaf C uptake to their eventual destination in above and belowground pools. Although mass balance approaches can be used to quantitatively asses tree C allocation, few studies so far have been able to provide direct empirical measurements of C allocation among component pools (**???**).

*Eucalyptus tree species as model for research*

Research on *Eucalyptus* trees is ecologically important for Australia as it is the most dominant tree genus (**???**). *Eucalyptus* forests are the continent’s most common forest type, covering about three-quarters of Australia’s native forests (92 million hectares) and occurring in all but the continent’s driest regions (**???**). *Eucalyptus* tree species are also economically important globally as they are commonly cultivated in plantations due to fast growth. Despite the fact that only a few *Eucalyptus* species have natural ranges outside continental Australia (**???**), *Eucalyptus* trees are grown in plantations in over 90 countries (**???**). This is because *Eucalyptus* species have been shown to exhibit both adaptive plasticity and genetic specialization to spatial variation in climate (**???**). Currently, the global plantation area of eucalypts totals nearly 20 million hectares, accounting for around 15 % of the world’s total plantation forests (**???**). 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

*Resource allocation in trees*

The distribution of assimilated C is a primary determinant of plant growth (**???**), yet our knowledge of the mechanisms by which allocation is regulated is poor (**???**). 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 (**???**). Biomass partitioning should not be confused with the allocation of newly fixed photosynthates to different organs, as the measured biomass at any time point represent the cumulative result of dynamic C allocation over time (**???**). This dynamic C allocation includes not only plant parts such as leaves, stems and roots but also respiration, exudation, turnover and transient C storage pools. 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 assessed in plants across “snapshots” in time, which should be done with caution (**???**). This is because of plants have developmental shifts in biomass partitioning, independent of resource supply, as they age (**???**, **???**). Additionally, supplies of light and soil resources fluctuate continuously, making equilibrium with allocation at any “snapshot” highly unlikely (**???**).

The allocation of photosynthetic C above and belowground is an important factor in terrestrial C cycling, yet our understanding of how global change impacts C allocation is incomplete (**???**, **???**). As accurately measuring tree C allocation remains a difficult task, especially belowground, drawing conclusions that are applicable to whole plants or ecosystems remains a challenge. Currently, the representation of C allocation lags behind photosynthesis (*An*) in process-based forest models (**???**, **???**, **???**). This lack of understanding of C allocation in trees is of major concern due to the potential for forest ecosystems to sequester C in a changing climate. This deficiency requires more empirical data to derive basic principles that drive patterns in tree C allocation in changing environements. However, this will require novel experiments and approaches to better quantify shifts in C allocation above and belowground in future studies.

*Coupling of photosynthesis and tree growth*

On short timescales, *An* and respiratory losses may not correlate with growth because of tissue C storage pools. On longer timescales, however, they determine net plant C balance and must correlate to growth. This had led to the long standing debate over how strongly plant growth is controlled by either source or sink activity (**???**, **???**). Studies manipulating either source activity (CO2 fumigation or defoliation) or sink activity (fruit removal, girdling or low growth temperatures) have not reached consensus when addressing this debate. This uncertainty arises from the difficulty in measuring the balance between C uptake and the fate of assimilated C among pools with long (biomass) or short (carbohydrate storage, respiration, exudation) retention times. When shifts in carbohydrate storage, tissue respiration or turnover rates occur, rates of C assimilation may not correlate with biomass production at a given time point (**???**, **???**, **???**). To assess this balance will likely require integration of empirical and modelling approaches to assess leaf physiological and whole plant responses to manipulations of source-sink activity. To address this knowledge gap, new approaches are needed to test how interactions between source and sink activity affect the fate of assimilate C across different temporal scales.

*Within canopy resource utilization*

Leaf photosynthesis has a saturating response with light and N is highly correlated with photosynthetic capacity. Consequently, it is commonly assumed that a limited availability of N should be distributed proportional to light availability within tree canopies. Observed canopy distribution of N is often less steep than optimal theory suggests, however, with shade leaves having more N than expected based on average light gradients (**???**). Additionally, constraints on water distribution from the soil to the upper canopy may negatively impact the distribution of photosynthetic N to canopy light availability (**???**, **???**). Whether insufficient hydraulic supply results in the observed sub-optimal canopy N gradients has yet to be empirically tested. Assessing leaf C gain as a function of light availability is also made difficult by frequent light fluctuations within a canopy, via sunflecks. Sunflecks cause temporal variation in PPFD that is not taken into account when considering what is optimal for a plant in terms of distributing resources along a gradient of light availability.

Leaves have been proposed to exhibit optimal physiological behavior in order to efficiently utilize and transport resources to maximize *An* (**???**). In trees, leaf physiology often focuses on full sun leaves and relationships between leaf physiological behavior and the availability of N, water and light between sun and shade leaves requires further attention. For example, gs has been hypothesized to be distributed within a canopy to utilize supplies of light, N and water to maximize *An* (**???**). In shade leaves, stomata might be expected to be more closed to efficiently use water with generally low *An*. To date, however, no clear picture has emerged on the relationship between gs and *An* within canopies (see **???**, **???**, **???**). As mesophyll conductance (gm) also limits *An*, complex relationships may exist between canopy light gradients, leaf N and gm. Unfortunately, a scarcity of values for gm within tree canopies (see **???**, **???**, **???**, **???**) hinders our ability to relate individual leaf physiological behavior to optimal canopy C uptake. As the CO2 drawdown from the atmosphere to the site of carboxylation includes gs and gm, relationships between *An* with light availability, N and water within canopies will require the integration of both physiological parameters in future experiments.

### Thesis Objectives

The overall research goal is to evaluate how trees adjust their growth and physiology to maximize resource uptake and C gain. Specifically, this PhD research addresses knowledge gaps of how tissue C allocation, source and sink regulation and resource distribution affect the coordination between *An* and whole tree growth. In order to investigate key mechanisms that drive patterns in resource allocation in trees this research was carried out across multiples scales, from leaf isotope discrimination across the photosynthetic CO2 flux pathway to tissue specific biomass partitioning to total belowground C allocation. Understanding how resource allocation is correlated with individual leaf physiological behavior within tree canopies is crucial in accurately determining the capacity for whole canopy C assimilation, which is the essential resource for tree growth. Aspects of this research use manipulations of key global change factors, including elevated CO2, warming and drought to investigate the plasticity of observed physiological and growth responses to future climate scenarios. An improved understanding of how C is allocated within trees will supply much needed empirical data for process-based forest models where C allocation is currently poorly represented.

This research focuses on two Australian tree species, *Eucalyptus tereticornis* and *Eucalyptus saligna*, which have important roles in both native forests ecosystems and as commercial plantation timber. *Eucalyptus tereticornis* is part of the critically endangered Cumberland Plain ecological community and *Eucalyptus saligna* is part of the critically endangered Blue Gum High Forest ecological community, with both communites having fragmented geographic distributions in the Sydney Basin bioregion (**???**). Both of these species are part of the “big nine” *Eucalyptus* species group which accounts for more than 90 % of planted *Eucalyptus* forests worldwide (**???**). As a result, the core findings of this PhD research have both conservation and commercial applications in addressing the productivity of these two important tree species in the face of global climate change. For example, considerable uncertainty remains as to the magnitude of CO2 fertilization on this continent as much of the vegetation is already under nutrient and/or water limitation (**???**).

This research was conducted using the state of the art Whole Tree Chamber experiment as well as a novel field-based seedlings container study at Western Sydney University. Using the two *Eucalyptus* tree species, fundamental principles of common optimization theories were tested at several growth stages. Leaf based data were combined with tissue biomass production and canopy C fluxes to develop a better understanding of how resources are allocated to optimize whole tree growth. Empirical data were also integrated with a seedling growth model to test how resource limitation impacts the coordination between A and growth. Specifically, this thesis aimed to address current knowledge gaps by answering the following main questions:

# References